Validation of Daily Periodicity of Otolith Increment Formation and Application for Analysis of Early Growth of Wild Juvenile Black Rockfish *Sebastes cheni*

Yasuhiro Kamimura, Ken-ichiro Mizuno, Tsutomu Noda, Koji Hiraoka, Hitoshi Tamaoki and Jun Shoji

Abstract: Daily periodicity of otolith increment formation was validated by the use of cultured fish in order to estimate the early growth rate of wild black rockfish *Sebastes cheni*. Daily increments started to be deposited at extrusion both on sagittae and lapilli. Mean growth rate \(0.38 \pm 0.08 \text{ mm day}^{-1}\) of wild juveniles \((52.2 \pm 4.8 \text{ mm in total length})\) collected in a seagrass *Zostera marina* bed in Miyagi Prefecture, Pacific coast of northern Japan, back-calculated based on the biological intercept method, was about double of those of cultured fish under similar temperatures \((0.16 \pm 0.06 \text{ and } 0.20 \pm 0.07 \text{ mm day}^{-1} \text{ at } 10 \text{ and } 12^\circ\text{C})\).

Key words: *Sebastes cheni*; Juvenile; Otolith increment; Early growth

Rockfishes (genus *Sebastes*) consist of more than 100 species and are widely distributed in the world’s oceans (Love et al. 2002). Some species have evolved a life history in which their larvae and juveniles strongly associate with substrates and vegetated habitats such as seagrass and macroalgal beds and reefs (Love et al. 1991, 2002). In previous field and laboratory studies, variability in habitat conditions such as temperature and vegetation have been considered as important determinants which affect early survival and recruitment in several *Sebastes* species (Boehlert 1981; Boehlert and Yoklavich 1983; Love et al. 1991, 2002; Hobson et al. 2001; Laidig et al. 2007).

A recent taxonomic review based on morphological and genetical analyses of *Sebastes* species found the former *S. inermis* included three congeners, *S. inermis*, *S. ventricosus* and *S. cheni* (Kai and Nakabo 2008). In previous studies before Kai and Nakabo (2008), *S. inermis* had been reported to be viviparous and highly dependent on vegetated habitats such as seagrass and macroalgal beds during the early life stages (Harada 1962; Nagasawa et al. 2000; Plaza et al. 2002; Pasten et al. 2003; Guido et al. 2004; Mizusawa et al. 2004). Larval and juvenile growth rate was estimated by the use of otolith daily increments (Plaza et al. 2001) as same as in other *Sebastes* species (Yoklavich and Boehlert 1987; Laidig et al. 1991; Kokita and Omori 1998). However, there are few studies which have worked on these three species separately (Kamimura and Shoji 2009; Kamimura et al. 2011) after the taxonomic review. Accumulating biological and ecological information on each species is an urgent subject indispensable for understanding the mechanism of recruitment fluctuation and sustainable use of the stock as fisheries resources.

The objective of present study is 1) to validate the daily periodicity of otolith increment formation in *S. cheni* using laboratory-raised fishes and 2) to estimate the early growth rate of wild fish by applying the results from the validation of daily periodicity of otolith increments formation and 3) to compare early growth rates between wild and cultured fishes. Wild black rockfish were collected in a seagrass bed on the Pacific coast of northern Japan and their growth rates were back-calculated by the use of the biological intercept method.

Rearing experiments using artificially-raised *S. cheni* juveniles (mean total length (TL) = 26.2 mm, \(n = 40\)) were conducted at Miyako Station, Tohoku National Fisheries Research Institute. Larvae were naturally extruded from an adult female (TL = 275 mm) landed on 18 February 2010 at Miyako Fisherman’s Association, Iwate Prefecture. Then larvae were introduced into 1,000 l black polycarbonate tanks on 6 May 2010 and were maintained at six temperatures \((10, 12, 14, 16, 18 \text{ and } 20^\circ\text{C})\) under a 10:14 (light:dark) photoperiod at a fish density about \(1.0 l^{-1}\). Each tank was provided with aeration and fish were fed with rotifer and brine shrimp until satiation four times a day. Twenty fish were sampled from...
each tank every seven days for three weeks and 40 fish at the end of the experiment (four weeks from the start) and were preserved in 10% seawater formalin solution, then TL was measured.

Validation of periodicity of otolith increment formation was conducted by the use of another lot that was naturally extruded in a 200 t tank with several adult fishes on 3 January 2009. Larvae were fed with rotifer and brine shrimp in a 200 l tank at natural temperature (10.5–12.0°C). Ten fish were sampled from the tank on 10, 20 and 30 days after the extrusion and were preserved in 90% ethanol. The right-side sagitta and lapillus were removed from each fish under a dissecting microscope and dried, then embedded in epoxy resin on a slide grass. Each otolith was ground by 2,000–10,000 grid lapping films until the nucleus was clearly visible. Otolith rings were counted from the extrusion check (Plaza et al. 2001) to the edge at 400–1,000 x magnification under a light microscope. Otolith ring counting was conducted three times and the mean values of the three counts were used as data.

Sampling for wild juveniles was conducted at a seagrass Zostera marina bed (38.332°N, 141.146°E) off Higashimatsushima, Miyagi Prefecture, northern Japan, on 10 June 2009. Fish were collected using a small seine (2 m × 1 m, 2 mm mesh) and a scoop net (0.3 × 0.3 m, 2 mm mesh), and were preserved in 90% ethanol for otolith analysis. In the laboratory, juvenile S. cheni were enumerated and were measured in TL (mm) to the nearest 0.1 mm. A total of 27 individuals were processed for otolith analysis in the same manner as in the cultured fish.

Growth analysis for wild S. cheni was carried out using the otolith daily ring measurement system (Ratoc System Engineering). In the present study, lapillus was used for the growth analysis due to higher visibility of increments although daily periodicity of increments used for the growth analysis due to higher visibility of increments. A measurement transect was set from the nucleus along the maximum radius. Number of increments on the lapillus was used as age (days after extrusion). Measurement of increment width was conducted from the nucleus to edge. A linear model was fitted to the relationship between otolith radius and TL. In addition, formation of both sagittae and lapilli were validated. A linear model was fitted to the relationship between the number of increments along the maximum radius. Number of increments on the lapillus was used as age (days after extrusion). Measurement of increment width was conducted from the nucleus to edge. A linear model was fitted to the relationship between otolith radius and TL. In addition, formation of both sagittae and lapilli were validated.

\[ L_a = L_c + (R_a - R_c)(L_c - L_a)(R_a - R_c)^{-1} \]

where \( L_a \) and \( L_c \) is fish size at age \( a \) and capture, \( R_a \) and \( R_c \) is otolith radius at age \( a \), capture and extrusion, and \( L_e \) is mean fish size at extrusion (cultured fish \( n = 30 \), 6.2 mm TL), respectively. Daily surface water temperature from December 2008 to May 2009 was obtained from an observation buoy of Miyagi Prefecture Fisheries Technology Institute (MPFTI) nearby the sampling site. Mean daily temperature from extrusion to capture, which each fish was expected to have experienced, was calculated for each fish.

Mean growth rate of wild S. cheni was compared with those of cultured fish at the same TL range. According to the mean TL at the start of the rearing experiment (26.2 mm TL), growth trajectories of wild fish were also established for the TL range larger than 26.1 mm. Then mean growth rates of wild fishes back-calculated for four weeks after the day at which fish TL reached 26.2 mm TL were compared with those of cultured fish for four weeks from the start of rearing experiments.

The relationships between the number of increments on sagittae (\( I_s \)) or lapilli (\( I_l \)) and age (\( A \), day) of cultured S. cheni were expressed by linear formulas as follows (Fig. 1):\footnote{Fig. 1. Relationships between the number of otolith increments (I) and days after extrusion (A) of cultured S. cheni. A, sagittae (\( I_s = 0.975A + 0.467, n = 30, r^2 = 0.988, P < 0.001 \)); B, lapilli (\( I_l = 0.950A + 0.600, n = 30, r^2 = 0.987, P < 0.001 \)). Ten fishes were analysed for each age.}

\begin{align*}
\text{Sagittae: } & I_s = 0.975A + 0.467 \quad (n = 30, r^2 = 0.988, P < 0.001) \\
\text{Lapilli: } & I_l = 0.950A + 0.600 \quad (n = 30, r^2 = 0.987, P < 0.001)
\end{align*}

The slopes of both regressions were not significantly different from 1.0 and intercept not significant from 0 (ANCOVA, \( P < 0.05 \)).
Mean (± SD) and range of TL of wild *S. cheni* used for the growth analysis were 52.2 (± 4.8) mm and 36.1–61.7 mm and those of extrusion date were 2 January 2009 (± 9.1 d) and 20 December 2008–21 January 2009. The relationship between otolith radius and TL (*L*), which was applied for the back-calculation of wild *S. cheni*, was expressed by a following linear regression:

\[ L = 0.136 R + 0.657 \ (n = 103, \ r^2 = 0.968, P < 0.0001) \]

Mean (± SD) back-calculated TL at age of wild fish increased from 26.2 to 36.8 ± 1.3 mm during four weeks, the period for the comparison of growth rate between wild and cultured fishes. Mean (± SD) daily growth rates of wild fish during the four weeks were 0.38 ± 0.08 mm day\(^{-1}\) (Fig. 2). Mean daily temperature which each wild fish were expected to have been experienced during the four weeks, estimated by the use of temperature of the observation buoy, was 10.8°C.

Mean (± SD) TLs of the cultured fish increased from 26.1 ± 1.6 to 30.7 ± 1.7 mm (10°C), 31.8 ± 2.1 mm (12°C), 32.9 ± 3.7 mm (14°C), 34.5 ± 4.9 mm (16°C), 35.2 ± 5.6 mm (18°C) and 36.6 ± 6.2 mm (20°C) for four weeks. Mean daily growth rates during the period were 0.16 ± 0.06 (10°C), 0.20 ± 0.07 (12°C), 0.24 ± 0.13 (14°C), 0.30 ± 0.17 (16°C), 0.32 ± 0.20 (18°C) and 0.37 ± 0.22 mm day\(^{-1}\) (20°C) (Fig. 2). The effect of temperature on the mean growth rate of cultured fish was significant (ANOVA followed by Tukey’s test for multiple comparison, *P* < 0.0001). There was a significant positive correlation between the mean growth rate (\(G\), mm day\(^{-1}\)) of cultured fish and temperature (\(T\), °C) tested in the present study (10–20°C) as follows:

\[ G = 0.0211 \times T - 0.0489 \ (n = 6, \ r^2 = 0.993, P < 0.0001) \]

Daily periodicity of otolith increment formation and timing of the first increment deposition have been validated in a variety of fish species (e.g. Pannella 1971; Campana and Neilson 1985). Otolith daily increments start to be deposited at the first feeding in many marine fish species for which larvae hatch from pelagic eggs, while deposition of a check mark at extrusion followed with daily increment have been reported in several viviparous *Sebastes* species (Laidig et al. 1991; Kokita and Omori 1998; Plaza et al. 2001). In the present study, timing of the first increment formation (at extrusion) and periodicity of increment formation (daily) were validated in both sagitta and lapillus of *S. cheni*.

According to a recent taxonomic review on *Sebastes* species by Kai and Nakabo (2008), which revealed that the former *S. inermis* consists of three species, *S. inermis*, *S. ventricosus* and *S. cheni*, previous reports on growth rates of larval and juvenile *S. inermis* (ca. 0.5 mm day\(^{-1}\); Plaza et al. 2002; Mizusawa et al. 2004) possibly reflected a composite of the growth rates of the two other species. In the present study, mean growth rates of wild *S. cheni* larvae and juveniles were estimated to be 0.38 mm day\(^{-1}\) based on validation of otolith daily increment formation using cultured fish.

Love et al. (1991) summarized growth rates of larvae and juveniles of 17 *Sebastes* species ranged between 0.12–0.72 mm day\(^{-1}\) with a mean growth rate of 0.29 mm day\(^{-1}\) for all species. In addition, mean growth rate of *S. diploproa* and *S. melanops* were reported to be 0.10–0.21 mm day\(^{-1}\) (10–20°C) and 0.09–0.31 mm day\(^{-1}\) (7–18°C), respectively, under laboratory conditions (Boehlert 1981; Boehlert and Yoklavich 1983). Based on these previous studies, *S. cheni* is concluded to exhibit a relatively higher growth rate during the larval and juvenile periods at similar temperatures among *Sebastes* species.

Generally, growth rates obtained from laboratory experiments need to be interpreted carefully since there are a variety of artificial biases in environmental conditions such as tank size, feeding condition and handling, which wild fishes do not experience in nature. In the present study, since the cultured fish were fed with invertebrate zooplankton prey until satiation four times per day, it is plausible that prey availability in the tank was not a restricting factor for their growth rate. Mean daily growth rate of wild fish (0.38 mm day\(^{-1}\) at 10.8°C) was about double of that of cultured fish under similar temperatures (0.16 and 0.20 mm day\(^{-1}\) at 10 and 12°C). In addition, standard deviation of the mean growth rate of wild fishes (0.38 ± 0.08 mm day\(^{-1}\) at 10.8°C) was smaller than those of cultured fishes (0.32 ± 0.20 mm day\(^{-1}\)).
day$^{-1}$ at 18°C, 0.37 ± 0.22 mm day$^{-1}$ at 20°C) which had a similar growth rate as wild fishes. Variability in body size tends to be more prominent under laboratory conditions compared to wild cohorts since there is no size-selective mortality (mostly due to predation: Houde 1987) in captive tanks. In nature, on the other hand, size-selective predation is considered as an important factor which affects length frequency distribution of the survivors of larvae and juveniles in many fish species (Meekan and Fortier 1996; Takasuka et al. 2003; Takahashi and Watanabe 2004; Shojo and Tanaka 2006; Plaza and Ishida 2008; Islam et al. 2010). Although seagrass beds are considered as important refuges from predators, recent field sampling and analysis of stomach contents of predators revealed piscivorous fish predator biomass increased at nighttime (Kinoshita et al. 2012). The higher growth rate at the same temperature and smaller deviation at the same growth rate of wild fish compared to those of cultured fish might reflect size-selective mortality in nature. Further analysis on environmental conditions and size- and growth-selective survival in nature (such as repeated sampling to capture the same cohort coupled with growth back-calculations using otolith daily increments) are needed to understand the mechanisms of survival processes of S. cheni.

Acknowledgments

We thank H. Aono and the staff of Miyako Station, National Research Institute of Tohoku National Fisheries Research Institute for supporting the experiments, MPFTI for providing temperature data, and S. Fukumoto, Geinan Fisherman’s Association, S. Iwasaki and the staff of Takehara Marine Science Station for helping field sampling.

References


