ISSN 0116-6514

This issue contains selected papers presented at the 8th Asian Fisheries Forum Kochi, November 2007

Asian Fisheries Science

The Journal of the Asian Fisheries Society

Volume 22 No. 2
June 2009

Asian Fisheries Science

The Journal of the Asian Fisheries Society

Volume 22, Number 2, June 2009

Articles

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Low Genetic Differentiation in the Populations of the Malabar Carp *Labeo dussumieri* as revealed by Allozymes, Microsatellites and RAPD

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Abstract

The population structure of *Labeo dussumieri*, an endangered and endemic cyprinid from three riverine locations in the Western Ghats, India was investigated using allozyme, microsatellite and RAPD markers. *L. dussumieri* samples were obtained from Meenachil, Manimala and Pamba River basins, Kerala. Fourteen (46.7%) out of 30 allozyme loci, seven microsatellite loci and 12 RAPD Operon decamers gave polymorphic pattern. Six allozyme loci (*AAT-2*, EST-4*, GLDH*, GPI-2*, G_6PDH** and *LDH-2**) and three microsatellite loci (*LdussG1*, *MFW19* and *Bgon22*) exhibited consistent significant deviation from Hardy-Weinberg Equilibrium expectations in different populations after probability level ($P<0.05$) was adjusted for sequential Bonferroni correction. All the three marker types demonstrated concordant results and various estimates revealed genetic variability within the subpopulations but surprisingly low level ($\theta = 0.0034$ to 0.0081) of genetic differentiation among *L. dussumieri* from different river samples. AMOVA analysis also indicated low differentiation among subpopulations. No evidence for a recent genetic bottleneck was observed in *L. dussumieri* populations based on allozyme and microsatellite data set analysis. Meenachil, Manimala and Pamba Rivers open in to the southern end of Vembanad Lake in Kerala and are connected to each other in the lower reaches through an extensive network of natural canals. Common ancestry in the prehistoric period; and possible mixing of fish populations resulting in high gene flow across the rivers through the lake and interconnecting canals could have been responsible for the lack of significant allelic heterogeneity among the *L. dussumieri* populations. The stocks from the three rivers do not require different management strategies and for propagation assisted river ranching programme of this species, large effective breeding population can be developed by mixing individuals from three rivers.

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Introduction

Labeo dussumieri (Valenciennes, 1842) a cultivable food fish, popularly known as 'Malabar Labeo'; or 'thooli' or 'pullan' (in Malayalam) or 'hiri kanaya' (in Sinhalese) and belonging to the family Cyprinidae is endemic to the west flowing rivers originating from southern part of the Western Ghats, India and lowlands of Sri Lanka (Day, 1878; Silas, 1953; Smith and Jiffry, 1986; Talwar and Jhingran, 1991; Pethiyagoda, 1991; Jayaram and Dhas, 2000). In India, the species is one of the highly esteemed food fishes and commands a higher price as compared to the Indian major carps, especially in Kerala State (Padmakumar et al., 2004). To date, stock assessment of the species have not been made in different rivers, hence there is no information about the current exploitable potential of Malabar Labeo. In recent years, there has been a massive hunt for the species from the wild in India and its occurrence became sparse in the rivers (Kurup, 1990, 1998). Similarly, Pethiyagoda (1991) recorded decline of this species in Sri Lanka because of competition with the exotic tilapia. The workshop on Conservation Assessment Management Plan (CAMP) to evaluate the status of freshwater fishes of India, held in 1997 categorized this species as 'endangered' based on IUCN criteria due to restricted distribution, loss of habitat, over-exploitation, destructive fishing practises and trade (CAMP, 1998; Ponniah and Gopalakrishnan, 2000). Hence, *L. dussumieri* was short-listed for taking up stock-specific, propagation assisted rehabilitation programme in rivers where it is naturally distributed. In connection with this, captive breeding and milt cryopreservation techniques have been developed in this species by the National Bureau of Fish Genetic Resources (NBFGR) in collaboration with the Regional Agricultural Research Station (RARS) of the Kerala Agricultural University, Kumarakom, Kerala (Gopalakrishnan et al., 2000; Padmakumar et al., 2004). In order to devise adequate conservation and management strategies for an endangered species, it is important to investigate its population history, geographical partitioning throughout its natural distributional range; and distribution of genetic diversity within and among populations through genetically controlled markers. This can also help in scientific planning of propagation assisted rehabilitation programmes and monitoring their impact on natural gene pool. Natural genetic resources also form the basis for selection of founder stocks for stock improvement programmes. Kurup (1990) studied the biology, bionomics and other related aspects of *L. dussumieri*. Genetic studies on this species have been limited to karyotyping (Nagpure et al., 2003). The present work is a part of the integrated plan covering different aspects including captive breeding, development of sperm cryopreservation protocols, documenting life history traits and information on genetic markers as well as stock structure of *L. dussumieri* in Indian rivers.

Identification of genetic markers with scorable alleles is prerequisite to generate stock structure data of any species (Ferguson et al., 1995). Genetic methods have great potential to distinguish distinct populations or stocks of fish species that cannot be

identified by morphological and meristic characters (Cadrin et al., 2005). Allozyme and microsatellite markers have been used independently or collectively to document genetic diversity and to draw inference about population structure in finfishes and shellfishes in natural environments (Muneer et al., 2009; Chauhan et al., 2007; Salini et al., 2004) and to unearth population level evolution in variety of vertebrates (Chistiakov et al., 2006). The development of species-specific microsatellite primers for PCR amplification of alleles can be expensive and time consuming, as it involves construction of genomic libraries, screening of clones with microsatellite sequences and designing microsatellite primers (Scribner et al., 1996). However, the flanking sequences of microsatellite loci are highly conserved among related taxa so that primers developed for one species can be used to amplify homologous loci in related species (Gopalakrishnan et al., 2004; Zardoya et al., 1996). Successful amplification of homologous microsatellite loci has been demonstrated in many cyprinid fishes (Lal et al., 2004; Gopalakrishnan et al., 2004; Mohindra et al., 2001). RAPD methodology which involves DNA amplification using arbitrary short sequences has also proved to be useful in discriminating species and in detecting genetic variation in cultures and natural populations of fishes (Muneer et al., 2008; Nagarajan et al., 2006). In the present study, allozymes, microsatellites and RAPD markers were used to investigate the genetic structure of three geographically isolated riverine populations of *L. dussumieri* in the Western Ghat Biodiversity Hotspot region of India. The work was taken up to assess genetic variation and to understand the scale of population structure of *L. dussumieri* across its natural distribution range with an ultimate objective to support the breeding and restocking programme of this species in Indian rivers for conservation purpose.

Figure 1. Map showing the collection sites (★) of *Labeo dussumieri* from Kerala

Materials and Methods

Sampling

A total of 198 specimens (66 each from three rivers) were collected during July 2003 to October 2006 from commercial riverine catches from three different rivers (Meenachil, Manimala and Pamba) in southern Kerala, India (Fig. 1; Table 1). The weight of the specimens ranged from 400 to 1100 grams. Sampling procedures were performed at actual site of collection. Liver tissue samples were taken and frozen immediately in liquid nitrogen ($^{-}$196°C) for allozyme analysis. Blood samples for DNA extraction were collected from the caudal vein using heparinized syringes, fixed in 95% ethanol in 1:5 (blood: ethanol) and transported to the laboratory on ice and stored at 4°C until used for genomic DNA extraction. Liver samples transported to the laboratory were stored at $^{-}$80°C until analysis.

Table 1. Sample size, location and sampling period of *Labeo dussumieri* from three riverine locations in Kerala, India.

River system	Collection Site	Sampling Date	No. of specimens	Total samples (N)
Meenachil River	Cheepunkal, Kidangoor 09^0 41' 19" N 76^0 38' 30" E	16.07.2003	10 [a]	66
		12.09.2004	16 [a]	
		15.11.2004	15 [a]	
		11.07.2005	15 [a]	
		06.10.2006	10 [a]	
Manimala River	Kavumbhagom, Tiruvalla 09^0 22' 04" N 76^0 35' 24" E	04.02.2005	07 [b]	66
		14.04.2005	28 [b]	
		07.04.2006	11 [b]	
		01.06.2006	03 [b]	
		16.07.2006	17 [b]	
Pamba River	Parumala, Chengannoor 09^0 19' 19" N 76^0 32' 12" E	04.07.2005	32 [c]	66
		27.02.2006	20 [c]	
		19.06.2006	14 [c]	

Common superscripts indicate the multiple data sets within rivers that were pooled after testing for absence of heterogeneity.

Allozyme analysis

Frozen liver samples (approximately 100mg) were homogenized in 250 mg/mL extraction buffer (0.17 M Sucrose, 0.2 M EDTA, 0.2 M Tris-HCl, pH 7.0). Homogenized samples were centrifuged for an hour at 12,000 rpm at 4°C and the supernatant was recentrifuged for 30 min. Allelic variation was investigated using 7% polyacrylamide gel electrophoresis. Electrophoresis was carried out at constant voltage 150 V at 4°C. A total 23 enzyme systems were examined and 15 enzymes yielded scorable activity (Table 2). Histochemical staining procedures outlined by Whitmore (1990) were used to visualize different alleles. Loci and alleles were designated following the nomenclature system of Shaklee et al. (1990a, b).

Table 2. Names of enzyme loci, enzyme commission (E.C.) number and observed alleles for allozyme analysis in *Labeo dussumieri*.

Enzymes	E. C. Number	No. of loci	Locus	Alleles	Monomorphic/ Polymorphic
Aspartate amino transferase	2.6.1.1	2	*AAT-1**	*100*	Monomorphic
			*AAT-2**	*100,108, 117*	Polymorphic
Creatine kinase	2.7.3.2.	2	*CK-1**	*100*	Monomorphic
			*CK-2**	*100*	Monomorphic
Esterase	3.1.1.-	7	*EST-1**	*086, 100*	Polymorphic
			*EST-2**	*100*	Monomorphic
			*EST-3**	*100*	Monomorphic
			*EST-4**	*096, 100*	Polymorphic
			*EST-5**	*100*	Monomorphic
			*EST-6**	*100*	Monomorphic
			*EST-7**	*100*	Monomorphic
Glucose dehydrogenase	1.1.1.47	1	*GLDH**	*086, 100*	Polymorphic
Glucose phosphate isomerase	5.3.1.9	2	*GPI-1**	*100*	Monomorphic
			*GPI-2**	*088, 100*	Polymorphic
Glucose-6-phosphate dehydrogenase	1.1.1.49	1	*G_6PDH**	*095, 100, 120*	Polymorphic
α-Glycerophosphate dehydrogenase	1.1.1.8	2	*αG_3PDH-1**	*100*	Monomorphic
			*αG_3PDH-2**	*080, 100*	Polymorphic
Glyceraldehyde-3-Phosphate dehydrogenase	1.2.1.12	1	*GAPDH**	*090,100*	Polymorphic
Lactate dehydrogenase	1.1.1.27	3	*LDH-1**	*100*	Monomorphic
			*LDH-2**	*077, 100, 150*	Polymorphic
			*LDH-3**	*100*	Monomorphic
Malate dehydrogenase	1.1.1.37	2	*MDH-1**	*100*	Monomorphic
			*MDH-2**	*100*	Monomorphic
Malic enzyme	1.1.1.40	1	*ME**	*100*	Monomorphic
Octonol dehydrogenase	1.1.1.73	1	*ODH**	*085, 100, 115*	Polymorphic
Phosphogluco mutase	5.4.2.2	2	*PGM-1**	*075, 100*	Polymorphic
			*PGM-2**	*078, 100*	Polymorphic
Superoxide dismutase	1.15.1.1	2	*SOD-1**	*100*	Monomorphic
			*SOD-2**	*100, 125*	Polymorphic
Xanthine dehydrogenase	1.1.1.204	1	*XDH**	*100, 108*	Polymorphic

Table 3. Characteristics of polymorphic microsatellite loci in *Labeo dussumieri*.

	Resource species					*Labeo dussumieri*			
Sl. No.	Species & Reference	Locus	Primer sequence (5' → 3')	Repeat motif	Ta (°C)	Repeat motif	Ta (°C)	No. of alleles#	NCBI GenBank Accession Number
1	*Catla catla* (Naish and Skibinski, 1998)	*LdussG1*	F: AGCAGGTTGAT CATTTCTCC R: TGCTGTGTTTCAAATGTTCC	(GATA)n ... (CCA)n	61	(GATA)n	54	4	AF517937
2	*Cyprinus carpio* (Crooijmans *et al*., 1997)	*MFW19*	F: GAATCCTCCATCATGCAAAC R: CAAACTCCACATTGTGCC	(CA)n	55	(GGA)n	52	5	DQ780025
		MFW26	F: CCCTGAGATAGAAACCACTG R: CACCATGCTTGGATGCAAAAG	(CA)n	55	(CA)n	57	7	EU272894
3	*Barbodes gonionotus* (Kamonrat *et al*., 2002)	*Bgon22*	F: TCTTGTTGATCACACGGACG R: ACAGATGGGGAAAGAGAGCA	(CCT)n	55	(CCT)n	55	5	EU272893
4	*Barbus barbus* (Chenuil *et al*., 1999)	*Barb37*	F: AAATACGCTCTCCTCATTAC R: GTACAAAAGCAAAAATAAATTA	(ATTT)n	50	(AAAT)n	50	4	DQ780024
5	*Labeo rohita* (Das *et al*., 2005)	*R3-1**	F:TATTCACCCCAAATCCATTA R:GACCCTTGTGCATAAGACC	(TG)n	58	(TG)n	58	4	DQ780026
		R6	F:TATCCTGGCTGAAAACTTTG R:CCCCTACAGGAACAACCAT	(TG)n	56	(TG)n	56	5	DQ780027

*Primer sequence of *R3* given by Das *et al*. (2005) was modified using PRIMER3 in the present study.

Microsatellite analysis

Genomic DNA was extracted from blood following a protocol modified from Ruzzante et al. (1996), using proteinase K, and phenol: chloroform. For microsatellite analysis to obtain polymorphic loci, 36 primers (microsatellite flanking regions) developed for cyprinid fishes *Catla catla* (Naish and Skibinski, 1998), *Cyprinus carpio* (Crooijmans et al., 1997), *Barbodes gonionotus* (Kamonrat et al., 2002; McConnell et al., 2001), *Barbus barbus* (Chenuil et al., 1999) *Labeo rohita* (Das et al., 2005), *Campostoma anomalum* (Dimsoski et al., 2000) and *Pimephales promelas* (Bessert and Orti, 2003) were examined for cross-priming in ten individuals of *L. dussumieri*. In this study, PCR amplification was performed in a 25 µl reaction mixture, that included 1 X PCR buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.01% gelatin), 0.2 mM of each dNTP, 2.0 mM of $MgCl_2$, 5 pmol of each primer, 1.5 U Taq DNA polymerase and 25-50 ng of template DNA. PCR (MJ Research PTC-200 thermal cycler) cycles were as follows (i) 1 cycle of denaturation of 94°C for 5 min, (ii) 25 cycles of denaturation at 94°C for 30 seconds relevant annealing temperature for 30 seconds, elongation at 72°C for 1 min, (iii) a final elongation of 1 cycle at 72°C for 4 min and stored at 4°C. PCR products were resolved through vertical non-denaturing polyacrylamide (19:1 acrylamide: bisacrylamide) gels electrophoresis (size 10.0 x 10.5 cm, Amersham Biosciences Ltd.). Electrophoresis was done with 1X TBE buffer for 5.30 hours at 10 v/cm at 4°C. Gel concentrations and annealing temperatures (Table 3) were optimized to obtain clear scorable allelic banding patterns. Amplified microsatellite loci were visualized via silver staining (silver staining kit, Amersham Biosciences, USA). Alleles were designated according to PCR product size, calculated relative to a molecular marker (pBR322 DNA/ *Msp*I digest) with Image master 1D Elite v3.01 (Amersham Biosciences, USA). A non-denaturing electrophoresis system has been found to provide the same resolution as that obtained with denaturing acrylamide gels and silver staining with the additional advantage of ease of use for analyzing large sample sizes (Wang et al., 2003). Moreover, Bovo et al. (1999) demonstrated that non-denaturing electrophoresis is not responsible for spurious or multiple bands in microsatellite analysis. Seven of the 36 loci screened (Table 3) gave clear scorable products with 4-7 alleles per locus. These seven polymorphic loci were finally analysed to confirm the occurrence of repeats through cloning and sequencing.The microsatellite products were run and eluted from 2% agarose gel, purified and ligated into TOPO vector (Invitrogen, Carlsbad, USA) and the ligation product was transformed into competent *Escherichia coli* DH5α cells. The transformed cells were cultured for 12-16hours at 37°C in LB media plate containing 50µg/mL ampicillin coated with 40mL X-gal (20mg/mL) and 4µL IPTG (200mg/mL). The transformants containing inserts were selected by blue/white screening (Sambrook et al., 1989). The recombinant plasmids were isolated by alkaline lysis method (Sambrook

et al., 1989) and were further purified through PEG precipitation for sequencing purpose. The sequencing was done in forward and reverse directions using ABI Prism Big Dye Terminator cycle sequencing ready reaction kit in the automated DNA sequencer ABI 3730 (Applied Biosystems, USA) according to manufacturers instructions. All microsatellite sequences isolated from *L. dussumieri* have been submitted to the NCBI GenBank (Fig. 3).

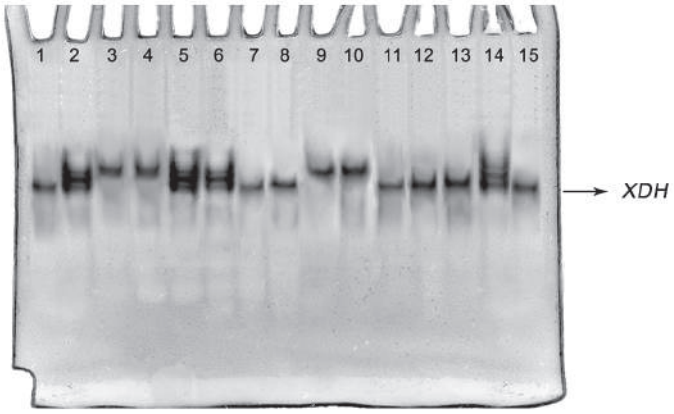

Figure 2. Xanthine dehydrogenase (*XDH*) pattern in *Labeo dussumieri*.
Lanes 1-5 samples from Meenachil, 6-10 Manimala and 11-15 Pamba Rivers.

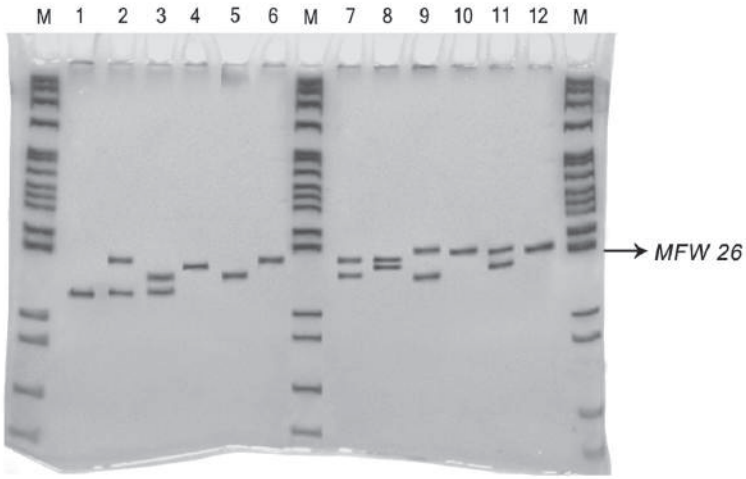

Figure 3. Microsatellite pattern of locus *MFW26* in *Labeo dussumieri*.
Lanes 1-4 samples from Meenachil, 5-8 Manimala and 9-12 Pamba Rivers.
M - molecular weight marker (pBR322 with *Msp*I cut)

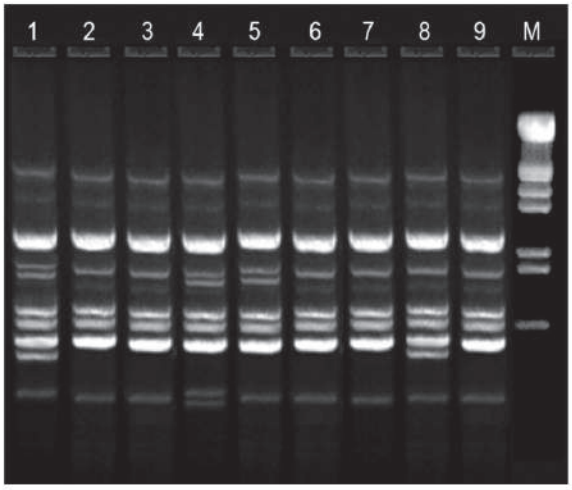

Figure 4. RAPD pattern of *Labeo dussumieri* with primer OPAH-09.
Lanes 1-3 samples from Meenachil, 4-6 Manimala and 7-9 Pamba Rivers.
M- molecular weight marker (λDNA with *Eco*R1 & *Hin*dIII double digest)

RAPD analysis

Eighty decamer primers from Operon Technologies, Alameda, USA (OPA1-20, OPAA1-20, OPAC1-20, OPAH1-10 and OPB1-10) were used for RAPD analysis. From these, 12 primers were selected for population genetic analysis taking into consideration of the repeatability, sharpness, intensity and polymorphic nature of the bands (Table 10). PCR amplifications were performed in a PTC 200 thermal cycler (M. J. Research, Inc., Watertown, USA) in 25µL reactions containing 1X reaction buffer [100mM Tris, 500mM KCl, 0.1% gelatin, pH9.0) with 1.5mM $MgCl_2$ (Genei, Bangalore, India), 6-8 pmoles of primer, 200 mM dNTPs, 2U *Taq* DNA polymerase (Genei, Bangalore, India] and 25ng of template DNA. To check DNA contamination, a negative control was made omitting template DNA from the reaction mixture. The reaction mixture was pre-heated at 95°C for 3 minutes followed by 40 cycles (94°C for 3 minutes, 40°C for 1.30 minutes and 72°C for 2 minutes). The reaction was then subjected to a final extension at 72^0C for 10 minutes. The resulting products were electrophoretically analysed through 1.5% agarose gels stained with ethidium bromide (5 µg/mL) in TBE buffer (90 mM Tris–borate and 2 mM EDTA, pH 8.0) and photographed using Image master VDS gel documentation system (Amersham Biosciences, USA). A known DNA size marker was run with every gel (λ DNA with *Eco*RI/*Hin*dIII double digest). The bands were designated according to the PCR product size in relation to the marker with Image master ID Elite v 3.01(Amersham Biosciences, USA).

Data Analysis

Individual fish genotypes at each allozyme and microsatellite locus were determined. These data were then analyzed for homogeneity between data sets for collections at different times within each river. Data sets within each river or neighboring tributaries that were not heterogenous (P>0.05) were later combined for further analysis for estimating genetic variation and differentiation parameters. A locus was considered polymorphic, if the frequency of the most common allele was less than or equal to 0.99 (Hartl and Clark, 1997). Allele frequencies and heterozygosity (observed and expected) values were calculated using Genetix ver. 4.05 software (Belkhir et al., 1997). Tests for conformity to Hardy–Weinberg expectations (probability test) and linkage disequilibrium were undertaken in Genepop ver.3.3d software (Raymond and Rousset, 1998). Fixation indices based on an infinite allele model (IAM, Kimura and Crow, 1964) and a stepwise mutation model (SMM, Kimura and Ohta, 1978) were estimated to determine the extent of population subdivision among samples in a quantitative way. Estimation of average F_{ST} and determining whether the values are significantly different from zero; and calculation of pair-wise population F_{ST} values (θ) of Weir and Cockerham (1984) and their significance levels, were carried out using Genetix ver. 4.05 software (Belkhir et al., 1997). Probability of è significantly deviating from zero was calculated using 1000 bootstraps. Under a SMM, estimates of R_{ST} (Slatkin, 1995) were made (only for microsatellite data - based on allele sizes) using the Genepop ver. 3.3d software. To correct for multiple simultaneous comparisons, sequential Bonferroni corrections were applied using a global significance level of 0.05 (Lessios, 1992). Microsatellite genotype data of the loci with known inbreeding coefficient or fixation indices ($+F_{IS}$) were tested for the expected frequency of null alleles according to Van Oosterhout et al. (2004, 2006) using MICRO-CHECKER (http:// www.microchecker.hull.ac.uk/) and thereafter analyzed for population differentiation. Genetic similarity/identity and distance between pairs of populations of *L. dussumieri* were estimated using POPGENE Version 1.31 (Yeh et al., 1999). Nei and Li's (1979) pair-wise genetic similarity (SI) among these specimens were computed and converted by POPGENE into genetic distance (GD) according to Hillis and Moritz's (1990) formula, GD = 1- SI. The partitioning of genetic variation among and within populations of *L. dussumieri* was calculated by hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) at 1000 permutations. The hierarchical components of genetic variation include (1) variance due to differences between individuals within a river; and (2) variance due to differences among populations. The AMOVA calculations were performed using ARLEQUIN v2.0 (Schneider et al., 2000; http://lgb.unige.ch/arlequin/). The Cornuet and Luikart (1996) programme BOTTLENECK ver. 1.2.02 was used to detect recent effective population size reduction (to assess the impact of population decline) using data from the allozymes under IAM and microsatellites under the more suitable two-phased model (TPM), in addition to IAM. BOTTLENECK detects past population reductions by testing for a transient

excess in measured heterozygosity compared with the heterozygosity expected at mutation-drift equilibrium (He>Heq). Excess in heterozygosity is generated because rare alleles are quickly lost due to drift during a bottleneck, but they contribute little to the expected heterozygosity (Luikart and Cornuet, 1998). To determine whether the 3 riverine populations of *L. dussumieri* exhibited a significant number of loci with gene diversity excess, "Wilcoxon Sign-Rank Tests" were employed in BOTTLENECK. In addition, a qualitative descriptor of the allele frequency distribution ("mode-shift" indicator) was also employed to discriminate bottlenecked populations from stable populations.

For the RAPD data, the images of gels were used to analyze the banding patterns. A binary matrix was produced whereby the presence or absence of each DNA fragment for each sample was recorded 1 or 0, respectively. Faint or poorly amplified fragments were excluded from the analysis as were fragments with very high (above 6500bp) or low (below 800bp) molecular weight. The analysis was based on few assumptions. First, all RAPD fragments scored represented 2-allele system, *i.e.*, presence (dominant) and absence (recessive) of bands. Second, fragments that migrated at the same position, had the same molecular weight, and stained with the same intensity were homologous bands from the same allele, and the alleles from different loci did not co-migrate. A third assumption was that the populations fit the Hardy-Weinberg equilibrium (Clark and Lanigan, 1993; Lynch and Milligan, 1994). From the binary matrix, the total number of RAPD fragments and polymorphic ones were calculated for each primer and for all primers. Genetic variability in three populations of *L. dussumieri* was estimated from the gene (allele) frequencies and percentage of polymorphic loci (%P) using POPGENE version 1.31 (Yeh et al., 1999). Tests for population differentiation were performed by calculation of G_{ST} and Nei's genetic distance between pairs of populations and for overall population using POPGENE version 1.31(Yeh et al., 1999).

Table 4. Distribution of dimeric *G_6PDH* genotypes in male and female *Labeo dussumieri* from different river systems.

Locus	Genotypes (Alleles & Rf value)	No. of individuals					
		Meenachil River		Manimala River		Pamba River	
		Male	Female	Male	Female	Male	Female
G_6PDH	*AA* (100/100)	14	09	18	08	10	14
	AB (095/100)	08	06	02	06	08	02
	AC (100/120)	06	03	04	03	03	01
	BB (095/095)	05	01	04	05	04	07
	BC (095/120)	04	03	01	02	02	02
	CC (120/120)	04	03	06	07	10	03

Table 5. Allozyme alleles and allele frequencies in *Labeo dussumieri* from three riverine populations and among populations.

Locus	Alleles	Meenachil River	Manimala River	Pamba River	Overall Populations
AAT-2	*100*	0.7270	0.7046	0.6800	0.7039
	108	0.1782	0.1811	0.1971	0.1855
	117	0.0948	0.1143	0.1229	0.1106
EST-1	*086*	0.3200	0.3214	0.4257	0.3557
	100	0.6800	0.6786	0.5743	0.6443
EST-4	*096*	0.4143	0.4495	0.4714	0.4451
	100	0.5857	0.5505	0.5286	0.5549
GLDH	*086*	0.4571	0.4143	0.3714	0.4143
	100	0.5429	0.5857	0.6286	0.5857
GPI-2	*088*	0.4071	0.3857	0.3753	0.3894
	100	0.5929	0.6143	0.6247	0.6106
G_6PDH	*095*	0.1400	0.0740	0.1000	0.1047
	100	0.7571	0.8143	0.8286	0.8000
	120	0.1029	0.1117	0.0714	0.0953
GAPDH	*090*	0.4071	0.4357	0.3714	0.4047
	100	0.5929	0.5643	0.6286	0.5953
αG_3PDH-2	*080*	0.2429	0.2643	0.2922	0.2665
	100	0.7571	0.7357	0.7078	0.7335
LDH-2	*077*	0.2000	0.2000	0.2714	0.2238
	100	0.5857	0.5143	0.5000	0.5333
	150	0.2143	0.2857	0.2286	0.2429
ODH	*085*	0.1571	0.1857	0.1714	0.1714
	100	0.5429	0.5143	0.6286	0.5619
	115	0.3000	0.3000	0.2000	0.2667
PGM-1	*075*	0.3714	0.4086	0.4143	0.3981
	100	0.6286	0.5914	0.5857	0.6019
PGM-2	*078*	0.4286	0.4445	0.4379	0.4370
	100	0.5714	0.5555	0.5621	0.5630
SOD-2	*100*	0.6357	0.6500	0.5857	0.6238
	125	0.3643	0.3500	0.4143	0.3762
XDH	*100*	0.5925	0.5643	0.6086	0.5885
	108	0.4075	0.4357	0.3914	0.4115

Number of specimens (n) = 66 from each river.

Results

Genetic variability

Of the 23 allozymes examined, 15 enzymes (30 loci) yielded scorable activity and of these 14 loci – *AAT-2*, EST-1*, EST-4*, GLDH*, GPI-2*, G_6PDH*, aG_3PDH-2*, GAPDH*, LDH-2*, ODH*, PGM-1*, PGM-2*, SOD-2** and *XDH** were polymorphic (46.7%) in *L. dussumieri* (Table 2; Fig. 2). Unlike in humans G_6PDH did not exhibit sex-linked inheritance in *L. dussumieri*. Both male and female specimens from all three rivers exhibited both homozygotes and heterozygotes. A sex-wise break up of G_6PDH is

given in Table 4. Seven out of the 36 microsatellite loci screened (Table 3; Fig. 3) gave clear scorable products with 4-7 alleles per locus. These seven polymorphic loci – *LdussG1* (AF517937), *MFW19* (DQ780025), *MFW26* (EU272894), *Bgon22* (EU272893), *Barb37* (DQ780024), *R3-1* (DQ780026), *R6* (DQ780027) - were confirmed to contain repeats and further considered for the population genetic analysis of *L. dussumieri.* Among these, two loci contained perfect repeats (*LdussG1* and *R3-1*) and sequence information of all the loci is presented in Fig. 5. The tandem repeats of five loci were same as that of the resource species while repeat motif of the locus *MFW19* and *Barb37* differed from that of the resource species. No significant genotype heterogeneity was observed between the multiple data sets (collections at different time intervals) within the rivers (Table 1). After combining the genotypic data from multiple data sets within each river, three data sets for the rivers - Meenachil, Manimala and Pamba were available for analysis of genetic variation and differentiation among *L. dussumieri* populations. Allele frequencies at polymorphic allozyme and microsatellite loci in *L. dussumieri* samples from the three riverine locations are presented in Tables 5 and 6 respectively. No population-specific alleles were observed for any allozyme or microsatellite locus. All the seven microsatellite loci exhibited considerable variation in all the sampled populations. In RAPD analysis, the PCR reagents were from the same source (GENEI Ltd., Bangalore, India) and the template DNA quantity and concentration were kept uniform across the samples (1 mL and circa 25 ng respectively per single reaction). This resulted in high level of reproducibility and sharpness of RAPD profiles in *L. dussumieri* even from the DNA sample that was stored at -20°C for more than six months, demonstrating the robustness of the technique (Fig. 4). A total of 117 random amplified DNA fragment from specimens of *L. dussumieri* were detected consistently with the 12 decamer primers in three populations. The size of the fragment ranged from 200 to 3000bp. The number of fragments generated per primer varied from 06 to 14 and altogether 65 bands (55.56%) were found to be polymorphic (Table 10). The average gene diversity (H) for each primer in each population ranged from 0.000 (OPA-07) to 0.2380 (OPAA-08) and the mean value for overall populations was 0.1848 (Table 14). No test for linkage disequilibrium was statistically significant (P>0.05) for any pair of allozyme or microsatellite or RAPD loci within each of the sample sites and when all samples were considered together.

Summary statistics for parameters of genetic variation at each allozyme and microsatellite locus and across all loci are given in Tables 7 and 8 respectively. Mean number of alleles per locus ranged from 1.326 to 1.342 for allozyme loci and 3.000 to 3.750 for microsatellite loci. Mean values of observed heterozygosity ranged from 0.1445 to 0.1898 for allozyme loci and from 0.4948 to 0.5360 for microsatellite loci respectively. Six allozyme loci (*AAT-2*, EST-4*, GLDH*, GPI-2*, G_6PDH** and *LDH-2**) and three microsatellite loci (*LdussG1, MFW19* and *Bgon22* in all samples from three rivers) (Tables 7 and 8) exhibited consistent significant deviation from Hardy-Weinberg

Equilibrium expectations in populations after probability level (P<0.05) was adjusted for sequential Bonferroni correction. Significant deviation at the *AAT-2** and *GLDH* loci was found in all samples from three rivers; at locus *EST-4** and *LDH-2** only in the Meenachil and Manimala samples; and for the remaining two loci (*GPI-2** and G_6PDH) only in the Manimala and Pamba samples. F_{IS} values greater than zero (+ve) indicating deficiency of heterozygotes was evident in these cases (Tables 7 and 8). The three microsatellite loci (*LdussG1*, *MFW19* and *Bgon22*) exhibiting $+F_{IS}$ values were tested

Table 6. Microsatellite alleles and allele frequencies in *Labeo dussumieri* from three riverine populations and overall populations.

Locus	Allele size (bp)	Meenachil	Manimala	Pamba	Overall Populations
LdussG1	079	0.2323	0.2986	0.2753	0.2687
	087	0.4357	0.3925	0.4250	0.4177
	099	0.1028	0.1106	0.1201	0.1112
	115	0.2292	0.1983	0.1796	0.2024
MFW19	160	0.1818	0.1397	0.1714	0.1643
	172	0.2528	0.2750	0.2365	0.2548
	187	0.3275	0.3249	0.3369	0.3298
	196	0.1454	0.1479	0.1297	0.1410
	205	0.0925	0.1125	0.1255	0.1101
MFW26	145	0.2375	0.2222	0.2426	0.2341
	149	0.0425	0.0532	0.0698	0.0552
	155	0.0125	0.1103	0.0700	0.0643
	163	0.2053	0.2217	0.2021	0.2097
	171	0.2592	0.1263	0.1915	0.1923
	185	0.1063	0.1542	0.1253	0.1286
	193	0.1367	0.1121	0.0987	0.1158
Barb37	146	0.0963	0.1382	0.1179	0.1175
	162	0.2626	0.3250	0.3123	0.2999
	182	0.3613	0.2635	0.2845	0.3031
	206	0.2798	0.2733	0.2853	0.2795
Bgon22	118	0.1964	0.1673	0.1855	0.1831
	127	0.0625	0.0526	0.0723	0.0624
	136	0.2432	0.2516	0.2614	0.2521
	145	0.3743	0.4099	0.3476	0.3773
	157	0.1236	0.1186	0.1332	0.1251
R3-1	109	0.2549	0.2613	0.2984	0.2715
	117	0.3152	0.3048	0.2902	0.3034
	133	0.2637	0.1994	0.2349	0.2327
	145	0.1662	0.2345	0.1765	0.1924
R6	166	0.0889	0.0867	0.0728	0.0828
	174	0.0497	0.0381	0.0129	0.0336
	182	0.2163	0.3250	0.2843	0.2752
	194	0.3653	0.2647	0.3347	0.3215
	198	0.2798	0.2855	0.2953	0.2869

Number of specimens (n) = 66 from each river.

for the occurrence of null alleles. The estimated null allele frequency using MICRO-CHECKER was not significant (P<0.05) at all the three tested loci in all populations (Table 9). There was also the absence of general excess of homozygotes over most of the allele size classes in all the three loci in different rivers and no instance of non-amplification of samples in repeated trials - all indicating the absence of null alleles and false homozygotes. Therefore, for population genetic analysis, information from all the seven microsatellite loci was considered.

LdussG1 (NCBI GenBank Accession # AF517937)

AAAGAATATT TGGGAAAAAG AACAGAGTTG CACCACAGGA AAGGAACA**GA**
TAGATAGATA GATAGATAGA TACAATTCA

Barb37 (NCBI GenBank Accession # DQ780024)

AAATACGCTC TCCTCATTAC TGTGG**AAATA AATAAAT**GTG G**AAATAAATA**
AATGTCT**AAA TAAATAAATA AATAAATAAA T**CTGGAAA**AA ATAAATAAAT**
AAGG**AAAT**AA **AATAAATAAA TAAAT**AATTT ATTTTTGCTT TTGTAC

MFW19 (NCBI GenBank Accession # DQ780025)

AGCCTAGGCT CGAGAAGCTT GTCGACGAAT TCAGATTACA GATGGGGAAA
GAGAGCATCC GAGCGTGACT GTACAATGAG TCTGCTAATT ACTTCATTCC
GGAGGAGGAG GAGGAGGAGG AGGAGGAGGA GCTCCGTGTG ATCAACAAGA
AATCACGAAT

R3-1 (lower locus) (NCBI GenBank Accession # DQ780026)

TATTCACCCC AAATCCATTA TCATTA**GTGT GTGTGTGTGT GTGTGTGTGT**
GTGTGTGAGA GCTGTCCTGG CCTGGGTGTT TGGCTGTGGT GGTCTTATGC
ACAAGGGTC

R6 (NCBI GenBank Accession # DQ780027)

TATCCTGGCT GAAAACTTTG CTGAAAACTT TGGAGGGAAA TG**GTGTGTGT**
CTC**GTGTGTG TGTGTGTGT**G ACGCTCAATG ATTTTCGGAA TCAGTAAAGT
AAAATTTTGT GTCTTTCTCA CAGCCTTTGT GGCTGTCTCC AGTGCAGGTG
ATGGTTGTTC CTGTAG

Bgon22 (NCBI GenBank Accession # EU272893)

TCTTGTTGAT CACACGGACG TCC**CCTCCT**T **CCTCCTCCTC CTCCTCCTCC**
TCCTCCTCCT CCTGGAATGG AATAATATTG ATACAGTGCT CGGATGCTCT
CTTTCCCCAT CTGTGTAA

MFW26 (NCBI GenBank Accession # EU272894)

CCCTGAGATA GAAACCACTG GACATTAATT AACAACCAGA TTAATTGTCT
TTTTAAA**CAC ACACACA**G**CA CACACACACA** A**CACACACAC ACACACACA**C
TGTGGAAATT TCCACGTTTC TTTTCTTTTG CATCCAAGCA TGGTG

Figure 5. Nucleotide sequences of seven microsatellite loci in *Labeo dussumieri*. Microsatellite regions are depicted in bold letters.

Population structure

The coefficient genetic differentiation (F_{ST}) under IAM model was consistent for both allozyme and microsatellite markers. The mean F_{ST} value across all the populations and all loci was 0.0034 (allozymes) and 0.0041 (microsatellites). Pair-wise comparisons of F_{ST} with probabilities of significance are given in Tables 11 and 12. Fixation indices under SMM Model (R_{ST}) for microsatellite loci were found to be comparable with F_{ST} values in pair-wise comparisons of samples (Table 12). The G_{ST} values were low for each RAPD primer across all populations (ranging from 0.0000 to 0.0115) and for overall populations (0.0081) (Table 13). Nei's (1978) unbiased genetic distance values estimated between pairs of three populations of *L. dussumieri* with allozymes, microsatellites and RAPD markers were also very low (Tables 14 and 15). AMOVA analysis revealed that 99.12% (allozymes) and 98.60% (microsatellites) variance was explained within populations/within rivers (Table 16). There was evidence of only very weak genetic differentiation among different populations of *L. dussumieri* sampled from three river basins.

Bottleneck analysis

The bottleneck results based on allozymes (IAM) and microsatellites (TPM) exhibited the expected L-shaped distribution indicating that all the populations followed mutation drift equilibrium (Fig. 6). The probability values (Sign and Wilcoxon Tests) also indicated absence of genetic bottleneck in *L. dussumieri* populations (Table 17).

Table 7. Parameters of genetic variation for the fourteen polymorphic allozyme loci in *Labeo dussumieri* from three different riverine locations.

Locus	Populations (n = 66 each)		
	Meenachil River	Manimala River	Pamba River
AAT-2			
H obs.	0.2429	0.3429	0.1857
H exp	0.3907	0.5710	0.2535
F_{IS}	+0.385	+0.406	+0.268
P_{HW}	<0.0001***	<0.0001***	<0.0001***
EST-1			
H obs.	0.4771	0.4272	0.5214
H exp	0.4800	0.4362	0.4996
F_{IS}	+0.005	+0.002	-0.139
P_{HW}	1.0000	1.0000	1.0000
EST-4			
H obs	0.0797	0.1856	0.4562
H exp	0.1868	0.3150	0.4984
F_{IS}	+0.284	+0.310	+0.090
P_{HW}	<0.0001***	<0.0001***	0.4799
GLDH			
H obs.	0.2143	0.1948	0.1429
H exp	0.3367	0.3082	0.3024
F_{IS}	+0.265	+0.323	+.0664
P_{HW}	<0.0001***	<0.0001***	<0.0001***
GPI-2			
H obs.	0.3976	0.2855	0.1143
H exp	0.4193	0.3976	0.2971
F_{IS}	+0.072	+0.227	+0.311
P_{HW}	0.4228	<0.0001***	<0.0001***

G_6PDH			
H obs	0.2585	0.2414	0.1714
H exp	0.3678	0.3253	0.2212
F_{IS}	+0.230	+0.451	+0.316
P_{HW}	0.0970	<0.0001***	<0.0001***
αG_3PDH-2			
H obs	0.2612	0.3000	0.3714
H exp	0.2678	0.3374	0.3770
F_{IS}	+0.009	+0.068	+0.002
P_{HW}	1.0000	0.6997	1.0000
GAPDH			
H obs	0.1811	0.2000	0.1823
H exp	0.1909	0.2323	0.1877
F_{IS}	+0.009	+0.032	+0.002
P_{HW}	1.0000	0.5099	1.0000
LDH-2			
H obs	0.2857	0.2571	0.3618
H exp	0.3298	0.2996	0.4853
F_{IS}	+0.151	+0.091	+0.241
P_{HW}	<0.0001***	<0.0001***	0.0513
ODH			
H obs	0.4755	0.3857	0.4213
H exp	0.4963	0.3696	0.4669
F_{IS}	+0.086	-0.134	+0.150
P_{HW}	0.0926	0.2209	0.1036
PGM-1			
H obs	0.2400	0.3429	0.1925
H exp	0.2669	0.3898	0.2053
F_{IS}	+0.077	+0.142	+0.099
P_{HW}	0.0121*	0.0143*	0.0201*
PGM-2			
H obs.	0.1857	0.4000	0.1400
H exp.	0.1898	0.4291	0.1705
F_{IS}	+0.001	+0.005	+0.090
P_{HW}	1.0000	1.0000	0.3118
SOD-2			
H obs.	0.4286	0.3000	0.3143
H exp.	0.4632	0.4415	0.4553
F_{IS}	+0.297	+0.226	+0.329
P_{HW}	0.0192*	0.0014*	0.0033*
XDH			
H obs	0.2909	0.4515	0.2432
H exp	0.3228	0.4917	0.2955
F_{IS}	+0.010	+0.028	+0.024
P_{HW}	0.3211	0.5278	0.4646
Mean overall loci			
H obs	0.1660	0.1898	0.1445
H exp	0.2128	0.2355	0.2135
Fis	---	---	---
$P_{(0.95)}$	0.2486	0.3142	0.3482
$P_{(0.99)}$	0.2468	0.3142	0.3482
A_n	1.3261	1.3354	1.3421

H obs. = Observed heterozygosity; H exp. = Expected heterozygosity; F_{IS} = Inbreeding coefficient; P_{HW} = Probability value of significant deviation from HWE; $P_{(0.95)}$ = Polymorphism at 0.95 criteria; $P_{(0.99)}$ = Polymorphism at 0.99 criteria; A_n = Mean number of alleles per locus; * = Significant at P<0.05; ***= Significant after Bonferroni adjustment.

Discussion

The present study reports the distribution and patterns of genetic variation in the natural population of *L. dussumieri* estimated from allozyme, microsatellite and RAPD markers. Ruzzante (1998) demonstrated that sample sizes larger than 50 individuals are adequate to minimize bias due to large number of alleles in microsatellite data and Silva and Russo (2000) inferred that sample size should be more than 30; in the present study sample size was 66 individuals per site in three localities analyzed. Therefore, estimates of population differentiation obtained, are unlikely to be confounded by small sample sizes.

Table 8. Parameters of genetic variation for the seven microsatellite loci in *Labeo dussumieri* from three different rivers.

Locus	Populations (n= 66 each)		
	Meenachil	Manimala	Pamba
LdussG1			
H obs.	0.6571	0.6310	0.5501
H exp.	0.7976	0.7832	0.6308
Fis	+0.3292	+0.2741	+0.3703
P_{HW}	<0. 0001***	<0. 0001***	<0. 0001***
MFW19			
H obs	0.6943	0.6927	0.5571
H exp	0.7652	0.7479	0.6604
Fis	+0.3344	+0.0470	+0.1631
P_{HW}	<0. 0001***	<0. 0001***	<0. 0001***
MFW26			
H obs	0.3336	0.2743	0.2571
H exp	0.3261	0.2767	0.2743
Fis	-0. 0123	+0.004	+0.0031
P_{HW}	0.9867	1.0000	0.8842
Barb37			
H obs	0.6098	0.5857	0.5671
H exp	0.5894	0.5539	0.6037
Fis	-0.0193	-0.0225	+0.0676
P_{HW}	0.9862	1.0000	0.0741
Bgon22			
H obs	0.3857	0.3180	0.3286
H exp	0.5291	0.4168	0.4321
Fis	+0.3748	+0.2143	+0.2827
P_{HW}	0.0111*	0.0122*	<0.0001***

R3-1			
H obs	0.7429	0.7387	0.6014
H exp	0.6871	0.7236	0.5644
Fis	-0.0743	-0.0254	-0.0834
P_{HW}	0.8072	1.0000	0.7828
R6			
H obs	0.6143	0.5802	0.6071
H exp	0.5949	0.5674	0.6098
Fis	-0.0318	-0.0022	+0.004
P_{HW}	0.9517	0.9687	1.0000
Mean Overall Loci			
H obs	0.4948	0.5360	0.5239
H exp	0.5067	0.5996	0.5619
Fis	---	---	---
$P_{(0.95)}$	0.8684	0.7896	0.7143
$P_{(0.99)}$	0.8684	0.7896	0.7143
A_n	3.7500	3.0000	3.3750

H obs = Observed heterozygosity; H exp = Expected heterozygosity; F_{IS} = Inbreeding coefficient; P_{HW} = Probability value of significant deviation from HWE; $P_{(0.95)}$ = Polymorphism at 0.95 criteria; $P_{(0.99)}$ = Polymorphism at 0.99 criteria; A_n = Mean number of alleles per locus; * = Significant at P<0.05; *** = Significant after Bonferroni adjustment.

Table 9. Summary statistics of null allele frequencies in microsatellite loci of *Labeo dussumieri.*

Microsatellite Locus	Populations showing positive F_{IS} values	Null allele frequency * (from MICRO-CHECKER)			
		Van Oosterhout	Chakraborty	Brooksfield 1	Brooksfield 2
LdussG1	Meenachil	0.0313	0.0402	0.0411	0.0411
	Manimala	0.0293	0.0278	0.0281	0.0281
	Pamba	0.0253	0.0189	0.0218	0.0218
MFW19	Meenachil	0.0043	0.0038	0.0048	0.0048
	Manimala	0.0116	0.0095	0.0108	0.0108
	Pamba	0.0163	0.0171	0.0188	0.0188
Bgon22	Meenachil	0.0380	0.0414	0.0373	0.0373
	Manimala	0.0013	0.0014	0.0017	0.0017
	Pamba	0.0025	0.0027	0.0032	0.0032

(* P< 0.05)

For allozyme loci, genetic variability in *L. dussumieri* was relatively high (mean H_o for overall loci 0.1445 - 0.1898), when compared with the values described for many freshwater teleosts (0.043 - Gyllensten, 1985; 0.046 - Ward et al., 1994). As reported for several vertebrates (Nevo et al., 1984) and plants (Frankham, 1996), populations of widespread species often show significantly higher heterozygosity

estimates than for population of species with more restricted distribution; as observed in the widespread and opportunistic European roach *Rutilus rutilus* (H_e= 0.0972 - 0.124; Bouvet et al., 1991) and mrigal, *Cirrhinus mrigala* in the Indian sub-continent (H_e= 0.1082 - 0.1224; Chauhan et al., 2007) in contrast to the endemic and rare species such as *Leuciscus* species (H_e= 0.0000 - 0.057). However, higher heterozygosity rates are also reported using allozyme markers in endemic teleosts with restricted distribution. Muneer et al. (2007) and Gopalakrishnan et al. (2006) in the endemic bagrid fish, *Horabagrus brachysoma* (H_o= 0.170 - 0.191); Musammilu (2008) in an endangered cyprinid, *Gonoproktopterus curmuca* (H_o= 0.145 - 0.156) and Tessier et al. (1995) in landlocked Atlantic salmon (H_e= 0.18 - 0.42) have recorded high heterozygosity rates as reported in the present study. Genetic variability estimates for *L. dussumieri* using microsatellite loci (H_o= 0.4948 - 0.5360; 4 - 7 alleles per locus) closely approximate the values reported for many freshwater fishes (H_o= 0.38 - 0.42; 2 - 7 alleles per locus; Chauhan et al., 2007). Estimate of gene diversity (H) is a measure of genetic variation with RAPD markers (Silas et al., 2005). In *L. dussumieri*, the H ranged from 0.0000 to 0.1998 with 12 decamers for overall populations and the values are in proximity to the gene diversity figures reported in other freshwater teleosts (0.132 - 0.215 in *G. curmuca*; Musammilu, 2008; 0.0558 - 0.1640 in *Tor malabaricus*; Silas et al., 2005). The percentage of polymorphism (%P) estimates (21.5 to 25.0%) with RAPD primers in *L. dussumieri* are also congruent with the values reported for many freshwater fishes (Musammilu, 2008; Silas et al., 2005; Cagigas et al., 1999).

Deviations from Hardy-Weinberg genotypic expectations were observed at some allozyme and microsatellite loci in the present study. One possible explanation for these observations, especially with the microsatellite loci is the presence of null alleles that do not amplify producing heterozygotes which cannot be distinguished (Van Oosterhout et al., 2004, 2006). But, the analysis of data using MICRO-CHECKER did not give any evidence for null allele homozygotes in *L. dussumieri* populations. Moreover, in *L. dussumieri*, significant departures from the Hardy-Weinberg equilibrium were found within samples across loci rather than within loci across most of the samples. Such a situation is not consistent with null alleles at these loci (Van Oosterhout et al., 2004; Musammilu, 2008) or null alleles were not present in significant frequency to be a major cause of observed heterozygotes deficit. Where homozygote excesses were detected, generally such deviations indicate that factors such as non-random mating, reduction in effective breeding populations or specific locus could be under selection pressure were the causes for the observed violations (Ferguson, 1995; Chauhan et al., 2007). Heterozygote deficits can also result from mixing of undetected genetically divergent stocks within the samples, referred to as Wahlund effect (Hartl and Clark, 1997). With respect to *L. dussumieri* fish escaping to rivers in large numbers from farms or hatcheries to create such a situation does not arise, as aquaculture and hatchery seed production of this species is yet to become a commercial activity in Kerala. Hence, the possible causes for the excess homozygosity levels can be speculated as over-

Table 10. The total number of RAPD fragments; number and percentage of polymorphic bands and average gene diversity for each and overall populations of *Labeo dussumieri.*

Primer Code	Meenachil River				Manimala River				Pamba River				Overall populations			
	Total no. of bands	No. of poly-morphic bands	% of poly-morphic bands (%p)	Average Gene diversity (H)	Total no. of bands	No. of poly-morphic bands	% of poly-morphic bands (%p)	Average Gene diversity (H)	Total no. of bands	No. of poly-morphic bands	% of poly-morphic bands (%p)	Average Gene diversity (H)	Total no. of bands	No. of poly-morphic bands	% of poly-morphic bands (%p)	Average Gene diversity (H)
OPA-07	08	0	00.00	0.0000	08	0	00.00	0.0000	08	0	00.00	0.0000	08	0	00.00	0.0000
OPA-08	12	3	25.00	0.0778	12	3	25.00	0.0778	12	3	25.00	0.0778	12	3	25.00	0.0778
OPA-09	08	2	20.00	0.0378	10	3	30.00	0.0424	10	3	30.00	0.0424	12	5	41.67	0.1584
OPA-10	06	1	16.67	0.0315	06	0	00.00	0.0000	06	1	16.67	0.0315	06	1	16.67	0.1370
OPAA-08	10	3	23.08	0.1051	10	2	20.00	0.2380	10	2	20.00	0.2380	10	5	50.00	0.1540
OPAA-12	07	2	28.57	0.0892	08	1	12.50	0.1241	06	0	00.00	0.0000	10	5	50.00	0.1540
OPAC-06	07	2	28.57	0.0892	07	2	28.57	0.0892	07	2	28.57	0.0567	07	2	28.57	0.0892
OPAC-14	07	2	28.57	0.0540	07	3	42.86	0.1889	07	3	42.86	0.1889	14	9	64.29	0.1998
OPAC-17	10	4	40.00	0.1047	10	4	40.00	0.1047	10	4	40.00	0.1047	10	6	60.00	0.1901
OPAH-09	10	3	30.00	0.0860	10	3	30.00	0.0860	10	3	30.00	0.0860	10	3	30.00	0.0860
OPB-05	06	2	33.33	0.0603	06	2	33.33	0.0603	06	2	33.33	0.0603	06	2	33.33	0.0603
OPB-08	07	2	28.57	0.0892	08	0	00.00	0.000	06	0	00.00	0.0000	10	2	20.00	0.0941
Total	98	26	--	--	102	23	--	--	98	23	--	--	115	43	--	--
Mean Primers	--	--	25.20	0.0687	--	--	21.86	0.0843	--	--	22.20	0.0739	--	--	34.96	0.1167

exploitation of the species over the years and habitat alteration leading to reduction in catches, ending with inbreeding, as reported by Kurup (1990) and CAMP (1998) and revealed from our constant interactions with the fishermen and local people during the study period.

Table 11. Pair-wise F_{ST} (θ) (above diagonal) and their significance levels (below diagonal) between riverine populations of *Labeo dussumieri* using allozyme markers.

Populations	Meenachil	Manimala	Pamba
Meenachil	—	0.0028	0.0064
Manimala	NS	—	0.0009
Pamba	NS	NS	—

NS = Not significant.

Comparable F_{ST} (θ) estimates from allozymes (0.0034), F_{ST} and R_{ST} values (0.0041 and 0.0048 respectively) from microsatellite loci and G_{ST} estimates 0.0081 from RAPD loci clearly indicate that the wild *L. dussumieri* populations are only weakly sub-structured and that only 0.34 to 0.81% of the total observed genetic variation is resulted from population differentiation. Wright (1978) and Hartl and Clark (1997) suggested that F_{ST} estimates in the range of 0.00 to 0.05 indicate only little genetic differentiation among populations. Ward et al. (1994) reviewed 49 freshwater species and observed F_{ST} estimates ranging from 0 to 74% with a mean of 22.2%. In their survey, 23 fish species out of 49 exhibited genetic differentiation (F_{ST}) ranging from 0 to 10% (Ward et al., 1994; Chauhan et al., 2007). Nei's (1978) genetic distance estimates between pair-wise populations of *L. dussumieri* were also low with all three classes of markers in the present study. AMOVA analysis of the data also did not indicate any significant genetic differentiation among the sampled populations of *L. dussumieri*. Comparing the selected morpho-meristic characters and life history parameters of *L. dussumieri* from Meenachil, Manimala, Pamba and Achankovil Rivers, Kurup (1990) also concluded that these populations constituted a homogeneous stock.

Table 12. Pair-wise Fisher's F_{ST} (θ) (above diagonal) and Rho-statistics R_{ST} (below diagonal) between riverine samples of *Labeo dussumieri* using microsatellite markers.

Populations	Meenachil	Manimala	Pamba
Meenachil	—	0.0047(NS)	0.0063(NS)
Manimala	0.0052(NS)	—	0.0012(NS)
Pamba	0.0069(NS)	0.0022(NS)	—

NS = Not significant.

It is crucial to identify populations that have undergone ancient or recent bottlenecks, because they may have been affected by the small population size through

demographic stochasticity, inbreeding or fixation of deleterious alleles, possibly leading to a reduced evolutionary potential and increased probability of extinction (So et al., 2006). Bottlenecked populations may exhibit gametic disequilibrium; reduced genetic diversity – particularly reduced allelic diversity and loss of rare or unique alleles; and increased heterozygosity relative to that expected at mutation drift equilibrium (Cornuet and Luikart, 1996). No evidence for a recent genetic bottleneck was observed in *L. dussumieri* populations based on allozyme and microsatellite data set analysis. Avise (2004) and So et al. (2006) opined that the ancient or recent bottlenecks will have little or no effect on the distribution of microsatellite genetic diversity and hence mitochondrial DNA markers may be a better choice for detecting genetic bottlenecks. However, allozyme and microsatellite markers were useful in identifying recent genetic bottlenecks in many freshwater teleosts, such as *G. curmuca* (Musammilu, 2008) and *H. brachysoma* (Muneer et al., 2009) and marine fishes like *Sardinella aurita* (Chikhi et al., 1998). While the present analysis based on allozyme and microsatellite loci did not pinpoint the evidence for a recent genetic bottleneck in *L. dussumieri* populations, it can be ascertained only through further studies employing mitochondrial DNA markers.

Table 13. Co-efficient of genetic differentiation (G_{ST}) using RAPD primers for overall populations of *Labeo dussumieri*.

Primer code	Sequence (5'- 3')	G_{ST}
OPA-07	GAAACGGGTG	0.0000
OPA-08	GTGACGTAGG	0.0075
OPA-09	GGGTAACGCC	0.0081
OPA-10	GTGATCGCAG	0.0096
OPAA-08	TCCGCAGTAG	0.0106
OPAA-12	GGACCTCTTG	0.0095
OPAC-06	CCAGAACGGA	0.0092
OPAC-14	GTCGGTTGTC	0.0115
OPAC-17	CCTGGAGCTT	0.0098
OPAH-09	AGAACCGAGG	0.0077
OPB-05	TGCGCCCTTC	0.0111
OPB-08	GTCCACACGG	0.0068
	Mean	0.0086
	Overall populations	0.0081

Genetic differentiation can be influenced by a number of evolutionary forces and their interaction that act on natural populations including; migration, random genetic drift, mutation etc (Hartl and Clark, 1997). Random genetic drift will tend to cause genetic differentiation, after subpopulations are fragmented and gene flow between them is either reduced or absent**.** *L. dussumieri* from different river basins sampled

here, are likely to have evolved from common ancestral gene pool. The genus *Labeo* is considered to have entered India during the Eocene (54-38 million years ago) following migration of Indo-Malayan fishes via the Indo-Brahma River, flowing westward from Assam in the north-east to the present-day Arabian Sea (Daniels, 2001). Migration of fishes that evolved during the Eocene (60 million years ago) continued until dismemberment of the Indo-Brahma River and formation of the modern river systems such as Indus, Ganga and Brahmaputra during the late Pleistocene. The Western Ghats were uplifted during the later half of the Eocene (circa 50 million years ago) even before India collided with mainland Asia. According to Radhakrishna (1993), before the uplift of the Western Ghats, there came into being the 67–68 million year old Deccan Traps and the 115-117 million year old Rajmahal Traps in peninsular India. These uplifted surfaces together with the Western Ghats and the Deccan Plateau were topographically ideal for a wide network of torrents, streams and rivers in peninsular India, providing conducive conditions for westward and southwestward migration of Malayan fishes (Radhakrishna, 1993). The still young and diversifying carps and catfishes found extensive habitats, as they were amongst the earliest colonizers invading peninsular India including Sri Lanka during the Eocene [Ceylon - which was a part Indian Peninsula up to the Miocene (24-5 million years ago) and again intermittently connected with the mainland upto the Holocene (10,000 years back)] (Silas, 1953). During the late Pleistocene (1.5-0.011million years ago), the Western Ghats came to be known more or less as they are today and the river capture, waterfalls and deep gorges gave rise to the present structure of watersheds in the region including Kerala (Daniels, 2001, Unnithan, 2001).

Table 14. Nei's pair-wise genetic distance using allozymes (above diagonal) and microsatellite markers (below diagonal) in *Labeo dussumieri*.

Populations	Meenachil	Manimala	Pamba
Meenachil River	****	0.0029	0.0032
Manimala River	0.0061	****	0.0014
Pamba River	0.0073	0.0041	****

The low genetic divergence among wild 'thooli' populations in spite of fragmentation may be as a result of the ongoing high gene flow among populations across the Meenachil, Manimala and Pamba Rivers. Originating from the Western Ghats, these rivers flow in close proximity, drain into the southern end of the Vembanad Lake and are connected to each other in the lower reaches through an extensive network of natural canals (Fig. 1). This could be the source of mixing of fish populations resulting in lack of significant allelic heterogeneity among the *L. dussumieri* populations. The southern end of the Vembanad Lake was converted into a freshwater lagoon following the construction of the Thanneermukkom Barrage in 1975 and Padmakumar et al. (2004) reported that this part of the lake as well as the interconnecting canals of the rivers act as a fish reserve, especially for the species such as *L. dussumieri*, *Puntius sarana*

subnasutus and *Horabagrus brachysoma*. Besides direct migration, a stepping stone model of migration that attributes effective gene flow to gene exchange among neighbors (Gaggiotti et al., 1999; Olivier et al., 2003) may also explain the lack of significant allelic heterogeneity, among thooli population in the river systems sampled here. The observed lack of private or locality specific allele at any of allozyme, microsatellite or RAPD loci argues in favour of effective ongoing gene flow. Therefore, common ancestry in the past and possible continuous exchange of individuals among rivers belonging to different river basins may explain the observed low levels of genetic differentiation among *L. dussumieri* populations. Comparable values for fixation indices based on the SMM (R_{ST}) and IAM (θ) estimates for microsatellite data indicate that the observed genetic structure of *L. dussumieri* population is likely to be of recent origin. In effect, there may have been insufficient time for isolation and mutational events to give rise to new alleles and unique genotypes as reported in *C. mrigala* (Chauhan et al., 2007).

Table 15. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) using RAPD markers in *Labeo dussumieri*.

Populations	Meenachil	Manimala	Pamba
Meenachil River	****	0.9923	0.9889
Manimala River	0.0077	****	0.9937
Pamba River	0.0111	0.0063	****

Allozyme, RAPD and microsatellite markers could be considered as random indicators to discriminate the three populations of *L. dussumieri*. Therefore, it would be of interest to compare the results obtained from the application of these three approaches to the same individuals. To date, only few studies have compared the results of allozymes with RAPD and microsatellites (Cagigas et al., 1999; Muneer, 2007, 2008, 2009; Musammilu, 2008). The three methods in the present study probably might have generated markers pertaining to different parts of thooli genome. But, all produced similar results and indicated only low genetic differentiation among the populations of *L. dussumieri*, indicating the robustness of the techniques applied. Although it was possible to gain a clear understanding of population structure using allozyme data alone, the use of more variable markers such as microsatellites and RAPDs could further confirm the analysis using allozymes which reinforced reliability of interpretations. These DNA techniques involved the examination of putative non-coding genes thought to be neutral, which permits high rates of mutation and lead not only to different alleles at each locus but also to an increase in the amount of genetic variation (Cagigas et al., 1999). Although the three techniques could not clearly discriminate the populations, microsatellites as a basic genetic tool overcome some of the disadvantages displayed by the other two. First, because specific primer development for a particular species

can be both time-consuming and costly, primers developed in one species can be used to amplify homologous loci in closely related species (Scribner et al., 1996). Second, many microsatellite loci are thought to be neutral (Zardoya et al., 1996) but some allozyme loci may be influenced by selection pressure, allowing only a few alleles at each locus (Musammilu, 2008). Furthermore, because *L. dussumieri* populations are under endangered category, killing specimens to collect liver and muscle for allozyme analysis becomes a significant inconvenience (fin clips and body slime may not give satisfactory results for all allozymes), which makes it advisable to adopt other techniques. Transportation of tissue samples from remote areas in liquid nitrogen and their subsequent storage in -85^{0}C freezer until further analysis are other disadvantages associated with allozyme analysis. The sampling for microsatellites and RAPD is usually non-lethal or minimum invasive unlike for allozymes. The RAPD methodology also involves some disadvantages compared with microsatellites. The dominant character of RAPDs makes it impossible to distinguish between homozygote and heterozygote of a particular fragment, and the comparison of bands across different gels often makes data scoring more difficult. Although reproducibility both within and among laboratories has been proved for RAPD polymorphisms (Cagigas et al., 1999; Muneer, 2008, 2009; Musammilu, 2008; Nagarajan et al., 2006; also in the present study) some confusion still exists regarding its application in population genetics especially of endangered species (basic assumption in RAPD analysis is, the populations fit the Hardy-Weinberg equilibrium). The apparent disadvantages of the allozyme and RAPD techniques further enhance the utility of microsatellites for the analysis of population genetic problems.

Table 16. Analysis of Molecular Variance (AMOVA) based on allozyme and microsatellite markers in three populations of *Labeo dussumieri*.

Sources of Variation	Degrees of freedom	Variance component	Percentage of Variation (%)	Fixation indices
		Allozymes		
Among populations (Among Rivers)	2	0.1423 (Va)	0.878	0.00878 (NS)
Within populations (Within River)	393	16.0610 (Vb)	99.122	---
Total	395	16.2033 (Vt)	---	---
		Microsatellites		
Among populations (Among Rivers)	2	0.2259 (Va)	1.40	0.014(NS)
Within populat ions (Within River)	393	15.9111(Vb)	98.60	
Total	395	16.137 (Vt)		

NS = Not significant.

However, microsatellites are not free from short comings. Non-specific amplification, presence of stutter bands and very high level of polymorphism demanding large sample sizes (to adequately characterize the genetic variation both within and among populations, to ensure that apparent differences among populations are not due to sampling error) are often encountered with microsatellites, complicating the genotyping and analysis. But in the present study, the number of alleles per locus was relatively less compared to other teleosts (Na-Nakorn et al., 1999). Also, the PCR conditions were optimized to overcome the problem of stutter bands and non-specific amplification in *L. dussumieri*. The non-denaturing PAGE coupled with silver staining could resolve the alleles up to 2 base pair difference in the present study.

Table 17. Bottleneck analysis in three populations of *Labeo dussumieri* with allozyme and microsatellite markers

	I A M (allozymes)			T P M (microsatellites)		
	Meenachil	Manimala	Pamba	Meenachil	Manimala	Pamba
Sign Test (P)	0.06923	0.07946	0.07300	0.62501	0.30974	0.57712
Wilcoxon Test (P)	0.07391	0.08391	0.07810	0.67712	0.56183	0.54688

The distribution of genetic variation evidenced from allozyme, microsatellite and RAPD data clearly indicate only low genetic differentiation among *Labeo dussumieri* populations in the rivers of the Western Ghats. Gene flow across river basins, after common ancestry, probably did not allow evolutionary forces to result in significant genetic differentiation. For management of wild thooli stocks, an important challenge will be to maintain high levels of genetic variation over time. Regulated water flows in the rivers will be crucial to maintain necessary large effective breeding population sizes that may be threatened due to habitat alteration. The stocks from the three rivers do not require different management strategies and for propagation assisted river ranching programme of this species, large effective breeding population can be developed by mixing individuals from three rivers.

Though the confirmed range of natural distribution of *L. dussumieri* is Kerala and Sri Lanka, Johal and Tandon (1979; quoted from Jayaram & Dhas, 2000) reported the species from East Punjab, which was afterwards treated as doubtful (Talwar and Jhingran, 1991). Another closely related species of *L. dussumieri* with dubious identity is *Labeo rajasthanicus* Datta and Majumdar 1970 from Jaisamand Lake, Rajasthan, India (Jayaram & Dhas, 2000). In an independent study in our laboratory, *L. rajasthanicus* specimens collected from its type locality exhibited overlapping morphological and meristic counts with that of *L. dussumieri* from Kerala (Raymond, 2006). Genetic

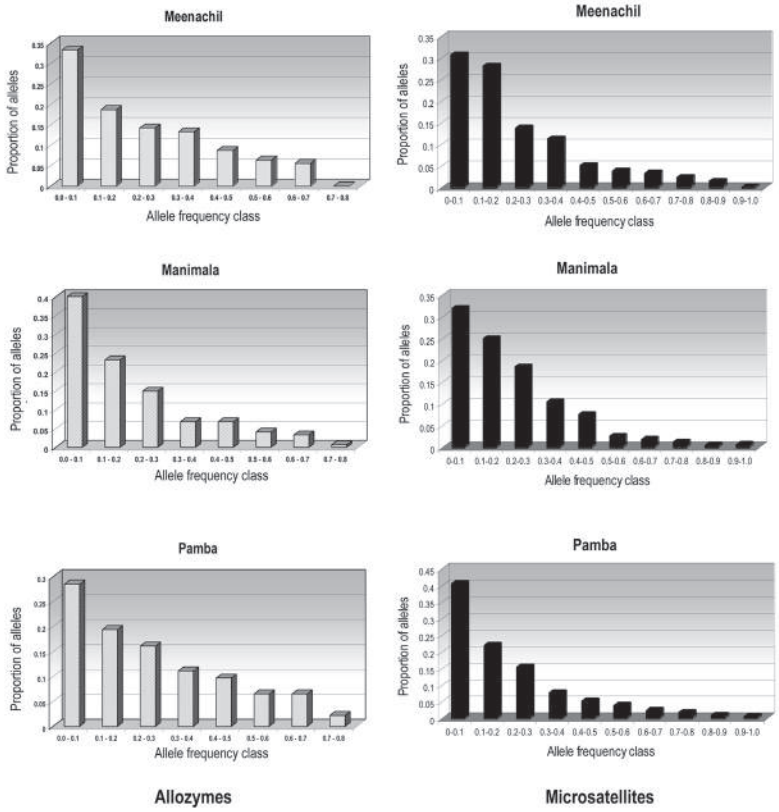

Figure 6. Qualitative "mode-shift" indicator test to discriminate bottlenecked populations of *Labeo dussumieri* from three rivers, based on allozyme and microsatellite allele frequency distribution.

divergence values between these two separate (?) species based on partial sequence information of mitochondrial 16SrRNA and Cyt*b* genes were very low (0.53% and 0.74% respectively; NCBI GenBank Accession #s DQ 520910 -13, DQ 520918 - 21; Raymond, 2006). Such low values of haplotype differences were rather unexpected especially since both species are separated by large geographical distances (~2500 km) and when there are no chances of migration and gene flow between Kerala and Rajasthan. Possibility of man-made introduction of *L. dussumieri*, even though the chances are remote, also needs to be explored. It is noted that the classic works like that of Hamilton-Buchanan (1822), McClelland (1839), Valenciennes (1847) and Day (1878) failed to make any mention about *L. rajasthanicus* or its closely related species *L. dussumieri* from Rajasthan (quoted from Jayaram & Dhas, 2000). In spite of the Indian and Sri Lankan land masses having been connected terrestrially from time to time upto the Holocene, the seperation of biotas of India and Sri Lanka in many cases is much more ancient (Silas, 1953; Bossuyt et al., 2004) and it would not be suprising if further research were to show the mainland and insular populations of *L. dussumieri* to be genetically distinct. The natural extension of the present study is to examine the finer scale of

phylogeography, population genetics and life history traits of these two species on a broad geographic range including Sri Lanka with intensive sampling not only to further investigate the evolutionary relationships, but also to assist in the management and recovery of rare and endangered populations.

Acknowledgements

The Indian Council of Agricultural Research (ICAR) and the National Agricultural Technology Project (NATP Sub-project MM-18), which supported the study financially, are gratefully acknowledged. The authors are grateful to Dr. S.P Singh (PI, NATP, NBFGR) for the encouragement and support. Technical assistance provided by Mr. Alfred Dourom is also duly acknowledged.

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Received: 05 February 2008; Accepted: 23 May 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Development of Loop-Mediated Isothermal Amplification (LAMP) Method for Rapid Detection of *Vibrio parahaemolyticus*

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Abstract

A technique for detecting *Vibrio parahaemolyticus* using a novel DNA amplification procedure designated loop-mediated isothermal amplification (LAMP) has been developed for the first time. A set of four primers, two outer and two inner primers, was designed specifically to recognize the thermolabile hemolysin gene (*tlh*) of *V. parahaemolyticus*. The LAMP reaction mix was optimized. The most optimal reaction temperature and time of the LAMP assay for the *tlh* gene was 60^0C and 60 min. Genomic DNAs from 28 bacterial strains including 14 *V. parahaemolyticus* strains were amplified using LAMP, and LAMP product was observed in other bacterial strains. The detection limit of this LAMP assay was approximately 90 fg. test tube^{-1} of *V. parahaemolyticus* genomic DNA and 24 cfu.mL^{-1} for pure cultures. In addition, this method was applied to detect noncultured artificially contaminated food samples. These results suggest that detection of *V. parahaemolyticus* by the LAMP assay is an effective and low-cost procedure with high specificity and sensitivity that requires no specialized equipment. This assay is expected to become a valuable tool for rapid detection and identification of *V. parahaemolyticus*.

Introduction

Vibrio parahaemolyticus is a gram-negative, halophilic bacterium that distributes worldwide in the estuarine and coastal environment, especially in fishes, shellfishes and seafood products. It has been considered as one of the most important pathogens for both human and aquacultured animals. This pathogen is a common cause of foodborne illnesses in many Asian countries, including China and Japan, and is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States (Su and Liu 2007). Nowadays, in China, the occurrence of the food poisoning caused by *V. parahaemolyticus* has increased remarkably, and this bacterium has become the leading foodborne pathogen of our country (Liu 2004). Thus, early and accurate detection of the pathogen is necessary for disease control.

Traditional cultivation methods including isolation and biochemical speciation

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is the primary tool for detection and identification of *V. parahaemolyticus*. These procedures are sophisticated and time-consuming generally taking at least 72 h. Recently, with the development of molecular biology, some powerful PCR-based techniques have been used for rapid detection of *V. parahaemolyticus* and the application for rapid diagnosis seems to be promising (Li et al. 2004; Ward and Bej 2006). These PCR-based methods are highly sensitive and specific, however, they are high-cost requiring sophisticated instruments for amplification and time-consuming because these methods usually require thermal cycling. Those methods are not in favor of rapid detection and diagnosis of this pathogen especially, for the on-the-spot test. Thus, a rapid, simple and cost-effective assay is needed to complement the current methods.

Notomi et al. (2000) recently reported a novel nucleic acid amplification method, designated loop-mediated isothermal amplification (LAMP), which amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions. LAMP relies on autocycling strand displacement DNA synthesis performed by a DNA polymerase with high strand displacement activity and a set of four specially designed primers (two inner and two outer primers). In the initial steps of the reaction, a stem-loop DNA structure is constructed as the starting material. In the later steps, one inner primer hybridizes to the loop on the LAMP product and initiates strand displacement DNA synthesis. This cycling reaction continues with accumulation of 10^9 copies of target within one hour (Notomi et al. 2000). Since it was first published in 2000, the LAMP method has been widely used in many fields, such as the rapid diagnosis of infectious diseases in clinic (Endo et al. 2004), qualitative and quantitative detection of some epidemic viruses (Imai et al. 2006) or bacteria (Hara-Kudo et al. 2005; Song et al. 2005; Yeh et al. 2005) and embryo sexing (Hirayama et al. 2006).

In the present study, we applied this recently developed loop mediated isothermal amplification method for the rapid detection of *V. parahaemolyticus* for the first time. LAMP specific primers were designed targeting the thermolabile hemolysin gene (*tlh*) of *V. parahaemolyticus*; the conditions of the assay were optimized; the specificity and sensitivity of the primers in the LAMP assay for detection of *V. parahaemolyticus* were determined and finally, the assay was applied to detect the pathogen in artificially contaminated food samples directly.

Materials and methods

Bacterial strains

Bacteria used in this study are listed in Table 1. The strains were from Institute of Microbiology of Chinese Academy of Science, Guangdong Institute of Microbiology in China, or isolated by our labs.

Table 1 List of bacterial strains used in this study and specificity of the LAMP primers

Bacterial species/strain	Medium	Growth temperature ^{0}C	LAMP
V. parahaemolyticus ATCC 33846	TCBS agar	30^{0}C	+
V. parahaemolyticus ATCC 33847	TCBS agar	30^{0}C	+
V. parahaemolyticus ATCC 17802	TCBS agar	37^{0}C	+
V. parahaemolyticus xq 1	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 2	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 3	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 4	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 5	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 6	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 7	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 8	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 9	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 10	TCBS agar	30^{0}C	+
V. parahaemolyticus xq 11	TCBS agar	30^{0}C	+
Vibrio campbellii ATCC 33864	TCBS agar	30^{0}C	—
Vibrio fluvialis ATCC 33809	TCBS agar	30^{0}C	—
Vibrio harveyi ATCC 33842	TCBS agar	30^{0}C	—
Staphylococcus aureus subsp. aureus 1.1361	Nutrient agar	37^{0}C	—
Salmonella sp. 1.1552	Nutrient agar	37^{0}C	—
Listeria innocua GIM 1.230	Nutrient agar	37^{0}C	—
Listeria welshimeri GIM 1.231	Nutrient agar	37^{0}C	—
Listeria monocytohenes GIM 1.228	Nutrient agar	37^{0}C	—
Listeria monocytohenes GIM 1.229	Nutrient agar	37^{0}C	—
Vibrio cholerae zhy1	TCBS agar	30^{0}C	—
Vibrio cholerae zhy2	TCBS agar	30^{0}C	—
Vibrio cholerae zhy3	TCBS agar	30^{0}C	—
Escherichia coli	Nutrient agar	37^{0}C	—
Shigella	Nutrient agar	37^{0}C	—

Template DNA preparation

One-milliliter bacterial suspension was centrifuged at 12000 rpm for five minutes, and the supernatant was discarded. Pellets suspended in 100 μl of sterile double water were boiled at 100^{0}C for ten minutes and immediate ice incubated for two minutes. After further centrifugation at 12000 rpm for five minutes, then supernatant was used as template DNA.

Design of LAMP primers

A set of species-specific LAMP primers comprising two outer and two inner primers was designed based on the highly conserved fragment of the *tlh* gene of *V. parahaemolyticus* (GenBank accession number M36437). The two outer primers were designated F3 and B3. The two inner primers were designated FIP and BIP. The FIP consisted of the F1c sequence (complementary to F1), a TTTT spacer and the F2 sequence. The BIP consisted of the B1c sequence (complementary to B1), a TTTT spacer and the B2 sequence. Primer sequences are shown in Table 2. Nucleotide sequences of targets for LAMP primers are illustrated in Fig. 1.

Table 2. Sequence of LAMP primers for specific amplification of the *tlh* gene (GenBank accession no M36437)

Name	Sequence	Location
FIP	5'-GCCCATTCCCAATCGGTCG-TTTT-CTATGTTTCGC TGTTGGTATCG-3'	(323～305) ～ (258～278)
BIP	5'-GTTCTACACCAACACGTCGCA-TTTT-TCGCCAAATCTAATGTTGCTTC-3'	(393～413) ～ (457～436)
F3	5'-CAGCACGCAAGAAAACCA-3'	(231～248)
B3	5'-ATTGTCAGCGGCGAAGAA-3'	(495～478)

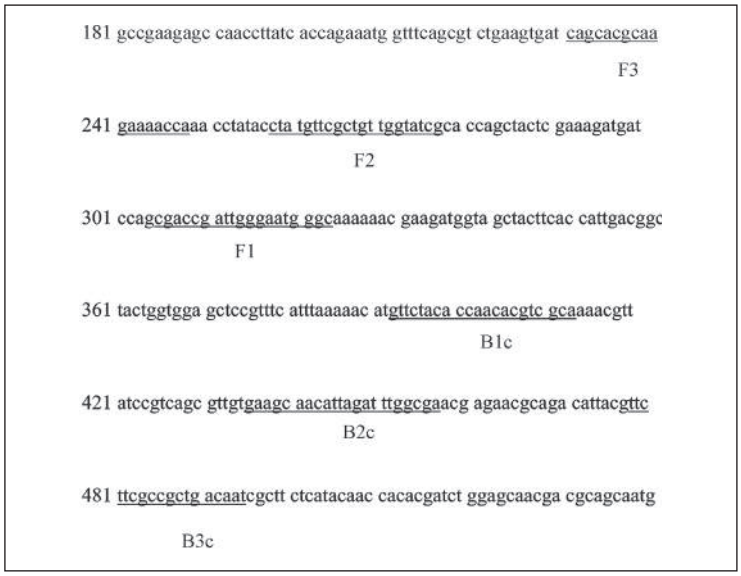

Figure 1. Nucleotide sequences of targets for LAMP primers on the *tlh* gene of *V. parahaemolyticus*.

LAMP reaction

The LAMP reaction was carried out in a 25 µl reaction mixture containing the following reagents with final concentrations: 8 mM $MgSO_4$, 1.0 mM dNTP, 0.8 M betaine (Sigma), 1.2 µM each of FIP and BIP primers, 0.2 µM each of F3 and B3 primers, 1 U *Bst*

DNA polymerase large fragment (New England Biolabs), 1x ThermoPol buffer and appropriate amount of template DNA. The reaction was carried out at 60^0C for one hour and inactivated at 80^0C for ten minutes (Notomi et al. 2000). Aliquots of 3 µl products were analyzed by 2% agarose gel electrophoresis and detected under UV light after ethidium bromide staining. The change in turbidity of the reaction mixture was, meanwhile, observed by naked eyes.

Specificity of LAMP primers

The specificity of the set of LAMP primers for the *tlh* gene of *V. parahaemolyticus* was determined by LAMP amplification of the genomic DNAs from 14 *V. parahaemolyticus* and 14 bacterial strains other than *V. parahaemolyticus* listed in Table 1.

Sensitivity of LAMP assay

The sensitivity of the assay was determined using genomic DNAs and pure cultures of *V. parahaemolyticus* standard strains. Genomic DNA from *V. parahaemolyticus* ATCC 33846 was extracted and 10-fold serially diluted in sterile double water. Aliquots of 2 µl each dilution were amplified by LAMP using the optimum reaction condition. A LAMP reaction mixture containing no template DNA was used as a negative control.

To determine the sensitivity of detection for pure cultures, overnight cultivated *V. parahaemolyticus* ATCC 33846 was quantitated by direct plating and 10-fold serially diluted. Aliquots of 1 mL each dilution was used to prepare template DNA and 2 µl template DNA of each dilution were amplified by LAMP using the optimum reaction condition. A LAMP reaction mixture containing no template DNA was used as a negative control.

Detection of V. parahaemolyticus in artificially contaminated food samples

Shrimp samples were purchased from a local seafood store. 10g of shrimp meat was added into 90 mL APW (peptone 10g, sodium chloride 10g, distilled water 1000 mL, pH 8.4), and homogenized in homogenizer for approimately 60 seconds to obtain the shrimp homogenate. Overnight cultivated *V. parahaemolyticus* ATCC 33846 suspensions (1 mL) were added into 10 mL shrimp homogenate to obtain artificially contaminated food samples. 1 mL of shrimp homogenate was used to prepare template DNA using Bacterial Genomic DNA Extraction Kit (Shanghai Sangon Biological Engineering Technology & Service CO., Ltd., China). LAMP amplification was performed under the optimum reaction condition. Template DNA extracted from uncontaminated shrimp homogenate was used as negative control.

Results

Standardization and optimization of LAMP assay

We initially standardized and optimized the LAMP assay for *V. parahaemolyticus*

detection using two outer and two inner primers from *tlh* gene and template DNAs from *V. parahaemolyticus* ATCC 33846 and ATCC 33847. The specific amplification generated the ladder-like pattern of bands (LAMP products) on agarose gel, and no band was observed in negative control (Fig. 2a). Besides, observing the LAMP reaction tubes by naked eyes, there was an increase in the turbidity of the reaction mixture with *V. parahaemolyticus* genomic DNA as the template DNA and no change was observed in the negative control tube; Centrifuged at 5000 rpm for several seconds, only the reaction tube with *V. parahaemolyticus* genomic DNA as the template DNA had white precipitate accumulating at the bottom of the tube (Fig. 2b).

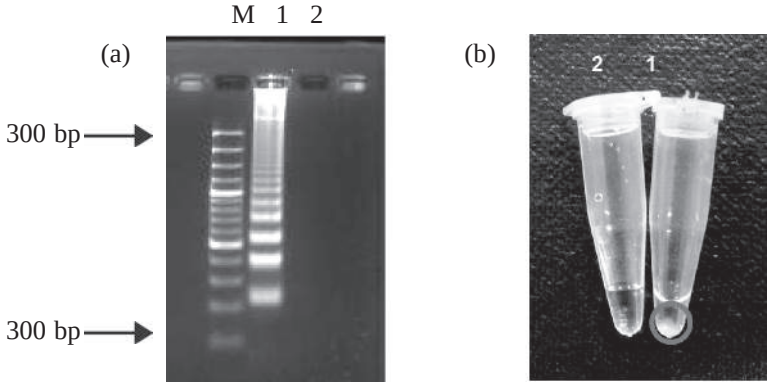

Figure 2. The LAMP result of *V. parahaemolyticus* standard strain.
M: 100 bp marker; 1: *V. parahaemolyticus* ATCC 33846; 2: negative control.

Effect of Mg^{2+} concentration

The LAMP reaction in the presence of various concentrations of Mg^{2+} was tested. We varied the Mg^{2+} concentrations from 2 to 18 mM to amplify the target DNA. Mg^{2+} concentration at 8 mM gave the maximal reaction product.

Effect of dNTPs concentration

The effect of dNTPs concentrations ranging from 0 to 1.8 mM on the LAMP reaction was tested. The results indicated that the optimal dNTPs concentration was 1.0 mM.

Effect of betaine concentration

The LAMP reaction in the presence of different concentrations of betaine ranging from 0 to 1.2 M was tested. It has been observed that increasing betaine concentration increased the amount of the LAMP reaction products and the concentration at 0.8 M had already had the optimal amplification.

Effect of primer ratio

Since a set of LAMP primers comprise two outer and two inner primers, the

effect of ratio of outer primers and inner primers on the LAMP reaction was determined. The LAMP reaction was performed in the primer ratios ranging from 1:1 to 1:8 and a primer ratio of 1:6 gave the maximal amplification.

Effect of temperature

The LAMP reaction was performed at temperatures ranging from 53 to 65^0C. When the reaction temperature reached 58^0C, the ladder-like pattern of bands was generated, and the temperature at 60^0C gave the best amplification result.

Effect of reaction length

The effect of reaction lengths from 30 to 60 min. on the LAMP reaction was tested with genomic DNAs of *V. parahaemolyticus* ATCC 33846 and ATCC 33847 as templates, respectively. When the reaction performed for 45 min., the typical ladder-like pattern of bands was generated. However, in later experiments, the reaction performing for 45 min. gave inconsistent and unstable results. Therefore, we chose 60 min. as the optimal reaction length.

As indicated above, the LAMP assay condition was optimized in a 25 µl reaction mixture as follows: 8 mM $MgSO_4$, 1.0 mM dNTP, 0.8 M betaine, 1.2 µM each of FIP and BIP, 0.2µM each of F3 and B3, 1 U *Bst* DNA polymerase large fragment, 1×ThermoPol buffer and appropriate amount of template genomic DNA. The reaction was carried out at 60^0C for 60 min.

Specificity of LAMP assay

The specificity of the LAMP primers for the *tlh* gene was tested in *V. parahaemolyticus* and other bacterial strains with a total amount of 28. All the 14 strains of *V. parahaemolyticus* were shown to be positive, whereas, all the other bacterial strains tested in this study were negative (Table 1). These results indicated that the primers were specific for detection of *V. parahaemolyticus.*

Sensitivity of LAMP assay

The sensitivity of the LAMP assay for detection of *V. parahaemolyticus* was determined using 10-fold serial dilutions. The concentration of genomic DNA extracted from *V. parahaemolyticus* ATCC 33846 was 449 $ng.\mu l^{-1}$. Aliquots of 2 µl each dilution were used as the templates of the LAMP amplification making the concentrations of templates ranging from 90 ng. test $tube^{-1}$ to 0.9 fg.test $tube^{-1}$. The detection limit of this LAMP assay for *V. parahaemolyticus* genomic DNA was around 90 fg. test $tube^{-1}$. The overnight cultivated *V. parahaemolyticus* ATCC 33846 was quantitated to be 2.39×10^7 $cfu.mL^{-1}$ by direct plating. The detection limit of this LAMP assay for *V. parahaemolyticus* pure cultures was approximately 24 $cfu.mL^{-1}$.

Detection of V. parahaemolyticus in artificially contaminated food samples

We applied the LAMP assay to detect *V. parahaemolyticus* in artificially contaminated shrimps. The typical ladder-like pattern of bands was observed in tubes with artificially contaminated shrimps as templates, and no amplicon was observed in negative control with uncontaminated shrimps as templates.

Discussion

In this study, we developed and optimized a LAMP assay for rapid detection and identification of *V. parahaemolyticus*. LAMP amplifies DNA under isothermal conditions, which uses a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA and a DNA polymerase with high strand displacement activity. The use of four primers (recognition of six distinct sequences) in the initial steps of LAMP and two primers (recognition of four distinct sequences) during the subsequent steps ensures high specificity for the target amplification. Thus, we can judge the presence and absence of the target gene by whether the amplification performs or not. On the basis of its special amplification mechanism, the final products of LAMP are a mixture of stem-loop DNAs with various stem lengths and cauliflower-like structures with multiple loops consisting of alternate inverted repeats of target sequence in the same strand, thus, the products generate unique ladder-like pattern of bands on agarose gel (Notomi et al. 2000). Furthermore, LAMP method is able to synthesize extremely large amount of DNA, so a large amount of by-product, pyrophosphate ion, is produced, yielding white precipitate of magnesium pyrophosphate in the reaction mixture. As the precipitate can easily be observed with naked eyes, detection of LAMP reaction can be done by judging the presence of accumulated precipitate after centrifugation and visual judgment of turbidity, which may be the most direct way to judge the nucleic acid in testing specimens being amplified by LAMP method or not (Mori et al. 2001). The LAMP assay established in this study performs under isothermal condition with a single temperature step at 60^0C for approximately one hour. Moreover, it conducts amplification and detection in one-step, so the LAMP assay can be done only using a water-bath that furnishes a constant temperature of 60^0C without usage of any other expensive or specialized equipment. When compared to PCR techniques, the LAMP assay is easier to perform, less time-consuming and lower cost.

In the present study, we chose *V. parahaemolyticus* species-specific gene- *tlh* gene as the target gene. A set of LAMP primers was designed specifically to recognize the *tlh* gene. In order to confirm specificity, the assay was conducted using *V. parahaemolyticus*, bacteria from *Vibrio* spp., and some other bacterial strains. DNA amplification was only observed in *V. parahaemolyticus*, but not other bacterial strains. This result suggests that this assay is specific for detecting *V. parahaemolyticus*.

Our LAMP assay was capable of detecting a minimum of 90 fg. test tube^{-1} for *V. parahaemolyticus* genomic DNA and 24 cfu.mL^{-1} for pure cultures. Whereas, the sensitivity of the Real-time PCR assay that recently published was 200 pg of *V. parahaemolyticus* genomic DNA and 10^4 cfu.mL^{-1} for pure cultures, and the sensitivity of conventional PCR previously reported was 2.4×10^2 cfu.mL^{-1} for pure cultures (Li et al. 2004; Ward and Bej 2006). Thus, compared to these other assays, the LAMP assay is shown to be more sensitive.

V. parahaemolyticus is an important seafood-borne pathogen throughout the world. Typically, human infections from this pathogen mainly result from the consumption of raw or undercooked seafoods. Furthermore, it is known that some components of food may inhabit DNA amplification (Hara-Kudo et al. 2005). Thus, in this study, the LAMP assay was applied to detect *V. parahaemolyticus* in artificially contaminated food samples. Artificially contaminated shrimp samples were detected *V. parahaemolyticus* positive by the LAMP assay, but not the samples that were not contaminated. This result demonstrates that the assay is capable to detect *V. parahaemolyticus* in noncultured food samples without prior isolation and biochemical speciation.

In conclusion, the LAMP assay standardized and optimized in the present study is specific and sensitive for rapid detection and identification of *V. parahaemolyticus* both in culture isolates and in food samples. When compared to traditional cultivation methods and PCR-based techniques, the LAMP assay is simpler, effective and less expensive that requires no specialized equipments. Therefore, the LAMP assay is expected to become a valuable tool for rapid detection and identification of *V. parahaemolyticus*, beneficial to both the seafood industry and consumer health.

Acknowledgement

This work was supported by Shanghai 'Promoting the Development of Agriculture through Science and Technology' Key Project Hu 2005 (4-2) and Hu 2006 (10-5), and by Shanghai Leading Academic Discipline Project T1102.

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Received: 31 December 2007; Accepted: 24 February 2009

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Characterization of Molluscan Muscle based on the Properties of Major Myofibrillar Protein Components

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Abstract

For the deeper understanding of the molluscan muscles for their effective utilization, the major protein components of molluscan mantle muscle, namely, myosin (heavy chain) and tropomyosin, were investigated from the viewpoint of structure-stability relationships. Molluscan myosin heavy chains are unique in that they have several additional residues in the rod region forming α-helical coiled-coil structure. On the other hand, molluscan tropomyosins clearly differed from other orthologous proteins, suggesting that the structural uniqueness gives rise to their characteristics, such as allergenicity. The amino acid sequence data, together with thermodynamic analysis data, could be useful to estimate the stability (or instability) of these protein components, and further the properties of the muscle *per se*.

Introduction

The phylum Mollusca (consisting of more than 110,000 species) has diverged in a variety of forms and ecological profiles, i.e., from bivalves, gastropods and further to cephalopods, which possess advanced brain and excellent locomotion system enabling their dexterous swimming at high speed, mainly by jet propulsion (Rokni and Hochner 2002, Takuwa-Kuroda et al. 2003). They are the highest class of the phylum, consisting of subclasses Coleoidea and Nautiloidae with 786 living species. They occur in all marine habitats of the world, and are great sources of protein from the sea, thus important for commercial fisheries and processing. Incidentally, the annual catch of mollusc in the world is around four million tons per year, and approximately 2.3 megatons for squids. For the effective utilization of the edible parts (mostly muscles) of molluscs, molecular approach to proteins consisting myofibril is worth intensive investigation.

Myosin and paramyosin are the two major components of molluscan muscle. Paramyosin does not show any biological activity, but takes a part in filament formation.

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However, myosin is directly involved in muscle contraction, using the chemical energy produced by ATP hydrolysis. Myosins are classified into eighteen different classes, though all the skeletal and obliquely striated muscle myosins belong to type II (Foth et al. 2006). Myosin is composed of two heavy chains of approximately 200 kDa and four light chains of approximately 20 kDa (Craig and Woodhead 2006). A globular head of molecule is called subfragment-1 (S-1), containing and N-terminal half of the heavy chain together with light chain subunits of approximately 20 kDa. Actin binding and ATP binding sites are also located in this domain. The fibrous part is referred to as a rod, consisting of a C-terminal half of the heavy chain alone. N- and C-terminal sides of the rod are called subfargment-2 (S-2) and light meromyosin (LMM), respectively. The rod portion forms a coiled-coil structure composed of two helices, which enables forming a thick filament under physiological conditions. In most cases, the properties of myosin are the major determinant for the stability of meat against heat and frozen storage. Toughness and water-holding capacity of meat are also greatly ascribed to the properties of the major proteins. For better understanding the properties of muscle, it is essential to characterize the major protein component, myosin.

Myofibrillar proteins including myosin and tropomyosin from fish and shellfish are generally less stable than the counterparts of higher vertebrates (Ogawa et al. 1993, Higuchi et al. 2002, Paredi et al. 2002, Li et al. 2003, Huang and Ochiai 2005). Myosin is also a very important factor for gel formation of fish meat (kamaboko) (Satoh et al. 2006). Myofibrillar (myosin) ATPase activity is considered to be a good quality indicator of fish meat paste (surimi) (Katoh et al. 1979). On the other hand, tropomyosin stabilizes actin filament and regulates muscle contraction (Yu and Ono 2006). Sequence data are available for molluscan myosin heavy chain and tropomyosin, while very scarce data are available for paramyosin. The rod portion of myosin has a coiled-coil structure composed of two α-helices (Root et al. 2006), and this is also true for tropomyosin, which has this structure throughout the entire molecule. The coiled-coil is considered to function as molecular motors propelled by electrostatic energy of ions (Jarosch 2005). Such unique structure makes it possible to characterize the structures of proteins, because the changes of α-helical content through thermal treatment or in the presence of denaturants can be monitored quite easily by circular dichroism analysis.

So far, most of the studies on molluscan muscle proteins have been focused on Ca^{2+}-regulated muscle contraction (Szent-Gyorgyi et al. 1999, Azzu et al. 2006), because these myosins are special in that the direct binding of Ca^{2+} to myosin light chain subunit regulates contraction. Primary structures have been revealed for some myosins and

tropomyosins (Matulef et al. 1998, Janes et al. 2000, Fujinoki et al. 2006), and collagen (Morales et al. 2000). In addition, invertebrate tropomyosins have also been identified as major allergens (Nakamura et al. 2005, Motoyama et al. 2006, Zhang et al. 2006). A few studies have dealt with the changes of biochemical characteristics of muscle proteins (Khaitlina et al. 1999, Hatzisisis et al. 2000, Inoue and et al. 2004, Kasamatsu et al. 2004).

In the present study, attempts were made to characterize the muscles from several molluscan species, taking the two representative muscle proteins, myosin heavy chain and tropomyosin as the markers for thermal stability of coiled-coil structure. These properties were discussed in relation with their amino acid sequences.

Materials and Methods

Live specimens of Japanese common squid *Todarodes pacificus*, Ommastrephidae, and common octopus *Octopus vulgaris*, Octopodidae, were purchased at a local wholesale market, immediately frozen with dry ice and transported to the laboratory. They were kept at -80ºC until used. Tropomyosin was isolated from the mantle muscle of *T. pacificus* and *O. vulgaris*. The acetone-dried muscle prepared according to the conventional method was extracted with 10 volumes of 1 M KCl. The supernatant obtained was subjected to isoelectric precipitation of tropomyosin by adjusting the pH to 4.5 by titrating 0.1 M HCl. Tropomyosin was further purified by ammonium sulfate fractionation between 40 ~ 50% saturation fractions. Protein concentration was determined by biuret method. DSC was performed using a microcalorimeter (model VP-DSC, MicroCal, Northampton, MA, USA) on the purified tropomyosin in a medium consisting of 10 mM sodium phosphate (pH 7.0), 0.1 M KCl, 1 mM dithiothreitol (DTT) and 0.01% NaN_3. The temperature range was from 5 to 80ºC. The increment of temperature was set to at 1ºC/min. Protein concentration was in the range from 1.0-1.5 mg/mL. DSC data were analyzed for determination of melting temperature (*T*m) using a software package Origin developed by MicroCal. Amino acid sequences of myosin heavy chain and tropomyosin were aligned with the software ClustalW. Phylogenetic tree was drawn on the basis of the amino acid sequences of α-tropomyosin using the neighbor-joining method.

Results and Discussion

Characterization of cephalopod myosins

Amino acid sequences of myosin heavy chain were compared between longfin inshore squid and bay scallop (Fig. 1).

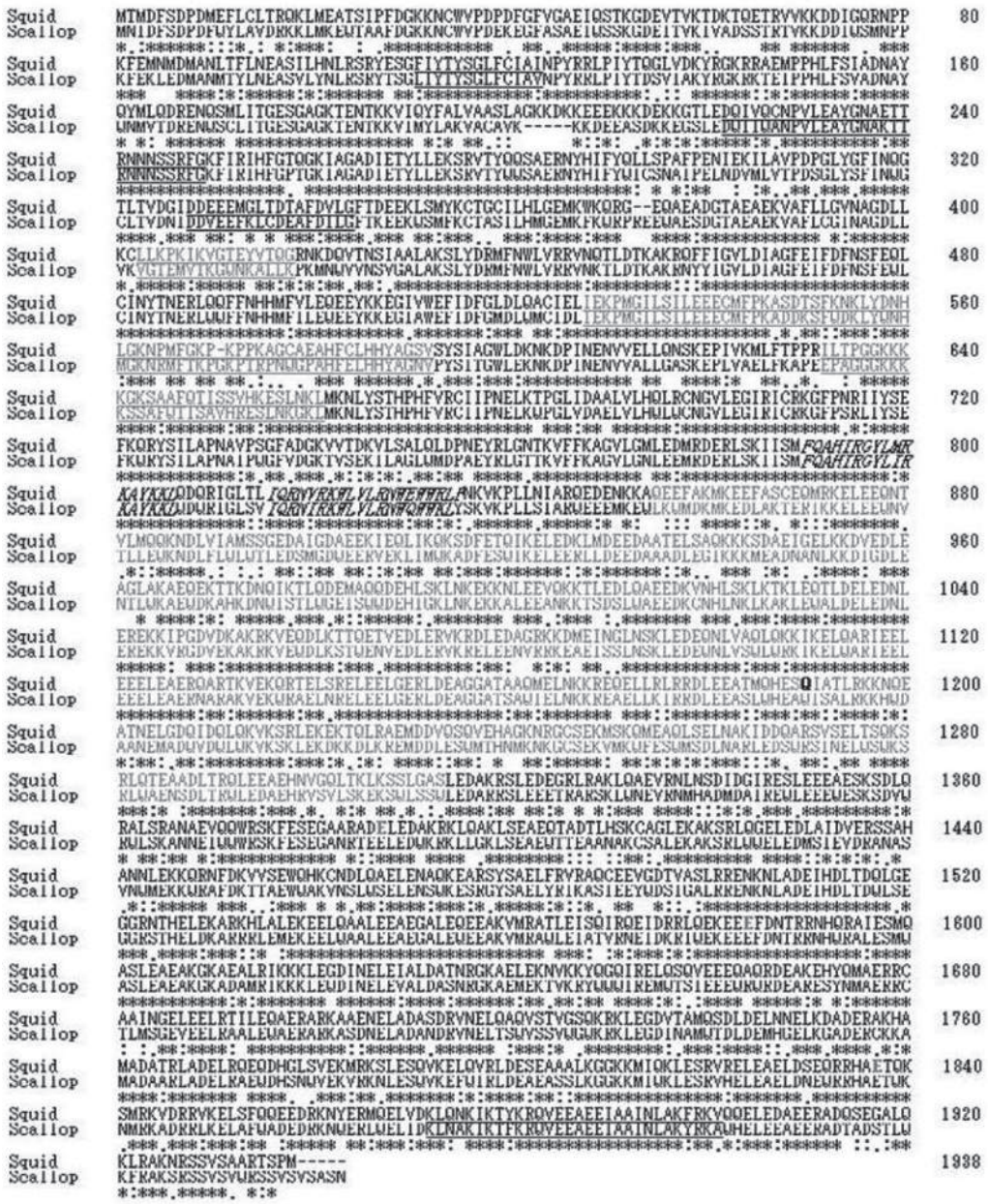

Figure 1**.** Alignment of the amino acid sequences of squid and scallop myosin heavy chains. The sequence data of longfin inshore squid *Loligo pealeii* (O44934) and bay scallop *Aequipecten irradians* (P24733) were aligned by Clustal W. The dentical residues are shown by asterisks, the conservative and semi-conservative replacements are shown by colons and semicolons. Gaps are indicated by hyphens. The residues consisting of the S1 and light meromyosin (LMM) regions are in black letters, while those of S2 region are in gray. ATP binding sites are underlined, whereas actin binding sites are in gray and underlined. The light chain binding sites are italicized. The assembly competent domain close to the N terminus is underlined. The skip residues are bold-faced. Symbols used; *, identical residues; : , conservative replacements; semi-conservative replacements, in comparison with the squid myosin heavy chain sequence.

In the figure, S-2 region connecting S-1 and LMM regions is shown by gray letters. Gaps were found in the head region of scallop heavy chain, while the tail of squid myosin heavy chain was a little shorter compared to scallop myosin. The sequence identity was about 87% throughout the molecule, though the identity varied among the regions of the molecule. In the rod regions of both myosins, four skip residues (as shown by bold-faced letters in the figure), which could perturb the regular coiled-coil structures, were specified.

The structure of the head portion (S-1) of myosin is very complicated. It is thus almost impossible to draw conclusive remarks on the sequence-stability relationship only by comparison of amino acid sequence. However, as described above, the rod portion of molluscan myosins give rise to characteristic sequence, suggesting the instability of this portion. It is established that the rod portion is involved in gel formation of meat, especially for the case of fish (Fukushima et al. 2003).

Myosin itself exists in a large amount in muscle, and thus is easily prepared. However, biochemical and thermodynamic data obtained from myosin are very complicated because this protein is a large molecule (~500 kDa) and is composed of quite different parts, namely, S-1 and rod. However, it requires a lot of labor to prepare S-1 or rod, because it is only possible after enzymatic cleavage of these portions. This is a drawback to the detailed study on structure-stability relationship of the myosin molecule. In contrast, it is much easier to prepare tropomyosin.

Characterization of cephalopod tropomyosins

As shown in Fig. 2A, tropom-yosin molecule is fibrous, forming α-helical coiled-coil structure almost throughout the entire molecule. The schematic diagram of the cross section of the coiled-coil structure is shown in Fig. 2B. In general, at the *a* and *d* positions, hydroph-obic residues tend to occupy to form a hydrophobic core to stabilize the coiled-coil (so-called 'a heptad repeat rule'). On the other hand, salt bridges tend to be formed between the *e* and *g* positions. As long as the tropomyosins so far studied are concerned, there are many exceptions to the localization of such amino acid residues, suggesting that the coiled-coil structure of tropomyosin is loosened at several regions.

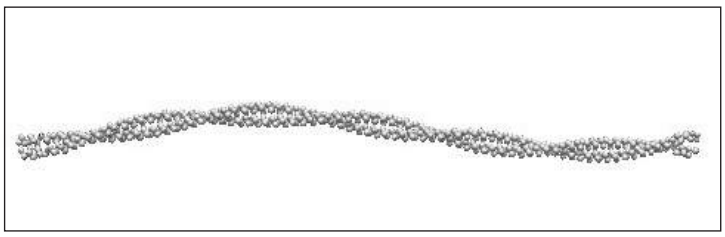

Figure 2A**.** Tertiary structure of tropomyosin molecule (PDB 2tma)

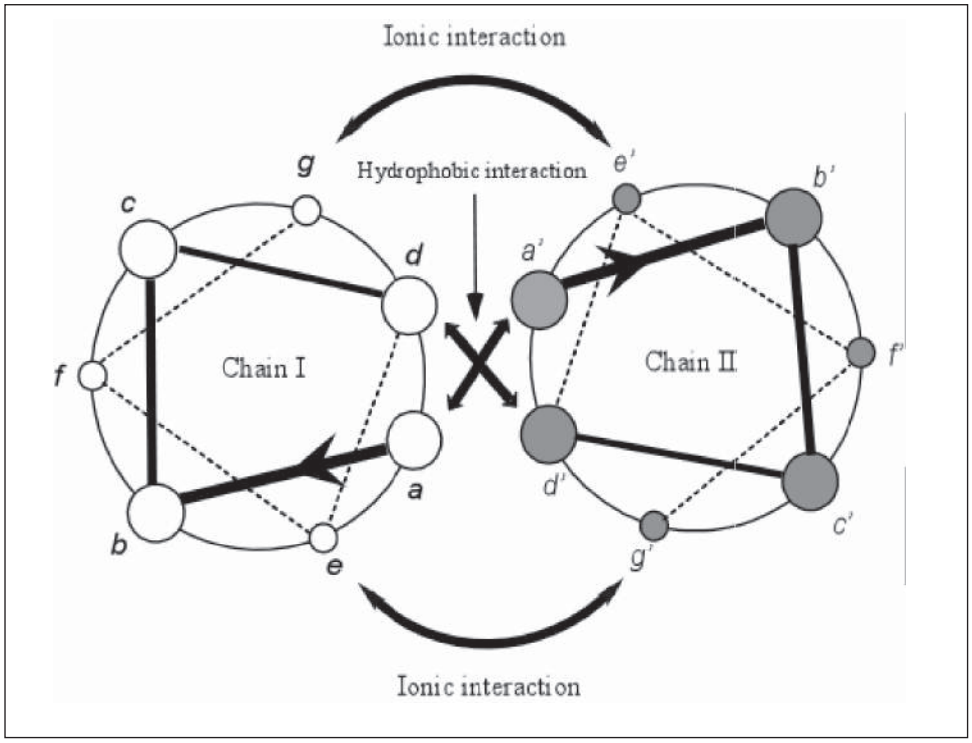

Figure 2B. The schematic diagram of the cross - section of coiled-coil structure

It is considered to be necessary for the function of this protein, namely, regulation of myosin-actin interaction during muscle contraction. In connection with this, molluscan tropomyosins tend to be extracted in water-soluble fraction, unlike vertebrate counterpart (Motoyama et al. 2006). The amino acid sequences of four molluscan tropomyosins were aligned with those from other sources (Fig. 3).

The organisms included Japanese common squid *T. pacificus*, common octopus *O. vulgaris*, and Japanese abalone *Haliotis diversicolor* (molluscs), lobster *Homarus americanus*, crab *Portunus sanguinolentus*, and prawn *Marsupenaeus japonicus*, (arthropods), amphioxus *Branchiostoma belcheri* (cephalochordate), white croaker *Pennahia argentata* and frog *Rana temporaria* (vertebrates). The sequences were headed with the heptad positions of the coiled-coil (*a* ~ *g*) corresponding to those in Fig. 2B. From Fig. 3, it is clear that there are so many amino acid replacements between tropomyosins from different phyla, though the heptad repeat rule is roughly true for all tropomyosins. Interestingly, the N-terminal eight residues are conserved for all tropomyosins, while the C-termini are not conserved so much.

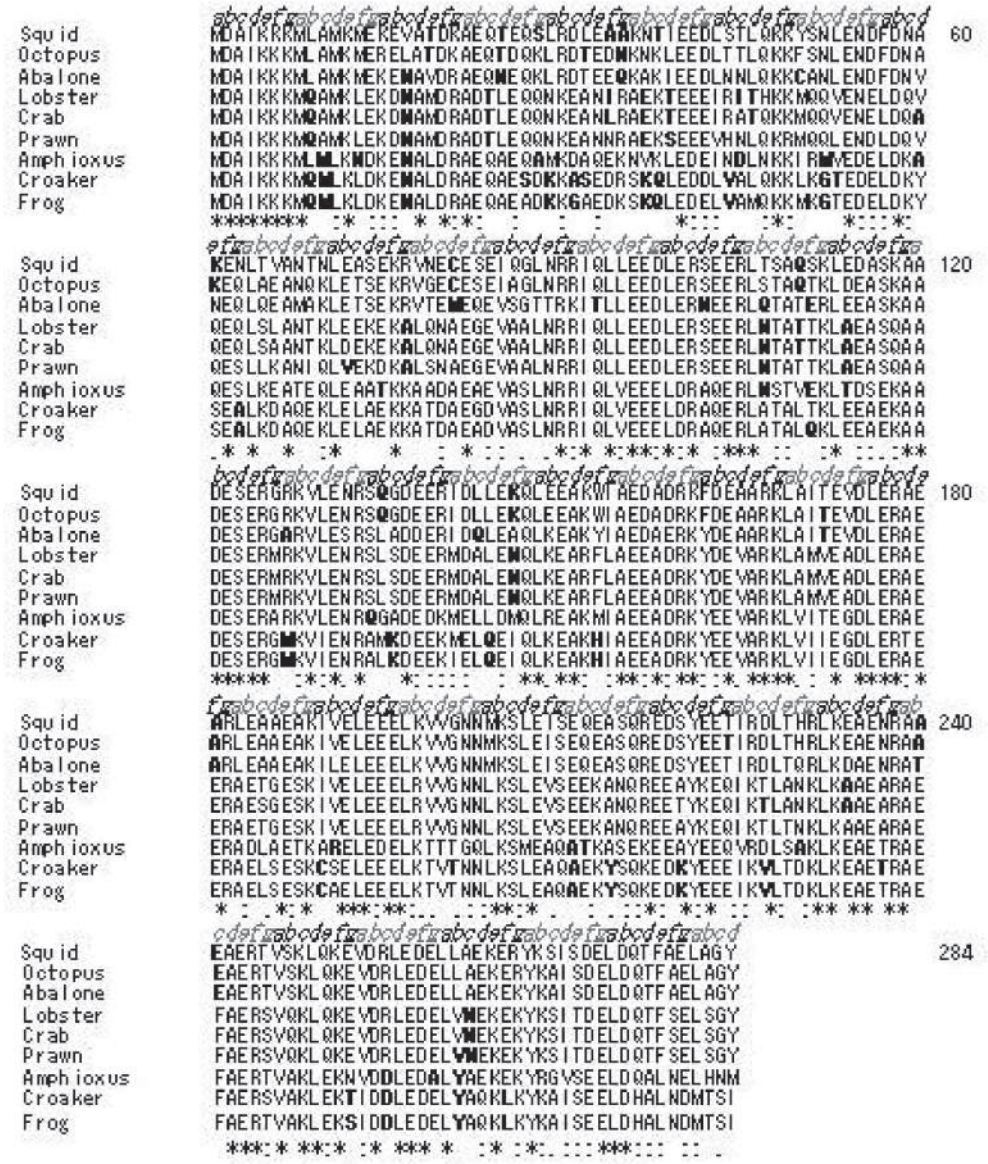

Figure 3. Alignment of amino acid sequences of four molluscan tropomyosins. Squid, Japanese common squid *Todarodes pacificus* (accession #Q2V0V2); octopus, common octopus (#Q2V0V0), Japanese abalone, *Haliotis diversicolor* (#Q9GZ71), lobster, *Homarus americanus* (#O44119), crab, blue crab *Portunus sanguinolentus* (#A1YYV6), prawn, *Penaeus japonicus* (#A2V731), amphioxus, *Branchiostoma belcheri* (#Q9NDS0), croaker, white croaker *Pennahia argentata* (#AB045645), frog, *Rana temporaria* (#P13105). The positions occupied with unique residues are boldfaced. Refer to the legend of Figure 1 for the symbols used.

Based on the amino acid sequences of these tropomyosins, a phylogenetic tree was drawn by the neighbor-joining method (Fig. 4).

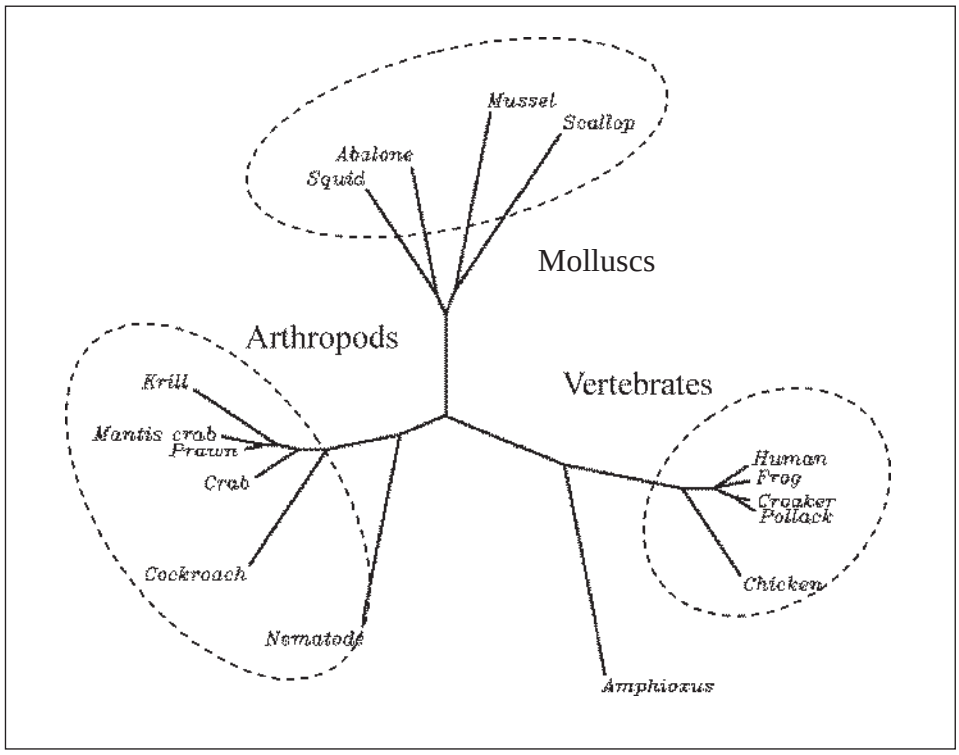

Figure 4. Unrooted phy-logenetic tree drawn based on the amino acid sequences of α tropomyosins by neighbor-joining method. The clusters of vertebrates, arthropods and molluscs are contained in circles separately. Squid, Japanese common squid *Todarodes pacificus* (accession #Q2V0V2); octopus, common octopus (#Q2V0V0), abalone, *Haliotis diversicolor* (#Q9GZ71), lobster, *Homarus americanus* (#O44119), crab, *Portunus sanguinolentus* (#A1YYV6), prawn, *Penaeus japonicus* (#A2V731), amphioxus, *Branchiostoma belcheri* (#Q9NDS0), croaker, *Pennahia argentata* (#AB045645), frog, (#P13105) *Rana temporaria.*

As a result, tropomyosins from different phyla formed clear clusters. It was suggested that molluscan tropomyosins have special structure, though it is not possible to predict the tertiary structure at present, because there is no proper template available. However, such characteristics might be related to the allergenicity of these tropomyosins.

Two species of molluscs, namely, common squid *Todarodes pacificus* and common octopus *Octopus vulgaris* were used for the preparation of tropomyosin. The sequence identities of these proteins were found to be higher than 70%. The parameter of thermal stability and DSC patterns were compared as shown in Fig. 5.

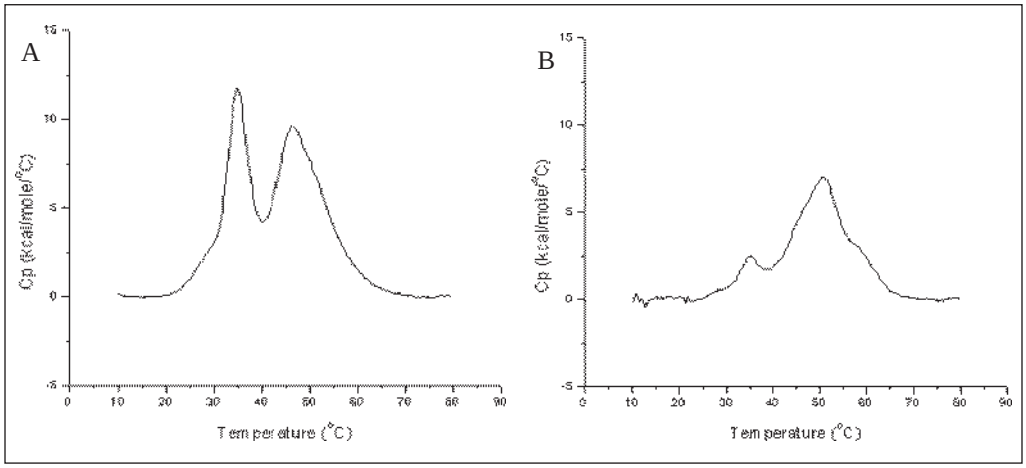

Figure 5. DSC patterns of squid *T. pacificus* tropomyosin. A, first scan; B, second scan. DSC analyses were performed in a medium consisting of 10 mM sodium phosphate (pH 7.0), 0.1 M KCl, 1 mM DTT, 0.01% NaN_3. The temperature range was set to 5-80ºC. The increment of temperature was set at 1ºC/min.

Squid tropomyosin showed a slightly higher transition temperature (*T*m) of 47.0ºC, than the octopus counterpart, whose *T*m value was 44.5ºC. The second scan of squid tropomyosin by DSC suggested that this protein was refolded even after the first thermal treatment which caused structure perturbation, though the endothermic peak at the lower temperature was not recognized in the second scan. Preliminary experiments showed that tropomyosins from scallop adductor and smooth muscles showed clearly different stability, with the *T*m values being 29.4 and 35.8ºC, respectively. Because smooth muscle (catch muscle) of the adductor has unique composition of proteins (Perreault-Micale and Szent-Gyorgyi 1996, Shelud'ko et al. 2001), tropomyosins from these two muscles seem to have adapted to respective physiological conditions.

These results suggest that molluscan muscle proteins are relatively stable. Incidentally, the *T*m values of fish tropomyosins are in the range of 26.4 ~ 46.5ºC (Huang and Ochiai 2005). Because molluscs are ectotherms, thus the stability of their proteins is greatly affected by the environmental temperature. Therefore, the proteins from cold-water inhabiting species are considered to be less stable compared to those from warm-water species. However, the stability of each protein component is to be examined for further discussion. So far several reports suggest that tropomyosin is a suitable model for the relationship between sequence, structure and function (Kluwe et al. 1995, Perry 2001, Miura-Yokota et al. 2005). This seems also to be true for molluscan counterparts.

The difference between the coiled-coils from myosin heavy chains and tropomyosins is that the former forms side-by-side filament under physiological condition, while the latter forms only head-to-tail polymerized filament and is soluble

even in water. The amino acid residues located at the surface of these proteins cause such a difference. Thermal stability is considered to depend on the stability of the hydrophobic core of these proteins.

It is very important to handle myosin during storage and processing of meat, because denaturation of this protein causes deterioration of meat as observed in decrease in solubility and water holding capacity. Filament formation ability largely affects such changes. To know the thermodynamic properties of such filamentous proteins is thus considered to be essential to optimize storage and processing conditions. Above all, the coiled-coil regions are excellent tools to monitor the structural changes of proteins.

Conclusion

Myosin heavy chain and tropomyosin from molluscan muscle, especially those from cephalopods were characterized based on their amino acid sequence and thermal denaturation profile. The sequence alignment of these proteins revealed the uniqueness of molluscan proteins. The coiled-coil regions of these proteins are excellent markers for their thermal stability. It could be helpful for optimizing the storage and processing conditions of molluscs.

Acknowledgment

The present study was defrayed in part by the research fund from the Ministry of Education, Science, Sport and Technology of Japan.

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Received: 28 December 2007; Accepted: 12 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Fish Production and Energy Requirement during Demersal and Aimed Midwater Trawling by Intermediate Range Freezer Trawler

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Abstract

This paper deals with the fish production and relative energy consumption in demersal and midwater trawling based on data derived from cruises of Intermediate range freezer trawler that operated in Indian waters during 1993-94. The trawler has length overall of 62.2 m, gross registered tonnage of 1898 and installed engine power of 2400 hp. Operations were conducted between 14° and 22° N lat., off west cost of India, within a depth range of 31 and 125 m, using 47.5 m four-seam bottom trawl rigged with bobbin gear and 70.0 m mid-water trawl. The present investigations have shown that significant improvements in landings were obtained during aimed midwater trawling, off west coast of India. The mean daily landings rose from an average of 5.66 t, during bottom trawling to 22.84 t, during midwater trawling, realising over 300% improvement in the landings that manifested in a significant reduction in the consumption of fuel per unit volume of fish landed by midwater trawling. Overall fuel consumption per kg fish landed by bottom trawling and midwater trawling worked to be 1.34 and 0.33 kg, showing a four-fold difference. The difference in daily fuel expenditure per unit volume of fish between bottom and aimed mid-water trawling was found to be highly significant statistically. As there is intense concentration of effort in the bottom trawl fisheries, it could be advantageous from the resource management perspective and also from the energy conservation point of view, to encourage diversification to midwater trawling, in a controlled manner without compromising on sustainability of resources. Stern trawler does not require any large-scale modifications in structure or deck layout, for undertaking midwater trawling. However, the vessel must be large enough, highly manoeuvrable and sufficiently powered to tow a large mouthed midwater trawl at speeds exceeding 4.5 knots; should be equipped with acoustic fish detection (sonar and echosounder) and trawl monitoring systems; and in addition, must have provision for handling and preserving large volume landings.

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Introduction

With the declaration of the Exclusive Economic Zone in 1986 over 2 million square kilometres extending to 200 nautical miles from coastline was brought under the exclusive national jurisdiction. This has restricted the free and open access of the distant water fishing fleet operated by developed nations such as Japan, Taiwan, Korea and erstwhile USSR, in Indian EEZ. The need for developing deep sea fishing industry for harvesting the extended area of jurisdiction was reflected in the formulation of deep sea policy during 1991 and subsequent reviews, in India (Anon 1977; Anon1996; Vijayakumaran & Haridas 1998).

Over 180 fishing vessels of 20 m L_{OA} and above and about 30 large vessels under joint venture / charter scheme were in operation in Indian waters in 1990s. The vessels under joint venture / chartered category were large trawlers equipped for undertaking demersal and/or midwater trawling, and long liners. The main objective of the deep sea fishing policy was modernisation of deep sea fishing sector of India, in a phased manner by acquisition of technology and expertise in order to harvest the under-utilised deep sea fishery resources in the Indian EEZ.

Single boat midwater trawling was developed in the late 1940s to capture pelagic shoaling fishes. Since then, the development and application has progressed at a great speed in different parts of the world. In midwater trawling, the gear must easily be manoeuvrable in accordance with the distribution of shoals of target species between surface and seabed. Successful midwater trawling require effective use of acoustic fish detection and net monitoring equipment, in order to guide the net accurately into the position of shoals. Both one-boat and two-boat midwater trawling are practised in commercial fisheries, in different parts of the world (Amos 1980; Brandt 1984; Sainsbury 1996).

Pelagic species are generally fast swimming. They form dense shoals during daytime and respond to stimuli collectively. Pelagic fishes posses well developed sight and hearing capabilities. Midwater trawling is most successful when shoals are dense and large; when fishes are less active due to low ambient temperature or physiological states such as nonfeeding, spawning or spent conditions; and when visibility is poor causing fish to react more slowly. Main design requirements for midwater trawls are high stability, large mouth opening, low turbulence and low drag. Midwater trawls require largest possible opening of the mouth, permitted by the available towing force of the vessel at the required towing speed, allowing roughly 30% margin of reserve power for gear manoeuvre during operations. The large mouth opening is usually achieved by the incorporation of large side panels. Wings are consequently reduced in size or absent altogether in the midwater trawl, unlike the bottom trawl. The mouth opening may be oval, circular or square depending on the design and rigging. In some designs of surface operated trawls, the lower panel is extended ahead of the head rope.

This is to counteract the tendency of pelagic fishes to dive downwards in response to disturbance caused by the approaching trawl (Scharfe 1969; Kristjonsson 1971; Amos 1980; Anon 1993).

Smooth water flow through the net is extremely important requirement in midwater trawls in order to prevent turbulence in the proximity of the trawl mouth. In order to achieve smooth water flow, midwater trawls are longer and more finely tapered with longer extension piece and codend, compared to bottom trawls. In addition, very large meshes are generally used in the wing and front trawl sections, which reduce the drag and improve the water flow. Most pelagic fishes are effectively herded into the small meshed hind part and codend by the large meshes used in the front trawl sections. Increase in mesh sizes of the front trawl sections is a major design improvement that has taken place in midwater trawl design. Mesh size of 200 mm used in earlier designs gradually increased to 3000 mm or more in large modern midwater trawls. Pelagic trawls wherein front sections are replaced with large meshes and ropes are technologically superior, in terms of drag reduction and increase in volume filtered (Brabant et al.1980). Fishes are generally subject to herding effect when they approach near the netting panel (Glass et al. 1993). Effective dimension of the net mouth thus, would be less by the distance from the panel at which the herding response is effective.

One of the important features of midwater trawl is the high towing speed required for catching fast-swimming pelagic fish. Size of the net and the resultant drag has to be matched with the vessel's available towing force at the towing speed effective for the target species. Drag of midwater trawl is primarily determined by twine surface area of the netting. Drag is also influenced by the shape and taper of the net and gear appurtenances such as floats, weights and sheer devices. Drag changes significantly with changes in towing speed (Reid 1977; Brandt 1984). The most popular otter board used for one-boat mid-water trawling is Suberkrub design (Suberkrub 1959). Suberkrub otter boards have high hydrodynamic efficiency with a sheer-drag ratio in excess of 6.0, high aspect ratio of 2:1, and is vertically cambered. Introduction of Suberkrub otter boards is a significant development in one-boat midwater trawling (Brandt 1971; FAO 1974).

Some of the earliest developmental stages in one-boat midwater trawling were the introduction of Larsson's phantom trawl; British Colombian trawl for herring; and Cobb pelagic trawl for Pacific hake (McNeely 1965; Brandt 1971; Anon 1993). Wider commercial acceptance of the technique took place after the successful introduction of the German one-boat midwater trawling system in 1960s and the simultaneous developments in the acoustic fish detection and net monitoring equipment. Success of the German one-boat trawling has proved that a midwater trawl of large mouth area

towed even at a slower speed could be more effective than a trawl with small mouth area towed at a greater speed (Scharfe 1969). Midwater trawl designs operated in different parts of the world tend to be similar in general features with regional variations in the structure and rigging of the gear components.

In aimed midwater trawling, the vessel is steamed towards the shoal of the target species after its location by sonar. At a reasonable distance from the target shoal, the gear is shot and its position under water is adjusted so as to take in the shoal. The fishing depth of the trawl is adjusted by varying vessel's speed and the length of the towing warps, either singly or in combination, for quicker response. The net monitor (net sonde) attached to the head rope of the trawl provides the data on the fishing depth, vertical opening of the net mouth and the catch entering the net, which are required for successful gear manoeuvre, based on data from sonar and echosounder. Additional sensors in net monitoring system could provide data on the horizontal spread of the trawl mouth and at otter boards, *in-situ* temperature and catch increment in the codend (Larsen 1989; Mross 1989; Allison 1971; Horn 1971).

In midwater trawl, towing tension is on the head rope and the vertical opening is primarily achieved by the depressor weights attached to the lower wing-ends. Floats attached to the head rope help in keeping the head rope clear during shooting and hauling operations. Thus, in midwater trawling, the combined length of the lower sweeps and bridles between wing-end and otter board are longer than the upper sweeps and bridles. In contrast, the towing tension is on the ground rope along the seabed in the bottom trawl and the vertical opening is achieved by lifting the head rope from the seabed by net design features and floatation. Towing speed varies with the target species. A towing speed of 2.5 -3.0 knots may be good enough for slow swimming target species while for fast swimming species towing speeds of 4.5 - 8.0 knots are used.

In India, a few studies have been conducted on midwater trawling from small trawlers (Perumal 1966; Sivan et al. 1979; Kartha & Sadanandan 1973; Mhalathkar et al. 1975; Mhalathkar et al. 1983). Indo-Norwegian Project conducted two-boat midwater trawling using midwater trawls of 17.6 m head rope from 9.76 m L_{OA} vessels, within 25 m depth zone off Kerala coast, during 1973-78. Single-boat midwater trawling was conducted off Kerala within 40 m depth, using 26.8 m x 26.8 m midwater trawl from 17 m L_{OA} (233 hp) and 19.81m L_{OA} (220 hp) vessel; 43.3m x 43.3 m midwater trawl from 23.8 m (480 hp) vessels and 36.1 m x 44.4 m midwater trawl from 28.0 m L_{OA} (400 hp) vessel, during 1973-75. Major landings during these operations were silver bellies, glass perches, clupieds, carangids and anchovies (Verghese 1975; Verghese & Nair 1975; Oommen 1989). Midwater trawling trials were conducted from *FORV Sagar Sampada* using three variations 46.4 m midwater trawls, rigged with 750 kg Lindholmen

pelagic otter boards (Kuttappan et al. 1989). Midwater rope trawls were operated successfully during Indo-Polish Industrial Fisheries Survey, along the north-west coast of India for pelagic resources such as horse mackerel, ribbonfish, elasmobranchs, pomfret, catfishes and carangids (Dwivedi et al. 1977). Investigations on midwater trawling for Antarctic Krill, were conducted during the First Indian Antarctic Krill Expedition 1995-'96 (FIKEX) (Boopendranath et al. 1999).

The relative operational energy consumption in fish production by demersal and aimed midwater trawling has not been studied so far in Indian waters. The objectives of the present study were (i) survey of the fishing gear, equipment and operation in an intermediate range freezer trawler operations; (ii) determination of daily production of the trawler by midwater and bottom trawling in the north-west coast of India; and (iii) estimation of fuel consumption and energy requirement per unit production of fish by aimed midwater trawling and bottom trawling.

Materials and Methods

Data on landings obtained during two cruises of Intermediate Range Freezer Trawler, which operated in Indian waters during 1993-94 were utilised for this study. The freezer trawler was designed for stern trawling using a bottom or midwater trawl and for onboard production of frozen fish packed in master cartons; production of fish meal and technical fish oil from nonfood fish; for storage of fish products and transportation of products to the port or transhipment to the transport ships (reefer vessels). The trawler had a length overall of 62.2 m and gross registered tonnage of 1898 t. It had an installed engine horsepower of 2400 hp, controllable pitch propeller in the steering nozzle and stern ramp for lowering and lifting of the fishing gear. Details of the vessel, equipment and fishing operations were collected during a cruise onboard in January 1994. The main particulars of the vessel, power plant and fishing equipment are given in Table 1.

Operations were conducted off west coast of India, between latitudes 14° and 22° N, within the depth range of 31 and 125 m (Fig. 1). Bottom trawl of 47.5 m headline length rigged with bobbin gear for rough bottom operation and oval otter boards were used for bottom trawling. Midwater trawl of 70.0 m headline length and suberkrub otter boards were used for aimed midwater trawling. Design details of bottom trawl and midwater trawl are given in Fig. 2 and 3, respectively. Vertical opening of the trawl mouth and horizontal opening between otter boards were measured by acoustic trawl monitoring equipment with sensor for vertical opening attached to the trawl headline and otter boards. Towing speed was measured using Doppler log.

Table 1. Main particulars of the Intermediate Range Freezer Trawler, engine and equipment

Vessel Details		
Length overall	:	62.2 m
Beam	:	13.8 m
Light draught	:	4.19 m
Load draught	:	5.21
GRT	:	1898 t
NRT	:	492 t
Sea autonomy under fuel reserve	:	34 days
Crew	:	35 men
Main Engines		
Type	:	Diesel 8VD 26/20 AL-2
Number of engines	:	2
Power	:	1200 hp (880 kW) at 1000 rpm
Specific fuel consumption per engine	:	166 $kg.hp.h^{-1}$
Auxiliary engine		
Number of engines	:	2
Power	:	846 hp (622 kW)
Specific fuel consumption	:	162 $g.hp.h^{-1}$
Emergency engine		
Number of engines	:	1
Power	:	102 hp (74 kW)
Specific fuel consumption	:	178 $g.hp.h^{-1}$
Fish Finding Devices		
Fish finder	:	1 no.; 19.7 kHz; range: 3000 m
Search light sonar	:	1 no.; 19.7 kHz; range 1500 m
Trawl monitoring system	:	1 no.; 25.5 kHz
Fishing Equipment		
Fishing gear		
Bottom trawl with appurtenances	:	2 nos
Midwater trawl with appurtenances	:	4 nos.
Otterboards- oval slotted type	:	6.5 m^2; 1750 kg each -1 set
Otterboards- suberkrub type	:	8.0 m^2; 2750 kg each - 2 sets

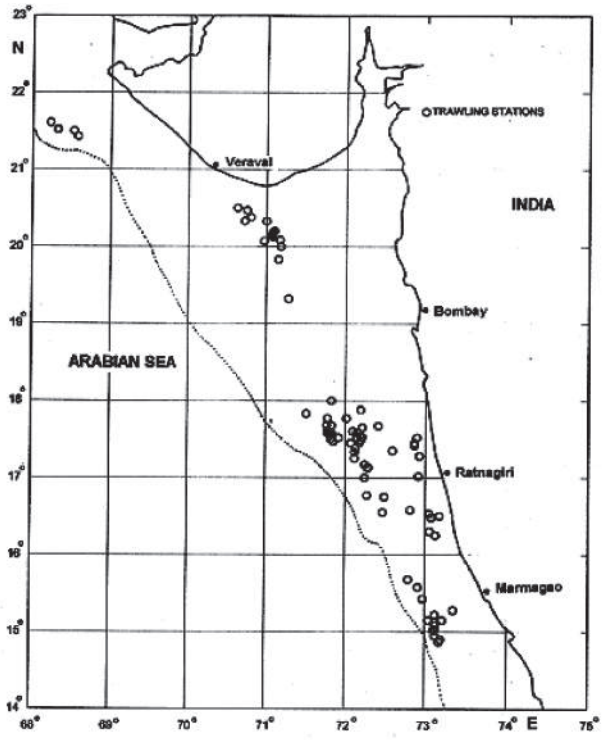

Figure 1. Trawling stations of intermediate range Freezer Trawler

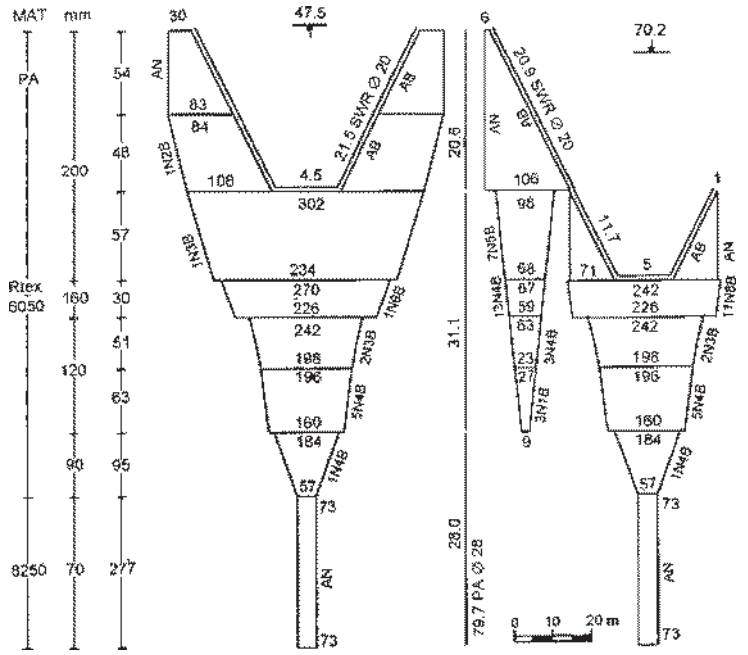

Figure 2. Design of 47.5 m bottom trawl.

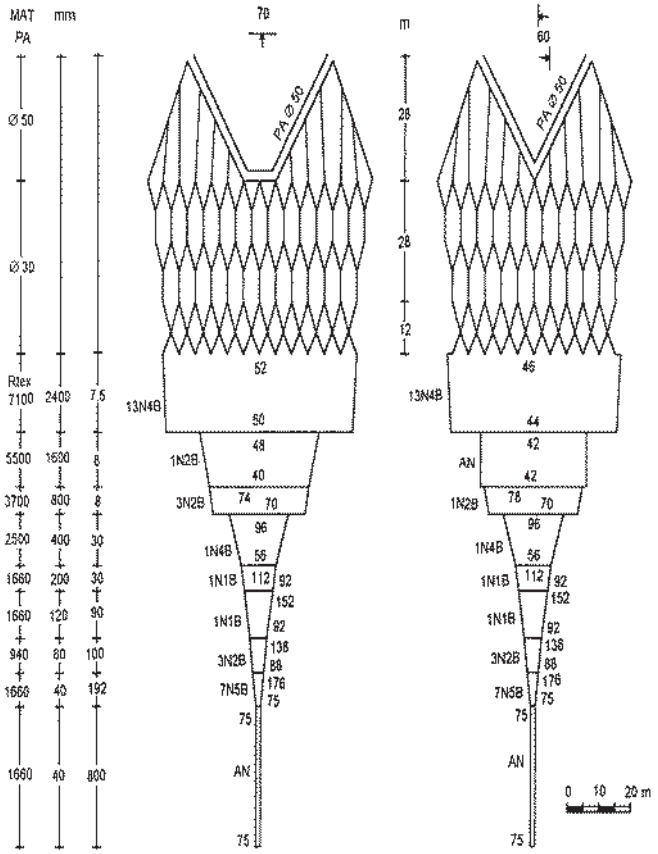

Figure 3. Design of 70.0 m midwater trawl

Average duration of tow for bottom trawling was 2.57 h and 2 to 4 hauls were taken in day. The mean towing speed was 4.23±0.24 knots. Seventy-five hauls spread over 24 days were taken during the period of study. Average duration of tow for aimed midwater trawling was 1.88 h and 2 to 3 hauls were taken per day. The mean towing speed was 4.33±0.16 knots. Seventeen aimed midwater trawling operations spread over 6 days, were conducted during the period of study. Details of operation and catch for bottom and aimed midwater trawling are given in Tables 2 and 3 respectively.

Table 2. Operational and catch details of the Intermediate Range Freezer Trawler

	Bottom trawling	Aimed midwater trawling
No of days	24	6
No of hauls	75	17
Depth range, m	50-121	31-125
Mean duration of hauls, h	2.57	1.88
Total catch, kg	135732	137051
Mean catch.day^{-1}, kg	5655.5	22841.8
SE of catch .day^{-1}	1461.9	7620.7

Table 3. Composition of landings by bottom trawling and aimed midwater trawling

Catch components	Catch (Kg)
Bottom trawling	
Pseudoscianids	1299
Pomfrets	2079
Squids & Cuttlefish	3156
Indian Mackerel	5265
Catfish	7402
Sciaenids	7798
Horse Mackerel	11610
Ribbon Fish	17604
Nemipterids	18369
Perches	34993
Miscellaneous	26157
Sub-total	135732
Aimed midwater trawling	
Scad	400
Horse Mackerel	135955
Miscellaneous	696
Sub-total	137051
Grand total	272783

Estimated duration of time spent for searching, shooting, towing and hauling for both bottom and aimed midwater trawling operations are given in Fig. 4.

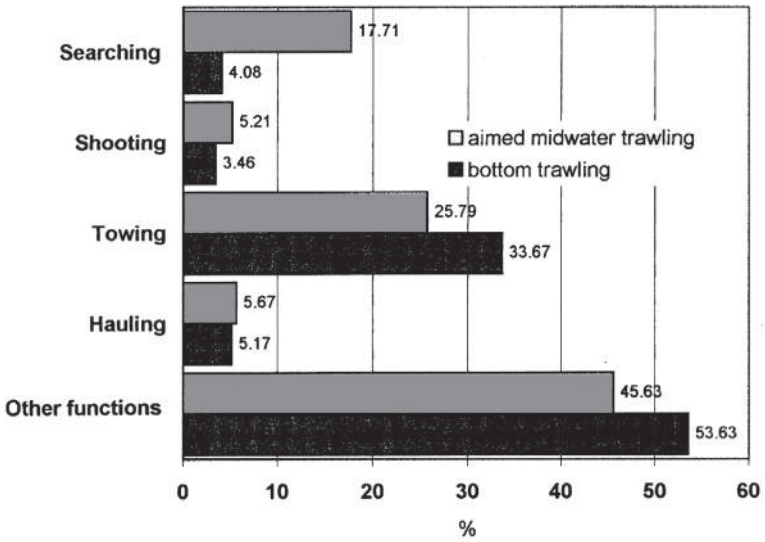

Figure 4. Time utilisation per day of Intermediate Range Freezer Trawler

Fuel consumption was estimated from the specific fuel consumption of the power plant for the estimated period of operation of the engines. Fuel consumption per unit volume of fish landed by both bottom trawling and aimed midwater trawling were determined from the data on the daily fuel consumption and landings. The daily fuel consumption per kg of fish landed were subjected to statistical analysis using Student *t*-test after logarithmic transformation of data, to determine if there is any significant difference between the values obtained for bottom trawling and aimed midwater trawling.

Results and Discussions

Total landings during the period of operations were 272.8 t, of which 135.7 t were landed during 24 days by bottom trawling and 137.1 t by aimed midwater trawling during 6 days of operations. Mean daily catch for bottom and aimed midwater trawling were, respectively, 5655.5 kg (SE: 1461.9) and 22841.8 kg (SE: 7620.7) (Table 2). Vertical opening of the bottom trawl was determined to be 6 m and horizontal opening between otter boards was 85 m. Midwater trawl attained a vertical opening of 45 m and horizontal opening between the otter boards was 160 m. Wing-end spread of midwater trawl was estimated to be 42 m.

Perches constituted 25.78% of the total landings by demersal trawling followed by nemipterids (13.53%), ribbonfish (12.97%), horse mackerel (8.55%), sciaenids (5.75%), cat fish (5.45%), Indian mackerel (3.88%), squids and cuttlefish (2.33%), pomfrets (1.53%), pseudosciaenids (0.96%) and miscellaneous fishes (19.27%). Over 99% of the landings by aimed midwater trawling was constituted by horse mackerel (*Megalaspis cordyla*) (Fig. 5). Scad (*Decapterus* sp.) and miscellaneous species contributed 0.29% and 0.51%, respectively (Table 3).

Figure 5. A catch of 22 tonnes of horse mackerel (*Megalaspis cordyla*), obtained during aimed midwater trawling, off west coast of India

Fuel consumption pattern for bottom trawling and midwater trawling, during the period of observations is given in Table 4. Total fuel consumed during the period of observations was 227.10 t of which 181.68 t was consumed during bottom trawling and 45.42 t during midwater trawling. Results of statistical analysis of the daily variation in the values of fuel consumption per kg of fish landed by bottom trawling and aimed midwater trawling are given in Table 5.

Table 4. Fuel consumption per unit volume of fish caught by bottom trawling and aimed midwater trawling

	Bottom trawling	Aimed midwater trawling
Total catch, kg	135732	137051
Fuel consumption, kg	181680	45420
Overall fuel consumption (kg fuel.kg fish$^{-1)}$	1.339	0.331

Table 5. Results of Student *t*-test of the variation in fuel consumption per unit volume of fish landed between bottom trawling and aimed midwater trawling, using log transformed data.

	Bottom trawling	Aimed midwater trawling
Mean	0.437	-0.231
Variance	0.293	0.389
Pooled variance	0.310	
Observations	24	6
df	28	
t - stat	2.626 (Significant at 0.01 level)	

The catch data showed large scale variations in the volume of total catch and catch composition. During bottom trawling operations, the vessel spent on an average 3.46% of the 24 h period for shooting, 33.67% for towing, 5.17% for hauling, 4.08% for ground shifting and 53.63% for fishing-independent functions. During aimed midwater trawling operations, the vessel spent on average 5.21% for shooting, 25.79% for towing, 5.67% for hauling, 17.71% for acoustic search for schools using search light sonar and 45.63% for other functions unrelated to fishing (Fig. 4).

Average fuel consumption was estimated to be 7.75 t.day^{-1}. Overall fuel consumption per kg fish landed by bottom trawling and midwater trawling worked to be 1.339 and 0.331 kg, showing a four-fold difference (Table 4). Daily values of fuel consumption per kg of fish landed ranged from a maximum of 37.83 kg to a minimum of 0.33 kg, with a mean value of 5.46 kg (SE: 1.61) and a median value of 2.70 kg, for bottom trawling. For midwater trawling, daily values of fuel consumption per kg of fish

landed ranged from a maximum of 5.36 kg to a minimum of 0.15 kg, with a mean value of 1.44 kg (SE: 0.85) and a median value of 0.32. Statistical analysis of the values of daily fuel consumption per unit volume of landed catch, has shown that the variation between the two types of operations is highly significant ($p < 0.01$; df: 28) (Table 5). There is about four-fold increase in the consumption of fuel used for unit volume of landings by bottom trawling compared to aimed midwater trawling operations.

Investigations on one-boat midwater trawling off the south-west coast by Integrated Fisheries Project, during 1973-85, from five large trawlers of 17.0-28.0 m L_{OA} (220-480 hp), have given encouraging results (Oommen, 1989). The overall catch rate realised was 102.8 $kg.h^{-1}$. The landings consisted of anchovies 22.3%, followed by glass perch (18.3%), carangids (11.8%), sardines (11.6%), silver bellies (8.6%), mackerel (0.5%) and other fishes (26.9%). Results of midwater trawling using rope trawl from *M.T. Muraena*, during Indo-Polish Industrial Survey, have shown that there is distinct possibility of catching sizeable quantity of horse mackerel, ribbon fish, pomfrets, catfish and carangids by midwater trawling from about 70-120 m along north-west coast of India (Anon 1979). Taking advantage of the diurnal migration, squid and cuttlefish can also be caught by midwater trawling (Joseph 1985).

Conclusions

The present investigations have shown that significant improvements in landings were obtained during aimed midwater trawling, off west coast of India. The mean daily landings rose from an average of 5.66 t, during bottom trawling to 22.84 t, during midwater trawling, realising over 300% improvement in the landings, which manifested in a significant reduction in the consumption of fuel per unit volume of fish landed by midwater trawling. As there is intense concentration of effort in the bottom trawl fisheries, it could be advantageous from the resource management perspective and also from the energy conservation point of view, to encourage diversification to midwater trawling, in a controlled manner without compromising on sustainability of resources. Stern trawler does not require any large-scale modifications in structure or deck layout, for undertaking midwater trawling. However, the vessel must be large enough, highly manoeuvrable and sufficiently powered to tow a large mouthed midwater trawl at speeds exceeding 4.5 knots; should be equipped with acoustic fish detection (sonar) and trawl monitoring systems (net monitor or net sonde); and, in addition, must have provision for handling and preserving high volume landings.

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Received: 19 December 2007; Accepted: 11 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Seasonal Variation in Semen Characteristics and Biochemical Composition of Seminal Plasma of Mrigal, *Cirrhinus mrigala* (Ham.)

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Abstract

The milt quality parameters such as percentage of spermatozoa motility, duration of motility, sperm concentration, and seminal plasma composition of milt of *Cirrhinus Mrigala* (*C. mrigala*) varied throughout the breeding season i.e. from April to September. The motility duration (second) was low in the beginning of season ie. April (42 ± 4.32) and recorded a peak of 97.5 ± 4.12 during July and again started declining and reached to 39 ± 4.76 at the end of the season. The mean pH values of seminal plasma ranged from 8.05 ± 0.19 to 8.6 ± 0.12 with a maximum pH in July. The osmolality of seminal plasma was low during beginning and end of breeding season. The highest osmolality of seminal plasma 291.5 mOsm kg^{-1} was observed during July. The milt yield mL kg^{-1}, spermatocrit (%), spermatozoa counts (nos/mL) observed in July were 13.9 ± 3.47, 82.5 ± 4.43, and 33.5 ± 1.4, respectively. The same parameters declined to 3.07 ± 0.76, 69.75 ± 4.78, and 14.3 ± 3.3 at the end of the breeding season. The following are the range of ion concentration during breeding season: Na 88.92 ± 22.22 to 140.5 ± 3.7 m Eq/L, K^+ 29.25 ± 5.0 to 52.3 ± 19.28 m Eq/L, and Cl 64.82 ± 3.60 to 174 ± 5.88 m Eq/L. The seasonal declines of Na and Cl ion levels were observed when seminal plasma osmolality values showed lower values. The mean range of total protein, cholesterol, and glucose were 0.105 ± 0.03 to 0.515 ± 0.05 g/dl, 8.52 ± 0.77 to 22.97 ± 2.98 mg/dl, and 0.525±0.05 to 1.83 ± 0.125mg/dl, respectively, during the spawning season. The semen characteristics and biochemical composition of mrigal will help in development of the basic knowledge and the strategies during artificial spawning programmes.

Introduction

Both male and female brood fish share equal responsibility for seed production. In brood fish farming, there has been more focus on female brood fish rather than male brood fish. The systematic studies on the functional efficacy of the teleostean testis with reference to carp are meagre. Several parameters have been documented to evaluate the milt quality including motility, spermatocrit, sperm density, fertilizing capacity

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osmolality and pH of seminal plasma, chemical composition of seminal plasma, enzymatic activity, and several others. Reports on such studies related to seasonal variation are fragmentary (Billard & Cosson 1992; Billard et al. 1995).

High quality of semen is important to the fisheries industry and laboratory research. There are many factors contributing to individual variation in sperm quality (Rana 1995) such as genetic variability among fish, rearing conditions, fish handling, sperm collection methods, storage of milt, and sperm activation conditions. The constitution of milt in terms of performance and numbers of spermatozoa, chemical composition, and osmolality varies interspecifically and even within the same individual with time. Therefore, the time of collection of milt is significant for successful cryopreservation. Individual and seasonal variability of gamete quality is well known for carps (Billard et al. 1995; Linhart et al. 1995; Christ et al. 1996). Correspondingly, Lubzens et al. 1997 and Linhart et al. 2000 indicated that this variability might also influence the motility and the success of fertilization after cryopreservation.

The biochemical composition of teleost milt has been studied by many workers over the years (Piironen & Hyvarinen 1983; Billard & Menezo 1984; Linhart et al. 1991; Billard et al. 1995b). The seminal plasma analysis includes inorganic constituents (Na^+, K^+, Ca^{2+}, Mg^{2+}) involved in the process of inhibition or activation of sperm motility (Morisawa et al. 1983; Morisawa 1985). Organic compounds such as triglycerides, glycerols, fatty acids, and glucose are found in seminal plasma (Lahnsteiner et al. 1993).

The Indian major carp, mrigal, *Cirrhinus mrigala* (Ham.) is a widely farmed species in the Indo-Gangetic floodplains of India, Bangladesh, and Pakistan. It is an important component of carp polyculture system. Mrigal occupies normally 30–40% in polyculture of Indian major carps consisting catla, rohu, and mrigal. Literatures on the seasonal variation in the biochemical composition of seminal plasma of Indian major carps are scanty. These parameters play an important role in the sperm of Indian major carps. To have controlled and successful production in aquaculture systems, it is necessary to have adequate knowledge of the physical and chemical characteristics of the semen of cultivated fishes. In the present study, an attempt has been made to evaluate the seasonal changes of semen characteristics and the biochemical composition of seminal plasma of mrigal, *C. mrigala* (Ham.) in detailed and systematic manner.

Materials and Methods

Carp brood husbandry practices

The brood fish of Indian major carp, *Cirrhinus mrigala* used in this study was reared in earthen ponds of 0.2 ha of the farm facility of the Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar (Lat. 20° 11’ 06"-20°11’ 45" N., Long.

85° 50'52" – 85°51' 35"E.), Orissa, India during October 2005 to August 2007.

Collection of semen

For the study of seasonal changes in the semen characteristics and biochemical composition of seminal plasma of *C. mrigala*, the milt was collected every month of the spawning season from two-year matured male brood fish (1.6 ± 0.4 kg) after five hour of intraperitoneal administration of hormone (Ovaprim, Salmon GnRH + domperidone, Syndel Laboratories, Canada) at a rate of 0.2 mL/kg body weight. Milt samples were collected in ice cooled and sterilized test tubes. During milt collection, attention was paid to prevent contamination by fecal matter, urine, blood, or scales; to provide enough oxygenation to the sperm by maintaining enough head space in the tubes, and to maintain the temperature of the collected semen at 4°C until further analysis. The collected semen was evaluated for sperm yield/kg body weight, motility, pH, spermatocrit percentage. sperm count, and biochemical composition of seminal plasma.

Motility assessment

Spermatozoa motility assessment was carried out by diluting milt with sterile water (1:100) at room temperature (31°C) on glass slide and was observed immediately under an inverted microscope (200X) (Zeiss, Germany) that is attached with a CCD camera. Estimation of spermatozoa motility was started immediately (approximately 10 s) after dilution and the movement was observed for 3 min. The motility was recorded in a computer using computer aided motility software (Biovis motility software, M/S Expert Vision Pvt. Ltd, India). The percentage of rapid, vigorous, and forward motility was observed and calculated in relation to the total number of observed (immotile and poorly motile) spermatozoa in each field of vision from the time activator was added until the motility up to 0.

Estimation of sperm count and percentage of spermatocrit

Spermatocrit values (packed sperm cells) of all the semen samples were determined by micro haematocrit centrifuge (Hermle, USA) immediately after collection of milt to avoid abnormal reading due to cellular swelling induced by CO_2 release (Wedemeyer & Yasutake 1977). All semen samples were assessed under a microscope (Zeiss, Germany) using a computer assisted semen analyzer (Biovis motility software) on a 20 µm micro cell counting chamber. The sperm count was carried out by diluting it 1000 times with an extender solution and adding 20 µl of mixture to the hemocytometer slide and observed under an inverted microscope. Sperm density was also determined by measuring spermatocrit value and also through microscopic sperm counting. Microhematocrit

capillary tubes (75 mm length and 1.2 mm diameter) were filled (approximately 75%) with semen and one end of each tube was sealed for tube centrifugation in a microhematocrit centrifuge at 10000 xg. Measurements were taken in triplicate for each sample, and the average of the three measurements was used for the results.

Measurement of osmolality

The seasonal variations of osmolality of seminal plasma were studied through the spawning season**.** The osmolality of seminal plasma was measured by an osmometer (Model 3250, Advanced Instruments Inc, Massachusetts-02062, USA) using a freezing point depression and expressed as mOsmos.Kg^{-1}

Measurement of pH

The pH of the seminal plasma of mrigal was examined every month during spawning season. During the month of July, the pH of seminal plasma of Indian major carp and exotic carp were also studied. The pH was measured using a laboratory pH meter.

Biochemical analysis of seminal plasma

Milt samples were centrifuged (10,000g, 10 min.), and the supernatant (seminal plasma) was collected in a sterile container and stored at -20°C for further analysis in the laboratory of Department of Biochemistry, S.C.B. Medical College and Hospital, Cuttack, India. All electrolytes, metabolites, and enzymes were determined using an automated system with adequate standards (Flexor-XL ISE, Netherlands). The following parameters were measured and expressed in the following units: albumin (g/dl), glucose (mg/dl) (Srikanth et al. 2004), urea, uric acid (Fei et al. 2006), cholesterol, triglycerides (Sullivan et al. 1985), bilirubin, urea, creatinine (mg/dl); alanine aminotransferase **(**GPT), aspartate aminotransferase **(**SGOT), chloride, potassium, sodium (mEq/l), albumin, and total protein (g/dl) (Kingsley 1939).

Results

The semen characteristics of mrigal showed a clear variation throughout the breeding season starting from April to September. The milt parameters are shown in Table 1.

Table 1. Seasonal changes in semen characteristics of mrigal, *C. mrigala*

	April	May	June	July	August	Sept
Milt yield (ml/kg)	2.65 ± 0.66^{d}	5.7± 1.42 ^{c}	12.3 ± 3.07^{b}	13.9± 3.47^{a}	9.5± 2.37^{b}	3.07± 0.76^{d}
Spermatocrit (%)	65.2 ± 4.81^{e}	75.2 ± 3.19^{c}	81.0 ± 3.16^{a}	83.40 ± 2.19 ^{a}	78.60 ± 2.60^{b}	69.8 ± 3.63^{d}
Sperm count (X 10^{9})/ml	10.30 ± 1.53^{d}	20.1 ± 3.13^{b}	31.18 ± 2.34^{a}	33.52 ± 2.73^{a}	19.6 ± 2.10^{b}	14.38 ± 2.95^{c}
Motility (%)	74.40± 5.17^{c}	85.0± 4.58 ^{b}	93.0 ± 3.31^{a}	92.2± 2.68 ^{a}	84.2 ± 3.70^{b}	77.0 ± 4.0 ^{c}
Motility duration (Seconds)	42.2 ± 3.76^{e}	55.6 ± 2.96 ^{d}	78.4± 3.50^{b}	97.0 ± 3.74^{a}	69.0 ± 2.91^{c}	39.2 ± 4.14 ^{e}
Osmolality (mOsm/kg^{-1})	252.0± 3.16^{e}	259.0± 5.61^{d}	273.8 ± 4.38^{b}	291.8± 4.81^{a}	267.0± 9.21^{b}	261.0 ± 4.06^{d}
pH	8.04 ± 0.16^{e}	8.22 ±0.14^{d}	8.40 ± 0.122 ^{b}	8.6 ± 0.12 ^{a}	8.3 ± 0.1 ^{b}	8.12 ± 0.1 ^{d}

Note: Values are expressed as Mean ± SD (n=27). Values having different superscripts differ significantly in a row

The volumes of milt obtained after hormone induction during the spawning season are shown in Fig. 1a**.** The maximum volume of milt yield (12.3 ± 2.27 kg-^{1}) was recorded in July, whereas the minimum milt yield was 2.66 ± 0.38 mL kg-^{1} in the beginning and in the end of the season in September (3.07 ± 0.69 mL kg-^{1}). The milt yield during different months of spawning season was significantly different.

The values of sperm concentration in different months of breeding season showed a clear variation (Fig. 1b). The sperm cell count ranged from 10.3 x 10^{9} mL^{-1} to 33.5 x 10^{9} mL^{-1} during spawning season. The maximum sperm count of 33.5 x 10^{9} mL^{-1} was recorded during the peak spawning season. A minimum sperm count was observed in the months of June and July during the spawning season. The seasonal variation in percentage

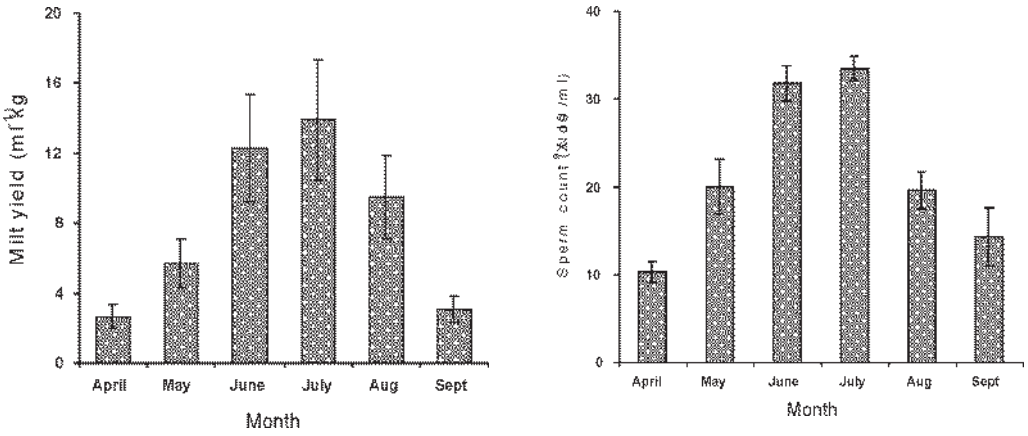

Figure 1. Seasonal variation in milt yield (a) and sperm count (b) in *C. mrigala*. Columns marked with the same letter are not significantly different at $P \leq 0.05$ (n=9)

of motile sperm and duration of motility during spawning periods are shown in Fig. 2 a and b. The maximum motility of 94% and 92% in the months of June and July, respectively, were observed. The percentage of motile sperm during the months of June and July was not significantly different. During other months, the motility percentage recorded was significantly different. The mean duration of sperm motility during milting period was in range from 39 ± 4.76 second to 97.5 ± 4.12 seconds. The maximum duration of motility was recorded in the month of July and minimum in the month of September (Fig. 2b).

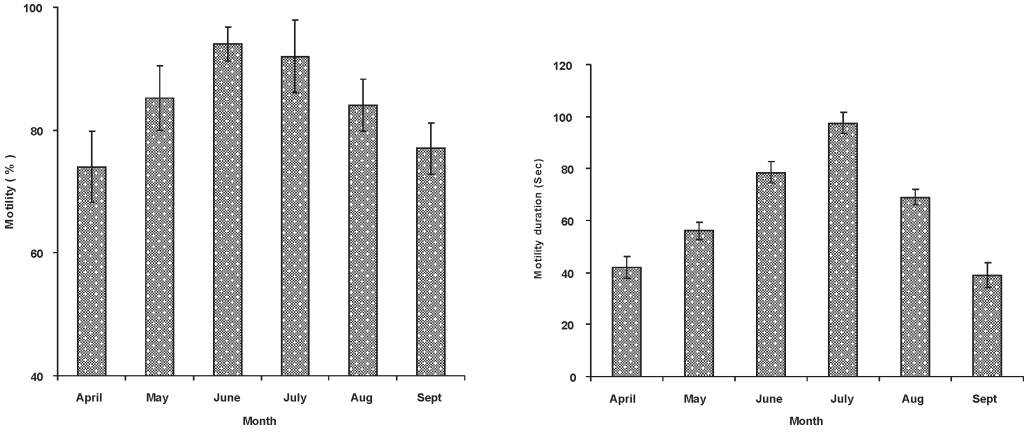

Figure 2. a & b. Seasonal variation in sperm motility (a) and duration of motile sperm (b) in *C. Mrigala* Columns marked with the same letter are not significantly different at $P < 0.05$ (n=9)

The spermatocrit values from April to September are shown in Fig. 3a. In the beginning of the breeding season, the spermatocrit value was minimum (65.2 ± 4.81%) and maximum value was observed in June (81 ± 3.16%) and July (83.40 ± 2.19%). The spermatocrit values during the month of May were 75%. The spermatocrit values were significantly different during the different months of breeding (milting) season.

The seminal plasma osmolality, which is the main factor that regulates sperm motility of mrigal, varied throughout the breeding period (Fig. 3b). The mean range of osmolality of seminal plasma observed from April to September was from 252.0 ± 3.16 mOsm kg^{-1} to 291.8 ± 4.81 mOsm kg^{-1}. The maximum osmolality was observed in the month of July and minimum was studied in the month of September. The mean osmolality of seminal plasma observed was 252, 259, 273, 291, 267, and 261 mOsmol kg^{-1} during April, May, June, July, August, and September, respectively. The osmolality of the seminal fluid studied in the milt of mrigal was significantly different during the different month of spawning season.

The pH values of seminal fluid of mrigal are shown in Fig. 3c. The mean pH

range of seminal plasma of *C. mrigala* was also recorded varying during the different month of spawning season. The pH of seminal fluid ranged from 8.04 ± 0.16 to 8.6 ± 0.12 (Fig. 3c). During April, May, June, July, August, and September, the pH was 8.0, 8.2, 8.4, 8.6, 8.3, and 8.12, respectively. The pH of seminal plasma during different months of breeding season was significantly different ($P < 0.05$).

The mean concentration of the seminal ions (Na, K, and Cl) in mrigal during different months is given in Table 2.

Table 2. Seasonal changes in biochemical composition of seminal plasma of mrigal, *C. mrigala*

Parameter	April	May	June	July	August	September
Protein(g/dl)	0.516 ± .043^{a}	0.108± 0.030^{c}	0.112±0.022^{c}	0.104 ± 0.029^{c}	0.414±0.029^{b}	0.112±0.022^{c}
Albumin (g/dl)	0.102±0.014^{c}	0.112± 0.016^{c}	0.107±0.019^{c}	0.212 ± 0.019^{a}	0.158±0.016^{b}	0.114±0.024^{c}
Creatinine (mg/dl)	0.702± 0.042^{e}	0.906± 0.220^{d}	2.216±0.116^{a}	1.730 ± 0.050^{b}	1.352±0.059^{c}	0.456±0.037^{f}
Total bilurin (mg/dl)	0.114 ±0.036^{b}	0.034± 0.015^{c}	0.102±0.022^{b}	0.034 ± 0.005^{c}	0.222±0.031^{a}	0.254±0.029^{a}
Urea (mg/dl)	7.020±0.303^{a}	3.160± 0.698^{b}	3.080±0.327^{b}	2.520 ± 0.389^{c}	3.460±0.296^{b}	2.480±0.130^{c}
Uric acid (mg/dl)	0.524± 0.055^{a}	0.294 ± 0.143^{b}	0.506±0.069^{a}	0.316 ± 0.037^{b}	0.434±0.053^{a}	0.536±0.047^{a}
HDLc(mg/dl	14.500±2.150^{c}	6.200± 2.049^{d}	20.60±2.509^{b}	24.82 ± 2.268^{a}	24.80±2.679^{a}	18.76±3.426^{b}
SGOT(IU/L)	36.580±1.613^{c}	44.820±1.200^{b}	87.32± 2.572^{a}	20.92 ± 1.466^{e}	22.36±1.352^{e}	32.70±1.036^{d}
SGPT(IU/L)	6.620 ±0.356^{c}	9.100± 1.072^{b}	6.360±0.736^{c}	5.860 ± 0.378^{c}	2.420±0.354^{d}	11.46±1.023^{a}
Na (mEq/L)	89.98 ± 7.40^{d}	107.42 ± 4.82^{c}	124.6 ±5.90^{b}	140.4 ±1.58^{a}	104.10±6.04^{c}	80.40 ± 3.33^{e}
K (mEq/L)	28.20±4.91^{d}	37.20 ± 3.63^{c}	40.76 ± 4.54^{c}	52.30 ± 16.7^{a}	48.84 ± 3.34^{a}	33.10 ± 2.41^{c}
Chloride (mEq/L)	64.86±3.11^{f}	103.46± 11.47^{d}	122.68 ± 4.32^{c}	174.2 ± 5.21^{a}	156.30 ± 3.19^{b}	93.12 ± 3.28^{e}
Glucose (mg/dl)	1.508 ±0.10^{b}	1.0 ± 0.20^{c}	0.52 ±0.04^{d}	0.626 ± 0.06^{d}	1.838 ± 0.10^{a}	1.538 ± 0.06^{b}
Cholesterol (mg/dl)	8.540 ± 0.67^{d}	7.16 ± 1.03^{d}	58.0 ± 2.91^{b}	56.68±2.58^{b}	22.98 ± 2.58^{c}	102.32 ±2.96^{a}
Tryglyceride (mg/dl)	12.02 ± 0.77 ^{c}	12.36 ± 3.43^{b}	20.92 ±1.71^{a}	10.016±0.99^{c}	19.48 ± 0.69^{a}	14.58 ± 1.24^{b}

Note: Values are expressed as Mean ± SD (n=27). Values having different superscripts differ significantly in a row

The mean concentration of sodium in the seminal plasma ranged between 80.45 ± 3.33 mEq/L to 140.4 ± 1.58 mEq.L^{-1} from April to September during milting period. The maximum concentration of sodium ion in seminal fluid was observed in July and a minimum was observed in September. The sodium concentration in the months of June and July was significantly different, whereas during the months of May and August, it was not significantly different (Fig. 4a.). The mean concentration of potassium ions in seminal fluid during milting period was in the range of 28.20 ± 4.91 mEq/L to 52.30 ± 16.7 mEq.L^{-1} Seasonal variation of potassium ions in seminal plasma was observed throughout the breeding period (Fig. 4b). The potassium concentration was

observed to be in increasing trend during the beginning of the season, and then it reached peak in July and started declining towards the end of the breeding season (September).

High concentration of chloride was observed during milting period from April to September that ranged from 64.86 ± 3.11 $mEq.L^{-1}$ to 174.2 ± 5.21 $mEq.L^{-1}$ (Fig. 4c). Increasing trend of the mean concentration of chloride in seminal fluid was observed initially reaching peak in July and chloride concentration declined till end of the September. The chloride concentration in the seminal fluid during different months of milting period was significantly different ($P < 0.05$).

The mean concentration of glucose, albumin, and protein of seminal plasma in *C. mrigala* during milting period was found varying throughout the season. The glucose in the seminal plasma was in a range from 0.52 ± 0.05 mg/dl to 1.83 ± 0.12 mg/dl (Fig. 5a), albumin was found in a range from 0.10 ± 0.01 mg/dl to 0.21 ± 0.02 mg/dl, (Fig. 5c), and Protein was recorded in a range of 0.105 ± 0.03 mg/dl to 0.515 ± 0.05 mg/dl (Fig. 5b) during the spawning season. The glucose concentration in the month of August is significantly different. The albumin concentration was also significantly different in the month of July. The protein concentration was not significantly different in May, June, July, and September.

The concentrations of cholesterol, triglyceride, and high density lipoprotein cholesterol (HDLc) in seminal fluid of *C. mrigala* varied throughout the milting period. During the period from April to September, the mean range of the concentration of cholesterol was observed to be from 7.1 ± 1.24 mg/dl to 102.3 ± 3.21 mg/dl (Fig. 6a.), triglyceride was observed to be from 9.97 ± 1.14 mg/dl to 14.475±1.41 mg/dl (Fig. 6b), and HDLc was found to be in a mean range of 6.0 ± 2.309 mg/dl to 25.0 ±3.05 mg/dl (Fig. 6c). The concentration of cholesterol in the seminal fluid was significantly different in September, whereas in June and July, it was not significantly different. The triglyceride concentration was not significantly different in June and August, whereas in April and July, it was also not significantly different. The concentration of HDLc was not significantly different in July and August

The concentrations of creatinine, SGPT, and SGOT during milting period are shown in Fig. 7 a, b, and c. The mean range of the concentration of creatinine, SGPT, and SGOT in seminal fluid was 0.457 ± 0.04 mg/dl to 2.21 ± 0.13 mg/dl, 2.412 ± 0.41 IU/L to 11.45 ± 0.18 IU/L, and 20.75 ± 1.63 IU/L to 87.32 ± 2.97, respectively, during the breeding season starting from April to September. Creatinine concentration was significantly different ($P < 0.05$) during different months of the season. The SGPT concentration was not significant during April, June, and July. However, SGOT concentration in the seminal fluid was significantly different in April, May, June, and September.

Variations were observed in the mean concentrations of total bilurin, urea, and

uric acid in the seminal plasma throughout the breeding season. The mean range of total bilurin in seminal fluid was from 035 ± 0.01 mg/dl to 0.257 ± 0.03 mg/dl (Fig. 8a.), mean concentration of urea was from 2.45 ± 0.13 mg/dl to 7.05 ± 0.341 mg/dl (Fig. 8b), and uric acid was from 0.28 ± 0.165 mg/dl to 0.535 ± 0.05 (Fig. 8c).

Discussion

The study of seasonal variation in milt characteristics and biochemical composition of Indian major carps is scanty. Billard et al. (1995), Linhart et al. (1995), and Christ et al. (1996) reported seasonal variation in quality of male gamete in some carps. Many other factors contributing to individual variation in sperm quality have been reported (Rana 1995; Rurangwa et al. 2004), and these factors are genetic variability among fish, rearing conditions, brood stress, sperm collection methods, and storage of milt and sperm activation conditions. The seasonal variation of gamete quality also influences motility and fertilization success (Lubzens et al. 1997; Linhart et al. 2000). Seasonal variation affects the keeping quality of spermatozoa in vivo (Baynes & Scott, 1987). Knowledge of physical and chemical constituents of spermatozoa and seminal fluid is a pre-requisite for the successful evaluation of the reproductive ability of different fish species. This may also lead to the better understanding of the mechanisms of fertilization and to detect anomalies. Changes in the quality of milt during the spawning season have been reported in teleosts (Billard et al. 1977).

In this study, the maximum milt yields were recorded in the peak of season and the yields declined toward the end of season. Similar variation in milt yield has also been reported by various workers in other fishes (Billard & Marcel, 1980). The variation in milt yield reported are due to the seasonal changes, the age of milter, the maintenance circumstances of the milters (Turkadov 1968; Ginzburg 1972; Kazakov 1978, 1979, 1981; Buyukhatipoglu & Holtz 1984; Piironen 1985), and the inducing agents (Billard & Marcel 1980; Wei & Crim 1983; Wohlfarth 1994; Lin et al. 1996). Billard & Marcel (1980) reported that injections of crude gonadotropin preparation of pike, carp, and partially purified salmon gonadropin in pike, *Esox lucius*, resulted in a significant increase in volume of milt compared with the control (Saline injected). In addition, they observed a significant increase in the collectable milt volume from the untreated males of common carp exposed to females undergoing ovulation after carp pituitary extract injection. This may be due to the release of sex pheromones i.e. C_{21} steroids.

The mean sperm count of milt during June and July was significant compared with the other months of reproductive season ($P < 0.05$). The sperm count declined as the spawning season advanced. Similar result has been observed by various workers. The sperm concentration increased during spermiation period in turbot, Atlantic halibut (Suquet et al. 1998) and decreased at the end of reproductive season of rainbow trout (Buyukhatipoglu & Holtz 1884). The spermatozoa concentration declines as the spawning

season advances in rainbow trout, *O. mykis* (Buyukhatipoglu & Holtz 1884; Billard & Marcel 1980; Wei & Crim, 1983; Wohlfarth 1994; Lin et al. 1996; Suquet et al. 1998) and carp, *Cyprinus carpio* (Christ et al. 1996; Lubzens et al. 1997), and Billard et al. (1977), Buyukhatipoglu & Holtz (1984) and Munkittrick & Moccia (1987) reported that sperm density declined as the season advanced (in rainbow trout).

The mean spermmatocrit value of milt studied during the month of July was significantly higher than other months during milting period in *C. mrigala*. The observation of spermatocrit value showed a clear seasonal variation during reproductive periods. Similar seasonal variation has also been reported in salmon (Piironen 1985) in rainbow trout (Munkittrick & Moccia 1987). Piironen & Hyvarinen (1983) observed that spermatocrit increased over the stripping season. Piironen 1985 reported seasonal variation of spermatocrit value in *Salmo salar.* The seasonal variation of spermatocrit value in Atlantic cod *Gadus morhua* has been reported (Rakitin et al. 1999).

The maximum motility of spermatozoa in mrigal was observed in June (94 ± 2.82%) and July (92 ± 5.88%). The mean duration of sperm motility during milting period was in range from 39 ± 4.76 sec to 97.5 ± 4.12 sec. The maximum duration of motility was recorded in July and minimum was recorded in September. Similar result has been reported in rainbow trout, brown trout (*Salmo trutta* [*S. trutta*]), brook trout *(Salvelinus fontanalis),* and Atlantic salmon (*Salmo salar*) (Benau & Terner 1980). During the peak spawning season, activated rainbow trout spermatozoa remained motile for 30–55 sec. By the end of the spawning season, the duration of the motility declined to 15 sec.

The mean pH value of the seminal plasma of *C. mrigala* during breeding season ranged from 8.05 ± 0.19 to 8.6 ± 0.12. The alkaline pH of the seminal plasma found in the mrigal is similar to the results observed by Billard (1981) who reported that alkaline pH gives better motility in rainbow trout spermatozoa. Optimum sperm motility has been reported at pH 9.0 in *Oncorhynchus mykiss* (Billard & Cosson, 1988) and *Scaphthalmus maximus* (Chauvaud et al. 1995) and pH 7.0 and 8.0 in *Cyprinus carpio* (Cosson et al. 1991). Alternation of the internal pH as possible mechanism interfering with motility was described for spermatozoa from different species.

In the present study, the osmolality of seminal plasma was found to be varying throughout the milting period from April to September. It has shown increasing trend until July (291.5 ± 5.5 mOsm kg $^{-1}$) and started declining towards the end of breeding season. These findings are in agreement with the observation of osmolality of seminal plasma by Kruger et al. (1984), Billard (1988), Aas et al. (1991), and Lahnsteiner et al. (1997) who reported variation of seminal osmolality in different seasons. Osmolality of seminal plasma of Atlantic salmon showed wide variation among 27 males ranging from 117 to 320 mOsmol kg^{-1}. Much variation of osmolality of seminal fluid observed

with extreme values 178–282 mOsm kg^{-1} by Kruger et al. (1984) for males were sampled at various times throughout the year. It was found that variations in the lower ranges were due to contamination of semen by urine (Perchec et al. 1995). The osmolality of semen had been studied in cyprinids (Cruea, 1969; Kruger et al. 1984; Billard & Cosson, 1992; Billard et al. 1995a, b; Lahnsteiner et al. 1996 Linhart et al. 1991, 2003a, b, c.). The osmotic pressure can vary between individuals, and this is correlated with the thinning (hydration) of the semen (Morisawa et al. 1979). In addition, variation in osmotic pressure observed in the literature might be due to hormonal induction of spermiation outside the natural reproductive season (Redondo Muller et al. 1991).

Seminal plasma of Fish contains mainly mineral compounds and low concentrations of organic substances. There is no information available on the seasonal changes in biochemical composition of seminal plasma of Indian major carps prior to and beyond spawning time. The composition of seminal fluid of fish has been reviewed by Billard & Cosson (1990) and Linhart et al. (1991). The constitution of milt in terms of performance and chemical composition varies interspecifically and even within the same individuals with time. The various available data show considerable intra- and inter-species variability in the composition of the seminal fluid. Ionic composition is reportedly changing during reproductive season (Linhart et al. 1992). It was reported that organic component of seminal plasma in *Salmo salar* underwent specific changes throughout the spawning season (Piironen 1985). Variation in the inorganic and organic composition of seminal plasma may affect the preservation properties of milt (Benau & Terner 1980; Piironen & Hyvarinen 1983, Kruger et al. 1984).

The Na^+ and K^+ concentration in the seminal plasma of *C. mrigala* is shown in Table 9. The high values of ions are believed to be responsible for the suppression of sperm motility. The Na^+ and K^+ concentrations of seminal plasma in mrigala were in the range of 80.45 ± 3.84 mEq/L to 140.5 ± 3.7 mEq/L and 29.25 ± 5.0 mEq/L to 52.3 ±19.28 mEq /L, respectively. The ionic composition in the range of 103–140 mM Na^+, 20–66 mM K^+ , 0.8–3.6 mM in Salmonid (Morisawa et al. 1983) and 94–107 mM Na^+, 39–78 mM K^+, 0.02–1.2 in cyprinids (Kruger et al. 1984) were reported. The seasonal variation of sodium and potassium concentrations in seminal plasma of cyprinids has been reported and similar results were obtained in the present experiments.

Limited information is available on the organic composition of the carp semen. The variability in organic composition of the seminal plasma is wide and changed according to season, to gonadotropin treatment given to stimulate spermiation, and during semen storage (Belova 1982; Kruger et.al. 1984). Some energetic substrates such as glucose and fructose are found in the seminal plasma and the sperm but in small amounts (Kruger et al. 1984) and are generally 10 times lower than in mammals (Ford & Rees 1990). Organic constituents of seminal plasma has been reported, and it was variable in the interspecies and the range ($mg.l^{-1}$) was 8-220 for glucose, 0-218 for fructose, 0-40

for cholesterol, 0-1316 for lipids, 35-391 for glycerol, 0.4-280 for protein, 84-136 for amino acids, and 12-136 for urea (Billard & Cosson 1990). The protein content is highly variable throughout the year (Billard et al. 1995). Similar result was also obtained in the protein content of seminal plasma, which showed variations throughout the season, and the range of amount of total protein was from 0.105 ± 0.03 to 0.515 ± 0.05 g/dl. In the present study, the mean concentrations of triglyceride and HDLc from April to September were found to be from 9.97 ± 1.14 mg/dl to 14.475 ± 1.41 mg/dl and 6.0 ± 2.309 mg/dl to 25.0 ± 3.05 mg/dl, respectively. The mean concentrations of creatinine, SGPT, and SGOT in seminal fluid (ranges) were 0.457 ± 0.04 mg/dl to 2.21 ± 0.13 mg/dl, 2.412 ± 0.41 IU/L to 11.45 ± 0.18 IU/L, and 20.75 ± 1.63 IU/L to 87.32 ± 2.97, respectively. Total bilurin in seminal fluid was 035 ± 0.01 mg/dl to 0.257 ± 0.03 mg/dl, and the mean concentration of urea was 2.45 ± 0.13 mg/dl to 7.05 ± 0.341 mg/dl and uric acid was 0.28 ± 0.165 mg/dl to 0.535 ± 0.05.

The mean ranges of cholesterol and glucose studied in the present experimental fish were 8.52 ± 0.77 to 22.97 ± 2.98 mg/dl and 0.525±0.05 to 1.83 ± 0.125 mg/dl, respectively during the spawning season. Kruger et al. (1984) reported that the cholesterol and glucose contents of carp seminal fluid were in the range from 0 to 40 mg/l and 9 to 100 mg/l, respectively. Stein & Bayrle (1985) found the highest glucose content in the seminal plasma of *S. trutta fario,* at 12.2 mg/100 mL compared with 3.7, 1.8, and 8.8 mg/100 mL for *Salmo gairdneri, S. trutta lacustris,* and *Coregonus* sps, respectively.

Although the milting in *C. mrigala* has been found to be started from April, it has two reproductive peaks i.e. June and July during the breeding season. The result indicates that in June and July, spermatocrit value, sperm count, milt volume, and duration of total spermatozoa motility were comparatively higher than in other months of spawning season, indicating a better quality of milt. Therefore, the information of the normal physical and chemical characteristics of seminal plasma of the *C. mrigala* presented in this study will help to optimize in selection of high quality male donors for aquaculture and artificial spawning performances.

Acknowledgment

This work was supported by a grant from the Indian Council of Agricultural Research, New Delhi, India and a Doctoral fellowship to first author by ICAR.

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Received: 27 December 2007; Accepted: 24 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Fish is an Integral Part of the Diet of the Rural Poor in Cambodia: Results from Fish Consumption Surveys

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Abstract

As part of a larger project on fish, nutrition and poverty in Cambodia, a survey was conducted to assess the consumption patterns of fish and other aquatic animals in poor rural households in three communes. One hundred and sixty three households were identified and selected in three ecological areas: Andong Snay Commune, Babong Commune, and Tuol Ampil Commune. The survey used recall interviews during three distinct seasons from March 2006 to February 2007: the dry season (March to April, 2006); the rainy season when many types of fishing are prohibited (August to September, 2006) and the rice harvesting and main fishing season (January to February, 2007). The results include detailed household and socio-economic data, fish species caught and consumed, and consumption of fish and fish products.

Introduction

Cambodia's freshwater capture fisheries probably contribute more to national food security and the economy than fisheries in most other countries in the world. The annual catch ranges between 290,000 to 430,000 t (Zalinge et al. 1998; Ahmed et al. 1998; Nao & Zalinge 2000; Department of Fisheries (DoF) 2001), making it the fourth largest in the world. The monetary value of the total fish catch ranges from US$ 250 to US$ 300 million (So & Nao 1999), which is 8 to 10% of the GDP of US$ 2,800 million (Ministry of Economic and Finance 1999). This enormous volume of fish is due in part to the high diversity of Cambodia's freshwater fisheries (Rainboth 1996). At the heart of this enormously rich fishery is the Tonle Sap Great Lake floodplain and the annual flood pulse driven by the Mekong river. The Tonle Sap varies in size from 2,500 km^2 in the dry season to 13,000 km^2 in wet season, including 4,800 km^2 of flood forest coverage, giving rise to a wide range of habitats for fish and other aquatic animals and plants. In addition to the wild capture fishery, small-scale aquaculture production has grown from

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1,610 t in 1984 to 15,000 t in 2000 (DoF 2001).

Many poor rural households are not able to produce enough rice to last an entire year. Fish and other aquatic animals and plants are generally available when rice is not. Fish provide an estimated 75% of the total animal protein intake for the population of rural Cambodia (Tickner 1996; Murshid 1998). In addition to protein, fish and other aquatic animals such as small fish, frogs, and snails are a major dietary source of fatty acids, minerals, and vitamins. However, not all species are of equal value in terms of nutrition. Postharvest handling, cooking habits and storage also affect nutritional value.

Fish and other aquatic animals and plants are important in poor rural households, both as a source of income and a source of good nutrition and health. However, the fisheries in Cambodia are under intense development pressure, and fisheries officials must increasingly justify their objections to development in other sectors in monetary and socio-economic terms. The objective of this study is to provide further data on the socio-economic dimensions of the rural people and to asses the consumption patterns of fish and other aquatic animals in rural poor households in Cambodia. These data will provide a basis for further research on nutrition, particularly in women and children, and research on markets in fish and fish products and how they may or may not connect the poor to the market chain.

Materials and Methods

Three different ecological areas were selected: An-dong Snay Commune (ASC) located in the Great Lake area (largest fishing area); Babong Commune (BBC) located in the lower Mekong (smaller fishing area) and Tuol Ampil Commune (TAC) located far from the main fishing areas (smallest fishing area) (Fig. 1). People in a total of 153 households were interviewed, roughly one-third from each Commune. The surveys were conducted during three distinct seasons: dry season (March to April 2006), rainy and closed fishing season (August to September 2006) and rice harvesting and fishing season (January to February 2007). A frequency and five days recall method was used; SPSS and MS Excel software were used to analyze the results.

Results

1- Socio-Economic Status of Rural Poor Households

Households ranged in size from three to fifteen members, with an average of six. In ASC and BBC, over 90% of household heads are men. In TAC, one of the poorest communities in Cambodia, the rate of male household heads drops to 75%. In rural Cambodia, people marry early and there are a large number of children under 14 years of age. A high percentage leave school to help earn income for the family, hence, levels

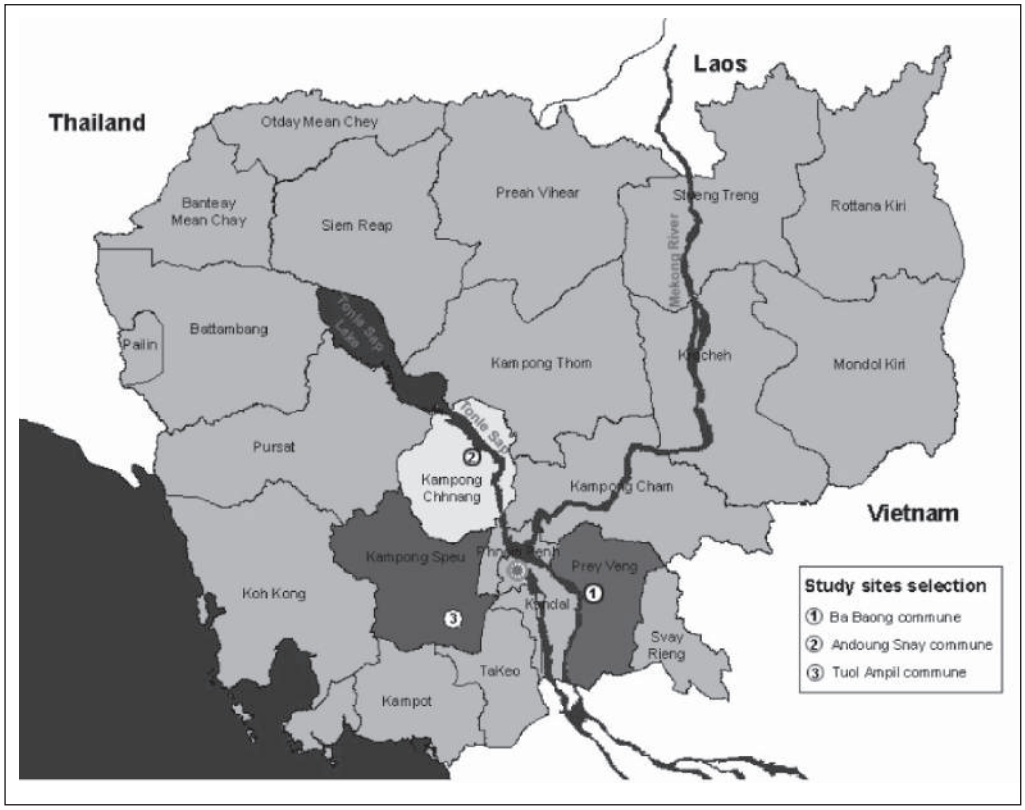

Figure 1. Location of surveyed communes: 1.BBC, 2. ASC and 3. TAC

of literacy is low. No household head had more than a secondary school education. Households in the target communes engage in a wide range of production and income generating activities. Just over half the households (53%) have land and can engage in farming. The main occupations are day labor (33%), fishing (31%), and small home-based businesses. Day labor is the main income for most households. Farming and fishing generally provides only enough for daily consumption. The average annual income of the household is 1.14 million riel (US$ 285.00). Average income was highest in Babong Commune. There were three basic types of dwelling observed. The poorest people live in houses made of bamboo poles with palm tree roofing. If people are a little better off, they can afford a house with thatched or even tin roofs. Just over 90% of the householders we surveyed live in the poorer type of house. Ninety-nine percent of the 153 households visited had no proper latrines. Most household members draw their water from a well of some sort and less than 30% boil their water before drinking. About half of the people in the households, we interviewed have access a commune health center. Unless health problems are serious, medications are bought at a local shop in the village and patients consult with the village health worker. Land holdings are small (0.041 ha), but two-thirds of the households owned a rice field and nearly

90% a small orchard. Fish ponds were rare in the survey households.

Most of the rural poor families depend on subsistence fishing. In TAC, 77% of all households engage in fishing, even though this area has the smallest fishing grounds, just over half in the largest fishing area (ASC), and 68% are involved in fishing in BBC. Fishing activity varies by season and geography determines the preferred location for fishing. In ASC, fishing households mainly fish in rice fields, followed by lakes and streams.

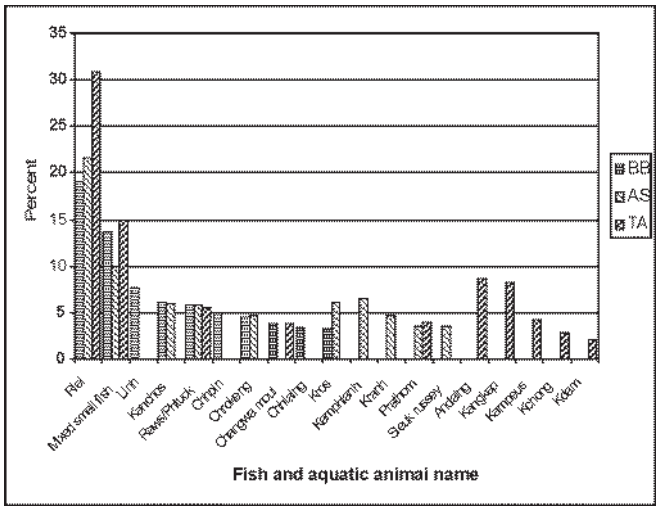

Figure 2. Top 10 most consume fish and others aquatic species in all round and survey communes (Scientific name is in Table 1.)

The same pattern holds in BBC, except they also have access to flooded forest. In TAC, people fish in rice fields, small ponds, and canals. The most common use of family fishing gears in BBC were hook long line, followed by gillnet and handle scooping baskets; in ASC were hand capture, followed by hook long line, and fish trap, and TAC were handle scooping basket, hand capture, and spear/knife. The five top most common caught species in BBC and ASC were fish whereas in TAC, within the top five caught species; three were aquatic animals (Fig. 2, Table 1).

Table 1. Local and scientific name of fish and others aquatic species

No.	Local name	Scientific name Fish species
1	Andaing	*Clarias macrocephalus*
2	Changwa moul	*Rasbora myersi*
3	Chhlaing	*Mystus nemurus*
4	Chhpin	*Hypsibarbus spp*
5	Chrokeng	*Puntioplites spp*
6	Kamphlanh	*Trichogaster spp*
7	Kanchos	*Mystus spp*

8	Kranh	*Anabas testudineus*
9	Kros	*Osteochilus hasselti*
10	Linh	*Thynnichthys thynnoides*
11	Prathom	*Pangasianodon hypophthalmus*
12	Raws/phtuok	*Channa striata*
13	Riel	*Henicorhynchus spp*
14	Sleuk russey	*Longiculter siahi*
Aquatic animal		
1	Kampeus	*Macrobrachium lanchesteri*
2	Kangkep	*Rana tigrina*
3	Kchong	*Pila ampullacea*
4	Kdam	*Somanniathelpusa brandti*

Household food and fish consumption

The mean household consumption of food items (gram of raw whole food/household/day) was calculated from the one week frequency data. The mean household rice consumption in all households was 2016.4 g household^{-1} day^{-1}, vegetable 765.1 g household^{-1} day^{-1}, fish and fish products 654.6 g household^{-1} day^{-1}, meat 71.9 g household^{-1} day^{-1} and fruit 222.2 g household^{-1} day^{-1}. The mean rice consumption was highest in TAC, the poorest commune, followed by ASC and BBC. People in households in all three communes said they ate rice everyday; and just over half said they consumed fish and vegetables every day of the week.

Household fish consumption was calculated using five-day recall questionnaires. Fish consumption was composed of fresh fish, other aquatic animals, and processed fish. The mean raw whole fish consumed was calculated as the sum of the weight of fresh fish and aquatic animal consumed and the weight of converted raw whole fish from processed fish consumed (Table 2). The mean raw whole fish consumed of rural poor people was 524.8g household^{-1} day^{-1} or 192.1kg household^{-1} year^{-1} or 32.0kg person^{-1} year^{-1}. Overall, mean total household fish consumption was high in ASC, reflected the access to fishing grounds. Overall household consumption of fresh fish was 285.1 g household^{-1} day^{-1}, aquatic animals was 78.2 g household^{-1} days-^{1} and processed fish was 93.5 g household^{-1} day^{-1}. The mean household consumption's proportion was fresh fish 62%, aquatic animals 17%, and processed fish 21%. Overall, 54% of rural poor householders said they consumed fresh fish and aquatic animals everyday. All rural household said they consumed fish paste and fish source everyday.

Table 2. Mean total household fish consumption

Commune	Mar-Apr, 06 (g/hh/d)	Aug-Sep, 06 (g/hh/d)	Jan-Feb, 07 (g/hh/d)	Mean (g/hh/d)	Mean (kg/hh/y)	Mean (kg/person/y)
ASC	638.6	555.9	701.7	632.1	231.3	38.6
BBC	463.5	476.4	568.8	502.9	184.1	30.7
TAC	394.9	495.9	430.5	440.0	161.1	26.9
All communes	498.4	509.6	566.6	524.8	192.1	32.0

hh-household

Among 10 most common consumed fish species the dominant were trey Riel, mixed small fish, and Ptuok, found in all communes, while other aquatic animals such as kangkep, kampeus, kchong, kdam was found only in TAC (Fig. 2). The results from cooking practices showed that fish soup with vegetable and grilled fish were the most common preparations.

Discussion

By all measures, the people in these household surveys are poor. They have low levels of literacy, poor access to health facilities, small land holdings or no land at all, not enough rice to last for even half the year, and low income. What they have is fish. Specifically, they rely on a relatively small number of species, most of which are 'whitefish' of low commercial value and varying nutritional value. The larger blackfish species such as climbing perch (trey Kranh) and snakehead (trey Ptuok) are usually sold for cash. Fish are both food and a source of income that can be exchanged for rice and other food products. The result of this study was very similar to fish consumption surveys conducted by Touch (1993), Ahmed et al. (1998), and Hortle et al. (2004); and much higher than FAO Food Balance Sheets (1998). The result of this study was lower than consumption figures in six provinces around Tonle Sap Lake, Kandal province, and Phnom Penh (between 22 and 68 kg per year of fresh fish and 10 and 24 kg per year of process fish). Some fishing communities in the Tonle Sap Lake area may consume 75.6 kg person^{-1} year^{-1} (Ahmed et al. 1998, Hortle et al. 2004). The national average fish consumption is in the range of 30 to 40 kg person^{-1} year^{-1}. These differences are due largely to the sample selection and location. The Mekong Committee figures are based on a basin-wide sample and the other studies included a wider range of income groups.

As in previous studies, these results also indicate that fish species consumed vary with fishing place and season. In another study, the most commonly consumed aquatic organisms are shrimps, crabs, and frogs as well as snakes, insects, and wading birds (Gregory et al. 1996). In the work studied by Shams and Hong (1998), the average distribution of aquatic animals in three districts of a central Cambodian province was 26% frogs, 22% crabs, 20% snails, 15% insects, 13% shrimps, and 4% snakes.

Most of the people in households, in our survey, engage in subsistence or family fishing, which involves many family members. Poor householders consume low-value fish and aquatic animals from rice fields and the wild and also buy low value fish in local markets. Fish consumption was high during the rice harvesting and fishing season, low at the end of the rainy season and the beginning of the closed fishing season, and lowest in the dry season. Rice field fishery ecosystems are known to be rich in aquatic resources derived from animals such as fish, small shrimps, crabs, snails, beetles, and from aquatic vegetables such as morning glory, lotus, and water lily. Processed fish products, such as fish paste and fish sauce play an important role in peoples' diets, especially during the dry season and in areas where there is less access to fishing grounds. Low price and ready availability of these products should be a high priority, along with more effective processing technology.

Conclusion

The results of this study indicate that poor people eat less fish overall, but eat fish more often than their more well-off neighbors. More well-off households can afford to supplement their diets with meat and other food items. Fish is a staple food item for the rural poor and central to their livelihood strategies. A major threat to rural poor livelihood strategies is the combination of increasing land pressure and decreasing access to common property resources (flooded rice fields, rivers, lakes, inundated forest, irrigation canals, and dikes) and pesticide pollution in rice fields and wetland areas. Many rural households are landless or have only small land holdings. As access to common property becomes more restricted, diminished, and priced out of reach, the poverty and vulnerability of rural poor can only intensify. Perhaps most importantly, the fish consumed by poor rural households are mostly migratory species. Any development initiative that impedes or alters migration routes will have an impact on the income and health of the poorest people in Cambodia.

A number of authors have indicated that aquaculture can be promoted to fill any gaps. This is unlikely given the size of the population that depends on wild fish and aquatic animals and plants, the volume of fish the wild capture fishery produces, and that fact that aquaculture requires an investment the poor can seldom afford.

Acknowledgement

The authors would like to thank the Council for Development Research (RUF), Ministry of Foreign Affairs of Denmark for the research and research capacity building project "The Role of Fish in Food and Nutrition Security in Developing Countries". We are also grateful to our colleagues, the project staff at IFReDI, and the commune heads of Andong Snay, Babong, and Tuol Ampil communes for their kind assistance. Thanks also to Mr. Terry Clayton for his assistance in editing the final draft of this paper.

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Received: 15 January 2007; Accepted: 21 November 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Removal of Heavy Metals from Water by the Direct Addition of Chitosan Prepared from Prawn and Squilla Shells

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Abstract

Chitosan was prepared from prawn shell (*Fenneropenaeus indicus*) and squilla (*Oratosquilla nepa*) and quality characteristics such as moisture, ash, viscosity and degree of deacetylation were determined. The efficacy of the prepared chitosan to remove heavy metals when directly added to water samples at 1% level was assessed. Water samples with the addition of known quantities of lead, zinc, cadmium, copper and iron and water samples collected from different locations in Vembanad Lake were used in this study. Significant reduction in the concentration of heavy metals was observed after treatment with chitosan. Chitosan from prawn shell was found to be more effective in the removal of heavy metals from water. Treatment with prawn chitosan removed 68% cadmium from spiked samples, whereas squilla chitosan removed 37%. In the case of water samples collected from Vembanad Lake, treatment with chitosan from prawn shell removed approximately 50% of cadmium and the same treatment resulted in removal of lead to non-detectable levels in all the samples.

Introduction

The phenomenal increase in the export of frozen prawn products from India presents the problem of a huge quantity of waste material comprising head and shell, which comes around 100000 tonnes annually on a rough estimate. A negligible portion of this waste is used directly as manure and the rest is being discarded. Direct disposal of such huge quantity of shell waste to water bodies or land often causes environmental problems; a scientific assessment of the impacts of such a large-scale discard is not yet carried out. Apart from prawn, squilla also constitutes considerable portion of trawler catch in certain seasons. *Oratosquilla nepa*, the species of squilla available in Indian water, is not generally used for human consumption because it does not contain much meat and hence almost entire catch is thrown back into the sea.

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An industrial product *viz.*, chitosan can be prepared by processing the waste of prawns, crabs and squilla. Chitosan finds extensive applications in food industries, wound healing, water purification, wine clarification, and photography and textile industries. Radhakrishnan & Prabhu (1971) had studied the preparation of chitosan from prawn waste by different methods for deacetylation of chitin using 50% aqueous KOH and equal volumes of 50% aqueous NaOH and ethyl alcohol. Later, Madhavan & Nair (1975) perfected a method for the preparation of chitosan from prawn waste. Madhavan et al. (1986) reported a modified method for deacetylation of chitin to produce chitosan by treatment with concentrated alkali. The metal binding property of chitosan prepared under different temperature conditions were studied by Madhavan & Nair (1978). Chitosan is a natural chelating polymer with diverse applications for the harmful chemicals and toxic heavy metals (Muzzarelli & Tubertini 1969; Yang 1984).

The content of lead, cadmium, and so on in the effluents from battery and metal plating industry can be reduced by treating the effluents with chitosan (Masri & Randfall 1978). In wastewater treatment, chitosan can also be used as chelating polymer for binding toxic heavy metals (Muzzarelli 1981). The role of chitin to remove Cd was demonstrated by Poirier & Cossa (1981). Chitosan can also be used as an agent to remove harmful chemicals from polluted streams and effluents (Chia 1994). Krishnamurthy & Frederick (2006) suggested that the biopolymers such as chitin, chitosan and modified starch could be used to remove or reduce the concentration of heavy metals from water. Chitosan is an excellent chelation polymer, which can be used for the purification of water intended for handling and processing food products (Das et al. 2003).

Vembanad Lake supports the rich aquatic fauna and the fields around the backwater are suitable for aquaculture. These areas support traditional, seasonal and perennial prawn fishery. The nutrients and pollutants introduced into the estuary control the distribution and abundance of less tolerant species in ecologically sensitive areas in the backwaters to a great extent. Cochin backwaters, considered one of the polluted estuaries in India, receive contaminated freshwater inputs and discharges of effluents and partially treated sewage from many points throughout its tidally mixed zone. The concentration of heavy metals is found to be higher in Cochin region of the lake owing to the discharge of effluents from Eloor industrial belt. The study by Priju & Narayana (2007) suggests that industrial effluents are the major source of metal enrichment (Cu, Ni, Zn and Cd) in the Vembanad lagoon system. Remani et al. (2004) reported that heavy metals such as Cu, Cd, Zn, Ni and Pb are heavily distributed in Chitrapuzha river which drains to the lake.

This study deals with the utilization of chitosan for the removal of heavy metal residues from spiked samples and natural water samples collected from selected locations in Vembanad Lake.

Materials and Methods

Water samples were collected from five different locations of Vembanad Lake *viz.*, Chambakkara, Kumbalangi, Cherai, Chellanam and Marine drive (locations I, II, III, IV and V, respectively). Standard solutions of Cd, Zn, Pb, Fe and Cu of 10 ppm concentration were prepared by diluting the stock solution (Merck, Germany).

Chitosan was derived from the shell of *Fenneropenaeus indicus* and squilla (*O. nepa*) by the method used in the study by Madhavan & Nair (1974). Chitosan powder at 1% level was directly added to the water samples kept at ambient temperature for 30 minutes. The samples were filtered and analysed with atomic absorption spectrophotometer (Varian AA 420, USA) in air-acetylene flame using the respective hollow cathode lamps (AOAC 1990). All the samples were analysed in triplicates, and the average value was noted.

Moisture and ash content were determined (AOAC 1975). Degree of deacetylation was determined by measuring the absorbance of chitosan solution in 1% acetic acid (Muzzarelli & Rochetti 1985) using spectrophotometer (Spectronic Genesys 5, Spectronic Instruments, Inc., Rochester, NY, USA). Viscosity of 1% chitosan solution (1 g of chitosan in 98 g of distilled water and 1 g of glacial acetic acid) was measured with Brookfield viscometer (LV DVE -230, Brookfield, Germany). AR grade acids and reagents were used for analyses.

Results and Discussion

Quality characteristics of chitosan used for this study are given in Table 1. Viscosity of prawn chitosan and squilla chitosan was 314.4 Cp and 213.14 Cp, respectively. Viscosity of prawn chitosan was found to be high compared to that of squilla chitosan. In the study by Thankappan & Madhavan (1995) the viscosity of chitosan obtained from *F. indicus* was 460, whereas in this study it was 314.4 Cp, which could be due to the reduced molecular weight because of the higher degree of deacetylation. The degree of deacetylation of the prawn chitosan (98.4%) was found to be higher than that of the squilla chitosan (75.5%). Ash content of prawn chitosan (0.72%) was also higher than that of the squilla chitosan (0.63%).

The result of treatment of standard water samples with prawn and squilla chitosan is given in Table 2. Prawn chitosan was found to have better capacity to remove heavy metals from the standard water samples compared with squilla chitosan. On treatment with prawn chitosan, maximum reduction (90%) was found in Cu concentration and similar reduction was also found in Zn concentration. When squilla chitosan was used only 50% reduction in Cu was observed. Under the same conditions, only 68% and 36% Cd was removed by prawn chitosan and squilla chitosan, respectively. In general, the retention of heavy metals was double in the case of water samples treated with

squilla chitosan. The effectiveness of prawn chitosan in removing heavy metals could be due to the higher degree of deacetylation (Table 1), which increases the binding sites for heavy metals. The free amino groups are abundant in chitosan, where the lone pair electrons of nitrogen bond with transition metal ions. The increase in the number of amino groups increases the capacity of chelation. Chitosan from different sources has different binding ability for heavy metals (Nair & Madhavan 1984; Chui et al. 1996).

Table 1. Quality characteristics of chitosan used for the study

Parameters	Prawn chitosan	Squilla chitosan
Moisture (%)	3.6 ± 0.12	3.2 ± 0.11
Degree of deacetylation (%)	98.4 ± 1.3	75.5 ± 0.8
Viscosity (Cp)	314.4 ± 11.4	213.1 ± 8.5
Ash (%)	0.72 ± 0.01	0.63 ± 0.01

Table 2. Concentration of heavy metals in spiked water samples after treatment with prawn and squilla chitosan

Heavy metal	Initial conc. (ppm)	Concentration after treatment (ppm)	
		Prawn chitosan	Squilla chitosan
Zn	10	1.126	5.261
Fe	10	2.889	5.571
Cu	10	1.027	2.931
Pb	10	2.120	5.90
Cd	10	3.213	6.320

The results of treatment of water samples collected from Vembanad Lake with prawn chitosan are given in Tables 3 and 4. The incidence of heavy metals indicated in two locations *viz*., water sample from locations I and V. The water samples contained all the five metals assessed. It is noteworthy that the sample collected from location V had the highest concentration of the metals under study. Zn was present in all the water samples collected and found to be within the range of 0.0059-0.026 ppm. Fe was found in the range of 0.321-1.242 ppm, which is the maximum among all the heavy metals studied, in all the samples collected from five locations. Cu was detected only in two locations. Substantial quantity of Pb was present in all the samples analysed (0.230-0.790 ppm) and Cd was in the range of 0.123-0.216 ppm.

Table 3. Removal of Zn, Pb and Cd in water samples from selected locations

Locations	Zn conc.(ppm)		Pb conc.(ppm)		Cd conc.(ppm)	
	Initial	After treatment	Initial	After treatment	Initial	After treatment
I	0.008	ND	0.450	ND	0.164	0.089
II	0.019	ND	0.690	ND	0.205	0.100
III	0.026	ND	0.230	ND	0.123	0.070
IV	0.0059	ND	0.703	ND	0.187	0.091
V	0.018	ND	0.790	ND	0.216	0.110

After allowing 30 minutes contact time of chitosan with water samples, Zn and Pb were reduced to non-detectable levels in all the five samples. Similarly, Cu was removed from both the samples to non-detectable level after the treatment. Fe was found in all the water samples and maximum Fe content (1.242 ppm) was found in the water sample collected from location V. The removal rate of iron was found to be varying from location to location. In the case of location I, the retention was 25%, whereas in location II it was approximately 10%. The different adsorption rates in each location could be due to the compositional variations and interactions of chemicals present in the sample. The concentration of Pb was significantly higher in water sample from location V. When treated with chitosan, Pb was reduced to non-detectable levels in all the samples. The concentration of Cd was highest in the water sample from location V. In all the samples studied nearly 50% of Cd was removed after treatment with chitosan.

Table 4. Removal of Fe and Cu in water samples from selected locations

Locations	Fe conc.(ppm)		Cu conc.(ppm)	
	Initial	After treatment	Initial	After treatment
I	0.474	0.103	0.016	ND
II	0.872	0.074	ND	ND
III	0.321	0.019	ND	ND
IV	0.358	0.017	ND	ND
V	1.242	0.233	0.017	ND

Chitosan derived from the shell waste of *F. indicus* was found to be effective compared to other chelating agents in removing heavy metals. Retention of the heavy metals after treatment with chitosan in the water samples was well below the maximum allowable concentration of water to be used in food industry (Table 5). However, 50% retention was observed in case of cadmium in all the samples.

Table 5. Maximum allowable concentration of selected heavy metals in drinking water (Lakshmanan 2007) [*WHO - World Health Organization; #USPHS - United States Public Health Service]

No.	Metal	*WHO (mg/L)	#USPHS (mg/L)
1	Zinc	15	Not exceeding 5
2	Lead	0.05	0.05
3	Cadmium	0.01	0.01
4	Copper	1.5	1
5	Iron	1	0.3

Acknowledgement

The authors are thankful to the Director, Central Institute of Fisheries Technology, for according permission to present the paper. The technical assistance rendered by Sh. Anish Kumar, K.C is gratefully acknowledged**.**

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Received: 31 December 2007; Accepted: 13 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Impact of Cyanobacterium, *Lyngbya semiplena* on Antioxidant Status of a Tropical Teleost *Oreochromis mossambicus* (Peters)

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Abstract

Biological antioxidants are compounds that protect biological system against the harmful effect of free radicals. The acetone extracts of the cyanobacterium, *Lyngbya semiplena* isolated from Cochin estuary, was found to act as an effective antioxidant in the oxidation system of emulsified linoleic acid *in vitro*. Antioxidant properties were expressed *in vivo* also. When the cyanobacterium was incorporated in the feed of ethanol-exposed *Oreochromis mossambicus*, it could protect the fish from lipid peroxidation and from subsequent tissue damage. Lipid peroxidation was assessed in terms of malondialdehyde, hydroperoxides and conjugated dienes. Antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathiones -transferase and non-enzymic antioxidant substance, glutathione in various tissues were also determined. Higher levels of lipid peroxidation were observed in the animal tissues on exposure to ethanol. However, there was a decrease in ethanol accentuated lipid peroxidation on co-treatment with cyanobacterial feed. Experimental diets could effectively bring down the requirement of defensive antioxidant enzymes in various tissues indicating that cyanobacteria could act as an antioxidant by scavenging the free radicals produced during ethanol exposure. *Lyngbya semiplena* is a food grade organism, highly nutritious and readily available from natural waters. These properties render it attractive for use in fish feed.

Introduction

Free radicals play a major role in the progression of a wide range of pathological disturbances and it can be scavenged by the addition or supplementation of antioxidants to food or to the biological system (Venkateswarlu et al. 2003). The role of dietary antioxidants and their potential benefits in health and disease have attracted great attention (Kehler & Smith 1994). The use of synthetic antioxidants has decreased due to their suspected activity as promoters of carcinogenesis (Namiki 1990). At present

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most of the researchers through out the world are interested in finding new and safe antioxidants from natural sources to prevent oxidative deterioration of food and to minimise oxidative damage to living cells (Pratt, 1992).

Cyanobacteria are sources of a wide variety of compounds with a potential of antioxidant activity. Like all photosynthesizing plants, cyanobacteria are exposed to a combination of light and high oxygen concentrations, which lead to the formation of free radicals and other strong oxidizing agents (Dykens et al. 1992). The elements of photosynthetic apparatus are especially vulnerable to photodynamic damage, because polyunsaturated fatty acids are important structural components of the thylakoid membrane (Sukenik et al. 1993). The absence of such damage in cyanobacteria, in spite of the proximity of the photosynthetically produced oxygen and suitable targets within the photosynthetic apparatus, suggests that these cells have protective antioxidant compounds and mechanisms. The cyanobacterial cells possess an antioxidant defence system, which causes removal of peroxides, free radicals such as superoxide anions ($O_2^{\cdot -}$) generated during photosynthesis and other metabolic process (Karni et al. 1984). Normally this system provides the conditions required for nitrogen fixation and other metabolic events by removing peroxides (Karni et al.1984). Therefore, screening and selection of cyanobacteria with high antioxidant property for producing formulated feed offers tremendous scope in aquaculture.

The importance of cyanobacteria in aquaculture is not surprising as they are the natural food source and feed additive in the commercial rearing of many aquatic animals (Aaronson et al. 1980; De La Noue and De Pauw 1988). Cyanobacteria are usually non-pathogenic and have high nutritive value, rich in carbohydrates, proteins lipids, minerals and vitamins (Cannell 1989). They are not only important as food source, but together with bacteria, they regulate the oxygen and CO_2 balance in the aquaculture systems (Pruder 1983). They also play a role in enhancing the quality of the animal species cultured (Borowitzka 1997). Recent research in natural products of cyanobacteria has made significant advances in aquaculture and they have been shown to produce a variety of compounds and some of them have been proved to possess biological activity of potential medicinal value (Kumar et al. 2003).

Considering the untapped potential of cyanobacteria in aquaculture, the aim of the present study was aimed to determine the antioxidant activity of cyanobacteria, *Lyngbya semiplena* against ethanol induced peroxidative damage in a teleost, Tilapia, *Oreochromis mossambicus.*

Materials and methods

Determination of antioxidant activity of Lyngbya semiplena in vitro

The cyanobacterium *Lyngbya semiplena* was isolated from water samples of

Cochin estuary and cultured in the laboratory using Allen and Nelson medium (Allen and Nelson 1910). The cultures were incubated at 25^{0}C with an illumination of 2000 lux for 30 days.

The cells were harvested at their exponential phase and extracted by continuous maceration with acetone (solvent: mycelia = 100:1, v/w) for 30 min. in a separatory funnel. The solvent layer was separated by passing it through Whatmann no.1 filter paper and evaporated to dryness in vacuum (Mitsuda et al. 1966).

The antioxidant activity of the crude acetone extracts in inhibiting linoleic acid peroxidation was assayed using the thiocyanate method (Yen & Chang 2003). 0.5mL methanol solution of the extract was mixed with linoleic acid emulsion (2.5 mL, 0.02M, pH 7.0) and phosphate buffer (2mL, 0.2M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.28 g of Tween-20 as emulsifier and 50mL phosphate buffer, and then the mixture was homogenised. Control containing all the above ingredients except cyanobacterial extract was also prepared. The reaction mixture was incubated at 37^{0}C to accelerate oxidation. The levels of oxidation were determined by measuring the absorbance at 500 nm after reaction with ferrous chloride and ammonium thiocyanate. The antioxidant activity was expressed as percentage of inhibition of peroxidation (IP%):

IP% = 1- (absorbance of sample at 500 nm)/(absorbance of control at 500 nm) x 100.

All tests were performed in triplicate and results averaged.

Maintenance of test organisms

Oreochromis mossambicus with an average weight of 15±3 g and an average length of 11±3 cm were collected from nearby ponds in and around Cochin, Kerala, India and from the culture ponds of Rice Research Institute, Vyttila, India. Collected fishes were immediately transported to the laboratory, using plastic carriers with the same pond water and were acclimated in dechlorinated waters in large tanks of 1000 L capacity. The water quality parameters were checked and maintained at the optimum level. Dissolved oxygen content was kept at 7.6 mg.L^{-1}; pH 7.5; temperature 26^{0}C and salinity at 0 ppt. The fishes were fed *ad libitum* with commercial feed from Higashimaru Pvt. Ltd. and were maintained in tanks for more than a week prior to the experiment. For experimentation the laboratory acclimated fishes were sorted into batches of six each and kept in fiber tanks of 30 L capacity. Water exchange was done daily and the fishes were maintained with adequate aeration.

Preparation of fish feed by incorporating live cyanobacteria

Experimental feed was prepared from the commercially available feed from Higashimaru Pvt. Ltd. The feed pellets were well powdered, mixed with adequate amount of water, autoclaved and then mixed thoroughly with 15% of live *Gloeocapsa*, having

high antioxidant property. Newly formulated diet was prepared in pellet form with the help of a laboratory pellet press and was allowed to air dry. Egg white, a natural binder was coated over the pellets to bind all the components of the feed together strongly.

Experimental feed named, F34, was prepared by mixing the cyanobacterial strain, *Lyngbya semiplena* (C34). Control feed was also made in the same way without incorporating cyanobacteria.

Effect of cyanobacteria in lipid peroxidation in vivo

Comparison of antioxidant status of the alcohol exposed fish fed with experimental diet and those given control diet was done by determining the level of antioxidants and antioxidant enzymes in the tissues. In order to assess long-term sub lethal toxicity of ethanol to the fish $1/10^{th}$ of the LC_{50} value was selected for treatment. A set of fishes supplied with experimental diets, but not exposed to ethanol was also tested for their antioxidant status.

Experimental design

The test organism, *O. mossambicus* were divided into four separate groups. Each group consisted of six fishes and the whole experiment was designed as follows:

Group I: Control feed (fishes fed with control diet)

Group II: Control feed + Ethanol (Ethanol treated fishes fed with control diet)

Group III: F34 (Fishes fed with *Lyngbya semiplena* incorporated diet)

Group IV: F34 + Ethanol (Ethanol treated fishes fed with *Lyngbya semiplena* incorporated diet)

The experimental animals were dosed for 21 days. Water exchange and ethanol dosage were done daily, so as to avoid any possible degradation or evaporation. They were fed on the same diet twice daily.

Preparation of tissue homogenate for biochemical analysis

The fishes were killed by pithing after the experimental period (21 days) and the tissues *viz.*, liver, gill, heart, muscle and kidney were removed from its body, wiped thoroughly using blotting paper to remove blood and other body fluids. They were washed, weighed and homogenised in ice-cold 0.1M Tris-HCl buffer of pH 7.4, using a glass tissue homogeniser. The homogenate was centrifuged at 5000 rpm for 10 minutes and supernatant was used for assessing lipid peroxidation.

Assessment of lipid peroxidation and antioxidant status of the fish

Lipid peroxidation was assessed in terms of malondialdehyde (Nihaeus & Samuelson 1968), hydroperoxides (Organisciak 1983) and conjugated dienes (Lee et al.

1982). Antioxidant enzymes such as superoxide dismutase (Kakkar et al. 1984), catalase (Machly & Chance 1955), glutathione peroxidase (Gromadzinska 1988), glutathione reductase (Bergemayer 1974) and glutathione-s-transferase (Gromadzinska 1988) and the non-enzymic antioxidant substance, glutathione (Ellman 1959) in various tissues such as heart, liver, gill, kidney and muscle were determined. The concentrations of enzymes and glutathione were estimated and expressed per milligram of protein in the corresponding tissues and therefore soluble protein content of the tissue extract was also measured by Lowry's method (Lowry et al. 1951) using bovine serum albumin as standard.

Statistical analysis

Variance analysis was done on all experimental data and statistical significance ($P<0.05$) of means of six replicates was judged by Duncan's New Multiple Range Test using SPSS (Statistical Package for Social Science) software (10.0).

Results

Lyngbya semiplena (C34), exhibited 58% inhibition of linoleic acid peroxidation (IP%), thereby suggesting its potential use as a value-added ingredient for stabilising food matrices against peroxidation reactions *in vivo*.

Superoxide dismutase (SOD) activity significantly increased ($P<0.05$) in-group II (ethanol treated) when compared to all other groups (Fig. 1).

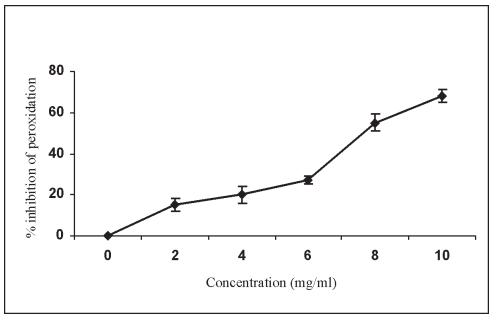

Figure 1. Antioxidant activity of *Lyngbya semiplena*

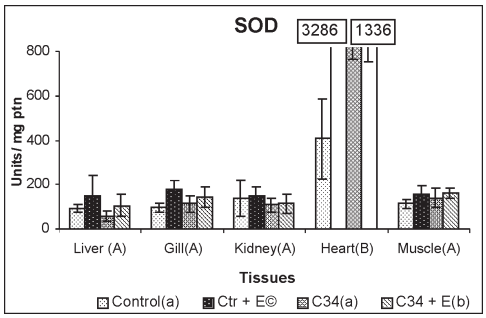

Figure 2. Activity of SOD in various tissues of the treated groups

But it was found that F34 could reduce the enhanced SOD activity due to ethanol exposure, more effectively. Similarly, ethanol exposure increased the levels of catalase, glutathione peroxidase (GPX), glutathione-s-transferase (GST), glutathione reductase (Gred), glutathione, malondialdehyde, hydroperoxides and conjugated dienes (CD). But they were found to be reduced to the normal level on treatment with experimental feed (Fig. 2-10).

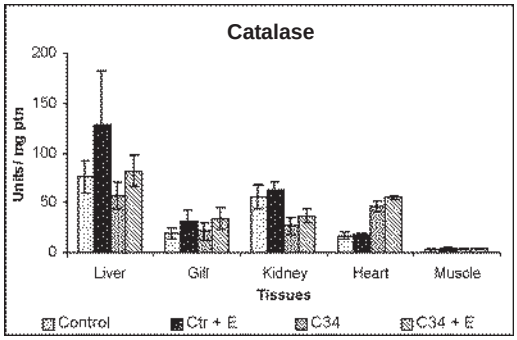

Figure 3. Activity of catalase in different tissues of the treated group

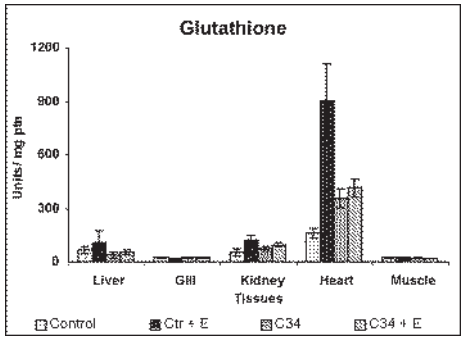

Figure 4. Level of glutathione in various tissues of the treated group

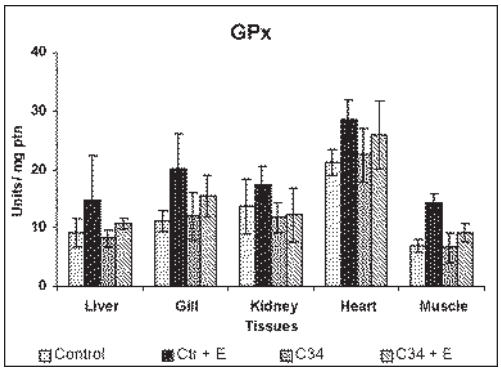

Figure 5. Concentration of Glutathione peroxidase in various tissues of the treated groups

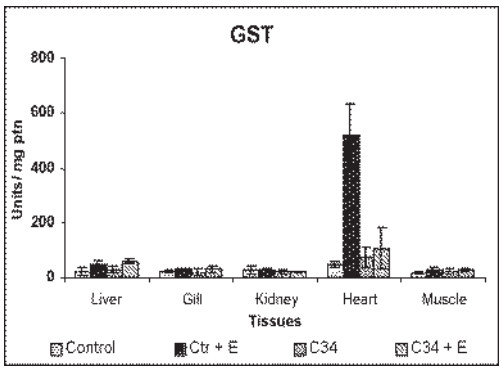

Figure 6. Concentration of Glutathione S-transferase in various tissues of the treated groups

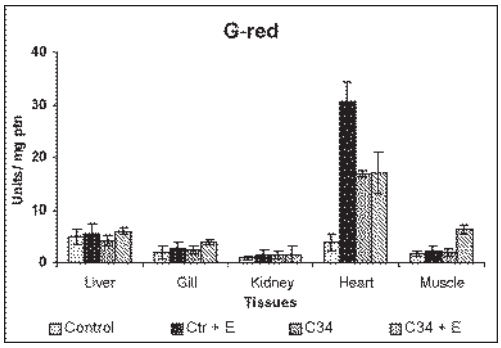

Figure 7. Concentration of Glutathione - reductase in various tissues of the treated groups

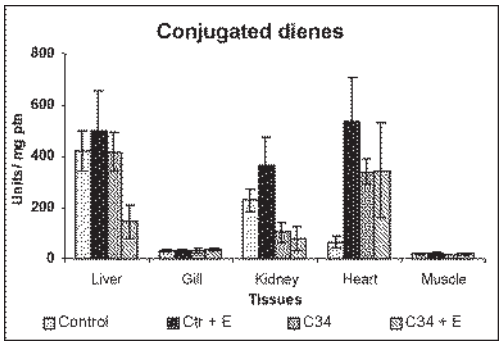

Figure 8. Concentration of conjugated dienes in various tissues of the treated groups

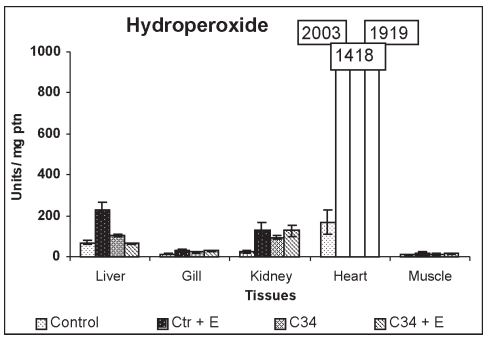

Figure 9. Level of Hydroperoxides in various tissues of the treated groups

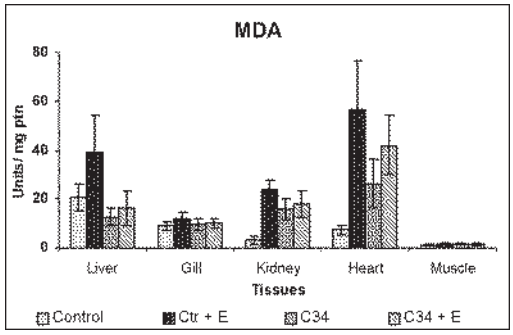

Figure 10. Level of MDA in various tissues of the treated groups

With regard to the antioxidant status of various tissues of the animal, results indicated that maximum level of SOD, glutathione, GST, Gred and hydroperoxides were observed in the heart tissue, whereas, GPX was high in both heart and gill. Catalase was maximum in liver followed by kidney. The concentration of CD was very high in both heart and liver followed by kidney. The concentrations of MDA were high in heart followed by liver and kidney. Muscle showed least activity for all the compounds studied.

Discussion

Cyanobacteria are sources of a wide variety of compounds with a potential of antioxidant activity. There are reports that β-carotene from algae could prevent cancer because of their antioxidant property (Schwartz & Shklar 1987; Fedkovic et al. 1993). It was shown that the algal extract was more effective on hamster cancer regression than by β-carotene alone suggesting a possible synergistic effect of the extract, as components, other than β-carotene, also have a decisive action in the oxidation inhibition (Schwartz & Shklar 1987). Some compounds such as vitamin C, phenols, amines and phospholipids from algae possess antioxidant activity (Tutour 1990). The levels of antioxidant compounds such as phenolic acids, tocopherols and carotenoids were determined from *Spirulina* (Miranda et al. 1998).

In the present study, a potent strain of cyanobacterium, *Lyngbya semiplena* that showed 58% of inhibition of lipid peroxidation was evaluated to test its efficacy in controlling tissue lipid peroxidation and the antioxidant status in experimental toxicity *in vivo*.

The effect of free oxygen radicals accumulation in cells under stress is lipid peroxidation via oxidation of unsaturated fattyacids leading to membrane damage and electrolyte leakage (Liu et al. 1987; Marschner 1995). Malondialdehyde, hydroperoxides and conjugated dienes are the major products of lipid peroxidation and therefore the level of these compounds in tissues can be taken as the index of lipid peroxidation. The

only mechanism which produces malondialdehyde in biological systems is lipid peroxidation.

SOD and catalase are the major antioxidant enzymes associated with scavenging the reactive oxygen species (ROS) (Marschner 1995). However, SOD detoxifies superoxide anion free radicals accompanying the formation of hydrogen peroxide (H_2O_2), which is very damaging to the nucleic acids and proteins (Fridovich 1986; Rabinowitch and Fridovich 1983) and can be eliminated by catalase and peroxidase (Marschner 1995; Scandalios 1990; Elstner and Osswald 1994). Glutathione reductase also plays a key role in oxidative stress by converting the oxidized glutathione (GSSG), to glutathione (GSH) and maintaining a high GSH/ GSSG ratio (Alscher 1989; Fadzilla et al. 1997). GSH is a major antioxidant that is known to protect cells from oxidative stress (Smith et al. 1990). Changes in processes that regulate GSH concentration and/or redox status are considered to be one of the important adaptive mechanisms of cells exposed to stressed conditions (Alscher 1989; Smith et al. 1990; Fadzilla et al. 1997).

In acute ethanol intoxication, liver microsomal metabolism of ethanol was accompanied by hydroxyl radical ($OH^{\cdot}$) generation by cytochrome p450 system. Hydroxyl radicals are responsible for the conversion of ethanol to acetaldehyde. The alcoholic liver injury appears to be generated by the effects of ethanol metabolism and the toxic effects of acetaldehyde, which may be mediated by acetaldehyde altered proteins (Ishak et al. 1991). There is no tissue storage of ethanol, and it reaches all organs of the body. In chronic lipid accumulation the liver cells become fibrotic and leads to impaired liver function. Ethanol increases triglycerides and cholesterol levels thus inducing imbalance in lipid metabolism in liver, heart, kidney and other organs and this could explain the reason for the increase in lipid peroxidation in these organs. Recently free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies (Salin and McCord 1975; Rowley and Halliwell 1983). Protection of cell membrane from lipid peroxidation has become a necessity to prevent, cure or delay of the aforesaid diseases.

In the present study, all antioxidant enzymes were stimulated on exposure to sublethal concentration of ethanol to *O.mossambicus*. Khan et al. 1997 and Balasubramanian et al. 2003 reported a similar observation of significant increase in lipid peroxidation in the tissues of mice that received ethanol. This may be a general adaptive defence response of the animal to toxic alcoholic environments (Karakoc et al. 1997; Sachin et al. 1997). The alcohol induced lipid peroxidation increased with experimental time. However, cyanobacterial diet (F34) significantly reduced the activities of antioxidant enzymes and the concentration of GSH in various tissues of Tilapia coincided with a decrease in concentration of MDA and a decrease in the formation of hydroperoxide and conjugated diene as well, suggesting that oxidative damage induced by alcohol be alleviated by the supplementation of cyanobacterial feed. The antioxidant

components of the feed restored the lipid peroxidation level to nearly those observed in control organisms.

The ability of cyanobacteria to protect the animal from ethanol-induced damage might be attributed to its direct antiperoxidative effect or may be due to its ability to restore the activity of antioxidants, superoxide dismutase and glutathione. *In vivo* experiment has proved that *Lyngbya semiplena* (C34) could act as a very good antioxidant in ethanol-induced Tilapia. The antioxidant effect and resultant protective ability of cyanobacteria may be attributed to the presence of natural compounds such as flavanoids, phenolic acids, vitamin A, vitamin E, vitamin C, phycocyanin, β-carotene and other carotenoid molecules (Miki 1991; Miranda et al. 1998; Bhat and Madyasta 2000) as they can reduce the damage caused by the free radicals. The specific component of the feed is able to reduce the levels of lipid peroxidation and restore the antioxidant status by enhancing acetaldehyde elimination and thus prevent the binding of acetaldehyde to cellular proteins and thereby exerts a protective effect in the animal.

It appears from our studies that the cyanobacteria exhibit its antioxidant role either directly by scavenging the oxidative species or indirectly by modulating the antioxidant levels. In addition, the chemical composition of the *Lyngbya semiplena* indicated that they have high nutritional value due to the presence of high contents of carbohydrates, proteins, lipids and pigments. The species is readily available from natural waters. Therefore, it will be profitable if this species could be cultured commercially for use as natural food source or feed additives in aquaculture and also as source of valuable chemicals such as antioxidant compounds.

Acknowledgements

The authors are grateful to the Department of Ocean Development, Govt. of India and the Ocean Science and Technology Cell, Annamalai University for providing research grant for conducting the study.

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Received: 31 December 2007; Accepted: 24 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Effects of Seed Extract of *Croton officinalis* (Alston) on the Antioxidant Status of *Oreochromis mossambicus* (Peters)

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Abstract

A number of plant products have been used as fish poisons from time immemorial since they are readily available and economical. Freshwater fish, *Oreochromis mossambicus*, were exposed to different concentrations of the fish poison, seeds of *Croton officinalis*, for 96 hours. The LC_{50} value for 96-hour exposure to toxin was found to be 1.18 ppm. A comparative investigation of the effect of toxin at different sub-lethal concentrations on activities of antioxidant enzymes Catalase, Superoxide dismutase, Glutathione peroxidase, Glutathione-S-Transferase, and on lipid peroxidation in gills, liver, heart, kidney, and muscle tissues were carried out. The antioxidant enzymes significantly increased at lower doses, 0.13 ppm and 0.25 ppm and thereby enabled the organism to overcome the oxidative stress induced by toxin. On exposure to 0.42 ppm of toxin, the levels of malondialdehyde, conjugated dienes, and hydroperoxides significantly increased with the corresponding decrease of antioxidant enzymes suggesting that a severe antioxidant stress was experienced by fish exposed to higher concentration of toxin.

Introduction

Oxidative stress potentially is experienced daily by all aerobic life when antioxidant defenses are overcome by pro-oxidant forces and is the basis of many physiological aberrations. Among the most used biomarkers of oxidative stress are antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione-S-transferase (GST). SOD lowers the steady state levels of O_2, CAT is mainly located in the peroxisomes and is responsible for the reduction of H_2O_2 produced from the metabolism of long chain fatty acids in peroxisomes. GST catalyzes the conjugation of xenobiotics with glutathione, whereas GPX catalyses the reduction of both H_2O_2 and lipid peroxides and is considered as an efficient protective enzyme against lipid peroxidation (LPO) (Winston & Di Giulio 1991). LPO is a molecular mechanism of cell injury leading to generation of peroxides and lipid hydroperoxides,

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which can decompose to yield a wide range of cytotoxic products, most of which are aldehydes like malondialdehyde (MDA), 4-hydroxynonenal (HNE) etc.

Naturally occurring fish poisons are widely used from time immemorial for easy harvesting and for the elimination of unwanted fishes in culture ponds. The present study focuses on a biochemical investigation of the effects of naturally occurring fish poison *Croton officinalis* (Alston) on the activities of antioxidant enzymes and on LPO in different tissues of a tropical teleost *Oreochromis mossambicus* (*O. mossambicus)* (Tilapia) adapted to freshwater.

Materials and Methods

Experimental design

The freshwater *O. mossambicus* (15 ± 3g) were collected from the culture ponds of Rice Research Institute, Vytilla, Kerala and were acclimated to laboratory conditions for a month. The characteristics of the water in tank is as follows: dissolved oxygen content was 7.8 ppm, hardness was below detectable amounts, pH 7.0 ± 0.37, temperature 26 ± 3°C, and salinity 0 ppt. Aqueous extract of seeds of *Croton officinalis* (collected from Pathanamthitta District, Kerala, India) was used as toxin for the experiment. For conducting the biochemical study, fishes were divided into four groups and were taken in four separate tanks with the first group serving as the control and the other three as toxin-treated groups that received a sub-lethal concentrations of toxin, 0.13 ppm (i.e. 1/10 of LC_{50}/96 h), 0.25 ppm (i.e. 1/5 of LC_{50}/96 h), and 0.42 ppm (i.e. 1/5 of LC_{50}/96 h). The experimental animals were dosed for 7 days.

Biochemical studies

The marker enzymes in oxidative stress i.e. the antioxidant enzymes were assayed and the products of LPO were estimated in the gill, liver, heart, kidney, and muscle of both control and toxin-treated groups.

Catalase (E.C.1.11.1.6) was assayed using the method of Maehly and Chance (1955). SOD (E.C.1.15.1.1) was assayed using the method of Kakkar et al. (1984). The assay of GPX (E.C. 1.11.1.9) was carried out by the method of Rotruck et al. (1973). GST (E.C.2.5.1.18) was assayed by the method of Beutler (1986). MDA was estimated by the method of Niehaus and Samuelson (1968). Conjugated diene (CD) was estimated by the method of Retnagol and Ghoshal (1966). Hydroperoxide (HP) was estimated by the method of Mair and Hall (1977). Protein was estimated by the method of Lowry et al. (1951).

Statistical analysis

Testing of statistical difference between test and control groups were carried out by two-way ANOVA (Tukey) using the software SPSS 10.0 package. p value < 0.05 was considered as significant.

Results

The results are presented in Table 1

Table 1. Effects of seeds of *Croton officinalis* on different tissues of *Oreochromis mossambicus*
[Values are mean ± SD from fish in each group]

Tissue	Enzyme	Control	0.13ppm	0.25ppm	0.42ppm
Gills	CAT*	$^{A}12.02 \pm 0.416^{d}$	$^{D}96.89 \pm 3.248^{d}$	$^{C}10.28 \pm 0.206^{d}$	$^{B}7.24 \pm 0.246^{d}$
	SOD**	$^{A}11.99 \pm 0.436^{e}$	$^{B}24.94 \pm 0.93^{e}$	$^{C}16.79 \pm 0.612^{e}$	$^{D}10.342 \pm 0.377^{e}$
	GPX#	$^{B}12.37 \pm 0.418^{c}$	$^{D}27.81 \pm 1^{c}$	$^{C}22.08 \pm 0.81^{c}$	$^{A}14.26 \pm 0.52^{c}$
	GST##	$^{A}27.94 \pm 1.07^{c}$	$^{D}191.46 \pm 7.036^{c}$	$^{C}79.64 \pm 2.77^{d}$	$^{B}58.38 \pm 2.236^{d}$
	MDA$	$^{A}0.018 \pm 0.004^{c}$	$^{B}0.091 \pm 0.001^{c}$	$^{C}0.035 \pm 0.003^{c}$	$^{D}0.095 \pm 0.005^{c}$
	CD$	$^{A}30.95 \pm 1.132^{c}$	$^{B}35.11 \pm 1.289^{c}$	$^{C}45.37 \pm 1.543^{c}$	$^{D}49.53 \pm 1.721^{c}$
	HP$	$^{A}14.83 \pm 0.505^{d}$	$^{B}17.29 \pm 0.599^{d}$	$^{C}25.84 \pm 0.868^{d}$	$^{D}35.35 \pm 1.262^{d}$
Liver	CAT*	$^{A}11.68 \pm 0.373^{c}$	$^{D}26.62 \pm 0.93^{c}$	$^{C}8.62 \pm 0.3^{c}$	$^{B}4.09 \pm 0.142^{c}$
	SOD**	$^{A}10.026 \pm 4.362^{d}$	$^{B}18.41 \pm 0.661^{d}$	$^{C}14.34 \pm 0.523^{d}$	$^{D}11.01 \pm 0.402^{d}$
	GPX#	$^{B}8.51 \pm 0.315^{a}$	$^{D}17.23 \pm 0.631^{a}$	$^{C}6.88 \pm 0.251^{a}$	$^{A}4.05 \pm 0.148^{a}$
	GST##	$^{A}35.08 \pm 1.291^{c}$	$^{D}88.39 \pm 3.01^{c}$	$^{C}83.96 \pm 3.063^{c}$	$^{B}56.58 \pm 2.066^{c}$
	MDA$	$^{A}0.062 \pm 0.007^{d}$	$^{B}0.146 \pm 0.003^{d}$	$^{C}0.109 \pm 0.005^{d}$	$^{D}0.304 \pm 0.006^{d}$
	CD$	$^{A}30.84 \pm 1.074^{d}$	$^{B}35.07 \pm 1.259^{d}$	$^{C}47.25 \pm 1.724^{d}$	$^{D}69.01 \pm 2.478^{d}$
	HP$	$^{A}27.26 \pm 1^{e}$	$^{B}28.96 \pm 1^{e}$	$^{C}34.52 \pm 1.177^{e}$	$^{D}52.06 \pm 1.809^{e}$
Heart	CAT*	$^{A}11.42 \pm 0.377^{e}$	$^{D}48.33 \pm 1.679^{e}$	$^{C}40.31 \pm 1.399^{e}$	$^{B}30.52 \pm 1.049^{e}$
	SOD**	$^{A}11.28 \pm 0.411^{c}$	$^{B}15.09 \pm 0.515^{c}$	$^{C}13.35 \pm 0.4^{c}$	$^{D}11.69 \pm 0.427^{c}$
	GPX#	$^{B}11.44 \pm 0.384^{b}$	$^{D}21.92 \pm 0.764^{b}$	$^{C}16.51 \pm 0.604^{b}$	$^{A}4.43 \pm 0.159^{b}$
	GST##	$^{A}19.38 \pm 0.742^{b}$	$^{D}45.6 \pm 1.641^{b}$	$^{C}35.21 \pm 1.292^{b}$	$^{B}21.87 \pm 0.804^{b}$
	MDA$	$^{A}0.021 \pm 0.005^{d}$	$^{A}0.113 \pm .0.002^{d}$	$^{C}0.028 \pm 0.01^{d}$	$^{D}0.127 \pm 0.015^{d}$
	CD$	$^{A}40.22 \pm 1.538^{d}$	$^{B}43.05 \pm 1.574^{d}$	$^{C}47.58 \pm 1.626^{d}$	$^{D}51.04 \pm 1.742^{d}$
	HP$	$^{A}7.9 \pm 0.164^{c}$	$^{B}9.54 \pm 0.323^{c}$	$^{C}12.36 \pm 0.424^{c}$	$^{D}15.7 \pm 0.538^{c}$
Kidney	CAT*	$^{A}4.14 \pm 0.15^{b}$	$^{D}14.12 \pm 0.48^{b}$	$^{C}6.34 \pm 0.216^{b}$	$^{B}2.57 \pm 0.087^{b}$
	SOD**	$^{A}10.58 \pm 0.386^{b}$	$^{B}12.13 \pm 0.44^{b}$	$^{C}11.62 \pm 0.403^{b}$	$^{D}9.54 \pm 0.348^{b}$
	GPX#	$^{B}12.58 \pm 0.45^{d}$	$^{D}67.02 \pm 2.328^{d}$	$^{C}18.37 \pm 0.626^{d}$	$^{A}9.74 \pm 0.391^{d}$
	GST##	$^{A}6.66 \pm 0.228^{a}$	$^{D}22.46 \pm 0.827^{a}$	$^{C}15.48 \pm 0.542^{a}$	$^{B}10.02 \pm 0.394^{a}$
	MDA$	$^{A}0.011 \pm 0.002^{a}$	$^{B}0.03 \pm 0.001^{a}$	$^{B}0.024 \pm 0.003^{a}$	$^{B}0.032 \pm 0.001^{a}$
	CD$	$^{A}8.8 \pm 0.305^{a}$	$^{B}12.07 \pm 0.418^{a}$	$^{C}17.67 \pm 0.646^{a}$	$^{D}34.15 \pm 1.184^{a}$
	HP$	$^{A}13.77 \pm 0.501^{b}$	$^{B}15.21 \pm 0.522^{b}$	$^{C}21.31 \pm 0.742^{b}$	$^{D}28.68 \pm 0.97^{b}$
Muscle	CAT*	$^{A}3.51 \pm 0.12^{a}$	$^{D}5.25 \pm 0.179^{a}$	$^{C}4.66 \pm 0.159^{a}$	$^{B}3.15 \pm 0.121^{as}$
	SOD**	$^{A}8.05 \pm 0.324^{a}$	$^{B}8.94 \pm 0.342^{a}$	$^{C}8.43 \pm 0.302^{a}$	$^{D}8.17 \pm 0.3^{a}$
	GPX#	$^{B}5.77 \pm 0.202^{b}$	$^{D}27.46 \pm 1^{b}$	$^{C}15.27 \pm 0.548^{b}$	$^{A}5.97 \pm 0.218^{b}$
	GST##	$^{A}7.26 \pm 0.252^{a}$	$^{D}28.75 \pm 1.052^{a}$	$^{C}12 \pm 0.438^{a}$	$^{B}9.71 \pm 0.357^{a}$
	MDA$	$^{A}0.062 \pm 0.004^{b}$	$^{B}0.055 \pm 0.004^{a}$	$^{C}0.025 \pm 0.005^{a}$	$^{D}0.141 \pm 0.007^{b}$
	CD$	$^{A}19.85 \pm 0.727^{b}$	$^{B}21.05 \pm 0.735^{b}$	$^{C}27.88 \pm 0.95^{b}$	$^{D}39.92 \pm 1.36^{b}$
	HP$	$^{A}5.67 \pm 0.197^{a}$	$^{B}7.05 \pm 0.244^{a}$	$^{C}10.59 \pm 0.357^{a}$	$^{D}13.11 \pm 0.459^{a}$

* one IU = Change in absorbance at 230 nm/min, expressed/mg protein
** Units/mg protein
\# µg of GSH/min/mg protein
\#\# nmoles of CDNB complexed/min/mg protein
$ mmol/100 g wet tissue

Control compared with toxin-treated groups are represented as values with lower case varies significantly ($P < 0.05$) between tissues and values with upper case varies significantly ($P < 0.05$) between concentrations.

Two-way ANOVA (Tukeys test) showed that there is significant difference ($p < 0.05$) between control and toxin-treated groups with respect to all the parameters tested.

Catalase activity in different concentrations of toxin-treated groups varied significantly compared to control. Among the tissues, CAT activity in gills ($p < 0.05$) was highest. Between different concentrations of toxin-treated groups, tissues at 0.13 ppm showed highest activity. ANOVA showed an overall significant change ($p < 0.05$) in SOD activity in gills, liver, heart, kidney, and muscle tissues. SOD activity was highly elevated in 0.13 ppm treated groups. All tissues at 0.42 ppm showed least activity. GPX activity was found to be highest in kidney at 0.13 ppm ($p < 0.05$) compared to control. Activity of GPX at 0.25 ppm and 0.42 ppm also showed significant increase ($p < 0.05$) compared to control. Among the tissues, all tissues at 0.13 ppm showed an increased GPX activity. Fishes exposed to 0.13 ppm of toxin showed highest GST activity ($p < 0.05$) compared to control. Comparison between tissues showed that gills at 0.13 ppm showed highest GST activity.

ANOVA showed an overall significant change ($p < 0.05$) in the level of MDA, CD, and HP compared to control. Comparison between different concentrations showed that tissues at 0.25 ppm showed highest level of MDA. Among the toxin-treated groups, CD and HP were found to be highest in 0.42 ppm treated group. No significant variation in the levels of CD and HP in muscle at 0.13 ppm was observed compared to control.

Discussion

All organisms posses effective mechanism to prevent and neutralize the free radical–induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, GSH, GPX, and GST. When the balance between reactive oxygen species (ROS) production and antioxidant defence is lost, oxidative stress results, which through a series of events deregulates the cellular functions leading to various pathological conditions. Any compound natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of damage.

Toxicity of oxygen is due to the production of oxygen-derived freeradicals, the most common ones being superoxide (O_2^-), hydroxyl free radical (OH^-), and the singlet oxygen. Under normal conditions also, free radicals are produced during several physiological processes. During mitochondrial respiration, 1%–5% free radicals are produced (Yau-Huei Wei 1998), and immune response by activated phagocytes (Babior et al. 1973) also produces free radicals. These normal levels of free radicals are scavenged

by the normal amounts of antioxidant enzymes. However, the substantial increase in the levels of these highly reactive radicals occurs when the animal is subjected to stress conditions like environmental chemicals or pollutants (Pedragas et al. 1993). This is reflected in the increased production of the antioxidant enzymes.

As reported by Kappus (1986) and Di Giulio et al. (1989), antioxidant defence consists of enzymes including SOD, CAT, GPX, and GST. One of the important features of these enzymes is their inducibility under conditions of oxidative stress (Akcha et al. 2000).

The results obtained in the present study reveal that SOD and CAT appeared to be significantly elevated in *O. mossambicus* exposed to 0.13 ppm concentration of toxin (aqueous extract of seeds of *Croton officinalis*) for 7 days. SOD and CAT are included in the primary antioxidant enzymes, which help in the detoxification of ROS formed from the toxin by decreasing the peroxide levels or by maintaining a steady supply of metabolic intermediates like glutathione (GSH) and NADPH (Kappus 1985). The tissue specific increase in CAT activity showed the following trend: Gills > Heart > Liver > Kidney > Muscle. The tissue specific increase in SOD activity showed the following trend: Gills > Liver > Heart> Kidney > Muscle. The increased activities of SOD and CAT in gills may be related to their physiological role in respiration. In fact, in fish, the extraction of oxygen from water occurs primarily at the gill surface and therefore the gills posses a more rapid and efficient enzymatic mechanism against increased levels of oxygen radicals (Afonso et al. 1996). The increase in tissue SOD activity suggests an increased generation of intracellular hydrogen peroxide that could be adequately detoxified by CAT activity, which was also significantly higher in gills, liver, heart, kidney, and muscle tissues of *O. mossambicus* exposed to 0.13 ppm of toxin for 7 days. Reduction of superoxide anion radicals by CAT prevents the formation of free radical intermediates from the toxin by oxygen reduction mechanisms (Reddy 1997).

GPX is characterized by its ability to reduce hydrogen peroxide and a large number of organic hydroperoxides. In the present study, GPX activity in different tissues showed the following trend: Kidney > Gills > Muscle > Heart > Liver. GPX and other glutathione metabolizing enzyme activities are strongly dependent on tissues, species, and developmental stage (Aceto et al. 1994). Glutathione was found in higher concentration in the kidney and has been directly or indirectly implicated in the maintenance of normal kidney function (Colowick et al. 1954), which may have resulted in increased GPX activity.

When compared to control, GST activity significantly increased in 0.13 ppm, 0.25 ppm, and 0.42 ppm. The maximum increase was seen in gills followed by liver, heart, muscle, and kidney. In the primary metabolism of xenobiotics, electrophilic reactive intermediates can be generated (Miller & Miller 1979; Selkisk et al. 1980). GST

constitutes a versatile mechanism against chemically induced damage. Gadagbui et al. (1996) also support the view that *O. mossambicus* is more likely to excrete xenobiotics as glutathione conjugates or mercapturic acids because of its high GST activity.

LPO, as measured by the concentration of MDA, did not significantly increase in tissues of fish exposed to 0.13 ppm and 0.42 ppm of toxin when compared to control group. This could be due to the increase of nonenzymatic antioxidants, as well as by the increased activity of GST, which can prevent the formation of MDA (Christophersen 1986).

On the other hand, activities of CAT, SOD, GPX, and GST were significantly inhibited in fish exposed to 0.42 ppm of toxin for 7 days. The decreased SOD and CAT activity observed in 0.42 ppm in toxin-treated experimental animal may be related to the increase in O_2^- production. CAT inhibition by O_2^- was previously described by Kono and Fridovich (1982). This shows that in experimental animal tissue dosed in 0.42 ppm, SOD, CAT, and GPX adaptive response were not enough to protect cells against the damage by oxyradicals generated from the toxin, whereas the levels of CD, HP, and MDA significantly increased in fish exposed to 0.42 ppm of toxin when compared to control group, the maximum increase was observed in liver followed by other tissues.

The foregoing results show that at lower doses, toxin-induced oxidative stress in *O. mossambicus* was overcome to a large extent by its antioxidant defence mechanism. In contrast, fishes treated at higher doses experienced LPO to a large extent, which is indicated by its high level of MDA, CD, and HP.

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Received: 31 December 2007; Accepted: 24 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Isolation and Characterization of Serum Immunoglobulins from kalbasu (*Labeo calbasu*) (Hamilton, 1822)

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Abstract

The Immunoglobulins (Ig) from the serum of kalbasu (*Labeo calbasu*, Cyprinidae) was isolated and characterized during the present investigation. Kalbasu of 500-800 g size were immunized with Bovine Berum Albumin (BSA) to prepare immune sera. The Ig was isolated from the immune sera by an immunoaffinity column of BSA-Sepharose 4B. The purity and homogeneity of the isolated sample were confirmed by observing a single band in native gradient polyacrylamide gel electrophoresis. This also indicated the presence of only single type of Ig in kalbasu. The molecular weight of native Ig molecule was determined from the gel to be ~857 kDa. In Sodium dodecyl sulphate polyacrylamide gel electrophoresis (E), the constituent heavy (H) and light (L) polypeptide chains of the Ig molecules were identified. There were only one type of H chain with a molecular weight of ~78 kDa and two types of L chain with molecular weights of ~27 kDa and ~26 kDa. The antiserum against the kalbasu Ig was raised in a rabbit and adsorbed with 10% kalbasu liver tissue homogenate in order to enhance its specificity. By an indirect ELISA standardized using this adsorbed rabbit antiserum, the normal serum Ig concentration in kalbasu was estimated to be ~2.82 mg.mL^{-1} (n=22), which is around 8% of the total serum proteins.

Introduction

Fish like higher vertebrates synthesize immunoglobulins (Ig) upon antigenic stimulation, which is secreted into blood circulation and also present in the bile and the skin mucus. The Ig have been isolated and specific structural characteristics investigated in a number of fish species (Pilstrom and Bengten 1996). In general, there is only one class of immunoglobulin in fish, which is considered analogous to mammalian IgM. Cartilagenous fishes (elasmobranchs) have been shown to possess a pentameric as well as a monomeric form of IgM; whereas, in bony fishes (teleosts) the molecule is tetrameric in nature, although low molecular weight IgM has also been reported in some species (Clem and McLean 1975). Presence of another class of immunoglobulin, homologue to mammalian IgD has also been reported from gene sequence studies in few teleostean

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species (Wilson et al. 1997; Hordvik et al. 1999; Stenvik and Jorgensen 2000). The tetrameric Ig in teleosts like mammalian IgM consist of heavy (H) and light (L) polypeptide chains. However, variations in both H and L chains as isotypes (Lobb et al. 1984; Lobb and Olson 1988; Sanchez and Dominguez 1991) and molecular weight variants (Watts et al. 2001; Swain et al. 2004; Grove et al. 2006) have been reported.

India is endowed with a wide variety of fish species. Carps constitute the bulk of the inland fish resources of India and kalbasu (*Labeo calbasu*) forms one important component in carp polyculture system. However, there are only limited studies on the immunoglobulin molecules of few indigenous species. Considering the importance of the species and the lack of information on its immune response, the present investigation was undertaken to isolate the serum Ig of kalbasu to characterize the molecules biochemically and produce rabbit antisera against these molecules for further application in immunological investigations.

Materials and methods

Production of immune serum in kalbasu

Experimental fish and their maintenance

Twenty-five numbers of adult kalbasu (*Labeo calbasu*) weighing 500-800g were collected from the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, India fish farm and maintained in tanks for immunizations. The animals were acclimatized for seven days prior to immunization.

Immunization of fish

Fourty mg of bovine serum albumin (BSA) was dissolved in 5 mL of tris buffered saline (TBS, 0.02 M Tris HCl with 0.15 M NaCl, pH 7.4) and emulsified with equal volume of Freund's complete adjuvent. Fishes were injected intraperitoneally with 800 µg BSA in 0.5 mL emulsion. Booster doses of BSA with Freund's incomplete adjuvant were administered on 14th and 28th days following the primary immunization. The fishes were bled through caudal vein on 42nd day of first injection in order to collect serum. The serum samples collected were pooled, aliquoted to 2 mL and preserved at -20°C for further experiments.

Isolation of kalbasu serum Ig by affinity chromatography

Kalbasu Ig was isolated by affinity chromatography on an immuno affinity column of BSA-sepharose 4B (Genei, India), according to the procedure of Swain et al. (2004). In brief, 4 mL of column matrix was packed and washed thoroughly with equilibration buffer (TBS). Three ml of of pooled kalbasu serum was filtered through a 0.45 µm filter, diluted 1:4 with TBS and slowly loaded into the column. The loaded serum sample

was then allowed to pass through the column slowly at a flow rate of 10 mL.h^{-1} and the column was washed with TBS till the absorbance of the flow at 280 nm dropped to baseline. The bound Ig in the column was then eluted with 10 mL of 0.1 M Glycine NaOH, pH 11.0 and was collected as a single fraction. The pH of the collected sample was immediately neutralized with 2 M Tris HCl, pH 2.5 and was dialysed against TBS over night at 4°C temperature. After dialysis the sample was concentrated by a centrifuge filter, vectaspin 3 (cut off 30 kDa) (Whatman, U.K) at 4000 x g at 4°C. The isolated sample was aliquoted and preserved at -20°C after determining the protein concentration.

Protein estimation

The total protein concentration of isolated immunoglobulin samples and serum samples were estimated by the dye binding method of Bradford (1976), using BSA as the standard.

Native gradient polyacrylamide gel electrophoresis (Native PAGE)

A native polyacrylamide gel electrophoresis on 2.8 to 22.5% acrylamide gradient was run to check the purity of the isolated Ig. The gels were analyzed with GS-800 densitometer and quantity one software (Bio-Rad, USA) and the molecular weight of the native kalbasu Ig was determined from six samples by comparing it with known molecular weight markers in the gel. Different molecular weight markers used were bovine IgM (950 kDa), thyroglobulin (660 kDa), apoferritin (480 kDa), catalase (250 kDa) and lactate dehydrogenase (146 kDa).

Sodium dodecyl sulphate polyacrylamide gel electrophoesis (SDS-PAGE)

SDS-PAGE was carried out according to the method of Laemmli (1970), in order to determine the types of heavy (H) and light (L) polypeptide chains present in kalbasu Ig molecules. The electrophoresis was run on a separating gel of 12% and a stacking gel of 5% acrylamide concentration. The molecular weight analysis was similarly carried out as for native gradient PAGE.

Production of anti-kalbasu Ig serum in rabbit

One healthy male rabbit procured from a local farm weighing around 1 kg was immunized with three injections of kalbasu Ig (300 µg·injection^{-1}) to raise anti-kalbasu Ig serum. The immunization schedule followed was similar to that mentioned earlier for immunization of fish. The rabbit was bled through the ear vein and the separated serum stored at -20°C.

Western blotting

The specificity of rabbit anti-kalbasu Ig serum was checked in western blotting.

Kalbasu serum and the purified Ig run in SDS–PAGE were electroblotted to a nitrocellulose paper (NCP) (Bio Rad, USA) at 100 V for 1 h. Half of the nitrocellulose paper was stained for 2 min. in 0.1% amido black protein staining solution containing 25% isopropanol and 10% acetic acid, and destained in a solution of 25% isopropanol and 10% acetic acid, to assess the transfer success. The other half of NCP to be processed for immunostaining with rabbit anti-kalbasu Ig serum diluted to 1:2000 in TBST and goat anti-rabbit IgG alkaline phosphatase conjugate (Genei, India) at a dilution of 1:1000 in TBST. Since the rabbit antiserum showed cross-reactions with some unrelated serum proteins, the antiserum was adsorbed overnight with kalbasu liver tissue homogenate (10%) at 1:1 ratio. The serum was centrifuged to remove the precipitate. The specificity of this adsorbed antiserum was checked again in western blot and used in subsequent ELISA experiment.

Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA was performed to determine the normal immunoglobulin concentrations in kalbasu serum samples. ELISA plates (Tarsons, India) were coated with 50 µl·well^{-1} of individual kalbasu serum at 1:64000 dilution (determined earlier by using one serum sample in dilutions of $2x10^3$ to $512x10^3$) in TBS. One dilution series of purified kalbasu Ig (1000 ng·mL^{-1} to 4 ng·mL^{-1}) was put in each plate as a reference standard. Each sample and standard was used in triplicate wells. After 3 h of incubation at 30°C, the plates were washed thrice with TBST (TBS with 0.05% Tween 20) at 5 min. interval. Blocking agent (5% skim milk powder in TBS) was added at 100 mL per well and incubated at 4°C over night. The plates were washed again and anti-kalbasu Ig rabbit serum was added at 1:10000 dilution (determined earlier by checker board titration) in 50 µl volume and incubated for 2 h at 30°C. The plates were washed again with TBST as mentioned before. Subsequently, goat anti rabbit IgG HRPO (horseradish peroxidase) conjugate (Genei, India) was added at 1:5000 dilution. After 2 h of incubation at 30°C and washing with TBST, 100 µl of substrate, trimethyl benzidine/hydrogen peroxide (TMB/ H_2O_2) (Genei, India) was added to each well. After 10 min. of incubation the colour reaction was stopped by adding 1 N H_2SO_4 at 50 µl per well. The absorbance was read at 450 nm in an automated ELISA reader (Model Sunrise, Tecan, Austria) against substrate control (only TMB/H_2O_2 substrate solution) and the results were analyzed by Magellan software. Absorbances obtained with standard Ig dilutions were plotted against the log concentration of Ig to get a standard curve. The Ig concentrations of individual serum samples were determined from the standard curve. A total number of 22 serum

samples from kalbasu (100-300 g size) were analyzed for Ig concentration. The total protein concentration of these serum samples were also estimated by the Bradford procedure mentioned earlier.

Results

Isolation of kalbasu Ig

The kalbasu Ig was eluted from BSA affinity column as a single continuous peak. The eluted fraction contained a protein concentration of 600-800 mg recovered from 3 mL of kalbasu immune sera.

Analysis of kalbasu Ig in native gradient PAGE

The purified kalbasu Ig sample showed a single band in native gradient PAGE analysis. The molecular weight of the kalbasu Ig was determined to be 857.09 ± 12.18 (SE) kDa (Fig. 1).

Analysis of the polypeptide chains of kalbasu Ig in SDS-PAGE

In SDS-PAGE four bands were observed (Fig. 2).

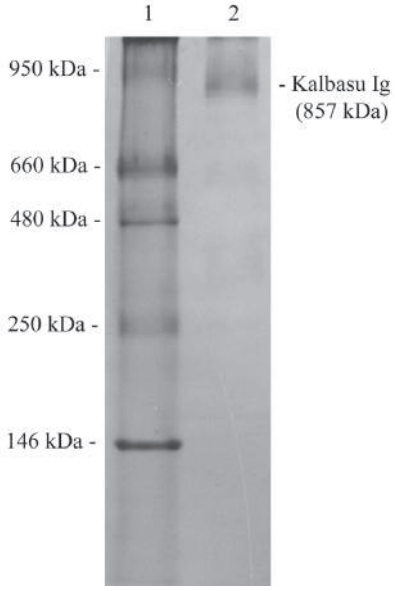

Figure 1. Analysis of purified kalbasu Ig in native gradient page: Lane 1. Molecular weight markers, Lane 2. kalbasu Ig.

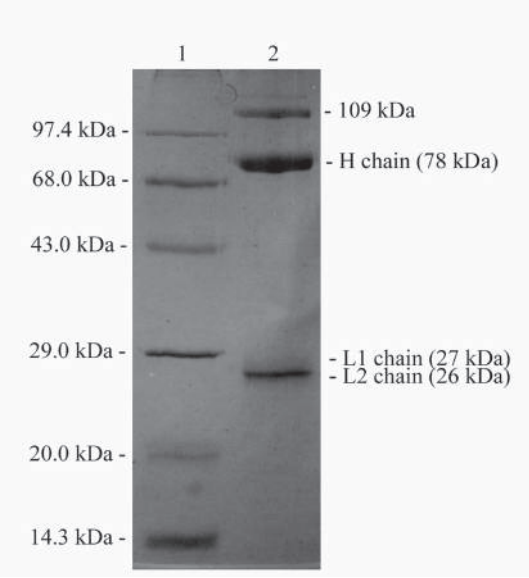

Figure 2. Analysis of kalbasu Ig in SDS-PAGE: Lane 1. Molecular weight markers, Lane 2. kalbasu Ig.

Based on the distance of migration one of these bands was identified as heavy (H) chain and the two other as light (L) chains. The molecular weights of these bands were calculated from the gel and found to be 78.31 ± 0.58 (SE) kDa for the heavy (H) chain and 27.39 ± 0.08 (SE) kDa and 25.94 ± 0.079 (SE) kDa for the two light chains. There was also a faint band observed above the heavy chain, whose molecular weight was determined to be 109.20 ± 0.81 (SE) kDa.

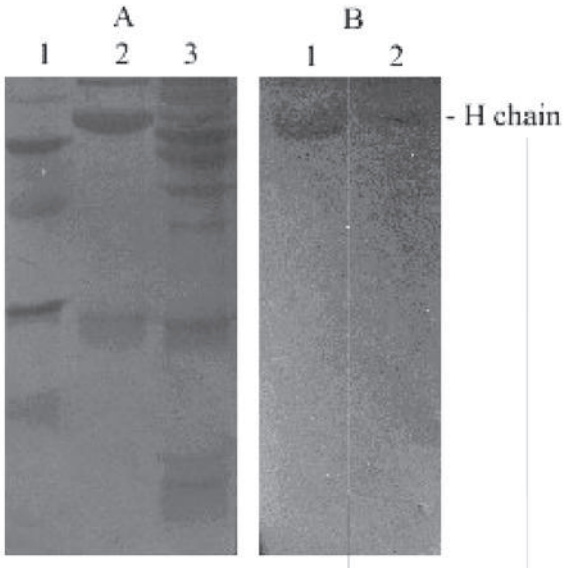

Figure 3. Specificity testing of adsorbed rabbit anti kalbasu Ig serum in western blotting: A. Amido black staining of the blot. Lane 1. Molecular weight marker, Lane 2. Purified kalbasu Ig, Lane 3. Kalbasu serum, B. Immunostaining of the blot. Lane 1. Purified kalbasu Ig, Lane 2. Kalbasu serum.

Specificity of rabbit antiKalbasu Ig serum in western blot

In western blot, the successful transfer of protein bands from SDS-PAGE gel could be assessed by staining the nitrocellulose paper with amido black (Fig. 3A). Immunoblotting with adsorbed rabbit anti-kalbasu Ig serum showed specific reaction with H chain of kalbasu Ig (Fig. 3B).

Quantitation of immunoglobulin concentrations in normal kalbasu serum

An indirect ELISA was standardized to determine the Ig concentration in kalbasu serum samples. Graph plotted using absorbance of serial two-fold dilution of normal kalbasu serum and purified kalbasu Ig showed parallelism (Fig. 4), which indicates the suitability of the system for determination of normal Ig concentration. The dilution of kalbasu serum samples was selected at 1:64000, so that the absorbance values fall within the linear part of the standard curve. The mean Ig concentration in 22 serum samples was quantitated to be 2.82 ± 0.22 (SE) $mg.mL^{-1}$. The average total protein concentration of these serum samples was estimated to be 36.22 ± 1.03 (SE) $mg.mL^{-1}$ and so, the normal Ig level constitutes approximately 8% of the total protein concentration.

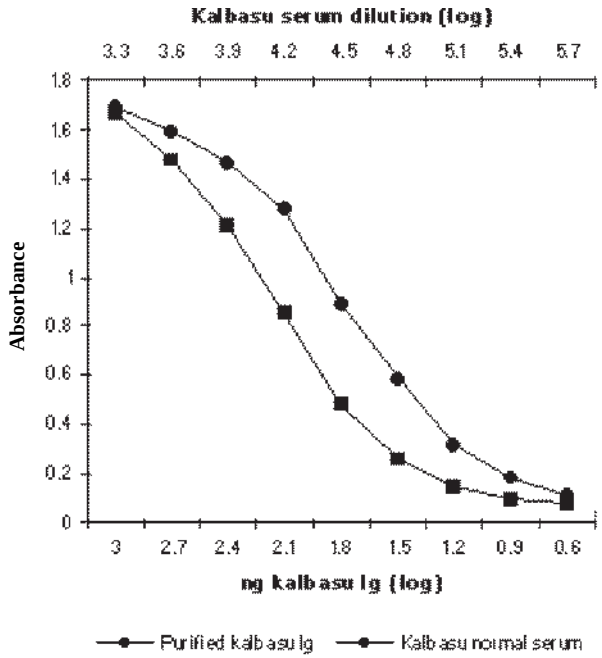

Figure 4. Standard curve used for quantitation of immunoglobulin in kalbasu serum by indirect ELISA.

Discussion

In the present study BSA affinity chromatography was used to isolate Ig from BSA immunized sera from kalbasu. BSA-immunoaffinity chromatography has been used successfully by several workers to purify Ig from other fish species (Phillips and Ourth 1986; Baldwin et al.1997; Swain et al. 2004). For isolation, the bound Ig from the column was finally eluted with a high pH buffer of 0.1 M glycine NaOH, pH 11.0 (Lim 1987), which gave a single continuous peak. The failure in initial attempt using low pH buffer prompted us using this high alkaline buffer, enabling elution of sufficient quantities (600-800 µg) of kalbasu Ig from the BSA affinity column.

In native gradient PAGE, the isolated Ig sample showed only one band that indicated the purity of the sample. The presence of the single band was also indicative of the existence of only single type of antibody molecule in kalbasu. In majority of the teleostean species, a single tetrameric serum Ig with a native molecular mass between 700 and 1000 kDa has been reported (Dacanay et al. 2006). In the present investigation also the kalbasu Ig was found to possess a molecular weight of ~857 kDa. Similar sized Ig have been reported in seabass *Dicentarchus labrax* (855 kDa) (Palenzuela et al. 1996). Based on the high molecular weight, the kalbasu Ig could be considered as IgM type, like other teleostean Ig.

In SDS-PAGE, the kalbasu Ig was reduced to produce one H chain type of ~78 kDa and two L chain types of ~27 kDa and ~26 kDa. Palenzuela et al. (1996) reported similar findings of a single H chain of ~78 kDa and two L chains of ~28.5 kDa and ~27.5 kDa for seabass (*D. labrax*). The molecular weight variants for L chain as found in our study, has also been reported for both H and L chains of several teleosts (Watts et al. 2001; Swain et al. 2004; Grove et al. 2006). Taking into account the molecular weights of H and L chains in the present study, the native kalbasu Ig of ~857 kDa possibly generates four monomeric immunoglobulin molecules of H_2L_2 configuration. Hence, the kalbasu Ig was confirmed to be tetrameric in nature as has been reported for other teleostean species (Ellis 2001). A faint band of ~109 kDa was also found in SDS-PAGE, which is possibly the unreduced H-L monomer, as could be calculated from the molecular weight of H and L chains.

In western blotting, the transfer efficiency could be demonstrated by amido black staining of the blot. In immunostaining of the membrane, the rabbit anti-kalbasu Ig serum reacted with the H chain as well as some non-specific serum proteins (not shown). After adsorption of rabbit serum to kalbasu liver cell homogenate, the specificity was found to be increased as it reacted only to the H chain of kalbasu Ig. Subsequently, this adsorbed antiserum was used for ELISA test.

In the present investigation, an indirect ELISA test was standardized with the rabbit anti kalbasu Ig serum to quantify the normal serum Ig concentration in kalbasu. The parallel sigmoid curves obtained with both purified Kalbasu Ig and normal kalbasu serum indicated that the test could be used to quantify Ig level in normal Kalbasu serum. ELISA has been used by others to measure the serum Ig concentrations in fish and its superiority over the traditional single radial immunodiffusion test has been implicated (Pomport-Castillon et al. 1997). An estimate of the normal serum Ig concentration in kalbasu weighing 100-300 g size was 2.82 $mg.mL^{-1}$, which was about 8% of the total serum protein concentration. In teleosts, the serum Ig concentration has been shown to vary between 2-7 $mg.mL^{-1}$ and the Ig concentration as percentage of total serum protein varies between 6-15 (Ellis 2001).

Conclusion

Kalbasu, *Labeo calbasu* Ig could be isolated to homogeneity by affinity chromatography and it could be proved that the kalbasu possesses only one type of high molecular weight tetrameric Ig similar to Ig of other teleosts. Polyclonal antisera raised against kalbasu Ig was found suitable for use in western blotting and ELISA and hence, may find applications in studying the immune response in kalbasu.

Acknowledgement

The authors gratefully acknowledge the Director, Central Institute of Freshwater Aquaculture, Bhubaneswar, India for providing necessary facilities for the study.

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Received: 23 November 2007; Accepted: 24 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Trawl Fishery of Juvenile Fishes along Mangalore-Malpe Coast of Karnataka and its Impact on Fish Stock

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Abstract

The article presents the quantitative and seasonal variation in percentage of juveniles in the commercial trawl fishery of Mangalore and Malpe in 2006 and discusses the possible impacts of juvenile fish fishery on fish stock in terms of quantity and value. Twenty finfishes and five shellfishes were identified in which considerable quantity of juveniles were caught, and the juvenile fishery is making notable impact on adult fishery. To understand the impact of the fishery of juveniles in a holistic manner, the species of juveniles caught by trawlers are categorized into three groups: I) both juveniles and adults are caught mainly by trawls II) juveniles of the species are caught in trawl, whereas their adults are targeted by gears other than trawl and III) juveniles of the species are caught by trawls regularly, but their adults are rarely caught or not figured in the fishery of the region. Detailed studies on the length-frequency distribution of important species were carried out to find juvenile percentage by weight, number, and months of abundance of juveniles. By statistical analysis (Thompson and Bell model), possible gain in weight and value of the resources, if the juveniles are not caught by trawls, was projected in category I and category II. In category I, the projection is made based on data available on *Nemipterus mesoprion* and the study shows that 7% increase in weight and 22% increase in value (286 lakh rupees) can be obtained if the juveniles of this species are not caught. In category II, *Scomberomorus commerson* is considered as an example for projection. Because the landings of the species by trawlers during 2006 was entirely formed of immature fishes, if the trawl avoids catching these juveniles, 20% increase in yield and 29% increase in value (406 lakh rupees) from the present level is projected by the study. The present article with these examples demonstrates the impact of juvenile fishery on the fish stock and fishery economy of the coast and concludes that since peak periods of specieswise juvenile exploitation is identified, by integrating these temporal data with the spatial data of juvenile fishery, management measures can be formulated and suggested so as to minimize the damages occurring to the commercial fishery due to juvenile exploitation.

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Introduction

Mangalore and Malpe fisheries harbours account for more than 53% of the total marine fish landing and 43% of trawl fisheries of Karnataka. The development of trawl fishery along this coast has been gradual since 1959. Over the years, fleet size of trawlers and trawling operation underwent qualitative and quantitative changes (Mohamed et al. 1998). This growth without doubt resulted in increased yields, employment and exports, it has also lead to excessive fishing pressure as a result of which juveniles of various fishes are exploited in large scale. A major quantity of the juvenile fishes are caught as 'bycatch' during some targeted fishery and are being discarded in the sea itself since it is of no edible value or it is economically not feasible to bring it to the shore. As the profitability from the trawl fishery declined due to stock-related and market-related reasons, there are indications that juveniles of some of the resources are becoming targeted fishery as an alternate source of income. World Wild life Fund pointed out that the problem related to the capture of immature fish reaches its acute level in the case of certain fisheries, with examples from Mediterranean waters, where the vessels completely focused on the capture of small fry and juveniles through using various gears in very shallow areas. Fishing carried out by trawling fleets relies on the capture of immature individuals, often falling far below the minimum legal size prescribed by EU rules (www.wwf.fi/www/uploads/doc/babyfishreport.doc). The present study is carried out to make an assessment of seasonal variations in the percentage of juveniles and to estimate the possible losses in terms of weight and value. Information on seasonal abundance of juvenile fishery of commercial species will help in deriving policies to reduce biological and financial loss due to juvenile fishery by formulating and introducing appropriate management measures.

To distinguish juveniles from adults in the fishery, few criteria are used in many parts of the world. Virginia Institute of Marine Sciences, USA, which is conducting regular juvenile surveys of fishes, especially of bluefish (*Pomatomus saltatrix*), term juveniles as young-of-year (YOY) i.e., fishes less than one year of age. (www.fisheries.vims.edu). It holds good for many temperate species, but in the case of tropical species, majority of which spawn within one year; hence, this criteria cannot be used. The definition, "juveniles are young fish, mostly similar in form to adult but not yet sexually mature" by Hubbs (1943) holds appropriate criteria for distinguishing juveniles of tropical fishes and it is used in the present study.

Materials and methods

Monthly estimates of catch of commercially important fishes landed at Mangalore and Malpe Fisheries Harbours were carried out based on weekly observations. Percentage of juveniles in each sample was estimated from the length-frequency distribution. The data thus obtained were used for calculating the percentage of juveniles in the fishery

by number and by weight. Monthly percentage of juvenile in the landing was also found using length-frequency distribution. Criteria given by Hubbs (1943) is considered for distinguishing juveniles from adults. Minimum size at maturity (MSM) was found from the maturity studies of individual species. The minimum size at which advanced matured ovary was found was considered as MSM. Length cohort studies were carried out on important species to find out yield and fishing mortality in different size groups. Projection of catch in single gear and multigear scenario was carried out by Thompson and Bell projection model (Thompson & Bell 1934) with the help of input data from length-cohort studies.

Results and discussion

At Mangalore and Malpe, 108 species of finfishes, 4 species of cephalopods, and 12 species of shrimps were landed by trawlers in 2006. Important groups in which considerable quantity of juveniles were landed were groupers, thread fin breams, whitefishes, soles, ribbonfishes, scianids, carangids, pomfrets, seerfishes and Indian mackerel. *Nemipterus mesoprion* (*N. mesoprion*) was the most important species in which high percentage of juveniles were found. The list of 20 commercially important finfish species and five important shellfishes (for which total landing of the species is <100 t in weight and juvenile component is <1% in composition) is given in the Table 1. During 2006, juvenile formed 22% by weight (2,914 t) and in *Nemipterus japonicus* (*N. japonicus*), juvenile formed 12% (696 t). In sole, *Cynoglossus macrostomus*, the juvenile percentage was 26% (1,119 t) and in whitefish, *Lactarius lactarius*, annual percentage of juveniles was 18% (97 t). In ribbonfish, *Trichurus lepturus*, 341 t of juveniles landed at Mangalore and Malpe in 2006. In carangid, *Decapterus ruselli*, juvenile percentage was 5% (118 t).

In management perspective, the juveniles caught by trawlers were categorized into three groups. (1) Juveniles of the species, adults of which is mainly caught by trawls. (2) Juveniles of the species, adults of which are mainly caught by gears other than trawls. (3) Juveniles of the species, whose adults are rarely seen or not seen in the fishery of the region. The categorization was carried out to specifically identify the impact of the juvenile fishery by trawl on the adult fishery by of same gear or any other gears so as to assess biological loss for the fishery as a holistic manner.

Among the important species, thread fin breams, whitefishes, soles, ribbonfishes, scianids, carangids and pomfrets are included under category I. In category II, seerfishes, Indian mackerel and oilsardines are included. Category III includes groupers, *Epinephelus* species especially *E. diacanthus* in which “protogyny” was reported (McIlwain et al. 2006).

Table 1. Trawl landing of commercially important resources with their juvenile contribution, at Mangalore and Malpe fisheries harbours in 2006.

Species			Juvenile landing			
Sl.no	Finfishes	Total landing (t)	Weight (t)	% in weight	% in number	Peak months & (%)
1	*Epinephelus diacanthus*	3646	3573	98	99	Oct (34%), Jan (23%)*
2	*Nemipterus mesoprion*	13386	2945	22	42	Dec (96%), Nov (86%)
3	*Cynoglossus macrostomus*	4599	1196	26	35	Feb (63%), Jan (60%)
4	*Nemipterus japonicus*	5780	694	12	35	Mar (58%), Feb (53%)
5	*Saurida tumbil*	3775	302	8		
6	*Trichurus lepturus*	25471	255	1	5	Jan (12%), Feb (12%)
7	*Scomberomorus commerson*	183	183	100	100	Mar (26%), Feb(18%)*
8	*Saurida undosquamis*	2118	169	8		
9	*Decapterus russelli*	2559	118	5	15	
10	*Decapterus macrosoma*	546	104	19		
11	*Lactarius lactarius*	553	99	18	36	
12	*Rastrelliger kanagurta*	2415	87	4		
13	*Epinephelus modestus*	397	48	12		
14	*Sardinella longiceps*	555	33	6		
15	*Leiognathus bindus*	572	29	5		
16	*Secutor insidator*	331	26	8		
17	*Parastromateus niger*	317	16	5		
18	*Otolithus rubber*	158	16	10		
19	*Priacanthus hamrur*	1361	14	1		
20	*Epinephelus epistictus*	113	14	12		
	Shell Fishes					
1	*Loligo duacelli*	6698	201	3	20	Apr (58%), Feb (16%)
2	*Sepia pharaonis*	6165	123	2	13	Mar (33%), Feb (32%)
3	*Metapenaeus dobsoni*	798	64	8	15	Dec (49%), Jan (10%)
4	*M.monoceros*	3025	61	2	7	May (22%), Mar (12%)
5	*S.choprai*	1014	20	2	6	Feb (16%), May (5%)

- 100% juveniles in all the months and percentage is monthly percentage of catch.

Category I

Finfishes: In category I, *N. mesoprion* was the most important species in which highest percentage of juveniles were found. During the year, juvenile formed 42% by number and 22% by weight (2,914 t). For the present study, the MSM 12.8 cm derived from maturity studies of the species was considered as criteria for distinguishing juveniles. Length-frequency distribution revealed that highest number of juveniles was found in December (96%) followed by November (86%). From January to April, juvenile represented in high proportions with percentage ranging from 50% to 69%.

In the case of *N. japonicus*, juvenile formed 35% by number and 12% by weight (696 t). MSM was found to be 13.8 cm. Highest percentage of juveniles were found during March (58%). In February, December and January, the percentages of juvenile in the fishery were high, 53%, 47% and 42%, respectively. Zacharia and Nataraja (2003) reported that *N. mesoprion* have an extended spawning season off Mangalore with major peak during October-December and a secondary peak during March-April. The bi-peak in juvenile abundance may be corresponding to these two peaks in spawning.

In sole, *Cynoglossus macrostomus*, the juvenile percentage was 35% by number and 26% by weight (1,119 t). MSM was found to be 11.5 cm, highest percentage of juveniles was found in February (63%). In January, April and September, the percentages of juvenile in the fishery were high and the percentages were 60%, 58%, and 49%, respectively.

In whitefish, *Lactarius lactarius*, annual percentage of juveniles was 35% by number and 18% by weight (97 t). The incidence of juveniles in the fishery was high in June, August and September, the percentages were 64%, 61% and 56%, respectively.

In ribbonfish, *Trichurus lepturus*, 341 t of juveniles landed at Mangalore and Malpe in 2006. Even though it only formed just above 1% by weight, it formed about 5% by number. In January, February and March, the percentages of juveniles were more than 12%.

In carangid, *Decapterus ruselli*, juvenile percentage was 15% by number and 5% by weight (118 t). Highest incidence of juveniles was noticed in January (48%) followed by October (38%) and November (19%).

Shellfishes: In shrimp, *Metapenaeus dobsoni*, juvenile percentage was 15% by number and 8% by weight (64 t). Highest incidence of juvenile percentage was observed in December (49%) followed by January (10%) and March (9%). In

M. monoceros, annual juvenile percentage was 7% by number and 2% by weight (61 t). Highest catch was observed in May 22%. In *Solenocera choprai*, annual percentage of juveniles was 6% by number and 2% by weight (20 t). The highest percentage of juveniles was observed in February (16%).

In cuttle fish, *Sepia pharaonis*, annual percentage of juveniles were 13% by number and 2% by weight (123 t). Highest juvenile landing was observed in March (33%) followed by February (32%) and May (26%). In squid, *Loligo duvaceli*, annual juvenile percentage was 20% by number and 3% by weight (201 t). Highest percentage of juveniles was observed in April (58%) and another peak was observed in October (30%).

Category II.

In category II, where adults are caught mainly by gears other than trawl, most important species is seerfish, *Scomberomorus commerson*, for which major gear for the commercial fishery is drift gillnet. In trawls, almost all the fishes caught were immature. In trawls, seerfish landing was183 t, which formed 16% of the seerfish landing in Mangalore-Malpe fisheries harbours. Although in all the months 100% of the catch was formed of juveniles, catch was highest in March (26%) followed by February, September, December and January with 18%, 13%, 10% and 9%, respectively. Muthiah & Pillai (2003) stated that out of 11.61 t million *S. commerson* landed by trawlers, only less than 1% get a chance to reproduce once before they were caught. In Indian mackerel and oil sardine, influence of juvenile fishery in trawl was found to be negligible when compared with total landing from the coast.

Category III

In category III, adults are rarely seen in the fishery but fishery for the juveniles is commercialized. In *Epinephelus diacanthus*, almost all the fishes caught were immature females (<F3stage), which by definition (Hubbs 1943) can be considered as juveniles. In this species, the assumption of impact of juvenile fishery is complicated because the species shows “protogyny” and no male was observed during the study. Big fishes were rarely caught, which were not available for maturity studies. Detailed study on the life history of the species has to be carried out to understand the impact of the capture of immature females in the population of this important resource.

Impact studies

Statistically, it is possible to project the difference in weight and value of the resources if the juveniles are not caught by trawls. In the first scenario in category 1, the juvenile fishery is damaging the adult catch in trawl itself, and in the second category, the losses are occurring in gears other than trawls, which are the major gear for those fishery. In the third category, the impact is to be studied in wide geological platform where no data is available on the adult fishery from the area of fishing of juveniles.

As an example in the first category, *N. mesoprion* catch was analyzed and results are given in tables. Here, Thompson and Bell predictive model is used from the results obtained through length cohort studies of the species for the year (Table 2). Yield in 2006 is found to be 13,347 t with a value of 1,258 lakh rupees. If the fishing mortality upto a size of MSM is reduced to zero (no juvenile fishing), the resulting yield is 14,293 t with an increase in weight of 7% and an increase in value of 23% (286 lakh rupees). The increased percentage for value reflects the increased value realized for bigger sized fishes.

Table 2: Thomson and Bell projection of catch and value of *Nempterus mesoprion* if juveniles are not exploited by trawlers.

Table 2.1.Yield and Value with present fishing mortality

Size class (cm)	Fishing Mortality	Average weight (g)	average price (Rs/kg)	Numbers	Total Mortality	Yield (t)	value 000' Rs.
4	0.00	1.3	2	870867	1.61	0	0
5	0.00	2.4	2	806678	1.61	0	0
6	0.02	3.9	2	744956	1.63	3	5
7	0.05	6.0	4	685179	1.66	11	43
8	0.11	8.8	4	626926	1.72	32	130
9	0.22	12.2	4	569502	1.83	85	341
10	0.43	16.4	4	511770	2.05	209	835
11	0.69	21.4	4	451677	2.30	400	1598
12	1.16	27.3	4	389608	2.77	762	3049
13	1.72	34.2	4	323031	3.33	1206	4825
14	2.08	42.1	10	254674	3.69	1473	14726
15	2.70	51.1	10	192584	4.31	1805	18051
16	2.60	61.2	10	136093	4.21	1562	15624
17	2.31	72.5	10	94727	3.93	1228	12280
18	2.67	85.1	10	65984	4.28	1213	12125
19	3.50	99.0	10	43115	5.11	1242	12421
20	2.60	114.3	10	24775	4.21	683	6833
21	2.58	131.0	16	15084	4.19	510	8167
22	3.92	149.2	16	8751	5.53	516	8254
23	2.86	169.0	16	3871	4.47	218	3483
24	2.22	190.4	16	1857	3.84	105	1679
25	1.87	213.4	16	906	3.48	55	883
26	0.30	238.2	16	424	1.92	6	100
27	1.61	264.7	16	259	3.22	23	361
						13347	**125813**

Table 2.2. Yield and Value with juvenile fishing mortality zero.

Size class (cm)	Fishing Mortality	Average weight (g)	average price (Rs/kg)	Numbers	Total Mortality	Yield (t)	value 000' Rs.
4	0	1.3	2	870867	1.61	0	0
5	0	2.4	2	806679	1.61	0	0
6	0	3.9	2	745025	1.61	0	0
7	0	6.0	4	685897	1.61	0	0
8	0	8.8	4	629290	1.61	0	0
9	0	12.2	4	575195	1.61	0	0
10	0	16.4	4	523606	1.61	0	0
11	0	21.4	4	474516	1.61	0	0
12	0	27.3	4	427915	1.61	0	0
13	0	34.2	4	383796	1.61	0	0
14	2.08	42.1	10	342151	3.69	1978	19784
15	2.70	51.1	10	258733	4.31	2425	24251
16	2.60	61.2	10	182839	4.21	2099	20990
17	2.31	72.5	10	127264	3.93	1650	16498
18	2.67	85.1	10	88648	4.28	1629	16290
19	3.50	99.0	10	57924	5.11	1669	16687
20	2.60	114.3	10	33285	4.21	918	9180
21	2.58	131.0	16	20265	4.19	686	10972
22	3.92	149.2	16	11757	5.53	693	11090
23	2.86	169.0	16	5201	4.47	292	4680
24	2.22	190.4	16	2495	3.84	141	2256
25	1.87	213.4	16	1217	3.48	74	1186
26	0.30	238.2	16	569	1.92	8	134
27	1.61	264.7	16	347	3.22	30	485
						14293	**154483**

In the second category, where adults are caught by gears other than trawls, *S. commerson* can be considered as a classic example. Here, length cohort analysis was conducted for pooled length-frequency data from trawl and gillnet. From the results of the analysis, size, group-wise mortality, population number are calculated and these values were used in Thomson and bell predictive model for projecting multigear scenario using length frequency data from trawl and gillnet separately (Table 3).

Table 3. Present Scenario and Thompson and Bell projection of catch and value of seerfish, if juveniles are not exploited by trawlers.

Scenarios	Present Yield	Projected Yield
Seerfish catch by gillnet (t)	1098	1541
Seerfish catch by trawl net (t)	189	
Total catch (t)	1287	
Increase in gillnet catch if there is no juvenile catch by trawlers (t)		253.6
Increase (%)		19.71
Value realized for seerfish catch in gillnet (Rs. lakh)	1279	
Value realized for seerfish catch in trawl net (Rs. lakh)	111	1796
Total	1390	
Increase in value of gillnet catch if there is no juvenile catch by trawlers (t)		406
Increase (%)		29.21

Pooled yield from gillnet and trawl during 2006 is 1,287 t with value of 1,390 lakh rupees and if the trawl is not catching the juveniles of seerfishes (if fishing mortality for seerfishes in trawl is zero), the projected yield in gillnet is 1541 t with an increase in weight of 20% and an increase in value of 29% (406 lakh rupees). Here also increased percentage for value than that of weight reflects the increased value realized for bigger sized seerfishes. Similar exercises of yield prediction by Thomson and Bell multifleet analysis were carried out by Muthiah et al. (2003) and projected that the total catch of *S. commerson* will slide down with increase in trawl effort from the present.

Conclusion

It is often argued that in tropical multispecies trawl fisheries, it is impossible to make policies for avoiding juvenile catches. However, by incorporating the knowledge about temporal and spatial juvenile abundance data, it is possible to formulate policies to reduce juvenile fishery. From the landing data, it is possible for us to identify the peak months of juvenile exploitation. The additional information required is about the spatial distribution of juvenile. Once these two sets of data are available, the scientists can understand the spatio-temporal distribution of juveniles of each species and fishermen can be advised to avoid specific geographical area in specific seasons to reduce the juvenile fishery. Since the trawlers are equipped with geographical positioning systems and most of our offshore fishing operations are targeted fishery according to market demand, these policies can be implemented under responsible fisheries guidelines, so as to minimize the damages occurring to the commercial fishery due to juvenile exploitation.

Acknowledgements

The authors thank Prof. (Dr.) Mohan Joseph Modayil, Director, C. M. F. R. I., Cochin for providing the facilities for carrying out the study. First author thanks Dr. C. Muthiah, Principal Scientist and Scientist-in-charge of Mangalore Research Centre of CMFRI for his constant encouragement and support.

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Received: 1 January 2008; Accepted: 24 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Chromosomal Studies on a Threatened Fish *Cyprinion semiplotus* (Teleostei: Cyprinidae) from Arunachal Pradesh

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Abstract

Cyprinion semiplotus (McClelland) (Fam: Cyprinidae), also known as Assamese Kingfish, is a minor carp that occurs naturally in the Hill Rivers of North East India. This species is recognized as threatened as its occurrence has declined considerably in the rivers of Arunachal Pradesh in recent times. Hence, the present study is undertaken to establish base chromosomal data for application in future conservation measures. Five live specimens were collected from a tributary of River Dikrong near Itanagar, Arunachal Pradesh. Samples were processed for chromosome preparation using a colchicine- KCl flame drying method (Khuda Bukhsh & Manna 1976). The diploid chromosome number (2n) for *C. semiplotus* was ascertained to be 50. The karyotype consisted of 12 metacentric, 8 submetacentric, 8 subtelocentric, and 22 telocentric chromosomes with a fundamental arm number (FN) 70. No sex chromosomes could be identified. This is the first report of a karyotype for *Cyprinion semiplotus*. The possible evolutionary significance of chromosome number is discussed.

Introduction

Cytogenetic studies of fish in recent years have grown in importance with regard to species characterization, evolution, and systematics. These studies are limited to approximately 10% of the total fish species known taxonomically across the world. The fish fauna in the northeastern states of India, particularly in Arunachal Pradesh, are well diversified in the Dikrong River and its tributaries. In this river system, 87 species of fish have been listed (Nath & Dey 2000). Among them, *Cyprinion semiplotus*, Cyprinidae, also known as the Assamese Kingfish, occurs in almost all rivers of the northeastern states of India. In recent years, its population has declined drastically due

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to various natural and anthropogenic stresses. A cytogenetical study on this endangered species was undertaken to determine the presence of natural genetic variation to aid future conservation programmes.

Materials and Methods

Five live specimens of *C. semiplotus* (Fig. 1) were collected from a tributary of Dikrong River near Itanagar, Arunachal Pradesh, using a cast net and the fish were acclimatized in an aquarium for a few hours

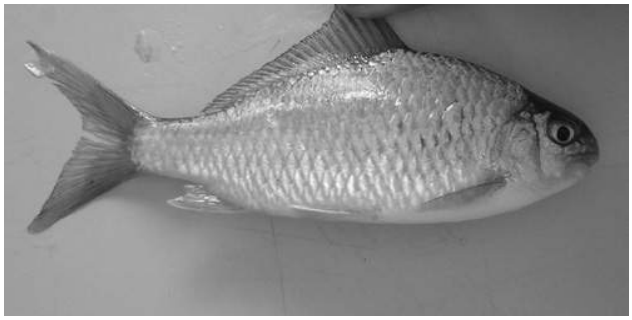

Figure 1. *Cyprinion semiplotus* (McClelland, 1839)

The sex of the specimens could not be identified, as specimens were not sexually matured. All the specimens were injected intraperitoneally with 0.05% Colchicine (1mL/ 100gm of body weight). After three hours the fish were sacrificed and the gill and kidney tissues were processed for chromosome preparation following KCl-Aceto methanol-flame drying method (Khuda Bukhsh & Manna 1976). Chromosome preparations were stained with 2% Giemsa in phosphate buffer (pH 6.8) and microscopically examined. The best metaphase spreads were identified and photographed consequently. For each fish 50 to 60 metaphase spreads were studied. Chromosome complements of three well spread metaphase spreads were measured individually and their centromeric indices and arm ratios were determined to ascribe the morphology, as suggested by Levan et al. (1964).

Results

Somatic metaphase complements contained 50 chromosomes in 52 cells out of the 60 cells studied. Thus, the diploid chromosome number in this species was ascertained to be 2n=50 (Fig. 2). Size of the individual chromosomes ranged from 4.27 to 2.0 µm. The karyotype consisted of 12 metacentric, 8 submetacentric, 8 subtelocentric, and 22 telocentric chromosomes with a fundamental arm number (FN) 70. No karyotype

variation was observed within sampled specimens. No sex chromosomes were identified in the karyotype (Fig. 3) because of the absence of any heteromorphic pair ((either in the form of differential staining or differential size) in unidentified sex of the specimens).

Figure 2. Metaphase complement

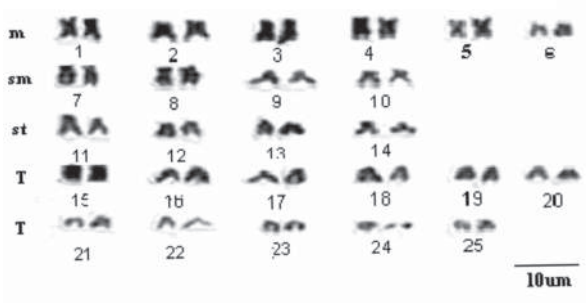

Figure 3. The karyotype of *Cyprinion semiplotus* consists of 2n=50

Discussion

This is the first report of karyotypic data for C*yprinion semiplotus*. Cytogenetic studies of a conspecific *Cyprinion macrostomus*, Cyprinidae, with Nucleolus Organizer Regions (NOR) were reported from Turkey (Yuksel & Gaffaroglu 2008). Diploid chromosome number for *C. macrostomus* was also 2n=50. However, the karyotype differed with 6 metacentric, 24 submetacentric, 12 subtelocentric, and 8 acrocentric chromosomes. In *C. semiplotus* more telocentric chromosomes were observed in comparison with *C. macrostomus* although the diploid chromosome number was the same. This suggests that Robertsonian rearrangements, and possibly pericentric inversions have influenced the karyotype evolution in the genus *Cyprinion*. Hence, both species possess a specific unique karyotype. Manna (1983; 1984) has studied the diploid chromosome number in most of the cyprinid species (70%) and suggested that the modal chromosome number in family Cyprinidae was 2n=50. The sex chromosomes have been detected only in a few species of fishes (Manna 1984). Khuda Bukhsh et al. (1986) observed female heterogamety (females (ZW) and male (WW)) in *Garra lamta* (Fam. Cyprinidae). Recent reviews on sex chromosome and sex determination in fish (Baroiller & D'Cotta 2001; Devlin & Nagahama 2002; Volf & Schartl 2002; Volff 2005) show various forms of genetic sex determination, which include both male heterogamety (males are XY and females are XX) and female heterogamety (males are ZZ and females are ZW). Sex determination mechanism in fish may have autosomal and polygenic influences. Recent studies on the medaka, *Oryzias latipes*, Adrianichthyidae, show a sex-determining gene, *DMY* (DM- domain gene on the Y chromosome). This gene on the Y chromosome is the master gene for male sex determination in the medaka (Matsuda 2005). Therefore, the medaka is expected to become a model species for studies on the

mechanism of sex determination in fishes. In the present study, the sex of the individuals could not be identified and in the karyotype no heteromorphic pair (either in the form of differential staining or differential size) were detected that can be defined as sex chromosomes. No explanation has been given in regards to the sex chromosome in *C. macrostomus* (Yuksel & Gaffaroglu 2008).

Conclusion

The basic karyotype for *C. semiplotus* was 2n=50, similar to that reported for *C. macrostomus* and 70% of other cyprinids. Karyotype evolution in this group of fish appears to have occurred largely through chromosome rearrangements and inversion events.

Acknowledgement

The authors are very thankful to the Principal, Dera Natung Govt. College, Itanagar, Arunachal Pradesh, for giving them permission to use their laboratory and to Mr. H. Sarma, Assistant Professor, of the same college for his help during the study. The authors also express their deep sense of gratitude to the authorities of 8th Asian Fisheries Forum for the poster presentation during the forum proceedings.

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Received: 31 December 2007; Accepted: 21 November 2008

Asian Fisheries Science **22** (2009): 505-510

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Influence of Phosphorus on Phytoplankton Diversity in a Shallow Eutrophic Reservoir

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Abstract

Seasonal changes in the algal diversity with respect to phosphorus load from sewage and dyeing effluent in a small eutrophic reservoir was investigated for a year with monthly samplings. An increase in phytoplankton count was observed ($R^2 = 0.6$) with rise in Phosphate concentrations. Maximum algal diversity (Shannon & Simpsons diversity indices, richness, and evenness) was observed during summer, April to July, when phosphate concentrations were at moderate level (0.074-0.163 mgL^{-1}). Excessive phosphate influx (1.060-1.170 mgL^{-1}) during rainy season, September to November, caused blooms of nonheterocystous assemblage of desmids, particularly of *Cosmarium sp* and myxophycean species, and a general decrease in algal diversity indices was observed.

Introduction

The importance of phosphorus (P) availability for phytoplankton growth is well established in many freshwater reservoirs and lakes (Jordan & Bender 1973; Schelske et al. 1974; Habib et al. 1997; Calijuri & Dos Santos 2001). Seasonal changes in phytoplankton community and numerical abundance in relation to the physicochemical parameters, particularly with P, occur in fresh water bodies, which signal the quality of the water. Hence, a study was made in Sulur tank with an area of 33.2 ha and volume of 0.506 m.cu.m, which is fed by Noyyal river originating from the Western ghats of South India, to observe the phytoplankton community as a bioindicator of the trophic status and health. In addition, to determine the seasonal succession of phytoplankton for the effective application of culture based fisheries. This tank is under the control of the town panchayat and regularly stocked by fingerlings of Indian major carps by the Sulur Fishermen Cooperative Society, Coimbatore (Tamil Nadu).

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Materials and Methods

Noyyal river receives large quantity of sewage effluent from Coimbatore city, having population of more than 30 lakh people, and the discharges from 180 dyeing and processing units located in the city (Fig. 1). It is reported that approximately 600 Megatonnes of garbage is being generated daily in Coimbatore Corporation. The river is also massively polluted by municipal wastes, soaps, and detergents from the Sulur town panchayat, which forms the chief sources of P to this tank. Frequent fish mortalities were observed during algal bloom season.

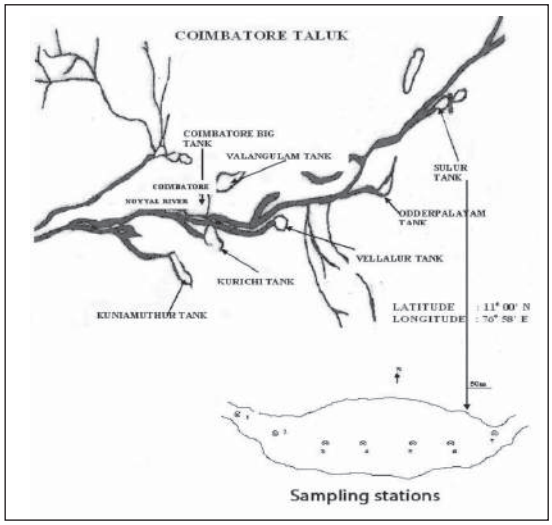

Figure 1. Map showing the Sulur tank location and sampling stations

Water samples were collected from surface waters at seven different places keeping equal distances. They were analyzed for available P as per the ascorbic acid method. Water, pH, alkalinity, and hardness were measured to observe major changes (APHA 1989).

Univariate analysis

The raw numerical abundance data for phytoplankton were collected from the samples. Analysis was performed to calculate species richness, diversity, and evenness index values using the PRIMER FIVE (Plymouth Routines in multivariate Ecological research) software package developed at the Plymouth laboratory, UK (Clarke & Warwick, 1994). Species richness was determined using Margaleff's index (d) that provides a measure of the number of species (S) present for a given number of individuals (N) according to the following equation:

$D = (S-1)/\log_2 N$.

Diversity index was calculated using the Shannon-Weiner (H') index:

$H' = \Sigma\, i\ pi\ (\log_2 pi)$, where pi is the proportion of the total count arising from the i^{th} species. Equitability, the evenness of the species distribution was determined using the Pielou's evenness index (J'):

J' = H' (observed) / H' max where H' max is the maximum possible diversity that could be achieved if all the species were equally abundant $=\log_2 (S)$

Simpsons diversity in the form of $\Delta^\circ = 1-\Sigma$ I (Xi (Xi-10 N9N-1)
All above indices were determined using the diverse routine within the PRIMER software package.

Results

The total algal cells increased with the increase in P concentration considering all the sites combined (Fig. 2).

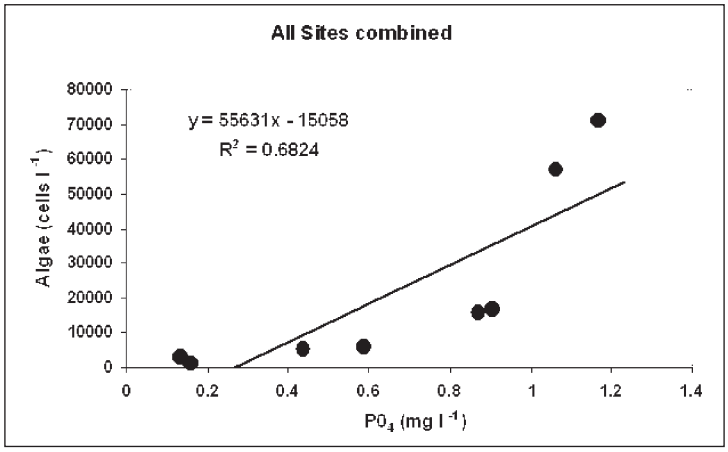

Figure 2. Correlation between phosphorus concentration and algal cells

The average concentration of available P increased from 0.159 mg L^{-1} (July) to 0.588 mg L^{-1} after a brief spell of rain in August. In proportion, plankton count raised from 1183 to 6046 Nos L^{-1}. During July and August only species of Chlorophyceae and Bacillariophyceae were present with scanty presence of *Microcystis sp*. A phenomenal increase in phytoplankton abundance comprising *Cosmarium sp* of Desmidaceae contributing 90% was observed, with little occurrence of other genera consequent to heavy rain, raising the P input to 1.06 mg L^{-1} during September (Fig. 3). In association with *Microcystis*, *Anabaena* and *Polycystis* also occurred. An apparent reduction in Bacillariophyceae and Chlorophyceae was also observed with the rise of desmidacean species *Cosmarium*, which eventually caused the bloom. When monsoon again brought a large quantity of sewage, the P level remained high (> 1.0 mg L^{-1}) even after dilution in October, November, and December. However, the highest record of plankton population (70884 Nos L^{-1}), of which the major count was represented by *Cosmarium sp*, induced dense algal bloom, covering the larger surface of the water during early monsoon in October, discolored the water and smelled obnoxious.

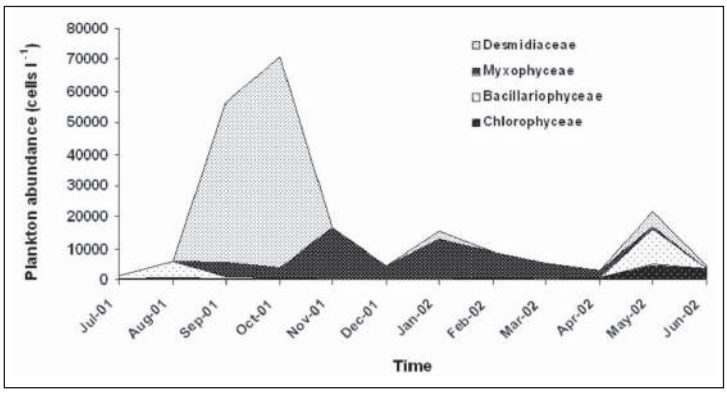

Figure 3. Plankton dynamics of Sulur tank.

A slight decrease in P shifted the algal diversity to *Spirulina sp* dominance in November, which persisted till March 2002 inspite of the P decline. However, with decreasing P concentration, the *Closterium sp* of Desmidiaceae and species of *Scenedesmus*, *Selenastrum*, and *Crucigenia* of Chlorophyceae appeared with declining total count. The P content registered 0.870 mg L^{-1} (January), 0.824 mg L^{-1} (February), and 0.437 mg L^{-1} (March) with corresponding plankton count of 10972, 7912, and 4712 NosL^{-1}, respectively. A further drop in P level (0.074 - 0.134 mg L^{-1}) during summer (April to June) reproduced algal spectrum, with representative species of Chlorophyceae and Bacillariophyceae. During June 2002, *Protococcus* sp appeared in the sample and were dominant (2596 Nos L^{-1}).

Water quality and algal indices

The entire period of study recorded alkaline pH (> 8.21). Surface temperature ranged from 25.9 to 30.5 °C. The highest conductivity value recorded was 5630 µmhos cm^{-1} in August before the monsoon set in and subsequently declined to 538 µmhos cm^{-1} immediately after monsoon by water dilution (Fig. 4). The dissolved oxygen (D.O.) depleted to 2.26 mg L^{-1} at surface when *Spirulina* sp bloom occurred, and there was increase in D.O. in the subsequent months (January to March) and again

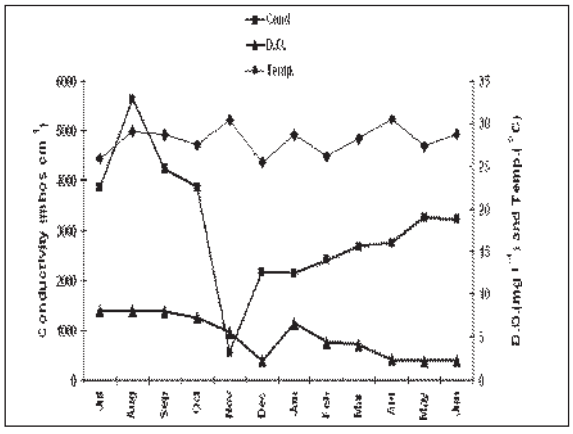

Figure 4. Physico-chemical qualities of water.

lowered to approximately 2.00 mgL^{-1} in summer.

The algal indices (Fig. 5) (Shannon, Margalef and Simpson indices) were higher during summer and pre-monsoon (March to July) and during other periods they were lower indicating the later periods comprised of monospecies of Myxophyceae, which outcompeted other algae during that time when the water was in lentic condition.

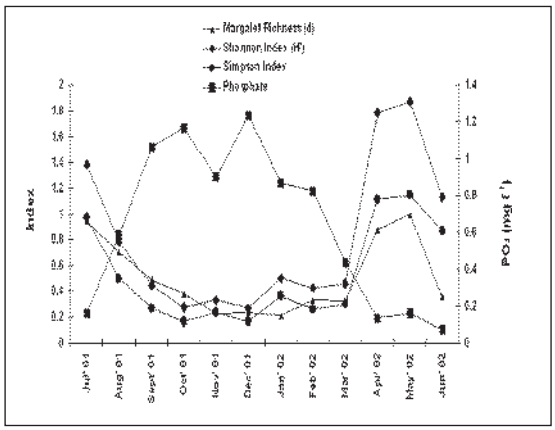

Figure 5. Algal indices and Phosphate concentration.

Discussion

Phosphorus supply is the principal determinant of phytoplankton standing crop and primary productivity in most lakes, a function modified by lake morphometry, light, lake depth, and other influences (Oglesby 1977; Ishida & Mitamura 1988). When it is further accompanied with high pH, increase in light, temperature, and stable water column, the blooms of blue green algae develop and it outcompetes other algae. It stimulated desmid assemblage and myxophycean blooms frequently and has been reported in P limiting freshwater systems (Schindler 1977; Habib et al 1997) and episodes of obnoxious blooms consequent to eutrophication have been recorded by many authors (Hecky & Kilham 1988; Anneville & Pelletier 2000; Bittencourt & Nascimento Moura 2001). The phytoplankton community of the fertilized lake was dominated by desmids, cryptophytes, and at times filamentous nonheterocystous cyanobacteria, *Lyngbya*, *Oscillatoria*, and *Pseudoanabaena* (Levine & Schindler 1989) indicating the role of phosphates in structuring phytoplankton communities in large mesocosms.

Pollution of the Sulur reservoir with anthropogenic phosphate input is the major problem, which caused desmids bloom during rainy season (September, October, & November). The continuous supply of P through sewage subsequently for three months has supported the Myxophyceae bloom and reduction of phosphate concentration during summer altered algal floristic composition. This clearly indicated that the phosphate flux was the principal factor forcing species change in this tank. In a hypertrophic water body due to fertilization recorded the Margalef index value ranging from 0.2 to 2.0 (Sigee 2005), whereas the index values of the present study also showed similar range. However, the intervention of nongovernmental organization based at Coimbatore reclaimed the reservoir from pollution by various management measures such as solid

waste management by Effective Microbial technology, removal of encroachment in the river stretch, desilting, and removal of water hyacinth.

Acknowledgement

The authors are thankful to the Director, Central Inland Fisheries Research Institute, Barrackpore, Kolkata, India for his constant encouragement and the facilities provided during the course of the study.

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Received: 30 December 2007; Accepted: 11 November 2008

Asian Fisheries Science **22** (2009): 511-519

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Smoke curing: A simple method of product development and value addition to low cost fish, *Gudusia chapra,* Clupeidae, from Hirakud Reservoir, India

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Abstract

Hirakud Reservoir is located in between Latitude $20^0$31'N to $21^0$45'N and Longitude 83^0 E to $85^0$10'E and covers a vast area of 74,592 hectares. It is also a source of high ichthyic diversity. Small sized low cost fish such as *Gudusia chapra,* Clupeidae, and *Rohtee cotio,* Cyprinidae, are sold in the local markets at cheaper price or preserved by curing. The small sized fish do not fetch any price to fishermen resulting in waste of time, energy, and under utilization. In an effort to improve the utilization of low cost fish for sustainable development, *Gudusia chapra* was used in the present study in which commercial and experimental smoke cured fish were subjected to quality analyses. The bacteriological, physical, biochemical, and sensory characteristics of *Gudusia chapra* smoke cured by hygienic and scientific methods using community fish smoking kiln (CoFiSmKi) designed and developed as a part of this study, are superior to same variety of commercial smoke cured fish. The CoFiSmKi's were installed in different remote fishing villages adjoining Hirakud Reservoir and fishers were given training cum demonstration on hygienic preparation of smoke cured fish.

Introduction

Hirakud, the largest Reservoir on Mahanadi River System, was taken up on the theme of Tennessee Valley project of America to control the devastating flood in Orissa due to Mahanadi River, power generation, and irrigation. Total area of the Hirakud reservoir quoted by Jhingran (1983) and Raghavachari and Rao (1984) is 74,592 hectares and 71,400 hectares, respectively. Geographically, Hirakud is situated in between Latitude $20^0$31'N to $21^0$45'N and Longitude 83^0E to $85^0$10'E.

Most of the fish catches of Hirakud Reservoir are consumed as fresh, sold in the

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local market at cheaper price, and all small sized commercially important as well as low cost varieties are dry/smoke cured by traditional methods (Prasad et al. 2005). The small sized low cost varieties of fish do not fetch any price to fishermen resulting in waste of time, energy, and under utilization of the fish catch. In any effort to improve utilization of low cost fish, it is important to study quality aspects such as physical, biochemical, microbiological, and sensory characteristics of fish processed by smoke curing and sold at different locations of Hirakud Reservoir. In the present study, a low cost variety fish namely *Gudusia chapra*, Clupeidae, smoke cured in different locations of Hirakud Reservoir was selected for quality assessment and was compared with the same variety smoke cured under controlled conditions. The aim was to find ways to improve quality so as to facilitate better utilization of low cost fish that is available as a product in lean season of fish catches, render fishermen to venture into reservoir to catch all varieties of fish, meet the protein requirements, and also to generate sustainable income to economically under privileged fisher folk. The community fish smoking kiln (CoFiSmKi) designed and developed as a part of this study was installed in different remote fishing villages adjoining Hirakud Reservoir. The purpose of this was to impart training cum demonstration to fishers on hygienic preparation of smoke cured fish for better utilization of low cost fish species.

Materials and Methods

Sample collection and location

Smoke cured *Gudusia chapra* was collected for analytical purpose from three different areas where smoke curing is prevalent: they include upper, middle, and lower reaches of Hirakud Reservoir. The location selection is similar to the studies of Fu et al. (2003).

Fresh *Gudusia chapra* was collected from the landing centres for experimental purpose. Samples were smoke-cured using CoFiSmKi, designed and fabricated at Burla Research Centre of Central Institute of Fisheries Technology (Prasad 2007).

Quality analyses of commercial smoke cured Gudusia chapra

Bacteriological methods

The smoked *Gudusia chapra* samples were collected from different locations of Hirakud Reservoir in self-locking new polythene bags (200 gauge, 500 g capacity). The bags were opened just before the collection of the sample to prevent external contamination to the product. Sampling for bacteriological analyses was carried out immediately after bringing it to the laboratory. A 10 g–smoked fish sample was weighed

in aseptic condition and was cut into small pieces with sterilized scissors under sterile conditions. The sample was aseptically triturated in sterile mortar and pestle using part of 90 mL normal saline, which was later mixed with the rest.

Appropriate dilution of the samples in sterile normal saline water were surface plated on plate count agar, violet bile salt glucose agar, Kenner Fecal or KF streptococcal agar and Baird-Parker agar for the enumeration of mesophilic aerobes, coliforms, faecal *streptococci* and *staphylococci* by following the standard procedures (ICMSF 1978; Mossel et al. 1978). Inoculated plates were incubated at 37^0C for 24 to 96 h, according to the type of enumerating bacteria. Colonies formed on the plates were counted and expressed as $\log_{10}$ CFU g^{-1} of the sample. Further, suspected colonies of *Staphylococcus aureus* were confirmed by coagulase positive test using rabbit plasma (Sanjeev and Surendran 1996).

Throughout the study, sterile normal saline (0.85% NaCl) was used as diluent. Chemicals and dehydrated media used in this study were of Qualigens (India) and Hi Media (India) make.

Physical and biochemical methods

The moisture, fats, and α NH_2 (alpha amino nitrogen) in smoked fish samples were estimated following AOAC (1995) methods. The total volatile nitrogen (TVN) in the cured fish samples was estimated by standard method (Conway 1947).

External quality of the smoke cured Gudusia chapra

The smoke cured *Gudusia chapra* samples collected from three different locations of Hirakud Reservoir were screened for percentage of miscellaneous fish (MF), percentage of extraneous material (EM) such as small parts of fire wood material used for smoking, small molluscs, by sieving and insect infestation and by manual methods using magnifying lenses. The MF or EM in smoked cured fish was assessed by standard method (Prasad et al. 2007),

$$\frac{\text{Total weight of MF/ EM}}{\text{Total weight of the specific sample}} \times 100 = \text{Percentage of MF/EM}$$

Sensory evaluation

The smoke cured *Gudusia chapra* were kept separately and evaluated for overall acceptability by a panel of 15 experienced panelists using a Ten-point Hedonic scale. The same panel was used throughout the study. The mean panel scores of each product were taken into consideration while assessing the quality. The quality of value added fish product collected from three regions of Hirakud Reservoir was assessed from the mean over all acceptability scores on source basis, assuming the Hedonic score 'Two'

to be the limit, below which the product was not acceptable.

Results and Discussion

Post-harvest Utilization

Most of the fish catches are consumed fresh but small sized fish and the other species that are commercially undervalued are sold at very low price or are smoke cured by traditional methods. As a part of better utilization of these groups of fish the commercial smoke cured *Gudusia chapra* samples collected from three different regions of Hirakud Reservoir *viz.*, upper, middle, and lower reaches and the same variety of fish smoke cured in controlled conditions were assessed. The various tests were conducted for bacteriological, physical, biochemical, external quality, and sensory characteristics. In tropical countries, annual losses of cured fish due to bacterial spoilage amount to two to three million tonnes (Clucas and Ward 1996). Hence, it is important to minimize the losses that can lead to better utilization of different varieties of fish that otherwise go waste.

Bacteriological quality of the smoked Gudusia chapra

Bacterial counts from all the smoked *Gudusia chapra* samples collected from different sources with mean values are shown in Table 1.

Table 1. Bacterial load in Low cost value added *Gudusia chapra* and their in comparison with same variety of fish processed under controlled conditions. All bacterial counts are expressed in log CFU g^{-1} of sample.

Source of smoke cured *G. chapra*	Total Viable Bacteria (TVB)	Coliforms	Faecal streptococci	Grouap D fecal strep-tococci	Total Staphy-lococci
Upper reaches of Hirakud Reservoir	5.67 (4.30 to 7.44)	2.63 (<1 to 4.15)	2.42 (<1 to 5.11)	1.90 (<1 to 5.30)	4.96 (3.10 to 6.86)
MID reaches Hirakud Reservoir	3.61 (3.15 to 5.53)	3.11 (<1 to 5.33)	3.29 (<1 to 7.12)	3.32 (<1 to 5.83)	3.87 (2.00 to 7.20)
Lower reaches of Hirakud Reservoir	6.08 (5.78 to 6.56)	0.23 (2.00 to 3.51)	3.26 (2.53 to 4.20)	2.81 (1.54 to 3.95)	4.78 (4.62 to 5.04)
Smoke cured under controlled conditions	2.48 (2.30 to 3.42)	<1	<1	<1	2.45 (1.94 to 2.95)

Mean and data given in parentheses are range of occurrence. When the occurrence of particular group of bacteria is not detected in the sample, the data is given as <1 taking into consideration experimental limitations. From each area an average of 15 fish samples were tested for comparison with fish processed under controlled conditions for value product development.

The smoke cured fish samples collected from lower reaches of Hirakud Reservoir harboured more number of total viable bacteria (TVB) in comparison with the other two groups. Fish or shellfish containing 8 log cycles g^{-1} are unfit for human consumption (Almas, 1981) and the TVB in the present study are within limits (Table 1). The occurrence of faecal *streptococci* in more numbers and from more sources than coliforms suggests that they are better indicators of hygienic conditions prevailing in postharvest handling and smoke curing places (Table 1). This corroborates findings of other works (Prasad and Seenayya, 1998; Prasad et al. 2007).

Consumption of fish and fishery products often results in staphylococcal food poisoning (Sanjeev and Surendran 1996). Though the mean staphylococcal counts were between 3 and 4 log CFU g^{-1}, in the range of occurrence in the samples of middle and upper reaches of Hirakud Reservoir it is slightly higher than the threshold level (6 log CFU g^{-1}) (Table 1) and is a potential health hazard (Bergdoll 1979). Sporadic occurrence of *staphylococci* from a wide variety of environmental sources has been reported (Kloos et al. 1992). In comparison with counts of different groups of bacteria in commercial samples of smoke cured *Gudusia chapra*, the one cured in controlled conditions harboured very less number, which indicates advantage of hygienic and scientific methods of curing the fish.

Physical and biochemical quality of smoke cured Gudusia chapra

Moisture is one of the important factors that determine the quality of smoke cured fish. In the present study, the mean moisture content of all the samples ranged from 5.7 to 15.9% (Table 2). In commercial smoke cured fish the mean fat content ranged from 7.6 to 13.7%, TVN from 73.6 to 153 mg %, αNH_2 from 183.6 to 295.6 mg % and peroxide values from 48.5 to 114.2 meq Kg^{-1}.fat. High TVN values were reported to correlate with high bacterial activity (Vanderzant et al. 1978). In the present study the commercial samples showed more TVN than that of experimental sample.

External quality of the smoked fish

The results of external quality of commercial smoke cured fish revealed that the presence of MF ranged from 0.69 to 5.5% in the samples. The MF identified included *Xenontodon cancilla* (Gourchana-Oriya name), *Pama pama* (Patharmundi-Oriya name), *Rohitee cotio* (Chilanti-Oriya name), *Ambasis nama* (Patponia-Oriya name) and *Puntius* spp. More than 50% of the samples contained EM ranging from 1.24 to 6.57%. The presence of MF in commercial smoke cured samples is similar to the observations made

Table 2. Physical and biochemical quality of commercial smoke cured *Gudusia chapra* in comparison with same variety of fish smoke cured under controlled conditions.

Source of smoke cured *G. chapra*	H_2O (%)	PV (meq Kg^{-1}.fat)	Alpha NH_2 (mg%)	TVN (mg%)	Fat (%). DWB
Upper reaches of Hirakud Reservoir (n=15)	15.93±10.8 (5.46 to 21.69)	114.2±60.99 (43.1 to 215.5)	295.6±131.64 (126.71 to 555.13)	153.00±56.36 (96.00 to 243.20)	7.58±2.57 (4.7 to 12.69)
Mid reaches Hirakud Reservoir (n=15)	5.72±1.80 (3.74 to 9.26)	86.22±57.11 (43.1 to 150.85)	251.33±169.99 (96.54 to 555.13)	104.00±25.16 (64.00 to144.00)	13.70±5.70 (6.01 to 18.21)
Lower reaches of Hirakud Reservoir (n=15)	5.98±1.05 (5.04 to 7.46)	48.46±27.07 (21.55 to 86.1)	183.6±94.91 (102.58 to 313.77)	73.6±9.05 (60.80 to 80.00)	10.57±7.76 (3.5 to 21.33)
Smoke cured under controlled conditions	8.46±1.13 (6.48 to 9.16)	35.63±18.88 (20.14 to 64.43)	146.23±78.45 (96.87 to 278.63)	59.35±7.22 (52.31 to 68.74)	12.95±4.97 (7.54 to 18.64)

Mean ± Standard deviation. Data given in parentheses are ranges. From each area an average of 15 fish samples were tested for comparison with fish processed under controlled conditions for value added product development.

earlier (Prasad et al. 2005). The composition of EM consists of large mud particles, small sticks, snails and burnt particles of algae. Insects were seen in four samples. All the samples contained broken pieces of fish. The percentage of other small fish in the product indicates inadequate care taken in postharvest handling. The percentage of EM indicates that care taken was insufficient during the smoking process and the level of insect infestation indicates the improper storage methods after product preparation, but prior to marketing (Prasad et al. 2007). On the contrary, the fish samples cured by scientific method, following good manufacturing practices, using CoFiSmKi are free from these problems. The results indicate the need for improvement in all quarters of postharvest handling till the product reaches the consumer. This is possible through training cum demonstration programmes.

Sensory Characteristics

The results of sensory evaluation of commercial and experimental smoke cured *Gudusia chapra* samples are shown in Table 3.

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Table 3. Sensory evaluation of commercial smoke cured *Gudusia chapra* in comparison with same variety of fish smoke cured under controlled conditions.

S. No.	Source of smoke cured *G. chapra*	Sensory evaluation scores*			
		Appearance	odour	Texture	Taste
1	Upper reaches of Hirakud Reservoir	6.65±0.29 (6.46 to 6.98)	6.57±0.40 (6.27 to 7.03)	6.51±0.18 (6.31 to 6.58)	6.65±0.07 (6.58 to 6.73)
2	Mid reaches Hirakud Reservoir	6.60±0.49 (6.23 to 71.5)	6.41±0.42 (6.08 to 6.88)	6.14±0.09 (6.04 to 6.19)	6.94±0.40 (6.58 to 7.38)
3	Lower reaches of Hirakud Reservoir	6.18±0.55 (5.85 to 6.81)	6.03±0.59 (5.35 to 6.42)	6.05± 0.36 (5.77 to 6.46)	6.20±0.36 (6.0 to 6.62)
4	Smoke cured under controlled conditions	9.26±0.31 (9.0 to 9.6)	9.0±0.26 (8.7 to 9.2)	9.4±0.36 (9.1 to 9.8)	9.6±0.44 (9.1 to 9.9)

Mean ± Standard deviation. Data given in parentheses are ranges. *The data represents the average of the sensory evaluation of 15 panelists who participated throughout the study. All the samples tested belonged to one variety of fish (*Gudusia chapra*) and had uniform shelf life of 7 days.

The overall mean score of 15 panelists of the commercial smoke cured samples are (~ six levels) good. However, it is excellent (nine) for the same variety of fish smoke cured in controlled conditions.

The present study indicates the need for improvement in postharvest handling of the product to upgrade overall quality, especially, the hygiene aspects in view of occurrence of *staphylococci* in high numbers to an extent hazardous to human health. In the present practice of smoke curing of fish, significant amount of firewood is collected from the forest that is resulting in deforestation (Mishra and Dash, 1984). After years of research the Burla Research Centre of Central Institute of Fisheries Technology has developed different models of CoFiSmKi's, which could be termed as Green kilns, that support not only the conservation of biodiversity of flora of Hirakud Reservoir area but also better utilization of fish. These kilns were installed in fishing hamlets adjoining Hirakud Reservoir and other parts of Orissa (Table 4).

Table 4. Details of installation of Community Fish Smoking Kiln (CoFiSmKi) in different remote fishing hamlets in Orissa

S.No	Location of CoFiSmKi (Burla Model) installation		Year of installation
	Fishing hamlet	District	
1	Sapne*	Sambalpur	2005
2	Rampaluga*	Jharsuguda	2005
3	Pujaripali* (Jhampali)	Jharsuguda	2005
4	Kurumkhel*	Bargarh	2005
5	Thebra*	Jharsuguda	2005
6	Mohammadpur*	Sambalpur	2006
7	Jagipali*	Sambalpur	2006
8	Sonutikara*	Sambalpur	2006
9	Balbuspur*	Sambalpur	2006
10	Jhikimiki sahi I.*	Deogarh	2006
11	jhikimiki sahi II.*	Deogarh	2006

*. Fishing hamlets under CIFT adoption.

In the same fishing villages, training cum demonstration programs were conducted on "Hygienic preparation of smoke cured freshwater fishes and prawns & use of eco-friendly fishing gear for sustainable fisheries". In the Green kilns the firewood used is very less in quantity in the initial stages of smoking and in the remaining period of fish curing, firewood is replaced with paddy husk or saw dust to generate smoke.

Acknowledgement

Authors are thankful to Dr. K. Devadasan, former Director, CIFT, Cochin, for his permission to present the findings in 8th Asian Fisheries Forum held at Cochin from 20th to 23rd November 2007.

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Received: 28 December 2007; Accepted: 12 December 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Quality Assessment of *Labeo rohita* and *Labeo calbasu* sold in Commercial Outlets of Sambalpur, Orissa, India

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Abstract

Bacteriological, biochemical, and sensory qualities of commercial sold *Labeo calbasu* and *Labeo rohita* were reported. The moisture, total volatile nitrogen (TVN), Peroxide Value and α-Amino nitrogen were 73.46%, 9.65 mg % N, 23.15 meq. kg^{-1} fat and 29.06 mg^{-100g}, respectively, for *L. calbasu* and 79.80%, 6.73 mg % N, 23.02 meq. kg^{-1} fat and 29.16 mg^{-100g}, respectively, for *L. rohita*. The bacteriological analysis of *L. calbasu* showed that total viable bacteria were at 4.82 log CFU g^{-1}, the sample was free from coliforms and faecal streptococci, although it harboured Group-D Streptococci and Staphylococci. The bacteriological analyses of L. rohita before and after washing indicate reduction in the counts of different bacteria groups by 1 to 2 log CFU g^{-1} of the sample. Variation in counts of different bacteria was also observed from edible meat portion to gut sample of the same variety of fish. The fresh *L. calbasu* and *L. rohita* scored an average of 9.75 and 9.2 in overall sensory evaluation by ten point Hedonic scale indicating the excellent quality. In addition, the cooked *L. calbasu* and *L. rohita* scored 8.58 and 7.9 out of ten points, respectively, which confirm that both the fishes were excellent in condition at the time of sale. In view of the occurrence of hygiene indicator bacteria more number of studies is necessitated.

Introduction

Fish form a rich source of animal protein available at an affordable price to all sections of the society and provide a means to tide over the nutritional difficulties of man. Importance of fish as a source of high quality, balanced, and easily digestible protein is well understood (Nair and Mathew 2000). Fish was one of the important items of bartered foods and was also exported outside the country in ancient times. From earning a few million rupees in initial years of Indian independence to a staggering gain of foreign exchange beyond billion dollars (US$) or 72,000 million rupees, fishing industry is playing a pivotal role in Indian economy. Fish is also providing jobs to more than four million people (Prasad and Seenayya 1998). Annual production of inland fish has increased from 0.2 to 2.8 million t (14 folds) during the last five decades. During

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this period fish production systems in the inland waters have expanded, diversified, intensified and technologically advanced. Inland open water systems such as rivers, estuaries, lagoons, flood plains, wetlands, and reservoirs contribute to nearly one million tons of fish (Sugunan 2002).

The information on quality of freshwater fish such as physical, biochemical, bacteriological, and sensory characteristics is scanty. The major food fishes of India include species of *Labeo, Catla, Cirrhina, Mystus, Wallago, Notopterus, and Ophiocephalus*. Importance of any fish as a food depends on its well-balanced chemical composition and it determines its nutritional quality (Shasini 2004). Reliable data on the nutritional quality of fish is therefore very essential in aiding the nutritionists and the technologists in dietary formulation, processing, and product development, as well as, nutrition labeling (Nair and Mathew 2000; Sahu 2004, Prasad et al. 2005).

In the light of the above, studies were undertaken on physical, biochemical, microbiological, and sensory characteristics of two freshwater *Labeo species viz*., *Labeo calbasu* and *Labeo rohita* sold in wet fish market of Sambalpur, Orissa. Both the *Labeo species* are common varieties of IMC that are sold in many parts of Orissa.

Materials and methods

Collection of sample

Fish samples were collected from the local outlets of Sambalpur, Orissa in fresh condition in sterile polythene bag (200 gauge) for immediate analysis in the laboratory.

Physical characters of fish.

Immediately after bringing the sample, length, breadth and weight was recorded in aseptic conditions.

Assessment of freshness of fish

Intellectron-Fish Tester VI (German make) was used to assess freshness of fish samples. The measuring positions (base of tail and abdominal cavity) for *L. calbasu* and *L. rohita* were as follows: at the head region below operculum, in the mid region above the abdomen and at the tail region above caudal fin. The measured data were expressed as the “degree of freshness scale”.

Sensory evaluation

For sensory evaluation of the raw and cooked fish, a group of expert panelists (15) were chosen and the same group carried out all the sensory evaluation throughout

the study. A small amount of fish samples were used for cooking in brine (2%) for five minutes. After bringing the temperature to ambient temperature, sensory evaluation tests were carried out (Sahu 2004; Shasini 2004). The sequence of observation included the general appearance, appearance of flesh, including, belly flaps, odour, and texture. The scoring ranged from a minimum of zero to maximum up to ten in a ten point Hedonic scale, where eight to ten was excellent, six to eight very good, four to six good, two to four average and two and below considered to be a spoiled case in which fishes are not fit for consumption. The statistical analysis of the data was carried out using standard methods (Visweswara Rao 1996).

Chemical composition

The fishes were analyzed for physical and biochemical quality parameters such as moisture, pH, fat, peroxide value (PV) and α-amino nitrogen (alpha NH_2) by standard methods (AOAC 1995). The Total Volatile Nitrogen (TVN) content of the fresh water fish samples were estimated by the method of Conway (1947).

Bacteriological examination of fish

Both the freshwater fish samples *Labeo calbasu* and *Labeo rohita* were screened for bacteriological quality that included total viable bacteria, coliforms, faecal streptococci, and staphylococci by standard methods (ICMSF 1978).

The bacteriological examination of the fresh water fish samples for total viable bacteria (TVB) were done by Miles and Mishra's method and spread plate method. The sterile poured plates were inoculated with sterile (Gamma irradiated) calibrated disposable pipettes (Volac-John Poulten Ltd, Barking, England).

Plate Count Agar (Standard Method Agar) was used to assess the load of TVB in the fish samples (ICMSF 1978).

Violet Red Bile Agar (1.2 percent) was used for the detection of coliforms present in the edible meat portion of the fish samples. Baird Parker Agar, a selective medium used for detection of coagulase positive staphylococci (ICMSF 1978). The media was sterilized in autoclave followed by addition of concentrated egg-yolk emulsion along with Potassium Tellurite solution and mixed well before pouring on the plates. KF Streptococcal Agar was used for the enumeration of faecal streptococci (ICMSF 1978). The required amount of KF Streptococcal Agar was boiled in distilled water to dissolve all the constituents followed by the addition of 1 mL (10%) of Triphenyl Tetrazolium Chloride to each 100 mL of sterile medium and mixed thoroughly. Kanamycin Aesculin Azide Agar, a selective media for the cultivation and isolation of Group-D streptococci was used.

Results and Discussion

The present study dealt on the quality aspects of the freshwater *Labeo calbasu* and *Labeo rohita*. The countries of distribution of *L. calbasu* and *L. rohita* include India, Pakistan, Bangladesh, Burma, Thailand, and South China (Sahu 2004). The principal freshwater *Labeo species*, consumed as food in India are *L. bata* (Bata), *L. boga* (Burmese fish or Jamuna fish), *L. boggut, L. calbasu* (Kalbasu), *L. dero* (Bongsa), *L. fimbriatus, L. gonius* (Cursa), *L. kontius, L. rohita* (Rohu) (Sashni 2004). The main *Labeo species* found in Orissa are *L. bata, L. boggut, L. calbasu* and *L. rohita* (Khanna 1999). In the present study, the fish used for experimental purposes were *Labeo calbasu* and *Labeo rohita*, which are bottom feeders, utilizing the decayed vegetation of benthic animals, plants and epiphytic plankton from the bottom of the pond. In Orissa, these two *Labeo species* are found in the river Mahanadi and its branches, and in ponds.

Physical characteristics of the fish

The length, breadth in three different regions of the body and weight of both the fish samples were taken in aseptic conditions**.** In the present study, the average (35 fishes) length of the fish *Labeo calbasu* (33.1 cm) was slightly higher to the observations made earlier (Bandhyopadhyay et al. 1985) in which the average length of *Labeo calbasu* was 30 cm. Whereas the length of *Labeo rohita* (33 cm) and weight (400g) were on lower side to the previous reports (Bandhyopadhyay et al.1985) where the average length and weight of *Labeo rohita* were 35 cm and 600g, respectively.

Freshness of fish samples

The samples of *Labeo calbasu* taken for experimental purpose showed maximum Intellectron reading at head portion (behind gills) (100), mid portion (belly region) (100) and end portion (caudal region) (100). The Intellectron reading for *Labeo rohita* were 72, 80 and 82 for head, mid and tail portions, respectively. The lowest indicator for freshness is '0' and the maximum is '100'. The readings of the present study indicate the freshness of both the fishes is of excellent category. In a study to assess the freshness of freshwater fish sold in wet fish market of Burla, the Intellectron readings varied from 17 to 75 and 47 to 72 for iced riverine/ reservoir fish and un-iced pond reared fish, respectively (Anon, 2004). In comparison with the above-mentioned study, the freshness of the fishes of the present study showed a marked improvement.

Sensory evaluation of fresh and cooked fish

Results of sensory characteristics of *L. calbasu* and *L. rohita*, of the present study are shown in Table 1.

Table 1. Physico-chemical quality characteristics of *Labeo rohita* : A comparison of present study with previous studies

Sl No	Quality parameters	Comparison of results with earlier studies			
		Present study		Sahoo (2006)*	Bandypadhyay et al. (1985)
		L. calbasu	*L. rohita*		
1	Moisture (%)	73.46	79.8	80.06	80.57
2	Total volatile nitrogen (mg%N)	9.65	6.73	13.4	8.8
3	Peroxide Value (m.e.q.Kg^{-1} fat)	23.15	23.02	33.32	18
4	αNH_2 (mg g-100)	29.06	29.16	53.41	30.00
5	Sensory evaluation (10 point hedonic scale)	9.75 (fresh) 8.58 (cooked)	9.2 (fresh) 7.9 (cooked)	7.5 (fresh) 7.2 (cooked)	9.9 (fresh) ND

ND: Not Done

In the present study the raw fish *L. calbasu* scored an average of 9.75, whereas *L. rohita* scored an average of 9.2 out of a ten point hedonic scale in overall appearance, flesh quality, odour and texture indicating the excellent quality. The average scoring of cooked *L. calbasu* and *L. rohita* was 8.58 and 7.9, respectively. This shows that the sensory quality of fishes of the present study were in excellent category.

Physical and Biochemical quality of the freshwater fishes

Details of different physical and biochemical quality analysis of *Labeo calbasu* and *Labeo rohita* collected from the local outlet are presented in Table 1. The compositions vary widely depending upon several factors such as species, size, sex, maturity and many others (Nair and Mathew 2000).

The moisture content of the fish *L. calbasu* was 73.46% while that of *L. rohita* was 79.80% (Table 1). The moisture content of *L. calbasu* is 2.5% (Jose Joseph 2002) and 7% (Bandhyopadhyay et al. 1985) lesser to the earlier reported data of the same variety of fish. Whereas the moisture content of *L. rohita* is 0.8% more to the earlier reports (Bandhyopadhyay et al. 1985). Jose Joseph (2002) reported the moisture in *L. rohita* in the range of 76.4 to 78.0%, which is slightly lower in comparison to the present study.

The fat content of the species *L. calbasu* and *L. rohita* were 0.85% and 4.44%, respectively, on dry weight basis. The earlier study of the same variety of fish, the fat percent varied from 1.80 to 4.20% (*L. rohita*) and 0.6% (*L. calbasu*) (Jose Joseph. 2002). The fat percent of *L. calbasu* of the present study is higher in comparison with same variety of fish reported by Jose Joseph (2002), while that of *L. rohita* is similar to

the higher range reported by the same author.

Determination of TVN forms the most widely used test for fish spoilage. The TVN content in the present samples of *L. calbasu* and *L. rohita* are 9.65 mg N% and 6.73 mg N%, respectively (Table 1). High TVN values were found to correlate with high bacterial activity and spoilage of fish (Sanjeev & Surendran, 1996). However, the level of TVN in the present study indicates that fishes sold in wet fish market of Sambalpur, are in good condition. According to Bandhyopadhyay et al. (1985) fresh *L. calbasu* and *L. rohita* showed the TVN of 8.8 and ≥ 9.0 mg N% indicating a lower value (in *L. calbasu*) and a higher value (in *L. rohita*) in comparison to the present study.

The PV of *L. calbasu* and *L. rohita* after storing the samples at -8^{0}C for three weeks and after subjecting the edible meat to ambient temperature for few hours were 23.15; 41.39 and 23.047; 36.90 meq/Kg fat, respectively (Table 1). The PV of other studies in *L. calbasu* was 18 meq/Kg fat and *L. rohita* was ≥ 30 meq/Kg fat (Bandhyopadhyay et al. 1985).

Similar results were also observed in the analyses of the edible meat portion of the fish for α-amino groups. The α-amino nitrogen in the samples of *L. calbasu* and *L. rohita* stored at -8^0 C for 3 weeks and when exposed to ambient temperature were 29.06; 43.42 and 29.16; 64.80 mg g-100, respectively.

Microbiological examination of fish

The results of bacteriological analysis *viz.*, total viable bacteria, coliforms, faecal streptococci and staphylococci of both the *Labeo species* are shown in the Table 2.

Table 2. Occurrence of total viable bacteria (TVB), hygiene indicator and other groups of bacteria in commercial sold *Labeo rohita*

No of samples	Bacterial counts before washing g^{-1}				
			Group D		
	TVB	C	FS	FS	Staphylococci
1	58×10^4	37×10^2	94×10^2	53×10^2	85×10^3
2	14×10^3	24×10^2	17×10^3	NG	40×10^2
3	27×10^3	23×10^2	98×10^2	18×10^2	78×10^3
4	34×10^3	28×10^2	87×10^2	25×10^2	93×10^3
5	31×10^3	19×10^4	92×10^2	34×10^2	27×10^4

No of samples	Bacterial counts after washing g^{-1}				
			Group D		
	TVB	C	FS	FS	Staphylococci
1	27×10^3	88×10^2	80×10^1	50×10^1	50×10^2
2	14×10^3	60×10^1	20×10^1	$<10^1$	30×10^2
3	19×10^3	43×10^2	16×10^1	80×10^1	CG
4	22×10^3	16×10^2	56×10^1	11×10^2	34×10^2
5	20×10^3	30×10^3	57×10^2	14×10^2	CG
No of samples	Bacterial counts of gut g^{-1}				
	TVB	C	Group D FS	FS	Staphylococci
1	88×10^5	30×10^5	$<10^1$	$<10^1$	$<10^1$
2	72×10^5	38×10^5	$<10^1$	26×10^2	60×10^2
3	24×10^4	18×10^3	$<10^1$	34×10^2	44×10^2
4	28×10^5	12×10^5	$<10^1$	42×10^2	61×10^2
5	22×10^4	24×10^3	20×10^1	33×10^2	10×10^2

TVB: total viable bacteria; CG: crowded growth; C: coliforms; Group D FS: Group D Faecal streptococci; FS: Faecal streptococci. Bacterial vounts are averages of triplicate determinations.

Where the occurrence was less than one log g^{-1}, the same projected as < 1 log g^{-1} owing to the experimental limitation that included the media employed, dilution factor, fastidious nature of some bacteria, pH, temperature, and size of inoculum. Fish is harvested from relatively cleaner environments, however, during subsequent handling spoilage and pathogenic bacteria come in contact with the fish (Chichester and Graham 1973; Prasad and Rao 1993). Hence, it is essential to screen the fish for spoilage and hygiene indicator bacteria.

Total viable bacteria

The TVB count in all fish samples varied three to four logs g^{-1}. In all the samples excepting the sample two the counts have decreased upon washing of the fish (Table 2). The TVB in gut of all the fish samples were higher by 1 to 2 log CFU g^{-1} in comparison with edible meat portion of the same sample. The fish flesh containing 8 log CFU g^{-1} TVB are considered unfit for human consumption (Alamas 1981) and the values in the present study is below the hazardous level.

Coliforms

Washing of the fish samples reduced the counts of coliforms and guts of all the

fish harboured high counts (Table 2). The presence of coliforms indicates level of hygiene of the product (Sahu 2006). Occurrence of coliforms in the guts could be due to feeding habits of the fish.

Group D faecal Streptococci

The counts of group D streptococci reduced from 1 to 2 log CFU g^{-1} in four out of five samples upon washing and the guts of the fish did not harbour this group of bacteria except in sample five where the occurrence is 200 CFU g^{-1} of the sample (Table 2).

Faecal streptococci

Washing of fish decreased number of streptococci. However, no significant difference is seen in their number in edible meat portion and in the guts (Table 2). The presence of faecal streptococci is hazardous to human health as they are implicated in infections of endocarditis in aged persons and pregnancy related problems in young women (Devriese 1992).

Staphylococci

The Staphylococcal count decreased from 1 to 2 log CFU g^{-1} upon washing the fish (Table 2). The fish samples harboured more number of staphylococci in edible meat portion than in guts (Table 2). The studies showed that fish and fishery products are good sources for staphylococcal food poisoning (Sanjeev and Surendran 1996). The acceptable level of staphylococcal counts in fish product is 6 log CFU g^{-1} and above (Bergdoll 1979) and in the present study the counts of staphylococci were below level of human health hazard.

Conclusions

The analysis of physicochemical quality parameters such as moisture, TVN, PV and α-Amino nitrogen of *Labeo calbasu* and *Labeo rohita* revealed that fishes were of good quality and on par with the quality of fishes of same varieties of other studies.

The raw fish *L. calbasu* and *L. rohita* scored an average of 9.75 and 9.2 in overall appearance, flesh quality, odour, and texture in the sensory evaluation by Hedonic scale indicating the excellent quality. And the cooked *L. calbasu* and *L. rohita* scored 8.58 and 7.9 out of a maximum of ten points, respectively, confirm that both the fishes were excellent in condition at the time of sale. The freshness test by using Intellectron-Fishtester VI showed that both the fishes were of excellent quality.

The bacteriological analysis before and after washing of the fish samples indicate reduction in the counts of different bacteria groups by 1 to 2 log CFU g^{-1} of the sample.

Variation in counts of different bacterial groups was also seen from edible meat portion to gut sample of the same variety of fish. In view of occurrence of hygiene indicator bacteria, more number of studies is necessitated especially in the absence of surveillance programs.

Acknowledgements

Authors wish to thank Dr. K Devadasan, Director, CIFT, Kochi, for permission to obtain guidance for M.Sc Dissertation work from Burla Research Centre of CIFT. M.M. Prasad, wishes to thank Director, CIFT, for permission to present the work at 8th Asian Fisheries Forum, Kochi.

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Received: 29 December 2007; Accepted: 31 October 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Antibiotic Susceptibility of Staphylococci Isolated from *Labeo rohita* sold in Burla Fish Market, Orissa

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Abstract

Screening of commercial sold freshwater *Labeo rohita* revealed that the staphylococcal count decreased from one to two log cycles/g upon washing the fish. The fish samples harboured more number of staphylococci in edible meat portion than in guts. The microscopic, biochemical and oxidative fermentations tests of the isolates by conventional and rapid detection methods revealed that they are *Staphylococcus haemolyticus*, *S. auricularius* and *S. caseolyticus*. When the isolates were compared with 45 different antibiotics the results varied from susceptible to resistant in comparison with American Type Culture Collection cultures. In comparison to Clinical Laboratory Standards Institute isolates both the staphylococcal test cultures were resistant to penicillin (G) and vancomycin. With other antibiotics *viz*., Pristinamycin, Ticarcillin/Clavulanic acid, Gatifloxacin, Clindamycin, Clarithromycin, Levofloxacin, Linezolid, Cefeprime, Erythromycin, Streptomycin, Fosfomycin, and Piperacillin/Tazobactam the results varied from intermediate to sensitive.

Introduction

Fish form a rich source of animal protein available at an affordable price to all sections of the society and provide a means to tide over the nutritional difficulties of man. Importance of fish as a source of high quality, balanced, and easily digestible protein is well understood (Nair and Mathew 2000). In the last six decades fish production systems in the inland waters have expanded, diversified, intensified, and technologically advanced. According to Sugunan's (2002) estimate one million tons of fish are available in the inland open water systems such as rivers, estuaries, lagoons, flood plains, wetlands, and reservoirs (Sugunan 2002). Fisheries play a very important role in the country's economy in generating much needed foreign exchange (nearly 72,000 million rupees), providing employment to millions of people and also in enhancing nutritional status of the people, especially, those who are residing in hinterlands (Sahu 2004).

The reports on quality analyses pertained to freshwater fish of *Labeo* spp., namely

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Labeo rohita (Shasini 2004) and *Labeo calbasu* (Sahu 2004) cautioned on the need for studies in view of occurrence of staphylococci in commercial fish samples of wet fish markets.

Staphylococci are inherently susceptible to most antibiotics except those with purely anti-Gram-negative spectra. However, staphylococci remain frequent causes of morbidity and mortality, having proved extremely adept at developing resistance, both by mutation and by DNA transfer. *Staphylococcus aureus* is a classical pathogen, causing infections at many sites (Waldvogel 1995; Lowry, 1998). Studies on antibiotic resistance of different species of Staphylococci were carried, but are confined to isolates obtained from cattle, cats, dogs, ducks, guinea pigs, horses, mink, pigeons, pigs, rabbits, and turkeys (Schwarz et al. 1998). Reports on antibiotic resistance of Staphylococci isolated from freshwater fish are scanty. Methicillin-resistant *Staphylococcus aureus* (MRSA) is well recognized as a major cause of infection in the healthcare setting but as a matter of great concern is now emerging in the community. The glycopeptides notably, vancomycin have traditionally been the mainstay of treatment of MRSA but overuse has led to the emergence of vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) (Appelbaum 2006).

In the light of the above, studies were undertaken on screening of freshwater *Labeo rohita* sold in wet fish market of Burla, Orissa, for staphylococci. The Fish samples were screened for staphylococci before and after cleaning of the same fish and also gut to see the difference between edible meat portion and gut of the fish. Besides carrying out quantitative estimation of staphylococci, the isolates of staphylococcal groups were characterized and were compared with 45 antibiotics.

Materials and methods

Collection of sample

Fish samples were collected from the local outlet of Burla in fresh condition in sterile polythene bag (200 gauge) for immediate analysis in the laboratory. The fish samples *Labeo rohita* collected from market of one source constituted one sample.

Screening of fresh Labeo rohita for staphylococci

Each sample weighed 20g fish and from this sampling was done for estimation of staphylococci. In screening the fish before and after washing, in edible meat portion and in gut for staphylococci, Baird Parker Agar was employed (ICMSF, 1978).

Characterization of the suspected staphylococcal isolate by rapid detection methods

The organisms to be identified were isolated by standard procedure on a common medium like Nutrient Agar or Soyabean Casein digest Agar. A single isolated colony was inoculated in brain Heart Infusion broth. A biochemical test kit for identification and differentiation of genus staphylococcus was employed in the present study. The kit uses standardized, colorimetric identification system utilizing twelve conventional biochemical tests *viz*., Voges-proskaeur's Esculin hydrolysis, PYR, ONPG, Arginine utilization, Glucose, Ribose, Arabinose, Sucrose, Sorbitol, Mannitol and Raffinose, which are based on the principle of pH change and substrate utilization. Each well was inoculated with 50 µL of the above test culture inoculum by surface inoculation method. On incubation, the metabolic changes of the test cultures resulted in change in colour in the media that was either visible spontaneously or after addition of a reagent. The cultures were also examined further for citrate utilization, arginine dihydrolase test, oxidase test, catalase test, haemolysis test, growth at high pH, and differentiation test of staphylococcus strain (Schleifer 1986).

Study of antibiotic sensitivity of staphylococcus strains isolated from Labeo rohita

The agar diffusion method using Mueller Hinton was followed for challenging test cultures against different antibiotics in different concentrations.

Results and Discussion

The original source of the fish samples is Hirakud Reservoir. After the harvest, the fishes are packed in bamboo baskets and are transported to the market in iced condition for further sale.

Staphylococcal examination of fresh Labeo rohita

The results of staphylococcal examination of edible meat portions of fish (skin and flesh) before and after washing and gut are shown in Table 1. The Staphylococcal count decreased from one to two logs on washing the fish. The fish samples harboured more number of staphylococci in edible meat portion than in guts. Fish is harvested from relatively cleaner environments, however, during post harvest handling, bacteria of spoilage type, hygiene indicator and human health hazard type come in contact with the fish (Prasad and Rao, 1993). This study has shown that simple washing of fish will reduce the bacterial load by one to two log cycles. The studies of Sanjeev and Surendran (1996) revealed that fish and fishery products are good sources for staphylococcal food poisoning. According to Bergdoll (1979) the acceptable level of staphylococcal counts in fish product is six logs and above, however, in the fish samples under study the counts of staphylococci were below the dangerous level to cause any human health hazard.

Table 1. Occurrence of staphylococci in commercially sold *Labeo rohita*

No of samples	Occurrence of staphylococci /g of the sample		
	Before washing	After washing	in gut
1	8.5×10^4	5.0×10^3	$<10^1$
2	4.0×10^3	3.0×10^3	6.0×10^3
3	7.8×10^4	OG	4.4×10^3
4	9.3×10^4	3.4×10^3	6.1×10^3
5	2.7×10^5	OG	1.0×10^3

Staphylococcal counts are averages of triplicate determinations. OG: Over growth

Characterization and identification of isolates

Gram's staining of the test culture confirmed that the isolates were Gram-positive cocci. The Voges-proskauer's esculin hydrolysis, PYR, ONPG, arginine utilization, oxidase, catalase, citrate utilization, lysine decarboxylase, haemolysis and growth at pH 9.6 varied between the isolates. The test culture was subjected to aerobic and anaerobic utilization of 21 different carbohydrates. In the present study, the test cultures utilized sucrose, maltose, D-trehlose, β-D fructose and with other carbohydrates such as lactose, raffinose, dextrose, maltose, salicin, sorbitol, lactose in both aerobic and anaerobic conditions the results varied between the isolates. The morphological, biochemical, and carbohydrate utilization tests of isolates from fish samples resemble *Staphylococcus haemolyticus*, *S. auricularius*, and *S. caseolyticus* (Schleifer 1986). Coagulase-negative staphylococci (CNS) include *Staphylococcus epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. auricularius*, *S. caseolyticus* and a number of other species. CNS are important as causes of line-associated infections in the immunosuppressed and account for many of the bacteraemic episodes in neutropenic patients (Hamory et al, 1987; Oppenheim 1998). Overall, CNS account for approximately 7 to 9% of bacteraemias reported to the Public Health Laboratory Service (Reacher et al. 2000) and are important also as causes of prosthetic valve endocarditis, being more frequent than *S. aureus* in this setting.

Antibiotic sensitivity of test culture

Staphylococci are ubiquitous in nature and *Staphylococus aureus* is the most pathogenic species. The spread of antibiotic resistance among *S. aureus* strains is a major concern in the treatment of staphylococcal infections (Ito et al. 2003). The spread of MRSA from the hospital to the community setting, coupled with the emergence of VISA and VRSA, has become a major cause of concern among clinicians and microbiologists (Appelbaum 2006).

Comparison of Staphylococcus isolates of the present study Staph I and Staph II to American Type Culture Collection cultures. In comparison to Clinical Laboratory Standards Institute (ATCC)

The response of test cultures when challenged with different antibiotics and the comparison with *S. aureus* of ATCC is shown in Table 2. For convenience of interpretation the range of antibiotic response ATCC is divided into mainly three categories. The interpretation zone between the lower and upper ranges was drawn and below the mean expressed as <, above the mean as >, less than equal to mean as ≤ and more than equal to mean ≥. Accordingly the Staph I test isolate was < to 80%, 15.6% towards ≥ and ≤ 4.4% to the antibiotics tested in comparison to the ATCC. Similarly Staph II test culture is < 77.77% and 33.33% towards ≤ to different antibiotics tested. Schwarz et al (1998) observed that majority of the staphylococcal isolates that have shown resistance to tetracycline was also resistant to one or more antibiotics. The isolates of the present study showed variation in comparison with ATCC cultures (Table 2) where one is resistant another one is sensitive to tetracycline. However, both were susceptible to chlortetracycline (Table 2). Reports indicate resistance of staphylococci to teicoplanin (Livemore, 2000). In the present study too the staphylococcal isolates were resistant to teicoplanin (Table 2).

Table 2. Antibiotics tested, code, level of concentration and the zones of interpretation

Sl No.	Antibiotic employed	Code	Conc (µ)	ATCC25923*
1	Nitrofurantoin	Nf	300	18-22(<,<)
2	Fusidic acid	Fc	30	26-37(<,<)
3	Sparfloxacin	Sc	5	27-33(<,<)
4	Pristinamycin	Pm	15	23-29(<,<)
5	Penicillin (G)	P	10 IU	26-37(≥,≥)
6	Moxifloxacin	Mo	5	28-35(<,<)
7	Sulphafurazole	Sf	300	24-34(<,<)
8	Furazolidone	Fr	50	18-22(<,<)
9	Amoxycillin	Am	10	28-36(<,≥)
10	Sulphaphenazole	Sp	200	24-34(<,<)
11	Tricarcillin/Clavulanic acid	Tc	75/10	29-37(≥,<)
12	Furaxone	Fx	100	18-22(<,<)
13	Chloramphenical	C	30	19-26(≥,≥)
14	Gatifloxacin	Gf	10	27-33(<,<)
15	Clindamycin	Cd	2	24-30(<,<)

16	Cefaclor	Cj	30	27-31(**<**,<)
17	Clarithromycin	Cw	15	26-32(**<**,<)
18	Ceftriaxone	Ci	10	22-28(**<**,<)
19	Levofloxacin	Le	5	25-30(**<**,<)
20	Cephotaxime	Ce	30	25-31(**<**,<)
21	Tetracycline	T	30	24-30(**≤**,≥)
22	Linezolid	Lz	30	27-31(**<**,<)
23	Cefeprime	Cpm	30	23-29(**<**,<)
24	Erythromycin	E	15	22-30(**<**,≥)
25	Vancomycin	V	30	17-21(**<**,<)
26	Pipemedic acid	Pa	30	13-19(**≥**,≥)
27	Sulphamethizole	Sm	300	24-34(**<**,<)
28	Amikacin	Ak	10	18-24(**<**,<)
29	Teicoplanin	Te	30	15-21(**<**,<)
30	Trimethoprim	Tr	30	19-26(**<**,<)
31	Ciprofloxacin	Cf	10	27-35(**<**,<)
32	Netillin	Nt	10	22-31(**<**,<)
33	Tobramycin	Tb	10	19-27(**<**,<)
34	Gentamycin	G	50	25-33(**<**,<)
35	Streptomycin	S	10	14-22(**<**,≥)
36	Norfloxacin	Nx	10	17-28(**≥**,≥)
37	Methanamine mandalate	Me	3	14-22(**<**,≥)
38	Ampicillin (Cloxacillin)	Ax	10	35-37(**<**,<)
39	Cephatoxime	Ce	10	25-31(**<**,<)
40	Floxidin	Fl	20	25-30(**<**,<)
41	Fosfomycin	Fo	50	25-33(**<**,≥)
42	Framycetin	F	100	18-24(**<**,<)
43	Chlortetracycline	Ct	30	19-28(**≥**,≥)
44	Pefloxacin	Pf	5	24-28(**<**,<)
45	Piperacillin/Tazobactam	Pt	100/10	27-36(**<**,<)

**Staphylococcus aureus* Parentheses: Staph1 in bold & Staph2 in normal. The interpretation zone between the lower and upper ranges was drawn and below the mean expressed as <, above the mean as >, less than equal to mean as ≤ and more than equal to mean ≥.

Comparison of Staphylococcus isolates of the present study Staph I and Staph II to CLSI (Clinical Laboratory Standards Institute) standards

In comparison with CLSI standards both the test isolates were sensitive to Ticarcillin/Clavulanic acid, Clarithromycin, Levofloxacin, Linezolid, Fosfomycin,

Piperacillin/Tazobactam, intermediate to Clindamycin, Cefeprime and resistant to penicillin (G) and Vancomycin (Table 3). With other antibiotics the response varied between the cultures.

Table 3. Results of antibiotics sensitivity of Staph I and II isolates of the present study in comparison to CLSI (Clinical Laboratory Standards Institute) tested *Staphylococcus aureus* type cultures

Sl No	Antibiotic employed	Code	Conc(µ)	Zone of interpretation in mm			Test results	
				Sensitive	Intermediate	Resistant	Staph1	Staph 2
1	Pristinamycin	Pm	15	19	16-18	15	Intermediate	Sensitive
2	Penicillin (G)	P	10 IU	29	20-27*	28	Resistant	Sensitive
3	Moxifloxacin	Mo	5	24	21-23	20	Intermediate	Resistant
4	Ticarcillin/Clavulanic acid	Tc	75/10	24-30*	23	14*	Sensitive	Sensitive
5	Gatifloxacin	Gf	10	23	20-22	19	Sensitive	Resistant
6	Clindamycin	Cd	2	21	15-20	14	Intermediate	Intermediate
7	Clarithromycin	Cw	15	18	14-17	13	Sensitive	Sensitive
8	Levofloxacin	Le	5	19	16-18	15	Sensitive	Sensitive
9	Linezolid	Lz	30	21	NA	NA	Sensitive	Sensitive
10	Cefeprime	Cpm	30	18	15-17	14	Intermediate	Intermediate
11	Erythromycin	E	15	23	14-22	13	Intermediate	Sensitive
12	Vancomycin	V	30	15	NA	NA	Resistant	Resistant
13	Streptomycin	S	10	15*	12-14*	11*	Intermediate	Sensitive
14	Fosfomycin	Fo	50	16*	13-15*	12*	Sensitive	Sensitive
15	Piperacillin/Tazobactam	Pt	100/10	18	18-20*	17	Sensitive	Sensitive

*Standards not pertain to *Staphylococcus aureus* type cultures. NA: Not available. In case of Vancomycin no zone is seen (total resistance)

Both the test cultures are resistant towards vancomycin. The first clinical isolate of VISA was identified in 1997, and these strains have now been reported worldwide (Hiramatsu et al. 1997). Slackening in hygiene can lead to drug resistance in *S. aureus* (Livermore 2000) and fish and fish curing environs all the more the source of drug resistant staphylococci owing to the unhygienic conditions. One of the important ways to tackle this problem is continuous monitoring of fish and fish curing environments for antibiotic resistant staphylococci. More recently, there have been reports of VRSA, which is even more alarming, as these isolates demonstrate complete vancomycin resistance (Kacica and McDonald, 2004). Antibiotic susceptibility studies in *Staphylococcus aureus* are gaining importance due to emergence of vancomycin resistance strains all over the world. The mechanisms underlying vancomycin resistance are not yet fully understood, changes to the bacterial cell wall the site of action of the

glycopeptides are believed to be key. Recent evidence also supports the transfer of genetic material among bacteria as contributing to the development of VRSA. Based on the cases identified to date, risk factors for the development of VRSA may include old age, compromised blood flow to the lower limbs, and the presence of chronic ulcers (Appelbaum 2006). In the absence of surveillance programs and possible limitations of automated and non-automated detection methods, many cases of VISA and VRSA infection go undetected. In this regard it shall be noted that immunocompromised and under nourished fisher folk, especially pregnant, lactating women, and old aged groups are more vulnerable to these kinds of infections. Hence, knowledge of antibiotic susceptibility pattern of the isolates is important for future studies.

Acknowledgements

First author wishes to thank Dr. K Devadasan, Director, CIFT, Kochi, for permission to obtain guidance for M.Sc., Dissertation work from Burla Research Centre of CIFT. M.M. Prasad, wish to thank the Director, CIFT, for permission to present the work at 8th Asian Fisheries Forum, Kochi.

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Received: 31 December 2007; Accepted: 31 October 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Development of a Cell Culture System From Gill Explants of the Grouper, *Epinephelus malabaricus* (Bloch and Shneider)

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Abstract

A cell culture system was developed successfully from gill explants of the Malabar grouper, *Epinephelus malabaricus*. Gill tissue samples aseptically excised from healthy juveniles of *E. malabaricus* were explanted in Leibovitz' L-15 medium supplemented with 0.07 M NaCl and 20% fetal bovine serum (FBS). A mixture of different types of cells emerged from the explants, and these cells were observed to spread and attach to the culture flasks from the second day onwards. Confluent monolayers comprising epithelioid as well as fibroblast-like cells were formed within ten days. The cells were found to grow well at 28 $\pm$ 2°C. The cell monolayers were subcultured by trypsinization and seeded into new flasks, which produced confluent monolayers comprising predominantly epithelioid-like cells in subsequent passages.

Introduction

In vitro cell culture systems are necessary for the isolation and characterization of viruses, the development of diagnostic reagents, the testing of therapeutics, and the production of materials for immunological and vaccination studies. Tissue culture and the development of cell lines from fish are of priority interest for pathogen detection and for studies in toxicology, carcinogenesis, cellular physiology, and genetic regulation and expression.

Groupers are important fish group widely used for mariculture in many countries in the Asian region. In recent years, with the rapid development of intensive aquaculture industry, infectious viral diseases have severely affected many high-valued fish species, including grouper, causing heavy economic losses. Iridovirus and nervous necrosis virus (fish nodavirus) are the two newly emerging viral pathogens that have been isolated and identified as the most important pathogens infecting grouper in the last decade. Outbreaks of iridoviral and nodaviral diseases in grouper have been reported in many countries (Hegde et al. 2002; Qin et al. 2003).

The establishment of healthy and sensitive fish cell lines is essential for isolation,

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identification, and characterization of infectious viruses from fish. More than 150 fish cell lines have been developed for virus isolation and propagation (Fryer & Lannan 1994). However, most of these cell lines are derived from freshwater or anadromous fish species and are not sensitive to the newly emerging marine fish viruses. The limited number of reports on viruses from marine fish compared with those from freshwater fish is due to the shortage of fish cell lines derived from marine fish. The study on marine fish cell lines has developed rapidly in recent years and at least 17 cell lines from tissues of commercially important marine fish have been described since 1980 (Fernandez et al. 1993a).

In India, successful marine fish cell culture systems have been developed only from the Asian sea bass, *Lates calcarifer* (Sahul Hameed et al. 2006; Lakra et al. 2006; Parameswaran et al. 2006a, b). Because cell cultures derived from the same species or a species closely related to that in which the disease occurs would be the most sensitive for virus isolation, cell lines derived from local species should be given high priority. The host and tissue specificity of virus underlines the need for developing cell lines from different species in different regions (Cheng et al. 1993).

In this context, development of grouper cell lines, anticipating problems such as viral disease outbreaks is very important and in the present study, an attempt was made to develop a cell culture system from gill explants of the Malabar grouper, *Epinephelus malabaricus.*

Materials and Methods

Preparation of fish and tissue collection

For initiating primary culture from gill, healthy juveniles of the grouper, *E. malabaricus* (average weight 62 $\pm$ 5 g) collected from the coastal waters of Cochin were used. Fishes were acclimatized in circular fiberglass tanks (having *in situ* biological filtration system holding 300 l of well-aerated and dechlorinated sea water of 30–32% salinity) for a period of about two weeks on a diet of marine shrimp/fish meat. Fishes were subsequently transferred to rectangular perspex tanks (90 cm x 60 cm x 45 cm) holding 50 l of well-aerated and dechlorinated seawater of 30% salinity in the Fish Pathology Laboratory of the Central Marine Fisheries Research Institute.

The fishes were starved for two days prior to killing for dissecting out the tissues and were maintained overnight in sterile, aerated seawater containing 1000 IU mL^{-1} penicillin and 1000 µg mL^{-1} streptomycin. Before killing, the fishes were tranquilized by plunging in iced water for 5 min., disinfected by immersing in sodium hypochlorite (500 ppm available chlorine) for 5 min., washed in sterile seawater, and swabbed with 70% ethyl alcohol. The gill tissue was aseptically excised and collected in sterile petridishes holding Leibovitz' L-15 (GIBCO) medium (serum free) containing 500 IU mL^{-1} penicillin and 500 µg mL^{-1} streptomycin. Tissue pieces were minced into small fragments using a sterile surgical scalpel and again washed in serum-free medium containing 500 IU mL^{-1} penicillin and 500 µg mL^{-1} streptomycin.

Explantation

The tissue pieces were resuspended in 2 mL of growth medium containing 20% fetal bovine serum, (FBS) (PAN Biotech, Germany), 200 IU mL^{-1} penicillin, 200 µg mL^{-1} streptomycin, and 0.25 µg mL^{-1} amphotercin B and were subsequently transferred to 25 cm^2 tissue culture flasks and distributed uniformly, and the flasks were incubated at 28 ± 2°C for 4-5 h. The medium was replaced with L-15 medium (pH 7.2 ± 0.2) containing 20% FBS, 100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin, and 0.125 µg mL^{-1} amphotercin B and incubated at 28 ± 2°C. The tissue explants were observed for growth and formation of monolayer of cells using an inverted microscope (Nikon TS 100).

Subculture and maintenance

Once confluent monolayers were formed in primary culture, cells were dislodged from the flask surface by treatment with 0.25% trypsin (0.25% trypsin and 0.2% EDTA in PBS). Two milliliters of fresh growth medium (L-15 containing 20% FBS, 100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin, and 0.125 µg mL^{-1} amphotercin B) was then added to neutralize the action of trypsin. The detached cells were then split into two portions, transferred to new tissue culture flasks, and incubated at 28 ± 2°C.

Results and Discussion

Explants of gill tissue readily got attached to the culture flask on incubation. Primary cultures initiated from gill explants showed promising results. Emergence of different types of cells from the attached gill explants was observed within a day (Fig. 1). Cells were observed to spread and attach to the culture flask from the second day onwards (Fig. 2). Growth of the cells was very fast, and the cells formed a confluent monolayer comprising epithelioid as well as fibroblast-like cells within ten days (Fig. 3).

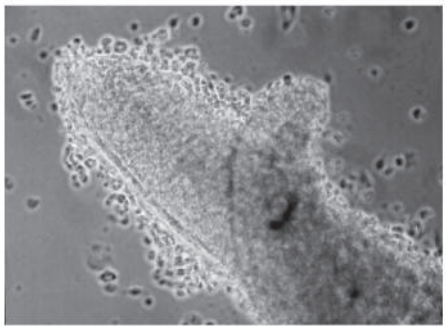

Figure 1. Cells emerging from the gill explants of *E. malabaricus* (X 100)

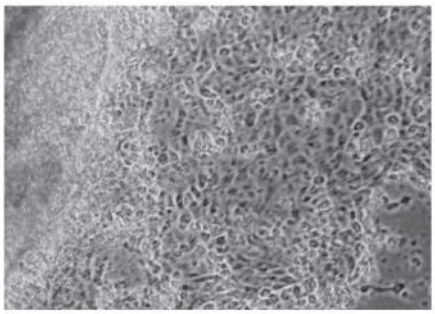

Figure 2. Spreading and attaching cells from the gill explant of *E. malabaricus* on day 2 post-explantation (X 100).

Trypsinization to detach the monolayer yielded individual cells along with cell clumps. The subcultured cells attached well to the flask surface and grew well (Fig. 4). The cell monolayers formed in the subcultured flasks (first passage) were successfully

harvested for passage by trypsinization, which produced confluent monolayers comprising predominantly epithelioid-like cells in subsequent subcultures (Fig. 5 and 6). The cell culture system developed has been successfully subcultured up to sixteenth passage level.

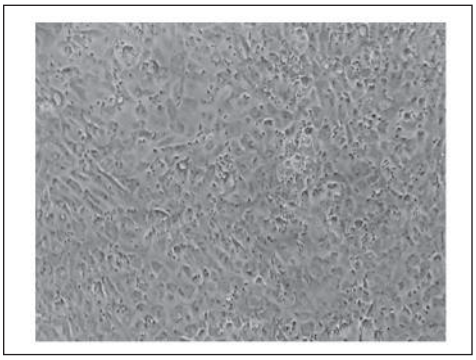

Figure 3. Cell monolayer formed from the gill explant of *E.malabaricus* in primary culture (X 100)

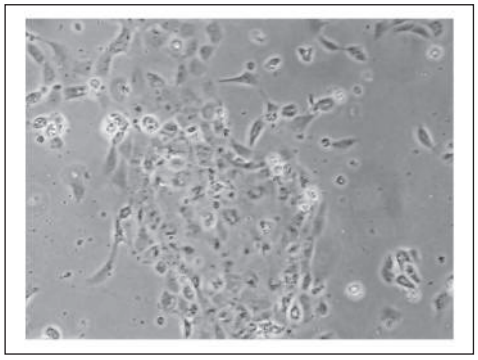

Figure 4. Attachment and formation of monolayer by the subcultured gill cells in the 1st passage (X 100)

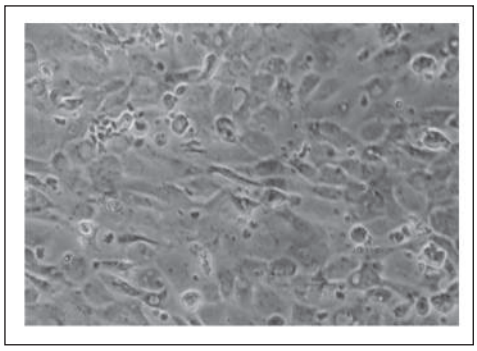

Figure 5. Complete monolayer formed by the subcultured gill cells in the 1st passage (X 100)

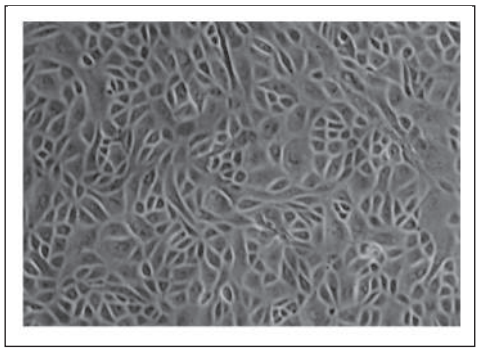

Figure 6. Cell culture system from gill explant of *E. malabaricus* at 6th passage (X 100)

In the present work, primary cell culture was developed from gill of *E. malabaricus* by means of explant technique, which has many advantages over the use of cell suspensions, such as speed, ease, maintenance of cell interactions and the avoidance of enzymatic digestion which can damage the cell surface (Parkinson & Yeudall 1992; Avella et al. 1994). Explants *in vitro* are supposed to be structurally closer to the organ *in vivo* than cultures obtained using cell suspensions.

In the present study, the Leibovitz's L-15 medium supplemented with 20% FBS supported good growth of the cells from gill tissue of *E. malabaricus*. The suitability of L-15 in supporting fish cell lines compared with that of other media has been documented by Fernandez et al. (1993a) when they compared the growth of many fish cell lines in different culture media at different temperature and sodium chloride concentrations.

Leibovitz medium was designed to maintain pH in the physiological range under normal atmosphere without added CO_2. Several researchers have studied the suitability of various mammalian and insect cell culture media and the required supplements for growth of fish cells. Among the various media tested [Leibovitz' L-15, Medium 199 (M-199) and Eagle's minimum essential medium (Eagle's MEM)], L-15 was found to be most suitable for the attachment and proliferation of cells (Lakra et al. 2005). Faster growth and better proliferation was noticed in cells cultured with L-15 medium at pH 7.4. The use of FBS at levels above 10% has been recommended for primary cultures, as well as for the initial passages, whereas an optimal concentration of 5% is enough in later stages (Sahul Hameed et al. 2006; Parameswaran et al. 2006a, b).

Lakra et al. (2006) observed optimum growth and attachment of sea bass caudal fin cell culture systems using 20% FBS and 1% fish serum. Homologous fish muscle extract and prawn muscle extract have been used for successful development of fish cell culture systems (Kumar et al. 1998; Lakra et al. 2006). In the present study, a successful primary culture was obtained using the tissue culture medium L-15 supplemented with 20% FBS without using fish serum/fish muscle extract. In general, growth and development of cell monolayer from gill tissue explants were good and easy to maintain.

Cells from the gill tissue of *E. malabaricus* grew well in L-15 with additional NaCl (0.07 M NaCl), which is needed for marine fish cells (Clem et al. 1961; Law et al. 1978; Li et al. 1984; Fernandez et al. 1993a). Clem et al. (1961) were the first to establish monolayer cell cultures from marine teleosts and obtained best results in commercial medium modified with 0.07 M NaCl. The JSKG cell line established from gonads of Japanese striped knife jaw, *Oplegnathus fasciatus* and PAS cell line from skin of purplish amberjack, *Seriola dumerili* were initiated at a higher NaCl concentration of 0.206 M but gradually adapted to a low NaCl concentration of 0.116 M after several subcultures (Fernandez et al. 1993 b). However, several authors have reported development of cell lines from marine fish without using increased NaCl concentrations in the cell culture medium (Chong et al. 1990; Chew-Lim et al. 1994; Chang et al. 2001).

Sahul Hameed et al. (2006) reported that for the establishment of SISK cell line from sea bass kidney, additional NaCl was not necessary. Similarly, the SISS cell line developed from spleen of Asian sea bass has good adaptation for growth in Leibovitz's L-15 without special requirements, such as NaCl addition (Parameswaran et al. 2006b).

In the present study, the gill tissue of *E. malabaricus* epithelial cells and fibroblast-like cells coexisted in the primary culture. However, as the culture progressed, epithelioid cells predominated in the subsequent subcultures. Chi et al. (1999) reported presence of both epithelial cells and fibroblast-like cells in primary culture of grouper fin (GF-1) cells. However, they reported that in subsequent subcultures, the fibroblast-like cells proliferated more rapidly than the epithelial cells and ultimately predominated. A similar morphological change has also been observed in orange spotted grouper *E. coicoides*

fin (GF-1) (Chi et al. 1999) and spleen (Qin et al. 2006) cells and in yellow grouper *E. awoara* fin (GF) and heart (GH) cells (Lai et al. 2003).

Many serum factors derived from platelets have a strong mitogenic effect on fibroblasts and also tend to inhibit epithelial proliferation, subsequently causing fibroblasts to overgrow in subcultures (Freshney, 1994). In general, a predomination of fibroblastic cells over epithelioid cells in cell cultures from fish has been reported (Lai et al. 2003). Production and maintenance of epithelioid cell line is reported to be comparatively difficult (Wang et al. 2003). However, in the present study, although epithelioid as well as fibroblast-like cells were present in the primary culture, epithelioid-like cells predominated as the culture progressed. The SF cell line developed from Asian sea bass fry consisted of both epithelial-like and fibroblast-like cells in initial subcultures. However, once the culture progressed, the predominant cell type was epithelial-like cells with small groups of fibroblast-like cells (Chang et al. 2001). Sahul Hameed et al. (2006) also observed both epithelial-like and fibroblast-like cells in initial subcultures and observed presence of only epithelial-type cells after 20 subcultures of the SISK cell line developed from sea bass kidney.

The results of the present study have clearly demonstrated good growth and formation of confluent monolayer of cells from gill tissue explants of *E. malabaricus*, which has been successfully subcultured. Gill tissue appears to be ideal for cell culture as it is easy to collect for use in cell culture. Hence, there is scope and prospect for development of cell line from gill tissue of *E. malabaricus.*

Acknowledgement

The authors express their deep sense of gratitude to Prof. (Dr.) Mohan Joseph Modayil for the facilities provided to carry out the study.

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Received: 31 December 2007; Accepted: 27 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Effect of Feeding Frequency on Growth Performance, Feed Efficiency and Bioenergetics of Golden Mahseer Early Fry

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Abstract

In the upland regions of India, the indigenous golden mahseer *Tor putitora* (Hamilton), is well recognized as one of the important game as well as food fish and for that reason in commercial fisheries too, it should occupy an important position. In the past, there has been a great decline in the fishery of golden mahseer and is now feared to be endangered. Thus, development of fisheries of this fish in impoundment waters will play a significant role in economy of the country because high mortality is associated with first feeding of golden mahseer in controlled conditions of hatchery. To enhance the growth of golden mahseer under culture systems, it becomes necessary to know about its feeding regime. Feed accounts major portion of the cost of fish culture system. A trial was conducted, to establish optimum feeding frequency, for rearing early fry of golden mahseer. The influence of feeding frequency on survival, growth performance, feed efficiency, protein efficiency ratio (PER), meal size and bioenergetic parameters was studied in the early fry of golden mahseer. Fish were stocked in tanks with flow through water system keeping three replicates for each treatment. Feeding was carried out four times a day, three times a day, two times a day and once a day for a period of 45 days. Frequency of feeding was found to significantly influence the growth parameters, feed efficiency, meal size and the bioenergetic parameters. Net weight gain, percent weight gain and specific growth rate (SGR) were significantly higher ($p < 0.05$) in fish fed three times a day than those fed one, two and four times a day. Feed conversion ratio (FCR), feed conversion efficiency (FCE) and PER were significantly better ($p < 0.05$) in fish fed three and four times a day compared with those fed one and two times a day. Meal size of 70% was significantly higher ($p < 0.05$) for fish fed three times a day compared to those fed one, two and four times a day. Fish fed three times a day had higher feeding rate 148.93 $Jg^{-1}.day^{-1}$, absorption rate 142.12 $Jg^{-1}.day^{-1}$ and absorption efficiency 95.43 $Jg^{-1}.day^{-1}$ compared with those fed with all other feeding frequencies. However, the percentage survival was independent of the treatments. This suggests that the best feeding frequency for golden mahseer early fry is three times a day.

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Introduction

Considered as one of the popular game fish with respected characteristics as a food fish, the golden mahseer *Tor putitora* (Hamilton) forms fisheries in the cold waters of Central Himalayan region of India. The coldwater regions of India previously could not be brought under the purview of aquaculture developments owing to various inherent problems related to degradation of aquatic habitat by anthropogenic activities, lack of scientific investigations pertaining to fish species, etc. Fortunately, in recent years, in support of golden mahseer, the biological investigations had already commenced and commendable success on production of stocking material through artificial fecundation have been achieved, and attempts are being made to assess their culture feasibilities (Tripathi 1977; Kohli et al. 2005; Joshi et al. 2007). Golden mahseer, an indigenous coldwater fish with desirable characteristics such as amenability to culture in captivity, capacity to accept supplementary feed and ability to tolerate wide range of environmental parameters, forms an interesting candidate for aquaculture in coldwater regions of India (Cordington 1939; Jhingran & Sehgal 1978; Chauhan et al. 2007). Thus the development of fisheries of this species will play a significant role in country's economy.

One of the major developmental constraints for golden mahseer aquaculture is lack of proper knowledge of larval growth and survivorship to the juvenile stage. The main constraints to intensify golden mahseer culture are the unpredictable and usually poor survival and growth rates of start feeding fry. Among the factors, which influence survival and growth, feeding could be considered as most relevant. To produce golden mahseer most efficiently, it is important to use appropriate diet as well as feeding strategies that will maximize growth rate and feeding efficiency. Nutritional research on golden mahseer has mainly focused on the development of diet (Mohan & Basade 2005; Keshavnath et al. 2007); less attention has been given to the practical issues of feed management. Feed management in aquaculture includes feed size, feeding rate, timing of feeding and feeding frequency (i.e. number of feedings per day). Among these issues, feeding frequency has been described for several species as a major factor influencing growth characteristics (Boujard & Leatherland 1992; Lee et al. 2000; Bolliet et al. 2001; Sanchez-Muros et al. 2003). The optimal number of feedings per day of a fixed daily ration may depend on species, age or size, environmental factors or food quality (Goddard 1996). Some researcher's findings demonstrate that the lowest food conversion and maximum growth rates can be achieved at the optimal feeding frequency (Andrews & Page 1975; Siraj et al. 1988). Feeding frequency, however, may affect food conversion, variability in fish size and loss of water-soluble nutrients from feed (Piper 1982). Suitable feeding frequency also affects the growth, survival and condition factor

of fish (Pfeiffer & Lovell 1990; Kayano et al. 1993; Vega et al. 1994; Sager & Winkelman 2006).

The aim of this study was to evaluate the effects of feeding frequency on the growth performance, survival, feed efficiency and bioenergetic parameters of golden mahseer early fry reared in flow through hatchery system.

Materials and Methods

Study site and experimental design

Golden mahseer fry produced at the Mahseer Seed Production Unit, Directorate of Coldwater Fisheries Research, Bhimtal, were procured for the study, and the trials were conducted at the same site. The test fish were treated with potassium permanganate and acclimatized to the feed and the rearing conditions before start of the experiment. The fry were randomly stocked in 12 experimental tanks (1.0 x 1.0 x 0.45 m; water depth 0.3 m) having flow through water system.

Feed and feeding

Fish were fed with the formulated feed (40% protein and 15% lipid) at a rate of 10% of biomass per feeding. Feed was delivered 1 (at 08:00 hours), 2 (0800 and 17:00 hours), 3 (08:00, 13:00 and 17:00 hours) and 4 (08:00, 1100, 14:00, 17:00 hours) times a day. Feed was given in circular feeding trays, one tray per unit. Daily at each feeding time, the uneaten feed was collected for weighing, and then the trays were rinsed and cleaned before placing the next feed. Faeces were collected by siphoning once in a day before adding fresh water. Feed rations were adjusted weekly, according to fish biomass and survival.

Chemical analysis

The biochemical composition of the experimental diets, test fish and faeces was analyzed using AOAC (Association of Analytical Chemists) (1995) methods in terms of dry matter, crude protein, crude fat, crude fibre, ash and nitrogen free extract. Gross energy content was calculated by applying standard conversion factors for fat (39.54 $k.Jg^{-1}$), protein (20.08 $k.Jg^{-1}$) and carbohydrates (17.15 $k.Jg^{-1}$) as recommended by Brett & Groves (1979).

Growth performance, feed efficiency and bioenergetic parameters

The average total length and body weight of fish were recorded initially and then subsequently at regular weekly intervals. On conclusion of the trial, the final total length

and body weight attained by the fish were recorded separately for all the replicates of each treatment. Growth performance, feed efficiency, survival and bioenergetic parameters (Charles et al. 1984) were determined in terms of net weight gain, percent weight gain, SGR, FCR, FCE, PER, meal size, feeding rate, absorption rate, absorption efficiency, conversion rate, conversion efficiency, metabolic rate and survivability.(SGR: specific growth rate = (Final weight – Initial weight)/days x 100. FCR: feed conversion ratio = Feed intake/Weight gain. FCE: feed conversion efficiency = (Weight gain/Feed intake) x 100. PER: protein efficiency ratio = Weight gain/Protein intake. Meal size (percentage of food consumed per feeding) = (Amount of food offered – Amount uneaten)/Amount of food offered x 100. Feeding rate = Energy consumed ($J.day^{-1}$)/ Initial live weight of fish (g). Absorption rate = Energy consumed – Energy of faeces ($J.day^{-1}$)/Initial live weight of fish (g). Absorption efficiency = Energy consumed – Energy of faeces (J)/Energy consumed (J) x 100. Conversion rate = Initial energy content of fish – Final energy content of fish ($J.day^{-1}$)/Initial live weight of fish (g). Conversion efficiency = Initial energy content of fish – Final energy content of fish (J)/Energy consumed (J) x 100. Metabolic rate = Absorption rate – Conversion rate.

Water quality management

Water quality parameters were monitored at regular weekly intervals as per standard methods (APHA 1998), and temperature being a crucial factor was monitored twice daily, in morning and evening throughout the experimental period. Water temperature ranged from 10-12^{o}C, pH 7.0-7.2, dissolved oxygen 8.2-8.6 $mg.L^{-1}$, free carbon dioxide 0-1.2 $mg.L^{-1}$, total alkalinity 90-96 $mg.L^{-1}$ and water flow rate was maintained at 0.5-1.0 $L.min^{-1}$.

Statistical analysis

One-way analysis of variance technique was used to test the difference between treatment means for the different feeding frequencies studied, and when significant difference ($p < 0.05$) was observed between treatments, further analysis was carried out using multiple range test at 5% level of significance (Snedecore & Cochran 1994).

Results

The growth performance, feed efficiency, bioenergetic parameters and survivability of golden mahseer fry increased with increase in feeding frequency from one meal per day to three meals per day and with further increase in feeding frequency to four meals per day the above parameters decreased (Table 1).

Table 1. Effect of feeding frequency on growth performance, feed efficiency and bioenergetic parameters of golden mahseer early fry

Parameters	No. of meals day^{-1}			
	1	2	3	4
Initial weight (mg)	191.00 ± 0.58	194.33 ± 0.33	191.00 ± 1.00	193.67 ± 0.33
Final weight (mg)	307.00 ± 3.15^{a}	330.5 ± 1.22^{b}	376.33 ± 0.88^{c}	370.67 ± 0.67^{c}
Net weight gain (mg)	116.00 ± 3.61^{a}	136.00 ± 1.00^{b}	185.33 ± 1.76^{c}	177.00 ± 0.58^{d}
Percent weight gain (%)	60.75 ± 1.94^{a}	69.98 ± 0.58^{b}	97.05 ± 1.40^{c}	91.39 ± 0.34^{d}
SGR (% day^{-1})	1.58 ± 0.04^{a}	1.77 ± 0.01^{b}	2.26 ± 0.03^{c}	2.16 ± 0.01^{d}
FCR	3.87 ± 0.10^{a}	3.76 ± 0.02^{a}	3.25 ± 0.03^{b}	3.22 ± 0.01^{b}
FCE (%)	25.89 ± 0.64^{a}	26.58 ± 0.15^{a}	30.78 ± 0.29^{b}	31.02 ± 0.09^{b}
PER	0.64 ± 0.01^{a}	0.65 ± 0.003^{a}	0.77 ± 0.01^{b}	0.76 ± 0.00^{b}
Meal size (%)	60.03 ± 0.04^{a}	65.01 ± 0.003^{b}	70.00 ± 0.003^{c}	67.49 ± 0.003^{d}
Feeding rate (Jg^{-1}·day^{-1})	112.06 ± 0.84^{a}	125.71 ± 0.28^{b}	148.93 ± 0.70^{c}	140.88 ± 0.16^{d}
Absorption rate (Jg^{-1}·day^{-1})	103.33 ± 1.27^{a}	117.99 ± 0.53^{b}	142.12 ± 0.67^{c}	133.65 ± 0.32^{d}
Absorption efficiency (%)	92.21 ± 0.45^{a}	93.86 ± 0.25^{b}	95.43 ± 0.20^{c}	94.87 ± 0.20^{c}
Conversion rate (Jg^{-1}·day^{-1})	2.31 ± 0.07^{a}	2.86 ± 0.02^{b}	4.26 ± 0.06^{c}	3.64 ± 0.01^{d}
Conversion efficiency (%)	4.90 ± 0.15^{a}	5.45 ± 0.04^{b}	6.82 ± 0.06^{c}	6.32 ± 0.02^{c}
Metabolic rate (Jg^{-1}·day^{-1})	101.14 ± 1.09^{a}	115.12 ± 0.51^{b}	137.86 ± 0.61^{c}	130.01 ± 0.31^{d}
Survival (%)	99.11 ± 0.44^{a}	98.67 ± 0.77^{a}	99.56 ± 0.44^{a}	98.67 ± 0.00^{a}

Data represent the mean ± SEM of three replicates. Values on the same line with different superscripts are significantly different ($p < 0.05$).

Net weight gain, percent weight gain and SGR were significantly higher ($p < 0.05$) in fish fed three times a day compared with those fed one, two and four times a day. FCR, FCE and PER showed no significant differences ($p > 0.05$) in fish fed three times and four times a day; however, these values were significantly better ($p < 0.05$) than fish fed one and two times a day. Meal size (% feed consumed per feeding) was also significantly higher ($p < 0.05$) in fish given feed three times a day compared with those fed one, two and four times a day. In fish fed three times a day, feeding rate (148.93 ± 0.70 $Jg^{-1}.day^{-1}$), absorption rate (142.12 ± 0.67 $Jg^{-1}.day^{-1}$) and conversion rate (4.26 ± 0.06 $Jg^{-1}.day^{-1}$) were significantly more ($p < 0.05$) than that for fish fed at all other feeding frequencies. Consequently, metabolic rate (137.86 ± 0.61 $Jg^{-1}.day^{-1}$) was also significantly greater ($p < 0.05$) in fish fed three times a day compared with those fed one, two and four times a day. Although the absorption efficiency and conversion efficiency showed no significant difference ($p > 0.05$) in fish fed three and four times a day, but they were significantly higher ($p < 0.05$) than those fish fed one and two times a day. Percentage survival was found to be independent of the treatment effects.

Discussion

In aquaculture, mostly the fishes are fed more than one meal per day (Thomassen & Fjaera 1996), and research on feeding frequency, although limited, has shown that increased feeding frequency results in increased growth rates (Charles et al. 1984; Tsevis et al. 1992; Hung & Storebakken 1994; Wang et al. 1998; Charles et al. 2006). Also in this study, significant increase in net weight gain, percent weight gain, SGR was observed with increase in feeding frequency from one meal per day to three meals per day. But with further increase in feeding frequency up to four meals per day, the growth parameters assessed were observed to decrease significantly compared with fish fed three meals per day. Investigations on other fish species have similarly revealed that growth generally increases with feeding frequency up to a given limit (Andrews & Page 1975; Grayton & Beamish 1977; Siraj et al. 1988; Tsevis et al. 1992; Wang et al. 1998; Charles et al. 2006). This suggests that three meals per day is the optimum feeding frequency for golden mahseer early fry. Optimum feeding frequency for different fish species varies. Growth rates were higher for common carp, *Cyprinus carpio* (Charles et al. 1984), rainbow trout, *Oncorhynchus mykiss* (Ruohonen et al. 1998) and tambaqui, *Colossoma macropomum* (Silva et al. 2007) when fed three meals per day. The most favourable feeding frequencies reported for various fish are one meal per day for *Channa striatus* (Sampath 1984), two meals per day for channel catfish, *Ictalurus punctatus* (Andrews & Page 1975), and juvenile sunshine bass, *Morone chrysops* x *M. saxatilis* (Webster et al. 2001), four meals per day for striped bass, *Morone saxatilis* (Powell 1973), six meals per day for ayu larvae, *Plecoglossus altivelis* (Cho et al. 2003), continuous feeding for African catfish, *Clarias lazera* (Hogendoorn 1981) and once every 48 hours for young grouper, *Epinephelus tauvina* (Chua & Teng 1978).

Dividing total daily feed, of 10% of body weight, into three meals probably increased the nutrient absorption capacity of fish from feed, as fish had access to nutrients more often during the day (Silva et al. 2007). Further it was observed that feeding frequency was strongly influenced by the time of gastric evacuation (Riche et al. 2004). Nile tilapia, *Oreochromis niloticus*, had an appetite 4 hours after food was offered; therefore a feeding management that offers meals every 4 hours is the best strategy for that species growth. In affirmation to this study, Silva et al. (2007) also observed that in tambaqui feeding frequency of three meals per day, provided at a median interval of 4 hours between meals during day light, offered better growth when food supplied on each meal was close enough to fish satiety, for example, 10% of body weight per day. In this study too when feed was given at 10% of body weight per day by 4 hour intervals with a feeding frequency of three meals per day during the day time, golden mahseer early fry grew better.

Feeding frequency had a significant effect on food consumption of golden mahseer early fry. The FCR, FCE and PER values were significantly better for golden mahseer that were fed at higher feeding frequencies of three to four meals per day than those fed at one to two meals per day. Meal size too increased with increase in feeding frequency being significantly more in fish fed three meals per day and reduced significantly with further increase in feeding frequency to four meals per day. Likewise Kayano et al. (1993) described lower FCR values for red-spotted grouper when fed at higher feeding frequencies, and Charles et al. (1984) accounted lower FCR in common carp fed at increased feeding frequencies. In other fish species too, food consumption was found to increase with feeding frequency but up to a given limit (Andrews & Page 1975; Grayton & Beamish 1977; Siraj et al. 1988; Tsevis et al. 1992; Wang et al. 1998; Charles et al. 2006).

Food intake is governed by hunger level or satiation level, which in turn depends on the amount of food remaining in the stomach (Brett 1971; Pandian 1975). *C. carpio* consumed maximum amount of feed when fed after deprivation of 8 hours; however, when fed after deprivation of 12, 24, 48 and 72 hours, the feed consumption decreased (Charles et al. 1984). Such a partial compensation for the infrequent meal was also observed in the catfish, *Heteropneustes fossilis* (Marian et al. 1982). In the sunfish, *Lepomis macrochirus*, Windell (1967) found that because of degenerative changes in the pyloric caecae, prolonged starvation decreased the food intake. When feeding frequency increased above three meals per day, the total intake of food per feeding dropped considerably due to the limited capacity of the stomach as most of the food in the stomach remained undigested (Charles et al. 1984). Moreover, frequent feeding forces food through the alimentary canal more quickly and causes incomplete digestion (Dawes 1930).

In this study, all the bioenergetic parameters, namely feeding rate, absorption rate, absorption efficiency, conversion rate, conversion efficiency and metabolic rate, were significantly affected by feeding frequency and were found to increase significantly with increase in feeding frequency up to three meals per day. However, at a higher feeding frequency of four meals per day compared with the optimum feeding frequency of three meals a day, feeding rate, absorption rate, conversion rate and metabolic rate decreased significantly, whereas absorption efficiency and conversion efficiency although declined but not significantly. Some researchers observed that in *C. carpio* (Charles et al. 1984), *C. striatus* (Sampath 1984) and *H. fossilis* (Marian et al. 1982), feeding frequency significantly influenced all the bioenergetic parameters except for absorption efficiency. Frequent feeding no doubt increased food intake and conversion; however, there was always a limit for intensive feeding (Charles et al. 1984). Because food consumption, growth and all the bioenergetic parameters were not significantly enhanced by increasing the number of meals from three to four times per day, as a result a feeding frequency of three meals per day at the ration size of 10% of body weight seems to be sufficient for maximal growth of the early fry of golden mahseer.

This study comprehends that manipulating feeding frequency can be an effective strategy of feed management for rearing of golden mahseer early fry, in view of the fact that feeding frequency significantly influenced their growth performance, feed efficiency and bioenergetics. The growth parameters – net weight gain, percent weight gain and SGR; the feed efficiency FCR, FCE, PER and meal size; and the bioenergetic parameters – feeding rate, absorption rate, absorption efficiency, conversion rate, conversion efficiency and metabolic rate were observed to be superior in case fish fed daily feed scheduled 10% of body weight (to satiation) divided into three meals. This demonstrates that golden mahseer early fry most efficiently used the feed when fed three times a day to satiation. Suggesting that for golden mahseer early fry feeding frequency of three meals per day is the most advantageous to achieve better growth performance with optimum feed efficiency under flow through rearing conditions.

Acknowledgement

The authors are thankful to the Director, Directorate of Coldwater Fisheries Research, Bhimtal, for constant encouragement and providing the facilities to conduct the study.

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Received: 23 November 2007; Accepted: 22 January 2009

Asian Fisheries Science **22** (2009): 561-567

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Effect of Different Substrata on the Growth and Survival of Green Mussel *Perna viridis* in Raft Culture at Ratnagiri (India)

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Abstract

The rens from two substrata *viz.*, nylon rope (2.5 m length and 24 mm diameter) and nylon strip (1.5 m length and 5 cm width) were prepared and suspended from wooden raft (6.32 x 2.7 m), anchored at depths 3.5-4.0 m in the Kalbadevi estuary (10°59'1''N and 73°10'25''E) at Ratnagiri. The seeds of *Perna viridis* with an average initial shell length of 31.1 ± 0.08 and 31.2 ± 0.07 mm were seeded in nylon rope and nylon strip rens, respectively. In this experiment, 10 replicates were used for each ren. The green mussel attained the shell length of 67.3± 0.7 and 65.5 ± 0.62 mm in 7 months for nylon rope and nylon strip rens, respectively. The average survival of 54.09% with an average production of 11.32 $kg.m^{-1}$ was obtained in nylon rope, whereas average survival of 35.26% with average production of 9.12 $kg.m^{-1}$ was obtained for nylon strip rens in 7 months. The growth of mussels on nylon rope and nylon strip ren was not significant, but survival and production of mussels were significantly higher in nylon rope compared with nylon strip ren during raft culture.

Introduction

Coir and other natural fibre ropes have been used extensively in mussel farming (Andreu 1968; Mason 1969; Maclean 1972). Their success has been attributed to their hairy and creviced nature. Most natural fibre ropes have a comparatively short life in seawater. Therefore, a synthetic substitute was used in the mussel farming industry.

In India, for culture of green mussel (*Perna viridis*, Mytilidae), coir rope (12 mm thickness) and nylon rope (14 mm thickness) was used by Appukuttan et al. (1998), whereas 14 mm diameter nylon rope and 20-25 diameter coir rope were used by Kuriakose (1980b). Parulekar (1980) used the strip of cotton mosquito curtain cloth (3 m length and 35 cm width) for attachment of mussel seeds on nylon rope (12 mm diameter) in raft culture. Ranade & Ranade (1980) used nylon ropes and coir ropes for the attachment of mussel seed. Narasimham (1980) observed high growth rate of green mussels on nylon ropes than natural beds.

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However, no study on performance of nylon rope and nylon strip has been carried out. Therefore, this paper describes the efficiency of these materials for growth, survival and production of mussels in raft culture.

Materials and Methods

Study area

Experiment was conducted from February 2002 to September 2002 at Kalbadevi estuary, Ratnagiri (10°59'1''N and 73°10'25''E) situated in Maharashtra state, along the West coast of India. The raft was anchored in the site protected from the strong winds and currents. The site had bottom of muddy sediments with mean depth of about 3.54 m at low tides.

Raft

The rectangular wooden floating raft (6.32 x 2.7 m) towed to a selected site with dugout canoe and moored at water depth of 3.5 m.

Collection of mussel seeds

Green mussel seeds were collected from Bhatye creek, about 8 km away from culture site. Mussel seeds were collected by hand picking method during the low tides in the month of February 2002. They were transported in wet gunny bags. Seeds were washed with fresh seawater to eliminate sand, detritus, predators, possible competitors and dead mussels before seeding.

Ren

Two types of ren materials *viz.*, nylon rope and nylon strip were used as substrata for the attachment of mussel seeds. Ten replications were used for each type of material.

Nylon rope ren

Each ren was prepared using the nylon rope of 2.5 m length and 24 mm diameter. The nylon rope was placed over the nylon net (mesh size = 18 mm) by leaving 0.7 m on one end and 0.3 m on another end for attaching the weight. Both ends of the nylon netting were tied to nylon rope using nylon thread. Mussel seeds were placed around the rope, and nylon netting was stitched to form the tube. At distal end of ren, stone ballast was attached to prevent it from becoming tangled with others.

Nylon strip ren

Each ren was prepared using nylon strip of 1.5 m length with 50 mm width. The nylon rope (3.5 m length with 4 mm diameter) was passed through nylon strip at regular interval of 30 cm in zigzag manner. Nylon strip along with nylon rope was placed on net

material. Both ends of nylon rope and nylon strip were tied with the net using nylon thread, leaving 1.2 m length of nylon rope at one end for suspending from raft and 0.3 m for attachment of stone at its distal end.

Seeding

Mussel seeds of an average initial shell length of 31.1 ± 0.08 and 31.2 ± 0.07 mm and average initial live weight of 2.51 ± 0.13 and 2.63 ± 0.09 g were seeded in nylon rope and nylon strip rens, respectively. Mussels were seeded at the rate of 1.67 $kg.m^{-1}$ for each ren.

Sampling

Samples were randomly collected from each replicate at interval of every month. The length was measured using vernier caliper to the nearest of 0.01 mm. Weight was recorded using 'Shimadzu' weighing balance with 0.01 g accuracy. Shell length was measured from the tip of the umbo to the posterior margin of the shell, shell height was measured in the greatest dorsoventral direction, and shell width was measured from side to side in the broadest region. Total live weight was recorded by weighing whole individual mussel; shell weight was recorded by removing meat and water from shell cavity; meat weight was recorded by removing meat from shell cavity, and dry meat weight was obtained from the soft tissue dried to a constant weight at 80°C using 'TEMPO' thermostat.

The initial and final numbers of mussels were recorded from each ren for estimation of survival. The mussel production (P) per meter rope was calculated using the following formula given by Karayucel & Karayucel (1999).

$$P = [N_t + N_{(t+1)} \times 2^1] \times (W_{(t+1)} - W_t)$$

where N_t is the number of mussel per meter of rope at the time of seeding, $N_{(t+1)}$ is the number of mussel per meter of rope at the time of harvest, W_t is the mean live weight at the time of seeding, and $W_{(t+1)}$ is the mean live weight at the time of harvest.

Water parameters

Water parameters such as water temperature, conductivity, total dissolved solids, pH, dissolved oxygen, carbon dioxide, salinity, total hardness, nitrite-nitrogen, nitrate-nitrogen, orthophosphate and silica were analysed by every 15 days during culture period following standard methods (APHA 1998).

Statistical analysis

Data on growth parameters, survival and production were analysed by Student's *t*-test (Snedecor & Cochran 1967).

Results

Water parameters

Water parameters such as temperature, conductivity, total dissolved solids, pH, dissolved oxygen, carbon dioxide, total hardness, nitrite-nitrogen, nitrate-nitrogen, orthophosphate and silica ranged from 26.5 to 30.5°C, 43.4 to 58.4 MS, 21.7 to 29.7 $g.L^{-1}$, 7.44 to 7.83, 4.8 to 5.8 $mg.L^{-1}$, 2.82 to 7.15 $mg.L^{-1}$, 6132 to 8001 $mg.L^{-1}$, 0.0079 to 0.025 $mg.L^{1}$, 0.425 to 1.1428 $mg.L^{-1}$, 0.0351 to 0.06 $\mu g.L^{-1}$ and 14 to 39.7 $\mu g.L^{-1}$, respectively. Salinity was fluctuating from 35 $g.L^{-1}$ in summer months (May-June, 2003) to 8 $g.L^{-1}$ in monsoon months (July-August, 2003).

Growth parameter

Average shell length, shell height, shell width, live weight, shell weight, wet meat weight and dry meat weight of green mussels reared for 7 months on nylon rope and nylon strip are given in Table 1. The growth parameters of the mussels reared on both the substrata show no significant difference ($p > 0.05$).

Table 1. Growth and survival of green mussel, cultured using different substrata in raft culture

	Parameters	Substrata	
		Nylon rope	Nylon strip
a.	Shell length		
	Initial average shell length (mm)	31.1 ± 0.81	31.2 ± 0.71
	Final average shell length (mm)	67.3 ± 0.70	65.5 ± 0.62
	Shell length increment (mm)	36.2	34.3
b.	Shell height		
	Initial average shell height (mm)	16.8 ± 0.39	16.7 ± 0.37
	Final average shell height (mm)	34.4 ± 0.45	33.7 ± 0.45
	Shell height increment (mm)	17.6	17.0
c.	Shell width		
	Initial average shell width (mm)	8.0 ± 0.21	8.1 ± 0.57
	Final average shell width (mm)	22.0 ± 0.47	21.6 ± 0.43
	Shell width increment (mm)	14.0	13.5
d.	Live weight		
	Initial average live weight (g)	2.52 ± 0.13	2.59 ± 0.11
	Final average live weight (g)	25.80 ± 0.81	23.89 ± 0.53
	Live weight gain (g)	23.28	21.30

e.	Shell weight		
	Initial average shell weight (g)	1.30 ± 0.11	1.36 ± 0.10
	Final average shell weight (g)	12.23 ± 0.45	10.96 ± 0.53
	Shell weight increment (g)	10.93	9.60
f.	Wet meat weight		
	Initial average wet meat weight (g)	0.61 ± 0.02	0.64 ± 0.03
	Final average wet meat weight (g)	7.53 ± 0.23	7.00 ± 0.11
	Wet meat weight increment (g)	6.92	6.36
g.	Dry meat weight		
	Initial average dry meat weight (g)	0.093 ± 0.03	0.095 0.011
	Final average dry meat weight (g)	1.26 ± 0.11	1.114 ± 0.07
	Dry meat weight increment (g)	1.167	1.029
h.	Survival		
	Initial average number per meter	635 ± 1.39	633 ± 1.39
	Final average number per meter	343.5 ± 12.11	223.2 ± 9.99
	Average percentage survival	54.09 ± 1.93	35.26 ± 1.56
i.	Production ($kg.m^{-1}$)	11.39 ± 0.14	9.12 ± 0.11

Survival

The nylon rope substrata resulted in significantly higher ($p < 0.05$) average survival of 54.09 ± 1.93% compared with the nylon strip substrata which yielded average survival of 35.26 ± 1.56% in 7 months.

Production

The mussel productions at the rate of 11.39 ± 0.14 $kg \times m^{-1}$ and 9.12 ± 0.11 $kg.m^{-1}$ were obtained from the substrata of nylon rope and nylon strip, respectively. The nylon rope yielded significantly higher ($p < 0.05$) production than nylon strip.

Discussion

In this study, green mussels (31.2 mm) attained the marketable size shell lengths of 63.3 and 62.3 mm on nylon rope and nylon strip substrata, respectively, within 5 months. After 5 months, the growth rate was retarded due to freshwater influx, resulting in final average shell length of 67.3 mm for the nylon rope and 65.5 mm for the nylon strip rens in 7 months. The growth in terms of shell length was higher in the present study compared with the study carried out by Appukuttan (1980).

In this study, green mussels attained the average shell width of 22.0 mm on the nylon rope and 21.6 mm on nylon strip. They attained the shell height of 34.4 and 33.7 mm on nylon rope and nylon strip, respectively, in 7 months. However, observations

on shell width and shell height were not reported in other studies.

Ranade et al. (1973) reported monthly weight gain of 3.0 g in *Mytilus viridis* cultured for 7 months. Qasim et al. (1977) reported the monthly weight gain of 11.3 g on nylon rope for *M. viridis*.

Green mussels attained the average final live weight of 8.33-8.48 and 8.39-8.50 g on rope substrata and onion bag respectively, within 8 months (Chaitanawisuti & Menasveta 1987). In this study, *P. viridis* attained the marketable size live weight of 22.45 and 20.97 g on the nylon rope and nylon strip, respectively, within 5 months. In 7 month culture period, they grew to size of 25.80 and 23.89 g, respectively, on nylon rope and nylon strip. The retarded growth during last 2 months is because of drop in salinity due to influx of freshwater. The growth obtained in this study is higher compared with that reported by Chaitanawisuti & Menasveta (1987).

During this study, the increment in the shell weight was of 10.93 and 9.60 g on the nylon rope and nylon strip substrata, respectively, within 7 months. However, Kuriakose (1980a) reported the growth rate in shell weight of 2.58 g.month^{-1} on nylon ropes for green mussel.

Kuriakose & Appukuttan (1980) recorded monthly growth rate in wet meat weight of 2.94 g on nylon ropes in 5 months. In this investigation, increment in the wet meat weight of 6.92 and 6.36 g was obtained on nylon rope and nylon strip substrata, respectively, within 7 months.

Dry meat weight increment was 1.16 and 1.03 g in 7 months for the substrata of nylon rope and nylon strip, respectively, during this study. However, no significant difference was found in growth parameters.

During this study, the final survival was found to be better on nylon rope compared with nylon strip in 7 months. It was found that because of continuous water current, the substrata, which is lighter than rope substrata, does not withstand against prevailing water current resulting slippage of mussels from nylon rope substrata.

The average per meter productions of 12 kg in 5-6 months (Kuriakose 1980a) and 6.9 kg within 5 months (Kuriakose & Appukuttan 1980) was observed on nylon rope. Ranade & Ranade (1980) recorded the production of 7 kg.m^{-1} in 6 months, and Parulekar (1980) reported average production of 18 kg.m^{-1} in 4½ months on nylon rope in raft culture method. In this study, the final production obtained was 11.39 and 9.12kg on nylon rope and nylon strip substrata for *P. viridis* in raft culture during summer months.

Conclusion

The nylon rope substratum is better compared with nylon strip substratum for growth, survival and production of *P. viridis* in raft culture system.

Acknowledgments

Authors are grateful to the authorities of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli and also to National Agricultural Technology Project on Mussel Mariculture for providing funds. Thanks to Dr. P.C. Raje, Associate Dean, College of Fisheries, Ratnagiri, for his valuable suggestions during this study.

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Received: 29 December 2007; Accepted: 26 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Prevalence of Fish Diseases in Sambalpur, Orissa, India

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Abstract

An assessment on prevalence of fish diseases in Sambalpur, Western Orissa, revealed the occurrence of six major diseases *viz*., fin rot, saprolegniosis, gill rot, *Ichthyophthirius* (white spot), costiasis and argulosis in this region. The percentage of occurrence varied from 4% to 16% in different fish farms surveyed. The study of physicochemical parameters of stocking and rearing ponds at Bomlai and Chiplima (Sambalpur, Orissa) revealed variations between the ponds. The bacteriological analyses of sediments collected from different points indicate occurrence of hygiene indicator bacteria at lower level. There is an increasing trend in counts of different bacterial groups in both sediments and in fish of stocking ponds from February to March correlating with increase in temperatures. The difference in bacterial counts of mesophilic aerobes, coliforms, faecal streptococci and group D streptococci and staphylococci varied from <1 to 2 log cfu.g^{-1} of the sample. The antibiotic susceptibility of aeromonads isolated from diseased *Cyprinus carpio* varied among and between the isolates from kidney, liver and skin lesions. Among the 17 antibiotics tested, all the isolates were susceptible to erythromycin and nitrofurantoin and resistant to cefaclor and vancomycin. This study inquires for better management of fishponds to avoid problems in future.

Introduction

Many Asian countries have a long tradition of aquaculture, and over 80% of fish produced by aquaculture comes from Asia where the production was 31.07 million metric tons valued nearly US $ 38.85 billion (FAO 2004). Nine of the top fourteen-aquaculture producers in the world are from Asia. Aquatic animal disease and environmental-related problems may cause annual losses of more than US $ 3 billion annually to aquaculture production in Asian countries (Shankar & Mohan 2002).

The freshwater aquaculture in India is mainly the cultivation of Indian major carps (IMC). The advent of 1980s has seen expansion of commercial carp culture in various parts of the country. Besides carp, small-scale cultivation of catfish (*Clarias batrachus*) and murrells (*Channa punctata*) is also practiced, and the aquarium

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trade is also attempting to establish itself as an industry. The main aim of commercial aquaculture is to boost production by intensification; hence, increased stocking density, fertilisation, feeding and use of chemicals and antibiotics have become common (Khatri 2004). Fish in freshwater systems are susceptible to a number of bacterial, viral and parasitic diseases. Large-scale mortalities of fish due to bacterial diseases reported all over the country. Among these diseases of bacterial origin, motile aeromonads play an important role in freshwater systems. Mass mortalities in India in major carps *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* due to motile aeromonad infection are reported (Karunasagar et al. 1986). Among these fishes, catla is most susceptible followed by mrigal and rohu. Aquaculture ponds receive inputs of organic matter such as uneaten feed, fertiliser and faeces. To assess the sediment quality, the techniques that are simple, rapid and practical on-site such as redox potential, pH, the hydrogen sulphide activity potential (pH_2S) and soluble ammonium nitrogen are used (Hussenot & Martin 1995). Measures such as sediment removal and water exchange only transfer pollution problems from pond environments to surrounding environments (Boyd 1997).

Reports on the prevalence diseases in freshwater fish and on physicochemical and the bacteriological quality of the pond sediments and fish of Western Orissa are scant. Hence, this study is undertaken to assess prevalence of different fish diseases in Sambalpur, Western Orissa. The bacteriological and physicochemical quality of freshwater fish rearing and stocking ponds located in different areas of Sambalpur districts, Orissa, was assessed. The physicochemical quality parameters were assessed for stocking ponds in the months of February and March to find whether there are any changes during the transition period and their bearing on the bacteriological quality of the sediments and fish. The aeromonads isolated from the diseased fish were tested for antibiotic resistance against 17 antibiotics.

Materials and Methods

Studies on prevalence fish disease in Sambalpur

A total of 25 fish farms (of both commercial and state government) were selected for disease assessment. From each pond, fish were harvested from different areas (average of four) and fish with symptoms of disease were brought to the laboratory for further examination.

Selection of fishponds for physicochemical and bacteriological quality assessment

Extensive survey was carried out in different farms located in Sambalpur district for assessment of physicochemical and bacteriological quality parameters. Among which pond selection was made from Bomlai and Chiplima fish farms. The selection of the farms ensured that both are far from each other (90 km), and neither the physicochemical

parameters nor the bacteriological quality parameters have any bearing on each other in view of the distance as well the source of water.

Description of the ponds

The Bomlai has built in area of 97.10 acres, whereas Chiplima has 151.06 acres and out of which Bomlai has 28.11 acres water spread area with 46 tanks. Among these tanks, 29 are nursery tanks in 5.01 acres, 7 rearing ponds in 3.01 acres and 10 stocking ponds in 20 acres. The Chiplima farm holds 62.5 acres of water-spread area that includes 7.5 acres of canals. The total numbers of tanks in the farms are 44 and out of which 12 are breeder tanks and 32 numbers nursery tanks. Each tank covers an area of 1.25 acres. Both farms are active in production in all spears from the inception. The study revealed that for the last 10 years both farms are active in production of spawn, fry and fish. In commercial farms, the tank size varied from half acre to one acre and number of tanks ranged from two to six in each farm.

Physicochemical quality parameters of the fishponds

Physicochemical parameters were obtained from rearing and stocking ponds of both the areas of study, i.e. Bomlai and Chiplima: oxidation-reduction potential (ORP-E_h), total dissolved solids (ppm), dissolved oxygen (ppm), temperature (°C), conductivity (mho) and salinity (ppt). All the parameters were analysed using Potable-Water and Soil Analyzer Kit (Naina Enterprises, Agra, India) (Khatri 2004).

Collection of fish samples

Fish sample was collected from the Bomlai and Chiplima rearing ponds in live oxygenated condition and was brought to the laboratory for observation and immediate analyses.

Diseased fish

Infected common carp was cleaned of the surface contaminants with sterile cotton swabs soaked in a chloroxylenol-based antiseptic. Skin lesions, gall bladder and kidney regions were sampled as described by Karunasagar et al. (1989). Samples were ground with sterile nutrient broth using a sterile mortar and pestle under aseptic conditions. A loop full of the sample was spread on M-*Aeromonas* selective agar plates and was incubated at 20°C and 36 ± 1°C for 24 h (Niewolak & Tucholski 2000).

Collection of sediments of fish rearing and stocking ponds

Top layer of the sediment was collected from different spots of different ponds. From each pond, samples were drawn from peripheral, mid and central regions in sterile

polythene bag (200 gauge) and were mixed thoroughly before making serial dilutions. Sterile normal saline (0.85% NaCl) was used as diluent throughout the study.

Microbiological methods

Miles–Mishra method was used for screening the sediment and fish samples using sterile (gamma irradiated) disposable pipettes (Volac-John Poulten Ltd, Barking, England) for quantitative estimation of different groups of bacteria (Khatri 2004). Spread plate method was also used for selective isolation of suspected bacteria for further purification and characterisation (Khatri 2004).

Suitable dilutions of the sediment and fish meat samples in sterile normal saline were surface plated on plate count agar, violet bile salt glucose agar, kanamycin aesculin azide agar and Baird-Parker agar for the enumeration of mesophilic aerobic bacteria, faecal coliforms, faecal streptococci and staphylococci according to the standard procedures (Bennet, 1984). Inoculated plates were incubated at 37°C for 24 h for mesophilic aerobes and faecal coliforms, 30 h for staphylococci and 48 h for faecal streptococci. In case of faecal streptococci, the incubation period was extended to 96 h till the very small colonies grew to a larger size for the count. M-*Aeromonas* selective agar was used (Havelaar et al. 1987) for screening the fish *Cyprinus carpio* based on the symptoms of the disease. In this medium, yellow (dextrin fermentation) colonies were picked and further tested for oxidase and trehalose fermentation. All the positive isolates were further characterised to confirm species level identification (Havelaar et al. 1987; Prasad et al. 1998).

Antibiotic sensitivity of the bacterial isolates

The antibiotic sensitivity of the *Aeromonas* spp. from the diseased fish was tested by agar diffusion method as described (Baur et al. 1966; Prasad et al. 1998). The bacteria were isolated from skin lesions: SSA^4B, SSA^4B (D), SSA^1B, SSA^1B (D), SSA^3S, SSA^3S (D), SSA^2S, SSA^2S (D); liver: LSA^1B, LSA^1B (D), LSP^1S, LSP^1S (D) and kidney: KSA^1S, KSA^1S (D), KSA^2B, KSA^2B (D) of the diseased fish.

The dehydrated media, reagents and antibiotic discs used in this study were from Hi Media (Mumbai, India) and chemicals from Qualigens (India).

Results and Discussion

Occurrence of fish disease

The major diseases identified were included fin rot, saprolegniosis, gill rot, *Ichthyophthirius* (white spot), costiasis and argulosis. The percentage of occurrence in different fish farms surveyed varied from 4% to 16% in this region.

The fin rot was seen in 12% of the farms from which samples were drawn. Saprolegniosis is prevalent mainly in IMC, especially in *Catla catla*, in Chiplima farm. This is seen in all stages of fish that included eggs, fry, fingerlings and fishes. Percentage of occurrence is seen in 4% of the farms. Gill rot is seen in farmed fish of both areas namely Bomlai and Chiplima, especially in the ponds polluted with organic matter. The percentage of occurrence is limited to 8% of the farms. *Ichthyophthirius* (white spot) and costiasis are seen in 16% of the farms of this region. The most affected fish varieties of costiasis were *L. rohita, C. catla, and C. mrigala.* This infection is seen in 16% of the farms surveyed. The prevalence of argulosis is seen in 12% of the farms surveyed (Fig.1). Argulosis infection is also seen in farmed freshwater carp, Rohu (*L. rohita*) in Andhra Pradesh. Ornamental fish *viz.*, twin tail barb and brass gold appear to be useful as biological agents for the control of *argulosis* in freshwater carps (Anon 2002).

Figure 1. *Labeo rohita* infected with argulosis, Chiplima Fish Farm, Sambalpur

This study indicates that disease problem is prevalent in the breeding tanks of Bomlai right from the beginning of the production, i.e. 1975. Similar problems are associated with farm at Chiplima too from the beginning of production, i.e. 1986. Besides these problems, Epizootic Ulcerative Syndrome (EUS) was major problem 10 years before, but the severity of the problem drastically reduced for the last few years. With regard to commercial fish farms too the disease problems were noted for last two decades, but the intensity of the diseases decreased. No large-scale mortalities were observed.

Studies on fish ponds located at Bomlai and Chiplima

Physicochemical and bacteriological quality of Bomlai and Chiplima fish rearing ponds

The physicochemical parameters of two rearing ponds of Bomlai and Chiplima (Table 1) indicate difference in temperature 4.22°C, pH 0.01, ORP 1.92, TDS 21.57, conductivity 0.13, DO_2 4.02, but the salinity of both farms found to be same. Presence

of less TDS in Chiplima resulted in high DO_2 content that is providing congenial conditions for growth of fish.

Table 1. Physicochemical and bacteriological quality parameters of fish rearing ponds at Bomlai and Chiplima (Data collected in Feb 2004)

Physicochemical parameters	Bomlai			Chiplima		
	Peripheral	Mid region	Central point	Peripheral	Mid region	Central point
Temperature (°C)	21.31 ± 0.22	21.02 ± 0.35	21.2 ± 0.14	16.68 ± 0.46	17.0 ±0.62	17.2 ± 0.14
pH	6.86 ± 0.31	7.07 ± 0.17	7.03 ± 0.01	6.54 ± 0.37	6.43 ±0.02	7.95 ± 0.07
ORP($-E_h$)	80.75 ± 3.77	80.75 ± 3.3	92.0 ± 1.41	84.75 ± 1.26	82.5 ±1.29	80.5 ± 0.71
TDS (ppm)	34.2 ± 0.40	34.75 ± 2.36	32.0 ± 0.00	12.5 ± 1.29	11.75 ±0.96	12.0 ± 0.00
Conductivity (mho)	0.83 ± 0.10	0.93 ± 0.1	0.90 ± 0.00	0.70 ± 0.00	0.78 ±0.05	0.80 ± 0.00
Salinity (ppt)	0.88 ± 0.10	0.88 ± 0.1	0.85 ± 0.07	0.80 ± 0.00	0.80 ±0.00	0.80 ± 0.00
DO_2 (ppm)	7.43 ± 1.01	6.93 ± 0.26	7.25 ± 0.07	11.4 ± 0.18	11.33 ±0.05	11.95 ± 0.07

ORP: Oxidation Reduction Potential; TDS: Total dissolved Solids. Data presented are averages of 4 ponds.

Bacteriological quality (counts in log cfu per g of the sample)				
	Fish	Sediment	Fish	Sediment
Mesophilic aerobes	3.52	3.4	4.6	5.2
Fecal coliforms	2.2	4.26	ND	ND
Streptococci	ND	ND	ND	ND
Group D fecal streptococci			4.00	2.96
Staphylococci	ND	4.08	4.23	ND

The bacteriological analysis of the sediments of the fish rearing ponds at Chiplima indicates that the tanks are free from hygiene indicating faecal coliforms but harboured group D faecal streptococci in the range of 3.08-3.30 log cycles with an average of 2.96 log cycles.g^{-1} of the sample. The mesophilic aerobic bacterial count ranged from 4.45 to 5.72 with an average of 5.20 log cycles.g^{-1} of the sample. The fish from the same pond contained group D faecal streptococci and staphylococci at 4.0 and 4.23 log cycles g^{-1} of the sample, respectively (Table 1).

The bacteriological analyses of the sediments of the fish ponds at Bomlai contained mesophilic aerobes in the range of 2.60-4.20 log cycles with an average of 3.52 log cycles.g^{-1} of the sample. The faecal coliforms occurred in the range of <1-2.60 log cycles were with an average of 2.20 log cycles.g^{-1} of the sample. However, all the sediment

samples were free from faecal streptococci and staphylococci. Overall load of different groups of bacteria is slightly higher in Chiplima than in Bomlai. Occurrence of coliforms in Bomlai fish rearing ponds could be due to external contamination.

Bacteriological quality and physicochemical parameters of the sediments and fish samples of Bomlai and Chiplima fish stocking ponds during transition period

The physicochemical parameters of the stocking ponds of two farms, i.e. Bomlai and Chiplima, recorded during transition period of winter to summer, i.e. February and March 2004, are provided in Tables 2 and 3.

Table 2. Physicochemical and bacteriological quality parameters of fish stocking ponds at Bomlai during transition period from winter to summer

Physicochemical parameters	Bomlai (Feb. 2004)			Chiplima (March 2004)		
	Peripheral	Mid region	Central point	Peripheral	Mid region	Central point
Temperature (°C)	21.05 ± 0.79	20.8 ± 0.45	20.1 ± 0.00	25.98 ±1.04	26.33 ± 1.04	26.55 ± 0.07
pH	6.57 ± 1.94	5.76 ± 0.78	9.8 ± 0.14	6.25 ±1.33	7.65 ± 1.33	9.0 ± 0.14
ORP($-E_h$)	54.75 ± 11.18	55.75 ± 6.70	42.0 ± 1.41	79.5 ±10.4	68.5 ± 10.41	63.5 ± 2.12
TDS (ppm)	27.5 ± 2.38	26.75 ± 0.96	27.1 ± 0.85	15.0 ±0.00	15.0 ± 0.00	15.0 ± 0.00
Conductivity (mho)	1.43 ± 0.49	1.4 ± 0.14	1.6 ± 0.00	0.2 ±0.00	0.2 ± 0.00	0.2 ± 0.00
Salinity (ppt)	1.15 ± 0.13	1.13 ± 0.15	0.8 ± 0.00	0.1 ±0.00	0.1 ± 0.00	0.1 ± 0.00
DO_2 (ppm)	8.05 ± 0.44	8.03 ± 0.41	9.00 ± 0.14	5.28 ±1.18	4.8 ± 1.18	6.15 ± 0.07

Bacteriological quality (counts in log cfu per g of the sample)

	Fish	Sediment	Fish	Sediment
Mesophilic aerobes	4.23	3.81	5.14	4.73
Fecal coliforms	2.22	ND	4.61	3.34
Streptococci	3.10	ND	4.15	ND
Group D fecal streptococci	3.40	2.86	3.60	2.92
Staphylococci	3.57	ND	4.12	ND

Table 3. Physicochemical and bacteriological quality parameters of fish stocking ponds at Chiplima during transition period from winter to summer

Physicochemical parameters	Chiplima (Feb. 2004)			Chiplima (March 2004)		
	Peripheral	Mid region	Central point	Peripheral	Mid region	Central point
Temperature (°C)	19.18 ± 0.17	19.35 ± 0.13	21.05 ± 0.07	27.93 ± 0.38	28.03 ± 0.29	28.30 ± 0.14
pH	7.64 ± 0.36	7.91 ± 0.35	6.0 ± 0.14	5.88 ± 0.50	5.78 ± 0.05	5.90 ± 0.00
ORP ($-E_h$)	58.25 ± 1.26	62.5 ± 1.29	68.5 ± 0.71	74.5 ± 7.05	73.5 ± 2.52	78.0 ± 1.41
TDS (ppm)	12.75 ± 1.71	16.75 ± 0.96	12.0 ± 0.41	15.25 ± 3.10	15.08 ± 2.56	20.0 ± 0.00
Conductivity (mho)	0.58 ± 0.10	0.58 ± 0.10	0.6 ± 0.00	0.3 ± 0.00	0.3 ± 0.00	0.3 ± 0.00
Salinity (ppt)	0.68 ± 0.05	0.63 ± 0.05	0.6 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
DO_2 (ppm)	10.28 ± 0.13	9.95 ± 0.13	10.35 ± 0.07	4.53 ± 0.35	4.63 ± 0.10	5.85 ± 0.07

ORP: Oxidation Reduction Potential; TDS: Total dissolved Solids. Data presented are averages of 4 samples.

Bacteriological quality (counts in log cfu per g of the sample)				
	Fish	Sediment	Fish	Sediment
Mesophilic aerobes	3.67	3.26	5.15	4.63
Fecal coliforms	3.93	3.34	4.45	3.51
Streptococci	2.07	ND	4.1	ND
Group D fecal streptococci	2.78	2.54	3.48	2.97
Staphylococci	3.78	ND	4.29	ND

During the period of February and March in Bomlai farm, increasing trend of temperature and ORP is seen and with the rest of the parameters a decreasing trend was observed which is marginal except for TDS. Similar trends were also observed in Chiplima except with TDS that has enhanced by nearly 3 ppm. Only the upper 5-10 cm of pond bottom soil influences water quality in ponds, and hence management standpoint, thus, the composition of this surface layer is of most importance (Boyd 1995). Low dissolved oxygen level is the major limiting water quality parameter in aquaculture systems. Chronically low-dissolved oxygen levels can reduce growth and feeding (Khatri 2004). In both areas under study the physicochemical quality of the pond water in both the stocking and rearing ponds, the TDS is high (>10 $mg.L^{-1}$). This can lead to low productivity of the ponds. The bacteriological quality of Bomlai showed an increasing

trend from February to March along with temperatures. The difference is more than 1 log cfu.g^{-1} of the sample in case faecal coliforms and faecal streptococci, nearly 1 log cfu.g^{-1} with mesophilic aerobes and less than 1 log cycle with group D faecal streptococci (Table 2). Similar trends were also seen in the study of the populations of indicator bacteria *viz.*, mesophilic, coliform and faecal streptococci together with relevant limnological parameters such as temperature, oxygen, BOD and chlorophyll-a revealed that during each season, populations of indicator bacteria increased with increasing water temperature, and maximal numbers of bacteria were recorded during the summer months (Markosova & Jezek 1994). This study corroborates the findings of the studies by Sivakami et al. (1996) and Al Harbi (2003) in which coliform organism found in sediment and fish samples.

The bacteriological quality of the sediment and the fish in Chiplima fish stocking ponds have also shown increasing trend from February to March with the increase in temperatures. In fish samples, the difference in increase is more than 1 log cfu.g^{-1} of the sample for mesophilic aerobes and streptococci. However, the difference in coliforms, group D streptococci and staphylococci from February to March is less than 1 log cfu.g^{-1} of the sample. In sediments of the Chiplima ponds, the difference in faecal streptococcal and group D streptococcal counts is also less than 1 log cfu.g^{-1} of the sample. This study agrees with other studies in which the counts of different groups of bacteria such as mesophilic aerobes, coliforms, streptococci and staphylococci of pond sediments and the fish tend to increase with increase in temperatures (Markosova & Jezek 1994; Kasai et al. 2002; Ogbulie & Cobiajuru 2003; Garcia et al. 2003).

Staphylococcus aureus is associated with eye infection in fish *Hypothalamichthys molitrix* (Shaw & Tyagi 1986). Catfish growing ponds contained faecal coliforms in the range of 14-1600 mL^{-1} of pond water (Shireman & Cichra 1994). This is higher than what is observed in this study. Environmental stress can trigger spread of dormant disease into full bloom resulting in large-scale mortality of fish (Wedemeyer et al. 1999).

Characterisation of the bacterial isolates from diseased fish (*C. carpio*) (Fig. 2) of this study revealed that they are *Aeromonas hydrophila* and *Aeromonas sobria*. Motile aeromonads also isolated from Epizootic Ulcerative Syndrome affected *Channa striatus* (Prasad et al. 1998). In this study, high temperatures (37˚C) were used for detection of different groups of bacteria in sediments, fish and aeromonads from diseased *C. carpio* and all groups of bacteria under screening could be detected. These results agree with the study on evaluation of the bacteria identified on broth agar in 20˚C and 37˚C, *viz.*, coli forms, faecal coli forms, faecal streptococci, *Aeromonas* sp. and *Salmonella* sp. in the muscles, skin and digestive tract content of common carp (*C. carpio*) (Niewolak & Tucholski 2000) in which the high temperatures favoured growth of all bacteria more, so *Aeromonas sp*. motile aeromonads are inherently associated with freshwater bodies and cultured fish production. At present, 10 different species are

recognised, but in moribund tropical fish, most commonly isolated species are *A. hydrophila* and *A. sobria* (Khatri 2004). Motile aeromonads are most commonly associated with bacteria found in fish infected with EUS (Lio-Po et al. 1991; Karunasagar & Karunasagar 1994; Prasad et al. 1998; Karunasagar et al. 2003; Golas et al. 2004).

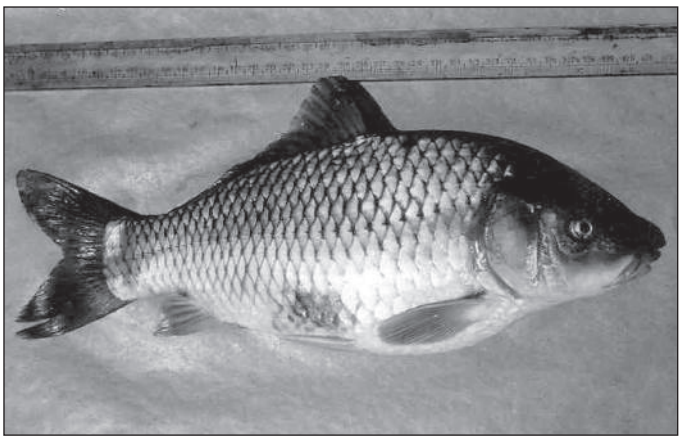

Figure 2. Cyprinus carpio with diseased symptoms

Antibiotic sensitivity of the bacterial isolates from diseased Common carp C. carpio

The sensitivity or resistance different isolates from different fish are shown in Table 4.

Table 4. Antibiotic sensitivity of the *Aeromonas hydrophila* isolated from the diseased fish

	Antibiotic tested																
Isolates	AN	AZ	CFC	CH	CLR	CL	E	G	K	NF	NR	OX	PP	P	SC	T	V
Concentration of the antibiotics in µg per disc except NF 300ml and NR 10U per disc																	
	30	15	30	30	15	2	15	10	30	300	10	5	100	10	5	30	30
KSA1S	S	S	R	S	I	R	S	S	I	I	S	S	R	R	S	S	R
KSA2B	S	I	R	S	R	R	S	I	I	R	S	I	R	R	S	S	R
LSA1B	R	S	R	I	R	R	S	R	R	S	S	S	R	R	R	I	R
LSP1S	I	S	R	S	I	R	S	I	R	R	S	S	R	R	S	R	R
SSA1B	S	S	R	S	I	R	S	I	S	R	S	S	R	R	S	S	R
SSA2S	S	S	R	S	S	R	S	S	I	R	S	S	R	R	S	S	R
SSA3S	S	S	R	S	S	R	S	I	I	I	S	S	R	R	R	S	R
SSA4B	S	S	R	S	I	R	S	I	R	R	S	I	R	R	S	R	R

Source of isolates; K-Kidney; L-Liver;S- Skin lesions. Antibiotics: AN:Amikacin, AZ:Azithromycin, CFC:Cefaclor, CH:Chloramphenicol, CLR: Clarithromycin, CL:Clindamycin, E: Erythromycin, G; Gentamycin, K: Kanamycin, NF:Nitrofurantoin, NR: Norfloxacin, OX:Ofloxacin, PP: Piperacillin, P: Penicillin G, SF: Sparfloxacin, T: Tetracycline, VV:Vancomycin. S: Susceptible; I: Intermediate; R: Resistant

The *Aeromonas* isolates from kidney (KSA^1S) of the diseased fish *C. carpio* showed susceptibility to 9 antibiotics, intermediate to 3 and resistant to 5 antibiotics out of 17 antibiotics tested. The other *Aeromonas* isolate from kidney (KSA^2B) was susceptible to six antibiotics, intermediate to four and resistant to seven antibiotics. The isolate from liver (LSA^1B) was susceptible to five antibiotics, intermediate to two and resistant to the remaining antibiotics. The second isolate from the liver (LSP^1S) was resistant to nearly 50% of the antibiotics, i.e. eight, intermediate to three and susceptible to six antibiotics. The isolates obtained from four different parts of affected area (skin lesions) were susceptible to more than 50% of the antibiotics tested and were resistant to few and the remaining fell into category of intermediate in the susceptibility (Table 4). Among the 17 antibiotics tested, all the isolates were susceptible to erythromycin and nitrofurantoin and resistant to cefaclor and vancomycin. Occurrence of antibiotic-resistant strains of *A. hydrophila* from fish samples from integrated fish farms in a Southeast Asian country was also reported (Twiddy & Reilly 1994).

Conclusion

This study revealed the occurrence of six major diseases *viz.*, fin rot, saprolegniosis, gill rot, *Ichthyophthirius* (white spot), costiasis and argulosis. This is helpful to develop preventive measures, control and spread of the diseases

The study on physicochemical parameters of stocking and rearing ponds at Bomlai and Chiplima revealed variations between the ponds and the seasons. In both areas under study the physicochemical quality of the sediments in both the stocking and rearing ponds, the TDS is high (>10 $mg.l^{-1}$). The pH is within the optimum levels, i.e. 6.5-8.5. The bacteriological analyses of sediments collected from different points indicate occurrence of hygiene indicator bacteria at lower level. The trend is an increase along with increase in temperatures of pond from February to March in all bacterial groups wherever the occurrence is noted both in sediments and fish. The antibiotic susceptibility of *A. hydrophila* isolated from diseased *C. carpio* varied among and between the isolates from kidney, liver and skin lesions. This result will act as baseline data for future studies. However, more studies are required to understand changing pattern of antibiotic resistance in environmental strains.

Acknowledgements

First author wish to thank Dr. K Devadasan, Director, CIFT, Kochi, for permission to guidance for M.Sc Dissertation work from Burla Research Centre of CIFT. M.M. Prasad wish to thank Director, CIFT, for permission to present of the work at 8th Asian Fisheries Forum, Kochi.

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Received: 31 December 2007; Accepted: 31 October 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Water Quality Characteristics of Two Derelict Water bodies of Aligarh, Uttar Pradesh, India

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Abstract

District Aligarh of Western U.P. in North India has diversified seasonal rain fed and sewage fed water bodies. Selected water bodies are ponds approximately 5 km away from university campus. These ponds seem to derelict as they are used just for collecting sewage water coming from the houses. Cattle use them for drinking water, bathing, and sometimes for grazing. The present study was carried out for 16 months from February 2001 to May 2002 and analysed various physicochemical parameters, namely temperature, dissolved oxygen, pH, transparency and nutrients. The study indicated that these water bodies are productive and they can be made useful for fish culture after adopting proper pond management strategies.

Introduction

India is rich in vivid types of lentic aqua-systems located in different geographical regions from the hot and dry arid zone to wet and humid zone of southern peninsula (Hosetti 2002). Aligarh, a district of Utter Pradesh in Northern India, is located in the central Ganga-Yamuna Doab at latitude 27°54'N and longitude 78°4'E. It experiences the tropical monsoon type of climate with marked North-East and South-West monsoon. Due to increasing population pressure in India it has become necessary to make use of more and more water bodies including derelict and waste water to fulfill the demand of protein requirements. The limnological characteristics of water of different regions are of immense practical value in fish culture programmes. Although lots of work has been done in the field of limnology in North India there is a lack of work done in these water bodies. Keeping in view the objective for use of such water bodies for fish culture it has become essential to analyze the water quality. Present study includes monitoring of various physicochemical and biological characteristics.

The selected water bodies are two ponds in Charrat village approximately 5 km away from university campus. At present these water bodies are used as drainage basin into which surface runoff water and sewage from surrounding catchment areas enters.

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Pond I is smaller approximately 0.26 ha in area than pond II which is approximately 1.03 ha. Depth varies from 0.52 to 0.96 m in pond I and 0.5 to 0.96 m in Pond II. The shoreline is irregular in both the ponds making the ponds very productive by increasing the opportunity of superimposition of photosynthetic zone upon decomposition zone.

Materials and Methods

For water quality analysis monthly sampling was done from these two water bodies for a period of 16 months from February 2001 to May 2002.Water sample was collected from surface and physicochemical and biological parameters were analyzed.

Transparency was recorded using standard Secchi disc method. Temperature of air and water was recorded with the help of mercury thermometer. Dissolved oxygen (D.O) was analysed by Winkler's modified technique (APHA 1992), carbon dioxide by titration method, and pH was determined with the help of Marconi's portable pen type digital pH meter. Alkalinity was determined by titration with a 100 mL water sample with 0.02 N sulphuric acid using phenolphthalein and methyl orange as indicators. Hardness was estimated by titration with 0.01 N EDTA solution using Muroxide as indicator (Trivedy and Goel 1984). The presence and amount of phosphates (PO_4-P) and nitrates (NO_3-N) were estimated following the method by Trivedy and Goel (1984) and Silica was determined by silicomolybdic acid (Ammonium Molybdate-yellow) method (Barnes 1959).

For phytoplankton analysis, monthly water samples (500 mL) were collected from each water body and fixed in 5 mL of Lugol's iodine solution (Edmondson 1959). After 24 hours the supernatant was discarded and concentrate was obtained. For zooplankton analysis every month 100 litres of water was filtered by passing it through plankton net made up of bolting silk cloth having mesh size of 30 µm, taking care that water was not disturbed much during the operation. Samples were then washed out into wide mouth bottles and were preserved by adding 5% formaldehyde solution to them. Further analysis was done by putting 1 mL of the fixed sample on a Sedgewick-Rafter cell, and studying it under an inverted microscope (Metzer). Counts were made as number of zooplankton per liter and number of phytoplankton of water sample. For qualitative analysis, the information given in Edmondson (1959), Needham and Needham (1962), Pennak (1978) and Tonapi (1980) were used. For most of the organisms encountered, the identification was made up to generic level.

Results and Discussions

Monthly variations in Secchi disc transparency, temperature, dissolved oxygen, and pH are given in Table 1.

Table 1. Monthly Variations in Air Temperature, Water Temperature, Transparency, pH and Dissolved Oxygen in Ponds I and II.

Months	Air temperature (ºC)		Water temperature (ºC)		Transparency (cm)		pH		Dissolved Oxygen (mg. L^{-1})	
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
February, 2001	18.3	18.3	15.5	16.5	38.5	38.0	9.0	8.7	6.2	4.2
March	25.5	25.5	23.5	24.0	37.0	34.0	9.1	9.0	8.0	6.8
April	25.5	25.5	24.0	23.5	34.0	30.5	8.4	8.4	7.6	6.2
May	36.0	36.0	34.0	34.0	36.5	36.0	8.6	8.3	6.0	3.6
June	39.0	39.0	35.0	35.0	32.0	32.0	8.3	8.6	7.2	5.0
July	38.0	38.0	35.0	36.0	28.5	18.0	8.3	8.6	5.6	4.6
August	36.0	36.0	32.0	32.0	18.0	16.5	8.3	8.5	6.0	3.4
September	30.0	30.0	28.0	27.0	22.0	26.5	8.4	8.7	8.0	4.4
October	30.0	30.0	28.0	28.0	28.0	28.0	8.3	8.5	6.0	4.0
November	29.0	29.0	25.5	25.5	38.5	38.5	8.4	8.6	4.6	2.6
December	22.0	22.0	18.5	18.5	32.5	36.0	8.6	8.4	5.0	3.8
January, 2002	17.0	17.0	16.0	16.0	30.7	27.0	9.1	9.1	8.4	7.2
February	15.0	15.0	14.0	14.5	36.5	38.0	8.6	8.3	4.8	5.2
March	23.0	23.0	21.5	21.0	38.5	34.0	8.7	8.8	4.0	3.8
April	25.0	25.0	23.0	22.5	26.5	23.5	8.8	8.7	6.4	6.6
May	32.0	32.0	28.5	28.5	28.0	24.5	8.5	8.8	5.2	6.2

It was found lowest in monsoon months, which might be due to entry of huge amount of suspended and colloidal matter, silt and clay into these water bodies along with the rain water from surrounding fields. Maximum transparency during winter might be due to settling of suspended materials. Water temperature in these shallow basins was closely related to ambient air temperature. The monthly water temperature changes follow the pattern in temperature change for Indian subcontinent. The fluctuations in D.O. content might be attributed to the fact that the concentrations and solubility of this gas in these waterbodies might be exposed to intense photosynthetic activity of

phytoplankton. Statistically, these two parameters show direct and positive correlation with oxygen D.O. Higher values during monsoon months in both the ponds were found to be mainly due to agitation of water caused by falling rainwater over the surface of the water body. Carbon dioxide was never detected. High pH above 8.3 in both ponds might be the probable cause of complete absence of carbon dioxide. Absence of carbon dioxide was also noted by Jhingran (1991). There is always an optimum range of pH for growth and survival of any organisms. In the present study, pH range fluctuated between 8.3 and 9.1. High pH in these ponds during certain months might be due to high photosynthetic activity. Monthly variation in alkalinity, hardness, phosphate, nitrates, and silica are given in Table 2.

Table 2. Monthly Variations in Total Alkalinity, Hardness, Phosphates, Nitrates and Silica in Ponds I and II.

Months	Total Alkalinity (mg/L)		Hardness (mg/L)		PO_4-P (mg/L)		NO_3-N (mg/L)		Silica (mg/L)	
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
February, 2001	360	384	290	230	0.36	0.27	0.12	0.07	0.240	0.133
March	400	389	270	170	1.65	1.14	0.13	0.08	0.223	0.195
April	380	350	290	200	1.74	1.74	0.11	0.10	0.351	0.326
May	285	204	500	500	1.14	0.91	0.18	0.15	0.638	0.726
June	350	280	380	360	1.60	0.93	0.21	0.20	1.310	0.285
July	320	298	280	260	0.98	0.98	0.16	0.12	1.162	1.100
August	300	128	142	140	1.10	0.91	0.18	0.10	1.096	1.196
September	340	190	160	141	0.97	1.14	0.19	0.09	0.564	0.651
October	300	185	164	234	0.85	1.16	0.15	0.09	0.832	0.928
November	261	167	232	240	0.62	0.53	0.11	0.18	0.675	0.077
December	370	180	290	270	0.67	0.57	0.13	0.05	0.787	0.833
January, 2002	380	380	380	220	1.53	0.58	0.21	0.06	0.916	0.326
February	300	213	276	234	0.73	0.98	0.10	0.07	0.216	0.252
March	170	225	320	312	1.05	0.80	0.11	0.11	0.833	0.598
April	250	295	350	360	1.06	1.01	0.13	0.13	0.429	0.326
May	220	295	326	312	1.01	1.18	0.16	0.16	0.351	0.226

Alkalinity ranges from 128 mg/L to 400 mg/L in both ponds. Jhingran (1991) has given the range 40 to 1000 mg/L for Indian waters. According to Alikunhi (1957) ponds

with alkalinity greater than 100 mg/L can be categorized as highly productive. Statistically, a positive correlation between alkalinity and phytoplankton population was obtained (Table 3). Hardness showed wide fluctuations (140-500mg/L).

Table 3. Statistical brief of various water quality parameters in ponds I and II.

Parameters		Parameters	Pond I	Pond II
Water Temperature	vs	PO4-P	0.328	0.331
		NO3-N	0.532*	0.674*
		Silica	0.549*	0.462
Dissolved Oxygen	vs	Phytoplankton	0.738*	0.570*
Total Alkalinity	vs	Zooplankton	0.792*	0.900*
PO_4-P	vs	Phytoplankton	0.595*	0.298
NO_3-	vs	Phytoplankton	0.614*	0.335
Phytoplankton	vs	Phytoplankton	0.422	-0.422
	vs	Zooplankton	0.969*	0.554*
	vs	Carbonates	0.67*	0.67*
	vs	Chloride	0.256	0.476
		Water-Temperature	0.036	0.136

* = Significant at 5% level

Higher concentration during May might be due to excessive evaporation. Higher values of hardness are due to detergent containing domestic wastes. Moreover, high hardness is the general characteristic of water bodies situated in Indian plains. Phosphates (PO_4-P) showed generally higher values except during winters (Table 2). Various environmental factors such as temperature, pH, and redox conditions can influence phosphorus cycling (Forsberg 1989). Incoming sewage water is the major source of phosphate. Generally, higher concentration of Nitrates (NO_3-N) was noted during summer and monsoon months (Table 2) in both ponds. The higher values of nitrates and phosphates during summer might be attributed to increased rate of decomposition of organic matter at high temperature, as well as, high rate of evaporation in these shallow water bodies. Values of dissolved silica during summer might be associated with regeneration of silicates from diatom frustule at high temperature. The trend of silica content proved that concentration increases at high temperature within the range for freshwaters (Wetzel 1983). Statistically, a positive correlation between water temperature and these nutrients was obtained (Table 3). Phytoplankton mainly comprises algae as these have suitably

adapted themselves to a planktonic mode of life. Five groups of planktonic algae are given in the order of abundance in Table 4 (a and b). Pond I showed minimum number (124 No./mL) in August 2001 and maximum number (217 No./mL) in January 2002, whereas pond II showed minimum number (83 No./mL) in November 2001 and maximum number (143 No./mL) in January 2002 (Table 4b). In the present study, it was noted that no single environmental factor was found responsible for the production of phytoplankton organisms but a number of factors acted together to bring forth the cumulative effect. Myxophyceae group is represented by following members: *Microcystis aeruginosa* contributed maximum (68 No./mL) during June 2001 in pond I and (56 No./mL) during February 2002 in pond II. This species showed its presence throughout the period of investigations. *Spirulina* was recorded in all the samples except during February 2001 and April-May, 2002 in pond I. In pond II, it was recorded only during July 2001 to February 2002. *Anabaena* showed its presence during February to July 2001, March 2002, May 2002 in pond I, during February to March 2001 and from August to December 2001 in pond II. *Agmenellum* also showed its presence throughout in all these ponds. Its maximum densities (17 No./mL and 12 No./mL) were recorded during April and February 2002 in ponds I and II. Chlorophyceae: In the present study, it is the second most abundant group of phytoplankton after myxophyceae (Tables 4 a,b). Members are *Crucigenia*, *Ankistrodesmus*, *Scenedesmus*, *Chlorella*, and *Protococcus* in pond I, *Ankistrodesmus*, *Protococcus*, and *Actinastrum* in pond II. The filamentous algae *Spirogyra* and *Ulothrix* show bimodal occurrence showing their presence during March to April 2001 and September to October 2001 in pond II and pond I showed the absence of *Spirogyra* during February to March 2001 and *Ulothrix* during May 2001, September to October 2001 and February to May 2002.

The genera noted to be absent during certain months appeared when conditions become favorable. In the present study, alkaline medium favors optimum growth of Chlorophyceae (Saha et al. 1985). The dominance of Chlorophyceae in pond I might also be due to comparatively high D.O. content than other ponds. Dhakar (1979) found that the green algae prefer waters with high D.O. content. Euglenophyceae*:* In the present study, euglenoids are represented by only two genera (*Euglena* sp. and *Phacus* sp.) throughout the study. The two species of *Euglena*, coexists without showing any competition and showed presence in good numbers throughout. During the period of investigations, *E. acus* and *E. deses* showed a range of 5 to 25 No./mL and 3 to 30.00 No./mL in pond I, 3 to 16 No./mL and 2 to 12 No./mL in pond II. *Phacus* sp. also showed continuous presence, fluctuating between 5 to 16 No./mL and 3 to 12 No./mL in ponds I and II, respectively. Less diversity and continuous presence of euglenoids in the present study might be due to richness of these waterbodies in terms of organic

Table-4a Distribution and Abundance of Phytoplankton population (No./ mL) in Pond I

Genera \ Months	Feb. 2001	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 2002	Feb.	March	April	May
MYXOPHYCEAE																
Microcystis	14	55	43	49	68	49	21	26	10	10	10	15	26	38	49	60
Spirulina	-	1	3	11	12	9	3	39	36	16	7	2	4	2	-	-
Anabaena	1	2	2	1	3	1	-	-	-	-	-	-	-	2	3	2
Agmenellum	5	9	7	17	7	4	2	4	5	4	3	3	10	15	16	14
Total	20	67	55	78	90	63	26	69	51	30	20	20	40	57	68	76
CHOLOROPHYCEAE																
Crucigenia	9	12	10	11	6	4	5	11	12	15	10	17	13	6	8	14
Pediastrum	1	2	3	-	1	2	2	1	-	1	2	3	1	2	1	2
Ankistrodesmus	12	18	11	4	13	11	4	7	14	9	12	18	18	10	9	4
Scenedesmus	4	6	5	7	7	6	5	4	5	4	6	10	8	5	6	8
Protococcus	12	18	9	10	12	16	10	16	18	18	19	24	17	10	9	10
Chlorella	5	3	2	1	2	3	2	2	3	8	6	8	4	2	2	3
Tetraspora	15	18	12	9	5	9	4	9	4	12	10	14	11	4	6	8
Actinastrum	6	3	3	2	2	5	3	7	8	7	3	9	7	4	6	9
Spirogyra	4	7	7	3	4	8	4	4	3	4	3	2	-	-	2	3
Ulothrix	2	4	5	-	2	3	1	-	-	2	1	1	-	-	-	-
Total	67	91	67	46	48	67	35	61	67	72	72	116	79	43	47	61
BACILLARIOPHYCEAE																
Navicula	2	3	6	14	18	16	18	11	12	11	10	10	8	8	6	6
Nitzschia	1	2	2	4	4	2	4	2	1	2	2	3	2	1	-	-
Synedra	-	-	1	3	2	4	1	3	2	2	2	3	2	4	3	2
Cyclotella	-	1	1	2	1	2	1	1	1	-	1	2	3	2	1	1
Amphora	2	3	2	2	3	3	2	1	1	-	1	2	2	3	3	2
Diatoma	1	2	1	1	2	1	2	1	-	-	-	1	1	-	1	1
Total	6	11	13	26	30	28	28	19	17	15	16	21	18	18	14	12
EUGLENOPHYCEAE																
Euglena acus	14	12	10	10	17	6	10	12	10	11	25	20	9	8	12	5
E.deses	12	6	7	6	5	10	11	10	12	10	10	30	6	7	10	3
Phacus sp.	16	5	10	8	7	8	9	16	8	10	10	13	6	6	14	6
Total	42	23	27	24	22	24	30	38	30	31	45	46	21	21	32	14
DESMIDIACEAE																
Closterium	5	6	5	6	4	6	4	3	5	3	12	10	5	2	13	6
Cosmarium	2	3	2	1	3	1	1	3	3	2	4	4	2	2	2	3
Total	7	9	7	7	7	7	5	6	8	5	16	14	7	4	15	9
Grand Total	142	201	199	181	197	189	124	193	173	153	169	217	165	143	176	172

Table - 4b Distribution and Abundance of Phytoplankton population (No./ mL) in Pond II.

Genera \ Months	Feb. 2001	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 2002	Feb.	March	April	May
MYXOPHYCEAE																
Microcystis	16	35	32	34	37	31	29	10	8	6	11	12	56	24	23	31
Spirulina	-	-	-	-	-	2	4	14	19	18	9	3	4	-	-	-
Anabaena	1	1	-	-	-	-	2	3	3	2	2	-	-	-	-	-
Agmenellum	3	4	3	4	7	5	6	7	6	6	7	5	12	2	5	5
Total	20	40	35	46	44	38	42	34	36	32	29	20	72	26	28	36
CHOLOROPHYCEAE																
Crucigenia	2	4	2	3	3	2	2	3	4	-	4	7	6	3	5	3
Pediastrum	1	1	-	-	-	1	-	2	1	-	-	2	1	-	-	-
Ankistrodesmus	5	7	4	3	4	3	3	5	4	3	5	10	6	4	5	3
Scenedesmus	2	3	3	2	2	1	1	2	1	1	-	3	2	3	4	5
Protococcus	4	7	5	5	4	5	4	9	7	4	2	19	10	7	6	4
Chlorella	1	2	2	1	1	2	1	2	3	1	-	4	1	2	2	1
Tetraspora	3	5	3	2	2	3	2	4	3	2	-	10	2	2	3	2
Actinastrum	4	12	4	4	3	2	3	5	5	3	2	14	3	6	6	3
Spirogyra	-	6	3	-	-	-	-	6	4	-	-	-	-	-	-	-
Ulothrix	-	2	2	-	-	-	-	2	3	-	-	-	-	-	-	-
Total	22	49	28	20	19	19	16	40	35	15	13	58	31	27	31	21
BACILLARIOPHYCEAE																
Navicula	3	2	5	16	4	15	10	10	12	12	18	6	5	7	6	3
Nitzschia	1	2	2	5	5	7	4	2	4	4	5	-	1	3	2	2
Synedra	-	1	1	4	-	8	5	2	2	2	3	2	1	3	2	-
Cyclotella	1	1	1	4	-	6	3	3	3	3	2	1	1	2	1	-
Amphora	-	-	1	1	-	2	-	1	2	2	1	-	-	-	-	-
Diatoma	-	-	-	1	-	-	-	1	-	-	-	-	-	1	-	-
Total	5	6	10	31	9	37	38	19	27	23	29	9	8	16	11	5
EUGLENOPHYCEAE																
Euglena acus	14	16	13	9	11	5	8	6	7	3	5	14	7	11	10	8
E.deses	7	11	6	4	10	3	5	3	4	2	2	12	3	10	10	6
Phacus sp.	7	10	5	4	7	4	3	6	4	3	6	12	3	7	9	6
Total	28	37	24	17	28	12	16	15	15	8	13	38	13	28	29	20
DESMIDIACEAE																
Closterium	9	8	8	6	4	4	5	7	12	3	4	14	6	6	16	15
Cosmarium	5	2	3	1	1	2	1	3	2	2	2	4	4	3	2	1
Total	14	10	11	7	5	6	6	10	14	5	6	18	10	9	18	16
Grand Total	89	142	108	121	105	113	102	118	127	83	90	143	134	106	117	98

matter and nutrients. Bacillariophyceae*:* This group is represented by *Navicula*, *Nitzschia*, *Amphora*, *Synedra*, *Diatoma,* and *Cyclotella*. All these have their own distribution pattern. Among these, *Navicula* was most dominant in pond I and II and showed its presence continuously. It was noticed that this group showed increase in abundance during summer at high temperature. Pearsall (1932) observed that diatoms occurred when the water was rich in nitrates, phosphate, and silicates. Desmidiaceae*: Closterium* sp. and *Cosmarium* sp., which are known to exhibit tolerance with the higher concentration of organic matter, were the only genera noted throughout the period of investigations. *Cosmarium* sp., was found to be less abundant in these ponds as compared to *Closterium* sp. Singh and Pandey (1991) recorded only two genera of desmids in polluted waterbody. Zooplankton: Reice and Wohlenberg (1993) have pointed out that the state of an aquatic system can not be truly understood without the knowledge of zooplankton due to its important role in food chain. Monthly abundance and distribution of different zooplankton species are given in Tables 5 (a & b).

Following group comprised zooplankton in these two ponds. Regarding seasonal fluctuations, zooplankton showed polymodal occurrence (several peaks of maxima) during early summer (March-April), monsoon and post monsoon (June-September) in the year 2001 and January, and April-May in the year 2002 in pond I. Pond II zooplankton showed maxima during summer (March) in the year 2001 and during winter (January) and again during summer (April) in the year 2002. Statistical analysis showed positive and significant correlation between zooplankton and phytoplankton density (Table 3). Rotifera*: Keratella tropica,* one of the most important rotifer species, occurred throughout the study in pond I. Pond II showed its absence during March, 2001, September to January 2002 and March 2002. *Testudinella* sp. was found throughout the study in Pond I. In pond II, it was recorded in the collections of May to October 2001 and January to April 2002. *Brachionus calyciflorus* was recorded throughout the study except in the month of January 2002 in pond I. In pond II, its absence was recorded from May to August 2001 and May 2002. *B. angularis* was recorded from Feb to June 2001 and from Sepember to January 2002 in pond I; from February to April 2001 and from September to April 2002 in pond II. Pennak (1978) remarked that *B. angularis* is often considered bicyclic or perennial. *Filinia longiseta* was observed throughout the year in both these ponds. Maximum density was recorded during July 2001 in pond I, during June 2001 in pond II. *Filinia longiseta* has also been reported by Pejler (1957) as a representative of eutrophic waters. *Notholca sp.* was recorded more frequently in pond I than in ponds II. *Lecane sp.* was encountered during February to July 2001, September to October 2001 and April to May 2002 in pond I, during February to March 2001, May to June 2001,

Table - 5a. Distribution and Abundance of Zooplankton population (No./ L) in Pond I.

Genera \ Months	Feb. 2001	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 2002	Feb.	March	April	May
CLADOCERA																
Daphnia carinata	16	32	31	22	5	16	10	28	23	22	30	52	26	15	12	10
Moina micrura	5	7	4	2	-	-	-	-	5	7	8	18	3	5	7	3
Ceriodaphnia cornuta	2	6	6	4	3	6	3	6	2	3	5	3	2	4	4	3
Leptodora sp.	1	-	-	-	2	2	1	1	-	-	-	1	-	1	-	2
Total	24	45	41	28	10	24	14	35	30	32	43	74	31	25	23	18
COPEPODA																
Cyclops sp.	6	15	10	6	13	10	4	9	3	4	5	4	6	5	8	10
Diaptomus sp.	4	1	8	7	7	6	5	7	2	2	2	3	5	4	7	6
Mesocyclops leuckarti	8	15	12	9	9	8	3	9	6	3	3	6	5	7	14	8
Total	18	40	30	22	29	24	12	25	11	9	10	13	16	16	29	24
ROTIFERA																
Brachionus angularis	2	12	13	18	3	-	-	1	12	10	6	9	-	-	-	-
B.calyciflorus	1	4	7	2	9	3	4	2	4	4	2	-	7	2	6	14
Testudinella sp.	2	2	1	2	3	1	1	3	2	2	2	2	3	1	2	2
Notholca sp.	1	2	2	3	2	1	-	2	1	1	2	1	2	1	2	1
Filinia longiseta	4	5	6	6	14	18	2	3	6	2	3	4	3	2	5	5
Keratella sp.	1	3	2	5	4	5	1	6	4	1	1	2	1	1	3	3
Lecane sp.	1	1	2	2	3	1	-	2	1	-	-	-	-	-	1	2
Total	12	30	33	36	38	29	8	28	29	20	16	18	16	7	19	27
OSTRACODA																
Cypris sp.	3	2	2	2	4	3	5	2	7	4	2	2	4	5	2	1
EGGS	6	2	3	2	8	9	3	7	8	2	5	10	3	2	10	10
NAUPLII	2	2	2	1	6	4	6	4	2	1	2	5	2	-	6	7
Grand Total	64	121	111	91	95	93	48	101	87	68	78	122	72	55	89	87

Table - 5b. Distribution and Abundance of Zooplankton population (No./ L) in Pond II.

Genera \ Months	Feb. 2001	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 2002	Feb.	March	April	May
CLADOCERA																
Daphnia carinata	22	24	13	8	7	5	9	10	16	14	16	28	19	17	18	6
Moina micrura	5	8	4	4	-	1	3	5	6	2	3	6	6	3	3	3
Ceriodaphnia cornuta	3	4	6	4	2	1	3	2	8	3	1	8	3	5	6	3
Leptodora sp.	1	1	3	1	2	1	3	5	2	1	-	2	3	3	1	1
Total	31	37	26	17	11	8	18	21	32	20	20	44	31	28	28	13
COPEPODA																
Cyclops sp.	4	6	9	8	7	8	6	3	5	2	3	5	4	2	7	8
Diaptomus sp.	3	4	7	2	4	3	4	2	2	1	2	2	3	1	5	2
Mesocyclops leuckarti	4	8	7	7	8	6	9	10	3	2	3	4	3	3	7	10
Total	11	18	23	17	19	17	19	15	10	5	7	11	10	6	19	20
ROTIFERA																
Brachionus angularis	8	4	3	-	-	-	-	3	4	6	5	9	8	3	6	-
B.calyciflorus	-	-	4	5	7	3	5	-	-	-	-	-	11	11	3	7
Testudinella sp.	-	-	-	2	3	3	2	1	1	-	-	1	2	1	2	-
Notholca sp.	-	-	-	1	1	-	-	-	-	-	-	-	-	-	1	1
Filinia longiseta	2	2	3	4	7	6	4	2	1	1	2	1	2	1	2	4
Keratella sp.	2	-	2	3	1	1	4	-	-	-	-	-	1	-	2	2
Lecane sp.	1	1	-	1	2	-	1	-	-	-	-	1	-	-	1	2
Total	13	7	12	16	21	13	16	6	6	7	7	12	14	6	17	17
OSTRACODA																
Cypris sp.	2	2	1	1	4	2	2	2	1	2	2	1	1	4	2	4
EGGS	8	6	4	6	7	2	2	3	4	5	7	8	6	4	3	4
NAUPLII	2	6	2	4	3	1	2	1	2	2	4	2	4	1	3	4
Grand Total	67	76	68	61	65	43	59	48	55	41	47	78	66	49	72	62

August 2001, January 2002, and April to May 2002, in pond II. Cladocera*: Daphnia carinata* is abundantly found throughout the study period contributing maximum (52 No./L) in pond I, (28 No./L) in pond II during January, 2002. *Moina micrura* was recorded in all these ponds, but found to be absent from June to September 2001 in pond I and during June 2001 in pond II. These species have been reported to be widespread in the plankton samples of ponds, lakes, and reservoirs of Northern India. *Ceriodaphnia cornuta* was noted throughout the study period in all these ponds. However, its density was always found to be lower than *Daphnia* spp. *Leptodora* sp. is a transparent crustacean and the largest of the cladocera (Cole 1983). It showed its occasional presence with one or two numbers per litre in both ponds. It's occasional presence and least density might be due to the fact that it rises at night and preys on other zooplankton, including *Daphnia*, but during the day it migrates toward deep waters and is not readily found (Cole 1983). It is also reported from freshwaters of Kashmir region (Sharma 2001). Copepoda: *Mesocyclops leuckarti* was found to be the most dominant in both ponds. It was recorded maximum (15 No./L) during March 2001 in pond I, (10 No./L) during September, 2001 and May 2002 in pond II. *Cyclops* sp. formed the second most abundant copepod genera and recorded in maximum numbers (15 No./L and 9 No./L during March, April 2001 in ponds I and II, respectively). *Diaptomus* sp. was recorded maximum (10 No./L and 7 No./L) during March and April and August 2001 in ponds I and II, respectively. This species always encountered in small numbers than other two species. Ostracoda*:* It is represented by *Cypris sp.* only in both ponds. Year round occurrence of eggs of rotifers and crustaceans and nauplii (Table 5 (a & b)) also indicate that these zooplankton (rotifers and crustaceans) are prolific and continuous breeders. The commonly occurring fish species in these ponds are *Channa punctatus*, *Wallago attu*, *Clarias batrachus*, *Heteropneustes fossilis*, *Puntius sophore*, and *Gambusia affinis*.

Conclusions

From the present study it has been concluded that these water bodies are productive. Presence of sufficient nutrients, suitable pH, phytoplankton, zooplankton, plants, and fishes is an indication of healthy and balanced ecosystem. Therefore, we can say that though these water bodies seem derelict they can be made useful for fish culture after adopting proper pond management strategies.

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Received: 31 December 2007; Accepted: 31 October 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Predicting Body Composition of Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Accurate prediction of proximate composition for a commercially cultured fish at a particular body weight could help to reduce overall feed waste, improve feed efficiency, and increase profitability. We studied the relationship between biochemical composition of Nile tilapia and its wet weight for the range of minimum and maximum body weight found in the published literature. We also tested the predictive value of regression equations. Logarithmic trends of the proximate composition showed a linear trend for tilapia up to 0.4 g. The trend formed a plateau for tilapia larger than 5 g. The slopes (*b*) for water, protein, fat, and ash contents as percent body-weight were -0.008, 0.003, 0.003, and 0.002 respectively. The slopes were close to "0" and did not change significantly after removing data from fishes smaller than 5 g in all four cases. Mean percent error of water (-0.145) and protein (-0.769) showed no differences between them. A large percent error mean for fat (-39.179) suggested presence of variations in fat contents to whole body weight. Our findings suggested no significant changes in percent water and percent protein over the life-span of Nile tilapia partially rejecting the null hypothesis that percent composition of Nile tilapia varies over their lifetime.

Introduction

Tilapias have recently gained importance as a valuable component of subsistence and commercial aquaculture production. In 2006, tilapia export to North America has surpassed that of catfish and has become the third most important cultured fish in the world followed by carps and Salmonids. Nile tilapia (*Oreochromis niloticus*) is the main culture species out of the nine tilapia species farmed around the world and is responsible for the significant increase in global tilapia production. Since 1990, global aquaculture production of Nile tilapia has increased almost seven folds and has been increasing exponentially since then (FAO 2005). Ever increasing demand for fish protein carries a lot of potential of future expansion of tilapia aquaculture to improve nutrition and livelihood status of the people in developing countries (Chowdhury and Bureau

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2006). Few species match tilapias in terms of their potential to become the cultured fish of future. A range of systems is suitable for tilapia, from low-input pond and paddy fields to intensive production in feedlot cages and cement tanks. Although genetic improvement indicated that overall tilapia production could be increased through the use of improved strains such as GIFT tilapia (ICLARM-ADB 1998), attaining an even and higher production standard has been a major challenge for the tilapia industry as a whole.

Studies of growth, nutrition, and bioenergetics of fish and other animals often rely on the ability of measuring body composition of individuals accurately. Whole body composition is a key parameter in determining protein for mass balance models used to estimate *N* losses from fish culture facilities (Cho et al. 1994; Cho and Bureau 1998; Silverstein et al. 1999; Cho 2004; Azevado et al. 2004; Dumas et al. 2007). Knowledge of the proximate composition of fish and factors affecting that composition allows assessment of fish health, determination of nutrient transfer efficiency from the feed to the fish, and makes it possible to predictably modify carcass composition (Shearer 1994). Accurate prediction of proximate composition for a commercially cultured fish at a particular body weight could help to reduce overall feed waste, improve feed efficiency, and increase profitability of the operation. Numerous studies analyzed carcass of the fish at the beginning and end of the experiment to determine the change in body composition (Ramseyer 2002), but few have attempted to predict the whole body composition from live body weight (e.g. Northern pike, Salam & Davies 1994). Paucity of information on determining whole body composition of Nile tilapia is one of the impediments because improved feed management efficiency could be attained with the predictability of whole body composition. Ramseyer (2002) predicted whole-fish nitrogen content for 60 fish species and six hybrids, including Nile tilapia, using regression analysis. However, maximum weight of Nile tilapia was 198 g for that calculation, making the developed equation impossible to be used for fish larger than 198 g as stated by Shearer (1994). Albeit a plenty of studies available on tilapia growth and nutrition in published literatures, none covered the whole range of body weight (from fry to market size) to predict proximate composition of the species.

Direct measurements of body composition involve sacrificing the animal, grinding the carcass, and taking a representative subsample for analysis of water, fat, protein, and ash content. An indirect measurement of body composition is thus desirable to avoid destruction of animals and to allow fish farmer's to adjust and optimize the feeding ration more frequently. Strong linear relationship between whole body protein and body weight have been observed for the rats (Miller and Weil 1963), carcass composition and whole body for white-tailed deer (Bois et al. 1997), and 14 species of fish (Groves 1970;

Shearer 1994). The objectives of the study were to: (1) determine whether water, protein, fat, and ash contents of Nile tilapia were related to its wet weight for the range of minimum and maximum body weight found in published literature; (2) determine the difference between the prediction results of log transformed data and absolute data; and (3) present regression equations for predicting fish whole body composition from fish live body weight.

Materials and methods

Whole-body composition and corresponding whole-body weight (WBW) values for Nile tilapia were collected from 34 articles published between 1982 and 2005. Most data were compiled through a manual search of Aquaculture (1983-2004), Aquaculture Research (1996-2005), and Journal of the World Aquaculture Society (1999-2002). Data were selected only from the studies where fish were fed at satiation or near satiation irrespective of the diet composition or types of feed, and where body composition contents were determined by the industry standard methods (AOAC 1990). Any data from studies with variable feeding regimes, or that included fertilization without feeding, or examined the effect of water quality were excluded. Data presented as dry matter content were converted to wet weight for the purpose of our study following Shearer's (1994) recommendation that proximate composition should be reported on a wet basis. A total of 224 samples were taken where the body weight ranged between 0.016 g to 559 g, water content ranged from 66.3% to 81.9%, protein ranged from 10.9% to 18.9%, fat content ranged from 4.9% to 13.0%, and ash content ranged from 1.4% to 7.5%. The mean temperatures ranged between 10°C and 31°C during these experiments.

Univariate linear regressions (ULR) were calculated by the least squares method with both untransformed and log_{10} transformed data. The ULR models for both regressions were

$$Y = b\mathrm{X} + \mathrm{c}\,, \qquad (1)$$

$$LnY = b\mathrm{LnX} + \mathrm{c}, \qquad (2)$$

where Y = untransformed amount (g) of water, protein, fat, and ash contents and X is fish wet weight. The $\log_{10}$ transformed data are presented as $log_{10}\,Y$ and $log_{10}\,X$, respectively. In a regression analysis, when the slope (b) equals 1, both components are increasing at the same rate. When $0<b<1$, then body weight is increasing faster than the component and when $b>1$, the component is making up an increasing portion of the fish. If $b=0$, then the component is not increasing, and if $b<0$, then the absolute amount of component is decreasing.

Absolute amount (g) and percent wet weight of water, protein, fat, and ash were analyzed against the absolute (g), and log_{10} transformed weight were analyzed against

the log_{10} transformed values of WBW. A null hypothesis that percent wet composition components varies significantly for Nile tilapia over its lifetime was tested by regressing percent wet weight of a proximate component against fish wet weight.

Residual plots were examined to assess the validity of the assumption of homogeneous error variance and to detect the presence of outliers. Normal probability plots were examined visually to ascertain whether or not the sample distribution was normal. Body composition predictions were validated by removing the tenth observation into an independent data set ("observed" values). A ULR equation was computed with the remaining data and used to predict body composition values ("predicted values") in the independent data set. A null hypothesis of equality between observed and predicted values was tested with a two-tailed pair sample *t*-test. The percentage error (PE) between observed and predicted values was calculated as

$$PE = \frac{\backslash observed - prediced /}{observed} X\ 100 \qquad (3)$$

Efficacy of using absolute values (g) and log $_{10-}$ transformed values for each proximate component were tested from the percent error of the regressions between each component and WBW.

Results

The logarithmic trends of the percent proximate components showed a linear trend for the fishes up to 0.4 g and formed a plateau for the fishes larger than 5 g (Fig. 1).

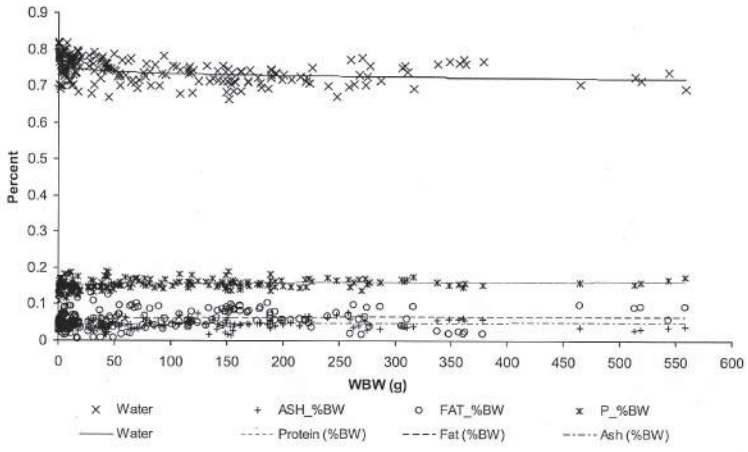

Figure 1. Percent composition of water, fat, ash and protein in relation to whole body weight (WBW) of Nile tilapia (*O.niloticus*)

The slopes (*b*) for percent of water, protein, fat, and ash contents were -0.008, 0.003, 0.003 and 0.002, respectively. In all four cases, the slopes were close to "0" and did not change significantly after removing data from fishes smaller than 5 g (Table 1). Mean PE of percent values of water (-0.145) and protein (-0.769) showed no differences between them. Mean PE was large for percent of fat (-39.179) indicating presence of variations in fat contents in relation to WBW. Regression equation of percent values of water, ash, fat and protein has very little or no value in predicting body composition except for fishes smaller than 1g (Eq. 3: Table1).

Table 1. Relationship of %water, %protein, %fat and %ash contents and whole body weight (WBW) of Nile tilapia; Eq. 1: 0.016-559g; Eq. 2: fishes smaller than 5g; Eq. 3: fishes smaller than 1g; Eq. 4: fishes larger than 5g.

	Water (%)			Protein (%)			Fat (%)			Ash (%)		
	$adjR^2$	*b*	a	$adjR^2$	*b*	a	$adjR^2$	*b*	a	$adjR^2$	*b*	a
Eq.1	0.22	-0.01***	0.77***	0.161	0.00***	0.14***	0.05	0.00***	0.05***	0.08	0.00***	0.04***
		±0.00	±0.00		±0.00	±0.00		±0.00	±0.00		±0.00	±0.00
Eq.2	0.43	-0.02***	0.76***	**0.23**	0.01***	0.14***	0.40	0.01***	0.05***	**0.18**	0.00***	0.04***
		±0.00	±0.00		±0.00	±0.00		±0.00	±0.00		±0.00	±0.00
Eq.3	**0.58**	-0.01***	0.78***	0.194	0.00^{ns}	0.14***	**0.43**	0.01**	0.05***	-0.08	0.00^{ns}	0.03***
		±0.00	±0.00		±0.00	±0.00		±0.00	±0.00		±0.00	±0.00
Eq.4	0.17	-0.01***	0.78***	0.09	0.00***	0.14***	0.05	0.01*	0.04***	0.00	0.00^{ns}	0.04***
		±0.00	±0.00		±0.00	±0.00		±0.00	±0.04		±0.00	±0.00

Note. *b* =coefficient and a = intercept. *** *P<0.0001*, ** *P<0.001*, * *P<0.01* of *t*- values; ^{ns} not significant

No differences were observed between the prediction results of absolute and log-transformed values of water, ash, and protein contents (Table 2).

In all these cases, PE values were relatively low and did not improve much after log-transformation. However, the PE values were much higher for the absolute ash-values and fat-values and were significantly reduced after logarithmic transformation. As a result, the adjusted R^2-values were also improved significantly from 0.89 to 0.98 for the ash and from 0.77 to 0.93 for the fat values.

Table 2. Comparison between the Univariate linear regressions (ULR) of absolute and log transformed values of water, protein, fat and ash contents with whole body weight (g).

	Regression with absolute values				Regression with log transformed values			
	$^1adjR^2$	^2b	^{2}a	PE	$^1adjR^2$	^2b	^{2}a	PE
Water	1.00***	0.73***	0.18	-15.26	1.00***	0.99***	-0.27***	-0.41
		±0.002	±0.338	±8.69		±0.001	±0.005	±0.401
Protein	0.99***	0.16***	-0.22	122.60	1.00***	1.02***	-1.95***	0.66
		±0.001	±0.119	±68.34		±0.003	0.012	±3.460
Fat	0.77***	0.07***	-0.25	589.82	0.93***	1.05***	-3.16***	-86.29
		±0.002	±0.351	±376.24		±0.019	±0.073	±78.510
Ash	0.89***	0.05***	0.10	-283.04	0.98***	1.04***	-3.32***	229.57
		±0.001	±0.158	±146.74		±0.01	±0.039	±194.210

* Significance of F-values from the analysis of variance (***<0.0001); Significance of *t* values (***<0.0001) & ± Standard error deviation from mean. PE is percent error mean Oi-Pi*100/Oi of each regression, b=coefficient and a = intercept of the regressions.

Water and protein content could be predicted from the absolute values by the equations $y = 0.7277WBW + 0.1812$ ($adj\text{-}R^2 = 0.998$) and $y = 0.1607WBW - 0.2194$ ($adj\text{-}R^2 = 0.995$), respectively (Fig. 2A and 2D).

We suggest to use log-transformed values to predict ash and fat content from WBW with the equations $y = WBW^{1.0421} \bullet 10^{-1.4426}$ ($adj\text{-}R^2 = 0.980$) $y = WBW^{1.0473} \bullet 10^{-1.375}$ ($adj\text{-}R^2 = 0.934$), respectively (Fig. 3B and 3C).

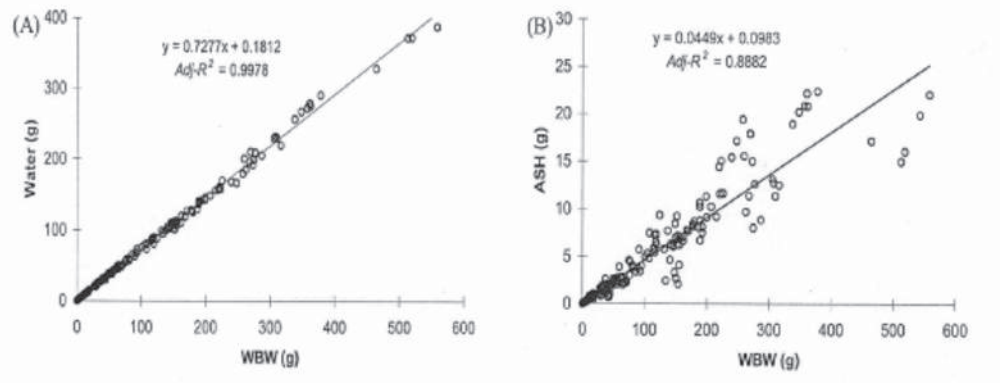

Figure 2. A) Relationship between absolute values of water and whole body weight
B) Relationship between absolute values of ash and whole body weight

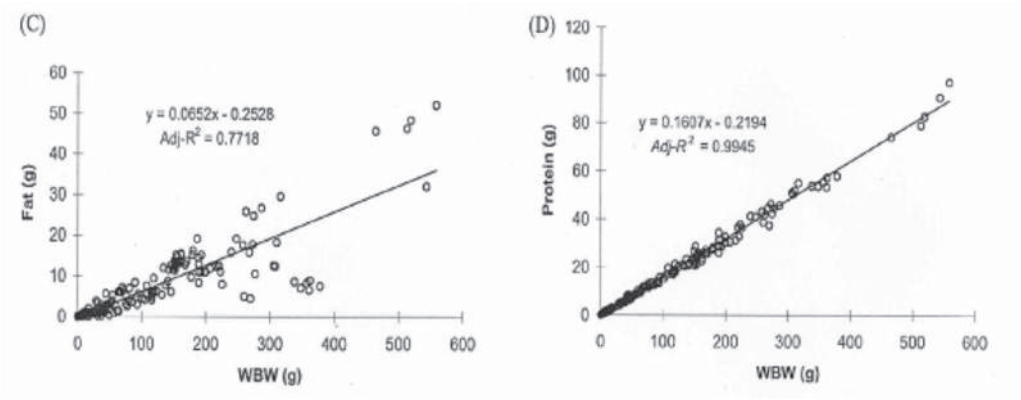

Figure 2. C) Relationship between absolute values of fat and whole body weight
D) Relationship between absolute values of protein and whole body weight

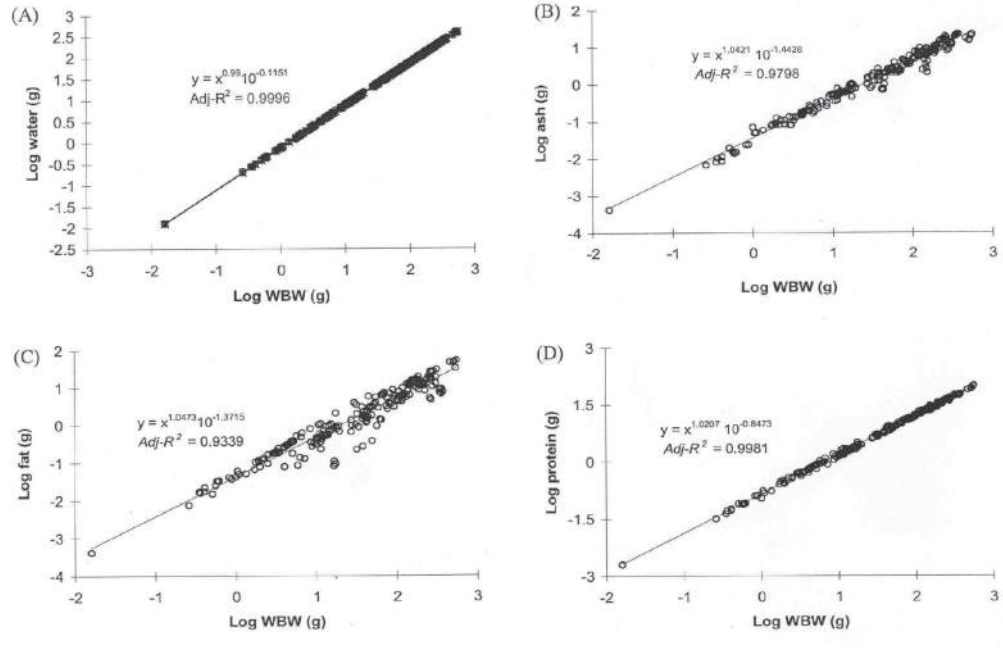

Figure 3:A) Relationship between log-transformed values of water and whole body weight
B) Relationship between log-transformed values of ash and whole body weight
C) Relationship between log-transformed values of fat and whole body weight
D) Relationship between log-transformed values of protein and whole body weight

Discussion

The relationships between nutrient intake and chemical and physical body composition are affected by a range of factors associated with nutrition, genotype, environment and stage of maturity (De Lange et al. 2003). Similar to the terrestrial animals (De Lange et al. 2003), our findings suggested a close association of water content in tilapia body with protein present in both the lean tissue and visceral organs. There were no significant changes in percent values of water and protein of Nile tilapia up to 600 g. Given these similarities, water can be accurately predicted from protein with a reasonable accuracy, using allometric relationships (Weis 2001; De Lange et al.2003).

Large variations in both the percent values of fat and ash contents were observed for tilapia larger than 400 g. Tilapia fed low-protein and low-energy diets, tends to burn body fat to compensate the energy requirement for maintenance. Overall growth rate is reduced increasing the percent protein content. Burned fat is usually replaced by water to maintain the body mass. Ali et al. (2005) observed similar changes in body composition in Rohu (*Labeo rohita* Hamilton), when fish were put in stress induced by fasting. This finding suggests proper adjustment of lipid content in tilapia diet to satisfy the energy requirement to maintain proper growth and suitable flesh quality. While adjusting lipid content in diet, it is important to understand the effect of genotypes in body lipid composition that varies between genotypes or even between strains (Garduno-Lugo et al. 2007).

Knowledge on tilapia body composition at different stages could help to determine the growth requirement for an essential element. Shearer (1984) observed the whole body composition of rainbow trout were homeostatically controlled and were affected by life-cycle stage, fish size, and reproductive state. The effect of reproductive stage on the body composition of commercially farmed tilapia is minimal due to the emphasis on the culture of all-male tilapia. Also genetic improvements of farmed tilapia showed that tilapia could reach market size i.e. upto 600 g before sexual maturity. In conclusion, it needs to be emphasized here that predicting body composition is only a preliminary step to determine dietary requirements for essential elements of fish. Apart from that it also facilitates to model fish growth and protein deposition rate at different dietary levels.

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Received: 23 November 2007; Accepted: 12 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Status of Elasmobranchs Fishery in Chennai, India

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Abstract

Catches of elasmobranchs in India showed an increasing trend from 27.4 thousand t in 1961 to 49 thousand t in 2006. During 2006, among the total elasmobranch catches throughout India, Tamil Nadu contributed substantially with 10.8 thousand tonnes. Observations on elasmobranchs fishery in Chennai for a period of 5 years from 2002–2006 was carried out. In Chennai fisheries harbor, annual elasmobranch catches varied from 489 t to 1735 t for the trawlnets and 194 t to 519 t for mechanized gillnets. In the same harbor, maximum catch of 2074 t of elasmobranchs was recorded in 2002. The contribution of elasmobranch i.e. 4.0%, 16.0% & 2.0% to the trawl, gillnet, and hooks and line (H&L), respectively, with the CPUE of 24.4, 136.7, and 1.3 kg in the respective gears were observed. Trawlers landed heavy catch of more than 100 t of elasmobranchs during June and July with the catch per hour (cph) of 1.4–1.6 kg. Gillnet catches were better during June-September, where monthly catch was above 35 t with CPUE of 203-287 kg. H&L landed good catch during February and March, where the catch was above 1 t with the CPUE of 3.3-4.0 kg.

Catch using trawlnets was dominated by sting rays (74.1%), whereas Carcharhinid sharks (51.1%) were dominant in the catch by mechanized gillnet. The elasmobranchs fishery in Chennai constituted 13 species of sharks, 13 species of rays, and 4 species of guitar fishes. Hammer head shark, *Sphyrna lewini* (*S. lewini*), was dominant among the sharks, with 33.8%, 35.0%, and 37.5% contribution in the trawlnet, mechanized gillnets, and H&L catches respectively, followed by *C.sorrah* and the bull shark *Carcharhinus leucas (C.leucas)*. Among the rays, the contribution of stingray *D. jenkinsii* to the catch was 38.7% using the trawlnets, 31.5% using the gillnet, and 57.8% using the H&L, followed by the lesser devil ray *Mobula diabolus* (*M. diabolus*). Of the four species of guitarfishes, *Rhynchobatus djeddensis* was dominant.

The range of size recorded for *D. jenkinsii* in the trawl catch was 150-1199 mm, whereas the range was from 950 to 2599 mm for *S. lewini* in the gillnet catch. A change in the pattern of fishery was observed during the study period. From 2003 onwards, decrease in the catch of devil ray *M. diabolus* (27.1-148.0 t) was observed. Increase in the catch of bull shark *C. leucas* (5.1–105.4 t) and thresher shark *Alopias vulpinus* (0.9-28.9 t) and decrease in the catch of milkshark *Rhizoprionodon acutus* and spadenose shark *Scoliodon laticatus* were also recorded. The price structure and export markets of various by-products are given.

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Introduction

Elasmobranchs consisting of sharks, sawfishes, rays, and skates form one of the largest marine fish resources, which are exploited by different types of gears such as trawlnet, mechanized (drift) gillnets, and hooks and line (H&L). There was gradual increase in the catch of elasmobranchs from 29401 t in 1961 to 69844 t in 1985. Thereafter, the catches remained approximately 70,000 t until 2005. The Elasmobranchs contributed approximately 4% of the India and 3% of the Tamil Nadu catches (Raje et al. 2002). Among the total elasmobranchs catches, 1.6% (823.6 t) of the catches were from Kasimedu, Chennai, and Tamil Nadu. The present study provides a detailed account of the exploitation of elasmobranch resources by analyzing the data collected from Kasimedu fisheries harbor, Chennai for the 5-year period from 2002–2006.

Materials and methods

Observations regarding the catches were carried out every week at Kasimedu fisheries harbor, Chennai, and data on catch, effort, and species composition were collected for the period from 2002 to 2006. The monthly and annual estimates of catches were calculated following the Stratified Multistage Random Sampling Design adopted by the Fishery Resource Assessment Division of Central Marine Fisheries Research Institute. Length frequency of dominant species of sharks and rays were also collected on the sampling days, and samples of stingray *D. imbricatus* were obtained for biological studies.

Results and discussion

Gear-wise catch

In Tamil Nadu, fishing ban is imposed on the operation of mechanized units from 16 April to 30 May every year. During 2002-2006, at Kasimedu fisheries harbour, Chennai, elasmobranchs contributed 4% (1160 t) to the total fish catch fluctuating between 717 t in 2004 and 2074 t in 2002. The contribution of sharks, rays, and guitar fishes were 23, 67 and 10%, respectively. Elasmobranchs were predominantly landed by trawls (72.5%), followed by gillnets and H&L (Table 1). Third quarter was more productive followed by last quarter (Table 2).

Table 1. Group-wise catch composition (tonnes) of Elasmobranchs during 2002 - 2006 at Chennai

Group	Trawl net						%
	2002	2003	2004	2005	2006	Average	
Sharks	161.7	118.6	68.5	83.8	104.2	107.4	12.8
Rays	1294.8	694.8	369.6	367.6	392.1	623.8	74.1
Guitar fishes	278.7	143.1	50.7	42.4	38.3	110.6	13.1
Total	1735.2	956.5	488.8	493.8	534.6	841.8	100
							72.5
	Gilnet						
	2002	2003	2004	2005	2006	Average	
Sharks	87.8	64.6	137.1	346.2	157.6	158.66	51.1
Rays	237.2	127	89.4	171.4	128.5	150.7	48.5
Guitar fishes	0	2.6	0.1	1	2.5	1.24	0.4
Total	325	194.2	226.6	518.6	288.6	310.6	100
							26.8
	Hooks & Line						
	2002	2003	2004	2005	2006	Average	
Sharks	8	2.6	0.9	1.9	0.4	2.76	34.7
Rays	6.3	13.8	0.7	5.3	0	5.22	65.3
Guitar fishes	0	0	0	0	0	0	0
Total	14.3	16.4	1.6	7.2	0.4	8	100
							0.7
Over all catch	2074.4	1167	717	1019.8	823.6	1160.4	100

Group	Mean catch (t) during 2002-2006	%
Sharks	268.8	23
Rays	779.7	67
Guitar fishes	11.9	10

Table 2. Quarter-wise mean catch (t) of elasmobranchs during 2002-2006 at Chennai

Quarter	Trawlnet	Gillnet	Hooks & Line	Total	%
I	188.7	72.8	4.2	265.7	22.9
II	147.2	63.6	0.6	211.4	18.2
III	274.9	132.9	1	407.4	35.2
IV	214.1	59.7	0.7	274.5	23.7
Total	824.9	329	6.5	1160.4	100

Trawlnet

At Kasimedu fisheries harbor, Chennai, daily and multiday trawlers land their catches. Maximum catches are recorded from multiday trawlers. During the period from 2002 to 2006, the monthly average catch of elasmobranchs varied from 18.1 t to 129.1 t with the CPUE of 15.1 to 28.7 kg for trawlers with the expended effort (units) varying from 1201 in April to 4503 in June. Highest and lowest monthly landings of 129.1 t and 18.1 t were recorded during June and April, respectively (Table 3).

Table 3. Month-wise average catch of elasmobranchs during 2002-2006 at Chennai

Trawlnet							
Month	Effort			Elasmobranchs			
	Units	AFH	Total fish catch (t)	Catch (t)	C/E (kg)	C/h (kg)	%
January	2823	52861	1600.7	69.1	24.5	1.3	4.3
February	2516	51975	1517.8	62.5	24.8	1.2	4.1
March	2747	58289	1558.3	57.1	20.8	1	3.7
April	1201	17846	469.5	18.1	15.1	1	3.9
May	0	0	0.0	0.0	0	0	0
June	4503	82549	3149.4	129.1	28.7	1.6	4.1
July	3972	73064	2443.0	105.7	26.6	1.4	4.3
August	3690	76101	2476.4	90.7	24.6	1.2	3.7
September	3174	67669	1999.6	77.0	24.3	1.1	3.9
October	2728	58681	1709.0	59.9	22	1	3.5
November	3095	67625	1832.4	84.6	27.3	1.3	4.6
December	3317	66414	1853.2	69.6	21	1	3.8
Total	33766	673074	20609.2	823.6	24.4	1.2	4

			Gill net				
January	176	4338	157.7	17.4	99	4	11
February	190	4456	183.9	24.3	127.8	5.5	13.2
March	349	9431	329.5	31.2	89.4	3.3	9.5
April	134	2682	98.3	16.2	121.3	6.1	16.5
May	547	1867	43.3	7.9	14.3	4.2	18.1
June	194	12791	264.0	39.5	203.5	3.1	15
July	162	4859	191.9	36.0	221.8	7.4	18.8
August	230	6065	244.9	49.5	215.1	8.2	20.2
September	165	5152	251.6	47.5	287.1	9.2	18.9
October	94	3264	102.5	15.0	160.4	4.6	14.7
November	50	1000	43.6	11.7	233.6	11.7	26.8
December	117	3725	147.6	33.0	282.5	8.9	22.3
Total	2407	59630	2058.9	329.1	136.7	5.5	16
			Hooks & Line				
January	268	1287	13.6	0.8	2.8	0.6	5.6
February	437	2342	20.7	1.7	4	0.7	8.4
March	513	2313	22.6	1.7	3.3	0.7	7.6
April	389	2257	39.3	0.5	1.3	0.2	1.3
May	145	578	9.2	0.0	0	0	0
June	257	1539	8.5	0.1	0.4	0.1	1.2
July	208	1335	9.7	0.1	0.4	0.1	0.9
August	957	4178	77.3	0.8	0.8	0.2	1
September	465	2784	62.5	0.1	0.2	0	0.1
October	285	1162	16.5	0.1	0.4	0.1	0.7
November	690	2913	16.7	0.3	0.4	0.1	1.6
December	320	1237	20.4	0.3	0.9	0.2	1.4
Total	4933	23925	317.0	6.5	1.3	0.3	2

Mechanized Gillnet

At Kasimedu fisheries harbor, Chennai, mechanized gillnets were operated throughout the year except November mainly for catching tunas, seer fish, and sharks. The mean monthly catch fluctuated between 7.8 t and 49.5 t with the CPUE of 14.3–287.1 kg. (Table 3).

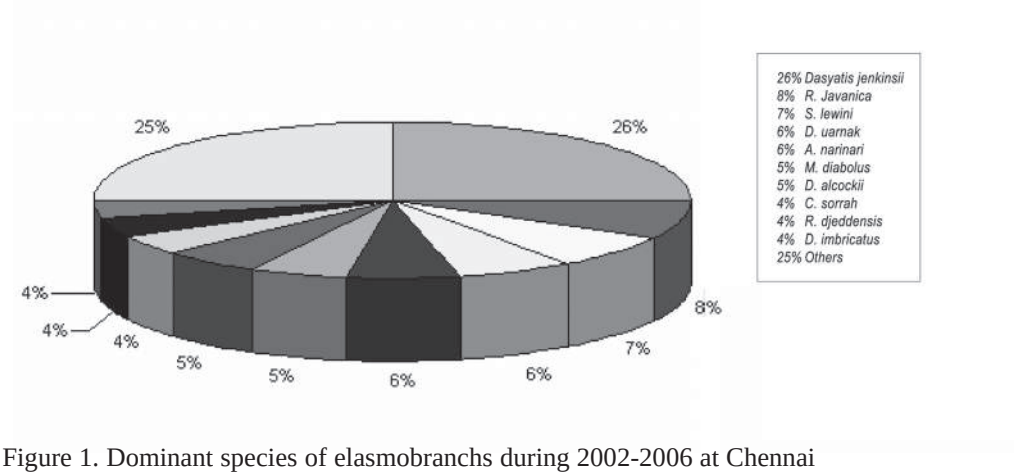

Figure 1. Dominant species of elasmobranchs during 2002-2006 at Chennai

Hooks and line

The elasmobranch catches landed by H&L were dominated by rays followed by sharks. The decreasing trend was discernible from 2002 to 2006. The mean monthly catch fluctuated between 0.08 t in September and 1.7 t in February (Table 3).

Species composition

Among the elasmobranchs, 13 species of sharks, 13 species of rays, and 4 species of guitarfishes were landed at Kasimedu fisheries harbor, Chennai. Of the 30 species that constituted the elasmobranch fishery at Chennai during 2002–2006, rays dominated the elasmobranch fishery with *D. jenkinsii* (25.1%) as the major species followed by *R. javanica* (Fig. 1).

Among 6 genera of rays *viz*., *Dasyatis, Aetobatus, Rhinoptera, Gymnura, Mobula,* and *Manta,* the sting rays *Dasyatis* spp. dominated the catch; 38.7%, 31.5%, and 58.2% of sharpnose stingray *D. jenkinsii* was caught by the trawl, the mechanized gillnet, and the H&L respectively. Among the sharks, the members of the family Carcharhinidae were predominant in the fishery. Eight genera of sharks namely, *Carcharinus, Rhizoprionodon, Sphyrna, Chiloscyllium, Iago, Alopias, Scoliodon, and Galeocerdo* were observed. The hammerhead shark *Sphyrna lewini* dominated the catch constituting 30.3%, 50.8%, and 35.1% in trawl, mechanized gillnet, and H&L catches, respectively, followed by *C. sorrah* and *Carcharhinus leucas* (*C. leucas*). Other dominant species were *Mobula diabolus.* Of the three genera *Rhina, Rhinobatos,* and *Rhynchobatos,* white-spotted shovelnose guitarfish *Rhynchobatos djiddensis* was dominant both in the trawl (42.1%) and gillnet (58.4%) catches (Table 4).

Table 4. Gear- wise species composition of elasmobranchs (t) during 2002-2006 at Chennai

Species	TRAWL NET							GILLNET							HOOKS & LINE							All gear %	% to elas mo
Shark	**2002**	**2003**	**2004**	**2005**	**2006**	**Average**	**%**	**2002**	**2003**	**2004**	**2005**	**2006**	**Average**	**%**	**2002**	**2003**	**2004**	**2005**	**2006**	**Ave rage**	**%**		
C. melanopterus	49.2	22.5	6.2	3.6	3.7	17.0	15.9	34.5	12.2	5.3	11.3	4.5	13.5	8.5	2.9	0.9	0.1	0.7	0.0	0.9	32.8	11.7	2.7
C. sorrah	7.7	12.5	11.2	20.4	24.4	15.2	14.2	0.0	12.0	51.4	80.4	24.6	33.7	21.2	0.0	0.0	0.2	0.1	0.1	0.1	2.7	16.2	4.2
C. limbatus	0.0	14.7	0.0	0.0	0.0	3.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.3
C.leucas	0.0	0.0	1.8	10.1	25.1	7.4	5.9	0.0	5.1	8.3	95.3	57.7	33.3	21.0	0.0	0.0	0.1	0.0	0.2	0.1	2.5	15.2	3.5
C. amblyrhynchoides	0.0	0.0	0.5	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C.brevipinna	0.0	0.0	0.0	0.4	2.7	0.5	0.6	0.0	0.0	0.0	2.7	2.4	1.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.1
R. acutus	34.4	11.0	10.0	22.2	21.4	19.8	18.5	21.4	10.4	8.8	4.9	6.5	10.4	6.5	1.8	0.5	0.0	0.2	0.1	0.5	18.5	11.4	2.6
S. lewini	53.5	40.0	21.9	13.8	13.3	30.3	28.2	31.6	23.2	42.1	110.4	46.5	50.8	32.0	2.3	1.2	0.4	0.9	0.0	1.0	35.1	30.5	7.1
C. griseum	8.2	4.3	1.7	3.2	1.2	3.7	3.5	0.0	0.0	0.0	2.4	0.4	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.4
I. oxyrinchus	0.0	4.5	5.8	0.4	0.1	2.2	2.0	0.0	0.8	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.2
A. vulpinus	8.7	0.0	3.7	2.5	4.4	3.8	3.6	0.0	0.9	20.2	26.4	12.2	11.9	7.5	0.9	0.0	0.1	0.0	0.0	0.2	8.9	5.9	1.4
S. lalicaudus	0.0	0.0	5.8	5.5	7.5	3.8	3.5	0.4	0.0	1.0	11.6	2.4	3.1	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.2	2.5	0.6
G. cuvieri	0.0	0.0	0.0	1.7	0.4	0.4	0.4	0.0	0.0	0.0	0.8	0.2	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1
Total	**181.7**	**118.6**	**68.5**	**83.8**	**104.2**	**107.3**	**100.0**	**87.8**	**84.6**	**137.1**	**346.2**	**157.6**	**158.7**	**100.0**	**8.0**	**2.6**	**0.9**	**1.9**	**0.4**	**2.8**	**100.0**	**100.0**	**23.2**
Ray																							0.0
Dasyatis jenkinsii	526.3	292.0	90.9	158.6	138.4	241.2	38.7	116.1	57.9	18.5	35.9	9.3	47.5	31.5	3.9	7.7	0.3	3.2	0.0	3.0	58.2	37.4	25.1
D.alcockii	119.4	55.3	60.0	13.1	22.4	54.1	8.7	0.0	9.7	16.5	2.0	2.2	6.1	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7	5.2
D.kuhli	39.9	9.5	9.9	11.7	8.5	15.9	2.5	13.3	0.4	0.4	0.7	0.2	3.0	2.0	0.1	1.3	0.0	0.4	0.0	0.4	6.7	2.5	1.7
D.imbricatus	84.0	34.9	27.5	34.9	26.8	41.6	6.7	2.8	3.5	2.2	2.5	0.0	2.2	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.6	3.8
D.uarnak	167.5	51.2	43.4	18.8	24.2	61.0	9.8	26.5	10.1	3.6	10.6	3.3	10.8	7.2	0.9	1.7	0.0	0.6	0.0	0.6	12.4	9.3	6.2
D.sephen	42.7	44.2	16.5	8.4	15.1	25.4	4.1	4.8	4.4	6.4	1.7	3.3	4.1	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8	2.5
D. bleekeri	2.5	12.9	17.5	2.2	9.7	9.0	1.4	0.0	2.9	0.4	2.9	0.7	1.4	0.9	0.0	0.7	0.0	0.0	0.0	0.1	2.8	1.3	0.9
D.zugei	9.1	0.0	3.7	0.0	0.0	2.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2
R. javanica	120.8	89.0	33.2	60.0	47.2	70.0	11.2	44.5	15.0	8.2	27.8	14.8	22.1	14.6	0.0	0.0	0.1	0.0	0.0	0.0	0.4	11.8	7.9
A. narinari	137.3	70.7	40.9	15.6	31.0	59.2	9.5	25.4	14.3	3.4	6.9	4.3	11.0	7.3	1.4	2.4	0.1	1.1	0.0	1.0	19.1	9.1	6.1
G. poecilura	45.3	13.5	13.5	11.4	4.4	17.6	2.8	2.9	3.2	3.0	2.1	0.5	2.3	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	1.7
M. diabolus	0.0	21.6	5.7	32.8	58.0	23.5	3.8	0.0	5.5	21.5	77.7	90.0	38.9	25.8	0.0	0.0	0.1	0.0	0.0	0.0	0.5	8.0	5.4
M. birostris	0.0	0.0	6.9	0.0	6.0	2.6	0.4	0.0	0.0	5.4	0.0	0.0	1.1	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.3
Others	0.0	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.8	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	**1294.8**	**694.8**	**369.0**	**367.6**	**392.1**	**623.8**	**100.0**	**237.2**	**127.0**	**89.4**	**171.4**	**128.5**	**150.7**	**100.0**	**8.3**	**13.8**	**0.7**	**5.3**	**0.0**	**5.2**	**100.0**	**100.0**	**67.2**
Guitarfish																							0.0
R. annylostoma	88.7	42.6	26.5	7.4	12.6	35.6	32.2	0.0	0.1	0.0	0.3	1.4	0.3	26.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	32.1	3.1
R. granulatus	75.1	32.1	12.5	8.4	4.5	26.5	24.0	0.0	0.0	0.0	0.5	0.0	0.1	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.8	2.3
R. djeddensis	114.9	68.4	11.6	22.9	14.9	46.5	42.1	0.0	2.5	0.1	0.2	0.7	0.7	58.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	42.3	4.1
R. obtusus	0.0	0.0	0.0	3.7	6.3	2.0	1.8	0.0	0.0	0.0	0.0	0.4	0.1	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	0.2
Total	**278.7**	**143.1**	**50.7**	**42.4**	**38.3**	**110.0**	**100.0**	**0.0**	**2.6**	**0.1**	**1.0**	**2.5**	**1.2**	**100.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**100.0**	**9.8**
Grand Total	**1735.2**	**956.5**	**488.8**	**493.8**	**534.6**	**841.8**		**325.0**	**194.2**	**226.5**	**518.7**	**288.5**	**310.6**		**14.3**	**16.4**	**1.6**	**7.2**	**0.4**	**8.0**			**100**

By products of elasmobranchs and their market at Chennai

The products of shark that have very high value such as fins, meat, liver oil, liver meal, cartilage, skin, and teeth and fins of guitarfishes and gill rakers of rays are exported from Chennai to Singapore, Thailand, Malaysia, and China. The price structure of the by-product is given in Table 5.

Table 5. Price structure of *Elasmobranchs* by products at Chennai

Product	Size (cm)	Price/kg (dried wt). (in rupees)
Fins of sharks and guitarfishes	Below 40 cm	Rs. 3500
	Above 40 cm	Rs. 4500
	Caudal fin 10–25cm	Rs. 7000
Hammerhead shark fin	Above 25cm	Rs. 4000
Shark teeth	.....	Rs. 1000
M.diabolus gill rakers	.....	Rs. 500

Conclusions

The study indicated that the catches of elasmobranchs decreased over the years from 2074 t in 2002 to 824 t in 2006. The peak landings were recorded during third quarter (July–September) as reported by Devadoss et al. (2000). In Tamil Nadu, 69% of the rays were caught by trawlers (Raje et al. 2002). In Chennai, rays contributed 67% in the elasmobranches catch. Contribution of sharks to the elasmobranchs catch was to the extent of 28% on the east coast (Raje et al. 2002) and at Chennai, it was 23%. Devadoss et al. (1989) reported that the landings of sharks by the gillnets have decreased from 71% during 1981–1985 to 59% during 2002–2006. Devadoss (1984) stated that the species of grey sharks *Carcharinus* spp. contributed 70 to 75% of the shark catch at Chennai, whereas during 2002–2006, *Carcharinus* spp. contribution decreased from 55% to 50%. Raje et al (2002) mentioned that the hammerhead shark *S. lewini* constituted only 12% of the sharks group in Tamil Nadu, whereas in the present study, it was recorded that the hammerhead shark (*S. lewini*) contribution has increased to 35% of the total sharks catch. The emerging small-scale fishery supported by the bull shark *C. leucas* (mostly of females) of Pulicat Lake, Chennai requires special investigation. Catches to the tune of 95 t and 58 t landed during 2005–2006 by trawlers and gillnetters. Eighty percent of the catches of *C.leucas* in Chennai was caught by mechanized gillnets. During the period of study, the landing of a female *C. leucas* with a total length of 3560 mm and weight of 320 kg during June 2005 at Kasimedu fisheries harbor, Chennai was reported. The present record of *C. leucas* is the largest recorded so for. (Rajapackiam et al. unpubl. data).

Pregnant sharks of *C. leucas* were often sighted off Pulicat Lake by the fishermen. This agrees well with the observations made by Devadoss et al. (1989). In November, because of unfavorable weather conditions, the gillnet operation by mechanized boats was stopped. During fishing ban by the mechanized boats, the fisherman operated gillnet in nonmechanized fiber boats. Therefore, the effort was more in operation resulting in low CPUE (14.3 kg).

A noteworthy observation was the quantity of *M.diabolus* catches landed by mechanized gillnets. The cow-nose ray *R. javanica* formed seasonal fishery during November–March. On several occasions, huge shoals formed by females were caught. A giant *Manta birostris* with 5.2 m disc width and weight of 1050 kg was landed by mechanized gillnet at Chennai fisheries harbor during April 2006. For the second time in Chennai fisheries harbor, two fan tail ray *Taeniura melanospila* landed in 2005 when the gillnet was used. The size of female was 150 mm with 70 kg, whereas for the male, it was 140 mm and 60 kg.

Acknowledgements

The authors express their sincere thanks to the Director, Central Marine Fisheries Research Institute, Cochin for permitting to publish this account and Dr.E.Vivakanandan, Head of the Demersal Fisheries division for his encouragement in this work.

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Received: 27 November 2007; Accepted: 04 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Deep-sea Teleostean Species-Diversity off the South West coast of India (7ºN - 10ºN lat.)

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Abstract

During the exploratory fishery survey conducted by M.F.V. Matsya Varshini of the FSI, Kochi base from February 2004 to April 2005 for assessing the abundance and distribution of the nonconventional deep-sea fin fish resources, an attempt has been made to prepare an inventory of the teleostean species-diversity in the 100 to 500 m depth zone off the south-west coast of India. A checklist of the 98 species belonging to 16 orders, 52 families, and 79 genera collected during the period along with the area and depth of collection is presented. The classification is based on Nelson (1984). Of the 98 species, 17 are identified to the generic level only. Even though this is not an exhaustive study, the presence of 16 orders and 98 species in the 100 to 500 m depth zone is a clear indicator of the rich teleostean species-diversity of this region. Global deep-sea demersal fish fauna is represented by 22 fish orders. Out of the 98 species recorded, 63 belong to the pre-perciform orders. The order Perciformes dominated the diversity with 29 species, followed by the order Lophiiformes (ten species) and order Scorpaeniformes (nine species). Families Myctophidae and Macrouridae with five species each topped in species diversity. Out of the 52 families, 22 families were represented by a single species. Families Cepolidae, Uranoscopidae, and Ariommatidae were represented in the 100-200 m depth zone only. A comparative account of the number of species recorded during previous surveys is furnished. Diversity indices using catch data collected through surveys of the south west coast of India have been worked out and explained in order to relate it with abundance indices. In future fishery surveys, emphasis must be given to exhaustive species-diversity studies and to make available specimens to facilitate the bar coding of the species.

Introduction

In India, deep-sea fishes are rapidly gaining importance as a potential resource, as the inshore fishery alone can no longer satisfy the growing demand for fish. Present trends in the landings indicate that most of our coastal fishery resources are either fully exploited or over exploited. Deep-sea sector beyond 100 m depth contour is considered as an important zone for the nonconventional fin fishes. Various surveys carried out by

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different agencies point out their presence in the above area. However, there is a lot of gap in the scientific knowledge of distribution and abundance of the above resource.

Biodiversity of deep- sea fishes of the world has always remained a challenge to eminent ichthyologists and taxonomists. Classical works of well-known ichthyologists and naturalists have thrown light into the peculiarities of these fish and has also given valuable information on the deep-sea ichthyodiversity of the world. The works of Day (1878); Gunther (1887); Alcock (1891, 1899); Goode and Bean (1895); Norman (1939); Marshall (1954,1974) and Smith and Heemstra (1986) are considered as important scientific contributions in the systematics of deep-sea fishes. Information on the biodiversity of unconventional fin fishes off the south west coast of India is based on the works of Samuel (1963), Tholasilingam et al. (1964); Silas and Prasad (1966); Oommen (1978,1980,1985); Joseph and John (1986); Balachandran and Nizar (1990); Khan et al (1996); and Venu and Kurup (2002). Identification of the important components of the resources and assessing their biomass are major prerequisites to formulate future plans for tapping them. Above knowledge about the resources helps the scientist and planners to recommend the sustainable yield and also the effort required to exploit the above stock. As a first step towards this an attempt has been made to understand the diversity of the deep-sea teleosts off the south west coast of India (7^0 N to 10^0N Lat.).

Materials and methods

M.F.V. Matsya Varshini, a purse-seiner cum stern trawler based at Kochi base of Fishery Survey of India conducts demersal trawl survey in south west coast, Wedge Bank and Gulf of Mannar. Exploratory fishing data of the above survey during the period between February 2004 and April 2005 is the base of this study. 100 to 500 m depth zone off the south west coast of India lying between 7^0N to 10^0N Latitude (Lat.) was the area of study. The vessel conducted 13 voyages during the period between February 2004 and April 2005. A total of 54 hauls have been made in the study area spending an effort of 60.33 hours. Out of the 54 hauls, eight hauls were made at 100 to 200 m depth strata and 46 hauls were made in the area between 200 and 500m depth zone. Distribution of hauls carried out during the period is shown in Fig. 1.

Detailed survey data during the months of March 2004, November 2004, and April 2005, during which the first author has participated onboard as scientist participant and cruise leader were utilized for estimating the species diversity index of the finfish

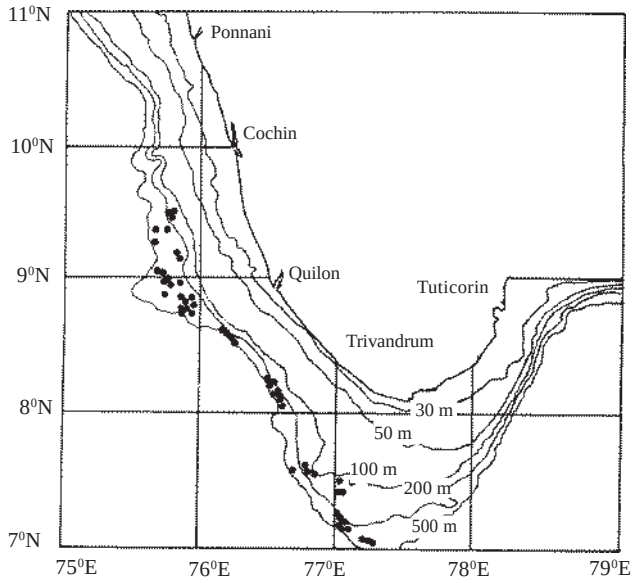

Figure1. Distribution of effort (hauls) during the period February 2004 to April 2005.

resources. A total of 24 hauls spending an effort of 28.58 hours have been carried out during the said period. A 45.6 m Expo model fish trawl and 45.12 m shrimp trawl were the gears used for the resource survey. Catches were sorted out-group wise/species wise immediately after each haul. The weight of each group/species was recorded to find out the species composition of the catches. Deck sampling procedures outlined by Pauly (1980) was followed to record the catches. Necessary entries were made in the catch data sheets for further analysis. Specimens collected from the haul were immediately photographed by using a digital camera. Specimens were identified up to species level by using standard references (Day 1878; Goode & Bean 1895; Alcock 1899; Munro 1955; Fischer & Bianchi 1984; and Smith & Heemstra 1986). Lat-wise diversity indices were estimated by following Shannon's diversity index (H) and Shannon's equitability (E_H) (Begon et al. 1996).

Results

During the period under study a total of 98 species belonging to 16 orders, 52 families, and 79 genera of non-conventional finfishes are recorded from the area. Out of the 98 species recorded from the area 63 species belong to pre-perciform orders. Except for the four species recorded from 100 to 200 m depth zone, all others are true deep-sea fishes. Out of the 52 families 22 are represented by single species. Family

Myctophidae and Macrouridae with 5 species each dominated over others in species richness. Check list of finfishes collected during the study is prepared following Nelson (1984). Areas of specimen collection, depth of collection, and total length of specimen used for the identification of the species are added in the checklist (Table. 1).

Table 1. Check List of Non-conventional Finfishes Collected

S.No	Species name	Common name	Area of collection Lat (N)/ long (E)	Depth (m)	Total length (cm)
(1)	(2)	(3)	(4)	(5)	(6)
INFRADIVISION: ELOPOMORPHA, ORDER: ANGUILLIFORMES, SUBORDER: ANGUILLOIDEI					
I	FAMILY: CONGRIDAE	Conger eels			
1	*Ariosoma* sp.	Conger	08^{0}52.5’ 75^{0}45.0’	340	29.3
2	*Bathycongrus guttatus* (Gunther, 1887)		09^{0}20.2’ 75^{0}44.4’	357	30.0
3	*Coloconger raniceps* Alcock, 1889	Frog head conger	09^{0}20.2’ 75^{0}44.4’	357	24.5
II	FAMILY: MURAENESOCIDAE	Pike congers			
4	*Gavialiceps taeniola* (Woodmason, in Alcock, 1889)		08^{0}15.7’ 76^{0}30.7’	455	39.9
III	FAMILY: NEMICHTHYIDAE	Snipe eels			
5	*Nemichthys acanthonotus* Alcock, 1894	Slender snipe eel	08^{0}34.5’ 76^{0}13.1’	282	57.0
INFRADIVISION: EUTELEOSTEI, SUPERORDER: PROTOCANTHOPTERYGII, ORDER: SALMONIFORMES; SUBORDER : ARGENTINOIDEI					
IV	FAMILY: ALEPOCEPHALIDAE	Slickheads			
6	*Rouleina squamilatera* (Alcock, 1898)	Blunt snout slickhead	08^{0}06.8’ 76^{0}39.9’	461	22.0
SUPERORDER: STERNOPTERYGII, ORDER: STOMIIFORMES, SUBORDER GONOSTOMATOIDEI					
V	FAMILY: STERNOPTYCHIDAE	Hatchetfishes			
7	*Polyipnus spinosus* Gunther, 1891		09^{0}20.2’ 75^{0}44.4’	357	6.4
VI	FAMILY: PHOTICHTHYIDAE	Lightfishes			
VII	FAMILY: CHAULIODONTIDAE	Viperfishes			
9	*Chauliodus sloani* Schneider, 1801	Sloan’s viperfish	08^{0}59.6’ 75^{0}46.3’	334	16.3
VIII	FAMILY: ASTRONESTHIDAE	Snaggletooths			
10	*Astronesthes martensii* Kluzinger, 1871	Astronesthid fish	09^{0}20.2’ 75^{0}44.4’	357	11.9
11	*Astronesthes trifibulatus* Gibbs, Amaoka &Haruta, 1984		08^{0}15.7’ 76^{0}30.7’	455	12.9
IX	FAMILY: MALACOSTEIDAE	Loosejaws			
12	*Photostomias* sp.		08^{0}15.7’ 76^{0}30.7’	455	17.8

Table 1 Continued

(1)	(2)	(3)	(4)	(5)	(6)
SUPERORDER: SCOPELOMORPHA, ORDER: ALULOPIFORMES, SUBORDER: AULOPOIDEI					
X	FAMILY: CHLOROPHTHALMIDAE	Greeneyes			
13	*Chlorophthalmus agassizi* Bonaparte, 18403	Short nose greeneye	09^{0}20.2' 75^{0}44.4'	357	19.2
14	*Chlorphthalmus bicornis* Norman, 1934	Spiny jaw greeneye	09^{0}20.2' 75^{0}44.4'	357	10.4
15	*Chlorophthalmus punctatus* Gilchrist, 1904	Spotted greeneye	08^{0}52.5' 75^{0}49.1'	336	9.1
SUBORDER ALEPISAUROIDEI					
XI	FAMILY: PARALEPIDIDAE	Barracudinas			
16	*Stemonosudis rothschildi* Richards, 1967		09^{0}20.2' 75^{0}44.4'	357	26.7
17	*Lestrolepis intermedia* (Poey, 1868)		09^{0}20.2' 75^{0}44.4'	357	16.5
18	*Neoscopelus macrolepidotus* Johnson, 1863	Large scaled lanternfish	09^{0}11.5' 75^{0}48.4'	372	17.2
XIII	FAMILY: MYCTOPHIDAE	Lanternfishes			
19	*Diaphus splendidus* (Brauer, 1904)		09^{0}20.2' 75^{0}44.4'	357	16.5
20	*Diaphus antonbruuni* Nafpaktitis, 1978		08^{0}14.2' 76^{0}32.4'	435	15.3
21	*Diaphus* sp.		08^{0}45.0' 75^{0}53.0'	410	7.8
22	*Diaphus* sp.		08^{0}08.4' 76^{0}36.4'	418	6.6
23	*Lampadena luminosa* (Garman, 1899)		08^{0}15.7' 76^{0}30.7'	455	12.1
SUPERORDER: PARACANTHOPTERYGII,ORDER: GADIFORMES,SUBORDER :GADOIDEI					
XIV	FAMILY: MORIDAE	Deep-sea cods			
24	*Physiculus argyropastus* Alcock, 1894		09^{0}20.2' 75^{0}44.4'	357	26.5
25	*Gadella* sp.		08^{0}52.5' 75^{0}45.0'	340	22.7
SUBORDER: MACROUROIDEI					
XV	FAMILY: MACROURIDAE	Grenadiers			
26	*Malacocephalus laevis* (Lowe, 1843)	Soft-head grenadier	09^{0}20.2' 75^{0}44.4'	357	27.8
27	*Malacocephalus* sp.		08^{0}14.2' 76^{0}32.2'	435	37.8
28	*Mesobius* sp.		08^{0}15.7' 76^{0}30.7'	455	13.2
29	*Coelorinchus quadricristatus* (Alcock, 1894)		08^{0}06.8' 76^{0}39.9'	461	21.0
30	*Coryphaenoides macrolophus* (Alcock, 1889)		08^{0}06.8' 76^{0}39.9'	461	15.3
ORDER: OPHIDIFORMES, SUBORDER: OPHIDIOIDEI					
XVI	FAMILY: OPHIDIIDAE	Cusk-eels			
31	*Neobythites macrops* (Gunther, 1889)		08^{0}15.7' 76^{0}30.7'	455	25.9
32	*Neobythites* sp.		08^{0}34.5' 76^{0}13.0'	340	14.3
33	*Hypopleuron caninum* Smith &Radcliffe, 1913		08^{0}34.5' 76^{0}13.0'	340	40.5
ORDER: LOPHIIFORMES, SUBORDER: LOPHIOIDEI					
XVII	FAMILY: LOPHIIDAE	Monks/Angler			
34	*Lophiodes mutilus* (Alcock, 1893)	Smooth angler	09^{0}11.5' 75^{0}48.4'	372	23.1
35	*Lophiodes* sp.	Angler	08^{0}52.5' 75^{0}45.0'	340	7.0

Table 1 Continued

(1)	(2)	(3)	(4)	(5)	(6)
SUBORDER: ANTENNAROIDEI					
XVIII	FAMILY: CHAUNACIDAE	Sea toads			
36	*Chaunax pictus* Lowe, 1846	Pink frog- mouth	09^{0}15.0' 75^{0}42.6'	369	18.7
37	*Chaunax endeavouri* Whitley, 1929	Coffinfish	08^{0}14.2' 76^{0}32.2'	435	21.1
38	*Chaunacops melanostomus* Caruso, 1989		08^{0}14.2' 76^{0}32.2'	435	5.1
XIX	FAMILY: OGCOCEPHALIDAE	Sea bats			
39	*Halieutaea coccinea* Alcock, 1889	Spiny sea bat	09^{0}20.2' 75^{0}44.4'	357	20.6
40	*Halieutaea nigra* Alcock, 1891		08^{0}50.2' 75^{0}56.8'	330	7.0
41	*Halieutaea stellata* (Vahl, 1797)	Starry hand fish	08^{0}50.2' 75^{0}56.8'	330	11.5
SUBORDER: CERATIOIDEI					
XX	FAMILY: DICERATIIDAE	Horned anglers			
42	***Ceratius (Diceratias) bispinosus* (Gunther, 1887)**	Two rod anglerfish	08^{0}14.2' 76^{0}32.2'	435	11.4
43	*Phrynichthys wedli* Pietschman, 1926		08^{0}11.6' 76^{0}32.2'	490	10.7
SUPERORDER: ACANTHOPTERYGII, SERIES: PERCOMORPHA, ORDER: LAMPRIFORMES,SUBORDER: ATELEOPODOIDEI					
XXI	FAMILY: ATELEOPODIDAE	Tadpole fishes			
44	*Ateleopus indicus* Alcock, 1891		08^{0}14.2' 76^{0}32.2'	435	34.2
ORDER: BERYCIFORMES,SUBORDER :BERYCOIDEI					
XXII	FAMILY: TRACHICHTHYIDAE	Slimeheads			
45	*Gephyroberyx darwini* (Johnson, 1866)	Darwin's slimehead	08^{0}14.2' 76^{0}32.2'	435	9.3
46	*Hoplostethus mediterraneus* Cuvier, 1829	Mediterranian slimehead	08^{0}14.2' 76^{0}32.2'	435	6.5
XXIII	FAMILY: BERYCIDAE	Berycids			
47	*Beryx splendens* Lowe, 1834	Slender beryx	09^{0}20.2' 75^{0}44.4'	357	15.2
XXIV	FAMILY: HOLOCENTRIDAE	Squirrelfishes			
48	*Ostichthys acanthorhinus* Randal, Soldier fishShimizu & Yamakava, 1982		08^{0}50.2' 75^{0}56.8'	330	13.5
SUBORDER:POLYMIXIOIDEI					
XXV	FAMILY: POLYMIXIIDAE	Beardfishes			
49	*Polymixia japonicus* Gunther, 1877	Silver eye	07^{0}08.2' 77^{0}04.8'	226	11.2
50	*Polymixia fusca* Kotthaus, 1970		07^{0}08.2' 77^{0}04.8'	226	10.3
ORDER :ZEIFORMES					
XXVI	FAMILY: ZEIDAE	Dories			
51	*Zenopsis conchifer* (Lowe, 1850)	Silver John dory	09^{0}20.2' 75^{0}44.4'	357	36.1
52	*Cyttopsis roseus* (Lowe, 1843)	Rosy dory	09^{0}20.2' 75^{0}44.4'	357	16.4
ORDER: SYNGNATHIFORMES, SUBORDER: AULOSTOMOIDEI					
XXVII	FAMILY: MACRORAMPHOSIDAE	Snipefishes			
53	Macroramphosus *sp.*		07^{0}08.2' 77^{0}04.8'	226	8.7

Table 1 Continued

(1)	(2)	(3)	(4)	(5)	(6)
SUBORDER: SYNGNATHOIDEI					
XXVIII	FAMILY: SYNGNATHIDAE	Pipefishes			
54	*Syngnathus acus* Linnaeus, 1758	Long snout pipefish	08^{0}50.2' 75^{0}56.8'	330	20.2
ORDER: SCORPAENIFORMES SUBORDER: SCORPAENOIDEI					
XXIX	FAMILY: SCORPAENIDAE	Scorpionfishes			
55	*Setarches quentheri* Johnson, 1862	Deep- water scorpion	08^{0}34.5' 76^{0}13.1'	282	10.2
56					0.6
	Setarches longimanus (Alcock, 1894)		09^{0}15.2' 75^{0}42.6'	369	0.6
57	*Ectreposebastes imus* Garman, 1899	Mid- water scorpion	08^{0}15.7' 76^{0}30.7'	455	11.2
XXX	FAMILY: TRIGLIDAE, SUBFAMILY: TRIGLINAE	Gurnards			
58	*Lepidotrigla* sp.		08^{0}59.6' 75^{0}46.3'	330	13.5
59	*Pterygotrigla hemisticta* (Temminck &Schlegel, 1842)	Black spotted gurnard	08^{0}59.6' 75^{0}46.3'	330	15.1
	SUBFAMILY: PERISTEDIINAE	Armoured gurnards			
60	*Satyrichthys adeni* (Lloyd, 1907)		08^{0}34.5' 76^{0}13.1'	282	29.9
61	*Satyrichthys* sp.		09^{0}15.2' 75^{0}42.6'	369	22.0
62	*Peristedion investigatoris* (Alcock, 1898)		09^{0}15.2' 75^{0}42.6'	369	12.4
63	*Peristedion halyi* (Day, 1888)		08^{0}34.5' 76^{0}13.1'	282	7.9
ORDER: PERCIFORMES, SUBORDER: PERCOIDEI					
XXXI	FAMILY: PERCICHTHYIDAE	Acropomatids			
64	*Acropoma japonicum* Gunther, 1859	Glowbelly	08^{0}59.6' 75^{0}46.3'	334	14.7
65	*Synagrops japonicus* (D'Oderelein, 1884)	Japanese splitfin	08^{0}06.8' 76^{0}39.9'	461	15.2
66	*Synagrops pellucidus* (Alcock, 1889)		07^{0}08.2' 77^{0}04.8'	226	10.2
67	*Neoscombrops annectens* Gilchrist, 1922	Scomber splitfin	08^{0}59.6' 75^{0}46.3'	334	12.3
XXXII	FAMILY: SERRANIDAE	Rock cods			
68	*Chelidoperca investigatoris* (Alcock, 1895)		07^{0}08.2' 77^{0}04.8'	226	11.2
XXXII	FAMILY:OSTRACOBERYCIDAE	shellskin alfonsinos			
69	*Ostracoberyx dorygenys* Fowler, 1934		08^{0}59.6' 75^{0}46.3'	330	8.7
XXXIV	FAMILY: EMMELICHTHYIDAE	Rovers			
70	*Emmelichthys nitidus*	Bonnet- mouth	08^{0}50.2' 75^{0}56.8'	330	20.5
XXXV	FAMILY: BATHYCLUPEIDAE	Bathyclupeids			
71	*Bathyclupea hoskynii* (Alcock, 1899)		09^{0}20.2' 75^{0}44.4'	357	12.9

Table 1 Continued

(1)	(2)	(3)	(4)	(5)	(6)
XXXVI	FAMILY: OWSTONIIDAE				
72	*Owstonia totomiensis* Taneka, 1908		07^{0}08.2' 77^{0}04.8'	226	36.8
XXXVII	FAMILY: CEPOLIDAE	Bandfishes			
73	*Acanthocepola limbata* (Valenciennes, 1835)	Bandfish	07^{0}33.8' 76^{0}50.3'	121	57.2
SUBORDER: TRACHINOIDEI					
XXXVIII	FAMILY:CHAMPSODONTIDAE	Gapers			
74	*Champsodon vorax* Gunther, 1867		08^{0}59.6' 75^{0}46.3'	330	5.9
XXXIX	FAMILY: URANOSCOPIDAE	Stargazers			
75	*Ichthyoscopus inermis* (Cuvier, 1829)		07^{0}33.8' 76^{0}50.3'	121	24.3
76	*Uranoscopus* sp.	Stargazer	07^{0}33.8' 76^{0}50.3'	121	19.7
77	*Xenocephalus elongatus elongates* (Temminck &Schlegel, 1843)		07^{0}08.8' 77^{0}04.3'	226	27.2
Xl	FAMILY: PERCOPHIDAE	Duckbills			
78	*Bemprops caudimacula* Steindachner, 1877		09^{0}20.2' 75^{0}44.4'	357	15.2
XLI	FAMILY: MUGILOIDIDAE	Sandsmelts			
79	*Parapercis* sp.		07^{0}08.2' 77^{0}04.8'	226	20.1
SUBORDER: CALLIONYMOIDEI					
XLII	FAMILY: CALLYONYMIDAE	Dragonets			
80	*Callionymus carebares* Alcock, 1890	Deep- water dragonet	09^{0}19.2' 75^{0}49.7'	249	12.8
SUBORDER :GOBIOIDEI					
XLIII	FAMILY: GOBIIDAE	Gobies			
81	*Gobius cometes* Alcock, 1899		08^{0}34.5' 76^{0}13.1'	282	10.1
SUBORDER: SCOMBROIDEI					
XLIV	FAMILY: GEMPYLIDAE	Snake mackerels			
82	Neoepinnula orientalis Sackfish(Gilchrist & Von Bonde, 1924)		09^{0}20.2' 75^{0}44.4'	357	17.4
83	*Ruvettus pretiosus* (Cocco, 1833)	Oilfish	09^{0}11.5' 75^{0}48.4'	372	33.9
84	*Promethichthys prometheus* (Cuvier, 1832)	Promethean escolar	09^{0}20.2' 75^{0}44.4'	357	16.8
	Rexea prometheoides (Bleeker, 1856)	Royal escolar	08^{0}14.2' 76^{0}32.2'	435	17.3
XLV	FAMILY: TRICHIURIDAE	Ribbon fishes			
86	*Benthodesmus elongatus* (Clarke, 1879)	Elongate frost fish	09^{0}15.2' 75^{0}42.6'	369	33.1
87	*Benthodesmus tenuis* (Gunther, 1877)	Slender frost fish	08^{0}43.2' 75^{0}58.4'	401	54.8
88	*Benthodesmus tuckeri* Parin & Becker, 1970	Tucker's frost fish	08^{0}43.2' 75^{0}58.4'	401	53.8

Table 1 Continued

(1)	(2)	(3)	(4)	(5)	(6)
89	*Trichiurus auriga* Klunzinger, 1884	Pearly hair tail	08^{0}06.8' 76^{0}39.9'	461	30.1
XLVI	FAMILY: CENTROLOPHIDAE	Ruffs /Medusafishes			
90	*Psenopsis cyanea* (Alcock, 1890)	Indian ruff	09^{0}20.2' 75^{0}44.4'	357	19.2
XLVII	FAMILY: NOMEIDAE	Drift fishes			
91	*Psenes squamiceps* (Lloyd, 1909)	Indian driftfish	08^{0}59.6' 75^{0}46.3'	334	18.1
XLVIII	FAMILY: ARIOMMATIDAE	Ariommatids			
92	*Ariomma indica (Day, 1870)*	Indian ariomma	07^{0}33.8' 76^{0}50.3'	121	14.6
ORDER: PLEURONECTIFORMES, SUBORDER: PLEURONECTOIDEI,					
XLIX	FAMILY: BOTHIDAE	Lefteye flounders			
93	*Citharichthys* sp.		09^{0}20.2' 75^{0}44.4'	357	14.9
94	*Chascanopsetta lugubris* Alcock, 1899	Pelican flounder	09^{0}15.2' 75^{0}42.6'	369	25.7
95	*Laeops macrophthalmus* (Alcock, 1889)		09^{0}11.5' 75^{0}48.4'	372	14.0
SUBORDER :SOLEOIDEI					
L	FAMILY: CYNOGLOSSIDAE	Tongue soles	08^{0}34.5' 76^{0}13.1'	282	9.7
96	*Symphurus* sp.				
ORDER: TETRAODONTIFORMES, SUBORDER :BALISTOIDEI					
LI	FAMILY: BALISTIDAE	Triggerfishes			
	SUBFAMILY:MONOCANTHINAE	Filefishes			
97	*Alutera scripta* Berry & Vogele, 1961	Scrawled filefish	08^{0}14.2' 76^{0}32.2'	435	45.4
SUBORDER: TETRAODONTOIDEI					
LII	FAMILY: TETRAODONTIDAE	Puffers			
98	*Amblyrhynchotes spinosissimus* (Regan, 1908)	Spiny blassops	07^{0}33.8' 76^{0}50.3'	121	12.0

In addition to the above, conventional finfishes like *Nemipterus* spp., *Saurida* spp., and *Priacanthus* spp. were also recorded from the 100 to 200 m depth zone. Latitude-wise species richness (S), Shannon's diversity index (H) and Shannon's equitability (E_H) and the Biomass estimated for selected species/groups (Sajeevan and Nair 2006) are furnished in Table 2.

Table 2. Latitudinal wise species diversity of nonconventional fin fishes off the S.W. Coast of India (7^0- 10^0N lat.) between 100 and 500 m depth.

Parameters	Lat. 7^0N-8^0N	Lat. 8^0N-9^0N	Lat. 9^0N-10^0N	Lat. 7^0N-10^0N
Species richness (S)	66	95	82	98
Shannon's diversity index (H)	1.429	1.662	2.493	1.957
Shannon's equitability (E_H)	0.341	0.366	0.564	0.427
Biomass (B) in tonnnes	52504.77	38219.04	7718.35	98442.16

Discussion

Global fish fauna comprise over 25000 species and of these10 to15% are found in the deep-sea environment. According to Cohen (1970) 1010 deep demersal fish and 1280 deep pelagic species are represented in the world ocean. Myers (1940) observed that Indo-Pacific fish fauna is the richest among the four tropical fish fauna. This is evident from the richness of the inshore fish fauna of the area. But only very little knowledge is available regarding the diversity of offshore fishes. Joseph and John (1986) reported that in contrast to the inshore region, the offshore region is poor in diversity represented by only a few species. Results of the present study, which recorded 98 species of non-conventional finfishes belonging to 16 orders, point out the richness of the offshore finfish diversity. This richness of the fauna becomes more clear when we consider the fact that there are only 22 orders of deep demersal fish fauna distributed all over the world (Helfman et al. 2003). As seen in Table 2, Lat. 8^0N to 9^0N dominates in species richness, but Lat. 9^0N to 10^0N dominates in the diversity and evenness. Diversity and biomass of non-conventional finfishes off the south west coast of India shows an inverse relationship. Diversity increases towards the northern latitudes but biomass decreases. Abundance of pearly hair tail *Trichiurus auriga*, Trichiuridae and Indian ruff *Psenopsis cyanea*, Centrolophidae in southern latitude may be a reason for the above phenomena.

A comparative statement of the number of deep-sea species recorded by different authors from the Indian region is furnished in the Table 3.

Oommen (1980) reported 63 species of fishes from the deep waters of the Quilon Bank. Bottom trawls were used for the above survey. Balachandran and Nizar (1990) reported 87 species of nonconventional finfishes from the Indian EEZ. Both bottom and pelagic trawls were used for this study. Khan et al. (1996) reported 34 species from the southeastern Arabian Sea. Demersal trawl nets were used for the above survey.

Table 3. Comparative statement of number of species of nonconventional deep-sea fin fishes recorded by different authors.

Authors	Area	Depth (m)	Number of species reported	Remarks
Oommen, 1980	QuilonBank (8^0-9^0N lat.)	175-370	63	Include 5 species of Elasmobranchs
Balachandran and Nizar, 1990	Indian EEZ	100-4524	87	Include both pelagic demersal deep-sea finfishes
Khan et al. 1996	South-eastern Arabian Sea (8^0-13^0N lat.)	170-777	34	Demersal fin fishes
Venu and Kurup, 2002	West coast of India (7^0-21^0N lat.)	201-750	23	Demersal fin fishes
Present study	South-west coast of India (7-10^0 N lat.)	100-500	98	Non-conventional demersal fin fishes

Venu and Kurup (2002) reported 23 species from the west coast of India. Bottom trawl nets were used for the survey. Major objective of all the above surveys was the study of distribution and abundance of the deep-sea finfish resources. Perhaps not much attention was paid to study the species diversity. The total number of 151 species were recorded by the above surveys from waters deeper than 100 m. Out of the 98 species of nonconventional fin fishes recorded during the present study, 56 species were not reported by the above authors. So the total number of nonconventional finfish species from the deeper waters of the Indian EEZ comes to 207. Alcock (1899) reported 169 deep-sea finfish species from the continental slopes of the Indian Ocean. Certainly there could be many more species that have not been represented in the samples or have been overlooked. An exhaustive search in http://www.fishbase.org, regarding the country-wise occurrence of deep-sea fishes show that out of the 98 species recorded during the present study, 30 species are not reported from the Indian EEZ by any of the previous authors.

Conclusion

The annual marine fish production in India remained static since 1997 when the production reached 2.97 million tonnes (CMFRI 2006). Further improvement in the landings can only be possible by targeting the harvest of under and unexploited resources

especially in depths beyond 100 m. The present study confirms the richness of diversity and abundance of deep-sea nonconventional fin fishes off the south west coast of India (7^0-10^0N lat.). Since the present study cannot be considered as an exhaustive effort to understand the species diversity of deep-sea teleost fishes, further surveys using different types of gears, covering the entire Indian EEZ are needed. In future fishery surveys, emphasis must be given to exhaustive species-diversity assessment and also to make available properly preserved materials to facilitate the barcoding of the different species.

Acknowledgement

The authors are thankful to Dr. D.D. Namboodiri, Dean, College of Fisheries, Panangad for the facilities. The first author is thankful to the Kerala Agricultural University, Trichur and Dr. K.P. Philip, Former Zonal Director, Fishery Survey of India, Kochi for the facilities provided.

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Received: 31 December 2007; Accepted: 08 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Vam Nao Deep Pools: A Critical Habitat for *Pangasius krempfi* and other Valuable Species in the Mekong Delta, Vietnam

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Abstract

A survey of deep pools in a certain part of the Mekong Delta, southern Vietnam identified 23 deep pools based on both local knowledge and bathymetric maps. Vam Nao areas with 3 deep pools are important habitats for many Mekong species due to capture of large and important fishes such as krempfi catfish (*Pangasius krempfi*, Pangasiidae); giant barb (*Catlocarpio siamensis*, Cyprinidae); small-scale croaker (*Boesemania microlepis*, Sciaenidae) and soldier river barb (*Cyclocheilichthys enoplos*, Cyprinidae). This indicated that Vam Nao deep pools are critical habitats for *Pangasius krempfi* and other important fish species in the Mekong delta. Important deep pools acting as refuge habitats or spawning habitats during the dry season should be designated as fish conservation zones to protect fish stocks. Moreover, fishing co-management should be the good solution to effective management as fishing regulation enforcement is likely impossible to apply in Mekong inland fisheries. In addition, quality and quantity of existing deep pools have been affected due to increased silt deposition that resulted from dam constructions and flood mitigation schemes. Hence, water management projects should assess their possible impacts to the fisheries before implementing

Introduction

A deep pool is defined as a relatively deep area that acts as a dry season refuge and a permanent habitat for a number of important fish species within the main river channels. Mekong fishes typically migrate from the main channels to seasonal flooded areas for feeding at the beginning of the flood season, they then move out of the flooded areas into main channels at the end of the monsoon and stay in deep pools during the dry season (Swerdrup-Jensen 2002). Hydrological regimes drive fish stocks in the lower Mekong Basin (Vu 2006 and Kurien et al. 2006). Fisheries in the Mekong delta are intimately linked to hydrological factors such as water level, flood duration, flood timing, river discharge, and rainfall (Vu 2006 and Kurien et al. 2006). Monsoon is responsible

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for the level of seasonal flooding. Seasonal flooded areas are considered as important feeding habitats for almost all Mekong fish species during the flood season (approximately five months). Fish move back into the river as water level recedes at the onset of the monsoon season. Deeper sections of the river or deep pools are important habitats for fish during the dry season. Locations and roles of deep pools in certain sections of the Mekong River have been documented by Viravong et al. (2006); Baird (2006); Chan et al. (2005) and Poulsen et al.(2002). They show that deep pools in the Mekong River and its tributaries are crucially important dry refuge habitats for fish assemblages that provide the recruitment for the Mekong fisheries. Fish sanctuaries are likely to be related to deep pools, which are important to fish stocks and fisheries in Laos, Thailand, Cambodia, and Vietnam (Baird 2006). Ninety-five deep pools (11 - 80 m deep) were identified in northern Cambodia and home for approximately 168 fish species during the dry season (Chan et al. 2005). In Lao PDR, there are at least 70 deep pools identified (Poulsen et al. 2002). Seven of them as important fish habitats were found in the Khone Falls (southern Laos PDR) area, based on interviews with fishers (Roberts and Baird 1995). Some of these deep pools were established as Fish Conservation Zone by local villagers because of awareness of the important habitats during the dry season (Baird et al. 1998).

However, there is little known about the deep pools in the Mekong delta, Vietnam. The location and importance of deep pools is not extensively known. Hence, there is no policy for protecting and managing fisheries in important deep pools, which are refuge habitats for many Mekong fish species. Moreover, deep pools in the upper Mekong River play an important role in maintaining the Mekong fisheries, which are important for many people in the Lower Mekong Basin in terms of employment and food security. Consequently, there is a need to examine deep pools in the Mekong delta. Objectives of the present study are to identify existing deep pools and examine the importance of existing deep pools to the fisheries.

Methods

Identification of deep pool

The present survey was investigated to identify existing deep pools along the mainstream of a certain part of the Mekong delta, southern Vietnam. Combination of two methods was used to identify deep pools in this survey. Local knowledge method was conducted first to gather basic information about existence of deep pools. For this an informal meeting with local fishery officers and experienced fishers was organized. They were asked to locate and draw a sketch map based on their experiences. Depth

and area of deep pools also were roughly estimated. Another approach for identifying deep pools was the use of bathymetric maps. Steps to identify deep pools were as follows: (1) define thalweg, which is a line following the lowest depths of a river; (2) plot distance against river depth; (3) subtract mean depth and (4) identify deep pools using zero-crossing method, which is based on the different depths between the deepest depth and mean depth. Current depths of identified deep pools were measured by an echo sounder JMC V-6202. In addition, based on bathymetric maps, area of each deep pool was calculated by using Microsoft AutoCAD.

Importance of deep pool to the local fisheries

One sixty seven local fishers were interviewed about their fishing activities in and around deep pools such as amount of fish catch, fishing gear, fishing habitat, fishing season, and importance of deep pools. At this stage, important deep pools to the local fisheries were recognised. In addition, local fishers usually brought their catch to the nearest markets to sell. Therefore, the daily amount of fish trading in those local markets close to those important deep pools, for one year, was monitored. Four fish vendors were chosen to monitor daily amount of fish capture trading by-species. Amount of fish trading of some important species was examined to see its distribution between seasons, particularly in the dry season, as important refuge habitats.

Results

Distribution of deep pools

Twenty-three deep pools are identified in the present survey (Fig. 1). Depth of the deepest pool is 44 m. There are two deep pools greater than 40 m deep; five deep pools greater than 30 m deep; nine deep pools greater than 20 m deep and the remaining greater than 10 m deep. The largest pool has 95 ha and one deep pool with 4 ha is the smallest pool. There are four deep pools with 50 - 70 ha; four deep pools with 30 - 50 ha and twelve deep pools with 10 - 30ha. Dominant identified deep pools ranged from 20 - 30 m deep and 10 - 30 ha.

The relationship between maximum depth and area of deep pools are shown in Fig. 2a. There is a difference between depth estimation based on bathymetric maps and echo-sounder measurement. If two estimates are the same, all points will place in the dashed line (Fig. 2b). However, they are many outlines around the dashed line. It indicates that morphological characteristics of existing deep pools have been changed over time. Hydrological regimes are the main factors influencing the morphological

characteristics of deep pools; moreover, the hydrological regimes vary from year to year in the Mekong River partly because of water management projects (dam constructions; flood mitigation schemes). Therefore, several deep pools have been affected by siltation, leading to change in its dimensions.

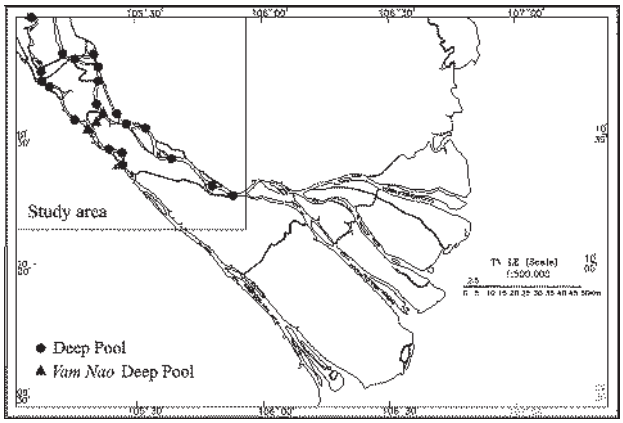

Figure 1. Distribution of identified deep pools in the Mekong delta

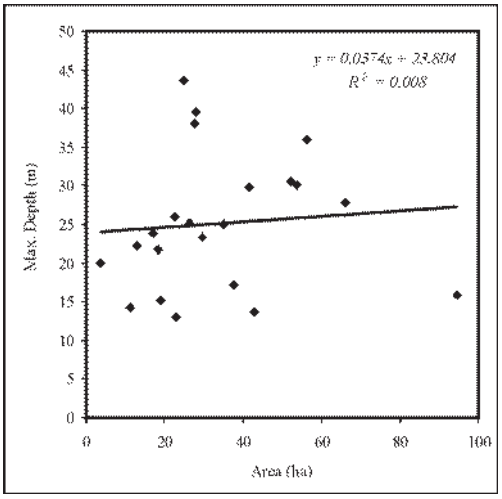

Figure 2a. Relationship between maximum depth and area of deep pools

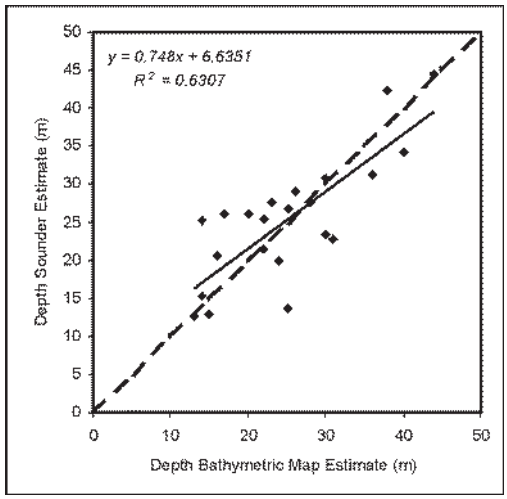

Figure. 2b. Difference between depth from bathymetric maps and echo-sounder estimate

Another reason for different depth estimations based on bathymetric maps and echo-sounder measurement is that sand in riverbed has been exploited heavily in a certain part of the river in recent years. This activity leads to changes in morphological characteristics in the riverbed and may have various impacts on the fisheries.

Importance of deep pools to the local fisheries:

Vam Nao area where there are three deep pools (Fig. 1) is claimed to be the most important fishing habitat. In addition, local fishers confirmed that amount of fish catch

in *Vam Nao* area is more than that in other fishing habitats, especially, fish species caught are usually large.

In addition, there are 19 fishing gear types operating in and around 23 deep pools. Gillnet and trawl nets are the most popular gears in the Mekong delta. Among those gears, there is one type of special gillnet called krempfi gillnet used to target large fish (140 - 180mm in diamond mesh-size), particularly, krempfi catfish (*Pangasius krempfi*, Pangasiidae). Moreover, there is a fishing community using krempfi gillnet (50 fishers) in *Vam Nao* area, whereas there is only one or two krempfi gillnet fishers in several deep pools remaining.

Total monthly amount of fish trading of four large and important species in one local market close to *Vam Nao* area with three important deep pools were examined with respect to its distribution and importance to the local fisheries. The first of two species are particularly important in the Mekong delta: krempfi catfish (*Pangasius krempfi*, Pangasiidae) and giant barb (*Catlocarpio siamensis*, Cyprinidae). The two species mainly distribute in *Vam Nao* area during the dry season (Fig. 3 and 4).

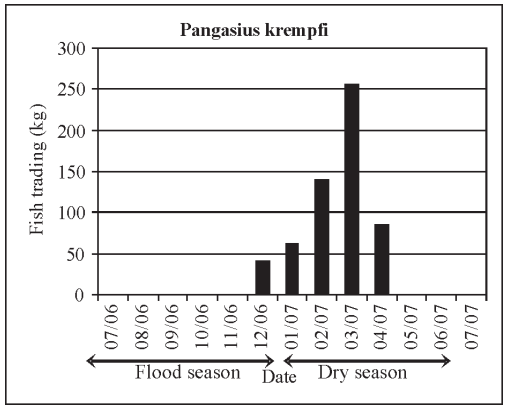

Figure 3. Total monthly amount of krempficatfish trading at a local market

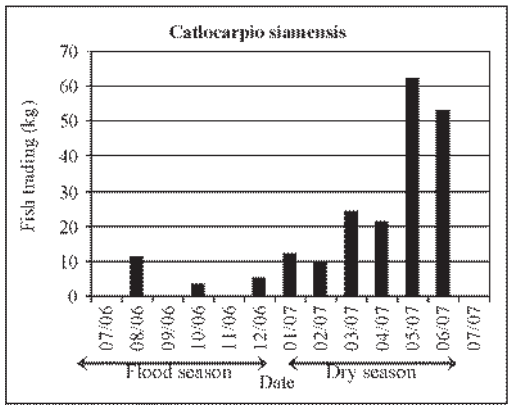

Figure 4. Total monthly amount of giant barb trading at a local market

Amount of fish trading of other two important species are shown in Fig. 5 and Fig. 6 namely, small-scale croaker (*Boesemania microlepis*, Sciaenidae) and soldier river barb (*Cyclocheilichthys enoplos*, Cyprinidae). As observed, the two species distribute all year round in *Vam Nao* area and there is higher fish trading during the flood season.

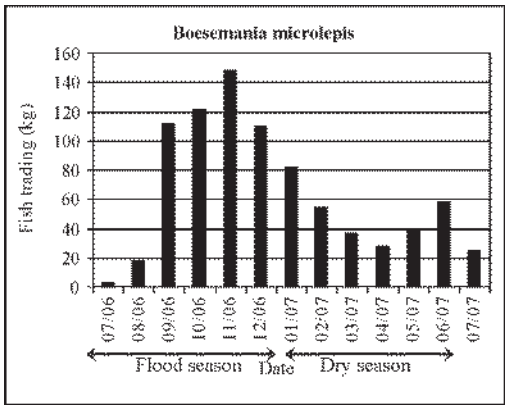

Figure 5. Total monthly amount of small-scale croaker trading at a local market

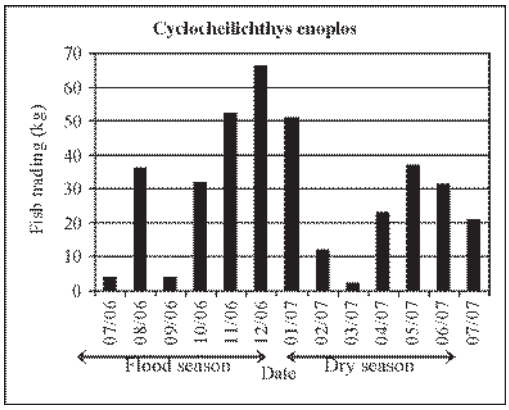

Figure 6. Total monthly amount of soldier river barb trading at a local market

Discussion

There are 23 deep pools identified in the upper part of the Mekong delta (Fig. 1). Number of deep pools in the Mekong delta is fewer than those in Cambodia (95 deep pools; Chan et al. 2005), also fewer than those in Laos (at least 70 deep pools; Poulsen et al. 2002). Deep pools areas in the Mekong delta are also smaller compared to other countries in the Lower Mekong Basin (LMB). Mekong delta is a downstream area and the final part of the LMB. Thus, difference of water level between the dry and the flood seasons in upstream is greater than that in the Mekong delta, downstream. For example, the difference in Phnom-Penh station (Cambodia) is approximately 7.5 - 9 m, while the difference in the Mekong delta is approximately 3 - 4 m. The higher the difference of water level between the dry and the flood seasons, the stronger is the water current. Stronger water current often scours much sediment in riverbed, probably resulting in deeper and more pools. Deep pools often occur at meander bends and straight alluvial channels because of secondary circulation and occur at constrictions and confluence zones in a channel because of flow convergence. There is no relationship between maximum depth and area among deep pools ($r^2 = 0.008$; $P=0.673$; Fig. 2). In addition, Chan et al. (2005) show that there is no relationship between area and depth of deep pools in Cambodia.

Morphological characteristics of existing deep pools have been changed over time (Fig. 3). The fact is that water management projects such as dam construction and flood mitigation schemes have affected the quality and quantity of deep pools due to increased silt deposition. For example, some deep pools have become shallower resulting from the Yali Dam in the upper part of the Sesan River Basin (Fisheries Office of

Ratanakiri Province, Cambodia, 2000). Similarly, there was a decrease of 7 – 8 m in one deep pool in Voen Say district. As a result, many catfish species disappeared in this area (Fisheries Office of Ratanakiri Province, Cambodia, 2000). Furthermore, construction of Theun-Hinboun Dam in Laos also showed the same results (Poulsen et al. 2002).

Several tentative indicators were developed to assess the important habitats within the Mekong River such as depth, current speed, substrate, slope, proximity to wetland forest, and occurrence of objects within the habitat (Baird et al. 1998). Depth and slope parameters related to morphological characteristics of deep pools are indicators to classify critical habitats. It implies that existence of deep pools play a crucial role in the Mekong River ecosystem. Deeper and larger pools are not always important habitats. For instance in the study, two pools are very deep (40 - 44 m), however, they are not considered to be critical habitats to the fisheries.

Vam Nao deep pools (Fig. 1) are admitted to be important habitats in the Mekong delta. The authors decided to monitor amount of fish trading in one local market for one year close to *Vam Nao* deep pools as local fishers often sell their catch at the nearest market. Krempfi catfish is a large species, which is only available during the dry season (Fig. 3). It indicates that this is a critical habitat for krempfi catfish. The largest amount of krempfi catfish trading occurs in March (255 kg month^{-1}). This time coincides with time of upstream migration for spawning. Although krempfi catfish migrate from South China Sea and Mekong delta estuarine to Khone Falls to spawn in June and July (Hogan et al. 2007), there are no adults caught in spawning condition in March in *Vam Nao* area. In addition, krempfi catfish is an anadromous species, similar to life history of salmon and undertake the longest migration routes among Mekong species (Hogan et al. 2007). After spawning, this species migrates back to the estuary and South China Sea; however, no fish are available in the local market during the flood season (Fig. 3). It shows that most of the adults may get caught when they migrate back to the sea.

Similarly, giant barb trading is mainly during the dry season (Fig. 4). Therefore, *Vam Nao* is an important habitat for this species during the dry season. Giant barb is found rarely in the Mekong River, particularly large fish. On the other hand, amount of fish trading of other two large and important species (small-scale croaker and soldier river barb) has the same pattern. The two species are available in the local market all year round, especially, more fish are caught at the end of the flood season (Fig. 5 & 6). Therefore, *Vam Nao* deep pools are also important habitats for those species. Water level starts receding at the end of the flood season, fish also move back to main channels and concentrate in deeper areas or deep pools within the river. Therefore, more fish are

caught at the end of the flood season, as local fishers are aware of the migration pattern and target migratory species. Thus, more fish are available at the local market at that time.

Deep pools in upstream of the Mekong River (Cambodia and Laos) are likely to be more important habitats than those in the Mekong delta, downstream. One of the reasons being that most Mekong white fish spawn in upstream, somewhere in Kratie Stung Treng (Cambodia) and around Khone Falls (Laos). Larvae and juveniles then drift downstream to flooded areas such as Mekong delta, Vietnam (Poulsen et al. 2002). Thus, spawning does not take place in the Mekong Delta for almost all Mekong white fish species. Another reason is that water level in the northern Cambodia and Laos during the dry season is very low and may expose to riverbed at certain sections of the river. Hence, deep pools upstream are more critical habitats for fish survival during the dry season.

Quality and quantity of existing deep pools have been affected due to increased silt deposition that resulted from dam constructions and flood mitigation schemes. Hence, water management projects should assess their possible impacts to the fisheries before implementing by using environmental impact assessment procedure.

Some species are highly migratory between countries, particularly, krempfi catfish. They depend on different habitats in different countries, especially, in the dry season where water level is low. Riparian countries of the Mekong Basin should take the issue into account to manage transboundary stocks. Therefore, important deep pools acting as refuge habitats or spawning habitats during the dry season should be designated as fish conservation zones to protect fish stocks. Moreover, fishing co-management should be the good solution to effective management as fishing regulation enforcement is likely impossible to apply in Mekong inland fisheries.

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Received: 07 December 2007; Accepted: 08 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Biological Diversity of the Green Mussel *Perna viridis* (L.), Mytilidae, Community from Bahadurgad Island off Malpe South West Coast of India

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Abstract

The biological diversity of the green mussel *Perna viridis* (L.), Mytilidae, community was studied at Bahadurgad Island off Malpe, near Udupi, south-west coast of India. A total of 4,262 individuals belonging to 94 species were recorded. The green mussel *Perna viridis* was rich in both number and biomass. The intertidal biodiversity of Bahadurgad Island was composed of algae (11 spp.), porifera (1 sp.), coelenterata (4 spp.), platyhelminthes (2 spp.), aschelminthes (1 sp.), ectoprocta (4 spp.), annelida (24 spp.), mollusca (22 spp.), arthropoda (23 spp.), echinodermata (1 sp.) and fish (1 sp.). Among polychaetes, the genera *Phyllodoce* with four species, *Eulalia* and *Perineries* with three species each and *Neries* with two species were encountered. Rest of the genera was represented by a single species each. Among crustaceans, the maximum species richness was contributed by decapoda comprising ten species of crabs, one species of shrimp (*Alpheus malabaricus*) and amphipoda (6 spp.), cirripedia (5 spp.) and isopoda (1 sp.). The genera *Modiolus* (*M. modiolus, Modiolus* sp.) and *Thias* (*T. rustica, T. tissoti*) were represented by two species each. Chitons were represented by *Acanthopluera granulata* and *Ischiochiton ruber.* Hydroid was represented by three species (*Clava leptosyla, Lovenella gracilis, Tubularia* sp.). Sea anemones with two species (*Haliplanella* sp., *Clava leptosyla*) were recorded. During the study period, *Chthamalus* sp. (1,566 ind.) was dominant, followed by *Perna viridis* (944 ind) and *Trochus radiatus* (42 ind.). The species diversity ($\log_e$, H') ranged from 1.4009 to 2.5296. The minimum (0.4119) and maximum (0.6466) values of evenness were recorded during monsoon (September 2006) and post-monsoon (December 2005) seasons, respectively. The species diversity was affected more by the distribution of individuals among species. Analysis of hierarchical cluster with complete linkage revealed the presence of two large groups. Group A was composed of coelenterates, gastropods, cirripedes, decapods, bivalves, amphipods, bryozoans, whereas group B consisted of algae, polyplacophora, polychaeta and others (porifera, platyhelminthes, aschelminthes, isopoda, echinodermata, fish). The Principle Component Analysis yield three components with Eigen value more than one. The component

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one (bryozoa, amphipoda, air temperature, water temperature, mussel bed temperature, pH, salinity and dissolved oxygen) accounted for 40.78% of variation, followed by component two (coelenterata, gastropoda, cirripedia, decapoda, porifera, aschelminthes, echindodermata, chordata) that accounted for 39.66% of variance and component three (algae, polychaetes, polyplacophora, bivalves, rainfall) accounted for 19.56% of variance.

Introduction

Island ecosystems are delicately balanced and have long been noted for their unique biological diversity. The isolation and small size of islands can be seen as both advantageous and disadvantageous. Because islands are isolated from the mainland, new species might be developed. But because of their small size, any imbalance can have a great impact. In recent days, the biodiversity of islands is subjected to disturbances and destruction. The introduction or removal of a species causes problems across the whole ecosystem. With the increasing rate of global change, islands represent some of the most fragile and vulnerable resources on the planet. Data on biological diversity is important to understand the magnitude of production (Leigh et al. 1987), energy pathways (Porter et al. 1996; Grall and Chavaud 2002; Raffaelli et al. 2003), ecosystem conservation (Widdows and Donkin 1992) and also to evaluate the environmental and anthropogenic effects (Lubchenco et al. 1984; Menge et al. 1986, Loot et al. 2005) on the biota. The composition, structure and diversity of marine mussel community have been documented (Suchanek 1979, 1980; Briggs 1982; Tsuchiya and Nishihira 1985; Commito 1987; Seed and Suchanek 1992; Lintas and Seed 1994). It is well known that large number of small animals inhabit in mussel patches, crevices of rocks, rock pools and on sessile organisms such as mussels (Hasomi 1967; Keith 1971; Paine 1971, 1976; Seed 1976; Dean 1981; Paine & Levin 1981; Roughgarden et al. 1988; Lohse 1993; Menge 1991, 1992; Menge et al. 1994; Petraitis et al. 2003). Scattered information is available on the composition of the intertidal regions of the Indian coast including some islands. There is a dearth of information on intertidal diversity of Bahadurgad Island off Malpe, near Udupi. Therefore, the present study was undertaken to study the intertidal biological diversity at Bahadurgad Island, one of the islands of St. Mary's group of islands off Malpe, near Udupi, south west coast of India.

Materials and methods

The study area consists of a small group of four islands collectively known as St. Mary's islands spread over a distance of 4 km from north to south off Malpe near

Udupi and has a rocky outcrop attaining a height of approximately 25 m (Naganna 1966). There is no human inhabitant in this island and tourists do not visit the island. Fishermen collect the large sized mussels from this island. Samples were collected from the intertidal habitat at Bahadurgad island (lat. 13º21'58'' N; long. 74º42'58'' E) during December 2005 (post-monsoon), March (summer), May (pre-monsoon) and September 2006 (monsoon). At the time of each sampling, air, water and mussel bed temperatures were recorded. The pH, salinity and dissolved oxygen of sea water were estimated (Strickland & Parsons 1968). The data on rainfall (mm) during the study period were obtained from the Office of the District Statistical Officer, Government of Karnataka, Udupi. At the study site, first algae were removed and the entire contents (mussels, sediment, fauna) were then removed from one square feet and brought to the laboratory. In the laboratory, mussels and associated fauna were separated. The density (ind. m^{-2}) of mussels was estimated. Subsequently, the fauna were segregated into major groups and preserved in 5% formaldehyde or 70% alcohol, identified and counted. Each colony / holdfast of algae, hydroids, bryozoans and molluscan egg mass was considered as one unit for calculation purposes. Shannon-Weaver density index (Shannon & Weiner 1949) and evenness (Pielou 1975) were calculated. The data were analysed by two methods, Principle Component Analysis (PCA) to find the number of components that can adequately explain the observed correlations among the observed variables and Cluster Analysis (CA) to elucidate the association of organisms (Davis 1973; Lewis-Bek 1994).

Results

A total of 94 species of sea weeds and marine fauna were recorded during the study period from the intertidal habitat of Bahadurgad Island and the data are presented in Table 1. A total of 11 species of seaweeds belonging to ten genera were recorded during the study period. Of these, the genus *Gracilaria* was represented by two species (*Gracilaria corticata, G. crassa,* Gracilariaceae). The maximum number of species (6 spp.) was recorded during the post-monsoon season followed by monsoon (4 spp.) and summer (3 spp.). Seaweeds were not recorded during the summer in the mussel community. A total of 83 species belonging to ten phyla comprising 4248 individuals of fauna were recorded during the course of the study period (Table 1). Aschelminthes were recorded only during pre-monsoon with two individuals. The maximum species richness was contributed by polychaeta (24 spp.) followed by arthropoda (23 spp.) and mollusca (22 spp.).

Table 1. Biological diversity recorded from Bahadurgad Island during the study period. Number represents the density (no. ft^{-2}).

Species	Post-monsoon (December '05)	Summer (March '06)	Pre-monsoon (May '06)	Monsoon (September '06)
Algae				
Acanthophora spicifera			1	
Centrocerks clavulatum			1	
Chaetomorpha antennina	1			2
Codium dicorticatum	1			
Enteromorpha compressa				1
Gigartina acicularis	1			1
Gracilaria corticata			1	
G. crassa	1			
Padina gymnospora	1			
Sargassum wightii	1			
Ulva fasciata				1
Phylum: Porifera				
Haliclona palmata	1	8	1	
Phylum: Coelenterata				
Clava leptostyla			49	
Haliplanella sp.	3		1	15
Lovenella gracilis	8			
Tubularia sp.	1			
Phylum: Platyhelminthes				
Gnesioceros sp.	1			
Prosthiostomum sp.	3			
Phylum: Aschelminthes				
Unidentified nematode			2	
Phylum: Ectoprocta				
Alcyonidium sp.			8	
Caberea sp.				22
Lichenopora hispida				1
Tricellaria peachii				3
Phylum: Annelida				
Eulalia (Eunida) sanguine	4			
E. albo-picta	8			

E. viridis	3			
Hydroides exaltatus			1	
Lopadorhynchus uncinatus	12			
Lumbrioneries notocerrata	2			
Mercierella enigmatica			6	
Nephthys inermis	3			
Neries (Ceratoneries) costae	6			
Neries sp.	4			
Perineries cultrifera	6			
P. nigropunctata	5		17	7
P. nunita		2		
Phyllodoce castanea		1	6	4
P. fristidii	9			
P. quadraticeps	9			
Phyllodoce sp.	3			
Platyneries coccinia	6			
Polymnia nebulosa	6			
Pomatoceros sp.			19	2
Psuedoneries gallapogenesis	9			
Sabella melanostigma	3			
Serpula vermicularis	1		1	2
Syllis gracilis	3			
Phlum: Mollusca				
Acanthopluera granulate	8		1	4
Acmaea sp.	6			
Babylonia spirata		1		
Branchiodontes modiolus				14
Bursa tuberculata	1			
Cantharus undosus		1		
Cardium setosum				1
Cellana radiate	10	3	3	26
Clypidina notate	2			
Cymatium aquatile		1		
Euchelus tricarinata		14	67	
Ischiochiton ruber	2			
Littorina undulate	1			

Modiolus modiolus		2		
Modiolus sp.	2			1
Morula nodulosa				2
Perna viridis	155	251	302	263
Thias rustica		1	7	
T. tissoti			7	3
Trochus radiates	5	1	14	2
Turbo brunneus	1			
Unidentified eggmass		1		
Phylum : Arthropoda				
Alpheus malabaricus	2	1	9	
Amphithoe inda		26	208	
Balanus amphitrite	1			
B. a. communis			16	
B. reticulates	1			11
B. variegates	5			5
Chthamalus sp.	112	26	846	582
Charybdis callianassanus			1	
Estius laevimanus	2	1		
Eurycarcinus orientalis				1
Grapsus strigosus			2	
Hyale hawaiensis		181	88	
Hyale sp. 1			192	
Hyale sp. 2		262	28	
Leptodius crassimanus		3	4	3
Leucothoe sp.			32	
Moera sp.			48	
Ozius rugulosus			1	
Platypodia cristata				2
sesarma occenica			26	
Sesarma quadrata		6		14
Xantho scabbarimus	7			4
Unidentified Cymathoid				4
Phylum: Echinodermata				
Ophiophragmus sp.	1			
Phylum : Chordata				
Aspidontus striatus		1		

The phyla coelenterata and bryozoa were represented by four species each followed by the polychaete worm with two species. Sponge (*Haliclona palmata*, Chalinidae), brittle star (*Ophiophragmus* sp.) and fish (*Aspidontus striatus*, Blenniidae) were represented by a single species each. Aschelminthes was represented by two species (*Prosthiostomum* sp., *Gnesioceros* sp.). The species richness and abundance of biological diversity is presented in Fig. 1. The maximum number of species was contributed by polychaeta (22.63%) followed by gastropoda (16.79%), decapoda (13.14%), algae (9.49%), cirripedia (7.3%), others (7.3%), bivalvia (6.57%) and amphipoda (6.57%). The remaining components were coelenterata (4.37%), bryozoa (2.92%) and polyplacophora (2.92%). The group others included porifera, platyhelminthes, aschelminthes, isopoda, echinodermata and fish. An examination of the numerical dominance in the major components in the mussel community showed that more than 95% were contributed together by arthropoda (64.73%) (i.e. cirripedia (37.66%), amphipoda (24.98%) and decapoda (2.09%)), mollusca (27.8%) (i.e. polyplacophora (0.35%), gastropoda (4.2%) and Bivalvia (23.25%)), and polychaeta (3.99%). Less than 5% was contributed together by coelenterata (1.81%), algae (0.33%) and others (0.54%).

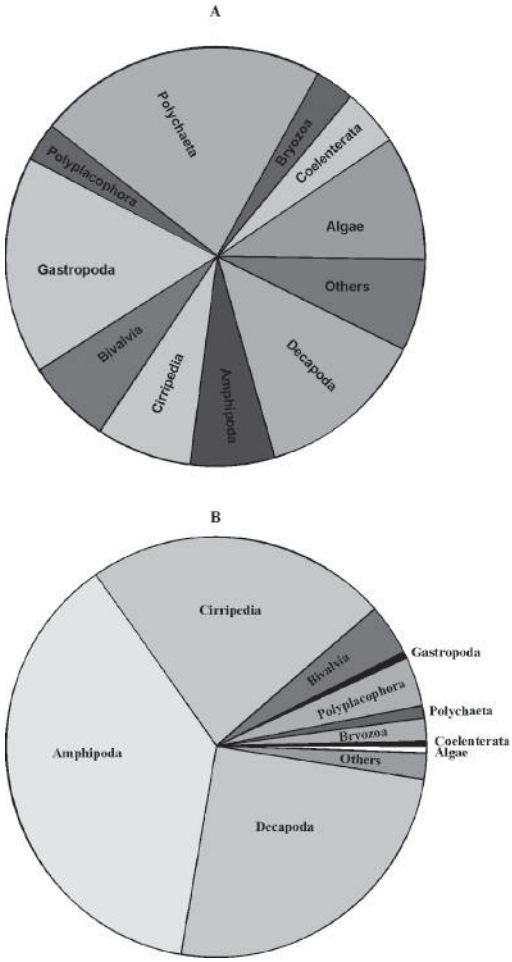

Figure 1. Species richness (A) and numerical abundance (B) of mussel community at Bahadurgad island. The group others included sponge, flat worms, round worms, echinoderms and fish

A total of 24 species belonging to 16 genera comprising 170 individuals of polychaetes were recorded during the study period. Among these, the genera *Phyllodoce* comprising of 4 species, *Eulalia* and *Perineries* with three species each, followed by

Neries with two species, were encountered. Rest of the genera was represented by a single species each. The maximum species richness was noticed during summer (19 spp.) and pre-monsoon (6 Spp.). However, during summer, only two species (*Perineries nunita, Phyllodoce castanea*) were encountered. The maximum density of polychaetes was noticed during post-monsoon season (102 ind.) followed by (50 ind.), monsoon (15 ind.) and summer (3 ind.). A total of 2763 individuals of crustaceans belonging to 23 species of 16 genera were recorded during the study period. Among these, the maximum species richness was contributed by decapoda comprising ten species of crabs and one species of shrimp (*Alpheus malabaricus*), followed by amphipoda (6 spp.), cirripedia (5 spp.) and one species of isopoda. The minimum and maximum densities of crustacea were 130 (post-monsoon) and 150 ind. ft $^{-2}$ (pre-monsoon), respectively. The genus *Balanus* was represented by four species, followed by *Hyale* (3 spp.) and *Sesarma* (2 spp.). Rest of the genera was represented by a single species each. The maximum species richness was registered (14 spp.) during pre-monsoon, whereas in other seasons, the richness was more or less the same (7-9 spp.)

A total of 21 species belonging to 19 genera comprising 1186 individuals of mollusca were recorded. Molluscan egg mass was recorded during summer. The genera *Modiolus* (*M. modiolus, Modiolus* sp.) and *Thias* (*T. rustica, T. tissoti*) were represented by two species each. Rest of the genera was represented by a single species each. Chitons were represented by *Acanthopluera granulata* and *Ischiochiton ruber.* A total of 14 species of gastropods, followed by five species of bivalves were encountered during the study period. The maximum density of gastropods was noticed during pre-monsoon (401 ind. ft $^{-2}$), followed by monsoon (316 ind. ft $^{-2}$), summer (276 ind. ft $^{-2}$) and post-monsoon (193 ind. ft $^{-2}$). *Perna viridis*, Mytilidae, *Cellana radiata,* Nacellidae and *Trochus radiatus,* Trochidae, were recorded during all the seasons. *Babylonia spirata, Bursa tuberculata, Cantharus undosus,* Buccinidae, *Cardium setosum,* Cardiidae, *Clypidina notata,* Fissurellidae Emarginulinae, *Cymatium aquatile,* Trichoniscoidea, *Ischiochiton rubber, Littorina undulata, Modiolus modiolus, Morula nodulosa* and *Turbo brunneus* were recorded only once during the study period. The maximum species richness was noticed during the post-monsoon season (11 spp.) followed by summer and monsoon with nine species each. Only seven species of mollusca were recorded during pre-monsoon. A total of four species comprising 77 individuals of coelenterata were recorded during the post-monsoon and monsoon seasons. Among coelenterates, hydroid was represented by three species (*Clava leptosyla, Lovenella gracilis, Tubularia* sp.). Sea anemones with two species (*Haliplanella* sp., *Clava leptosyla*) were recorded during pre-monsoon (49 ind.). During summer, coelenterates, platyhelminthes, aschelminthes and ectoprocta were not encountered in the mussel bed. Among bryozoans,

Caberea sp., *Lichenopora hispida* and *Tricellaria peachii* were recorded only during monsoon, whereas *Alcyonidium* sp. was recorded during pre-monsoon season only. During post-monsoon and summer seasons, bryozoans were not encountered in the community.

Seasonal variation in the species diversity (Shannon-Weaver index, H'), the distribution of individuals among species (evenness) and number of species (H' max) are presented in Fig. 2. During the study period, the maximum species diversity was observed during the post-monsoon (H' = 2.5296) when 449 individuals belonging to 50 species were recorded, followed by pre-monsoon (H' = 2.0682) when 2016 individuals were distributed among 35 species. In summer and monsoon, the H' were 1.6017 and 1.4009 natural bel, respectively. During summer and monsoon seasons, a total of 794 individuals belonging to 22 species and 1003 individuals belonging to 30 species were recorded. The minimum (0.4119) and maximum (0.6466) evenness (J') were noticed during monsoon and post monsoon

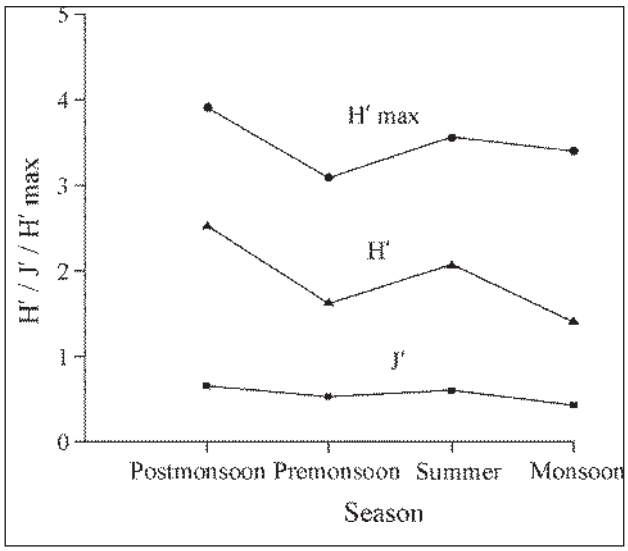

Figure 2. Seasonal variation in species diversity (H'), evenness (J') and H' max.

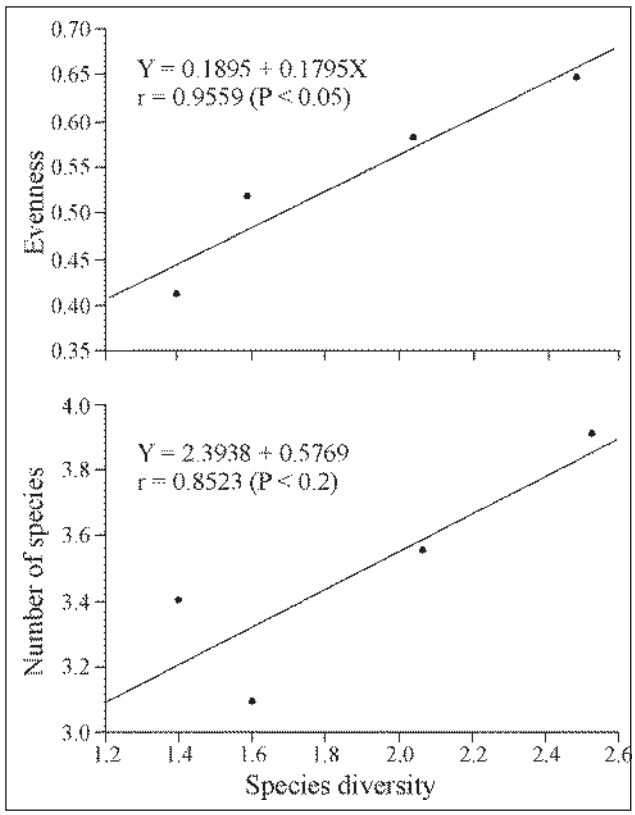

Figure 3. Relation between species diversity, evenness and species diversity and number of species.

seasons, respectively. During summer and pre-monsoon seasons, the evenness values were 0.5182 and 0.5817, respectively. The number of species (H' max) was minimum in summer (3.091) and maximum in post-monsoon season (3.912). Relationship between species diversity and evenness and species diversity and log number of species (H' max) is depicted in Fig 3. The linear relationship between species diversity and evenness and species diversity and number of species were Y = 0.1895 + 0.1795X (r = 0.9559, P <0.005) and Y = 2.3938 + 0.5769X (r = 0.8523, P <0.2), respectively. Analysis of hierarchical cluster with complete linkage revealed the presence of two large groups (Fig 4).

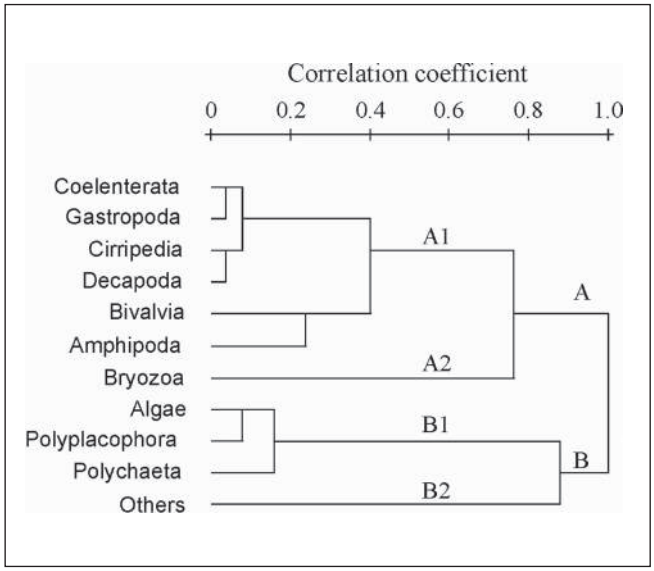

Figure 4. Dendrogram using complete linkage for major groups of mussel community.

Group A, with three subgroups A1 (coelenterata, gastro poda, cirripedia, deca poda, bivalvia, amphi poda) and A2 (bryozoa) and Group B with two subgroups B1 (algae, polyplacophora, poly chaeta) and B2 (others including porifera, platyhelminthes, aschelminthes, isopoda, echinodermata and fish). The PCA yield more detailed picture of the interaction of biological diversity and environmental parameters (Fig 5).

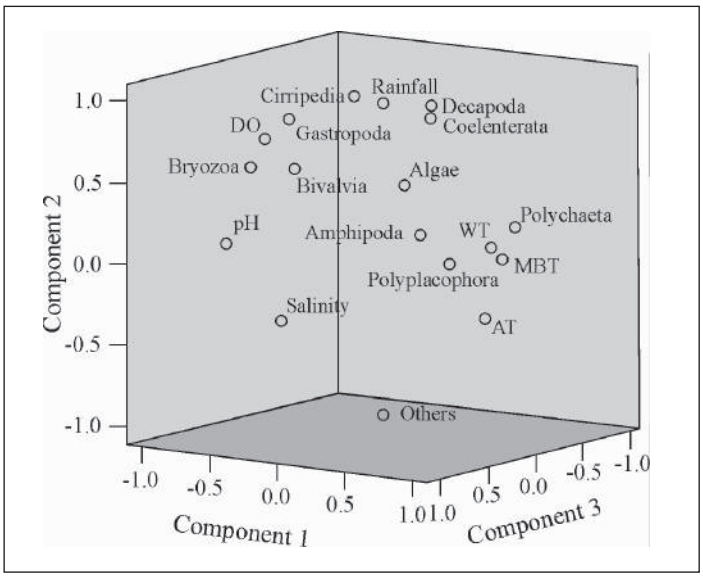

Figure 5. Component plot in rotated space.

A total of three components showed Eigen value more than one in the present study. The component one (bryozoa, amphipoda, air temperature, water temperature, mussel bed temperature, pH, salinity, dissolved oxygen, rainfall) accounted for 40.78% of variation, followed by component two (coelenterata, gastropoda, cirripedia, decapoda, porifera, aschelminthes, isopoda, echindodermata, chordata) which accounted for 39.66% of variance and component three (algae, polychaetes, polyplacophora, bivalves) accounted for 19.56% of variance.

Discussion

A good deal of information on associated flora and fauna of mussels from temperate region is available. Kanter (1977) and Suchanek (1979) reported a total of 200 species of invertebrates in *Mytilus californianus* bed. Briggs (1982) reported a total of 34 species of associated organisms with *M. edulis* in Ireland waters. Tsuchiya and Nishihira (1985) reported 69 species of invertebrates in mussel patch at Asamushi, Japan. Lintas and Seed (1994) have recorded a total of 59 taxa in the beds consisting of *M. edulis* and *M. californianus*. Thippeswamy (1990) reported 258 species of associated organisms of green mussel, *Perna viridis* microhabitat at Someshwar, near Mangalore, India. During the present study, a total of 94 species comprising sea weeds, invertebrates, and fish were collected at the study site. Of these, 11 species of algae belonging to ten genera and 87 species of fauna comprising 3277 individuals were recorded. Among 11 species of seaweeds recorded, nine species were encountered only once, whereas two species (*Chaetomorpha antennina, Gigartina acicularis*) were recorded twice. A total of six species (*Chaetomorpha antennina, Codium dicorticatum, Gigartina acicularis, Gracilaria crassa, Padina gymnospora* and *Sargassum wightii*), three species (*Acanthophora spicifera, Centrocerks clavulatum, Gracilaria corticata*) and four species (*Chaetomorpha antennina, Enteromorpha compressa, Gigartina acicularis, Ulva fasciata*) of algae were recorded during post-monsoon, pre-monsoon, and monsoon seasons, respectively. Seaweed was not represented during summer. A large number of algal species in post-monsoon and low number in winter have been reported in Paradise point at Karachi (Fatima 1996).

One species of sponge *Haliclona palmata* was recorded in all the seasons, except monsoon, in the present study. Coelenterates were found in all seasons, except during summer. One species of nematode was observed only in summer season. During summer and monsoon seasons 2 spp. and 1 sp. of ectoprocta, respectively, were recorded. Polychaetes were found in all seasons. They were numerically abundant in post-monsoon followed by pre-monsoon. Only two species of polychaetes were recorded in summer. Molluscs were recorded during the entire study period. The numerical dominance was

noticed in pre-monsoon followed by monsoon. Arthropods were associated with mussel community during the entire study period. Among arthropods, barnacles were numerically abundant and their peak of abundance coincided with the stable and protective adult mussel bed. Large number of barnacles, (*Chthamalus* sp.) settle on the large mussel shells, thus utilizing the mussel microhabitat and hence, high abundance. The numerical abundance was noticed in pre-monsoon and monsoon seasons.

Number of species and even distribution of individuals per species are the two important components of species diversity, which increases with increased number of species and more even distribution of individuals per species. Therefore, diversity measures both species richness and species evenness (distribution of individuals among species) in the community (Pielou 1975). During the study period, the values (using natural log) of Shannon-Weaver index varied from 1.4009 (monsoon) to 2.5296 (post-monsoon). Tsuchiya & Nishihira (1985) reported the values of species diversity (using $\log_2$) of 3.154, 4.274, 4.245 and 3.537 for young, peripheral, central and old mussel patches at Asamushi, Japan.

During monsoon a total of 1003 individuals were distributed among 30 species. However, during this season density of *P. viridis* and *Chthamalus* sp. were 263 and 582, respectively. Rest of the individuals (158) were distributed among 28 species. Diversity was low (1.4009) during monsoon season when large number of *P. viridis* and *Chthamalus* sp. settled. Therefore, the low diversity index was due to low evenness (0.4119). During post-monsoon, 449 individuals were distributed among 50 species, the H' being 2.5296. During this season the highest density of *P. viridis* (155 ind.) and *Chthamalus* sp. (112 ind.) was noticed. The rest of 182 individuals were distributed among 48 species. The estimated J' was 0.6466 that indicated a more even distribution of individuals among species during this season. This concept is further supported by the linear regression relationship ($Y = 0.1895 + 0.1795X$, $r = 0.9559$, $P < 0.05$) between H' and J' (Fig. 3). Thus, the diversity is affected by the distribution of individuals among species. During the study period, the species richness varied from 22 (summer) to 50 (post-monsoon) species. Species diversity of mussel community during summer and post-monsoon were 1.6017 and 2.5296, respectively. During pre-monsoon and monsoon seasons the values of Shannon-Weaver index were 2.0682 and 1.4009 natural bel, respectively. The diversity values were indirectly proportional to the species richness in summer (22 spp.) and monsoon (30 spp.). Therefore, the diversity is poorly correlated with species richness. The regression equation between H' and H' max was $Y = 2.3938 + 0.5769X$ ($r = 0.0.8523$, $P < 0.2$). Therefore, it can be concluded that the diversity is affected more by the even distribution of individuals among species than species richness and number of species.

The hierarchical CA showed the close interaction between the groups (Fig. 4). Coelenterata, gastropoda, cirripedia, and decapoda form one subgroup. Coelenterates and cirripedes grow on gastropod shells and also on decapods. Coelenterates grow on bivalves. Gastropods and decapods (crabs) have prey-predator relationship with bivalves. Gastropods are also significant predators on mussels and choose their prey on the basis of profitability (i.e. the potential energy gain from a food item relative to handling time), which increases with prey size (Seed 1996). He further reported that gastropods could consume up to two mussels (1-3 cm in shell length) per week in summer, at the height of their predatory activity. Predation by crabs on plantigrades of mussels has been demonstrated by Harger (1972) and Rovero et al. (2000). Bryozoa formed a separate subgroup and it is dependent on mussels for space and food. Algae, polyplacophora, polychaeta and others (sponge, round worm, isopod, sea star and fish) formed a separate group. Chitons coexist with algae and polychaetes. Many polychaetes consume fresh algae (Joseph 1978), isopods and some polychaetes are omnivores (Levington 1982). Mussels are dominant competitors, they are able to exploit their resources, quickly outcompete other competitors, dominate the available space and ultimately reduce the species diversity on the rocky substrate. The interaction of environmental factors on the community composition was analyzed using PCA (Fig. 5). The component one included nine Eigen vectors (bryozoa, amphipoda, air temperature, water temperature, mussel bed temperature, pH, salinity, dissolved oxygen, rainfall). The components two and three included only Eigen vectors of biological variables. The component two included five Eigen vectors such as coelenterata, gastropoda, cirripedia, decapoda and others (porifera, aschelminthes, isopoda, echindodermata, chordata) whereas the component three included four Eigen vectors (algae, polychaetes, polyplacophora, bivalves) of biological variables. The vectors such as amphipods, air, water and mussel bed temperatures of component one, others of component two algae, polychaetes and polyplacophora of component three show negative values. The effect of the environmental factors (except rainfall) was noticed more on algae, bryozoans and amphipods. Rainfall affected the population of coelenterates, polychaetes, chitons, gastropods, bivalves, cirripedes, decapods and others. Intertidal systems are dynamic environments, constantly subjecting their inhabitants to varying conditions of temperature, humidity and salinity, as well as physical disturbance from storms and wave action (Creese & Kingsford 1998; Madarasz, unpubl. data). While varying levels of temperature, salinity and nutrients reaching intertidal communities could result from hydrodynamic regimes, the dynamics of intertidal populations are also closely linked to biological processes.

Acknowledgement

First author is grateful to University Grants Commission, Government of India for the fellowship under Faculty Improvement Programme and to St. Aloysius College, Mangalore for deputation.

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Received: 01 January 2008; Accepted: 22 November 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Effect of Hydrology on Fish Catch of Trawl net Fishery in the Mekong Delta

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Abstract

The Mekong River Commission monitored daily fish catches of five trawl net fishers in the Mekong delta for three years period (2003 to 2005). Hydrological parameters, including water level, discharge, and rainfall were recorded daily in the *Chau Doc* station. Simple linear regression was used to describe the statistical relationship between catch per unit effort (CPUE) and hydrological parameters, based on method of least squares. Catch per unit effort exhibited a strong seasonal variation, with the highest CPUE in November (wet season), when most fish move from flooded areas back to the mainstream, and lowest catch in the dry season. The peak in mean monthly CPUE lagged behind that of mean monthly hydrological parameters for a period of one to two months. Linear regression analysis showed that the mean monthly CPUE correlated most with mean monthly water level of the previous month ($r=0.71$; $p<0.0001$), discharge of two previous months ($r=0.72$; $p<0.0001$) and rainfall of one previous month ($r=0.56$; $p=0.002$). Lateral and downstream fish migrations were believed to be the main causes affecting those relationships. Fishery regulations should, therefore, be managed in relation to hydrological regime conditions to maintain the fisheries as ecologically sustainable. Moreover, any river flow modification may be detrimental to fish stocks and, therefore, to the fisheries. As a result, any activity that regulates river flow must be taken into account.

Introduction

Seasonal flooding is a common natural feature in the lower Mekong Basin. Water level is low during the dry season (January to June); therefore, fish mainly stay in the main channels. But, water level increases at the beginning of the flood season (July to December) and creates a large flooded area, which is productive and a important feeding habitat for fish and other aquatic organisms (Sverdrup-Jensen 2002). In addition, most Mekong fish species are migratory; they migrate upstream for spawning (Poulsen et al. 2002). Their eggs and larvae drift downstream with the water current and spread out into flooded areas further downstream in Cambodia and the Mekong delta for feeding. Fish move back to the main channels at the onset of the dry season (Poulsen et al. 2004).

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Hydrological regimes influence river fisheries around the world (De Graaf et al. 1999; 2003a; Welcomme 1985, 2003; Wolter & Menzel 2005). According to Welcomme (1975) both fish populations and catch are strongly correlated with hydrological regime in Africa floodplains. He suggested that fish catch can be predicted by calculating a regression equation based on hydrological indices (water level and river discharge) and fish production. In a later study, Welcomme (2003) observed hydrological factors, environmental degradation and fishing pressure also affect fish abundance. Similarly, inland fisheries production was strongly associated with flood pulses in Bangladesh and around the world (De Graaf 2003a).

Several studies investigating the relationship between river flow and fish catch in the lower Mekong Basin have been conducted, but mainly in Cambodia such as Zalinge et al. (2003); Hortle et al. (2004 & 2005); Pengbun et al. (2005). They found that there was a positive relationship between fish catch and water level. Moreover, Hortle et al. (2004) show that catches of bagnet (stationary trawl net) in the Tonle Sap River in 2003 to 2004 decreased by 47% compared with high flood year (2002 to 2003), as low water level and short flooding period partly contributed to the decline during that period.

There has been an increase in conflict over water resources for hydropower, irrigation, and river navigation. Recently, the *Nam Theun* 2 hydropower project to construct a dam located in the lower Mekong tributary (Laos) is believed to carry significant risks in relation to the environment and social structure, but the financing for the project has been signed by the World Bank on 3 May 2005 (Starr 2005).

Identification of hydrological factors that affect fish abundance in rivers and floodplains is vital to river ecosystems for their conservation and management. Furthermore, hydrological factors can act as environmental indicators for environmental impact assessment including progress towards sustainable development. These indicators can be used to assess the ecosystem health and possibly to forecast future changes in the environment. It would be a valuable tool for both fishers and fishery managers if fish catch could be predicted in correlation with hydrological indicators.

Characteristics of most Mekong fish species are fast growth and early maturity. They are much more sensitive to changes in environmental conditions than to fishing pressure (Tran et al. 2001). Consequently, there is a need to examine the effects of hydrological regimes on fish catch to improve understanding of flood regimes in relation to fish catch in conservation and management purposes in the Mekong delta, Vietnam. Objectives of the present study were to measure the relationship between hydrological variables and fish catch of trawl net, a popular fishing gear in the Mekong delta.

Methods

Hydrological parameters were measured on a daily basis at *Chau Doc* station, upper part of the Mekong delta (Fig. 1) including water level, river discharge, and rainfall. These three parameters are considered to have potential impacts on fish catch.

We monitored five trawl net fishers on a daily basis for three years period (2003 to 2005) in the Mekong delta. A data collection form was designed to gather information mainly on fishing effort and fish catch by species. Every fisher was asked to recode the information in the form before selling his/her catch to local markets.

Simple regression was used to describe the statistical relationship between a response catch per unit effort (CPUE) and one predictor (hydrological parameter). The level of relationship between dependent and independent variables were determined using the correlation coefficient (r). The strength of the correlation was determined by the "closeness" of the coefficient to ±1. The coefficient of determination value indicates the proportion of the variability in one observation that is accounted for by variability in another.

To determine the probability of a true relationship between depen-dent variable and independent variable, t-tests were performed on dependent variables (water level, river discharge, and rainfall) and independent variables(CPUE) by testing for a significant departure of the regression line slope from zero. The level of significance was 0.05 for all statistical tests. Moreover, CPUE and hydrological variables may not correlate together in the same time. Thus, lag time is taken into consideration to find the best correlation between CPUE and hydrological parameters.

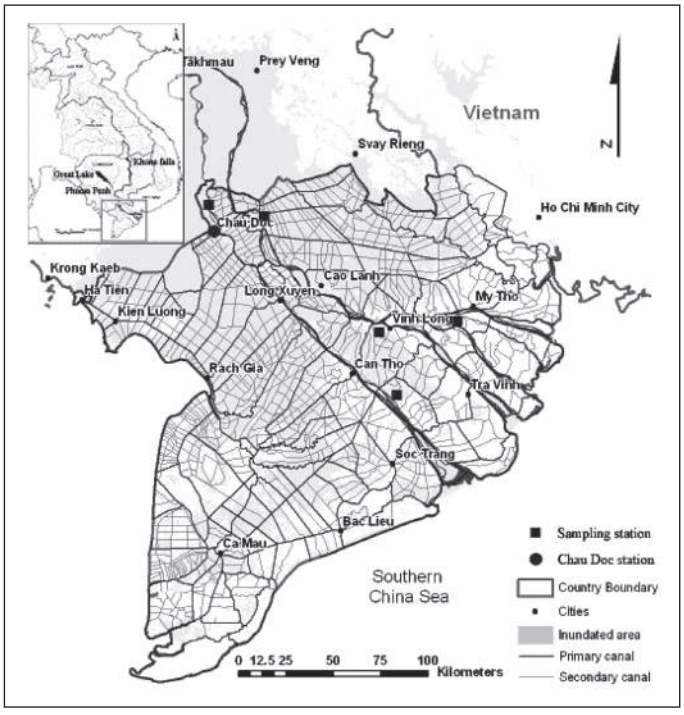

Figure 1: Sampling station in the Mekong delta

Results

Both catch rates and hydrological parameters are seasonal. Catch per unit effort and hydrological variables correlated closely, however, the peak in CPUE lags behind that of hydrological variables for a period of one to two months (Fig. 2).

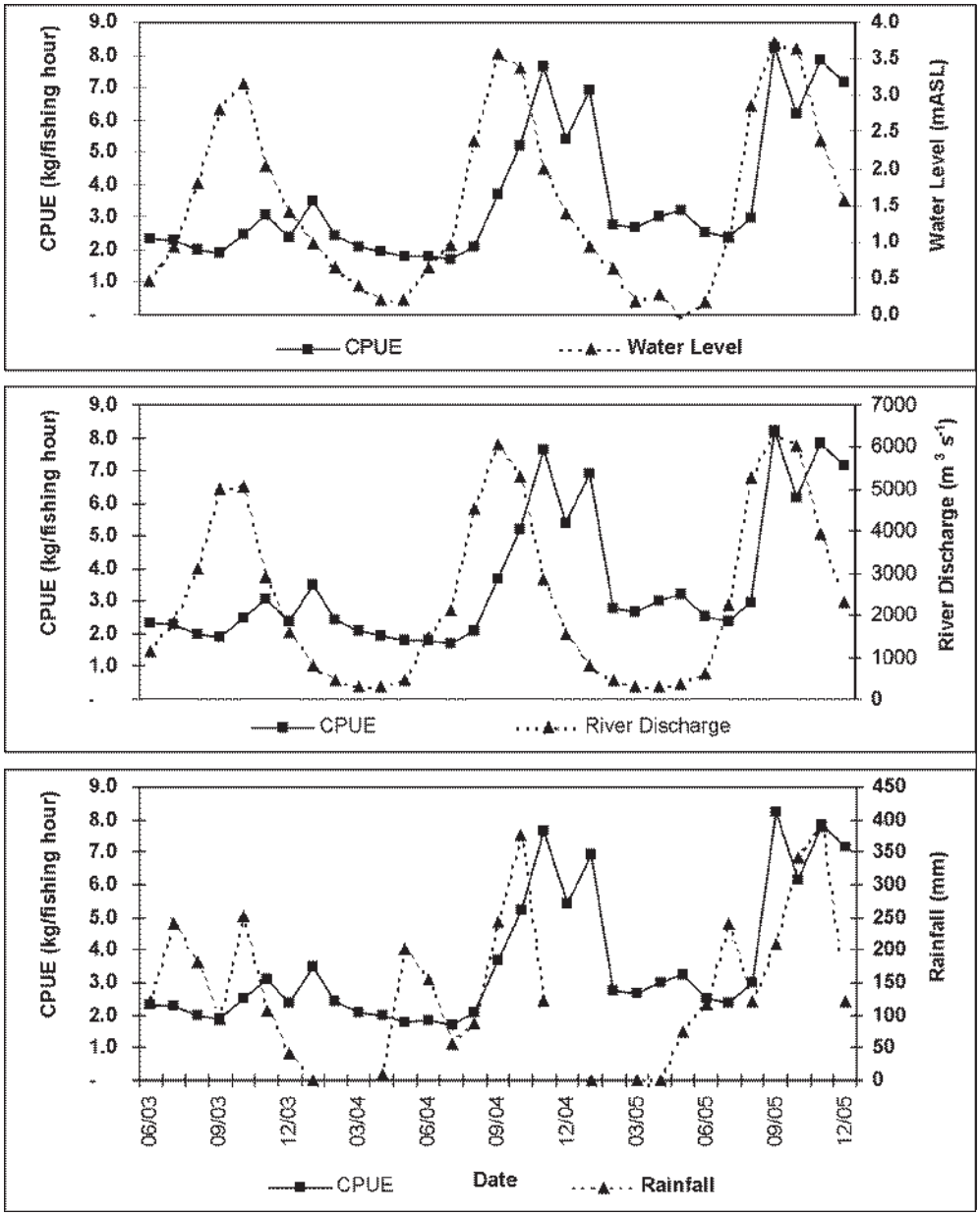

Figure 2: Comparison between mean monthly CPUE and mean monthly water level above sea level (top); river discharge (middle) and rainfall (bottom)

A statistical representation showed that all three hydrological variables (water level, discharge, and rainfall) have a significant influence on catch rates. The level of correlation between CPUE and water level of previous month is the strongest ($r=0.71$; $p<0.0001$) among different lag times. Similarly, there is closest relationship between CPUE and river discharge of two previous months ($r=0.72$; $p<0.0001$) and correlation between CPUE and rainfall of previous month is the strongest ($r=0.56$; $p=0.022$; Table 1). Similarly, river discharge also has a close correlation to CPUE (Fig. 2). The CPUE correlated most with discharge of two previous months (Table 1). The level of correlation between CPUE and rainfall is weaker compared to water level and discharge due to high variation in rainfall.

Table 1. Relationship between mean monthly CPUE and mean monthly hydrological parameters of different lag times, including Pearson correlation (r) and p=value.

Lag time	Hydrological Parameters		
	Water Level	River Discharge	Rainfall
The same month	*r=0.45; p=0.011*	*r=0.36; p=0.046*	*r=0.33; p=0.096*
Previous month	*r=0.71; p<0.0001*	*r=0.67; p<0.0001*	*r=0.56; p=0.022*
Two previous months	*r=0.69; p<0.0001*	*r=0.72; p<0.0001*	*r=0.49; p=0.010*
Three previous months	*r=0.52; p=0.003*	*r=0.61; p<0.0001*	*r=0.43; p=0.026*
Four previous months	*r=0.15; p=0.424*	*r=0.31; p=0.086*	*r=0.20; p=0.336*
Five previous months	*r= -0.24; p=0.196*	*r= -0.07; p=0.712*	*r=0.04; p=0.833*

Discussion

The trend of water level and river discharge are seasonal and almost the same, therefore, the linear relationship analysis showed that there was a strong relationship between water level and discharge ($r=0.98$; $p<0.0001$) and effects of water level on the fisheries should be relatively similar to those of river discharge. Moreover, measurement of water level is easier and cheaper than that of river discharge, therefore, river discharge can be relatively predicted from water level in case of limited budget and personnel.

Maximum water level occurs in September/October while catch rates are high during the late flood season (September to December), behind the peak of water level in a period of time (one to two months). It is noted that floodplains in the Mekong delta

are not permanently flooded but flooded only during the flood season. Fish grow quickly in flooded areas due to increased availability of food and move back to main channels (Poulsen et al. 2002). Local fishers are aware of this fish behaviour, and consequently fishing frequency at that time is very high. As a result, catch rates are seasonal and higher than that in the dry season. Therefore, the overall trends of both water level and CPUE are similar; thus, they show a strong relationship between them.

Fish catch in Bangladesh floodplain fisheries showed a strong correlation between annual yield and flood levels (De Graaf 2003a, 2003b). The yields of wet years or high water level years (1998/1999) were 20 to 25% higher than average, while the yields were only 2 to 8% of the average in extremely dry years, low water level years (1992/1993; De Graaf 2003a). Similarly, higher fish catch was found in the River Tocantins (a sub-basin of the Amazon basin) and in the floodplain lake on the northern bank of the Amazon River during the wet season (Cetra & Petrere 2001; Cerdeira et al. 2000).

From the linear regression equation between CPUE and water level (CPUE=1.2 water level of the previous month+1.8), a decrease in water level of one metre is predicted to result in a loss of 1.2 kg fish per fishing hour. The model predicts a nil catch when water level declines to 1.5 m below sea level at the *Chau Doc* station. When this situation occurs, seawater may penetrate the entire Mekong delta, and freshwater fishes either die or move further upstream.

A strong positive relationship between CPUE and discharge was found, which is consistent with the results of previous studies (Schlosser and Ebel 1989, Bunt 1991, Claire et al. 1991, Growns and James 2005, Rowell et al. 2005). This is the strongest relationship among hydrological variables (r=0.72; Table 1). The most important role of river discharge is the transportation of eggs or larvae from upstream (Laos and north Cambodia) into floodplains downstream (Phnom Penh and Mekong delta). As previously noted, nearly all fish species migrate upstream to spawn at the beginning of the flood season, yet their eggs and larvae drift downstream with the water current. If the flow rates are not strong enough, eggs and larvae may not be brought into productive floodplains downstream and may be exposed to unfavourable feeding conditions in the mainstream with high water speed. During high flow conditions, the increased sediment load can contribute to higher fish production (Koponen et al. 2003). However, several studies found a negative relationship between fish catch and discharge for American shad (*Alosa sapidissima*; Crecco & Savoy 1984), young-of-the-year flannel mouth sucker (*Catostomus latipinnis*), pink salmon (*Oncorhynchus gorbuscha*) and yellow stone cutthroat trout (*Salmo clarkilewisi*) Bulkley & Benson 1962). It is believed that increased freshwater flow rates can cause injury to larvae and quickly push eggs or larvae back

out to sea where they perish, accounting for a gradual decrease in their abundance.

CPUE was also positively correlated with rainfall of previous month (r=0.56; p=0.002). The first local rain, late in the dry season, is considered to be an important factor triggering fish migration for spawning (Nguyen et al. 2005). This migration may contribute to establishing the relationship between CPUE and rainfall. Also, rainfall had a positive correlation with fish catch in the Senegal River, Niger, and in the Chad Basins (Welcomme 2003).

Fish larvae depend upon seasonal flooded areas as important nursery habitats along the Mekong River. Hence, fish larvae are particularly sensitive to flooding. Unfortunately, flood controls or flood mitigation schemes are widely popular in the Mekong delta (Zalinge et al. 2003). However, these activities clearly prevent fish larvae entering the productive flooded areas. Similar results were recorded in a temperate floodplain-river ecosystem in the upper Yazoo River basin. Hydrological factors had a strong influence on fish stocks, and these factors were more important than the effect of climatic factors such as air temperature, rainfall, number of frost-free days on fish stocks (Jackson & Ye 2000).

As discussed previously, hydrological factors of a month (m) strongly affects catch rates in the following month (m+1 or m+2). This implies that the impact of hydrological factors on CPUE cannot result from spawning since almost Mekong species require at least one year to reach sexual maturity and entering the fishery. Moreover, catch of bagnet fishery in Cambodia is related to water level in the same year (Pengbun et al. 2005). This suggests that the impact of water level, discharge, and rainfall on spawning is less important than other factors, such as migration, for two reasons. First, maximum flood levels occur in September/October and then gradually recede, therefore all most all fish in flooded areas migrate back to the mainstream (lateral migrations). Catchability should increase at this time because of the increase in fish density. The change in hydrological factors is the main stimulus for migration and the peak of CPUE follows the peak of hydrological factors at a lag of one or two months.

Second, it is believed fish below Khone Falls and from the Great Lake (Fig. 1) migrate downstream and take about one or two months to reach the Mekong delta and then enter the fisheries. Consequently, these downstream migrations influence the relationship between CPUE and hydrological factors at a lag of one to two months. This hypothesis is based on a study which estimated 20 days for the Siamese mud carp (*Cirrhinus siamensis,* Cyprinidae) to migrate upstream from Phnom Penh to Khone falls (Fig. 1; Baird et al. 2003). As a result, fisheries in Vietnam are likely to depend upon Cambodia's fisheries, as many species migrate between countries. Cooperation

between countries within the Mekong Basin should be made to manage the Mekong fisheries at sustainable exploitation levels.

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Received: 07 December 2007; Accepted: 18 December 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Break-even Analysis and Profitability of Aquaculture Practices in India

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Abstract

Economics of different aquaculture practices in India were worked out on annual basis and the break-even analysis has been done to compute the price required at a given level of production to cover all costs. While the shrimp-oriented aquaculture industry in India recorded exceptional growth for the last three decades in spite of its high exposure to risk and uncertainties, the farming/culture of various other species has not picked up to the expected level enabling the optimum use of potential areas suitable for aquaculture. The break-even price for the tiger shrimp through semi-intensive culture system is worked out at Rs.161/kg and Rs. 126/kg by improved extensive method, while it fetches market sales price of Rs. 350 to 400/kg. White shrimp culture is less risky and the break-even price worked out to Rs.166 /kg in semi-intensive and Rs. 88/kg in improved extensive culture while it obtains market sales price of Rs. 300-350/kg. Break-even price of other farming systems like crab culture worked out to Rs. 107/kg and crab fattening to Rs. 173/kg while the market sales price of crab is Rs. 250/kg. In mussel culture, break-even price worked out to Rs. 3.35/kg (market sales price Rs.8/kg) while that of seaweeds (*Gracilaria edulis)* worked out to Rs. 7328/tonne in dried form (market sales price of dried seaweed is Rs.6000/tonne). The net profit varies for different systems of aquaculture from Rs.49,060/ha for traditional paddy cum prawn filtration system, Rs. 11.15 lakh/ha for crab culture and Rs.14.99 lakh/ha for crab fattening, Rs. 23.94 lakh/ha for pearl culture, Rs.9.48 lakh/ha/year to Rs.6.03 lakh/ha/year for longline mussel culture in Karnataka and Kerala, Rs 1.85 lakh/ha for rack and ren culture of edible oysters in Kerala and Rs.0.58/ha for the rope culture of *Gracilaria edulis*. The paper concludes that there is ample scope and feasibility for developing an integrated approach in the aquaculture practices in India. Other development strategies suggested for promoting aquaculture include introduction of legal framework for regulating all the types of aquaculture, delineating effective marketing strategies and development of parallel marketing avenues especially in the domestic market.

Introduction

Aquaculture has emerged as one of the fastest growing food farming systems at global level with enormous potential for further development. Although India with a

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production of approximately 2.3 million tonnes per annum is the second largest aquaculture producer, its contribution is hardly 5% of the global production. However, the country is endowed with a long coastline of 8129 km, 1.2 million hectares of potential brackish water area and 0.5 million sq.km of continental shelf with diverse ecosystems, offering vast scope for development and diversification of coastal aquaculture.

In India, the traditional system of coastal aquaculture is practised in approximately 50,000 hectares in the low lying brackishwater areas of West Bengal, Kerala, Karnataka, and Goa without disturbing the ecological equilibrium. Besides the brackish water area, the shallow coastal region upto a depth of 30 meters are suitable for sea farming of approximately 73 species of various marine organisms of potential drug/pharmaceutical importance including finfishes (food fishes and ornamental fishes), crustaceans, molluscs, seaweeds and sea cucumber (Devaraj and Appukkuttan 2000).

Inspite of the availability of different technologies for diversified farming practices of various candidate species, the coastal aquaculture in India is dominated by shrimp culture and in this process hardly 13% of the potential area is currently utilized. The high demand and the consequent price appreciation of shrimps in the international market led to its growth of production. While the shrimp-oriented aquaculture industry in India recorded exceptional growth for the last three decades, the farming/culture of other suitable species in different localities has not picked up to the expected level to enable the optimum use of potential areas suitable for aquaculture. Rational use of aquatic resources by judicious application of sustainable and diversified farming practices can ensure food security, increased employment opportunities, enhanced foreign exchange earnings and socio-economic upliftment of coastal rural poor.

Materials and Methods

Both primary and secondary data have been collected and utilised for the present analysis. Economics of different culture systems were given on annual basis as the duration of different practices varies from four months to one year. While working out the economics, the total cost indicated was the sum of annual fixed cost and annual operating cost. Operating costs include all those costs, which are incurred only when the farms are under operation and fixed costs are those incurred even if there is no culture operation. The fixed cost includes the interest on initial investment, depreciation of the permanent assets and insurance premium. The information collected from various publications has been updated by substituting the current input and output prices (2006-07). Similarly, the average yield and earnings per hectare for all type of aquaculture systems has also been worked out or projected irrespective of existing/optimum size of farms advisable and presented only to enable easy assessment of comparative efficiency.

Break-even analysis has been used to assess the economic feasibility of the culture systems along with other indicators like net profit generated, annual fixed cost, and annual operating cost . The rate of return for each culture practice was calculated using the formula

RR (%) = [(NR+R)/Ci] x 100,

where NR is the net profit, Ci is the capital investment and R is the rate of interest on capital investment.

The break-even point is the point at which revenue is exactly equal to costs and hence no profit is made and no losses are incurred. The selling price, fixed costs or operating costs will not remain constant resulting in a change in the breakeven. Hence, these should be calculated on a regular basis to reflect changes in costs and prices and in order to maintain profitability.

The break-even production was calculated using the formula

BP = TFC/ (Average farm-gate price per unit- Average variable cost per unit)

Where BP is the break-even production and TFC is the total fixed cost.

Results and Discussion

Shrimp culture

The level of production of any commodity is determined by their price realization through market mechanism. Coastal aquaculture in India is also not an exception to this. High demand and unit value realization of shrimps, especially cultured shrimps in the export market has led to the increase in the production of penaeid shrimps through aquaculture. The proliferation of shrimp farming activity, in particular with the advent high input systems, helped in increasing the per capita yield and consequent economic returns to the farmers.

The economics of various shrimp farming systems in India have been worked out at different locations of the country (Jayagopal and Sathiadhas 1993; Usha Rani et al. 1993; Panikkar et al. 1995; Jayaraman et al. 1996; MPEDA 1998; Prasad 1999). In the present analysis, the key economic indicators such as rate of return and break-even price were calculated for all types of shrimp farming systems existing in the country. The indicative economics of semi-intensive farming of both the tiger shrimp *Penaeus monodon* and the white shrimp *Fenneropenaeus indicus* are given in Table 1. The data clearly shows that the annual net profit obtained from the farming of tiger shrimp is better than that of white shrimp. The break-even price for the tiger shrimp through

semi-intensive culture system is Rs.161/kg whereas that of the white shrimp is Rs.166/kg. But the risk factor is lesser in *F. indicus* culture due to the robust nature of the species than that of *P. monodon.* There is not much difference in the capital investment and infrastructure facilities for the culture of both the species. The annual net profit for tiger shrimp culture is worked out at Rs.8.36 lakh/ha with a rate of return of 98%, whereas the net profit for white shrimp culture is worked out at Rs.4.43 lakh/ha/year with a rate of return of 66% to the capital investment.

Table 1. Break-even analysis of shrimp culture systems in India (2006-07)

Type of farming system		Semi intensive		Improved extensive		Extensive
Species cultured		Tiger shrimp	White shrimp	Tiger s shrimp	White shrimp	Tiger shrimp
Annual fixed cost	(Rs. lakh)	1.94	1.58	0.772	0.574	0.227
Annual operating cost	(Rs. lakh)	7.70	7.24	2.12	0.882	1.37
Total costs	(Rs. Lakh)	9.64	8.82	2.892	1.456	1.597
Annual production	(Kg)	6000	5300	2400	2000	2324
Farm gate price	(Rs.)	350	300	400	350	150
Annual net operating profit	(Rs. Lakh/ha)	10.30	6.01	6.28	5.12	2.12
Annual net Profit	(Rs. lakh)	8.36	4.43	5.508	4.236	1.892
Break-even production	(Kg)	875	967	248	188	250
Break-even price	(Rs./Kg)	161	166	126	88	69
Rate of return	(%)	98	66	165	169	150
Input output ratio		1.87	1.50	2.91	3.40	2.19

Very high profit levels from the improved extensive culture of *Penaeus monodon* and *Fenneropenaeus indicus* in Tamil Nadu were recorded. The annual net profit worked out at the present level for *P. monodon* is Rs.5.51 lakh/ha and that for *F. indicus* is Rs.4.24 lakh/ha. The break-even price for shrimps cultured by improved extensive method is lesser than that by the semi-intensive method. It is Rs.121/kg and Rs.88/kg for *P. monodon* and *F. indicus,* respectively. The farm gate price is also lesser for *F. indicus* (Rs. 300/kg) compared to that of *P. monodon* (Rs.350/kg) and this makes the difference in their rate of return by 4%.

The capital investment, as well as, the operating costs are also lesser in the improved extensive system of production compared to semi-intensive shrimp farming. An economic analysis of extensive shrimp farming for *P. monodon* in Andhra Pradesh has shown that the break-even price (Rs.69/kg) is still lesser than that of improved

extensive farming. But the net profit is comparatively low with Rs.1.89 lakh/ha/annum with a rate of return of 150% to the capital investment. In this culture system also, the major component of the operating costs is the feed cost (61%) followed by salaries and wages (12%). The factor-product relationship in prawn farming has been analysed by Kumar and Panikkar (1993) and they found that the level of production could be increased by using more efficient feed and better feed management.

Integration of paddy and shrimp farming

Integrated farming is the best means of increasing productivity from a unit area of land or water body. Integrated farming with crop, livestock and finfish or shellfish has been found to be a rewarding offer, helping to augment production of highly valued fish with minimum level of inputs. This is made possible by way of recycling of wastes/byproducts of one crop as input for the production of another.

In India, integrated farming is done in the *Bheries* of West Bengal, the *Pokkali* fields of Kerala, the *Khazen* lands of Goa and the *Khar* lands of coastal Karnataka, where during the rainy season, saline resistant traditional varieties of rice are grown. After the harvest of paddy, during the summer months, shrimp seeds along with other finfish seeds entering the fields with tidal water, are entrapped, grown for a period and harvested. In Kerala, during this period, a special variety of paddy called '*Pokkali*', which is tolerant to 6-8 ppt salinity, is grown in these fields. Different species of shrimps together with fishes such as *Mugil* spp. *Chanos chanos*, *Etroplus suratensis*, *Megalops cyprinoides*, *Lates calcarifer* and *Oreochromis mossambicus* enter into the fields with the inflow of tidal water. The shrimp is the dominant one, but in most of the areas it is the commercially least important species like *Metapenaeus dobsoni* and *M. monoceros*, which dominates in comparison to the commercially important species like *Fenneropenaeus indicus* and *Penaeus mondon*. Regular thinning of crop by filtration is carried out in every new moon and full moon period. Final harvesting will be done at the end of the season by operating sluice net and cast net. The prawn yield from the traditional practice usually varies between 500-1000 kg/ha depending upon the location of the field in relation to the bar-mouth, the distance from main water body, the size of the feeder canal, and the position of the sluice (Sathiadhas et al. 2000). In Kerala, out of 65,000 ha of brackish water area, more than 13,000 ha is under the traditional paddy cum prawn filtration system.

Table 2. Economics of traditional system of prawn culture in *pokkali* field at Vypeen, Kerala (2006-07)

Economic Indicators		Amount/Production
Operating costs	(Rs.)	14,840
Average Annual Production	(kg/ha)	736
Gross Revenue	(Rs.)	63,900
Net profit	(Rs.)	49,060

Economic analysis of a paddy cum prawn culture system in Kerala showed that with a shrimp production of 700-800 kg/ha, a net profit of Rs.49,060/ha/crop is obtained from prawn filtration per hectare after 4-5 months. George (1980) worked out a production of 11,754 kg from a 16 ha filtration farm while Sathiadhas et al. (1989) worked out a production of only 8,590 kg from 17.11 ha filtration farm at Vypeen. The capital investment required is the cost of land and most of the farmers conduct this culture operation in leased lands which are normally having an area of more than five hectares. Rotation of prawn filtration with paddy is found to be the most eco-friendly farming system in this region. The total annual net profit from prawn culture works out at Rs.49,060/ha (Table 2).

Crab culture/fattening

In India, crabs used for domestic consumption, as well as, for exports are largely obtained from capture fisheries. The demand for live crabs in foreign market has prompted India to make efforts in large scale growing of mud crabs in confinements. Mud crab farming is picking up in recent years in states like Andhra Pradesh, Tamil Nadu and Kerala. The only constraint is the nonavailability of enough stocking materials for culture and fattening. It is therefore imperative that concerted efforts are needed to develop commercial hatchery for adequate and sustained supply of baby crabs to make mud crab farming an organized industry.

Sathiadhas and Najmudeen (2004) reviewed the economic efficiency of mudcrab farming/fattening under different production systems and evaluated its techno-economic performance by comparing them with semi-intensive shrimp farming. Net annual profit from the fattening system is Rs.14.99 lakh/ha and that of culture is only Rs.11.15 lakh/ha (Table 3). Annual profit from crab fattening with composite culture with fish and shrimp is approximately Rs.8.17 lakh/ha. Fattening system provides the highest rate of return of 244% followed by culture system (189%), which is much higher than that obtained for all types of shrimp farming. Crab fattening with composite culture provides a rate of return of 145%.

Table 3. Break-even analysis of mud crab culture, fattening and crab fattening with composite shrimp/fish culture systems in Kerala, India (2006-07).

Particulars		Crab culture	Crab fattening	Crab fattening &Composite fish prawn farming
Annual fixed cost	(Rs. lakh/ha)	1.50	1.50	1.40
Annual operating cost	(Rs. lakh/ha)	5.25	24.09	12.77
Total costs	(Rs. lakh/ha)	6.75	25.59	14.17
Annual production	(kg/ha/year)			
Crabs		3,150	12,715	8,000
Prawns				2,125
Fishes				1,958
Annual Revenue	(Rs.lakh/ha/year)			
Crabs		17.90	40.58	20.00
Prawns				1.45
Fishes				0.89
Annual net operating profit	(Rs. Lakh/ha)	12.65	16.49	9.57
Annual net profit	(Rs. lakh/ha/year)	11.15	14.99	8.17
Break-even Price	(Rs/kg)	107	173	—
Break-even production	(kg)	1125	1357	1549
Rate of return	(%)	189	244	145
Input output ratio		2.65	1.59	1.58

Even though the net profit and other indicators are higher in fattening system, the break-even price of crabs is higher (Rs.173/kg) compared to the culture system (Rs.107/kg). The economic analysis conducted in other places of the country also indicates its higher profitability as compared to other forms of aquaculture (Kathiravel et al. 1997; Anil and Suseelan 2001). Major reason for enhanced economic efficiency of crab culture and fattening is that it can be done with minimal initial requirements.

Molluscan farming

The major molluscan species suitable for coastal mariculture with high growth rate in India are mussels, oysters, clams and pearl oysters. At global level, molluscs contribute 18% to the total aquaculture production. If appropriate economical and sustainable culture technologies are applied, all these bivalves could be produced in large scale with much profit. Groups of farmers in the coastal area with the technology developed by the Central Marine Fisheries Research Institute (CMFRI) have taken up

the mussel culture as a small scale farming activity with good profit. The Institute has also developed appropriate technology for edible oyster culture (*Crassostrea madrasensis*) in brackishwater areas and was found successful for commercialization. Another major technological achievement in bivalve mariculture has been the indigenously developed method for the culture and production of pearl, from the pearl oyster, *Pinctada fucata*. Recent attempt to culture pearl oysters in onshore tanks by the Institute has also proved to be successful.

Pearl culture

Pearls were considered to be the first precious gem known to mankind. India is endowed with the natural resources of the pearl oyster in the Gulf of Mannar and the Gulf of Kutch. The major pearl producing oysters in India are *Pinctada fucata* distributed in the Gulf of Mannar and Gulf of Kutch and the blacklip pearl oyster *P. margaretifera* in the Andaman and Nicobar Islands.

The pearls are classified into A, B, and C grades according to their quality, uniformity of coating and shape. The A grade pearls are sold at the rate of Rs.1500/g, B grade Rs.1000/g and C grade Rs.500/g. Even though India had the distinction of producing cultured pearls in 1973 itself, it could not produce cultured pearls for world trade. Pearl culture is a long-term investment and huge profits can be made in a successful culture operation, as there is great demand for pearls.

The economics of pearl culture differ between the culture methods and regions. The estimated production from a 6×6 m floating raft culture is 1849 pearls/crop (Velayudhan 1995). Economic analysis of onshore pearl culture has been carried out at Visakhapatnam (Rao and Devaraj 1996; Rao 2005) and that of shallow water cage culture has been worked out at Vizhinjam, Kerala (Achari et al. 1998). The projected annual net profit from the open sea raft culture is very high against huge investments in the tune of Rs.67.40 lakh/ha . The break-even price for the pearls produced through open sea raft culture is worked out at Rs.33/pearl, if the culture is expanded to one hectare area (Table 4). For the present economic analysis, production rate from shallow bottom cage culture is estimated at about 1350,000 pearls/ha @ 700 pearls/cage, with a break-even price of Rs.29/pearl. Achari et al. (1998) estimated a production of 1000 pearls/ cage of 65x64x64 cm^2 size and this estimate is much higher than that of the other two culture systems.

Table 4. Break-even analysis of pearl oyster culture systems in India

Economic Indicators		Open sea raft culture	Shallow bottom cage culture	Onshore marine culture
Annual fixed cost	(Rs. lakh)	37.85	8.55	8.98
Annual operating cost	(Rs. lakh)	76.79	378.99	18.00
Total costs	(Rs. lakh)	114.64	387.54	26.98
Average annual production	(No. of pearls)	3,50,000	1350000	100000
Break-even price	(Rs./pearl)	33	29	27

The break-even price of pearls from onshore pearl culture worked out at Visakhapatnam is Rs.27/pearl with a production of 1,00,000 pearls/ha. In all the three culture methods, the cost of labour is the major operating expenditure. Normally in the case of marine pearls, the pearls are sold on the basis of their weight rather than on the basis of numbers. Estimates on the production of pearls on weight basis are required to work out the real profit from all the above mentioned culture systems. Rao (2005) has estimated an annual pearl production of 27,000 g/4000 m^2 from onshore tank culture of pearl oysters at Visakhapatnam with a net profit of 23.94 $lakh^{-1}.ha^{-1}.year^{-1}$ considering the culture period as 24 months. The economics of all the pearl culture systems worked out here are based on the experimental results and estimations obtained by research institutions of the country. In the case of open sea mariculture practices there are legal and other issues involved which may lead to higher cost of production than that of the projected figures.

Mussel culture

Culture of mussels gives the highest production rate among the edible molluscs. In India, two species of marine mussels (Green mussel *Perna viridis* and the Brown mussel *Perna indica*) are distributed in the rocky coastal areas where they support traditional sustenance fishery. Mussel culture was mainly done by raft method and long line method. As mussels are a relatively low priced product, export of mussel product from India is relatively a recent enterprise and the quantities are not substantial. Besides generation of alternative employment to fishermen, entrepreneurs can earn substantial profit by adopting mussel farming technologies. Diversification of products i.e. value added products may help in further spreading of mussel farming and its successful adoption.

With a culture duration of five to six months, two crops can be harvested in a

year from the longline method. The estimated production of mussels from one meter rope is 10-12.5 kg/crop. From a longline unit of 360 m^2 a total production of 54,720 kg shell-on mussels can be obtained, of which 40% will be the meat (Kuriakose and Appukkuttan 1996). Economics of the longline mussel culture has been worked out at Byndoor, Karnataka (Mohamed et al. 1998) and in Kerala (Velayudhan et al. 1998) by the Central Marine Fisheries Research Institute (CMFRI). The projected economics per hectare in the present analysis shows that the break-even price is higher in Kerala (Rs.4.8/kg) than that of Karnataka (Rs.3.35/kg). The cost of production in Kerala is high mainly due to high cost of labour and seed. An annual net profit of Rs.9.48 lakh/ha can be obtained from culture at Byndoor, whereas the net profit worked out in Kerala is approximately Rs.6.03 lakh/ha/year (Table 5).

Table 5. Break-even analysis of long line mussel culture systems in India

Economic Indicators		Karnataka	Kerala
Annual fixed cost	(Rs. lakh)	4.03	1.87
Annual operating cost	(Rs. lakh)	15.02	11.42
Total costs	(Rs. lakh)	19.05	13.29
Average annual production	(tonnes)	360	276
Sale price	(Rs./Kg shell on)	8	7
Annual net profit	(Rs. Lakh)	9.48	6.03
Break-even production	(Tonnes)	105	65
Break-even price	(Rs./Kg)	3.35	4.80
Rate of return	(%)	141	163

Edible oyster farming

The edible oysters are widely distributed along the Indian coast. Of the six species of oysters belonging to the family Ostriedae, *Crassostrea madrasensis* is the dominant, having a wide distribution in the major backwaters in shallow coastal regions of India. Apart from the edibility of the meat, the shells have various industrial and agricultural uses. The indicative economics of the rack and ren culture of edible oysters in Kerala worked out by CMFRI in 300 m^2 area and the projected economics per hectare area are shown in Table 6. An annual net profit of Rs. 1.83 lakh/ha can be obtained from the rack and ren culture with a capital investment of only Rs.3.05 lakh/ha. The rate of return worked out for culture in one hectare area is 72%.

Table 6. Break-even analysis of edible oyster culture by rack and ren method carried out in the backwaters of Kerala.

Economic Indicators		Amount/production
Annual fixed cost	(Rs. lakh)	1.60
Annual operating cost	(Rs. lakh)	3.10
Total costs	(Rs. lakh)	4.70
Average annual production	(kg)	10,200
Farm gate price of heat shucked meat	(Rs/kg.)	60
Net profit	(Rs. lakh)	1.85
Break-even production (heat shucked meat)	(kg)	5404
Break-even price for oyster with shell	(Rs./tonne)	3686
Break-even price for heat shucked meat	(Rs/kg)	46.10
Rate of Return	(%)	72

Seaweed culture

There is a very good demand for certain seaweeds in foreign countries, which are now under-exploited or unexploited in our country. Hence, they may be exploited and exported to earn foreign exchange to the nation. The bays and creeks present in the open shore along the east and west coast, lagoons and corals of the southwest coast of Tamil Nadu, Andaman Nicobar Islands and Atolls of Lakshwadeep have immense potential for cultivation of seaweeds, where commercial culture could be undertaken by the seaweed utilisers and the entrepreneurs. Since 1972, the CMFRI has been engaged in the cultivation of several economically important seaweeds and the method of cultivation of *Gracilaria edulis*, a fast growing species with minimum seed material has been standardized (Chennubhotla and Kaliaperumal 1998).

The economic analysis of the rope culture of *G. edulis* along the coast of peninsular India indicates that an annual profit of Rs.58,000/ha/year can be obtained from 4 successive crops with an operating cost of Rs.1,52,000/ha/year (Table 7). The break-even price was worked out at Rs.7,238/tonne of dry seaweed. While in Minicoy Lagoon, the input requirement is less and the production rate is high and the net profit is also higher than which worked out for the culture along the Peninsular Indian coast (Chennubhotla 1996).

Table 7. Break-even analysis of culture of seaweed *Gracilaria edulis* at Mandapam, Tamil Nadu.

Economic Indicators		Amount/production
Annual operating cost	(Rs. lakh)	1.52
Average annual production	(Tonnes)	21
Sale price of dry G. *edulis*	(Rs./tonne)	10,000
Annual net profit from 4 crops	(Rs. lakh)	0.58
Break-even price for wet seaweed	(Rs./tonne)	1,810
Break-even price for dry seaweed	(Rs/tonne)	7,238

Conclusion

The techno-economic evaluation of various aquaculture/mariculture practices reveals that there is ample scope for their product diversification rather than confining only to shrimp farming. The issues such as excess stocking rates beyond the carrying capacity, disease outbreaks and the possible environmental impacts in the fast pace of the development of shrimp farms necessitate the adoption of alternatives such as crabs and finfishes for brackishwater culture. The increasing export demand of crabs coupled with high price in the international market has stimulated the crab culture and fattening especially, in the states of Kerala and Tamil Nadu. The projected economics of open sea and onshore pearl culture practices should be further tested by transferring the technical know-how to the private farms for the commercial production. There is still great demand for pearls in the export market, which fetches the highest unit value. In addition, the feasibility of integrated shrimp culture with bivalves and finfishes are to be worked out and propagated to obtain maximum output through an eco-friendly system of farming. The concept of "Fishery estates" in the potential regions of this country with the introduction of diversified and integrated shrimp/finfish culture systems side by side should be promoted to accelerate the development of aquaculture industry. Special promotion incentives along with legal rights should be offered to the investors in these fishery estates to promote open sea mariculture.

There is need for introduction of legal framework for regulating all the types of aquaculture. The socio-economic acceptability of the inhabitants of the region also has to be taken into consideration before initiating such programmes. Promotion of eco-friendly coastal aquaculture is vital for generating employment and higher income for coastal fishermen. It is imperative to create proper marketing strategy for export marketing of aquaculture products in view of increasing aquaculture production and create parallel marketing avenues, especially in the domestic markets. Transformative

changes were brought about in the attitude of the fishermen through extension. The transfer of technology efforts has helped to mitigate rural poverty by empowering the resource poor fishermen with access to technology and through employment generation activity and skill upgradation. Success achieved by the participating farmers has formed a model for others to start trying these technologies in their ponds that can contribute to social equity and environmental sustainability.

Acknowledgements

The authors thank Prof. (Dr). Mohan Joseph Modayil, Director, CMFRI, Kochi, for his constant encouragements and support for the preparation of this manuscript.

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Received: 31 December 2007; Accepted: 28 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Simulation Model for Evaluating the Response of Management Options on the Demersal Resources of Tamil Nadu Coast

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Abstract

The response of different management options on demersal fish catch in Tamil Nadu was examined using simulation model and time series data on catch and effort of demersal resources in Tamil Nadu during 1989-2005. For the simulation study surplus, production model and spectral models were used to simulate effort, yield and biomass. Genetic algorithm was used to estimate parameters of surplus production model. Effort, biomass and yield were simulated for the period 2006 to 2015 under different levels of effort such as reducing by 25%, 50%, and 75% of present level increasing by 25%, 50%, 75% and 100% of present level and also for the present level. The simulation results revealed that when the level of exploitation is kept at 25%, 50%, 75%, and 100% of the present level, the yield falls below the maximum sustainable yield (MSY) level and the biomass is kept above its MSY level. When the exploitation level is increased by 25% of the present level of exploitation, the yield falls below the MSY level in the years up to 2013 and the biomass remains above that at MSY level. But at this level of exploitation, the yield falls above the MSY level and the biomass falls below its MSY level for the years 2014 and 2015. The optimum exploitation level was worked out as 91.25% of the present level of exploitation.

Introduction

Systems analysis and simulation techniques have been applied in marine fisheries management as a tool to assist resource managers for evaluating proposed management actions. Using information available about the fishery and related aspects, simulation models attempt to estimate resulting future changes due to implementation of different management options. Prior knowledge about the effect of implementation of these management options on the fishery resources is very much essential to implement the correct management measure. A quantitative assessment of the effect of different management options on the fishery resources is possible through simulation modeling of the system. Here, a simulation study was conducted to examine the effects of restrictions imposed on fishing effort on the demersal fishery resources of Tamil Nadu, India.

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With regard to marine fish production, the state of Tamil Nadu stands fourth among the maritime states, after Kerala, Gujarat, and Maharashtra. During the period 2001-2005, the estimate of average annual landings in the state is 3,55,451 t (tonnes), which accounts for 14.45% of the total production in the country. The average demersal landing during 2001-2005 is 1,08,561 t, which is about 30.91% of the marine fish landings in the state. Important demersal resources, based on landings in the state, are silverbellies (30.88%), perches (29.49%), elasmobranchs (14.14%), croakers (7.64%), goatfishes (5.54%), lizardfishes (4.06%), catfishes (2.68%), and pomfrets (2.48%). Important gears that caught demersal resources are mechanized trawlnet, mechanized hooks and lines, multiday trawlnet, outboard hooks and lines, and outboard gillnets. On an average during 2001-2005, about 61.07% of the demersal landings were by mechanized trawlnets, 14.74% by outboard gillnets, 6.8% by outboard hooks and lines, and 5.93% by multiday trawlnets.

Application of simulation model into fisheries research was considered by many authors. Grant et al. (1981) gave a generalized bio-economic simulation model for annual-crop fisheries and demonstrated its use in marine fisheries management. George & Grant (1983) described a stochastic simulation model for the dynamics of brown shrimp (*Penaeus aztecus*) in Galveston Bay, Texas. Parker (1986) used data from the Celtic sea and formulated a dynamic simulation model to describe the accumulation of chlorophyll within the thermocline. Carothers & Grant (1987) explored the relationship between recruitment seasonality and ordination of alternative management policies through a general stochastic simulation model.

Ackley (1995) developed a simulation model of the Bering Sea fishery as a quantitative means for estimating the impacts of management actions on catch and bycatch. Christensen (1998) constructed two mass-balance trophic models to describe the Gulf of Thailand ecosystem and validated the dynamic simulation model, *Ecosim*, to predict ecosystem level changes following changes in fishing pressure. Senina et al. (1999) developed a stochastic simulation model for the community of competing anchovy *Engraulis encrasicolus* and sprat *Clupeonella delicatula* in the Azov Sea in Russia and investigated their extinction risk on the basis of time series of population abundance and environmental factors that influence reproduction.

Beare et al. (2000) examined the potential of real-time performance indicators in the Australian northern prawn fishery using a stochastic optimal control model of the fishery. Chen et al. (2000) developed a fuzzy logic model with genetic algorithm for analyzing stock-recruitment relationships of southeast Alaska pink salmon (*Oncorhynchus gorbuscha*) and West Coast Island Pacific herring (*Clupea pallasii*) stocks. Azadivar et al. (2002) used simulation-based optimization to determine an area management policy with optimal fishing rate for the sea scallop resources of Georges Bank in Northwest Atlantic Ocean. Mishra et al. (2002) developed a bio-energetic

dynamic simulation model for the growth of penaeid shrimps based on an existing model on tilapia growth, and it was consisting of submodels for molting, feed consumption, digestion and biosynthesis, energy metabolism, oxidation, and growth. Prager (2002) made a comparison of results from logistic and generalized surplus production models by the simulation of stock of swordfish *Xiphias gladius* in the North Atlantic Ocean. Schnute & Haigh (2003) used a simulation model based on compound binomial-gamma distribution to assist the planning and design of ground fish trawl survey.

Materials and Methods

The basic surplus production model (Schaefer 1954) is used for calculation of biomass, fishing mortality, and yield in the simulation. The model is given by

$$\frac{dB_t}{dt} = rB_t(1-\frac{B_t}{K}) - F_t B_t$$

where B_t is the biomass at time *t* (year), *r* is the intrinsic rate of increase of the stock, *K* is the carrying capacity, and F_t is the fishing mortality rate. The recursive expressions for calculating biomass and yield given by Prager (1994), based on the model parameters initial biomass B_0, carrying capacity *K*, intrinsic growth rate *r,* and catchability coefficient *q* using time series data on catch and effort, were followed in this study. The maximum sustainable yield (MSY), biomass at MSY, fishing mortality that generates MSY, and the fishing effort corresponding to MSY were estimated (Prager 1994) as given below.

$$MSY = \frac{Kr}{4}; \qquad B_{MSY} = \frac{K}{2};\ F_{MSY} = \frac{r}{2};\quad f_{MSY} = \frac{r}{2q}$$

A genetic algorithm developed was used for estimation of the parameters of the surplus production model using time series data on catch and effort of demersal resources of Tamil Nadu during 1989-2005 obtained from the database of the Central Marine Fisheries Research Institute, Cochin. Effort series for demersal catch were computed using the effort of mechanized trawlnet, mechanized hooks and lines, multiday trawlnet, outboard hooks and lines, and outboard gillnets, which mainly catch demersal resources. Management options can be introduced only on the effort series on hours of operation of the gears. For simulating effort series for future years, spectral time series models were adopted by estimating model parameters and residual variance using time series data on effort of these gears. The spectral model used has the expression

$$y_t = a_0 + \sum_{i=1}^{k}\left[a_i Sin(2\pi\lambda_i t) + b_i Cos(2\pi\lambda_i t)\right] + \varepsilon_t .$$

The error term ε_t was assumed to be from a normal distribution with zero mean and constant variance for simulation purpose. For implementation of the genetic algorithm, spectral model estimation and simulation of effort, biomass, fishing mortality, and yield computer software were developed in-house in C++. For the prediction of

future biomass and yield, the effort scenario was simulated first, which was then used for the yield forecast. The simulation of effort was carried out as follows. From the spectral model that was exclusively fitted on the effort time series, the mean effort for a future year was computed with the parameters. Then, the distributional aspect of effort sequence was assumed to be normal with standard error estimated from the effort series. With each of the predicted mean effort, 1000 numbers of simulated normal values with appropriate error were added to get the sequence of 1000 effort values for each year of forecast. For each value of the simulated effort, biomass, fishing mortality, and yield were calculated using the estimated surplus production model, and averages of these quantities were recorded. Such simulations were carried out for each of the future years from 2006 to 2015. Restrictions on hours of operations were introduced by multiplying the simulated effort by a suitable factor before calculation of biomass, fishing mortality, and yield.

Results and Discussion

Estimates of parameters of surplus production model obtained through the genetic algorithm using time series data on catch and effort for demersal resources of Tamil Nadu and the estimates of spectral model parameters used for modeling effort series are given in Table 1.

Table 1. Estimates of parameters of surplus production model and spectral model

Parameter	Estimate	No	Frequency	Periodogram	Sine term	Cos term
B_0	197369.24	1	0.0588	141493	-16603.51	-1191.87
K	474003.06	2	0.1176	53593	4792.94	4096.61
r	1.268522	3	0.2353	43819	138.69	-5153.34
q	0.000004028	4	0.4706	33695	2143.46	-3334.68
		5	0.3529	29502	3375.61	807.13
		6	0.1765	29134	-3427.17	-48.35

The estimate of MSY for demersal resources in Tamil Nadu calculated using the estimates of surplus production model parameters is 1,50,320 t (tonnes), estimate of biomass corresponding to the MSY level is 2,37,001 t, estimate of fishing mortality rate at MSY level is 0.6343, and estimate of effort corresponding to MSY level is 1,57,463 hours of operation. Plot of the biomass and yield calculated using the estimated surplus production model along with the observed catch is given in Fig. 1.

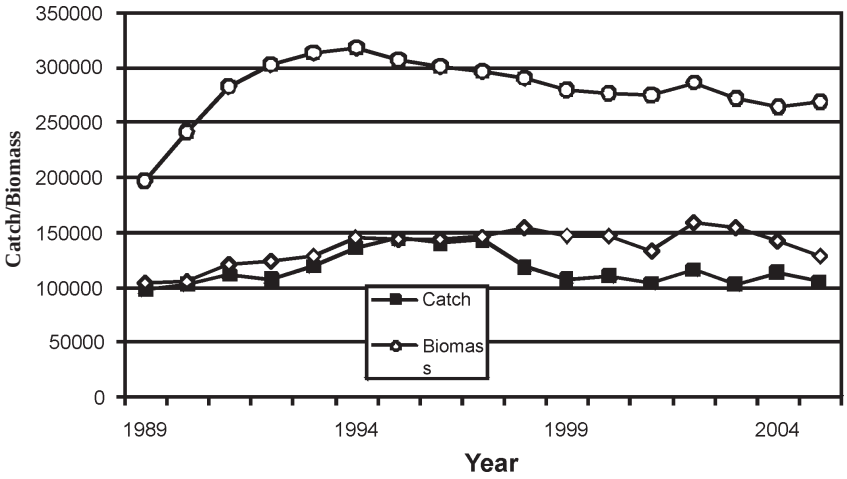

Figure 1. Plot of observed catch along with yield and biomass computed using the estimated surplus production model for the demersal resources of Tamil Nadu

The observed effort series and the corresponding fitted series based on spectral model are shown in Fig. 2.

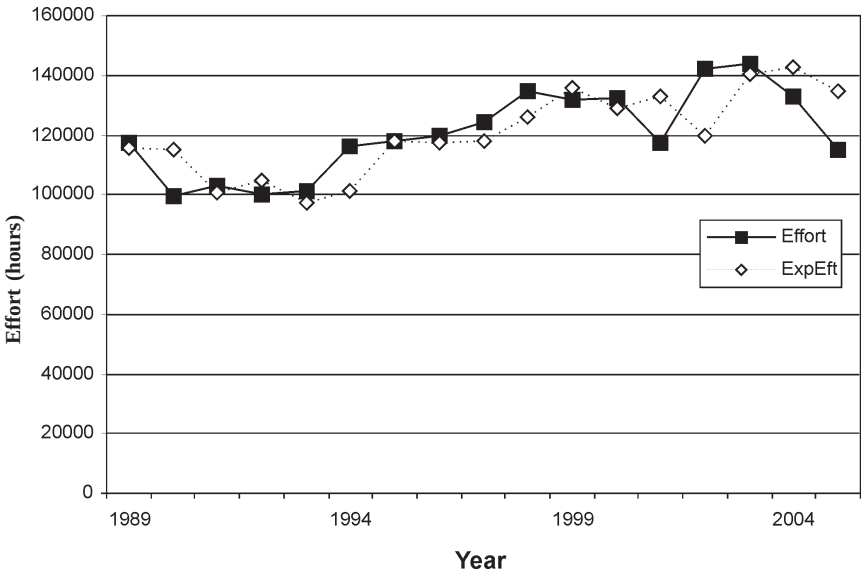

Figure 2. Plot of observed effort and effort computed using the fitted spectral model

Estimates of fishing mortality rate, biomass and expected yield based on the fitted model are given in Table 2. During the period 1996-2005, the observed demersal catch is below the MSY level for all the years. The average annual demersal catch during this period is 1,16,433 t, which is far below the MSY.

Table 2. Estimates of fishing mortality, biomass and yield calculated based on the fitted surplus production model

Year	F (t)	Biomass	Yield
1989	0.473234	197369	104625
1990	0.399908	241940	105903
1991	0.414505	283894	122132
1992	0.402365	303107	124592
1993	0.407327	314682	129121
1994	0.467437	318752	146056
1995	0.474535	307751	144375
1996	0.482095	301587	144229
1997	0.500229	297330	147151
1998	0.54349	291747	155137
1999	0.531442	280622	148316
2000	0.533488	277885	147742
2001	0.473576	276195	133727
2002	0.57314	287251	159957
2003	0.580032	272828	155651
2004	0.534568	264854	143015
2005	0.464775	269660	129503

Simulation of the demersal fishery for Tamil Nadu was carried out by generating effort series using the estimated spectral model with a normal error term having zero mean and constant variance for different levels of effort such as 25%, 50%, 75%, 100%, 125%, 150%, 175%, and 200% of the current level of exploitation. One thousand such simulations were made, and for each simulation mortality, biomass and yield series were generated for the period from 2006 to 2015, and the averages of the results of simulation are given in the Table 3 for different levels of exploitation.

Table 3. Fishing mortality, biomass, and yield simulated for different levels of exploitation for the demersal resources of Tamil Nadu

	25%			50%		
Year	Mortality	Biomass	Yield	Mortality	Biomass	Yield
2006	0.1159	285781	38593	0.2317	285781	73688
2007	0.1016	371239	40196	0.2031	344048	73760

2008	0.1051	413569	44362	0.2102	377674	80781
2009	0.0981	427884	42339	0.1964	389098	77275
2010	0.1018	434374	44273	0.2035	396596	80816
2011	0.1187	435474	51396	0.2375	397485	93285
2012	0.1180	431483	50833	0.2362	389532	91658
2013	0.1186	430410	51014	0.2370	387087	91570
2014	0.1269	429913	54367	0.2537	386040	97283
2015	0.1370	427652	58320	0.2740	381652	103555

		75%			100%	
Year	Mortality	Biomass	Yield	Mortality	Biomass	Yield
2006	0.3469	285781	105430	0.4633	285781	134510
2007	0.3045	318418	101202	0.4063	293948	122982
2008	0.3153	343045	109590	0.4212	309636	131340
2009	0.2949	351005	104837	0.3934	313588	125046
2010	0.3046	358863	109436	0.4068	321286	130782
2011	0.3553	359684	125458	0.4750	321686	148879
2012	0.3543	348409	122584	0.4719	307401	143597
2013	0.3549	344311	121834	0.4745	301972	142475
2014	0.3807	342547	128981	0.5079	299064	149543
2015	0.4105	336112	135812	0.5480	290967	155829

		125%			150%	
Year	Mortality	Biomass	Yield	Mortality	Biomass	Yield
2006	0.5787	285781	160554	0.6950	285781	184192
2007	0.5077	271105	139567	0.6087	249494	151339
2008	0.5252	277971	145925	0.6322	248077	155058
2009	0.4909	277862	138228	0.5891	243114	144869
2010	0.5089	284586	144681	0.6099	248306	151036
2011	0.5933	284193	162999	0.7125	247244	168734
2012	0.5898	267600	155467	0.7077	228717	158443
2013	0.5919	260518	152901	0.7118	220087	154535
2014	0.6344	256684	159380	0.7603	214778	158799
2015	0.6854	247033	164023	0.8223	204133	160935

Plots of observed catch, estimated average biomass, and average yield for different exploitation levels are shown in Fig. 3 and 4.

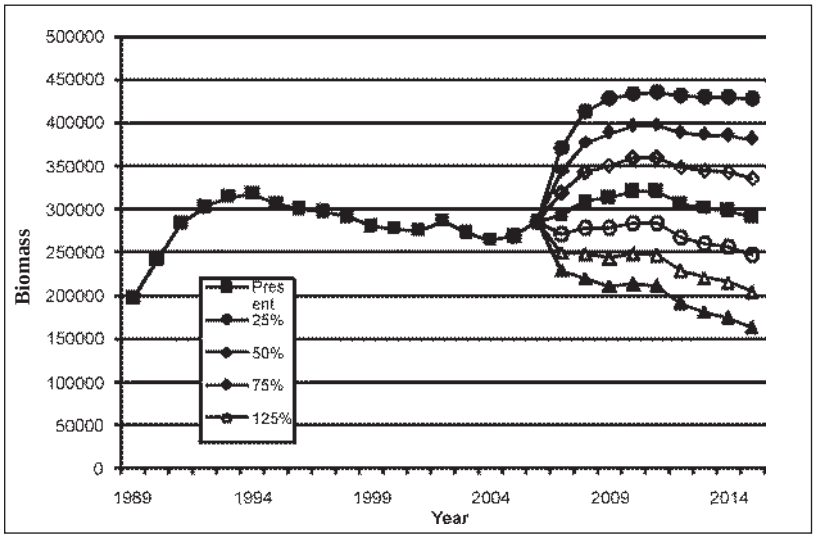

Figure 3. Simulated biomass for demersal fishery in Tamil Nadu for the period up to 2015 for different exploitation levels (as percentages of present level)

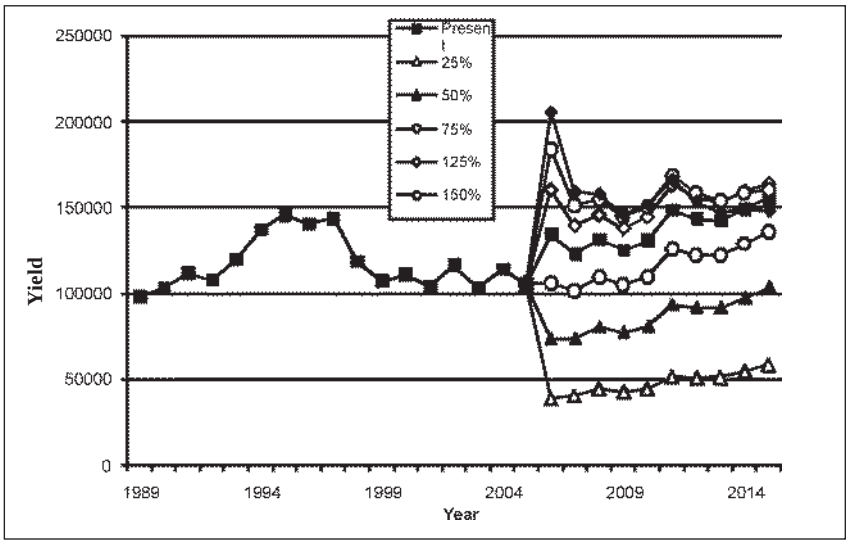

Figure 4. Simulated yield for demersal fishery in Tamil Nadu for the period up to 2015 for different exploitation levels (as percentages of present level)

When the fishery was simulated for the period 2006-2015 maintaining the present level of exploitation, the average annual yield obtained for the period was 1,38,498 t which is below the MSY and the average biomass obtained for the period was 3,04,533 t. The yield obtained in individual years is below the MSY except for the year 2015 in

which the expected annual yield is 1,55,829 t. The expected minimum yield was 1,22,982 t for the year 2007. In the case when the exploitation level was reduced to 75% of the present level, the expected yield in all the years from 2006 to 2015 was found to fall below MSY with 1,16,516 t, as the average annual yield for the period and the average annual biomass for the period was 3,38,818 t. The maximum yield expected was 1,35,812 t for the year 2015, and the minimum expected was 1,01,202 t in 2007. Simulation results were also obtained by keeping the exploitation level at 125% and 150% of the present level. The simulated average yield during 2006-2015 for 125% level was 1,52,373 t and for 150% level was 1,58,794 t both falling above MSY level. The maximum expected yield for these two exploitation levels was 1,64,023 t in 2015 and 1,84,192 t in 2006, respectively. In these cases, the expected average annual yields were above MSY in most of the years. The annual average biomass during the period 2006-2015 in the two cases was 2,71,333 and 2,38,973 t, respectively.

From the above results, it was observed that the optimum level of exploitation is between 75% of the present level and the present level of exploitation (100%). To work out the optimum level of exploitation that will retain all the years expected yield below the MSY level, further simulations were carried out for finer divisions of levels of exploitations, and it was found that at 91.25% of the present level of exploitation, the expected yields for all the years form 2006 to 2015 are below MSY.

Acknowledgment

The authors wish to express their gratitude to Prof. (Dr.) Mohan Joseph Modayil, Director, Central Marine Fisheries Research Institute, Cochin, for providing facilities necessary for the conduct of the simulation study.

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Received: 31 December 2007; Accepted: 26 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

An Appraisal of the Marine Fisheries of Orissa, India

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Abstract

This paper presents the trend of marine fish landings of Orissa during the period 1975-2006. The marine fish landings of Orissa indicated a general increasing trend from 16,804 t in 1975 to 1, 01,500 t during 2005 contributing to an average of 2.6% of the all India marine fish landings. Demersal fishery resources including crustaceans and molluscs dominated the landings ranging between 6575 t during 1977 to 56,556 t during 2005. The pelagic fishes contributed to 8497 t during 1977 to 44472 t during 2005. A total of six types of mechanized and motorized gear each and seven types of non-motorized gears were operated off Orissa during 2005 & 2006.The total effort both in units and Actual Fishing Hours (AFH) was greater during 2005 (5.91 Lakh units & 35 Lakh AFH) than during 2006 (5.49 Lakh units & 31 Lakh AFH). While motorized gears expended more effort in terms of units, mechanized gears had put in more AFH during both the years. Gear wise, all the resources were landed more by mechanized gears followed by motorized gears and non-motorized gears. Resource wise, pelagic fishes were contributed mainly by carangids (21%), ribbon fishes (19.5%) and other clupeids (13.5%). The major contributors of demersal resources were croakers (35%), pomfrets (17%), catfishes (16%), and silver bellies (5.4%). Crustacean resources were dominated by penaeid prawns (78%) followed by non-penaeid prawns (11.6%) and crabs (9.25%). Seasonally, all major resources were landed more during October to December and January to March period.

Introduction

The state of Orissa (lat. 17.75ºN & 22.5ºN; Long 81.5ºE & 87.6ºE) has a coast line of 480 km and a continental shelf area of 25000 km^2. The state has six maritime districts namely Balasore (80 Km), Bhadrak (50 Km), Kendrapara (68 Km) Jagatsinghpur (67 Km), Puri (155 Km) and Ganjam (60 Km) (Fig. 1). The marine fishery of Orissa assumes importance both in relation to domestic market and export earning. The coastal and offshore waters off Orissa form a rich abode of many a quality pelagic and demersal resources. According to Reuben et al. (1989), in order of richness of bottom trawl fishery resources along the northeast coast of India, Orissa ranks first followed by Andhra Pradesh and West Bengal. Though the fishery potential of Orissa had been assessed through several exploratory surveys (Sheriff 1961; Krishnamoorthy 1976; Sekharan 1973; Sekharan et al. 1973; Joseph et al. 1976; Appa Rao 1978; Appa Rao and

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Krishnamoorthi 1983; Reuben et al. 1989; Menon et al. 1996) the trend of marine fish landings through commercial landings had been less attempted (Dharmaraja & Philipose 1977; Scariah et al. 1987; CMFRI 1995). This paper is an attempt on the appraisal of marine fisheries of Orissa based on commercial landings with particular emphasis on the current exploitation and future potential.

Figure 1. Major fish landing centres in Orissa*.

Materials and Methods

The catch and effort data on marine fisheries of Orissa are obtained from FRA Division of CMFRI, Kochi. Gear-wise landing data for the period between 2005 and 2006 are analyzed with special reference to seasonal abundance of major groups landed in mechanized trawlers and motorized gill netters. Catch per hour is reckoned for evaluating seasonal abundance.

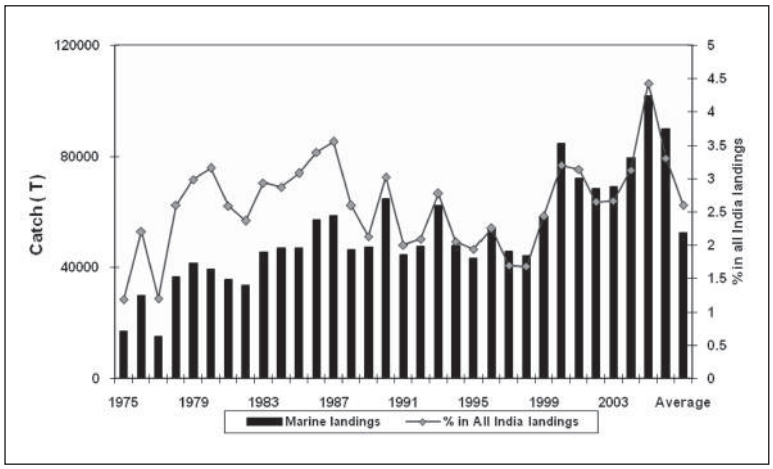

Figure 2a. Marine fish landings off Orissa & its % in All India marine landings during 1975 to 2006

Results and Discussion

Exploitation (Fig. 2a,b)

The marine fish landings in Orissa during 1975 to 2006 indicated a general increasing trend from 16804 t during 1975 to 101500 t during 2005 (Fig. 2a). The landings of Orissa formed 1.18% of the all India marine landings during 1975 to 4.42% during 2005, the average being 2.60%.

The demersal resources including crustaceans and molluscs dominated the total landings ranging between 6575 t during 1977 and 56556 t during 2005 (Fig. 2b).

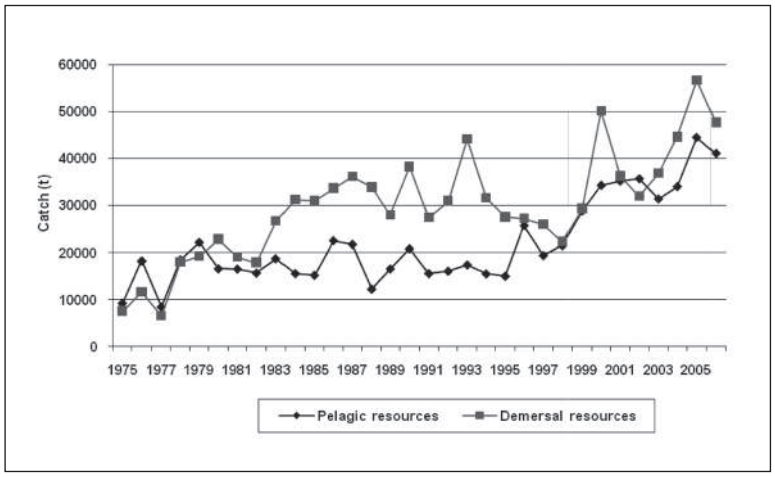

Figure 2b. Pelagic and demersal resources landed off Orissa during 1975 to 2006

The pelagic resources ranged between 8497 t during 1977 and 44472 t during 2005.

According to Scariah et al. (1987), the pelagic fish landings off Orissa indicated a declining trend from 18245 t during 1976 to 15600 t in 1984, with a corresponding decline in effort input by the non-mechanized units from 29823 units in 1976 to 17553 units in 1984. The present study shows that during the same period, the demersal resources indicated an increase in landing from 11578 t in 1976 to 31173 t in 1984. This may be because of the increase in contribution by the mechanized sector from 1980 onwards when mechanization started gathering momentum in Orissa. From 1985 to 2006 also, the contribution of demersal resources was on the higher side ranging between 31070 t in 1985 and 56556 t in 2005 when compared to their pelagic counter part and this may also be due to the wider continental shelf off Orissa in addition to multiday trawl operation by the mechanized trawlers. Likewise, during 1993 to 98 period, the demersal finfish landings had declined from 38740 t to 18608 t with a corresponding decline in mechanized trawler units operation from 1,12,000 t to 38000 t and that of mechanized gill netters from 1,18,000 t to 68,000 t (Srinath et al., 2003). But the pelagic

resources indicated a fluctuating pattern ranging from 15037 t during 1995 to 21488 t during 1998, which may be attributed to the increased motorized units operation from 10000 m to 2,82,000 m (Srinath et al. 2003).

***Fishery during 2005 and 2006*:** With a view to analyse the recent trend in landings, the catch and effort data during 2005 and 2006 were studied in detail.

***Fishing crafts and gears*:** Orissa state has a total of 57 landing centers spread over 641 fishing villages. According a latest estimate off Orissa, there are a total of 3577 mechanized, 4719 motorized, and 15,444 non-motorized fishing crafts operated by a total of 74,980 active fisher folk (CMFRI, 2007a).

***Effort expended during 2005 and 2006*:** A total of 19 types of gears were operated off Orissa under the mechanized, motorized, and nonmotorized categories (Table 1).

Table 1. Particulars of crafts & gears operated off Orissa during 2005 & 2006

Crafts	Gears
Mechanised	Single day Trawlers(MTN), MultiDay Trawlers(MDTN), Mechanised Gill Netters(MGN), Mechanised Drift Netters(MDN), Mechanised Drift Gil Netters(MDGN), Mechanised Hooks & Line (MHL)
Motorised	Out Board Gill Netters(OBGN), Outboard Drift Netters(OBDN), OutBoard Bottom Set Gill Netters(OBBGN), Out Board Hooks & Line(OBHL), Out Board Ring Netters (OBRN), OutBoard Ring Seine(OBRS)
Non motorised	Non Motorised Gill Netters(NMGN), Non motorised Hooks & Line(NMHL), Non Motorised Shore Seine(NMSS), Non Motorised Boat Seine(NMBS), Non Motorised Drift Netters(NMDN), Non Motorised Bottom Set Gill Netters(NMBGN), Non Motorised Ring Netters(NMRN)

Particulars regarding unit effort and actual fishing hours (AFH) expended off Orissa during 2005 and 2006 are presented in Fig. 3a & b.

Effort in units (Fig. 3a): During 2005, a total of 0.69 lakhs mechanized units were operated followed by 2.80 lakh units of motorized gears and 2.42 lakh units of non-motorized gears. The total units expended amounted to 5.91 lakhs units. During 2006, mechanized, motorized, and non-motorized gears operated 0.56 lakhs, 2.95 lakhs and 1.98 lakh units, respectively, the total effort input being 5.49 lakh units (Fig. 3a).

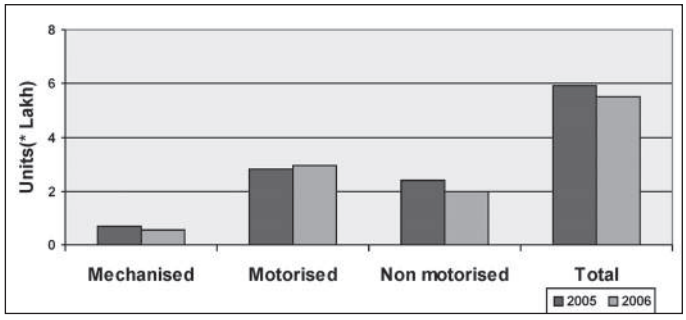

Figure 3a. Effort (units) expended off Orissa during 2005 & 2006

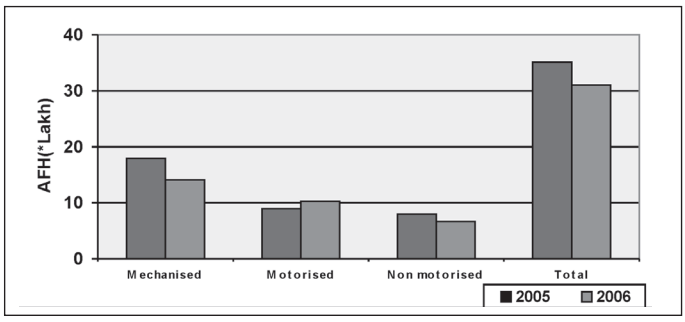

Figure 3b. Actual fishing Hours expended off Orissa during 2005 & 2006

Effort in AFH (Fig. 3b): During 2005, the mechanized gears expended an effort of 18.03 lakhs followed by 8.96 lakhs hrs by motorized gears and 8.03 lakhs hrs by non-motorized gears.

During 2006, the mechanized gears had put in an effort of 13.9 lakh hrs while motorized gears expended 10.34 lakh hrs. The effort expended by non-motorized gears was 6.71 lakh hrs (Fig. 3b). The total fishing hours expended during 2005 & 2006 amounted to 35 lakhs and 31 lakhs, respectively.

It may be noticed that the total effort both in units and AFH expended is more during 2005 than during 2006. However, among the different groups of gears, motorized gears were found to have put in more effort in units while mechanized gears had expended more fishing hours during both the years that may be attributed to the operation of multiday trawlers by mechanized units.

Fishery Particulars regarding the landings of various resources off Orissa for the years 2005 & 2006 are given in Table 2.

Table 2. Major fishery resources landed off Orissa during 2005 & 2006

Resources/Years	2005		2006	
	Catch (t)	%	Catch(t)	%
Pelagic fishes	43791	43.14	41081	45.86
Demersal fishes	38750	38.18	34017	37.97
Crustaceans	17293	17.04	13094	14.62
Molluscs	1194	1.18	514	0.57
Miscellaneous	472	0.47	880	0.98
Total	101500		89586	

During 2005, pelagic fishes contributed to a total of 43791 t (43%) while demersal fishes formed 38750 t (38%). Crustacean resources brought a landing of 17293 t (17%) with molluscs (1194 t; 1.2%) and miscellaneous items (472 t; 0.47%) contributing to the rest of the catches. The total catch amounted to 101500 t.

During 2006, the total catch of 89586 t was contributed by the pelagic fishes (41081 t; 45.86%), demersal fishes (34017 t; 38%), crustaceans (13094 t; 14.62%), molluscs (514 t; 0.57%) and miscellaneous items (880 t; 1%).

***Gearwise landings*:** Average catch and percentage of resources landed in mechanized, motorized and non-motorized gears during 2005 & 2006 are given in Fig. 4.

Pelagic fishes were contributed maximum by mechanized gears (24,171 t; 57%) followed by motorized gears (12757 t; 30%) and non-motorized gears (5508 t; 13%). Demersal fishes also were landed the maximum in mechanized gears (27145 t; 74.6%) followed by motorized gears (7463 t; 20.5%) and non-motorized gears (1776 t; 5%). Crustacean resources were landed the maximum in mechanized gears 14098 t; 93%) followed by motorized gears (594 t; 4%) non-motorized gears (503 t; 3%). Molluscan resources also were landed the maximum by mechanized gears (840 t; 98%) with lesser representation in motorized gears (7 t; 0.75%) and non-motorized gears (6 t; 0.61%).

An evaluation of gear wise landings during 2005 and 2006 show that both pelagic and demersal resources were landed more in mechanized gears contributing to 57% and 75%, respectively. During 2005 and 2006, the mechanized trawlers operated 27422 and 21115 units and 1432131 and 1122201 AFH while the mechanized gill netters operated 40976 and 31239 units and 369018 and 253269 AFH (CMFRI, 2007b). While the contribution of demersal resources in mechanized gears can be attributed more to

operations of mechanized trawlers, the increased landings of pelagic resources also by mechanized gears may be due to the operation of mechanized gill netters.

***Resource wise landings*:**

Pelagic resources: Average percent composition of pelagic, demersal, and crustaceans resources landed during 2005 & 2006 are depicted in Fig. 5a-c.

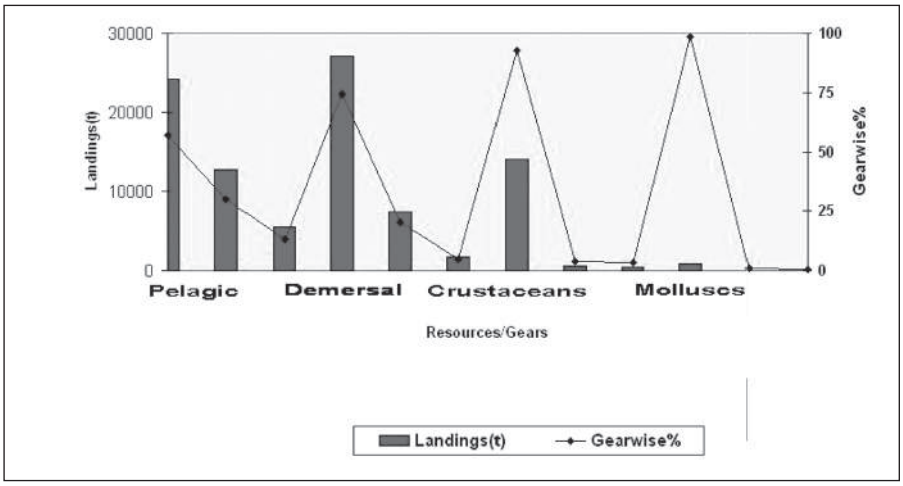

Figure 4. Gearwise landings (t) of major resources off Orissa during 2005 & 2006 (Average)

Among pelagic fishes, carangids (20.58%), ribbon fishes (19.54%) and other clupeids (13.47%), were the major resources landed followed by other sardines (9.23%), *Stoleph-orus* spp., (6.6%), Indian mackerel (6.64%), *Setipinna* spp (5.71%) and Seer fishes (5.2%) Fig. 5a.

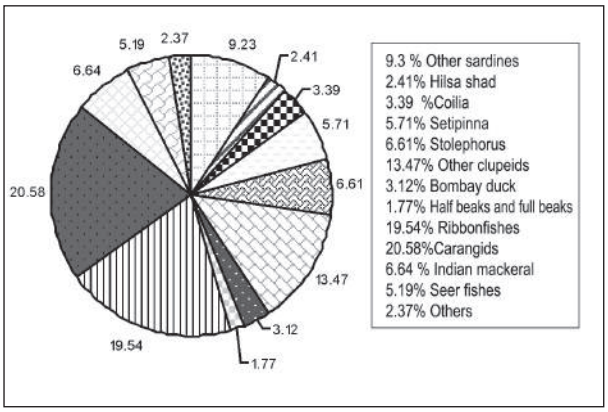

Figure 5a. Average % composition of pelagic resources landed off Orissa during 2005 & 2006

Among demersals, croakers (35%), pomfrets (17%), catfishes (16%), silver bellies (5.36%), goat fishes (4.66%) and flat fishes (4.22%) were the major groups landed (Fig. 5b). Among crustaceans, penaeid (78.2%), non penaeid prawn (11.64%), crabs(9) and stomatopods were the major groups landed (Fig. 5c).

Major species landed :

Among pelagic fishes, major carangids represented in the landings were *Megalaspis cordyla, Caranx ignobilis, C.malabaricus, Decapterus russelli, D macrosoma* and *Selar crumenophthalmus*. Major ribbon fish species landed were *Trichiurus lepturus, Lepturacanthus savala, Eupleurogrammus intermedius* and *E.muticus*. Lesser sardines were represented more by *Sardinella gibbosa, S.fimbriata and S.brachysoma* (CMFRI. 2007).

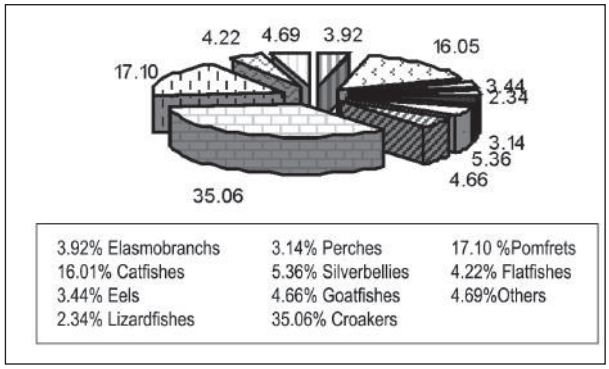

Figure 5b. Average % composition of demersal resources landed off Orissa during 2005 & 2006

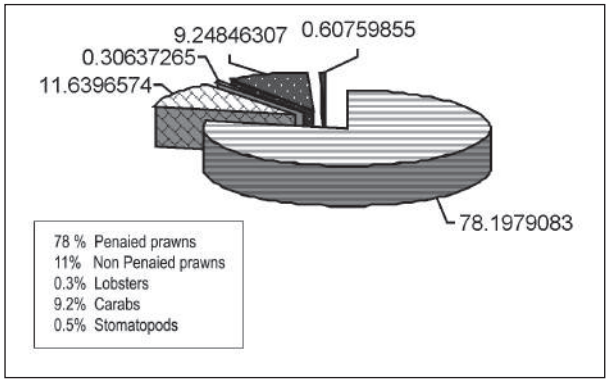

Figure 5c. Average % contribution of crustacean resources off Orissa during 2005 & 2006

Among demersal fishes, catfishes were represented by species such as *Tachysurus thalassinus, T.tenuispinis,* and *T.jella* while the dominant croakers landed were *Otolithoides biauritus, Protonibea diacanthus, Johnius carutta, Otolithus argenteus* and *O.ruber*.

Among crustaceans penaeid prawns were represented by *Parapeneopsis stylifera, Metapenaeus dobsoni, M.monoceros, M.affinis, Solenocera crassicornis, S.choprai and S.indica,. S.hextii, and Fenneropenaeus indicus.* Molluscan resources were chiefly represented by cephalopods.

Resource wise landings in the present study shows that pelagic groups like ribbon fishes and carangids and demersal groups such as croakers, catfishes, and pomfrets were the major groups landed. Exploratory surveys along Orissa coast had indicated that resources such as carangids and catfishes yielded high catch rates of 500 kg/hr at 19º26'N, 85º09'E at 90 m depth and 1500 Kg/hr off 19º19'N, 85º15'E (at 62 m depth) (Menon et al. 1996). Vijayakumar and Naik (1991) had reported of high abundance of catfishes along north east coast within 51-100 m depth. Sivakami et al. (1996) had

reported that Orissa coast is a potential zone for carangids. Nair et al. (1996) had obtained a catch rate of 493 kg/hr of ribbon fishes from 100 m depth off south Orissa coast. Off Andhra-Orissa coast, mackerel was abundant in the 51-100 m depth with a catch rate of 109 Kg/hr (Anon, 1987). Reuben et al. (1989) had noticed very rich grounds for mackerel off 19ºN, 80ºE, 20ºN, 87ºE and 20ºN, 88ºE and for sciaenids at 19ºN, 84ºE areas. They had also observed that non demersal groups like mackerel and carangids were exploited by the traditional sector much below their potential leaving scope for harvesting the surplus stocks from waters beyond 40 m depth. Thus, it may be concluded that the shelf waters off Orissa have a rich potential of both pelagic and demersal fishes probably by virtue of a wider continental shelf of trawlable muddy/sandy bottom with the coast of Orissa characterized by several estuarine systems of higher productivity.

According to Whitehead (1973) and Rao (1973), the distribution of Oil sardine (*Sardinella longiceps* Val.) along the east coast of India is mainly off Tamil Nadu and Andhra Pradesh where they form stray catches. Antony Raja (1969) includes the coast of Orissa for the occurrence of oil sardine "under verification". Ramasomayajulu and Dhana Raju (1985) have confirmed its occurrence in this region and Scariah et al. (1987) had reported of a landing of 539 t of oil sardine off Orissa coast during 1984. Pillai et al. (2003) had observed that in the north eastern states of West Bengal and Orissa, a new fishery for oil sardine has emerged from a position of no landings. In the present study, oil sardine formed 86 t and 196 t during 2005 and 2006 contributing to an average of 0.35% of the pelagic fish landing off Orissa thereby confirming their distribution off Orissa coast. They were landed in outboard gill net, ringnet, non-motorized gillnets & trawl net.

***Seasonal abundance*:** Catch rate (Kg/hr) of dominant groups of various resources landed during 2005 & 2006 in mechanized trawlers and motorized gill netters is depicted in Fig. 6a-l.

Pelagic fishes:

Mechanized trawlers:

Ribbon fishes: During 2005, ribbon fishes had the peak landings during January (7.03 Kg/hr), March (14.35 Kg/hr), November (9 Kg/hr) and December (9 Kg/hr). In addition during 2006, peak landings were observed during January (6.04 Kg/hr), November (10 Kg/hr) and December (17.63 Kg/hr) (Fig. 6a).

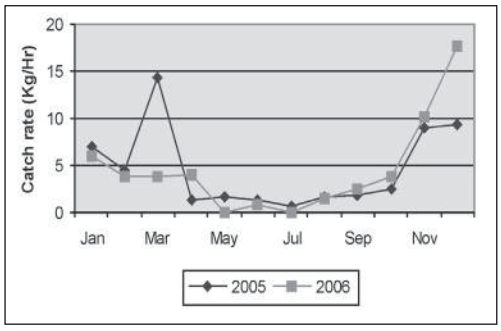

Figure 6a.Seasonal abundance (C/hr) of Ribbon fishes landed off Orissa in mechanised tralwers during 2005 & 2006

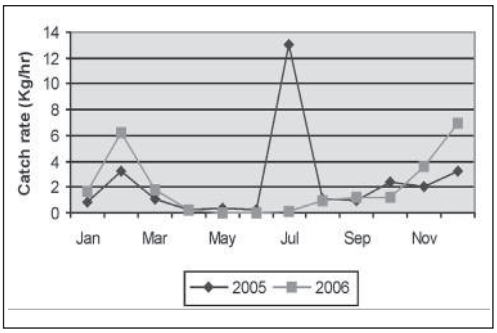

Figure 6b.Seasonal abundance(C/hr) of Carangids landed off Orissa in mechanised trawlers during 2005 & 2006

Carangids: Carangids were landed the maximum during July 2005 (13 Kg/hr) and during February (6.28 kg/hr) and December (7 Kg/hr) during 2006 (Fig. 6b).

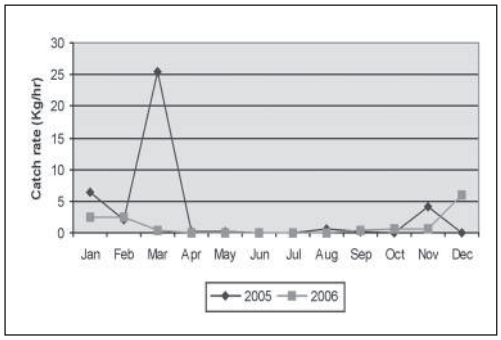

Figure 6c. Seasonal abundance of mackeral landed in mechanised trawlers off Oissa during 2005 & 2006

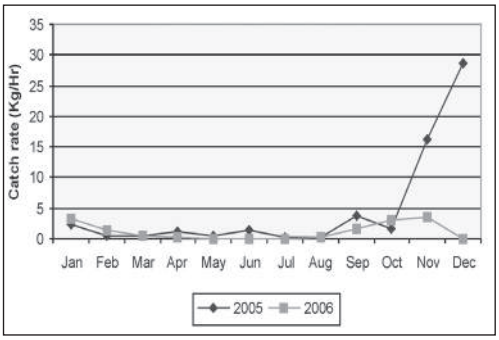

Figure 6d. Seasonal abundance (C/Hr) of Ribbon fishes landed off Orissa in motorised gill netters during 2005 & 2006

Mackeral: Indian mackerel were landed the maximum during January (6.36 Kg/hr), March (25.48 Kg/hr) and November (4.13 Kg/hr) during 2005 while during 2006, catch rates were generally less with peaks during January (2.55 Kg/hr), February (2.5 Kg/hr) and December (6 Kg/hr) (Fig. 6c).

Motorized gill netters:

Ribbon fishes: During 2005, ribbon fishes were landed the maximum during November (16.11 Kg/hr) and December (28.67 Kg/hr) while during 2006, peak landings were noticed during January (3.18 Kg/hr) and November (3.61 Kg/hr) (Fig. 6d).

Carangids: During 2005, carangids were landed the maximum during January (7 Kg/hr), March (31.31 Kg/hr), April (12.6 Kg/hr) June (7.32 Kg/hr), August (20.35 Kg/hr)

October (20.2 Kg/hr) November (20.2 Kg/hr) and December (13.4 Kg/hr). During 2006, peak landings were noticed during January (6.52 Kg/hr), February (12.92 Kg/hr) and June (5.24 Kg/hr) (Fig. 6e).

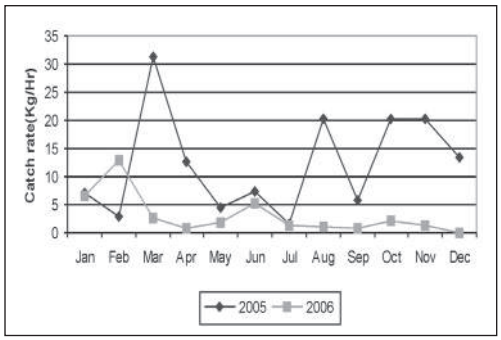

Figure 6e. Seasonal abundance (C/Hr) of Carangids landed in motorised gill netters off Orissa during 2005 & 2006

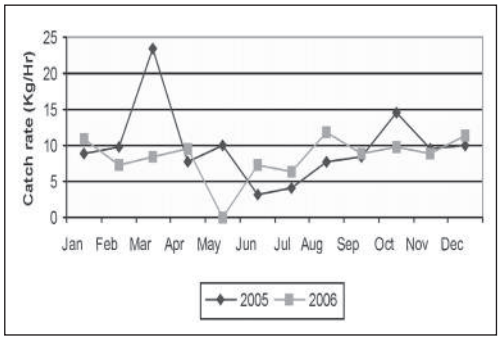

Figure 6f. Seasonal abundance of Croakers landed in mechanised trawlers off Orissa during 2005 & 2006

Demersal fishes:

Mechanized trawlers: Croakers: In mechanized trawlers, croakers generally brought good catch rates particularly during March (23.51 Kg/hr) and during October 2005 (14.45 Kg/hr) and during August (11.81 Kg/hr) and December 2006 (11.42 Kg/hr) (Fig. 6f).

Catfishes: Catfish landings were generally less in mechanized gears with peak only during November 2006 (18.66 Kg/hr) (Fig. 6g).

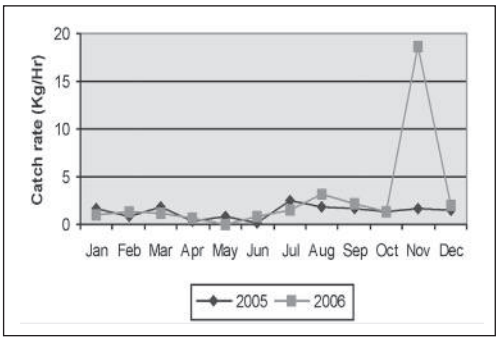

Figure 6g. Seasonal abundance (C/Hr) of catfishes landed off Orissa in mechanised trawlers during 2005 & 2006

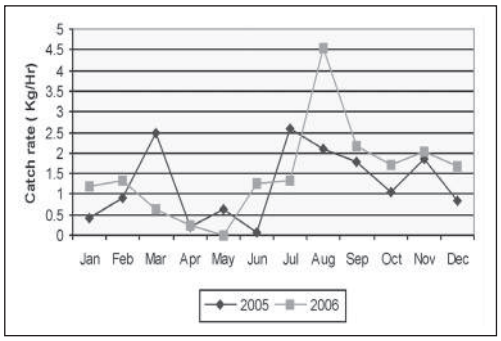

Figure 6h. Seasonal abundance (C/Hr) of Pomfrets landed in mechanised trawlers off Orissa during 2005 & 2006

Pomfrets: Pomfret landings in mechanized trawlers were high during March (2.5 Kg/hr), July (2.6 Kg/hr) and during November 2005(1.84 Kg/hr). During 2006, good catch

rates were obtained during August (4.54 Kg/hr), September (2.16 Kg/hr) and November (2 Kg/hr) (Fig. 6h).

Motorized gill netters:

Croakers*:* Compared to mechanized trawlers, landings of croakers in motorized gill netters was less with peak during March (9.06 Kg/hr), August (6.47 Kg/hr) and October (12 Kg/hr) during 2005. During 2006, peak catch rates were obtained during February (2.42 Kg/hr), June (2.11 kg/hr) and July (4.76 Kg/hr) (Fig. 6i).

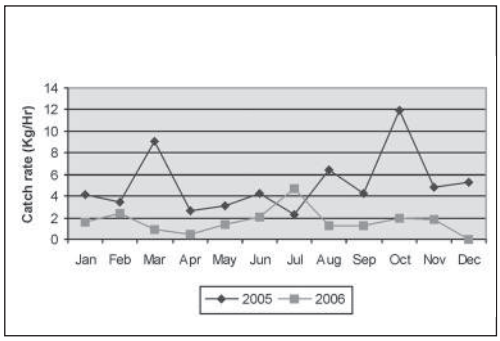

Figure 6i. Seasonal abundance of Croakers landed in motorised gill netters off Orissa during 2005 & 2006.

Catfishes: Catfish landings in motorized gill netters indicated a peak during January (7.8 Kg/hr), March (5.51 Kg/hr), June (6.1 Kg/hr), August (6.01 Kg/hr) and October (5.5 Kg/hr) (Fig. 6j).

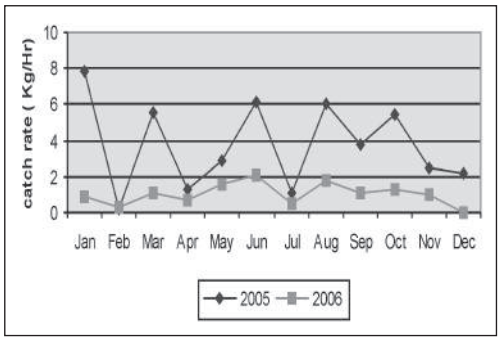

Figure 6j. Seasonal abundance of Catfishes (C/Hr) off orissa in motorised gill netters during 2005 & 2006

Pomfrets: Pomfrets brought good landings only during March (24.83 Kg/hr), May (9.8 Kg/hr) August (8.09 Kg/hr), September (9.53 Kg/hr) and October 2005 (25.6 Kg/hr) (Fig. 6k).

***Crustaceans*:**
***Mechanized trawlers*:**

Penaeid prawns*:* Penaeid prawns in mechanized trawlers was generally good with peak during January (9.21 Kg/hr), July (16.93 Kg/hr), October (11.31 kg/hr) November (9.81 Kg/hr) and December (8.04 Kg/hr) during 2005. During 2006, peak landings were obtained during January (7.74 Kg/hr), April (6.58 Kg/hr), June (10.48 Kg/hr), November (11.42 Kg/hr) and December (14.05 Kg/hr) (Fig. 6l). In motorized gears the catch of penaeid prawns was negligible.

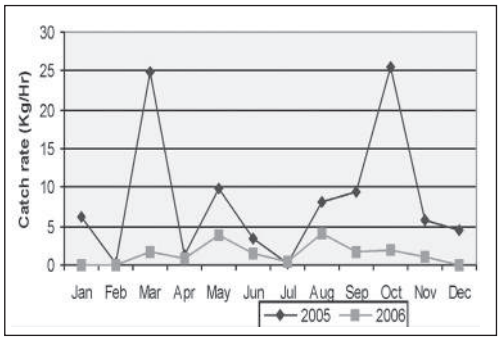

Figure 6k. Seasonal abundance of Pomfrets landed off Orissa in motorised gill netters during 2005 & 2006

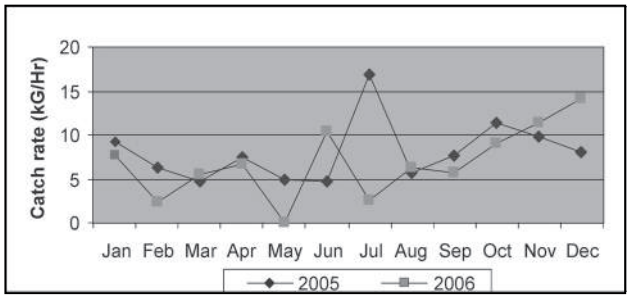

Figure 6l. Seasonal abundance (C/Hr) of penaeid prawns landed in mechanised trawlers off Orissa during 2005 & 2006

Seasonally, major resources such as ribbon fish, mackerel, croakers & catfish had brought higher catch rates during October to December & January to March period, while other resources such as carangids were landed more during February, April, June, and August. Penaeid prawns also brought good landings during January, July & October to December. Sekharan et al (1973) have found that catfishes are dominant in > 30 m depth during March-June. Scariah et al (1987) have observed that during 1875 to 84, off Orissa coast the highest landings was during fourth quarter (October to December) followed by first quarter (January to March), when planktivorus fishes such as sardines, pomfrets, etc contributed to more than 33%. According to Sankaranarayanan & Reddy (1968), there is evidence of upwelling in the North Western Bay of Bengal in January. Mathew et al (1996) have observed higher concentration of zooplankton in North West Bay of Bengal off Chilka Lake & Paradeep area during January and that the most productive period was during the North East monsoon (October to January) followed by premonsoon (February to May). The above authors have concluded that the fresh water influx and the high nutrient load towards the head of the Bay (Qasim 1977) coupled with the south westerly current and the prevailing wind pattern along with the effect of upwelling would have caused a piling up of standing crop in the coastal waters between 18º N & 20º N. It is therefore logical to believe that the peak landings of planktivorous fishes off Orissa during the north east monsoon season during October to December have a bearing on the proliferation of zooplankton population because plankton production is funneled either by pelagic or demersal groups into fish production (Sheldon et al. 1975).

Acknowledgements:

The authors wish to express their gratitude to Prof. (Dr). Mohan Joseph Modayil, Director, CMFRI, Kochi for suggesting this topic and necessary improvements made and to Dr.E.Vivekanandan, Head, Demersal Fisheries Division, CMFRI, Kochi, for the encouragements. Thanks are also due to Dr.M.Srinath, Head, FRA Division for providing the catch and effort data. The help rendered by Smt.P. K. Seetha, Senior Technical Assistant in preparing the manuscript is duly acknowledged.

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Received: 23 November 2007; Accepted: 10 December 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

PCR Cloning and Partial Sequencing of *rtx*A Gene of non-O1 / non-O139 *Vibrio cholerae* Isolated from Gold Fish *Carassius auratus* in India

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Abstract

A total of 16 non-O1/ non-O139 *Vibrio cholerae* isolates were obtained from ornamental fish collected from aquarium traders located in four metro cities of India. All the isolates were confirmed by amplifying the 300 bp fragment in the 16S-23S rRNA intergenic spacer regions. Amplification of 431 bp fragment of gene, which resulted in detectable levels of PCR product, was achieved with a minimum of 8 CFU/mL of *V. cholerae*. The detection limit for *rtxA* gene by PCR amplification of genomic DNA was 20 picogram. A 431 bp fragment of the *rtxA* gene of *V. cholerae* was cloned and sequenced (NCBI accession number EU714289). Nucleotide sequence analysis data of the *rtxA* gene showed that about 96% identical to those of *V. cholerae* RTX toxin gene cluster.

Introduction

Vibrio cholerae is an important cause of diarrhea in many parts of Asia and Africa. *V. cholerae* is an autochthonous inhabitant of brackishwater and estuarine systems (Colwell et al. 1977). Toxigenic *V. cholerae* O1 and *V. cholerae* O139 are etiological agents of epidemic cholera. However, both *V. cholerae* O1 strains that do not produce cholera toxin, i.e., that are nontoxigenic, and non-O1/non-O139 strains have also been associated with cholera, gastroenteritis, septicemia, and/or extraintestinal infections (Mukhopadhyay et al. 1995). Conventional methods used to detect and classify cholera-causing vibrios isolated from clinical and environmental samples require several days to complete and involve culture in alkaline peptone water, thiosulfate citrate bile sucrose agar, slide agglutination with specific antisera, and assay for production of cholera toxin (Sakazaki 1992). Molecular methods, including PCR and DNA-DNA hybridization performed with probes specific for *V. cholerae*, provide more reliable identification (Chun et al. 1999) but have limitations because of cost and the facilities required for analysis; these limitations are particularly significant for field studies involving large numbers of samples.

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Although cholera toxin (CT) is clearly the most important causative factor for cholera, CT deficient isolates of *V. cholerae* also elicit mild to severe diarrhea and other reactogenic symptoms in human, indicating that other toxins are likely to contribute to the pathogenesis of the disease (Coster et al. 1995). A novel toxin in *V. cholerae* that belongs to the RTX (repeat in toxin) family of toxins, which are generally produced by several pathogenic gram-negative bacteria, was recently discovered (Chow et al. 2001). Lin et al. (1999) proposed that the *V. cholerae RTXA* (VcRTX-A) toxin, might play a role in the gastrointestinal virulence property of *V. cholerae*. A total of 16 non-O1 and non-O139 *V. cholerae* were isolated from aquarium fish, koi carp and gold fish in India during the previous study (Swaminathan et al. 2007).

In this study, rapid detection and confirmation of *V. cholerae* non-O1 and non-O139 isolated from koi carp and goldfish by amplification and sequencing of a 431 bp fragment of the *rtxA* gene of *Vibrio cholerae* was carried out.

Materials and methods

Source of the isolates

A total of 16 *V. cholerae* non-O1 and non-O139 serogroups isolated from different regions of India were included in our study. All the isolates were obtained from koi *Cyprinus carpio* koi, goldfish *Carassius auratus* (L.) during 2004-05. All the isolates were subsequently examined and characterized. The identity of the isolates was confirmed by serotyping at the National Institute for Cholera and Enteric Diseases , Kolkata, India.

Identification of V. cholerae by PCR

Identification and confirmation of isolated *V. cholerae* was done by amplification of a fragment of 16S-23S rRNA intergenic spacer region (ISR) of *V. cholerae* as described by Chun et al. (1999).

Construction of specific PCR primers

The *rtxA* sequences of these *V. cholerae* was obtained by accessing the nucleotide sequence database (GenBank accession # AF119150). The data were examined with multiple alignment analysis (CLUSTAL W), the specific region for bacteria was identified, and the specificity was confirmed by FASTA. Specific PCR primers for identification of *V. cholerae* were designed from the sequence of *rtxA* using Laser gene 6 software. Primers were tested for specificity among different bacterial isolates. The cross-reactivity of *V. cholerae rtxA* gene primers were checked by NCBI–BLAST and by genomic DNA amplification of other bacteria, i.e. *Escherichia coli, Salmonella arizonae, Pseudomonas alcaligenes, Aeromomas hydrophila, Edwardsiella tarda, Staphylococcus aureus* and *Flavobacterium* sp.

Preparation of PCR samples

Bacterial pellets were diluted in sterile saline prior to lysis by 10 min. of boiling in a water bath. Bacterial genomic DNA isolation of all *V. cholerae* was according to Hiney et al. (1992). The nucleic acid preparation was finally suspended in 50 µl of TE buffer. The isolated nucleic acid was qualified and quantified at 260 nm and 260/280 ratios, respectively.

PCR amplification

The PCR amplification was performed using the primers designed in this study. We used 10 ng of genomic DNA, 50 pmoles of primers, 100 µ moles of each dNTP's and 2 mM of $MgCl_2$. Samples were subjected to 35 cycles of amplification (94^0C for 2 min., 64^0C for 1 min. and 72^0C for 3 min. on a Master cycler (Eppendorf). 10 µl of the reaction mixture was then analyzed by submarine gel electrophoresis in 1.2% agarose.

Cloning and Sequencing of 431 bp fragment of V. cholerae rtx A gene

A fragment of 431 bp of *V. cholerae rtxA* gene was amplified using designed primer. The DNA band of interest was excised and purified from the gel at position 431 bp and was ligated to pCR 2.1 TOPO cloning vector and transformed into DH5 α *E. coli* strain (Invitrogen). The recombinant clones were confirmed by PCR using designed primer. The two terminal sequences of the cloned genes fragment was determined with the ABI PRISM Dye primer cycle sequencing ready reaction kit and ABI 377 DNA auto sequencing machine by using M13sequence primers.

Sensitivity of the PCR

For determining the sensitivity of the PCR 10-fold dilutions (8 X 10^{-5} to 8 cells) were tested. When nucleic acids were used, the sensitivity of the PCR was determined by amplifying 5µl of 10-fold serial dilutions (20ng to 2pg). PCR amplification was performed with a DNA thermal cycler as described before.

Results

Primer pairs was designed on the basis of the nucleotide sequence of the *rtxA* downloaded from NCBI and used to amplify target sequences in genomic DNA from 16 isolates of *V. cholerae* and yielded a product of the expected 431 bp size for *V. cholerae* (Fig. 1). Similar amplification of the expected product size was not observed for the other bacteria *viz., Escherichia coli, Salmonella arizonae, Pseudomonas alcaligenes, Aeromomas hydrophila, Edwardsiella tarda, Staphylococcus aureus* and *Flavobacterium* sp when the primer pairs were used. All strains of *V. cholerae* isolated in our laboratory were confirmed and a PCR amplification product of the expected length (431 bp) was obtained (Fig.1).

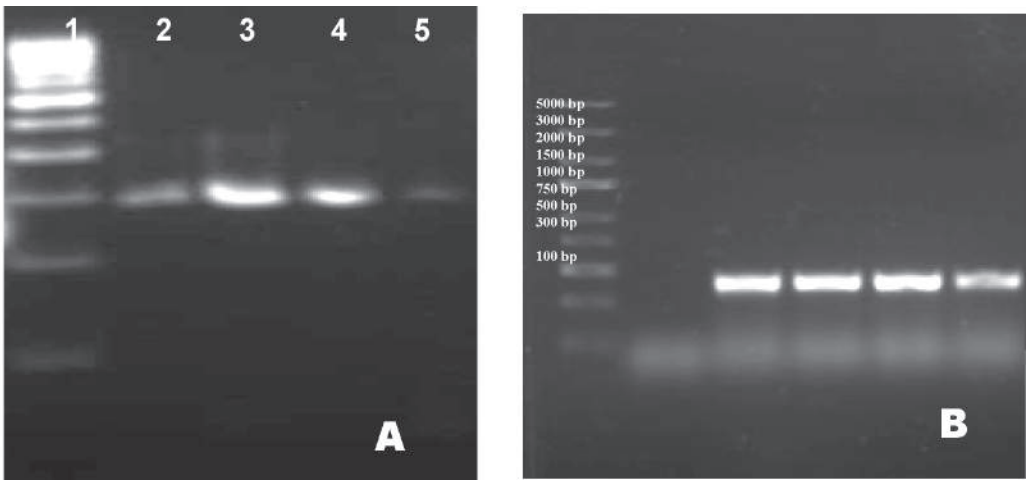

Figure 1. Detection of non - O1 and non - O139 *Vibrio cholerae* by PCR

A- Confirmation of *V. cholerae* by amplification of the fragment of 16S-23S ISR by PCR.
Lane 1 - 100bp ladder, lane 2-5-16S-23S ISR (300bp).
B-Detection of *V. cholerae* by amplification of the fragment (417bp). of *rtx*A gene by PCR.
Lane 1 – Generuler DNA Ladder (Fermentas), Lane 2 -- Negative control Lane 3 – Positive control
lane 4 - 6 – Positive test samples

The 431 bp fragment of *rtxA* of *V. cholerae* was cloned in PCR 2.1 TOPO cloning vector for sequencing. The cloned 431 bp fragment of the *rtxA* was sequenced and comparison of this sequence with other sequences from DDBJ/EMBL/Genbank was made. Comparing the 431 bp fragment of the *V. cholerae* isolates 96% homology with *rtxA* sequences of *V. cholerae* strains *viz.*, AE003852.1, AF119150.1, was found. The sequence of the 431 bp fragment of *rtxA* of *V. cholerae* was deposited in the NCBI GenBank (EU714289).

Amplification which resulted in detectable levels of PCR product was achieved when a minimum of 8 CFU/mL of *V. cholerae* were lysed, on the basis of an average of five repeated testing of viable cells and PCR assays. The minimum amount of purified DNA in the reaction mixture needed to obtain a detectable PCR product was 20 pg. The lower limit of detection of *V. cholerae* bacterial cells or isolated DNA by PCR was examined for all the strains of *V. cholerae*. A suspension of cells of the isolates was diluted and processed. A PCR amplification product could not be obtained when a sample with more than 10^6 CFU was used in the assay, probably because of accumulation of soluble cell products inhibitory to PCR. Isolated DNA from *V. cholerae* was serially diluted in saline and used as a template. The minimum amount of purified DNA in the reaction mixture needed to obtain a detectable PCR product was 20 pg.

In the study, we used the PCR technique for the identification of the *rtxA* genes of *V. cholerae* and the specificity of the primers used here is noteworthy, and can reach high sensitivity. The detection limit for *rtxA* gene from *V. cholerae* was 20 pg.

Discussion

This study was undertaken to explore the possibility of contamination of *V. cholerae* serogroups O1 and O139, the most important causative organisms for cholera and also potential public health importance, by isolating these organisms from body surface, gill and intestine of common ornamental fishes. *V. cholerae* is often transmitted by water but fish or fish products that have been in contact with contaminated water or faeces from infected persons also frequently serve as a source of infection (Colwell et al. 1977). *V. cholerae* O1 or O139 were not isolated from body surface swabs, gills and intestine of these common table fishes. Dalsgaard et al. (1995) isolated 143 *V. cholerae* non-O1 strains from shrimp farms in Thailand, and characterized and grouped by ribotyping. All the 16 isolates of *V. cholerae* isolated in our laboratory were confirmed and a PCR amplification product of the expected length (431 bp) of *rtxA* gene was obtained. Nonepidemic *V. cholerae* non-O1 serogroup strains, which cause only sporadic, milder cases of diarrhea, do secrete the RTX cytotoxins but do not secrete CT (Chow et al. 2001). The sequence of the 431bp fragment of the *rtxA* gene had showed 96% homology with the other *rtxA* gene sequences of the *V. cholerae*, *viz.*, AE003852, AF119150.

Non-O1/ non-O139 *V. cholerae* strains can no longer be ignored. The rationale for continuous monitoring is based on the emergence of serogroup O139 (Bengal) in Bangladesh (CT positive) and Argentina (CT negative), each of which clearly evolved independently. The sixth pandemic, the seventh pandemic, and U.S. Gulf Coast isolates represent three different clones, each independently evolved from environmental non-O1 *V. cholerae* isolates (Karaolis et al. 1995). Finally, the emergence of a new clone of the *V. cholerae* O1 El Tor in Calcutta, India (Sharma et al. 1997) has been reported. The possibility exists that those additional new strains of toxigenic *V. cholerae* with epidemic potential may emerge in the future. While CT is a principal virulence factor for *V. cholerae*, the contribution of the RTX toxins to its pathogenesis requires further investigation.

In previous study it was described that the rtx gene was absent only from the *V. cholerae* classical O1 serogroup strain, which has greater epidemic potential than strains of the other serogroups, despite its displacement by the El Tor biotype since the seventh pandemic (Chow et al. 2001). Therefore, nonepidemic *V. cholerae* non-O1 serogroup strains, which cause only sporadic, milder cases of diarrhea, does secrete the RTX cytotoxins but do not secrete CT. Further investigation is required to determine the role of RTX in the pathogenicity of non-O1 /non-O139 *V. cholerae.*

Conclusion

The 431 bp fragment of *rtxA* of *V. cholerae* was amplified by the primer designed and the PCR product was cloned in pCR 2.1 TOPO cloning vector for sequencing. The cloned 431 bp fragment of the *rtxA* was sequenced and comparison of this sequence with other sequences from DDBJ/EMBL/Genbank was made. The sequence of the

431 bp fragment of *rtxA* of *V. cholerae* was deposited in the NCBI GenBank (EU714289). The sensitivity and specificity of the primer designed to detect the rtxA gene fragment of *V. cholerae* were checked. The primer could identify 8 CFU/mL *V. cholerae* and 20 pg of purified DNA of *V. cholerae*.

Acknowledgement

The authors are grateful to Dr S. Ayyapan,, DDG (Fy), ICAR, New Delhi for the encouragement.

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Received: 31 December 2007; Accepted: 06 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Employment Scenario and Labour Migration in Marine Fisheries

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Abstract

Employment status and opportunities in marine fisheries sector increased over the years inspite of growing mechanization and incessant replacement of labour intensive fishing technologies. Fish, being a highly perishable product, needs the services of several people for its fast movement from catching point to consuming point without deteriorating its quality. It provides employment not only to fisherfolk in fishing villages, but also to those hailing from adjoining as well as interior regions. The present study attempts to assess the manpower employed in active fishing as well as in secondary and tertiary sectors both from coastal villages and other regions. Macro level employment status has been worked out based on the well established assumption that every 5 kg of marine fish produced provides employment to one person in the harvesting and another 1.2 persons in the post harvest sector (Sathiadhas et al. 1997). The study indicates that about 12.5 lakh people are involved in active fishing in India while the postharvest sector including export and domestic marketing employs about 15 lakh and in tertiary sector there are around 2 lakh people. Among these, 71 percent of active fishers, 50 percent of secondary sector workers and 42 percent in the tertiary sector are inhabitants of coastal fishing villages. In secondary sector, around 30 percent are women workers of which 81 percent are residents of fishing villages in the coastal belt. There is ample scope of development of employment potential of secondary and tertiary sectors in view of globalization of economy. An additional export of almost 1 lakh tonnes of value added products in our marine exports could easily corner about Rs. 1500 crores of forex earnings and generate regular employment opportunity for about 35,000 fisherfolk.

Technological changes in fishing coupled with the widespread use of electronic gadgets like mobile phones and GPS have promoted migration of fisherfolk in search of better catch and earnings. A case study of socio economic dimensions of migrant fisher folk who are natives of Colachel, Thoothoor, and Vallavilai regions of Kanyakumari District of Tamil Nadu was carried out for which primary data were collected by the help of pre-structured schedules. Migratory fishing is having definite implications upon the social and economic milieu of migrants as well as on the migrants' families who are left back at their native place. Factors inducing migration among these fisherfolk include high demand for shark in the international market coupled with its earning potential, accessibility to landing points, and berthing facilities and better price

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realization. Constraints faced by in-country migrants include fluctuating returns resulting in insufficient income and indebtedness, frequent clashes with locals of landing center in other states, exploitation of migrant fishing units in other states, forced sales, delay in payment of sale proceeds, missing of fishing boats/fishermen and ergonomic problems due to long fishing trips without adequate facilities. Foreign migrants face problems like detention due to crossing maritime borders, withholding of passports and other documents, ill treatment from the sponsors and exploitation due to ignorance of fishermen.

Introduction

Fisheries sector has been faring high in terms of its forex earning potential and employability of vast majority of coastal community in the primary, secondary, and tertiary sectors associated with fishing. However, the plight of marine fisherfolk amplified by inequitable distribution of earnings leading to indebtedness and marginalization is a much debated issue for the planners and policy makers. Improvements in technology has led to unbridled capital investment in this sector and has attracted more and more people from the adjacent coastal transects who necessarily do not belong to the fishing community. Seafood exports from India is exploring new heights with increasing opportunities for value addition and branding of products. This has led to mushrooming of export units employing large number of skilled and unskilled workers. Further over the years, there is increase in the coastal fisher population inducing more and more people in fishing and allied activities. Disguised unemployment is rampant in all sectors since earnings from marine fisheries are not proportionate to the increase in stakeholders. This has instigated labour migration induced by the earning potential in the distant waters coupled with limited resources in their vicinity. The study attempts to estimate the trend in labour force employed in active fishing in marine fisheries over the years, the sectoral distribution of labour force, and extent of migration. This paper also analyses the case of Tamil fishermen of Kanyakumari coast who relocate to different places for fishing, the factors that induce migration and problems associated with it.

Materials and Methods

The study has been done widely depending on primary and secondary sources of information. The data about labour requirement for each type of craft–gear combinations in the primary sector and the number of persons employed in secondary sector activities were collected from the 20 sample units at selected centres. Detailed household survey to assess the socio economic implications of migration were conducted during 2006 in villages of Thoothoor, Colachel, and Vallavilai along Kanyakumari coast. Secondary sources of information include catch figures and marine fisheries census reports of CMFRI for various years.

Results and Discussion

Coastal population dynamics and employment in active fishing

The marine fisheries census of CMFRI has estimated coastal population and manpower employed in active fishing and related sectors from time to time. The active fishers in coastal villages in marine fisheries have been increasing over the years at a compound growth rate of 3.13 percent (Table 1) almost in consonance with population growth of 3 percent. Marine fisheries is recognized as a sunrise sector and the prospects

Table 1. Growth of active fishermen in coastal fishing villages over years (1961-62 to 2005)

State	1961-62	1973-77	1980	2005	Compound annual growth rate of active fishers (%)	Compound annual population growth rate (%)
West Bengal	9434 (26)	15076 (25)	19756 (24)	70750 (26)	8.88**	4.8 **
Orissa			30724 (26)	121282 (27)	9.59	5.53
Andhra Pradesh	47700 (35)	64592 (27)	83903 (26)	138614 (27)	2.45	3.03
Tamil Nadu	56586 (26)	68317 (24)	96500 (24)	206908 (26)	3.76*	3.42 *
Pondichery		3785 (23)	5512 (22)	10341 (24)	3.41*	3.26 *
Kerala	74241 (22)	80898 (21)	131101 (20)	140222 (23)	1.46	1.35
Karnataka	8963 (17)	21740 (22)	25005 (22)	37632 (22)	3.31	2.76
Maharashtra	20698 (20)	41539 (21)	72074 (23)		2.88	2.59
Gujarat	11732 (14)	22518 (18)	83322 (26)	36527 (24)	4.56	3.16
Goa	NA	4067 (27)	8871 (22)	2515 (24)	2.44 #	3.35 #
Daman & Diu	NA			5868 (20)		
Total	229354 (24)	322532 (22)	437899 (23)	889528 (25)	3.13	3

** Growth rate of last 15 years
* Growth rate of last 30 years
\# Combined growth rate of Goa, Daman and Diu

of foreign exchange earnings and employability is attracting more and more people into active fishing and allied sectors. Although there was a general increase in active fishers in all the maritime states, high variation was observed in relation to increase in total population. Active fishers increased more than proportionate to the growth in population in the states of West Bengal, Orissa, Karnataka, and Gujarat. Conversely, there was lesser growth rate of active fishers compared to population growth in Andhra Pradesh and UT of Daman and Diu. However, there was not much change in the proportion of active fishers in the total population as the overall share of active fishers increased from 24 percent in 1961-62 to 25 percent in 2005).

Sectoral distribution of active fishers

Human resource utilization in marine fisheries covers not only the coastal fisherfolk but also the adjacent and some times distant residents also. It was estimated that roughly 12.5 lakh people are involved in active fishing while 15 lakh involve in secondary and about 2 lakh in tertiary sectors. On an average, 5 kg of marine fish produced gives employment to one in harvesting and 1.2 persons in post harvest sector. The sectoral distribution of employment pattern of active fishers over the years is presented in Table 2.

There is a clear shift of employment pattern towards mechanised and motorized sectors. The active fishers in the mechanised segment increased from 24 percent in 1980-81 to 35 percent in 2004-05 and motorized segment from 17 percent in 1997-98 to 32 percent in 2004-05. The share of active fishers in the non-mechanised segment decreased from 75 percent in 1980-81 to 34 percent in 2004-05.

Table 2. Sectoral distribution and growth of active fishermen in India

Active fishermen in	1980-81	1997-98	2003-04	2004-05
Mechanised sector	114000(24)	200000(20)	412596(34)	430931(35)
Motorised sector	—	170000(17)	442581(36)	401577(32)
Non Mechanised sector	348000(75)	650000(64)	365360(30)	415312(34)
Total	462000	1020000	1220577	1247820

Source: Various marine fisheries census of CMFRI

While there is considerable sectoral shift by active fishers in marine fisheries, the extent of their dependence on fishing activities remain almost stable with increase in population (Table 3). The total active fishers in marine fisheries and full time fishers doubled during the past 15 years. The increase in part time fishers were more than other categories (137 percent) with 13 percent of the total active fishers engaged in part time fishing compared to the earlier 11 percent.

Table 3. Change in involvement in active fishing over the years

Year	Full time	Part time	Occasional	Total
1980	357703(82)	49654(11)	30542(7)	437899(100)
2005	717999(81)	117628(13)	53901(6)	889528(100)
Growth (percent)	101	137	76	103

Current employment scenario of marine fisheries in India

In India, marine fisheries sector employs around two million people of which 12.47 lakh people are in active fishing, 14.97 lakh in secondary sector avocations and two lakhs in tertiary sector. Out of the total employed, 59 percent of them hail from the coastal fishing villages alone. It is observed that most of the sea faring fishers also live in the nearby coastal villages. Seventy one percent of those employed in primary sector reside in coastal fishing villages (Table 4). Similarly, 51 percent of secondary sector workers and 42 percent of tertiary sector workers are from the fishing villages. The export orientation of marine fisheries sector has led to mushrooming of seafood export units doing varied activities like peeling, curing, preprocessing, processing and packing. These units have high employment potential and employ women in large numbers. Women in nonfishing areas also are attracted to such jobs that have resulted in overcrowding effect leading to low wage rate. In secondary sector, around 30 percent are women workers of which 81 percent are residents of fishing villages in the coastal belt. The tertiary sector undertakes fishery allied activities in which nonfishermen dominate.

Table 4. Employment pattern in marine fisheries and coastal fishing villages (2005)

	Marine fisheries sector	Coastal fishing villages	
	Total number of people employed(lakh)	Number of people employed (lakh)	percentage of total employment
Primary	12.47	8.89	71
Secondary	14.97	7.56	51
Women in secondary sector	4.49	3.65	81
Tertiary	2.00	0.83	42
Total	29.44	17.28	59

The state wise break up of the total people employed in primary and secondary sector in marine fisheries is given in Table 5. Fisherfolk from the coastal fishing villages form a part of the total labour employed in marine fisheries. The primary sector workforce in marine fisheries was estimated on the basis of average employment pattern in the fishing crafts in the respective states. More than 90 percent of people from coastal villages are involved in active fishing in the states of Orissa, Andhra Pradesh, and Tamil Nadu and rest comes from adjacent villages and even from other states. In states like Karnataka, Goa, Maharashtra, and Gujarat less than half of the active fishermen are from fishing villages.

Table 5. State wise employment pattern in marine fisheries and coastal fishing villages (2005)

	(A) Total employed in marine fisheries		**(B)** Inhabitants of coastal fishing villages		percent of (B) to (A)	
State	Primary	Secondary	Primary	Secondary	Primary	Secondary
West Bengal	1,12,144	1,34,573	70,750	57,741	63	43
Orissa	1,34,669	1,61,603	1,21,282	1,52,534	90	94
Andhra Pradesh	1,47,289	176,747	1,38,614	1,52,892	94	87
Tamil Nadu	2,25,102	2,70,122	2,06,908	1,04,509	92	39
Pondichery	18,461	22,153	10,341	10,095	56	46
Kerala	1,94,816	2,33,779	1,40,222	71,074	72	30
Karnataka	1,05,721	1,26,865	37,632	45,699	36	36
Goa	16,237	19,484	2,515	3,382	15	17
Maharashtra	1,36,628	1,63,954	72,074	81,780	53	50
Gujarat	1,56,753	1,88,104	83,322	75,082	53	40
Total	12,47,820	14,97,384	8,89,528*	7,56,391*	71	51

(* Total includes figures for Daman and Diu)

There are lots of people in the adjacent coastal transects and interior regions who find employment in fishing related fields, as the share of inhabitants of fishing villages to total secondary employment in marine fisheries ranges from 17 to 94 percent. It was found that Tamilnadu employs maximum people in the primary and secondary sector in marine fisheries. The estimated primary and secondary employment in marine fisheries does not incorporate the employment in Andaman & Nicobar Islands, Lakshadweep, and Daman and Diu. Hence, the actual employment in marine fisheries is likely to be more than the current estimate.

Occupational profile of coastal fisherfolk in maritime states in India

Workers Population Ratio in coastal fishing villages

The Workers Population Ratio (WPR) connotes employability of workers as ratio to total population (Table 6). The WPR of adult population in marine fisheries sector shows that 75.39 percent of the adult population is employed. In Orissa, workers exceed the adult population and WPR of total population was 64.21. The lowest WPR to adult population was seen in Daman and Diu (UT) and Kerala. In Kerala, the low WPR may be due to lower participation of women in fisheries sector. It is found that most of the women in fisheries sector in Kerala are confined to secondary sector on peeling and fish vending activities. Highest labour participation was seen in Orissa followed by Andhra Pradesh.

Table 6. Labour participation of coastal fishing population in marine fisheries (2005)

State	Total employed	WPR (adult population)	WPR (total population)
West Bengal	130,459	79.80	48.40
Orissa	289,175	106.73	64.21
Andhra Pradesh	300,233	99.00	58.87
Tamil Nadu	324,234	60.22	41.02
Pondichery	22,133	75.19	51.44
Kerala	224,606	52.59	37.30
Karnataka	90,831	73.67	53.14
Goa	6,399	81.89	59.98
Maharashtra	164,579	74.47	51.53
Gujarat	168,794	88.44	52.22
Daman and Diu	7,549	42.38	25.76
Total	1,728,992	75.39	49.13

The overall dependency ratio of marine fisherfolk in India is estimated to be 2.04 denoting that every person working in marine fisheries sector supports two persons. It varies across the states from 1.56 (Orissa) to 3.88 (Daman and Diu). Among those employed in marine fisheries, most of them are active fishermen while 43.75 percent are in secondary sector occupations and 4.80 percent are involved in other activities including tertiary sector (Table 7). However, majority of those employed in marine fisheries are in secondary sector in the states of Orissa, Andhra Pradesh on the East Coast and Karnataka, Goa, Maharashtra, and Gujarat on the West coast. Coupled with the intensity of marine fishing more people are involved in active fishing in

Tamil Nadu, Kerala, Andhra Pradesh, and Orissa. The quality concerns after the WTO, wide spread consumer preference and increased price for value added products in the international markets have increased the scope of secondary sector in fisheries. These developments have led to improvement in handling and processing facilities adjacent to export units adding to the employability. The fisherfolk employment in other sectors ranged from 1.03 (Daman and Diu) to 8.26 percent (Karnataka) in different states.

Table 7. State wise occupational pattern of coastal fisherfolk in India (2005)

State	Number of fisherfolk engaged in			
	Primary Sector	Secondary sectors	Other sector	Total
West Bengal	70,750 (54.23)	57741 (44.26)	1,968 (1.51)	130,459 (100)
Orissa	121,282 (41.94)	152,534 (52.75)	15,359 (5.31)	289,175 (100)
Andhra Pradesh	138,614 (46.17)	152,892 (50.92)	8,727 (2.91)	300,233 (100)
Tamil Nadu	206,908 (63.81)	104,509 (32.23)	12,817 (3.95)	324,234 (100)
Pondichery	10,341 (46.72)	10,095 (45.61)	1697 (7.67)	22,133 (100)
Kerala	140,222 (62.43)	71,074 (31.64)	13,310 (5.93)	224,606 (100)
Karnataka	37,632 (41.43)	45,699 (50.31)	7,500 (8.26)	90,831 (100)
Goa	2,515 (39.30)	3,382 (52.85)	502 (7.84)	6,399 (100)
Maharashtra	72,074 (43.79)	81,780 (49.69)	10725 (6.52)	164,579 (100)
Gujarat	83,322 (49.36)	75,082 (44.48)	10,390 (6.16)	168,794 (100)
Daman and Diu	5,868 (77.73)	1,603 (21.23)	78 (1.03)	7,549 (100)
Total	889,528 (51.45)	756,391 (43.75)	83,073 (4.80)	1,728,992 (100)

* *Figures in parenthesis denote percentage to total*

Labour involvement in secondary sector

The state wise break up of the secondary sector activities in marine fisheries is given in Table 8. In West Bengal, Andhra Pradesh, and Gujarat majority employed in secondary sector are engaged as contract labourers at landing centres to retail points. The major occupation of fisherfolk engaged in secondary sector is marketing of fish in Maharashtra (53.59 percent), Goa (49.91 percent), Tamil Nadu (34.57 percent) and UT's Pondichery (63.33 percent) and Daman and Diu (54.90 percent). Both marketing of fish and contract labourers are predominant in Orissa (20.78 percent, 24.77 percent), Kerala (25.29 percent, 24.26 percent) and Karnataka (31.35 percent, 30.73 percent). In Orissa, Andhra Pradesh, and Maharashtra, curing/processing was taken up by significant portion of workforce within the secondary sector. In Kerala peeling work was predominantly undertaken in the secondary sector, mostly by women, due to the existence of more number of export units.

Table 8. State wise employment pattern in secondary sector in coastal villages (2005)

State	Marketing of fish	Making/repairing net	Curing/ Processing	Peeling	Labourers	Others	Total
West Bengal	5237 (9.07)	15326 (26.54)	4705 (8.15)	478 (0.83)	26151 (45.29)	5844 (10.12)	57741 (100)
Orissa	31691 (20.78)	40252 (26.39)	27849 (18.26)	3167 (2.08)	37781 (24.77)	11794 (7.73)	152534 (100)
Andhra Pradesh	34337 (22.46)	23926 (15.65)	28319 (18.52)	2996 (1.96)	55372 (36.22)	7942 (5.19)	152892 (100)
Tamil Nadu	36126 (34.57)	19051 (18.23)	6250 (5.98)	2107 (2.02)	25657 (24.55)	15318 (14.66)	104509 (100)
Pondichery	6393 (63.33)	630 (6.24)	364 (3.61)	5 (0.05)	714 (7.07)	1989 (19.70)	10095 (100)
Kerala	17976 (25.29)	9560 (13.45)	3881 (5.46)	8057 (11.34)	17242 (24.26)	14358 (20.20)	71074 (100)
Karnataka	14327 (31.35)	7876 (17.23)	3342 (7.31)	581 (1.27)	14043 (30.73)	5530 (12.10)	45699 (100)
Goa	1688 (49.91)	479 (14.16)	0	0	515 (15.23)	700 (20.70)	3382 (100)
Maharashtra	43822 (53.59)	9086 (11.11)	9209 (11.26)	1439 (1.76)	11565 (14.14)	6659 (8.14)	81780 (100)
Gujarat	14885 (19.82)	13452 (17.92)	3212 (4.28)	4310 (5.74)	31366 (41.78)	7857 (10.46)	75082 (100)
Daman and Diu	880 (54.90)	80 (4.99)	11 (0.69)	3 (0.19)	256 (15.97)	373 (23.27)	1603 (100)
Total	207362 (27.41)	139718 (18.47)	87142 (11.52)	23143 (3.06)	220662 (29.17)	78364 (10.36)	756391 (100)

** Figures in parenthesis denotes percentage to total*

Women in marine fisheries: A cross section of secondary sector employment

The activity wise occupational structure of fisherfolk engaged in secondary sector is given in Table 9. Fish marketing and labourers for various activities from the landing centre to retail points provide employment to more than 56 percent of the fisherfolk engaged in secondary sector. While marketing is dominated by females (MF ratio (Male Female ratio) of 2.8), labour in the secondary sector is done by men (MF ratio of 0.4). Curing /processing and peeling are undertaken by women (MF ratio of 3.1 and 3.4, respectively). Male female participation in secondary sector is almost equal denoted by the ratio of 0.9. Of the fisher population engaged in secondary sector, women accounted for 48 percent of the work force in marketing, curing/processing and peeling sectors.

Table 9. Gender wise occupational structure in secondary sector (2005)

Sl. No	Activity in secondary sector	Number of fisherfolk involved			Male female participation Ratio
		Male	Female	Total	
1	Marketing	54670 (26.36)	152692 (73.64)	207362 (100)	2.8
2	Making/repairing of net	111661 (79.92)	28057 (20.08)	13971 8(100)	0.3
3	Curing/processing	21211 (24.34)	65931 (75.66)	87142 (100)	3.1
4	Peeling	5251 (22.69)	78921 (77.31)	23143 (100)	3.4
5	Labourer	153431 (69.53)	67231 (30.47)	220662 (100)	0.4
6	Others	44704 (57.05)	33660 (42.95)	78364 (100)	0.8
	Total	390928 (51.68)	365463 (48.32)	756391 (100)	0.9

** Figures in parenthesis denote percentage to total*

Labour migration – Case of Tamil fisherfolk of Kanyakumari coast

The term migration is presented as a delimitated theme among spatial mobility, which is defined in congruence with the changes in the geographical location occupations of the respondent (Richard, 1969). The tendency of moving from place to place or from one occupation to another may be due to various reasons *viz.*, geographic, demographic, and ethnographic; differences in skills, knowledge, and abilities; demand and supply conditions of resources; socio-economic characteristics; political and religious forces;

employment potential and market orientation. These tendencies, unevenly distributed within and among places, create inequalities with the community and results in a series of interlinked economic and social consequences.

The growth pattern in favour of mechanised and motorised sectors along with increased capital investments in these sectors have absorbed a major chunk of active fishermen, thus resulting in large-scale disguised unemployment within the sectors. Ultimately, this has resulted in the emergence of spatially mobile fishermen groups, which could be termed as migrant fisher folk. They may be either temporary or permanent settlers. One example of such migrant fisher folk who are fishing off the Kerala coast is fishermen of Tamil Nadu. Most of the migrants hail from the fishing villages of Thoothoor, Neerodi, Marthandamthurai, Vallavilai, Eraviputhenthurai, Chinnathurai, Thoothoor, Colachel, Poothurai, and Eraiyumanthurai. These fishers go for multi-day fishing throughout the year and anchor at any of the landing centres or harbours off the Kerala coast. The migratory pattern of shark fishers in Thoothoor, Vallavilai, and Colachel regions in Tamil Nadu showed that 35 percent were non migrants (local fishing) and 65 percent were migrants (in-country and foreign). Among the in-country migrant shark fishers, 85 percent relocate to coasts of Kerala (Cochin, Vizhinjam, Kollam, Kozhikode, Kannur, and Kasargode), Karnataka (Mangalore, Malpe and Gangoli), Goa, Maharashtra, and Gujarat. Foreign migration constituted 15 percent of total migratory fishers and they go for fishing along the coasts of Qatar, Saudi Arabia, Dubai, and Doha. The non-migratory units had average investment in fishing units ranging from Rs. 4000 for non-mechanised to Rs. 13.5 lakh for mechanised units. Technological advancement has facilitated fish finding devices like ecosounder and GPS and communication technologies to facilitate market intelligence. The capital investment in migratory units has increased considerably due to increase in size of vessel with storage capacity and use of electronic gadgets and telecommunication devises. For the migratory units (Multi-day mechanised fishing units), average investment varies from Rs. 15 lakh to Rs. 30 lakh.

There are several problems faced by these migrant fisherfolk that need to be addressed. The study focuses on the constraints of different stakeholders in the migratory pattern, including in-country migrants, foreign migrants, families of migrants and marine fisheries sector as a whole.

Demographic profile of "shark fishers"

Local fisherfolk encounter many problems that induce migration. Major lacunae are insufficient landing and berthing facilities, inability to realise competitive prices in the local market due to lack of common landing points, rivalry and frequent conflicts among villages in resource sharing, restrictions in fishing, vulnerability, and debt trap

of local money lenders and traders. High demand for shark in international market and its earning potential induces the fishermen to migrate according to availability of fish stocks. Accessibility to landing points and berthing facilities in other states/countries is another factor responsible for migration.

Among the respondents, 62 percent are literate with 95 percent of them having education till the primary level and 5 percent till secondary level. Various studies reveal that migration has helped the migrant households avoid hunger, starvation, and death and it (migration) became a vital livelihood strategy, though it failed to evaluate the economic status of all migrating households (Sundari 2005; Vijay 2002; Standing 1985). The housing pattern of the migrant fisherfolk is better than the nonmigrants. While most of the non-migratory fishers lived in *kutcha* houses of 125 to 300 sq.ft, in-country migrants lived in tiled/concrete houses of 500-700 sq.ft. The most affluent category is the foreign migrants who built 850-1200 sq.ft concrete houses.

Constraints of in-country migrants

The prospects of migration are attracting more and more people, but there are inherent problems associated with foreign and in-country migration. The common problems encountered by the shark fishermen of Thoothoor were identified in the study

- 86 percent of respondents opined that seasonal nature of fisheries does not ensure moderate/high returns. Though good returns are reaped in some of the seasons, there are lean seasons also in which catch is very low that they are forced to borrow. Informal borrowing results in debt trap for 79 percent of the respondents, channelising the revenue from bumper harvests to settlement of debtors. They are often forced to borrow for next journey repeating this cycle
- Frequent clashes with locals of the landing centers in other states was experienced by 44 percent of respondents
- Exploitation was fleet by 38 percent of migrant fishers due to ignorance of language and other factors
- 63 percent of them said that there was collection of high rent for lodging and price discrimination in purchasing fishing requisites and other essentials in the other states
- 43 percent of respondents admitted that traders advance money before voyage and they are bound to forced sales at lower prices
- Unnecessary delay in payment of sale proceeds by the merchants was experienced by 43 percent

- Reports of missing of fishing boats/fishermen occurred frequently in migratory fishing and 33 percent of respondents said that there is lack of Government initiatives in tracing them
- Ergonomic problems due to long fishing trips without adequate facilities was experienced by 72 percent of them
- 69 percent of them said that quality of fish is usually affected because of lengthy voyages and absence of proper storage facilities

Constraints of families of migrant fishers

Continuous absence of fishermen from their families increases the burden of housewives in looking after the families and caring of children. Irregular inflow of remittances to households affects the socio-economic milieu of the families. Most often, families borrow for day to day expenses as fishermen return only by a month or more as experienced by 65 percent of respondents. Women counterparts had to mobilize working capital for next round of fishing when male members are away in case of 58 percent of respondents. 71 percent of respondents said that debtors pose frequent enquires and force families for repayment in absence of male members. Occasional missing or non-traceability creates tension in families (37 percent). 82 percent of fishermen returning after voyage have to prepare for next voyage and have no time for family matters. Irregular income forces female counterparts to take up alternative avocations in case of 58 percent of respondents.

Perils of foreign migrants

Though the earning potential of foreign migration is higher, there are more severe problems associated with it. Fish workers are often treated as slaves by sponsors, boat owners, and public. Exploitation due to illiteracy is also rampant. While sharing is existent in the foreign vessels also, the fishers rarely know the actual price of fish sold in the market. Lack of facilities in fishing units affects the quantum of catch. Detention by neighbouring countries while fishing due to crossing of maritime borders is common in overseas fishing due to ignorance. Agreements are not honoured by sponsors/boat owners and passports and other documents are frequently impounded. In addition, if the fishers face any threat from the sponsors, there is no accessibility to the Indian High Commission/Embassy to address their problems.

Consequences of migration

The adverse consequences of migration in marine fisheries sector include capital deepening of fishing crafts. The motorization of boats has increased the economic burden

of the traditional fishing units; consequently polarization of fishermen into owners and workers has taken place; also fishing efforts get concentrated in selected regions. Due to increased mechanization and with the introduction of nylon nets in the place of hand-made nets, women have lost their major source of living which has forced them to shift to non-fishing avocations for alternative income earning.

Labour in the traditional sector moves towards modern fishing technologies; the movement resulting in a situation in which the traditional fishing sector gets integrated into the global economy and labour loses its traditional skills. Reorganization and work and division of labour have resulted in the disappearance of the traditional sharing system and the emergence of an inequitable distribution of incomes among participants in the fishing activity.

Conclusion

The economic development in the coastal belt is not in congruence with the other regions and the socio economic status remains backward compared to other sectors. The fishing population has grown over the years; inducing more and more people in primary, secondary, and tertiary sectors. Disguised unemployment is rampant with the increased earning potential due to introduction of labour saving and capital intensive mechanised fishing units. The non-mechanized sector is slowly being phased out, driving the labourers to other sectors, leading to overcrowding and resultant low per capita earnings. It was estimated that a total of 29.44 lakh people are employed in marine fisheries sector of which about 12.5 lakh people are involved in active fishing, 15 lakh in secondary sector and 2 lakh in tertiary sector. Out of those employed in marine fisheries sector, the coastal fisherfolk accounts for 71 percent in primary sector, 50 percent in secondary sector and 42 percent in tertiary sector. The case study of migratory fisherfolk highlights their common problems and their families. The high earning potential of shark in domestic and international markets induce the fishers for migratory fishing in search of fishing grounds. However, fisherfolk encounter several problems during the long duration trips away from their homes, creating social tension for families and poor ergonomics for themselves without realizing much out of the trip due to debt bondage. The families, especially the women households have to run the families with seasonal income from male members after trips that are often trapped with non-formal sources of debt. There are frequent conflicts among the migrants and local fisherfolk. It requires high priority to evolve strategies to protect the rights of migratory fisherfolk both in-country and foreign. In addition, fisheries infrastructure in their native places also should be developed. Concrete and comprehensive long term policies for ocean development in terms of resource exploitation, conservation and regulation, domestic and export

marketing, aquaculture and mariculture, human resource utilization and management are to be evolved and implemented in a phased manner for the balanced and sustainable development of marine fishery sector in our country. It is required to frame policies to suit the changing fishing methods and evolve programmes for capacity building of fisherfolk and their families associated with migratory fishing.

Acknowledgement

We express our heartfelt thanks to Dr. Mohan Joseph Modayil, Director, CMFRI for the constant encouragement in the preparation of this paper. Thanks are also due to all Technical Staff who have contributed in the collection of data.

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Received: 31 December 2007; Accepted: 21 November 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Pesticide-induced Histopathological Changes in the Freshwater Fishes of Kuttanand, Kerala-A Tool to Assess Water Quality and the Health Status of Fishes

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Abstract

Kuttanad, the rice bowl of Kerala, is a region where overdose application of pesticide is prevalent during the punja cultivation periods. According to the data compiled by Kuttanad Water Balance Study Project, 485 tonnes of pesticides were applied in Kuttanad on an annual basis of which 370 tonnes were used for the punja crop alone (KWBSP, 1990). In such a degraded aquatic environment, particularly where pollutants occur at chronic sublethal concentrations, changes in the structure and functions of aquatic organisms occur more frequently than their mass mortality. Therefore, one of the possible methods of assessing the effects of pollutants on fresh water fish inhabiting this ecosystem is to examine their organs for morphological changes. In fishes, apart from lethal effects of pesticides and the consequent mortality of eggs, larvae, and adults, their prolonged exposure in sublethal concentration may also result in reproductive abnormality, stock recruitment, deformities of eggs and larvae, retardation of hatchling percentage and body abnormalities. In the present study, a tool developed by Bernet et al. (1999) is used to assess the histopathological conditions; hence, histopathology is used as a tool to assess the health status of two freshwater fishes of Kuttanad, *viz*., *Etroplus suratensis* and *Anabas testudineus*. The organ index calculated based on various reaction patterns of the different organs of fishes exposed to sublethal concentrations of monocrotophos for a period of 30 days showed that gills were severely affected, liver was moderately affected, and kidney was the mildly affected organ, irrespective of fish species. Histopathology provides evidences of adaptation to degeneration, and histopathological alterations can be used as biomarkers of environmental pollution by organic chemicals. Histological changes in fish gill should become a rapid "early warning system" for water quality assessment in sublethal and chronic situations, as the toxicants induce changes at lower levels of biological organization prior to organismic changes.

Introduction

Kuttanad, the rice bowl of Kerala, India, is a region where overdose application of pesticide is prevalent during the punja cultivation periods. Traditionally, there are three cropping seasons for paddy in Kerala, the virippu, mundakan, and punja seasons. Punja crop, the traditional crop of Kuttanad, is sown in November to December and the

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harvest takes place by the end of March. The peak period of the pest damage, particularly by the brown plant hopper, is from February to March, which not only reduces the yield but also entails additional expenses for pesticides. There is no systematic crop surveillance and therefore, farmers arbitrarily apply pesticides at regular intervals. These ways of treatments are ineffective as well as unwanted and can cause severe damage to Kuttanad ecosystem. (KWBSP 1990).

According to the data compiled by Kuttanad Water Balance Study Project, 485 tonnes of pesticides were applied in Kuttanad on an annual basis of which 370 tonnes were used for the puncha crop alone (KWBSP 1990). Dimecron, Monocrotophos, Henosan, Thymet, Fernoxan, and Nuvacron are the major components of the pesticides being used in Kuttanad. In such a degraded aquatic environment, particularly where pollutants occur at chronic sublethal concentrations, changes in the structure and functions of aquatic organisms occur more frequently than their mass mortality. Therefore, one of the possible methods of assessing the effects of pollutants on fresh water fish inhabiting this ecosystem is to examine their organs for morphological changes.

In fishes, apart from lethal effects of pesticides and the consequent mortality of eggs, larvae, and adults, their prolonged exposure in sublethal concentration may also result in reproductive abnormality, stock recruitment, deformities of eggs and larvae, retardation of hatchling percentage, and body abnormalities. Evidence of retardation of natural propagation of fishes is already discernible in Kuttanad due to very low yield registered from these regions (Kurup et al. 1990). Hence, water pollution can lead to different changes, ranging from biochemical alterations in single cell into changes in whole populations. In general, the end points used in toxicity studies are mortality, survival, and growth with acute toxicity tests. These parameters are quite appropriate, but for long-term sublethal concentrations, these relevant parameters are difficult to ascertain. In the past, there were no tools to measure the magnitude of histopathological conditions in the affected organs. However, at present, many tools are available. Hence, in the present study, histopathological parameters are used to assess the nature and magnitude of toxic effects of pesticides that are being widely applied in the paddy fields of Kuttnad. This analysis is “user friendly” for the field investigator (Hinton 1993).

The advantage of histopathology as a biomarker lies in its intermediate location with regard to the level of biological organization (Adams et al. 1989). Histological changes appear as a medium-term response to sublethal stressors, and histology provides a rapid method to detect the effects of irritants, especially chronic ones in various tissues and organs (John et al. 1993). Histopathological analysis yields data on a number of organs and permits localization of lesions within specific cell types. With a thorough prior knowledge of normal anatomy, the investigator can use histological analysis to detect alterations in tissues and organs caused by exposure to toxicants. When concentration of a toxicant is sufficient to result only in cellular injury and not death, sublethal (adaptive) changes can be observed in affected cells.

sublethal (adaptive) changes can be observed in affected cells.

The exposure of fish to chemical contaminants is likely to induce a number of lesions in body organs like gills, liver, and kidney. These organs are suitable for histological examination to determine the effect of extent of pollution (Hinton 1993). Gills exhibit large surfaces, which are subjected to direct and permanent contact with potential irritants. Liver plays a key role in metabolism and subsequent excretion of xenobiotics and is also the site of vitellogenin production. Kidney is important for the maintenance of a stable internal environment and partially involved in the metabolism of xenobiotics (Hinton 1993).

In the above-mentioned conditions, it is felt that a study on the pesticide-induced histopathological changes in selected fishes would be helpful in bringing out the lethal effect caused to fish health due to ubiquitous application of pesticides and henceforth establishing the necessity for a judicious use of pesticides in agriculture in future.

Materials and methods

Juveniles of *Etroplus maculatus* (*E. maculatus*) and *Anabas testudineus* (*A. testudineus*) were collected from pollution-free ponds from the natural habitat. These fishes (size 47.5 ± 9.0 mm and 71.5 ± 6.0 mm in total length and 330 ± 80 mg and 750 ± 150 mg in weight, respectively) were acclimatized to the laboratory conditions for 14 days prior to the bioassay. During these periods, they were fed *ad libitum* once a day on fresh clam meat. The experiments on the lethal and sublethal toxicity of monocrotophos- an organophosphate pesticide-on the juveniles of *E. maculatus* and *A. testudineus*, the true denizens of Kuttanad paddy fields, were conducted for 48 hours and 30 days, respectively, during the period of investigation.

Based on the LC_{50} values (Mercy et al. 2000), five nominal concentrations of the pesticide were selected for sublethal toxicity studies. Maximum and minimum sublethal concentrations were chosen based on Konar (1969) and Sprague (1973). The experimental fishes were exposed to such sublethal concentrations for a period of 30 days. The concentrations of pesticides used for each sublethal exposure are given in Table 1.

Table1. Forty-eight-hour LC_{50} values and sublethal concentrations chosen for the experiment

Fish species	Pesticide	48-hr.LC_{50} (ppm)	Sublethal concentrations ($mg.L^{-1}$)					
Etroplus maculatus	Monocrotophos	3.36	0.0	0.1	0.3	0.6	1.0	1.5
Anabas testudineus		102.59	0.0	2.0	5.0	10.0	18.0	36.0

Sublethal exposure was carried out in a static system where water and pesticide medium were renewed every 24 hours to obtain the desired pesticide concentration. A control free of pesticide was also maintained in each experiment. All the treatments were made in triplicates. Ten healthy fishes, each of the target species, chosen at random from the acclimated stock were reared in 32 litres of water in seasoned cement cisterns. The ratio of the animal wet-weight to water volume ranged from 0.4899 to 2.7875 gm.L^{-1}. The tanks were covered with plastic mesh nets to prevent the escape of the fishes by jumping. All the experiments were conducted in ambient temperature (28 ± 2°C). The dissolved oxygen, pH, and temperature in the different treatments were measured immediately before and after the pesticide inoculation. After 30 days, i.e. after the termination of the experiments, five specimens from each of the treated as well as the control group were killed and the target organs such as the gills, liver, and kidney were dissected out and fixed immediately in Bouin's fluid. Histological sections were prepared based on standard procedures and stained using hematoxylin and eosin. Each organ is observed for its detailed histology. Same species of fishes were collected from the paddy fields of Kuttanad during the months of February and March, and histological preparations were carried out for the target organs and were observed for their histopathological lesions.

In the present study, histopathological conditions of different organs were assessed based on Bernet et al. (1999) who classified the histopathological changes of each organ into five reaction patterns (Table 2). Each pattern includes several alterations with respect to either functional unit of the organ or entire organ. Calculation of the index values was based on an importance factor (*w*) and score value (*a*).

Importance factor (w)

The relevance of a lesion depends on its pathological importance, i.e. how it affects organ function and the ability of the fish to survive. This is taken into account by an importance factor assigned to every alteration listed in the histological description.

The alterations are classified into three important factors:

1) Minimal pathological importance, the lesion is easily reversible as exposure to irritants ends; 2) Moderate pathological importance, the lesion is reversible in most cases if the stressor is neutralized; and 3) Marked pathological importance, the lesion is generally irreversible, leading to partial or total loss of the organ function.

Score value (*a*) Every alteration is assessed using a score ranging from 0 to 6, depending on the degree and extent of alteration: (0) unchanged; (2) mild occurrence; (4) moderate occurrence; and (6) severe occurrence (diffuse lesion). Intermediate values were also considered.

Table 2. Histopathological assessment tools for 3 fish organs (i.e. gills, liver, and kidney). An importance factor ($W_{org\ rp\ alt}$) ranging from 1 to 3 is assigned to every alteration: it is composed of the respective organ (org), the reaction pattern (rp), and the alteration (alt)*. # Extracted from Bernet et al. (1999)

Reaction pattern	Functional unit of the tissue	Alteration #	Importance factor	Score value	Index
Gills		Hemorrhage/hyperemia			
Circulatory disturbances		/aneurysm	WGC1 = 1	aGC1	IGC
		Intercellular edema	WGC2 = 1	aGC2	
Regressive changes	Epithelium	Architectural and structural alterations	WGR1 = 1	aGR1	IGR
		Plasma alterations	WGR2 = 1	aGR2	
		Deposits	WGR3 = 1	aGR3	
		Nuclear alterations	WGR4 = 2	aGR4	
		Atrophy	WGR5 = 2	aGR5	
		Necrosis	WGR6 = 3	aGR6	
		Rupture of the pillar cells			
	Supporting tissue	Architectural and structural alterations	WGR7 = 1	aGR7	
		Plasma alterations	WGR8 = 1	aGR8	
		Deposits	WGR9 = 1	aGR9	
		Nuclear alterations	WGR10 = 2	aGR10	
		Atrophy	WGR11 = 2	aGR11	
		Necrosis	WGR12 = 3	aGR12	
Progressive changes	Epithelium	Hypertrophy	WGP1 = 1	aGP1	IGP
		Hyperplasia	WGP2 = 2	aGP2	
	Supporting tissue	Hypertrophy	WGP3 = 1	aGP3	
		Hyperplasia	WGP4 = 2	aGP4	
Inflammation		Exudate	WGI1 = 1	aGI1	IG1
		Activation of RES	WGI2 = 1	aGI2	
		Infilteration	WGI3 = 2	aGI3	
Tumor		Benign tumor	WGT1 = 2	aGT1	IGT
		Malignant tumor	WGT2 = 3	aGT2	
					IG
Liver		Hemorrhage/hyperemia	WLC1 = 1	aLC1	ILC
Circulatory disturbances		/aneurysm			
		Intercellular edema	WLC2 = 1	aLC2	
Regressive changes	Liver tissue	Architectural and structural alterations	WLR1 = 1	aLR1	ILR
		Plasma alterations	WLR2 = 1	aLR2	
		Deposits	WLR3 = 1	aLR3	

Table 2. Continued..

		Nuclear alterations	WLR4 = 2	aLR4	
		Atrophy	WLR5 = 2	aLR5	
		Necrosis	WLR6 = 3	aLR6	
		Vacuolar degeneration			
	Interstitial tissue	Architectural and	WLR7 = 1	aLR7	
		structural alterations			
		Plasma alterations	WLR8 = 1	aLR8	
		Deposits	WLR9 = 1	aLR9	
		Nuclear alterations	WLR10 = 2	aLR10	
		Atrophy	WLR11 = 2	aLR11	
		Necrosis	WLR12 = 3	aLR12	
	Bile duct	Architectural and			
		structural alterations	WLR13 = 1	aLR13	
		Plasma alterations	WLR14 = 1	aLR14	
		Deposits	WLR15 = 1	aLR15	
		Nuclear alterations	WLR16 = 2	aLR16	
		Atrophy	WLR17 = 2	aLR17	
		Necrosis	WLR18 = 3	aLR18	
Progressive					
changes	Liver tissue	Hypertrophy	WLP1 = 1	aLP1	ILP
		Hyperplasia	WLP2 = 2	aLP2	
	Interstitial tissue	Hypertrophy	WLP3 = 1	aLP3	
		Hyperplasia	WLP4 = 2	aLP4	
	Bile dudct	Hypertrophy	WLP5 = 1	aLP5	
		Hyperplasia	WLP6 = 2	aLP6	
		Wall proliferation of bile ducts or ductules			
Inflammation		Exudate	WLI1 = 1	aLI1	IL1
		Activation of RES	WLI2 = 1	aLI2	
		Infilteration	WLI3 = 2	aLI3	
Tumor		Benign tumor	WLT1 = 2	aLT1	ILT
		Malignant tumor	WLT2 = 3	aLT2	
					IL
Kidney					
Circulatory		Hemorrhage/hyperemia			
disturbances		/aneurysm	WKC1 = 1	aKC1	IKC
		Intercellular edema	WKC2 = 1	aKC2	

Table 2. Continued..

Regressive changes	Tubule	Architectural and structural alterations	WKR1 = 1	aKR1	IKR
		Plasma alterations	WKR2 = 1	aKR2	
		Deposits	WKR3 = 1	aKR3	
		Nuclear alterations	WKR4 = 2	aKR4	
		Atrophy	WKR5 = 2	aKR5	
		Necrosis	WKR6 = 3	aKR6	
	Glomerulus	Architectural and structural alterations	WKR7 = 1	aKR7	
		Plasma alterations	WKR8 = 1	aKR8	
		Deposits	WKR9 = 1	aKR9	
		Nuclear alterations	WKR10 = 2	aKR10	
		Atrophy	WKR11 = 2	aKR11	
		Necrosis	WKR12 = 3	aKR12	
	Interstitial tissue	Architectural and structural alterations	WKR13 = 1	aKR13	
		Plasma alterations	WKR14 = 1	aKR14	
		Deposits	WKR15 = 1	aKR15	
		Nuclear alterations	WKR16 = 2	aKR16	
		Atrophy	WKR17 = 2	aKR17	
		Necrosis	WKR18 = 3	aKR18	
Progressive changes	Tubule	Hypertrophy	WKP1 = 1	aKP1	IKP
		Hyperplasia	WKP2 = 2	aKP2	
	Glomerulus	Hypertrophy	WKP3 = 1	aKP3	
		Hyperplasia	WKP4 = 2	aKP4	
		Thickening of Bowman's capsular membrane			
	Interstitial tissue	Hypertrophy	WKP5 = 1	aKP5	
		Hyperplasia	WKP6 = 2	aKP6	
Inflammation		Exudate	WKI1 = 1	aKI1	IK1
		Activation of RES	WKI2 = 1	aKI2	
		Infilteration	WKI3 = 2	aKI3	
Tumor		Benign tumor	WKT1 = 2	aKT1	IKT
		Malignant tumor	WKT2 = 3	aKT2	

Mathematical calculation of lesion indices:

1. Reaction index of an organ ($I_{org\ rp}$)

Only the lesions within one organ are studied, the following indices are applicable.

$$I_{org\ rp} = \sum_{alt} (a_{org\ rp\ alt} \times w_{org\ rp\ alt}),$$

where org = organ; rp = reaction pattern (constant); alt = alteration; a = score value; w = importance factor. The quality of the lesion in an organ is expressed by the reaction index.

2. Organ index (I_{org})

$$I_{org} = \sum_{rp} \sum_{alt} (a_{org\ rp\ alt} \times w_{org\ rp\ alt})$$

(Abbreviations same as in reaction index formula). This index represents the degree of damage to an organ

3. Total index (Tot-I)

$$Tot\text{-}I = \sum_{org} \sum_{rp} \sum_{alt} (a_{org\ rp\ alt} \times w_{org\ rp\ alt})$$

(Abbreviations same as in reaction index formula). This index represents a measure of the overall health status based on the histological lesions.

Results

The organ index calculated based on various reaction patterns of the different organs showed that gills were severely affected, liver was moderately affected, and kidney was the mildly affected organ, irrespective of fish species (Table 3–13).

Fishes of same species collected from Kuttanad also showed similar pattern except for *A. testudineus* in which liver was less damaged than kidney (Table 10–11). The total index indicated the overall health status of the fishes in each concentration and in Kuttanad. There was a gradual decrease in the health status of fish according to the increase in concentration of pesticide

Table 3. Organ index values of the gills of *E. maculatus* exposed to monocrotophos (following Bernet et al. 1999)

Concentrations	0.0 ppm					0.1 ppm					0.3 ppm					0.6 ppm					1.0 ppm					1.5 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WGC1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	-	-	4/4	2/2	2/2	4/4	4/4	2/2	4/4	6/6	6/6	4/4	4/4	4/4
WGC2=1	-	-	-	-	-	6/6	4/4	2/2	2/2	-	4/4	2/2	4/4	4/4	2/2	2/2	4/4	4/4	2/2	4/4	4/4	2/2	4/4	4/4	2/2	4/4	4/4	4/4	4/4	4/4
WGR2=1	-	-	-	-	-	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	-	-	-	-	-	4/4	2/2	2/2	2/2	2/2	4/4	2/2	2/2	2/2	2/2
WGR5=2	-	-	-	-	-	-	8/4	4/2	8/4	-	8/4	4/2	4/2	4/2	-	8/4	8/4	4/2	4/2	-	-	-	-	-	-	12/6	8/4	8/4	8/4	4/2
WGR6=3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12/4	6/2	6/2	12/4	-	12/4	12/4	12/4	12/4	12/4
WGR7=1	-	-	-	-	-	-	2/2	-	-	-	2/2	-	-	-	-	4/4	-	2/2	2/2	2/2	6/6	4/4	4/4	6/6	4/4	6/6	4/4	6/6	4/4	6/6
WGR11=2	-	-	-	-	-	8/4	4/2	4/2	-	-	4/2	8/4	4/2	-	8/4	12/6	8/4	4/2	8/4	4/2	12/6	12/6	8/4	8/4	12/6	8/4	12/6	12/6	8/4	8/4
WGP1=1	2/2	2/2	-	-	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	-	-	2/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WGP2=2	4/2	-	4/2	-	-	4/2	8/4	4/2	4/2	8/4	8/4	12/6	12/6	4/2	4/2	4/2	4/2	8/4	8/4	4/2	12/6	8/4	8/4	12/6	8/4	4/2	8/4	8/4	4/2	4/2
WGP4=2	-	-	-	-	-	-	-	-	-	-	12/6	8/4	4/2	4/2	8/4	12/6	8/4	4/2	4/2	8/4	8/4	8/4	4/2	4/2	4/2	8/4	12/6	8/4	12/6	12/6
WGT1=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	-	-	-
Organ index of each fish	6	2	4	-	2	22	30	18	18	12	42	38	30	18	26	46	32	26	32	24	60	46	46	50	36	68	68	64	58	56
Mean organ index of 5 fishes	2.8					20.0					30.8					32.0					46.4					62.8				

Denominator value denotes the score value: Numerator value = (score value x importance factor) WGC1 = 1 means importance factor = 1.

Table 4. Organ index values of the liver of *E. maculatus* exposed to monocrotophos (following Bernet et al. 1999)

Concentra-tions	0.0 ppm					0.1 ppm					0.3 ppm					0.6 ppm					1.0 ppm					1.5 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WLR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	2/2	-	-	-	-	-	-	2/2	-	-	-	-	-
WLR2=1	-	-	-	-	-	-	-	-	-	-	2/2	-	2/2	2/2	-	4/4	-	4/4	2/2	2/2	4/4	6/6	4/4	6/6	4/4	6/6	6/6	4/4	6/6	4/4
WLR4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	-	-	4/2	8/4	4/2	8/4	8/4	8/4	8/4	4/2	8/4	4/2
WLR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	4/2	-	8/4	-
Organ index of each fish	-	-	-	-	-	-	-	-	-	-	2	-	2	2	-	8	2	10	2	2	8	14	8	14	16	18	18	8	22	8
Mean organ index of 5 fishes			0					0					1.2					4.8					12.0					14.8		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WLC1 = 1 means importance factor = 1.

Table 5. Organ index values of the liver of *A. testudinues* exposed to monocrotophos (following Bernet et al., 1999)

Concentrations	Control					2.0 ppm					5.0 ppm					10.0 ppm					18.0ppm					36.0 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WLC1=1	-	-	-	-	-	2/2	-	-	-	-	-	2/2	-	2/2	-	-	2/2	2/2	-	2/2	2/2	4/4	-	4/4	-	2/2	2/2	2/2	4/4	2/2
WLR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	-	2/2	-	-
WLR2=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	4/4	2/2	6/6	2/2	4/4	2/2	6/6	4/4	2/2
WLR4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	4/2	-	8/4	-	-	4/2	4/2	-	-
WLR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	8/4	-	4/2	12/6	-
Organ Index of each fish	-	-	-	-	-	2	-	-	-	-	-	2	-	2	-	-	2	2	-	2	10	12	2	22	2	16	8	18	20	4
Mean organ index of 5 fishes			0				0.4					0.8					1.2						9.6					13.2		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WLC1 = 1 means importance factor = 1

Table 6. Organ index values of the kidney of *E. maculatus* exposed to monocrotophos (following Bernet et al., 1999)

Concentrations	0.0 ppm					0.1 ppm					0.3 ppm					0.6 ppm					1.0 ppm					1.5 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WKC2=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	2/2	-	4/4	2/2	4/4	6/6	4/4	2/2	2/2
WKR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	-	2/2	-	2/2	-	-	-	-	-	-	-	-	-	-
WKR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	8/4	-	8/4	12/6	8/4	2/2	2/2
Organ index of each fish	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2	-	2	8	2	4	12	2	12	18	12	4	4
Mean organ index of 5 fishes			0					0					0					1.2					5.6					10.0		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WKC1 = 1 means importance factor = 1.

Table 7. Organ index values of the kidney of *A. testudineus* exposed to monocrotophos (following Bernet et al., 1999)

Concentrations	0.0 ppm					2.0 ppm					5.0 ppm					10.0 ppm					18.0 ppm					36.0 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WKC2=1	-	-	-	-	-	-		-	-	-	-	-	-	-	-	2/2	-	4/4	-	2/2	2/2	4/4	2/2	4/4	4/4	6/6	4/4	6/6	6/6	2/2
WKR5=2	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	4/2	"	4/2	"	"	"	"	"	"	"	8/4	4/2	"	4/2	"
WKP2=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	4/2	4/2	4/2	4/2	12/6	8/4	8/4	8/4	12/6
Organ index of each fish	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	6	"	8	"	2	6	8	6	8	8	26	16	14	18	14
Mean organ index of 5 fishes			0					0					0					3.2					7.2					17.6		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WKC2 = 1 means importance factor = 1.

Table 8. Organ index values of the gills of *A. testudineus* exposed to monocrotophos (following Bernet et al. 1999)

Concentrations	0.0 ppm					2.0 ppm					5.0 ppm					10.0 ppm					18.0ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																									
WGC2=1	-	-	-	-	-	2/2	2/2	2/2	-	2/2	2/2	2/2	4/4	2/2	4/4	4/4	2/2	4/4	2/2	4/4	6/6	4/4	2/2	6/6	4/4
WGR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WGR5=2	-	-	-	-	-	-	-	-	-	-	4/2	4/2	-	4/2	4/2	4/2	4/2	4/2	4/2	4/2	4/2	4/2	8/4	4/2	4/2
WGR7=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	2/2	-	4/4	2/2	4/4	2/2	-	2/2	-
WGR11=2	-	-	-	-	-	-	-	-	-	-	8/4	4/2	-	4/2	4/2	8/4	8/4	-	4/2	4/2	8/4	8/4	12/6	4/2	4/2
WGP1=1	2/2	2/2	-	-	-	2/2	-	2/2	2/2	-	6/6	2/2	2/2	4/4	2/2	2/2	4/4	2/2	2/2	4/4	-	-	-	-	-
WGP2=2	4/2	-	-	4/2	4/2	4/2	4/2	-	-	4/2	-	-	-	-	-	8/4	4/2	4/2	4/2	-	8/4	4/2	4/2	8/4	4/2
WGP4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	-	4/4	2/2	2/2	4/4	2/2	2/2	-	2/2
Organ index of each fish	6	2	-	4	4	8	6	4	2	6	20	12	6	14	14	30	24	18	22	20	34	24	28	24	18
Mean organ Index of 5 fishes			3.2					5.2					13.2					22.8					25.6		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WGC1 = 1 means importance factor = 1.

Table 9. Organ index values of the gills of *E. maculatus* and *A. testudineus* collected from the paddy fields of Kuttanad (following Bernet et al., 1999)

Fish species	*E. maculatus*										*A. testudineus*									
No. of fishes	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Alterations																				
WGC1=1	4/4	2/2	4/4	6/6	2/2	2/2	4/4	4/4	6/6	4/4	4/4	2/2	-	2/2	4/4	-	4/4	4/4	2/2	-
WGC2=1	4/4	4/4	2/2	2/2	4/4	4/4	2/2	4/4	2/2	4/4	6/6	4/4	6/6	2/2	6/6	4/4	4/4	6/6	4/4	2/2
WGR5=2	8/4	8/4	4/2	4/2	12/6	8/4	8/4	4/2	12/6	12/6	8/4	12/6	4/2	12/6	-	4/2	8/4	8/4	12/6	-
WGR6=3	12/4	18/6	12/4	6/2	6/2	12/4	12/4	6/2	12/4	12/4	12/4	6/2	6/2	-	12/4	12/4	12/4	6/2	6/2	6/2
WGR7=1	6/6	4/4	4/4	2/2	4/4	4/4	4/4	2/2	6/6	4/4	6/6	4/4	6/6	4/4	4/4	4/4	2/2	6/6	2/2	6/6
WGR11=2	12/6	4/2	8/4	4/2	4/2	8/4	12/6	12/6	8/4	4/2	8/4	-	4/2	4/2	8/4	8/4	4/2	8/4	-	8/4
WGP2=2	12/6	8/4	8/4	12/6	8/4	12/6	12/6	12/6	8/4	4/2	8/4	12/6	-	4/2	12/6	12/6	8/4	4/2	12/6	8/4
Organ index of each fish	58	48	42	36	40	50	54	44	54	44	52	40	26	28	46	44	42	42	38	30
Mean organ index of 10 fishes	47.0										38.8									

Denominator value denotes the score value: Numerator value = (score value x importance factor). WGC1 = 1 means importance factor = 1.

Table 10. Organ index values of the liver of *E. maculatus* and *A. testudineus* collected from the paddy fields of Kuttanad (following Bernet et al., 1999)

Fish species	*E. maculatus*										*A. testudeneus*									
No of fishes	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Alterations																				
WLC1=1	2/2	-	2/2	-	-	-	2/2	2/2	2/2	-	-	-	2/2	2/2	2/2	4/4	4/4	-	2/2	-
WLR2=1	6/6	4/4	4/4	2/2	2/2	4/4	6/6	6/6	6/6	2/2	-	-	-	-	-	-	-	-	-	-
WLR4=2	8/4	4/2	4/2	-	-	4/2	8/4	8/4	8/4	-	4/2	8/4	4/2	4/2	4/2	8/4	8/4	4/2	4/2	8/4
WLR5=2	4/2	-	-	4/2	4/2	-	4/2	4/2	-	4/2	-	-	-	-	-	-	-	-	-	-
WLR6=3	6/2	-	-	-	-	-	6/2	6/2	6/2	-	-	-	-	-	-	-	-	-	-	-
WLT1=2	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	-	-	8/4	-	8/4	-	-
Organ index of each fish	26	8	10	6	6	8	26	26	22	6	8	8	10	6	6	20	12	12	6	8
Mean organ Index of 10 fishes	13.6										9.6									

Denominator value denotes the score value: Numerator value = (score value x importance factor). WLC1 = 1 means importance factor = 1.

Table 11. Organ index values of the Kidney of *E. maculatus* and *A. testudineus* collected from the paddy fields of Kuttanad (following Bernet et al., 1999)

Fish species	*E. maculatus*										*A. testudeneus*									
No of fishes	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Alterations																				
WKC2=1	4/4	2/2	-	4/4	4/4	2/2	4/4	2/2	2/2	2/2	2/2	4/4	-	6/6	4/4	2/2	-	-	-	2/2
WKR5=2	8/4	-	-	4/2	4/2	-	-	4/2	4/2	4/2	4/2	12/6	4/2	8/4	-	-	4/2	-	4/2	-
WKP2=2	4/2	4/2	8/4	4/2	4/2	4/2	4/2	4/2	4/2	-	12/6	12/6	8/4	12/6	4/2	-	-	4/2	8/4	-
Organ Index of each fish	16	6	8	12	12	6	8	10	10	6	18	28	12	26	8	2	4	4	12	2
Mean organ index of 10 fishes	9.4										11.6									

Denominator value denotes the score value: Numerator value = (score value x importance factor). W_{KC2}= 1 means importance factor = 1.

Table 12. Total index of *E. maculatus* and *A. testudineus* exposed to different sublethal concentrations of monocrotophos based on the organ index.

Fish	Treatment (ppm)	Organ Index			Total Index
		Gill	Liver	Kidney	
E. maculatus	0.0	2.8	0	0	2.8
	0.1	20.0	0	0	20.0
	0.3	30.8	1.2	0	32.0
	0.6	32.0	4.8	1.2	38.0
	1.0	46.4	12.0	5.6	64.0
	1.5	62.8	14.8	10.0	87.6
A. testudineus	0.0	3.2	0	0	3.2
	2.0	5.2	0.4	0	5.6
	5.0	13.2	0.8	0	14.0
	10.0	22.8	1.2	3.2	27.2
	18.0	25.6	9.6	7.2	42.4
	36.0	33.2	13.2	17.6	64

Table 13. Total index of *E. maculatus* and *A. testudineus* collected from Kuttanad based on the organ index

Fish Species	Organ Index			Total Index
	Gill	Liver	Kidney	
E. maculatus	47.0	13.6	9.4	70.0
A. testudineus	38.8	9.6	11.6	60.0

Discussion

The organ indices are used for calculating the total index, which gives the health status of an organism in particular, under altered environmental condition. In the present study, the total index showed the health status of fishes in each sublethal concentration and in the field conditions. The health status became worse in the higher sublethal concentrations of both the fishes treated with monocrotophos. The total index of *E. maculatus* collected from Kuttanad is 70, which is comparable to the total index value of *E. maculatus* exposed to sublethal concentrations of 1.0 ppm monocrotophos (total index 64). The total index value of *A. testudineus* collected from Kuttanad is 60, which is comparable to the total index value of *A. testudineus* exposed to sublethal concentrations of 36 ppm monocrotophos (total index 63.4).

It is evident from the index value that the gills are in the irreversibly damaged condition as per Poleksic & Mitrovic-Tutundzic (1994). This irreversible condition in the gills of *E. maculatus* and *A. testudineus* may be due to the chronic microtoxicosis (sublethal effects) as a result of toxicants in the medium. Thus, gill degeneration can be considered as a factor, which seriously impairs the viability of organisms, while this may not represent a hazard to the life of the individual. It has great importance as far as the survival of the population is concerned. Szakolczai et al. (1994) have also reported that structural changes in gills can be considered suitable to monitor the level of environmental contamination, especially the sublethal and chronic effects of pollutants, particularly in those cases where other methods of assessments are not satisfactory, and to compliment the assessment of the average level of pollution. It should be emphasized that fish gill can maintain their vital functions even when some lamellae are heavily damaged. However, chronic exposure of these gill lamellae to pesticides will lead to the histological degeneration of the irreversible condition that will lead to functional disturbance or dysfunction of the organ. This gradually leads to mortality and in turn affects the population of the ecosystem. Hence, the present study carried out on the natural population supports the view of Poleksic & Mitrovic-Tutundzic (1994) that histological changes in fish gills should become one of the methods used for assessment of water quality in sublethal and chronic situations.

The histopathological changes are one of the most sensitive parameters for the evaluation of chronic toxicity test effects and thus also for the derivation of Maximum Allowable Toxicant Concentration as reported by Poleksic & Mitrovic-Tutundzic (1994). Moreover, the sublethal concentrations may become lethal for populations confronted

with additional stresses. This should be taken into serious consideration when evaluating the effects of mixtures of toxicants in fresh water fish under natural conditions.

In the natural ecosystem, fishes are exposed simultaneously to more than one biocide or contaminant because some chemicals are applied continuously and are highly persistent or others are applied as combinations to increase the efficiency or reduce costs (Marking 1977). Kuttanad is an area where the pesticides and weedicides are applied simultaneously or intermittently. The problems of toxicity of mixtures of pesticides on fish have been recognized only recently and notable among the studies are those of Macek (1977); Fabacher et al. (1976); Nair et al. (2000). While studying the individual and combined acute lethal toxicity of monocrotophos and 2,4-D on the juveniles of *E. suratensis*, a highly favored food fish of Kuttanad region, Nair et al. (2000) reported that a strictly additive nature of their combined toxicity and the sequential and even simultaneous use in the ecosystem increases the potential for pollution. Significant increase in sensitivity could be achieved from histological studies when compared with routine parameters like survival and mortality. Histopathology provides evidences of adaptation to degeneration, and this certainly represents the major advantage of the use of histopathological alterations as biomarkers of environmental pollution by organic chemicals.

Therefore, it is proposed that the histological changes in fish gill should become one of the methods used for assessment of water quality in sublethal and chronic situations as the toxicants induce changes at lower levels of biological organization occurring prior to organismic changes. It should therefore provide a rapid "early warning system" as suggested by Moore (1985).

Acknowledgement

The Science, Technology, Environment Committee, Kerala State (STEC), which supported this study financially, is gratefully acknowledged. The authors are grateful to the Dean, College of Fisheries, Kochi for providing facilities to carry out the work.

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Received: 31 December 2007; Accepted: 04 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Effects of Stocking Biomass on Growth, Survival, and Production of the two Sizes of Clam *Meretrix lyrata* Cultured in the Intertidal Areas

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Abstract

The triplicate experiment had been conducted in 50 m^2 plots randomly placed in the intertidal areas to evaluate the effects of stocking biomass on survival, growth performance, and quality of clam *Meretrix lyrata*. The two stocking sizes (Mean ± SD, cm) at shell length of 1.0 ± 0.2 and 1.7 ± 0.1 were scattered at different biomass: 0.05, 0.1, 0.2, 0.3 $kg.m^{-2}$ and 0.34, 0.68, 1.36, 2.03 $kg.m^{-2}$ and named as T1, T2, T3, T4 and T5, T6, T7, T8, respectively. Results show that meat ratio of the clam were similar regardless of different stocking biomass. The fatty acids were rich in highly unsaturated fatty acids especially docosahexaenoic acid but were variable. In contrast, growth and survival of the clam were strongly affected by the stocking biomass in which the lower stocking biomass resulted in higher specific growth rate (SGR) and survival rate. The biomass gained therefore reduced accordingly with increase in the stocking biomass, although the increase of final production was evident. However, SGR and survival of the treatments T1, T2, and T3 were not significantly different, suggesting the highest net profit and investment return of the treatment T3. Therefore, the stocking biomass of 0.2 $kg.m^2$ was recommended to maximize profit of the clam cultivation.

Introduction

The mollusk production has been increasing steadily during the last two decades (Gibbs 2004) and has reached the total production of 13.25 mmt, which accounts for 23.3% of total world aquaculture production in 2004 (Tacon & Halwart 2006). Among mollusk species, the bivalve shellfish are not only the favorable seafood but also are regarded as the most ecologically efficient forms of aquaculture because these are low trophic–level animals. Besides, bivalve shellfish are filter feeders, which can also be used as biofilter for improvement of water quality (Shpigel & Blaylock 1991; Shpigel et al. 1993; Shpigel et al. 1997; Mazzola & Sara 2001) and thus contribute to the sustainable aquaculture development.

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Clams belong to bivalve shellfish but they are different from the others by dwelling on the bottom. Researches have been carried out on the production of various clam species (Shpigel & Spencer 1996; Cigarrýa & Fernandez 2000; Zhang & Yan 2006) and the use of clam for improvement of water quality (Shpigel & Fridman 1990; Jara-Jara et al. 1997). In Vietnam, the endogenous brackish water clam *Meretrix lyrata* (*M. lyrata*) is an emerging cultured species for coastal aquaculture because this is favorable seafood in the national and international markets. *M. lyrata* distributes naturally in the intertidal of southern coast and known as "Ngheu Ben Tre" because the exploited production mostly comes from Ben Tre province, south of Vietnam. Recently, due to high consumption demand, *M. lyrata* is being cultivated and expanded to the northern coastal provinces such as in Nam Dinh, Thanh Hoa, Nghe An, and Ha Tinh. However, the clam production still is very unstable and unpredictable due to poor management. The technical information on clam culture still is very limited. Therefore, it is necessary to study the establishment of a standard protocol to enhance the production and profit of clam culture.

Among the factors that affect growth and production, feed and feeding of clam have been regarded as the most important factors. Researches recently have revealed that feed clearance rate have positive relationship with body size, and within a range of food concentration, their feeding can be strongly affected by substrata (Zhuang & Wang 2004) and by salinity or diurnal rhythm (Zhuang 2006). For maximizing production and profit, Zhang & Yan (2006) described a new three-phase culture method for Manila clam farming in China. In this method, the seed was artificially produced indoor during winter and the grow-out phase was conducted in the intertidal with appropriate stocking size, stocking density, and substrate. In the intertidal areas where the feed are naturally dependent, uncontrollable, and variable, stocking biomass becomes an important factor to increase the growth and production. The objective of this research was to evaluate the effect of stocking biomass of the two sizes of *M. lyrata* on growth performance and survival to enhance the production and profit of cultivation. The other parameters within the culture system cannot be altered as it is a natural ecosystem highly connected to capture fisheries, which is one of the key industries for the fishery community.

Materials and Methods

The experiment had been conducted in the intertidal areas that belong to Hau Loc District, Thanh Hoa Province. There were 24 plots of 50 m^2 each, separated by plastic mesh and randomly allocated for eight treatments (three replicates each). The small clam seeds at shell length of 1.0 ± 0.2 cm were scattered at four different biomass: 0.05, 0.10, 0.20, and 0.30 $kg.m^{-2}$ and named as T1, T2, T3, and T4, respectively. The bigger size of clam seed at a shell length of 1.7 ± 0.1 cm was stocked at 4 different stocking biomass: 0.34, 0.68, 1.36, and 2.03 $kg.m^2$ and named as T5, T6, T7, and T8, respectively. This experiment was terminated after 165 days of rearing. Environment factors such as temperature (thermal meter), DO, pH (Oxyguard), turbidity (Sechi disk), and salinity

(Refractometer) of water in the experiment site were daily monitored at 3 designated points within the experimental area.

Growth of clam, expressed in mean of shell length (cm) and mean of live weight (g), was determined by random sampling (n = 30) and was measured every fortnight. The daily specific growth rate (SGR) was calculated using the following formula (Jara-Jara et al. 1997):

$$SGR(\%.day^{-1}) = 100*(LnW_f-LnW_i)/t.$$

Where W_i and W_f are mean of initial weight and final weight, respectively, and t is number of experiment days. Size variation of the clam was evaluated according to Wang et al. (1998) in which the mean of three replicates of the coefficient of variation (CV) was used to examine the inter-individual variation among the clam in each treatment: CV(%) = 100*SD/M, where M is mean of live weight, and SD is standard deviation of the clam in each treatment. The meat ratio (% of meat weight: total live weight) of clam was conducted by separating the meat content of random samples (n = 20). The excess water was removed by placing the sample on tissue paper.

At the end of experiment, clam was randomly sampled and preserved in Liquid Nitrogen Biological Container (YDS-3, -196^{o}C) for the analysis of fatty acids. The fatty acids content expressed in mg.g^{-1} dry weight was first extracted by placing the clam in a 35-mL glass tube with a teflon lined screw caps and 5 mL of methanol/toluene mixture (3:2 v/v) was added; then, 0.1 mL of internal standard solution containing 4.78 mg.mL^{-1} 20:2(n-6) fatty acid dissolved in iso-octane was added. The freshly prepared acetylchloride/methanol mixture (1:20 v/v) then was added as the esterification reagent. The tube was flushed with nitrogen gas and closed tightly before carefully shaking and was placed in a boiling water bath (100^{o}C). After one hour, the tube was cooled, 5 mL of distilled water and 5 mL of hexane were added, and the upper layer was separated by centrifugation. The combined hexane phase was dried by filtration process carried out in a flask using the anhydrous sodium sulphate filter, and the FAMEs were finally dissolved in 0.5 mL iso-octane and transferred in a 2-mL glass vial for injection in Finnigan Trace GC untra with capillary column BP-70 (50m x 0.32mm x 0.25µm). All data of the treatments were tested for significant differences ($p < 0.05$) using One-way ANOVA followed by Turky test for multiple comparisons of means. The data are expressed as Average ± SD, and the statistical analysis was carried out using GraphPad Prism version 4.0 and Microsoft Office EXCEL for Window.

Results and Discussion

Environment conditions of the experiments

The experiment site situated the intertidal areas near the estuary where the clams

have been already cultivated for recent years. The environment factors such as DO, water temperature, pH, and salinity (Table 1) were regarded as the best conditions for clam development. The high levels of salinity fluctuation are typical for estuary ecological conditions. The average water temperature was 23.59 ± 2.40°C, relatively low compared with the normal water temperature in the south of Vietnam, where *M. lyrata* is naturally distributed. This mean clam is not affected by the marked variation and good growth and survival rate were noticed. However, low water temperature might affect growth performance, and the growth and survival of *M. lyrata* might be not as high as the ones cultivated in the south of Vietnam. As Soudanta et al. (2004) has described, in the Manila clam cultured in four rearing sites that were selected for their varied ecological characteristics, it was observed that the environmental conditions influenced the physiological and immunological parameters."

Table 1. Environment conditions in the experiment site

Parameters	DO (ppm)	Water temperature (°C)	pH	Salinity (ppt)	Turbidity (cm)
Average ± SD	6.25 ± 0.42	23.59 ± 2.40		25.65 ± 2.84	9.05 ± 3.13
Maximum	7.66	31.00	8.99	31.00	20.00
Minimum	5.50	19.50	7.21	20.00	5.00

Growth performance

The growth performance of the two stocking sizes of *M. lyrata* at different stocking biomass expressed in specific growth rate, final shell length, and final live weight as well as size variation are shown in Tables 2 and 3.

Table 2. Growth performance of clam at stocking size of 1.0 cm

Treatments	T1	T2	T3	T4
SGR	1.25 ± 0.05^{a}	1.13 ± 0.05^{a}	1.08 ± 0.10^{ab}	0.94 ± 0.37^{b}
Final length (cm)	2.04 ± 0.13^{a}	2.01 ± 0.09^{ab}	1.95 ± 0.10^{b}	1.95 ± 0.11^{b}
Final weight (g)	5.92 ± 1.08^{a}	5.76 ± 0.81^{ab}	5.46 ± 0.76^{ab}	5.30 ± 0.85^{b}
% of meat.total weight	15.87 ± 1.00^{a}	15.48 ± 2.72^{a}	15.53 ± 1.02^{a}	15.15 ± 5.47^{a}
CV% (weight)	28.72 ± 2.55^{a}	23.07 ± 0.24^{b}	23.73 ± 1.55^{b}	27.78 ± 2.11^{ab}

Value (Mean ± SD) followed by different superscript letters within a row are significantly different ($P < 0.05$). T1, T2, T3, and T4 are treatments of clam cultured at 0.05, 0.1, 0.2, and 0.3 kg.m^{-2}, respectively. SGR = daily specific growth rate; CV = coefficient of variation

Table 3. Growth performance of clam at stocking size of 1.7 cm

Treatments	T5	T6	T7	T8
SGR	0.62 ± 0.04^{a}	0.46 ± 0.03^{b}	0.33 ± 0.02^{c}	0.32 ± 0.02^{cd}
Final length (mm)	2.36 ± 0.17^{ab}	2.40 ± 0.10	2.32 ± 0.11^{bc}	2.27 ± 0.10^{c}
Final weight (g)	9.24 ± 1.20^{a}	9.33 ± 0.95^{a}	8.90 ± 1.12^{a}	8.21 ± 1.01^{b}
% of meat.total weight	14.53 ± 1.89^{a}	15.78 ± 2.35^{a}	16.53 ± 0.62^{a}	15.48 ± 1.31^{a}
CV% (weight)	22.3 ± 0.45^{a}	19.05 ± 5.16^{a}	18.69 ± 3.36^{a}	22.73 ± 4.16^{a}

Value (Mean ± SD) followed by different superscript letters within a row are significantly different ($p < 0.05$). T5, T6, T7, and T8 are treatments of clam cultured at 0.34, 0.68, 1.36, and 2.06 $kg.m^{-2}$ respectively. SGR = daily specific growth rate; CV = coefficient of variation.

For the small-size group, there was no significant difference in specific growth rate and final weight among T1, T2, and T3 treatments (Table 2), indicating that growth of the clams were not affected by the stocking biomass below 0.2 $kg.m^{-2}$. The final size of *M. lyrata* was more variable at low (T1) and high (T4) stocking density compared with the medium (T2 and T3) ones. The meat yield expressed in percentage of meat per total weight, which is regarded as the most valuable part of the clams, was not significantly different ($p > 0.05$) in all treatments

The growth of *M. lyrata* at stocking size of 1.7 cm significantly reduced with an increase in the stocking biomass (Table 3). At high stocking biomass (T7 and T8), the SGRs were relatively low and were not significantly different. The final length and final weight of the treatment T8 were significantly smaller compared with others. However, the size variation was not affected by different stocking biomass.

In general, at younger stage, the animal has better growth rate. In the case of clam, at the same stocking biomass, the small-size clam (1.0 cm) grew much better than the large-size clam (1.7 cm). In the intertidal areas, the natural feed and environmental factors are uncontrollable and are dependent of nature. Dynamics of tide, wave, and current create the availability of algae, organic matter that is regarded as feed for clam. However, because clam is filter feeder and passively dwells on the bottom, the amount of biomass decreases beyond certain level and hence the natural feed might not be enough for its growth. Moreover, in treatments of the same size organisms, increasing biomass led to the increase in the competition of other environmental conditions such as habitat, DO, and increased accumulation of metabolic wastes, that is feces, which is regarded as a detriment for the growth of clam (Yan et al. 2006). It was also observed that at the same temperature, the clearance rate and ingestion rate of clam increased exponentially with an increase in the size (Zhuang and Wang, 2004). Results of the

growth performance (Table 3) indicated that at high stocking biomass (more than 0.3 kg.m^{-2}), the growth could be inhibited, and the growth rate significantly reduced with an increase in the biomass. In addition, it was noted that winter is not an appropriate culture period because the water temperature is normally low and does not support the growth of *M. lyrata*, the tropical species.

Survival

The stocking biomass influenced the survival rate in both the sizes of clam. Survival was very high in the low stocking biomass treatment (T1) and was almost similar in the treatments T2 and T3. As seen in Fig. 1 the treatment T1 was significantly different ($p < 0.05$) from the treatment T4.

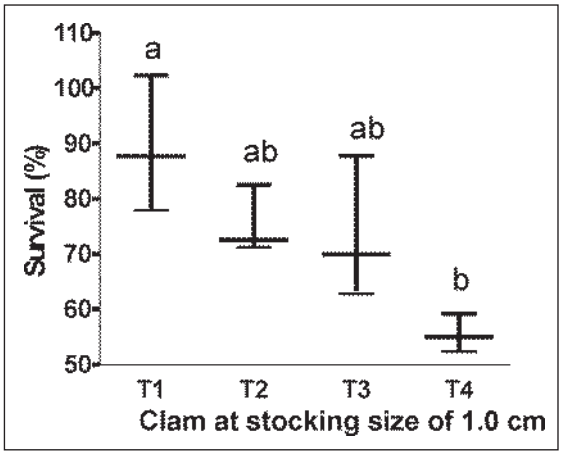

Figure 1. Survival of clam size 1.0 cm rearing at different stocking biomass.(Value (Average ± SD) followed by different superscript letters are significantly different ($p < 0.05$). T1, T2, T3, and T4 are treatments of clam cultured at 0.05, 0.1, 0.2, and 0.3 kg.m^{-2}, respectively).

In the larger stocking groups, survival of the treatment T5 was highest followed by the treatment T6. Survival of the treatment T7 and treatment T8 were very low and were not significantly different (Fig. 2). On the other hand, the results presented in Fig. 1 and 2 also indicate that the clam survival is not only affected by the stocking biomass but also affected by the stocking density. The environmental condition and food availability could be explained as the main reasons for the impact of the stocking biomass on survival rate.

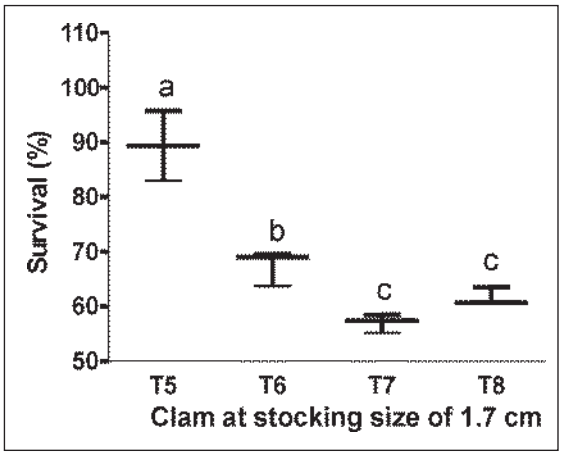

Figure 2. Survival of clam size 1.7 cm rearing at different stocking biomass. (Value (Average ± SD) followed by different superscript letters are significantly different ($P<0.05$). T5, T6, T7 and T8 are treatments of clam size 1.7cm cultured at 0.34, 0.68, 1.36 and 2.06 kg.m-2 respectively).

Stocking size had been detected to affect survival of the Manila clam in which the small-size group showed higher mortality; however, substrata or predators are

not the only reason for this high mortality rate (Cigarrýa & Fernandez 2000), and the normal stocking size of this species for intertidal cultivation was 1.0 cm (Zhang & Yan 2006). In our trial, at same stocking biomass (0.30 and 0.34 kg.m^{-2}), survival rate of treatment T4 (1.0 cm) was very low (55%) compared with survival rate of 90% of the treatment T5 (1.7 cm). Within the same size 1.7 cm, the treatment T7 and T8 had relatively low survival rate compared with the treatment T5 and T6, suggesting that those stocking biomass were too high for the development of clam.

Production and quality

Both growth and survival was considered for the estimation of the production of clam. There was a positive relationship between the clam production and stocking biomass, although the growth and survival were negatively affected. Among the small stocking size group, the final production increased accordingly with the gain in biomass and no significant difference ($p > 0.05$) was detected between T1 and T2 and T3 and T4 (Table 4). The percentage of biomass gained, in contrast, showed a decreasing trend as the stocking biomass increased. No significant difference was detected between T1 and T4. This is due the fact that the increase in biomass negatively affected the growth and survival of the clams.

Table 4. Biomass production of clam at stocking size of 1.0 cm

Treatments	T1	T2	T3	T4
Final production (ton.ha^{-1})	4.14 ± 0.57^{a}	6.82 ± 0.56^{a}	12.62 ± 2.16^{b}	14.84 ± 0.91^{b}
Biomass gained (ton.ha^{-1})	3.62 ± 0.57^{a}	5.78 ± 0.56^{a}	10.54 ± 2.16^{b}	11.72 ± 0.91^{b}
% of biomass gained	697.1 ± 109.4^{a}	555.8 ± 53.6^{ab}	506.9 ± 104.0^{ab}	375.8 ± 29.3^{b}

Value (Mean ± SD) followed by different superscript letters within a row are significantly different ($p < 0.05$). T1, T2, T3, and T4 are treatments of clam cultured at 0.05, 0.1, 0.2, and 0.3 kg.m^{-2}, respectively

In the larger stocking size (1.7 cm), the final production of the clam significantly increased with an increase in the stocking biomass ($p < 0.05$). The percentage of biomass gained, in contrast, decreased with an increase in the stocking biomass in T5, T6, and T7 (Table 5). However, no significant difference ($p > 0.05$) was observed in the biomass gained in the treatment T5 and T6 and percentage of biomass gained in the treatments

T7 and T8. In both size groups, the increase in biomass certainly had a negative impact on the net production.

Table 5. Biomass production of clam at stocking size of 1.7 cm

Treatments	T5	T6	T7	T8
Final production (ton.ha^{-1})	9.49 ± 0.68^{a}	14.46 ± 0.69^{b}	23.58 ± 0.68^{c}	34.80 ± 1.00^{d}
Biomass gained (ton.ha^{-1})	6.10 ± 0.68^{a}	7.68 ± 0.69^{a}	10.02 ± 0.69^{b}	14.46 ± 0.99^{c}
% of biomass gained	180.0 ± 20.0^{a}	113.3 ± 10.1^{b}	73.9 ± 5.1^{c}	71.1 ± 4.8^{c}

Value (Mean ± SD) followed by different superscript letters within a row are significantly different ($p < 0.05$). T5, T6, T7, and T8 are treatments of clam cultured at 0.34, 0.68, 1.36, and 2.06 kg.m^{-2}, respectively.

The high value of percentage of biomass gained confirmed that the stocking biomass was barrier for the development of clam. However, the increase in the biomass gained as well as final production indicated that the determination of appropriate stocking biomass is important for the production of clam. The economic calculation therefore is vital to optimize investment benefit.

Fatty acid profile

There was difference in the fatty acid profiles between treatments regardless of different stocking biomass. The total FAME varies from 134.4 to 193.7 mg.g^{-1} dry weight (Table 6). However, the presence of high content of HUFA especially DHA content (29.00 to 62.77 mg.g^{-1} dry weight) indicated the value of clam as seafood product. The variation observed in the amount of fatty acids in clam may be attributed to the development of ovary and/or growing development stage when the fatty acids normally accumulate. Our result confirmed the variation in the amount of fatty acid of clam *Ruditapes decussatus* reared in sea water and effluent from a fish farm in Galicia (Jara-Jara et al. 1997). The variation in the amount of fatty acid and the factors affecting this variation need a further research.

Economic evaluation

The estimation of the economic benefit of clam cultured in the intertidal areas is shown in Table 7. The net profit is calculated based on the output cost, input cost, and price of the clam.

Table 6. Fatty acids of clam cultured at different stocking sizes and different stocking biomass

Fatty Acids	T1	T2	T3	T4	T5	T6	T7	T8
14:00	0.58	-	-	1.07	-	0.59	2.52	6.35
16:00	44.26	42.67	78.27	21.63	47.07	84.63	33.54	33.94
16:1(n-7)	9.85	-	3.53	7.88	-	0.75	10.94	11.71
17:00	0.19	-	-	0.89	-	-	1.94	1.22
17:1(n-7)	-	-	-	-	-	-	3.39	7.71
18:00	4.63	15.63	22	23.98	16.82	7.84	10.08	10.72
18:1(n-9)	63.02	39.79	26.83	29.68	49.38	33.41	27.18	31.94
18:1(n-7)	-	-	-	5.31	6.33	-	-	-
18:2(n-6)t	0.41	8.19	-	1.06	-	-	2.35	13.74
18:3(n-3)	-	-	-	0.54	-	-	1.1	5.16
20:1(n-9)	-	7.83	-	0.52	8.18	-	-	-
20:4(n-6)	1.11	-	7.72	2.98	5.06	2.72	3.54	8.9
20:4(n-3)	-	-	-	0.31	-	-	-	-
20:5(n-3)	4.45	3.11	-	5.95	6.2	0.97	7.96	3.29
24:00:00	-	-	-	1.17	-	-	-	-
22:5(n-6)	-	-	-	-	-	-	1.56	-
22:5(n-3)	-	3	4.96	1.85	-	-	2.46	-
22:6(n-3)	45.78	29	33.62	29.65	27.58	62.77	30.4	30.0
Sum (n-3)	50.23	35.11	38.58	37.76	33.78	63.74	40.82	30.29
Sum (n-6)	0.11	0	7.72	2.98	5.06	2.72	5.1	8.9
Sum HUFA	50.34	35.11	46.3	40.74	38.84	66.46	45.92	42.19
Total FAME	174.3	149.2	176.9	134.4	166.6	193.7	139	166.1

Value = $mg.g^{-1}$ dry weight; T1, T2, T3 and T4 are treatments of clam cultured at 0.05, 0.1, 0.2 and 0.3 $kg.m^{-2}$ respectively; T5, T6, T7 and T8 are treatments of clam size 1.7cm cultured at 0.34, 0.68, 1.36 and 2.06 $kg.m^{-2}$ respectively.

Table 7. Economical evaluation of the two stocking size of clam rearing at different stocking biomass

Stocking size	Shell length 1.0 cm				Shell length 1.7 cm			
Treatments	T1	T2	T3	T4	T5	T6	T7	T8
Stocking biomass (ton.ha^{-1})	0.50	1.00	2.00	3.00	3.40	6.80	13.60	20.40
Final production (ton.ha^{-1})	4.14	6.82	12.62	14.84	9.49	14.46	23.58	34.80
Input (mill VND.ha^{-1})*								
Cost for seed (1)	17.50	35.00	70.00	105.00	61.20	122.40	244.80	367.20
Mesh and fencing	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30
Labor cost	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20
Hut for daily monitoring	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Land lease	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Harvesting cost (B*2%)	0.99	1.64	3.03	3.56	2.28	3.47	5.66	8.35
Total input (A)	37.99	56.14	92.53	128.06	82.98	145.37	269.96	395.05
Output (mill VND.ha^{-1} with assumption price of 12 mill VND.ton^{-1} for all harvested clam)*								
Total output (B)	49.72	81.82	151.44	178.08	113.90	173.52	282.96	417.60
Net profit (A - B)	11.72	25.68	58.91	50.02	30.93	28.15	13.00	22.55
Rate of investment return (%)	30.85	45.75	63.67	39.06	37.27	19.36	4.82	5.71

(1) The seed cost was 0.035 mill VND.kg^{-1} size 1.0 cm and 0.018 mill VND.kg^{-1} size 1.7 cm

The main cost in *M. lyrata* cultivation was the expense in seed purchase. Cost of seed ranged between 46% and 81% in small-size seed (1.0 cm) for the four treatments (T1, T2, T3, and T4). As all other costs are fixed, the increase in stocking biomass increased the total cost invested. Although total production increased with the increase in stocking biomass, the economic analysis clearly indicated that the net profit decreased beyond the level of 2 ton.ha^{-1} stocking biomass (T3). The treatment T4 with the stocking density of 3 ton.ha^{-1} yielded lesser net profit compared with the treatment T3. This can be explained by the higher proportion of seed cost while the biomass gained was lower due to less growth and survival. Therefore, the stocking biomass of 2 ton.ha^{-1} is recommended for *M. lyrata* at stocking size of 1.0 cm.

For the treatment T5, T6, T7, and T8, cost of seed increased from 73.8% to 92.9%. Because the price of seed was higher than the price of harvested clam, while the biomass gained reduced accordingly with increase in stocking biomass, the net profit was reduced and relatively lower compared with the 1-cm seed stocking treatments. We suggested that the clam size more than 1.7 cm should not be cultured at stocking biomass more than 6.8 ton.ha^{-1}.

Conclusions

The result of this experiment indicated that *M. lyrata* grow very well in the intertidal areas in north coast of Vietnam during winter at water temperature of 23.59 ± 2.40°C. The stocking biomass had strong effect on growth performance and survival of clam. For the stocking seed at shell length of 1.7 cm, among 4 different stocking biomass 0.34, 0.68, 1.36, and 2,04 $kg.m^{-2}$, the higher biomass, the lower growth performance as well as the lower survival, which eventually resulted in reduction in the net profit even the final biomass were increasing. For the small seed at shell length of 1.0 cm, among stocking biomass of 0.05, 0.1, 0.2, and 0.3 $kg.m^{-2}$, the lower stocking biomass resulted in better growth performance. The survival rate of the stocking biomass of 0.3 $kg.m^{-2}$, however, was significantly lower than the others, and therefore, the highest net profit as well as investment return was obtained at the stocking biomass of 0.2 $kg.m^{-2}$. We recommend using this stocking biomass to maximize the profit of the cultivation.

Quality of the clam expressed as the meat ratio of clam was similar regardless of different stocking size or stocking biomass. In addition, the fatty acids of clam were rich in HUFAs especially DHA and EPA but also were showed differences between the treatments. This might be related to the natural feed availability or the different development stages of maturation; further research on this issue need to be addressed.

Acknowledgments

This research is a part of the collaboration project VIE 027/05 “Development of clam culture for improvement and diversification of livelihoods of the poor coastal communities in Central Vietnam” between the Aquaculture Research Sub-Institute for North Central (ARSINC-RIA1), Vietnam and the South Australian Research and Development Institute (SARDI), Australia. The project was funded by the AusAIDs through the Collaboration of Agriculture and Rural Development (CARD) program.

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Received: 31 December 2007; Accepted: 11 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Genetic Characterization of *Aeromonas hydrophila* using Protein Profiling and RAPD PCR

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Abstract

Genetic relationship among *Aeromonas hydrophila* isolates from fish and traditional brackishwater farms were evaluated through Randomly Amplified Polymorphic DNA (RAPD) analysis as well as Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS PAGE) profile of cellular proteins. PCR amplification of the DNA from the bacterial isolates using five random primers (OPA-01, 03, 04, 05 and 10) produced 46 amplicons, which were scorable as distinct bands on agarose gel electrophoresis. Absolute polymorphism of RAPD profile was evident with a unique pattern for each isolate, indicating its usefulness as an ideal tool for evaluation of genetic heterogeneity within the species, which are not revealed by other methods like morphological and biochemical characterization and cellular protein profile. Cellular protein profile did not reveal significant polymorphism as all the isolates revealed uniform pattern indicating its usefulness as a tool for species level identification. Four protein bands of molecular size *viz.*, 19.5 kDa, 25.6 kDa, 29 kDa and 65.6 kDa were shared by all the isolates in the study.

Introduction

Aquaculture has grown significantly during the past decade. However, the aquaculture industry faces several problems that have to be solved before it can achieve and sustain significant growth as an industry. Disease is one of the major threats faced by this industry.

Role of the motile aeromonad, *Aeromonas hydrophila* as an etiological agent in fish and shellfish disease is highly significant. Although the outcome of disease by this secondary pathogen mostly depends on the presence of a primary pathogen, it is well recognized as a primary pathogen also (Miyata et al.1995).

Aeromonas hydrophila (Chester 1901); Stanier (1943) is a gram negative, facultatively anaerobic, motile, waterborne bacterium with wide distribution. They have long been known to be pathogenic to cold and warm-blooded fauna. Since 1960 the

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potential of these organisms to cause diseases in human has been recognized. In humans and other mammals, *A.hydrophila* has been reported as the causative agent of numerous manifestations associated with gastroenteritis, systemic infections and bacterial endocarditis. In amphibians, reptiles, and fishes, these organisms have reportedly been associated with necrotic septicaemic and ulcerative diseases (Austin and Austin 1993; Inglis 1993; Olafse et al. 1993). Though the exact role of *A.hydrophila* in disease still remains in a state of flux, it is accepted as an opportunistic pathogen of homeothermic and poiekilothermic hosts.

To determine the health risks in aquatic environment associated with the exposure to *A. hydrophila*, epidemiological tracking is required. Conventional methods used for bacterial identification are tiresome, time consuming and not fit for mass scale screening of bacteria. Microbial molecular genetics is gaining popularity in recent times as an essential tool in the classification of the microbes.

Arbitrary/Random primed PCR amplification of DNA (AP-PCR/RAPD) has been increasingly reported as a method for the genetic characterization of microorganisms. The advantages of this method for the characterization of *Listeria monocytogenes, Vibrio vulnificus, Aeromonas salmonicida* and *Bacillus thuringiensis* have already been reported by Lawrence et al. (1993), Covadonga et al. (1998), O'hlei et al. (2000) and Pattanayak et al. (2001) respectively. Cellular proteins are the expressions of genetic information coded in the DNA and therefore the protein profile reveals the functional genetic diversity. Mc Lean et al. (1993) and Das et al. (2005) have observed that protein profiling has application in the characterization, classification and identification of bacterial isolates.

In the present study, the genetic heterogeneity and phylogenetic relationship among *Aeromonas hydrophila* isolates from the tissues of moribund fishes (*Etroplus suratensis)* and the waters of traditional brackishwater farms in the Cochin region of Kerala State (along south west coast of India) were evaluated using the RAPD profile and the cellular protein profile resolved through Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS PAGE)

Materials and methods

Bacterial Isolates

Twenty-eight isolates of *Aeromonas hydrophila* from the tissues of moribund fishes (*Etroplus suratensis)* and the waters of traditional brackishwater farms in Cochin were used in this study. Isolation of *Aeromonas hydrophila* was done using selective medium, Rimler Shotts agar (Hi Media). The plates were incubated at room temperature (RT 28°C for 48 hours). All cultures were identified to the species level using differential biochemical tests (Table 1). Selected *Aeromonas hydrophila* colonies were subcultured in peptone water for further molecular characterization.

RAPD-PCR Analysis

DNA Extraction

The protocol developed by Murray and Thompson (1980) with modifications was used for isolation of DNA from broth culture of the bacteria at log phase. The modified protocol without the use of proteinase K has given good result, yielding quality DNA of approximately 20 μg from a 2 mL bacterial culture.

Primers

A panel of 20 numbers of decamer random primers from M/S Operon Technologies, designated as OPA-1 to OPA-20 was used for PCR amplification of the bacterial DNA template. Five out of these 20 primers, *viz*., OPA-01, OPA-03, OPA-04, OPA-05 and OPA-10 were selected for final screening as they only generated several reproducible amplicons that could be resolved as distinct bands by agarose gel electrophoresis.

PCR amplification and resolution of RAPD markers

PCR reactions has been optimized for important parameters such as annealing temperature, concentration of $MgCl_2$, template DNA, *Taq* DNA polymerase, dNTP's and primers. The PCR reaction components consists of 200 mm dNTP, 7.5 pico moles of primer, 2 units of Taq DNA polymerase enzyme, assay buffer with working concentration of 1.5 mM $Mgcl_2$, 20-30 ng template DNA in an assay volume of 25mL. These concentrations were determined by a series of preliminary standardizing experiments.

Thermal cycling was performed with Perkin-Elmer thermocycler (GeneAmp PCR System 2400). Each of the 35 PCR cycles standardized for this work consisted of denaturation of DNA at 92°C for one minute, primer annealing at 37°C for one minute and primer extension at 72°C for one minute. All PCR samples were subjected to an initial two minutes denaturation for 94°C and post amplification extension at 72°C for ten minutes. PCR products were stored at -20°C until electrophoresis was performed.

The PCR amplification products were resolved by carrying out electrophoresis using a 1.5% agarose gel, stained with ethidium bromide. The marker used was λ DNA cut with HindIII/EcoRI. The DNA bands were visualized and documented using a Vilber Lourmat Gel documentation system.

Phylogenetic Analysis

Amplified DNA fragments resolved as bands on the gels were used to generate the data matrix by giving scores of zero and one for the absence or presence of bands, respectively, at each band position. The data matrix of individual primers was finally

joined to form a single matrix. Parameters like overall amplicon frequency at each locus, number and percentage of polymorphic loci, Nei's gene diversity and Nei's original measures of genetic identity and genetic distances between the isolates were estimated from the RAPD data using the POPGENE under Microsoft Windows 3.11 (Yeh et al. 1997).

Bacterial Protein Isolation and SDS PAGE

Aeromonas hydrophila were harvested from 2 ml broth culture during the post logarithmic phase by spinning at 10000 rpm, 4°C, 10 minutes in 1.5mL eppendorf tubes in a refrigerated high speed centrifuge. The supernatant was drained off and 100 µl of Tris, EDTA, Glucose buffer (pH 8) containing 5mg/mL lysozyme was added to each of the pellets and vortexed in a vortex mixture machine. This was incubated at 4°C for 15minutes, mixing gently every five minutes. The cell suspension was then centrifuged at 10000 rpm, 4°C, 10 minutes and the supernatant was collected in eppendorf tubes and stored at -20°C for further use. SDS PAGE carried out in the present study was on the lines of Laemmeli et al. (1970).

Results

Aeromonas hydrophila appeared as flat yellow colonies with entire margin in the Rimler shotts medium. In primary characterization tests, they were gram negative, rod shaped, motile, oxidase positive, fermentative, 0/129 resistant and novobiocin resisitant, suggesting that colonies are aeromonads. All isolates were confirmed to the species level *Aeromonas hydrophila* by differential tests given in Table 1.

Table 1. Characteristics of bacteria isolated in the study.

Test	Characteristics	Test	Characteristics
Gram stain	+	Ornithine decarboxylase	+
Shape	Rods	H2S production	+
Motility	+	Arabinose	
Oxidase	+	Indole	+
Glucose APW	+	Voges Proskauer	+
Glucose O/F	Fermentative	Gelatin hydrolysis	+
Simmons citrate	+	Catalase	+
ONPG for α galactosidase	+	Antibiotic sensitivity	
Arginine dihydrolase	+	Ampicillin	Resistant
Lysine decarboxylase	+	Chloramphenicol	Sensitive

RAPD Profile

The thermal cycle regime optimized for the PCR consisted of denaturation at 92°C, primer annealing at 37°C and primer extension at 72°C, and each step for duration of one minute. In our attempt to reduce the background banding, the annealing temperature was increased to 55ºC, and it gave clear reproducible bands. Amplification of the DNA from each of the 28 isolates with the five primers named earlier produced a total of 46 amplicons, which were consistent and appeared as distinct bands on agarose gel after electrophoresis. The RAPD fingerprints of the isolates generated by these random primers OPA-01 and OPA-10 are given in Fig. (1-2).

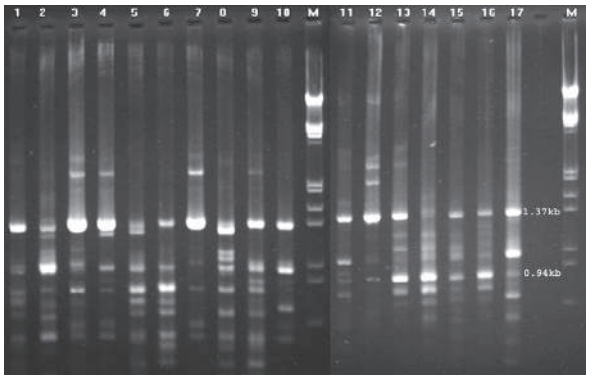

Figure 1. RAPD pattern of *A.hydrophila* isolates generated by primer OPA-01. Lane 1-17: Ah1-Ah17 isolates; M:Marker.

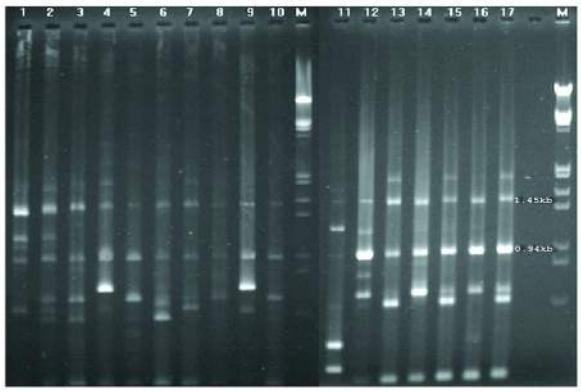

Figure 2. RAPD pattern of *A.hydrophila* isolates generated by primer OPA-10. Lane 1-17: Ah1-Ah17 isolates; M: Marker.

The amplicons ranging from 2.5 kb on the higher side to approximately 0.5kb on the lower side (as determined from the relative mobility of marker DNA *viz*., λ DNA cut with HindIII /EcoR I) were only considered for the POPGENE analysis.

The OPA-01 produced 12 amplicons, which were scorable as distinct bands in the gel. Whereas two of the amplicons were produced by all the isolates, ten amplicons showed variation between isolates. The amplicons shared by all the isolates were 0.94 kb and 1.37 kb size. Seven amplicons were generated by OPA-03, which were within the range of 1.584kb to 0.83kb. OPA-04 produced ten amplicons ranging in size from 2.5kb to 0.55kb. OPA-05 produced a total of six amplicons and OPA-10 produced 11 numbers of amplicons with size ranging from 1.9 kb to 0.5 kb. With primer OPA-10, 0.94 kb and 1.45 kb amplicon was common to all the isolates.

Polymorphism of the RAPD pattern was quite apparent among the different isolates. The RAPD fingerprint pattern was unique for each of the isolates. Comparison of the amplicons at each of the 46 loci indicated that all but four were polymorphic. The non-polymorphic loci were of primers OPA-01 and OPA-10. However, perusal of the banding pattern indicates that there were a number of fragments, which were homogenous among many of the isolates. The POPGENE analysis indicated that the overall polymorphism was 95.65%. All the primers except OPA-01 and OPA-10 were highly discriminatory. The two monomorphic loci each of OPA-01 and OPA -10, with fixed allele *viz*., 1.37 kb, 0.94 kb and 1.45 kb, 0.94 kb respectively, are common to *A. hydrophila.* These fragments may be utilized for species-specific marker development, only if amplification of other aeromonads are also examined with these primers OPA-01 and OPA –10.

Similarity Index, Genetic Distance and Phylogenetic Relationships

The average similarity index between *Aeromonas hydrophila* isolates was calculated as Nei's original measures of genetic identity and genetic distance, considering all the amplicons resulting from all the primers. The coefficient of genetic identities ranged from 0.413 to 0.848, most of them were of high magnitude. Estimates of genetic distances were of lower magnitude. However, no correlation in genetic distance/similarity could be made between isolates from moribund fishes and those from brackishwater farms.

Cellular Protein profile

Soluble cellular proteins extracted from the isolates and resolved through SDS-PAGE analysis are presented in Fig. 3.

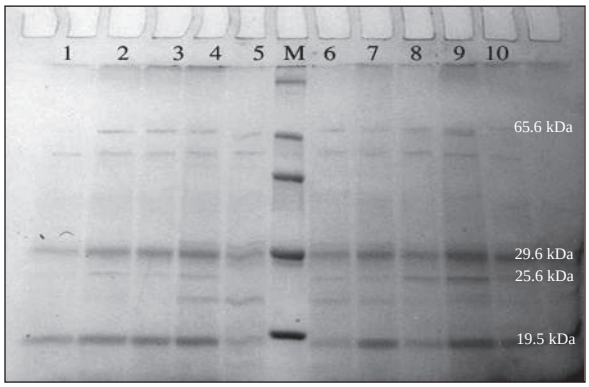

Figure 3.Cellular protein profile of *A.hydrohila* resolved through SDS PAGE. Lane 1-10:Ah1-Ah10 isolates; M: Marker.

No significant polymorphism was evident through PAGE as all the isolates revealed more or less uniform profile. Four protein bands of molecular size *viz.,* 19.5 kDa, 25.6 kDa, 29 kDa and 65.6 kDa were shared by all the isolates in the study.

Discussion

Epidemiological tracking of *A. hydrophila* of aquaculture systems requires evaluation of genetic diversity and phylogenetic relationship among the isolates.

Since the conventional methods are time consuming compared to the molecular tools, the potential of RAPD analysis and the cellular protein profile for characterization and evaluation of heterogeneity in *A. hydrophila* was assessed.

The study revealed that the RAPD profiles are highly discriminatory between all the isolates. RAPD has wide genomic coverage unlike amplification using specific primers and hence it has higher discrimination. RAPD techniques have many advantages over the conventional methods and other molecular techniques. It does not require any prior information on the sequence of the DNA being characterized. The RAPD technique requires the least quantity of DNA among the various techniques. Miyata et al. (1995) observed that the DNA required for RAPD is less than one hundredth of the amount required for other methods. We were able to generate reproducible RAPD profiles with as little as 20ng of DNA per PCR reaction in the study.

The DNA fingerprint pattern based on RAPD profiles revealed considerable amounts of polymorphism. Each isolate produced a unique pattern leading to absolute polymorphism. Comparison of individual amplicons revealed considerable variation between isolates by way of variations in size and number of amplified fragments. The polymorphism of the amplified fragments in this study was very high (95.65%) with only four amplicons out of 46 being shared by all the isolates. Thus, the discriminating ability of the RAPD fingerprint observed in this study make it an ideal tool for evaluating population genetic diversity of the microbial populations within species. Molecular characterization of *A.hydrophila* strains from Japan by Miyata et al. (1995) also revealed considerable heterogeneity within the species. Results of this study corroborate the observation by other workers that motile aeromonads are genetically diverse (MacInnes and Trust, 1979) and antigenically very heterogenous (Mulla and Millership 1993). RAPD-PCR fingerprints have been used for typing and differentiation of bacteria and increasingly, for the study of genetic relationships between strains and species of microorganisms, plants and animals. The analysis of such fingerprints is based upon the assumption that co-migration of amplicons does not occur and that any given band contains a single amplicon. Co-migration of RAPD-PCR amplicons from *A. hydrophila* has been discussed earlier by Oakey et al. 1998. However, this was not observed in the present study.

This study points towards the presence of considerable genetic heterogeneity among the isolates even though they belong to the same species and are phenotypically homogenous as revealed by morphological and biochemical characterizations. This is quite possible. The morphological and biochemical characterization depend on the coding region of DNA, and any sequence changes in the regions are subjected to natural selection and culling. Very few of the variants, which have selective advantage, are only allowed to get established in the population and result in heterogeneity. Whereas, major portion of the DNA in the cell is the non-coding region that can accumulate genetic variation as

they are not subjected to natural selection and therefore, accumulate variations, which can be detected by nucleic acid based techniques.

As expected in the light of the above statement cellular protein profile resolved through SDS- PAGE could not reveal intraspecies variation, as the profiles of all the isolates were more or less uniform. Hanninen et al. (1995) also reported a similar condition in *A.salmonida* from 28 Finnish strains with uniform SDS pattern among all the isolates studied, whereas the genetic differentiation could be generated with RAPD markers. Das et al.(2005) also has reported the use of SDS PAGE in Protein fingerprinting of *A.hydrophila* and they have revealed the genetic similarity between strains and reference strain (MTCC 646) .They have observed unique clear and distinct bands in different local and reference strains of *A.hydrophila*, which they have suggested as suitable for molecular identification. These protein bands were of molecular weight 19.5, 23.5, 25.6, 32.4, 36.1, 41.2, 65.6,71.3, 72.9 and 86.2 kDa. Among the common fragments determined in our study, bands of molecular size *viz.*, 19.5 kDa, 25.6 kDa, 29 kDa and 65.6 kDa were shared by all the *A. hydrophila* isolates.

Conclusion

Search for species-specific amplicons that could be used as molecular markers of *A.* hydrophila pointed towards the amplicons generated by OPA-1 and OPA-10. However, the species specificity can be confirmed only by checking these primes with isolates of other aeromonads. The occurrence of large number of RAPD genotypes in the species indicating intraspecific genetic diversity which remain hidden with other methods of characterization especially, cellular protein profiling, reflect the potential and sensitivity of this approach for population genetic and systematic studies. Hadrys et al. (1992) has made a similar conclusion after carrying out RAPD studies in crabs. The results show that protein fingerprinting has the potential to differentiate *Aeromonas* species, but the low variation indicates that this technique is not efficient for the characterization of strains within a species. Conversely, RAPD fingerprinting allows the identification of strains but the high variability limits its potential as an aiding method for species identification.

Acknowledgement

The authors thank Dr. Mohan Joseph Modayil, Director CMFRI, Cochin, for providing necessary facilities and Dr. R. Paulraj, Principal Scientist, for encouragement. The author acknowledges CIFE/ICAR for the financial assistance received during the tenure of this work.

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Received: 31 December 2007; Accepted: 28 February 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Inter-sectoral Disparity and Marginalization in Marine Fisheries in India

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Abstract

Fisheries policy in the eleventh plan aims at sustainable economic growth, with due concerns on food and nutritional security and supply side responses. The plan accords overarching priorities on bridging the sharpening divides and increasing disparities in all sectors. The socio-economic framework of the fishing community with structural changes in coastal sector needs successful design and implementation of development programs. This article highlights the sectoral growth of fishing units and their capital investment over the years, change in ownership pattern of means of production, earnings, sectoral disparity, and inequity among marine fisher folk in India. Base material for the analysis includes primary data collected from selected centers of maritime states in India and secondary data on marine fisheries census of CMFRI and other relevant publications.

There has been sizeable growth of 70% in the mechanized fishing units and about 200% growth in motorized sector that are technically efficient (over the last 12 years until 2005). However, there has been a downtrend of 43% in the nonmechanized units (traditional sector) denoting a gradual phasing out of less efficient units. The improved socio-economic status of fishers is reflected by increase in literacy level, reduction in dropouts, and improvement in housing type. The proportion of owner operators in marine fisheries declined over the years with the increasing capital requirement for possessing motorized and mechanized fishing units. The fishermen involved in active fishing is more than the absorbing capacity of the fisheries sector leading to disguised unemployment and has led to lower per capita production, increased pressure on fishing, which results in juvenile catch, large level discards, and thus ultimately causing serious threats to resource sustainability and environmental stability. The nonmechanized sector is providing about 33% of the employment in active fishing, yet harvesting hardly 7% of the annual landings, whereas mechanized segment that employs 34% harvests 70% of total catch creating wide inter-sectoral income disparity. The annual per capita catch of fisher folk in mechanized segment is more than twice as those of the per capita catch of the motorized segment and nine times of the per capita catch of the nonmechanized (traditional sector) segment clearly signifying growing inter-sectoral disparity in distribution of economic gains. Average annual per capita earnings of fishing laborer range from Rs.13,200 for a motorized *dingi* with bagnet to

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Rs. 1,27,200 for a mechanized purse seiner. Significant variation is also observed even within groups of crafts namely trawlers, gillnetters, purseseiners, motorized, and traditional crafts. The analysis indicate that there is high incidence of poverty in the coastal rural sector explicitly revealing that majority of these people still could not get much of the benefits of the economic development taken place in our country.

Introduction

Fisheries get importance in planners' agenda in view of the fact that it supports a significant section of population recognized as backward sections of society. Fisheries play a pivotal role in our national development programs either on growth terms, contributing to foreign exchange earnings and domestic consumption, or on equity terms in formulation of poverty reduction strategies. The main objectives of fisheries and aquaculture programs of Government of India during the plan periods have been towards enhancing production and productivity, increasing the export of marine products, promoting sustainable development through responsible fisheries, generation of employment, and enhancing welfare of fisher folk and improvement of their socio-economic status. The socio-economic framework of the community with its structural changes over the years is the base for formulating plans of action focused on target population. The successful design and implementation of development programs are hampered by lack of such information. Further, impact and trickle down effects of the previous efforts by developmental agencies can be gauged by the changes in socio-economic indicators of beneficiaries. Several studies have highlighted the micro and macrolevel socio-economic conditions of fishermen in our country (Desai & Baichwal 1960; Gurtner 1960; Sen 1973; Prakasam 1974; George 1974; Selvaraj 1975; Amarasiri Desilva 1977; Lawson 1977; Panikkar 1980; Sathiadhas & Venkitaraman 1981; Shanbhu Dayal 1981; Pietersz 1983; Platteau 1984; Prasada Rao & Kumar 1984; Krishna Srinath 1987; Sathiadhas & Panikkar 1988; Korakkandy Ramakrishnan 1994). Various studies carried out in the context of a developing country like India point out several problems including marginalization of traditional fisher folk, decreasing ownership of crafts and gears, increasing capital intensity, and declining productivity.

Materials and Methods

Comprehensive usage of data, both secondary and primary, is attempted in this article. The primary database consists of cost and earnings data of different types of fishing units, collected systematically from all the maritime states in India by the Socio Economic Evaluation and Technology Transfer Division of Central Marine Fisheries Research Institute. Secondary data from Marine Fisheries Census of CMFRI and various publications cited herein are also used in the preparation of this article.

Results and Discussion

Socioeconomic profile of coastal fishers

The marine fishery resources of India comprise 2.02 million sq km of Exclusive Economic Zone with a continental shelf area of 4,91,000 sq. km. Amongst the different maritime states, Gujarat has the longest coast line of 1600 Kms followed by Tamil Nadu (1076 Kms) and Andhra Pradesh (974 Kms). There are 641 fishing villages in Orissa followed by Tamil Nadu (581) and Andhra Pradesh (498). However, with regard to basic fish landing facilities, Tamil Nadu ranks first with 352 centers followed by Andhra Pradesh (271) and Kerala (178). The marine fisher population is concentrated in the East coast of India (59%) constituting West Bengal, Orissa, Andhra Pradesh, and Tamil Nadu. In the West coast, 17% of fisher men population is from Kerala alone. Among the maritime states, fisher population is highest in Tamil Nadu (22%) followed by Kerala. A similar trend is observed in case of distribution of fisher families across the states. An average fisher household in India has a family size of five, ranging from four in Andhra Pradesh, Tamil Nadu, and Pondichery to six in Karnataka and Daman and Diu (Table 1). The coastal fishing villages in India are thickly populated as the fishermen prefer to stay along the coast line owing to the access to the sea. The Coastal Zone Regulations are not strictly adhered to at times with the reluctance of fishers to move away from proximity to the sea. Among the ten maritime states, Kerala is the most densely populated (population per village) state in India (2713 people per village).

Table 1. Profile of Marine Fishermen Population in India (2005)

State	Fishermen population	Number of fishermen families	Average Family size	Average population/ fishing village
West Bengal	269,565	53,816	5	779
Orissa	450,391	86,352	5	703
Andhra Pradesh	509,991	129,246	4	1024
Tamil Nadu	790,408	192,152	4	1360
Pondichery	43,028	11,541	4	1537
Kerala	602,234	120,486	5	2713
Karnataka	170,914	30,176	6	1096
Goa	10,668	1,963	5	274
Maharashtra	319,397	65,313	5	787
Gujarat	323,215	59,889	5	1229
Daman and Diu	29,305	5,278	6	1332
Total	35,19,116	756,212	5	1099

The literacy rate among fisher folk in maritime states of India was found to be 56.50% in 2005. (Table 2). In all maritime states, the literacy rate for coastal population is much lesser than the State averages indicating their poor social development index adding to their vulnerability. Among the maritime states, Kerala ranks first in literacy of marine fisher folk with 72.84%, which is also lower than State literacy rate of 90.86% (Census 2001). It is observed that 50.70% of the fisher folk (excluding children) are educated up to primary level, followed by 39.40% upto secondary and 9.90% above secondary level education. In contrast to previous trend of huge drop outs from education after primary level, above 50% of the fisher folk studied beyond primary level. This shows that once fisher folk get exposed to education, they are inclined to get educated to higher levels as seen in most of the maritime states provided there is availability of educational infrastructural facilities.

Table 2 Literacy status of marine fisher folk in India (2005)

State	Literacy rate		Status of Education			
	State Average (2001)	Coastal sector (2005)	Primary	Secondary	Above secondary	Total
West Bengal	68.64	45.65	83,301 *(67.70)*	33,734 *(27.41)*	6,018 *(4.89)*	123,053 *(100)*
Orissa	63.08	47.88	142,005 *(65.84)*	56,879 *(26.37)*	16,783 *(7.78)*	215,667 *(100)*
Andhra Pradesh	60.47	32.47	111,403 *(67.27)*	45,827 *(27.67)*	8,384 *(5.06)*	165,614 *(100)*
Tamil Nadu	73.45	66.75	260,088 *(49.30)*	206,257 *(39.10)*	61,229 *(11.61)*	527,574 *(100)*
Pondichery	81.24	63.18	12,763 *(46.95)*	10,904 *(40.11)*	3,518 *(12.94)*	27,185 *(100)*
Kerala	90.86	72.84	171,470 *(39.09)*	218,704 *(49.86)*	48,493 *(11.05)*	438,667 *(100)*
Karnataka	66.64	69.93	52,572 *(43.98)*	49,606 *(41.50)*	17,346 *(14.51)*	119,524 *(100)*
Goa	82.01	69.12	1,691 *(22.93)*	4,581 *(62.12)*	1,102 *(14.94)*	7,374 *(100)*
Maharashtra	76.88	67.04	94,303 *(44.04)*	97,446 *(45.51)*	22,368 *(10.45)*	214,117 *(100)*
Gujarat	69.14	40.93	70,658 *(53.40)*	52,088 *(39.37)*	9,560 *(7.23)*	132,306 *(100)*
Daman and Diu	78.18	58.28	7,760 *(45.44)*	7,273 *(42.59)*	2,045 *(11.97)*	17,078 *(100)*
Total	64.84	56.50	1,008,014 *(50.70)*	783,299 *(39.40)*	196,846 *(9.90)*	1,988,159 *(100)*

* *Figures in parenthesis denote percentage to total*

The overall literacy status doubled from 18.57% in 1980 to 56.50% in 2005. The improved socio-economic status of fishers is reflected by increase in literacy level (Table 3). The situations in the past have improved that almost half of the population could access education facilities. Among the educated persons, only 20% were able to have higher education beyond primary level in 1980, whereas at present, the situation has improved that almost half of them study above primary level.

Table 3. Change in educational status of fisher folk in India over the years

Educational status	Primary	Secondary	Above secondary	Not educated	Total	Literacy rate
1980	280987	56998	13489	1541442	1892916	18.57
Percentage to total	14.84	3.01	0.71	81.43	100	
2005	1008014	783299	196846	1530957	3519116	56.50
Percentage to total	28.64	22.26	5.59	43.50	100	

Source: Marine Fisheries Census of CMFRI, 1980 and 2005

The overall dependency ratio of marine fisher folk in India is estimated to be 2.04 denoting that every person working in marine fisheries sector supports two persons (Table 4). The dependency ratio varies across the states from 1.56 (Orissa) to 3.88 (Daman and Diu). Among those employed in marine fisheries, most of them are active fishermen, whereas 43.75% are involved in secondary sector and 4.80% in tertiary sector.

Table 4. Occupational profile of coastal fisher folk in India (2005)

State	Number of fisher folk engaged in			Dependency ratio	
	Primary Sector	Secondary sector	Tertiary sector	Total	ratio
West Bengal	70,750 *(54.23)*	57741*(44.26)*	1,968*(1.51)*	130,459*(100)*	2.07
Orissa	121,282*(41.94)*	152,534*(52.75)*	15,359*(5.31)*	289,175*(100)*	1.56
Andhra Pradesh	138,614*(46.17)*	152,892*(50.92)*	8,727*(2.91)*	300,233*(100)*	1.70
Tamil Nadu	206,908*(63.81)*	104,509*(32.23)*	12,817*(3.95)*	324,234*(100)*	2.44
Pondichery	10,341*(46.72)*	10,095*(45.61)*	1697*(7.67)*	22,133*(100)*	1.94
Kerala	140,222*(62.43)*	71,074*(31.64)*	13,310*(5.93)*	224,606*(100)*	2.68
Karnataka	37,632*(41.43)*	45,699*(50.31)*	7,500*(8.26)*	90,831*(100)*	1.88
Goa	2,515*(39.30)*	3,382*(52.85)*	502*(7.84)*	6,399*(100)*	1.67
Maharashtra	72,074*(43.79)*	81,780*(49.69)*	10725*(6.52)*	164,579*(100)*	1.94
Gujarat	83,322*(49.36)*	75,082*(44.48)*	10,390*(6.16)*	168,794*(100)*	1.91
Daman and Diu	5,868*(77.73)*	1,603*(21.23)*	78*(1.03)*	7,549*(100)*	3.88
Total	889,528*(51.45)*	756,391*(43.75)*	83,073*(4.80)*	1,728,992*(100)*	2.04

* Figures in parenthesis denote percentage to total

Structural changes in fishing fleets and ownership pattern

There is a definite trend of decline of non-mechanized boats in recent years. However, there is a clear increase in motorized and mechanized boats due to their better technical efficiency. In mechanized sector itself, growth rate of trawlers is increasing at a faster rate, especially boats with OAL of 15 m and above, suited for multiday fishing. Many of our existing mechanized boats have now started operating even beyond 100 m depth resorting to multiday fishing, and the current trend is to go for higher OAL fitted with engines of higher horsepower.

Table 5. Growth rate of marine fishing units in India (1961–1962 to 2004–2005)

Year	SECTOR							
	Non mechanized		Motorized		Mechanized		Total	
	Number	Growth Rate (%)	Number	Growth Rate (%)	Number	Growth Rate (%)	Number	Growth Rate (%)
1961-62	90424	—	0	—	0	—	90424	—
1973-77	106480	18	0	—	8086	—	114566	27
1980-81	137000	29	0	—	19013	135	156013	36
1993-94	182096	33	26171		34571	82	216667	39
1997-98	160000	-12	32000	22	47000	36	239000	10
2004-05	104270	-35	75591	136	58911	25	238772	-0.10

The trends indicate the possible phasing out of non-mechanized units at least in certain regions, which ultimately reflected a negative growth of 35% during 1997–1998 to 2003–2004 (Table 5). This downtrend is compensated in the motorized sector implying large-scale motorization of existing traditional crafts. When the technical efficiency of a particular gear is better than the other, the lesser efficient gears gradually disappear from the operation (Sathiadhas 1998). Mechanized units displayed a major boom during 1980s and 1990s. The growth rates were 135% and 147%, respectively, in 1980 and 1997 due to diversification and extended area of operation. However, the growth rate of mechanized crafts has reduced to the level of 25% in 2005 (Table 6).

Table 6. Ownership of fishing units per active fishermen/fishermen households in India

Particulars	1961-62	1973-77	1980	2005
Total number of units	90424	114566	144030	238772
Active fishermen	229354	322532	437899	1247820
Ownership by active fishermen	39	36	33	19

Source: Marine Fisheries Census of CMFRI, 1961–1962, 1973–1977, 1980, and 2005

In the open access marine fisheries, mode of ownership on means of production by fisher folk greatly influences the occupational pattern and socio-economic status. In India, hardly 19% of active fishermen in marine fisheries sector have ownership on craft and gear in 2005 (Table 6). The proportion of owner operators in marine fisheries declined over the years with the increasing capital requirement for motorized and mechanized fishing units. The ownership of craft and gears by fisher folk declined over the years from 39% to 33% (1961–1962 to 1980), and it has sharply reduced to 19% in 2005. Currently, 14% in mechanized sector, 19% in motorized sector, and 25% in traditional sector have ownership of crafts and gears. This phenomenon is not only due to increasing capital requirement but also due to low disposable income available with fisher folk for investment. Most of the non-motorized units operate as family enterprises not even realizing the full operating cost.

Increasing Capital Investment in Fisheries sector

Capital investment in marine capture fishery comprises of investment in fishing equipments, which includes hull, engine, gears, and other accessories. The gross capital investment on marine capture fishing sector during 2004–2005 is estimated to be Rs.11,328 crores comprising Rs.9724 crores in mechanized, Rs.1009 crores in motorized, and Rs.595 crores in non-mechanized sector (Table 7).

Table 7. Estimated capital investment in crafts and gears in India (1997, 1998 and 2005)

Category	Investment (Rs. Crore)	
	1997–1998	2004 - 2005
a) Mechanized sector		
Trawlers	1879	8289
Purse-seiners	134	189
Gillnetters	255	725
Dolnetters	49	258
Others	72	263
Sub total	**2388**	**9724**
b) Motorized sector		
Dugout canoes	31	13
Catamarans	48	89
Plank-built boats	188	455
Others	188	452
Sub total	**456**	**1009**
b) Nonmechanized		
Dugout canoes	218	46
Catamarans	236	141
Plank Built Boats	420	396
Others	49	12
Sub total	**923**	**595**
Deep sea fishing vessels	**350**	-
TOTAL	**4117**	**11328**

In India, the ownership of fishing equipments was mostly in private sector. The per capita investment on fishing equipments per active fisherman worked out to Rs. 2,25,651 in 2005 in mechanized sector compared with Rs. 1,25,689 in 1997–1998 (Table 8). In the motorized sector, the per capita investment per active fisherman declined from Rs. 26,835 in 1997–1998 to Rs. 25,126 in 2005. In case of non-mechanized sector, the per capita investment marginally increased to Rs.14,266 in 2005 from Rs.13,979 in 1997–1998. This can be attributed to the increased proportion of fiber coating on the existing traditional crafts, purchase of FRP boats, and marine plywood boats.

Table 8. Per capita investment on fishing equipments per active fishermen in India – 1997–1998 and 2005 (Rs.)

Sector	1997-1998*	2005
Mechanized	1,25,689	2,25,651
Motorized	26,835	25,126
Nonmechanized	13,979	14,266
Overall	40,363	90,654

Economics of different types of Fishing Units

Estimated costs and earnings of different craft-gear combinations are given in Table 9. Among the mechanized category, purse seines with 15 mt OAL engaged in multiday fishing (2–5 days) had the highest net operating income per trip (Rs. 42,382) and gross earnings (Rs. 1,15,025). Similarly, the trawlers with single-day operation had the lowest operating income (Rs. 537) among mechanized sector. Among trawlers, the highest gross earnings and net operating income was reported from multiday units (6 and above days). Among gillnets, multidays units (6 and above) also reported high earnings.

Within the motorized sector, canoes with ring seines had the highest and plank-built boats with gillnet had the lowest net operating income per trip. Catamarans with hooks and lines that operate with minimum costs (Rs. 420) had a lower net income (Rs. 150) in the non-mechanized sector. Dugout canoes/shore seines had the highest income (Rs. 1,250) among non-motorized category. On an average, almost all types of fishing units have a surplus net operating income. However, in each category, there is a number of less efficient units running on losses. Further, for non-mechanized (traditional sector) units, the major component of the operating cost is wages to laborers, which is usually shared depending on gross revenue.

Per Capita Earnings of a Fishing Laborer

The per capita earnings of a fishing laborer in a year is given in Table 10. It can

Table 9. Costs and earnings of different craft gear combinations per trip (2003–2004)

Type of craft-gear combination	OAL (mt)	Gross Earnings (Rs)	Operating Costs (Rs)	Net Operating Income (Rs)
Mechanized				
Trawlers				
Single day units	12	2474	1937	537
Multiday units (2–5 days)	14	23351	17648	5703
Multiday units (6 and above)	15	44575	27934	16641
Gillnetters				
Single day units	10	2564	1072	1492
Multiday units (2–5 days)	13	21054	14716	6338
Multiday units (6 and above)	14	61870	40150	21720
Purseseiners				
Single day	10	34682	13548	21134
Multiday units (2–5 days)	15	115025	72643	42382
Dolnetter (Single Day)	13	2586	1231	1355
Motorized				
Plankbuilt Boats with gillnet	8	1950	1470	480
Canoes with gillnets	9	6590	5500	1090
Fiber-glass boats with gillnet	10	1490	940	550
Catamarans with gillnet	10	3530	3000	530
Canoes with ring seines	8	24000	20000	4000
Canoes with minitrawl	7	1720	1100	620
Fiber-glass boats with hooks and lines	8	2380	1160	1220
Dingi/bag net units	10	2450	1500	950
Non mechanized				
Catamarans with gillnet	4	735	525	210
Fiber-glass boats with gillnet	9	900	575	325
Dugout canoes/Shoreseines	8	7500	6250	1250
Catamarans with hooks and Lines	4	570	420	150

be observed that the physical productivity of worker per unit of capital invested has declined steeply, which is a phenomenon characteristic of the open access resources subject to increased commercialization (Kurien & Paul 2001). The annual per capita earnings of fishing laborers was the highest for purse seines (Rs.1,27,200) engaged in multiday fishing (2–5 days) and lowest for trawlers of same category (Rs.16,800). Although per day earnings per trip were the lowest for single-day trawlers (Rs.120), their annual earnings were higher than multiday trawler units as they could operate 240 trips in a year (28,800). In case of gillnetters, the annual per capita earnings of the single day units were higher than that of multiday units, although the per capita earnings of multiday units in this category worked out to be the second highest among the

mechanized units.

Table 10. Per capita earnings of a Fishing Laborer (2003–2004)

Type of craft-gear Combination	Earnings Per trip (Rs)	No. of trips	Annual per capita earnings (Rs)
	Mechanized		
Trawler			
Single day	120	240	28800
Multiday units (2–5 days)	280	60	16800
Multiday units (6 and above)	650	36	23400
Gillnetters			
Single day	300	240	72000
Multiday units (2–5 days)	350	60	21000
Multiday units (6 and above)	1680	36	60480
Purseseiners			
Single day	500	240	120000
Multiday units (2–5 days)	2120	60	127200
Dolnetter/Dol net (Single day)	90	240	21600
	Motorized		
Plankbuilt Boats/gillnet	194	230	44620
Country crafts/gillnets	200	220	44000
Fibreboats/gillnet	100	240	24000
Catamarans/gillnet	150	200	30000
Countrycrafts/ring seines	100	200	20000
Countrycrafts / minitrawl	75	180	13500
Fiberboats/hooks and lines	100	240	24000
Dingi/bag net	60	220	13200
	Nonmechanized		
Catamarans with gillnet	200	200	40000
Dugout canoes/Shore seines	100	180	18000
Country crafts with gillnets	120	240	28800
Catamarans with Hooks and Lines	80	240	19200

Among the motorized fishing units, plankbuilt boats/gillnets had the highest annual per capita earnings (Rs. 44,620) and the lowest was recorded for dingi/bag net units (Rs. 13,200). Catamarans with gillnet fetched Rs. 40,000 as gross per capita earnings in the year in the nonmechanized (traditional) sector.

Intersectoral disparities in marine fisheries sector

Every 100 kg of fish produced from marine fisheries provide full-time employment for 20 persons in the harvesting sector and another 24 persons in the postharvest sector and one person in the tertiary sector. Earlier studies (Sathiadhas et al. 1997) confirmed

that altogether 10.2 lakhs people are involved in active fishing and 12 lakhs people are involved in preharvest and postharvest sector of marine fisheries during 1995. During 2003–2004, 12.20 lakhs people were employed in active fishing in the primary sector and another 15 lakhs in the preharvest and postharvest sector in the secondary sector, and one lakh people were employed in the tertiary sector. The recent estimates have confirmed that 12.49 lakhs people are employed in the primary sector. The Marine Fisheries Census of CMFRI, 2005 has estimated that 8.89 lakhs people are involved in active fishing alone in the coastal villages of India. According to the Marine Fisheries Census of 2005, there are 58911 mechanized units, 75591 motorized units, and 104270 nonmechanized (traditional) units. Average number of sea faring persons is 6 in a trawler, 9 in a Gillnetter, 20 in purse seiners and liners, 5 in dolnetters, and 9 in others. In case of motorized units, the number of persons range from 2 in a motorized catamaran to 40 in shore seiners, and the average number is assumed to be 5. In case of nonmechanized (traditional sector) units, number of crew varies between 2 for catamaran to 44 for shore seiners. The average number of crew in nonmechanized (traditional sector) craft is assumed to be 4. Thus, it is estimated that additional 3,60,060 persons are also involved in active fishing from adjacent areas to the coastal belt.

The proportion of catch by mechanized sector as a whole increased from 40% during 1980 to 68% in 1997 and again declined to 66% in 2003 (Table 9). Currently, the share of mechanized sector is 70% of the catch. At the same time, the number of active fishermen depending on mechanized fisheries increased from 1.14 lakhs to 2 lakhs and again increased to 4.3 lakhs, respectively, during the same period. It should be noted that the annual per capita production of active fisherman during the period has initially increased from 5260 kg in 1980 to 8130 kg in 1997 and declined to 4175 in 2003 and 3701 kg in 2005. It is highly evident that the increase in share of production in the sector is taken away by the increase in number of crafts and proportionate increase in the number of fisher folk depending on the sector. The annual average production per unit has come down to an all time low value of 27 tonnes. This clearly indicates the high prevalence of disguised unemployment in the mechanized fisheries sector.

In motorized segment also, the similar trend was observed. The annual production per unit is declining over the years from 13 tonnes in 1997–1998 to 7 tonnes in 2005. In case of annual per capita production per active fisherman, it almost halved to 1320 kg in 2005 from 2390 kg in 1997–1998. The ownership of means of production per active fisherman regained its earlier position in 2005 (19%) after a decline to 12% in 2003–2004. The nonmechanized (traditional sector) segment has experienced significant reduction in the share of production as well as gross earnings. The share of nonmechanized (traditional sector) sector in marine fish production reduced from 60% in 1980–1981 to 7% in 2005. Similar trend was observed in case of average annual production with a decrease from 6.57 tonnes in 1980–1981 to 1.6 tonnes in 2005. The

Table 11. Structural changes in socio-economic parameters in non-mechanized, motorized, and mechanized sector (1980–1981 to 2005)

Item	1980 –1981	1997 –1998	2004 –2005
Mechanized			
Marine fish production (%)	40	68	70
Average annual production per unit (in tonnes)	32	33	27
Annual per capita production/active fishermen (in Kg)	5260	8130	3701
Ownership of means of production by active fishermen (%)	17	24	14
Active fishermen	114000	200000	430931
Motorized			
Marine fish production (%)	—	19	23
Average annual production per unit (in tonnes)	—	13	7
Annual per capita production/active fishermen (in Kg)	—	2390	1320
Ownership of means of production by active fishermen (%)	—	19	19
Active fishermen	—	170000	401577
Nonmechanized (traditional sector)			
Marine fish production (%)	60	13	7
Average annual production per unit (in tonnes)	6.57	1.7	1.6
Annual per capita production/active fishermen (Kg)	2590	420	408
Ownership of means of production by active fishermen (%)	39	25	25
Active fishermen	348000	650000	415312
Total			
Average annual production per unit (in tonnes)	9.6	9.6	9.6
Annual per capita production/active fishermen (in Kg)	3247	2254	1837
Ownership of means of production by active fishermen (%)	34	23	19
Active fishermen	462000	1020000	1247820

annual per capita production per active fisherman suffered utmost decline from 2590 kg in 1980–1981 to 408 kg in 2005. There has been a slight increase in ownership of means of production by active fishermen in 2005 (25%) after a steep decline from 39% in 1980–1981 to 21% in 2003–2004. The pressure for employment in active fishing is increasing more than proportionate to the harvestable yield in the open access marine fisheries. The fishermen involved in fishing is more than the absorbing capacity and has led to lower per capita production, juvenile fishing, and large scale discards and causes serious threats to resource sustainability and environmental stability. Further intensive mechanization in the marine sector has led to increase in production but has ultimately marginalized the traditional nonmechanized (traditional) sector. There is a wide disparity in income between those engaged in different sectors. It may be noted that still nonmechanized sector is providing about 33% of the employment in active

fishing, yet harvesting hardly 7% of the annual landings, whereas mechanized segment, which employs 34% harvests 70% of total catch. The annual per capita catch of fisher folk in mechanized segment is more than twice as that of the per capita catch of the motorized segment and nine times of the per capita catch of the nonmechanized (traditional sector) segment. These phenomenon results in marginalization of the indigenous nonmotorized sector by the motorized and mechanized sectors and frequently create conflicts among fishers.

Employment in fisheries sector has undergone rapid structural changes during the last few decades. Among those engaged in the mechanized sector, 75% work in trawl fisheries and the remaining 25% in other sectors. In the case of motorized sector, 50% are engaged in ring seine fishery alone. There is a wide intrasectoral disparity in income between those engaged in various craft gear combinations within each sector. The number of annual fishing days per worker reveals that the level of employment for hired laborers as well as those not having sufficient equipment is low and they are very much underemployed. The seasonal nature of fishery and the risk and uncertainties associated with marine fishing entangled the fishermen in the low-income trap. The poor economic condition coupled with the less availability of finance from the institutional agencies compel them to sustain with less equipped fishing implements, which in turn results in diminishing returns (Table 11).

Conclusion

Marine fishing industry in India has continuously recorded increase in private capital investment. The private capital investment on fishing equipments alone increased from about Rs. 4117 crores in 1997–1998 to Rs. 11, 328 crores in 2004–2005. The labor class in active fishing is increasing more than proportionate to their demand resulting in disguised unemployment. It is seen that hardly 19% of the active fishermen in India have ownership of fishing implements. Inequitable distribution of income is continuously increasing, further widening the gap between the rich and poor in the coastal economy. Along with the mounting inequity in harvesting open access resources, there are constraints like depletion of resources necessitating conservation strategies to sustain the marine wealth. In this context, policy interventions are essential to provide alternative avocations in agriculture, aquaculture, and other coastal-zone-based employment opportunities instead of increasing pressure to harvest more and more marine fish resources. Finance plays a crucial role in accelerating any business activity/economic development, and fisheries sector is not an exception. The extent of indebtedness and the average outstanding debt per indebted households are comparatively less among fishermen as per the figures of institutional sources, but the affairs of the fisher folk are really grim as they are virtually gripped in the hands of noninstitutional agencies, namely the money lenders and traders for which legitimate data sources do not exist. This is because of the inherent problems in the functioning of the institutional agencies, which need to be reviewed. Special coastal area development programs offering easy credit

availability for entrepreneurial activities for the surplus labor may check the disguised unemployment, intersectoral disparity, and poverty among the coastal fisher folk in India.

Acknowledgements

I hereby express my sincere thanks to Prof. (Dr). Mohan Joseph Modayil, Director, CMFRI, Kochi, for his kind encouragement and support for preparation of this article.

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Received: 31 December 2007; Accepted: 11 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Analysis of Seasonal Variation of Indian Frozen Shrimp in the European Union Market

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Abstract

Fish, particularly shrimp, is becoming a staple food in the diet of the Europeans. The boost in aquaculture production has led to enhanced frozen shrimp exports from India during the past decade. After Japan and US, European Union (EU) has been found to be an emerging market for Indian frozen shrimp products. During 2006, the value of frozen shrimp exports to EU was 337.93 million US dollars amounting to 57,554 tonnes in terms of volume.

Analyzing the seasonal pattern in the prices realized by the commodity in an international market will enable strategic planning for maximum gains. This article presents the analysis of seasonal variation in prices according to various size grades of Headless (HL) Black Tiger in the EU shrimp market. The prices realized for HL Black Tiger shrimp exports to the EU during 2006 was in the range of 6.30 to 11.30 $ per kg for the grade 16/20, 5.10 to 10.00 $ per kg for the grade 21/25, and 2.95 to 9.80 $ per kg for the grade 26/30. A sharp decline in the prices was realized in the export of HL Black Tiger to EU over the past few years. The reasons for the price fluctuations have been identified. The seasonal variation in the prices during 2006 was studied, and it was found that for the popular grades like Black Tiger shrimp, the seasonal indices were high during the third quarter of the year. Lowest prices were realized during the first quarter of 2006. Our export strategy should be based on the seasonal demand for the products with more stress on value addition with an Eco label.

Introduction

India is one of the major shrimp producing countries in the world. The boost in aquaculture production in India during the early 90's has led to enhanced production of shrimp, and Andhra Pradesh is a leading producer of shrimp in India with a production of more than 70,000 metric tonnes during 2005–2006 out of total production of 1.4 lakh metric tonnes during the same year. After Japan and US, European Union (EU) has been found as an emerging market for Indian frozen shrimp products. During 2006, India exported 53,216 tonnes of frozen shrimp to the EU valued at 332.5 million US

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dollars. Belgium is the largest market for Indian frozen shrimp among the EU countries, and during 2006, India had a market share of 18.1% (Anon 2006) at this market. Imports of shrimp by Italy from India have increased to 43% in the current year thereby making India as the leading Asian supplier to Belgium.

More than 90 countries export frozen shrimp to the EU countries. India exports frozen shrimp to 13 countries in the EU. It exports shrimp products in Peeled Deveined (PD), Headless (HL), Cooked, Blanched, and Individually Quick Frozen (IQF) forms. Black Tiger is one of the major varieties of frozen shrimp exported to the EU from various ports of India. Recently, the EU had to reject products of Pakistani origin re-exported by Indian companies. This happened after the EU imposed a ban, for quality reasons, on Pakistani shrimp in March 2007.

The price of a commodity depends on the demand and supply for it in a particular market. Generally, it is observed that over the past few years, there is a sharp decline in prices of frozen shrimp products in all the major markets. The prices of HL Black Tiger of popular grades were analyzed with the aim to understand how the seasonal variation is reflected in the prices of Indian frozen shrimp in the EU market. This article presents analysis of the prices of Indian frozen shrimp exports to the EU market and suggests effective strategic measures that will ensure a steady market for Indian products in EU.

Material and methods

Weekly price data of prices of shrimp traded at the EU shrimp market for different varieties and grades were collected for the period 2001 to 2006 from published data of MPEDA and pooled to get monthly data. Analysis was carried out for the product forms and grades of shrimp where continuous data was available for these years. In addition, data was collected from UN Commodity trade statistics database on quantity and value of frozen shrimp exports. The seasonal variation in prices was studied by calculating the monthly indices for selected grades-16/20, 21/25, 26/30 and 31/40. Seasonal indices were calculated using standard procedure (Krishna Rao 1972; Waugh et al. 1969). In addition, analysis of the prices of HL Black Tiger originating from different ports in various markets was compared using Analysis of Variance technique.

Results and Discussion

There were 95 types of products exported to EU market as frozen shrimp. It was observed that these products were in the forms of HL shrimp, PD shrimp, Peeled Undeveined shrimp (PUD), and IQF shrimp. The major importing countries in the EU

are UK, Belgium, Germany, France, Netherlands, Italy, and Spain. The main ports from which frozen shrimp were exported are Visakhapatnam, Tuticorin, Chennai, Kochi, Mumbai, and Veraval. To the EU market, Brown, Pink, White, and Black Tiger varieties are exported.

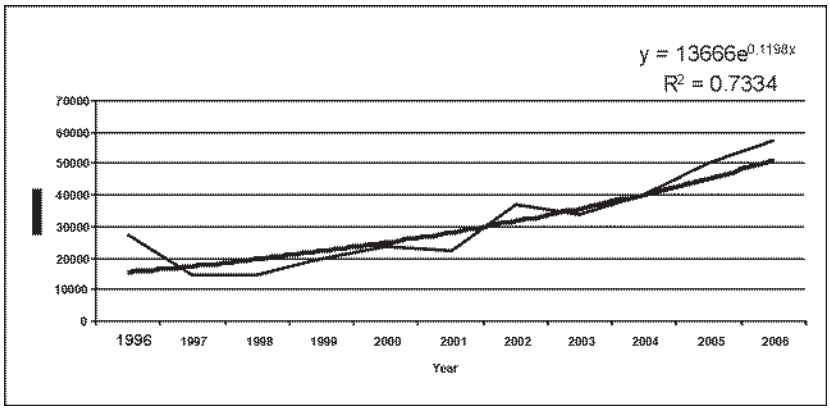

Figure 1. Shrimp exports to European Union.

Fig. 1 shows that the volume of shrimp exports to the EU has increased exponentially ($R^2 = 0.73$) during the past decade. As mentioned earlier, among the EU countries, Belgium is the largest market for Indian frozen shrimp and 32.08% of value realized from out of the total shrimp exports to EU was from this country. The next largest market for Indian frozen shrimp among the EU countries is UK (25.82%) followed by France (10.8%).

The data pertaining to weekly prices of HL Black Tiger shrimp in popular grades realized in the EU market during 2001 to 2006 were compiled. Table 1 gives the monthly average prices in dollar per kg realized by HL frozen shrimp from India in the grade 21/25 exported to EU during 2001 to 2006. It was found that there was a sharp decline in the prices until 2004 and there appears slight stability in 2005. The trend was similar in the case of other popular grades of HL shrimp *viz*., 16/20, 25/30, and 31/40.

It was observed that the price fall was drastic from 2002 onwards. The percentage variation in prices in comparison with previous year was ranging from 23 to 30%.

Similarly, analysis of the prices of frozen shrimp exports in the form of PD in the popular grades *viz.*, 26/30, 31/40, and 41/50 was carried out, and in general, decrease in prices was realized over the years. Table 2 gives the average prices of PD frozen shrimp exported to EU for the popular grade 26/30.

Table 1. Average prices of Headless frozen shrimp (Grade 21/25) exported to EU countries

Month	Average price ($ per kg)					
	2001	2002	2003	2004	2005	2006
January	12.60	8.65	8.97	8.35	7.79	5.60
February	13.04	9.54	9.35	6.93	8.03	-
March	13.56	9.64	8.09	8.00	8.45	-
April	11.92	9.09	8.00	7.11	8.59	9.50
May	12.05	9.25	8.62	7.88	7.82	8.80
June	-	-	8.55	7.25	7.53	7.10
July	7.00	8.77	8.73	8.63	8.48	7.88
August	-	7.50	9.01	7.74	7.69	8.03
September	8.10	8.33	9.26	7.55	8.27	9.60
October	7.47	8.85	8.23	7.88	8.33	-
November	-	9.38	7.66	7.97	7.60	8.69
December	8.13	8.90	5.46	7.96	7.80	9.50

Table 2. Average prices of Peeled Deveined frozen shrimp (Grade 26/30) exported to EU countries.

Month	Average price ($ per kg)					
	2001	2002	2003	2004	2005	2006
January	14.60	10.33	10.05	8.53	8.80	7.1
February	13.90	-	9.30	8.44	9.02	7.32
March	-	-	9.63	8.64	8.98	8.04
April	-	9.02	9.74	8.54	9.26	7.8
May	11.65	9.63	9.14	9.19	9.19	8.92
June	-	10.25	9.18	8.70	8.72	8.9
July	-	9.63	8.78	7.92	7.73	9.5
August	12.30	8.35	9.52	8.76	7.68	8.62
September	-	-	8.98	8.95	7.96	8.93
October	9.40	11.70	7.67	8.70	7.79	9.1
November	8.70	11.37	8.55	8.90	7.81	9.33
December	8.90	11.45	-	9.01	7.68	9.73

The analysis of prices of IQF shrimp in the popular grades 100/200 and 200/500 did not reveal any decrease in prices over the years (Table 3).

Table 3. Average prices of IQF shrimp (Grade 100/200) exported to EU countries.

Month	Average price ($ per kg)				
	2001	2002	2003	2004	2005
January	-	2.10	2.73	4.30	3.50
February	-	-	3.72	-	2.80
March	-	2.10	3.60	3.80	3.72
April	-	2.90	3.68	3.20	4.10
May	3.90	2.35	4.09	2.90	3.47
June	3.74	2.90	3.64	-	3.65
July	3.50	-	3.82	3.31	3.63
August	-	2.82	3.87	3.25	3.99
September	3.45	3.43	3.90	3.47	3.85
October	3.80	3.53	3.10	3.54	3.73
November	-	3.64	4.01	2.96	4.00
December	3.15	-	-	3.10	3.88

A cursory look at the prices of frozen shrimp, in general, in the major forms like HL and PD reveals that a sharp decline in prices is realized over the years. In the case of IQF shrimp, no appreciable change is observed during the past years. The supply of shrimp from developing countries has increased over the years and is exceeding the actual demand leading to fall in prices.

When the antidumping investigations were initiated against the warm water frozen shrimp of Indian origin and five other countries, there was an apprehension that shrimp trade will be affected and there will be an impact on the prices. To explore whether the antidumping investigations and subsequent slapping of duties on Indian shrimp exports to US had any effect on the prices of shrimp realized in the EU market, the average monthly prices of popular varieties of frozen shrimp during 2006 was analyzed. Black Tiger is a popular variety of shrimp exported in large volumes to all the major markets especially in EU and USA. It is exported to EU in HL, PD, PUD and Cooked forms. The monthly prices of HL Black Tiger realized in the EU market during 2006 were computed by pooling the weekly prices for the popular grades. Fig. 2 depicts the pattern of average monthly prices of HL Black Tiger shrimp realized during 2006 in the popular grades 16/20, 21/25, 26/30, and 31/40.

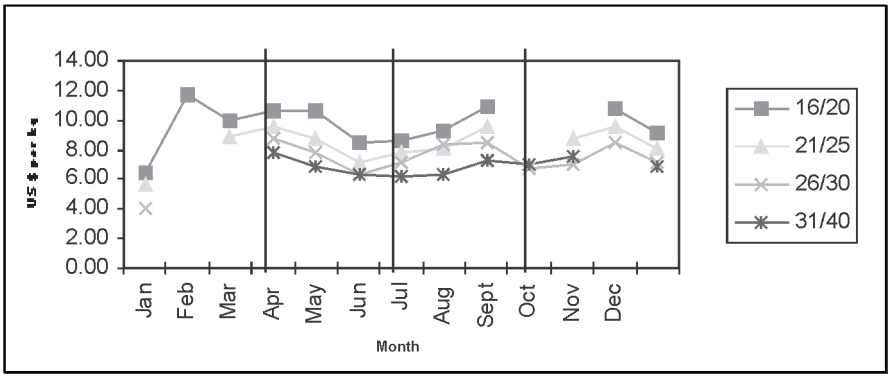

Figure 2. Monthly average prices of HL BT shrimp exports in EU market during 2006

The seasonal indices were calculated for grades 16/20, 21/25, 26/30, and 31/40 of HL Black Tiger shrimp exports to EU. Table 4 gives the gradewise average monthly prices of the Indian frozen shrimp to EU pertaining to the variety Black Tiger exported in the HL form. Table 5 gives the monthly seasonal indices of prices of HL Black Tiger shrimp for the grades 16/20, 21/25, 26/30, and 31/40 pooled over the past five years.

The percentage variation between the maximum and minimum indices ranged from 26 to 38%. The indices were high from February to October for all the grades. Comparatively lower indices were reported during November to January. Fish consumption tends to drop during summer and increase toward the end of the year-possibly because of the tradition of eating fish in the Christmas holidays. However, contrary to what one might expect, the prices fell during this period.

For the grade 16/20, the seasonal indices varied from 0.87 to 1.12, the maximum being during the month of April. For the grade 21/25, the maximum index was computed during April, and it varied from 0.8 to 1.12. In addition, in the case of grade 26/30, the maximum index was computed during the month of April and the range of the seasonal index was from 0.84 to 1.21. Seasonal index varied from 0.90 to 1.27 in the case of grade 31/40 with maximum computed during October.

It was observed that during 2006, average prices of frozen shrimp exported from Chennai and Tuticorin was $4.53 per kg and $4.3 per kg, which was more than the prices realized from Kochi, Kollam and Mangalore ports ($3.59, $3.25 and $3.9 per kg, respectively). The fact that products from these ports are able to bargain better prices has to be further explored.

From the data, it was observed that in the popular grades 16/20, 21/25, 26/30 and 31/40, the prices of HL Black Tiger shrimp exports to various countries varied significantly ($p < 0.05$). In addition, the port of origin of the export had also significant impact on the prices ($p < 0.01$) with $R^2 = 0.93$. Particularly, higher prices were realized from UK compared to Belgium during the same period.

Table 4. Average monthly prices of Indian frozen shrimp (Black Tiger) in EU (US $ per kg)

	January	February	March	April	May	June	July	August	September	October	November	December	Overall
	8.84	10.11	10.61	10.60	9.51	9.13	9.64	9.44	9.30	8.93	9.40	8.20	9.45
16/20	(±3.19)	(±2.98)	(±0.90)	(±0.28)	(±1.78)	(±1.72)	(±1.83)	(±1.85)	(±1.23)	(±2.30)	(±0.00)	(±2.77)	(±2.23)
	8.03	8.80	9.32	9.50	8.43	7.86	8.88	8.28	9.00	9.47	6.83	7.79	8.5
21/25	(±2.46	(±2.79)	(±1.15)	(±0.62)	(±1.08)	(±1.45)	(±1.36)	(±1.76)	(±1.02)	(±2.13)	(±2.63)	(±2.23)	(±1.86)
	6.74	8.73	8.33	9.38	7.80	6.52	7.77	8.04	7.39	8.15	7.44	6.93	7.73
26/30	(±2.50	(±1.39)	(±1.03)	(±3.00)	(±1.00)	(±1.22)	(±1.03)	(±0.93)	(±2.14)	(±1.83)	(±1.68)	(±2.00)	(±1.80)
	6.06	6.96	7.26	6.71	7.30	6.39	6.88	6.66	6.79	8.60	6.73	6.09	6.76
31/40	(±2.46	(±2.50)	(±0.84)	(±2.16)	(±0.70)	(±1.00)	(±0.85)	(±0.98)	(±0.72)	(±2.83)	(±1.09)	(±1.95)	(±1.52)

- Figures in parentheses indicate standard deviation

Table 5. Seasonal indices of prices of Indian frozen shrimp (Headless Black Tiger)

	January	February	March	April	May	June	July	August	September	October	November	December
16/20	0.94	1.07	1.12	1.12	1.01	0.97	1.02	1.00	0.98	0.95	0.99	0.87
21/25	0.94	1.04	1.10	1.12	0.99	0.92	1.04	0.97	1.06	1.11	0.80	0.92
26/30	0.87	1.13	1.08	1.21	1.01	0.84	1.01	1.04	0.96	1.05	0.96	0.90
31/40	0.90	1.03	1.07	0.99	1.08	0.94	1.02	0.99	1.00	1.27	0.99	0.90

Moreover, for HL Black Tiger exports, a comparison of prices realized from USA, Japan, and EU revealed that Japan has always offered competitive prices. EU imports shrimp of a much lower price in comparison with the other markets ($p < 0.05$). This can be explained by the fact that EU imports more of coldwater shrimp, which is generally smaller and lower in price than the tropical shrimp. Fig. 3 gives the monthly average prices of HL Black Tiger shrimp exports to the major markets during 2006 for the grade 16/20.

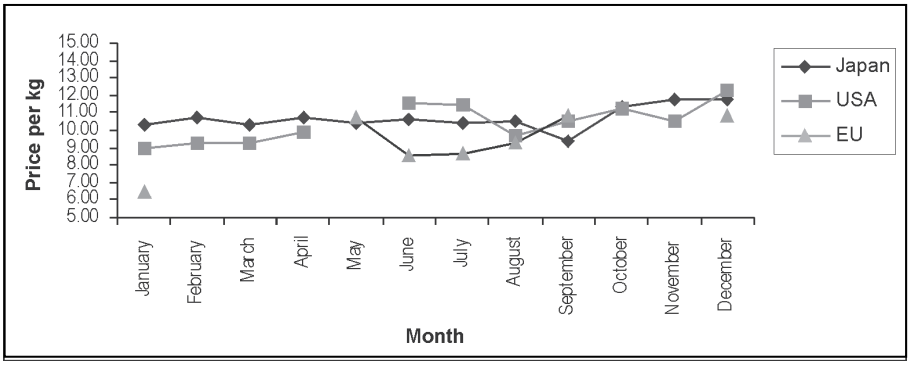

Figure 3. Monthly average prices of HLBT shrimp exports at major markets Grade 16/20 during 2006.

Conclusion

The EU is India's largest trading partner in seafood exports. The EU process regulations are very stringent and the exporters incur additional costs to abide by these regulations. In spite of this, India is able to export large quantities of seafood to the EU during the last few years. However, it is seen that shrimp trade has not grown in value terms in the last years. This is because the supply exceeds demand. There are some opportunities to improve the prices realized such as diversification of markets and more exports of value-added shrimp like butterfly cut, ring presentation, and coated products. Moreover, highlighting the quality of the product through a geographical denomination of origin or ecolabeling will certainly boost up prices. EU market depends on the value of Euro and future economic growth. At present, Euro is strong on US dollar making the EU market attractive for trade. Newer markets like Southeast Asia, Latin America, and China have to be explored. For example, China is the seventh largest shrimp importer and some of these shrimp goes for reprocessing but an increasing share stays in the country.

The prices of Indian frozen shrimp to EU exhibit a seasonal pattern. The seasonal demand for the product must be taken into account while developing marketing strategies so as to realize better prices. Increased domestic demand should be kept in mind while

sending various products into a market. It is also necessary to maintain stringent quality standards to compete with other supplying countries and for being able to sell our product at a premium. India does not have a single seafood ecolabel. Measures should be taken to ensure that Indian products go with a single ecolabel to obtain a sustainable price premium. Market research on consumer's tastes and preferences could further improve India's recent export performance.

Finally, the phenomenon of brand names in the retail trade in frozen fish products should be kept in mind. There is a dual tendency: on one hand, there is one brand name that captures almost half the sales; on other hand, distributor's brands (or no-name products) also account for a very high percentage.

Acknowledgements

The authors are grateful to Director, CIFT for granting permission to present the paper in the 8th Asian Fisheries Forum, Cochin.

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Received: 31 December 2007; Accepted: 06 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Controlled Breeding and Larval Rearing Techniques of Marine Ornamental Fishes

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Abstract

International trade of marine ornamental fishes has been expanding rapidly in recent years, and the fact that nearly 98% of the species traded are collected from reef habitats is of vital concern for the conservation of the fragile coral reef ecosystem. Hence, it is widely accepted that the ultimate answer to a long-term sustainable trade of marine ornamental fishes is only through the development of hatchery production technologies. The techniques for broodstock development, breeding and seed production of three species of damsel fishes *viz.* the three spot damsel, *Dascyllus trimaculatus*, the humbug damsel, *Dascyllus aruanus* and the blue damsel, *Pomacentrus caeruleus*, were developed and standardised, which can be scaled up for commercial level production. Broodstock development was done in one-tonne Fiber Reinforced Plastic (FRP) tanks with biological filter and by feeding with natural feeds. The size range of broodstock fish of *D. trimaculatus*, *D. aruanus* and *P. caeruleus* were 9-10, 7-8 and 7-9 cm, respectively. The number of eggs per spawning ranged from 5000 to 15000. The interval between two successive spawnings ranged from 3 to 14 days. The eggs were attached either on the sides of the broodstock tank or on the substratum provided in the broodstock tank. Parental care by the male was noted. Hatching occurred on the evening of the fourth day of incubation. The larvae were altricial type with no mouth opening at the time of hatching for *D. trimaculatus* and *D. aruanus*. The larvae of *P. caeruleus* were with mouth opening at the time of hatching. The length range of newly hatched larvae was 1.5-2.5 mm and the range of mouth opening was 150-200 µ.

Larviculture was done in five-tonne capacity FRP tank by employing greenwater technique. Copepod nauplii were used as the starter feed and after about two weeks when the mouth opening of the larvae had reached around 450 µ, newly hatched *Artemia* nauplii were supplemented. The metamorphosis period ranged from 20 to 40 days. Several batches of the three species were hatchery produced, and the technique can be scaled up for commercial level production for ornamental fish trade.

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Introduction

It has been reported that nearly 1500 species of marine ornamental fishes are traded globally, and most of these species are associated with coral reefs. Nearly 98% of the marine ornamental fishes marketed are wild collected from coral reefs of tropical countries. The fishing methods, which destroy the fragile corals, and over harvesting of the species in demand are the vital problems associated with the trade. It is widely accepted that the ultimate answer to a long-term sustainable trade of marine ornamental trade can be achieved only through the development of hatchery production technologies (Colette et al. 2003). In this context, it is imperative to develop commercially viable seed production techniques of important species, which are in demand for the long-term sustainability of the trade.

Among the commercially traded families of reef fishes, Pomacentridae dominate, accounting for nearly 43%. The damsels contain approximately 235 species worldwide (Allen 1991). The most widely traded pomacentrids in the international market in the recent past include the humbug damsel (*Dascyllus aruanus)*, the three spot damsel (*Dascyllus trimaculatus*) and the blue damsel (*Pomacentrus caeruleus)*. Methodologies can be scaled up to commercial level production for breeding; seed production of these three species of damsel fishes was developed and several trials of seed production were carried out.

Materials and Methods

Broodstock development

Fishes collected (6-8 nos) by traps were introduced in one-tonne FRP tanks for broodstock development. These tanks were fitted with biological filters to maintain the water quality to the optimum level. The filtration rate was about 200 $l.hour^{-1}$. The range of water quality parameters of the broodstock tanks were as follows:

Temperature –25ºC-29.5ºC	pH – 8.3-8.6
Salinity – 28-31 ppt	Dissolved oxygen – 4.5-5.1 $mL.l^{-1}$

Water in the broodstock tanks was exchanged @ 30% once in a week. The broodstock tanks were kept under translucent roofing to reduce the light intensity. Feeding of the fishes was done once in a day @ 5-10% of the body weight. Finely chopped fishes, shrimps and molluscan meat were given as feed to the broodstock fishes. Substrata were provided in the broodstock tanks for the attachment of eggs during spawning.

Live feed culture

Live feeds such as microalgae and copepods were cultured separately to maintain required densities of greenwater and zooplankton in the larval rearing tanks to feed the

damsel fish larvae during initial larval phase. Pure cultures of microalgae *Nanochloropsis* sp. were maintained in indoor culture rooms by employing standard methods. These cultures were then scaled up in outdoor algal production facility to the required volume.

Hatching and larval rearing

The substratum with egg clutch was transferred to the larval-rearing tanks containing seawater having the same physicochemical characteristics of the parent tank. A gentle airflow was created over the eggs by placing an air stone near to the egg clutch, and egg clutch was left in darkness. Hatching took place on the night of third day of incubation. In some cases, the eggs were hatched in the broodstock tank, and the newly hatched larvae were introduced into larviculture tanks. Larval rearing was carried out in five-tonne FRP tanks. The inner side of the tank was light blue in colour in order to have a better contrast between the live feed and the surroundings. The range of water quality parameters in the larviculture system were as follows:

Temperature – 27ºC-31.5ºC pH – 7.5-8.6

Salinity – 28-34 ppt; Dissolved oxygen – 4.5-5.1 $mL.L^{1}$

Greenwater technique using microalgae *Nannochloropsis* was adopted for the larval rearing of damselfishes. The adults of two species of copepods *viz.*, *Euterpina acutifrons* and *Pseudodiaptomus serricaudatus* were inoculated into the greenwater at a density of 50 l^{-1}. When the copepods have started their growth phase, as was noted by counting the number of egg-bearing copepods and nauplii per 50 mL, the newly hatched larvae were introduced into these tanks. Approximately 2000 larvae of each species were introduced into the respective tanks. Larviculture was done in water with different cell counts of microalgae and the larval survival was noted on 20 day of post hatch (dph) (in the case of blue damsel on 15 dph). One set of experiment was conducted by employing copepods alone as live feed and another set was conducted by employing copepods and rotifers together as live feed. The density of live feeds in the tanks was examined everyday and adjusted to the desired level by adding from the cultures, maintained separately. The range of values given under each set is based on the results of three trials.

Results

Broodstock development and spawning

All the three species of fishes spawned in captivity after 4-8 months of maintenance in the broodstock tanks. Prior to spawning, the parent fishes actively cleaned the site for attaching the eggs by rubbing it with their pelvic fins and picking off any loose particles or algae with their mouths. During spawning, females attached their eggs on the cleaned site, which were immediately fertilised by the males. Spawning occurred

during the morning hours. The development of egg took place in 3 days at 28˚C. During this period, the parent fishes took care of the eggs by protecting them and by fanning them with the pectoral fins and tail.

D. trimaculatus and *D. aruanus* are dioecious, and the mature fish ranged in size 9-10 cm and 7-8 cm total length (TL), respectively. In a single spawning, 8000-10000 eggs in the case of the former and 12000 to 15000 eggs in the case of latter were present. The eggs were attached either to the sides of the tanks or on the substrata provided inside the broodstock tanks. The average periodicity of spawning was 2 weeks. Parental care by the male was noted. The eggs were oval in shape.

P. caeruleus is protogynous and polygamous. The mature fish ranged in total length from 7 to 9 cm. Approximately 5000-6000 eggs were present in a single spawning. The eggs were attached on the substrata provided inside the broodstock tanks. The average periodicity of spawning ranged between 3 and 12 days. Parental care by the male was noted. The eggs were oval in shape.

Larval rearing

D. trimaculatus & *D. aruanus:* Larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larvae was 2.5 mm and 2.4mm, respectively. The larvae were transferred to five-ton capacity round FRP tanks in which mixed culture of two species of copepods *viz.*, *P. serricaudatus* and *E. acutifrons* were maintained in greenwater. Mouth opening was formed on the second day, and the gape measured around 150 µm in *D. trimaculates* and 160 µm in *D. aruanus*. The larvae started feeding from the third day of hatching. The results of the larviculture systems experimented with copepods as live feed and the combination of copepods and rotifer as live feeds are given in Tables 1-4. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the greenwater was maintained at a range of 1 x 10^5 cells - 6 x 10^5 cells mL^{-1}. After 20 days when the average size of the larvae had reached around 4 mm with average mouth gape of around 450 µm, freshly hatched *Artemia* nauplii were fed *ad libitum*. Thereafter, no mortality was noted. The larvae started metamorphosing from 35^{th} day of hatching and all the larvae metamorphosed by 40^{th} day. The just metamorphosed young one measured from 12 to 13 mm in length. In the case of *D. aruamus*, the metamorphosis started from 25 dph and completed by 31 dph, young ones measured 8.0-8.5 mm in length.

Table 1. Larviculture of *D. trimaculatus* with copepods

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos. mL^{-50}	Range of nauplii nos. mL^{-50}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	1-2	2-4	0-1%
2	$1x10^5$ - $6x10^5$	7-97	35-203	3-4%
3	$1x10^6$ - $6x10^6$	2-4	2-6	0-2%

Table 2. Larviculture of *D. trimaculatus* with combination feed of copepods and rotifers

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Range of rotifers nos. mL^{-1}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	Nil	Nil	2-8	Nil
2	$1x10^5$ - $6x10^5$	Nil	Nil	4-14	Nil
3	$1x10^6$ - $6x10^6$	Nil	Nil	8-20	Nil

Table 3. Larviculture of *D. aruanus* with copepods as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg bearing copepods nos. mL^{-50}	Range of nauplii nos. mL^{-50}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	1-2	2-5	0-1%
2	$1x10^5$ - $6x10^5$	1-109	3-273	3-8%
3	$1x10^6$ - $6x10^6$	1-4	2-8	0-3%

Table 4. Larviculture of *D. aruanus* with copepods and rotifers as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Range of rotifers nos. mL^{-1}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	Nil	Nil	2-10	Nil
2	$1x10^5$ - $6x10^5$	Nil	Nil	6-12	Nil
3	$1x10^6$ - $6x10^6$	0-1	Nil	10-20	Nil

***P. caeruleus**:* The newly hatched larvae measured approximately 1.2 mm with an average mouth gape of 200 µ. The larvae were transferred to five-tonne capacity FRP tanks in which greenwater was developed and a mixed culture of copepods (*P. serricaudatus* and *E. acutifrons*) was maintained. The results of the larviculture trials with copepods and combination of copepods and rotifer as live feeds are given in Tables 5 and 6, respectively. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the greenwater was maintained at a range of 1 x 10^5 cells-6 x 10^5 cells mL^{-1}. After 15 days, freshly hatched *Artemia* nauplii were also supplemented. Thereafter, no mortality was noted. The larvae started metamorphosing from the 17th day and by 21st day all of them metamorphosed. The average length of just metamorphosed juvenile was 21 mm.

Table 5. Larviculture of *P. caeruleus* with copepods as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Larval survival (20 dph)
1	1 x 10^4 - 6 x 10^4	0-2	0-2	0-1%
2	1 x 10^5 - 6 x 10^5	7-41	23-132	3-4%
3	1 x 10^6 - 6 x 10^6	2-4	1-4	0-2%

Table 6. Larviculture of *P. caeruleus* with combination of copepods and rotifers as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Range of rotifers nos. mL^{-1}	Larval survival (20 dph)
1	1 x 10^4 - 6 x 10^4	Nil	Nil	5-10	Nil
2	1 x 10^5 - 6 x 10^5	Nil	Nil	8-18	Nil
3	1 x 10^6 - 6 x 10^6	0-1	Nil	10-18	Nil

Discussion

The global marine ornamental fish trade has been increasing and hence in recent years, research and development on breeding and seed production of marine ornamental fishes has also gained momentum. It is well understood that the key factors for the successful larviculture of marine finfishes depend chiefly on the appropriate size and nutritional quality of live feeds employed. Among the marine ornamental fishes, the first success was achieved in the breeding and seed production of clownfishes, as their larviculture protocols are comparatively easy (Hoff 1996). In India also the first success

was in the development of hatchery techniques of clownfishes (Gopakumar et al. 2001; Ignatius et al. 2001; Madhu and Rema 2002). Experimental success was also obtained in the breeding and larval rearing of damselfishes (Gopakumar et al. 2002). Olivetto et al. (2003) reported successful larval rearing of the pomacentrid *Chrysiptera parasema*. The most critical aspect of larviculture of pomacentrids other than clownfishes is the underdeveloped state of larvae at hatching and the consequent problems of starter feed. The three species of damselfishes studied were with altricial type of larvae and the mouth gape of newly hatched larvae ranged from 150 to 200 µ. In trials on feeding with the available strain of the rotifer *Brachionus rotundiformis* as starter feed, the larvae survival was not successful. The co-feeding of the selected two species of copepods *viz.*, *P. serricaudatus* and *E. acutifrons* in greenwater along with larvae yielded positive results. The small size of the first naupliar stages of the copepods employed and the availability of different sizes of nauplii during the initial phase of larviculture would have sustained the first exogenous feeding of the larvae. The initial stages of nauplii noted in the larviculture system measured from 60 to 80 µ, which is suited for the first feeding of the larvae. The high EPA, DHA and ARA content of copepods also would have facilitated the larval survival and growth (Stottrup 2003).

The maintenance of copepods in multiplicative phase in the larviculture system is the crucial factor for the survival of the larvae. An optimum cell count of greenwater was found to be required for the larval rearing which is found to be $1 \times 10^5 - 6 \times 10^5$ mL^{-1}. The cell count range of $1 \times 10^4 - 6 \times 10^4$ cells. mL^{-1} would have been too low for the multiplication of the copepods. The cell count range of 1×10^6-6×10^6 appears to be too high as it would have affected the filter feeding of the copepods. Hence, the cell count range $1 \times 10^5 - 6 \times 10^5$ cells mL^{-1} appears to be optimum for multiplication as was indicated by the maximum number of egg-bearing copepods and nauplii. The naupliar count alone cannot be taken as an indicator of multiplication due to the fact that most of the newly hatched nauplii will be fed by the larvae. The better survival of the larvae can be directly attributed to the availability of freshly hatched nauplii, which was indicated by the abundance of egg-bearing copepods and nauplii in the larviculture system. It is believed that survival rates could be further enhanced if the copepods in the larviculture system could be kept at optimum production level. The optimum cell count of 1×10^5-6×10^5 mL^1 for greenwater was maintained in the larval system by adding fresh cultures of phytoplankton after checking the cell density.

The larviculture trials with copepods and rotifers as live feeds were not successful. The rotifers multiplied rapidly by parthenogenesis and filled the system. The copepods being sexually reproducing could not keep pace with the rotifer multiplication and were rapidly eliminated from the system. The larvae of the species experimented were unable to accept rotifers as starter feed which resulted in total mortality of the larvae. It is also noted that the critical phase of larviculture was over by 15-20 dph. After 15-20 dph, the

mouth gape had reached around 450 µ and can be fed with freshly hatched *Artemia* nauplii. The absence of any mortality from this stage onwards indicated that if the larvae could be fed with suitable feed initially, the larviculture of these species could be accomplished easily with conventional live feeds.

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Received: 07 December 2007; Accepted: 13 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Indian Shrimp Trade: Reflections and Prospects in the Post–WTO Era

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Abstract

Indian fisheries sector in view of its potential contribution to national income, nutritional security, employment opportunities, social objectives, and export earnings plays an important role in the socio-economic development of the country. The marine products exports contributed a whopping 7245 crores of foreign exchange to the exchequer during 2005–2006, which is one-third of the total agricultural exports and 1.5 percent of the total GDP. Shrimps continue to be the predominant item in our marine export claiming about 29 and 67 percent in quantity and value terms share, respectively. Until recently, India depended heavily on one product (shrimp) and one market (Japan) for its marine products export and thus there is a need for product and market diversification. Shrimp export from India to the United States is also posing serious concern based on the restriction placed on the ground that these are not caught using turtle-excluding devices and with proper antidumping measures. The present study is an attempt to address the significance of the shrimp trade in the Indian seafood export basket based on the data collected for the period from 1979 to 2005 from different sources. The different export parameters like the growth, instability, competitiveness, dynamics of changes, integration, impediments faced, and prospects in the post-WTO framework are analyzed using econometric tools. The results of the study indicated that the trade liberalization initiated during 1991 had embarked improvement in the Indian shrimp export. However, recently, there is erosion in the competitiveness of Indian shrimp trade. Nevertheless, there are issues of concern due to the competitiveness, instability, and rejections on quality grounds. Infrastructure development, creating brand image, adoption of HACCP guidelines, value addition and antidumping measures, horizontal integration by ploughing in more area under shrimp farming considering the vast potential of unexploited brackish-water resources are the core issues, which need to be addressed.

Introduction

Indian fisheries sector, in view of its potential contribution to national income, nutritional security, employment opportunities, social objectives, and export earnings plays an important role in the socio-economic development of the country. Export earnings are presently valued to be more than Rs. 7,250 crores from a volume of 5.2 lakh tonnes. In addition, it provides direct and indirect employment and dependency

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for more than 14 million people in the country. With an estimated production potential of 8.4 million tonnes, the present level of production in the country is 6.57 million tonnes with an annual growth rate of about 6 percent with 60 and 40 percent contribution, respectively, from the inland and marine sector**.** Japan, USA, European Union, South East Asia, and Middle East are the five major markets for Indian seafood export**.**

Among the different species, shrimps continue to be the predominant item in our marine export accounting for about 28 and 60 percent share in quantity and value terms, respectively. Internationally traded fisheries products are characterized by a high degree of heterogenity, reflecting the wide range of species and of processing techniques (Chand, Ramesh. 1997). The seafood industry in many countries is undergoing a rapid change to process more and more ready to cook and ready to eat items in convenient packs. Indian seafood industry, by and large, still remains as a supplier of raw materials to the preprocessors in foreign countries and 90 percent of raw materials are exported in bulk packs, which is the prime reason for the drastic reduction in the unit value realization (Salim 2002).

India depends heavily on one product (shrimp) and one market (Japan) for its marine products export, and thus, there is a need for product and market diversification. India's predominant position in shrimp market is being eroded due to the sudden spurt in farmed shrimp production in China, Indonesia, Thailand, and Vietnam (Datta and Chakrabarti 2001).

The broad objectives of this investigation are to study the export performance and potential of Indian shrimp under the trade-liberalized economy. However, the specific objectives are as follows: To assess the dynamics of changes in export of Indian shrimp, to decompose the growth and instability of Indian shrimp exports and also to assess the market potential and opportunities in shrimp exports.

Materials and Methods

The secondary data pertaining to export quantity, export, export unit value, domestic price, and international price of major marine products at different markets were gathered from the various publications of Marine Products Exports Development Authority (MPEDA), Ministry of Commerce, Government of India, and the data pertaining to macroeconomic indicators like Gross National Income of the importing countries and exchange rate, etc were collected from various published Governmental and Non-Governmental sources for the study period.

The study was based on secondary data covering a period of thirty-two years, starting from 1975 to 2006. The study period was divided into two segments *viz*., preliberalization (1975 to 1990) and postliberalization (1991 to 2006) periods. This grouping was carried out to compare the export performance of the Indian shrimp export

in the preliberalization and postliberalization periods. The growth, instability, direction of export, demand-supply elasticities of exports, and competitiveness were analyzed, and the analytical tools used in this study are discussed below

Tools of Analysis

A. Analysis of Growth

The growth in quantity exported, export value, and unit value realized from exports were analyzed using the exponential growth function of the form,

$$Y = ab^t e_{t,} \quad (1)$$

where Y = dependent variable for which growth rate was estimated; a = Intercept; b = Regression coefficient; t = Time variable; e = Error term

The compound growth rate was obtained for the logarithmic form of the equation (A) and is given below.

$$\text{In } Y = \text{Ln } a + t \text{ Ln } b. \quad (2)$$

Then, the compound growth rate (r) was computed by using the relationship

$$r = (\text{Anti Ln of } b - 1) \times 100 \quad (3)$$

B. Decomposition Model

The decomposition model of Hazell & Peter (1982) was used to find the source of growth and variability in Indian marine products exports. The export quantity and export unit values were first detrended using the linear relations of the form

$$z_t = a + b + e_t, \quad (4)$$

where z_t denotes the dependent variable (export quantity and export unit value); t = time variable; and e_t = random variable residual with zero mean and variance σ^2. After detrending the data, the residuals were centered on the export mean export quantity and export unit value resulting in the detrended time series data of the form

$$z^*_t = e_t + \bar{z}, \quad (G)$$

where $\bar{z}$ = mean of export quantity/unit value; z^*_t = detrended export quantity or unit value.

The detrended values were subjected to the following analysis

$$EV = EQ.\ EUV \quad (5)$$

EV = The export value of shrimp products

EQ = The export quantity of shrimp products

EUV = The export unit value of shrimp products

The variance of the export value (*V* (*EV*)) is expressed as follows:

$$V(EV) = \overline{EQ}^2 \; V(EUV) + \overline{EUV}^2 + V(EQ) + Cov(EQ, EUV) - Cov(EQ, EUV)^2 + R, \quad .(6)$$

where and = the mean export quantity and mean export unit value; *R* = the residual term, which is expected to be small

It is apparent from the above expression that *V (EV)* is not only a function of the variances in export quantity and unit value but also a function of the mean export quantity and unit value and of the covariance's between quantity and unit value. Evidently, a change in any one period of these components would lead to a change in *V (EV*) between these two periods, and similarly, average export value *E (EV*) can be expressed as follows:

$$E(EV) = \overline{EQ} \; \overline{EUV} + COV\{EQ.EUV\} \ldots(7)$$

It was affected by the changes in the covariances between export quantity and unit value and also by the changes in the mean export quantity and unit value. The objective of the decomposition analysis is to partition the changes in the *V (EV)* and *E (EV)* between the two periods into constituent parts, which could be attributed separately to changes in the mean, variances, and covariance of export quantity and export unit value, which is

$$E(EV_I) = \overline{EQ}\overline{EUV}_1 + COV\{EQ_I.EUV_I\} \ldots(8)$$

$$E(EV_{II}) = \overline{EQ}_{II}\overline{EUV}_{II} + COV\{EQ_{II}.EUV_{II}\} \ldots(9)$$

Each variable in the second period could be expressed as the counterpart in the first and the change in the variable between the two.

For example,

$\overline{EQ_{II}} = \overline{EQ_I} + \Delta\overline{EQ}$ and $\Delta\overline{EQ_{II}} = \overline{EQ_{II}} - \overline{EQ_I}$. Therefore,

$$E(EV_{II}) = (\overline{EQ_I} + \Delta\overline{EQ})\;(\overline{EUV_1} + \Delta\overline{EUV}) + Cov(EQ_I.EUV_I) + \Delta Cov(EQ.EUV) \ldots(10)$$

The change in the average export value *[*Δ *E (EV*)] was then obtained by subtracting equation (K) from (M).

This was reduced to

$$\Delta E(EV) = E(EV_{II}) - E(EV_I)$$

$$= \overline{EQ_1}\;\Delta\overline{EUV} + EUV.\Delta\;\overline{EQ} + \Delta\;\overline{EQ}\;.\Delta\overline{EUV} + \Delta\,\mathrm{Cov}(EQ,EUV)\ldots(11)$$

Table 1. Components of Change in Average Export Value

Sl.No	Source of Change	Symbol	Components of Change
1	Change in mean export value	$\Delta\overline{EUV}$	$\overline{EQ}$, $\Delta\overline{EUV}$
2	Change in mean export quantity	$\Delta\,\overline{EQ}$	$\overline{EUV}$, $\Delta\ \overline{EQ}$
3	Interaction between changes in (1) and (2)	$\frac{\Delta\overline{EUV}\,\Delta}{\overline{EQ}}$	$\Delta\overline{EUV}\,\Delta\ \overline{EQ}$
4	Change in EQ-EUV covariance	ΔCov(EQ,EUV)	Δ Cov(EQ,EUV)

Table 2. Components of Change in the Variance of Export Value

	Source of Change		Components of Change
	Description		Symbol
1	Change in Mean EUV	$\Delta\overline{EUV}$	$2\ \overline{EQ_1}\ \Delta EUV\ \text{Cov}(EQ_1,EUV_1) +$ $([2\ \overline{EUV}_1\Delta\ \Delta\overline{EUV}) + (\Delta\overline{EUV})^2]\ \text{V}(EQ_1)$
2	Change in mean EQ	$\Delta\,\overline{EQ}$	$2\ \overline{EUV}_1\Delta\,\overline{EQ}\ \text{Cov}(EQ_1,EUV_1) + [2\ \overline{EQ_1}\,\Delta$ $\overline{EQ}\ + (\Delta\ \overline{EQ})^2]\ \text{V}(EUV_1)$
3	Change in EUV variance	ΔV(EUV)	$(\overline{EQ_1})^2\Delta\text{V}(EUV)$
4	Change in EQ variance	ΔV(EQ)	$(\overline{EUV}_1)^2\ \Delta\text{V}(EQ)$
5	Interaction between changes in mean EUV and EQ	$\Delta\overline{EUV}\,\Delta\ \overline{EQ}$	$2\,\Delta\overline{EUV}\,\Delta\,\overline{EQ}\ \text{Cov}(EQ_1,EUV_1)$
6	Changes in EQ-EUV Covariance	Δ Cov(EQ,EUV)	$[2\ \overline{EQ_1}\ \overline{EUV}_1 - 2\ \text{Cov}(EQ_1,EUV_1)]$ Δ Cov(EQ,EUV)–[Cov(EQ,EUV)]^2
7	Interaction between changes in mean EQ and EUV covariance	$\Delta\ \overline{EQ}\ \Delta\text{V}(EUV)$	$[2\,\overline{EQ}\,\Delta\,\overline{EQ}\ -(\Delta\,\overline{EQ}\ )^2]\,\Delta\text{V}(EUV)$
8	Interaction between changes in mean EUV and EQ covariance	$\Delta\overline{EUV}\,\Delta\text{V}(EQ)$	$[2\,\overline{EUV}\ \Delta\overline{EUV} - (\Delta\overline{EUV})^2]\Delta\text{V}(EQ)$
9	Interaction between changes in mean EQ and EUV and changes in EQ–EUV covariances	$\Delta\overline{EUV}\,\Delta\,\overline{EQ}$ Δ Cov(EQ,EUV)	$[2\,\overline{EUV}_1\Delta\ \ \overline{EQ}\ + 2(\ \ \overline{EQ_1}\ \Delta\overline{EUV}) + 2\Delta$ $\overline{EQ}\ \ \Delta\overline{EUV}\ ]\ \Delta$ Cov(EQ,EUV)
10	Change in residual	ΔR	Δ V(EQ,EUV) – Sum of other components

Where $\overline{EQ}_1 \Delta\overline{EUV}$ and $\overline{EUV}_1 \Delta\overline{EQ}$ arose form the changes in mean export unit value and mean export quantity. They are called as the pure effects, as they arose even when no other sources of change.

$\Delta\overline{EUV}$ was an interaction effect, which occurred from the simultaneous occurrence of changes in mean export unit value and mean export quantity. Obviously, this term will be zero if either the mean export value or the mean export quantity remains unchanged.

$\Delta COV\ (EQ.EUV)$ occurred from the changes in the variability of the export quantity or export unit value.

Since

$$COV\ (EQ, EUV) = \rho\ [V\ (EQ)\ V\ (EUV)]^{1/2}, \quad (12)$$

where ρ is the correlation coefficient, then it can be observed that $\Delta COV\ (EQ.EUV)$ occurred from the changes in the variances of export quantity and unit value and from the changes in the correlation between the two.

The changes in the variance of export value *V (EV)* can be decomposed in an analogous way. The components of the change in the variance of export value are given below. Thus, there are 10 sources of changes in export value variance; four of these are changes in mean export unit value, changes in mean export quantity, interaction between changes in mean export quantity and mean export unit value, and changes in the export quantity–unit value variance, which are similar to that of Table 1. However, changes in export value variance had also occurred from the changes in the variances of export quantity and unit value and from changes in interaction terms between all these components.

C. Export Instability

Instability in export is expected to hamper the process of economic development. This analysis was used to find out the fluctuations in export of major marine products during preliberalization and postliberalization periods. (Begum, S. and A.F.M. Shamsuddin, 1998) To study the export instability, Coppock's instability index was used to estimate the variation in the export of shrimp, which algebraically is expressed as the following estimable form:

$$\text{V Log} = \frac{\sum_{i=1}^{n} \left(\frac{\log X_{t+1}}{X_t} - m\right)^2}{N}. \quad (13)$$

$$\text{The instability index} = (\text{antilog}\sqrt{\text{V log.g}} - 1) \times 100, \quad (14)$$

where

X_t = Value of exports in year t or volume of exports in year t

N = Number of years – 1

m = The arithmetic mean of the difference between the logs of X_t and X_{t+1} etc.

V log = Logarithmic variance of the series

D. Dynamics of the Structural Change in Exports

The structural change in export of major marine products was examined by estimating the transitional probability using Markov-chain model. This econometric analysis not only helps to know the trend in sustaining existing market but also helps to know the shift in shares from one country to another over a period of time.

Results and Discussion

Export Growth of Indian Shrimp

The growth patterns in the export of shrimp from India during the pre-liberalisation (1975-1990) and post-liberalisation (1991-2006) period in both quantity and values are furnished in Table 3.

Table 3. Export growth of Indian shrimp products

Year	Pre-liberalization (1975–1990	Post-liberalization (1991–2006)
Total		
Quantity (tonnes)	3.72**(1.84)	7.04** (2.37)
Value (Rs)	3.48* (1.62)	11.72** (3.48)
Value (US $)	3.44** (1.92)	5.89** (1.81)
Unit Value (Rs)	–0.28* (-0.14)	4.37(–0.02)
Frozen Shrimp		
Quantity (tonnes)	1.21* (0.89)	5.15** (2.67)
Value (Rs)	2.10* (0.97)	5.08** (2.36)
Value (US $)	2.07* (1.10)	5.11**(2.42)
Unit Value (Rs)	1.24 (0.82)	–0.07* (–0.03)

Figures in parenthesis indicate the standard errors of the estimates; ** indicates 1% level of significance; * indicates 5% level of significance.

The growth rate of marine products in terms of quantity, value in rupees, dollar, and unit value for the different commodities are estimated in Table 3. The

commoditywise export of marine products indicated that the postliberalization period performed better than the preliberalization period with respect to quantity, value in rupee, and US dollar terms with 7.04, 11.72, and 5.89 percent, respectively. The export basket during the postliberalization was characterized by the dominance of diversification of frozen squids, cuttlefish fresh, and frozen fishes compared with the high-valued species (shrimps and lobster), which resulted in the increased realization of prices.

Frozen shrimp, the largest value component, registered a 5.15 percent growth in quantity and 5.08 percent in value terms. However, the unit value registered a decline of –0.07 percent during the postliberalization period, which can be attributed to lower unit value realization and the price-making behavior of the buyer in the export markets.

Decomposition Analysis

Decomposition analysis was done for decomposing the sources of growth on average export value and variance of export value of Indian marine products. In addition, the decomposition of the sources of growth in average export value and variance of the export value were analyzed.

The results of the decomposition analysis of the components of change in the average export value and variance of fish exports are given in Tables 4 and 5. The components of changes in the export value of Indian shrimp in terms of change in mean export quantity and mean export unit value and their variability besides the interaction effect are given in Table 5.

Table 4. Decomposition analysis of the components of change in average export value of Indian fish exports

Sl. No:	Source of Change	Percentage Share
1	Change in Mean Export Unit Value	2.43
2	Change in Mean Export Quantity	93.08
3	Interaction between changes in (1) and (2)	4.67
4	Change in EQ-EUV covariance	-0.18

The results indicated that the contribution of change in mean export quantity was the highest among the other components of change, which accounted for 93.08 percent of the increase in average export value. This was as expected because the export quantity had recorded significant higher growth rates during both the period, whereas the export unit value recorded a negative growth rate during the postliberalization period. The changes in the covariance between the mean export quantity and the mean export unit value accounted 0.18 percent decrease in the mean export value. The changes in the covariances could have occurred through the changes in the variance of export quantity and export unit value. With regard to interaction effect, the export quantity was benefited to a small extent (4.67 percent) from both mean export quantity and mean export unit

value, which indicated that the increase in export value paved way for an increase in export quantity.

Table 5. Decomposition analysis of the components of change in the variance of export value of Indian marine products

Sl. No:	Source of Change in Variance Description	Percentage Share
1	Change in Mean EUV	–0.17
2	Change in Mean EQ	27.59
3	Change in EUV Variance	0.18
4	Change in EQ Variance	74.36
5	Interaction between changes in mean EUV and EQ	–0.14
6	Changes in EQ-EUV Covariance	–3.85
7	Interaction between changes in mean EQ and EUV covariance	0.84
8	Interaction between changes in mean EUV and EQ Covariance	5.35
9	Interaction between changes in mean EQ and EUV and changes in EQ-EUV Covariance	–9.32
10	Change in residual	5.16

The change in variability of export quantity accounted for 27.59 percent in the variance of export value. The coefficient of variation was worked out at 15.5 percent and 23 percent, respectively, during the preliberalization and postliberalization periods. The change in the variance of export quantity was the important source in increasing the export value variance to the extent of 74.26 percent. The change in the covariance between mean export quantity and mean export unit value was –3.82 percent, showing that the variability effect of both the mean export quantity and mean export unit value reduced the instability of export value variance to a small extent, thus generating a stabilizing effect among all other components of change.

The effect of interaction term was also important in determining the stability of the export value and when added together contributed 6 percent of the increases in the variance of total export value. The interaction terms arose in part from the change in mean export unit value and export quantity covariance and had induced a change in the behavior of the exporters, which affected the mean or variance of the export quantity and had led to the instability of the export value.

The results of the decomposition analysis of the components of change in the average export value and variance of Indian shrimp are given in Tables 6 and 7.

Table 6. Decomposition analysis of the components of change in average export value of frozen shrimp

Sl. No:	Source of Change	Percentage Share
1	Change in Mean Export Unit Value	30.85
2	Change in Mean Export Quantity	40.62
3	Interaction between changes in (1) and (2)	27.64
4	Change in EQ-EUV covariance	-0.89

The results indicated that the contribution of change in mean export quantity was the highest among all other components of change with 40.62 percent accountability for the increased in average export value. The change in mean export unit value accounted for 30.85 percent followed by 27.64 percent contributed by the interaction between the mean export unit value and mean export quantity. The contribution of mean export quantity and mean export unit value as the dominant sources of change in average export value of frozen shrimp is as expected as they registered significant growth with higher instability among export quantity and export unit value.

The components of change that affected the stability of export value are shown in Table 7. The effect of interaction terms is the most important in determining the stability of export and accounted for about 65 percent of the increase in the variance of total export value. The interaction terms arouse from the changes in mean export unit value and export quantity covariance, mean export quantity and export unit value covariances, and interaction between them. In addition, the export quantity variance, mean export quantity, and mean export unit value variance contributed 14.64, 13.01, and 9.27 per cent, respectively, in determining the stability of export value.

Thus, it could be summarized that the change in mean export quantity, mean export unit value, and interaction between mean export quantity and mean export unit value are the major sources of changes in determining the average export value of frozen shrimp, where the stability of export value depends more on the interaction terms (65 percent) rather than the individual components.

Export Instability

The export performance of a market during a period was also measured based on the extent of variability or fluctuations in addition to the point of view in the increase in quantity, value, and unit value. Thus, Coppocks instability index was used to study the degree of instability in quantity, value, and unit value of marine products export from India during the two period's *viz*., preliberalization and postliberalization for the different commodities and markets, and the estimated instability indices.

Table 7. Decomposition analyses of the components of change in the variance of export value of frozen shrimp

Sl. No:	Source of Change in Variance	
	Description	Percentage Share
1	Change in Mean EUV	1.84
2	Change in Mean EQ	13.01
3	Change in EUV Variance	9.27
4	Change in EQ Variance	14.64
5	Interaction between changes in mean EUV and EQ	0.78
6	Changes in EQ-EUV Covariance	6.24
7	Interaction between changes in mean EQ and EUV covariance	24.18
8	Interaction between changes in mean EUV and EQ Covariance	26.54
9	Interaction between changes in mean EQ and EUV and changes in EQ-EUV Covariance	15.89
10	Change in residual	-12.39

Export Instability of Indian shrimp

The instability indices of Indian shrimp export were analyzed using the Coppocks Instability Index, and the results are given in Table 8.

Table 8. Instability indices of Indian shrimp export

Year	Preliberalization (1975–1990)	Postliberalization (1991–2006)
Total		
Quantity (tonnes)	12.34	22.82
Value (Rs)	16.04	26.83
Value ($)	12.98	25.19
Unit Value (Rs)	10.15	18.39
Frozen Shrimp		
Quantity (tonnes)	7.15	12.18
Value (Rs)	18.12	23.46
Value ($)	18.25	23.42
Unit Value (Rs)	14.31	16.14

The results indicated that the degree of instability was more pronounced during the postliberalization period with 22.82, 26.83, and 18.39, respectively, in terms of quantity, value, and unit value even though more growth was associated. Some of the reasons that can be attributed to the growing instability is the increasing number of trading partners, fluctuations in the Japanese economy and frozen shrimp registered higher export quantity variation (12.18 percent) during postliberalization period compared with preliberalization period (7.15 percent), suggesting that there exist severe competition among the different exporters and the exports are very much responsive to the prices. In addition, the essentiality of a buyers market and lesser number of importers paved the way for higher instability.

Thus, it could be noted that the post liberalization period generated a higher degree of instability for frozen shrimp. The analysis suggested the need for diversification of commodities, which would reduce the degree of instability.

Structural Change in Shrimp Export

The dynamics in the directions of export and changing pattern in the trade of major marine products from India by shift in export shares from one country to another over a period of time were analyzed using the Markov chain model.

The estimated transitional probability matrix of Indian frozen shrimp export in quantity during the preliberalization period is presented in Table 9. The transitional probability gives a broad indication of the change in the direction of trade of frozen shrimp export from India over a period of 12 years .The major countries importing Indian frozen shrimp consistently included Japan, USA, and European Union accounting more than 80 percent in quantity and value. The export to remaining countries was pooled under 'Others.'

Table 9. Transitional probability matrix of Indian frozen shrimp export during preliberalization period

Importing countries	Japan	EU	USA	Others
JAPAN	0.8072	0.0000	0.1833	0.0095
EU	0.0000	0.7512	0.1369	0.1119
USA	0.5564	0.1247	0.3189	0.0000
Others	0.0000	0.8687	0.0000	0.1313

Table 9 revealed that Japan and European Union were the stable Indian frozen shrimp export markets, which have been most stable during preliberalization period as reflected by the high probability of retention of 0.80 and 0.75, respectively. The results indicated that the probability that Japan retained its export share from one period to another was about 81 percent during 1975–1990 and that of the European Union was 75 percent. The higher retention of Japan was reinforced by high probability of transfer from United States (0.556) and that of European Union market were reinforced by high probability of transfer from USA (0.124) and 'Others' (0.868), respectively. The probability of retention of frozen shrimp by United States was found to be 0.31 between the periods from 1975-1990. There was small probability of loss from Japan and European Union markets to US market at 0.183 and 0.136, respectively. The probability of retention of 'Others' was found to be 0.131 with high probability of transfer from European Union (0.111). Others include South East Asian Countries and Middle East.

The estimated transitional probability matrix of Indian frozen shrimp export in quantity during the postliberalization period is presented in Table 10. The Table shows that Japan and European Union were the stable Indian frozen shrimp export markets, which have been most stable during postliberalization period as reflected by the high probability of retention of 0.756 and 0.465, respectively. The results indicated that the probability that Japan retained its export share from one period to another was about 75.6 percent and that of the European Union was 46.5 percent during 1975–1990. The higher retention of the Japan is reinforced by higher probability of transfer from European Union (0.365), whereas that of European Union was reinforced by high probability of transfer from United States (0.605). The probability of retention of United States was 0.328 with high probability of transfer of 0.158 from Japan and 0.219 from 'Others' even after losing 0.605 to European Union.

Table 10. Transitional probability matrix of Indian frozen shrimp export during postliberalization period

Importing countries	Japan	EU	USA	Others
Japan	0.7562	0.0000	0.1584	0.0855
EU	0.3656	0.4652	0.0809	0.0884
USA	0.0000	0.6054	0.3283	0.0663
Others	0.0910	0.0678	0.2196	0.6216

Thus, it can be concluded that the frozen shrimp market remained more or less stable with Japan as the major trading partner followed by European Union and United States. It is significant to note that the 'Others' gained sizeable probability of retention during the postliberalization period (0.621) compared with preliberalization period (0.131). The presence of 'Others' indicates the emergence of newer trading partners with India.

Market Potential and Opportunities
Export Demand and Supply Elasticity

Based on the export demand and supply function, the export demand supply equations for the shrimp were estimated using 2-stage least square (2SLS) estimates, and the results are discussed below. The price and the income elasticities obtained from the results would indicate whether the shrimp exports enjoy a competitive advantage in terms of higher price and income elasticities.

The demand and supply elasticities for frozen shrimp export to major countries using the 2SLS estimates during the preliberalization period are given in Table 11. The results indicated that the price elasticities of the export demand were significant at 1 percent level for Japan, United States, and UK. The price elasticity worked out to be 0.94, -1.93, and -0.35 for Japan, United States, and UK, respectively. It implied that 10 percent increase in the price had led to 9.4 percent increase in the quantity demanded for Japan, a reduction in 13 percent and 3.5 percent for the quantity demanded in United States and UK, respectively. The price elasticities estimated of USA and UK is in concordance with the neo classical theory of demand that the quantity demanded is inversely related with price rise.

Table 11. Demand and supply elasticities for shrimp exports to major markets during preliberalization period

Countries	Demand Elasticity		Supply Elasticity
	Price	Income	Price
Japan	0.94**	-0.50**	0.42**
USA	-1.30**	0.24**	0.05
Germany	-2.43	0.62**	0.38
UK	-0.35**	0.48**	0.48**
Spain	0.29	0.58	0.68

n = 15 **one percent level of significance

The income elasticity for Japan, USA and Germany was worked out at 0.50, 0.24 and 0.62, respectively, and was significant at one percent level. The results implied that one percent in income would increase the quantity demanded by 0.5, 0.24 and 0.62 percent. The price elasticities of supply worked out to be 0.42 and 0.48, respectively, for Japan and UK, which implied that one percent increase in the price would increase the supply by 0.42 and 0.48 percent, respectively.

The demand and supply elasticities for frozen shrimp export to major countries using the 2SLS estimates during the postliberalization period are given in Table 12. The results indicated that the price elasticities of export demand were significant at one per

cent level for USA, UK, Spain, and Italy. The price elasticities worked out to be -2.69, -4.47, -0.15, and -0.18 for USA, UK, Spain, and Italy, respectively. The price elasticities for Japan were found to be 0.16, which indicated that the quantity demanded increased with price rise based on the fact that Japan continues to be the largest importer and consumer of fish. The results obtained for the other countries were supporting the neo classical theory of demand which states that one percent increase in the price would decrease the quantity demanded by the level corresponding to the elasticities. The income elasticities was the highest for UK with 3.98, which indicated that one percent increase in the income would increase the quantity demanded by 3.98 percent at one percent level of significance. The price elasticities of supply were the highest for Spain followed by Japan, USA, and Italy at 4.13, 2.75, 2.07, and 0.89 percent, respectively. This indicated that one percent increase in the price of frozen shrimp would increase the quantity supplied at the corresponding price elasticities of supply.

Table 12. Demand and supply elasticities for shrimp exports to major markets during postliberalization period

Countries	Demand Elasticity		Supply Elasticity
	Price	Income	Price
Japan	0.16**	0.02**	2.75**
USA	-2.69**	0.88**	0.89*
UK	-4.47**	3.98**	0.11
Spain	-0.15**	0.15	4.13*
Italy	-0.18**	0.50**	2.07**

n = 15 **1% level of significance *5% level of significance

Nominal Protection Coefficient

In accordance with the theory of comparative advantage in international trade and against the backdrop of liberalization, it becomes imperative to analyses the export competitiveness of major marine products. In the present study, an attempt was made to analyze the competitiveness of frozen shrimp (Balassa 1965). The estimated nominal protection coefficient (NPC) for frozen shrimp during the preliberalization and postliberalization periods is depicted below in Table 13.

It was found that the NPC was less than one clearly indicating the competitiveness of Indian shrimp in the world market. The average NPC for the preliberalization and postliberalization periods was estimated to be 0.86 and 0.62, respectively. The increase in competitiveness of the Indian shrimp in the postliberalization period is due to the emergence of more number of markets like European Union and USA, and the resultant demand generated a premium price for shrimp in the world market.

Table 13. Nominal Protection Coefficient (NPC) for Frozen Shrimp

Year	Domestic Price (US $)	Domestic Reference Price (US $)	International Reference Price (US $)	Nominal Protection Coefficient
Period I (1975-1990)	8.26	8.93	10.81	0.86
Period II (1991-2006)	7.54	8.13	13.02	0.62
2003	7.52	8.17	16.24	0.50
2004	7.86	8.54	12.75	0.61
2005	8.64	9.29	13.5	0.69

However, the situation of late is changing due to the increase in domestic prices and poor crustacean landings, scarcity of raw material, and higher input requirement in the form of processing charges, electricity etc. The competitiveness of Indian shrimp is declining in the recent years with the NPC calculated at 0.61 and 0.69 in 2004 and 2005, respectively.

Conclusions

The study concludes with the following findings:

- The analysis of growth indicated that there exists a decline in the unit value realization of shrimp exports during the postliberalization period
- The decomposition analysis for frozen shrimp shows that the change in mean export quantity, mean export unit value, and interaction between mean export quantity and mean export unit value are the major sources of changes in determining the average export value of frozen shrimp. The postliberalization period generated a higher degree of instability for frozen shrimp.
- Japan and European Union were the stable Indian frozen shrimp export markets during preliberalization period and postliberalization period as reflected by the high probability of retention. Thus, it can be concluded that the frozen shrimp market remained more or less stable with Japan as the major trading partner followed by European Union and United States
- The demand and supply elasticities for frozen shrimp export to major countries using the 2SLS estimates during the preliberalization period indicated that the price elasticities of export demand were significant at 1 percent level for Japan, United States, and UK. The results indicate that Japan continues to be inelastic

for price rise, whereas United States and UK are sensitive to changes in the price rise.

- The NPC analysis of frozen shrimp indicated that the competitiveness during the postliberalization period decreased, and the poor competitiveness of the shrimp could be attributed to the decreased landings and lower international price realizations.

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Received: 31 December 2007; Accepted: 06 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

An Analysis of Capital Formation in Fisheries Sector in India

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Abstract

The fisheries sector occupies an important place in the socio-economic development of the country. The sector generates income and employment, provides livelihood and nutritional security to a large section of economically backward population, and stimulates growth for a number of subsidiary industries in the country, besides being a source of valuable foreign exchange earnings. In view of the increasing importance of fisheries sector in the national economy, the study was undertaken to examine the trends of capital formation in fisheries sector and its share in total economy. To examine the share of fisheries sector in the total as well as agricultural outlay, the contribution of central sector, centrally sponsored and states schemes for fisheries development, and the investment in fisheries research, the secondary data were compiled and analyzed. To examine the growth in fisheries Gross Fixed Capital Formation (GFCF) as well as Gross Domestic Product (GDP), the trend lines were fitted with the exponential function to estimate the compound growth rates different periods. The elasticity of fish GDP with respect to fish GFCF was also estimated using log-linear relationships.

The study revealed that the share of fisheries in agriculture outlay increased from 1.74% during first plan to 5.62% during sixth plan and then declined to 3.7% during tenth plan. The share of GFCF in fisheries sector in total GFCF was almost constant around half a percent between 1970-1971 to 1985-1986 and then started increasing at a steady pace during 1985-1986 to 2002-2003 at constant prices, whereas at current prices, it was hovering around 0.6% up to 1995-1996, and it reached a high of 1.12% during 2003-2004. The contribution of fish to total GDP is hovering around 1% at 1993-1994 prices (constant prices) since 1970-1971. On the other hand, at current prices, the contribution of fish to total GDP was increasing from 0.63% in 1970-1971 to 1.2% in 2003-2004. The study depicts that the growth in fisheries GFCF has been maintaining a high level of around 9.5% during eighties and nineties. However, during seventies, the growth of fisheries GFCF was of the order of around 5.4%. If one considers the overall period from 1970-1971 to 2003-2004, it was found that the total growth of fisheries GFCF was around 8%. The lower rate of growth over the whole period may be attributed to the nearly stagnant trend of fisheries GFCF during seventies. The study concludes that the investment in fisheries research has been increasing all through the plan periods and the Government is giving some importance to this sector. However, there is still scope for more public investment in fisheries research to realize the potential gains of research.

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Introduction

The fisheries sector occupies an important place in the socio-economic development of the country. The sector generates income and employment, provides livelihood and nutritional security to a large section of economically backward population, and stimulates growth for a number of subsidiary industries in the country, besides being a source of valuable foreign exchange earnings. Fisheries contributed about 1% of the total GDP and 5.3% of the GDP from agriculture sector in 2005–2006. Fishing, aquaculture, and a host of allied activities provide a source of livelihood to over 14 million people. The exports of marine products from the country during 2005-2006 reached US $1435 million accounting for 14% of exports of agriculture and allied products.

Indian fishery is an important component of the global fisheries, with India being the fourth largest producer of fish in the world and second in inland fish production. India's share in the world production has increased from 3.7% in 1950 to 4.1% in 1991 and 4.4% in 2000. Further, India's share in the world export of fish products was 2.3% in 2005. After a decline in 2003-2004, it had picked up in subsequent years and grew by 6.3% in April-October 2006. European Union accounted for the largest share of India's export of marine products, followed by US and Japan.

The country is endowed with an Exclusive Economic Zone (EEZ) extending to 20.2 lakh sq. km with a continental shelf area of about 5.3 lakh sq. km having about 8118 km coastal length with some of the richest fishing grounds in the world. The estimated potential for fish production from marine and inland water bodies is about 3.9 and 4.5 million tons, respectively. The main inland fishery resources include about 12.4 lakh ha of brackish water area, 24.14 lakh ha of fresh water ponds and tanks, 7.98 lakh ha lakes, and 29.07 lakh ha reservoirs, besides about 1.96 lakh km of rivers and canals. However, the fish production of 6.50 million tons was much below than the projected production of 7.75 million tons in the year 2005–2006 by the working group on fisheries for the tenth five-year plan. During the last decade, the marine fish production has reached a plateau. Most of the major commercially exploited stocks are showing signs of over exploitation (Ayyappan 2006). On the contrary, demand for sea food has been growing in domestic markets as well as in overseas markets in view of its high quality. Keeping this in view, the Government of India formulated a comprehensive Marine Fishing Policy in 2004 to fulfill the national objectives of augmenting marine fish production on a sustainable level. National Fisheries Development Board has also been set up by government of India in 2006 to realize the untapped potential of fishery sector through research and development including biotechnology.

Capital formation refers to the net additions to the (physical) capital stock in an accounting period or to the value of the amount of increase of the capital stock. Capital formation is often used as an abbreviation for GFCF. A large number of studies have been undertaken for studying the capital formation in agriculture and allied activities

during the last two decades (Mishra and Chand 1995; Chand 2001). However, at disaggregated level, a detailed study on capital formation in fishery sector has not been undertaken so far. In view of the increasing importance of this sector in the national economy, this study was undertaken to examine the trends of capital formation in fisheries sector and its share in total economy and suggest suitable policy measures for sustainability of this sector.

Material and Methods

To study the five-year plan-wise pattern of investment for fisheries development in the country, the share of fisheries sector in the total as well as agricultural outlay were examined using the secondary data on total, agricultural, and fisheries outlays collected from various issues of Economic Survey and Hand Book of Fisheries Statistics. The secondary data on scheme-wise outlay and expenditure for fisheries development were also compiled under different five-year plans from the Hand Book of Fisheries Statistics and analyzed to study the contribution of central sector, centrally sponsored and states schemes for fisheries development. Further, to examine the investment in fisheries research, the outlay for agriculture and fisheries research were compiled and analyzed. The time series data on the total, agricultural, and fisheries gross domestic product (GDP) as well as GFCF in total, agriculture, and fisheries sectors from 1970-1971 to 2003-2004 were collected from various issues of National Accounts Statistics. The triennium averages centered at the mid-point of the triennia were compiled and tabular analysis was carried out for meaningful conclusions.

To examine the growth in fisheries GFCF as well as GDP, the trend lines were fitted with the exponential function to estimate the compound growth rates for historical period (1970-1971 to 2003-2004) and three decadal periods (1970-1971 to 1979-1980, 1980-1981 to 1989-1990, and 1990-1991 to 1999-2000). To see precisely the year in which deceleration in fisheries growth started, the growth rates were estimated between fixed base 1990-1991 and by extending the terminal year from 1995–1996 onwards. To estimate the elasticity of fish GDP with respect to fish GFCF, log-linear relationships between fish GDP and GFCF were fitted for historical as well as three decadal periods. To study the sudden decrease during the later period, the model was fitted for two sub-periods *viz*., 1990-1991 to 1995-1996 and 1996-1997 to 2003-2004. Further, to identify the year of deceleration in elasticity, the model was fitted between fixed base 1990-1991 and by extending the terminal year from 1995-1996 onwards.

Results and Discussion

Plan-wise outlay and expenditure for fisheries development

The outlay on agriculture and allied sectors (agriculture, forestry, and fishing), fisheries subsector, and total outlay during the five-year plan periods is presented in Table 1. It is seen from the table that the share of agriculture sector outlay in total outlay was continuously decreasing during the five-year plans from nearly 15% under first five-year plan to 3.9% under tenth plan. On the other hand, the share of fisheries subsector

outlay in total outlay initially increased from 0.26% under first plan to 0.52% under fourth plan and then started declining and decreased up to 0.19% during tenth plan. However, the share of fisheries outlay in agriculture outlay increased from first plan (1.74%) to sixth plan (5.62%) and then declined to 3.7% during the tenth plan, although the Working Group on Agricultural Research and Education for the Tenth Five-Year Plan recommended that the budgetary allocation to fisheries sector should be enhanced to 9% of total agricultural allocation in X Plan (Planning Commission 2001b). It shows that although the importance of fisheries in agriculture sector was increasingly felt up to sixth plan only, the fisheries still continues to be a neglected sector in national policies. Even now, this sector has got an important place in India's export basket.

Table 1. Outlay for Fisheries Development during Five-Year Plans

(Rs. in Crores)

Plan	Total Outlay	Outlay for Agriculture and Allied Sector	Outlay for Fisheries Sector	Share of Fisheries Sector (%)		Share of Agriculture to Total Outlay (%)
				Total Outlay	Agriculture Outlay	
I (1951-1956)	1960	294	5.13	0.26	1.74	15.00
II (1956-1961)	4600	529	12.26	0.27	2.32	11.50
III (1961-1966)	7500	1068	28.27	0.38	2.65	14.24
IV (1969-1974)	15902	2728	82.68	0.52	3.03	17.16
V (1974-1979)	39322	4302	151.24	0.38	3.52	10.94
VI (1980-1985)	97500	6609	371.14	0.38	5.62	6.78
VII (1985-1990)	180000	10524	546.54	0.30	5.19	5.85
VIII (1992-1997)	434100	22467	1232.82	0.28	5.49	5.18
IX (1997-2002)	859200	42462	2070.00	0.24	4.88	4.94
X (2002-2007)	1525639	58933	2060.54*	0.19	3.70	3.86

Source: Economic Survey – Different issues
* Hand book of Fisheries Statistics, 2004

Scheme-wise outlay and expenditure for fisheries development

An overview of scheme-wise outlay for fisheries development under different plans is presented in Table 2. The table depicts the outlay under three heads: central sector schemes, state schemes, and centrally sponsored schemes (from fourth plan onwards). Up to the third five-year plan, the centrally sponsored schemes were part of central sector schemes. It is also seen that the share of state schemes in total fish outlay decreased from first plan (81%) to sixth plan (53%) and then fluctuated between

60% in seventh plan to 64% in tenth five-year plan. Under central schemes, the share of centrally sponsored schemes increased from 7% under fourth plan and 27% under tenth plan. A careful observation of Table 1 and 2 reveals that the central government has played an important role in the development of fisheries sector as there is a direct relationship between central sector schemes outlay and share of fisheries in agricultural outlay.

Table 2. Scheme-wise Outlay for Fisheries Development over Five-Year Plans

(Rs. Crore)

Plans	Central Sector Schemes	Centrally Sponsored Schemes	State Schemes	Total
First Plan	1.00* (19.49)	-	4.13(80.51)	5.13 (100.00)
Second Plan	3.73* (30.42)	-	8.53 (69.58)	12.26 (100.00)
Third Plan	6.72* (23.77)	-	21.55 (76.23)	28.27 (100.00)
Annual Plans (1966-69)	15.30* (36.25)	-	26.91 (63.75)	42.21 (100.00)
Fourth Plan	28.00 (33.87)	6.00 (7.26)	48.68 (58.88)	82.68 (100.00)
Fifth Plan	51.05 (33.76)	17.00 (11.24)	83.19 (55.02)	151.21 (100.00)
Sixth Plan	137.10 (36.94)	36.62 (9.87)	197.42 (53.19)	371.14 (100.00)
Seventh Plan	156.58 (28.65)	60.75 (11.12)	329.19 (60.23)	546.52 (100.00)
Annual Plans (1990-92)	25.45 (8.69)	55.16(18.84)	212.13 (72.46)	292.74 (100.00)
Eighth Plan	139.00 (11.53)	300.00 (24.89)	766.39 (63.58)	1205.39 (100.00)
Ninth Plan	240.00 (11.60)	560.00(27.06)	1269.78(61.35)	2069.70 (100.00)
Tenth Plan	175.00 (8.49)	565.00(27.42)	1320.54(64.09)	2060.54 (100.00)

* includes centrally sponsored schemes outlay.
Note: Figures in the parentheses are the percentages to total
Source: Hand book of Fishery Statistics, 2004.

Table 3. depicts scheme-wise expenditure under different five-year plans. It can be observed that the expenditure on fisheries development was impressively increasing over the various five-year plans. It ranged from around Rs. 3 crores during the first plan period to Rs.1414 crores during the ninth plan period at current prices. However, the share of state's schemes was decreasing over the plans except the third and the ninth plan. It was around 86% during the first plan and decreased to 62% in the eighth plan. On the other hand, the share of expenditure under centrally sponsored schemes was increasing impressively from fourth plan (10%) to 24% during eighth plan. However, it declined to 19% during the ninth plan period.

Table 3. Scheme-wise Expenditure for Fisheries Development over Plans

Plans	Scheme-wise Expenditure (Rs. Crore)				Percentage expenditure to outlay			
	Central Sector	Centrally Sponsored	State Schemes	Total	Central Sector Schemes	Centrally Sponsored Schemes	State Schemes	Total
First Plan	0.38* (13.67)	-	2.4 (86.33)	2.78 (100)	38.00*	-	58.11	54.19
Second Plan	1.8* (19.87)	-	7.26 (80.13)	9.06	48.26*	-	85.11	73.90
Third Plan	3.03* (12.99)	-	20.29 (87.01)	23.32	45.09*	-	94.15	82.49
Annual Plans (1966-69)	9.04* (27.67)	-	23.63 (72.33)	32.67	59.08*	-	87.81	77.40
Fourth Plan	8.11 (14.99)	5.17 (9.55)	40.83 (75.46)	54.11	28.96	86.17	83.87	65.45
Fifth Plan	39.93 (34.66)	4.07 (3.53)	71.21 (61.81)	115.21	78.22	23.94	85.60	76.19
Sixth Plan	75.54 (26.33)	28.8 (10.04)	182.61 (63.64)	286.95	55.10	78.65	92.50	77.32
Seventh Plan	116.93 (24.48)	53.26 (11.15)	307.4 (64.36)	477.59	74.68	87.67	93.38	87.39
Annual Plans (1990-1992)	16.48 (6.06)	43.73 (16.07)	211.9 (77.87)	272.11	64.75	79.28	99.89	92.95
Eighth Plan	161.01 (14.40)	268.02 (23.96)	689.43 (61.64)	1118.5	115.83	89.34	89.96	92.79
Ninth Plan	124.97 (8.84)	273.18 (19.31)	1016.26 (71.85)	1414.4	52.07	48.78	80.03	68.34

* includes centrally sponsored schemes outlay.
Note: Figures in the parentheses are the percentages to total
Source: Hand book of Fishery Statistics, 2004.

Table 3 also presents plan-wise proportion of expenditure to outlay for fishery development. There was poor utilization of total outlay in fisheries development except the seventh and eighth five-year plans (87% and 93%, respectively). It is very disturbing to observe that the outlay utilization has declined to 68% during ninth plan from a record of 93% during eighth plan period. Further, the share of expenditure in total outlay was as low as 54% and 65% during first and fourth plan periods, respectively. Moreover,

the central sector and centrally sponsored schemes performed impressively only during seventh and eighth plan periods. On the other hand, the performance of states schemes was very good in the utilization of total outlay for fisheries development except first five-year plan (58%). The impressive performance of central schemes in the utilization of outlay for fisheries development has been translated into impressive growth in GDP of this sector (Table 8). It can be inferred from the above discussion that there is an urgent need to first increase the central outlay and secondly to better utilize the allocated outlay under the central schemes for the faster development of fisheries in the country to meet the growing domestic demand and to exploit the opportunities for becoming an important player in the global market.

Outlay for fisheries research

Research is an important component for sustaining fish production and productivity along with maintaining international standards necessary for fish quality assurance. There is a great need for research in the area of aquaculture and marine biotechnology for strengthening the gap in the areas of fish health and disease diagnostics, transgenic aspects, cell and tissue culture, etc.

The plan-wise outlay of fisheries research is presented in Table 4. It is seen that the outlay for research in agriculture and allied sectors had increased phenomenally ever since the fourth five-year plan. It was around Rs.85 crores during the fourth plan and had increased to Rs.5050 crores in the tenth plan. On the same lines, the outlay for fisheries research has also increased manifolds from Rs.2.25 crores in the fourth plan to Rs.157 crores in the tenth plan. The proportionate share of outlay for fisheries research in total fisheries outlay had more than doubled from the fourth plan to the ninth plan, whereas the share of Indian Council of Agricultural Research (ICAR) and Department of Agricultural Research and Education (DARE) in total agriculture outlay had increased at a slower pace during the same period. On the other hand, the share of agricultural research outlay to total outlay had been decreasing over the various plan periods. It is important to note that the internal rate of return to investment on fisheries research and development was found to be very high (42% to 55%) and benefit-cost ratio was also found to be very impressive (2.1 to 3.4) under different Total Factor Productivity (TFP) Scenarios (Kumar 2004). Thus, one can conclude that the investment in fisheries research has been increasing all through the plan periods and the Government is giving some importance to this sector. However, there is still scope for more capital formation in fisheries research to realize the potential gains of research in this sector.

Table 4. Plan-wise Outlays for Fisheries Research

(Rs. in Crores)

Plans	Outlay for ICAR and DARE	Outlay for Fisheries Research	% of ICAR reasearch outlay to total fisheries Outlay	% of fisheries reasearch outlay to total fisheries Outlay	% of fisheries reasearch outlay to total ICAR outlay
Fourth Plan	85.00	2.25	3.12	2.72	2.7
Fifth Plan	153.56	9.60	3.57	6.35	6.2
Sixth Plan	340.00	15.75	5.14	4.24	4.6
Seventh Plan	448.00	18.25	4.26	3.34	4.0
Eighth Plan	1300.00	65.00	5.79	5.27	5.0
Ninth Plan	2100.00	125.00	4.95	6.04	6.0
Tenth Plan	5050.00*	157.14*	8.57	7.63	3.1

Source: Planning Commission, Government of India, 2001

* Total plan outlay for Indian Council of Agricultural Research and Department of Agricultural Research and Education

Capital formation in fisheries

The pace and pattern of fisheries development is to a great extent conditioned by the growth of the infrastructure facilities. Infrastructure plays a critical role on both input and output sides. On the input front, it helps to ensure timely, adequate, and quality input delivery to the farmers, whereas on the output front, it helps to integrate local markets with national and international markets. Therefore, an adequate and efficient infrastructure system is essential for realizing the potential of the sector. Fig. 1 shows the trend in capital formation in fisheries sector during the last three decades. It is observed that the index of capital formation in fisheries was almost stagnant till 1980-1981 and then continued increasing steadily and surpassed the index of agriculture capital formation (Ag GFCF) during the year 1995-1996.

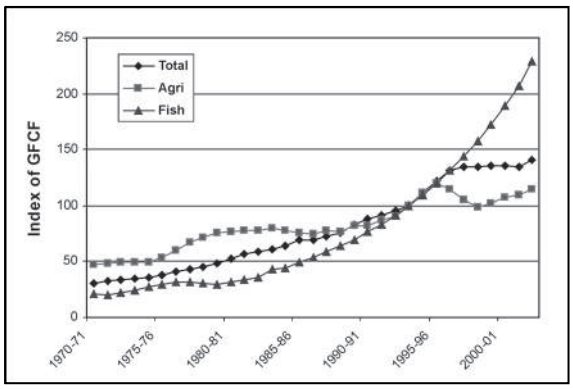

Figure 1. Trend of Capital Formation in Fisheries and Agriculture (Base TE: 1993-94 = 100)

A perusal of Table 5 reveals the percent share of GFCF in fisheries sector to total GFCF as well as Ag GFCF at constant and current prices. It can be observed that the share of GFCF in fisheries sector in total GFCF was almost constant around half a percent between 1970–1971 and 1985–1986 and then started increasing at a steady pace during 1985–1986 to 2002–2003 at constant prices, whereas at current prices, it was hovering around 0.6% up to 1995–1996 and it reached a high of 1.12% during 2003–2004. The share of fish GFCF to Ag GFCF was subdued during the seventies as fishery was practiced as a subsistence activity by fishermen community with little or no use of external inputs. However, it showed a rising trend ever since 1980–1981 and reached a high of 14% during 2002–2003. In contrast, the share of GFCF in agriculture sector to total GFCF has always been on the decline ever since 1970–1971 and reached as low as 7.6% during 2000–2001.

Table 5. Capital Formation for Fisheries Development (Rs. Crores)

Periods	Total GFCF	Ag GFCF	Fish GFCF	% Ag GFCF to Total GFCF	% Fish GFCF to Total GFCF	% Fish GFCF to Ag GFCF
At 1993–1994 prices						
1970-71	54369	8196	261	15.07	0.48	3.18
1975-76	68509	9401	373	13.72	0.54	3.96
1980-81	95370	13491	390	14.15	0.41	2.89
1985-86	125683	13232	614	10.53	0.49	4.64
1990-91	160452	14360	957	8.95	0.60	6.67
1995-96	221720	20850	1507	9.40	0.68	7.23
2000-01	246664	18695	2370	7.58	0.96	12.68
2002-03	255101	20103	2874	7.88	1.13	14.30
At Current price						
1970-71	6445	947	39	14.70	0.61	4.15
1975-76	13938	1857	84	13.32	0.60	4.51
1980-81	29577	3765	133	12.73	0.45	3.52
1985-86	62750	6064	377	9.66	0.60	6.22
1990-91	125605	10934	818	8.70	0.65	7.48
1995-96	291223	20523	1813	7.05	0.62	8.83
2000-01	351162	28747	3193	8.19	0.91	11.11
2003-04	414957	36151	4661	8.71	1.12	12.89

Source: National Accounts Statistics – Various Issues

Contribution of fisheries sector to gross domestic product

The share of fisheries gross domestic product (Fish GDP) to total GDP and agricultural and allied activities (Ag GDP) is presented in Table 6. The contribution of fish GDP to total GDP is hovering around 1% at 1993-1994 prices (constant prices) since 1970-1971. On the other hand, at current prices, the contribution of fish GDP to total GDP was increasing from 0.63% in 1970-1971 to 1.2% in 2003-2004. As a result, the contribution of fish GDP to Ag GDP was increasing at current as well as constant prices. These developments indicate that the fisheries sector is poised to grow further in the near future. During the same period, the growth rate of fisheries GDP has been declining (Table 8). Further, the growth in the total fish production during the liberalization period (1990-1991 to 1995-1996) and the post liberalization period (1996-1997 to 2003-2004) had decreased from 5.16% to 2.78%, respectively. This decline was due to declining growth especially in marine fish production during the same periods, which decreased from 3.25% to 0.16%. There was also a declining trend in inland fish production during the said periods. This clearly shows that there has been a virtual stagnation in the marine fish production due to overexploitation.

Table 6. Contribution of Fisheries Sector to Gross Domestic Product in India (Rs in crores)

Periods	GDP	Ag GDP	Fish GDP	Percentage contribution of		
				Ag GDP to Total GDP (%)	Fish GDP to Total GDP (%)	Fish GDP to Ag GDP (%)
At 1993–1994 Prices						
1970-71	292560	133429	3043	45.61	1.04	2.28
1975-76	335887	143779	3741	42.81	1.11	2.60
1980-81	400164	156041	3947	38.99	0.99	2.53
1985-86	514108	185706	5229	36.12	1.02	2.82
1990-91	683688	219030	6919	32.04	1.01	3.16
1995-96	902559	260691	9807	28.88	1.09	3.76
2000-01	1204968	292772	11552	24.30	0.96	3.95
2002-03	1338952	299557	13059	22.37	0.98	4.36
At Current Prices						
1970-71	42279	19397	267	45.88	0.63	1.38
1975-76	76124	31595	567	41.50	0.74	1.79
1980-81	130386	49233	943	37.76	0.72	1.91
1985-86	250170	84327	2160	33.71	0.86	2.56
1990-91	512687	160788	4868	31.36	0.95	3.03
1995-96	1077959	314827	12184	29.21	1.13	3.87
2000-01	1915437	480337	22465	25.08	1.17	4.68
2003-04	2285382	531238	26321	23.25	1.15	4.95

Source: National Accounts Statistics – Various Issues

Capital formation and gross domestic product in fisheries

The share of fisheries capital formation to fisheries GDP had been increasing at a slower pace up to the year 1980-1981 and then continued to increase at a steady pace (Table 7). The proportion of total GFCF to total GDP was around 19% during 1970-1971 and 2002-2003 at constant prices. However, it had grown to 24% during 1980-1981 and stayed at same proportion up to 1995-1996 and then started declining. At current prices, this proportion was continuously increasing throughout the period from 1970-1971 to 2003-2004 except 1990-1991 and 2000-2001.

Table 7. Share of Capital Formation in Fisheries to Gross Domestic Product in India

(Percent)

Periods	GFCF to GDP	Ag GFCF to Ag GDP	Fish GFCF to Fish GDP	Fish GFCF to Total GDP	Fish GFCF to Ag GDP
At 1993-994 Prices					
1970-71	18.58	6.14	8.57	0.09	0.20
1975-76	20.40	6.54	9.96	0.11	0.26
1980-81	23.83	8.65	9.87	0.10	0.25
1985-86	24.45	7.13	11.74	0.12	0.33
1990-91	23.47	6.56	13.84	0.14	0.44
1995-96	24.57	8.00	15.37	0.17	0.58
2000-01	20.47	6.39	20.51	0.20	0.81
2002-03	19.05	6.71	22.01	0.21	0.96
Current Prices					
1970-71	15.24	4.88	14.73	0.09	0.20
1975-76	18.31	5.88	14.76	0.11	0.26
1980-81	22.68	7.65	14.07	0.10	0.27
1985-86	25.08	7.19	17.47	0.15	0.45
1990-91	24.50	6.80	16.80	0.16	0.51
1995-96	27.02	6.52	14.88	0.17	0.58
2000-01	18.33	5.98	14.21	0.17	0.66
2003-04	18.16	6.80	17.71	0.20	0.88

Growth in Capital formation and gross domestic product in fisheries

Table 8 reveals that the growth in fisheries GFCF has been maintaining a high level of around 9.5% during eighties and nineties. However, during seventies, the growth of fisheries GFCF was of the order of around 5.4%. If one considers the overall period

from 1970-1971 to 2003-2004, it was found that the total growth of fisheries GFCF was around 8%. This lower rate of growth over the whole period may be attributed to the declining trend of fisheries GFCF during seventies. On the other hand, the GFCF in the agriculture sector has been very low (2.8%) over the whole period *viz.,* 1970-1971 to 2003-2004. Although the growth in agricultural GFCF has been on the decline ever since 1990-1991 onwards, the growth in fisheries GFCF has maintained almost a uniform trend of 9.5%.

Table 8. Growth in Fisheries GFCF and GDP at Constant Prices (percent)

Particulars	Growth Rates of GFCF			Growth Rates of GDP		
	Total	Agri.	Fishery	Total	Agri.	Fisheries
Historical Period						
(1970-71 to 2003-04)	5.34	2.77	7.94	5.06	2.85	4.87
Decadal Period						
1970-71 to 1979-80	5.20	5.83	5.44	3.58	2.10	2.86
1980-81 to 1989-90	4.78	0.12	9.53	5.41	3.13	5.74
1990-91 to 1999-00	5.68	2.42	9.50	6.16	3.19	5.43
Liberalization Period						
1990-91 to 1995-96	6.62	7.94	9.51	5.72	3.57	7.46
1990-91 to 1996-97	7.05	6.71	9.50	5.99	3.45	7.15
1990-91 to 1997-98	6.84	4.89	9.51	6.13	3.47	6.55
1990-91 to 1998-99	6.49	2.56	9.51	6.16	3.38	5.90
1990-91 to 1999-00	5.68	2.42	9.50	6.16	3.19	5.43
1990-91 to 2000-01	5.20	1.76	9.49	6.12	2.99	5.15
1990-91 to 2001-02	4.71	1.71	9.49	6.07	2.98	5.07
1990-91 to 2002-03	4.01	1.61	9.49	5.97	2.63	5.09
1990-91 to 2003-04	4.20	1.92	9.54	6.00	2.74	5.07

Table 8 presents the compound growth rates in fish GDP during the last three decades as well as during the post liberalization period. It can be observed that historical growth in fish GDP was higher than the growth in Ag GDP. However, it was only

marginally lower than the growth in total GDP. The decade-wise analysis showed that growth in fish GDP was higher than the Ag GDP in all the three decades. However, growth in fish GDP was found to be higher only during eighties compared with total GDP. Decade-wise comparison of growth in fish GDP revealed that the growth was impressive during eighties (5.74%) and nineties (5.43%) in comparison with the seventies (2.86%). The study of growth in fish GDP during the liberalization phase reveals that during the early period of liberalization (1990-1991 to 1995-1996), there was a steady growth (7.46%) in contrast to preliberalization phase (1980-1981 to 1989-1990).

To see precisely in which year deceleration in fisheries growth started, the growth rates were estimated between fixed base 1990-1991 and extending the terminal year from 1995-1996 onwards. The results also reveal that the growth rate of fish GDP was the highest during the period 1990-1991 to 1995-1996. There is a continuous deceleration in the growth rate after this period till 2000-2001 beyond which the growth has almost stagnated at around 5.07%. No doubt, there was a deceleration in growth in fish GDP beyond 1990-1991: 1995-1996 period, yet the growth was higher than the growth rates in agriculture during the respective periods. Further, the rate of growth in total economy turned out to be higher than the growth in fisheries sector after the period beyond 1990-1991 to 1998-1999. It is important to note that during the period 1996-1997 to 2003-2004, the growth in fish production had decreased to 2.78% from 5.16%, which is the production during 1990-1991 to 1995-1996, and this decline can be attributed to the decline in marine fish production during the same periods, which decreased to 0.16% from 3.25%.

Relationship between fisheries gross domestic product and capital formation

Table 9 presents the results of log-linear relationship between fish GDP and GFCF in fisheries sector for different periods. The model explained the variation in fish GDP by capital formation in this sector to a considerable extent. GFCF was explaining the maximum variation in the GDP (to the extent of 99%), as this variable represents collectively the influence of all other variables like investment on fishing crafts and gears, investment on hatcheries, ponds development, and other infrastructural development variables like landing centers, cold storage facilities, transportation etc. The elasticity of fish GDP with respect to fish GFCF was found to be significant for all the periods under consideration.

Table 9. Elasticity of Fish GDP with respect to Fish GFCF during Different Periods

Period	Constant	Elasticity	t-value	R^2
Historical Period				
(1970/71-2003/04)	4.579	0.620	51.70	98.8
Decadal Period				
1970/71-1979/80	5.745	0.420	7.141	86.4
1980/81-1989/90	4.542	0.623	11.315	94.1
1990/91-1999/00	4.852	0.586	9.807	92.3
Liberalization Period				
1990/91-1995/96	3.366	0.796	15.936	98.4
1996/97-2003/04	5.867	0.451	7.002	89.1
1990/91-1996/97	3.433	0.787	22.018	99.0
1990/91-1997/98	3.796	0.735	18.369	98.3
1990/91-1998/99	4.53	0.632	9.387	92.6
1990/91-1999/00	4.852	0.586	9.807	92.3
1990/91-2000/01	5.081	0.554	10.606	92.6
1990/91-2001/02	5.144	0.545	12.462	94.0
1990/91-2002/03	5.127	0.548	14.801	95.2
1990/91-2003/04	5.176	0.541	17.108	96.1

The perusal of the table reveals that the response of fish GFCF to fish GDP was poor during seventies (0.42), whereas it increased during eighties to 0.62 and again decreased to 0.59 during the period nineties. To study this sudden decrease during the later period, the model was fitted for two subperiods *viz.*, 1990-1991 to 1995-1996 and 1996-1997 to 2003-2004. It was surprising to observe that during the first half of the nineties, there was a significant increase in the elasticity coefficient (0.8). This may be due to the effect of liberalization policies initiated in 1991. During this period, greater emphasis was laid on the development of inland fish production including brackishwater and freshwater aquaculture because of its export potential and high value. There was considerable private investment because of the favorable policy environment. However, owing to production mismanagement and regulatory problems, the production growth started tapering off and as a result, the elasticity coefficient decreased to 0.45 during the period 1996-1997 to 2003-2004. Further, to identify the year of deceleration in elasticity, the model was fitted between fixed base 1990-1991 and extending the terminal year

from 1995–1996 onwards. It was observed that the response of fish GFCF to fish GDP started decelerating from the year 1996–1997.

Conclusions

The study concluded that the central government has played an important role in the development of fisheries sector as there is a direct relationship between central sector schemes outlay and share of fisheries in agricultural outlay. The scheme-wise expenditure under different five-year plans depicts that the expenditure on fisheries development was impressively increasing over the various five-year plans. Although, the growth in fish GDP during the period 1990-1991 to 1995-1996 was steady (7.46%), there was a continuous deceleration in the growth rate after this period till 2000–2001 (5.07%).

Thus, there is an urgent need to increase the central outlay and utilize the allocated outlay efficiently under central schemes for the faster development of fisheries in the country to meet the growing domestic demand and to exploit the opportunities for becoming an important player in the global market. The investment in fisheries research has been increasing all through the plan periods, and the Government is giving some importance to this sector. However, there is still scope for more public investment in fisheries research to realize the potential gains of research. Further, increasing public and private investment is also needed for strengthening infrastructure for diversifying fisheries activities to enhance fish production, productivity, and export. Private sector investment in fisheries can also play an important role in seed and feed production, adopting existing technologies for higher production, human resources development, postharvest management, and marketing.

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Received: 23 November 2007; Accepted: 06 March 2009

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Open Water Farming of Pearlspot *Etroplus suratensis* (Bloch) in Low-Volume Cages

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Abstract

Pearlspot, *Etroplus suratensis* (Bloch) seeds of size 6.0-50.0 g were stocked @ 200 m^{-3} in low-volume cages of size 2 m^3 in open waters of mean depth 2 m and moderate water flow (> 0.05 $m.second^{-1}$) in Vembenad lake, on the south west coast of India. The fish was fed with 'Higashi' brand commercial sinking pellets of 20% crude protein. Average fish production was 26.76±9.308 (range 17.80-44.40) $kg.2\ m^{-3}$ in 205.3 ± 60.983 days. The stocked fish exhibited absolute growth rate of 0.50-0.90 $g.day^{-1}$ and maximum survival from 45 to 100%. The fish was observed to attain an average size of 163.76 ± 40.214g and a maximum size of 350-480 g at harvest. The specific growth rate ranged from 0.27 to 0.76% day^{-1}. Maximum production was achieved in cages with highest stocking density (230 m^{-3}), and the over all food conversion ratio was 3.52. Length–weight relationship indicated the general well-being of the cage-reared fish compared to that from natural catches. Net cages stocked with pearlspot were almost devoid of fouling and mesh clogging algae as the fish was observed to feed on the filamentous algae attached to the cage structure. These observations indicate the role of pearlspot as a 'scraping' species. Higher concentration of organic carbon in sediments and nutrients, especially nitrates in waters just under the cages indicates the essential need for restricting nutrient loading from cages to below the environmental carrying capacity.

Introduction

Sustainable intensification of food production through aquaculture calls for exploration of new systems of farming and diversification of species. In the context that water will be at a premium and its shortages are becoming critical, multiple use of water systems is also gaining attention world wide (FAO 2006). With reduction in facilities for production, the shift is from low-value species, such as cyprinids, to high-value species. Pearlspot, *Etroplus suratensis* is a high-value, non-tilapian cichlid, indigenous to peninsular India and Srilanka. It is a brackish water fish that has become naturally acclimatised to freshwaters. The fish feeds predominantly on filamentous algae and detritus in nature. Owing to good palatability, omnivorous feeding habits and hardy nature, it is greatly suited to aquaculture. Culture of commercially important fishes in enclosures in open water bodies is an accepted strategy that ensures high production

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and when adopted judiciously, it can promote parallel enhancement of natural fisheries (Hu & Liu 1997; Welcomme & Bartley 1997). The present study pertains to the evaluation of production performance of pearlspot, *E. suratensis*, in cage enclosures in open waters in the Vembenad estuarine system, on the south west coast of India.

Materials and Methods

Experimental cages were set up near Thaneermukkom and Muhamma in Vembenad lake (Lat. 9˚28´ & 10˚ 10´N and Long. 76˚13´ & 76˚31´E) on the south west coast of India, approximately 50 km south of Cochin near the National Waterway No. III (Fig. 1). Rectangular cages of size 1.70 x 1.20 x 1 m made of soft polythene webbing and hard HDPE square meshes of 15-17 mm were fabricated and used. The soft cages were kept in shape by sinkers and anchors. coconut reapers, which are considered water resistant, were used as cage frames for hard cages. The cages were fixed on to floating pontoons attached with a walkway in site I, Muhamma (M1, M2) and on bamboo rafts in site II, Thanneermukkom (T1-T8). The cages were positioned in open waters 500 m away from the shore and water depth 2 m, with moderate water flow (0.05 m.second^{-1}) and good wave action. The submerged volume of the cages was invariably 1 m^3. To avoid loss of feed pellets, the floor of the net cages was covered with 0.5 mm fine mesh netting. The cages were moored on to the lake bottom using cement concrete anchors of 25-30 kg. A cluster of eight replicate cages at site I and 32 cages at site II were used for the study. The rectangular cages were placed in such a way that the longer sides faced the water current, which effectively increased water exchange. To protect the cage installation from floating weeds, bamboo barricade was provided for each cluster of cages. Seeds of pearlspots, *Etroplus suratensis*, produced in the Regional Agricultural Research Station, Kumarakom were nursery reared to appropriate size in net cages (8 mm mesh) and were acclimatised to cage environment and stocked. A stocking density of 200 No. cage^{-1} was maintained in all the cages, and when the size of the seed was bigger, their number was reduced to rationalise the initial biomass. Cage mesh size was 15-17 mm, determined based on the head girth and the

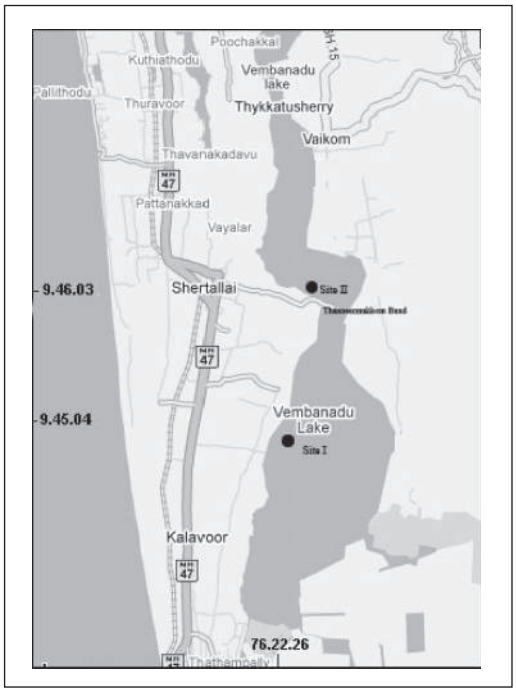

Figure 1. Experimental sites in the Vembanad lake

size of seed, following the formula, A = 0.026 x TL, where A is the stretched length of the mesh and TL is the total length of the fish fingerling. The stocking size of fishes varied from 6.0 to 50.0 g. Prior to stocking, all the fishes were disinfected by a prophylactic bath in povidone iodine solution (50 ppm) for 15 minutes. The stocked fishes were fed on 'Higashi' brand dry sinking pellets of size 2.5 x 5.0 mm and crude protein 20%. Daily feed was adjusted to size of fish, calculated at 5% of the biomass and at approximately 70-80% of satiation. Fish was trained to congregate near the feeding point, and feeding was performed manually twice a day. Every month the feed ration was modified after ascertaining the fish biomass and rate of consumption. As a protection from the sun, the top portion of the cage surface was covered by shading cloth. Fish was bulk harvested as it reached a minimum marketable size after 130-330 days. The survival rate, mean weight, specific growth rate (SGR = 100 x (*ln* mean final body weight - *ln* mean initial body weight)/duration), absolute growth rate (AGR; g/fish/day), production rate (kg.m^{-3}) and food conversion ratio (FCR) were determined. Average size of the fish was determined monthly by weighing and measuring 10% of the fish in each cage. Critical water quality parameters such as temperature, transparency, pH, dissolved oxygen (DO) and salinity were monitored at monthly intervals. Organic carbon content of the bottom sediments was estimated after Buchanan (1971) and nutrients, PO_4-P, NO_2-N and NO_3-N of the waters after Strickland & Parsons (1968). Water samples were collected from the cage site and open lake locations 1 km away from the cage installation for analysis. The primary productivity of the experimental plot was assessed by dark and light bottle method.

Results

Mean survival of fish in cages varied from 45% to 100% (Table 1). In a few cases, the retrieval exceeded the initial stocking density, owing to auto stocking of the cages by natural entry of seeds from the surrounding waters. The AGR of the fish in cages was 0.72 ± 0.141 (0.50-0.90 g. fish^{-1}.day^{-1}). The mean SGR was 0.50 ± 0.134% day^{-1} (0.27-0.76). The fish was observed to attain an average size of 163.76 ± 40.214 g (112-222g) and a maximum size of 480 g. The stocking density was not found to affect the mean size. Average fish production was 26.76 ± 9.308 kg/ 2 m^3 cage (17.80- 44.40) in 205.3 ± 60.983 (130-330) days. Fish yield of 38 kg/2 m^3/182 days was achieved at highest stocking density (230 m^{-3}). Final biomass increased to 44.0 kg.cage^{-1} when the fish was retained for a period of 330 days. Total production of fish in cages increased with the increase of initial biomass and stocking size. The overall FCR was 3.52 (2.91-4.61) in the replicated cages. The length–weight relationship of fish harvested from cages (Fig. 2) was compared with identically sized catches from the open lake (Fig. 3). It indicated that the cage-reared fishes were in a better condition compared with fish collected from natural catches.

Table 1. Growth performance of pearlspot, *Etroplus suratensis* in cages of Vembenad Lake.

Parameters	Site I		Site II							
	M1	M2	T1	T2	T3	T4	T5	T6	T7	T8
Cage size (m^3)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Stocking Density ($No.m^{-3}$)	110	230	200	200	200	200	200	200	200	200
Period of rearing (days)	166	182	130	130	197	207	214	231	266	330
Mean size at stocking										
Weight (g)	50.0	32.0	27.0	27.0	6.0	12.0	6.0	6.0	12.0	27.0
Length (cm)	9.0	8.0	10.1	10.1	6.3	7.8	6.2	6.3	7.8	10.1
Mean size at harvest										
Weight (g)	200.0	185.7	127.2	111.7	184	167	127	115	198	222
Length (cm)	20.0	19.0	15.2	14.2	17.7	18.0	15.8	16	16.3	18.9
Maximum size at harvest										
Weight (g)	350	325	200	210	410	320	275	225	300	480
Length (cm)	21.0	20.0	20	18.0	24.0	23.0	21.0	20	20	25
Total Biomass ($kg.cage^{-1}$)	24.10	38.00	20.9	19.4	31.3	32.7	21.1	17.9	17.8	44.4
SGR (%)	0.363	0.420	0.518	0.474	0.755	0.552	0.619	0.555	0.458	0.277
AGR ($g. fish^{-1}. day^{-1}$)	0.90	0.84	0.77	0.65	0.9	0.75	0.56	0.50	0.70	0.59

Critical water quality parameters such as water temperature, transparency, pH, DO and nutrient levels in the cage site did not show any significant variation compared with open lake location (Table 2). Water temperature varied from 27°C to 30.5°C at the cage site. The surface and bottom waters did not exhibit any significant difference, Secchi disc transparency fluctuated between 86.50 ± 35.41 and 114.25 ± 17.98 cm. Water was more turbid during monsoon, owing to monsoonal turbulence and riverine inflow. Similarly, transparency was also reduced during postmonsoon months, associated with increased algal production.

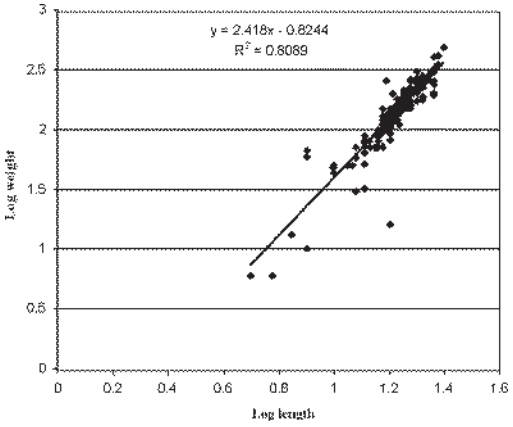

Figure 2. Length weight relationship of *E. suratensis* from cages

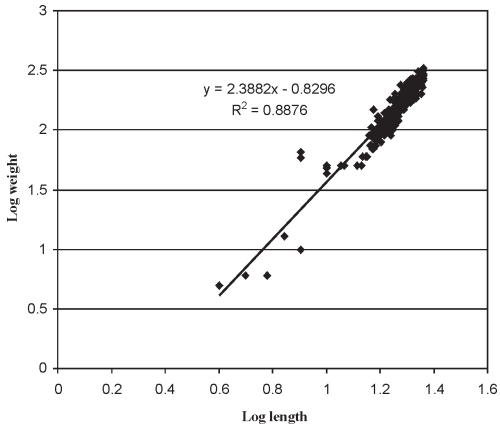

Figure 3. Length weight relationship of *E. suratensis* from natural water

Table 2. Water quality parameters in the cage culture sites and open lakes

Parameters	Cage culture site				Open lake	
	Site I		Site II			
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Depth(cm)	177.00 ± 20.03	135.0 - 190	219.3 ± 20.7	174 - 242	131.2±18.52	100 - 154
Transparency(cm)	86.50 ± 35.41	50.0 - 170.0	114.25±17.98	90 - 150	106.4±35.79	35.5 - 150
Temperature(^{0}C) Air	28.20 ± 0.42	28.0 - 29.0	30.0 ± 1.54	26.0 - 31.0	29.67±1.91	26.3 - 33.2
Water	27.30 ± 0.48	27.0 -28.0	28.50 ± 1.15	27.0 - 30.5	28.89±1.31	26.7 -30.8
pH	6.95 ± 0.16	6.5 -7.0	7.17 ± 0.25	7.0 - 7.5	6.58± 0.53	5.80 - 7.60
Salinity (ppt)	1.28 ± 1.26	0.06 -3.47	1.73 ±2.90	0.08-7.07	1.82± 2.16	0.06 - 6.42
Dissolved Oxygen(mg. l^{-1})						
Surface	8.37 ± 1.26	6.4 -10.1	8.48 ± 0.66	7.6 - 10.0	7.56±1.34	4 .0 - 8.6
Bottom	7.81 ± 1.37	6.4 - 9.9	8.04 ± 0.65	7.2 - 9.6	6.84±1.15	4.2 - 8.0
Primary productivity						
GPP (mg C. m^{-3}. $hour^{-1}$)	126.82±54.06	30 - 210	96.11± 37.89	53.33 - 154.29	66.84±32.82	24 - 120
NPP (mg C. m^{-3}. $hour^{-1}$)	65.38±52.68	15 -202.5	44.31± 27.30	13.32 - 90.57	43.77±26.26	0 - 80.0
Organic carbon (%)	1.06±0.65	0.06 - 1.93	2.60± 2.45	0.72 -6.64	1.37±0.68	0.3 - 2.7

The DO levels at the cage site were moderate with monthly variation ranging from 6.4 to 10.10 mg. l^{-1} for surface and from 6.4 to 9.9 mg. l^{-1} in bottom waters. In the open lake locations, DO varied between 4.0 and 8.6 mg. l^{-1}. Salinity ranged from nil to 3.5 ppt during the period of study. Salinity variations were less pronounced at site I situated upstream the salinity regulator at Thanneermukkom. In site II, located on the seaward side, salinity levels were high and variations more pronounced Organic carbon percentage in sediments below the cages was found to increase perceptibly, and it ranged from 1.06 ± 0.65 to 2.60 ± 2.45%. Organic content in sediments at the cage site increased perceptibly from initial levels, 0.5 to 2%. The gross primary productivity at the cage sites was also higher (96.11 ± 37.89 to 126.82 ± 54.06 mg C. m^{-3} $hour^{-1}$) than that in the open lake location (66.84 ± 32.82 mg C. m^{-3} $hour^{-1}$) away from cages. Nitrite concentration in water increased from 0.006 to 0.068 µg. l^{-1}, during the culture period. However, the maximum concentration of nitrite at site I (4 µg. l^{-1}) was lesser than its highest concentration (28 µg. l^{-1}) at the adjacent open lake location, observed during monsoon. The nitrate concentration also increased sharply at the cage site during the farming period compared with its initial levels, its maximum concentration was 15.2 µg. l^{-1} compared with 4.0 µg. l^{-1} at the adjacent open water location.

Discussion

Growth performance of pearlspots (*E. suratensis)* to an average size of 200 g in 166 days and maximum size of 350 g is remarkable in the context that pearlspots are generally considered slow-growing species, growing hardly to 120-130 g in pond conditions (Thampy 1980). Apparently, unlike in ponds, the production capacity of fish in cages is not limited by water quality or feed inputs (Rowland et al. 2004). The attainment of an average size of 127 g in 130 days in open water cages indicates the versatility of pearspots for cage culture. Apparently, production of fish in cages increased with increase in initial stocking size and stocking density. The results indicate that even at low stocking densities, higher stocking size results in a reasonably better fish yield. This implies that for maximizing production in cage farming, it is essential to ensure an optimum individual size and biomass. The results show that an initial stocking size of 30-50 g will be good if the commercial target is to market pearlspot at 200-250g in 6-8 months. Survival rates are also very good when stocking size is high. The observed biomass gain of 0.9 $g.day^{-1}$ for pearlspot under cage culture in open waters is apparently linked to high stocking density employed in the study. High stocking rate coupled with heavy feeding contribute to enhanced production in cage fish farming. The results also reveal that pearlspot adapts well to captivity in low-volume cages. Probably being a schooling species, the fish tolerate such crowding in cages.

The net cages stocked with pearlspots were almost devoid of algal growth and mesh clogging, a common problem encountered in cage fish farming. This is apparently due to the algal browsing behaviour of pearlspots. Studies on food preference of

E. suratensis (Devaraj et al. 1975; De Silva et al. 1984; Keshava et al. 1988; Jayaprakas & Padmanabhan 1985; Bindu & Padmakumar 2008) indicate that the fish is essentially an algal grazer subsisting mostly on filamentous algae and detritus. Probably, the cage enclosures functioned as a substratum for the growth of filamentous algae, which became an additional source of food for the fish in cages. The fish apparently consumed large quantities of mesh clogging algae by nibbling or scrapping (Keenleyside 1979; Yamaoka 1991). The semicircular nature of the mouth and minimum protractibility of the lips are adaptations for such a complex food capturing process (Geetha et al. 1990). This observation shows that pearlspots can be employed as a 'scraping' species in cage culture systems.

Earlier studies on cage farming in India were mostly exploratory, and the species used were either carps or murrells (Parameswaran 1993). Being a high-valued species, locally fetching almost six times market value compared with carps, the study points to the immense possibilities of farming of pearlspot in net cages. Unlike carps, *Etroplus* is not a jumping species, and being very gentle they do not damage the cage netting during sampling or at harvest. The laterally compressed body shape foils easy escape of the fish from cages and also permits larger mesh size and a better water turn over for the cages. From the length–weight relationship of this species, it obvious that the cage-reared fishes attain a higher biomass increment (b = 2.418; $p<0.05$) with reference to length compared with fish from natural waters (b = 2.388; $p<0.05$). Apparently, this is a reflection of the superior well-being and adaptability of the fishes to cage farming.

Accumulation of organic matter and decomposition of feed residues affect the oxygen availability and oxygen consumption of the fish in cages (Jiwyam & Chareontesprasit 2001). The DO levels and transparency have been reported to go very low levels near cages (Vargasmachuca et al. 2007). In the present study, high oxygen levels observed at the cage site indicates that caged fishes were not constrained by oxygen in such large open waters. The DO concentration in fish cages was apparently dependant on rate of exchange of water (Li & Xu 1988). In sediments below the cage, the organic carbon percentage was found to increase from its initial levels and so also the nitrate concentration in the waters. This has contributed to high primary productivity in the lake waters. It could therefore be inferred that in oligotrophic water bodies cage culture shall be of help to enhance fish production while in eutrophic waters, cage farming beyond limits can lead to severe problem of pollution. In common carp cages at Philippines, Beveridge (1996) observed 90% of the phosphate was lost to waters. In Chinese cage culture system, Li (1994) reported that 27% of the N and 14% of P introduced as fish feed were only utilized by the fishes. In salmon cage culture in Europe, Kautsky & Folke (1989) reported that 75% of N and 77% of P introduced as feed were reportedly lost to the water. Beveridge (1984) put forth a predictive model for fish production by keeping water quality within acceptable limits. This indicates the dire need to maintain an appropriate cage-to-open water ratio, for restricting nutrient loading.

Experiments on cage culture in India have been mostly exploratory and the yield rates range from 0.7 to 1.3 kg.m^{-3}.month^{-1} (Bandhyopadhyay 2003) and a net fish production of 16.03 kg.m^{-3}.year^{-1} (Kumaraiah 2006) has also been reported. However, very high productivity has been reported by authors elsewhere (Li 1994; Hu & Liu 1997; Zainal & Effendi 1998).

The relatively promising performance of pearlspot in the present study is apparently due to species attributes, superior quality of the feed utilized and the high environmental resilience offered by the vast water body. The food conversion efficiency achieved in the present study is comparable to that reported earlier in India (Dehadri 1975; Kumaraiah et al. 1986; Sukumaran et al. 1986). When feed with higher protein percentage (35-40%) is utilized, over all FCR up to 1.3-2.0 has been reported by some workers (Luchini & Quiris 1990; Rowland et al. 2004). The impressive growth performance of pearlspots indicates the tremendous potentials of this species for cage farming in open waters. Being a brand cuisine of the backwater tourism and with high market demand, cage culture of pearlspots offer great promise. The stimulated algal production in waters outside the cages though in very small scale warrants the need for setting limits while popularizing commercial cage farming.

Acknowledgement

We place on record our deep sense of gratitude to Mr. K.R. Viswambharan I.A.S. Hon. Vice Chancellor, Kerala Agricultural University for encouragement and support. We are deeply indebted to Dr. D. Alexander, Director of Research, Kerala Agricultural University for whole hearted support and encouragement. We are grateful to Kerala State Council for Science Technology and Environment (KSCSTE) for financial support.

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Received: 31 December 2007; Accepted: 11 November 2008

Asian Fisheries Society, Selangor, Malaysia
Available online at www.asianfisheriessociety.org

Mapping of Fisheries Resources at *Panchayat* Level using GIS

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Abstract

The availability of information on resources is of primary importance in the planning and development process and for taking appropriate policy decisions. This is more apt in ecologically fragile areas like the coastal zones. The vulnerability of the coastal zone was evident through the devastating impact of the Tsunami in 2004. To make the process of retrieval and analysis of the data more perceivable, interactive, and easy to visualize, it is ideal to put it on a Geographic Information System (GIS) platform. This article highlights the development of GIS-based fisheries resource maps of Chellanam *panchayat* in the Ernakulam district, Kerala, India. The information incorporated in this map was generated using GPS and field level surveys and analyzed and interpreted using suitable geoprocessing tools of ArcGIS, an *ESRI* software of GIS, used for the development of the GIS models. In all, three resource specific maps were developed besides other figures and tables, generated out of survey data and of GIS analysis.

Introduction

An Information System is the systematic and organized way of providing information that can aid the decision making process. The system utilizes computer hardware, software, manual procedures, models etc. for analysis using databases. In short, Information System is the organized collection of computerized data stored in such a way that dissemination of information from the system will be rapid, accurate, and timely (Burrough 1986). This enable the users in decision making, which is based on comprehensive scientifically collected and analyzed data. In short, Information System is the culmination of development of databases of the information generated for a system using hardware, software, procedures, and data pertaining to the system.

Geographic Information System (GIS) is one such information system built on spatial and nonspatial data. Spatial data require geographical references and projections to make the data meaningful. GIS provides information in a geographic platform so that the information of an earth surface will be more precise and meaningful and easy to visualize (Goodchild et al. 2007). GIS is the totality of all process of the development of the information right from data collection. Although the concept of GIS is not new, the computerization of geographic information is of recent origin.

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Thus, GIS is the combination of spatial and nonspatial data, analytical methods, computer hardware, software, and personnel; these are all organized to automate, manage, analyze, update, and deliver information in a meaningful geographic platform.

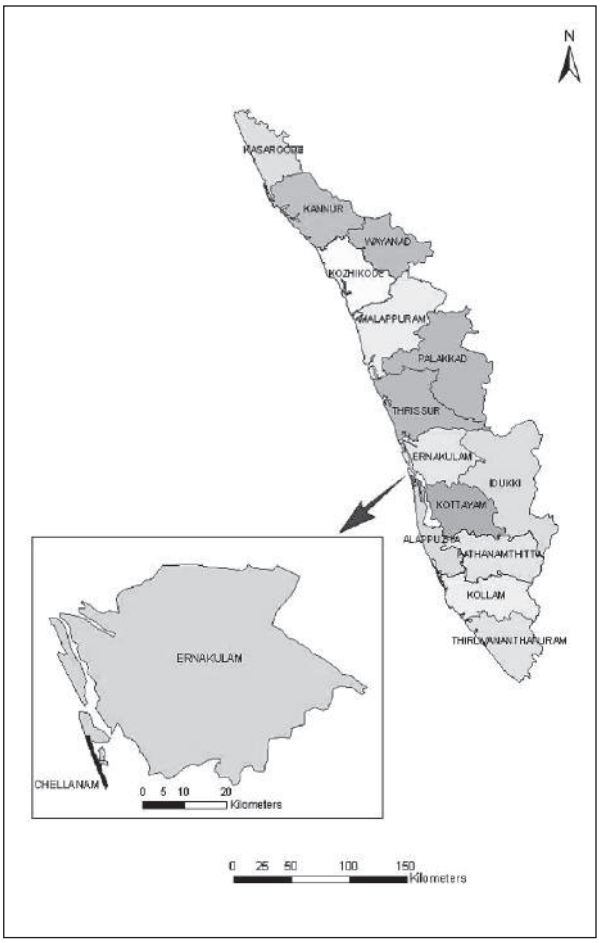

Figure 1. Chellanam grama Panchayat : Location Map

Decentralization of power and micro level planning with people's initiative and participation in the development process has been in focus since the eighth five-year plan in India. The 72nd and 73rd Amendments of 1991 in the Constitution of India ensure the establishment of *Panchayat* Raj system of administration at the district, block, and village levels (Chattopadhyay et al. 1999). A *panchayat* is a village level governance unit. The *panchayat* is supposed to be empowered with respect to preparation of plan for social and economic justice and implementation of plans to achieve these goals. This task of plan preparation and execution requires detailed technical and socio-organizational and economic input at various levels. Information with regard to resource availability, resource use, information gaps, constraints and potentials need to be generated at different levels for various plans (Dalal-Clayton and Dent 2001). An initiative in the direction of resource mapping at village level was taken up in 1990s in the state of Kerala, India in collaboration with various organizations. A pilot project was financially supported by the Department of Science and Technology, Government of India to cover 25 *panchayats* across the state of Kerala to shape the methodology. The program emphasizes the importance of warehousing of information at *panchayat* level for all planning at grass root level. A project on resource mapping of locations, which has bearing on national heritage in the Ernakulam district of Kerala, India, was also carried out in a GIS platform by Centre for Studies in Culture and Heritage, Cochin (Pisharody et al. 2005).

It is estimated that 60% of the rural poor in India are inhabited in the coastal areas. This emphasizes the fact that development of resource database at *panchayat* level, especially in the vulnerable coastal areas deserves high degree of priority in any national level developmental planning in general and fisheries development programs in particular. The devastating impact of Tsunami in 2004, along the coastal belt of South India has focused on the need for generating a resource database of coastal villages.

The present study is an attempt to generate an information base of natural resources and man-made infrastructure facilities pertinent to fisheries development of Chellanam *panchayat*, a coastal *panchayat* situated in the south–west part of Ernakulam district of Kerala, India (Fig. 1) using GIS technology.

Materials and Methods

The study was conducted in 2006–2007. Secondary data regarding the population of the *panchayat* and boundary details were collected from secondary sources like the Chellanam *Panchayat* Office. The area was identified and the boundaries of Chellanam *Panchayat* as in 1988 were demarcated from the topographic map (toposheet) of 1993 (No.58/C/5/SW and 58/C/5/NW of Survey of India on scale 1:25000). Boundary points of the *panchayat* were located after discussion with the village officials. Based on the toposheet and the details of the border points collected through field survey, the boundary map and other resource maps of the Chellanam *panchayat* were digitized and created in GIS platform as given below.

Map creation and Vectorization

The toposheets of 1993, which include Chellanam *panchayat*, were scanned and the boundary map of Chellanam *panchayat* were digitized with the help of Identified control points and boundary points. The map was georeferenced with the help of toposheet and some landmark points of the *panchaya*t whose latitude and longitude values were collected using Global Positioning System (GPS), so as to assign each point of the area the corresponding latitude and longitude using the georeferencing tools in ArcGIS 9.0. Thus, base map was prepared by vectorizing different thematic layers like landing centers, infrastructural facilities (fish processing centers, markets, etc.) roads, canals, land-water resources (land use) etc.

Development of Database

A personal geodatabase was created using the *Arc Catalog* application of ArcGIS software to warehouse all spatial and attribute data pertaining to the *panchayat*. Feature dataset is created in the geodatabase. Different feature classes with polygon, line and

point geometries were also created in the feature data set as per the requirements of each theme. The study area boundary, land-water resources (landuse of the *panchayat area)*, were vectorized as polygon feature classes, the roads and canals as line feature classes and resource points like landing center, processing units, hatchery units, markets, etc. as point feature classes. These feature classes were vectorized using the *ArcMap* tools of ArcGIS. With the help of GPS handset, the fisheries resource points (fish landing centers) and fisheries infrastructure points (man-made infrastructure facilities pertinent to fisheries development) of Chellanam *panchayat* were located by measuring the latitude and longitude values of the geographic position of the respective resource. The corresponding attribute data for each feature class were collected through field level survey and were compiled and fed into the geodatabase of the GIS model. The topological rules were applied to the datasets and validated and the errors were eliminated by editing the erred feature classes. These feature classes of the feature data set incorporated in the

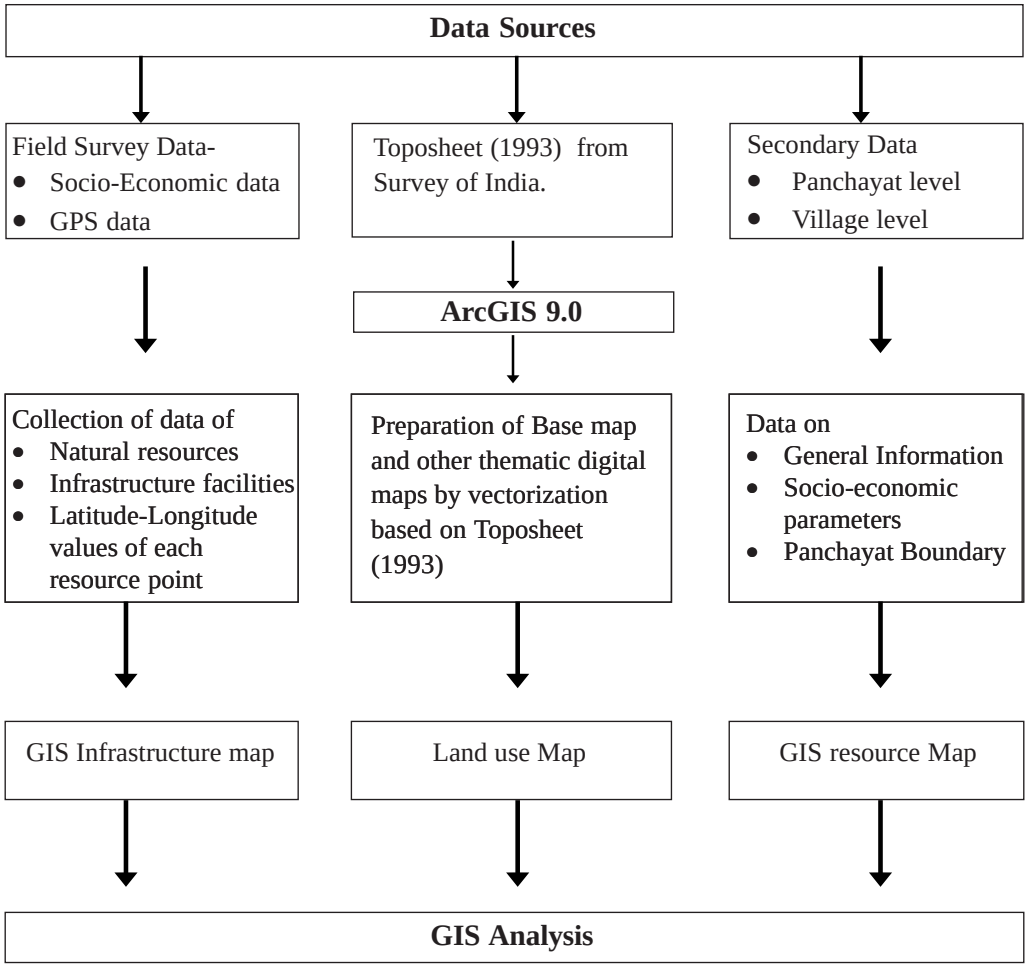

Figure 2. Flow Chart showing development of GIS model

personal geodatabase is used to develop the GIS resource models *viz.*, land-water resource maps, fish landing center map and infrastructure map of Chellanam *panchayat*.

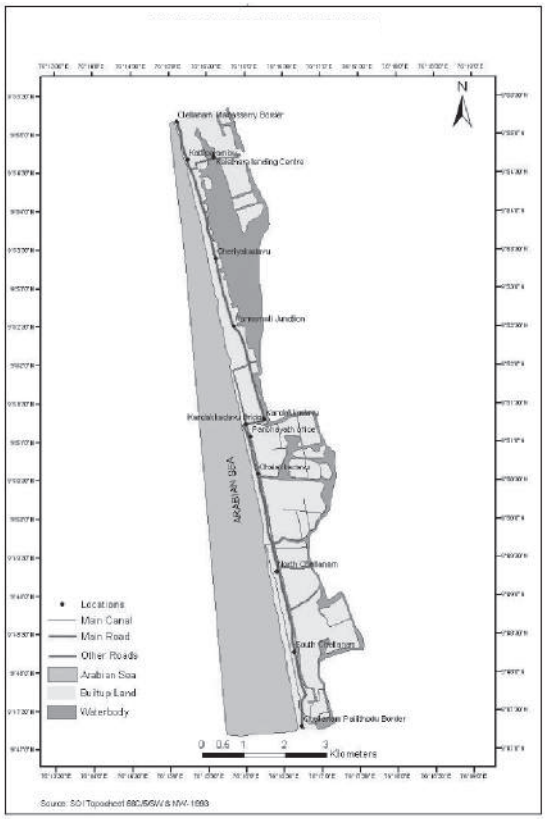

Figure 3. Chellanam Grama Panchayat : Study Area.

GIS Analysis

Based on the landuse map generated, the total area of the Chellanam *panchayat*, the land and water resources of the *panchayat* etc. were estimated quantitatively using GIS analysis tools of ArcGIS. ArcGIS software developed by Environmental Systems Research Institute, Inc. ESRI (Inc.), USA is versatile GIS software, which has different software tools to develop various GIS models and to carry out suitable GIS analysis to generate complete information about the geographical area under study. The procedure adopted for this study has been diagrammatically represented in the flow chart (Fig. 2).

Results and Discussion

Chellanam *panchayat* (Fig. 1) is a coastal strip with an area of 19.37 sq.km situated in the south west coast of Ernakulam district of Kerala, India (Anon 2006-07), with latitude 9° 47'- 9° 56'N and longitude 76° 15' -76° 17'E. It has a coastal length of 16.5 km and the breadth about 1.5 km. (Anon 2006-07). It is flanked by the Arabian Sea in the West and Kumbalangi and Ezhupunna *Panchayat*s in the East and shares boundaries with the Cochin Corporation in the North and Alappuzha district of Kerala in the South (Anon 2006-07). It is a typical coastal *panchayat* endowed with natural resources like water bodies, canals, and low lying paddy fields used for fish farming. The *panchayat* area map created after vectorization and overlaying necessary thematic layers developed in the GIS platform is presented in Fig. 3.

Similarly, a fisheries resource map showing the 9 fish landing centers of Chellanam

panchayat was developed.

Infrastructure map showing the major man-made fisheries infrastructure facilities available in the *panchayat* was also mapped. In all 19 establishments that included fish processing units, markets, hatcheries, drying units, and fishing implements and accessory stores were mapped. The map is presented in Fig. 4.

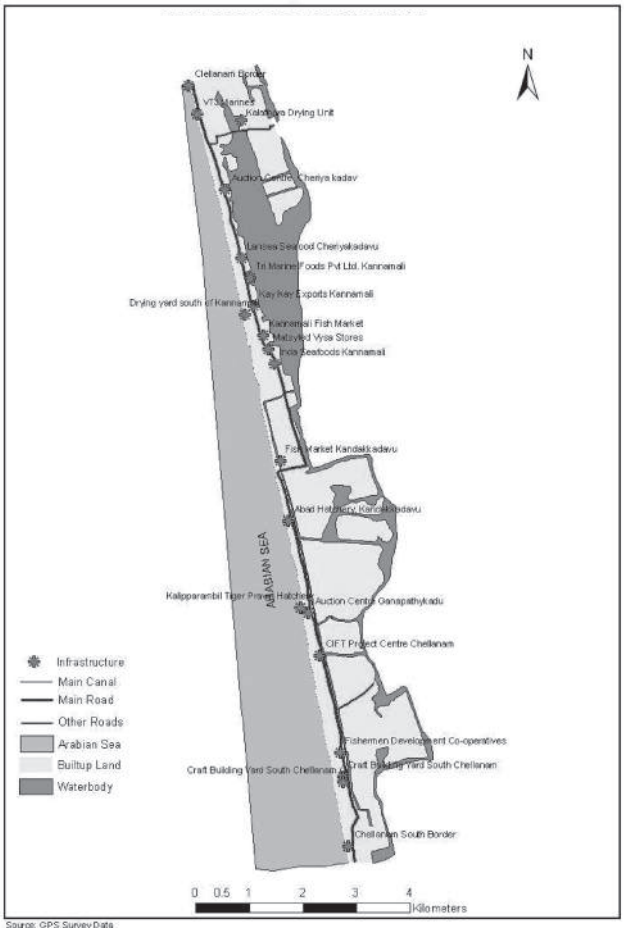

Figure 4. Chellanam Grama Fisheries infrastructural facilities

A land-use map showing the major land and water resources of the *panchayat* were also generated based on the data pertains to the year 1988. The total area of the Chellanam *panchayat* during 1988 was estimated using GIS analysis. The land and water resources, which had been classified in the categories *viz.*, Built-up area/ Mixed crops, Paddy field/ aquaculture area, and water body, as per the top sheet, were also estimated. The analysis estimated that the Chellanam *panchayat* has a total area of 19.32 sq.km of which the built-up/mixed crop area is 5.85 sq.km, water body is 5.53 sq.km, and paddy field/aquaculture area is 7.95 sq.km (Table 1).

The land-water resources *viz.*, Built-up area/Mixed crop, Water body, and Paddy field/aquaculture area are, respectively, 30%, 29% and 41% of the total area of the *panchayat*. This figure shows that besides marine capture fishery, Chellanam *panchayat* has immense potential of aquaculture fisheries also, as majority of the land use is paddy field/aquaculture.

Table 1. Distribution of Land and Water Resources of Chellanam *Panchayat*

Land and Water Resources	Area (km^2)
Built up Area/Mixed crop	5.85
Water body	5.53
Paddy field/aquaculture area	7.95
Total Area	19.32

Conclusion

Through this study, a digital map of Chellanam *panchayat* in the Ernakulam district of Kerala state in India was developed. The major advantage of such a map over conventional paper maps is immense. The different resource points of the *panchayat* can be visualized and it is also useful in estimating different natural resources. The models developed can also be used to do further spatial and geographic analysis. The changes in resource use, especially land and water, can be studied by comparing the existing pattern with the toposheet estimated one. A number of need-based maps can be generated that can be used for appropriate modelling studies to catalyze viable fisheries developmental programs of the *panchayat* area. Constant updating of the data into the geodatabase can help in updating maps as well, that can be tailored to meet location specific needs, including development needs. Several value added information systems like Decision Support System (DSS), Management Information System (MIS), and Expert System (ES) to facilitate micro level planning of development programs of the geographical area under study can also be generated.

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Received: 31 December 2007; Accepted: 24 February 2009

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(proceedings)	Ronquillo, I.A. 1974. A review of the roundscad fishery of the Philippines. Proceedings Indo-Pacific Fisheries Council 15 (3):351-375.
(book chapter)	Smith, I. and K.C. Chong. 1984. Southeast Asian milkfish culture: economic status and prospects. *In*: Advances in milkfish biology and culture (ed. J.V. Juario, R.P. Ferraris and L.V. Benitez), pp. 1-20. Island Publishing House, Manila.
(journal article)*	Widodo, J. 1991. Maturity and spawning of shortfin scad (*Decapterus macrosoma*) (Carangidae) of the Java Sea. Asian Fisheries Science 4:245-252. * Journal titles should be given in full.

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