Early Life History of Clupeoid Fishes in Ariake Sound, Shimabara Bay, Japan

日本有明海鲱亚目鱼类早期生活史

有明海におけるニシン亜目魚類の初期生活史

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September 2021

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Summary

Shimabara Bay is the largest tidal flat region with the highest tidal range in Kyushu Island, southwestern Japan, of which the Ariake Sound is the innermost part, and contains brackish, highly turbid water with strong tidal currents. Previous studies in the sound revealed the inner estuaries as an important spawning and nursery ground clupeoid fishes, i.e., *Coilia nasus* (Engraulidae), *Ilisha elongata* (Pristigasteridae), *Konosirus punctatus* and *Sardinella zunasi* (Clupeidae). *Coilia nasus* is an endemic species, *I. elongata* and *S. zunasi* are likely local stocks in Shimabara Bay. These species have similar spawning seasons and grounds, while their larvae inhabit from spring to summer. It is speculated that some larval niche isolation should occur to avoid competition within the estuaries.

The ontogenetic descriptions of *I. elongata*, *K. punctatus* and *S. zunasi* were reviewed. *Ilisha elongata* revealed several unique characteristics, such as two chorions in the egg and a more anterior pelvic bud (fin) position. These characteristics contrast strikingly with the clupeid fishes, suggesting that this species belongs to the Pristigasteridae and not to the Clupeidae.

Ichthyoplankton surveys collected in March, May, July and November 2019 showed that *I. elongata* spawned just off river mouths in summer and attained peak spawning in June. The larvae with elliptical eyes migrated inside the rivers, where the waters were more turbid. Thus, the spawning and nursery grounds of *I. elongata* almost entirely overlapped with those of the clupeid *S. zunasi* both temporally and spatially, which may result in larval competition between the two species.

Surveys conducted in July 2016 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 dispersed from the surface to the middle layers. The findings suggest the existence of niche competition or 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.

Survey conducted in May 2006 and May 2019 showed that *K. punctatus* and *S. zunasi* spawned almost over the sound, and the larvae were aggregated in the innerpart of the sound. Of those, huge *K. punctatus* larvae seemed to use a beach as their nursery ground, and *S. zunasi* larvae were abundant in some river estuaries. There, however, is likely a competition of larval niche during early larval period between two species in the estuaries. A comparison of the vertical distributions of the two species in the estuary showed that *K. punctatus* larvae were aggregated in the surface layers, while *S. zunasi* larvae remained spread throughout the water column during all tidal phases. Thus, examining differences in larval mouth sizes suggested to reveal a possibility of feeding competition and preference for early larval periods in some estuaries.

The result of otolith examination and egg distribution showed that the spawning period of *I. elongata*, *K. punctatus* and *S. zunasi* was extended from May to July, April to May, and May to July, respectively. The larvae of *S. zunasi* have the highest growth rate, followed by *I. elongata* and *K. punctatus*. Growth rate of these species from the littoral zone of a beach, and various river differed significantly. The differentiation of growth rate between the three clupeoid fishes may be attributed to water temperature, food availability and specific difference.

This study reviewed the ontogeny of *I. elongata*, *K. punctatus* and *S. zunasi* in Ariake Sound; discussed the taxonomic position of *I. elongata*; revealed the egg and larva distribution of clupeoids in spring and summer; estimated the spawning period and growth rates; clarified the niche competition and segregation between closely relative species, the influence of building a dike across the Isahaya inlet, and the importance of Ariake Sound as spawning and nursery ground for clupeoid fishes.

General introduction

The suborder Clupeoidei consists of four families (Pristigasteridae, Engraulidae, Chirocentridae, Clupeidae), 91 genera, more than 400 species worldwide, of which 17 Clupeidae, 9 Engraulidae, 1 Chirocentridae species occur in Japanese waters (Nakabo 2013, Nelson et al., 2016). The clupeoid fishes are prime important to fisheries, accounted for 24% of the world fish catch, of which the largest clupeoid catches come from the southeast Pacific in 1982 (Whitehead 1985). However, the world fisheries production is declining. The fish catches were also decreasing in Ariake Sound, Shimabara Bay, Japan (Kuroda et al. 2002, Terada & Ito 2017, Jia et al. 2018).

The largest area of tidal flats with the highest tidal range in Japan is born by Shimabara Bay, of which Ariake Sound, as the most innerpart, are brackish, highly turbid water with strong tidal currents (Inoue 1980, Yagi et al. 2011). In Ariake Sound, many works have been done on the early life histories of fishes, including community composition of larval and juvenile in the inner estuary of the sound, and littoral zone of beach (Hibino et al. 2002, Yagi et al. 2011).

The importance of upper estuary in Ariake Sound as a nursery ground has been demonstrated for diverse fish, including clupeoid fish, i.e., *Coilia nasus*, *Ilisha elongata, Konosirus punctatus* and *Sardinella zunasi*, which lead their early life history stages there, from spring to summer, with the periods overlapping between the species (Yagi 2010, Yagi et al. 2011). *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). The early life histories of the engraulid *C. nasus* and the clupeid *S. zunasi*, both of which are abundant in summer, have been studied in Ariake Sound (Takita 1966, 1967, Ishida & Tsukahara 1972, Yagi 2010, Simanjuntak et al. 2015, Simanjuntak 2016, Ito et al. 2018). The eggs and larvae of the clupeid *K. punctatus* are present from late winter to early summer in various waters in Japan and the Korean Peninsula, but they have not been reported in Ariake Sound (Yoshida 1937, Matsushita & Nose 1974, Kawasaki et al. 2006). Although the reproductive ecology of *I. elongata* has been examined in Ariake Sound and the Seto Inland Sea (Zhang & Takita 2007, Zhang et al. 2009, Masui et al. 2016), its early life history has only been investigated sporadically in the Geum estuary in western Korea, around the Hangzhou estuary in central China, and in Ariake Sound. Accordingly, little is known about the distribution of the egg and larval stages of this species in any region (Yabe 1938, Sha & Ruan 1981, Yagi et al. 2011). It

is speculated that some niche isolation should occur to avoid larval competition within the estuaries.

In this paper, the ontogeny of *I. elongata*, *K. punctatus* and *S. zunasi* in the sound were reviewed, their egg and larval distributions were examined and compared to better understand the distribution dynamics and various utilization of spawning and nursery ground of clupeoid fishes. Furthermore, age and growth of three species larvae were examined using otolith to better understand its early life history.

General materials and methods

Study area: Shimabara Bay is s semi-enclosed and a relatively well-mixed bay with the largest tidal flat region and the highest tidal range in Kyushu Island, southwestern Japan, of which the Ariake Sound is the innermost part, and contains brackish, highly turbid water with strong tidal currents (Fig. 1) (Inoue 1980, Yagi et al. 2011).   Samples were collected at ca. 45 stations in May 2006, July 2016 and March, May, July and November 2019 (Table 1).
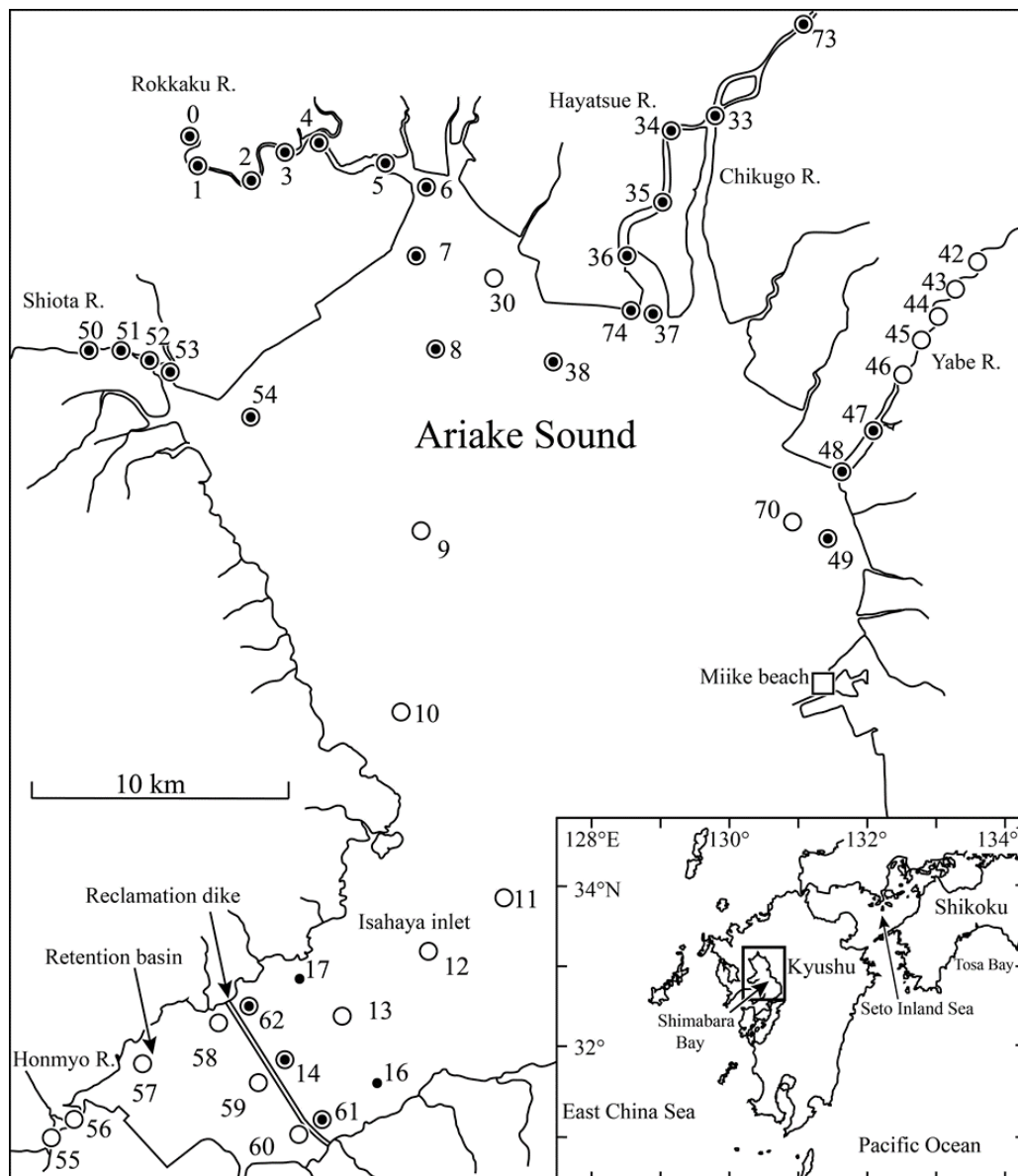
Fig. 1.   Map showing stations where fish were collected in Ariake Sound, Japan.   Open circles (Stns. 0–14, 30, 33–38, 42–62, 70, 73, 74), solid circles (Stns. 0–8, 14, 16, 17, 33–38, 47–54, 61, 62, 73, 74) and the open square (Miike beach) indicate the stations where collections were made by a larva net for pelagic eggs and larvae, beam trawl for demersal juveniles and seine net for larvae and juveniles entering the littoral zone, respectively.

Float

Depth recorder

Mesh size; 0.5 or 1 mm

Float

1 or 1.3 m

3 m

3 m

a

Weight

Flow meter

Float

0.25 m

0.05 m

Mesh size: 1 mm

Flow meter

1.5 m

3.8 m

b

Float

1.5 m

Tickle chain

Ground rope

Mesh size; 2 mm

0.3 m

c

Ground sheet

Mesh size; 1 mm

Float

1 m

70 cm

80 cm

d

Weight

4 m
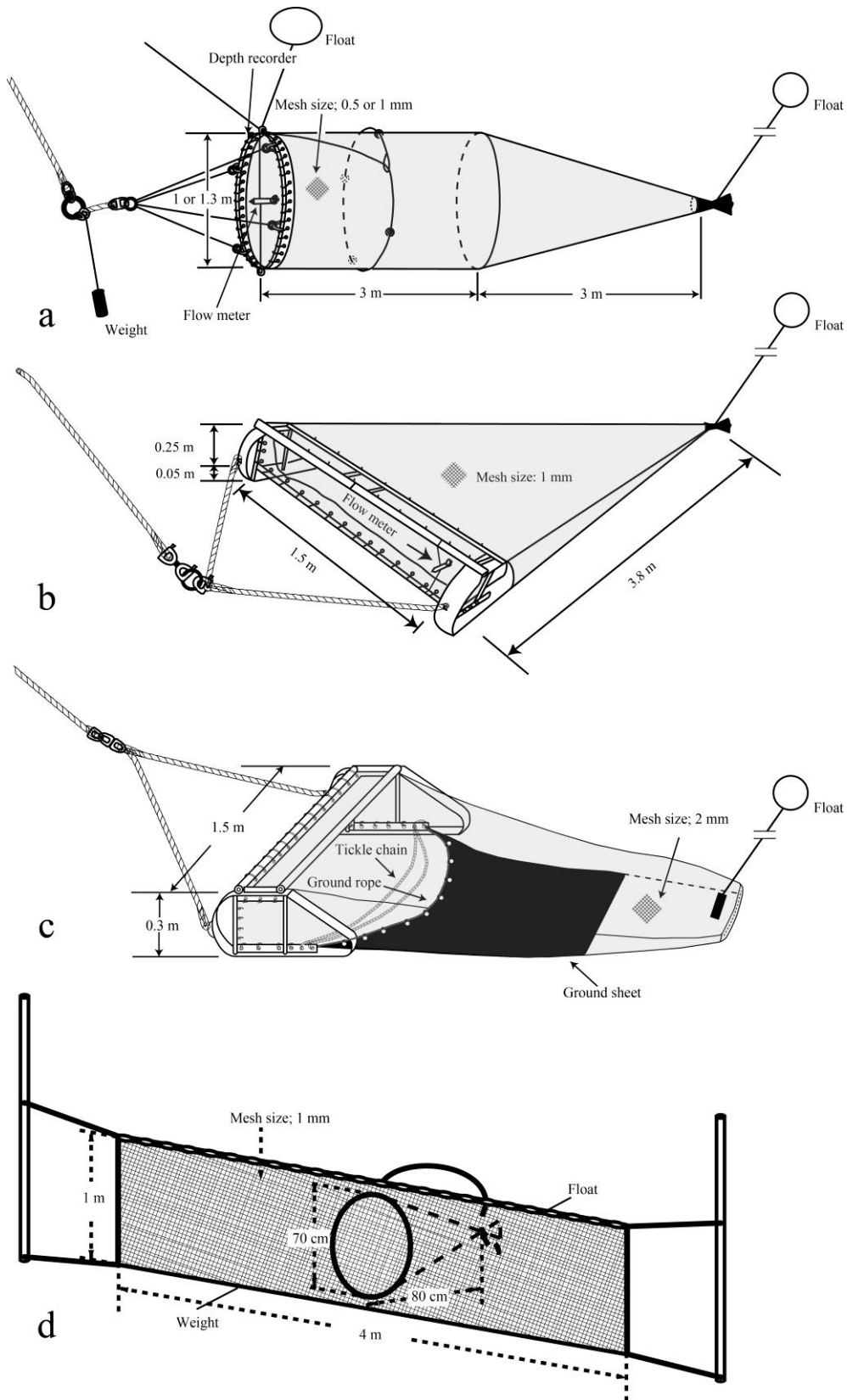
Fig. 2. Four types of gears used in the present study. **a**: system of surface, middle, and oblique tows by larva nets, the discrete-depth horizontal towing method were followed Zhong et al. (2003) to avoid contaminations with other layer samples; **b**: near-bottom net for near-bottom tows; **c**: beam trawl for bottom tows; **d**: seine net.

Table 1. Survey schedule in Ariake Sound in the present study. [1]: spring tide; [2]: only surveyed for water parameters; [3]: tidal collection; [4]: tidal collection from discrete layers.

Year	Date		Station	
			Flood tide	Ebb tide
2006	May	12		8^{*2}, 9, 10^{*2}, 11, 12, 14
		13^{*1}	1, 2^{*3}, 3, 4^{*3}, 5	2^{*3}, 4^{*3}, 6
		14	6^{*4}, 7^{*4}, 8^{*4}	6^{*4}, 7^{*4}
		15	33–36	37, 74
2016	July	19		55–60
		20^{*1}	7^{*4}	7^{*4}
		21	17, 61, 62	7, 9–13, 16
		22	6, 8, 50–52, 54	4, 5, 53
		23	33–38, 73	
		24	0–3	
2019	March	20	17	9–13, 16
		21^{*1}	Miike	42–49
		22	33–36	6–8, 37, 38
		23	50-54	3–5
		24	0-2	
	May	16		9–13, 16, 17, 61, 62
		17	8, Miike	43–49
		18	33, 73	34–38, 6, 7
		19^{*1}	50–52, 54	3–5, 53
		20	0–2	
	July	2		55–60
		3^{*1}	17, 62	16, 61
		14		9–13, 50–54
		15	Miike	6–8, 42–45, 47–49
		16	37, 38	4, 5, 33–36, 73
		17^{*1}	0–3	
	November	11	8–13, 16, 17, 61, 62	
		12^{*1}	33, 34, 73	6, 7, 35–38
		13	1–5	
		14	70^{*3}	70^{*3}
		15	30^{*3}	30^{*3}

Sampling gear: Pelagic eggs and larvae were collected by surface, middle and oblique tows using a larva net with a 1.3-m mouth diameter (Fig. 2a).   The mesh apertures of the nets were 0.5 mm at the sea stations (outside river: Stns. 6–14, 30, 37, 38, 48, 49, 53, 54, 61, 62 and 70 in Fig. 1), and 1.0 mm at river stations (inside river: Stns. 0–5, 33–36, 42–47, 50–52, 73 and 74) to avoid clogging of the net by suspended particles.   Additionally, a larva net with a 1-m mouth diameter (0.5-mm mesh aperture) was towed horizontally at the surface in the retention basin (Stns. 55–60 in Fig. 1) because of shallow depths of ca. 2 m.

Near-bottom larvae were collected by a specialized beam trawl (width, 1.5 m; height, 0.25 m; 1-mm mesh aperture) (Fig. 2b) (Aljamali et al. 2006, Yagi et al. 2009) of the near-bottom layer.

Beam trawl (width, 1.5 m; height, 0.3 m; 2-mm mesh aperture) (Fig. 2c), modified according to the description of Kuipers (1975) for collecting demersal juveniles.

A small seine net (1 × 4 m, 1-mm mesh aperture) (Kinoshita et al. 1988) (Fig. 2d) was also used to collect larvae and juveniles that migrated to the littoral zone at Miike beach.

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 larva net were measured using a divers watch (Log Memory 1473, Casio in 2006) or a depth recorder (Mark5, Alec Electronics in 2016; DEFI-D10, JFE Advantech in 2019) attached to the nets.   The towing distances (m) of the beam trawl and seine net was monitored using a GPS (Colorado 300, Garmin in 2006 and 2016; GPSMAP 64s, Garmin in May 2019).

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

$$N_{LN} = (n \times d \times 100)/(A \times D)$$

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;

$$N_{BT, SN} = (n \times 100)/(w \times D)$$

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

To compare fairly the standing stock in the surface, middle and near-bottom layers in July 2016, n·1000 m^{-3} was calculated with the following formula for the densities of individuals:

$$N_{LN, NBN} = (n \times 1000)/(A \times D)$$

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.

Sample processing: All samples were initially fixed with ca. 10% seawater formalin. Eggs and larvae were immediately sorted and preserved in 10% formalin and 99% ethanol, respectively. Eggs and larvae of were identified by egg chorion; oil globule to egg diameter proportions; the perivitelline space for eggs; the myomere counts and pigmentation for larvae; the anal ray counts for larvae and juveniles. *Coilia nasus* were identified according to Takita (1967) and Simanjuntak (2016); *Ilisha elongata* according to Yabe (1938), Uchida (1958), Mito (1966) and Sha & Ruan (1981); *Konosirus punctatus* according to Nakai (1948), Uchida (1958), Mito (1961) and Kuroda et al. (1983); *Sardinella zunasi* according to Uchida (1958) and Takita (1966).

The developmental stages of eggs, larvae and juveniles were counted 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 (BL) (notochord length for preflexion and flexion larvae, and standard length for postflexion larvae and juveniles).

The early life history of *C. nasus* in Ariake Sound has been well-studied by Simanjuntak (2016), the ontogeny was not reviewed and some data of his study were discussed in this dissertation.

Observation of physical parameter: At each sampling station, temperature (°C) and salinity were measured at 0.5 m intervals from the surface to the bottom using an STD (AST500-P, Alec Electronics), and turbidity was measured at 1 m intervals from the surface to the bottom using a Water Quality Checker (WQC-22A, TOA DDK) in 2006. In 2016 and 2019, the temperature (°C), salinity and turbidity were observed at 0.5 m intervals from the surface to the bottom using a Compact-CTD (ASTD687, JFE Alec in 2016; ASTD102, JFE Advantech in 2019). 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) in July 2016.

Chapter 1. Ontogeny

1.1. Introduction

Recently, *Ilisha elongata* was reassigned to the family Pristigasteridae (subfamily Pelloninae) (Nelson et al. 2016).   *Konosirus punctatus* and *Sardinella zunasi* still belong to the family Clupeidae.   Although once considered a subfamily of the family Clupeidae (Nelson 1967), Nelson (1970) and Grande (1985) elevated it to the rank of superfamily owing to the unique characteristics of these fish.   Di Dario (2002) determined this superfamily to be a basal group of Clupeoidei. There is no doubt that this group is at least independent from Clupeidae, being farthest from Pristigasteridae, as reported by Whitehead (1985).   However, Nakabo (2013) did not adopt the Pristigasteridae still used Clupeidae as the family of *I. elongata*.

In the inner estuaries of Ariake Sound, *Coilia nasus*, *I. elongata*, *K. punctatus* and *S. zunasi* lead their early life history stages there, from spring to summer, with the periods overlapping between the species.   The ontogeny of the *C. nasus* and *S. zunasi* have been studied in Ariake Sound (Takita 1966, 1967, Simanjuntak 2016).   The ontogeny of the *I. elongata* and *K. punctatus* have been studied in other water, but they have not been reported in Ariake Sound (Yoshida 1937, Yabe 1938, Kuwatani et al. 1956, 1958, Uchida 1958, Mito 1966, Sha & Ruan 1981).   These clupeoid fishes have similar larval morphological characteristics, i.e., slender body, small head, straight and long intestine, and scarce pigmentation (Uchida 1963).

In this chapter, to better understand their early life history, we reviewed the ontogeny of *I. elongata*, *K. punctatus* and *S. zunasi*, because the previous works were problematic or insufficient. Furthermore, we try to find egg and larva characteristics, which may indicate that *I. elongata* belongs to the Pristigasteridae or Clupeidae.

1.2. Materials and methods

Fish eggs and larvae were collected in the Ariake Sound in July 2016, March, May, July and November 2019.   A total of 100 eggs of three species were measured their egg diameter and oil globule diameter.   In addition, the yolk diameter of *Konosirus punctatus* and *Sardinella zunasi* were also measured to compare their wideness of perivitelline space.   A total of 100 larvae of three species were measured their eye diameter, body depth, body, pre-pelvic, preanal, head and snout

lengths according to the work of Okiyama (2014).　To ascertain whether there was any difference in prey preference between the three clupeoid fishes, a total of 50 *Ilisha elongata*, 100 *K. punctatus* and 128 *S. zunasi* larvae were randomly selected and their mouth size (Shirota 1970) was measured. The data of mouth sizes were evaluated for heterogeneity of variances using ANCOVA between the three species (significance level of $\alpha = 0.001$).　Statistical analyses were performed in SPSS 19.

All drawings and measurements were made using a camera lucida and ocular micrometer attached to a stereomicroscope.　A representative series of specimens used in this study have been deposited in the Usa Institute of Marine Biology, Kochi University.


1.3. Results

1.3.1. *Ilisha elongata* (Pristigasteridae)

The pelagic eggs are spherical, 1.70–2.20 mm (principally 1.8–2.1 mm) in diameter, with a single, yellowish, relatively large oil globule 0.30–0.44 mm in diameter.　The egg has moderate perivitelline space, a largely segmented yolk, and clear, smooth chorions, which are uniquely double. Pigments are utterly absent during the early stages (Fig. 3a, b), after an embryo tail is freed from the yolk, distinctive scattered melanophores begin to form dorsally on the occipital, shoulder, and oil globule, and ventrally on the separating point of the tail from the yolk (Fig. 3c).

In the larvae (ca. 5 mm) which are still consuming the yolk, the mouth, oil globule, anus and pectoral fin does not open yet, is present at the end of the yolk, opens at ca. 79% BL, and buds, respectively.　Patches of small, scattered melanophores are distributed on the frontal head, dorsal shoulder, ventral abdomen, back oil globule, and ventrally just before the anus.　The eye remains unpigmented (Fig. 3d).

The long, straight gut reaches 77–80% BL during the preflexion and flexion stages, and subsequently, the anus migrates gradually frontward until it reaches ca. 68% BL in ca. 22 mm juveniles (Figs. 3, 4a).　Initially, the head length, snout length and body depth are ca. 15, 3 and 8% BL, respectively, and gradually increase to ca. 24, 6 and 15% BL, respectively, by ca. 22 mm juvenile (Figs. 3, 4b–d).　The eye is elliptical (long/short axes of 1.3–1.7) and subsequently becomes more round (long/short axes of ca. 1.1) with growth until entering the juvenile period (Figs. 3, 4f).
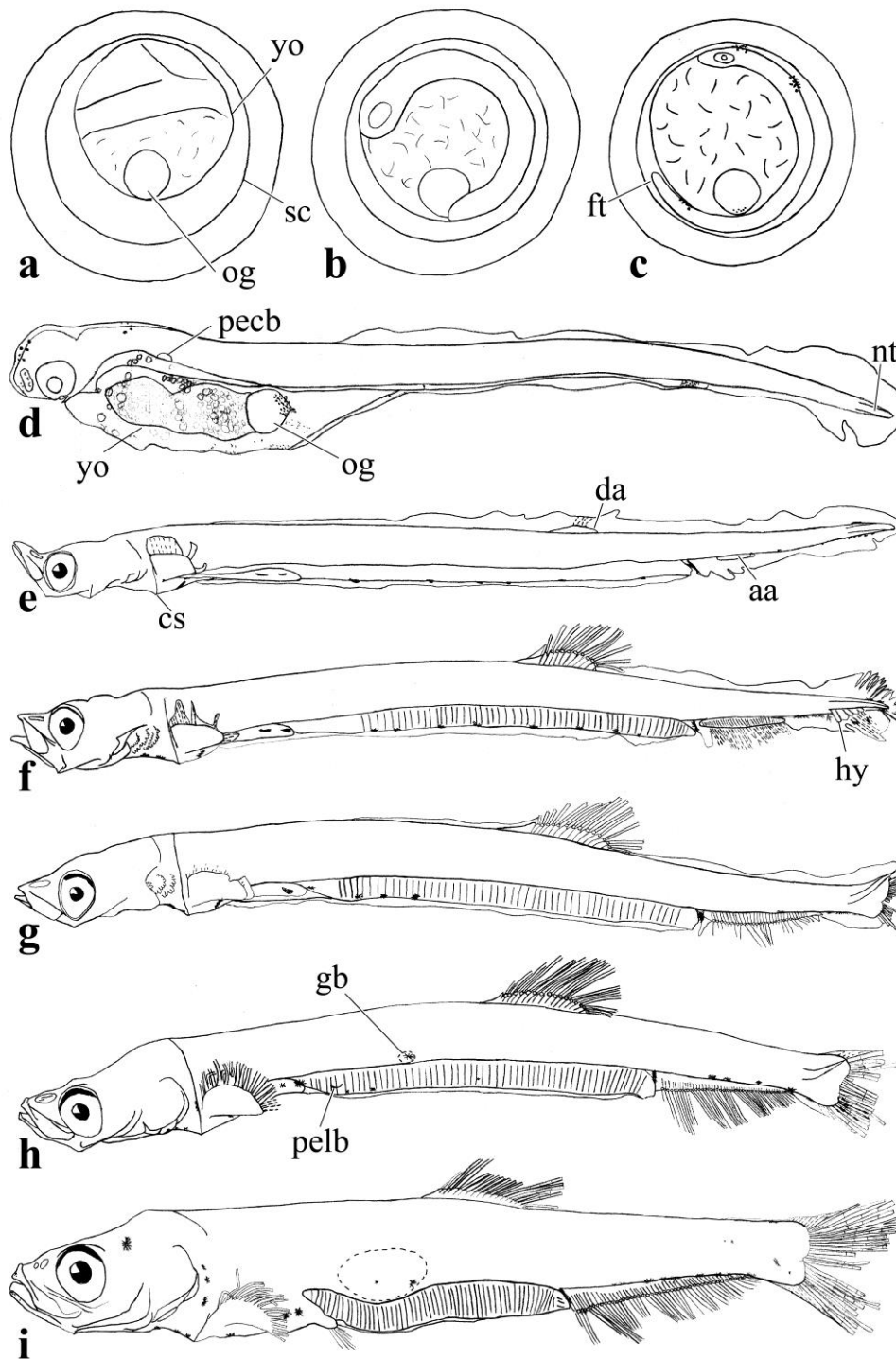
Fig. 3. Developmental stages of *Ilisha elongata* collected in the present study. **a**: A-stage egg, 2.06 mm dia.; **b**: B-stage egg, 2.06 mm dia.; **c**: C-stage egg, 1.92 mm dia.; **d**: 5.1 mm BL yolk-sac larva; **e**: 8.3 mm BL preflexion larva; **f**: 12.2 mm BL flexion larva; **g**: 13.9 mm BL flexion larva; **h**: 17.6 mm BL postflexion larva; **i**: 22.2 mm BL juvenile. *aa*: anal anlagen; *cs*: cleithral symphysis; *da*: dorsal anlagen; *ft*: free tail; *gb*: gas bladder; *hy*: hypural; *og*: oil globule; *pecb*: pectoral bud; *pelb*: pelvic bud; *sc*: secondary chorion; *yo*: yolk. (note: egg drawings are somewhat schematic)
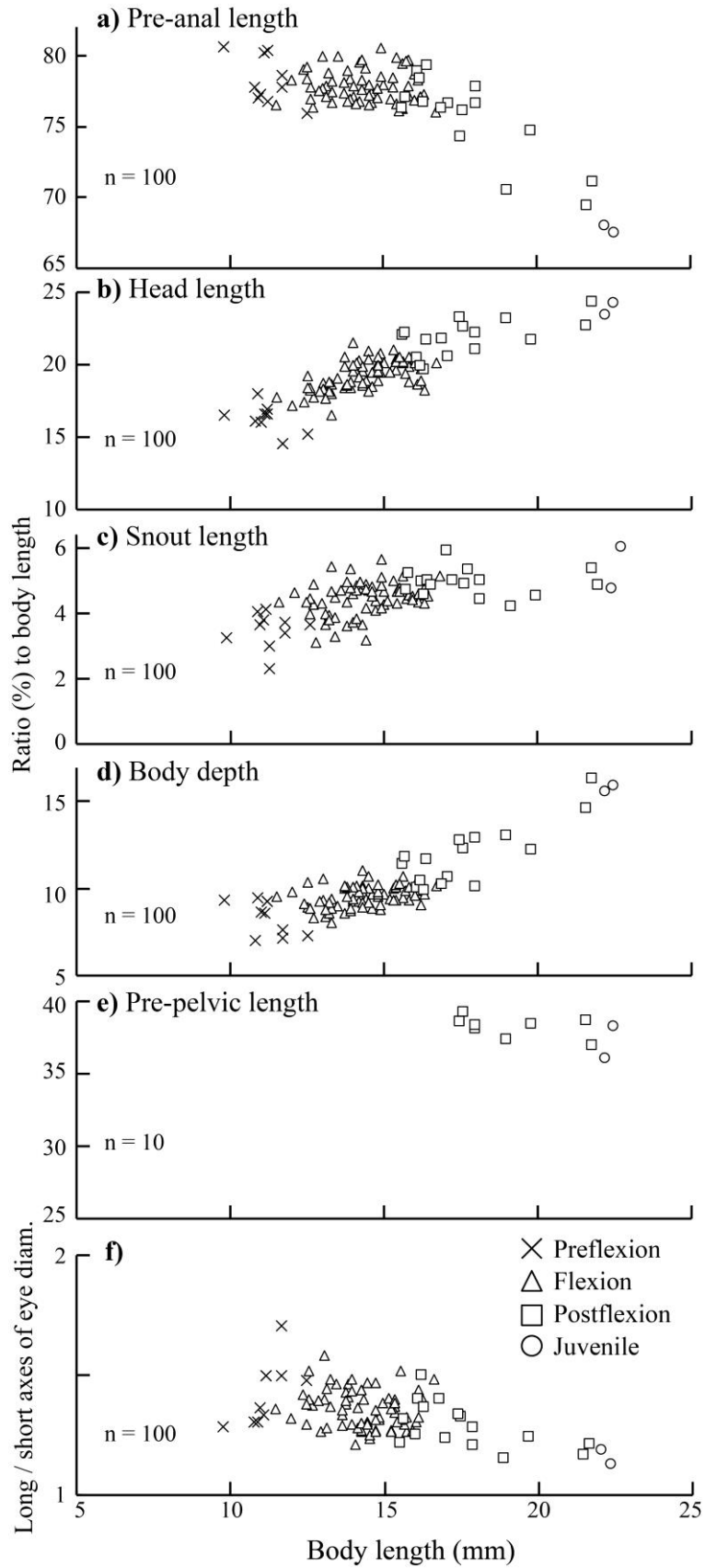
Fig. 4. Ontogenetical morphometries in various parts of *Ilisha elongata* collected in the present study.

The ca. 8 mm larva bears no hypurals, which begin to differentiate at ca. 9 mm. Some hypurals start to push up the notochord tip at ca. 12 mm (Fig. 3e, f). Notochord flexion is almost complete at ca. 14 mm (Fig. 3g). Dorsal and anal anlagen are present at ca. 8 mm, and the number of myomeres between the dorsal termination and anal origin is 10 (Fig. 3e). Dorsal fin rays develop earlier than anal rays with both rays being completely developed by ca. 18 mm larva (Fig. 3e–h). The pelvic bud is present at ca. 39% BL in ca. 17 mm postflexion larva, hardly migrating by ca. 22 mm juvenile with a completed pelvic fin (Figs. 3h, i, 4e).

Externally the visible gas bladder is differentiated with small pigments in ca. 18 mm postflexion larvae, thereafter being considerably expanded in ca. 22 mm juveniles (Fig. 3h, i).

Melanophores are distributed posteriorly to the cleithral symphysis, laterally on the foregut, irregularly along the ventral margin of the hindgut, above the rectum, on the posterior anal anlagen, the ventral margin of the caudal peduncle, the dorsal margin, and the bottom of the undifferentiated hypurals with the notochord tip between, the latter being positioned more along the caudal rays with more developed upper and lower lobes (Fig. 3e). When starting notochord flexion, the melanophores are also distributed anterior to the cleithral symphysis (Fig. 3f). Just prior to flexion completion, melanophores are absent along the hindgut and on the dorsal notochord tip (Fig. 3g). After postflexion, small melanophores appear along the cleithrum, and increase in number during the juvenile period (Fig. 3h, i). Melanophores occur on the frontal and ventral mouth tip, the dentary in two rows, dorsal margin of the caudal peduncle, and internally on the otic and hypural region in juveniles (Fig. 3i).

1.3.2. *Konosirus punctatus* (Clupeidae)

The pelagic eggs are spherical, 1.18–1.50 mm (principally 1.2–1.3 mm) in diameter, with a single, yellowish oil globule 0.10–0.18 mm in diameter. The egg has a wide perivitelline space, a segmented yolk 0.74–0.90 mm in diameter, and a clear, smooth chorion. Pigments are utterly absent during the early stages (Fig. 5a, b), after an embryo tail is freed from the yolk, small melanophores begin to form dorsally on the body (Fig. 5c).

In the larvae (ca. 4 mm) which are still consuming the yolk, the mouth, oil globule, anus and pectoral fin does not open yet, is present at the posterior of the yolk, opens at ca. 86% BL, and buds,

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respectively.　Small melanophores are distributed on the frontal head, dorsally along the body, ventral abdomen, ventrally just before the anus, and the dorsal and ventral margin of notochord tip. The eye remains unpigmented (Fig. 5d).

　　The long, straight gut are ca. 85% BL in ca. 5 mm preflexion larvae, the anus migrates gradually backward until it reaches ca. 88–91% BL during flexion larvae, and subsequently, the anus migrates gradually frontward until it reaches ca. 85% BL in ca. 15 mm postflexion larvae (Figs. 5, 6a). Initially, the head length, snout length and body depth are ca. 12, 2 and 8% BL, respectively, and
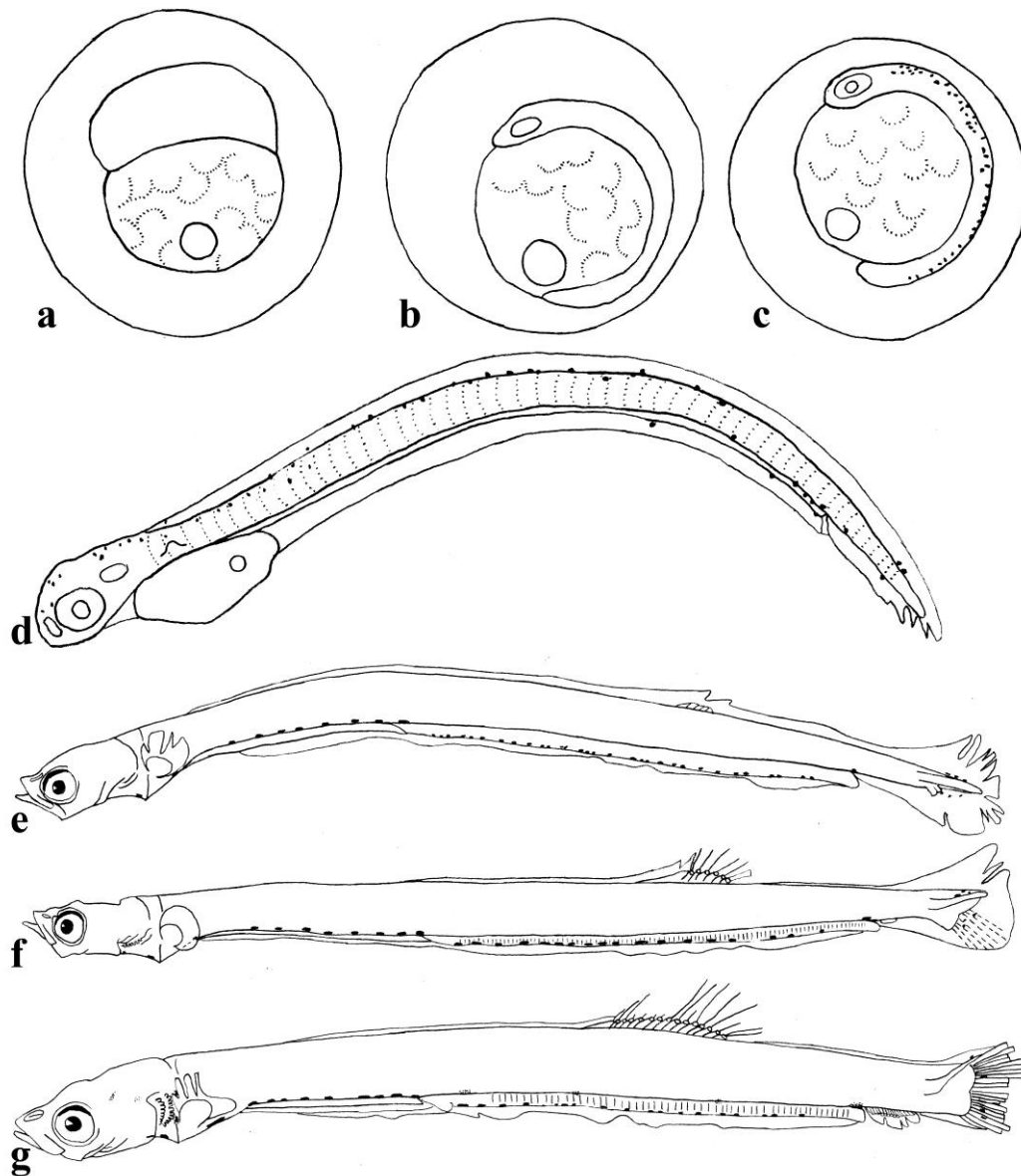
Fig. 5.　Developmental stages of *Konosirus punctatus* collected in the present study.　**a**: A-stage egg, 1.28 mm dia.; **b**: B-stage egg, 1.30 mm dia.; **c**: C-stage egg, 1.22 mm dia.; **d**: 4.2 mm BL yolk-sac larva; **e**: 7.6 mm BL preflexion larva; **f**: 9.9 mm BL flexion larva; **g**: 13.7 mm BL postflexion larva. (note: egg drawings are somewhat schematic)
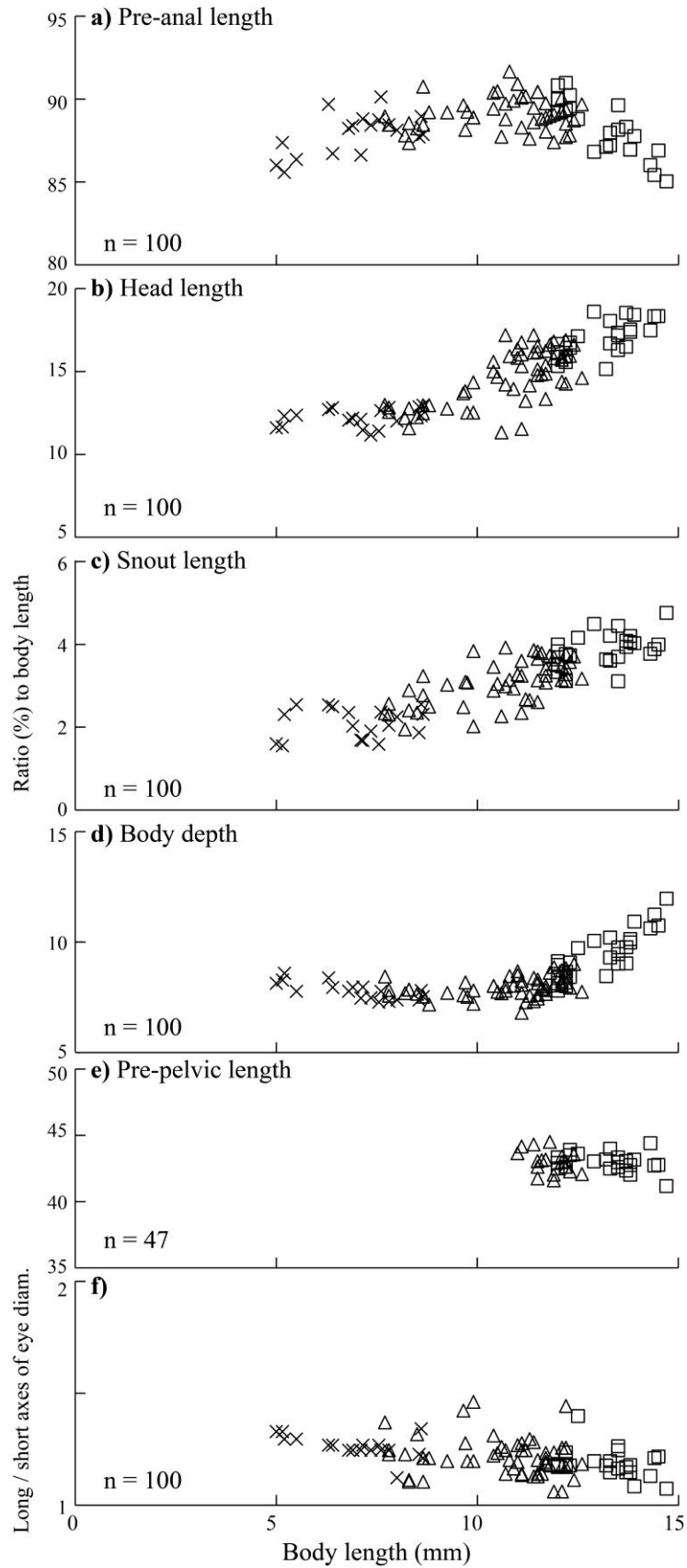
Fig. 6. Ontogenetical morphometries in various parts of *Konosirus punctatus* collected in the present study. Pattern of developmental stages of larvae is the same as in Fig. 4.

begin to increase at ca. 10 mm flexion larvae, increase to ca. 18, 5 and 12% BL, respectively, by ca. 15 mm postflexion larvae (Figs. 5, 6b–d).   The eye is elliptical (long/short axes of 1.2) and subsequently becomes more round (long/short axes of ca. 1.1) with growth by ca. 15 mm postflexion larvae (Figs. 5, 6f).

The ca. 4 mm larva bears no hypurals, which begin to differentiate at ca. 7 mm.   Some hypurals start to push up the notochord tip at ca. 8 mm.   Notochord flexion is almost complete at ca. 12 mm.   Dorsal and anal anlagen are present at ca. 8 mm and 10 mm, respectively.   Dorsal fin rays develop earlier than anal rays with dorsal rays being completely developed, anal rays being incompletely by ca. 14 mm larva (Fig. 5d–g).   The pelvic bud is present at ca. 43% BL in ca. 11 mm flexion larva, hardly migrating by ca. 14 mm postflexion larva with an incomplete pelvic fin (Figs. 5g, 6e).

Melanophores are distributed anteriorly and posteriorly to the cleithral symphysis, dorsally on the foregut, irregularly along the ventral margin of the hindgut, above the rectum, the dorsal margin, and the bottom of the undifferentiated hypurals with the notochord tip between, the latter being positioned more along the caudal rays with more developed upper and lower lobes (Fig. 5e, f). After postflexion, small melanophores appear along the cleithrum, and internally on the otic region and over the hindgut (Fig. 5g).


1.3.3. *Sardinella zunasi* (Clupeidae)

The pelagic eggs are spherical, 1.42–1.76 mm (principally 1.5–1.7 mm) in diameter, with a single, yellowish oil globule 0.08–0.14 mm in diameter.   The egg has a wider perivitelline space than *Konosirus punctatus* (29–44% vs. 52–64% egg diameter), a segmented yolk 0.60–0.76 mm in diameter, and a clear, smooth chorion. Pigments begin to form dorsally on the body at B-stages (Fig. 7a, b).

In the larvae (ca. 4 mm) which are still consuming the yolk, the mouth, anus and pectoral fin opens at ca. 80% BL, and forms, respectively.   Small melanophores are distributed dorsally on the foregut, irregularly along the ventral margin of the hindgut, above the rectum.   The eye has been pigmented (Fig. 7c).

The long, straight gut are ca. 85% BL in ca. 5 mm preflexion larvae, the anus migrates gradually

backward until it reaches ca. 87–90% BL during flexion stages, and subsequently, the anus migrates gradually frontward until it reaches ca. 82% BL in ca. 15 mm postflexion larvae (Figs. 7, 8a). Initially, the head length, snout length and body depth are ca. 12, 2 and 8% BL, respectively, and begin to increase at ca. 10 mm flexion larvae, increase to ca. 18, 5 and 11% BL, respectively, by ca. 15 mm postflexion larvae (Figs. 7, 8b–d).　The eye is elliptical (long/short axes of 1.2–1.4) and subsequently becomes more round (long/short axes of ca. 1.1) with growth by ca. 15 mm postflexion larvae (Figs. 7, 8f).
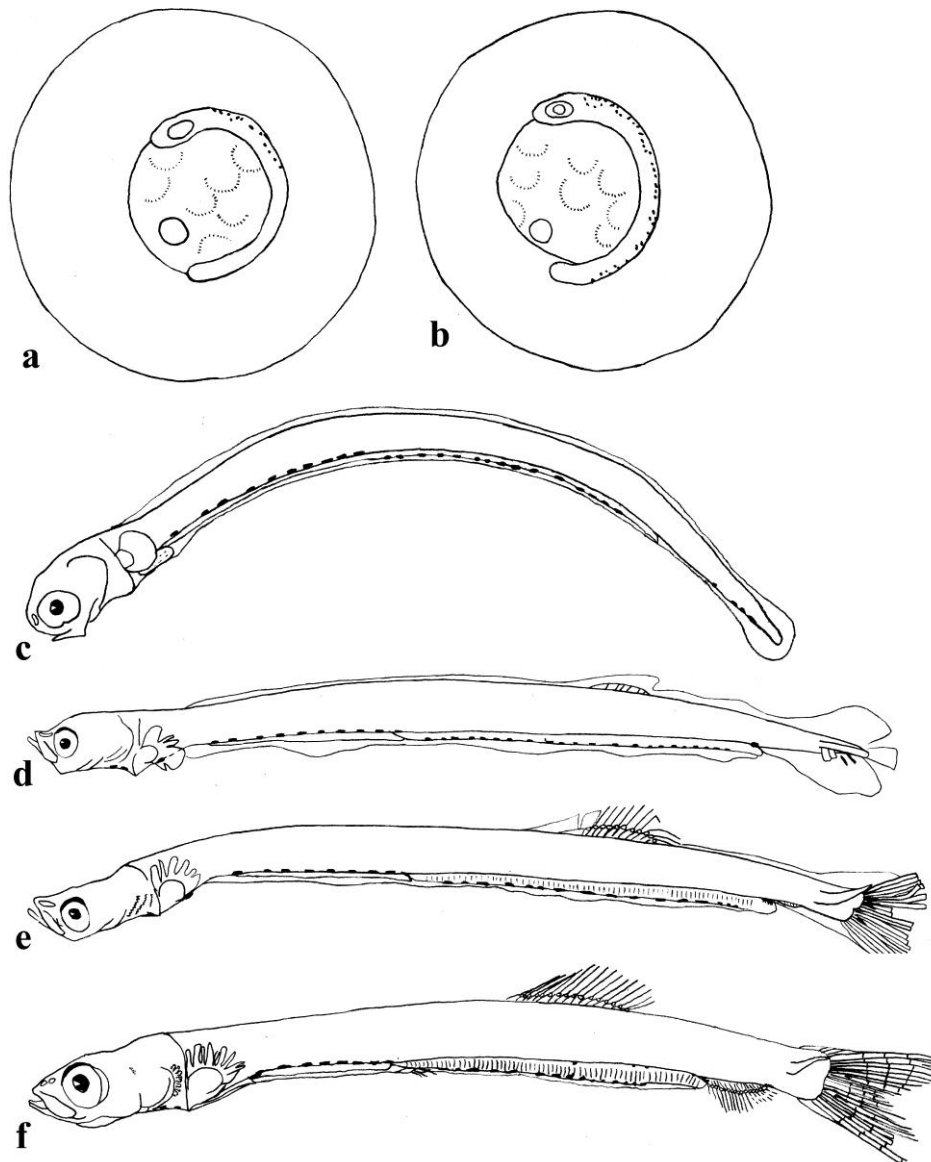

Fig. 7.　Developmental stages of *Sardinella zunasi* collected in the present study.　**a**: B-stage egg, 1.72 mm dia.; **b**: C-stage egg, 1.64 mm dia.; **c**: 3.9 mm BL yolk-sac larva; **d**: 7.5 mm BL preflexion larva; **e**: 10.0 mm BL flexion larva; **f**: 14.3 mm BL postflexion larva. (note: egg drawings are somewhat schematic)
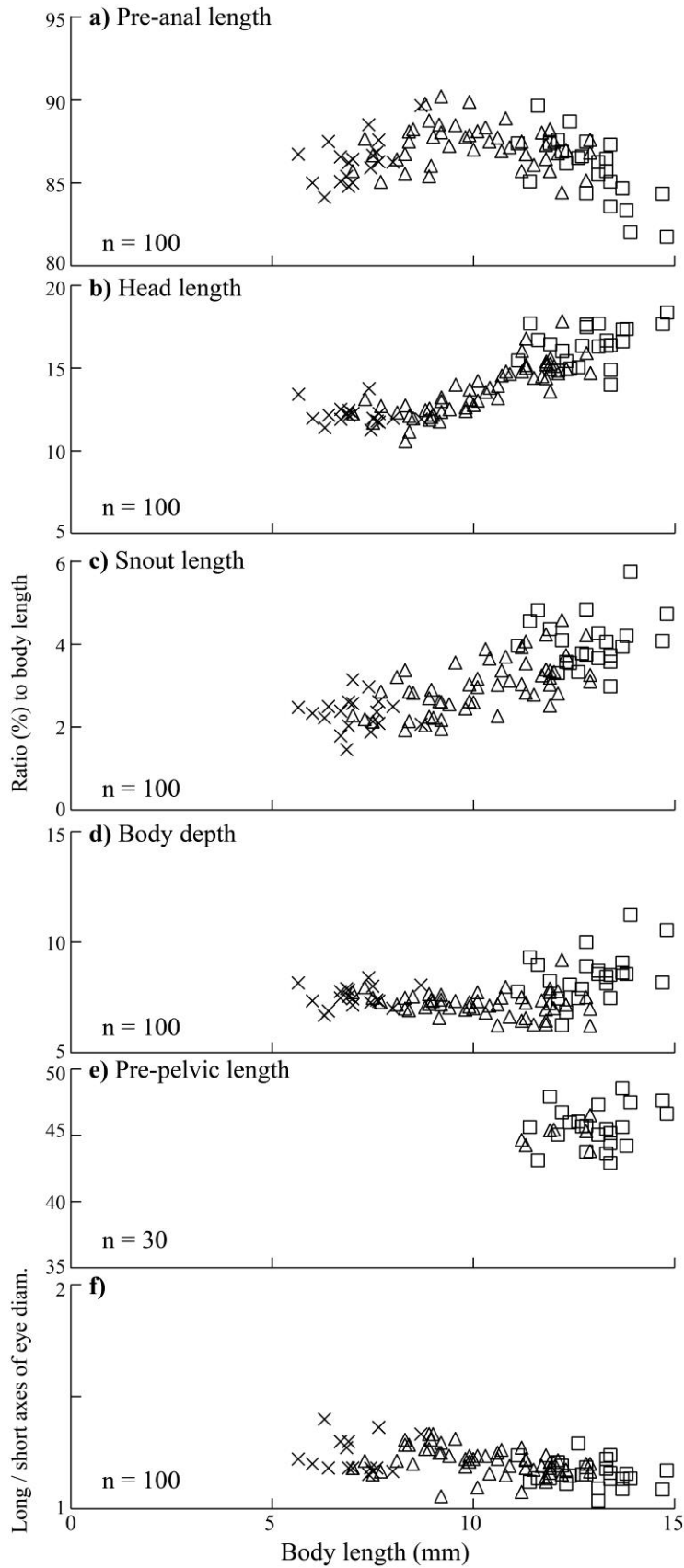
Fig. 8. Ontogenetical morphometries in various parts of *Sardinella zunasi* collected in the present study.　Pattern of developmental stages of larvae is the same as in Fig. 4.

The ca. 4 mm larva bears no hypurals, which begin to differentiate at ca. 7 mm. Some hypurals start to push up the notochord tip at ca. 8 mm. Notochord flexion is almost complete at ca. 12 mm. Dorsal and anal anlagen are present at ca. 7 mm and 10 mm, respectively. Dorsal fin rays develop earlier than anal rays with dorsal rays being completely developed, anal rays being incompletely by ca. 14 mm larva (Fig. 7d–f). The pelvic bud is present at ca. 45% BL in ca. 11 mm flexion larva, hardly migrating by ca. 14 mm postflexion larva with an incomplete pelvic fin (Figs. 7f, 8e).

Melanophores are distributed anteriorly and posteriorly to the cleithral symphysis, dorsally on the foregut, irregularly along the ventral margin of the hindgut, above the rectum, and the bottom of the undifferentiated hypurals with the notochord tip between, the latter being positioned more along the caudal rays with more developed upper and lower lobes (Fig. 7d, e). After postflexion, melanophores appear internally on the otic region, over the hindgut and the ventral margin of the caudal peduncle just posterior to the end of anal fin (Fig. 7f).
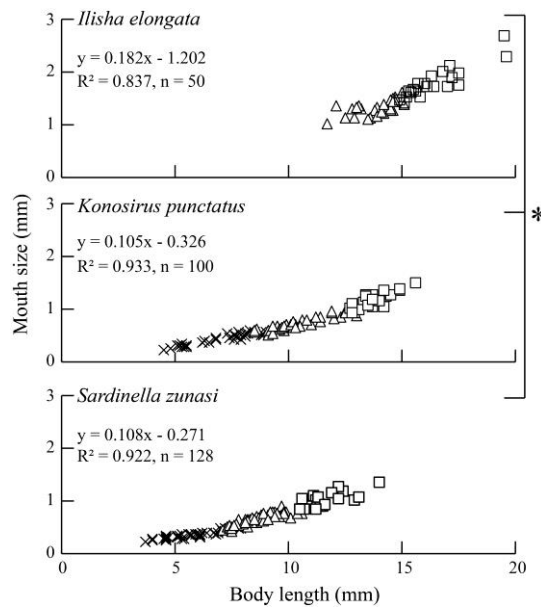
Fig. 9. Comparison of increment of mouth size with growth between *Ilisha elongata*, *Konosirus punctatus* and *Sardinella zunasi*. *Significant at α = 0.001 between different species.

1.3.4. Comparison of mouth sizes

When the mouth sizes of *Ilisha elongata*, *Konosirus punctatus* and *Sardinella zunasi* were compared, significant differentiation could be recognized, but overlapped between *K. punctatus* and *S. zunasi* during preflexion stages (Fig. 9).

1.4. Discussion

Delsman (1930) collected certain eggs from the coasts of the western Java Sea and hatched them, and he provisionally identified these eggs and hatched larvae as *Ilisha elongata* by their characteristics, because it was the commoner *Ilisha* species along the north coast of the western Java Sea.   However, this identification is likely suspect, because more than five species of *Ilisha* are distributed around that district (Carpenter & Niem 1999).   Sha & Ruan (1981) detailed the ontogeny of eggs to juveniles, where the eggs were likely caught in the field, but information regarding whether the subsequent larvae and juveniles were collected from field or reared is missing. Furthermore, regarding the relative positions of the dorsal and anal fins, these two fins in the 14.91 mm postflexion larvae in Plate 2 in Sha & Ruan (1981) are evidently closer together than those in the report by Uchida (1958) and our specimens (Fig. 3).   Accordingly, that specimen is not *I. elongata* but is probably the engraulid *Setipinna tenuifilis* (Valenciennes), as reported by Young et al. (1995).

Compared with the pelagic, oil-globuliferous eggs and larvae of *Konosirus punctatus*, *Sardinella zunasi* (Figs. 5, 7) and various other clupeid fishes (Uchida 1958, Takita 1966, Noichi 2014), *I. elongata* bears two chorions, a much larger oil globule, narrower perivitelline space in their eggs, and a much more anteriorly positioned pelvic bud (fin) and gas bladder (Figs. 3, 4).   These characteristics may indicate that *I. elongata* belongs to the Pristigasteridae and not to Clupeidae.

For identification of the *K. punctatus* and *S. zunasi*, It is useful to use the perivitelline space for eggs; and the pigmentation on the dorsal margin of notochord tip for larvae (Figs. 5, 7).

The larvae of three species bore elliptical (narrow) eyes, which are also found in the larvae of the oxudercids *Odontamblyopus lacepedii* and *Tridentiger barbatus*, which are endemic to Ariake Sound (Tojima 2020), and found especially in mesopelagic myctophid fish (Moser et al. 1984), and clupeids from Lake Tanganyika (Tshibangu & Kinoshita 1995), likely suggesting adaptation to low-light environments, such as turbid waters, the deep sea and deep lakes.   Uchida (1958) made no references to the above significant morphological characteristics.

Assessments of auxotrophic differentiation based on mouth size (Fig. 9) seemed to reveal a possibility of feeding competition for *K. punctatus* and *S. zunasi*, while the feeding preferences likely varies between *I. elongata* and other two species.

Chapter 2. Egg and larva distribution

2.1. Distribution of early stages of *Ilisha elongata*

2.1.1. Introduction

In the western Pacific, *Ilisha elongata* is widespread in the Java Sea, along the northern coast of Australia to Queensland, and from the South and East China Seas to Peter the Great Bay, i.e., from tropical to cooler humid waters (Whitehead 1985, Mohsin & Ambak 1996, Isa et al. 1998, Carpenter & Niem 1999). In Japan, *I. elongata* is present from Okinawa to western Niigata facing the Japan Sea, and to eastern Hokkaido facing the Pacific, principally in Shimabara Bay, Kyushu, and the Seto Inland Sea (Uchida 1958, Whitehead 1985, Yamada et al. 2007, Aonuma & Yagishita 2013).

Accordingly, little is known about the distribution of the egg and larval stages of this species in any region (Yabe 1938, Sha & Ruan 1981, Yagi et al. 2011).

In this part, we examined egg and larval distributions of *I. elongata* in Ariake Sound to better understand its early life history. Furthermore, we assessed its early stages to understand the distribution dynamics.

2.1.2. Materials and methods

Early-stage fish specimens were collected in the Ariake Sound in Shimabara Bay during each spring tide in March, May, July and November 2019 (Table 1). Pelagic eggs and larvae were collected by larva nets at the Stns. 0–13, 30, 33–38, 42–62, 70 and 73 (Fig. 1). Beam trawl conducted at Stns. 0–8, 16, 17, 33–38, 47–54, 61, 62 and 73 for collecting demersal juveniles. A small seine net was also used to collect larvae and juveniles that migrated to the littoral zone at Miike beach during all months except November 2019.

2.1.3. Results

Of four parameters (Fig. 10), only temperatures exhibited seasonal variations, and showed small differences between rivers, estuaries and the open bay. Drastic haloclines were observed between the estuary and the upper reach of the rivers in all seasons, caused by the tidal exchange of water. Salinity in July was relatively lower than that in other months, because the survey was
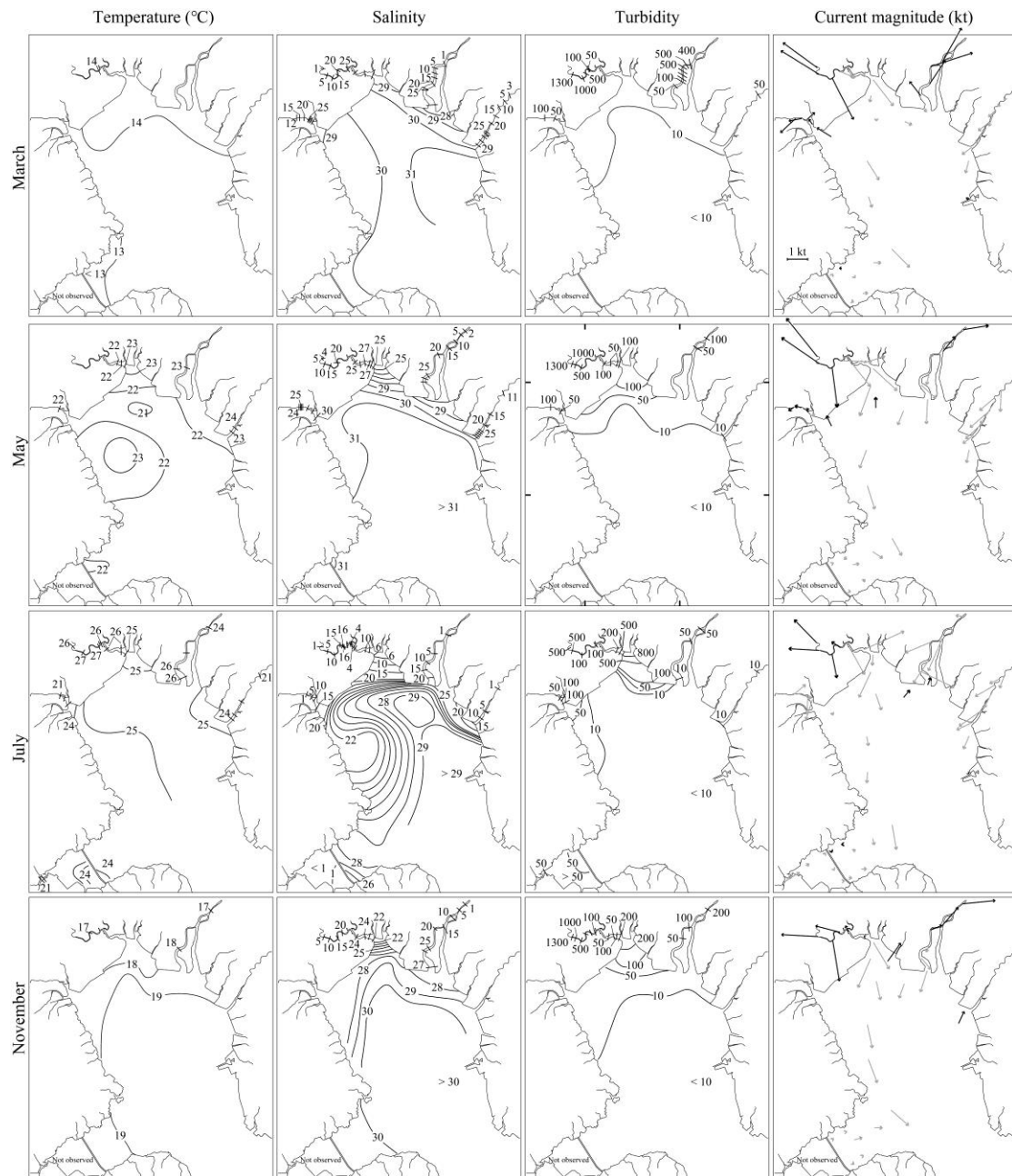
Fig. 10. Horizontal distribution of water temperature, salinity, turbidity (at 0.5 m depth) and current magnitude (at 1 m depth) when fish were collected at each station in March, May, July and November 2019. Solid and shaded arrows indicate flood and ebb tides, respectively.

conducted at the conclusion of the rainy season. While it was notably less turbid in the open bay and Isahaya inlet in all seasons, waters around estuaries were more turbid in the Rokkaku and Hayatsue Rivers than in the Shiota and Yabe Rivers; turbidities were highest in the Rokkaku and lowest in the Yabe Rivers. Currents more than 1.5 kt were frequently measured inside and outside the estuaries during flood or ebb tides, but the velocities were constantly low inside the Isahaya inlet. Miike beach was similar to the open bay with regards to temperature, salinity and turbidity, but

velocity was weaker.

A total of 493 eggs, 416 larvae (4.4–21.8 mm, with a modal size at 14–15 mm), and two juveniles (22.2–22.7 mm) of *Ilisha elongata* were collected by the larva net, and a total of 13 larvae (11.5–19.3 mm, with a modal size at 13–14 mm) and four juveniles (22.5–82.7 mm) were collected by the beam trawl (Fig. 11).   No eggs, larvae or juveniles were present at Miike beach in March, May, July.   Most eggs were collected in May, being utterly absent in March and November (Fig. 12).   November collections only yielded three juveniles (40.9–82.7 mm), one from each of Stns. 5, 7 and 38, by the beam trawl (Fig. 11).   Eggs were typically distributed around estuaries, of which the Shiota River produced the most abundant yield.   Some yolk-sac larvae were found around the Shiota and Rokkaku estuaries in May.   Thereafter, more developed larvae were more widely and abundantly distributed around all the estuaries, with the Hayatsue River having the highest density of larvae.   Compared with eggs, larvae migrated further into the upper reaches of most of the rivers. Although the beam trawl was only able to collect a few larvae and juveniles, which tended to be larger than those collected by the larva net, their spatial distribution was nearly the same as the larvae caught by the larva net.
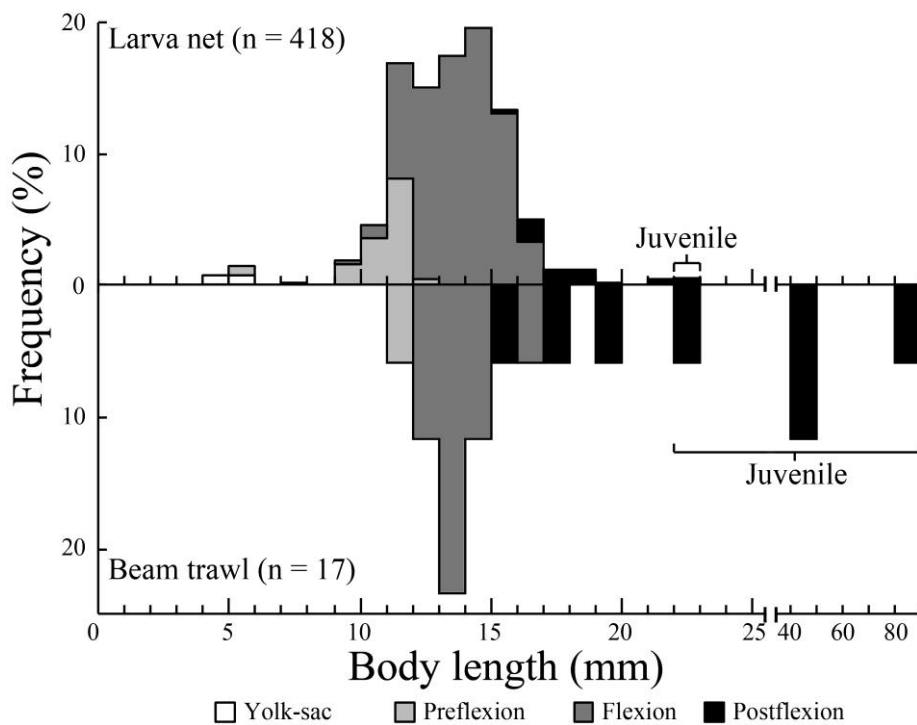
Fig. 11.   Comparison of size and developmental stage frequencies of *Ilisha elongata* between larva net (upper) and beam trawl (lower) collection in Ariake Sound.   Juveniles larger than 40 mm BL were captured in November.
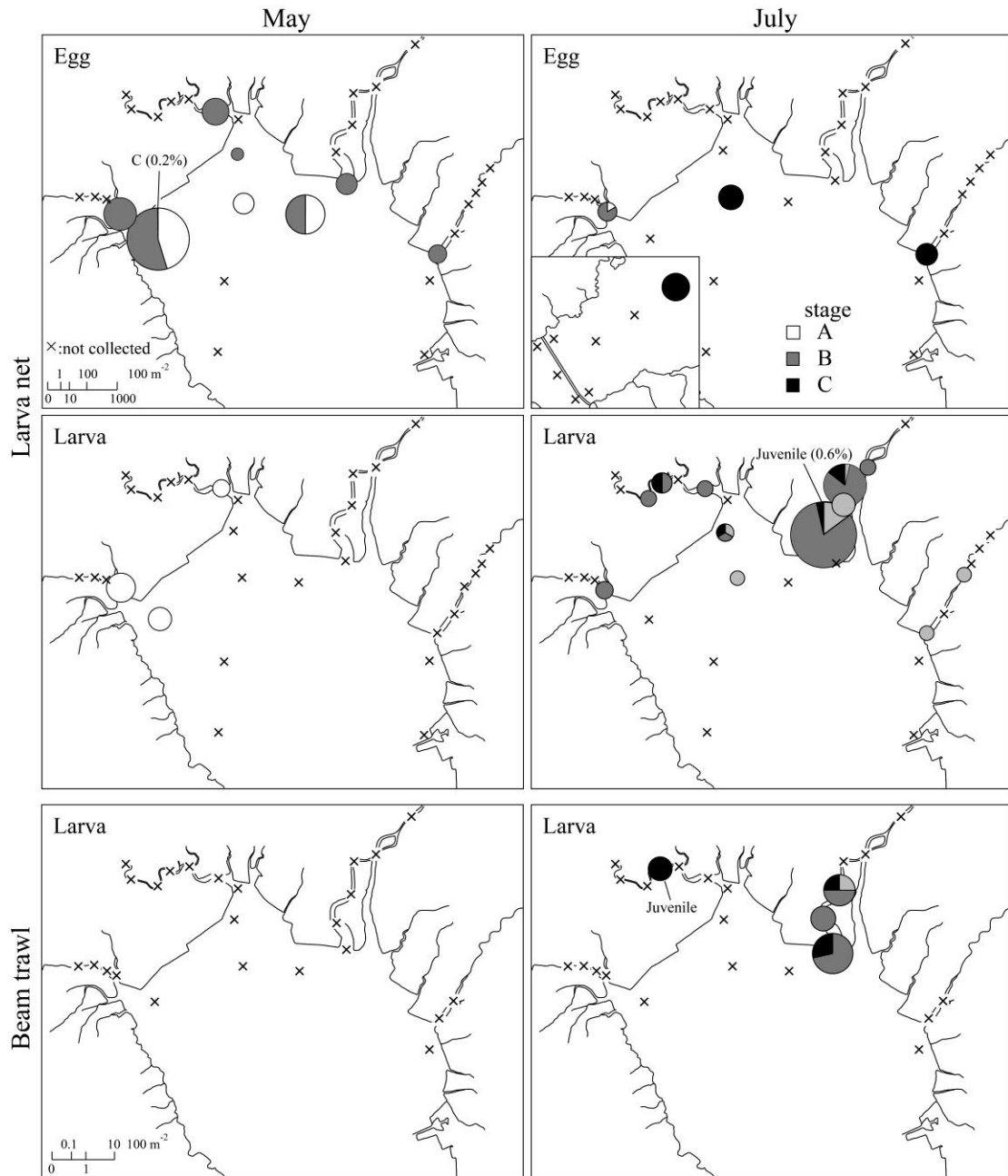
Fig. 12. Horizontal distribution of the early stage of *Ilisha elongata* collected by larva net and beam trawl in Ariake Sound in May and July 2019. The diameter of each circle is drawn in proportion to the square root of its density (n·100 m^{-2}). Pattern of developmental stages of larvae is the same as in Fig.11.

2.2. Comparison of early-stage distribution between *Ilisha elongata* and *Sardinella zunasi* in summer

2.2.1. Introduction

Although there is considerable information about the early life history of *Coilia nasus* (Takita 1967, Ishida & Tsukahara 1972, Yagi 2010, Simanjuntak et al. 2015, Simanjuntak 2016, Ito et al.

2018), *Konosirus punctatus* (Yoshida 1937, Matsushita & Nose 1974, Kawasaki et al. 2006, Yagi 2010) and *Sardinella zunasi* (Takita 1966, Yagi 2010) in Ariake Sound and other Japanese waters, only Yagi (2010) has examined the life history of *Ilisha 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 and trophic differences in their use of the nursery grounds in Shimabara Bay.

2.2.2. Materials and methods

Ilisha 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 larva nets at Stns. 0–13, 16, 17, 33–38, 50–62 and 73 (Fig. 13).   To collect demersal juveniles, beam trawling was performed at Stns. 0–8, 16, 17, 33–38, 50–54, 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, a specialized beam trawl in the near-bottom layer, and a beam trawl on the bottom at Stn. 7 (Figs. 2, 13), 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.

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 larval sizes 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 $\alpha = 0.01$.

2.2.3. 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
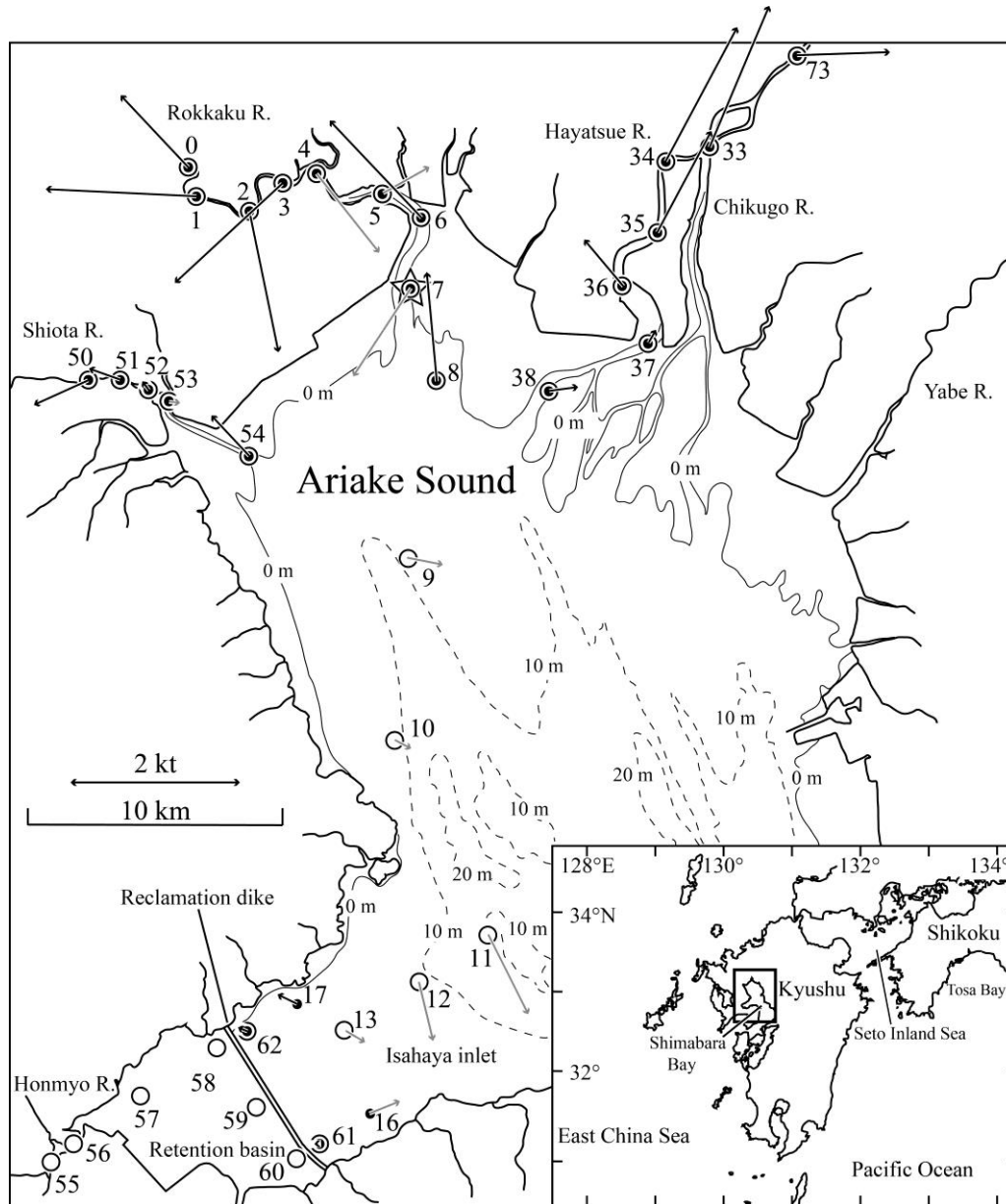
Fig. 13.   Chart showing stations where early stage fishes were collected in Ariake Sound, Shimabara Bay, July 2016.   Sampling of pelagic and demersal specimens was performed using a larva net (open circles, Stns. 0–13, 16–17, 33–38, 50–62, 73), and beam trawling (solid circles, Stns. 0–8, 16–17, 33–38, 61–62, 73), of these river stations: Stns. 0–5, 33–36, 50–52 and 73; estuary stations: Stns. 6–8, 37, 38, 53 and 54; open bay stations: Stns. 9–11.   Tidal sampling by discrete layer tows was performed from flood to ebb tides at Stn. 7 (star).   Solid (flood tide) and shaded (ebb tide) arrows show tidal direction and speed (kt) at a depth of 1 m when specimens were collected at each station.   0 m-lines indicate the level of the lowest spring tide.
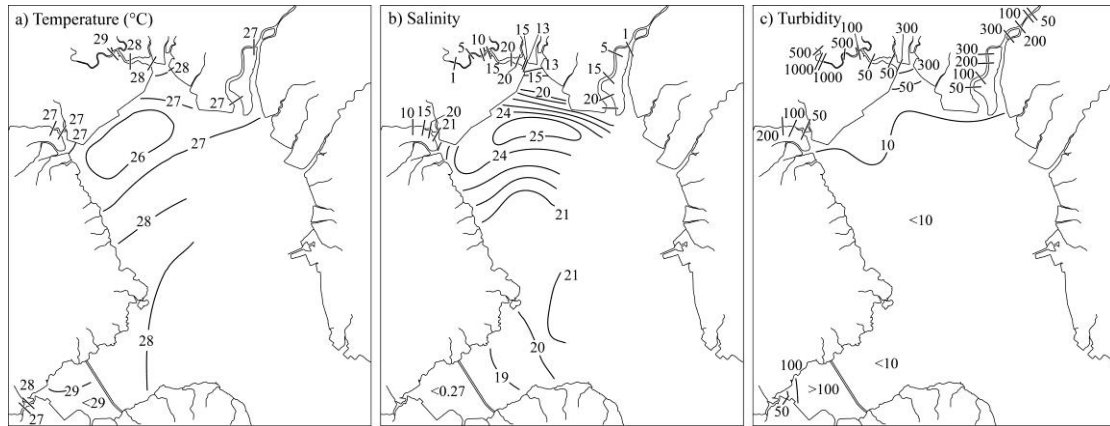
Fig. 14. Horizontal distribution of temperature (°C), salinity and turbidity at each station when fishes were collected in July 2016.
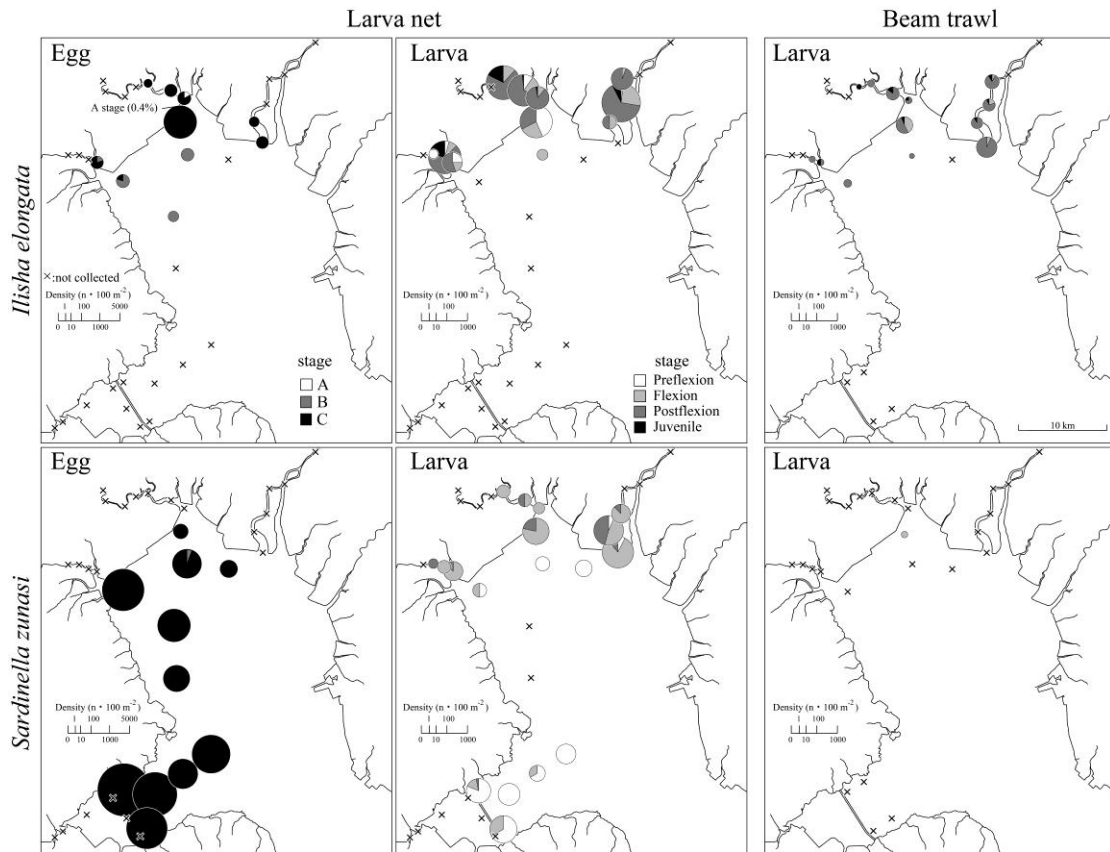
Fig. 15. Horizontal distribution of the early stages of *Ilisha elongata* and *Sardinella zunasi* collected by larva net and beam trawling in Ariake Sound in July 2016. The diameter of each circle is drawn in proportion to the square root of density (n·100 m^{-2}).

temperatures than the other rivers (Fig. 14a). 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. 14b). 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. 14b).   Turbidities were considerably higher near the estuaries than in the open bay and at the Isahaya inlet (Fig. 14c), 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. 13).

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. 15).

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. 15).

Tidal distribution: Dominant tidal currents had a marked effect on the vertical structure of the water column (Fig. 16).   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.   Turbidities 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. 17a).   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 at the flexion stage) and 24 juveniles
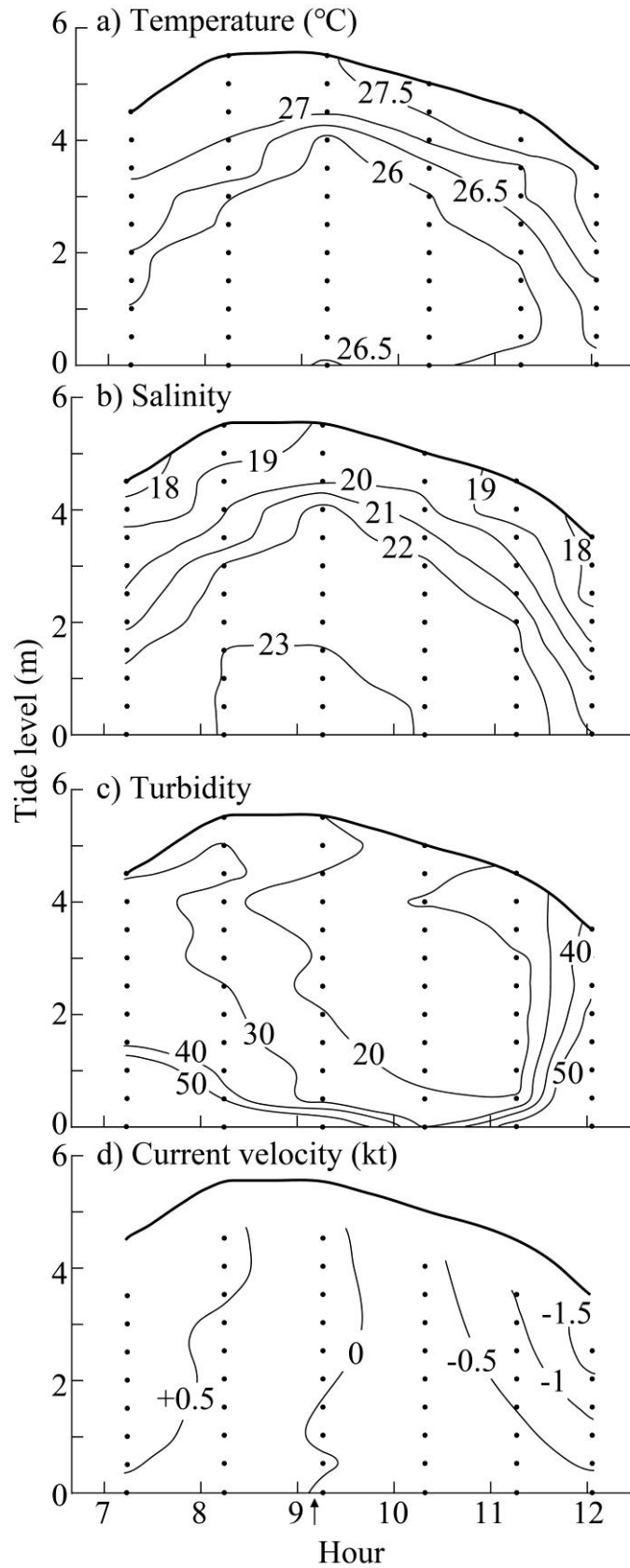
Fig. 16. Vertical profiles of physical parameters at Stn. 7 with the tidal level on 20 July 2016. Flood and ebb tide velocities are denoted as + and –, respectively. The solid arrow indicates the hour at high tide.
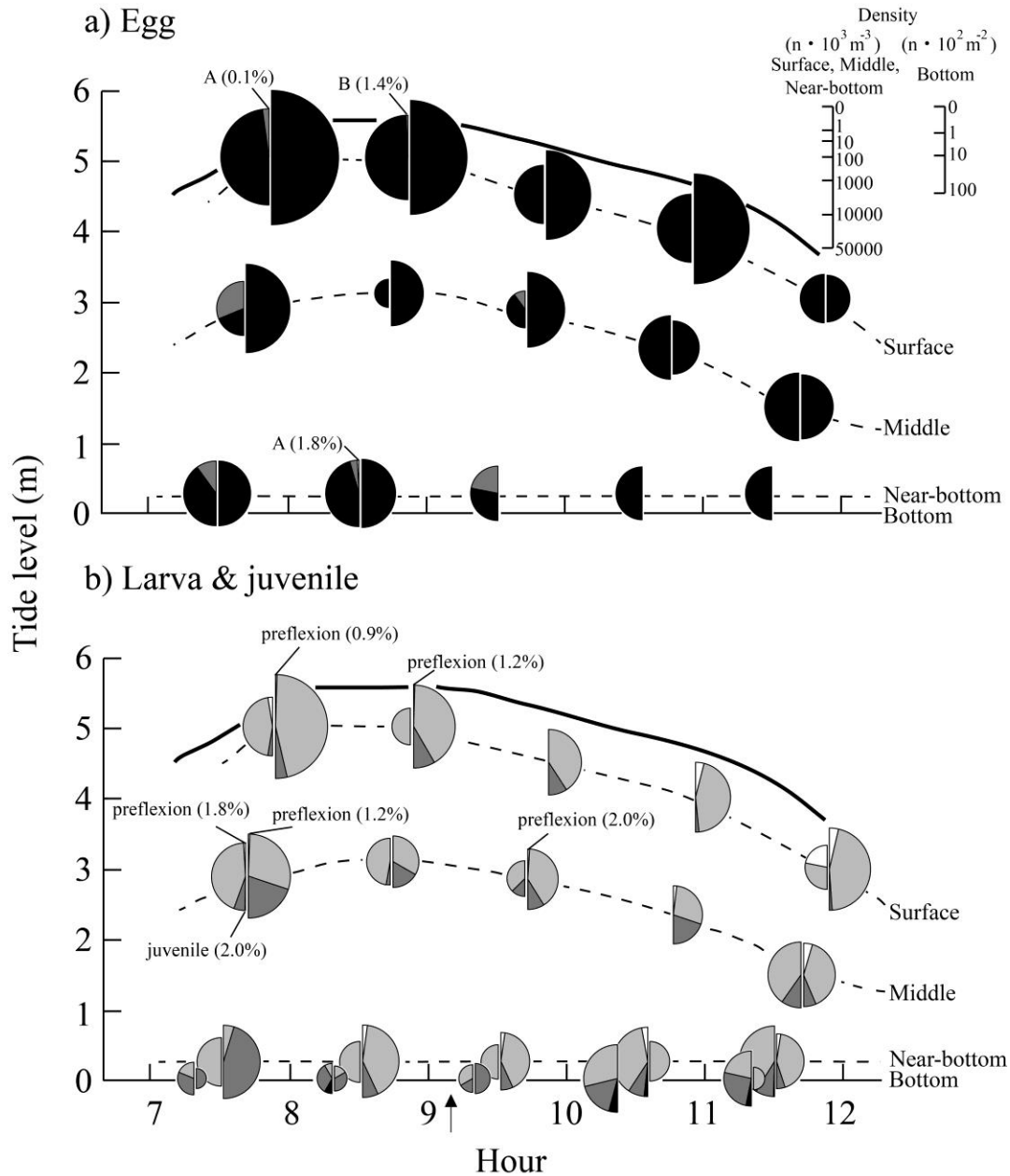
Fig. 17. Comparison of tidal changes and early vertical distribution between *Ilisha elongata* (left half) and *Sardinella zunasi* (right half). The diameter of each half is drawn in proportion to the cube root of density (n·1000 m^{-3}) for the surface, middle and near-bottom layer samples, and the square root of density (n·100 m^{-2}) for the bottom samples. The pattern of developmental stages is the same as that in Fig. 15. The solid arrow indicates the hour at high tide.

(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. 18). 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
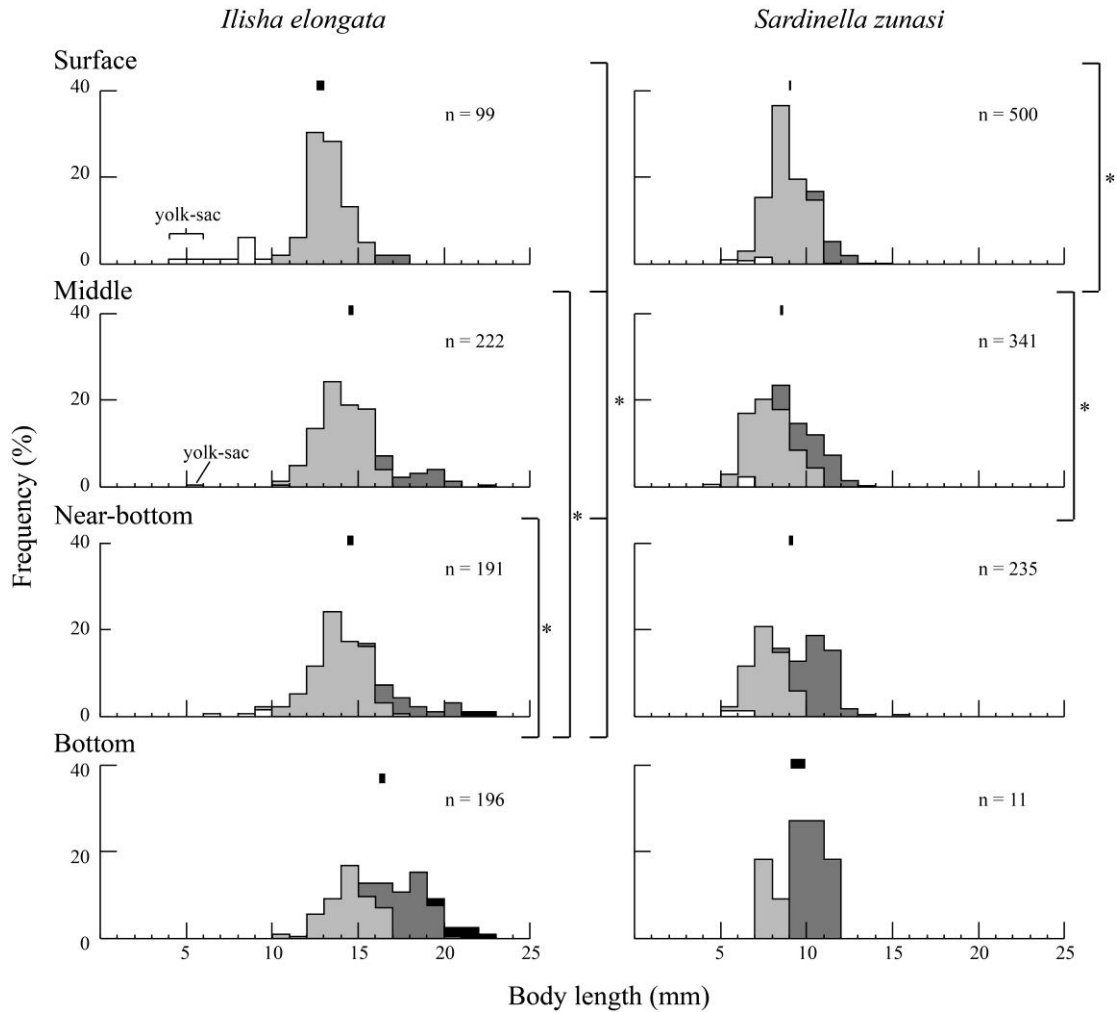
Fig. 18. Comparison of the size and development-stage frequencies for *Ilisha elongata* and *Sardinella zunasi* in different layers. The thick horizontal bar above each histogram denotes mean body length (±SE). The pattern of larval developmental stages is the same as that in Fig. 15. *Significant at α = 0.01 between different layers.

when the ebb flows were highest (Fig. 16d), resulting in larvae being dispersed throughout all layers (Fig. 17b). 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. 17b).

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. 19).   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. 19).
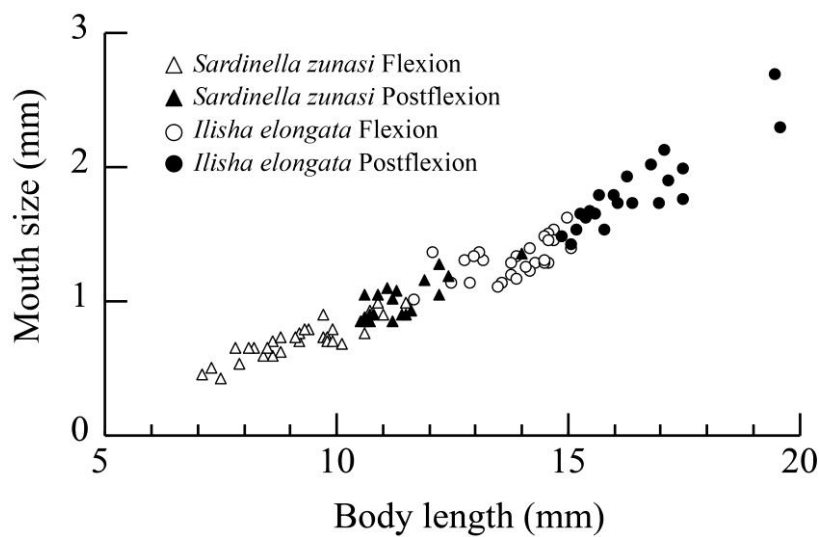
Fig. 19.   Comparison of increment of mouth size with growth and development in *Ilisha elongata* and *Sardinella zunasi* larvae.

2.3. Comparison of early-stage distribution between *Konosirus punctatus* and *Sardinella zunasi* in spring

2.3.1. Introduction

Spawning of clupeoid fishes in the Ariake Sound occurs mainly in spring in *Konosirus punctatus*, in summer in *Coilia nasus* and *Ilisha elongata*, and in both seasons in *Sardinella zunasi* (Takita 1966, 1967, 1978, Wang et al. 2021a, b).   Consequently, in the inner estuaries of the bay,

larvae of *K. punctatus* and *S. zunasi* occur both sympatrically and in abundance in spring, while larvae of *C. nasus*, *I. elongata* and *S. zunasi* occur both sympatrically and in abundance in summer (Yagi 2010, Yagi et al. 2011, Wang et al. 2021a, b).   Due to food isolation and slight differences in distribution, larval niche competition was not considered to be intense between *C. nasus*, *I. elongata* and *S. zunasi* in summer (Wang et al. 2021a, b); however, the extent of larval niche competition between *K. punctatus* and *S. zunasi* in spring has not yet been clarified.

This study therefore compared the horizontal and vertical distribution of *K. punctatus* and *S. zunasi* over tidal cycles to clarify the potential for spatial and trophic differences in their use of nursery grounds in Shimabara Bay.

2.3.2. Materials and methods

Konosirus punctatus 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 May 2006 and May 2019 (Table 1).   Pelagic eggs and larvae were collected by larva nets at Stns. 0–14, 33–38, 43–54, 61, 62, 73 and 74 (Fig. 20).   To collect demersal juveniles, beam trawling was performed at Stns. 0–8, 14, 16, 17, 33–38, 47–54, 61, 62, 73 and 74.   A small seine net was also used to collect larvae and juveniles that immigrated into the littoral zone at Miike beach in May 2019.

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, and a beam trawl on the bottom at Stns. 6–8 (Figs. 2, 20), located outside the mouth of the Rokkaku River.   Two and three sets of the three categorized collections were made at Stns. 6, 8 and 7, respectively, from flood to ebb tides on 14 May 2006.

To ascertain whether there was any difference in size preference of prey between the two fish species, a maximum of 100 specimens of each species, collected from the two species most overlapped stations (Stns. 48 and 54), were randomly selected and their mouth size (Shirota 1970) was measured.   The data of mouth sizes were evaluated for heterogeneity of variances using ANCOVA between *K. punctaus* and *S. zunasi* (significance level of $\alpha = 0.001$).
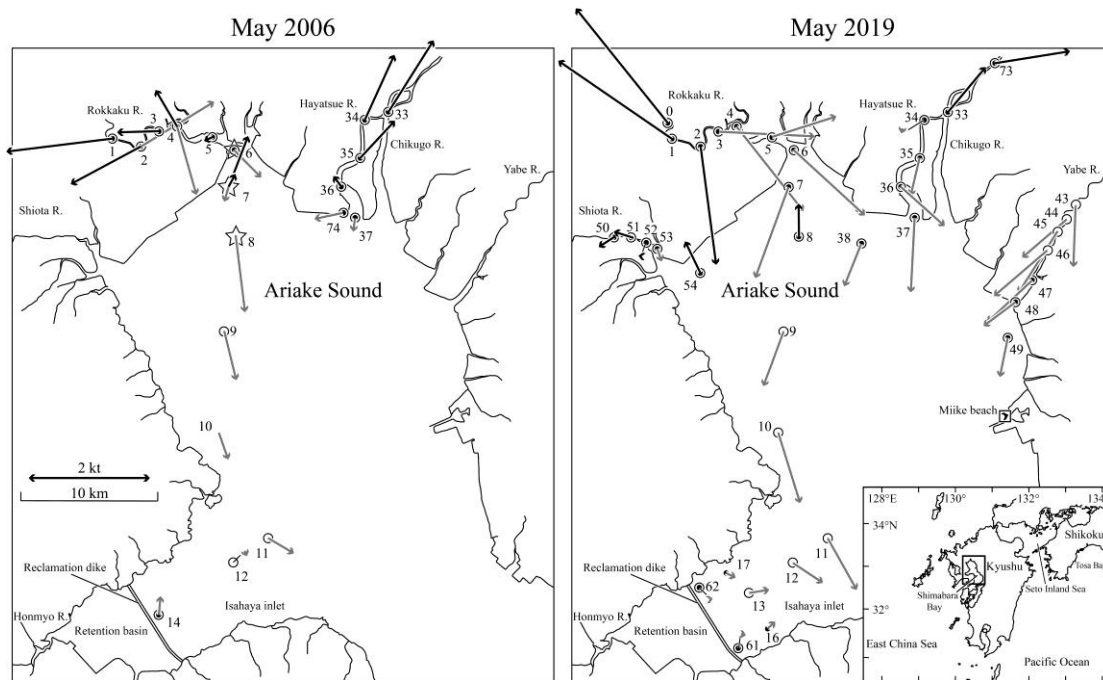
Fig. 20. Chart showing stations where early stage fishes were collected in Ariake Sound, Shimabara Bay, May 2006 and May 2019. Open circles (Stns. 0–14, 33–38, 43–54, 61, 62, 73, 74), solid circles (Stns. 0–8, 14, 16, 17, 33–38, 47–54, 61, 62, 73, 74) and an open square (Miike beach) indicate the stations where collections were made by a larva net for pelagic eggs and larvae, beam trawl for demersal juveniles and seine net for larvae and juveniles immigrating the littoral zone, respectively. Tidal sampling by discrete layer tows was performed from flood to ebb tides at Stns. 6–8 (star) in May 2006. Solid (flood tide) and shaded (ebb tide) arrows show tidal direction and speed (kt) at a depth of 1 m when samples were collected at each station.

2.3.3. Results

Horizontal distribution in the sound: Small differences were observed in the temperatures of the sea water and the river water, although the Rokkaku and Yabe River had slightly higher temperatures than the other rivers in May 2006 and May 2019, respectively (Fig. 21). Marked haloclines were observed between the estuary and the upper river reaches, and these are attributed to the tidal exchange of water. The lower salinity was observed in Isahaya inlet, May 2006 because the southern and northern water gates of the dike intermittently opened from 7 to 13 May and 9 May, respectively (from Nagasaki Station, Kyushu Regional Agricultural Administration Office, 2006, personal communication). While turbidity was low in the open bay and Isahaya inlet, waters around estuaries were very turbid. In May 2006, waters around estuaries were the most and least turbid in the Rokkaku and Hayatsue Rivers, respectively. In May 2019, waters around estuaries
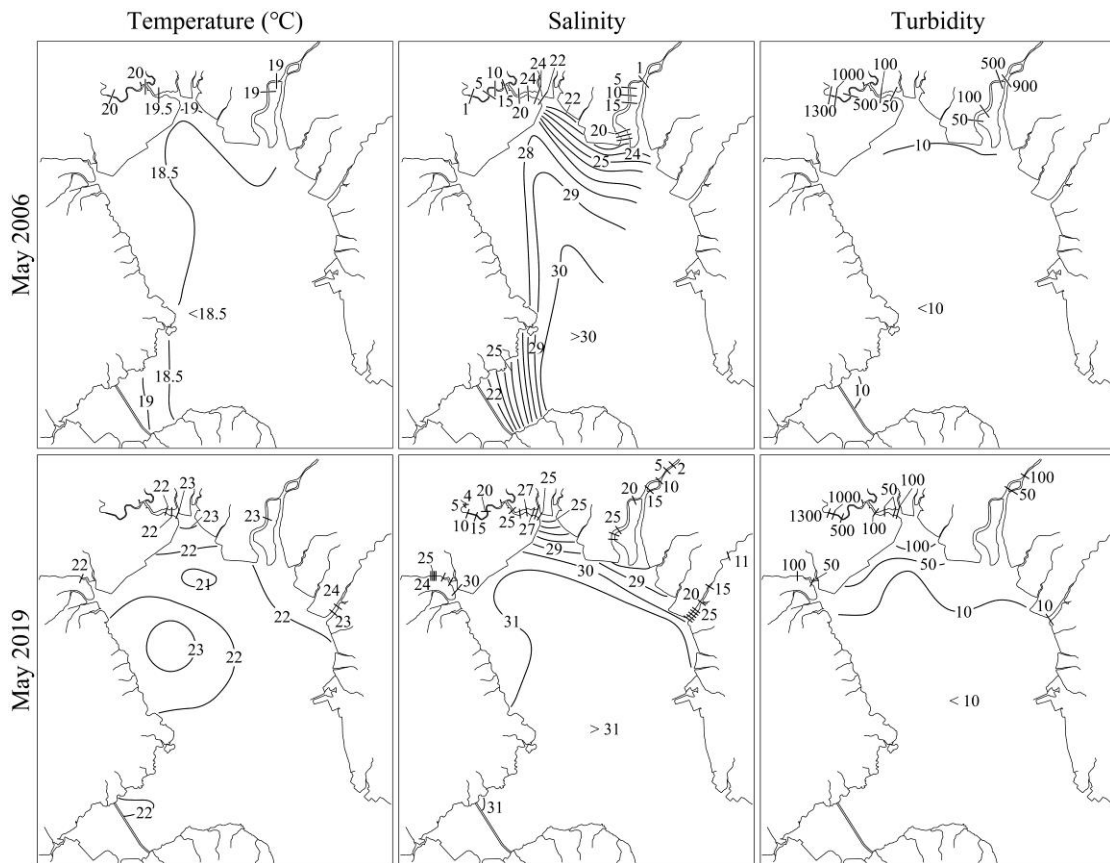
Fig. 21. Horizontal distribution of physical parameters at 0.5 m depth (turbidity measured at 1 m depth in May 2006) at each station when fishes were collected in May 2006 and May 2019, respectively.

were the most and least turbid in the Rokkaku and Yabe Rivers, respectively. Current velocities >1.5 kt were frequently measured in the Rokkaku River, and were remarkably weaker in the Isahaya inlet, Shiota River and Miike beach. In the open bay, Hayatsue and Yabe Rivers, it was observed ranged generally from 1 to 1.5 kt (Fig. 20).

In May 2006, a total of 9,684 *Konosirus punctatus* and 1,955 *Sardinella zunasi* eggs, chiefly at the A-stage of development, were collected from the Isahaya inlet to the inner estuaries, in which the *K. punctatus* than *S. zunasi* eggs were more abundant, and the latter were utterly absent within the Rokkaku and Hayatsue River (Fig. 22).

A total of A total of 1,452 *K. punctatus* larvae (2.7–14.7 mm with a modal size at 10–11 mm) and 99 *S. zunasi* larvae (3.1–8.3 mm with a modal size at 3–5 mm) were collected by the larva net, while the beam trawl captured neither *K. punctatus* nor *S. zunasi* larvae in any stations (Figs. 22, 23). Compared with eggs, *K. punctatus* larvae migrated further into the upper reaches of Hayatsu River. The *S. zunasi* larvae were considerably scantier than *K. punctatus* larvae, being mainly
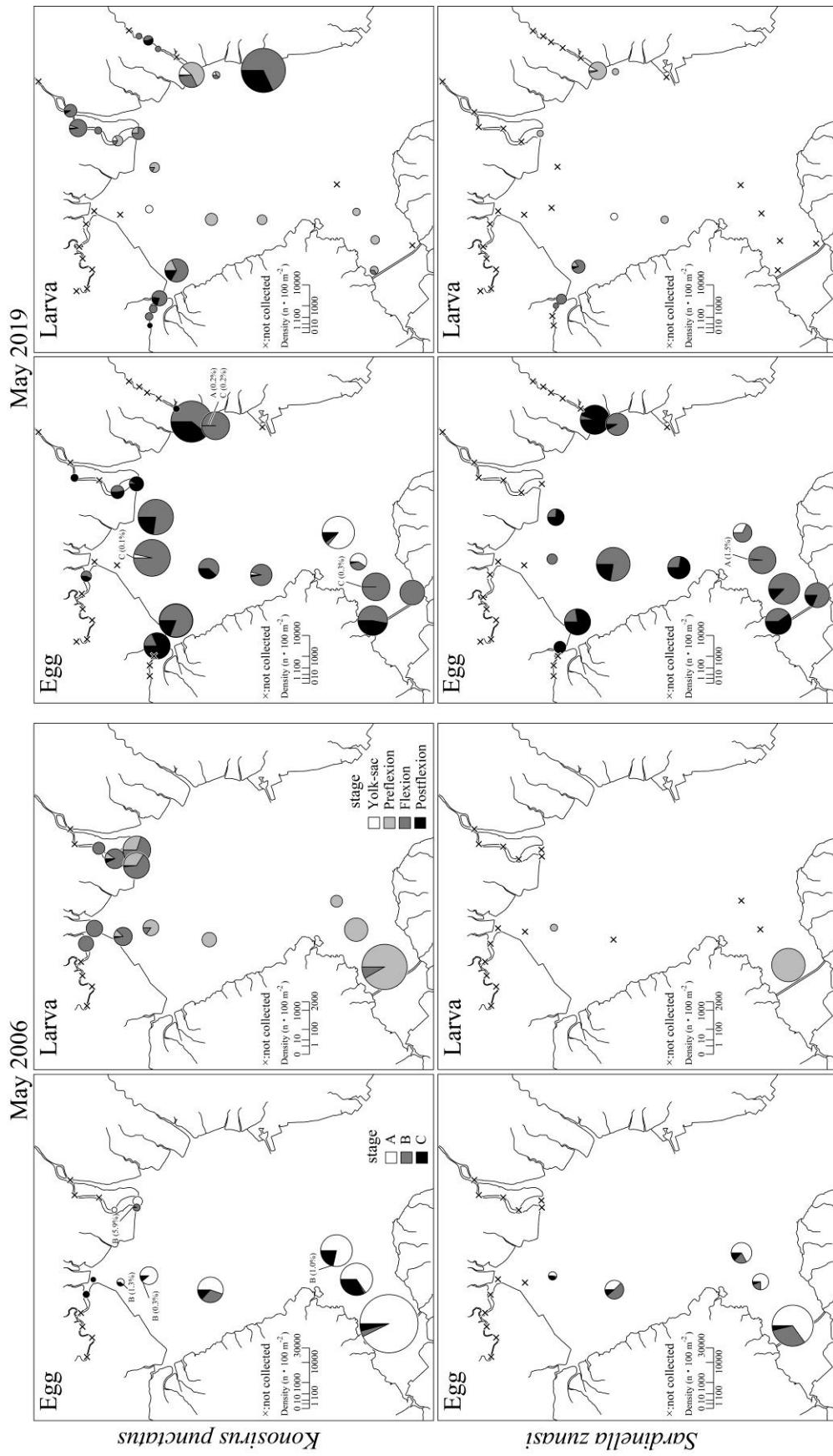
Fig. 22. Horizontal distribution of eggs and larvae of *Konosirus punctatus* and *Sardinella zunasi* collected by larva net and seine net in Ariake Sound, May 2006 and May 2019, respectively. The diameter of each circle is drawn in proportion to the square root of density (n·100 m^{-2}).
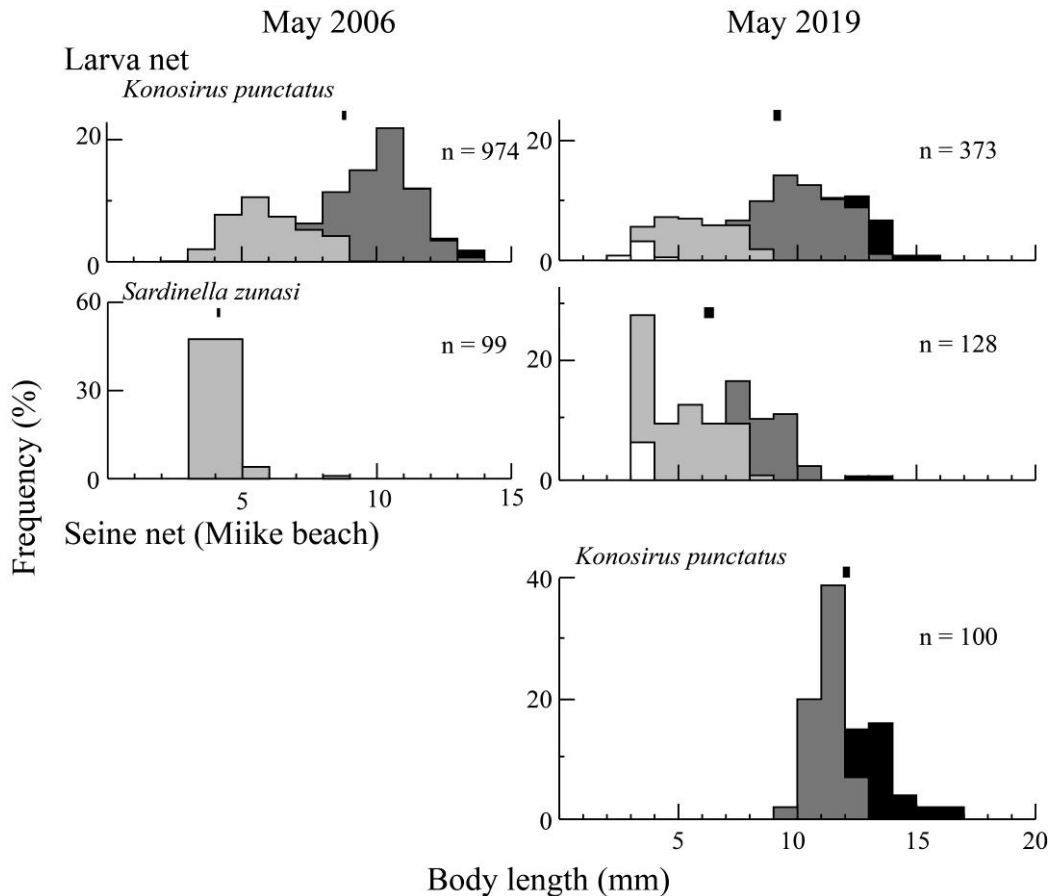
Fig. 23. Comparison of the size and developmental stage frequencies between *Konosirus punctatus* and *Sardinella zunasi* collected in Ariake Sound, May 2016 and May 2019, respectively. Thick bars on each histogram denote mean values (±SE) of body length. The pattern of larval developmental stages is the same as in Fig. 22.

distributed in the Isahaya inlet.

In May 2019, a total of 9,804 *Konosirus punctatus* and 3,780 *Sardinella zunasi* eggs, chiefly at the B and C-stages of development, were collected from the Isahaya inlet to the inner estuaries (Stns.8, 38, 48, 49, 53 and 54), where the *K. punctatus* were more aggregated than the *S. zunasi* eggs. The latter eggs were not observed in the Rokkaku and Hayatsue Rivers. There, however, were dominantly A-stages eggs of *K. punctatus* only near the mouth of Isahaya inlet (Stns. 11 and 12) (Fig. 22).

A total of 817 *K. punctatus* larvae (2.9–15.5 mm with a modal size at 9–10 mm) and 128 *S. zunasi* larvae (3.1–13.1 mm with a modal size at 3–4 mm) were collected using the larva net, but neither *K. punctatus* nor *S. zunasi* larvae were collected at any of the stations by beam trawling (Figs. 22, 23). Compared to eggs, *K. punctatus* larvae were found further upstream in rivers except the

Rokkaku River, being scarce in the Isahaya inlet.   The *S. zunasi* larvae were considerably scantier than *K. punctatus* larvae, being distributed almost in the mouth of the Shiota and Yabe Rivers, and absent in the Isahaya inlet.   A total of 61,282 *K. punctatus* larvae [14,419· haul^{-1} (ca. 50 m distance), mainly at the flexion stage, 9.4–16.4 mm with a modal size at 11–12 mm] were collected in the littoral zone of Miike beach, but no *S. zunasi* larvae occurred at this site (Figs. 22, 23).

Tidal distribution: Dominant tidal currents had a marked effect on the vertical structure of the water column at Stns. 6–8 (Fig. 24).   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.   Turbidities 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 1,363 *K. punctatus* and 42 *S. zunasi* eggs almost at the A-stage of development were collected in the surface and middle layers of Stns. 6–8 and Stn. 8, respectively.   The highest density of *K. punctatus* eggs were found in the Stn. 8 (Fig. 25).   The *K. punctatus* eggs were homogenously dispersed during flood tides, with absence in the surface layer after high tide, while the *S. zunasi* eggs were not collected in the middle layer during flood tide, and distributed vertically at high tide.

A total of 592 *K. punctatus* larvae (2.7–13.9 mm, chiefly at the flexion stage), and only 5 *S. zunasi* larvae (3.2–8.3 mm, all at the preflexion stage) were collected at Stns. 6–8 and Stn. 8,
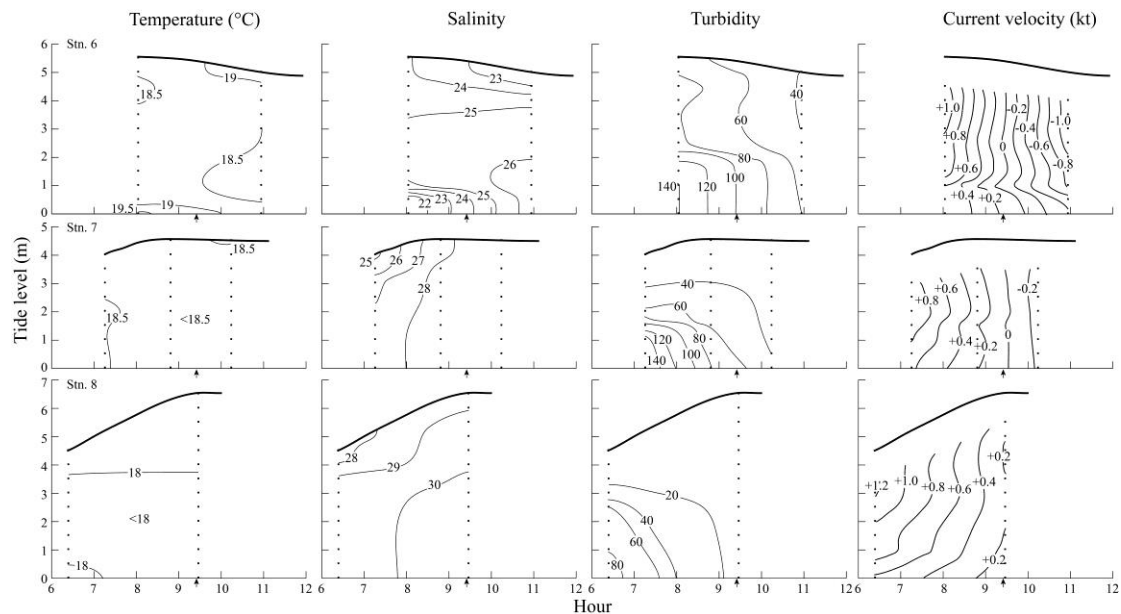
Fig. 24.   Vertical profiles of physical parameters at Stn. 6–8 with the tidal level on 14 May 2006.   Flood and ebb tide velocities are denoted as + and –, respectively.   The solid arrow indicates the hour at high tide.
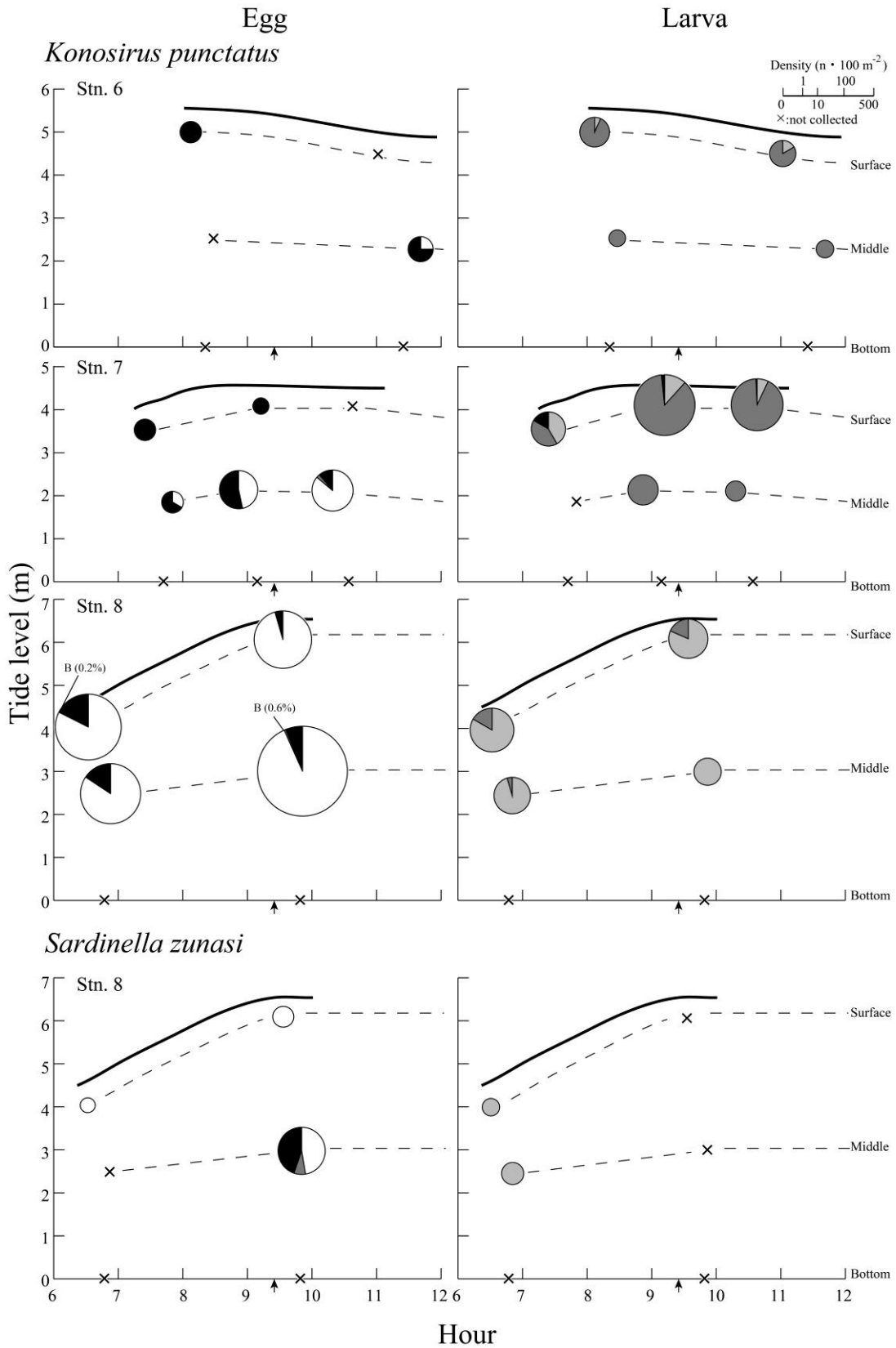
Fig. 25. Comparison of tidal changes and early vertical distribution between *Konosirus punctatus* and *Sardinella zunasi* at Stns. 6–8. The diameter of each circle is drawn in proportion to the square root of density (n·100 m⁻²). The pattern of developmental stages is the same as that in Fig. 22. The solid arrow indicates the hour at high tide.

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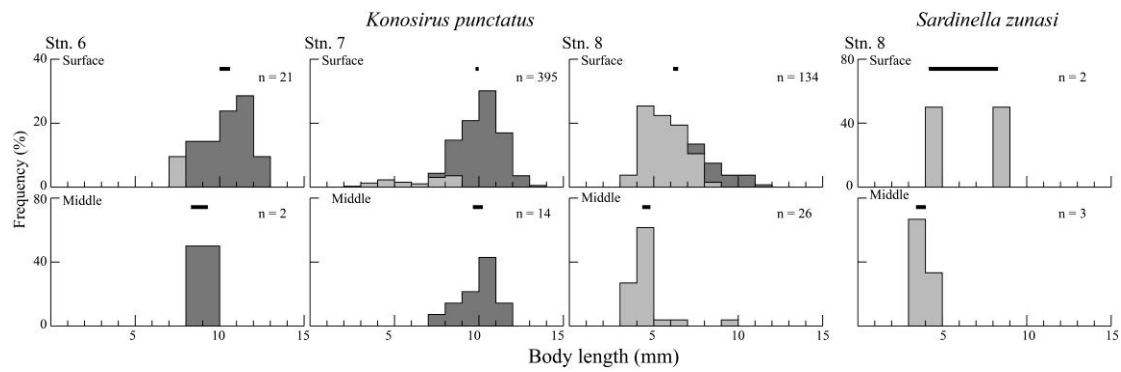
Fig. 26. Comparison of the size and development-stage frequencies for *Konosirus punctatus* and *Sardinella zunasi* in different layers of Stn. 6–8. The thick horizontal bar above each histogram denotes mean body length (±SE). The pattern of larval developmental stages is the same as that in Fig. 22.

respectively (Figs. 25, 26). In *K. punctatus*, the modal size was 11–12 mm at the surface, 8–10 mm in the middle layer of Stn. 6, 10–11 mm on Stn. 7, 4–5 mm on Stn. 8, respectively, implying that the size of larvae decreased with the distance from the river mouth. The *K. punctatus* larvae were more abundant in the surface than middle layer during all tidal phases.

Comparison of mouth size: A comparison of the mouth sizes of the two species revealed a significant difference between larvae at the flexion stage, but no such difference was observed at the preflexion or postflexion stages in both species (Fig. 27).
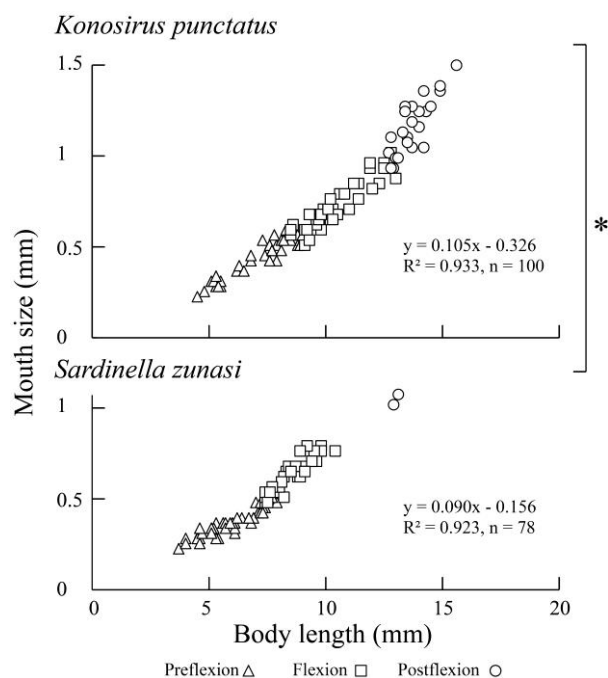
Fig. 27. Comparison of increment of mouth size with growth and development in *Konosirus punctatus* and *Sardinella zunasi*. *Significant at α = 0.001 between the two species.

2.4. Discussion

2.4.1. Distribution of early stages of *Ilisha elongata*

Ilisha elongata eggs and larvae were more abundant in May and July, respectively. Temperatures at which eggs and larvae were collected ranged from 20.8 to 25.5°C (generally ca. 22°C) and from 21.6 to 27.3°C (generally 24–27°C), respectively. The salinities ranged from 13.6 to 31.2 (mainly 29–31) and from 1.2 to 30.9 (mainly 3–16), respectively (Figs. 10, 12). Considering these physical phenomena, it is likely that this fish temporally spawns with the most actively in June, and spatially just off the river mouth. This spawning period is almost in accordance with the results estimated by GSI and histological examinations in Ariake Sound (Zhang et al. 2009). In the Seto Inland Sea, which is another water body with high productivity, *I. elongata* was estimated to spawn from June to August, according to GSI observations (Masui et al. 2016), likely one month later than in Ariake Sound, and this might be attributable to differences in seasonal temperatures between the two locations.

In a western Korean estuary facing the Yellow Sea, *I. elongata* spawned in the upper reaches (salinity: 22–23), ca. 10 km from the river mouth (Yabe 1938). In Ariake Sound, salinities are less than 22 over the 2–8 km upper reaches from all river mouths (Fig. 10). These differences in salinity may be attributable to differences in maximum tidal distances, which is ca. 10 m in western Korea vs. ca. 6 m in Ariake Sound (Inoue 1980, Okada 1987). Thus, it is likely that the Korean spawners migrate to within the river with the greater flood tide.

In Ariake Sound, more larvae (chiefly flexion stage) than eggs were distributed inside most of the rivers (Fig. 12). This suggests that *I. elongata* larvae migrate upstream during development. In comparison with the larva net, the beam trawl barely collected any larvae, but it was able to collect a few large juveniles (Figs. 11, 12). The extremely low numbers of juveniles caught suggests that *I. elongata* do not remain in this habitat after reaching juvenile stage. No larvae or juveniles were found in the littoral zone of a tidal flat (Miike beach) (Fig. 12) and they were absent throughout the year (Hibino et al. 2002). Takita et al. (2003) reported that juveniles and adults were collected more offshore. These facts suggest that *I. elongata* juveniles and adult are distributed in offshore waters, rarely migrating inshore.

The larvae of *Konosirus punctatus* were widespread throughout Ariake Sound, including in the

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open bay area, estuaries and littoral zone of Miike beach, where large numbers of larvae have been found in May and June (Hibino et al. 2002, Yagi et al. 2011), so that Miike beach is likely used as their main nursery ground.   In other waters such as Tosa Bay, Shikoku, *K. punctatus* is the most abundant of the spring larval ichthyofauna along the surf zones of sandy beaches (Senta & Kinoshita 1985, Kinoshita 1993).   Hence, there seems to be very little competition for niches between larval *I. elongata* and *K. punctatus*, with only slight temporal and spatial overlap.   *Coilia nasus* breed not in the Shiota and Yabe Rivers, but in the Rokkaku and Hayatsue Rivers, spawning in the near-freshwater of the upper reaches (Stns. 0, 1 and 73 in Fig. 1) (Takita 1967, Simanjuntak 2016). After hatching, ebb tides disperse the larvae to the lower reaches (Stns. 6 and 7 in Fig. 1). Therefore, *I. elongata* can encounter *C. nasus* during the larval period.   Thus, it is likely that niche competition between these two species occurs in the lower reaches of the rivers.   Takita (1966) and Yagi (2010) reported that *Sardinella zunasi* uses the estuary off river mouths, so that the spawning and nursery grounds of *S. zunasi* and *I. elongata* almost entirely overlap both temporally and spatially.   Differences in larval diet may be necessary to avoid competition between the two species. Further studies on the larval feeding ecology and the vertical distribution of these clupeoid fish are needed to sufficiently understand niche partitioning among these species in Ariake Sound.

2.4.2. Early-stage distribution of *Ilisha elongata* and *Sardinella zunasi* in summer

Larva net tows showed a marked difference in the 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. 15).   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 showed that after the juvenile period, *I. elongata* settles near estuaries, while *S. zunasi* continues a pelagic life.   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. 15) and tidal tows (Fig. 17a) 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 and 36 hr at 23–26°C and 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. 14a, 16a), 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. 2021a).

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. 17b, 18).   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 aggerated at the surface and are dispersed vertically because of the strongly mixed flood tide (Fig. 16d).   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. 17b, 18).   This implies that *S. zunasi* leads a pelagic life that 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. 19) 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, Calanoida), while *S. zunasi* appeared to prey on *Oithona davisae* Ferrari & Orsi, 1984 (Oithonidae, 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.

2.4.3. Early-stage distribution of *Konosirus punctatus* and *Sardinella zunasi* in spring

Difference in the horizontal distribution of two species eggs shows that *Konosirus punctatus* than *Sardinella zunasi* is more euryhaline spawner, because the former eggs were distributed also around waters of salinity< 31 in May 2019 (Figs. 21, 22). The eggs of both species were primarily at B and C-stages of development in May 2019. Previous incubation studies showed that eggs reached the B-stage after 19 and 21 hr at 17–20°C and 15–20°C in *K. punctatus* and *S. zunasi*, respectively (Kuwatani et al. 1956, Takita 1966). Considering that the water temperatures of approximately 22°C in May 2019 (Fig. 21), accumulated temperatures for incubation, seldom occurrence of A-stage, and surveys conducted in the morning suggests that both species spawn in the evening. The presence of A and C-stage eggs at water temperatures of approximately 19°C in May 2006 also support the estimation that spawning occurs in the evening. Furthermore, about 27°C temperature in July 2019, rising 5°C from May 2019, made *S. zunasi* most of eggs develop to C-stage (Wang et al. 2021b), therefore this species is an evening spawner for any seasons in Ariake Sound.

In *S.zunasi*, although there is little differentiation in the horizontal distribution scale of the eggs between May 2006, May 2019 (the present study) and July 2019 (Wang et al. 2021b), the larvae were hardly distributed in the Rokkaku and Hayatsue Rivers in May. In summer of 2005 and 2019, a rather number of *S. zunasi* larvae occurred in both the Rokkaku and Hayatsue estuaries (Yagi et al. 2011, Wang et al. 2021b), but there were no or a few larvae in these two estuaries in May 2006

and May 2019 of the present study.   The reason for this difference is considered to be a seasonal variation.   In case of the Isahaya inlet, there may be a large larval mortality from unknown cause, and also in July 2019, the Isahaya inelt hardly could accelerate to develop *S. zunasi* larvae (Wang et al. 2021b).   Furthermore, the Isahaya inlet was annually yield no more than a few larvae of this species in summer of 2004–2011 (Takeuchi 2012).   Nevertheless, *S. zunasi* larvae were formerly predominately abundant in the Honmyo estuary (Stn. 55 in Fig. 1) in June 1979 (Kinoshita 2007, Takeuchi 2012).   These facts likely suggest that the Isahaya inlet became inappropriate to a nursery ground for *S. zunasi* after building a dike across the inlet blocking the Honmyo River (Fig. 1) in 1997.   However, some *S. zunasi* larvae were collected when the water was brackish in the Isahaya inlet after the water gates of the intermittently opened in May 2006 (Figs. 21, 22).

Compared to the eggs, the *K. punctatus* larvae migrated upstream in Shiota, Hayatsue and Yabe Rivers, becoming more euryhaline.   However, few *K. punctatus* larvae were collected in the Rokkaku River, suggesting that the extremely turbid waters of the Rokkaku River have an adverse effect on larval migration.   In this study, a large number of *K. punctatus* larvae were collected in Miike beach.   This finding was corroborated by Hibino et al. (2002) who demonstrated that *K. punctatus* larvae were the most abundant fish larvae in Miike beach.   In other areas, such as Tosa Bay in Shikoku, *K. punctatus* has been reported to be one the most abundant along surf zones of sandy beaches in spring (Kinoshita 1993, Fujita 2005).   These findings imply that *K. punctatus* larvae use shallow coastal areas such as Miike beach as nursery grounds, rather than inner estuaries in the Ariake Sound.   Indeed, Kinoshita's (2007) and Takeuchi's (2012) data of 1979–1980 showed that the *K. punctatus* larvae were relatively uncommon in the Honmyo estuary, which has waters as turbid as those of Rokkaku estuary (data not shown).   This phenomenon was observed in 2004–2009 (Yagi et al. 2011, Takeuchi 2012) until the present, indicating a nature of *K. punctatus* oneself.

According to the vertical distribution of *K. punctatus*, the larvae were mainly aggregated in the surface, while the *S. zunasi* were vertical distributed throughout most layers during all tidal phases (Wang et al. 2021b).   Thus, while there may be niche competition in the surface layers between the two species.

Although the main larval character differed between *K. punctatus* and *S. zunasi*, these two species were sympatrically distributed during the flexion and preflexion stages in the Yabe and

Shiota estuaries, respectively (Fig. 22). Assessments of auxotrophic differentiation based on mouth size (Fig. 27) seemed to reveal a possibility of feeding competition and preference for flexion and preflexion larval periods in some estuaries, respectively. Wang et al. (2021b) found that the spatial distribution of *S. zunasi* and *Ilisha elongata* larvae was horizontally concordant, but that their vertical distributions varied during the ebb tide in summer in the Ariake Sound. At the time, the authors proposed that this difference in the vertical behavior of both species was to avoid competition. Future studies should examine the feeding habits of *S. zunasi* and *K. punctatus* in Ariake Bay and clarify how interactions between these two species in different layers are affected by tidal cycles.

Chapter 3. Spawning and growth

3.1. Introduction

The ring increments in the otolith are widely used for age determination and to estimate the growth patterns of fishes during early life stages (Takahashi et al. 2014).   For clupeoid fishes, estimates of spawning date and growth patterns of larvae were based on an examination of microstructural increments on the sagittal otolith (Djumanto 2005, Simanjuntak 2016).   The otoliths of *Ilisha elongata*, *Konosirus punctatus* and *Sardinella zunasi* were examined to better understanding the spawning period and growth rates of three clupeoid larvae in Ariake Sound. Although the information of daily periodicity of ring formation in these three species is limited, it was concluded that otolith increments of other clupeoids were formed daily (Methot 1981, Lough et al, 1982, Tsuji & Aoyama 1984, Hayashi et al. 1989, Hayashi & Kawaguchi 1994).

3.2. Materials and methods

A maximum of 30 specimens of *Ilisha elongata*, *Konosirus punctatus* and *Sardinella zunasi* from the Miike beach, Isahaya inlet and each river in May and July 2019, respectively, was randomly selected for age estimation.   Although there were scarce *I. elongata* and some *S. zunai* larvae occurring in May 2019, the specimens were too small and/or initially preserved in 10% formalin solution, the otoliths probably dissolved.   Only one *S. zunasi* larva collected in May was used. The left and right side of sagitta (Fig. 28) were removed from the specimens under a dissecting microscope and fixed on a microscope slide face up with epoxy resin and covered by cover glass. For each otolith, increments outside the nucleus were counted three times under a light microscope at 400–600x magnification.   The mean ring count was used for age (days).   The hatching dates of each specimens were estimated from the age and back calculating from the collection date.

The relationships between body size and age of each species in various areas were examined with regression analysis.   The growth of three species and each species in various areas were analyzed using ANCOVA to determine whether growth rate (slope) differed.   The significance level for all tests were 0.001.
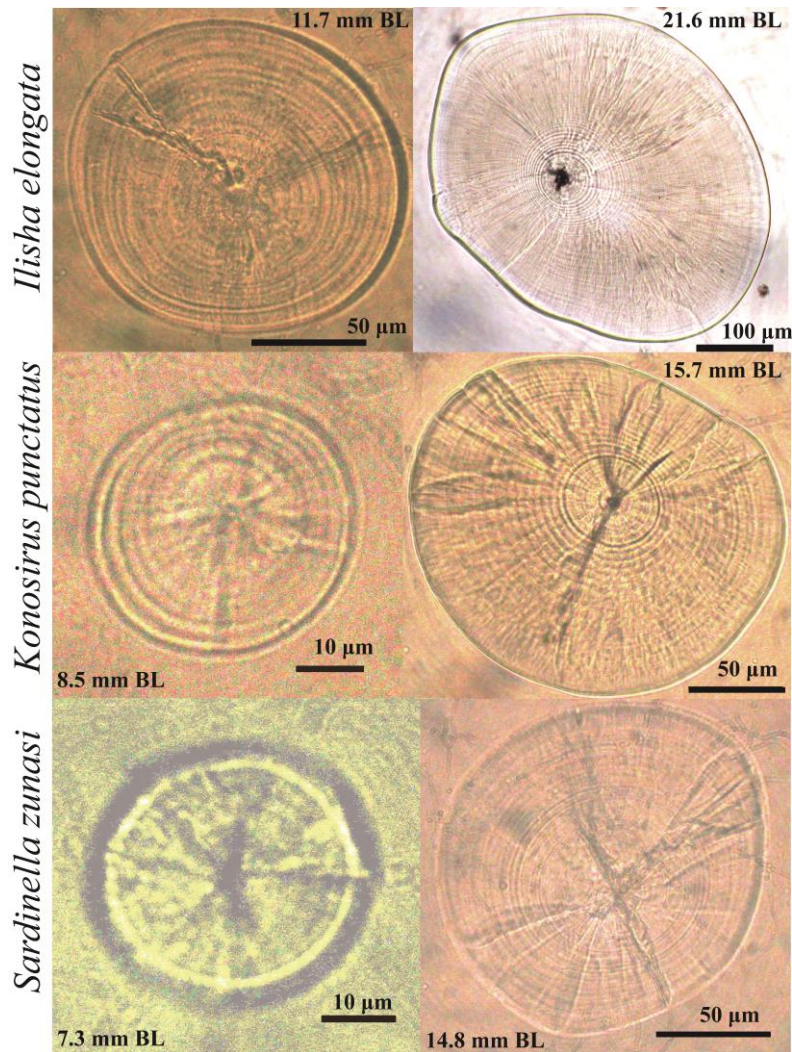
Fig. 28. Sagitta from *Ilisha elongata*, *Konosirus punctatus* and *Sardinella zunasi*. The body length of larvae and scales are indicated.

3.3. Results

3.3.1. Spawning period

The hatching dates of *Ilisha elongata*, which were collected in July, were estimated to extend from middle June to early July with the prominent peak appearing in late June (Fig. 29). According to the yolk-sac larvae distribution of *I. elongata* in May (Fig. 12), the hatching dates of *I. elongata* might be extend from middle May to middle July. The hatching dates of *Konosirus punctatus*, which were collected in May, were estimated to extend from middle April to middle May with the prominent peak appearing in middle May. The hatching dates of *Sardinella zunasi*, which were collected in May and July, were estimated to extend from middle May to early July with the prominent peak appearing in early July.
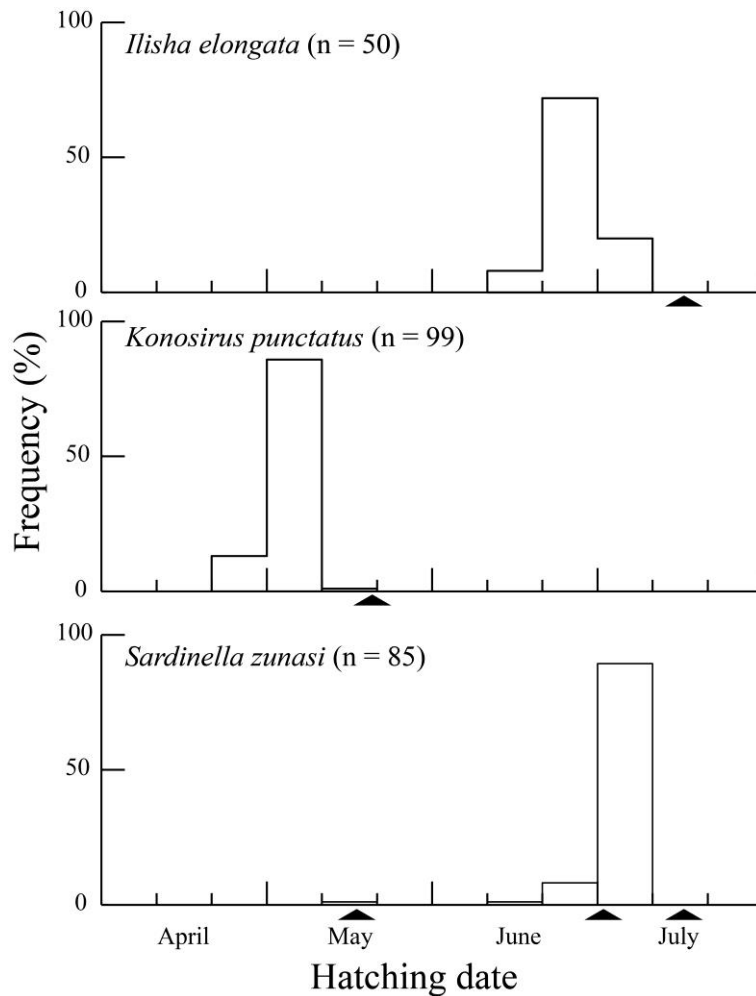
Fig. 29.   Comparison of hatching date distributions between *Ilisha elongata*, *Konosirus punctatus* and *Sardinella zunasi*.   Solid triangle indicated collection date.

3.3.2. Growth rates

The regression of body length and age showed the linear function as the best fit explaining ca. 87, 87 and 85% of the total variation of *Ilisha elongata*, *Konosirus punctatus*, and *Sardinella zunasi*, respectively (Fig. 30).   Based on the linear function, the growth rate of *I. elongata*, *K. punctatus*, and *S. zunasi* are ca. 0.45, 0.40 and 0.51 mm $\cdot$ day^{-1}, respectively.   Growth rate of these three species from the Miike beach, and various river differed significantly (Fig. 31) (Table 2).   The highest growth rate was observed in *S. zunasi* in Hayatsue River and the lowest in *K. punctatus* in Shiota River.   For *K. punctatus* and *S. zunasi* larvae, the highest growth rates were in the Yabe River and Hayatsu River, while the lowest in the Shiota River and Miike beach, respectively.
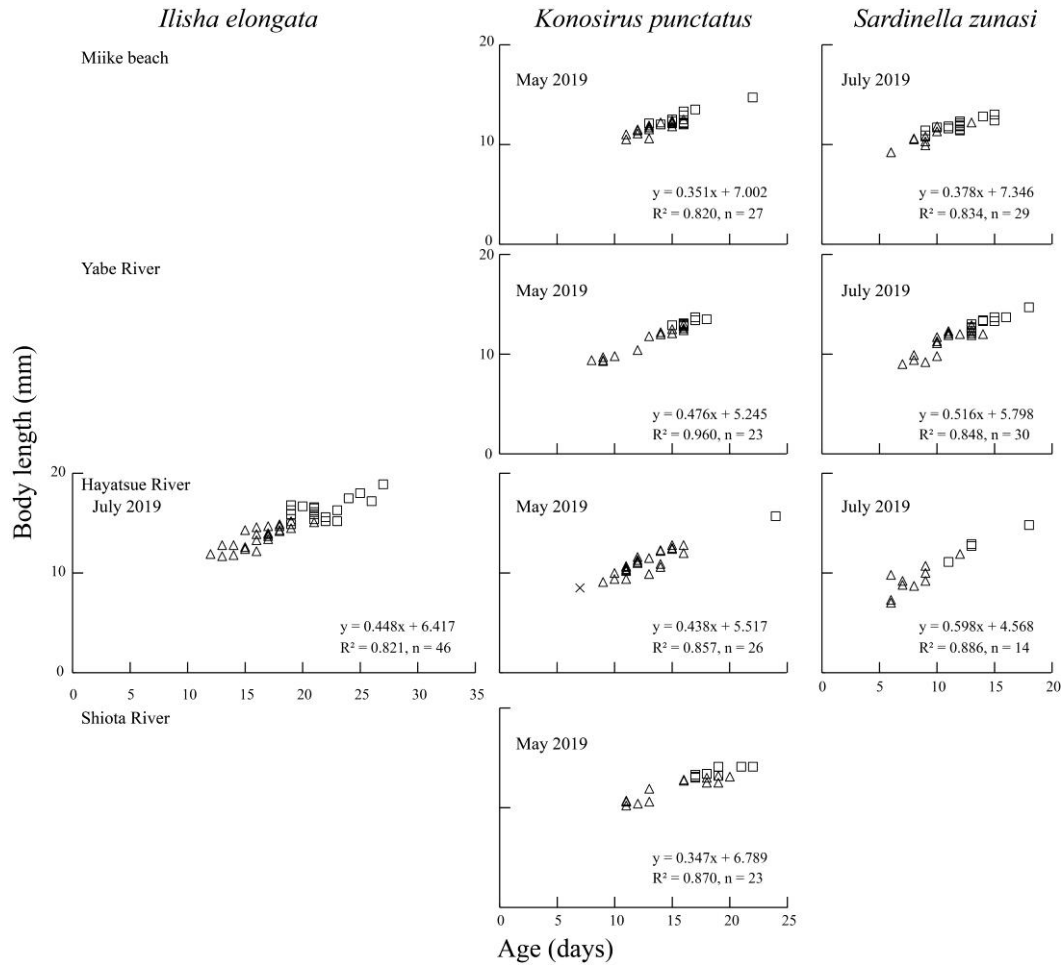
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3.4. Discussion

The spawning period of *Ilisha elongata* is from May to July (Fig. 29), and almost in accordance with the results estimated by GSI and histological examinations in Ariake Sound (Zhang et al. 2009). The spawning period of *Konosirus punctatus* is also accorded with the results of GSI examinations by Takita (1978) in Ariake Sound. According to Takita (1966), the spawning season of *Sardinella zunasi* seems to extend from April to August, longer than our observation. Further age estimation using otolith should do by more specimens collected in other months.

Compared to the growth rates of *I. elongata* and *S. zunasi* in July, the *K. punctatus* in May has the lowest growth rates (Fig. 30), with the lower water temperature than July (Fig. 10). Temperature is a factor for promoting the growth of fish, Djumanto (2005) reported that *Engraulis japonicus* (Engraulidae) has highest growth rate in summer, lowest growth rate in winter with the lowest water temperature. The growth rates of *S. zunasi* are higher than those of *I. elongata* in July, being likely attributed to specific difference. The *S. zunasi* larvae can be accelerated the development and enhanced their locomotory ability. Thus, it is likely that the developed larvae and juveniles of *S.zunasi* has already immigrated to other areas by faster growth.
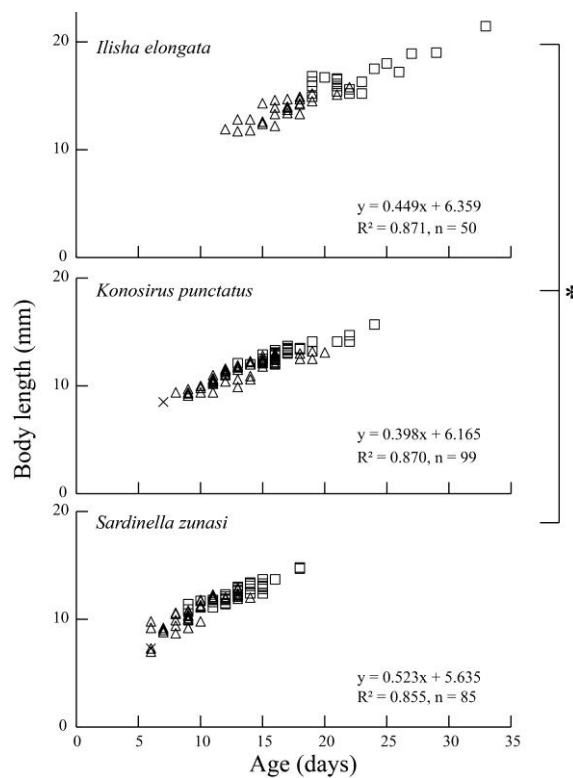
Fig. 30. Comparison of relationships between body length and age of *Ilisha elongata*, *Konosirus punctatus*, and *Sardinella zunasi* collected in May and July 2019. *Significant at $\alpha = 0.001$.

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Ilisha elongata *Konosirus punctatus* *Sardinella zunasi*

Miike beach

May 2019

y = 0.351x + 7.002
R² = 0.820, n = 27

July 2019

y = 0.378x + 7.346
R² = 0.834, n = 29

Yabe River

May 2019

y = 0.476x + 5.245
R² = 0.960, n = 23

July 2019

y = 0.516x + 5.798
R² = 0.848, n = 30

Hayatsue River
July 2019

y = 0.448x + 6.417
R² = 0.821, n = 46

May 2019

y = 0.438x + 5.517
R² = 0.857, n = 26

July 2019

y = 0.598x + 4.568
R² = 0.886, n = 14

Shiota River

May 2019

y = 0.347x + 6.789
R² = 0.870, n = 23

Body length (mm)

Age (days)

Fig. 31. Comparison of relationships between body length and age of *Ilisha elongata* (July 2019), *Konosirus punctatus* (May 2019), and *Sardinella zunasi* (July 2019) collected in Miike beach, Yabe, Hayatsue and Shiota Rivers.

Table 2. Results of ANCOVA for difference of relationships between body length and age of three clupeoid larvae between areas. IE, *Ilisha elongata*; KP, *Konosirus punctatus*; SZ, *Sardinella zunasi*. HR, Hayatsue River; MB, Miike beach; SR, Shiota River; YB, Yabe River. *Significant at α = 0.001.

Species	Area	KP				SZ		
		MB	YR	HR	SR	MB	YR	HR
IE	HR	*	*	*	*	*	*	*
KP	MB		*	*	*	*	*	*
	YR			*	*	*	*	*
	HR				*	*	*	*
	SR					*	*	*
SZ	MB						*	*
	YB							*

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The growth rates of *K. punctatus* and *S. zunasi* varied between the Miike beach and various rivers (Fig. 31).　Simanjuntak (2016) reported that the growth rate of *Coilia nasus* in the Isahaya retention basin was tended to faster than other river estuaries specimens.　The *C. nasus* larvae in the retention basin fed mainly on cladocera, while calanoid copepod in the river estuaries. Therefore, food availability is another factor affected the growth rate.　The differences of growth rate in same species may be due to the feeding preference, feeding habit of each species in the different areas should be examined in the near future.

Chapter 4. General discussion

Niche competition or segregation: According to the spawning period, egg and larva distribution, the spawning and nursery grounds of *Konosirus punctatus*, *Sardinella zunasi*, *Ilisha elongata* and *Coilia nasus* were showed in the Fig. 32.

Coilia nasus spawned in the near-freshwater of the upper reaches of the rivers in summer. After hatching, ebb tides disperse the larvae to the lower reaches (Simanjuntak 2016). Compared to other species larva distribution, it is likely that less niche competition occurs in the low reaches of rivers between *C. nasus* and other clupeoid fishes.

In summer, the *I. elongata* and *S. zunasi* larva distribution were accorded in the inner estuaries. According to the observation of tidal collection in different layers, the *I. elongata* larvae were dispersed from the surface to the near-bottom layers during the flood tide while they moved to near-bottom layer and bottom to avoid strong current in the surface and middle layer after the high tide. While *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 phase. There may be pelagic and lower demersal competition between the two speices. Furthermore, isolation in terms of prey preference is considered to occur in these fishes based on the differentiation of mouth sizes and anecdotal observations (Yagi 2010). Therefore, niche competition between larval *I. elongata* and *S. zunasi* in the inner estuary environments of Ariake Sound is considered to be less likely.

In spring, although *K. punctatus* and *S. zunasi* used the littoral zone of Miike beach and inner estuaries as their main nursery ground, respectively, a part of the two species larvae were overlapped around the mouth of the rivers. The vertical distribution of the two species mainly overlapped in the surface layer during all tidal phases. Thus, the larval niche competition between the two species should be likely less. Such niche isolation between closely related species may facilitate the coexistence of these sympatric species.

Assessments of auxotrophic differentiation based on mouth size seemed to reveal a possibility of feeding competition and preference for flexion and preflexion larval periods in some estuaries, respectively. The feeding habits should be examined to clarify the possible niche segregation between these clupeoid fishes in the near future.

Biological diversity: The *C. nasus* larvae and juveniles distributed over wide estuaries and Isahaya
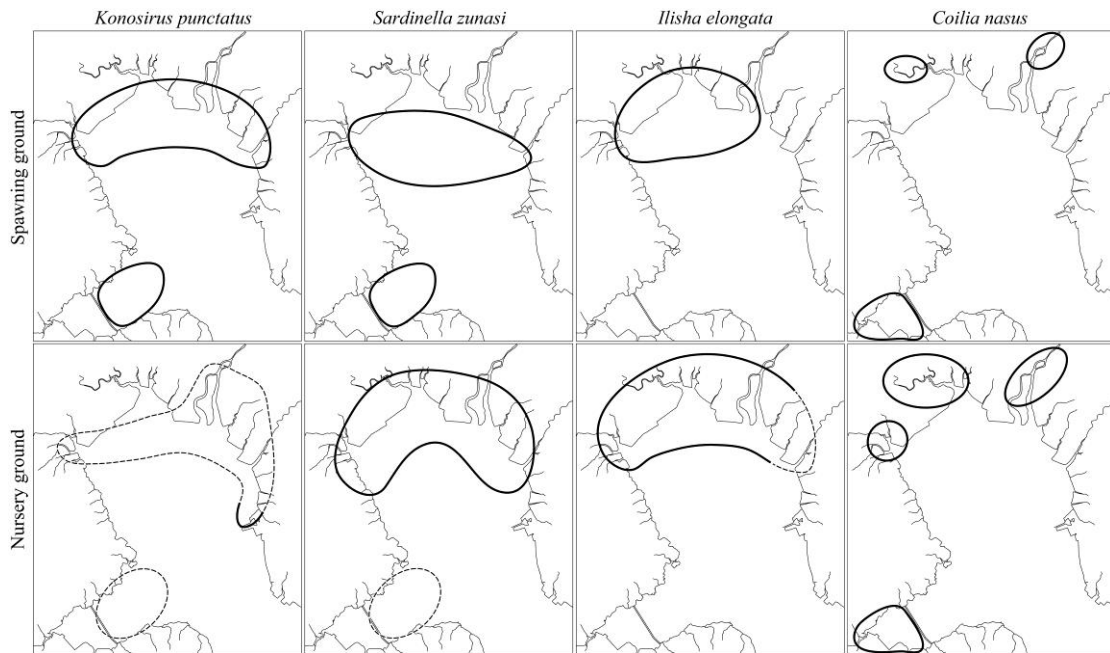
Fig. 32. A conceptual model of spawning and nursery grounds of four clupeoid fishes in Ariake Sound. Solid line areas indicate distinctive grounds, and broken ones, minor or uncertain grounds.

retention basin, it may suggest that this fish has a highly tolerable to salinity. The *I. elongata*, *K. punctatus* and *S. zunasi* also showed some tolerance to the salinity, might be an important factor for a wide geographical distribution of these fishes in Ariake Sound. Furthermore, the growth rates of each fishes varied in the different areas of the sound. Therefore, there are bio- diversity occurring in the early life histories of clupeoid in Ariake Sound.

Influence of building a dike across the Isahaya inlet: Before the construction of a dike across the Isahaya inlet blocking the Honmyo River in 1997, the Honmyo River estuary had an environment like those of inner estuaries. Kinoshita (2007) reported about 40 years ago the Honmyo River estuary was an important nursery ground for fishes, especially endemic and restricted species. The *S. zunasi* larvae were predominant in Honmyo River estuary in June 1979, but only a few larvae could be collected in the Isahaya inlet after the construction of the dike (Yagi et al. 2011, Takeuchi 2012). These facts likely suggest that the Isahaya inlet became inappropriate for *S. zunasi* to use the inlet as a nursery ground after building the dike in 1997. However, some *S. zunasi* larvae occurred when the water was brackish after the water gates intermittently opened in May 2006. It seems that the environment of this area had an environment like those of inner estuaries again, after getting freshwater replenishment. The opening period of water gates should be discussed by the

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management of dike, fishery and agriculture group for sustainable utilization. The Isahaya inelt reclamation project resulted in the loss of 1550 ha of tidal flats, and is one of the possible reasons for the decrease in the tide and tidal current (Manda & Matsuoka 2006, Jia et al. 2018). In addition, decrease in the tidal amplitude and current contribute to the sedimentation of floating mud. The accelerated sedimentation of floating mud will increase the transparency of seawater (Hayami et al. 2015), which might cause high predation rate for early stages of fish. On the other hand, there were no *C. nasus* eggs and larvae collected in the Honmyo River 40 years ago (Kinoshita 2007), while the retention basin became a new spawning and nursery ground for *C. nasus* (Simanjuntak et al. 2015, Simanjuntak 2016). Many construction projects have been conducted in Asia to satisfy the requirements of urban, agricultural, and industrial use, and caused some environmental issues, such as water pollution, deterioration of the ecosystem and the aquatic environment (Li et al. 2014, Hayami & Hamada 2016). The effects of those construction projects on fishes also need to be investigated.

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