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EXPERIMENTAL LARVAL DEVELOPMENT OF PENAEDAE SHRIMP, *TRACHYPENAEUS CURVIROSTRIS* (STIMPSON, (1860) FROM EGYPTIAN MEDITERRANEAN COAST

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Key words: Trachypenaeus curvirostris, laboratory reared Larvae, description of larval developmental stages.

ABSTRACT

Gravid females of *Trachypenaeus curvirostris* were collected from Abu-Kir fishing area, near Alexandria city, and spawned in NIOF hatchery by natural means. This larvae were reared from hatching to postlarval stage at 27^{o}C and 40 ppt. The complete larval development and metamorphosis into post larvae was described. The larvae were reared in a fiberglass tanks (280L). Larvae from the first zoeal stages to the third zoeal stage were fed on algae; a mixture of *Nannochloropsis oculata, cheatoceros gracilis* and *Tetraselmis chii.* Larvae from mysis stage to the first post larval stage were fed on Rotifers and newly hatched nauplii of Artemia. Two naupliar stages, three protozoeal stages, three mysis stages and one post larval stage were described and illustrated with measurements of body length of each stage as well as substage. After about 24 days following hatching on the basis of morphological characteristics, larval stages of *T. curvirostries* could be distinguished from parallel stages of closely related species of the family Penaeidae. Shape of the growth curve, depending on the increase in body length of the different stages and substages during the rearing period were estimated. Preliminary rearing trials during the nursery phase for a period of 60 days were described.

1. INTRODUCTION

Trachypenaeus curvirostries (Stimpson, 1860), is a small marine penaeid species with hard, pubescent shell, found both in coastal and deep sea waters. The small-sized shrimp species of the family Penaeidae in Egyptian shrimp landed catch, such as *Metapenaeus stebbingi*, *Trachypenaeus curvirostris* and *Parapenaeus longirostris*. They constitute the main bulk of shrimp yield (about 40 ~ 50% of total shrimp catch) from the period after regulation of the Nile flow up till now. A clear change of species composition and distribution of these species is obvious Wadie and Abdel Razek (1985). While the larger-size species such as *Penaeus japonicus, Panaeus kerathurus, Penaeus semisulcatus* and *Metapenaeus monoceros* showed a sharp decrease in the total shrimp yield a long the fishing area of Egyptian Mediterranean coast.

Trachypenaeus curvirostris is considered to be of Indo-west pacific origin. Successively recorded in Turkey, Egypt Dowidar and Ramadan, (1972); entered the eastern Mediterranean through the Suez Canal (Fig. 1) The first Mediterranean record of *Trachypenaeus curvirostris* by Steinitz (1929) in Israel. It is also recorded in east Africa, Madagascar, Red Sea to Arabian sea, India, Malaysia, Hong Kong, Taiwan, Korea, Japan and northern Australia Holthuis, (1980).

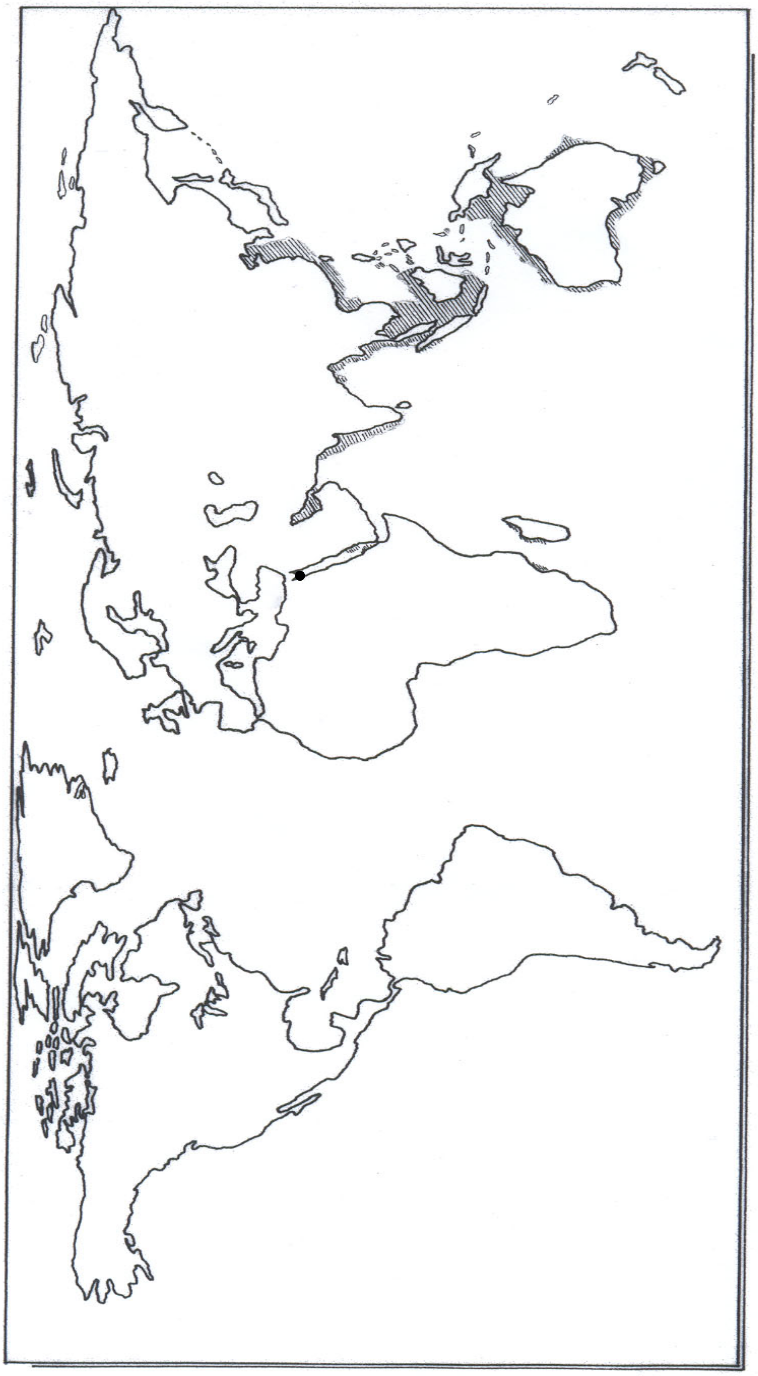

Fig. (1): Geographical distribution of *Trachypenaeus curvirostris* (Stimpson 1860), (FAO)

Because of the small size of shrimps of this species, their the commercial importance is usually minor, but recently, their importance increased in Egyptian shrimp market with increasing with increasing demand on them.

According to FAO (1999) the total catch reported for this species in the world was 403027t. The landing countries in this respect were China (400786t) and Taiwan produce a considerable catch of this species (2241t). Some studies on its distribution, migration, demographic structure and reproductive biology were reported by Cha *et al.* (2004) in the yellow sea of Korea. Very little is known about the early life history of this species. Some preliminary trials were done to describe the complete early developmental stages of *Trachypenaeus curvirostris* based on planktonic samples, George and Paulinos (1973) and Paulinose (1982a). However these studies did not cover the whole developmental stages.

Other trials through spawning of *Trachypenaeus curvirostris* were done. However, they failed to get all the larval stages [Ishikawa and Imabayashi (1991)] and Ronquillo and Saisho (1992). Ronquillo and Saisho, (1995), reported a complete and detailed description and illustration of early larval stages of laboratory-reared *Trachypenaeus curvirostris* in Kyushn Iland, Japan. The generic identification and larval description of *Trachypenaeus curvirostris* in northwestern gulf of Mexico was reported by Cook (1964).

The present study describes a complete, early larval stages of laboratory reared *Trachypenaeus curvirostris* in Egyptian waters with observations on its larval growth rate during the metamorphosis and nursery stages.

2. MATERIALS AND METHODS

2.1. Collection of Gravid females

Gravid females with a complete ripe olive/green ovaries of *Trachypenaeus curvirostris* were taken by commercial trawlers off Abu-Kir fishing area near Alexandria City in 50-100 m depth. Small numbers of samples (10-20) of gravid females were used for spawning experiments during three successive years from 2003, 2004 to 2005, usually during spawning season annually from May-September. These were maintained on deck in plastic container (50L) in running sea water and transported to the NIOF shrimp hatchery.

2.2. Spawning

Several gravid female shrimps were placed in groups of 10-20 females in 280L rectangular fiber glass tanks filled with filtered seawater and aerated vigorously. The spawned eggs were incubated in the same spawning tanks after taking out the spent spawners. The spawned eggs were counted and incubated at 26-27^{o}C and 39-40 ppt.

Samples of eggs and hatched nauplii were taken as well as daily samples of protozoea and mysis stages throughout the period of development.

Each sample was placed directly into 4% buffered formalin in which larvae at different developmental stages were stored in excellent conditions until required for furthur study.

2.3. Larval rearing

Nauplii did not require feeding, but as soon as protozoea appeared in the culture, mixed algal culture were added four times daily as a food source. A mixture of *Chaetoceros gracilis*, *Tetraselmis chii* and *Nannochloropsis oculata* cultured in an enriching culture medium (NIOF algal culture lab). An equivalent amount of water was removed from the outer tank prior to this addition.

2.4. Larval collection and illustration

All descriptions were made from preserved material. These were then photomicrographed, observations as well as sketch drawings were made at 64X or more with aid of a camera lucida attached to a euromex compound microscope.

Measurements were taken in mm calibrated using a micrometer eyepiece.

Description of the appendages depended on the external parts of the body from the ventral view of each metamorphosed stage without dissecting the larval sample. Body length of nauplii were measured between the apical and caudal margins, excluding furcal spines, in the mid-line. Body length of the protozoea, mysis and post larval stages were measured from the anterior margin of the carapace with rostrum to the posterior margin of the telson in the mid-line.

Nomenclature is based on that used by Hudinaga (1942); Cook (1964); Fielder *et al.* (1975) and Ronquillo and Saisho (1995).

2.5. Growth of nursery phase

Experimental rearing of larvae from first post larvae to a period of 60 days under laboratory conditions in fiber glass tanks was done. Hatched Artemia nauplii and minced clam meat in addition to prepared dried artificial diet Taha (2000) were used as a daily feeding regime. Records of total length (to the nearest mm) and weight were to the nearest mg) taken for each larval stage. Water quality conditions were constant during all periods of the experiments.

3. RESULTS

3.1. Embryonic development

Gravid females of *Trachypenaeus curvirostris* spawned during night, the newly released oocytes were irregular inshape and they were observed at the bottom of the aquarium. Eggs hatched directly in the morning after about 10 hours of spawning after and also a very few segmented eggs could be observed. The average diameter of the fertilized eggs could be determined. The average diameter of the fertilized eggs just before hatching was about 0.14 mm and the diameter of the yolk 0.09 mm tiny and opaque in shape as in Fig. (2a) & Plate (1) A.

3.2. Development of larvae and duration of the larval stages:

<u>First nauplius</u> stage (Fig. 2b, N-1), Plate (1) B

Body length 0.17 mm duration one day.

Body pear shaped, unsegmented, simple; three pairs of appendages arising from anterior portion of body, 1st antenna, 2nd antenna and mandible. A median ocellus is located near the anterior end. Posterior and post naupliar region in rounded and armed with a pair of caudal spines of equal length extending straight posteriorly. All setae on oppendages are simple at this stage.

Antennule; uniramous, slightly shorter than body, with 2 short ventrolateral setae and 1 long dorsolateral seta. Appendages lack spines or processes such as would be utilized for feeding purposes.

Antenna; biramous endopod with 3 long terminal satae; exopod with 2 short ventrolateral setae and 2 long terminal setae.

Mandible; biramous; with 4 terminal setae and 1 subterminal setae endopod and exopod with 2 long terminal and 1 long subterminal setae.

<u>Second nauplius</u> stage (Fig. 2c, N-2), Plate (1) C

Body length 0.24 mm (duration one day)

The shape remains essentially the same as in the previous stage except for elongation of the post naupliar region. Outline of developing appendages is evident at the ventral side of the body. The caudal spine formula is now 2+2 due to the addition of small spines.

Antennule; three ventrolateral setae (1 medium and 2 short); 3 terminal and 1 medium dorsalateral seta.

Antenna; endopod with 2 short simple ventrolateral and 3 terminal setae; exopod with 3 long ventrolateral setae and 4 terminal setae.

Mandible; as in previous stage.

First protozoea stage (Fig. 3a, PZ-1) & Plate (2) A

The day following the previous stage N_2, first protozoea appeared and it passes through 4 intermoult growth stages having the some morphological & morphometric characteristics. It increased in body length only.

The body length ranges from 0.26 to 0.32 mm. The protozoea is characterized by a large prominant, carapace followed by a slender segmented thorax and an abdomen which is also segmented. There are two pairs of prominent appendages arising from the anterior part of the body. The first unbranched, and the second branched. Carapace with median notch between two globular frontal organs, covers the sessile compound eye. The terminal body segment is large, broadly bifurcate posteriorly.

Antenna I; consists of three major segments: a basal segment with five subsegments, bearing one short seta; second segment possesses one ventrolateral and 2 posterolateral setae; distal segment with 5 terminal setae and 1 subterminal.

Antenna II; Protopod 2-segmented. Endopod 2-segmented with four long, terminal setae. Exopod segmented with 4 setae on the terminal segment.

Maxilla II, Maxilliped I and Maxilliped II, biramous and larger than maxilla.

Telson; broadly bifurcates with 7+7 furcal spines.

Second protozoea stage (Fig. 3b, PZ-2) & Plate (2) B

Body length ranges from 0.42 to 0.48 mm and it takes three days for intermoult growth between the 1st & 2nd protozoea. The most clear features of this substage are: stalked compound eyes free from carapace; rostrum projecting venturally and segmented abdomen. Frontal papilla present on inner margin of each eye.

Antenna I; segmentation as in protozoea I, distal segment with 7 setae.

Second antenna; as in protozoea I.

Maxilla II; protopod with setae on first to fifth lobes. Endopod and exopod as in protozaea I

Maxilliped I & Maxilliped II; endopod and exopod as in protozoea I.

Telson: increasing in size although having the same morphometric characters larger than in protozoeal I.

Third protozoea stage (Fig. 3c, PZ-3) & Plate (2) C

Body length of varies between 0.58 to 0.66 mm and it takes 2 days for intermoult growth to be completed. Rostrum slightly curved downwards. Carapace covers the first five thoracic somites. Frontal papilla still persists on occular margin of each stalked eye. Pereopods are still rudimentary and biramous. The abdomen is divided into six segments including the telson. Uropods present externally. Biramous uropods shorter than telson, small anal spine present.

Pereopods rudimentary, but biramous and prominent. Antenna I; consisting of 4 major segments with spines.

Antenna II; as in protozoea I.

Pereopods undeveloped; biramous; protopod present; setae absent.

First Mysis stage (Fig. 4a, M-1) & Plate (3) A

Body length ranges from 0.76 to 1.23 mm and it takes four days for intermoult growth i.e. after about 11 days from hatching, the larvae metamorphose into mysis stage. This stage exihibts a similar shape of the adult form. Perieopods are still undeveloped and the exopods are mainly used for swimming. Carapace covers the thoracic somites. The short rostrum does not reach beyond the eye and bears no dorsal rostral tooth. The first five abdominal segments are of similar size; the sixth is much longer. Median dorsal spines occur on fourth to sixth abdominal segment.

Antenna I; 3 major segments, first segment with rudiment of statocyst.

Antenna II; protopod 2 segmented; basis with spine. Endopod and exopod unsegmented. Pereopods; undeveloped, five pairs of similar form. Exopods unsegmented, longer than endopod.

Uropods and telson; endopod with 14 marginal setae, exopod with 16 marginal setae.

Telson cleft terminally with 6 pairs of spines posteriorly and 2 pairs laterally.

<u>Second mysis</u> stage (Fig. 4b, M-2) & Plate (3) B

Body length ranges from 1.36 to 1.53 mm and it takes 4 days for intermoult growth. The characteristics of this substage are the following:

The short rostrum bears 2 dorsal rostral tooth; rostrum is shorter than eye.

Antenna I; Peduncle 3- segmented; first segment with 1 statocyst, 1 basal spine and each segment with a number of marginal setae.

Antenna II; endopod 3 segmented. Exopod with marginal setae. Pereiopods; endopod of first to third chelate, endopods of fourth and fifth pereopod are chelate, with marginal setae.

Pleopods; two-segmented and biramous. Uropods and telson, protopod as in mysis I. endopod with 18 marginal setae. Exopod with 1 outer spine and 17 marginal setae. Telson flattened terminally with 6 pairs of spines. Dark spots were observed on protopod and endopod of each side.

<u>Third mysis</u> stage (Fig. 4c, M-3) & Plate (3) C

Body length ranges from 1.56 to 1.72 mm and it takes four days for intermoult growth. The distinguishing characters of this substage are as follows;

Presence of developed biramous pleopods with apical setae; 10 segmented antennal endopod; and 2 dorsal rostral teeth.

Pereopods; endopods segmented; propodus and dactyl of first 3 pairs of pereopods modified to form a chela.

Uropods and talson; protopod as in mysis II; endopod with 17 marginal setae. Exopod with 1 outer spine and 16 marginal setae. Telson with 2 pairs of lateral spines and 8 terminal spines on posterior margin.

<u>First post larvae</u> stage (Fig. 5, PL-1) & Plate (4)

Body length of about 1.79 mm on the average. This stage is reached after 24 days after hatching. At this stage the first five pairs of biramous pleopods are now functional swimmerets. The swimming function shifts from thorax in mysis stage to abdomen in postlarva. Rostrum bears 3 dorsal rostral teeth. Pereopods; endopod 5-segmented; propods and dactyl of first 3 pairs of pereopods modified to form chela.

Antenna I; peduncle 3- segmented.

Antenna II; basis with 1 spine, endopod with (2) segmented protopod and (10) segmented flagellum with numerous setae. Exopod with 1 distro-lateral spine on outer margin with 20 marginal satae.

Pleopods, biramous, 2- segmented distal segments with long (8) setae.

Uropod and telson; endopod with 16 marginal setae. Exopod with 1 outer spine and 23 marginal setae. Telson rounded posteriorly with 2 pairs of lateral spines and 10 terminal spines.

Fig. (6) describes the rate of increase in body length of the different metamorphosed stages of *Trachypenaeus curvirostris* from egg to the first post larvae in the laboratory rearing experiment during August-September, 2005.

While Fig. (7) shows the growth in length and in weight during the nursery period, and shows data collected from rearing laboratory experiment from first post larval to 3 months period for *Trachypenaeus curvirostris*.

4- DISCUSSION

Several papers have been published regarding the larval development and morphology of some species assigned to the genus *Trachypenaeus* Alcock, 1901 as mentioned by Ronquillo & Saisho (1995). The external morphological characteristics of the larvae, of *Trachypenaeus curvirostris* described in this paper are closely related to the same species described by Ronquillo and Saisho (1995) in Japan, specially with the naupliar and protozoeal stages. In the present study, only two naupliar substages were recorded not six nauplii as in the other penaeid species. Cook (1964) reported that within a given developmental stage, the size ranges of penaeid larvae as a whole are extremely variable and the possibility also exists that larvae (and post larvae) of the same species grow dissimilarly at different times of the year.

The pattern of embryonic development of *Trachypenaeus curvirostris* is similar to that described by Ronquillo and Saisho (1995) due to the presence of a wide perivitelline space and occurrence of embryonized nauplius and protozoea stages. But the main difference between the two studies is the general pattern and number of the intermoult growth of the substages of early larval development of *Trachypenaeus curvirostris*.

Hudinaga (1942) found that protozoeal stages of *P. japonicus* Bate exhibited intermolt growth, the occurrence of which may also be true for other stages as suggested for nauplii of *Xiphopeneus sp.* by Renfro and Cook (1963), and in the present study on *Trachypenaeus curvirostris* in protozoeal and mysis stages.

Examples of departure from this sequence are provided by the larvae of *Sicyonia brevirostris* which, when reared in the laboratory, appeared to pass through four mysis substages, and by those of *Parapenaeus sp.* which, as determined from sample material, also have at least four sub stages Cook (1964). Such apparent anomalies suggest that descriptions of penaeid larvae obtained either from rearing experiments or plankton samples must be viewed with caution until more is known about the effects of environmental factors on early growth and morphology.

With aid of Hudinaga studies (1942) on the *P. japonicus* larval stages and substages and the suggested key to stages and substages of penaeid larvae and early post larvae presented by Cook (1964) also with the help of the provided detailed description and illustrations of the complete early larval stages of laboratory-reared *Trachypenaeus curvirostris* reported by Ronquillo and Saisho (1995), the present description was done. Some differences were observed within the three mysis substages and first post larvae such as the number of marginal setae of uropods as well as the number of terminal spines of telson. Also the presence of the biramous pleopods was observed in second and third mysis substages as well as in first post larvae of *Trachypenaeus curvirostris* under study, while it was reported as a uniramous by Ronquillo and Saisho (1995) in Japan. The number of the dorsal teeth of rostrum in Mysis substages showed some differences also.

The present study is the first complete illustration of the early developmental stages of *Trachypenaeus curvirostris* inhabiting Egyptian Mediterranean waters and there is a need to establish a reliable key based on laboratory reared penaeid larvae of known parentage in this area.

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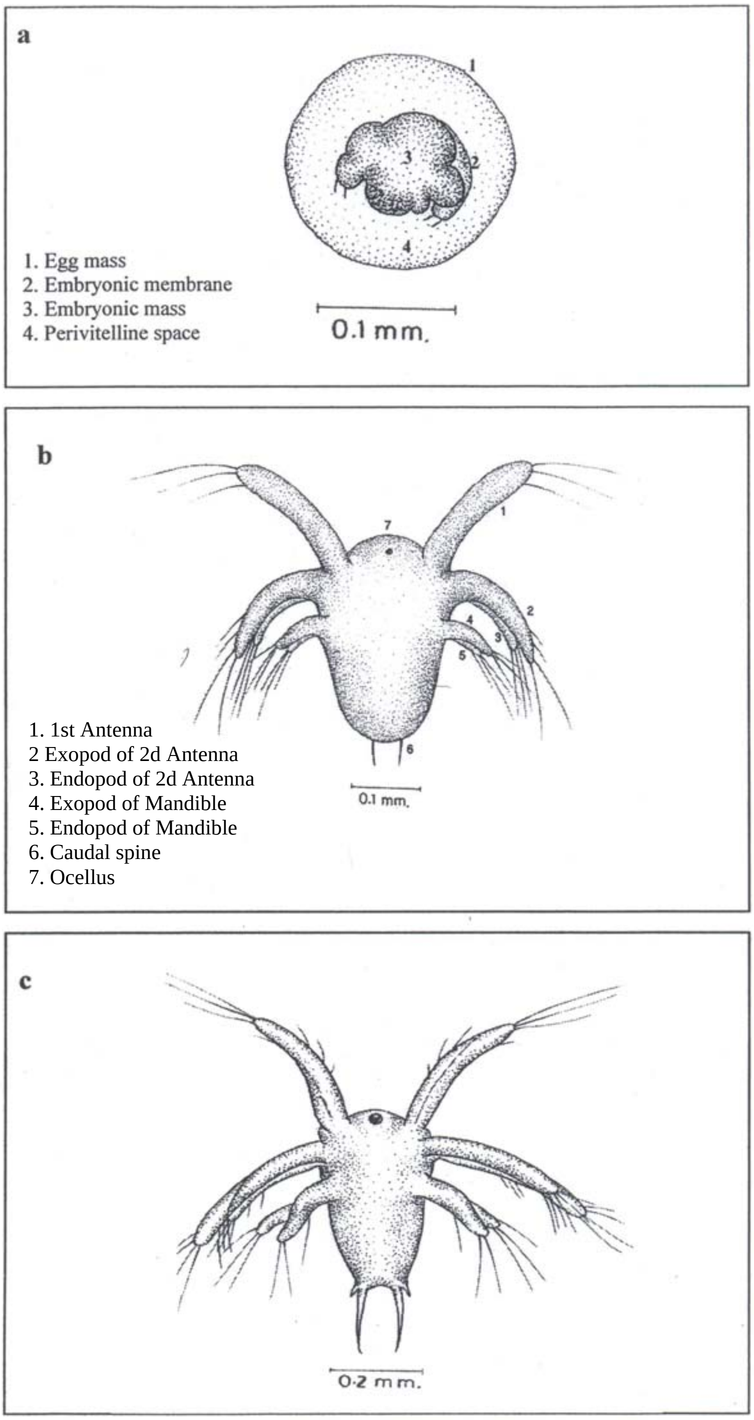


Fig. 2. *Trachypeneaus curvirostris* (Stimpson, 1860). a, fertilized egg; b, first nauplius; c, second nauplius (ventral view)

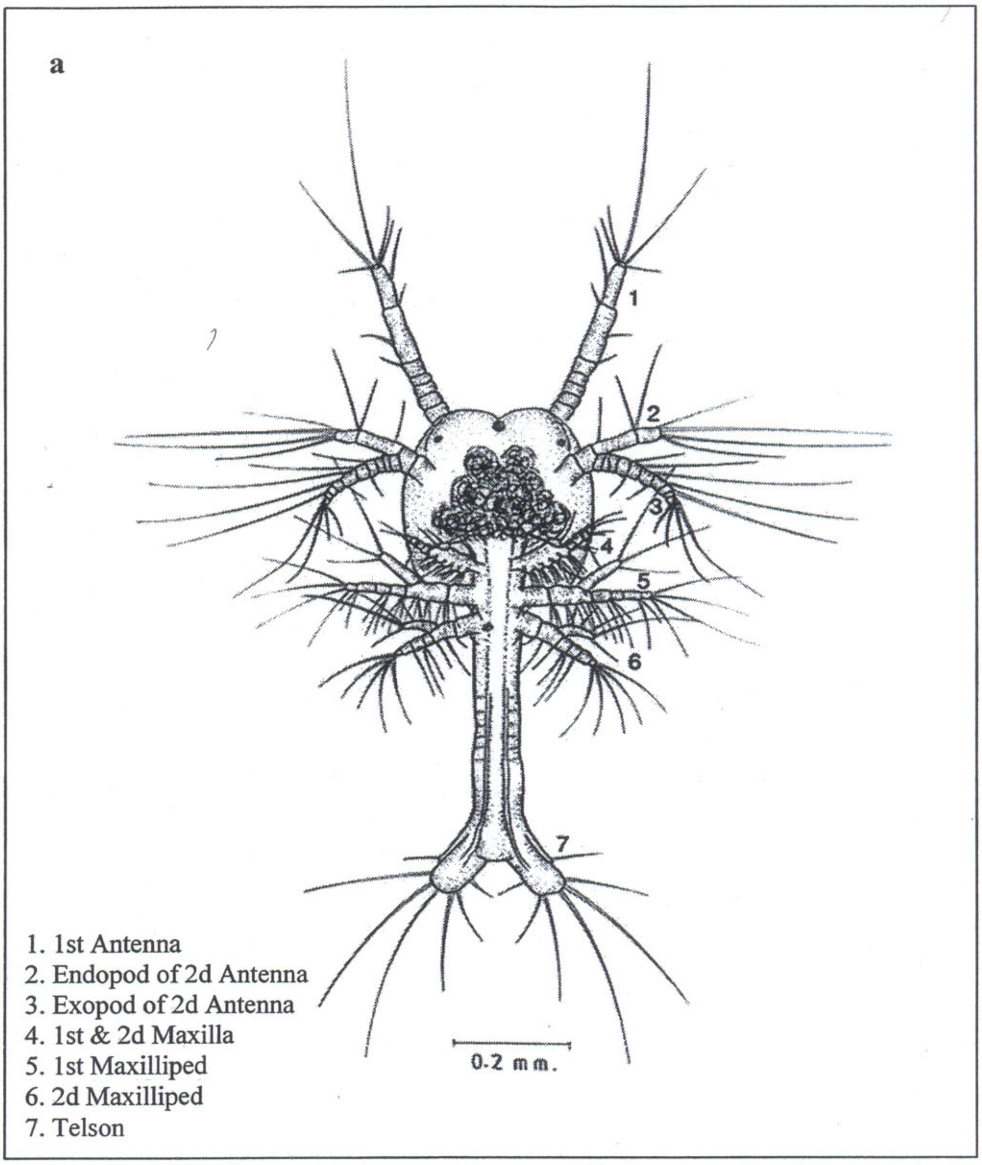


Fig. 3a. *Trachypeneaus curvirostris* (Stimpson, 1860) first protozoea (ventral view)

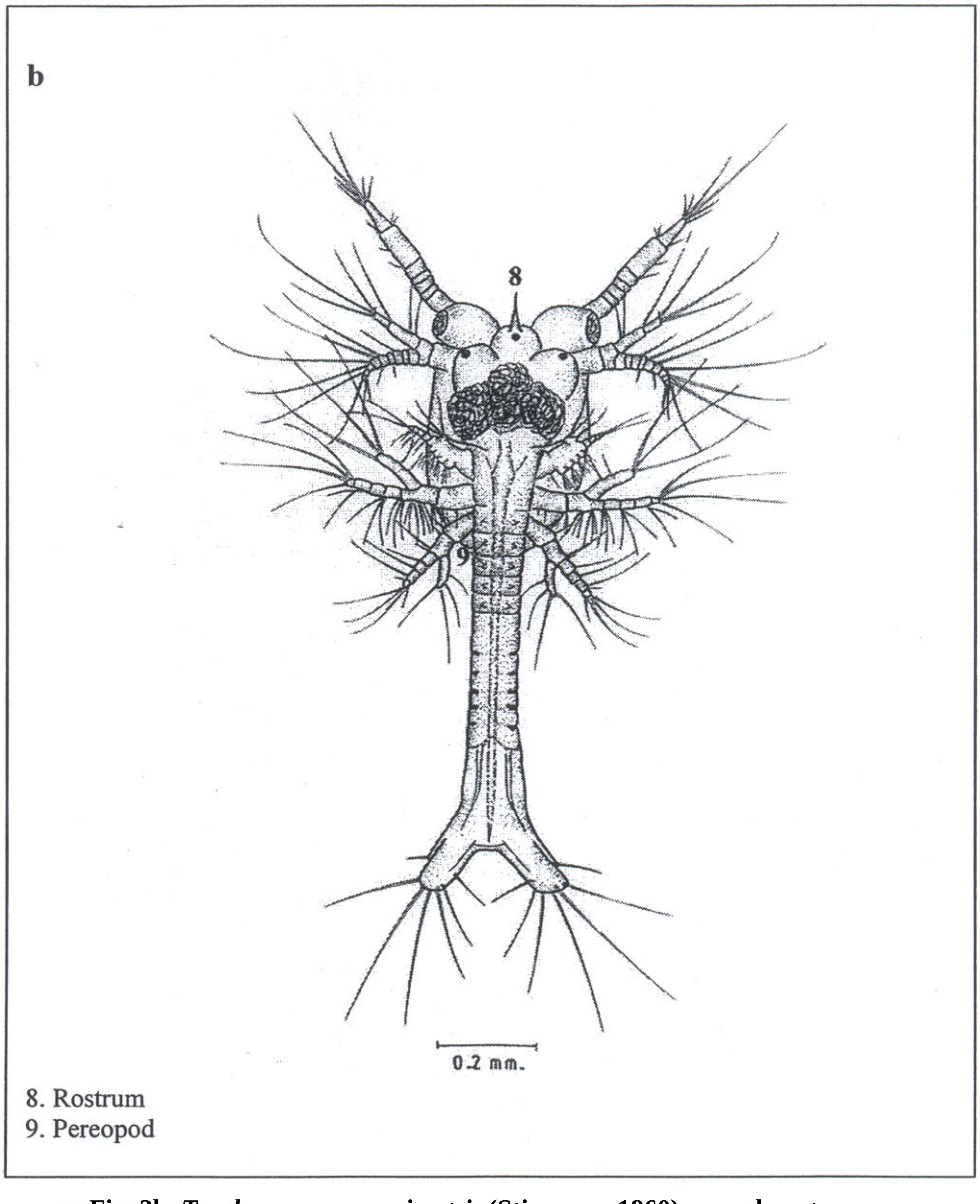


Fig. 3b. ***Trachypeneaus curvirostris*** **(Stimpson, 1860) second protozoea (ventral view)**

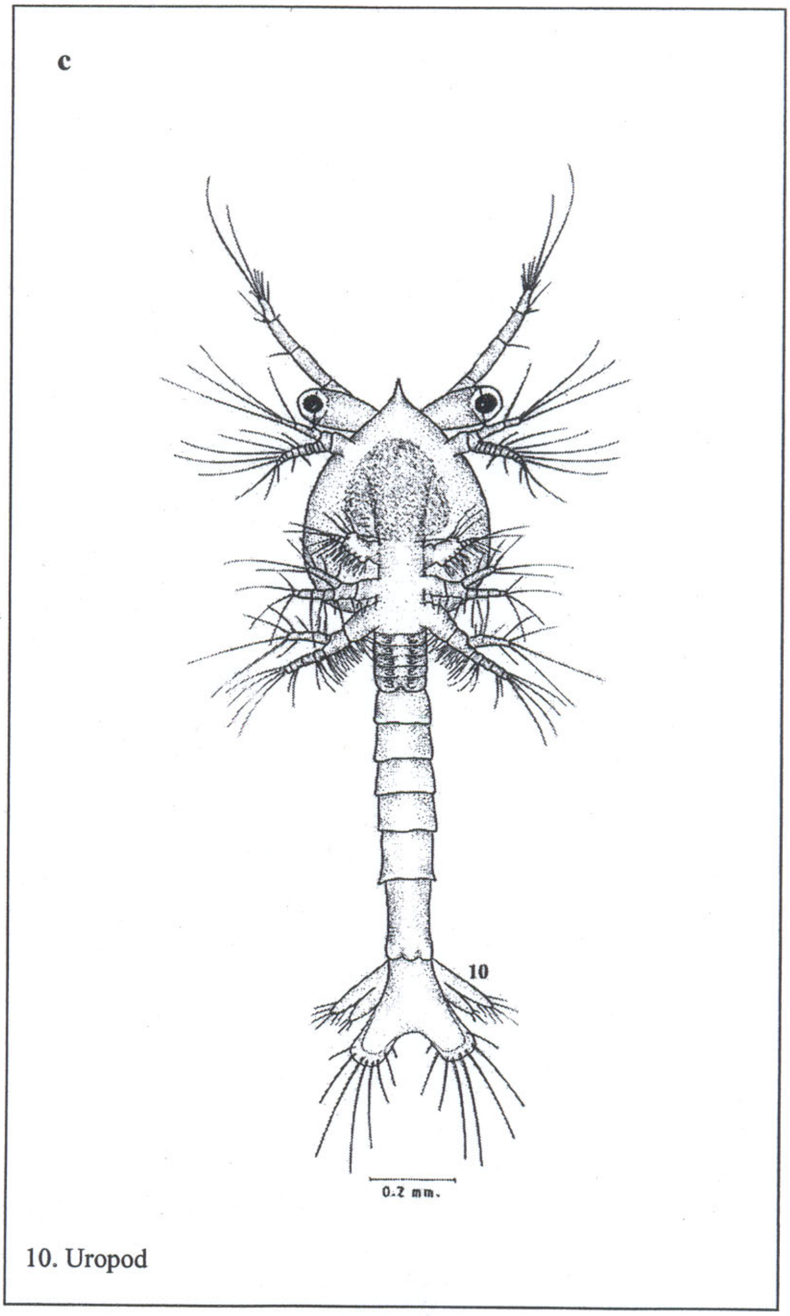


Fig. 3c. *Trachypeneaus curvirostris* (Stimpson, 1860) third protozoea (ventral view)

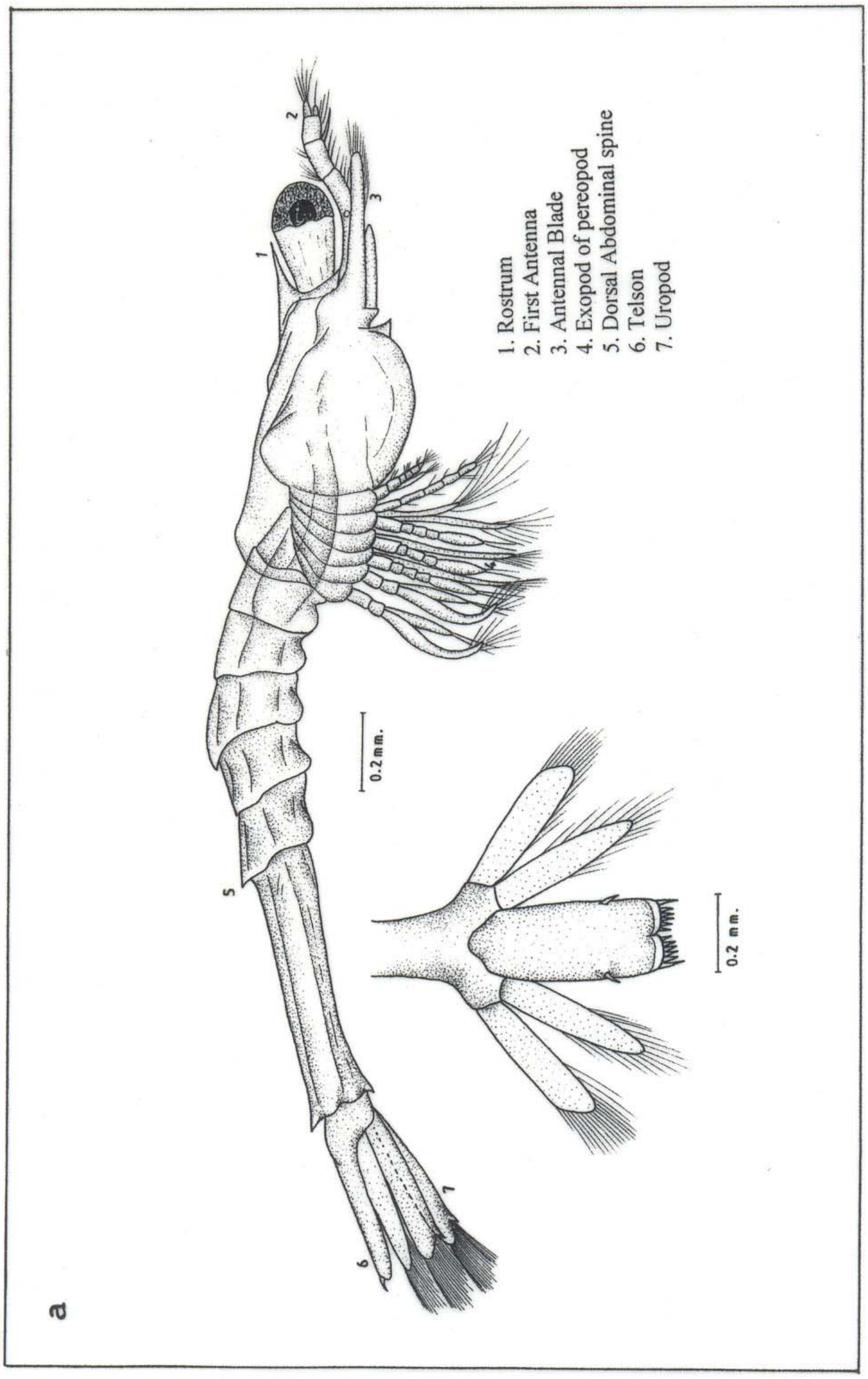


Fig. 4a. ***Trachypeneaus curvirostris*** **(Stimpson, 1860) first mysis stage (lateral view)**

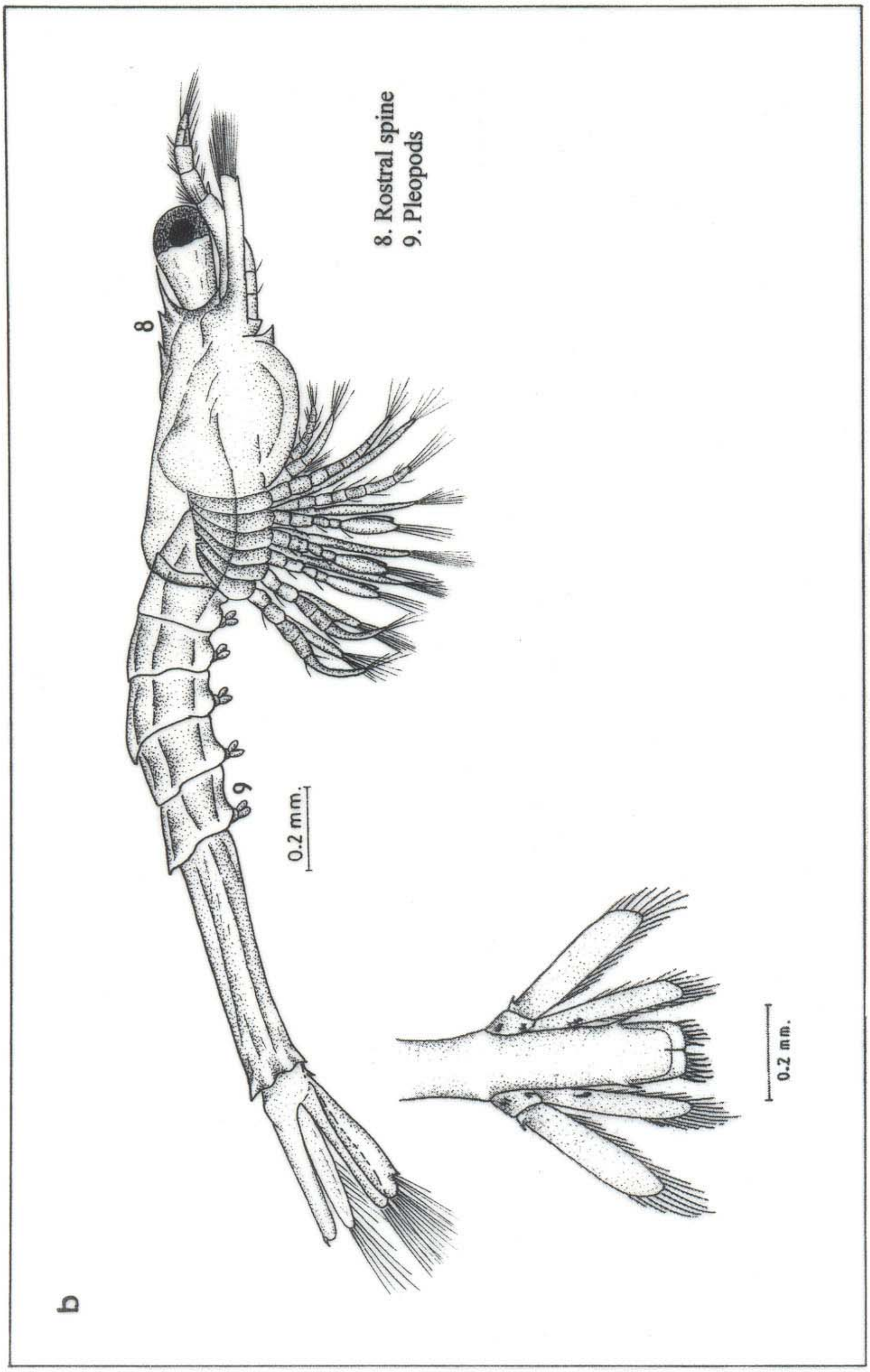


Fig. 4b. *Trachypeneaus curvirostris* (Stimpson, 1860) second mysis stage (lateral view)

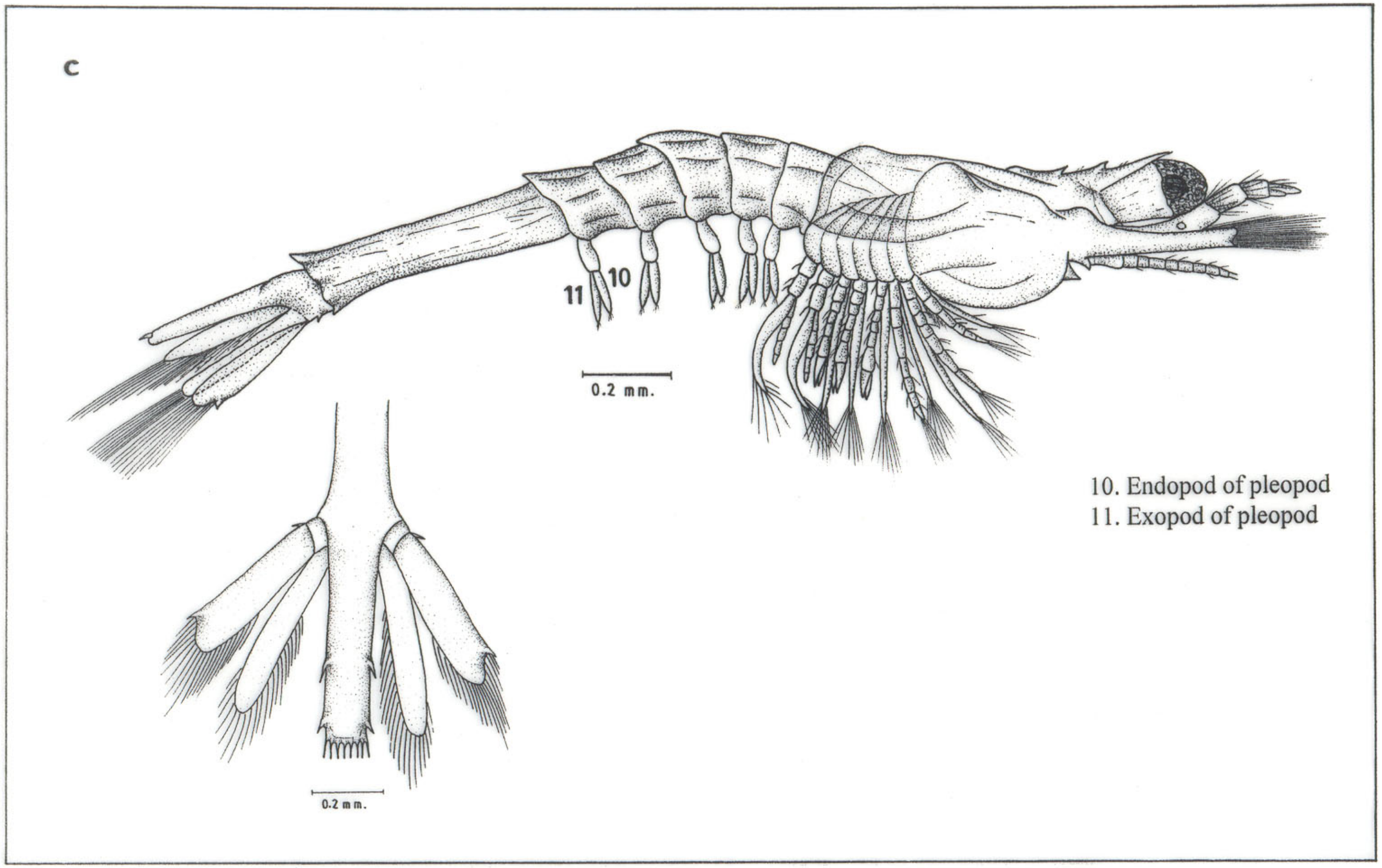


Fig. 4c. ***Trachypeneaus curvirostris*** **(Stimpson, 1860) third mysis stage (lateral view)**

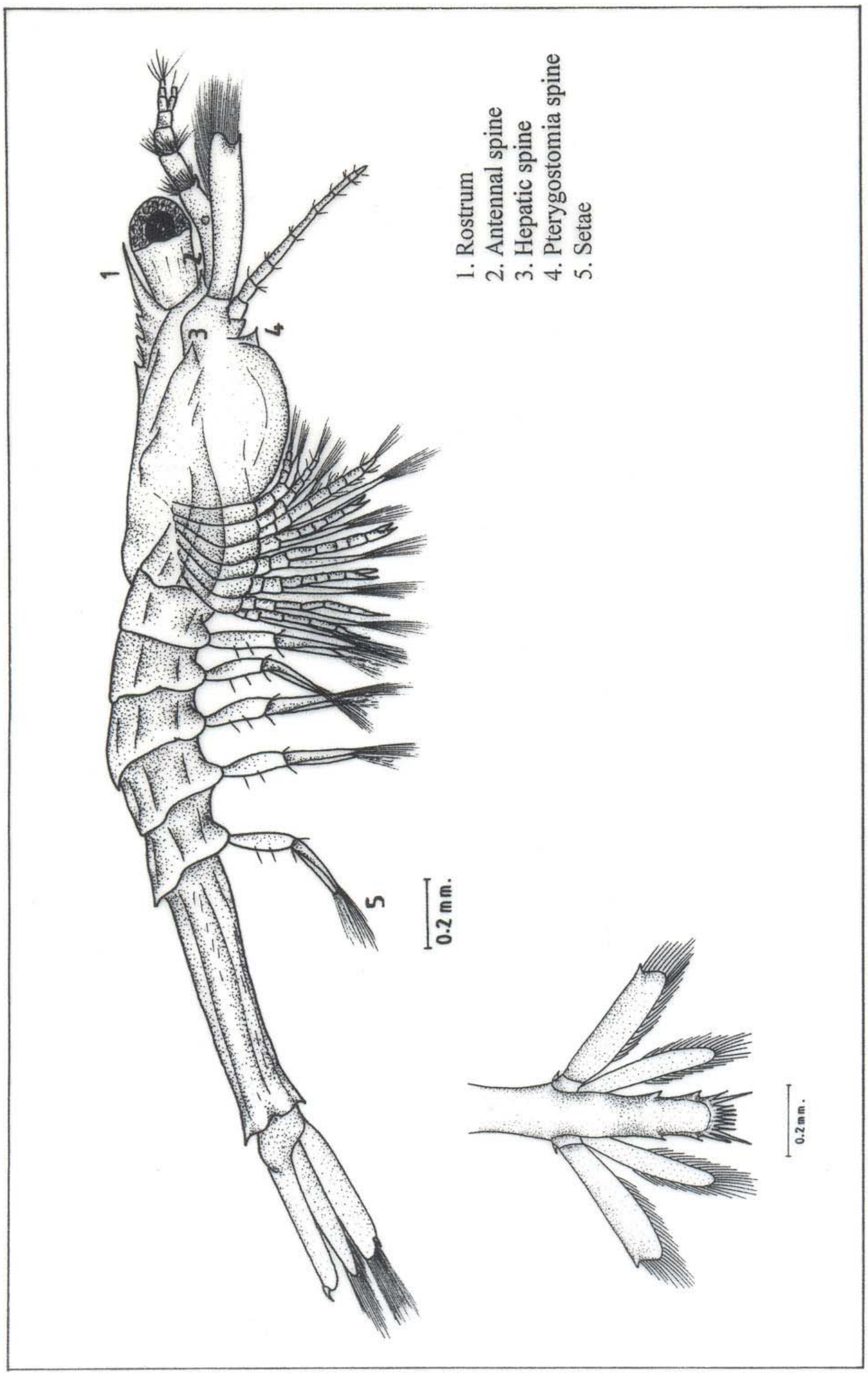


Fig. 5. ***Trachypeneaus curvirostris*** **(Stimpson, 1860) first postlarva (lateral view)**

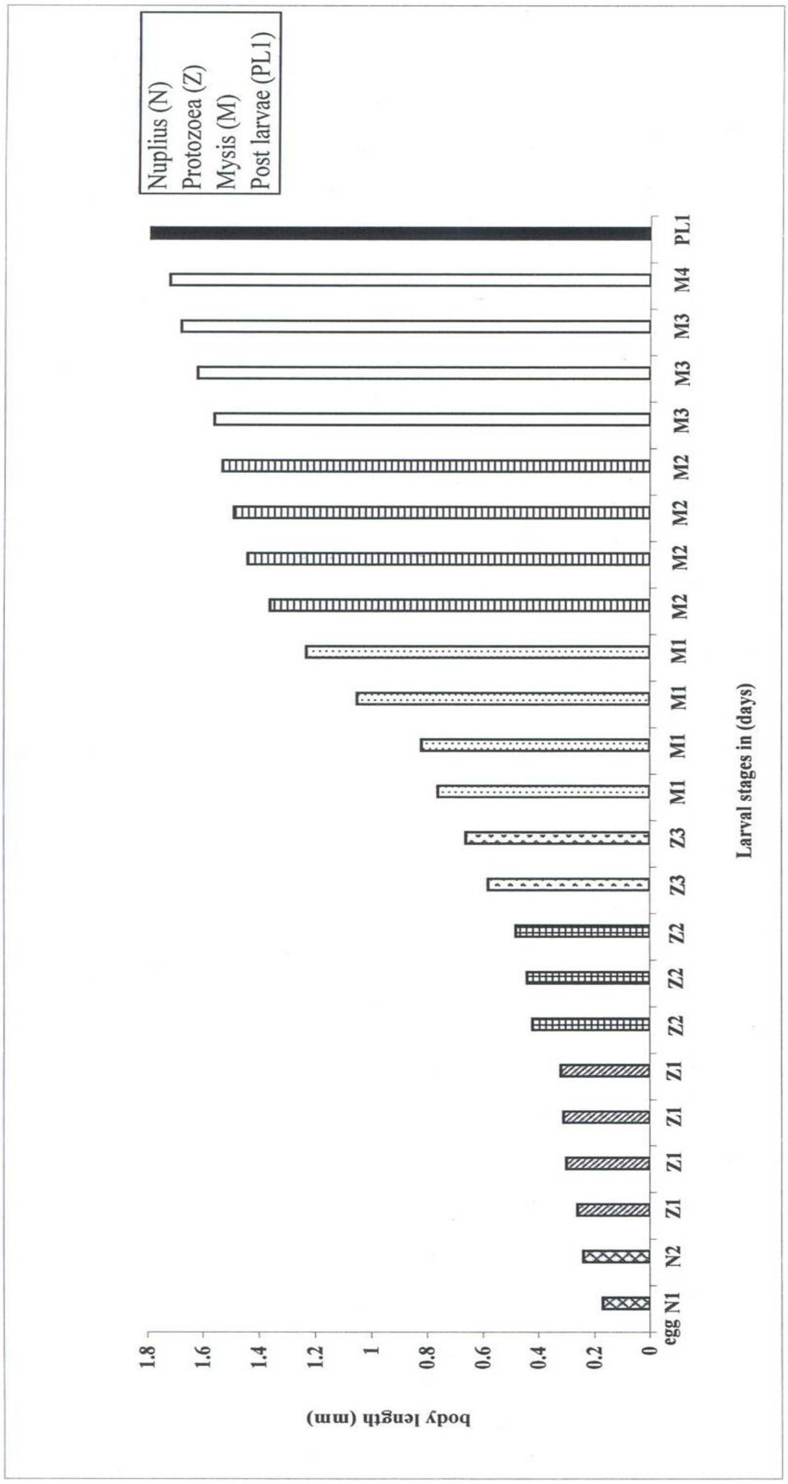


Fig. 6. Growth in length during the Metamorphosed stages of *T. curvirostries* reared in laboratory

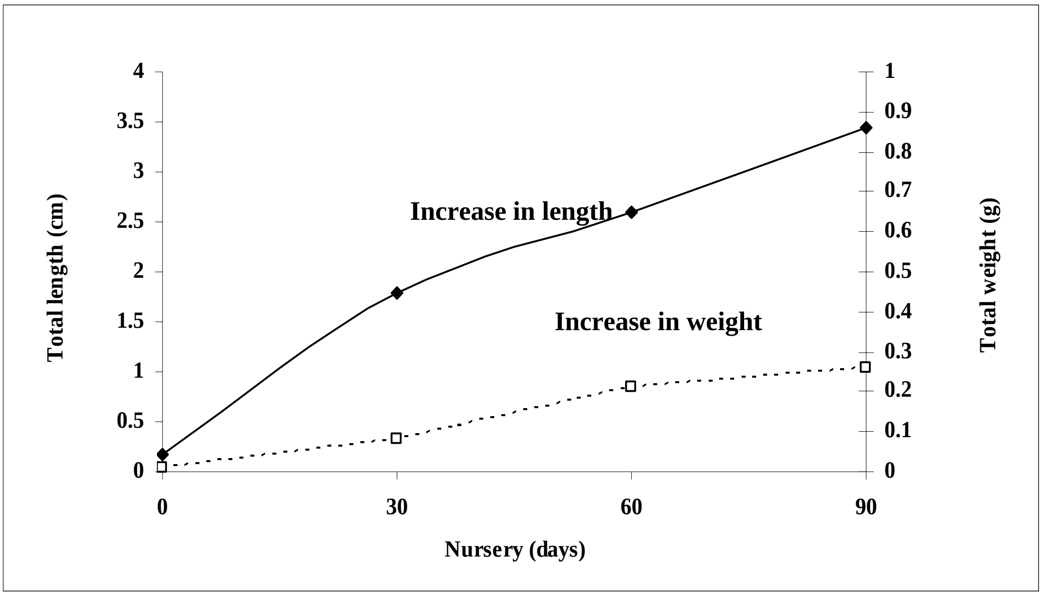


Fig. 7. The growth in length and in weight during the nursery period (1st PL →3 months) of *T. curvirostris* reared in laboratory

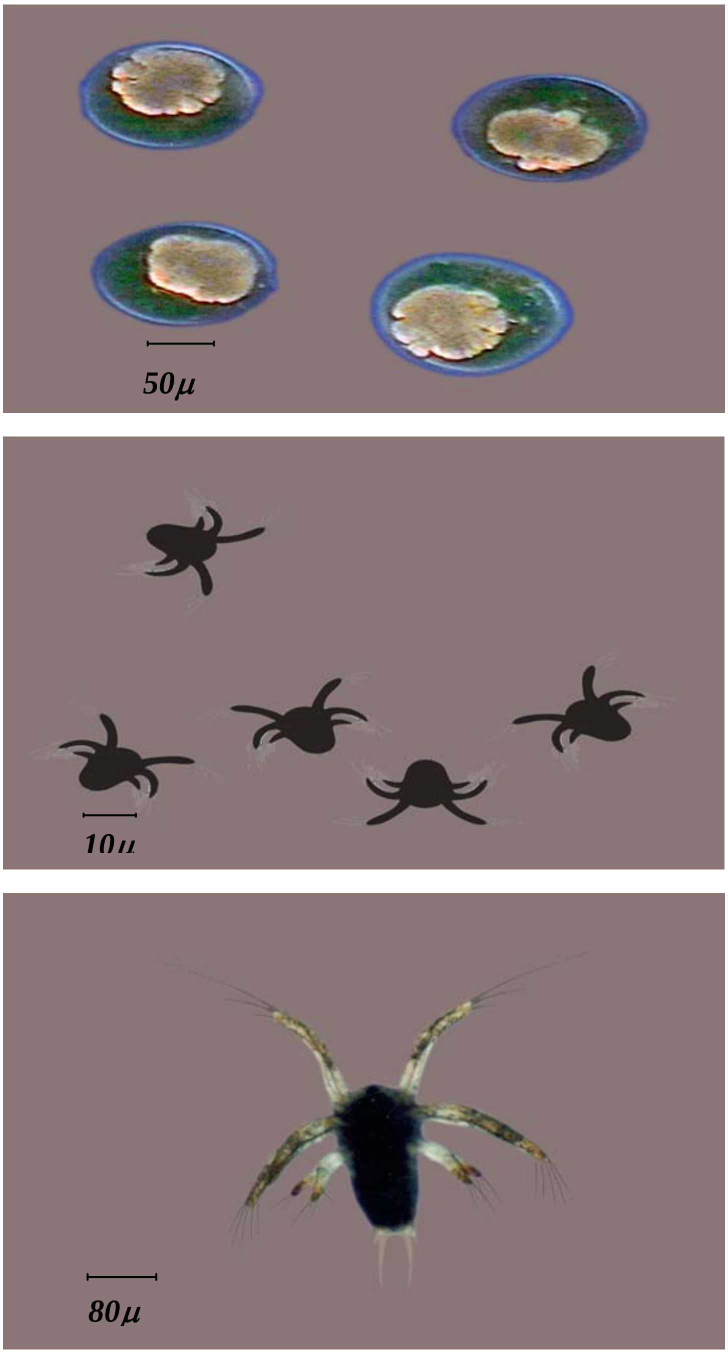
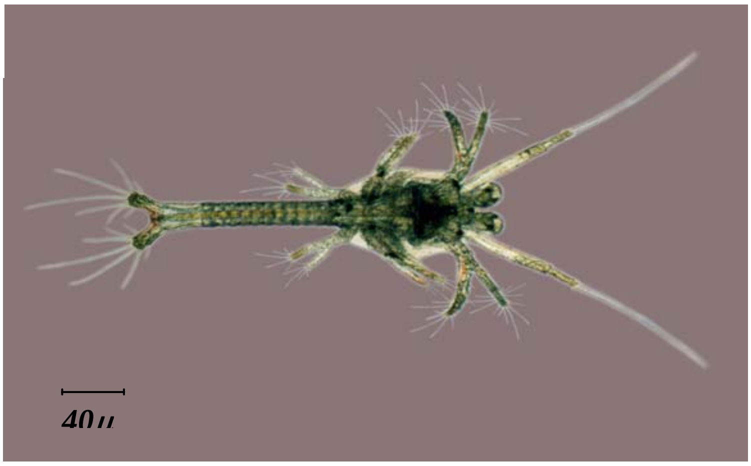


Plate (1): Trachypenaeus curvirostris (Stimpson, 1860). A; fertilized eggs; B, first Nauplius; C, second nauplius.

A

B



C

Plate (2): *Trachypeneaus curvirostris* (Stimpson 1860). A, First protozoea; B, second protozoea; C, Third protozoea.

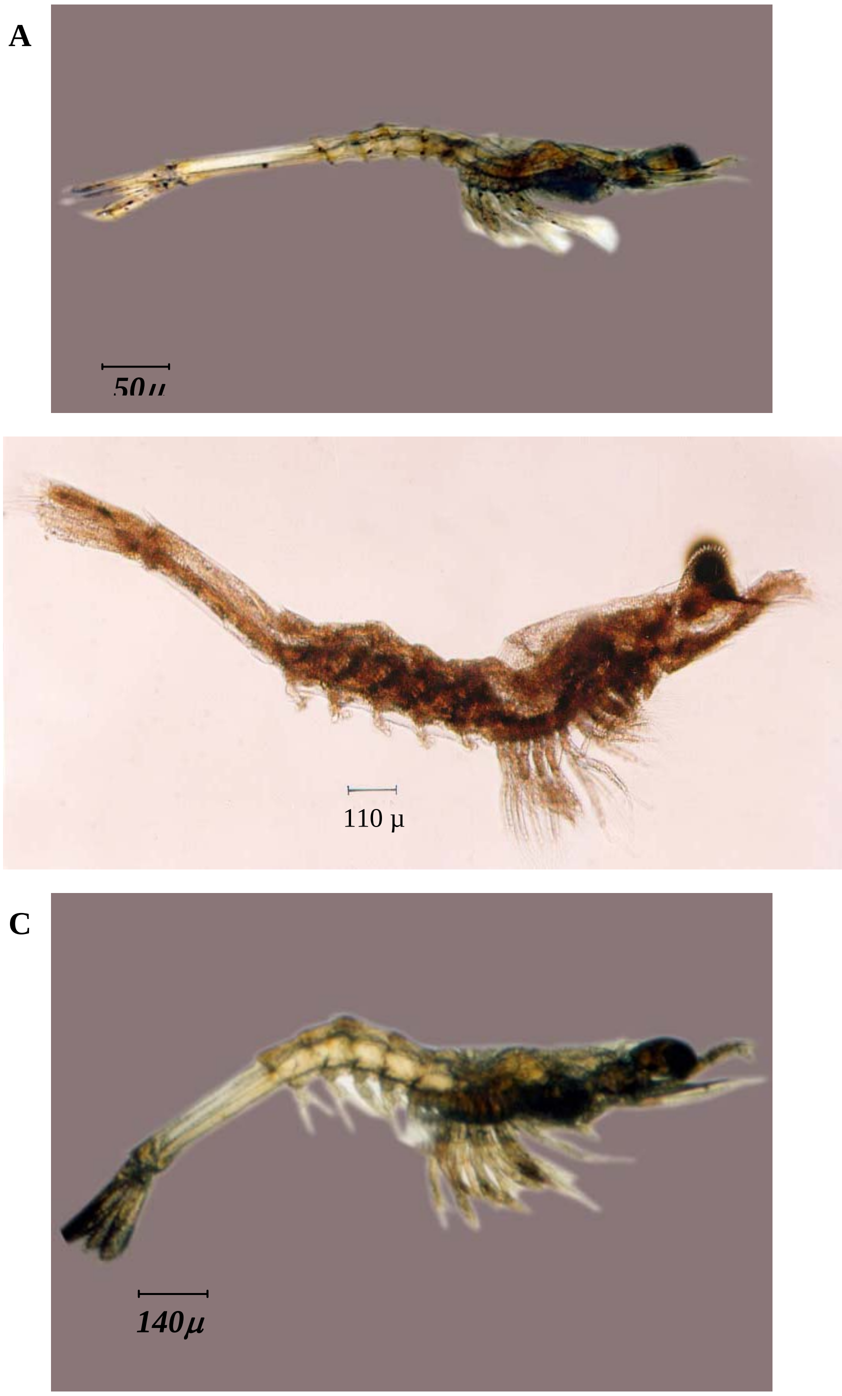


Plate (3): ***Trachypenaeus curvirostris*** **(Stimpson, 1860). A, First mysis; B, Second mysis; C, Third mysis**

Plate (4): Post Larva of *Trachypenaeus curvirostris* (Stimpson, 1860) reared in the laboratory (24 day after hatching).

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