SPAWNING AND REARING OF THE PENAEID PRAWN, *METAPENAEUS AFFINIS* (H. MILNE EDWARDS) IN THE LABORATORY

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ABSTRACT

The results of two experiments on the spawning and large-scale rearing of *Metapenaeus affinis* under laboratory conditions are reported. The nauplius larva hatched out about 8 hours after spawning while protozoea I, mysis I and post-larva I were attained after 46 hours, 189 hours and 322 hours respectively. While the nauplii thrived on the reserve yolk, protozoea larvae were fed, with varying effects, with cultures of unicellular algae viz., *Synechocystes marina* and *Tetraselmis gracilis* and the diatom, *Thalassiosira* sp. *Thalassiosira* sp., was found to be the best food in protozoea stage. In mysis stage, they were fed with *Artemia* larvae, whereas, in post-larval stages artificial diets were given as food. The morphological features of the different larval stages are described in detail.

INTRODUCTION

Successful spawning and mass-rearing of the larvae of the important penaeid prawns under controlled conditions have become an urgent necessity in the face of our intensive programme of culturing them in commercial scale. Rao and Kathirvel (1973) reported the spawning of *Metapenaeus dobsoni* in brackishwater medium. Raje and Ranade (1975a and 1975b) described the spawning and larval development in the laboratory of *Penaeus merguiensis* and *Metapenaeus monoceros*, and Thomas *et al* (1975) reported the spawning and early development of *Parapenaeopsis stylifera*. However, mass-culture techniques have not been so far developed in this country. Attempts were, therefore, made successfully to rear the larvae of some commercially important species of penaeids in large scale in the laboratory and the results achieved in rearing *M. affinis* is presented in this paper.

Earlier information on the larvae of *M. affinis* is limited to the reports of Hudinaga (1941) of 6 naupliar stages obtained as a result of his rearing these larvae in captivity, and Mohamed *et al* (1968) and Rao (1974) of the first post-larvae and three mysis stages respectively based on their plankton collections. The present communication completely covers the larval development of this species.

EXPERIMENT I

Four gravid female prawns, one *Metapenaeus affinis*, two *M. dobsoni* and one *Parapenaeopsis acclivirostris*, were picked out from a trawl catch obtained from 20-22 m depth off Cochin on 14-3-1975. They were immediately transferred to a plastic tub containing sea water from the same locality. The temperature of the water in the tub was 31.2°C, same as the surface temperature of the fishing ground. These prawns were brought to the laboratory at 15.30 hrs the same day. In the laboratory the animals were kept in separate tubs containing fresh sea water having the initial temperature of 29.8°C and salinity, 33.45 ppm. Sodium salt of EDTA was added to the water at the rate of 1 g| 100 l and tubs were kept undisturbed at room temperature which ranged between 29.2°C and 30.6°C.

All the four species spawned at about midnight of the same day. At 06.00 hrs the next day, eggs were observed in large numbers at the bottom of all the tubs. The eggs were in an advanced stage of development with well-formed active nauplii inside. The mothers were then removed from these tubs. Although the subsequent development of all the three species were studied in detail, only that of *M. affinis* is described in the following account. The development of the other species will be dealt with separately.

Observations

At 06.00 hrs all the viable eggs of *M. affinis* were well-developed with nauplii actively moving inside the egg membrane. The microscopic observations on random samples showed that percentage of such viable eggs were high and there were very few dead eggs. At 07.30 hrs the nauplii started emerging out of the eggs. The process of hatching was the same as in *P. stylifera*, as observed by Thomas *et al* (1975); the larvae made vigorous riggling movements till a portion of the egg membrane gave way. As soon as the larvae have hatched out they began swimming actively to the surface of the water column. The larvae were positively phototactic; they showed the tendency to crowd in a place of greatest illumination. Taking advantage of this behaviour, the larvae were siphoned out with the help of a table-lamp and transferred to various glass troughs. In order to avoid any overcrowding at a single place due to uneven illumination the troughs were kept covered with black paper all the time except when it was necessary to keep them exposed for the purpose of observation. The nauplius I metamorphosed to nauplius II at 10.00 hrs. After about 4 h, at about 14.00 hrs, these moulted to nauplius III. Nauplius IV and nauplius V were reached at 18.00 hrs and 22.00 hrs, respectively. The last substage, nauplius VI, was reached at 06.00 hrs on 16-3-1975, at about 30 h after spawning. No food was offered during this entire period as the nauplii characteristically thrive on the yolk retained for the purpose.

The last naupliar stage, nauplius VI, remained for 16 h without a visible change, a period very long when compared to that of the earlier stages, evidently to accomplish the great morphological changes in the next stage, Protozoea I. Protozoea I emerged at 22.00 hrs, 46 h after spawning. The larva in this stage gained more length and was more agile. They swam about steadily unlike the naupliar stages (which moved about in short jerks) and started feeding. From Protozoea I onwards, the larvae were fed with cultured unicellular algae, *viz.*, *Synechocystes marina* and *Tetraselmis gracilis*. They were feeding on these algae actively, as evidenced from the greenish colour of the alimentary tract and the greenish threads of feacal matter trailing behind many of them. However, mortality in this stage was high. A postmortem examination revealed that most of the dead larvae had their appendages apparently got immobile obviously due to lots of fine particles entangled on the plumose setae. This was probably resulted from the constant stirring of the water during the artificial aeration. Only very few larvae survived this stage.

The Mysis-I larvae were attained 189 h after spawning. With their cephalic region turned downwards they swam actively up and down in the trough. The freshly hatched nauplii of the brine shrimp, *Artemia* sp., were offered as food on which the larvae at this stage were feeding voraciously. Mysis II was noticed at 09.00 hrs on 24-3-1975 and they moulted to Mysis III at 10.00 hrs on 26-3-1975 (274 h after spawning). Mysis III metamorphosed to Post-larva I at 10.00 hrs on 28-3-1975, taking a total of 322 h (13 days and 10 h) after spawning. Rearing was continued up to Post-larva IV and the interval between Post-larva I and II, and II and III was about a day each.

Experiment II

Subsequently, another gravid female measuring 118 mm in total length, collected from the same locality on 25-3-1975, also spawned in the laboratory.

Observations

The animal started spawning at 22.00 hrs on the same day and completed the process of spawning by 22.45 hrs. Most of the eggs in the ovary appeared to have spawned. The hatching of nauplius I started at 08.00 hrs on the next day morning (26-3-1975). They were removed and kept in a number of troughs as in the previous experiment. The nauplius VI metamorphosed to Protozoea I in 60 h, taking 14 h more than the time taken in Experiment I (See Table 1). In this experiment, the protozoea larvae were fed only with cultures of the diatom, *Thalassiosira* sp., which yielded a very high rate of survival (90%) of the larvae. The colour of the digestive tract and the long strips of metabolic waste trailing behind the larvae were indicative of the acceptability of the diatom beyond doubt. The efficacy of this diatom as a feed, at this stage of development, was evident from the decrease in the time taken (91 h) by the protozoeal stages in this ex-

TABLE 1. *Duration of the different larval stages during metamorphosis of* M. affinis *in the laboratory.*

Larval stages	1st Experiment			2nd Experiment			Average duration after spawning (hrs)
	Date	Time (hrs)	Duration after spawning (hrs)	Date	Time (nrs)	Duration after spawning (hrs)	
Spawning	14-3-'75	24.00	00.00	25-3-'75	22.00	00.00	00.00
Nauplius I	15-3-'75	07.00	07.30	26-3-'75	08.00	10.00	08.60
Nauplius II	15-3-'75	10.00	10.00	26-3-'75	11.00	13.00	11.50
Nauplius III	15-3-'75	14.00	14.00	26-3-'75	15.00	17.00	15.30
Nauplius IV	15-3-'75	18.00	18.00	26-3-'75	22.00	24.00	21.00
Nauplius V	15-3-'75	22.00	22.30	27-3-'75	09.45	35.45	28.88
Nauplius VI	16-3-'75	06.00	30.00	27-3-'75	20.00	46.00	38.00
Protozoea I	16-3-'75	22.00	46.00	28-3-'75	10.00	60.00	53.00
Protozoea II	19-3-'75	00.00	96.00	30-3-'75	10.00	108.00	102.00
Protozoea III	21-3-'75	07.00	151.00	31-3-'75	11.00	133.00	142.00
Mysis I	22-3-'75	21.00	189.00	1-4-'75	10.00	151.00	170.50
Mysis II	24-3-'75	09.00	225.00	3-4-'75	09.00	203.00	214.00
Mysis III	26-3-'75	10.00	274.00	4-4-'75	16.00	234.00	254.00
Post-larva I	28-3-'75	10.00	322.00	6-4-'75	15.00	281.00	301.50
Post-larva II	29-3-'75	10.00	346.00	7-4-'75	16.00	306.00	326.00
Post-larva III	30-3-'75	09.00	369.00	11-4-'75	10.00	396.00	382.50
Post-larva IV	3-4-'75	10.00	466.00	14-4-'75	10.00	468.00	467.00
Post-larva V	—	—	—	16-4-'75	10.00	516.00	—
Post-larva VI	—	—	—	18-4-'75	10.00	564.00	—
Post-larva VII	—	—	—	22-4-'75	09.00	659.00	—

periment to metamorphose to Mysis I when compared to the previous experiment (when it took 143 h to reach Mysis I). Postlarva I was reared further on artificial feed and after passing through six more postlarval stages they reached juvenile stage 34 days after spawning.

LARVAL STAGES

Informations on early larval and post larva - I stages of *M. affinis* are available through the works of Hudinaga (1941) and Mohamed *et al* (1968) respectively. In the present study 6 naupliar, 3 protozoeal, 3 mysis and 7 postlarval stages were recognised and the descriptions of the same are given below:

Egg: (Fig. 1, a): diameter — 0.23-0.25 mm.

Eggs are spherical in shape, with a clear perivitelline space; embryonic mass measures 0.14 mm. Fully developed nauplius (Fig. 1, b) is visible in the late stages.

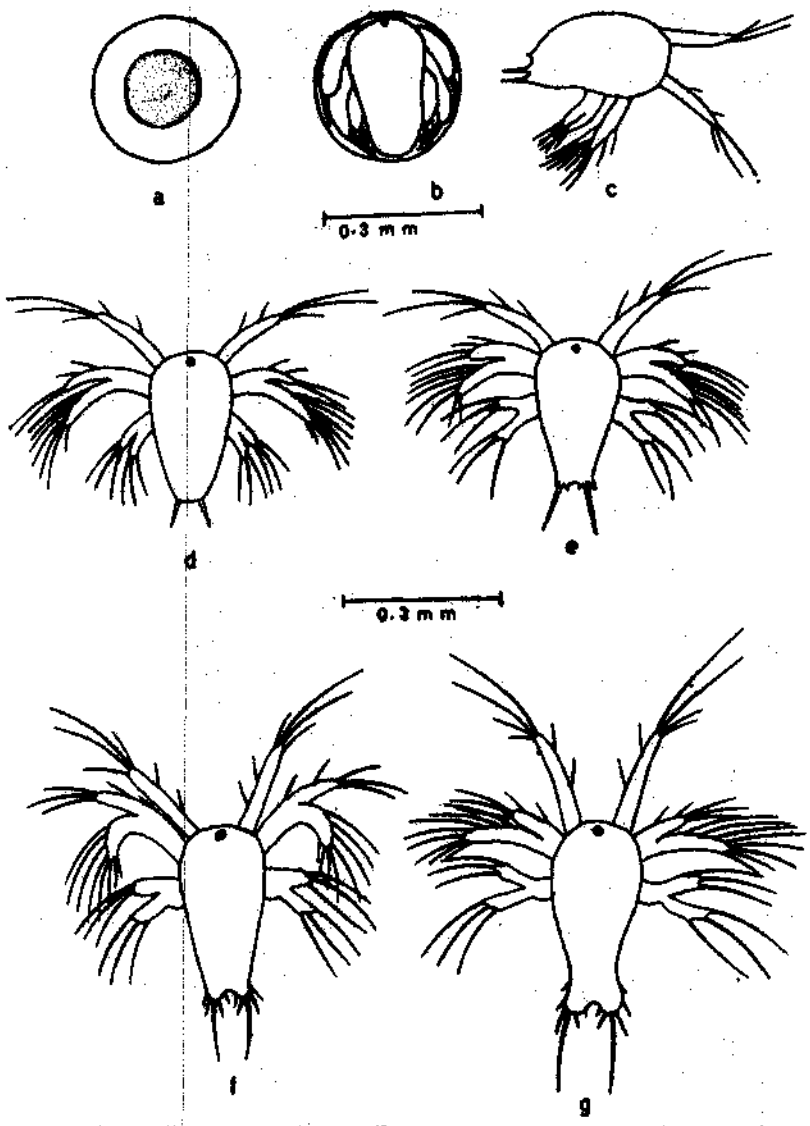

FIG. 1. Egg and naupliar stages of *M. affinis*: a, egg — segmentation completed. b, egg with nauplius inside. c, Nauplius I — dorsal view. d, Nauplius II — dorasal view. e, Nauplius III — dorsal view. g, Nauplius V — dorsal view.

Duration of development inside the egg: 8-10 hrs.

Nauplius I (Fig. 1, c): Total length (TL)- 0.21-0.25 mm;
Greatest width (GBW)- 0.11 mm.

Body of the larva is pyriform with median eye, posterior pair of spines and a minute posterodorsal spine. Paired uniramous antennules, biramous anten-

nae and mandibles present. Antennules with 2 setae on the innerside and 3 at the tip; endopod of antennae with 2 terminal and 2 lateral setae while exopod with 3 terminal and 4 lateral setae; each rami of mandible carrying 3 setae.

Duration of the stage: 2½-3 hrs.

Nauplius II (Fig. 1, d): TL — 0.25-0.26 mm; GBW — 0.13 mm.

The posterodorsal spine disappears. Caudal fork with 2 spines. Setae on the appendages become plumose. No increase of setation on appendages.

Duration of the stage: 4 hrs.

Nauplius III (Fig. 1, e): TL — 0.26-0.28 mm; GBW — 0.15 mm.

Body elongates and posterior end develops a notch between the furcae. Number of spines and setation on the appendages remains same Each furca with 3 spines.

Duration of the stage: 4-7 hrs.

Nauplius IV (Fig. 1, f): TL — 0.30-0.36 mm; GBW — 0.17 mm.

Body of the larvae further elongates posteriorly and caudal furcae develop 2 more pairs of spines. One more pair of setae added to the tip of antennules. Exopods of antennae develop one more seta at the tip and an additional setae on the lateral border of endopod. Mandibles as in the previous substage; rudiments of 2 pairs of maxilla and first 2 pairs of maxilliped appear. Mandibles develop prominent swellings at the bases.

Duration of the stage: 4½-11½ hrs.

Nauplius V (Fig. 1, g): TL — 0.37-0.39 mm; GBW — 0.19 mm.

Posterior region of larvae further elongates and furcae get more differentiated, bearing 6 pairs of spines. Basal swellings of mandibles enlarge further. Setation same as in Nauplius IV. Buds of thoracic legs become larger.

Duration of the stage: 7½-10 hrs.

Nauplius VI (Fig. 2, a): TL — 0.39-0.42 mm; GBW — 0.22 mm.

Body of the larvae gets still elongated. Number of furcal spines increase to 7 on each lobe; frontal organ appears in this stage; segmentation on the appendagaes not clearly visible, annular indentations present; more setae appear on the lateral border of the antennules. Other structures remain unaltered.

Duration of the stage: 14-16 hrs.

Protozoea I (Fig. 2, b): TL — 0.71-0.78 mm; Carapace length (CL) — 0.35-0.38 mm; Carapace width (CW) — 0.31-0.34 mm.

Body, transparent with oval carapace, 6-segmented thorax and unsegmented abdomen, ending in a pair of furcae. One pair of antennules, antennae, mandibles, maxillae and maxillipeds present. Third maxillipeds not fully developed; naupliar eye persists and frontal organs still present. *Antennule* (Fig. 2,c) — uniramous with 3 segments of which tip of distal segment bear one long and two short setae, in addition to three lateral ones. *Antenna* (Fig. 2, d) — biramous with 2-joined protopod and 8-segmented exopod, bearing 9 plumose setae, along its inner margin and tip and also 2 on its outer margin; endopod 2-segmented, distal segment with 5 plumose setae at its apex and proximal segment with 3 setae on its inner margin. *Mandible* (Fig. 2, e) — asymmetrical, devoid of exopod and endopod; masticatory process strongly serrated. *Maxilla* I (Fig. 2, f) — with 3-segmented endopod, bilobed protopod and papilla-like exopod; endopod bears 2, 2 and 4 setae respectively on each of its segments from basal to distal. Exopod with 4 setae; anterior lobes of protopod with 4 stout setae and posterior one with 6 stout setae. *Maxilla* II (Fig. 2, g) — endopod 4-segmented, distal segment having 3 and other with 2 long setae; protopod with 5 lobes, 1st, 2nd and 4th with 3 setae, 3rd one with 2 and 5th with 5 setae; endopod small, bearing 5 plumose setae. *Maxilliped* I (Fig. 2, h) — biramous with 4-segmented endopod, 2-segmented protopod and unsegmented exopod; distal segment of endopod with 5 long setae and proximal one with 3 and others with 1-2 long setae; protopod with 6-8 long setae; exopod with 7 long plumose setae along its outer and distal margins. *Maxilliped* II (Fig. 2, i) — similar to that of 1st maxilliped; but with less setae on proto-, endo- and exopodites. *Maxilliped* III — rudimentary. Telson bearing 7 spines on each lobe. *Colouration* — dark-brown, spot-like chromatophores present at the base of antenna; branching reddish brown chromatophores present in the middle of each lobe of telson.

Duration of the stage: 48-74 hrs.

Protozoea II (Fig. 3, a): TL — 1.16-1.20 mm; CL — 0.41-0.46 mm; CW — 0.39-0.41 mm.

The notable feature of this stage is the presence of stalked eyes, rostrum, small supraorbital spines and segmented abdomen. *Antennule* (Fig. 3, b) — segmentation more clear; distal segment bears 1 small and 3 long setae. *Antenna* (Fig. 3, c) — exopod 10-segmented, 11 long plumose setae along its inner margin and 2 short plumose setae on the outer margin. *Mandible* (Fig. 3, d) — number of large teeth on the cutting edge increased. No significant changes in the structure of *Maxilla* I (Fig. 3, e), *Maxilla* II (Fig. 3, f), *Maxilliped* I (Fig. 3, g) and *Maxilliped* II (Fig. 3, h). *Maxilliped* III (Fig. 3, i) — small, biramous and unsegmented; exopod with 3 long plumose setae at its distal end. Telson same as in the previous sub-stage. *Colouration* — dark-brown, branched chromatophores present at the base of antenna; a dark-brown, band-like chromatophores at the posterior border of carapace; branched brownish ones in the middle of caudal lobes.

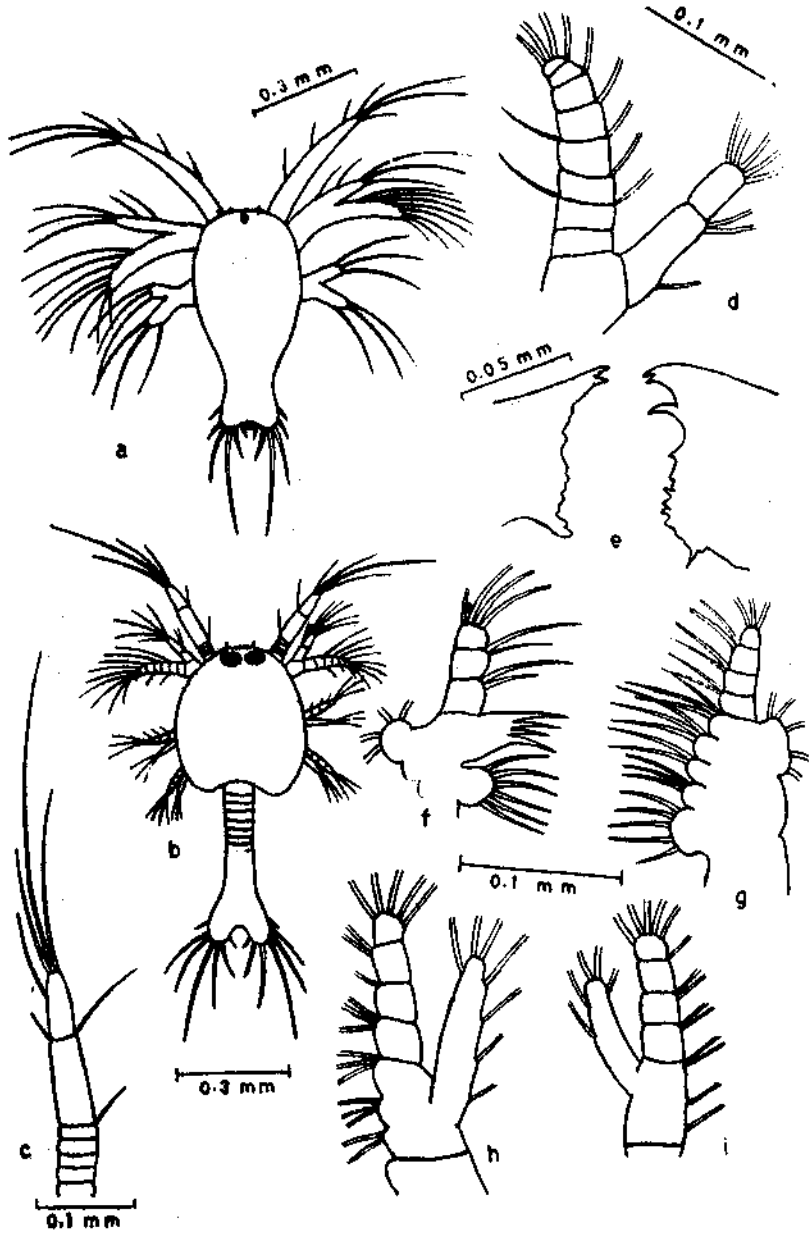

FIG. 2. Naupliar and protozoeal stages of *M. affinis*: a, Nauplius VI — dorsal view. b, Protozoea I — dorsal view. c, antennule of Protozoea I. d, antenna. e, mandible. f, maxilla I. g, maxilla II. h, maxilliped I. i, maxilliped II.

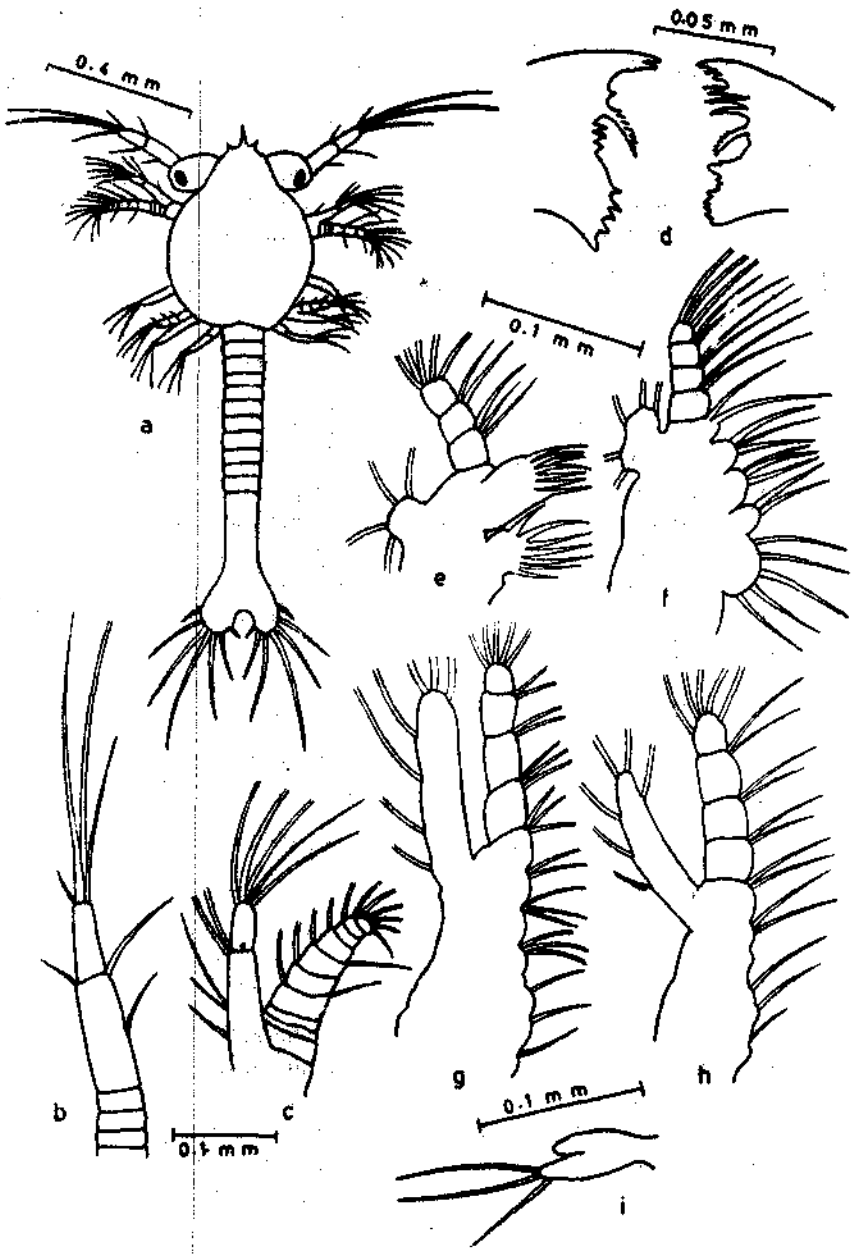

FIG. 3. Protozoea II of *M. affinis*: a, dorsal view. b, antennule. c, antenna. d, mandible. e, maxilla I. f, maxilla II. g, maxilliped I. h, maxilliped II. i, maxilliped III.

Duration of the stage: 25-31 hrs.

Protozoea III (Fig. 4, a): TL — 1.86-1.88 mm; CL — 0.73 mm; CW — 0.39-0.42 mm.

Presence of a median dorsal spine on each abdominal segment and development of biramous uropods are characteristic features of this stage. Carapace with more pronounced supraorbital spines and rostrum. Abdomen 6-segmented and 5th and 6th segments with lateral spines. *Antennule* (Fig. 4, b) — 3-segmented, proximal one devoid of subsegments; distal segment with 2 aesthetes and 2 long plumose setae at its apex. *Antenna* (Fig. 4, c) — exopod 10-segmented with 11 long plumose setae on the inner and distal margin and 2 on the outer margin. *Mandible* (Fig. 4, d), *Maxilla* I (Fig. 4, e), *Maxilla* II (Fig. 4, f), *Maxilliped* I (Fig. 4, g) and *Maxilliped* II (Fig. 4, h) remain essentially same as in previous substage. *Maxilliped* III (Fig. 4, i) — unsegmented and biramous, bearing 3 long plumose setae each on exopod and endopod; 5 pairs of small, biramous buds developed on thoracic legs (Fig. 4, j); uropods biramous exopod longer than endopod. *Colouration* — one pair each of branched orange-red chromatophores at the bifurcation of endopod, exopod and posterior border of telson; reddish spot-like chromatophores at the lateral side of abdominal segments.

Duration of the stage: 23-30 hrs.

Mysis I: TL — 2.29-2.67 mm; CL — 0.83-0.99 mm.

Mysis II: TL — 3.02-3.30 mm; CL — 0.96-1.01 mm.

Mysis III: TL — 3.49-3.60 mm; CL — 1.05-1.09 mm.

Mysis I, II and III stages have been described by Rao (1974) and the present material agrees with the descriptions given by him.

Post-larva I: TL — 3.44-3.79 mm; CL — 1.21-1.26 mm.

Material on hand agrees in all essential characters with the descriptions given by Mohamed *et al* (1968). Rostrum small, projecting a little beyond frontal border of carapace, with 2 longer and 2 smaller spines and a minute epigastric. Antennal and hepatic spines well developed. No appreciable change in the mouth parts from those of Mysis III; pereiopods without exopods and 1-3 chelate; pleopods well formed, uniramous, 3-segmented, distal segment with setae; no spines on 5th abdominal segment; telson with 2 pairs of lateral and 5 pairs of posterior spines.

Advanced Post-larvae

In the present investigations, the following series of postlarvae measuring from 3.44 to 15.12 mm in total length were encountered:

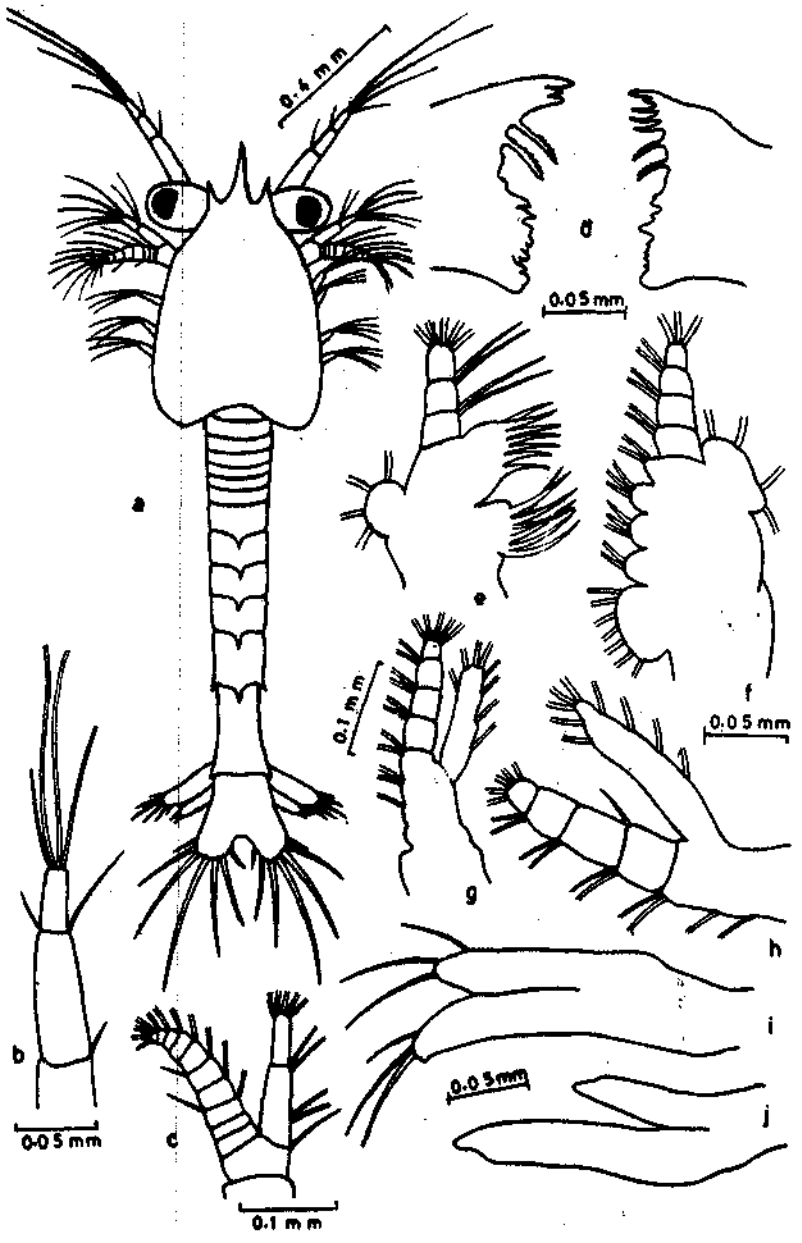

FIG. 4. Protozoea III of *M. affinis*: a, dorsal view, b, antennule. c, antenna. d, mandible. e, maxilla I. f, maxilla II. g, maxilliped I. h, maxilliped II. i, maxilliped III. j, buds of 1st pair of pereiopod.

Substages	Total length (mm)	Carapace length (mm)	No. of spines on the rostrum
I	3.44- 3.79	1.21-1.26	3
II	4.58- 5.16	1.41-1.56	3
III	4.81- 5.01	1.63-1.88	4
IV	5.19- 7.31	2.22-2.51	5
V	8.76- 9.38	2.69-3.25	6
VI	11.28-13.50	3.80-4.50	7
VII	13.80-15.12	4.70-4.95	7

Rostrum in post-larval substages I and II short, not exceeding beyond eye. In Post-larva IV, rostrum extends to one-fourth and in Post-larva V and VI to middle eye; ventral margin slightly cancave and tip curved down, so that the rostrum becomes partially hidden when viewed laterally; number of dorsal teeth on rostrum (Fig. 5, g-1) increases. No appreciable changes in carapace, except that the hepatic and antennal spines become prominant at each moult; 6th abdominal segment remains as the longest in all substages. Median dorsal spine

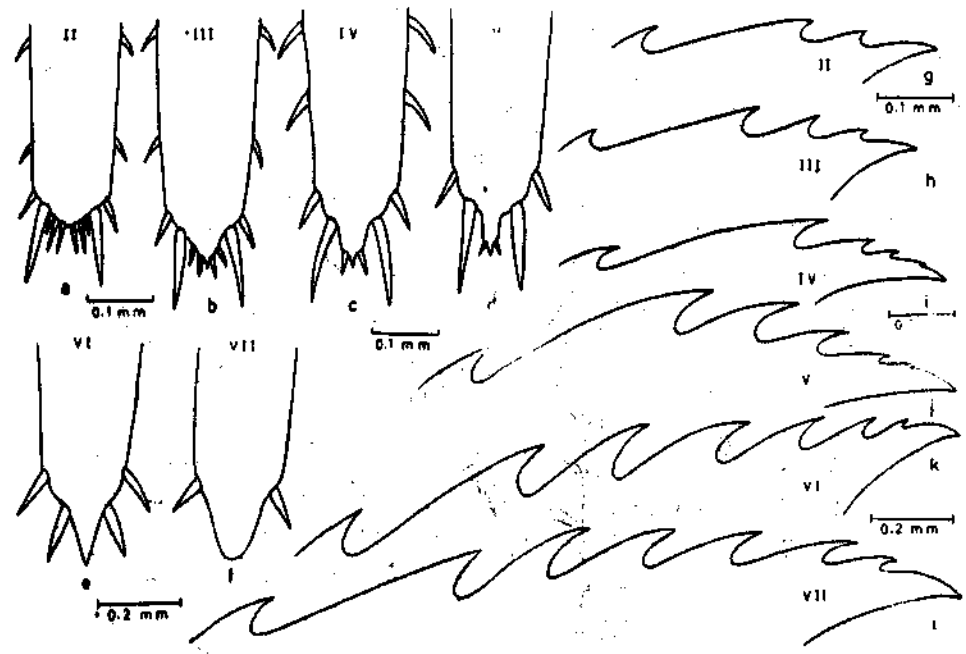

FIG. 5. Post-larvae of *M. affinis*: a, telson of Post-larva II. b, telson of Post-larva III. c, telson of Post-larva IV. d, telson of Post-larva V. e, telson of Post-larva VI. f, telson of Post-larva VII. g, rostrum of Post-larva II. h, rostrum of Post-larva III. i, rostrum of Post-larva IV. j, rostrum of Post-larva V. k, rostrum of Post-larva VI. l, rostrum of Post-larva VII.

on this segment become smaller at each moult, to be represented by a sharp point in Post-larva VII. Telson (Fig. 5, a-f) and uropod in Post-larva II — posterior margin of telson with 6 spines, median 2 of which larger; spines on posterolateral corner longest and those on lateral margin smaller. In Post-larva III, posterior margin becomes a triangular process and the posteromedian spines now reduced to 4 in number, placed on the tapering border of this process. In the next two substages, only one pair of median spines present in the conspicuous triangular process on the posterior margin. In Post-larva VI, spines on tapering border disappears and the tip of telson ends in an acute spinous process with 2 long subterminal spines which disappear completely in Post-larva VII; lateral spines, however, still persist though very small in size. Uropods unchanged throughout the post-larval development, except for increase in size and addition of setae at each moult.

Conclusion

The sequence of development of *M. affinis* from egg to post-larvae observed in the laboratory agrees in general with that of *M. dobsoni*, described by Menon (1951) and Rao (1974), and that of *M. monoceros* described by Raje and Ranade (1975b). In *M. affinis* the time taken by the egg to hatch after spawning is 8-10 h which is in agreement with that of *P. stylifera* (Thomas *et al* 1975) and *P. acclivirostris* and *M. dobsoni* (Thomas *et al*, MS a and b). However, it is reported that the eggs of *P. japonicus* (Hudinaga, 1942), *P. monodon*, *P. semisulcatus* and *M. monoceros* (Liao *et al*, 1969a and 1969b) take 12-14 h at temperature ranging from 27° to 30.8°C. It may be possible that the higher temperature under which the present experiments were carried out (30.4° to 30.6°C) was responsible for the reducing of the time taken for hatching.

These experiments have also demonstrated the technical possibility of mass culturing the post-larvae of this commercially important species under controlled conditions, through the various sensitive larval stages of nauplii, protozoea and mysis, from the spawning achieved of the gravid females collected from the wild. Feeding the protozoeal stages with the diatom, *Thalassiosira* sp., and mysis with the nauplii of brine shrimp was proved to be effective both for survival and growth of the prawn in both the experiments. Therefore, with the maintainence side by side, of sustained cultures of the diatom and the brine shrimp, which is rather easy to achieve, it now appears possible to develop hatcheries of this prawn for supplying necessary seeds for stocking in culture ponds.

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