Biology of Flat Toadfish, *Colletteichthys dussumieri* (Valenciennes, 1837) of Cochin Estuary

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in
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Declaration

I hereby declare that this thesis entitled "**Biology of Flat Toadfish, *Colletteichthys dussumieri* (Valenciennes, 1837) of Cochin Estuary**", is a genuine record of the research work done by me under the scientific supervision of **Dr. K. Y. Mohammed Salih,** Professor Rtd, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Kochi- 16 and that this has not previously formed the basis of the award of any degree, diploma or associateship in any University.

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Certificate

This is to certify that the thesis entitled "**Biology of Flat Toadfish, *Colletteichthys dussumieri* (Valenciennes, 1837) of Cochin Estuary**", is an authentic record of the research work carried out by Smt. Roja Sebastian, under my scientific supervision and guidance in the School of Marine Sciences, Cochin University of Science and Technology, in partial fulfillment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology and that no part thereof has been presented before for the award of any other degree, diploma or associateship in any University.

Prof. Dr. K.Y. Mohammed Salih
Supervising Guide

Dedicated to the memories of

My loving Daddy

whose Love, Prayers, Sacrifice, Care & Support

have enabled me to pursue and fulfill my dreams…..

"**Dad,** your guiding hand on my shoulder will remain with me forever."

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Roja Sebastian

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Chapter 1

GENERAL INTRODUCTION

Contents

1.1 Introduction

Life evolved in the oceans and consequently, the diversity of taxa that live there is enormous. Among these, fishes have been ecological dominants in aquatic habitats through much of the history of complex life. They are excellent showcases of the evolutionary process, exemplifying the intimate relationship between form and function, between habitat and adaptation. By any measure, fishes are among the world's most important natural resources. Additionally, with over 25,000 known species, the biodiversity and ecological roles of fishes are being increasingly recognized in aquatic conservation, ecosystem management, restoration and aquatic environmental regulation (Ormerod, 2003).

Cochin estuary, a part of the extensive estuarine system of backwaters on the south west coast of India, is a tropical positive estuarine system which is situated at the tip of the northern Vembanad Lake, and is the largest estuary in the state of Kerala, extending between 9º 40' and 10º 12' N and 76º 10' and 76º 30'E with its northern boundary at Azheekode and southern boundary at

Thannirmukkam bund. The salinity gradient in the Cochin Backwaters supports diverse species of flora and fauna, according to their tolerance for saline environment. This tropical estuary with high productivity acts as nursery ground for many species of marine and estuarine fin fishes and molluscs and crustaceans. Low lying swamps and tidal creeks, dominated by sparse patches of mangroves with their nutrient rich physical environment, support larvae and juveniles of many commercially important species. The areas of backwaters with fine sediments and rich organic matter supports abundant and diverse benthic fauna. According to the influence of the southwest monsoon and other associated meteorological conditions, the year may be conveniently split into three well-defined periods with characteristic hydrographic conditions i.e., monsoon (June – September), post-monsoon (October - January) and pre-monsoon (February – May). The changes in the hydrology of backwaters controlled by the seasons play an important role in regulating the migrant fauna of the estuary (Menon *et al*., 2000). The fishes of tropical estuaries are subject to a range of interactions of physical-chemical and biological processes that determine their patterns of occurrence, distribution and movement (Blaber, 2000). Hence it is desirable to study the various aspects of its biology.

The biology of fish, and in particular its growth and reproductive biology, has been the subject of vast study for many decades. In recent years, 4000-5000 original research papers have been published annually in over 400 journals covering all aspects of fish biology (Cvancara, 1992). Knowledge of fish biology, and the principal factors which determine growth and body composition, is important when considering the role of fish as a source of nutrition.

The Batrachoidiformes commonly referred to as toadfishes (or frogfishes in Australia), are a group of small to medium-sized bottom dwelling fishes which inhabit the warmer waters of coastal regions of America, Europe, Africa and India. They are found worldwide between about 51ºN and 45ºS along continents in marine and brackish waters, occasionally entering rivers, with several freshwater species in South America. They are found from the shoreline down to a depth of at least 366 m, often lying buried in the sand or mud, under rocks or coral heads and debris, hiding in crevices and burrows, where they function as ambush predators feeding on crabs, shrimps, molluscs, sea urchins and fishes. Toadfishes are hardy and are able to survive for hours after being removed from the water. They are also experts in camouflage. Their ability to change colour to lighter or darker shades at will and their mottled pattern makes them difficult to see. Toadfishes are said to be quiet vicious and will snap at almost anything upon the slightest provocation. Toadfishes do not school, but they are gregarious and tend to congregate together (Halstead, 1970). They have limited dispersal ability because of their demersal eggs which lack pelagic larvae. Compared to other fishes, they are sluggish in nature.

Though toadfish are not commercially exploited, they are consumed on a small scale by local fishermen but usually end up as a source of fishmeal and oil. A few smaller toadfishes from brackish-water habitats have been exported as fresh-water aquarium fishes. Some batrachoids are venomous. However, the greatest interest of these fishes to biotoxicologists is their unique and highly developed venom organs (Halstead, 1970). Some Batrachoid species have traditionally been used as laboratory animals in the field of physiology (Hopkins *et al.*, 1997; Gilmour *et al*., 1998; Perry *et al*., 1998; Paert *et al*., 1999), toxicology (Gutierrez *et al*., 1978; Sinovcic *et al*., 1980; Sarasquete *et al*.,1982), ethology (Ament *et al*.,1997; Bass, 1998), neurobiology (Rabbitt *et al*., 1995; Fine *et al*., 1996; Hirsch *et al*., 1998), cardiology (Benitez *et al*., 1994a, b; Coucelo *et al*., 1996), biomedicine (Lopes-Ferreira *et al*., 2000, 2004; Smith and Wheeler, 2006) and endocrinology (Fine *et al*., 1996; Knapp *et al*., 1999). Toadfishes are one of the best-studied groups for understanding vocal communication in fishes (Rice and Bass, 2009). The scientific demand for toadfish has spawned to what may be the world's smallest fishery (Mensinger and Tubbs, 2006).

The flat toadfish, *Colletteichthys dussumieri* (Valenciennes, 1837) is a sedentary and solitary species that lives partly buried in soft sand and mud or concealed in rock crevices, in coral reefs or in sea grass or weedy bottoms and in tidal pools (Randall, 1995). They are found in the Persian Gulf and along the coasts of Pakistan, India and Srilanka (Greenfield, 2006). They prefer high saline waters (Kurup and Samuel, 1985). Though they have no commercial importance in fisheries, but significantly sound management of vegetated coastal resources relies on the basic knowledge on the biology of the species, including information on population structure. Such information influences the development of management strategies and strategies for conserving biodiversity. Moreover, the flesh of *C. dussumieri* is said to have ethno-medicinal uses for the cure of asthma (personal information). Aim of the present study is to provide the first detailed information on various aspects of biology of the species, *Colletteichthys dussumieri* of Cochin estuary.

1.2 Literature review

Till recently most of the publications on the toadfishes refers to taxonomy and systematics and few reports on biological aspects of some species. Aside from references to *Colletteichthys dussumieri* in purely systematic papers (Greenfield, 2006; Greenfield *et al.*, 2008) and another regarding morphometrics (Roja *et al*., 2010), no information is available on any of the aspects of the species. The natural history of only four species has been studied in any detail: *Opsanus tau* (Gudger, 1910; Gray and Winn, 1961; Wilson *et al.*, 1982), *Opsanus beta* (Breder, 1941; Tavolga, 1958, Serafy *et al.,* 1997, Malca *et al*., 2009), *Porichthys notatus* (Hubbs, 1920; Arora, 1948) and *Halobatrachus didactylus* (Palazon-Fernandez *et al*., 2001; Pereira *et al*., 2011), the biology of *C. dussumieri* remains unknown.

Taxonomy forms the very basis of all biological research. Taxonomic documentation is only the first step in understanding our biodiversity. In fact, it is the step without which other research is impossible. Most information about toadfishes refers to taxonomy and systematics and some of the representative publications are those of: Collette, 1966; Greenfield and Greenfield, 1973; Collette and Russo, 1981; Greenfield *et al*., 1994; Collette, 1995; Randall, 1995; Greenfield, 1996; Greenfield, 1997; Greenfield, 1998; Greenfield, 1999 and Collette *et al*., 2006.

Biometric studies are useful for the identification of a fish species and for detecting variations in the fish population. Biometry reflects the proportionate growth of different body parts and the influence of environmental factors in a particular habitat. Roja *et al*. (2010) observed discrepancies in meristic and morphological characters of *C. dussumieri* from estuarine waters of India. Dovel (1960) studied the variation in size and morphological changes that take place during the prolarval growing period and metamorphosis to the young stage of *Opsanus tau*. Costa *et al*. (2003), analysed the Lusitanian toadfish, *Halobatrachus didactylus* from six different localities in terms of morphometric and meristic characters in order to investigate the hypothesis of population fragmentation on the

Portuguese coast. Marques *et al*. (2005) studied the variation in bilateral asymmetry of the Lusitanian toadfish along the Portuguese coast. Argyriou *et al*. (2006) recorded the morphometric characters of *H. didactylus* from waters of the Ionian Sea, Western Greece. Marques *et al*. (2006) assessed the differentiation of *H. didactylus* along the Portuguese coast considering morphological characters (20 morphometric and 16 meristic) and genetic markers (10 allozymes, 11 loci).

Food and feeding habit of the fish in the estuary is of great importance to understand their niche, behavioral patterns, life history, growth and management of commercially important fisheries (Bal and Rao, 1984). A few scientists have dealt with the aspect of food composition and feeding habits of toadfishes. Hubbs (1920) reported that the nocturnally active toadfish, *Porichthys notatus* feed on small crustacean larvae, other zooplankton and small fishes. Linton (1901) noted that alimentary canal of *Opsanus tau* was chiefly filled with crustacean and molluscan remains and the bones and scales of fishes. Gudger (1910) reported that *O. tau* had more preference for blue crab. The food and feeding habits of oyster toadfish near Solomons was assessed by Schwartz and Dutcher (1963). Food habits of *O. tau* in New Jersey waters were studied by McDermott (1965). Feeding and growth by the sessile larvae of *Porichthys notatus* was investigated by Crane (1981). Wilson *et al.* (1982) analyzed the feeding habits of the oyster toadfish, *Opsanus tau* in South Carolina. Hoffman and Robertson (1983) studied food and feeding habits of two Caribbean reef toadfishes namely, *Amphichthys cryptocentrus* and *Sanopus barbatus.* Granado and Gonzalez (1988) studied the dietary habits of *Amphichthys cryptocentrus*. Mensinger and Tubbs (2006) examined the effects of temperature and diet on the growth of captive year 0 specimens of *Opsanus tau*.

Detailed investigations on the reproductive biology of a few species of toadfishes are available from different geographical localities. The functions and histology of the yolk-sac of the young toadfish, *Batrachus tau* was studied by Ryder (1890). Gudger (1910) gave a detailed description on the fertilization and embryonic development of oyster toadfish, *Opsanus tau.* Observations on the habits and early life history of plain midshipman (Batrachoididae), *Porichthys notatus* was made by Arora (1948). Hoffman (1963**)** gave a detailed investigation of the gross and microscopic anatomy and seasonal changes of the reproductive system of male toadfish, *Opsanus tau*. While studying the reproductive ecology and sound production of the toadfish, *Opsanus tau,* Gray and Winn (1961) found a protracted spawning season of the species in the Chesapeake Bay. Hoffman (1963) also analysed the accessory glands and their ducts in the reproductive system of the male toad fish, *O. tau*. Hoffman and Robertson (1983) studied the foraging and reproduction of the two Caribbean Reef toadfishes, *Amphichthys cryptocentrus* and *Sanopus barbatus*. According to them, egg size and number of eggs in the ovaries of the species were similar to those of other toadfishes. Granado and Gonzalez (1988) studied the reproduction and larval development of *Amphichthys cryptocentrus* from the islands of Margarita and Cubagua, Venezuela. Their study was focused on sex ratio, maturity stages, and minimum length at first maturation and fecundity. They identified 5 maturity scale for the species. Annual variations in fecundity, egg size and condition of the plainfin midshipman (*Porichthys notatus*) were evaluated by DeMartini (1990). Gonzalez De Canales *et al.* (1992) studied histological and histochemical characteristics in *Halobatrachus didactylus* (Schneider, 1801) during oogenesis. Rosety *et al.* (1992) analysed the biochemical parameters during reproduction of the toadfish, *Halobatrachus didactylus* (Schneider, 1801).

Palazon-Fernandez *et al*. (2001) worked on some basic reproductive traits (sex ratio, size at sexual maturity, spawning period and fecundity) of *Halobatrachus didactylus*. The morphology of the genital apparatus of two batrachoid species, *Opsanus tau* and *Porichthys notatus*, was studied by Barni *et al*. (2001). The anatomical organization of the female reproductive apparatus was similar in both species but differences were observed in the rhythm of gametogenesis with individual oocyte production asynchronous in *O. tau* and group synchronous in *P. notatus*. Fine *et al.* (2004) studied the seasonal variation in androgen levels in the oyster toadfish. This study quantified gonad development and plasma androgens in males and females throughout a seasonal cycle to relate them to the prolonged reproductive cycle and to quantitative changes in boatwhistle parameters. Habitat, abundance and size at maturity of scarecrow toadfish, *Opsanus phobetron* at Bimini, Bahamas were studied by Newman *et al*. (2004). The presence of large numbers of scarecrow toadfish including mature females, when the water temperature was >22° C, suggests that the species is a successful breeding tropical population and not a glacial relict. Barimo *et al*. (2007) conducted field studies in Florida Bay to examine physiological, ecological and behavioural characteristics of the gulf toadfish, *Opsanus beta*, in relation to nitrogen metabolism, habitat usage, and spawning. Sisneros *et al.* (2009) investigated the morphometric changes associated with the reproductive cycle and behaviour of the intertidal –nesting, male plainfin midshipman *Porichthys notatus*.

Age information forms the basis for calculations of growth rate, mortality rate and productivity, ranking it among the most influential of biological variables. Calculations as simple as that of growth rate, or as complex as that of virtual population analysis, all require age data, since any rate calculation requires an age or elapsed time term (Campana, 2001).

Age and growth in the batrachoididae family have been studied using various methods. Schwartz and Dutcher (1963) employed vertebrae to estimate age in Maryland population of toadfish (*O. tau*) and discerned 12 age groups with sexual difference in growth. Wilson *et al.* (1982) used otoliths to assess the age structure of a south Carolina population and found most of the toadfish (*O. tau*) were <6 year old with no sexual difference in growth. Radtke *et al.* (1985) determined somatic and otolith growth in the oyster toadfish (*O. tau*). Serafy *et al.* (1997) used the length frequency distribution to ascertain the growth of *Opsanus beta* in Biscayne Bay, Florida. Vianna *et al.* (2000) estimated the growth and mortality of *Porichthys porosissimus* employing length frequency analysis. Malca *et al.* (2009) determined the age and growth of Gulf toadfish, *Opsanus beta* based on otolith increment analysis. The estimated ages of males and females ranged from <1 year to 6 and 5 years, respectively. Age, growth and mortality of *Halobatrachus didactylus* was investigated by Palazon-Fernandez *et al.* (2010) using otoliths.

The condition factor (K) (Le Cren, 1951) is a quantitative parameter of the well-being state of the fish and reflects recent feeding conditions. This factor varies according to influences of physiologic factors, fluctuating according to different stages of the development. Anderson and Neumann (1996) refer to length/weight data of population, as basic parameters for any monitoring study of fisheries, since it provides important information concerning the structure and function of populations. Wilbur and Robinson (1960) presented linear regression equations for length, weight and girth relations of *Opsanus tau*. Organ – body weight relationship in *O. tau* was studied by Robinson *et al*. (1960). Swartz and Van Engel (1968) re-examined the mathematical relations between length, weight and girth in the toadfish, *O. tau*. Wilson *et al.* (1982) observed no detectable differences in the growth rate or size of age classes of *O. tau* in South Carolina. Similar observations were made by Radtke *et al*. (1985) for *O. tau.* Muto *et al*. (2000) reported a positive allometric growth for *Porichthys porosissimus.* Vianna *et al.* (2000) investigated the length-weight relationship and relative condition factor of *Porichthys porosissimus*. Palazon-Fernandez *et al*. (2001) assessed the length-weight relationship and condition factor of *Halobatrachus didactylus*.

For better utilization and processing of new resources analysis for proximate chemical composition and nutritional components becomes a prerequisite, especially in case of new varieties of sea food hitherto not analyzed. An understanding of the composition is vital to evaluate each species of fish in terms of quality. Histochemical and biochemical aspects of the lipids of the female toadfish, *Halobatrachus didactylus*, during its annual reproductive cycle were studied by Munoz-Cueto *et al*. (1996). Investigation on chemical composition of fish from Indian waters has been reported by many workers. Some of the recent studies are as follows. John and Hameed (1995) studied the biochemical composition of *Nemipterus japonicus* and *Nemipterus mesoprion* in relation to maturity cycle. Mohanty and Samantray (1996) studied the biochemical composition of juvenile *Channa striatus* and associated the data with the reproductive cycle and water temperature. Shendge and Mane (2007) correlated seasonal variation in the biochemical compoisition of cyprinid fish, *Cirrhinus reba* (Hamilton) with the reproductive cycle. Changes in biochemical composition of muscles of an Indian major carp, *Labeo rohita* in influence of age was investigated by Gangwar *et al*. (2007). Nutritive value of *Botia berdmorei* and *Lepidocephalus guntea*, endemic in the water bodies of Manipur (India) has been studied by Sarojnalini (2010). The nutritive value of six important commercial fishes from India was validated by Ravichandran *et al*. (2011) and the nutritive parameters included protein, fatty acid, carbohydrate and moisture.

From the foregoing account it is seen that so far no attempt has been made to study the various aspects of biology of flat toadfish, *Colletteichthys dussumieri*. Though not important as a food fish, the species is an interesting batrachoid on account of its peculiar mode of life, habitat and parental care. Therefore, it was thought worthwhile to scrutinize in detail the various aspects of its biology.

1.3 Objectives of the study

- To study the systematics of the fish, *Colletteichthys dussumieri*
- To study the growth of morphometric variables in relation to total length
- To analyse the food and feeding habits
- To determine the fecundity and factors influencing reproduction
- To calculate the age and growth of the fish by otolith analysis
- To determine length – weight relationship and condition factor
- To analyse the proximate biochemical constituent to elucidate its nutritional status

1.4 General organization of the thesis

The thesis is organized into nine chapters.

First chapter comprises of general introduction, importance of the present study, review of works done on family Batrachoididae, the objectives of present study and the general organization of thesis.

The salient features of *C. dussumieri* together with its systematic position are described in the **second chapter**. A key for identification of species is also included.

The **third chapter** examines the morphometric characters in order to determine changes with growth and differences between sexes.

Information on the qualitative and quantitative aspects of food composition in relation to sex and season, relative length of gut, seasonal variation in feeding intensity and gasto-somatic index are presented in the **fourth chapter**.

The **fifth chapter** incorporates various aspects of reproduction. The dynamics of spermatogenesis and oogenesis of the fish species are illustrated with the help of the histological studies of ovary and testis in different stages of maturity. Maturity stages of males and females, monthly percentage occurrence of fish with gonads in different stages of maturity, pattern of progression of ova during different months, gonado-somatic index, minimum length at first maturity, sex ratio and fecundity and its relationship to various body parameters are the various reproductive and biological aspects discussed in this chapter.

Estimation of age and growth characteristics, worked out separately for male and female populations by otolith analysis are dealt in **chapter six**. Validating the annual periodicity of growth zone formation by performing a marginal increment analysis, determination of growth parameters, natural mortality, longevity and growth performance index are also presented.

The **seventh chapter** put forth the relationship between total length (mm) and body weight (g) in both the sexes. This chapter also describes the seasonal and size- wise variation of relative condition factor (Kn) and Ponderal index (K) of the fish.

The **eighth chapter** evaluates the nutritive value of the species by analyzing the proximate composition. Seasonal variations in protein, lipid, carbohydrate and moisture contents were estimated.

Finally, in **ninth chapter** results from the whole study are summarized.

In general, each chapter is subdivided into brief introduction, materials and methods, results and discussion. Table, graphs and photographs are inserted at appropriate places. The relevant references pertaining to the above chapters have been given at the end.

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Chapter 2

SYSTEMATICS OF *COLLETTEICHTHYS DUSSUMIERI* (VALENCIENNES, 1837)

Contents

2.1 Introduction

Batrachoididae, or toadfish, is the sole family in the order Batrachoidiformes (Haplodoci). These small to medium-sized fishes are easily recognized by their characteristic shape, with a large, broad, flattened head, often with barbels and /or fleshy flaps around their large mouths, and a tapering body.

The systematics and identifying characteristics of batrachoid fishes have been discussed by Day (1865, 1876), Jordan and Evermann (1896-1900), Gilbert and Starks (1904), Bean and Weed (1910), Meek and Hildebrand (1923), Jordan *et al*. (1930), Hubbs and Schultz (1939), Fowler (1936), Smith (1952, 1961), Mendis (1954), Marshall (1964), Cervigon (1966), Collette (1966), Hutchins (1981, 1984), Randall (1995) and Greenfield *et al.* (2008).

The first toadfish to be described was *Cottus grunniens* (now known as *Allenbatrachus grunniens*) by Linnaeus and the other *Gadus tau* (Linnaeus, 1766) (now in genus *Opsanus*). Ogilby (1908) was the first to revise the family Batrachoididae, recognizing ten genera and 35 species. Miranda –

Ribeiro (1915) erected the family Thalassophrynidae for *Thalassophryne* and *Thalassothia* and the family Porichthyidae for Porichthys. In a key to the genera, Smith (1952) recognized three subfamilies: Batrachoidinae, Porichthyinae and Thalassophryninae. He also documented 20 genera. Collette (1966) positioned two genera (*Daector* and *Thalassophryne*) in subfamily Thalassophryninae. The subfamily Porichthyinae contains genus *Aphos* and *Porichthys* (Walker and Rosenblatt, 1988). The remaining toadfish genera have been placed in the Batrachoidinae (Greenfield, 2006). Since Smith's (1952) summary, Roux and Whitley (1972) described the genus *Perulibatrachus*, Greenfield *et al*. (1994) described *Bifax*, Collette (1995) described *Potamobatrachus*, Greenfield (1997) described *Allenbatrachus* and Greenfield (2006) described *Vladichthys* and *Colletteichthys*.

Currently the Family Batrachoididae (Greek, batrachos = frog) is represented by 25 genera and 78 species (Greenfield *et al.*, 2008). Four subfamilies of toadfishes are recognized: Porichthynae, Thalassophryninae, Batrachoidinae and Halophryninae.

2.1.1 Systematic Position (Greenfield *et al.*, 2008)

Body usually scale less (small cycloid scales in some); head large with eyes more dorsal than lateral; mouth large and bordered by premaxilla and maxilla; pore (foramen) in axil of pectoral fin in some; pelvic fin jugular (in front of pectorals), with one spine and two or three soft rays; three pairs of gills; gill membrane broadly joined to isthmus; branchiostegal rays six; four or five pectoral radials; swimbladder present; upper hypurals with peculiar intervertebral like basal articulation with rest of caudal skeleton; no ribs, epiotics or intercalars; no pyloric caeca.

…………order Batrachoidiformes

Small to medium – sized fishes of characteristic shape. Head broad and flattened, often with barbels and / or fleshy flaps around jaws; opercle and subopercle with spines. Mouth large, terminal, and slightly protrusible; moderately strong teeth present in jaws and on the roof of mouth. Glandular tissue may be present in opercular region and pectoral-fin axil. Gill openings small, restricted to sides of body. Two separate dorsal fins; first dorsal fin with II or III spines; second dorsal fin long, with 15 to 25 soft rays. Anal fin somewhat shorter than second dorsal fin, with 12 to 28 soft rays. Pectoral fins large and broad - based. Pelvic fins jugular in position, with 1 spine and 1 to 3 soft rays. Skin scaly or naked. Lateral system very well developed, lateral line either single or multiple. Number of vertebrae ranging from 25 to 47. Colour: variable; back and sides usually brownish, often with spots, saddles, bars or other markings.

…………family Batrachoididae

Three dorsal- fin spines; no hollow dorsal and opercular spines connected to venom glands; one or two subopercular spines and one to three filaments; body with or without scales; axillary pore behind pectoral fins present or absent; lacks photophores and canine teeth; no foramina in median process of pelvic bone; median process of pelvic bone not joined to pelvic bone along its entire length; ventral edge of ceratohyal square where it joins epihyal; dorsal edge of quadrate not flat all the way across where it meets the metapterygoid.

…………subfamily Halophryninae

2.1.2 Key to genera of Batrachoididae (subfamily: Halophryninae)

(Greenfield *et al.*, 2008)

Body completely naked; maxillary flaps absent; axillary foramen or pocket at top of pectoral–fin axil; soft dorsal-fin rays 19-24, usually fewer than 24; supraorbital tentacles present and others on head; anterior nasal tentacle not elongate; opening at top of pectoral-fin axil a funnel shaped pit with glandular tissue inside and extending from ventral margin onto axil; lower gill opening well below lower pectoral-fin base; sub- opercle with two spines, upper one large and lower one smaller

…………*Colletteichthys*

Body completely naked; maxillary flaps absent; no axillary foramen or pocket; fewer than three subopercular spines; fewer than 24 dorsal-fin rays; teeth conical or blunt; two subopercular spines; supraorbital tentacle or tentacles present; gill openings less or greater than pectoral-fin base; head more pointed and flattened with lower jaw protruding; eye diameter less than snout length; interorbital width greater than eye diameter; gill opening either at or below pectoral-fin base.

…………*Allenbatrachus*

Body completely naked; maxillary flaps absent; axillary foramen or pocket at top of pectoral-fin axil; soft dorsal-fin rays 19-24, usually fewer than 24; one subopercular spine and two filaments; supraorbital tentacles absent and few on head; anterior nasal tentacle long.

…………*Austrobatrachus*

With scales extending forward to first dorsal-fin base; funnel-shaped pocket present on upper part of pectoral-fin axil; accessory pectoral-fin radial not ossified; two subopercular spines and two filaments; prominent tentacles above eyes; anterior nostril with single pointed tentacle; anal-fin rays 13-14; pectoral fin spotted.

…………*Barchatus*

Body completely naked; maxillary flaps absent; axillary foramen or pocket at top of pectoral-fin axil; soft dorsal-fin rays 19-24, usually fewer than 24; supraorbital tentacles present and others on head; anterior nasal tentacle not elongate; opening at top of pectoral-fin axial a distinct round hole, not funnel shaped and lacking glandular tissue on ventral margin; lower gill opening at lower pectoral-fin base; subopercle with one strong spine

…………*Batrachomoeus*

Body completely naked; maxillary flaps absent; no axillary foramen or pocket; fewer than three subopercular spines; fewer than 24 dorsal-fin rays; teeth conical or blunt; two subopercular spines; no tentacles above eye; gill openings not less than pectoral-fin base; pelvic fins not reaching vent; head shallow, depressed, 17% or less standard length; eye less than interior orbital width.

…………*Batrichthys*

Body completely naked; a flap with an eye spot at end of maxilla on each side of mouth.

…………*Bifax*

Body with at least some scales (may be embedded and difficult to see); no small, round, foramen in pectoral-fin axil, but a funnel shaped pocket might be present; pectoral axil without a pocket; anal-fin rays 18 or fewer, nasal barbels present.

…………*Chatrabus*

Body with at least some scales (may be embedded and difficult to see); small, round foramen present on upper part of pectoral axil beneath upper edge of opercular membrane (fewer than 24 dorsal-fin rays; no tentacle above eye).

…………*Halobatrachus*

Body completely naked; maxillary flaps absent; no axillary foramen or pocket; fewer than three subopercular spines; fewer than 24 dorsal-fin rays; teeth conical or blunt; two subopercular spines; supraorbital tentacle or tentacles present; gill openings less or greater than pectoral-fin base; head rounded with lower and upper jaws about equally terminal; eye diameter greater than snout length; interorbital width equal to or less than eye diameter; gill opening clearly above lower margin of pectoral-fin base.

…………*Halophryne*

Body with at least some scales (may be embedded and difficult to see); no small, round, foramen in pectoral-fin axil, but a funnel-shaped pocket might be present; a more or less funnel-shaped pocket (deep or shallow) present on upper part of pectoral-fin axil; no obvious tentacles above eyes.

…………*Perulibatrachus*

Body with at least some scales (may be embedded and difficult to see); no small, round, foramen in pectoral-fin axil, but a funnel-shaped pocket might be present; a more or less funnel-shaped pocket (deep or shallow) present on upper part of pectoral-fin axil; one or more prominent tentacles above eye; scales on body restricted to posterior half; anal-fin rays 15-17; pectoral fin without spots; anterior nostril with a large tuft of tentacles.

…………*Riekertia*

Body completely naked; maxillary flaps absent; no axillary foramen or pocket; fewer than three subopercular spines; fewer than 24 dorsal-fin rays; teeth conical or blunt; one subopercular spine; dorsal-fin rays14-17; anal-fin rays 11-13; upper lateral - line pores 25-31; lower lateral- line pores 23-31; epaxial trunk musculature extending forward to cover entire dorsocranium behind orbits

…………*Triathalassothia*

2.2 Description of the species

Colletteichthys dussumieri is the only species in the genus *Colletteichthys* (Greenfield, 2006*)*

Synonyms

Batrachus dussumieri (Valenciennes,1837)
Austrobatrachus dussumieri (Valenciennes, 1837)

Common name

Flat Toadfish

Classification

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Superphylum: Osteichthyes

Class: Actinopterygii

Subclass: Neopterygii

Superorder: Paracanthopterygii

Order: Batrachoidiformes

Family: Batrachoididae
Subfamily: Halophryninae
Genus**:** *Colletteichthys*
Species: *dussumieri* (Valenciennes, 1837)

Distinctive characters of species *Colletteichthys dussumieri* (Greenfield, 2006)

Three solid dorsal – fin spines without venom glands ; three solid opercular and one short subopercular spine, often with a small second point below; two subopercular filaments; upper accessory pectoral – fin radial totally ossified; three lateral lines present, the upper with 43-53 pores, the middle with about six, and the lower with 26- 30; no photophores ; no scales; a funnel- shaped pit at top of pectoral-fin axil, with glandular tissue inside and extending from ventral pit margin onto axil; interorbital areas not crossed by conspicuous skin ridges; head into standard length - 2.4 to 2.8 times; two rows of pointed teeth in anterior portion of lower jaw; sides of lower jaw with single row of pointed teeth; upper jaw with three rows of pointed teeth anteriorly, two rows on side, grading into single row posteriorly; vomer and palatine with single row of pointed teeth; dorsal-fin elements III- 19-22; anal-fin rays 15-17; pectoral- fin rays 21-24; vertebrae 27.

Colour pattern: light brown, shading to white ventrally, with four, irregular, branching, dark brown bars on body and dark bands and blotches on head and fin.

The above mentioned characters are those described by Greenfield (2006).

According to Randall (1995), upper lateral line of this fish possess only 33-41 pores. The maximum length attained by the fish was reported to be 27cm (Hutchins, 1984; Randall, 1995). In the present study, presence of 50-55 upper lateral line pores, 8 middle lateral line pores, 30-38 lower lateral line pores and 29 vertebrae were observed. The maximum length attained by fish was found to be 30.5 cm (Roja *et al*., 2010).

C. dussumieri is generally seen hiding in crevices and burrows and also prefers muddy bottom. Kurup and Samuel (1985) reported it to be resident species confined to high saline areas.

2.3 Earlier reports (In India and World)

A persual of available literature (Table 2.1) revealed that the northern Indian Ocean species described by Valenciennes (1837) as *Batrachus dussumieri* has in the past been placed in the South African genus *Austrobatrachus* (Smith, 1949); however, this species was later included in new genus, *Colletteichthys.*

Valenciennes, in Cuvier and Valenciennes (1837) described *Batrachus dussumieri* from Malabar, India. Day (1876), in his classical work on the 'Fishes of India', has given the systematic account of this species as *Batrachus grunniens* (Bloch.) (Hutchins, 1981). Bhimachar and Venkataraman (1952), while studying the inshore fish population of the Malabar Coast, reported the species as *Batrachus grunniens* (Bloch.). Smith (1949) described the genus *Austrobatrachus* for the South African species *Pseudobatrachus foedus* Smith, 1947. Menon (1963) utilized Smith's genus for *B. dussumieri*; with the exception of Nagabhushanam and Rama Rao (1970) who used *Halophyrne*; the species has been referred to as *A. dussumieri* (Hutchins, 1981, 1984; Randall, 1995; Carpenter *et al.*, 1997). While comparing *dussumieri* with *Austrobatrachus foedus,* Greenfield (2006) observed wide variation in their morphology and described a new genus *Colletteichthys* for the species and later placed it in the subfamily Halophyryninae (Greenfield *et al*., 2008).

Table 2.1. The previous reports of *Colletteichthys dussumieri* are as follows:

Batrachus dussumieri	Valenciennes, 1837. Cuvier, G. and Valenciennes, A., 1837. *Histoire naturelle des poissons. Tome douzieme.Suite du Livre quatorzieme. Gobioides. Livre quinzieme. Acanthopterygiens a pectorals pediculees,* vol.12. Levrault, Paris, France. pp.507. Günther, A. 1861. Catalogue of the acanthopterygian fishes in the collection of the British Museum. Vol.3. The Trustees of the British Museum, London.
Batrachus grunniens	Day, F., 1876. The fishes of India; being a natural history of the fishes known to inhabit the seas and freshwaters of India, Burma, and Ceylon with descriptions of the subclasses, orders, families, genera and species. Pt. 2: 169-368. B. Quaritch, London. Seas of India to the Malay Archipelago Regan, C. T., 1905. Of fishes from the Persian Gulf, the Sea of Oman and Karachi, collected by Mr. F.W. Townsend. *J. Bombay Nat. Hist*., 16(2): 318-333. Munro, I. S. R., 1955. The marine and freshwater fishes of Ceylon. Department of External Affairs, Canberra. pp.124. Coastal waters Blegvad and Löppenthin, 1944. Fishes of the Iranian Gulf. *Danish Sc. Inv. Iran,* Copenhague, pt. III, pp.247.

	Mahdi. 1971. Addition to the marine fish fauna of Iraq. *Iraq Nat. Hist. Mus. Pub.*, 28: 1-43, pls. 1-16. Kuronuma and Abe, 1972. Fishes of Kuwait. Kuwait Inst. Sci. Res., Kuwait: I-XIV pp. 1-123. figs. 1-37, pls. 1-20. Arabian Gulf and Indian Ocean to Ceylon. Rather common in the western seas in the Gulf. Kuronuma and Abe, 1986. Fishes of the Arabian Gulf. Kuwait: Kuwait Institute for Scientific Research, Kuwait. The International Academic Printing Co. Ltd. pp. 356. Arabian Gulf, then eastward to Coast of India and East Indies, thence, northward to Bay of Siam.
Austrobatrachus dussumieri	Menon, 1963. Taxonomy of the Indian frogfishes (Fam. Batrachoididae). LABDEV, J.S.T., Kanpur, 1(pages not numbered). Hutchins, 1981. Nomenclature status of the toadfishes of India. *Copeia.* (2) pp. 336-341. India, Red sea, Persian Gulf and Sri Lanka. Hutchins, 1984. Batrachoididae. In W. Fischer and G. Bianchi, eds., *FAO species Identification sheets for Fishery Purposes. Western Indian Ocean fishing area 51*. Vol. I. FAO, Rome, Italy. 4 pages (unnumbered). "Gulf" and along the Coasts of Pakistan, India and Sri Lanka. Kurup and Samuel, 1985. Fish and fishery resources of the Vembanad Lake. In: Proc. harvest and post harvest technology of fish. Society of Fisheries Technologists: 77-82. Vembanad Lake.

	Randall, 1995. Coastal fishes of Oman. University of Hawai'i press, Honolulu, Hawaii, USA. P.389. Arabian Gulf of India (Malabar, the type locality) and Srilanka. Carpenter *et al.*, 1997. The corals and coral reef fishes of Kuwait. Kuwait Institute for Scientific Research, Safat, Kuwait. P.166. Coral reefs of Kuwait. Kapoor, *et al.*, 2002. Fish biodiversity of India. National Bureau of Fish Genetic Resources Lucknow, India. pp. 775.
Halophryne dussumieri	Nagabhushanam and Rama Rao, 1970. A review of the taxonomy of the Indian frogfishes (Family Batrachoididae). *J. Bombay Nat. Hist. Soc.,* 67: 339-344.
Colletteichthys dussumieri	Greenfield, 2006. Two new toadfish genera (Teleostei: Batrachoididae). *Proceedings of the California Academy of Sciences*. Ser.4, 57 (32): 945-954. Roja *et al.*, 2010. First record of the flat toadfish *Colletteichthys dussumieri* (Batrachoidiformes: Batrachoididae) from estuarine waters of India. *Marine Biodiversity Records*, 3; e56.

It is well known that only two species of toadfishes (Batrachoididae) inhabit Indian waters (Day, 1876; De Beaufort, 1962; Menon 1963; Nagabhushanam and Rama Rao, 1970). *Colletteichthys dussumieri* is easily distinguished from *Allenbatrachus grunniens* by the presence of a prominent foramen in the upper portion of the pectoral axil. However their identities are uncertain as a variety of names has been employed by the authors indicated above (Table 2.2.). Day (1876) used *Batrachus grunniens* (Linnaeus) and *B. gangene* (Hamilton), De Beaufort (1962) referred to *Halophryne trispinosus* (Günther) and *H. gangene,* Menon (1963) used *Austrobatrachus dussumieri* (Valenciennes) and *Batrichthys grunniens*, and Nagabhushanam and Rao (1970) preferred *Halophryne dussumieri* and *H. gangene*.

In the tenth edition of his Systema Naturae, Linnaeus (1758) described *Cottus grunniens.* This species was stated to inhabit American coasts. Bloch and Schneider (1801) placed *Cottus grunniens* in a new genus *Batrachus* and gave the distribution as "in India utraque" (= on both sides of India). Hamilton (1822) described *Batrachoides gangene* from the Ganges River and the specie was characterized by lacking a pectoral axillary foramen, must be relegated to the synonymy of the former species. This leaves Valenciennes's (1837) *Batrachus dussumieri* as the oldest available name for the species possessing a foramen in the pectoral axil described from the Malabar Coast of India. He also re-described *B. grunniens* based on specimens from the Indian Ocean. Gunther (1861) published concise descriptions of both *Batrachus grunniens* from East Indian Seas and *Batrachus dussumieri* from west India. He placed *Batrachoides gangene* in the synonymy of *B. grunniens.* In addition, he described *Batrachus trispinosus,* a new species from Bombay, Singapore and Penang based on specimens from the last two localities. Day (1876) provided descriptions and figures of *Batrachus grunniens* and *Batrachus gangene*. He listed *B. trispinosus* and *B. dussumieri* in the synonymy of *B. grunniens*. The distribution for *B. grunniens* was given as “Seas of India … to the Malay Archipelago" and for *B. gangene* "Estuaries of the Ganges and other large Indian and Burmese rivers." De Beaufort (1962) recognized *Halophryne trispinosus* and *H. gangene* as occurring along the coasts of India.

Menon (1963) gave descriptions of *Austrobatrachus dussumieri* and *Batrichthys grunniens*, employing South African genera (Smith, 1952). He placed *Batrachoides gangene* in the synonymy of the latter species on the basis that there was no mention of a pectoral axillary foramen in the original description of *Cottus grunniens*. Menon (1963) also included *Batrachus trispinosus* as a synonym of *Austrobatrachus dussumieri*. Nagabhushanam and Rao (1970) recognized *Halophryne dussumieri* and *H. gangene*. They placed *Batrachus trispinosus* in the synonymy of *H. dussumieri*.

Hutchins (1981) unraveled the complex nomenclature of the toad fish species of India and correctly determined that the species described by Linnaeus (1758) as *Cottus grunniens* had priority over *Batrachoides gangene* described by Hamilton (1822). Hutchins (1981) followed Menon (1963) and recognized *Austrobatrachus dussumieri* (Valenciennes) (dorsal III, 20-22; anal 16-17; pectoral 22-24) and *Batrichthys grunniens* (Linnaeus) (dorsal III, 20; anal 16-18; pectoral 21) as the valid names for the two species of indian batrachoidid fishes. The distribution of the former species was given as India, Red Sea, Persian Gulf and Srilanka and estuarine areas of the Ganges River, Singapore and the Philippines for the latter. As a part of revision of the batrachoidid genera, Greenfield (Greenfield, 1997; 2006) re-described the two and placed them in genus *Colletteichthys* and *Allenbatrachus* respectively. Greenfield (2008) assigned both these genus in the new subfamily Halophryninae. Thus the names of the two species of toadfish found in Indian waters are *Colletteichthys dussumieri* (Arabian Gulf to India and Srilanka) and *Allenbatrachus grunniens* (Estuarine areas of the Ganges River (India) eastward to Borneo and the Philippines, including the gulf of Thailand) respectively.

Table 2.2. Nomenclatural status of the toadfishes of India

	Colletteichthys dussumieri	***Allenbatrachus grunniens***
Linnaeus(1758)	-----	*Cottus grunniens* America, Mediterranean sea
Bloch and Schneider (1801)	-----	*Batrachus grunniens* "in India utraque"(= on both sides of India).
Hamilton(1822)	-----	*Batrachoides gangene* Ganges River
Valenciennes(1837)	*Batrachus dussumieri* Malabar	-----
Günther(1861)	*Batrachus dussumieri* India	*Batrachus grunniens* East Indian Seas
Day (1876)	*Batrachus grunniens* Seas of India to the Malay Archipelago	*Batrachus gangene* (Hamilton) Estuaries of the Ganges and other large Indian and Burmese rivers
De Beaufort(1962)	*Halophryne trispinosus*	*Halophryne gangene* Coasts of India
Menon (1963)	*Austrobatrachus dussumieri*	*Batrichthys grunniens*
Nagabhushanam and Rama Rao(1970)	*Halophryne dussumieri*	*Halophryne gangene*
Hutchins (1981)	*Austrobatrachus dussumieri*	*Batrichthys grunniens*
Greenfield(1997)	-----	*Allenbatrachus grunniens* India eastward to Philippines
Greenfield (2006)	*Colletteichthys dussumieri* Western Indian Ocean: Found in the Persian Gulf and along the Coasts of Pakistan, India and Srilanka.	-----

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Chapter 3

MORPHOMETRICS

Contents

3.1 Introduction

Taxonomic identification is the pioneer step in the study of a species. Among different methods used, morphological techniques are considered to be the earliest and authentic method for the identification of species (Nayman, 1965). Quantitative morphological techniques have traditionally been used for the classification of fishes into respective hierarchical group (family, genus and species). Taxonomic information is vital to associated research in areas such as marine biology, ecology, conservation and fisheries management (Cadrin, 2000; Cabral *et al*., 2003; Tzeng, 2004; Doherty and McCarthy, 2004).

Morphological characters are the ones used in the identification of fishes. The countable characters of a fish are collectively called as meristic (e.g. myomeres, vertebrae, fin rays) and the measurable characters as morphometrics. These characters are more superficial as well as more variable and hence these characters are among the most commonly used ones for differentiation of species and populations. Morphometric and meristic studies have provided useful results for identifying marine fish stocks, describing their spatial distribution and for measuring discreteness and relationships among stocks (Ihssen *et al*., 1981; Melvin *et al*., 1992; Turan *et al.*, 2004, 2005). Phenotypic, specifically morphometric, analysis is useful, however, for demonstrating the degree of intraspecific variation within a population (Murphy *et al*., 2007).

The morphometry of fishes is amongst the most easily perceivable means of assessing the evolutionary adaptation of a species to its environment (Kovac *et al.,* 1999). Animals with the same morphometric characteristics are often assumed to constitute a stock and morphometric variations between stocks can provide a basis for stock structure and are useful for studying short-term, environmentally induced variation, for example, in fisheries management (Begg *et al.*, 1999; Avsar, 1994; Cadrin, 2000).

Different populations of the same species of a fish are known to differ morphologically through genetic differences or owing to differences in the ecological conditions, when the structure, shape and form in question are fixed throughout life (Chondar, 1973). Geographical isolation can result in the development of different morphological features between fish populations because the interactive effects of environment, selection and genetics on individual ontogenies produce morphometric differences within a species (Cadrin, 2000). Patterns of geographic variation in phenotypic traits among wide-ranging coastal marine fishes often suggest the influence of environmental factors and local habitats (Corti and Crosetti, 1996).

The use of morphometrics has gained wide acceptance in the contemporary biological scene. As such it is increasingly used as a necessary complement to molecular studies due its low budget requirement and acceptable resolving power of discrimination.

In India, a number of studies have been performed on this subject for marine and freshwater fishes. The most important ones are those of Pillay (1951, 1957), Radhakrishnan (1957), Sarojini (1957), Tandon (1962), Chaterjee *et al*. (1977), Seshagiri Rao (1981), Silas *et al.* (1985) and Devi *et al.* (1991).

Published works on the morphometry of toad fishes are scanty. The present study aims to investigate the interrelationships of various morphometric characters of *Colletteichthys dussumieri*, their growth rates in relation to total length and to provide the population characteristic of the species in Cochin estuary.

3.2 Materials and methods

Samples for the present study were collected during May to August 2008 from Cochin estuary. Fifty five specimens of *C. dussumieri* (34 males and 21 females) in the size range 119 - 299 mm total length were examined for morphometric analyses. Fresh specimens were measured for morphometric characters to the nearest millimeter using a divider and a measuring board. The following fifteen morphometric characters were obtained for each fish (Fig 3.1.).

Total length (T.L): The distance from tip of the snout to the tip of longest ray of caudal fin.

Standard length (Std.L): The distance from the tip of the snout to the end of hypural plate.

Head length (H.L): The distance from tip of the snout to the posterior point of opercular membrane.

Snout length (S.L): The distance from the tip of upper jaw to the front margin of the orbit.

Post-orbital length (P.O.L): The distance from hind margin of orbit to the tip of opercular membrane.

Inter-orbital length (I.O.L): The distance between the dorsal margins of the eyes.

Eye diameter (E.D): The greatest horizontal distance between the free orbital rims.

Pre-first dorsal fin (P.ID): The distance from the tip of the snout to the anterior end of the first dorsal fin base.

Pre-second dorsal fin (P.IID): The distance from the tip of the snout to the anterior end of the second dorsal fin base.

Pre-pectoral length (P.Pc.L): The distance from the tip of the snout to the insertion of the pectoral fin.

Pre-pelvic length (P.Pv.L): The distance from the tip of the snout to the insertion of the pelvic fin.

Pre-anal length (P.A.L): The distance from the tip of the snout to the insertion of the anal fin.

Body depth (B.Dep): The distance from the anterior end of first dorsal fin to the ventral surface of the fish at deepest part.

Depth through anal fin (Dep.A): The distance from the anterior end of second dorsal fin to the anterior end of the anal fin.

Caudal peduncle depth (C.Pd.Dep): The minimum distance between the dorsal and ventral edges of the caudal peduncle.

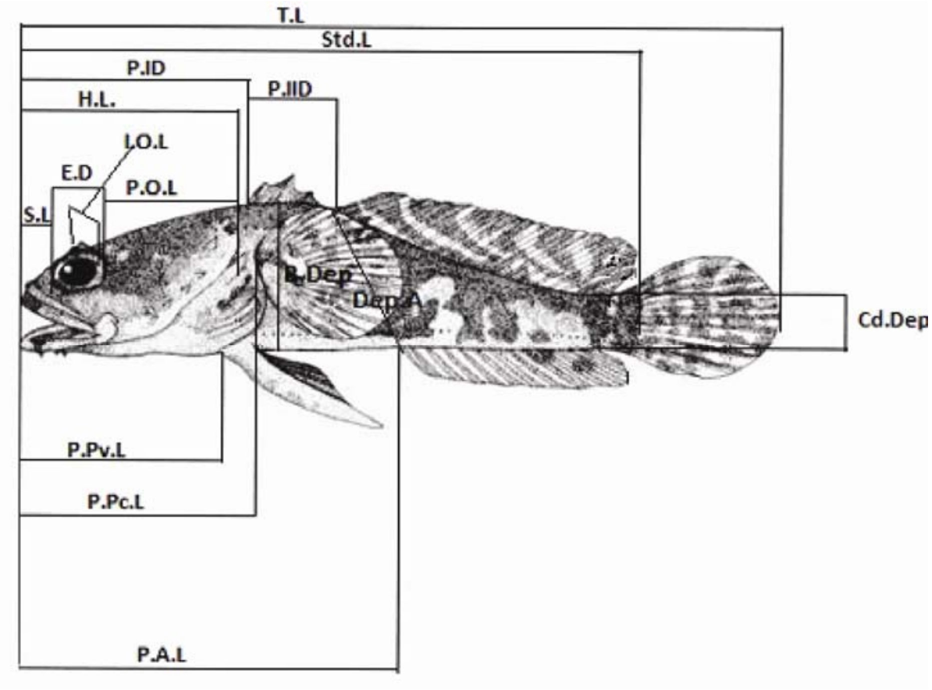


Fig.3.1. Schematic illustration showing morphometric features of *C. dussumieri* selected for the study.

In order to establish the morphometric characteristics of the stock of *C. dussumieri* of Cochin estuary, the degree of association between various variables (measurements), correlation coefficient (r) between these morphometric measurements were calculated. Since a significant correlation was found between total length (T.L) and other measurements, the morphometric characters were plotted against total length to check if they were adequately described by a straight-line relationship. The relationships were analysed using a standard linear regression expression and was applied separately for males and females. The statistical relationships between total length and various body characters were derived through the regression equation:

$$Y = a + bX$$

Where ‘X’ denotes total length as independent variable and ‘Y’ the other dependent variables, ‘a’ and ‘b’ are constants (the intercept and the slope of the regression line respectively). The goodness of fit of the relationship between the variables was derived from the coefficient of correlation.

The morphometric dimensions (expressed as per cent T.L) were plotted against T.L. To analyse their allometric relationships with T.L, the variables and T.L were $\log_{10}$ transformed and the regression slopes calculated. The significance of the slope was tested by means of t-test (Zar, 2005). The morphometric variables were then divided into three types: positive allometry (+A), when the slope (allometry coefficient) was significantly >1·0 and the proportional variable increased relative to T.L; negative allometry (-A), when the slope was significantly <1·0 and the proportional variable decreased relative to T.L; and isometry (I) when the slope showed a non-significant difference from 1·0, indicating direct proportionality between the variable and T.L.

To examine differences in morphometric dimensions between males and females, the regression slopes of each variable versus total length (T.L) were tested by means of Students’t-tests. The test statistic is:

$$t = \frac{b1 - b2}{Sb1 - Sb2}$$

Where ‘b’ is the slope of the regression line, and ‘Sb’ is the standard error of ‘b’.

3.3 Results

The mean with standard error, range (minimum and maximum values) of each measurement were calculated against each character as presented in

Table 3.1. The correlation matrix between various measurements is presented in Table 3.2. for male and Table 3.3. for female. Established correlation coefficients (r) showed a highly significant correlation ($P<0.01$) in both the sexes except for the values for snout length in females ($p<0.05$). The highest correlation coefficient was observed between total length and standard length in both male ($r = 0.996$) and in female ($r = 0.988$). The lowest was between snout length and eye diameter in male ($r = 0.497$) and snout length and pre-pelvic length in female ($r = 0 .339$).

The results of statistical analysis on morphometric characters are summarized in Table 3.4. Scatter plots of various regression lines are illustrated in Fig.3.2a - Fig.3.2e for male and Fig.3.3a – Fig.3.2e for female. It was found that all the body measurements showed a linear relationship against total length.

Standard length on Total length

The regression equation of standard length on total length is represented as $Y = -2.6107 + 0.832 X$ and $Y = 3.457 + 0.7964 X$ for male and female respectively. The regression coefficient ‘b’ was significant ($P<0.01$) with an average rate of 0.83 mm increase in standard length in male and 0.80 mm in female per 1 mm of total length. There is a high degree of positive correlation between standard length and total length with ‘r’ value being 0.996 in male and 0.988 in female.

Head length on Total length

The linear relationship of head length with total length is derived by regression equation $Y = 0.478 + 0.3373 X$ for male and $Y = -1.438 + 0.3459 X$ for female. The regression coefficient ‘b’ was significant ($P <0.01$) in both the sexes with an average rate of 0.34 mm increase in head length in male and 0.35 mm in female per 1mm of total length. The degree of association between the two is high and positive with ‘r’ value being 0.971 in male and 0.949 in female.

Snout length on Total length

The relationship between snout length and total length was linear with correlation coefficients ‘r’, 0.663 in male and 0.670 in female. The regression equation is given as $Y = 2.157 + 0.064 X$ and $Y = 1.271 + 0.072 X$ in male and female, respectively. The regression coefficient ‘b’ values were found to be significant ($P < 0.01$) with an average rate of 0.06 mm and 0.07 mm increase in snout length with 1mm increase in total length.

Post-orbital length on Total length

The estimated equation was $Y = 2.467 + 0.2109 X$ for male and $Y = -3.686 + 0.2328 X$ for female. The ‘b’ values were found to be significant ($P < 0.01$) in both male and female, with an average rate of 0.21 mm and 0.23 mm increase in post- orbital length for 1 mm increase in total length of male and female respectively. The degree of association between the two is high and positive with ‘r’ value being 0.968 in male and 0.952 in female.

Inter-orbital length on Total length

The linear relationship between the inter-orbital length and total length may be expressed as $Y = -6.0803 + 0.141 X$ and $Y = -6.7264 + 0.1413 X$ for male and female respectively. The ‘b’ values were found to be significant ($P < 0.01$) in both male and female with an average rate of 0.14 mm increase in inter – orbital length with 1 mm increase in total length. High positive correlation was calculated with ‘r’ values of 0.923 and 0.855 in male and female respectively.

Eye diameter on Total length

The relationship was found to be linear as is clear from the regression equation Y =1.192 + 0.0442 X for male and Y = 2.872 + 0.0389 X for female, with significant 'b' value ($P < 0.01$) with an average rate of 0.044 mm increase in eye diameter for every 1 mm increase in total length in male and 0.039 mm in female. Correlation coefficients 'r' was calculated to be 0.847 and 0.831 in male and female respectively.

Pre-first dorsal length on Total length

The estimated equation Y = -1.042 + 0.3146 X was considered for male and Y = - 4.1869 + 0.3351 X for female. The regression coefficient 'b' was significant ($P < 0.01$) with an average rate of 0.315 mm increase in pre-first dorsal length per 1mm of total length in male and 0.335 mm in female respectively. The degree of association between the two is high and positive with 'r' value being 0.946 in male and 0.942 in female.

Pre-second dorsal length on Total length

The relationship between pre-second dorsal length and total length was linear with correlation coefficients 'r', 0.942 in male and 0.950 in female. The regression equation can be given as Y = 4.703 + 0. 3843 X and Y = -5.0245 + 0.4366 X for male and female respectively. The regression coefficient 'b' values were found to be significant ($P<0.01$) with an average rate of 0.38 mm and 0.44 mm increase in pre-second dorsal length with 1mm increase in total length.

Pre-pectoral length on Total length

Pre-pectoral fin length showed a linear relationship with total length and the estimated equation derived was Y = -1.0814 + 0.294 X in male and Y = 5.494 + 0.2614 X in female. The estimated 'b' value was significant

(P<0.01) in both the sexes. The average rate of the increment of pre-pectoral fin length was 0.29 mm and 0.26 mm for every 1 mm increase in total length in male and female respectively. The degree of association between the two is high and positive with ‘r’ value being 0.899 in male and 0.862 in female.

Pre-pelvic length on Total length

The linear relationship of pre-pelvic fin length with total length is derived by regression equation Y = 5.796 + 0.2153 X and Y = -8.098 + 0.279 X for male and female respectively. The equation infers that pre-pelvic length increases by 0.22 mm in male and 0.28 mm in female for every 1 mm increase in total length. Pre-pelvic length showed high positive correlation with total length, with ‘r’ value 0.793 in male and 0.824 in female.

Pre-anal length on Total length

The regression of pre-anal length on total length is given as Y = -10.271 + 0.5658 X and Y = -6.180 + 0.5531 X with ‘r’ values of 0.955 and 0.961 for male and female respectively. Significant ‘b’ values (P <0.01) were found in both the sexes with an average rate of 0.566 mm and increase in pre-anal length for every 1 mm increase in total length in male and 0.55 mm in female.

Depth through first dorsal length on Total length

The relationship between depth through first dorsal fin length and total length was linear with correlation coefficients ‘r’, 0.810 in male and 0.752 in female. The regression equation considered was Y = -10.57 + 0.276 X and Y = -7.879 + 0.2688 X in male and female respectively. The regression coefficient ‘b’ values were found to be significant (P<0.01) with an average rate of 0.28 mm and 0.27 mm increase in depth through first dorsal fin length with 1 mm increase in total length.

Depth through second dorsal length on Total length

The estimated equation were Y = - 4.227 + 0.236 X for male and Y = -7.515 + 0.2567 X for female. The 'b' values were found to be significant ($P < 0.01$) in both male and female with an average rate of 0.24 mm and 0.26 mm increase in depth through second dorsal length with 1 mm increase in total length for male and female respectively. The degree of association between the two is high and positive with 'r' value being 0.904 in male and 0.892 in female.

Caudal peduncle length on Total length

The equation derived for caudal peduncle length and total length was Y = -0.0142 + 0.0828 X for male and Y = 2.1972 + 0.0733 X for female. The regression coefficient 'b' was significant ($P<0.01$) with an average rate of 0.08 mm increase in caudal peduncle length in male and 0.07 mm in female per 1 mm of total length. Like other characters, there is a high degree of positive correlation between caudal peduncle length and total length with 'r' value being 0.948 in male and 0.900 in female.

In males, two dimensions (inter – orbital length and body depth) revealed a positive allometric (+A) relationship and one (eye diameter) had a negative allometric (-A) relationship with T.L (t-test, d.f = 32, $P<0{\cdot}05$) (Table 3.5). Eleven dimensions (standard length, head length, snout length, post-orbital length, pre-first dorsal fin, pre-second dorsal fin, pre-pectoral length, pre-pelvic length, pre-anal length, depth through anal fin and caudal peduncle depth) had an isometric relationship with T.L ($P>0{\cdot}05$) (Table 3.5). In females, three measurements (inter- orbital length, pre-pelvic length and body depth) had a +A relationship and three measurements (eye diameter, pre-pectoral length and caudal peduncle depth) had a -A relationship with TL (t-test, d.f = 19, P<0·05) (Table 3.5). Eight dimensions (standard length, head length, snout length, post-orbital length, pre-first dorsal fin, pre-second dorsal fin, pre-anal length and depth through anal fin) increased isometrically with T.L (P>0·05) (Table 3.5).

Difference between slopes of various regression lines of male and female is shown in Table 3.6. Comparisons of slopes estimated in above relationships showed significant difference between male and female at 1% level (P<0.01) in the following morphometric characters: eye diameter (E.D), pre-second dorsal fin (P.IID) and pre-pelvic length (P.Pv.L), while standard length (Std.L), post-orbital length (P.O.L), pre-first dorsal fin (P.ID), pre-pectoral length (P.Pc.L), pre-anal length (P.A.L), depth through anal fin (Dep.A) length and caudal peduncle depth (C.Pd.Dep) exhibited significant difference at 5% level (P<0.05). Significant difference between male and female (P>0.05) were not exhibited in case of relationship between head length (H.L), snout length (S.L), inter-orbital length (I.O.L) and body depth (B.Dep) to that of total length.

3.4 Discussion

Analysis of the morphometric characters is very important for clarifying the identity of any fish species (Wadie *et al*., 1987). The present study on the morphometric characters of *C. dussumieri* revealed that all the fourteen measurements of the external parts of the fish have a linear association with total length. This finding was drawn from the value of correlation coefficient ‘r’ which was highly significant (P<0.01) in all the relationships in male and female. Although the measurements related to snout length (P< 0.05 in female) followed by eye diameter showed the least values of correlation coefficient with other characters, still their correlation was significant at the same significance level in both the sexes.

Morphological variations should be attributable to body shape differences and should not be related to the relative size of the fish to avoid misinterpretation of the results (Strauss, 1985). Several univariate and multivariate techniques are used to nullify the effect of size and regression analysis is one among them. The regression analysis in the present study revealed that the growth is exhibiting very similar pattern in both male and female with slight but significant differences in the values of growth rate of different characters in relation to total length. The highest growth rate was observed in case of standard length, 0.83 mm and 0.80 mm for male and female respectively. Next to standard length are pre-anal length (0.566 mm, 0.553mm) and pre-second dorsal fin length (0.384 mm, 0.437 mm). Eye diameter was the slowest growing character with growth rate of 0.04 mm and 0.039 mm in male and female respectively.

Verep *et al*. (2006) demonstrated that eye diameter was the most variable in *Barbus taurics*, whereas the least variability was found in pre-dorsal distance. Negi and Nautiyal (2002) inferred that the parameters showing most significant linear regression in relation to total length in *Barilius* sp were snout length and least depth of caudal peduncle. The body parameters standard length and pre-dorsal length were observed to be least significant in both sexes. The biometric analysis of pipefish species considered snout morphology as the most important character followed by the dorsal fin length and eye dimension (Gürkan, 2008). Moazzam *et al*. (2004) obtained strong correlation between total length and other parameters and the highest was with pre-anal length in *Hilsa kelee*. Regression analysis on the morphometric characters of *Pagrus pagrus* (Minos *et al.*, 2008) revealed that standard length, post-orbital distance and pre-orbital distance were significantly different in males and females.

These characters exhibited different growth patterns between sexes. The only insignificant one was head length. Saker *et al.* (2004) indicated high growth rate in standard length and fork length while caudal depth and inter dorsal space showed sluggish growth rate in *Megalaspis cordyla*.

Investigation on the morphology of Lusitanian toadfish, *Halobatrachus didactylus* (Costa *et al*., 2003) confirmed the relevance of morphometric characters (head width, inter-orbital width and pelvic fin length) for a good separation of population on Portuguese coast. They found no significance on the above characters between sexes. Similar to the above study, present finding on the inter-orbital length indicated no significant difference between sexes. This result is again affirmed by the results of Marques *et al.* (2005). The independent t-test indicated no statistical differences between males and females ($P > 0.05$) for inter-orbital distance. In the present study, body dimensions including pre-pelvic fin length, pre-first dorsal fin length and post - orbital length had a higher rate of change in females but other characters had a higher growth rate in males.

In *C. dussumieri*, from 14 proportional dimensions, two in male and three in female represented a +A relationship with T.L while one and three had a -A relationship in males and females respectively. Both sexes followed a positive allometry for inter - orbital length and body depth while negative allometry for eye diameter. Males showed an isometric (I) growth pattern for pre-pelvic length, pre-pectoral length and caudal peduncle depth. However, females showed an accelerated (+A) growth rate for pre-pelvic length and a retarded (-A) growth pattern for pre-pectoral distance and caudal peduncle depth respectively. These results indicate a sexual dimorphism of the species. Marr (1955) affirmed that the ratio between various body parts and increase in length at different stages of life might not exhibit constant relative growth. Tandon (1962) stated that the ratio between various parts of males and females in the fish species studied by them were different. Minos *et al*. (2008) stated that the dimorphic characters in *Pagrus pagrus* may reflect the adaptation of males and females to different social or/ and reproductive roles rather than different niche utilization as both sexes were grown under the same artificial environment (rearing conditions).

Isometric relationships observed among various parts of body measurements of male (standard length, head length, snout length, post-orbital length, pre-first dorsal fin, pre-second dorsal fin, pre-pectoral length, pre-pelvic length, pre-anal length, depth through anal fin and caudal peduncle depth) and female (standard length, head length, snout length, post-orbital length, pre-first dorsal fin, pre-second dorsal fin, pre-anal length and depth through anal fin) of *C. dussumieri* suggest that these parameters increased proportionally with increasing total length thereby maintaining a symmetric form. This is in agreement with findings of Jaiswar *et al.*, 2004; Naeem and Salam, 2005; and Zafar *et al.*, 2002. On the other hand, Reiss (1989) proposed that organisms are not usually isometric, even when organized on similar patterns. Instead, certain proportions change in a regular fashion. Even within a single growth stage, different parts of the body of a fish may grow at different rates (Ricker, 1979). Some investigators correlated this fact with the variations in ecological conditions of habitats or variation in the physiology of the animals, or both. Geographical variation has already been documented by various workers for many fishes. According to Barlow (1961), these differences are caused by the fact that variation in shape is usually more related to genetic causes while meristic characters are much more dependent on environmental fluctuations. Observed disparity between populations and sexes were attributed to genetic and environmental cause (Jaiswar *et al.*, 2004; Saini *et al.*, 2008; Todd *et al*., 1981).

The simple method for testing hypotheses about equality of two population’s regression coefficient ‘b’ involves the use of students ‘t’ test. This test was applied to verify the significance of difference in morphometric characters of males and females. The ‘t’ test showed significant differences between male and female in all the characters except for head length, snout length, inter-orbital length and body depth (Table 3.6).

The analysis of morphometric variables demonstrated a substantial degree of differences between the sexes. The study suggests that male and female of *Colletteichthys dussumieri* have similar values of growth rate of the examined measurements. Nevertheless, they followed diverse pattern of growth.

Table 3.1. Various examined morphometric measurements in males and females of *C. dussumieri*

Morphometric Characters	Male			Female		
	Mean ±SE	Minimum	Maximum	Mean ±SE	Minimum	Maximum
T.L	230.38 ± 6.24	119	299	194.48 ± 5.55	142	233
Std.L	189.06 ± 5.21	95	246	158.33 ± 4.48	116	188
H.L	78.19 ± 2.17	38.5	103	65.83 ± 2.03	48	81
S.L	16.90 ± 0.60	7	24	15.19 ± 0.59	10.5	20
P.O.L	51.06 ± 1.36	27	65	41.60 ± 1.36	31	53
I.O.L	26.31 ± 0.95	11	40	20.76 ± 0.92	12	28
E.D	11.37 ± 0.326	7	16	10.44 ± 0.259	7.5	12
P.ID.L	71.43 ± 2.075	35.50	92.50	60.98 ± 1.975	42.50	72.50
P.IID.L	93.24 ± 2.039	47	119	79.88 ± 2.55	56	98
P.Pc.L	66.62 ± 2.129	31	93	56.33 ± 1.68	40	71
P.Pv.L	55.40 ± 1.69	23.50	75	46.93 ± 1.88	32	58
P.A.L	120.09 ± 3.696	61	182	101.38 ± 3.195	71	129
B.Dep	53.07 ± 2.128	23	82	44.40 ± 1.98	30.50	64.50
Dep.A	50.15 ± 1.63	22	75	42.40 ± 1.60	29	55
C.Pd.Dep	19.06 ± 0.544	10	25	16.45 ± 0.452	12	21

Table 3.2. Correlation matrix between various morphometric measurements in males of *C. dussumieri*

		T.L	Std.L	H.L	S.L	P.O.L	I.O.L	E.D	P.ID	P.IID	P.P.cL	P.Pv.L	P.A.L	B.Dep	Dep.A	C.Pd.Dep
T.L	**Correlation**	1														
Std.L	**Correlation**	.996(**)	1													
H.L	**Correlation**	.971(**)	.966(**)	1												
S.L	**Correlation**	.663(**)	.659(**)	.692(**)	1											
P.O.L	**Correlation**	.968(**)	.963(**)	.967(**)	.645(**)	1										
I.O.L	**Correlation**	.923(**)	.914(**)	.895(**)	.700(**)	.908(**)	1									
E.D	**Correlation**	.847(**)	.832(**)	.831(**)	.497(**)	.861(**)	.811(**)	1								
P.ID	**Correlation**	.946(**)	.938(**)	.953(**)	.706(**)	.927(**)	.844(**)	.750(**)	1							
P.IID	**Correlation**	.942(**)	.933(**)	.951(**)	.749(**)	.917(**)	.850(**)	.748(**)	.978(**)	1						
P.Pc.L	**Correlation**	.899(**)	.886(**)	.913(**)	.634(**)	.872(**)	.783(**)	.727(**)	.900(**)	.899(**)	1					
P.Pv.L	**Correlation**	.793(**)	.797(**)	.835(**)	.565(**)	.785(**)	.646(**)	.604(**)	.810(**)	.844(**)	.835(**)	1				
P.A.L	**Correlation**	.955(**)	.948(**)	.946(**)	.679(**)	.924(**)	.914(**)	.793(**)	.922(**)	.939(**)	.920(**)	.805(**)	1			
B.Dep	**Correlation**	.810(**)	.812(**)	.792(**)	.689(**)	.791(**)	.815(**)	.692(**)	.816(**)	.817(**)	.716(**)	.610(**)	.826(**)	1		
Dep.A	**Correlation**	.904(**)	.891(**)	.899(**)	.688(**)	.907(**)	.909(**)	.753(**)	.911(**)	.905(**)	.871(**)	.754(**)	.934(**)	.841(**)	1	
C.Pd.Dep	**Correlation**	.948(**)	.943(**)	.917(**)	.679(**)	.940(**)	.920(**)	.773(**)	.945(**)	.925(**)	.852(**)	.695(**)	.927(**)	.844(**)	.941(**)	1

** Correlation is significant at the 0.01 level (2-tailed).

Table 3.3. Correlation matrix between various morphometric measurements in females of *C. dussumieri*

		T.L	**Std.L**	**H.L**	**S.L**	**P.O.L**	**I.O.L**	**E.D**	**P.ID**	**P.IID**	**P.Pc.L**	**P.Pv.L**	**P.A.L**	**B.Dep**	**Dep.A**	**C.Pd.Dep**
T.L	**Correlation**	1														
Std.L	**Correlation**	.988(**)	1													
H.L	**Correlation**	.949(**)	.937(**)	1												
S.L	**Correlation**	.670(**)	.658(**)	.589(**)	1											
P.O.L	**Correlation**	.952(**)	.925(**)	.954(**)	.662(**)	1										
I.O.L	**Correlation**	.855(**)	.854(**)	.869(**)	.466(*)	.872(**)	1									
E. D	**Correlation**	.831(**)	.828(**)	.779(**)	.512(*)	.781(**)	.766(**)	1								
P.ID	**Correlation**	.942(**)	.930(**)	.952(**)	.667(**)	.935(**)	.896(**)	.811(**)	1							
P.IID	**Correlation**	.950(**)	.943(**)	.950(**)	.592(**)	.914(**)	.920(**)	.813(**)	.981(**)	1						
P.Pc.L	**Correlation**	.862(**)	.860(**)	.937(**)	.427(*)	.869(**)	.832(**)	.679(**)	.861(**)	.882(**)	1					
P.Pv.L	**Correlation**	.824(**)	.822(**)	.909(**)	.339(*)	.830(**)	.819(**)	.706(**)	.838(**)	.870(**)	.936(**)	1				
P.A.L	**Correlation**	.961(**)	.967(**)	.905(**)	.645(**)	.917(**)	.832(**)	.870(**)	.903(**)	.912(**)	.820(**)	.826(**)	1			
B.Dep	**Correlation**	.752(**)	.781(**)	.752(**)	.655(**)	.720(**)	.788(**)	.583(**)	.781(**)	.821(**)	.738(**)	.678(**)	.757(**)	1		
Dep.A	**Correlation**	.892(**)	.889(**)	.889(**)	.660(**)	.858(**)	.754(**)	.720(**)	.853(**)	.876(**)	.804(**)	.777(**)	.903(**)	.813(**)	1	
C.Pd.Dep	**Correlation**	.900(**)	.873(**)	.903(**)	.525(*)	.873(**)	.842(**)	.785(**)	.903(**)	.929(**)	.852(**)	.798(**)	.835(**)	.715(**)	.842(**)	1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Table 3.4. Summary of statistical analysis of males and females of *C. dussumieri*

Morphometric measurements	Male			Female		
(mm)	a	b	r	a	b	r
Std.L	-2.611	0.832	0.996	3.457	0.796	0.988
H.L	0.478	0.337	0.971	-1.438	0.346	0.949
S.L	2.157	0.064	0.663	1.271	0.072	0.670
P.O.L	2.467	0.211	0.968	-3.687	0.233	0.952
I.O.L	-6.080	0.141	0.923	-6.726	0.141	0.855
E.D	1.192	0.044	0.847	2.872	0.039	0.831
P.ID	-1.042	0.315	0.946	-4.187	0.335	0.942
P.IID	4.703	0.384	0.942	-5.024	0.437	0.950
P.Pc.L	-1.081	0.294	0.899	5.494	0.261	0.862
P.Pv.L	5.796	0.215	0.793	-8.098	0.279	0.824
P.A.L	-10.271	0.566	0.955	-6.18	0.553	0.961
B.Dep	-10.570	0.276	0.810	-7.88	0.269	0.752
Dep.A	-4.227	0.236	0.904	-7.515	0.257	0.892
C.PdDep	-0.014	0.083	0.948	2.197	0.073	0.900

Table 3.5. Ontogenetic changes in morphometric measurements for males and females *C. dussumieri*

Dimension	Males				
	Log a	b	SE(b)	r^2	Increment
Std.L	-0.14871	1.027	0.0142	0.9938	I
HL	-0.4684	0.999	0.0413	0.9481	I
S.L	-1.029	0.953	0.165	0.509	I
P.O.L	-0.5118	0.939	0.0382	0.9497	I
I.O.L	-1.490	1.231	0.0826	0.8739	+A*
E.D	-0.5804	0.699	0.0426	0.8936	-A*
P.I.D	-0.5603	1.022	0.0572	0.9087	I
P.II.D	-0.2938	0.958	0.05696	0.898	I
P.Pc.L	-0.596	1.024	0.0819	0.830	I
P.Pv.L	-0.5826	0.984	0.1151	0.695	I
P.A.L	-0.3726	1.038	0.0479	0.936	I
B.Dep	-1.540	1.377	0.0977	0.8612	+A*
Dep.A	-0.869	1.087	0.0813	0.8479	I
C.Pd.Dep	-1.0437	0.983	0.0523	0.9169	I

Dimension	Females				
	Log a	b	SE(b)	r^2	Increment
Std.L	-0.0422	0.979	0.0315	0.9807	I
HL	-0.5079	1.016	0.0760	0.9038	I
S.L	-0.9171	0.915	0.2219	0.4724	I
P.O.L	-0.7933	1.054	0.0828	0.8949	I
I.O.L	-1.6116	1.282	0.1033	0.8901	+A*
E.D	-0.7373	0.767	0.1038	0.7418	-A*
P.I.D	-0.6841	1.079	0.0845	0.8955	I
P.II.D	-0.5386	1.066	0.078	0.9077	I
P.Pc.L	-0.0404	0.777	0.1076	0.7333	-A*
P.Pv.L	-1.2252	1.265	0.0841	0.9220	+A*
P.A.L	-0.4407	1.069	0.0619	0.9399	I
B.Dep	-1.2737	1.284	0.1001	0.896	+A*
Dep.A	-1.3087	1.166	0.1299	0.8092	I
C.Pd.Dep	-0.6258	0.801	0.1010	0.7679	-A*

Values given (log *a*+*b*) are from the equation log (dimension)=log *a*+*b* log (*TL*). Increment patterns are: +A, positive allometry; -A, negative allometry; I, isometry; * P<0.5

Table 3.6. Comparision between regression coefficient in males and females of *C. dussumieri*

Morphometric Measurements (mm)	Male		Female		t	Significance
	b1	Sb1	b2	Sb2		
Std.L	0.832	0.013	0.796	0.028	-2.400	* *
H.L	0.337	0.015	0.346	0.026	0.818	NS
S.L	0.064	0.013	0.072	0.018	1.518	NS
P.O.L	0.211	0.01	0.233	0.017	3.143	* *
I.O.L	0.141	0.01	0.141	0.02	0.000	NS
E.D	0.044	0.005	0.039	0.006	-5.270	* * *
P.ID	0.315	0.019	0.335	0.027	2.500	* *
P.IID	0.384	0.024	0.437	0.033	5.889	* * *
P.Pc.L	0.294	0.025	0.261	0.035	-3.300	* *
P.Pv.L	0.215	0.029	0.279	0.044	4.267	* * *
P.A.L	0.566	0.031	0.553	0.036	-2.600	* *
B.Dep	0.276	0.035	0.269	0.054	-0.368	NS
Dep.A	0.236	0.02	0.257	0.03	2.100	* *
C.Pd.Dep	0.083	0.005	0.073	0.008	-3.163	* *

(t, 2, 53 DF) *** $P < 0.001$, ** $P < 0.05$

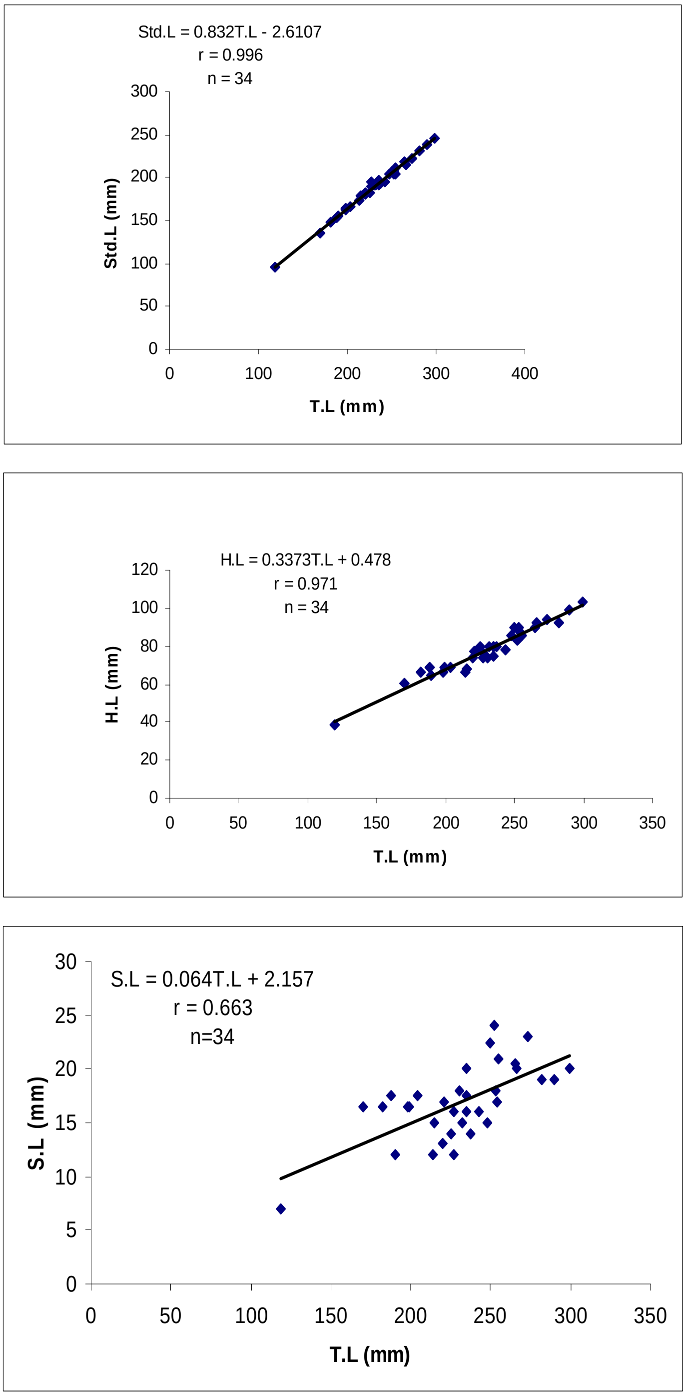


Fig.3.2a. Relationship between various morphometric measurements and total length in males of *C. dussumieri*

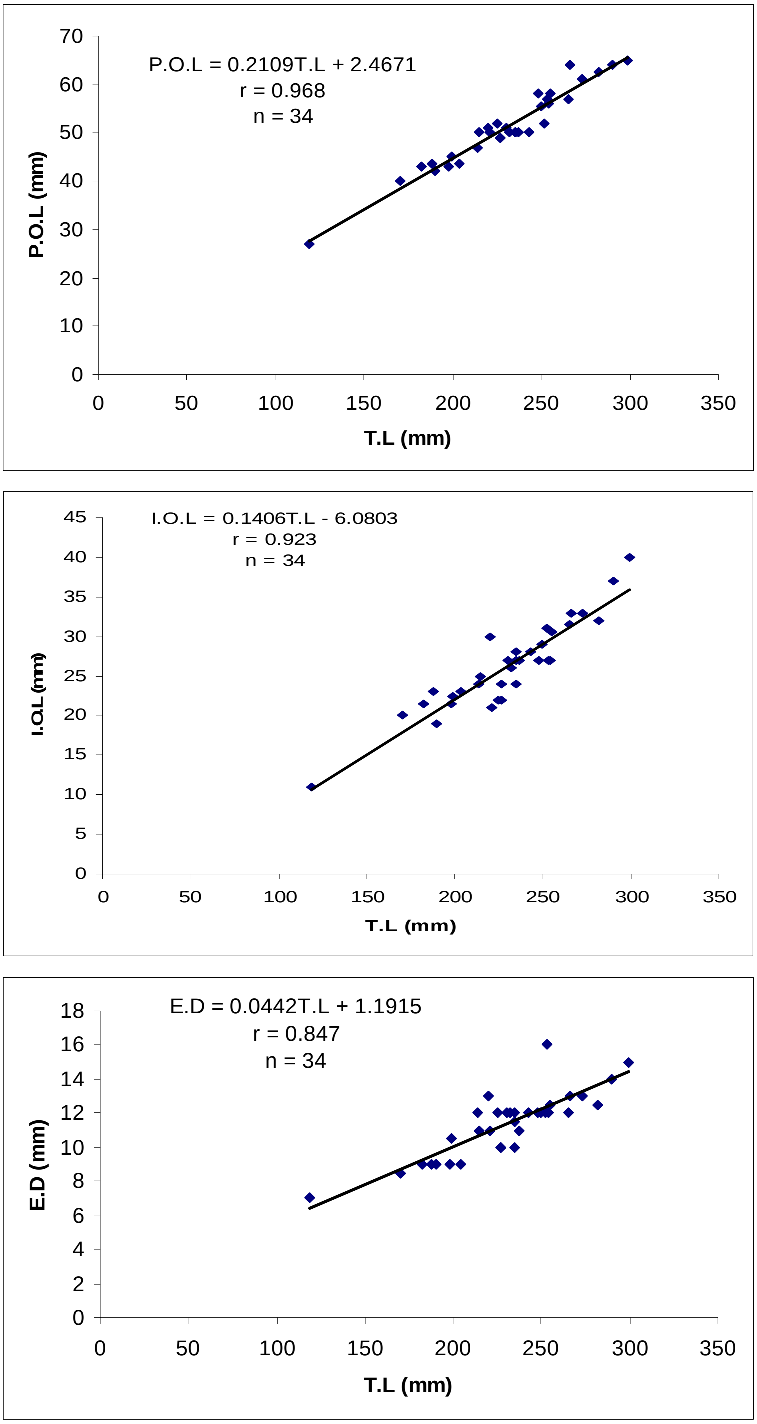
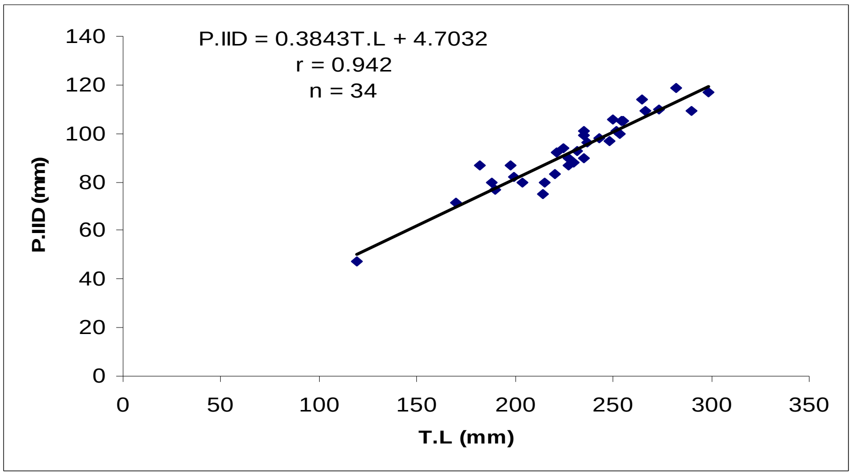
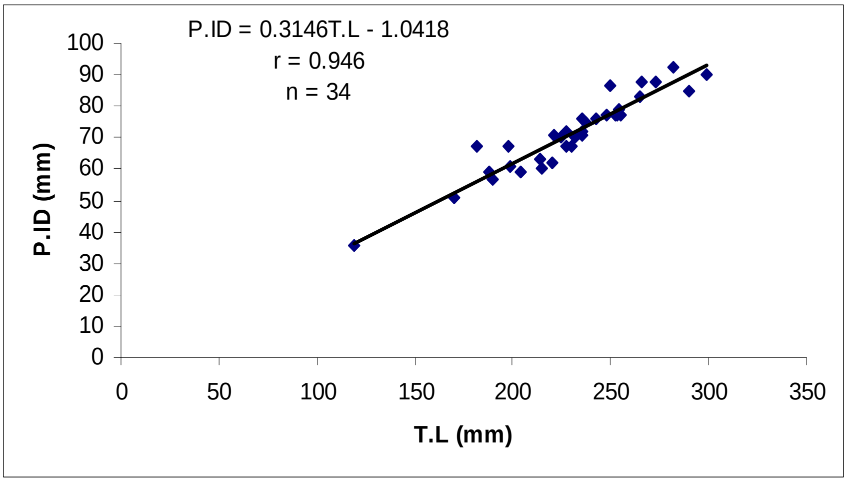


Fig.3.2b. Relationship between various morphometric measurements and total length in males of *C. dussumieri*





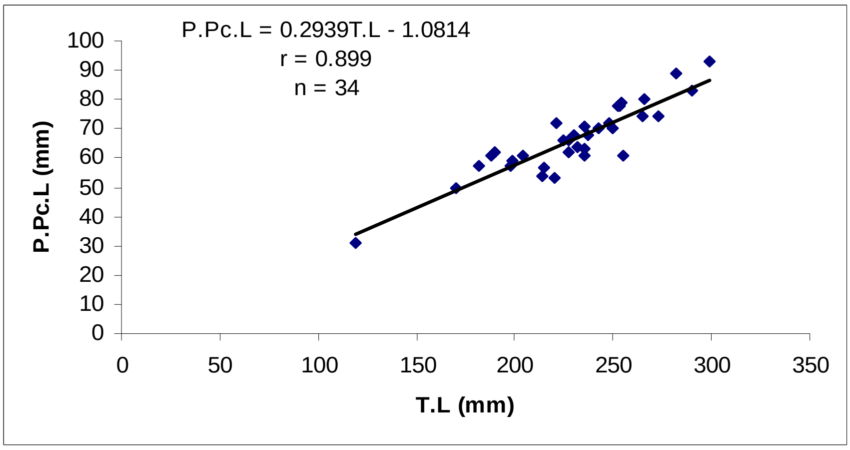


Fig.3.2c. Relationship between various morphometric measurements and total length in males of *C. dussumieri*

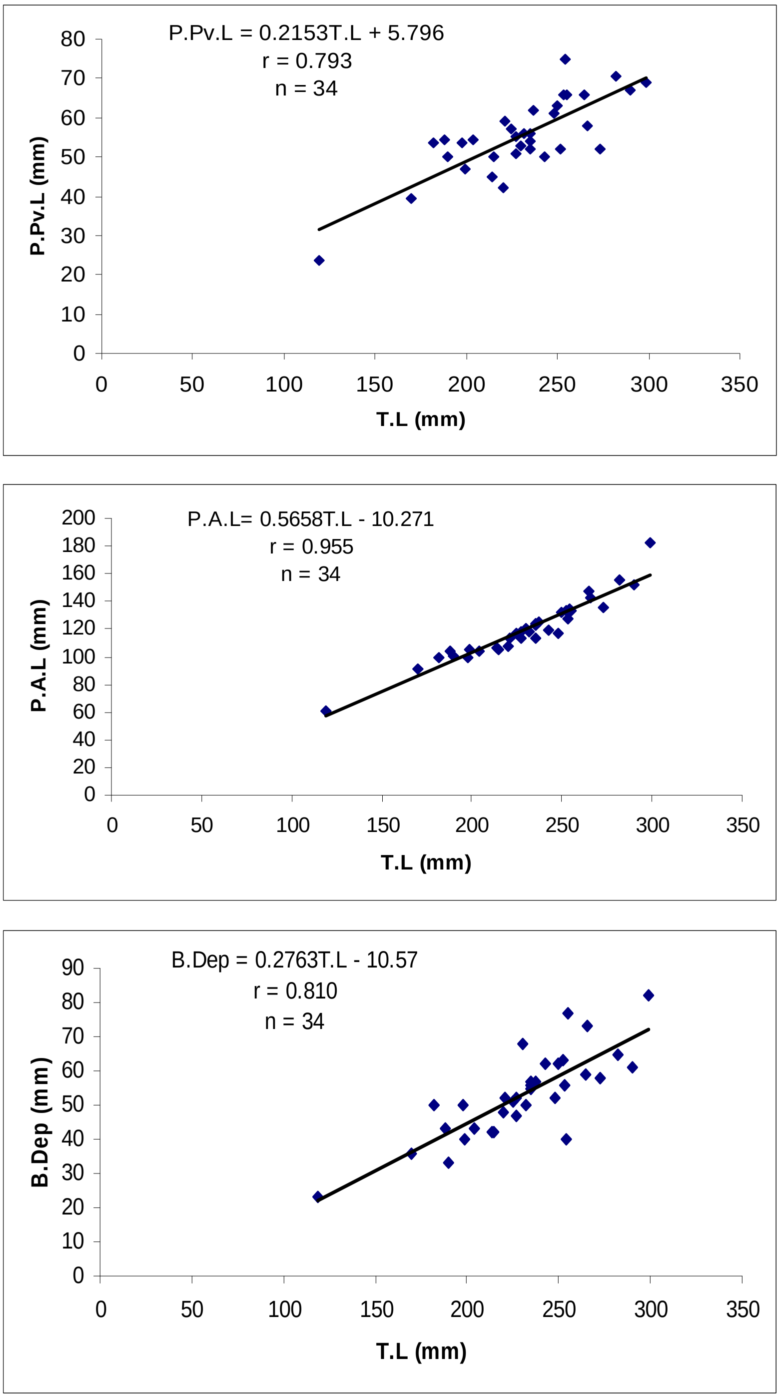
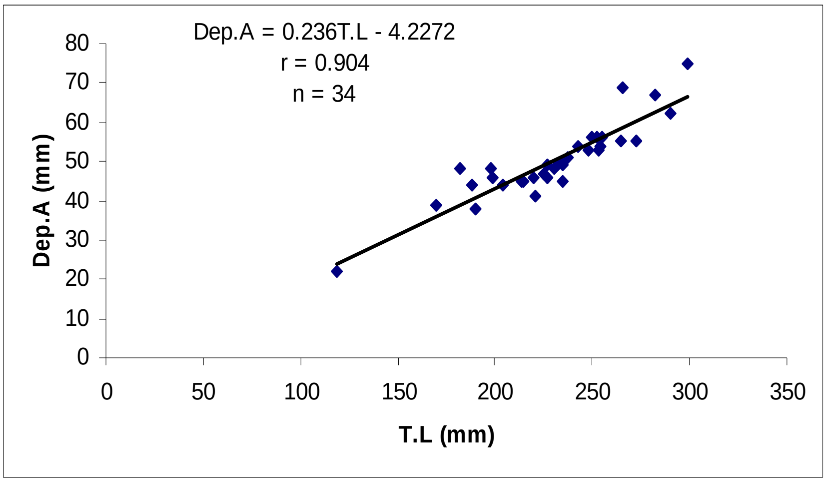


Fig.3.2d. Relationship between various morphometric measurements and total length in males of *C. dussumieri*



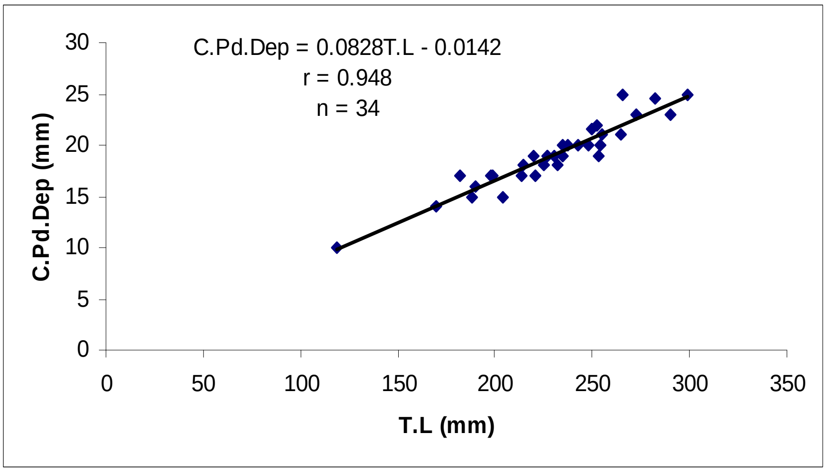


Fig.3.2e. Relationship between various morphometric measurements and total length in males of *C. dussumieri*

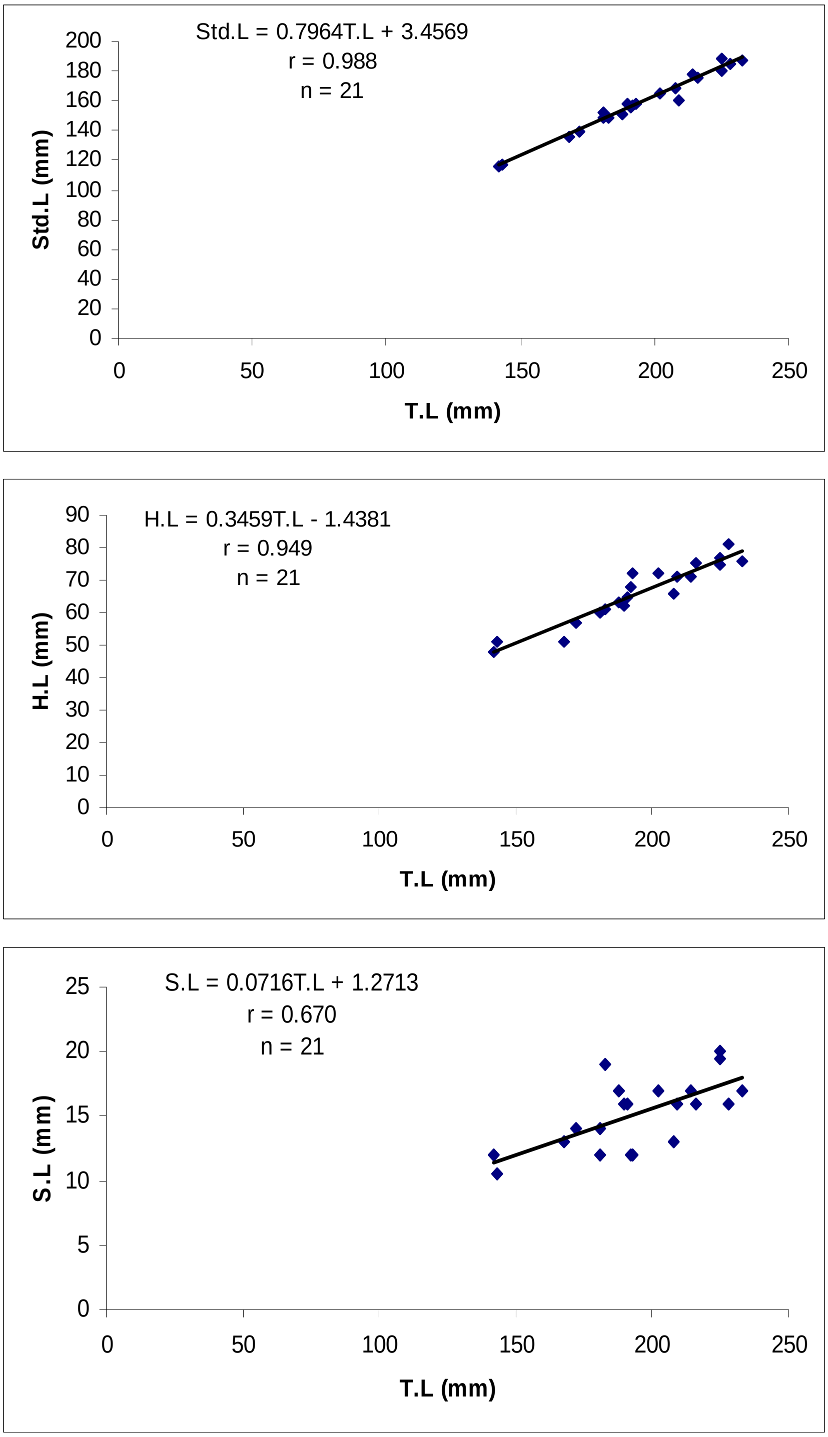
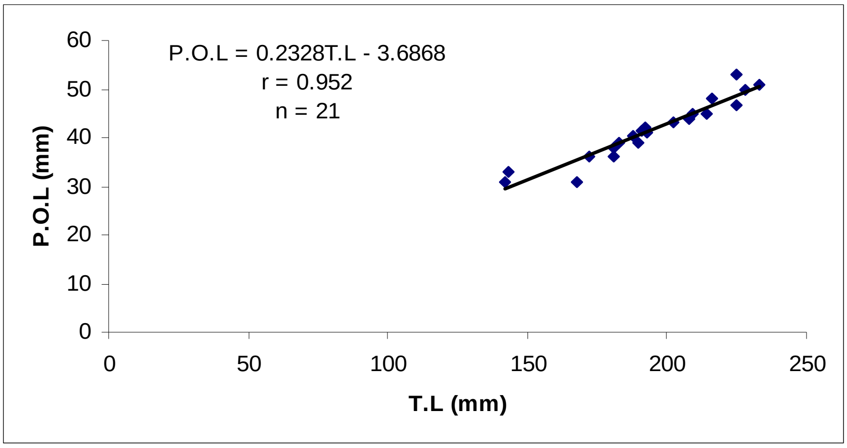
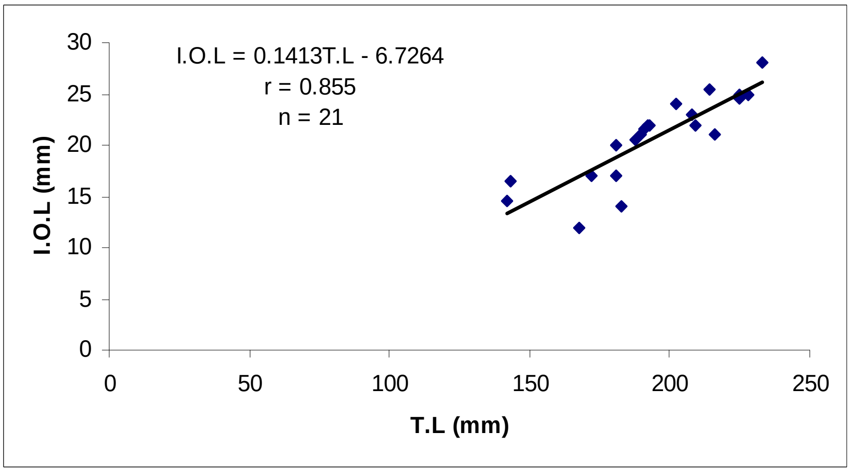


Fig.3.3a. Relationship between various morphometric measurements and total length in females of *C. dussumieri*





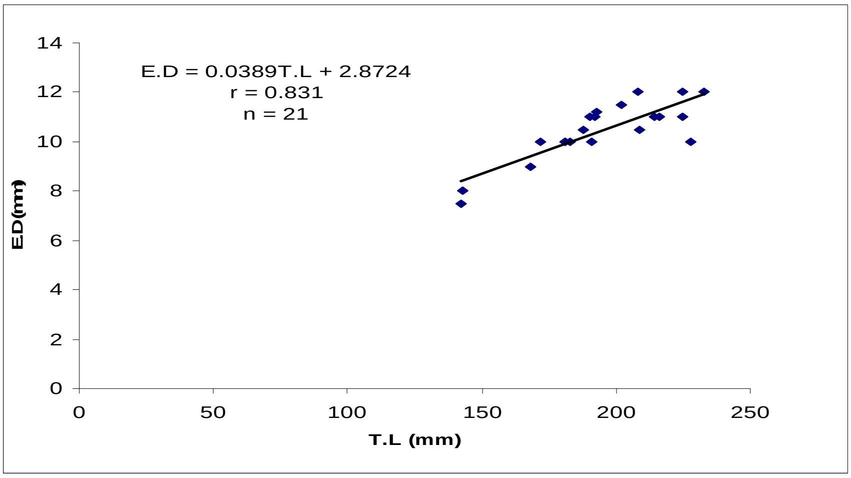


Fig.3.3b. Relationship between various morphometric measurements and total length in females of *C. dussumieri*

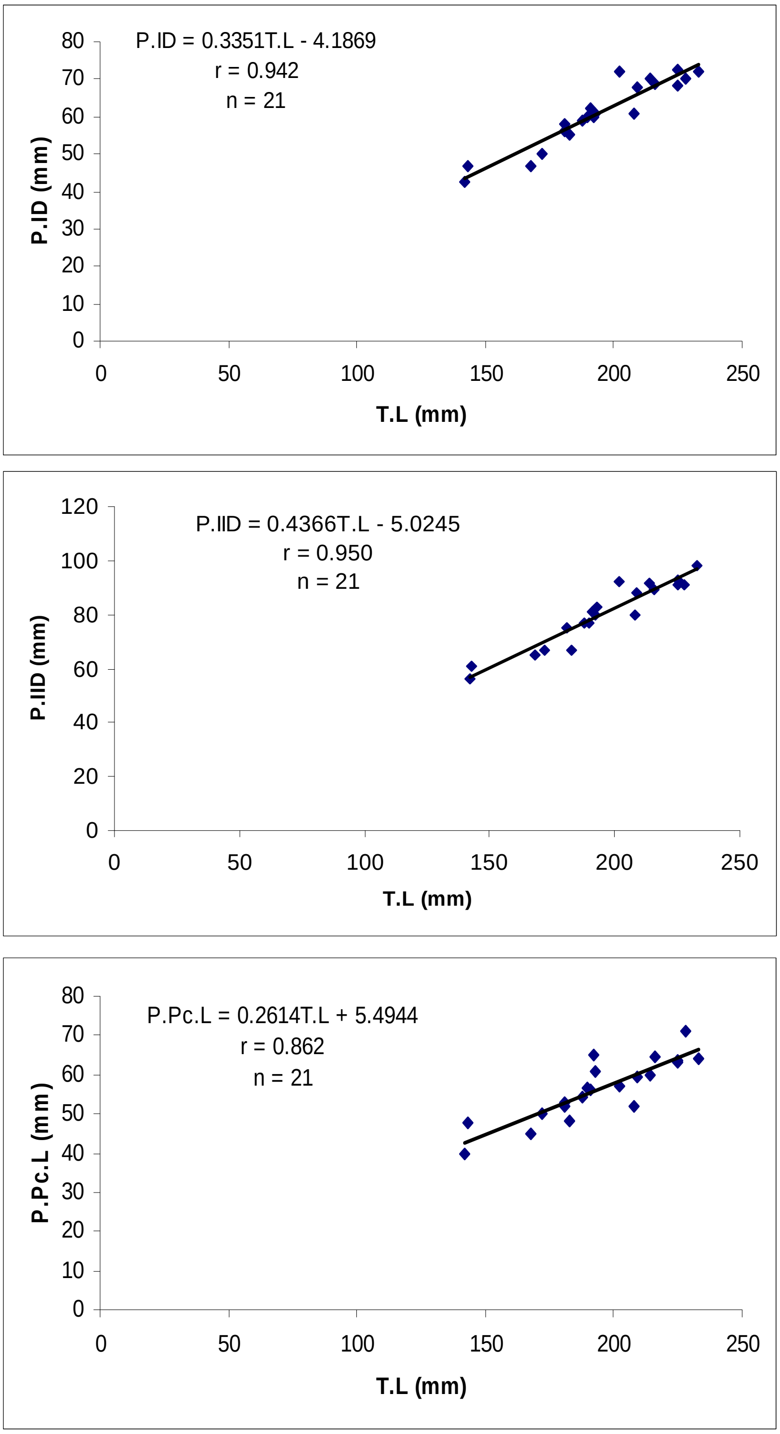
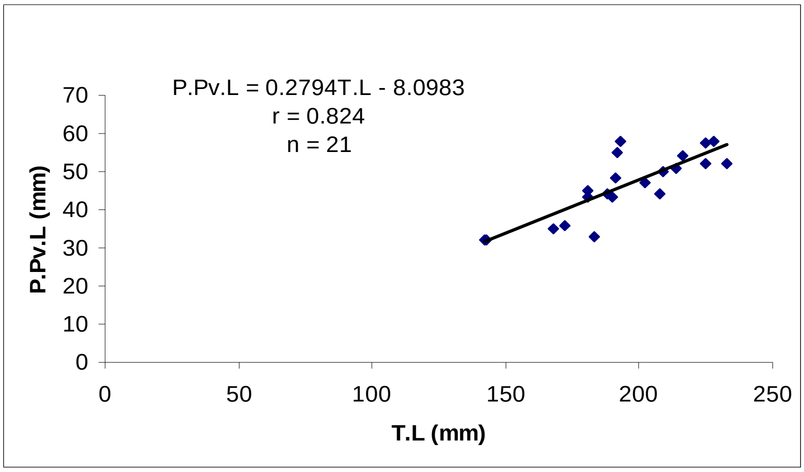
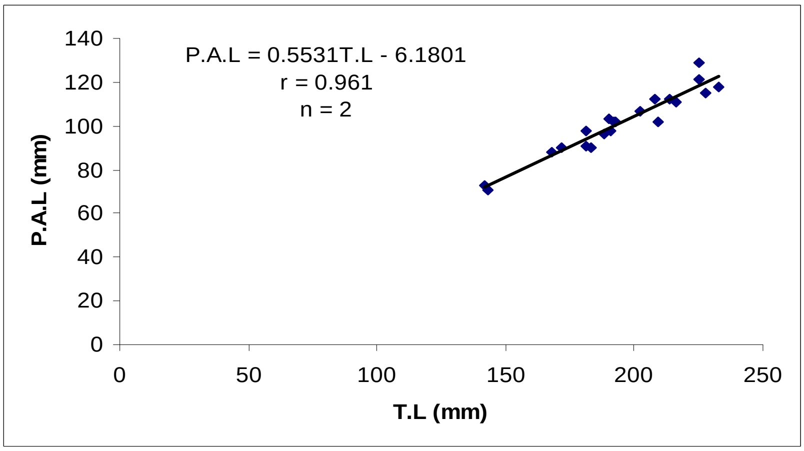


Fig.3.3c. Relationship between various morphometric measurements and total length in females of *C. dussumieri*





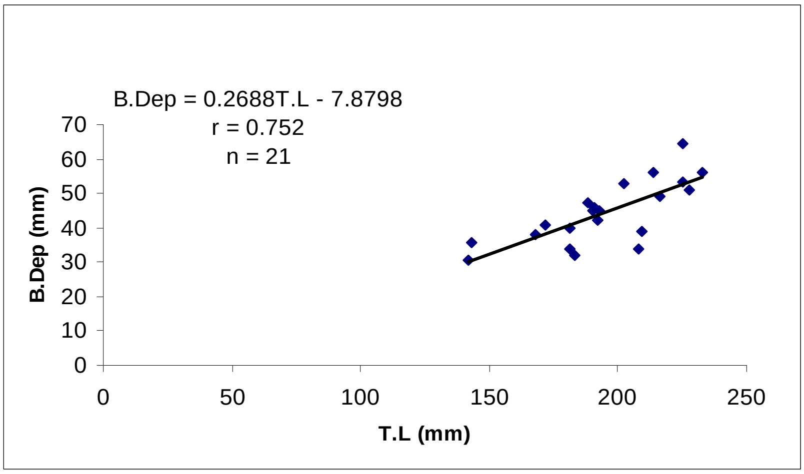


Fig.3.3d. Relationship between various morphometric measurements and total length in females of *C. dussumieri*

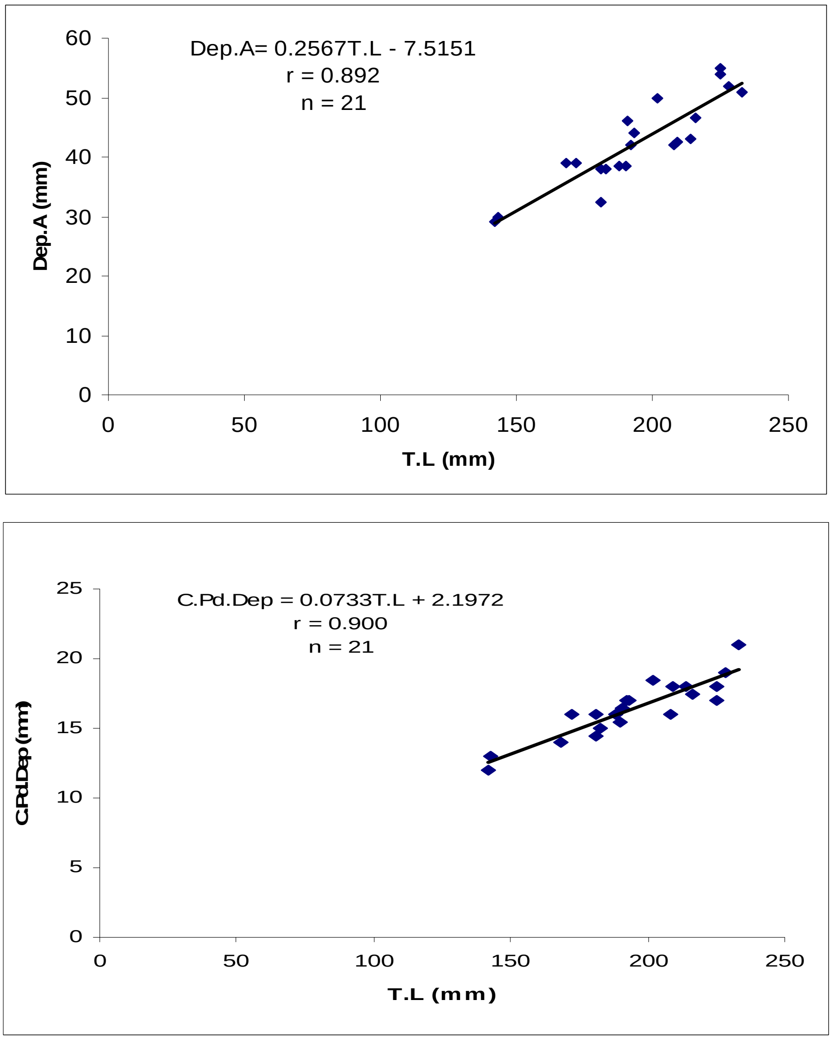


Fig.3.3e. Relationship between various morphometric measurements and total length in females of *C. dussumieri*

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Chapter 4

FOOD AND FEEDING

4.1 Introduction

Every animal requires energy for growth, maintenance, reproduction and other metabolic activities-all takes place at the expense of the energy which enters the organism in the form of food. Hence food can be considered as the most essential prerequisite for the growth and survival of all living forms. Fish just like any other organism require food in order to perform its biological processes. Food forms the basis to all function of an individual fish as well as the population and differs considerably in the preference to what they consume. Feeding is one of the main concerns of daily living in fishes and the fish devotes large portion of its energy searching for food. Feeding and searching for food are factors, which regulate or at least influence the distribution, migration and growth of fish (Papaconstantinou and Caragitsou, 1992).

The study of food and feeding habits of fishes help in understanding various aspects of their biology like migration, growth, maturation, spawning and seasonal variations in biochemical composition. Accurate description of the diets and feeding habits provides the basis for understanding the trophic interactions in aquatic food webs (Vander

Zanden *et al.*, 2000, Vander Zanden and Rasmussen, 2002) and also help to determine the niche in the ecosystem, the preferred food items and the food spectrum overlapping with that of co-existing fishes (Basudha and Vishwanath, 1999). Hence, it is desirable to study the food and feeding habits as a part of fish biology.

Fish species in tropical regions are found to be highly complex with many species coexisting in the same environment. This high diversity is governed by localized environmental disturbances and preference for different microhabitat and food items. The study of the food of a species by the analysis of the stomach content gives an idea about its diet, determining its trophic level in the ecosystem and its possible dependence on other species. Moreover, the possible variations in its diet depending on certain parameters such as the depth where it is located, the time of the day and the season of the year can also be determined. This can give information about the position of an individual while eating as well as its function as a part of the biocoenosis (Amezaga, 1988).

The success of effort to introduce and cultivate any new species, however, will depend on quantitative information about the food and feeding habit, nutritional requirements and trophic ecology of that species. This helps to determine cultivation of desirable species combinations in culture systems with minimum interspecies competition for the natural food. It also provides vital clues in developing supplementary feed for the species. Therefore, a study of food and feeding habits of fishes is very important in any fisheries management programme.

As the nature of food depends to a great extent upon the nature of environment, the problem is interesting from specific, as well as ecological point of view. The food and feeding habit of fishes vary from season to season. Seasonal change in temperature not only influence food consumption and rate of digestion but also quality and quantity of available food organisms. The diets of most fish species changes with age and growth. The time and extent of changes in food and feeding habits varies from species to species and often with changes in the life style or habitat (Blaber, 2000). Without knowledge of the food requirements, feeding behaviour pattern, and predator - prey relationships, it is not possible to understand the predicted changes that might result from any natural or anthropogenic intervention.

An extensive work has been done on the food and feeding ecology of many fish species all over the world. Some of the outstanding works in this field are those of Hynes (1950), Hyslop (1980), Fuentes (1982), Russel (1983), Gunn and Milward (1985), Kuo and Shao (1991), Said *et al.* (1994), Weerts *et al.* (1997), Crabtree *et al*. (1998), Rizkalla *et al.* (1999), Dadzie *et al.*(2000a), Terrats *et al.* (2000), Hajisamae *et al*.(2003), Pyka and Kolman (2003), Bachok (2004), De Melo *et al*. (2004), Mamun *et al.* (2004), Castriota *et al.* (2005a, b), Beltrano *et al*. (2006), Blay *et al.* (2006), Bahou *et al.* (2007), Bascinar and Saglam (2009), Shalloof and Khalifa (2009) and Yem *et al*. (2009).

A number of scientists have also studied various aspects of food and feeding habits of several fish species from Indian waters. (Pillay, 1952; Menon and Chacko, 1956; Venkataraman, 1960; Natarajan and Jhingran, 1963; Dhulkhed, 1963; Mojumder, 1969; Kagwade, 1971; Patnaik and Jena, 1976; Pati, 1983; Khumar and Siddiqui, 1989; Manikyala and Srinivasa, 1991; Vasudevappa and James, 1992; Khan and Fatima, 1994; Badrudeen and Mahadevan, 1996; Serajuddin *et al.*, 1998; Kiran and Puttaiah, 2004;

Arockia Raj *et al.* 2004; Manojkumar, 2004, 2007; Serajuddin and Ali, 2005; Sreeraj *et al.* 2006; Sudhakar and Shameem, 2006; Prabha and Manjulatha, 2008; Sarkar and Deepak, 2009; Sivadas and Bhaskaran, 2009.)

The present chapter deals with food and feeding of *C. dussumieri.*

4.2 Materials and methods

Specimens for the present study were collected from Cochin estuary using gill net during October 2003 - September 2005. The study is based on 467 specimens, 219 females (95 - 246 mm TL) and 248 males (94 - 305 mm TL). The specimens were properly cleaned in the laboratory and the total length, total weight, sex, stage of maturity and degree of stomach fullness were recorded. The stomach of each fish was dissected out and the food was preserved in 5% formaldehyde for further study.

The degree of stomach fullness was assessed on the basis of distension of stomach folds (Rao, 1964). Depending on the extent of distension of the stomach and the amount of food in it, the stomachs were designated as gorged, full, 3/4 full, 1/2 full, 1/4 full, barley full and empty. Numerical values of 100, 75, 50, 25, 12, 6 and 0 (zero) were assigned respectively to the above categories (Khan and Hoda, 1993). Stomachs were classified as heavily fed (gorged, full and ¾ full), moderately fed (½ full and ¼ full) and poorly fed (barely full and empty) following the method used by Manoj Kumar (2007).

Generally, the stomach is considered for evaluating the food and feeding, however in case of herbivorous fish the alimentary canal is very long and retain most of the food for prolonged period. In the present species the alimentary canal is short; hence the stomach was only considered for study.

The feeding intensity or the degree of feeding is related with season, maturity, spawning and availability of food materials. It is determined by calculating the gastro- somatic index (Ga.S.I). The gastro-somatic index was calculated to investigate monthly as well as size-wise variations in feeding intensity, using the method adopted by Desai (1970).

$$\text{Ga.S.I} = \frac{\text{Weight of the stomach contents}}{\text{Total weight of the fish}} \times 100$$

The length of the gut was measured and the relative length of the gut (RLG) was computed for the different length groups by the method of Al-Hussaini (1949).

$$\text{RLG} = \frac{\text{Length of the gut}}{\text{Total length of the body}}$$

The percentage occurrence of food item was analyzed by the method of Hynes (1950). The relative importance of the items was judged by the 'Index of Preponderance' as given by Natarajan and Jhingran (1962), which combines both volumetric and frequency of occurrence methods giving rise to accuracy in grading various food elements, was employed to evaluate the food preferences of males and females. The Index of Preponderance was resolved by the equation:

$$\text{I} = \frac{\text{ViOi}}{\sum \text{ViOi}} \times 100$$

Where,

I = Index of Preponderance of the food item.

Vi = Percentage of volume index of the food item.

Oi = Percentage of occurrence index of the food item.

The points (volumetric) method as suggested by Pillay (1952) was employed for the estimation of various food items. The fullness of stomach was also taken into account in the allotment of points (Frost, 1943). The points gained by each food item altered proportionally to the total points allocated for the stomach. The food elements were identified as far as possible up to the species or up to the genus or family, depending upon the completeness of the organism and the extent of digestion.

4.3 Results

In the toadfishes a variety of modifications seem to be associated with feeding. Head broad and flattened; eyes on top of head, upward-directed; the pectoral fins are large with a broad base; the pelvic fins are well forward under the body and have a fibrous pad along the front border of the leading ray. The mouth of *C. dussumieri* is large (wide), terminal, and slightly protrusible; jaws equal or the lower projecting; moderately strong teeth present in jaws and on roof of mouth. Two rows of pointed teeth in anterior portion of lower jaw; sides of the lower jaw with a single row of pointed teeth; upper jaw with three rows of pointed teeth anteriorly, two rows on side, grading into a single row posteriorly ; vomer and palatine with a single row of pointed teeth. Pharyngeal teeths are short, conical and sharp-pointed. Gill openings are small and are restricted to sides of the body, just in front of pectoral-fin base. Gill rakers are short and snout. The oesophagus is equipped with well developed longitudinal folds to permit distensibility. It is short, muscular with a wide lumen at its connection with the stomach on one end and the pharynx on the other. The fine, mainly longitudinal folds of the oesophagus became coarse folds in the stomach. Stomach is large and muscular and the intestine is relatively short and straight (Fig.4.1a). The intestine runs straight to anus.

The liver is bilobed and compact structure. The left lobe is larger than the right lobe and partly separated from each other. It is yellowish brown in colour. Between lobes of the liver a gall bladder is present.

The relative gut length (RLG) in different groups of *C. dussumieri* ranged from 0.819 to 0.97 in males and 0.817 to 0.914 in females (Fig 4.1b).

4.3.1 General diet composition of *C. dussumieri*

The various food items recorded from the stomach of *C. dussumieri* during the study period are presented in Table 4.1. Examination of stomach contents revealed that food items could be assorted into seven categories namely fish, prawn, crab, isopod, squilla, mollusca and semi digested matter.

Prawns were the most predominant dietary item recorded from the stomach of the fish round the year. Fish was found to show preference mainly to penaeid prawns like *Metapenaeus dobsoni*, *Penaeus indicus* and *Metapenaeus monoceros*.

Crabs formed the second dominant food items in *C. dussumieri.* Crabs were mainly represented by Juveniles of *Charybdis* sp. and *Scylla serrata.*

Fries of teleost fishes regularly encountered in the stomach of the *C. dussumieri*, belonged to *Leiognathus* sp., *Stolephorous* sp., *Anchoviella* sp. and *Sardinella* sp. Of the different species of fishes preferred, *Leiognathus* sp. has been found to be highly relished by the specie.

Among molluscs, bivalves like *Musculista senhousia* and *Sunetta scripta* occurred in minor quantities.

Isopods and *Squilla* spp. formed 0.29 to 0.3% and 0.32 to 3.12% total volume of stomach contents (Fig 4.2a and 2b).

Semi digested matter comprised of unidentified animal matter, which are in a semi digested state, formed 4.46 to 4.67% of the stomach contents (Fig 4.2a and Fig 4.2b).

4.3.2 Variation in the diet composition of males and females

The food of males and females were analyzed separately to find out the differences, if any. The percentage composition of different food items of males and females are given in Figs.4.2a and Fig. 4.2b respectively.

The study revealed that the food preferences of males and females were similar with variations in the magnitude of different food items consumed. The preference for crabs was found to be higher in males (35.12%) than in females (21.74%). Prawns formed the favourite group of food in both males (37.6 %) and females (35.99 %). Fishes, with respective value of 28.42%, were more abundant in females as against 19.1% in males. Molluscs and squilla, with respective values of 5.79% and 3.12%, were more abundant in females as against 2.9% molluscs and 0.32% squilla in males. Isopods formed a minor category in both sexes. The quantity of semi digested matter constituted almost similar in both males and females.

4.3.3 Seasonal variations in the diet of males and females

The monthly variation in the diet composition of males and females of *C. dussumieri*, based on index of preponderance values, for the years 2003 - ’04 and 2004 - ’05 are given in Tables 4.2, 4.3, 4.4 and 4.5. It revealed that percentage of composition of different food items varied in different months according to their availability and preference of fish.

Crustaceans were present in the stomach throughout the year and formed the bulk of the fish diet.

During 2003-’04, prawns formed a regular food item throughout the year in males with highest occurrence in August with an index value of 81.28 and minimum in October, the value being 8.1 (Table 4.2). Juveniles of the crabs formed 27.31% of the annual average food composition and were present during the entire period. The highest index value was recorded in the month of July (76.11) and the lowest was in August (3.59). Squilla and isopods were rare in the food. Squilla was observed only in March and May with an index values of 4.73 and 0.856 respectively. Isopod was present in March (3.41), April (1.07) and September (0.159). Fishes were recorded throughout the year, and ranked third in the diet registering the highest index value of 79.35 in October and lowest value of 2.13 in November. Molluscs were encountered in the food during the months of December, February - May and July, with highest occurrence in March (8.22) and lowest in July (0.655).

According to the index, crabs were the most preferred food item of males during 2004-’05. The item gained high index values during March – September with a peak value in July (70.51) whereas index values of prawns dominated during October to February gaining an elevated value of 57.42 in December. The incidence of squilla was noticed during the months of April (0.68), May (0.19) and July (0.36) while isopods were recorded only in February (0.81). Fishes formed the third dominant food item in all months. Maximum index was in March (28.29) and minimum in December (3.34). Molluscs were not represented during the months of November and December. Semi digested matter formed 2.09% and 6.23% of the annual average food composition of 2003- ’04 and 2004-’05 respectively.

Prawns formed considerable portion of the diet of females during 2003-’04 (Table 4.4). They gained high index value of 76.13 during the month of May and least value of 6.06 by July. Fishes were found in significant quantities during December and February. Crabs appeared as an important component in the diet during most of the months, 59.09 (January) and 3.58 (May) being the highest and lowest index values recorded. But they were absent in November. Molluscs were absent in October, November and August, with maximum index value of 50.51 in July. Squilla formed a small portion of the diet during the months of February, April, July and August. Isopods with very low index value were present in the stomach only in September. During 2004-’05, prawns were copious in the diet in April (70.54) (Table 4.5). Fishes were more abundant during the second year with highest values in August (59.52) and minimum in April (7.75). Index values of crabs varied between 8.31 (February) to 51.28 (March). Squilla was encountered in diet only during the month of January (19.11). Isopods were recorded only in October- November, February and June months. Molluscs contributed 7.17 % to the annual average food composition. These were absent in October – December, April and August – September. The index values of molluscs ranged from 1.11 in May to 44.59 in January. The highest quantity of semi-digested food was found in September (27.56) and December (10.11) in 2003-’04 and 2004-’05 respectively.

In males, prawns constituted the main food while crabs ranked next in importance during 2003-’04. But this was reversed during 2004-’05. Fish formed the third preferred item during both years. During 2003-’04 and 2004-’05, in females, prawns were the most preferred food item while fish were next in importance. Crabs occupied third position in both years.

Molluscs ranked fourth position in both males and females in throughout the period of investigation. Squilla and isopods were of minor importance in the diet of *C. dussumieri.*

4.3.4 Feeding Intensity

4.3.4.1 Guts in different degrees of fullness

Monthly fluctuations in the percentage occurrence of stomachs in different degrees of fullness in males and females of *C. dussumieri* during the years 2003-’04 and 2004-’05 are depicted in Figs. 4.3, 4.4, 4.5 and 4.6 respectively.

In males, ‘gorged’ stomachs were present in March, April and June during 2003-’04. The incidence of ‘full’ stomachs was noticed in December and February – July with high percentage in May (33.3%) and June (33.3%). Fishes with ‘¾ full’ stomachs were observed throughout the year except in December and May with a dominance in August (50%). Predominance of ‘½ full’ stomachs was seen in July (60%), but were absent in January and June. ‘¼ full’ stomachs were not encountered in November and June – July with elevated percentage in December (44.44). Stomachs which were ‘barely full’ were absent during April - August while empty stomachs were lacking from January – August with raised percentage in November (33.3%). During 2004-’05, ‘gorged’ stomachs were perceived form March to July while fishes with ‘full’ stomachs occurred from February –June. Dominance of ‘gorged’ and ‘full’ stomachs were seen in April with percentage values of 25% and 37.5% respectively whereas the presence of ‘¾ full’ stomachs were observed in all months except October, with maximum percentage of occurrence in May (40.9%). The occurrence of ‘½ full’ stomachs were recorded throughout the year with highest percentage in October (62.5%). Fishes with ‘¼ full’ stomachs were observed throughout the year except April. ‘Barely full’ stomachs were noticed during November, January – March and July with preponderance in February (21.43%). The highest percentage of empty stomachs was recorded in November (35.29%) and the lowest in September (10%). Male fishes with ‘gorged’ and ‘full’ stomachs were totally absent in October, November, August and September whereas gorged’ , ‘full’, ‘¾ full’, ‘½ full’, ‘¼ full’ and ‘barely full’ stomachs was recorded in March during both the years. The highest incidence of ‘empty’ stomachs was encountered in November during both the years.

During 2003-‘04, female fishes with ‘gorged’ stomachs were observed during February to April and August with a highest frequency of 12.5% in August. Prevalence of ‘full’ stomachs were discerned in March and May – July and ‘¾ full’ stomachs in February, May and September. Dominance of ‘full’ stomachs was seen in June (66.6%) and July (66.6%) and ‘¾ full’ in February (33.3%). Fishes with ‘½ full’ stomachs were encountered during all the months whereas ‘¼ full’ stomachs were absent during November – January and June –July. ‘½ full’ stomachs were abundant in May (38.46%) and August and least in January (12.5%). Highest percentage of occurrence of ‘¼ full’ stomachs were noticed in March (26.08%) while it was slight in February (6.66%). Fishes with ‘barely full’ stomachs were not encountered during November and June - July while empty guts were observed from October – November, January – May and August –September with the maximum percentage in November (66.66%). During 2004-‘05, ‘gorged’ stomachs were discerned in March (20%) and May (12.5%) while fishes with ‘full’ stomachs January, April – June and September with utmost percentage in January and June (33.3%) and minimum in May and September (12.5%). Frequency of ‘¾ full’ stomachs were evident from February - March, May, July and September with peak percentage in July and lowest in September (12.5%). '½ full' stomachs were recorded in all months except July and August and were copious in February (66.6%) and scarce in September (12.5%). Incidence of '¼ full' stomachs were invariably high during July and August (50%) followed by November (42.85%) and January (33.3%). However, '¼ full' stomachs were absent during February and June. Fishes with 'barely full' stomachs were absent during January – March, May and July with foremost occurrence in August (50%) and minor percentage in November (9.52%). Empty stomachs were observed during October - December, May and September with predominance in November (38.09%). Fishes with 'gorged', 'full' and '¾ full' stomachs were totally absent in October, November and December while presence of '½ full', '¼ full', 'barely full' and 'empty' stomachs was recorded in October during both the years. The highest frequency of 'empty' stomachs was encountered in November during both the years.

4.3.4.2 Gasto-Somatic Index (Ga.SI)

Monthly fluctuations in gastro-somatic index of male and female *C. dussumieri* during 2003-'04 and 2004-'05 are shown in Figs. 4.7 - 4.8 respectively. The pattern of variation was almost similar in males during both the years. The Ga.S.I values gradually increased from December to March and were fairly high. During 2003-'04, a decrease in Ga.S.I was observed on October and the lowest value of 0.86 was recorded in November. In December the index value started to increase steadily from 0.92 until May when a peak value of 3.24 was recorded. In June there was a slight drop in the Ga.S.I to 3.17, followed by a sharp decline in July and it continued till September. On the other hand, during 2004-'05, minimum value of 0.76 was discerned in November and the value peaked up to 2.2 in

April, then a gradual decrease was followed till September. In females, during 2003-’04, least value of 0.72 was recorded in November, was followed by a gradual increase in the Ga.S.I, reaching the crest value in May (3.06). This was followed by a gradual decline up to September. This pattern was almost same during 2004-’05, except for the maximum value recorded in March (2.08).

Lengthwise variation in Ga.S.I of males and females is depicted in Fig.4.9. In males, the higher Ga.S.I value of 3.25 was found in the smallest size group of 91-100 mm TL, thenceforth a gradual decreasing trend was discernable upto 120 mm TL. A peak Ga.S.I value of 4.25 was registered in 131-140 mm TL size group; thereafter a steady decline in values was noticed up to 191-200 mm TL group. High Ga.S.I value was observed in male fishes in 201-210 mm TL, followed by an intermittent increase and decrease in the values. The lowest Ga.S.I value (0.53) was recorded in the largest size group of 301-310 mm TL. In females, 91-100 mm TL size group attained the uppermost Ga.S.I value of 4.17. This was followed by an irregular raise and shrink in the values up to 241-250 mm TL. The lowest Ga.S.I value (0.92) was noticed in 161-170 mm TL size group.

It is worth noticing that males and females follow almost similar trends in feeding intensity as indicated by gastro-somatic index during the two years with minor variations (Fig.4.10 and 4.11). The only striking difference was the intense feeding in males in April 2004-’05, in contrast, the feeding intensity of female was high during the month of March.

4.4 Discussion

Knowledge of feeding regimes of fish species is of great importance in understanding their ecological interaction (Alberto *et al*., 2003). The feeding behaviour is a species characteristics formed during its evolutionary history (Nikolsky, 1963). The structure of the alimentary canal and external morphology are greatly influenced by ecology of the food and feeding regimes (Thomas, 1962; Sinha, 1968). The digestive apparatus of fishes show marked diversity in its morphology and function. Although the alimentary canal differs considerably among species, it shows some basic structural similarities. For a given species, the overall morphology is chiefly related to the nature of food, feeding habits, body size, shape and sex (Kapoor *et al*., 1975; Smith, 1989). A large and varied group of fishes including toadfishes has flattened itself against the substrate. Presumably this flattening provides a way of fading into the bottom for defensive and sometimes, for attack, purposes (Gosline, 1996). Flattened heads, large pectoral fins and modified pelvic fins that allow them to adhere to the bottom, support their benthic ambush predatory pattern. According to Gosline (1996), toadfishes use a camouflage - and - ambush technique to capture their larger prey and for it they must swim directly forward over the substrate. Structural changes of the feeding apparatus could enable the exploitation of different food sources and influence food selectivity (Wainwright, 1988). Modification in the position, shape and the size of the mouth as well as the dentition in various species of fishes are correlated with the nature of food and the manner in which it is obtained. Carnivorous fish generally have a large mouth that can open wide and is endowed with an array of pointed teeth, which are sometimes curved towards the back to keep hold of their prey. Omnivorous and herbivorous fish have a smaller mouth, with flat teeth ideally suited to grinding food. Fishes with smaller mouths are good at sucking in their prey, whereas fishes with larger mouths are better at grasping prey from the side (Alexander, 1967). The gap and mouth of toadfishes are very wide but shallow. The toadfishes eat items on or slightly above the substrate (Gosline, 1996). The large, terminal, and slightly protrusible mouth and strong and pointed teeth of *C. dussumieri* is well adapted to suit its carnivorous feeding habit. The pharyngeal teeth are adapted to perform mastication (Gosline, 1996). The ability to protrude the jaw confers an advantage in specific circumstances such as obtaining benthic prey or food from otherwise inaccessible places (Alexander, 1967; Osse, 1985). Gill rakers, forward-directed projections on the inner margins of the gill arches, vary in size and number depending on a fish's diet (Wootton, 1990) and were used as a comparative trait. The number and structure of the gill-rakers can vary considerably, from a few small hard tubercles in predatory fishes to a complex network of numerous rakers in fishes which feeds on planktonic larvae. *C. dussumieri* showed few, short gill rackers which is a probable indication that it only serve to prevent the escape of the prey from oro-branchial cavity. The size of the stomach in fishes is closely related to the feeding behavoiur. In fishes which swallow large prey, or else consume large amount of food at the same time, the stomach is usually large (Nikolsky, 1963). Well developed musculature of the stomach helps in accommodating prey larger than stomach (Subla, 1970). Large, muscular and distensible stomach in *C. dussumieri* is highly developed for carnivorous mode of life.

The length of the intestine of the fish depends upon the feeding habits. Modification in the intestine (short, moderate, lengthy or coiled) is an indication of its feeding habit- carnivore, omnivore or herbivore (Fukusho, 1969). According to Fryer and Iles (1972), the length of the intestine is clearly related to the trophic status of the species, and its length is ordered in the following way: carnivores < omnivores < herbivores < detritivores. Junger *et al.* (1989) stated that short intestines indicate a tendency to carnivory. Carnivorous fishes normally have short and more or less straight intestine. This is because the meat gets digested more easily (Pandey and Shukla, 2005; Serajuddin and Ali, 2005), whereas in herbivores fishes the intestine is long and highly coiled because the vegetable food items take more time for digestion. The intermediate condition is found in omnivores. The short and straight intestine of *C. dussumieri* appears to indicate that this fish is a carnivore (Plate 4.1a).

The gross morphology of the intestinal tract appears to be linked to the feeding habits of individual fish species. Jacobshagen (1913) found a direct correlation between the nature of the food and length of the intestine. According to him, carnivorous fishes have short intestine and plant and mud feeders have long intestines. Though Suyehiro (1942) mentioned some exceptions to this generalisation one must admit that it generally holds good. Barrington (1957) suggested that more than one factor is responsible for determining the relative length of the alimentary canal. Al-Hussaini (1949) opined that the length of the gut is compensated by the average mucosal area and a short gut may be compensated by longer mucosal folds. Sinha and Moitra (1975) found that the nature of the food ingested by a fish largely determines the length of the gut and the number and nature of the mucosal folds. Longer gut lengths (larger ratios) are typical of herbivores that ingest fibrous plant foods that resist digestion, whereas carnivores have shorter systems (smaller ratios) adapted to processing mostly high-quality foods (Wootton, 1990). Low relative gut length (RLG) is indicative of carnivory while greater RLG of herbivory. According to Jobling (1995), the ratio of intestinal length to body length is usually less than unity in carnivorous species, being lower for piscivorous than for more generalized carnivores that feed on molluscs, worms, crustaceans and insects. In omnivores the ratio may increase to around 2-3, and the ratio is even higher in herbivores and those fish species which consumes diets with a high roughage content. Relative intestinal lengths summarized by Al-Hussaini (1949) and Kapoor *et al*. (1975) range from 0.5 to 2.4 for carnivores, 0.8 to 4 for omnivores, and 2 to 21 for herbivores. In all dietary categories, both maximal and minimal values for relative intestinal length tended to increase with body size, but absolute increase was much greater for the herbivores than the omnivores and greater for the omnivores than the carnivores (Kramer and Bryant, 1995). Granado and Gonzalez (1988) studied the dietary habits of *Amphichthys cryptocentrus* (Batrachoididae). An intestinal index showed that this species remained carnivorous during juvenile and adult stages. In the present investigation, RLG values of 0.81 to 0.97 in males and 0.80 to 0.914 in females indicate the tendency towards carnivory. In *C. dussumieri*, the RLG values in both sexes gradually increased with increase in total length of the body.

The analysis of stomach contents *of C. dussumieri* from Cochin estuary revealed that there exist a strong preference towards some food items in both males and females. The crustaceans, especially prawns formed the principal food item, frequently consumed by all fishes irrespective of sex and size, followed by crabs. Teleost fishes and molluscs were the respective 3rd and 4th preferred category of food of males. On the other hand, in females, fishes formed the 2nd most favourite food while crabs and molluscs occupied 3rd and 4th positions respectively. On the basis of relationship between the fishes and their food (feeding preferences), Nikolsky (1963) divided food of fishes into four categories. (a) Basic foods – comprising major parts of gut content. (b) Secondary food – frequently found in the gut, but in small quantities. (c) Obligatory food – found in the absence of basic food (d) Incidental food - found rarely in the gut. In accordance with the above categorization, prawns, crabs and fishes could be discerned as the basic food while molluscs formed the obligatory food and incidental food was constituted by isopods and squilla spp. in both males and females.

Based on the extent of variation in the selection of food, Nikolsky (1963) classified fishes as (a) euryphagic – feeding on a variety of food (b) stenophagic - feeding on few selected types of food and (c) monophagic – feeding only on a single type of food. *C. dussumieri* was found to be selective in its feeding habit. Their food comprises of a narrow spectrum, preferring selected groups of crustaceans and fishes as its main food item with a considerably low consumption of bivalve mollusc and therefore, could be categorized as stenophagous in nature. Blay *et al*. (2006) opined that juveniles of the cassava croaker (*P. senegalensis*) and the bigeye grunt (*B. auritus*) are stenophagous, as they fed on a narrow range of food items consisting of larvae and fingerlings of fish, shrimps and cuttlefish. Their preference for a particular food item among this food spectrum suggests possible food resource partitioning between the two species as a means to minimize competition. According to Santhosh and Biswas (2010), spiny eels (*Macrognathus pancalus, M. aral*) are stenophagic and carnivorous and the adult feed on earthworms, insects, micro crustaceans, and larvae of other aquatic invertebrates.

On the basis of character of food consumed, Das and Moitra (1956, 1963) applied an improved scheme for the classification of fishes from Uttar Pradesh. Accordingly, the categories are: (a) Herbivorous – 75% of food comprise of plants (b) Omnivorous – plant and animal foods are approximately 50% - 50%, neither is less than 10% - 15% (c) Carnivorous -

animal foods constitute of about 75%. Later two more categories were added: (a) Herbi-omnivorous – greater amount of plant foods (b) Carni - omnivorous – greater amount of animal foods. Based on the above categorization, it appears that *C. dussuimieri* belong to the carnivorous group.

Based on the trophic niches fishes occupy in the water, Das and Moitra (1955) divided the fishes into 3 categories: (a) plankton eating surface feeders (b) column or mid feeders (c) bottom feeders. The bottom feeders scrap the surface of bottom stones, rocks to collect the detritus. There are other fishes that, in fact, suck the bottom mud and debris. Most of the bottom feeders are benthophagous and detritophagous; others have special adaptations to feed upon the phytobenthos, zoobenthos and mobile foods. Surface feeders are mainly omnivorous or carnivorous while mid- and bottom – feeders are herbivores, omnivores or carnivores (Das and Moitra, 1955). Although *C. dussumieri* has a terminal mouth, inappropriate for bottom-feeding, on the basis of the results of gut content analysis and other morpho-anatomical features of the fish species, especially its well-developed and protractible lips, large pectoral fins and modified pelvic fins that allow them to adhere to the bottom and allows it to obtain food without changing its general body position, it can reasonably be asserted that *C. dussumieri* is a bottom feeder.

Toadfishes, *Opsanus tau* (Linton, 1901; Gudger, 1910; McDermott, 1965; Phillips and Swears, 1979) and *Porichthys notatus* (Hubbs, 1920) have been reported to consume a variety of foods including crustaceans, mollusc and fishes. Granado and Gonzalez (1988) studied the dietary habits of *Amphichthys cryptocentrus* (Batrachoididae). According to them, the fish feed on small crustaceans and molluscs. While studying the food and feeding of Caribbean reef toadfishes namely *Amphichthys cryptocentrus* and *Sanopus barbatus*, Hoffman and Robertson (1983) reported that both fed primarily on long-spined urchin, *Diadema antilarum* indicating a narrow food preference of the two species. Food taken by the oyster toadfish, *Opsanus tau* primarily consisted of mud crabs. Squid, stone crabs, anchovy and juvenile crabs were incidental foods (Wilson *et al.,* 1982). This was similar to that reported by Schwartz and Dutcher (1963). Toad fish *Opsanus tau* was known to readily consume brachyuran crabs, including blue crabs (Schwartz and Dutcher 1963; McDermott 1965; Wilson *et al.,* 1982). According to Mensinger and Tubbs (2006), the juveniles of *O. tau* resembles the adults in everything but size and they possessed a voracious appetite for moving prey such as brine shrimp, mysids, small crabs and fish. Based on the field observations, 15% to 60% of toadfish had empty stomachs, indicating that wild toadfish do not feed daily (Schwartz and Dutcher, 1963; Wilson *et al*., 1982).

From the present findings it is noted that the food and feeding habit of *C. dussumieri* is the same in both sexes, but there was conspicuous variance in the percentage of occurrences of different food items. Monthly variation in the gut contents confirmed that both the sexes have identical feeding habits, consuming the same food items, but with slight variations in magnitude. As seen from the results, it could be inferred that the major portion of the diet comprised of prawns, crabs and fishes almost throughout the period of investigation. It appeared that among the major food items, a decrease in any of the category was duly compensated by another group. During both the years, males consumed prawns and crabs abundantly and fishes in relatively small quantities whereas the females showed a reverse order. Slight variations were also observed to the minor components of the diet in males and females during both the years. There was no variation in the diet of *C. dussumieri* in relation to sex, size and season. The high density of the available prey species within each designated food taxon was taken as the most plausible explanation (Stephenson *et al*., 1970).

Fishes with heavily fed (gorged, full and ¾ full), moderately fed (½ full and ¼ full) and poorly fed (barely full and empty) stomachs were observed during most of the months. Male fishes with heavily fed and moderately fed stomachs during 2003-'04 and 2004-'05 indicates that it is an active feeder whereas females with heavily fed and moderately fed stomachs were noticed during 2003-'04 and in 2004-'05, were moderately fed as high proportions of moderately fed stomachs. The emptiness percentage and stomach fullness indices are very important to assess feeding intensity (Prabha and Manjulatha, 2008). Feeding intensity is negatively related to the percentage of empty stomachs (Bowman and Bowman, 1980). Data on the feeding intensity revealed that the occurrence of empty stomachs in females was more compared to males which are indicative of a poor feeding. The feeding intensity of a fish is related to its stage of maturity, reproductive state and the availability of food items in its environment (Maddock and Burton, 1999; Sivakami, 1996; Kiran and Puttaiah, 2004). Breeding periodicity was found to have profound influence on the feeding rate in *C. dussumieri.* Most active feeding period was found in the gonad maturing and ripening stages, which is the second and the third stage of the sexual cycle. This suggests that, at this stage, the fish feed more voraciously because of a higher energy demand associated with gonad development. Feeding intensity was found to be less during the pre-spawning and spawning periods in females as indicated by the low gastro-somatic index and preponderance of guts with low degrees of fullness. Low feeding activity was observed during July to December. Just after spawning, from January onward, when the largest number of spent individuals was encountered, feeding intensity increased considerably. High feeding intensity observed during March - June might be attributed to increased feeding activity to meet the demand for more energy in spawning season. Observations based on high feeding intensity in spent fish may be suggestive of high food requirements for building up of the gonads. In males, feeding activity pattern followed almost similar to that of female. Feeding activity was very low in November and high during April - June. High feeding activity pointed to the extra requirements of energy and low feeding to the exhaustion caused by spawning. Low feeding in male could also be attributed to the cessation of feeding of males, since they provide parental care to the developing embryos (Vasudevappa and James, 1980, 1992). The low pre-spawning feeding intensity seen in females might be ascribed to the matured gonads, permitting limited space in the abdominal cavity for intake of food. Malhotra (1967) reported that fasting in *Botia birdi* during the pre-spawning stage might be due to the pressure exerted by the voluminous ovary against the alimentary canal. The occurrence of poor feeding coincident with peak breeding in other fishes has been reported by Karekar and Bal (1958), Thomas (1969), Desai (1970), Pisolkar and Karamchandani (1981), Khan *et al.* (1988), Kurup (1993) and Bhuiyan *et al.* (2006). According to Kiran and Puttaiah (2004), other reasons for empty stomachs are regurgitation (Pillai, 1952), periodicities in feeding, availability of food, digestibility, physiological reasons, health factor and low metabolic activity.

Gastro-somatic index indicated high feeding intensity in immature and juvenile fish than the mature ones. This may be attributed to the very high food requirements in the young and fast growing fish (Kuthalingam, 1967; Pati, 1980; Armstrong *et al*., 1992; Sivakami, 1996). Small-sized fish actively and more frequently feed than larger ones. This phenomenon has been recorded in majority of fish species (Nikolsky, 1963). The variations in the feeding intensity of both sexes of the fish were observed to be in the same pattern. However, males consumed more food than females. Higher feeding intensity in males compared to females has been reported by Khumar and Siddiqui (1989) in *Labeo calbasu* and Rao and Rao (1991) in *Nemipterus japonicus.* This might be due to the fact that more physiological stress laid down on the females than in males during spawning period of the fish. Maximum feeding intensity was observed in both sexes in maturing stage (III), as they approach the size at first maturity. Similar result was reported by Serajuddin and Ali (2005) in *Macrognathus pancalus*.

Influence of feeding intensity on condition factor was evident during some of the months in both sexes of *C. dussumieri*. This aspect has been dealt with in detail in Chapter 7 on ‘Length-weight relationship and condition factor’.

The present study indicated that *C. dussumieri* showed a stenophagism by selecting few organisms in their diet despite the presence of large number of organisms in the habitat. The results revealed that the fish is “Carnivorous-stenophagic-bottom feeder”. It is obvious that composition and preferences of food are the same in both sexes. But observations on the average amount of feeding in both sexes show that males exhibit a little higher feeding intensity than females. The study of natural diets of fish species is very useful approach for understanding aspect of the species biology and ecology, towards a more sustainable management of their stocks and the development of conservation measures (La Mesa *et al*., 2007; Kitsos *et al*., 2008).

Table 4.1. Index of Preponderance value of different food items in the stomach of *C. dussumieri*

Sl.No.	Food item	Index Value		
1	Fish	26.26	22.06	24.16
2	Prawn	42.32	32.08	37.20
3	Crab	23.45	35.47	29.46
4	Isopod	0.21	0.29	0.25
5	Squilla	0.58	0.85	0.715
6	Mollusca	4.32	4.98	4.65
7	Semi digested matter	2.86	4.27	3.565

Table 4.2. Monthly index of preponderance values of different food items in males of *C. dussumieri* during 2003 – '04

	October	November	December	January	February	March	April	May	June	July	August	September	Average
No. of specimens	7	6	9	3	12	21	11	12	3	5	4	13	
Food items	Index of preponderance												
Fish	79.35	2.13	46.98	12.195	21.96	40.96	19.597	9.93	6.61	8.02	14.34	39.11	25.1
Prawn	8.1	51.06	24.35	45.122	53.51	19.71	26.173	65.49	77.45	13.75	81.28	48.09	42.84
Crab	12.145	45.39	25.22	42.68	17.64	15.36	50.74	20.54	15.94	76.11	3.59	2.38	27.31
Mollusc	0.00	0.00	3.45	0.00	3.92	8.22	2.42	2.97	0.00	0.66	0.00	0.00	1.8
Squilla	0.00	0.00	0.00	0.00	0.00	4.73	0.00	0.856	0.00	0.00	0.00	0.00	0.47
Isopod	0.00	0.00	0.00	0.00	0.00	3.41	1.07	0.00	0.00	0.00	0.00	0.159	0.39
Semi digested matter	0.405	1.42	0.00	0.00	2.97	7.61	0.00	0.228	0.00	1.47	0.79	10.255	2.09

Table 4.3. Monthly index of preponderance values of different food items in males of *C. dussumieri* during 2004 – '05

	October	November	December	January	February	March	April	May	June	July	August	September	Average
No. of specimens	8	18	9	12	14	12	8	22	7	14	9	10	
Food items	**Index of preponderance**												
Fish	4.34	10.59	3.34	18.38	22.15	28.29	18.05	17.59	3.59	5.14	7.53	11.79	12.57
Prawn	49.22	45.48	57.42	50.33	36.79	30.02	30.2	7.49	25.42	12.76	29.33	26.74	33.43
Crab	4.825	38.54	39.24	26.36	27.29	35.15	47.02	62.88	69.49	70.51	57.01	59.31	44.8
Mollusc	6.03	0.00	0.00	1.73	6.48	0.32	2.03	7.59	1.02	2.37	5.42	0.58	2.8
Squilla	0.00	0.00	0.00	0.00	0.00	0.00	0.68	0.19	0.00	0.36	0.00	0.00	0.103
Isopod	0.00	0.00	0.00	0.00	0.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.068
Semi digested matter	35.59	5.39	0.00	3.195	6.48	6.22	2.02	4.26	0.48	8.86	0.71	1.58	6.23

Table 4.4. Monthly index of preponderance values of different food items in females of *C. dussumieri* during 2003 – '04

	October	November	December	January	February	March	April	May	June	July	August	September	Average
No. of specimens	19	3	4	8	15	23	15	26	3	3	8	13	
Food items	**Index of preponderance**												
Fish	12.69	42.86	69.66	15.15	48.68	19.59	6.64	19.42	39.44	25.25	18.07	11.73	27.42
Prawn	60.91	57.14	16.85	18.18	18.075	53.6	42.99	76.13	52.78	6.06	73.52	25.33	41.8
Crab	20.81	0.00	11.24	59.09	30.65	19.44	28.36	3.58	7.22	15.15	4.68	34.84	19.59
Mollusc	0.00	0.00	2.25	7.58	0.104	1.41	19.00	0.372	0.56	50.51	0.00	0.267	6.84
Squilla	0.00	0.00	0.00	0.00	0.924	0.00	0.604	0.00	0.00	3.03	3.73	0.00	0.69
Isopod	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.267	0.02
Semi digested matter	5.59	0.00	0.00	1.567	5.96	2.41	0.503	0.00	0.00	0.00	0.00	27.56	3.64

Table 4.5. Monthly index of preponderance values of different food items in females of *C. dussumieri* during 2004 – '05

	October	November	December	January	February	March	April	May	June	July	August	September	Average
No. of specimens	15	21	6	3	3	5	5	8	3	2	2	8	
Food items	Index of preponderance												
Fish	30.94	30.94	55.55	9.55	78.95	30.77	7.75	24.94	24.31	17.14	59.52	8.19	31.55
Prawn	33.97	33.97	15.15	1.27	8.31	5.13	70.54	60.17	48.61	22..86	11.91	56.72	30.72
Crab	30.33	30.33	19.19	25.48	8.31	51.28	21.71	13.78	13.89	35.72	28.71	35.09	26.14
Mollusc	0.00	0.00	0.00	44.59	2.77	12.82	0.00	1.11	7.64	17.14	0.00	0.00	7.17
Squilla	0.00	0.00	0.00	19.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.59
Isopod	0.92	0.92	0.00	0.00	1.66	0.00	0.00	0.00	2.78	0.00	0.00	0.00	0.523
Semi digested matter	3.84	3.84	10.11	0.00	0.00	0.00	0.00	0.00	2.77	7.14	0.00	0.00	2.308

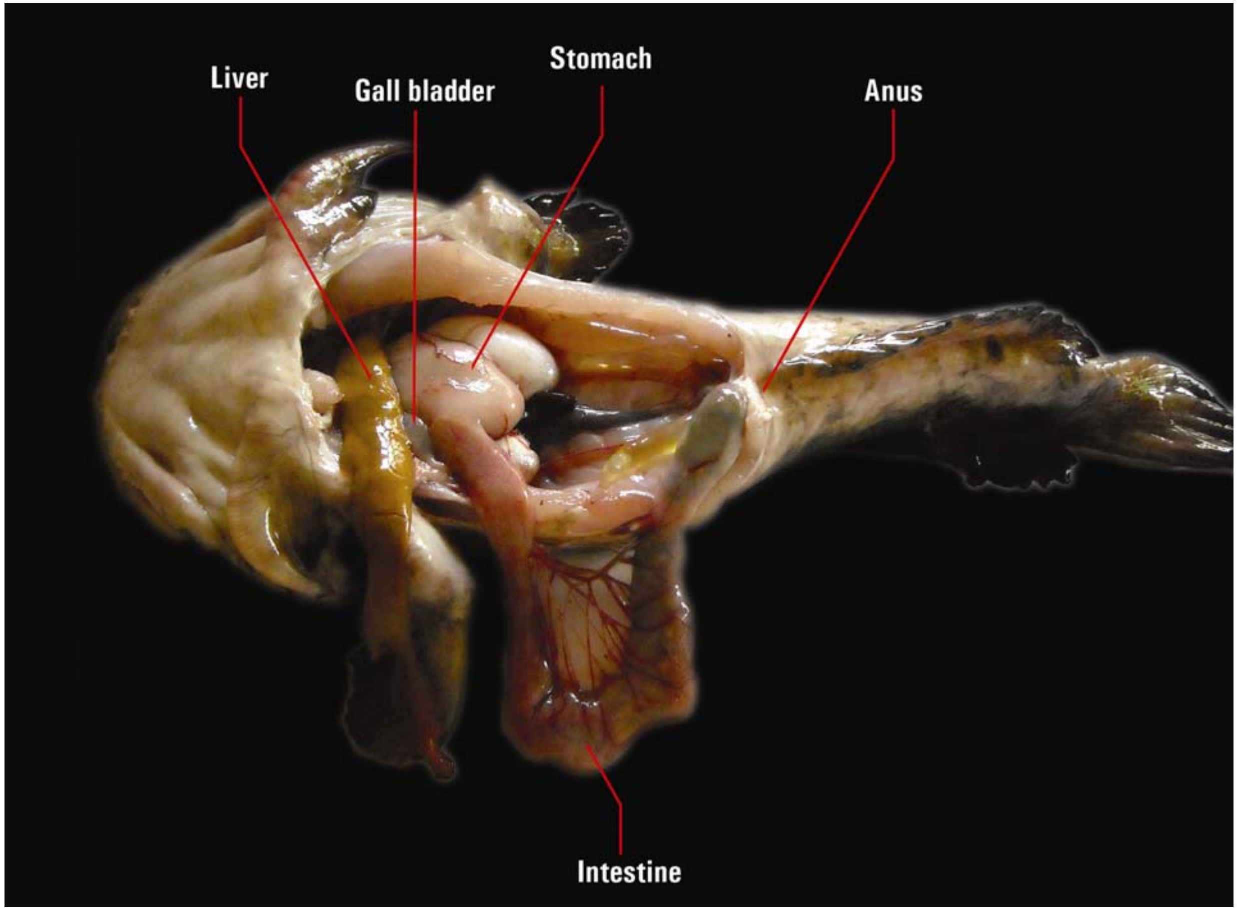

Fig. 4.1a. Alimentary canal of *Colletteichthys dussumieri*

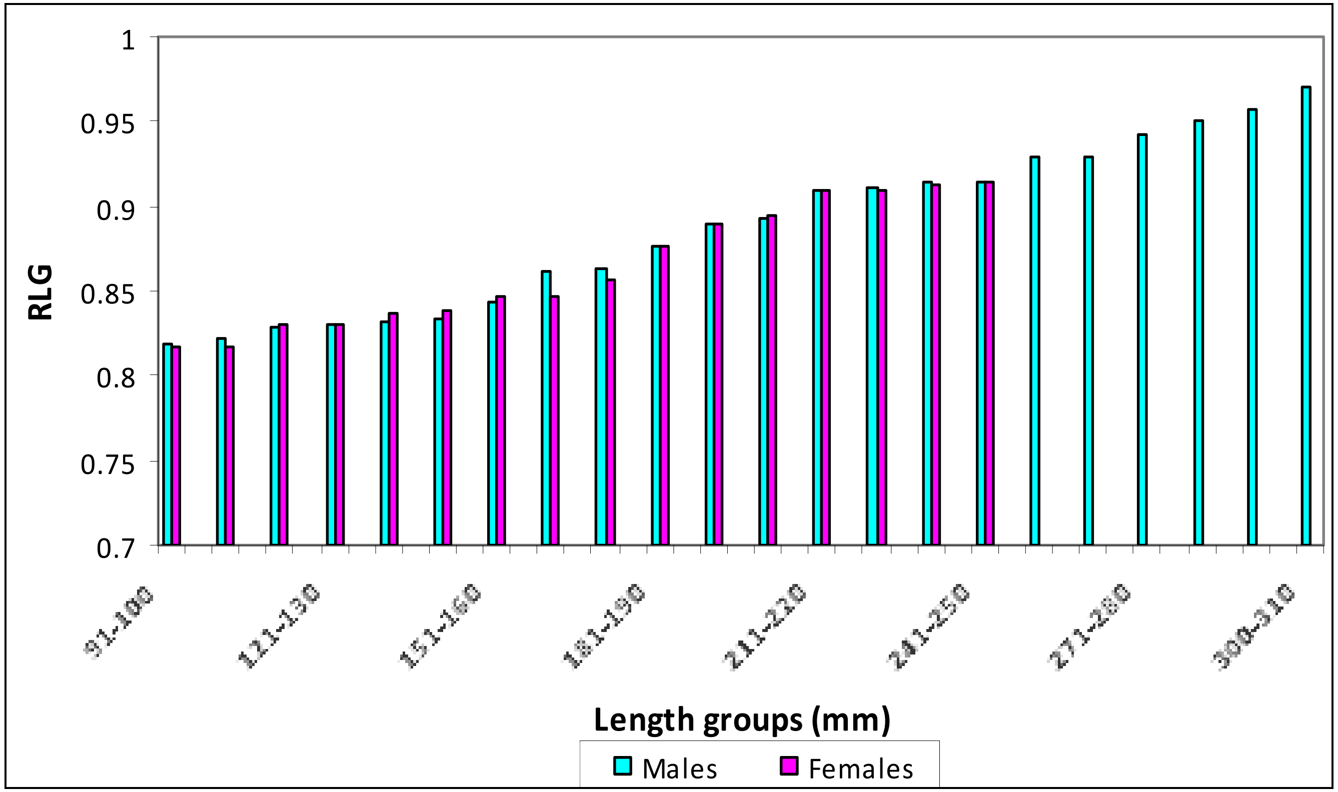

Fig. 4.1b. Variation in relative gut length in different length groups of *C. dussumieri*

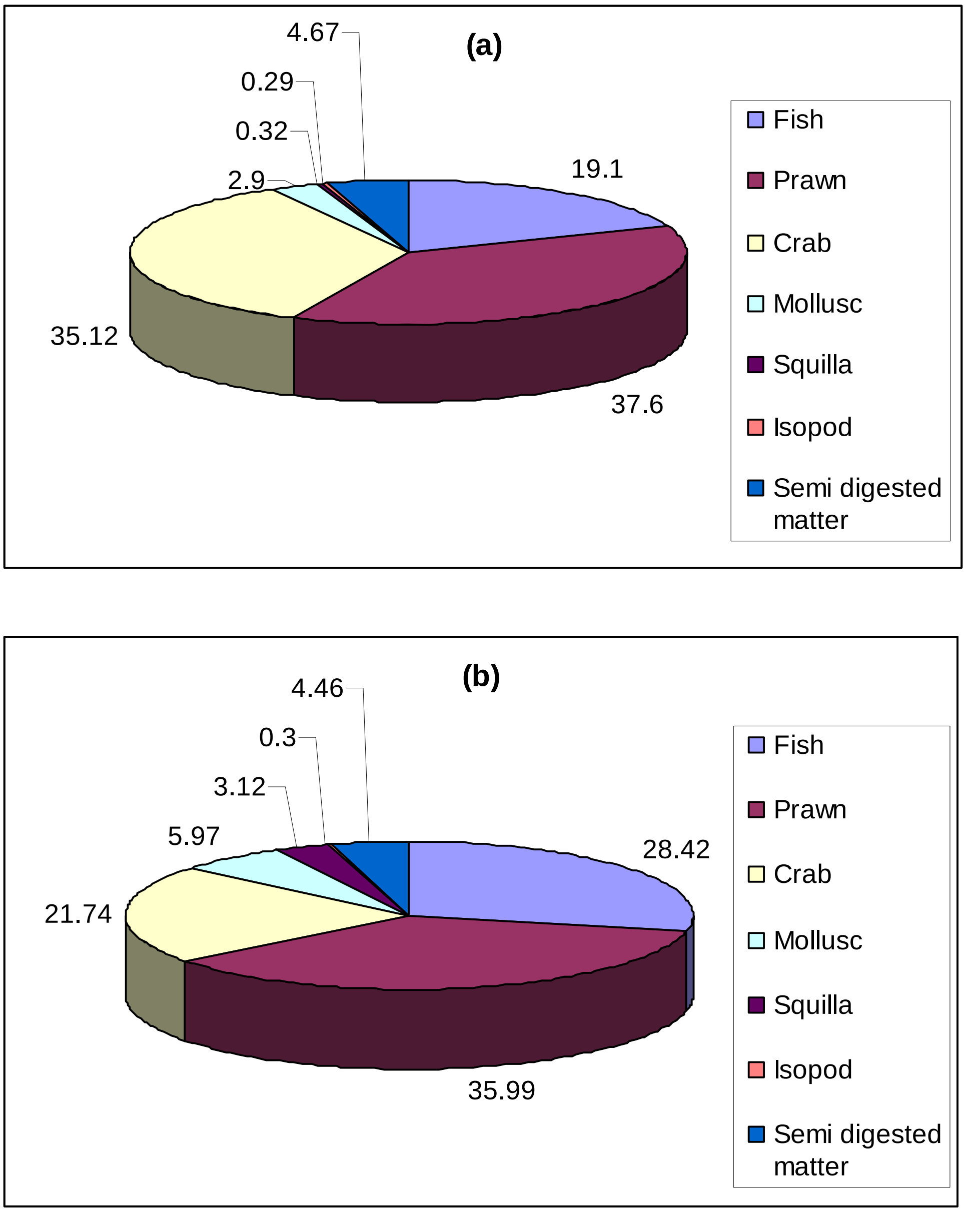

Fig.4.2. Diet composition of (a) males and (b) females of *C. dussumieri* (Pooled for 2003-04 and 2004-05)

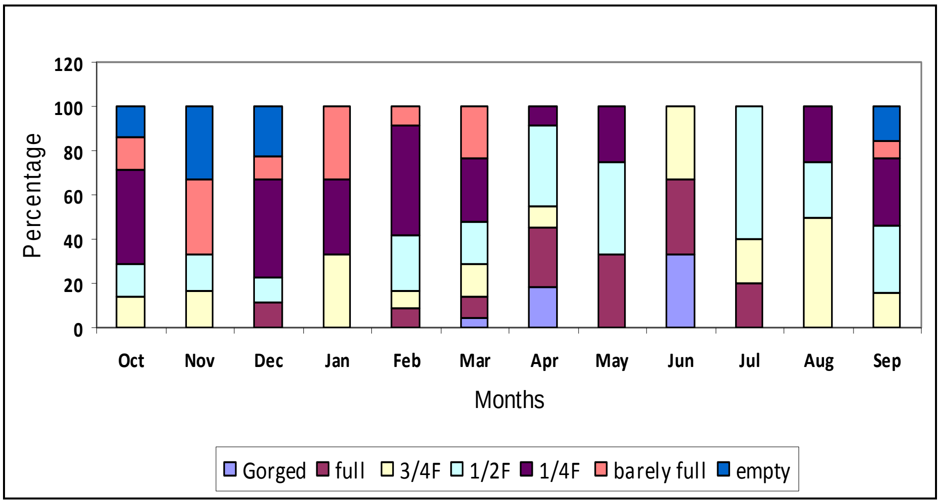

Fig.4.3. Percentage occurrence of stomach in different degrees of fullness in males of *C. dussumieri* during October 2003 – September 2004

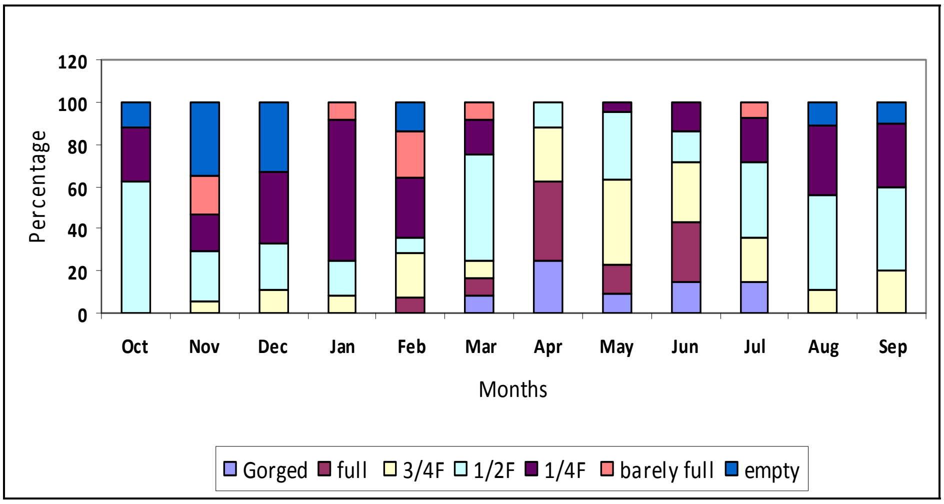

Fig.4.4. Percentage occurrence of stomach in different degrees of fullness in males of *C. dussumieri* during October 2004 – September 2005

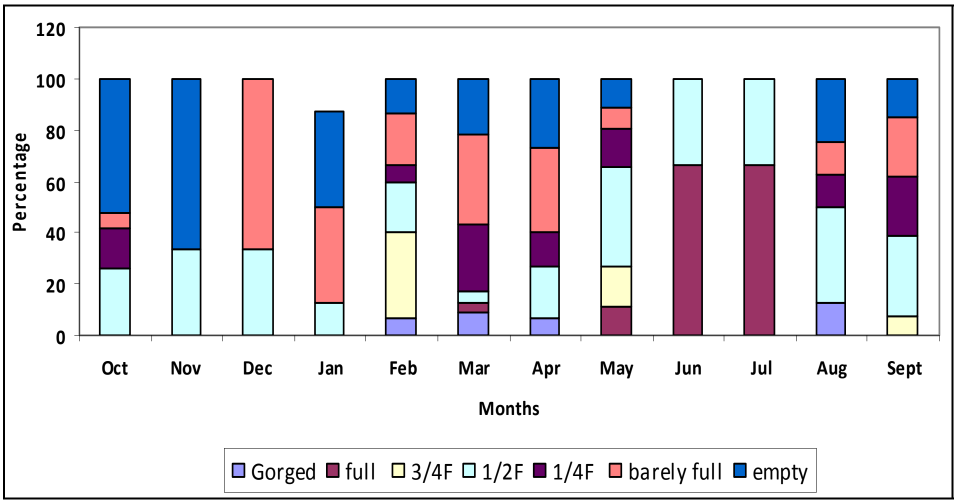

Fig.4.5. Percentage occurrence of stomach in different degrees of fullness in females of *C. dussumieri* during October 2003 – September 2004

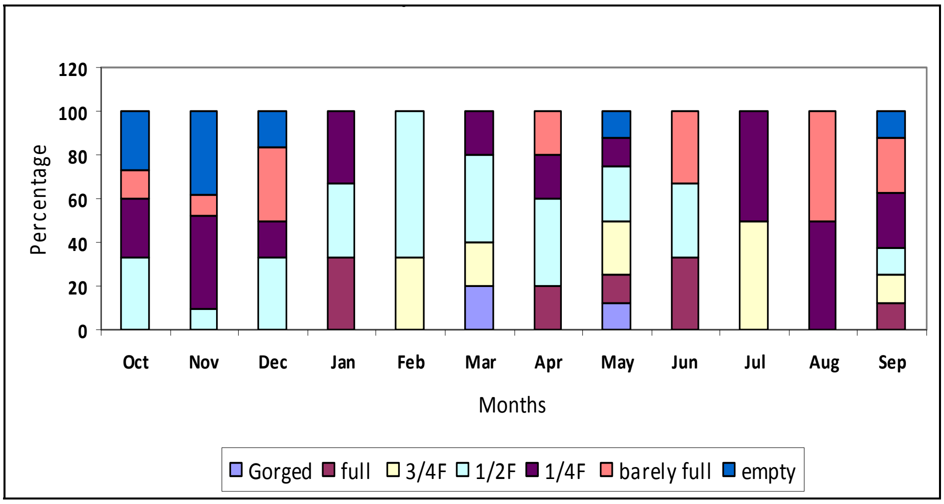

Fig.4.6. Percentage occurrence of stomach in different degrees of fullness in females of *C. dussumieri* during October 2004 – September 2005

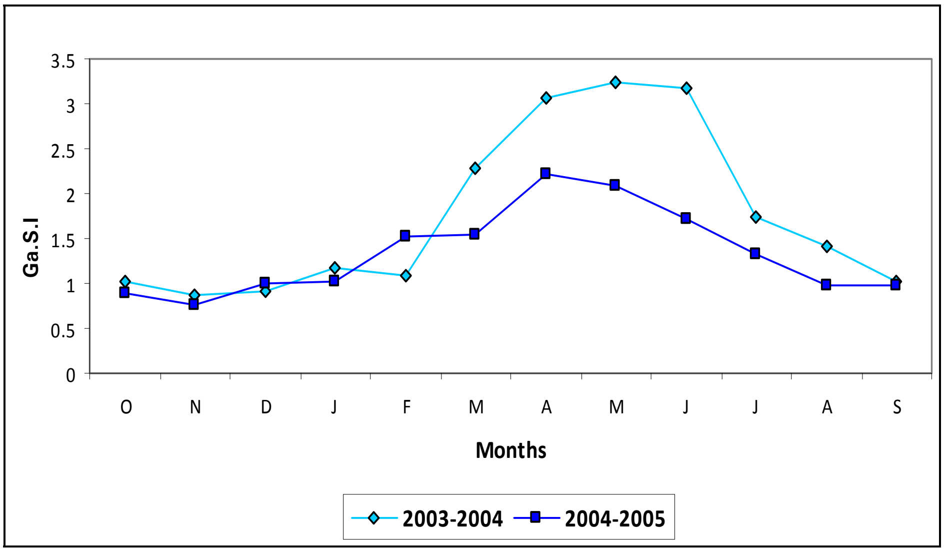

Fig.4.7. Monthly variation in gastro-somatic index of males of *C. dussumieri*

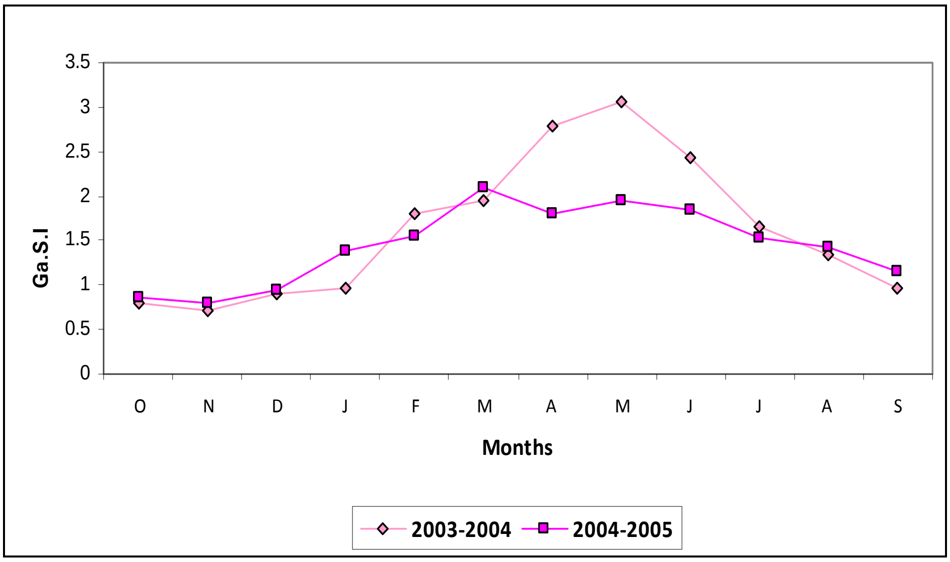

Fig.4.8. Monthly variation in gastro-somatic index of females of *C. dussumieri*

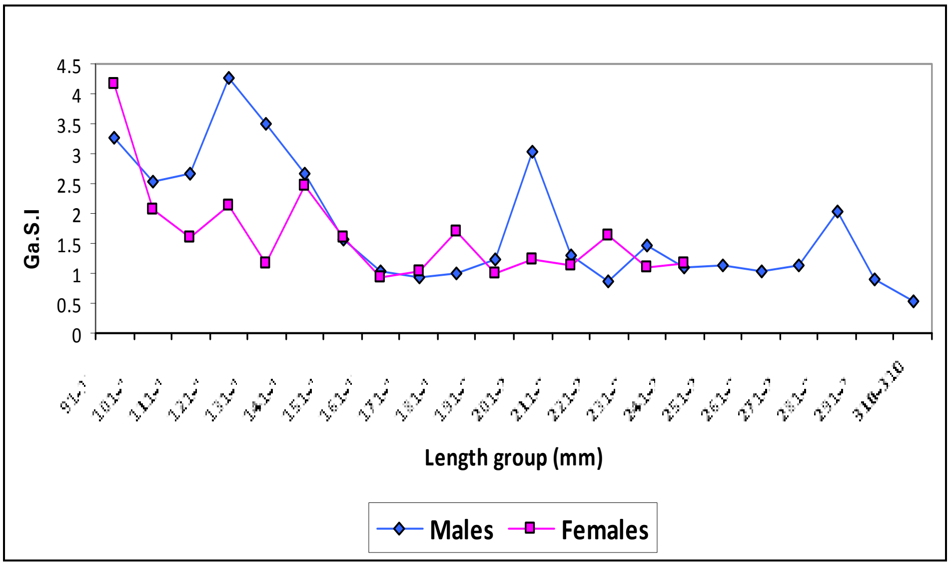

Fig.4.9. Lengthwise variation in gastro-somatic index of *C. dussumieri*

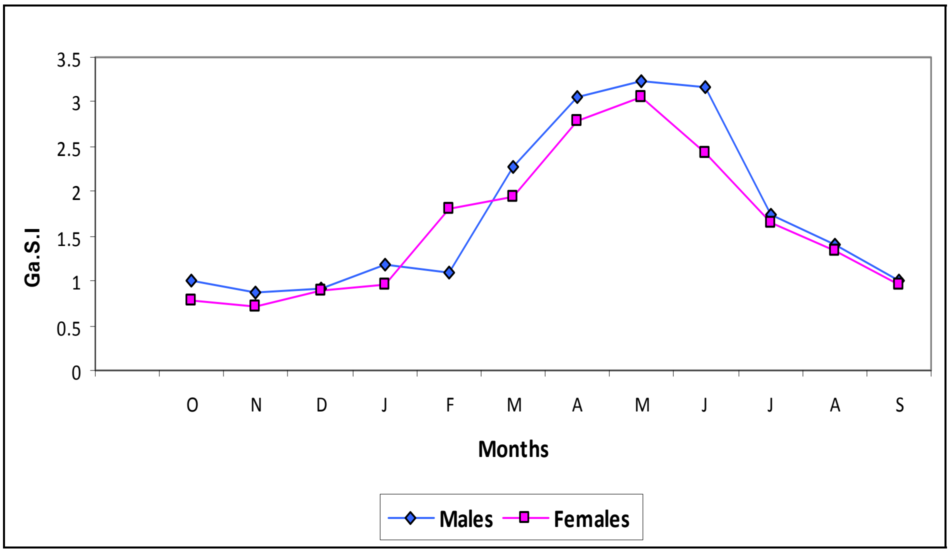

Fig.4.10. Monthly variation in gastro-somatic index of *C. dussumieri* during October 2003 – September 2004

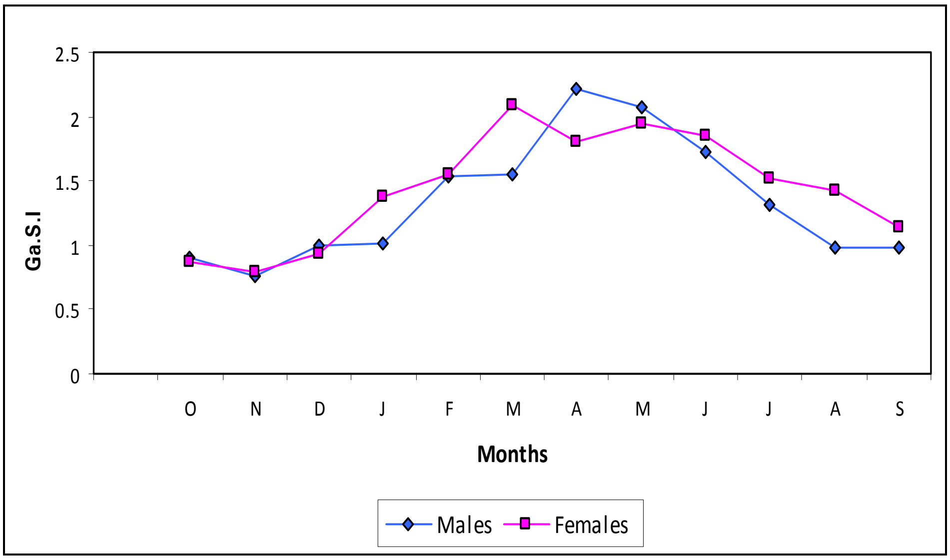

Fig.4.11. Monthly variation in gastro-somatic index of *C. dussumieri* during October 2004 – September 2005

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Chapter 5

REPRODUCTION

Contents

5.1 Introduction

Reproduction is at once the most conservative and yet the most adaptive of functions in the propagation and evolution of species. It is a dynamic metabolic activity in most fishes and it involves sequential changes in the germ cells. The success of any fish species is ultimately determined by the ability of its members to reproduce successfully in a fluctuating environment and thereby to maintain the viable population (Moyle and Czech, 1988). Therefore the reproductive strategy as reflected in anatomical, behavioural, physiological, and energetic adaptations is an essential commitment to future generations. It is also a continuous development process throughout ontogeny, requiring energetic, ecological, anatomical, biochemical and endocrinological adaptations. This group of vertebrates has adopted diversified reproductive strategies in that a vast majority of them breed seasonally and a few continuously. Whatever their reproductive strategy, there exists a common principle for completion of reproduction (Sundararaj, 1981; Tyler and Sumpter, 1996).

In nature, reproduction is closely correlated with environmental conditions (Sundararaj and Vasal, 1976; Davies *et al*., 1999) particularly temperature, day length and food supply. These environmental factors have the greatest influence upon the gonadal development initiation and fecundity of the species. Moreover, establishment of extensive data bases on reproductive parameters with corresponding data on abiotic factors enables the study of causal relationships between reproductive potential and environmental variation. This leads to a better understanding of observed fluctuations in reproductive output and enhances our ability to estimate recruitment (Kraus *et al*., 2002).

Information on the reproductive biology of the candidate species is very much essential for the development of aquaculture industry. In order to proceed with the artificial means of reproduction and to produce good quality eggs, it is necessary to have basic information on reproductive biology of the species. The aquaculturist will need to provide both the proximate and ultimate environmental cues that sustain the reproductive cycle. Studies on reproduction, including the assessment of maturation cycle, size at first maturity, sex ratio, spawning periodicity, fecundity, reproductive load etc of a fish species, permit quantification of the reproductive capacity of individual fish and are an essential prerequisite for a rational and sustainable management of fisheries. These studies are essentially meant for elucidating both short term and long term variations in the production of broods which are finally recruited in the population as exploitable stocks.

Studies on the reproductive biology of commercially important fishes from Indian waters have been successfully carried out by many authors. Amongst them, the notable contributions are of Gokhale, 1957;

Dharmamba, 1959; Nair, 1959; Qasim and Qayyum, 1961; Belsare, 1962 ; Sathyanesan, 1962 ; Talwar, 1962; Pillay and Rao, 1962; Balan, 1965; Malhotra, 1967; Rao, 1967; Sam Bennet, 1967; Bhatt, 1968; Dhulkhed, 1968; Kagwade, 1968; Devadoss, 1969; Desai, 1973; Qasim, 1973a; Geevarghese and John, 1983; Jayasankar, 1990; Goswami and Dasgupta, 2004; Raje *et al*., 2004; Manojkumar, 2005; Sahayak, 2005; Vinci *et al*., 2005; Raje, 2006; Ali and Prasad, 2007; Suresh *et al*., 2007; Zacharia and Jayabalan, 2007; Euphrasia and Kurup, 2008 ; Joshi, 2008 and Rao and Krishnan, 2009.

A through review of available literature showed that hitherto no information is available on the reproductive biology of *C. dussumieri*. Therefore a detailed investigation on various aspects of reproduction was carried out in the present study. This study covered maturity stages, development of ova to maturity, spawning season, gonado somatic index, size at first maturity, sex ratio and fecundity of *C. dussumieri* from Cochin estuary.

5.2 Materials and methods

Samples of *Colletteichthys dussumieri* were collected from Cochin estuary at monthly intervals from October 2003 to September 2005 by using gillnets of mesh size 50 mm and 70 mm. The sample consisted of 248 males and 221 females ranging in total length from 95 to 305 mm and 94 to 246 mm and weight between 15.5 to 706 gm and between 14.9 to 275 gm. After blotting off water, total length (TL) of each fish was measured to the nearest 1 mm; total weight (TW) was recorded to the nearest 0.1g. Each fish was then dissected to determine sex (presence of ovaries or testes) and gonad development. The gonads were removed, wiped, weighed and measured for length. Maturity stages of each gonad were recorded. The gonads were dissected out and preserved in 4 % neutral buffered formaldehyde for further studies.

Stages of maturity of gonads were determined on the basis of morphological appearance (macroscopic observations) as well as microscopic observations. Macroscopic characteristics were defined based on vascular irrigation, size, color and proportion of oocytes per development stages and rate of abdominal cavity occupied by ovaries. In the case of testes, besides the general appearance, colour, size etc., the extent of lobulation of the edges was used for determining the stage. Microscopic observations were based on the presence and predominance of different types of germinative lineage cells (oocytes I, II, III and IV) and on the presence/absence of empty follicles. While macroscopic observations were based on fresh samples, microscopic observations were based on materials preserved in formaldehyde and histological observations.

For histological studies, the fresh gonad samples were fixed in Bouin’s solution for at least 24 hours. Gonad tissue was dehydrated and cleared in ethanol and xylene respectively. The tissues were then embedded in paraffin and 4-6 μm-thick sections were using a microtome. The sections were mounted on microscope slides and stained using hematoxylin / eosin following the procedure given in Weesner (1960). This conventional technique was followed for processing testes and immature and spent ovaries. Since routine wax- embedding method leads to crumbling and collapse of yolk-laden oocytes, yolky oocytes were processed following the double embedding method of Khoo (1979) as modified by Gopalakrishnan (1991).

The spawning season was inferred from the study of the relative frequencies of gonad maturity stages and monthly variation in gonadosomatic index through the study period.

The most common five-point maturity scale, ideal for most of the tropical spawners as developed by Qasim (1973a), Belsare (1962) and Nunez and Duponchelle (2009) was adopted to classify the maturity stages of gonads.

For ova maturation studies, ovary hardened in 4% formaldehyde, was carried out following the method of Clark (1934). The ova-diameter measurements were taken under microscope with an ocular micrometer at a magnification which gave a value of 0.0261 mm to each micrometer division (o.m.d.) and larger ova are measured by means of a Vernier dial caliper. Samples of ova from anterior, middle and posterior regions of both the lobes of ovary were examined. As there was no difference in the size of ova from different parts of the ovary, the ova were sampled from the middle region of the ovary only. A total of 150 ovaries at different maturity stages were analysed and from each ovary the diameters of about 100 ova were measured. The ova diameter from different ovaries of identical stage were pooled and grouped into 5 omd intervals for plotting the graph. One ocular micrometer division (o.m.d) was equal to 26 µm.

Fecundity studies were based on 20 mature females of *C. dussumieri* in the size range of 169 - 230 mm and weight range of 100 - 336 gm. Formalin preserved ovary of known weight, was transferred to modified Gilson’s fluid (Simpson, 1951) and kept for two days. To allow a better penetration of the fixative and to make the membrane breakdown easier and in order to disperse the oocytes the ovaries were frequently shaken. Fecundity was estimated based on all mature ova present in fish ovary of stage III and IV.

The mathematical relationship of fecundity with other parameters like total body length, total body weight, ovary length and ovary weight was worked out by the least square method, $F= aX^b$ where, F = fecundity, a = constant, X = variable (total body length, total body weight, ovary length or ovary weight) and b = correlation coefficient. The exponential relationship was transformed into a straight line logarithmic form based on the equation,

$$\text{Log } F = \log a + b\text{Log}X$$

Gonadosomatic index (GSI) was calculated month-wise for males and females, applying the formula of June (1953) and Yuen (1955).

$$\text{GSI} = \frac{\text{Weight of the gonad}}{\text{Weight of fish}} \times 100$$

To estimate the minimum length at first maturity (Lm), males and females were grouped separately into 10 mm class interval and fish in stage III and above were considered mature. Maturity curves were drawn to the scatter plots to estimate the length at which 50% of fish mature.

Sex ratio was calculated for different months and size groups of the fish. The sex ratio in each month and at each 10mm length interval with a hypothetical 1:1 ratio were tested for significance by using Chi-square formula (Snedecor and Cochran, 1967).

$$\chi^2 = \frac{\Sigma(\text{O - E})^2}{\text{E}}$$

Where,

O = observed number of males an females in each month/ length group

E = expected number of males an females in each month/ length group

5.3 Results

Like most of other teleostean, the reproductive glands (testes of males and ovaries of females) of *C. dussumieri* are typically paired structures.

The gonads are elongated, paired and rounded bodies, suspended from the dorsal wall of the coelomic cavity by a short mesovarium in females or a mesorchium in males. The right and left lobes are usually unequal in size (left ovarian lobe is relatively larger than the right). They are situated on either side of the air bladder, ventral to the kidneys in the posterior region of the abdominal cavity. Gonoducts are present in both sexes. Females have a common oviduct arising from the ovarian wall in proximity to the most posterior part of the ovaries and open in the genital pore immediately behind the anus. In males two main testicular ducts (Grier *et al*., 1980), each running along the testis length, fuse in a common sperm duct shortly before reaching the urogenital opening. A pair of sexually dimorphic fan- like structures is present in males of this species. These organs evaginate from the distal portion of the main testicular ducts, just posterior to the testis and anterior to the sperm duct.

5.3.1 Gametogenesis

Gametogenesis involves alteration of primordial germ cells into mature gametes passing through a series of cytological stages. Spermatogenesis is the evolution of spermatozoa from sperm mother cells (spermatogonia) while oogenesis is the process of transformation of oogonia into ripe egg, both processes involving complicated changes occurring in ooplasm as well as germinal vesicle (nucleus).

5.3.1.1 Spermatogenesis

Spermatogenesis in this species appears to be similar to that reported for many other teleosts. It occurs within individual compartments or cysts which line the tubule walls. Each compartment contains similar germ cells, all showing a specific stage of spermatogenesis. Six distinct stages of spermatogenesis have been recognized and described.

a) Primary spermatogonia (Stage I)

Stage I cells are usually large, irregularly shaped with an indistinct nucleus. The nucleus contains lightly stained granules of various sizes and shapes and a prominent nucleolus. The ooplasm is slightly granular and shows less affinity to basic dyes like haematoxylin. Cell membranes are indistinct and compartments or cyst boundaries are hardly visible at this stage (Fig.5.1).

b) Secondary Spermatogonia (Stage II)

Stage II cells form by at least two divisions of stage I cell. At this time the cyst membrane is clearly defined and each cyst contains many cells. These cells are smaller than their predecessors. The nuclei are spherical, smaller and contain darkly stained granules. The ooplasm which stains darker than that of the stage I cell contains scattered, variably sized granules (Fig.5.1).

c) Primary Spermatocyte (stage III)

Stage III cells are characterized by having a smaller nucleus. Granules appear larger in size and uniformly fill the nuclear area. Nuclear material is obviously more condensed and stain purple with haematoxylin-eosin (Fig.5.2).

d) Secondary Spermatocyte (Stage IV)

Stage IV cells form by maturation and gradual change of the stage III cells. The nuclei show no further decrease in size nor does there appear to be any further division at this time. Rather, there is a slight reduction in cell volume and more strikingly a condensation of nuclear chromatin material so that it appears as a homogenous body. The major differences between stage III and stage IV cells are in the staining density of the nucleus and the slight reduction in size of the stage IV cell (Fig.5.2).

e) Spermatids (Stage V)

The stage IV cells divide to form the more abundant stage V cells. The nuclear chromatin material appears to have migrated to the periphery of the nucleus leaving a clear central area. The ultimate distribution of the nuclear material in this stage varies considerably from a half shell- form to a hollow sphere but there are always unstained areas in the center. The amount of nuclear material and the cell volume is about one half the sizes of the stage IV cells (Fig.5.3).

f) Spermatozoa (Stage VI)

Stage VI cells are apparently mature sperm. The transformation of spermatids into spermatozoa is termed spermiogenesis. No further division appears to have taken place from stage V to stage VI, and the nuclear material has condensed to a darkly stained perfect sphere without the clear areas seen in the previous stage. Spermatozoa have dense spherical heads with distinct long tails. Presence of two tails is characteristic of toad fish sperm (Fig.5.4).

During and immediately preceding the spawning season, the lumina of the tubules are found to be greatly dilated and filled with masses of mature sperm (Fig.5.5). The cyst membrane bursts, releasing the sperm into the lumen of the seminiferous tubule.

5.3.1.2 Oogenesis

Each ovary is covered externally with a thin and delicate layer of peritoneum beneath which lies the thick tunica albuginea, containing blood vessels, connective tissue cells and smooth muscle fibres. The innermost layer is the germinal epithelium which projects into the lumen of the ovary (ovocoel) in the form of lamellae. These ovigerous lamellae are the seat for the development of oocytes, which are visible in various stages of development. The germ cells or oogonia are found in clusters on these lamellae. Each oogonium in *C. dussumieri* passes through the following stages in the process of maturation to form a ripe ovum.

I. Previtellogenesis

Primary growth phase: This is the initial phase of oocyte growth, since the formation of the oocyte from the oogonia. It can be divided into two stages.

a) Chromatin nucleolus stage: The oocytes are highly basophilic and are characterized by centrally placed nucleus with a distinct nucleolus together with chromatin network. The basophilic ooplasm is a homogenous, undifferentiated thin layer surrounding the nucleus (Fig.5.6).

b) Perinucleolus stage: Concomitant with oocyte growth, the nucleus increases in size, and the multiple nucleoli become located around the periphery of the nucleus. They are strongly basophilic in nature. In early perinucleolus stage (Fig.5.7a) the nucleoli are found mainly scattered in the chromatin meshwork and the oocyte is surrounded by monolayer of simple squamous follicular cells which are interrupted at some places. More nucleoli move to the periphery of the nucleus in the late perinucleolar stage, some are still in the centre of the nucleus (Fig. 5. 7b). There is conspicuous increase in the size of the oocyte. The ooplasm acquires a granular structure and gradually loses its good affinity to haematoxylin and tends to be stained only faintly therewith. Just outside the nuclear membrane, a spherical deeply-stained body, the “yolk nucleus” or “Balbiani bodies”, appears in the ooplasm. Later it migrates to cortical ooplasm and gradually disappears.

II. Vitellogenesis

Secondary growth phase: A general increase in the size of the oocyte takes place as a result of elaboration of vitelline substances; hence, this phase is also referred to as the vitellogenic phase.

a) Cortical alveoli (Yolk vesicle) stage: This stage is characterised by the appearance of clear vesicles or vacuoles (cortical alveoli) in the ooplasm. The vesicles start to accumulate from the periphery of the oocyte. The nucleoli still persist as in perinucleolar stage. Convolution of nuclear membrane begins. The oocyte with these features is said to be in the early yolk vesicle stage (Fig.5.8a). Later the yolk vesicles increase in size and number until they occupy the entire ooplasm. The ooplasm appears as vacuolated structure as it remains unstained with basic dyes. The nuclear walls become wavier in outline and the nucleoli become reduced in size. The follicular layer around the oocyte is complete. Zona radiata develops from the peripheral zone of ooplasm and the oocyte is in the late yolk vesicle stage (Fig.5.8b).

b) Yolk globule stage: Heavy deposition of yolk begins at early yolk globule stage (Fig.5.9a). Further growth of oocyte takes place. There is increase in the number of yolk vesicle. These are spread in the entire ooplasm. Appearance of yolk in the form of minute granules in the extra vesicular ooplasm can be frequently observed. They are seen in the peripheral region first. Between the ooplasm and the follicular layer or zona granulosa, a vitelline membrane or zona radiata is quite distinct. Nucleolar extrusion also continues at this stage.The late yoke globule stage (Fig.5.9b) is characterised by the extensive deposition of the yolk globules in the whole of ooplasm. Then they invade the yolk vesicles which appear to be displaced towards the periphery of the ooplasm. The nucleus is very much reduced at this stage. Accumulation of yolk facilitates rapid growth of the oocyte. The whole ooplasm becomes impregnated with yolk granules later on. At the final stage of vitellogenesis, some of the granules fuse to form larger globules. A thin layer of fibroblast known as theca is also distinguished outside the follicular layer.

c) Migratory nucleus stage: The yolk granules reach maximum size. They are fairly large in size because of the frequent fusion. The yolk vesicles also fuse and acquire bigger size. Larger yolk vesicles are mostly in the centre of the oocyte. The nucleus looses its circular shape. This is accompanied by the dissolution of nuclear membrane and the gradual migration of the nucleus towards the animal pole takes place. Nucleoli are few in number. Some yolk vesicles are seen along the periphery of the oocyte forming cortical alveoli (Fig.5.10).

d) Ripe ovum stage: The ripe ovum is larger in size, yellowish in colour. Enormous accumulation of yolk granules and globules may be seen in the ooplasm. The ripe ovum is surrounded by three layers: the internal layer is known as zona radiata, middle layer is the follicular layer and the outer most layer is called the theca. The zona radiata layer has minute radial canals. The nucleus of the oocyte is pushed by the yolk towards the peripheral ooplasm. The follicular cells become glandular and secrete some substance to form a protective layer zona pellucida around the oocyte membrane. When ovulation takes place, follicular membranes rupture leading to the release of ripe ova into the ovarian lumen for spawning (Fig. 5.11).

In addition to above described features, two more structures were identified. These are:

Postovulatory follicles: The follicular membrane remains in the ovary after the liberation of the oocytes into the surrounding medium, tending to collapse due to the mechanical pressure. They appear as shrinkage and distorted structures with no cells in their lumen (initially) and are present during the period of spawning (Fig. 5.12).

Atretic follicles: Some post vitellogenic oocytes fail to undergo maturation and degenerate. In these oocytes yolk loses its structural integrity. The cytoplasmic inclusions lose their organization. The zona radiata forms a number of folds and break at several points, and the oocyte is invaded by phagocytic cells (Fig. 5.13).

5.3.2 Stages of maturation

Degree of maturation and the morphology of the gonad in different stages of maturity of *C. dussumieri.*

Stage	Degree of maturation	Ova diameter (mm)	Description of the gonads
I	Immature virgins / Resting	< 234µm	Ovaries thin, thread like pale in colour, occupying 1/4th of the body cavity of fish (Fig.5.14a). Oocytes under magnification appeared transparent and with a central nucleus (Fig. 5.14b). In ''resting'' or recrudescence period diameter of the ovary and thickness of the ovarian wall are larger. Most of the largest oocytes in this class appeared spherical; however the smaller ones were pear shaped. Left lobe slightly longer than the right one. Testis is thin, slender (thread like), transparent and pale in colour (Fig. 5.16a and Fig. 5.16b). Occupy more than ½ of body cavity. Left slightly longer than the right one. Cysts contained only spermatogonia.
II	Maturing virgins / Recovered spents	234-522µm	Ovaries become considerably larger, cylindrical and increase in weight and volume. Yellowish white in colour; with minute opaque whitish / yellowish eggs visible to the naked eyes (Fig.5.15a). Ovary occupied more than half of the body cavity. Two lobes are unequal in size; the left lobe is longer than right. Under microscope, the oocytes were spherical and translucent, and central portion of the eggs appeared darker (Fig.5.15b). Testes become enlarge, flat, increase in weight and volume, and creamy white in colour. Left

			lobe is longer than the right one (Fig. 5.16c and Fig.5.16d). Well defined seminiferous lobules with spermatogonia and spermatocytes. Seminiferous cysts of dividing cells appear.
III	Mature/ Ripening	Small opaque-522-1044µm Large opaque- 1044– 3100 µm	Reddish brown or yellow; Ovary occupying 3/4th or more of the body cavity (Fig.5.15c). Ovarian wall becomes thin, ova round, opaque and appear as dark bodies under microscope (Fig.5.15d). Testis extending more than ½ -3/4th of the body cavity. Testes are longer and wider, light pinkish and thicker in size and look more vascular (Fig. 5.16e and Fig. 5.16f). Seminiferous lobules with spermatogonia as well as seminiferous cysts of actively dividing spermatocytes, spermatids and spermatozoa.
IV	Ripe	3100-6240µm	Ovary becomes more enlarged occupying almost entire body cavity (Fig.5.15e), with large number of big, turgid, spherical, translucent, yellowish orange ripe ova (Fig. 5.15f). and with numerous blood vessels ramifying over their surface. Testes become soft turgid pinkish red and increase in weight and volume. Blood capillaries prominent. Seminal vesciles are bulged due to sperm storage (Fig. 5.16g and Fig. 5.16h). Seminiferous cysts very much reduced. The entire lumen filled with motile spermatozoa.

V	Spent		Bruised ovary. Purple in colour and very flaccid, shrinked and sac like, reduced in volume. Ovary wall thick and blood capillaries are big. Gonad shrunken having loose walls (Fig.5.15g). Ovary contains ripped unspawned darkened eggs and a large number of small ova and empty follicles (Fig. 5.15h). Testes occupy more than 3/4th of the visceral cavity. Testes seem to be dorsoventrally flattened, become flabby, thin and dull white in colour (Fig. 5.16i and Fig5.16j). The lumen of seminiferous lobules contains residual spermatozoa and empty spaces.

5.3.3 Monthly percentage occurrence of *C. dussumieri* with gonads in different stages of maturity during 2003-'04 and 2004-'05

The month wise percentage occurrence of the different stages of maturity in males and females during 2003-'04 and 2004-'05 are presented in Figs.5.17a and 5.17b.

Immature/resting males (Stage I) occurred from March to May with a predominance in April (100%) during 2003-'04 whereas during 2004-'05, they were encountered from February to June. Maturing virgins / recovered spent individuals (Stage II) made their appearance in May and continued up to July with highest contribution of 100% in June during 2003-'04 and its occurrence extended to August during 2004-'05, scoring 100% in July. Ripening males (Stage III) were observed from July to September during 2003-'04, showing preponderance in August with 100% while in the succeeding year, October and August to September marked their occurrence. Ripe males (Stage IV) were observed from October to January and September. Their maximum occurrence during 2003-'04 was recorded in October (100%). During 2004-'05 they were totally absent in September. Individuals with V Stage (spent) of maturity were recorded in November to March with a peak in February (100%) during 2003-'04 whereas during 2004-'05, they were found to occur from December to March with utmost incidence in February (95%).

It was discerned that there was apparently not much differences in the month wise occurrences of different stages of maturity between the sexes in both years. During both the years, females in the immature/ resting stage were observed from March to June with April and May contributing 100% incidence during 2003-'04 and April alone in the succeeding year. Specimens in Stage II started appearing in June and continued till August with peak in July (95% occurrence during 2003-'04 while 100% in 2004-'05). But they made their appearance in May during 2004-'05. Ripening females (Stage III) were encountered from July to September with predominance in September during both the years. But they were totally absent in July during 2004-'05. Gravid females (Stage IV) were observed from September to January in both the years and both years registered 100% prevalence in October. Individuals with V Stage of maturity were recorded from the catches from November to March with peak in March (76%) during 2003-'04 and in February (100%) in the succeeding year.

5.3.4 Pattern of progression of ova during different months

Ova diameter frequency of *C. dussumieri* during January to December is given in Fig. 5.18.

All oocytes measuring less than (generally 234µm) 260µm were immature. In the next batch of oocyte above 260µm, yolk deposition had just commenced and was found to be maturing ones. These oocytes on further deposition of yolk became opaque and represented the ripening batch of eggs having a size range of 522-3100µm. The yolk- laden mature eggs showed immense increase in size to become large hyaline eggs, within the range 3100-6240µm, represented the eggs to be spawned immediately. The pattern of progression of ova during different months showed the predominance of immature ova (<130-260µm) during March and April. Oocytes up to 780µm appeared in May with a major mode at 391- 520µm (maturing). Thereafter, the progression of ova was very rapid with the result that ripening oocytes were very prominent in June with the mode shifting to 781-910µm and a minor mode at 261-390µm. A major mode at 911- 1040µm and minor mode 391-520µm in July and with few eggs entering the late ripening stage was discerned. During August, ripening ova developed further and formed a major modes at 911-1040µm, while the maturing ova with a mode at 651-780µm. In addition a new batch of maturing ova with mode at 261-390µm made its appearance. The mature/ripening group of ova grew further and reached a mode greater than 3100µm and formed the ripe ova and was ready for spawning. These were noticed during September, October, November and December. Thereafter, the ova size diminished in January and February with multiple modes. Immature, maturing and ripening ova were noticed in February with a major mode at 261-390µm and a minor mode at 781-910µm. Excluding March and April, ripening oocytes having 522-3100µm diameter were observed in the ovaries in varying proportions. Largest oocytes having greater than 3100µm diameter were encountered only during September to December. January also recorded low frequency of ripe eggs.

5.3.5 Gonadosomatic Index

The monthly fluctuations in the mean values of gonadosomatic index (GSI) for males and females during October 2003 to September 2005 are depicted in Fig.5.19 and Fig.5.20. During 2003-'04, the gonadosomatic index of male showed maximum values in October (1.228) followed by November (0.827) and December (0.514). In the subsequent months the values declined and showed a steady trend till July except for a slight increase in March (0.1475). The minimum value of 0.053 was discerned in February. The values again shot up in August (0.448) and September (0.4099). The trend was more or less the same during 2004-'05 except for the slight variations in the values. Females showed distinct seasonality in GSI values similar to those of males. In the case of females, the higher index values occurred in October to January with a peak in November (11.899) and thereafter declined sharply in February (1.02) during 2003-'04. Low values were observed from March to July and there were more or less concurrent fluctuations in the GSI values during this period. Mean GSI values were again higher in August and September. During 2004-’05 also, the females exhibited similar trend except for the high GSI value in February.

5.3.6 Minimum size at first maturity

The percentage occurrence of the different stages of maturity in each of the length groups of males and females are presented in Tables 5.1 and 5.2 respectively. Fig. 5.21 shows the graphical representation of the data with regard to males and females of C. *dussumieri*. Stage I and II of both testes and ovary were arbitrarily considered immature and the subsequent stages mature.

Table 5.1 showed that the smallest mature male belonged to the 131-140 mm (131 mm) total length group. While Table 5.2., illustrated that the smallest of the female specimens procured (141-150 mm (141 mm) length group) were mature. If the length at which 50% of the fishes are mature can be considered as the minimum length at first maturity (Kagwade, 1968), as represented in Fig. 5.21., the smallest mature males are of 182 mm length (181-190 mm TL) and females are of 151 mm (151-160 mm TL) length. Thus the size of the smallest mature male is less than that of the mature female.

5.3.7 Sex-ratio

In *C. dussumieri*, sexes could not be distinguished externally. To find out the sex- ratio during different months and in different size groups, 248 males (94-305 mm TL) and 221 females (95-247 mm TL) were examined. The data on monthly distribution of sex ratio pooled for 2003-'04 and 2004-'05 is depicted in Table 5.3. Sexes did not differ significantly (P>0.05) from 1:1 except in October when females were favoured significantly, and July when males predominated. The pooled average for the whole period shows M: F ratio as 100: 89, indicating marginal domination of male population which was proved as insignificant at 5% level when the sex ratio was subjected to chi-square test.

The data on sex ratio in relation to size group is given in Table 5.4. The number of males and females were more or less equal at 91-140 mm length groups. Females were predominant in the fishery from 141-210 mm TL and thereafter males became dominant. Beyond 251 mm TL, only males were encountered. Chi- square values indicated that there was significant variation from 1:1 ratio in the size groups from 141-150 mm TL, 161-200 mm TL, 211-220 mm TL and 231-250 mm TL whereas those between

91-140 mm TL, 151-160 mm TL, 201-210 mm TL and 221-230 mm TL did not skew significantly from the expected values. The chi- square value for the overall sex ratio showed that the variation in length groups were insignificant ($P>0.05$).

5.3.8 Fecundity

Fecundity is determined as the total number of ova shed in the spawning season by the ripe fish. Statistical analysis was conducted to elicit the relationships between fecundity (F), total length of fish (TL), total weight of fish (W), weight of ovary (OW), and length of ovary (OL) and the results are depicted in Figs.5.22 - 5.27. Fecundity is presented in Table 5.5. The maximum fecundity (859) was observed in a fish measuring 214 mm in total length and weighing 250 gm. The lowest fecundity was noticed in a 187 mm fish weighing 122 gm and the fecundity of which was 150 ova.

The logarithmic relationship between fecundity (F) and total length (TL) of fish was linear (Fig. 5.22). The calculated relationship was:

$$\text{Log } F = -7.2786 + 4.2497 \log TL$$

The correlation coefficient ($r = 0.767$) indicated a significant ($p < 0.01$) relationship.

The fecundity and body weight also showed a linear relationship (Fig. 5.23). The regression equation of fecundity (F) on body weight (W) is expressed by the formula,

$$\text{Log } F = -0.3075 + 1.2496 \log W$$

The correlation coefficient (r) was 0.844 indicating fairly high significant ($P < 0.01$) relationship between the two variables.

Relationships between fecundity and ovary length (Fig.5.24) and fecundity and ovary weight (Fig.5.25) were linear. The equations that relate these parameters are the following:

$$\text{Log F} = 0.1564 + 2.155 \log \text{OL} \ (r = 0.834)$$

$$\text{Log F} = 1.6287 + 0.6427 \text{ OW} \ (r = 0.771)$$

Scatter diagram of ovarian weight (OW) on body length (TL) and body weight (W) of *C. dussumieri* has been shown in Fig.5.26 and Fig.5.27 respectively, and the regression equation was found to be represented by:

$$\text{Log OW} = -6.9521 + 3.6014 \log \text{TL} \ (r = 0.542)$$

$$\text{Log OW} = -1.6775 + 1.3438 \log \text{W} \ (r = 0.756)$$

The deviation of exponential or 'b' values were analyzed against '3' in the case of length and against unit or '1' in respect of weight using t-test and the results are presented in Table 5.6. According to the results, estimated 'b' values were of all parameters expect that of ovary length and ovary weight, did not deviate significantly from '3' and '1'.

The results of the statistical analysis carried out to test the significance of correlation coefficient 'r' are represented in Table 5.7. Among the various body parameters, highest correlation existed between fecundity and body weight (W) (0.844) and the lowest correlation was observed between ovary weight and total length of the fish (TL) (0.542). All body parameters were found significantly correlated to fecundity.

5.4 Discussion

The male and the female reproductive systems of *Colletteicthys dussumieri* are similar anatomically and histologically to other batrachoid species. The female reproductive apparatus is common to many other teleost species (Nagahama, 1983). In contrast the male sperm transport system in *C. dussumieri* is different from the majority of teleosts (Grier *et al.*, 1980; Nagahama, 1983). First it has testicular efferent ductus, a feature uncommon among species with the unrestricted testis type (Grier, 1981; Rasotto and Sadovy, 1995), and second it has a pair of accessory organs, representing evaginations of the main testicular ducts as reported in *Opsanus tau* and *Porichthys notatus* (Barni *et al*., 2001).

Spermatogenesis and Oogenesis in this species appears to be similar to that reported for many other teleosts. Six distinct stages in spermatogenesis (viz. the primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa) as observed in *C. dussumieri*, have been recognized and described in other teleosts such as *Opsanus tau* (Hoffmann, 1963*), Oligosarcus hepsetus* (Santos *et al*., 2006) and *Lophiosilurus alexandri* (Marcelo *et al*., 2007). Double tails are characteristics of the sperm of toadfish (Hoffman, 1963) and similar observations were obtained in *C. dussumieri*. However, the existing literature on spermiogenesis producing sperm with two flagella were identified and described in few teleost families such as Bagridae (Yasuzumy, 1971), Batrachoididae (Hoffman, 1963; Stanley, 1965), Myctophidae (Mattei and Mattei, 1976), Apoginidae (Mattei and Mattei, 1984), Loarcidae (Yao *et al.*, 1995) and Cichlidae (Matos *et al*., 2002).

Detailed information on the ovarian growth and development in fishes has been studied by many authors (Matthews, 1938; Hayashi, 1972; Shackley and King, 1977; Nagahama, 1983; Ezzat *et al*., 1994; Tyler and Sumpter, 1996; Colombo and Grandi, 1996; Yoneda *et al*., 1998; Srijunngam and Wattanasirmkit, 2001; Arocha, 2002; Stark and Somerton, 2002; Gulsoy *et al*., 2006; Juchno *et al*., 2007; Jose and Jose, 2008; Koc *et al*., 2008; Mahmoud, 2009; Nunez and Duponchelle, 2009). Several Indian investigators have contributed to this aspect of fishery biology and some of those are Gokhale, 1957; Belsare, 1962; Sathyanesan, 1962 ; Gopal Dutt, 1964; Nayyar, 1964; Guraya *et al*., 1975; Malhotra *et al*., 1978; Agarwal *et al*., 1992 and Arockiaraj *et al.,* 2004. The results of the present study on oogenesis in *C. dussumieri* lend support to the findings of the above authors.

The oogonia pass through eight maturation stages to form the ripe ova. Regarding the production of new crop of oocytes, it has been stated that they are formed from the follicle cells (Wheeler, 1924 and Yamamoto, 1956). However some authors are of opinion that the new oocytes develop from the germinal epithelium covering the lamellae (Bara, 1960 and Arruda, 1988). Ramos (1983) suggested that these oocytes originate from residual oogonia. In *C. dussumieri* distinct nests of oogonia are observed in addition to immature oocytes at the resting stage of the ovaries. Therefore it can be speculated that the germinal epithelium is responsible for the new crop of oogonia. However, the residual oogonia also serve as a part of the parent stock for the succeeding cycle.

The morphohistological changes observed during the oocyte growth (previtellogenesis and vitellogenesis) include the formation of extra nucleoli, appearance of nucleolar extrusion; origin and subsequent disappearance of the yolk nucleus; formation of cortical alveoli; yolk bodies and development of follicular wall.

Considerable opinion exists about the exact origin, growth and function of the yolk nucleus. Some workers assign its origin to nuclear activity, which passes through the nuclear membrane to invade the cytoplasm (Wheeler, 1924; Sathyanesan, 1959; Malhotra *et al.* 1978) and others believe it to be cytoplasmic in origin (Chaudhry, 1949; Nayyar, 1964). In *C. dussumieri*, the yolk nucleus appears adjacent to the nuclear membrane and gradually moves towards the periphery of oocyte and finally disappears as the growth advances. The appearance of yolk nucleus of Balbiani followed immediately by cytoplasmic zonation in the oocytes, however, indicates the role the former possibly plays in the maturation of the oocytes. This view is more or less consistent with that of Belsare (1962), Bhargava (1971) and Malhotra *et al.* (1978).

The rhythm of deposition of yolk inclusions in the oocyte of fish differs from species to species. Dadzie (1974) observed that in *Tilapia mossambica*, lipid vesicles (cortical alveoli) and yolk granules appeared in the oocyte at the same time. In the present study, cortical alveoli were the first type of yolk inclusion to appear in the vitellogenic oocytes, their appearance marked the onset of vitellogenesis. This is in accordance with the findings of Shackley and King, 1977; Dadzie *et al.,* 2000b; Srijunngam and Wattanasirmkit, 2001 and Mahmoud, 2009.

With the advancement of maturity the previtellogenic oocytes become vitellogenic with the deposition of yolk and the follicle wall (oocyte envelope) seems more developed with clear differentiation into theca, follicular epithelium and zona radiata. Similarly, the oocyte of *C. dussumieri* is also covered by these 3 layers.

The post ovulatory follicle has been widely described in oviparous teleosts (Goldberg *et al.,* 1984; Agarwal *et al*., 1992). Oocyte after attaining full maturity, ovulation takes place. The theca and granulose cell layers are left behind after the discharge of ova and the structure formed is referred to as the post-ovulatory follicle or discharge follicle or ovulation scar or corpus luteum of ovulation. In *C. dussumieri*, the ovary of spawning and spent phase showed a number of postovulatory follicles (POF) in various stages of their development and degeneration.

The process of degeneration (atresia) is a consistent feature of teleost ovary (Agarwal, 1988). Some of the previtellogenic and vitellogenic yolky oocytes fail either to reach the ripe egg stage or to get spawned. Instead they get degenerated and are resorbed. It may affect the oocyte at any stage of development, more frequently in maturing and unovulated mature oocytes (Rastogi, 1969). The onset of atresia has been said to be related to age, stage of the reproductive cycle, environmental conditions (season, temperature, light, availability of food etc.), and the general health of the individual animal (Saidapur, 1978). In teleostean species, occurrence of atretic oocyte is a common phenomenon of pre-spawning, spawning and post-spawning ovaries (Guraya, *et al*. 1975). Atretic oocytes were encountered in *C. dussumieri* during pre-spawning, spawning and post-spawning periods. The remnants of the atretic oocytes are seen as groups of yellow pigment cells.

The oocytes growth was similar in the teleosts. In most of the teleosts, the progress of oogenesis might be in four, five, six and eight stages. The oocytes development in the *C. dussumieri* was manifested in a series of changes, occurring in eight stages.

Studies on reproduction, including the assessment of size at maturity, fecundity, duration of reproductive season, daily spawning behaviour and spawning fraction, permit quantification of the reproductive capacity of individual fish. Results of the two years data have shown that as far as occurrence of gonads in different stages of maturity is concerned, both the males and females followed almost similar trend.

Coastal or estuarine fishes in the tropics and sub-tropics are mainly serial spawners with a protracted spawning season (Longhurst and Pauly, 1987) in contrast to species in temperate regions. The stimuli of the onset of reproduction in any tropical estuarine fishes are less clear than fresh water and tropical reef species. This is perhaps because of the general variety of biotic and abiotic influences of marine, estuarine and freshwater origin that come together in this environment (Blaber, 2000). However, spawning usually occur at a time when environmental conditions are most favorable for larval survival and development. Temperature plays an important role in triggering spawning in tropics. It is clear from Fig. 5.17a and Fig. 5.17b that mature gonads were discerned from late September to January. However, the percentage frequency of mature gonads was higher during October to December. Fully spent individuals were observed from November to March and the highest percentage was recorded in February. This suggests that *C. dussumieri* has a prolonged spawning season extending from September to January. The highest spawning activity was observed during October and November in both the sexes. Based on the results of the present study, it can well be concluded that *C. dussumieri* inhabiting Cochin estuary spawns once in a year, spawning takes place during September to January with intense spawning in October - November in females and October - December in males. Similarly Fine *et al.* (2004) discerned a protracted spawning season in oyster toadfish, *Opsanus tau*, in the Chesapeake Bay, ranging from May to the end of July. Spawning season of *Halobatrachus didactylus* were found to extend from March to August, with a peak in May - June (Palazon-Fernandez *et al*., 2001). This further affirmed the prolonged spawning period in the species.

Hickling and Rutenberg (1936) propounded the theory that vital information regarding the spawning habits of fish may be obtained by the investigation of a mature ovary. According to them the number of peaks of ova in a mature ovary gives a clue to the number of spawnings per individual. Clark (1934) inferred that multiple modes occurring in ova diameter frequency polygons of the Californian sardine, *Sardine caerulea* were suggestive of more than one spawning per season by a particular fish. This method has been successfully applied for delineating the spawning period of many Indian fishes by several authors (Prabhu, 1956; Nair, 1959; Talwar, 1962; Bennet, 1967; Geevarghese and John, 1983; Zacharia and Jayabalan, 2007; Rao and Krishnan, 2009).

In the present study it was observed that the ova- diameter increased from 26 to 6240µm along with the progression of the maturity stage. Measurement of ova-diameter and their frequency polygon distribution at different times of the months in a year was a common method in determining the maturity cycle of the fish (Macer, 1974). The progressive change observed in the in the intra-ovarian diameter for a period not less than a year can give an idea of the spawning periodicity of the fish studies (Biswas, 1993). According to Qasim and Qayyum (1961) in fishes which possess single group of oocytes during the breeding season, the cycle of spawning in each individual occurs only once a year and the state of maturity at any given time is fairly uniform throughout the population. From the percentage occurrence of the mature ova in different months it is inferred that in *C. dussumieri*, the mature ova shows a single peak suggesting that they spawn once in a year and the breeding season extends from September to January with a peak from October to November. It is inferred that since there is only one mode formed by the big maturing ova and as it is sharply separated from the rest of the stock of eggs, spawning in this species is restricted to a definite period and most probably each individual spawns only once in a year. The complete demarcation of a group of ova in the mature ovary of *C. dussumieri* further supports the observation that individual fish spawns only once in a year.

The diameter of the oocytes is related to the reproductive behavior and it is not dependent on the species body size (Vazzoler, 1996). Species that display parental care behaviour have larger oocytes, while smaller oocytes characterize species that do not (Nakatami *et al*., 2001). Normando *et al.* (2009) reported that the elliptic shape and large size of vitellogenic oocytes of *Cichla kelberi* was related to the attachment mode of eggs to the substratum and to parental care behaviour. The size registered for the oocytes of *C. dussumieri* suggests that this species does have parental care and possess adhesive disc.

The ratio between the body weight and the weight of the gonad shows the status of the ovary in terms of maturity and denotes the phase of reproductive cycle. The cycle of maturation and monthly variation of gonadosomatic ratio provides good indication of the extent of development of gonad with respect to the time of year. Gonad staging on a descriptive scale allows a rapid qualitative assessment of the breeding state and gonad weight gives a quantitative record of changes in the gonad condition (Crossland, 1977). The GSI has been widely used as an indicator of the fish spawning period, but its use in reproductive biology studies is more suitable when associated with other reproduction indicators such as macroscopic and histological techniques. This is important for males, since differences in size and weight are less conspicuous than in females (Chaves, 1991). GSI values of both males and females followed more or less the same trend. Low GSI values in March, April and May is concomitant with a period of early development of gonads and spent fishes. The slight high values observed from June, July and August reflected a diversity of gonad stages including a large number of maturing (II stage) and ripening (III stage) gonads. The peak GSI values encountered in November coincided with the highest incidence of ripe males and gravid females. During spawning season, the GSI show a sinking due to the release of the gonadal products. Hence breeding season ensues the months with maximal GSI. The GSI values of females were much higher than males implying a greater proportion in body reserves were allocated to the gonads (Chatzifotis *et al*., 2004). In *C. dussumieri*, the sudden drop in the values in December is indicative of the termination of spawning season. The conclusion drawn earlier that *C. dussumieri* spawns once in a year can be further substantiated by a single peak of GSI in November.

Teleostean fishes have been observed to exhibit different types of spawning habits by Hickling and Rutenberg (1936) and De Jong (1939). Prabhu (1956) and Karekar and Bal(1960) have classified fishes based on their spawning pattern. Fish can have fractionated or total spawning. In the former, oocytes mature in sets and are released at different times during a long reproductive period, while in the latter all oocytes mature synchronously and are released during a short reproductive period (Vazzoler, 1996). It would thus appear that *C. dussumieri* was found to breed once in a year in Cochin estuary with ovaries cointaining prominent single group of maturing oocytes and exhibit total spawning.

The size at first maturity depends on the nature of the environment in which the population of concern lives (Moyle and Czech, 1988). Knowledge on the size at first maturity of fish helps to predicts harvestable size of the fish. Hence it has great benefits in fishery as well as aquaculture. Size at sexual maturity between populations of the same species varies due to growth rates, fishing removals, food availability, and hydrological conditions (Chapman *et al*., 1996; Hood and Johnson, 2000; Potts and Manooch, 2001).

The point of inflexion in the curve of Kn values plotted against length is indicative of the length at which sexual maturity starts (Hart, 1946; Pillay, 1954). The present investigation supports this observation as the point of inflexion was seen in the size group 121-130 mm TL for both sexes. The size at first maturity of male and female was estimated at 131mm TL and 141 mm TL respectively. Thus, males attain sexual maturity at a smaller length than the females. A similar observation was made in *Halobatrachus didactylus* (Palazon-Fernandez *et al*., 2001) and *Amphichthys cryptocentrus* (Granado and Gonzalez, 1988) in family Batrachoididae. Males began maturing earlier than females, a fact that has been attributed to gains in fecundity with size (Stearns, 1992). Such observations had been reported in fishes such as *Caranx kalla* (Kagwade, 1968); *Silurus glanis* (Alp *et a*l., 2004); *Decapterus russelli* (Manoj kumar, 2005); *Lactarius lactarius* (Zacharia and Jayabalan, 2007); *Amblypharyngodon mola* (Suresh *et al*., 2007). The first appearance of ripe and spent individuals in 181-190 mm group in males and 151 – 160 mm size group in females of *C. dussumieri* suggests that this roughly corresponds to the minimum size group at which the females and males attain ripeness and start spawning. Length at fifty percent maturity (minimum size at maturity) for males was greater than for females, which may indicate that after first maturation females reduce growth and allocate energy mainly for the production of gametes while males, with a smaller reproductive effort, continue growing (Palazon-Fernandez *et al.*, 2001). In *C. dussumieri* the males are larger (longer and heavier) in size than females. The maximum size of the males and females encountered during the present investigation is 305 mm and 246 mm respectively. The difference in the size at first maturity and the maximum size attained in the two sexes may be attributed due to differential growth rates between sexes. Female fish normally have higher energy requirements for reproduction than males, causing them to have a slower growth rate. Bowering (1976) pointed out that differences in growth between sexes are the result of genetics that determines the physiology and behaviour of the fish.

In fish, sex ratio varies considerably from species to species but in the majority of species it is close to 1:1. It differs from one population to another of the same species and may vary from year to year in the same population (Nikolsky, 1963). Any deviation from this ratio may indicate the dominance of one sex over the other. This happens because of differential behaviour of sexes, environmental conditions, fishing, etc. (Bal and Rao, 1984). The determination of sex-ratio in a population enables one to follow the movement of sexes in relation to season (Kagwade, 1968). The monthly distribution of two sexes in *C. dussumieri* did not show a significant difference in the distribution of both sexes except in July when males were dominant over females, October when females were dominant over males. However, the overall male: female ratio was 100: 89. The Chi-square value did not show any significant difference indicating equal distribution of both sexes. The greater number of females compared to males in October, coincident with the reproductive peak. This could be attributed to the fact that, as in other batrachoid species such as *Halobatrachus didactylus*, *Opsanus beta, Opsanus tau* and *Amphichthys cryptocentrus*, males actively guard the eggs, and maintain residence in the nests until young attain the free- swimming stage (Palazon-Fernandez *et al.*, 2001; Breder, 1941; Gray and Winn, 1961; Hoffman and Robertson, 1983), making them less exposed to capture. Dominance of males in July may be related to predominance of maturing males of a particular size. Costa *et al.* (1995) obtained an overall 1:1 sex ratio for *Halobatrachus didactylus* in the Mira estuary. Granado and Gonzalez (1988) also reported a 1:1 ratio for *Amphichthys cryptocentrus*, in tropical waters. Palazon-Fernandez *et al.* (2001) noticed 1:1 sex ratio in *Halobatrachus didactylus* in all months except in May and February during the periods of investigation. The overall male to female ratio in *Osteogeneiosus militaris* were found to be 100 : 95 indicating more or less equal distribution in that fish (Raje, 2006). Unequal distribution of sexes (sex ratio 10:17) in *Tachysurus caelatus* was attributed to differential behaviour of the males, which probably move in separate shoals after spawning towards shallow water (Raje, 2006). Thus the deviation from normal 1:1 sex ratio might be due to the partial segregation of mature fish through their habitat preference (Parish *et al.*, 1986) and differences between sexes in behaviour (Baglin, 1982), differential fishing (Kesteven, 1942), differences in growth rate between fishes (Qasim, 1973a), differences in age and size at maturity (Reynolds, 1974) and differences in morphology and physiological activity (Baglin, 1982). Therefore, unequal distribution of sexes may be related either to any one or to a combination of several factors stated above.

In the present study, size related differences in sex ratios were observed. Significant deviations from parity usually occurred among the medium and largest sizes, with females predominating the medium while the largest sizes were disproportionately male. Similar observations were obtained in *Halobatrachus didactylus* (Palazon-Fernandez *et al.*, 2001). The differences observed in the size-specific sex ratio of the toadfish have been reported for other fish species (Turner *et al.*, 1983; Stergiou *et al.*, 1996). In *Nemipterus peronii,* (Said *et al.*, 1994) females predominated the population at small sizes while males at larger sizes and the study indicated the existence of sex-specific differences in growth, with males growing faster than females. Difference in size-specific sex ratio have been related to sexual differences in growth, mortality, energetic cost of reproduction, and differential migration or spatial segregation by sex (Sadovy and Shapiro, 1987; Said *et al.*,1994; Stergiou *et al.*, 1996) or sex- reversal (Said *et al.*, 1994). Qasim (1966) has attributed sexual difference in the rate of growth to the preponderance of one sex, and according to him the preponderant sex attains a bigger size. In *C. dussumieri*, higher rate of growth and size do explain the preponderance of one sex since the minimum size at first maturity and the maximum size of the individual is greater in males.

Fecundity is the most common measure of reproductive potential in fishes. Fecundity of the individual fish is determined from the total number of mature ova that are destined to be shed at the ensuing spawning season. Fecundity information of a species is essential for estimating seed production capacity and spawning population of the species concerned. Marked differences in fecundity among species often reflect different reproductive strategies (Pitcher and Hart, 1982; Wootton, 1984; Murua and

Saborido-Rey, 2003). Within a given species, fecundity may vary as a result of different adaptations to environmental habitats (Witthames *et al.,* 1995). Even within a stock, fecundity is known to vary annually, undergo long-term changes (Rijnsdorp, 1991; Kjesbu *et al.,* 1998). Moreover, knowledge about fecundity of a fish is essential for evaluating the commercial potentialities of its stock, life history, practical culture and actual management of the fishery (Lagler *et al*., 1967).

Very little information is available on the fecundity of toadfishes. As for other batrachoid species, fecundity in *C. dussumieri* is very low. Hoffman and Robertson (1983) estimated the fecundity of *Sanopus barbatus* which varied from 245 to 499 ova. Granado and Gonzalez (1988) reported the fecundity of *Amphichthys cryptocentrus* with a range of 45-467 ova. Arora (1948) observed 20 to 800 ova in *Porichthys notatus*. In the present study, fecundity of *C. dussumieri* varied from 150 to 859. As for other batrachoid species, fecundity in *C. dussumieri* is very low. This must be compensated for by a high survival of eggs and juvenile fish that results from males guarding the nests until young are free- swimming. The high amount of yolk, and the great egg sizes may also be the mechanisms to assure a high survival rate in embryos and larvae and such instances has been reported by Palazon-Fernandez *et al.* (2001) for *Halobatrachus didactylus* (Batrachoididae) and Yanez-Arancibia *et al*. (1976) and Palazon-Fernandez *et al*. (1994) for the Ariidae, *Galeichthys caerulescens* and *Bagre marinus*, respectively. Raje (2006) reported 44 to 81 eggs and 27 to 61 eggs in *Tachysurus caelatus* and *Osteogeneiosus militaris* respectively. Dan (1977) observed 28 to 82 eggs in *Tachysurus tenuispinis.* Fecundity of *Noemacheilus montanus* was estimated to vary from 500 to 1005 (Bahuguna and Khatri, 2009). The fecundity of *Mastacembelus pancalus* (Rahman and Miah, 2009) was found to vary from 881 to 1182 eggs.

Fecundity of any species of fish depends not only upon the size and age of the fish, but also on the size of the egg. Fish that produce larger eggs are generally less fecund than those producing smaller eggs (Tyler and Sumpter, 1996). According to Svardson (1949) and Lagler *et al*. (1967) larger larvae produced from larger eggs have a better chance in natural selection than smaller larvae produced from smaller eggs. Batrachoids release adhesive, large eggs in a single layer (Breder and Rosen, 1966). Low fecundities, large eggs have been reported in other batrachoidids (*Opsanus tau*: Gudger, 1910; *Opsanus beta*: Breder, 1941; *Porichthys porossisimus*: Lane, 1967; *Batrachoides* sp.: Collette and Russo, 1981). The fecundity estimates of *C. dussumieri* and other batrachoids show that these species have a reproductive strategy common to other fish families with parental care, i.e. they produce a small number of large eggs. This also implies that *C. dussumieri* ova are likely to give rise to large hardy larvae.

Variation was observed in the fecundity of paired ovaries. In the present study, left portion of the ovary of *C. dussumieri* were found to be larger and contained higher number of eggs than the right one. This was further confirmed by similar findings of Palazon-Fernandez *et al*. (2001) in *Halobatrachus didactylus* (Batrachoididae). Hartman and Conkle (1960) observed that in 90 percent of cases, the left ovaries of salmon hold more eggs than the right side. Hence the number of eggs produced is typical of a species, even though, as with egg size, intraspecific genetic variation, age body size, environmental conditions and nutrition may increase the variance in number (Bagenal, 1969; Wootton, 1973; Townshend and Wootton, 1984; Flemming and Gross, 1990; Bromage *et al.,* 1991).

The knowledge of fecundity and its relationship with the body measurements make it possible to estimate the number of eggs that are likely to be spawned by the fish. Diverse relationships were found to exist between the fecundity and various parameters. Studies on fecundity and its relationships with various body parameters viz. total length, total weight, ovary length and ovary weight are useful in increasing the yield of fish species, stock management and assessment in any water body (Bahuguna and khatri, 2009). The relationship between length and fecundity differ in different species of fishes. Fecundity has been shown to increase as square (Clark, 1934) or cube of length (Simpson, 1951; Bagenal, 1957; Pillay, 1958) or a fourth power of length (Farran, 1938) or more than a fourth power of length (Varghese, 1980). In the present study, the exponential value of *C. dussumieri* was observed to be 4.249 indicating that fecundity increases at a rate above the fourth power of length. But t-test on 'b' value revealed that it did not deviate significantly from the value of '3'. This value was higher than the exponential value (b= 2.85) (Chapter 7) in length – weight relationship suggesting that the fecundity increased at a rate higher than the rate of increase of body weight in relation to length.

Chonder (1977), Singh *et al.* (1982), Singh and Srivastava (1982), Somdutt and Kumar (2004) and Joshi (2008) have observed linear relationship between fecundity and total length. Fecundity was found to have a linear relationship to body weight. The 'b' value of 1.250 did not significantly deviate from unity. In other words, the number of ova increased in proportion to body weight. A linear relationship between the fecundity and the fish weight has been reported in *Trichiurus patului* (Gupta, 1968), *Puntius sarana* (Sinha, 1975), *Puntius chilinoides* (Singh *et al.*, 1982), and *Puntius sarana* (Somdutt and Kumar, 2004). The increase in fecundity with body length and weight is consistent with the observations made in Batrachoididae (Palazon-Fernandez *et al.*, 2001; Granado and

Gonzalez, 1988) and other fish species (Gartner, 1993; Barbin and McCleave, 1997). Jhingran (1961) found a proportional increase in fecundity with increase in length and weight of the Gangetic anchovy, *Setipinna phasa*. Rita kumari and Nair (1979) observed that in *Noemacheilus triangularis,* the number of eggs increases with the size of the fish. In the present study, the coefficient of determination (r^2) indicated that approximately 59% of the variation in fecundity was associated with body length. The correlation of fecundity on body weight was highly closer than for length as nearly 71% of the variation in egg production was explained by changes in body weight. Similarly, as gauged by the magnitude of 'r^2' values, fecundity of *Porichthys notatus* (Batrachoididae) was more closely related to somatic weight than body length (DeMartini, 1990). Mathur (1964) observed that egg production in *Hilsa ilisha* is somewhat more highly correlated with weight than length of fish. Dobriyal (1988) observed more association between fecundity and body weight than body length in *Hypoththalmichthys molitrix*. The relationship of fecundity with ovary length (OL) was found to be linear. But 't'test on exponential value (b=2.155) indicated that it deviates significantly from the value of '3', revealing that as fecundity increases, ovary length decreases. In most fishes, the fecundity is related to ovary weight linearly or at a power almost equal to unity. This signifies that the number of eggs in the ovaries increases in proportion to the weight of the fish and also to the weight of its gonads. But in the present study, a non-linear relationship between fecundity and gonad weight exist, although there was a straight-line relationship between fecundity and body weight. Similar observation was made by Bagenal (1957) in long rough dab. According to him, heavier gonads produce fewer eggs than do the lighter ones, so the eggs are presumably larger and heavier in heavy gonads. This is in close conformity to the present findings.

The coefficient of correlation of the various statistical relationships derived between fecundity, body length, body weight, ovary length and ovary weight revealed significant relation between fecundity and the body parameters. The highest degree of correlation was seen between fecundity and body weight. It is well known that the weight of ovaries of a fish is mainly influenced by the ova contained in them. The ‘r’ values between ovary weight and body length and ovary weight and body weight exhibited a fair correlation between the variables, but body weight was more closely related to ovary weight than length, as observed in *Carassius auratus* by Delahunty and De Vlaming (1980).

Assessment of fecundity have paramount importance in fisheries management as it provides knowledge about the number of offspring produced in a season and the reproductive capacity of the species (Qasim and Qayyum, 1963). The fecundity and its relation to the size (length and weight) of a fish make it possible to estimate the number of eggs likely to be liberated (Chondar, 1977). From the multivariate analysis, body weight was identified as the most appropriate predictor of ovarian egg count, explaining 71% of the variance. In this study, the fecundity of the *C. dussumieri*, increased in proportion to total length (TL) and body weight (BW) and these results would be invaluable in enumerating the fecundity without sacrificing the specimen.

Table 5.1. Maturity stages (in %) in different length groups of males of *C. dussumieri*

MATURITY STAGES

Length group (mm)	I	II	III	IV	V
91 -100	33.33	66.67			
101 - 110	33.33	66.67			
111 - 120	20	80			
121 - 130	17.5	82.5			
131 - 140	25	65	10		
141 - 150	16.67	70.83	12.5		
151 - 160	12.5	62.5	25		
161 - 170		72.5	16.67	10.83	
171 - 180		57.28	30.22	12.5	
181 - 190		44.8	30.2	14.5	10.5
191 - 200		11.5	36.36	36.36	15.78
201 - 210			28.57	64.28	7.15
211 - 220			25.66	43.58	30.76
221 - 230			51.52	33.33	15.15
231 - 240			17.15	60	22.85
241 - 250			30.43	56.53	13.04
251 - 260			38.46	23.08	38.46
261 - 270			27.28	36.36	36.36
271 - 280			20	40	40
281 - 290				100	
291 - 300			33.33		66.67
301 - 310			50		50

Table 5.2. Maturity stages (in %) in different length groups of females of *C. dussumieri*

MATURITY STAGES

Length group (mm)	I	II	III	IV	V
91 -100	100				
101 - 110	100				
111 - 120	100				
121 - 130	75	25			
131 - 140	12.5	87.5			
141 - 150	21.4	50	28.6		
151 - 160		41.67	16.67	25	16.67
161 - 170		33.33	27.78	22.22	16.67
171 - 180		27.78	22.22	33.33	16.67
181 - 190		26.08	4.34	30.44	39.14
191 - 200		22.86	28.57	28.57	20
201 - 210		9.09	36.36	31.82	22.73
211 - 220			30.78	34.61	34.61
221 - 230			60	20	20
231 - 240				50	50
241 - 250				50	50

Table 5.3 Monthly Distribution of sex ratio and chi - square test in *C. dussumieri* pooled for 2003 -'04 and 2004 -'05

Month	No. of specimens	Males	Females	Sex- ratio (M:F)	Proportion of males	Chi-square	Significance at 5% level	D.F.
October	49	15	34	2.27	0.306	7.367	S	1
November	47	23	24	1.04	0.489	0.021	NS	1
December	28	18	10	0.56	0.643	2.286	NS	1
January	26	15	11	0.73	0.577	0.615	NS	1
February	44	26	18	0.69	0.591	1.455	NS	1
March	61	33	28	0.85	0.541	0.410	NS	1
April	39	19	20	1.05	0.487	0.026	NS	1
May	68	34	34	1.00	0.500	0.000	NS	1
June	16	10	6	0.60	0.625	1.000	NS	1
July	24	19	5	0.26	0.792	8.167	S	1
August	23	13	10	0.77	0.565	0.391	NS	1
September	44	23	21	0.91	0.523	0.091	NS	1
Total	**469**	**248**	**221**	**0.89**	**0.529**	**1.554**	**NS**	**1**

NS : Not significant S : Significant

Table 5.4. Lengthwise distribution of sex ratio and chi - square test in *C. dussumieri* pooled for 2003 -'04 and 2004 -'05

Length group	No. of specimens	Males	Females	Sex- ratio (M:F)	Chi- square	Significance at 5% level	D.F.
91 - 100	7	3	4	1.33	0.143	NS	1
101 - 110	6	3	3	1.00	0.000	NS	1
111 - 120	13	5	8	1.60	0.692	NS	1
121 - 130	8	4	4	1.00	0.000	NS	1
131 - 140	9	4	5	1.25	0.111	NS	1
141 - 150	19	5	14	2.80	4.263	S	1
151 - 160	20	8	12	1.50	0.800	NS	1
161 - 170	24	6	18	3.00	6.000	S	1
171 - 180	25	7	18	2.57	4.840	S	1
181 - 190	34	9	25	2.78	7.529	S	1
191 - 200	46	11	35	3.18	12.522	S	1
201 - 210	36	14	22	1.57	1.778	NS	1
211 - 220	60	39	21	0.54	5.400	S	1
221 - 230	55	33	22	0.67	2.200	NS	1
231 - 240	43	35	8	0.23	16.953	S	1
241 - 250	24	22	2	0.09	16.667	S	1
251 - 260	13	13	0				
261 - 270	11	11	0				
271 - 280	7	7	0				
281 - 290	4	4	0				
291 - 300	3	3	0				
301 - 310	2	2	0				
Total	**469**	**248**	**221**	**0.89**	**1.554**	**NS**	**1**

NS : Not significant S : Significant

Table 5.5. Fecundity of *C. dussumieri*

Length of fish (mm)	Weight of fish (gm)	Weight of ovary (gm)	Length of ovary (mm)	Fecundity
169	100	15.45	9.90	207
172	116	12.30	9.50	194
179	136	19.25	11.90	244
183	128	20.25	11.30	239
185	120	12.12	10.20	155
187	122	11.53	9.40	150
193	148	12.65	10.50	223
195	170	12.81	10.60	213
195	194	37.80	15.80	379
196	180	25.95	13.70	294
197	154	23.05	11.50	275
199	134	10.40	10.40	234
202	188	13.35	10.30	423
204	178	15.60	11.60	320
205	218	30.25	12.00	331
208	216	32.55	13.00	446
211	178	25.35	12.40	278
214	250	74.05	16.90	859
225	336	50.80	15.20	478
230	230	20.52	14.50	611

Table 5.6. Statistical analysis to test deviation from Cube law

	b	df	Sb	t = b-3/Sb	t = b-1/Sb	Probablity
F vs TL	4.250	19	0.837	1.4934		NS
F vs W	1.250	19	0.187		1.3368	NS
F vs OL	2.155	19	0.335	-2.522		$P < 0.05$
F vs OW	0.643	19	0.125		-2.856	$P < 0.05$
OW vs TL	3.6014	19	1.3158	0.4570		NS
OW vs W	1.3438	19	0.2738		1.2556	NS

NS = not significant

Table 5.7. Statistical analysis to test the significance of correlation coefficient

	r	t	Probablity
F vs TL	0.767	5.071	$P < 0.01$
F vs W	0.844	6.676	$P < 0.01$
F vs OL	0.834	6.413	$P < 0.01$
F vs OW	0.771	5.136	$P < 0.01$
OW vs TL	0.542	2.736	$P < 0.01$
OW vs W	0.756	4.9	$P < 0.01$

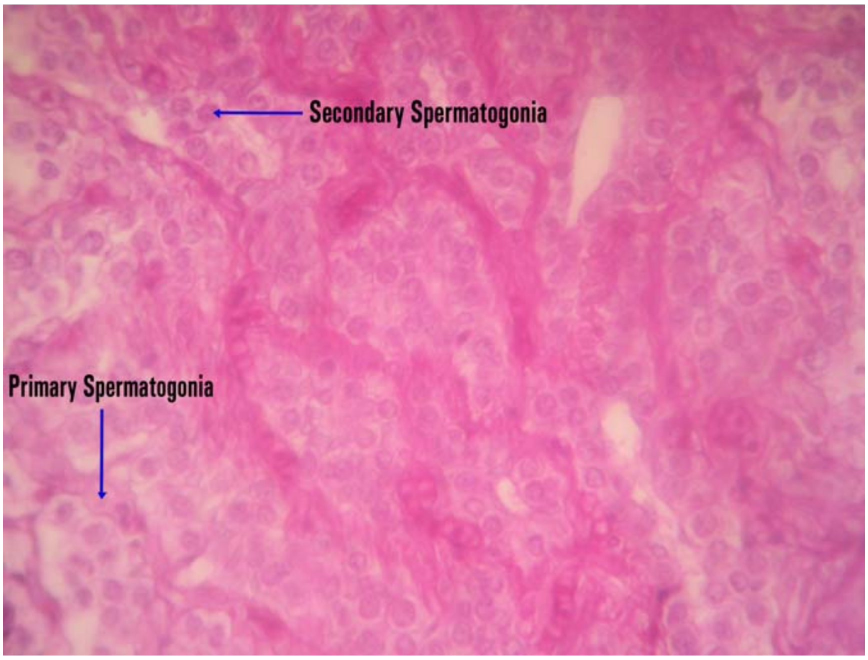


Fig.5.1. T.S. of testis showing primary and secondary spermatogonia

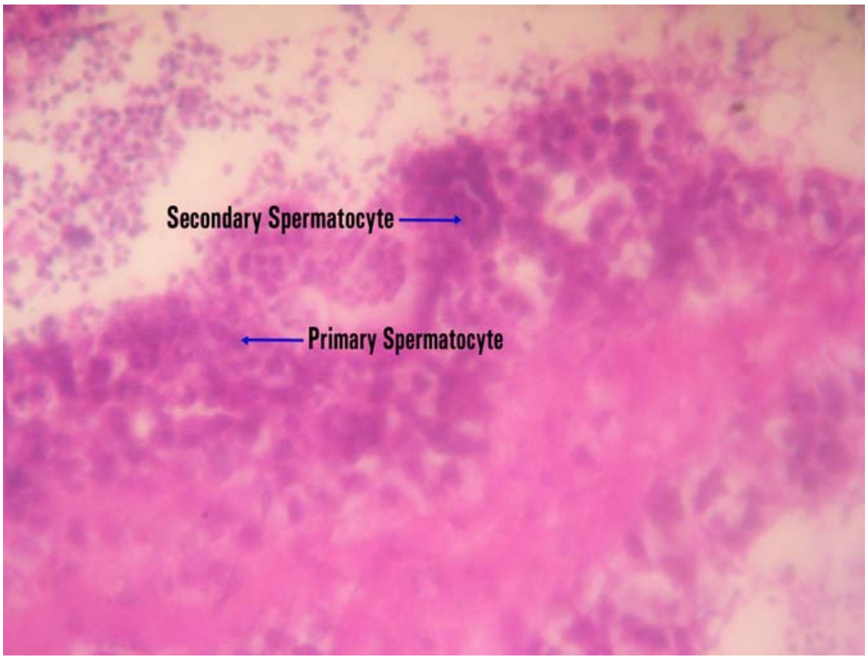


Fig.5.2. T.S. of testis showing primary and secondary spermatocytes

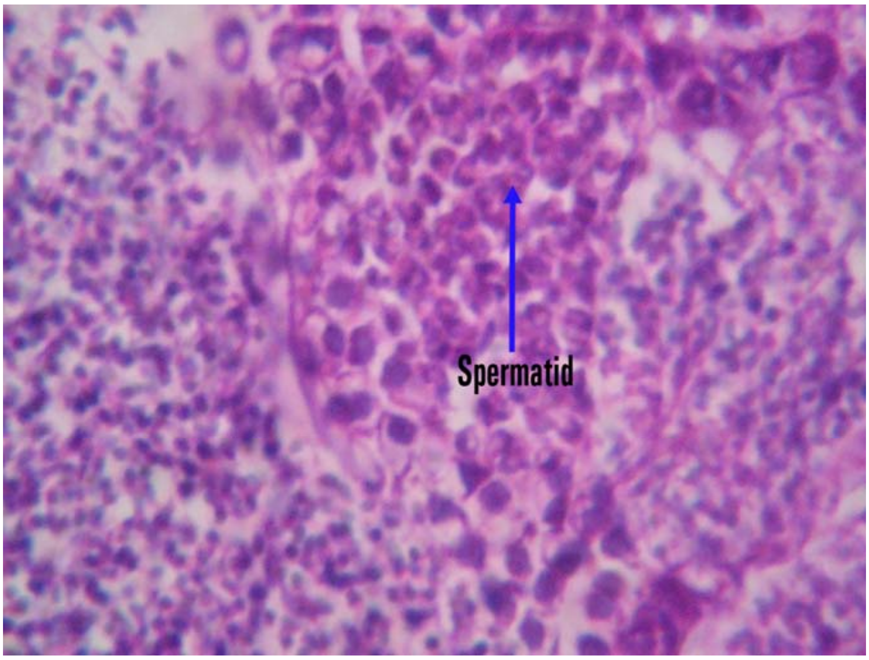


Fig.5.3. T.S. of testis showing spermatid

Fig.5.4. Bifalgellate spermatozoa

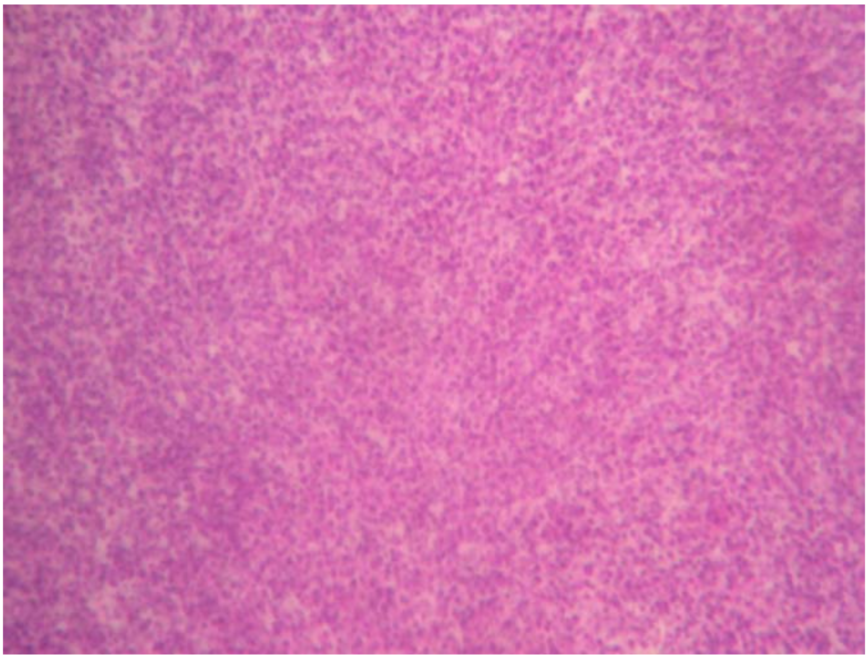


Fig.5.5. T.S. of testis showing mass of spermatozoa

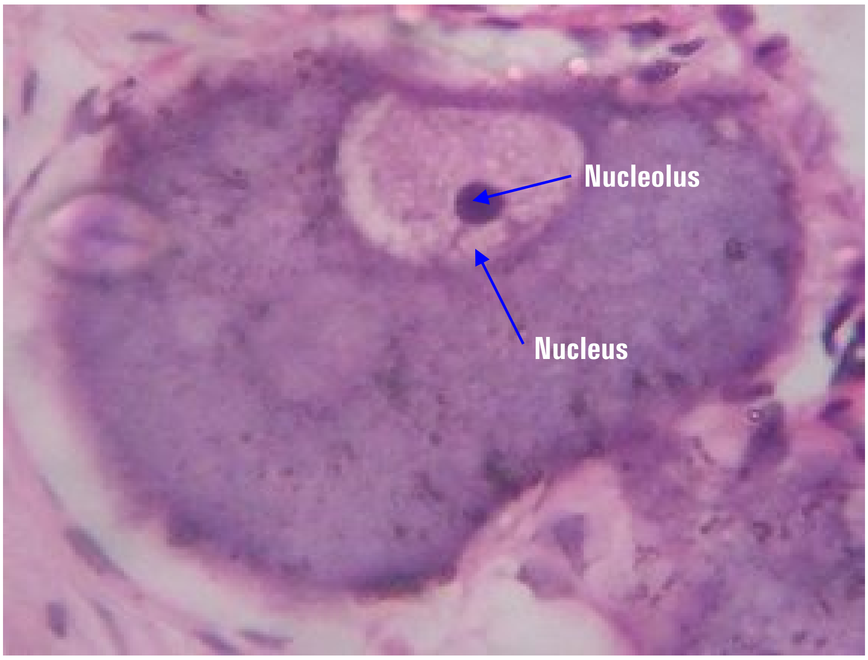


Fig.5.6. Chromatin nucleolus stage

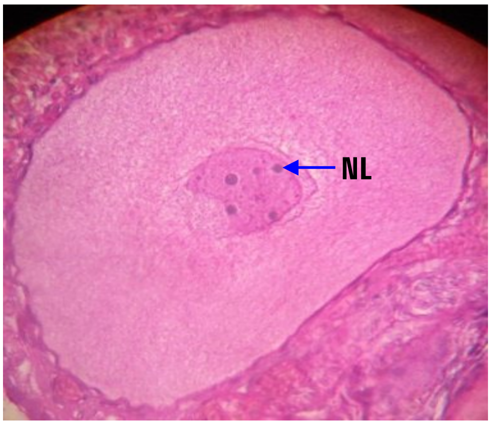


Fig.5.7a. Early perinucleolus stage

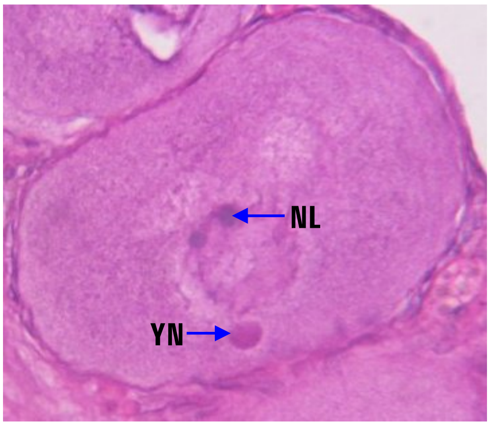


Fig.5.7b. Late perinucleolus stage

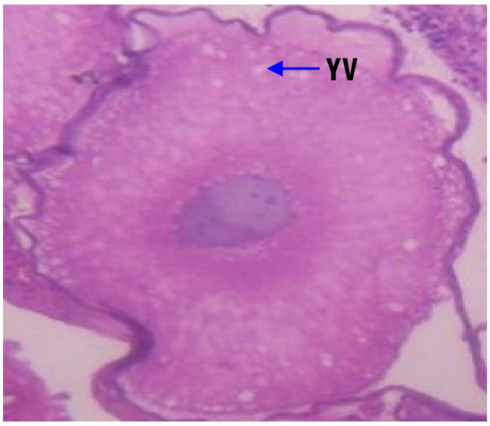


Fig.5.8a. Early yolk vesicle stage

Fig.5.8b. Late yolk vesicle stage

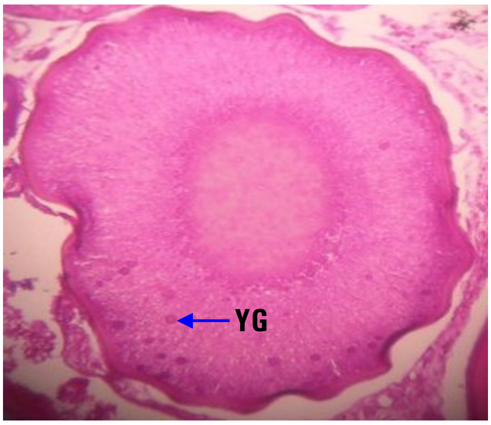


Fig.5.9a. Early yolk globule stage

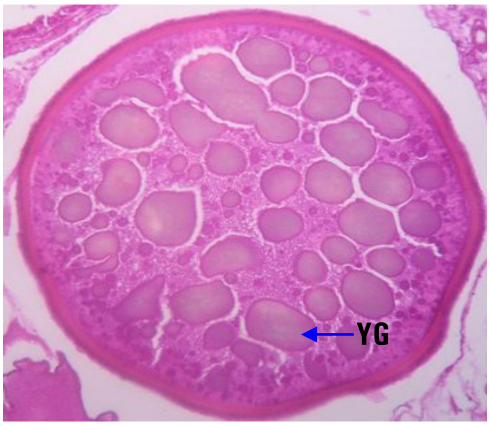
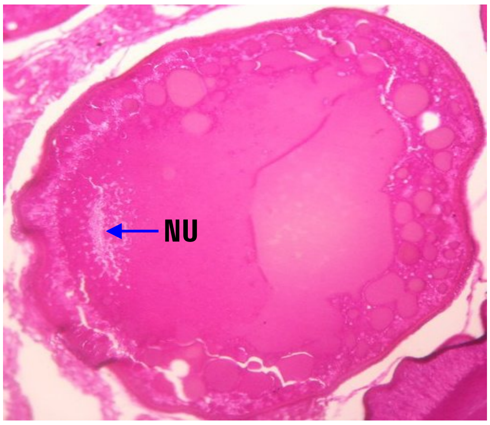


Fig.5.9b. Late yolk globule stage

NL : **Nucleolus**
YN: **Yolk Nucleus**
YV : **Yolk Vesicle**
YG: **Yolk Globule**



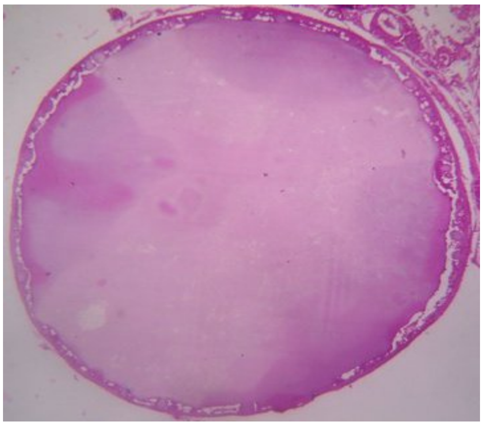
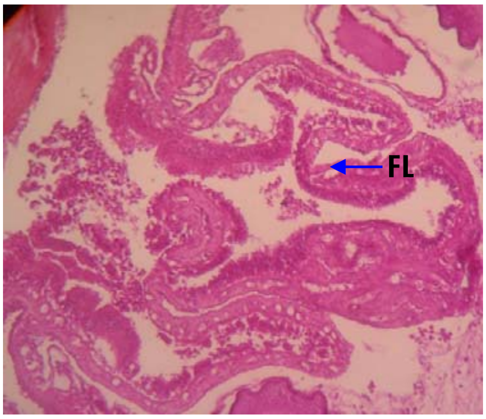


Fig.5.10. Migratory nucleus stage

Fig.5.11. Ripe egg



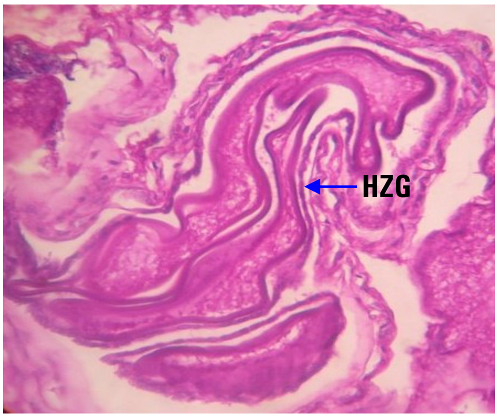


Fig.5.12. Postovulatory follicles

Fig.5.13. Atretic follicles

NU : Nucleus **FL: Follicular Layer** **HZG: Hypertrophied Zona Granulosa**

Fig.5.14a. Resting ovary (stage 1)

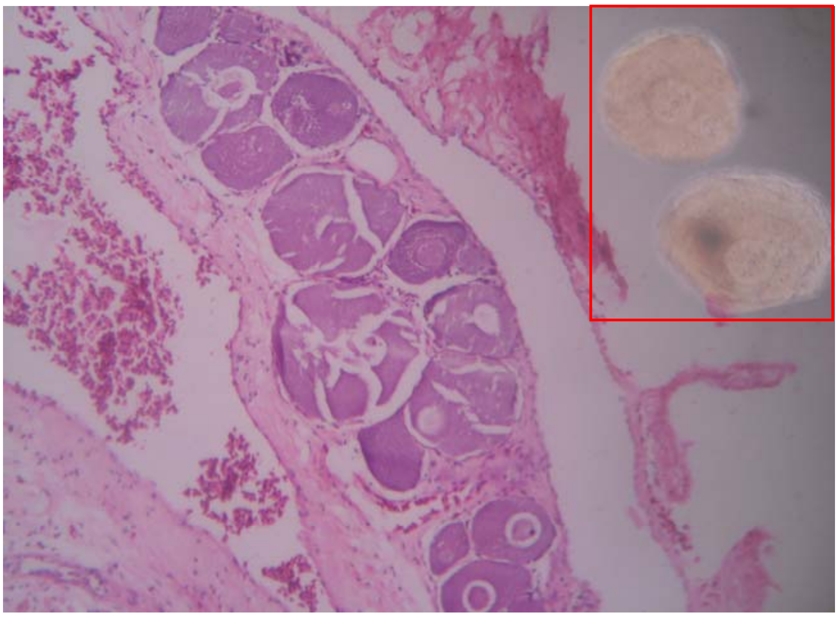


Fig.5.14b. Resting ovary

Fig.5.15a. Maturing ovary (stage 2)

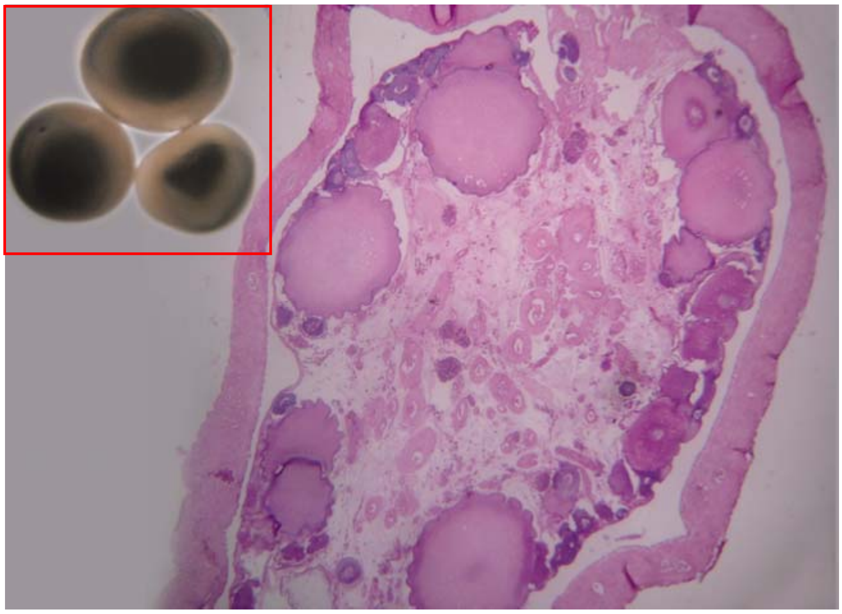


Fig.5.15b. Maturing ovary

Fig.5.15c. Ripening ovary (stage 3)

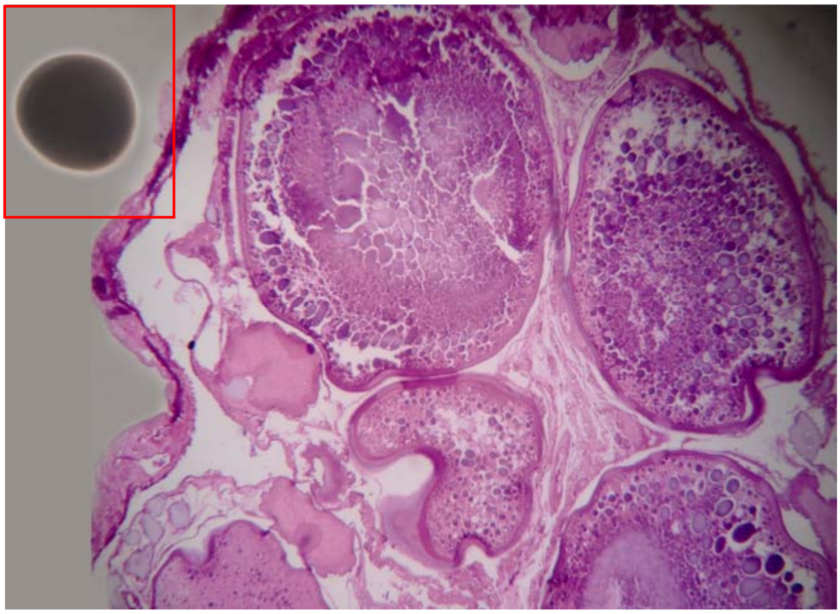


Fig.5.15d. Ripening ovary

Fig.5.15e. Ripe ovary (stage 4)

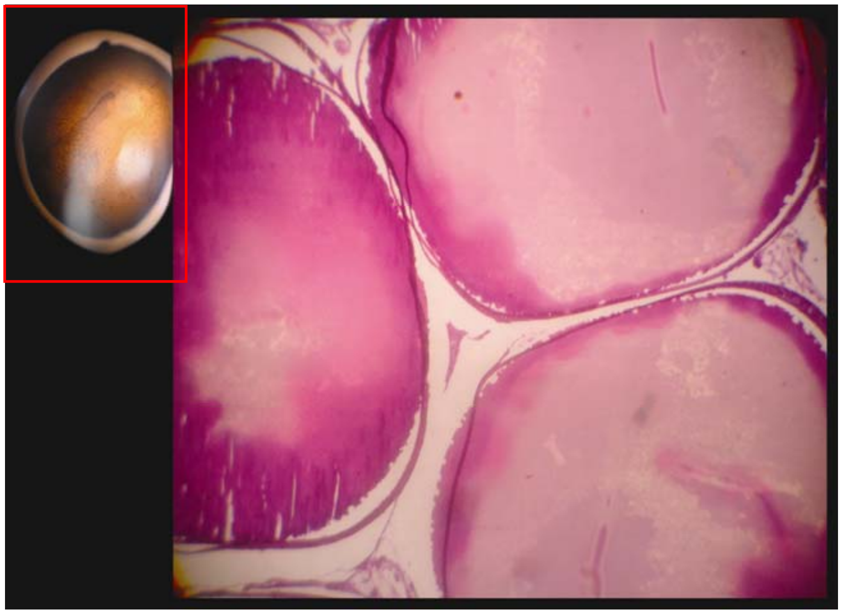


Fig.5.15f. Ripe ovary

Fig.5.15g. Spent ovary (stage 5)

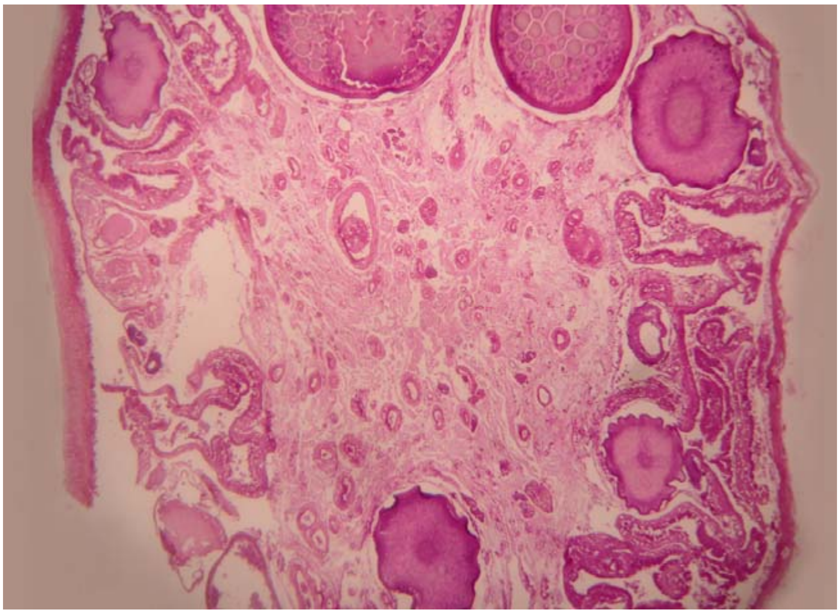


Fig.5.15h. Spent ovary

Fig.5.16a. Immature/ Resting testis (stage 1)

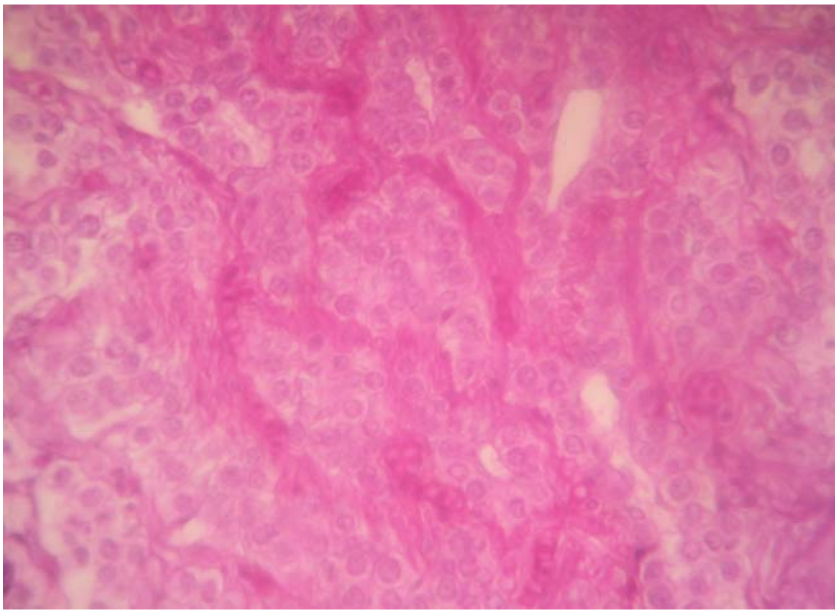


Fig.5.16b. Immature/Resting testis

Fig.5.16c. Maturing testis (stage 2)

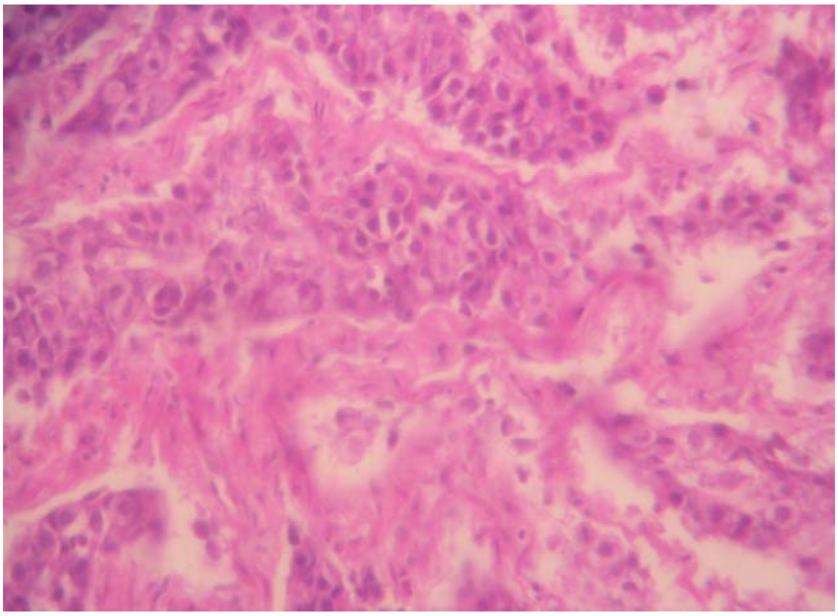


Fig.5.16d. Maturing testis

Fig.5.16e. Ripening testis (stage 3)

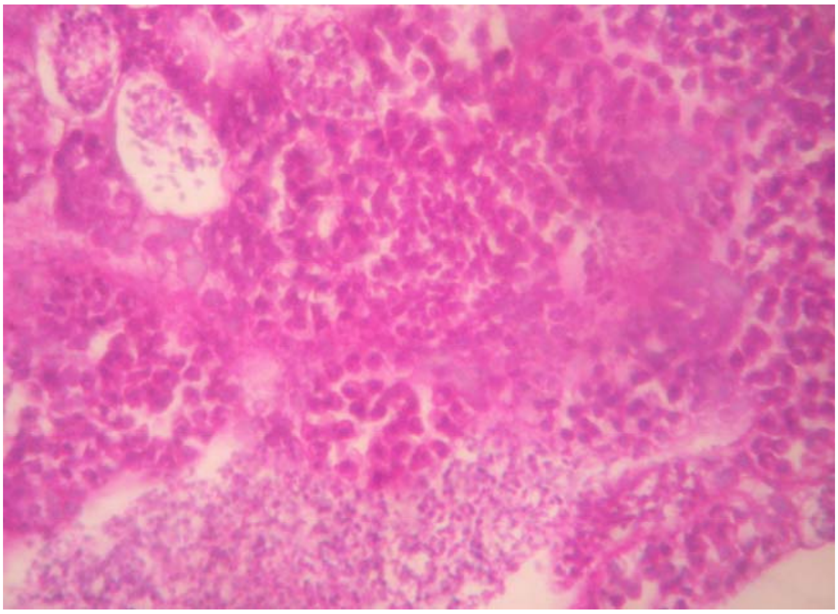


Fig.5.16f. Ripening testis

Fig.5.16g. Ripe testis(stage 4)

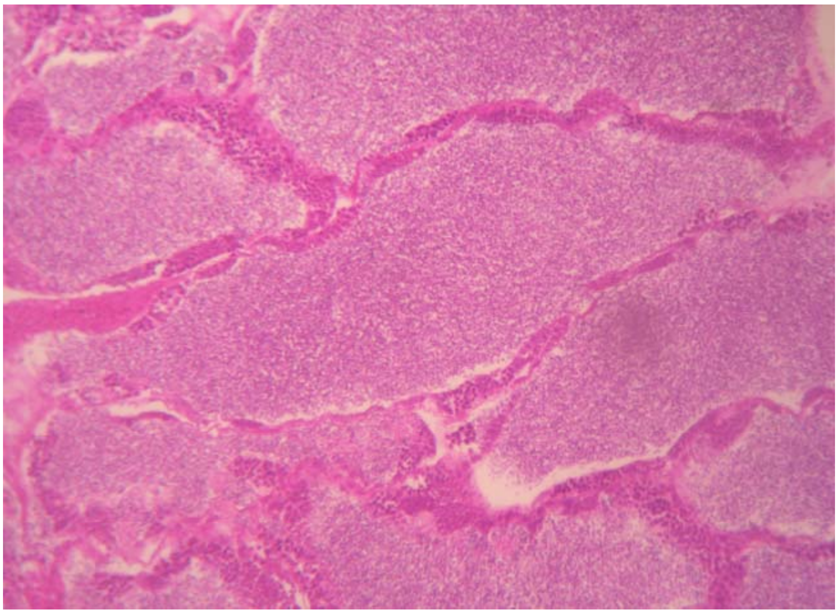


Fig.5.16h. Ripe testis

Fig.5.16i. Spent testis (stage 5)

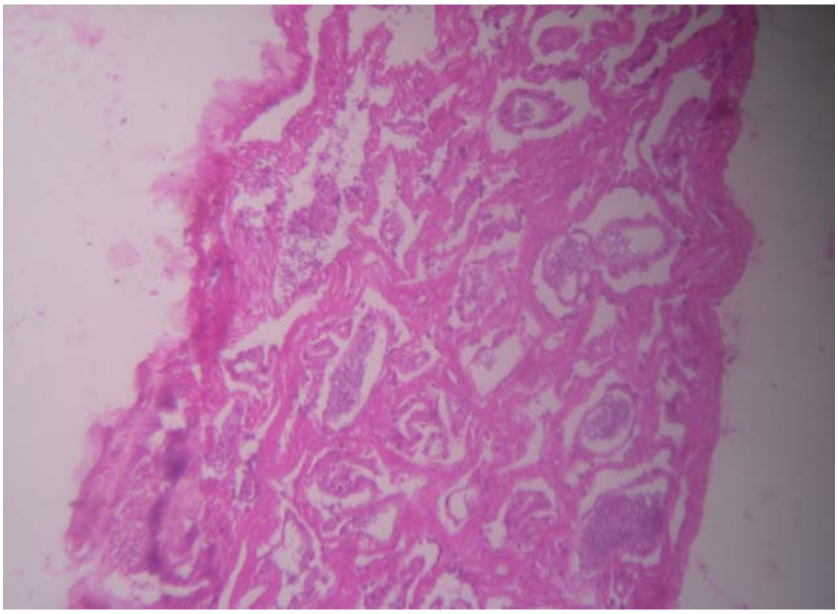


Fig.5.16j. Spent testis

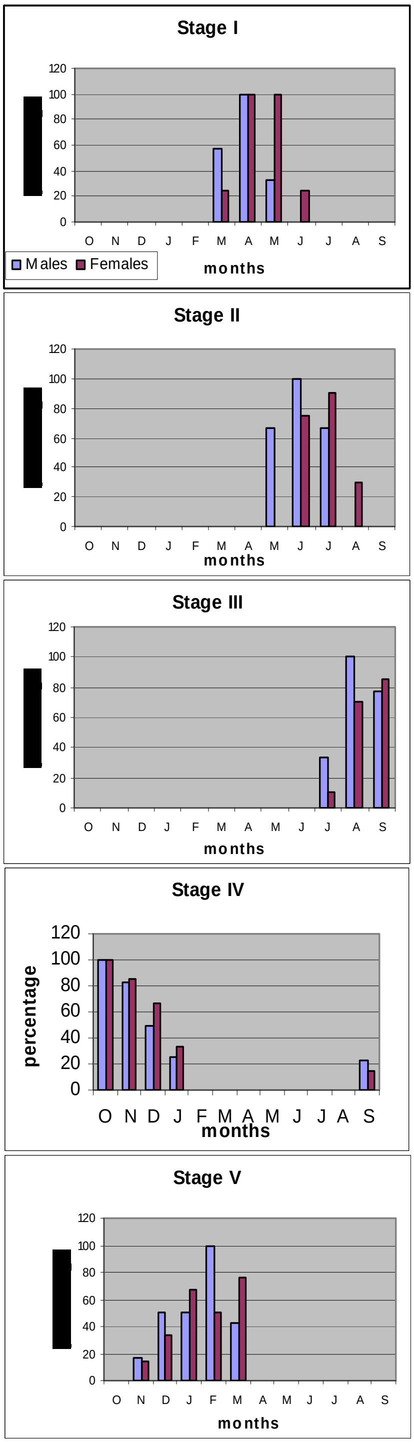


Fig. 5.17a. Monthly percentage occurrence of gonads of *C. dussumieri* in different stages of maturity during 2003-'04

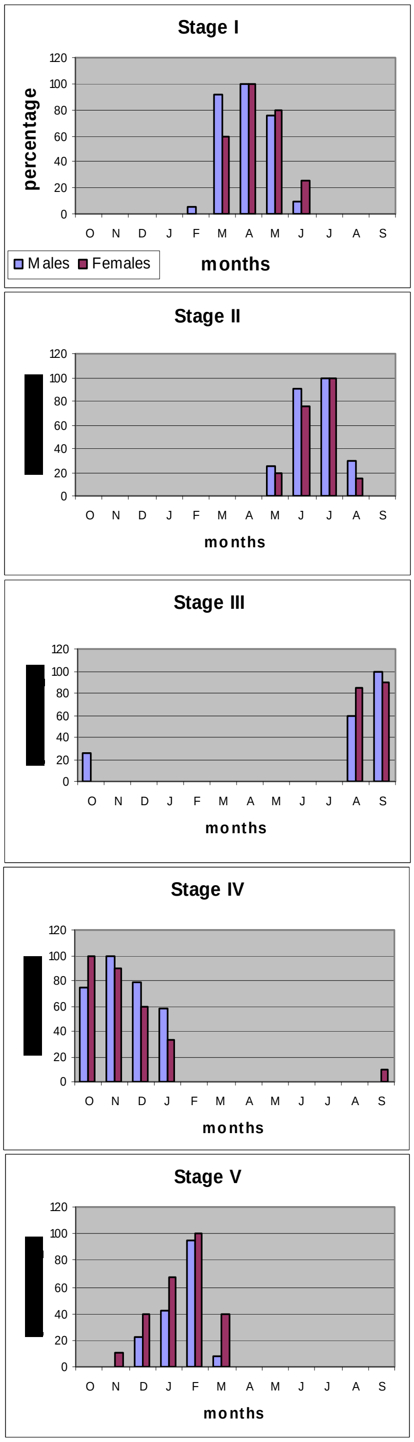
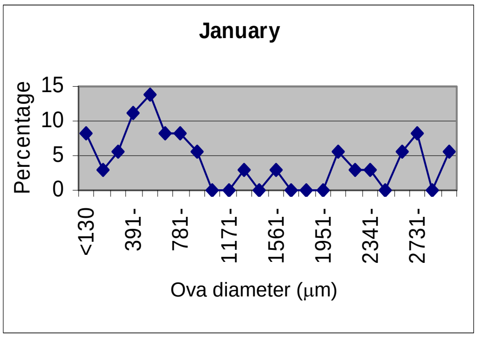
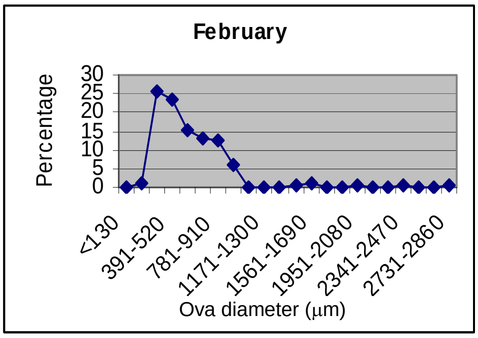
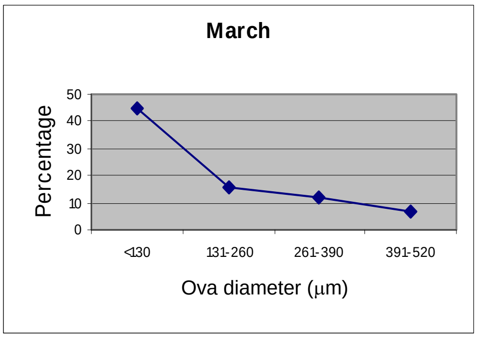
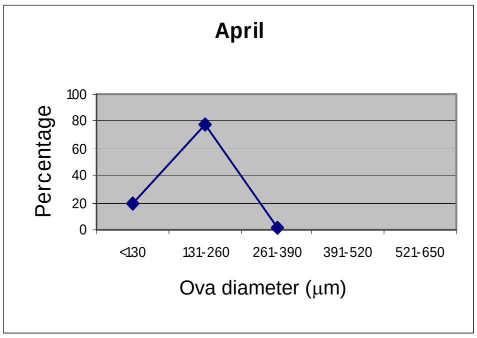


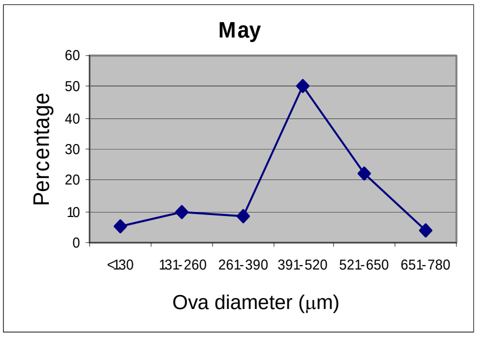
Fig. 5.17b. Monthly percentage occurrence of gonads of *C. dussumieri* in different stages of maturity during 2004-'05

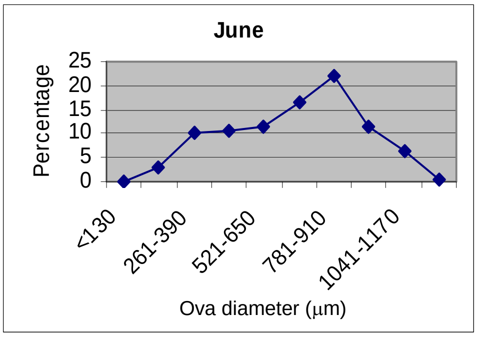

January
Percentage
15
10
5
0
<130
391-
781-
1171-
1561-
1951-
2341-
2731-
Ova diameter (μm)


February
Percentage
30
25
20
15
10
5
0
<130
391-520
781-910
1171-1300
1561-1690
1951-2080
2341-2470
2731-2860
Ova diameter (μm)


March
Percentage
50
40
30
20
10
0
<130
131-260
261-390
391-520
Ova diameter (μm)


April
Percentage
100
80
60
40
20
0
<130
131-260
261-390
391-520
521-650
Ova diameter (μm)


May
Percentage
60
50
40
30
20
10
0
<130
131-260
261-390
391-520
521-650
651-780
Ova diameter (μm)


June
Percentage
25
20
15
10
5
0
<130
261-390
521-650
781-910
1041-1170
Ova diameter (μm)

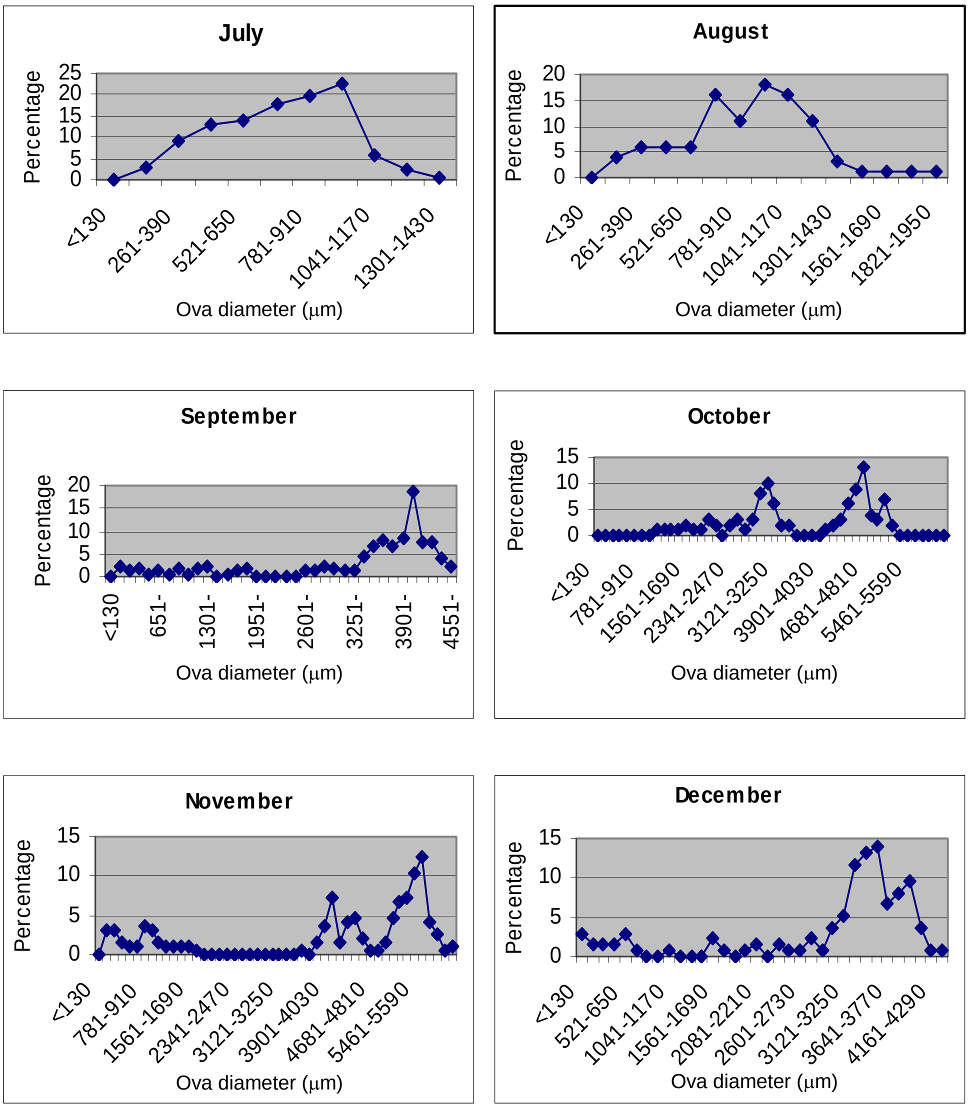


Fig. 5.18. Monthly variation in ova diameter percentage frequency of *C. dussumieri*

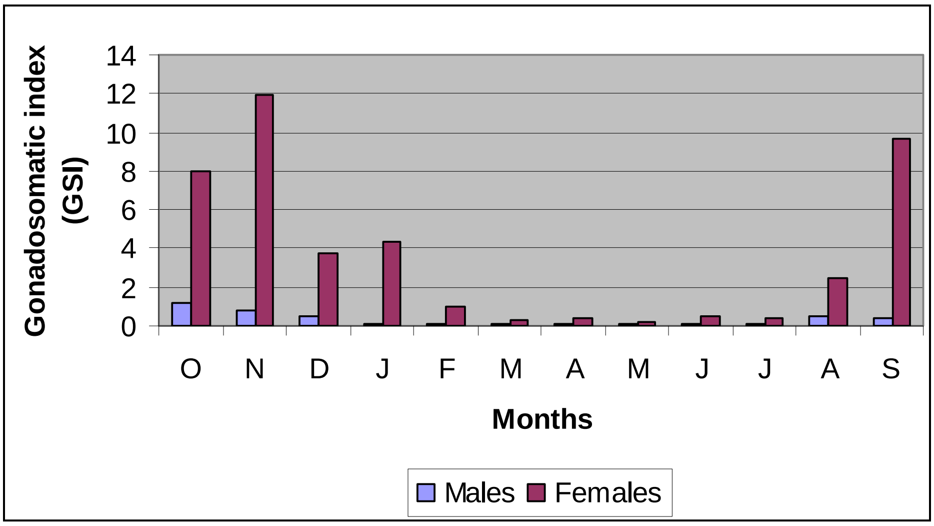


Fig. 5.19. Monthly variation of gonadosomatic index in *C. dussumieri* during 2003-'04

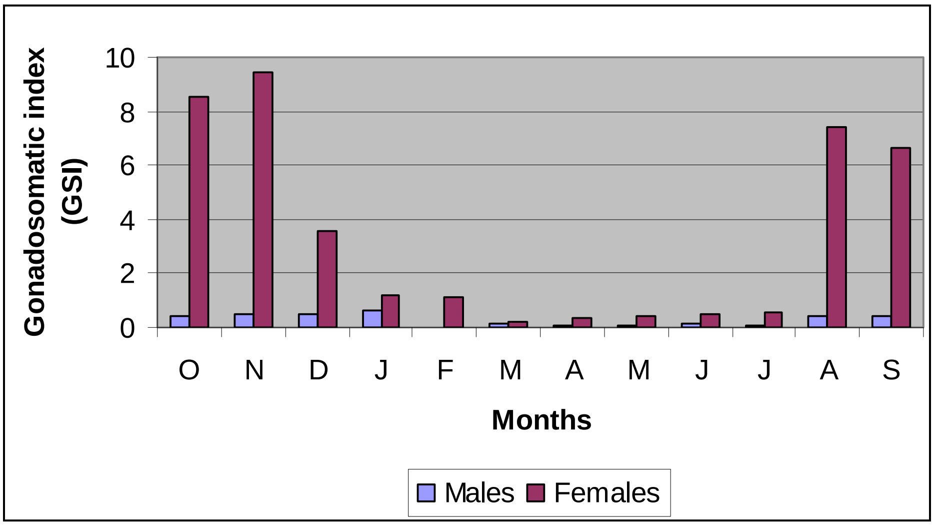


Fig. 5.20. Monthly variation of gonadosomatic index in *C. dussumieri* during 2004-'05

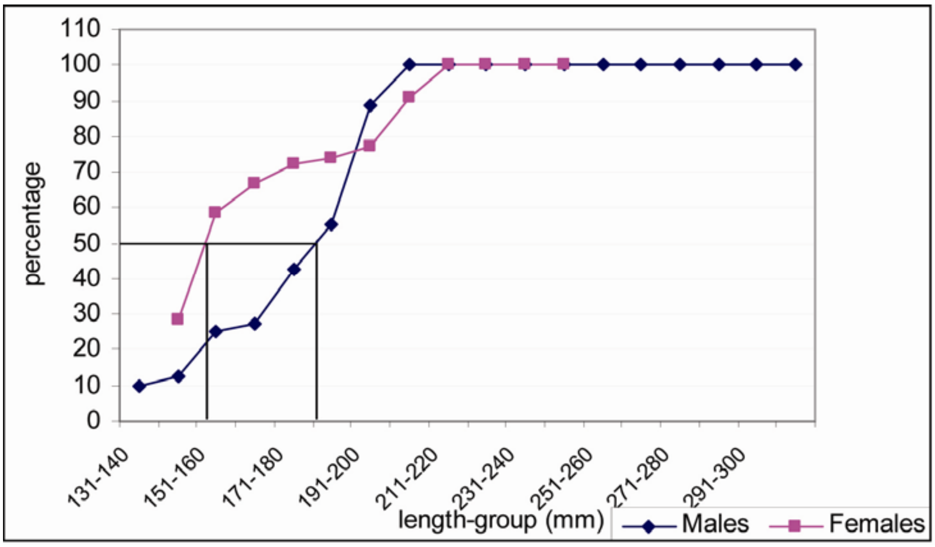


Fig.5.21. Percentage occurrence of mature males and females in *C. dussumieri*

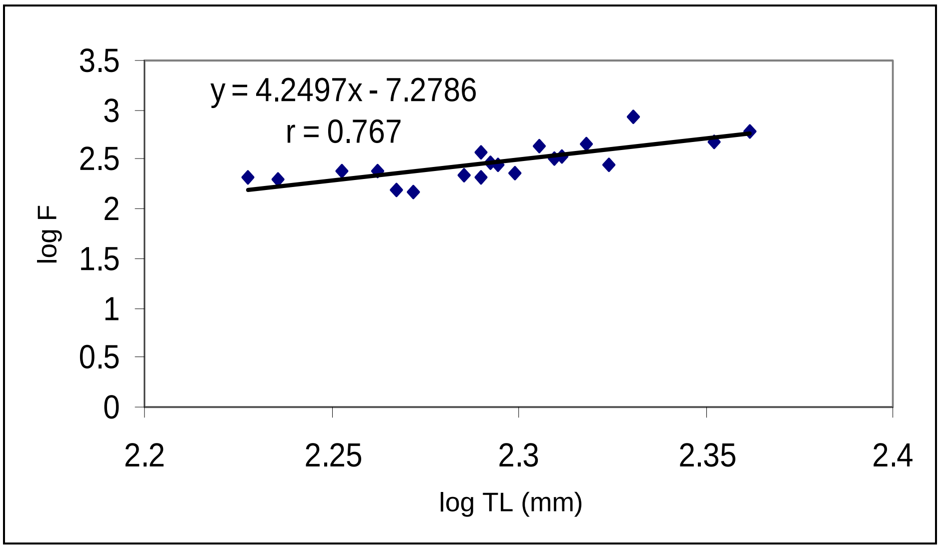


Fig.5.22. Relationship between fecundity and total length

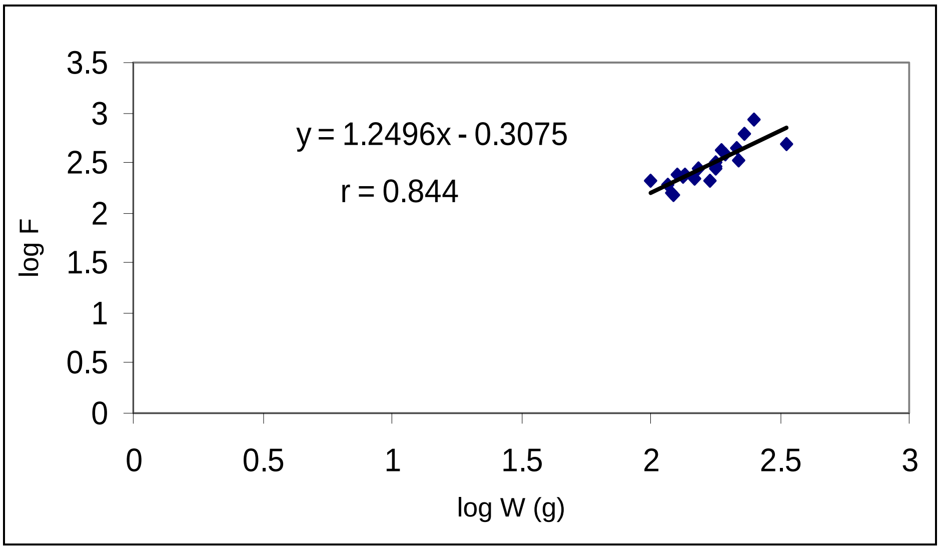


Fig. 5.23. Relationship between fecundity and body weight

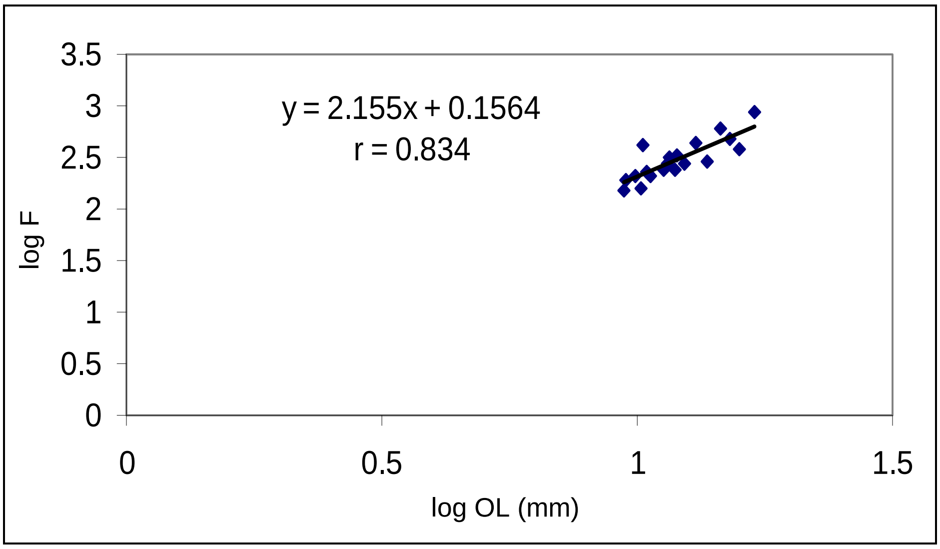


Fig. 5.24. Relationship between fecundity and ovary length

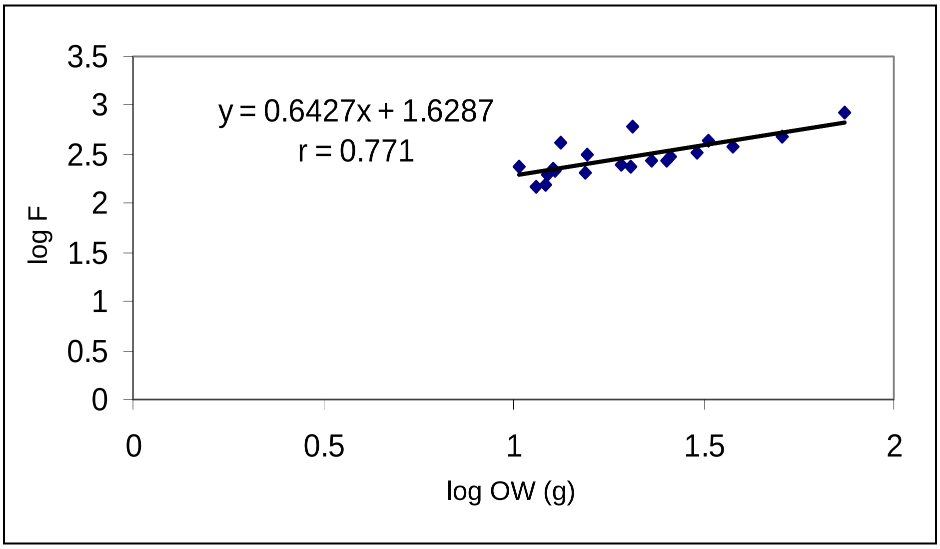


Fig. 5.25. Relationship between fecundity and ovary weight

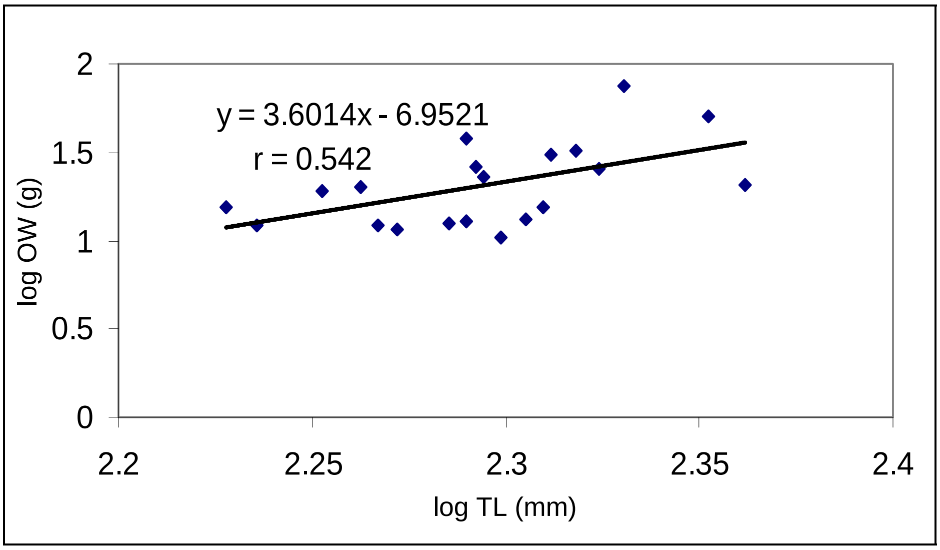


Fig. 5.26. Relationship between total length of fish and ovary weight

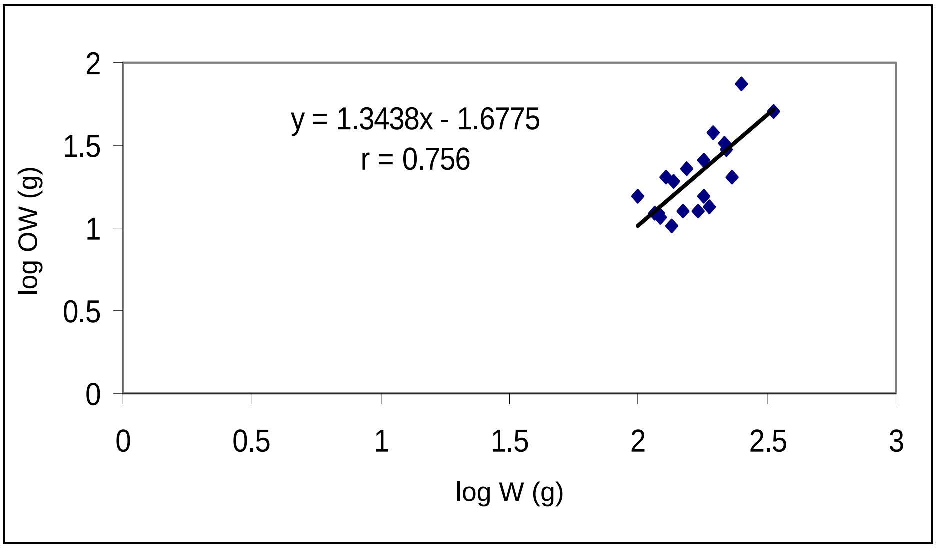


Fig. 5.27. Relationship between body weight of fish and ovary weight

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Chapter 6

AGE AND GROWTH

Contents

6.1 Introduction

With the growth, the fish attains age, and the age and growth are very closely related.

The determination of fish age and growth is fundamental in fisheries biology and management. Such age-determined parameters as mortality and growth underlie the population dynamics models used in fishery analyses. Age studies can furnish other basic data such as stock age structure, age at first maturity, spawning frequency, individual and stock responses to changes in the habitat, recruitment success, etc. Age and growth data also permit the determination of population changes due to fishing activities. According to Qasim (1973b), the main purpose of the study of growth is to determine the amount of fish that can be produced in terms of quantity (weight) in a body of water in relation to time.

One of the significant aspects for getting accurate data on fish biology and population dynamics is to determine the age with lowest error (Chalanchuk, 1984). There are two methods by which the age of fish can be determined. The first is the direct method, which is accomplished through direct measurements of growth rate of specific specimens extrapolated to the stock as a whole. By rearing the fish in artificial enclosures or by marking them in their natural environment and subsequent recapture (tag-recapture studies- Morales- Nin, 1992; Das, 1994) are the two direct estimation method. Age data estimated by this method is definite and reliable; furthermore, it generally establishes a basis for validation studies (Beamish and Mcfarlane, 1983). The marking method is limited in use due to it being costly and time-consuming.

The second one, indirect method is accomplished in two ways which are used as counterchecks to each other. One method in age determination is by Peterson's or the length-frequency analysis. However, it is considered a reliable method when the samples are representative of a fish population which has a short life, fast growth and reproduction once in a year (Casselman, 1987; Baker and Timmons, 1991). The other method used in the aging of fish is the examination of bony structures such as scales, otoliths, vertebral centra, dorsal and pectoral spines etc. (anatomical method) at least three times by multiple readers. Both methods have been widely employed for growth and ageing studies of temperate fishes and yielded good results. Until recently, these techniques were not employed for tropical fish growth studies.

Calcified structures have been used to estimate growth and age for a great diversity of fishes (Bagenal and Tesch, 1978; Pannella, 1980; Secor *et al.*, 1995). In temperate regions, growth checks on hard structures usually correlate with responses to changes in water temperature or photoperiod (Beckman and Wilson, 1995). At tropical latitudes, variation in temperature and photoperiod is less pronounced and it is presumed that tropical fish lack skeletal growth characters amenable to age and growth analyses. Consequently, there are few reported studies of tropical fish growth based on skeletal structures, and most of these are studies are on fishes of marine origin (Fowler, 1995).

Determination of age by examination of the hard anatomical structures is the preferred method as it is least prone to subjective interpretation and tagging artifacts (Brothers, 1982). In temperate regions, fisheries management largely relies upon this technique for determining age, and the otoliths have emerged as the most-often preferred anatomical structure (Bagenal, 1974; Manooch, 1982; Pentilla and Dery, 1988). Until recently, the methods were not used for tropical fish growth studies for two reasons. First, tropical fish were assumed to lack seasonal growth patterns. This was thought to result in poorly developed growth marks in the hard parts (Brothers, 1980). Second, tropical fishes were believed to lack seasonality in recruitment. Protracted recruitment would result in skewed and bimodal length distributions of cohorts. In the absence of a distinct recruitment season, it is difficult to employ the length frequency method for growth studies. However, Longhurst and Pauly (1987) pointed out that in most tropical fishes growth follows predictable seasonal patterns, which can be detected using length frequency data, or by the analysis of seasonal bands in the otoliths.

Among the numerous hard structures used for age estimation (scale, otolith, vertebrae, etc.), the otolith is generally considered to be the best for estimating the age of many teleost fishes (Campana and Thorrold, 2001). However, annulus formation in otoliths requires validation before it can be used to estimate the age of the fish (Beamish and McFarlane, 1983; Campana, 2001).

The physiological functions of fish follow dial environmental changes and individuals undergo cycles of rest and activity that can be registered on their otoliths (Radtke and Morales- Nin, 1989). Discontinuities in otoliths also appear to be related to ontogeny and habitat changes (Pannella, 1971). Otoliths continue to grow under conditions of food deprivation (Marshall and Parker, 1982; Campana, 1983; Neilson and Geen, 1984) while fish stressed by exertion (Campana, 1983) or by exposure to low pH (Geen *et al*., 1985) do not show evidence of otolith resorption. Otoliths are often the first calcified structures that appear during the early development of teleosts.

Otoliths have several advantages over scales and spines for estimating age because otoliths are not subjected to resorption and their growth is acellular rather than by ossification (Secor *et al*., 1995). Higher precision of age estimates from otoliths has led several authors to recommended otoliths over other structures for estimating age (Boxrucker, 1986; Sharp and Bernard, 1988; Hoxmeier *et al*., 2001).

Otoliths have been used to estimate age in fish such as channichthyids which lack scales (Kock and Everson, 1998). However, the growth rings are known to be more distinct and clear on the otolith than in other structure of the fish. Therefore, they are extensively used to determine the age in fishes (Jennings *et al*., 2001; David and Pancharatna, 2003). This method of aging is based on the fact that successive rings are formed as the fish grows in age. Moreover, otolith elemental analyses give an exact idea on the migratory behaviour of the fishes and environmental stress experienced by it due to pollution and / or other factors. Such data can be incorporated into the fishery management plans and appropriate conservation strategies can be put forth for those fishes facing serious threat of extinction (Radhakrishnan *et al*., 2009).

Since there is definite periodicity in the seasons of the temperate region, the growth rings or checks are more or less clear and easy to interpret. Similar growth checks have been noticed in hard skeletal parts of tropical and subtropical fishes and among the main references on the subject are the works of Radhakrishnan (1954, 1957), Seshappa and Bhimachar (1955), Pantulu and Singh (1962), Qasim (1973b), Buesa (1987), Fowler (1990), Seshappa (1999), Pilling *et al.* (2000), Ambrosio *et al.* (2003), Caldow and Wellington (2003), Pilling *et al.* (2003), Ismen (2005), Grandcourt *et al*. (2006), Nolf *et al.* (2006), Zekeria *et al.* (2006), Lin and Tzeng (2009) and Shamsan and Ansari (2010a). This work represents the first comprehensive study on age, growth, and mortality of a population of *C. dussumieri* from Cochin estuary based on age estimates from otoliths and contributes to management of these stocks.

6.2 Otolith

The use of otoliths as indicators of fish age has now reached the century landmark, starting with Reibisch's observations of otolith annuli in 1899. In 1971, Pannella's discovery of daily growth increments helped propel the interpretation of otolith microstructure into the mainstream of fish biology.

Otoliths ("ear stones") are structures located in the inner ear cavity of all teleost fish. All teleost fishes have three pairs of otoliths: the *asteriscae, lapillae* and *sagittae*. The sagitta is the largest of the three otoliths and is generally used in studies of age estimation in teleosts. Each sagittal otolith is enclosed in a fluid filled sac called the sacculus within the otic capsule of the head. The otic capsules are situated on either side of the posterior portion of the brain. Otoliths are acellular and avascular. Otoliths are complex polycrystalline bodies, composed of needle shaped crystals of calcium carbonate in the form of aragonite, radiating outwards in three dimensions from a centrally located nucleus and passing through a network of fibrous collagen like protein called otolin. Otoliths act as sound receptors and also play a role in balance and orientation. Otolith size and shape, particularly of the sagittae, varies greatly among species. Size and shape of the sagittae are related to their function of sound detection in fish (Popper and Lu, 2000).

Seasonal changes in the fish's growth rate are reflected in the otolith. Materials are deposited on the otolith from the endolymph, the fluid that surrounds the otolith. The deposits are formed in bands of alternating optical density, which appear either opaque or hyaline (translucent) under reflected light. The alternating zones on the otolith are due to the differences in the amount of protein (otolin) in the zones and shape of the aragonite crystals. The opaque zones in the otoliths are formed during the period of fast growth and the hyaline zone is laid down usually during the period of slow growth. These zones are also called rings.

6.3 Materials and methods

A total of 351 individuals (199 males: 63 mm – 305 mm TL and 152 females: 82mm -246 mm TL) of *Colletteichthys dussumieri* were collected from Cochin estuary using gill net (55 mm, 70 mm) and hook and line between October 2004 and September 2005. Total length (TL, mm), standard length (SL, mm) and total weight (W, 0.1 g) of each individual were measured. The individuals were sexed by macroscopic examination of the gonads as males and females. The otoliths were removed from the cranium by means of a transverse incision through the head using a sharp knife. Once the incision has been made, and the semi-circular canals exposed, the sagitta was removed with a fine forceps. Sagittal otoliths were removed, cleaned and stored dry in code-numbered vials.

Otoliths were placed in glycerine for one to two weeks to clear the otolith which will allow for improved reading. The left sagitta was mainly used for age determination; the right sagitta was used only if the left one was damaged. Otoliths were placed in water and were observed with a compound microscope (4x - 10x) using reflected light on a black background. Otolith exhibited alternating opaque (broad, white) and hyaline or translucent (narrow, clear) bands. The distal portion of each translucent zone was considered the annulus. The areas of complete growth for each year were represented by the combination of one opaque zone and a subsequent translucent zone. The total number of translucent zones was recorded in order to assign an estimated age to each fish. The linear measurements of each otolith were taken with an ocular micrometer and it included: the otolith length (OL: the longest dimension between the anterior and posterior edges), the dorsal otolith radius (OR: the dimension from the focus (F) to the dorsal edge), the width (WL: the dimension from the dorsal to the ventral edge) and the distance from the focus to the outer margin of each translucent zone (ring radius, r_n) on the dorsal radius (Fig. 6.1). All measurements were made with the concave side of otoliths facing down, to ensure that the anterior and posterior edge of the otolith were in the same focal plane. Otoliths were weighed (OW) to the nearest 0.01 mg. Each otolith was examined thrice at different times and an accurate count was taken for the analysis of growth characteristics.

The relationship between fish total length-at- capture (TL_c), otolith length (OL), and otolith weight (OW) were statistically assessed using regression analysis and calculating the correlation coefficient 'r' (Zar, 2005). The relationship of fish total length-at-capture (TL_c) and otolith radius- at- capture (OR_c) were analyzed with linear regression using untransformed data. The linear equation was $TL_c = a + b\ OR_c$.

Characterization of the otolith edge was used for inferences on the period of annulus formation (Newman and Dunk, 2003). Marginal increment analysis was used to validate the periodicity of the formation of the growth increments as well as the number of rings formed. The proportion of samples with opaque / translucent margins was calculated for each month and used to infer the timing and periodicity of increment formation. The marginal increment ratio (MIR) is expressed as the following equation and was used to establish the period of ring formation:

$$MIR = (R_C - R_L) \times (R_L - R_{L-1})^{-1}$$

Where,

R_C = otolith radius

R_L = distance from the focus to the outer edge of outermost transparent zone

R_{L-1} = distance from the focus to the outer edge of the penultimate translucent zone.

The mean MIR and the standard deviation were computed for each month by sex for all ages combined and also for each age separately. An analysis of variance (ANOVA) was used to detect significant differences by sex, age group and month of capture. The Post- Hoc multiple comparison test was used to detect which pair wise differences among treatments were significant. Means (± S.D.) were plotted against month of capture, the minima indicating the month of annulus formation.

The back-calculated total lengths at each age were determined from the body proportional equation (Francis, 1990):

$$L_A = [(a+bR_A)/\ (a+bR_C)]\ L_C$$

Where,

L_A = back- calculated total length to annulus A

R_A = otolith radius to annulus

R_C = total otolith radius at a time of capture

L_C = total length at time of capture

a = intercept from the linear total length-otolith radius regression

b = slope from the linear total length- otolith radius regression.

Theoretical growth parameters for females and males were estimated by fitting the back-calculated length-at-age to the von Bertalanffy (1938) growth function (VBGF).

$$L_t = L_\infty\ [1\text{-exp}\ (-K\ (t\text{-}t_0))]$$

L_t = Length at age t

L_∞ = Predicted asymptotic length (maximum length which the fish tend to attain)

e = base of Neparian or natural logarithm

k = Brody growth coefficient, a measure of the rate at which length approaches L_∞

t = age of the fish

t_o = arbitrary origin of growth curve or theoretical age when fish length = 0.

Growth parameters (L_∞, K and t_0) were estimated graphically by plotting the Ford (1933) - Walford (1946) graph as described in Ricker (1975).

Differences in von Bertalanffy growth curves were tested by means of the Chow test (Chow, 1960). This test is an application of the F-test, commonly used to test structural change in a regression model attributable to variations in some of or all the parameters. In the case of two groups, it requires the sum of squared errors from three regressions, one for each group (ess_1 and ess_2) and one for the pooled data (ess_c pool). Thus,

The formula for the "Chow test" of this constraint is

$$F = \frac{\dfrac{\text{ess\_c} - (\text{ess\_1} + \text{ess\_2})}{k}}{\dfrac{\text{ess\_1} + \text{ess\_2}}{\text{N\_1} + \text{N\_2} - 2 * k}}$$

Where,

ess_1 and ess_2 are the error sum of squares from the separate regressions

ess_c is the error sum of squares from the pooled (constrained) regression,

k is the number or estimated parameters

N_1 and N_2 are the number of observations in the two groups.

Longevity was calculated from Taylor's equation (Taylor, 1958):

$$A_{0.95} = t_0 + \frac{2.996}{K}$$

Where $A_{0.95}$ is the lifespan or age required to reach 95% of the final length (L_∞), and K and t_0 are von Bertalanffy growth parameters.

The growth performance index (Φ') of *C. dussumieri* was computed according to the formula of Pauly and Munro (1984) as:

$$\Phi' = \text{Log}_{10}\,K + 2\,\text{Log}_{10}\,L_{\infty}$$

Where, K is the growth constant and L_{∞} is the asymptotic length derived from the von Bertalanffy growth curve.

Natural mortality (M) was estimated based on Taylor's formulae (Taylor, 1958)

$$M = \frac{2.996K}{2.996 + Kt_0}$$

Where, K and t_0 are parameters of the von Bertalanffy growth equation.

6.4 Results

6.4.1 Relationship between total length and various parameters (total length, weight and radius) of otolith

The relationship between fish total length at capture and length and weight of the otolith for both sexes is illustrated in Fig. 6.1a - Fig. 6.1b and Fig.6.2a – Fig.6.2b.

The regression analysis based on least square method revealed that there was a highly significant association between total length of fish and length and weight of the otolith of male ($r = 0.926$ and 0.868, $p < 0.001$) and female ($r = 0.898$ and 0.804, $p < 0.001$) respectively. The regression equations were given as:

Male

$$Y\,(T.L_C) = -22.728 + 38.954\,X\,(O.L)$$

$$Y\,(T.L_C) = 128.63 + 2775.7\,X\,(O.W)$$

Female

$Y\ (T.L_C) = -12.065 + 34.742\ X\ (O.L)$

$Y\ (T.L_C) = 130.69 + 2345.2\ X\ (O.W)$

Where $T.L_C$ is total length- at- capture, O.L is length of otolith and O.W denoted the weight of otolith.

Somatic growth ($T.L_C$) and otolith growth (O.L) were strongly linearly related in both males ($R^2 = 0.858$) and females ($R^2 = 0.806$).

Fig.6.3a. and Fig.6.3b. shows the sex-specific relationship between fish total length -at- capture ($T.L_C$) and otolith radius -at- capture ($O.R_C$).

Male $Y\ (T.L_C) = 28.949 + 71.877\ X\ (O.R_C)$ $r = 0.942, R^2 = 0.8891$

Female $Y\ (T.L_C) = 34.585 + 66.154\ X\ (O.R_C)$ $r = 0.903, R^2 = 0.815$

6.4.2 Growth check on otolith

Otolith displayed well-defined alternating thin translucent zones and wide opaque zones under reflected light (Fig.6.4, Fig.6.4a – Fig.6.4i). An opaque zone combined with a translucent zone was interpreted as one year’s growth. Formation of growth increments followed a seasonal pattern (Fig.6.5a - Fig.6.5c). The translucent zones begin to develop in June and continued until February and the proportion of otolith with translucent margins was highest in September to November, coinciding with peak spawning (chapter 5). A marked decline in January and February, followed by an absence of translucent margins from March to May indicates cessation of translucent annulus formation and the onset of opaque annulus formation beginning in June. The opaque zones were laid down mainly from February to June when growth is faster. The data suggest that only one opaque and one translucent zone are laid down per year and represent valid annual growth increments.

6.4.3 Marginal increment analysis

The changes with time of the rate of marginal increment $(R_C - R_L)$ x $(R_L - R_{L-1})^{-1}$ were measured to determine the time of annulus formation. The monthly mean MIR (sexes and all ages combined) showed a single minimum in September to November (Fig.6.6a - Fig.6.6c). The spawning of this fish, judged from monthly changes in gonad indices, lasts from September to January, with the highest intensity in October and November. This pattern indicated the formation of one annulus per year in September to October. Similar pattern was exhibited by monthly means of MIR when plotted by sex, and for the different ages respectively. ANOVA detected significant differences during the year (df = 11, 339; $p<0.001$) (Table 6.1.) and for age-group (df = 8, 342; $p<0.001$) (Table 6.2.). The Post-Hoc Test showed that the MIR in September – November was significantly lower than the rest of the year, providing further evidence that annulus formation occurred yearly in September – November (Table 6.4). Comparison of MIR between age groups by means of Post Hoc Turkey HSD test revealed differences between age groups 3 and 4 (Table 6.5). The t-test did not show significant differences for the sexes. The number of annuli represents the years from birth.

Age distributions for the sexes are exhibited in Fig.6.7. The maximum age of the sampled toadfish was 9 years for males and 7 years for females. In the present study, females less than 2 years were poorly represented in the sample. Age distribution was almost similar for both sexes and was skewed to the left. The population was dominated by 3 to 5 year old fishes. Fishes younger than 2 years and older than 6 years were poorly represented. After age 4, males dominated the older age classes.

6.4.4 Back-calculated total length

The mean back – calculated lengths – at –age for 1 to 9 year old specimens are listed in Table 6.6a and 6.6b. Growth increments were higher during the first year of life (mean 39.9 mm and 41.0 mm L_T for males and females, respectively). After that, the growth rates of both sexes slowed appreciably. Males and females showed similar mean lengths until age 3; afterwards, males grew faster and consequently reached larger sizes than females. The differences become more pronounced with increasing age (Table 6.6a - Table 6.6b); Fig.6.9).

6.4.5 Estimation of growth parameters

Table 6.7. shows the various parameters of VBGE. The Various parameters were found to be $L\infty = 351$ mm and $t0 = -0.81$ and $k = 0.168$ /year and $L\infty = 303$mm and $t0 = -0.65$ and $k = 0.216$ /year were obtained by graphical method (Fig. 6.8a - Fig. 6.8d) for males and females respectively. The von Bertalanffy growth parameter estimates are listed in Table 6.7.

The von Bertalanffy (1938) growth equations, fitted to back-calculated lengths- at- age for the last annulus were

Male : $L_t = 351\ [1\text{-}e\ (-0.168(t-(-0.81))]$
Female : $L_t = 303\ [1\text{-}e\ (-0.216(t-(-0.65))]$

The theoretical length at different ages as calculated by this equation showed a very close agreement with those estimated by otolith method (back-calculated lengths) (Table 6.8). Subsequently, the growth curve of *C. dussumieri* is shown in Fig.6.9.

Results of the Chow test comparing growth curves among sexes reveal that growth profiles were significantly different between sexes (F= 12.60866 ; df= 2, 357; P<0.001) of the specie (Table 6.9).

The estimated life span (longevity) of the investigated species was 17.02 years and 13.2 years for males and females respectively. The value of the growth performance index for the specie was 4.32 and 4.30 for males and females respectively (Table 6.10).

Estimates of natural mortality indicated that females (0.23) had higher mortality rates than males (0.18) (Table 6.10).

6.5 Discussion

In the present study, sagittal otoliths were determined to be valid structures for age and growth studies in *C. dussumieri.* They satisfy the criteria stressed by Van Oosten (1929) and Williams and Bedford (1974):- a) Otoliths can readily be located and removed, and are relatively easy to prepare, store and read; b) a recognizable pattern can be seen in the otoliths; c) growth of the otoliths was proportional to the overall growth of the fish; d) the number of growth zones increased with an increase in fish size and marking pattern was similar for fishes of different ages; e) growth check marks formed once a year and at approximately the same time; and f) back-calculated lengths-at- age agree with observed lengths-at-capture.

Otoliths were successfully used by Wilson *et al*., (1982) and Radtke *et al*., (1985) to assess age structure in the oyster toadfish *Opsanus tau*. Radtke considered otoliths as useful structures for age and mortality estimations in this species. Studies on the age and growth of Gulf toadfish (*Opsanus beta)* (Malca *et al*., 2009) and *Halobatrachus didactylus*

(Palazon-Fernandez *et al*., 2010) revealed that sagittal otoliths as valid structures for age and growth studies, similar to the results found in *Opsanus tau* (Wilson *et al*., 1982). In *C. dussumieri* otoliths exhibited a well- defined and consistent mark pattern consisting of one narrow translucent zone deposited during slow growth and one broad opaque white zone formed during fast growth.

Ring validation in age and growth studies is essential in regions where climatic conditions are less variable, resulting in less clear otolith marks (Beamish and McFarlane, 1983; Jepsen *et al*., 1999). The timing of annulus formation in tropical fishes is not well understood, but Fowler (1995) noted that most tropical marine species share a pattern similar to temperate fishes; fast growth synchronous with opaque zone deposition. Although the difficulty of resolving the margins of otolith increments for MIR analysis may influence its validity (Campana, 2001), it has been useful for validating the annual periodicity of the annulus in numerous fish species (Takita *et al*., 1993; Colloca *et al*., 2003; Boudaya *et al*., 2008; Lin and Tzeng, 2009; Palazon-Fernandez *et al*., 2010). Annual deposition of an opaque and a hyaline band in the otoliths of tropical fishes has been recorded for *Lutjanus kasmira* (Morales-Nin and Ralston, 1990), surgeonfishes, parrotfishes (Choat and Axe, 1996) and *chaetodon larvatus* (Zekeria, 2006). In reviews of otolith studies in tropical latitudes, Beckman and Wilson (1995) found that opaque zone deposition occurs during period of increased growth, whereas the corresponding translucent zone is formed during periods of low metabolic activity. The marginal increment analysis in this study showed that one opaque and one translucent zone are formed each year. The thin translucent bands, considered annuli, corresponding to the period of slow growth begins in June and is completed in October - November, coincident with peak spawning. Translucent zone formation during slow growth has been reported for sagittae of several other teleosts (Rao, 1966; Jayaprakash, 1976; Sunder and Subla, 1984; Brothers and Mathews,1987; Admassu and Casselman, 2000; Waldron and Kerstan, 2001; Yoneda *et al.*, 2002; Colloca *et al.*, 2003; Boudaya *et al.*, 2008).

Otolith growth and zone formation might be under the control of a combination of factors (environmental variation and physiological effects) which may vary among species (Pannella, 1971; Morales-Nin, 2000). Apposition marks in bony structures of tropical species has been associated with the reproductive process (Bowering, 1978). Admassu and Casselman (2000) interpreted the formation of translucent bi-annuli in otoliths of young *Oreochromis niloticus* to coincide with minimum water temperature, spawning associated loss in condition and presumably with reduction in the quantity and quality of the food consumed by the fish. Gjoesaeter *et al.* (1984) and Jepsen *et al.* (1999), analyzing tropical fish, related the existence of growth synchronization and spawning to reduced growth rates. In tropical latitudes, temporal matching of the floods and environmental factors associated with the reproductive process and its influence on the rate of food consumption makes it difficult to identify the factor responsible for ring formation. Even when ring formation occurs outside of the spawning period, it has been at least partially caused by reproductive cycle demands (Ambrosio *et al.*, 2003). Gonadal maturation, fall in water temperature and parental care are the main determinant factors of slow growth for *Gymnogeophagus lacustris* (Hartz *et al.*, 1998). The periodicity of translucent zone formation and its relation with reproductive activity observed in *C. dussumieri*, have also been reported in other fish species such as the 'ghol', *Pseudosciaena diacanthus* (Lacepede)

(Rao, 1966); two-banded seabream, *Diplodus vulgaris* (Pajuelo and Lorenzo, 2003); spotted seatrout, *Cynoscion nebulosus* (Ihde and Chittenden, 2003); spotted goatfish, *Pseudupeneus maculatus* (Santana *et al*., 2006); striped mullet, *Mugil platanus* (Castro *et al*., 2009). A possible explanation for this would be that, during the spawning season, energy may be allocated primarily for gamete production instead of somatic growth. Further more, during the spawning season male toadfishes may decrease their feeding activity while they guard nests and take care of the eggs and fry. This hypothesis, although plausible for adult fish, cannot explain the formation of annuli in young immature fish. So annulus formation is not necessarily a direct consequence of the reproductive activity, and must also be related to seasonal patterns of growth rather than reproduction. Similar observations are reported in other toadfish species like *Halobatrachus didactylus* (Palazon-Fernandez *et al*., 2010 and *Opsanus beta* (Barimo *et al*., 2007; Malca *et al*., 2009). Many factors other than spawning can affect growth rates of fish, including seasonal changes in environmental factors, mainly water temperatures, photoperiod, feeding regime and related effects on metabolism, as observed in other fish species (Campana and Neilson, 1985; Campana, 1984; Morales – Nin and Ralston, 1990; Bullock *et al*., 1992; Newman *et al.,* 2000; Pajuelo and Lorenzo, 2003). Present results also imply that translucent bands coincide with a period of decrease in temperature, reduced feeding (chapter 4) and lower body condition (depletion of peritoneal fat reserves) (Chapter 7 and 8).

In some fish populations, when growth over the lifetime is measured, stanzas are observed, with sudden changes in growth rate between the stanzas. The most commonly reported growth rate stanzas are those related to larval metamorphosis, with different growth patterns, pre- and post metamorphosis. Other well-described stanzas are related to physiological change or to maturation (Saborido-Rey *et al*., 2004). In *C. dussumieri*, the growth rate stanzas precede and follow an age of 3 or 4 years (as revealed from Table 6.5 and Fig.6.9). This can be attributed to maturation and physiological change. In redfish, *Sebastes mentella*, the growth rate stanzas were observed at an age of 5 or 6 years. Saborido-Rey *et al.* (2004) related the alteration to progressive shift from a more pelagic to a more demersal habitat with age and to change in feeding behaviour.

Of the 200 males and 157 females sampled, males were predominant in specimens greater than 5 years old. The data also suggest a difference in maximum length and age attained by the sexes. The oldest male collected was 9 years old, whereas the oldest female was 7 years old. Examination of the otoliths of oyster toadfish in South Carolina, revealed that ages ranged from <1 to 8 year, with a median age of 3 year (Wilson *et al*., 1982). The estimated ages of males and females of *Opsanus beta* ranged from <1 year to 6 and 5 years, respectively (Malca *et al.,* 2009). Schwartz and Dutcher (1963) and Radtke *et al*. (1985) in *Opsanus tau* and Palazon-Fernandez *et al*. (2010) in *Halobatrachus didactylus* reported maximum ages of 12 and 9 years for males and females toadfishes respectively. Radtke *et al*. (1985) concluded that the differences in sex-specific numbers by age for *O. tau* resulted from females experiencing a higher mortality rate as a result of the increased energy investment for gametes in females.

Toadfishes are medium-lived fishes, and the maximum age for each sex must be near the maximum age for the species owing to the low fishing pressure on the population. *C. dussumieri* reach sexual maturity at 131 mm TL for males and 141 mm TL for females (refer Chapter 5), which correspond to 2 and >2 years of age for males and females respectively. Similarly, *Halobatrachus didactylus* reach sexual maturity at 160 mm TL for males and 191 mm TL for females, (Palazon-Fernandez *et al*., 2001), which correspond to 2 and 3 years of age for males and females respectively (Palazon-Fernandez *et al*., 2010). Gudger (1910) confirmed that toadfish do not spawn until age 2. Similarly Wilson *et al*. (1982) reported that *O. tau* became sexually matured between 2 to 7 years.

Present study revealed that the longevity of *C. dussumieri* males was greater than that of the females and the males attained a larger size than females. Similar phenomenon was observed for other batrachoid species. Schwartz and Dutcher (1963) and Radtke *et al*. (1985) found similar differences between the sexes for *O. tau* caught in Maryland and York river respectively, with females growing at a lower rate and attaining younger ages than males. Wilson *et al*. (1982) noted no sex related differences in growth for a population of *O. tau* in South Carolina, although they found differences in maximum age attained by the sexes, which were lower for females. Palazon-Fernandez *et al*. (2010) also established similar differences in length between the sexes in *Halobatrachus didactylus*. He opined that the slowed growth observed in females compared with males is likely due to differences in energy budgets during the reproductive season. Ota *et al*. (2010) observed similar male-biased sexual size dimorphism in cichlid, *Lamprologus callipterus*. Sexual size dimorphism (SSD) is common in both plants and animals, and it reflects the adaptation of males and females to their different reproductive roles (Fairbairn, 1997).

Age validation is essential to accurate age estimation and for accurate age interpretation, both otolith and fish growth must exhibit a continuous relationship (Fowler and Short, 1998). However, the relationship between otolith growth and somatic growth is often complicated by factors such as ambient temperatures and available food resources. The results obtained using the back-calculation method are very satisfactory and demonstrate the validity of using otoliths for estimating the growth of the flat toadfish. As ring formation is regular, the otoliths can be used for age determination and because the fish length and otolith size are closely correlated, it is judged as valid to permit the use of measurements to previously formed marks to back calculate the growth history (Campana, 1990; Francis, 1990). Similar results were reported for *O. tau* by Radtke *et al.* (1985) and for *Halobatrachus didactylus* by Palazon-Fernandez *et al.* (2010). They found a strong association between otolith dimension and body growth and stated that otolith length could be chosen as the best parameter for estimation of fish total length.

A method of validating growth parameters involves the comparison of growth performance index (Φ') in terms of growth in length with other estimates obtained for the same or a closely similar species (Gayanilo and Pauly, 1997). The von Bertalanffy growth parameter estimates of *C. dussumieri* obtained in the present study were compared with the status of those of other batrachoididae recorded in the previous studies (Table 6.11). The growth performance index of *C. dussumieri* obtained in the present study falls within the values mentioned of those estimated for other members of the batrachoid family. Even though differences can be observed between the population parameters, the similarity of the Φ' values indicates the existence of a similar growth pattern. According to Pauly and Munro (1984), related species present similar values of Φ' and each taxa may have a particular distribution of values, different from other taxa, and can be described by its mean value, i.e. the value of this parameter for species from a particular family should be closer to its average than to the average value of another family. The parameter $\Phi' = \mathrm{Log}_{10} K + 2\ \mathrm{Log}_{10} L_\infty$ can be used as it has been found that similar species with different growth parameters can have similar Φ' estimates.

Moreover, differences in growth patterns can be the result of differences in genetic structure and / or differences in temperature, density of food and diseases (Pauly, 1994; Wootton, 1990).

The values of natural mortality (M) of *C. dussumieri* obtained in the present study were similar to the figures recorded for other batrachiod species in the previous studies (Table 6.11) except for *O. tau* females, which have a higher mortality (M = 0.41) than males of the same species, and to the estimations made for the rest of the batrachoids. The present study estimated a higher natural mortality rate for females than for males. This further substantiated the studies of Radtke *et al.* (1985, *O. tau*) and Palazon-Fernandez *et al.* (2010, *Halobatrachus didactylus*). Although variation in mortality can be explained by such diverse factors as age, density, illnesses, parasites, food supply, abundance of predators, water temperature, fishery pressure, sex and size (Vetter, 1988), according to Palazon-Fernandez *et al.* (2010), this may be caused by increased predation due to their smaller size, to behavioural or distributional differences that make them more susceptible to predation or fishing effort, or to the existence of higher environmental pressures acting on the females. Another possibility is that they simply reach senescence before males.

In conclusion, whole–view otolith examination can provide a precise method for determining age and back-calculated lengths of *C. dussumieri*. This study provides the first detailed estimates of age, growth and mortality rate for the specie. Future research should focus on the environmental and internal factors affecting their growth.

Table 6.1. Analysis of variance on monthly mean MIR of *C. dussumieri*

Descriptives (Monthwise) MIR

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
October	39	.0474	.09792	.01568	.0157	.0792	.00	.37
November	25	.1559	.16940	.03388	.0860	.2259	.00	.70
December	9	.1935	.20088	.06696	.0390	.3479	.00	.50
January	17	.2114	.17093	.04146	.1235	.2993	.00	.56
February	34	.2062	.22204	.03808	.1287	.2836	.00	1.08
March	50	.2100	.17347	.02453	.1607	.2593	.00	1.08
April	27	.3266	.19944	.03838	.2477	.4055	.03	.83
May	61	.3662	.22455	.02875	.3087	.4237	.05	.99
June	10	.4748	.30762	.09728	.2548	.6949	.10	1.13
July	20	.5332	.39310	.08790	.3492	.7172	.00	1.25
August	18	.3163	.22749	.05362	.2032	.4294	.00	.67
September	41	.1229	.19591	.03060	.0611	.1847	-.06	.67
Total	351	.2447	.24525	.01309	.2190	.2705	-.06	1.25

ANOVA MIR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.844	11	.531	11.844	< .001
Within Groups	15.207	339	.045		
Total	21.052	350			

Significant

Table 6.2. Analysis of variance on age group mean MIR of *C. dussumieri*

Descriptives (age –group) MIR

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	10	.1454	.09812	.03103	.0752	.2156	.03	.31
2.00	25	.1648	.15982	.03196	.0989	.2308	.00	.75
3.00	90	.1888	.17903	.01887	.1513	.2263	-.06	.69
4.00	93	.3321	.24556	.02546	.2815	.3827	.00	1.11
5.00	76	.2194	.25630	.02940	.1608	.2779	.00	1.08
6.00	30	.2656	.32197	.05878	.1453	.3858	.00	1.25
7.00	10	.1962	.32487	.10273	-.0362	.4286	.00	1.00
8.00	8	.2833	.39271	.13884	-.0451	.6116	.00	1.13
9.00	9	.3978	.23230	.07743	.2192	.5763	.00	.83
Total	351	.2447	.24525	.01309	.2190	.2705	-.06	1.25

ANOVA MIR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.558	8	.195	3.416	.001
Within Groups	19.494	342	.057		
Total	21.052	350			

Significant at 0.1% level

Table 6.3. T- test on MIR of males and females of *C. dussumieri*

	Sex	N	Mean	Std. Deviation	t	df	Sig. (2-tailed)
MIR	**Males**	199	.2348	.24576	-.863	349	.389
	Females	152	.2577	.24478			

Not Significant

Table 6.4. Post Hoc Test - Multiple Comparisons to test differences in monthwise variation in MIR of *C. dussumieri*

Dependent Variable: MIR
Tukey HSD

(I) Month	J) Month	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
October	November	-.1085	.05426	.693	-.2871	.0701
	December	-.1460	.07832	.780	-.4038	.1117
	January	-.1640	.06155	.249	-.3666	.0386
	February	-.1587	.04970	.066	-.3223	.0048
	March	-.1626(*)	.04525	.019	-.3115	-.0137
	April	-.2792(*)	.05303	.000	-.4537	-.1047
	May	-.3188(*)	.04342	.000	-.4617	-.1758
	June	-.4274(*)	.07507	.000	-.6745	-.1803
	July	-.4858(*)	.05825	.000	-.6775	-.2941
	August	-.2689(*)	.06035	.001	-.4675	-.0703
November	September	-.0755	.04737	.911	-.2314	.0804
	October	.1085	.05426	.693	-.0701	.2871
	December	-.0375	.08233	1.000	-.3085	.2335
	January	-.0555	.06658	1.000	-.2746	.1636
	February	-.0502	.05580	.999	-.2339	.1334
	March	-.0541	.05188	.997	-.2248	.1166
	April	-.1707	.05879	.144	-.3642	.0228
	May	-.2102(*)	.05030	.002	-.3758	-.0447
	June	-.3189(*)	.07925	.004	-.5797	-.0581
	July	-.3773(*)	.06354	.000	-.5864	-.1682
	August	-.1604	.06547	.376	-.3758	.0551
	September	.0330	.05374	1.000	-.1438	.2099
September	October	.0755	.04737	.911	-.0804	.2314
	November	-.0330	.05374	1.000	-.2099	.1438
	December	-.0706	.07796	.999	-.3271	.1860
	January	-.0885	.06110	.953	-.2896	.1126
	February	-.0833	.04913	.870	-.2450	.0784
	March	-.0871	.04462	.725	-.2340	.0597
	April	-.2037(*)	.05249	.007	-.3765	-.0310
	May	-.2433(*)	.04277	.000	-.3840	-.1025
	June	-.3519(*)	.07470	.000	-.5978	-.1061
	July	-.4103(*)	.05777	.000	-.6004	-.2202
	August	-.1934	.05989	.060	-.3905	.0037

* The mean difference is significant at the .05 level.

Table 6.5. Post Hoc test - Multiple comparisons to test the differences in MIR of age groups of *C. dussumieri*

Dependent Variable: MIR
Tukey HSD

(I) Age	(J) Month	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3.00	**1.00**	.0434	.07958	1.000	-.2050	.2918
	2.00	.0240	.05398	1.000	-.1445	.1925
	4.00	**-.1433(*)**	.03530	.002	-.2535	-.0331
	5.00	-.0306	.03719	.996	-.1467	.0855
	6.00	-.0768	.05033	.843	-.2339	.0804
	7.00	-.0074	.07958	1.000	-.2558	.2411
	8.00	-.0944	.08808	.978	-.3694	.1805
	9.00	-.2090	.08347	.234	-.4695	.0516
4.00	**1.00**	.1867	.07945	.316	-.0614	.4347
	2.00	.1672	.05379	.052	-.0007	.3351
	3.00	**.1433(*)**	.03530	.002	.0331	.2535
	5.00	.1127	.03692	.061	-.0025	.2280
	6.00	.0665	.05013	.923	-.0900	.2230
	7.00	.1359	.07945	.740	-.1121	.3839
	8.00	.0488	.08797	1.000	-.2258	.3234
	9.00	-.0657	.08334	.997	-.3259	.1945

* The mean difference is significant at the 0.05 level.

Table 6.6a. Mean observed and back-calculated lengths-at-ages for male *C. dussumieri*

Age class (years)	N	Observed Mean L_T	Annulus								
			I	II	III	IV	V	VI	VII	VIII	IX
1	10	87.1	79.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	11	129.2	85.6	123.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	35	175.2	93.2	133.2	168.7	0.0	0.0	0.0	0.0	0.0	0.0
4	42	213.2	92.4	135.5	173.3	206.2	0.0	0.0	0.0	0.0	0.0
5	48	233.2	91.5	131.4	165.4	197.3	227.3	0.0	0.0	0.0	0.0
6	27	247.1	92.0	130.8	162.7	191.1	217.2	232.4	0.0	0.0	0.0
7	9	263.3	92.2	128.0	154.6	181.7	209.0	234.1	258.7	0.0	0.0
8	8	271.4	93.1	120.5	148.3	174.4	199.2	225.4	244.6	265.5	0.0
9	9	292.3	96.6	127.8	151.4	176.4	197.7	222.1	244.4	264.5	284.7
Mean			91.4	131.3	165.5	195.1	218.1	229.9	249.4	265.0	284.7
Increment				39.9	34.2	29.7	23.0	11.8	19.5	15.6	19.7

Table 6.6b. Mean observed and back-calculated lengths-at-ages for female *C. dussumieri*

Age class (years)	N	Observed Mean L_T	Annulus						
			I	II	III	IV	V	VI	VII
1	5	86.2	78.7	0.0	0.0	0.0	0.0	0.0	0.0
2	14	123.4	83.7	118.2	0.0	0.0	0.0	0.0	0.0
3	55	172.2	88.1	130.3	166.3	0.0	0.0	0.0	0.0
4	51	202.4	90.5	132.2	167.2	192.3	0.0	0.0	0.0
5	27	221.6	89.5	128.8	160.7	185.1	216.5	0.0	0.0
6	4	233.0	90.4	121.6	156.1	178.3	208.5	230.6	0.0
7	1	246.0	92.7	115.6	145.6	166.5	191.4	217.8	246.0
Mean			88.3	129.3	165.1	189.0	214.8	228.1	246.0
Increment				41.0	35.8	23.9	25.7	13.3	17.9

Table 6.7. Growth parameters derived from the von Bertalanffy growth function

Parameter	Males	Females
L_∞	351	303
k	0.168	0.216
t_0	-0.81	-0.65
n	200	157

Table 6.8. Mean observed, back-calculated (B-C) and theoretical total lengths for male and female

Age	Males			Females		
	Observed	B-C	Theoretical	Observed	B-C	Theoretical
1.0	87.1	91.4	92.03	86.2	88.3	90.7
2.0	129.2	131.3	132.08	123.4	129.3	131.9
3.0	175.2	165.5	165.9	172.2	165.1	165.2
4.0	213.2	195.1	194.6	202.4	189.0	191.9
5.0	233.2	218.1	218.8	221.6	214.8	213.5
6.0	247.1	229.9	239.2	233.0	228.1	230.9
7.0	263.3	249.4	256.5	246.0	246.0	244.9
8.0	271.4	265.0	271.1			
9.0	292.3	284.7	283.5			

Table 6.9. Results of the Chow test comparing growth curves among sexes

ANOVA^b (Pooled)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	687991.288	1	687991.288	2167.454	.000^a
	Residual	113953.454	359	317.419		
	Total	801944.742	360			

ANOVA^b (Male)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	418301.159	1	418301.159	1166.576	.000^a
	Residual	70997.221	198	358.572		
	Total	489298.380	199			

ANOVA^b (Female)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	199894.849	1	199894.849	896.870	.000^a
	Residual	35437.995	155	222.880		
	Total	235332.845	156			

Ess_c =113953.454, ess_1 = 70997.221 and ess_2= 35437.995, k=2 and N1=200, N2= 156

Hence f statistic is given by F= 12.60866

P value is given by **0.0000051.** Hence we reject the null hypothesis that the regression coefficients are equal and we need to consider separate regression for both male and female.

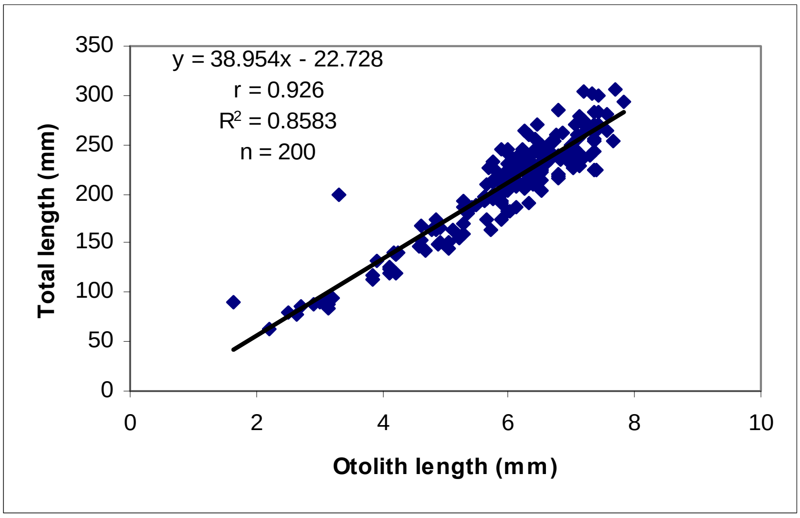
Table 6.10. Estimated life span, growth performance index and natural mortality rates for male and female of *C. dussumieri*

Sex	Life span	Growth performance index	Natural mortality
Males	17.02	4.32	0.18
Females	13.2	4.30	0.23

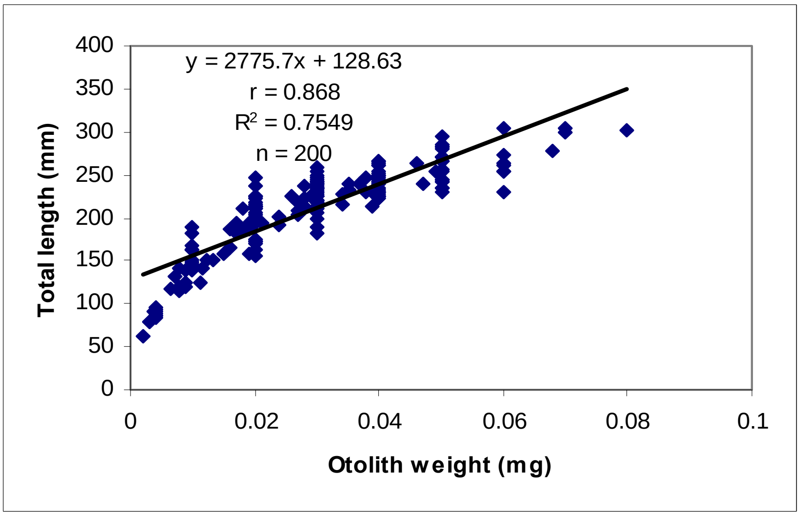
Table 6.11. Comparisons of estimates of the von Bertalanffy growth parameters, Φ' value and natural mortality rates reported by various authors for some batrachoid species

Species	L_∞	K	t_0	Φ'[1]	M[2]	Investigators
Opsanus beta (♂&♀)	300	0.168	-0.09	4.18	0.17	Serafy *et al.,* 1997
Opsanus beta (♂&♀)	300	0.218	-0.27	4.29	0.22	Serafy *et al.,* 1997
Opsanus tau ♂	407.5	0.15	-0.33	4.40	0.15	Radtke et al., 1985
Opsanus tau ♀	271.5	0.39	-0.44	4.46	0.41	Radtke et al., 1985
Halobatrachus didactylus ♂	477.1	0.15	-0.59	4.53	0.15	*Palazon-Fernandez et al.,* 2010
Halobatrachus didactylus ♀	363.7	0.20	-0.75	4.42	0.21	*Palazon-Fernandez et al.,* 2010
C. dussumieri ♂	351	0.168	-0.81	4.32	0.18	This study
C. dussumieri ♀	303	0.216	-0.65	4.30	0.23	This study

[1] values calculated from author's data; [2] values calculated from author's data using the equation of Taylor (1958)

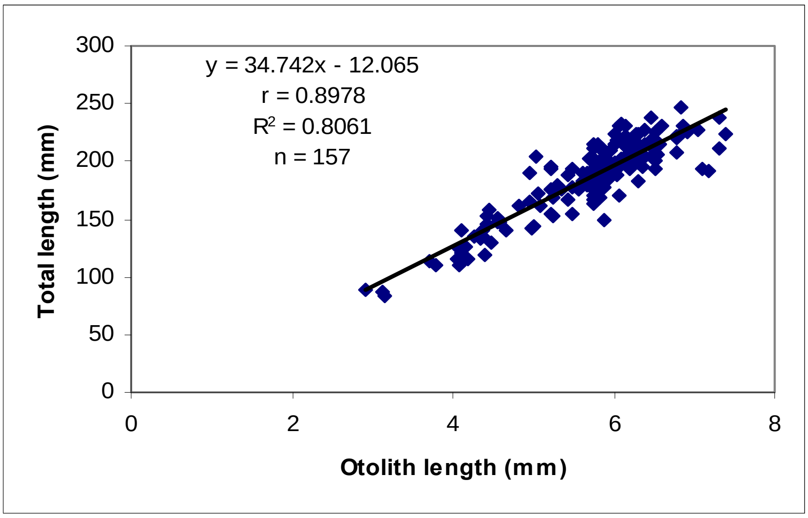


(a) Length of the otolith

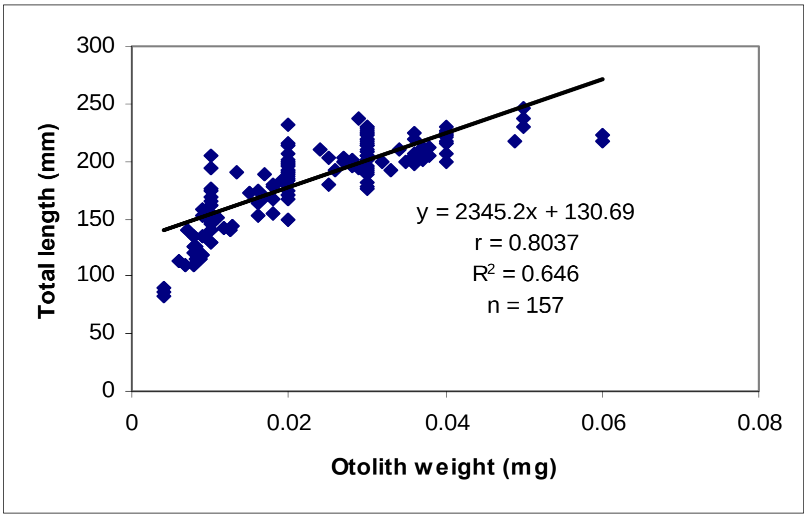


(b) Weight of the otolith

Fig. 6.1. Relationship between total length – at- capture of the fish (Male)



(a) Length of the otolith



(b) Weight of the otolith

Fig. 6.2. Relationship between total length – at- capture of the fish (Female)

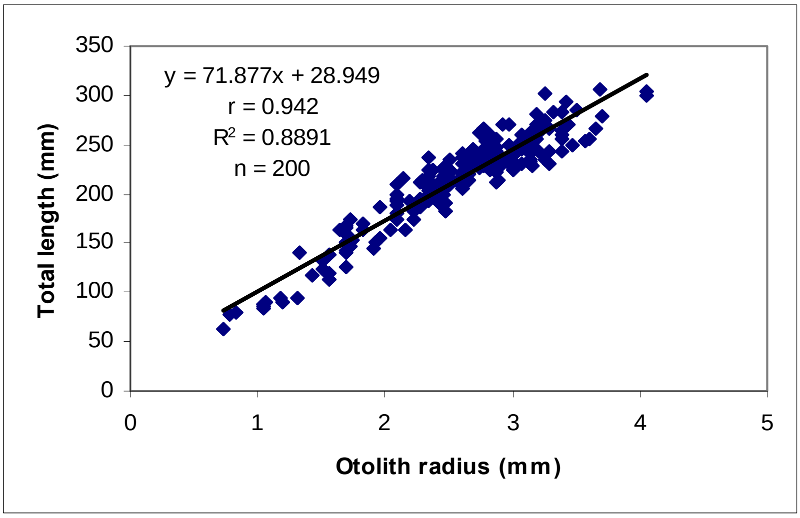


Fig.6.3.a. Relationship between fish total length -at- capture (T.L$_C$) and otolith radius -at- capture (O.R$_C$) (Male).

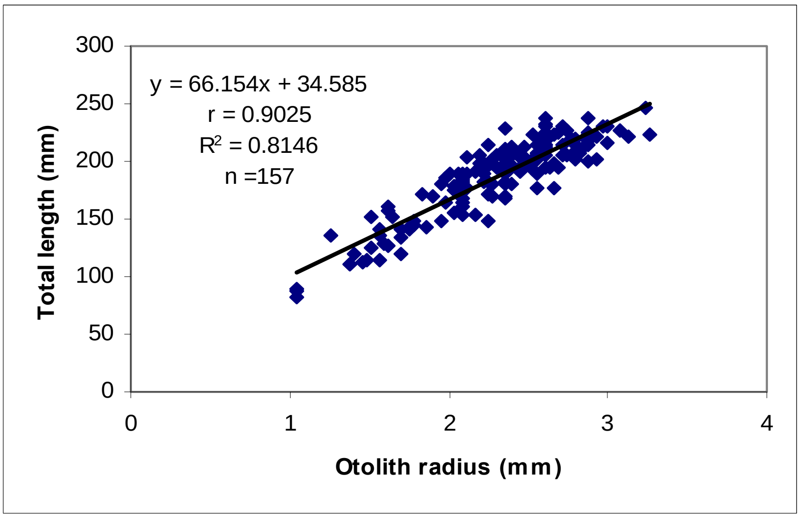


Fig.6.3.b. Relationship between fish total length -at- capture (T.L$_C$) and otolith radius -at- capture (O.R$_C$) (Female).

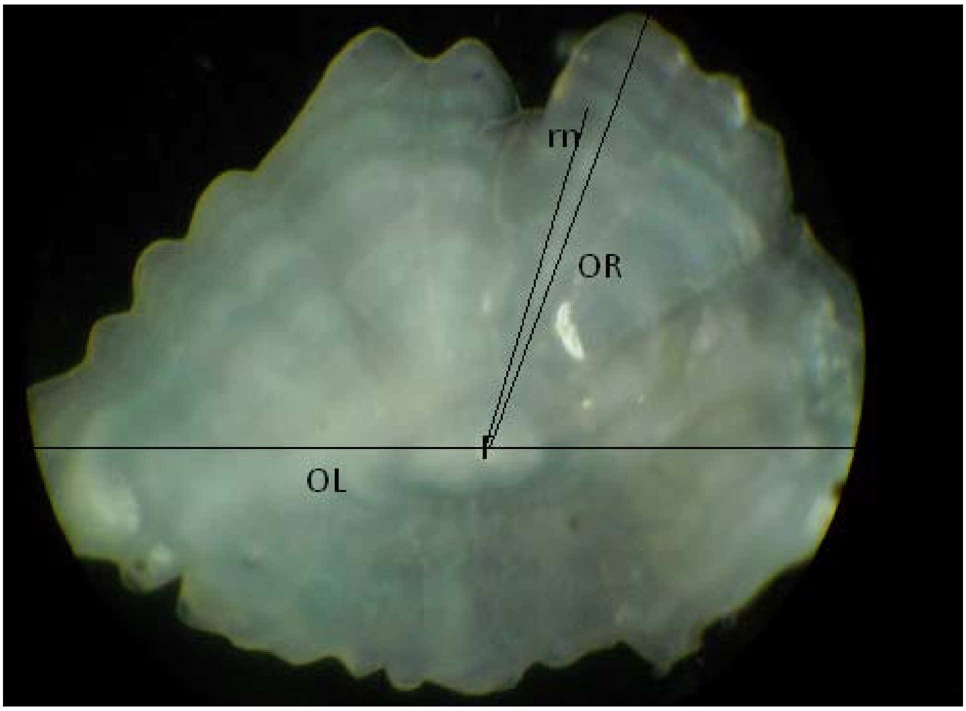
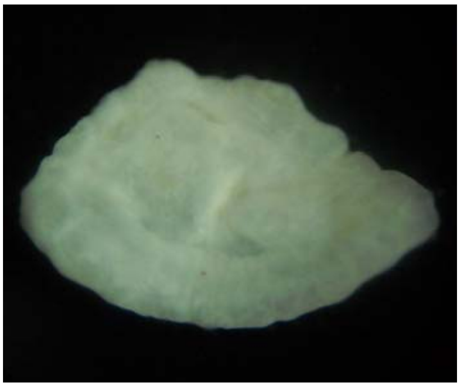


Fig.6.4. Photomicrograph of a sagittal otolith of *C. dussumieri* showing growth increments. OL – Otolith Length, OR- Otolith radius, rn- ring radius



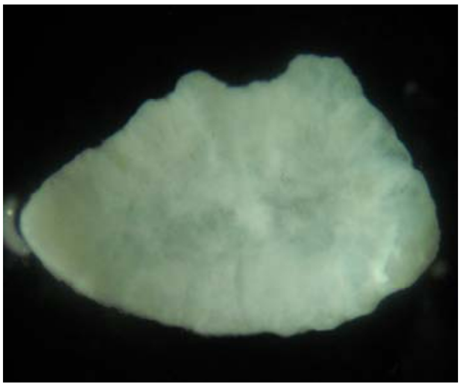


Fig. 6.4a. Otolith from 89 mm TL *C. dussumieri* in age group 1

Fig. 6.4b. Otolith from 141 mm TL *C. dussumieri* in age group 2

Fig. 6.4c. Otolith from 163 mm TL *C. dussumieri* in age group 3

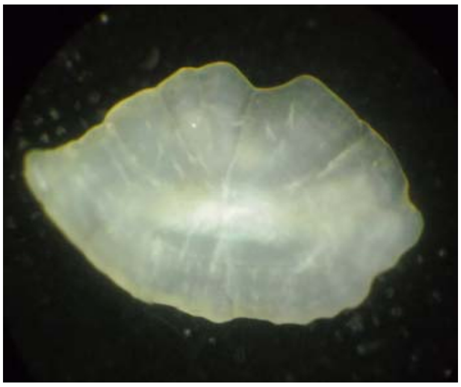


Fig. 6.4d. Otolith from 194 mm TL *C. dussumieri* in age group 4

Fig. 6.4e. Otolith from 214 mm TL *C. dussumieri* in age group 5

Fig. 6.4f. Otolith from 240 mm TL *C. dussumieri* in age group 6

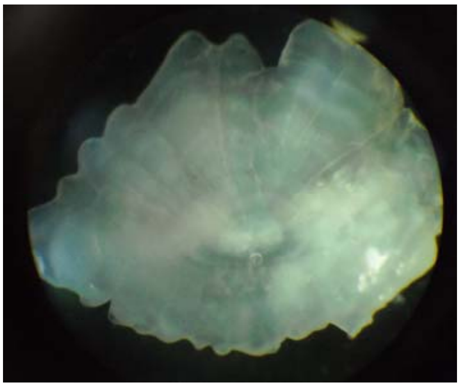


Fig. 6.4e. Otolith from 264 mm TL *C. dussumieri* in age group 7

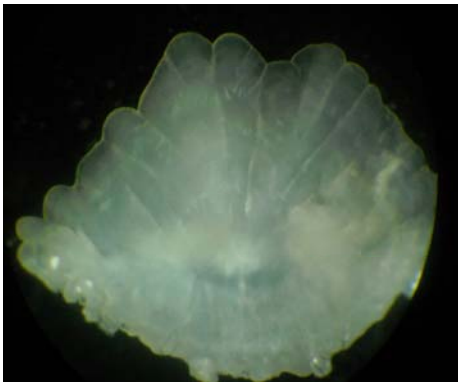


Fig. 6.4f. Otolith from 270 mm TL *C. dussumieri* in age group 8

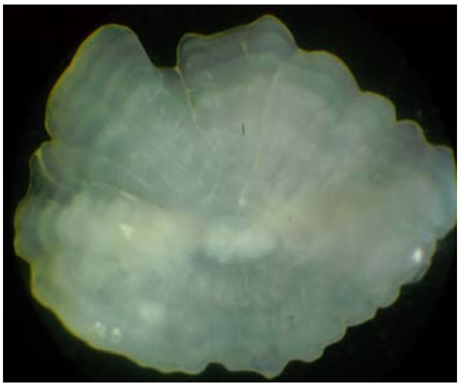


Fig. 6.4f. Otolith from 284 mm TL *C. dussumieri* in age group 9

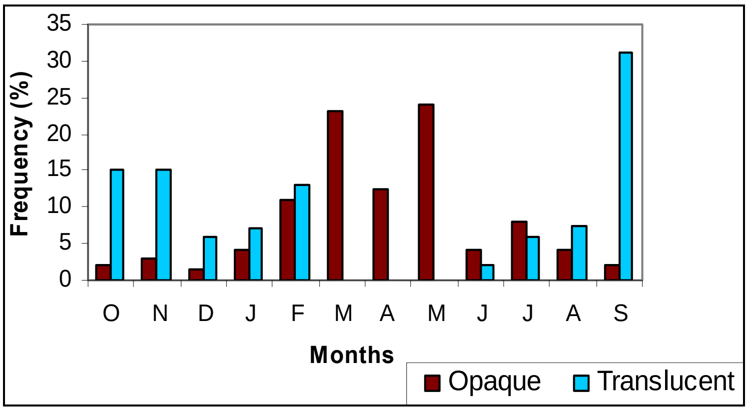


Fig. 6.5a. Monthly frequency of opaque and translucent margins of otoliths of males of *C. dussumieri*

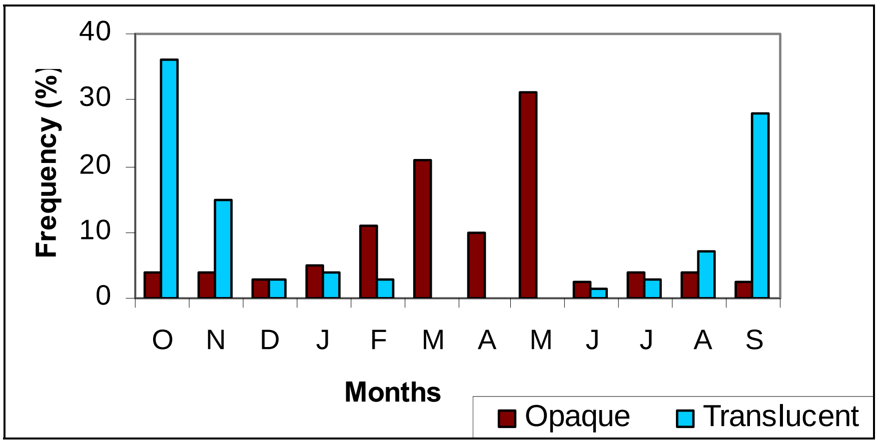


Fig. 6.5b. Monthly frequency of opaque and translucent margins of otoliths of females of *C. dussumieri*

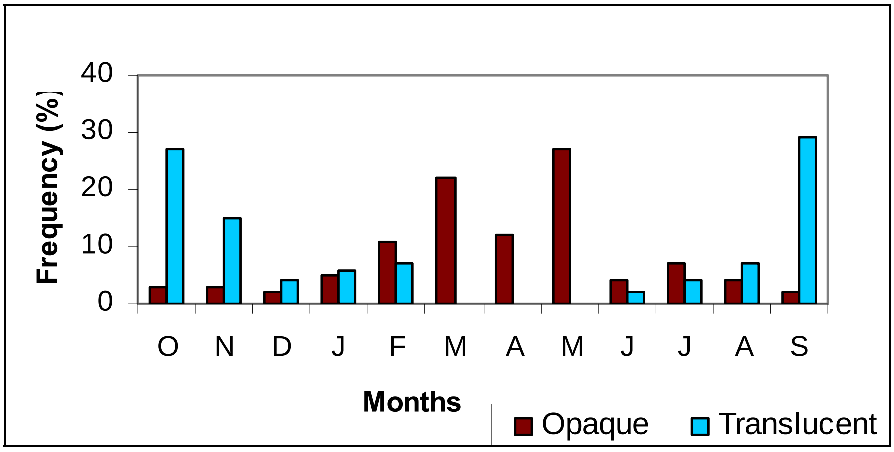


Fig. 6.5c. Monthly frequency of opaque and translucent margins of otoliths of pooled specimens of *C. dussumieri*

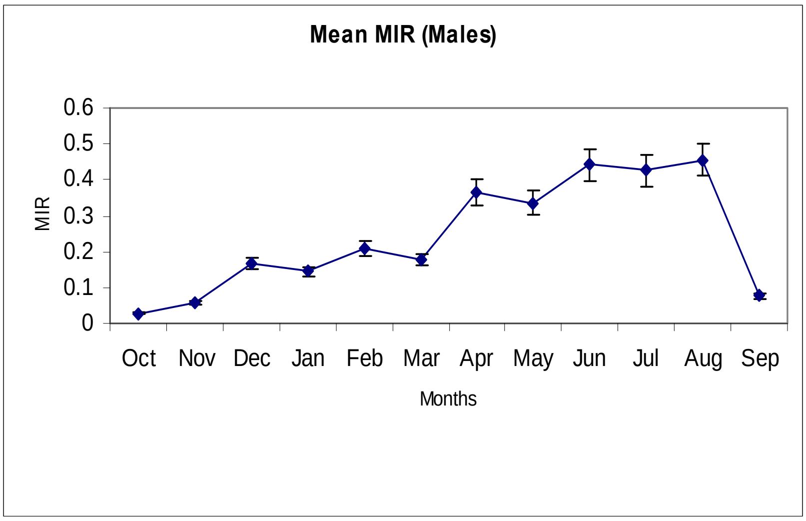


Fig.6.6a. Mean marginal increment in otoliths of *C. dussumieri* by month (Males). The error bars represent ± standard error (SE).

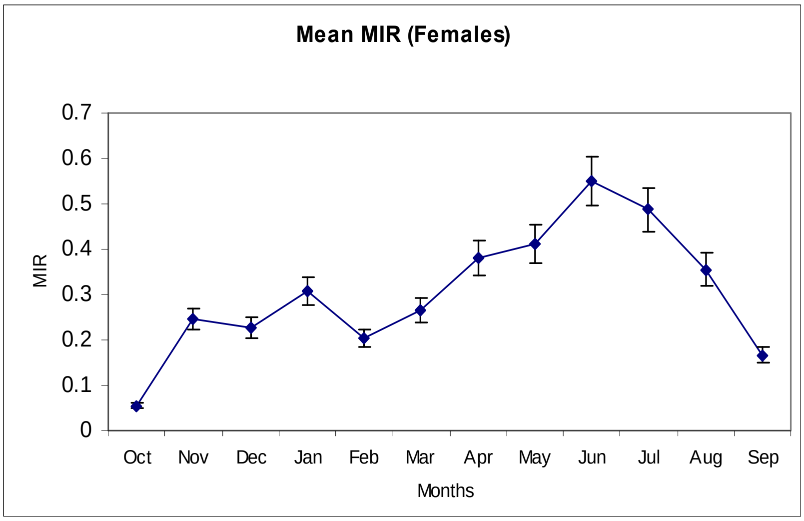


Fig.6.6b. Mean marginal increment in otoliths of *C. dussumieri* by month (Females). The error bars represent ± standard error (SE).

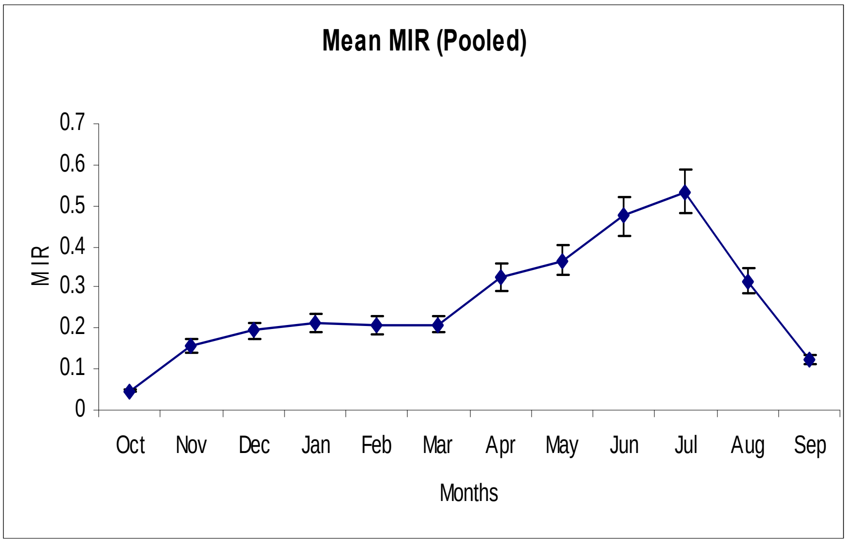


Fig.6.6c. Mean marginal increment in otoliths of *C. dussumieri* by month (pooled). The error bars represent ± standard error (SE).

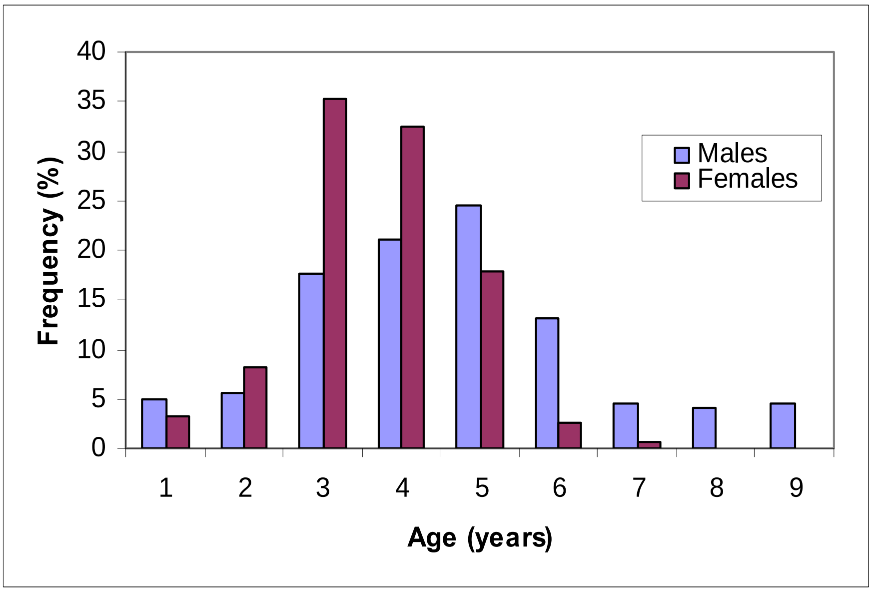
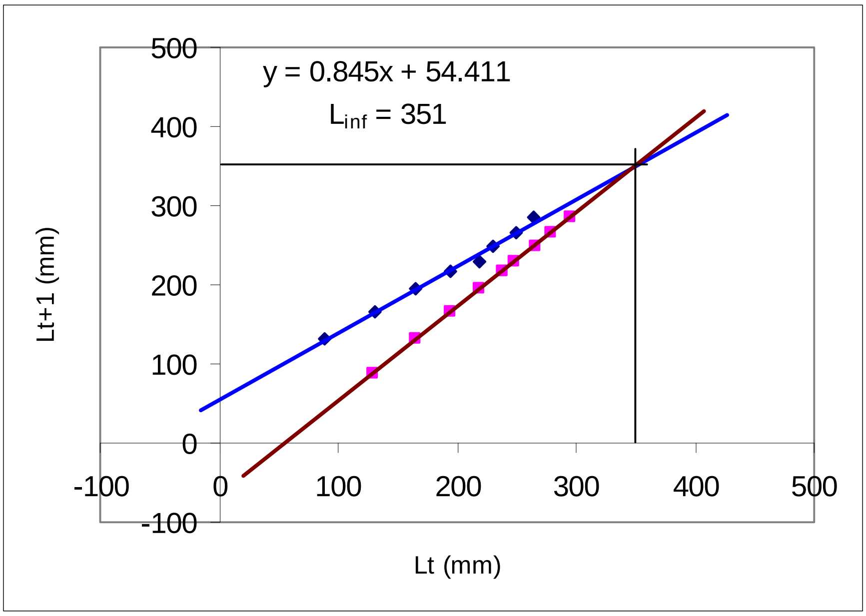
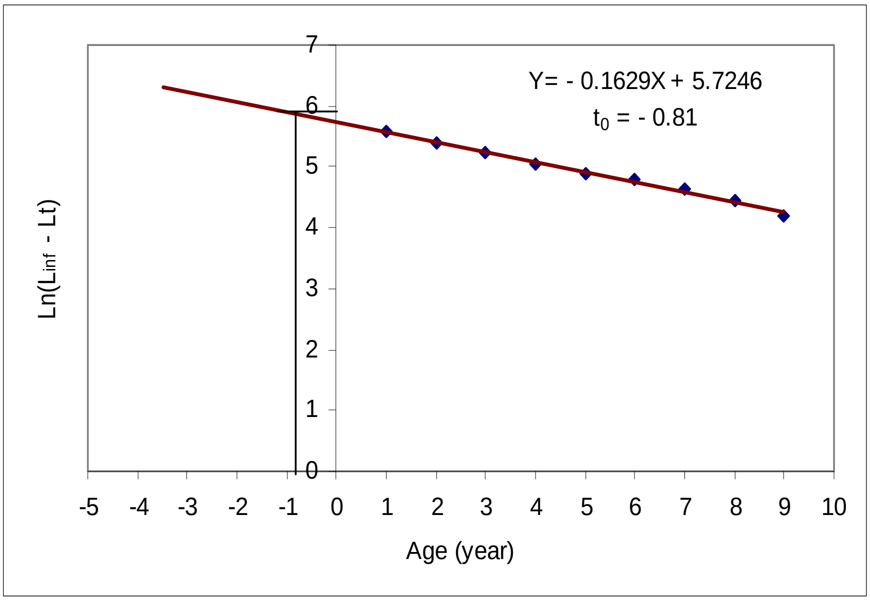


Fig.6.7. Age distribution for male and female *C. dussumieri*

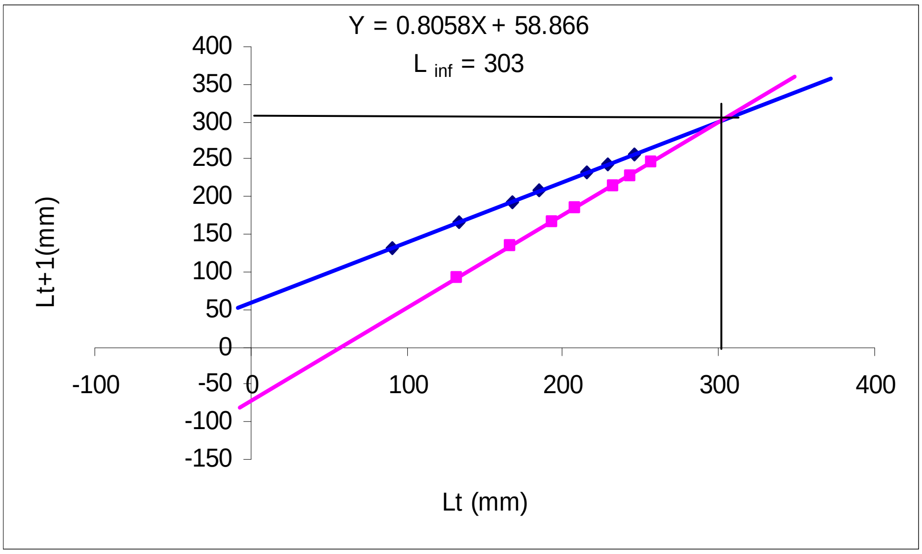


(a)

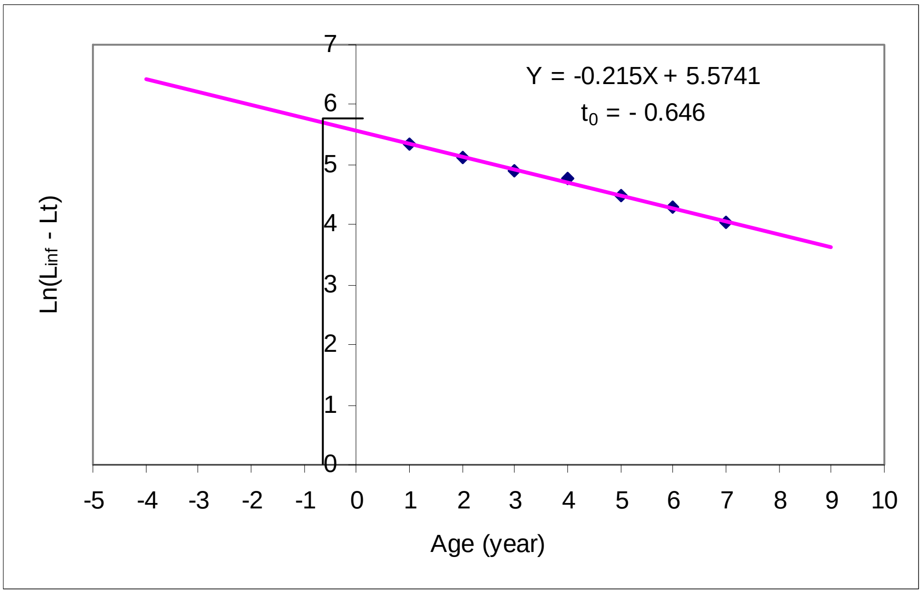


(b)

Fig. 6.8. Ford-Walford Graph for male *C. dussumieri*: (a) L∞, (b) t_0



(c)



(d)

Fig. 6.8. Ford-Walford Graph for female *C. dussumieri*: (c) $L\infty$, (d) t_0

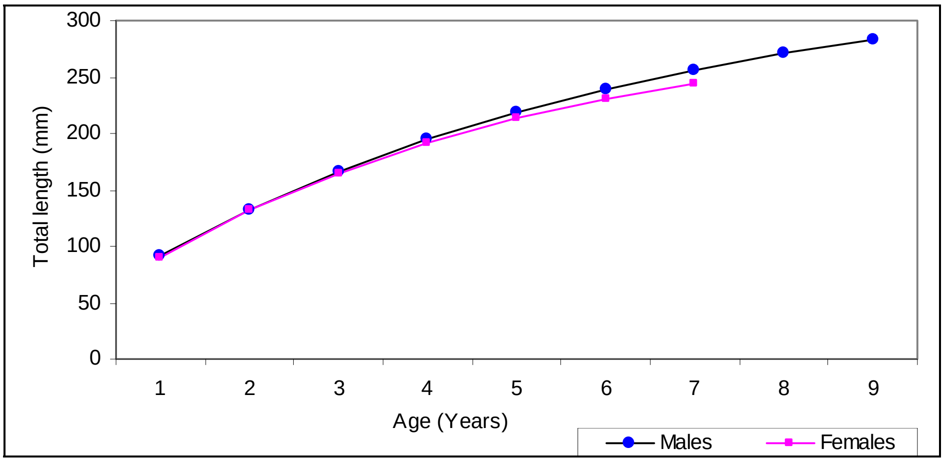


Fig. 6.9. von Bertalanffy growth curve derived from back calculated age-at-lengths for male and female *C. dussumieri*

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Chapter 7

LENGTH – WEIGHT RELATIONSHIP AND CONDITIONS FACTOR

Contents

7.1 Introduction

Growth is the process of increase or progressive development of an organism. Typically, growth can be defined as the change in size (length, weight) over time. Increment in size is due to conversion of food matter into building mass of body by the process of nutrition. Many factors influence the growth of a fish. Among these more common determinants are the amount and size of available food, the number of fish utilizing same food source, temperature, oxygen and other water-quality factors; the size, age and sexual maturity of the fish.

Growth of fish can be considered as no more than the individual production of mass. The growth process is specific for each species of fish. However, it can differ in the same species inhabiting different geographical locations and is easily influenced by several biotic and abiotic factors. Growth is a specific adaptive property, ensured by the unity of the species and its environment (Nikolsky, 1963).

Every animal in its life exhibit growth both in length and increase in weight, the relationship between these two has both applied and basic importance. The length - weight relationship is one of the standard methods yielding authentic biological information with two objectives: firstly, it establishes the mathematical relationship between the two variables, length and weight, so that unknown variable can be readily computed from the known variable. Secondly, to know the variations from the expected weight, for the known length groups, this in turn reflects its fatness, general well being, gonad development and suitability of environment of the fish (Le Cren, 1951).

Length- weight (L-W) relationships can be used to (i) estimate weight from length of individual fish and for length classes of fish, (ii) to estimate standing-crop biomass when the length frequency distribution is known (Martin - Smith, 1996; Petrakis and Stergiou, 1995), (iii) to convert growth -in- length equations to growth-in-weight for prediction of weight-at-age and use in stock assessment models (Pauly, 1993), (iv) to calculate condition indices (Safran, 1992; Petrakis and Stergiou, 1995) and (v) for life history and morphological comparisons of populations from different regions (Petrakis and Stergiou, 1995).

Length – weight relationship is determined by collecting data on both the length and the weight of the fish in different phases of life and calculating the relationship existing between the two by the formula

$$W = a L^{b}$$

‘W’ is the weight of the fish, ‘L’ is the length of the fish and ‘a’ and ‘b’ are constants to be determined empirically from the data.

In fishes, weight is an exponential function of length (or any linear dimension); under conditions of isometric growth, the regression follows the cube law (Rounsefell and Everhart, 1953; Lagler, 1956; Ricker, 1958). But in reality, the actual relationship between the variables, length and weight may depart from this, either due to environmental conditions or condition of fish (Le Cren, 1951). In nature, the body proportions of a fish continually change with ageing. The form and specific gravity do not remain constant throughout the life history of the fish, which often causes the regression coefficient of weight on of length, depart from 3. In such cases the value of the exponent 'b' in the parabolic equation may lie between 2.5 and 4 (Hile, 1936; Martin, 1949).

The exact relationship between length and weight differs among species of fish according to their inherited body shape and within a species according to the condition (robustness) of individual fish. But, condition is variable and dynamic. Individual fish within the same sample vary considerably and the average condition of each population varies seasonally and yearly. Sex and gonad development are other important variables in some species. The condition factor is an index reflecting interactions between biotic and abiotic factors in the physiological condition of fishes. It shows the population's welfare during the various stages of the life cycle (Angelescu *et al.*, 1958).

Condition of fish in general is an expression of relative fatness of fish. The relative robustness, or degree of well-being, of a fish is expressed by "coefficient of condition," denoted by 'K' (also known as Fulton's condition factor, or length-weight factor, or Ponderal Index). Variations in a fish's coefficient of condition primarily reflect state of sexual maturity and degree of nourishment. Condition values may also vary with fish age, season and in some species, with sex. K- factor vary with species and size, but larger values generally are indicative of better fish condition. If fish does not undergo the cube law, the 'K' value is directly affected by length, age, maturity, feeding intensity and other factors. In order to eliminate the effect of these factors on the 'K' value, Le Cren (1951) suggested the calculation of relative condition factor 'Kn' which does so only if the exponent value is equal to 3. Thus K factor measures the variations from an ideal fish which holds the cube law while Kn measures the individual deviation from the expected weight derived from the length-weight relationship. The relative condition factor has an expectation of one and the deviation from one will yield information such as differences in the nutritive level and the effect of physico- chemical factors on the life cycle of organisms. So the study of the condition factor ('K') and relative condition factor ('Kn') is thus important for understanding the life cycle of fish species and contributes to adequate management of these species and therefore, to the maintenance of equilibrium in the ecosystem.

The importance of length- weight relationship and condition factor of fishes has inspired a large number of works in different parts of the world to analyze this relationship in both marine and freshwater fishes. Studies on the length – weight relationship and relative condition factor in the fishes of the family Batrachoididae are very few in number. The most notable contributions in this regard are that of Wilbur and Robinson (1960); Schwartz and Dutcher (1963); McDermott (1965); Richard and Willard (1968); Radtke *et al*. (1985) and Palazon-Fernandez *et al*. (2001).

The determination of the exact nature of the relationship that exists between length-weight and condition factor of fishes has been recognized as an important part of fishery biological studies. No information is available on the length - weight relationship and condition factor of *Colletteichthys dussumieri.* In view of this practical utility, the present study was undertaken to elucidate the pattern of growth and general well-being of this fish species from the Cochin estuary.

7.2 Materials and methods

Monthly samples were collected from Cochin estuary using gill net (55 mm, 70 mm) and also by applying hook and line. Total length and weight of the fishes were recorded to the nearest 1.0 mm and 1.0 g, respectively. Sex was determined by macroscopic examination of the gonads. The study is based on the length and weight data of 467 specimens, 219 females of the length range 95 mm - 246 mm (weight 15.05 gm – 275 gm) and 248 males of the length range 94 mm - 305 mm (weight 15.50 gm- 500 gm) collected during the period from October 2003 to September 2005.

The method suggested by Le Cren (1951) was followed to compute the length and weight relationship. Accordingly, the length – weight relationship can be expressed as:

$$W = a\,L^b$$

Where W and L are weight (g) and length (mm) of the fish respectively and ‘a’ and ‘b’ are two constants (initial growth index and regression constants respectively).

When expressed logarithmically the above equation becomes a straight line of the formula:

$$\text{Log } W = \log a + b \log L,$$

The constants 'a' represents the point at which the regression line intercepts the y- axis and 'b' the slope of the regression line were estimated by the method of least square (Snedecor and Cochran, 1967). The regression of log weight on log length was first calculated independently for males and females and then for the species. The significance of regression was assessed by ANOVA. The regression coefficients of the sexes were compared by the analysis of covariance (ANCOVA) to establish the variations in the 'b' values, if any, between them. The significance of difference, in the estimate of 'b' in males , females and pooled data of sexes from the expected value of 3 (isometric growth) was tested by Bailey's t – test (Snedecor and Cochran, 1967) as given by the formula,

$$t = \frac{b - 3}{Sb}$$

b = regression coefficient of log transformed data

Sb = standard error of b

The t-test (Snedecor and Cochran, 1967) on 'r' values reveals whether significant correlation exists between length and weight.

Condition factor K, a measure of the well-being or plumpness of a fish, was calculated according to the equation presented in Carlander (1977):

$$K = \frac{W \times 10^5}{L^3}$$

Where W is the weight of the fish in grams and L is the total length of the fish in millimeters. The number 10^5 is a scaling factor when metric units are used (i.e., grams and millimeters) and is used to bring the value of K near unity.

The relative condition factor, introduced by Le Cren (1951) was calculated using the formula:

$Kn = W / \hat{W}$ Where,

W is the observed weight and $\hat{W}$ is the calculated weight, and was calculated based on the length- weight relation regression equation.

K and Kn was calculated for different month and size wisely for both the sexes. The average value of each month irrespective of size was considered. The average K and Kn for each 10 mm length group was also calculated and plotted against the respective length groups.

7.3 Result

The mathematical relationship between total length and weight of male and female of *C. dussumieri* obtained by logarithmic regression equations are as follows:

Male : $\text{Log } W = -4.646 + 2.96 \text{ Log } L$
Female : $\text{Log } W = -4.372 + 2.85 \text{ Log } L$

Such relationship was depicted in Fig.7.1 and 7.2. Their corresponding parabolic equations can be expressed as:

Male : $W= 0.0004426\ L^{2.96}$
Female : $W= 0.0002355\ L^{2.85}$

The correlation coefficients ‘r’, 0.953 for male and 0.934 for female was found to be significant ($P< 0.001$) in both instances indicating good correlation between length and weight. The results of ANOVA on regression of males and females are presented in Tables 7.2 and 7.3 respectively. The length- weight regressions were found to be highly significant in both sexes ($P<0.001$). The errors in the regression coefficients (Table. 7.1) were minimum in the case of both sexes. The results of the analysis of covariance (ANCOVA) are shown in Table 7.4. The values of the slope ('b') for males and females exhibited a significant difference (F value = 6.166**,** df: 1, 467, Significant at 5%), thereby indicating heterogeneity of the samples. Hence, pair wise comparison between males and females were carried out using students 't' test (Zar, 2005). The results (Table 7.5) show that 'b' value is significantly different ($P< 0.01$) between sexes. Hence, pooling of data to provide a single equation expressing the length- weight relationship of *C. dussumieri* will not be justifiable, thus necessitating fitting up of separate equations for males and females.

The significance of variation in the estimates of regression coefficient value 'b' from '3' was tested using 't' test (Table.7.1), the value of 't' for both the sexes and pooled were as:

Male = 0.67	$P>0.05$	not significant.
Female = 2.04	$P<0.05$	significant.
Pooled = 2.51	$P<0.05$	significant

The fluctuations noticed in Kn values of males and females during 2003-'04 and 2004-'05 are represented in Figs.7.3 and 7.4 respectively. In 2003- '04, the Kn values of males showed highest peak of 1.368 in May followed by October and August sharing similar value of 1.1545 succeeded by a value of 1.1368 in February. Lowest Kn value was observed in November (0.6115). The males showed Kn values less than 1 in December, January and June. Whereas in 2004-'05 the maximum Kn value was registered in April (1.686) and lowest in November (0.7339). Excluding December, February and August all other months had Kn values greater than 1. Incidentally, the values of ponderal index (K) (Fig. 7.6) were found in conformity to the Kn values during both the years.

During 2003-’04, females registered maximum Kn value of 1.2672 was registered in May followed by 1.0876 in September. A sharp inflexion occurred in November thus recording the lowest value of 0.655. During 2003 – ’04, females showed low Kn values in October. A sharp inflexion occurred in November, recording the lowest value of 0.655. A gradual increase in Kn value from December to February was followed by a decrease in value by March. It again shot up in April thenceforth showed a rapid increase registering a value of 1.2672 in May. This was succeeded by steady decline in values till September. During 2004 - ’05 utmost value of 1.2043 was recorded in March, accompanied by May with a value of 1.1423. August (0.5501) registered the least Kn value during 2004 -’05. The seasonal variations in the values of ponderal index (K) (Fig. 7.7) were similar to that of relative condition factor (Kn).

The average Kn values for different length groups of 10 mm interval with respect to males and females are depicted in Fig.7.5. In case of males, lowest Kn value (0.7208) was observed in 161 – 171 mm size class. Diminished Kn value (<1) was noticed in 91-100 mm, 121-130 mm, 141-150 mm and 191-210 mm length classes. Rest of the length classes indicated higher values (>1). From 221-260 mm, Kn values swayed between 1.02-1.03. In females, summit Kn value of 1.49 was found in 91 – 100 mm length class. Elevated Kn values (>1) were perceived in 101-120 mm, 131-160 mm, 191-200 mm, and 211-240 mm length group. The ponderal index (K) showed the same trend as that of relative condition factor (Fig. 7.8).

The seasonal variation in Kn values of males and females showed almost similar trend during 2003-’04, both scoring a peak value in May and inferior value in November. During 2004 - ’05 lowest value was observed in November in male whereas August in female. April accompanied by March earned apex Kn value in male but in females, March and May registered high Kn values. A scrutiny of the lengthwise variation in relative condition factor exhibited high kn values in smaller fishes in both sexes. In larger fishes it showed more or less static values. In males, high Kn values were observed in the length group from 101-140 mm, 151-160 mm and 171-190 mm TL, showing a slight dominance in condition over the females. Length groups 221-260 mm TL attained a steady value in male.

Sex-wise analysis of Kn values revealed that the mean Kn values in males (1.032 and 1.017) were higher than those of females (0.996 and 0.979) for both the years.

Comparing the Kn values with GSI revealed that there exist a direct correlation between the two variables in both sexes i.e., $r = 0.69$, $P=0.012$ for females and $r = 0.59$, $P = .043$ for males. Coincidence of Kn and GSI in female and male was depicted in Fig.7.9 and 7.10., respectively. The correlation between Kn values and Ga.S.I was also found to be significant in both sexes, $r = 0.70$, $P=.0106$ for females and $r = 0.715$, $P = .008$ for males and their corresponding relationships are shown in Fig.7.11 and 7.12 respectively.

7.4 Discussion

The ‘r’ values showed a fine correlation between length and weight in *Colletteichthys dussumieri*. The regression coefficient of male (2.96) was found to be higher than in female (2.85). From this trend, it may be presumed that males gain more weight with the increase in length and subsequently age than females. The difference was significant as revealed from the analysis of covariance. Since, there was significant difference between slopes of regression lines in both sexes, it was necessary to have separate regression equation to express the length - weight relationship in *Colletteichthys dussumieri* from Cochin estuary.

According to Wootton (1990), if the fish retains the same shape and its specific gravity remains unchanged during lifetime, it is growing isometrically and the value of exponent ‘b’ would be exactly 3.0. A value significantly larger or smaller than 3.0 indicates allometric growth. A value less than 3.0 shows that the fish becomes lighter (negative allometric) and greater than 3.0 indicate that the fish becomes heavier (positive allometric) for a particular length as it increases in size (Wootton, 1990). Although the ‘b’ values were almost equal to the expected value ‘3’ for ideal fish in both the sexes, the ‘t’ test clearly showed no departure from cube law in male. This suggests isometric growth ($b=3$) in male and a negatively allometric pattern ($b<3$) in female which indicate that the rate of increase in body length is not proportional to the rate of increase in body weight. The results for pooled value of both sexes also gave a negative allometric growth ($b<3$). Since, the difference between the slopes of the regression of male and female was significant ($P<0.05$), it reflects a divergence in growth pattern in both the sexes. This change may be due to a number of factors including season, habitat, gonad maturity, sex, diet, stomach fullness, health, preservation techniques and locality (Bagenal and Tesch, 1978; Froese, 2006). Such differences in values ‘b’ can be ascribed to one or a combination of most of the factors including differences in the number of specimens examined, area / season effects and distinctions in the observed length ranges of the specimens caught, to which duration of sample collection can be added as well (Moutopoulos and Stergiou, 2002). According to Jhingran (1968) and Frosta *et al.* (2004) the slope value ‘b’ indicates the rate of weight gain relative to growth in length and varies among different populations of the same species or within the same species. The correlation coefficients indicate the degree of association between length and weight of the fish. The high values of correlation coefficients in both sexes revealed that there is perfect relationship between the two variables in this species.

Information on the length – weight relationship of toadfishes divulges that many of them deviate from cube law. Wilbur and Robinson (1960) presented linear regression equations for length, weight and girth relations of *Opsanus tau*. Schwartz and Dutcher (1963) and McDermott (1965) observed that males grew considerably larger than females in the same species. Richard and Willard (1968) re-examined the difference in the maximum length of males and females and the mathematical relationship between length, weight and girth of the above species. The toadfish showed isometric growth and analysis of covariance (ANCOVA) indicated sexual dimorphism in the girth-length relation. According to them, the difference might be due to the difference in size range or it might reflect seasonal or ecological variations in the growth of the species. Moyle and Cech (1988) indicated that the differences in length between the sexes are the most common form of sexual dimorphism among fishes. Wilson *et al.* (1982) noted no sex related difference in growth for a population of *O. tau* in Carolina, although they found differences in maximum age attained by the sexes, which were lower for females. Muto *et al.* (2000) estimated the ‘b’ value ($b = 3.314$, $P = 0.01$, $r^2 = 0.987$) of *Porichthys porosissimus*

(Batrachoididae) and revealed that it significantly differed from '3'. Palazon-Fernandez *et al.* (2001) studied the length – weight relationship of *Halobatrachus didactylus* (Schnieder, 1801). Equations for the sexes differed (male-Wt = 6, 9390 x $^{10\text{-}6}$ $L_t{}^{3.1510}$, Female-Wt = 4, 5941 x $^{10\text{-}6}$ $L_t{}^{3.2284}$, t = -2.70; $p<0.01$) and exponents of the two regression showed a positive allometry (t = 9.39, $p<0.01$ for males and t = 9.53; $p<0.01$ for females). Length-weight relationships for Brazilian estuarine fishes along a latitudinal gradient by Joyeux *et al.* (2009) disclosed that *Batrachoides surinamensis* (Bloch & Schneider, 1801) (b = 3.172, P = 0.0000) and *Thalassophryne maculosa* (Gunther, 1861) (b = 3.279, P = 0.0001) presented positive allometry for the species. All these earlier reports corroborate the present findings on the length-weight relationship in *C. dussumieri* in which significant departure of 'b' value from the isometric value of 3 was noticed in respect of both female and pooled sample. Negative allometric growth for females and the pooled sample, exhibited that they tend to become thinner as they grow larger. It was concluded that LWR followed cube law only in male of *C. dussumieri.*

Females of *C. dussumieri* were found to become lighter for their length as they grew larger as evident from the disparity in 'b' values. Similar trend has been observed in other teleost fishes too. Mohanraj (2008) while reporting on the length-weight relationships of *Upeneus sundaicus* and *Upeneus tragula* from Gulf of Mannar, stated that males of both species showed isometric growth while the females showed negative allometric growth and separate regression equations have been proposed for both male and female of *U. sundaicus* and *U. tragula.* Soomro *et al.* (2007) observed that in *Eutropiichthyes vacha* (Schilbeidae: Siluriformes), the values for allometric coefficient b of the LWR were close to isometric value for male (b= 3.159) and combined values for both sexes (b=3.053). However, it suggested negative allometric growth for female (b=2.973). Length-weight relationships described for ten important demersal and pelagic fish species caught with bottom trawl and midwater trawl by Kalayci *et al.* (2007), exposed that the growth was negative allometric (b<3, P<0.05) for overall samples of *Gobius niger, Engraulis encrasicolus*, *Sprattus sprattus* and *Pomatomus saltatrix*. They opied that functional regression "b" value represents the body form, and it is directly related to the weight affected by ecological factors such as temperature, food supply, spawning conditions and other factors, such as sex, age, fishing time, area and fishing vessels. The values of *b* = 2.790 and 2.880 recorded for *Clarias gariepinus* and *Illisha africana* respectively showed that the rate of increase in body length is not proportional to the rate of increase in body weight (Fafioye and Oluajo, 2005). The length-weight relationships in *Spicara maena* (Linnaeus, 1758) revealed that males were heavier than females for a given length. This may be explained by protogynous hermaphroditism, because females predominated in smaller size classes and males larger ones (Cycek *et al.*, 2007). Laghari *et al.* (2009) found that the values of regression co-efficient in *Rita rita* was ideal in case of male and combined sexes (b= 3.87 and 3.56) respectively, while in case of female the regression co-efficient values showed satisfactory growth (b= 2.34). According to him, the value of "b" may differ depending upon feeding, sex and maturity state. The results of the present study are in conformity to the above findings.

The increase in weight of any individual was not due to a single factor but various factors (Townsend *et al.*, 2003). The factors could be either intrinsic or extrinsic, or both and favoured the changes of the growth parameters (length and weight) of the fish (Youson *et al*., 1993; Altinok and Grizzle, 2001). The seasonal storage and utilization of lipid reserves are important in fish metabolic activities and reproduction (Guillemot *et al*., 1985). Feeding intensity was found to be higher in males, season wise as well as size wise (Chapter 4). Males are heavier than females of the same length. It may be inferred from the data on the higher regression coefficients in males of *C. dussumieri*, have better robustness than females. This may be due to the fact that the females have to divert a considerable part of the energy for oogenesis and females spend more energy than the males during the breeding.

Hile (1936) found that the exponent 'b' usually lies between 2.5 and 4.0 and 3 is the ideal value of 'b' (isometric growth) while deviation from 3 shows the allometric growth. Allen (1938) suggested that the value of 'b' remains constant at 3.0 for an ideal fish. Departure from the cubic relation has been recorded by Le Cren (1951). Beverton and Holt (1957) recorded that cubic relationship between length and weight existed and suggested that the value of 'b' is almost always near to 3.0. Ricker (1958) observed that a fair number of species seem to approach this ideal. All allometric coefficients (b) estimated in this study were within the expected range 2.5-3.5. According to Pauly and Gayanilo (1997), 'b' values may range from 2.5 to 3.5 suggesting that result of this study is valid. With regard to *C. dussumieri,* the present study provides the first LWR estimates for the genus.

The condition of fish is subjected to variations with a number of factors including reproductive cycles (Le Cren, 1951; Babu and Nair, 1983; Nural Amin, 2001) and availability of foods (Rounsefell and Everhart, 1953; Shafi and Quddus, 1974; Anibeze, 2000; Morato *et al*., 2001). Such fluctuations may also be influenced by the environmental factors, age and the physiological state of the fish (Brown, 1957). The study showed that there was a definite seasonal cycle in the Kn of both sexes of *C. dussumieri*. The high values of Kn during February to June may be attributed directly to feeding activity, with peak in May during 2003-'04 in both male and female and April and March in 2004-'05 for males and females respectively , coinciding with the number of the spent fishes. This may be related to the increase in the feeding intensity of the spent fishes to rebuild their body reserves. This resulted in sharp increase of Kn at the end of spawning and during post spawning period.

The gradual decline in Kn from October to January with minimum value in November coincided with the peak spawning season. This may be due to decline in feeding intensity by the spawner fishes. Condition and feeding activity decrease in the spawning time (Maddock and Burton, 1999). Lizama and Ambrosio (2002) confirmed that lowest Kn values during the more developed gonadal stages may mean resource transfer to the gonads during the reproductive period. According to Da Costa and Araujo (2003), relatively lower Kn values are usually due to the fact that a larger part of the energy is allocated for certain activities such as growth and emptying of ovaries. But moderate Kn values recorded for males in October, August to September during 2003-'04 and October and September in 2004-'05 coincided with the occurrence of increasing gonadosomatic index (GSI) and moderate feeding (Ga.S.I.). But in females, this was observed in August to September during 2003-'04 and September to October in 2004-'05. The correlation between decreased fat deposition in the visceral cavity and decreased condition has been documented in the present study. In many fishes, reserves are used primarily in reproduction

(Shchepkin, 1971; Wootton and Evans, 1976). The increased sexual ripeness and decreased condition has been reasonably well documented in the literature, with increased reproductive activity and depletion of bodily reserves (Stewart, 1988). In tropical fish, condition factor can decrease during the spawning season due to a loss in body weight of approximately 10% (Garcia-Cagide *et al.*, 1983). Thus it appears that reproduction cycle in *C. dussumieri* is related to the variations in the condition factor.

In the present investigation, a comparison of monthly variation of Kn with gastro and gonado somatic indices revealed a highly significant correlation between Kn and gastro somatic index in both the sexes. This suggests that feeding intensity may be the main but not the only factor responsible for the monthly variation in Kn in *C. dussumieri*. It seems that there is an interrelation between feeding intensity and reproduction and these two factors are the most important that influence the Kn. Baragi and James (1980) found it difficult to explain the changes in the condition of the Sciaenid, *Johneops osseus* based on the intake of food and sexual cycle. They suggested that this could depend on several other unknown factors. Seasonal variation in Kn is influenzed by the gonadal development, feeding activity and several other factors (Doddamani *et al.*, 2001). Anibeze (2000) reported that the increased Kn values in *Heterobranchus longifilis* during rains have been attributed to the food availability and gonadal development. In *Labeo boga*, Pervin and Mortuza (2008) confirmed that the fluctuations in Kn value between the sexes was due to several reasons, such as feeding intensity, gravid condition of female or other factors. Mohanraj (2008) inferred that the Kn values obtained less than 1.0 in certain months among male and females *Upeneus sundaicus* and *Upeneus tragula* may be due to poor feeding, breeding or non-availability of suitable food items in the ambient environment. Youson *et al.* (1993) established that in *Petromyzon marinus,* fluctuations in condition factor were influenced by temperature and photoperiod. Studies on Kn of *Catla catla* by Sachidanandamurthy and Yajurvedi (2008) provided an evidence for the fact that water quality parameters in undesirable range interfere with the growth and well being of fish. The present study on the condition of *C. dussumieri* suggests that Kn values were influenced by feeding intensity and reproductive cycle.

With regard to size groups, the fishes belonging to size groups 91-100 to 111-120 mm TL and 91-100 mm TL to 141-150 mm TL in the case of males and females comprised mostly of immature and maturing have high Kn values. This is expected because of high feeding intensity in these size groups. The point of inflection in the Kn curve of different length groups indicates the length at which sexual maturity of fish starts (Hart, 1946). In males and females of *C. dussumieri*, this point, which may be indicative of the commencement of maturation, lies at 121-130 mm TL. The first sharp increase of Kn at 181-190 mm and 151-160 mm length in males and females respectively, may reflect the length at first maturity (see Chapter 5). The high Kn values observed in 131-140 mm TL and 151-160 mm TL size group of males might be due to the occurrence of large number of maturing and mature individuals within that particular range. Lower Kn values beyond 121-130 mm TL size class indicated the appearance of spent individuals. In females, the increase in Kn values after the size group 161-170 mm TL was slower than that in males, might be due to differences in energy budgets during the reproductive season. Palazon-Fernandez *et al.* (2001) reported that Lusitanian toadfish, *Halobatrachus didactylus* reached sexual maturity at 160 mm L_t for males and 191 mm L_t for females.

According to them, males and females of *H. didactylus* matured at different lengths and opined that size at maturity for males was greater than that of females, which may indicate that after first maturation females reduce growth and allocate energy mainly for the production of gametes while males, with a small reproductive effort, continue growing. Similar was reported in toad fish, *Amphichthys cryptocentrus* by Granado and Gonzalez (1988). The above explanation seems to be appropriate in the case of *C. dussumieri.*

An evaluation of the ponderal index (K) and relative condition factor (Kn) values revealed that in the case of both males and females, the two values were closely co-ordinated. The results of the present findings strongly corroborated with the earlier findings that the ponderal index is applicable only if the fish obeys the cube law in its length-weight relationship (Le Cren, 1951). Males, which follow cube law, depicted exactly the same trend in K and Kn values while females, having 'b' value slightly lower than 3 showed the same nature of variation in the values.

Sex-wise analysis of Kn values revealed that males had relatively higher mean Kn values than females (mean 1.0244 ± 0.1555 SE and mean 0.9879 ± 0.1635 SE, respectively). The relative condition factor (Kn) is an expression used to assess the condition of fish, and Kn value 1 or more than 1 is considered as well being of fish (Sachidanandamurthy and Yajurvedi, 2008). Sivashanthini and Abeyrami (2003) reported relatively higher mean Kn values in males than females of *Gerres oblongus* (mean 1.6300 ± 0.0452 SE and mean 1.4109 ± 0.03906 SE, respectively). Joadder (2009) found the mean Kn for males and females of *Glossogobius giuris* to be 1.0555 and 1.0046, respectively and concluded that males were in a slight better condition. Investigation on the mean Kn values of soldierfish, *Myripristis murdjan* (Anbalagan *et al*., 2009) revealed that the values range from 2.0 - 3.9 in male and 2.2 - 2.3 in females respectively. Mohanraj (2008) inferred from the data on the relative condition factor that the males of *Upeneus sundaicus* and *Upeneus tragula* had better robustness than the females. Higher Kn values noticed in males of *C. dussumieri* in the present study suggest that the males are in better condition when compared to females.

The present study provides baseline information on the length-weight relationship and relative condition for *C. dussumieri.* An isometric growth pattern is pronounced only in males. Feeding activity and reproduction cycle are the main factors influencing the condition of the species. It may be concluded that the growth of males is quiet satisfactory and the overall growth performance of males show better growth in relation to weight increment than females.

Table 7.1. Statistical details showing number of fish studied (n), intercept (log a), regression coefficient (b), standard error of b (Sb), correlation coefficient (r) and results of Bailey's t-test on "b" and t- test on correlation coefficient (r)

	n	log a	b	Sb	t	P	r	t	P
Males	248	- 4.6462	2.96	0.0601	0.67	P>0.05	0.9529	49.27	P<0.01
Females	219	- 4.3723	2.85	0.0738	2.04	P<0.05	0.9344	38.64	P<0.01
Pooled	467	- 4.4698	2.89	0.0447	2.51	P<0.05	0.9487	4411.4	P<0.01

Table 7.2. Analysis of variance on the regression of the length-weight relationship in males of *C. dussumieri*

	SS	df	MS	F	P
Regression	30.155	1	30.155	2429.693	P<0.001*
Residual	3.053	246	.012		
Total	33.209	247			

*** Significant at 0.1% level**

Table 7.3. Analysis of variance on the regression of the length-weight relationship in females of *C. dussumieri*

	SS	df	MS	F	P
Regression	17.395	1	17.395	1492.047	P<0.001*
Residual	2.530	217	.012		
Total	19.925	218			

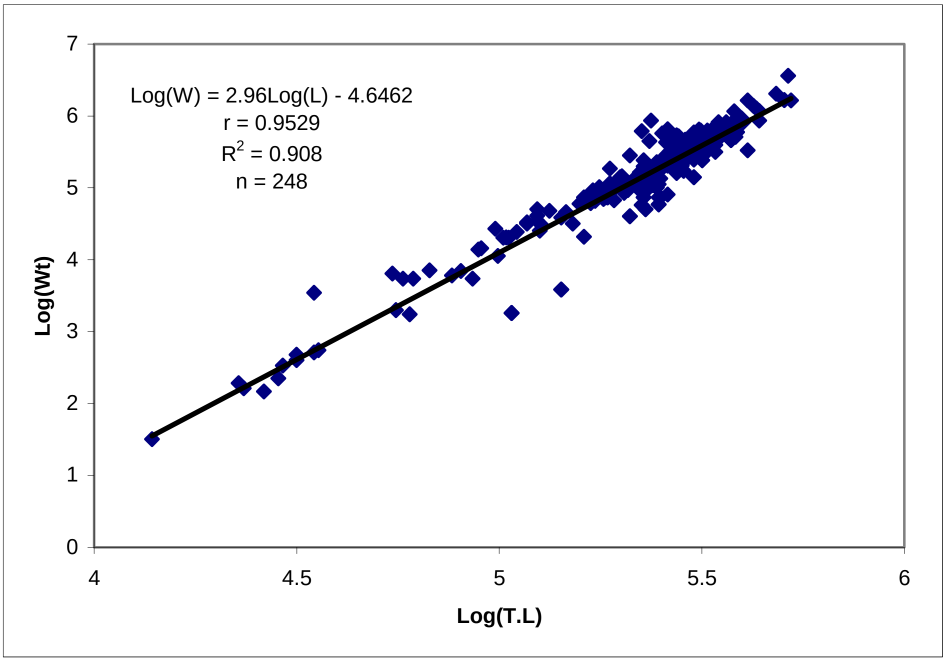
*** Significant at 0.1% level**

Table 7.4. Comparison of regression lines of males and females of *C. dussumieri* by ANCOVA

Source of variation	Degree of freedom	Regression coefficient	Deviation from regression		
			Degree of freedom	Sum of squares	Mean square
Male	248	2.960	247	3.053	0.012
Female	219	2.850	218	2.530	0.012
Total	-	-	465	5.583	0.012
Pooled within	467		466	5.657	0.012
Difference between slopes			1	0.074	0.074
Comparison of slope, (df: 1,467) **F = 6.166** (Significant at 5%)					

Table 7.5. Result of pair wise comparison of regression coefficients of males and females of *C. dussumieri* using t-test

Between	df	t	P
Males - females	465	24.44	Significant



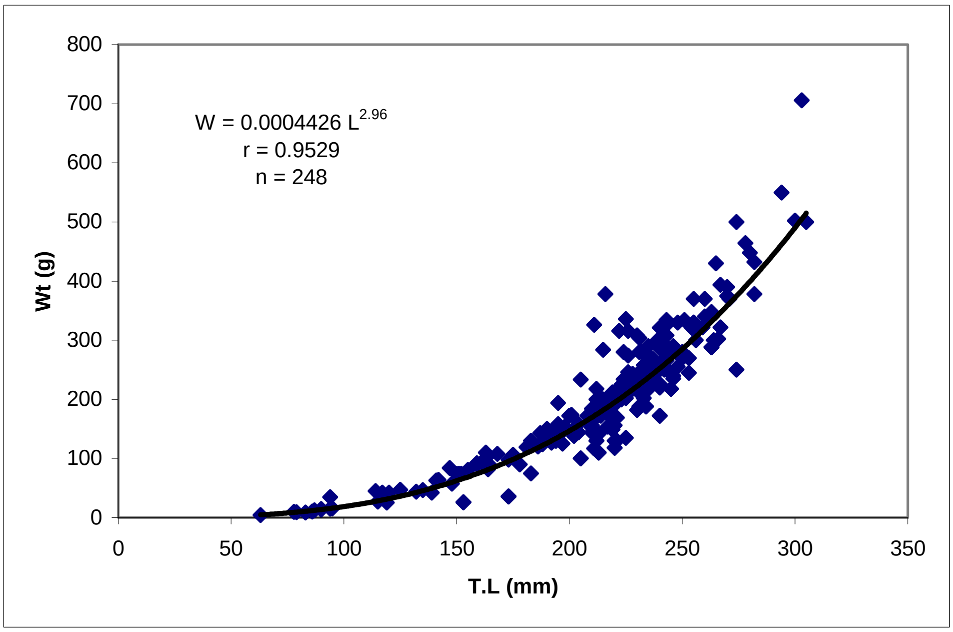
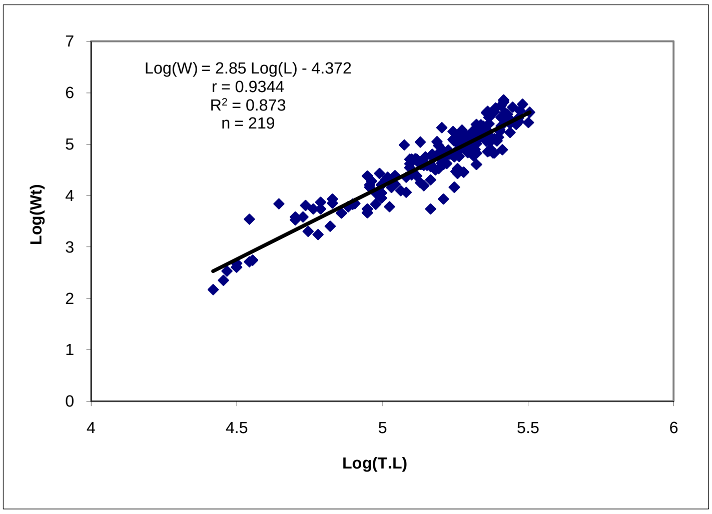


Fig.7.1. Length-Weight relationship in males of *C. dussumieri*.



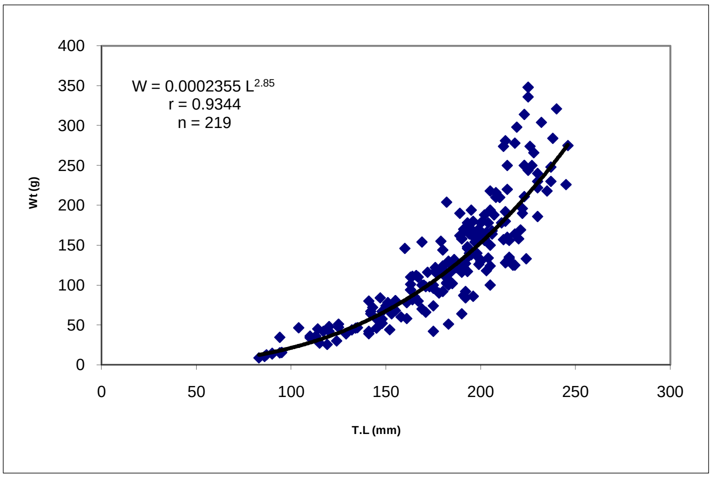


Fig.7.2. Length – weight relationship in females of *C. dussumieri*

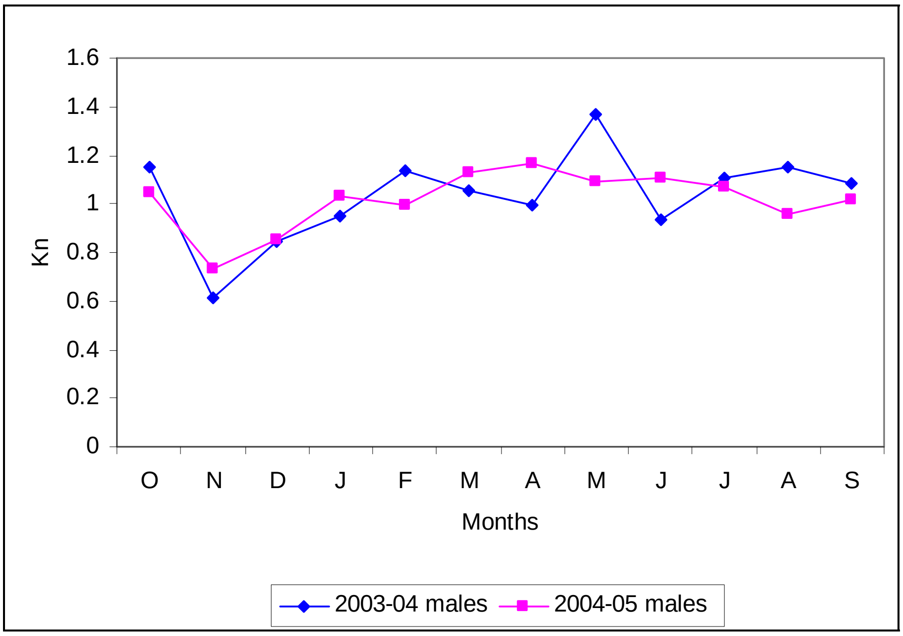


Fig.7.3. Seasonal variation in relative condition factor (Kn) of *C. dussumieri*

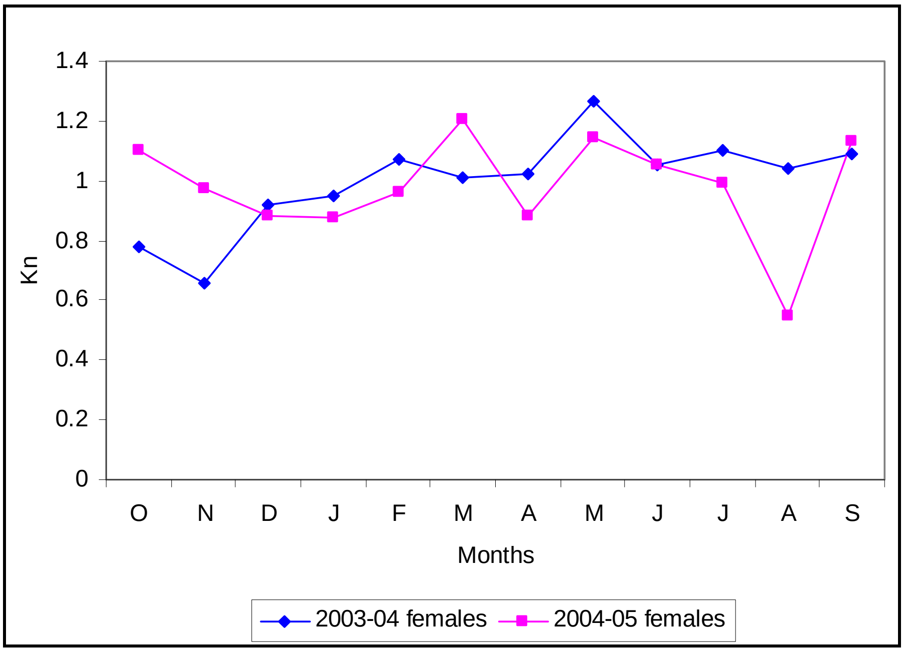


Fig.7.4. Seasonal variation in relative condition factor (Kn) of *C. dussumieri*

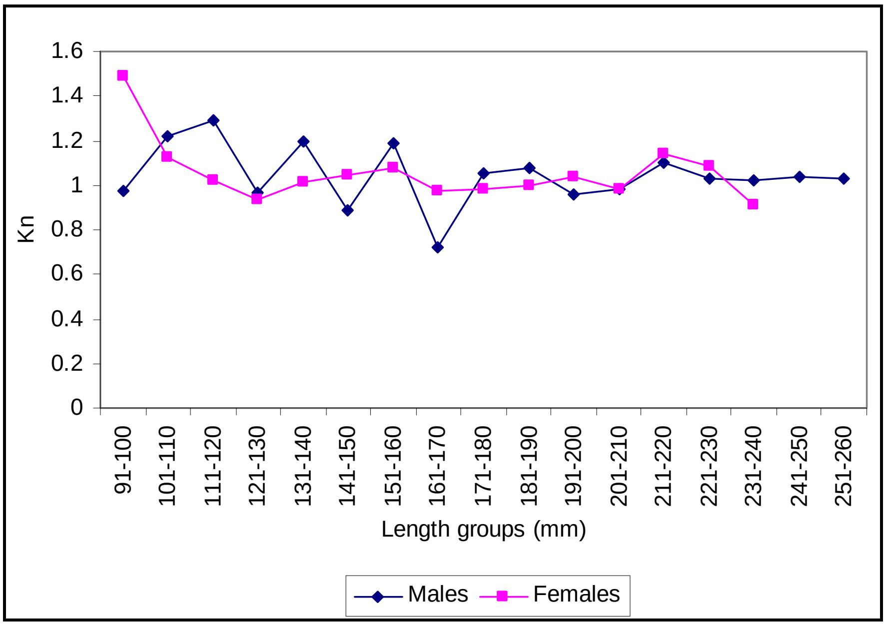


Fig.7.5. Lengthwise variation in relative condition factor (Kn) of *C. dussumieri*

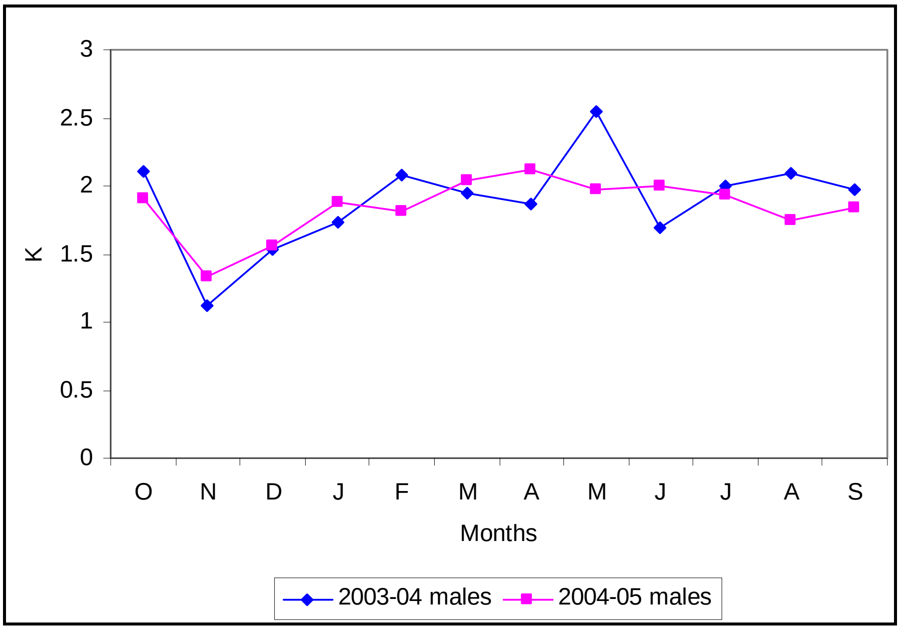


Fig.7.6. Seasonal variation in ponderal index (K) of *C. dussumieri*

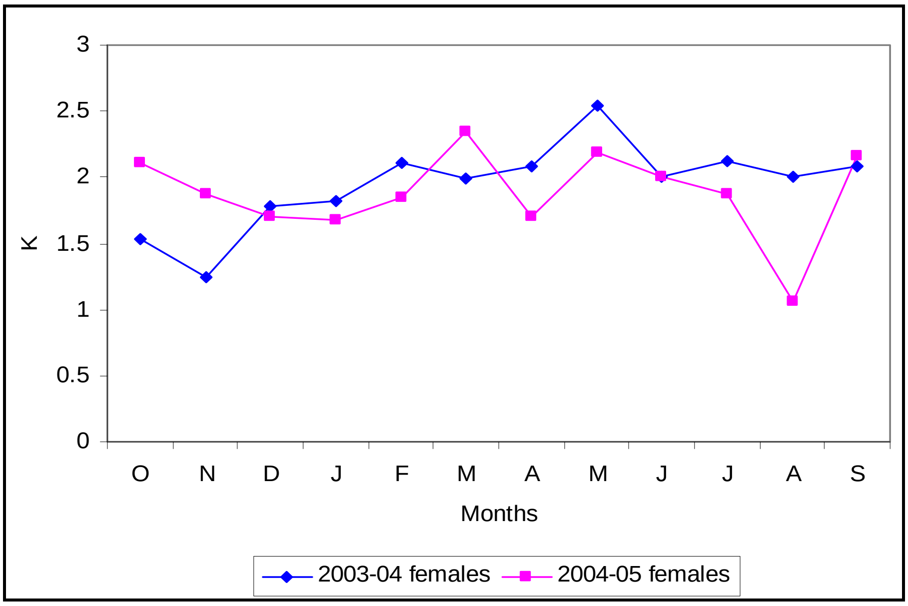


Fig.7.7. Seasonal variation in ponderal index (K) of *C. dussumieri*

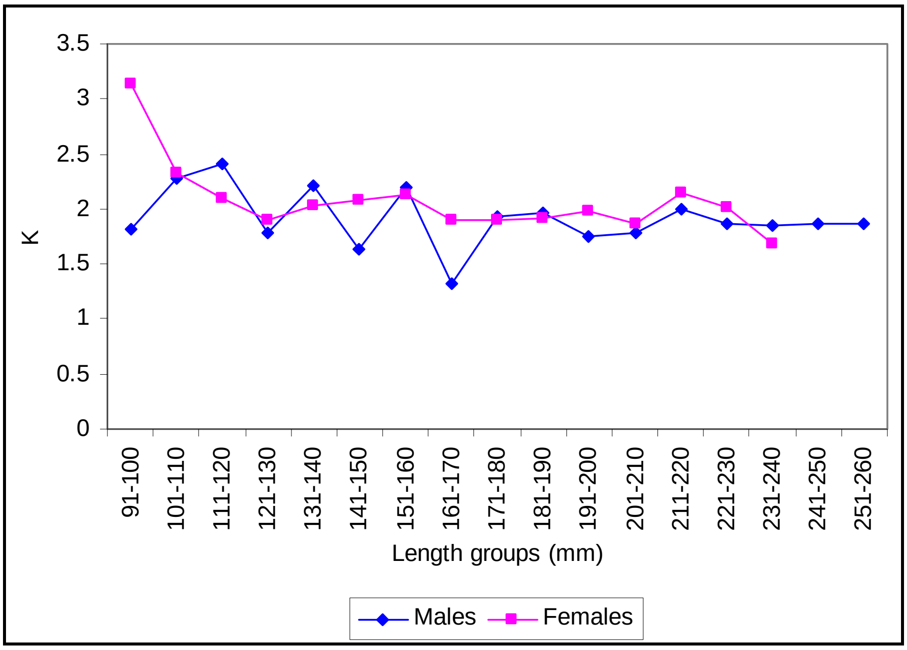


Fig.7.8. Lengthwise variation in ponderal index (K) of *C. dussumieri*

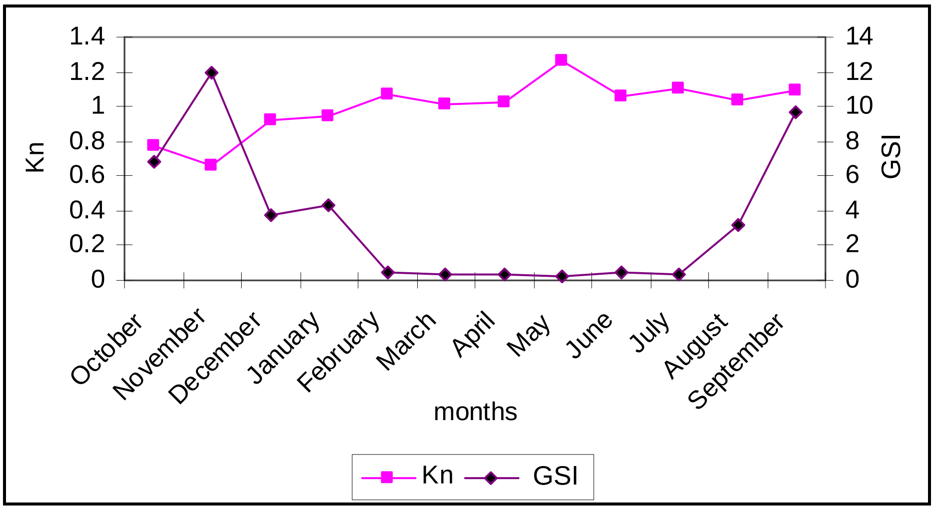


Fig.7.9. Kn-GSI (female 2003 – '04)

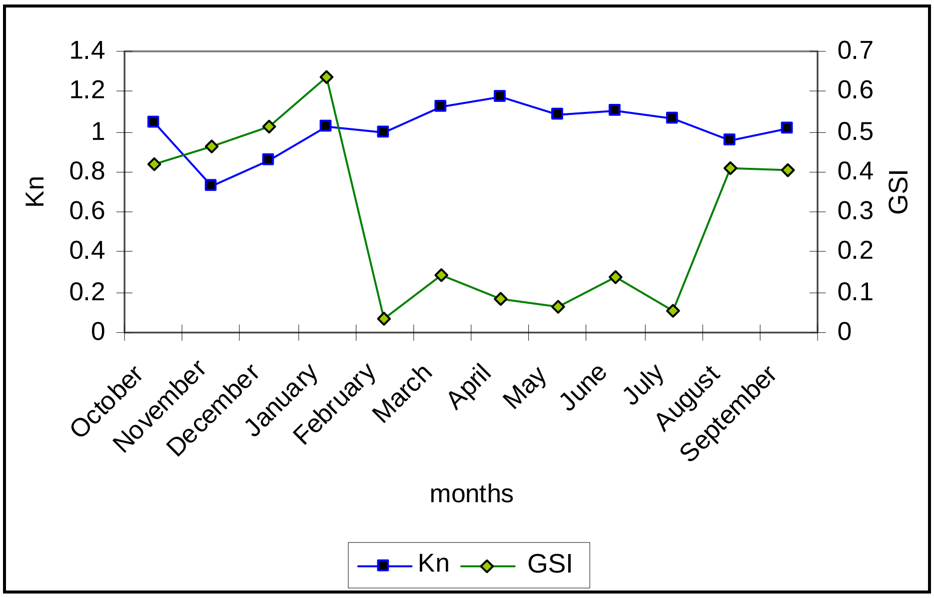


Fig.7.10. Kn-GSI (male 2003 – '04)

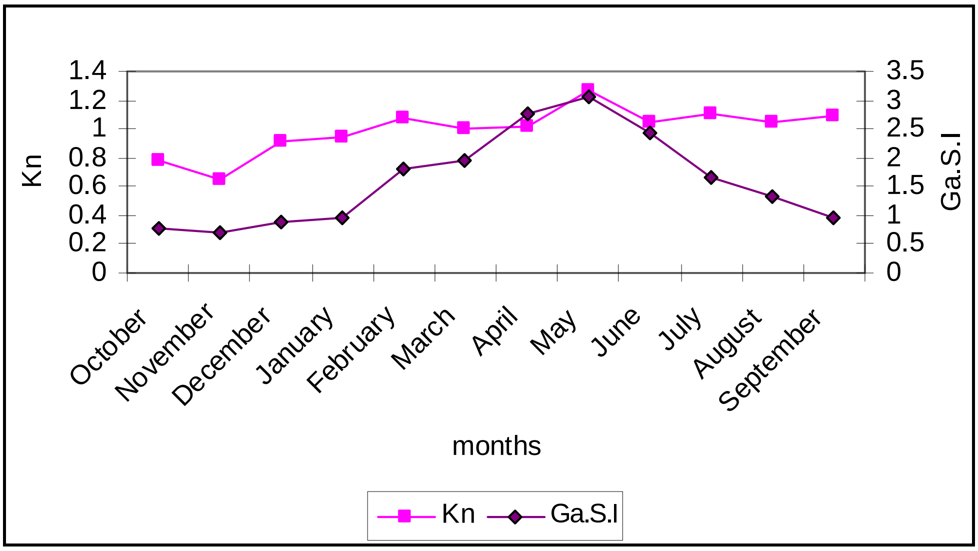


Fig. 7.11. Kn-Ga.S.I (female 2003 – '04)

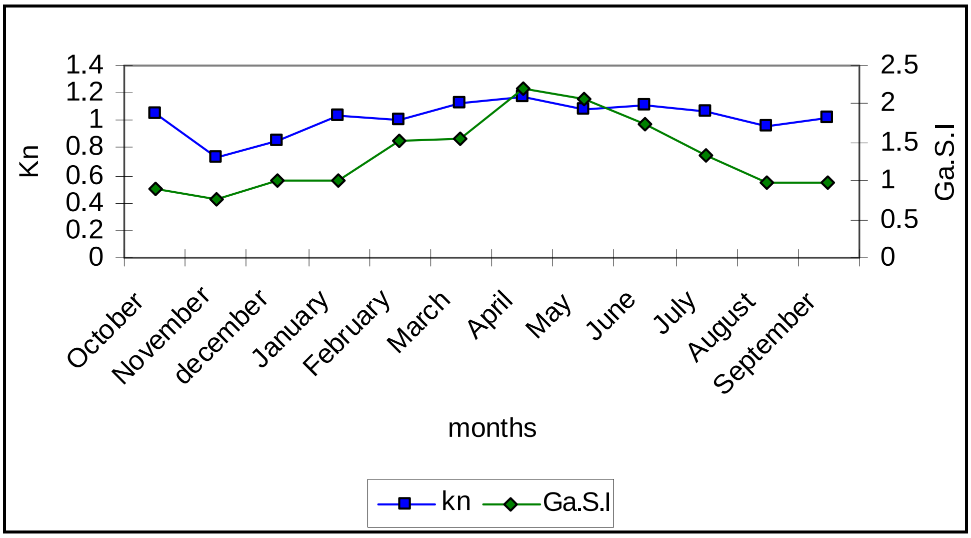


Fig.7.12. Kn-Ga.S.I (male 2003 – '04)

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Chapter 8

BIOCHEMICAL COMPOSITION

Contents

8.1 Introduction

The increase in human population that led to shortage of animal protein sources all over the world has directed the attention to fish as rapid and healthy compensatory source of good quality animal protein. Fishes are quite different from the other animal food sources, because they provide calories with high quality proteins, which contain all essential amino acids in easily digestable form. So they are beneficial nutrition sources (Weatherley and Gill, 1998). Fish mainly assimilates proteins in its muscles. Fish protein has relatively high digestibility and is considered to have high biological and growth promoting value (Shekhar *et al*., 2004). In terms of nutrition, fish is considered as a rich source of protein, good quality fat and micronutrients (Stansby, 1962).

Biochemical studies of fish tissue are of considerable interest for their specificity in relation to the food values of the fish and for the evaluation of their physiological needs at different periods of life. It is also necessary to have the data on the composition of fish in order to make the best use of it as food and also to develop the technology of processing fish and fish products. Generally changes in chemical composition of body have been known to reflect storage or depletion of energy reserves. The values of body composition in fishes vary considerably within and between species, with fish size, sexual condition, feeding, time of the year and activity (Weatherly and gill, 1987). Food composition, environment and genetic trait are also known to influence chemical composition of fish (Oni *et al.*, 1983).

Health benefits of fish meat has been studied extensively and there are reports which confirm its preventive effects against cardiovascular diseases and some types of cancer, including colon, breast and prostate cancer (Rose and Connoll, 1993; Marchioli, 2001; Sidhu, 2003). These effects are largely attributable to the polyunsaturated fatty acids (PUFA) found in fish oils especially the n-3 family including the eicosapentaenoic acid (EPA or 20:5 n-3), the docosapentaenoic acid (DPA or 22:5 n-3) and the docosahexaenoic acid (DHA or 22:6 n-3) which are not synthesised in the human body but their inclusion in human diets is essential (Alasalvar *et al.*, 2002). It is also reported that n-3 PUFA have been recognized as an important component with beneficial properties for the improvement of visual function (Carlson and Werkman, 1996) and also for the prevention of atherosclerosis and thrombosis (Calder, 2003). Although PUFA composition may vary among different fish species of both fresh water and marine origins (Rahman *et a*l., 1995), it is important for human health, to increase the consumption of fish and its products (Sargent, 1997). Moreover bioactive peptides isolated from various fish protein hydrolysates have been shown to exhibit antihypertensive, antithrombotic, immunomodulatory and antioxidative activities (Kim and Mendis, 2006).

Proximate composition of a number of marine, freshwater and brackishwater fishes has been reported (Ramaiyan *et al.*, 1976; Chandrasekhar and Deosthale, 1994; Vishwanath, 1996; Gopakumar, 1997; Nair and Suseela, 2000; Shekhar *et al.*, 2004; Ali *et al.*, 2005; Majumdar and Basu, 2009; Mandal *et al.*, 2010; Shamsan and Ansari, 2010b). However, hitherto no information is available on the biochemical composition of *C. dussumieri.* Hence a pioneer investigation on the biochemical components of this species was taken up to evaluate its nutritional quality.

8.2 Materials and methods

Samples of *C. dussumieri* were collected from Cochin estuary during the period from January 2004 to December 2005. The specimens (200 – 230 mm TL of similar maturity stage) were properly cleaned in the laboratory and the total length, total weight and sex and maturity stages were determined. For biochemical analysis, a portion of the muscle from the widest part of the body (devoid of bones) after removal of skin was taken from males and female separately and used for moisture, protein, fat and carbohydrate determination.

Estimation of Moisture content

Estimation of the moisture content was carried out by drying the pre-weighed wet samples at 60 - 80 °C in a thermostat until a stable weight was reached. The difference in weight was calculated and expressed as percentage moisture content of the sample. Percentage was calculated by the following formula.

$$\text{Percentage (\%) of moisture} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

Estimation of Lipid

Total lipid was estimated by the method of Barnes and Blackstock (1973).

Reagents

Chloroform – Methanol (2:1) mixture, 0.9% NaCl

Procedure

0.5 gm of wet tissue was homogenized in a homogenizer and added 10 ml of chloroform-methanol mixture and mixed well. The homogenate was filtered through a Whatman No.1 filter (wetted with solvent) and purified by shaking with 0.2 volumes of 0.9% aqueous NaCl. It was then transferred to a small separating funnel and after shaking, the biphasic mixture was allowed to stand overnight at 40^0C. The lower phase was then separated, and the volume adjusted to 10 ml by the addition of chloroform. The extracts were transferred to clean and dry sample tube and allowed to dry in a vaccum dessicator. The tube and contents were then weighed and the lipid content was expressed as percentage by the following formula

$$\text{Lipid \% (wet body weight)} = \frac{\text{Weight of lipid (mg)}}{\text{Weight of the sample (mg)}} \times 100$$

Estimation of protein

Protein was estimated by the method of Lowry *et al.* (1951).

Reagents

0.1 N NaOH, Alkaline copper reagent, Folin - Ciocalteau phenol reagent, standard protein solution, Trichloro acetic acid (TCA).

Procedure

After isolating the tissues, 2% homogenates were prepared in 0.33M cold sucrose solution. The homogenates were centrifuged at 1000 x g for 15 minutes. To the supernatant equal volume of 10% TCA was added to precipitate the proteins. It was allowed to stand for 30 minutes at room temperature and centrifuged at 1000 x g for 15 minutes. The sediment was dissolved in 1 ml of 0.1N NaOH. After suitable dilution a known volume of the solution was mixed with 5.0 ml of alkaline copper reagent. Mixed well by shaking vigorously. After 10 minutes, 0.5 ml of Folin – Ciocalteau phenol reagent was added and mixed well. The tubes were kept for another 30 minutes. The absorbance was measured at 500nm, in a spectrophotometer (Hitachi). The system devoid of sample was used as the blank. Bovine serum albumin was used as standard. The values were expressed as mg/g Wet wt. of tissue and the concentrations were expressed as percentage.

Estimation of total carbohydrate

The total carbohydrate was estimated by the method of Carroll *et al.* (1956).

Reagents

10% Trichloro acetic acid (TCA), Anthrone reagent

Procedure

0.5% homogenate of muscle tissue was prepared in 10% Trichloro acetic acid (TCA). The homogenates were centrifuged at 3000 x g for 15 minutes. To 0.2 ml of the supernatant, 5 ml of anthrone reagent was added and boiled for 15 minutes. The tubes were cooled and absorbance measured at 620 nm in a spectrophotometer using blank, which consists 10% TCA and anthrone in the same proportion. The values were expressed as mg of glucose/g Wet wt. of tissue and the results were expressed in percentage. All the values of biochemical components were expressed as percentage wet weight.

Triplicate determinations were carried out on each chemical analysis and mean values were calculated. Analysis of variance (ANOVA) was employed to find out significant differences, if any, in the protein, lipid and carbohydrate contents between males and females of *C. dussumieri*. The relationships between the four biochemical components were tested by computing the correlation coefficient (r). The higher the value of ‘r’, (the closer to unity) the stronger the relation is.

Triplicate determinations were carried out on each chemical analysis and mean values were calculated.

8.3 Results

The proximate composition of males and females of *C. dussumieri* is represented in Table 8.1. Moisture forms the major component of the biochemical composition with an annual average value of 79.75 % and 79.80 % in males and females respectively throughout the investigation period. Protein content of males and females were estimated to be 18.34 % and 18.24 % respectively. Lipid and carbohydrate constituted 1.69% and 0.34% in males but it was 1.63% and 0.28% in females.

The month wise profile of proximate composition of muscle of *C. dussumieri* is given in Table 8.2. The seasonal variation of moisture was in the range of 76.2 – 82.70 % in males and 75.95 – 83% in females. Monthly variations showed highest values of percent moisture in December (82.70% in male, 83% in female) and January (81.63% in male, 82.31% in female) for both the sexes. Whereas, the lowest values for the both sexes were recorded in June (76.2% in male, 75.95% in female) and the decreased values were discerned from May to September. Protein content varied from 15.90 (February) to 20.84% (September) in males and 15.63

(February) to 19.74% (August) for females. In males, seasonal variation of lipid content ranged between 1.31% in February and 2.06% in September. While in females it was from1.32% in February and 2.14% in September. Carbohydrate occurs in a very minute quantity in the fish tissues. Monthly variation showed that carbohydrate fluctuated between 0.15% to 0.48% in males and 0.14% to 0.43% in females.

Analysis of variance on protein, lipid and carbohydrate (Tables 8.3 - 8.5) proved to be insignificant among the sexes.

Table 8.6 illustrates the interrelation between the four components based on correlation coefficient and it derived the following observations.

a) An inverse relationship (negative) exists between moisture and protein contents which were tested to be significant ($p< 0.05$) for both the sexes.

b) A negative relationship exists between moisture and lipid contents, which were detected to be significant ($p< 0.05$) for both the sexes.

c) A strong positive relationship exists between protein and lipid contents, which were tested to be highly significant ($p<0.01$) for males and significant ($p< 0.05$) for females.

d) Other relations such as: moisture/carbohydrate, protein/carbohydrate, lipid/carbohydrate proved to be all insignificant.

8.4 Discussion

The knowledge of chemical composition of any edible organism is extremely important since the nutritive value is reflected in its biochemical contents. The principal constituents of fish and mammals are the same. In general, the biochemical composition of the whole body indicates the fish quality. The principle constituents are water (66 – 84%), protein (15 – 24%), lipids (0.1 – 22%), minerals (0.8 – 2%) and sugar in very minute quantity (0.3%) at maximum value in fishes (Jacquot, 1961). According to Nair and Suseela (2000), the proximate composition of Indian fishes ranges between 65 – 90% water; 10 – 22% protein, 01 – 20% lipid and 0.5–05% minerals.

In the present investigation, the values obtained for C. *dussumieri* were estimated to be in the above range that is, 75.95 – 83% moisture, 15.63 - 20.84% protein, 1.31 - 2.14% fat and 0.14 - 0.47% carbohydrates, respectively. According to Muraleedharan *et al*. (1996), the chemical composition of fish varied from 16-21% proteins, 0.1-25% lipids, 0.4-1.5% ash and moisture 60-81% with extremes of 96%. Abdulrahman and D'Souza (2008) determined the chemical composition of eight species of marine fishes. The moisture content varied between 63.3- 76.0g/100g, protein content between 17.9 - 21.8 g/100g, fat content between 1.1-16.1 g/100g and carbohydrate was absent or negligible ie, between 0.0-0.4g/100 g wet wt. Suseno *et al*. (2010) recorded 70.1 - 72.1% water, 23.0 - 24.8% protein, 1.9 - 4.1% fat, 0 - 1.75% carbohydrate and 1.7 - 2.4% ash in ten species of deep sea fishes. According to Kingston and Venkataramani (1994), knowledge of biochemical composition of fish is of great help in evaluating its nutritive value.

Generally changes in chemical body composition have been known to reflect storage or depletion of energy reserves. Waters (1982) reported that seasonal variation in chemical composition is due to an alternate accumulation and expenditure of fat and protein. The values of body composition in fishes varies considerably within and between species

(Jacquot, 1961), with fish size, sexual condition, feeding, time of the year, activity (Weatherly and gill, 1987; Muraleedharan *et al*., 1996), food composition, environment and genetic trait (Oni *et al*., 1983). Marked changes were observed in the biochemical composition of the muscle of *C. dussumieri* during different seasons of the year, which may be the result of the processes mentioned above.

It is an accepted fact that there exists a strong link between the main energy yielding reserves like fat and protein to the water, the ‘milieu’ of all biological functions. An increase in proportion of one of these constituents leads to the decrease of the other, so that the sum remains approximately constant (Love, 1970). Being the largest constituent of all tissues and the substratum in which all other components are incorporated, any change in the concentration of water in any tissue should have some bearing in the biochemical function occurring in the animal. There must be some internal adjustment for any alteration that happens to the water level. The percentage of water is good indicator of its relative contents of energy, protein and lipids. The lower the percentage of water, greater the lipids and protein contents and higher the energy density of the fish (Gopakumar, 1998; Dempson *et al*., 2004; Aberoumad and Pourshafi, 2010)

Seasonal variation in the moisture content of the body muscles of *C. dussumieri* showed higher values in the moisture content from January – April which coincided with the increase in water salinity. Low moisture was recorded in May. However, with the onset of monsoon, a sharp decrease in value was noticed in June and it continued till September. Elevated values were observed from October to December (Post-monsoon), with the advancement in maturation and spawning activity. The moisture content was higher in females than in males.

The moisture was inversely related to lipid content in the present study. According to FAO, 1999, moisture and lipid contents in fish fillets are inversely related and their sum is approximately 80% with other components accounting for the remaining 20%. This inverse relationship has also been reported in marine fishes such as, *Rastrelliger kanagurta* (Venkataraman and Chari, 1951); *Pseudosciaena aneus and Johnius carutta* (Rao, 1967); *Sparus aurata* (Wassef and Shehata,1991); *Mullus barbatus* (Lloret *et al.,* 2007) and freshwater fishes *Wallagonia attu* (Jafri, 1969), *Ophicephalus punctatus* (Jafri and Khawaja, 1968) and *Clarius batrachus* (Bano, 1977). Majumdar and Basu (2009) obtained a value of -0.987 for the correlation coefficient (r) between moisture and fat content indicating an inverse relationship existing between the two in Indian shad, *Tenualosa ilisha.* Hanna (1984) reported that generally there was an increase in fat and less water content in flesh with increasing size (i.e., increasing age) in *Variola louti.* whereas, Shekhar *et al.* (2004) reported that moisture content did not significantly differ according to season in *Labeo rohita*, and was low when other constituents (Lipid, protein and carbohydrate) were high. Higher percent of moisture during pre-monsoon and post-monsoon when the lipid content was low can be ascribed to similar rapid fall in lipid content during spawning season. This reciprocal relationship between moisture and fat was more pronounced in both males and females.

Islam and Joadder (2005) reported that moisture content of *Glossogobius giuris* increased during summer and late autumn, which coincided with the breeding season of the fish. Seasonal variation in the moisture content of the body muscles of *Sillago sihama* (Shamsan and Ansari, 2010b) showed that the increase in moisture content from January -

May coincided with the increase in water salinity. Further raise in moisture from July until October, with the advancement in maturation and spawning activity could be ascribed to similar rapid fall in lipid content during spawning season and the moisture was inversely related to lipid content. Similarly, in the present study, high values of moisture were discerned during the late post-monsoon (late spawning period) and first half of pre-monsoon when feeding intensity was low (refer chapter 4). The results of the present study revealed that changes in moisture content in the muscle of *C. dussumieri* could be attributed to changes in lipid level directly and to spawning and feeding intensity indirectly.

An inverse relationship between water and protein in the tissues has been documented by many authors. Merayo (1996) reported that during maturation of the gonad in bib (*Trisopterus luscus* L.), the muscle water content increases to the highest levels and the muscle protein content decreases. This was also found in cod, *Gadus morhua*, by Love (1960), who suggested that the decrease in protein and increase in water content of the muscle were the results of gonadal growth taking place at the expense of muscle protein reserves. However, the rising of muscle moisture in *Pylamis* Sp. (El-Sayed, 1979 quoted by Merayo, 1996) during spawning period were assumed to be of endocrine origin. Gonadotrophic hormones increase pre-spawning, therefore, it could be expected that consequent increase in water content might be observed during that period. Analysis of correlation in bib indicated that there was a clear inverse correlation between muscle water and muscle protein of male and female. A negative relationship was known to exist between protein and moisture contents in females and young ones of *Sparus aurata* (Wassef and Shehata, 1991).

Similarly, a significant inverse relationship was observed in both males and females of *C. dussumieri*.

Lipids are the primary energy storage material in fish (Love, 1970; Adams, 1999; Tocher, 2003). Fish store the lipids in various organs; particularly in muscles and liver. Lipid composition and distribution between and within tissues in fish vary from species to species and are influenced by seasonal and dietary variations (Ackman, 1980; Henderson and Tocher, 1987). Thus, the lipid content of a fish indicates the surplus energy available for future maintenance, growth, and reproduction. Lipid content is a good index of future survival in some species (Simpkins *et a*l., 2003) and a strong indicator of reproductive potential in some fish stocks (Marshall *et al*., 1999). Lipid content is an important aspect affecting the flesh taste in many species (Robb *et al*., 2002). The concentration of lipid varies considerably in different parts of the body of the fish (Love, 1970). The lipid content in the body of the fish changes depending on the time of the year (Dawson and Grimm, 1980), environmental conditions (Gill and Weatherly, 1984), stage of maturity of the gonads (Craig, 1977), state of nutrition (Elliot, 1976) and age (Parker and Vanstone, 1966). In fact, total lipid and its composition in fish vary more than any other nutrient component (Thakur *et al*., 2003).

The lipid content and fatty acid composition of Indian marine fish species has been previously reported (Nair and Gopakumar, 1978). The range of lipid content in edible parts is approximately 0.5 to 18%. This depends on seasonal variation in feeding habits and regional differences in basic foods and nutrients (Bulliyya *et al*., 1997). Depending on the level of fat contents, fish can be grouped into four categories: lean fish (<2%), low fat (2-4%), medium fat (4-8%) and high fat (>8%) fish (Ackman, 1989).

Accordingly, *C. dussumieri* having an average 1.63-1.69% fat content can be grouped into the first category (lean fish).

A rise in the fat content of *C. dussumieri* muscles before spawning followed by a gradual decline during spawning was noticed. This is due to low feeding and mobilization of the constituent to gonad development. Reduction in the lipid content during the spawning season has been recorded in *Bregmaceros mclellandi* (Parulekar and Bal, 1969) and M*ugil cephalus* (Das, 1978). Chidambaram *et al.* (1952) observed an increase in fat values before spawning followed by a fall after spawning in the muscle of mackerel. Solomon *et al.* (1984) reported that the body lipid content of rose bitterling, showed an initial increase before the start of spawning, stayed high throughout the spawning season and dropped low at the end of the spawning season and this pointed towards a parallel relationship of fattening cycle with gonadal cycle. The fat content of common carp reached the lowest level in the spent stage, indicating its utilization in spawning activity (Masurkar and Pai, 1979).

Venkataraman and Chari (1951) opined that a plankton rich food increases the fat content on fishes. Dealing with the red mullet, *Upeneus indicus,* Ramaswamy (1955) has also dealt on the influence on the fat value of food on that of fish. Anney (1988) reported lower values of fat in the carnivorous fish *Megalops cyprinoides* when compared to *Scatophagus argus*, an omnivore. The low lipid content value in *Tilapia guineensis* and *Tilapia melanotheron* might be as a result of the environment, species and the type of diet the fishes feed on (Abimbola *et al*., 2010). Omotosho (1996) found that lean flesh with a significant low fat content in *Dasyatis margarita*, might be a reflection of the influence of the food taken and also its physiological inability to store an appreciable quantity of fat in the body. It was reported that active fish stored their lipids in muscle tissues; but mostly inactive fish living at the bottom of water store their lipids in liver (Castell *et al.*, 1972). The results of the present study corroborate the above findings as *C. dussumieri* showed sluggish bottom dwelling habits. Low fat content of the muscle is recognized due to their carnivorous feeding nature (refer chapter 4).

Fish protein contains all essential amino acids which are easy to digest. The protein digested and assimilated is mostly incorporated in the muscles of the fish (Dabhade *et al.*, 2009). Ali (2001) reported that protein content, which is a vital constituent of living cells, tends to vary relatively little in healthy fish unless drawn upon during particular demands of reproduction or during food deprivation periods. Similar to fat, the protein content in the body of fishes change depending on time of year, environmental condition, stage of maturity of the gonads, state of nutrition and age.

Protein was the most dominant biochemical constituent in the muscle of *C. dussumieri*. Protein values were observed to increase from March and reached the peak value in September when maximum numbers of mature fishes were found. Decline in protein content were noticed from October (spawning period) to February, coincided with post-spawning period. Such depletion in muscle-protein during spawning period has been reported in many fishes (Love, 1970). Selvaraj (1984) reported depletion in muscle protein in *Ilisha melastoma*. According to him, it might be due to the fact that the build-up of gonad is often accomplished at the expense of body-proteins. Jafri and Khawaja (1968) reported protein cycle in *Ophicephalus punctatus* and showed correlation between feeding and spawning. Shreni (1980) stated that the protein cycle in fishes (*Heteropneustes fossili*) can be synchronized with maturity of fishes than feeding. Decline of protein has also been reported by Srikar *et al.* (1979) in *Clarias batracus*; Somavanshi

(1983) in *Garra mullya*; and Luzzana *et al.* (1996) in *Coregonid bondella.* According to Dabhade *et al.* (2009), in *Channa gachua* muscle protein started declining gradually during spawning and post-spawning phases. This decline of muscle protein was attributed to its transfer into ovaries to meet energy requirement of fish during spawning and post spawning phases. In the present study muscle protein content in *C. dussumieri* can be correlated with the phases of maturity and spawning when the gonads were ripe and decline during spawning and post-spawning period.

Omotosho (1996) opined that a relative high protein recorded for *Dasyatis margarita* could be associated with its high preference for shrimps as its main diet. In *Clarius gariepinus*, (Osibona *et al.*, 2009) high tissue protein content resulted from the equally high protein content of their diets (fish items, crustaceans, molluscs, algae and diatoms). Similarly in *C. dussumieri,* high protein content is due to its preferences in food, feeding mainly on crustaceans (refer chapter 4).

Beamish *et al.* (1979) recorded a large fluctuation in the proximate composition of the muscle, gonad, liver and whole body of various stages in the adult phase of the life cycle of the anadromous sea lamprey which was related to feeding, migration and reproduction of the animal. The data indicated that protein, as well as lipid was extensively catabolized during the spawning period. Vijayakumaran (1979) observed increase in the energy reserve, in the form of lipids and proteins and its depletion due to spawning in *Ambasis gymnocephalus*. Bhuiyan *et al.* (2003) reported higher fat and protein content in ripe and gravid fish whereas a low level of fat and protein was recorded in spent and young fish. The feeding intensity does not appear to have any effect on the protein content. Well–marked seasonal variations were observed in the concentration of different chemical constituents in *Clarius* *batrachus* and these changes have been attributed to factors like feeding and gonad maturation. The low values recorded for protein and fat during the spawning period presumably indicate mobilization of these constituents towards gonad development. Similarly, an increase in the concentration of these constituents during the post-spawning months might be due to the recovery of the fish from the strenuous act of spawning (Bano, 1977). Similarly in *Sparus aurata* (Wassef and Shehata, 1991), the lower values of lipids and protein contents of adults in winter months, particularly December, which coincided with the peak of spawning probably due to the consumption of such materials for gonadal development. Hence, a positive relationship was found between lipid and protein contents, which were detected to be significant for females of *Sparus aurata* (Wassef and Shehata, 1991). Intensive feeding in *C. dussumieri* in April to June and moderate feeding in July to September (pre-spawning- refer Chapter 5) coincides with relatively high values of lipid and protein. With the commencement of spawning season in October, feeding activity also decreased and remained so until December. There was a parallel decrease in the muscle lipid and proteins during spawning season. These could be attributed to less food intake and recruitment of these constituents towards gonad development. Likewise in the present study, a highly significant parallel relationship exists between lipid and protein contents for both male and female sexes.

Carbohydrates formed a minor percentage of the total composition of the muscle. Carbohydrate content was slightly high in *C. dussumieri* male than the female. Nargis (2006) reported similar findings in *Anabas testudineus*. The low values of carbohydrates recorded in the present study supports the view that carbohydrate plays an insignificant role as energy reserve in aquatic animals (Love, 1970). Vijayakumaran (1979) stated that carbohydrate plays a minor part in the energy reserves of *Ambassis gymnocephalus* and the depletion due to spawning is also negligible when compared to lipid and protein. Selvaraj (1984) reported maximum concentration of carbohydrate in the liver (2.0 - 2.25%) and minimum content in the muscle (0.2 - 0.5% wet weight) of *Ilisha melastoma* and since it is in very low concentration in the muscle, liver and gonad, he affirmed that its role in the mobilization of energy during maturation and spawning may be negligible.

The importance of a species depends on its nutritional value. Stansby and Olcott (1963) classified the fishes into five types based on the fat and protein contents in the muscle.

Category	Type	Oil Content (%)	Protein Content (%)
A	Low oil- high protein	Less than 5	15-20
B	Medium oil- high protein	5-15	15-20
C	High oil – low protein	More than 15	Less than 15
D	Low oil- very high protein	Less than 5	More than 20
E	Low oil- low protein	Less than 5	Less than 5

According to the present results, *C. dussumieri* can be included under the category A, the oil content recorded was under 5% (1.31% – 2.14 %) and the maximum protein content recorded was 20.84%. Despite the low economic value of batrachoid fishes, it could be concluded that *C. dussumieri* can be referred to as high protein, low-oil, bony fish and variation in biochemical composition in present study seems to be governed by spawning cycle and feeding activity.

Table 8.1. Proximate composition in males and females of *C. dussumieri*

	Moisture %	Protein %	Lipid %	Carbohydrate %
Males	79.75	18.34	1.69	0.34
Females	79.80	18.24	1.63	0.28

Table 8.2. Seasonal variations in proximate composition of males and females of *C. dussumieri*

MONTHS	MALES				FEMALES			
	Moisture %	Protein %	Lipid %	Carbohydrate %	Moisture %	Protein %	Lipid %	Carbohydrate %
January	81.63	17.44	1.51	0.36	82.31	16.05	1.33	0.32
February	81.22	15.90	1.31	0.47	81.52	15.63	1.32	0.24
March	80.23	17.42	1.61	0.38	80.81	18.14	1.57	0.34
April	80.01	18.14	1.65	0.34	80.26	18.36	1.63	0.27
May	79.20	19.21	1.76	0.41	79.9	19.49	1.64	0.43
June	76.20	19.88	1.85	0.28	75.95	19.61	1.77	0.34
July	77.70	19.19	1.77	0.33	76.00	19.30	1.74	0.35
August	78.80	20.58	1.86	0.45	78.40	19.74	1.81	0.20
Sept.	78.46	20.84	2.06	0.48	79.20	19.53	2.14	0.24
October	80.00	16.74	1.83	0.18	80.20	17.31	1.79	0.28
Nov.	80.81	18.14	1.55	0.15	80.10	18.62	1.36	0.17
Dec.	82.7	16.57	1.46	0.22	83.00	17.09	1.43	0.14

Table 8.3. Analysis of variance on protein content in the muscle tissue of males and females of *C. dussumieri*

Source of variation	SS	df	MS	F	Significance F	p
Between Groups	0.283837	1	0.283837	0.112029	4.300949	Insignificant
Within Groups	55.73936	22	2.533607			
Total	**56.0232**	**23**				

Table 8.4. Analysis of variance on lipid content in the muscle tissue of males and females of *C. dussumieri*

Source of variation	SS	df	MS	F	Significance F	p
Between Groups	1295.511	1	1295.511	0.987847	4.300949	Insignificant
Within Groups	28851.88	22	1311.449			
Total	**30147.39**	**23**				

Table 8.5. Analysis of variance on carbohydrate content in the muscle tissue of males and females of *C. dussumieri*

Source of variation	SS	df	MS	F	Significance F	p
Between Groups	0.026004	1	0.026004	1.961513	4.300949	Insignificant
Within Groups	0.291658	22	0.013257			
Total	**0.317663**	**23**				

Table 8.6. Interrelation between muscle's moisture, protein, lipids and carbohydrate contents for *C. dussumieri*

Sex	Variables	r	$p \leq 0.05$	Significance
Male	a) Moisture / Protein	-0.774	*	S
	b) Moisture / Lipid	-0.762	*	S
	c) Moisture / Carbohydrate	-0.199	-	IS
	d) Protein / Lipid	+0.829	**	S
	e) Protein / Carbohydrate	+0.352	-	IS
	f) Lipid / Carbohydrate	+0.159	-	IS
Female	a) Moisture / Protein	-0.758	*	S
	b) Moisture / Lipid	-0.601	*	IS
	c) Moisture / Carbohydrate	-0.366	-	IS
	d) Protein / Lipid	+0.705	*	S
	e) Protein / Carbohydrate	+0.228	-	IS
	f) Lipid / Carbohydrate	+0.165	-	IS

r = correlation coefficient, $p \leq 0.01$ significant (**), $p \leq 0.05$ significant (*),
IS = insignificant, S = Significant

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Chapter 9

SUMMARY AND CONCLUSION

This is the first study investigating detailed biological aspects of flat toadfish, *Colletteichthys dussumieri* of Cochin estuary. They have no commercial importance in fisheries but significantly sound management of vegetated coastal resources relies on the basic knowledge on the biology of the species, including information on population structure. The study was undertaken for a period of twenty four months from October 2003 to September 2005. The highlights of the results are as follows:

Systematics

A detailed description of *Colletteichthys dussumieri* is furnished along with their synonyms. Discrepancies in meristic (lateral line pores and vertebrae) characters were observed.

Morphometrics

Growth variability in fourteen morphometric characters of *C. dussumieri* were evaluated and linear regression analysis was applied to calculate growth rate of each variable in relation to total length. The equations explaning each morphometric variable versus total length of males and females are:

Standard length	Y = -2.6107 + 0.832 X	Y = 3.457 + 0.7964 X
Head length	Y = 0.478 + 0.3373 X	Y = -1.438 + 0.3459 X
Snout length	Y = 2.157 + 0.064 X	Y = 1.271 + 0.072 X
Postorbital length	Y = 2.467 + 0.2109 X	Y = -3.686 + 0.2328 X
Interorbital length	Y = -6.0803 + 0.141 X	Y = -6.7264 + 0.1413 X
Eye diameter	Y =1.192 + 0.0442 X	Y = 2.872 + 0.0389 X
Pre first dorsal fin	Y = -1.042 + 0.3146 X	Y = -4.1869 + 0.3351 X
Pre second dorsal fin	Y = 4.703 + 0. 3843 X	Y = -5.0245 + 0.4366 X
Pre pectoral length	Y = -1.0814 + 0.294 X	Y = 5.494 + 0.2614 X
Pre pelvic length	Y = 5.796 + 0.2153 X	Y = -8.098 + 0.279 X
Pre anal length	Y = -10.271 + 0.5658 X	Y = -6.180 + 0.5531 X
Body depth	Y = -10.57 + 0.276 X	Y = -7.879 + 0.2688 X
Depth through anal fin	Y = -4.227 + 0.236 X	Y = -7.515 + 0.2567 X
Caudal peduncle depth	Y = -0.0142 + 0.0828 X	Y = 2.1972 + 0.0733 X

(X denotes total length and Y the other variables)

Established correlation coefficients (r) showed positive significant correlation ($P<0.01$) in both the sexes except for the values for snout length in females ($p<0.05$). The highest correlation coefficient was observed between total length and standard length ($r = 0.996$) in male and ($r = 0.988$) in female. The lowest was between snout length and eye diameter ($r = 0.497$) in male and snout length and pre-pelvic length (r = 0 .339) in female. Comparison of regressions of the characters between male and female showed significant differences in all the characters except for head length, snout length, inter-orbital length and body depth. So the analysis of morphometric variables demonstrated a substantial degree of differences between the sexes concerning either the growth pattern or the rate of change of some variables.

Food and Feeding

Study on food and feeding habits of *C. dussumieri* pointed towards its carnivorous nature. Analysis of its gut content based on frequency of occurrence, index of preponderance and relative gut length measurements revealed that crustaceans mainly prawns and crabs and teleostean fishes make up the bulk of the diet. At times bivalve molluscs were also encountered in gut. It is apparent that composition and preferences of food are the same in both sexes. But observations on the average amount of feeding in both sexes show that males have a little higher feeding intensity than females.

Feeding intensity was high in early maturity stage and was relatively lower in fish with ripening gonads. High feeding intensity was observed in immature and juvenile fish in comparison to the matured ones. The present study indicated that C. *dussumieri* is a stenophagic fish feeding on a few selected organisms despite the presence of large number of organisms in their vicinity. The results revealed that the fish is “Carnivorous-stenophagic-bottom feeder”.

Reproduction

In the light of the morphological and histological changes of gonads, five maturity stages were established in *C. dussumieri* :- (a) immature / resting, (b) maturing virgins / recovering spents, (c) mature / ripening, (d) ripe and (e) spent. The complete demarcation of a group of ova in the mature ovary and gonadosomatic index of *C. dussumieri* gives the impression that individual fish spawns only once in a year between September to January with a peak in October to November.

Size at 1st maturity was 131 mm TL for males and 141 mm TL for females. Size at 50% maturity was estimated to be 182 mm (181-190mm)

TL for males and 151mm (151-160 mm) TL for females, corresponding to ≥2 years old. The overall male: female ratio was 100:89. The Chi-square value did not show any significant difference indicating equal distribution of both sexes.

Fecundity of *C. dussumieri* varied from 150 to 859. The coefficient of correlation of the various statistical relationships derived between fecundity, body length, body weight, ovary length and ovary weight revealed significant relation between fecundity and other body parameters, indicating *C. dussumieri* to be a low fecund fish.

Age and growth

Age, growth and mortality of *C. dussumieri*, were determined by examination of the whole sagittal otoliths. Marginal increment analysis of otoliths revealed that increments formed on the otoliths were deposited once a year, translucent formed between September and November coincident with the maximum reproductive peak, while the opaque zone formed mainly from February to June when growth is faster. Maximum age limit for males and females were 9 and 7 years respectively. Males matured at an age of approximately 2 years and females took a little more than 2 years. Fish total length and otolith radius were strongly related in both males (R^2 = 0.889) and females (R^2 = 0.815). The von Bertalanffy growth curve was used to describe growth. The parameters were derived from back-calculated length-at-age. Significant differences in growth parameters were found between sexes (in males $L\infty$=351 mm, K= 0.168 and t_0 = -0.81; in females $L\infty$=303mm, K= 0.216 and t_0 = -0.65). Longevity of males was greater than that of the females and males reached larger sizes than females. The growth performance index of the specie falls within the values mentioned of those estimated for other members of the batrachoid family. Natural mortality rate was found to be higher in females than in males.

Length-weight relationship and condition factor

The length –weight relationship was calculated separately for males and females.

Male: $\text{Log } W = -4.646 + 2.96 \text{ Log } L$
Female: $\text{Log } W = -4.372 + 2.85 \text{ Log } L$

The correlation coefficients ‘r’ 0.953 for male and 0.934 for female were found to be significant ($p < 0.001$) in both instances indicating good correlation between length and weight. Covariance analysis for length-weight relationships of male and female fishes revealed that there exists a significant variation in this aspect between male and female fishes. By testing (students ‘t’ test) the regression coefficients against the isometric value of 3, it was found that for males the growth pattern agrees with the isometric growth formula while in the case of the females the cubic formula was not found to be a proper representation of the length-weight relationship.

The seasonal variation in Kn values of males and females showed almost similar trend. Sex-wise analysis of Kn values revealed that the mean Kn values in males (1.032 and 1.017) were higher than those of females (0.996 and 0.979) for both the years. The ponderal index (K) showed the same trend as that of relative condition factor. Feeding activity and reproductive cycle are the main factors influencing the condition of the species. The relative condition of fish shows seasonal variation, with males generally being in better condition than the females.

The growth of males is quiet satisfactory and the overall growth performance of males show better growth in relation to weight increment than females.

Biochemical composition

Sex-wise seasonal variation in the proximate composition of the muscles of *C. dussumieri* were estimated. On fresh weight basis, percentage protein ranged from 15.90 - 20.84%, lipids from 1.31-2.06%, moisture 76.2 - 82.70% and carbohydrate from 0.15-0.48% in males. While in females, protein ranged from 15.63 - 19.74%, lipid 1.32 - 2.14%, moisture 75.95 - 83% and carbohydrate 0.14 - 0.43%. The results revealed that moisture content was inversely related to protein and lipid content. A highly significant parallel relationship exists between lipid and protein contents for both male and female sexes. The carbohydrate does not contribute much to the reserve in the body. According to the present results, the oil content recorded was less than 5% (1.31% – 2.14%) and the maximum protein content recorded was 20.84%. Despite the low economic value of batrachoid fishes, it could be concluded that *C. dussumieri* can be referred to as high protein and low-oil bony fish. Variations in proximate composition in present study seem to be governed by spawning cycle and feeding activity.

It is a well known fact that the knowledge on fish biology particularly morphometry, food and feeding habit, reproduction, age and growth, length-weight relationship, condition factor etc. is of utmost importance, not only to fill up the lacuna of our present day academic knowledge but also in the utility of the knowledge in increasing the technological efficiencies of the fishery entrepreneurs for evolving judicious pisciculture management. Poor understanding of the biology of fishes in a fishery management could lead to dramatic changes in the biological attitudes and productivity of species (Smith *et al.*, 1991). It is, therefore, essential that an appropriately weighted and conservative approach be adapted for the management of *C. dussumieri* of Cochin estuary. It is also paramount and essential in the long term that morphometric, meristic and genetic data be gathered quickly and disseminated on the specific populations, upon which assessments are made, in order to track changes which will affect the stability of the population over time. The genetics of fish oocytes is at present a much neglected yet vital area of study. Hence, studies focusing on the molecular mechanisms controlling oocyte growth and development are to be initiated. Information on early embryonic and larval development and organogeny is of critical importance in understanding the basic biology of a particular species and their dietary needs and environmental preferences (Borcato *et al.*, 2004). Consequently, studies on the detailed embryonic and larval development of *C. dussumieri* are indispensable. Further studies are required on the ethno- medicinal value of the species with regard to asthma cure. The present study provides the first baseline data on various aspects of biology of *Colletteichthys dussumieri* of Cochin estuary.

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