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## Intraspecific behaviors and major cheliped sexual dimorphism in three congeneric callianassid shrimp

Received: 19 March 2004 / Accepted: 10 August 2004 / Published online: 17 September 2004  
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**Abstract** Decapod callianassid shrimps are usually solitary occupants of their burrows. They are known to show distinct sexual dimorphism of the major cheliped, which is used as a weapon for intraspecific fighting. Three species of *Nihonotrypaea* occur in an estuary in southern Japan; they consist of two tidal flat species (*N. harmandi*; *N. japonica*) and one boulder beach species (*N. petalura*), with maximum population densities of 1,400, 340, and 12 m<sup>-2</sup>, respectively. The major cheliped size and total length of shrimp were recorded from each population. The degrees of major cheliped sexual dimorphism were ordered as *N. harmandi* > *N. japonica* > *N. petalura*. In the laboratory, intra- and intersexual behaviors at forced encounters between two shrimps were recorded, for the former behavior throughout the year and the latter in the non-breeding season. At their intersected burrows, the shrimps either fought or retreated or filled the burrow crack. Males interacted aggressively with each other, with the intensity being *N. petalura* > *N. harmandi* ≈ *N. japonica*. Females of the tidal flat species were non-aggressive, while those of *N. petalura* were as aggressive with each other as were males. Intersexually, males of all species and females of *N. petalura* were much less aggressive than intrasexually. In *N. petalura* only, burrow-sharing behavior between sexes occasionally occurred. The interspecific difference in these behaviors is in parallel with the degree of major cheliped sexual dimorphism. Different intensities of intrasexual competition for mates could have been imposed by the different population densities of these species.

### Introduction

Decapod thalassinidean shrimps of the family Callianassidae are common burrow-dwelling macro-invertebrates, occurring from estuarine intertidal to marine subtidal soft sediments (Griffis and Suchanek 1991; Dworschak 2000; Felder 2001). One of the peculiar morphological characters of callianassid shrimps is a distinct sexual dimorphism in the major cheliped after maturation, with that of the male becoming larger and more massive. The regression equations and/or curves for such allometric growth patterns have been described for a number of species (Hailstone and Stephenson 1961; Devine 1966; Dworschak and Pervesler 1988; Felder and Lovett 1989; Rowden and Jones 1994; Dumbauld et al. 1996; Labadie and Palmer 1996; Berkenbusch and Rowden 1998; Dworschak 1998; Souza et al. 1998; Nates and Felder 1999). It is a generally accepted view that sexual dimorphism of the cheliped in decapods is a consequence of their widespread use by the male in combat, display, and courtship (e.g. Hartnoll 1974). Antagonistic behaviors have often been noted in casual observations of several callianassid shrimps when the same or opposite sex were encountered in intersected burrows in aquaria (MacGinitie 1934; Griffis 1988; Felder and Lovett 1989; Witbaard and Duineveld 1989; Rodrigues and Hödl 1990; Rowden and Jones 1994; Tamaki et al. 1997). It was reasonably assumed by callianassid researchers that such antagonistic behavior, irrespective of the opponent's sex, is related to intense competition for burrow space; except for a few examples, such as *Callianassa filholi* on intertidal sandflats (Devine 1966) and *Neotrypaea biffari* on boulder beaches (MacGinitie and MacGinitie 1968; Ricketts et al. 1985), callianassid shrimps are characterized by solitary habits. It was also often assumed that competition for females between males was a driving force to evolve the latter's enlarged major cheliped (i.e. intrasexual selection), leading to female-biased sex ratios after maturation as a consequence of severe combats between males

Communicated by T. Ikeda, Hakodate

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in the populations of several species (Felder and Lovett 1989; Dumbauld et al. 1996; Tamaki et al. 1997; Pezzuto 1998; Souza et al. 1998; Nates and Felder 1999; Shimizu and Rodrigues 2000). However, virtually no systematically designed and quantitative observations of intraspecific antagonistic behaviors between shrimps of the same or opposite sexes have been made to substantiate the above assumptions. Only a short abstract is available on the intraspecific behavioral interactions in *Neotrypaea californiensis* and *N. gigas*, and it provides no substantial data (Griffis 1988). Even when all thalassinidean shrimps are considered, only brief descriptions from outcomes of forced encounters between two individuals are found for three species (Berrill 1975; Coelho 2001).

In Japanese waters, three callianassid species of the genus *Nihonotrypaea*, formerly assigned to the genus *Callianassa* (see Manning and Tamaki 1998), are commonly found in intertidal habitats (Tamaki 2003): *N. harmandi* (Bouvier, 1901), *N. japonica* (Ortmann, 1891), and *N. petalura* (Stimpson, 1860). Note that in papers by A. Tamaki and his colleagues published before 1998, the name *C. japonica* was incorrectly applied to *N. harmandi* (see Tamaki 2003). Of the three species, both *N. harmandi* and *N. japonica* are distributed in bare sandflats, whereas *N. petalura* is found on boulder beaches (Tamaki et al. 1999). In the Ariake Sound estuarine system, western Kyushu, Japan (the system ranges from Ariake Sound, via Tachibana Bay, to the coastal waters of the East China Sea), it is estimated that *N. petalura* makes up only 6% of the total number of shrimps of *Nihonotrypaea* (Tamaki and Miyabe 2000). Furthermore, it is evident that *N. harmandi* and *N. petalura* are distributed along the coastline facing the East China Sea, across Tachibana Bay, to the outermost one-third of Ariake Sound, whereas *N. japonica* occurs mostly along the coastline of the middle one-third of Ariake Sound (Tamaki et al. 1999). Although *N. harmandi* and *N. japonica* attain densities of up to 1,400 and 340 m<sup>-2</sup>, respectively (Tamaki et al. 1997; Flach and Tamaki 2001; Wardiatno et al. 2003), *N. petalura* exists more sparsely in sand patches surrounded by boulders, with mean densities of 5.7–12.0 m<sup>-2</sup> (Shimoda and Tamaki 2004).

To date intraspecific antagonistic behaviors between shrimps, irrespective of the opponent sex, have been observed in aquaria for all species of *Nihonotrypaea* (Tamaki et al. 1997, personal observation); burrow-sharing behavior between sexes was occasionally noted for *N. petalura* only (K. Kubo and A. Tamaki, personal observation). These observations appear to be in parallel with those for three species of the genus *Neotrypaea* distributed on the west coast of North America, i.e. *N. californiensis* and *N. gigas* inhabiting bare tidal flats (MacGinitie 1934; Griffis 1988; Griffis and Chavez 1988; Dumbauld et al. 1996) and *N. bifari* inhabiting boulder beaches (MacGinitie and MacGinitie 1968; Ricketts et al. 1985).

The aim of the present article is to make comparisons between the three species of *Nihonotrypaea* as to their intra- and intersexual antagonistic or coexistent behav-

iors in aquaria and to relate these behaviors to the degree of sexual dimorphism of the major cheliped. Possible patterns between behaviors and cheliped morphology are postulated to reflect the density of each species in its respective natural setting. Information on basic biology and life-history traits of the three species used in conducting the experiments comes from Tamaki et al. (1996) and Tamaki et al. (1997) for *N. harmandi*; Y. Wardiatno and A. Tamaki (unpublished data) for *N. japonica*; and K. Kubo and A. Tamaki (unpublished data) for *N. petalura*.

## Materials and methods

### Measurement of body size and major cheliped related to growth

The specimens to be measured were collected and fixed in 10% neutralized seawater–formalin, as follows: (1) *Nihonotrypaea harmandi*—from the Tomioka Bay sandflat (32°31'N; 130°02'E) at the northwestern corner of Amakusa-Shimoshima Island (see Tamaki et al. 1997) from May 1992 to October 2003; (2) *N. japonica*—from the Shirakawa sandflat (32°47'N; 130°36'E) at the mouth of the Shirakawa River in Kumamoto City (see Wardiatno et al. 2003) from April 1999 to August 2003; and (3) *N. petalura*—from the Kuchinotsu-Cho boulder beach (32°36'N; 130°12'E) at the southernmost part of Shimabara Peninsula (see Shimoda and Tamaki 2004) from June 1997 to February 2002. Only those specimens unparasitized by the rhizocephalan *Polysaccus japonicus* (in *N. harmandi*) or by the bopyrid isopod *Pseudione longicauda* (in *N. japonica* and *N. petalura*) were selected in the laboratory. Sex was determined according to the presence (in females) or absence (in males) of the second pair of pleopods. As a measure of body size, total length (TL) was recorded along the mid-dorsal curvature from the tip of the rostrum to the posterior margin of the telson, to the nearest 0.1 mm under a stereomicroscope. As a measure of major cheliped length (MCL), the distance between two tangential lines, one drawn to the most proximal projection point on the carpus and the other to the most distal downward curving point on the dactylus, was recorded to the nearest 0.1 mm

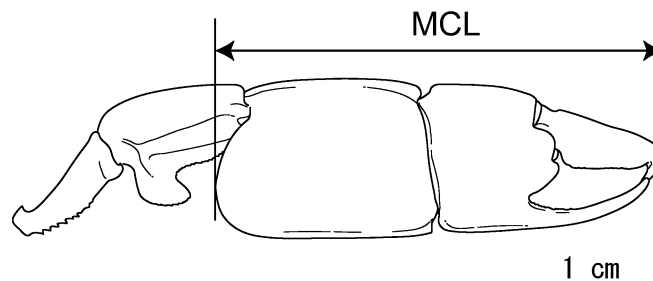
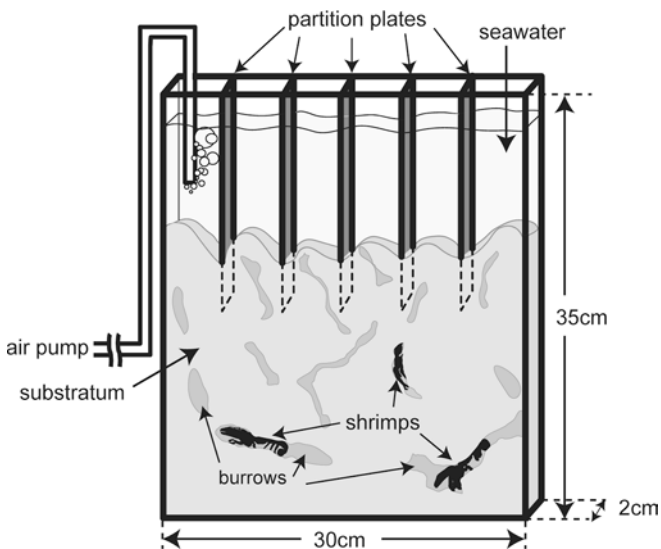


Fig. 1 *Nihonotrypaea harmandi*. Major cheliped of a male of 39.1 mm TL, illustrating the part measured for major cheliped length (MCL)

(Fig. 1). Major cheliped wet weight (MCWW) was determined to the nearest 0.1 mg after removing this appendage at the proximal base of the ischium and blotting it with tissue paper. In the measurements of MCL and MCWW, different sets of specimens were used for each species.

#### Observation of intra- and intersexual behaviors in each species

Specimens used for behavioral observations were collected from the above-mentioned locations from October 2002 to August 2003, except for *N. harmandi*, which was taken from the Oh-é sandflat (32°37'N; 130°14'30"E) near the Kuchinotsu-Cho boulder beach. Each shrimp was brought separately to the laboratory and maintained in a 100-ml plastic bottle sealed with a 1-mm mesh nylon net under gently running seawater until use in the experiment. The dimensions of the acrylic aquaria used for the observations were of six types: (1–3) 30 cm (width)×35 cm (height)×1 or 1.5 or 2 cm (thickness), (4 and 5) 10 cm×35 cm×1 or 1.5 cm, and (6) 15 cm×35 cm×2 cm (Fig. 2). The sediment that was collected from each shrimp collection site was filled from one-half to two-thirds of the aquarium height. In the case of *N. petalura*, the boulders, the cobbles, and some of the pebbles were removed due to the size limitation of aquaria (see Shimoda and Tamaki 2004). Depending on shrimp body widths, one of the aquaria with the “closest-fit” thickness was selected for efficient observation. Several partition plates were inserted to a depth of 2–5 cm in equal distances from each other. A single shrimp with no damage to its major cheliped was introduced into each compartment. Most shrimps reached the bot-



**Fig. 2** Acrylic aquarium for the observation of shrimp behavior, with one shrimp in each compartment initially introduced from above and with two video-camera recorders, one close to the front and the other one at the rear of the observation panels. Other types of aquaria were 10 or 15 cm in width and 1 or 1.5 cm in thickness

tom of the aquaria within 24 h. After the shrimp made their burrows to some extent about 12 h later, the interaction between any two shrimp was recorded whenever their extending burrows intersected. Records were made using digital video-camera recorders (Sony DCR-TRV 9, 30, and 50) set close to both observation panels under fluorescent lamps in the room. After the termination of each experiment, the shrimp were retrieved and fixed for the determination of sex and TL. In some cases, however, shrimps were kept alive for later reuse (up to three times, compiled in the last paragraph). In the field, encounters between shrimp of various sizes with the same or opposite sexes can occur. But the present experiments were conducted in accordance with all three following rules.

**Encounter rule 1.** Only adult shrimp with TL greater than the smallest mature one were used. It is generally accepted that the smallest mature male is the one at a point from which its major cheliped length begins to markedly increase against TL (Hailstone and Stephenson 1961; Devine 1966; Dworschak and Pervesler 1988; Felder and Lovett 1989; Rowden and Jones 1994; Dumbauld et al. 1996; Labadie and Palmer 1996; Berkenbusch and Rowden 1998; Dworschak 1998; Souza et al. 1998; Nates and Felder 1999). Such TL values are 20 mm in *N. harmandi*, 32.5 mm in *N. japonica*, and 24.8 mm in *N. petalura*. For females of *N. harmandi* and *N. petalura*, the smallest mature ones were defined as the smallest ovigerous ones, i.e. 17.9 and 25.7 mm, respectively. Although most females of *N. japonica* used in the experiment were larger than the smallest ovigerous one (32.4 mm TL), 8.2% of the shrimps fell between this size and 23.6 mm TL (i.e. that of the smallest one with a well-developed ovary). Inclusion of these small females was required to secure a sufficient number of specimens.

**Encounter rule 2.** The observations of the encounters between shrimps of the same sexes were made in both breeding and non-breeding seasons, whereas those between shrimps of the opposite sexes were only carried out in the non-breeding season, to eliminate any possible behaviors associated with copulation (see Candisani et al. 2001). The breeding seasons are from June to October in *N. harmandi*, from the end of February to

early November in *N. japonica*, and from the end of April to early November in *N. petalura*. Thus, the experiments were conducted in the following periods: (1) from December 2002 to June 2003 for the same sexes of *N. harmandi*; (2) from December 2002 to May 2003 for the opposite sexes of *N. harmandi*; (3) from November 2002 to July 2003 for the same sexes of *N. japonica*; (4) from the end of November 2002 to mid-February 2003 for the opposite sexes of *N. japonica*; (5) from October 2002 to August 2003 for the same sexes of *N. petalura*; and (6) from mid-November 2002 to the end of March 2003 for the opposite sexes of *N. petalura*.

Encounter rule 3. The encounter between any two shrimps was limited to that between shrimps with a similar TL. Labadie and Palmer (1996) suggested that the chelipeds of *Neotrypaea californiensis* were used for sustained grappling with similar-sized conspecifics. Although care was taken to select such a combination of shrimps at the start of the experiment, only results for the sets of actual shrimp TL ratios of <1.3 were used for statistical analyses. This critical value was determined rather arbitrarily to secure a sufficient number of encounters. The mean ( $\pm$ SD) and range of TL ratios (the number of encounters) was  $1.10 \pm 0.07$  and 1.00–1.28 ( $n=80$ ) for *N. harmandi*,  $1.11 \pm 0.08$  and 1.00–1.29 ( $n=73$ ) for *N. japonica*, and  $1.09 \pm 0.07$  and 1.00–1.27 ( $n=67$ ) for *N. petalura*. The total number of shrimps and their TL values (mean  $\pm$ SD; range) used in the experiments were as follows: (1) 85 males of *N. harmandi* ( $41.0 \pm 6.2$  mm; 27.1–56.8 mm), of these, 10 were used twice; (2) 75 females of *N. harmandi* ( $36.1 \pm 5.2$  mm; 24.2–46.7 mm), of these, 9 were used twice, and 1, three times; (3) 75 males of *N. japonica* ( $45.2 \pm 10.9$  mm; 23.9–66.1 mm), of these, 10 were used twice, and 3, three times; (4) 71 females of *N. japonica* ( $51.5 \pm 10.0$  mm; 26.6–63.4 mm), of these 7 were used twice, and 1, three times;

and (6) 69 females of *N. petalura* ( $39.7 \pm 6.1$  mm; 27.2–53.0 mm), of these, 10 were used twice, and 3, three times. When any shrimp was used twice or three times, care was taken not to make an encounter against the identical opponent.

## Results

Characteristics of major cheliped dimorphism in the three species

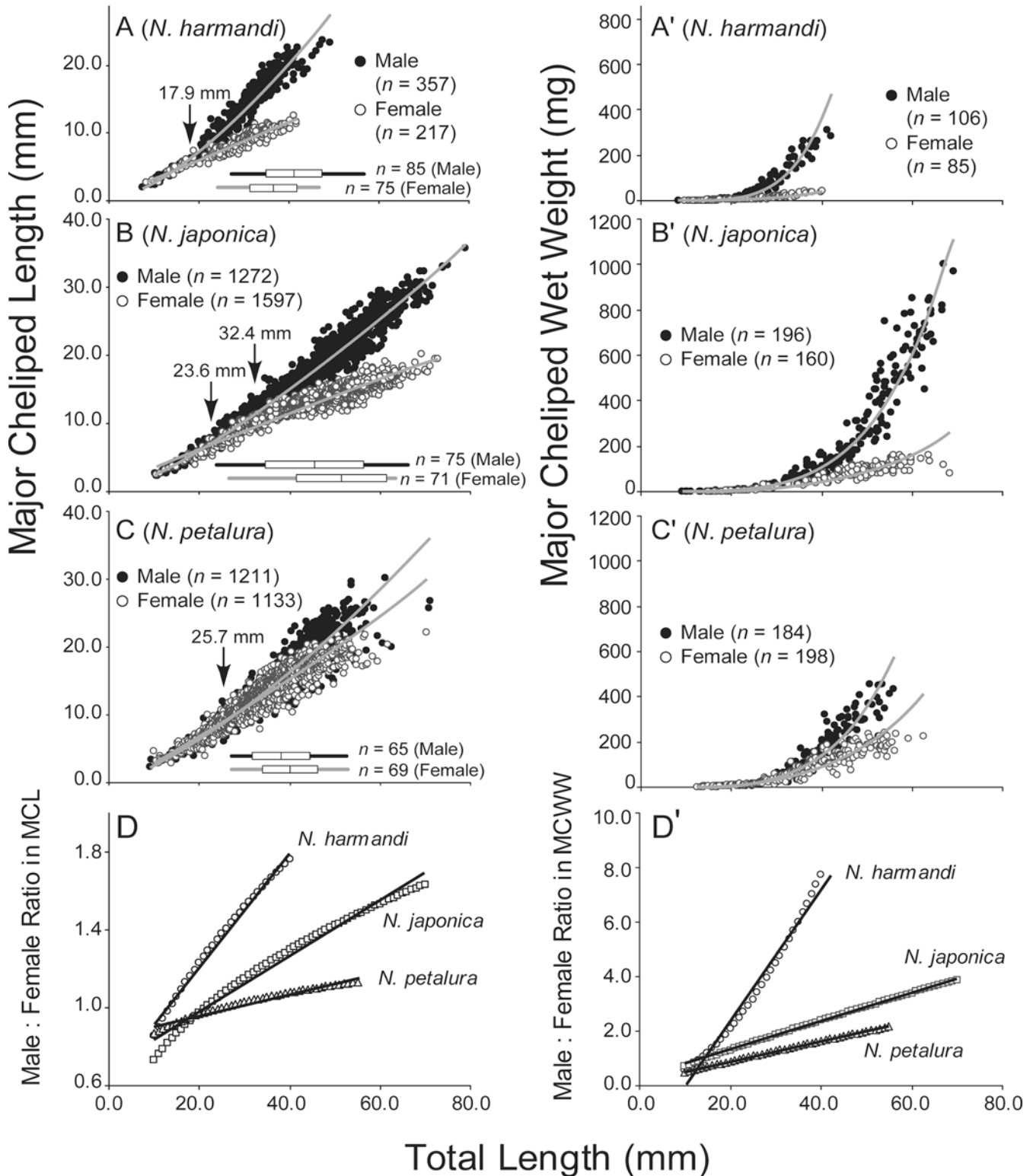
### MCL

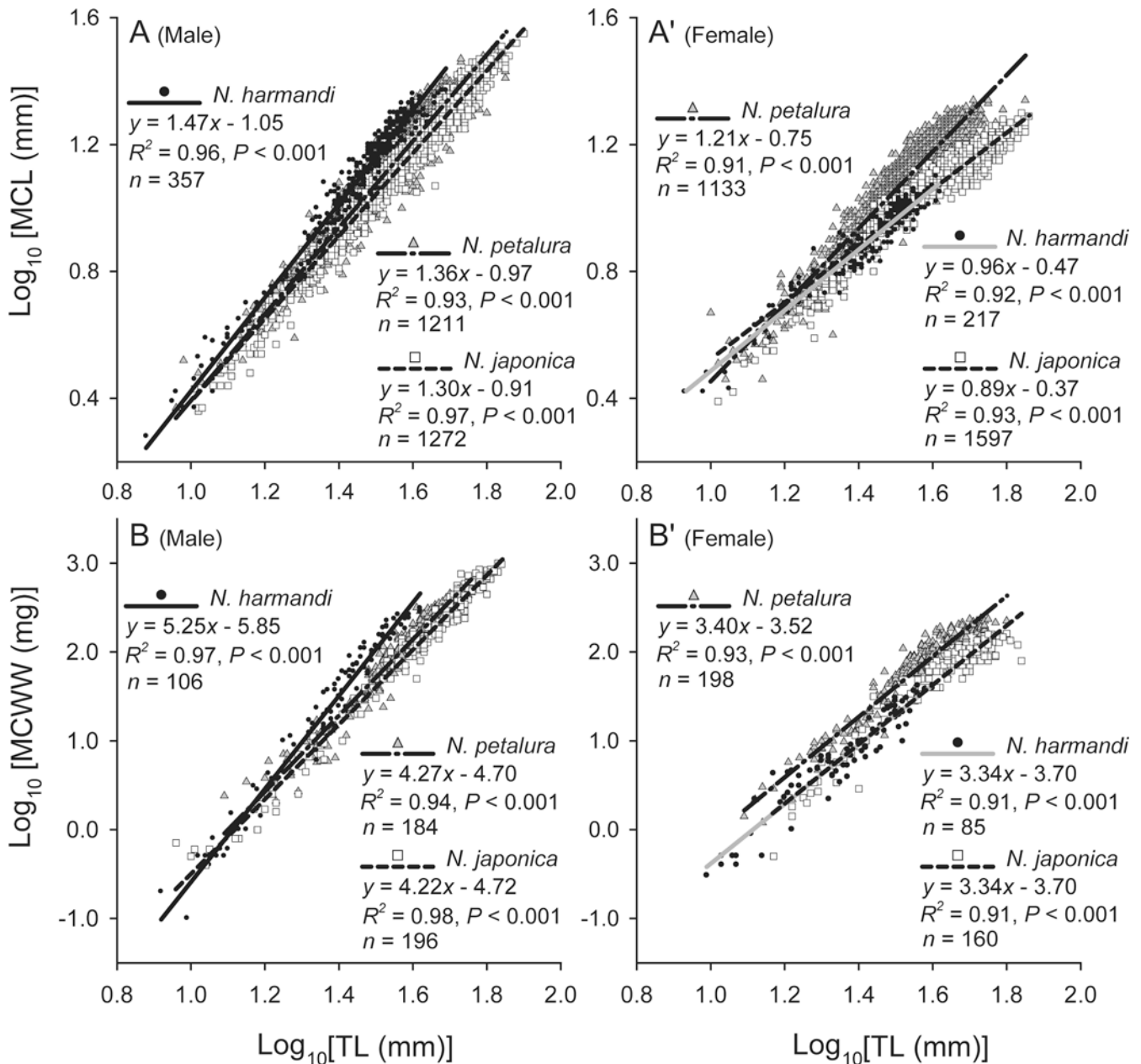
The data on MCL (mm) against TL (mm) for the three species of *Nihonotrypaea* are shown in Fig. 3A, B, C. The power function ( $y = ax^b$ ) was fitted to the plots in each figure (SPSS 2002a); both variables were transformed into the logarithm to the base 10, with the linear regression equations (Fig. 4A, A'). Based on these linear regression equations, analysis of covariance (ANCOVA) was performed for significant differences between sexes in each species (SPSS 2002b). In all species, the slope of the linear regression line for males was significantly steeper than that for females (*N. harmandi*:  $F_{1, 570} = 402.5$ ,  $P < 0.001$ ; *N. japonica*:  $F_{1, 2,865} = 2,053.4$ ,  $P < 0.001$ ; *N. petalura*:  $F_{1, 2,340} = 100.0$ ,  $P < 0.001$ ). The ratios of male MCL to female MCL (abbreviated as  $MCL_{ratio}$ ) against TL for the three species are shown in Fig. 3D. The plots in this figure were derived from the data on the power functions mentioned above, with every 1-mm increase in TL from 10 mm to the almost maximum value for each species, i.e. 40 mm for *N. harmandi*, 70 mm for *N. japonica*, and 55 mm for *N. petalura*. The linear regression equations fitted to these plots were as follows: (1)  $MCL_{ratio} = 0.030TL + 0.617$  ( $n = 31$ ,  $R^2 = 0.99$ ,  $P < 0.001$ ) for *N. harmandi*; (2)  $MCL_{ratio} = 0.014TL + 0.691$  ( $n = 61$ ,  $R^2 = 0.98$ ,  $P < 0.001$ ) for *N. japonica*; and (3)  $MCL_{ratio} = 0.006TL + 0.849$  ( $n = 46$ ,  $R^2 = 0.96$ ,  $P < 0.001$ ) for *N. petalura*. The difference in the slope values of the regression lines among

Fig. 3A–D; A'–D' *Nihonotrypaea harmandi*, *N. japonica*, *N. petalura*. A–C and A'–C': relationships between major cheliped length (A–C) or wet weight (A'–C') and total length of shrimp, with curves for the nonlinear regression equations (power functions); the log-transformed equations are given in Fig. 4. Different sets of specimens were used between A–C and A'–C'. The arrows in panels A and C and the right one in panel B indicate the smallest ovigerous female, and the left one in panel B indicates the smallest female with a well-developed ovary. The horizontal bars and boxes in panels A–C show the range, mean, and  $\pm$ SD of the specimens used for behavioral observations. D and D' Relationships between male to female ratio in major cheliped length (D) or wet weight (D') and total length of shrimp, with lines for the linear regression equations given in the first section of the "Results"

the three species was significant ( $F_{2, 132} = 736.1$ ,  $P < 0.001$ ; ANCOVA). The Bonferroni's multiple comparison test detected a significant difference ( $\alpha = 0.05/3$ ) between every pair of the three species: (1)  $F_{1, 88} = 480.1$ ,  $P < 0.001$  for the *N. harmandi* and *N. japonica* pair; (2)  $F_{1, 73} = 3,113.0$ ,  $P < 0.001$  for the *N. harmandi* and *N. petalura* pair; and (3)  $F_{1, 103} = 503.0$ ,  $P < 0.001$  for the

*N. japonica* and *N. petalura* pair. The slope values were ordered as  $N. harmandi \gg N. japonica > N. petalura$ , with the maximum  $MCL_{ratio}$  values at 40 mm TL being 1.8, 1.3, and 1.1, respectively. The relationship between  $\log_{10}MCL$  and  $\log_{10}TL$  (Fig. 4A, A') can also be used to find interspecific differences in the linear regression equations between the males or between the females.





**Fig. 4A, B; A', B'** *Nihonotrypaea harmandi*, *N. japonica*, *N. petalura*. Log-log plots between major cheliped length (A, A') or wet weight (B, B') and total length of shrimp, with linear regression lines, based on the same set of data as in Fig. 3

The difference in the slope values of the regression lines among the three species was significant both between the males ( $F_{2, 2,834} = 49.8$ ,  $P < 0.001$ ) and between the females ( $F_{2, 2,941} = 336.0$ ,  $P < 0.001$ ). The Bonferroni's multiple comparison test detected a significant difference ( $\alpha = 0.05/3$ ) between every pair of the three species: (1)  $F_{1, 1,625} = 120.0$ ,  $P < 0.001$  for males of the *N. harmandi* and *N. japonica* pair; (2)  $F_{1, 1,564} = 31.3$ ,  $P < 0.001$  for males of the *N. harmandi* and *N. petalura* pair; (3)  $F_{1, 2,479} = 22.2$ ,  $P < 0.001$  for males of the *N. japonica* and *N. petalura* pair; (4)  $F_{1, 1,810} = 13.1$ ,  $P < 0.001$  for females of the *N. harmandi* and *N. japonica* pair; (5)

$F_{1, 1,346} = 79.0$ ,  $P < 0.001$  for females of the *N. harmandi* and *N. petalura* pair; and (6)  $F_{1, 2,726} = 651.3$ ,  $P < 0.001$  for females of the *N. japonica* and *N. petalura* pair. Between the males, the slope values were ordered as *N. harmandi* (1.47)  $\gg$  *N. petalura* (1.36)  $>$  *N. japonica* (1.30). By contrast, between the females, the slope values were ordered as *N. petalura* (1.21)  $\gg$  *N. harmandi* (0.96)  $>$  *N. japonica* (0.89).

#### MCWW

The data on MCWW (mg) against TL (mm) for the three species of *Nihonotrypaea* are shown in Fig. 3A', B', C'. The power function ( $y = ax^b$ ) was fitted to the plots in each figure (SPSS 2002a); both variables were transformed into the logarithm to the base 10, with the

linear regression equations (Fig. 4B, B'). Based on these linear regression equations, the ANCOVA was performed for significant differences between sexes in each species (SPSS 2002b). In all species, the slope of the linear regression line for males was significantly steeper than that for females (*N. harmandi*:  $F_{1, 187} = 169.3$ ,  $P < 0.001$ ; *N. japonica*:  $F_{1, 352} = 106.0$ ,  $P < 0.001$ ; *N. petalura*:  $F_{1, 378} = 64.2$ ,  $P < 0.001$ ). The ratios of male MCWW to female MCWW (abbreviated as  $MCWW_{ratio}$ ) against TL for the three species are shown in Fig. 3D'. The plots in this figure were derived from the data on the power functions mentioned above, just as for MCL. The linear regression equations fitted to these plots were as follows: (1)  $MCWW_{ratio} = 0.240TL - 2.486$  ( $n = 31$ ,  $R^2 = 0.98$ ,  $P < 0.001$ ) for *N. harmandi*; (2)  $MCWW_{ratio} = 0.053TL + 0.253$  ( $n = 61$ ,  $R^2 = 0.999$ ,  $P < 0.001$ ) for *N. japonica*; and (3)  $MCWW_{ratio} = 0.037TL + 0.156$  ( $n = 46$ ,  $R^2 = 0.999$ ,  $P < 0.001$ ) for *N. petalura*. The difference in the slope values of the regression lines among the three species was significant ( $F_{2, 132} = 1,842.7$ ,  $P < 0.001$ ; ANCOVA). The Bonferroni's multiple comparison test detected a significant difference ( $\alpha = 0.05/3$ ) between every pair of the three species: (1)  $F_{1, 88} = 2,227.2$ ,  $P < 0.001$  for the *N. harmandi* and *N. japonica* pair; (2)  $F_{1, 73} = 1,898.7$ ,  $P < 0.001$  for the *N. harmandi* and *N. petalura* pair; and (3)  $F_{1, 103} = 2,757.2$ ,  $P < 0.001$  for the *N. japonica* and *N. petalura* pair. The slope values were ordered as *N. harmandi*  $>>$  *N. japonica*  $>$  *N. petalura*, with the maximum  $MCWW_{ratio}$  values at 40 mm TL being 7.1, 2.4, and 1.6, respectively. The relationship between  $\log_{10}MCWW$  and  $\log_{10}TL$  (Fig. 4B, B') can also be used to find interspecific differences in the linear regression equations between the males or between the females. Between the males, the difference in the slope values of the regression lines among the three species was significant ( $F_{2, 480} = 60.7$ ,  $P < 0.001$ ). The Bonferroni's multiple comparison test detected a significant difference ( $\alpha = 0.05/3$ ) between two pairs of the three species but not between the other one: (1)  $F_{1, 298} = 120.5$ ,  $P < 0.001$  for the *N. harmandi* and *N. japonica* pair; (2)  $F_{1, 286} = 66.3$ ,  $P < 0.001$  for the *N. harmandi* and *N. petalura* pair; and (3)  $F_{1, 376} = 0.3$ ,  $P = 0.61$  for the *N. japonica* and *N. petalura* pair. The slope values were ordered as *N. harmandi* (5.25)  $>$  *N. petalura* (4.27)  $\approx$  *N. japonica* (4.22). In pair 3, a small difference in the  $y$ -intercept values of the regression lines [ $-4.68$  (*N. petalura*) versus  $-4.71$  (*N. japonica*)] was significant ( $F_{1, 377} = 55.0$ ,  $P < 0.001$ ). Between the females, the difference in the slope values of the regression lines among the three species was not significant ( $F_{2, 437} = 0.2$ ,  $P = 0.80$ ), while that in the  $y$ -intercept values was significant ( $F_{2, 439} = 262.6$ ,  $P < 0.001$ ). For the latter case, the Bonferroni's multiple comparison test detected a significant difference ( $\alpha = 0.05/3$ ) between two pairs of the three species but not between the other one: (4)  $t_{439} = 0.01$ ,  $P = 0.99$  for the *N. harmandi* and *N. japonica* pair; (5)  $t_{439} = 15.39$ ,

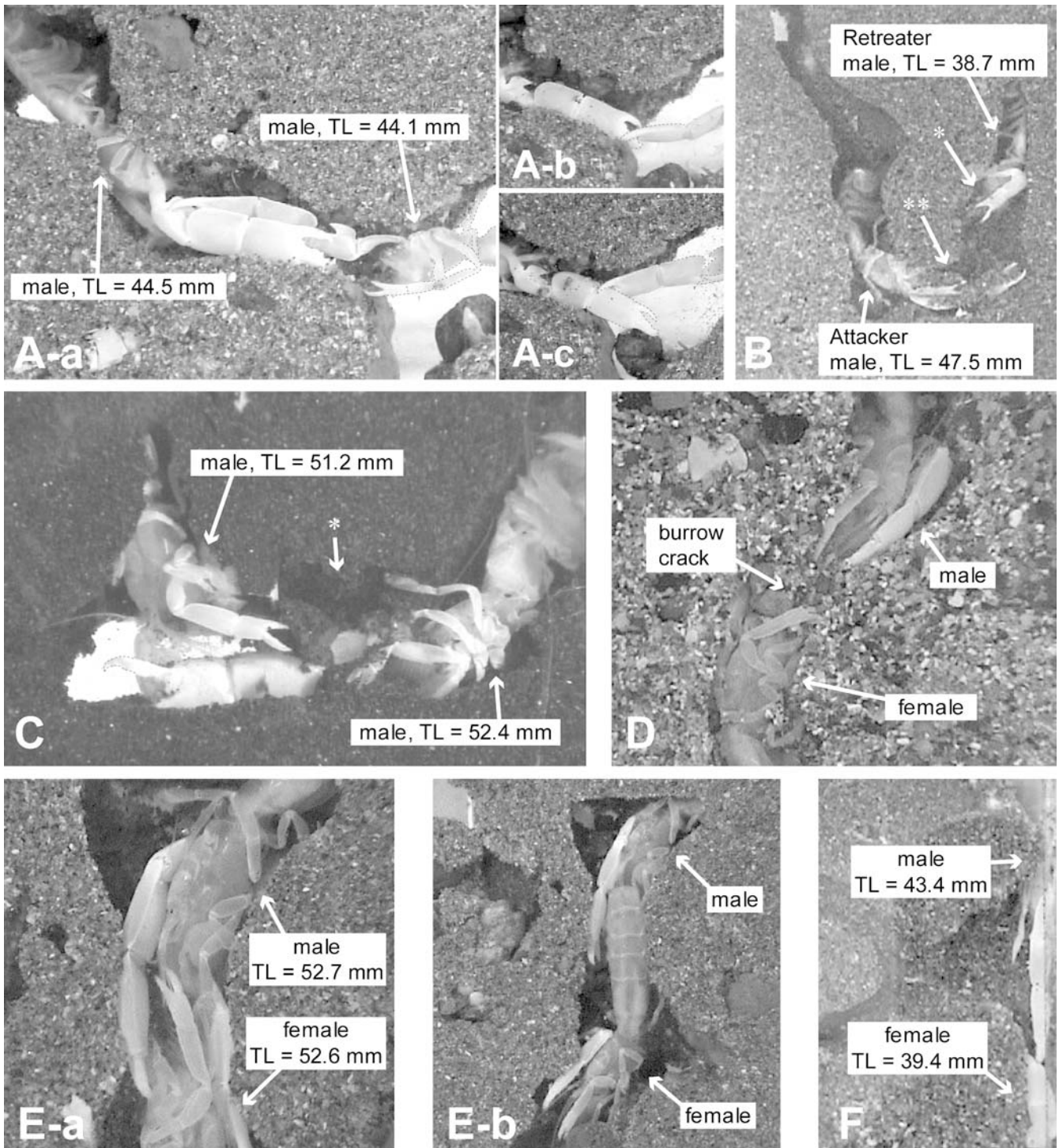
$P < 0.001$  for the *N. harmandi* and *N. petalura* pair; and (6)  $t_{439} = 19.93$ ,  $P < 0.001$  for the *N. japonica* and *N. petalura* pair. The  $y$ -intercept values were ordered as *N. petalura* ( $-3.52$ )  $>$  *N. harmandi* ( $-3.70$ ) = *N. japonica* ( $-3.70$ ).

#### Terminology of shrimp behaviors and associated events at encounters

Tens of cases that satisfied the three encounter rules listed in the "Materials and methods" were recorded for each species. The number of cases were 80 for *N. harmandi* (32 between males, 27 between females, and 21 between sexes), 73 for *N. japonica* (29 between males, 27 between females, and 17 between sexes), and 67 for *N. petalura* (22 between males, 24 between females, and 21 between sexes). The interactions between two individuals could be categorized into four groups: (1) "reciprocal attacking" (Fig. 5A-a, b, c, C); (2) "one-way attacking" by the "attacker", with withdrawal of the "retreater" showing no signs of counterattacking throughout (Fig. 5B); (3) "mutual burrow-crack filling" between the two burrows, with no attacking of each other (Fig. 5D); and (4) "burrow sharing", cohabiting and moving about freely in a connected burrow (Fig. 5E-a, b, F). Hereafter, the categories 1, 2, and 3 are collectively called "antagonistic behaviors"; categories 1 and 2 are called "fighting" and category 3 is a peaceful solution. The retreater in one-way attacking and both individuals in mutual burrow-crack filling are collectively called "non-attackers". Detailed descriptions on the constituent behaviors of the above interactions and the definitions of associated terms are given in the next paragraphs and the next section. Category 4 was occasionally observed only between sexes of *N. petalura*, whose detailed behaviors are described in the last section.

#### Attacking

Immediately after their encounter, an attacker protrudes its major cheliped toward the opponent, grappling the merus (Fig. 5A-a) or the dactyl (Fig. 5A-b) or the junction between the propodus and the carpus (Fig. 5A-c) of the latter's major cheliped with its chela. Such a grappling act is often unsuccessful. In a few cases both shrimp have been observed to grapple each other's minor chelipeds with their major chelipeds. The grappling of a main body part such as the carapace or the abdomen is rare. Also in some cases an attacker simply protrudes its major cheliped, grappling nothing. During reciprocal attacking the shrimp swiftly go back and forth or stop in response to each other's movement, with the tips of their pleopods directed forward and vibrated quickly from time to time.



#### *Mutual burrow-crack filling*

Both shrimp fill a crack generated at the intersection between their burrows, either by pushing sediment with their third maxillipeds, minor chelipeds, and second pereopods (“bulldozing” sensu Stamhuis et al. 1996) or by lifting and carrying sediment with their third maxillipeds, minor chelipeds, and second pereopods (“lifting” and “carrying” sensu Stamhuis et al. 1996) from

rearward to the crack to tamp the dumped sediment there with their third maxillipeds and second pereopods (“tamping” sensu Stamhuis et al. 1996) (Fig. 5D).

#### Termination of fighting

Fighting ended in a tie or a win and loss, as defined below. The total numbers of cases for which the





**Fig. 5A–F** *Nihonotrypaea petalura* (A, B, D, E, F), *N. japonica* (C). Typical antagonistic or coexistent behaviors of two shrimps at encounters. For terminology and details of behaviors, see text in the “Results”. **A** “Reciprocal attacking” between the same combination of males: **a** the left one is grappling the merus of the larger cheliped of the other one with its chela, **b** both are grappling each other’s dactyl of the larger cheliped, and **c** the right one is grappling the junction between the propodus and the carpus of the left one’s larger cheliped with its chela. **B** “One-way attacking” between males: immediately after the shrimps encountered at the point with *double asterisks*, the right one withdrew to make a block at the point with a *single asterisk* (“burrow blocking”). **C** “Reciprocal attacking” between males: the ceiling of the intersected burrows (*asterisk*) is accidentally collapsing in the course of shrimp violent acts, which has resulted in the separation of the shrimps (“tie” outcome). **D** “Mutual burrow-crack filling” between two individuals of opposite sex (total length, not measured). **E** “Burrow sharing” between the same combination of a male and a female: **a** “tender touching” (face to face), male lacking its minor cheliped and **b** “tender touching” (from behind). **F** “Tender pushing” (face to face) in burrow sharing between two individuals of opposite sex

outcomes could be determined (due to the visibility of shrimp behaviors) were 20 for *N. harmandi*, 21 for *N. japonica*, and 45 for *N. petalura*.

### Tie

In the course of violent reciprocal attacking, the two shrimps are separated by accidental collapse of a part of their burrow walls. In a few cases both shrimp actively break their burrow walls with their minor chelipeds to make a block between them (“burrow blocking”), while defending the opponents’ attacking by grappling of each other’s major chelipeds. Subsequently, each shrimp tamps a new burrow wall, utilizing the collapsed sediment (Fig. 5C).

### Win and loss

In reciprocal or one-way attacking, the attacker and the retreator become the “winner” and the “loser”, respectively, at the time when either, the latter draws away (often swiftly) and breaks its own burrow wall with the use of its major and minor chelipeds and second pereopods to make a block just in front of the former (burrow blocking) (Fig. 5B), or, the former simply drives the latter out of the burrow onto the sediment surface (“driven out”).

### Duration of antagonistic behaviors

The duration of fighting or mutual burrow-crack filling was measured for the three species. The termination of the latter was defined as the cessation of the burrow-crack-filling acts by both individuals. Only those cases for which a complete recording from beginning to end had been made were analyzed. The mean ( $\pm$ SD,  $n$ =number of all intra- and intersexual encounters

combined) duration of fighting and of mutual burrow-crack filling for *N. harmandi* were  $320 \pm 272$  s ( $n=17$ ) and  $901 \pm 1,621$  s ( $n=44$ ), respectively, with their difference being significant ( $0.001 < P < 0.01$ ; a two-tailed Mann–Whitney *U*-test; SPSS 2002a). The same parameters for *N. japonica* were  $383 \pm 368$  s ( $n=19$ ) and  $900 \pm 856$  s ( $n=41$ ), with a significant difference ( $0.001 < P < 0.01$ ). Those parameters for *N. petalura* were  $289 \pm 456$  s ( $n=41$ ) and  $393 \pm 196$  s ( $n=41$ ), with a significant difference ( $0.001 < P < 0.01$ ). The difference in the duration of fighting among the three species was not significant ( $P=0.39$ ; Kruskal–Wallis test), but that in the duration of mutual burrow-crack filling was close to significance ( $P=0.0503$ ). For the latter case, the Steel–Dwass’ multiple comparison test detected a significant difference only between *N. japonica* and *N. petalura* ( $0.01 < P < 0.05$ ), with the duration in the former species lasting longer.

### Frequency of intra- and intersexual fighting and mutual burrow-crack filling

The frequencies of fighting and mutual burrow-crack filling in all encounters between males, between females, and between sexes in the three species are summarized in

**Table 1** *Nihonotrypaea harmandi*, *N. japonica*, *N. petalura*. Frequencies of “fighting” and “mutual burrow-crack filling” in all encounters between males, between females, and between individuals of opposite sex. The two figures *in parentheses* under fighting show the number of cases of “reciprocal attacking” and of “one-way attacking”, respectively. The frequency of “burrow sharing” in *N. petalura* is not included. The difference in the proportional frequencies between the two behaviors was tested for significance by a two-tailed binomial test ( $\alpha=0.05$ )

	Frequency		
	Fighting	Mutual burrow-crack filling	Significance
<i>N. harmandi</i>			
Male–male	14 (13, 1)	18	$P=0.60$
Female–female	2 (2, 0)	25	$P < 0.001$
Male–female	4 (2, 2)	17	$0.001 < P < 0.01$
<i>N. japonica</i>			
Male–male	16 (15, 1)	13	$P=0.71$
Female–female	1 (1, 0)	26	$P < 0.001$
Male–female	4 (2, 2)	13	$0.01 < P < 0.05$
<i>N. petalura</i>			
Male–male	20 (18, 2)	2	$P < 0.001$
Female–female	18 (15, 3)	6	$0.01 < P < 0.05$
Male–female	7 (5, 2)	11	$P=0.48$

**Table 1.** The difference in the proportional frequencies between the two behaviors was tested for significance by a two-tailed binomial test based on the null hypothesis of the equal share (50%) by each behavior (Zar 1999). The patterns for *N. harmandi* and *N. japonica* were quite similar. Firstly, between males, the proportional frequencies of the two behaviors were not significantly different, with that of fighting being about half (44% or 55%). Secondly, in fighting between males, reciprocal attacking occurred in almost all cases (93% or 94%). Thirdly, both between females and between sexes, the proportional frequency of fighting was significantly lower than that of mutual burrow-crack filling, accounting for only 4% or 7% between females and 19% or 24% between sexes. Finally, in the two cases of one-way attacking between sexes in each species, males were the attackers. By contrast, the pattern for *N. petalura* was different. Firstly, not only between males, but also between females, the proportional frequency of fighting (91% and 75%, respectively) was significantly higher than that of mutual burrow-crack filling. Secondly, between sexes, the proportional frequencies of the two behaviors were not significantly different, with that of fighting being about 39%. Thirdly, in both intra-sexual and intersexual fighting, reciprocal attacking predominated (from 71% to 90%). Finally, in one of the two one-way attackings between sexes, the female was the attacker, with its TL and MCL greater than those of the male. Of all encounters in the three species, this was

the single case in which a female acted as the attacker against a male.

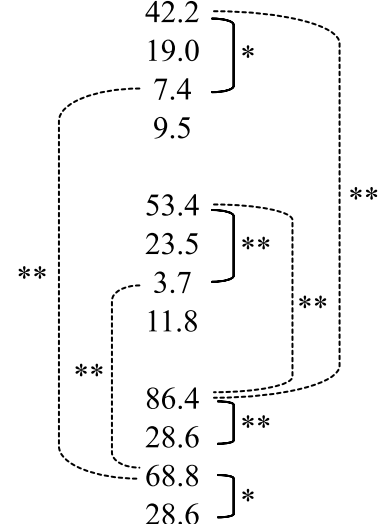
Comparison of intra- and intersexual aggressiveness between the three species

The aggressiveness of an individual of each sex against an opponent of either sex can further be evaluated by calculating the percentage of individuals that exhibited attacking behavior in all individuals involved in fighting and mutual burrow-crack filling (plus burrow sharing in *N. petalura*). The “aggressiveness index (%)” is defined as the number of attackers×100/the number of both attackers and non-attackers, where the number of attackers ( $n_A$ ) equals 2×the number of cases of reciprocal attacking + the number of cases of one-way attacking and the number of non-attackers ( $n_{NA}$ ) equals the number of cases of one-way attacking + 2×the number of cases of mutual burrow-crack filling (+ 2×the number of cases of burrow sharing in *N. petalura*). The aggressiveness indices are provided for the four encounter categories in each species (Table 2): (1) for a male against another male, (2) for a male against a female, (3) for a female against another female, and (4) for a female against a male. Furthermore, a two-tailed test comparing two proportions was performed for a significant difference in the indices between categories 1 and 2, between 3 and 4, between 1 and 3, and between 2 and 4

**Table 2** *Nihonotrypaea harmandi*, *N.japonica*, *N. petalura*. Intra- and interspecific comparisons of “aggressiveness indices” defined as the percentage of “attackers” of all individuals involved in “fighting” and mutual “burrow-crack filling” (plus “burrow sharing” in *N.petalura*). In the intraspecific comparisons, a two-tailed test comparing two proportions was performed for a significant difference between categories 1 and 2, between 3 and 4, between 1

and 3, and between 2 and 4, with significantly different pairs connected by *solid lines* ( $\alpha$  for Bonferroni’s multiple comparison test=0.05/4). Likewise, in the interspecific comparisons, the test was performed between each pair of the three species for respective category, with the significantly different pairs connected by *dashed lines* ( $\alpha$  for Bonferroni’s multiple comparison test=0.05/3); \*0.001 < P < 0.01; \*\*P < 0.001

	Number of individuals		Aggressiveness Index (%)
	Attacker	Non-Attacker	
<i>N. harmandi</i>			
(1) Male against another male	27	37	42.2
(2) Male against a female	4	17	19.0
(3) Female against another female	4	50	7.4
(4) Female against a male	2	19	9.5
<i>N. japonica</i>			
(1) Male against another male	31	27	53.4
(2) Male against a female	4	13	23.5
(3) Female against another female	2	52	3.7
(4) Female against a male	2	15	11.8
<i>N. petalura</i>			
(1) Male against another male	38	6	86.4
(2) Male against a female	6	15	28.6
(3) Female against another female	33	15	68.8
(4) Female against a male	6	15	28.6



(Zar 1999). The continuity-corrected test statistic is:  $Z_C = \{|p_i - p_j| - 1/2(1/n_i + 1/n_j)\} \{p(1-p)/n_i + p(1-p)/n_j\}^{-1/2}$ , where the subscripts *i* and *j* stand for the two categories compared,  $n_i = (n_A + n_{NA})_i$ ,  $p_i = (n_A)_i/n_i$ ,  $p = (n_i p_i + n_j p_j)/(n_i + n_j)$ , and  $Z_C$  is defined as zero in the case of  $|p_i - p_j| - 1/2(1/n_i + 1/n_j) \leq 0$ . The significance level was determined according to Bonferroni's multiple comparison procedure ( $\alpha = 0.05/4$ ). The characteristics in each species are summarized, as follows:

1. *N. harmandi* and *N. japonica*. In both species, a male acted as the attacker in about 40% or 50% of the encounters against another male. Against a female, that proportion decreased to about 20%, though the differences between categories 1 and 2 in Table 2 were not statistically significant. In *N. harmandi*, a female acted as the attacker in slightly < 10% of the encounters against both another female and a male, with no significant difference. In *N. japonica*, a female acted as the attacker in slightly < 5% of the encounters against another female and in slightly > 10% of the cases against a male, with no significant difference. In both species, the difference between the two intrasexual aggressiveness indices was significant, but not between the two intersexual ones.
2. *N. petalura*. The intrasexual aggressiveness indices were quite high in both sexes (about 90% between males and about 70% between females), with no significant difference. The intersexual aggressiveness indices were lower in both sexes (identical values of 29%), with a significant reduction from the intrasexual to the intersexual indices in each sex.

In each species, the aggressiveness indices between males were not significantly different between the non-breeding and breeding seasons; 36.1% versus 50.0%,  $Z_c = 0.86$ ,  $P = 0.39$  for *N. harmandi*, 40.9% versus 61.1%,  $Z_c = 1.23$ ,  $P = 0.22$  for *N. japonica*, and 91.7% versus 80.0%,  $Z_c = 0.68$ ,  $P = 0.50$  for *N. petalura*. In *N. petalura*, those indices between females were not significantly different between the two seasons, either; 60.7% versus 77.8%,  $Z_c = 0.88$ ,  $P = 0.38$ . Thus, intrasexually, males of *N. harmandi* and *N. japonica* and both sexes of *N. petalura* were aggressive to the same degree throughout the year.

The data in Table 2 were also used to make inter-specific comparisons in pairs for the four encounter categories listed in the first paragraph, whereby the significance level for the multiplicity was also determined according to the Bonferroni procedure ( $\alpha = 0.05/3$ ). In category 1 (a male against another male), the aggressiveness indices were ordered as *N. petalura* (86%) > *N. japonica* (53%)  $\approx$  *N. harmandi* (42%), with only the differences between the former and each of the latter two significant (both  $P < 0.001$ ). In category 3 (a female against another female), a significantly much stronger aggressiveness of *N. petalura* (69%) was detected than for *N. harmandi* (7%) and *N. japonica* (4%) (both  $P < 0.001$ ). The aggressiveness indices between the latter two species were not significantly different. In the two

categories of intersexual encounters (i.e. 2 and 4), there were no significant differences between any pair of the three species.

Details of the outcomes of fighting and their frequencies

Table 3 summarizes the frequency of the outcomes of fighting divided by their mechanisms (columns) and processes (rows). Reciprocal attacking ended in tie in most cases in all species (100% in *N. harmandi*, 89% in *N. japonica*, and 82% in *N. petalura*). As mentioned earlier, in *N. petalura* exhibiting one-way attacking, a male was a loser against a female, with the burrow-blocking mechanism. This was the single case in which any male became a loser in all of the cases of intersexual fighting in the three species (eight cases of reciprocal attacking and six of one-way attacking).

Of all cases of fighting combined, 15% (= 3/20), 24% (= 5/21), and 27% (= 12/45) ended in win and loss in *N. harmandi*, *N. japonica*, and *N. petalura*, respectively, in which the individuals were neither wounded nor killed. Of all cases, 5% (= 1/20), 5% (= 1/21), and 13% (= 6/45) ended in being driven out for the losers in *N. harmandi*, *N. japonica*, and *N. petalura*, respectively. Any

**Table 3** *Nihonotrypaea harmandi*, *N. japonica*, *N. petalura*. Frequency of the outcomes of "fighting" divided by mechanisms (columns) and processes (rows). The rightmost column includes either unconfirmed mechanisms or unconfirmed outcomes, with the asterisk indicating the confirmed win and loss. The sex of the winners is shown in parentheses

	Tie	Win and loss		Unconfirmed
		Burrow collapse	Burrow blocking	
<i>N. harmandi</i>				
Reciprocal attacking				
Male-male	13			
Female-female	2			
Male-female	2			
One-way attacking				
Male-male			1	
Female-female				
Male-female		1 (male)		1* (male)
<i>N. japonica</i>				
Reciprocal attacking				
Male-male	14		1	
Female-female	1			
Male-female	1	1 (male)		
One-way attacking				
Male-male				1*
Female-female				
Male-female		1 (male)		1* (male)
<i>N. petalura</i>				
Reciprocal attacking				
Male-male	13	1	3	1
Female-female	13		1	1
Male-female	5			
One-way attacking				
Male-male		1	1	
Female-female		2	1	
Male-female		1 (female)		1* (male)

death of shrimp that could occur in the field may be due to fish predation on such driven-out losers exposed on the substratum surface or to heat stress during tidal exposure periods in the summer (Tamaki et al. 1997; Shimoda and Tamaki, personal observation).

In fighting with the win and loss outcomes in all species, the winners were larger than the losers in most cases (75% of all cases combined). In *N. harmandi*, the two winners were larger than their losers in both TL (ratio = 1.14 and 1.26) and MCL (ratio = 1.11 and 1.87), while the one (male) was smaller in TL (ratio = 0.94), but larger in MCL (ratio = 1.20) than its loser (female). In *N. japonica*, three winners were larger than their losers in both TL (ratio = 1.05 to 1.29) and MCL (ratio = 1.06 to 1.54). Another one (male) was larger in TL (ratio = 1.02), but smaller in MCL (ratio = 0.95) than its loser (female). The other one (male) was smaller in TL (ratio = 0.95), but larger in MCL (ratio = 1.06) than its loser (male). In *N. petalura*, nine winners were larger than their losers in both TL (ratio = 1.01 to 1.23) and MCL (ratio = 1.02 to 1.70); in the single case in which a female defeated a male, the TL and MCL ratios were 1.16 and 1.29, respectively. The other three winners (two between males and one between females) were smaller than their losers in both TL (ratio = 0.85 to 0.97) and MCL (ratio = 0.83 to 0.94).

In all cases of fighting between two shrimp with their TL ratios > 1.3 (i.e. out of encounter rule 3 in "Materials and methods";  $n=1$  in *N. harmandi*;  $n=2$  in *N. japonica*;  $n=8$  in *N. petalura*), the winners were larger than their losers.

#### Details of burrow sharing behavior in *N. petalura*

Burrow sharing between sexes of *N. petalura* occurred in 3 of the 21 encounters that met the three encounter rules given in "Materials and methods" (cases 1–3 in

Table 4), in one of the eight encounters that met only rules 1 and 2 (case 4), and in one of the seven encounters that took place during the breeding season (case 5). Thus, burrow sharing occurred at a rate of 12.5–14.3% of the encounters. The definitions of some peculiar constituent behaviors of shrimp in a shared burrow are given below:

"Tender touching".	Both individuals sometimes gently contact each other face to face by placing and swiftly moving their antennules, antennae, third maxillipeds, minor chelipeds, and second pereopods on the other's body (Fig. 5E-a). Such contact, using the same appendices as above, is also made by gently touching the rear part of a shrimp of either sex from behind (Fig. 5E-b). After tender touching, the two shrimps either pass each other or move back peacefully.
"Tender pushing".	The male sometimes protrudes its major cheliped rather slowly and pushes the female gently either face to face or from behind (Fig. 5F) to simply go ahead.
"Tender pulling".	The male sometimes holds the rear part of the female abdomen with its minor chelipeds and second and third pereopods to draw the latter closer and simply go over it. Sometimes the female holds on to the burrow floor against such pulling.

In case 1, the recording of behaviors was terminated by accidental collapse of the burrow, which separated the shrimps. In cases 2 and 3, we terminated it ourselves, after which burrow sharing continued for at least 2–3 h. In case 4, we also terminated the recording, and, about 3 h later, the female was driven out. Through all cases, two or four constituent behaviors were recorded each, with tender touching (face to face) and tender

**Table 4** *Nihonotrypaea petalura*. Frequencies of constituent behaviors of burrow sharing and their mean ( $\pm$  SD) durations (parentheses). Cases 1–4 and 5 were recorded in the non-breeding and breeding seasons, respectively

	Case 1	Case 2	Case 3	Case 4	Case 5
Male TL (mm), female TL (mm), ratio	43.4, 39.4, 1.10	52.7, 52.6, 1.00	40.2, 40.6, 1.01	33.7, 45.8, 1.36	32.1, 40.9, 1.27
Constituent behavior					
Tender touching					
Face to face	4 (16.8 $\pm$ 14.2 s)	5 (13.0 $\pm$ 8.1 s)	17 (72.1 $\pm$ 42.9 s)	7 (39.1 $\pm$ 52.6 s)	14 (13.2 $\pm$ 9.2 s)
From behind female by male	1 (24 s)	6 (13.8 $\pm$ 5.2 s)	1 (24 s)	1 (16 s)	3 (52.0 $\pm$ 83.1 s)
From behind male by female				1 (8 s)	2 (10 s, 8 s)
Tender pushing	8 (9.3 $\pm$ 5.4 s)	1 (4 s)			
Tender pulling	1 (47 s)	1 (17 s)		5 (34.0 $\pm$ 14.1 s)	
Total time of observation (s)	4,456	5,918	7,187	2,937	8,539
All constituent behaviors combined					
Time (s)	238	169	1,250	468	374
Percentage in total time (%)	5.3	2.9	17.4	15.9	4.4

touching (from behind female by male) occurring in common. In terms of both frequency and duration, tender touching (face to face) took place most consistently. The duration summed for all constituent behaviors accounted for 3–17% of the entire duration of the recording. The female collected for case 5 had carried eyed eggs, with a well-developed ovary ready for the next egg deposition (cf. Tamaki et al. 1996). After releasing larvae by 0900 hours on 7 June 2003, it molted at around 1400 hours on 9 June. Immediately, it was put in an aquarium together with a male. Their burrow sharing began 5,036 s later and lasted for 8,539 s, during which only tender touching (face to face, 14 times) and tender touching (from behind each other, 5 times) took place, occupying 4% of the entire period, but no copulation happened. Then, they left each other by making a new burrow wall between them, after which the female never deposited eggs during the month prior to the end of observations.

## Discussion

Except for a few cases of burrow sharing behavior between sexes in *Nihonotrypaea petalura*, all individuals of the three species exhibited solitary behavior. Antagonism was manifested by either fighting or mutual burrow-crack filling, the latter lasting significantly longer. It is noteworthy that the degrees of intrasexual aggressiveness were quite different between the tidal flat species (*N. harmandi*, *N. japonica*) and the boulder beach species (*N. petalura*) (Tables 1, 2). Summarizing the results in the tables, it can be concluded that: (1) males are aggressive against males in all species, with the intensity ordered as  $N. petalura > N. harmandi \approx N. japonica$ ; (2) females are basically non-aggressive against females in *N. harmandi* and *N. japonica*, while, intrasexually in *N. petalura*, females are as aggressive as males; (3) males are less aggressive against females than against males in all species; (4) females are as non-aggressive against males as against females in *N. harmandi* and *N. japonica*; and (5) in *N. petalura*, females are less aggressive against males than against females. Furthermore, as regards the outcomes of fighting (Table 3), it is noteworthy that: (1) no individuals were wounded and directly killed; (2) the larger individuals, generally with the larger major chelipeds (Fig. 3), were at an advantage in most cases; and (3) the proportion of the driven-out cases in fighting was rather low (5–13%). The above feature 1 is different from fatal combats reported for several thalassinidean shrimps (Berrill 1975; Tunberg 1986; Rodrigues and Hödl 1990; Rowden and Jones 1994; Coelho 2001). From feature 2, it could be deduced that the much smaller individuals may not dare challenge the larger opponents in the first place (cf. Labadie and Palmer 1996). Feature 3, which is a possible fatal factor under natural conditions, raises an interesting question for further study, i.e. after maturation, is any biased sex ratio with growth of individuals actually brought about

in a local population of each species as a consequence of fighting, and to what extent?

The differences in the degree of intrasexual aggressiveness among the three species of *Nihonotrypaea* were in parallel with those in the absolute size and sexual dimorphism in the shrimp major cheliped, which was used as the sole weapon in fighting (Fig. 5A–D). It is noteworthy that the female major cheliped of *N. petalura* was much larger than those of *N. harmandi* and *N. japonica* in both length and mass (Fig. 4A', B') and that the major cheliped size of female *N. petalura* was closest to that of males (Fig. 3D, D'). In fact, the female major cheliped of *N. petalura* was so effective as to even defeat a male of a nearly equivalent body size. By contrast, the female major chelipeds of the other two species were much smaller and lighter than those of the males (Fig. 3D, D'), and they appear to be of little use as a weapon. Among the three species, the male major cheliped of *N. petalura* was intermediate in both length and mass, while that of *N. japonica* was the smallest (Fig. 4A, A'). This would be derived from the phylogenetic relationships of the three species and/or the constraints of their contrasting habitat conditions (see Wardiatno et al. 2003; Shimoda and Tamaki 2004). Interestingly, between the two tidal-flat species, the male major cheliped size (both length and mass) was significantly much greater in *N. harmandi* than in *N. japonica* (Fig. 4A, B), in contrast to the little or no difference between chelipeds of the females (Fig. 4A', B'). This was reflected by a significantly higher degree of sexual dimorphism in the major cheliped in *N. harmandi* (Fig. 3D, D').

The interspecific differences in the intra- and intersexual aggressiveness between the three species of *Nihonotrypaea* in relation to those in the degree of major cheliped dimorphism could have evolved from potentially different intensities of intrasexual competition for mates performed under natural population densities. In the Ariake Sound estuarine system, the maximum mean densities of adults of the three species so far recorded were 1,440 m<sup>-2</sup> for *N. harmandi* (on the Tomioka Bay sandflat: Flach and Tamaki 2001), 343 m<sup>-2</sup> for *N. japonica* (on the Shirakawa sandflat: Wardiatno et al. 2003), and 12 m<sup>-2</sup> for *N. petalura* (on the Kuchinotsu-Cho boulder beach: Shimoda and Tamaki 2004). In addition to the extremely low density of *N. petalura* on a square meter basis, actually, individual burrows are distributed on isolated sand patches surrounded by boulders, in which only a few conspecifics, for example two or three, exist (see Fig. 1 in Shimoda and Tamaki 2004). In such a situation, severe intraspecific competition for mates could occur not only between males, but also between females. This intrasexual selection might have been a driving force to evolve the severe aggressiveness accompanied with the enlarged major cheliped as a weapon in both sexes. By contrast, in the tidal flats, with much higher population densities, females should find less difficulty in securing mates. In such a situation, females could maximize their fitness by just sitting and

waiting for males to come, with their acquired energy directed not into fighting (and enlarging their major chelipeds), but into ovarian development. For males, intraspecific competition for mates could be more intense in *N. harmandi* than in *N. japonica*, with the maximum density four times higher in the former population, which could further be related to its more distinct sexual dimorphism in the major cheliped (Fig. 3D, D').

The burrow-sharing behavior of *N. petalura* (Fig. 5E, F; Table 4) could also be understandable in light of a selective force to secure mates by both sexes under their low-density situation. This might have further accelerated intrasexual selection in each sex. Interestingly, this behavior was observed even in the non-breeding season. In their reviews on the biology and ecology of intersexual pair-bonding species in decapods, Salmon (1983) and Asakura (1998) pointed out the species' severe intrasexual aggressiveness associated with less distinct sexual dimorphism in the major cheliped as compared with those in species under the mating system of polygyny or promiscuity. Of the three species of a callianassid genus *Neotrypaea* distributed on the west coast of North America, *N. biffari* can be regarded as an analogue to *N. petalura* in terms of habitat (MacGinitie and MacGinitie 1968; Shimoda and Tamaki 2004). MacGinitie and MacGinitie (1968) mentioned that adults of *N. biffari* always lived in pairs in the field. No records of intraspecific antagonistic behaviors of this species have been published. Of 58 resin casts of burrows of *N. petalura* collected from the field, no one contained two adult shrimps in it, with only a single shrimp or none in exactly the half casts for each (Shimoda and Tamaki 2004). In fact, the present laboratory observations revealed that the frequency and duration of burrow sharing were rather limited (Table 4). Thus, the mating system of *N. petalura* would not be strict monogyny. In thalassinidean shrimps as a whole, a contrasting combination of intrasexual aggressive and intersexual pair-bonding behaviors has been observed for *Neaxius vivesi*, *Axianassa australis*, and *Pomatogebia operculata* [see Berrill (1975) for the former; Coelho (2001) for the latter two]. Coelho (2001) recorded that in their burrow sharing, shrimp of both sexes of these species exhibited recognition display either face to face (both species) or from behind, similar to the tender touching behavior observed for *N. petalura* (Fig. 5E). The maximum population densities of *N. vivesi*, *A. australis*, and *P. operculata* seem to be as low as  $8\text{ m}^{-2}$  (Berrill 1975), up to  $7\text{ m}^{-2}$  (Dworschak and Rodrigues 1997; Felder 2001), and  $0.006 (= 5/780)\text{ m}^{-2}$  (Scott et al. 1988), respectively.

The present behavioral observations on and morphometries of the three species of *Nihonotrypaea* could be used to explain the change in the sex ratio with individual growth after maturation in several local populations. Toward this goal, it is necessary to examine the mating system of each species. First of all, the recording of intersexual interactions between

shrimps in the breeding season will be needed. However, it is extremely difficult to investigate the mating system of thalassinidean shrimps, and virtually nothing is known about this group (e.g. Candisani et al. 2001). Another possible step would be to map the spatial arrangement of individual male and female burrows, with the determination of their occupants' body size and female sexual receptivity in selected local populations. Through this procedure, a more informative measure of population density than a gross mean, such as mean crowding (Lloyd 1967), could be related to the change in the population sex ratio (cf. Shuster and Wade 2003).

**Acknowledgements** We wish to thank R. Hirohashi, N. Obayashi, K. Harada, and R. Fujiwara for their assistance in the field work, G. Itani for the identification of the bopyrid isopod on *Nihonotrypaea japonica*, and A. Asakura for copies of literature. This study was supported by the Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research 12640618, 13854006, and 15570018 to A.T.

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