Available online at http://www.journalijdr.com

IJDR

International Journal of
DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 4, Issue, 2, pp. 377-383, February, 2014

Full Length Research Article

EARLY LARVAL STAGES OF THE SPINY LOBSTERS, *Panulirus homarus, Panulirus versicolor* AND *Panulirus ornatus* CULTURED UNDER LABORATORY CONDITIONS

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ARTICLE INFO

Article History:
Received 10th November, 2013
Received in revised form
28th December, 2013
Accepted 04th January, 2014
Published online 21st February, 2014

Key words:
Phyllosoma,
Salinity tolerance,
Panulirus homarus,
Panulirus versicolor,
Panulirus ornatus

ABSTRACT

Early developmental stages of the phyllosoma larvae of three tropical spiny lobsters, *Panulirus homarus*, *Panulirus versicolor*, and *Panulirus ornatus* have been described by rearing them. *P. homarus* phyllosoma grew to the V stage in 46-61 days. *P. ornatus* larva reached the IV stage in 32-36 days, while *P. versicolor* attained the same stage in 30-38 days. More than one or two moults were recorded between different stages, such as after II stage, in all the three lobsters. Early stages of phyllosoma larvae of *P. homarus* could be successfully reared in low salinity of 28 psu. The problems caused by epibionts like *Leucothrix sp*., *Zoothamnium sp*., *Acinetasp*., *Epistylis sp*. and *Vorticella sp*. are discussed. The features of the phyllosoma larvae were compared with the descriptions reported from plankton collections.

INTRODUCTION

The completion of life cycle of a spiny lobster, *Jassus lalandi* was first achieved by Kittaka (1988). Since then, full larval development has been achieved in many species of temperate and subtropical lobsters (Kittaka 1994). The period of larval development in spiny lobsters varies from 4 months to more than 12 months in captivity in different species (Kittaka 1988, 1994). In India, Prasad and Thampi, (1959) were the first to describe the first stage phyllosoma larvae of *Panulirus homarus* and *Panulirus ornatus* bred in the laboratory. Later Radhakrishnan and Vijayakumaran (1986, 1995) and Vijayakumaran and Radhakrishnan (1986) described various stages of phyllosoma larvae of *P. homarus* reared in captivity and their feeding and other requirements. Duggan and McKinnon (2003) also reported the early developmental larval stages of *P.ornatus* under laboratory conditions. Despite the number of studies addressing the larval biology (Dickey-Collas *et al* 2000a: Briggs *et al*., 2002: Rotlland *et al*., 2004: dos Santos and Peliz, 2005: Patrica *et al*., 2009). This paper reports the rearing of the early phyllosoma stages of three commercially important spiny lobsters in India, *P. homarus, P. versicolor* and *P. ornatus* and attempts to find out if the early phyllosoma larvae of these lobsters could be distinguished from one another by any special characters.

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MATERIALS AND METHODS

Wild breeders of *P. homarus*, *P. versicolor* and *P. ornatus* were reared in 100-250 l fibreglass tanks and fed the green mussel, *Pernav iridis* and the clam, *Donax cuneatus* at the Kovalam field laboratory of the Central Marine Fisheries Research Institute, Chennai, India. During November-December, due to north east monsoon rains, the salinity of the source seawater reduced to 20 to 25 psu, from the normal 32-35 psu, and was made up to 32 psu in the breeder tanks by adding raw common salt. The number of phyllosoma larvae released was estimated by taking 10 sub samples from the

breeding tank. Healthy and active larvae which swim towards a light source were collected for rearing. The larvae were reared in U-shaped fiberglass tanks of 30 and 80 l capacity, with a screen of 100µ mesh at the middle to prevent escape of live feed and larvae. The tanks were connected to a recirculation system (with upwelling water circulation) with biological filter, cartridge filters (10, 5 and 1µ) and an Ultra Violet filter. A stocking density of 10/l freshly hatched larvae was followed after initial experiments in which up to 100/l were stocked. Freshly hatched *Artemia* nauplii, at a rate of 6-7/ ml, were fed to the larvae daily in the morning after removing debris and dead larvae by siphoning from the bottom of the tank. The nauplii and phyllosoma were often found sticking to the screen due to water circulation and the screen was cleaned daily. Once a week, the tank was emptied, cleaned and dried and the screen was replaced. Dead larvae and moults were examined daily to note the developmental stages, feeding and health status. The U-shaped tanks were also used as static system with aeration for larval rearing. Other systems used for rearing were large (3-5 l) and small (500 ml) beakers, 2-8 l round aquaria with thin glass and small plastic containers. Small beakers and plastic containers were used for rearing larvae individually.

Salinity, temperature, pH and Dissolved Oxygen (DO) of rearing water were checked daily in the morning while alkalinity, ammonia, nitrite and nitrate were measured once a week in the larval rearing systems. The larvae were reared in 32 to 35psu salinity except in one of the experiments where the larvae of *P. homarus* were reared in 28 psu salinity made up by diluting stored sweater of 35 psu. Freshly hatched *Artemia* nauplii were the main feed used. When the larvae reached III stage 2-3 day old *Artemia* nauplii (fed with mixed phytoplankton) were also fed to them along with the freshly hatched ones. Shark liver oil, squid oil, cod liver oil and a commercial enrichment medium, "Super Selco" (Inve Inc., Belgium) were used to enrich the freshly hatched *Artemia* nauplii with ω3 Highly Unsaturated Fatty Acids (HUFA). Freshly hatched nauplii were kept in strongly aerated enrichment medium for six hours prior to feeding. Green mussel gonad, polychaete worm, N*ereis* sp., and commercially available frozen marine C*yclops* were also tried as feed for phyllosoma larvae.

Malachite green (10ppm), formalin (25ppm), turmeric powder (1ppm) and streptomycin (0.5 –1ppm) were used for dip treatment at frequent intervals to disinfect the larvae with epicommensals like *Zoothamnium* sp., *Acinata* sp., *Epistylis* sp., *Vorticella* sp., and the filamentous bacterium, *Leucothrix* sp. For microbial monitoring, Total Plate Count (TPC) and Thiosulphate-Citrate-Bile Salts-Sucrose Agar (TCBS) plate count (for *Vibrio*) in the storage and larval tanks in the recirculating system and *Artemia* hatching tanks were monitored at weekly intervals. The sequence of developmental stages of *P. cygnus, P. homarus, P. ornatus and P. versicolor* described by Berry (1971), Phillips and Sastry (1980), Prasad *et al*. (1975) and Radhakrishnan and Vijayakumaran (1995) were followed for determining the stages. Total Length (TL) and Carapace Length (CL) were measured from the anterior tip of the carapace between the eyestalks to the posterior end of the abdomen in the midline and to the posterior margin of the carapace respectively. Maximum width of carapace (CW) was also noted for each larva.

RESULTS

The egg development was completed in all the three species of lobsters in 22-30 days at temperatures of 25-30^{0}C. Hatching of phyllosoma larvae occurred during night or early morning and in all but one instance, larvae were released as fully developed phyllosoma in all three species of lobsters. Week larvae were released on many occasions. One of the *P. homarus* breeders released a whole batch of over 100,000 larvae as what appeared to be "naupliosomas", because of their highly folded and curved appendages but in reality were stage 1 phyllosoma tamorphologically, because they had a uniramous second antenna and fully developed maxilliped and periopods**.** The "naupliosoma" had all the features of the phyllosoma, but the appendages were folded and curved and allowed little movement. Few "naupliosoma" larvae were kept individually and they were observed to stretch their appendages over a period of 10-12 hours to assume the form of phyllosoma. No moulting was involved in the process. However, none of the "naupliosoma" survived more than 24 hours.

The salinity in the larval rearing tanks ranged from 32-35 psu and low survival was recorded when the salinity was raised by adding common salt. The larvae reared in 28 psu salinity had good survival and normal development. The pH ranged from 7.7 to 8.2 and temperature from 25 to 31^{0}C in the rearing system. DO values generally ranged from 4-5ml/l. Mass mortality of *P. homarus* larvae was recorded when the DO level fell below 1ml/l in the U shaped tanks in the reirculating system in one of the experiments. The ammonia level was below 0.1 ppm and the nitrate and nitrite levels were below 0.05 ppm. The alkalinity ranged from 100-110 mg $CaCO_3$ in the larval rearing system. *P. versicolor* and *P. ornatus* larvae survived up to 42-43 days while *P. homarus* larvae could be reared up to 61 days. The enriched *Artemia* nauplii were fed only to larvae reared individually and the commercial enrichment medium proved to be better than fish oils. Freshly hatched *Artemia* nauplii were the best feed among all the feeds tried. Mussel gonad and frozen marine cyclops were also consumed by larvae from III stage onwards but these feeds get entangled in the setae in the exopods of the periopods hampering the movement of larvae. Epicommensal infestation was more in larvae fed with such feeds.

The protozoan, *Zoothamnium* sp. and the filamentous bacterium, *Leucothrix* sp. were the dominant epicommensals, while *Acinata* sp., *Epistylis* sp., and *Vorticella* sp. were also recorded occasionally on the larvae. Ten-minute dip in 10ppm malachite green and bath treatment with 0.05-ppm streptomycin were used to control epicommensals on the larvae. The bacterial load especially that of *Vibrio sp.*, was high and progressively increased from 2.4 x $10^2$to 1.2 x 10^4 CFU/ml in later stages of rearing. Instances of mortality of larvae were high with high *Vibrio* load. *P. ornatus* larva reached the IV stage in 32-36 days, while *P. versicolor* attained the same stage in 30-38 days. *P. homarus* grew to the V stage in 46-61 days. Larvae of all lobsters reached the III stage after two moults, but three moults were involved between III and IV stages. In *P. homarus*, which was grown to Vb stage, two moults were recorded in IV and V stages. The instars within a stage have been identified as a, b and c, like IIIa, IIIb and IIIc.

Table 1. Bacterial load in lobster hatchery system

Medium used	Total Plate count (TPL)			
	Brood stock tank	Upwelling tank with water circulation	Upwelling tank without water circulation	Artemia hatching tank
Nutrient Agar	2.3×10^2 to 4.6×10^3	2.34×10^2 to 2.2×10^3	2×10^3 to 9.1×10^3	15×10^3 to 7×10^4
TCBS Medium	0 to 6×10^3	2.4×10^2 to 1.2×10^4	1.35×10^2 to 18.9×10^3	4.2×10^3 to 4.5×10^4

Table 2. Growth parameters of early larval stages of *P. homarus* (The data on Planktonic larvae after Prasad et al., 1975) Values expressed as mean ±SD

Stage	Carapace length (mm)	Carapace Width (mm)	Total length (mm) (Range in parentheses)		Growth factor	
			Reared	Planktonic	Reared	Planktonic
I	0.77±0.03	0.71±0.01	1.45±0.04 (1.38-1.52)	1.70±0.42 (1.4-2.00)	-	
II	1.15±0.03	0.96±0.02	2.01±0.03 (1.81-2.06)	2.5±0.57 (2.1-2.9)	1.39	1.47
IIIa	1.33±0.02	1.07±0.03	2.23±0.03 (2.12-2.27)	-	1.12	-
IIIb	1.77±0.03	1.39±0.02	2.82±0.03 (2.79-2.92)	-	1.26	-
IIIc	1.85±0.03	1.36±0.03	2.84±0.02 (2.57-3.01)	3.35±0.78 (2.8-3.9)	1.01	1.50
IVa	2.15±0.02	1.45±0.03	3.19±0.01 (3.17-3.22)	-	1.12	-
IVb	2.48±0.02	1.64±0.03	3.63±0.04 (3.59-3.69)	5.15±2.19 (3.6-6.7)	1.14	1.49

Table 3. Growth parameters of early larval stages of *P. versicolor*. (The data on planktonic larvae after Prasad et al., 1975) Values expressed as mean ± SD

Stage	Carapace length (mm) Reared	Carapace Width (mm) Reared	Total length (mm) (Range in parentheses)		Growth Factor	
			Reared	Planktonic	Reared	Planktonic
I	0.81±0.01	0.77±0.02	1.50±0.03 (1.45-1.58)	1.65±0.21 (1.5-1.8)	-	-
II	1.12±0.03	0.98±0.04	1.96±0.16 (1.73-2.04)	2.4±0.0.71 (1.9-2.9)	1.31	1.45
IIIa	1.35±0.02	1.08±0.03	2.15±0.04 (2.11-2.25)	-	1.07	-
IIIb	1.72±0.03	1.30±0.03	2.69±0.05 (2.61-2.75)	-	1.25	-
IIIc	1.79±0.03	1.21±0.02	2.78±0.02 (2.65-2.98)	3.55±0.78 (3.0-4.1)	1.03	1.48
IVa	2.01±0.07	1.41±0.04	3.22±0.02 (3.16-3.24)	4.85±0.92 (4.2-5.5)	1.16	1.37

Table 4. Growth parameters of early larval stages of *P. ornatus*. (The data on planktonic larvae after Prasad et al., 1975) Values expressed as mean ±SD

Stage	Carapace length (mm) Reared	Carapace Width (mm) Reared	Total length (mm) (Range in parentheses)		Growth Factor Planktonic	
			Reared	Planktonic	Reared	Planktonic
I	0.79±0.03	0.76±0.01	1.54±0.03 (1.49-1.58)	1.65±0.35 (1.4-1.90	-	-
II	1.22±0.06	0.98±0.04	1.82±0.21 (1.73-1.84)	2.6±0.35 (2-3.2)	1.21	1.57
IIIa	1.40±0.09	1.17±0.13	2.23±0.14 (2.19-2.49)	-	1.28	-
IIIb	1.86±0.13	1.26±0.06	2.78±0.13 (2.61-2.87)	-	1.25	-
IIIc	2.41±0.05	1.65±0.05	3.46±0.05 (3.40-3.51)	3.9±0.85 3.3-4.5)	1.29	1.50
IVa	2.44±0.07	1.68±0.04	3.58±0.03 (3.53-3.60)	5.8±1.70 (4.6-7.0)	1.03	1.49

Table 5. Carapace Length (CL) to Carapace Width (CW) ratio and cumulative intermoult period of phyllosoma larvae of *P. homarus, P. versicolor* and *P. ornatus* reared in the laboratory Values expressed as mean ±SD

Stage	CL/CW ratio			Stage	Cumulative intermoult period (days) (Range in Parenthesis)		
	P. h	*P. v*	*P. o*		*P. h*	*P. v*	*P. o*
I	1.08	1.05	1.04	I-II	8.6±2.0 (7-10)	8.5±1.9 (8-11)	8.2±1.9 (7-10)
II	1.20	1.14	1.24	II-IIIa	14.6±1.9 (13-20)	15.2±1.8 (14-20)	14.5±1.9 (13-19)
IIIa	1.24	1.25	1.20	IIIa-IIIb	22.4±1.1 (21-25)	22.6±1.4 (21-24)	21.4±1.1 (19-23)
IIIb	1.27	1.32	1.48	IIIb-IIIc	25.7±1.2 (26-30)	26.8±1.2 (25-32)	25.6±1.2 (26-29)
IIIc	1.36	1.48	1.46	IIIc-IVa	33.5±1.4 (32-38)	35.0±2.8 (33-36)	34.3±1.3 (32-36)
IVa		1.43	1.45				

Table 6. Changes in successive phyllosomaa stages in Coaxal and exopodal spines and number of setae on the apical segment of maxilla 2 and pairs of swimming setae on exopods of periopods in *P. homarus, P. versicolor* and *P. ornatus* reared in the laboratory

Stage	No. of setae on Maxilla 2	No. Coaxal spines (Dorsal (DCV) and Ventral (VCV)), Sub exopodal spines (SEV) and No. of pairs of setae on exopods of Maxillipid (MX) and Periopods (P)					
		MX 3	P1	P2	P3	P4	P5
I	4	3, VCS	5, VCS, SCS	5, VCS, SCS	Exobud	-	-
II	4	3, VCS, SES	6-7, VCS, SES	6-7, VCS, SES	Exobud VCS, ES	-	-
IIIa	4	4, VCS, SES	7, VCS, SES	7, VCS, SES	3, VCS, SES	-	-
IIIb	4	4, VCS, SES, DCS	8, VCS, SES, DCS	8, VCS, SES, DCS	4, VCS, VES, DCS	P bud	-
IIIc	4	5, VCS, SES, DCS	9, VCS, SES, DCS	9 VCS, SES, DCS	5, VCS, SES, DCS	P bud	-
IVa	4	5, VCS, SES, DCS	10, VCS, SES, DCS	10, VCS, SES, DCS	6, VCS, SES, DCS	P biramous	-

The CL, TL, CW and growth factor of various stages of phyllosoma larvae of *P. homarus, P. versicolor*and *P. ornatus* and the TL of the above species reported from plankton collections by Prasad *et al*. (1975) are given in Tables 1, 2 and 3 respectively. The growth factor is expressed as the ratio of increase in TL after a moult in an instar/stage over the previous instar/stage. CL/CW ratios of the larvae of the three species and the intermoult periods are given in Table 4. The intermoult durations are similar in all the three species. In the first stage, the CL/CW ratio of *P. homarus* was slightly higher than that of the other two species. In all the three species the cephalic shield is oval shaped but slightly concave antero-laterally with a very obtuse medial point at the posterior end.

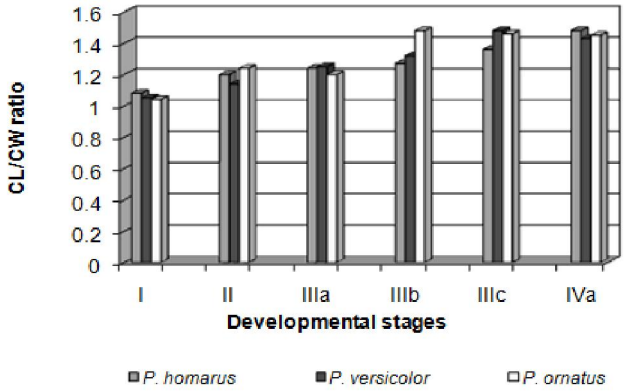


Figure 1. carapace length/carapace width ratio in phyllosoma larvae of *P. homarus, P. versicolor* and *P. ornatus*

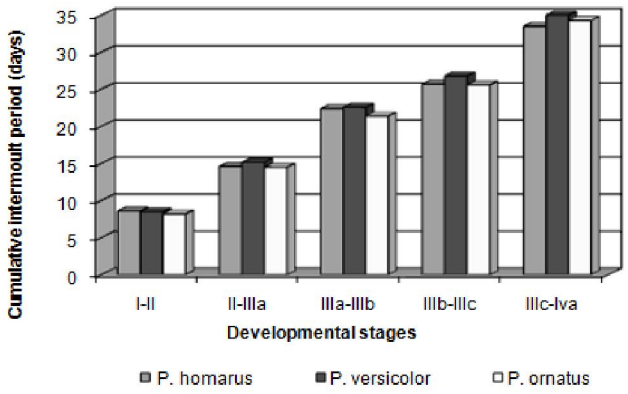
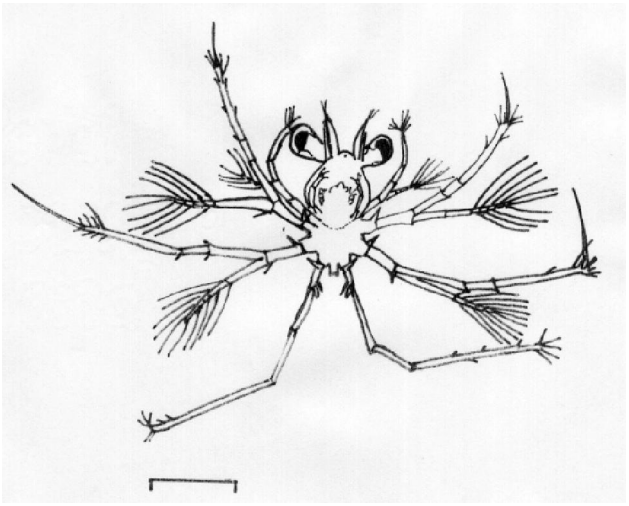
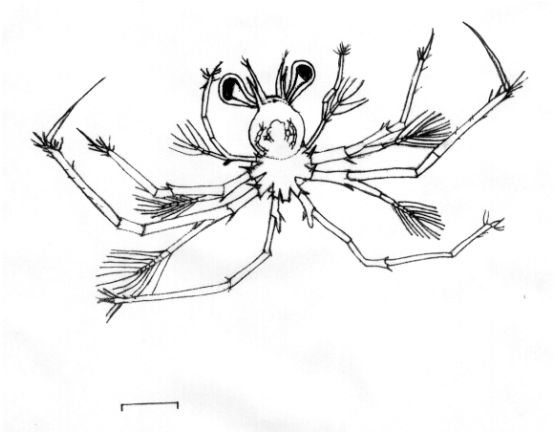


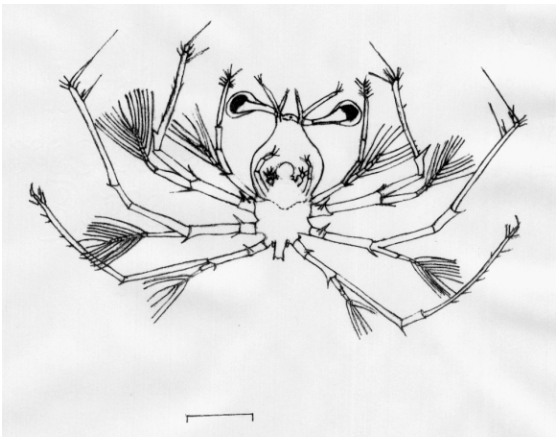
Figure 2. Cumulative intermoult periods in phyllosoma larvae of *P. homarus, P. versicolor* and *P. ornatus*



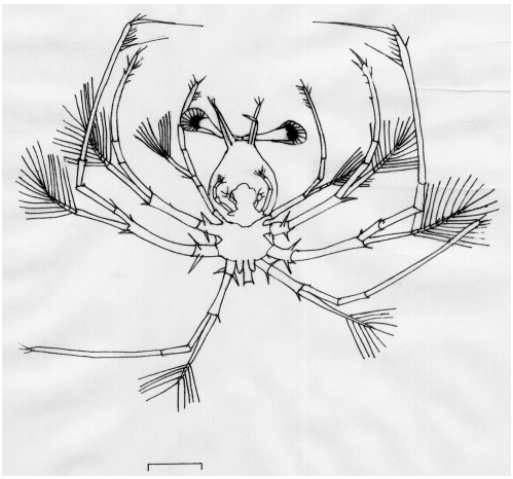
a. Stage I



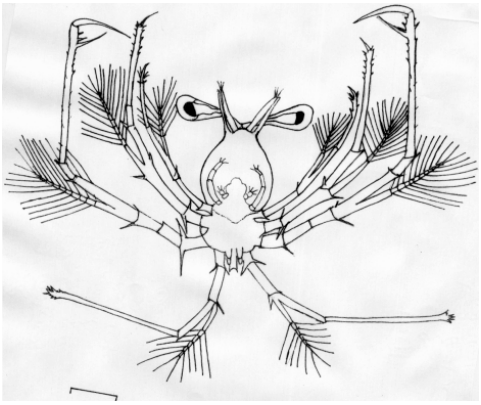
b. Stage II



c. Stage IIIa

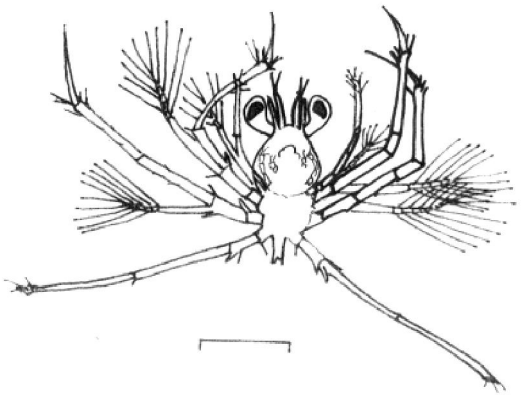


d. Stage IIIc

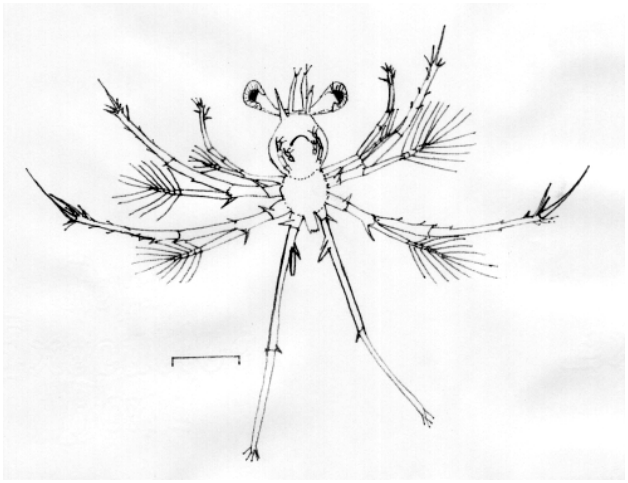


e. Stage IV

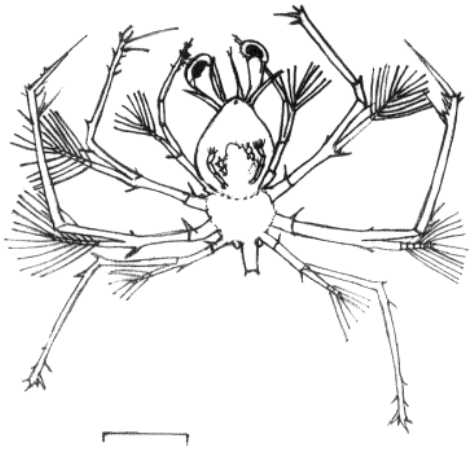
Figure 3. *P. homarus*; Phyllosoma stages



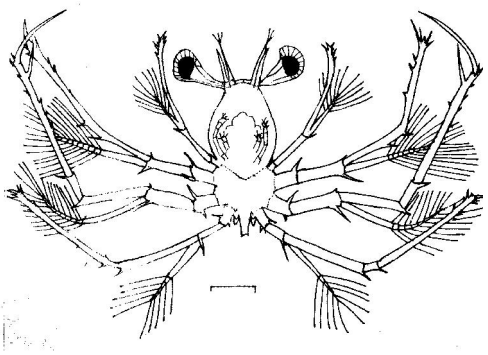
a. Stage I



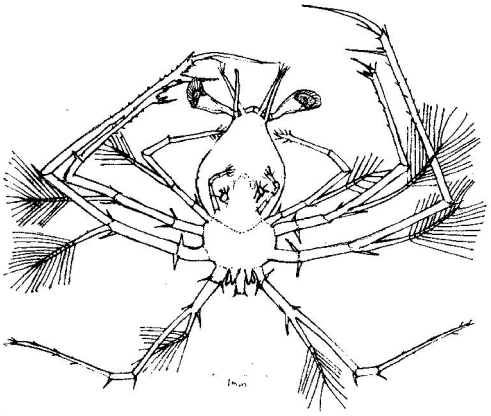
b. Stage II



c. Stage IIIa

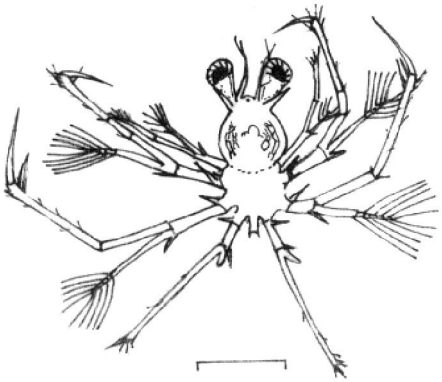


d. Stage IIIc

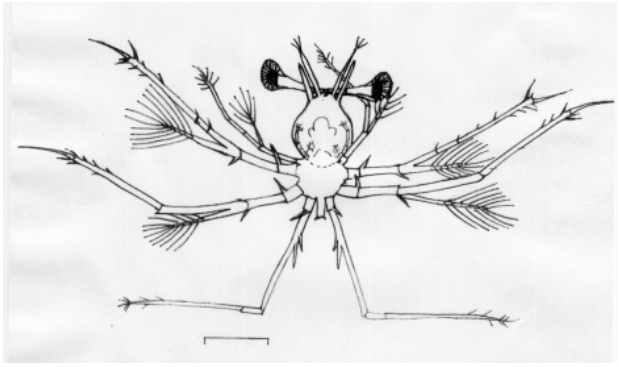


e. Stage IV

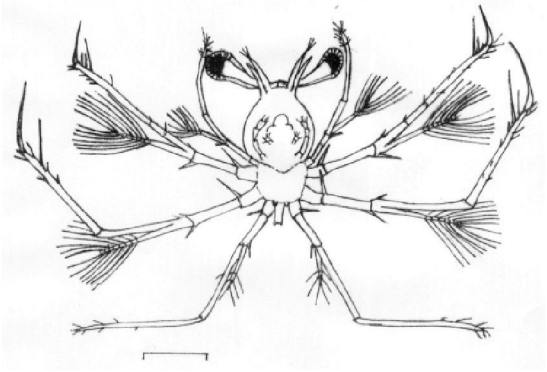
Figure 4. *P.ornatus*; Phyllosoma Stages



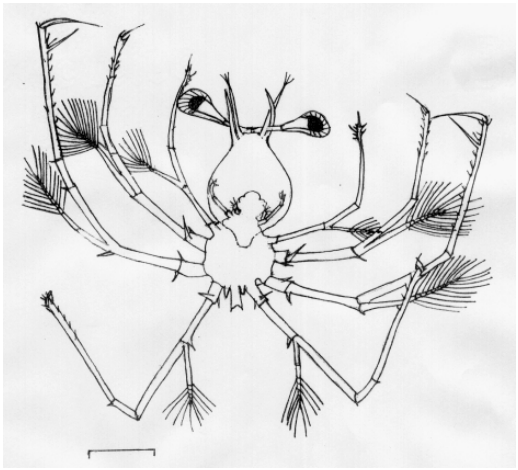
a. Stage I



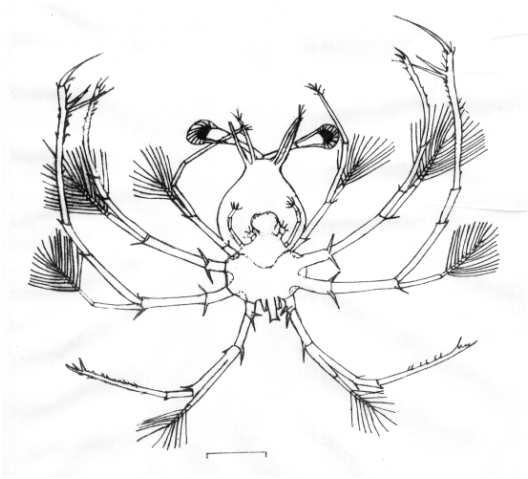
b. Stage II



c. Stage IIIa



d. Stage IIIc



e. Stage IV

Figure 5. *P. versicolor*: Phyllosoma stages

The antero-lateral region becomes progressively more concave just below the insertion of the antennae. The postero-medial point becomes progressively more pronounced and acute as illustrated in Figures 3 - 5. Principal changes in successive phyllosoma stages of the three species are given in Table 5 and in figures 3-5. There are no perceptible variations in the features of the larvae of the three species in the four early stages and sub stages.

DISCUSSION

Coastal areas in the Indian peninsula are subjected to seasonal changes in salinity due to discharge of large quantities of freshwater during the south west monsoon (May to September) in the west coast and the north east monsoon (October to December) in the east coast. Salinity as low as 20 psu has been recorded during November in the coastal waters near Chennai. The spiny lobsters inhabit near shore waters and are subjected to the temporary fluctuation in salinity. As these lobsters breed throughout the year, the phyllosoma larvae also might be exposed to lower salinities. The normal moulting and growth recorded for early phyllosoma larvae in lower salinity of 28 psu demonstrate their adaptive capability to survive in lower salinities. Larval stages named as "naupliosoma", "prenaupliosoma" and "prephyllosoma" have been described for a few spiny lobsters (Phillips and Sastry, 1980). The naupliosoma lasts only few hours and moults to stage I phyllosoma. Deshmukh (1968) described a stage distinct from naupliosoma and phyllosoma in freshly hatched *P. dasypus* (= *homarus*) and termed it "prephyllosoma". Prasad and Thampi (1959) also observed this form in *P. burgeri* (= *homarus*). The prephyllosoma was distinctly free living and lasted about three hours after which it moulted to the stageI phyllosoma larva, but all the larvae released were not in the prephyllosoma stage. Vijayakumaran and Radhakrishnan (unpublished) observed release of "naupliosoma" similar to the ones reported in this study in *P. homarus* and believed that this was the result of premature release due to some stress or weakness to complete the larval release and that these larvae never survived. The release of a whole batch of larvae which looked like naupliosoma but were really classifiable as stage 1 phyllosoma with uniramous antennae and fully developed maxilliped and periopods, supports the view that the naupliosoma is an embryonic, and not free living stage in the development of *P. homarus*. Most of the larvae from II stage onwards tend to remain at the bottom of the tank and many of these are handled daily while removing debris and dead larvae by siphoning the bottom. During individual rearing also, the larvae were transferred twice daily while changing water and cleaning the containers.

The handling stresses, infestation of the larvae with epicommensals, and high vibrio load in later stages were some of the reasons for larval mortality. Berry (1974), Prasad and Thampi (1959), and Prasad *et al*. (1975) reported the difficulty in identifying early larval stages of the spiny lobsters *P. homarus, P. versicolor* and *P. ornatus* as there are similarities between early stage larvae of these species. The only difference appeared to be the number of setae in the apical segment of the second maxilla. 5 setae were recorded in II maxilla in the IV stage in *P. homarus* and *P. versicolor*, while 6 in III stage and 10 in IV stage were recorded for *P. ornatus* larva by these authors. The present observation differs with description of Prasad *et al* (1975), in that only 4 setae were recorded in the apical segment in the II maxilla even in IV stage and this was similar in all three species and could not be taken as the identification feature of any species. The other difference from the description of Prasad *et al.,* (1975) was the size of the larvae in the plankton collection. Radhakrishnan and Vijayakumaran (1995) also reported similar size differences between the reared and field-collected *P. homarus* larvae. Berry (1974) observed that there was similarity in stages 5-9 and 11 of *P. ornatus* from South China Sea described by Johnson (1971) and *P. homarus* from the Natal coast.

The two appear to be indistinguishable except that the dorsal coaxal spine appears in stage 3 in *P. homarus* and apparently in stage 8 in *P. ornatus*. The observation of Johnson (1971) on the dorsal coxal spine in *P. ornatus* is contrary to the observation in this study and that of Prasad *et al*. (1975) that the dorsal coxal spine appears in stage III in all three species of *P. homarus, P. versicolor and P. ornatus.* The present observation differs from that of Prasad *et al*. (1975) in that the dorsal coxal spine does not appear even in IV stage in the III maxilliped and the first periopod in these species. The Presence of Sub-exopodal spine in periopods 1-4 in *P. versicolor* (Johnson, 1971) and only in 1-3 in *P. homarus*and *P. ornatus* could be described as the identifying feature of *P. versicolor*. This could not be compared in this study since the larvae were grown to only IV stage and the fourth leg was only in the initial stages of development. Description of phyllosoma

larval stages with distinctive features for individual species is required for identification of the larvae from plankton collections to understand distribution, settlement and recruitment to the fishery. It was extremely difficult to pinpoint distinguishing features of early larval stages of *P. homarus, P. versicolor* and *P. ornatus* from hatchery reared phyllosoma larvae.

Acknowledgement

We thank Dr. P.V. Sreenivasan and Dr. E. Vivekanandan, Principal Scientists, Central Marine Fisheries Research Institute, Madras Research Centre, Chennai for critically reviewing the manuscript. Our thanks are also due to Mr. M. Kathirvel, Principal Scientist, Central Institute of Brackish water Aquaculture, Chennai for the help in preparing the drawing of the larvae.

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