Doctoral Dissertation

Taxonomy and life history of two catadromous species of *Hediste* (Nereididae, Annelida) in eastern Asia

（東アジアの降河回遊性カワゴカイ属 2 種の分類と生活史）

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Contents

Abstract

In this dissertation, the details of the catadromous life history of *Hediste diadroma*, which is a common species in Japanese estuaries, are summarized. After that, an undescribed species of *Hediste* (*H.* sp.), which seems to exhibit a similar catadromous life history, is taxonomically described based on Korean specimens.

Chapter 1 is an introduction. Circumboreal distributions of five nominal species of *Hediste* in the north temperate zones are summarized. The importance of those species in an estuary ecosystem is explained. Though these five species are morphologically very similar to one another, there are marked differences in their reproductive and developmental characteristics, with species adopting one of two contrasting life cycle forms: catadromous or estuary-resident. This dissertation focuses on *H. diadroma* exhibiting a typical catadromous life history, and *H.* sp. probably exhibiting the same life history.

In Chapter 2, study on the larval recruitment process in the early phase of the catadromous life history of *H. diadroma*, which is widely distributed throughout Japan, is summarized. Phototaxis of planktonic larvae, produced by artificial fertilization, changed drastically from positive to negative during the 3-chaetiger nectochaeta stage, 3.5–4.0 days after fertilization. During this stage, three kinds of appendages appeared, and the bands of cilia used for locomotion were reduced; these results suggest that larvae shifted from the pelagic to the demersal stage at approximately 4.0 days after fertilization. The demersal stage seems to be effective at keeping the larvae around a river mouth, preventing them from washing out to sea. Field sampling took place in the lower reaches of the estuary, planktonic larvae of, mainly, 5- to 8-chaetiger stages were collected in evening high tides during the spring tides in April and May. During the

same period, benthic juveniles of 5- or higher chaetiger stages were collected at low tide from intertidal flats from a wide area of the estuary that adults inhabited. The present study shed light on the less-known larval recruitment process of *H. diadroma*, which seems to consist of the following three steps: (1) pelagic stage, which lasted from the trochophore to early 3-chaetiger nectochaeta stages for a period of a few days, (2) demersal stage, which lasted from the late 3-chaetiger nectochaeta to 5- to 8-chaetiger nectochaeta stages for a period of about a month, (3) upstream migration at the 5- to 8-chaetiger nectochaeta stages with rising tides and their settlement in tidal flats in a wide area of the estuary.

In Chapter 3, an undescribed *H.* sp. is taxonomically described based on immature individuals collected from the freshwater and the upper reaches of the estuary of the Han River in Korea, and mature individuals collected from the lower reaches of the same river during reproductive swarming. In an immature stage, *H.* sp. is morphologically distinguishable from *H. japonica* due to the presence of a digitate lobe at the tip of the postchaetal ligules only in anterior chaetigers, and the absence of homogomph falcigers, but indistinguishable from *H. diadrom* and *H. atoka*. However, *H.* sp. is distinguishable from all other five congeners in a sexually mature stage, where it shows a unique epitokous metamorphosis (addition of many homogomph spinigers and enlargement of notopodial dorsal ligule in middle and posterior parapodia) during reproductive swarming. The morphological characteristics (paragnath numbers on the proboscis and morphologies of parapodia and ordinary chaetae) of the atokous specimens well agree with those of the epitokous specimens. Our results show that (1) three catadromous species of *Hediste* (*H. japonica*, *H. doadroma*, *H.* sp.) are distributed in eastern Asia, and (2) epitokous metamorphosis is species specific in these three cadromous species.

Chapter 4 is a general discussion. The migratory characteristics of catadromous species of *Hediste* are compared with those of other catadromous macrobenthos and fishes which were relatively well studied. Finally, remaining problems and perspective of the study about catadromous species of *Hediste* are presented.

Chapter 1

Introduction

Estuarine nereidid polychaetes of the genus *Hediste* show a circumboreal distribution in the north temperate zones of the Pacific and Atlantic coasts and consist of the following five nominal species (Sato 1999, 2004, 2017): *H. diversicolor* (O. F. Müller, 1776) distributed along both the Northeast and Northwest Atlantic (Smith 1977), *H. limnicola* (Johnson, 1903) distributed along the Northeast Pacific (Smith 1958), and *H. japonica* (Izuka, 1908), *H. diadroma* Sato & Nakashima, 2003, and *H. atoka* Sato & Nakashima, 2003 (Sato & Nakashima 2003) distributed in Asia along the Northwest Pacific.

Although these five species are morphologically very similar to one another, there are marked differences in their reproductive and developmental characteristics, with species adopting one of two contrasting life cycle forms: catadromous or estuary-resident (Sato 2017).

In this dissertation, the details of the catadromous life history of *Hediste diadroma*, which is a common species in Japanese estuaries, are summarized. After that, an undescribed species of *Hediste* (*H.* sp.), which seems to exhibit a similar catadromous life history, is taxonomically described based on Korean specimens.

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Chapter 2

Larval recruitment process in the catadromous life history of *Hediste diadroma* in an estuary in Kagoshima Bay, Southern Japan

Introduction

Estuarine nereidid polychaetes of the genus *Hediste* show a circumboreal distribution in the north temperate zones of the Pacific and Atlantic coasts and consist of the following five nominal species (Sato 1999, 2004, 2017): *H. diversicolor* (O. F. Müller, 1776) distributed along both the Northeast and Northwest Atlantic (Smith 1977), *H. limnicola* (Johnson, 1903) distributed along the Northeast Pacific (Smith 1958), and *H. japonica* (Izuka, 1908), *H. diadroma* Sato & Nakashima, 2003, and *H. atoka* Sato & Nakashima, 2003 (Sato & Nakashima 2003) distributed in Asia along the Northwest Pacific. These species often attain high density and biomass in estuarine habitats (Sato & Nakashima 2003, Hanafiah et al. 2006) and have ecologically important functions in nutrient cycling in an estuary ecosystem (Tsuchiya & Kurihara 1979, Sayama & Kurihara 1983, Kikuchi 1986).

Although these five species are morphologically very similar to one another, there are marked differences in their reproductive and developmental characteristics, with species adopting one of two contrasting life cycle forms: catadromous or estuary-resident (Sato 2017). The life cycle of the catadromous form is characterized by migration between adult habitats with low salinity and larval habitats with high salinity, with reproductive swarming of mature adults in combination with species-specific epitokous metamorphosis, and a planktic larval phase in early development. The catadromous life cycle form is adopted only by two Asian species, *H. diadroma* and *H. japonica* (Sato 2017). The estuary-resident life cycle is characterized by the completion of the life cycle within low-salinity regions, without epitokous metamorphosis and reproductive swarming of adults, and lacking a true planktic larval phase in early development. This form is widespread in both Pacific (*H. atoka* and *H. limnicola*) and Atlantic species (*H. diversicolor*). *Hediste limnicola* has highly specialized reproductive characteristics such as hermaphroditism, self-fertilization, and viviparity (Smith 1950).

Hediste diadroma, a species with a catadromous life cycle, is commonly distributed in Japan and often coexists with *H. atoka*, a species with an estuary-resident life cycle (Sato & Nakashima 2003, Tosuji & Sato 2012). Recently, invader populations of *H. diadroma* were discovered along North American Pacific coasts, replacing the native congener *H. limnicola* (Nishizawa et al. 2014, Tosuji & Furota 2016). Early previous studies on the reproduction and life history of this species were carried out under the species name of *Nereis japonica* by Kagawa (1955), and that of the small-egg type (or form) of *Neanthes japonica* by Sato & Tsuchiya (1987, 1991), Kikuchi (1998) and Sato (1999).

The reproductive swarming of *H. diadroma* usually occurs from January to April throughout Japan (Sato & Tsuchiya 1987, Sato & Nakashima 2003, Kikuchi & Yasuda 2006), with some exceptional records in November in Nakatsu, western Seto Inland Sea

(Sato et al. unpublished data), December in the Ariake Sea (Hanafiah et al. 2006), and June in Usujiri, Hokkaido (Sato & Nakashima 2003). Reproductive swarming occurs immediately after the night high tide during spring tides, and the swarming mature adults are transported by downstream water currents of the ebb tide. They release the eggs or sperm into the ambient water around the river mouth, where fertilization occurs and the fertilized eggs sink to the bottom. The species has a one-year lifespan and adults die after spawning. The eggs are small (130–170 μm in diameter) and develop into free-swimming trochophore larvae under relatively high salinity environments with a favorable salinity range of 22–30 (Sato & Tsuchiya 1987, 1991, Tosuji & Sato 2006). After a long planktic larval life that lasts a month or more, nectochaeta larvae are transported upstream by rising tides and settle in the adults' habitats in the upper reaches of low salinity estuaries (Kagawa 1955).

Of particular interest is how the planktic larvae of *H. diadroma* prevent themselves from being washed out to sea and when and how they migrate upstream to settle into the adult habitats of estuaries. Though Kagawa (1955) reported that planktic larvae of *H. diadroma* metamorphose to benthic juveniles at the 6-chaetiger stage during their migration into an estuary, the details of larval behavior and morphological change during larval recruitment remain unclear. Our aim in the present study was to examine the larval retention and recruitment process in the catadromous life cycle of *H. diadroma*, by carrying out field surveys and laboratory behavioral and morphological observations.

Materials and Methods

Study site

The Omoigawa river flows into the inner part of Kagoshima Bay, Aira City, Kagoshima Prefecture, Southern Japan (Fig. 1). The estuarine intertidal flats exist along the lower reaches of the river from the river mouth (300 m wide) to 3 km upstream. Two euryhaline nereidid species, *Hediste diadroma* and *H. atoka*, dominate the macrobenthic community in most of the estuarine tidal flats except around the river mouth. The reproductive swarming of mature adults of *H. diadroma* has been observed around the river mouth from late February to April (Sato & Tsuchiya 1987, Sato & Nakashima 2003, our unpublished data). The upstream migration of larvae of *H. diadroma* on rising tides seems to occur mostly in April or May in the Omoigawa river (Sato & Tsuchiya 1987, 1991). On the other hand, the sympatric congener *H. atoka* seems to reproduce mainly during autumn (late September to October), although a few mature adults are found throughout almost the entire year in Kagoshima Prefecture (Sato 2017).

This estuary is the type locality of *H. diadroma* described by Sato & Nakashima (2003).

Field survey and sampling

Field surveys were conducted at the following six stations in the estuary: Sta. 1 at the river mouth (31°42'28"N 130°37'16"E); Sta. 2 approximately 0.5 km upstream from the river mouth, under the Shigetomi-bashi bridge (31°42'34"N 130°37'05"E); Sta. 3 approximately 1 km upstream from the river mouth, under the Aokizuru-bashi bridge (31°42'53"N 130°37'13"E); Sta. 4 approximately 2 km upstream from the river mouth, under the Kusumoto-bashi bridge (31°43'12"N 130°36'49"E); Sta. 5 approximately 2.5 km upstream from the river mouth, under the Inari-bashi bridge (31°43'10"N

130°36'34"E); Sta. 6 approximately 3 km upstream from the river mouth, under the Shinkai-bashi bridge (31°43'01"N 130°36'22"E).

Planktic larvae of *H. diadroma* were collected at Stas. 2–5 in evening rising tides during the spring tides of April and May 1989 (based on unpublished data by Yoshifumi Kuroki), 2013, and 2015, by the following two methods: (1) collection of larvae using 20 m horizontal tows of a plankton net (63 µm mesh, 30 cm mouth diameter) from the center of the bridge at Stas. 2–5, 0.6–2.6 h before the evening high tide on April 6 (high tide level: 350 cm, moon age: 29.4) and 21 (322 cm, 15) and May 7 (328 cm, 1.6), 1989 and at Sta. 2, 1.5 and 1.0 h before high tide on April 26, 2013 (352 cm, 15.7); (2) collection of larvae by filtering 120 L surface-water collected using a 6 L bucket at the riverside through the same plankton net at Sta. 2, 0.5 h before high tide on April 26, 2013 and 1.5, 1.0, and 0 h before high tide on April 18, 2015 (348 cm, 28.7). During each collection, two or three replicated samples were collected: in order to observe the morphology of larvae, one of the samples was fixed in the field in 10% formalin containing Rose Bengal stain and the others were brought alive to the laboratory for molecular species identification (see below). From the formalin fixed plankton samples, larvae of *Hediste* were sorted under a stereomicroscope (SMZ1000; Nikon, Japan) and transferred to 80% ethanol for preservation.

To examine the process of larval settlement, benthic juveniles were obtained from sediment samples in tidal flats at low tides in March, April, and May of 1989 (based on unpublished data by Yoshifumi Kuroki) and 2013. Sediment samples were collected from the bottom surface to 5 cm depth at Stas. 2–5 by using a core sampler (7 cm in diameter) on March 6 (level of the closest high tide: 315 cm, moon age: 27.8), April 6 (343 cm, 29.4), April 21 (317 cm, 15.0), and May 7 (333 cm, 1.6), 1989. Sediment samples were collected from the bottom surface to 3 cm depth at Stas. 1–5 by using a core sampler (3 cm in diameter) on April 27 (344 cm, 16.7) and May 29 (324 cm, 19.1), 2013. These samples were fixed in the field in 10% formalin containing Rose Bengal stain. The sediment samples were stirred in a container with tap water and the water was sieved with a 149 µm mesh. This work was repeated several times. All the residual material on the mesh was transferred in tap water, from which the juveniles of *H. diadroma* were sorted under the stereomicroscope and preserved in 80% ethanol.

The salinity gradient of the river water in the estuary of the Omoigawa river was examined around high tide times on November 3, 1986 (high tide level: 340 cm, moon age: 0.9, spring tide) (based on unpublished data by Kazuhiko Iwanaga), January 29 (326 cm, 2.3, spring tide) and February 1 (299 cm, 5.3), 1990 (based on unpublished data by Yoshifumi Kuroki). The salinity of the surface and bottom layers at the riverside or the center of the bridges at Stas. 1–6 was measured in the period between 30 min before and after high tide by using a salinometer (UC77; Central Kagaku, Japan; or Type MC5; EIL, England). The salinity and temperature of surface and bottom water at Sta. 2 were measured during larval sampling in 2013 and 2015 by using an SCT meter (Model 650; YSI, USA). The salinity gradient of the interstitial water in the sediment was also examined while sampling for benthic juveniles around low tide on May 29, 2013 (level of the closest high tide: 324 cm, moon age: 19.1). The salinity of the interstitial water that drained into the holes dug in the sediments was measured with a refractometer (S/Mill; Atago, Japan).

The measured values of the high tide level at the Kagoshima Port (approximately 15 km away from the sampling sites) for the sampling dates were obtained from the database of the Japan Meteorological Agency (2019).

Identification of planktic larvae species

Hediste diadroma is morphologically indistinguishable from *H. atoka* when they are still sexually immature (Sato & Nakashima 2003). In order to identify the species of the planktic larvae, the species-specific multiplex PCR method (Tosuji & Sato 2012) was used. Living worms (0.5–2 mm in body width), which were obtained by rearing the planktic larvae in the laboratory for about two months, were fixed in 80% ethanol for DNA analysis. Prior to DNA extraction, ethanol preserved samples were placed in a phosphate-buffered saline (PBS) solution for 30 min to remove the ethanol. DNA was extracted by using a Whatman FTA Elute Micro Card (GE Healthcare, NJ, USA). Multiplex PCR was performed by using a Multiplex PCR Kit (Qiagen, Germany) with Q-solution at a final concentration of 0.5 × 0.125 μM of each of eight primers. The PCR cycling regime was as follows: initial denaturation at 95°C for 15 min, then 40 cycles at 94°C for 30 s, at 60°C for 60 s, at 72°C for 60 s, and a final 10-min extension at 72°C. The PCR products were then loaded on a 3% agarose gel (Agarose 21; Nippon Gene, Japan). The two species of *Hediste* were distinguished by their electrophoretic patterns that showed different molecular sizes of the species-specific PCR products. The *H. atoka*-specific band was expected at 135 bp and the *H. diadroma*-specific band was expected at 260 bp.

All of the seven specimens collected during the three samplings of April 26, 2013 were identified as *H. diadroma*. Among a total of 62 specimens collected during the three samplings of April 18, 2015, most of them (95%) were identified as *H. diadroma*, except for 3 individuals identified as *H. atoka.* In the present study, therefore, all the planktic and benthic 3- to 18-chaetiger nectochaeta specimens (see Result section) were regarded as *H. diadroma*.

A cohort of planktic larvae consisting of 3- to 8-chaetiger nectochaetes is defined here as a pre-settling recruiting cohort. Another cohort of 9- to 18-chaetiger nectochaetes is defined as a post-settling dispersing cohort, regarded as temporal swimming juveniles, which seem to disperse during rising tides as has been observed in *H. atoka* (Kikuchi 1998, Kikuchi & Yasuda 2006). The youngest benthic specimens of the 5- to 8-chaetiger stages that are comparable to the planktic larvae of the pre-settling recruiting cohort were regarded as just settled juveniles.

Observation of larval behavior

Sexually mature worms of *H. diadroma* were collected with a scoop net from the river mouth of the Omoigawa river from reproductive swarms near the surface after the night high tide at 21:00 on March 21, 2015. The live mature adults were brought to the laboratory where they were used for artificial fertilization, according to the method described in Tosuji & Sato (2006). Gametes were obtained by pressing each adult with forceps in a petri dish. The eggs were inseminated with sperm suspension (final sperm concentration: 10^{-5} dilution of dry sperm) in an aerated aquarium (length: 16 cm; width: 9 cm; height: 9 cm) containing seawater diluted to a salinity of 28.

Sixty hours after insemination, a part of the active swimming larvae was fixed in 10% formalin for morphological observation. The remaining larvae were reared at room temperature (17–24°C) in the aquarium and placed on the window side where the sunlight reached them. The location and phototactic behavior of swimming larvae were observed 3.0, 3.5, 4.0, 4.5, and 5.0 days after insemination. Some of the larvae were fixed every time for morphological observation. An LED light source (TIKKA XP2;

Petzl, France) was used for the observation of the phototaxis of swimming larvae at night time. Aeration was temporarily stopped for 3 min so as not to interfere with water flow and the light source was applied horizontally from one side of the aquarium in order to remove the influence of geotaxis.

Morphological observation

The morphology of the preserved larval and juvenile specimens obtained from the field and through artificial rearing were examined under a compound microscope (Optiphot-2; Nikon, Japan). Since the parapodial lobe of chaetiger 1 of the planktic larva changes to the second peristomial cirri of the settled juvenile (Kagawa 1955), the segment with second peristomial cirri of the settled juvenile was treated as chaetiger 1. Photographs were taken with a film camera (FDX-35; Nikon, Japan) on the compound microscope for whole body images.

The preserved specimens were dehydrated in a graded ethanol series, transferred to pure 2-methyl-2-propanol (tert-butyl alcohol), and dried by using a vacuum freeze dryer (VFD-21; Vacuum Device, Japan) in order to be scanned via electron microscopy. Then, they were mounted on aluminum stubs and coated with gold palladium (Ion Coater IB-2; Eiko, Japan). Observations were performed with a scanning electron microscope (SEM) (Miniscope TM-1000S; Hitachi, Japan).

The number of chaetae in both the right and left parapodia of chaetiger 1, the length of the longest chaetae in chaetiger 2, and the length of anal cirri were quantitatively examined under the compound microscope by using the 5- and 6-chaetiger planktic larvae collected at Sta. 2 on April 26, 2013 and the 5- to 19- chaetiger benthic juveniles collected at Stas. 1–4 on the next day (April 27, 2013). Differences in these characteristics between developmental stages were statistically tested by using the Wilcoxon–Mann–Whitney test or the Steel–Dwass test.

Results

Morphology of larvae and juveniles

Early planktic larvae

In the early 3-chaetiger nectochaetes (3 and 3.5 days after insemination) (n=24), the prostomial antennae, peristomial cirri, and anal cirri were usually not yet developed (Figs. 2B, 3A, C), though any one of these appendages appeared in 29% of them. The bands of motile cilia remained at this stage (Fig. 3C). In the late 3-chaetiger nectochaetes (4 and 4.5 days after insemination) (n=31), all of the three appendages were usually developed (Figs. 2C, 3D), though the prostomial antennae or the peristomial cirri were lacking in 16% of them. The bands of motile cilia were reduced (Fig. 3D). In the latest 3-chaetiger nectochaetes (5 days after insemination) (n=12), all three appendages were well developed (Fig. 2D).

Chaetal morphology was examined by the SEM observation. In the 3-chaetiger planktic larvae (Fig. 3A), two kinds of chaetae were found; the long larva-specific homogomph spinigers having a fine spine on the collar of the shaft and a relatively long blade with unique coarse serration (Fig. 3E) were present in all chaetigers, whereas one or two simple capillaries (Figs. 3F, G) were found in chaetiger 2.

Recruiting nectochaetes before and after settlement

The following three major morphological changes occurred during settlement at the 5- to 8-chaetiger stages: 1) modification of parapodial lobes to second peristomial cirri, accompanied by a decrease of chaetae in chaetiger 1; 2) chaeta length shortening in all chaetigers; 3) elongation of anal cirri (Fig. 4).

The number of chaetae of chaetiger 1 (average of right and left) of the 5- and 6-chaetiger benthic juveniles (average ± SD: 3.2 ± 1.5 and 1.5 ± 1.4, respectively) (Fig. 4D) was significantly smaller than that of the 5- and 6-chaetiger planktic larvae (7.6 ± 3.3 and 10.3 ± 1.2, respectively) (Figs. 4A–C) (Wilcoxon–Mann–Whitney test, $p < 0.005$; Fig. 5A). The number of chaetae of the 5-chaetiger planktic larvae collected 1.5 h before high tide (10.5 ± 2.9) (Fig. 4A) was significantly larger than that of larvae collected 1.0 and 0.5 h before high tide (7.0 ± 2.7 and 5.7 ± 2.4, respectively) (Figs. 4B, C) (Steel–Dwass test, $p < 0.03$). The chaetae of chaetiger 1 were completely detached during the 6- to 10-chaetiger stages, except in a 13-chaetiger juvenile that retained a few chaetae.

The length of the longest chaetae in chaetiger 2 of the 5- and 6-chaetiger benthic juveniles (150 ± 16 μm and 154 ± 16 μm, respectively) (Fig. 4D) was significantly shorter than that of the 5- and 6-chaetiger planktic larvae (196 ± 28 μm and 222 ± 28 μm, respectively) (Figs. 4A–C) (Wilcoxon–Mann–Whitney test, $p < 0.007$; Fig. 5B). The length of the longest chaetae of the 5-chaetiger planktic larvae collected 1.5 h before high tide (210 ± 30 μm) (Fig. 4A) was not significantly different from that of larvae collected 1.0 and 0.5 h before high tide (195 ± 19 μm and 185 ± 24 μm, respectively) (Figs. 4B, C) (Steel–Dwass test, $p > 0.2$).

The length of anal cirri of the 5- and 6-chaetiger benthic juveniles (73 ± 19 μm and 93 ± 21 μm, respectively) (Fig. 4D) was significantly longer than that of the 5- and 6-chaetiger planktic larvae (31 ± 8 μm and 31 ± 4 μm, respectively) (Figs. 4A–C) (Wilcoxon–Mann–Whitney test, $p < 0.006$; Fig. 5C). The length of anal cirri of the 5-

chaetiger planktic larvae collected 1.5 h before high tide (31 ± 6 μm) (Fig. 4A) was not significantly different from that of larvae collected 1.0 and 0.5 h before high tide (34 ± 9 μm and 28 ± 7 μm, respectively) (Figs. 4B, C) (Steel–Dwass test, $p > 0.4$).

In the planktic larvae of 6-chaetiger (Fig. 3B) and 8-chaetiger stages, five kinds of chaetae were found: the larva-specific homogomph spinigers (Fig. 3E) were present in chaetigers 1–4; a simple capillary (Figs. 3F, G) was present in chaetigers 2 or 3; the following three kinds of chaetae were present in chaetigers 2–8: (1) larva-specific homogomph falcigers having a fine spine on the collar of the shaft and a relatively long blade with ordinary serration (Fig. 3H), (2) ordinary heterogomph falcigers having a short blade with ordinary serration (Fig. 3I), and (3) ordinary homogomph spinigers having a relatively long blade with ordinary serration (Fig. 3J).

Larval behavior in early planktic phases

Each fertilized egg sank to the bottom of the aquarium, secreting a jelly layer. Embryonic development occurred within the jelly layer. Approximately 2 days after insemination, the trochophores hatched out of the jelly layer and began swimming as planktic larvae.

The active swimming larvae at the metatrochophore (Fig. 2A) and early 3-chaetiger nectochaeta stages (Fig. 2B) (2.5 and 3–3.5 days after insemination), showed positive phototactic behavior toward the LED light or sunny side. In contrast, the late 3-chaetiger nectochaeta larvae (4–5 days after insemination) (Figs. 2C, D) showed negative phototactic behavior away from the LED light or sunny side; they displayed crawling behavior on and swimming behavior around the bottom.

Developmental stages and abundance of recruited larvae and juveniles

Late planktic larvae migrating upstream

In 1989, a total of 165 nectochaetes of the 3- to 18-chaetiger stages were obtained by net tows at Stas. 2–5 on April 6 and 21 and May 7 (Fig. 6). A pre-settling recruiting cohort of planktic larvae consisting of 3- to 8-chaetiger nectochaetes appeared at all stations on April 6 with the highest tide level (350 cm) and at Stas. 2 and 3 on April 21 and May 7. Larvae of this cohort were most abundant at Stas. 2 (n=53) and 3 (n=65) on April 6, with 5- and 6-chaetiger nectochaetes dominating. The youngest larvae of the 3-chaetiger stage were found at Sta. 4 on April 6 and Sta. 2 on May 7. Another post-settling dispersing cohort of a small number of larger nectochaetes (9- to 18-chaetiger stages) was found at Stas. 2 (n=2) and 3 (n=1) on April 6, at Sta. 2 (n=1) on April 21, and at Stas. 3 (n=7) and 4 (n=6) on May 7.

In 2013, a total of 47 nectochaeta larvae of the 5- to 7-chaetiger stages, all belonging to the pre-settling recruiting cohort, were obtained by sampling at Sta. 2 on April 26 (Figs. 7A–C), consisted mostly of 5-chaetiger nectochaetes (89%).

In 2015, a total of 99 nectochaeta larvae of the 5- to 8- chaetiger stages, all belonging to the pre-settling recruiting cohort, were obtained by sampling at Sta. 2 on April 18 (Figs. 7D–F), consisted mostly of 7-chaetiger nectochaetes (73%). The number of larvae increased from the first sampling (9 ind. 120 L^{-1}) to the third one (65).

Benthic juveniles after settlement

A total of 1894 benthic juveniles of the 5- and higher chaetiger stages were obtained from the sediment samples collected at Stas. 2–5 from March to May 1989 (Fig. 8). On March 6, the just settled juveniles of the 5- to 8-chaetiger stages appeared most abundantly (98182 m^{-2}) at Sta. 3 with the ones belonging to the 7- and 8-chaetiger stages being most dominant, while they did not appear at Sta. 5. One month later, on April 6 when the level of the closest high tide was the highest (343 cm), they appeared at all stations; their densities at Stas. 2 (4416 m^{-2}) and 3 (6234 m^{-2}) were higher than at Stas. 4 (2338 m^{-2}) and 5 (519 m^{-2}), though all the densities were much lower than those recorded on March 6. Half a month later, on April 21, they appeared only at Sta. 2 (5455 m^{-2}). Another half a month later, on May 7, they also appeared only at Sta. 2 in their lowest density (519 m^{-2}).

A total of 215 benthic juveniles of the 5- and higher chaetiger stages were obtained from the sediment samples collected at Stas. 1–5 from April and May 2013 (Fig. 9). On April 27, the just settled juveniles of the 5- to 8-chaetiger stages appeared at Stas. 1–4 with the highest density at Sta. 3 (13211 m^{-2}), where the juveniles belonging to the 6-chaetiger stage were the most dominant, while they did not appear at Sta. 5. Another cohort, consisting of 9- to 17-chaetiger juveniles, was found in Stas. 1–4. One month later, on May 29, the just settled juveniles appeared at Stas. 2–4, where the salinity of interstitial water was 3–22 (Fig. 10); the juveniles had the highest density at Sta. 4 (3303 m^{-2}) and did not appear at Stas. 1 (salinity: 23) and 5 (0). Their densities were much lower than those on April 27.

Salinity gradient in the estuary of the Omoigawa river

The salinity of the surface and bottom layers was relatively high in the lower reaches (Stas. 1–3) with values of 17–33 and 25–33, respectively (Fig. 10). The salinity of the bottom layer decreased gradually or rapidly between Stas. 3 and 4 depending on the tidal strength. Saline water did not reach the upper reaches of the estuary (Stas. 4–6) in neap tide (less than approximately 300 cm in high-tide level).

The salinity of the interstitial water in the sediment was also relatively high in Stas. 1–3 (22–23) and low in Stas. 4 and 5 (3 and 0, respectively) (Fig. 10).

The salinity (temperature) of the surface and bottom water during larval sampling at the center of the bridge of Sta. 2 on April 26, 2013 were 34 (16.5–17.1°C) and 34 (17.2–16.5°C), respectively. The salinity of the surface water during the sampling at the riverside of Sta. 2 on the same date were 32–35 (17–17.6°C). The salinity of the surface and bottom water during the sampling at the riverside of Sta. 2 on April 18, 2015 were 26–32 (18.6–20.2°C) and 27–32 (18.6–20.1°C), respectively.

Discussion

Significance of larval behavior at the early stage

Previous studies revealed that the trochophores of *Hediste diadroma* hatch out and start a free-swimming larval life after embryonic development within a jelly layer on the bottom and that both trochophores and metatrochophores show positive phototaxis (Sato & Tsuchiya 1991, Sato 1999, Tosuji & Sato 2006). Our results are in agreement with previous findings on the topic, while also showing that larval phototaxis changed drastically from positive to negative during the 3-chaetiger nectochaeta stage, between 3.5 and 4.0 days after fertilization; this is when the three kinds of larval appendages (prostomial antennae, first peristomial cirri, and anal cirri) that seem to function as sensory organs (Purschke 2005), began to grow when the larval ciliary bands reduced. This result suggests that larvae shifted from the pelagic to the demersal stage during the 3-chaetiger nectochaeta stage. The switch of larval phototaxis from positive to negative has also been observed in sedentary polychaetes such as *Hydroides ezoensis* and *Spirobranchus kraussii* (Miura & Kajihara 1984).

Sato & Tsuchiya (1991) and Tosuji & Sato (2006) demonstrated that lipid droplets, which were situated in the anterior body of trochophores and metatrochophores, migrated to the middle body at the early 3-chaetiger nectochaeta stage and disappeared at the late 3-chaetiger nectochaeta stage, when prostomial antennae, first peristomial cirri, and anal cirri appeared and the larvae began to feed. The disappearance of lipid droplets also seems to assist larvae in their pelagic to demersal shift; Yokouchi (1990) reported that pelagic nectochaeta larvae of the Japanese population of "*Neanthes virens* (Sars, 1835)", which seems to be *Alitta dyamusi* (Izuka, 1912) according to the recent taxonomic revision (Villalobos-Guerrero & Bakken 2018), gradually sink to the bottom layer due to a decrease in lipid droplets with a specific gravity smaller than that of seawater, although a drastic change in larval phototaxis was not detected during their larval development.

Consequently, the planktic larvae of *H. diadroma* likely spend most of their life in the bottom layer around a river mouth as demersal inhabitants during the late 3-chaetiger to 5- to 8-chaetiger nectochaeta stages. In general, estuaries have a two-layer circulation system, with residual tidal currents having a net outflow in the surface layer and a net inflow in the bottom layer (Forward & Tankersley 2001). Thus, the demersal stage seems to be effective at keeping the larvae around the river mouth, preventing them from washing out to sea. In fact, dense planktic larvae of estuarine crustaceans (Bousfield 1955, Suzuki & Kikuchi 1990, Uno & Nakano 2002, Kusuda et al. 2006) and mollusks (Nelson & Perkins 1931, Carriker 1951, 1967, Baker 2003) have been found in the bottom layer. Toba et al. (2012) reported that small larvae of Manila clam

Ruditapes philippinarum were abundant in the surface and middle layers, whereas large larvae were mainly sampled from the bottom layer in the coastal sites of Tokyo Bay, Japan. The daily vertical migration of larvae synchronized with tidal rhythms, which seems to be effective for their retention in estuaries, has been observed in the crab *Rhithropanopeus harrisii* (Cronin & Forward 1979, Forward & Cronin 1980, Cronin 1982) and the oyster *Crassostrea virginica* (Wood & Hargis 1971). It is unknown whether such daily vertical migration occurs in the larvae of *H. diadroma* during their demersal stage.

Upstream migration and settlement at the late stage of larval development

Our results indicate that planktic larvae of, mainly, 5- to 8-chaetiger nectochaetes of *H. diadroma* were transported upstream by rising tides as the pre-settling recruiting cohort into the adult habitat; this suggests that the nectochaetes may have been ready to survive in the low-salinity environment in the upper reaches of the estuary. A relatively high salinity (more than 20) is essential for the early development of *H. diadroma* (Kagawa 1955, Sato & Tauchiya 1987, Tosuji & Sato 2006). Kagawa (1955) reported that planktic larvae gained viability in low salinity (as low as 6), when they reached the 6-chaetiger nectochaeta stage. The present study demonstrated that the youngest benthic juveniles of the 5- to 8-chaetiger stages, which were comparable to the pre-settling recruiting cohort of planktic larvae, were collected from the sediment samples in tidal flats from a wide area of the estuary. This result indicates that the settlement may occur at the 5- to 8-chaetiger stages just after the upstream larval migration at the same stages. The larger juveniles of the 9- to 17-chaetiger stages, which were found in Stas. 1–4 in 2013 (Fig. 9), seemed to have settled during the previous spring tide (half a month earlier) and grown up.

Tidal strength (high-tide levels) seems to determine how far the migrating larvae are transported upstream in the estuary. Our data show that densities of both the pre-settling recruiting cohort of planktic larvae and the just settled 5- to 8-chaetiger benthic juveniles were higher in the lower (Stas. 2, 3) than in the upper reaches (Stas. 4, 5) of the estuary (Figs. 6, 8). Since high-tide levels vary daily depending on the semi-lunar tidal rhythm, the rising seawater reaches the upper reaches of the estuary less frequently (Fig. 10). Therefore, the opportunities for larval settlement seem to be fewer in the upper reaches of the estuary.

Our results suggest that the upstream larval migration may depend on the upstream movement of seawater (salt wedge) during the rising tide. Such behavior is well known as flood-tide transport for decapod larvae in estuarine areas (Epifanio 1988, Forward & Tankersley 2001, Gibson 2003); megalopa larvae of the Japanese mitten crab *Eriocheir japonica*, a typical catadromous species, migrate upstream from the sea and settle in the uppermost reaches of an estuary during a single flood tide, preferably at night around the spring tides (Kobayashi & Archdale 2016).

Our results on the post-settling dispersing cohort of the 9- to 18-chaetiger stages in the plankton samples (Fig. 6) suggest that once the larvae of the 5- to 8-chaetiger stages are settled in the lower reaches of the estuary, a part of the young juveniles may float again in the water column and may be transported into the upper reaches by rising tides, probably avoiding the extreme high density of the lower reaches. Kikuchi (1998) and Kikuchi & Yasuda (2006) reported that temporal swimming juveniles of *H. atoka* of, mainly, 4- to 7-chaetiger stages actively disperse within an estuary during rising tides.

Morphological changes during larval settlement

Kagawa (1955) first examined the early development of *H. diadroma* and described the outline of the larval morphological changes around the 6-chaetiger stage as follows: (1) detachment of long chaetae, (2) modification of the parapodial lobes in chaetiger 1 to the second peristomial cirri. Our findings agree with the results of Kagawa (1955) and provide further clarity on the details of the morphological changes in *H. diadroma*. In addition, I described anew the elongation of the anal cirri after the settlement, which the drawing of Kagawa (1955) already implied.

Regarding chaetal morphology, I found that chaetae of the 3-chaetiger larvae of *H. diadroma* consist of the larva-specific long homogomph spinigers and simple capillaries, which have not been found in adults (Sato & Nakashima 2003). I also discovered that three kinds of chaetae (the larva-specific homogomph falcigers, ordinary heterogomph falcigers, and ordinary homogomph spinigers) were added in chaetiger 2 onwards in the 6- and 8-chaetiger larvae, while the larva-specific homogomph spinigers remained in chaetigers 1–3. Wilson (1932) reported that nectochaetes of the nereidid polychaete *Nereis pelagica*, in the 3- and higher chaetiger stages, have the three kinds of larva-specific chaetae that are the same as those of *H. diadroma*. Gustus & Cloney (1973) reported that 3-chaetiger nectochaetes of another nereidid, *N. vexillosa*, have the two kinds of larva-specific chaetae (homogomph spinigers and homogomph falcigers) that are the same as those of *H. diadroma.*

I found that the number of chaetae in chaetiger 1 decreased and the length of chaetae shortened during settlement. This may be caused by the larva-specific long homogomph spinigers being mostly detached during settlement. Our results also suggest that the detachment of the larva-specific spinigers may begin prior to settlement. Though long chaetae seem to benefit planktic larvae in defending themselves against predators (Pennington & Chia 1984) and in increasing the flotation area of the larvae (Fauchald 1974, Sato & Tsuchiya 1991), they seem to be useless in the benthic life after settlement. It is unknown when the larva-specific homogomph falcigers are detached.

Conclusion

The present study clarified the less-known larval recruitment process in the catadromous life cycle of *H. diadroma*. Based on the present and previous studies (Kagawa 1955, Sato & Tsuchiya 1987, 1991, Kikuchi 1998, Tosuji & Sato 2006, Kikuchi & Yasuda 2006), the entire life history of *H. diadroma* can be summarized as follows (Fig. 11): (1) Reproductive swarming of mature adults toward the sea accompanied by epitokous metamorphosis and spawning around the river mouth with positive phototaxis; (2) death of adults after spawning; (3) the sinking of fertilized eggs to the bottom; (4) larvae hatching and entering the pelagic stage, which lasted from the trochophore to early 3-chaetiger nectochaeta stages (a few days) and was characterized by active larval swimming with positive phototaxis; (5) entering the demersal larval stage, which lasted from the late 3-chaetiger to 5- to 8-chaetiger nectochaeta stages (about a month) and was characterized by larval crawling behavior on and swimming around the bottom, with negative phototaxis; and (6) upstream migration of the 5- to 8-chaetiger nectochaetes on rising tides and their settlement in tidal flats in a wide area of the estuary, including a low-salinity regime and accompanied by the settlement metamorphosis.

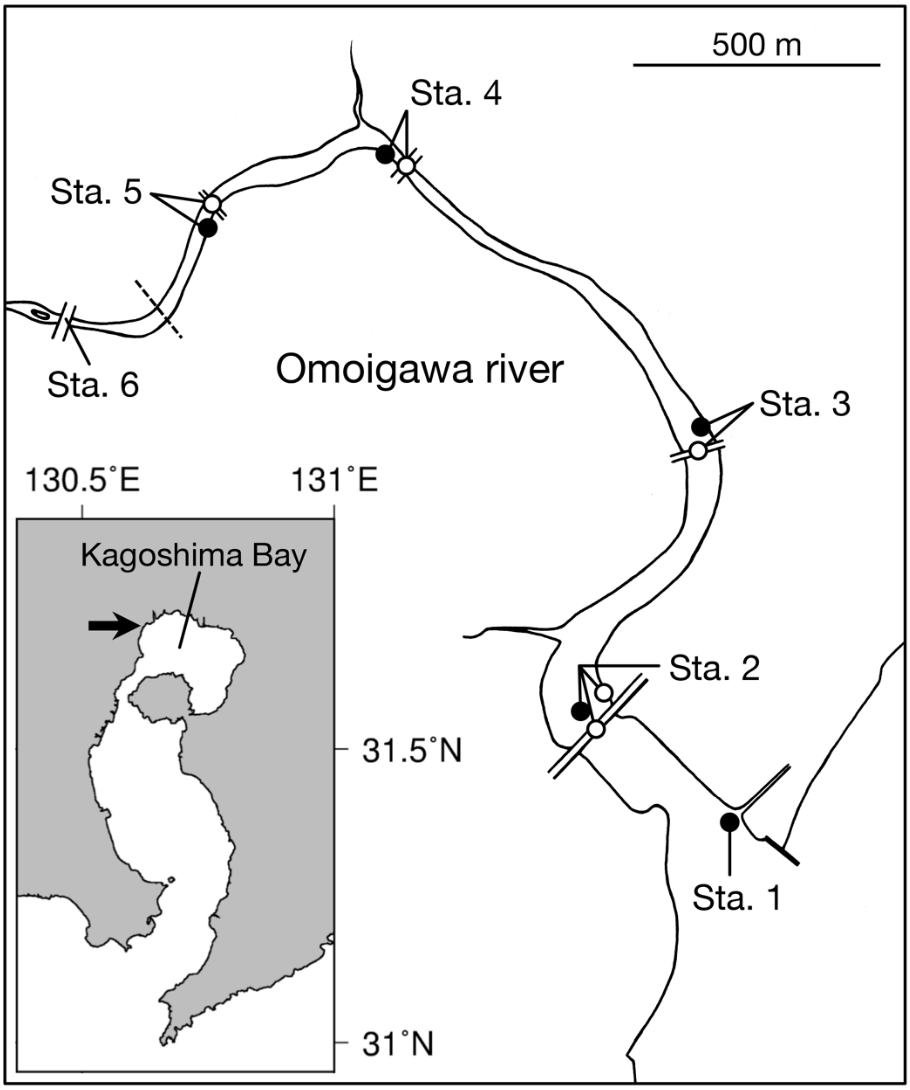

Fig. 1. Location of the study sites (Stas. 1–6) in the Omoigawa river estuary in the inner part of Kagoshima Bay, Southern Japan. Open circles, sampling sites for planktic larvae at high tides; closed circles, sampling sites for benthic juveniles at low tides. Dashed line indicates the upper limit of the saltwater intrusion.

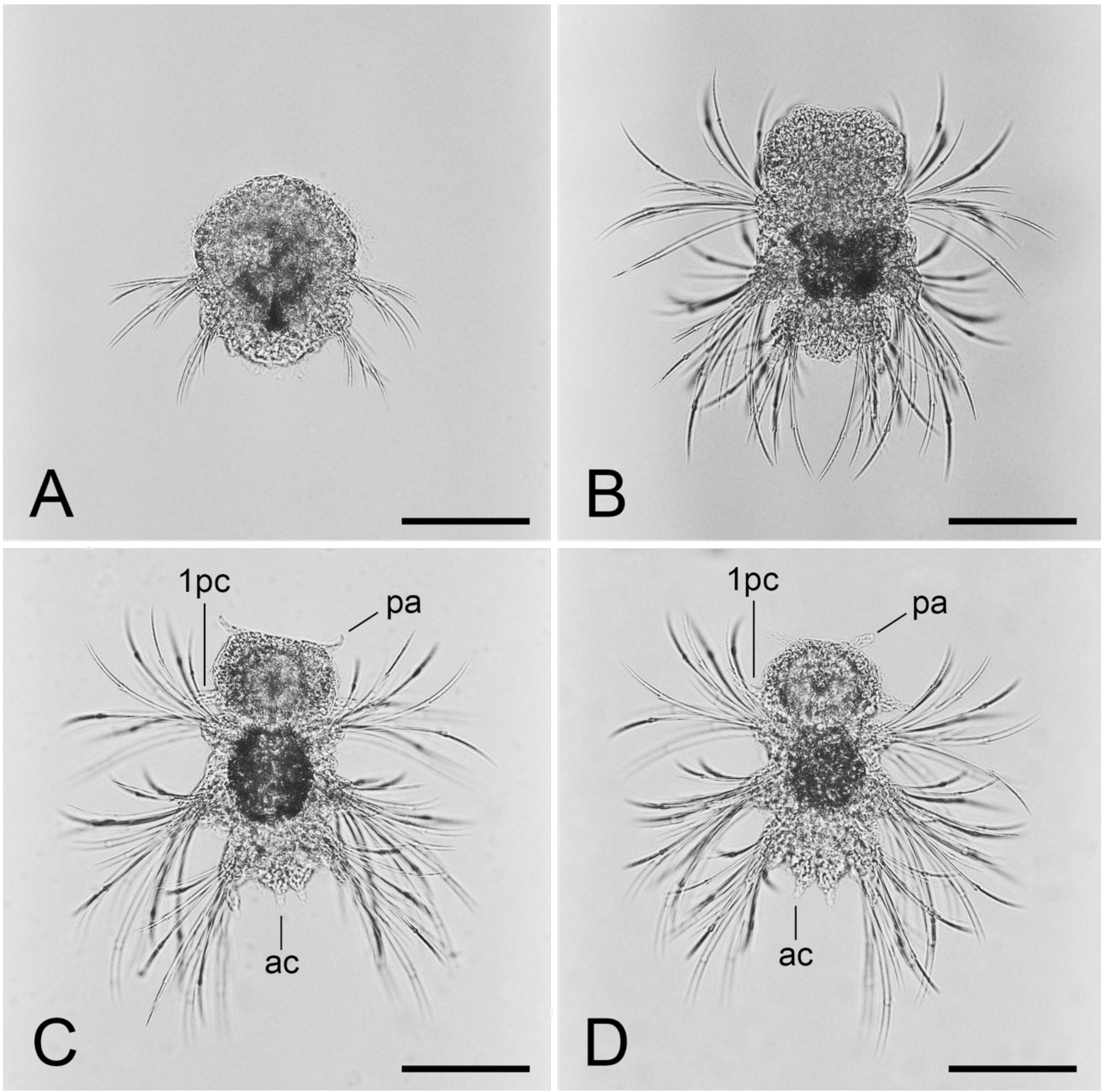

Fig. 2. Swimming larvae of *Hediste diadroma* produced by artificial fertilization. (A) Metatrochophore (2.5 days after insemination); (B) early 3-chaetiger nectochaeta (3.5 days); (C) late 3-chaetiger nectochaeta (4.5 days); (D) late 3-chaetiger nectochaeta (5 days). pa, prostomial antennae; 1pc, first peristomial cirri; ac, anal cirri. Scale bars: 100 µm.

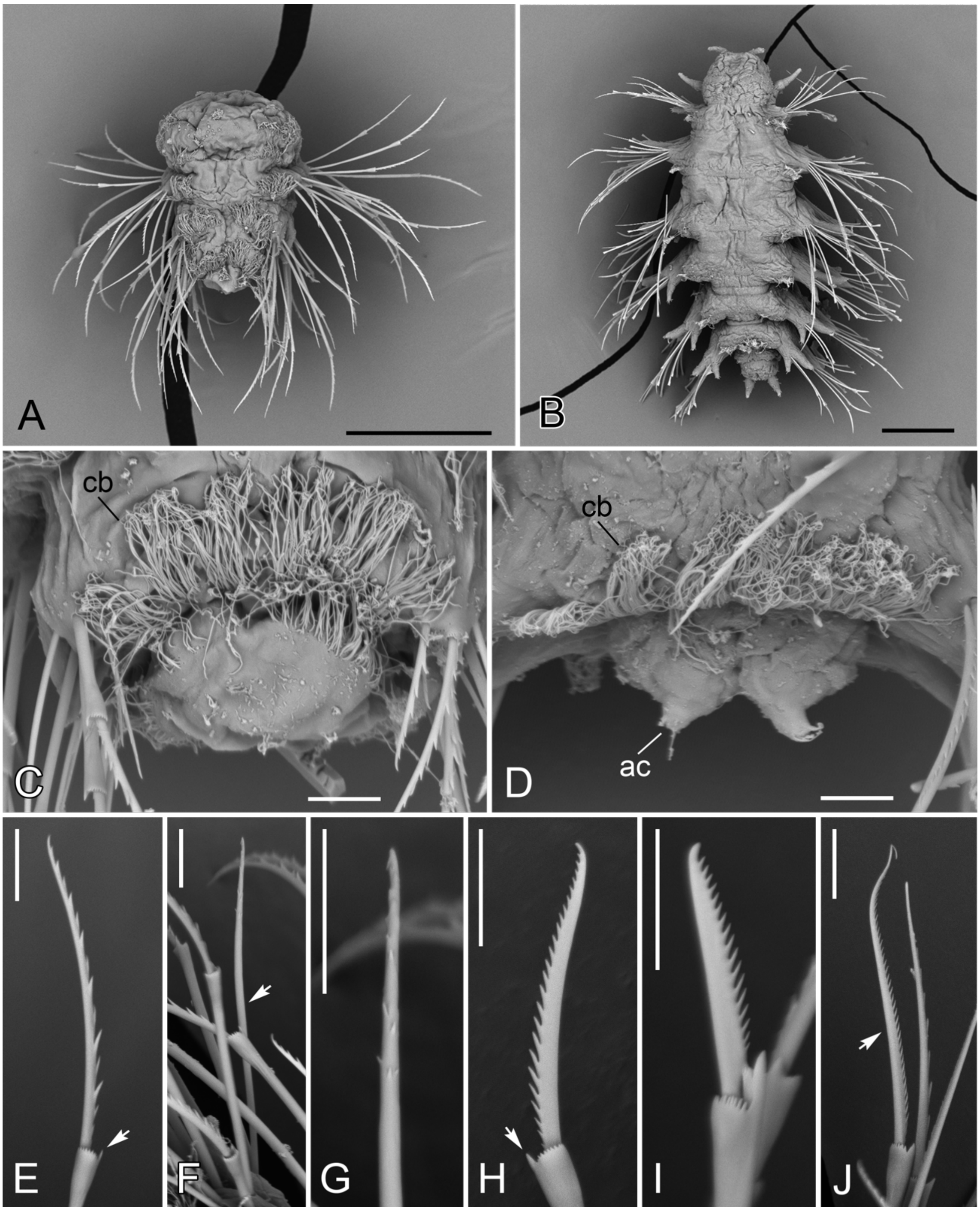

Fig. 3. Scanning electron micrographs of the planktic nectochaeta larvae of *Hediste diadroma.* (A) An early 3-chaetiger nectochaete produced by artificial fertilization (3 days after insemination); (B) a planktic 6-chaetiger nectochaete (dorsal view) collected at Sta. 2 on April 18, 2015; (C) posterior end of an early 3-chaetiger nectochaete (3

days); (D) posterior end of a late 3-chaetiger nectochaete (4 days); (E) larva-specific homogomph spiniger having a fine spine (arrow) on the collar of the shaft and a relatively long blade with unique coarse serration from chaetiger 1 in (A); (F) simple capillary (arrow) from chaetiger 2 in (A); (G) enlargement of tip of the simple capillary in (F); (H) larva-specific homogomph falciger having a fine spine (arrow) on the collar of the shaft and a relatively long blade with ordinary serration from chaetiger 6 in a planktic 8-chaetiger nectochaete; (I) ordinary heterogomph falciger from chaetiger 6 in (B); (J) ordinary homogomph spiniger (arrow) from chaetiger 4 in a planktic 8-chaetiger nectochaete. ac, anal cirri; cb, ciliary band. Scale bars, 100 µm for (A, B); 10 µm for (C–J).

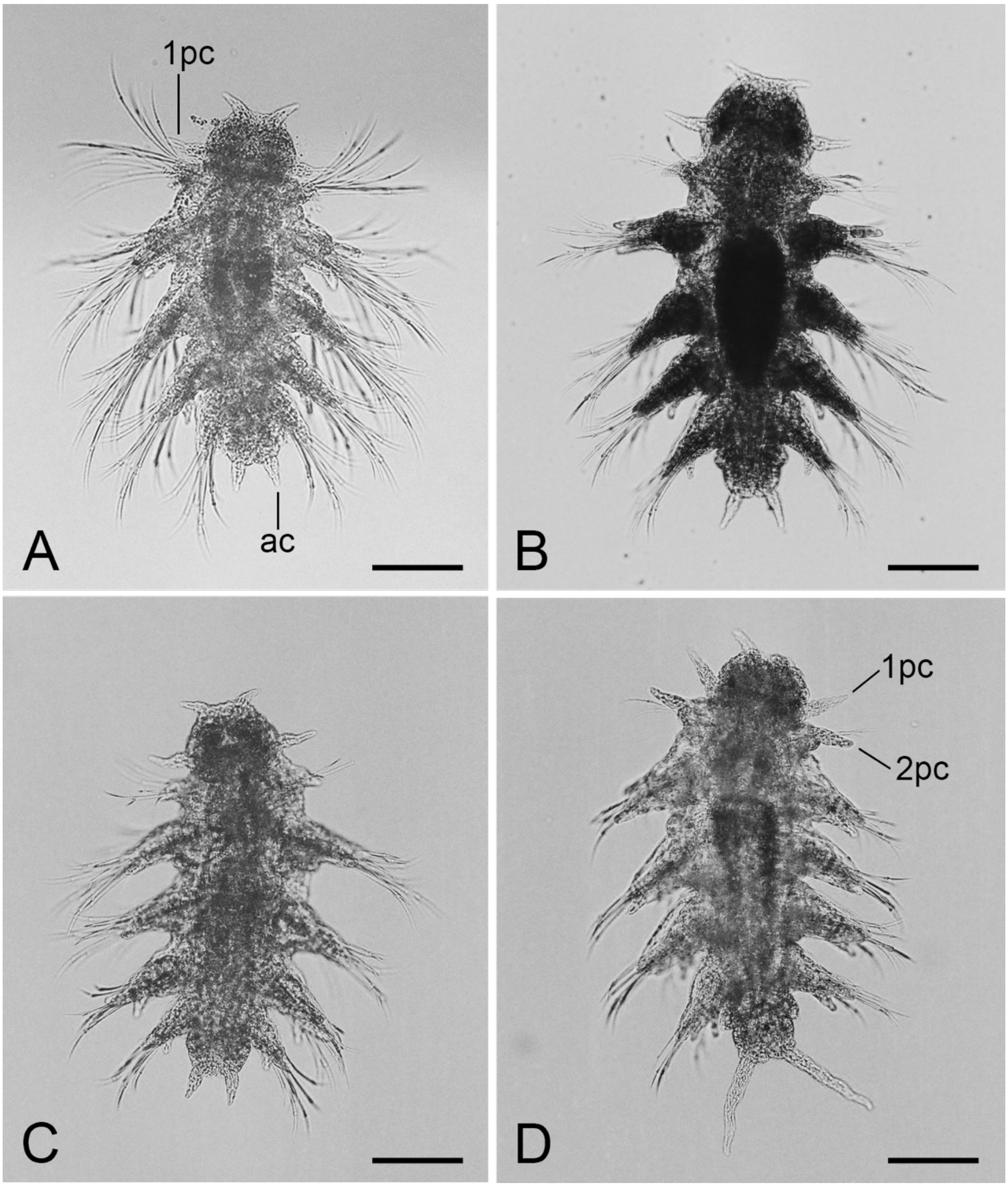

Fig. 4. Planktic 5-chaetiger larvae (A–C) and a benthic 5-chaetiger juvenile (D) of *Hediste diadroma* collected in the Omoigawa river estuary. Planktic larvae were collected at Sta. 2, 1.5 (A), 1.0 (B), and 0.5 h (C) before high tide on April 26, 2013. A benthic juvenile (D) was collected from intertidal flats at Sta. 3 the next day (April 27, 2013). 1pc, first peristomial cirri; 2pc, second peristomial cirri; ac, anal cirri. Scale bars: 100 μm.

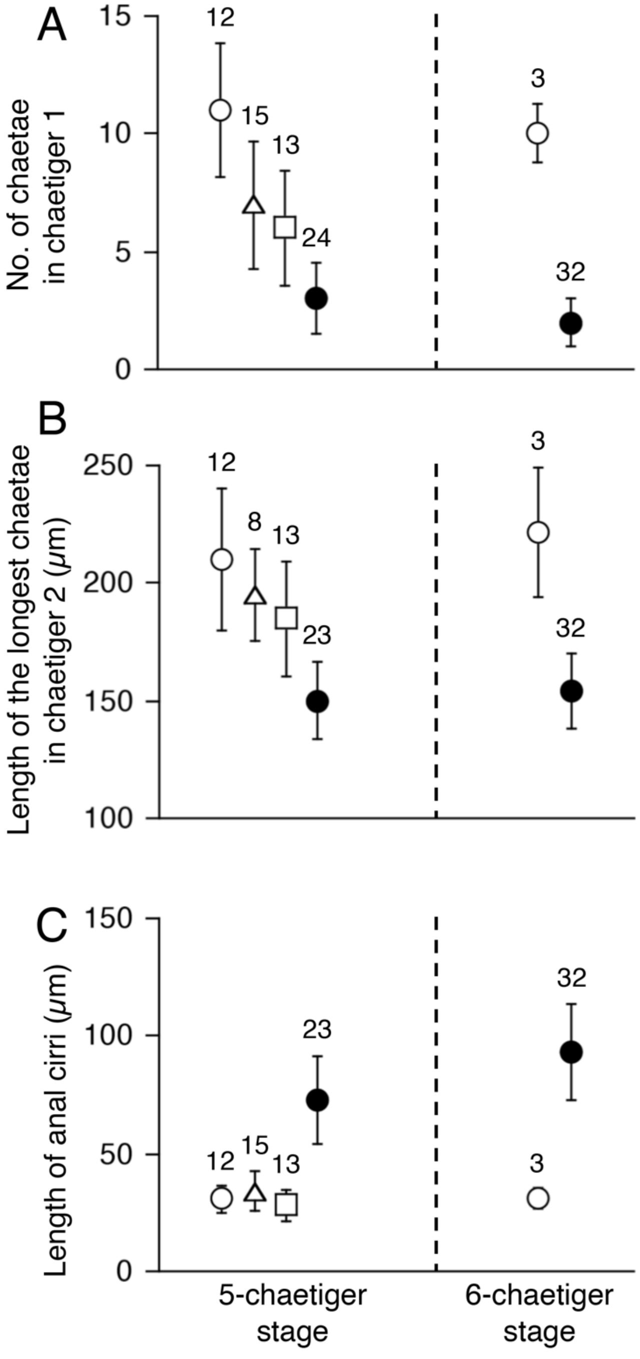

Fig. 5. Comparison of three morphological characteristics of the 5- and 6-chaetiger nectochaetes of *Hediste diadroma* between planktic larvae before settlement (open symbols) and benthic juveniles after settlement (closed circles). (A) Number of chaetae in chaetiger 1; (B) length of the longest chaetae in chaetiger 2; (C) length of anal cirri.

Planktic larvae were collected 1.5 (open circles), 1.0 (open triangles), and 0.5 (open squares) h before the evening high tide on April 26, 2013 at Sta. 2. Benthic juveniles were collected at Stas. 1–4 at low tide the next day (April 27, 2013). Averages and SD bars are shown. The number above each data point indicates the number of specimens examined.

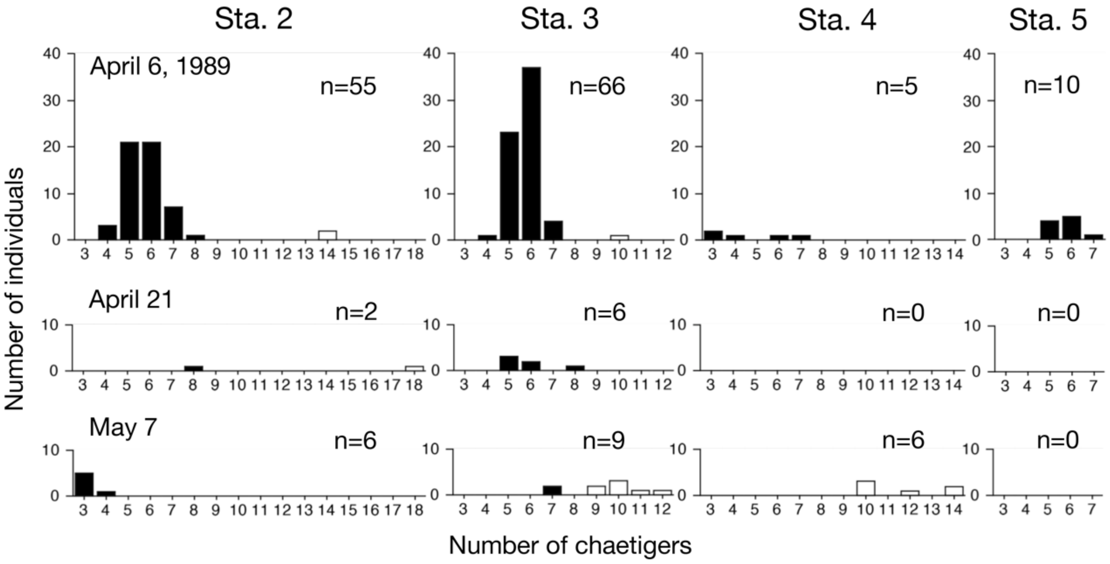

Fig. 6. Frequency distributions of numbers of the planktic nectochaetes of the 3- to 18-chaetiger stages of *Hediste diadroma*, collected by 20 m horizontal tows of a net from the bridge at each of Stas. 2–5 in the evening rising tides on April 6 and 21 and May 7, 1989. Black-shaded bars indicate the pre-settling recruiting cohort of the planktic larvae consisting of 3- to 8-chaetiger nectochaetes. White bars indicate the post-settling dispersing cohort of 9- to 18-chaetiger nectochaetes, which were assessed as not being true planktic larvae but temporal swimming juveniles.

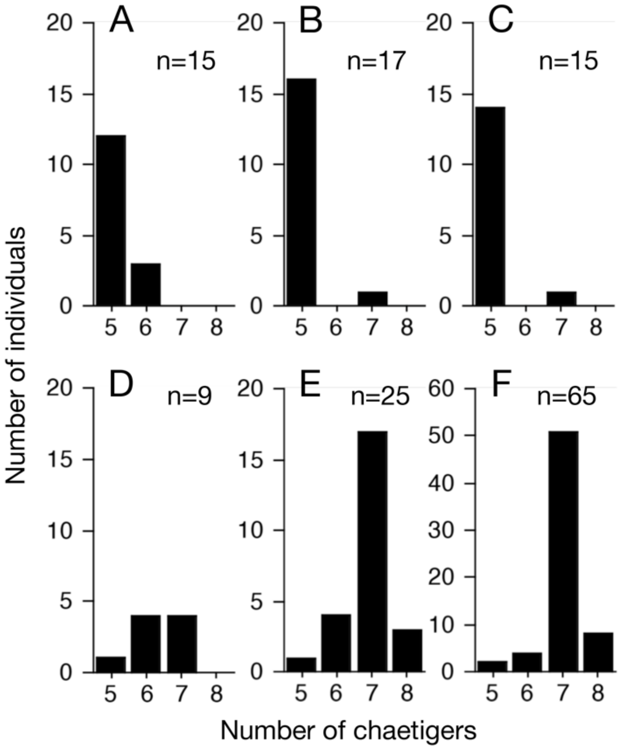

Fig. 7. Frequency distributions of numbers of the planktic nectochaetes of the 5- to 8-chaetiger stages of *Hediste diadroma*, collected at Sta. 2 on April 26, 2013 (A–C) and April 18, 2015 (D–F). In 2013, they were collected by 20 m horizontal tows of a net from the bridge, 1.5 (A) and 1.0 h (B) before high tide and by filtering a 120 L water sample with a net at the riverside 0.5 h before high tide (C). In 2015, they were collected by filtering a 120 L water sample with a net at the riverside, 1.5 (D), 1.0 (E), and 0 h (F) before high tide.

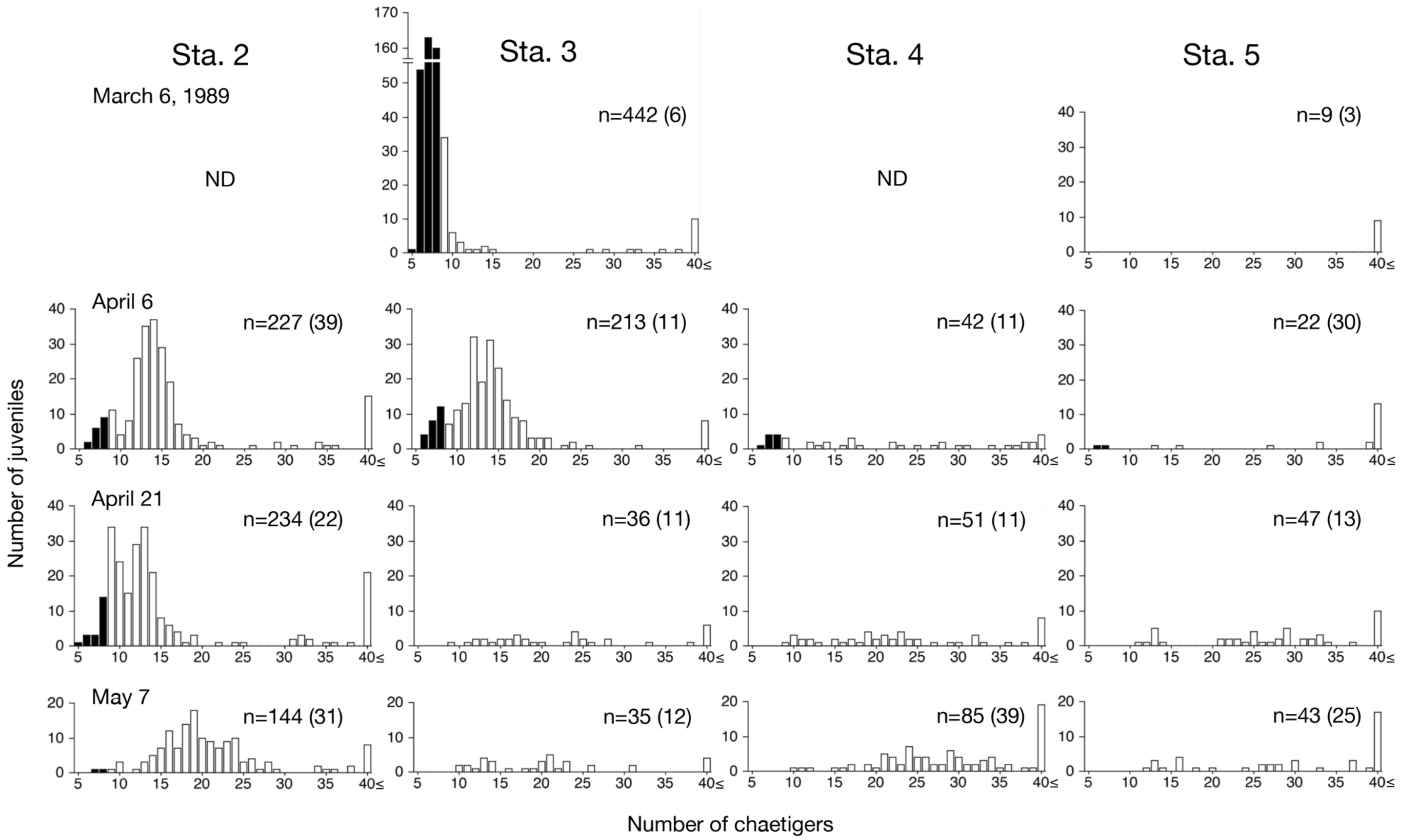

Fig. 8. Frequency distributions of numbers of the benthic juveniles collected from intertidal flats at Stas. 2–5 by using a core sampler (7 cm in diameter, one sample each) at low tides on March 6, April 6 and 21, and May 7, 1989. Black-shaded bars indicate the youngest 5- to 8-chaetiger juveniles, which were regarded as just settled juveniles of *Hediste diadroma*. White bars indicate the 9- and higher chaetiger juveniles, which seemed to consist mostly of juveniles of *H. diadroma* settled around the previous spring tides, together with some individuals of the sympatric congener (*H. atoka*). In each graph, the total number of available juveniles is shown, together with unavailable incomplete specimens (anterior fragments with less than 40 chaetigers) in parentheses.

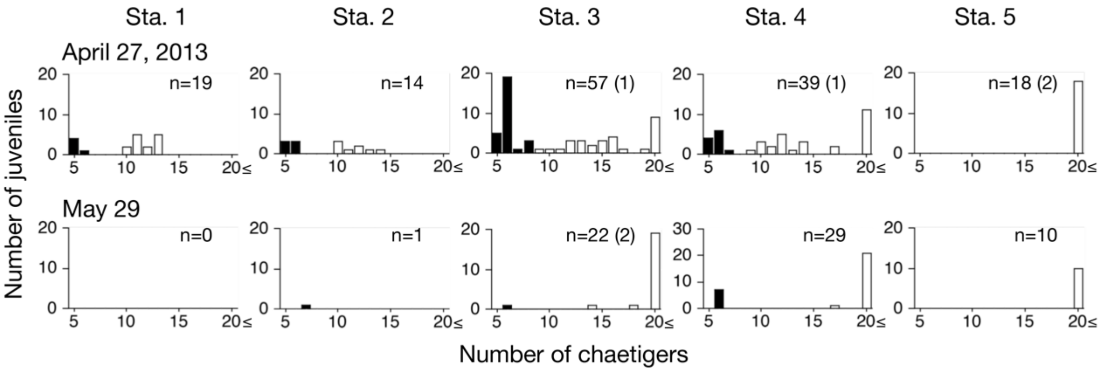

Fig. 9. Frequency distributions, based on pooled data from three samples, of numbers of the benthic juveniles collected from intertidal flats at Stas. 1–5 by using a core sampler (3 cm in diameter, three samples each) at low tides on April 27 and May 29, 2013. Black-shaded bars indicate the youngest 5- to 8-chaetiger juveniles, which were regarded as just settled juveniles of *Hediste diadroma*. White bars indicate the 9- and higher chaetiger juveniles, which seemed to consist mostly of juveniles of *H. diadroma* settled around the previous spring tides, together with some individuals of the sympatric congener (*H. atoka*). In each graph, the total number of available juveniles is shown, together with unavailable incomplete specimens (anterior fragments with less than 40 chaetigers) in parentheses.

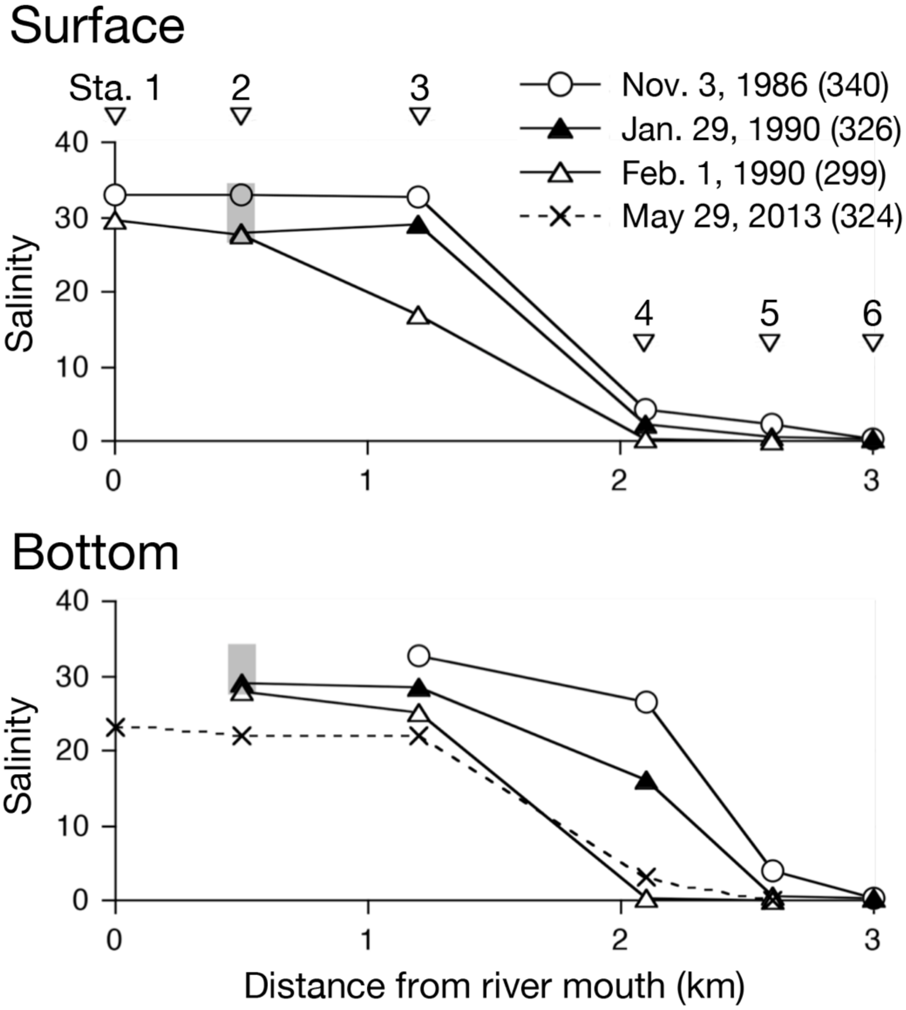

Fig. 10. Salinity gradient of the river water (solid lines) at surface and bottom layers at high tides with various tidal levels in 1986 and 1990 in the estuary of the Omoigawa river. Data of the interstitial water at a low tide on May 23, 2013 are also shown (a dashed line). Vertical bars at Sta. 2 indicate salinity ranges based on pooled data obtained during the larval samplings at high tides on April 26, 2013 and April 18, 2015 (high-tide level: 352 cm and 348 cm, respectively). The measured values of the high-tide levels of the measurement dates are shown in parentheses (cm).

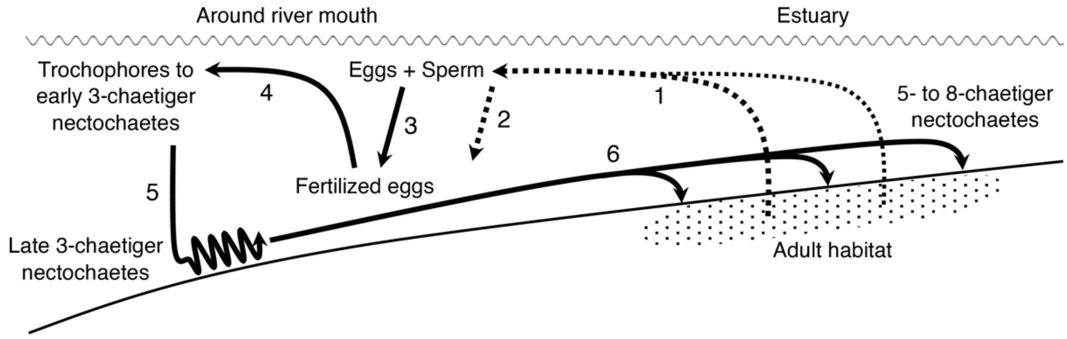

Fig. 11. Schematic diagram of the catadromous life cycle of *Hediste diadroma* in an estuary. (1) Reproductive swarming of mature adults toward the sea and spawning around the river mouth; (2) death of adults after spawning; (3) the sinking of fertilized eggs to the bottom; (4) larvae hatching and entering the pelagic stage, which lasted from the trochophore to early 3-chaetiger nectochaeta stages (a period of a few days); (5) entering the demersal stage, which lasted from the late 3-chaetiger to 5- to 8-chaetiger nectochaeta stages (about a month); (6) larval upstream migration at the 5- to 8-chaetiger nectochaeta stages and their settlement in tidal flats in a wide area of the estuary.

Chapter 3

Description of an undescribed species of *Hediste* newly found from the Han River estuary in Korea

Since this chapter contains taxonomic species descriptions, this published version gives a digest instead of the description.

Digest

In this chapter, an undescribed species of *Hediste* is taxonomically described based on immature individuals collected from the freshwater and the upper reaches of the estuary of the Han River in Korea, and mature individuals collected from the lower reaches of the same river during reproductive swarming. In an immature stage, *H.* sp. is morphologically distinguishable from *H. japonica*, but indistinguishable from *H. diadrom* and *H. atoka*. However, *H.* sp. is distinguishable from all other five congeners in a sexually mature stage. These results show that (1) three catadromous species of *Hediste* (*H. japonica*, *H. doadroma*, *H.* sp.) are distributed in eastern Asia, and (2) epitokous metamorphosis is species specific in these three cadromous species.

Chapter 4

General Discussion

Summary of the present study

The present study shed light on the less-known larval recruitment process in the catadromous life cycle of *Hediste diadroma*, which seems to consist of the pelagic stage, demersal stage, and upstream migration and their settlement in an estuary. The present study also revealed that presence of the undescribed species of *Hediste* (*H.* sp.), which seems to exhibit a similar catadromous life history.

My results show that (1) four species of *Hediste* are distributed in eastern Asia: three species (*H. japonica*, *H. doadroma*, and *H.* sp.) with the typical or non-typical catadromous life history and one species (*H. atoka*) with the estuary-resident life history, and (2) epitokous metamorphosis is species specific in these three cadromous species.

Comparison of life histories in Asian *Hediste*

Based on the present and previous studies (Izuka 1908, Kagawa 1955, Sato & Tsuchiya 1987, 1991, Sato 1999, Sato & Nakashima 2003, Tosuji & Sato 2006, Sato & Sattmann 2009, Tosuji & Sato 2010, 2012, Nishizawa et al. 2014, Tosuji & Furota 2016, Tosuji et al. 2019), the reproductive characteristics and distribution of four species of Asian *Hediste* can be summarized as follows: (1) *H. atoka*: benthic reproduction without epitokous metamorphosis, direct development with 200–250 μm in egg diameter, widely distributed throughout Japan (from Hokkaido to Ishigaki-jima Island) and Korea (both the east and west coasts); (2) *H. diadroma*: swarming with marked epitokous metamorphosis (addition of epitoke-specific sesquigomph spinigers in middle chaetigers), 1-month planktic development with 130–170 µm in egg diameter, distributed in Japan (from Hokkaido to Kagoshima) and North American Pacific coasts (human-mediated introduction); (3) *H. japonica*: swarming with slight epitokous metamorphosis (increase in number of homogomph spinigers), 10-day planktic development with 180–210 µm in egg diameter, distributed in Japan (Ariake Sea) and Korea (throughout west coast); (4) *H.* sp. (undescribed species): swarming with marked epitokous metamorphosis (addition of many homogomph spinigers and enlargement of notopodial dorsal ligule in middle and posterior chaetigers), developmental mode unknown with around 200 µm in egg diameter, endemic to Han River Estuary in Korea.

Based on the topology of a phylogenetic analysis using the combined data set of 16S rDNA and COI, Tosuji et al. (2019) concluded that the epitoky of *Hediste*, which is markedly different from the typical heteronereis form prevailing in many marine nereidids, seems to have evolved independently three times in *H. japonica*, *H. diadroma*, and *H.* sp. only in Asia. My results on the morphology of *H.* sp. well support the previous conclusion.

In *H. diadroma*, pre-mature swimming adults has been observed from several estuaries in Japan in December to March (Hanafiah et al. 2006; Sato et al. unpublished data). These findings suggest that at least a part of the pre-mature adults swim to the surface and are transported downstream by the ebb before true swarming (Sato 2017). It is an interesting issue whether such pre-mature swimming occurs in the adults of *H.* sp. inhabiting in the upper reaches of the estuary. In either case, the unique epitokous metamorphosis (addition of many homogomph spinigers and enlargement of notopodial dorsal ligule in middle and posterior parapodia) of *H.* sp. may have an adaptive significance for increasing the swimming ability during the downward migration.

Characteristics of downward and upward migration in the catadromous life cycle of nereidid polychaetes

Results of the present and previous studies (Kagawa 1955, Wu et al. 1985, Sato & Tsuchiya 1987, Hanafiah et al. 2006) show that the catadromous migration of nereidid polychaetes (*Hediste* spp. and *Tylorrhynchus* spp.) strongly depends on the movement of tidal current during both upward and downward migration, while other catadromous aquatic animal such as the Japanese mitten crab *Eriocheir japonica* and the Japanese eel *Anguilla japonica* depend on the tidal current only during upward migration (Kaifu et al. 2010, Kobayashi & Archdale 2016). Therefore, the large tidal magnitude seems to be necessary for maintenance of the catadromous life cycle in the nereidid polychaetes. In fact, *Hediste* sp. were found from the Han River estuary that is located the east coast of the Yellow Sea, where the tidal magnitude is the greatest in Asia yielding the maximum tidal range of more than 9 m. The present distribution of *H. japonica* is restricted to mudflats in the Ariake Sea, where the tidal magnitude is the greatest in Japan yielding the maximum tidal range of more than 6 m (Sato & Nakashima 2003). This fact may be one of the reasons why the catadromous form of nereidid polychaetes is endemic to Asia. Sato (2017) point out that the geographic characteristics of the Asian estuaries may benefit the evolution and maintenance of the catadromous life cycle; because of the dominance of steep sloping rivers in the mountainous coasts, the Asian coasts seem to be rich in small-sized estuaries (e.g., most Japanese estuaries are less than 30 km long), where the distance between estuaries (adult habitat) and the sea (larval habitat) is short enough for the adult downward and larval upward migrations in the catadromous life cycle.

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