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Role of bioluminescent organ in *Photopectoralis bindus* (Valenciennes, 1835) (Family: Leiognathidae) from Kerala coast

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Original Article

Abstract

Seasonal and length group based changes in the development of gonads and Light Organ (LO) were studied in orangefin ponyfish, *Photopectoralis bindus.* Light organ shows sexual dimorphism with males having larger LO compared to conspecific females belonged to the same length group. Gonadosomatic Index (GSI) and Percentage Light organ weight (PLW) were used to find out the possible synchronization in the gonad and light organ enlargement in both sexes. A significant positive correlation was observed between GSI and PLW in males but not in females; it can be suggested that gonad and light organ development correlate only in males during the breeding seasons irrespective of the length of the fish, whereas, the light organ remained the same size in all sexually mature females irrespective of the length of the fish and breeding seasons. The result suggested the possible role of bioluminescence in mate recognition in this silverbelly species as the same was reported for other species of silverbellies. It can be explained in terms of sexual selection imposed by reproductive success.

***Keywords**: Photopectoralis bindus, gonadosomatic index, percentage light organ weight, symbiotic bioluminescence, leiognathidae*

Introduction

Leiognathids are facultative schoolers inhabiting sandy coastline and mud-bottomed coastal bay and estuarine environments in the tropical and sub-tropical Indo-Pacific Ocean (Jones, 1985). They are capable of emitting a bright light from their ventral surface. About 40 species from seven genera are recognized as valid (Froese and Pauly, 2009), and in these small bioluminescent fish, the source of light is a facultative symbiotic marine luminous bacteria, *Photobacterium leiognathi* (Reichelt *et al*., 1977) maintained within an internal light organ, surrounding and communicating with the oesophagous (Dunlap and McFall-Ngai, 1987). Most leiognathid species possess sexually dimorphic light organ (Sparks *et al*., 2005; Ikejima *et al*., 2008). Males possess enlarged light organs compared with females, and in some species, the morphological differences in light organ system are so extreme that not only the light organ size, but also the area of the transparent skin patch or stripe become distinct in males (Ikejima, *et al*., 2008). Bioluminescence in leiognathids plays roles in intra- and interspecies signaling in *Photoplagios elongatus* (Sasaki *et al*., 2003). Such studies are not reported from *Photopectoralis bindus* from Kerala coast. Hence, present study investigated the sexually dimorphic light organ and associated external dimorphic traits in orange fin pony fish *P. bindus*. The work is also aimed to test the possible coupling between sexual maturity and secondary development of the light organ.

Material and methods

Sexually mature males and females (only fishes above the length at first maturity) of *P. bindus* were used for the study. Specimens were collected from major landing centers located in the coastal districts of Kerala, Vizhinjam (Triruvananthapuram Dist.), Neendakara (Kollam Dist.), Thoppumpadi (Ernakulam Dist.), Azhikkode-Munanbam (Thrissur Dist.) and Beypore (Kozhikode Dist) during 2005 to 2007 (Fig. 1). Apart from these, fish were collected from the minor landing center at Chettuwa (Thrissur Dist.). Specimens were brought to the laboratory on ice and total length (TL, 1mm) and body weight (BW, 0.01 g) of each specimen were measured. The fishes were dissected to note the sex and stage of maturity.

Spawning season and length at first maturity were determined from the microscopic and macroscopic examination of gonads of various stages of maturity. Maturity curves were drawn to estimate the average length at first maturity for the species by plotting the percentage of mature fish obtained in 80 mm to 130 mm TL.

In the present study, the report of Balan (1963) on the reproductive biology of *P. bindus* (Syn. *Leiognathus bindus*) from the Calicut region along the Kerala coast was also referred. A total of 2020 specimens; 958 males and 1062 females of *P. bindus* (males TL > 80 mm and females - TL > 85 mm) were used in the study.

The gonads were removed and the circumoesophageal light organ was enucleated with a part of oesophagus, and then oesophageal tissues connecting to the light organ were carefully removed using forceps and dissecting scissors under a binocular microscope. The opaque membrane (melanophore rich muscular shutter) covering the light organ was not removed to avoid making damage and loss of bacterial cells from the light organ. As an operational unit to demonstrate gonad development, Gonadosomatic Index (GSI) was calculated using the equation GSI $= 10^2$ (GW/BW) where, 'GW' is the gonad weight (gm) and 'BW' is the body weight (gm). GSI is also useful to explain the state of maturity and intensity of spawning (James, 1967). Percentage of light organ weight to body weight was calculated to depict the secondary development of the light organ by the

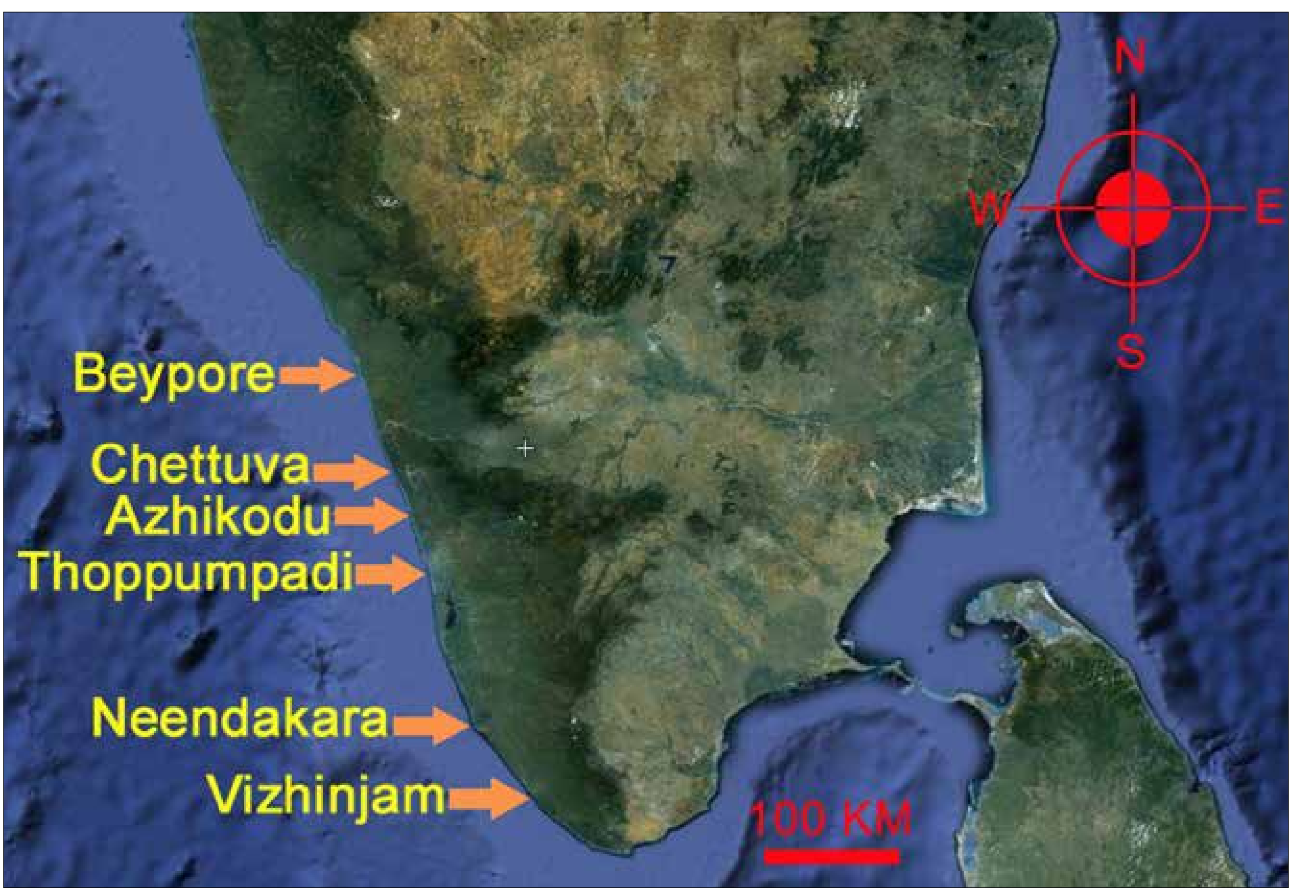

Fig. 1. Collection localities of silverbellies

following equation, percentage light organ weight, PLW = 10^2 (LW/BW), where, 'LW' is the light organ weight (gm) and 'BW' is the body weight (gm). Correlation between both mean GSI and PLW in each sex group was tested with Pearson's correlation. The fishes were grouped into 10mm length groups and the relationship between monthly mean PLW and GSI for each length group of males and females was tested with Pearson's correlation. One way ANOVA and Post Hoc Test (Tukey) was performed using SPSS 2013 statistical software to find out whether the difference in the mean GSI and PLW of various months (Months of the study period were grouped according to the maturity stages of the gonads and the GSI).

October to March - group 1 (mature, ripe gonads, higher GSI indicate breeding season), April to September -group 2 (lower GSI and immature and maturing gonads). Fishes were divided into 3 length groups in both sexes; 80- 99 mm (group 1), 100-119 mm (group 2) and 120-129 mm (group 3) based on length at maturity to find out significance at $p=0.05$.

Similarly, GSI and PLW were also estimated for males and females belonging to different maturity stages. The presence of external dimorphism if any in association with the presence of internal light organ was determined by visual observation.

Results and discussion

In *P. bindus*, the dorsal side of the light organ is covered with a darkly pigmented retractable muscular membrane. Dull creamy white coloured glandular part is visible ventrally with retracting the muscular membrane. A very distinct internal light organ dimorphism and associated external modification, specific to males were noticed in this species (Fig. 2). Light organ of sexually mature males attained a peculiar triangular shape due to the hyper trophication of dorsolateral lobes (both right and left lobes); whereas, females belonged to the same length group possessed a rather simple round light organ. In males, the enlarged right lobe of the light organ is projecting into the silvery lining of the gas bladder while the left lobe is facing to transparent muscles on the cheek region. In both sexes, the pigmented membrane of light organ was provided with an orange tinge along its ventral margin (Fig. 3). In males, a clear, transparent area was seen at the base of pectoral fin through which the internal darkly pigmented membrane of the enlarged light organ lobe was visible. In females, there was no such detectable transparency in the pectoral fin base (Fig. 4).

Gonad and Light organ development

For males and females, a highly significant difference was obtained between the mean GSI values of two month groups

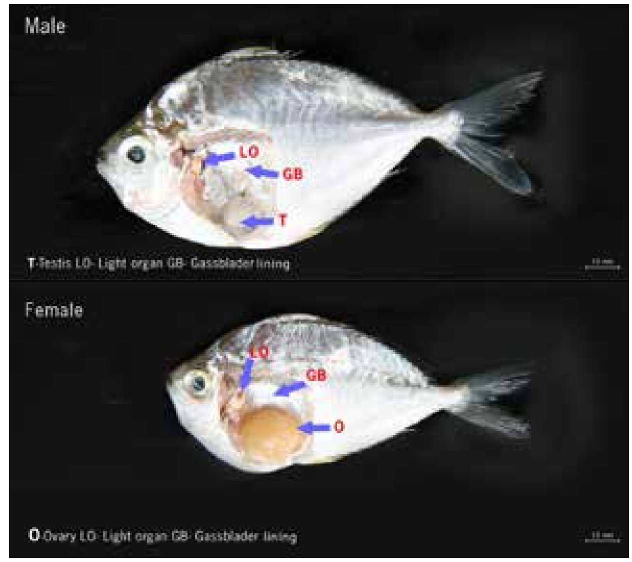

Fig. 2. Light organ dimorphism in *Photopectoralis bindus*

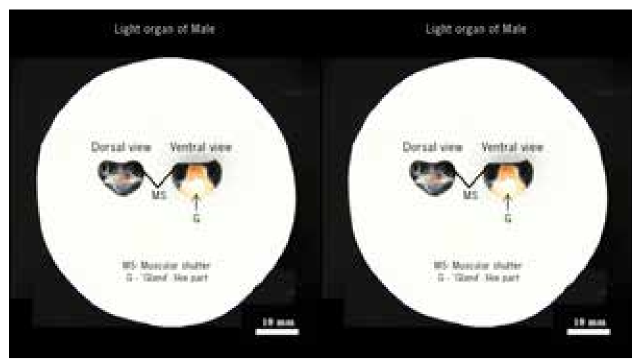

Fig. 3. Light organ of male and female *P. bindus*

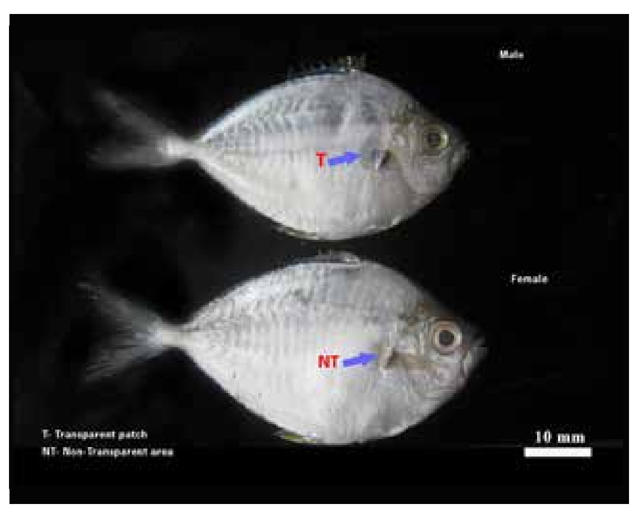

Fig. 4. Sexually dimorphic male and female of *P. bindus*

[F = 54.8.09 at p = 0.01 (Males); F = 58.499 at p = 0.01 (females)]. Whereas, GSI of males and females belonging to various length groups (classified into 3) did not show any significant difference. These results of macroscopic and

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Table 1. Monthly mean GSI and PLW (mean ± Sd) of sexually mature males and females of *P. bindus* from March 2005 to February 2007 (pooled) from Kerala coast

Month	No. of fish examined		GSI		PLW	
	Male	Female	Male	Female	Male	Female
Mar	65	68	2.05±1.32	2.1±1.2	1.72±1.0	0.31±1.6
Apr	75	80	0.73± 1.2	1.11±1.3	0.32±1.3	0.32±2.2
May	84	82	0.98±1.3	0.85±1.3	0.48±1.3	0.31±1.2
Jun	92	106	0.42±1.58	0.75±2.2	0.52±2.5	0.30±1.6
July	106	120	0.65±2.03	0.70±1.5	0.62±1.6	0.29±1.1
Aug	125	105	0.33±1.56	0.68±2.5	0.23±1.6	0.30±1.5
Sep	56	65	1.10±2.53	1.03±2.6	0.80±2.5	0.30±1.3
Oct	51	75	1.81±1.26	1.81±1.3	1.43±1.0	0.29±1.0
Nov	89	92	2.41±4.26	2.93±1.6	1.68±2.2	0.28±1.6
Dec	62	71	3.22±3.5	3.65±2.5	2.11±1.2	0.29±0.3
Jan	122	112	4.07±1.56	3.77±1.6	2.26±1.5	0.27±0.3
Feb	78	80	4.01±2.36	4.19±1.5	2.31±1.6	0.27±1.2

Table 2. Mean GSI and PLW of sexually mature specimens of male *P. bindus* belong to various length groups from March 2005 to February 2007 (pooled) from Kerala coast

Month	Length groups (in mm)									
	80-89		90-99		100-109		110-119		120-129	
	GSI	PLW	GSI	PLW	GSI	PLW	GSI	PLW	GSI	PLW
Mar	2.61	2.31	2.23	1.36	2.82	3.56	3.20	3.62	3.56	2.64
Apr	0.83	0.86	0.96	0.46	1.22	1.03	2.82	2.06	1.62	1.03
May	0.98	0.65	1.02	0.56	1.58	0.86	1.56	0.85	1.46	1.03
Jun	0.22	0.56	1.26	0.63	1.62	0.66	1.33	0.55	1.56	1.00
July	0.45	0.85	1.52	0.56	1.71	0.58	1.26	0.73	2.06	0.95
Aug	1.03	0.75	1.53	0.75	1.23	0.65	1.63	0.56	1.95	0.65
Sep	1.00	1.13	1.46	1.96	1.61	0.63	1.81	0.66	1.83	0.81
Oct	1.61	1.65	1.02	1.10	1.63	1.26	1.73	0.93	1.82	2.03
Nov	2.71	2.81	2.86	2.74	2.88	3.35	2.92	2.56	2.95	2.96
Dec	2.82	3.41	2.65	3.52	2.94	3.65	3.56	3.63	3.25	2.85
Jan	2.66	3.36	2.31	3.56	3.16	3.85	3.61	3.85	3.93	3.86
Feb	3.12	3.25	2.52	3.62	3.34	3.41	3.88	3.91	3.82	3.92
Average	1.67	1.80	1.77	1.74	2.15	1.96	2.44	1.99	2.48	1.98
n	65		102		120		73		81	

Table 3. Mean GSI and PLW of sexually mature specimens of female *P. bindus* belong to Various length groups from March 2005 to February 2007 (pooled) from Kerala coast

Month	Length groups									
	80-89		90-99		100-109		110-119		120-129	
	GSI	PLW	GSI	PLW	GSI	PLW	GSI	PLW	GSI	PLW
Mar	2.13	0.31	2.06	0.31	2.98	0.29	3.12	0.29	3.45	0.28
Apr	1.05	0.34	1.56	0.30	1.01	0.28	1.05	0.29	1.02	0.28
May	1.15	0.32	1.85	0.32	1.15	0.32	1.05	0.31	0.76	0.29
Jun	1.09	0.33	1.46	0.29	1.65	0.31	1.28	0.33	1.25	0.33
July	1.30	0.31	1.12	0.31	0.73	0.33	1.16	0.28	1.16	0.32
Aug	0.98	0.32	1.16	0.29	1.25	0.32	1.56	0.27	1.28	0.32
Sep	1.89	0.33	1.06	0.30	1.56	0.34	0.98	0.31	1.31	0.32

Oct	2.23	0.32	1.12	0.31	1.23	0.33	1.26	0.31	1.66	0.29
Nov	3.52	0.32	3.21	0.31	3.05	0.31	2.99	0.27	3.06	0.28
Dec	3.24	0.31	2.65	0.31	3.03	0.29	3.35	0.29	3.26	0.31
Jan	3.41	0.30	2.85	0.30	3.15	0.28	3.15	0.29	2.86	0.33
Feb	2.98	0.30	3.15	0.31	3.74	0.31	2.98	0.31	3.56	0.29
Average	2.08	0.31	1.93	0.31	2.04	0.31	1.99	0.29	2.05	0.30
n	45		52		102		140		73	

microscopic observations of gonads and ova diameter measurements clearly indicated that *P. bindus* along the coastal waters of Kerala (South west coast of India) has a single prolonged breeding seasons *i.e*, group 1 month (October to March). This is similar to the breeding period of *P. bindus* reported from the South - West coast of peninsular India (Balan, 1963). Monthly mean GSI and PLW of males and females of *P. bindus* is shown in Table. 1.

In males, the difference between mean PLW of two month groups were highly significant at $p = 0.01$ level ($F = 53.704$) whereas, that of females were not significant ($F = 5.990$). Difference between mean GSI and mean PLW of males and females belonging to different length groups were not statistically significant (Table 2 and 3). GSI and PLW of females belonging to various length classes and various maturity stages indicated a negative correlation whereas; a highly significant positive correlation is obtained for males belonging to various length groups and at various stages of maturity (Table 4 and 5). Males exhibited a highly significant positive correlation between GSI and PLW during various months of the year ($r = 0.980$ at $p = 0.01$) whereas, significant negative correlation ($r = -0.701$ at $p = 0.05$) was obtained for females (Fig. 5 and 6 for males and females respectively). A significant difference was obtained between the mean GSI ($F = 8.902$ at $p = 0.096$) and mean PLW of males ($F = 12.487$, at $p = 0.072$) of various stages of maturity whereas, difference between the mean GSI ($F = 11.608$, $p = 0.076$) of females at different maturity stages was significant but that of mean PLW was not significant ($F = 1.00$, not significant).

The above result clearly indicated that, only males exhibited

Table 4. Correlation between monthly mean GSI and PLW of males and females of various length classes of *P. bindus* (Pearson's correlation)

Length group mm	Correlation coefficient 'r'	Level of significance	significance	Length group	Correlation coefficient 'r'	Level of significance	Significant or nor significant
	Males				females		
80-89	0.919	0.01	Significant	80-89	-0.614	-	negative correlation
90-99	0.966	0.01	Significant	90-99	0.336	-	Not significant
100-109	0.925	0.01	Significant	100-109	-0.485	-	Not significant
110-119	0.925	0.01	Significant	110-119	-0.292	-	Not significant
120-129	0.935	0.01	Significant	120-129	-0.293	-	Not significant

Table 5. Mean GSI and PLW (mean ± Sd) with correlation coefficients of males and females of *P. bindus* at different stages of maturity

Maturity stage of gonad	GSI (mean ±sd)	PLW (mean ±sd)	Correlation coefficient	Level of significance	Significant or not significant
Males (Testis)					
Immature	0.73±0.35	0.32±0.65	0.993	P = 0.01	Highly significant
Maturing	1.82±0.26	1.40±.023			
Mature	2.82±1.23	2.00±0.12			
Ripe	3.06±0.5	2.21±0.23			
Females (ovary)					
Immature	0.82±0.23	0.31±0.06	-0.783	Negative correlation	Not significant
Maturing	1.90±0.15	0.30±0.03			
Mature	3.26±0.06	0.30±0.05			
Ripe	4.08±0.08	0.30±0.06			

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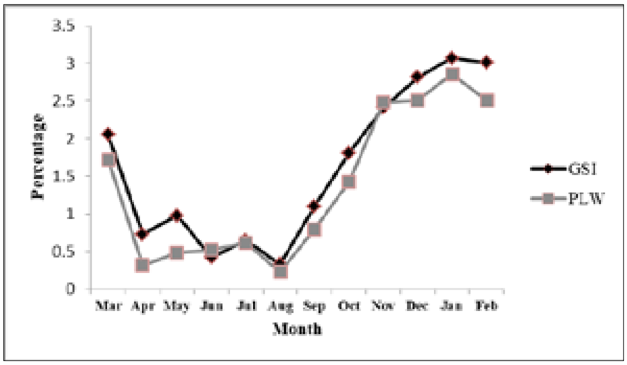

Fig. 5. Relationship between GSI and PLW of male *P. bindus* from March 2005 to February 2007 (pooled) from Kerala coast

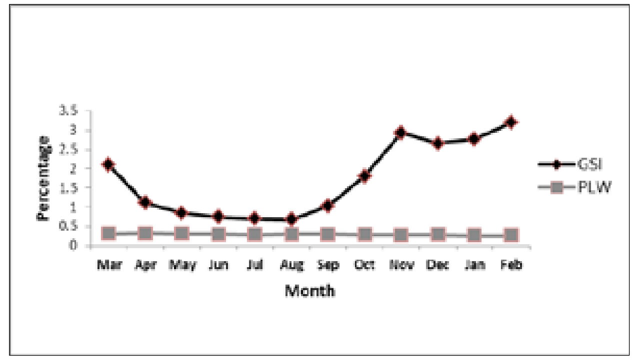

Fig. 6. Relationship between GSI and PLW of female *P. bindus* from March 2005 to February 2007 (pooled) from Kerala coast

enlargement of light organ in connection with breeding season irrespective of the total length of the fish, whereas, light organ retained the same size, shape (round) and almost same weight in all females examined irrespective of the total length and maturity stage. The enlarged right lobe of light organ is directly facing to the silver lined reflective gas bladder through a window like opening. In males, due to hypertrophication of the dorso-lateral lobes, light organ attains a distinct triangular shape which is morphologically different from the simple round light organ of females. Even though, Jayabalan (1986) reported sexual dimorphism in *Leiognathus bindus,* he did not explain the internal shape dimorphism in the light organ of males and females of this species. Jayabalan (1989), Kimura *et al.* (2003) and Sparks and Dunlap (2004) reported the internal dimorphism in the light organ of some pony fish species. The result of the study strongly supported the hypothesis that the gonad development and light organ enlargement coincides only in males irrespective of the total length of the fish. Hence, it can be suggested that the male light organ in these leiognathids might play a role in reproduction mostly by producing species specific and sex specific mate attraction and/or recognition visual signals.

Jayabalan and Ramamoorthi (1982) reported that in, *Secutor insidiator* bacterial luminescence helps to lure or attract potent mate. Jayabalan (1986) also suggested the possible role of sex specific luminescence in attracting opposite sex in *P. bindus*. Recently, Sasaki, *et al.* (2003) made field observation on bioluminescent signaling of *Photoplagios elongatus*, used for intra-species communication during breeding season (June through August). Azuma, *et al.* (2005) worked with *L. nuchalis* from Japan, and reported that the light emitting signal was detected only in the spawning season. McFall Ngai and Dunlap (2005) reported the internal, external or both forms of light organ sexual dimorphism in 14 species of leiognathids and suggested a sexual recognition function of dimorphic light organ. Sparks *et al.* (2005) reported that the male pony fishes use species-specific patterns of lateral luminescence signaling to attract mates. Recently, Chakrabarty, *et al.* (2011) suggested that, the leiognathid subfamily Gazzinae comprises two-thirds of ponyfish species which are sexually dimorphic with regard to features of the light organ system and supposed to utilize it for species specific luminescence display. However, aforesaid authors did not conduct any detailed research on the possible synchronization of gonad and light organ development in these pony fishes to support this hypothesis.

A clear evidence for the synchronized development of gonad and bioluminescent light organ in a highly sexually dimorphic pony fish, *Photoplagios rivulatus* was reported for the first time by Ikejima, *et al.* (2008). Their result strongly supports the functional coupling between reproduction and bioluminescence in this ponyfish species. Ikejima, *et al.* (2008) reported the presence of a male specific rectangular flank patch for the emission of light which act as an externally sexually dimorphic trait by conducting nonparametric test. In the present work, this is rectified by applying one way ANOVA test along with Post-Hoc result to precisely find out where exactly is the significant difference between the mean GSI and mean PLW of fishes at various maturity stages, mature fishes belonging to various length groups and mean GSI and PLW of fishes during breeding and non-breeding seasons of the year. The present study (although direct field observation was lacking) together with the behavioral evidence as observed by Sasaki, *et al.* (2003) and Azuma, *et al.* (2005) in other silverbelly species also, strongly support the original presumption that the bioluminescence plays a role in reproduction in leiognathids.

Bioluminescence serves as a tool of visual communication in fish and shown to use it extensively in the context of mate choice Kingston, *et al.* (2003). Widder (2010) reported that many marine fish species uses bioluminescent light for mate recognition and attraction. In Stomiiforms and myctophids, the light organ of males are often considerably enlarged compared to conspecific females (Herring, 2007).

The present observation agreed well with the observation of Herring (2007) that, in the sea, structures associated with bioluminescence may show a marked sexual dimorphism and used to generate bioluminescent sexual signals. In addition, leiognathids inhabit turbid coastal waters with poor visibility and are often captured in mixed assemblages of several species (Sparks, *et al*., 2005). It can be inferred from the present result that, sexually dimorphic light organ and synchronized development of gonad and light organ in *P. bindus* helps for the males to emit species - specific patterns of luminescence to attract mates, and also enables to maintain reproductive isolation in the turbid coastal environments. It can be suggested that light organ dimorphism has permitted a number of morphologically similar forms to coexist and maintain species fidelity, especially in habitats with extremely poor visibility.

Light organ and sexual dimorphism study is the first of this kind from Kerala coast. Result of this study clearly proved the hypothesis that, light organ and gonad development correlated only in male pony fishes species; *Photopectoralis bindus.* This study would definitely help to continue further research in this field which awaits much exploration and scientific findings. Finally, it is suggested that, attention should be given to the migratory patterns and ecological features which determine the location and size of commercially exploitable leiognathid concentration. The leiognathid stocks, however, tend to be more rapidly reduced in size than the total demersal stock of the area where trawling activities are intense. This is probably due to the occurrence of bulk of these fishes in very shallow waters near estuaries and over muddy bottom. Such areas which often also yield good shrimp catches, generally tend to attract an over proportionate number of trawlers and other gears. Hence proper management measures should be taken for protecting silverbelly stock from depletion.

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