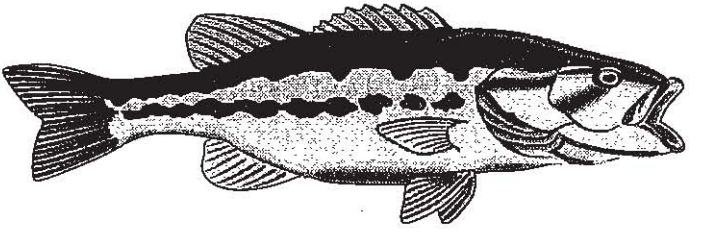
"GUTSHOP '96"

Feeding Ecology and Nutrition in Fish

Symposium Proceedings

Don MacKinlay

Karl Shearer



International Congress on the Biology of Fishes

San Francisco State University July 14-18, 1996

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Feeding Ecology and Nutrition in Fish Symposium Proceedings

Don MacKinlay
Karl Shearer

Physiology Section AFS *American Fisheries Society*

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PREFACE

Twenty years have passed since the first GUTSHOP was held in Astoria, Oregon in October 1976. Although I don't have access to the proceedings from GUTSHOP 76, I was able to obtain those from GUTSHOP 78 in which there were 26 papers grouped into the following topics :

Methodology and Taxonomy	6	Competition	5
Statistical Analysis	7	Modeling	2
Trophic Structure	6		

As I prepare this brief introduction to GUTSHOP 96 there have been 31 oral and poster papers submitted which have been placed into the following categories:

Gut Morphology and Digestion	7	Feeding Ecology	8
Modeling and Methodology	7	Nutrition	9

Based on the titles of the papers submitted in 1996 it appears that modeling and methodology still represent major areas of interest on the other hand, there appears to have been a reduction in the number of studies examining the taxonomy of food organisms and conversely, an increased interest in digestion. The titles this year also include nutrition papers that cover both food (natural prey and field studies) and feed (artificial diets and controlled laboratory experiments).

Finally, while earlier GUTSHOPS consisted primarily of contributions of local origin, GUTSHOP 96 as part of the International Congress on the Biology of Fishes, truly reflects the international interest in "Fish Food Habits Studies".

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CONGRESS ACKNOWLEDGEMENTS

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Don MacKinlay

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Gut Morphology and Digestion

DIGESTIVE ENZYMES AS AN INDICATOR OF FEEDING ECOLOGY OF WILD FISH

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Introduction

The comparison of the activities of some enzymes involved in cavital and membrane-linked digestion in various fish species has demonstrated the correlation between the type of feeding and the level of the enzymatic activity. Carnivorous fish have as a rule high protease activity while omnivorous and herbivorous fish demonstrates high carbohydrase activity (Ugolev & Kuz'mina, 1993a, 1994). In recent years it has been established a relationship between the properties of fish digestive enzymes and environmental temperature which determined by many conditions in particular by temperature range of fish feeding (Ugolev & Kuz'mina, 1993b). The aim of this investigation is to study the correlation between food spectrum or biochemical food composition, motive feeding activity, as well as temperature conditions of fish feeding and a number of their enzyme characteristics.

For the purpose of revealing an indicator of fish feeding ecology 37 species of sea and freshwater cartilaginous, sturgeons and bony fishes were studied. It was investigated two basic ichthyocenoses, namely the Black Sea fish community and the Volga river fish community. Both the sea and freshwater fish species belong to ecological groups of different feeding mode - ichthyophages, planktophages, benthophages, phytoplankto-benthophages, phyto-benthophages, euryphages, plankto-ichthyophages and ichthyo-benthophages (Svetovidov, 1964; Poddubny & Gallat, 1995).

Results

Effect of food spectrum and biochemical food composition. Different species of fish show insignificant variation in the proteinases activity (Fig.1). Proteolytic activity in cartilaginous varies from 1.1+0.5 μmol/min in benthophage buckler skate to 3.2+0.9 μmol/min in ichthyophage spiny dogfish. At the same time the variation of enzyme activity in marine teleosts had a greater range with a minimal level found in benthophage ichthyophage flounder (0.5 μmol/min) and maximal one found in plankto-ichthyophage Black Sea Scad (4.5 μmol/min). For most of the studied fishes the values were 2.0-4.0 μmol/min (red mullet, whiting, black umber, high-body pickerel, gray mullet, gilheat, rocking, barfish, bluefish) while for there benthophage species (flounder, comber, ratan goby) it less than 2.0 μmol/min and for another three ichthyophages (scorpion, Black Sea scad, toad goby) over 4.0 μmol/min. The total proteolytic activity of intestinal mucosa

in freshwater fish was higher. In teleost it changed from 2.2+0.5 μmol/min for benthophages carp and roach to 6.0+0.8 μmol/min for ichthyophage pike. In the most benthophage and ichthyophage - benthophage species the activity level varied from 3.0 to 5.0 μmol/min. In sturgeons the total proteolytic activity was rather high too (3.9+0.5 μmol/min for sturgeon and 6.1+0.7 μmol/min for sterlet).

The activity of carbohydrases varied considerably in different species especially teleosts, depending on their food. In particular in marine teleosts the amylolytic activity varied from 0.7+0.2 μmol/min for ichthyophage whiting to 4.1+0.5 μmol/min for phyto-benthophage gilthead. However the relationship between carbohydrases activity and the type of feeding was not strongly pronounced in this fish group. The amylolytic activity in the intestinal mucosa of freshwater teleosts varied more considerably: from 0.2+0.1 μmol/min for ichthyophage pike to 12+0.2 μmol/min for phyto-benthophage crucian-carp (Fig.2). It is interesting that the acivity level rose gradually in the folloing order: ichthyophages → ichthyophages-benthophages → benthophages → phyto-benthophages.

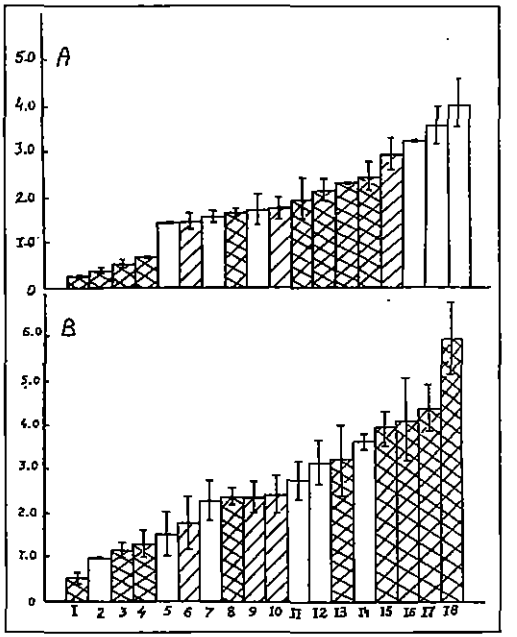

Figure 1. Total amilolytic (A) and total proteolytic activity in some species in the Black Sea fish.

Vertical, enzyme activity, μmol × g^{-1} × min^{-1}, A: 1-spiny dogfish, 2-buckler - skate, 3-stingray, 4-rockling, 5-whiting, 6-gray mullet, 7-comber, 8-bluefish, 9-Black Sea scad, 10-black umber, 11-gilthead, 12-high-body pickerel, 13-red mullet, 14-barfish, 15-toad goby, 16-ratan goby, 17-scorpion, 18-flounder. B: 1-flounder, 2-comber, 3-stingray, 4-buckler skate, 5-ratan goby, 6-spiny dogfish, 7-red mullet, 8-whiting, 9-black umber, 10-high-body pickerel, 11-gray mullet, 12-gilthead, 13-rockling, 14-barfish, 15-bluefish, 16-scorpion, 17-toad goby.

The most interspecies differences were found for protease activity carbohydrase activity ratio (stingray - 0.4, buckler skate - 2.1, spiny dogfish - 6.5; sturgeon - 0.9, sterlet - 2.2). In some marine teleost phyto-benthophage and benthophages this index varied from 0.16 to 0.89; in the other (benthophages, plankto-ichthyophages and bentho-ichthyophages) this index changed from 1.3 to 2.3. In ichthyophages the ratio of protease/carbohydrase activity was close to 3.5. However the most differences were demonstrated for freshwater teleosts. Thus, in this fish group protease/carbohydrase ratio increased in the following order: roach -0.5, bream -1.3, perch -3.5, burbot - 5.3, pike -30. As it is seen, this index in the ichthyophage pike is 60 - fold higher than in the phytoplankto-benthophage roach. Analyses of α-amylase activity have shown the range of variability in different species, with the maximum activity (carp) 41.4 mg/min exceeding by 70-100 times the minimal activity (sander and pike), 0.9+0.1 and 1.2+0.2 mg/min (Ugolev & Kuz'mina, 1994). The range of variability of disaccharidases activity is several fold lower: the maximum are 22.5-fold higher than minimum for sucrase (the white bream - pike) and 4.1-fold higher for maltase (crucian carp - pike). Thus, the most significant changes in the level of the

enzymatic activity effected by the feeding spectrum have been demonstrated for enzymes realizing of the initial stages of carbohydrate hydrolysis.

In a number of cases the type of feeding correlates with regulatory properties of enzymes. In particular, tributyrin causes the inhibition of carbohydrase activity in benthophages (up to 60%) and stimulation of one in ichthyophages and ichthyophage-benthophages (up to +120%). The same agent may cause the opposite effect for alkaline phosphatase in the same fish species (Fig.3). As the indicator of fish feeding intensity, the comparison of the same enzyme activity under standard temperature (for example 20°C) as well as the coefficient of enzyme activity variation may be used. Most values of enzyme activity and least values of variation coefficient testify about good feeding condition and high feeding intensity of fish population (Kuz'mina, 1980).

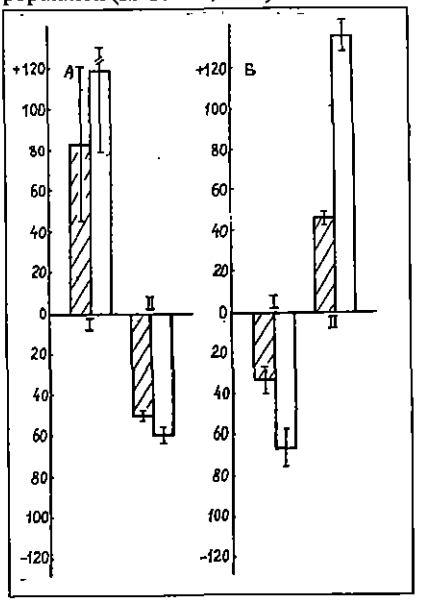

Figure 3. Effect of tributyrin on intestinal mucosa sucrase (I) and alkaline phosphatase (II) activity in burbot (A) and bream (B).
Vertical, stimulation (+) or inhibition (-), % of control, light columns, 0°C, hatched columns, 20°C.

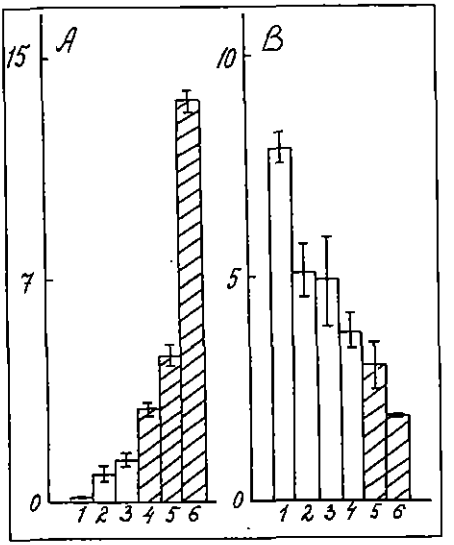

Figure 2. Total amylolytic (A) and total proteolytic (B) activities of intestinal mucosa in some freshwater fish. Vertical, enzyme activity, μmol × g^{-1} × min^{-1}, A: 1-pike, 2-burbot, 3-perch, 4-bream, 5-roach, 6-crucian carp. B: 1-pike, 2-asp, 3-burbot, 4-sheatfish, 5-bream, 6-carp.

Motive feeding activity. The study of α-amylase activity in the blood in some freshwater species shows that in some fish its level is lower than 1 (burbot - 0.30+0.05, safrefish - 0.33+0.03, bream - 0.52+0.05, blue bream - 0.69+0.12, white bream - 0.73+0.15, crucian carp 0.81+0.15, roach - 0.98+0.16 mg × ml^{-1} × min^{-1}, in other fish is higher than 1 (pike 1.13+0.32, pike-perh 1.18+0.14, ruff - 1.33+0.23, ide - 1.42+0.42, perch - 1.49+0.10 mg × ml^{-1} × min^{-1}. The most interspecies differences were found for the ratio blood α-amylase activity/intestine mucosa α-amylase activity. This index is 0.33 for blue bream, 0.57 for safrefish, 0.72 for bream, 1.24 for burbot, 3.79 for perch, 5.57 for pike perch and 6.20 for pike. As it can be seen the fishes with less motive feeding activity have low values of this index, while fishes with more motive feeding activity have high values of the index.

Temperature condition of fish feeding. The comparison of some characteristics of various hydrolases in fish with different type of feeding shows that some of them may be indicator of feeding behaviour. The most differences of temperature characteristics were revealed for enzyme realizing the initial stages of carbohydrate and protein degradation. In particular α-amylase temperature optimum is 40 °C for benthophages and planktophages and 30 °C for ichthyophages and ichthyophages-benthophages. The relative enzyme activity in the zone of low and physiological temperatures in the former is 10-30% of the maximal activity, in the latter is 50-90% of the maximal one (Fig.4). The temperature optimum of enzymes hydrolizing various proteins lies in the zone of high temperature (50-60 °C). However the relative enzyme activity values of proteolysis in zone low and physiological temperatures in typical and facultative predators significantly differs from those of typical bethophages and planctophages. In the former group the relative activity of enzyme realizing initial stages of proteolysis (pepsin) is 50-90% from maximal activity, in the latter group the same index is 5-20% only. One more important characteristic is the apparent energy of activation of some enzymes. It was established that E_{act} values of α-amylase differed in predatory and non predatory fish (Fig.5). In particular, in benthophages and planktophages E_{act} values changed from 9.4 kcal/mol (blue bream) to 11.3 kcal/mol (bream) in the temperature zone 10-30 °C, while in typical and facultative predators it changed from 1.5 kcal/mol (pike) to 3.6 kcal/mol (perch) in summer. It is interesting that this indicator in the zone 0-10 °C changes from 9.1 kcal/mol (bream) to 11.4 kcal/mol in fishes of the former group and from 2.2 kcal/mol (pike perch) to 6.7 kcal/mol (pike) in fishes of the latter group in winter. E_{act} values of proteases realizing

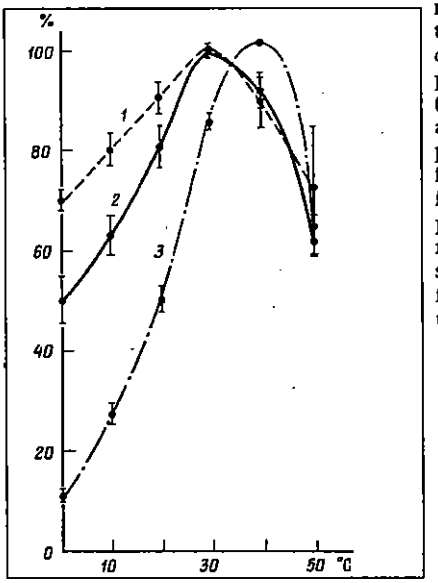

Figure 4. Effect of temperature on α-amylase activity of intestinal mucosa in pike-perch (1), safrefish (2) and bream (3).
Ordinate, enzyme activity, % of maximal one. Abscissa, temperature, °C.

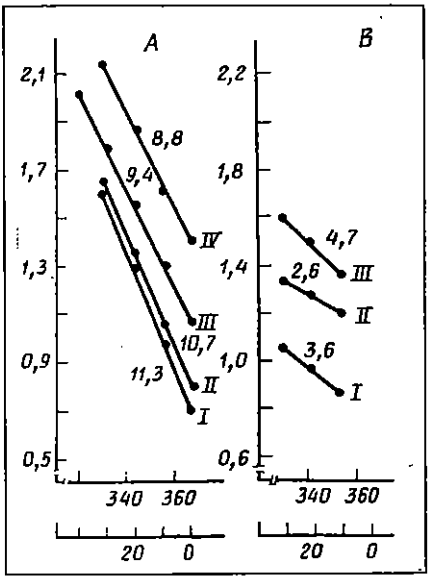

Figure 5. Arrhenius plots for intestinal mucosa α-amylase in some freshwater fish.
Ordinate, lg (V × 10^2) where V is a rate of enzymatic reaction. Abscissa, 1/T × 10^5 is value inversely proportional to absolute temperature (bottom line is temperature, °C).

initial stages of proteolysis differ more significantly. For pepsin of most predators the physiological temperature zone is characterised by very low values of E_{act} 1.1-1.5 kcal/mol, while for trypsin of most benthophages and planktophages the same temperature zone is characterised by high values of E_{act} closed 10 kcal/mol. Hence, low E_{act} values of these enzymes testify to a predator type of fish feeding but high E_{act} values testify to a non predatory type of fish feeding. Some information about it may be received using K_m and V values too. Under the same conditions the enzymes of predators have less K_m values than those of non predators. K_m values of sucrase are 28.6 mM and 14.8 mM in pike but 45.7 mM and 24.1 mM under 0^0 and 20 ^{0}C correspondinly. The same regularity is revealed for intestinal mucosa alkaline phosphatase in different fish species with similar type of feeding. It is known, that interspecies differences of V values are similar to those of enzyme activity (Ugolev & Kuz'mina, 1993a,b).

Thus, many characteristics of fish digestive enzymes are fine adapted to the spectrum, biochemical food composition, motive feeding activity and feeding intensity as well as to environment temperature of active exogenic fish feeding. This phenomenon is observed for various taxonomic groups of marine and freshwater fish during all its history life. In spite of intraspecies variability in some of them mean values are effective indicators of feeding ecology of wild fish. Composition of different enzyme characteristics let get more complete information about this important problem.

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THE INFLUENCE OF STARVATION ON THE CARBOHYDRATE TRANSPORT IN CARP GUT

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Functional topograpy of carbohydrate absorption in carp gut

In the present time the regularities of carbohydrate membrane hydrolysis and transport in vertebrate have been studied sufficiently (Ugolev,Kuz' mina,1993). At the same time, the data on distribution of transport activities in the fish intestine are discrepant. The maximal level of the glucose transport was found in the proximal part of the several marine and freshwater fish (Ferraris,Ahearn,1984). In the works of the other authors the glucose transport rate was shown as maximal in the middle or distal parts of fish intestine. In the most cases the maximum of the transport activities in the distal part of intestine was found in the omnivorous and the carnivorous fish species with comparatively short gut. Such discrepant data may be due to transport peculiarities in different fish species or dissimilar research methods. So far as the mucosa mass and diameter of gut vary along the intestinal length, the way of transport activity expression (mmol/l, mmol/sm,mmol /g wet mass) can influence the results of investigations.

As the functional topography of the carbohydrate absorption in the carp intestine is not clearly understood and in the most works the authors deal with the transport of free monomers, mainly glucose, the absorption of free monomers as well as hydrolises-released monomers along the intestine of carp ***Cyprinus carpio L.*** is investigated in the present work. The accumulation of hexose in the mucosa of intestinal strips in vitro under incubation in equivalent 10mmol solutions of glucose, galactose, fructose, maltose, saccharose, and solution of starch (1.8g/l) at 20 °C during 60 min was studied. Proximo-distal gradient of the free and hydrolysis-released monomers accumulation was found in carp intestine (Figure 1). The maximal level of glucose, galactose, maltose and saccharose accumulation was shown in the 7th-8th segments (fructose in 12th) of the gut. The maximal accumulation of starch was found in the 7th and 12th segments of the intestine (mmol/l).

As the diameter and the mucosa mass in the carp intestine decreased in the distal direction, the hexose accumulation in mmol per the mass of an intestinal portion was estimated. In this case the proximo-distal gradient of the carbohydrate accumulation differed from the above. The maximal level of the free monomers accumulation was in the first intestinal segment. The maxima of the disaccharides accumulations were in the 1th and 8th segments. The accumulation of glucose as a product of starch hydrolysis was the highest in the 10th gut segment.

The foregoing demonstrates that the estimation of the carbohydrate accumulation per unit of mucosa mass discovers the significant role of the distal intestinal part in the

transport process, that may be considered as adaptation to the reduction of the gut relative length in fish as compared with the higher vertebrate animals. The estimation of the accumulation per gut segment mass reveals the important role of the proximal intestinal part due to the maximal mucosal mass of this intestinal part in benthophage carp.

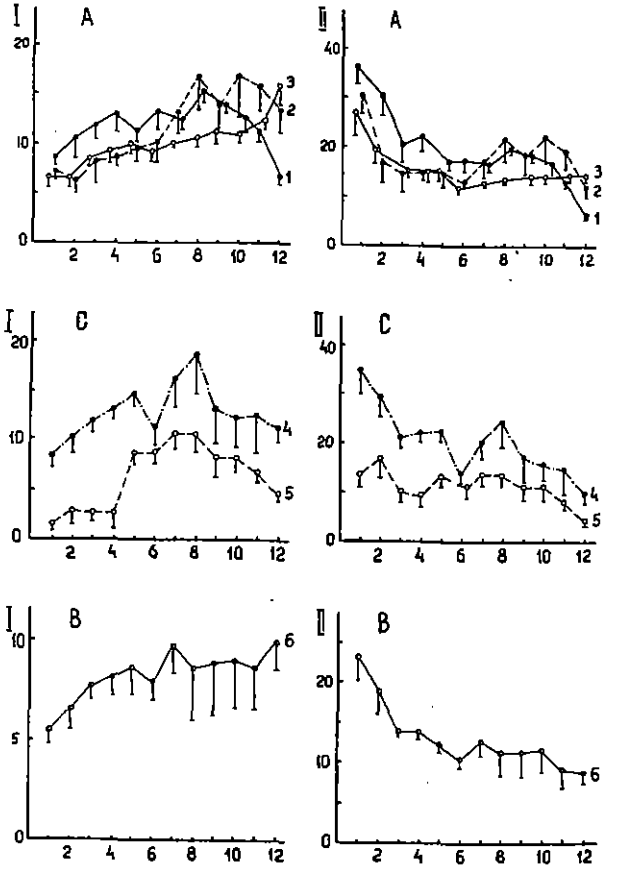

Figure 1. Accumulation of glucose(1), galactose(2), fructose(3), maltose(4), saccharose(5) and starch(6) by various segments of carp intestine at 20 °C during 60 min. Abscissa, segments of the carp intestine; ordinate, I - hexose concentration in the intestinal mucosa (mmol/l), II - hexose quantity (mmol/mucosa mass of intestinal segment). N = 6-12.

The effect of starvation on carbohydrate transport in carp.

The significant individual variability of the functional topography depends on an animal age, its functional condition, food composition and another factors in the

higher vertebrate animals has been shown (Kushak,1983). One of the main important factors, influencing the efficiency of the hydrolytic and transport system function is a degree of fish feeding. The model "satiety-starvation" is more frequently used. At present it is known that the starvation is accompanied by a series of functional and structural reorganizations. However the data of these experiments are contradictory. In some works the starvation increases the nutrition hydrolysis , but the other authors show the decrease of intensity of the process. Such descrepancy in the data to a larger extent depends on methods of enzyme and transport activity determination and the duration of starvation. For example, the mass and thickness of fish intestine decreases significantly during the period of winter starvation (McLeese,Moon,1989). It may be one of the reason for an increase of glucose transport expressed in mmol/g mass of an intestine in starving fish. However, the main effect of starvation on glucose transport is assosiated with the lack of luminal nutrients (Karasov,Diamond,1983). The investigation of the carbohydrates accumulation in carp intestine exposed to 48 hour starvation has shown the level of glucose and galactose as well as of monomers of maltose and saccharose hydrolysis to be lower along the intestine length in starving than in feeding fish (Figure 2). At this the most significant changes occured in the proximal segments of intestine. The maximal level of the above carbohydrates was 1.5-2 times as low in starving than in feeding fish. Early the similar changes in glucose and galactose transport under starvation were observed in rats, hamsters, mice and turtles (Ugolev,1972).

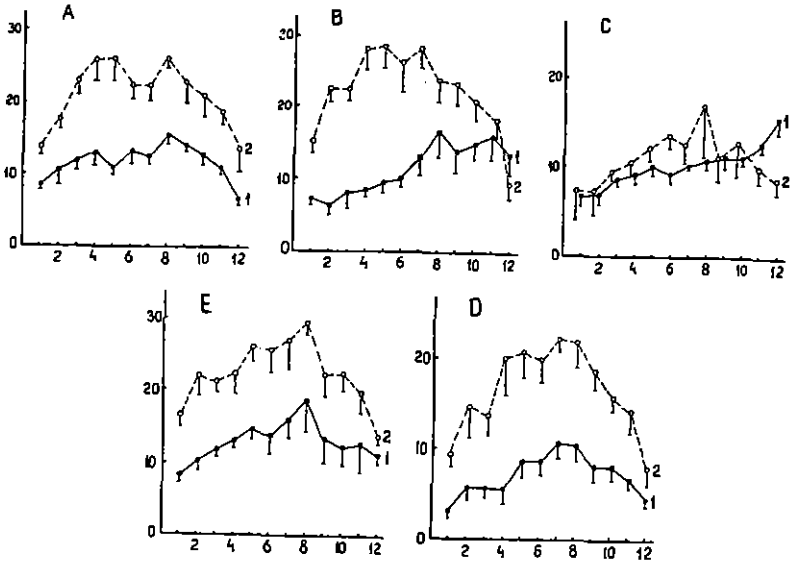

Figure 2. The influence of starvation (48 h) on the hexose accumulation in the carp intestinal strips under incubation in equivalent solutions of glucose (A), galactose (B), fructose (C), maltose (E) and saccharose (D) in feeding (1) and starving (2) fish. Abscissa, segments of the carp intestine; ordinata, hexose concentration in the intestinal mucose (mmol/l). N = 4-9.

So the starvation of fish like of many vertebrate animals not only changes the intensity of transport but proximal-distal gradient of carbohydrate accumulation along the intestinal length.

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EFFECT OF LIVE FOOD, ARTIFICIAL AND MIXED DIET ON THE SURVIVAL, GROWTH AND DIGESTIVE ENZYME ACTIVITIES OF *CLARIAS GARIEPINUS* (BURCHELL) LARVAE

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Abstract

This study was conducted to determine the effect of live food (*Artemia* nauplii), artificial and mixed diets on the survival, growth and digestive enzyme activities of African catfish, *Clarias gariepinus*, larvae. Newly hatched catfish larvae were stocked at 15 larvae l^{-1} in nine 15L glass aquaria for growth study and six 200L circular fiberglass tanks for enzyme study. All diets were randomly assigned and given *ad libitum* between 2-4 times a day for 16 days.

The highest mean survival rate was observed among larvae that fed on mixed diet (78.8%), followed by those fed on *Artemia* (73.4%) and artificial diet (63.0%). The mixed diet also gave the best growth for catfish larvae. However, the larval growth for those fed on *Artemia* and artificial diet were nearly identical and significantly lower ($P<0.05$) than those fed on mixed diet.

The study also showed that the highest total amylase, trypsin and chymotrypsin activities were found in larvae which fed on mixed diet. This was followed by those fed on artificial diet and live food, respectively. However, the patterns of specific content of digestive enzymes were nearly identical for both *Artemia* and artificial diet fed larvae. This indicated that *C. gariepinus* larvae could equally and efficiently digest both live and formulated feed.

Keywords: *Clarias gariepinus* larvae, growth, digestive enzymes

Introduction

Clarias gariepinus was introduced to Malaysia in the early 1980s (Thalathiah and Ibrahim, 1992). Since then, it has become one of the most popular commercially cultured freshwater

catfishes. Although a specific larval diet for *C. gariepinus* has been developed (Uys and Hecht, 1985), it is not locally available. Most of the hatchery operators use *Artemia* nauplii during the larval rearing as there are conflicting findings by Uys and Hecht (1985) and Van Damme *et al.* (1990) on the use of artificial diets. This study was conducted to evaluate a locally available commercial larval diet and to determine the effects of live food, artificial and mixed diets on the survival, growth and digestive enzyme activities of *C. gariepinus* larvae. Knowledge on the digestive capability of fish larvae would be useful in developing a specific larval diet (Uys and Hecht, 1987) and feeding strategies (Verreth and Segner, 1995) for that specie.

Materials and methods

This study was conducted in nine 15L glass aquaria for growth measurement and six 200L circular fiberglass tanks for enzyme samples. Newly hatched catfish larvae were stocked in all tanks at 15 larvae l^{-1}. The tanks were randomly assigned to three different diets i.e. live food (*Artemia* nauplii, Bio-Marine Brand), a commercial artificial larval diet (Gold Coin Brand) and a mixed live-artificial diet. Artificial feed was given 4 times a day (0800,1200,1600,2000H) while live food was given 2 times a day (0800 and 1600H). For the mixed diet, artificial diet was given 10 min earlier than the live food. Larvae were fed *ad libitum* from the second day of rearing (Uys and Hecht, 1985).

Larvae were sampled at every other day for total length and weight measurements, and enzyme activities. All enzyme samples were kept at -80°C until assayed. Trypsin, chymotrypsin and amylase activities were respectively determined using TAME, BTEE (Rick, 1974a,b) and corn starch (Rick and Stegbauer, 1974) as substrates in international unit (U) $larvae^{-1}$ or mg^{-1} body weight following the modified microtechniques described by Kamarudin *et al.* (1994).

Results and Discussion

Survival and Growth

The survival rate and growth of larvae fed on the different diets are shown in Fig. 1 and Table 1. The mixed diet produced the highest survival rate and significantly higher growth ($P<0.05$) for *C. gariepinus* than the other diets. This study showed that the artificial diet performed as well as the live food but with a lower survival rate. The growth results of the present study were comparable to those of the earlier works (Uys and Hecht, 1985; Verreth and Van Tongeren, 1989).

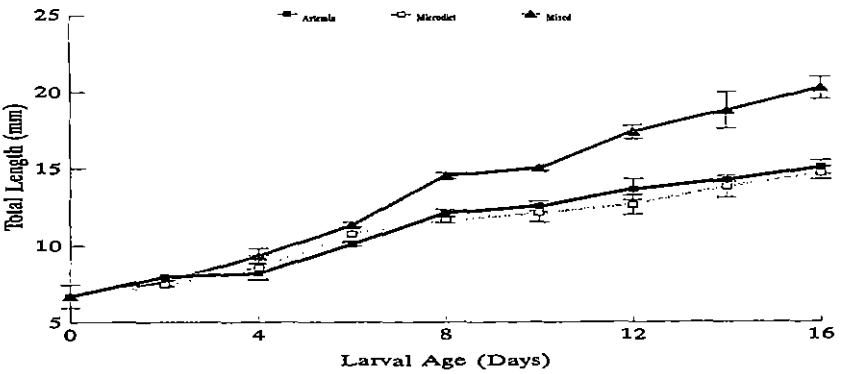

Fig. 1 Growth of *C. gariepinus* larvae fed on different diets

Table 1. Mean survival rate, body weight and specific growth rate (SGR) of *C. gariepinus* larvae fed on different diets*.

Diet	Survival (%)	Wet Body Weight (mg)			SGR (% d^{-1})
		Initial	Final	Gain	
Artemia	73.4±2.0 a	2.67±0.01 a	25.90±3.11 b	23.23±3.11 b	14.2±0.7 b
Microdiet	63.0±4.0 b	2.67±0.01 a	21.03±1.27 b	18.36±1.27 b	12.9±0.4 b
Mixed	78.8±3.3 a	2.67±0.01 a	66.73±6.77 a	64.06±6.77 a	20.1±0.6 a

* Means within the same column and followed by a similar letter are significantly different (P>0.05).

Digestive Enzyme Activities

Generally, total trypsin activity increased after Day 6 and was the highest in larvae fed on the mixed diet (Fig. 2). In contrast, specific trypsin content was initially high in all treatments and decreased as the larvae developed. However, sharp peaks of trypsin activity were observed at Day 8 especially for larvae fed on artificial and mixed diets.

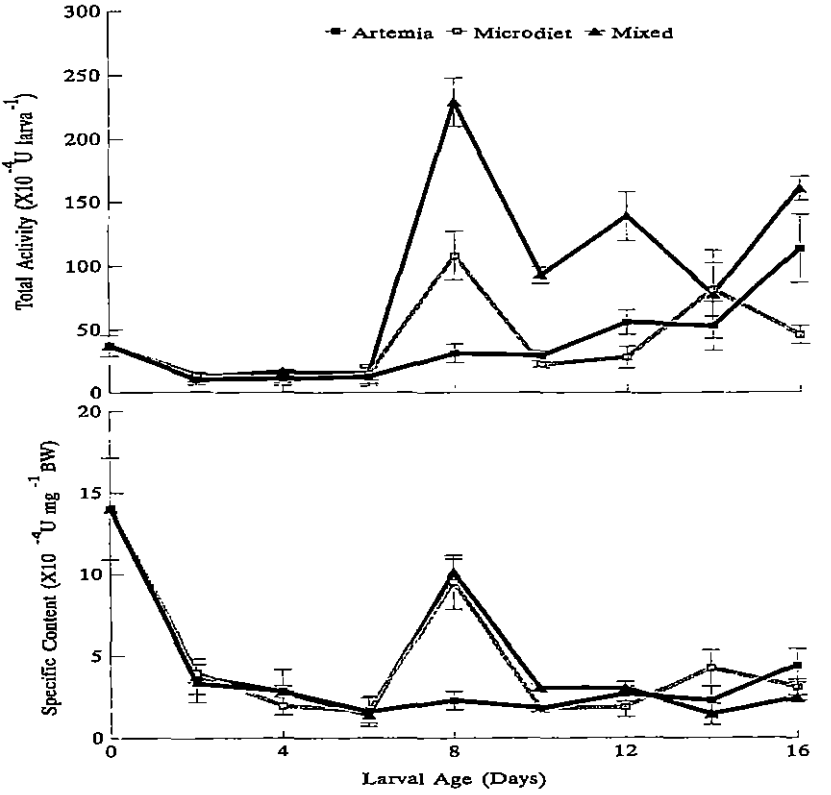

Fig. 2 Trypsin activity in developing *C. gariepinus* larvae fed on different diets

Total chymotrypsin activity also increased with larval development. The highest activity was observed among those fed on the mixed diet, followed by those fed on artificial diet and live food, respectively. Similar to trypsin activity, specific chymotrypsin content in all larvae was initially high and decreased with as larvae developed (Fig. 3). After Day 6, the activity started to increase to a peak at Day 10. In general, the chymotrypsin activity in larvae fed on mixed and artificial diets were higher than the activity in those fed on live food.

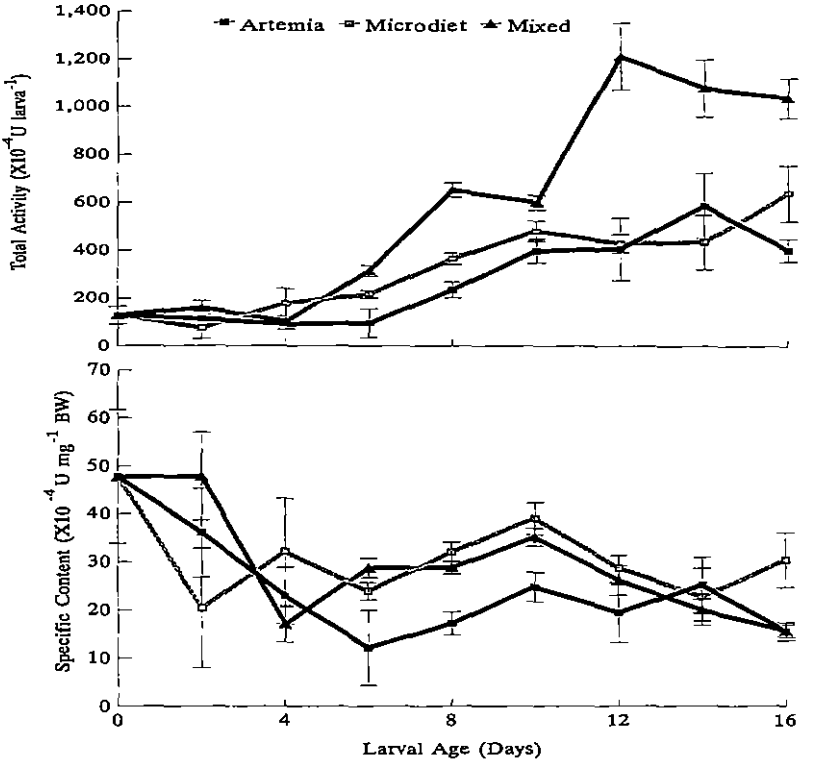

Fig. 3 Chymotrypsin activity in developing *C. gariepinus* larvae fed on different diets

The activities (specific content) of proteolytic enzymes in *C. gariepinus* larvae dropped after first feeding. Similar drop was observed in *Macrobrachium rosenbergii* larvae (Kamarudin *et al.*, 1994). However, these activities peaked when the larvae were 8-10 days old. These peaks coincided with the earliest weaning time recommended for *C. gariepinus* larvae (Verreth and Van Tongeren, 1989; Van Damme *et al.*, 1990). Chymotrypsin activity in *C. gariepinus* larvae remained higher than trypsin activity throughout the study. Similar observation was made in striped bass larvae (Baragi and Lovell, 1986).

In general, total amylase activity increased as larvae developed especially among those fed on the mixed diet. Specific amylase content was low at hatch and suddenly peaked at Day 2 (Fig. 4) which coincided with the first feeding. Later, amylase activity generally decreased with development although the activity was higher in those fed on artificial diet in the first 6 days.

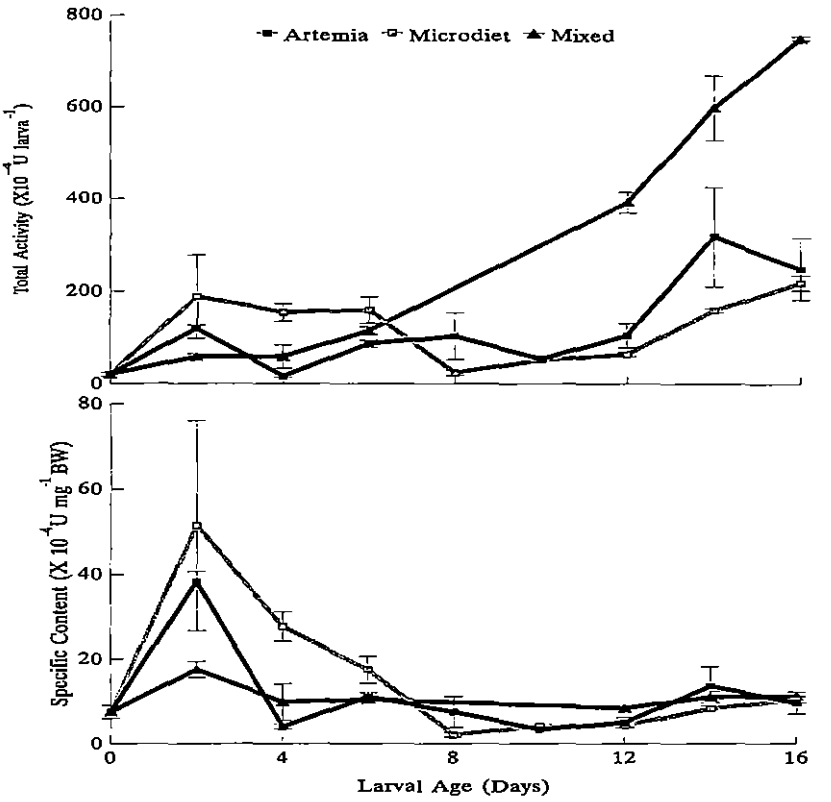

Fig. 4 Amylase activity in developing *C. gariepinus* larvae fed on different diets

Zambonino Infante and Cahu (1994) reported that feeding artificial diet resulted in higher amylase and proteolytic enzymes (except trypsin) in *Dicentrarchus labrax* larvae. Similar patterns were also seen in the present study. Based on growth and digestive enzyme activities, this study suggested that *C. gariepinus* larvae were very capable to adapt themselves to their diet.

Acknowledgment

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DIETARY AND MORPHOLOGICAL DEVELOPMENT IN FOUR SPECIES OF SIGANIDAE AT GREEN ISLAND REEF: IMPLICATIONS FOR FOOD AVAILABILITY AND DIGESTIVE CAPABILITIES

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Introduction

Herbivorous fishes of the family Siganidae occur in shallow waters throughout the tropical and subtropical Indo-Pacific region (Woodland, 1990). There are 27 species in the family: on a taxonomic basis they are divided into two subgenera, but on an ecological basis they are better divided into species that form pairs as adults and species which school throughout life (Woodland, 1990). The literature focusses on the schooling species and their aquaculture potential (Lam, 1974; Popper and Gundermann, 1975). It is rare for species from a taxonomically uniform family to exhibit both pairing and schooling behaviours, yet there have been no comparative studies of the two lifestyles.

At Green Island Reef, juveniles of *Siganus doliatus*, *S. fuscescens*, *S. lineatus* and *S. punctatus* are present in schools in the seagrass beds on the reef flat. As adults,*S. doliatus* and *S. punctatus* form pairs, while *S. fuscescens* form small schools (<20) and *S. lineatus* form large schools (20-100). Adult fish are found primarily in the coral areas, but adults of the schooling species have been observed migrating to the seagrass beds on the reef flat to feed. Based on these observations it was hypothesized that there might be dietary differentiation both within and between species.

Juvenile siganids appear morphologically similar, but during development superficial differences in morphology between pairing and schooling species become apparent. Generally, the schooling species attain a larger terminal size than the pairing species (Woodland, 1990), although *S. punctatus* is an exception. Particularly, *S. doliatus* and *S. punctatus* appear deeper bodied with protuberant snouts while *S. fuscescens* and *S. lineatus* appear streamlined with blunter heads. Of interest is the extent of these morphological differences and their relevance to the diets of the respective species. Less noticeable are internal differences in morphology. Digestion in herbivorous fishes is poorly understood, but it is generally accepted that they have a relative gut length >3 (Odum, 1970) and that an increase in relative gut length is associated with an increase in the capability to digest macroalgae (Benavides *et al.*, 1994). It has also been suggested that pyloric caecae serve to expand the effective surface area of the gut (Buddington, 1987). If there is dietary differentiation within this family, it is possible that there may also be differences in gut morphology (Murie, 1994).

The primary aim of this research was to quantify the diets of four species of rabbitfish that are present on Green Island Reef, describing changes through ontogeny and making comparisons between pairing and schooling species. In addition, morphometric measurements were taken in order to describe the main anatomical features that are relevant to feeding and digestion - the head and gut. The ultimate objective was to interrelate these aspects of biology and ecology.

Materials and Methods

Green Island is a vegetated coral cay located 27km off the coast near Cairns. The reef flat extends several kilometres southeast of the island, with a lagoonal area to the northeast. There is a continuous coral ledge around the front (weather side) of the reef and the edge of the navigation channel, with large patch reefs on the sheltered side and smaller patch reefs in the lagoon. There

are extensive seagrass beds in the lagoonal area and on the reef flat, making this an atypical location for the region. Although close associations between seagrass beds and reefs are common in the Caribbean (Ogden and Zieman, 1977; Parrish, 1989), most seagrass beds in the Great Barrier Reef region are coastal, while the reefs are further offshore.

Specimens of *S. doliatus, S. fuscescens, S. lineatus* and *S. punctatus* were collected from the various subhabitats between November 1993 and January 1996. Adult fish were captured with a speargun while using SCUBA, killed immediately and placed on ice. The gut was removed and unravelled, and the following sections measured: oesophagus, anterior stomach, pyloric stomach, the intestine as far as the "s" bend (Suyehiro, 1942) and the intestine from the bend to the anus. Pyloric caecae were also counted. The gut was preserved to allow stomach contents analysis at a later date, and the frame of the fish was frozen. Juvenile fish were captured by dragging a beach seine net through the seagrass beds on the reef flat, and frozen within 15 minutes of capture. Their small size required that they be dissected in the laboratory under a microscope. Again the gut was removed and unravelled, and the sections measured. The number of specimens taken was approximately in proportion to the abundance of the relevant size classes of the various species. The data were analysed using graphing and regression techniques.

Stomach contents (proportions) were quantified using the line intercept method proposed by Jones (1968). To reduce the number of variables while maximising the amount of information retained, items were assigned to 16 taxonomic and structural categories. The data were analyzed using Canonical Discriminant Analysis (CDA), which analyzes the multivariate means of designated groups (juveniles and adults of the four species), based on the original variables, and calculates new canonical axes which best demonstrate the separation between those means. Groups are standardised to unit within-sample variance, and the calculations are weighted by sample size. It includes information on the contributions of the original variables to the new axes. It is a pattern-seeking technique, and as such the interpretation is subjective.

Measurements aimed at describing the mouth and the shape of the head were made in the laboratory. The height of the gape and the depth and length of the head were measured with callipers. The depth of the head was measured in two places: behind the eye and at the base of the first doral spine (D1). These measurements were analysed using regression techniques.

Results

The results of the CDA of the stomach contents are shown in Figure 1. This figure is in two parts, graph A showing canonical axes 1 and 2, and graph B showing canonical axes 3 and 2. These first three canonical axes account for 86.8% of the total sample variation. Each graph is accompanied by a biplot showing which of the original variables are contributing most to the separation between the points, and in which direction.

Canonical axis 1 separates the adult *S. doliatus* from the juveniles and the rest of the adults. This is the most marked separation, and is influenced primarily by the occurrence of of *Dictyota spp.* and other membraneous phaeophytes in their diet. These algae are rare in the diets of juveniles and adults of other species. Small rhodophytic algae make a contribution to this separation, but are also consumed by the juveniles. In addition, membraneous rhodophytes, large rhodophytes and chlorophytes add minor contributions to the separation, as does the presence of unidentified calcareous material in the stomachs of the juveniles and some of the other adults.

Canonical axis 2 is the main axis of separation between the adults and juveniles. The main features of the juvenile diets are filamentous rhodophytes, phaeophytes and chlorophytes, as well as small fleshy rhodophytes. In contrast, seagrasses and blue-green filamentous algae from the genus *Lyngbya* were the most important components in the diets of adult *S. fuscescens* and *S. lineatus*. Seagrass is also present in the diet of adult *S. punctatus* . On this axis, adult *S. doliatus* are closer to the juveniles than the other adults as they do not eat seagrasses or *Lyngbya spp.*

Canonical axis 3 provides further distinction within the adults of the different species, and also within the juveniles. It is influenced primarily by the presence of animal material in the diet, and also by fleshy phaeophytes. The separation of both adult and juvenile *S. punctatus* is enhanced by the former, and the position of *S. doliatus* is influenced by the latter.

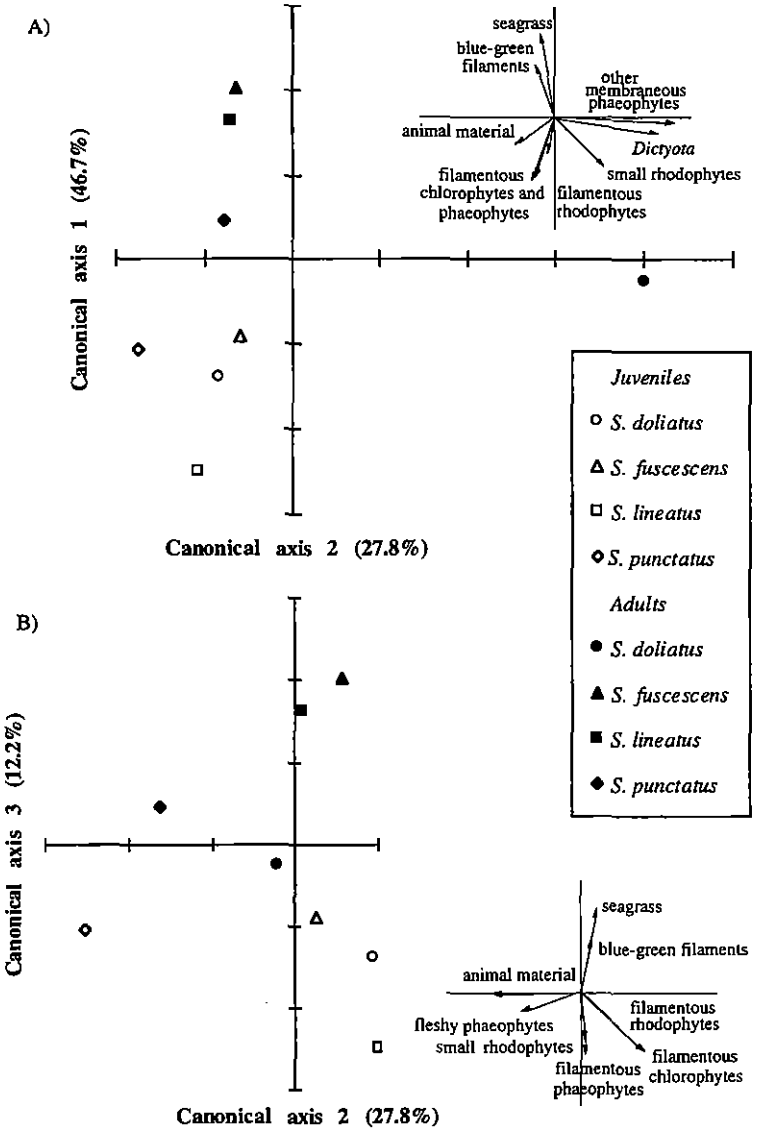


Figure 1. Canonical Discriminant Analysis of diet data.
These plots show the means of both juvenile and adult groups for the four species as listed. Plot A shows their positions on Canonical Axes 1 and 2, which are responsible for 74.5% of the variation in the data. Plot B shows their positions on Canonical Axes 3 and 2, which are responsible for 40% of the variation in the data. These three axes together account for 86.8% of the variation in the data. The associated biplots illustrate the main food variables affecting the separation of the means, and the way in which they are contributing to each of the canonical axes.

S. doliatus has the most diverse diet among the juveniles, with individuals consuming material from an average of 8.6 out of the 16 food categories. The filamentous algae, small rhodophytes and the unidentified calcareous material that are common to all juveniles are predominant, but membraneous and larger fleshy rhodophytes also feature. Adult *S. doliatus* have the broadest diet of all groups examined. On average, individuals had consumed food from 10.3 categories, and this contributes to their obvious separation on the main canonical axis. Many of these categories are eaten throughout life, but dietary expansion occurs through the inclusion of *Dictyota spp.* (21%) and other tougher membraneous phaeophytes (11%).

S. punctatus adults retain dietary characteristics similar to those of the juveniles, as shown by the proximity of their group means on both canonical plots. Animal material is sometimes present in negligible proportions in the stomachs of essentially herbivorous fishes, and usually appears to have been ingested accidentally along with targetted plant material. In *S. punctatus* however, sessile invertebrates such as colonial ascidians and sponges comprise nearly 20%, on average, of the stomach contents of both juveniles and adults. Juveniles and adults both utilise 6 or 7 food categories, the distinguishing features being the calcareous material present in the juvenile diet, and the presence of 33% membraneous rhodophytes and 19% seagrass in the adult diet.

Differences between juvenile and adult diets are more noticeable in the schooling species. *S. fuscescens* maintains dietary diversity, utilising 6 or 7 of the food categories, but the categories targetted change with age. The diet of juvenile *S. fuscescens* is similar to that of other juveniles, featuring filamentous algae, rhodophytes and the unidentified calcareous material. In contrast, 47.5% of the diet of the adults is made up of seagrasses, while the blue-green alga *Lyngbya spp.* comprises 21.5%. *S. lineatus* undergo a similar change in target food categories, switching from a primarily algal diet as juveniles to a mainly seagrass (46.5%) and *Lyngbya spp.* (12.5%) diet as adults. They also reduce the overall diversity of their diets, going from feeding on an average of 4.8 categories to utilising only 3.

Figure 2. Relative gut length through ontogeny
Regressions, with 95% confidence intervals, of total gut length against standard length for the four species. Slope values are given in the equations, along with r^2 values.

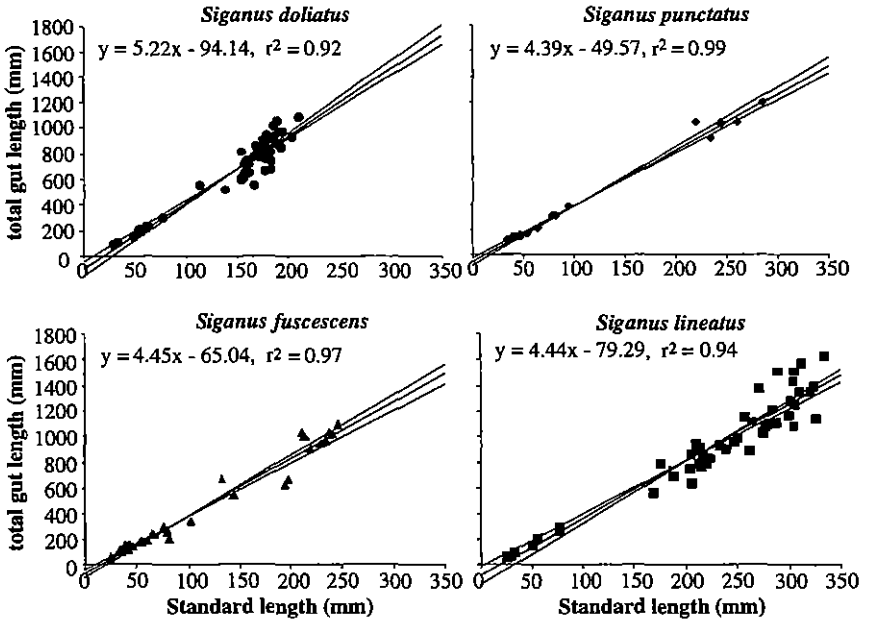


Figure 2 is a series of regression plots of gut length against standard length for the four species. Gut length shows a strong linear relationship with standard length, with r^2 values between 0.92 and 0.99. The slopes of the regression lines, indicating the rate at which the gut is lengthening relative to the length of the body, are significant. *S. doliatus* shows the greatest rate of relative increase at 5.2, and the other species are all at approximately 4.4. Table 1 shows the increases in trelative gut length between juveniles and adults for the four species. The juveniles start at approximately 3 - 3.6, which increases to between 4 and 4.6. It appears that the pairing species have slightly longer relative gut lengths than the schooling species, regardless of terminal size.

Table 1. Relative gut length (±S.E.) for juveniles and adults of the four species.

Species	Juvenile Rel. Gut Length	Adult Rel. Gut Length
S. doliatus	3.59 ± 0.08	4.64 ± 0.07
S. fuscescens	3.10 ± 0.13	4.15 ± 0.16
S. lineatus	2.99 ± 0.14	4.06 ± 0.08
S. punctatus	3.39 ± 0.13	4.22 ± 0.15

Figure 3 examines the distributions of pyloric caecae in juveniles and adults of the four species. Number of caecae per fish ranged from 4 to 7. For all four species, an increase in the number of caecae with age is obvious, as the distributions shift to the right. What is also apparent is that the pairing species have more caecae on average at a given age than the schooling species, and that the maximum number of caecae is lower for the schooling species.

Figure 3. Development of caecae through ontogeny
Frequency histograms of the number of caecae in juvenile and adult fish of the four species.

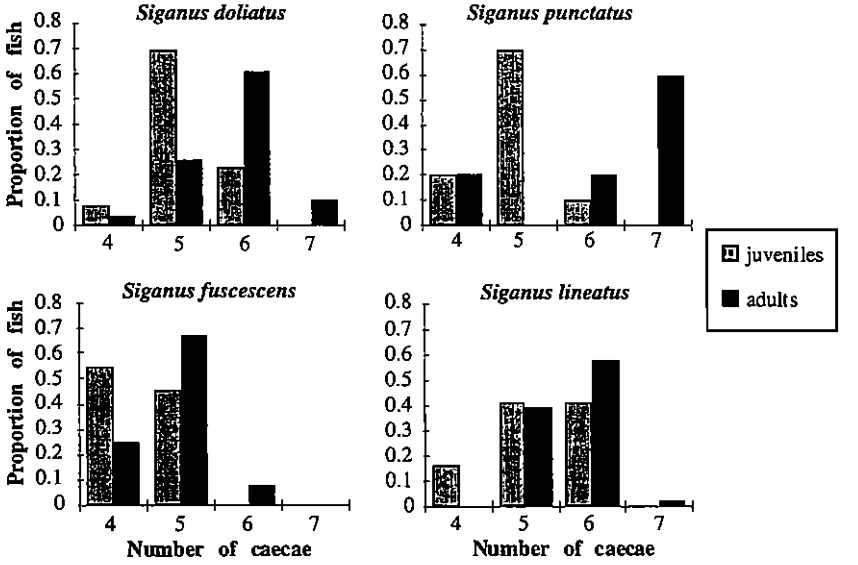


Table 2. Mean length (mm ± S.E.) of the largest caecum in juvenile and adult fish of the four species.

Species	Largest caecum - Juvenile	Largest caecum - Adult
S. doliatus	10.24 ± 0.68	29.10 ± 0.75
S. fuscescens	7.51 ± 0.75	32.46 ± 3.05
S. lineatus	7.95 ± 0.39	55.52 ± 2.68
S. punctatus	13.45 ± 2.23	57.80 ± 5.40

Table 2 gives details of the mean length of the largest caecum per fish. It is clear that the caecae continue growing as the fish grows, and that their ultimate size is related to the terminal size of the fish. Amongst the juveniles, the pairing species seem to have longer caecae, but amongst the adults, the largest caecae are found in *S. punctatus* and *S. lineatus*, the fish with the largest terminal size.

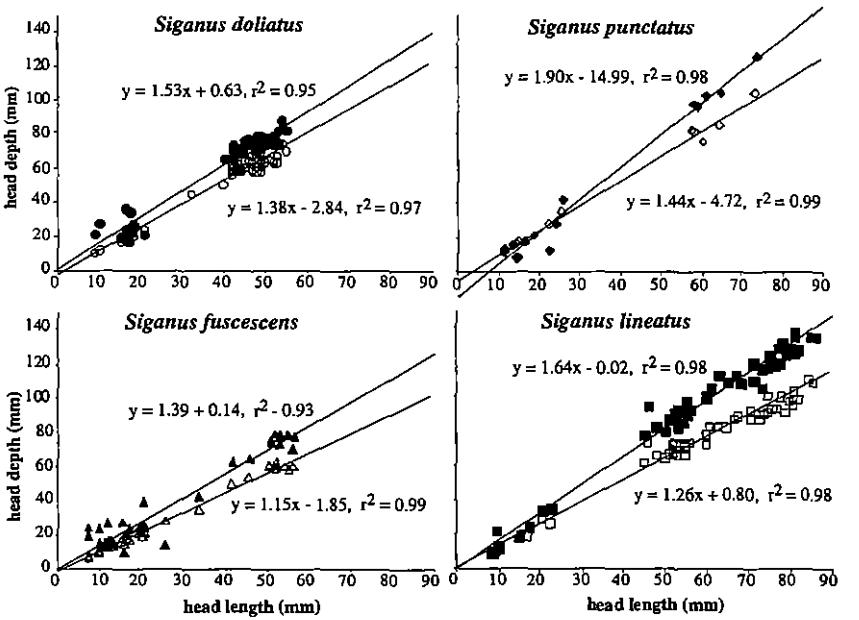
Table three outlines a series regressions of gape height against head length for the four species, and includes mean values for the actual gapes as an indication of the scale being discussed. Gape shows good correlation with head length, producing r^2 values between 0.72 and 0.94. The relationship shows low relative growth however, and seems to be consistent between species, with all the slopes having values approximating 0.2. Juveniles have gapes in the range of 3 - 6mm, while adults have gapes of 6 - 18 mm. Gape is again related to terminal size, with *S. punctatus* and *S. lineatus* having larger gapes than *S. fuscescens* and *S. doliatus*. Of the two large species, *S. punctatus* seems to have a smaller gape.

Table 3. Gape regression parameters and group means (mm ± S.E.)

Species	Slope	95% C.I	r^2	Juvenile mean	Adult mean
S. doliatus	0.20	0.17 - 0.23	0.72	4.58 ± 0.24	10.92 ± 0.26
S. fuscescens	0.22	0.20 - 0.24	0.94	3.81 ± 0.29	11.71 ± 0.57
S. lineatus	0.21	0.18 - 0.23	0.82	3.35 ± 0.49	15.07 ± 0.49
S. punctatus	0.19	0.16 - 0.22	0.93	3.98 ± 0.54	12.4 ± 1.03

Figure 4 is a series of double regressions, which plot two measures of head depth against head length for the four species. The aim of these plots was to demonstrate the relative difference between these two measures (one taken behind the eye and the other at the first dorsal spine) with size as an indication of head shape through ontogeny.

Figure 4. Ontogenetic changes in relative head depth
These regressions plot the two head depth measurements against head length. The filled symbols are the depth at the first dorsal spine (D1), while the open ones are the depth posterior to eye. Confidence intervals have been excluded for clarity.



The divergence of these initially similar measurements with increasing head length indicates a steepening of the head profile through ontogeny. Little divergence and low slope values for both these lines, such as are displayed by *S. fuscescens* , are indicative of a streamlined profile as the head is not very deep relative to its length. Adults of this species are most similar in morphology to the juveniles. The other species all have greater slope values and/or greater divergence. *S. punctatus* has the highest slope values and the greatest divergence, and is observably the species with the steepest head profile and more protuberant snout. *S. doliatus* and *S. lineatus* are in between these two. The deep body of S. lineatus results in its high upper slope value, but the lower of the two lines has a relatively lower value, and describes a blunter head than might be expected.

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STRUCTURE AND FUNCTION OF THE INTESTINAL EPITHELIUM IN FRESHWATER TELEOSTS WITH DIFFERENT TYPES OF FEEDING

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The mucosa of the intestine is of great importance in digestive, resorptive, and metabolic processes in different animals. The morphological organization of the digestive tract is related to their fishes type of feeding (Kapoor et. al., 1975). However, data on the fine structure of the intestinal epithelium in fish with various types of feeding are lacking. The aim of the present investigation was to examine the ultrastructure of the intestinal epithelium and gut functional organization in three species of fishes (the pike, the burbot and the bream) with different types of feeding.

In all fish species the epithelium of the intestine mainly consists of elongated columnar enterocytes and goblet cells. The apical part of the enterocyte plasmalemma bears numerous microvilli, forming the brush border. The length and number of the enterocyte microvilli in the different parts of fish intestine are given in Table 1.

Table 1. Dimensions of the microvilli of the enterocyte brush border in pike, burbot and bream,mean±S.E. (Kuperman & Kuz'mina,1994)

Species		Height of microvilli (μm)	Diameter of microvilli (μm)	No, μm^{-2}
Pike	1	0.60±0.02	0.14±0.04	52.7±2.7
	2	0.79±0.04	0.11±0.003	62.3±7.0
	3	0.92±0.07	0.10±0.05	20.8±4.8
Burbot	1	0.50±0.02	0.12±0.01	32.4±7.1
	2	1.22±0.06	0.12±0.03	46.0±7.9
	3	1.08±0.04	0.13±0.005	35.2±7.1
Bream	1	1.88±0.05	0.09±0.01	47.9±4.2
	2	1.89±0.02	0.12±0.003	48.8±4.4
	3	0.94±0.07	0.13±0.01	45.7±5.8

1, Anterior; 2, middle; 3, posterior part of the intestine

Functional topography of the fish intestine was studied for pike and bream in detail. The analysis of the data obtained suggests that different enzymatic activities were distributed along both fish intestines in an irregular manner (Table 2). A significant decrease of enzyme activities was mostly observed in the distal intestinal segment in bream. In pike the variability of the proximo-distal gradients was revealed. In particular the total amylolytic activity was maximal in the distal segment; α-amylase, maltase, sucrase and all peptidases were maximal in the middle segment; and alkaline phosphatase was maximal in the proximal segment. These interspecies differences were probably caused by different gradients of the intestinal mucosa mass. Thus, mucosa mass of the middle and distal intestinal segments in both species was almost equal, while the mucosa mass of the proximal one in bream was 2-fold larger than that in pike.

In the same time it is important to know the standard values ($\mu mol \cdot g^{-1} min^{-1}$) of enzyme activity in each intestinal segment. In this case the character of proximo-distal gradients of the same ensymes is changed. In particular, the differences between enzyme activities in various intestinal segments are reduced in both species especially in bream. In some cases (peptidases) the maxiomum of enzyme activity is observed in the middle part of both fish intestines; in other cases it is revealed in the distal one (total amylolytic activity and sucrase). Maltase, α-amylase and alkaline phasphatase activities were maximal in the proximal part of intestine of bream, but only alkaline phasphatase was maximal in that of pike. Proximal-distal gradients of the activity of carbohydrases and alkaline phasphatase in burbot are similar to those in pike.

Table 2. Distribution of enzyme activities along the intestine in pike (upper values) and bream (botton values) μ mol/min, N=5

Enzyme	Intestinal segments		
	Proximal	Middle	Distal
Total amylolytic	0.28±0.01	0.23 ±0.01	0.42±0.01
activity (Starch)	1.70±0.02	0.86 ±0.02	0.65±0.02
α-amylase*	0.95±0.01	1.15±0.03	0.88±0.02
(Starch)	12.00±0.46	5.07±0.12	3.29±0.07
Maltase	0.56±0.01	0.69±0.01	0.46±0.01
(Maltose)	3.40±0.04	1.53±0.02	0.96±0.02
Sucrase	0.01±0.001	0.04±0.001	0.03±0.001
(Saccharose)	0.40±0.001	0.14±0.004	0.27±0.001
Alkaline phosphatase	0.13±0.002	0.07±0.001	0.04±0.001
Sodium p-nitrophenyl phosphate	0.27±0.004	0.07±0.001	0.03 ±0.001
Peptidases**			
(Triglycyl-glycine)	1.34±0.04	2.39±0.05	1.68±0.06
	2.30±0.04	0.86±0.01	0.37±0.004
(Diglycyl-glycine)	2.29±0.01	4.60±0.12	2.45±0.07
	3.50±0.14	1.49±0.02	0.44±0.01
(Glycyl-glycine)	7.00±0.21	8.09±0.34	5.04±0.28
	11.00±0.23	3.06±0.03	2.24±0.02
(Glycyl-L-valine)	5.88±0.08	9.20±0.27	4.55±0.08
	280.00±5.70	90.00±1.00	60.79±1.10

* mg/min ** The assays by L.F.Smirnova. In the branches is a substrate.

In spite of the similarities between the basic structural organization of the intestinal epithelium of the three species of freshwater teleost fishes, there are a number of

peculiarities in their ultrastructure. The most substantial differences were discovered in the exterior structure of the enterocyte brush border. Thus, the height of microvilli of the same part of the intestine in the three species of fish differed considerably. The height of microvilli of the anterior part of burbot intestine was significantly smaller ($P<0.001$) than of pike and bream. The length of microvilli in the middle section of the pike intestine was much smaller, than in the burbot ($P<0.001$) and especially in the bream ($P<0.001$). The differences between the values of these parameters were significant only in the posterior parts of all fish species.

In previous work it has been shown that the anatomical structure of the intestine depends on feeding habits of the fish, in particular the relative length of the intestine depends upon the nature of the food (Kapoor et. al., 1975). Thereis probably a link between feeding habits and the structure of the enterocyte brush border.

It was also demonstrated that the quantitative characteristic of the brush border in certainly dependent on the localization of the enterocytes in different parts of the fish intestine. The maximal heigth of microvilli in the pike and the burbot is observed in the anterior section, while in the bream it is in the posterior part. In the bream, which starves in the winter degenerative changes have been recorded in the intestinal epithelium. During the period of starvation the number of celluar organells and their electron density decreased. These data are evidence of the marked influence of feeding intensity on the ultrastructure of the intestinal mucosa in fish.

Thus, there have been revealed differences in morphology, ultrastructure and the activity of enzymes in the intestine of bream burbot and pike. However, these differences are more prominent between the typical bentophag-bream and the predators-bakbot and pike than between the latter two species. The differences observed are probably connected with the larger length of the intestine in bream and can be considered as the structural and functional adaptation to the type of feeding.

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EFFECT OF PH, TEMPERATURE AND SOME POLLUTANTS ON FISH DIGESTIVE ENZYMES IN NATURE AND EXPERIMENTS

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Effect of pH, temperature and some pollutants on fish digestive enzymes in experiments

It is known, that temperature, pH and different modificators may change the digestive enzyme activities (Ugolev & Kuz'mina,1993). At the same time it was demonstrated that the temperature influences the effect of various modificators as well as pH-function of fish digestive enzymes. The degree of the effect depends on the species of fish, chemical structure of modificators, medium temperature etc. (Kuz'mina & Nevalenny,1983; Kuz'mina,1984,1987; Golovanova et al, 1994). However, in our previous works an integral investigation of the above factors was not made. The present work is aimed at the study of the effect of temperature, pH and some pollutants (dichlorvos, naphthalene and cadmium) on proteolytic, amylolytic and sucrase activities of intestinal mucosa in some species of freshwater fish,namely, bream, Abramis brama, zander, Stizostedion lucioperca, perch, Perca fluviatilis, pike, Esox lucius and blue bream, Abramis ballerus in vitro experiments. Total proteolytic activity was estimated according to an increase of tyrosine by the modified method of Anson (1938), total amylolytic and sucrase activities by the modified method of Nelson (Ugolev & Iezuitova,1969). The casein (10 g/l), starch (18 g/l) and saccharose (50 mM) were used as substrates. For preparation of the homogenates the Ringer solution (pH 5.0, 7.4 and 8.5) without phosphorus ions was used. Preincubation of homogenates with $CdSO_4$ (50 mg/l of total cadmium) was carried out during 60 min at 0^0, 10^0, 20^0 and 37 °C, at the above pH values.

The data on mono-, bi- and polyfactor effects of pH, temperature and cadmium testify that in the most of cases the cadmium (but not dichlorvos and naphthalene) effects only slightly the proteolytic activity (Table 1). The increase of pH from 7.4 to 8.5 causes the growth of enzyme activity in bream at all temperature values, in zander and perch at 10^0 - 37 °C only. The decrease of pH from 7.4 to 5.0 as a rule significantly diminishes the enzyme activity especially in zander and perch in all temperature ranges. In the presence of cadmium this effect is became stronger at pH 5.0 and is reduced at pH 8.5. The level of intestinal mucosa proteolytic activity grows significantly with temperature in all fish species. However a degree of the increase of enzyme activity in the temperature range from 0^0 to 37 °C is different in various pH zones due to various Q_{10} values. In bream it is larger at pH 7.4 and in zander and perch at pH 8.5. At the same time both the temperature and the cadmium are less effective then the temperature itself. Thus the change of temperature from 20 °C to 0 °C at pH 7.4 decreases proteolytic activity in 5.4, 4.0 and 3.3-fold in bream, zander and perch correspondingly. The change of pH from 7.4 to 5.0 at temperature 20 °C decreases significantly the enzyme activity (6.6 and 3.4 - fold) in zander

and perch only. The most changes are observed under polyfactor effects of pH 5.0, temperature 0 °C and cadmium. In this case the proteolytic activity is reduced 7.7, 8.8 and 7.3-fold in bream, zander and perch accordingly. In alkaline zone of pH this influence is approximately two times as less.

Table 1.

Effect of temperature, pH and cadmium on intestinal mucosa proteolytic activity in some fish species, $\mu mol \times g^{-1} \times min^{-1}$, N=5-8

Temperature, °C	Cadmium concentration, mg Cd/l	pH 5.0	pH 7.4	pH 8.5
		Bream		
0	0	0.08-0.01	0.10-0.05	0.28-0.05
	50	0.07-0.04	0.07-0.02	0.17-0.01
10	0	0.24-0.07	0.28-0.04	0.63-0.24
	50	0.21-0.03	0.18-0.06	0.26-0.13
20	0	0.46-0.12	0.54-0.06	1.20-0.55
	50	0.30-0.07	0.48-0.10	0.92-0.14
37	0	1.32-0.30	4.05-0.82	3.30-2.52
	50	0.89-0.15	3.00-0.76	2.53-2.13
		Zander		
0	0	0.42-0.09	0.87-0.12	0.86-0.11
	50	0.39-0.10	0.63-0.17	0.77-0.11
10	0	0.45-0.18	1.73-0.29	2.11-0.35
	50	0.41-0.12	1.68-0.30	2.07-0.28
20	0	0.52-0.16	3.44-0.83	4.50-0.53
	50	0.63-0.16	3.25-0.50	3.67-0.70
37	0	1.08-0.26	6.66-0.94	0.29-0.46
	50	1.07-0.21	6.32-0.36	9.66-0.74
		Perch		
0	0	0.20-0.11	0.82-0.22	0.52-0.21
	50	0.37-0.08	0.45-0.19	0.59-0.16
10	0	0.54-0.07	1.15-0.16	1.93-0.19
	50	0.78-0.11	1.22-0.25	1.67-0.14
20	0	0.79-0.16	2.70-0.22	3.11-0.19
	50	0.91-0.19	2.15-0.31	1.89-0.18
37	0	1.59-0.27	7.89-0.47	8.07-0.74
	50	1.93-0.24	5.96-0.61	6.48-0.78

The effects of pH, temperature and cadmium (not dichlorvos or naphtalene) on total amylolytic activity are observed (Table 2). The enzyme activities are 2-4 times lower at 0 °C then at 20 °C and pH 5.0 then at pH 7.4 in all fish species. Cadmium significantly decreases enzyme activity in bream at pH 7.4,in zander and perch at pH 8.5. The simultaneous changes in temperature ($20^0 \rightarrow 0$ °C) and ($7.4 \rightarrow 5.0$) reduce the total amylolytic activity 7 times in bream and 3 times in zander and perch. The above enzyme activity is 3-7 times as low in fish studied under polyfactor effects of pH 5.0, 0 °C and cadmium then at pH 7.4, 20 °C,lack of cadmium . But these changes are due to bifactor effect of temperature and pH mainly. Mono-, bi- and polyfactor effects of pH, temperature and cadmium on succharase activity of intestinal mucosa in bream and zander are similar to those of total amylolytic activity, but are weaker. The same regularities of all enzymes studied were observed in pike and blue bream.

Table 2.

Effect of temperature, pH and cadmium on intestinal mucosa total amylolytic activity in some fish species, μmol × g^{-1} × min^{-1}, N=5-8

Temperature, °C	Cadmium concentration, mg Cd/l	pH 5.0	pH 7.4	pH 8.5
Bream				
0	0	1.38 ± 0.09	2.55 ± 0.17	2.12 ± 0.07
	50	1.30 ± 0.15	2.37 ± 0.20	2.28 ± 0.22
10	0	1.61 ± 0.16	4.60 ± 0.21	3.68 ± 0.33
	50	1.59 ± 0.15	3.57 ± 0.29	3.24 ± 0.23
20	0	2.51 ± 0.26	9.62 ± 0.63	7.10 ± 0.58
	50	2.52 ± 0.25	8.00 ± 0.59	6.34 ± 0.40
Zander				
0	0	0.20 ± 0.01	0.34 ± 0.03	0.35 ± 0.03
	50	0.17 ± 0.01	0.32 ± 0.03	0.28 ± 0.04
10	0	0.22 ± 0.02	0.43 ± 0.03	0.49 ± 0.04
	50	0.21 ± 0.02	0.42 ± 0.05	0.38 ± 0.01
20	0	0.30 ± 0.02	0.62 ± 0.05	0.65 ± 0.05
	50	0.28 ± 0.02	0.59 ± 0.07	0.49 ± 0.06
Perch				
0	0	1.42 ± 0.07	1.91 ± 0.02	2.18 ± 0.11
	50	1.46 ± 0.02	1.89 ± 0.06	2.08 ± 0.04
10	0	1.67 ± 0.03	2.81 ± 0.07	3.31 ± 0.09
	50	1.69 ± 0.02	2.75 ± 0.10	3.13 ± 0.12
20	0	2.09 ± 0.06	3.67 ± 0.15	4.52 ± 0.14
	50	1.91 ± 0.06	3.40 ± 0.05	3.89 ± 0.11

Effect of pH and seasonal temperature on perch enzyme activity in nature.

Intestine enzyme activity of perch from 5 acidic and 1 neutral lakes has been investigated using two methodical aproaches. In the first case the enzyme activity is determined by a standard method (for 1 g of enzyme active preparation). In the second case the enzyme activity is calculated for real intestine mucosa and intestine content mass. In all seasons especially in spring and autumn the size and mass of perch from neutral lake are higher than in acidic lakes due to the presence of large fish specimens. In all seasons except for the winter the total proteolytic activity of intestinal mucosa in perch from neutral lake is 1.8-3.4 times higher than in acidic lakes (Table 3). Total amylolytic activity in perch from the neutral lake is significantly higher than in acidic lakes in spring and autumn, the sucrase activity - only in spring ($P < 0.05$). The effect of cadmium on the enzyme activities studied hasn't been found in perch from both neutral and acidic lakes. The differences observed are probably conditioned by the largest differences of fish feed intensity estimated as ratio of intestine content mass to empty intestine mass in this period (1.95±0.9 and 0.34±0,06 corespondently). The spectrum of food objects and feeding intensity dynamic differ too. In the neutral lake the predators prevail over benthophages, in acidic lakes were only benthophages.The maximum feeding activity in the first case are observed in spring, in the second case in summer (Komov & Zhgareva,1995). The differences of real enzyme activity (in all mucosa and all intestine content) between perches from neutral and acidic lakes became still more considerable as compared with those estimated by a standard method. Thus, the acidification of lakes causes changes in perch type of feeding and intestine enzyme activity.

Table 3.

Enzyme activity of intestinal mucosa in perch from neutral and acidic lakes, μmol × g^{-1} × min^{-1}, N=5-10

Season temperature,	Lake pH	Cadmium concentrarion, mg/l	Total proteolytic activity	Total amylolytic activity	Sucrase activity
Spring 10 °C	7.0	0	6.18 ± 1.23	3.49 ± 0.20	0.64 ± 0.06
		50	5.74 ± 1.14	2.91 ± 0.21	0.53 ± 0.06
	4.5	0	2.06 ± 0.56	2.56 ± 0.17	0.38 ± 0.04
		50	1.84 ± 0.55	2.28 ± 0.18	0.25 ± 0.04
Summer 20 °C	7.0	0	3.98 ± 1.87	2.12 ± 0.17	0.25 ± 0.04
		50	3.29 ± 1.03	2.77 ± 0.39	0.26 ± 0.07
	4.5	0	2.13 ± 0.28	2.11 ± 0.16	0.35 ± 0.11
		50	2.19 ± 0.39	2.14 ± 0.16	0.30 ± 0.13
Augtumn 10 °C	7.0	0	2.53 ± 1.12	3.44 ± 0.26	0.32 ± 0.09
		50	2.04 ± 0.84	2.87 ± 0.17	0.30 ± 0.08
	4.5	0	0.83 ± 0.26	2.46 ± 0.17	0.40 ± 0.05
		50	0.74 ± 0.14	2.18 ± 0.19	0.42 ± 0.06

So, the monoeffect of temperature or pH on digestive enzymes is shown. But the enzyme activities to a larger extend are influenced by a complex effect of these factors in all fish studied in nature and experiment. In the most of cases cadmium didn't cause any changes in enzyme activity.

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DIET AND DIGESTIVE EFFICIENCY OF ZEBRAPERCH (*HERMOSILLA AZUREA*) AN HERBIVOROUS KYPHOSID FISH OF SOUTHERN CALIFORNIA MARINE WATERS

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Diet

Herbivorous fishes in tropical and subtropical marine waters often eat algae containing secondary metabolites that act a chemical defenses. Included in this group are members of the family Kyphosidae, several of which in the genus *Kyphosus* appear to feed mainly on brown algae with chemical defenses (Randall, 1983; Rimmer and Wiebe, 1987; Steinberg, 1989). In the present work we proposed first to test the hypothesis that the zebraperch (*Hermosilla azurea*), a little-studied temperate-zone kyphosid, also consumes chemically defended brown algae as a major part of its diet.

To test this hypothesis, zebraperch were captured by spear and gillnet off Santa Catalina Island between July 1993 and April 1994. Digestive tracts from the esophagus to the anus were removed and their lengths measured. Stomachs were separated from the rest of the digestive tract, and their contents fixed in 10% buffered formalin, sorted to the lowest possible taxon and then dried to a constant weight to determine the contribution of each algal species to the diet of the fish.

Results of the stomach content analysis showed that zebraperch ate 17 different species of algae (Table 1), with the red algae *Polysiphonia* spp. comprising nearly two-thirds of the diet by dry weight and occurring in more than 75% of the stomachs.

These results do not support our hypothesis that zebraperch eat chemically defended brown algae as do their tropical relatives. Zebraperch diets consisted mainly of red algae not known to contain defensive chemicals (Hay and Fenical, 1988). A small proportion of the fish's diet did include the brown alga *Halidrys dioica*, which is known to contain secondary compounds that act to deter invertebrate herbivores (Steinberg, 1985). Therefore, zebraperch appear to eat chemically defended algae occasionally but more often choose to eat algae with weak or no chemical defenses.

Table 1. Diet of zebraperch based on stomach content analysis of individuals (n=60) captured at Santa Catalina Island.

Dietary Item	Frequency of occurrence	% of total (dry weight)
Red algae (Rhodophyta)		
Polysiphonia spp.	78.3	63.8
Chondracanthus canaliculatus	42.4	11.6
Ceramium spp.	5.0	5.0
Pterocladia capillacea	8.3	4.6
Gelidium coulteri	8.3	1.2
Corallina officinalis	16.7	<1.0
Cryptopleura crispa	1.7	<1.0
Hypnea valentiae	1.7	<1.0
Liagora californica	11.3	<1.0
Rhodoglossum affine	3.3	<1.0
Brown algae (Phaeophyta)		
Halidrys dioica	10.0	3.7
Ectocarpaceae	5.0	2.4
Cylindrocarpus rugosus	16.7	1.7
Green algae (Chloropohyta)		
Enteromorpha spp.	6.7	2.3
Chaetomorpha linum	10.0	1.7
Codium fragile	1.7	<1.0
Ulva lobata	3.3	<1.0

Digestive Efficiency

In this part of the work, we hypothesized that zebraperch can digest nondietary brown algae that contain secondary chemicals and that occur abundantly in the fish's habitat as efficiently as they digest dietary red and green algae. We based this hypothesis on the expectation that zebraperch retain the ability to digest chemically defended brown algae even though not part of the fish's diet because their tropical relatives (*Kyphosus* spp.) consume and apparently digest such algae. Our goal was to test the hypothesis by comparing the efficiencies with which zebraperch digest three species of dietary red and green algae and three species of nondietary brown algae, each of the latter know to contain measurable concentrations of secondary metabolites (Table 2).

The digestive efficiency of zebraperch was determined by feeding individual fish (n=10) each alga and calculating the total amount of organic matter, carbon, nitrogen, and protein assimilated from the alga. Efficiencies were calculated by comparing the amounts of these constitutents in the food and feces using ash as an assumed nonabsorbed marker. Each nondietary alga was fed to the fish by first anesthetizing the fish in MS-222 and then placing the food chopped to bite size into the stomach using forceps and a glass rod. The fish revived and swam normally within a few minutes after the procedure. The effect of the procedure on digestive efficiency was assessed by comparing the digestive values obtained for a dietary alga (*Chondracanthus canaliculatus*) when fed to the fish naturally and when force-fed to the fish under anesthesia.

Table 2. Dietary and nondietary algae used (and to be used) in the digestive efficiency experiments with zebraperch.

Algal species	Order/division	Secondary metabolites
Dietary		
Chondracanthus canaliculatus	Gigartinales/Rhodophyta	Halophenolics
Mazzaella leptorhynchos	Gigartinales/Rhodophyta	Unknown
Ulva lobata	Ulotrichales/Chlorophyta	Unknown
Nondietary		
Sargassum muticum	Fucales/Phaeophyta	Terpenoids and Phlorotannins
*Macrocystis pyrifera**	Laminariales/Phaeophyta	Terpenoids and Phlorotannins
*Pachydictyon coriaceum**	Dictyotales/Phaeophyta	Terpenoids and Phlorotannins

* In progress

The food, food material used as a control for loss of nutrients in the tanks, and fecal material were ground to a fine powder and stored in screwtop vials until the constituent analyses were performed. Carbon and nitrogen contents of the food and fecal material were determined by the Marine Science Analytical Laboratory at the University of California, Santa Barbara. Protein content was assayed using the dye Coomassie Brilliant Blue G-250 following Neighbors and Horn (1991). Ash and organic content of the ground samples were determined by burning triplicate subsamples (>50mg) for six hours at 550°C in a muffle furnace following Montgomery and Gerking (1980).

Results of the experiments designed to compare the digestive efficiency of zebraperch when fed a dietary alga under normal conditions and when force-fed the same alga under anesthesia showed a significant difference ($P<0.05$) only for carbon digestion. In this case, the anesthetized fish assimilated significantly more carbon than the naturally fed fish (Table 3).

Table 3. Digestive efficiency of zebraperch (n=10) for carbon, nitrogen, and protein when fed the dietary alga *Chondracanthus canaliculatus* normally (N) and when force-fed the alga under anesthesia (A). The same superscript letter in a column indicates no significant difference ($P>0.05$) between the two treatments.

	Mean digestive efficiency (%)		
Alga	Carbon	Nitrogen	Protein
Chondracanthus canaliculatus (N)	73.6^a	77.6^a	85.9^a
Chondracanthus canaliculatus (A)	89.2^b	86.0^a	88.1^a

Results obtained to date show that zebraperch can digest carbon, nitrogen, and protein form the nondietary alga *Sargassum muticum* with an efficiency equal or greater than that for the three species of dietary algae (Table 4).

Table 4. Digestive efficiency of zebraperch for carbon, nitrogen, and protein when fed three dietary algae (D) and one nondietary algae (ND). The same superscript letters in a column indicates no significant differences (P>0.05) in digestive values among the different algae.

	Mean digestive efficiency (%)		
Alga	Carbon	Nitrogen	Protein
Chondracanthus canaliculatus	73.7[a]	77.7[a]	85.9[a]
Mazzaella leptorhynchos	74.5[a]	73.7[a]	88.4[a]
Ulva lobata	82.7[a,b]	79.1[b]	83.2[a]
Sargassum muticum	82.9[b]	80.7[a,b]	94.9[b]

The results of the dietary component of this project do not support our hypothesis that zebraperch consume chemically defended brown algae as a major part of its diet but rather showed that zebraperch eat a wide variety of algae in their shallow subtidal habitat, mainly red algae and some green algae both with apparently weak or no chemical defenses. Brown algae made up only a small proportion of the stomach contents. The data obtained to date on digestive efficiency, however, do support our second hypothesis that zebraperch can assimilate nutritional constituents from nondietary brown algae as efficiently as from dietary red and green algae. Zebraperch digested nutrients from a brown alga just as well as they did from three dietary algae. Digestive efficiency experiments on two other nondietary brown algae are in progress.

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Modeling and Methodology

INFERRING ECOLOGICAL RELATIONSHIPS FROM THE EDGES OF SCATTER DIAGRAMS: A COMPARISON OF LEAST SQUARES AND QUANTILE REGRESSION TECHNIQUES

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Introduction

The ability to make quantitative predictions based on the interdependence of two variables is a central theme in ecology. The usefulness of scatter diagrams to illustrate associative relationships and to aid in the interpretation of statistical analysis of bivariate data is well recognized (Freedman et al. 1978, Iman 1994). In addition to demonstrating the relationship between two variables, scatter diagrams provide a graphical representation of variability in the data. The boundaries of this variability are potentially useful in explaining various ecological phenomena. Until recently, however, estimates of the slopes of these boundaries were primarily fit by eye, while statistical techniques yielding quantitative estimates of the slopes of upper and lower bounds of scatter diagrams have been underutilized (see Maller et al. 1983 and Blackburn et al. 1992). This is despite the fact that hypothesis testing requires quantitative measures of the magnitude of these relationships.

When examining predator-prey interactions, knowledge of the range of prey sizes included in the diet of a growing predator is critical in order to estimate parameters accurately and design appropriate foraging models. Scatter diagrams have been especially prominent in studies linking predator and prey body size wherein they have been used to illustrate ontogenetic patterns in prey size use by a variety of predator taxa. For animal taxa, regression analyses normally indicate an increase in mean prey size with predator size. However, mean prey size does not usually increase proportionately with predator size and substantial variability exists in most cases (Cohen et al. 1993).

The examination of scatter diagrams commonly reveals one of two patterns in prey use by predators. One pattern demonstrates a similar prey size range for both small and large predators with minimum and maximum prey sizes showing analogous patterns of increase with predator size. The second common pattern depicts ontogenetic increases in prey size range consumed by predators, which results from the consumption of nearly constant minimum prey sizes as predators grow coupled with relatively steep increases in maximum prey size. The existence of the latter pattern of prey use has led to the postulation of theories involving potential competitive advantages for larger predators (Wilson 1975).

Analyses of the diets of piscivorous fish predators also indicate a general increase in prey size with predator growth (Emerson et al. 1994). This increase has been attributed to ontogenetic increases in predator mouth size, swimming speed, and visual acuity (Keast and Webb 1966, Werner 1979, Persson 1990). However, analogous to other animal taxa, examination of predator size-prey size scatter diagrams often reveals patterns of constant minimum prey size coupled with increases in maximum prey size leading to wider prey size ranges for larger predators (Fig. 1). Therefore, testable differences may exist between the relationships of minimum and maximum prey size with predator size. We utilize predator size- prey size data for several piscivorous fishes to illustrate the strengths and weaknesses of two regression techniques in estimating the slopes of upper and lower bounds of scatter diagrams. These techniques are, however, widely applicable to other ecological disciplines.

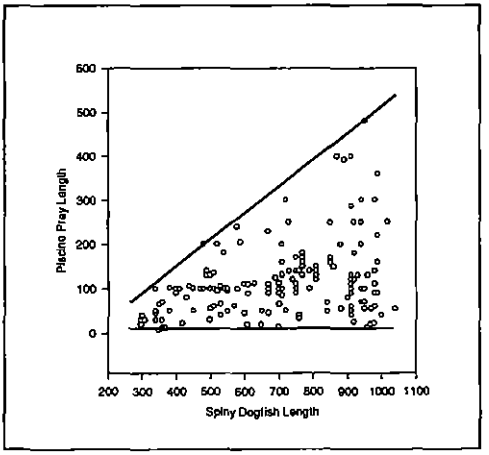

Fig. 1. Plot of piscine prey length vs. predator length for spiny dogfish (*Squalus acanthias*). Lines representing minimum and maximum prey size are fit by eye.

Blackburn et al. (1992) employed a least-squares (LS) regression technique to estimate the slopes of the upper bounds of animal abundance vs. body size scatter plots. Observations were grouped into a number of equal (n) size classes and the uppermost points within each size class were used to estimate the LS regression slope of the upper bound. The authors concluded that this approach was adequate for estimating the slopes of the boundaries of polygonal relationships.

In this paper we extend the LS regression technique described by Blackburn et al. (1992) to estimate minimum as well as maximum slopes of predator-prey scatter diagrams. We make several modifications to this LS regression technique and compare results for consistency. We describe and modify a technique based on a least absolute values regression model and comparisons are made between the two regression techniques for validity of slope estimates.

Methods

Six independent data sets each consisting of body length information for a specific piscivorous fish predator-prey assemblage were used to compare regression techniques. A diverse group of fish was chosen with individual species representing freshwater habitats (tigerfish *Hydrocynus brevis*), marine pelagic environments (bluefish *Pomatomus saltatrix*), as well as benthic (winter skate *Raja ocellata*) and demersal (red hake *Urophycis chuss*; white hake *U. tenuis*; and European hake *Merluccius merluccius*) marine habitats.

Four modifications were made to the central philosophy of the LS regression technique originally described by Blackburn et al. (1992). Modifications differed in the methods applied to divide the data into a number of equal size classes. Data were either separated into size classes containing an equal number of observations or partitioned into size classes representing equal increments of the independent variable (predator length measured in millimeters (mm)). Modifications also differed in the x and y coordinates used to estimate the regression slope. LS regression slopes were estimated using either the actual predator-prey pairs or using the median or midpoint predator length (x) paired with the actual minimum or maximum prey length (y) within each size class.

A quantile regression technique (StataCorp. 1995) based on a least absolute values model was applied to the same six predator-prey data sets. The regression model is fit by minimizing the sum of the absolute values of the residuals (Harris 1950, Bloomfield and Steiger 1980, Rousseeuw and Leroy 1987), rather than the sum of the squares of the residuals as in ordinary LS regression models. Regression models based on least absolute values criteria are resistant to extreme outlying values in the y-direction (Narula and Wellington 1982, Rousseeuw and Leroy 1987) to the extent that model fit is unaffected by changes in y-values so long as the signs of the residuals are maintained (Bloomfield and Steiger 1983). Moreover, regression models that minimize the sum of absolute deviations are particularly effective when using non-parametric estimators of location (e. g. medians, quantiles) (Koenker and Bassett 1978).

Three modifications of the quantile-based regression technique were tested. Modification one (1) consisted of no alterations with respect to the x and y variables. Regression models were fit to the actual undivided data. Modification two (2) initially partitioned the data into a number of size classes containing an equal number of observations and quantile regression slopes were estimated using actual y-values and median x-values of the size classes. Modification three (3) separated the data into size classes of equal increments of predator length (mm) and slopes were then estimated using actual y-values and midpoint x-values of the size classes. Estimates of minimum (10^{th} quantiles) and maximum (90^{th} quantiles) slope were tested for homogeneity across modifications for each predator-prey assemblage. Estimates of slopes generated by quantile regression techniques were then tested for homogeneity against estimates of slopes generated by LS regression techniques.

To examine the effect of size class number on estimates of LS regression slope, we partitioned data from four predator-prey assemblages into size classes containing an equal number of observations and estimated regression slopes using the actual minimum and maximum x-y pairs within each size class. Estimates of minimum and maximum slope were generated for approximately 10, 20, 30, 40, and 50 size classes and observed trends examined. The data sets used in this analysis consisted of predator-prey body length information for four species of piscivorous fish separate from the six species used in the analyses described earlier. The predator group is diverse and includes a freshwater piscivore (walleye *Stizostedion vitreum*), a predatory reef species (coral trout *Plectropomus leopardus*), a marine, demersal piscivore (Atlantic cod *Gadus morhua*) and an elasmobranch (spiny dogfish *Squalus acanthias*).

Results

The four modifications of the LS regression procedure produced significant ($p < 0.05$) slope estimates for only 33% of the applications (16 out of 48) which were equally divided between estimates of minimum (8 out of 24) and maximum (8 out of 24) slope. Results within species were highly variable across modifications with standard errors of significant regressions averaging 29% of the values of the slope estimates.

For the same six predator - prey assemblages, the three modifications of the quantile regression technique generated significant ($p < 0.05$) slope estimates for 72% of the applications (26 out of 36) with all but one estimate of maximum slope being significant (17 out of 18) and half of the estimates of minimum slope being significant (9 out of 18). In contrast to modifications of the LS regression procedure, results within species were consistent across modifications with standard errors of significant regressions averaging 18% of the values of the slope estimates.

Estimates of minimum and maximum LS regression slopes did not significantly increase or decrease with increasing number of size classes for four predator-prey assemblages with the exception of a significant increase in estimates of minimum slope for Atlantic cod ($F = 3.453$; $p < 0.025$); however, obvious trends were evident for each species. Estimates of maximum slopes showed a decreasing pattern, whereas estimates of minimum slopes tended to increase with increasing numbers of size classes. Further, probability values of the regression coefficients decreased with increasing number of size classes across species, especially for estimates of minimum slope.

Discussion

Within species comparisons made across applications of modifications to the LS regression technique yielded a high degree of variability among estimates of minimum and maximum slopes. Although only one of five comparisons between significant LS slope estimates produced statistically detectable differences, results were generally inconsistent. One or more LS regression modifications often produced significant estimates of minimum or maximum slope, whereas the remaining modifications failed to detect a significant relationship between minimum or maximum prey length and predator length. Moreover, LS regression modifications that produced significant estimates of minimum slope frequently failed to produce significant estimates of maximum slope for the same data set.

Results of applications of quantile regression modifications, on the other hand, were less variable across modifications within species and were generally consistent. No comparisons among significant quantile regression slope estimates produced statistical differences. Further, all quantile regression modifications that generated significant estimates of minimum slope also generated significant estimates of maximum slope for the same data set.

Tests for differences among slope estimates across modifications of both regression techniques were performed for maximum prey length consumed by tigerfish, as well as for both minimum and maximum prey length consumed by bluefish and European hake. Tests were performed only between significant slope estimates generated by the two regression techniques. Results indicated no statistically significant differences in slope estimates between regression techniques. However, we argue that this result is a by-product of large estimates of regression coefficient standard errors, especially for those coefficients estimated by LS regression modifications, which negate observed differences. Dismissal of observable differences between regression techniques may lead to spurious conclusions about the role of a predictor variable in structuring the boundaries of a dependent variable.

Contrary to findings of Blackburn et al. (1992), we observed a declining trend in estimates of maximum slope with an increasing number of size classes. Moreover, a slight increase in estimates of minimum slope was evident across data sets. In addition, probability values of significance tests tended to decrease as the number of size classes increased. Based on regression principles (Draper and Smith 1981), these results would be expected. As the number of size classes increases, more of the data are being used to estimate the slopes of upper and lower bounds. As more data are used, estimates of minimum and maximum slope should converge toward the estimate of the slope of the regression estimating the mean, which is generated when each observation represents one size class. In other words, the number of size classes is limited to the number of observations in the data set and any regression generated with this maximum number of size classes is simply a regression through all observations, or the mean regression. Hence, the estimate of a regression slope generated from data partitioned into size classes will approach the estimate of the mean regression slope as size class number increases. Further, as the number of observations used to estimate the regression increases, the amount of variation explained by the regression model should increase, thus decreasing the amount of unexplained variation. Therefore, increasing sample size would be expected to generate lower probability values for significance tests, as seen here.

The results of this study indicate that estimates of LS regression slopes may depend upon four factors which are subject to arbitrary decision-making by the investigator. The first is the method of data partitioning, which was shown to affect both regression significance levels and values of slope estimates. Second is the decision of which x-y pairs to use in fitting the regression model. This decision may also affect significance levels and values of slope estimates. The third factor involves the number of size classes used. Our results suggest that as the number of size classes increases, estimates of minimum and maximum slope will converge toward the mean regression slope. Hence, decisions regarding size class number may considerably affect regression coefficient values and consequently, conclusions drawn from those values. The fourth factor involves the diagnosis of outliers (Barnett and Lewis 1994) (especially in the y-direction) and the decision to include or remove them from the regression model. The LS regression technique applied in this study appeared to be most sensitive to extreme y-values, therefore decisions concerning outliers could prove critical in estimation of upper and lower bounds of scatter diagrams when employing this technique.

The quantile regression technique based on a least absolute values model produced results that were markedly more consistent than those produced by the LS technique. The procedure is designed to be applied to unpartitioned data sets (StataCorp. 1995) (i. e., no partitioning of the data is necessary as with the LS procedure) and data sets were partitioned for two of the modifications only for the comparative purposes of this study. However, results remained consistent despite data partitioning and irregardless of the methods of data partitioning. When the quantile regression procedure is employed as designed, with no data partitioning, the arbitrary decisions associated with the LS regression procedure are removed. The only decision exposed to the subjectivity of the investigator is the choice of quantiles used to represent minimum and maximum slope. We chose the 10^{th} and 90^{th} quantiles to represent minimum and maximum prey size consumed by a piscivorous fish predator. Fish predator-prey scatter diagrams are inherently variable, thus we felt these choices would provide conservative estimates of the scope of the diet. This work was focused on estimation of slopes of the boundaries of scatter diagrams; however, the regression technique will estimate quantiles with values between 0 and 100. The estimation of regression slopes for quantiles other than minimum, median, and maximum may provide insight into the nature of various ecological phenomena. In general, quantile choice will be dependent upon available ecological information and the nature of the research question.

Regression techniques based on least absolute values models have been successfully employed in

various areas of research (Mudrov et al. 1968, Blattberg and Sargent 1971, Taylor 1974). Recently, regression models based on least absolute deviations have been used to estimate quantiles for relationships between fish standing stock and habitat variables (Terrell et al. 1996). The authors concluded that application of quantile regression techniques may prove valuable in determining habitat variables that limit standing stock of fishes. Our results indicate that quantile regression procedures based on least absolute values models are robust with respect to extreme outlying y-values and sparseness contained within data sets relative to regression procedures based on LS models. We recommend application of quantile regression based procedures for analyses directed at ecological phenomena associated with the boundaries of scatter diagrams. Quantile regression techniques based on least absolute values models are, however, no less sensitive than ordinary LS regression models to extreme outliers in the x-direction (leverage points) (Rousseeuw and Leroy 1987), and investigators should be cautious when applying regression models to data sets containing outlying x-values.

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SEASONAL DAILY RATION ESTIMATES OF WALLEYE POLLOCK (*Theragra chalcogramma*) IN THE EASTERN BERING SEA FROM A BIOENERGETICS MODEL

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Introduction

Walleye pollock (*Theragra chalcogramma*) supports the world's largest single species fishery and comprises 67% of the total groundfish biomass in the eastern Bering Sea (Wespestad, 1993). The stock assessments used to manage this resource rely primarily on catch-age analysis of fishery data, and forecasting year-class strength is based primarily on a survey index of age 1 walleye pollock (Wespestad and Dawson, 1992). Better understanding of the sources and magnitude of juvenile mortality would enhance the reliability of management forecasts that rely on incomplete knowledge of factors influencing spawner-recruit relationships. Integration of predation mortality into stock assessment procedures is accomplished through multispecies models (Bromley, 1994) and, recently, the stock synthesis model (Livingston and Methot, 1994).

The major identified source of predation mortality for juvenile walleye pollock in the eastern Bering Sea is cannibalism by adult walleye pollock (Livingston, *et al.* 1986; Livingston, 1991; Livingston *et al.*, 1993). Estimates of the predation mortality exerted by the adults on juvenile walleye pollock and other economically important species in the eastern Bering Sea are based on estimates of daily ration and diet composition (Dwyer *et al.*, 1987; Livingston, 1991; Livingston *et al.*, 1993). Gastric evacuation models and observed mean stomach fullness have been used to estimate seasonal daily ration by size (Livingston *et al.*, 1986; Dwyer *et al.*, 1987), but the resulting high conversion efficiencies suggest that these estimates are biased low (Livingston *et al.*, 1986). Possible sources of bias may include undetected regurgitation, reduced stomach contents weight from preservatives,

and depressed gastric evacuation from forced feeding (Dwyer *et al.*, 1987). Livingston (1991) estimated more realistic daily rations by size, based on observed growth increments and a generalized food conversion efficiency for fish. However, these were annual rations and did not take into account seasonal changes in growth and other variables that could cause within-year ration changes. Because walleye pollock cannibalism varies by season (Dwyer *et al.*, 1987), a bioenergetics model that accounts for seasonal variation in growth, water temperature, and prey energy content could improve the estimates of daily ration (Livingston, 1991).

A bioenergetics model of individual fish growth (Hewett and Johnson, 1992) was parameterized for walleye pollock by Buckley and Livingston (1994) based primarily on the laboratory work of Paul (1986) and Smith *et al.* (1986; 1988; 1989). In this paper we simulated laboratory feeding experiments to corroborate the model's performance, estimated the daily ration of walleye pollock by season and age in the eastern Bering Sea using the bioenergetics model, and discussed what improvements to the bioenergetics model may be the most effective.

Methods and Materials

Bioenergetics models are based on the balanced energy equation which states that consumed energy must be egested, excreted, or used for respiration, reproduction or growth (Hewett and Johnson, 1992). The basic unit of the model's daily accounting of energy input and output is the calorie, which is translated to and from weight dependent processes using the caloric density (calories per gram wet weight, or cal/g) of the predator and the prey. In this application we specify estimates of seasonal growth, maximum consumption, respiration, egestion and excretion, and the balanced energy equation is iteratively solved for the proportion of the maximum consumption (a weight and temperature specific value). The proportion of the maximum consumption allowed by the bioenergetics model cannot be less than 0 or more than 2.

Submodel choice and parameterization of the bioenergetic functions (internal parameters) are described in Buckley and Livingston (1994). However, early applications indicated that the theoretical maximum consumption allowed by the model was too low. Therefore, the parameter CA (the theoretical maximum daily consumption of a 1 g fish at 0°C) was increased from 0.02878 g/g/d to 0.4 g/g/d. The equations and parameter values used are summarized in Table 1 with parameter symbols consistent with Hewett and Johnson (1992). The model was parameterized to predict ration from growth patterns in the northwest and southeast areas of the eastern Bering Sea shelf because previous studies (Lynde *et al.*, 1986; Hinckley, 1987) indicated different growth patterns for walleye pollock in these two subareas. Other differences between the northwest and southeast areas of the eastern Bering Sea shelf included water temperature, diet composition, and spawning date. The energy lost to spawning was assumed to be the same in the two areas, but the proportion of body weight spawned was different because of the seasonal fluctuations in caloric density (Buckley and Livingston, 1994).

In order to corroborate model performance, simulations of laboratory feeding trials were performed that predict consumption from the observed growth. The predicted total consumption, daily ration and gross conversion efficiency (growth/consumption) were compared to the reported laboratory results. In the simulations it was assumed that spawning did not occur, predator caloric density was 1400 cal/g, and the temperature was constant. The daily ration in the laboratory trials was calculated using average daily consumption and initial weight (Smith *et al.*, 1988), and was compared to the daily ration on the first day in the model simulations.

The walleye pollock growth data input into the model was derived in three ways from individual size-at-age observations sampled from commercial fishing vessels throughout the year. First, the average quarterly weight-at-age was calculated for ages 2 through 8 in the northwest area and ages 2 through 10 in the southeast area (Buckley and Livingston, 1994). The extreme negative winter growth in the

Table 1. Equations and parameter values for the bioenergetics model of walleye pollock in the eastern Bering Sea; W = weight (g) and T = temperature (°C).

Consumption: $C = CA * W^{CB} * e^{(CQ * T)} * P$

Respiration: $R = (RA * W^{RB} * f(T) * ACT) + (SDA * (C - F))$
where $f(T) = V^X * e^{(X * (1 - V))}$
and $V = (RTM - T)/(RTM - RTO)$
$X = (Z^2 * (1 + (1 + 40/Y)^{0.5})^2)/400$
$Z = \ln(RQ) * (RTM - RTO)$
$Y = \ln(RQ) * (RTM - RTO + 2)$

Egestion : $F = FA * C$

Excretion : $U = UA * (C - F)$

Parameter	Description	Value
C	specific daily feeding rate (g/g/d)	
P	constant proportion of maximum consumption	
CA	intercept of the allometric function	0.4
CB	slope of the allometric function	- 0.6292
CQ	temperature dependence coefficient	0.3763
R	specific rate of respiration ($g\text{-}O_2/g/d$)	
RA(<100 g)	intercept of the allometric function	0.0069
(100-299 g)		0.0066
(300-400 g)		0.0063
(>400 g)		0.0061
RB	slope of the allometric function	-0.26
RQ(<100 g)	temperature dependence coefficient	6.04
(100-199 g)		4.5
(300-400 g)		3.0
(>400 g)		1.8
RTO	temperature at highest respiration	9.0
RTM	lethal water temperature	16.0
ACT	multiplier of measured respiration level	1.3
SDA	proportion of assimilated energy lost to specific dynamic action	0.125
FA	proportion of consumed energy egested	0.20
UA	proportion of absorbed energy excreted	0.11

Reproduction:	Southeast	Northwest
age of first spawn	4	4
spawning date	1 April	15 July
proportion of energy spawned (= -126 cal/g)	0.127	0.101

southeast area was assumed to result from seasonal movement patterns (Lynde *et al.*, 1986) and/or difficulties in ageing pollock during this time period, and an assumption of no weight-growth was made for this season and area. Second, a seasonally oscillating von Bertalanffy growth function (Pauly *et al.*, 1992) was fit to monthly mean length-at-age data with a period of zero length-growth from August through January for ages 1 through 10+. The fitted monthly lengths were converted to weights using seasonal length-weight relationships from the data, and a three-point running average was used to smooth the monthly weights. Third, the same fitted monthly lengths were converted to weights using a single length-weight conversion. The ration estimates were presented as a percentage of the predator's body weight per day (% bw/d).

Results and Discussion

Our model calculated the total food consumption, daily ration and gross conversion efficiency by walleye pollock to be very close to that observed under laboratory conditions (Table 2). The feeding trial from Smith *et al.* (1988) was conducted at a constant temperature and a specific diet. The high gross conversion efficiency in this feeding trial and simulation was the result of feeding the walleye pollock a very high energy food, fillets of Pacific herring (*Clupea harengus pallasi*, 1992 cal/g). The simulations and the feeding trials presented by Yoshida and Sakurai (1984) yielded similar results even though the temperatures were not constant in the feeding trials and the diet consisted of unknown proportions of squid (*Todarodes pacificus*), sand lance (*Ammodytes personatus*) and juvenile walleye pollock. The caloric density of the diet in these feeding trials was estimated to be 1250 cal/g based on values reported in the literature (Perez, 1994; Smith *et al.*, 1986; Steimle and Terranova, 1985; Thayer *et al.*, 1973). By manipulating the caloric density of the diet in the model,

Table 2. Simulation of laboratory feeding trials of walleye pollock using a bioenergetics model to estimate consumption, daily ration, and gross conversion efficiency.

Reference	Temp. (°C)	Time (d)	Prey caloric density (cal/g)	Predator weight Start (g)	Predator weight End[a] (g)	Cumulative consumption (g)	Daily ration (%bw)	Gross conversion efficiency (g/g)
Smith *et al.*, 1988:								
(feeding trial)	5.00	30	1992	352	398.5	93.98[b]	0.89	46.0%
(simulation)						96.86	0.90	48.0%
Yoshida and Sakurai, 1984:								
(feeding trial)	4.28	365	unknown	446	641	1586	0.97[c]	12.3%[c]
(simulation)			1215			1575.19	0.90	12.4%
(simulation)			1250			1530.36	0.87	12.7%
(feeding trial)	11.15	365	unknown	533	728	1792	0.90[c]	10.9%[c]
(simulation)			1215			1805.47	0.87	10.8%
(simulation)			1250			1754.15	0.84	11.1%

[a]The end weights were calculated from data given in the references as a rate of weight gain per day or per year.
[b]The cumulative consumption was calculated from the reported rate of daily consumption given in Smith *et al.* (1988).
[c]The daily ration and conversion efficiency were calculated from the cumulative consumption reported in Yoshida and Sakurai (1984).

we were able to nearly duplicate the feeding trial consumption rates when the caloric density of the diet was 1215 cal/g. The similarity of the simulation and the laboratory feeding trial at 11.15°C was especially encouraging because the model parameters were established by experiments using temperatures less than 8°C.

Our first attempts to fit the proportion of the maximum consumption to the observed growth in the eastern Bering Sea were unsuccessful because the proportion required to achieve the specified growth was greater than 2 in seasons of high growth. The model only allows consumption to be 0 to 2 times the theoretical maximum consumption. Assuming the specified growth rates were realistic, the theoretical maximum consumption calculated using the parameters in Buckley and Livingston (1994) was too low. Three factors probably contributed to the low theoretical maximum consumption. First, walleye pollock were fed a very high-energy food, fillets of Pacific herring, during the maximum consumption versus body weight feeding trials (Smith *et al.*, 1986), and gastric evacuation of high-energy diets has been shown to be slower than that of high-energy diets for rainbow trout (*Oncorhynchus mykiss*) and marine flatfish (Jobling, 1987). Second, in the feeding trials to assess the effect of temperature on maximum consumption, the walleye pollock held at lower temperatures were fed a higher-energy diet, thus the maximum consumption could be underestimated at lower temperatures like those used in the model (1.79°C to 4.56°C). Third, the effect of temperature on consumption has only been determined for very small walleye pollock (mean weight = 49 g; personal communication, A.J. Paul, Institute of Marine Science, Seward Marine Center, P.O. Box 730, Seward, Alaska 99664), and this effect probably decreases with fish-size (Buckley and Livingston, 1994) causing an underestimation of consumption especially for larger walleye pollock at lower temperatures.

We attempted to correct for the apparent underestimation of maximum consumption by elevating the parameter CA from 0.02878 to 0.4, allowing the model to predict consumption based on observed growth. Increasing CA did not affect the model's calculation of the amount of food required to achieve the specified growth, it only changed the theoretical maximum consumption, and therefore, the proportion of the maximum consumption that was required. We do not suggest that CA = 0.4 is a realistic parameter value because it implies that a hypothetical 1 g pollock at 0°C consumes 0.4 g/g/d or 40% bw/d. The parameters that describe consumption can be very influential when forecasting growth from feeding rates (Kitchell *et al.*, 1977), and more research into the temperature effect on maximum consumption of large walleye pollock is required before the model can be realistically applied in this fashion. In our application, the calculated maximum consumption is irrelevant as long as the proportion of the maximum consumption is between 0 and 2.

The pattern of rations predicted by the bioenergetics model from average growth patterns was high in spring and summer, and low in autumn and winter, and was consistent among all ages in the southeast and northwest areas of the eastern Bering Sea shelf (Figures 1a and 1b). We believe this pattern is generally accurate based on prey availability in the eastern Bering Sea (Cooney, 1981; Dwyer *et al.*, 1987), temperature variations (Chen, 1983), day-length variations and winter-acclimated reduced hunger as has been suggested for other species (Johnston and Battram, 1993). A similar pattern in consumption rates was generated from all three treatments of the size-at-age data with the period of high consumption rates extending from March or April through August or September. Chen (1983) considered the main feeding season to extend from June through October. Variations in the period and amplitude of the seasonal feeding pattern may vary considerably among years and ages based on patterns of observed growth (Chen, 1983).

Ration estimates from our seasonal bioenergetics model of walleye pollock range from 0.0% bw/d to 3.9% bw/d, while ration estimates found in the literature range from 0.05% bw/d to 6.4% bw/d (Dwyer *et al.*, 1987; Springer, 1992). Dwyer *et al.* (1987) estimated rations by season for two size classes of walleye pollock in the northwest and southeast areas of the eastern Bering Sea shelf (ranging from 0.05% bw/d to 0.62% bw/d) based on mean stomach fullness and gastric evacuation

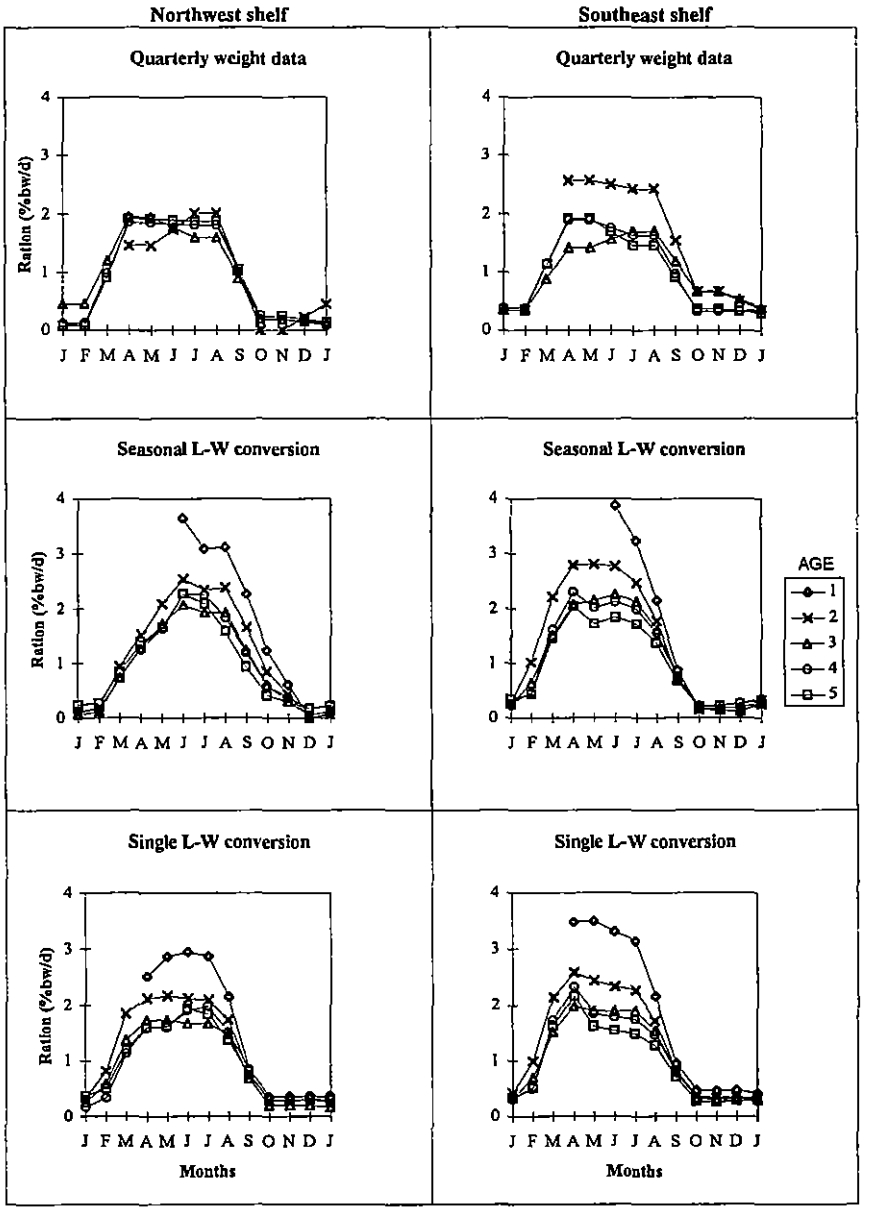

Figure 1a. Bioenergetics model estimates of walleye pollock ration as a percentage of body weight per day (% bw/d) by age for each month of the year in two subareas of the eastern Bering Sea shelf for three different treatments of size-at-age data. (Walleye pollock ages 1 through 5)

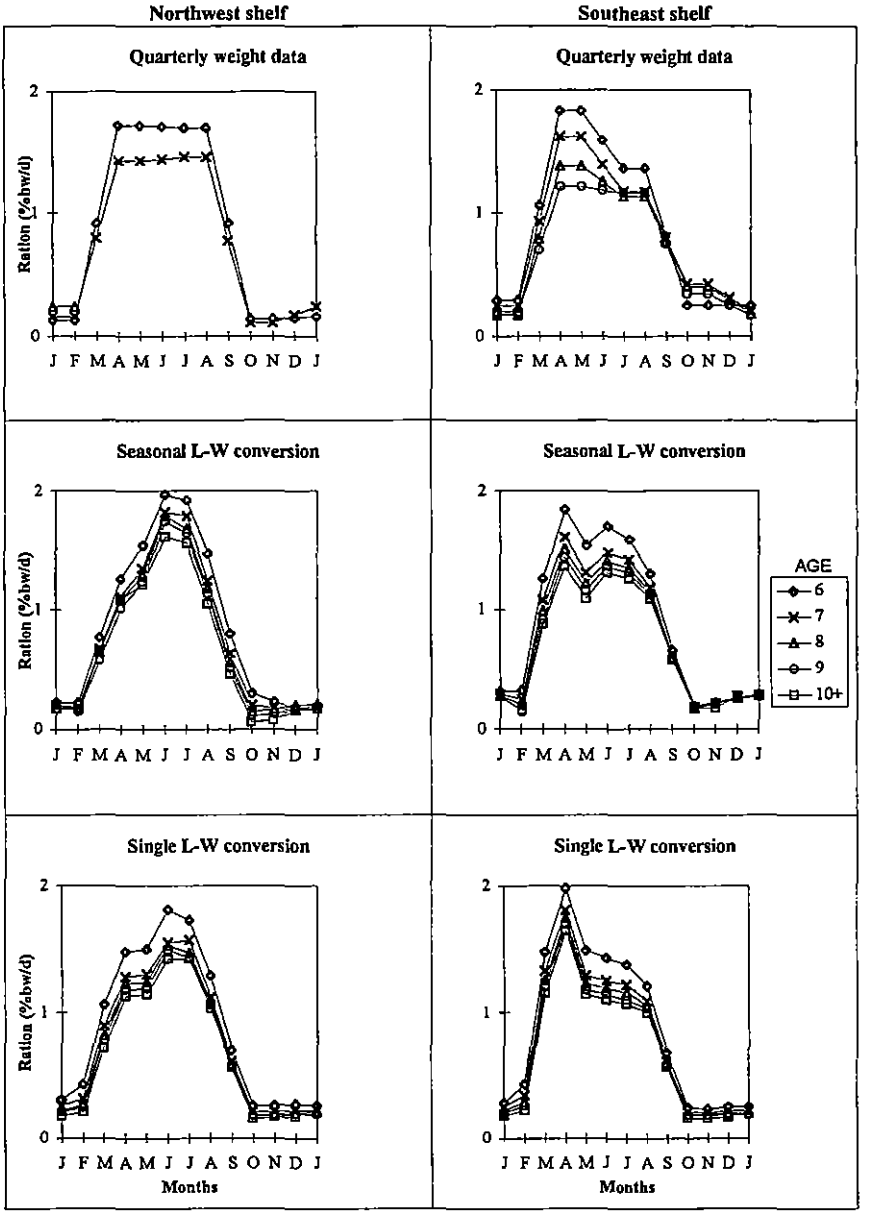

Figure 1b. Bioenergetics model estimates of walleye pollock ration as a percentage of body weight per day (% bw/d) by age for each month of the year in two subareas of the eastern Bering Sea shelf for three different treatments of size-at-age data. (Walleye pollock ages 6 through 10+)

rates, but the resulting conversion efficiencies suggest that these estimates are biased low (Livingston *et al.*, 1986).

The rations estimated using the bioenergetics model may also be biased because of uncertainties about activity costs and external variables (Ney, 1993) which result from a general lack of knowledge about the activity and movement patterns of walleye pollock on daily, seasonal and lifetime scales (Buckley and Livingston, 1994). The activity level of aquaria-held walleye pollock is probably less than the activity level of walleye pollock in the eastern Bering Sea. Therefore, if the model accurately simulates walleye pollock at a reduced activity level (and therefore a reduced consumption level to achieve the observed growth) then the model would underestimate natural consumption rates. Biotelemetry studies of walleye pollock could improve the estimates of their thermal history and improve our knowledge about the activity level of walleye pollock in the eastern Bering Sea relative to aquaria-held individuals. Ontogenetic movements of walleye pollock between the northwest and southeast subareas of the eastern Bering Sea may cause bias in estimated growth rates, and therefore in model-estimated consumption (Buckley and Livingston, 1994). Movement and growth patterns can be clarified through tagging studies, but past attempts to tag walleye pollock have met with little success (Paul *et al.*, 1990). Recent experimentation with coded wire tags indicates that survival and retention rates, and the potential for recovery, may be high enough to warrant a large-scale coded wire tagging program for walleye pollock (NRC and NMT, 1996).

Complete validation of this bioenergetics model may be impossible (Ney, 1993) and corroboration with other estimates of seasonal daily rations of walleye pollock may not be very informative because of the problems associated with those estimates. However, the strength of using a bioenergetics model is that it combines laboratory derived physiological parameters, which are probably not too biased, with field measures of external variables that (although they may be biased or unrefined) are probably less biased than field measures of mean stomach fullness.

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ON THE GROWTH OF THE MEDITERRANEAN HAKE (*Merluccius merluccius L.*) FROM THE SANTA POLA BAY (S.E.SPAIN)

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Introduction

The hake, *Merluccius merluccius* (Linnaeus, 1758), is a widely distributed groundfish species on the continental shelf and slope off Europe and in the Mediterranean Sea at depths between 30 and 1 000 m, though it is most abundant between 70 and 370 m. The species carries out daily vertical feeding migrations, staying close to the bottom in the daytime and rising off the bottom to adopt a midwater habit at night, and is a target species in trawl fisheries.

Materials and methods

The data were compiled from monthly samples collected from landings by the commercial fleet at the Santa Pola (Figure 1) fish wharf in 1991, 1992, and 1993. A total of 72 samples, two per month, were collected from January 1991 to December 1993, contributing a total of 20 806 individuals, mesuring its individual total length at the nearest lower cm; in each month some individuals were intensively sampled; total length in cm; total weight in g; gutted weight in g; and girth around the base of the pectoral fins in cm; separately by sexes, were recorded from a total of 2 889 individuals.

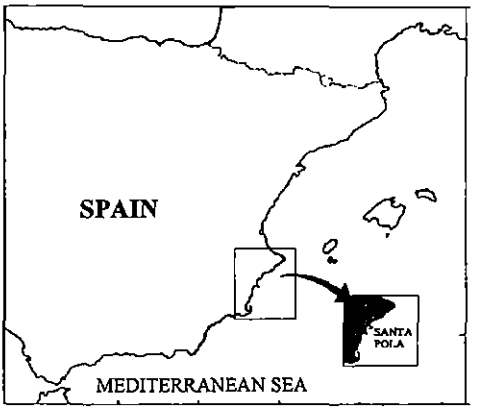

Figure 1.- Situation of the area of study

The relationships between certain parameters considered were determined. For total length:total weight and girth:total weight, the data was expressed by a power equation of the form $W = a*L^b$. For the other parameter relationships, total weight:gutted weight and total length:girth, the data was adjusted by linear regression: $Y = a + bX$. The relationships were established separately for males, females, individuals of undetermined sex, and the population as a whole.

The age-length relationship was computed from size frequencies. Monthly data samples were expanded to the total number of individuals caught per month by the fleet, and grouped by 2-cm size class, separated by sexes using the sex ratio by size, and smoothed over three classes; the pool of individuals for which sex could not be determined was divided equally between males and females. The Von Bertalanffy growth function (VBGF) $L_t = L_\infty (1 - e^{-k(t-t0)})$ was employed as growth expression and the growth parameters ($L\infty$, K and t_0) estimated using the ELEFAN (Gayanilo et al. 1988) and FISHPARM (Prager et al. 1987) automatic computer programs. Estimations of the "best combination" of the VBGF parameters were done, looking to optimize the values of the goodness index (Rn), and giving a value of $t_1 = 12$ cm to determine $\hat{k}$. The method of Bhattacharia (MPA mode of the ELEFAN program) was applied to the data quarterly and the results were then applied to the FISHPARM program.

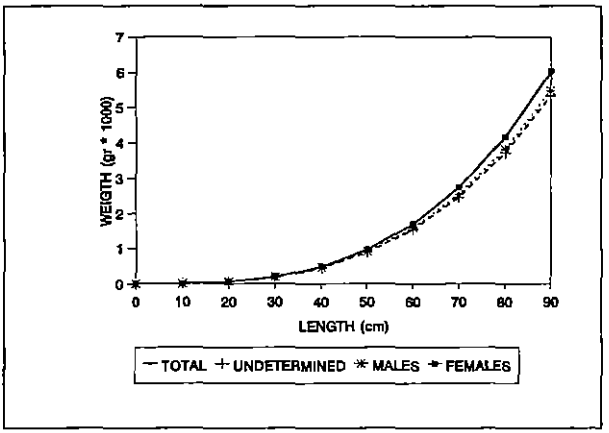

Figure 2. Curves of relative growth (Length-Weigth) for the hake by sex.

Results

The results for the relationships between the various biometric parameters for hake established in this study have been presented as follows: The **length-weight** relationships are presented in Table I, resulting that the coefficient b significantly differ than 3 in all cases except in males, which is graphically represented in Figure 2, showing that the curves of the females and total poulation overlap each other and, on the other hand, undetermined and males also overlap;

TABLE I.- Parameters of relative growth (Legth-Weigth relationship: Weigth = a * Length ^{b}) calculated for the different groups (undetermined, males, females and total) of *Merluccius merluccius*; significance levels = ***<0.001, **<0.01, *<0.05 y NS<0.1 in a "t"Test.

Group	a	b	err.b	signif.	r2	n	range
males	0.006	3.05	0.02941	NS	0.96	502	13.5-52.5
females	0.0048	3.12	0.01259	***	0.99	955	11.5-68.0
undeterm.	0.0056	3.06	0.01681	***	0.96	1 369	4.0-32.5
total	0.0048	3.12	0.00477	***	0.99	2 826	4.0-68.0

The **girth-weight** relationships are presented in Table II, show a significant difference between b and 3 in all cases except in the undetermined, showing that its growth curves (Figure 3) coincide between those of males and females, yielding lesser weights at the same girth than undetermined and total population; the **girth-length** relationships are presented in Table III, resulting a relation between parameters in the form that the girth is nearly the half of the length; and the **gutted weight-total weight** relationships are shown in Table IV.

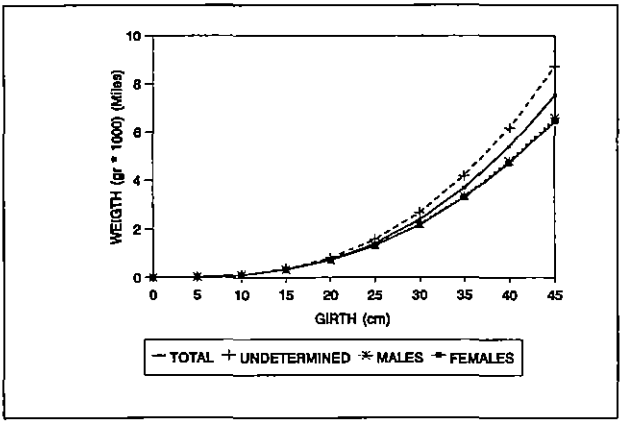

Figure 3. Curves of relative growth (Girth-Weigth) in hake by sex.

TABLE II.- Parameters of relative growth (Girth-Total Weigth relationship: Weigth = a * Girth^b) calculated for the different groups (undetermined, males, females and total) of *Merluccius merluccius*; significance levels = ***<0.001, **<0.01, *<0.05 y NS<0.1 in a "t"Test.

Group	a	b	err.b	signif.	r2	n	range
males	0.210	2.72	0.03390	***	0.93	437	13.5-52.5
females	0.214	2.71	0.01683	***	0.96	819	11.5-68.0
undeterm.	0.125	2.93	0.02464	NS	0.91	1 230	4.0-32.5
total	0.141	2.86	0.00675	***	0.99	2 486	4.0-68.0

TABLE III.- Parameters of relative growth (Length-Girth relationship: Girth = a + b Length) calculated for the different groups (undetermined, males, females and total) of *Merluccius merluccius*.

Group	a	b	r2	n	range
males	-0.289	0.42	0.86	437	13.5-52.5
females	-1.552	0.48	0.92	819	11.5-68.5
undeterm.	0.080	0.38	0.90	1 230	4.0-32.5
total	-0.995	0.46	0.97	2 486	4.0-68.5

The size-weight relationship values shown, were similar to the values calculated by other workers in the Western Mediterranean, see Quesada (1991), resulting in general that the values of b were higher than 3. Differences between group sizes were small and were mainly ascribable to the fact that the size ranges for the individuals of undetermined sex and for males were smaller than the size range for females; for the segment of the size range covered by all three

of these groups the growth curves overlaps, suggesting that any differences were not significant. The value of the allometric coefficient *b* was significantly greater than 3, indicative of a slightly positive allometry in the growth of hake in the Mediterranean over its entire life span, except for males in which case were not different from 3 being its growth isometric, resulting that females were more robust than males.

TABLE IV.- Parameters of relative growth (Gutted Weigth-Total Weigth relationship: Gutted Weigth = a + b Total Weigth) calculated for the different groups (undetermined, males, females and total) of *Merluccius merluccius*.

Group	a	b	r2	n	range
males	2.11	0.88	0.96	502	13.5-52.5
females	5.91	0.85	0.99	955	11.5-68.0
undeterm.	1.50	0.80	0.94	956	4.0-32.5
total	3.22	0.86	0.99	2 413	4.0-68.0

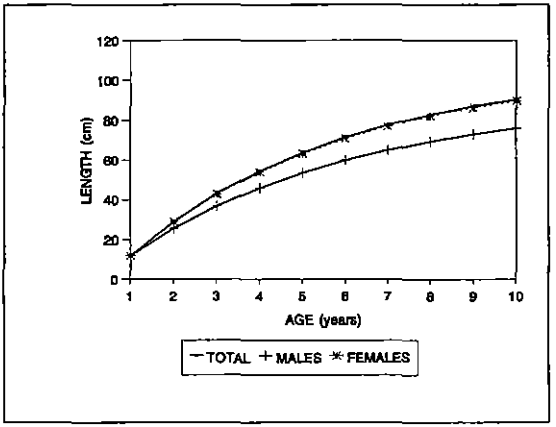

Figure 4. Curves of the absolute growth (Length-Age) of hake, using the ELEFAN program

The **age-length** relationship result shows, in general, a growth rate higher than accepted until now: The results of the differents estimations yielded by the ELEFAN program are shown in Table V, showing in Figure 4 the growth curves; for the parameters calculated by the use of the FISHPARM program, the results of the differnt estimations done, appear in Table VI, while the absolute growth curve appears in Figure 5. The results for the calculations of the VBGF parameter values indicate that the two programs yielded comparable estimates. Growth differences between the sexes appeared in the second year of life, and the K values produced by the programs differed, the ELEFAN estimate being higher. In both cases the curves of the females and total population overlaps, being higer than the males ones.

TABLE V.- Results of the growth parameters (length- age relationship) of the VBGF ($L_t = L_\infty (1 - e^{-k(t-t0)})$) obtained using the ELEFAN program, for the different groups (males, females and total) in *Merluccius merluccius*.

Group	L_∞	K	t_0	Rn
males	90	0.19	0.24	0.108
females	105	0.20	0.39	0.142
total	106	0.20	0.40	0.132

TABLE VI.- Results of the growth parameters (length- age relationship) of the VBGF ($L_t = L_\infty (1 - e^{-K(t-t0)})$) obtained using the FISHPARM program, for the different groups (males, females and total) in *Merluccius merluccius*.

Group	L_∞	K	t_0
males	73.3	0.172	- 0.108
females	99.7	0.153	0.264
total	113.2	0.123	0.137

We recomend for its application, as a result of this work, the use of the FISHPARM parameters, due to the low values of the goodness index (Rn) obtained in the calculations of the ELEFAN growth parameters. In general the age of the individuals caught by trawl did not exceed 7-8 years old, implying that the species not live longer than 10 years.

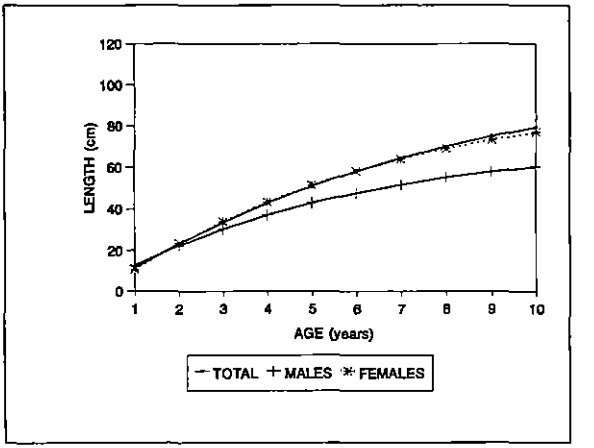

Figure 5. Curves of absolute growth (Length-Age) in hake using the FISHPARM program.

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SAMPLE SIZE AND DATA ANALYSIS:

ARE WE CHARACTERIZING AND COMPARING DIET PROPERLY?

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Abstract

We reviewed over 200 papers that compared primarily fish diets between species, sites, and sample dates. Precise descriptions of each diet are necessary in order to make comparisons between them. Yet none of the studies we reviewed provided any estimates of precision. We advocate the use of cumulative prey curves (performance tests) for determining whether enough samples have been collected to describe diet precisely and for subsequent comparisons. The studies we reviewed used indices, correlations, and parametric statistics to compare diets, and many claimed to find no difference between diets. We used a power analysis technique to determine that a majority of those papers would have found a difference between diets had they increased their sample size only slightly. We also advocate using individual guts as a sample unit, thereby providing for the estimate of standard deviation for a given index, thus increasing its utility in determining if differences exist, especially if traditional parametric statistics cannot be applied. This does not, however, make indices strong inference tools. Particularly shocking was our observation that many studies used only indices to compare diet, without statistical analysis, and explicitly stated that "significant" differences existed between diets.

Introduction

In studies of fish feeding habits, it is often useful to ask whether the diets of two species differ, how much they differ, and whether these differences are statistically significant. Similarly, these questions can be extended to comparisons within a species between or among different locations, times, sexes, or age classes of fish. In order to make such comparisons, however, it is necessary to be sure that each diet being compared is described adequately, or precisely. Once comparisons have been made, studies often claim that no differences between diets exist. However, often the researchers have not collected enough samples to detect any differences, if a difference really exists. Non-significant results may actually have been significant had only a few more samples been collected. This error was made because the test performed lacked power.

A Basic Review of Power Analysis

The power of a test is defined as the probability of correctly rejecting a null hypothesis, and is mathematically equal to $1-\beta$, where β is the probability of not rejecting a false null hypothesis (Type II error). Furthermore β is related to α, the probability of incorrectly rejecting a null hypothesis (Type I error). Mostly by convention, we generally set α at 0.05, meaning we are

willing to reject a null hypothesis that is not false 5% of the time (see Zar, 1984; Sokal and Rohlf, 1995). The extent to which β errors occur, however, is often ignored.

Traditionally, *a posteriori* power analysis techniques are used to determine the power, or 1-β, level of a test after it has been performed. To determine the expected power of a test that has yet to be performed, *a priori* techniques are employed. In both cases, β will depend on several factors: variability of the data (precision), effect size (the difference to be detected), sample size, and α. Given a certain sample size, effect size, and precision, as one changes α, β will vary inversely. The equations for power can be manipulated so that one can solve for any one of the above factors. In doing so, the α and β levels can be set by the researcher, and the researcher can then determine, for example, how many samples are necessary to detect a difference (effect size) of *d*. Power analysis is infinitely useful for planning experiments and sampling designs, as well as determining the validity of a studies results.

It is the purpose of this review to elucidate problems in the current literature regarding the lack of precision in dietary descriptions and power in dietary comparisons. We provide a detailed description of a method for evaluating the precision of the data sets to be compared. We determine the proportion of recently published studies that were supported by a sufficient sample size for the comparisons made and conclusions drawn. We present *a priori* power techniques for assessing the number of samples needed to detect a difference between diets given the stated effect size, and compare those values to the actual number of samples (i.e. guts) collected. Consideration is given to effect size, which is important for determining when these predictions of sample size are relevant. Finally, we describe techniques for increasing the inferential strength of diet indices, typically used to compare diets.

Methods

Published laboratory and field studies that compared diets between fish species, size classes, sites studied, and experimental groups were used to evaluate precision and sample size used for detecting differences in diet composition. Studies were chosen from published papers located using a standard literature data base (Aquatic Sciences and Fisheries Abstracts). Studies were targeted in the data base using the key words diet, food, feeding, or analysis, and selected based on the material contained in the abstract. We were looking specifically for mention of the type of analysis used in the study, and an indication of how much of the data were presented. More than 200 papers were subsequently scrutinized in the course of this study.

Precision in Diet Description

None of the original ≈200 studies utilized any technique for determining if an adequate number of samples had been collected to precisely describe diet prior to performing comparisons. Therefore, we felt compelled to re-describe a technique for performing such evaluations. We recommend the use of cumulative prey curves (or performance curves; see also Elliott, 1971; Hurturbia, 1973; Cailliet, 1977; Hoffman, 1979; Cailliet et al., 1986). These are based on the fact that as sample sizes increase, variation (and species richness) tends to decrease, and thus the curve reaches an asymptote as new prey types are being introduced into the diet only rarely.

Cumulative prey curves are created by plotting the cumulative number of prey types (i.e. unique items) against the cumulative number of guts analyzed. Or, as mathematically described:

$$S_n f(n) \ ,$$

where S is the number of prey types found for a given number of guts analyzed (n). Variations of this technique can be found in Hurturbia (1973) and Hoffman (1979), where diversity indices (H) are recalculated as new guts are added to the sample size (n), and each subsequent H_n is plotted against n. Curves can be plotted in the original order of gut analysis, or by randomizing. Guts are generally opened and the contents quantified in a haphazard order (usually the order of collection, which is often correlated with some other feature of the sampling design). It is

important to randomize the order of analysis to prevent bias. Numerous randomizations can be performed, from which a mean number of new prey types can be determined for each consecutive gut, and plotted. Standard deviations can be calculated providing a measure of the variation in number of new prey types throughout the analysis. This allows one to see not only if the curve reaches an asymptote, but the variability of the asymptotic region.

As an example of the technique, we constructed cumulative prey curves from three sample data sets (collected by LAF and GMC). Curves were plotted using both original and randomized gut orders, allowing for comparison of the asymptotic nature of the curves.

Assessing Sample Size Sufficiency in Making Comparisons

We used an *a priori* power analysis technique described by Cohen (1988) to determine if adequate sample sizes were being used to ascertain diet differences between study groups. The technique (described herein) requires that diet data be presented as either actual numerical values or as proportions of total diet for each variable quantified (i.e. actual numbers of a diet item found in fish from one site versus another). Because such raw data are rarely provided, and few papers supplied graphs that allowed the extraction of such values, only 55 studies of the original ≈200 presented enough data to be analyzed with the power analysis technique. Of these, it was necessary for the author(s) to have stated implicitly that *no difference* was detected between diets studied (the corollary being that if a difference in diet was detected, clearly the sample size used was large enough). Thus, for this analysis, 41 separate, independent comparisons were evaluated.

In these 41 studies, data were more often presented as proportions, rather than actual values. Proportions were expressed as either percent number (%N; quantity of a prey type relative to the total quantity of prey collected), percent frequency of occurrence (%FO; number of predators in which a prey type appeared in relative to the total number of predators), or a combination, such as Index of Relative Importance (IRI; [(%N + %V) x %FO], Pinkas et al., 1971). These values were compared using a variety of standard diet indices (i.e. Percent Similarity Index, Hutchinson's Niche Breadth, Morisita's Index of Similarity; see Silver, 1975, Cailliet and Barry, 1978, Krebs, 1989). Occasionally, proportions and actual data values were also compared using parametric t-tests and ANOVA, as well as correlation (usually Spearman's ranked correlation; see Zar, 1984).

To our knowledge, the *a priori* power analysis techniques used in our study have not previously been used to determine sample size sufficiency for food habits studies. As previously mentioned, such techniques are generally used to determine the power of a test to be performed. We use *a priori* techniques here to evaluate tests already performed, as they are the only type possible given the data obtained from the studies we reviewed (this is discussed further in the next paragraphs). According to convention, we set α=0.05 and β= 0.20 (see Zar, 1984). Given these values and the effect sizes measured in the studies being critiqued, we calculated the sample size necessary ($\hat{n}$) to detect a significant difference according to the formulas outlined here.

For diet comparisons using indices:

$$\hat{n} = \frac{1570}{100 \times h^2}, \qquad \text{(Eq. 1)}$$

where the value 1570 comes from Cohen's (1988) tables for the α and β levels set. The tables are specific to this particular equation. The symbol h represents the difference between the arcsine-transformed proportions being compared by the index. When multiple values were compared in a study (i.e. proportions of several different diet items across sites), we selected the largest difference (effect size) presented, assuming this would be the easiest difference to detect and the most forgiving test of sample size sufficiency. To determine the validity of using Equation 1, we used control data sets (collected by the authors, some of which are the same data as used in the cumulative prey curves, see Results) and compared $\hat{n}$ calculated Equation 1 to $\hat{n}$

calculated by a more traditional formula that incorporates variance (Cohen, 1988; Krebs, 1989):

$$\hat{n} = \frac{(Z\alpha + Z\beta)^2 \times s^2}{d^2}, \qquad \text{(Eq. 2)}$$

where s^2 is the variance, d is the untransformed difference between the proportions being compared, and Z_α and Z_β are the z-scores for the α and β values set (for $\alpha = 0.05$, $Z_\alpha = 1.9600$; for $\beta = 0.20$, $Z_\beta = 0.8500$). Equation 2 was otherwise impossible for us to utilize since variance is not usually provided in studies using indices to compare diet. For the control datasets, %N of a diet item was the measure compared between diets.

This same method for estimating $\hat{n}$ (Eq. 2; see also Winer, 1971; Sokal and Rohlf, 1995) was used to evaluate a second category of diet comparisons; those using parametric tests (t-test or ANOVA; for the latter, choosing one pair of values within the ANOVA according to the largest difference criterion used above). The type of analysis used (t-test or ANOVA) was simply a category to separate the original 200+ papers and for choosing the best power analysis technique from those available. (Note: our technique does not calculate the power of the statistical test *per se* since we are not using any value obtained from the statistical test. We are using the means and standard deviations provided by the authors to evaluate the sufficiency of their dataset. Although *a posteriori* power analysis techniques can be used to determine virtually the same information, we chose *a priori* techniques for their simplicity and to be consistent with the evaluation technique used for index-based studies.) Data sets that violate the assumptions of parametric statistics to a "moderate" degree do not affect the validity of the sample size predictions (Cohen, 1988), so the test can also be used to evaluate studies that used non-parametric (ranked) tests if desired (assuming that non-parametric tests were chosen because the assumptions of parametric statistics were violated).

A second method described by Cohen (1988) was used to determine if an adequate sample size had been used in a third category of studies, those relying on correlation tests to determine if diet was different. It is necessary to understand at this point that if two diets are significantly correlated, they are significantly *similar*. However, finding significance, as mentioned above, necessarily implies that the sample size used in the test was sufficient. Thus, for correlations, we scrutinized studies that failed to find a significant correlation given the sample size used. These studies, therefore, imply that there are differences between the measures of diet being compared. It should be noted that only the null hypotheses have changed, the concept of needing a sufficient sample size to perform a given test, remains the same. The formula is:

$$\hat{n} = \frac{1573 - 3}{100 \times z^2} + 3 \qquad \text{(Eq. 3)}$$

As in the previous formula, 1573 is from Cohen's table for the a and b levels set, and z is the Fisher transformed correlation coefficient (r). This test was designed for use with the Pearson product-moment correlation, but violating the assumptions of homogeneity or homoscedasticity to a "moderate" degree does not affect the validity of the power estimates (Cohen, 1988). Therefore, we used the test to evaluate sample size in studies using both Spearman's rank correlation and Kendall's tau.

In each case, because the equations provided are designed to estimate sample size needed for a given comparison, the value $\hat{n}$ is the number of samples needed at each site, of each species, or each age class. (e.g. to compare two sites, one must have $\hat{n}$ samples from site one, and the same number of samples, $\hat{n}$, from site two). Many studies, however, did not obtain the same number of samples for each diet. Therefore, estimated sample sizes were contrasted with the actual sample sizes of the two statistical populations being compared (i.e. n_1 and n_2), resulting in two contrasts per index, parametric statistic, or correlation ($\hat{n}$ vs. n_1 and $\hat{n}$ vs. n_2).

Results and Discussion

Precision in Diet Description

When plotted in the order in which guts were analyzed (Fig. 1a, c, e), the cumulative prey curves for all three sample data sets leveled off, indicating that no new prey types are being found in the diet. However, notice that when gut order was randomized, two of the three data sets took many more guts to approach an asymptote (Fig. 1d, f), and in one case, the standard deviation remains quite large (Fig. 1d). This suggests that sample order may indeed cause a bias and its removal by randomizing may provide for a more reliable estimate of the sample sizes needed to describe diet precisely. Although clearly more conservative, randomizing can often provide an additional advantage, as new prey types can suddenly pop up in the diet after the quantification of tens or even hundreds of gut contents. Although not shown, randomization can smooth the terminal end of the curve, as these items, although appearing in the diet at the end of the sampling period, are actually quite rare overall.

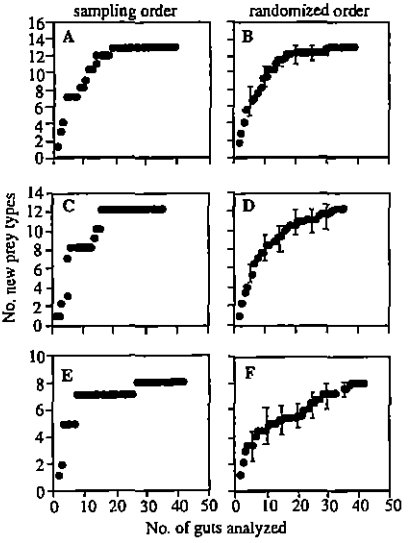

Figure 1. Cumulative prey curves for three different data sets. On the left side of the figure the cumulative number of prey items is plotted against the number of guts analyzed (a, c, e). The order of gut analysis is the actual sampling order. One the right side, for each of the three data sets, the order of gut analysis has been randomized five times, and a mean number of new prey items calculated for those five orders of analysis (b, d, f). For every tenth gut, the standard deviation has been added.

Since a key to high power in a statistical or other type of comparison is low variability, such techniques are important precursors for the statistical techniques described in this paper. However, the body of fish feeding literature seems to be entirely free of such verification. As stated earlier, none of the original ≈200 studies utilized cumulative prey curves, or any technique for determining if an adequate number of samples had been collected to precisely describe diet and perform any subsequent comparisons. Cumulative prey curves, however, do not replace the need to assess sample size sufficiency for performing subsequent diet comparisons.

Assessing Sample Size Sufficiency in Making Comparisons

Surprisingly, few of the studies critiqued here had collected enough samples to truly determine that diets were not different. Only one out of 13 indices (Fig. 2) used to indicate no difference between diets had a sample size (n) exceeding that estimated by power analysis ($\hat{n}$; Eq. 1) for bothdiets being compared (n_1 and n_2; Fig. 2). Thus, for only one index had enough samples been collected to detect the stated difference (effect size), and a difference had not, in fact, been found by the researchers. This finding is validated by $\hat{n}$ calculated using our second method (Eq. 2)

for our control data set (Table 1), which shows that the prediction of $\hat{n}$ is consistent with that predicted by more traditional methods when the necessary information (standard deviation) is available. Though the latter method appears to be less conservative, for both comparisons evaluated, actual n values were still much smaller than $\hat{n}$ (Table 1).

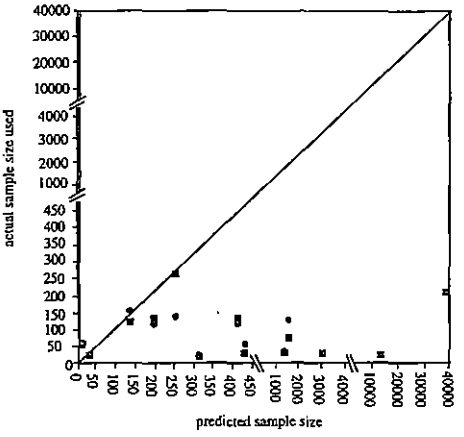

Figure 2. Plots comparing actual sample size (n_1 as ■; n_2 as ●) to predicted sample size ($\hat{n}$) as calculated using Equation 1. In each case, the actual values shown were the sample sizes for two dietary datasets compared using standard indices (points for a single comparison are paired vertically on the graph because $\hat{n}$ estimated for each dataset is the same for a given comparison; 2 datasets per comparison). The 45° line shown indicates where on the graph, actual sample size matches the predicted sample size necessary to detect a difference in diet. Studies with sufficient sample size have both points above the line shown. Breaks in the axes indicate where a series of numbers has been skipped to facilitate viewing all $\hat{n}$ simultaneously.

Table 1. Predicted sample sizes ($\hat{n}$) given by Equations 1 and 2 as compared to actual sample sizes collected for the two diets being compared in each study (n_1 and n_2). The data are from the control dataset and give only a limited picture of how well the two methods compare in their predictions of $\hat{n}$.

Eq. 1	Eq. 2	n_1	n_2
13314	3787	19	19
3054	2201	19	19
430	253	25	48
368	197	39 [1]	35 * [2]
368	213	35 [2]	41 * [3]

* not shown in Figure 2

[1] first data set shown in Figure 1

[2] second data set shown in Figure 1, used to test effect size of 3.7% (a difference not thought to be significant in the original study) in making comparisons with the first data set. Since Equation 1 does not incorporate variance, it will provide the same $\hat{n}$ for each data set given the effect size of 3.7%.

[3] third data set shown in Figure 1, used here to make diet comparisons with the second data set, tested using the same effect size (3.7%, thus giving the same value for Eq. 1), but with the variation (s^2) more typical of that data set (Eq. 2). Notice that more samples are needed to make a comparison with the same effect size when using data sets 2 and 3 (Eq. 2; $\hat{n}$=213), which both had only slightly asymptotic cumulative prey curves, than 1 and 2 (Eq. 2; $\hat{n}$=197).

Similarly, two out of 14 studies using t-test/ANOVA to indicate no significant difference between diets (n_1 and n_2) used the sample sizes necessary to detect such a difference (Fig. 3). This means that a difference may have existed, but the analysis simply lacked the statistical power to detect the difference due to low sample sizes. The difference was called non-significant due to this lack of power.

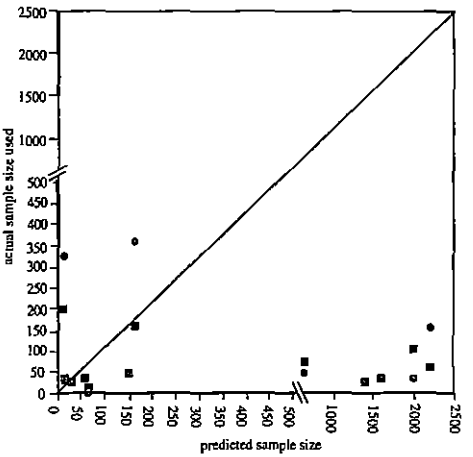

Figure 3. Plots comparing actual sample size (n_1 as ■; n_2 as ●) to predicted sample size ($\hat{n}$) as calculated using Equation 2. In each case, the actual values shown were the sample sizes for two dietary datasets compared using t-tests or ANOVA (points for a single comparison are paired vertically on the graph because $\hat{n}$ estimated for each dataset is the same for a given comparison; 2 datasets per comparison). Details are as in Figure 2.

Only three of 14 non-significant correlations used sample sizes that exceeded $\hat{n}$ predicted by Cohen's second method (Eq. 3; Fig. 4). As above, a significant correlation may have existed, the analysis simply lacked the power to detect it. Only when sample sizes approach $\hat{n}$ can a powerful correlation be performed and confidence be placed in the conclusions drawn.

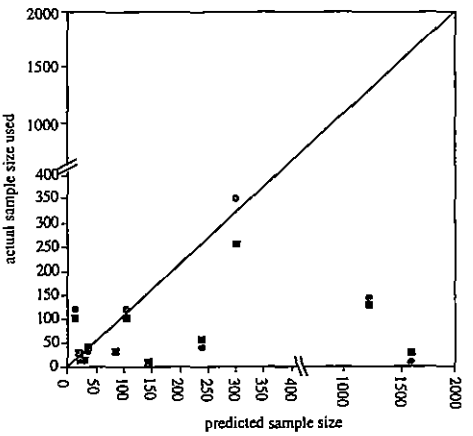

Figure 4. Comparisons of actual sample size (n_1 as ■; n_2 as ●) to predicted sample size ($\hat{n}$) as calculated using Equation 3. In each case, the actual values shown were the sample sizes for two dietary datasets compared using correlation (points for a single comparison are paired vertically on the graph because $\hat{n}$ estimated for each dataset is the same for a given comparison; 2 datasets per comparison). Details are as in Figure 2.

It should be noted that for most of the studies that we evaluated, the $\hat{n}$ estimates were only slightly larger than the actual number of samples collected. Thus, had only a few more samples been collected, a difference might have been found. Had no difference been detected with that sample size ($\hat{n}$), the power of the test would have been extremely high (approaching 100%), and great confidence could have been placed in the results. This result is encouraging since it

suggests that dietary studies are amenable to powerful statistical comparisons at reasonable sample sizes.

In a few of the studies, however, the $\hat{n}$ estimates were unreasonably high (over 10,000, see Fig. 2). In these cases, the sample size required ($\hat{n}$) is considered unachievable and a difference is likely to never be detected. Certainly most studies are undertaken with finite resources, the most limiting of which is usually time. In such cases, to collect the number of samples predicted by power analysis is close to impossible and perhaps ridiculous. The desired effect size is usually extremely small, and this small effect size is strongly influencing the power of the test. Under such circumstances, we maintain that the only recourse is to understand the limitations of the data and state any conclusions with the proper degree of confidence. Johnson (1995) points out that nearly null hypotheses actually are false, it only takes enough samples to find this statistically. The biological relevance of such small differences must be considered (see Yoccoz, 1991). It is necessary to evaluate what such differences between diets would mean for the conclusions of the study relative to its objective and desired interpretations and conclusions. If the effect size is close to or lower than the natural variability in diet found within one or both of the populations, time and samples could be better allocated to different questions.

Data Analysis

Finally, since most of the original ≈200 studies utilized indices, we would like to stress the importance of quantifying the variation around any given index. This is achieved by treating individual guts as sample units (or individual net tows, whatever level is most appropriate in maintaining independence among units), and calculating a mean value for %N or %FO of a prey type across all guts collected in a site, season, or species (whatever the unit of comparison). A similar technique is proposed by Smith (1985), whereby variance is estimated for overlap indices. A second multinomial technique has been described for estimating variance associated with proportions used in indices, however, the assumption of independently and identically distributed datasets must be met (i.e. it is only valid if data are not clustered; see Smith, 1985). The advantage of estimating either variance or standard deviation is there is a clearer picture of the distribution of data, allowing for clearer interpretations.

In selecting which index to choose, consider McDonald and Green's (1983) finding that there may be problems with indices that incorporate several measures of diet, like the IRI. Values like %N and %FO were highly correlated in their study of benthic soft-bottom and demersal fish predators. They argued that compound indices are difficult to interpret and analyze statistically, and require extra, redundant work. Multiple measures may be necessary, however, when prey items differ in size, as the choice of either abundance or weight, for example, may bias the estimate of one prey type's contribution to the diet (Cailliet et al., 1986; McDonald and Green, 1983).

In spite of the increased inferential power of an index with variation, it is not a statistic based on probability, and inferences *per se* cannot be made (see Platt, 1964). That is, *significant* differences cannot be inferred. Of the original ≈200 papers reviewed, a shocking proportion lacked inferential statistics of any kind (these generally refer to parametric statistics but non-parametric and non-distributional based statistics have some inferential power), yet stated that diets differed *significantly*.

Some studies did compliment the use of indices with parametric statistics, which is highly recommended if possible. Parametric statistics lend rigor to any study, and when accompanied by traditional methods of power analysis (see Zar, 1988), the level of confidence to place in the conclusions is easily determined. Because of the particular problems associated with proportional diet data (data values expressed as proportions), multivariate techniques have been recommended (see Crow, 1979; Ellison, 1979). Data sets, however, are likely to be heavily weighted with zeros, leading to problems of heterogeneity of variances among statistical populations, and seriously skewed distributions (see Cailliet and Barry, 1977; Underwood, 1981; Clarke and Warwick, 1994). Recently, non-parametric techniques have been described (see Crow, 1979 for

non-parametric multivariate statistics; or Clarke and Warwick, 1994 for ANOSIM), and may prove useful for making comparisons (note: see Potvin and Roff, 1993; Johnson, 1995; Garson and Moser, 1995; Smith, 1995 for a discussion of non-parametric *versus* parametric statistics). With these stipulations in mind, it not surprising that the majority of studies we reviewed relied on indices alone for making comparisons. Indices, as such, have no stated requirements of independent data sets, or distributional nature. However, the conclusions one draws are only as good as the data on which they are based. Thus, we reemphasize the need for precision in the data set, and sufficient sample size for powerful comparisons. In Cailliet and Barry's (1979) paper in which indices were finally collected into one paper and their use in diet analysis described, they paraphrased Horn (1966) when saying "Indices...are only appropriate in situations in which there is implicit confidence that the proportions of items in each category are adequately characterized" Fifteen years later, we re-emphasize this condition.

There is no substitute for enough samples in reaching conclusions about the available data, whether you are using indices or statistics to make comparisons. In the last decade, power analysis has given us the techniques to evaluate the strength of a statistical test, usually determined directly by sampling effort. Those techniques have now provided us with a means of evaluating non-statistically based comparisons (indices). We strongly recommend their use in determining the strength of conclusions drawn from comparisons of data sets. In addition, we re-emphasize the necessity of using cumulative prey curve technique for determining when enough samples have been collected for precisely describing the data sets to be compared. It is only through such techniques that we will strengthen this area of comparative ecology.

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ESTIMATION OF DAILY RATION IN AGE-O WALLEYES: APPLICATION OF A LOW-COST/LOW EFFORT MODEL IN FISH ECOLOGY STUDIES

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Abstract

I used a regression approach to develop a multiperiod model for pond-reared age-0 (16.0-37.2 mm, total length) walleyes. The multiperiod model predicts average 24-h food weights (Fs) from two to three point estimates of food weight (Ft) in walleye guts determined over a 3-6 h period (versus 24-h collections required by standard approaches such as the Elliot and Persson method). Fs is multiplied by evacuation rate and again by 24-h to yield daily ration. I corroborated multiperiod model predictions of daily ration against independent estimates of daily ration derived via the Elliot and Persson model (E-P model). Accuracy tests involving pooled error distributions around Fs indicated high-model accuracy with 93-100% of predicted Fs within 2 SE's of observed Fs when Ft from one to three time periods were entered into the regression model. Independent model predictions of daily ration for walleye were within 0.9-16.3% (mean = 6.8%) of E-P estimates. The multiperiod model can increase the temporal and spatial scope of age-0 walleye food consumption studies because accurate estimates of daily ration can be obtained from only two to three fish collections made over a 3-6 h period.

Introduction

Field estimates of fish daily ration are commonly derived directly via measuring feeding periodicity over 24-h (Eggers, 1977; Elliot and Persson, 1978; Boisclair and Leggett, 1988), or indirectly via bioenergetics models (Lyons and Magnuson, 1987; Hewett and Stewart, 1989). Methods which rely on feeding periodicity data are labor and cost intensive, requiring 24-h association with sampling sites. Consequently, the spatial and temporal resolutions of food consumption studies are often compromised (Nakashima and Leggett, 1978; Boisclair and Leggett, 1988; Ney, 1990; Hayward et al., 1991). Bioenergetics models are parameter intensive, requiring from 15-30 parameters that describe temperature- and weight-dependent components of consumption, metabolism, and waste losses. Bioenergetics models will predict consumption if temperature and growth are known (Ney, 1990; 1993). Errors are associated with each parameter (Bartell et al., 1986), and the accuracy of consumption estimates derived via bioenergetics models have often been questioned (Minton and McLean, 1982; Boisclair and Leggett, 1989; Wahl and Stein, 1991). Species- or age-specific physiological differences dictate that parameters of bioenergetics models be developed or modified for each species (Hewett and Johnson, 1992). Bioenergetic models developed for adults of a particular species may not be applicable to larvae and juveniles (Post, 1990; Madon and Culver, 1993). In light of the limitations associated with existing methods for estimating fish daily ration, alternative approaches are highly desired.

A low-effort technique was developed by Hayward et al. (1991) to estimate daily ration of juvenile and adult yellow perch, *Perca flavescens*, in Lake Erie. This technique was later extended to estimate average daily food weight in reservoir populations of larval gizzard shad, *Dorosoma cepedianum* (Hayward and Hiebert, 1993). The low-effort model is based on the premise that within any 24-h feeding period, inherent relationships exist between time-specific point estimates of food weight in fish guts (Ft) and the 24-h average food weight (Fs) (Hayward and Hiebert, 1993). Consequently, if strong correlations exist between Ft and Fs for 3-4 consecutive time periods over several 24-h feeding periods, a multiperiod model can be developed to accurately estimate Fs from just 3-4 estimates of Ft collected over a 6-9 h period (Hayward and Hiebert, 1993). Model predictions of Fs can then be multiplied by evacuation rate and again by 24-h to yield daily ration (Eggers, 1977; Hayward and Hiebert, 1993).

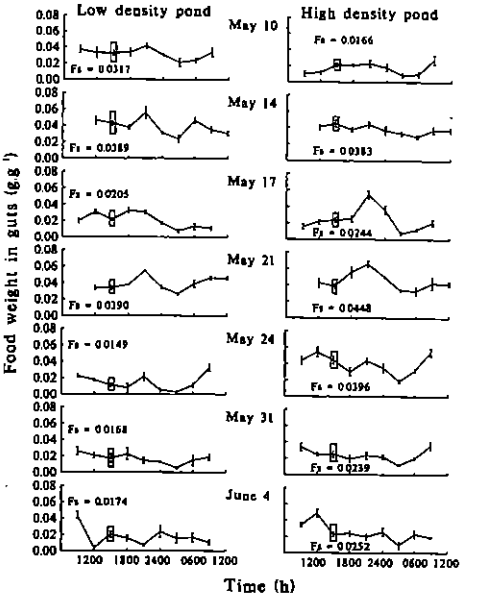

Figure 1. Diel variation of food weights in guts of age-0 walleyes in two model ponds. The box indicates point estimates of food weight (Ft ± SE) determined at 1500 h that were regressed on values of average daily food weights (Fs) for each date. Suchregressions of Fs on Ft were determined for all other time periods.

The above approach offers a promising means of reducing effort while increasing the temporal and spatial scope of fish energetic studies. However, multiperiod models for other fish species need to be developed and tested to fully evaluate the applicability of such an approach to fish energetic studies in general (Hayward and Hiebert, 1993). Hayward and Heibert (1993) have suggested analysis of existing 24-h feeding data as one way to develop and test multiperiod models for other fish species.

In this study, I developed and tested a multiperiod model (Hayward and Hiebert, 1993) for pond-reared age-0 walleyes, *Stizostedion vitreum*, using a series of 24-h feeding data that were collected as part of a larger study on walleye energetics (Madon and Culver 1993). Diel feeding data collected from two ponds on seven separate dates during May-June 1990, were used to develop regressions for the multiperiod model. A set of diel feeding data collected from another pond were used to provide independent tests of Fs and daily ration estimates predicted by the model.

Materials and Methods

Field and laboratory procedures.- I used 24-h feeding data from experiments that were conducted in ponds at the St. Mary's Fish Hatchery, St. Mary's, Ohio, as part of a study to develop a bioenergetics model for larval and juvenile walleyes (Madon and Culver, 1993). Ponds were filled with water from nearby Grand Lake, and therefore contained a natural assemblage of

zooplankton. Details on pond filling, stocking, and diel sampling of fish for analysis of food weight in guts are explained elsewhere (Madon, 1993; Madon and Culver, 1993).

Age-0 walleyes were collected at 3-h intervals over a 24-h period (0900-0900 hours or 1200-1200 hours) from three ponds between May 7-June 4, 1990 (Table 1). The 3-h time periods are: 0900, 1200, 1500, 1800, 2100, 2400, 0300, and 0600 hours. For each sampling date, gut contents of walleyes collected from one low density (25 fish•m^{-3}) and one high density (50 fish•m^{-3}) pond were weighed (methodology described in Madon and Culver, 1993) to generate time-specific estimates of food weight in guts (Ft) and average 24-h food weights (Fs). Average 24-h food weights in age-0 walleye guts were assumed to be accurately reflected by the mean of nine point estimates (Hayward and Hiebert 1993). Data on Fs and Ft collected from these two ponds, henceforth called model ponds, were used to generate regressions for the multiperiod model. Data on Fs and Ft were similarly estimated from walleyes collected from another low density (25 fish•m^{-3}) pond, henceforth called the test pond, to provide independent corroborations of Fs values predicted by the multiperiod model.

Table 1. Wet weights (mg, ± SE) of age-0 walleyes during May - June, 1990, in model ponds (low density = 25 fish•m^{-3}; high density = 50 fish•m^{-3}) and an independent test pond (low density = 25 fish•m^{-3}). Fish total lengths ranged from 16.0 - 37.2 mm, and 16.0 - 34.8 mm, in the low and high density model ponds, respectively, and from 14.3 - 35.0 mm in the test pond.

Date	Wet weights (SE)		
	Model ponds		Independent test pond
	Low density	High density	Low density
May 7	–	–	17.6 (0.5)
May 10	35.7 (0.9)	33.3 (0.6)	31.3 (0.8)
May 14	54.9 (1.2)	47.2 (0.6)	45.2 (1.1)
May 17	76.8 (2.2)	62.9 (1.2)	63.8 (1.6)
May 21	124.1 (2.5)	94.0 (2.2)	101.2 (2.5)
May 24	165.3 (3.8)	132.7 (2.2)	141.5 (4.1)
May 31	287.0 (4.5)	229.1 (5.3)	286.7 (10.8)
June 4	350.6 (5.1)	283.3 (6.0)	–

Multiperiod model development.- I used procedures described by Hayward and Hiebert (1993) to develop a multiperiod model for age-0 walleyes. First, for each 3-h time period, I developed linear regressions of Fs on Ft determined over all sampling dates. Next, I constructed a multiperiod regression model that estimated Fs from any of three 3-h time periods. Criteria for choosing these three 3-h time periods were (1) that they were consecutive in time, and (2) r^2 of linear regressions for each time period were greater than 0.70. Data of Fs and Ft from the three 3-h time periods were used to construct the multiperiod model using a stepwise procedure with backward selection, accompanied by residual analysis to select independent variables (Hayward and Hiebert 1993). The multiperiod model was used to predict Fs by incorporating point estimates of food weight (Ft) of any one, two, or three consecutive 3-h time periods included by the model. When point estimates of Ft from more than one time period were used, Fs values were generated separately for each time period and then averaged.

Multiperiod model corroboration.- Two sets of procedures were used to corroborate the multiperiod regression model. The first test involving model corroboration within regression domains used a jackknife procedure (described in Hayward and Hiebert, 1993).

The second corroboration procedure involved comparisons of multiperiod model predictions of Fs

values for walleyes in the test pond with observed Fs values. This procedure tested for the ability of the model to predict Fs accurately for walleyes in an independent location, when Ft data from one to three 3-h time periods collected in that location were entered into the model. I conducted partitioning mean square error (MSE), Bonferroni joint confidence intervals and the reliability index tests to assess the degree to which values of Fs predicted by the model matched observed values (see Rice and Cochran, 1984 for further details on these tests).

Walleye Daily Ration.- I used the Elliot and Persson (1978) method (E-P) to estimate daily ration (g of food consumed•g^{-1} wet weight of fish•d^{-1}) of walleyes in the test pond for each sampling date. The E-P procedure is a widely used method that provides standard estimates of daily ration against which all other estimates of food consumption may be compared (Cochran, 1979; Boisclair and Leggett, 1988; Hayward et al., 1991). The E-P method requires information on gut evacuation rates and diel variation in feeding to estimate daily ration. Temperature- and weight-specific gut evacuation rates were predicted from a gut evacuation rate multiple regression model developed for age-0 walleyes (Madon and Culver, 1993), and combined with diel estimates of food weight (collected at 3-h intervals over 24-h) to yield E-P daily ration estimates for each sampling date. I calculated proportional errors which represented 95% confidence intervals (CI) around each E-P daily ration estimate (Zar, 1974; Sokal and Rohlf, 1981; Post 1990). Daily ration values were estimated by multiplying predicted Fs by evacuation rates and by 24 h (Eggers, 1977). Estimated daily ration values were corroborated if they fell within 95 % CI of observed daily ration values.

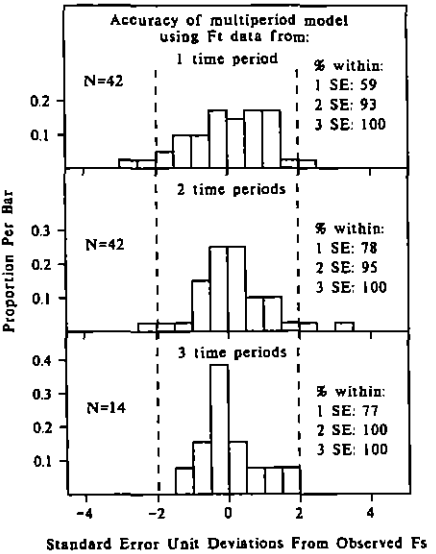

Figure 2. Error distributions that indicate the accuracy of the multiperiod model when point estimates of food weight (Ft) in age-0 walleye guts were included from one to three time periods to estimate average daily food weight (Fs). Each error distribution reveals the proportions of standard error units that model predictions of Fs deviate from observed Fs values.

Results and Discussion

Diel feeding cycles and the multiperiod model.- The amplitude of point estimates of food weights (Ft) in walleye guts ranged widely between test ponds and sampling dates (Fig. 1) due to differences in food availability (Madon, 1993). As a result, average daily food weights (Fs) showed date- and site-specific differences (Fig. 1). Correlations between Fs and Ft for age-0 walleye were significant for all time periods (Table 2), although the strongest correlations ($r^2 >$ 0.70) were for time periods between 1500-0600 h. Hayward and Hiebert (1993) indicated that strong correlations between Fs and Ft can be expected in fishes which adhere to consistent feeding patterns across days, and that correlations would be insignificant during periods when feeding ceases. Unlike larval gizzard shad which feed during daylight but cease feeding at night (Hayward and Hiebert, 1993), age-0 walleyes in this study never ceased feeding during the night and exhibited less consistent feeding patterns (Fig. 1). Highly significant correlations between 2400-0300 h were observed in this study (Table 1) because walleye fed during the night. The

variable feeding patterns exhibited by walleye in this study also explains why correlations between Fs and Ft were weaker ($r^2 < 0.70$) for 0900 and 1200 h (Table 1).

Strong correlations of Fs and Ft between 1500 and 0600 h indicated that Fs could be estimated from a point estimate of Ft from any one of six time periods (Table 1). For the multiperiod model however, I chose Fs and Ft data from 1500, 1800, and 2100 h with correlations (r^2) ranging from 0.707-0.871 (Table 1).

The multiperiod regression model developed over these three time periods was,

$$Fs = 0.006 + 0.765 \cdot Ft + 10.333 \cdot Ft^2 - 0.608 \cdot Ft^2 \cdot t,$$

where, Fs is the regression estimate of Fs; Ft is as previously defined; t is the time of day (in 24-h time) that a point estimate of food weight in walleye guts was made; $r^2 = 0.812$, $F_{(3, 38)} = 53.1$, $P < 0.0001$, $N = 42$.

Table 2. Linear regressions ($Fs = \alpha + \beta \cdot Ft$) relating time-specific point estimates of food weight (Ft, $g \cdot g^{-1}$) to estimates of average 24-h food weights (Fs) in guts of age-0 walleyes collected from one high density (50 $fish \cdot m^{-3}$) and one low density (25 $fish \cdot m^{-3}$) fish ponds. Asterix indicates the three consecutive time periods from which Fs and Ft data were chosen to construct the multiperiod model.

Time Period	Model Coefficient		Regression Statistic			
	α (SE)	β (SE)	N	r^2	F	P
0900	0.010 (0.005)	0.594 (0.153)	14	0.556	15.0	0.002
1200	0.011 (0.004)	0.551 (0.124)	14	0.620	19.6	0.001
1500 *	0.002 (0.003)	0.890 (0.099)	14	0.871	81.0	<0.001
1800 *	0.006 (0.004)	0.758 (0.129)	14	0.743	34.7	<0.001
2100 *	0.011 (0.004)	0.483 (0.090)	14	0.707	29.0	<0.001
2400	0.007 (0.004)	0.780 (0.126)	14	0.762	38.4	<0.001
0300	0.013 (0.003)	0.900 (0.132)	14	0.794	46.4	<0.001
0600	0.009 (0.003)	0.815 (0.123)	14	0.787	44.3	<0.001

Multiperiod model corroboration.- The multiperiod model shown above was reconfigured for each sampling date by suppressing all Fs and Ft data in both test ponds for that date. Fs was then predicted for the date for which data were suppressed using Ft from one up to three time periods. Error distributions (Fig. 2) were normal (Shapiro-Wilk test for $N < 50$, $p > 0.05$), and indicated no bias tendency in regression predictions of Fs (t-test, $P > 0.05$). Accuracy of Fs values predicted by the multiperiod model increased as the number of time periods included in the model increased, although the model met the accuracy criterion (> 90% of predicted Fs within $\pm$ 2 SE of observed Fs) even when only one time period was included (Fig. 2). When Ft data from any one to three time periods were included, from 93 to 100% of Fs values predicted by the model were within 2 SE of observed values (Fig. 2). These results indicate that multiperiod model could predict Fs accurately by including Ft determined from a minimum of only one fish collection made at either 1500, 1800, or 2100 h at each site. These results differ from those of Hayward and Hiebert (1993) who reported that the performance of their multiperiod model for larval gizzard shad met a similar accuracy criterion only when Ft data from three or four time periods were included in the model. Therefore, this regression approach is even more robust when applied to age-0 walleyes.

I used the above multiperiod model derived for walleyes in two model ponds to predict Fs for walleyes reared in an independent test pond. Fs values predicted by the model from one to three time-specific point estimates of Ft were corroborated versus observed values of Fs (Figs. 3-5)

using partitioning of MSE, Bonferroni joint confidence intervals, and reliability index tests (Table 3).

Table 3. Partitioning of mean square error into systematic (mean and slope) and random (residual) proportions for relationships between observed average daily food weight, Fs, and Fs predicted via the multiperiod model for age-0 walleyes in the test pond. Fs was predicted by incorporating time-specific food weights (Ft) measured in walleyes collected from the test pond from 1, 2 and 3 time periods into the multiperiod model. Bonferroni joint confidence intervals (CI) for the null hypothesis of an intercept (β_0) of 0 and a slope (β_1) of 1, and a reliability index ($k > 1$) indication the degree to which model predictions are within observed values, are given for each comparison of model predictions to observed values.

Predicted Fs using Ft from:	Source of Error			Bonferroni Joint CI		k
	Mean	Slope	Residual	$\beta_0 \pm 95\%$ CI	$\beta_1 \pm 95\%$ CI	
1 Time Period						
1500 h	0.006	0.001	0.993	0.006 ± 0.007	0.819 ± 2.047	1.15
1800 h	0.003	0.001	0.996	0.007 ± 0.009	0.713 ± 0.302	1.21
2100 h	0.012	0.021	0.967	0.005 ± 0.012	0.860 ± 0.404	1.27
2 Time Periods						
1500 & 1800 h	0.000	0.013	0.987	0.007 ± 0.005	0.766 ± 0.169	1.15
1500 & 2100 h	0.008	0.002	0.990	0.006 ± 0.006	0.839 ± 0.204	1.16
1800 & 2100 h	0.001	0.000	0.999	0.006 ± 0.009	0.786 ± 0.287	1.20
3 Time Periods						
1500, 1800 & 2100	0.002	0.007	0.991	0.006 ± 0.008	0.800 ± 0.200	1.15

This procedure tested for the model's ability to accurately predict Fs for walleyes in other locations. Partitioning of MSE for all combinations of time-periods revealed that any deviations of predicted values of Fs from observed values were largely due to random variations (proportion of MSE due to random error >0.967) and not due to errors in the mean and slope of regressions of predicted versus observed Fs (Table 3; Figs. 3-5). Bonferroni joint confidence intervals for all time-period combinations, except the 1500 & 1800 (2 time period) combination, revealed that intercepts and slopes of regressions of predicted Fs on observed Fs were not different from 0 and 1, respectively (Table 3). These results indicated that regressions of predicted Fs on observed Fs were not significantly different from the 1:1 line (Figs. 3-5). The reliability index ranged from 1.15 to 1.27 over all time period combinations tested (Table 3) and indicated that predicted values of Fs were in close agreement with observed values (Rice and Cochran, 1984; Madon and Culver, 1993). Mean predicted values of Fs were within 0.8-

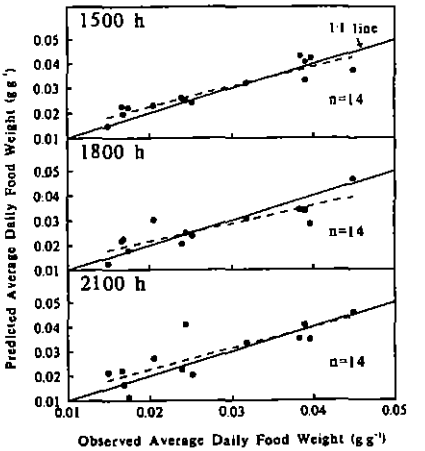

Figure 3. Relationships between average daily food weight (Fs) observed in the test pond and Fs predicted by the multiperiod model when a point estimate of food weight (Ft) from any one time period (1500, 1800, or 2100 h) was included in the model. Errors are not systematic if values fall along a 1:1 line (solid line). Broken lines indicate least squares regressions.

5.0 % of mean observed values over all time period combinations. Thus, the multiperiod model derived in this study accurately predicted Fs for walleyes at another location. These results are consistent with those of Hayward et al. (1991) and Hayward and Hiebert (1993) who showed that multiperiod models derived from data collected at one location are capable of predicting Fs in fish at other independent locations.

Walleye daily ration.- I compared values of daily ration of walleyes estimated from multiperiod model predictions of Fs (henceforth called predicted daily ration) with daily ration values derived via the standard E-P procedure (henceforth called E-P daily ration) for walleyes in the test pond. Predicted daily ration estimates were all within 95% CI's around E-P estimates across all dates when Ft data from three time periods were used in the model (Fig. 6), and deviated 0.9-16.3% (mean = 6.8%) from E-P estimates. Hayward et al. (1991) have similarly shown good agreement of daily ration estimates derived via the multiperiod approach with E-P estimates for yellow perch. Predicted daily ration estimates fell outside 95% CI of E-P estimates for May 7 and May 31, and May 7, when Ft from one time period, and 2 time periods, respectively, were used in the model (Fig. 6). It is noted that May 7 represents a date which lies outside the period (May 10 - June 4) when data were collected to develop the multiperiod model (Table 1). Consequently, walleyes in the test pond on May 7 were of approximately half the mean weight of those in the model ponds on May 10 (Table 1). This comparison then tests for the model's ability to accurately predict daily ration for dates and fish sizes outside those over which the model was constructed, and indicates that data from a minimum of three time periods must be included to obtain accurate estimates of daily ration in such cases.

Conclusions.- Previous tests have demonstrated that the multiperiod regression approach accurately predicts Fs for yellow perch (Hayward et al., 1991) and larval gizzard shad (Hayward and Hiebert 1993). This study demonstrates that the approach

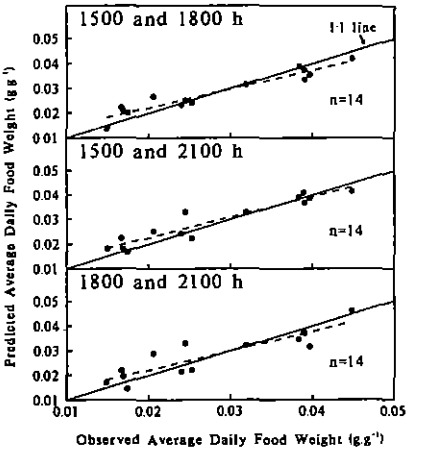

Figure 4. Relationships between average daily food weight (Fs) observed in the test pond and Fs predicted by the multiperiod model when point estimates of food weight (Ft) from any two time periods (1500 & 1800 h, 1500 & 2100 h, and 1800 & 2100 h) were included in the model. Errors are not systematic if values fall along a 1:1 line (solid line). Broken lines indicate least squares regressions. See Table 3 for statistical tests.

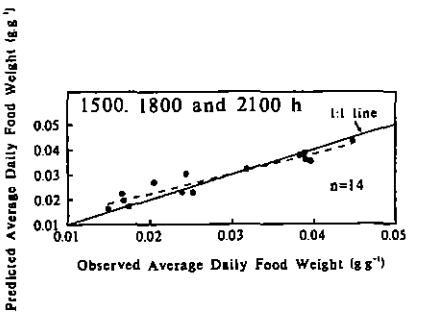

Figure 5. Relationships between average daily food weight (Fs) observed in the test pond and Fs predicted by the multiperiod model when point estimates of food weight (Ft) from all three time periods (1500, 1800, and 2100 h) were included in the model. Errors are not systematic if values fall along a 1:1 line (solid line). Broken lines indicate least squares regressions. See Table 3 for statistical tests.

also works for age-0 walleye. This is the first study that demonstrates that a multiperiod model developed from fish populations in one system can be used for conspecific populations in another system. However, the ability of the multiperiod model to provide accurate estimates of average daily food weights across years was not tested, and this application of the model for age-0 walleye warrants further study. Multiperiod models developed for yellow perch and larval gizzard shad have shown abilities to make accurate estimates of average daily food weights across years.

The multiperiod model for walleyes (16.0-37.2 mm, TL) derived in this study provides accurate estimates of average daily food weight from one to three point estimates of food weight in guts of walleye determined at 1500, 1800, and 2100 h. Accuracy of Fs estimates increased as the number of time periods entered into the model increased. This study showed that estimates of average daily food weight can be converted to accurate estimates of daily ration for age-0 walleye.

The regression approach shows tremendous promise as a way to increase the spatial and temporal scope of field studies of food consumption by age-0 walleye. For example, depending on the degree of accuracy desired, two to three fish collections could be made over a period of a 3-6 h, although three fish collections are recommended if estimates of daily ration for walleye slightly outside the 16.0-37.2 mm size range are required. Because daily ration estimates for age-0 walleyes could be generated in a 3-6 h time span, more frequent estimates of food consumption at multiple sites would be possible.

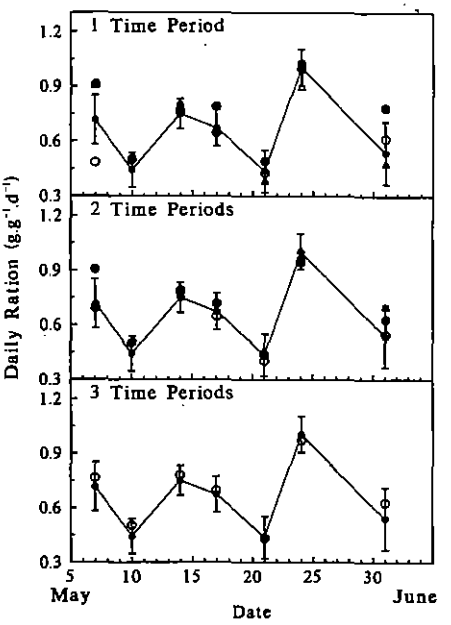

Figure 6. Comparisons of age-0 walleye daily ration ± 95% CI derived via the Elliot and Persson (E-P) method (filled circles, solid line) with daily ration estimates derived from multiperiod model predictions of average food weights (Fs) when point estimates of food weight (Ft) from one to three time periods were included in the model. Symbols in each case represent the following time periods:- 1 Time Period: 1500 h (open circle), 1800 h (open triangle), 2100 h (asterisk); 2 Time Periods: 1500 & 1800 h (open circle), 1500 & 2100 h (open triangle), 1800 & 2100 h (asterisk); 3 Time Periods: 1500, 1800, and 2100 h (open circle).

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SPATIAL PATTERNS OF SALMON PREY, FEEDING AND GROWTH IN THE NORTHEAST PACIFIC OCEAN

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Abstract

We describe results from analyses of data sets on zooplankton biomass and salmon diets and describe the development of a spatially-explicit model to represent feeding and growth processes of Pacific salmon in the Northeast Pacific Ocean. Our research objective was to define the effective prey patch size for salmon (over scales from 100 km to > 2000 km) and describe how salmon feeding and growth is influenced by patterns of sea surface temperature (SST) and prey resources. We compiled data on sea surface temperature (SST), zooplankton biomass, and salmon diets in the Northeast Pacific during spring and summer of 1962. We found evidence of spatial-, temperature- and prey-dependence on salmon feeding. We found evidence of meso ($\leq$ 500 km) and gyre- (>1000 km) scale patch structures in prey (zooplankton) biomass and predator (salmon) feeding. Based on these data sets, we developed algorithms to simulate feeding and growth of salmon on the high seas. We computed spatially-explicit growth rate potential for a representative 500 g sockeye salmon *Oncorhynchus nerka* to provide a visual "snapshot" of growth conditions extant in the Northeast Pacific Ocean during spring and early summer of 1962. Growth rate potential appeared to be maximized in the center of the Alaskan Gyre. We also discovered that growth rate potential declines sharply within the southern zone of their distribution, at approximately the latitude where salmon abundance has been observed to decline by one to two orders of magnitude. This latter pattern suggests that bioenergetic mechanisms may give rise to this apparent "zone of intolerance" for salmon. We describe potential future applications of the model to better understand effects of oceanographic regime shifts and broad scale climate change on salmon growth and mortality processes.

Introduction

Patterns of prey distribution, feeding and growth processes of Pacific salmon in off-shore marine environments are poorly understood. Because of logistical difficulties in sampling and experimentation on the high-seas, very few directed field efforts have been conducted to try to gain insight into biological mechanisms regulating growth and survival of salmon in this environment. Rates of growth and survival, and the factors affecting them, are a critical uncertainty that continues to frustrate attempts at predicting production trends in Pacific salmon stocks. We have only a rudimentary understanding of critical high seas processes that may be affecting salmon growth and production, such as the extent of predator-prey overlap, the effective patch size of prey resources for salmon, and how SST and prey density interact to influence rates of salmon feeding and growth. Some of these processes may be responsible for the long term reduction in mean size of British Columbia salmon (Hinch et al. 1996, Cox and Hinch, in review). Many of these questions are of critical importance to basic science, as well as to developing better practical management policies. By explicitly addressing these issues, we may gain a more comprehensive understanding of growth and, ultimately, survival of these and other important stocks during their high seas life history stage.

We contend that much can be gained through re-analysis of past data bases involving extensive biological sampling, coupled with the application of state-of-the-art approaches in statistics, modeling and data visualization that can offer fresh insights into patterns in the data. Our objective is to address the following questions: 1) Are there detectable "patches" of zooplankton, on the scale of 100 to 2000 km, on the high-seas?, and 2) How are salmon feeding and growth affected by SST and the spatial distribution of these prey resources? We provide below a description of the relevant data sets, our analytical methods, and some preliminary results that address these questions.

Data Sources and Analytical Methods

We compiled data on sea surface temperatures (SST, COADS data base), zooplankton biomass (LeBrasseur 1965a), and salmon diets (LeBrasseur 1965b). The latter two data sets were based on archived data records from the Pacific Biological Station at Nanaimo, British Columbia. The period of biological sampling occurred during 1956-1964. The sampling was extensive in space (lat. 40-60°N, long. 120-160°W) and time (1957-64, all seasons). We focused data analysis on the biological measures of prey (zooplankton) and predator (salmon) during 1962 and tested for significant spatial patterns and responses along latitudinal environmental gradients. Zooplankton was measured using NORPAC nets with a 330-351 micron mesh. The nets were hauled from 150 m to the surface at 1 m per second. Details of laboratory methods can be found in LeBrasseur (1965a). Salmon for the diet survey were collected using gill net sets. Additional details of sampling protocol can be found in LeBrasseur (1965b). We report here results for pink salmon *O. gorbuscha* and sockeye salmon. For each individual salmon in the diet survey, we expressed stomach fullness as a percentage of body weight (wet weight stomach contents/whole body wet weight X 100). We discarded data from any stations where less than three individuals of a given species were collected. We computed an average and standard error for stomach fullness at each sampling station. We applied Mantel's tests (Mantel 1967) to detect spatial dependence in the zooplankton biomass and salmon stomach fullness data, and developed correlograms using distance class intervals to gain insight into the nature of the spatial patch structure. We computed a salmon growth index at each station by subtracting metabolic costs expressed in wet weight grams (basal and active metabolism, SDA, and egestion and excretion) from the mean wet weight of observed stomach contents (see Beauchamp et al. 1989 for description of general approach). This growth index was expressed as a loss (negative) or gain (positive) in

weight to the individual in units of percent of body weight. We explored the effect of SST and latitude on zooplankton biomass, stomach fullness and this growth index.

Patterns in Zooplankton Prey and Salmon Stomach Fullness

We found evidence of spatial patterns (i.e. data were spatially autocorrelated) in a majority of the zooplankton biomass data sets. There appeared to be two consistent scales at which spatial dependence was evident. We detected meso- (≤ 500 km) and gyre-scale (> 1000 km) spatial dependence (Fig. 1). These results are consistent with the hypothesis that meso-scale physical structures (e.g. eddies) may lead to the formation of meso-scale food "patches", while dominant offshore current fields within the Gulf of Alaska may provide a physical template for the distribution of zooplankton at much larger scales of observation.

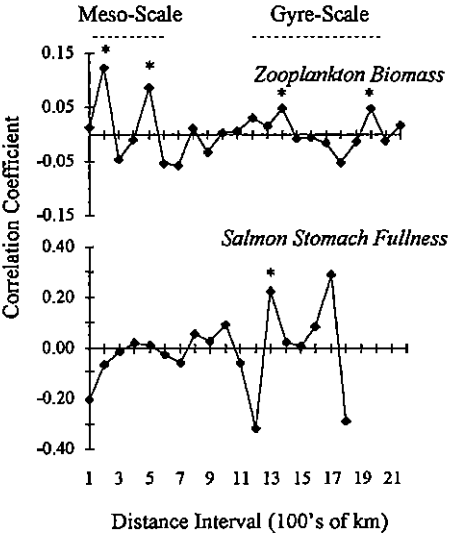

Figure 1. Correlograms describing meso- (≤ 500 km) and gyre (>1000 km) scale dependent patch structures in zooplankton biomass and pink salmon stomach fullness during May-June 1962 in the Northeast Pacific Ocean. High positive correlation coefficients (* = significance at p = 0.05) indicate coherence in response variables at sampling stations separated at a given distance interval. Note coherence in gyre-scale patterns between prey biomass and predator feeding.

Analyses of pink and sockeye salmon stomach fullness during spring and summer of 1962 indicated spatial similarities at a scale of approximately 1200 km (results for pink salmon shown in Fig. 1). The coherence in the data at this scale suggests that predators are responding to the general gyre-scale patterns detected in the prey "landscape". We also found a four- to five-fold reduction in stomach fullness measures between approximately 9 to 10 °C SST for sockeye salmon and between 10 to 12 °C SST for pink salmon. We also discovered a reduction in stomach contents below lat. 50°N, which was coincident with a reduction in zooplankton biomass (Fig. 2). By transforming the stomach contents to a growth index, we discovered a marked reduction in growth below 50°N. Only one positive growth index value was observed below 47°N. This is the approximate location of the sharp southern limit in the distribution of salmon in this region. We used the results of these analyses to develop temperature- and prey-dependent foraging functions that were applied in the spatially-explicit growth model described below.

Spatial Patterns in Predicted Growth

We used a software shell (Walter et al., in review) that represents the Northeast Pacific as a two dimensional spatial grid composed of cells of dimension 1° lat. by 1° long. We interpolated data on SST and zooplankton biomass across two dimensions to estimate the

thermal properties and prey biomass within each cell in the grid. We input these cell-specific values into a coupled bioenergetic and foraging model to estimate growth rate potential (sensu Brandt et al. 1992) for a representative 500 g sockeye salmon during June 1962. This method provides a visual "snapshot" of growth conditions across the Northeast Pacific.

The greatest potential for growth during the spring appeared to be centered in the mid- to lower-region of the Alaskan Gyre (Fig. 3). This central region appeared to correspond to an optimal mix of sufficient levels of prey abundance and preferable thermal properties. A very sharp reduction in growth rate potential is evident in the southern latitudes, where growth rate declines from a high of nearly 2% body weight per day to -0.5% body weight per day along a transect of approximately 300 km. This feature arises from the interaction between levels of zooplankton biomass and temperature-dependent metabolic demands (see also Fig. 2). This may provide a plausible hypothesis to explain the sharp "thermal" limits in the oceanic distribution of salmon as described by Welch et al. (1995). This apparent "zone of intolerance" may arise from a suite of behavioral traits that have been selected to optimize growth rates. In Fig. 3, one can also discern an area in the center of the gyre that supports relatively high growth rate potential for salmon, surrounded by concentric "rings" of lower potential. These general patterns in growth rate potential emerge from the results described above identifying the importance of prey abundance and temperature on salmon feeding.

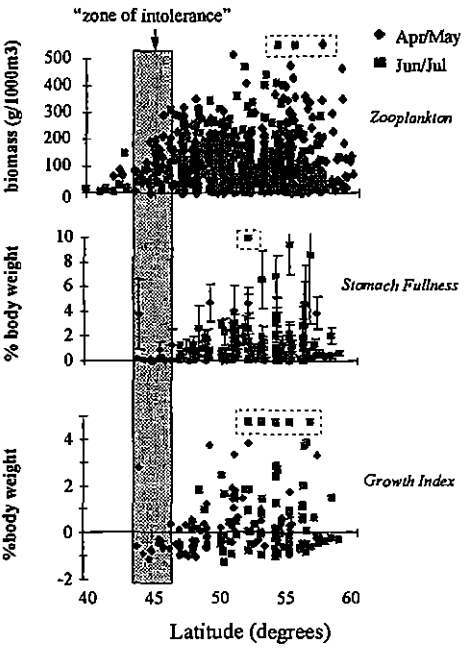

Figure 2. Latitudinal gradients in salmon prey biomass, and stomach fullness and computed growth index for pink salmon in the Northeast Pacific Ocean. Data were collected during the spring and summer of 1962. The top panel is zooplankton biomass presumed to be available to foraging salmon, and the stomach fullness and growth index in the bottom two panels serve as measures of feeding and growth responses across latitude. The shaded bar represents the approximate latitudinal zone over which salmon biomass has been observed to decline sharply. Values within dashed lines are off the scale indicated on the axes.

Discussion

The present model represents the product of collaborative efforts by physical, biological and fisheries oceanographers, along with expertise in computer software design and data visualization. This effort represents a rare, successful marriage between biology and technology, and will allow new insights into the ecology of Pacific salmon in coastal and high seas environments. These efforts will culminate in explicit predictions in spatial distribution and growth rates that can be rigorously tested with innovative field programs employing new

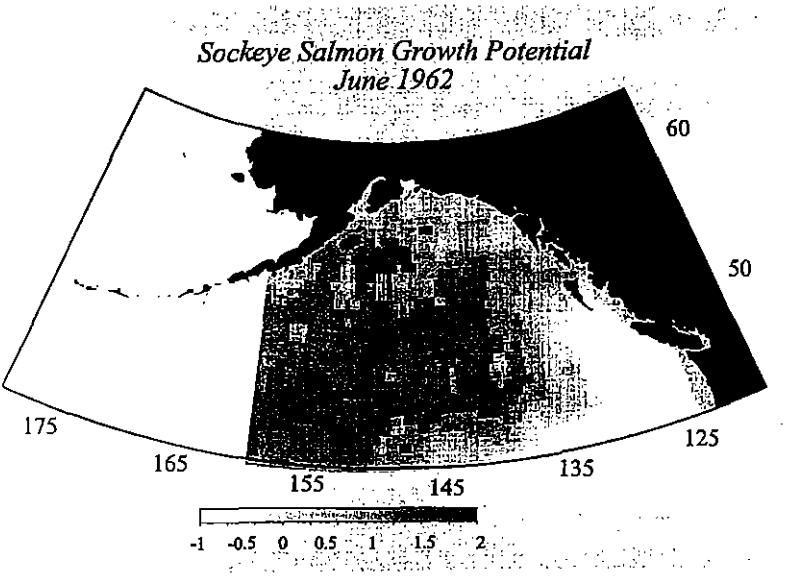

Figure 3. Spatially-explicit growth rate potential for a representative 500 g sockeye salmon across the Northeast Pacific Ocean during June 1962. Growth is in units percent body weight per day. Note region that supports high growth potential near the center of the gyre, and the concentric "rings" of poor growth potential surrounding it, and the sharp reduction in growth potential at southern latitudes.

sampling technologies such as acoustics and smart tags (e.g. MacLennan and Simmonds 1992, DeLong et al. 1992).

We are presently evaluating the effects of the 1977 regime shift on the spatial patterns and growth responses of salmon in this region. Brodeur and Ware (1992) reported an approximate doubling of zooplankton biomass and a marked shift in the spatial distribution of zooplankton throughout the Northeast Pacific Ocean. We intend to evaluate how these shifts in prey distribution may have influenced predator-prey overlap and salmon growth processes. Coincident with these changes was a substantial increase in abundance of salmon and other nekton (Brodeur and Ware 1994) which suggests top down processes may have become more important in recent years. We hope to evaluate the relative importance of these top down and bottom up food web dynamics in regulating growth and production rates of salmon on the high seas.

Although much of what was described above addresses issues related to growth, we intend to apply the model to gain insights into mechanisms responsible for regulating mortality, a critical uncertainty in managing these stocks across the Pacific Rim. We have begun to explore the trade-off between growth and mortality in Pacific salmon using techniques of dynamic programming (Scandol et al., in review). If indeed the critical period in the Pacific salmon life history is during the first month or months at sea, we could gain insights into mechanisms of this process by hindcasting growth conditions in past years. There is a substantial literature

that supports the notion that mortality is strongly size dependent, and hence growth conditions experienced by juveniles during this period may serve as an accurate predictor of year class strength. We are presently working with Canada's Department of Fisheries and Oceans and the Pacific Salmon Commission to compile data sets on smolt migration timing, size distribution, abundance and subsequent survival rates for select salmon stocks. This information, coupled with our model, may allow us to better explain the observed variance in smolt-to-adult survival. We hope the present effort can be successful at accomplishing this goal to help improve salmon conservation efforts and develop better policies for stock management.

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Feeding Ecology

NON-INDIGENOUS SPECIES CAUSE MAJOR SHIFTS IN THE FOOD-BASE OF ESTUARINE-DEPENDENT FISHES

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Abstract

We analysed the diet of juvenile English sole (*Pleuronectes vetulus*) and juvenile starry flounder (*Platichthys stellatus*) to determine the numerical contribution of non-indigenous macroinvertebrate species (hereafter alien species) on demersal food webs. Fish were collected in the Alsea Bay and Yaquina Bay estuaries (Oregon, USA) during the summer 1993. Alien prey consist mainly of crustaceans, polychaetes and clams similar to native prey. English sole and starry flounder consumed proportionally more native prey in Alsea Bay and nearly equal native and alien prey in Yaquina Bay. Alien species consisted, respectively, of 1/3 to 1/2 of the diet of English sole and starry flounder by numbers in both estuaries. Thus, alien species have greatly altered the food webs for estuarine-dependent demersal fish. How these shifts in prey consumption affect the growth, survival and recruitment of native estuarine-dependent fishes remains unknown.

Introduction

A common assumption in many feeding studies of fish is that interspecific differences in prey selection within an assemblage result from coevolution of species (Moyle et al. 1982). However, alien species may be particularly important in the Pacific coast of North America, where at least 160 non-indigenous invertebrates and fish species in 11 phyla may have been introduced by humans into estuaries, bays and harbors (Carlton 1979, Cohen and Carlton 1995).

The principal recent source of alien organisms in the Northeast Pacific estuaries may be from discharge of ballast water from ships (Carlton and Geller 1993). However, fouling on hulls of ships and importations of the Atlantic oyster (*Crassostrea virginica*) and the Japanese oyster (*Crassostrea gigas*) for culture in the Northeast Pacific coast of USA and Canada were also

major vectors for the spread of alien species into estuaries and bays (Carlton 1979). Despite the large number of inadvertently introduced aquatic species and their rapid recent spread (e.g., Elton 1958, Lachner et al. 1970, Baltz 1991, Carlton and Geller 1993, Cohen and Carlton 1995), the ecological effects of introduced species in estuaries are poorly known (e.g., Posey 1988, Nichols et al. 1990). Anecdotal information from northern San Francisco Bay suggests that native fish rely little on alien prey (Carlton 1979).

English sole and starry flounder occur in many Northeast Pacific estuaries during their juvenile stage (Monaco et al. 1990), where they depend on epifauna and infauna for food. Castillo et al. (1995) found that juvenile fishes (English sole *Pleuronectes vetulus*; starry flounder *Platichthys stellatus*; staghorn sculpin *Leptocottus armatus*; and chinook salmon *Oncorhynchus tshawytscha*) preyed upon at least one alien macrobenthic species in the Alsea Bay and Yaquina Bay estuaries.

The objectives of the present study are to: (1) Measure the relative abundance of native and alien macroinvertebrate species in the diet of juvenile English sole and starry flounder in the Alsea Bay and Yaquina Bay estuaries, and (2) Compare the occurrence and numerical importance of native and introduced macroinvertebrate species in the diet of English sole and starry flounder. This is the first study of the relative importance of native and introduced prey items in the diets of estuarine-dependent fishes.

Study Sites and Methods

The Alsea Bay and Yaquina Bay estuaries were selected as study sites for their geographic proximity and similar morphology. Yaquina Bay is located 192 km south of the Columbia River estuary. Nearly 35 % of its 15.8 km^2 surface area at mean high water (MHW) is intertidal (Hamilton 1973). Alsea Bay is located 25 km south of Yaquina Bay. Approximately 46 % of its 8.7 km^2 surface area at MHW is intertidal (Hamilton 1973). Only Yaquina Bay has received ballast water traffic. Both estuaries have been used for culture of introduced oysters (Carlton 1979). Unlike Yaquina Bay, oyster culture was discontinued in Alsea Bay in the 1930's (Anja Robinson, Hatfield Marine Science Center, OR, pers. comm. 1995).

Juvenile English sole and starry flounder were collected in each estuary at six intertidal areas using a beach seine (32 m x 1.8 m and 0.8 cm stretched mesh size) between July and September 1993. We sampled during daylight hours. Sites were selected to occur between salinities ranging between 34-35 ppt in low estuarine sections and 1-2 ppt. in upstream areas. Flatfish used for gut content analyses were grouped by species and estuary (Table 1). Captured fish were given a lethal dose of MS-222 (200 mg/l). A 10% solution of buffered formalin was then injected into the coelomic cavity to fix prey items. Feeding habits of English sole were based on stomach contents. The combined number of prey items in the stomach and the anterior 1/3 of the intestine were analysed in the starry flounder since stomach fullness in this species was often low.

Individual prey items were identified to the lowest taxonomic level and usually to species level. Classification of species into native and alien was based on reported species introductions (Carlton 1979, Carlton and Geller 1993) and criteria for detecting alien species (Chapman 1988). Species of unknown geographical origin were classified as "cryptogenic" (Carlton 1982, Carlton 1996). We computed the percentage of occurrence of prey species in gut contents according to their origin (i.e., native, alien, and cryptogenic) as the average percentage of occurrence for all species within each origin type. The total numerical contribution of prey species according to origin was computed as the sum of the mean number of prey having the

same origin type.

We computed ranks of relative abundance for the 13 most numerous prey items. Bivalves and their attendant siphons were classified as different prey. Polychaete parts and capitellid parts of unknown species were also grouped as different prey. We computed diet overlap of flatfish by dividing the common number of prey found (between flatfish species in a given estuary, or between estuaries for a given flatfish species) by the total number of prey compared. Diet overlap indices were computed separately for all identified macrobenthic species and for the 13 most abundant prey items. In the later case, unknown fragments of polychaetes and capitellids were excluded from these computations to prevent a potential overestimation of diet overlap indices.

Results

Similar numbers of each flatfish species were collected between estuaries (Table 1). Bivalves, crustaceans and polychaetes were the most common prey of English sole and starry flounder in both Alsea Bay and Yaquina Bay and most prey species were native. Bivalves, crustaceans and polychaetes each included at least one introduced species (Table 2). Greater numbers of native and alien prey species in both species of flatfish were found in Yaquina Bay (alien = 9; native = 26) than in Alsea Bay (alien = 5; native = 19). The diet overlap based on all identified macroinvertebrate species (Table 2) showed similar prey overlap of flatfish between Alsea Bay and Yaquina Bay (English sole: 0.47; starry flounder: 0.50). However, the diet overlap between English sole and starry flounder was greater in Yaquina Bay (0.50) than in Alsea Bay (0.41).

Table 1. Total number, mean total length - standard deviation (SD), and total length range of juvenile English sole and starry flounder collected from the Alsea and Yaquina Bay estuaries during summer 1993.

Species Estuary	Number of fish	Mean fish length - SD (cm)	Fish length range (cm)
English sole:			
Alsea Bay	135	6.82 - 1.42	3.6 - 10.0
Yaquina Bay	113	7.08 - 1.37	2.4 - 10.1
Starry flounder:			
Alsea Bay	61	9.05 - 4.33	3.6 - 23.2
Yaquina Bay	61	13.26 - 4.95	5.4 - 23.4

At least three alien species were found within in the 13 most abundant prey items of both species of flatfish in Alsea and Yaquina Bay (Table 3). The diet overlap based on main prey items (Table 3), showed that the prey overlap of flatfish between estuaries was greater for starry flounder (0.44) than for English sole (0.29). Interestingly, the diet overlap between English sole and starry flounder was again greater in Yaquina Bay (0.53) than in Alsea Bay (0.26). Such

Table 2. Benthic macroinvertebrate species found in the diet of juvenile English sole (E) and starry flounder (S) in the Alsea Bay and Yaquina Bay estuaries. Species origins are: native (N), alien - Atlantic (A), Japan (J) and Asia (I) - and cryptogenic (C). Mechanisms of introduction are: oyster (O), fouling of ship hulls (F), and ballast water (B). Sources of introduction in the U.S. West Coast estuaries are based on: (1) Carlton (1979), (2) (Carlton, J.T. Williams College, CT, pers. comm. 1996), and (3) Carlton and Geller (1993).

Species	Estuary				Origin / Mechanism
	Alsea		Yaquina		
Bivalvia					
Clinocardium nuttallii	E		E	S	N
Cryptomya californica	E	S	E	S	N
Macoma balthica	E	S	E	S	N
Mya arenaria	E	S	E	S	A / O[1]
Mysella tumida	E			S	N
Transennella tantilla			E		N
Crustacea					
- Amphipoda					
Allorchestes angusta			E		N
Ampithoe laceriosa				S	N
Ampithoe valida			E		A /(F and/or O)[1]
Corophium acherusicum			E	S	A /(F and/or O)[1]
Corophium salmonis	E	S	E	S	N
Corophium spinicorne		S	E	S	N
Corophium brevis			E		N
Eobrolgus spinosus	E	S	E	S	A / O[2]
Eogammarus confervicolus	E		E	S	N
- Cumacea					
Cumella vulgaris	E	S	E	S	N
Nippoleucon hinumensis	E	S	E	S	J / B[3]
- Decapoda					
Callianassa californiensis	E		E	S	N
Crangon franciscorum		S	E	S	N
Hemigrapsus oregonensis	E				N
Upogebia pugettensis	E			S	N
- Tanaidacea					
Leptochelia dubia	E		E	S	C
Pancolus californiensis	E		E		C
Sinelobus stanfordi	E		E		C
Polychaeta					
Amaeana occidentalis			E		N
Anaitides mucosa			E		C
Armandia brevis	E		E		N
Dorvillea rudolphi			E		C
Eteone californica	E	S	E	S	N
Eteone longa			E		N
Glycinde polygnatha	E		E		N
Glycinde armigera			E		N
Heteromastus filiformis				S	A / O[1]
Hobsonia florida	E	S	E	S	A / O?[2]
Malacoceros fuliginosus			E		N
Manayunkia aestuarina			E	S	C
Mediomastus californiensis	E		E	S	N
Nephtys caeca	E				N
Nereis limnicola	E	S		S	N
Owenia fusiformis	E		E		C
Paraonella platybranchia				S	N
Polydora proboscidea			E		N
Pseudopolydora kempi	E	S	E	S	I / (F and/or O)[1]
Pygospio californica	E				N
Pygospio elegans	E	S	E	S	C
Streblospio benedicti			E	S	A / (F and/or O)[1]
Tharyx parvus			E	S	C

Table 3. Ranking of mean numerical importance of the 13 major prey items consumed by juvenile English sole (3.A) and starry flounder (3.B) in the Alsea and Yaquina Bay estuaries between July and September 1993. Numbers of prey items decline from rank 1 to 13. Alien species are indicated in bold, and cryptogenic species are indicated by an asterisk. No significant Spearman rank correlations were found between species or estuaries.

3.A

	Main prey of English sole	
Rank	Alsea Bay	Yaquina Bay
1	*Macoma balthica* (siphon)	Harpacticoida
2	Harpacticoida	***Streblospio benedicti***
3	*Pygospio elegans* *	***Nippoleucon hinumensis***
4	*Corophium salmonis*	*Macoma balthica* (siphon)
5	*Armandia brevis*	*Corophium salmonis*
6	*Cumella vulgaris*	*Leptochelia dubia* *
7	Ostracoda	*Polydora proboscidea*
8	Polychaeta (parts)	***Pseudopolydora kempi***
9	***Nippoleucon hinumensis***	Capitellidae (parts)
10	Capitellidae (parts)	Polychaeta (parts)
11	***Mya arenaria***	*Corophium brevis*
12	Oligochaeta	*Pygospio elegans* *
13	***Hobsonia florida***	*Corophium spinicorne*

3.B

	Main prey of starry flounder	
Rank	Alsea Bay	Yaquina Bay
1	Harpacticoida	*Corophium salmonis*
2	*Corophium salmonis*	***Streblospio benedicti***
3	Chironomidae	***Hobsonia florida***
4	*Nereis limnicola*	*Macoma balthica* (siphon)
5	***Hobsonia florida***	***Pseudopolydora kempi***
6	Diptera (pupae)	*Corophium spinicorne*
7	*Corophium spinicorne*	*Leptochelia dubia* *
8	***Mya arenaria*** (siphon)	*Manayunkia aestuarina* *
9	*Macoma balthica* (siphon)	***Nippoleucon hinumensis***
10	Ostracoda	**Mya arenaria**
11	*Macoma balthica*	Harpacticoida
12	***Pseudopolydora kempi***	Polychaeta (part)
13	*Cryptomya californica*	Chironomidae

consistent pattern suggests greater differences in prey availability and/or prey selection between flatfish species in Alsea Bay. On the other hand, the alien polychaete *Streblospio benedicti* was a major prey for English sole and starry flounder in Yaquina Bay, yet this prey was absent from the flatfish diet in Alsea Bay.

A higher mean percentage of occurrence of native prey items were found in Alsea Bay in both English sole and starry flounder, and a higher mean percentage of alien species were found in both species fromYaquina Bay (Figure 1). Similar total numbers of alien prey species were found in the diet of both species in Alsea Bay and Yaquina Bay, but English sole consumed a greater diversity of native prey than starry flounder (Figure 1). Unlike the prey items in Yaquina Bay, native species in the diet of both species of flatfish in Alsea Bay showed higher percentage of occurrences and higher total numbers when compared to alien species.

Native prey consumed by both species of flatfish outnumbered alien prey in Yaquina Bay, but alien prey had a higher mean occurrence of alien prey relative to native prey. Cryptogenic species in the diet of both species of flatfish had lower average occurrences than either native or alien prey. Native species were the predominant food source of English sole and starry flounder in Alsea Bay. However, both species of flatfish preyed upon similar ratios of native and alien prey in Yaquina Bay (Figure 2).

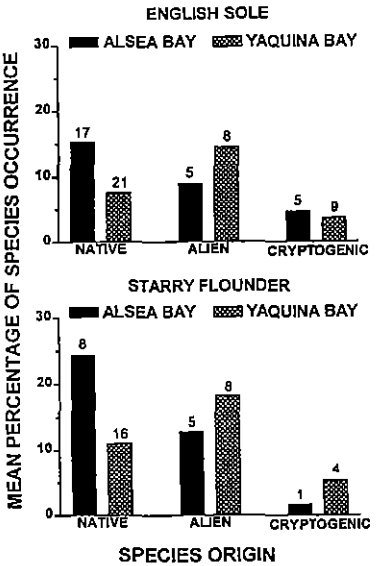

Figure 1. Mean percentage of prey occurrences by species' origin in diets of juvenile English sole and starry flounder. The number of species in each category is indicated.

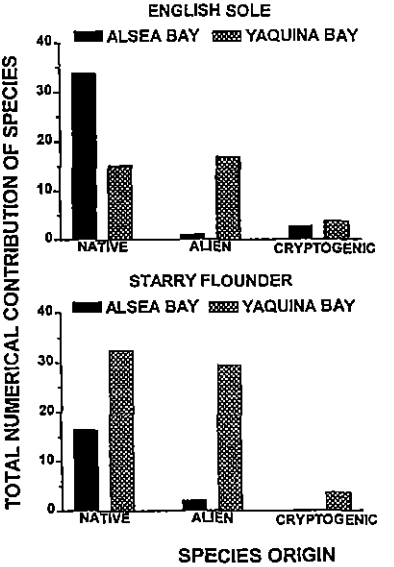

Figure 2. Total numerical contribution of prey by species' origin in the diets of juvenile English sole and starry flounder. The number of species in each category is shown in Figure 1.

Discussion

Alien species are a major food source for juvenile English sole and starry flounder in Yaquina Bay and Alsea Bay. Demersal fish food webs have been substantially altered in both estuaries, particularly in the case of starry flounder. The higher proportions of alien prey found in Yaquina Bay may be due almost entirely to species introduced by oyster culture and fouling on hulls of ships.

Differences in the spatial distribution of English sole and starry flounder may affect their feeding habits. The polychaetes such as *Hobsonia florida* and *Nereis limnicola*, and diptera (including chironomids) were more common in upstream areas where salinities were less than 15 ppt and where starry flounder were more abundant than English sole. In contrast, the cryptogenic cosmopolitan polychaete *Pygospio elegans* were more common in the diet of English sole collected at intertidal areas with salinities over 30 ppt .

More macroinvertebrate species could be added to the list of native or alien prey as cryptogenic species are resolved. The *Capitella* species complex, a group of polychaete species usually erroneously referred to as "*Capitella capitata*" (Grassle and Grassle 1976), was a minor cryptogenic component in the diet of juvenile English sole in Alsea Bay and Yaquina Bay and in the diet of juvenile starry flounder in Yaquina Bay. The taxonomy, ecology and evolution of meibenthos are too poorly documented to allow determinations of their origins. Predictably, meiobenthic prey, mainly harpacticoid copepods (Table 3), were more important in the diets of smaller fish (less than 8 cm total length) of both English sole and starry flounder. Fish prey were found only in two cases - one northern anchovy *Engraulis mordax* in Yaquina Bay and one shiner perch *Cymatogaster aggregata* in Alsea Bay - and both are native species consumed by starry flounder.

The size ranges of the starry flounder are broader that those of English sole in our samples (Table 1). We used age-length relationships for English sole (Rosenberg 1982) and starry flounder (Campana 1984) to estimate the ages of English sole and starry flounder. Such age-length relationships suggest that starry flounder may have a longer estuarine rearing period (c.a., 3 years) than English sole (c.a., 1 year). Consequently, starry flounder may experience greater shifts in food sources than English sole in estuarine intertidal areas.

These data strongly indicate that alien species have altered Pacific Northwest food webs. Whether the reported species introductions have significantly altered the growth, survival, and recruitment of estuarine-dependent fishes however remains to be determined. Critical information needed for such determination includes the food quality, availability and abundance of alien species relative to endemic species. These data are lacking. Given the declines in catches of starry flounder and other species of fish in Oregon (Berry et al. 1980, Lukas and Carter 1994), such information should be valuable.

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DIFFERENTIAL SURVIVAL OF CHINOOK SALMON RELEASED INTO TWO ADJACENT BUT DISSIMILAR FJORDS

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Abstract: The Salmonid Enhancement Program released chinook salmon smolts into two adjacent fjords near Vancouver, after three weeks of rearing in sea cages. Vertical haul net sampling indicated that zooplankton production in Howe Sound was dominated by large crustaceans (copepods, mysids, euphausids) whereas Indian Arm was dominated by large gelatinous animals (*Cyanea, Aurelia*). The survival of the salmon released in Howe Sound was much higher that that of smolts released in Indian Arm, suggesting that the gelatinous-dominated ecosystem is not conducive to supporting juvenile salmon populations.

Introduction

The harbor of the city of Vancouver lies between two fjords (Figure 1) that have similar glacial genesis but quite different ecological makeups. Indian Arm, a northern extension to the east of the harbor in Burrard Inlet, is approximately 22 km long and 1 km wide. The average basin depth is 200 m, with a 26 m deep sill at its mouth. Howe Sound is located west of Vancouver and extends approximately 42 km north with a width of from 2 km at the northern end to 18 km at the southern end. There are a number of large islands in Howe Sound that take up a large proportion of the area. Mean depth in Howe Sound is 325 m and there is a 30 m sill across the fjord.

The Salmonid Enhancement Program of Fisheries and Oceans Canada has been releasing chinook salmon into the two fjords as part of a sea cage rearing program that has been operated to increase the survival of smolts over those released directly into the rivers feeding the fjords. Subsamples of all groups of fish released are coded-wire tagged in a coastwide program to monitor survival and contribution rates from different stocks.

Methods

To collect zooplankton samples, a SKOR net with 30μm mesh was lowered to a depth of 30 m and then drawn quickly back to the surface. A 'downrigger' winch with special heavy-duty cable and a 5 kg weight allowed for speedy recovery and vertical transect of the net. The samples were washed into 40 ml DR plastic vials and fixed with 10% formalin. Samples were collect monthly from April

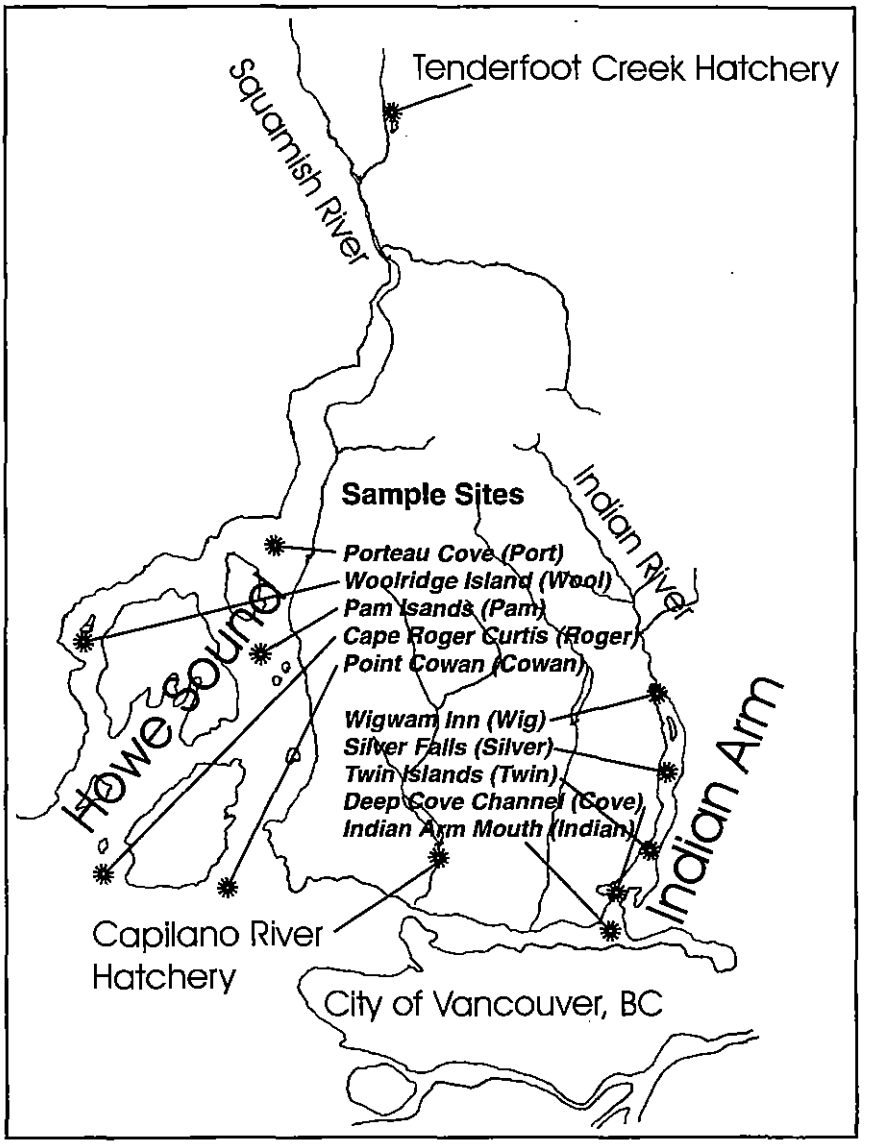

Figure 1. Map showing the location of the hatcheries, fjords and zooplankton sampling sites used in the chinook salmon sea cage release program conducted near Vancouver, BC.

to December 1991, but this paper will only deal with the samples between April and August, when the bulk of the salmon juvenile rearing occurs.

In the lab, all samples were transferred from the formalin fixative to a sugar/borax/formalin solution for long-term storage (Unesco, 1968). Samples were then split into equal portions, one being used for species identification and the other for volume and weight analysis. This analysis involved separating the gelatinous from the arthropod plankton by washing the sample over a 1.0 mm mesh screen, which retained the jellies and let the arthropods go through. These fractions were allowed to settle for 4 hr in conical volumetric flasks and the settled volume was recorded. The gelatinous fraction was placed on aluminum weighing trays and the arthropod fraction was drained and placed on pre-weighed/ashed filter paper. Both were weighed and placed in a drying oven for 24 hrs at 80°C. After dry weight was recorded, the samples were placed in a muffle furnace at 400°C for 4 hrs, after which the ash weight was recorded.

Periodic surveys of the fjords were also conducted using a colour depth sounder (fish finder), that could be focused to various depth ranges. The sounder clearly showed the profiles of large sea jellies, as well as the location of the halo/thermocline that resulted from an outflow of warm fresh water that overlay the cooler, deeper, saline water. In addition, visual estimates of the abundance of large jelly fish were made at locations where weather and water conditions permitted.

The chinook salmon smolts came from the Capilano River and Tenderfoot Creek hatcheries, where they had been raised to approximately the 4 g size and tagged by injecting 1.0 mm binary, magnetized coded wires into their nose cartilages. Their adipose fins were clipped to serve as an indicator that a coded-wire tag was present. The fish were vaccinated for vibriosis at the hatcheries. They were transported to the sea cage sites in Indian Arm and Howe Sound and reared for three weeks prior to release, which was accomplished by simply removing the nets that had enclosed them. This seawater rearing period was meant to acclimatize the fish to seawater and allow them to complete the smolting process without being vulnerable to predators.

Recovery of tagged fish occurred through the Mark Recovery Program (MRP) (Kuhn, 1988), an international, coast-wide effort to sample the commercial, indian and sport fisheries in the eastern Pacific Ocean. Tags returned from escapement of adults into spawning streams and from returns to hatcheries are also accounted for. As part of the MRP, the tags that are recovered are entered into a database where they are adjusted for sampling rate, the number of fish tagged and the number of fish in the released population. The data base calculates the survival rate for that tag group.

Results and Discussion

Zooplankton Abundance

The aquatic ecosystem in Indian Arm is clearly dominated by gelatinous plankton, as indicated by the volumes and dry weights (Figure 2) of samples taken in April to June, when hatchery chinook salmon smolts are released (and when peak migration to the ocean for wild-spawned juveniles occurs). Conversely, these figures show that arthropods (mostly crustaceans) dominate the aquatic ecosystem of Howe Sound. The peak productivity in 1991 occurred in May but oceanic conditions vary from year to year and the peak can be anywhere in the April-to-June time frame.

The gelatinous plankton in Indian Arm not only included a large number of small hydromedusae such as *Obelia, Phialidium* and *Aequorea* in the net samples, but also an extraordinary population of large scyphomedusae, such as *Cyanea* and *Aurelia*, that were too large to be sampled by the

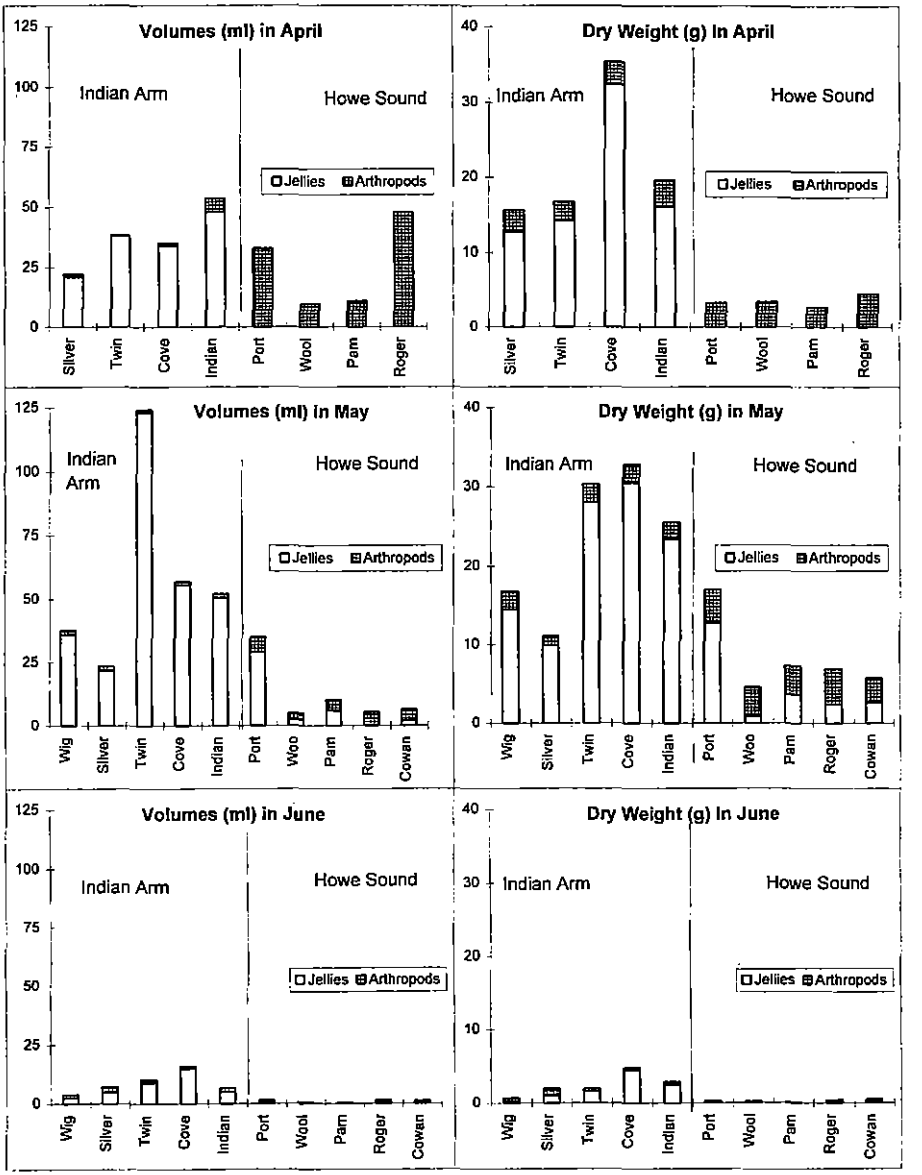

Figure 2. Volumes and dry weights of zooplankton of two types collected in Indian Arm and Howe Sound in April to June,1991.

vertical haul of the SKOR net. During June to September, specimens of *Cyanea* over 1.0 m in diameter were commonly seen. During one east-west transect of Indian Arm in June, a mono-layer of dinner-plate-sized (150-250 mm) *Aurelia* could be seen (and detected on the fish finder) stretching across the entire fjord at 3 m deep, the depth of the thermo/halocline. The total standing stock of the gelatinous plankton in Indian Arm could not be estimated by our infrequent and patchy sampling, but it was clearly far greater than that indicated by the net sampling. The other fraction of the plankton samples in Indian Arm were small crustaceans, such as harpacticoid and calanoid copepods, decapod zoae and ostracods.

The only gelatinous plankton seen in Howe Sound were small forms, such as *Obelia* and *Aquorea*, and also some ctenophores, mostly *Pleurobrachia*. The crustaceans in Howe Sound were mostly large forms: gammarid, hyperiid and caprellid amphipods, mycids, and larger forms of calanoid and harpacticoid copepods. We have also caught very large (2-3 cm) *Euphausia pacifica* in Howe Sound, but not with this sample gear.

Adult Salmon Survivals

The adult survivals from hatchery releases in the 1980's from both the Tenderfoot and Capilano Hatcheries were in the range below 0.6% (Figure 3). The first release from Capilano Hatchery into Indian Arm survived marginally better than the release into the Capilano River at the hatchery, but subsequent releases did as poorly as river releases. On the contrary, the first release from Tenderfoot Hatchery into Howe Sound, and subsequent releases, fared much better than the releases into various locations in the Squamish River watershed.

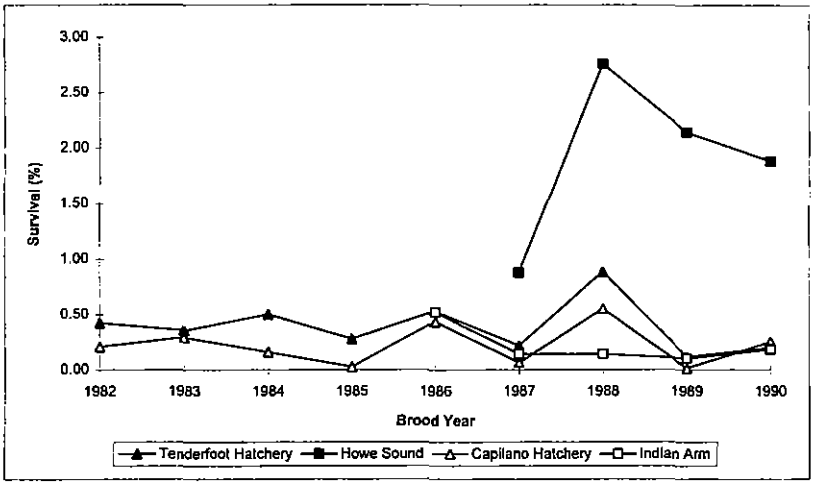

Figure 3. Survival rates from releases of chinook salmon smolts from two hatcheries and of fish from the same hatcheries released after short-term rearing at sea cage sites in nearby fjords. The triangles represent the hatchery releases and the boxes represent the sea cage groups. Open icons are from Tenderfoot Hatchery and black icons are from Capilano Hatchery.

As a consequence of the zooplankton abundance and type shown by the studies reported in this paper, we reduced the number of chinook smolts being released into Indian Arm (from a maximum of about 3 million in 1989) in subsequent years and eventually virtually eliminated such releases.

This was before the salmon survival rates were confirmed with adult return data. We also increased the releases into Howe Sound from 400 thousand to 1.5 million.

Indian Arm is generally more productive of zooplankton than Howe Sound, but productive in animals that are not of use to sustaining fish-based productivity. The classic paper of Greves and Parsons (1977) that speculates that a change in the amount and kind of nutrient loading to a marine ecosystem can favor either a fish-sustaining or sea jelly-dominant trophic chain, from phytoplankton to large predators, may explain both the apparent difference in zooplankton populations in Howe Sound and Indian Arm, and also the consequences for fish rearing. The high zooplankton biomass in Indian Arm may be explained by the complete urbanization of its access to the sea through the Vancouver city area. Prior to the 1920's, Indian Arm streams supported large runs of salmon. Raw sewage was poured into the harbour from the city from the 1890's to the 1970's, and many of Indian Arm's beaches are still closed for periods every summer because of high coliform bacteria counts. This excessive nutrient enrichment probably shifted the trophic food chain to the gelatinous domination seen in this study. Now that nutrient loading into the Indian Arm water source has decreased dramatically because of divergence of all sewage outlets to treatment plants that discharge elsewhere, we wonder if there is any way that the aquatic ecosystem can be turned back to become fish-sustaining again.

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KINEMATICS OF FEEDING IN THE SWELLSHARK, *CEPHALOSCYLLIUM VENTRIOSUM*: EVIDENCE FOR BIDIRECTIONAL FLOW

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Abstract

The kinematics of prey capture and transport were investigated in one-year old swellsharks, *Cephaloscyllium ventriosum* (Scyliorhinidae: Carchariniformes). Prey capture in these sharks generally consisted of one or more ram-dominated capture bites, a resting phase during which the food was held in the teeth of the shark, and then suction-dominated prey transport. Interestingly, during capture and early transport, most of the water taken in appears to be forced back out the mouth anteriorly. This type of water movement is referred to as bidirectional, as opposed to the unidirectional flow found typically in feeding teleosts, where the water enters the mouth and continues posteriorly out the gill openings after prey capture. It is unknown if this behavior is unique to swellsharks. It is the aim of this paper to describe prey capture, focusing specifically on this water movement, and investigate parallels to other aquatic feeding vertebrates known to use bidirectional feeding.

Introduction

As comparative studies become more prominent in the field of functional morphology, one of the striking conclusions is that even taxa that differ markedly in their morphology use remarkably similar kinematic patterns for performing certain behaviors (Lauder and Prendergast, 1992). These behaviors include feeding in an aquatic medium, which has been studied and compared in fishes, salamanders, and turtles among others (see for example Lauder and Prendergast, 1992; Reilly and Lauder 1992). It has been suggested that the aquatic medium imposes certain constraints on feeding due to the water's density and viscosity that result in similarities among feeding behaviors across even distantly related taxa.

Sharks are an extremely successful and diverse group of vertebrates. In general, however, they possess many ancestral features of the jaw relative to the more derived jaw structures found in the well-described bony fishes for prey capture. In spite of this, it is specific aspects of their morphology allow them to perform quite specialized behaviors during feeding. The "primitive" hyostylic jaw attachment allows for cranial kinesis and upper jaw protraction during feeding (Moss, 1972). Upper jaw mobility is thought to provide for greater dexterity in obtaining small food items and an addition of force (provided by the greater contribution of the upper jaw) when cutting large food items (Moss, 1972). With these observations in mind, the question should be asked: do sharks, specifically swellsharks, fit into the highly stereotyped set of feeding behaviors described for other aquatic feeding organisms?

It is the aim of this paper to describe the basic pattern of prey capture and transport in the swellshark. During the study, it was noted that bidirectional water flow seemed to occur during prey transport, and occasionally even during prey capture. This particular feature of prey capture and transport will specifically addressed relative to other bidirectionally feeding aquatic vertebrates.

Methods

Prior to experiments, sharks were housed together in a 400 L tank, and maintained at a temperature of 18±2°C feeding on the same prey as used in the experiments. Five individuals of an average total length of 30.0 cm (range: 23.6 - 37.0 cm) were video-taped feeding on prey items (pieces of fish, described below) in filming tanks (20 gallon aquaria) maintained at the same mean temperature as the holding tanks (18±0.5 °C). This same apparatus was used previously by Gibb (1995) who provides further details on this equipment. Data were collected by video-taping the shark capturing and transporting the prey item at 250 fs^{-1} (fields per second) using a NAC HSV-500 high-speed video system. Two video cameras were used simultaneously to tape two views, lateral and ventral, in order to observe and measure the movement of selected points on the head and body. The ventral view was obtained by aiming a camera at a front-surface mirror angled at 45° and placed beneath the tank. The lateral and posterior images were scaled equivalently using marked grids and a reference scale placed in the tank at the location of the sharks head (prior to beginning experiments). Output from the two cameras was combined on a split screen. The tank was lit from the side by a high-intensity 600W photo lamp.

Prey items were offered to a shark by placing the piece of fish on the base of the filming tank and allowing the shark to approach the prey and subsequently capture it. To account for differently sized sharks, sizes of prey offered were scaled to the diameter of the shark's mouth. Only video sequences in which all the pertinent features of the head and jaws were clearly visible in both the lateral and ventral views were used. For measurement consistency, all attempts were made to use only sequences in which the shark remained perpendicular to the cameras.

Video sequences were analyzed frame by frame using a custom digitizing program. Frames were downloaded at 0.024 sec intervals, starting at three intervals (0.072 sec) prior to mouth opening (time 0), and increasing to 0.012 sec intervals just prior to and throughout mouth opening and closure. Set landmarks on the shark's head and jaws were digitized allowing for the subsequent calculation of pertinent lengths and angles (Fig. 1). The front of the orbit was always the most reliably digitized point, and therefore used to determine relative movement of the variables around it (see Fig. 1B, lateral view). Distances from the front of the orbit to the upper jaw tip, lower jaw tip, hyoid, jaw articulation, and origin of the "water bulge" seen forming during capture and transport (described in detail later) were calculated. These lengths were set to a starting value of zero by subtracting the resting orbit-to-point distance (prior to the beginning of prey capture) from any distances subsequently measured, thus absolute movements of each point were determined. Head thickness was measured at the first gill arch and at the pectoral fin insertion (see Fig. 1B). Body height off the bottom

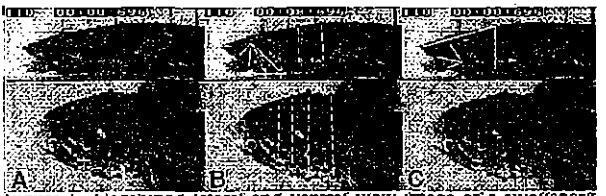

Figure 1. Combined lateral and ventral view image of a swellshark with a captured prey item from the NAC high-speed video with digitized landmarks shown (A), the subsequent lengths measured from the landmarks (B), and the subsequent angles measured from the landmarks (C). The arrows in the center image (B) indicate the directions of movement that were measured from frame to frame, the points marked by arrows are the snout, hyoid, and the point at which a bulge of water was observed to form. In B, solid lines indicate distances from the eye that were subsequently zeroed to the starting position to quantify movements of the end points. Dashed lines indicate linear distances that were absolute. See text for additional details.

was also determined. From the ventral view (Fig. 1B), head widths were measured at the labial cartilages, first gill arch, and pectoral fin insertion. Jaw width was also determined by measuring from jaw articulation to jaw articulation. Head angle, upper jaw angle and gape angle were also calculated (see Fig. 1C). Predator-prey distance was also determined, but will be discussed only briefly in this paper.

Results

Prey capture in these sharks generally consisted of one or more ram-dominated (meaning the prey item remained stationary during prey capture and the shark physically moved forward to overtake the prey item) capture bites, a resting phase during which the food was held in the teeth of the shark, and then suction-dominated prey transport (Fig. 2). Once food was offered, it sometimes had be presented quite close to the snout of the shark to initiate a feeding response. In other cases, the shark would accelerate its body along the bottom of the tank to bite the prey item. Little to no movement of the prey item was observed. Mouth opening did not begin until the shark was very close to the prey item. It was then often the case that the shark swam with its mouth opened maximally (often with a gape angle greater than 90°) until the upper jaw was positioned directly over the prey item. As a result of this movement, the lower jaw often actually contacted the prey item before mouth closure was initiated and the subsequent capture bite occurred. Clear upper jaw protrusion could be seen during the bite (movement of the jaw away from the skull), however, the magnitude was so small that it was not always separable from digitizing error in the analysis. Mouth closure either lead to subsequent biting events, or the bite was held and the shark entered the holding phase. All sharks eventually entered a holding phase if bites were used to capture the prey item. A small portion of strikes were recorded where the prey item was taken directly into the mouth without a capture bite. During the holding phase, the shark remained relatively motionless, with the exception of repeated observations of snout lifting such that the entire anterior portion of the shark was lifted and rotated posteriorly, and the shark sat propped up on its pectoral fins (not visible in Fig. 2). The hyoid may stay depressed throughout this phase. Re-opening of the mouth

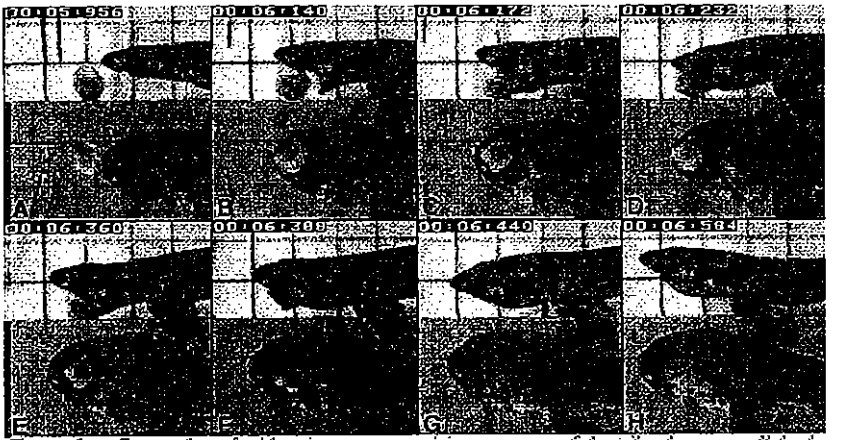

Figure 2. Composite of video images comprising a successful strike by a swellshark. Numbers in the upper left corner of each image depict a time code (min:sec:ms), providing an indication of the relative speed of the feeding event. Frames A-D represent prey capture, with hyoid protrusion evident by frame C. Notice that the prey item maintains position, with the shark changing position to overtake it. The bite is complete in D, and the shark has entered the holding phase described in the text. In frame E, transport is beginning, the mouth is opening again and the prey will be taken into the mouth (F). In G, the mouth is closed, and the ventral surface of the shark's branchial region can be seen expanded by a bulge of water taken in during prey transport. This bulge can be seen moving anteriorly, and starting to exit the mouth in H.

signified the initiation of prey transport. During transport, the mouth was rapidly opened (much more rapidly than during prey capture), and the prey item was taken into the mouth presumably by suction, although in some feeding events sharks were still moving forward due to the momentum of the prey capture event. Mouth closure also occurred rapidly, at which time a bulge could be seen on the ventral surface of the shark's branchial region (below the gill openings). This bulge could be observed to move anteriorly, and disappear as the mouth opened again. It is presumed that this bulge was due to water in the oral cavity, and hence referred to as the water bulge throughout this paper. A similar bulge was observed in some, but not all, prey captures. Video images of swellsharks feeding on dye perfused prey items confirmed that this bulge movement did in fact reflect a net movement of water into and out of the mouth (Fig. 3). Thus, bidirectional feeding does appear to be occurring in swellsharks. During mouth closure in prey transport, the shark may rotate its head anteriorly again, sometimes at an angle even smaller than the angle at the start of prey capture. During this movement, the shark appears to bend its head such that the gills are compressed (see Fig. 3, panel D, this position in still evident even at this time in the strike). The timing of this compression activity appears to be correlated with the ability to pass water out of the gills (unidirectionally), however, the mechanism by which this is achieved, and the actual function of this apparent gill compression is unknown. The kinematics of the feeding events described are shown in Figure 4.

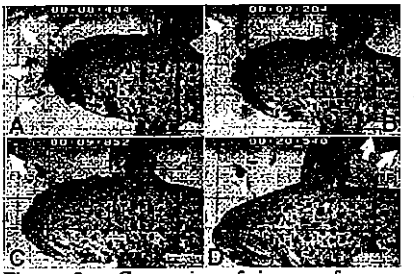

Figure 3. Composite of images from a successful strike on a piece of fish soaked with blue food coloring. In A, mouth closing is occurring, and dye can be seen to exit the mouth. At the bite (B), as the shark is entering the holding phase, the most dye can be seen to exit the mouth. In C, some dye can be seen exiting the gills, however, the majority of the gill-exiting dye is not seen until D, a full 11 seconds later.

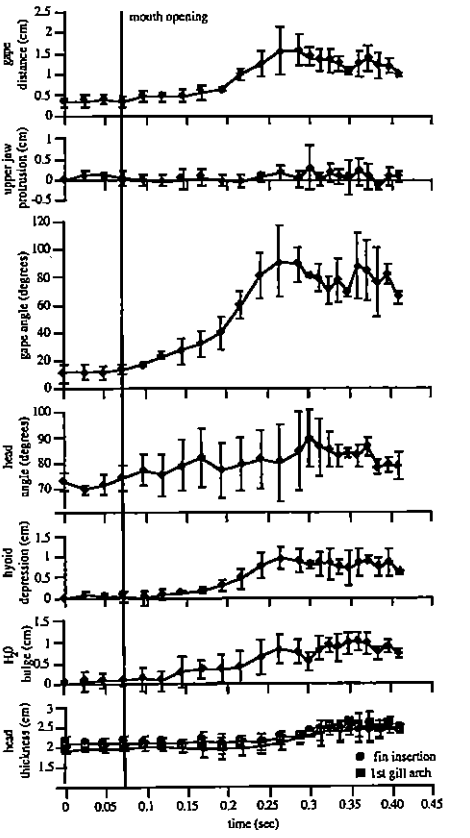

Figure 4. Kinematic plots of selected variables from those digitized during prey capture. Values are means (±SD) for four strikes from a single representative individual. Recall that upper jaw protraction, hyoid depression, and the position of the water bulge are zeroed to the resting value, the value at time zero. Time zero is three intervals (0.072 sec) prior to mouth opening, indicated on the plots. These plots end at the start of the holding period, providing a relative estimate of the position of the features of the head and jaw as this individual enters that phase of feeding.

Notice that for prey capture mouth opening (increased gap distance) appears to slightly precede the start of hyoid depression. Peak gape coincides with maximum gape angle (as it must considering how they are measured), but also maximum hyoid depression and water bulge formation. The hyoid and the water bulge can be seen both depressing and retracting (moving posteriorly) in video footage. The kinematic value plotted encompasses both directional movements. Examination of data on dorsoventral hyoid and water bulge movement alone indicates no noticeable difference (including magnitude) from the trend shown. Peak upper jaw protraction occurs slightly after peak gape, and seems to coincide with maximum head angle. In the swellshark upper jaw protrusion is directed primarily ventrally (relative to the snout), and little anterior movement of the jaw away from the skull could be detected (note: this isolated ventrally directed movement has also been referred to as protraction or depression in the literature). Head thickness increases at both the first gill arch and the pectoral fin insertion at roughly the same time as head angle begins to decrease again. Hyoid protrusion and the position of the water bulge remain remarkably constant throughout this period of increased head thickness. Although the holding phase is not included in Figure 4, the ending positions of each of the variables are nearly identical to the starting position as the shark entered the prey transport phase (Fig. 5). After the initiation of prey transport, gape distance and angle can be seen to increase as the prey item is taken into the mouth fully. Mouth opening during transport occurs much faster than during prey capture (≈0.02 sec *vs.* 0.20 sec). Maximum gape distance and gape angle during transport are smaller then seen in prey capture. Gape distance changes parallel gape angle changes. Although not shown, the angle created between the lower jaw and the ventral surface of the shark posterior to the jaw (lower jaw angle) was calculated and changes were exactly 180° out of phase with changes in gape angle (i.e. the lower jaw angle is at a minimum when gape angle is at its maximum). This indicates that head angle is not contributing to jaw closure such that gape distance is reduced when the position

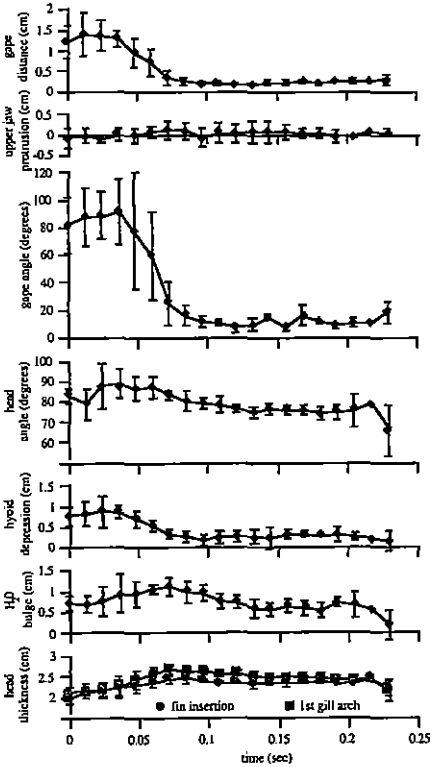

Figure 5. Kinematic plots of selected variables (same as Fig. 4) during prey transport. Values are means (±SD) for four transports (the same four feeding events used in Fig. 4) from the single representative individual. Notice that gape distance does not start at zero, as transport begins with the food item held in the teeth, at the end of the holding period. Also note that although head angle has increased slightly, the other variables are quite near their values at the end of prey capture, entering the holding phase, in Figure 4.

of the lower jaw is still at a maximum (as seen in the snapping turtle *Chelydra*, Lauder and Prendergast, 1992). Peak gape appears to occur coincident with or slightly before maximum hyoid depression (like prey capture). Maximum head angle occurs after peak gape and maximum head angle and plateaus around this time. Maximum water bulge formation is observed after peak gape and maximum hyoid depression, and appears highly correlated with an additional increase (beyond the maximum seen in prey capture) in head thickness. During mouth closure, gape distance does not decrease all the way to zero in many cases as the upper and lower jaws do not meet precisely and the presence of the water and food item in the mouth seem to contribute to pulling the jaws apart slightly. Mouth closure appears to be more rapid during transport than during prey capture (≈0.8 sec *vs.* 1 sec). The hyoid remains slightly depressed even after the mouth has closed.

Discussion

The general kinematic pattern described here for the swellshark is quite similar to that described for feeding requiem sharks (Carchariniformes: Carcharinidae) described by Frazzetta and Prange (1987). As a prey item is approached, the head is rotated dorsally while the lower jaw is depressed. The capture bite is achieved primarily through lower jaw elevation, however, upper jaw protrusion may assist in the bite, possibly even increasing the rate of jaw closure (Moss, 1972; Frazzetta and Prange, 1987). Cranial or head rotation ventrally does appear to contribute to jaw closing as it occurs simultaneously with gape reduction. However, in sharks its movement is complimentary, not contradictory, to the movement of the lower jaw, and does not appear to be a primary regulator of jaw closure as seen in other organisms (i.e. *Chelydra*, Lauder and Prendergast, 1992) where the lower jaw remains stationary of even moves ventrally while the head also rotates ventrally. Multiple bites are common in prey capture for Carchariniformes (P. Motta and C.D. Wilga, pers. comm.), however captures that lack a bite and consist entirely of a single event where the prey is completely engulfed also have been observed (Frazzetta and Prange, 1987). Prey transport as a separate feature of feeding, however, is poorly described for carchariniform sharks.

Like the findings of Gillis and Lauder (1995), there were distinct differences between prey capture and transport in the swellsharks studied here. Certainly the holding phase described serves to completely separate the two activities into discrete units of activity for analysis. However, distinct kinematic differences also exist. Movements of many kinematic variables occurred much more rapidly but also to smaller extremes during prey transport. In the swellshark, the mouth opened and closed more rapidly during prey transport, and variables like gape distance reached smaller maxima. This same pattern is seen in teleost fishes (Gillis and Lauder, 1995). Also analogous to teleosts, hyoid depression reached similar maxima during transport and prey capture, as did head angle. In this study, however, upper jaw protrusion was smaller or practically non-existent in prey transport in contrast to much larger jaw protrusion during transport versus capture in teleosts (Gillis and Lauder, 1995). This latter finding seems to support the notion that jaw protrusion in carchariniform sharks functions to enhance biting during prey capture.

The most notable event during prey transport, however, was the occurrence of bidirectional water movement. Bidirectional flow has been described in several salamanders (Lauder and Shaffer, 1986; Reilly and Lauder, 1992) and turtles (Lauder and Prendergast, 1992). Its occurrence has been correlated with a lack of or very reduced gill openings that prevent water from passing posteriorly out of the mouth (unidirectionally). Swellsharks have quite large gill openings, and a clearly developed system of buccal pumping for respiration (pers. obs.). Swellsharks can be seen methodically pumping water over the gills prior to a prey capture event. This "warm-up period" is thought to serve the shark by allowing it to taste the food and then decide whether to strike (T. Tricas and D. Nelson, pers. comm.). Multiple personal observations support this theory, as stationary sharks begin actively pumping water over the gills within minutes of the prey being placed in the tank, and will then accelerate and strike, or turn and swim away. Clearly, water flow is altered during prey capture and transport such that it is redirected out the slightly opened mouth (open due to the prey lodged in the teeth after capture, or reopened following transport), and does not pass posteriorly out the gill openings until well after transport occurs. The key characteristic of unidirectional feeding is the occurrence of water exiting out the gills posteriorly before the mouth has closed (Reilly and Lauder, 1992).

Several parallels can be drawn between the kinematics of bidirectionally feeding salamanders and turtles, and swellsharks. In the salamanders *Amphiuma* and *Cryptobranchus*, head angle is strongly depressed (to below the starting value at time 0) during mouth closure, thereby contributing to gape closure. Rapid hyoid depression does not begin until near peak gape. While the hyoid is protracted, the mouth reopens, and water can be seen emerging from the mouth (Reilly and Lauder, 1992). In the snapping turtle *Chelydra*, head angle is reduced such that it is the primary regulator of jaw closure. As alluded to earlier, the head begins to bend on vertebral column ventrally while the lower jaw is still depressing, thus gape distance is held constant while lower jaw angle decreases. As the lower jaw begins to reverse direction, it quickly meets the upper jaw, brought into position by the severely reduced head angle. Rapid hyoid depression does not begin until after the mouth starts to open, and remains depressed while the mouth reopens slightly to let water out (Lauder and Prendergast, 1992). In swellsharks, as mentioned above, we see these same patterns: head angle contribution to mouth closure, rapid hyoid movement after mouth opening, prolonged hyoid depression, and mouth reopening to allow water to exit.

The consequence of these kinematic patterns to bidirectional flow is uncertain. The head angle's contribution to mouth closure may help to rapidly close the mouth before the water rebounds, taking the prey item back out of the mouth again. Clearly, this is assisted in sharks by upper jaw protrusion. Reduction of head angle may contribute to the compression of the gill arches observed in the video footage of feeding swellsharks. As mentioned previously, the timing of this compression appears to be correlated with the ability to pass water out of the gills (unidirectionally). Multiple instances of head bending such that the gills appear to be compressed are often observed after mouth closure in prey transport. However, the mechanism by which this is achieved, and the actual function of this apparent gill compression is unknown. It would appear that prolonged hyoid depression may facilitate, or be a consequence of, holding onto the water that was taken in with the prey item. Certainly, mouth reopening is required to allow the water to exit the buccal cavity. Nonetheless, is it uncertain exactly how water is prevented from exiting the gills during feeding. Comparing the kinematic and motor patterns of respiration/buccal pumping and bidirectional water flow in the swellsharks is clearly necessary to understand bidirectional water movement and why it might be utilized over the unidirectional flow pattern used in respiration.

Acknowledgments

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DIET OF PIKE, *ESOX LUCIUS* L., FROM LAKE PEIPSI (ESTONIA) IN 1995

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1. Introduction

Pike is the most common fish in the lakes of Estonia. This fish species is unpretentious and resistant to several unfavourable living conditions, particularly to poor oxygen conditions. Pike is also very tolerant to low pH and to high organic matter content (Pihu, 1993). The ecological significance of pike as a top predator in Estonian lakes is great.

Lake Peipsi (Fig. 1) as a large waterbody, poor in higher vegetation, is not a very suitable habitat for pike. Its number in L. Peipsi is relatively small, which is also due to the scarcity to flooded spawning areas in spring (Yefimova, 1966). According to official data pike constituted 1.4 % of fish catches in the Estonian part of L. Peipsi in 1995. The total catch of pike in the last three years has been about 30 t. The legal size of this fish in the lake is 40 cm, and the commercial part of its population consists of about ten generations.

The population of pike in L. Peipsi was studied with respect to the diet composition in 1995. The frequency of occurrence and number of food objects per individual are given. Size-related changes in the diet of pike are discussed.

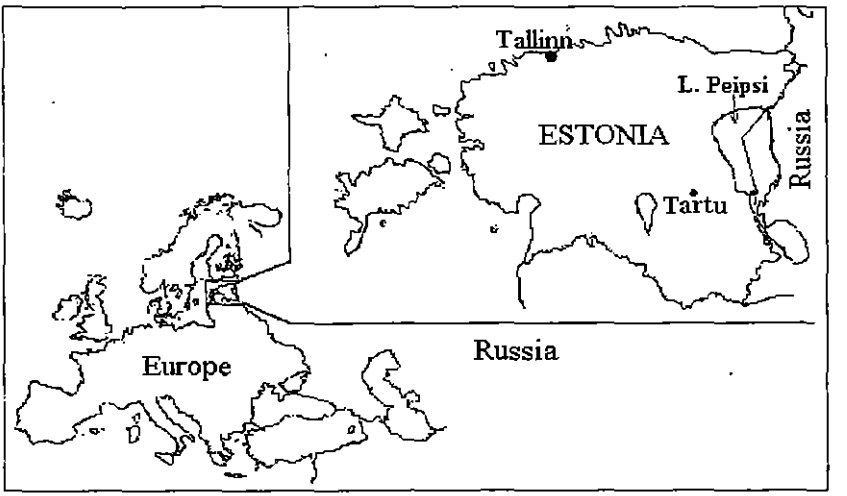

Fig. 1. Location of L. Peipsi.

2. Study area

The total surface area of L. Peipsi (in the broad sense) is 3,558 km^2, average depth 7.1 m, maximum depth 15.3 m. The lake is located on the border of Estonia and Russia (Fig. 1). This paper considers its northernmost and largest part, L. Peipsi *s. s.*, with a surface area of 2,670 km^2, average depth 8.3 m and maximum depth 12.9 m (Kupcov & Arukaèvu, 1983). L. Peipsi is a eutrophic waterbody with alkaline water (pH 7.6-8.4) (Timm *et al.*, 1994). Water transparency has not exceeded 1.2 -- 2.7 m in recent years (Timm, 1993). The ice-free period lasts usually from April till November.

L. Peipsi belongs to smelt-bream lakes; due to eutrophication during the last decades it has obtained features of a pikeperch lake. According to official data the total catch of fishes in the Estonian part of L. Peipsi made up 1,624 tons in 1994 and 2,132 tons in 1995.

The share of valuable fishes, mostly the inhabitants of eutrophic waterbodies such as bream *Abramis brama* (L.), pike *Esox lucius* L., pikeperch *Stizostedion lucioperca* (L.), perch *Perca fluviatilis* L. and the inhabitants of oligotrophic waterbodies such as whitefish *Coregonus lavaretus maraenoides* Poljakow and lake smelt *Osmerus eperlanus eperlanus* m. *spirinchus* Pallas was quite large in experimental catches, constituting 89% of the total catch of fishes in 1994. Among them pike made up 2 % (Fig. 2).

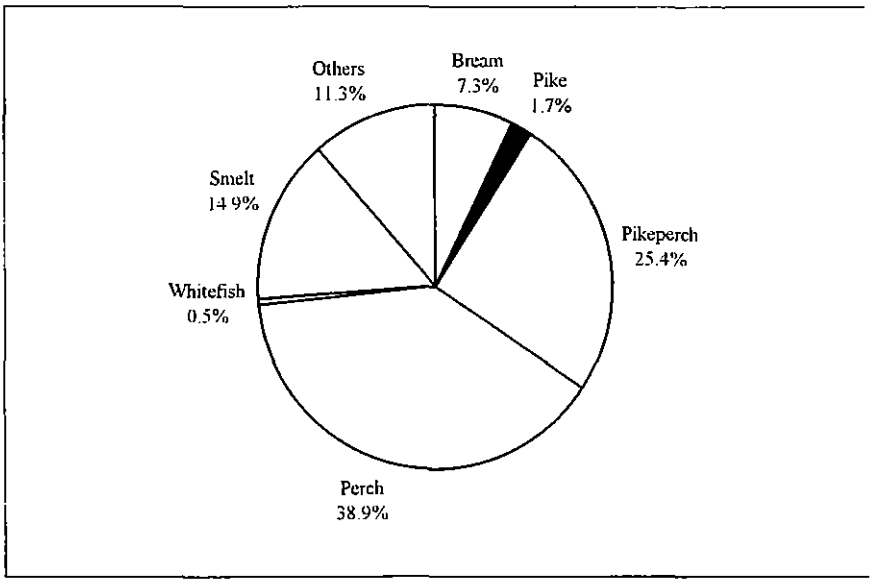

Fig. 2. Weight share of pike and other valuable fishes in experimental catches in L. Peipsi in 1994.

3. Material and methods

The material was collected from the open part of L. Peipsi *s. s.* from July till October 1995. Fishes were caught with the experimental Danish seine (mesh size 18-22 mm in the cod-end) and the trawl (mesh size 10-14 mm in the cod-end). All caught pikes, a total of 64 fishes with a standard

length (Sl) of 28-105 cm were dissected and the stomach content analyzed. The length of fishes was measured with the accuracy of 1 cm.
Prey fishes or their remains were counted, measured and identified. Some specimens of partly digested prey fishes, not recognizable by external morphology were not identified to the species.
The diet was assessed on the basis of the stomach content and was expressed as prey frequency of occurrence (i. e. the percentage of all fish examined in which that prey species occurred) and as percentage prey number (the number of each prey species expressed as a percentage of all observed prey).

4. Results

4.1. The composition of the diet

Pike turns into a piscivorous predator during the first summer. This fish consumes different food organisms in Lake Peipsi. Its diet included at least 5 prey fish species: perch, ruffe, smelt, roach and pikeperch. Invertebrates were not found in pikes' stomachs. About 40% of the examined stomachs were empty. Smelt, perch and ruffe were the most frequently consumed species (Fig 3)

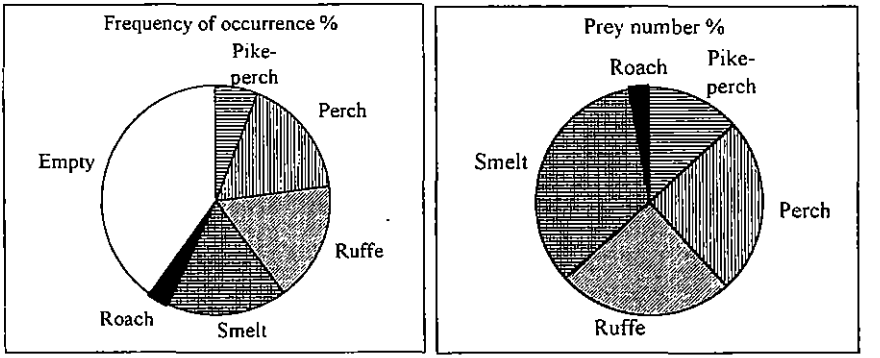

Fig 3. Frequency of occurrence (%) and percentage of prey fish species (in number) in the diet of pike from Lake Peipsi in 1995.

All dissected stomachs contained on the average 1.2 prey fishes, whereas fed fishes had swallowed on the average 2.1 prey items. Smelt dominated in the diet numerically (34%), followed by perch and ruffe (both 25% in number) (Fig 3).

4.2. Size-related changes

Smelt and perch were the commonest fish species in the diet of small pike (28-53 cm) (Figs. 4, 5). They dominated both by frequency of occurrence (25 %) and numerically: the fraction of smelt formed 41 % and that of perch 35 % of all consumed fishes. The part of smelt decreased numerically in the food of larger pikes.
The empty stomach occurred most frequently (65%) in pike Sl = 54-79 cm (Fig. 5). In other length groups the share of empty stomachs made up around 30%. Ruffe was the commonest prey species (54.5% in number and 17% in occurrence) in pike Sl = 54-79 cm.
Pikeperch appeared in the food of the largest (Sl >80 cm) pikes, while it was the most abundant prey species (39 % in number) in this length group.
Predation pressure of pike on the roach population is weak in all length groups.

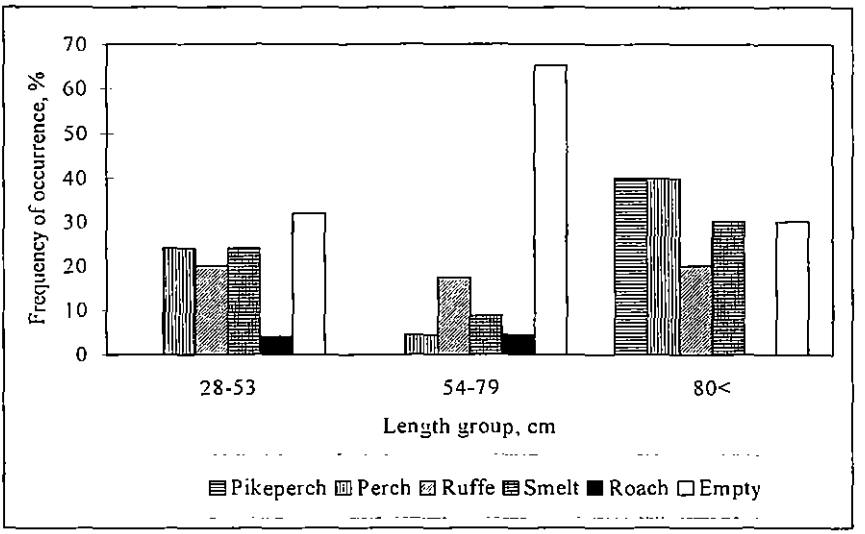

Fig. 4. The frequency of occurrence of prey fish species in the diet of pike from Lake Peipsi in 1995.

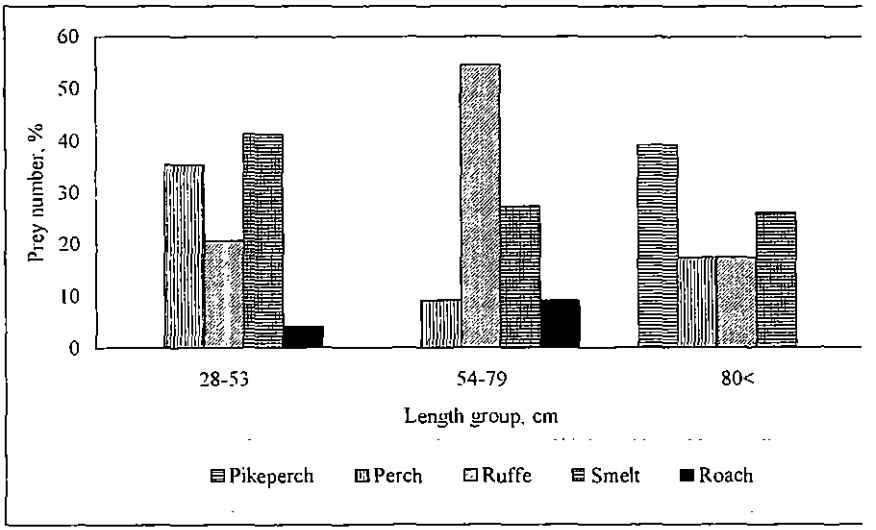

Fig. 5. The percentage of prey fish species in number in the diet of pike from Lake Peipsi in 1995

5. Discussion

Pike are opportunistic in their feeding habits. It has been suggested that they can change their prey selection relatively rapidly in response to changes in the abundance and vulnerability of prey species (Adams, 1991).

A comparison of the diet of pike from L. Peipsi in 1995 with data from 1960-1963 (Pihu, 1966) demonstrates slight shifts in prey choice. The diet of pike included at least 16 prey fish species; among them smelt, perch, ruffe, roach dominated in prey occurrence and number in 1960-1963. Smelt formed a major part in the diet of smaller pike in 1960-1963 as well as in 1995. The role of roach, as well as of bleak, vendace and burbot has decreased during recent decades. At the same time, the share of pikeperch in the food of pike has increased in connection with the growing abundance of the pikeperch population in the lake.

Pikeperch constituted only 0.2-0.3% (about 19 t) of total catch in 1960-1963, but 17-27% (380-470 t) in 1989-1995. During the 1960s pike consumed only single pikeperch fry, whereas in 1995 this prey species dominated in the food of large (SI >80 cm) pikes. At the same time, the abundance of the pike population has decreased in the lake. The catch of pike made up about 190 t (5-6 % of total annual catch) in 1958-1968, but only 30-93 t (1.5-3,5% of total annual catch) in 1989-1995

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FEEDING OF PIKEPERCH, *STIZOSTEDION LUCIOPERCA* (L.) IN LAKE PEIPSI (ESTONIA)

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1. Introduction

Pikeperch as a fastidious fish inhabits the best eutrophic and hypertrophic lakes in Estonia, which are relatively large and deep, with a rather high pH and fish productivity. As the lakes are located mostly in cultivated areas, they are exposed to agricultural pollution. Pikeperch avoids closed lakes and is quite sensitive to winter anoxia (Pihu, 1993).

Pikeperch has become one of the most important valuable commercial fishes in Lake Peipsi. Owing to its high commercial value and vulnerability to fishery, pikeperch is at the same time the most endangered fish species in the lake.

Lake Peipsi (also L. Peipus in older German literature and L. Chudskoje in Russian) is one of the largest inland waterbodies in Europe (Fig. 1).

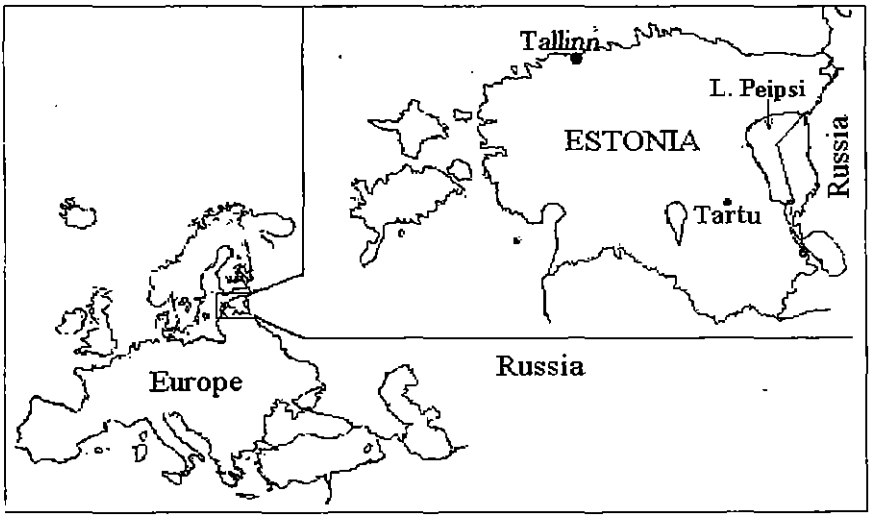

Fig. 1. Location of Lake Peipsi.

It occupies the fourth place after the lakes of Ladoga, Onega and Vänern and is shared between the territories of Estonia and Russia.

L. Peipsi belongs to smelt-bream lakes; due to eutrophication during the last decades it has obtained features of a pikeperch lake. Its fish productivity as well as catches (about 25-35

kg/ha/year) are high; in this respect L. Peipsi exceeds all the large lakes of North Europe (Pihu, 1983). About 2/3 of its surface area and about 60% of the catch of fish belong to Russia, the rest to Estonia.

The population of pikeperch was studied considering the composition of the diet. The frequency of occurrence and number of food items per individual are presented. Size-related changes in the diet of pikeperch are discussed.

2. Study area

The total surface area of L. Peipsi (in the broad sense) is 3,558 km^2, average depth 7.1 m, maximum depth 15.3 (Kupcov & Arukaèvu, 1983). The lake consists of three parts: the large and deep northern part, Lake Peipsi *s.s.*; the southern part, Lake Pihkva, and Lake Lämmijärv which connects them. This paper deals with L. Peipsi *s.s.* Its surface area is 2,670 km^2, average depth 8.3 m, maximum depth 12.9 m (Kupcov & Arukaèvu, 1983). It is a eutrophic waterbody with alkaline water (usually pH 7.6-8.4) (Timm *et al.*, 1994). Water transparency has not exceeded 1.2 -- 2.7 m in recent years (Timm, 1993). Ice appears usually in November and melts in April. The average temperature of the surface water layer during the ice free period is about 7.3° C (Uleksina, Filatova, 1983).

According to the present data L. Peipsi and the streams falling into it serve as a habitat for 34 fish species. The occurrence of fish species typical of oligotrophic waterbodies such as lake smelt *Osmerus eperlanus eperlanus* m. *spirinchus* Pallas and vendace *Coregonus albula* (L.) is quite high. According to official data the total catch of fishes in the Estonian part of L. Peipsi made up 1,624 tons in 1994 and 2,132 tons in 1995. The principal fishes in commercial catches are lake smelt, perch *Perca fluviatilis* L., pikeperch, bream *Abramis brama* (L.) and pike *Esox lucius* L. (Fig. 2).

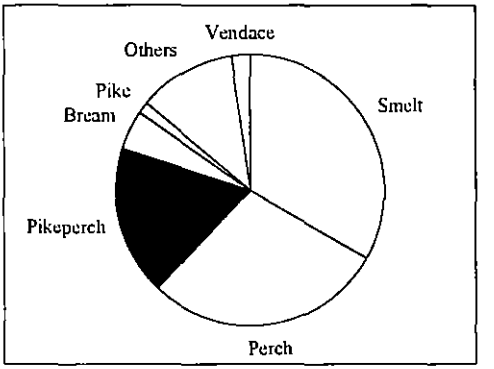

Fig. 2. Weight share of pikeperch and other valuable fishes in commercial catches from L. Peipsi in 1995

Fishes are caught from L. Peipsi mostly with Danish seines and large gill nets. According to an official agreement between Estonia and Russia the number of Danish seines allowed to use in L. Peipsi in 1994--1995 was 40: 20 on the Estonian side, 20 on the Russian side. Mesh size in the cod-end of the commercial seine is 80 mm stretched, and mesh size of gill nets is 140 mm. The legal size of pikeperch in the lake is 40 cm.

3. Material and methods

The material was collected from the open part of L. Peipsi *s.s.* from April to October 1995. Fishes were caught with the experimental Danish seine (mesh size 18-22 mm in the cod-end) or experimental trawl (mesh size 10-14 mm) mostly in the morning hours and dissected immediately. A total of 342 fishes were examined during the study period (Table 1). All fishes were measured with the accuracy of 1 cm. Prey fishes or their remains were counted, identified and measured.

Two different measures are used for the description of the stomach content: frequency of occurrence and abundance of prey fish. The frequency of occurrence (FO) is defined as the number of fishes in which a prey item occurs expressed as a fraction (%) of the total number of the examined predators. Fishes with empty stomachs were included.

Table 1. Number and measurements of examined pikeperch

Month	Standard length (Sl), cm				
	<19	20-29	30-39	40-49	50<
April			4	9	5
May			8	15	1
June			2	3	1
July		3	5	7	
August	80	2	1	28	5
September	69	27	15	43	6

4. Results

4.1. The composition of the diet

Pikeperch turns into a predator at the end of the first summer. The diet of pikeperch contained at least 6 species of prey fishes: smelt, ruffe, perch, vendace, roach, pikeperch. In addition, a shell of *Dreissena polymorpha* was found in the stomach of one pikeperch. More than half of the examined fishes had consumed smelt (FO = 51.5%), followed by ruffe (22.5%) and perch (9%). FO of other prey fishes was less than 2%. 20% of the dissected stomachs were empty.

4.2. Size-related changes

The first prey item for pikeperch is smelt fry. According to our data fishes with Sl < 15 had consumed only smelt (Table 2). With increasing body size the diet of pikeperch was enriched with ruffe and fry of perch. Fishes in the length group 15-19 cm began to take ruffe. Cannibalism occurred in the same length group. Two pikeperches (Sl = 19 cm and 19.5 cm) caught in September had both taken a pikeperch of Sl ≅ 8 cm. The remains of perch occurred in the stomach of specimens with a length over 30 cm. The remains of vendace, roach and pikeperch were found in the stomachs of larger pikeperch too. A comparison of the diet of pikeperch of different size demonstrated a shift in prey choice.

Table 2. Frequency of occurrence (%) of several prey fishes in the diet of pikeperch from L. Peipsi in 1995

Pikeperch		Prey fish					
Length, cm	n	Perch	Ruffe	Smelt	Vendace	Others	Empty
<15	53			94.3			5.7
15-19	96		11.5	65.6		2.1	20.8
20-24	17		17.7	64.7			17.6
25-29	18		29.4	41.2			29.4
30-34	14	21.4	21.4	35.7			35.7
35-39	21	14.3	47.6	33.3	4.8		28.6
40-44	72	16.7	41.7	31.9	1.4	4.2	12.5
45-49	33	21.2	33.3	21.2	3.0	12.2	27.3
50-72	18	38.9	5.6	22.2	5.6	39.0	22.2
Average		9.4	22.5	51.5	1.2	2.0	19.9

With the increasing size of pikeperch the FO of smelt in its food decreased gradually. At the same time the FO of ruffe increased till the length of the predator reached 35-39 cm (FO_{max} = 48%).

while in larger length groups it decreased again noticeably, constituting only 6% in specimens Sl > 50 cm. Predation pressure of pikeperch of Sl > 30 cm on perch increased gradually with the growth of the predator. Large specimens (Sl > 50 cm) consumed perch most frequently (Table 2).
When comparing different pikeperch length groups, the share of fishes with empty stomachs is quite varied. Among young fishes (age 1+, Sl < 15 cm) 95% of specimens had taken food. The most inactive consumers were fishes of Sl = 25-39 cm.
The diet of pikeperch reveals evident size related changes: larger specimens prefer larger prey fish species, and their diet composition is more diverse. The maximum size of the prey caught appeared to be related mainly to predator gape dimensions.

4.3. Number and size of consumed prey fishes

Smelt was the most abundantly consumed species (Table 3), whereas all size groups of pikeperch feed on it. Ruffe is the second rate prey organism, and it was consumed most heavily by pikeperch of Sl = 40-44 cm. The average number of prey fishes per one stomach in different predators size groups is different. As a rule, bigger specimens had consumed more prey items. For example, pikeperch of Sl = 15-19 cm had engulfed on an average 0.13 ruffes or 0.77 smelts, whereas in the stomachs of fishes of Sl = 40-44 cm we found the remains of 1.1 ruffes or 1.33 smelts.

Table 3. Number of engulfed prey fishes per one pikeperch

	Prey fish					
Length group, cm	Pikeperch	Roach	Vendance	Perch	Ruffe	Smelt
<15						0.98
15-19					0.13	0.77
20-24					0.29	0.76
25-29					0.28	0.56
30-34				0.36	0.43	0.86
35-39				0.24	0.62	0.67
40-44			0.01	0.26	1.10	1.33
45-49		0.09	0.03	0.24	0.64	1.03
50-72	0.06	0.28	0.00	0.50	0.06	2.06
Average	0,01	0,02	0,01	0,13	0,42	1,0

The size variation of consumed prey fishes was not very high. The smallest prey fish (ruffe) found in the stomach of pikeperch had a length of 3 cm, the largest one (perch and roach), 12 cm. There was a considerable correlation between measurements of the prey fish and the predator. As a rule, bigger pikeperch consumed bigger prey fishes.

4.4. Seasonal changes

According to our data there is no significant difference between the frequency of occurrence of prey fishes in the diet of pikeperch in April-June and July-September ($F_{calc.} << F_{tab.}$). A significant difference occurred neither between the number of empty stomachs of pikeperch in spring and autumn periods ($F_{calc.} << F_{tab.}$). In both cases the relative number of stomachs without food constituted about 20% (Table 3). Our data showed that the feeding intensity of pikeperch during the whole ice free period is approximately the same.

5. Discussion

Pikeperch is economically the most important species of both brakishwater bays and inland waterbodies in many Baltic areas. This species is heavily exploited all over L. Peipsi.

In L. Peipsi the main food of pikeperch fingerlings up to a length of 3-5 cm consists of *Leptodora*. Later, they start to prey on the larvae and fry of smelt. Larger pikeperch consumes almost all available fish: smelt, roach, perch, bleak, ruffe (Shirkova, 1966; Erm, 1981).
According to our data pikeperch in L. Peipsi fed mainly on smelt, ruffe and perch, whereas the diet of small specimens (Sl < 15 cm) consisted only of smelt. With increasing body size the diet of pikeperch diversifies with ruffe and fry of perch being added. The fractions of other prey fish (roach, vendace, pikeperch) were small.
In lakes of southern Finland pikeperch consumed mainly smelt and perch, but they fed also on roach and bleak (Peltonen & Ruuhijärvi, 1995) like in L. Peipsi.

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A STUDY OF FOOD AND FEEDING OF GREY MULLETS IN THE SOUTHERN OF THE CASPIAN SEA.

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Introduction

There are many factors that potentially influence both the amount and type of food found in the digestive tracts of fish, including the diel cycle, seasonal changes, size of fish, food availability, and differential digestion rates (Bowen and Allanson, 1982).

A great deal has been published about the food of the mullet (Mugilidae) and it has been summarized by Pillay (1953), Thomson (1963, 1966), Odum (1968 *a*, *b*, 1970), Hickling (1970), Zismann, *et al.*, (1975). Mullet have been described variously as vegetarian, omnivorous, planktophagous, and as devouring small crustaceans. They have been named "mud eaters" by Zenkevich (1963), "iliophagous" by Pillay (1953), "detritus feeders" by Rajan (1964), "algal feeders" by Hiatt (1944), "feeders on micro and meio benthos" by Hickling (1970), "interface feeders" by Odum (1970), "deposit feeders" by Fagade and Olaniyan (1973) and "soft bottom feeders" by Blaber (1976).

Differences in diet are quite evident according to age and size of the fish. A study of the stomach contents of *Liza aurata* and *L. ramada* revealed an ontogenetic change in the diet (15-40 mm SL, 4-5 months old) (Vallet, *et al.*, 1970). Young individuals fed on Copepoda, Amphipoda and Nematoda, indicating an essentially carnivorous diet. However, older fish (larger than 50 mm SL) feed on diatoms, plant fragments, sand and mud, indicating that they were mainly herbivorous and detritivorous.

That juvenile mullet are initially carnivorous and planktonic feeders and the adults detritovoros has also been noted by Hickling (1970); and Odum (1970). It has been suggested that ingested sand acts as a grinding paste for the degradation of plant cell walls in the pyloric portion of the stomach (Thomson, 1966).

The change of food from animal to plant material is not sudden but gradual. Copepoda and Cladocera appear to be the most important components in the diet of mullet up to 30 mm, with insect larvae, algae and detritus also consumed. Over 60 mm total length, the stomach contents include Polychaeta, Cladocera, Insecta, *Sagitta*, detritus, blue-green algae, diatoms and a small amount of sand (Anon, 1976).

The Caspian Sea's grey mullet (*L. aurata* and *L. saliens*) were introduced from the Black Sea to the Caspian Sea between 1930 to 1934 (Nikolskii, 1961). The suitable environment has enabled the population to increase in numbers rapidly. Nowadays they provide one of the pricipal fishing resources, especially in the southern part of the Caspian Sea.

The aim of present study was to determine any difference between the diet of juvenile and adult grey mullet in the southern Caspian sea and to observe any differences between the diets of *L. aurata* and *L. saliens* in this region. The number of pyloric caeca were also recorded.

MATERIALS AND METHODS

Specimens

Specimens of *Liza aurata* and *Liza saliens* were collected during 1993-1995 by traditional beach seine from four fishing areas with 91 sample sites along the Iranian side of the Caspian Sea;Anzali, Kiashahr, Babolsar, and Bandar Torkman. A subsample (n= 1280, 10% of the total sample) were examind and were comprised of 713 L. aurata and 567 L. saliens. The age of the specimens were estimated using scales.

Methods

The stomach contents were obtained after killing the fish. Stomachs were removed, the number of puloric caeca for each specimen was counted and the stomach contents were weighed and preserved in 4% formalin for subsequant identification. Prey items were identified to as low a toxonomic level as possible.

The diet was determined by two mwthods: (a) frequency of occurrence, and b) percent composition by number. The frequency of occurrence (F) of prey items was calculated by the following equation: F (%) = number of fish which food item occurred × 100 / total number of fish

Food items were quantified by Numerical percentage (N) using the following equation:

$$N\ (\%) = \text{number of organism per fish} \times 100 / \text{total number of organisms}$$

Both diet analysis were calculated for different length classes for both species.

RESULTS

Number of pyloric caeca

The results show that the number of pyloric caeca in both species varied. In *Liza aurata* the range varied from 6-10, although most of the fish had 8 pyloric caeca (Table 1).

Table 1: Frequency of specific number of pyloric caeca in *Liza aurata* in different fishing areas along the Iranian side of the Caspian Sea

Area	1	2	3	4
No. of pyloric cecae	Anzali	Kiashahr	Babolsar	Bandar Torkman
6	3	1	3	0
7	43	12	22	29
8	131	75	99	143
9	41	21	26	53
10	2	1	2	6

In *Liza saliens*, the range of number of pyloric caeca was between 6-9, although most of the fish had 7 (Table 2).

Table 2: Frequency of specific number of pyloric cecae in *Liza saliens* in different fishing areas along the Iranian side of the Caspian Sea

Area	1	2	3	4
No. of pyloric cecae	Anzali	Kiashahr	Babolsar	Bandar Torkman
6	10	8	13	9
7	95	59	63	172
8	29	18	31	51
9	-	2	3	4

Frequency of occurrence (F value in %)

In all four fishing areas, the major components of the stomach contents of *Liza aurata* and *Liza saliens* were fine sand and detritus. However, there were differences relating to the age: while adult fish had a considerably higher amount of fine sand and detritus juvenile fish had a very low amount and in many cases none.

Results of frequency of occurrence show that for *Liza aurata*, in all fishing areas and in length class 10.1 to 20 cm, all organisms were present. Calanoida were the only organisms that were present in all the length classes. The sum of the percentage of occurrence of organisms in all length classes is given in Table 3. As can be seen in this table, Foraminifera had the maximum percentage of occurrence whereas Ostracoda and Nematoda had the minimum.

Table 3: Frequency of occurrence (%) of different organisms in the stomach content of *Liza aurata* in different length classes in all four fishing areas.

Organisms	Bivalvia	Foramini-fera	Ostracoda	Nematoda	Calanoida	Cyclopoida	Eggs*	*Nereis*	Number of fish
length (cm)									
0-10	-	-	50	-	100	50	-	-	2
10.1-20	41	29	18	6	35	12	6	18	17
20.1-30	50	41	-	-	38	-	9	19	248
30.1-40	42	57	-	-	34	15	-	23	233
40.1-50	33	23	7	17	13	-	30	47	30
F value(%)	45	46	1	1	35	7	6	22	Total 530

* These eggs belong to the calanoida and other organisms but not to the fish.

Results of frequency of occurrence show that for *Liza saliens*, in all fishing areas and in most length groups, Bivalvia and Foraminifera were present. The sum of the percentage of occurrence of

organisms in all length classes is given in Table 4. As can be seen in this table, Bivalvia and Foraminifera had the maximum percentage of occurrence whereas Nematoda had the minimum.

Table 4: Frequency of occurrence (%) of different organisms in the stomach content of *Liza saliens* in different length classes in all four fishing areas.

Organisms	Bivalvia	Foramini-fera	Ostracoda	Nematoda	Calanoida	Cyclopoida	Eggs	*Nereis*	Number of fish
length (cm)									
0-10	-	-	43	-	87	38	-	-	60
10.1-20	71	64	16	7	56	31	-	-	45
20.1-30	65	59	-	-	-	-	20	34	158
30.1-40	83	60	-	23	-	-	28	68	40
F value(%)	55	49	11	4	25	12	14	26	Total 303

In general the results illustrate that Bivalvia, Foraminifera, Calanoida, and *Nereis* had high percentage of occurrence in both *Liza aurata* and *Liza saliens*. On the other hand, Ostracoda, Nematoda, Cyclopoida, had low percentage of occurrence. However, the percentage of occurrence of Ostracoda, Nematoda and eggs in *Liza saliens* were higher than in *Liza aurata* (Table 5).

Table 5: Frequency of occurrence (%) of different organisms in the stomach content of *Liza aurata* and *Liza saliens* along the Iranian side of the Caspian Sea.

Organisms	Bivalvia	Foramini-fera	Ostracoda	Nematoda	Calanoida	Cyclopoida	Eggs	Nereis	Number of fish
Species									
F value(%) *L. aurata*	45	46	1	1	35	7	6	22	530
F value(%) *L. saliens*	55	49	11	4	25	12	14	26	303

Numerical percentage (N value in %)

The results showed that from a total of 57992 organisms which were counted in the stomach contents of *Liza aurata* more than half were Bivalvia and about one third were Calanoida in all four fishing areas (Table 6).

Table 6: Numerical percentage (%) of different organisms in the stomach content of *Liza aurata* in different length classes in all fishing areas.

Organisms	Bivalvia	Foramini-fera	Ostracoda	Nematoda	Calanoida	Cyclopoida	Eggs	*Nereis*	Total Number of
FL (cm)									organisms
0-10	-	-	67	-	309	27	-	-	403
10.1-20	1514	511	146	15	4468	55	72	14	6795
20.1-30	15346	2301	-	-	3247	-	112	71	21077
30.1-40	13723	2732	-	-	2534	76	-	220	19285
40.1-50	400	623	42	43	2599	-	991	98	4796
N value(%)	53	11	0.4	0.1	23	0.3	2	0.7	57992

Results show that from a total of 33403 organisms which were counted in the stomach contents of *Liza saliens*, Bivalvia and Calanoida were the most important prey whereas Nematoda and *Nereis* had the minimum (Table 7).

Table 7: Numerical percentage (%) of different organisms in the stomach content of *Liza saliens* in different length classes in all fishing areas.

Organisms	Bivalvia	Foramini-fera	Ostracoda	Nematoda	Calanoida	Cyclopoida	Eggs	*Nereis*	Total Number of
FL (cm)									organisms
0-10	-	-	1239	-	6985	1190	-	-	9414
10.1-20	2647	231	119	144	3669	190	-	-	7000
20.1-30	11353	969	-	-	-	-	120	110	12552
30.1-40	2648	371	-	191	-	-	874	353	4437
N value(%)	50	5	5	1	32	4	3	1	33403

The results show that the consumption of Bivalvia was similar in *Liza aurata* and *Liza saliens*. However, the consumption of Foraminifera by *Liza aurata* was higher than by *Liza saliens* and the consumption of Calanoida by *Liza saliens* was higher than by *Liza aurata*. Consumption of Ostracoda, Nematoda, Cyclopoida, eggs and *Nereis* by *Liza saliens* was higher than by *Liza aurata* (Table 8).

Table 8: Numerical percentage (%) of different organisms in the stomach content of *Liza aurata* and *Liza saliens* along the Iranian side of the Caspian Sea.

Organisms	Bivalvia	Foramini-fera	Ostracoda	Nematoda	Calanoida	Cyclopoida	Eggs	*Nereis*	Total Number of
Species									organisms
N value(%) *L. aurata*	53	11	0.4	0.1	23	0.3	2	0.7	57992
N value(%) *L. saliens*	50	5	5	1	32	4	3	1	33403

DISCUSSION

Number of Pyloric caeca

The comparison between the results of the present study and those of previous studies with respect to the number of pyloric caeca shows that there are great variation between them. The range start varies from 3-5 to 8-9.

Some authors (Gunther, 1861 and Nikolskii, 1961) have reported that there is no intraspecific variation in the number of pyloric caeca. However, other authors, such as Perlmutter (1957), Trewavas (1972), and Oren (1981), have observed that there such variation in the number of pyloric caeca does occur.

With respect to *Liza aurata* the present results are in agreement with those of Oren (1981), and partially in agreement with those of Gunther (1861), Perlmutter (1957), and Nikolskii (1961). With respect to *Liza saliens* the present results are in agreement with Oren (1981) about the variation of the number of the pyloric caeca within the species. However, the present study does not support the most frequent number of pyloric caeca which is 8. Moreover, the present study with respect to *Liza saliens* is not in agreement with other previous studies. In the present study, all the sampled fish (*Liza saliens*) had 3 elongated pyloric caeca which was used to identify this species from *Liza aurata*. In *Liza aurata* all pyloric caeca were in the same length. The comparison of the results of present and previous studies are given in Table 9.

Table 9: Comparison between the number of pyloric cecae of grey mullet in the present and previous studies.

Studies	Gunther (1861)	Perlmutter (1957)	Nikolskii (1961)	Trewavas (1972)	Oren (1981)	Present (1996)
Fish species						
Liza aurata	8	8-9	8	-	6-11 mostly 8	6-10 mostly 8
Liza saliens	8	3-5	8	3-5	6-9 mostly 8	6-9 mostly 7

Frequency of occurrence (F value in %)

The stomach content of all the adult fish *Liza aurata* and *Liza saliens* in all the fishing areas are predominantly full of fine sand particles, plant and animal origin detritus. However, beside the sand and detritus, considerable number of organisms were taken in by the fish.

The predominant prey item which occurred in all the fishing areas and in the largest number of the fish stomach contents *Liza aurata* and *Liza saliens* was bivalvia. The next predominant prey item which occurred in all the fishing areas and in the largest number of the fish stomach contents *Liza aurata* and *Liza saliens* was Foraminifera. Another predominant prey item which occurred in all the fishing areas and in the considerable number of the fish stomach contents *Liza aurata* and *Liza saliens* was Calanoida. The other food items have not occurred in very many fish stomach content.

The results do show that grey mullet species which live in the Caspian Sea somehow select the food items. On the lower length class (juvenile class) Ostracoda, Calanoida, and cyclopoida seems to appear more than other organisms. However, On the adult fish classes of both species, Bivalvia, Foraminifera, and *Nereis* seems to occur more than other species.

Numerical percentage (N value %)

The results of frequency of occurrence alone can not be based for observing the exact value of food items of fish then further analysis of numerical percentage of food items was done to achieve somehow an accurate estimation of the value of food items in the diet of Caspian Sea grey mullet species.

The predominant prey item which had the highest number in all the fishing areas and in the largest number of the fish stomach contents *Liza aurata* and *Liza saliens* was bivalvia. The next predominant prey item which had the higher number than other organisms in all the fishing areas and in the large number of the fish stomach contents *Liza aurata* and *Liza saliens* was Calanoida. Another predominant prey item which had quite a lot of number in all the fishing areas and in the considerable number of the fish stomach contents *Liza aurata* and *Liza saliens* was Foraminifera. The other food items have not had considerable number in very many fish stomach contents.

CONCLUSIONS

1- The number of pyloric cecae varies between and within *Liza aurata* and *Liza saliens* in the southern part of the Caspian Sea. In *Liza aurata* it varies from 6 to 10 (mostly 8) and in *Liza saliens* it varies from 6 to 9 (mostly 7).

2- Large amounts of sand particles and detritus were observed in the stomach contents of all fish.

3- Using the frequency of occurrence method, the predominant food items for both mullet species were Bivalvia, Foraminifera and Calanoida

4- According to numerical percentage method the predominant food items for both mullet species were Bivalvia, Calanoida, and Foraminifera .

5- Grey mullet species of the southern part of the Caspian Sea seems to select the food items. Juveniles prefer to consume more Ostracoda, Calanoida, and Cyclopoida. Adults prefer to feed more on Bivalvia, Foraminifera, and *Nereis*.

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GROWTH STUDIES ON RABBITFISH *SIGANUS CANALICULATUS* PARK (F:SIGANIDAE) IN THE ARABIAN GULF NEAR DAMMAM, SAUDI ARABIA.

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ABSTRACT

Age was determined using both scale-reading and length frequency distribution methods.Growth rates in length and weight are estimated.von Bertalanffy's growth equation was also fitted and their parameters are evaluated.Three formulae are derived to represent weight/length relationship for males, females and sexes combined.Condition factor values are also computed which showed monthly variations that are mainly linked with feeding intensity and breeding cycle.

INTRODUCTION

Siganids, herbivorous fishes associated with coral reefs, are commercially important in the Arabian Gulf region (Al Ghais, 1993).Three Siganus species are recorded along the Saudi coasts (Abdulhady, 1995), of which whitespotted *S.canaliculatus* is the most abundant and popular food fish.Besides, there has been increasing interest in siganids culture in the area, as well as in other areas of the world (Nelson *et al.*, 1992).The present study descibes the growth rates of *S.canaliculatus* with the aim of management of their fishery and development of their culture.

RESULTS AND DISCUSSION

1. Growth in length :

1.1.Scales Interpretation Method

Age was determined by examining minute, cycliod embeded scales from the tail-peduncle region (Hussein, 1986). The regression equation: $L = 2.7886 + 22.1006\ S$ ($n=440$, $r^2=0.995$) was obtained to represent the relation between anterior scale radius (S,mm) and total fish length (L, cm).Back calculated lengths at the end of each year of life, annual increment and specific growth rate are calculated, firstly for separate sexes. Sex variations are tested (ANOVA) to be insinificant ($p<0.05$), then all data were pooled (Table 1).Maximum annual increment (11.3 cm) was attaind by the end of first year of life, then tend to decrease with age.

Table (1): Comparison of calculated length for sexes combined at different years of life from two methods for age determination with the theoretical lengths.

Age	L. frequency method			Scale method			von Bertalanffy's method		
(y)	L^	Increment	% increase	L^	Incr.	% increase	Lt	Incr.	% increase
0	-	-	-	11.30	11.30	34.24	11.18	11.18	33.98
1	15.7	-	0.476	16.23	4.93	14.94	16.14	4.96	15.08
2	19.87	4.17	0.126	20.11	3.88	11.76	20.19	4.05	12.31
3	24.35	4.48	0.136	23.75	3.64	11.03	23.5	3.31	10.06
4	27.0	2.65	0.08	26.54	2.79	8.45	26.21	2.71	8.24
5	29.53	2.53	0.077	28.72	2.18	6.61	28.42	2.21	6.72

von Bertalanffy growth papameters for *S.canaliculatus* proved to be : L_∞ = 38.48 cm ; K = 0.2014 ; t_o= -0.74 yr for males & L_∞ = 38.22 cm ; K = 0.2064 ; t_o = -0.71 yr for females. The von Bertalanffy growth equation was used to evaluate the hypothetical lengths of fish at age as compared with the back-calculated lengths (Table 1).

1.2.Length/Frequency distribution Analysis

By applying a modified probability method of Cassie (1954 & 1963), Fig. (1) had resulted which shows 6 age classes on length/frequency distibution graph (n=2024,df=32).The only females two oldest age groups are discarded.The agreement between the mean lengths of age groups and the corresponding back-calculated is confirmed (Table 1). The validity of scales for ageing such tropical species can be assessed for the first time. Growth rate of the present work, is relatively slower than that recorded by Al Ghais (1993) for the same species, but based on vertebral circuli.

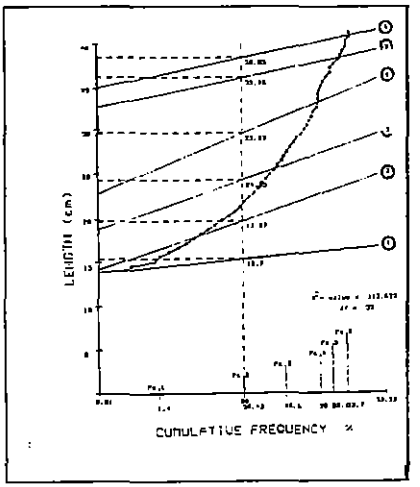

Fig. (1). Probability paper analysis of length frequency distribution of 2024 *S. canaliculatus*. Ringed Nos. = age classes.

2.Weight/Length relationship

The following equations are derived for *S.canaliculatus* using total length (L, cm) and weight (W, g):

for males $W = 0.03496\ L^{2.7242}$ (1)

(n=1056, r=0.9984)

for females $W = 0.0311\ L^{2.7751}$ (2)

(n=968 , r=0.9982)

Sex variations are tested (ANOVA) to be insignificant ($p<0.05$), accordingly,

for both $W = 0.0343\ L^{2.7352}$ (3)

(n=2024, r=0.9979)

3.Condition Factor (K)

Variations of mean K values with fish length (Fig 2) shows a decreasing trend with the increase in size.Mean K, for both sexes = 1.48 (Fulton's);1.2 (Clark's) & 0.99 (relative condition K_n). Comparatively lower values are recorded in June (postspawning), particularly for females, and in September due to the decreased feeding intensity (Fig 3).

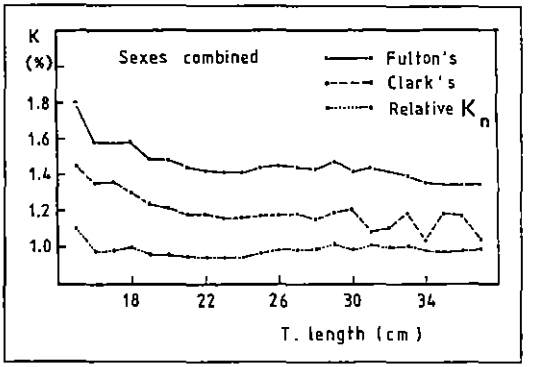

Fig. (2). Variations of conditon factor with fish length for *S. canaliculatus*.

4.Growth in weight

Having the weight/length formulae 1,2 & 3, it is possible to evaluate average calculated weights corresonding to average back-calculated lengths in Table (1). Annual increment and specific growth rate are also computed (Table 2).The highest weight increment (71 g) as well as % increase (14.6%) were attained in the third year of life. However, fish can reach a marketable size (=126 g) at the end of their second year of life. W_∞ was computed to be 727.9 for males and 765.2 g for females.

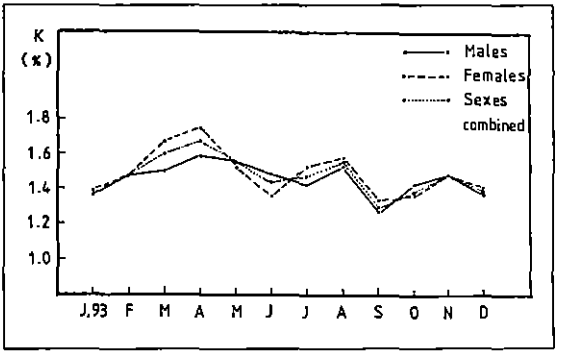

Fig. (3). Monthly variations of condition factor for *S. canaliculatus*.

Table (2): Percentage and relative increase in weight with age for *S. canaliculatus* in the Arabian Gulf

Age (y)	Males				Females				Sexes combined			
	Calc. wt (g)	Increment (g)	% increase*	Relative** increase (%)	Calc. wt (g)	Increment (g)	% increase	Relative increase (%)	Calc. wt (g)	Increment (g)	% increase	Relative increase(%)
0	25.90	25.90	5.41	100.00	25.84	25.84	3.00	100.00	26.08	26.08	5.33	100
1	69.00	43.10	9.00	166.41	70.95	45.11	5.23	174.57	70.08	44.00	9.00	168.71
2	124.68	55.68	11.62	80.70	127.71	56.76	6.58	80.0	125.87	55.79	11.40	79.61
3	193.84	69.16	14.43	55.47	203.39	75.70	8.78	59.27	197.01	71.14	14.55	56.52
4	261.14	67.56	14.10	34.85	278.79	75.40	8.75	37.07	267.90	70.89	14.50	35.98
5	326.75	65.35	13.64	25.02	347.99	69.20	8.03	24.82	333.93	66.03	13.51	24.65

* In relation to the calculated weight at the last year of life.

** In relation to the weight attained for the preceding year.

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Nutrition

LARGE EYES AS INDICATORS OF REDUCED GROWTH.

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Introduction

Lundberg (1875) noted in an investigation of the herring (*Clupea harengus)* in the Baltic Archipelago, that small herring had relatively large eyes. Generally the relative eye diameter of herring in the Baltic Sea seem to increase from the south to the north, while the mean length and growth rate seem to decrease (Parmanne, 1990).
Other examples of a relation between location and eye size are capelin (*Mallotus villosus*) from different locations in Canadian Atlantic waters (Sharp *et al.*, 1978), Indian carp (*Label dero*) in three rivers with different ecological conditions (Nasar *et al.*, 1983), *Coregonus peled* introduced in two Mongolian lakes (Dulmaa & Penaz, 1986) and charr (*Salvelinus leucomaenis*) in a Japanese river and a lake (Takami & Kinoshita, 1990).
In some cases have the morphological differences, including eye size, within a species, been so characteristic that the species has been separated into two forms. This have been shown for two forms of Alaska pollack (*Theragra chalcogramma*) (Kim & Huh, 1978), two types of Japanese jack mackerel (*Trachurus japonicus*) (Suda *et al.* 1987), and two forms of the salmon, *Oncorhyncus rhodurus* called the amago salmon and the biwa salmon (Fujioka, 1988).

Pankhurst (1989) found evidence among diurnal fish that species of small size generally develops large eyes. He also found that individuals of the New Zealand sweep (*Scorpis lineolatus*) and spotty (*Notolabrus celidotus)* subjected to a prolonged period of darkness in a tank, showed proportionally larger eyes than normal fish of similar body size. The large eye size could be an adaption to the low light intensity in the tank, but it could also be an effect of a reduced growth rate due to low prey density or low prey visibility (Pankhurst, 1992).

These studies all demonstrated differences in eye size, but only the studies by Kim & Huh (1978) and Pankhurst (1992), seem to relate the different eye size to differences in growth rate.

Further evidence that eye size can be dependent upon growth rate was provided by Pankhurst & Montgomery (1994). They performed a study of relative eye diameter and growth of rainbow trout *Oncorhynchus mykiss* in a controlled feeding experiment, and in two naturally occurring populations. Their results clearly demonstrated that slow growing fish can develop relatively larger eyes than normally growing fish. Based on their data it can be calculated that the eye diameter in the slow growing (low feed) group was approx. 20% larger than for fish of the same length in the fast growing (high feed) group.

It should therefore be investigated whether differences in eye size generally are reflections of differences in growth rate

The present study presents another example of a possible linkage between eye size and some indicators of reduced growth rate in the Africa fresh water sardine, *Limnothrissa miodon* in Lake Kariba, Zimbabwe.
L. miodon is naturally occurring in Lake Tanganyika and introduced into the artificial Lake Kariba, where it grows to only about half its normal length in Lake Tanganyika. Because of difficulties in age determinations using otolith readings (Chifamba, 1992) or cohort modal progression analysis, it is still unresolved whether the small size is due to a high mortality e.g. after spawning, or a cessation of growth. The difference in size was observed before a fishery began, and is therefore probably a function of the large differences between the two lakes. These differences and some possible explanations for the smaller size are described by Marshall (1993).

During a study of the feeding biology of *L. miodon* in Lake Kariba (Paulsen, 1994) it was accidentally observed that some individuals had remarkably larger eyes than others. In the absence of an established method to perform a direct age (and thereby growth rate) determination it was decided to try to relate the eye size to two known indicators of reduced growth, otolith size and mesenteric fat content.
Several authors have recently demonstrated a relation between otolith size and growth rate. Otolith growth seem to be influenced by an age dependent component and an growth dependent component. Relatively large otoliths indicates a reduced growth rate (Secor and Dean, 1989).
Mesenteric fat content has been widely used, also in clupeids (Rajasilta, 1992), as an indicator of nutritional status. Long term differences in mesenteric fat content is assumed to affect growth rate. Short term fluctuations, as a result of e.g. variations in food availability or gonad formation, could temporarily impair the link to growth rate and thereby to otolith and eye size.

Material and Methods

L. miodon were sampled during nighttime from a commercial fishing boat using a 9m diameter circular lift-net and mercury lamps for light attraction of fish. During daytime *L. miodon* was sampled using a mid-water trawl (8m opening) from a research vessel. Fishing was carried out at depths from 0 - 30m. Samples were taken both at near-shore and at open-water locations on the lake. Informations from the commercial fishery indicates that there is no systematic variation between locations. The samples are therefore assumed to be representative for the population of *L. miodon*. In the period June - August 1992 400 *L. miodon* were sampled from a total of 48 lifts and 10 trawl hauls. The total body length, sex, eye diameter and fat index were registered for each individual. In July 1993 further 64 individuals were sampled from a total of 8 trawl hauls, and measurement of otolith size included in the analysis. The fish were immediately frozen (1992) or short term preserved in 4% formalin. The preservations had no effect on total body length.

The parameters were registered as follows:

Length. Measured as "total length" to the nearest mm below.
Eye diameter. The mean maximum diameter of both eyes measured across the eye cavity, within intervals of 0.1 mm, using a microscope eyepiece ruler.
Otolith diameter. The mean maximum diameter of both sagitta otoliths, measured within intervals of 0.01 mm, using a microscope eyepiece ruler.
Mesenteric fat. Expressed as a five step index for the content of fat around the interior organs. 0= No fat. 1= A thin string of fat along intestine. 2= Wider, removable string of fat along intestine, small amount of fat around stomach. 3= Fat around stomach removable in one piece. 4= All organs covered by fat.

The normal eye size and otolith size at different length were calculated from linear 1. order regressions. In order to identify deviations from the normal size, the "residual eye diameter" and

"residual otolith diameter" were calculated for each individual as the residual of the observed diameter to the value predicted by the regression model.

Statistical analysis. Regressions were performed as ordinary 1. order least-squares regressions. Regression lines were tested for linearity, slope different from zero, and differences between regression lines according to the methods described in Fowler & Cohen (1992). 95% confidence limits on the figures are calculated using the "SigmaPlot®" software (Jandel Scientific).
As fat content and gonad development stage were registered on relative scales, a statistical test suitable for observations on an ordinal scale had to be used. The relation between eye diameter (otolith diameter) and fat content was analysed using "Jonckheeres test for ordinal observations" (Siegel & Castellan, 1988). This test is basically an extension of the Kruskall-Wallis test to include the extra informations of an ordered scale.

Results.

Fig. 1 shows the relation between eye diameter and total body length for the 1992 and 1993 samples. The lines do not deviate significantly from a straight line. As a deviation primarily would affect the largest and the smallest individuals and 90% of the observations are in the length range 55-77mm, it was decided to accept the regression line for calculation of normal eye size.
When comparing fish of the same length there seem to be a considerable variation in eye diameter. The eye diameter ranged from 74% to 145% of the diameter predicted by the regression model for the 400 individuals sampled in 1992. The length to eye diameter relationship differed significantly ($p<0.05$) between the two sampling years. (1992: $y= -0.179 + 0.0716x$, $r^2= 0.52$, N=400, 1993: $y= -0.854 + 0.0882x$, $r^2= 0.58$, N= 64).
Fig. 2 shows the relation between otolith diameter and total body length for the 1993 samples. Proportionality between fish length and otolith size is generally used when calculating growth rate dependent deviations in otolith size (Campana, 1990). In this study the diameter ranged from 84% to 112% of the diameter predicted by the otolith diameter - body length regression model. ($y= 0.118 + 0.0245x$, $r^2= 0.71$, N= 64).

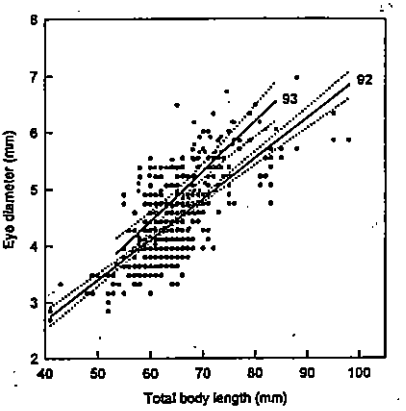

Figure 1. Total body length - eye diameter relation for *L. miodon*. 1992 (o) and 1993 (•). Regression lines with 95% confidence limits.

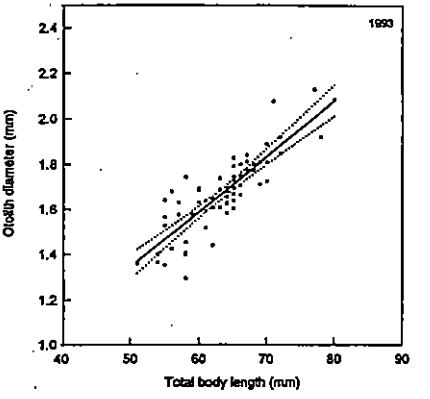

Figure 2. Total body length - otolith diameter relation for *L. miodon* 1993. Regression line with 95% confidence limits.

To investigate whether individuals with relatively large eyes also had relatively large otoliths, the two parameters were plotted against each other (Fig. 3). A regression line was calculated . The regression line is tested for linearity and the slope is significantly ($p<0.05$) different from zero indicating a covariation of the deviations from the predicted diameters of both otoliths and eyes. Both parameters are functions of fish length and a low precision in length measurements would in it self generate a covariation. The observed deviations from predicted diameter are, however, far larger than could be explained by inaccuracies in length measurements.

Fig. 4 and 5 show the relation between fat index and eye size, expressed as % of the predicted values from the regression model for the 1992 and 1993 datasets. The mean values are shown with number of observations and standard deviation.

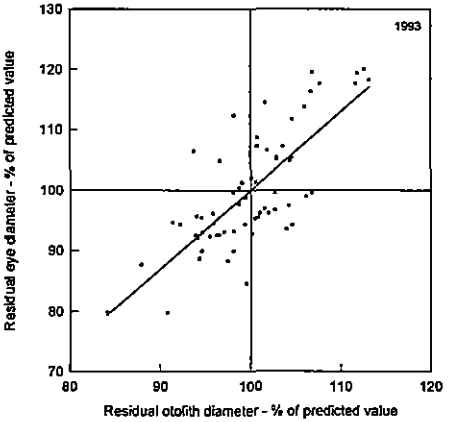

Figure 3. Relative otolith diameter - relative eye diameter for *L. miodon* 1993. Regression line with 95% confidence limits ($y= -29.840 + 1.298x$, $r^2= 0.58$, $N= 64$).

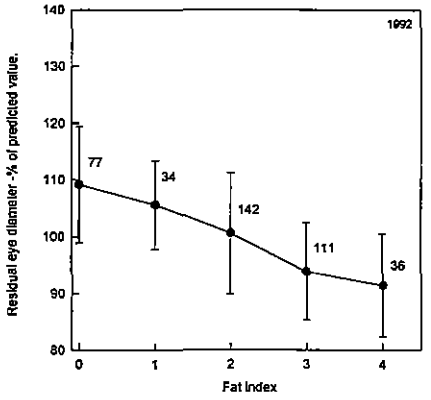

Figure 4. Fat index - eye diameter as % of predicted from the regression model for *L. miodon* 1992. Mean values ± SD. Number of individuals in the fat index groups are indicated (N= 400).

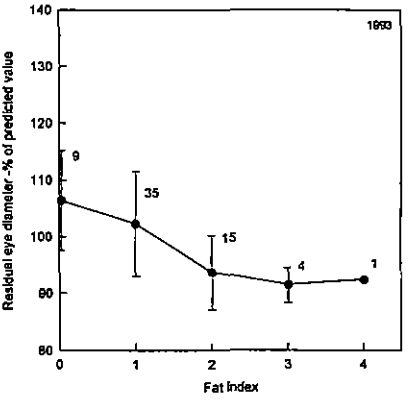

Figure 5. Fat index - eye diameter as % or predicted from the regression model for *L. miodon* 1993. Mean values ± SD. Number of individuals in the fat index groups are indicated (N= 64).

The relationship between eye diameter and fat index is significant ($p<0.05$) using Jonckheeres test for ordered data. Both for 1992 and 1993 the eye diameter residuals decreased from approx. 110% of the predicted diameter in individuals with fat index 0 to approx. 90% of the predicted in individuals with fat index 3 and 4. If the effect of fat index is included in the regression model it increases the correlation coefficient (r^2) in the eye diameter - body length regression (Fig. 1) from

0.52 to 0.66 in 1992 and from 0.58 to 0.71 in 1993. Comparison of the 1992 and 1993 dataset shows that 146 of 400 individuals (37%) in 1992, and only 5 out of 64 individuals (8%) in 1993, had a fat index of 3 or 4. The higher fat index in 1992 is therefore in agreement with the observation of generally smaller eyes in the 1992 dataset (Figure 1).

Fig. 6 show the relation between fat index and otolith diameter, expressed as % of the predicted values from the regression model for the 1993 dataset. The mean values are shown with number of observations and standard deviation.
The decrease in eye diameter with increasing mesenteric fat content is significant ($p<0.05$).

Discussion

The eye diameter of *L. miodon* in Lake Kariba was observed to vary from 74% to 145% of the normal diameter for fish of the same length in a sample of 400 fish. This variation is equal to a variation in the area of the eye of almost four times. Is this variation just "random variation" or is it related to some specific life conditions ? Other studies have shown that eye size may vary with location, and a recent study (Pankhurst and Mongomery, 1994) has demonstrated a direct relation to fish growth rate. This situation seem to be comparable to the observations of a variation in otolith size, and the now well documented relation between otolith size and growth rate. The observations in this study of a covariation between relative otolith size and relative eye size suggests that individuals with large otoliths or large eyes could be particularly slow growing individuals.

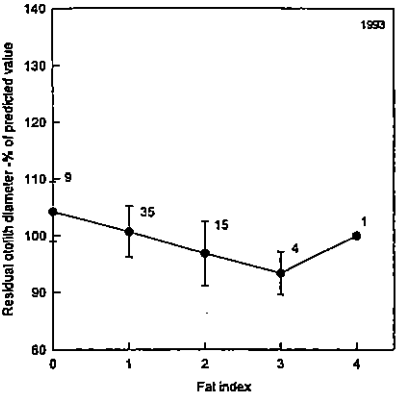

Figure 6. Fat index - otolith diameter as % of predicted from the regression model for *L. miodon* 1993. Mean values ± SD. Number of individuals in the fat index groups are indicated (N= 64).

Registration of mesenteric fat content also showed a clear relation to relative eye size. Individuals with low fat content had significantly larger eyes than individuals with high fat content. Also the fish in the 1992 samples had significantly less fat than the fish in the 1993 samples, and significantly larger eyes.
This observation supports the hypothesis of a linkage to growth. Other explanations are, however, also possible. Sex differences and gonad development stage were also registered in connection with this study, without any indication of a relation to eye size or mesenteric fat content. A relation was found between mesenteric fat content and otolith size with low fat individuals having larger otoliths than high fat individuals, supporting the view that fat content is related to growth rate.
If it is assumed that the eye size in *L. miodon* is related to growth rate on could consider the biological implications of this finding. A visual inspection of the large eyed individuals reveals that it is only the eyes, and not the whole head which is particularly large. This is in contrast with the situation eg. in old gadoids which develops large head in relation to body size. The large eyes could be an expression of a high priority of an organ, vital for a selective plankton feeding fish as *L. miodon*, which has been shown to be feeding primarily during dawn and dusk (Paulsen, 1994).

The present study provides evidence of a linkage between eye size, otolith size and mesenteric fat content, which all, independently, has been shown to be related to growth rate. In the absence of a

link to variations in growth rate. One could therefore ask whether there is a specific reason to expect a high variation in growth rate ?
The catches of *L. miodon* in Lake Kariba generally consists of individuals of a very narrow size range. This length distribution does not seem to change much between years, over the year, between locations or between different fishing gears. A possible explanation for the apparent accumulation of 50-70 mm fish could be that fish in this size range more or less stops growing, leading to individuals of the same length with different age and nutritional condition.
The Lake Kariba population of *L. miodon* offers a unique opportunity to study a naturally growth stunted population, and morphological expressions of a reduced growth rate may be easier to find here than in most other places. The findings in other studies, of a relation between eye size or otolith size and differences in physical conditions, ecological conditions, between areas or between different forms of the same species may therefore, at least partly, be explained by differences in growth rate.

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THE ROLE OF ADIPOSITY IN APPETITE CONTROL IN JUVENILE CHINOOK SALMON (*Oncorhynchus tshawytscha*)

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Abstract

Two sequential experiments were conducted to determine if adiposity affected feed intake in juvenile chinook salmon (*Oncorhynchus tshawytscha*) with different nutritional histories. Fry were fed high fat (23%) or low fat (3%) diets at high (satiation) and low (one half satiation) ration levels for 7 months prior to the start of the intake experiment. This pre treatment produced fish averaging 22 g with 11.3% (high fat diet) and 5.4% (low fat diet) body fat when fed to satiation or, 11 g with 8.1% (high fat diet) and 4.0% (low fat diet) body fat when fed at one-half satiation. Exp. 1 was a 2 x 2 factorial design where duplicate groups of 20 fish from the 22 g groups were fed high (20.3%) or low (2.5%) fat diets twice daily to satiation six days/week for 3 weeks. Daily feed intake was recorded. The same protocol was used in Exp. 2 on fish (40 fish/tank) from the smaller 11 g groups. Feed intakes on day one and cumulative feed intakes after 21 days were compared using Two-Way ANOVA with initial whole body fat and dietary fat as the independent variables. Effects were considered significant at $P<0.05$. In both experiments, high body fat and low dietary fat led to significantly lower feed intake on day one. After 21 days of feeding however, only the effect of high body fat was significant, indicating that fish adjusted to the low fat diets. Our results show that in both fast and slow growing juvenile chinook salmon, adiposity plays a role in regulation of feed intake.

Introduction

Appetite in fish appears to be under multifactorial control (Peter, 1979; Vahl, 1979; Fletcher, 1984). Although the physiological control of appetite is not well understood, regulators such as stomach distention and concentration of circulating nutrients have been identified (NRC, 1987) Recently, studies designed to examine the role of adiposity (body fat level) on regulation of appetite in fish have shown a negative correlation between adiposity and voluntary food intake (Metcalfe and Thorpe, 1992; Jobling and Miglavs, 1993). These studies have used starvation to produce fish differing in adiposity, thus confounding the roles of adiposity and starvation induced compensatory growth on feed intake. Our experiment was designed to determine if the level of adiposity affected feed intake in juvenile chinook salmon (*Oncorhynchus tshawytscha*) not subjected to starvation. Additionally, the effect of dietary fat level on food intake was examined.

Materials and Methods

Pretreatment

From first feeding, groups of 400 fish were reared in 750 l tanks supplied with flow through de-chlorinated municipal water. The fish were reared on natural photoperiod; water temperatures varied seasonally from 8 to 16 °C. Fish were fed diets containing 23% fat and 65% protein (high fat) or 3% fat and 85% protein (low fat) diets. Two groups were pair fed the high and low fat diets to satiation (based on intake of the group eating the least feed) and two other groups of fish were fed these diets at half this amount. This feeding regime produced four groups of fish: large fish (22 g) with 11.3±2.1% or 5.4±1.8% body fat; and small fish (11g), with 7.0±1.2% or 3.3±0.2% body fat. This phase of the experiment lasted 7 months.

Experiment 1

To test the effects of adiposity and dietary fat on food intake, we employed a 2 X 2 factorial design crossing the body fat level treatment (high or low body fat) with dietary fat level treatment (high or low) to produce four groups. Each treatment combination was replicated. In Exp. 1 we used fish with a mean weight of 22 g at the start of the experiment. The fish were moved from the flow through water system to a recirculating system one day prior to the start of the experiment. This resulted in the fish being exposed to a decrease in water temperature (13 to 11°C) and an increase in pH (6.5 to 7.8). The fish were fed twice daily, 9 AM and 1 PM, 6 d/w for 21 d. Pellets were presented a few at a time as long as fish continued to feed. After feeding ceased, uneaten pellets were removed from the tank using a siphon. These were counted and the weight of this feed was subtracted from the feed fed based on an average prefeeding pellet weight. Daily feed intake was recorded.

Experiment 2

The protocol for experiment 2 was the same as for experiment 1, except small high and low fat fish (11 g) were used. These fish had been acclimated to the recirculation system for 30 days prior to the start of the experiment. Water quality remained stable (11 °C and pH 7.0).

The diets fed in the feed intake study (Table 1) were high or low in lipids (16 and 4%) and the low lipid diet contained 12% alpha cellulose to produce diets containing equal amounts of protein (65%).

Table 1. Composition of the experimental diets fed to juvenile chinook salmon to examine the effect of body fat and dietary fat on short term feed intake.

Ingredient	Low fat	High fat
	% dry weight	
Fish meal[1]	628	628
Gelatin	100	100
Wheat gluten	50	50
Fish oil	40	160
Vitamin C	1	1
Choline Cloride	10	10
CaH_2PO_4	10	10
Trace minerals	1	1
Vitamin mix	15	15
MgO	5	5
Alpha-cellulose	120	0

[1] Fish muscle meal, 95% protein, 1% fat, 4% ash.

Analysis

Initial fat determinations on fish were performed on 10 individual fish from each of the four pretreatment groups. Final fat determinations were performed on pooled samples of five fish from each tank. Fish were dried to constant weight at 105 °C, ground and extracted using a

Soxhlet extractor with dimethychloride as the solvent. Feed intakes on day one and cumulative feed intakes after 21 days were compared using Two-Way ANOVA (Statview™, Abacus Concepts, Berkeley, CA, 1992) with initial whole body fat (high or low) and dietary fat (high or low) as the independent variables. Effects were considered significant at $P<0.05$.

Results

Experiment 1

Feed consumption in experiment 1 was lower than expected based on normal intake observed for juvenile chinook salmon in previous experiments using flow-through municipal water. This was attributed to the fish being subjected to a change in water quality. No mortality occurred during the experiment. Comparison of feed intakes on day 1 (Table 2) indicated that feed intake was significantly influenced by fish body fat. Although the effect of dietary fat was not significant, both high and low body fat fish ate considerably less of the low fat diet. After 21 d of feeding , the low body fat fish had consumed significantly more of both the high and low fat diets than the high body fat fish (Table 2).

Table 2. Initial and final weights and body fat, feed intake on day 1, total feed intake and feed efficiency of juvenile chinook salmon fed high (20.3%) and low (2.5%) fat diets for 21 days (Experiment 1). For all descriptive statistics, n=2.

Body Fat	Diet Fat	Initial Weight [1] (g)	Final Weight [1] (g)	Initial Fat [2] (%)	Final Fat [3] (%)
High	High	22.7±0.1	21.4±2.5	11.3±2.1	12.0±0.1
High	Low	22.5±0.3	21.4±0.4	11.3±2.1	12.9±1.0
Low	High	22.6±0.1	24.4±0.2	5.4±1.8	6.9±0.2
Low	Low	22.7±0.1	22.7±0.2	5.4±1.8	6.1±0.1
Probability	df				
Body Fat	1		0.08		0.0001
Dietary Fat	1		0.38		0.85
Interaction	1		0.36		0.07

Body Fat	Diet Fat	Feed Eaten Day 1 [4](g)	Feed Eaten Total [4](g)	Gain/Feed x100
High	High	1.7±1.1	25.3±23.3	NC[5]
High	Low	1.4±0.3	11.1±6.6	NC[5]
Low	High	9.3±0.4	50.2±3.9	66±10
Low	Low	6.0±1.5	37.2±4.3	0±5
Probability	df			
Body Fat	1	0.0009	0.04	0.20
Dietary Fat	1	0.06	0.20	0.90
Interaction	1	0.08	0.95	0.89

[1] Total weight of fish in tank/number of fish.

[2] Estimated from 10 individual analysis from a common pool of high or low fat fish.

[3] Based on tank means, 5 individual analysis/tank.

[4] Dry weight basis, intake /tank.

[5] Fish lost weight.

Experiment 2

Feed consumption in experiment 2 (~1% body weight/d) was considerably higher than in experiment 1 (Table 3). We attribute this to the fish being acclimated to the recirculation system. No mortality occured during the experiment. On day one the low body fat fish consumed significantly more feed than the high body fat fish and both groups consumed significantly more of the high fat diet. After 21 days low body fat fish had consumed

significantly more feed than the high body fat fish (Fig 1). The highest cumulative feed consumption occurred in the low body fat fish fed the low fat diet. Mean fish weight increased in all treatments, and body fat levels increased in all groups except the high body fat fish fed the low fat diet. Feed efficiency (gain/feed) was significantly higher on the high fat diet but was not affected by the level of body fat.

Table 3. Initial and final weights and body fat, feed intake on day 1, total feed intake and feed efficiency of juvenile chinook salmon fed high (20.3%) and low (2.5%) fat diets for 21 days (Experiment 2). For all descriptive statistics, n=2.

Body Fat	Diet Fat	Initial Weight [1] (g)	Final Weight [1] (g)	Initial Fat [2] (%)	Final Fat [3] (%)
High	High	10.6±0.1	11.7±0.2	7.0±1.2	9.2±0.6
High	Low	10.5±0.3	11.0±0.3	7.0±1.2	7.1±0.2
Low	High	10.5±0.1	12.0±0.1	3.3±0.2	5.0±0.8
Low	Low	10.6±0.3	11.6±0.4	3.3±0.2	4.2±0.4
Probability	df				
Body Fat	1		0.06		0.0008
Dietary Fat	1		0.04		0.02
Interaction	1		0.38		0.16

Body Fat	Diet Fat	Feed Eaten Day 1 [4](g)	Feed Eaten Total [4](g)	Gain/Feed x100
High	High	10.9±0.1	63.6±4.6	85.7±9.4
High	Low	8.8±1.8	47.0±2.7	42.7±8.8
Low	High	20.7±3.0	81.8±1.6	88.1±22.5
Low	Low	15.4±1.1	89.7±4.9	53.1±3.6
Probability	df			
Body Fat	1	0.003	0.003	0.50
Dietary Fat	1	0.05	0.17	0.01
Interaction	1	0.30	0.01	0.72

[1] Total weight of fish in tank/number of fish.

[2] Estimated from 10 individual analysis from a common pool of high or low fat fish.

[3] Based on tank means, 5 individual analysis/tank.

[4] Dry weight basis, intake /tank.

Discussion

During the pretreatment portion of the experiment the amount of feed fed to all the groups was determined by the group fed to satiation and eating the least amount of feed. Our observation was that the high fat fish had the least appetite, and that low fat fish would have consumed additional feed. This was expected since numerous reports have shown that fish will eat more of a less energy dense diet up to the limits of their stomach capacity (Lee and Putnam, 1973; Bromley and Adkins, 1984; Hilton, et al., 1983). Metcalfe and Thorpe (1992) and Jobling and Miglavs, (1993) reported that in juvenile Atlantic salmon (Salmo salar) and Arctic charr (Salvelinus alpinus) fatter fish had reduced appetite compared to starved lean fish. Jobling and Miglavs (1993) reported that feed intake in 6.5% body fat fish was about 50% of that of fish previously starved 4% body fat fish. The results of both our experiments clearly show that even without a starvation period, adiposity affects appetite in juvenile chinook salmon.

The effect of dietary fat level on feed intake was less clear. Initially, high fat diets were consumed in greater quantities, but some adjustment to the low energy density diet was observed in experiment 2 (Fig. 1).

In our experiments differences in appetite were of a similar magnitude between large (22g) fish with 11.3 and 5.4%, and small fish with 7.0 and 3.3% body fat despite different rates of growth (fast vs.slow). Therefore, adiposity was a major factor regulating feed intake. This

suggests that growth may actually be retarded if a feeding regime or diet results in high body fat levels in juvenile salmonids.

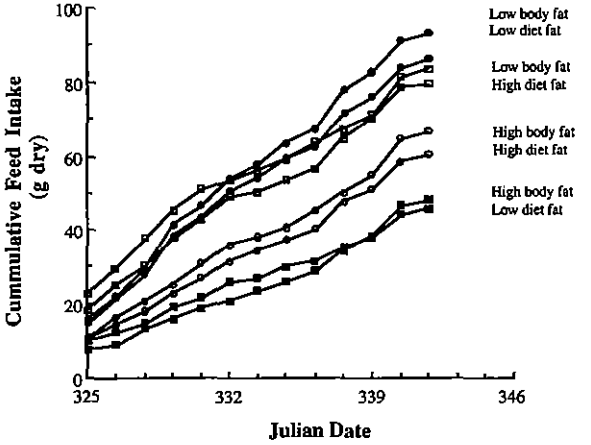

Figure 1. Experiment 2, cumulative feed intake of juvenile chinook salmon (11 g) with high (7.0%) or low (3.3%) body fat, fed high (20.3%) or low (2.5%) fat diets.

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REPRODUCTIVE CYCLING AND CONTROL IN SOME NW ATLANTIC TELEOSTS

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Abstract

Variations in reproductive cycling and their control can be related to seasonal food availability, and muscle quality. Variants studied, in marine teleosts from the NW Atlantic, include short-season spawners and batch-spawners as well as fish reputed to be semelparous (single-time spawning).

Introduction

Cycles of reproduction for Northern teleosts, whether fresh-water or marine can be classified in various ways. One of the major classifications (Cole 1954) deals with whether spawning is normally repeated in an individual from year to year (iteroparous) or whether spawning is terminal event in the life-cycle (semelparous). For females it is accepted that oogenesis may be completely synchronous, group-synchronous or asynchronous (Marza 1938, Harder 1975, Wallace & Selman 1981). It is expected that semelparous females will have completely synchronous oocyte development, and it is implied that semelparous females in the final phases of oogenesis should not have immature oocytes because all oocytes should be committed to the terminal reproduction. It also makes sense that semelparous fish will have the option to draw heavily on body reserves in the last phases of gamete development if such fish are going to die shortly after reproduction anyway they might as well commit most of their energy to gamete development and only reserve sufficient to complete the final gamete deposition phase.

For iteroparous fish in habitats with highly seasonal food supply a problem of survival compatible with energy allocation to reproduction has perhaps been solved by various mechanisms in the cycles. One option is finely adjusting numbers of gametes produced both at the species and the individual level. The ultimate option is to totally omit reproduction in a facultative manner (Burton 1994) so that fish only reproduce if they have a good chance of post-spawning survival. For fish such as winter flounder, *Pleuronectes americanus*, which has to sustain a long winter fast at least in the Northern part of its range, the strategy of facultative spawning omission makes it possible for individual fish to "choose" whether to risk committing energy to a spawning season.

However winter flounder is an unusual flatfish in that it has short-season spawning, certainly for the females, in that all the evidence is that females deposit all their eggs in a very short time, probably much less than a week. This contrasts with other Northern flatfish such as yellowtail, *P.ferrugineus*, halibut, *Hippoglossus hippoglossus*, and American plaice (long rough dab in European waters), *Hippoglossoides platessoides*, all of which are confidently reported to be batch-spawners. Such batch-spawners will deposit sub-sets of the mature oocytes at intervals over a period of about 1 month or more to give an extended spawning period, with several advantages. One of these advantages is perhaps increasing the chance of survival for at least some of the offspring which may hatch at a better time than earlier or later groups. Another

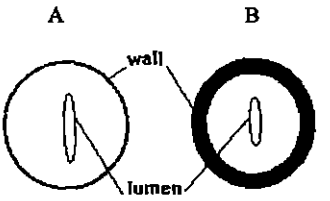
advantage is that if the final stages of maturation include considerable increase in size with pressure on the abdomen, then batch spawning staggers the effect and makes possible a larger overall production of oocytes. A considerable disadvantage would be the effect an extended spawning period would have on options for feeding. A possible effect would be a down-grading of white muscle protein content to provide materials for batch-spawners as is implied by Roff (1982).

Cycles examined

Recently, in addition to studies on winter flounder (Burton & Idler 1984, 1987a, 1987b; Burton 1991a, 1991b, 1994) and earlier work looking at aspects of salmonid reproduction (Burton et al 1985), several other NW Atlantic teleost cycles have been examined in conjunction with thesis work and summer employment of several students and assistants. Gametogenesis of male and female cod was studied (Burton et al 1996); oogenesis of American plaice is being examined in association with muscle depletion (Maddock & Burton 1995, 1996), and the reproductive cycle of capelin *Mallotus villosus* has been scrutinized (Flynn & Burton 1995, 1996) in the light of strong suggestions that it is semelparous. I am just beginning (with two students) to investigate the life cycle of the NW Atlantic "turbot", *Reinhardtius hippoglossoides*, a fish which is supposed to migrate North and spawn in deep water off the Davis Strait (Scott & Scott 1988), which makes knowledge of reproduction somewhat inaccessible. Another feature of this fish is that it has been recently under increasing commercial pressure and it has the interesting biological feature that males seem to be very rare.

Results

Winter flounder seems be an extreme case of a reproductive cycle that may be quite typical for NW Atlantic teleosts whether they be batch-spawners (cod, most Northern flatfish except winter flounder) or short-season spawners like winter flounder. In all these fish whether batch-spawners or spawners over a very compressed time period the females at least seem to have a very protracted oogenesis that may be subject to very fine control of timing and several switches and adjustments related to nutrition. Because both cod and flatfish have similar cystovarian ovaries with substantial outer muscle layers which apparently get irreversibly thicker (Fig.1) after the first spawning (Burton & Idler 1987a, Burton et al 1996) it should be reasonably easy to identify adults at certain seasons, and particularly non-spawning adults.



A.Immature B.Post-spawning or non-reproductive adult

Figure1. Changes in the cystovarian ovary after spawning

It is certain that winter flounder, and perhaps cod, and probably many of the other flatfish do not necessarily spawn every year after the first spawning (Burton 1994, Burton et al 1996) and it is clear that spawning omission can be primarily related to recent poor feeding conditions. Experiments (Burton 1991a,b, 1994) have confirmed that winter flounder reproduction is very

sensitive to feeding conditions and that winter flounder have a critical period, close to the normal spawning season, during which nutritional status can influence whether a fish will begin or maintain gametogenesis for the next spawning season. In studying factors controlling this "decision" it is interesting that Burton & Burton (1989) noted that non-reproductive adults at the normal spawning time showed evidence of some seasonal steroidogenesis. Recent examination of pituitaries of some non-reproductive fish shows that there is some synthetic activity in the DR (distinct region Burton et al 1981) normally associated with reproductive fish and the production of carbohydrate-rich gonadotropins.

Winter flounder, because it has a very short spawning period is easier to study, from the point of the inter-relationships between reproduction and nutrition and early gametogenesis, than the batch-spawners which have an extended spawning season and at least the possibility of variable late recruitment into vitellogenesis (indeterminate gametogenesis). Studies on American plaice, a fish noted for its propensity to sacrifice muscle condition (Templeman & Andrews 1956, Roff 1982) are ongoing, with timing of gametogenesis (Maddock & Burton 1996) and development of jellied muscle (Maddock & Burton 1995) compared to the situation in winter flounder (Burton & Idler 1984, Maddock & Burton 1994). The main interest on the nutrition-reproduction interaction is whether American plaice does undergo late vitellogenesis which draws on muscle protein (Maddock & Burton 1996). For batch-spawners generally there may be problems with nutrition in that protracted spawning can interfere with feeding, which may necessitate drawing on somatic stores. Winter flounder which, off Newfoundland, undergoes a protracted winter fast (Kennedy & Steele 1971, Fletcher & King 1980) but does start feeding both in the wild and in the laboratory before spawning, even though the temperatures may be below the temperatures at which flounder generally stop feeding in the late fall, then stops feeding for a very short time (about a week) around the spawning time for females (unpublished records, Burton).

Capelin, *Mallotus villosus*, has the general reputation (Nakashima 1987, Shelton et al 1993) of being a one-season spawner, ie is semelparous. The post-spawning ovaries however (Flynn & Burton 1995, 1996) contain immature oocytes and it is possible to keep females, though not males, alive after spawning. Such post-spawned females will develop vitellogenic oocytes for the next spawning season (Flynn & Burton 1995, 1996) so it is evident that at least the females are not semelparous. The situation with the males remains puzzling. I have not been able to persuade them to resume feeding after spawning, whereas the females feed readily on *Artemia*. However it is entirely possible that they die partly at least partly because the spawning period for males is protracted (Templeman 1948). Conversely, the females have a short spawning (Templeman 1948) period similar to that of winter flounder. It will probably not be possible to use simple ovarian structure to detect repeat-spawners in wild females because the ovary is not of the cystovarian type. Neither is it of the simple gymnovarian type such as is found in salmonids (Flynn & Burton 1996). Detection of repeat-spawning males in the wild would be possible if the testicular structure changes with spawning as occurs with winter flounder (Burton & Idler 1987a). As male capelin are generally larger and more robust than females it was somewhat surprising to find that females survive much better than males, even though Templeman (1948) and more recently Shackell et al (1993) had indicated this could be so. This life cycle requires more study, particularly as this small species is such an important prey item in the NW Atlantic.

Work with turbot (Greenland halibut *Reinhardtius hippoglossoides*) has just started, with initial data collection already showing we are going to need considerable revision of published accounts of where the fish spawn. It is also evident that clearly identifiable males continue to be very rare in samples collected.

Conclusions

For fish with cystovarian ovaries it may be possible to obtain sound data on spawning omission for adult females in the wild populations of the NW Atlantic. For any fish, marine or freshwater, which is subject to nutritional constraints because of short feeding seasons perhaps compounded by long spawning periods there are various adaptations that may have arisen. Nutritionally controlled and irregular spawning omission for individuals is one possibility while for others it may be possible to maintain batch-spawning by short-term sacrifice of white muscle. Surprisingly however the small pelagic capelin females do not deplete their soma to the point of obligate semelparity unlike the Pacific coast and much larger fish of the *Onchorhynchus* grouping in the same taxon (Protacanthopterygii). Although I do not have firm data yet on the batch-spawning flatfish there is evidence in the literature (Pitt 1966, Bagenal 1957, Templeman & Andrews 1956) that some of these flatfish may show periodic spawning omission as individuals. Cod, *Gadus morhua*, is also a batch-spawner, and although it may show some muscle depletion (Love 1960) it seems more likely that it responds to poor nutrition by down-grading its fecundity (also found for flatfish species, Burton 1994), and by spawning omission (Burton et al 1996), also reported for Barents Sea cod by Oganesyan (1993).

The inter-relationship of muscle as protein (or fat) store, and the fine control of seasonal allocation or depletion of materials to or from gonads and muscle, may be shifted towards conservation of soma in the case of the winter flounder or muscle depletion in the case of American plaice.

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PROTEIN METABOLISM IN FISH: COMPARATIVE TURNOVER RATES

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INTRODUCTION

When we look at an animal growing we are seeing the balance between the animal's rates of protein synthesis and protein degradation. Therefore, in order to understand what is controlling growth rates we need measurements of the rates of protein synthesis and growth; if we wish to go further and relate protein growth and protein synthesis rates to, for example, the amount of amino acid ingested, we need to know in the same individuals, rates of food consumption and digestive efficiency. As measurements of protein growth and protein synthesis are normally carried out on individuals, increasingly protein metabolism studies are being preceeded by measurements of protein and energy absorption of individual fish which may have been held in groups (e.g. Carter *et al.* 1993; McCarthy *et al.* 1994).

FOOD CONSUMPTION

Rates of food consumption of the individual fish or of the group as a whole and the dynamics of the distribution of food between individuals are turning out to be key measurements in understanding food conversion efficiency. If we take a group of Atlantic salmon being fed a reduced ration as an example, at one extreme, the dominant animals, although gaining the highest proportion of the available food may also be expending energy at a high rate in order to maintain their dominant position. The most subordinate animals will be eating less and may be forced by hunger to occasionally compete for food with more dominant animals and may suffer fin damage as a result. However, the reduced activity levels of the subordinate fish may promote increased conversion eficiency of the food they acquire. We are only just beginning to be able to understand social hierarchies in groups of fish and the likely trade offs between dominance, food acquisiton, energy expenditure, growth rates and possible stress and disease resistance.

PROTEIN SYNTHESIS METHODOLOGY

The majority of studies that have measured rates of protein synthesis in fish have used a single flooding dose injection (Garlick *et al.* 1980) of a radiolabelled amino acid, commonly ^{3}H-phenylalanine, and have measured the incorporation of radiolabel into body protein over a known incubation time. The methodology, necessary validation criteria and a thorough review of the available data is provided by Houlihan *et al.* (1995a, b & c). Recently our attention has turned

to measurements of protein synthesis using stable isotopes, principally ^{15}N, which has been used extensively in the study of protein synthesis in mammals (reviewed in Houlihan *et al.* 1995a & b). Our approach has been to utilise ^{15}N-phenylalanine to make single terminal measurements of protein synthesis using the flooding dose technique (Owen *et al.* unpublished data, 1995) and to use make non-invasive measurements of protein synthesis using ^{15}N protein.

We have adapted the stochastic end-point model to measure rates of protein synthesis in fish following ingestion of ^{15}N protein (Carter *et al.* 1994), using the simple single pool model of Waterlow *et al.* (1978). The theoretical assumptions of the model and the methodology employed have been described in detail in Carter *et al.* (1994) and Houlihan *et al.* (1995b). Initial results from our laboratory on the protein metabolism of rainbow trout indicate that protein synthesis rates obtained from feeding ^{15}N-enriched protein and collecting ammonia are similar to those obtained with radiolabelled amino acids (Table 1). Carter *et al.* (1994) reported a mean fractional rate of synthesis of 2.30 ± 0.67 %.day^{-1} in 100g rainbow trout measured over the first 24 h following a ^{15}N-labelled meal. Following a flooding dose injection of L-[2,6-^{3}H]-phenylalanine to measure protein synthesis in fish of a similar size, the relationship between fractional rates of protein consumption (k_c, gramme of protein consumed per gramme of fish protein expressed as a percent per day) and synthesis (k_s) for feeding rainbow trout (40-100g:14°C) was described by $k_s = 1.69 + 0.45\ k_c$ (McCarthy *et al.* 1994). This relationship predicted a mean ks of 2.60 %.day^{-1} for the trout used in the ^{15}N experiment which was similar to that calculated from the stable isotope analysis. Similar rates of protein synthesis were also found in the white muscle of rainbow trout of a similar size (Table 1). These synthesis rates were obtained following a flooding dose injection of ^{3}H-phenylalanine (incubation period of 3 to 6 hours) and from a terminal measurement of the incorporation of ^{15}N-protein into white muscle protein 48 hours after a meal. The similarity between whole-animal and white muscle rates of protein synthesis measured over 2 days using a stochastic end-point model and over a few hours, as by the flooding dose method, provides confidence in the results obtained using the two techniques. Also, the similarity in measuring rates of protein synthesis using the two techniques further supports the validity of calculating rates of protein degradation as the difference between synthesis and growth (Millward *et al.* 1975) and therefore, the estimates of protein turnover made for fish (*e.g.* Carter *et al.* 1993; Houlihan *et al.* 1994; MCarthy *et al.* 1994).

Feeding ^{15}N enriched protein and collecting the excreted ammonia in laboratory studies is advantageous because it allows non-invasive and non-destructive measurements of protein synthesis which can be repeated on the same animal (Owen *et al.* 1995; Carter *et al.* unpublished data 1993). Therefore the use of stable isotopes should be used increasingly in the study of protein synthesis in fish. This would enable us to answer questions such as, how do changes in environmental conditions affect the protein turnover of the same fish? Furthermore, being able to 'track' the same fish provides the opportunity to measure ontogenic changes in protein synthesis and construct synthesis/weight relationships for the same fish; the only assumption that we need to make is on the protein-nitrogen content of the fish. The application of stable isotopes to measure rates of protein synthesis in the field is discussed below.

Diurnal cycling

Studies with individual animals have shown that the postprandial increase in oxygen consumpion is accompanied by a stimulation in the rates of protein synthesis and an increase in ammonia excretion. Results from our laboratory with ^{15}N suggest that the ammonia that initially appears after a meal is not derived from the amino acids that were ingested (Owen *et al.*, unpublished data 1995); there may be an obligatory amino acid oxidation of currently held amino acid to supply energy for protein synthesis before the arrival in the tissues of the absorbed amino acids.

Table 1. A comparison of whole body and white muscle fractional rates of protein synthesis (k_s, %.day^{-1}) in rainbow trout, *Oncorhynchus mykiss*, measured by different methods. Wt = body weight (g) and T°C = water temperature (°C)

k_s (%.day^{-1})	Method	Wt	T°C	Ref
a) Whole body				
2.3 ± 1.9[a]	Caudal vein infusion (L-U[^{14}C]-Leu)	80g	10°C	1
2.5 ± 1.0	Flooding dose injection (L-[2,6 3H]-phe)	100g	10°C	2, 3
2.3 ± 0.7	Fed ^{15}N-protein	117g	12°C	4, 5
b) White muscle				
1.5 ± 1.0	Flooding dose Injection (L-[2,6 ^{3}H]-phe)	100	10°C	3
2.5 ± 0.6*	Fed ^{15}N-protein	117g	12°C	5

a = synthesis rates calculated using the specific radioactivity of the plasma as an estimate of the free pool specific radioactivity
* = synthesis rates calculated from killing fish 48 h after feeding and measuring the incorporation of ^{15}N-protein into muscle protein.
(1 = Fauconneau & Arnal 1985; 2 = McCarthy *et al.* 1994; 3 = McCarthy, unpublished data, 1991; 4 = Carter *et al.* 1994; 5 = Owen unpublished data, 1993)

Efficiency of retention of synthesised proteins

The efficiency of retention of synthesised protein (protein growth * 100/protein synthesis) seems to be a key indicator of strategies of protein metabolism (Table 2). High efficiencies of retention of synthesised proteins indicate reduced protein degradation rates and hence low turnover rates in growing animals. At first sight it looks as though invertebrate animals have higher efficiencies of retention of synthesised proteins compared with most fish and with the mammalian examples cited in Table 2. Further studies will reveal whether invertebrates have minimized protein turnover in order to maximise growth rates but presumably with the loss of the advantages that protein turnover brings.

In a recent review of protein metabolism in fish larvae we concluded that although weight-specific growth rates are high, rates of protein synthesis, amounts of free amino acids and RNA concentrations are not exceptional considering the small size of the organisms and in fact fish larvae seem to be following scaling relationships established for larger fish (Houlihan *et al.* 1995c). There was little evidence that rapidly growing fish larvae or juvenile fish sacrifice protein turnover in order to maximize retention efficiencies of synthesized protein; i.e. fish larvae are not exhibiting the invertebrate strategy described above. However, it would be surprising if examples of reduced turnover growth were not found in some rapidly growing

Table 2. The efficiency of retention of synthesized protein (k_g/k_s, %, whole-body protein growth *100/whole-body protein synthesis) for various endotherms and ectotherms. The sources of these data are provided in Houlihan *et al.* (1995b).

Species	k_g/k_s	Weight
a) Endotherms		
Pig	15	32 kg
Lamb	26	12.2 kg
Rat	20	211 g
Chicken	24	138 g
b) Ectotherms		
Mussel	92	10 g
	70	10 g
Octopus	95	150 g
Brown Tiger Prawn	48 - 93	5 g
Herring larvae	50	0.05 g
Nase	50	0.045 g
Rainbow Trout	35 - 69	0.2 g
Mossmbique Tilapia	30 - 40	0.0 g
	30 - 40	0.1 g
	30 - 40	1.0 g
	30 - 40	10 g
Sea bass	30 - 60	2.3 g
	47	3.5 g
	77	8 g
Goldfish	69	14 g
Grass carp	54	23 g
Turbot	33	50 g
Common carp	25	30-60 g
Rainbow Trout	45	51 g
	53	51 g
	35	70 g
Plaice	51	60 g
Dab	43	250 g
Salmon	32	180 g
Cod	42	300 g

species. In the remainder of the fish species in Table 2, retention efficiencies are mainly between 30 and 50 percent. At the moment it is not possible to clearly relate these different efficiencies to different growth rates, environmental conditions, temperature etc. However, the value of protein degradation may permit both rapid adaptation to environmental perturbations as well as the removal of damaged or abnormal proteins which may be harmful (Kirkwood 1981).

Energy cost of protein synthesis

Although it is recognied that making proteins consumes energy there is still a a degree of uncertainty as to the contribution that protein synthesis makes to resting oxygen consumption. Polypeptide synthesis proceeds through three stages - the formation of the initiation complex that contains two ribosomal subunits, secondly the process of peptide chain elongation and finally the process of termination. From ATP utilization in these processes it can be estimated that 40 mmole of ATP are needed for the synthesis of each gram of protein. In place of the conventional energy budget where the emphasis has been on the components such as energy used in maintenance, specific dynamic action, activity, growth and reproduction it is now possible to calculate at least one of the costs of living - the proportion of the ingested energy that is used for the synthesis of proteins. In inactive animals it seems that protein synthesis represents between 20 and 40% of the total oxygen consumption. In ectotherms where 55% of the absorbed energy is used for respiration (Jobling 1994) protein synthesis may account for 15% of the total energy ingested (calculated from Carter *et al.* 1993).

Biochemical correlates of protein synthesis and growth

One important question in protein metabolism in fish concerns the extent of turnover in naturally occuring populations. The data thus far discussed have been drawn from laboratory studies: how can these techniques and results be applied to fish in the wild?. One strategy that has been used is to estimate the growth rates of wild fish populations is to compare growth correlates from laboratory studies with measurement of these correlates from wild animals. In this approach laboratory growth studies are conducted under experimental conditions that seek to mimic the environmental conditions experienced by the fish. Through manipulation of the amount of food offered, a range of individual growth rates are generated for the species of interest: tissue samples are then taken for measurement of the likely growth correlates. Samples from the wild population are compared with those generated from the laboratory and likely growth rates of the wild animals are estimated (reviewed in Houlihan *et al.* 1993). We have used this approach in our laboratory to estimate the growth rates of saithe, *Pollachius virens* L. from the wild (Mathers *et al.* 1992a & b) using muscle RNA concentrations (RNA to protein; RNA to DNA and RNA/g wet weight) and aerobic enzyme activities (Citrate synthase; Cytochrome c oxidase and Lactate dehydrogenase). Recent work has also shown a correlation between epaxial muscle ornithine decarboxylase activity and growth rate (Benfey *et al.* 1994): measurement of this enzyme activity in wild fish, compared to laboratory-reared animals may also be a suitable correlate of recent growth rates in the wild. This leaves the question of the rates of protein synthesis in wild populations. To our knowledge there have been no attempts to estimate rates of protein synthesis of fish from the wild using laboratory-derived calibrations. Studies from our laboratory have shown that tissue and whole-animal RNA to protein ratios (μg RNA/mg protein) are correlated with both rates of protein synthesis and growth for a number of fish species (e.g. Houlihan *et al.* 1989, 1993, 1994). We would suggest that measurement of RNA to protein ratios in wild animals, together with careful laboratory calibration, may provide valuable estimates of both growth and protein synthesis in wild fish. It has also been suggested that measurement of muscle ornithine decarboxylase activity may also be a possible biochemical correlate of protein synthesis (Arndt *et al.* 1994).

STABLE ISOTOPES: LINKING LABORATORY AND FIELD?

We have recently turned our attention to the use of stable isotopes to estimate rates of protein synthesis in the field and we would suggest that there are two possible approaches. Firstly, the use of ^{15}N-phenylalanine, administered as a single flooding dose, would allow wild-caught fish to be injected and held in net pens for a known incubation time. The use of the stable isotope-labelled amino acid removes the danger of radioactive contamination in the field and allows the use of an established technique to measure rates of protein synthesis. However, it is possible that the synthesis rates obtained may not be valid due to the stress of capture, injection and confinement. A second approach may be to use seasonal changes in tissue ^{15}N/^{14}N ratios to estimate tissue turnover rates and hence protein synthesis in wild fish. We have recently examined seasonal changes in the ^{15}N/^{14}N composition of Flounder, *Platichthys flesus* L., and its major prey items in the Ythan estuary (Aberdeenshire, N.E. Scotland). We are currently examining the possibility of using this data to model rates of protein synthesis in wild fish populations.

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MUSCLE PROTEIN SYNTHESIS AND PROTEIN DEPOSITION IN RAINBOW TROUT (*Oncorhynchus mykiss* Walbaum)

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ABSTRACT

White muscle accounts for 30-60% of the body mass of a fish: this presentation examines the relationship between white muscle and whole body rates of protein synthesis and growth and the contribution that the white muscle makes to whole body protein metabolism in rainbow trout. Linear relationships were found between protein consumption and the rates of protein synthesis and protein growth in the white muscle and whole body. The results indicated that 0.65 and 0.17 g of protein were synthesised and 0.24 and 0.12 g of protein deposited per gramme of protein consumed in the whole body and white muscle respectively. The white muscle accounted for 25% and 45% of daily whole body protein synthesis and deposition respectively.

INTRODUCTION

In all animals there is a continual cycle of synthesis and degradation of protein, with growth occurring when the rate of protein synthesis exceeds the turnover rate (Houlihan *et al.* 1995). Following the development of the flooding dose technique by Garlick *et al.* (1980) to measure rates of protein synthesis, early studies in fish concentrated on examining tissue-specific differences in the rates of protein synthesis, degradation and growth (e.g. Houlihan *et al.* 1988). Recently the relationship between consumption, protein synthesis and growth in fish has been examined, with particular attention being paid to the anabolic stimulation of protein synthesis and the retention efficiency of synthesised proteins as growth (Houlihan *et al.* 1988; Carter *et al.* 1993b, 1994; McCarthy *et al.* 1994). The white muscle accounts for 30-60% of the body mass of a fish (Fauconneau & Arnal 1985; Houlihan *et al.* 1988; Carter *et al.* 1933a; McCarthy unpublished data). In the white muscle the fractional rates of protein synthesis and degradation, the percentage of the protein mass synthesised or broken down per day, are lower compared to other body tissues such as the liver and gill (Houlihan *et al.* 1988). However, as a result of its large mass relative to other body tissues the white muscle makes a major contribution to the total

absolute daily rate of protein synthesis and deposition. The aims of this study were, (i) to examine the anabolic stimulation of white muscle and whole body rates of protein synthesis in juvenile rainbow trout, (ii) to examine the contribution made by the white muscle to whole body rates of protein synthesis and growth and, (iii) to compare these results with the available data for ectothermic and endothermic vertebrates.

Methods

The full experimental protocol for this study has been published previously (McCarthy *et al.* 1994) and only a brief description is given here. Three groups of 24 rainbow trout were reared for 73 days in freshwater (350 l tanks; 40 l/h water turnover; 7.7°C, range 5 - 11°C) and fed either a 0.5. 1.0 or 2.0 % Body mass/day meal (Aqualine Trout Fingerling) in a single daily meal and a single group of 20 fish were starved. The initial mass of the experimental animals was 40.8 g (± 0.8 SEM, n = 92). Fish from the same stock group (43.3 g ± 2.3 SEM, n= 20) were killed at the start of the experiment in order to estimate the initial protein content of the experimental animals for the calculation of protein growth rates (see below). Individual food consumption rates were measured on four occasions (days 27, 55, 64 and 72) using X-radiography (McCarthy *et al.* 1993, 1994) and the data used to calculate protein consumption rates (g protein/day).

At the end of the experiment, white muscle and whole body rates of protein synthesis were measured in nine fish from each of the three ration groups and in 10 starved fish using the flooding dose technique (Garlick *et al.* 1980, Houlihan *et al.* 1995). The fish (58.3 g ± 3.3 SEM, n = 37) were fed at 0700 and 3h later were injected via the caudal blood vessels, without anaesthesia, with a solution containing 135 mM L-phenylalanine and [L-2,6-^{3}H]phenylalanine (Specific radioactivity 1250 ± 40 SEM, n=4, dpm/nmole). Following a known incorporation period in freshwater (10°C, mean 278 min ± 5 SEM, n = 37), each fish was killed (by a sharp blow to the head and transection of the spinal cord), frozen in liquid nitrogen and stored at -70°C until analysis. Triplicate white muscle and whole body samples were taken in order to measure the free pool and protein-bound specific radioactivity and protein content as outlined in Houlihan *et al.* (1995) in order to calculate white muscle and whole body absolute rates of protein synthesis (g protein/day). White muscle and whole body protein growth rates (g protein/day) were calculated for each fish using the measured final protein content and the estimated initial protein content as outlined in McCarthy *et al.* (1994).

Results and Discussion

a) Method validation

Due to the range of incorporation times (228 - 345 min), the data were grouped at 30 minute intervals to examine the pattern of flooding of the free pool over time and the incorporation of radiolabel into body protein. Following a single flooding dose injection, the free phenylalanine concentration in the white muscle free pool remained elevated and stable (ANOVA, $p<0.05$) over the course of the incubation (Table 1) and exhibited a 12 fold elevation above basal level (74 nmole phe/g wet weight, Carter *et al.* 1995). The specific radioactivity of the white muscle free pool remained elevated over the course of the incubation. The mean S_a values for each time interval were not significantly different from each other or from the phenylalanine-specific radioactivity of the injection solution (ANOVA, $p<0.05$). The mean white muscle S_a was 1091±101 dpm/nmole or 87.3% (±1.3) of the specific radioactivity of the injection solution. Linear regression analysis revealed that the incorporation of [^{3}H]phenylalanine into both the white muscle and the whole body protein pools was linear over time:

White Muscle: $S_b = 0.01*t - 0.05$ ($R^2=0.835$, $n=5$, $p<0.05$)
Whole Body: $S_b = 0.02*t - 0.18$ ($R^2=0.842$, $n=5$, $p<0.05$)

where t is the time in minutes following injection.

The use of the flooding dose technique is based on several assumptions which have been recently reviewed by Houlihan *et al.* (1995). Briefly, these assumptions are (i) that the flooding dose of phenylalanine does not affect the rate of protein synthesis, (ii) the flooding dose results in a rapid elevation and stabilisation of the phenylalanine-specific radioactivity in the body free amino acid pools to a similar level as the injection solution over the incorporation period and (iii) that the labelling of body protein with the radiolabel is linear over the incorporation period. Previous studies in fish and rats have shown that a single flooding dose injection of [^{3}H]phenylalanine does not affect the rate of protein synthesis (reviewed in Houlihan *et al.* 1995). The time course results of this study meet the second and third assumptions, and are agreement with previous studies (reviewed in Houlihan *et al.* 1995) and therefore validate the synthesis values obtained in this paper.

Table 1. White muscle free pool phenylalanine concentration (WM Phe, nmole/g wet wt) and free pool phenylalanine-specific radioactivity (WM S_a, dpm/nmole) and white muscle (WM S_b, dpm/nmole) and whole body protein-bound phenylalanine-specific radioactivity (WB S_b, dpm/nmole) for 60 g rainbow trout following a single flooding dose injection of [^{3}H]phenylalanine (specific radioactivity, 1250 ± 40 dpm/nmole). The data are grouped for 30 minute intervals and are presented as the mean value ± SEM.

Time	n	WM Phe	WM S_a	WM S_b	WB S_b
210-239	4	883±50	1149±33	1.11±0.05	3.28±0.56
240-269	12	863±46	1132±29	1.50±0.21	4.16±0.45
270-299	13	803±79	1269±23	1.63±0.30	4.93±0.56
300-329	4	856±19	1007±57	1.78±0.19	4.53±0.73
330-359	4	956±73	1172±60	1.81±0.57	5.71±0.92

b) CONSUMPTION-SYNTHESIS-GROWTH RELATIONS

Significant linear correlations were found between protein consumption (A_r, g protein/day) and white muscle and whole body protein synthesis (A_s, g protein/day) and between protein consumption and white muscle and whole body protein growth (A_g, g protein/day) (Figure 1):

Consumption-synthesis
Whole Body: $A_s = 0.65*A_r - 0.07$ ($R^2=0.853$, $n=37$, $p<0.001$)
White muscle: $A_s = 0.17*A_r - 0.01$ ($R^2=0.694$, $n=37$, $p<0.001$)

Consumption-growth
Whole Body: $A_g = 0.24*A_r - 0.01$ ($R^2=0.833$, $n=37$, $p<0.001$)
White muscle: $A_g = 0.12*A_r - 0.01$ ($R^2=0.698$, $n=37$, $p<0.001$)

The regression analysis indicated that for every gramme of protein consumed (i) 0.17 and 0.65 g of protein were synthesised and (ii) 0.12 and 0.24 g of protein were deposited in the white muscle and whole body respectively. The efficiency of retention of synthesised protein as growth (A_g*100/A_s) was 72.3% (±5.9) in the white muscle and 22.0% (±2.7) in the whole body (data not shown).

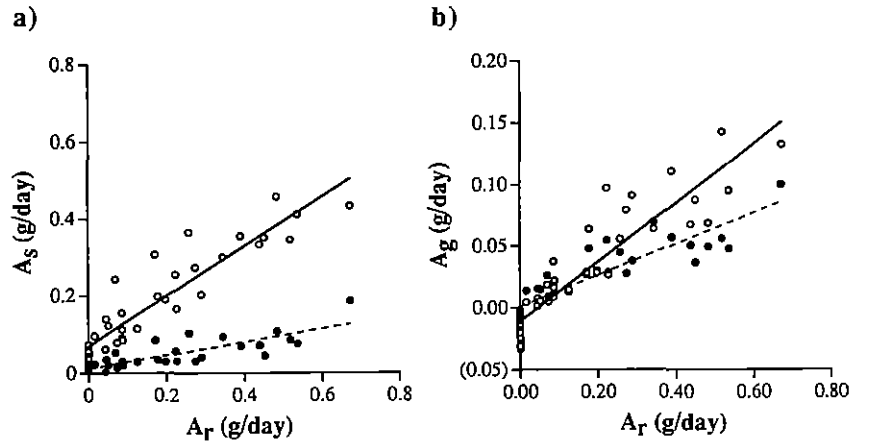

Figure 1. (a) Scatter plot showing the relation between protein consumption (g protein/day) and protein synthesis (g protein/day) in the white muscle (closed circles) and whole body (open circles) of rainbow trout (*O. mykiss* Walbaum). (b) Scatter plot showing the relation between protein consumption (g protein/day) and protein growth (g protein/day) in the white muscle (closed circles) and whole body (open circles) of rainbow trout (*O. mykiss* Walbaum).

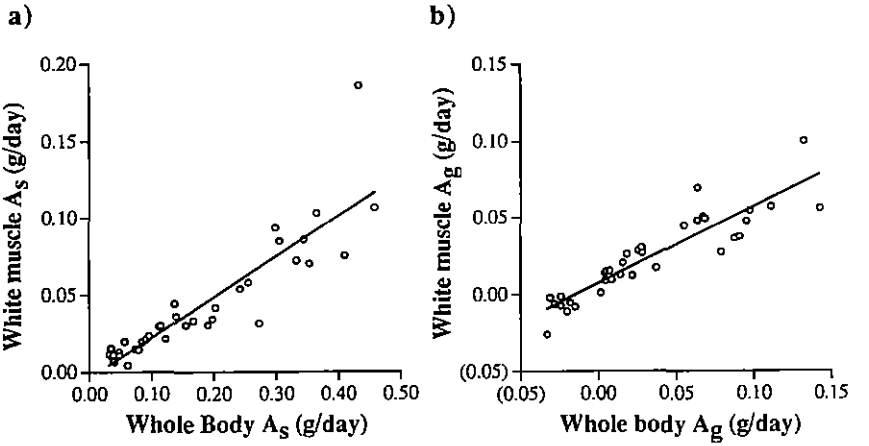

Figure 2. (a) Scatter plot showing the relation between protein synthesis (g protein/day) in the whole body and white muscle of rainbow trout (*O. mykiss* Walbaum). (b) Scatter plot showing the relation between protein growth (g protein/day) in the whole body and white muscle of rainbow trout (*O. mykiss* Walbaum).

The proportion of protein synthesised in the whole body per gramme of protein consumed are in close agreement with published values for rainbow trout of a similar size. Carter *et al.* (1994) report an anabolic stimulation of 0.83g of protein synthesised per gramme of protein consumed in 100 g rainbow trout fed a 2% B.W./day meal of the same commercial diet at 14°C. From the data published by Fauconneau and Arnal (1985) for 98 g rainbow trout at 10°C we have calculated that for every gramme of protein consumed (i) 0.25 and 0.74 g of protein were synthesised and (ii) 0.13 and 0.22 g of protein were deposited in the white muscle and whole body respectively. In fish, the white muscle is characterised by having the highest retention efficiency of synthesised protein of the tissues/organs in the body with efficiencies in the order of 50-70% (this study, Fauconneau & Arnal 1985; Houlihan *et al.* 1988; Carter *et al.* 1993a). The retention efficiency in the whole body is lower and values between 14 and 37 % have been reported for fish (this study, Fauconneau & Arnal 1985; Houlihan *et al.* 1988; Carter *et al.* 1993b, McCarthy *et al.* 1994),

c) WHITE MUSCLE-WHOLE BODY RELATIONS

Significant linear correlations were found between rates of protein synthesis (A_s, g protein/day) in the white muscle and whole body and between rates of protein growth (A_g, g protein/day) in the white muscle and whole body (Figure 2):

Synthesis: $WM = 0.25*WB - 0.01$ ($R^2=0.780$, $n=37$, $p<0.001$)
Growth: $WM = 0.45*WB + 0.01$ ($R^2=0.690$, $n=37$, $p<0.001$)

where WM and WB are the white muscle and whole body rates respectively. The regression analysis indicated that, expressed on an absolute basis (g protein/day), the white muscle accounted for 25 % of daily protein synthesis and 45 % of daily protein growth in this study.

Table 2. (a) Percentage contribution (%) of muscle protein synthesis (g protein/day) to whole body protein synthesis (g protein/day) for several animal species. (* = preruminant lambs, 7-8d old). (b) Percentage contribution (%) of muscle protein growth (g protein/day) to whole body protein growth (g protein/day) for several animal species.

	Species	Body mass	%	Reference
A)	Sea bass	10 g	21	McCarthy *et al.* (unpubl)
	Grass carp	23 g	28	Carter *et al.* (1993a and b)
	Rainbow trout	40-100 g	25	This study
	Rainbow trout	98 g	33	Fauconneau & Arnal (1985)
	Atlantic cod	300 g	26	Houlihan *et al.* (1988)
	Sparrow	25-30 g	32-35	Murphy & Taruscio (1995)
	Lamb*	4.5 kg	29	Attaix *et al.* (1988)
	Rat	100-130 g	25	Preedy *et al.* (1983)
	Rat	100 g	19	Garlick *et al.* (1976)
	Pig	76 kg	42	Garlick *et al.* (1976)
	Cow	376 kg	14	Lobley *et al.* (1980)
B)	Grass carp	23 g	35	Carter *et al.* (1993a and b)
	Rainbow trout	40-100 g	45	This study
	Rainbow trout	98 g	58	Fauconneau & Arnal (1985)
	Atlantic cod	300 g	42	Houlihan *et al.* (1988)

The percentage contribution made by muscle protein synthesis and growth to whole body protein synthesis and growth for a number of animal species are shown in Table 2. The available data suggests that in growing non-ruminant animals reared under excess feeding conditions, the white muscle provides a fairly constant contribution to whole body protein synthesis. The available data on the contribution made by the white muscle to protein deposition in the whole body indicates the substantial contribution (35 - 60 %) made by white muscle to whole body protein deposition.

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EFFECT OF FEEDING VERSUS STARVATION ON DNA SYNTHESIS IN RAINBOW TROUT FRY

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ABSTRACT

The DNA synthetic activities of 73-day old rainbow trout, *Oncorhynchus mykiss*, fed and starved for 29 days were quantified in individual blood cells using flow cytometry. The fraction of cells at the G2 and S stages during the cell division cycle, representing potential for cell proliferation, growth and condition parameters (condition factor and specific growth rate) were always greater in fed fry than in starved fry.

INTRODUCTION

The RNA/DNA ratio has been used as an indicator of the physiological condition of aquatic organisms, particularly the growth and nutritional status of fish larvae (Blow, 1987; Buckley and Lough, 1987; Clemmesen, 1993, 94; Westerman and Holt, 1988, 1994; Canino, 1994). Poor nutritional condition contributes to low protein synthesis, slow growth, and thus, results in a low RNA/DNA ratio (Chung et al., 1993). The analytical techniques for determining RNA and DNA concentrations have been well established for pooled fish larvae and also sensitive assays have been developed for a single fish larvae (Karstein and Wallenburger, 1972, 1977; Calderon and Buckley, 1993; Clemmesen, 1994; Canino and Calderon, 1995). Individual fish tissues may respond differently to physiological condition or nutritional status (Chung et al., 1988, 1993). Thus, measurement of the RNA/DNA ratio of the cells of individual tissues may provide a more accurate index of physiological condition than whole fish homogenate. The growth rate of

different organs may vary, and therefore, interpretation of the RNA/DNA ratio in whole fish homogenate as an index of condition may be difficult (Theilacker and Shen, 1993). Most studies carried out to date have quantified nucleic acid concentrations of individual tissues, a single whole fish or pooled fish larvae. Few studies have been performed to determine nucleic acid concentrations in single cell types from fish larvae (Theilacker and Shen, 1993).

To refine nucleic acid analysis of a single cell types, we utilized flow cytometry (Theilacker and Shen, 1993) to quantify DNA in the erythrocytes of rainbow trout, *Oncorhynchus mykiss*, larvae. These cells were chosen because they are present in quantity, free of contamination from other tissues and already exist in a suspension of single cells suitable for flow cytometry (Darzynkiewicz, 1991). The objective of this study was to determine condition factor, specific growth rate, and DNA synthetic activities during the G2 and S stages of the cell division cycle, and to use these values, as indicators of the physiological condition of fed or starved rainbow trout.

MATERIAL AND METHODS

Rainbow trout fry were purchased in October 1994 from Spring Valley Trout Farm, Langley, British Columbia and were acclimated to laboratory conditions. Water temperature was ±10°C. The 79-day old fry were cultured during 29 days in a flow through system at the West Vancouver Laboratory. The fry were fed *ad libitum* with trout chow #3 or fasted during the experimental period. The total wet weight and total length of 20 fry were measured at 4, 9, 14, 20, and 29-day intervals in each experiment. Fry blood sampled with glass capillary by heart puncture, about 1-3 μmL, was transferred into a 1.5-mL centrifuge tube filled with 200 μL cryoprotectant on ice. The cryoprotectant was prepared with 1 mL fetal bovine serum, 1 mL dimethyl sulfoxide (DMSO), and 4 mL Eagle Minimum Essential Solution Medium (MEM). We dissociated blood cells by pipetting about 10 times in cryoprotectant, and then, immediately added an equal volume of 0.08N HCl on ice to ensure nucleic acid stability in the cell preparation (Theilacker and Shen, 1993). The fry were frozen on dry ice and the blood samples treated with cryoprotectant were kept at -20°C until nucleic acid concentrations were determined by flow cytometry using the Coulter Epics XL.

The fluorochrome, acridine orange solution was added to the thawed sample prior to flow cytometry (Protocols 21, 1978).

Condition factor (K) was calculated by $K = (W \times 10{,}000) / L3$ (Busacker et al., 1990), where L is standard length in mm and W is wet weight in mg. Specific growth rate (SGR) was obtained by $SGR = (\ln W2 - \ln W1) \times 100 / (t2 - t1)$, where W1 and t1 were total wet weight and time at the beginning of experiment and W2 and t2 final wet weight and time (Ricker, 1979).

RESULTS AND DISCUSION

Average total wet weight of the fry increased from 612.4 to 1558.6 mg for those fed *ad*

libitum, and decreased to 385.8 mg for those fasted during 29 days (Fig. 1). Average body length increments were from 39.7 mm to 52.3 mm for those fed continuously and almost similar values without much change for those fasted (Fig. 2). Therefore, condition factor (K) was maintained at approximately 10 for those which were fed and dropped to less than 7 for those maintained without any food for 29 days (Fig. 3). Specific growth rate (SGR) varied from about 6 to 9 mg g-1 day-1 for those fed *ad libitum*; however, negative growth occurred in those fasted 9 days to 29 days (Fig. 4). The percentage of blood cells

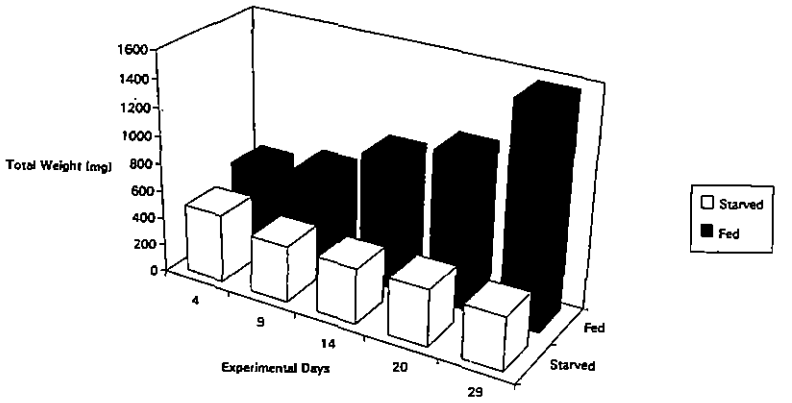

Figure. 1. Total wet weight change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.

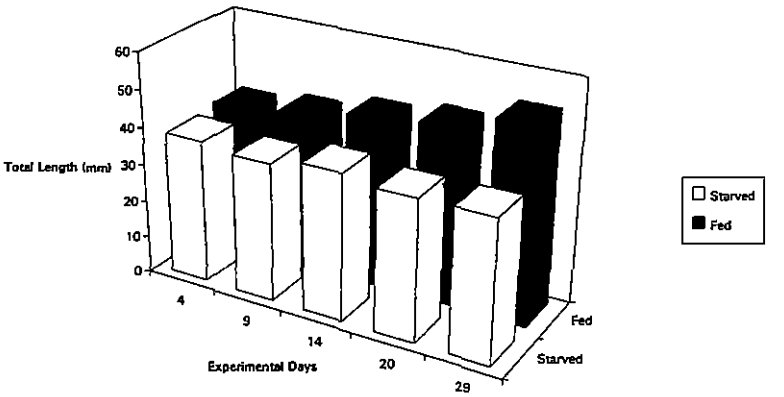

Figure 2. Total length change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.

In the G2 and S stages of the cell division cycle were 1.52-3.13% for those fed continuously and ranged from 0.28 to 2.1% for those fasted for 29 days (Fig. 5). However, percentage of cells undergoing division was always higher in those fed than in those fasted during entire experimental period. The Student t-test results indicated that all

parameters tested were significantly different ($p < 0.05$) between those fed and starved, except for total body length.

The percentages of cells in the G2 and S stages of the cell division cycle are considered to be independent from external factors, and dependent upon intracellular activities (Hartwell and Weinert, 1989; Murray and Kirschner, 1989). Therefore the fraction of

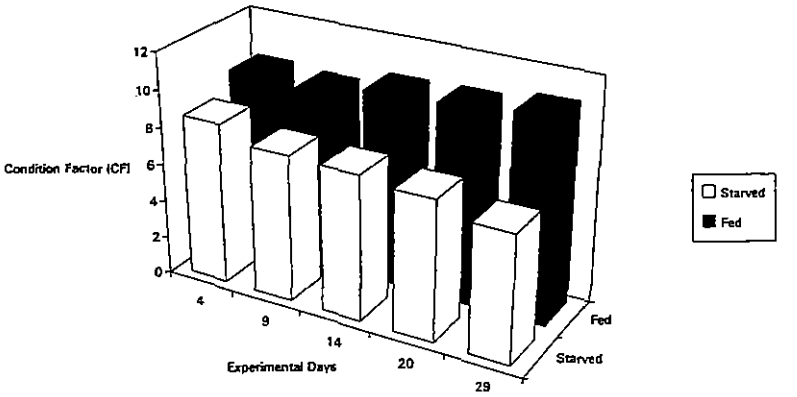

Figure 3. Condition factor change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.

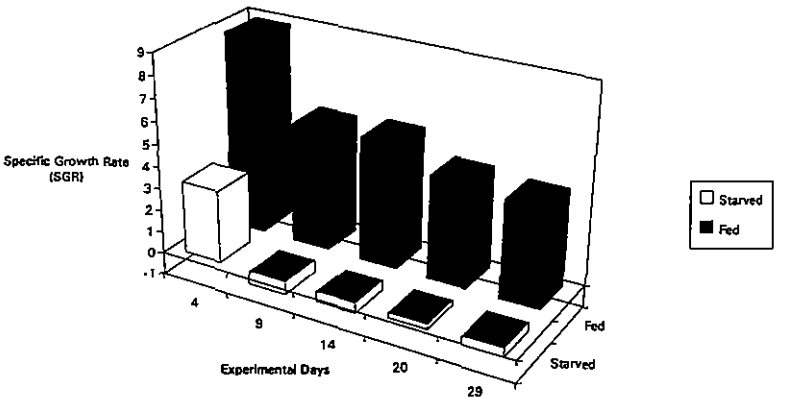

Figure. 4. Specific growth rate change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.

cells in the G2 and S stages of cell division is an indicator of the numbers of dividing cells under varying nutritional or physiological conditions. The percentages of dividing blood cells showed that the growth and nutritional conditions of the rainbow trout fry tested are well correlated with the G2 and S stages of cell proliferation.

CONCLUSION

The total body wet weight and total length changes, condition factor, specific growth rate, and percentage of blood cells in the S2 and G stages of the cell cycle indicated that nutritional status is an important factor which determines the physiological condition of rainbow trout fry. In this study we compared fry exposed to extreme conditions i.e. satiation versus starvation. It remains to be determined whether measurement of DNA synthesis can detect differences in condition after measurable moderate changes in nutritional status.

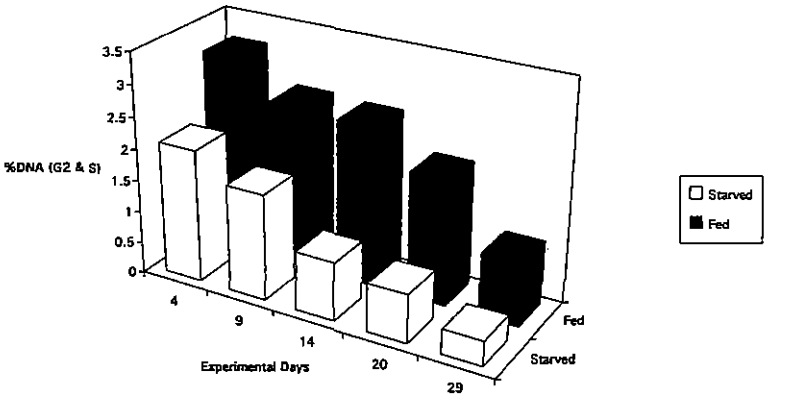

Figure 5. Percentages of the DNA synthesis activities during the G2 and S stages of the cell division cycle in rainbow trout, *Oncorhynchus mykiss*, fed and starved for 29 days.

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FEEDING EFFECTS ON RNA/DNA RATIO IN JUVENILES OF COROCORO GRUNT (*Orthopristis ruber)*

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ABSTRACT

Orthopristis ruber (42.41 ± 3.2 mm) was cultivated during 30 days at 27°C under three feeding regimes: fed with *Donax sp.* at 5% of its wet weight, 3% of bivalves plus 2% of NaA zeolite, and refed with *Donax* after 9 days starving. RNA and DNA concentrations of white muscle, using fluorometric method, were measured every three days.The RNA/DNA ratio was higher in fed fish than in those starved, confirming that feeding has an influence on the RNA/DNA levels and physiological condition of this species.

INTRODUCTION

The cellular RNA concentrations change immediately in response to the changes in food regime, while the DNA concentrations remain relatively constant. Thus, RNA/DNA ratio is a useful indicator of physiological condition in fish. The rate of growth may be limited by food availability (Chung *et. al.*, 1991; McMillan and Houlihan, 1993; Mather *et. al.*, 1993; Malloy and Targett, 1994). It has been shown that the fish starved and further re-fed *ad libitum* yield a sudden growth, and often reach the initial level or at least a compensatory growth. The physiological basis of this phenomenon has not been demonstrated, but it is known that animals subjected to starvation and then re-fed can eat a larger amount of food and are more efficient in transforming the food into energy than those kept on a normal food regime (Miglavs and Jobling, 1989). On the other hand, aquaculturists want to obtain a larger, heavier fish for commercial purposes and have therefore used a complementary element in the fish diet to disminish the cost and time of growth in these organisms. Sodalite has been used as a dietetic complement, since 1965 (Mumpton and Fishman, 1977). Japanese aquaculturists initiated to use it. Zeolites have been used in animal diets because of their property of slowing down the passage of nutrients in the digestive system and also their capability of ionic exchange with ammonium ion. Furthermore it has been shown that zeolite aggregated to animal diet not only gives significant increases in body weight gained by unit of food consumed, but also favours some aspects in animal health, controlling diarrhea and other intestinal disorders. In this sense, the aim of this study is to obtain information of instantaneous and individual growth from fish in different food regimes while measuring RNA and DNA

concentrations in white muscle using fluorometric technique and expressed as RNA:DNA index.

MATERIAL AND METHODS

The experiment was done with juvenile specimens of Corocoro grunt (*Orthopristis ruber*), collected by seine in Turpialito Bay, Gulf of Cariaco, Sucre State, Venezuela. The standard length was 42.41 ± 3.2 mm. Afterwards the fish were held in acuaria for acclimation to 27°C during four weeks. The bioassays were performed in three different food regimes: a) fed with 5% of chipichipi *Donax* sp., b) starved and then re-fed with 5% of chipichipi, and c) continuosly fed with 3% of chipichipi plus 2% of zeolite (type NaA) synthesized in the laboratory. Eight fish were taken every three days during the feeding and fasting periods and every day during the delayed feeding experiment (three days) and then every three days for eighteen days more. Immediately they were frozen with dry ice and stored at -17°C until nucleic acid concentrations were measured. The fluorometric method proposed by Karsten and Wollenberger (1972, 1977) was used with a modification in ribhonuclease concentration, increased from 25 µg/ml to 100 µ/ml and incubation time from 20 minutes to 30 minutes in order to be sure that all the RNA was hydrolized by the enzime. For protein determinations the Bradford (1976) method, using comassie blue with a calibration curve prepared with bovine albumin serum, was employed.

RESULTS AND DISCUSSION

The results obtained on the instantaneous growth rate measured by the RNA/DNA index during the experimental period showed that the RNA/DNA ratio was higher in fed fish than in starved fish (Table 1).

In starved fish, the RNA/DNA ratio resulted in a significant decrease during three further days (from 3.60±0.17 to 2.21±0.04; $p<0.01$, Table 2), accentuated more by the ninth day with a level of less than one fourth of its initial level (0.56±0.01). This was probably due to the fish using their reservoir of muscle proteins to maintain their primordial functions instead of using that energy for growth. This has been demonstrated with the decreasing of their muscle protein and RNA concentrations (Table 1). When the fish were re-fed, this index increased during the first three days of the re-feeding period and reached more than half of its initial value (1.94±0.06), recovering initial value by the fourth day of re-feeding (4.56±1.76). The increase in RNA concentrations may imply a higher protein synthesis. This finding agrees with previous results: the RNA/DNA ratio may be affected by food availability (Bulow, 1981, 1987; Clemmesen, 1988; Chung *et al.*, 1991; Segnini *et al.*, 1994). These studies also indicate that changes in the ratio can be detected from the beginning of starvation, and during starvation the body uses this protein to maintain necessary activities and to replace vital tissues.

DNA concentration value per unit weight in starved fish increased (from 0.26±0.01 to 0.69±0.01) by the ninth day of starvation. DNA concentration decreases may be due to the increase in the number of cells per unit weight (Bulow, 1987).

On other hand, the RNA/DNA ratio of fish fed with bivalves showed a similar tendency to those fed with bivalves plus zeolites type NaA until the eighteenth day. At the end of the experiment the fish fed with a complement of zeolite type NaA had a higher ratio (11.69±0.23), indicating that the growth pattern is favoured by this feeding condition, and the

organisms have a tendency to grow more effectively because of higher RNA synthesis for zeolite type NaA + bivalves (3.07±0.03) than with only bivalves (2.59± 0.03). This fact implies a higher protein synthesis and faster growth, probably due to zeolite advantages, which help intestinal absorption of nutrients by slowing the speed of food passage through the digestive tract and thus favouring the metabolic absorption of mineral by increasing food conversion. This fact has been demonstrated by many authors (Escurra and Pérez, 1984; Yokohama, 1984; Waldroup,1984; Soca, 1993)

Table 1. Mean values of RNA, DNA and Proteins concentrations (µg/mg) and the RNA/DNA ratio in *Orthopristis ruber* fed continuosly with chipichipi, fasted and re-fed with chipichipi, and fed continuosly with chipichipi + NaA zeolite for 30 days.

Day	RNA (µg/mg)	DNA (µg/mg)	Proteins (µg/mg)	RNA/DNA
Fed with chipichipi only				
0	0.90±0.01	0.29±0.01	209.25± 3.96	3.04±0.08
03	0.78±0.01	0.29±0.01	213.82± 3.44	2.71±0.06
06	0.86±0.01	0.25±0.01	203.15± 3.73	3.39±0.20
09	1.81±0.06	0.33±0.01	237.21± 2.10	5.54±0.15
10	1.15±0.02	0.29±0.01	256.31± 2.28	4.01±0.01
11	1.17±0.01	0.27±0.01	268.13± 2.67	4.27±0.14
12	1.81±0.04	0.28±0.01	287.93± 7.55	6.64±0.56
15	1.89±0.05	0.31±0.03	290.42± 2.86	6.20±0.86
18	2.02±0.06	0.29±0.04	330.82± 3.64	6.96±0.75
21	2.47±0.03	0.33±0.01	337.69± 4.53	7.45±0.30
24	2.46±0.03	0.34±0.01	402.14± 8.74	7.29±0.16
27	2.49±0.01	0.33±0.01	406.62± 2.86	7.60±0.28
30	2.59±0.03	0.34±0.02	408.27± 3.67	7.74±0.33
Starved and re-fed with chipichipi only				
0	0.95±0.03	0.26±0.01	229.11± 5.75	3.60±0.17
03	0.76±0.01	0.34±0.01	151.93±18.92	2.21±0.04
06	0.61±0.01	0.49±0.01	72.68± 8.38	1.23±0.05
09	0.38±0.01	0.69±0.01	20.92± 2.88	0.56±0.01
10	0.58±0.01	0.35±0.03	110.66± 4.86	1.66±0.16
11	0.53±0.02	0.28±0.02	158.55± 5.68	1.94±0.06
12	0.80±0.05	0.32±0.01	211.96± 6.33	2.48±0.25
15	0.93±0.01	0.23±0.06	296.24± 2.66	4.56±1.76
18	1.50±0.01	0.30±0.01	314.98± 4.35	4.96±0.12
21	1.95±0.08	0.30±0.01	355.21± 3.78	6.53±0.11
24	2.43±0.02	0.32±0.01	414.50± 3.93	7.57±0.22
27	2.49±0.01	0.33±0.01	417.11± 4.99	7.62±0.21
30	2.52± 0.01	0.33±0.01	430.37± 9.34	7.65±0.18

Table 1 (continued).

Day	RNA (µg/mg)	DNA (µg/mg)	Proteins (µg/mg)	RNA/DNA
Fed with chipichipi and NaA zeolite				
0	0.89±0.01	0.30±0.01	212.12± 2.09	3.02±0.04
03	0.78±0.03	0.34±0.01	288.39± 4.81	2.31±0.13
06	0.84±0.01	0.28±0.02	311.51±12.95	3.04±0.16
09	0.92±0.01	0.25±0.01	343.01±10.08	3.67±0.10
10	1.38±0.05	0.30±0.02	369.33± 8.00	4.64±0.34
11	1.78±0.07	0.34±0.01	381.93± 3.32	5.21±0.22
12	2.01±0.04	0.37±0.01	408.78± 9.04	5.46±0.05
15	2.09±0.01	0.35±0.01	425.59± 5.80	6.05±0.09
18	1.96±0.04	0.26±0.01	413.16±11.13	7.43±0.44
21	2.13±0.03	0.26±0.01	440.41± 5.64	8.24±0.18
24	2.59±0.01	0.29±0.01	530.61±10.79	8.80±0.16
27	2.74±0.03	0.29±0.01	566.10± 9.54	9.44±0.25
30	3.07±0.02	0.26±0.01	654.26±15.56	11.69±0.23

Table 2. Results (F-values) of ANOVA for *Orthopristis ruber* fed, starved and re-fed during the experimental period.

Day	Fed vs Starved-Refed		Fed vs Fed (NaA Zeolite)	
	RNA/DNA	Protein	RNA/DNA	Protein
0	26.32 **	24.26 **	0.19 NS	1.23 NS
03	139.26 **	31.08 **	24.10 **	477.45 **
06	322.19 **	766.13 **	5.49 *	135.28 **
09	3178.95 **	11024.26 **	325.06 **	316.60 **
10	607.29 **	2205.76 **	9.66 **	554.27 **
11	698.47 **	915.89 **	38.21 **	2143.71 **
12	139.04 **	178.04 **	13.40 *	315.72 **
15	2.10 NS	6.65 *	0.09 NS	1310.92 **
18	20.96 **	23.41 **	0.86 NS	148.30 **
21	24.65 **	26.42 **	15.08 **	604.04 **
24	3.13 NS	4.99 *	136.79 **	256.71 **
27	0.004NS	9.97 **	72.14 **	769.29 **
30	0.15 NS	14.55 **	283.75 **	7 09.99 **

NS: Not significant
*: 5% level of significance
**: 1% level of significance

CONCLUSSION

From the results it can be concluded that it is posible to monitor the growth of fish measuring RNA/DNA ratio, which indicates a useful index for evaluating nutritional status and a physiological condition of this species. Also it is able to infer that feeding with zeolite type NaA as a dietetic complement in corocoro grunt has a positive influence on RNA/DNA ratio, indicating that this complementary food regime is better than food regime without it.

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Effect of Ration. Temperature and Body Weight on Growth of Black Porgy , *Sparus macrocephalus* (B.)

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Growth of black porgy, *Sparus macrocephalus*, was studied experimentally at four ration levels from starvation to *ad libitum* and four temperature ranging from 14.8 to 26.8℃. At maximum rations, the specific growth rate (SGR) increased with increased temperature and was negative, linear function of body weight. The relationship between SGR and ration levels was a decelerating curve. The method of stepwise regression was used to develop the predictive models for the specific growth rates. Maintenance rations and optimum rations both increased with increased temperature. The relationship between maintenance、optimum rations and body weight was influenced by temperature. Conversion efficiencies increased with ration from zero at maintenance ration to a peak at the optimum ration, then declined with further increased ration.

Ⅰ. INTRODUCTION

It is known that growth rate of fish can vary in response to temperature and food availability, and perhaps less obviously, to light, oxygen and other variables. This property of variable growth in fish can be referred to as plasticity (Weatherley, 1990). The effects of environmental factors on growth can be predicted for effective management of fisheries. A bioenergetics model of growth, offering a powerful potentially tool, should predict the relationship between growth rate and these factors (Brett, 1979, Elliott, 1979).

This paper reports an experimental study on the growth of black porgy, *Sparus macrocephalus* (B.), a important mariculture species in China and Japan, in relation to ration, body weight and temperature. Empirical regression models were also developed to describe the quantitative relationship between the growth of the black porgy and these factors. The models provide an alternative technique for predicting the growth of this species in natural population (Allen & Wootton, 1982). In addition, the maintenance and optimum rations and conversion efficiencies were discussed.

Ⅱ. MATERIALS AND METHODS

Black porgy used for the feeding-growth experiments were collected from artificial

breeding in spring 1994. The experiments were carried out from August 1 to November 11, 1994 at mean temperature of 26.8, 24.4, 20.1 and 14.8℃ respectively. At each temperature, there were four ration levels ranging from starvation to *ad libitum*. The fish were starved for 24h before the start of an experiment. On the first day of each experiment, the fish were blotted of excess seawater and weighed two to six fish of similar weight and transferred to individual plastic tanks containing 80 l seawater. The experimental fish were fed the prescribed weight of thawed *Ammodytes personatus* twice daily at 9:00 and 16:00. Uneaten food was collected 1h later and reweighed. Each experiment last 20 days. At the end of experiments, the fish were starved 24h and weighed. The carcase were dried at 65℃ to constant weight and reweighed. During the experiments, several weighed samples of food were collected and dried. The energy content of fish and food were determined using Phillipson Oxygen Bomb Calorimeter. At the start of each experiment, eight fish were killed and provided estimates of the initial dry matter and energy content.

Ⅲ. RESULTS

The specific growth rate in dry body weight was calculated as

$$SGR_D=100(\ln W_t-\ln W_o)/t$$

Where W_t is the final and W_o the initial dry weight of fish, and t is the number of days(=20). Specific growth rate in energy (SGR_E) were calculated similarly. Mean values of specific growth rates of black porgy at each ration and temperature are shown in Table 1 .

Tab.I. The specific growth rate and conversion efficiency of the black porgy in relation to ration level

T	Initial weight (g)		Number	Ration(% B.W/day)		SGR_E(%/day)		SGR_D(%/day)		K_D(%)		K_{DN}(%)	
(℃)	mean	S.E	of fish	mean	S.E	mean	S.E	mean	S.E	mean	S.E	mean	S.E
26.8	7.70	1.04	36	7.90	0.32	2.8538	0.3847	2.9677	0.3186	36.62	5.24	53.23	8.93
	6.41	1.90	36	6.67	1.06	1.4611	0.4246	1.6809	0.3706	24.29	3.93	37.73	4.94
	6.73	1.85	35	3.39	0.21	-1.2446	0.2381	-0.4195	0.2483	-	-	-	-
	7.74	1.19	24	0	-	-2.8969	0.4269	-1.7695	0.1643	-	-	-	-
24.4	24.12	4.37	24	7.58	0.75	1.9801	0.2877	2.0568	0.2789	27.44	8.44	42.92	13.83
	20.95	6.45	22	4.07	0.77	-0.2799	0.0993	0.4222	0.0966	10.34	2.08	31.78	13.91
	20.43	6.18	22	2.78	0.34	-1.2058	0.1551	-0.3301	0.1235	-	-	-	-
	23.56	9.91	24	0	-	-3.4039	0.0209	-2.1548	0.2077	-	-	-	-
20.1	37.63	16.25	12	5.64	0.27	2.3504	0.2362	2.0559	0.2337	37.02	5.45	57.93	8.39
	35.97	8.94	12	4.32	0.53	1.2863	0.1705	1.0703	0.1750	24.55	0.91	42.70	2.55
	38.40	15.52	11	2.48	0.10	-0.2369	0.0990	-0.0539	0.1759	-	-	-	-
	40.78	14.66	12	0	-	-2.5541	0.0792	-1.7124	0.2234	-	-	-	-
14.8	48.52	12.81	18	2.18	0.37	0.2170	0.0341	0.2422	0.0190	7.84	1.47	24.92	3.60
	58.94	10.24	18	2.60	0.26	-0.6773	0.2019	-0.4541	0.1162	-	-	-	-
	51.70	11.90	18	1.57	0.19	-1.2760	0.3538	-1.0683	0.1571	-	-	-	-
	49.57	5.33	18	0	0	-3.4066	0.3060	-2.4353	0.2764	-	-	-	-

At maximum rations, specific growth rate increased with increased temperature. The regression models describing the relationships are following:

$$SGR_D=-1.228(\pm 1.169)+0.961(\pm 0.383)\ln T \quad (R^2=0.8667) \qquad (1)$$

$$SGR_E=-10.004(\pm 1.459)+3.895(\pm 0.478)\ln T \quad (R^2=0.9178) \qquad (2)$$

In these equations SGR is specific growth rate (%/day) in either dry weight or energy, and T is temperature (℃); standard errors of parameters are given in parentheses.

At 20.1℃, specific growth rate negatively correlated to body weight and the linear regression between specific growth rate in dry weight and dry body weight was described in the equation below:

$$SGR_D=3.46(\pm 0.152)-0.060(\pm 0.010)W \quad (R=-0.9502) \qquad (3)$$

At each temperature, the specific growth rate increased curvilinearly with ration levels (Fig.1), starting from a negative value at starvation. At all temperatures, ration level had a significant effect on the specific growth rates and regressions describing these relationship were set up using the method of Allen & Wootton (1982):

$$SGR=a+b\ln(RL+1)$$

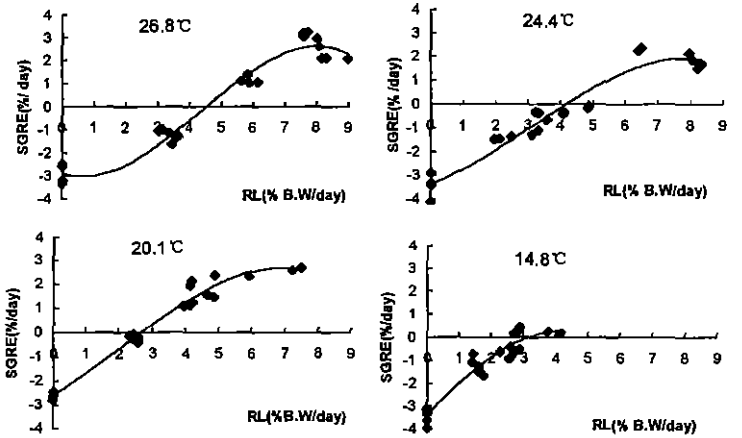

Fig.1 Relationship between specific growth rate in energy (%/day) and ration (% body weight/day) for black porgy

Where a and b are constants, RL the ration level as a percentage of mean dry body weight per day. The parameters of regression equations were exhibited in Table II.

Empirical models to predict specific growth rate were developed by using the following as predictor variables: ln(RL+1), where RL is ration level as percentage of mean dry body weight per day, lnW, where W is mean dry body weight in g. and lnT, where T is temperature in ℃(Allen &

Tab.II. The regression relating specific growth rate (SGR_D and SGR_E) to ration level in black porgy

Tempe-	SGR_D			SGR_E		
rature(℃)	a	b	r	a	b	r
26.8	-2.3101 ± 0.4068	2.0207 ± 0.2334	0.8885	-3.5513 ± 0.4847	2.4837 ± 0.2781	0.8942
24.4	-2.3088 ± 0.1634	1.8395 ± 0.1089	0.9635	-3.6777 ± 0.2456	2.3206 ± 0.1037	0.9494
20.1	-1.8736 ± 0.1549	1.8489 ± 0.1103	0.9630	-2.7224 ± 0.1736	2.4504 ± 0.1236	0.9731
14.8	-2.4832 ± 0.1227	1.7055 ± 0.1147	0.9537	-3.4111 ± 0.1506	2.3320 ± 0.1408	0.9622

Wootton, 1982), All possible interaction terms were initially included in the full model which was fitted to data, using the backward regression procedure to reduce the number of predictor variables where possible. The resulting models were:

$$SGR_D=-2.105+3.052\ \ln W+1.934\ln(RL+1)-1.005\ \ln T\ln W \quad (R^2=0.8810) \quad (4)$$

$$SGR_E=-3.645+5.040\ \ln W+2.423\ \ln(RL+1)-1.5589\ \ln T\ \ln W \quad (R^2=0.8831) \quad (5)$$

The multiple correlation coefficients and partial correlation coefficients of both models shown significant and ration level was most sensitive to specific growth rate among these terms. The relationship between specific growth rate and body weight were complicated and determined by temperature.

At the maintenance ration, there is not net change in the weight or the total energy content. Maintenance rations were estimated from the regression models (4) and (5) relating specific growth rate to ration levels, temperature, dry body weight and their interaction terms. The equation for the maintenance ration for growth in energy content (R_{mainE}) was

$$R_{mainE}=4.500W^{(0.644\ln T-2.080)}-1 \quad (6)$$

The equation for the maintenance ration for growth in dry body weight (R_{mainD}) was

$$R_{mainD}=2.969W^{(0.519\ln T-1.578)}-1 \quad (7)$$

Where R_{mainE} and R_{mainD} were expressed as percentages of dry body weight per day. Both maintenance rations increased with increased temperature curvilinearly.

The optimum ration is the ration at which the ratio of growth rate to ration is a maximum. The optimum rations for the black porgy were estimated from the models of specific growth rate using the method of Allen & Wootton (1982). The equation for the optimum ration for growth in energy content (R_{optE}) was

$$R_{optE}=12.234W^{(0.644\ln T-2.080)}-1 \quad (8)$$

The equation for the optimum ration for growth in dry body weight (R_{optD}) was

$$R_{optD}=8.070W^{(0.519\ln T-1.578)}-1 \quad (9)$$

Where R_{optE} and R_{optD} were expressed as percentages of dry body weight per day. Both optimum rations increased with increased temperature. The comparison of maintenance and optimum rations demonstrated that there were consistent trends in these two types of model.

The gross and net conversion efficiencies for dry weight were shown in table I . The gross conversion efficiency (K_D) was calculated as

$$K_D=100(W_t-W_0)/C$$

where W_t is the final and W_0 the initial dry body weight, and C is the total food intake in dry weight. The net conversion efficiency (K_{DN}) was calculated as

$$K_{DN}=100(W_t-W_0)/(C-C_{main})$$

where C_{main} is the food intake in dry weight deduced from maintenance ration model

(equation 7).

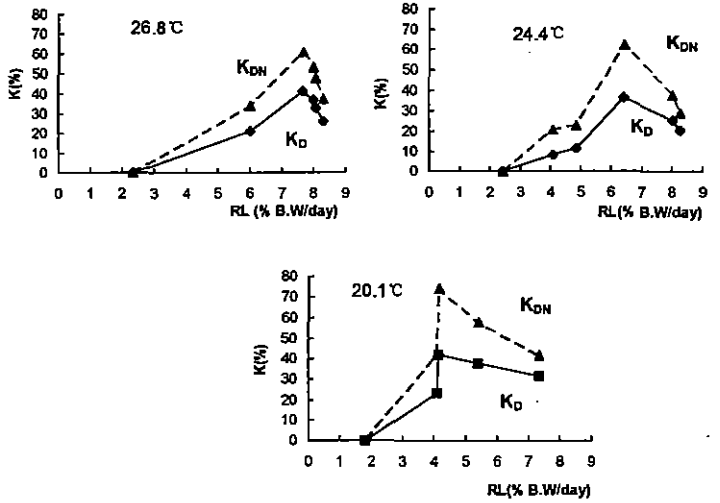

Fig.2 Relationship between mean conversion efficiencies and ration (% body weight/day) for black porgy at different temperature

The mean value of K_D and K_{DN} plotted against rations are shown in Fig .2. The curves started from the maintenance rations, where the conversion efficiencies are, by defination, zero. and reached the peak, the optimum ration, and then declined with further increased ration. The gross and net conversion efficiencies demonstrated similar trends at each temperature

IV DISCUSSIONS

There were several studies on the effects of environmental factors on growth rate and the effects of ration, temperature and body weight were remarked among these factors (see review by Webb, 1978; Brett, 1979; Elliott, 1979,1982; Weathereley & Gill, 1987; Weatherley, 1990). When food were abundant, the growth rate of fish generally deceased with increased body weight (Elliott, 1979; Jobling,1983). At maximum ration, the specific growth rate of black porgy were negative, linear function of body weight at 20.1℃.

The effect of temperature on growth rate reflect inconsistent in the fish studies and growth rate at maximum rations is usually highest at an intermediate temperature called the optimum temperature for growth. The specific growth rate increased with temperature below this temperature and the result turned out contrary over this temperature (Brett, 1979; Elliott, 1979; Cui & Wootton,

1988). The optimum temperature for growth were not found in several experiments so that the growth rate at maximum ration positively correlated to temperature (Smith, 1986; Cui & Wootton, 1987). In this study, at maximum ration the multiple regression equations relating specific growth rate to dry body weight, temperature and their interaction term were obtained using stepwise regression:

$$SGR_E=-10.004+3.896\ \ln T \quad (R^2=0.7399) \qquad (9)$$
$$SGR_D=-10.816+4.154\ \ln T \quad (R^2=0.8353) \qquad (10)$$

It was indicated that specific growth rate increased with increased temperature and temperature was more sensitive to growth rate than body weight.

Food supply is probably the most potent factor affecting the growth in fish (Brown, 1957), while other factors, either abiotic or biotic, only indirectly affect growth because they affect feeding and metabolism (Klaoudatos & Apostolopoulos, 1986). At a given temperature, growth rate is a decelerating, curvilinear function of ration in several species (Elliott, 1979; Cui & Wootton, 1988). At a high ration a further increase in ration result in little or no increase in growth rate and the maximum ration was defined as the ration above which growth rate did not increase (Bertt et al., 1969). This study on black porgy demonstrated the similar result with other researches. From equation (4) and SGR-RL model (SGR=a+b ln (RL+1)), the coefficient equations were obtained:

$$a=-3.6450+(5.0397-1.5589\ \ln T)\ln W \qquad (11)$$
$$b=2.4233 \qquad (12)$$

The study on southern catfish (Silurus meridionalis) (Xie, 1989) indicated that maintenance metabolic rate (a) decreased with increased body weight and increased with temperature, and the net energy conversion index (b) positively correlated to temperature. In this study, the relationship between a and temperature shown similar trend with southern catfish and the relationship between a and body weight was in fluenced by temperature. The coefficient b in black porgy was constant confirmed by the slope in regression relating specific growth rate in energy content to ration levels (Table II).

Empirical models relating growth rate to ration, body weight and temperature were developed in several studies. Elliott (1979) suggested that ration should be expressed as a percentage of maximum ration. Smith et al. (1986) set up a empirical models on the growth of walleye pollock (Theragra chalcogramma) in which the rations. were expressed in calories and % B.W. However, the expression of a percentage of body weight was a better predictor of growth rate in minnows, sticklebacks and juvenile rockfish (Cui & Wootton, 1988; Allen & Wootton, 1982, Bochlert & Yoklavich, 1983). In the present the determination coefficients of the models were high so that the models were useful tools for prediction of growth rate of black porgy.

Equations relating maintenance and optimum rations to body weight and temperature were deduced from the empirical models for growth rate. These equations predicted that both maintenance and optimum rations increased with increased temperature. The same trend has been observed in other studies (Brett, 1979; Elliott, 1982; Allen & Wootton, 1982; Cui & Wootton, 1988). It was also indicated that, in this study, the maintenance and optimum ration

increased with increased body weight over 20.9℃ and were inverse function of body weight below that temperature. The relationship to temperature of maintenance and optimum ration were predicted in Fig.3. Both ration levels were sensitive to temperature.

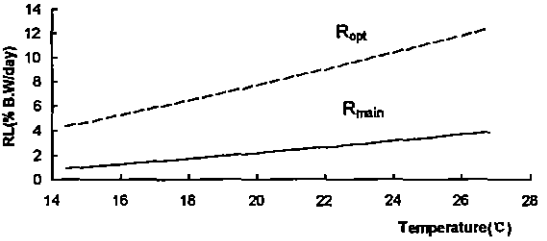

Fig.3 Predicted relationship to temperature of maintenance and optimum rations (% body weight/day) for black porgy weighing 10g (dry weight)
------optimum ration; _____maintenance ration

As ration increased above the maintenance ration, the gross conversion efficiencies of black porgy increased up to a maximum at the optimum ration and then declined with a further increment of ration. This relationship confirmed the general pattern of research in other species (Brett, 1979; Elliott, 1979; Weatherley & Gill, 1987, Cui & Wootton, 1988).

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FLESH PIGMENTATION OF *Clarias gariepinus* (Burchell 1822): UPTAKE AND DEPOSITION OF DIETARY CAROTENOIDS

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Abstract

Flesh colour enhancement by inclusion of cartotenoids in the diet of the African sharptooth catfish, *Clarias gariepinus* (Burchell 1822), was studied. Fish were fed diets supplemented with either beta carotene, carophyll red (canthaxanthin), carophyll pink (astaxanthin) or an algal source of astaxanthin (*Hematococcus pluvialis*). Catfish were fed for 29 days at an active ingredient level of 100 mg/kg. After samples of muscle revealed some but not distinct colour uptake, levels in the feed were increased to 250 mg/Kg and feeding continued for another 31 days. After 60 days of feeding there were significant visual differences between carotenoid fed fish and control fish fed a diet without colouring agents. Spectophotometric measurements of acetone extract from muscle samples confirmed visual observations and showed that the flesh of catfish fed carophyll pink contained the most colour, about 4 times that of control fish, and double that of fish fed on beta carotene, carophyll red and *H. pluvialis*.

Introduction

Fish are not capable of synthesizing their own carotenoid pigments, and therefore depends on introduction of pigments via the food (Schiedt *et al.* 1985; Simpson and Kamata 1979). The technique of introducing pigments into flesh and skin of many cultured fish via their food is in use worldwide and is considered obligatory to maintain product value and consumer acceptance (Ellis 1979). For example, skin colour of hobby fish determines the

value of the product and salmon farmers are forced to add pigments to fish diets so their product is similar to wild caught salmon (Smith *et al.* 1992, Ellis 1979; Simpson and Kamata 1979). However, the naturally uncoloured flesh of other species such as the channel catfish is preferred by the consumer, eliminating the need for colour enhancement (Lovell 1984). African catfish, *Clarias gariepinus*, is considered a medium quality fish and is generally marketted as a grey or pale fleshed product. In the present work, an attempt has been made to achieve a pinkish flesh, aiming at improving catfish quality. The pinkish meat could be marketed smoked or as fresh fillet.

The purpose of this experiment was to study the effects of the carotenoids beta carotene, astaxanthin and canthaxanthin, supplied in the diet, on flesh colouration of the African sharptooth catfish.

Materials and Methods

Groups of 7 juvenile catfish (avg weight 225 g), reared from eggs, were stocked into one of 4 experimental tanks and left for one week to acclimate. The experimental tanks (84 L; 50x70x24 cm) were within a 1200 L recirculation system which included a water purification unit and received 100 mls min-1 of fresh water. Tanks were provided with aeration and water temperature was 27°C.

Diets were prepared by fine-grinding (AEG, Germany) pigment-free, commercial fish feed pellets, mixing in colouring agent, adding water, and extruding (WLS Loser, GmbH Co., Germany) to make 2 mm (dia) x 5 mm (length) pellets which were subsequently air dried. Three colouring agents, carophyll pink (astaxanthin), beta-carotene, and carophyll red (canthaxanthin; all Hoffman La Roche) were added at concentrations of 100 $mg \cdot Kg^{-1}$ (active ingredient concentration) and a fourth colour free diet was prepared (control). Fish were fed at a rate of 4% of biomass four times a day.

After 31 days of feeding, one fish from each group was killed and samples of muscle and skin collected. Samples were compared visually, for differences in colouration, by a panel of 4 people . Observations at this time indicated that some pigment had been deposited in the flesh, but differences were not distinct. Subsequently, feeding of experimental diets continued and the concentration of pigment was increased to 250 $mg \cdot Kg^{-1}$. At this time a fourth experimental group was introduced and fed on a diet containing extract of the microalgae *Haematococcus pluvialis* (Fan *et al.* 1995; Boussiba and Vonshak 1991). The *H. pluvialis* contained 4% astaxanthin and cell walls had been disrupted prior to addition into the feed, which was prepared to give a final astaxanthin concentration of 250 $mg \cdot Kg^{-1}$. Feeding of the

4 experimental diets continued for another 29 days, after which 3 fish were sampled from each treatment. Subsequently, all fish were fed the control feed and muscle and skin samples of one fish were collected after 10, 20 and 30 days.

Before sampling, fish were moved from the recirculating system to a fresh water flow through system and food was withheld. After 2 days in fresh water, they were placed in a slurry of ice water for 1 hr, then removed, quickly decapitated, gutted and flesh samples collected from the dorsal musculature and stored at -30°C for subsequent analysis. At the time of sampling, there was also a visual comparison of the extent of colouration in muscle samples from different treatments .

Spectrophotometric quantification of colouration of muscle and skin was done by measuring the absorbency at 470 μm following acetone extraction. Prior to extraction samples were freeze dried and ground to a fine powder with mortar and pestle. The relative amount of colour was expressed on a sample dry weight basis.

Results

Fish in all groups grew during the experiment, indicating that food containing pigment was consumed and utilized. The specific growth rate and food conversion efficiency are shown in Table 1.

Table 1. Specific growth rate and food conversion efficiency of catfish fed different diets

Treatment	Specific Growth Rate (% / day)	Food Conversion Efficiency
Control	1.82	0.44
Carophyll pink	1.52	0.49
Carophyll red	0.83	0.20
Beta Carotene	1.18	0.34
H. pluvialis	0.89	0.33

Visual assessment of the muscle samples indicated that carotenoid pigments had been stored in the muscle but probably not the skin of catfish. The differences between treatments were still visually apparent up to 30 days after feeding of carotenoids had stopped.

Spectrophotometric measurements of the extract of muscle tissue confirmed visual observations with fish fed astaxanthin showing the greatest degree of flesh colouration (Figure 1).

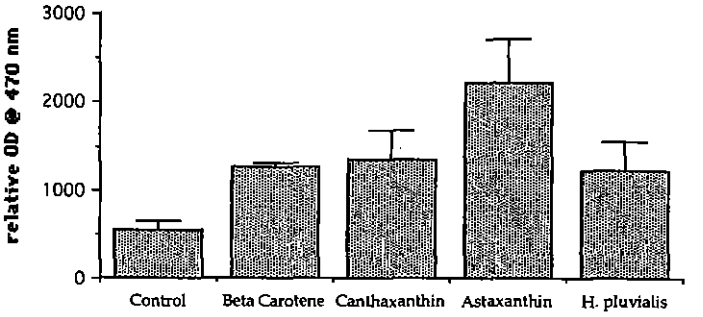

Figure 1. Weight adjusted relative OD at 470 nm of muscle samples of *C. gariepinus* fed diets supplemented with carotenoids.

Discussion

This study shows that carotenoid pigment, when supplied in the feed, is taken up and deposited in the flesh of *C. gariepinus*. This preliminary study suggests that the levels of pigment required to give a desirable muscle colouration may be higher than is required in trout or salmon (Torrissen 1985). Further studies manipulating dose and feeding duration are required before recommendations can be made to aquaculturists.

The uptake/deposition of the 3 different carotenoids in catfish are similar to the results of experiments on salmonids (Schiedt *et al.* 1985) as beta carotene did not result in as much flesh colouration as carophyll pink. Muscle colouration in catfish fed carophyll red was also less than those fed carophyll pink and these findings are similar to those of Bjerkeng et. al. (1990) showing canthaxanthin uptake less efficient than astaxanthin uptake in rainbow trout. Deposited carotenoids also appeared to be quite stable in catfish flesh with little depletion after 30 days, a finding similar to that of Choubert (1985) with rainbow trout.

The reason that fish fed on a natural source of astaxanthin (*H. pluvialis*) were less coloured than those fed synthetic astaxanthin (carophyll pink) may be the duration of carotenoid feeding as those on carophyll pink were fed for an additional month at 100 mg/Kg. It may also be that the naturally occurring astaxanthin in *H. pluvialis* is less available to catfish compared to carophyll pink because of digestibility differences or degradation of the

astaxanthin through oxidation (Bubrick, 1991). Less efficient uptake of astaxanthin from *H pluvialis* compared to carophyll pink has been shown in rainbow trout (Sommer *et al.* 1991).

In conclusion, this study shows that *C. gariepinus* absorb dietary carotenoids, resulting in improved flesh colouration. A further study will quantify the specific feeding procedures (eg. dose and duration) required to produce a higher value product.

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