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Larval development of the western school prawn *Metapenaeus dalli* Racek, 1957 (Crustacea: Decapoda: Penaeidae) reared in the laboratory

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Abstract

The six Naupliar, three Protozoa, three Mysis and first post-larval forms of the western school prawn *Metapenaeus dalli* Racek, 1957 were cultured in the laboratory. These stages were described in detail and compared to those of other metapenaeids. The ontogenetic development occurred in 12 days at 26°C, with both the growth rate and morphological patterns of development in *M. dalli* broadly following those recorded for other metapenaeids. Differences were found between *M. dalli* and other

metapenaeids at corresponding stages of larval development, with these being the number, location and composition of individual setae and other minor spinal development.

KEYWORDS: Morphology, planktonic, Penaeidae, Australia

Introduction

The morphological development of many of the ~29 metapenaeid species, which occur exclusively throughout the Indo-West Pacific (De Grave and Fransen 2011; De Grave 2014), have yet to be fully described. The larval life of such species is short, less than two weeks depending on rearing conditions, but is relatively complex. Larvae metamorphose through three stages – Nauplius, Protozoa and Mysis – before reaching the post-larval stage (Dall et al. 1990). Moreover, each stage can also include multiple sub-stages, which are accompanied by ontogenetic changes in morphology, swimming and feeding behaviour (FAO 1978; Dall et al. 1990; Jones et al. 1997).

Studies of larval morphology have traditionally been combined with laboratory rearing techniques, due to difficulties associated with developing reference material from the natural environment.

Although genotypic identification has been developed in many penaeid species (Vaseeharan et al. 2013), and in *Metapenaeus affinis* H. Milne Edwards, 1837 (Lakra et al. 2010) and *Metapenaeus dobsoni* Miers, 1878 (Mishra et al. 2009), this method requires the taxonomic identification of reference stock prior to development of genetic markers. However, historical literature is often inconsistent in its reporting of the larval metapenaeid taxonomy required for such identifications.

Species such as *Metapenaeus ensis* De Haan, 1844 (Leong et al. 1992; Ronquillo and Saisho 1993), *Metapenaeus joyneri* Miers, 1880 (Lee and Lee 1968), *Metapenaeus macleayi* Haswell, 1879 (Preston 1985), *Metapenaeus bennettiae* Racek & Dall, 1965 (Preston 1985) and *Metapenaeus brevicornis* H. Milne Edwards, 1837 (Teng 1971; Rao 1979) have their larval development described (or partially described); however, many of these lack descriptions of either whole appendages or details of the position, number and arrangement of setae on them. Characterising larval development in detail is crucial both for developing and comparing aquaculture rearing techniques and distinguishing between

congeners with overlapping geographical distributions (Rothlisberg et al. 1983; Jackson and Rothlisberg 1994).

One species that has yet to be described is the western school prawn *Metapenaeus dalli* Racek, 1957. This species is highly prized by recreational fishers and was once also the target for a commercial fishery within the Swan-Canning estuary before stock levels declined markedly (Smith 2006; Smithwick et al. 2001; Tweedley et al. 2014). Most recently, *M. dalli* has been targeted for restocking of the Swan-Canning fishery. The distribution of this species is known to overlap with those of 10 other metapenaeids, namely *M. affinis*, *Metapenaeus anchistus* de Man, 1920, *M. brevicornis*, *M. dobsoni*, *Metapenaeus elegans* de Man, 1907, *M. ensis*, *M. moyebi*, *Metapenaeus papuensis* Racek & Dall, 1965, *Metapenaeus endeavouri* Schmitt, 1927 and *Metapenaeus suluensis* Racek & Dall, 1965 (De Grave, 2014). Territorial ranges of *M. dalli* extend from southern Java in Indonesia to within Australian waters from Darwin in the north, along the coastline of Western Australia to Cape Naturaliste in the south (Grey et al. 1983). While this species is found in inshore marine waters in latitudes above 31°S, it is only found in estuaries below this latitude and is believed to complete its entire life cycle within these systems (Potter et al. 1986, 1989) and is thus regarded as a solely estuarine species (Potter et al. 2015a, 2015b).

The aims of this study are (1) to provide a comprehensive, well-documented and systematic description of the morphological development of three planktonic stages (Nauplius, Protozoa and Mysis) and the first benthic sub-stage (post larva) of *M. dalli*; and (2) to compare and contrast discriminatory features with other previously described metapenaeids.

Materials and methods

Brood stock collection

Ovigerous female *M. dalli* were collected at night from the Swan-Canning Estuary (31°56'50"S 115°54'58"E) in Perth, south-western Australia, during March 2014, using a hand trawl net 1.5 m

high, 4 m wide and constructed from 9 mm mesh. Retained individuals were disinfected with 1 ppm formaldehyde for 30 minutes, then immediately transported to the laboratory and placed in aerated holding tanks overnight (FAO 1978). Females were stocked into 300 L conical base tanks at a density of up to 40 per tank for one to four days. The tanks were filled with seawater at a salinity of ~33 drawn from a bore through a limestone filter, accessing nearshore marine water, aerated constantly and maintained at a temperature of ~26°C. The base of each tank was fitted with a fine grate that allowed eggs to pass through, separating them from brood stock to prevent any potential cannibalism. After spawning, eggs were collected on 48 µm and 63 µm screens and rinsed, sub-sampled for counting and re-suspended in 300 L of aerated 1 µm filtered seawater. Egg viability was assessed both visually and by hatch rate, with greater than 75% hatch rate considered suitable for use in the study (FAO 2007).

Larval culture

After spawning, hatched Nauplii were collected and stocked at 250 Nauplii L⁻¹ (D'Souza and Kelly 2000) in three 6 L flat-bottom cylinder glass culture vessels with 1 µm filtered seawater. Temperature was maintained at 26 ± 0.5°C by housing culture vessels in 115 L temperature-controlled water baths, heated with pre-calibrated Eheim Jager 150 W aquarium water heaters and monitored with Thermocron TCS temperature loggers every 10 minutes. Salinity was monitored daily using an ATAGO PAL-03S digital refractometer. Vessels were exposed to 3.5 µmol photons m⁻² s⁻¹ white incidence light with 12:12 h light:dark photoperiod. The larval cultures were aerated from the base to provide vertical mixing as well as oxygenation.

When the larvae reached Protozoa I, culture vessels were fed two microalgae strains obtained from the Australian National Algal Culture Collection, Commonwealth Scientific and Industrial Research Organization (CSIRO), Marine & Atmospheric Research Hobart, Tasmania. A chlorophyte, *Tetraselmis chuii* (CS-26), and a diatom, *Chaetoceros muelleri* (CS-176), were fed in a daily ration at a density of 3 × 10⁴ cells mL⁻¹ and 9 × 10⁴ cells mL⁻¹, respectively. Both cultures were maintained under a 12:12 h light:dark photoperiod with 180 µmol photons m⁻² s⁻¹ white incidence light from fluorescent lights, in 15 L culture vessels with ambient salinity of ~33 and ~25°C temperature. Each

species was cultured in Guillard's F2 medium, with sodium metasilicate added at 30 g L^{-1} for the diatom *C. muelleri* (Guillard and Ryther 1962). To avoid carbon limitation, food-grade carbon dioxide (CO_2) was injected and maintained at a pH of 7.4–7.7. Feed concentration was maintained daily by counting cells from sampled culture water with a Neubauer haemocytometer until the post-larval stage. The volume of water in the culture vessels was topped up to 6 L daily with a combination of microalgae feed and fresh seawater. The gut contents of the larvae were also briefly examined microscopically to confirm that the algal cells were being ingested (D'Souza and Kelly 2000).

Sampling frequency, fixing, staining and measurements

Routine sampling commenced when the newly hatched Nauplii were transferred from spawning tanks to the 6 L culture vessels, and continued every 6 h until Protozoa I larvae were observed at ~48 h. Subsequently, samples were taken every 24 h until post larvae I were observed after ~12 days. At each sampling period, single 200 mL subsamples were taken from each culture vessel, screened over $43 \mu\text{m}$ nylon mesh and subsequently fixed in 10% tetraborate-buffered formaldehyde, until staining and mounting occurred.

Fixed samples were initially removed from tetraborate-buffered formaldehyde and placed into polyvinyl lactophenol (PVL) medium (Gray and Wess 1950) with a few drops of 1% Chlorazol Black E stain for 24–48 h before examination, following the methods described by Rothlisberg et al. (1983).

Samples were then removed from PVL stain medium and placed into fresh PVL medium on a single concave slide for examination under a Leica Dialux 22 compound microscope. Transverse imagery was taken with a top-mounted Tucsen 9 MP camera and downloaded with TSVIEW 7 software. Images were then traced by hand and scanned for digital labelling and placement in figures. For each figure, Adobe Illustrator CS6 was used to refine the images so that the morphological details could be clearly presented. For each sub-stage, a minimum of 15 individuals were examined to describe morphological features, with 10 individuals measured for mean \pm standard deviation (SD) total length and carapace length. Measurements were calculated using the ruler tool in Adobe Photoshop CS6, with calibration determined by images of a micrometer. Total length measures were taken from the

most anterior point of the carapace, excluding appendages, to the most posterior tip of the tail, excluding spines; carapace length was taken from the same anterior point to the posterior end of the carapace.

Identification of taxonomic characters

Stages of larval development and taxonomic characters of individual appendages were identified using descriptions by Leong et al. (1992) and by Dall et al. (1990). The approximate appearance of larval stages Nauplius (Figure 1a–b), Protozoa (Figure 1c–d) and Mysis and post larva (Figure 1e–f) and the location of individual appendages with labels are shown below (Figure 1). Seta arrangement across appendages was generally given from the distal to the proximal end (outermost to innermost), with the most distal point considered as the terminal end. One exception was for the first antenna from the Protozoa stage onwards, where it was defined from the proximal to the distal end. This was due to the change in the morphology of the coxa and basis, affecting the continuity of the descriptions. Where setae were arranged across multiple segments or lobes, arrangement was given as the number of setae per segment. For example, arrangement for a four-lobed appendage was given as a+b+c+d setae from distal to proximal. Descriptions of mandibles involved incisor and molar processes with the number of teeth observed. The positions of the incisor and molar processes, along with the positions of teeth, are also shown, in Figure 1g. Molars were identified as broad oval-shaped grinding plates covering half of the mandible process, incisors as large claw-like peaks in the process at the extremity to the molar and teeth as smaller bristle-like processes between the incisor and molar process.

Results

Duration of larval development

The larval development of *M. dalli* comprised six Naupliar, three Protozoa and three Mysis stages before metamorphosing to the post-larval stage. The development lasted ~12 days in total under the

conditions used in this study, with various time frames for each sub-stage and their total and carapace lengths given in Table 1.

Description of larval development

Nauplius

Morphological development during this stage can be subdivided into six Naupliar sub-stages (Figures 2–7). Both body and appendage forms are relatively consistent, with an eyespot present prior to the development of stalked eyes in the Protozoa II sub-stage. Identifiable changes are apparent in abdominal shape, mandible protrusion and the number and type of setae. Posterior-end morphology is distinct, with a median notch from Nauplius V onwards separating two groups of spines exhibiting bilateral symmetry. Body length also increases through development. The first antenna consists of a uniramous appendage; second antennae and mandible are both biramous. Each appendage is lightly segmented, often difficult to observe until the Protozoa stage.

Nauplius I (N I). N I (Figure 2a) trunk semi-ovoid, anterior half enlarged, posterior end rounded with two spines; all setae are simple.

First antenna (Figure 2b) five segmented, with four terminal setae and seta on third, fourth and fifth segments.

Second antenna (Figure 2c) biramous, coxa two-segmented, basis unsegmented with seta; endopod three-segmented with two terminal setae, seta on second and third segments; exopod four segmented with three terminal setae, two setae at join to second segment and seta at join to third segment.

Mandibles (Figure 2d) biramous, coxa and basis unsegmented; both endopod and exopod two segmented, each has two terminal setae and seta laterally on first segment.

Nauplius II (N II). N II (Figure 3a) trunk similar to N I, with posterior spines outwardly protruding.

First antenna (Figure 3b) has four terminal setae, two setae on third segment, seta on fourth and fifth segment. Longest terminal seta is plumose; all others are simple.

Second antenna (Figure 3c): coxa and basis as per N I; endopod has two terminal plumose setae, simple seta on second and third segment; exopod has three terminal plumose setae and simple terminal seta, plumose seta at joins of second and third segments.

Mandibles (Figure 3d): setal position unchanged from N I, setae are plumose.

Nauplius III (N III). N III (Figure 4a) Posterior has four spines, outermost exhibiting a claw shape. Inner spines are one-third longer than N II.

First antenna (Figure 4b): four terminal setae, longest two terminal setae are plumose, three simple setae on third segment, simple seta on fourth segment.

Second antenna (Figure 4c): coxa, basis and endopod as per N II; exopod has three terminal plumose setae, one is bifurcated, a simple terminal seta, two plumose setae on second segment and a plumose seta on third segment.

Mandible (Figure 4d): mandibular protrusion forms on basis; endopod and exopod as per N II.

Nauplius IV (N IV). N IV (Figure 5a) trunk is elongated, with two groups of four spines posterior. The two longest spines are twice as long as those in N III.

First antenna (Figure 5b) six segmented, four terminal setae, longest two terminal setae plumose, simple seta at join of third and fourth segment and simple seta on fourth, fifth and sixth segments.

Second antenna (Figure 5c): endopod four segmented, three terminal plumose setae, simple seta at join of second, third and fourth segments; exopod five segmented, three terminal plumose setae, one is bifurcated, simple terminal seta, plumose seta on second segment, two plumose setae on third segment.

Mandible (Figure 5d): mandibular protrusion now turned inward.

Nauplius V (N V). N V (Figure 6a) posterior trunk elongated with median notch forming in the posterior end, separating two groups of spines. Each group has two plumose spines and four that are

simple. Abdominal section (i.e. below mandible) now exhibits precursory appendages of later stages of development.

First antenna (Figure 6b) has three terminal plumose setae, one simple terminal seta, simple seta at joins of second, fourth, fifth and sixth segments.

Second antenna (Figure 6c): endopod has three terminal plumose setae, one simple terminal seta, simple seta on second and fourth segments; exopod has four terminal plumose setae, one is bifurcated, plumose seta at joins of second third and fourth segments.

Mandible (Figure 6d) unchanged from N IV.

Nauplius VI (N VI). N VI (Figure 7a) trunk as per N V, but with more precursory appendages in the abdominal section. Posterior end now exhibits three terminal plumose spines and three simple spines on each side of the median notch.

First antenna (Figure 7b) has four terminal plumose setae, plumose seta at joins of second, fourth, fifth and sixth segments.

Second antenna (Figure 7c): endopod has three terminal plumose setae, simple seta at joins of third, fourth and basis segments; exopod has two terminal plumose setae, plumose seta at join with second segment, two plumose setae at join to third segment, plumose seta on third and fourth segments, one simple seta on fifth segment. Bifurcation is now lost on terminal seta.

Mandible (Figure 7d) unchanged from N IV.

Protozoa

The Protozoa stage is defined by three distinct sub-stages common to penaeid prawns (Figures 8–10). External morphological changes from the Nauplius are present in the form of a carapace and abdominal somites, with maxillae, maxillipeds and mandibles protected ventrally by the carapace. The Nauplius eyespot is replaced by a pair of eyes, sessile in Protozoa I, with eyestalks forming in Protozoa II. Pereiopods are formed at Protozoa III. The telson and number of abdominal somites changes between sub-stages. The first and second maxillae and mandibles are partially covered by the

carapace and thus are not immediately recognisable in observation. Formation of the mandibular process is important for the switch from endogenous feeding of the Nauplius stages to exogenous feeding of marine microalgae from Protozoa I. Formation of a rostral spine occurs from Protozoa II, and dorso-median spines on the abdominal somites in Protozoa III.

Protozoa I (PZ I). In PZ I (Figure 8a), rostral spine and eyestalks are yet to form. Seven abdominal somites exist anterior to a telson that appears similar to that of the posterior section of N VI.

First antenna (Figure 8b) is uniramous with coxa, basis and endopod; antenna has three articulations proximal to the coxa, with single seta at the most distal articulation; two setae, one mid-section on the coxa and another at the join to the basis; another one on the basis; endopod has five setae, two of which are terminal; all setae are simple.

Second antenna (Figure 8c) is biramous with protopod, endopod and exopod. Endopod has a single fused segment, with five terminal setae and six setae arranged three in the middle and three near the join to protopod; exopod has 10 segments, with four terminal setae, inner seta at each join to the sixth segment and two outer seta at the join to the fourth and seventh segments; setae on the endopod and exopod are plumose.

Mandible (Figure 8d) consists of left and right ventral process, located between the second antenna and the first maxilla. Each process has both incisor and molar processes, with left mandible possessing one tooth and right mandible two teeth.

First maxilla (Figure 8e) has protopod with coxal and basial endites. Coxal endite with seven setae, five are plumodenticulate (leaf-like), flanked by two plumose setae; basial endite with seven cuspidate (pointed) setae, continuing through until Mysis III sub-stage; endopod has three segments with five terminal setae, and five others in two pairs and a single arrangement from distal to proximal end (henceforth denoted as 2+2+1); scaphognathite is small and knob-like with four setae; exopod and endopod have plumose setae.

Second maxilla (Figure 8f) has coxial and basial endites bilobed; coxial endite with 6+2 plumose setae, basial endite with 2+2 plumose setae; endopod has five segments with three terminal +

2+2+2+1 setae. Two terminal setae are simple, the rest are plumose; scaphognathite small and rounded with five plumose setae.

First maxilliped (Figure 8g) has protopod, endopod and exopod; protopod has coxa with five setae and basis with three setae; endopod has five segments with five terminal + 2+2+3+3 setae; exopod has four terminal setae, with three setae positioned along the outer margin. All setae are plumose.

Second maxilliped (Figure 8h) has protopod, endopod and exopod; protopod has coxa with three setae and basis two setae; endopod has four segments, with five terminal + 2+2+2 setae; exopod has three terminal setae and three positioned along the outer margin. All setae are plumose.

Third maxilliped (Figure 8i) has two segments with two terminal plumose setae.

Telson (Figure 8a) has a deep notch with seven spines each side, all of which are plumose.

Protozoa II (PZ II). In PZ II (Figure 9a), rostrum and eyestalks take form; two supraorbital spines are observed; three abdominal somites are added, with one covered by the carapace which begins to extend posteriorly. Third maxilliped now has endopod and exopod (Figure 9e).

First antenna (Figure 9b) now has four articulations prior to formation of the coxa; a single seta on coxa at join to the most distal articulation; single seta mid-section on the coxa, three at the join to the basis; basis has two setae at join to endopod; endopod has five terminal setae; all setae are simple.

Second antenna (Figure 9c): endopod now two segmented, with five terminal + 3+2+1 setae; exopod now 12 segmented, with four terminal setae, inner seta at each join to the seventh segment, outer seta at the fourth and seventh segment; setae on both endopod and exopod plumose.

Mandible (Figure 9d): left mandible possesses one tooth and right mandible five teeth.

First maxilla (Figure 9e) is unchanged from PZ I.

Second maxilla (Figure 9f): coxial endite is bilobed with 3+7 plumose setae; basal endite trilobed with 3+3+5 plumose setae; endopod is unchanged; scaphognathite slightly increased in size.

First maxilliped (Figure 9g): coxa eight setae and basis three setae, respectively; endopod and exopod unchanged. All setae are plumose.

Second maxilliped (Figure 9h) coxa two setae and basis three setae respectively; endopod and exopod unchanged. All setae are plumose.

Third maxilliped (Figure 9i) is biramous; both endopod and exopod have three terminal plumose setae each.

Telson (Figure 9a) longest spine is now one-third longer in comparison to PZ I.

Protozoa III (PZ III). In PZ III (Figure 10a), rostrum and eyestalks continue to develop; presence of 10 abdominal somites ending in a tail fan with a telson and precursory uropods; posterior five abdominal somites have dorso-median spines and the anterior two somites are now covered by the carapace.

First antenna (Figure 10b) coxa has lost its articulations, with two setae at the proximal end of the coxa and one seta halfway to basis; basis has five setae at the proximal join and two at the join with the endopod; endopod with five terminal setae. All setae are simple.

Second antenna (Figure 10c): endopod is unchanged from PZ II; exopod has five terminal setae, with outer seta at joins of the second, third, fourth, fifth, seventh and ninth segments, and inner seta at the joins of the fourth and seventh segments; a small tooth-like structure also appears at the proximal end of the exopod.

Mandible (Figure 10d): left mandible two teeth and right mandible six teeth.

First maxilla (Figure 10e) is unchanged from Protozoa II.

Second maxilla (Figure 10f): coxial endite is bilobed with 3+8 plumose setae; basal endite trilobed with 3+5+5 plumose setae; endopod is unchanged; scaphognathite is enlarged into a mushroom shape.

First maxilliped (Figure 10g) has coxa with eight setae and basis with six setae; endopod has four segments and five terminal + 2+2+3+3 setae; exopod has four terminal setae, four positioned along the outer and one on the inner margin. All setae are plumose.

Second maxilliped (Figure 10h) has coxa with two setae and basis with two outer setae and one inner seta; endopod now has five terminal + 2+2+2 setae; exopod has three terminal setae and three setae along the outer margin. All setae are plumose.

Third maxilliped (Figure 10i): endopod has three terminal setae and one seta at the join to the second segment; exopod is two segmented with three terminal setae. All setae are plumose.

Pereiopods (Figure 10j) begin to form as five pairs, with each pair positioned ventrally on each of the five anterior thoracic somites; each is rudimentary, biramous and has no setae.

Telson (Figure 10a) is now flanked by two pairs of uropods from PZ II; outer pair is major, biramous and located at the dorsal end of the tail section, with six small terminal spines each; inner pair is minor and bare.

Mysis and post larvae

There are three sub-stages of Mysis development (Figures 11–13) prior to the first post-larval stage (Figure 14). Morphology changes drastically from the Protozoa form, beginning to demonstrate precursory features of the adult. Both the carapace and thoracic sections lengthen at each sub-stage, as shown in the measures of carapace and total length. From Mysis I, an antennal spine is formed as a precursor to the rostrum. The posterior section of the carapace extends to cover the first two thoracic somites, including newly formed pereiopods; exposed thoracic segments have singular dorso-median spines.

From Mysis II, one pterygostomian spine is formed on either side of the anteroventral points of the carapace. All Mysis sub-stages have two pairs of antennae, with both distal rami of antennae segmented. In addition, there are one pair of mandibles, two pairs of maxillae, three pairs of maxillipeds, five pairs of pereiopods and a tail fan with a telson and two pairs of uropods. Of those

pereiopods, the first three pairs are morphologically similar, with the third pereiopod being the largest. The final two pereiopods are also morphologically similar to each other; thus, only the third and fifth pereiopods are described in this section. Five pairs of pleopods appear in the third Mysis stage without setae, which develop moderate setation during the first post-larval form. A dorso-ventral spine protrudes from the protopod and two more spines over each pair of uropods.

Behaviourally, these forms begin to become increasingly nektonic, eventually becoming capable of capturing prey items such as zooplankton, whilst changing to an omnivorous diet. During these Mysis stages they switch from being planktonic and phototactic to increasingly benthic dwellers.

Mysis I (M I). In M I (Figure 11a), antennal spine is formed, but without teeth.

First antenna (Figure 11b) uniramous with major and minor rami at terminal end; coxa has three plumose setae and the early formation of an antennal spine on the inner marginal line; basis has three setae at coxa join and one halfway to endopod; endopod with three setae at join to basis, one halfway to rami and three at join to rami; minor ramus has two terminal plumose setae, major ramus has seven terminal simple setae.

Second antenna (Figure 11c) has changed markedly from PZ III morphology (Figure 10b), with a reduction in segmentation and number and composition of setae. Appendage is biramous with protopod and endopod. Exopod flattens from Mysis I to become an antennal scale; therefore, endopod and exopod results are reversed in text. Exopod has 11 plumose setae positioned marginally; small endopod has three terminal setae and three simple setae positioned laterally.

Mandible (Figure 11d): left mandible three teeth and right mandible seven teeth.

First maxilla (Figure 11e) is unchanged from PZ III (Fig. 17e) apart from the basal endite, which now has 10 cuspidate setae, of which one is opposed.

Second maxilla (Figure 11f): coxial endite is bilobed with 4+8 plumose setae; basal endite trilobed with 2+5+5 plumose setae; endopod four segmented, with three terminal + 2+2+2 setae, two terminal setae are simple; scaphognathite begins to enlarge with eight plumose setae.

First maxilliped (Figure 11g) coxa has five plumose setae and basis seven plumose setae; endopod four-segmented with five terminal + 2+2+3+3 setae, four terminal setae are simple; exopod has four terminal plumose setae and three plumose setae along the outer margin, all are plumose.

Second maxilliped (Figure 11h) coxa six plumose setae and basis three plumose setae; endopod has five terminal setae + 2+3+4+4 setae, four terminal setae are simple, arranged as 2+2+3+3 outer setae and 1+1+1 inner setae. This differs from the PZ III unilateral arrangement; exopod has four terminal plumose setae and three setae along the outer margin.

Third maxilliped (Figure 11i) biramous; single simple seta on basis. This appendage changed significantly from PZ III, with additional segmentation and overall size; endopod is five segmented and has five terminal + 3+1+2+2+2 setae, arranged as 2+0+1+2+2 outer and 1+1+1 inner setae. Plumose seta at the joins of the fourth, fifth and basal segments; exopod two segmented, with six terminal plumose setae. Plumose seta on the outer and inner margin.

Third pereopod (Figure 11j) is biramous and single segmented, five terminal plumose setae on endopod and three terminal plumose setae on exopod.

Fifth pereopod (Figure 11k) is biramous and single segmented, four terminal plumose setae on the endopod and three terminal plumose setae on exopod.

Telson (Figure 11 l) now flanked by two sets of uropods in their adult position dorso-laterally to the telson; outer uropod is two-segmented, with a posterodorsal spine at the join between the two segments on each uropod. Each outer uropod has 11 marginal furcal spines and one posterolateral spine in the outermost position; inner uropod has eight marginal furcal spines; telson with deepening notch in centre and 6+6 furcal spines, with 2+2 spines positioned laterally approximately two-thirds posterior.

***Mysis II* (Figure 11b).** In M II, body is more elongated than at M I and pleopods beginning to form, but without structure seen in later Mysis sub-stages.

First antenna (Figure 12b): coxa has four marginal plumose setae; basis has six plumose setae at coxa join and two halfway to endopod; endopod with five plumose setae at join to basis and five at join to rami; major and minor rami are unchanged from M I.

Second antenna (Figure 12c): protopod develops an antennal spine at the join to the endopod; Exopod has 15 plumose setae positioned marginally; small endopod has three simple setae along the outer margin.

Mandible (Figure 12d) left mandible two teeth and right mandible seven teeth (obscured from view).

First maxilla (Figure 12e): scaphognathite has now lost all setae from M I. Protopod and endopod are unchanged.

Second maxilla (Figure 12f): scaphognathite enlarged with 12 plumose setae.

First maxilliped (Figure 12g) coxa four plumose setae and basis six plumose setae; endopod has five terminal setae + 2+2+4+4 setae, with four terminal setae simple. Setae are arranged as 2+2+3+3 outer and 1+1 inner; exopod has six terminal plumose setae and one plumose seta along the outer margin.

Second maxilliped (Figure 12h): coxa has four plumose setae and basis has two; endopod now has five segments with five terminal setae + 3+0+3+4+3 setae arranged 2+0+2+3+2 outer and 1+1+1 inner setae. Only the distal inner seta and four terminal setae are simple; exopod has four terminal plumose setae and one plumose seta on the outer margin.

Third maxilliped (Figure 12i): one simple seta on basis; endopod has five terminal + 2+1+1+2+2 outer setae and 2+2+1 inner setae, with two outer and two inner setae simple; exopod has six terminal plumose setae and two setae positioned laterally.

Third pereopod (Figure 12j): endopod has four terminal plumose setae and three plumose setae on the outer margin; exopod is now two segmented with three terminal plumose setae. Rudimentary chelae begin to form at the distal end of the endopod.

Fifth pereopod (Figure 12k): endopod has four terminal plumose setae; exopod has three terminal plumose setae; endopod is now two segmented.

Telson (Figure 12l) unchanged from M I; outer uropods have 12 marginal furcal spines and one posterolateral spine as per M I; inner uropods with nine marginal furcal spines.

Mysis III (M III). In M III (Figure 13a), body continues to elongate as per mean measures. Pleopods begin to form as five pairs on the five posterior abdominal somites. Rostrum now has an epigastric tooth and a first rostral tooth.

First antenna (Figure 13b): coxa has five plumose setae; basis has seven plumose setae at coxa join and two halfway to endopod; endopod with six plumose setae at join to basis and six at join to rami; minor ramus has three terminal plumose setae, major ramus has seven terminal simple setae.

Second antenna (Figure 13c): exopod has 16 plumose setae positioned marginally; endopod is now two segmented with no setae.

Mandible (Figure 13d) has a left mandible possessing two teeth, and right mandible six teeth.

First maxilla (Figure 13e): coxial endite setae become simple setae.

Second maxilla (Figure 13f) largely unchanged from M I, except for an enlarged scaphognathite with 13 plumose setae.

First maxilliped (Figure 13g): protopod and endopod are unchanged from M II; exopod has five terminal plumose setae and one plumose seta on the outer margin.

Second maxilliped (Figure 13h): coxa has two plumose setae and basis has four; endopod now has five terminal + 3+0+3+4+3 setae arranged as +2+0+2+3+2 outer setae and 1+0+1+1+1 inner setae, four of the terminal setae are simple; exopod has four terminal plumose setae and one plumose seta on the outer margin.

Third maxilliped (Figure 13i): basis has one seta at the endopod join; endopod has 18 setae arranged as five terminal + 2+1+1+2+2 outer setae and 2+2+1 inner setae; exopod is two segmented with six terminal plumose setae.

Third pereopod (Figure 13j): endopod is two segmented, with six terminal plumose setae; Exopod is four segmented with three terminal plumose setae, two outer plumose setae at the second segment join and one inner (obscured from view) at the join to the fourth segment.

Fifth pereopod (Figure 13k) protopod has two simple setae; endopod has five segments with five plumose setae arranged one terminal + 2+1+1; exopod has five terminal plumose setae.

Pleopods (Figure 13l) are two segmented without setae.

Telson (Figure 13m) unchanged from M I; outer uropods have 14 marginal furcal spines and one posterolateral spine; inner uropods with 13 marginal furcal spines.

Post larvae I (PL I). After the Nauplius, Protozoa and Mysis larval stages, the morphology of the post-larval stage begins to resemble the adult form (Figure 14a).

First antenna (Figure 14b): coxa has 14 plumose setae, with antennal spine forming a more defined point; basis has eight plumose setae at coxa join and five halfway to endopod; endopod with seven plumose setae at join to basis and six at join to rami; minor ramus has three terminal plumose setae, major ramus is now two segmented and has four terminal simple setae and four setae positioned laterally, three of which are plumose.

Second antenna (Figure 14c) protopod has two simple setae, one located mid-section and another at the join to the antennal scale; antennal scale has 20 plumose setae positioned marginally, with five additional simple setae positioned adjacent to the marginal line; endopod has five segments with four terminal + 2+4+1+3 short and fine simple setae.

Mandible (Figure 14d): left mandible three teeth and right mandible nine teeth.

First maxilla (Figure 14e): coxial and basial endites are extended; setae as per M III.

Second maxilla (Figure 14f) coxial endite has 4+9 plumose setae; basial endite has 2+5+5 plumose setae; endopod unchanged from M I; scaphognathite enlarged with 23 plumose setae.

First maxilliped (Figure 14g): protopod and endopod are same as M III except for two setae of the inner endopod being lost; exopod is unchanged.

Second maxilliped (Figure 14h) basis now has three setae; endopod has five terminal setae +3+3+4+4 setae, arranged as 2+0+2+3+3 outer setae and 1+0+1+1+1 inner setae, four of the terminal setae are simple; exopod has four terminal plumose setae and one seta on the outer margin. All setae plumose.

Third maxilliped (Figure 14i): endopod is now six segmented and has five terminal + 4+3+2+3+4+2 plumose setae, arranged as 2+1+1+2+2+2 outer setae and 2+2+1+1+2 inner setae; exopod has six terminal plumose setae.

Third pereopod (Figure 14j) has six terminal setae on the endopod; exopod has six setae arranged two terminal + 2+2; all setae are plumose; chelae on the first three pereopods are now functional.

Fifth pereopod (Figure 14k): coxa and basis have simple seta; endopod and exopod are unchanged.

Pleopods (Figure 14l) are elongated from M III with six short terminal simple setae.

Telson (Figure 14m) unchanged from M I; outer uropods have 15 marginal furcal spines and one posterolateral spine; inner uropods with 14 marginal furcal spines; median notch is faintly cleft.

Discussion

The larval development of *M dalli* can be subdivided into six Naupliar, three Protozoa and three Mysis stages before individuals metamorphose into the post-larval form. This ontogenetic development occurs in ~12 days at 26°C, with both the growth rate and morphological patterns of development in *M. dalli* described here showing, at a broad level, similarity among congeners. However, the number, location and composition of individual setae and other minor spinal developments vary among species at corresponding ontogenetic stages. Differentiation between metapenaeid species was found to be effective when comparing the number and combinations of the first and second antennae setal arrangement (Table 2). Differentiation of each species was possible at

each sub-stage via this method, with subtle differences in the number of setae through the Nauplii and Protozoa sub-stages, and major differences throughout the Mysis and post-larval sub-stages. An example of this is the comparison of *M. dalli* and *M. moyebi*, where at the N I stage, there were seven setae observed on the first antenna of *M. dalli* and five on that of *M. moyebi*. In addition, there were four setae on the endopod and six setae on the exopod of *M. dalli*, while *M. moyebi* had five setae on each of the endopod and exopod. Unfortunately, comparisons of *M. dalli* with other studies and species proved difficult, however, as many studies showed incomplete work or lacked appropriate detail. Examples of this are given for the first and second antennae on *M. moyebi* (Nandakumar et al., 1989) and *M. monoceros* (Muthu et al., 1979) in Table 2, whereby these works failed to describe these appendages. In addition, comparison of size at each sub-stage between species is prohibitive, due largely to the effects that regional specificity and different culturing techniques can have on growth rates of penaeids (Dall et al. 1990).

Differentiation between these species in the wild for distribution and/or abundance studies would prove even more difficult and time consuming without a methodology based on comprehensive frameworks with well-defined criteria. Few examples exist of such approaches, except for works based on the second Protozoa sub-stage of the penaeid prawns *Penaeus esculentus* Haswell, 1879, *Penaeus latisulcatus* Kishinouye, 1896, *Penaeus merguensis* de Man, 1888, *Penaeus semisulcatus* De Haan, 1844 (Rothlisberg et al. 1983), and *M. ensis* with *M. endeavouri* Protozoa (Jackson and Rothlisberg 1994). These studies of distribution and abundance were successfully achieved with larvae from studies in the Gulf of Carpentaria. This involved the use of between 14 and 17 individual discrimination factors (see Rothlisberg et al. 1983; Jackson and Rothlisberg 1994), focusing on length and width of individual segments of the first and second antennae, and total and carapace length, using comprehensive larval reference collections within clearly defined research zones. This has yet to be applied to other species or regions, however, largely due to limited availability of detailed larval descriptions and endemic reference material, and due to the high degree of overlap among species in both morphometric and meristic characters. Genetic methods of identification may be successful, but markers for *M. dalli* have yet to be determined. Application of morphological techniques for

distinguishing *M. dalli* may be possible in areas such as Western Australia, where only three species of metapenaeids occur. However, due to complex overlapping distributions of many congeneric species, regions such as Indonesia and the Northern Territory would require more comprehensive reference material to distinguish *M. dalli* from other species.

Overall, this study will support further works detailing endemic population patterns of penaeid prawns along the Australian coastline and in Indonesia, as well as provide supporting material for the development of commercial aquaculture of this and related species in the future.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure 1. Morphological characters of penaeid larval stages adapted from Dall et al. (1990); (a) Nauplius I dorsal view; (b) Nauplius I lateral view; (c) Protozoaea II dorsal view; (d) Protozoaea II anterior section ventral view; (e) Mysis and post larva I lateral view; (f) Mysis and post larval tail fan. (g) Right mandible process of Protozoaea, Mysis and post-larva sub-stages. Abbreviated labels include: End, endopod; Ex, exopod; 1st Ant, first antenna; 2nd Ant, second antenna; Mn, mandible; Rst sp, rostral spine; Ab somite, abdominal somite; 1st Mx, first maxilla; 2nd Mx, second maxilla; 1st Mxp, first maxilliped; 2nd Mxp, second maxilliped; 3rd Mxp, third maxilliped; 1st per, first pereopod; 3rd per, third pereopod; 5th per, fifth pereopod. © Elsevier, license no. 3686250188613.

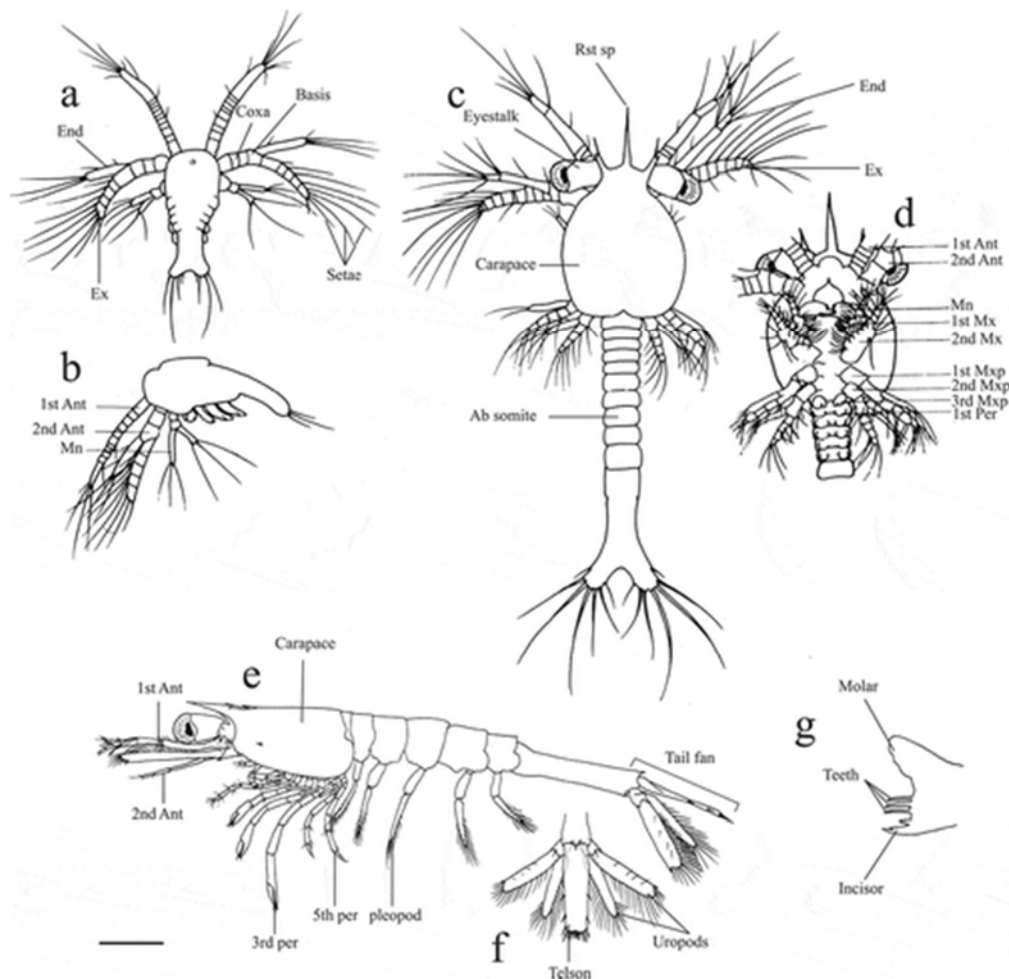


Figure 2. *Metapenaeus dalli* Nauplius I (a) ventral view; (b) first antenna; (c) second antenna; (d) mandible. Endopod (End.) and exopod (Ex.) denoted on second antennae and mandible. Scale bar = 0.1 mm.

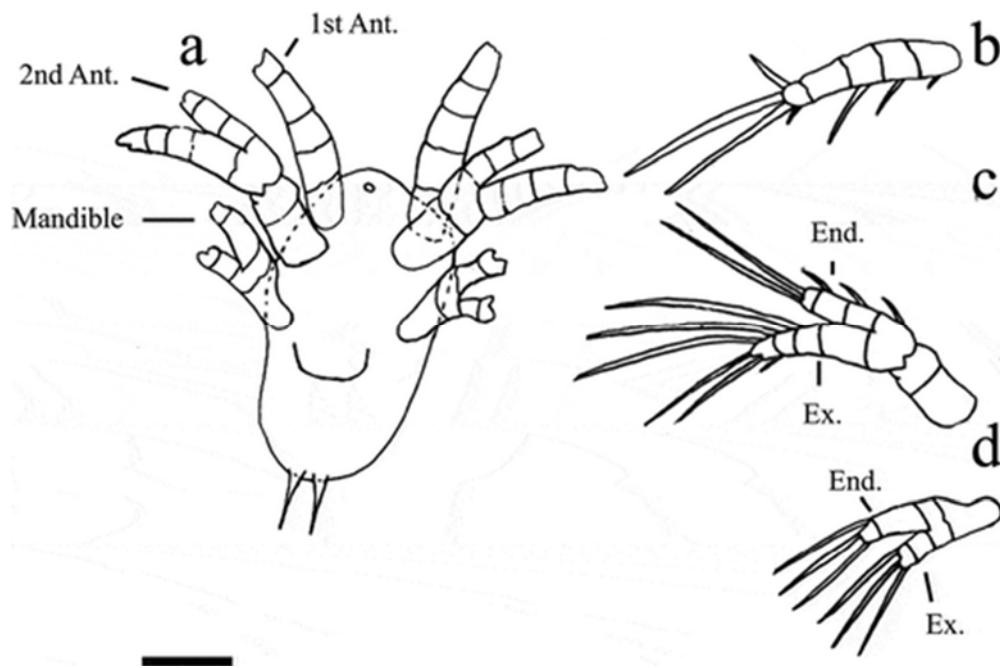


Figure 3. *Metapenaeus dalli* Nauplius II (a) ventral view; (b) first antenna; (c) second antenna; (d) mandible. Scale bar = 0.1 mm.

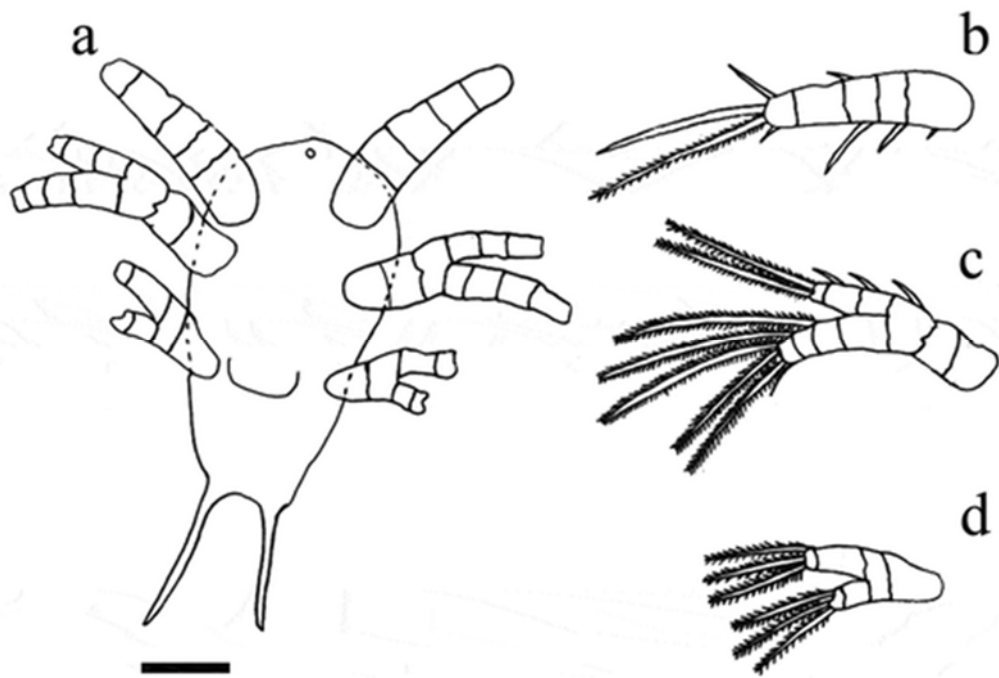


Figure 4. *Metapenaeus dalli* Nauplius III (a) ventral view; (b) first antenna; (c) second antenna; (d) mandible. Scale bar = 0.1 mm.

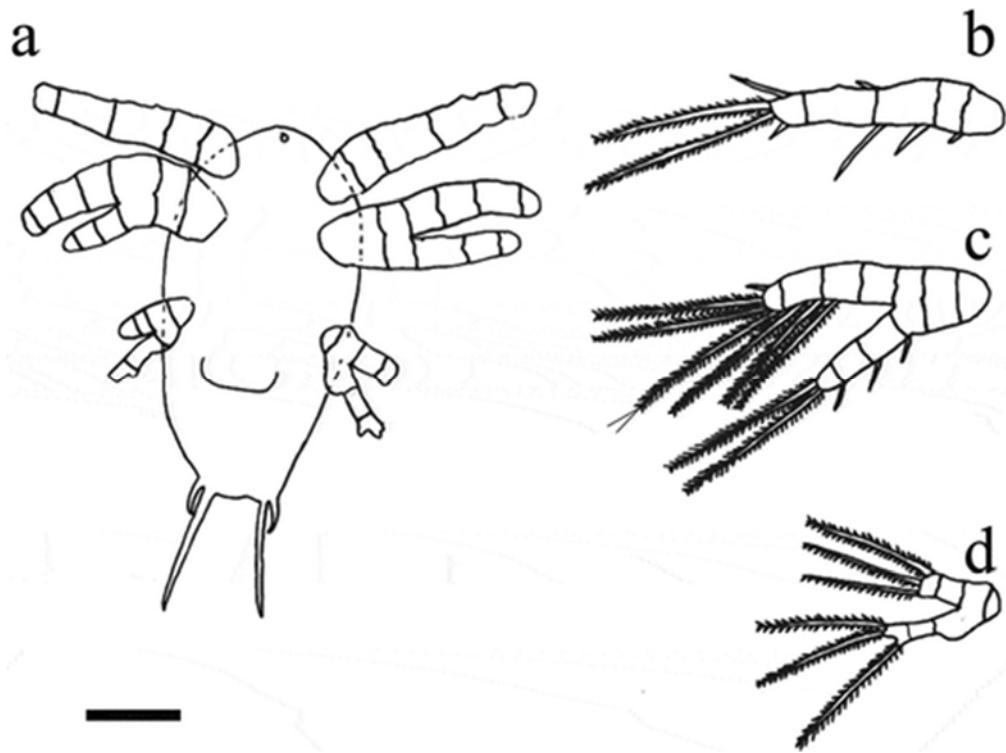


Figure 5. *Metapenaeus dalli* Nauplius IV (a) ventral view; (b) first antenna; (c) second antenna; (d) mandible. Scale bar = 0.1 mm.

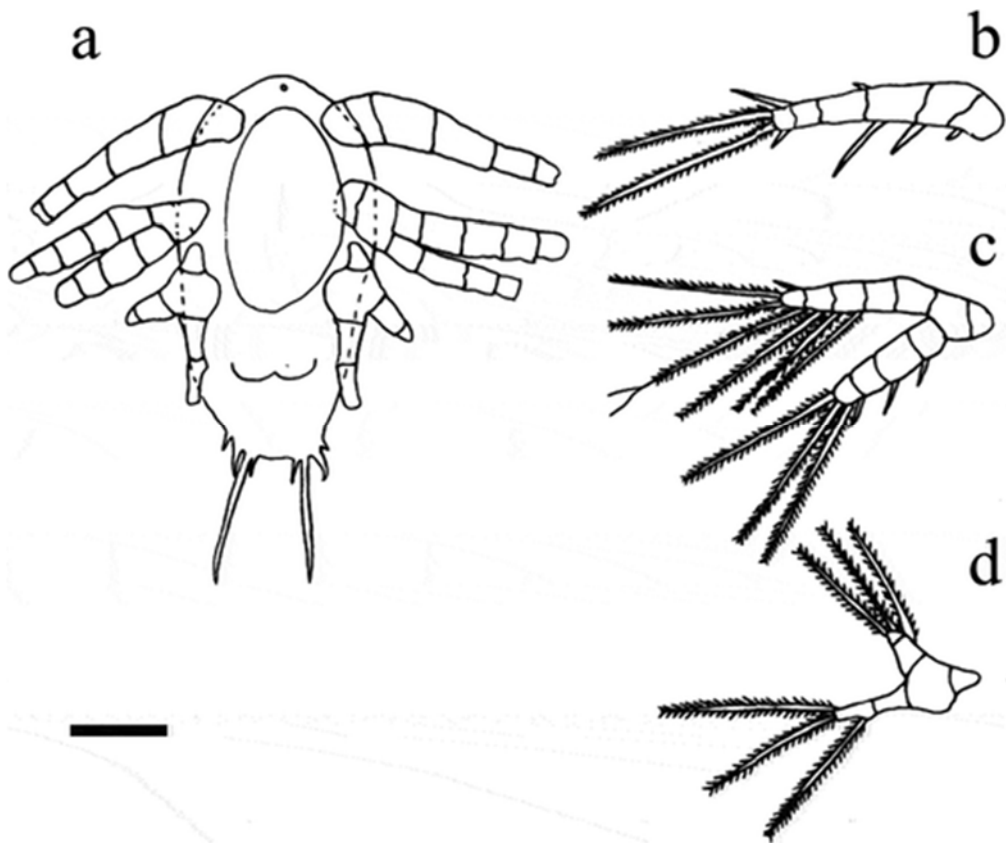


Figure 6. *Metapenaeus dalli* Nauplius V (a) ventral view; (b) first antenna; (c) second antenna; (d) mandible. Scale bar = 0.1 mm.

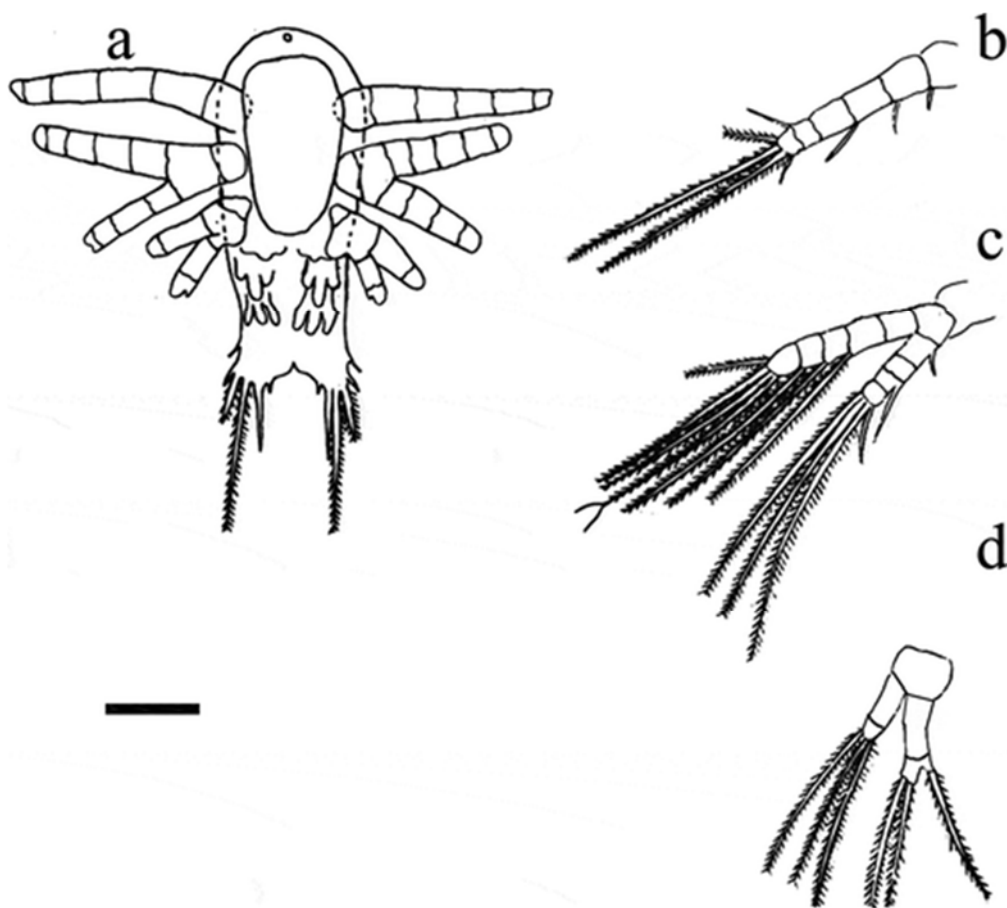


Figure 7. *Metapenaeus dalli* Nauplius VI (a) ventral view; (b) first antenna; (c) second antenna; (d) mandible. Scale bar = 0.1 mm.

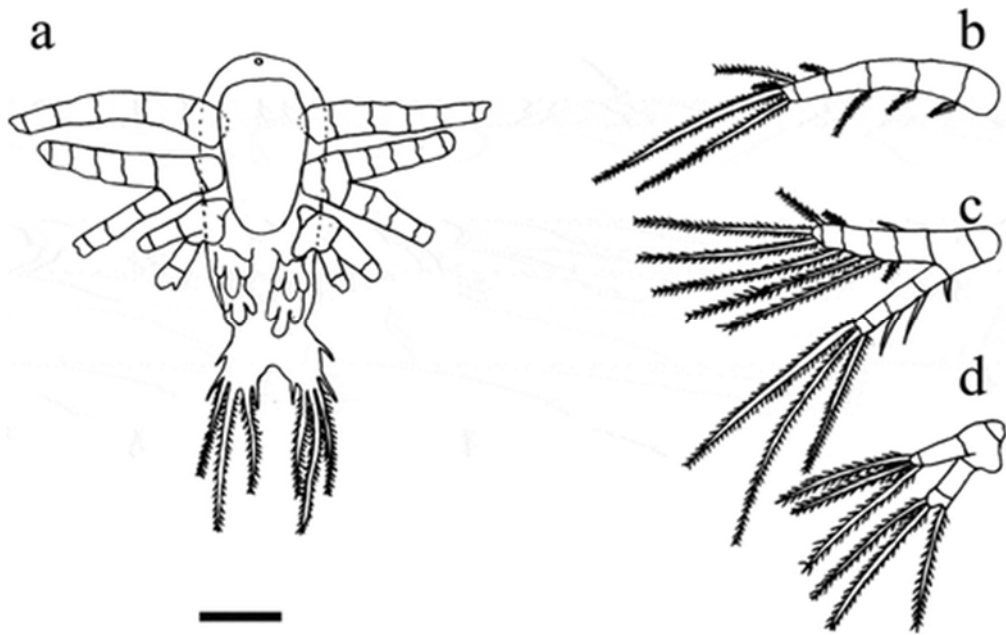


Figure 8. *Metapenaeus dalli* Protozoa I (a) dorsal view; (b) first antenna; (c) second antenna; (d) mandible; (e) first maxilla; (f) second maxilla; (g) first maxilliped; (h) second maxilliped; (i) third maxilliped. Scale bars: a–c, g–i = 0.1 mm; d–f = 0.05 mm.

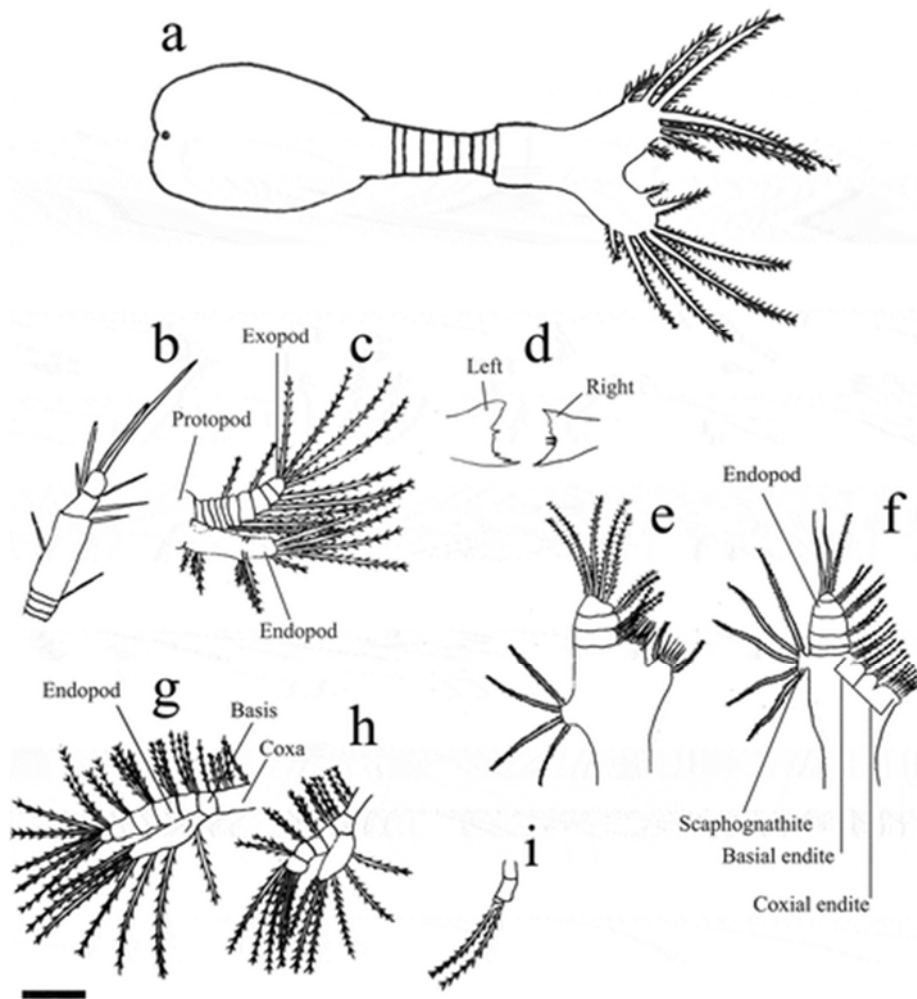


Figure 9. *Metapenaeus dalli* Protozoa II (a) dorsal view; (b) first antenna; (c) second antenna; (d) mandible; (e) first maxilla; (f) second maxilla; (g) first maxilliped; (h) second maxilliped; (i) third maxilliped. Scale bars: a–c, g–i = 0.1 mm; d–f = 0.05 mm.

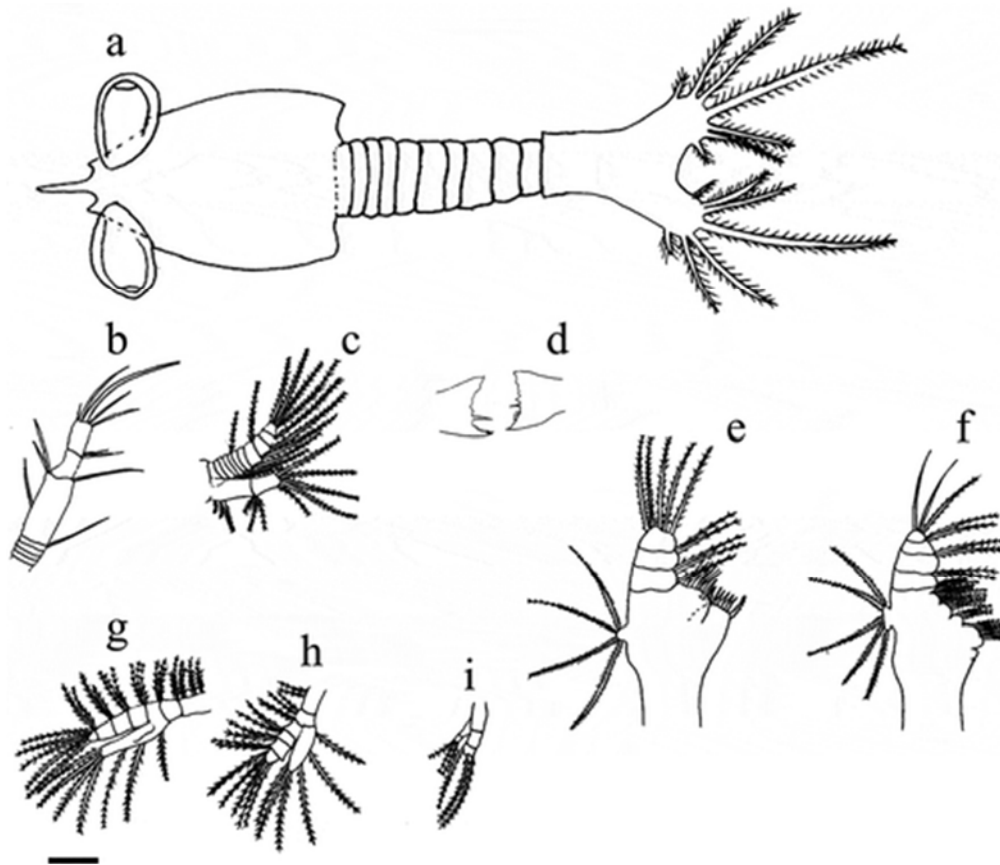


Figure 10. *Metapenaeus dalli* Protozoa III (a) dorsal view; (b) first antenna; (c) second antenna; (d) mandible; (e) first maxilla; (f) second maxilla; (g) first maxilliped; (h) second maxilliped; (i) third maxilliped; (j) periopod. Scale bars: a–c, g–i = 0.1 mm; d–f = 0.05 mm.

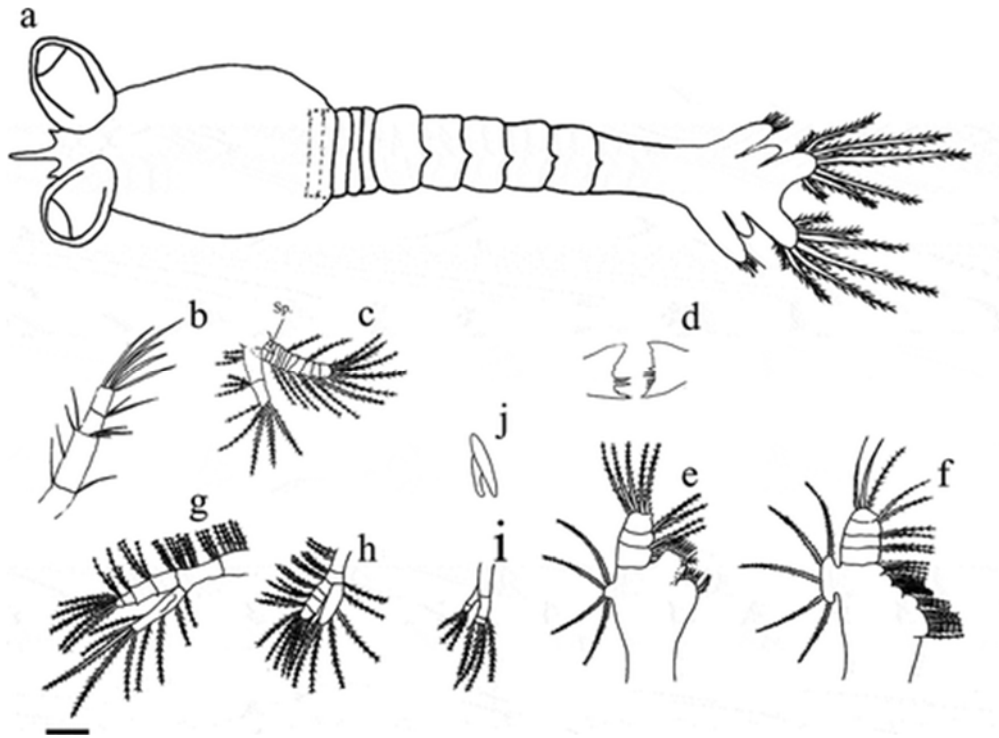


Figure 11. *Metapenaeus dalli* Mysis I (a) lateral view; (b) first antenna; (c) second antenna; (d) mandible; (e) first maxilla; (f) second maxilla; (g) first maxilliped; (h) second maxilliped; (i) third maxilliped; (j) third pereopod; (k) fifth pereopod; (l) telson and uropods. Scale bars: a–c, g–l = 0.1 mm; d–f = 0.05 mm.

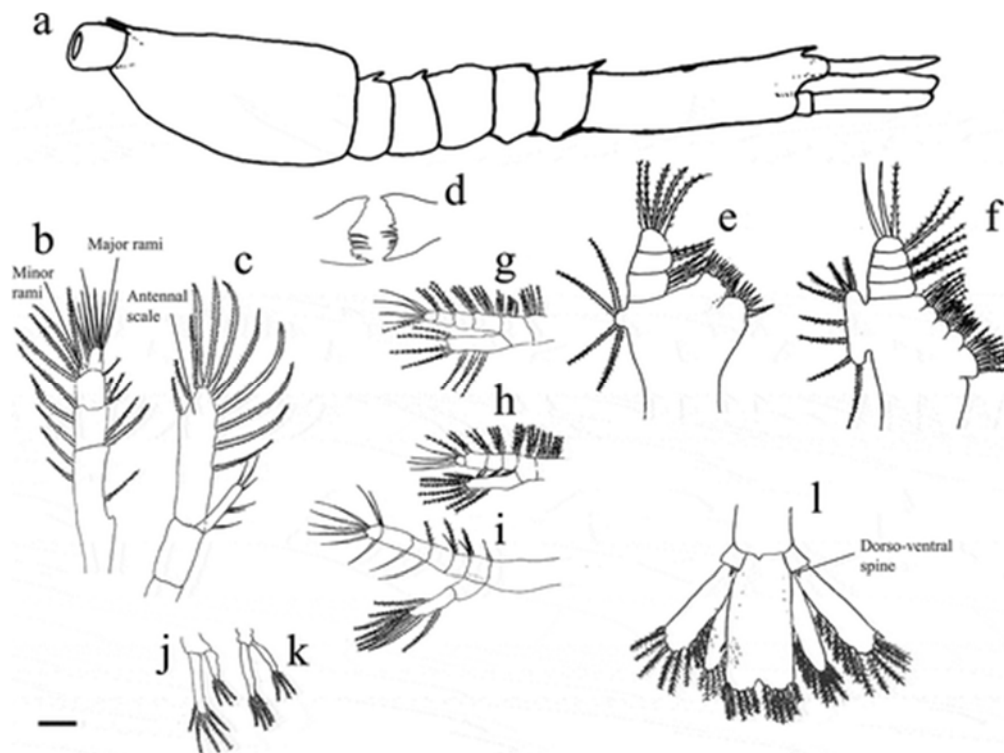


Figure 12. *Metapenaeus dalli* Mysis II (a) lateral view; (b) first antenna; (c) second antenna; (d) mandible; (e) first maxilla; (f) second maxilla; (g) first maxilliped; (h) second maxilliped; (i) third maxilliped; (j) third pereopod; (k) fifth pereopod; (l) telson and uropods. Abbreviated label (Pt. sp.) is pterygostomain spine. Scale bars: a–c, g–l = 0.1 mm; d–f = 0.05 mm.

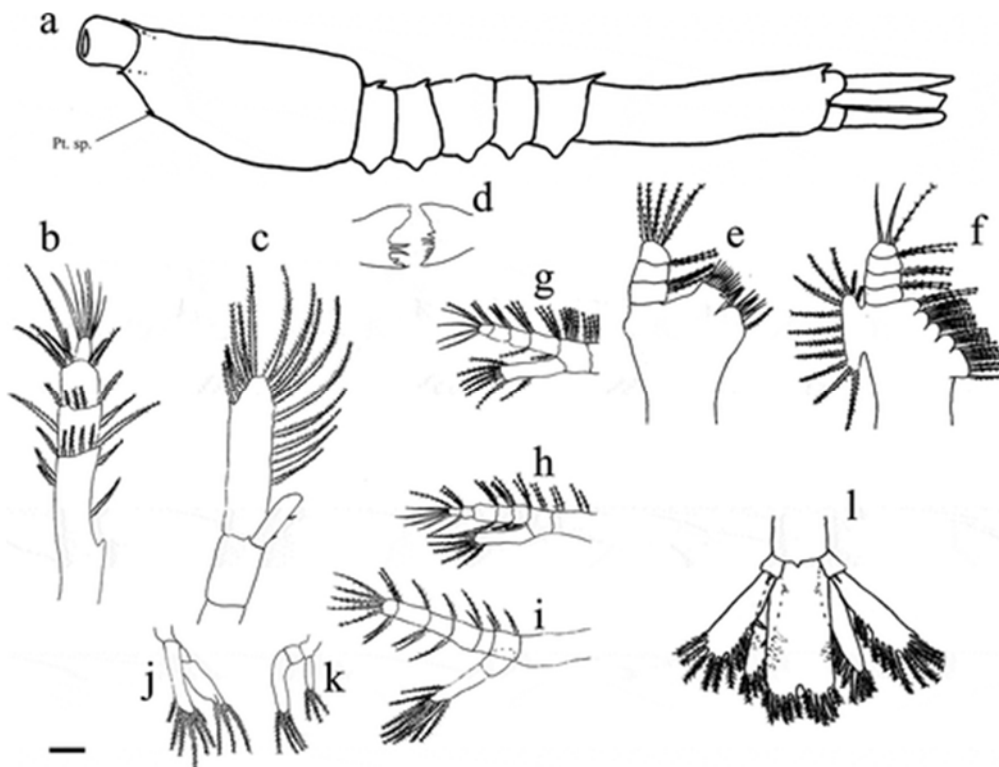


Figure 13. *Metapenaeus dalli* Mysis III (a) lateral view; (b) first antenna; (c) second antenna; (d) mandible; (e) first maxilla; (f) second maxilla; (g) first maxilliped; (h) second maxilliped; (i) third maxilliped; (j) third pereopod; (k) fifth pereopod; (l) pleopods; (m) telson and uropods. Scale bars: a–c, g–m = 0.1 mm; d–f = 0.05 mm.

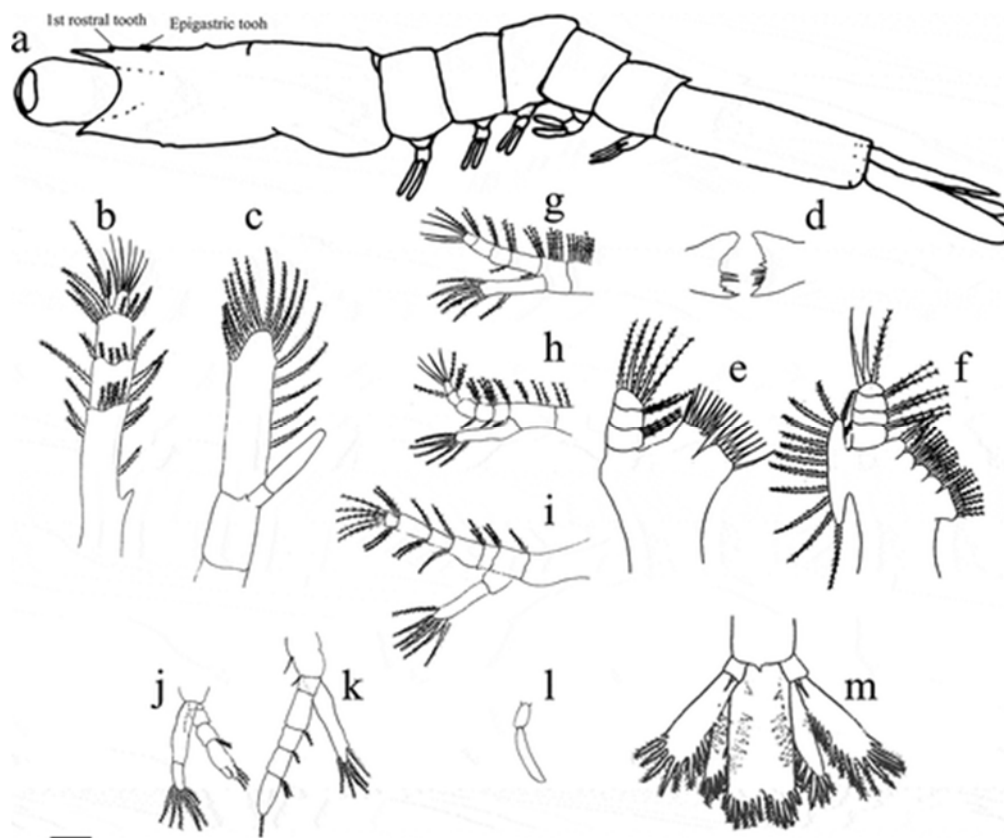


Figure 14. *Metapenaeus dalli* Post larvae I (a) lateral view; (b) first antenna; (c) second antenna; (d) mandible; (e) first maxilla; (f) second maxilla; (g) first maxilliped; (h) second maxilliped; (i) third maxilliped; (j) third pereopod; (k) fifth pereopod; (l) pleopods; (m) telson and uropods. Scale bars: a-c, g-m = 0.1 mm; d-f = 0.05 mm.

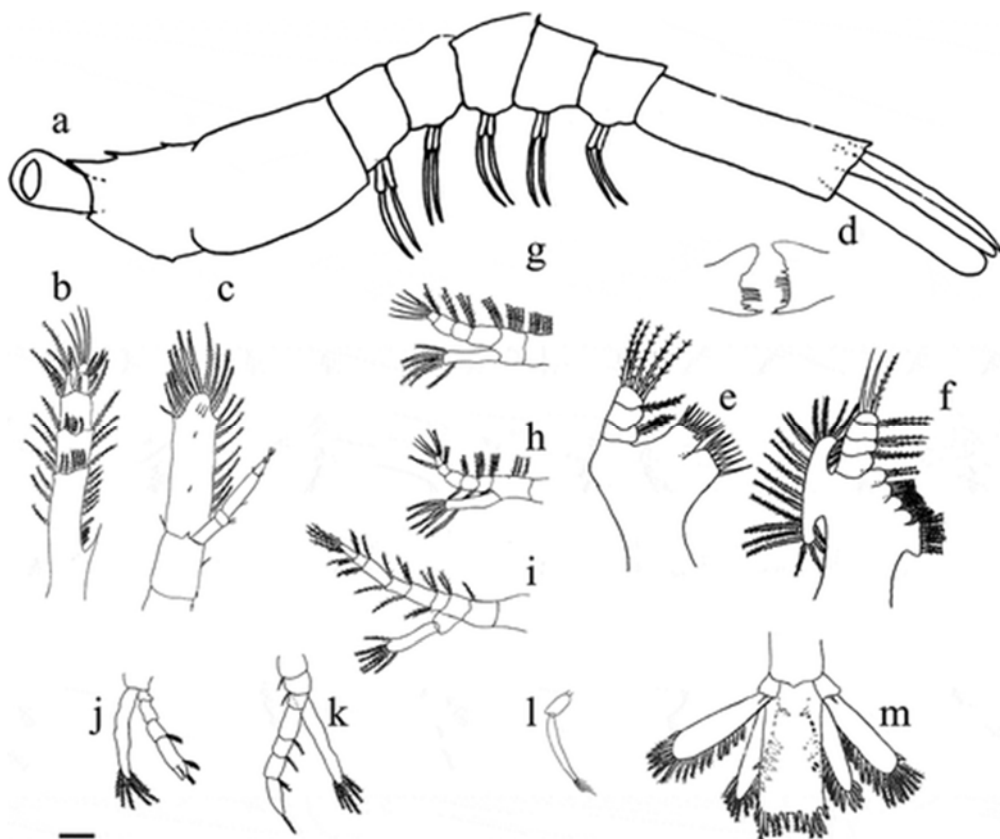


Table 1. Approximate length and duration of larval sub-stages in the development of *Metapenaeus dalli*, from hatching through to Post larvae I under conditions used in this study (see methods).

Nauplius duration is given as time taken to complete all six sub-stages only.

Larval stage	Sub-stage	Approximate duration (days)	Total length (mm ± SD)	Carapace length (mm ± SD)
Nauplius	I		0.300 ± 0.017	
	II		0.302 ± 0.016	
	III		0.310 ± 0.023	
	IV		0.370 ± 0.022	
	V		0.401 ± 0.019	
	VI	2	0.405 ± 0.019	
Protozoea	I	1	0.746 ± 0.034	0.348 ± 0.012
	II	1	1.178 ± 0.091	0.462 ± 0.043
	III	2	1.824 ± 0.069	0.720 ± 0.031
Mysis	I	1	2.422 ± 0.090	0.862 ± 0.027
	II	1	2.557 ± 0.079	0.869 ± 0.028
	III	2	2.81 ± 0.067	0.899 ± 0.027
Post larvae	I	1	2.984 ± 0.060	0.913 ± 0.022

SD: standard deviation

Table 2. Comparison of the number of setae on the first and second antennae of *Metapenaeus dalli* and six other species of metapenaeids reared in other laboratory studies. Biramous second antenna is defined by setae on the endopod and exopod, with exopod representing the antennal scale during Mysis and post-larval stages.

Stage	Character	<i>M. dalli</i>	<i>M. ensis</i>	<i>M. affinis</i>	<i>M. moyebi</i>	<i>M. monoceros</i>	<i>M. dobsoni</i>
		This study	Ronquillo & Saisho (1993)	Hassan (1980)	Nandakumar et al. (1989)	Mohamed et al. (1979)	Muthu et al. (1979)
N I	1 st ant.	7	6	6	5	–	7
	2 nd ant. End/Ex	4/6	4/5	5/5	5/5	5/6	4/5
N II	1 st ant.	8	7	7	6	–	7
	2 nd ant. End/Ex	4/6	5/6	5/6	5/6	5/6	4/6
N III	1 st ant.	8	7	7	6	–	7
	2 nd ant. End/Ex	4/7	5/7	5/7	5/7	5/7	5/7
N IV	1 st ant.	8	8	7	–	–	7
	2 nd ant. End/Ex	6/7	6/8	5/9	–	5/8	5/8
N V	1 st ant.	8	9	7	6	–	6
	2 nd ant. End/Ex	6/7	6/9	6/10	5/8	5/9	5/9
N VI	1 st ant.	8	10	8	7	–	9
	2 nd ant. End/Ex	6/9	6/10	8/12	5/9	7/9	6/9
PZ I	1 st ant.	9	10	11	9	10	8
	2 nd ant. End/Ex	11/11	12/12	10/12	11/12	11/12	11/12
PZ II	1 st ant.	12	11	11	–	10	8
	2 nd ant. End/Ex	11/12	12/12	10/13	11/12	11/12	11/12
PZ III	1 st ant.	15	12	14	–	11	12
	2 nd ant. End/Ex	11/13	12/12	10/13	11/12	11/12	11/12
M I	1 st ant.	23	34–35	24	–	23	–
	2 nd ant. End/Ex	6/11	6/11	3/10	–	5/11	5/11
M II	1 st ant.	30	37–38	28	27	–	–
	2 nd ant. End/Ex	3/15	6/17	3/15	-/14	0/14	0/13
M III	1 st ant.	35	61	30	40–41	32	–
	2 nd ant. End/Ex	0/16	6/21	3/17	-/15	0/16	0/15
PL I	Ex. setae	51	51	42	53	50	58–59
	2 nd ant. End/Ex	14/25	13/27	14/28	-/26	6/22–25	12/23