

Distribution, reproductive biology and biochemical composition of *Rhopalophthalmus indicus* (Crustacea: Mysida) from a tropical estuary (Cochin backwater) in India

Biju A*, Gireesh R and Panampunnayil S.U

National Institute of Oceanography, Regional Centre, Dr. Salim Ali Road, Ernakulam north P.O, Cochin-18, Kerala, India

*Corresponding author email address: bijuanio75@gmail.com

Telephone: +91 0484 2390814

Fax: +91 484 2390618

Running head: Biology of *Rhopalophthalmus indicus*

Abstract

Distribution, reproductive biology and biochemical composition of *Rhopalophthalmus indicus* Pillai were investigated base on samples collected over a period of one year from Cochin backwater. *Rhopalophthalmus indicus* Pillai was recorded through out the year with peak abundance during pre-monsoon. The population density was influenced by Chlorophyll *a*, dissolved oxygen, salinity and water temperature. The species showed periodicity in the abundance and produce more than one generation per year. The number of embryos carried by a single female ranged from 6-13, and was correlated with female body length ($P > 0.05$), tending to increase with the size of the female. Egg size varies between 0.42-0.47 mm, and was independent of female size. Both males and females attain sexual maturity at a length of 8.4 mm. Seasonality is observed in biochemical composition, as mature males and females had higher protein contents, immature stages contained high carbohydrate content and brooding females accumulated more lipid.

Keywords: Mysida, *Rhopalophthalmus indicus*, developmental stages, protein, carbohydrate, lipid, Absorption spectroscopy

INTRODUCTION

Mysid crustaceans are one of the major components of estuarine and coastal zooplankton communities and play a key role in structuring estuarine communities (Mees & Jones, 1997). They occupy a wide variety of aquatic environment and are ubiquitous member of the estuarine ecosystem. Due to their high densities in estuaries, mysid plays an important role as a resource for many organisms that use estuaries as nurseries. In general, mysids are omnivores, feeding on detritus, zooplankton and phytoplankton, as such form a link between microbial producers and secondary consumers (Webb, 1973) and are responsible for the remineralization of a large portion of the refractile detritus (Fockedey & Mees, 1997). As an energy converter at different trophic levels, significance of mysids in the ecosystem has been greatly underestimated. Therefore, the analysis of biochemical composition in mysids is important, to understand the nutritional value and flow of energy at different trophic level. Life history characteristic of mysid species can vary considerably from one habitat to another (Mauchline, 1980) and the knowledge of local population is essential for subsequent studies on related scientific field (Hanamura *et al.*, 2009).

Mysid related fisheries based on several mixed group of species are present in regions of China, Korea, and south eastern countries (Omori, 1978), where they used for making shrimp paste, sauces and preserved food for human consumption. In some areas of India (Chilka lake and Konkan region), mysids have been harvested for human consumption, but it is not commercially exploited. To judge their viability as fishery commodity the whole aspects of the species should be studied to get required information about age, maturity, life span and nutritive quality. *Rhopalophthalmus indicus* is a common mysid in the west coast of India (Biju, 2009). In Cochin backwaters, this species particularly abundant and their biomass occasionally exceeds than other zooplankton. Recently, individuals of this species were observed in guts of several abundant fish species (Biju, unpublished data). At present there is no information is available for biology and ecology of this species. Considering its ecological importance, the present study aims to elucidate the distribution, reproductive biology and biochemical composition of *Rhopalophthalmus indicus* Pillai based on 12-month survey on a tropical estuary, Cochin backwaters, in India.

Materials and methods

Study area

The Cochin Backwater system, a large basin of brackish water, is one of the largest estuaries in India, extending between 9° 40' 12" and 10° 10' 46" N and 76° 09' 52" and 76° 23' 57" E. Details of study area have already been reported (Biju *et al.*, 2009) and hence are not dealt with here.

Sampling procedure and data analysis

Samples were collected as a part of the studies on “Ecosystem Modeling of Cochin backwaters” in the period March 2003- February 2004. Weekly Samples were taken for one full year cycle covering pre-monsoon (February-May), monsoon (June –September) and post-monsoon (October-January). Zooplankton was collected before dawn from surface waters using a Working Party (WP) net (mesh size 0.2 mm, mouth area 0.6 m²) fitted with a flow meter to estimate the volume of water filtered. The net was hauled for 10 min. at the surface using a small boat at a speed of approximately 2 knots. Samples were preserved in 4% formaldehyde. At each station, surface water samples were collected using a clean plastic bucket and measured temperature, salinity (Digi Autosalinometer), dissolved oxygen (Winkler methods), pH (pH meter), and chlorophyll *a* (Strickland & Parsons, 1972).

In the laboratory, mysids were sorted from the samples and classified according to the degree of development of secondary sexual characteristics (Mauchline, 1980). Samples with high mysid numbers were subsampled with Folsom splitter. In the other samples all *R. indicus* were counted and subjected to detailed analysis. After sexed and measured, for each category the following criteria were used: juveniles- individuals without secondary sexual characteristics; adult males- well-developed lobus masculinus; immature males- lobus masculinus present but not yet setose; adult females- well-developed marsupium; immature females- incompletely developed marsupium. Adult females with or without eggs or larvae in the marsupium were separated into a different category; female with egg, female with eyeless larvae, female with eyed larvae and females with fully exposed marsupium (spent females).

Total body length is given as the distance between the anterior margin of the carapace and the apex of the telson, and was measured using a binocular microscope fitted with a micrometer eyepiece. In the same manner, eyeless larvae and eyed larvae were measured from the anterior to the posterior end when straightened (Hanamura, 1999). The egg diameter was measured along the longest. Brood size (number of eggs or larvae) was determined only for those females with an unruptured marsupium. To study the marsupial development, 52 brooding females collected during pre-monsoon period (February - May) were examined.

Analytical methods

Samples for biochemical analysis were collected during pre-monsoon (March 2007) and monsoon period (June 2007). The samples were transported in 10 L containers to the laboratory, where mysids were identified and sorted out at species level as mature males, immature males, spent females, females with embryos (brooding female), immature females and juveniles. The specimens were then left in filtered sea water (for 12-24hrs.) to evacuate their guts. Samples from each group

were lyophilized and refrigerated at -30°C; smaller portions of this material were later weighed and used for the determination of biochemical constituents (analytical replicates) (Azeiteoro *et al.*, 2001).

Proteins quantification was conducted according to the Folin phenol method (Lowry *et al.*, 1951) using bovine albumin as standard. The quantification of total carbohydrates was performed according to the colorimetric method using phenol and sulphuric acid (Dubois *et al.*, 1956), using glucose as standard. Lipid extraction was carried out according to Bligh and Dyer (1959) by direct elution with chloroform and methanol (1: 2 v: v). The extracted lipids were dried at 80°C (20 min.) and determined spectrophotometrically after carbonization at 18°C in concentrated sulphuric acid (Marsh & Weinstein, 1966) with tripalmitine solution used as a standard. All analyses were performed in triplicate.

Data analysis

Multiple regression analysis (SPSS-10) was employed to assess the predictability of population density on the physico-chemical variables.

The model used for the purpose was,

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5$$

Where Y = population density, x_1 = chlorophyll, x_2 = dissolved oxygen, x_3 = salinity, x_4 = pH, x_5 = water temperature. The significance of the fitted regression was tested using ANOVA.

RESULTS

Relation with environmental factors

Rhopalophthalmus indicus thrives in the estuary through out the year and tolerates temperature and salinity of 28-32.5°C and 1.84-29.16 respectively. A significant correlation was observed between population density of *R. indicus* and salinity ($P < 0.05$) (Figure 2). The fitted multiple regression model for the data was found to be $Y = -1322.681 + 63.631 x_1 + 6.856 x_2 - 135.959 x_3 + 56.304 x_4 + 0.644 x_5$. Statistical analysis revealed that pH did not have much influence on population density of *R. indicus*, while chlorophyll *a*, dissolved oxygen salinity and water temperature, had apparently some influence on their distribution. The fitted regression model for the data is significant ($R^2 = 0.482$) as can be seen from the ANOVA (Table 1). Details of the environmental properties of Cochin backwaters during the study period are given by Biju (Biju, 2009).

DISTRIBUTION PATTERN

The distribution of *Rhopalophthalmus indicus* fluctuated greatly in each sampling stations (Figure 3). The highest population density was observed at S2 station (avg. 1077.3 ± 1272.7 ind./1000m³), while marked reduction in S3 (avg. 109.2 ± 99.9 ind./1000m³) and completely absent in S1. *R. indicus* occurred through out the study period. There was a clear seasonal variation in the distribution of *R. indicus* during the present study. Compared to that of other seasons, the high abundance of *R.indicus* occurred in pre-monsoon period (February- May) (65.9% of the total population) with an average density of 779.6 ± 552.5 ind./1000m³ at S2 and 73.6 ± 97.2 ind./1000m³ at S3. All the developmental stages were observed from February to May, except spent females in February, with comparatively high density and comprised of 13.4% immature males 19.3% mature males, 9.2% spent females, 16.3% females with eyed larvae, 5.4% females with eyeless larvae, 8.7% females with eggs, 7.4% immature females and 20.6% juveniles.

With the onset summer monsoon (June), a clear decrease in the density of *R. indicus* was observed with an average density of 103.3 ± 188.2 ind./1000m³ at S2 and 7.1 ± 19.8 ind./1000m³ at S3. Only 12.4% of the total population of *R. indicus* occurred in the monsoon period (June-September) of which 12.8% immature males, 31.8% mature males, 5.2% spent females, 11.7% females with eyed larvae, 6.3% females with eggs, 20.3% immature females and 11.9% juveniles.

During the post-monsoon period (October-January) 21.7% of *R. indicus* occurred with an average density of 224.8 ± 252.4 ind./1000m³ at S2 and 32.2 ± 58.3 ind./1000m³ at S3 and was constituted by 19.7% immature males 28.8% mature males, 5.6% females with egg, 32.7% immature females and 13.2% juveniles. Spent females, females with eyed larvae and females with eyeless larvae were absent in this period. October to December months showed more or less similar pattern of distribution. The monthly percentage shows that juveniles dominated most of the samples and the percentage composition of each life stages of *R. indicus* varied with seasons (Figure 4).

Reproduction and development

Brood characteristics

The periodical one year examination on number of embryos or larvae in the marsupium of different sized females was carried out. The smallest brood (6) recorded was that of a female in the size class 8-9 mm and the largest brood (13) was that of a female in the size class 10.6-11 mm. The brood size was positively correlated with female body length ($P < 0.05$) (Figure 5). Size variation of eggs ranged from 0.42 to 0.47 mm, irrespective of female length. The smallest brooding female (8.3

mm body length) was observed in May with brood size between 6 and 7 larvae per marsupium. The largest brood size found was 13 larvae in a female with body length of 10.7 mm, caught in March.

Marsupial development

The complete larval development of mysids takes place within the brood pouch of females and can be divided into three phases; eggs or early embryos (Stage-1), eyeless larvae (Stages 2-6) and eyed larvae (Stages 7-9). Stage 9 larvae are categorized as free living larvae. Sub divisions of above mentioned last two phases were particularly based on morphological changes, in which nine stages can be recognized in the marsupial development (Figure 6a-i). Morphological features of different larval stages are given in Table 2. On observation of 52 - berried females, 17% are females with eggs (early embryo), 52.8% with eyeless larvae and 30.2% eyed larvae.

Post-marsupial development

Total length of the smallest free-swimming juvenile was 2.5 mm and the largest juvenile measured 5.6 mm. Immature stages exist in the size range of 5.4 to 9.2 mm, even though males mature at 8.6 mm and can be easily distinguished by the absence of setae on masculins in antennules. Immature females are in the size range of 5.4 to 8.6 mm in which formation of marsupium started and the oostegites present as four separate lamellae (anterior and posterior) but did not join ventrally. As body size increases, the oostegites become larger and fringed with setae while posterior pair of lamellae tightly overlaps anterior pair to form a compact pouch. The appearance of eggs in the marsupium was observed, when the body attained 8.5 to 10.2 mm length. The eyeless and eyed larvae were observed in the size range 8.3 to 10.4 mm and 9.3 to 10.7 mm respectively. Spent females had a size range of 10 to 10.8 mm. Females were larger than males. Both sexes attain their greatest size during pre-monsoon period (Table 3), where male ranges to 10.3 mm and female 10.8 mm in total length.

Biochemical composition

Rhopalophthalmus indicus showed variation in their biochemical composition during pre-monsoon and monsoon period (Table 4). Protein was the primary body component in all the life stages. Protein content in immature males and females, were lower than in adult males and females. The amount of total carbohydrate was exceedingly low and immature individuals showed higher carbohydrate proportion than mature male and females. High lipid contents were occurred in females especially in brooding females.

DISCUSSION

Being a complex ecosystem, the abundance and distribution of mysids in Cochin backwater depends upon the combination of environmental factors. In the present study, *Rhopalophthalmus indicus* showed heterogeneity in their distribution, which may be apparently related with the influence of physical factors. From the regression analysis, it is evident that except pH, other environmental parameters like chlorophyll *a*, salinity, water temperature, and dissolved oxygen have much influence on the distribution and abundance of *R. indicus* in the Cochin backwater. Many workers described the effect of environmental factors on the distribution of mysids in different areas (Greenwood *et al.*, 1989; Baldo *et al.*, 2001; Pothoven *et al.*, 2004; Grabe *et al.*, 2004; Hanamura *et al.*, 2009; Biju *et al.*, 2009). In the present study, the population density shows considerable variation in all stations. This may be correlated with the particular geographical conditions of the stations. Even though the breeding peak was invariably observed in the pre-monsoon period, the population density was completely absent in the harbour mouth station (S1) and comparatively low in shipping channel station (S3). This may be due to the effect of tidal currents in that particular area. Many reports discussed the influence of tidal currents on the distribution of zooplankton and mysids (Hill, 1991; Hough & Naylor, 1992; Moffat & Jones, 1993; Schlacher & Wooldridge 1994, Rost *et al.*, 1998).

Similar to its congeneric species, *R. terranatalis* O. Tattersall (Wooldridge, 1986) and *R. mediterraneus* Nouvel (Baldo *et al.*, 2001), *R. indicus* was observed throughout the year and higher reproductive rates during the warm pre-monsoon period. It is the evidence of periodicity in the abundance of *R. indicus* in the Cochin backwaters. The species abundance of *R. indicus* significantly correlated with salinity. All life stages, especially high abundance gravid females were observed in high saline pre-monsoon period may be related with their breeding peak. The abundance of brooding females during pre-monsoon period indicates that salinity plays an important role in the development of marsupium. The observation of more ovigerous females of the mysid *Neomysis integer* was interpreted as the selection of optimal salinities for embryonic development (Hough & Naylor, 1992). Similar high saline segregation of adult females of *R. mediterraneus* observed in the Guadalquivir Estuary (Baldo *et al.*, 2001). In the present study, brooding females are present till the onset of monsoon, after that brooding females appeared only during offset of post-monsoon while large number of juveniles are present in most of the months (except August, September and October). This suggests that the disappearance of brooding females during these months may be related to the migration of low saline water to high saline areas for the selection of optimal salinities for embryonic development. Generally, gravid females seem to have a lesser degree of osmotic regulation in their marsupial fluid than in their haemolymph (McLusky & Heard, 1971). Similar

seasonal movements have been widely documented for coastal mysid populations (Azeiteiro *et al.*, 1999) and this type of movements may have an important salinity related reproductive significance (Greenwood *et al.*, 1989). The effect of salinity in determining mysid distribution in estuaries has been widely documented for other species (Wooldridge, 1986; Moffat & Jones, 1993; Köpcke & Kaush, 1996). The occurrence of brooding females and juveniles during pre-monsoon and offset of post-monsoon suggest that similar to its congeneric species *R. mediterraneus* and *R. terranatalis*, have more than one generation per year (Baldo *et al.*, 2001; Wooldridge, 1986), although the longevity of single individual lives is not known; only additional laboratory observations on growth and brood production help to determine the number of generations and life span of individuals (Mauchline, 1980).

Mysids larval development takes place entirely within the marsupium of female suggests that these organisms are ideal for developmental studies (Dahl, 1977). Larval development within the marsupium of female consists of three different phases/stages. Various workers used different terminology to the three phases of marsupial development of mysids (Nair, 1939; Jepsen, 1965; Mauchline, 1980, Wittmann, 1981, Cuzin-Roudy & Tchernigovtzeff, 1985; Johnston *et al.*, 1997; Nunzio *et al.*, 1994; Quddusi & Tirmizi, 1995). They also differentiate these three phase into different stages based on their morphological changes. In the present study we observed *R. indicus* pass through nine larval stages (Table 2), is comparable with that of recent studies on this species reared in laboratory (Biju, Unpublished data). Earlier report reveals that, duration of each stage varied with species (Johnston *et al.*, 1997). In the present study, eyeless larvae had five morphologically different larval development stages, which suggest that, this phase might have taken longest duration in the larval development of *R. indicus*. In mysid, duration of incubation period is also varying with species. The shortest duration (4 days) of marsupial development is reported for *Mesopodopsis orientalis* W. Tattersall, while the longest (270 days) developmental reported in *Mysis relicta* Loven, a cold-water species (Lasenby & Langford, 1972). The duration of marsupial development is related to ambient temperature and salinity which are species specific (Nair, 1939; Berril, 1971; Lasenby & Langford, 1972). In general, in colder temperature the length of incubation period is greater than under warmer conditions. Wittmann (1984) reported that water temperature play an important role in the ecophysiology of mysids.

Biochemical composition

Analysis shows seasonal variation in biochemical composition of *Rhopalophthalmus indicus*. This is apparently relates to individual's nutritional status and breeding cycle (Pastorinho *et al.*, 2003). Comparatively high biochemical compositions observed during pre-monsoon period may be

related with breeding of *R. indicus* in the Cochin backwater. Protein was the principal component and main metabolic reserve in all life stages. The present results were also consistent with another species obtained for *Mesopodopsis orientalis* collected from Cochin Backwaters (Biju *et al.*, 2009). High abundance, euryhaline nature, high nutritive value, and short life cycle of this species may make it suitable for bioassay tests, and it may also have potential use in aquaculture field, because of their biochemical composition can satisfy the nutritious needs of a wide variety of animals, and are also complies with the recommendations of FAO (1989).

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Figure captions

Fig. 1. Location of the three stations, S1-S3, In the Cochin backwaters, India.

Fig. 2. Relationship between salinity and population density of *Rhopalophthalmus indicus* Pillai in Cochin backwater.

Fig. 3. Seasonal variations of *Rhopalophthalmus indicus* Pillai in Cochin backwaters.

Fig. 4. Composition of the population of *Rhopalophthalmus indicus* Pillai by age groups in Cochin backwaters. IM, immature males; MM- mature males; SF, spent females; ED, females with eyed larvae; EL, females with eyeless larvae; EG, females with egg; IF, immature female; J, juveniles.

Fig. 5. Relationship between total length of females and brood size in *Rhopalophthalmus indicus* Pillai in Cochin backwater.

Fig. 6. Different life stages during larval development of *Rhopalophthalmus indicus* Pillai in Cochin backwater (a) Stage 1; (b) Stage 2; (c) Stage 3; (d) Stage 4; (e) Stage 5; (f) Stage 6 ; (g) Stage 7 ; (h) Stage 8 ; (i) Stage 9.

Table 1

Results of ANOVA for environmental parameters with population density of *Rhopalophthalmus indicus* Pillai.

Source	SS	df	MS	F	P value
Regression	4933374	5	986674.872	20.69	$p < 0.001$
Residual	5293379	111	47688.095		
Total	1022673	116			

SS, sum of square; df, degree of freedom; MS, mean square; F, table value

Table 2. Morphological variations during marsupial developments of *Rhopalophthalmus indicus* Pillai

	Develo- pmental stages	Morphological characters	Body length (mm)
Embryo	S1	The yolk globules were more or less spherical or somewhat polygonal. (Fig.6a)	0.5- 0.6
	S2	Abdomen protruding from the egg, larvae look likes a “comma”. Four tube like or conical structures appear in the middle of the body (Fig.6b).	1-1.2
	S3	Yolk mass markedly concentrated towards the anterior region, length of tube like structures were found to be increase (rudimentary antenna and antennules), posterior end became more pointed (Fig.6c).	1.3-1.5
Eyeless larvae	S4	The anterior part bend inwards, posterior part of the abdomen becomes narrow. Marking of the thoracic appendages appeared (Fig.6d).	1.5-1.7
	S5	The posterior part of the abdomen becomes narrower, the size of the anterior region was markedly reduced, rudiments of thoracic appendages became clearer and the body segmentation go to started (Fig.6e).	1.6-1.8
	S6	The thoracic appendages were free, length of antennae and antennules were increased. Yolk was fully concentrated in the anterior region, as segmentation progressed. Formation of exopod and endopod started in the uropod. Posterior pointed end of the body became some what rounded (Fig.6f).	1.8-1.9
Eyed larvae	S7	Development of eyestalk with a patch of cornea on its tip was observed. Antennae and antennules became clearer and have more length. Body segmentation became clear. The anterio dorsal region had a very prominent bulge of yolk. Endopod and exopod were separated from uropod with setae on the posterior regions. Setae also occurred on the tips of the thoracic appendages. Telson appeared without spines (Fig.6g).	2-2.2
	S8	Body became more or less straight. Eyestalk was more developed, cornea become more thickened and clear. Antennae and antennules were clearly observed. The amount of yolk present in the anterior part got reduced markedly. The length of thoracic appendages increased. The abdominal region becomes quite clear, the eyes were clearly pigmented and segmentation was completed. Pair of small bud like structures (rudimentary pleopod) occurred in the abdominal segments (Fig.6h).	2-2.2
	S9	The size of the individuals increased, yolk got completely encircled in the digestive tract. Statocyst also appeared in this stage. The appendages were quite developed and larvae become miniature of the adult (Fig.6i).	2.5

Table 3

Variations in length (mm) of the various age groups of *Rhopalophthalmus indicus* Pillai in different months.

Month	IM	MM	SF	ED	EL	EG	IF	J
March '03	6.5-8.9	6.6-8.9	10-10.5	10.3-10.7	8.5-9.5	8.5-10	7.4-7.8	3.2-4.3
April	7.5-9	9.8-10.3	10.2-10.8	9.3-10.2	9-9.2	8.8-10.2	5.7-8.2	3-5.2
May	7.2-8.4	9.3-9.5	10-10.3	9.8-10	8.3-9.2	8.6-9.9	5.8-8	2.5-4.7
June	6.3-8	9.7-9.8	-	9-9.4	-	9-9.7	6-7.2	3-5.6
July	6.3-9	9.2-10	-	-	-	-	5.9-6.7	4.2-5
August	-	8.8-9.7	-	-	-	-	5.4-5.9	-
September	6.1-8	-	-	-	-	-	5.5-6.3	-
October	6.9-8.7	-	-	-	-	-	-	-
November	5.8-8.6	9-9.4	-	-	-	-	5.8-6.7	3.2-5
December	6.8-8.2	8.6-10.3	-	-	-	-	6.3-8.4	3.4-4.8
January'04	6.9-7.8	8.8-10.2	-	-	-	9.2-9.6	8.2-8.6	2.5-5.2
February	6.7-9.2	9-9.2	-	-	9.2-10.4	8.9-9.7	5.6-8.3	3.4-4

IM, immature males; MM- mature males; SF, spent females; ED, females with eyed larvae; EL, females with eyeless larvae; EG, females with egg; IF, immature female; J, juveniles.
(- absent).

Table 4

Biochemical composition (%dry wt) of the various developmental stages of *Rhopalophthalmus indicus* Pillai during pre-monsoon and monsoon period from Cochin backwaters.

Developmental stages	Protein		Carbohydrate		Lipid	
	PM	M	PM	M	PM	M
Mature male	73.01±1.32	70.90±2.37	6.64±0.77	5.42±1.45	13.65±2.39	11.36±4.21
Immature male	72.38±1.52	64.36±1.60	8.00±4.41	7.22±2.36	13.73±1.97	13.00±2.30
Spent female	73.68±1.15	-	5.44±1.90	-	15.37±3.29	-
Brooding female	73.23±1.37	69.59±1.56	6.27±1.47	5.98±2.21	17.48±1.64	16.98±0.70
Immature female	71.83±1.46	66.59±2.63	6.76±0.98	6.49±1.37	16.79±2.35	15.97±1.52
Juveniles	70.64±1.24	64.30±1.30	5.93±2.77	4.67±2.36	14.65±3.22	14.23±2.45

± Standard deviation; PM- pre-monsoon; M- monsoon; - absent

Figure 1

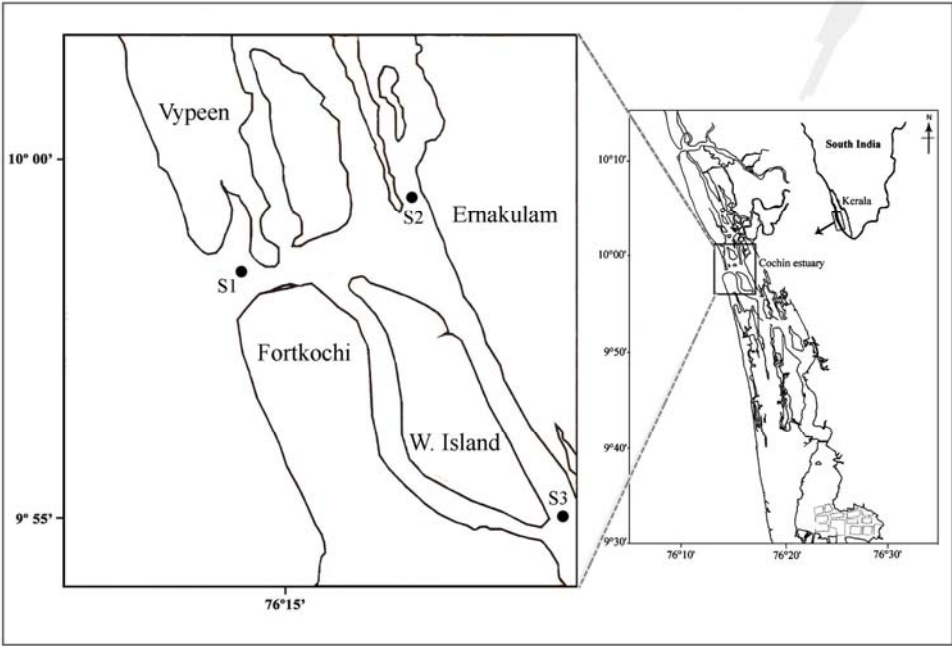


Figure 2

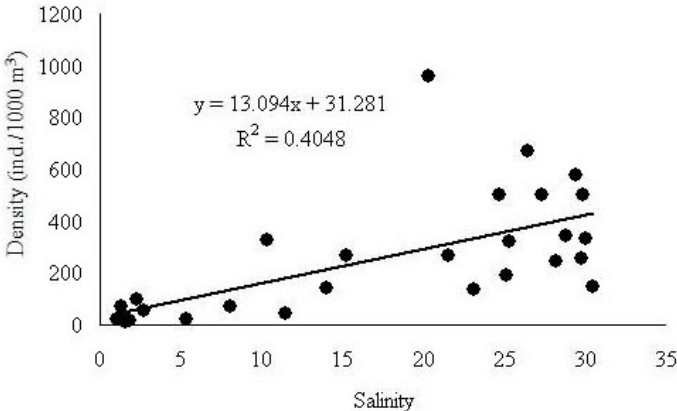


Figure 3

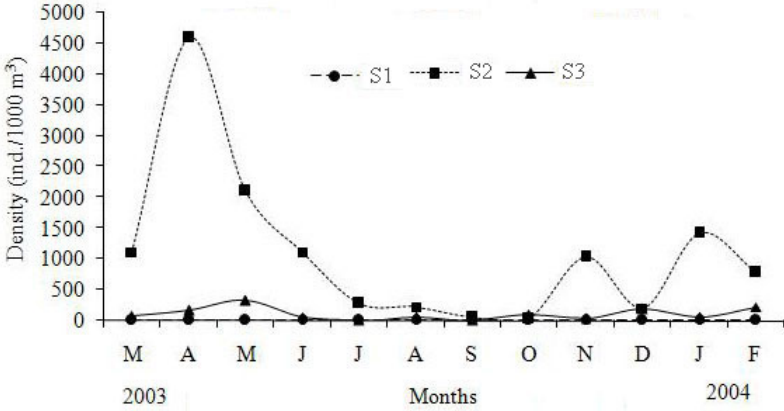


Figure 4

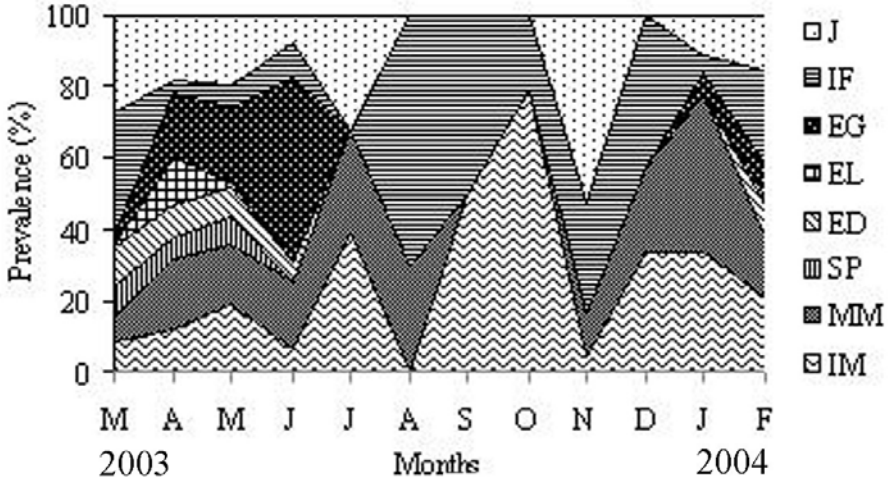


Figure 5

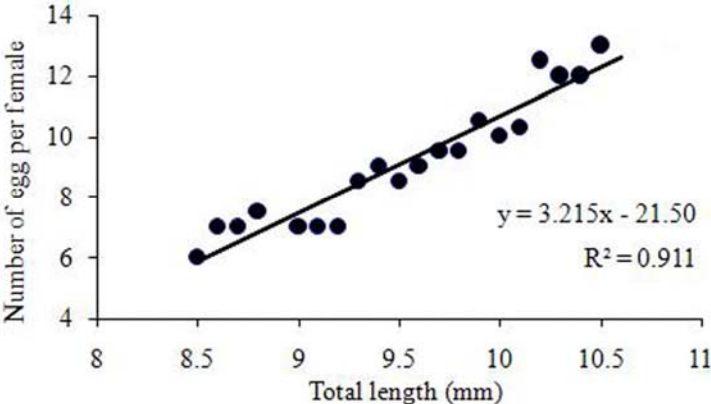


Figure 6

