Comparison of larval distribution in two clupeoid fishes (*Ilisha elongata* and *Sardinella zunasi*) in the inner estuaries of Ariake Sound, Shimabara Bay, Japan

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**Abstract:** In early life history, the clupeoid fishes, *Ilisha elongata* (Pristigasteridae) and *Sardinella zunasi* (Clupeidae) have similar spawning seasons and nurseries from spring to summer in the Ariake Sound, Shimabara Bay, Japan. Surveys conducted using larva nets and a beam trawl in July showed that the horizontal egg and larva distributions of *I. elongata* and *S. zunasi* both overlapped considerably in the inner estuaries; however, *I. elongata* settled in the estuaries while *S. zunasi* retained a pelagic distribution. A comparison of the vertical distributions of the two species in the estuary showed that *I. elongata* larvae were dispersed from the surface to near-bottom layers during the flood tide, and moved downwards to avoid being swept out of the estuary after the high tide, while *S. zunasi* larvae remained spread throughout the water column in all tidal phases. The findings suggest the existence of niche segregation during the pelagic larval period in the two species. Thus, by examining differences in larval mouth sizes, which are much larger in *I. elongata* than in *S. zunasi*, it appears that prey preference varies between the two species, prey specialization occurs, and there is no niche competition between the two species.

**Key words:** Ariake Sound, estuary, *Ilisha elongata*, larval distribution,* Sardinella zunasi*

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**Introduction**

Shimabara Bay is the largest tidal flat region with the highest tidal range in Japan. The Ariake Sound, which is located in the innermost part of the bay, is characterized by having brackish, highly turbid water with strong tidal currents (Inoue 1980, Yagi et al. 2011). The importance of this upper estuary as a nursery ground has been demonstrated in a wide variety of fish species, including clupeoid fishes, such as *Coilia nasus* Temminck & Schlegel, 1846 (Engraulidae), *Konosirus punctatus* (Temminck & Schlegel, 1846), *Sardinella zunasi* (Bleecker, 1854) (Clupeidae), and *Ilisha elongata* (Bennett, 1830) (Pristigasteridae). These species have similar spawning seasons and grounds as clupeoid fishes, and their larvae are present from spring to summer (Yagi 2010, Yagi et al. 2011). Clupeoid fishes have common larval morphological characteristics, i.e., slender body, small head, straight and long intestine, and scarce pigmentation (Uchida 1963). *Coilia nasus* is an endemic species (Uchida & Tsukahara 1955) and *S. zunasi* and *I. elongata* are likely local stocks in Shimabara Bay (Takita 1980, 2000).

Although there is considerable information about the early life history of *C. nasus* (Takita 1967, Ishida & Tsukahara 1972, Yagi 2010, Simanjuntak et al. 2015, Simanjuntak 2016, Ito et al. 2018), *K. punctatus* (Yoshida 1937, Matsushita & Nose 1974, Kawasaki et al. 2006, Yagi 2010) and
S. zunasi (Takita 1966, Yagi 2010) in Ariake Sound and other Japanese waters, only Yagi (2010) has examined the life history of I. elongata. When we examined the egg and larva distributions of this species in the Ariake Sound, we found that I. elongata and S. zunasi larvae have almost the same life history patterns and a sympatric distribution in the estuary. These findings suggested the existence of niche competition or some segregation between the two species.

This study compared the horizontal and vertical distribution of I. elongata and S. zunasi over tidal cycles to examine the potential for spatial differences in their use of the nursery grounds in Shimabara Bay.

Materials and Methods

Illisha elongata and Sardinella zunasi eggs and larvae were collected in Ariake Sound, Shimabara Bay in the morning (7:00–12:00) at around spring tide (full moon) in July 2016 (Table 1). Pelagic eggs and larvae were collected by oblique tows using a larva net with a mouth diameter of 1.3 m. The mesh apertures of the nets were 0.5 mm at the sea stations (outside river and river mouth: Stns. 6–13, 37, 38, 35, 54, 61 and 62 in Fig. 1), and 1 mm at river stations (inside river: Stns. 0–5, 33–36, 50–52 and 73) to avoid clogging of the net by suspended particles. In addition, where depths were shallower than 2 m, a larva net with a mouth diameter of 1 m (0.5-mm mesh aperture) was towed horizontally at the surface inside the retention basin (Stns. 55–60). To collect demersal juveniles, beam trawling (width, 1.5 m; height, 0.25 m; 2-mm mesh aperture) was performed using a modification of the method described by Kuipers (1975) at Stns. 0–8, 16, 17, 33–38, 50–52, 61, 62 and 73.

To examine differences in the size and vertical distribution of the two species relative to the tidal phase, discrete-depth horizontal tows in the surface and middle layers were conducted with a larva net (1.3-m mouth diameter, 0.5-mm mesh aperture), a specialized beam trawl (width, 1.5 m; height, 0.25 m; 1-mm mesh aperture) (Aljamali et al. 2006, Yagi et al. 2009) in the near-bottom layer, and a beam trawl on the bottom at Stn. 7 (Fig. 1), located just outside the mouth of the Rokkaku River. Five sets of the four categorized collections were made from flood to ebb tides on 20 July 2016.

The towing distances (m) of the larva net and near-bottom net were measured by a flow meter (2030R, General Oceanics) attached to the nets. The towing depths (m) of the larva net were measured using a depth recorder (Mark5, Alec Electronics). The towing distance of the beam trawl was monitored using a GPS (Colorado 300, Garmin).

The number of individuals (n) was converted to density (N: inds. 100 m$^{-2}$) using the following equations:

\[ N_{LN} = \frac{n \times d \times 100}{A \times D} \]

where \(A\) is the area (m$^2$) of the larva net, \(D\) is the towing distance (m), and \(d\) is the towing depth (m) for the larva net;

\[ N_{BF} = \frac{n \times 100}{w \times D} \]

where \(w\) is the width (m) of the beam trawl, and \(D\) is the towing distance (m) for the beam trawl.

To compare fairly the standing stock in the surface, middle and near-bottom layers, inds. 1000 m$^{-3}$ was calculated with the following formula for the densities of individuals:

\[ N_{LN, NBN} = \frac{n \times 1000}{(A \times D)} \]

where \(A\) is the area (m$^2$) of the larva net and near-bottom net, \(D\) is the towing distance (m) for the larva net and near-bottom net.

All samples were initially fixed with ca. 10% field-water formalin. Eggs and larvae were immediately sorted and preserved in 10% formalin and 99% ethanol, respectively. Eggs and larvae of I. elongata were identified according to Uchida (1958) and Wang et al. (2021), and S. zunasi eggs and larvae were identified according to Uchida (1958) and Takita (1966). Diameters of pelagic, spherical eggs before preservation are 2.2–2.5 mm in I. elongata (Uchida 1958), 1.74–1.91 mm in S. zunasi (Takita 1966). The developmental stages of eggs, larvae and juveniles were assessed based on the ontogeny of the embryo [stage A (before embryo differentiation), B (starting embryo to parting tail tip from yolk), C (end of B to hatching), Nakai 1962], notochord tip and completion of fin rays (Kendall et al. 1984), respectively. Unlabeled lengths indicate body length (notochord length for preflexion larvae and flexion larvae, and standard length for postflexion larvae and juveniles). To ascertain whether there was any difference in size preference of prey between the two fish species, a maximum of 50 specimens of each species, collected from the middle layer at Stn. 7 at 07:42 on 20 July, were randomly selected and their mouth size (Shirota 1970) was measured. The mean body lengths were plotted against water layer and the data on body lengths were compared by one-way ANOVA using the Games-Howell post hoc multiple comparison test using a significance level of $\alpha=0.05$.

At each sampling station, temperature (°C), salinity and turbidity (NTU) were observed at 0.5 m intervals from the

| Date (July) | Flood tide | Ebb tide |
|------------|------------|----------|
| 19         |            | 55–60    |
| 20*        | 7**        |          |
| 21         | 17, 61, 62 | 7, 9–13, 16 |
| 22         | 6, 8, 50–52, 54 | 4, 5, 53 |
| 23         | 33–38, 73  |          |
| 24         | 0–3        |          |

*: spring tide, **: tidal collection from discrete layers. See Wang et al. (2021) about the information on sampling stations.
surface to the bottom using a Compact-CTD (ASTD687, JFE Alec). The current velocity was measured with an ADCP (WHSZ1200-I-UG12, RD Instruments) at 0.5-m depth intervals from a depth of 1 m to the bottom, except for the stations inside the retention basin (Stns. 55–60 in Fig. 1).
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Fig. 2. Horizontal distribution of temperature (°C), salinity and turbidity (NTU) at 0.5 m depth of each station when fishes were collected in July 2016.

Fig. 3. Horizontal distribution of the early stages of Ilisha elongata and Sardinella zunasi collected by larva net and beam trawling in Ariake Sound in July 2016. The diameter of each circle is drawn in proportion to the square root of density (inds. 100 m$^{-2}$). Crosses represent the absence of species.
Results

Horizontal distribution in the sound

Small differences were observed in the temperatures of the sea water and the river water, although the Rokkaku River and the retention basin had slightly higher temperatures than the other rivers (Fig. 2a). Marked haloclines were observed between the estuary (Stns. 7, 37 and 54) and the upper river reaches (Stns. 0, 50 and 73), and these are attributed to the tidal exchange of water (Fig. 2b). Salinity measurements showed that torrential rains in June and July resulted in the water in the bay being more brackish than normal. In addition, the water in the retention basin was almost entirely fresh (Fig. 2b). Turbidities were considerably higher near the estuaries than in the open bay and at the Isahaya inlet (Fig. 2c), with the highest values observed in the Rokkaku estuary. Current velocities >1.5 kt were frequently measured both inside and outside the estuaries, but the current velocities were constantly low inside the Isahaya inlet (Fig. 1).

The *Ilisha elongata* and *Sardinella zunasi* eggs, both being mostly C-stage, were distributed around the estuaries (Stns. 6–8, 37, 38, 53 and 54) including the lower reaches of rivers (Stns. 4 and 5) for the former, and widely from Isahaya inlet to most inner waters, being absent inside rivers, for the latter. Neither of the two species eggs were present inside the reclamation dike (Fig. 3).

The *I. elongata* larvae were abundant in the estuaries and lower reaches of three rivers, with most being at the postflexion stage of development. Compared to eggs, larval densities were higher in all rivers. Beam trawl samples revealed that postflexion larvae and juveniles of *I. elongata* were distributed from the estuaries to the lower river reaches (Stns. 3–5, 34–36 and 52).

The *S. zunasi* larvae, principally in the flexion and preflexion stages, were distributed separately in the inner estuaries and in Isahaya inlet, respectively. Subsequently, few larvae were collected in the beam trawls of the sound (Fig. 3).

Tidal distribution

Dominant tidal currents had a marked effect on the vertical structure of the water column (Fig. 4). Although the current velocity was marginally stronger at the surface than in the bottom layers during both flood and ebb tides, the current flowed up and down, mixing vertically. Turbidity also increased at current velocities >0.5 kt during both tides. Weak stratification of water temperature and salinity gradients was observed in the water column.

A total of 2,794 *I. elongata* and 20,235 *S. zunasi* eggs, almost all at the C-stage of development, were collected in the surface, middle and near-bottom layers. The *I. elongata* eggs were homogenously dispersed (i.e., from the surface to the near-bottom layers) during both tides, with densities decreasing slightly after high tide (Fig. 5a). However, while *S. zunasi* eggs were distributed vertically through most of the layers during flood tides, they were not collected in the near-bottom layer after high tide.

A total of 870 *I. elongata* larvae (4.6–20.7 mm, chiefly...
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At the flexion stage) and 24 juveniles (19.6–22.7 mm), and 5,265 S. zunasi larvae (4.7–15.5 mm, chiefly at the flexion stage) were collected in the five sets of samples (Fig. 6). During the flood tide, the I. elongata larvae were dispersed from the surface to the near-bottom layers, but after high tide, few if any larvae were collected in the surface and middle layers. However, earlier larvae returned to these two layers when the ebb flows were highest (Fig. 4d), resulting in larvae being dispersed throughout all layers (Fig. 5b). Conversely, S. zunasi larvae were dispersed from the surface to near-bottom layers at all tidal phases.

Beam trawl findings showed that larvae and juveniles of I. elongata were more abundant than those of S. zunasi, especially during ebb tide (Fig. 5b).

In I. elongata, other than between the middle and near-bottom layer (p=0.999), size distributions were statistically significant between four layers (p<0.001), with the modal size being 12–13 mm at the surface, 13–14 mm in the middle and near-bottom layers, and 14–15 and 18–19 mm on the bottom, implying that the size of larvae and juveniles increased slightly with depth. Postflexion larvae and juveniles were more common in the deeper layers, with approximately half of all postflexion larvae and juveniles found on the bottom.

In S. zunasi, there being statistical significance between the surface and middle (p<0.001), and the middle and near-bottom layers (p=0.002) in size distribution, the mode was 8–9 mm for the flexion stage in the surface and middle layers, which decreased to 7–8 mm in the near-bottom layer; the peak was bimodal, i.e., the mode of the flexion group was 7–8 mm, while that of the postflexion group was 10–11 mm. Only 11 larvae were collected at the bottom in five sets of tidal collections.

Larval sizes in individuals occurring in the middle layer at flood tide were distinctively larger in I. elongata than in S. zunasi, overlapping only between 12–14 mm (Fig. 7). Furthermore, when the mouth sizes of the two species were compared, those of I. elongata larvae were almost always larger than those of S. zunasi, with very little overlap being observed between the species (Fig. 7).

**Discussion**

Larva net tows revealed a marked difference in the hori-
horizontal distribution of *Ilisha elongata* and *Sardinella zunasi* eggs and larvae; *I. elongata* were concentrated in inner estuaries, while *S. zunasi* were distributed from the inner estuaries to the Isahaya inlet (Fig. 3). Based on egg distributions, it appeared that *I. elongata* and *S. zunasi* primarily spawn below the mouth of the Rokkaku River and in the Isahaya inlet, respectively. The distribution of the pelagic larvae of both species overlapped in the inner estuaries, with *S. zunasi* being less developed in the Isahaya inlet than in the inner estuaries. The presence of undeveloped larvae suggests that the Isahaya inlet is suboptimal for early life stage of *S. zunasi*. Beam trawl surveys revealed that after the juvenile period, *I. elongata* settles near estuaries, while *S. zunasi* continues a pelagic life style. It therefore appears that both species have a sympatric distribution around inner estuaries during the pelagic larval period.

The eggs of both *I. elongata* and *S. zunasi* collected by oblique (Fig. 3) and tidal tows (Fig. 5a) were almost all at the C-stage of development, suggesting that spawning in
these two species occurs over a limited period. Previous incubation studies showed that eggs reached hatching and the C-stage of development after 30 hrs at 23–26°C and 36 hrs at 15–20°C in I. elongata and S. zunasi, respectively (Yabe 1938, Takita 1966). Considering that the water temperature in the present study was approximately 27°C (Figs. 2a, 4a), accumulated temperatures for incubation, the absence of early developmental stages, and specimens present in the morning suggests that both species spawn in the evening. In I. elongata, the presence of A and B-stage eggs at water temperatures of approximately 22°C in May also support the estimation that spawning occurs in the evening (Wang et al. 2021).

In I. elongata, the larvae were dispersed from the surface to the near-bottom layers during the flood tide while they were scarce or absent at the surface and more abundant in the near-bottom layer after the high tide. Furthermore, the density of demersal larvae and juveniles increased suddenly during the ebb tide (Figs. 5b, 6). Unlike general selective tidal stream transport (Jager 1999, Forward & Tankersley 2001), it is likely that the larvae of this species do not need to be aggregated at the surface and are dispersed vertically because of the strongly mixed flood tide (Fig. 4d). Strongly mixed currents usually occur in the inner estuaries of Ariake Sound during both flood and ebb tides (Yagi et al. 2009, 2011). By employing this behavior in response to tidal flows, the larvae and juveniles may avoid being swept out of the estuary into the open bay and remain in the estuaries, which they use as nursery grounds. Regarding returning larvae when the tide was at its highest ebb flow, they were likely at too early a developmental stage with insufficient locomotory ability to remain in the estuary.

On the other hand, S. zunasi larvae were homogenously dispersed from the surface to the near-bottom layers and few settled on the bottom during any of the tidal phases (Figs. 5b, 6). This implies that S. zunasi leads a pelagic life and is more dependent on tidal currents, which results in larvae and juveniles being dispersed outside estuaries.

Thus, while there may be pelagic competition between the two species, demersal competition is considered to be lower. Assessments of auxotrophic differentiation based on mouth size (Fig. 7) revealed that their preferences for prey likely varies between I. elongata and S. zunasi larvae. According to anecdotal observations by Yagi (2010), copepod prey of I. elongata larvae included Pseudodiaptomus inopinus Burckhardt, 1913 (Pseudodiaptomidae, Calanoidea), while S. zunasi appeared to prey on Oithona davisi (P. Orsi, 1984 (Oithoniidae, Cyclopoida). Of these copepods, the former is markedly larger than the latter (Hirakawa 1997, Nishida 1997). Although distribution of larval I. elongata and S. zunasi overlapped with each other, prey and niche competition are not likely to have occurred in the sampling period due to marked differences between the two co-occurring species in terms of body length distribution and/or mouth size. Such niche isolation between closely related species may facilitate the coexistence of these sympatric species.

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