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PELAGIC LARVAL DURATION AND MORPHOLOGY AT RECRUITMENT OF *STIPHODON PERCNOPTERYGIONUS* (GOBIIDAE: SICYDIINAE)

Nozomi Yamasaki* and **Ken Maeda**
Graduate School of Engineering and Science, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Nakagami-gun, Okinawa 903-0213, Japan
Email: stiphodon@hotmail.com
*(*Corresponding Author)*

Katsunori Tachihara
Laboratory of Fisheries Biology & Coral Reef Studies, Faculty of Science, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Nakagami-gun, Okinawa 903-0213, Japan
Email: ktachiha@sci.u-ryukyu.ac.jp

***ABSTRACT.* –** We examined the pelagic larval duration (PLD) of *Stiphodon percnopterygionus* (Gobiidae: Sicydiinae) using the sagittal otoliths of pelagic larvae, settled larvae and juveniles before or after recruitment into streams on Okinawa Island, Southern Japan. We also provide descriptions of larval morphologies. Pelagic larvae [13.5 - 14.2 mm in standard length (SL)] just before recruitment were collected at the surf zones of beaches near mouths of streams. The larvae had a transparent body, a conspicuous swim bladder and emarginate caudal fin. Compared with the pelagic larvae, settled larvae (12.7 - 13.6 mm SL) collected at freshwater areas of streams were more pigmented, but they were still essentially transparent with an emarginate caudal fin. The PLD of *S. percnopterygionus* was estimated to be 78 - 146 days (mean ± SD = 99 ± 16 days; n = 45).

***KEY WORDS.* –** Otolith, pelagic larval duration, recruitment, Sicydiinae, *Stiphodon*.

INTRODUCTION

Adult *Stiphodon percnopterygionus* are found in riffles and pools from the upper to middle reaches of streams (Iwata, 1989; Watson & Chen, 1998) where they spawn (Yamasaki & Tachihara, 2006). Sicydiine gobies, including *S. percnopterygionus*, have an amphidromous life cycle, in which hatched larvae are swept out to sea immediately where they develop as pelagic larvae before recruitment into streams (McDowall, 2004). Little is known about their pelagic larval duration (PLD) (Bell et al., 1995; Radtke & Kinzie, 1996; Shen et al., 1998; Radtke et al., 2001) which is a key part of their biology.

Otoliths of teleost fishes are located in the membranous labyrinth of the inner ear and contain a large amount of ecological information about the individual. In previous studies, otoliths were used in two different ways to estimate the PLD of amphidromous gobies. Radke & Kinzie (1996), Radtke et al. (1988, 2001), Bell et al. (1995) and Shen et al. (1998) estimated the PLD of amphidromous gobies from the number of daily growth increments from the core to the check mark in the otoliths. This is based on the assumption that the check mark was deposited at recruitment. Another method used to estimate the PLD of amphidromous gobies is to count the daily growth increments from the core to the edge of otoliths from larvae just before or after recruitment into streams (Bell et al., 1995; Radtke et al., 2001).

The purpose of the present study are as follows: 1) to examine the larval morphology of *S. percnopterygionus*; 2) to validate the daily periodicity of otolith increments for *S. percnopterygionus* using an alizarin complexone marking technique and 3) to determine the PLD by counting the daily growth increments from the core to the check mark in the otoliths, or by counting of daily growth increments from the core to the edge in the otoliths of larvae just before and after recruitment.

MATERIALS AND METHODS

***Collection of pelagic larvae.* –** Using a small seine net (mesh size, 1.0 mm; height, 0.8 m; width, 3.5 m; cod end with a depth of 1 m and diameter of 0.7 m; sinkers attached along the bottom edge), pelagic larvae (n = 8) were collected in the surf zones of the sandy beach at Sedake and the stony beach at Aritsu on Okinawa Island, Southern Japan. These beaches were conterminous with the stream mouths of the Sedake and Aritsu Streams respectively (Fig. 1). Poles were set on both

sides of the net and the net was hauled parallel to the shoreline at a depth of 0.2 to 1.0 m. All collections were carried out at night (1 to 4 nights per month) from December 2003 to December 2004 at random tidal stages. On each night, the net was hauled for 30 to 400 m at Sedake Beach and/or Aritsu Beach. The salinity and water temperature at both beaches ranged from 14 - 35 ppt and 17.7 - 29.9°C, respectively. All specimens were brought to the laboratory after chilled fixation. Their standard length (SL) in mm was measured using a micrometer under a stereomicroscope (SMZ800, Nikon). After extraction of the sagittal otolith, all specimens were fixed in 10% formalin. Two pelagic larvae specimens used for sketching were fixed without extraction of the sagittal otolith. An additional specimen (13.8 mm SL) collected in the surf zone at Sedake Beach on 14 August 2004 was kept in the laboratory for 8 months to confirm the larval identification of the species.

Collection of settled larvae and juveniles. – Eleven settled larvae and 44 juveniles were collected using a hand net at the Aha and Teima Streams on Okinawa Island (Fig. 1) from October 2001 to November 2002. The sampling sites were freshwater areas and were located approximately 1.8 and 3.3 km upstream from their mouths, respectively. After SL measurements, all specimens were fixed in 99% ethanol. In addition, a settled larva used for sketching was collected at a freshwater area located approximately 0.8 km upstream from the mouth of the Yona Stream on Okinawa Island (Fig. 1) in April 2004 and was fixed in 10% formalin.

The developmental stages were classified as "pelagic larva", "settled larva", "juvenile" and "adult" using the classification systems of Leis & Carson-Ewart (2000) and Maeda & Tachihara (2005). Transparent larvae collected from water column of the surf zones using the small seine net were classified as pelagic larvae. The pigmented larvae collected from the bottom of freshwater streams using the hand net were classified as settled larvae. The immature fish that had lost all temporary specializations for pelagic life, such as a less pigmented body and emarginate caudal fin were classified as juveniles and specimens > 20 mm SL were classified as adults, because both males and females mature at approximately 20 mm SL (Yamasaki & Tachihara, 2006).

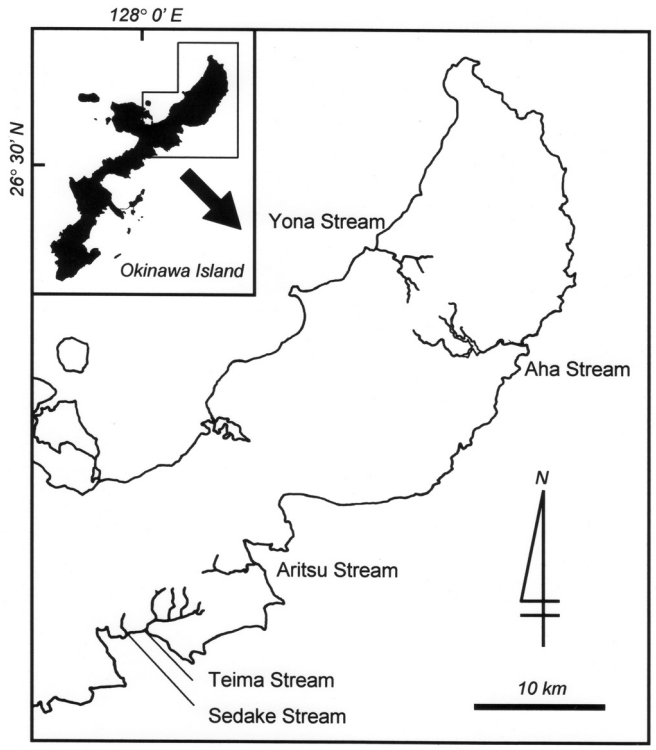

Fig. 1. Map showing the sampling sites of *Stiphodon percnopterygionus* on Okinawa Island. The beaches where the pelagic larvae were collected are conterminous with the mouths of Aritsu and Sedake Streams.

Otolith increment periodicity. – To validate the daily periodicity of otolith increments, 3 settled larvae collected in the Aha Stream were kept in a 5 L plastic container containing freshwater for a day and were then treated with otolith-marking methods using alizarin complexone (ALC). The larvae were transferred into a 2 L plastic bottle containing the ALC solution (50 mgL^{-1}) for 24 hours. The larvae were then kept in an 30 L aquarium for 9 days before further treatment with the ALC solution for 24 hours. After an additional 40 days of holding in the aquarium, the otoliths of the 3 larvae were extracted and embedded on a glass slide using clear nail varnish. The presence and location of the 2 alizarin-stained bands were confirmed under a light microscope (MICROPHOT-FXA, Nikon) fitted with a ultra-violet light source. The number of increments between the 2 alizarin-stained bands was counted in photographs taken with a digital camera (COOLPIX5400, Nikon) attached to a light microscope (ECLIPSE E600, Nikon) and compared with the interval between ALC treatments (i.e. 9 days).

Otolith increment analysis. – The sagittal otoliths of the pelagic larvae, settled larvae and juveniles were extracted under a stereomicroscope and embedded on glass slides using clear nail varnish. There was no need for polishing or cleaning the otoliths. The increments from the core to the edge and from the core to the check mark (Fig. 2) in the otoliths of the pelagic larvae, settled larvae and juveniles were counted in photographs taken by the digital camera attached to the light microscope. The birth months of the pelagic larvae, settled larvae and juveniles were calculated from the sampling dates and the number of increments from the core to the edge in

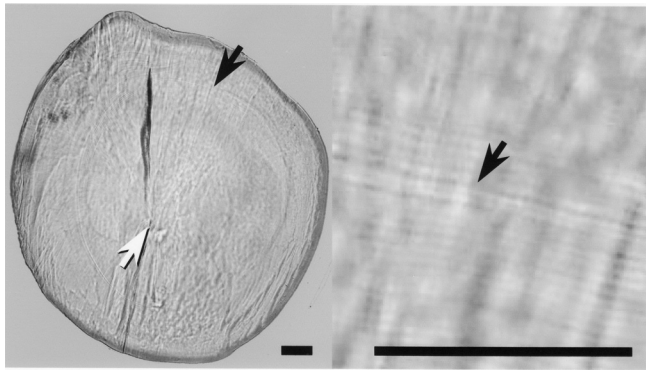

Fig. 2. Sagittal otolith of *Stiphodon percnopterygionus* juvenile (18.1 mm SL) collected at Aha Stream on Okinawa Island in November 2001. Left = entire otolith; Right = magnification of area around check mark; white arrow = otolith core; black arrow = the check mark. Scale bars = 0.05 mm.

Table 1. Sampling location, sampling month, standard length and ventral pigmentation of type A and type B *Stiphodon* larvae collected in the surf zones of two beaches on Okinawa Island.

Stiphodon Types	Sampling Location	Sampling Month	n	Standard Length (mm)		Ventral Pigmentation
				Range	Mean ± SD	
Type A	Sedake, Aritsu	May, August, November	4	13.5 - 14.2	13.9 ± 0.3	Present
Type B	Aritsu	April, June	4	12.3 - 15.3	13.5 ± 1.3	Absent

n = number of *Stiphodon* larvae collected.

their otoliths. It is not known when the first increment was deposited in the otolith of *S. percnopterygionus* but we assumed that it was deposited at hatching (Bell et al., 1995). Adult specimens (> 20 mm SL) were not included in the counts of increments because the increments at the otolith edge of adults were unclear and hence uncountable.

RESULTS

***Description of pelagic larvae and settled larvae.* –** Two types of pelagic larvae of *Stiphodon* were collected in the surf zones of Sedake Beach and Aritsu Beach in April, May, June, August and November 2004. All of the larvae had transparent bodies, conspicuous swim bladders immediately anterior to the anus and emarginate caudal fins. Fin ray counts of the pelagic larvae were as follows: dorsal fin, V ~ VI - I, 10; anal fin, I, 10; pectoral fin, 14; pelvic fin, I, 5. The pelagic larvae had conspicuous external melanophores at the tip of both the upper and lower jaws, as well as on the dorsolateral part of the caudal fin base. The pelagic larvae also had conspicuous internal melanophores above the posterior end of the anal fin base towards the lateral midline. Additionally, the pelagic larvae had conspicuous erythrophores with internal melanophores above the posterior end of the anal fin base toward the lateral midline and with external melanophores on the caudal fin base. These features were common to both types of pelagic larvae. Type A larvae (13.5 - 14.2 mm SL; mean ± SD = 13.9 ± 0.3 mm SL; n = 4), which were collected at Aritsu Beach and Sedake Beach in May, August and November 2004, had ventral pigmentation (a single mid-ventral row of melanophores reaching from the origin of the pelvic disk to the middle of the pelvic disk, a double row originating at the origin and extending past the edge of the disk, a triple row on either side along and on the anal fin, as well as a single row along the ventral midline of the caudal peduncle) (Table 1 & Fig. 3). In contrast, type B larvae (12.3 - 15.3 mm SL; mean ± SD = 13.5 ± 1.3 mm SL; n = 4), which were collected at Aritsu Beach in April and June 2004, had no ventral pigmentation (Table 1 & Fig. 4). One specimen of a type A pelagic larva (13.8 mm SL) collected at the surf zone of Sedake Beach grew to 21.6 mm SL after eight months of rearing and was identified as *S. percnopterygionus* using the classification system of Watson & Chen (1998). We were unable to identify the type B pelagic larvae as we did not rear any specimens. Hence, hereafter, we refer to type A larvae as *S. percnopterygionus* and type B larvae as *Stiphodon* sp.

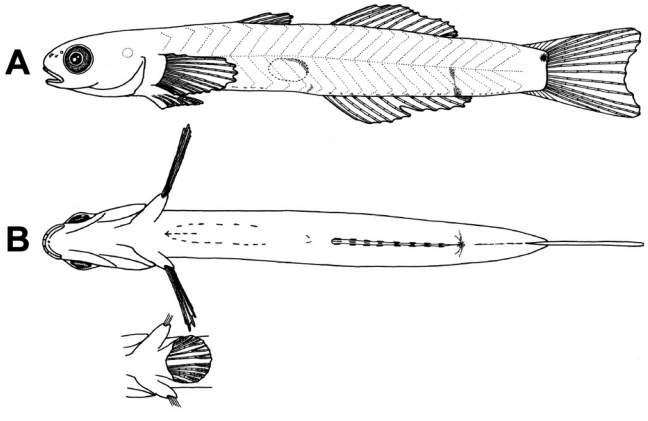

Fig. 3. Pelagic larvae of *Stiphodon* type A (14.0 mm SL) collected in the surf zone of Aritsu Beach on 29 May 2004. A) lateral view, B) ventral view. Pelvic disc was drawn separately to show the ventral pigmentation.

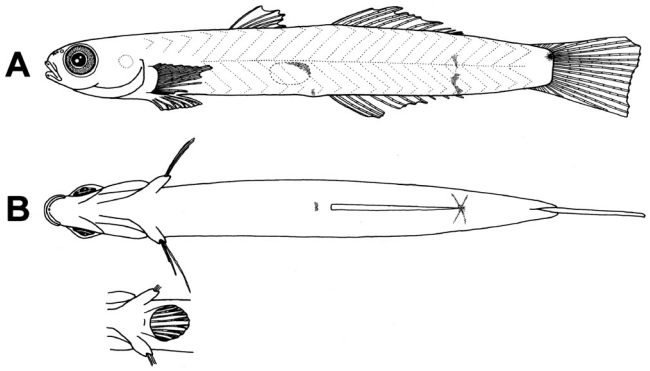

Fig. 4. Pelagic larvae of *Stiphodon* type B (15.3 mm SL) collected in the surf zone of Aritsu Beach on 12 April 2004. A) lateral view, B) ventral view. Pelvic disc was drawn separately to show the ventral pigmentation.

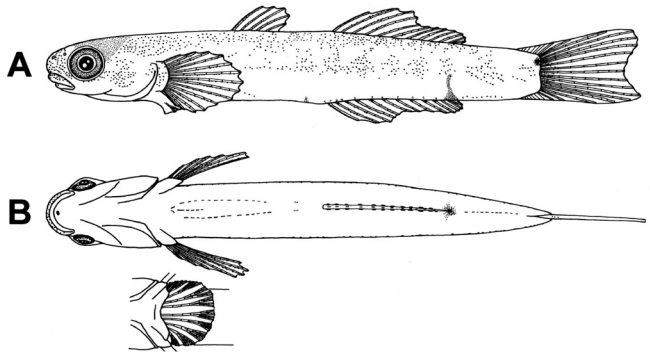

Fig. 5. Newly-settled larva (12.2 mm SL) of *Stiphodon percnopterygionus* collected at Yona Stream on 29 April 2004. A) lateral view, B) ventral view. Pelvic disc was drawn separately to show the ventral pigmentation.

Table 2. Stage, environment, standard length, age, number of fish with check mark and pelagic larval duration (PLD) based on the increments from the core to the edge and PLD based on the increments from the core to the check mark of pelagic larvae, settled larvae and juveniles of *Stiphodon percnopterygionus*.

Stage	Environment	No. of Fish	Range of Standard Length (mm)	Age (days)	No. of Fish Displaying Check Mark	PLD (days) Based on the Increments to the Edge		PLD (days) Based on the Increments to the Check Mark	
						Range	Mean ± SD	Range	Mean ± SD
Pelagic larvae	Surf zone	2	13.5 - 14.2	98 - 110	0	98 - 110	-	-	-
Settled larvae	Freshwater area	10	12.7 - 13.6	83 - 123	7	83 - 99	93 ± 9	80 - 110	94 ± 12
Juveniles	Freshwater area	33	14.0 - 19.6	103 - 182	33	-	-	78 - 146	100 ± 18

Settled larvae (12.2 - 13.6 mm SL; n = 11) of *S. percnopterygionus* were collected in the Aha and Yona Streams in June, July, September and October 2002 and April 2004. Compared with pelagic larvae (type A), the settled larvae were more pigmented although they were still essentially transparent. The caudal fin of settled larvae was emarginate (Fig. 5) and no erythrophores were observed in the settled larvae. There are no settled larvae without ventral pigmentation such as in type B of pelagic larvae.

Validation of daily increments in otoliths. – All the otoliths of the three settled larvae of *S. percnopterygionus* subjected to the ALC treatment had two bands stained with ALC (ALC checks). The two bands were observed under UV light and the mean number of rings (increments) between the two alizarin-stained bands was 8.7 (9, 9, 8; n = 3; Fig. 6) and this matches the number of interval days between the two ALC treatments (i.e. 9 days).

Age of larvae and juveniles. – In addition to the four pelagic larvae collected at the beaches and 11 settled larvae collected in the streams, 44 juvenile *S. percnopterygionus* were collected in the streams from October 2001 to November 2002. The daily growth increments from the core to the edge of the otoliths were countable in two pelagic larvae (13.5 and 14.2 mm SL, respectively), 10 settled larvae (12.7 - 13.6 mm SL) and 33 juveniles (14.0 - 19.6 mm SL). Age was estimated as 98 - 110 days for these pelagic larvae, 83 - 123 days for settled larvae and 103 - 182 days for juveniles, respectively (Table 2). An obvious check mark was found in the otoliths of all juveniles and seven settled larvae. Their PLD were estimated by counting the daily growth increments from the core to the check mark. No obvious check marks were found in the otoliths of pelagic larvae as they were at a stage just before settlement or were newly-settled. Their PLD were estimated by counting the daily growth increments from the core to the edge.

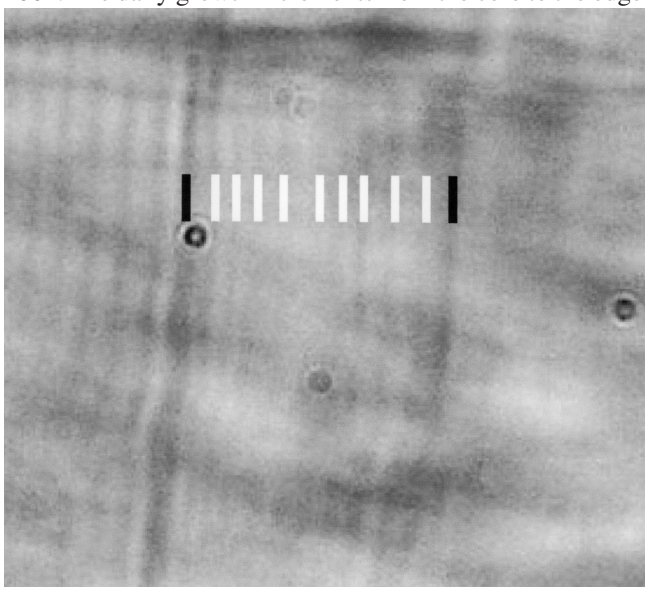

Fig. 6. Sagittal otolith of *Stiphodon percnopterygionus* immersed in an alizarin complexone solution twice with a nine-day interval. Black bars = increments stained with alizarin complexone; white bars = unmarked increments.

Pelagic larval duration. – PLD was estimated as 98 - 110 days for pelagic larvae (n = 2), 80 - 110 days for settled larvae (n = 10) and 78 - 146 days for juveniles (n = 33) (Table 2). Thus, the PLD of *S. percnopterygionus* was estimated as 78 - 146 days (mean ± SD = 99 ± 16 days; n = 45).

The estimated birth months of *S. percnopterygionus* (based on the pelagic larvae, settled larvae and juveniles) were February to August, October and November. We found differences in PLD between different birth months: specimens born in February or March had a longer PLD than specimens born in the other months (Table 3).

DISCUSSION

Morphology. – This is the first study to examine the morphology of *Stiphodon* larvae. The sicydiine gobies collected in the seagrass beds and beaches of Lombok Island, Indonesia (Sicydiine type B of Harada & Suharti, 2000) also had similar developmental stages, sizes, proportions and pigment patterns to those observed in the present study. In spite of the similarities between the larvae of *S. percnopterygionus* and Sicydiine type B of Harada & Suharti (2000), the latter had the following pigmentation patterns that we did not observed in *S. percnopterygionus*: one stellate melanophore on the ventral part of the foregut and scattered melanophores on the dorsal part of the midbrain.

All of the present *S. percnopterygionus* larvae that we collected in the surf zones were pelagic larvae with transparent bodies, a conspicuous swim bladder and complete fin rays.

Table 3. Range and mean of pelagic larval duration (PLD) of *Stiphodon percnopterygionus*, by birth month.

Birth Month	No. of Fish	PLD (days)	
		Range	Mean ± SD
January	0	-	-
February	5	93 - 146	114 ± 20
March	8	89 - 128	110 ± 12
April	7	84 - 121	99 ± 13
May	2	95 - 96	96 ± 1
June	10	78 - 130	97 ± 20
July	5	80 - 106	91 ± 11
August	6	81 - 105	90 ± 10
September	0	-	-
October	1	85	-
November	1	84	-
December	0	-	-

We did not find any more-developed larvae (such as the newly-settled larvae collected in the freshwater area) or less-developed larvae (such as pre-flexion larvae, with fanfold, or without complete fin rays) in these areas. This suggests that *S. percnopterygionus* larvae stay in the surf zones for a short period just before recruitment into the streams.

***Validation of daily increments in otoliths.* –** In the present study, the number of increments between the two alizarin-stained bands (mean, 8.7; n = 3) coincided with the number of days in the interval between the ALC treatments (i.e. nine days), confirming the daily periodicity of increment formation, at least for the period from settled larva to juvenile. As we used settled larvae to confirm the daily periodicity of increments, we could not confirm if daily increments occur before settlement. However, the increments before settlement are probably also deposited daily, because there have been no reports of differences in the deposition cycle of otolith increments between pre-settlement larvae and post-settlement larvae.

***Pelagic larval duration.* –** In present study, the PLD of *S. percnopterygionus* was estimated as 78 - 146 days (mean: 99 days). This PLD is longer than the one to two months reported for marine gobies (Brother et al., 1983; Shafer, 1998) but it corresponds to the PLD reported for other sicydiine gobies (Bell et al., 1995; Radtke & Kinzie, 1996; Shen et al., 1998; Radtke et al., 2001).

The PLD of present specimens born in February or March were longer than those of specimens born in the other months. Although we did not observed clear seasonal differences in PLD (probably because of the random collection method and small sample size), it appears that PLD was longer for larvae born in colder months. We expect that further studies may reveal clear seasonal changes in PLD of *S. percnopterygionus*.

The estimated birth months based on the present specimens were February to August, October and November. This disagrees with their reported spawning season of May to December on Okinawa Island (Yamasaki & Tachihara, 2006). The Kuroshio Current flowing Northeastward near Okinawa Island may explain this inconsistency between the spawning season on Okinawa Island and the estimated birth months of the present specimens. We hypothesize that some of the *S. percnopterygionus* recruits on Okinawa Island were carried by the Kuroshio Current from Southern regions where *S. percnopterygionus* spawns for longer periods. However, this hypothesis is based purely on the inconsistency between the spawning season and the present estimated birth months and requires confirmation by further detailed studies of larval dispersal of gobioid fish that includes *S. percnopterygionus*.

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