

Life history of the copepod *Hemicyclops gomsoensis* (Poecilostomatoida, Clausidiidae) associated with decapod burrows in the Tama-River estuary, central Japan

HIROSHI ITOH¹ & SHUHEI NISHIDA^{2*}

¹ Suidosha Co. Ltd, 8–11–11 Ikuta, Tama, Kawasaki 214–0038, Japan

² Ocean Research Institute, University of Tokyo, 1–15–1 Minamidai, Nakano, Tokyo 164–8639, Japan

Received 16 March 2007; Accepted 16 May 2007

Abstract: A 17-month field survey and laboratory experiments were conducted to investigate the life cycle, seasonal population fluctuations, and salinity tolerance in the poecilostomatoid copepod *Hemicyclops gomsoensis* associated with the burrows of the mud shrimp *Upogebia major* and 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 burrows, it was revealed that *H. gomsoensis* is planktonic during the naupliar stages and settles on the bottom during the first copepodid stage to inhabit the burrows of *U. major* and, to a lesser extent, those of *M. japonicus*. While females carrying egg-sacs were present throughout the year, the copepods' reproduction took place mainly during early summer to autumn with a successive decrease from autumn to winter. Occasionally the copepod populations in the burrows suffered from severe flushes of river water that led to salinity decreases in the burrow water to fatal levels, but usually the salinity in the burrow was within optimal levels and permitted recovery and maintenance of the populations.

Key words: life cycle, mud-flat, salinity tolerance, seasonal change, *Upogebia*

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 (CI) 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). On the basis of the morphological characters of the adults, particularly those of the mandibles, and their association with benthic burrows,

it has been suggested that the genus represents an evolutionary link between true parasitic copepods and those with a more free-living life style in the Poecilostomatoida (Ho 1991, Huys & Boxshall 1991, see also Boxshall & Halsey 2004 for discussion on the systematic position of Poecilostomatoida).

The burrowing activities of various benthic invertebrates in intertidal and subtidal zones have received special attention with respect to their role in controlling the physico-chemical properties of sediments and biological production of the areas (e.g. Koike & Mukai 1983, Ziebis et al. 1996, Berkenbusch & Rowden 2003, Kinoshita et al. 2003, James et al. 2005). In addition, it has progressively become clearer that these burrows provide diverse organisms with living habitats, and that such associations underpin the presence of highly diverse assemblages in areas of considerable environmental fluctuation, such as intertidal zones and estuaries (e.g. MacGinitie 1935, MacGinitie & MacGinitie 1949, Dobbs & Guckert 1988, Sato et al. 2001, Itani 2004, Anker et al. 2005, Koller et al. 2006).

The ecological characteristics and the systematic position of *Hemicyclops*, together with the importance of invertebrate burrows as described above, suggest that the genus is

* Corresponding author: Shuhei Nishida; E-mail, nishida@ori.u-tokyo.ac.jp

an interesting group to increase our understanding of the burrow-associated life style and its relationship to species diversity in intertidal communities. However, our knowledge of these copepods is 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).

In the Tama-River estuary, central Japan, 5 species of *Hemicyclops* have been recorded from the burrows of invertebrates. Of these *Hemicyclops gomsoensis* Ho & Kim is the most abundant (Itoh 2001). This species was first described in Korea (Ho & Kim 1991) from the burrows of the ocyropodid crab *Macrophthalmus japonicus* (de Haan) which is also common in the estuaries of northeastern Japan and forms burrows in muddy tidal flats (Ono 1965, Kurihara et al. 1989). In the Tama-River estuary *H. gomsoensis* has been collected from the burrows of the mud shrimp *Upogebia major* (de Haan) as well as from those of *M. japonicus* (Itoh & Nishida 1998, Itoh 2001). *Upogebia major* are common in the lower intertidal to subtidal zones of the coasts from Hokkaido to Kyushu, Japan, as well as in the Yellow-Sea and Taiwan (Miyake 1982). Their burrows are fairly large and inhabited by various animals, such as phoronid worms, decapods, gobies, and copepods (Kinoshita 2002, Itani 2004).

The present study is based on a 17-month observation in the Tama-River estuary, and investigates (1) the morphology and habitats of *H. gomsoensis* throughout its life cycle, (2) seasonal changes in their abundance and population structure at different sites and with different hosts, and (3) their salinity tolerance with respect to environmental changes within the host burrows. Their food habit is also examined in an attempt to provide supplemental information for discussion and to stimulate further research. As far as we are aware, this is the first account following a field population of a *Hemicyclops* species throughout their life cycle.

Materials and Methods

Study site

The Tama River is among the biggest of the rivers flowing into Tokyo Bay, with an average discharge of $36.6 \text{ m}^3 \text{ s}^{-1}$ and a width of ca. 500 m at its mouth (Ministry of Land, Infrastructure and Transport: <http://www.keihin.ktr.mlit.go.jp/english/index.htm>). The present study sites are located on a mud-flat ($35^\circ 32' \text{N}$, $139^\circ 46' \text{E}$) bordering the right bank of the river facing downstream (Fig. 1). Sites UM (Upstream-*Macrophthalmus*) and UU (Upstream-*Upogebia*) are located 4.2 km upstream from the river mouth where burrows of *Macrophthalmus japonicus* and *Upogebia major*, respectively, are abundant. Site DU (Downstream-*Upogebia*) is located 2.7 km from the river mouth and is dominated by *U. major*. With respect to the tidal levels, Site UM is located between the mean sea level and the mean high water level of neap tide (MHWN) and has reed bushes on its bank side, while Sites UU and DU are between the mean low water of neap tide (MLWN) and the extreme low water of spring tide (ELWS). Sampling for the planktonic stages was done at 0.5 m-deep points offshore of Sites UU and DU, referred to as Sites U and D, respectively.

Life cycle and developmental sages

As a basis for the study of population structure, the morphological changes of *Hemicyclops gomsoensis* were followed through all developmental stages. Adult females that carried egg sacs were collected with a pipette (see next section) from the burrows of *M. japonicus* at Site UM on 26 July 1996. Egg sacs were removed from the copepods' body and kept at 20°C in a 50-ml Petri dish containing 30-ml river water that was collected from Site U and filtered through a $20\text{-}\mu\text{m}$ nylon sieve. The filtered river water contained dinoflagellates (mainly *Prorocentrum* spp.) at high concentrations, hence no additional foods were supplied. Specimens representing all 6 naupliar stages (NI-NVI) were obtained from this culture. All copepodid stages were col-

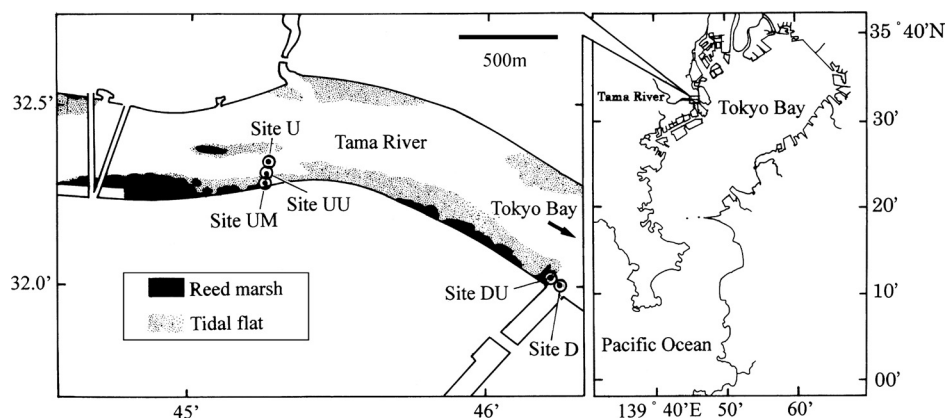


Fig. 1. Map of study sites. Sites U and D: plankton sampling. Sites UU and DU: sampling from burrows of *Upogebia major*. Site UM: sampling from burrows of *Macrophthalmus japonicus*.

lected with a hand pump (see next section) from the burrows of *U. major* at Site UU on 26 July 1996. The 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, length and width of prosome and urosome for the copepodids) were measured under a compound microscope.

Seasonal change of population structure

Populations of *H. gomsoensis* in the Tama-River estuary were investigated monthly from January 1999 to May 2000 within 2 h before and after low spring tides.

Plankton samples were collected at Sites U and D 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 were fixed and preserved in 2% buffered formaldehyde/river-water. While the mesh size of 100 μm may be not fine enough to retain younger nauplii (body width of NI: ca. 60 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. gomsoensis* were sorted and enumerated under a compound or a stereo microscope. For Site D, however, only the copepodids were examined, due to the great abundance of many other marine species that made nauplii identification at the species level impossible. The enumerated values 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. gomsoensis* were sampled from the burrows of *M. japonicus* (Site UM) and *U. major* (Sites UU and DU). The burrows of *M. japonicus* were easily distinguished from those of *U. major* by the large diameter (usually >ca. 5 cm) of their entrance and reduced diameter just beneath the entrance. In the *Upogebia* burrows the entrance was much narrower (usually <ca. 1 cm) but with a much larger space beneath the entrance. The *Macrophthalmus* burrows are usually constructed obliquely relative to the mud surface with a J-shape and a length of up to ca. 50 cm, reaching to a depth of 30–40 cm from the mud surface (Wada 2000; see also Lee & Koh, 1994). The total volume of each burrow usually contained less than 500 ml of water and they were often interconnected with the burrows of conspecific crabs, another crab *Ilyoplax pusilla* (de Haan), or polychaetes. Considering this structural feature, we sampled 10 burrows that appeared to have few connections with other burrows by digging the substrate by hand and sucking up the entire aqueous content of the burrows with a large (50 mL) plastic pipette.

Upogebia burrows were Y-shaped and fairly large; the reported maximum dimensions are 208 cm in depth, 3 cm in diameter, and 1700 mL in volume in Tokyo Bay (Kinoshita 2002). It was not possible to recover the entire aqueous content of each burrow, so copepods were collected from

10 burrows at each site by sucking up 1000-ml of water per burrow with a plastic hand pump (for cleaning home aquaria). Burrows were selected so that at least 30 cm length of the intake pipe of the pump was able to be inserted.

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).

The water samples containing the copepods were kept unfixed in plastic bottles, brought back to the laboratory, and left for 1–2 h. After confirming the presence of copepods creeping on the settled sediments, drops of concentrated formaldehyde (37%) were added for fixation. The supernatant was gently stirred with a glass stick so that the copepods were suspended in the water, which was then filtered through a 100 μm sieve. The copepods were sorted from this sample, identified to the species level, and the number of each developmental stage and sex were counted. For each station the counts from the 10 burrows were converted to abundances per burrow for the *Macrophthalmus* burrows (Site UM) and to abundances per 1000 mL for the *Upogebia* burrows (Sites UU and DU). The prosome length of adult females and males was measured to the nearest 10 μm for as many as 50 copepods of each sex. The presence/absence of egg sacs and spermatophores was also recorded in the females.

One-way analysis of variance (ANOVA) was performed to test for seasonal differences in copepod abundance in the burrows at each site, while the differences in abundance and prosome length between sites at each sampling occasion were examined by the Student's *t*-test, except for the prosome-length data that showed a bimodal distribution and where the Kruskal-Wallis test was applied. 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. Wilcoxon's signed-ranks test or Kruskal-Wallis test was applied to test for differences in temperature, salinity, abundance of planktonic populations, ratios of adults to total copepods, and sex ratios between sites throughout the study period.

Stage duration and salinity tolerance

For estimating stage durations, CI copepods of *H. gomsoensis* were collected from the study site on 6 June and 1 August 1999 and individually reared in a micro-plate well containing 20 mL of river water (salinity: ca. 20 psu) from the study site under a light regime of 500 lux with a L:D cycle of 12:12 h. A total of 48 copepods were reared at both 15°C and 25°C. Epiphytic microalgae, mostly pennate diatoms, that were grown in a one-week incubation of a <50- μm filtrate of the river water on micro-plate wells, were supplied as food. The rearing water was not changed

throughout the experiment, and the developmental stage of each copepod was examined daily at 1800 h.

The survival of *H. gomsoensis* at different salinity levels was examined for adult females. Copepods of the CV stage were collected at Site U on 6 June and 1 August 1999 and individually reared in micro-plate wells containing 20 mL of river water (salinity: ca. 20 psu) from the same site under a light regime of 500 lux with a L:D cycle of 12:12 h at 25°C. Epiphytic microalgae, prepared as above, were supplied as food. Twenty copepods, within 4 d after final molt, were individually introduced into a 6-ml glass vial containing a mixture of seawater and river water each of the following salinity levels: 0, 2, 5, 10, 15, 20, and 30 psu. The original seawater (salinity: 32 psu) was collected from Kawasaki Harbor near the mouth of the Tama River, while the river water (salinity: 0 psu) was from a site 25 km upstream from the river mouth. The copepods were kept at 25°C in darkness without food, and their life status was recorded daily at 1800 h. In addition, to examine the influence of salinity on egg-sac formation, 20 adult females without egg-sacs, sampled from the study site, were individually introduced into vials with salinity levels of 5, 10, 15, 20, and 30 psu, and reared at 25°C in the dark with a supply of epiphytic microalgae prepared as above. The presence/absence of egg sacs on each copepod was examined daily at 1800 h. The copepods reared at 5 psu did not form egg sacs at all. These copepods were transferred after one week of incubation into 15-psu vials and examined for their egg-sac formation thereafter.

The copepods' survival at different salinity levels was analyzed by one-way ANOVA, while the data on egg-sac formation was analyzed by two-way ANOVA without replication, after square-root-transformation of the original data, followed by Tukey's post-hoc test.

Food habits

A qualitative analysis of food habits of *H. gomsoensis* was made on the specimens collected at Site UU from the *Upogebia* burrows with a hand pump, as described above, on 4 June and 2 July 2000. A 1000-mL water sample from the burrow was fixed immediately with buffered formaldehyde at a final concentration of 2%. From the original sample, adult specimens were sorted out under a stereo microscope and guts with recognizable material were removed from the prosome with dissecting needles. Each gut was put in a drop of distilled water on a slide and the contents were manipulated with needles, covered with a glass slip, and examined under a compound microscope for the occurrence, types, taxonomic categories, and/or sizes of items.

Results

Life cycle and developmental stages

From the rearing experiment and field sampling, it was

confirmed that the post-embryonic life of *Hemicyclops gomsoensis* 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 1995, 1997), and hence are not described in detail here. Each stage was larger than the corresponding stage in the co-occurring species *H. japonicus* (Itoh & Nishida 1995, 1997) without an overlap in ranges at any stage, providing a good aid in identification of stages/species. The body length (mm) of each stage was as follows ($n=10$): NI, 0.119 ± 0.003 ($\bar{x} \pm \text{SD}$); NII, 0.143 ± 0.002 ; NIII, 0.164 ± 0.003 ; NIV, 0.185 ± 0.006 ; NV, 0.217 ± 0.005 ; NVI, 0.252 ± 0.005 ; CI, 0.585 ± 0.011 ; CII, 0.644 ± 0.020 ; CIII, 0.861 ± 0.039 ; CIV, 1.129 ± 0.048 ; CV (female), 1.461 ± 0.061 ; CV (male), 1.451 ± 0.038 ; CVI (female), 1.646 ± 0.105 ; CVI (male), 1.737 ± 0.068 . The other species common at the study site, *Hemicyclops spinulosus* Itoh & Nishida, 1998, can be distinguished from *H. gomsoensis* by the following characters (Fig. 2; Itoh, unpublished observation). In the naupliar stages, *H. spinulosus* has a triangular process and two pairs of spinules on the mid-posterior margin of the labrum, but *H. gomsoensis* has only one pair of spinules in the same area. In the copepodid stages, the inner-most setae of the caudal rami are shorter than the outer-most setae in *H. spinulosus*, but more than twice as long as the outer-most setae in *H. gomsoensis*.

Seasonal change of population structure

Environmental conditions

The water temperature (Fig. 3) showed a trend similar among all sites (Sites UM, UU, DU, U, D), with low values in winter and high values in summer ranging from 8.5°C (Jan. 1999) to 30.3°C (Aug. 1999) without significant differences between the sites (Kruskal-Wallis test, $p>0.05$).

The salinity showed a trend to be higher in winter than in summer at all sites with occasional peaks (June and September) and depressions (March–May and July–August) corresponding to fluctuations in river discharge and, in contrast to the temperature, salinity levels differed considerably among sites (Fig. 3). Throughout the year, in the *Upogebia* burrows, the salinity was higher at downstream (Site DU) than at upstream sites (Site UU), and in the upstream sites it was higher in the *Upogebia* burrows (Site UU) than in the *Macrophthalmus* burrows (Site UM) (Wilcoxon's test, $p<0.05$), with the average salinity during 1999 being 17.2 psu at Site UU, 23.2 psu at Site DU, and 14.7 psu at Site UM. In July 1999 the minimum salinity during the study period (2.0 psu) was recorded at Site UM, while it was still at higher levels (7.4 and 16.5 psu) in the *Upogebia* burrows,

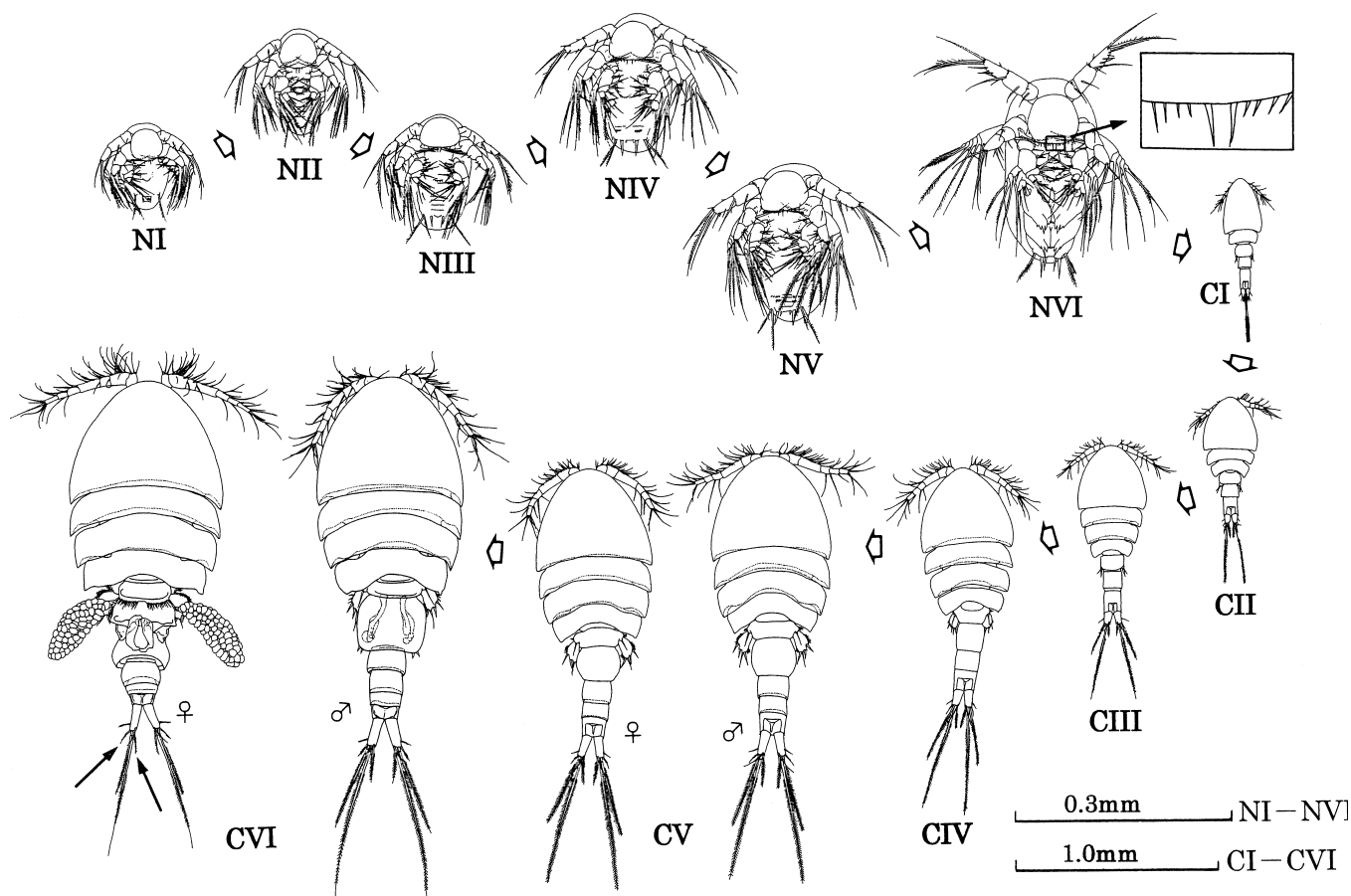


Fig. 2. Post-embryonic development of *Hemicyclops gomsoensis*. Characters discriminating *H. gomsoensis* from a co-occurring species *H. spinulosus* are indicated for nauplius (NVI: inset) and copepodid (CVI: arrows) stages (see text for further explanation).

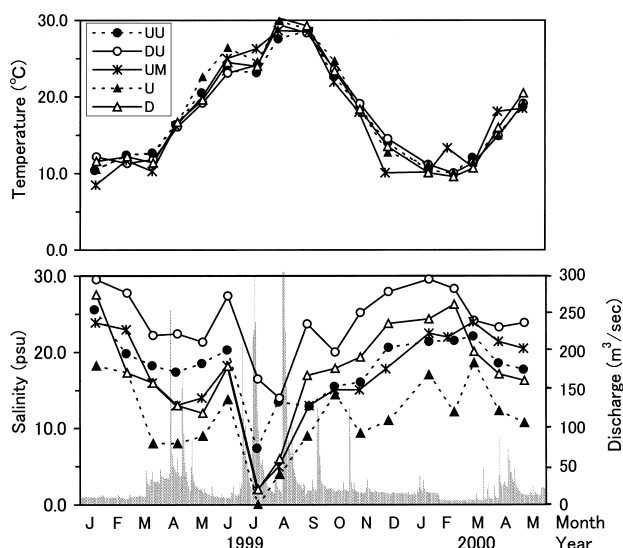


Fig. 3. Water temperature and salinity in burrows of *Upogebia major* (Sites UU and DU), *Macrophthalmus japonicus* (Site UM), and river water (Sites U and D) from January 1999 to May 2000. Discharge at Ishihara Gauging Station in the Tama River is also shown (Ministry of Land, Infrastructure and Transport).

despite the much lower salinity of the river water at Sites U (0.8 psu) and D (1.3 psu).

Planktonic populations

The plankton-net samples contained developmental stages NI–NVI and CI–CIII. The nauplii occurred in January and from May to December 1999 and in April and May 2000, with high densities in May and June 1999 and May 2000 at Site U (Fig. 4A). Copepods in the CI stage comprised more than 99% of the total number of copepodids at Sites U and D, and showed a seasonal pattern similar to that for the nauplii at Site U, except in May 2000 at Site D where no copepodids were collected (Fig. 4B). High densities of >30 copepods m^{-3} were observed at Site U in June, August, November 1999 and May 2000, while at Site D the densities were below 10 indiv. m^{-3} throughout the study period, except in September 1999 when an extremely high density (61 indiv. m^{-3}) was observed. Except during this month, the density of the CI stage was significantly higher at Site U than at Site D (t -test, $p < 0.05$).

Benthic populations

Copepodids of *H. gomsoensis* were collected year-round from the burrows of both *Upogebia* and *Macrophthalmus*,

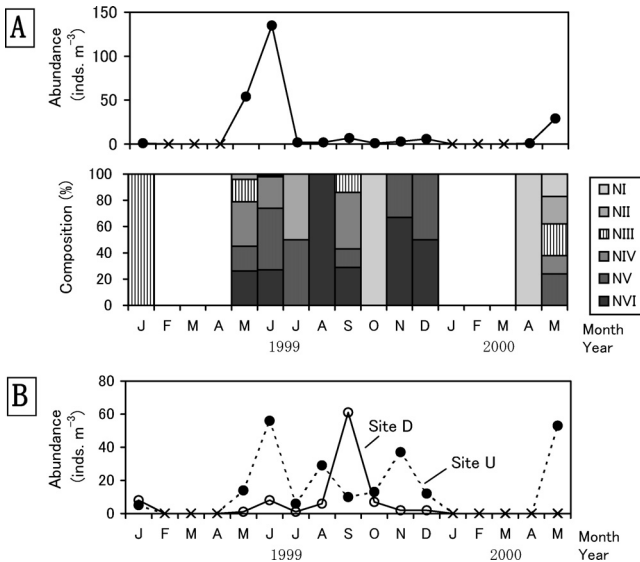


Fig. 4. Abundance and population structure of planktonic stages of *Hemicyclops gomsoensis* from January 1999 to May 2000. A: naupliar stages at Site U. B: copepodid stage I at Site U (solid circle) and D (open circle). “×” indicates non-occurrence of copepods.

while nauplii were not collected at all. Copepod abundances in the *Upogebia* burrows showed significant seasonal variation both at Sites UU (one-way ANOVA, $F_{16, 153}=34.65$, $p<0.001$) and DU ($F_{16, 153}=10.96$, $p<0.001$), with significantly higher densities from June to November than from January to May (post-hoc test, $p<0.01$) at Site UU, and at Site DU higher in July and August than from January to May ($p<0.01$) (Fig. 5A, B). The copepod density was significantly higher at Site UU than at DU throughout most of the high-density season (Fig. 5A, t -test, $p<0.05$). The contribution of adult specimens to the total number of copepods showed high values in February–April, July, October and November 1999, and in February–April 2000 without significant differences between the two sites (Wilcoxon’s test, $p>0.05$).

The copepod abundance in the *Macrophthalmus* burrows (Site UM) also showed significant seasonal variation (one-way ANOVA, $F_{16, 153}=48.06$, $p<0.001$), with high densities in June–September 1999 and a marked decrease in July (Fig. 5C). Density was low in the other months, with significantly higher densities in June–September than in January–May (post-hoc test, $p<0.01$). In particular, during January–April of both years there were some burrows from which not even a single copepod was collected (Fig. 5C). The contribution of adult specimens to the total number of copepods during June–December 1999 was not significantly different from the values at Site UU but was significantly lower than at Site DU (Wilcoxon’s test, $p<0.05$).

The sex ratio (percentage of males to total copepods of a particular stage) of CVs in the *Upogebia* burrows (Sites UU and DU) was below 25% during January–April, at 25–75% during May–December 1999, and decreased again to below

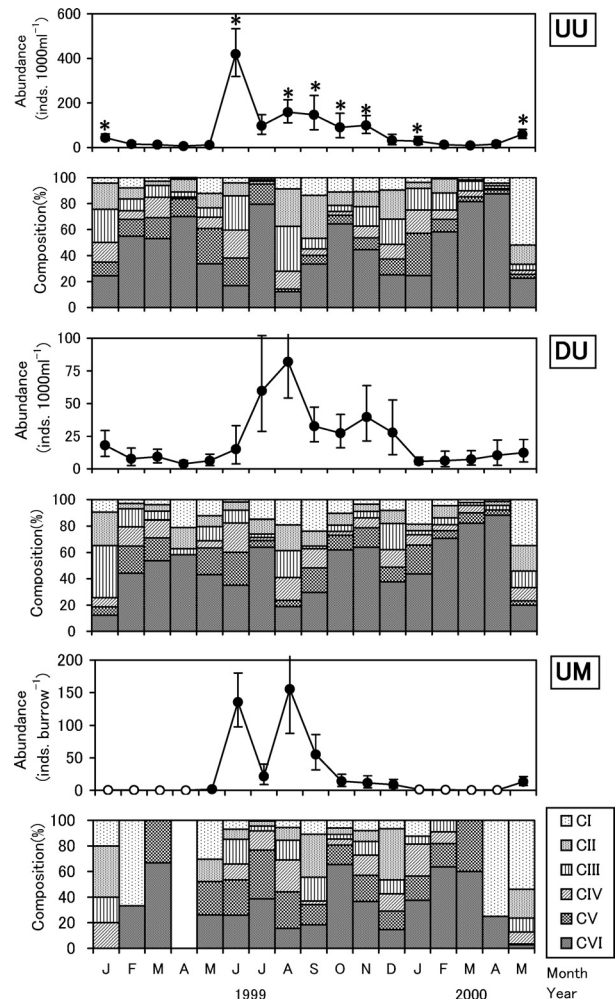


Fig. 5. Abundance and population structure of copepodid stages of *Hemicyclops gomsoensis* from January 1999 to May 2000 at different sites (UU, DU, and UM). Asterisks indicate a significant difference between Sites UU and DU. Open circles indicate presence of burrows without *H. gomsoensis*. Error bars indicate 95% confidence intervals.

25% in February 2000 (Fig. 6). In the adults it was around 50% throughout the year, except for a slight increase in January or February. Owing to the scarcity of collected specimens, the sex ratio in the *Macrophthalmus* burrows (Site UM) was estimated only for June–December 1999, where it was 25–50% in the CVs and 50–75% in the adults. It was significantly lower in the CVs (Wilcoxon’s test, $p<0.05$) and higher in the adults ($p<0.05$) than in the *Upogebia* burrows (Site UU).

The percentage of females carrying egg-sacs compared with the total number of adult females in the *Upogebia* burrows was above 30% for most months, but decreased in January, February and/or August; no egg-carrying females were collected in February 1999 or January 2000 at Site DU (Fig. 7). In the *Macrophthalmus* burrows (Site UM) there was a marked decrease in the ratio in August 1999. The percentage of females without spermatophores on the

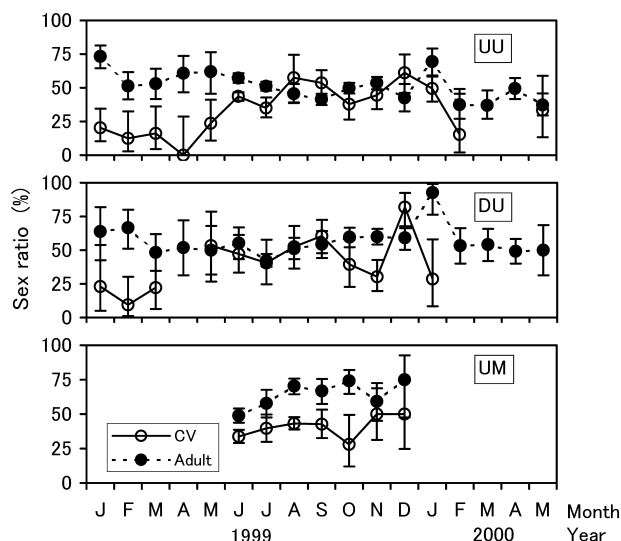


Fig. 6. Seasonal change in sex ratios (number of males/total number of males and females) of *Hemicyclops gomsoensis* at different sites (UU, DU, and UM). Error bars indicate 95% confidence intervals.

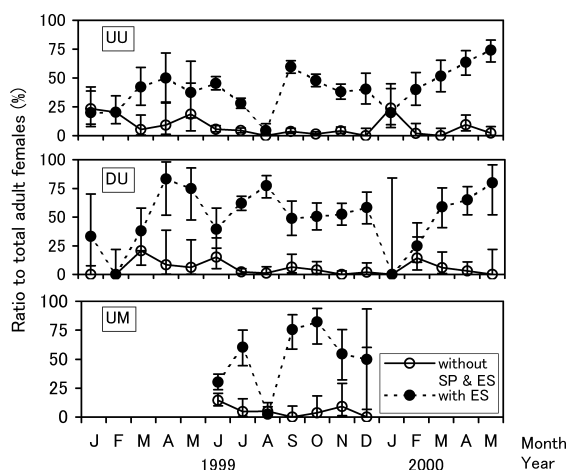


Fig. 7. Seasonal changes in ratios of females carrying egg-sacs and those without egg-sacs (ES) and spermatophores (SP) to adult females of *Hemicyclops gomsoensis* at different sites (UU, DU, and UM). Error bars indicate 95% confidence intervals.

genital double-somite was below 25% throughout the year.

The prosome length in both females and males of *H. gomsoensis* from the *Upogebia* burrows showed significant seasonal variation (one-way ANOVA; female, $F_{16, 708} = 127.72$, $p < 0.001$; male, $F_{16, 588} = 90.10$, $p < 0.001$), with higher values from winter to spring than from summer to autumn (Fig. 8). They were significantly larger at Site DU than at Site UU for two thirds of the study period (t -test or Kruskal-Wallis test, $p < 0.05$) (Fig. 8).

The prosome length of copepods in the *Macrophthalmus* burrows (Site UM) was analyzed only during June–December 1999, and showed significant seasonal variation in both females (one-way ANOVA, $F_{6, 238} = 56.00$, $p < 0.001$) and males ($F_{6, 286} = 48.87$, $p < 0.001$) with a de-

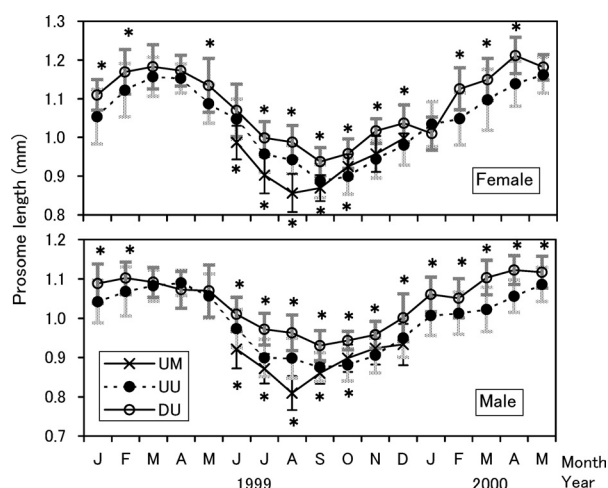


Fig. 8. Seasonal change in mean prosome length of *Hemicyclops gomsoensis*. Error bars indicate SD. Asterisks above and below the bars indicate significant differences of mean prosome length between Sites UU and DU, and between Sites UU and UM, respectively.

Table 1. Durations (days: mean \pm SD) of copepodid stages (CI–CVI) of *Hemicyclops gomsoensis* maintained at 15°C and 25°C. The values for CVI indicate days to death after final molt.

Stage	15°C		25°C	
	Female (n=4)	Male (n=3)	Female (n=4)	Male (n=1)
CI	30.0 \pm 18.3	23.7 \pm 15.9	6.0 \pm 3.4	6
CII	8.5 \pm 2.7	8.3 \pm 2.9	2.8 \pm 0.5	7
CIII	9.3 \pm 5.1	23.3 \pm 17.2	3.5 \pm 0.6	2
CIV	21.3 \pm 13.3	22.3 \pm 8.5	6.0 \pm 2.7	6
CV	25.5 \pm 11.7	28.7 \pm 18.5	5.8 \pm 1.0	7
CI–V	94.5 \pm 20.9	106.0 \pm 20.8	24.0 \pm 4.9	28
CVI	44.5 \pm 22.2	60.0 \pm 15.9	23.3 \pm 3.0	32

pression around August (Fig. 8). The mean prosome length was significantly longer at Site UU than at Site UM in both sexes during June–September (t -test or Kruskal-Wallis test, $p < 0.05$), but was not significantly different between these sites during November–December.

Stage duration and salinity tolerance

Out of the 48 CIs reared for stage-duration determination, 4 females and 3 males grew to adults at 15°C, with 4 females and 1 male at 25°C. The survival rate was less than 15% in both conditions. Most copepods died during the CI without further molt, and only 50% and 31% molted to CII at 15°C and 25°C, respectively. Most of the copepod deaths occurred when the copepods crept up the wall of the rearing vessel and got out of the water, which is similar to the observation by Gurney (1944) during his experiment with the *Saphirella*-like copepodids. There were considerable variations in the durations both among and within stages, and between sexes and temperatures (Table 1). The total aver-

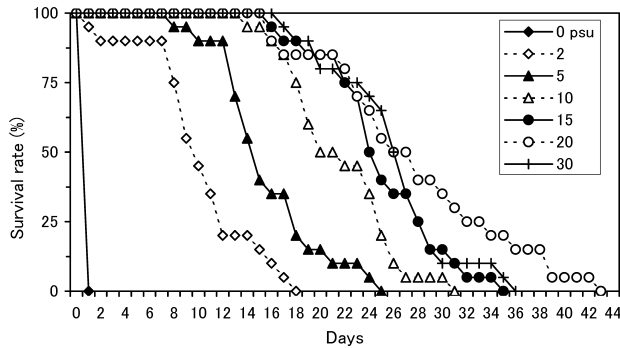


Fig. 9. Survival rates (%) of adult females of *Hemicyclops gomsoensis* at different salinity levels at 25°C.

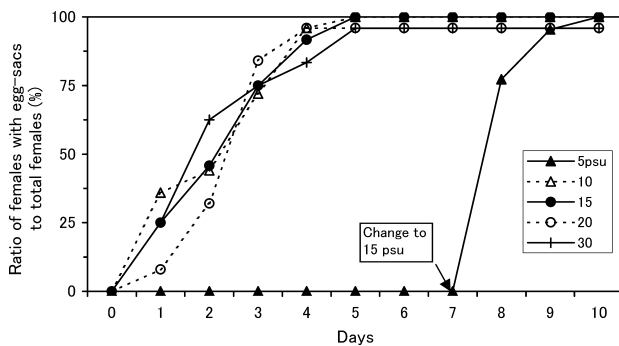


Fig. 10. Egg-sac formation rates (%) of *Hemicyclops gomsoensis* at different salinity levels at 25°C. The copepods in water of 5 psu salinity were transferred to 15 psu on day 7.

age duration until female maturity, including the days after capture of CI, was significantly longer at 15°C (94.5 d) than at 25°C (24.0 d) (t -test, $p < 0.01$).

Copepod survival was significantly affected by salinity (one-way ANOVA, $F_{6, 133} = 579.9$, $p < 0.001$). There were significant differences in mean survival rates between all salinity levels (post-hoc test, $p < 0.01$) except for between 15, 20 and 30 psu ($p > 0.05$) (Fig. 9). All adult females introduced into freshwater died within 15 min. In the treatments with salinities of 2 psu or higher, more than 90% of the copepods lived for 1 week or more, but a sudden decrease was observed on Day 8 at 2 psu, and Day 13 at 5 psu. The number of days taken to reach a cumulative death rate of 50% of the copepods was 9.5 d at 2 psu, 14.5 d at 5 psu, 20 d at 10 psu, 24 d at 15 psu, and 26 d at 20 and 30 psu. The longest survival time of 43 d was recorded at 20 psu.

More than 75% of copepods carried egg-sacs within 3 d of introduction into waters with salinities of 10 psu or higher, and there were no significant differences in the proportion of females carrying egg-sacs between the treatments with salinities of 10, 15, 20, and 30 psu (post-hoc test, $p > 0.05$) (Fig. 10). At 5 psu, no copepods produced egg-sacs within 7 d after introduction. More than 75% of these copepods produced egg-sacs within 1 d after transfer to 15-psu water.

Table 2. Frequency (%) of occurrence and size (μm) of items found in guts of *Hemicyclops gomsoensis* collected from burrows of *Upogebia major*.

Date	4 June 2000	4 June 2000	2 July 2000
Sex	Female	Male	Female
<i>n</i>	10	9	10
Body Length (mm)	1.53–1.93	1.61–2.02	1.45–1.77
Centric diatoms (diameter)	40.0 (17–58)	0.0	60.0 (17–28)
Pennate diatoms (length)	100.0 (14–236)	66.7 (24–176)	70.0 (30–210)
Dinoflagellates (largest dimension)	20.0 (22)	0.0	0.0
Nematodes (largest dimension)	10.0 (>215)	0.0	0.0
Copepod nauplii (body length)	30.0 (161)	0.0	10.0
Copepodids	0.0	0.0	20.0
Unidentified crustaceans	10.0	11.1	10.0
Sand grains	80.0	77.8	80.0
Unidentified remains	0.0	33.3	0.0

Food habits

The gut contents of *H. gomsoensis* from the *Upogebia* burrows are shown in Table 2. The most frequently observed prey items were diatoms and sand grains, followed by copepod nauplii, dinoflagellates, and nematodes. The diatoms were dominated by centric diatoms of the genus *Cyclotella* and pennate diatoms of the genera *Naviculla* and *Surirella*, but also included large-sized species ($>200 \mu\text{m}$) of the pennate genera *Gyrosigma* and *Pleurosigma*. On the basis of morphological features of appendages and other body parts, the copepod nauplii were ascribed to the Harpacticoida, while the copepodids belonged to CII stages of the Cyclopoida.

Discussion

Life cycle and habitats

The present observations, based on the sampling of planktonic- and benthic populations, have demonstrated that the life cycle of *Hemicyclops gomsoensis* comprises an earlier pelagic phase (NI-CI) and a later benthic, burrow-inhabiting phase (CI-CVI), and that CI is the transitional stage from a pelagic to benthic life style. The question still remains as to the potential importance of mud-flat surfaces outside the burrows as a benthic habitat, as this was not investigated during the present study. However, the common, year-round occurrence of copepodids in the burrows and the negligible number of copepodids of CII or later stages in the plankton samples ($<1\%$ of total copepodids), together with a higher risk of predation by fish on the mud surface than in the burrows (authors' unpublished observa-

tion), suggest that the burrows of *Upogelia major*, followed by those of *Macrophthalmus japonicus*, are major, if not the only, benthic habitats of *H. gomsoensis* in the Tama-River estuary.

It should be noted that together with *Hemicyclops gomsoensis*, another congener *Hemicyclops spinulosus* was collected from the *Macrophthalmus* burrows, but their abundance was nearly one order of magnitude less than that of the former (data not shown), and it was essentially absent from the *Upogebia* burrows (Itoh 2001). Itoh (2001) found *H. spinulosus* attaching to the body surface and the burrow wall of the polychaete *Tylorrhynchus heterochaetus* (Quatrefores) in the study site and suggested a close association between this copepod and the polychaete. Since the burrows of *M. japonicus* are often interconnected with polychaete burrows, it is suggested that the occurrence of *H. spinulosus* from the *Macrophthalmus* burrows might be due to an accidental collection from interconnected polychaete burrows when sucking the burrow water out with a large pipette. The life history and population fluctuations of *H. spinulosus* will be dealt with elsewhere (Itoh & Nishida, in preparation).

Seasonal variation

In the present study the following coincident or sequential patterns were observed between the seasonal variations in the pelagic- (Fig. 4) and the benthic (burrow-inhabiting) populations (Fig. 5): an abrupt increase of nauplii (i.e. pelagic stages) during May and June followed by an increase of benthic copepodids during June–August; a decrease of both nauplii and benthic copepodids in the following autumn; and a continued low abundance of both populations through winter to May. These observations, together with the presence of all copepodid stages and adult females with egg-sacs throughout the year, indicate that the main reproductive season of *H. gomsoensis* at the study sites is from early summer to autumn, while reproduction itself takes place throughout the year. There is also a discrepancy in seasonal patterns in the pelagic- and the benthic populations, in that the former totally disappeared during January–April, while the latter sustained their population in the *Upogebia* burrows year round. This is attributable to the combined effect of dispersion and behavior of the nauplii after release from the adults and the sampling method applied in this study, such as: (1) dispersion of nauplii after release by physical processes, such as river flow and turbulence, may have decreased their density to below the detection limit of the net sampling; (2) the nauplii may have a behavioral adaptation against passive dispersion, such as submergence into near-bottom “saline wedges” and topographical gaps to maintain their position, and this may result in a patchy distribution; and (3) the mesh size (100 μm) of the net may have failed to collect a significant part of the younger stages. Even taking into account this discrepancy, however, the highly coherent patterns between the pelagic

and benthic populations suggest that they are closely linked and that the majority of the population is self-sustained through some kind of recruitment mechanism, while a part of the population may disperse into the Bay water to settle at other habitats outside their original estuary (Itoh & Nishida 1991).

The present estimates of the stage durations (Table 1) should be viewed with caution because of the high mortalities during the experiments and the large variation among and within stages that may partly be attributable to the experimental conditions applied in this study that were considerably different from those in situ. Under this limitation, if we assume an average duration from the CIs to adult females of 24.0 d at 25°C and a duration from NI to CI of 9 d at 20°C (Itoh, unpublished data), a maximum average generation time from April to November may be roughly estimated at 1 month, which implies the presence of at least 7 generations per year. In addition, the average duration from CIs to adult females of 94.6 d at 15°C and the continued presence of the largest-size population from February to June (data not shown) would indicate presence of an overwintering generation that was born in November–December and slowly grew to be adults in the next spring, with a generation time of 6–7 months. If this is the case, a minimum of 8 generations per year may be the case.

Environmental conditions in the burrows

According to the present experiment on the salinity-tolerance of *H. gomsoensis*, a drastic reduction in survival rate of starved females occurred at salinity levels of 5 psu or less, and the egg production of fed females also ceased at 5 psu, suggesting that the threshold salinity levels for both survival and egg production may be between 5 and 10 psu. On the basis of these observations, the following relationships may be assumed to occur between fluctuations in river-flow and/or habitat salinity and the copepod populations: (1) a severe flush will sweep the planktonic populations out to sea; (2) slight to moderate reduction of salinity in the burrows (down to ca. 10 psu) will have little detrimental effect on the copepod population; (3) severe reduction of salinity in the burrows (down to <5 psu) will result in copepod death and cessation of egg production. The effect of the first process may have been evident in the observation in July 1999, at the time of a severe flush, resulting in a marked decrease of nauplii and CIs in the planktonic phase at both upstream and downstream sites (Fig. 4). This coincided with an elevated proportion of adult copepods in the burrows (Fig. 5A, B), that can be ascribed to a possible reduced recruitment rate of the CI stage from the pelagic population. In the same month there was a considerable reduction in the populations of both early copepodids and adults in the burrows of *M. japonicus* and this may have been due to a severe reduction in salinity in the burrows to <5 psu (Fig. 3), representing the third process proposed above. The marked reduction in the ratio of egg-sac carry-

ing females to adult females in August 1999 at Sites UU and UM (Fig. 7) may also reflect the above reduction in salinity in the burrows in the previous month. It should be mentioned that a more marked reduction in the percentage of females carrying egg-sacs was observed for a longer period during the preliminary survey for the present study: in 1998 the salinity in the *Macrophthalmus* burrows was less than 5 psu from August through October, corresponding to a continual high flow rate of river water and a near-zero ratio of egg-carrying females to adult females during that period (Itoh, unpublished observation).

Relationships of prosome length to temperature and salinity

A multiple-regression analysis applied to the mean prosome length (PL, mm) of *H. gomsoensis* from burrows of both *U. major* and *M. japonicus* (combined) indicates a significant relationship of PL with temperature (T , °C) and salinity (S , psu):

$$\text{Female: PL} = -0.0063 T + 0.0051 S + 1.0520, \\ (r=0.64, n=44, p<0.05),$$

$$\text{Male: PL} = -0.0051 T + 0.0057 S + 0.9746, \\ (r=0.64, n=44, p<0.05).$$

This indicates that temperature and salinity are subequally related to the prosome length but with different trends, negative in temperature and positive in salinity. The negative relationship between temperature and body length is consistent with what has been reported in many pelagic copepods that, under sufficient food supply, copepods grow to achieve larger body size under lower temperature regimes (e.g. Deevey 1960, McLaren 1974, see Mauchline 1998 for review). This has been ascribed to a reduction in inter-molt durations under higher temperatures resulting in reduced growth during each stage, and this has also been assumed to be a general rule in the Crustacea (Kurata 1962).

As for the relationships between salinity and body length, Kimoto et al. (1986) reported that the prosome length and specific growth rates of the brackish-water copepod *Sinocalanus tenellus* (Kikuchi) were highest at 15 psu on the basis of rearing experiments under different salinities. They hypothesized that non-optimum salinities led to energy losses caused by physiological stresses, such as from the copepods' control of osmotic pressure and respiration, resulting in reduced growth rates. The significant differences in the prosome lengths of *H. gomsoensis* between those in the burrows of *U. major* at the downstream site (DU), the upstream site (UU) and those in the burrows of *M. japonicus* (UM), in order of decreasing prosome lengths and which occurred through most of the year (Fig. 8), coincide with decreasing salinity levels in the burrows in that same order, and hence appear to reflect the copepods' responses to different salinity conditions. The salinity-toler-

ance experiment suggests that the optimum salinity, with the least physiological stress to the copepods, may exist somewhere between 15 and 30 psu. The large temporal fluctuations of salinity in the burrows, regardless of the host species or location, make it difficult to apply the experimental results to the field populations. It is noted, however, that the incidences of salinities measured outside this range (15–30 psu) were 5, 3, and 1 time at Sites UM, UU, and DU, respectively, out of the 17 total observation measurements. This may indicate the possible magnitude of low-salinity stress in the burrows in that order, suggesting that salinity could be a potential factor controlling the observed size differences in the copepods among the study sites, although this awaits more direct experimental evidence.

Population fluctuations at different sites

During most of the study period the population density of *H. gomsoensis* was significantly higher at upstream- (Site UU) rather than downstream sites (Site DU). This was particularly evident in summer and autumn when a difference of a factor of 5 or more was sustained. Prosome length, however, showed the reverse trend, being shorter at Site UU than at Site DU. Seasonal fluctuations in temperature at these sites did not differ significantly, in contrast to the marked differences in salinity levels (Fig. 3). This poses a quandary: why was the population density at Site DU lower than at Site UU, despite the similar temperature conditions between them, and the similar or, perhaps even more optimum/stable salinity levels at the former site? Possible explanations may include the influence of factors that were not examined in the present study, such as the following. First, dispersion and retention mechanisms of pelagic populations, as widely reported in estuaries (Lance 1962, Grindley 1964, Wooldridge & Erasmus 1980), might have caused differences in the number of CI copepodids that were recruited into the burrow populations at different sites. This is suggested from the higher densities of CI copepods in the water column at Site U than at Site D during most of the study period (Fig. 4B). This coincides with the presence of high-salinity (>20 psu) water intermittently penetrating the near-bottom layer of the estuary in the upstream direction (Itoh 2001), which might have enhanced accumulation of the CI copepods in the water around the upstream site. Secondly, differences in food conditions, if any, might have differently influenced the growth and survival of the upstream- and the downstream populations, but no information is presently available to investigate this possibility.

The *Macrophthalmus* burrows (Site UM) showed marked differences from the *Upogebia* burrows (Site UU) in that the population decrease of copepods was more severe after the flush in July 1999, possibly resulting from a decrease in salinity to a fatal level (2.0 psu), and that there were burrows without any copepods from January to April both in 1999 and 2000. This is attributable to the structure of the *Macrophthalmus* burrows, which are more susceptible to

environmental changes than those of *Upogebia*. For example, (1) much smaller volume (<ca. 500 mL, Itoh, unpublished observation), (2) degradation of the burrow in colder seasons due to reduced activity of the crabs (Itoh, unpublished observation), (3) shorter life of the burrows [on the analogy of the crab, *Helice tridens* (De Haan), in which 50% of newly constructed burrows are degraded within 4 d (Takeda & Kurihara 1987)], and (4) possible predation by gobies associated with the *Macrophthalmus* burrows (Itoh & Nishida, in preparation).

Food habits

The present observations indicate that *H. gomsoensis* at the study sites contained a wide range of micro- and meiobenthos, as well as detrital particles, in their guts. Epiphytic diatoms and sand grains occurred in high frequencies, followed by dinoflagellates, nematodes, and copepods. To our knowledge, this is the first report of the food habit of *Hemicyclops* spp. In contrast, there are reports on other poecilostomatoid copepods that directly feed on the mucous (in *Paranthesius anemoniae* Claus, *Ostrincola koe* Tanaka, *Conchylurus quintus* Tanaka, *Modiolicola bifida* Tanaka) or blood (*Selioides bocqueti* Carton) from the hosts or tubes of the hosts [*Myxomolgus myxicolae* (Bocquet and Stock), *M. proximus* Humes and Stock] (Gotto 1979). The associations of these copepods may be viewed as parasitism, and feeding is an essential factor necessitating their association with the hosts. The gut contents of *H. gomsoensis* in the present study, however, contained micro- and meiobenthos that are common in estuarine mud-flats (e.g. McLusky 1994) as well as in the burrows (Itani 2004, Anker et al. 2005, Koller et al. 2006), hence the availability of nutritional resources in the burrows appears to be a facultative condition for the copepods, and this is consistent with the successful growth and reproduction of the copepods fed pennate diatoms throughout their life cycle (this study, Itoh & Nishida 1995, 1997). On the other hand, organic contents comparable to, or even higher than, those of the mud-flat sediments outside have been reported from decapod burrows, as indicated by the chlorophyll *a*, carbon contents and/or bacterial abundance in the burrow wall and in the sediments (Dobbs & Guckert 1988, Berkenbusch & Rowden 2003, Kinoshita et al. 2003, Anker et al. 2005), and this appears to fulfill the necessary food availability conditions for the copepods.

Conclusion

The life cycle of *Hemicyclops gomsoensis* in the Tama-River estuary, as investigated in the present study, may be summarized as follows. While the ovigerous females were present throughout the year, their reproduction took place mainly during early summer to autumn. The nauplii hatched from the females are planktonic and develop to the first copepodid stage (C1s) in the river water. The C1s then

settle to the bottom to inhabit the burrows of *Upogebia major* and *Macrophthalmus japonicus*, the former being the major habitat of the local population, and grow to be adults, feeding on microorganisms and organic particles in the burrows. Occasionally the copepod populations in the burrows suffered from severe influxes of river water that led to salinity decreases in the burrow water to fatal levels, but usually the salinity in the burrows was within optimal levels that permitted recovery and maintenance of the population. From these and other observations it is suggested that *H. gomsoensis* takes advantage of the burrows in various respects, such as for refuge from adverse physical conditions and predators (Itoh & Nishida, in preparation), food resources (see also Kinoshita et al. 2003), population maintenance, and mate finding. However, it is still unknown whether the association only benefits the copepods, or whether there might be some kind of symbiotic relationship between the copepods and the hosts or with other associating organisms, inviting future studies.

Acknowledgements

We thank Ms. Y. Sasaki for her help in the field sampling and the laboratory experiments. Special thanks are due to Drs. K. Kinoshita and G. Itani for information and discussion on the ecology of thalassinidean shrimps and their associates. Thanks are also due to Dr. J.-S. Ho and an anonymous reviewer for their constructive comments on the manuscript. This study was partly supported by grants from the Japan Society for the Promotion of Science for the Multilateral Cooperative Research Program (Coastal Oceanography).

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