Hydrobiologia (2006) 560:175–196
DOI 10.1007/s10750-005-1448-y

Primary Research Paper

Early developmental stages of the green tiger prawn, *Penaeus semisulcatus* de Haan (Crustacea, Decapoda, Penaeidae)

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Received 10 June 2005; in revised form 12 October 2005; accepted 22 October 2005

Key words: Penaeus semisulcatus, penaeid shrimp, developmental stages, larval taxonomy, crustacean morphology, shrimp aquaculture

Abstract

Gravid females of *Penaeus semisulcatus* were spawned in the laboratory by natural means. The embryos were documented and the larvae were reared from hatching to postlarval stage at 28.2–30.0 °C and 33.5–34.5 g kg^{-1} salinity for about 10 days (223 h 55 min). Six naupliar stages, three protozoea stages, three mysis stages and the first postlarval stage were described and illustrated. The larvae were fed only with microalgae *Tetraselmis tetrathele* and *Chaetoceros gracilis* from first protozoea until the second mysis, with about 90% survival rate; from the third mysis until the first postlarva they were fed with similar microalgae coupled with rotifer *Brachionus plicatilis* and *Artemia* nauplii. The embryonic and larval stages of *P. semisulcatus* are generally similar to those of other closely related species in the family Penaeidae, such as *Melicertus canaliculatus*, *Fenneropenaeus merguiensis*, and *Marsupenaeus japonicus*, except for the size and structure of diagnostic characters, setation of appendages and duration of metamorphoses. The change in the feeding habit during ontogeny was related to morphological transformation of the feeding apparatus of larvae and postlarvae. This paper is the first comprehensive and complete account of the early developmental stages of *P. semisulcatus*.

Introduction

The green tiger prawn, *Penaeus semisulcatus* de Haan, is one of the most common and commercially important marine shrimp in the Indo-West Pacific region. Although the postlarval and juvenile stages are usually found in brackish estuarine areas, the adult stage of this species is caught mainly by commercial trawlers in the open sea and by fish corrals set in coastal waters. This large penaeid species grows poorly in brackish water fishponds.

This species inhabits mud, sandy-mud or sandy-grit substrates on the continental shelf from the coastline to depths of about 130 m, but is most abundant in waters shallower than 60 m (Yu & Chan, 1986; Hayashi, 1992). Penaeus semisulcatus can form small shoals and is predominantly nocturnal, burying in the substrate in daytime. It is mostly fished at night-time, but is also fished by daytime in some areas (Holthuis, 1980).

Based on published literatures (Holthuis, 1980; Baba et al., 1986; Yu & Chan, 1986; Hayashi, 1992), *P. semisulcatus* has wide geographic distribution ranging from the waters of south and east Africa to India and Sri Lanka, including the Red Sea, Persian Gulf and Western Madagascar. Further east it

extends as far as Korea, Japan, the Philippines, New Guinea and Northern Australia. It entered the Eastern Mediterranean through the Suez Canal and is now found along the coasts of Egypt, Israel, Lebanon, Syria and southern Turkey.

Liao & Huang (1972) spawned *P. semisulcatus* in Taiwan and provided preliminary information concerning the culture of larvae under controlled conditions. Courties (1976) spawned *P. semisulcatus* from Madagascar, and illustrated six naupliar and first protozoeal stages only. Hassan (1982) spawned *P. semisulcatus* from the Arabian Gulf and described 5 naupliar, 3 protozoeal, 3 mysis, and 1 postlarval stages.

This study was conducted to illustrate and describe fully the embryonic and complete larval development of the Pacific *P. semisulcatus*, and to compare its external larval morphological characters with closely related species of penaeid shrimps such as *Melicertus canaliculatus* (Oliver), *Fenneropenaeus merguiensis* (De Man) and *Marsupenaeus japonicus* (Bate) that are found in Indo-West Pacific region.

Materials and methods

Collection, identification and spawning

Several gravid *P. semisulcatus* with well-developed olive green ovaries were selected and purchased from Azumacho Fisheries Cooperative in Nagashima, Japan. The shrimps were caught in the southern Yatsuhiro Sea (32°13′ N, 130°13′ E), Southwest of Kyushu Island, Japan, by fishermen using gill nets. *Penaeus semisulcatus* was identified by comparing its morphological characters with published descriptions (Miyake, 1982; Motoh & Buri, 1984; Baba et al., 1986; Yu & Chan, 1986; Hayashi, 1992). The carapace length of the spent spawners ($N = 18$) ranged from 45.8 to 50.5 mm; body weight ranged from 66.9 to 78.1 g; and body length from 169.5 to 180 mm. The spawning and larval rearing experiments were conducted at the Fisheries Experimental Station of Kagoshima University. Gravid shrimps were spawned in well-aerated 100-l and 500-l capacity fiberglass tanks with filtered seawater.

Egg incubation and larval rearing

The physico-chemical parameters were monitored closely. The spawned eggs were counted and incubated at 28.0–29.1 °C and 33.5–34.5 g kg^{-1} salinity, pH 8.10–8.25 and 6.05–6.33 mg l^{-1} of dissolved oxygen. As embryonic development progressed, samples were taken, measured, photomicrographed, and preserved in 4% buffered formalin (Omori and Ikeda, 1984).

The larvae from first protozoea until first postlarval stages were fed daily with 50 000 cells ml^{-1} of *Tetraselmis tetrathele* (West) Butcher and 100 000 cells ml^{-1} of *Chaetoceros gracilis* Schütt (Ronquillo & Yamasaki, 1986). *Tetraselmis tetrathele* was cultured using Yashima medium (Hirata, 1980); while *C. gracilis* was cultured by enriching the culture medium with 300 mg l^{-1} of KNO_3, 30 mg l^{-1} of $NaH_2PO_4 \cdot 2H_2O$, 15 mg l^{-1} of Na_2SiO_3 and 30 mg l^{-1} of Clewat-32 (Ronquillo & Saisho, 1993, 1995, 1997; Ronquillo et al., 1997). *Brachionus plicatilis* Müller were cultured using the technique of Hirata (1980). Starting from mysis-3 stage, *B. plicatilis* (20 ind. ml^{-1}) and newly hatched *Artemia nauplii* (5 ind. ml^{-1}) were also fed to the larvae until postlarval stage. Algal cultures, which reached logarithmic phase, were harvested and fed to the larvae. Survival rate of each larval substage was based on an average of 5 aliquot samples.

Larval collection, preservation and illustrations

Larval samples were collected, narcotized using Gohar's method (Steedman, 1976), and preserved in 4% buffered formalin and glycerol at 8:2 ratio for observation and illustrations (Ronquillo & Saisho, 1993).

Morphological descriptions and illustrations were made from preserved or newly narcotized larvae ($N \geq 10$ per brood) from 3 spawning batches using a camera lucida. Measurements were taken from preserved samples ($N \geq 10$ each from 3 broods) using a micrometer eyepiece. Appendages were dissected out from each larval substage with a small amount of solution and stained with either Chlorazol Black-E or methylene blue (Omori & Ikeda, 1984) to enhance ultrastructures. The type specimens were deposited in UPV Museum of the University of the Philippines in the Visayas, Iloilo, Philippines.

Nomenclature, measurement and description

The taxonomic names were adopted from the publication of Perez Farfante and Kensley (1997). Published terminologies for penaeid embryonic development (Hudinaga, 1942; Anderson, 1973, 1979, 1982), larval stages (Williamson, 1982; Dall et al., 1990), external morphology (Cook & Murphy, 1971; McLaughlin, 1980, 1982; Felgenhauer, 1992; Stachowitsch, 1992), and setal classification (Watling, 1989) were used in the description. The type of setules are described in the text for each appendage. Only representative setules are shown in the figures. Larval measurements (Ronquillo & Saisho, 1993, 1995, 1997) are defined as follows: body length (bl) of nauplius, from anterior to posterior end of the body excluding furcal spines; body length (bl) of protozoea, from the base of rostrum to the apex of telson excluding furcal spines; body length (bl) of mysis and postlarva, from the post-orbital margin of carapace to the tip of telson with abdomen extended in a straight line; carapace length (cl), the distance between the post-orbital margin and the medial posterior border of the carapace; body width (bw), the greatest distance across the dorsal or ventral surface; furcal spine length (fsl), from the base of the longest furcal spine to its apex; *N*, number of specimens examined. Measurements are given in mean values with standard deviation in parentheses. The furcal spine formula is represented by the number of spines on left and right processes of the bifurcated telson. The setal formula was established on the number of setae from proximal to distal segment.

Results and description

Spawning and development

Spawnings were observed between 20:00 and 24:00 h. Depending upon the size of breeder ($N = 18$, cl = 45.8–50.5 mm, bl = 169.5–180 mm, bw = 66.9–78.1 g) and condition of ovary, fecundities ranged between 93 000 to 366 000 eggs. Table 1 summarizes the chronology of the embryonic development of *P. semisulcatus*; while Figure 1 and 2 illustrate the series of its embryonic development. Egg diameter ($N \geq 20$ for each stage) increased from 0.29 mm during spawning time to 0.30 mm just before hatching. About 98.5% of larvae emerged 14 h and 10 min after spawning time at 28.0–28.2.1 °C and 34.5 g kg^{-1} salinity (see Table 1), after puncturing the external membrane with their posterior spines and sliding out by extending their appendages (Fig. 2e).

Table 1. Chronology of embryonic development of *Penaeus semisulcatus* at 28.2 ± 0.2 °C, pH 8.20–8.32, and 34.5 g kg^{-1} salinity

Cumulative time (h:min)	Stage
00:00	Spawn
00:08	Sperm Penetration Stage
00:08	Formation of First Polar Body
00:19	Formation of Fertilization Membrane
00:23	Formation of Second Polar Body
00:32	First Cleavage (2-cell Stage)
00:48	Second Cleavage (4-cell Stage)
00:58	Third Cleavage (8-cell Stage)
01:15	Fourth Cleavage (16-cell Stage)
01:30	Fifth Cleavage
01:48	Sixth Cleavage
02:07	Seventh Cleavage
02:36	Blastula
04:32	Gastrula
05:12	Antennal Limb Bud
06:20	Mandibular Limb Bud
06:35	Antennular Limb Bud
08:05	Biramous Antenna and Mandible
11:10	Elongation of Spines and Setae
14:05	Movement of Limbs
14:10	Emergence from Egg Membrane

The larvae passed through 6 naupliar, 3 protozoeal, and 3 mysis stages before reaching first postlarval stage in a minimum of about 9 days (224 h) from hatching at 28.2–30.0 °C and 33.5–34.5 g kg^{-1} salinity (see Table 2).

Description of larval stages

First nauplius (N-1) (Fig. 3A and D)
bl = 0.317 mm (0.012); bw = 0.166 mm (0.007); fsl = 0.113 mm (0.007); $N = 35$.

Body is pyriform, unsegmented and bears 3 pairs of natatory appendages: uniramous antennule, biramous antenna and mandible (Fig. 3A and D). A labrum projects ventrally from anterior

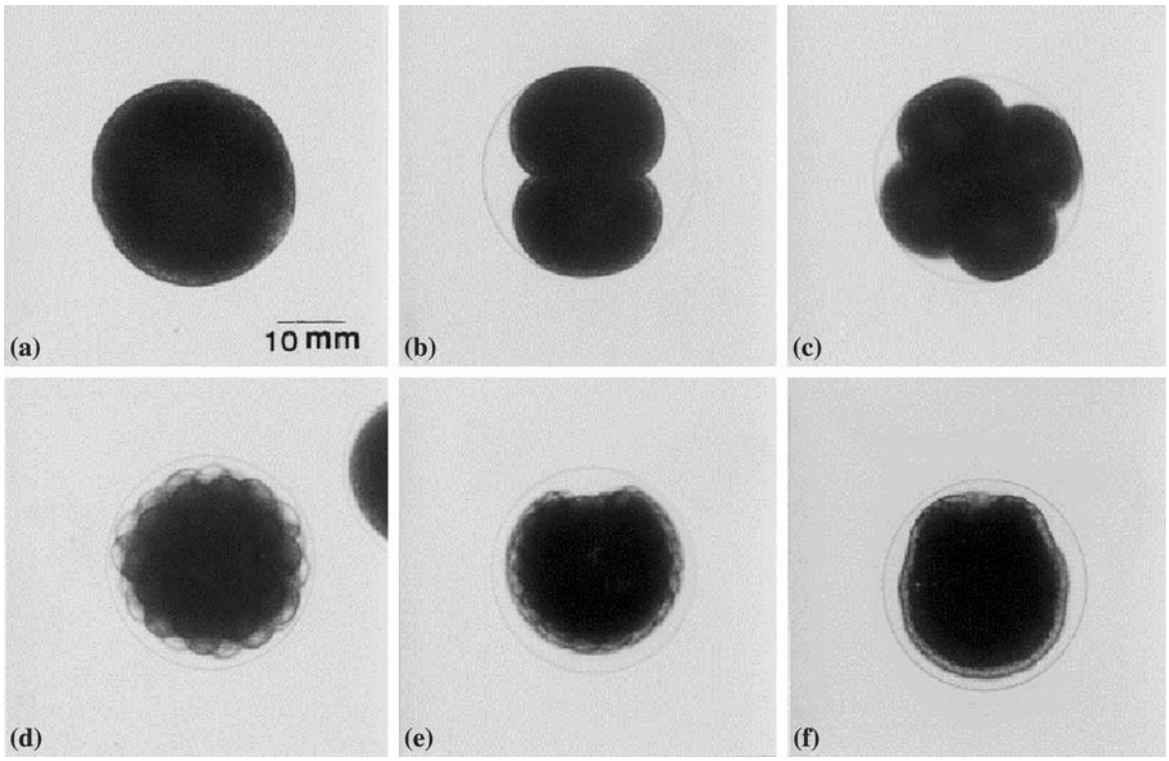

Figure 1. Embryonic development of *Penaeus semisulcatus* at 28.2 ± 0.2 °C, pH 8.20–8.32 and 34.5 g kg^{-1} salinity. (a) Newly Spawned Egg (00:00); (b) Two-cell Stage (00:32); (c) Four-cell Stage (00:48); (d) Morula Stage (02:07); (e) Blastula Stage (02:36); (f) Gastrula Stage (04:32).

end and a median ocellus is located near the anterior end. A tiny median spine (lost after first moult) is present postero-dorsally and together with a pair of furcal spines extending posteriorly makes the furcal spine formula 1 + 1 + 1. All setae are simple at this stage.

Antennule. Uniramous, slightly shorter than body, with 3 ventrolateral setae (1 short and 2 median), 1 long dorsolateral seta, and 2 long terminal setae (plus a small spike).

Antenna. Biramous; endopod with 2 medium ventrolateral setae and 2 long terminal setae; exopod with 3 long ventrolateral setae and 2 long terminal setae.

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

Second nauplius (N-2) (Fig. 3B and E)
bl = 0.344 mm (0.022); bw = 0.186 mm (0.008); fsl = 0.137 mm (0.010); $N = 30$.

Body is similar to preceding stage except for the absence of the tiny median posterodorsal spine (Fig. 3B and E). Long and moderate setae of this substage and subsequent substages are plumose. The longest terminal seta of the antennal exopod is bifid apically from this substage until Nauplius-6. Furcal spine formula is 1 + 1.

Antennule. Two medium simple ventrolateral setae; 3 terminal setae (2 medium simple and 1 long plumose); one medium simple dorsolateral seta.

Antenna. Endopod with 2 short simple ventrolateral setae and 2 long plumose terminal setae plus a small spike; exopod with 3 ventrolateral setae (1 medium simple and 2 long plumose), 3 terminal setae (1 long plumobifid, 1 long plumose and 1 short simple).

Mandible. As in previous stage, except all setae are plumose.

Third nauplius (N-3) (Fig. 3C and F)
bl = 0.387 mm (0.017); bw = 0.191 mm (0.009); fsl = 0.202 mm (0.016); $N = 48$.

The shape remains essentially the same as in the previous stage except for the elongation of the abdominal region (Fig. 3C and F). Outline of

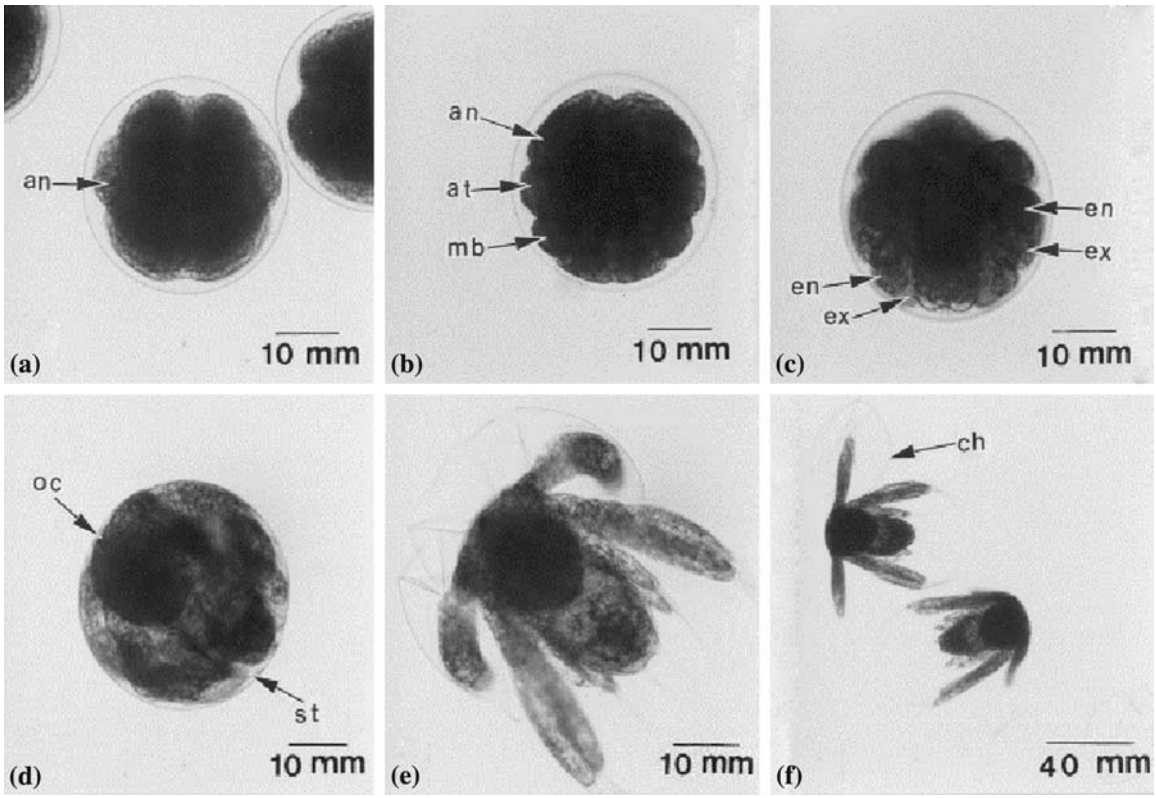

Figure 2. Embryonic development of *Penaeus semisulcatus* at 28.2 ± 0.2 °C, pH 8.20–8.32 and 34.5 g kg^{-1} salinity. (a) Antennal Limb Bud Stage (05:12); (b) Mandibular Limb Bud Stage (06:20); (c) Biramous Antenna and Mandible (08:05); (d) Elongation of Setae and Spine (11:10); (e) Hatching Nauplius (14:10); (f) Newly Hatched Nauplius Beside Empty Chorion. Abbreviations: an, antenna; at, antennule; la, labrum; mb, mandible; oc, ocellus; st, seta; en, endopod; ex, exopod; ch, chorion.

Table 2. Chronology of larval development of *Penaeus semisulcatus* at 28.0–30.0 °C, pH 8.20–8.32 and 33.5–34.5 g kg^{-1} salinity

Cumulative time (h:min)	Stage
00:00	First Nauplius
03:20	Second Nauplius
09:20	Third Nauplius
15:50	Fourth Nauplius
21:20	Fifth Nauplius
27:20	Sixth Nauplius
47:20	First Protozoea
78:50	Second Protozoea
123:50	Third Protozoea
152:30	First Mysis
176:20	Second Mysis
199:50	Third Mysis
223:55	First Postlarva

developing appendages is evident at the ventral side of the body. A depression separates two developing furcal processes. The furcal spine formula is now 2 + 2, 2 + 3, or 3 + 3 due to the addition of small spines.

Antennule. Three ventrolateral setae (1 spike and 2 medium simple); 3 terminal setae (1 short and 2 long plumose).

Antenna. Endopod with 2 short simple ventrolateral setae and 3 terminal setae (1 short simple and 2 long plumose); exopod with 4 ventrolateral setae (3 long plumose and 1 plumo-bifid), 3 terminal setae (1 short, 1 medium simple and 1 long plumose).

Mandible. Unchanged.

Fourth nauplius (N-4) (Fig. 4A and D)

bl = 0.415 mm (0.021); bw = 0.197 mm (0.010); fsl = 0.206 mm (0.011); $N = 30$.

Abdominal region is more slender and flattened dorsoventrally (Fig. 4A and D). Furcal spine formula is 4 + 4 or 4 + 5. Rudiments of abdominal

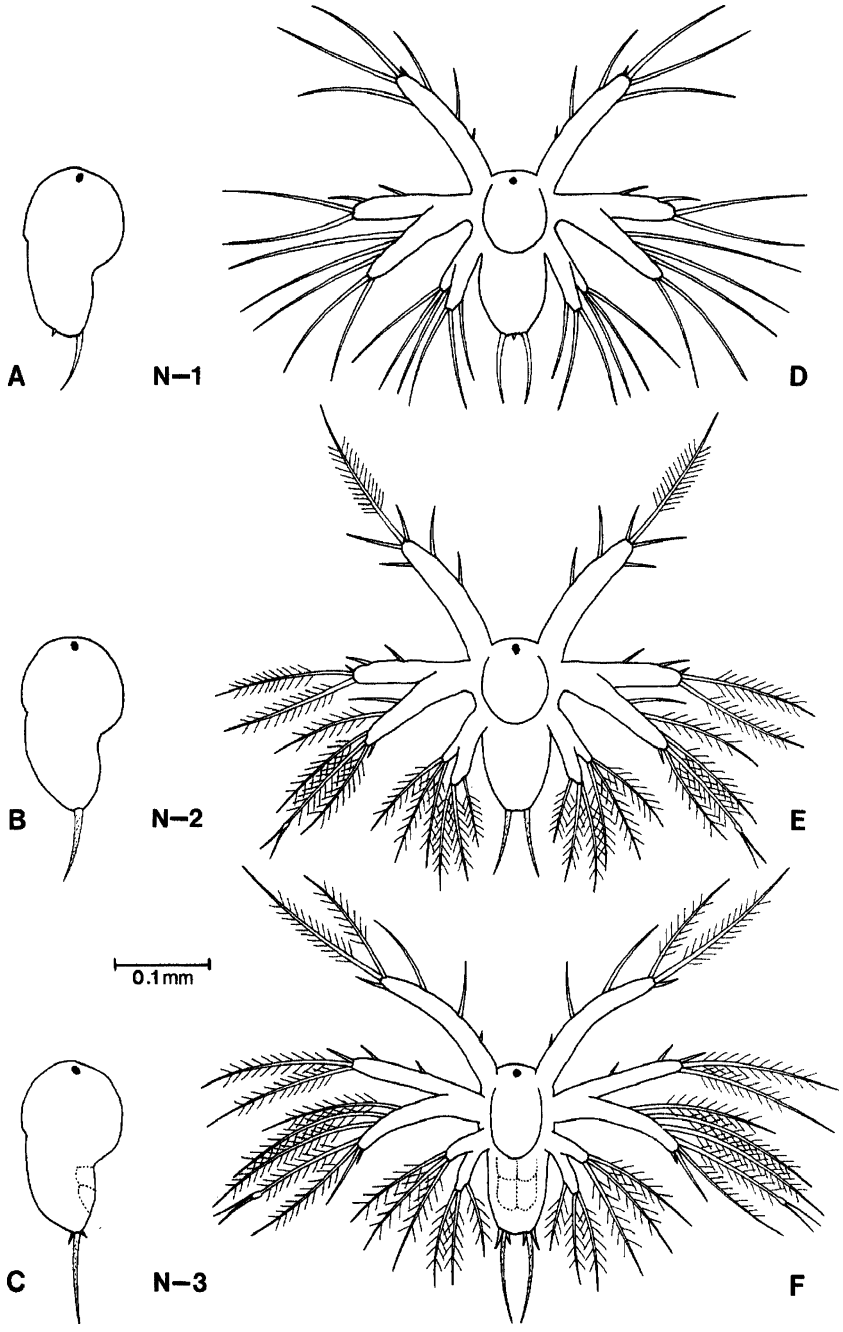

Figure 3. Naupliar stages of *Penaeus semisulcatus*. (A–C), lateral view excluding appendages; (D–F), ventral view; N-1, first nauplius; N-2, second nauplius; N-3, third nauplius.

limbs can be seen through the cuticle posterior to labrum. Segmentation of antennule and antennules indistinct.

Antennule. Three ventrolateral setae (2 medium simple and 1 short); 3 terminal setae (2 long plumose and 1 medium simple).

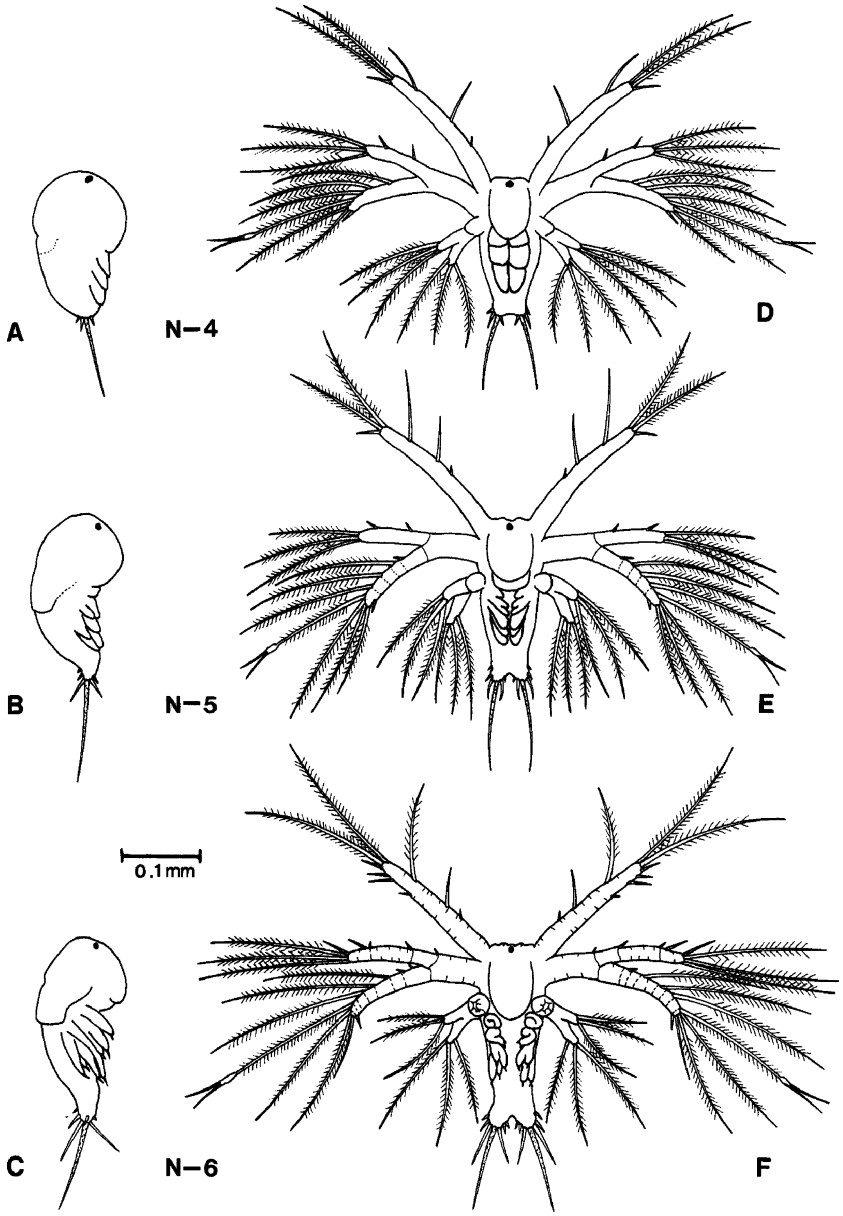

Figure 4. Naupliar stages of *Penaeus semisulcatus*. (A–C), lateral view excluding appendages; (D–F), ventral view; N-4, fourth nauplius; N-5, fifth nauplius; N-6, sixth nauplius.

Antenna. Endopod with 2–3 short simple lateral setae and 3 long plumose terminal setae; exopod with 4 ventrolateral setae (3 long plumose and 1 plumo-bifid) and 3 terminal setae (2 long plumose and 1 short simple).

Mandible. Unchanged.

Fifth nauplius (N-5) (Fig. 4B and E)

bl = 0.447 mm (0.016); bw = 0.201 mm (0.005); fsl = 0.235 mm (0.025); $N = 30$.

The outline of the developing carapace can be seen on the dorsal surface of the body. Abdominal region has become longer and more slender

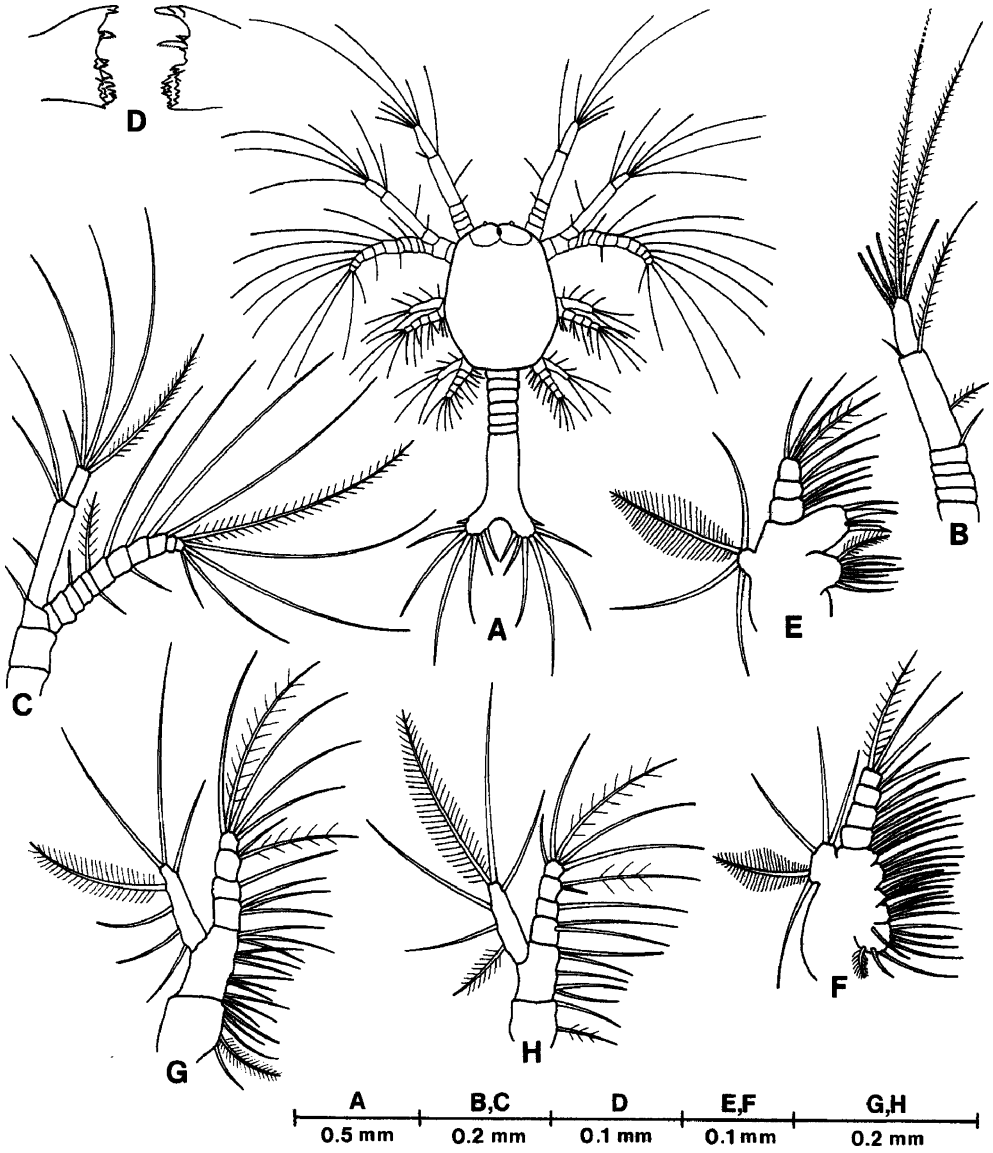

Figure 5. First protozoea of *Penaeus semisulcatus*. (A) dorsal view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped I; (H) maxilliped II.

(Fig. 4B and E). The maxilla and first and second maxillipeds are free from the body but do not possess setae. Furcal spine formula is now 5 + 5, 5 + 6, or 6 + 6.

Antennule. Three ventrolateral setae (2 medium simple and 1 short simple); 3 terminal setae (2 long plumose and 1 short simple).

Antenna. Endopod with 2 short simple lateral setae and 3 long plumose terminal setae; exopod with 5 ventrolateral setae (1 long plumo-bifid, 3 long plumose and 1 short simple) and 3 long plumose terminal setae and 1 short simple dorsolateral seta.

Mandible. As in previous stage, except for the swelling of basal nodule (gnathobase).

Sixth nauplius (N-6) (Fig. 4C and F)

bl = 0.578 mm (0.037); bw = 0.207 mm (0.007); fsl = 0.270 mm (0.015); $N = 30$.

The developing carapace is very distinct and the body is more elongated (Fig. 4C and F). The antennule and antenna possess annular indentions. A pair of frontal organs appears anterior to ocellus. Four pairs of appendages following the mandible are biramous non-articulated structures with primordial setae. The furcal spine formula is now 6+7 or 7+7.

Antennule. Three ventrolateral setae (1 long plumose, 1 medium simple and 1 short simple); 3 terminal setae (2 long plumose and 1 short simple); 3 dorsolateral setae (2 short simple and 1 spike).

Antenna. Endopod with 4 short ventrolateral setae and 4 terminal setae (3 long plumose and 1 medium simple); exopod with 6 ventrolateral setae (1 long plumo-bifid, 3 long plumose, 1 short simple and 1 spike) and 4 terminal setae (2 long plumose, 1 medium simple and 1 short simple).

Mandible. As in previous stage, except for enlargement and serration of gnathobase.

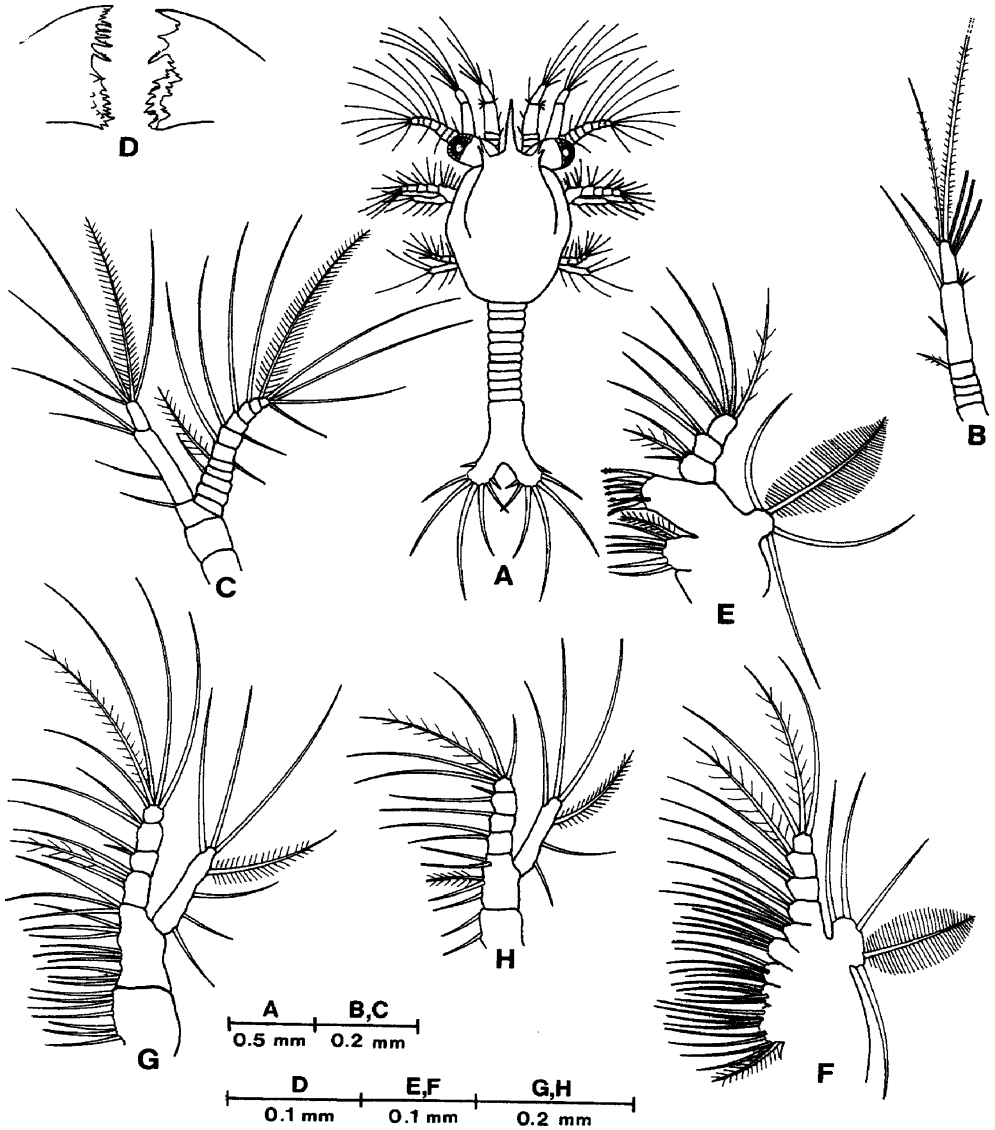

Figure 6. Second protozoea of *Penaeus semisulcatus*. (A) dorsal view; (B) antennule; (C) antenna; (D), mandible; (E) maxillule; (F) maxilla; (G) maxilliped I; (H) maxilliped II.

First protozoea (Fig. 5)
bl = 1.20 mm (0.158); cl = 0.505 mm (0.013); cw = 0.405 mm (0.015); $N = 30$.

After a minimum of 36 h 05 min from hatching at 29.8 °C and 31.0 g kg^{-1} (maximum: 47 h 30 min at 28.3 °C and 33.5 g kg^{-1} salinity), the larvae metamorphosed into protozoea (Fig. 5A). The body is differentiated into cephalothorax and abdomen. A loose-fitting carapace, with medium notch between two globular frontal organs, covers sessile compound eyes.

This is the first feeding stage and the abdominal limb pairs (maxillae and first and second maxillipeds) become specialized feeding apparatus in association with well-developed mandibular gnathobases, which have already lost the endopod and

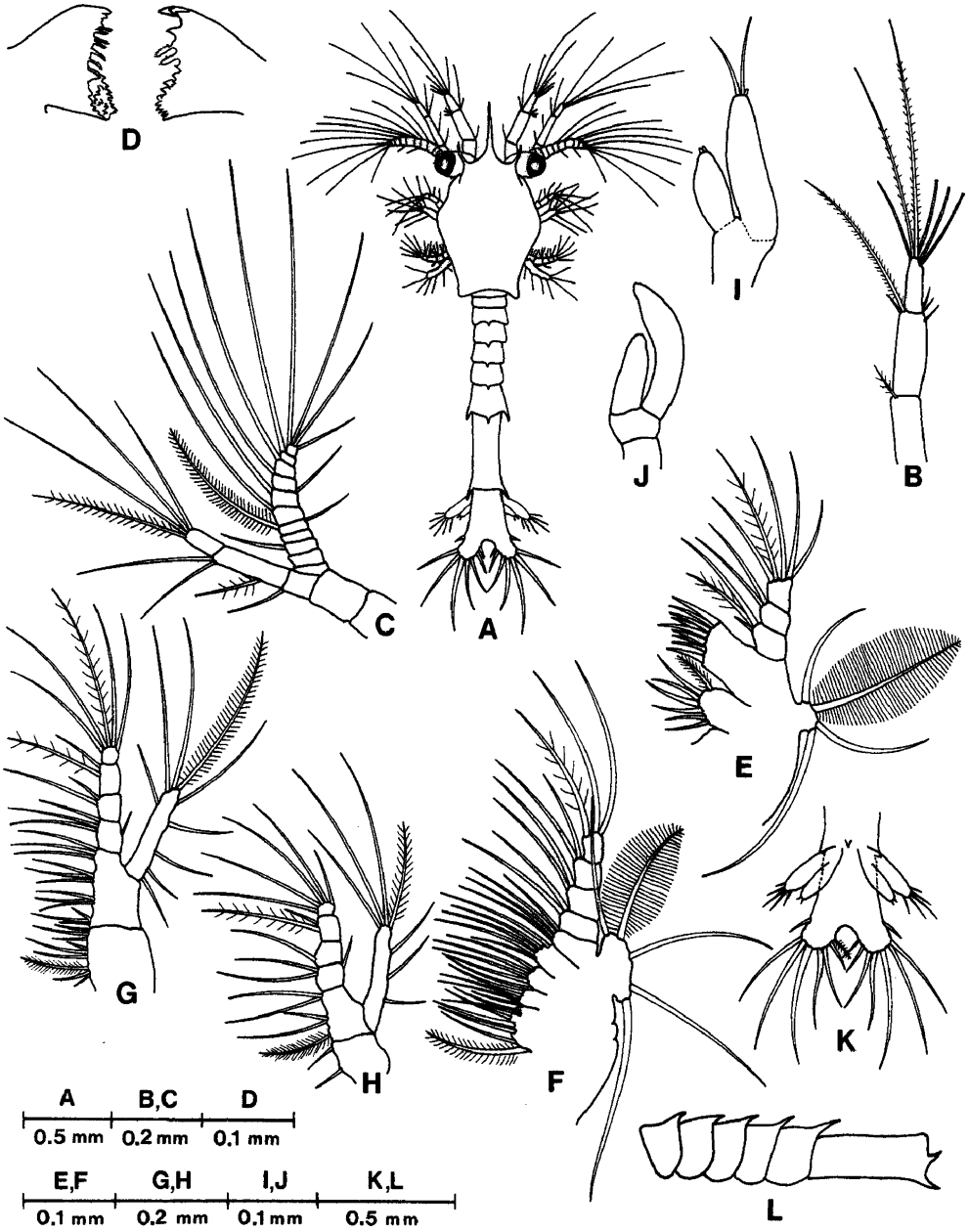

Figure 7. Third protozoea of *Penaeus semisulcatus*. (A) dorsal view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped I; (H) maxilliped II; (I) maxilliped III; (J) pereopod I; (K) telson and uropod; (L) side view of abdomen.

exopod. The labrum has an anterior short spine and posterior to it are two lobes of paragnaths with short bristles on their inner margins. Third maxilliped is absent on thoracopods.

Characteristics of appendages are as follows.

Antennule (Fig. 5B): Consists of three major segments; basal segment is divided into five subsegments, bearing 1 short serrulate seta; second

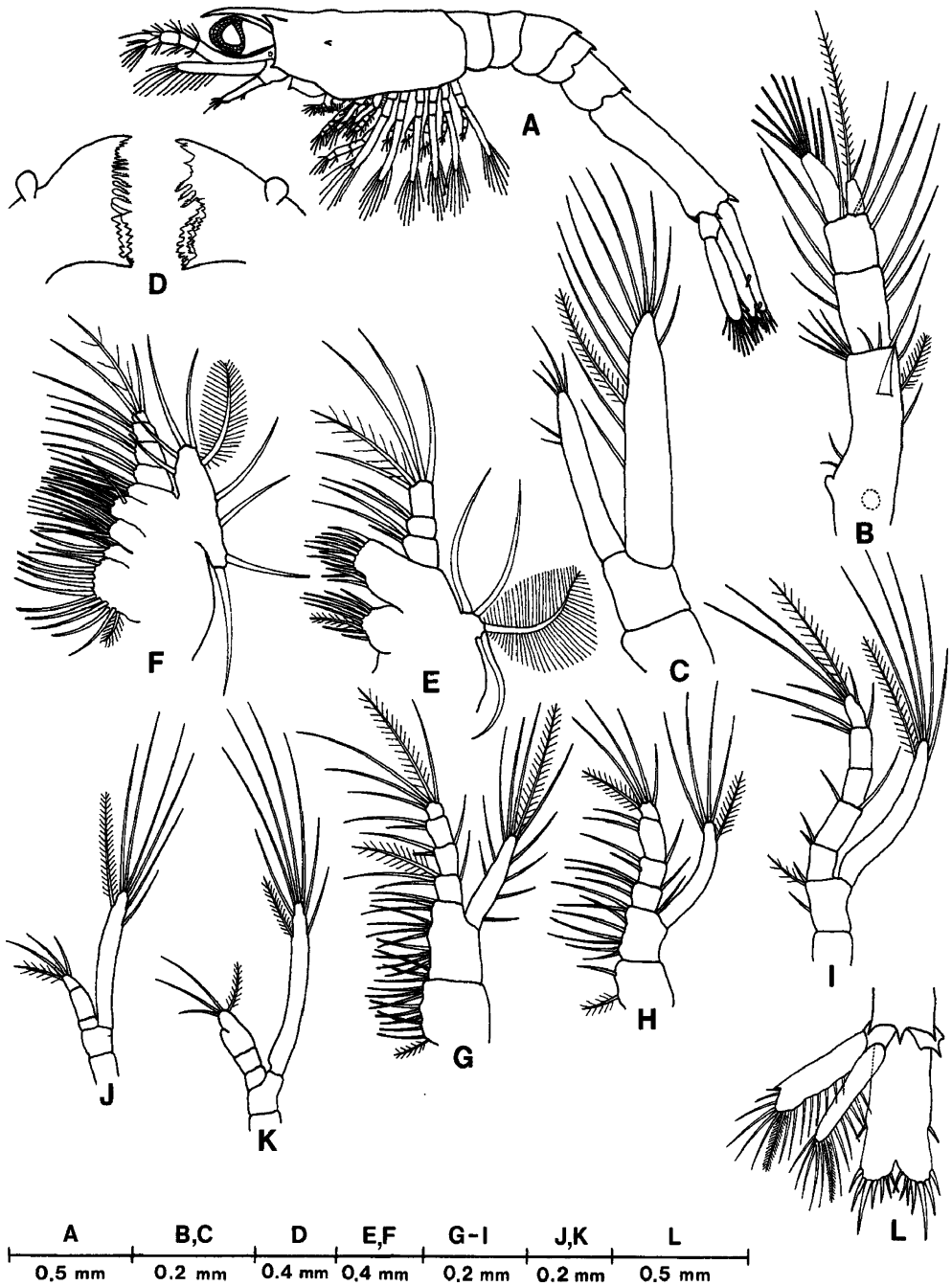

Figure 8. First mysis of *Penaeus semisulcatus*. (A) side view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped I; (H) maxilliped II; (I) maxilliped III; (J) pereopod I; (K) pereopod V; (L) telson and uropod.

segment possesses 1 ventrolateral and 2 posterolateral setae; distal segment with 6 terminal setae (2 long sparsely plumose and 4 aesthetascs).

Antenna (Fig. 5C): Protopod 3-segmented with 1 anterolateral plumose seta. Endopod 2-segmented: proximal segment with 1 lateral and 2 posterolateral plumose setae; distal segment with 1 short simple and 4 long plumose setae. Exopod 11-segmented with 13 plumose setae.

Mandible (Fig. 5D): Exopod and endopod lost but possesses distinct molar and incisor processes. Right incisor bears 1 slender serrated seta and 2 cuspidate teeth; left incisor with 1 short serrated seta; molar with numerous smaller conate and uncinate teeth.

Maxillule (Fig. 5E): Protopod with coxal endite of 7 stout setae (1 simple and 6 plumose) and basal endite of 4 stout setae (2 simple, 2 denticulate).

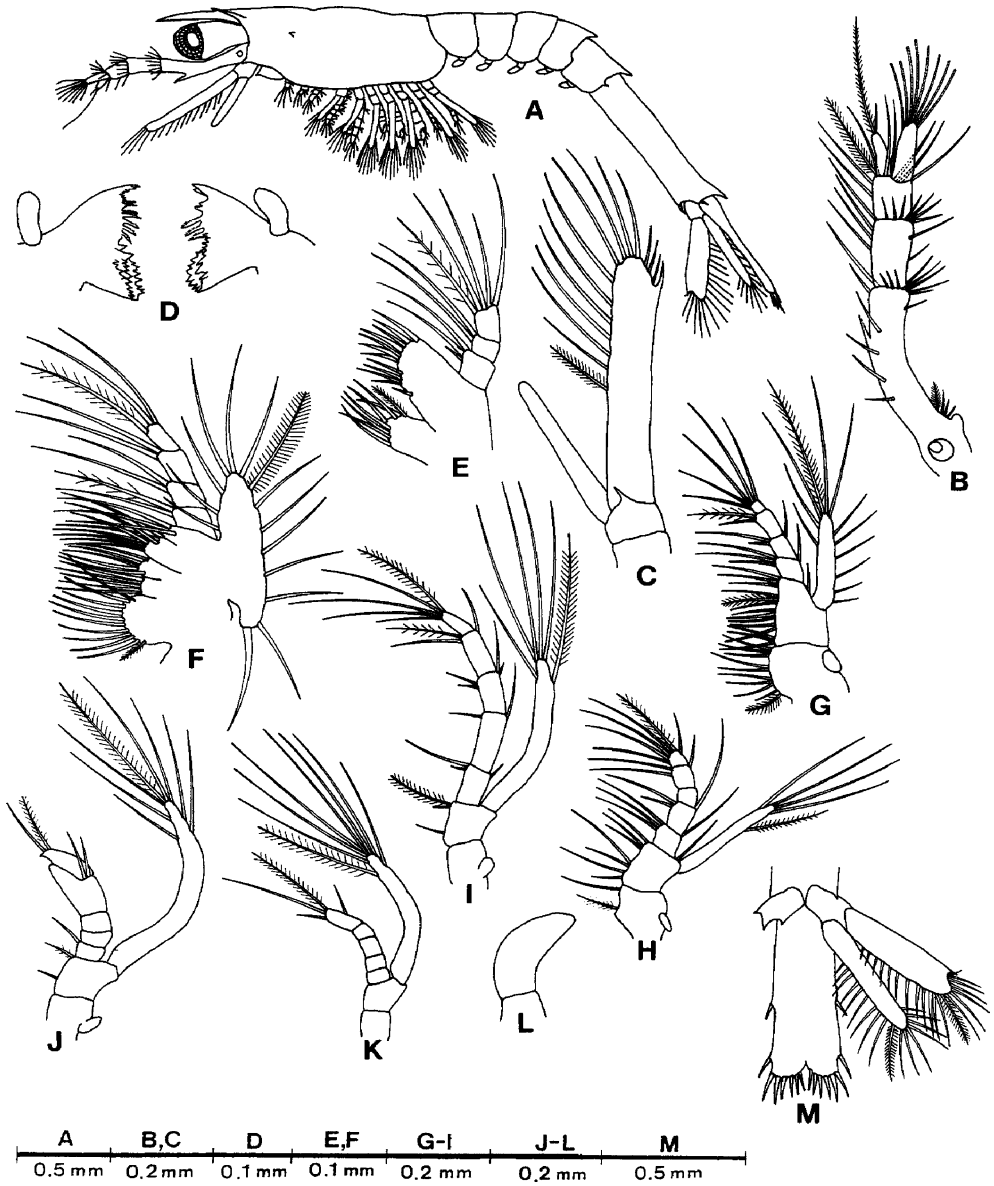

Figure 9. Second mysis of *Penaeus semisulcatus*. (A) side view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped I; (H) maxilliped II; (I) maxilliped III; (J) pereopod I; (K) pereopod V; (L) telson and uropod.

Endopod of 3 segments has setation of 3+2+5. Small knob-like exopod bears 4 plumose setae.

Maxilla (Fig. 5F): Protopod with 5 lobed endites; proximal (coxal) lobe has 8 plumose setae and other 4 distal (basal) lobes with 4 plumose setae each. Endopod 4-segmented with plumose setation of 2+2+2+3. Exopod ovoid with 5 plumose setae.

First maxilliped (Fig. 5G): Biramous and longer than maxilla. Protopod 2-segmented; coxa with 5 plumose setae on inner surface; basis with 12 plumose setae on inner surface. Endopod

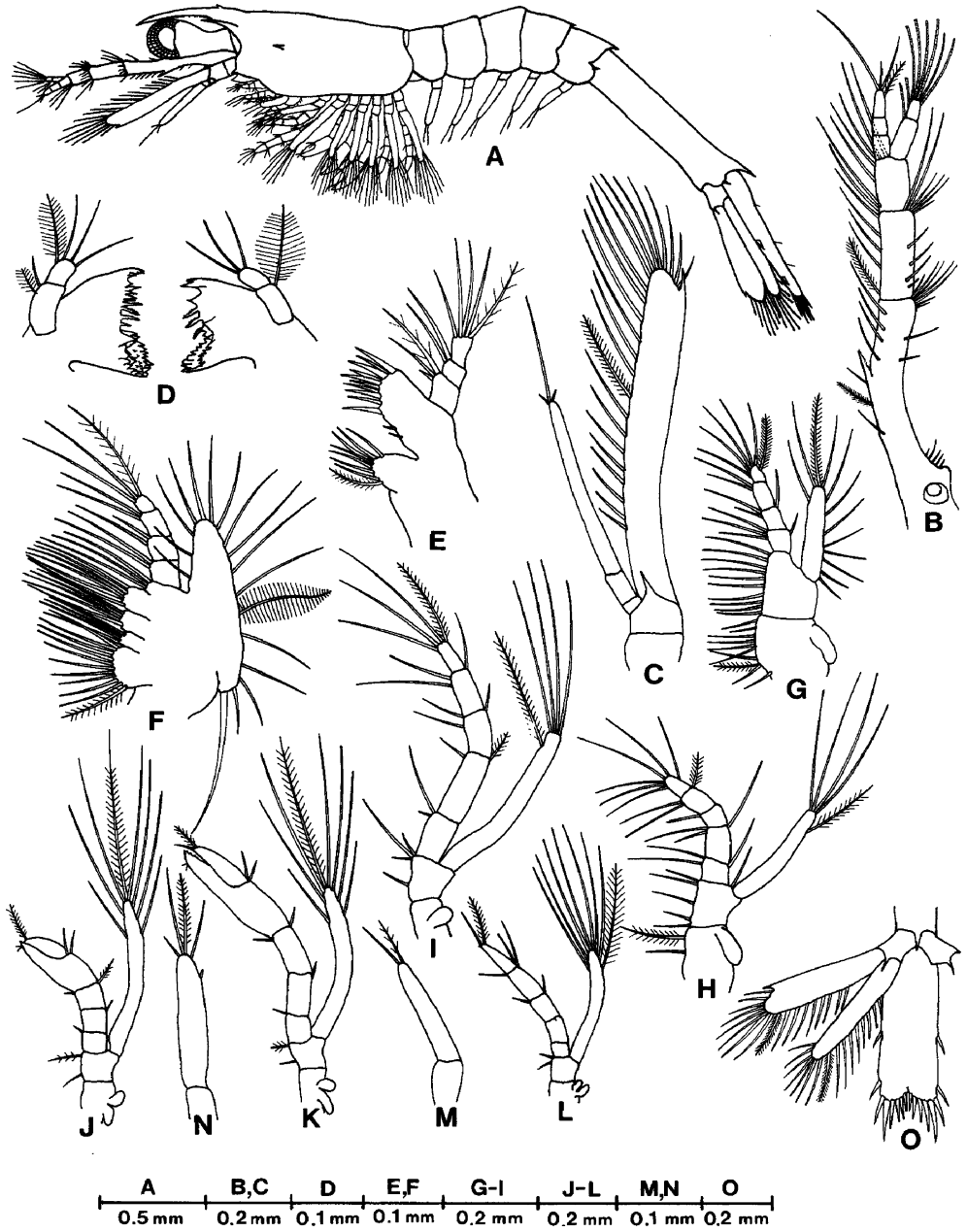

Figure 10. Third mysis of *Penaeus semisulcatus*. (A) side view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped I; (H) maxilliped II; (I) maxilliped III; (J) pereopod I; (K) pereopod III; (L) pereopod V; (M) pleopod I; (N) pleopod V; (O) telson and uropod.

4-segmented with 3 + 1 + 2 + 5 plumose setation. Exopod with 7 plumose setae.

Second maxilliped (Fig. 5H): Protopod 2-segmented; coxa with 2 plumose setae and basis with 5 plumose setae. Endopod 4-segmented with plumose setation of 2 + 1 + 2 + 5. Exopod has 6 plumose setae.

Telson (Fig. 5A): Broadly bifurcate with 7 + 7 furcal spines. All spines plumose, except the simple outermost pair.

Second protozoea (Fig. 6)

bl = 1.506 mm (0.158); cl = 0.618 mm (0.009); cw = 0.541 mm (0.017); $N = 30$.

The prominent features of this substage (Fig. 6A) are: stalked compound eyes free from carapace; ventrally projecting rostrum; a pair of bifurcated supraorbital spines; and segmented abdomen. Frontal organs are absent in this and succeeding stages. Third maxilliped is still absent.

Characteristics of appendages are as follows.

Antennule (Fig. 6B): Segmentation as in first protozoea. Basal segment bears 1 plumose seta; second segment with 5 setae (1 long plumose and 4 medium plumose); distal segment with 6 setae (2 long plumose and 4 aesthetascs).

Antenna (Fig. 6C): As in first protozoea.

Mandible (Fig. 6D): Asymmetrical. Left incisor with 5 slender serrated setae; right incisor with 1 serrated seta and 1 prominent cuspidate tooth. Molar with numerous smaller conate and uncinate teeth.

Maxillule (Fig. 6E): As in first protozoea, except basal endite with 7 stout denticulate setae.

Maxilla (Fig. 6F): Protopod with 9,4,5,4,3(or 4) plumose setae on first to fifth lobes, respectively. Endopod and exopod as in first protozoea.

First maxilliped (Fig. 6G): Coxa with 7 plumose setae on inner surface; basis with 12 plumose setae on inner surface. Endopod and exopod as in first protozoea.

Second maxilliped (Fig. 6H): As in first protozoea.

Telson (Fig. 6A): As in first protozoea but slightly larger.

Third protozoea (Fig. 7)

bl = 2.589 mm (0.141); cl = 0.749 mm (0.043); cw = 0.608 mm (0.034); $N = 30$.

Rostrum curves slightly downwards between a pair of non-bifurcated supraorbital spines (Fig. 7A). Carapace covers the first 5 thoracic somites. Third maxilliped and pereopods are rudimentary and biramous. Abdomen is divided into 6 segments including the telson. Each of the first 5 segments possesses a dorsomedian spine on its posterior margin (Fig. 7L). The sixth segment bears 1 postero-ventral spine and a pair of mid-lateral spines. A pair of biramous uropods (Fig. 7K), originates from posteroventral edge of the sixth abdominal segment.

Characteristics of appendages are as follows.

Antennule (Fig. 7B): Consisting of 3 major segments: first segment with 1 distal plumose seta; second segment with 5 setae (1 long plumose and 4 simple); third segment with 6 setae (4 aesthetascs and 2 long plumose).

Antenna (Fig. 7C): As in second protozoea.

Mandible (Fig. 7D): Asymmetrical. Left incisor with 6 slender serrated setae; right incisor with 2 serrated setae and 1 prominent cuspidate tooth. Molar with numerous inner smaller conate and outer uncinate teeth.

Maxillule (Fig. 7E): As in second protozoea. Coxal endite with 7 stout setae (1 simple, 2 plumose and 4 plumodenticulate); basal endite 9 stout setae (2 simple and 2 plumose).

Maxilla (Fig. 7F): As in second protozoea.

First maxilliped (Fig. 7G): Endopod with setation of 4 + 2 + 2 + 5 on first to fourth segments, respectively. Exopod with 9 plumose setae. Coxa with 9 plumose setae; basis with 12 plumose setae.

Second maxilliped (Fig. 7H): Endopod with setation of 3 + 1 + 2 + 5; exopod with 7 plumose setae. Coxa with 2 plumose setae; basis with 5–6 plumose setae.

Third maxilliped (Fig. 7I): Not fully developed; endopod unsegmented with 3 terminal setae (2 long plumose and 1 primordial); exopod with 2 terminal primordial setae.

Pereopods (Fig. 7J): Undeveloped; protopod present; seta absent.

Telson and Uropods (Fig. 7K): Biramous uropods shorter than telson, bearing 5 plumose setae on exopod and 2 primordial setae on endopod. Telson with 1 simple and 7 plumose setae on each lobe. Small anal spine is present.

First mysis (Fig. 8)

bl = 2.913 mm (0.073); cl = 0.804 mm (0.014); $N = 35$

After a minimum of ~6 days (152 h 30 min) at 28.5–30.0 °C and 33.5–34.0 g kg^{-1} salinity (maximum: 160 h 30 min at 27.5–29.1 °C and 33.5–34.5 g kg^{-1} salinity) from hatching, the larvae metamorphosed into mysis stage (Fig. 8A). This stage is a progressive metamorphosis toward juvenile adult form. Thoracopods (mandible, maxillae and maxillipeds) retain their feeding and ingestive function. Pereopods are still undeveloped and the exopods are mainly used for swimming. Carapace closely covers the thoracic somites and bears pairs of supraorbital, antennal, and hepatic spines. Rostrum is naked and reaches beyond the eye.

The first 5 abdominal segments are more or less similar in size; the sixth is much longer. Median dorsal spines occur on third to sixth segments. Sixth segment with pairs of postero-lateral and ventral spines on each side and an anal spine on posterior border.

Characteristics of appendages are as follows.

Antennule (Fig. 8B): Consisting of 3 major segments, bearing 2 unsegmented rami: first (basal) segment with rudiment of statocyst, 1 prominent serrated ventromedian spine and 12 plumose setae; second with 6 plumose setae; third with 5 plumose setae. Outer ramus with 6 terminal and 2 subterminal aesthetascs; inner ramus with 1 long plumose and 1 short simple setae.

Antenna (Fig. 8C): Protopod 2-segmented; endopod unsegmented with 6 plumose setae. Exopod (scaphocerite) blade-like, with 12 plumose setae on margin.

Mandible (Fig. 8D): Asymmetrical; small palp present. Left incisor process with 7 slender serrated setae; right incisor with 3 serrated setae and 1 prominent cuspidate tooth. Molar process with numerous inner smaller conate and outer uncinate teeth.

Maxillule (Fig. 8E): Basal endite with 8 setae (2 simple, 2 plumose, and 4 plumo-denticulate); coxal endite with 12 setae (2 simple and 10 denticulate). Endopod and exopod as in third protozoea.

Maxilla (Fig. 8F): Protopod with 11, 4–5, 5–7, 6, 4 plumose setae on first to fifth lobes, respectively. Endopod with setation of 2 + 2 + 2 + 3. Exopod (scaphognathite) unsegmented, with 9 plumose setae.

First maxilliped (Fig. 8G): Coxa and basis with 10 and 14–15 plumose setae, respectively. Endopod with setation of 4 + 3 + 2 + 5. Exopod unsegmented, with 10–11 plumose setae.

Second maxilliped (Fig. 8H): Coxa and basis with 2 and 8 plumose setae, respectively. Endopod with setation of 4 + 3 + 2 + 5. Exopod with 5 plumose setae.

Third maxilliped (Fig. 8I): Fully developed. Coxa and basis with 0 and 3 plumose setae, respectively. Endopod with setation of 1(2) + 2 + 0 + 3 + 5. Exopod with 6 plumose setae.

Pereopods (Fig. 8J–K): Undeveloped. First 3 pairs (Fig. 8K) with chela formation and slightly larger than the last 2 pairs (Fig. 8J). Protopod 2-segmented, without seta. Endopod 3-segmented with 4–5 (first to third pereopods) or 4 (fourth and fifth pereopods) plumose setae on the distal segment. Exopods unsegmented, longer than endopod, with 7 or 8 plumose setae.

Uropods and Telson (Fig. 8L): Protopod with 1 spine on ventral margin. Endopod and exopod blade-like. Endopod with 12–13 plumose marginal setae. Exopod with 1 outer spine and 15–16 plumose marginal setae. Telson cleft terminally with 8 pairs of spines; 1 pair inserted laterally and 7 pairs posteriorly.

Second mysis (Fig. 9)

bl = 3.29 mm (0.119); cl = 0.91 mm (0.059); $N = 35$.

The distinguishing features of this substage (Fig. 9A) are: presence of 2-segmented pleopods and absence of dorso-median spine on third abdominal segment.

Characteristics of appendages are as follows.

Antennule (Fig. 9B): Peduncle 3-segmented; first segment with 1 statocyst, 1 blunt protrusion, 1 prominent serrated ventromedian spine and 20 plumose setae; second segment with 12–13 plumose setae; third segment with 9–10 plumose setae. Outer ramus with 6 terminal and 2 subterminal aesthetascs; inner ramus with 1 long plumose and 1 short simple setae.

Antenna (Fig. 9C): Basis with 1 spine. Endopod unsegmented without any seta. Exopod with 1 latero-distal spine on outer margin and 16 plumose, marginal setae.

Mandible (Fig. 9D): Unsegmented palp present. Left incisor process with 7 slender serrated

setae; right incisor with 3 serrated setae and 1 denticulate tooth. Molar process with numerous smaller inner conate and outer uncinate teeth.

Maxillule (Fig. 9E): Coxal endite with 8 setae (2 simple, 2 plumose, 4 plumodenticulate) and basal endite with 12–13 setae (2–3 simple and 10 denticulate). Endopod as in first mysis. Exopod absent.

Maxilla (Fig. 9F): Protopod with 11, 4–5, 5–7, 6, 4 plumose setae on first to fifth lobes, respectively. Endopod as in first mysis. Exopod with 15 plumose setae.

First maxilliped (Fig. 9G): As in first mysis. Coxa with epipod or mastigobranchial plate.

Second maxilliped (Fig. 9H): Coxa and basis with 2 and 6–8 plumose setae, respectively. Coxa with epipod. Endopod with setation of $4+3+0+3+6$. Exopod with 5 plumose setae.

Third maxilliped (Fig. 9I): Coxa naked with epipod; basis with 3 plumose setae. Endopod with setation of $(1–3)+2+4+3+5$. Exopod with 5–6 plumose setae.

Pereopods (Fig. 9J–K): Protopod 2-segmented, with 0–2 plumose setae on basis. Endopod of first to third pereopods 5-segmented with plumose setation of $1+0+0+(2–3)+2$ (Fig. 9J). Endopod of fourth and fifth pereopods 5-segmented with setation of $0+0+0+0+4$ plumose setae (Fig. 9K). 1st to 3rd pereopods with non-functional chelae. Exopods with 7–8 plumose setae.

Pleopods (Fig. 9L): Rudimentary, uniramous and 2-segmented.

Uropods and Telson (Fig. 9M): Protopod as in first mysis. Endopod with 17 plumose marginal setae. Exopod with 1 outer spine and 19 plumose marginal setae. Median notch of the telson less pronounced than in the preceding stage. Telson bears 8 pairs of spines: 2 pairs inserted laterally and 6 pairs posteriorly.

Third mysis (Fig. 10)

bl = 3.92 mm (0.147); cl = 0.93 mm (0.047); $N=35$.

The distinguishing characteristic features of this substage (Fig. 10A) are: presence of developed pleopods with 2–5 short apical setae (Fig. 10N and M); 3-segmented antennal endopod and a dorsal tooth on the rostrum. The armature of the abdomen and uropods are almost similar to the preceding substage.

Characteristics of appendages are as follows.

Antennule (Fig. 10B): Peduncle 3-segmented; first segment with statocyst, 1 basal spine, 1 prominent serrated ventromedian spine and 22–25 plumose setae; second segment with 14–16 plumose setae; third segment with 7–9 plumose setae. Outer ramus 2-segmented with 6 terminal and 2 subterminal aesthetascs; inner ramus 3-segmented with 1 long and 3 medium plumose setae.

Antenna (Fig. 10C): Protopod 2-segmented, with 1 spine. Endopod 3-segmented with 1 long and 2 short apical plumose setae. Exopod with 1 disto-lateral spine on outer margin and 23 marginal plumose setae.

Mandible (Fig. 10D): Segmented palp present; proximal segment with 1–2 plumose setae; distal segment with 3–4 apical plumose setae. Left incisor process with 7 serrated setae; right incisor with 3 serrated setae and 1 denticulate tooth. Molar process with numerous smaller conate teeth.

Maxillule (Fig. 10E): As in second mysis.

Maxilla (Fig. 10F): Protopod and endopod as in second mysis. Exopod unsegmented with 17 plumose setae.

First maxilliped (Fig. 10G): As in second mysis.

Second maxilliped (Fig. 10H): As in second mysis.

Third maxilliped (Fig. 10I): Protopod and exopod as in second mysis. Endopod with setation of $2+2+3+3+5$.

Pereopods (Fig. 10J–L): Coxa with epipods; basis with 2 plumose setae. Endopod 5-segmented; first to fifth segments with 1,1,2,2,(1–2) plumose setae, respectively on first to third pereopods (Fig. 10J and K); 0,0,2,3,2 plumose setae on fourth and fifth pereopod (Fig. 10L); propodus and dactylus of first 3 pairs of pereopods modified to form a chela. Exopod with 7–8 plumose setae.

Pleopods (Fig. 10M and N): Two-segmented with 2–4 short simple apical setae.

Uropods and Telson (Fig. 10O): Protopod as in second mysis. Endopod with 17 plumose marginal setae. Exopod with 1 outer spine and 15 plumose marginal setae. Telson as in second mysis.

First postlarva (Fig. 11)

bl = 5.324 mm (0.126); cl = 1.393 mm (0.041); $N=35$.

At this stage (Fig. 11A), the mouthparts and maxillipeds assume their basic adult form. The

chelae of first 3 pairs of pereopods are functional; and the pleopods are now well-developed swimmerets. The swimming function shifts from thorax in mysis stage to abdomen in postlarva. Exopods of pereopods and maxillipeds are not degenerated or lost.

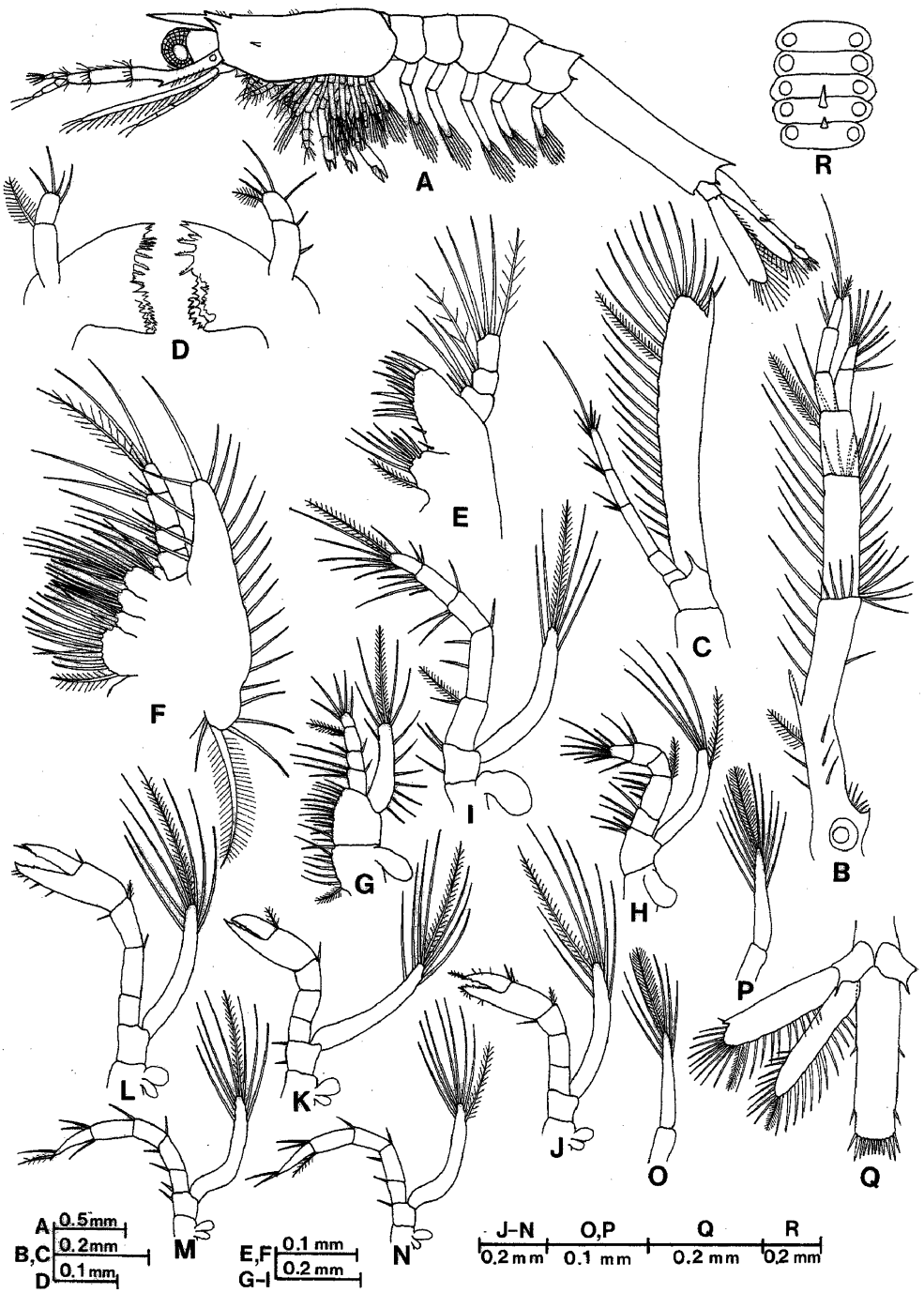

Figure 11. First postlarva of *Penaeus semisulcatus*. (A) side view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped I; (H) maxilliped II; (I) maxilliped III; (J) pereopod I; (K) pereopod II; (L) pereopod III; (M) pereopod IV; (N) pereopod V; (O) pleopod I; (P) pleopod V; (Q) sternal plate; (R) telson and uropod.

The carapace (Fig. 11A) bears pairs of supraorbital, antennal, and hepatic spines. Rostrum with 2 dorsal teeth is longer than the eye length. The dorsomedian and the midlateral spines on the abdominal segments are visible only at the fifth and sixth segments. The sternal plate (Fig. 11R) has 1 long median spine in the fourth segment and 1 short median spine in the fifth segment.

Characteristics of appendages are as follows.

Antennule (Fig. 11B): Peduncle 3-segmented; first segment with 1 statocyst, 1 basal spine, 1 prominent serrated ventromedian spine and 26 plumose setae; second segment with 20 plumose setae; third segment with 4–11 plumose setae. Outer ramus 2-segmented with 9 aesthetascs; inner ramus 3-segmented with 2 distal setae on second segment and 5 terminal setae on third segment.

Table 3. Comparison of larval morphological characteristics of *Penaeus semisulcatus*, *Melicertus canaliculatus*, *Fenneropenaeus merguiensis*, and *Marsupenaeus japonicus*

Stage	Character	*Penaeus semisulcatus* (Present authors)	*Melicertus canaliculatus* (Choy, 1984)	*Fenneropenaeus merguiensis* (Motoh & Buri, 1979)	*Marsupenaeus japonicus* (Hudinaga, 1942)
Egg	Diameter (mm)	0.29–0.30	0.26–0.28	0.27	0.26–0.28
Nauplius-1	Antennular setae	6	5	6	7
	Antennal setation[a]	5/5	4/6	4/5	5/5
Nauplius-2	Bifurcated setal tip of Antennal exopod	+	–	–	–
	Antennular setae	6	5	6	7
	Antennal setation[a]	5/6	5/7	5/6	5/6
Nauplius-3	Antennular setae	6	6	6	7
	Antennal setation[a]	5/7	5/8	5/7	5/7
Nauplius-4	Antennular setae	6	6	5	7
	Antennal setation[a]	5/8	5/8	5/7	5/9
Nauplius-5	Antennular setae	7	6	5	7
	Antennal setation[a]	6/9	6/8	5/8	6/10
Nauplius-6	Antennular setae	9	7	7	8
	Antennal setation[a]	7/11	7/12	6/10	7/12
Protozoea-1	Endopodal setation [b] of 1st maxilliped	2+1+2+5	2+2+2+4	3+1+2+5	3+2+2+5
	Endopodal setation of 2nd maxilliped	3+1+2+5	2+2+2+4	2+1+2+5	1+1+1+5
Protozoea-2	Supra-orbital spine	bifurcated at tip	bifurcated	bifurcated at base	bifurcated at tip
Protozoea-3	Exopodal setae of maxilla	7	4	5	6
Mysis-1	Endopodal setation [b] of 2nd maxilliped	4+3+1+4	3+3+2+4	4+3+2+5	4+3+2+5
Mysis-2	Rostral spine	0	1	0	1
	Endopodal setation [b] of 2nd maxilliped	3+2+0+2+5	2+3+2+2+4	4+3+0+2+5	–
Mysis-3	Rostral spine	1	2	0	2
	Endopodal setation of 3rd maxilliped	3+2+1+3+4	2+2+0+2+5	2+2+3+3+4	1+2+3+4+6
Postlarva-1	Rostral spine	2 or 3	2 or 3	0	3
	Carapace length (mm)	1.39	1.60	1.60	1.36

[a]Endopod/exopod.
[b]Setation is arranged from proximal to distal segment.

Antenna (Fig. 11C): Protopod with 1 prominent spine. Endopod 5-segmented with 10 plumose setae. Exopod (scaphocerite) with 1 disto-lateral spine on outer margin and 25–26 plumose marginal setae.

Mandible (Fig. 11D): Palp 2-segmented; proximal segment with 1–2 plumose setae; distal segment with 4–5 plumose setae. Left and right incisors as in third mysis.

Maxillule (Fig. 11E): Proximal and distal endites of protopod with 8 and 11 plumodenticulate setae, respectively. Endopod with 3 + 2 + 5 setation.

Maxilla (Fig. 11F): Protopod with 12, 3, 6, 6, 3 plumose setae on first to fifth lobes, respectively. Endopod with 2 + 2 + 2 + 3 setation. Exopod (scaphognathite) unsegmented, with 23 plumose setae.

First maxilliped (Fig. 11G): Coxa and basis with 8 and 15–16 plumose setae, respectively. Coxa with epipod or mastigobranchial plate. Endopod with 4 + 3 + 2 + 5 plumose setation. Exopod unsegmented, with 7 plumose setae.

Second maxilliped (Fig. 11H): Coxa with epipod and basis with 3 plumose setae. Endopod with 3 + 5 + 0 + 3 + 7 plumose setation. Exopod with 5 plumose setae.

Third maxilliped (Fig. 11I): Fully developed. Coxa with epipod and basis with 3 plumose setae. Endopod with 2 + 3 + 3 + 3 + 7 plumose setation. Exopod with 6 plumose setae.

Pereopods (Fig. 11J–N): Coxa with 2 epipods; basis with 0–2 plumose setae. Endopod 5-segmented; propodus and dactylus of first 3 pairs of pereopods modified to form a chela; first to fifth segments with 0 + 0 + 2 + 9 + 4 setation on first pereopod (Fig. 11J); 0 + 0 + 1 + 1 + 0 on second pereopod (Fig. 11K); 0 + 1 + 2 + 7 + 1 on third pereopod (Fig. 11L); (1–2) + 2 + 1 + 3 + 2 on fourth and fifth pereopods (Fig. 11M and N). Exopod with 8 plumose setae.

Pleopods (Fig. 11O and P): Two-segmented; 4 pairs of plumose setae on first to third pleopods (Fig. 11O); 5–6 pairs on fourth and fifth pleopods (Fig. 11P).

Uropods and Telson (Fig. 11Q): Endopod with 22 plumose marginal setae. Exopod with 1 outer spine and 21–22 plumose marginal setae. Telson bears 12 spines terminally and 2 pairs of spines laterally.

Discussion and conclusion

This is the first time that a complete and comprehensive account of the early developmental stages of *Penaeus semisulcatus* has been reported.

Embryonic and larval development

The pattern of embryonic development of *P. semisulcatus* is similar to *Metapenaeus ensis* (Ronquillo & Saisho, 1993) and *Marsupenaeus japonicus* (Hudinaga, 1942); but different from those of *Trachypenaeus curvirostris* (Ronquillo & Saisho, 1992) and *Metapenaeopsis barbata* due to the absence of large perivitellin space and non-occurrence of embryonized nauplius and protozoea stages. The duration of embryonic development and hatching is similar to those reported by Hassan (1982) for *P. semisulcatus* and Hudinaga (1942) for *M. japonicus*.

The pattern of larval development of *P. semisulcatus* is similar to other penaeid larvae of known parentage, which have been spawned in the laboratory (Dall et al., 1990). Hassan (1982) reported only five naupliar substages of *P. semisulcatus* that he spawned from the Arabian Gulf. The six naupliar substages of the Japanese *P. semisulcatus* found in the present study are similar to the findings of Courties (1976) for the naupliar larvae of *P. semisulcatus* from Madagascar. In the present study, the moulting to the Nauplius-2 substage was observed about 3 h 20 min after hatching at 28.5–30.0 °C and 33.5–34.5 g kg^{-1} salinity; and the morphological changes observed for Nauplius-2 larvae were the plumose setation and the absence of postero-dorsal spine on Nauplius-1. The result of the present study is similar to the six naupliar stages of *P. semisulcatus* in Madagascar as reported by Courties (1976).

Morphological difference

The Nauplius-2 to Nauplius-5 larvae of Hassan (1982) corresponds respectively with the Nauplius-3 to Nauplius-6 larvae in the present study and in the report of Courties (1976). The additional morphological characters found in the naupliar substages in this study are the presence of postero-dorsal spine and simple setae on Nauplius-1, and the plumo-bifid setae on the exopod of the antenna

of Nauplius-2 to Nauplius-6. The phylogenetic significance of this taxonomic character has been discussed in previous reports (Ronquillo & Saisho, 1993; 1995; 1997).

Hassan (1982) reported that the furcal spine formula of first mysis until first postlarval stage is 9 + 9; but in this study the furcal spine formula is 8 + 8. There seems to be a morphological difference between the larvae of *P. semisulcatus* in the Arabian Gulf and in western Pacific region. The difference could be attributed to geographic or genetic variations.

In Table 3, the larvae of *P. semisulcatus* are compared with those of *Fenneropenaeus merguiensis* (Motoh and Buri, 1979), *Melicertus canaliculatus* (Choy, 1984) and *Marsupenaeus japonicus* (Hudinaga, 1942). The egg diameter of *P. semisulcatus* (0.29–0.30 mm) is slightly larger than those of *F. merguiensis* (0.27 mm), *M. canaliculatus* (0.26–0.28 mm) and *M. japonicus* (0.26–0.28 mm). The naupliar substages of *P. semisulcatus* can be differentiated from other congeners through the setal type and formula on antennule and antenna.

The protozoeal substages of the congeners can be differentiated through the endopodal, exopodal and protopodal setation of the antennae, maxillae and maxillipeds including the position of bifurcation in the supraorbital spines of protozoea- 2.

The mysis and the first-postlarval stages can be differentiated by the presence of rostral teeth, spination of carapace and setation of maxillipeds.

This study shows that even in the earlier developmental stages, the larvae of P. semisulcatus can be distinctly differentiated from the larvae of other closely related penaeid species in the Indo-West Pacific region, i.e., *Fenneropenaeus*, *Penaeus*, *Melicertus* and *Marsupenaeus*.

Larval rearing

The use of *Chaetoceros* and *Tetraselmis* in penaeid shrimp hatchery management has been strongly endorsed by Ronquillo et al. (1997). The use of exclusively algal diets for larval rearing of penaeid shrimps is well documented (Gopalakrishnan, 1976; Emmerson, 1980; Kuban et al., 1985; Ronquillo & Yamasaki, 1985; Sanchez, 1986; Chu, 1989; Ronquillo & Saisho, 1993, 1995, 1997; Ronquillo et al., 1997; D'Souza & Kelly, 2000). Previous reports (Parsons et al., 1961; Ogino, 1963; Ackman et al., 1968; Watanabe et al., 1983; Fukusho et al., 1984; Whyte, 1987) have shown that phytoplankters have high nutritional content and are an ideal live feed diet for larval shrimps.

Ronquillo & Saisho (1993) discussed the relationship between the morphological changes in the feeding apparatus with the shift in feeding habit of penaeid shrimp larvae from filter feeder into raptorial omnivore as the planktonic larvae adapt to benthic life.

By correlating the morphological structures of the feeding apparatus (maxillae, maxillipeds, mandibles and pereopods) with the food and feeding habits, including growth and survival of different larval and postlarval stages, it could be concluded that the larvae of *P. semisulcatus* are filter feeders until mysis-2, and could greatly benefit from an inexpensive but efficient diet of diatoms (*Chaetoceros*) and prasinophyte (*Tetraselmis*). When *P. semisulcatus* moulted to mysis-3, the pereopods became fully functional chelae for grasping, and the larvae started to prefer larger prey and became cannibalistic. This is the stage where zooplankton like *Artemia* nauplii and *Brachionus spp*. must be introduced to improve the survival rate in the hatchery production of this species.

Acknowledgements

We extend our appreciation to Drs. Akihiko Shinomiya and Shusaku Kadowaki for the use of facilities in the Fisheries Research Laboratory of Kagoshima University. Access to the excellent facilities of the Third Department of Internal Medicine, Kagoshima University, and the technical help of Dr. Alicia Tashiro-Ronquillo during the preparation of the manuscript are gratefully acknowledged. Thanks are also due to Mr Yutaka Mizuno, Station Head of Tarumizu Fisheries Research Station, for *Chaetoceros gracilis* inoculum, and to Dr. Shigehisa Yamasaki for *Tetraselmis tetrathele* inoculum. We are grateful to Dr. Hiroshi Suzuki for offering valuable comments on this paper. Thanks to Dalyce Epp for production assistance

References

Ackman, R. G., C. S. Tocher & J. McLacklan, 1968. Marine phytoplankter fatty acids. Journal of the Fisheries Research Board of Canada 25: 1603–1620.

Anderson, D. T., 1973. Embryonic and Phylogeny in Annelids and Arthropods . Pergamon Press, Oxford.

Anderson, D. T., 1979. Embryos, fate maps, and phylogeny of arthropods. In Gupta, A. P. (ed.), Arthropod Phylogeny. Van Nostrand Reinhold, New York, 59–105.

Anderson, D. T., 1982. Embryology. In Abele, L. G. (ed.), The Biology of Crustacea. Vol. 2, Academic Press, London, 1–41.

Baba, K., K. Hayashi & M. Toriyama, 1986. Decapod Crustaceans from Continental Shelf and Slopes around Japan. Japan Fisheries Resource Conservation Association, Tokyo.

Choy, S. C., 1984. Larval development of *Penaeus (Melicertus) canaliculatus* (Oliver, 1811) reared in the laboratory (Decapoda, Natantia). Crustaceana 46: 1–22.

Chu, K. H., 1989. *Chaetoceros gracilis* as the exclusive feed for the larvae and postlarvae of the shrimp *Metapenaeus ensis*. Aquaculture 83: 281–287.

Cook, H. L. & M. A. Murphy, 1971. Early developmental stages of the brown shrimp, *Penaeus aztecus* Ives, reared in the laboratory. Fishery Bulletin 69: 223–239.

Courties, C., 1976. Description des premiers stades larvaires de trois crevettes peneides pechees a Madagascar: *Penaeus indicus* H. Milne Edwards; *Penaeus semisulcatus* De Haan; *Metapenaeus monoceros* (Fabricius). Cahiers Orstom Oceanographie 14: 49–70.

Dall, W., B. J. Hill, P. C. Rothlisberg & D. J. Sharples, 1990. The biology of the Penaeidae. In Blaxter, J. H. S. & A. J. Southward (eds), Advances in Marine Biology. Vol. 27, Academic Press, New York and London, 1–489.

D'Souza, F. M. L. & G. J. Kelly, 2000. Effects of a diet of a nitrogen-limited alga (*Tetraselmis suecica*) on growth, survival and biochemical composition of tiger prawn (*Penaeus semisulcatus*) larvae. Aquaculture 181: 311–329.

Emmerson, W. D., 1980. Ingestion, growth and development of *Penaeus indicus* larvae as a function of *Thalassiosira weissflogii* cell concentration. Marine Biology 58: 65–73.

Felgenhauer, B. E., 1992. External anatomy and integumentary structures. In Harrison, F. W. & A. G. Humes (eds), Microscopic Anatomy of Invertebrates. Vol. 10, Wiley-Liss, New York, 7–43.

Fukusho, K., M. Okauchi, S. Nuraini, A. Tsujigado & T. Watanabe, 1984. Food value of a rotifer *Brachionus plicatilis*, cultured with *Tetraselmis tetrathele* for larvae of red sea bream *Pagrus major*. Bulletin of Japanese Society of Scientific Fisheries 50: 1439–1444.

Gopalakrishnan, K., 1976. Larval rearing of the red shrimp, *Penaeus marginatus* (Crustacea). Aquaculture 9: 145–154.

Hassan, H., 1982. The larval development of *Penaeus semisulcatus* de Haan, 1850 (Decapoda, Penaeidae) reared in the laboratory. Journal of Plankton Research 4: 1–18.

Hayashi, K. I., 1992. Dendobranchiata Crustaceans from Japanese Waters . Seibutsu Kenkyusha, Tokyo.

Hirata, H., 1980. Culture methods of marine rotifer, *Brachionus plicatilis*. Mini Review and Data File of Fisheries Research (Kagoshima University) 1: 27–46.

Holthuis, L. B., 1980. FAO species catalogue. Vol. 1. Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries. FAO Fisheries Synopsis 125: 1–162.

Hudinaga, M., 1942. Reproduction, development and rearing of *Penaeus japonicus* Bate. Japanese Journal of Zoology 10: 305–393.

Kuban, F. D., A. L. Lawrence & J. A. Wilkenfield, 1985. Survival, metamorphosis and growth of larvae from four penaeid species fed six food combinations. Aquaculture 47: 151–162.

Liao, I. C. & T. L. Huang, 1972. Experiment on the propagation and culture of prawns in Taiwan. In Pillai, T. V. R. (ed.), Coastal Aquaculture in the Indo-Pacific Region. Fishing News Book, West Byfleet, England, 328–354.

McLaughlin, P. A., 1980. Comparative Morphology of Recent Crustacea. W.H. Freeman, San Francisco, CA.

McLaughlin, P. A., 1982. Comparative morphology of crustacean appendages. In Abele, L. G. (ed.), The Biology of Crustacea. Vol. 2, Academic Press, New York, 197–256.

Miyake, S., 1982. Macrura, Anomura, and Stomatopoda. Japanese Crustacean Decapods and Stomatopods in Color . Hoikusha, Tokyo.

Motoh, H. & P. Buri, 1979. Larvae of decapod crustacea of the Philippines. 4. Larval development of the banana prawn, *Penaeus merguiensis* reared in the laboratory. Bulletin of Japanese Society of Scientific Fisheries 45: 1217–1235.

Motoh, H. & P. Buri, 1984. Studies on the penaeoid prawns of the Philippines. Researches on Crustacea 13(14): 1–120.

Ogino, C., 1963. Studies on the chemical composition of some natural food of aquatic animals. Bulletin of Japanese Society of Scientific Fisheries 29: 459–462.

Omori, M. & T. Ikeda, 1984. Methods in Marine Zooplankton Ecology . John Wiley & Sons, New York.

Parsons, T. R., K. Stephens & J. D. H. Strickland, 1961. On the chemical composition of eleven species of marine phytoplankters. Journal of the Fisheries Research Board of Canada 18: 1001–1016.

Perez Farfante, I. & B. Kensley, 1997. Penaeoid and Sergestoid shrimps and prawns of the World. Keys and diagnoses for the families and general. Memoires Du Museum National D'Histoire Naturelle, Zoologie 175: 1–223.

Ronquillo, J. D. & T. Saisho, 1992. Occurrence of embryonized nauplius and protozoea stages in southern rough shrimp, *Trachypenaeus curvirostris* (Stimpson, 1860) (Decapoda, Penaeidae). Researches on Crustacea 21: 47–58.

Ronquillo, J. D. & T. Saisho, 1993. Early developmental stages of greasyback shrimp, *Metapenaeus ensis* (de Haan, 1844) (Crustacea, Decapoda, Penaeidae). Journal of Plankton Research 15: 1177–1206.

Ronquillo, J. D. & T. Saisho, 1995. Early developmental stages of southern rough shrimp, *Trachypenaeus curvirostris* (Stimpson, 1860) (Decapoda, Penaeidae) reared in the laboratory. Crustaceana 68: 833–863.

Ronquillo, J. D. & T. Saisho, 1997. Larval development of *Metapenaeopsis barbata* (de Haan, 1844) (Crustacea: Decapoda: Penaeidae). Marine and Freshwater Research 48: 401–414.

Ronquillo, J. D., T Saisho & S. Yamasaki, 1997. Culture of *Tetraselmis tetrathele* and its utilization in the hatchery

production of different penaeid shrimps in Asia. Hydrobiologia 368: 237–244.

Ronquillo, J. D. & S. Yamasaki, 1986. Cultivation of the prasinophyte *Tetraselmis tetrathele* in relation to the seed production of kuruma prawn, *Penaeus japonicus*. Mini Review and Data File of Fisheries Research (Kagoshima University) 4: 77–87.

Sanchez, M. R., 1986. Rearing of mysid stages of *Penaeus vannamei* fed with cultured algae of three species. Aquaculture 58: 139–144.

Stachowitsch, M., 1992. The invertebrates: an illustrated glossary . Wiley-Liss, New York.

Steedman, H. F., 1976. Zooplankton Fixation and Preservation. Monographs on Oceanographic Methodology 4. UNESCO, Paris.

Watanabe, T., C. Kitajima & S. Fujita, 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34: 115–143.

Watling, L., 1989. A classification system for crustacean setae based on the homology concept. In Felgenhauer, B. E. L. Watling, & A. B. Thistle (eds), Crustacean Issues. Vol. 6, A.A. Balkema, Rotterdam, 16–26.

Whyte, J. N. C., 1987. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture 60: 231–241.

Williamson, D. I., 1982. Larval morphology and diversity. In Abele, L. G. (ed.), The Biology of Crustacea. Vol. 2, Academic Press, New York, 43–110.

Yu, H. P. & T. Y. Chan, 1986. The Illustrated Penaeoid Prawns of Taiwan . SMC, Taipei.