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Short Note

Validation of otolith daily increments in the amphidromous goby *Sicyopterus japonicus*

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Abstract — To determine the periodicity of growth increment deposition in the otoliths of newly recruited larvae of the amphidromous goby *Sicyopterus japonicus*, an otolith validation experiment was performed. Larval gobies collected at the river mouth of the Ota River, Wakayama, Japan were immersed in an alizarin complexone (ALC) solution to mark the timing in their otoliths. After being held under natural light conditions for 15 days, they were treated with ALC again to make the second time mark. The number of growth increments between two ALC marks in the otoliths was 14.1 on average, which roughly coincided with the number of days between two ALC treatments. This experiment validated the daily periodicity of otolith growth increment deposition in *S. japonicus* showing that the otolith increments of the species can be used for age determination with daily precision.

Key words: *Sicyopterus japonicus*, amphidromy, otolith, daily increment, ALC mark, daily ring

Introduction

Sicyopterus japonicus (Teleostei: Gobiidae: Sicydiinae) is an amphidromous goby (McDowall, 1988) that spawns in freshwater, and their newly hatched larvae migrate downstream to the sea where they have their larval life before migrating back to rivers to grow and reproduce (Iida et al. 2009). The subfamily Sicydiinae includes about 110 species that all have amphidromous life histories (Froese and Pauly 2009). Their oceanic larval duration (OLD) has been reported to be as long as 50 to 270 days (Bell et al. 1995, Radtke et al. 2001, Hoareau et al. 2007, Yamasaki et al. 2007, Lord et al. 2010). For *S. japonicus* that distribute from Taiwan to Fukushima, northern part of Japan, their OLD was reported to be more than 130 days (Iida et al. 2008, Shen and Tzeng 2008). Such a long OLD in Sicydiinae fishes is considered to show a tendency for their necessity of the oceanic larval life (Watanabe et al. 2006, Iida et al. 2008, 2009). However, those studies of *S. japonicus* have been done based on assumption of daily deposition of otolith growth increments, although a few validation studies on the deposition rate of otolith increments have been done in two sicydiine species *Sicyopterus lagocephalus* on La Réunion Island (Hoareau et al. 2007) and *Stiphodon percnopterygionus* in Okinawa (Yamasaki et al. 2007).

The objective of this study was to validate the daily deposition of the growth increments in the otoliths of *S. japonicus* using an alizarin complexone (ALC) time marking technique.

Materials and Methods

Newly recruited larvae of *Sicyopterus japonicus* (N>200, ca. 30 mm in total length) were collected by a square lift net in the mouth of the Ota River, Wakayama, Japan on April 20, 2008. These larvae were brought alive to the laboratory, where they were acclimated in a 20 l aerated freshwater tank. An otolith marking method was applied after Tsukamoto (1988). Randomly selected 62 larvae were transferred into another 20 l fresh water aquarium containing a 20 mg/l solution of alizarin complexone (ALC) in well water and were held there for 24 h. Although six individuals died during ALC immersion, the surviving 56 larvae were kept afterwards in freshwater in a 20 l aquarium for 15 days before the second treatment with the ALC solution for 24 h. After an additional rearing for 6 days in the aquarium, all the specimens were sacrificed and preserved in 99% ethanol. By allowing 6 additional days of potential otolith growth, the difficulty in discriminating the last few increments that were formed at the otolith edge (the edge effect) using optical microscope techniques could be eliminated (Campana and Neilson 1985). The aquarium was kept under natural light conditions and with a water temperature at ca. 19°C. Fish were not fed during the experiment to avoid the additional effect of feeding on otolith increment deposition and because no feeding behavior was observed in the estuary during their recruitment to river.

Randomly selected 30 specimens were used for otolith analysis. A sagittal otolith was extracted from each fish and mounted on a glass slide with euparal (Chroma-Gesellschaft

Schmid GmbH & Co.), and examined at 50–500× under an optical microscope and was photographed using a digital camera (Nikon, digital camera DXM1200F). The location of the fluorescent ALC mark was determined under UV light using an optical microscope (Nikon, Optiphoto-2) fitted with a fluorescence attachment (Nikon, EFD2), and the otolith microstructure was photographed at a magnification of ×400 using a digital camera. The center of ALC marks were assumed as the start and the end of the experiment. The number of increments between the two ALC marks was counted in the photographs.

To verify that otolith observations made without grinding were accurate, some otoliths were checked after grinding. Randomly selected 3 otoliths were embedded in epoxy resin (Epofix, Struers) and then ground to expose the edge, using a grinding machine equipped with a diamond cup wheel (Discoplan-TS, Struers), and further polished with OP-S (Struers) on an automated polishing wheel (Planopol-V, Struers). These three otoliths were observed under transmitted light microscope and it was confirmed that the observed position of ALC marks was not different before and after grinding.

To compare the number of rings between ALC marks and the days of rearing duration, a chi-square test was carried out. Significant difference was determined at the 0.05 probability level.

Results and Discussion

Sicyopterus japonicus larvae were successfully marked with an alizarin complexone (ALC) solution (Fig. 1). Of the 30 otoliths examined, 20 otoliths with clear ALC marks were selected and the number of rings between the two ALC marks was counted. The mean number of increments was 14.1, ranging from 12 to 16 (Fig. 2). The number of observed rings and the days of rearing duration were not significantly different from 1 : 1 (chi-square test, $p>0.05$). This indicated that the increments observed in the sagittal otoliths of *S. japonicus* larvae were deposited daily. A slightly lower number of otolith increments observed in ALC marked fish might be a result of some physiological stress during ALC treatment that appear to stop daily deposition for a few days, while two individuals with 16 increments might be counting error for narrow faint increments. It is generally believed that the wild fish deposit clearer otolith increments than those of reared fish because the wild fish experience a stronger environmental rhythm to control the daily deposition of otolith increments than fish under rearing conditions (Campana and Neilson 1985). The experiment was done under unfed condition, and this might be one factor contributing to a slightly lower number of increments.

This result was consistent with the daily increment deposition in the otoliths of other Sicydiine gobies, *Sicyopterus lagocephalus* (Hoareau et al. 2007) and *Stiphodon percnopterygionus* (Yamasaki et al. 2007), although there was no description about the experimental temperature in these studies. We conducted our experiment at 19°C, which was chosen as the water temperature because it was the same as temperature at the surf zone near the river mouth of the Ota River in April. Although the early life history of the Sicydiine gobies in the ocean is totally unknown because of only a few collection data, and therefore, the water temperature they experience in the ocean is unclear, the experimental temperature of 19°C used in the study appears not to be so different from their natural environmental condition, at least during their recruiting season in spring. Therefore the result of the present study might be almost the same as that of in natural condition. We could conclude that *S. japonicus* has daily incre-

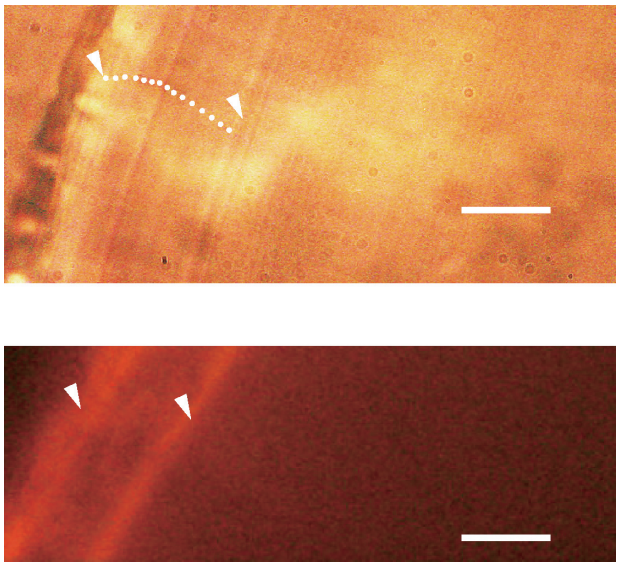

Fig. 1. Photographs of a sagittal otolith of a larval *Sicyopterus japonicus* reared for 15 days following the first alizarin complexone (ALC) mark, and for an additional 6 days after the second ALC mark. Top panel was viewed in visible light and bottom panel in fluorescent light. Scale bars indicate 10 μm.

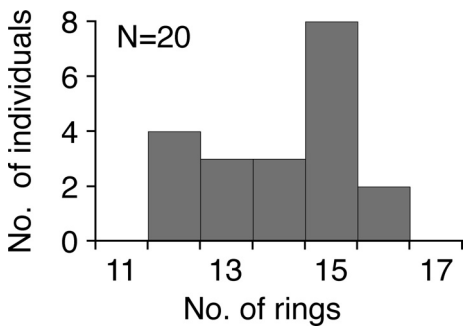

Fig. 2. Frequency distribution of number of rings between the two alizarin complexone marks in sagittal otoliths of *Sicyopterus japonicus* larvae that were reared for 15 days between two ALC treatments.

ment deposition in their larval saggital otoliths and that increment counts can be used for the estimation of oceanic larval duration (OLD) with daily precision.

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