Sexual Dimorphism and Reproduction of the Amphipod *Hyale crassicornis* Haswell (Gammaridea: Hyalidae)

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Kwok-Ho Tsoi and Ka-Hou Chu (2005) Sexual dimorphism and reproduction of the amphipod *Hyale crassicornis* Haswell (Gammaridea: Hyalidae). *Zoological Studies* **44**(3): 382-392.   In the present study, we explored the sexual maturation, sexual dimorphism, and fecundity of a hyalid amphipod *Hyale crassicornis* in the laboratory.   This amphipod passed through 11 to 21 instars, and the mean lifespan of males and females was 178 and 175 d, respectively.   Males and females attained sexual competency between instars 5 and 7 at an age of 34~44 d, and between instars 5 to 8 at an age of 38~53 d, respectively.   Precopulatory pairing behavior was the criterion used for the onset of sexual maturity.   Nine of 10 characters studied exhibited sexual dimorphism. Transitions of growth phases can be revealed from the allometric relationships of the sexually dimorphic gnathopods 1 and 2 to body length.   Male gnathopods 2 grew in 3 phases, while female gnathopods 1 and 2 grew in 2 phases.   Each female generated a total of 2 to 17 (mean, 6) broods.   Brood size ranged from 3 to 33 (mean, 13) offspring and was positively correlated with the body length of the female.   Each female produced 16 to 290 offspring throughout its lifespan. http://zoolstud.sinica.edu.tw/Journals/44.3/382.pdf

Key words: Amphipod, *Hyale crassicornis*, Hyalidae, Growth, Reproduction.

Sexual dimorphism is often strong in amphipods (Barnard 1975).   The sexes of gammarideans can usually be distinguished by differences in secondary sexual characters, including body size, gnathopods (Moore and Wong 1996), and antennae (Barnard 1971, McLaughlin 1980). Development of these secondary sexual characters reflects sexual differentiation and maturation (Hartnoll 1982).   The growth of variable character Y (a sexual character measurement) relative to reference character X (body length measurement) is expressed by the allometric equation, $Y = aX^b$, in which the constant "a" represents the proportional factor and "b" the allometric coefficient (Huxley 1924).   Any change in level of allometry (allometric coefficient) indicates a variation in the relative growth rate and hence reveals a growth-phase transition from the sexually immature phase to pre-mature or mature phases (Charniaux-Cotton 1957, Hartnoll 1978).   For instance, a change in the allometric relationship between the flagellar length of antenna 2 and body length is a good indicator of sexual maturity in male *Orchomene nanus* (Moore and Wong 1996).

The onset of precopulatory pairing behavior (involving males grasping and carrying females), which can be induced by a series of pheromonal stimulations (Borowsky 1990), occurs in some amphipods once sexual maturity is attained. Precopulatory pairing behavior indicates the maturity of males and females.   As sperm cannot be stored in female amphipods, mature males pair with females until the completion of ovulation and fertilization (Borowsky 1990).   Because this behavior is energetically costly (Calow 1979, Moore 1986a) and highly risky (Strong 1973), males begin to pair with females only when ovulation is about to occur following molting (Iribarne et al. 1995).   A study of *Corophium volutator* showed that mature males selected females on the basis

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of their time to receptivity rather than their body size (Forbes et al. 1996).

Hyale crassicornis (Gammaridea: Talitroidea: Hyalidae) is distributed in intertidal areas of Kiama, New South Wales, Cape Naturaliste and Western Australia (Barnard 1974). Information on the spatial distribution of this amphipod species in Southeast Asia is still limited. In Hong Kong waters, particular *Hyale* species are recognized (Jiang and Zhou 1982, Moore 1986b, Ren 1994), but these do not include *H. crassicornis*. However we have collected this species from the intertidal zone in eastern waters of Hong Kong. It is commonly found beneath rocks and pebbles, and is often associated with the seaweed *Ulva lactuca*. The present study reports the growth pattern and reproductive biology of *H. crassicornis*. Such information will enhance our understanding of the biology of this species found in Southeast Asia.

MATERIALS AND METHODS

Culture

The amphipod *H. crassicornis* was collected from Tolo Harbor, Hong Kong and reared in 3 outdoor culture tanks at the Marine Science Laboratory, The Chinese University of Hong Kong. Each tank contained 3000 L of natural seawater with the turnover rate of ~2600 L/d. For the growth study, ovigerous females were randomly collected from the tanks. No more than 2 freshly released juveniles were taken from each female. Each juvenile was individually reared in a 100 ml glass beaker filled with 80 ml of artificial seawater (Instant Ocean®, Aquarium Systems, Mentor, USA). Every 2 d, the water temperature, salinity, pH, and dissolved oxygen content of the experimental vessels were monitored, and about 40 ml of the culture water was replaced with freshly prepared and well-aerated artificial seawater. Each individual was fed a small piece (with a volume of 1~2 mm^3) of freeze-dried krill after water replacement. Since this amphipod was found to live and graze on the green alga *U. lactuca*, a piece of thallus (10 cm^2) was also provided as shelter and a supplementary food source for the amphipods. The thallus piece was replaced once it had been severely grazed. Any fecal matter, food residue, exuviae, and undesirable algae were removed during the water exchange. Throughout the experimental period, the range of water temperature was recorded as 24~26°C, salinity as 30~33 ppt, pH

values as 6.5~8.2, and dissolved oxygen contents as 4.9~7.0 mg/L. A 14:10 h light: dark regime at a light intensity of between 857 and 1212 lux was maintained.

Growth

Twenty-five amphipods (including 9 males and 16 females) were successfully reared throughout their lifespan and examined daily for molting. Parameters including body length (from the rostrum to the posterodistal end of urosomite 3 in a naturally curved posture), length and articular numbers of left antennae 2 (from the posterior end of the peduncle to the anterior tip of the flagellum), and propodus length of the left gnathopods 1 and 2 (the linear distance between 2 adjacent joints of the dactylus and carpus) were measured 1 d after molting. For these measurements, the amphipod was placed on a glass slide and covered with a small sheet of thin plastic film. Each amphipod was examined for no more than 2 min to minimize any effect of hypoxia and environmental stress. The image of the amphipod was recorded using a compound microscope (Nikon model SE, Nikon Corporation, Tokyo, Japan) and a color camera set (Teli® CCD CS 5110, Tokyo Electronic Industry, Tokyo, Japan), and the various body parameters were measured to 10^{-2} mm with a video-based image analyzing program (Quantimet 500C, Leica, Wetzler, Germany). Data collections were limited to instar 15 for males and instar 18 for females because there were only 2 males and 3 females which survived beyond these specified instars.

Sexual maturation

Another batch of newly hatched juveniles (including 11 males and 13 females) were collected and reared under standardized laboratory conditions as described above. Once an experimental juvenile attained instar 4 or sexual differentiation, another sexually mature individual of the opposite sex and of similar size was collected from the outdoor culture tanks and introduced into the experimental vessel for rearing with the juvenile. The pair was fed with 2 pieces of krill flesh with a volume of 1 mm^3. A small piece of *U. lactuca* thallus was provided as shelter and a supplementary food source. Maintenance was carried out as described above.

Each pair of amphipods was examined daily for molting, precopulatory pairing, and juvenile release. The 1st occurrence of precopulatory pair-

ing behavior and production of a clutch shortly after the behavior indicated that such experimental amphipods (of either sex) had attained sexual maturity. In total, 24 experimental amphipods, including 11 males and 13 females were successfully reared until maturity. The 13 females were continuously observed throughout their entire lifespan. Occurrences of all pairing behaviors and numbers of juveniles released from each brood were recorded until the death of the females.

Fecundity

A 3rd batch of ovigerous females was collected from the outdoor culture tanks for elucidating the relationship between fecundity and body length. Water temperatures were recorded as 23~25°C, salinity as 32~33 ppt, pH values as 8~9,

and dissolved oxygen content as > 7.0 mg/L. In total, 102 females carrying early (stage I) eggs were screened. Numbers of stage I eggs (Sainte-Marie et al. 1990, Jones and Wigham 1993) liberated from the marsupium were counted under a dissecting microscope, and the body lengths of these females were measured. A regression line of brood size versus female body length was obtained using the method of least squares.

Statistical analysis

The data were analyzed with linear regression, Student's *t*-test, two-factor analysis of variance (ANOVA), and Tukey test according to Zar (1996). The test statistics of the growth phase lines was calculated based on the method described by Somerton (1980). The computer

Table 1. Lifespan and the total number of instars recorded in *Hyale crassicornis* (SD, standard deviation)

Male

Individual	1	2	3	4	5	6	7	8	9								Mean	SD
Lifespan (d)	262	164	248	191	199	167	111	136	121								177.7	52.9
(instar)	19	13	15	15	16	15	12	12	12								14.3	2.3

Female

Individual	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Mean	SD
Lifespan (d)	170	201	211	159	254	150	99	143	143	258	213	138	122	185	197	150	174.6	45.2
(instar)	17	17	18	18	21	15	13	15	16	18	21	13	18	20	17	11	16.8	2.8

Table 2. Summary of sexually dimorphic characters in *Hyale crassicornis*. Instar refers to the number of instars at which the parameter of the 2 sexes began to deviate (Tukey test after 2-factor ANOVA with sex and instar as factors)

Parameters	Sexual dimorphism	Instar	*p* value
Body length (BL)	male > female	7	= 0.016
Head length (HL)	male > female	7	= 0.021
Eye length (EL)*	male = female	~	= 0.536
Length of antenna 1 (AL1)	male > female	7	= 0.001
Length of antenna 2 (AL2)	male > female	6	= 0.005
Number of articles on antenna 1 (AA1)	male > female	7	= 0.030
Number of articles on antenna 2 (AA2)	male > female	7	= 0.011
Propodus length of gnathopod 1 (GL1)	male > female	6	= 0.008
Propodus length of gnathopod 2 (GL2)	male > female	4	= 0.006
Merus length of pereopod 7 (MP7)	male > female	7	= 0.016

*No significant deviation between the 2 sexes was found at all developmental stages.

software programs SigmaStat version 2.03 (SPSS, Chicago, USA) and SigmaPlot 2000 (SPSS, Chicago, USA) were employed for calculations and graphical presentation in the data analysis.

RESULTS

Lifespan

The mean lifespans of the 9 males and 16 females were 178 ± 53 (mean ± SD) and 175 ± 45 d, respectively (Table 1). The 2 values did not significantly differ (Student's *t*-test; *p* = 0.878). The maximum lifespans of males and females were 262 and 258 d, respectively. The mean lifespans of the males and females encompassed 14 ± 2 and 17 ± 3 instars, respectively. These 2 values significantly differed (*p* = 0.041). The maximum numbers of instars reached by males and females were 19 and 21, respectively.

Growth and sexual dimorphism

Nine of 10 characters studied exhibited sexual dimorphism (Table 2). Sexual differentiation was first detected in propodus length of gnathopod 2 at instar 4 (Tukey test; *p* = 0.006). This secondary sexual character is thus a potential indicator with which to differentiate sexes at an early stage in the life of this amphipod.

1. Body length (BL)

The mean BL of males was significantly greater than that of females (two-factor ANOVA;
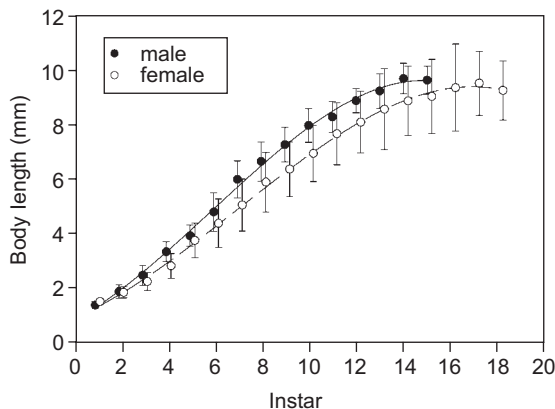
Fig. 1. Body length of *Hyale crassicornis* at different instars. *N* = 9 (male), and 16 (female). Error bars represent the standard deviation.

with sex and instar as factors; *p* < 0.001) (Fig. 1). Deviation between the 2 sexes became significant at instar 7 (Tukey test; *p* = 0.016). The maximum BL was 11.02 mm for a male at instar 16 (data not shown) and 11.38 mm for a female at instar 17.
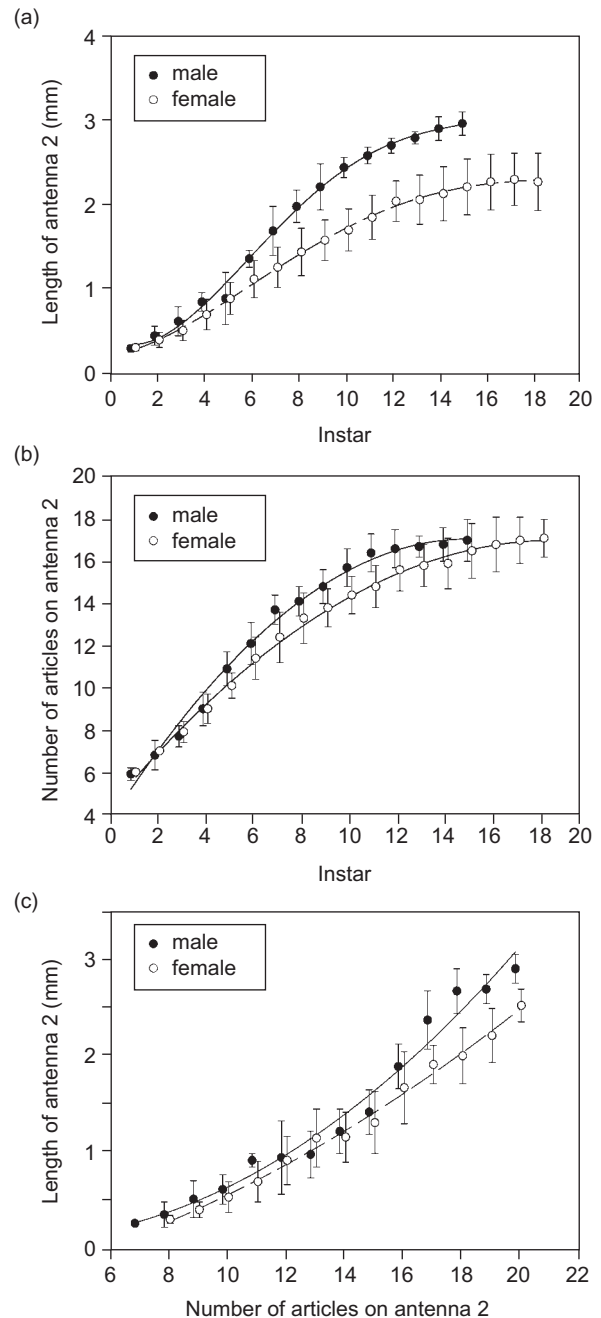
Fig. 2. (a) Length of antenna 2 (AL2) at different instars. (b) Number of articles on antenna 2 (AA2). (c) Relationship between AL2 and AA2. Regression equations of the best-fit curves in males and females were $Y = 0.01X^2 - 0.06X + 0.17$ ($r^2 = 0.977$) and $Y = 0.01X^2 + 0.06X - 0.47$ ($r^2 = 0.993$), respectively. Error bars represent the standard deviation.

## 2. Length of antenna 2 (AL2)

The mean AL2 (Fig. 2a) and the number of articles on antenna 2 (AA2) (Fig. 2b) were significantly greater in males than in females (2-factor ANOVA, sex/instar; $p < 0.001$). Antenna 2 exhibited more-pronounced sexual dimorphism than antenna 1 (data not shown). Deviation of AL2 between the 2 sexes became significant at instar 6 (Tukey test; $p = 0.005$). A non-linear relationship between AL2 and AA2 was observed (Fig. 2c). This relative growth showed that AL2 of males was significantly greater than that of females (2-factor ANOVA, sex/AA2; $p < 0.001$). The articular length of the 2 sexes did not significantly differ (Tukey test; $p > 0.05$) when the number of articles was below 16. The male AL2 became longer than the female AL2 from the article-16 stage (equivalent to instar 7 or older) onwards (Tukey test; $p = 0.002$).

3. Propodus length of gnathopod 2 (GL2)

The GL2 (Fig. 3a) and propodus length of gnathopod 1 (GL1) (data not shown) were significantly longer in males than in females (2-factor ANOVA, sex/instar; $p < 0.001$). Deviation between the 2 sexes became significant at instar 4 (Tukey test; $p = 0.006$) for GL2 and at instar 6 ($p = 0.008$) for GL1. Gnathopod 2 exhibited the strongest sexually dimorphic characteristic among all of the characters studied. The ratio of GL2/GL1 in males increased from 1.0 for the 1st 3 instars to a peak at 2.6 for instar 12 (Fig. 3b). This ratio in females slightly increased from 1.1 to the highest value of 1.4 at instar 18. In females, it did not exceed 1.5 at any instar.

Sexual maturation
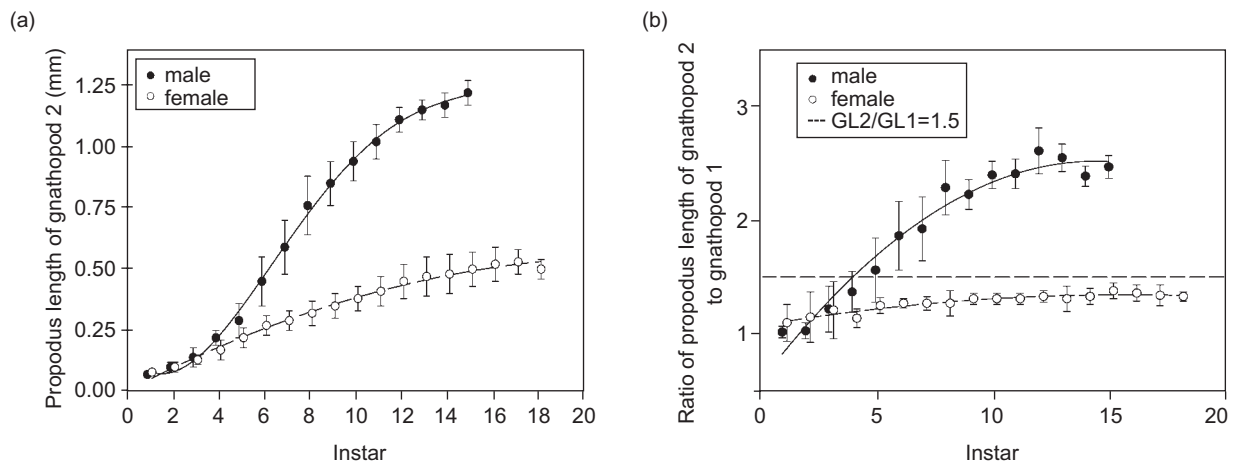
Fig. 3. (a) Propodus length of gnathopod 2 (GL2) at different instars. (b) Variation of the ratio of GL2/propodus length of left gnathopod 1 (GL1). Regression equations of the best-fit curves in males and females were $Y = -9 \times 10^{-3}X^2 + 0.267X + 0.595$ ($r^2 = 0.967$) and $Y = -1 \times 10^{-3}X^2 + 0.034X + 1.078$ ($r^2 = 0.911$), respectively. Error bars represent the standard deviation.

Table 3. Appearance of precopulatory pairing behavior in pairs of *Hyale crassicornis* (SD, standard deviation)

Male

Individual	1	2	3	4	5	6	7	8	9	10	11			Mean	SD
No. of instars	6	7	6	5	7	7	6	6	7	6	6			6.3	0.6
Age (day)	42	44	37	34	38	43	38	39	40	35	41			39.2	3.2
Body length (mm)	5.26	5.30	5.35	4.69	4.45	5.53	4.50	4.32	5.52	3.86	6.15			4.99	0.68

Female

Individual	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean	SD
No. of instars	8	7	6	6	6	6	6	6	8	5	8	7	7	6.6	1.0
Age (day)	46	40	45	53	38	43	41	45	40	38	41	45	45	43.1	4.1
Body length (mm)	5.04	4.62	4.29	6.13	5.22	5.56	4.72	4.92	4.60	4.16	5.14	4.98	4.33	4.90	0.55

Precopulatory pairing behavior in females began between instars 5 and 8, at an age of between 38 and 53 d (Table 3). All females had a fully developed marsupium at the 1st incidence of pairing. The marsupium was formed by the overlapping of 4 pairs of bilateral and spoon-like oostegites with entanglement of the fringing brood setae. The oostegites began to develop in the lateroventral region of pereon segments 2 to 5 during instars 3 to 4. In males, precopulatory pairing behavior began between instar 5 and 7, at an age of between 34 and 44 d. Males attained maturity at a significantly earlier age than did females (Student's *t*-test; *p* = 0.018). However, there was no significant difference between the 2 sexes in sexual maturity in terms of instar number (*p* = 0.443) or body length (*p* = 0.702).

The allometric relationships of gnathopods 1 and 2 to body length were determined. In males, only 1 growth phase was observed for GL1, while the growth of GL2 was differentiated into 3 phases (Fig. 4a). A statistical test based on the residual sum of squares about the phase lines showed that it was not significant to use 2 lines to describe the data of the male GL1 (*p* = 0.326), but the resulting 3 lines fit the data significantly better than a single line (*p* < 0.0001). The allometric coefficient of the best-fit regression line of GL1 (b = 1.01) did not deviate from unity (Student's *t*-test; *p* = 0.425), indicating that GL1 grew isometrically. In the 3 growth phases of GL2, it grew with positive allometry in the immature and mature phases with both coefficients exceeding unity (Student's *t*-test; p < 0.0001), and with the allometric coefficient of the mature phase (b = 1.64) greater than that of the immature phase (b = 1.28) (Student's *t*-test; *p* < 0.0001). The transition from the immature phase to the mature phase occurred at a BL of about 3~4 mm (equivalent to instar 4~5). However, once the BL reached 8 mm (equivalent to instar 9~10 or older), GL2 grew in a post-mature phase, and the allometry became negative (b = 0.82) with a coefficient smaller than unity (Student's *t*-test; *p* = 0.0016).

In females, both the GL2 (Fig. 4b) and GL1 (Fig. 4c) grew in 2 growth phases. The resulting 2 lines fit the data better than a single line for both GL2 (*p* < 0.0001) and GL1 (*p* = 0.0001). The allometric coefficients of the immature and mature phases significantly differed (*p* < 0.0001) for both the GL2 and GL1. Growth of the GL2 switched from the immature phase with positive allometry (b = 1.16) exceeding unity (Student's *t*-test; *p* = 0.0005) to the mature phase with negative allome-
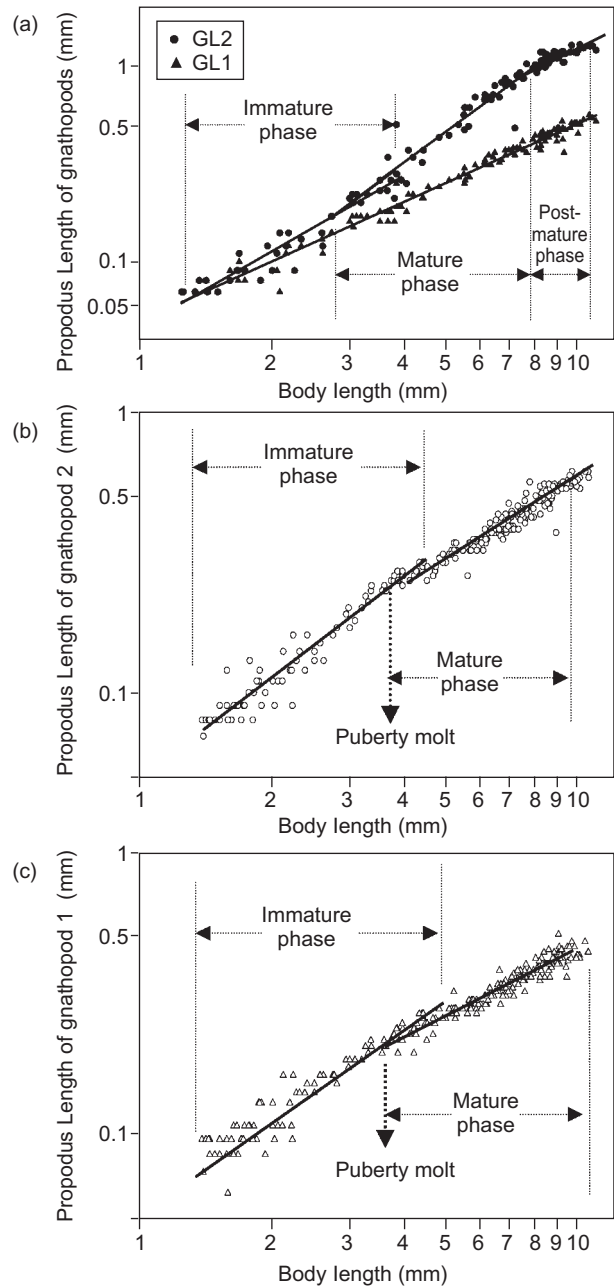
Fig. 4. (a) Relative growth of propodus length in gnathopod 1 (GL1) and gnathopod 2 (GL2) to BL in males (*N* = 120). Regression equations of the best-fit line for GL1 was $\log_{10}GL1$ = $1.01\log_{10}BL$ - 1.30 (r^2 = 0.980). For GL2, regression equations of the best-fit lines for immature, mature, and post-mature phases were $\log_{10}GL2 = 1.28\log_{10}BL$ - 1.34 (r^2 = 0.925), $\log_{10}GL2 = 1.64\log_{10}BL$ - 1.48 (r^2 = 0.857), and $\log_{10}GL2 = 0.82\log_{10}BL$ - 0.74 (r^2 = 0.666), respectively. (b) Relative growth of GL2 to BL in females (*N* = 253). Regression equations of the best-fit lines for immature phase and mature phase were $\log_{10}GL2 = 1.16\log_{10}BL$ - 1.30 (r^2 = 0.936) and $\log_{10}GL2$ = $0.86\log_{10}BL$ - 1.13 (r^2 = 0.944), respectively. (c) Relative growth of GL1 to BL in females. Regression equations of the best-fit lines for the immature phase and mature phase were $\log_{10}GL1 = 0.99\log_{10}BL$ - 1.31 (r^2 = 0.923) and $\log_{10}GL1$ = $0.78\log_{10}BL$ - 1.19 (r^2 = 0.921), respectively.

try (b = 0.86) of smaller than unity (Student's *t*-test; *p* < 0.0001) at a BL of 3.8~4.5 mm (equivalent to instars 5~8). At a similar BL and life stage as did GL2, GL1 growth shifted from the immature phase with isometry (b = 0.99) not different from unity (Student's *t*-test; *p* = 0.413) to the mature phase with negative allometry (b = 0.78) of smaller than unity (Student's *t*-test; *p* < 0.0001).

Fecundity

The total number of broods generated throughout the lifetime of the laboratory-reared females varied from 2 to 17 (mean, 6.2). Females produced 3~33 (mean, 13.2) juveniles in each brood (Fig. 5a). In total, 16 to 290 offspring were produced throughout the lifetime of females (Fig. 5b). The mean and median fecundity values were 81 and 54 juveniles, respectively. The high variation in lifetime fecundities of these females was associated with their lifespan. Three females which died after carrying 2 broods produced an average of 17 offspring only. In comparison, 2 long-lived females with 15 and 17 broods each produced a total of 190 and 290 juveniles, respectively.

Females collected from the outdoor tanks produced 2~20 (mean, 9.8) juveniles per brood. This value was significantly lower than the corresponding value for laboratory-reared animals (Student's *t*-test; *p* < 0.001). Brood size was linearly correlated to body length (both in logarithmic scales) for ovigerous females (*p* < 0.0001) (Fig. 6).

DISCUSSION

Sexual dimorphism and maturation

This study shows that male *H. crassicornis* has significantly greater body size, longer antennae, and more-robust gnathopods than does the female. These sexually dimorphic characters occur in males for precopulatory pairing. Both sexes of *H. crassicornis* can grow up to 11 mm in body length. Mature males have a larger body size than mature females of the same instar. Body length is sexually dimorphic in other amphipod species such as *Caprella gorgonia* (Lewbel 1978), *C. danilevskii* (Takeuchi and Hirano 1991), and *Orchomene nanus* (Moore and Wong 1996). Larger males have been found to have a smaller chance of losing the female than smaller males during pairing (Iribarne et al. 1995). This selective advantage was evidenced in *Gammarus pulex* such that larger males paired with females sooner (Elwood et al. 1987) and for longer times (Ward 1983) than did smaller males. Studies on *Eogammarus oclairi* showed that a larger male has a higher chance of either outcompeting a smaller male for access to a female or taking over a female in pre-copula with a smaller male (Iribarne et al. 1996). In contrast, the size of females exhibits no selective advantage in pair formation. For instance, males of *Corophium volutator* select mates based on the time to receptivity, not on the size of the female (Forbes et al. 1996).

Barnard (1971) found that male amphipods have longer antennae than females. The well-
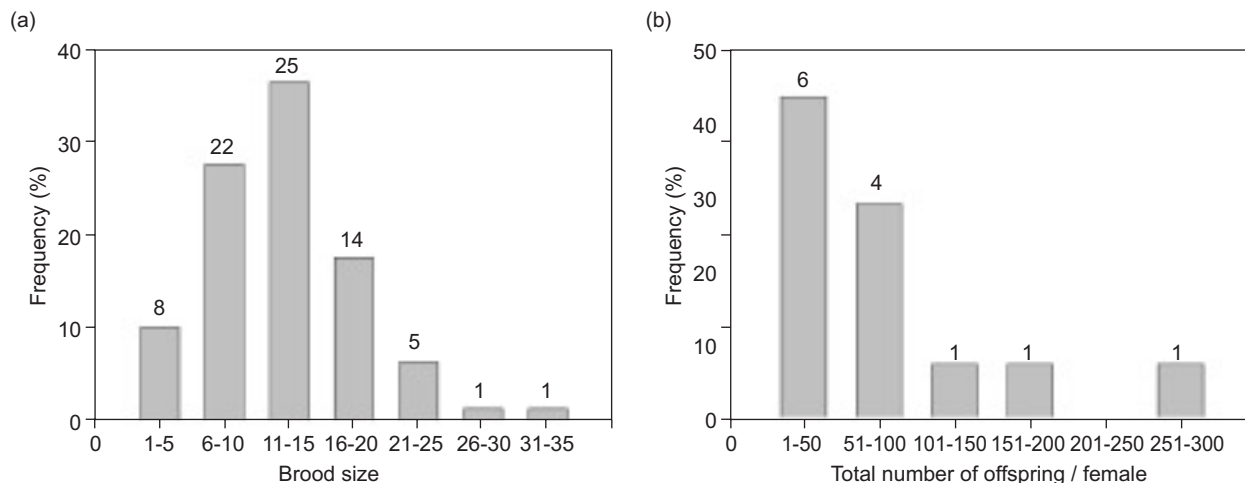
Fig. 5. Fecundity of laboratory-reared females (*N* = 13). (a) Frequency distribution of brood size. The number of broods is shown at the top of each bar. (b) Frequency distribution of total number of offspring generated throughout the lifetime of 13 females. The number of females is shown above each bar.

developed antennae 2 of the males are important for detection of sex pheromones released from receptive females for mate location recognition (Dahl et al. 1970, Borowsky 1990). Moore and Wong (1996) reported that flagella on antennae 2 of male *Orchomene nanus* become more elongated after sexual differentiation. The elongation is associated with a higher increment rate in the number of articles in the post-maturation stage. Males of *Talitrus saltator* have longer antennae 2 than do females due to the presence of more articles (Scapini et al. 1999). This study found that males of *H. crassicornis* have longer antennae 2 than do females at the same instar. The deviation was associated with a greater number of articles on the antennae of males than of females. Furthermore, the relationship between antennal length and articular numbers revealed that the articular length of males was greater than that of females when the article number reached 16 or greater (equivalent to instars 7~8 or older). Increases in articular length and number contributed to the lengthening of antennae 2 in the mature phase of males.

Gnathopods 2 are generally larger, more complex, and strongly ornamented in male amphipods (Barnard 1971). The enlarged gnathopods 2 of males have generally been claimed to be used in clasping the thoracic region of the coxal plates of females during precopulatory pairing (Borowsky 1984). However, gnathopods 2 of male *Gammarus duebeni* are used for stroking and brushing the female's head (Hartnoll and

Fig. 6. Linear correlation between brood size (stage I eggs) and body length (BL) of females (*N* = 102) on a $\log_{10}$ scale. Regression equation of the best-fit line was $\log_{10} Brood\ size = 3.01 \log_{10} BL - 1.59$ ($r^2 = 0.564$).

Smith 1978), and this brushing behavior may be associated with enhancing the flow of female pheromones in *G. palustris* (Borowsky 1990). In *Caprella gorgonia*, the enlarged gnathopod 2 of the male carries a poison spine that is possibly used against other males (Lewbel 1978). The size of gnathopod 2 was regarded by Hiwatari and Kajihara (1988) as an index for sexual differentiation in *Hyale barbicornis* during sexual maturation. In the present study, the enlargement of gnathopod 2 in *H. crassicornis* was indicated by the relative growth ratio of the propodus lengths of gnathopod 2 to gnathopod 1. This ratio is suggested to be an effective index revealing sexual dimorphism of this species. This ratio in male *H. crassicornis* increased from 1.0 in the 1st 3 instars to a maximum of 2.6 in instar 12, while that of the female only increased slightly from 1.1 in instar 1 to 1.4 in instar 18.

Direct observations of the occurrence of precopulatory pairing behavior revealed that males of *H. crassicornis* attain sexual competency between instars 5 and 7 at an age of 34~44 (mean, 39) d. Females acquire sexual competency between instars 5 and 8, at an age of 38~53 (mean, 43) d. However, another hyalid, *H. barbicornis*, reaches sexual maturity between instars 8 and 12 (equivalent to an age of about 35 d) in the male and between instars 9 and 13 in the female (at an age of about 49 d) at 24°C (Hiwatari and Kajihara 1988). Another gammaridean, *Orchomene nanus*, sexually matures at instar 8 (Moore and Wong 1996). A caprellidean, *Caprella danilevskii*, attains maturity at instar 7 (Takeuchi and Hirano 1991). In this study, the results obtained from direct observations of the occurrence of precopulatory pairing behavior are concordant to the results obtained from the relative growth patterns of gnathopods. In female *H. crassicornis*, the allometric relationships of propodus length on gnathopod 1 or gnathopod 2 to body length show that the pubertal molt occurred at between instars 5 and 8. Both characters of the female showed an abrupt decline in extent of allometry after the pubertal molt. Such a pubertal molt might also be used to differentiate the 2 growth phases occurring in *Pseudoprotella phasma* (Harrison 1940).

In males, no distinctive pubertal molt was observed in the growth of GL1 while 3 growth phases were differentiated in the growth of GL2. At instars 4~5, a transition from the immature phase to the mature phase occurred. The occurrence of this pubertal molt was concordant to the timing of the 1st appearance of the pre-copula
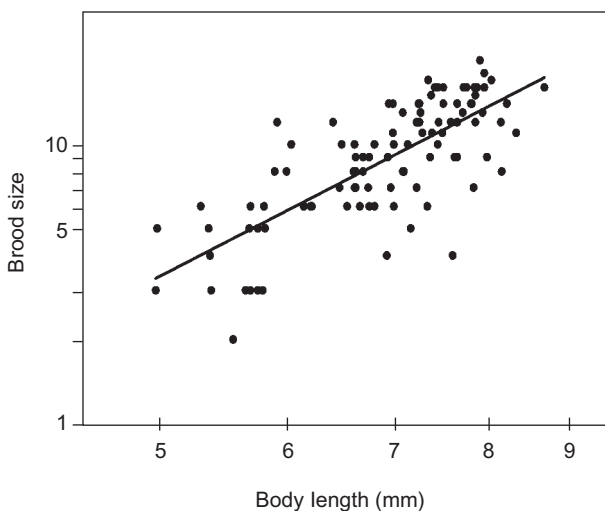
(instars 5~7). Following the pubertal molt, the post-pubertal molt occurred at instars 9~10 or an older stage and could be discriminated into mature and post-mature phases. In both sexes of *Orchestia gammarellus*, 3 growth phases can also be differentiated. However, the immature phase (but not the mature phase as found in *H. crassicornis*) is separated into 2 phases by the presence of a pre-pubertal molt (Charniaux-Cotton 1957).

Fecundity and morphometric relationship

Laboratory-reared females of *H. crassicornis* produced larger broods than females collected from semi-cultured outdoor tanks. Laboratory-reared females produced an average of 13 and a maximum of 33 offspring in a brood. The average and maximum brood sizes of the females collected from outdoor tanks were 10 and 20, respectively. Variation in brood size may be associated with differences in the living conditions of the females. The laboratory culture was maintained in a standardized and stable environment. Animals were provided with a continuous food supply and were not exposed to predators. Such conditions favor higher fecundity in amphipods (Einarson 1993). In contrast, food limitation and a relatively unstable environment may lower the fecundity and reproductive effort of amphipods living in the field. In addition, dark-colored eggs in the brood pouch often make the female more visible to predators (Van Dolah and Bird 1980) and thus a large brood size may result in a higher risk of predation (Sainte-Marie et al. 1990). Thus extrapolations based on the lifetime fecundity of laboratory-reared females might lead to an overestimation when applied to wild populations.

Nelson (1980) and Van Dolah and Bird (1980) contributed informative reviews on the reproductive output of selected amphipod species. The reproductive outputs of these amphipods are highly variable. Brood size may vary from 1 egg in *Seborgia minima* (Bousfield 1970) to more than 400 in *Gammaracanthus loricatus* (Steele and Steele 1976). Our study showed that laboratory-reared females of *H. crassicornis* produced 3 to 33 (mean, 13.2) offspring in each brood. Each female produced a total of 16 to 290 (mean, 81) offspring in 2 to 17 (mean, 6.2) broods over its lifespan. The reproductive output of *H. crassicornis* is comparable to those of other species. A female *H. barbicornis* produced 11.1 juveniles per brood and 47.6 offspring in 4.3 broods at 24°C (Hiwatari and Kajihara 1988). *Hyale nilssoni* may produce 15 eggs per brood with the maximum capacity reaching 183 eggs from 15 broods (Moore 1986a). Two offshore benthic oedicerotids, *Westwoodilla caecula* and *Monoculodes packardi*, respectively produced 3 and 2 successive broods, with an average of 9.2 and 36.4 eggs per brood (Beare and Moore 1998). A female *Orchomene nanus* might produce a maximum of 4 broods and a total of 27~52 juveniles throughout its life (Moore and Wong 1996). A mesopelagic amphipod, *Cyphocaris challengeri*, produced 20 to 65 eggs per brood (Yamada and Ikeda 2000).

Brood size increases with body size among female amphipods (Steele and Steele 1991). A linear correlation between mean brood size and maternal female size has been well documented in many amphipods, e.g., *Gammarus lawrencianus* (Steele and Steele 1970), *H. nilssoni* (Moore 1986a), *Orchestia gammarellus* (Persson 1999), and *Pectenogammarus planicrurus* (Bell and Fish 1996). Larger females generally carry more eggs than smaller ones because the length, and hence the embryo-carrying capacity of each tubular ovary, is proportional to body size (Sheader 1977a, Jones and Wigham 1993). Each tubular ovary lies dorsolaterly to the midgut, and a single linear row of oocytes is found inside each tubular ovary (Schmitz 1992). We have also observed that females of *H. crassicornis* possess a linear row of oocytes inside each tubular ovary, which accounts for the linear correlation between brood size and body length. In contrast, a non-linear correlation was found in species with non-tubular ovaries. For example, the brood size of the hyperiid *Parathemisto gaudichaudi* varied approximately with the square of the female's body length because the ovaries of this species are made up of flattened plates rather than tubular strands (Sheader 1977b). However, studies on some amphipods such as the mesopelagic hyperiid *Primno abyssalis* (Ikeda 1995) and the benthic oedicerotid *Monoculodes packardi* (Beare and Moore 1998) showed no association between brood size and maternal size.

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