

**Otolith Increment Formation and Planktonic Larval  
Duration of a Temperate Damsel fish,  
*Chromis notatus notatus***

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The early life history of tropical damselfishes has been well studied in the field, with age estimation techniques utilizing otolith daily increments being established (e.g., Thresher et al., 1989). Otoliths of *Amphiprion clarkii* and *Pomacentrus amboinensis* have a check mark, which is associated with settlement (Thresher et al., 1989). The check mark has been used to estimate the duration of larvae in the plankton and their pre- and post-settlement growth rates (e.g., Wellington and Victor, 1989; Thorrold and Milicich, 1990). However, little is known about the early life history of temperate damselfishes.

The present study deals with a temperate species, *Chromis notatus notatus*, which occurs around Japan to the East China Sea and attains 12 cm in total length (TL). The time of first otolith increment deposition was determined and daily periodicity of increments in the juvenile stage validated. In addition, the microstructure of otolith increments was examined and ages of juveniles estimated for comparison of the planktonic larval duration with that of other damselfishes.

**Materials and Methods**

Spawning of *Chromis notatus notatus* at rocky reefs off Tsuyazaki, northern Kyushu, Japan (33°47'N, 130°29'E) occurs from the middle of June to the middle of August (Nakazono et al., 1979). Underwater observations at the same site in 1988 confirmed the above spawning period, newly settled juveniles first being observed on July 28. An egg mass of *C. n. notatus* collected at Tsuyazaki on August 6, 1991, hatched in a 30 l aquarium on the same day. Newly-hatched larvae were fixed in 95% ethanol. Ten

specimens, immersed in glycerine in order to see otolith increments clearly, were slightly squashed under a cover glass on a glass slide. Otolith diameters were measured and numbers of increments counted by light microscope.

Seventeen settled juveniles (10.5–19.1 mm TL) were captured by hand net in the field on October 21, 1988 and September 25, 1989 and used to validate the periodicity of otolith increment formation. The specimens were immersed without food in an aerated solution of Alizarin Complexone (1,2-dihydroxy-anthraquinone-3-yl-methylamine-N,N-diacetic acid, ALC) (150 ppm) in sea water on the day following capture. Subsequently, the fishes were kept in an outdoor aquarium, being fed once per day on sea-urchin ovaries and carp food. Six juveniles captured in 1988 were fixed 5 days after ALC immersion. The remaining 11 juveniles (captured in 1989) were again immersed in ALC solution, 4 or 5 days after initial immersion, and were fixed 3 or 4 days later in 95% ethanol. Water temperature in the aquarium was 19.5–20.3°C in 1988 and 24.4–25.7°C in 1989. The sagittae were removed from each specimen and mounted in epoxy resin on microscope slides. Otoliths were ground in the sagittal plane with water-proof, 1500 grit abrasive paper until the increments near the edge became clear, after which they were viewed under fluorescent UV light. Counts were made of the number of increments between the fluorescent band (corresponding to the time of ALC treatment) and the edge of the otolith in the 1988 trial and between the two bands in the 1989 trial.

An additional 25 juveniles (9.7–15.0 mm TL), thought to have settled shortly before, were captured by hand net on August 1 and 14, September 3 and October 7, 1988 and preserved in 95% ethanol. Sagittae were removed and mounted on microscope slides in epoxy resin, then ground to the core in the transverse plane with water-proof, 1500 grit abrasive paper. Maximum otolith diameters and increment widths were measured, and the number of increments counted. For observation of the microstructure of the sagittal increments by scanning electron microscope (SEM), the otolith of one individual (13.2 mm TL) was etched with 0.05 N HCl for 40 seconds and vacuum-coated with gold (400 Å).

The fishes and their otoliths used in this study are deposited at the Fish Section, National Science Museum, Tokyo.

## Results

Newly-hatched larvae of *Chromis notatus notatus* were  $2.03 \pm 0.08$  mm TL ( $\bar{x}$  and SD,  $n = 10$ ). Sagittae and lapilli, but not asterisci, were found in each fish (Fig. 1). The longest diameter recorded for the sagittae was  $11.6 \pm 1.0 \mu\text{m}$  ( $n = 10$ ) and that for lapilli,  $11.7 \pm 1.0 \mu\text{m}$  ( $n = 10$ ). One or two increment(s) were detected in both otoliths (Table 1).

Pink-stained bands on the otoliths of juveniles that had been immersed in 150 ppm ALC solution could be seen under UV light. In five cases, it was impossible to count the increments because they were unclear or their widths too narrow. In 10 out of 12 sagittae, the number of increments outside the band or between the two bands matched the number of rearing days (Table 2). These results showed that increments in the sagittae of juvenile *C. n. notatus* were formed daily.

Figure 2 shows a transverse plane of a sagitta of *C. n. notatus* (13.2 mm TL), viewed by SEM. The radius of the core at the center of the plane measured  $1.8 \mu\text{m}$ . Each otolith increment was composed of a wide, light band and an adjoining, narrower dark band. Forty-seven increments were counted from the core to the edge along the long axis, two being inside the radius corresponding to that of the sagitta of a newly-hatched larva ( $5.8 \mu\text{m}$ ). Because the first two increments were thought to have been formed before hatching, the post-hatching age of the fish was estimated to be 45 days. Increment widths along the long axis increased considerably at the 23rd increment, those before the transition being fine and weak, and those after it being relatively thick and clear.

The total length and sagittal radius measured along the long axis of 25 juvenile *C. n. notatus* were positively correlated ( $r = 0.871$ , Fig. 3). Otolith increments could be counted from the core to the edge only in 15 specimens, because some otoliths were cracked or had ambiguous increments. A transition from fine, weak to thick, clear increments was observed in all otoliths. The number of increments between the  $5.8 \mu\text{m}$  radius from the core (see above) and increment transition was  $22.2 \pm 4.8$  (17–34), with  $22.1 \pm 2.4$  (15–26) increments after transition ( $n = 15$ ). The age of the 15 juveniles, according to their increment numbers, was estimated to be  $44.1 \pm 4.4$  (36–52) days, their total length being  $12.8 \pm 1.3$  (9.7–14.4) mm TL. Six juveniles captured on August 1, three days following the first appearance

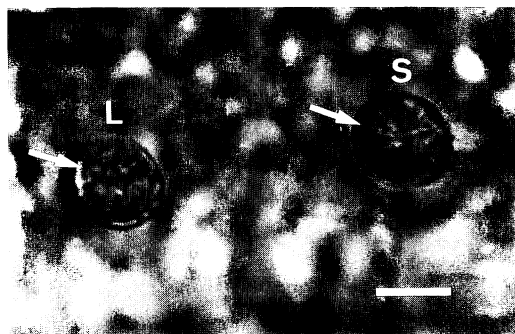


Fig. 1. Otolith of a newly-hatched larva of *Chromis notatus notatus* (2.03 mm TL). S—sagitta; L—lapillus. Arrow indicates an increment. Scale bar =  $10 \mu\text{m}$ .

Table 1. Number of increment(s) in sagittae and lapilli of newly-hatched larvae of *Chromis notatus notatus*

Number of increments	Number of individuals	
	Sagitta	Lapillus
1	6	8
2	4	2

Table 2. Number of increments in sagittae between fluorescent band and edge of otolith (\*) or between two fluorescent bands in *Chromis notatus notatus*

Total length (mm)	Marking interval (days)	Number of increments
19.1	5*	5
18.5	5*	5
18.1	5*	3
17.5	5*	5
17.2	5*	u
15.9	5*	ug
12.7	5	u
12.1	5	u
12.0	5	5
11.5	5	5
11.5	5	4
12.7	4	4
11.8	4	4
11.5	4	4
11.3	4	u
11.2	4	4
10.5	4	4

u—uncountable; ug—unsuccessful graining.

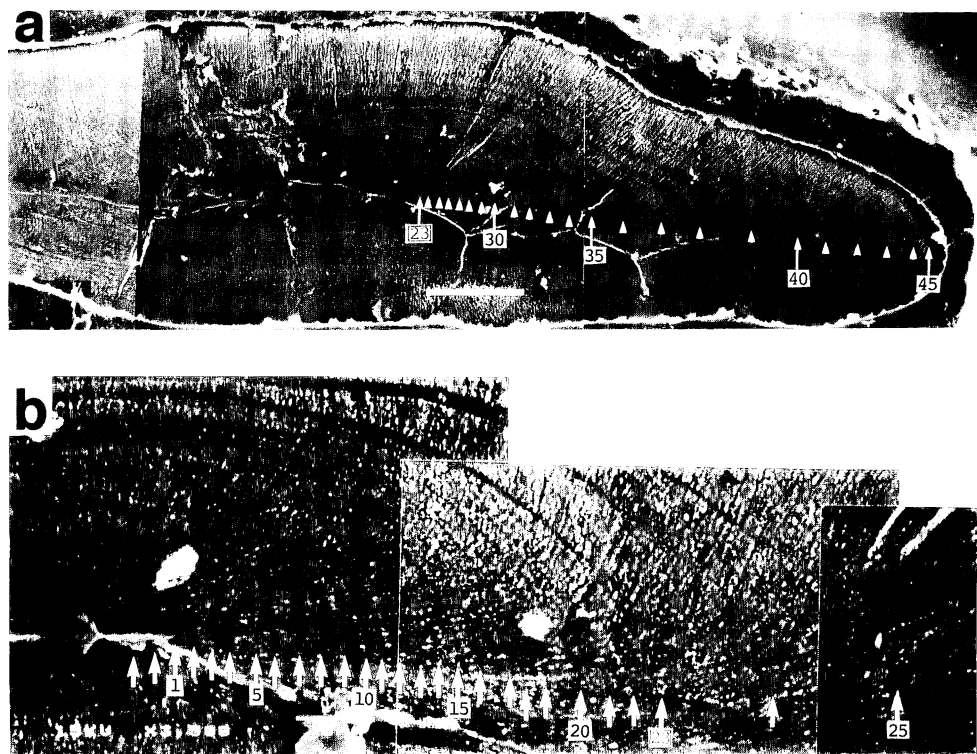


Fig. 2. SEM photograph of the sagittal otolith of a juvenile *Chromis notatus notatus* (13.2 mm TL). a) The transverse plane. Scale bar = 50  $\mu$ m; b) higher magnification from the core to increment transition. Scale bar = 5  $\mu$ m. Arrow indicates an increment.

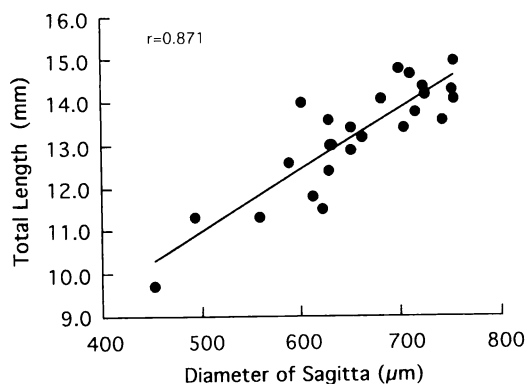


Fig. 3. Relationship between sagitta diameter and TL of *Chromis notatus notatus*.

of newly-settled juveniles were  $47.5 \pm 2.7$  (45–52) days old, being  $12.4 \pm 1.1$  (11.3–13.6) mm TL. The youngest and smallest specimen (36 days old, 9.7 mm TL) was captured on August 14. This specimen had been captured in open water near the reef and had

incomplete body melanization. The other juveniles, which had been captured close to the substrate, had advanced body melanization with a white spot at the end of the dorsal fin, as found in adult *C. n. notatus*.

### Discussion

The time of formation of the first otolith growth increment differs between species. This study revealed that the first increment in *Chromis notatus notatus* was formed before hatching. Such otolith formation has also been reported in the temperate wrasse, *Halichoeres tenuispinis*, by Kishiro and Nakazono (1991). In tropical damselfishes, e.g. *Amphiprion clarkii* and *Stegastes planifrons*, increments begin to form on the day of hatching (Thresher et al., 1989; Wellington and Victor, 1989), and in the milkfish, *Chanos chanos*, two days after hatching (Tzeng and Yu, 1988). Therefore, the timing of the formation of the first increment must be considered, if the correct age is to be estimated.

Juvenile *C. n. notatus* captured three days after the first appearance of newly settled juveniles were  $47.5 \pm 2.7$  (45–52) days old. The fish are thought to have been planktonic for at least 44.5 days, even if all had spent three days on the reef. Such planktonic duration is longer than that of any of the other 133 species of tropical damselfishes (0–37.4 days) studied by Thresher et al. (1989) and Wellington and Victor (1989). One possible reason for their longer planktonic life is that *C. n. notatus* exists in a temperate area, where water temperatures are generally lower (20–27°C during June to October at Tsuyazaki). There is room for argument, however, regarding planktonic larval duration of *C. n. notatus*, since it may vary both spatially (Thresher et al., 1989; Thorrold and Milicich, 1990) and temporally. The youngest specimen, which was captured in free water near the reef, was 36 days old and had incomplete body melanization. It is thought to have been about to settle on the reef.

The increment width along the long axis increased drastically following increment transition in *C. n. notatus*. Some transition in otolith microstructure has been shown to correspond to settlement; viz. the sudden decrease in increment width in *Pomacentrus coelestis* (Thorrold and Milicich, 1990), and the abrupt disappearance of previously prominent dark lines delineating each increment in *Halichoeres bivittatus* (Victor, 1982). However, increment transition in juvenile *C. n. notatus* does not correspond to settlement, because 15–26 subsequent increments were counted. A positive correlation occurred between total length and otolith diameter in settled *C. n. notatus* juveniles. Should there be a similar correlation during the planktonic larval stage, it suggests that the growth rate increased after increment transition. Further study of the change in life history responsible for increment transition is necessary.

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#### スズメダイ仔稚魚の耳石輪紋形成と浮遊期間

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福岡県津屋崎町の岩礁域で採集したスズメダイの卵塊より得た孵化仔魚と、同岩礁域で採集した稚魚を用いて耳石の輪紋構造を観察した。孵化直後の仔魚の扁平石の長径は  $11.6\mu\text{m}$  で、既に1本又は2本の輪紋が形成されていた。また、稚魚をアリザリン・コンプレクソン 150 ppm 海水溶液に24時間浸漬して、耳石輪紋に標識をつけた後に飼育したところ、標識の外側の輪紋数は飼育日数に一致し、耳石輪紋は日周輪であることが確認された。よって、本種の日齢は孵化仔魚の耳石半径より外側の輪紋数と一致する。稚魚の耳石輪紋は17–34本目から著しく広がっていたが、これは着底前に起こると考えられた。スズメダイの浮遊期間は少なくとも44.5日あると推測され、これまでに多く報告されている熱帯性のスズメダイ科魚類の浮遊期間より長いことが明らかとなった。

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