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

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**Abstract:** The recruitment process of planktic larvae of the nereidid polychaete *Hediste diadroma*, which has a catadromous life cycle, was examined in an estuary in Kagoshima Bay, Southern Japan. Our laboratory experiments showed that the phototaxis of the planktic larvae changed drastically from positive to negative during the 3-chaetiger nectochaeta, 3.5–4.0 days after fertilization. During this stage, the prostomial antennae, first peristomial cirri, and anal cirri appeared and the ciliary bands used for swimming were reduced; these results suggest that larvae shifted from pelagic to demersal stage at approximately 4.0 days after fertilization. Field sampling took place in the estuary, where reproductive swarming of mature adults was observed in late February to April; planktic larvae of, mainly, 5- to 8-chaetiger stages were collected in evening high tides during the spring tides of April and May 1989, 2013, and 2015. During the same period, 5- or higher chaetiger benthic juveniles were collected at low tide from intertidal flats from a wide area of the estuary where adults inhabited. The morphology of benthic juveniles of 5- and 6-chaetiger stages differed from that of planktic larvae of the same stages in the following characteristics: (1) the long larva-specific spinigers were lost; (2) the second peristomial cirri, originating from the parapodial lobes of chaetiger 1 of the planktic larvae, appeared; and (3) the anal cirri were elongated. Our results indicate that 5- to 8-chaetiger nectochaeta are critical phases for the successful settlement of planktic larvae, which come back into an estuary with rising tides.

**Key words:** metamorphosis, nectochaeta, phototaxis, settlement, upstream migration

**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, Tosui & Sato 2012). Recently, invader populations of H. diadroma were discovered along North American Pacific coasts, replacing the native congeners H. limnicola (Nishizawa et al. 2014, Tosui & 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, Tosui & 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). 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°51′42″E), Sta. 2 at 3 km upstream, Sta. 3 at 2 km upstream, Sta. 4 at 1 km upstream, Sta. 5 at 500 m upstream, and Sta. 6 at 300 m upstream.
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, 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 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 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), January 29 (326 cm, 2.3, spring tide) and February 1 (299 cm, 5.3), 1990. 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, we used the species-specific multiplex PCR method (Tosuji & Sato 2012). 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. ato-ka* (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 mount-
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 anal cirri of the 5- and 6-chaetiger benthic juveniles (73 ± 19 µm and 93 ± 21 µm, respectively) (Fig. 3D) 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 1–4; a simple capillary (Figs. 3F, G) was 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 recruitment of *Hediste diadroma*

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 posi-
tive 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.

![Image](image-url)
Larval recruitment of *Hediste diadroma* 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 (9818 ind. 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 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 (1321 ind. 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 agree-
ment 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.

**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.
Larval recruitment of *Hediste diadroma* 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

![Frequency distributions for different stations](image)

**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.

![Frequency distributions for different stations](image)

**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.
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, we described anew the elongation of the anal cirri after the settlement, which the drawing of Kagawa (1955) already implied.

Regarding chaetal morphology, we found that chaetae of the 3-chaetiger larvae of H. diadroma consist of
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The larva-specific long homogomph spinigers and simple capillaries, which have not been found in adults (Sato & Nakashima 2003). We 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*.

We 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 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 trophophore 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.

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References

Baker P (2003) Two species of oyster larvae show different depth distributions in a shallow, well-mixed estuary. J Shellfish Res 22: 733–736.

Bousfield EL (1955) Ecological control of the occurrence of barnacles in the Miramichi estuary. Natl Mus Can Bull 137: 1–69.

Carriker MR (1951) Ecological observations on the distribution of oyster larvae in New Jersey estuaries. Ecol Monogr 21: 19–38.

Carriker MR (1967) Ecology of estuarine benthic invertebrates: a perspective. In: Estuaries (ed Rauff GH). American Association for the Advancement of Science Publication, Washington, pp. 442–487.

Cronin TW (1982) Estuarine retention of larvae of the crab...
Rhithropanopeus harrissi. Est Coast Shelf Sci 15: 207–220.
Cronin TW, Forward Jr RB (1979) Tidal vertical migration: an endogenous rhythm in estuarine crab larvae. Science 205: 1020–1022.
Epifanio CE (1988) Transport of invertebrate larvae between estuaries and the continental shelf. Am Fish Soc Symp 3: 104–114.
Faulchald K (1974) Polychaete phylogeny: a problem in protostome evolution. Syst Zool 23: 493–506.
Forward Jr RB, Cronin TW (1980) Tidal rhythms of activity and phototaxis of an estuarine crab larva. Biol Bull 158: 295–303.
Forward Jr RB, Tankersley RA (2001) Selective tidal-stream transport of marine animals. Oceanogr Mar Biol Ann Rev 39: 305–353.
Gibson RN (2003) Go with the flow: tidal migration in marine animals. Hydrobiologia 503: 153–161.
Gustus RM, Cloney RA (1973) Ultrastructure of the larval compound setae of the polychaete Nereis vexillosa Grube. J Morphol 140: 355–366.
Hanafiah Z, Sato M, Nakashima H, Tosuji H (2006) Reproductive swarming of sympatric nereid polychaetes in an estuary of the Omuta-gawa River in Kyushu, Japan, with special reference to simultaneous swarming of two Hediste species. Zool Sci 23: 205–217.
Japan Meteorological Agency (2019). Tidal observation data. Available at: https://www.data.jma.go.jp/gmd/kaiyou/db/tide/genbo/index.php (accessed on 29 September 2019)
Kagawa Y (1955) Note on the optimum salinities, studied in the adult and larva of the brackish-water polychaete worm, Nereis japonica. J Gakugei Coll Tokushima Univ Nat Sci 6: 11–16. (in Japanese with English abstract)
Kikuchi E (1986) Contribution of the polychaete, Neanthes japonica (Izuka), to the oxygen uptake and carbon dioxide production of an intertidal mud-flat of the Nanakita River estuary, Japan. J Exp Mar Biol Ecol 97: 81–93.
Kikuchi E (1998) Life history and dispersal pattern of the polychaete worm Neanthes japonica (Izuka) in the Nanakita River Estuary, Miyagi Prefecture. Jpn J Limnol 59: 125–146.
Kikuchi E, Yasuda K (2006) Comparison of the life cycles of two sympatric estuarine polychaetes, Hediste diadroma and H. atoka (Polychaeta: Nereididae), in the Nanakita River estuary, northeastern Japan. Limnology 7: 103–115.
Kobayashi S, Archdale MV (2016) Migration process of megalopae of the Japanese mitten crab Eriocheir japonica (De Haan) from open sea to tidal river. Estuar Coast 39: 864–854.
Kusuda T, Iyooka H, Il-kweun OH, Wasada Y (2006) Characteristics on habitat of larvae of Detritanotus japonicus in the Kita River. Doboku Gakkai Ronbunshu G 62: 325–331. (in Japanese with English abstract)
Miura T, Kajihara T (1984) An ecological study of the life histories of two Japanese serpulid worms, Hydroids ezoensis and Pomatoleios kraussii. In: Proceedings of the First International Polychaete Conference (ed Hutchings PA). The Linnean Society of New South Wales, Sydney, pp. 338–354.
Nelson TC, Perkins EB (1931) Annual report of the department of biology, for the year ending June 30, 1930. New Jersey Agr Exptl Sta Bull 522: 1–47.
Nishizawa R, Sato M, Furota T, Tosuji H (2014) Cryptic invasion of northeast Pacific estuaries by the Asian polychaete, Hediste diadroma (Nereididae). Mar Biol 161: 187–194.
Pennington JT, Chia FS (1984) Morphological and behavioral defenses of trophophore larvae of Sabellaria cementaria (Polychaeta) against four planktic predators. Biol Bull 167: 168–175.
Purschke G (2005) Sense organs in polychaeta (Annelida). Hydrobiologia 535/536: 53–78.
Sato M (1999) Divergence of reproductive and developmental characteristics in Hediste (Polychaeta: Nereididae). Hydrobiologia 402: 129–143.
Sato M (2004) Diversity of polychaetes and environments in tidal flats: a study on the Hediste species group (Nereididae). Fish. 76: 122–133. (in Japanese with English abstract)
Sato M (2017) Nereididae (Annelida) in Japan, with special reference to life-history differentiation among estuarine species. In: Species Diversity of Animals in Japan (eds Motokawa M, Kajihara H). Springer Japan, Tokyo, pp 477–512.
Sato M, Nakashima A (2003) A review of Asian Hediste species complex (Nereididae, Polychaeta) with descriptions of two new species and a redescription of Hediste japonica (Izuka, 1908). Zool J Linn Soc 137: 403–445.
Sato M, Tsuchiya M (1987) Reproductive behavior and salinity favorable for early development in two types of the brackish-water polychaete Neanthes japonica (Izuka), Benthos Res 31: 29–42.
Sato M, Tsuchiya M (1991) Two patterns of early development in nereidid polychaetes keying out to Neanthes japonica (Izuka). Ophelia Suppl 5: 371–382.
Sayama M, Kurihara Y (1983) Relationship between burrowing activity of the polychaetous annelid, Neanthes japonica (Izuka) and nitrification-denitrification processes in the sediments. J Exp Mar Biol Ecol 72: 233–241.
Smith RI (1958) Physiological and reproductive adaptations of Nereis diversicolor to life in the Baltic Sea and adjacent waters. In: Essays on Polychaetous Annelids in Memory of Dr. Olga Hartman (eds Reish DJ, Faulchald K). Allan Hancock Foundation, University of Southern California, Los Angeles, pp. 373–390.
Suzuki H, Kikuchi T (1990) Spatial distribution and recruitment of pelagic larvae of sand bubbler crab, Scopimera globosa. La mer, 28: 172–179.
Toba M, Yamakawa H, Shouji N, Kobayashi Y (2012) Vertical distribution of Manila clam Ruditapes philippinarum larvae characterized through year-round observations in Tokyo Bay, Japan. Nippon Suisan Gakkaishi, 78: 1135–1148. (in Japanese with English abstract)
Tosuji H, Furota T (2016) Molecular evidence for the expansion of the Asian cryptic invader Hediste diadroma (Nereididae: Annelida) into the northeast Pacific habitats of the native H. limnicola. Zool Sci 33: 162–169.
Tosuji H, Sato M (2006) Salinity favorable for early development and gamete compatibility in two sympatric estuarine species of the genus Hediste (Polychaeta: Nereididae) in the Ariake Sea, Japan. Mar Biol 148: 529–539.
Tosuji H, Sato M (2012) A simple method to identify Hediste sibling species (Polychaeta: Nereididae) using multiplex PCR amplification of the mitochondrial 16S rRNA gene. Plankton Benthos Res 7: 195–202.

Tsuchiya M, Kurihara Y (1979) The feeding habits and food sources of the deposit-feeding polychaete, Neanthes japonica (Izuka). J Exp Mar Biol Ecol 36: 79–89.

Uno K, Nakano S (2002) Effect of salinity on habitat of larvae of Uca around the Yoshino River mouth. Ann J Hydraul Eng JSCE, JSCE 46: 1175–1180. (in Japanese with English abstract)

Villalobos-Guerrero TF, Bakken T (2018) Revision of the Alitta virens species complex (Annelida: Nereididae) from the North Pacific Ocean. Zootaxa 4483: 201–257.

Wilson DP (1932) The development of Nereis pelagica Linnaeus. J Mar Biol Assoc UK 18: 203–217.

Wood L, Hargis Jr WJ (1971) Transport of bivalve larvae in a tidal estuary. In: Proceedings of the Fourth European Marine Biology Symposium (ed Crisp DJ). Cambridge University Press, Cambridge, pp. 29–44.

Yokouchi K (1990) Ontogenetic change in photoresponses during larval development of the sandworm (Neanthes viens (Sars)). Bull Tohoku Natl Fish Res Inst 52: 79–86. (in Japanese with English abstract)