SYNOPSIS

Leiognathids (Order: Perciformes, Family: Leiognathidae) commonly called silverbellies or pony fish are benthopelagic fish occupying an important position in the demersal fishery resource of our country. Silverbellies are widely distributed in the coastal water of tropical and sub-tropical regions. Even though, a little quantity is consumed in fresh condition, silverbellies are favourite item in dry fish and other fish byproduct industry of our country. These morphologically conservative fish often pose many taxonomic problems to fish taxonomists which lead to many misidentifications that plague the family for years. They often found in mixed assemblages of a few to several species and inhabit the turbid coastal and estuarine waters; occasionally ascends into freshwater reaches of rivers. This fish species are specially noticed for the 'chirping' sound production, slippery body due to mucus secretion, protracted mouth tube and emission of symbiotic bioluminescent light from an internally located light organ. It is one of the most commercially important 'by-catch' fish species in fishery industries due to high quantity of bone and fatless flesh that provide plenty of calcium and protein sources.

OBJECTIVES OF THE STUDY

A survey on diversity of silverbelly community of Kerala coast along with a detailed analysis of food and feeding habits, reproductive biology, length - weight relationship and light organ and sexual dimorphism in some dominant leiognathid species available are aimed in this study. Present study was conducted during March 2005- February 2007 along Kerala coast (South West coast of India).

1. To gather information on the current systematic account of Leiognathidae by preparing keys for identification of genera and species. Species identification was reaffirmed based on their meristic counts and morphometric characteristics.

2. A detailed study on the diet and feeding habits of three leiognathid species; *Gazza minuta*, *Leiognathus brevirostris* and *Secutor insidiator* is aimed. Study also focused on comparative mouth morphology in these 3 co-occurring species as well as to quantify the degree of overlap in the diet.

Seasonal and ontogenetic variations in the feeding intensity and types of prey items consumed by the fish is also aimed with a view to get information on the functional role of these fishes in their ecosystem.

3. To compute length –weight relationship equation for 3 species; *Gazza minuta*, *Leiognathus brevirostris* and *Secutor insidiator* in order to get information on the type of growth exhibited by them. Study also aimed to investigate the relative condition factor of these 3 species in a month and length group basis.

4. Study is aimed to get information on the breeding season, spawning frequency, fecundity and relative fecundity, size at first maturity and sex ratio of 3 silverbelly species; *Gazza minuta*, *Leiognathus brevirostris* and *Secutor insidiator* which would provide management options for the protection of its spawning stock and to ensure a sustained yield.

5. Presence of bioluminescence in silverbellies forced to conduct a study on the inter and intra specific variation in the light organ of 3 leiognathids; *Leiognathus brevirostris*, *Photopectoralis bindus* (syn. *Leiognathus bindus*) and *Secutor insidiator.* Study aimed to investigate the internal light organ dimorphism as well as any associated sexual dimorphism in these species. Owing to an enlarged light organ in male leiognathids; the present study is intended to test the hypothesis that in male leiognathids development of gonad and secondary development of light organ is synchronized.

MATERIALS AND METHODS

1. SYSTEMATICS

Leiognathids belonging to different genera and species were collected from major landing centers located in the coastal districts of Kerala; Vizhinjam (Triruvananthapuram dist.), Neendakara (Kollam dist.), Thoppumpadi (Ernakulam dist.), Azhikkode – Munanbam, Chettuwa (Thrissur dist.), and Beypore (Kozhikode dist). Colour description of the species was based on fresh specimens. Morphometrics and meristics were studied using preserved specimens (6% formaldehyde). For identification of each species, standard description given by various authors was consulted along

with the current information furnished by databases such as FAO's SPECIESDAB and Fish base online publication. 14 morphometric variables were measured for males and females of each species and expressed a percentage of total length of fish. Distribution recorded only in the Indian region is given for each species.

2. FOOD AND FEEDING HABIT

Food and feeding habits of *Leiognathus brevirostris*, *Gazza minuta* and *Secutor insidiator* were studied. Both qualitative and quantitative analyses of diet were carried out using weighed and preserved stomachs (4% formalin).

2.1 Qualitative analysis of diet: Each stomach was emptied into a petridish and examined under a binocular microscope and identified the food items up to the possible taxonomic level depending on the state of digestion.

2.2 Quantitative analysis of diet: Analysis was done using frequency of occurrence and numerical methods. Ontogenetic (size related change in feeding) and seasonal changes in the diet was analysed.

2.3 Feeding intensity: Based on the degree of distension of stomach and the amount of food it contained. The stomachs were classified as gorged, full, ¾ full, ½ full, ¼ full and empty and the data were pooled and classified as poorly fed (¼ full and empty) and actively fed (½ full, ¾ full, full and gorged). Stomach fullness data were analysed to study the feeding activity and to measure the intensity of feeding in relation to season and size for both sexes.

Statistical analysis - Contingency table analysis: Non-parametric two way contingency table by Chi-square test was applied to test for independence between prey groups in the diet of fish and seasons and prey groups in the diet and length groups of fish. The same test was used to test for independence between feeding intensity and seasons and feeding intensity and various length groups of fish.

2.4 Gastro-somatic index (GSI): GSI was recorded during various months using the equation, GSI = 100 (weight of stomach)/weight of fish.

2.5 Feeding Index: Points were allotted to 'gorged', 'full', ' ¾ full ', ' ½ full', ' ¼ full', and 'empty' stomachs as 25, 20, 15, 10, 5 and 0. Feeding index = (P * 100) / ∑ X * N), where, P = total points allotted , X = points allotted to a gorged gut (in the present study, 25) and N = number of guts observed. The denominator (X * N) actually represents to total points possible. The month and length group based determination of feeding index was calculated for both sexes in three silverbelly species.

2.6 Comparative mouth morphology study: The following variables were measured: Total length (TL) and head length (HL) were measured to the nearest 1mm; the length of the mouth tube (tube length - TuL) when the jaws were fully extended, the horizontal and vertical length of mouth tube opening (mouth gape) were measured using a vernier caliper. The ratio of the tube length to head length (TuL/HL) and other characters associated with feeding, such as number of gill rakers and the size of teeth, were also examined. The month wise data on food items for two years were pooled and various dietary items were broadly categorized as plankton, benthos and other items.

2.7 Diet overlap: The dietary overlap of each species pair was calculated by simplified Morisita index or Morisita-Horn index using the equation

$$C_H = \frac{2\,(\sum Pij\ Pik)}{\sum P^2ij + \sum P^2ik}$$

Where C_H = Morisita-Horn index of overlap between species i and k; Pij = proportion of food item i of the total food used by the species j; Pik = proportion of food item i of the total food used by the species k.

3. LENGTH-WEIGHT RELATIONSHIP AND RELATIVE CONDITION FACTOR

3.1 Length-weight regression equation: Computed for males and females of three silverbelly species; *Leiognathus brevirostris, Gazza minuta* and *Secutor insidiator.* The specimens were sexed and total length (TL) and weight of each specimen were measured after blot drying with filter paper. The parameters 'a' and 'b' of length weight relationship of the three species were estimated by Ordinary Least Square method (OLS). $W = a\ X^{b}\ e^{u}$ where, W = body weight, X = the variable (total length), a = constant, b = regression coefficient and u = random disturbance term (which

measures the impact of all other factors which are not included in the model). Regression analysis was performed using SPSS – 13 Statistical software. The exponential relationship was transformed into a straight line relationship based on logarithms by following the equation, ln W = a + b ln L + u where, ' ln ' denotes the natural logarithm, where ln L (log L) is the independent and ln W (log W) is the dependent variables. T-test was used to determine whether the coefficient 'b' was significantly different from 3 (to gather information on whether growth follows isometric or allometric pattern) using the formula,

T = (b -B)/sb, where, B = 3, b = regression coefficient and sb = standard error of b. Significance of difference between the regression coefficients of the sexes at 5% level was tested by ANCOVA

3.2 Relative condition factor: Kn = W/w, where 'W' represents observed weight and 'w' calculated weight derived from the length- weight relationship equation. Monthly and length group based Kn values were calculated for males and females of 3 pony fish species.

Statistical Analysis: One way ANOVA and Post Hoc Test (Tukey) was performed to find out whether the difference in the monthly mean Kn values of males and females belonging to different length groups are significant or not at p = 0.01 or 0.05 for 3 silverbelly species.

4. REPRODUCTIVE BIOLOGY

4.1 Quantification of ovaries into different stages of maturity: Reproductive biology of 3 silverbelly species; *Leiognathus brevirostris*, *Gazza minuta* and *Secutor insidiator* were studied. Each fish was measured for its total length and weighed to nearest 0.01 gm. Macroscopic and microscopic examinations of ovaries were carried out. Ovaries were also examined histologically and a five maturity stage classification was adopted.

4.2. Size at first maturity: Maturity curves were drawn to estimate the average length at first maturity for three species.

4.3 Spawning frequency: Ova diameter measurements were recorded using an occular micrometer to understand the duration and frequency of spawning

4.4 Fecundity: Gravimetric sub sampling method was used. Estimation of fecundity is based on

the mature ovaries (penultimate stage of ripeness) as all the ova destined to be spawned during the ensuing season are mature in such ovaries. All mature ova having a fully yolked structure were counted under the microscope

Absolute fecundity = Weight of the ovary X Average number of eggs in the subsamples/ Average weight of the subsamples

4.5 Relative fecundity: Absolute fecundity calculated was expressed in terms of unit body weight, unit body length and unit ovary weight by the ordinary least square method (OLS),

$F = a X^{b} e^{u}$ where, F = fecundity, X = the variables (total length, body weight and ovary weight), a = constant, b = regression coefficient and u = random disturbance term (which measures the impact of all other factors which are not included in the model). Multiple regression analysis was also performed to get the combined influence of these three variables on the fecundity of the fish.

$F = A\ TL^{b1}\ BW^{b2}\ OW^{b3}\ e^{u}$

$\ln F = a + b_1 \ln TL + b_2 \ln BW + b_3 \ln OW + u$

where, b1, b2 and b3 are the regression coefficients of total length, body weight and ovary weight respectively (TL = Total length, BW = Body weight and OW = Ovary weight).

4.6 Gonado-somatic index (GSI): GSI = (Weight of gonad/Weight of fish) X 100

The monthly mean oocyte diameter and monthly mean GSI were compared using Pearson's correlation in three species.

4.7 Sex ratio: The sex-ratio distribution was studied to test whether the observed sex ratio in each month differed significantly from the expected ratio. Chi-square test (X^2 test) was employed to observe the deviation from the hypothetical 1: 1 ratio (null hypothesis).

$X^2 = \sum (O-E)^2 / E$ where, O = observed frequency of males and females and E = expected frequency of males and females.

5. LIGHT ORGAN AND SEXUAL DIMORPHISM IN LEIOGNATHIDS

Sexually mature males and females (only fishes above the length at first maturity) of *Leiognathus brevirostris*, *Secutor insidiator* and *Photopectoralis bindus* were used for the study.

The gonads were removed and the circumoesophageal light organ was dissected out. Gonads and light organs were weighted in an electronic balance (GW, LW, 0.001 g). Gonadosomatic Index (GSI) was calculated; GSI = 10^2 (GW/BW) and Percentage light organ weight to body weight (PLW) was calculated to depict the secondary development of the light organ by the following equation: PLW = 10^2 (LW/BW) where, 'LW' is the light organ weight (gm) and 'BW' is the body weight (gm).

Statistical Analysis: Correlation between monthly mean GSI and mean PLW in males and females was tested with Pearson's correlation. Synchronization in the gonad and light organ development was studied in three silverbelly species, One Way ANOVA and Post Hoc Test (Tukey) was performed which determines the difference in mean GSI and mean PLW of various month groups and lengths groups are significant or not at p = 0.01 or 0.05 level (Months of the study period were grouped into various categories according to the maturity stages of the gonads and the GSI of each species). Presence of sexual dimorphism if any in association with presence of internal light organ was determined by eye.

RESULT AND CONCLUSION

SYSTEMATICS: The relationship of family leiognathidae is described. 14 species of leiognathids belonging to 6 genera were collected and morphometrics of males and females were recorded. Fin formula was worked out for each species. While many pony fish species are widely distributed along the Kerala coast, some others recorded only stray occurrence.

FOOD AND FEEDING HABITS: *Gazza minuta* is predatory, preying upon small pelagic fishes apart from post-larval and juvenile shrimps, worms and various miscellaneous items. *Leiognathus brevirostris* is a planktophagus and benthophagus carnivore feeding upon 15 dietary items; whereas *Secutor insidiator* is basically planktophagus (zooplankton and phytoplankton) and the fish is a pelagic surface feeder. Significant seasonal variation is observed in the prey types consumed by *Gazza minuta* but not in *Leiognathus brevirostris* and *Secutor insidaitor*. Trophic spectrum of *Gazza minuta* and *Leiognathus brevirostris* showed significant ontogenetic variation (p = 0.05)

which can be explained as a mean to reduce competition for food between juveniles and adults. However, *Secutor insidiator* did not show any significant change in the trophic spectrum as the fish grow in size.

Gazza minuta showed reduced feeding intensity and feeding index during monsoon season, which can be explained due to active spawning act of this fish along the Kerala coast during monsoon season. Similarly, *Leiognathus brevirostris* exhibited significant seasonal variation ($p = 0.01$) in the feeding intensity with reduced feeding during active spawning period and intense feeding during pre and post spawning periods. However, *Leiognathus brevirostris* did not show any significant ontogenetic variation in feeding intensity. *Secutor insidiator* did not show significant seasonal variation in the feeding intensity however, exhibited a significant difference ($p = 0.01$) in the feeding intensity of fishes belonging to different length groups.

Comparative mouth morphology in three species indicated that, the least protrusible, horizontally directed and widest mouth tube opening along with the presence of curved, sharp, caniniform teeth in both jaws, and very few gill rakers of *Gazza minuta* is designed to handle large and actively moving prey whereas, the highest degree of jaw protrusion in an upward direction, smallest mouth tube opening, small rudimentary teeth and greatest number of gill rakers in *Secutor insidiator* is associated with planktivorus diet. Intermediate degree of jaw protrusion in a downward direction, small weak and rudimentary teeth in *Leiognathus brevirostris* facilitates consumption of 50% plankton and almost equal proportion of benthos and other prey types. Due to peculiarities in their mouth morphology, diet overlap study indicated only moderate and low level of dietary overlap between these silverbelly species. It can be suggested that variously modified feeding apparatus and low or moderate diet overlap might favour the co-existence of these 3 silverbelly species in the same localities along Kerala coast.

LENGTH - WEIGHT RELATIONSHIP AND RELATIVE CONDITION FACTOR: LWR equation was computed for three species of silverbellies. *Leiognathus brevirostris* males showed isomeric growth whereas females showed positive allometric growth pattern. Both sexes of *Gazza*

minuta followed isometric growth of an ideal fish whereas males and females of *Secutor insidiator* showed positive allometric growth. Analysis of covariance revealed a significant difference in the 'b' estimates of males and females of *Gazza minuta* and *Secutor insidiator* which necessitated separate LWR equations for males and females in these two species. However, for *Leiognathus brevirostris*, a common LWR equation was computed as the difference in the 'b' estimates of males and females did not show significant difference. Kn values of males and females of three silverbelly species showed a rise during the breeding season of the fish and fall during peak spawning period. Present also showed the role of good feeding environment in determining the condition of three silvebelly species.

REPRODUCTIVE BIOLOGY: *Leiognathus brevirostris* spawns for a short period twice in a year with short duration, from February to April and from September to November off Kerala coast. *Secutor insidiator* breeds two times in a year; a prolonged breeding season from May to September and a short breeding season from January to March. *Gazza minuta* breeds only once in a year but with prolonged duration (from April to October). Highly significant ($p = 0.01$) positive correlation was obtained between the mean oocyte diameter and gonado-somatic index in three species. Present study indicated that spawning of *Gazza minuta* and *Secutor insidiator* is correlated with south- west monsoon while spawning of *Leiognathus brevirostris* takes place during pre and post monsoon months along the coast of Kerala. It is observed that, the mature and ripe specimens of *Gazza minuta* and *Secutor insidiator* were obtained from deep water trawl landing during breeding season. Hence it is reasonable to think that these two species might be moving to deep waters for spawning while *Leiognathus brevirostris* is reported to be a close inshore species, breeds in near shore waters hence this species avoid monsoon season for spawning in order to overcome the turbulant surface waters and rough weather during monsoon.

The size at first maturity (50%) recorded for females of *Leiognathus brevirostris*, *Gazza minuta* and *Secutor insidiator* were 75 mm, 78 mm and 70 mm respectively. *Gazza minuta* showed the highest fecundity among these 3 silverbelly species. In *Leiognathus brevirostris* and

Gazza minuta, a good fit was obtained for log fecundity and log total length whereas, in *Secutor insidiator*, the best fit was obtained for log fecundity and log body weight than other two variables. While multiple regression analysis indicated that in *Leiognathus brevirostris* only total length contributed significantly to explaining the variance in fecundity; in *Gazza minuta* both total length and body weight and in *Secutor insidiator* all three variables contributed significantly to explaining the variance in fecundity. Apart from these variables other factors such as age and /or environmental factors might contribute considerably to the variation in fecundity in these pony fish species.

LIGHT ORGAN AND SEXUAL DIMORPHISM: Light organ is a simple 'gland like' structure encircling the oesophagus in *Leiognathus brevirostris*, *Secutor insidiator* and *Photopectoralis bindus* however; interspecific and intraspecific size and shape specificity were observed in them. In both sexes of *Leiognathus brevirostris*, light organ is 'donut shaped', in *Secutor insidiator*, it is 'dombbell shaped' and in male *Photopectoralis bindus*, it is triangular shaped and in females light organ is circular in shape. Males of these 3 species exhibited hypertrophication of the dorsolateral lobe or lobes of the light organ owing to its enlarged size compared to conspecific females.

Sexual dimorphism was observed in two species in association with the internal dimorphic light organ. In males of *Leiognathus brevirostris* and *Photopectoralis bindus*, a specific transparency is noticed at the pectoral fin base which is lacking in females. A curious case of sexual dichromatism was observed in *Leiognathus brevirostris* with adult males showing more brilliantly coloured fins than females belonging to same length group. Sexual dimorphism is not observed in *Secutor insidiator* even though internal light organ is dimorphic in sexes.

Synchronization in the gonad and light organ development is noticed only in males of 3 pony fish species. A significant positive correlation is obtained for monthly mean GSI (gonadosomatic index) and PLW (percentage light organ weight) only in males of 3 silverbelly species. Males of three species showed a significant difference in the mean PLW and mean GSI during various months grouped according to the breeding and non-breeding seasons of the fish. However, there is no significant difference in the mean GSI and mean PLW of fishes belonging to

different length groups (in three species). In females, there is no significant correlation between mean GSI and PLW during various months and does not show any significant difference between monthly mean GSI and monthly mean PLW. In females of these 3 species, light organ is a rather simple structure throughout the life which does not show any further enlargement in connection with the breeding season of fish. However, in males of these three species, light organ clearly showed hypertrophication in size and weight in connection with the enlargement of gonads. Enlarged light organ in males in connection with breeding season can be explained on the basis of sexual selection pressure imposed on the bioluminescent light of varying intensity and frequency emitted by males for the purpose of mate recognition and/or attraction in a rather heterogenous shoal. This result would provide scientific evidence for the predicted reproductive role of male light organ in a shoal forming fish which often contain more than one species and inhabit turbid coastal waters with poor visibility for maintaining species fidelity and thus leads to reproductive success. This study would definitely help to continue further research in this field which awaits much exploration and scientific findings.