Ontogeny of robust tonguefish (*Cynoglossus robustus*) larvae with initially a single, subsequently double-dorsal elongated fin rays in Osaka Bay, eastern Seto Inland Sea, Japan

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Abstract: A total of 40 robust tonguefish (*Cynoglossus robustus*) larvae [2.1–10.2 mm body length (BL)] collected from Osaka Bay, eastern Seto Inland Sea were investigated. Those larvae that were smaller than 4 mm BL possessed a single filament-like elongate fin ray emitting from the most anterior dorsal fin. In larvae of between 4 and 5 mm BL we subsequently observed a second short fin ray, next to the first one. At the preflexion stage, 6.5–7 mm BL, the longer of the elongated fin rays extended to about 60% of BL and the shorter one extended to approximately 30–40% of the length of the longer one. These specimens did not develop transverse bands of melanophores laterally on their tails. These characteristics are the same as those presented in hatchery-reared larvae of the same tonguefish. Osaka Bay is also home to larvae from *C. interruptus* and *C. joyneri*, both with double elongated dorsal fin rays. These larvae can be easily distinguished from larval *C. robustus* by counting the myomeres, dorsal and anal fin rays, and by observing the pigmentation pattern on the tail.

Key words: *Cynoglossus robustus*, larva, morphological characteristic, Osaka Bay, tonguefish

Robust tonguefish *Cynoglossus robustus* Günther, 1873 are commonly found along the southern coasts of Japan, and it is one of the most commercially important tonguefish found in the Seto Inland Sea (Kusakabe 2011, Mototani et al. 2014). In Japan, we find species of cynoglossid larvae with one, two, or six elongated anterior rays in the dorsal fin (Minami 2014). Of these, *C. robustus* larvae are described as having a somewhat flag-like single elongated ray (Fujita & Uchida 1957, Minami 2014) and obscure transverse pigment bands on the lateral surface of the tail (Minami 2014). However, Hirooku et al. (2013) reported that the initial elongated fin ray increases to two rays in hatchery-reared *C. robustus*. Further, Omi et al. (2019) reported that cynoglossid larvae collected in Osaka Bay, that had the characteristic numbers of dorsal and anal fin rays of adult *C. robustus*, had double elongated fin rays and no pigment bands on the lateral surfaces of their tails. In Fujita & Uchida (1957) and Hirooku et al. (2013), the morphology of hatchery-reared *C. robustus* larvae was reported, while Omi et al. (2019) only looked at the morphology of wild larvae larger than 9.5 mm standard length (SL). The ontogeny of wild *C. robustus* larvae is still unclear, so herein we describe the ontogeny of *C. robustus* larvae on the basis of wild-caught specimens from Osaka Bay.

Larvae were collected using a larval net (net mouth diameter 45 cm; mesh size 0.335 mm) from the R/V Osaka, which belongs to the Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture. Sampling was undertaken at 20 stations in Osaka Bay, the eastern Seto Inland Sea, once a month between 2009 and 2018 (Fig. 1). Vertical tows using the net started at 1 m above the seafloor and ended at the surface. However, at deeper stations, where the sea floor was deeper than 50 m, we set a maximum vertical tow distance of 50 m for our collections. Samples were immediately fixed in 5–10% formalin seawater on board and finally preserved in 10% buffered formalin.

Because Kusakabe (2011) revealed that the spawning season for *Cynoglossus robustus* in Osaka Bay is from June to August, we only examined the samples collected from June to November each year for descriptions of larval development. Cynoglossid larvae were sorted based on the work of Minami (2014) and developmentally staged according to the guidelines established by Kendall et al. (1984). Following stage estimation, we measured their body length (BL) including notochord length for preflexion and flexion larvae and standard length in postflexion larvae. We measured preanal length

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(PAL) from the tip of the head to the start of the anal fin and body depth (BD) at the start of the anal fin. We also counted the number of myomeres and completed fin rays. Larvae with one elongated fin ray on the dorsal fin were identified as *C. robustus* by Fujita & Uchida (1957) and Hirooku et al. (2013). Omi et al. (2019) reported that cynoglossid larvae from Osaka Bay, with double elongated dorsal fin rays belonged to one of three species, *Cynoglossus interruptus* Günther, 1880, *Cynoglossus joyneri* Günther, 1878 and *C. robustus*. Omi et al. (2019) counted the myomeres and grouped specimens with 50 or less into one of the first two species mentioned above and those with 58–59 as the last species, respectively. Using this information, we classified larvae with double elongated fin rays, and 55 or more myomeres as *C. robustus*. A representative series of the specimens used in this study were deposited in the Usa Institute of Marine Biology, Kochi University (UKU502011–502014). A total of 40 specimens of *C. robustus* larvae were investigated, with a BL that ranged from 2.1–10.2 mm, and 55–59 myomeres, countable in the larvae larger than 4.2 mm (Table 1).

Preflexion larvae, specimens smaller than 4 mm BL, had a single elongated dorsal fin ray (Table 1, Fig. 2a). The bud of the second elongated fin ray appeared just behind the first ray at 4–5 mm BL in preflexion larvae (Fig. 2b). In 6.5–7.0 mm BL preflexion larvae, the first elongated fin ray extended to about 60% of BL and the second elongated fin ray reached about 30–40% of the length of the first one (Fig. 2c, 2d). Common dorsal and anal fin rays began to differentiate from the posterior ends along the tail at 7 mm BL (Fig. 2d). Notochord flexion began between 7–9 mm BL. In 9.6 mm BL post flexion larva, all dorsal, anal and caudal fin rays had formed (Fig. 2e, Omi et al. 2019). The number of completed dorsal and anal fin rays was between 125–131 and 99–102, respectively (Table 1). In all specimens, the pectoral fin was large and paddle-shaped but no fin rays developed and the right-eye never migrated. The gut was coiled and gradually protruded beyond the ventral body margin. The rostral hook was well developed during the flexing of the notochord. The BD/BL ratio was 3.4–15.3% with deepening at the tail. The PAL/BL ratio was relatively stable during the larval period at 25.5–36.5%. We observed clusters and longitudinal series of melanophores along the dorsal and ventral margins of the tail. We also saw small melanophores scattered on the upper opercular margin, cleithral symphysis, ventral gut, anus and

![Image](image_url)

**Fig. 1.** Ichthyoplankton sampling stations in Osaka Bay. Solid triangles indicate the stations sampled with a larva net between 2009 and 2018.

| Number of elongated anterior dorsal fin rays | BL (mm) | Count of myomeres | Number of completed fin rays | BD/BL (%) | PAL/BL (%) | Number of specimens |
|---------------------------------------------|--------|-------------------|-----------------------------|-----------|------------|---------------------|
| 1                                           | 2.1–4.0| —                 | —                           | 3.4–6.1   | 25.5–34.2  | 25                  |
| 2                                           | 4.2–10.2| 55–59            | 125–131                     | 4.8–15.3  | 27.8–36.5  | 15                  |

**Fig. 2.** Developmental stages of *Cynoglossus robustus* from Osaka Bay. a: 2.7 mm BL preflexion larva (UKU502011); b: 4.5 mm BL preflexion larva (UKU502012); c: 6.5 mm BL preflexion larva (UKU502013); d: 7.0 mm BL preflexion larva (UKU502014); e: 9.6 mm BL postflexion larva from Omi et al. 2019 (UKU502010).
elongated dorsal fin rays. During this study we did not see any pigment bands on the lateral surfaces of the tail.

In our wild Cynoglossus robustus larvae, a single elongated dorsal fin ray was observed in the head region of specimens under 4 mm BL. The second elongated fin ray appeared just behind the first one. This unique phenomenon has previously been observed in hatchery-reared C. robustus (Hirooku et al. 2013). Studies on the ontogeny of Cynoglossus larvae, C. interruptus, C. joyneri, C. lighti Norman, 1925, C. gracilis Günther, 1873, C. itinus (Snyder 1909), C. macrostomus Norman, 1928, and C. zanzibarensis Norman, 1939, all with double dorsal fin rays, did not report increases in the elongated dorsal fin rays like we observed in C. robustus (Dileep 1989, Uyeda & Sasaki 2000, Wood 2003, Yagi et al. 2009, Omi et al. 2019). Therefore, it is likely that this specialized ontogeny only occurs in C. robustus.

The maximum size of the Cynoglossus robustus pelagic larvae from this study was 10.2 mm BL (Table 1). This is in line with the minimum size (9.6 mm BL) of the settled juvenile specimens in Omi et al. (2017). Furthermore, no larvae with a migrating right eye were collected in this study. Rapid metamorphosis has been observed in Cynoglossus abbreviates (about 37 hours) and Cynoglossus capensis (a single night) (Brownell 1979, Fujita et al. 1986). This suggests that eye migration in C. robustus is synchronized with their settlement and occurs rapidly.

There were no pigment bands on the tail of the Cynoglossus robustus larvae in this study. However, C. robustus larvae described by Minami (2014) did have obscure transverse bands, and they maintained the single elongated dorsal fin ray up to 10.1 mm BL. It is likely that other cynoglossid larvae were identified as C. robustus in Minami (2014). In Osaka Bay, Cynoglossus interruptus and Cynoglossus joyneri larvae appear sympatrically with C. robustus and they have double elongated dorsal rays (Omi et al. 2019). This study is the first that describes a way to distinguish C. robustus larvae with double elongated dorsal fin rays from the other two Cynoglossus larvae using the following characteristics: myomeres ≤50, dorsal 115 and anal rays ≥88 for C. interruptus and C. joyneri, respectively, and a row of pigmentation along the lateral midline for C. interruptus and scattered pigmentation, near the center of the tail for C. joyneri.

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