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EARLY LARVAL DEVELOPMENT OF THE SPINY LOBSTER PANULIRUS HOMARUS (LINNAEUS, 1758) REARED IN THE LABORATORY

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ABSTRACT

Phyllosoma larvae of the spiny lobster, *Panulirus homarus* were hatched and reared in the laboratory on a diet of *Artemia salina* nauplii. The larvae were reared in individual as well as in mass culture systems. The temperature of the rearing water ranged from 26 to 29° C and salinity from 34 to 35‰. The larvae reared individually moulted nine times and reached the sixth stage in sixty days. Mean total length of the newly hatched larva was 1.48 mm and stage VI larva measured an average of 4.87 mm. The early stages of phyllosoma larva of *P. homarus* are morphologically similar to those of other tropical species. Larvae infested with sedentary ectoparasites were effectively treated with 10 ppm malachite green. Change in feeding habits resulted in mortality of the larvae in the sixth stage.

RÉSUMÉ

Des larves phyllosomes de la langouste Panulirus homarus ont éclos, puis ont été élevées au laboratoire grâce à un régime de nauplies d'Artemia salina. Les larves ont été élevées aussi bien individuellement qu'en masse. La température de l'eau d'élevage était de 26 à 29°C et la salinité de 34 à 35‰. Les larves élevées individuellement ont mué 9 fois et ont atteint le sixième stade en soixante jours. La longueur totale moyenne des larves nouvellement écloses était de 1,48 mm et la larve au stade VI mesurait en moyenne 4,87 mm. Les premiers stades de la larve phyllosome de P. homarus sont morphologiquement semblables à ceux des autres espèces tropicales. Les infestées d'ectoparasites sédentaires ont été effectivement traitées avec 10 ppm de vert malachite. La modification du régime alimentaire a abouti à la mortalité des larves au sixième stade.

INTRODUCTION

Attempts at rearing phyllosoma larvae of spiny lobsters through their entire life cycle have been unsuccessful due to difficulties in providing suitable diets in the later stages of development (Saisho, 1962; Ong, 1967; Dexter, 1972). The first success in rearing a palinurid lobster from egg to puerulus stage was achieved by Kittaka (1988) and Kittaka et al., (1988a, 1988b).

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Among the six species of spiny lobsters occurring in Indian waters, *Panulirus homarus* (Linnaeus, 1758) is commercially exploited along the coast of southern India. The present paper describes and illustrates the early larval development of this species in the laboratory.

Although infestation of the cultured larvae by various microepibionts has been a major problem, especially in mass culture systems, there is little information on these aspects of phyllosoma larvae. An attempt has thus been made to study the effect of a chemical agent on filamentous protozoans infecting the phyllosoma larvae of *P. homarus*.

MATERIAL AND METHODS

An ovigerous female of *P. homarus*, 57.3 mm in carapace length, was obtained off Kovalam, Madras, southeast coast of India. It was maintained in a 250 l fibreglass tank containing filtered seawater, in the laboratory. The larvae were visible through the transparent shell of the dark-brown coloured eggs. The water was moderately acrated and the lobster was fed with fresh clam meat. Hatching occurred during the night and the young directly emerged as phyllosoma larvae. Water temperature in the spawning tank at the time of hatching was 24.2°C and salinity 32.8‰. Once the eggs had hatched, the female was removed.

Phyllosoma larvae are positively phototactic and healthy larvae are attracted towards the light source. For mass rearing of the larvae, 150 larvae each were transferred to six 5 l (10×30 cm) glass troughs containing 3 l of filtered (through 1 µm mesh) seawater. For individual rearing, each larva was pipetted and placed in a translucent cup (150 ml) with filtered seawater. The larvae were fed *Artemia* nauplii daily. Individual larvae were transferred to newly prepared cups every day. Phyllosomas were exposed to ambient photoperiod conditions in the laboratory. Temperature of seawater in the rearing containers ranged from 26 to 29°C and salinity was 34 to 35‰. The moults were recorded and five larvae each were preserved in 7% buffered formalin after each moult.

The sequence of developmental stages proposed for the phyllosoma larvae of *Panulirus cygnus* George, 1962 (cf. Phillips & Sastry, 1980) has been followed for delineating the stages in the present study. Total length (TL) and carapace length (CL) are measured from the anterior tip of the carapace between the cyestalks to the posterior end of the abdomen in the midline and the posterior margin of the carapace, respectively. Maximum width of the carapace was considered as the carapace width (CW).

The effect of malachite green was examined on the stalked protozoans infecting the phyllosoma larvae at 5, 10, and 20 ppm concentrations. The larvae, in groups of five, were maintained in plastic cups (150 ml) and were exposed for 5, 10, and 15 minutes to each of these concentrations. After exposure, they were released back into the rearing cups and were examined

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with regard to the condition of the infestation as well as to that of the larvae. The malachite green treatment was continued for five days.

RESULTS

In the laboratory, phyllosoma larvae reared individually moulted nine times and progressed through six developmental stages. The larvae moulted once at stages I and II, three times at stage III, and twice at stages IV and V. The intermediary stages between two numbered stages, have been assigned to a substage (table I). The larvae undergo a series of morphological changes. In stage I larvae, the eyestalk is unsegmented (fig. 1), whereas in stage II, it becomes segmented (fig. 2). The exopod of the third pereiopod becomes setose in stage III (fig. 3). The fourth pereiopod, which appears in stage III, becomes biramous in stage IV (fig. 4). In stage V, the exopod of pereiopod 4 becomes setose (fig. 5), and in stage VI, the antennule becomes four-segmented (fig. 6). In addition to these changes, a pair of setae was added on the exopod of pereiopods 1-3, with each moult (table II).

The intermoult period of the larvae was relatively consistent in the early stages of development. However, the duration of later stages was increasingly variable. While the difference in intermoult period of stage I larvae was 2 days (minimum: 8 days, and maximum: 10 days), variation in cumulative intermoult period was 12 days for the larvae which moulted from stage Vb to VI. The larvae moulted through the entire range of stages within a minimum period of 52 days and a maximum of 64 days. Few of the larvae survived for a maximum of 70 days. There was a progressive reduction in the numbers of larvae after each moult and only 8% survived till after the ninth moult (VI stage, table III).

Larvae in mass culture moulted to stage II in 12-14 days, compared to 8-10

Table I

Carapace length, carapace width, total length, and growth of laboratory reared and planktonic phyllosoma larvae of *Panulirus homarus* (Linnaeus, 1758)

	Carapace length (mm)	Carapace width (mm)	Range in TL (mm)		Mean T	TL (mm)	Growth factor	
Stage			Reared	Planktonic (Prasad et al., 1975)	Reared	Planktonic	Reared	Planktonic
<u>I</u>	0.78	0.72	1.45-1.50	1.40-2.00	1.48 ± 0.01	1.70 ± 0.42		_
II	1.15	0.93	1.84-2.04	2.10 - 2.90	1.94 ± 0.14	2.50 ± 0.57	1.31	1.47
IIIa	1.31	1.05	2.10-2.26	—	2.21 ± 0.34	-	1.13	_
IIIb	1.76	1.35	2.72-2.90	-	2.79 ± 0.56	_	1.26	—
IIIc	1.80	1.25	2.58 - 3.06	2.80 - 3.90	2.82 ± 0.34	3.35 ± 0.78	1.01	1.34
IVa	2.15	1.47	3.18-3.21	—	3.19 ± 0.02	-	1.14	-
IVb	2.48	1.64	3.59-3.68	3.60-6.70	3.63 ± 0.06	5.15 ± 2.19	1.14	1.54
Va	2.80	1.83	3.87-4.06	_	3.96 ± 0.96	_	1.09	
Vb	3.20	2.15	4.53-4.84	5.60 - 8.40	4.69 ± 0.22	7.00 ± 2.01	1.19	1.36
VI	3.56	2.33	4.71-5.03	6.20-11.0	4.87 ± 0.23	8.60 ± 3.39	1.04	1.23



Fig. 1. Phyllosoma larva of Panulirus homarus (Linnaeus, 1758), stage I, ventral view.



Fig. 2. Phyllosoma larva of Panulirus homarus (Linnacus, 1758), stage II, ventral view.



Fig. 3. Phyllosoma larva of Panulirus homarus (Linnaeus, 1758), stage III, ventral view.

TABLE II

Number of sctae on the apical segment of maxilla 2, and pairs of swimming setae on the various exopods, in various phyllosoma stages of *Panulirus homarus* (Linnacus, 1758) reared in the laboratory

	Mean total	Setae on	Number of pairs of swimming setae on exopod:						
Stage	(mm)	maxilla 2	Maxilliped 3	Leg 1	Leg 2	Leg 3	Leg 4	Leg 5	
I	1.48	4	3	5	5	bud	0	0	
П	1.94	4	3	6-7	6-7	bud	0	0	
IIIa	2.21	4	4	7	7	3	0	0	
IIIb	2.79	4	4	8	8	4	bud	0	
IIIc	2.82	4	5	9	9	5	bud	0	
IVa	3.19	4	5	10	10	6	biramous	0	
IVb	3.63	4	6	11-12	11-12	7	biramous	0	
Va	3.96	5	7	13	13	8	3-4	bud	
Vb	4.69	5	9	14	14	9-10	5	bud	
VI	4.87	7	10-11	14-15	14-15	10-11	7	bud	



Fig. 4. Phyllosoma larva of Panulirus homarus (Linnaeus, 1758), stage IV, ventral view.



Fig. 5. Phyllosoma larva of Panulirus homarus (Linnaeus, 1758), stage V, ventral view.



Fig. 6. Phyllosoma larva of Panulirus homarus (Linnaeus, 1758), stage VI, ventral view.

days for individually held larvae, and survival was only 23% after the first moult. The major cause of mortality in mass culture was infestation by filamentous microepibionts, mainly stalked, *Vorticella*-like protozoans. The dense colonies that grow on the appendages immobilized the larvae and prevented them from normal swimming and feeding activities. Infestation was noticed on the fourth day after hatching, as small colonies on the pereiopods. In the present study, the seawater was not UV sterilized. The infected larvae were used in the experiments on malachite green treatment.

Infected larvae exposed to 5 ppm concentration of malachite green for a maximum exposure of 15 minutes still had colonies in an active state, even after termination of the experiment. Larvae exposed to 10 ppm concentration for 10 minutes were devoid of the infestation on the third day. After exposure for 10 minutes in 10 ppm concentration, the cup-like structures of the Protozoa changed into a globular shape and were stained greenish blue. On the third day, the cups disappeared and only the stalks remained on the pereiopods. The larvae were active once again and moulted to the next stage.

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TABLE III

	Cumalative in		
Stage	Range	Mean (± S.D.)	- Percent survival
<u> </u>	8-10	8.7 ± 1.95	96
II – IIIa	13-18	14.9 ± 1.95	80
IIIa IIIb	19-22	20.3 ± 1.16	64
IIIb – IIIc	25-29	26.6 ± 1.26	57
IIIc – IVa	32-36	33.7 ± 1.25	48
IVa - IVb	36-45	40.2 ± 2.82	35
IVb – Va	39-50	46.4 ± 3.06	25
Va – Vb	43-57	53.2 ± 4.54	15
Vb – VI	52-64	60.0 ± 3.33	8

Cumulative intermoult period (± S.D.) and percent survival of the phyllosoma larvae of *Panulirus homarus* (Linnaeus, 1758), reared in the laboratory

DISCUSSION

The phyllosoma larvae of the tropical spiny lobster Panulirus homarus have been successfully reared up to the sixth stage on an exclusive diet of recently hatched Artemia salina (Linnaeus, 1758). Although early stage larvac readily feed on freshly hatched Artemia nauplii, late stage larvae seem to have difficulty in catching them. Dexter (1972) found that phyllosoma larvae of Panulirus interruptus (Randall, 1840) prefer large food items such as fish larvae, chaetognaths, and hydromedusae. Kittaka (1988a, 1988b) fed phyllosomas successfully with various sizes of mussel meat to suit the feeding habits of the larvae at different phases of the larval life history. In addition to quality, the optimum quantity of food required for maximum growth of the larvae also needs to be understood. Saisho (1966) found shortening of the intermoult period in phyllosoma larvae of Panulirus japonicus (Von Siebold, 1824) by increasing the density of nauplii given as feed. Vijayakumaran & Radhakkrishnan (1986) found that at optimum food density, phyllosoma larvae of P. homarus moulted only two times to metamorphose from stage III to stage IV as opposed to thrice in the present study. The high mortality in later stages can probably be attributed to the inability of the larvae to catch sufficient prev items to sustain growth.

Mortality of larvae in mass culture was mainly due to infection by *Vorticella*like protozoans. Since starved larvae were not infected, *Artemia* nauplii were suspected to be the source of infection. It is also possible that high larval density (50 larvae/l) resulting in increased interaction of the larvae, would have led to fast spreading of the disease causing organism. Furthermore, palinurid lobster larvae have a prolonged intermoult period which is disadvantageous, at least in laboratory conditions, as it provides sufficient time for microepibionts to grow and so to immobilize them (Ong, 1967). A contributing cause for occurrence of microepibionts was believed to be high levels of phosphate and nitrate in the culture water (Stewart, 1980).

Morphologically, the early phyllosoma larva of *P. homarus* is almost similar to that of other tropical species. Among those three tropical species, i.e., *P. homarus, Panulirus polyphagus* (Herbst, 1793) and *P. interruptus*, the early larvae of which have been reared in the laboratory, *P. homarus* is slightly larger than the other two species in corresponding stages. But, there are specific differences in the sequence of development among the various species. Until now, the phyllosoma larva of *P. homarus* is known only from descriptions based on preserved plankton material (Berry, 1974; Prasad et al., 1975). Preliminary comparison of the laboratory reared specimens with larvae from the plankton shows, that larvae from nature are always larger than those reared in the laboratory (table I). However, there are no major differences in morphology between the planktonic material and the laboratory reared larvae, as far as our observations indicate.

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REFERENCES

- BERRY, P.F., 1974. Palinurid and scyllarid lobster larvae of the Natal coast, South Africa. Oceanogr. Res. Inst. (Durban), Invest. Rep., **34**: 1-44.
- DEXTER, D.M., 1972. Molting and growth in laboratory reared phyllosomes of the California spiny lobster, *Panulirus interruptus*. Calif. Fish. Game, **58**: 107-115.
- Киттака, J., 1988. Culture of the palinurid *Jasus lalandii* from cgg to puerulus. Bull. Japanese Soc. scient. Fish., **54** (1): 87-94.
- Киттака, J. & E. Iкедами, 1988 (cf. a). Culture of the palinurid Palinurus elephas from egg to puerulus. Bull. Japanese Soc. scient. Fish., **54** (7): 1149-1154.
- KITTAKA, J., M. IWAI & M. YOSHIMURA, 1988 (cf. b). Culture of a hybrid of spiny lobster genus *Jasus* from egg stage to puerulus. Bull. Japanese scient. Soc. Fish., **54** (3): 413-418.
- ONG, K.S., 1967. A preliminary study of the early larval development of the spiny lobster *Panulirus* polyphagus (Herbst). Malay. Agric. Journ., **46** (2): 183-190.
- PHILLIPS, B.F. & A.N. SASTRY, 1980. Larval ecology. In: J.S. COBB & B.F. PHILLIPS (eds.), The biology and management of lobsters, 2: 11-57. (Academic Press, New York).
- PRASAD, R.R., P.R.S. TAMPI & M.J. GEORGE, 1975. Phyllosoma larvae from the Indian Ocean collected by the Dana expedition 1928-1930. Journ. mar. biol. Assoc. India, 17: 56-107.
- ROBERTSON, P.B., 1968. The complete larval development of the sand lobster, *Scyllarus americanus* (Smith), (Decapoda, Scyllaridac) in the laboratory, with notes on larvae from the plankton. Bull. mar. Sci., **18**: 294-342.
- SAISHO, T., 1962. Notes on the early development of phyllosoma of Panulirus japonicus. Mem. Fac. Fish. Kagoshima Univ., 1: 18-23. [In Japanese, with English abstract, tables, and legends].
- , 1966. A note on the phyllosoma stages of spiny lobster. Inform. Bull. Planktol. Japan, 13: 69-71. [In Japanese, with English abstract, tables, and legends].
- STEWART, J.E., 1980. Diseases. In: J.S. COBB & B.F. PHILLIPS, The biology and management of lobsters, 2: 301-342. (Academic Press, New York).
- VIJAYAKUMARAN, M. & E.V. RADHAKRISHNAN, 1986. Effects of food density on feeding and moulting of phyllosoma larvae of the spiny lobster *Panulirus homarus*. Proc. Symp. coastal Aquaculture, **4**: 1281-1285.