Life history of the copepod *Hemicyclops spinulosus* (Poecilostomatoida, Clausidiidae) associated with crab burrows with notes on male polymorphism and precopulatory mate guarding

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**Abstract:** A 25-month field survey was conducted to investigate the life cycle and seasonal population fluctuations in the poecilostomatoid copepod *Hemicyclops spinulosus* in the burrows of the ocypodid crab *Macrophthalmus japonicus* in the mud-flats of the Tama-River estuary, central Japan. On the basis of sample collections in the water column and from the crab burrows, it was confirmed that *H. spinulosus* is planktonic during the naupliar stages and settles on the bottom during the first copepodid stage to inhabit the burrows. Furthermore, the copepods’ reproduction took place mainly during early summer to autumn with a successive decrease from autumn to winter. A supplementary observation on the burrows of the polychaete *Tylorrhynchus heterochaetus* suggested that these burrows are another important habitat of *H. spinulosus*. There were additional discoveries of male polymorphism and precopulatory mate guarding behavior by males, suggesting an adaptation in the reproductive strategy of this copepod to their narrow habitat spaces and low population densities, in contrast to the congeneric species *H. gomsoensis*, which co-occurs in the estuary but attains much larger population sizes and is associated with hosts having much larger burrow spaces.

**Key words:** Life cycle, male polymorphism, mud-flat, polychaete burrow, seasonal change

**Introduction**

Poecilostomatoid copepods of the genus *Hemicyclops* have been reported from intertidal and subtidal zones, including estuarine mud-flats, all around the world with the exception of polar regions (Humes 1984) and ca. 40 species are currently recognized (Vervoort & Ramirez 1966, Itoh & Nishida 2002, Mulyadi 2005). Many species of *Hemicyclops* occur on the body surface or in the burrows of benthic invertebrates, such as thalassinidean decapods and polychaetes, during their copepodid stages (Humes 1984). The first copepodids (CIs) of some species have also been collected from the water column, often predominating in local plankton assemblages (Itoh & Nishida 1991), and these have sometimes been called “*Saphirella*-like copepodids”, referring to the specialized body form that is shared by some other members of the Clausidiidae and related families (e.g. Gooding 1963, 1988, Izawa 1986, Kim & Ho 1992, Itoh & Nishida 1995, Itoh 2006).

The ecological characteristics and the systematic position of *Hemicyclops*, together with the ecological importance of invertebrate burrowers, suggest that the genus is an interesting group to increase our understanding of the burrow-associated life style and its relationship to species diversity in intertidal communities (Itoh & Nishida 2007). However, until recently our knowledge of these copepods had been essentially limited to taxonomic descriptions, geographic distributions, morphology of post-embryonic developmental stages, and fragmentary descriptive remarks on their relationships with the host species (see Itoh 2001 for review).

Among the species of *Hemicyclops* commonly distributed in the mud-flats of the Tama-River estuary, the life cycle and seasonal fluctuation of *H. gomsoensis* Ho & Kim, the most abundant species inhabiting the burrows of the mud shrimp *Upogebia major* (de Haan) and the ocypodid crab *Macrophthalmus japonicus* (de Haan), have been investigated by Itoh and Nishida (2007). The present study deals with *H. spinulosus* Itoh & Nishida, the second-most abundant species in the area (Itoh 2001, Itoh & Nishida 2007), mainly on the basis of a 25-month observation on the field populations in the water column and from the burrows of *M. japonicus*, a major benthic habitat of the cope-
pod as presumed in the initial stage of the study (Itoh 2001, Itoh & Nishida 2007). In addition, at a later stage of the sampling program we found that the copepods are also associated with the polychaete Tylorrhynchus heterochaetus (Quatrefages), the burrows of which frequently are in contact with Macrophthalmus burrows. Hence a collection was made from the polychaete burrows for a comparison of the population structure of the copepods between the hosts. We also describe, for the first time in Hemicylops, two types (medium- and large sized) of non-typical males and precopulatory mate guarding behavior, and discuss their significance in the copepod’s reproduction.

Materials and Methods

Study site

The Tama River is among the biggest of the rivers flowing into Tokyo Bay, with an average discharge of 36.6 m³ s⁻¹ and a width of ca. 500 m at its mouth (Ministry of Land, Infrastructure and Transport, Japan: http://www.keihin.ktr.mlit.go.jp/english/index.htm). The present study sites are located on a mud-flat (35°32’N, 139°46’E) bordering the right bank of the river (Fig. 1). Site M is located 4.2 km upstream from the river mouth where burrows of Macrophthalmus japonicus are abundant, between the mean sea level and the mean high water level of neap tide (MHWN), and has reed bushes on its bank side. Sampling for the planktonic stages was done at 0.5 m-depth offshore of Site M, referred to as Site P.

Life cycle and developmental stages

As a basis for the study of population structure, the morphological changes of Hemicylops spinulosus were followed through all developmental stages. Adult females carrying egg sacs were collected with a pipette (see next section) from the burrows of M. japonicus at Site M on 16 May 1998. Egg sacs were removed and kept at 20°C in a 50-mL Petri dish containing 30-mL river water that was collected from Site P and filtered through a 20-μm nylon sieve. The filtered river water contained dinoflagellates (mainly Prorocentrum spp.) at high concentration, hence no additional foods were supplied. Specimens representing all 6 naupliar stages (NI-NVI) were obtained from this culture. The copepodid stages collected from the burrows of M. japonicus on 12 June (for CI-CIV) and 9 October 1999 (for CV-CVI) were examined. All specimens were fixed and preserved in 2% formaldehyde/river-water buffered with sodium tetraborate. The morphology of specimens representing each stage was examined and illustrated, and body dimensions (body length and width for the nauplii; body length, from the anterior margin of prosome to the posterior margin of the caudal ramus excluding the caudal setae, for the copepodids) were measured under a compound microscope.

Seasonal change of population structure

Populations of H. spinulosus in the Tama-River estuary were investigated monthly from May 1998 to May 2000 within 2 h before and after low spring tides. Plankton samples were collected at Site P by towing a hand net (mouth diameter, 20 cm; mesh size, 100 μm) 3 times along the shoreline for a distance of 10 m, then fixed and preserved in 2% buffered formaldehyde/river-water. While a mesh size of 100 μm may not be fine enough to retain younger nauplii (body width of NI: ca. 80 μm), it was not feasible to use finer mesh due to the abundance of suspended particles in the river water, which would have caused serious clogging of the net. The nauplii and copepodids of H. spinulosus were sorted and enumerated under a compound or a stereo microscope. The counts were converted to abundance per cubic meter applying the mouth area and towing distance of the net and assuming 100% filtration efficiency.

The benthic populations of H. spinulosus were sampled from the burrows of M. japonicus, identified, and counted as described in Itoh and Nishida (2007). Briefly, we sampled 10 burrows that appeared to have few connections with burrows of other benthic animals, such as conspecific crabs,
or another crab *Hypolax pusilla* (de Haan), by digging the substrate by hand and sucking the entire aqueous content of the burrows with a large (50 mL) plastic pipette. The copepods were sorted from this sample, identified to the species level and the numbers of each developmental stage and sex were counted. The counts from the burrows were converted to abundances per burrow. The body length and width, and prosome length (PL) of adult females and males were measured to the nearest 10 μm for all specimens. The presence/absence of egg sacs and spermatophores was also recorded in the females.

The temperature of the offshore- and burrow water was measured with a mercury thermometer on site, while salinity of the water sample was determined in the laboratory with a salinometer (DigiAuto-3G; Tsurumi Seiki Co. Ltd).

**Populations in polychaete burrows**

The copepodids of *H. spinulosus* on the body surface and in the burrows of polychaetes were first encountered on 6 November, 1999 at Site M. The polychaetes were first collected by chance during the copepod sampling from crab burrows. The burrows of polychaetes had small entrances on the mud surface, with diameters of <5 mm. It was impossible to identify the polychaete species by the shape of their entrances. We inserted a narrow spade into the mud substrate below the entrances to dig out the polychaetes. On the next day at the same site 10 polychaetes each with and without *H. spinulosus* attached to their bodies were collected with their burrow-wall sediments and fixed individually in vials containing 4% formaldehyde/river-water. In the laboratory the polychaetes were identified and the copepods were examined under a stereo microscope for stage, sex (including male polymorphs), presence of egg sacs and/or spermatophores, and male polymorphism. In addition, we observed copepods that appeared to perform precopulatory mate guarding (Boxshall 1990, Shimanaga 1998). Hence some copepods were kept alive, brought back to the laboratory, and the behavior of CV females and CVI males was observed in a 50-mL glass vial containing river water from Site P with a supply of benthic algae (mostly pennate diatoms) from Site M.

**Statistical analyses**

One-way analysis of variance (ANOVA) was performed to test for seasonal differences in copepod abundance in the *Macrothalthalma* burrows. To equalize variance and normalize distribution, the abundance data were square-root \((x+0.5)\)-transformed prior to the analyses. Where significant differences in the ANOVA were detected, Tukey’s post-hoc test was applied to identify sources of variation. The PL of females, sex ratios, and the ratios of the females carrying egg-sacs were pooled for every 2 months and for 2 years. One-way ANOVA was applied to test for seasonal difference in the PL of females, but not for males owing to the small sample size. The differences of copepod abundance between the crab- and polychaete burrows were examined by Mann-Whitney’s *U*-test, while differences in the stage composition, sex ratios, and the ratios of females carrying spermatophores were examined by the Chi-square test. Wilcoxon’s signed-ranks test was applied to test for differences in temperature and salinity between sites throughout the study period.

**Results**

**Life cycle and developmental stages**

From the rearing experiment and field sampling, it was confirmed that the post-embryonic life of *Hemicyclops spinulosus* comprises 6 naupliar and 6 copepodid stages, wherein the CI is the *Saphirella*-like copepodid and the CVI is the adult (Fig. 2). The male and female are distinguishable at CV and CVI. Each naupliar stage is distinguished from the others by the number of caudal setae and/or spines, while copepodid stages are distinguished from each other by the number of body somites. These sequential changes in body structure are similar to those reported for *Hemicyclops japonicus* Itoh & Nishida (Itoh & Nishida 1995, 1997), and hence are not described in detail here. Each stage was larger than the corresponding stage in *H. japonicus* (Itoh & Nishida 1995, 1997) without an overlap in ranges in any stage, providing a good aid to identification of stages/species. The body length (mm) of each stage was as follows (n = 10): NI, 0.136 ± 0.001 (mean ± SD); NII, 0.155 ± 0.003; NIII, 0.171 ± 0.006; NIV, 0.194 ± 0.007; NV, 0.225 ± 0.007; NVI, 0.259 ± 0.003; CV, 0.503 ± 0.014; CLI, 0.546 ± 0.028; CII, 0.736 ± 0.045; CIV, 0.968 ± 0.026; CV (female), 1.345 ± 0.049; CV (male), 1.245 ± 0.072; CVI (female), 1.722 ± 0.071; CVI (male, but excluding ‘non-typical’ males: see the section Male polymorphism), 1.510 ± 0.118. The differences between *H. spinulosus* and *H. gomsoensis*, another co-occurring congener at the study site, are described in Itoh and Nishida (2007, see also Fig. 2).

Although no mention was made of the body color in the original description of *H. spinulosus* (Itoh & Nishida 1998), the bodies of the CIV-CVIs observed in the present study are bright red when alive (Fig. 11), which makes it easy to distinguish the copepods from the host and substrates, as well as from other congeneric species, while the nauplii and CIs are almost colorless; no definitive observation has been made on the CI-CIIIs. In contrast, the CIV-CVIs of *H. gomsoensis* are almost colorless except for the presence of brown pigments that are mostly restricted to the thoracic somites.

**Seasonal change of population structure**

**Environmental conditions**

The water temperature both in the burrows and of the river water (Fig. 3) showed peaks in July-August and troughs in December-January, ranging from 8.5°C to
29.6°C, with no significant difference between the burrow- and river water (Wilcoxon’s test, \( p > 0.05 \)).

The salinity in the burrows ranged from 1.2 to 24.0 with a trend to be higher in winter than in summer with occasional peaks and depressions, corresponding to the fluctuations in river discharge (Fig. 3). The salinity in the burrows was significantly higher than in the river water (Wilcoxon’s test, \( p < 0.01 \)), with minimal values (<3) recorded in August–October 1998 and July–August 1999.

**Planktonic populations**

The plankton-net samples contained only the NI-NVI and CI copepods. They occurred only in May–August 1998, April–October 1999, and May 2000, with high densities (>20 copepods m\(^{-3}\)) in July 1998, May and August 1999, and May 2000 (Fig. 4A).

**Benthic populations**

Copepodids of *H. spinulosus* were collected year-round from the *Macrophthalmus* burrows, while nauplii were not collected at all. Copepod abundances showed significant seasonal variation (one-way ANOVA, \( F_{2,225} = 4.47, p < 0.001 \)). In the first year (May 1998–April 1999), copepod abundance was significantly higher in July than in June, September, and from November to April; it was also significantly higher in May, August, and October than in December (post-hoc test, \( p < 0.05 \)). In the second year (May 1999–May 2000), they were significantly more abundant in September than in May 1999, January, March and May 2000 (\( p < 0.05 \)). The mean abundance tended to increase from early summer to autumn with a successive decrease from autumn to winter (Fig. 4B), and the peaks of mean abundance corresponded with those of the planktonic populations (Fig. 4A) with a one-month delay or less.
During May–August 1998 and May–September 1999, CI-CIII comprised \( \frac{1}{2} \) of the total benthic population. Particularly in July 1998 and May and August 1999 when the pelagic population showed its highest abundance, CI alone contributed \( \frac{1}{2} \) to the benthic population (Fig. 4B). During September 1998–April 1999 and October 1999–May 2000, on the other hand, CIV -CVI comprised \( \frac{2}{3} \) of the total population, showing a gradual advance of stages from CIV to CVI in the latter period.

The sex ratio (percentage of males to total copepods in CV/CVI) averaged 22.8% in CVs and 29.4% in the adults, with the trend to decrease in high-temperature seasons, and this was particularly evident in the adults, with ratios of \( \frac{1}{2} \) in May–June/July–August (Fig. 5).

Females carrying egg-sacs occurred in May–October 1998, March–September 1999, and May 2000 (Fig. 4B), comprising \( \frac{2}{3} \) of the total number of adult females in May–June and July–August (Fig. 6). The percentage of females without spermatophores on the genital double-somite showed a reverse trend, being \( \frac{1}{5} \) in May–June and July–August but 33% and 20% in January–February and November–December, respectively (Fig. 6).

The PL of adult females, averaged for two months, ranged from 0.93 to 0.97 mm showing no significant seasonal variation (one-way ANOVA; female, \( F_{5,162} = 2.27, p = 0.136 \)), but in May–June females with PL of \( \geq 1.05 \) mm were more abundant than in the other months, comprising 28.9% of the total females (Fig. 7).

**Male polymorphism**

Sixty-three CVI males of *H. spinulosus* that coincided with the original description (Itoh & Nishida 1998) were collected from *Macrophthalmus* burrows during the whole study period. In addition, two types of CVI males that differed from the original description in size and structure of the maxilliped and the leg-1 endopod were encountered. Hereafter, the smallest males that agree with the original description are referred to as the “small-type”, the largest males with well-developed maxillipeds as the “large-type”, and males of intermediate sizes as the “medial-type” (Fig. 8).

A total of 6 medial-type males (Fig. 8C) were collected, one each in May and October 1998, and February, April, August, and November 1999. Their body lengths and widths were 1.96–2.30 mm (mean: 2.13 mm) and 0.74–0.85 mm (0.78 mm), respectively, and were larger without a range overlap, than those of the small-type males which
measured 1.30–1.67 mm (1.51 mm, n/H11005 10) and 0.55–0.60 mm (0.57 mm, n/H11005 10). Both in the small- and medial-type males there is a process at the base of each inner seta on the second and third segments of the leg-1 endopod. These processes are directed inward in the small-type (Fig. 8B: arrowheads) but directed distally in the medial-type males (Fig. 8D: arrowheads). The maxilliped of the medial-type males (Fig. 9B) resembles those of the small-type males (Fig. 9A) in having an elongated terminal claw which is subequal to the second segment but differs in having a marked depression on the inner margin of the second segment (Fig. 9B: arrow). In addition, the two setae at the basal 1/3 of the claw are much longer (Fig. 9B: arrowheads) than those in both the small- and large-type males (Fig. 9A, C: arrowheads).

Only a single specimen of the large-type male, with a body length of 2.66 mm and width of 1.05 mm, was found in October 1998 (Fig. 8E). It lacks the processes at the basal part of the inner setae on the second- and third segment of the leg-1 endopod (Fig. 8F: arrowheads), and has a well-developed maxilliped with a large process on the inner-distal margin of the second segment (Fig. 9C: arrow) and a thick terminal claw which is shorter than the second segment (Fig. 9C).

These three types of males are assumed to be conspecific, since, except for their body size and the features of the maxilliped and leg-1 endopod, there are no marked differences between these types in other features, while there are marked differences between co-occurring species in the morphology of the posterior part of the labrum: the bilateral rows of setae in *H. spinulosus* (Fig. 10A–C) are lacking in *H. gomsoensis* (Fig. 10D) and *H. tanakai* Itoh & Nishida (Fig. 10E); the continuous tooth-row on the posterior margin in *H. spinulosus* is discontinuous at the midpoint in *H. ctenidis* Ho & Kim (Fig. 10F); the anterior extension of the medial granular area in *H. spinulosus* is lacking in *H. ctenidis*. All these features are shared by the three types of males of *H. spinulosus*.

**Populations from polychaete burrows**

**Abundance and population structure**

All polychaetes from November 1999 with which *H. spinulosus* were associated (n/H11005 10) were *Tylorrhynchus heterochaetus* while no *Hemicyclops* species were found from another polychaete, *Hediste* sp. (n/H11005 10), which was dominant at the study site (Nishi & Tanaka 2006), either from their body surface or from the burrows. The burrows of both species were fairly narrow, less than 5 mm in diameter throughout their lengths; the burrows of *Tylorrhynchus* reached much deeper (40–60 cm) than those of *Hediste* (<20 cm). Many of the *H. spinulosus* were attached to the body surface of *Tylorrhynchus*, while others were attached to the burrow wall (Fig. 11). Since the burrows were so narrow, it was impossible to determine on which side the copepods had attached, either on the polychaete or on the burrow wall, before the burrow was dug out and the polychaete exposed. Hence both types of association will hereafter be collectively referred to as “in the burrows”.

The total number of copepodids per *Tylorrhynchus* burrow ranged from 5 to 24 (mean: 14.9) showing no significant difference with those from the *Macrophthalmus* burrows (U-test, p/H11022 0.05) (Fig. 12). In the *Tylorrhynchus* burrows the adults comprised 47.1% and both CVs and CIVs comprised >20%, with the younger stages being minor constituents, while CIs-CIIIs comprised 27.7% in the *Macrophthalmus* burrows, showing a significant difference in the stage composition between the hosts (\( \chi^2 = 35.96, \) DF=3, p/H11005 0.001). Sex ratios averaged 33.8% in the adults and 15.6% in the CVs in the *Tylorrhynchus* burrows, while those in the *Macrophthalmus* burrows were 45.9% and 36.8%, respectively, showing no significant difference between the hosts (p/H11022 0.05). The percentage of females without spermatophores to the total females was 36.7% in the *Tylorrhynchus* burrows, while that in the *Macrophthalmus* burrows was 20.0%, with no significant difference between the hosts (p/H11022 0.05). There were no females carrying egg-sacs in the burrows of either host.

A total of 23 small-type, one medial-type, and one large-type male was collected from the *Tylorrhynchus* burrows,
Fig. 8. Whole animal (A, C, E) and leg-1 endopod (B, D, F) in three male-types of *Hemicyclops spinulosus*. A, B, small-type; C, D, medial-type; E, F, large-type. The processes at the base of each inner seta on second and third segments of leg-1 endopod are directed inward in small-type males (B: arrowheads) but are directed distally in medial-type males (D: arrowheads). These processes are lacking in large-type males (F: arrowheads).
while 16 small-type and one medial-type males were found in the *Macrophthalmus* burrows in November 1999. The small-type males contributed 92.0% and 94.1% of the total in the *Tylorrhynchus* - and *Macrophthalmus* burrows, respectively.

**Precopulatory mate guarding**

In November 1999, two small-type males each holding a CIV (sex unknown) or a female CV were collected, one from each of two *Tylorrhynchus* burrows. In fixed and preserved condition, the male was mounted on the dorsal side of the urosome of the CV female and clasped the female with the terminal claws of its maxillipeds located between the fifth pedigerous somite and the genital double-somite of the female (Fig. 13).

A small-type male and a CV female were put in a 50-mL vial and their behavior was observed. The male clasped the female within 5 h, and continued to clasp until releasing the female between 65 and 77 h after the start of the observation when the female had already molted into CVI with a spermatophore attached to the anterior part of the genital double-somite (see Fig. 2), and the cast-off skin remained in the vial.

**Discussion**

**Life cycle and habitats**

The present observations, based on sampling planktonic- and benthic populations, confirmed that the life cycle of *Hemicyclops spinulosus* comprises an early pelagic phase (NI-CI) and a late benthic, burrow-inhabiting phase (CI-CVI), and that CI is the transitional stage from pelagic to benthic life style, as established for *H. gomsoensis*, the major benthic habitat of which is the burrow of *Upogebia major* (Itoh & Nishida 2007).

In the present study we targeted the crab *Macrophthalmus japonicus* as a major host of *H. spinulosus*, since the copepod was first described from the burrows of this crab (Itoh & Nishida 1998). Later on, however, appreciable numbers of the copepods were found in the burrows of the polychaete *Tylorrhynchus heterochaetus*. *Hemicyclops spinulosus* were observed to attach to the body surfaces of *T. heterochaetus* (Fig. 11) but not to those of *M. japonicus*. Association with polychaetes is known in *Hemicyclops arenicolae* Gooding, *H. adhaerens* (Williams), and *H. ctenidis* (Gooding 1960, Gooding 1963, Ho & Kim 1990), all of which have been collected from washings of the poly-
chaetes’ bodies. For the occurrence of this copepod from the burrows of *Macrophthalmus japonicus* and *Tylorrhynchus heterochaetus* in November 1999. Error bars indicate 95% confidence intervals.

Fig. 11. Photographs showing *Hemicylops spinulosus* attached to the body surface (white arrows) and burrow wall (green arrows) of the polychaete *Tylorrhynchus heterochaetus*.

Fig. 12. Abundance and population structure of *Hemicylops spinulosus* from burrows of *Macrophthalmus japonicus* and *Tylorrhynchus heterochaetus* in November 1999. Error bars indicate 95% confidence intervals.

For the occurrence of this copepod from the *Macrophthalmus* burrows, the following cases may be proposed: (1) the copepods might have originated only from the crab burrows that had connections with the polychaete burrows, hence might have been collected by sucking the water of the crab burrows with a large pipette; (2) the copepods are inhabiting the crab burrows, as well as those of the polychaete. The first case is unlikely, since comparable abundances of copepods were collected from both types of burrows (Fig. 12), although the copepods from the crab burrows may have contained by-catch from the polychaete burrows (Itoh & Nishida 2007). In addition, *T. heterochaetus* leave their burrows for the water column in order to reproduce in late autumn. These are known as the “Japanese Palolo” (Izuka 1903), and the posterior halves of the polychaetes’ bodies are left in the burrows, finally decomposing and the burrows disappear consequently (Itoh unpublished observation). In contrast, the burrows of *M. japonicus* are present throughout the year, providing the copepods with a habitat to maintain their populations during the absence of the *Tylorrhynchus* burrows, this being consistent with the second case outlined above. It appears that *H. spinulosus* takes advantage of the different conditions provided by the two hosts, both with considerably different biology/ecology, e.g. in burrow space, food availability, predation risk, physicochemical properties of burrow water, and seasonal availability of the burrow itself, as suggested by the different stage composition of the copepods between the hosts (Fig. 12), while there was no apparent difference in the copepod coloration between the hosts.

Among the above 3 species of *Hemicylops* associated with polychaetes, *H. adhaerens* has been reported from the
body surfaces of the mud shrimp *Upogebia affinis* Say as well as from the polychaete *Nereis virens* Sars (Gooding 1963), suggesting the copepod associates with multiple hosts as in the present species. *Hemicyclops spinulosus* were not collected from the burrows of the polychaete *Hediste* sp. that were abundant at the study site (Nishi & Tanaka 2006). The burrows of *Hediste* sp. are located within 20 cm from the mud surface, in contrast to those of *Tylorrhynchus* which reach 40–60 cm in depth (Furota 1996), and appear to be less suitable for *H. spinulosus*. Perhaps this is because of their structural instability under more turbulent conditions in shallower than in deeper sediment layers.

The bright-red color of *H. spinulosus* is of particular interest, since it appears to be confined to this species and is not found in other congeners occurring in the Tama-River estuary, suggesting an ecological and/or physiological specificity in the species. The color expands over the whole body and appears to derive from the body fluid of the copepod rather than from its gut contents. Furthermore, the copepod’s feeding appendages are of the scraping type, lacking any structure for sucking the body fluid of the host (Itoh & Nishida 1998). Hence the origin of the color being from the blood of the host polychaete due to feeding is unlikely. The color is reminiscent of that known from various crustaceans that possess/synthesize hemoglobin in habitats with low-oxygen conditions, including some copepods (e.g. Fox 1957, Hourdez et al. 2000). It is possible that *H. spinulosus* possesses hemoglobin, which may be highly advantageous in the low-oxygen environment in the mud-flat sediments (e.g. Satoh et al. 2007), but this awaits confirmation.

**Seasonal fluctuation of population structure**

In the study site, females carrying egg-sacs were present only during March–October (Fig. 4B), and pelagic stages (NI-NVI and CI) were collected only during April–October (Fig. 4A), suggesting that reproduction of *H. spinulosus*
took place mainly from spring to autumn. In spring the CIs from the *Macrophthalmus* burrows were first collected in May, followed by all copepodid stages and adults in June, as observed in 1999 (Fig. 4B). This leads to an estimated developmental time from the CI to the adult of one month or less, and the possibility of five generations from May to October, assuming a mean generation time of one month during this period. In addition, the gradual change in stage composition from October 1999 to May 2000 (Fig. 4B) and the presence of large adult females (PL = 1.05 mm) in May–June (Fig. 7) suggest the presence of an over-wintering generation with a life span of seven months or more, leading to an estimate of six generations per year.

While the gross pattern of seasonal fluctuation, i.e. higher abundance in summer than in winter, was similar in planktonic- and benthic populations, there was a sharp contrast between them. All the abrupt decreases in abundance of the planktonic population in August–October 1998 and July and September 1999 (Fig. 4A) corresponded with considerable increases in river flow (Fig. 3), which may have flushed a considerable part of the planktonic population out of the estuary. However, the benthic population did not show significant reductions corresponding with these flushes, in spite of the considerable decrease in the salinity of the burrow water (down to <5: Fig. 4B). This is in contrast to the marked reduction in the benthic population of *H. gomoensis* in the *Macrophthalmus* burrows when the salinity decreased to <5 (Itoh & Nishida 2007), suggesting that *H. spinulosus* is more tolerant to lower salinities than is *H. gomoensis*. This is consistent with the observations in *H. spinulosus* that appreciable numbers of females carrying egg-sacs were present throughout the summer (Figs 4B, 6) and that higher abundances of CIs were present at more upstream sites (Itoh 2001).

**Sex ratio, male polymorphism, and precopulatory mate guarding**

The sex ratio (percentage of males) of *H. spinulosus* during the study period averaged 29.4% in the adults and 22.8% in the CVs, being far lower than in *H. gomoensis* (ca. 50%: Itoh & Nishida 2007). In addition, there were three types of males that differed in body length and in the shape of the maxilliped and the leg-1 endopod; the smallest type was most abundant and showed precopulatory mate guarding behavior; male polymorphism and precopulatory mate guarding were hitherto unknown in *Hemicylops* species.

Each of the above traits is known in other families/genera of the order Poecilostomatoida (Table 1): low sex ratios in the sabelliphilids *Paranthessius anemoniae* and *Modiolicola bifida*, the clausidiids *Hemicyclops ctenidis* and *Conchyliurus quintus*, the myicolids *Ostrincola koe* and *Midicola pontica*; male polymorphism in *M. pontica*; precopulatory mate guarding in the pseudanthessiid *Pseudanthessius tortuosus*, the rhynchomolgid *Pennatulicola*

### Table 1. Reports (marked “O”) of low sex ratio, male polymorphism, and precopulatory mate guarding in poecilostomatoid copepods.

| Family and species | Host | Low sex ratio | Male polymorphism | Precopulatory mate guarding | Reference |
|--------------------|------|---------------|-------------------|-----------------------------|-----------|
| Sabelliphilidae     |      |               |                   |                             |           |
| *Paranthessius anemoniae* |      |               |                   |                             |           |
| Claus              | Snake-locks anemone: *Anemonia sulcata* (Pennant) | O | — | — | Briggs 1976 |
| *Modiolicola bifida* Tanaka | Manila clam: *Ruditapes philippinarum* (Adams & Reeve) | O | — | — | Ko 1969 |
| Clausidiidae       |      |               |                   |                             |           |
| *Hemicyclops ctenidis* | *Nereis: Hediste* sp. (as *Neanthes japonica* (Izuka)) | O | — | — | Kim & Ho 1992 |
| *Hemicyclops spinulosus* Itoh & Nishida | *Polychaeta, Tylorrhynchus heterochaetus* and crab, *Macrophthalmus japonicus* | O | O | O | This Study |
| *Conchyliurus quintus* Tanaka | Manila clam | O | — | — | Ko 1969 |
| Myicolidae         |      |               |                   |                             |           |
| *Ostrincola koe* Tanaka | Manila clam | O | — | — | Ko 1969 |
| *Midicola pontica* Sowinsky | Blue mussel: *Mytilus galloprovincialis* Lamarck | O | O | O | Do et al. 1984, Do & Kajihara 1986 |
| Pseudanthessiidae  |      |               |                   |                             |           |
| *Pseudanthessius tortuosus* Stock, Humes & Gooding | Amphinomid polychaete | — | — | O | Gotto 1979 |
| Rhynchomolgidae    |      |               |                   |                             |           |
| *Pennatulicola pterophilus* (Stock) | Pennatulid sea pen | — | — | O | Gotto 1979 |
pterophilus and M. pontica. Of these copepods H. ctenidis and C. quinatus belong to the same family as H. spinulosus but all other species are, more-or-less, at distant phylogenetic positions from H. spinulosus (see Ho 1991, Huys & Boxshall 1991).

Among the above copepods, M. pontica possesses all three attributes found in H. spinulosus, i.e. low sex ratios, male polymorphism, and precopulatory mate guarding. In addition, there is experimental evidence that sex and type of males (the “atypical male” with higher swimming ability to move between hosts than the “typical male”) in M. pontica are determined epigenetically according to presence/absence and/or sex of conspecific copepods that have preoccupied the mantle cavity of the host (Mytilus galloprovincialis) at the time the copepods enter the host (Do et al. 1984, Do & Kajihara 1986). Such a mechanism appears to enhance the probability of male-female encounters in semi-enclosed microhabitats and thereby enhances reproductive success (Do et al. 1984). A similar mechanism may apply in H. spinulosus inhabiting polychaete- and crab burrows, which are similar to the mantle cavity of bivalves in that all have fairly limited spaces formed in the sediments.

Precopulatory mate guarding is known in animal species for which the mating period of females is relatively limited (Ridley 1983) and is widespread among podpole copepods (Boxshall 1990). We have been unable to find this behavior in H. gomsoensis (Ioth unpublished data) which have much larger population densities within a single host (e.g. >100 copepods per burrow in November 1999) than in H. spinulosus (15 copepods per Tylorrhynchus burrow; 10 copepods per Macrophthalmus burrow), suggesting that precopulatory mate guarding may be advantageous to males in securing mates in low-density populations. On the other hand, since this behavior necessitates a high investment of time by the males, lowered mating activity of males, such as in low temperature, would result in increased numbers of unmated adult females that had not experienced mate guarding. This is consistent with the increased proportion of females without spermatophores in winter in the present study. In this respect, the medial- and large-type males, whose maxillipedes appear suitable to clasp the laterally-expanded genital double-somites of adult females, may be engaged exclusively in mating only with adult females without precopulatory guarding.

Hemicyclops spinulosus are planktonic during the naupliar stages, shift to burrows at the CI stage (Saphirella-like copepods), and live in the burrows until adult, a life style shared by H. gomsoensis, and perhaps by many other congeners. However, the present study also demonstrated considerable differences between the two species in their hosts and in attributes related to reproduction. Species diversity in Hemicyclops is assumed to be a result of adaptive radiation to utilize diverse hosts and their burrows as habitats (Humes 1984, Huys & Boxshall 1991) in estuarine mudflats, which are highly productive but subject to considerable environmental changes. The planktonic early period is essential in such a habitat to secure dispersion and gene flow among local populations, and this trait, coupled with the use of burrows, appears to comprise the basic life history pattern common in this genus. On the other hand, diverse morphology and spatial scales of the burrows and the ecology of the hosts, which are directly related to the encounter probability of prospective mates, may have resulted in the marked differences between species in attributes related to reproduction, as observed in the present study.

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