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Development in culture of larval spotted spiny lobster *Panulirus guttatus* (Latreille, 1804) (Decapoda: Achelata: Palinuridae)

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ABSTRACT

There is little information on the early life history of the spotted spiny lobster *Panulirus guttatus* (Latreille, 1804), an obligate reef resident, despite its growing importance as a fishery resource in the Caribbean and as a significant predator. We cultured newly-hatched *P. guttatus* larvae (phyllosomata) in the laboratory for the first time, and the growth, survival, and morphological descriptions are reported through 324 days after hatch (DAH). Phyllosomata were cultured at 25 °C in a flow-through seawater system within a series of custom 80 l plankton-kreisel tanks and provided with ongrown *Artemia* and mussel gonad. Mean body length (BL) of phyllosomata was 1.70 mm ($N = 10$) at hatch and increased linearly to 22.20 mm at 226 DAH ($N = 3$). Morphological characters from a total of 164 sampled phyllosomata were ascribed to nine distinct developmental stages (stages I-IX), and described and illustrated. Although no final stage phyllosomata (stage X) were obtained, the BL in the final stage was extrapolated at 39.6 mm using a Gompertz function, expressing the relationship between phyllosoma stages and BL. The total duration of phyllosomata for *P. guttatus* was estimated at 410 d, with the 5th and 95th percentiles at 334 and 526 d, respectively. Our data suggest that *P. guttatus* has a larger body size in the final larval stage and a substantial pelagic larval duration compared with other related panulirid lobsters in its geographic range. The described morphological and biological attributes associated with the early-life history for this species can inform future studies, and add value to models of distribution and population connectivity.

Key Words: aquaculture, early life-history, Gompertz function, Guinea lobster, kreisel, phyllosoma

INTRODUCTION

The spotted spiny, or Guinea, lobster, *Panulirus guttatus* (Latreille, 1804) is an obligate reef inhabitant found throughout the shallow waters of the southern Gulf of Mexico and the Caribbean from Bermuda to Suriname (Holthuis, 1991). This common and wide-ranging species, although a target in artisanal harvesting activities (Evans & Lockwood, 1994; Sharp *et al.*, 1997) is not currently threatened by large-scale fishing operations; however, localized declines may be occurring and are cause for concern (Acosta & Robertson, 2003; Wynne & Côté, 2007; Butler *et al.*, 2011a). Importantly, there exists little biological information on early-life history characteristics, especially details relevant to the pelagic larval phase (i.e. phyllosoma) and duration for this species. Larval studies of the more economically-significant congener *Panulirus argus* Latreille, 1804 typcially do not distinguish between other panulirud larvae (i.e., phyllosomata) and potentially obscure observations and studies of the numerically less abundant *P. guttatus* (Yeung & McGowan, 1991; Canto-García *et al.*, 2016). The lack of differentiation between these species for ecological and fisheries studies is likely confusing given the striking differences in size and morphology of the subsequent post-larval (puerulus) phase (Lyons & Hunt, 1997). While molecular methodologies can, for the most

part, unambiguously differentiate adults and larvae of *P. argus*, *P. guttatus*, and *P. laevicauda* Latreille, 1817 (e.g., Silberman & Walsh, 1992), only one formal study of the phyllosoma phase of *P. guttatus* describes the morphological features of middle- and late-stage phyllosoma stages (stages VI to X) from specimens collected in waters around Cuba (Baisre & Alfonso, 1994). Other biological traits during the pelagic larval phase (e.g., pelagic larval duration, growth rate) have never been reported for *P. guttatus*. The parametrization of such biological data are critical for furthering our understanding of the early-life history of this complex larval phase and help contribute much needed knowledge for differentiating this species from others (*P. argus* and *P. laevicauda*) throughout its range.

Studies on the culture of the phyllosoma of palinurid (spiny) lobsters have long been successful in better discerning the full phyllosoma phase as well as to produce juveniles for aquaculture-related applications (e.g., Matsuda & Takenouchi, 2007; Phillips & Matsuda, 2011). Several spiny lobster species have so far been the subject of such studies, and culture techniques for larval palinurid lobsters have progressed substantially, thereby yielding increased survivorship and overall production of these larval lobsters in controlled environments (Goldstein & Nelson, 2011; Jensen *et al.*, 2011; Phillips & Matsuda, 2011; Fitzgibbon & Battaglene, 2012). It still remains unclear if culturing *P. guttatus* from eggs through their putative phyllosoma stages is compatible with current culture technology and methodologies in the laboratory. Given some of the life-history traits for this diminutive species, including an adult growth rate that is comparable to other *Panulirus* lobsters (Hunt & Lyons, 1986; Negrete-Soto *et al.*, 2002; Robertson & Butler, 2003), there is the possibility that this species has potential as a candidate for aquaculture.

We cultured newly hatched phyllosomata of *P. guttatus* using techniques modified from those used in *P. japonicus* (Matsuda *et al.*, 2006) and *P. argus* (Goldstein *et al.*, 2008) and obtained stage IX phyllosomata, or the sub-final stage in the entire phyllosoma phase, based on the staging criteria for *Panulirus argus* (Goldstein *et al.*, 2008). We describe the growth, survival, and morphological characteristics that contribute to the future elucidation of the early-life history phase of *P. guttatus* to clarify taxonomic descriptions, provide data inputs for subsequent biophysical modeling, and create a knowledge base for the potential closed-system aquaculture for this species.

MATERIALS AND METHODS

Terminology

In our descriptions, "instar" refers to the intermolt period between two successive ecdyses, and "stage" denotes one or more specific morphological characteristics unique to the phyllosoma. A stage can therefore comprise one or more instars (Mikami & Greenwood, 1997) depending on its duration. For clarity, "phyllosoma" (plural "phyllosomata") is a particular pelagic larval life-history phase specific to palinurid (spiny) and scyllarid (slipper) lobsters. This term may be used interchangeably with the term "larva." Descriptions of setae follow those of previous characterizations of phyllosomata (Matsuda *et al.*, 2006; Goldstein *et al.*, 2008) as well as the general classification system developed by Garm (2004) where applicable.

Sources of adults and phyllosomata

Adult male and female spiny lobsters (52 mm average carapace length (CL)) were collected by divers from the barrier reef in the Florida Keys in April 2014; lobsters were then transported in coolers by boat to the Florida Fish and Wildlife Conservation Commission wet-laboratory in Marathon, Florida. Animals were placed into a 1500 l flow-through holding tank with artificial shelters and fed live *Batillaria* sp. (Gastropoda), frozen fish, shrimp, or squid daily *ad libitium*. After eight weeks, three females (two with external eggs and one without external eggs) and four males were placed into plastic bags infused with 100% oxygen, packed into styrofoam boxes, and transported by airfreight from Miami, FL, USA to Osaka, Japan. Upon their arrival in Japan (21 June), the lobsters were placed in an oxygenated 200 l tank and transported by automobile to Mie Prefecture Fisheries Research Institute, Mie. The adult lobsters were held in a 1,000 l fiberglass flow-through tank at a temperature of 24.0 ± 0.3 °C and fed fresh mussel (*Mytilus galloprovincialis* Lamarck, 1819) and frozen krill. One of the two females that were carrying external eggs dropped her eggs in a few days after arrival in Japan, while another female with external eggs continued to hold her eggs as they developed through to hatching. Just prior to hatching, the female lobster with extruded eggs (46.4 mm CL) was isolated in a 100 l black polyethylene tank so that we could collect phyllosomata for culture. On the morning of 28 June 28, ~ 200,000 newly hatched phyllosomata were observed at the tank's surface. A total of 800 phyllosomata from those larvae that showed a strong positive swimming response to overhead lights were collected at the surface using a small glass collection vessel and subsequently used in our culturing system.

Culture tank and system design

A drum-shaped tank, "plankton-kreisel" (hereon "kreisel)", was used for the culture of phyllosomata culture (Fig. 1). The kreisel was originally designed for culturing zooplankton by Greve (1968) and is routinely used to rear and exhibit gelatinous zooplankton at public aquariums (Raskoff *et al.*, 2003; Goldstein & Nelson, 2011). The tank used for this study was constructed from a modified design by Horita (2007); the tank was made of clear acrylic and had a working volume of 80 l (80 cm diameter, 16 cm wide). The operational flow circuitry for this tank was customized for this study as follows: fresh seawater, which was filtered through 0.2 µm membrane filters and then temperature-controlled at ~ 25 °C, entered the tank through a 12 mm diameter polyvinyl chloride (PVC) pipe, to which a faucet cup was fitted at the inlet, with a flow rate of ~ 1.3–1.5 l min^{-1}. Accordingly, the turnover of culture seawater was calculated at 100 – 113% of the total volume h^{-1}. The inlet was set at the center of the tank to improve flow in the center of the tank relative to the well-circulating perimeter of the tank. The temperature of seawater in the tank was measured daily (25.1 ± 0.4 °C) throughout the rearing cycle.

Water currents were generated by seawater driven from the middle of a drain pipe at a flow rate of 1.7–1.9 l min^{-1} through three narrow pipes (5 mm diameter) from just beneath the surface using a magnetic drive pump (REI-SEA, RSD-10A; Iwaki Co., Tokyo, Japan). The drain pipe was mounted upward on the PVC cleanout adaptor with a plug (10 cm diameter) set at the middle of the lateral side of the tank to drain excess water and to regulate overall water level. A mesh screen (0.7–5.0 mm mesh) was fitted on the inner side of the cleanout adaptor to prevent larvae from escaping.

Phyllosomata were transferred regularly, until they reached ~ 15 mm BL, from used kreisel tanks to clean tanks using a 1 m siphon hose (3–5 cm diameter), extended between the opening mouths at the tops of each tank; each kreisel was thoroughly cleaned once per week using a dilute (5%) bleach (sodium hypochlorite) solution. We implemented this practice during the dark phase in the laboratory so that phyllosomata more readily swam across the siphon hose into the clean tank within a few hours. At BLs > 15 mm, phyllosomata were transferred individually using glass transfer spoons into clean kreisel tanks.

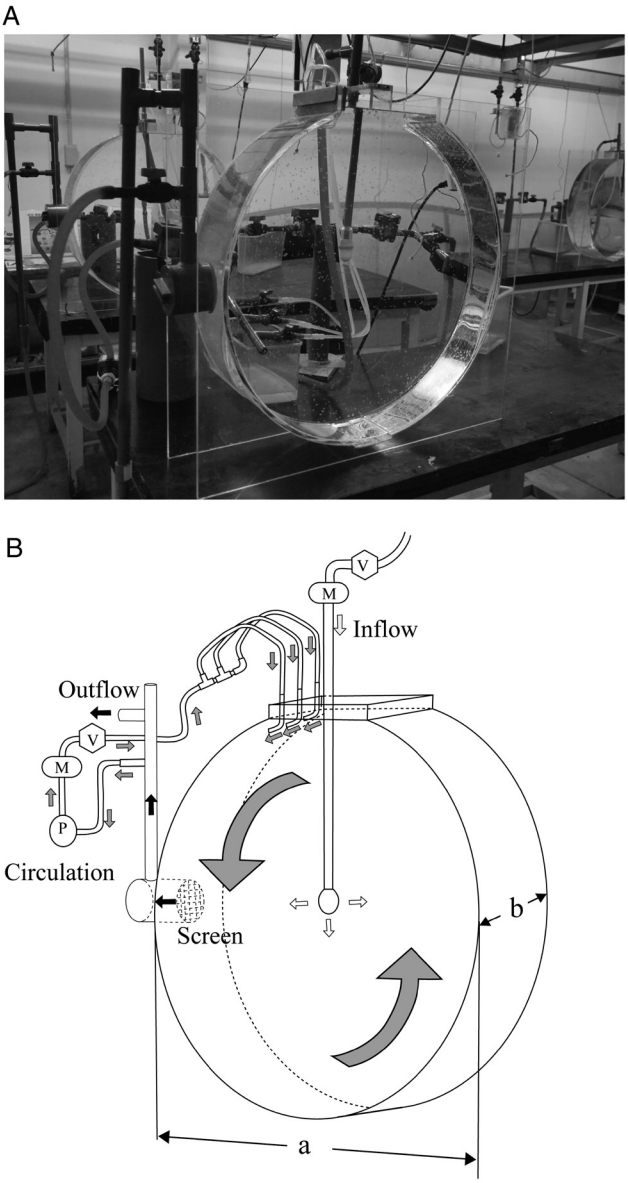

Figure 1. Kreisel tank design and setup used for the culture of *Panulirus guttatus* phyllosomata (**A**). Culture systems with the kreisel tank (**B**). The tank was constructed of clear acrylic (7 mm thick) with a working volume of 80 l. The diameter (a) was 80 cm and the width (b) 16 cm. Filtered seawater enters the tank at the center through a polyvinyl chloride pipe (PVC), to which a faucet cup was fitted at the inlet. Water current was generated by injecting recirculating seawater through narrow three pipes (5 mm diameter) from beneath the surface with a magnetic drive pump (P). The flow rates of the fresh seawater and the recirculating seawater are controlled using valves (V) and flowmeters (M). A nylon screen is set at the entrance of drain pipe to prevent larvae from escaping.

Culture of phyllosomata

Cultured phyllosomata from the first to the fourth instar were fed live brine shrimp (~ 1.0 mm BL) daily, hatched from dried egg cysts from the Great Salt Lake (Salt Creek, Salt Lake City, UT, USA) and were cultured in tandem with the diatom (*Phaeodactylum tricornutum*) for 3–5 d, at a density of 0.5 individuals ml^{-1}. Upon reaching the fifth instar, phyllosomata were fed a combination of juvenile or adult *Artemia* cultured with the diatom, along with finely minced and washed mussel (*M. galloprovincialis*) gonad. *Artemia* density was decreased to 0.05 individuals ml^{-1} as phyllosomata developed. Approximately 50–60 pieces (1 to ~ 4 mm^3) of minced mussel gonad were prepared and fed once daily to each kreisel. Dead *Artemia* and uneaten mussel gonad were removed using a small siphon hose before they were replaced with fresh material daily.

Seawater salinity ranged 33–35 psu, and lighting was controlled using full-spectrum overhead fluorescent bulbs equipped with electric timers with photoperiods regulated at 14 h light:10 h dark. Light intensity during the light phase measured ~ 1 μmol m^{-2} s^{-1}, except for ~ 2 h at 5 μmol m^{-2} s^{-1} to remove uneaten food and exuviae.

Survival rate (S) for this trial was calculated as follows:

$$S(\%) = 100 \times \prod_{i=0}^{n-1} Si, \quad (1)$$

where S_i is the survival rate during the period from the ith sampling to the (i+1)th sampling for morphological observations.

Sampling and measurements

A total of 10 phyllosomata were sampled at hatch and 10 phyllosomata of each instar from the 2nd to the 5th instar were also sampled from the kreisel a few days after they molted to their respective instar. Because the number of instars for each phyllosoma could not be recognized beyond the 5th instar due to the large individual variability in the intermolt period, 10 phyllosomata were randomly sampled every two weeks between 44–142 d DAH. Beyond 142 DAH, 5 phyllosomata were sampled every two weeks between 156–184 DAH. Thereafter, 19 phyllosomata were sampled at irregular intervals. A combined total of 164 phyllosomata were sampled and used for morphological observations. All specimens were fixed in 10% buffered formalin and then archive-preserved in 70% ethanol.

Body dimensions of specimens sampled were measured before fixation as follows (Fig. 2): body length (BL) from the anterior margin of the cephalic shield between the eyestalks to the posterior end of the pleon; cephalic shield length (CL) from the anterior margin between the eyestalks to the posterior margin of the cephalic shield; cephalic shield width (CW) measured at the widest section of the cephalic shield; thorax width (TW) at the widest section of the thorax; abdominal length (AL) from a level line with the base of the pleon to the posterior end of the pleon; and eyestalk length (EL) from the anterior margin of the cephalic shield to the segmentation between eyestalk and eye. The length between the bases of 2nd maxilliped (a) and the distance (b) from the midpoint between the coxal segments of the 2nd maxilliped to the anterior margin of the mouth parts were measured and expressed as b/a, as defined by Johnson (1968) and described by Baisre & Alfonso (1994).

Whole body and appendages of the preserved phyllosomata were quantified according to the methodology of Matsuda & Yamakawa (2000). The presence of the sternal spines located near the base of maxillipeds and pereiopods were additionally noted, because wild *P. guttatus* phyllosomata captured by Baisre & Alfonso (1994) contained these spines *in situ*. Mandibles were not described in this study since their small structure precluded accurate and detailed drawings.

Each developmental stage was assigned (stages I to X) to each of the 164 preserved specimens based on the criteria for *P. argus* phyllosomata given by Goldstein *et al.* (2008) (Supplementary material Table S1). Measurements and drawings of all phyllosomata were made using a Nikon profile projector (model V-12A: Nikon, Tokyo, Japan) and later digitized using Adobe Illustrator CS2 (Adobe Systems, San Jose, CA, USA).

Statistical evaluation

Because BL increased linearly over the course of DAH (see below), the relationship between BL and DAH was assumed to be expressed with the following linear function:

$$BL = a \times DAH + b, \tag{2}$$

where a and b are parameters to be estimated.

Normal distribution $N(\hat{L}_i, \hat{\sigma}_i^2)$ was assumed for describing the random error of BL, where $\hat{L}_i$ is the estimated BL for the ith data and $\hat{\sigma}_i$ is the standard deviation. It is assumed here that $\hat{\sigma}_i$ is proportional to the following linear function of DAH_i:

$$\hat{\sigma}_i = c \times DAH_i, \tag{3}$$

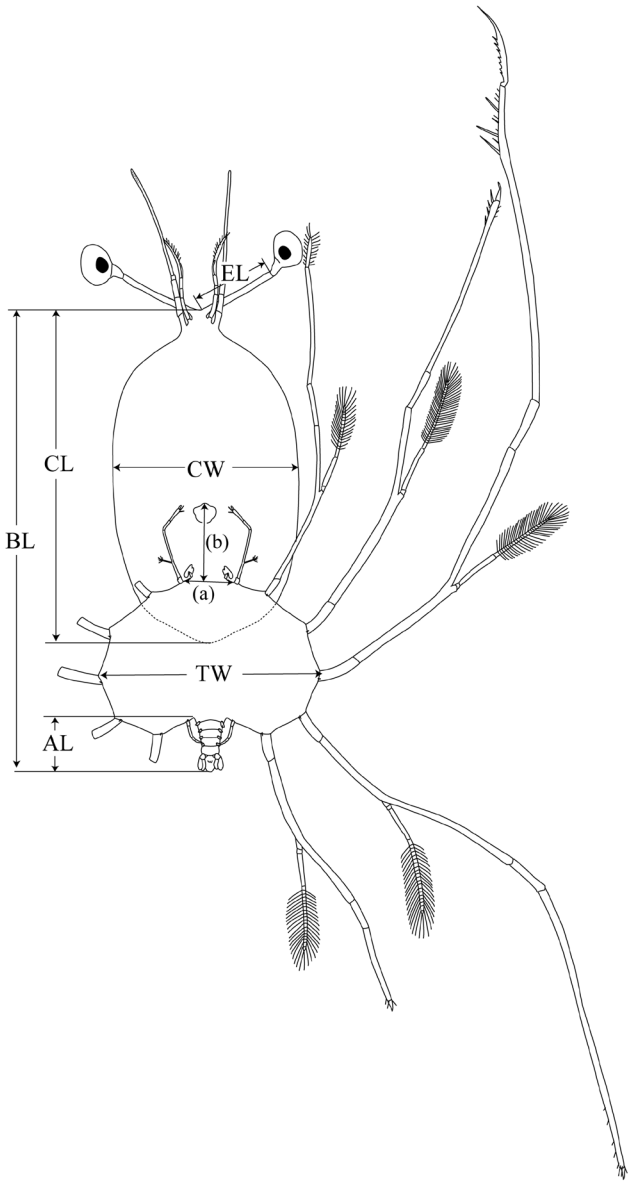

Figure 2. Measurements of phyllosomata of *Panulirus guttatus*. BL, body length; CL, cephalic shield length; CW, cephalic shield width; TW, thorax width; AL, pleon length; EL, eyestalk length (a), length between the coxal segments of the 2nd maxilliped; (b), length from the midpoint of (a) to the anterior margin of the mouthparts.

where c is a parameter to be estimated. The values of $\hat{L} \pm 1.96\hat{\sigma}$ represent the 2.5 and 97.5 percentiles of BL at each DAH, respectively.

The parameters (a, b, and c) were estimated simultaneously by maximizing the following log-likelihood function with the aid of a non-linear optimization software,

$$\varphi = -\sum_{i=1}^{i_{\max}} \left(\frac{1}{2} \ln 2\pi + \ln \hat{\sigma}_i \right) - \sum_{i=1}^{i_{\max}} \frac{(L_i - \hat{L}_i)^2}{2\hat{\sigma}_i^2}, \tag{4}$$

The relationship between phyllosoma stages and BL for *P. argus* was expressed by fitting these data to the Gompertz equation (Yamakawa & Matsuda, unpublished data). This function relates an asymmetric growth model, where growth is slower at the beginning and the latter phase than at the mid-phase (Otterlei *et al.*, 1999). The Gompertz function was thus fitted for the data of *P. guttatus* larvae as follows:

$$BL = d \times \exp\left[-\exp\left\{ -e\,(S - f) \right\} \right], \tag{5}$$

Where S is the number of phyllosomata stages, and d, e and f are parameters to be estimated.

The parameters were estimated by the least squares method.

RESULTS

Our culture of phyllosomata of *P. guttatus* progressed favorably through 50 DAH with a survival rate of 97% (Fig. 3); molting complications led to some mortality through frequent incurved phyllosomata. Larvae with slight body deformations, however, could still capture food and these deformations did not appear to cause mortality. When larvae with body deformations molted to the next instar, the distortion tended to be more serious than the previous one and was implicated as a cause of mortality. The survival rates of phyllosomata thus decreased gradually to 88% at 100 DAH, 70% at 150 DAH, and 36% at 200 DAH (Fig. 3). The phyllosoma culture was terminated at 324 DAH, when the last individual died due to molt complications.

The mean BL for the 1st instar (newly hatched) phyllosomata was 1.70 mm (1.66–1.74 mm, $N = 10$). The BL of phyllosomata sampled for measurements increased linearly over the larval culture period (Fig. 4); the mean BL was 11.47 mm at 100 DAH (9.40–13.25 mm, $N = 10$), and 23.00 mm at 198 DAH (22.00–24.20 mm, $N = 3$). The BL range of phyllosomata measured at each sampling interval increased with age. The estimated

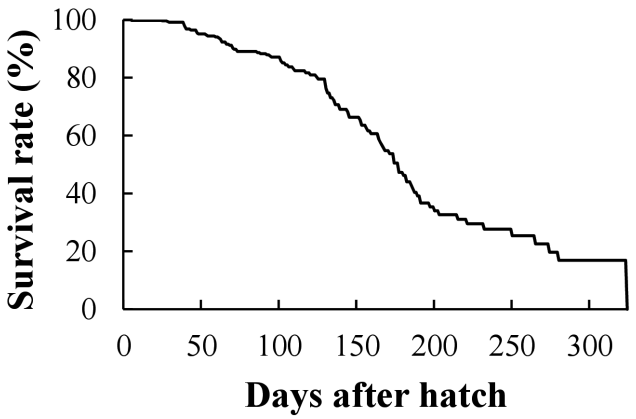

Figure 3. The survival rate of the cultured phyllosomata of *Panulirus guttatus* dropped sharply to zero from 16.9% at 324 d after hatch because most larvae that had been alive at 280 d after hatch were sampled toward the end of the culture.

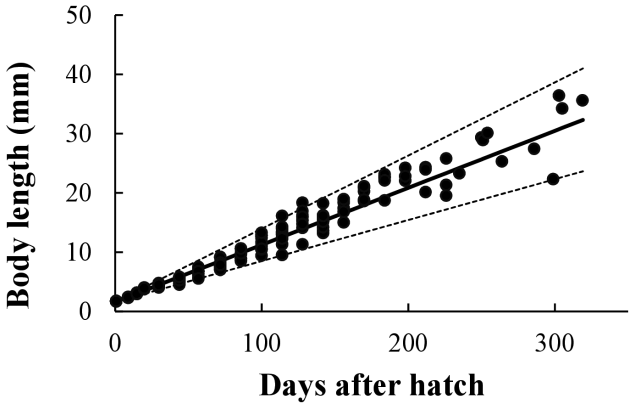

Figure 4. Relationship between body length (BL) and days after hatch for the cultured phyllosomata of *Panulirus guttatus*. The solid line and the dashed lines indicate the estimated linear relationship and the estimated 5% and 95% percentiles of BL at each day, respectively.

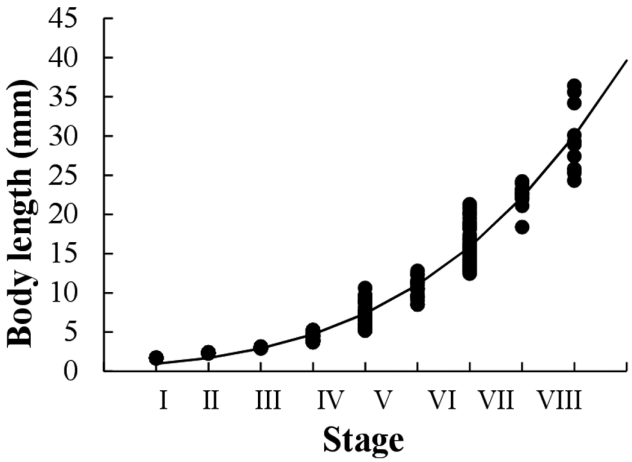

Figure 5. Relationship between developmental stages and body length for the cultured phyllosomata of *Panulirus guttatus*. The solid line indicates the relationship curve calculated by the Gomperz function. See the methods section for a detailed explanation of the equation.

relationships between BL and DAH and between the 2.5 and 97.5 percentiles of BL and DAH are also shown in Figure 4. The BL and the expected 2.5 and 97.5 percentiles of BL to DAH were expressed using the linear functions (2) and (3), respectively, and the parameters (*a*, *b* and *c*) of these functions were estimated at 0.0963, 1.6034, and 0.0139.

We assigned nine distinct developmental stages (I-IX) based on the morphology of 164 sampled phyllosomata. The final phyllosoma stage (stage X) could not be observed. A summary of the developmental traits and descriptions of the larvae are given in Supplementary material Tables S2, S3, Supplementary material Text S4, and Figures S6-S14 (captions to these figures in Supplementary material Text S5). The relationship between the phyllosomata stages and BLs for *P. guttatus* were expressed in a Gompertz time-series function (5) (Fig. 5). The parameters (*d*, *e*, and *f*) of the function were estimated at 665.9741, 0.0937, and 21.0754, respectively.

DISCUSSION

The goal of this study was to culture and describe all larval stages for the spotted spiny lobster, *Panulirus guttatus*. We were successful in documenting all (stages I-IX) but the final stage for *P. guttatus* phyllosomata while also presenting the details of a novel plankton-kreisel culture system. This tank system has many advantages for the culture of complex and hydrodynamically-suited animals such as phyllosomata including the continued suspension of these larvae in the water column with continuous vertically-rotating water currents. These tank attributes tended to minimize mechanical stress and decrease larval aggregation and entanglement (Goldstein & Nelson, 2011; herein). Water current dynamics in kreisels also help to disperse food items (e.g., *Artemia* and small pieces of mussel gonad) that enhance larval-food encounter rates (Phillips & Matsuda, 2011). Just as important, this tank design has the potential to decrease the probability of molt failures due to the patterns of water current. For example, in a similar species, *P. japonicas*, the success rate of metamorphosis from the final phyllosoma stage to the post-larval (i.e., puerulus) stage was higher in the plankton-kreisel (85%) than in the circular tank without continuous vertically-rotating water currents (38%) (Phillips & Matsuda, 2011). This result was probably induced in part, by the absorption of oxygen across the phyllosoma body surface, facilitated by ample water currents over each larva. This, in turn, had a favorable effect on the success of molting throughout our study period. From a design perspective, this type of tank has rudimentary construction and is easy to operate, making it a very suitable platform for the culture of phyllosomata.

P. guttatus phyllosomata cultured within our plankton-kreisel system revealed high survival until 50 DAH (> 90 %), but molt-related mortality occurred frequently thereafter, regardless of the positive effect of the kreisel tank on the success of observed molting. Consequently, no phyllosomata reached the final phyllosoma stage (stage X), even though the culture lasted > 300 days and the largest phyllosoma that was obtained in this study was 34.6 mm in BL. At this time, phyllosoma cultures of *P. japonicus* within the same tanks as *P. guttatus* larvae were in good condition and survivorship reached ~ 60 % through until the post-larval stage.

Duration of phyllosomata

A total of 164 phyllosomata of *P. guttatus* were sampled for morphological observations and assigned to nine distinct stages (I–IX) plus stage X, a total of 10 stages, based on the same criteria given for the phyllosomata of *P. argus* (Goldstein *et al.*, 2008). The relationship between the phyllosoma stages (I-IX) and their associated BLs for each stage were expressed with the Gompertz time series function (Fig.5). For example, the BL of the final-observed stage phyllosomata, which have bilobed gill buds on the coxae of 1st to 4th pereiopods, was estimated at 39.6 mm, extrapolated from this equation. Furthermore, the theoretical BL of the 5 and 95 percentiles of BL at each day post-hatch were expressed by the linear functions (2) and (3). From these functions and the estimated BL of the final stage phyllosomata, the period of time from hatch to the final phyllosoma stage was estimated at 395 days within the 5th and 95th percentiles of 319 and 511 days, respectively. Assuming that the duration of the final phyllosoma stage of *P. guttatus* is ~ 15 d in accordance with the observation that the final stage of *P. argus* consisted of a single instar and the mean duration was 15 d ($N = 6$; Goldstein *et al.*, unpublished data), the total duration of the phyllosoma phase of *P. guttatus* was estimated at 410 d, with 5 and 95 percentiles being 334 and 526 d. This pelagic duration is fairly protracted compared to other species of *Panulirus*. (Goldstein *et al.*, 2008; Phillips & Matsuda, 2011).

There are currently three extant species of spiny lobsters (species of *Panulirus*) in the Caribbean Sea and neighboring coastal waters: *P. argus*, *P. guttatus*, and *P. laevicauda* (Holthuis, 1991). The duration of the phyllosoma phase of *P. argus* is 140–198 days (mean = 174 d) in the laboratory and the BL of the final observed phyllosoma stage was 25.10–32.73 mm (mean = 28.78 mm; Goldstein *et al.*, 2008). Although the development of phyllosomata

and the duration of the larval stages of *P. laevicauda* have not been fully described, this species appears to have small phyllosomata similarly to *P. argus* (Baisre & Alfonso, 1994), which allowed us to suggest that *P. laevicauda* also has a comparatively short larval phase. When the larvae of *P. guttatus* are compared to those of *P. argus* and *P. laevicauda*, *P. guttatus* has a comparatively lengthy planktonic phase concurrent with a larger larval body size. Although there is a possibility that these traits in *P. guttatus* phyllosomata may reflect an adaptive strategy for dispersal and survival in the pelagic realm, we cannot validate this assumption due to the lack of knowledge on the distribution of the larvae of *P. guttatus* and their metamorphosis and recruitment in nature. Our knowledge of the transport and settlement dynamics in the phyllosomata of an obligate reef lobster such as *P. guttatus* is limited at best (Sharp *et al.*, 1997) and an extended larval duration may be a strategy to stave off sub-optimal conditions or "patchy" settlement sites (e.g., patch reefs) before larval competency is achieved (Gebauer *et al.*, 2003). Subsequently, such differences in the larval traits among these three spiny lobsters will draw increasing attention with respect to the interspecific relationship of spiny lobsters throughout their range.

Phyllosoma stages

Baisre & Alfonso (1994) described the phyllosoma stages VI to X of *P. guttatus* based on specimens collected in coastal waters around Cuba, although they did not ascribe the staging criteria for their specimens. Their phyllosomata wholly resemble our specimens and their stages VI-X appear to correspond with our stages VI through IX, respectively. We must recognize that there are likely morphological differences between their phyllosomata and ours (e.g., length of eyestalk, shape of apical region of antenna, ratio between CW and CL and the developments of exopod of 2nd maxilliped and abdominal segmentation). Specifically, the length of the eyestalk of their stage X was 3.5–3.7 mm, compared with our Stage IX at 4.85–6.5 mm. The values of CW/CL in the stage X phyllosomata described by Baisre &Alfonso (1994) were 0.60.69, while our stage X ranged 0.52–0.56. Their stages IX and X had noticeable spatulate tips in the antennae, whereas the apical region of the antenna in our corresponding stages did not develop such spatula-shaped antennae, remaining in a semi-spatulate state. While the identifications by Baisre & Alfonso (1994) are probably reliable based on their descriptions, the morphological discrepancies between the phyllosomata could be a developmental artifact resulting from a laboratory-reared environment. Late-stage phyllosomata of *P. japonicus* reared in culture have narrower cephalic shields than their wild counterparts (Matsuda, 2005). Furthermore, the pueruli of *P. guttatus* have antennae with spatulate apices (Briones-Fourzán & McWilliam, 1997), which clearly indicates that later stage larvae of this lobster also have antennae with spatulate apices, an attribute observed in other palinurid species as well (McWilliam, 1995). Wild-caught final stage phyllosomata of *P. ornatus* (Fabricius, 1798) have antennae with noticeable spatulate tips (McWilliam & Phillips, 1992) and laboratory-reared *P. ornatus* phyllosomata were also described as having antennae with spatulate tips (Smith *et al.*, 2009); however, the degree of spatulate shape in reared phyllosomata appear to be smaller than those from their *in-situ* counterparts. The profile of the spatulate shape is possibly influenced by changes in developmental settings. Discrepancies in the developmental rates of the exopod of the 2nd maxilliped and the abdominal segmentation have not been observed in the phyllosomata of the species of *Panulirus*. Further studies are needed to confirm the effects of laboratory-based environments on the development of these characters and their comparison to wild-caught specimens. The ability to culture and document laboratory-reared larvae does add a substantial advantage in parameterizing known metrics that include developmental age, growth rates, and larval duration. These data are especially essential for furthering our ability to better predict larval distributions for this species in coupled bio-physical models for ecological and fisheries-related applications, including stock management (e.g., Butler *et al.*, 2011b; Bradford *et al.*, 2015, Whomersley *et al.*, 2018).

A comprehensive phylogenetic analysis of the species of Panulirus through nucleotide sequence data (16S and COI) revealed that *P. guttatus* along with its congeners *P. argus*, *P. echinatus* Smith, 1869, and *P. penicillatus* (Olivier, 1791) are closely related, comprising a sister clade (Ptacek *et al.*, 2001). McWilliam (1995) presented an evolutionary sequence of several species-groups in the late phyllosoma phase of *Panulirus* and assigned the phyllosomata of *P. guttatus* into group 2B, within 4 groups and 2 subgroups based on 6 distinct morphological characters: the apical region of antennal flagellum, ventral coxal spine, dorsal coxal spine, subexopodal spine in pereiopods, sternal spine on the thorax, and width of the cephalic shield width relative to thorax width (Baisre & Alfonso, 1994). Although in the later larvae of Baisre & Alfonso (1994) the ventral coxal, dorsal coxal, and subexopodal spines in the pereiopods were absent, their larvae had sternal spines on the thorax near the coxae of the 1st to 5th pereiopods, consistent with our phyllosomata. According to the grouping of the species of *Panulirus* by McWilliam (1995), the phyllosomata of *P. argus* and *P. laevicauda* belong to group 1 and *P. guttatus* to group 4. Our study then confirms that the later phyllosoma stages of these three lobsters are morphologically distinguishable from each other on the basis of the six characters that McWilliam (1995) described.

A new understanding of the larval duration and confirmation of comparable morphological differences between the phyllosomata of *Panulirus* spp. can facilitate future studies on the fisheries, ecology, and population source and sink dynamics of *P. guttatus*. Although *P. guttatus* is a target of directed fisheries in selected regions of its range, prevention of local extinctions remains a concern (Butler *et al.*, 2011a). *Panulirus guttatus* is also a key top-down predator in Caribbean coral reefs (e.g., Briones-Fourzán & Lozano-Alvarez, 2013; Butler & Kintzing, 2016) and likely plays an important role in modulating the presence and abundance of some gastropods like the corallivorous snail *Coralliophila abbreviata* (Lamarck, 1816) (Williams *et al.*, 2014). The morphological and biological attributes associated with the early-life history for this species have generated informative data for future culturing studies, and for acquiring better resolution as it pertains to population connectivity, and ultimately towards a more informed conservation-centric framework for the management of lobster fisheries.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Key to phyllosoma stages of *P. guttatus*.
S2 Table. Summary of characters of phyllosomata of P. *guttatus*.
S3 Table. Morphometric size comparisons for phyllosomata of *P. guttatus* reared in the laboratory.
S4 Text. Description of phyllosoma stages of *P. guttatus*.
S5 Text. Captions for Figs. S6–S14.
S6 Figure. Phyllosoma stage I of *P. guttatus*
S7 Figure. Phyllosoma stage II of *P. guttatus*.
S8 Figure. Phyllosoma stage III of *P. guttatus*.
S9 Figure. Phyllosoma stage IV of *P. guttatus*.
S10 Figure. Phyllosoma stage V of *P. guttatus*.
S11 Figure. Phyllosoma stage VI of *P. guttatus*.
S12 Figure. Phyllosoma stage VII of *P. guttatus*.
S13 Figure. Phyllosoma stage VIII of *P. guttatus*.
S14 Figure. Phyllosoma stage IX of *P. guttatus*.

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Fig. 7

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Fig. 8

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Fig 9

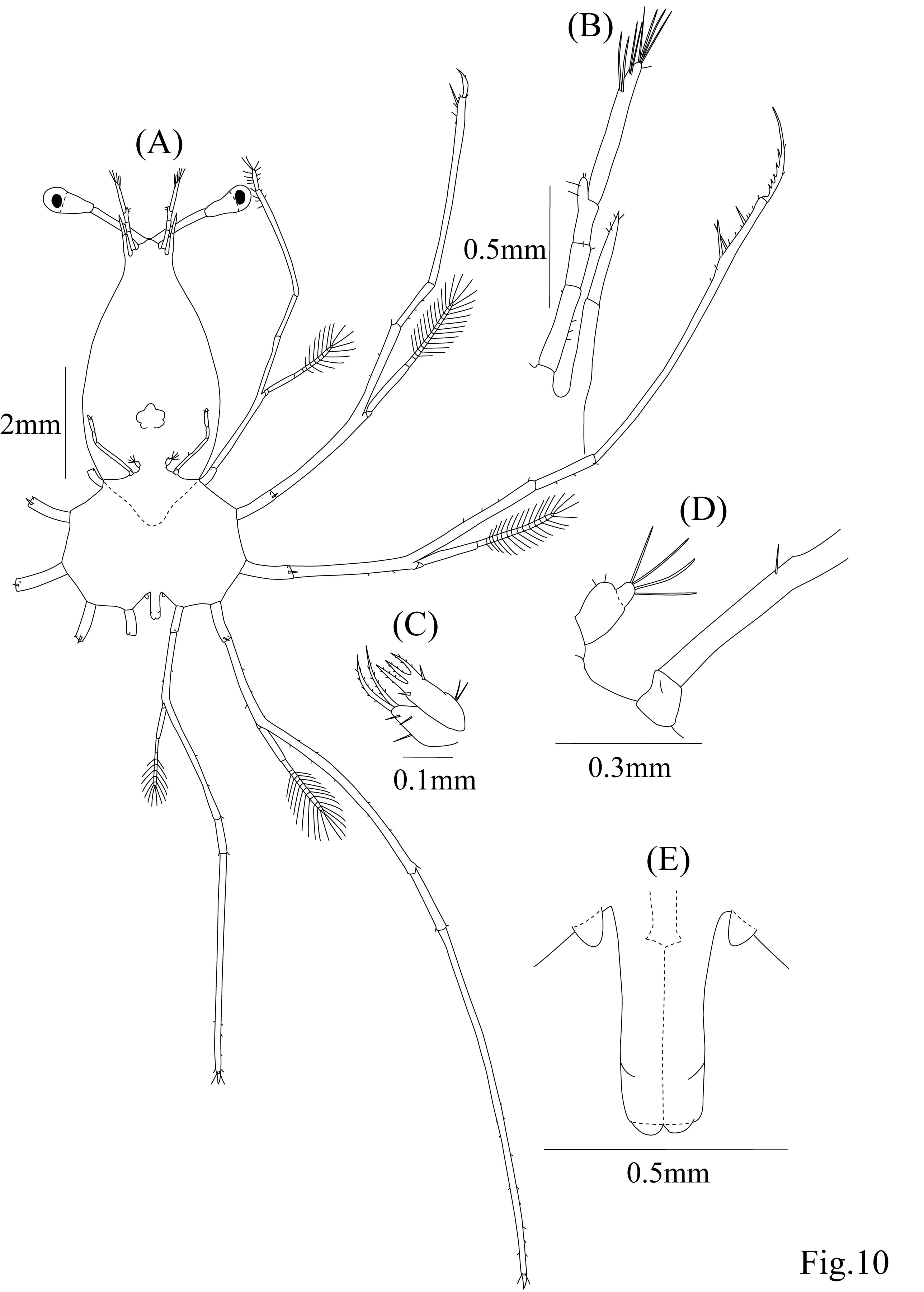

Fig.10

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Fig.11

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Fig.12

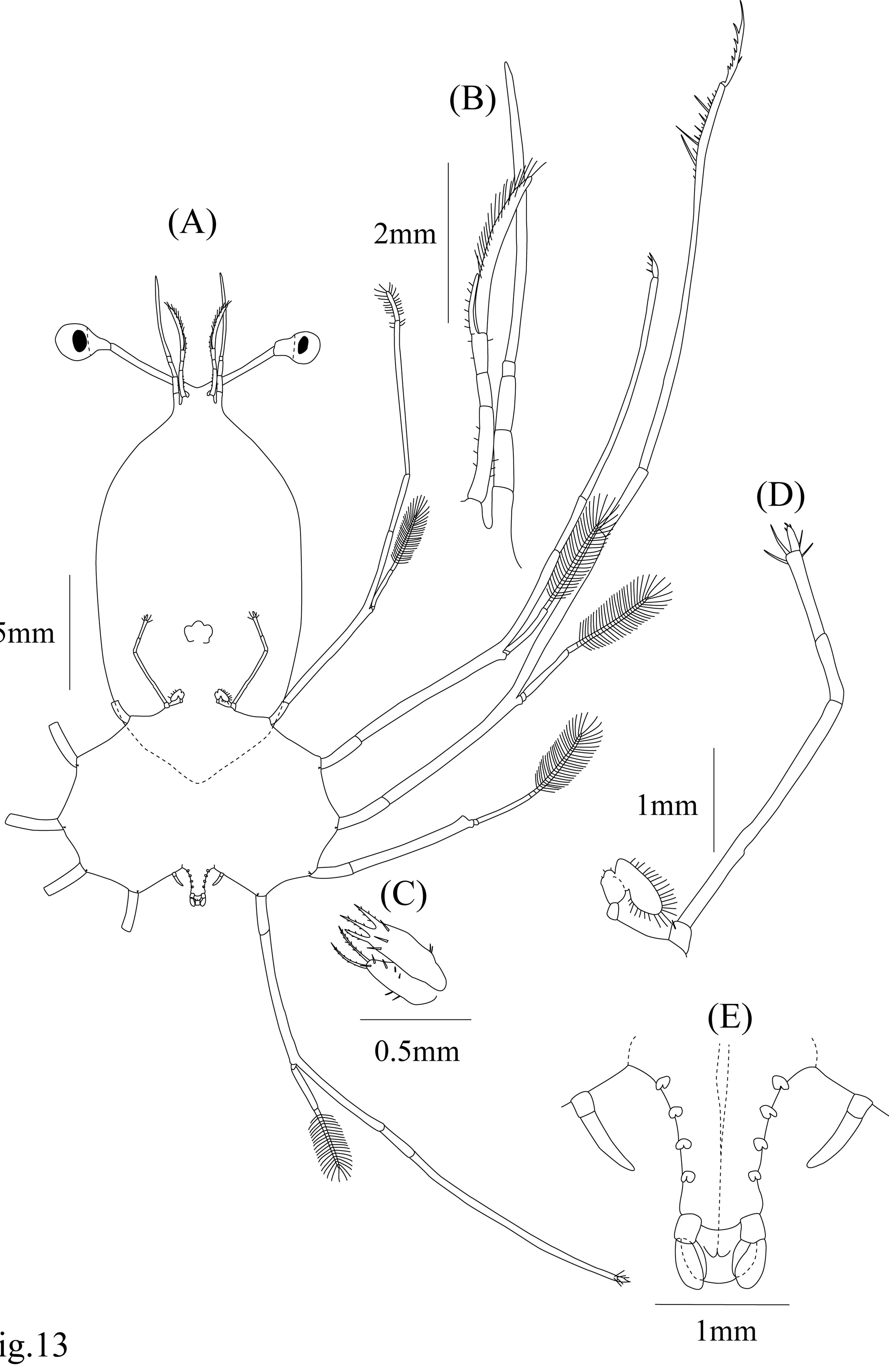

Fig.13

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Fig.14

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Fig.6