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Post-embryonic development and reproduction of *Pseudodiaptomus annandalei* (Copepoda: Calanoida)

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Abstract: This paper describes the developmental stages, rates of post-embryonic development, and reproduction of the calanoid copepod *Pseudodiaptomus annandalei* at different temperatures. Copepods used in the experiment were reared as a batch culture in the laboratory. The developmental stages were divided into 6 naupliar (NI, NII, NIII, NIV, NV, NVI) and 6 copepodid stages (CI, CII, CIII, CIV, CV, CVI), the last being the adult. The dimorphic stages were CIV, CV and CVI. The first naupliar stage had a very short duration and lacked the terminal spine that was common in the other naupliar stages. Dramatic changes in the morphology of the developmental stages took place during the transition from the last naupliar stage to the first copepodid stage. Ovigerous females carried a pair of ovisacs containing 4–14 eggs. Eggs of females at 15°C did not hatch. Females isolated from males at 20, 25, 28, and 33°C produced no more than two clutches of viable eggs. In contrast, females paired with males at a 1:1 ratio produced 9 clutches of viable eggs within 11 days. Stage duration was significantly longer at 20°C but was similar at 25, 28, and 33°C. Temperature did not appear to affect the body length of copepods. The growth coefficient did not differ significantly among higher temperature treatments (25, 28, and 33°C).

Key words: *Pseudodiaptomus annandalei*, developmental stages, temperature, reproduction.

Introduction

The use of adult stage for the sole purpose of taxonomic classification is a very established rule. On the other hand, post-embryonic morphology provides structural and stage-dependent characters different from those of adults and could be a very useful reference for other studies such as population dynamics. The typical copepod life cycle is comprised of six naupliar and five copepodid stages preceding the adult stage. Each stage is separated by a molt and development is determinate, with no molting in the adult (Boxshall, 1992). Development of the different stages involves determination of the intermoult periods and the associated growth factors such as temperature and food. Although modes of development have already been established for many copepod species, it is sad to note that *Pseudodiaptomus annandalei*, Sewell, 1919, has remained unstudied.

The occurrence of *Pseudodiaptomus annandalei* has been reported in Batangas, Philippines and Lake Kolleru, India. Adult *P. annandalei* collected from both areas were recorded and classified taxonomically by Walter (1986, 1987) for the first time. *P. annandalei* was observed to be abundant in several aquaculture ponds in the Philippines (Golez, unpublished data). Although attempts to use this species for aquaculture seem to be successful (Doi et al. 1997; Toledo et al. 1997), constraints in propagation techniques lie in the poor understanding of its biology. We need to examine not only their developmental stages but also their reproductive biology in order to understand their population dynamics.

This paper describes the developmental stages of *P. annandalei*, contributing to basic knowledge on their biology that will lead to an understanding of their population dynamics. We also examined the effects of temperature on the post-embryonic development and reproduction of *P. annandalei*, along with mate limitation on the reproductive potential of females. Reproductive biology in other species of *Pseudodiaptomus* has been reported by Uye et al. (1982), Jerling & Wooldridge (1991) and Liang & Uye (1997). However, none of these reports dealt with the spawning bi-

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ology individually.

Materials and Methods

Collection of copepods in a pond

Pseudodiaptomus annandalei were collected from a small (500 m^2) and shallow (average depth=85 cm) brackish-water pond (salinity: ca. 18 psu) in Panay Island, Philippines. A seine net with 60 μm mesh was operated manually along the surface down to the bottom of the pond being careful not to disturb the sediments. Collection of copepods was done in early morning before dawn. The timing of collection was based on reports that *Pseudodiaptomus* follow a diel vertical migration pattern—staying on the bottom during the day, migrating into the water column at night, and remaining evenly distributed until dawn (Walter 1986, Kouassi et al. 2001). *P. annandalei* were transported to the laboratory, sorted and reared in 250-L fiberglass tanks for about 14 days.

Culture and experiment procedures

P. annandalei that had been collected previously from the brackish-water pond and reared in the laboratory in the Philippines as described above, were transported to Japan. Copepods were reared in filtered (GF/F) seawater collected from Sagami Bay, Japan. The salinity was adjusted to 20 psu by diluting with de-ionized water. *P. annandalei* were reared through to several generations as a batch culture on a mixed diet of *Tetraselmis tetrathele* and *Isochrysis galbana* ($\sim$10 $\mu g\,L^{-1}$ chlorophyll *a*) in an incubator with temperature of 25°C and 12L : 12D light cycle. The copepods were transferred into freshly-prepared rearing water with the same diet and concentration as previously described, at 3-day intervals.

Experiment A: Observation of post-embryonic development

Twenty ovigerous females were isolated from the batch culture (at 25°C). These copepods were kept in a 1000 ml beaker and incubated at 25°C. After 24 hours, nauplii that hatched out were separated from the females by filtering the contents of the beaker with a 250 μm plankton net, by which females were retained in the net. Two hundred newly-hatched nauplii were pipetted and reared further to adult stages in a 1000 mL beaker. Five to ten copepods were taken randomly at 12 to 24-hour intervals for morphological observation of the different stages of development, body length measurement and determination of development time for each stage. The sampling interval was recorded and used to estimate the stage duration. Body length of nauplii was measured from the most anterior part down to the end of the body excluding the terminal spine (total body length). In copepodid stages, however, length was measured from the most anterior part of the cephalosome down to the end of the furcal spine. Body length was measured to the nearest 0.01 mm with an ocular micrometer. Nikon Optiphot and Olympus Bx60 microscopes were used to visualize and record the different stages and to sketch the morphology of the copepods. Development time was determined by observing the copepods at 12-hour intervals

Experiment B: Observation of spawning pattern

Fifty adult females were isolated from the batch culture and were incubated in a 1000 mL beaker for 24 hours. Two sets of experiments were done to evaluate the effects of temperature and mate limitation on the spawning of *P. annandalei*. Females that became ovigerous (bearing ovisacs) were isolated individually into 10-mL micro-dishes and incubated at 15, 20, 25, 28, and 33°C (n=6 per treatment). In a separate set of a 6-well micro-dish, a male and female pairs were allocated to each well and kept in a 28°C incubator. Copepods were provided with the same food at the same concentrations as in the batch culture. Their spawning pattern was observed daily for 11 days; the frequency of viable egg production and non-viable egg production, and the number of nauplii that hatched out, were assessed. Differences in nauplii production among treatments were evaluated using Fisher's Least Significant Difference Test (Systat 8.0, SPSS, Chicago, USA). Statistical differences was considered to be significant at $p<0.05$.

Experiment C: Post-embryonic development at different temperatures

Newly hatched nauplii were separated from ovigerous females by the same method as described in Experiment A. Fifty nauplii were reared in 100 mL beakers at different temperatures (20, 25, 28, 33°C). Other rearing conditions were the same as those in the batch culture. Acclimation of copepods that were originally incubated at 25°C was done for 1–2 hours to let them adapt to the target temperature. This was done by transferring copepods from one incubator with a specific temperature to another (e.g. 25°C→23°C→20°C; 25°C→28°C; 25°C→28°C→31°C →33°C). Three to five individuals were taken from each container daily for estimations of stage duration and measurement of body length. The 12 stages of development were designated with numerals 1, 2, 3,...,12 for stages NI, NII, NIII,....CVI, respectively. Because a constant rate of development occurred in all the post-embryonic stages, the rates of development at each temperature were estimated using the linear function:

$$T=a+bs$$

where T is time, s is the developmental stage, a is a constant and b is the slope which also represents stage duration.

Growth of *P. annandalei* was measured in terms of body length. Nauplii were measured from the most anterior to the posterior end of the body, excluding the caudal armatures.

Body length of copepodids was expressed in terms of prosome length. Body length was measured to the nearest 0.01 mm with an ocular micrometer. Growth was expressed by the growth coefficient, in terms of length:

$$L=L_o e^{gs}$$

where L is the length of the adult, L_o is the length of NI, g is the growth coefficient, and s is the developmental stage.

Results

The following descriptions for each developmental stage were based on the incubation conditions at 25°C.

Morphology of the stages

The life cycle of *P. annandalei* was divided into 6 naupliar (NI, NII, NIII, NIV, NV, NVI), 5 copepodid (CI, CII, CIII, CIV, CV) and an adult (CVI) stage. Sexual dimorphism starts from CIV toward the adult stage where males and females can be distinguished through their antennule and 5th swimming legs.

Nauplius I (NI): The first naupliar stage hatched 18 hours after spawning. The body was symmetrical, egg-shaped and with an average length of 0.15±0.018 mm. The caudal armature was composed of 2 setae with the same length. The antennule had 2 segments (Fig. 1a).

Nauplius II (NII): N I molted to NII stage 21 hours after hatching. In ventral view, the body shape was almost the same as that in NI but more elongated. The average body length was 0.174±0.11 mm. The caudal armature was composed of 1 sharp spine and 1 seta that were of the same length. Antennule had 3 segments (Fig. 1b).

Nauplius III (NIII): At thirty-three hours (33 hour) after hatching, NII reached the NIII stage. The body was pear-like in shape and had a narrow thoracic end. Average body length was 0.19±0.005 mm. Caudal armature was composed of 1 spine and 1 seta parallel to each other, and 1 fine seta slightly curved and extending outward. These caudal armature elements were almost of the same length. Rudiments of the 1st maxilla were formed (Fig. 1c).

Nauplius IV (NIV): NIII molted to NIV stage 45 hours after hatching. The body shape was more elongated than in NIII. Body length was 0.22±0.01 mm. Length of the caudal armature elements changed proportionately (sharp spine> seta>fine seta). Budding coxa of the 1st maxilla had 1 seta (Fig. 1d).

Nauplius V (NV): NIV developed to NV stage 74 hours after hatching. The body became more elongated and narrower at the thoracic end than in NIV. Length of the body was 0.28±0.004 mm. The length proportions of caudal armature elements remained the same as in NIV. The 1st maxilla had 2 segments (Fig. 1e, f).

Nauplius VI (NVI): Molting to the last naupliar stage (NVI) took place around 96 hours after hatching. The body became more elongated than NV and measured 0.31±0.11 mm. The terminal sharp spine became greatly reduced relative to the seta and also became two-segmented. Additional feeding appendage (maxilla 2) was formed. Rudiments of the 1st and 2nd swimming legs (P1 and P2) appeared (Fig. 1g, h).

Copepodid I (CI): There was a dramatic change in the morphology from the last naupliar stage from which the copepod molted to the first copepodid stage (CI) around 110 hours after hatching. The total body length was 0.44± 0.005 mm. The head and the 1st pedigerous somite were fused. The 4th and 5th pedigerous somites were fused in this stage and also in all copepodid stages. Two pairs of swimming legs, and 4 pairs of setae on each of the caudal rami were developed. Urosome had 2 segments (Fig. 2a, b).

Copepodid II (CII): CI molted to CII 141 hours after hatching. It grew longer (0.56±0.01 mm) than CI, and had 3 pairs of swimming legs and 4 pairs of setae on each of the caudal rami. The urosome had 2 segments as in CI (Fig. 2c, d).

Copepodid III (CIII): Molting to CIII took place 165 hours after hatching. The body length was 0.65±0.04 mm. Copepods in this stage had 4 pairs of swimming legs and 6 setae on each of the caudal rami. Urosome was 2-segmented as in CI and CII (Fig. 2e, f).

Copepodid IV (CIV): CIII molted to CIV 217 hours after hatching. This stage was characterized by the presence of 5 pairs of swimming legs, and 6 setae each on the left and the right caudal rami. Urosome of both male and female had 3 segments.

Male: Body length was 0.74±0.04 mm. The asymmetrical fifth swimming leg (P5) was thick and peg-like in lateral view (Fig. 2g, h). The exopod of both left and right legs had 1 lateral spine and 2 terminal spines (Fig. 3a).

Female: Body length was 0.91±0.03 mm. The 5th swimming leg (P5) was symmetrical (Fig. 3e), thinner than that of the male and was peg-like in lateral view (Fig 2m, n). Both the left and the right 5th swimming leg bear 1 spine on the outer margin and 2 terminal spines (Fig. 3e).

Copepodid V (CV): *P. annandalei* developed into CV about 240 hours after hatching. P5 was almost identical in the respective sexes as that in CIV but was bigger and longer and with more setae and spines. The caudal rami had the same number of setae as in CIV.

Male: Body length was 0.78±0.08 mm. The urosome had 4 segments. P5 was thicker than in CIV in lateral view (Fig. 2i, j). The exopod of both the left and right swimming legs had 2 lateral spines and 1 terminal spine. The endopod of the left P5 became more elongated (Fig. 3b).

Female: Body length was 1.00±0.04 mm. Urosome was 3-segmented. The 5th swimming leg (P5) was thicker and longer than that of CIV in lateral view (Fig. 2o, p). Left and right P5 had 1 lateral, 2 medial and 2 thick terminal spines. A pair of setae extending outward formed near the base of P5 (Fig. 3f).

Copepodid VI (Adult): *P. annandalei* finally reached the adult stage around 264 hours after hatching. The 5th swim-

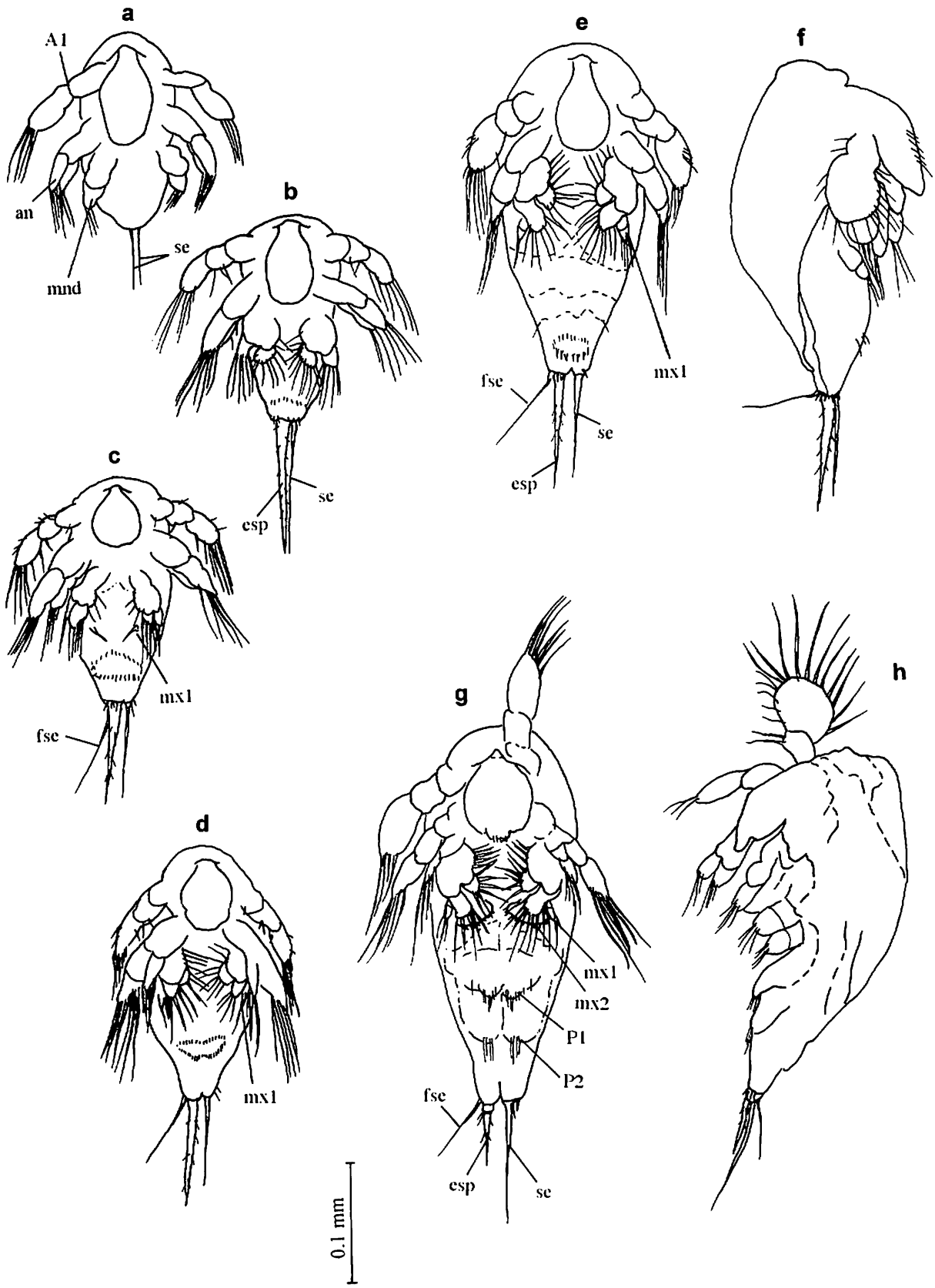

Fig. 1. Naupliar stages: a—NI; b—NII; c—NIII; d—NIV; e, f—NV (ventral and lateral views); g, h—NVI (ventral and lateral views); A1—antennule; an—antenna; mnd—mandible; mx—maxilla; se—seta; fse—furcal spine; esp—terminal spine; mx1—first maxilla; mx2—second maxilla; P1—first swimming leg; P2—second swimming leg.

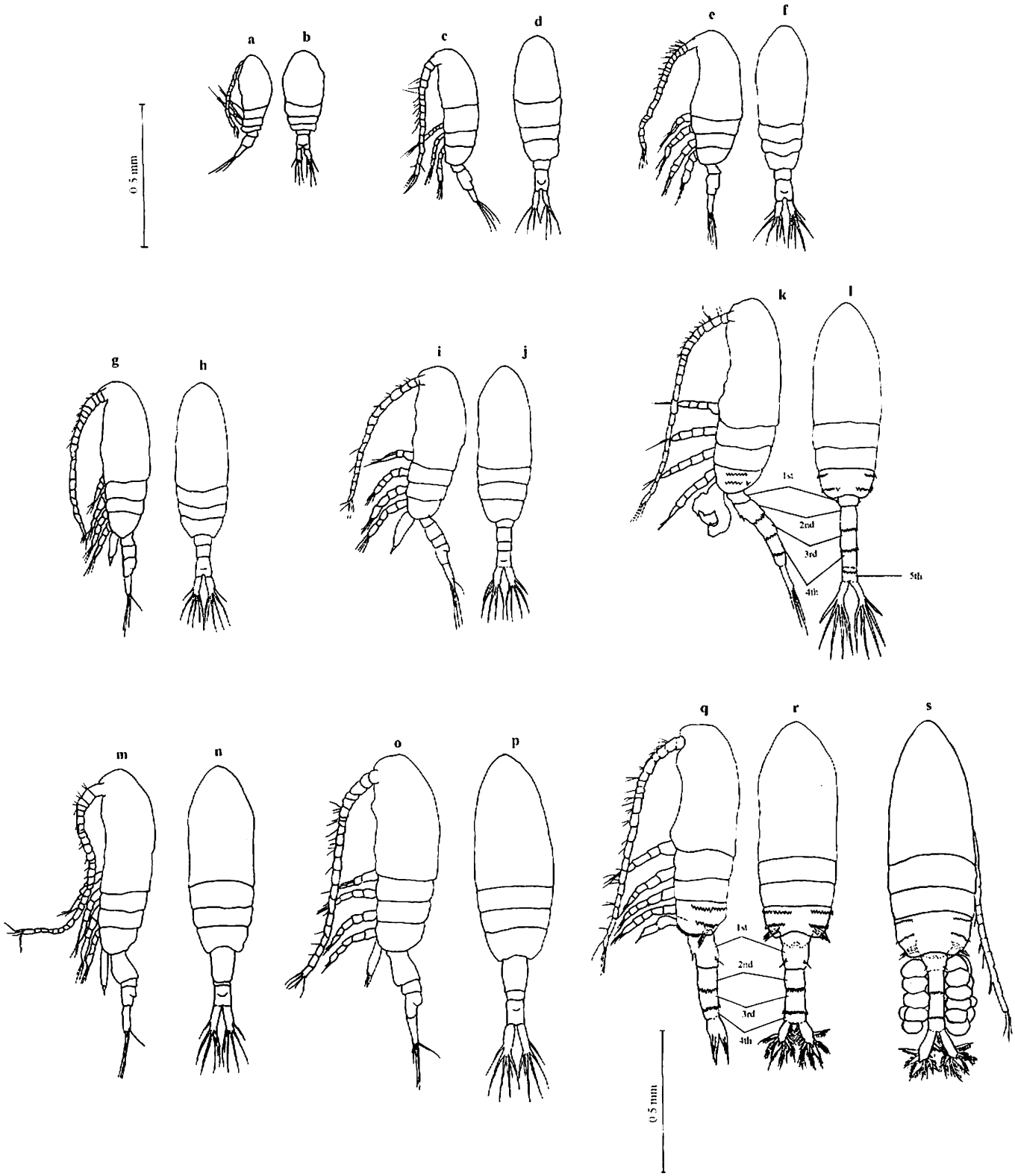

Fig. 2. Copepodid and adult stages: a, b—CI; c, d—CII; e, f—CIII; g, h—CIV male (lateral and dorsal views); i, j—CV male; k, l—CVI (adult male); m, n—CIV female; o, p—CV female; q, r—CVI (adult female); s—ovigerous female.

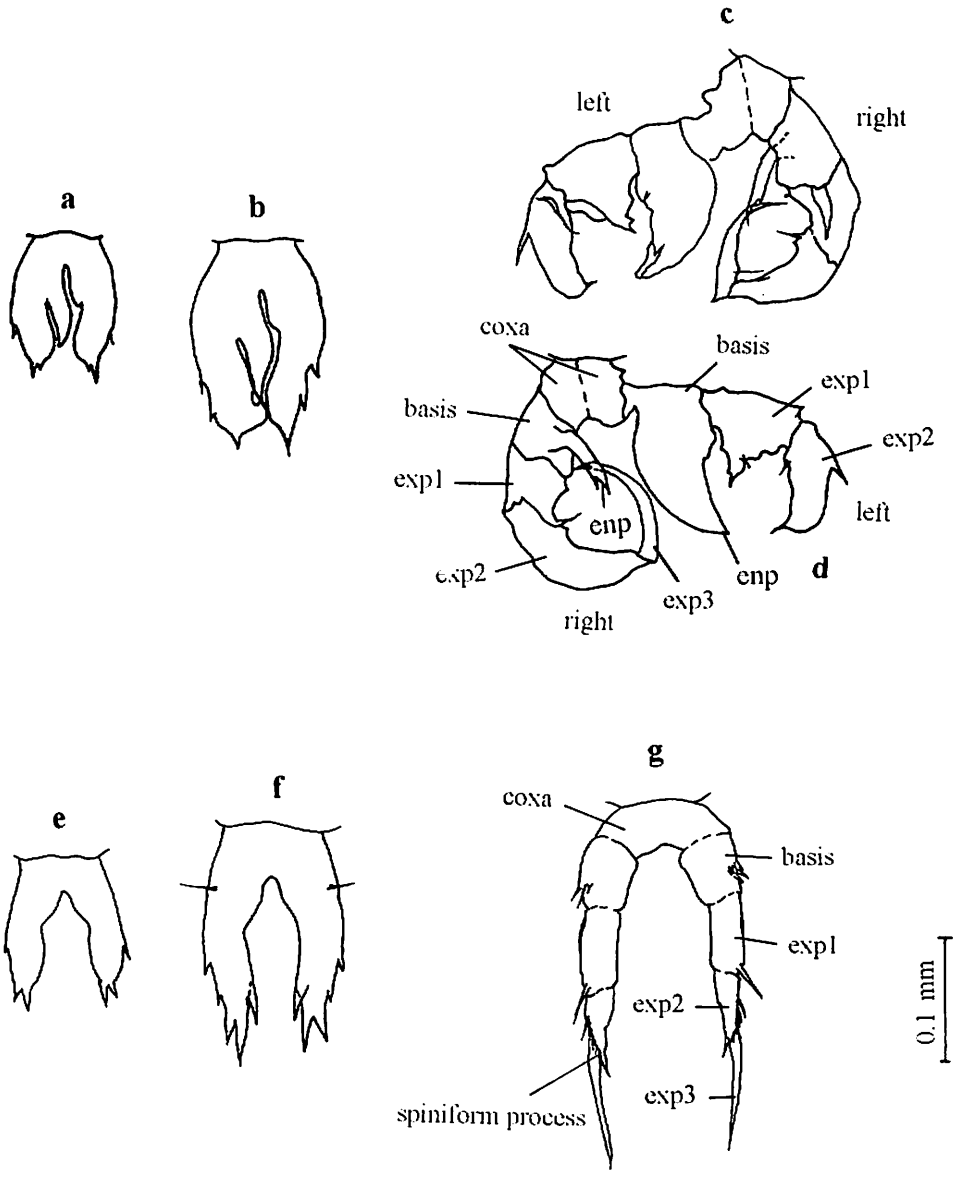

Fig. 3. Fifth swimming legs: a—CIV male; b—CV male; c—CVI male (posterior view); d—CVI male (anterior view); e—CIV female; f—CV female; g—CVI female; exp1—first exopod; exp2—second exopod; exp3—third exopod; enp—endopod.

ming leg (P5) was very morphologically different compared to P5 in CV. The caudal rami had 6 setae.

Male: Body length was 1.05±0.04 mm. Urosome was 5-segmented. The last dorsal thoracic segment had a pair of small spines and 2 rows of fine spinules; at dorsal view, 2nd, 3rd, and 4th urosomal segments had a very fine row of spinules (Fig. 2k, l). The antennule (A1) was asymmetrical—left A1 with 22 segments; right A1 with 21–22 and geniculation between the 18th and 19th segments (Fig. 4a). P5 was asymmetrical, divided into many segments and complicated shapes; inner side of right leg had a rectangular-shaped and unsegmented endopod; exopod was composed of 3 segments; the 3rd segment was sickle-shaped and had 1 spine; end of right endopod was slightly bifurcate while the left endopod formed a large projecting process (Fig. 3c, d).

Female: Body length was 1.10±0.02 mm. The urosome had 4 segments. Ovigerous individuals carried a pair of eggsacs (Fig. 2s) containing 4 to 22 eggs. Antennule (A1) was symmetrical and with 22 segments (Fig. 4b). The last thoracic segment had 2 rows of spinules on the dorsal side; 2nd and 3rd urosomal segments with very fine spinules on the dorsal end side; ventral outer margin of 5th thoracic segment was lined with serrated spinules that were clearly observable in lateral view. The 1st urosomal segment had a large fold on the ventral side and it also had a pair of stout spines extending laterally. Just after molting to the adult stage, the origin of the 1st urosomal segment had 3 spines. However, at full maturity, the 3 spines became fused and turned into a large distinct spine. It was also observed that the thin 3rd furcal seta became thick at full maturity (Fig. 2q, r). The overall shape of P5 was slender; outer margin of

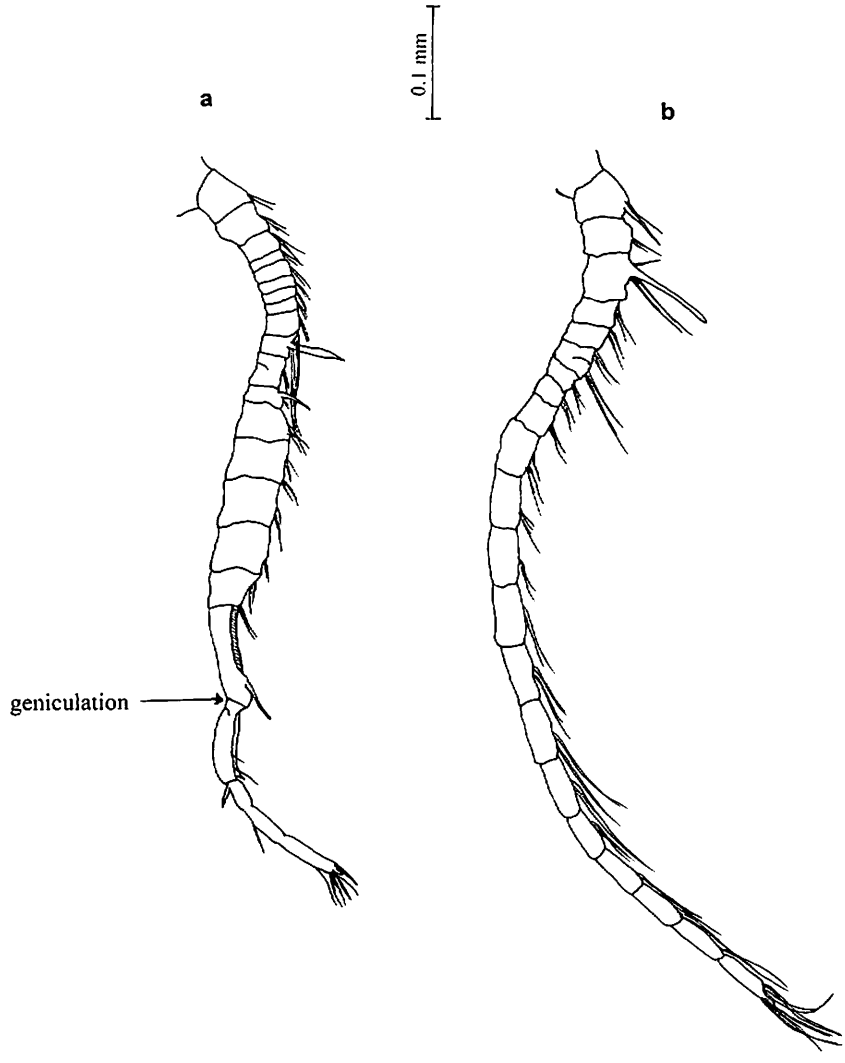

Fig. 4. Right antennule of male (a) and female (b); geniculation in male's right antennule was found between the18th and 19th segments.

basis had 5 spines; spinules appeared between the 1st and 2nd exopodal segments; the end of the 2nd exopodal segment bore a spiniform process; 3rd exopodal segment formed a long, narrow and sharp structure (Fig. 3g).

Spawning pattern and production of nauplii

Eggs of females incubated at 15°C did not hatch out while those at 20, 25, 28 and 33°C hatched successfully. All females at 20°C spent 4 to 5 days to hatch out 2 clutches of viable eggs while it only took 2 to 3 days for females at 25, 28, and 33°C. Total egg production per female at all temperatures ranged from 8 to 20 with an average of 4 to 10 eggs per clutch (Table 1). Egg production of females incubated at 20°C was significantly lower compared to females at higher temperatures ($p<0.05$, Fisher's Least Significant Difference Test). Females generally shed off the succeeding clutches of eggs after the second clutch hatched out.

Females paired with males in a 1:1 ratio produced 9 clutches of viable eggs in 11 days. All of these clutches of eggs hatched out successfully within 24 hours from spawning. The average number of nauplii produced per clutch was 14±1.76 (SD). Nauplii production per female was 133±14.1 for the 11 day period (Table 1). Production of nauplii by females paired with males was continuous from Day 1 to Day 6 but ceased on Day 7 (Fig. 5). No females bore any eggsacs on Day 6. On Day 7, however, females extruded eggsacs and nauplii production resumed on Day 8. In contrast to females with mates, females that were isolated from males produced only two clutches of viable eggs (Table 1, Fig. 5). After successful hatching of the second clutch, the succeeding clutches of eggs were aborted or shed off within 48 hours and never hatched out.

Post-embryonic development at different temperatures

P. annandalei were able to develop to the adult stage at all temperatures tested. Stage durations were similar in all

Table 1. Number of clutches and nauplii produced by *P. annandalei* females which were individually isolated or paired with a male in micro-dishes at different temperatures. Duration of each experiment was 11 days. Values with different superscripts are significantly different (Fisher's Least Significant Difference Test, $p<0.05$).

Temperature (°C)	Number of Females	Total Number of Clutches	Number of Viable Clutches	Avg. Nauplii Production per Clutch	Total Nauplii Production per Female
Isolated Female					
15	6	1	0	0	0^a
20	6	3	2	4	8 ± 2.52^b
25	6	8	2	10	20 ± 1.01^c
28	6	8	2	10	19 ± 3.21^c
33	6	8	2	10	19 ± 2.08^c
Female with mate*					
28	5	9	9	14±1.76	133±14.1

* Each female was paired with amale. Nauplii production in these females was not included in the statistical analysis.

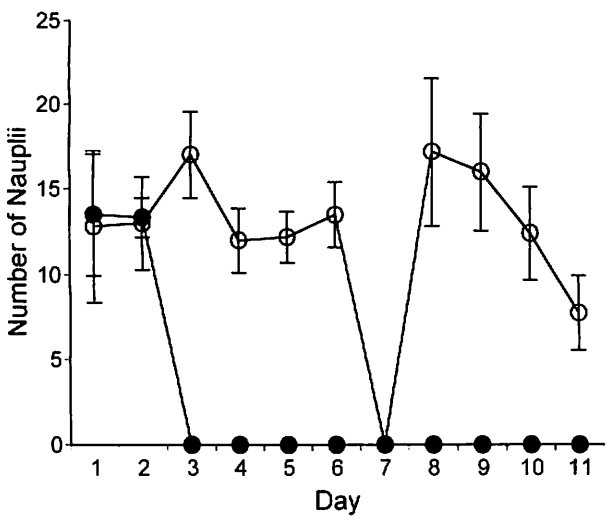

Fig. 5. Nauplii production of isolated females (●) and females paired with males (○). Error bar denotes standard deviation.

stages except in the first naupliar stage which was very short. The post-embryonic development of copepods at 20, 25, 28, and 33°C, were regressed well according to the following linear equations, respectively:

$$T_{20}=-2.75+1.77S \quad (n=45;\ r^2=0.96),$$
$$T_{25}=-0.78+0.9S \quad (n=33;\ r^2=0.97),$$
$$T_{28}=-0.97+0.85S \quad (n=32;\ r^2=0.93),$$
$$T_{33}=-0.68+0.9S \quad (n=26;\ r^2=0.98).$$

where T (day) is the time at which a particular stage was attained, and S is the developmental stage. The subscript to T denotes temperature. From the linear functions above, the adult stage was attained in 19, 10, 9.2, and 10.1 days at 20, 25, 28, and 33°C, respectively (Fig. 6A). Assuming a constant rate of development for all stages, stage-specific duration was similar at 25 (0.9), 28 (0.85) and 33°C (0.9) and longer at 20°C (1.77) ($p<0.05$, Comparison of Slopes)

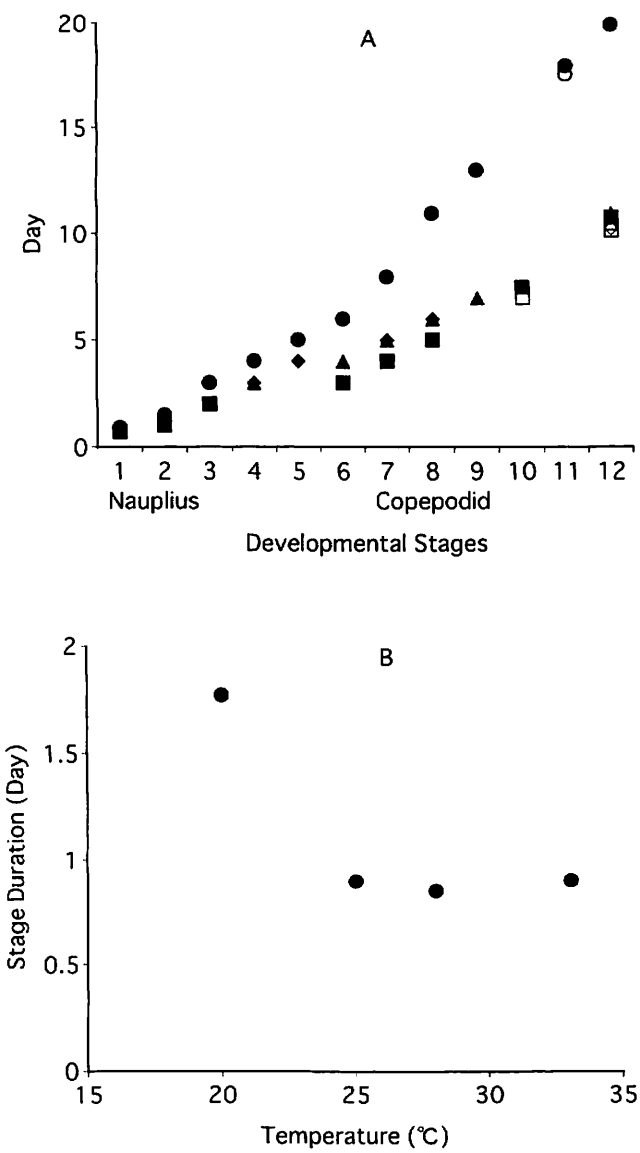

Fig. 6. A: Development of *P. annandalei*; (◆) 20°C; (■) 25°C; (▲) 28°C; (●) 33°C; B: stage duration at different temperatures.

Table 2. Body length (prosome length in the case of CIV to CVI stages) of *P. annandalei* reared at different temperatures. No significant differences among treatments were found (Comparison of Slopes, $p<0.05$). Values are means±standard error. Blank in the column means no data.

Developmental Stages	Temperature (°C)			
	20	25	28	33
NI	0.15±0.001 (n=3)			
NII	0.18±0.001 (n=5)	0.18±0.01 (n=5)	0.18±0.02 (n=5)	0.18±0.01 (n=5)
NIII	0.22±0.001 (n=5)	0.22±0.01 (n=3)	0.25±0.03 (n=3)	0.24±0.01 (n=5)
NIV	0.24±0.02 (n=3)	0.28±0.004 (n=5)		0.29±0.02 (n=3)
NV	0.27±0.11 (n=3)			0.31±0.01 (n=3)
NVI	0.33±0.02 (n=3)	0.32±0.001 (n=3)	0.32±0.02 (n=3)	
CI	0.38±0.02 (n=4)	0.33±0.005 (n=3)	0.33±0.001 (n=3)	0.38±0.04 (n=3)
CII	0.45±0.09 (n=3)	0.45±0.04 (n=4)	0.36±0.05 (n=3)	0.48±0.001 (n=3)
CIII	0.52±0.02 (n=3)	0.5±0.01 (n=3)		
CIV♂			0.54±0.04 (n=3)	
CIV♀			0.61±0.04 (n=3)	
CV♂	0.64±0.01 (n=3)			
CV♀	0.69±0.06 (n=3)		0.7±0.001 (n=3)	
CVI♂	0.72±0.01 (n=4)	0.7±0.01 (n=3)	0.68±0.01 (n=3)	
CVI♀	0.8±0.001 (n=3)	0.8±0.01 (n=4)	0.8±0.02 (n=3)	0.75±0.01 (n=4)
Growth Coef. (g)*	0.15	0.14	0.14	0.11

* Growth coefficients were derived from the slope of the exponential equation ($L=L_o e^{gt}$) as described in the Materials and Methods.

(Fig. 6B). Males tend to develop faster than females (Fig. 6A). Females at 25, 28, and 33°C started carrying ovisacs two days after reaching maturity while those at 20°C started spawning 1 day later. Hatching of nauplii occurred within 24 hours from spawning at all temperatures.

Body length in some post-embryonic stages varied to some extent at different temperatures. However, based on the exponential regressions of body length, the growth coefficient, g, did not vary significantly among temperatures 25°C (0.14), 28°C (0.14), and 33°C (0.11) ($p<0.05$, Comparison of Slopes) (Table 2).

Discussion

In the present study *Pseudodiaptomus annandalei*, released its first naupliar stage, with very short duration (ca. 3 hours), at 25°C. Uye & Onbe (1975) also reported their observation of the first naupliar stage of *P. marinus*. There are contrary reports that the first naupliar stage in other diaptomids is brooded within the egg sac (Johnson 1948; Jacobs 1961; Ummerkutty 1964; Grice 1969; Alvarez & Kewalramani 1970; Björnberg 1972). These authors could have missed the emergence of the first naupliar stage from the ovisac due to the very short duration of NI.

Morphological differences in the naupliar stages lie mostly in segmentation of the antennule, development of the feeding appendages, and proportion of the terminal elements. The most dramatic alteration in body form of *P. annandalei* occurs at the transition from the last naupliar stage (NVI) to the first copepodid stage (CI) at which segmentation, size and appendage structure change substantially. Specifically, new body segments or somites are formed by lengthening of the anal segment and subsequent constriction of the proximal portion (Hulsemann, 1991). Copepodid IV and V had slight morphological differences in the swimming legs. These copepodid stages, however, were far different from the adult, especially in the modification of the male's 5th swimming legs. Walter (1987) distinguished *P. annandalei* from other species of *Pseudodiaptomus* by the presence of laterally directed spines on the first urosomal segment and the presence of serrated spines at the ventral edge of the last prosomal segment in adult females. We observed a significant change in this laterally directed spine of the adult female during early and full maturation. This spine on the lateral side of the urosome appeared to be 3 spines clumped together right after molting to the adult stage. However, these spines became fused as the copepod progresses towards maturity. This change could be a preparatory stage for mating. That is, fused spines could be an indication that a female is matured and ready to mate.

Mating behavior in planktonic copepods (Blades & Youngbluth 1979; Buskey 1998) and in several species of *Pseudodiaptomus* (Jacoby & Youngbluth 1983) has been described. Fertilization of matured oocytes in pseudodiaptomids is accomplished by copulation when the male transfers one or more spermatophores to the genital somite of the female. However, only one spermatophore can fertilize the eggs (Jacoby & Youngbluth 1983). In the experiment, multiple spermatophore attachment in females paired with males was never observed, although this is common in ovigerous females collected from ponds in the Philippines (Golez, unpublished data) where some females bear several

spermatophores. Multiple spermatophore attachment is common in heterospecific mating (Jacoby & Youngbluth 1983) which is highly likely to occur in the field.

There are a number of studies relating egg production and hatching rate to temperature in calanoid copepods, some of which were reported by Corkett & McLaren (1970), Williamson & Butler (1987), White & Roman (1992), McLaren & Leonard (1995), and Liang & Uye (1997). In the present study, the effect of temperature on reproduction of *P. annandalei* was assessed in terms of viable egg production (eggs that hatched out successfully). Hatching of the eggs was deterred at 15°C and delayed for 1 day at 20°C relative to the other higher temperatures, reflecting the adverse effect of low temperature on egg hatching. Low temperatures also prolonged the ovigerous condition of the female, while their ovaries still bear dark matured oocytes. These matured oocytes may have been fertilized initially but were unable to hatch due to low temperatures. Viable egg production was restricted to only two clutches in females isolated from males, whereas, it was continuous in females paired with males. This implies that the presence of males is crucial in sustaining nauplii production. Like other pseudodiaptomids, reproduction in *P. annandalei* is multimating and thus the presence of males is very important (Williamson & Butler 1987; Wilson & Parrish 1971; Jacobs 1961). Females paired with males continuously spawned viable eggs. However, the matured oocytes were fully exhausted by multiple or repeated mating within 7 days. The resting phase or the period when females have exhausted their matured oocytes and thus ceased extruding eggs was extremely short (1 day). Resumption of spawning on Day 8 may imply that fertilization and maturation of the oocytes can take place within just a short period at high temperatures (28°C in this experiment).

Faster development of copepods reared at temperatures 25, 28, and 33°C than that at 20°C is supported by many reports on the inverse relationship between temperature and stage duration (Uye et al. 1982; Jerling & Wooldridge 1991; Takahashi & Ohno 1996; Liang & Uye 1997; Campbell et al. 2001). In the present study, the rates of development at 25, 28, and 33°C were almost the same. This may reflect the rates of development of *P. annandalei* in aquaculture ponds in the Philippines where year-round water temperatures ranged within 24–36°C (Golez, 2000).

Differences in body length between treatments did not vary significantly. As reported by Hada et al. (1986) on populations of *Sinocalanus tenellus* in a brackish water pond in Japan, little correlation could be found between body size and environmental temperature in situ. Klein-Breteler & Gonzalez (1982) found that food concentration had a greater influence on body size than temperature. Evans (1981) found that 83% of the variance of body size in *Temora longicornis* in the North Sea was explained by the abundance of the diatom Thalassiosira and only 7% by the temperature. Food, as expressed by chlorophyll *a* concentrations in the present experiment, was above $10\,\mu g\,l^{-1}$. This food level was considered to be in excess, since average chlorophyll a in the pond where *P. annandalei* was collected is usually below $10\,\mu g\,l^{-1}$. Body length of the copepods did not vary at different temperatures, probably because our experiments were not continued through several generations of *P. annandalei*. Alternatively, the range of temperature (25–33°C) used in the experiment must be within the range for optimum growth of *P. annandalei*.

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