

# Occurrence Patterns and Ontogenetic Intervals of *Eutaeniichthys gilli* (Gobiidae) in Obitsu-gawa River Estuary, Tokyo Bay, Central Japan

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**Abstract:** Occurrence patterns and ontogenetic intervals based on the development of swimming and feeding functions were investigated on the endangered goby, *Eutaeniichthys gilli*, collected from Obitsu-gawa River estuary in the inner Tokyo Bay. A small seine net and two set nets were used for the samplings at the main stream from May 2005 to April 2006 and at the creek on the tideland from July 2009 to June 2010. Individual numbers of *E. gilli* collected were 94 ranging from 2.8 to 34.5 mm BL with the mean  $\pm$  SD =  $7.7 \pm 3.9$  mm BL and 7 from 6.4 to 33.9 mm BL with  $17.1 \pm 12.3$  mm BL at the tidal flat in the lower and middle streams, respectively, although no specimen was collected at the upper stream. On the other hand, 1,127 individuals of 10.1–39.5 mm BL with  $27.5 \pm 7.2$  mm BL ( $n = 464$ ) were collected from the creek. Based on the development of swimming- and feeding-related characters of 3.5–39.1 mm BL ( $n = 100$ ) cleared and stained specimens, the following three swimming and feeding phases were recognized: caudal fin propulsion from 3.5 to 5.0 mm BL, whole body propulsion from 5.0 to 10–11 mm BL, and functional, juvenile swimming over 10–11 mm BL; sucking and biting from 3.5 to 5–6 mm BL, improved sucking and biting abilities from 5–6 to 10–12 mm BL, and functional, juvenile feeding over 10–12 mm BL. Based on the occurrence patterns and functional development, the spawning ground is considered to be formed in the tideland including the creek of Obitsu-gawa River estuary, and the hatched larvae are drifted and dispersed toward the sea. The forehead tidal flat at the lower stream is one of nurseries for early stage larvae. No nursery and growing habitats are formed in the main stream, but the juveniles of about 8 mm BL and larger migrate shoreward to and grow in the tideland of the Obitsu-gawa River estuary. This study revealed that Obitsu-gawa River estuary is almost the only major spawning ground and provides nursery and growing habitats for *E. gilli* in the inner Tokyo Bay.

**Keywords :** goby, habitat shift, occurrence pattern, functional development

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## 1. Introduction

The gobiid *Eutaeniichthys gilli* is widely distributed in coastal areas from Aomori to Iriomote-jima in Japan, Korea and the Yellow Sea, and inhabits in and around burrows of mud shrimps (e. g., *Upogebia* spp.) on soft bottom ti-

dal flats in estuaries (AKIHITO *et al.*, 2000; SUZUKI AND SHIBUKAWA, 2004; HENMI *et al.*, 2014). The species is one of the endangered species and designated as NT, near threatened, by Ministry of the Environment, Government of Japan, because of a rapid decrease of estuarine tidal flats by reclamation (JAPAN MINISTRY OF THE ENVIRONMENT, 2019). Various studies have been conducted regarding the life history of the species such as maturation and spawning, morphological development, growth and feeding habits (DOTSU, 1955; ZAMA, 1999; HENMI *et al.*, 2014). However, the functional development of larvae and juveniles of the species remains unclear.

As reported in many studies, the environment of Tokyo Bay has been deteriorated since 1960's, by anthropogenic impacts during the period of rapid economic growth. SHIMIZU (1999) reported that 20% of coastal areas in the inner Tokyo Bay have been reclaimed and the shorelines have been changed to concrete seawalls. Since 1980's, environmental restoration has been undergone by constructing artificial tidal flats in the inner Tokyo Bay (NAKASE *et al.*, 2009). Many studies have been conducted on fishes inhabiting artificial and/or natural tidal flats in the inner Tokyo Bay for knowing whether or not the tidal flats function as habitats for fishes (KANOU *et al.*, 2000; HERMOSILLA *et al.*, 2012a). Under these circumstances, remained natural and constructed artificial tidal flats have been evaluated as a nursery ground for many fish species (NASU *et al.*, 1996; KUWABARA *et al.*, 2003; KOHNO *et al.*, 2008; MURASE *et al.*, 2014; MURAI *et al.*, 2016). However, *Eutaeniichthys gilli* has been reported occurring sporadically in the inner Tokyo Bay and abundant only in Obitsu-gawa River estuary (KANOU *et al.*, 2000; IWATA and HOSOTANI, 2005; KOHNO *et al.*, 2014), where the tideland contributes a significant portion of what is still considered as a natural system in the inner Tokyo Bay (HERMOSILLA

*et al.*, 2012a, b).

This study is therefore conducted to establish the ontogenetic intervals during the early life history of *Eutaeniichthys gilli* based on the development of swimming- and feeding-related characters. The occurrence patterns are also investigated in Obitsu-gawa River estuary. The relationships between ontogenetic intervals and occurrence patterns are examined to elucidate how the species utilizes the estuary during its life history, which would be useful information for planning conservation of this species in the inner Tokyo Bay.

## 2. Material and methods

Two types of sampling gear were used for fish sampling in this study as follows. A 0.8 mm mesh-size small seine net (cf., KANOU *et al.*, 2002) was monthly towed at three stations (upper, middle and lower stations in Fig. 1) along the main stream of Obitsu-gawa River from May to December 2005 for all the stations and additionally from March and April 2006 for the upper and middle stations. The small seine net was towed parallel to the river bank at day time over a distance of 20 m and depths between 0.5 and 1 m. The opening of net was adjusted to be 4 m, and thus the sampling area was 80 m<sup>2</sup>. Two 0.33 mm mesh-size small set nets were set at three stations (solid circles in Fig.1) along the small, natural tidal creek situated on the tideland in the north of Obitsu-gawa River estuary with its east channel opening towards the river and the west channel into the bay (Fig. 1) from July 2009 to June 2010. The tidal creek has an average depth of 20 cm and width of 4 m at low tide. The 0.33 mm mesh-size set net extends 2 m laterally with 50 cm in height, and at the center a mouth of 50 cm square on a side opens and tapers posteriorly into a conical net of 1.2 m in length (see HERMOSILLA *et al.*, 2012b). The set net was instal-

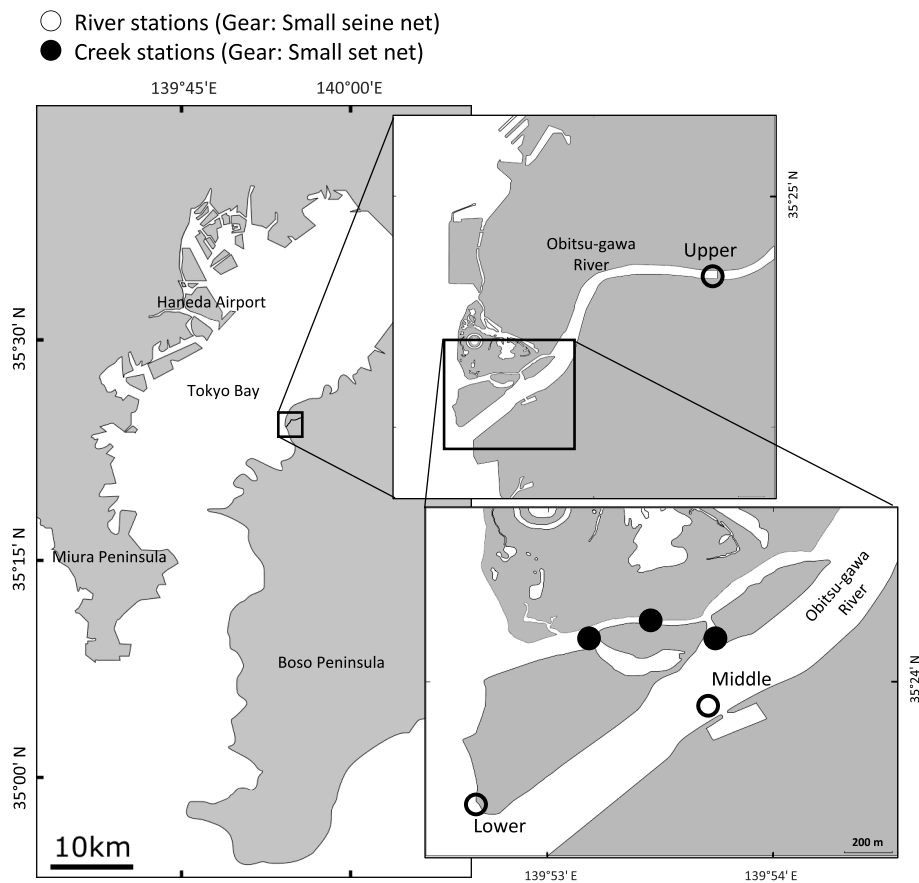


Fig. 1 Map showing the locations of sampling stations in Obitsu-gawa River in the inner Tokyo Bay. Sampling gears are small seine net (Open circles) and small set net (Solid circles).

led at two points along the tidal creek covering an area of 10 m<sup>2</sup> for each station. One net blocked the incoming water while the other was fixed 2 m away to collect fish. Trapped fishes between the set nets were collected and caught by carefully sweeping the water just above the substrate towards one side of the net by two persons using a polyethylene board. Water temperature and salinity were measured after the samplings in the respective three main river and tidal creek stations.

All the samples were fixed with 5–10% formalin in the field and later preserved in 70% ethanol

in the laboratory. *Eutaeniichthys gilli* specimens were identified following NAKABO (2013) and OKIYAMA (2014). In this study, all *E. gilli* collected from three stations in the creek were processed together, and a total of 464 individuals were extracted randomly for the measurement of body length (BL: *sense* LEIS and TRNSKI, 1989), being measured to the nearest 0.1 mm. The BL measurement was, on the other hand, done for all the specimens collected from the main river stations.

Additionally, 100 specimens (3.5–39.1 mm BL) selected from all the specimens were cleared and

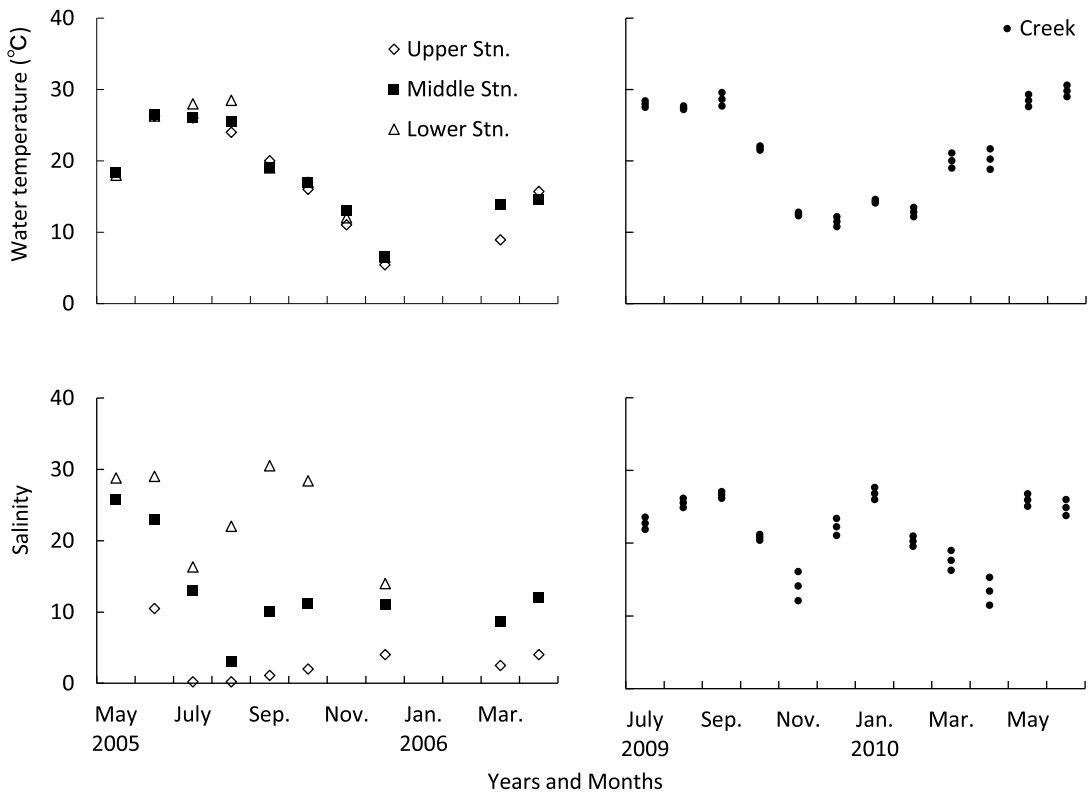


Fig. 2 Water temperature and salinities of the sampling stations in Obitsu-gawa River in the inner Tokyo Bay.

stained by following the method of POTTHOFF (1984) for morphological studies to establish the ontogenetic intervals. The histogram method of developmental events by 1.0 mm BL fish-size intervals employed by SAKAI (1990) and the key characters method (cf., KOHNO AND SOTA, 1998) were both applied to determine the ontogenetic intervals.

### 3. Results

#### 3.1 Physical conditions of water

Water temperatures showed the same tendency as increasing during the summer season and decreasing during the winter season in all the sampling stations, and no remarkable different tendencies were found between the stations in

each of the main stream and tideland (Fig. 2). The highest water temperature, 28.5 and 30.6 °C, was recorded at the lower station in August 2005 and at the creek in June 2010, respectively, and the lowest, 5.4 and 10.8 °C, at the upper station in December 2005 and at the creek in December 2009, respectively.

The salinity varied from 14.0 to 30.5 with the mean  $\pm$  SD =  $24.1 \pm 6.2$  at the lower station, from 3.0 to 25.8 with  $13.1 \pm 6.7$  at the middle station and from 0.2 to 10.5 with  $3.1 \pm 3.1$  at the upper station in the main stream (Fig. 2). In the creek stations, the salinity varied from 11.5 to 27.7 with  $21.8 \pm 4.6$ .

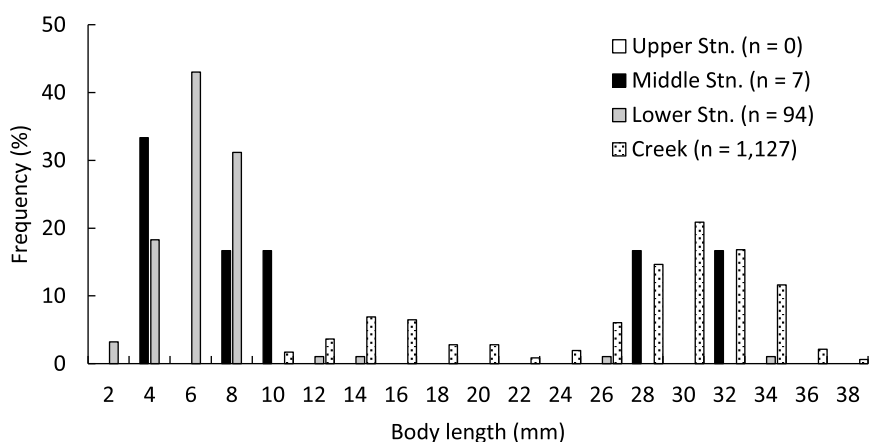


Fig. 3 Size composition of *Eutaenichthys gilli* collected from three main river and tidal creek stations in Obitsu-gawa River in the inner Tokyo Bay.

### 3.2 Occurrence patterns

A total of 1,228 *Eutaenichthys gilli* were collected from the Obitsu-gawa River estuary (Fig. 3), 101 individuals (2.8–34.5 mm BL with the mean  $\pm$  SD =  $8.0 \pm 4.7$  mm BL) being collected from the main river stations and 1,127 (10.1–39.5 mm BL with  $27.5 \pm 7.2$  mm BL;  $n = 464$ ) from the creek.

The 101 specimens collected from the main river stations consisted of 94 and 7 individuals from the lower and middle stations, respectively, and no specimens were collected from the upper station. The size of specimens ranged from 2.8 to 34.5 mm BL (mean  $\pm$  SD =  $7.7 \pm 3.9$  mm BL) in the lower station and from 6.4 to 33.9 mm BL ( $17.1 \pm 12.3$  mm BL) in the middle station (Fig. 3). In the lower station, almost all specimens, 93 in number, occurred in August, and the remained one in October, while in the middle station seven specimens occurred sporadically in March, May, July and October.

The size of specimens collected from the creek stations ranged from 10.1 to 39.5 mm BL (mean  $\pm$  SD =  $27.5 \pm 7.2$  mm BL;  $n = 464$ ) with the mode being in the size of 30.0–31.9 mm BL

(Fig. 3). *Eutaenichthys gilli* occurred throughout the year during the sampling period in the creek (Fig. 4). Specimens of about 15 mm BL and smaller started appearing in July, increased in number in October and November and were continued to appear until February. The mode of appearance shifted from February to April, and specimens of about 27–30 mm BL appeared continuously until November.

### 3.3 Morphological development

#### 3.3.1 Swimming-related characters

*Flexion of the notochord end:* The flexion of notochord end was first evident at the smallest specimen of 3.5 mm BL with the angle of  $17^\circ$  (Fig. 5A). Notochord flexion was complete in the specimens of 4.6 mm BL and larger with the stable angle of  $35\text{--}45^\circ$ .

*Caudal fin supports and fin rays:* Two cartilaginous buds of hypurals 1 + 2 and 3 were detected at the smallest specimen of 3.5 mm BL. The cartilaginous buds of epurals 1 and 2, hypural 4, parhypural as well as those of the hemal and neural spines of the future pleural centra 2 and 3 were evident at 4.9 mm BL. Additionally, the hemal-

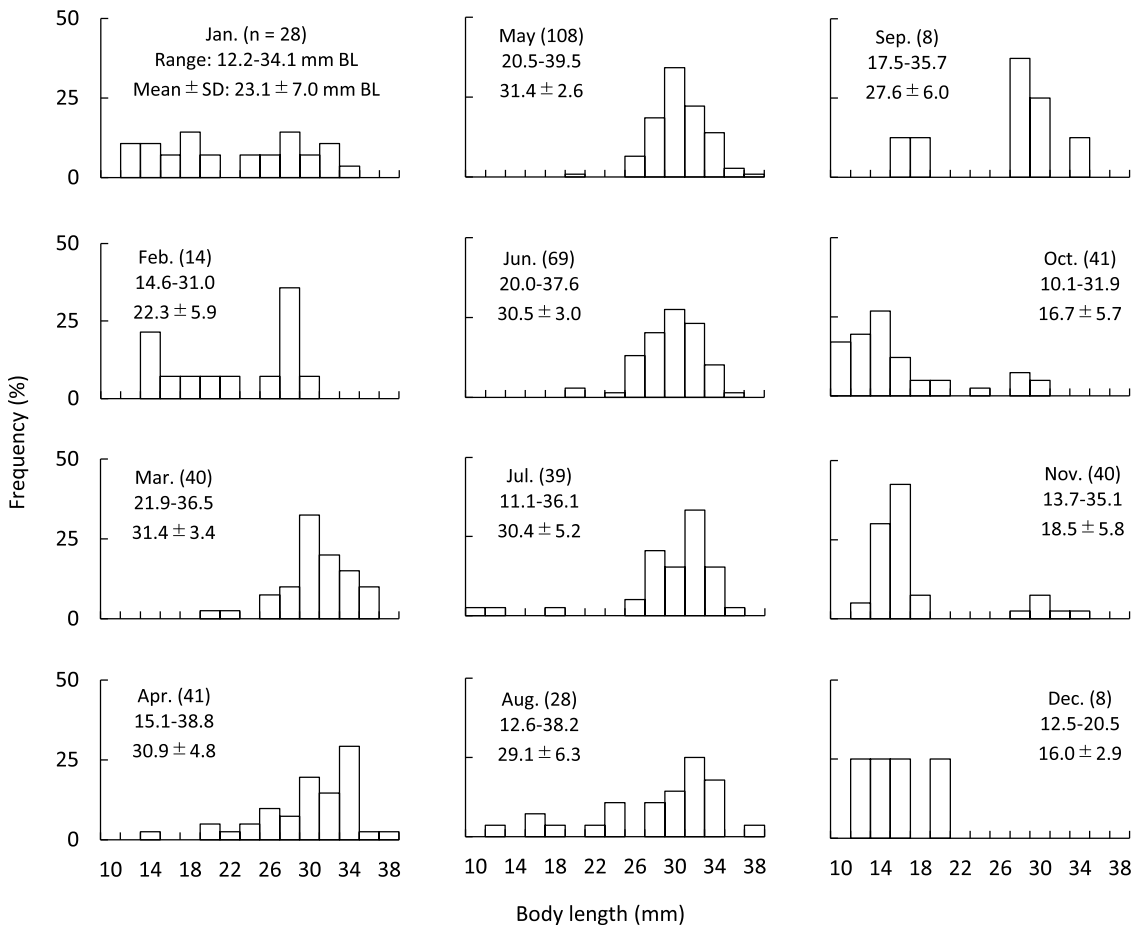


Fig. 4 Monthly changes of the size composition of *Eutaenichthys gilli* collected from the tidal creek in Obitsu-gawa River in the inner Tokyo Bay.

and neural-spine cartilages of the future pleural centrum 3 as well as the cartilaginous bud of hypural 5 were detected at 7 mm BL. The ossification was first observed on hypural 1 + 2 at 8.4 mm BL then followed by hemal arches, hypurals 3 + 4 and parhypural at 8.8 mm BL, 9.0 mm BL and 10.4 mm BL, respectively. At 10.6 mm BL, hypural 5 and epurals 1 and 2 started ossifying and thus all the cartilaginous elements started ossifying. The bony urostyle was observed at the smallest specimen of 3.5 mm BL.

The principal caudal fin rays were first dis-

cerned at 3.5 mm BL, when nine rays were observed (Fig. 5B). The adult complement of 6 + 7 principal caudal fin rays was attained at 6.6 mm BL.

*Dorsal fin supports and fin rays:* Two pterygiophores of the first dorsal fin were evident at 5.5 mm BL, and the adult complement of three cartilaginous pterygiophores were observed at 7.0 mm BL. All pterygiophores were noted ossifying at 10.5 mm BL.

Cartilaginous pterygiophores of the second dorsal fin were first observed at 4.9 mm BL with

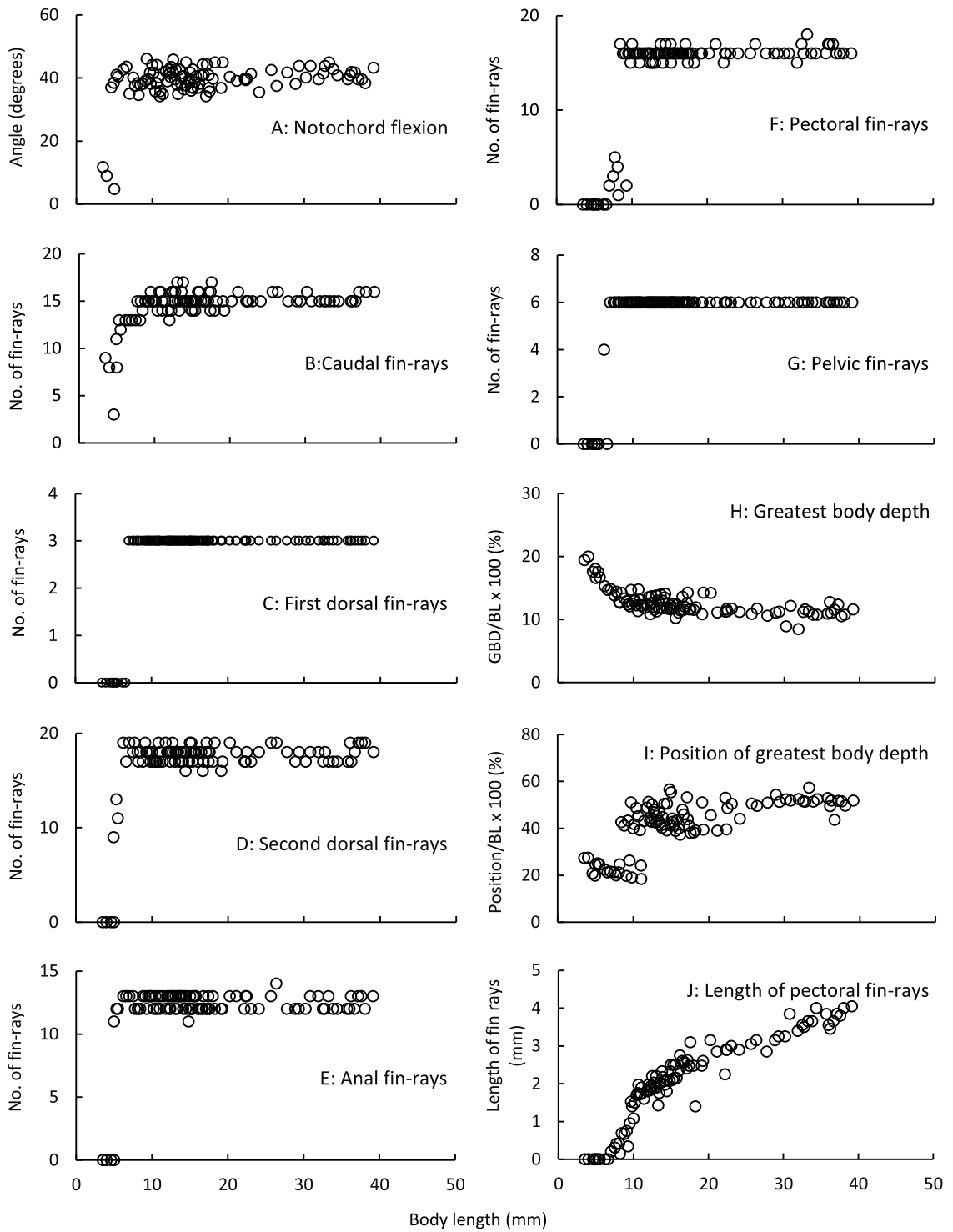


Fig. 5 Changes in swimming-related characters with growth in *Eutaenichthys gilli*.

17 in number. The adult complement of 19 pterygiophores was attained at 6.2 mm BL. Five cartilaginous pterygiophores of second dorsal fin started ossifying at 10.2 mm BL and all were noted at 15.8 mm BL.

Three spines of the first dorsal fin were attained at 7.0 mm BL (Fig. 5C). In the second dorsal fin, nine soft rays were first observed at 4.9 mm BL (Fig. 5D), and the adult complement of 19 fin rays was attained at 6.2 mm BL.

*Anal fin supports and fin rays:* The cartilaginous pterygiophores of anal fin were first discerned at 4.9 mm BL, when ten in number were observed. The number reached the adult complement of 13 at 7.0 mm BL. Five pterygiophores started ossifying at 9.3 mm BL, and the ossification was noted in all pterygiophores at 16 mm BL.

Anal fin rays were first evident at 4.9 mm BL, when eleven soft rays were observed (Fig. 5E). The adult complement of 13 rays was attained at 7.0 mm BL.

*Pectoral fin supports and fin rays:* The pectoral fin supports of coraco-scapular cartilage and a rod-shaped bony cleithrum were observed at the smallest specimen of 3.5 mm BL. The posttemporal, supracleithrum and a bladelike cartilage, which later grew into actinosts, were observed at 4.9 mm BL, and thus all the pectoral fin supports were observed. The ossification was first observed on the coracoid at 10.2 mm BL, then followed by the actinosts and scapula at 10.6 and 13.3 mm BL, respectively.

Two rays of pectoral fin were first observed at 7.0 mm BL (Fig. 5F). The adult complement of 17 rays was attained at 8.4 mm BL.

*Pelvic fin supports and fin rays:* The pelvic fin support, the basipterygium, was first discerned at 4.9 mm BL, and the ossification started at 9.5 mm BL.

The pelvic fin rays were first discerned at 6.2

mm BL, when four rays were observed (Fig. 5G). The adult complement of six rays was observed at 7.0 mm BL.

*Vertebra:* The smallest specimen examined, 3.5 mm BL, possessed 38 centra and cartilaginous 35 neural- and 15 hemal-spines. The adult complement of 38 neural- and hemal-spines was attained at 7.0 mm BL, as well as some of them started ossifying. All of hemal- and neural-spines were observed ossifying at 8.8 mm BL.

*Maximum body depth and its position:* The ratio of the maximum body depth to BL was 19% in the smallest specimen of 3.5 mm BL (Fig. 5H). The ratios decreased gradually and became stable at 10–15% at about 10.0 mm BL.

The ratio of position of maximum body depth was about 20 % in the specimens of 3.5–11.0 mm BL (Fig. 5I). The ratio leaped up during the size ranging from 8.4 to 11.4 mm BL, and then became stable from 40% to 50%.

*Length of pectoral fin-rays:* The pectoral fin length was 0.21 mm when the first pectoral fin ray was observed at 7.0 mm BL (Fig. 5J). Two flexion points were observed at about 10 and 20 mm BL, after which the increase rate becoming slower.

### 3.3.2 Feeding-related characters

*Mouth width:* The mouth opened in all specimens examined, and the mouth width was 0.4 mm in the smallest specimen of 3.5 mm BL (Fig. 6A). The mouth width increased rapidly up to 10.4 mm BL and more or less slowly thereafter.

*Jaw structure:* The smallest specimen of 3.5 mm BL possessed the upper jaw composed of the maxilla and premaxilla. The lower jaw of the same specimen was composed of the dentary, angular and Meckel's cartilage, the retroarticular being first observed at 4.0 mm BL.

*Jaw teeth:* The smallest specimen of 3.5 mm BL possessed six upper jaw teeth (Fig. 6B). The



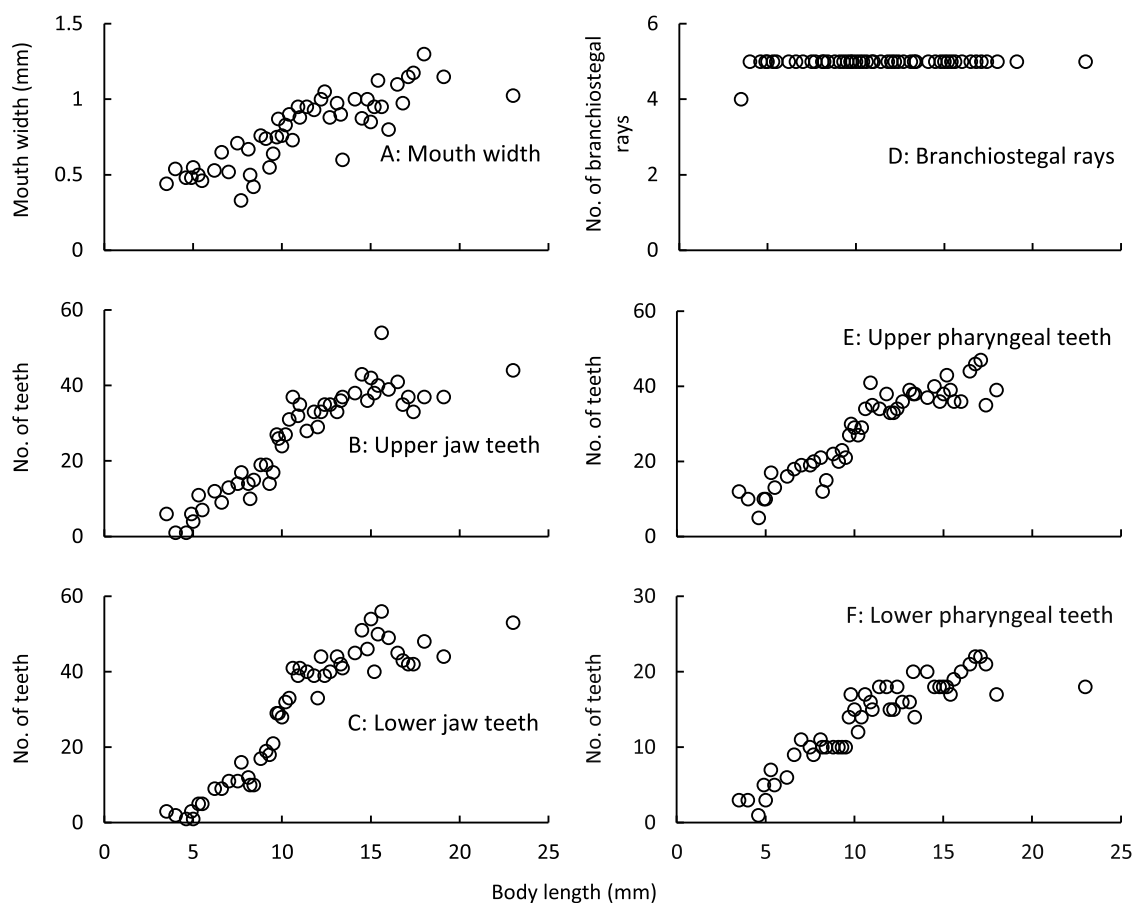


Fig. 6 Changes in feeding-related characters with growth in *Eutaenichthys gilli*.

number of upper jaw teeth then increased slowly with a leap up flexion point at about 9.5 mm BL up to about 16 mm BL, after which the number becoming more or less stable.

The smallest specimen of 3.5 mm BL possessed three lower jaw teeth (Fig. 6C). The number of lower jaw teeth increased rapidly and leaped up at about 10 mm BL. The number became more or less stable between 40 and 55 thereafter.

*Suspensorium*: The hyomandibular-symplectic and palatoquadrate cartilages were discerned at the smallest specimen of 3.5 mm BL. The ossification started on the symplectic and quadrate at

4.6 mm BL and 5.0 mm BL, respectively. The bony endopterygoid was first observed at 4.9 mm BL. The cartilaginous metapterygoid was first observed at 7.5 mm BL, and the hyomandibular, palatine and metapterygoid started ossifying at 8.8 mm BL, 9.1 mm BL and 9.7 mm BL, respectively.

*Hyoid and branchiostegal rays*: The smallest specimen of 3.5 mm BL possessed the hypohyal, ceratohyal-epihyal and interhyal cartilages, and the basihyal was discerned at 4.0 mm BL. The ossification was first observed at the smallest specimen of 3.5 mm BL on the ceratohyal and followed by hypohyal and basihyal at 5.5 mm BL.

The interhyal and epihyal started ossifying at 8.8 mm BL.

Four branchiostegal rays were first observed at 3.5 mm BL, and the adult complement of five rays was attained at 4.0 mm BL (Fig. 6D).

*Pharyngeal teeth:* The smallest specimen of 3.5 mm BL possessed 12 upper pharyngeal teeth (Fig. 6E). The number increased until about 10 mm BL, when the number leaped up and became more or less stable thereafter.

Three lower pharyngeal teeth were observed at the smallest specimen of 3.5 mm BL (Fig. 6F). The number increased more or less rapidly until about 10 mm BL and became more or less stable thereafter.

*Opercular bones:* The opercle, preopercle and subopercle were observed at the smallest specimen of 3.5 mm BL. The interopercle appeared at 4.9 mm BL.

## 4. Discussion

### 4.1 Development phases

#### 4.1.1 Developmental phases of swimming function

Based on the development of swimming-related characters shown in Figure 7, the following three phases were recognized in the specimens of *Eutaeniichthys gilli*.

*The phase of caudal fin propulsion (from 3.5 to 5.0 mm BL):* The completion of notochord flexion and appearances of caudal fin supports and fin rays were observed in this phase, which indicate that the beating of caudal fin produces propulsion (KOHNO *et al.*, 1983). The completion of vertebral centra in number would reinforce the power of beating. DOTSU (1955) suggested that the newly hatched larvae of *Eutaeniichthys gilli* (about 3.6 mm in total length) possessed a more or less strong swimming ability when comparing with other gobiids.

*The phase of whole body propulsion (from 5.0*

*mm BL to 10.0–11.0 mm BL):* This phase is divided into two sub-phases, pre-whole and whole-body propulsion sub-phases, at about 7.0 mm BL. Dorsal and anal fin rays and fin supports started appearing and became complete in number during the former sub-phase; these characters are considered to reinforce the body axis and thus allow larvae to swim powerfully by propagating the beat of the whole of the body posterior to generate propulsion (OMORI *et al.*, 1996). In larvae of 7.0 mm BL and larger, the neural and hemal spines and the dorsal and anal fin supports started ossifying; these characters prevent the larvae from rolling caused by whole-body beating (GOSLINE, 1971). Morphologically, the smallest juvenile having the adult fin-ray number was 8.4 mm BL at the whole-body propulsion sub-phase.

*The phase of functional, juvenile swimming (over 10.0–11.0 mm BL):* All characters concerning the swimming function were completed in number and started ossifying by this phase. Moreover, the greatest body depth and its position became stable, indicating the improvement of swimming ability (ALEEV, 1963). Therefore, juveniles larger than 10–11 mm BL were considered to have acquired the functional swimming mode.

#### 4.1.2 Developmental phases of feeding function

Based on the development of feeding-related characters shown in Figure 8, the following three phases were recognized in the specimens of *Eutaeniichthys gilli*.

*The phase of sucking and biting (from 3.5 mm BL to 5.0–6.0 mm BL):* The gape elements of maxilla, premaxilla, dentary and angular were equipped in the smallest larva of 3.5 mm BL, indicating that the larva had the gape opening and closing ability (SHINAGAWA *et al.*, 2002). The

branchiostegal rays, opercular bones and elements of suspensorium and hyoid arch appeared and became complete in number during this phase, indicating that the negative pressure for sucking food organisms could be generated (KOHNO *et al.*, 1997). In addition, jaw and pharyngeal teeth, which function to bite/capture and to pass the captured food to the digestive tract, respectively (GOSLINE, 1971), appeared during this phase. Therefore, the larvae in this phase are considered to have both the sucking and biting abilities.

*The phase of improved sucking and biting ability (from 5.0–6.0 mm BL to 10.0–12.0 mm BL):* All elements of suspensorium and hyoid arch appeared in this phase, indicating the sucking ability of the larvae in this phase improved (TAMURA *et al.*, 2013). Furthermore, the number of jaw teeth became stable in this phase, indicating the biting ability also improved.

*The phase of functional, juvenile feeding (over 10.0–12.0 mm BL):* All the characters concerning the feeding function became complete not only in number but also ossification. The number of pharyngeal teeth also became stable, and thus the functional feeding mode was considered to be attained in specimens over 10.0–12.0 mm BL.

#### 4.2 Habitat shifts with development

This study revealed that nearly all the larvae of *Eutaeniichthys gilli* smaller than 10 mm BL were collected at the lower stream station of Obitsu-gawa River (Figs. 1, 3), with a mode of 6.0–7.9 mm BL. The larvae smaller than 5 mm BL would swim using caudal fin propulsion, and those from 5 to 10 mm BL would swim more actively using whole the body (Fig. 7). These results suggest that the larvae of 5–10 mm BL would actively migrate shoreward. DOTSU (1955) reported that the newly hatched larva of 3.6 mm in total length, TL, vigorously swam in the mid-

water of a glass-jar.

However, few specimens of *Eutaeniichthys gilli* were collected at the middle stream station and no specimen at the upper stream (Fig. 3), indicating that *E. gilli* does not utilize the main stream of Obitsu-gawa River as a habitat. EGUCHI *et al.* (2008) indicated that the distribution of *E. gilli* does not expand to tidal-reach upper stream areas but is restricted to river mouths.

The shoreward migrating larvae occurred at the tideland in the north of Obitsu-gawa main river, and the smallest specimen collected at the creek in the tideland was 10.1 mm BL (Fig. 3), which being a juvenile morphologically by having the complete number of fin rays, with the first peak of 14.0–15.9 mm BL. These juveniles occurring in the tideland were considered to attain the functional swimming and feeding modes (Figs. 7, 8), and thus they would swim actively toward the tideland. HENMI *et al.* (2014) reported that the size of *Eutaeniichthys gilli* collected at a tidal flat in Kochi Prefecture ranged from 10.6 to 36.8 mm BL and that the smallest one was judged as a juvenile by having pelvic fins. DOTSU (1955) also suggested that the juvenile stage would start at 11.2 mm TL, which is converted to 10.2 mm BL by using the result of HENMI *et al.* (2014).

A second peak or a mode of size was observed at 30.0–31.9 mm with the largest one being 39.5 mm BL in this study (Fig. 3). KANOU *et al.* (2004) examined feeding habits of *Eutaeniichthys gilli* juveniles of 15–31 mm BL, which corresponding to the size range between the two peaks in this study, collected from the inner Tokyo Bay and reported that the juveniles were grouped into a small benthic and epiphytic crustacean feeder. The maximum size of *E. gilli* was reported as 47 mm TL by Dotsu (1955: 40.7 mm BL judged from the figure by HENMI *et al.*, 2014), 43.9 mm BL (ARAO, 2005: collected at a tideland in Mie

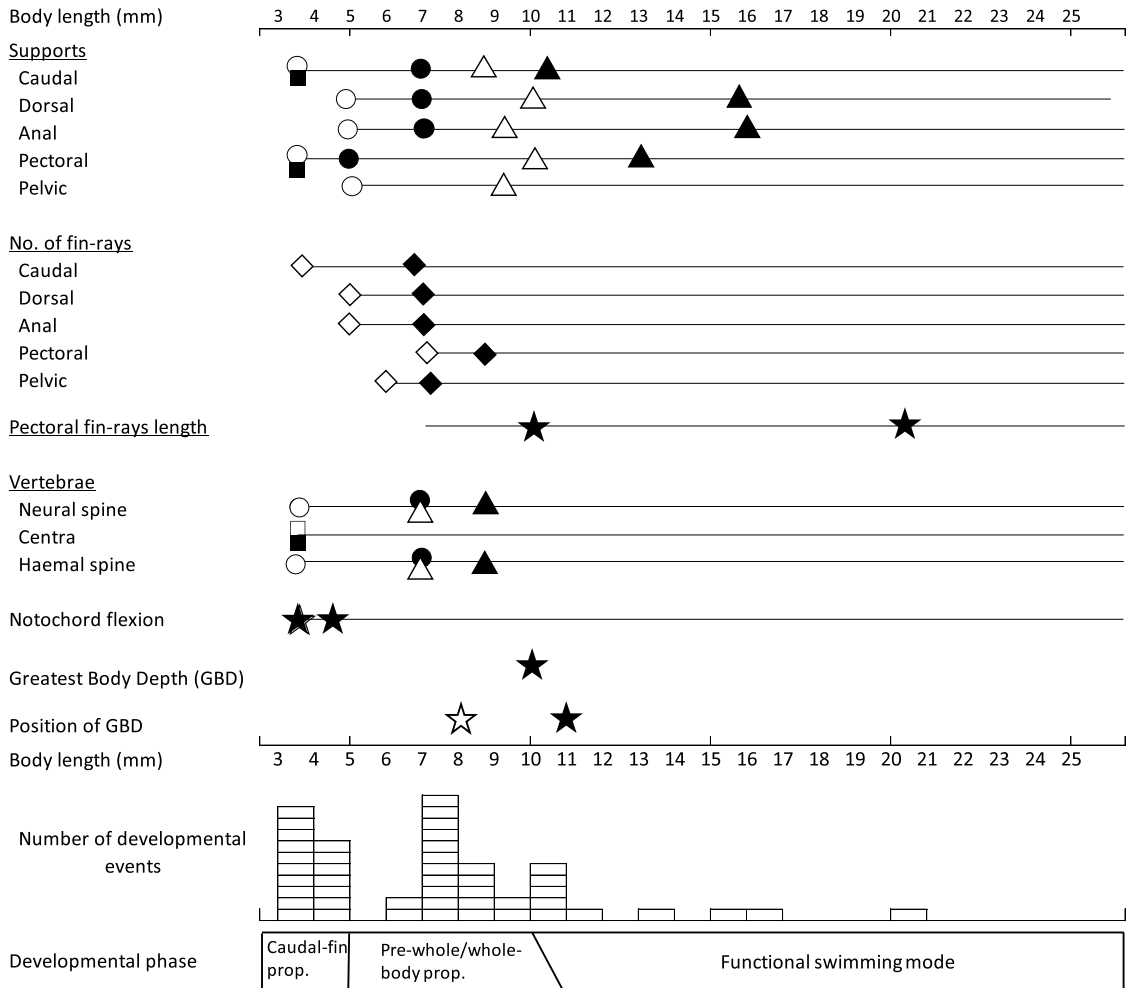


Fig. 7 Schematic representation of the development of swimming-related characters with growth in *Eutaenichthys gilli* collected from Obitsu-gawa river in the inner Tokyo Bay. ○: cartilaginous elements start appearing; ●: all cartilaginous elements start appearing; □: bony elements start appearing; ■: all bony elements start appearing; △: cartilaginous elements start ossifying; ▲: all cartilaginous elements start ossifying; ◇: fin rays start appearing; ◆: fin rays become complete in number; ☆: notochord end starts to flex; ★: flexion points of morphometric characters are observed, notochord flexion become complete.

Prefecture) and 36.8 mm BL (HENMI *et al.*, 2014). Although the mature size was reported as 35 mm TL (30.3 mm BL) by DOTSU (1955), specimens of 27.0 mm BL and larger collected by HENMI *et al.* (2014) had matured eggs. It is also well known that *E. gilli* utilizes burrows of mud shrimps such as *Upogebia yokoyai*, *U. major* and

*Nihonotrypaea japonica* not only for spawning but for inhabiting (DOTSU, 1955; SUZUKI AND SHIBUKAWA, 2004; HENMI *et al.*, 2014), although the degree of dependence on burrows would be lower in *E. gilli* than in other gobiids such as *Gymnogobius macrognathos* (EGUCHI *et al.*, 2008). In Obitsu-gawa River estuary including the tide-

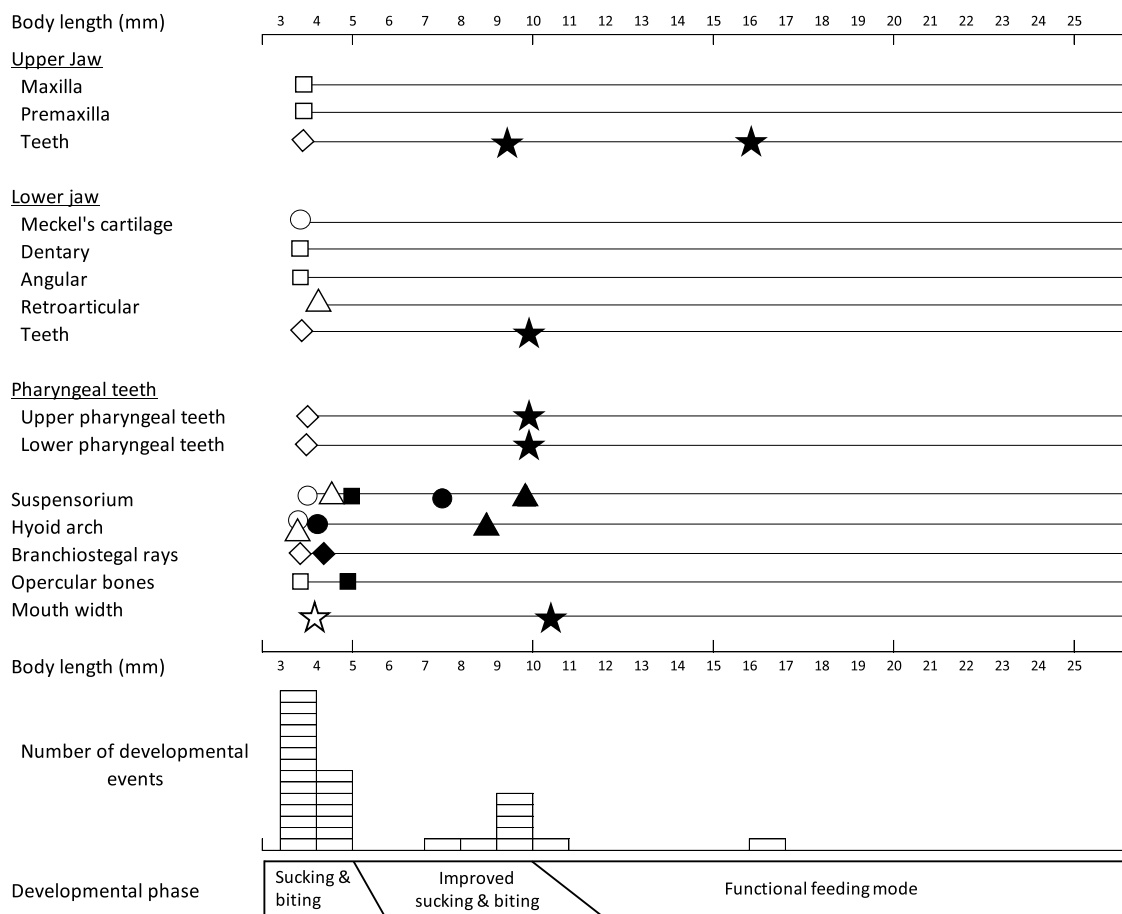


Fig. 8 Schematic representation of the development of feeding-related characters with growth in *Eutaeniichthys gilli* collected from Obitsu-gawa river in the inner Tokyo Bay. ○: cartilaginous elements start appearing; ●: all cartilaginous elements start appearing; □: bony elements start appearing; ■: all bony elements start appearing; △: cartilaginous elements start ossifying; ▲: all cartilaginous elements start ossifying; ◇: teeth and branchial rays start appearing; ◆: branchial rays become complete in number; ☆: mouth opens and premaxilla start appearing; ★: flexion points of teeth number and upper jaw length are observed.

land, the mud shrimp *Nihonotrypaea japonica* and *Upogebia major*, are known to be commonly distributed (KOBAYASHI *et al.*, 2003; ITANI, 2004). These results indicate that it is highly possible that the specimens of about 27 mm BL and larger collected in this study at the creek on the tideland of Obitsu-gawa River estuary would participate in the spawning.

The size of DOTSU's (1955) newly hatched lar-

va, 3.6 mm TL, corresponding to 3.37 mm BL measured from his figure, was measured under the fresh condition. Applying the result of Fukuhara (1979), who pointed out that the shrinkage rate from fresh condition to 3-5 % neutral formalin preserved for one month was 15 % in the BL of newly hatched Japanese red sea bream *Pagrus major* larvae, the size of newly hatched, formalin preserved *Eutaeniichthys gilli* larva is

considered to be 2.86 mm BL, corresponding to the smallest larva collected at the tidal flat of lower stream station in this study, 2.8 mm BL. However, the larvae of 3.9 mm BL and smaller numbered only 3 out of 94 larvae collected at the station in this study (Fig. 3). Furthermore, no larger specimen was collected in the lower stream station. Therefore, it seems improbable that the forehead tidal flat at the lower stream of Obitsu-gawa River would be a spawning ground of *E. gilli*. Rather, the spawning ground would be formed at the tideland in Obitsu-gawa River estuary, and the hatched larvae are considered to be drifted toward the sea by tidal currents and dispersed by coastal currents.

#### 4.3 Importance of Obitsu-gawa River estuary

This study demonstrated that the Obitsu-gawa River estuary including not only river tidal flats but the tideland located in the north of the river mouth provides the spawning ground and nursery and growth habitats for *Eutaeniichthys gilli*.

Nursery and/or growing habitats are observed in such other areas of the inner Tokyo Bay as an artificial tidal flat of Kasai Rinkai Park and a natural tidal flat formed along the artificial Keihin-jima Island. However, most of the specimens collected from these areas are smaller than about 15 mm BL, and individual numbers are less than about 20 during sampling periods varying from 1 to 5 years (KUWABARA *et al.*, 2003; YAMANE *et al.*, 2004; KOHNO *et al.*, 2014; UMEDA AND KOHNO, 2017). Furthermore, no *Eutaeniichthys gilli* specimen was collected at the tidal flat of Keihin-jima Island by NASU *et al.* (1996) and MOTOKI *et al.* (2009). Although KOHNO *et al.* (2014) collected 29 individuals of *E. gilli* at a forehead artificial sandy beach in Haneda during the years of 2006–2013, the size varied from 4.4 to 10.9 mm BL, and 18.7 and 30.1 mm BL.

These results suggest that capacities as nursery/growing habitats in these areas would be small for *E. gilli* when comparing with those in Obitsu-gawa River estuary.

Individuals of 30 mm BL and larger as a mature size of *Eutaeniichthys gilli* were sporadically collected at an intertidal mudflat (33–43 mm BL: KANOU *et al.*, 2005) and tide pools (30–40 mm BL: KANOU *et al.*, 2018) in the Tama-gawa River estuary, although the individual numbers collected are 4 and 9, respectively. SUGAWARA *et al.* (2018) collected one specimen of 35.1 mm BL at a *Zostera* zone of Futtsu in the inner Tokyo Bay. These areas are considered not to be a major spawning ground of *E. gilli*.

In conclusion, *Eutaeniichthys gilli* depends whole the life history on the Obitsu-gawa River estuary, which is almost the only major spawning ground for the species in the inner Tokyo Bay. It is therefore important to protect and conserve the Obitsu-gawa River estuary.

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