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ORIGINAL ARTICLE

Biology

Age and growth of crimson sea bream *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan

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Abstract

Age and growth of crimson sea bream *Evynnis tumifrons* were studied using samples collected from October 1999 to August 2013 off the southwestern coast of Kyushu, Japan. Ring marks (outer edges of opaque zones) on the 1805 transversely sectioned otoliths were counted and seasonality in their deposition was validated by marginal increment. Our results revealed that one ring mark was formed per year from late spring to early summer. Assuming December as the birth month, ages were assigned to every individual according to the number of ring marks and the value of marginal increments. Growth was estimated by fitting the von Bertalanffy growth function to the length-at-age and weight-at-age data. The estimated growth curves did not differ significantly between the sexes, and the growth curve of the pooled data was $L_t = 271(1 - \exp(-0.604(t + 0.193)))$ for length-at-age and $W_t = 519(1 - \exp(-0.484(t + 0.625)))^3$ for weight-at-age. The maximum age observed was 15 years for females and 16 years for males.

Keywords Age · *Evynnis tumifrons* · Growth · Otolith

Introduction

The sea breams of Sparidae are excellent food fish and are of high commercial importance (Iwatsuki 2009). This family belongs to the perch-like fishes (Perciformes) that comprise more than 100 species (Boufersaoui et al. 2018). Approximately 13 sea bream species are distributed in the coastal waters of Japan (Hayashi and Hagiwara 2013); one of these is the crimson sea bream *Evynnis tumifrons*. This species is endemic to the coastal waters of China, Hong Kong, Japan, South Korea and Taiwan (Akazaki 1962, 1984; Iwatsuki et al. 2007, 2014), and is found on the rocky reefs, gravel and sandy bottoms of the continental shelves (Hayashi and Hagiwara 2013). It feeds on a wide range of benthic invertebrates and fishes (Iwatsuki et al. 2014), and it was reported that its bathymetrical distribution varies according to the ontogenetic development, where the juveniles are commonly found at shallow depths (around 10 m) and the adult stages are in the deeper waters (50–100 m).

In Japan, *E. tumifrons* is a commercially important fish species (Kudoh and Yamaoka 2004) and is caught mainly by gill nets, surrounding seine nets and angling. Its age and growth have been studied in areas off Akita, Niigata, Fukuoka, Ibaraki, and Kochi and Miyazaki (Yamada et al. 2007). These localities are widely distributed from the northern to southern regions of Japan, but the southwestern region is not included.

Furthermore, the above-mentioned studies determined age and growth of *E. tumifrons* using scales, while some studies have criticized the use of scales due to the underestimation of ages in old fish by this character (Beamish and McFarlane 1983; Carlander 1987). On the other hand, transversely sectioned otoliths have been recommended as the age determination character because otoliths continue to grow towards the internal (proximal) side as fish age (Beamish and McFarlane 1983; Casselman 1987; Abecasis et al. 2008) and annuli on transversely sectioned otoliths are clearly exhibited (Masuda and Noro 2003; Masuda et al. 2003; Lee et al. 2009; Piddocke et al. 2015). This age

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determination character has been applied to many fish species such as *Platycephalus indicus* (Masuda et al. 2000), *Rhabdosargus sarba* (Radebe et al. 2002), *Nemipterus bathybius* (Granada et al. 2004), *Sillago aeolus* (Rahman and Tachihara 2005), *Gerres equulus* (Iqbal et al. 2006), *Lutjanus fulviflammus* (Shimose et al. 2009), *Pseudopleuronectes yokohamae* (Lee et al. 2009), *Mora mora* and *Epigonus telescopus* (Vieira et al. 2013), *Scolopsis monogramma* (Akita and Tachihara 2014) and *Trachurus japonica* (Yoda et al. 2014), but has not yet been applied to *E. tumifrons*. Hence, existing knowledge on the life history characteristics of *E. tumifrons* is insufficient.

The aim of the present study is to describe the age and growth of *E. tumifrons* using transversely sectioned otolith for the first time with samples collected off the southwestern coast of Kyushu, Japan. The results derived are indispensable for the development of measures to improve the management of this commercially important fish species.

Materials and methods

Sampling and measurement

Samples of *E. tumifrons* were mainly collected at Eguchi Fisheries Cooperative, Hioki city, Kagoshima Prefecture, southern Japan, from April 2012 to August 2013. The fishers belonging to this cooperative caught the *E. tumifrons* using gill nets and surrounding seine nets in the area of 31°33′–31°39′ N and 130°13′–130°20′ E off Hioki city on the southwestern coast of Kyushu, Japan (Fig. 1a). After the fish were landed, the cooperative's staff sorted them into eight different weight categories. We sampled specimens of various body sizes from these categories once a month, and a total of 1599 specimens (794 females and 805 males) were collected. In addition, to compensate for the insufficient number of the small-sized individuals collected at Eguchi Fisheries Cooperative, a total of 206 (73 females, 57 males and 76 sexually unknown) small fish caught by set net off Kasasa town (Fig. 1b) in 2004 and with Danish seine and gill nets off

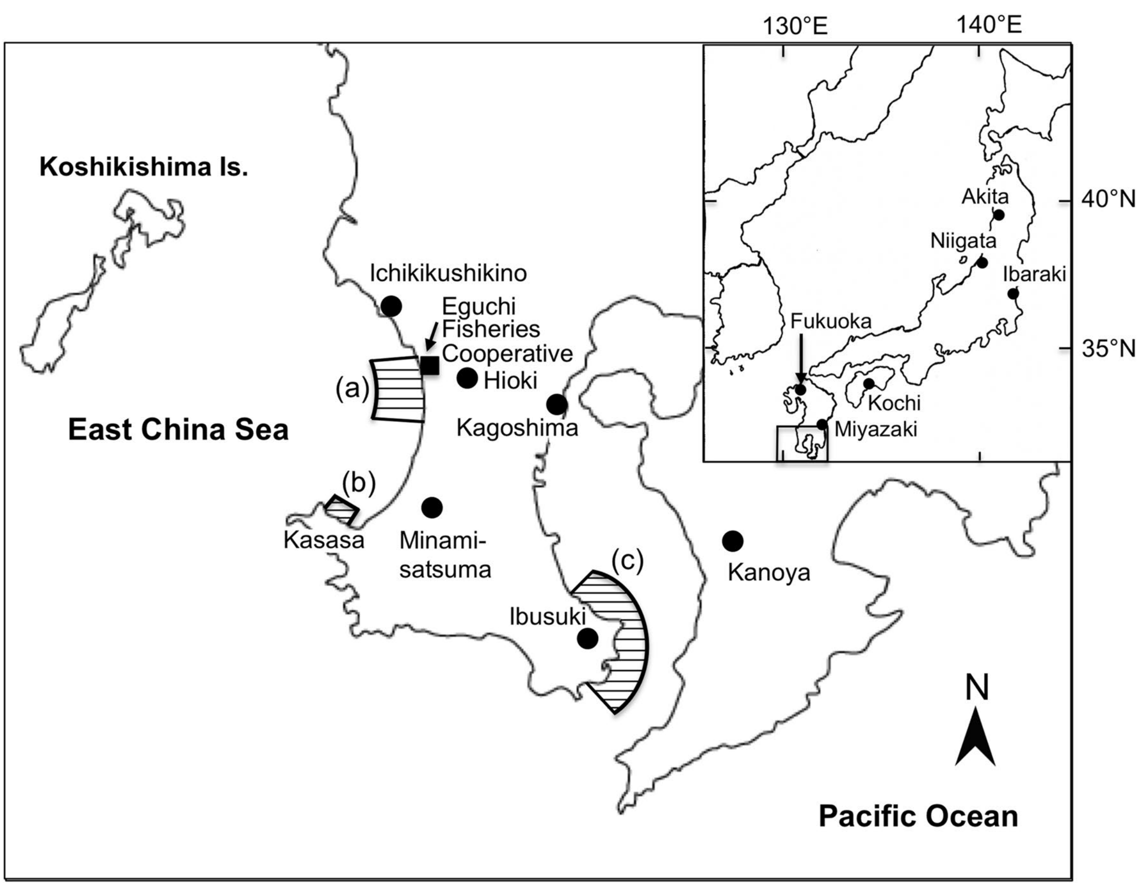

Fig. 1 Map of the sampling sites (shaded areas) of *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan. **a** Off Hioki city, **b** off Kasasa town, Minami Satsuma, and **c** off Ibusuki city

Ibusuki city (Fig. 1c) during the period from 1999 to 2004 were used for the analysis.

Fish collected were immediately chilled in ice and transported to the Laboratory of the Fisheries Biology of Faculty of Fisheries, Kagoshima University. Fish were measured for fork length (FL) on the measuring board to the nearest 1 mm and body weight (BW) on the electronic balance (UX6200H, Shimadzu, Kyoto, Japan) to the nearest 0.01 g, and then sexed through visual observation of the gonads.

Preparation of transversely sectioned otoliths

Otoliths were extracted from the fish head, washed with tap water and kept in a multi-well plate in a dried condition. The left otoliths were embedded in epoxy resin and transversely cut into 2-mm-thick sections through the focus using a micro-cutter (type MC-201, Maruto, Tokyo, Japan). The sectioned otoliths were polished using a grinder (type 9820, Makita, Tokyo, Japan) towards the focus, leaving a consequent thickness of 0.25 mm. The polished otoliths were then mounted on a slide glass with nail enamel (Masuda and Noro 2003; Granada et al. 2004; Iqbal et al. 2006).

Counting and validation of ring marks

The number of ring marks (outer edges of the opaque zones) on sectioned otoliths was counted from the focus to the dorsal tip of the sulcus acusticus using a microscope (Leica MZ 12.5, Leica Microsystems, Heerbrugg, Switzerland) under a transmitted light at 32× magnification (Fig. 2a). Otoliths that were difficult to count were excluded from the analysis.

To validate the seasonality in the deposition of ring marks (outer edge of opaque zones) in sectioned otoliths, the marginal increment (MI), i.e., the distance between the outer edge of the outermost opaque zone and the periphery, was measured at 32× magnification and expressed as either (1) a proportion of the distance between the focus and the outer edge of the opaque zone when only one opaque zone was present, or (2) a proportion of the distance between the outer edges of the two outermost opaque zones when two or more opaque zones were present.

Assignment of age

Monthly changes in the gonadosomatic index and the occurrence of the mature or spawned females collected off the southwestern coast of Kyushu, Japan have shown that the spawning season lasts from November to May, with a peak

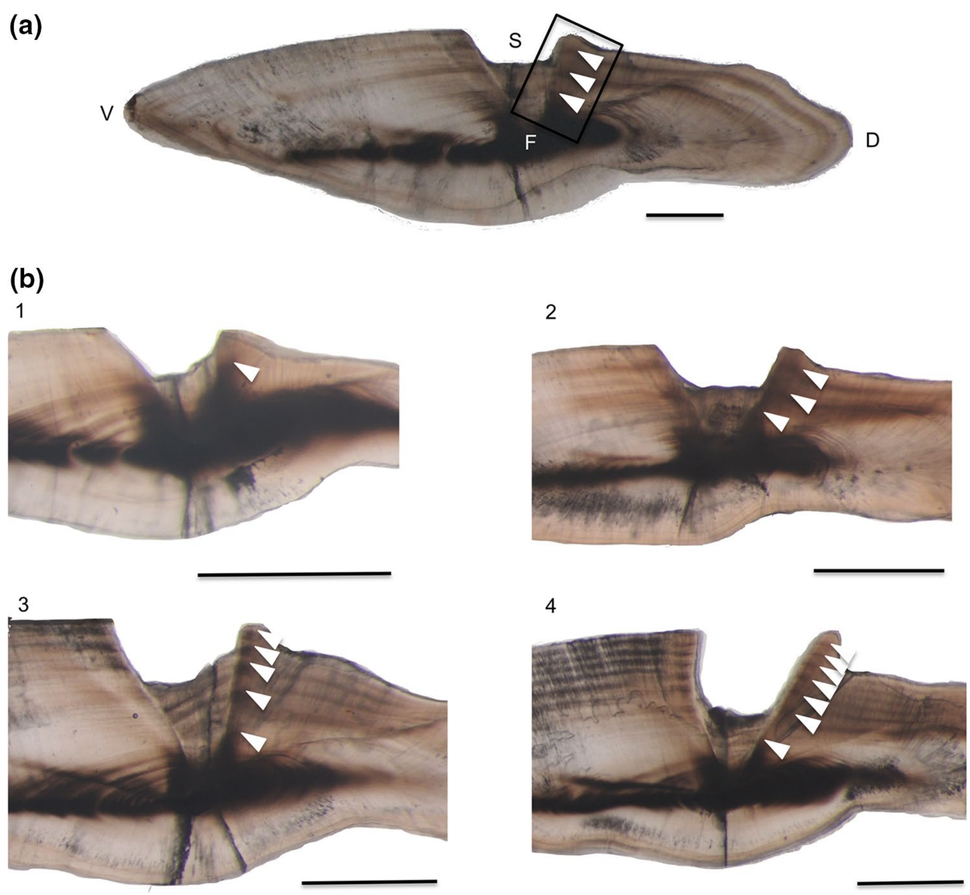

Fig. 2 **a** Counting and measuring area (within a rectangle) of ring marks on sectioned otolith of *Evynnis tumifrons*. *F* focus, *S* sulcus acusticus, *V* ventral side, *D* dorsal side. Open triangles indicate ring marks. **b** Ring marks (open triangles) on transverse sections of otoliths of the four representative specimens of *Evynnis tumifrons*. **b1** 135 mm fork length (FL) male with one ring mark; **b2**, 221 mm FL female with three ring marks; **b3** 260 mm FL male with five ring marks; **b4**, 255 mm FL female with seven ring marks. Bar = 1 mm

in November or December (Havimana et al. 2020). Assuming that the birth month was December based on this result, ages were assigned to every individual according to the number of ring marks, the degree of MI values (i.e., high or low) and the time elapsed from the birth month to the capture month. For example, for the fish captured in June (6 months after December), an age of 0.5 (=6/12) years was assigned to individuals with one new ring mark of low MI value or no ring mark, while for the individuals with a second new ring mark of low MI value or one old ring mark of high MI value, an age of 1.5 (=1+6/12) years was assigned. The same assignment of age was applied for older fish. In the following month of July, 0.08 (=1/12) was added to the age (Masuda et al. 2003).

Growth analysis

The 76 individuals whose sex was unknown were alternatively treated as female or male under the assumption of a sex ratio of 1:1, and were incorporated into sexually known female or male data. The von Bertalanffy growth equation was fitted to the length-at-age and weight-at-age data based on the least-squares method using the curve-fitting function of the computer software (DeltaGraph 7, Salt Lake City, UT, USA). The equation was expressed as $L_t = L_\infty(1 - \exp(-K(t - t_0)))$ for length and $W_t = W_\infty(1 - \exp(-K(t - t_0)))^3$ for weight, where L_t and W_t are the fork length (mm) and body weight (g) at age t (year), L_∞ and W_∞ are the asymptotic fork length and body weight, K is the growth coefficient and t_0 is the hypothetical age when length or weight will be zero.

The F test was conducted to compare the growth curves between the sexes using the formula provided by Akamine (2010):

$$F = \left[(S_p - S_f - S_m)/r\right] / \left[\left(S_f + S_m\right) / \left(n_f + n_m - 2r\right)\right],$$

where S_p is the residual sum of squares (RSS) for both sexes (pooled data), S_f is the RSS for females, S_m is the RSS for males, n_f is the sample size of females, n_m is the sample size of males and r is the number of parameters.

Results

Ring formation period

Clear opaque and translucent zones were observed alternately from the core region to the terminal edge of the transversely sectioned otoliths (Fig. 2b). The opaque zones of *E. tumifrons* were classed as type A as described by Katayama (2018), and the distance between the ring marks (outer edge of the opaque zones) appeared to be smaller from the focus towards the otolith's margin. A total of 1790 out of 1805 (99.2%) otoliths had countable ring marks; thus, the transversely sectioned otolith was regarded as a good calcified structure from which to estimate the age of *E. tumifrons*.

The monthly changes in marginal increment (MI) were analyzed to estimate the ring formation period. The occurrence of low MI values suggested that the new ring marks were recently formed, and high values suggested that new ring marks were not yet formed. The period of coexistence of low and high MI values was therefore considered as the ring formation period. Looking at the results for females (Fig. 3a), the coexistence period of low and high MI values was observed in June in the one-ring group and from May to July in the two-ring group. In the three-, four- and 5–15-ring groups, it was from June to July. For males (Fig. 3b), a similar trend was observed in June in the one-ring group, from May to July in the two-ring group, from June to July in the three-ring group, and in July in the four-ring group. Based on this information, rings were estimated to form from May to July and were considered as annuli in *E. tumifrons.*

Length–frequency distribution

The monthly changes in length–frequency distribution by age for both sexes are shown in Fig. 4. Assuming that the birth month was December, monthly occurrences of the assigned ages (0–6 ≤ years old) against the fork length were demonstrated. Clear peaks were observed in the young age groups of 0, 1 and 2 years but not in age ≥ 3 years. The 0-year-old fish first appeared in March, with fork length ranging from 36 to 46 mm. In December, they turned 1 year old, with FL ranging from 107 to 143 mm, and became the major component of the catches in April and subsequent months. In the following December, these 1-year-old fish turned 2 years old, and FL ranged from 180 to 233 mm; this transition of age continues throughout the life of the fish.

Growth

The von Bertalanffy growth function estimated from the length-at-age of the females was $L_t = 272$ $L_t = 272(1 - \exp(-0.599(t + 0.189)))$ ($n = 896$, $r^2 = 0.907$) and that of the males was $L_t = 270(1 - \exp(-0.610(t + 0.197)))$ ($n = 894$, $r^2 = 0.909$), with no significant difference between the sexes ($P > 0.05$). For the body weight-at-age, the estimate was $W_t = 527(1 - \exp(-0.479(t + 0.621)))^3$ ($n = 896$, $r^2 = 0.856$) for the females and $W_t = 509(1 - \exp(-0.493(t + 0.618)))^3$ ($n = 894$, $r^2 = 0.852$) for the males, with no significant difference between the sexes ($P > 0.05$). Hence, the von Bertalanffy growth function of the pooled data of the length-at-age was $L_t = 271(1 - \exp(-0.604(t + 0.193)))$ ($n = 1790$, $r^2 = 0.862$) (Fig. 5a) and that of the weight-at-age was

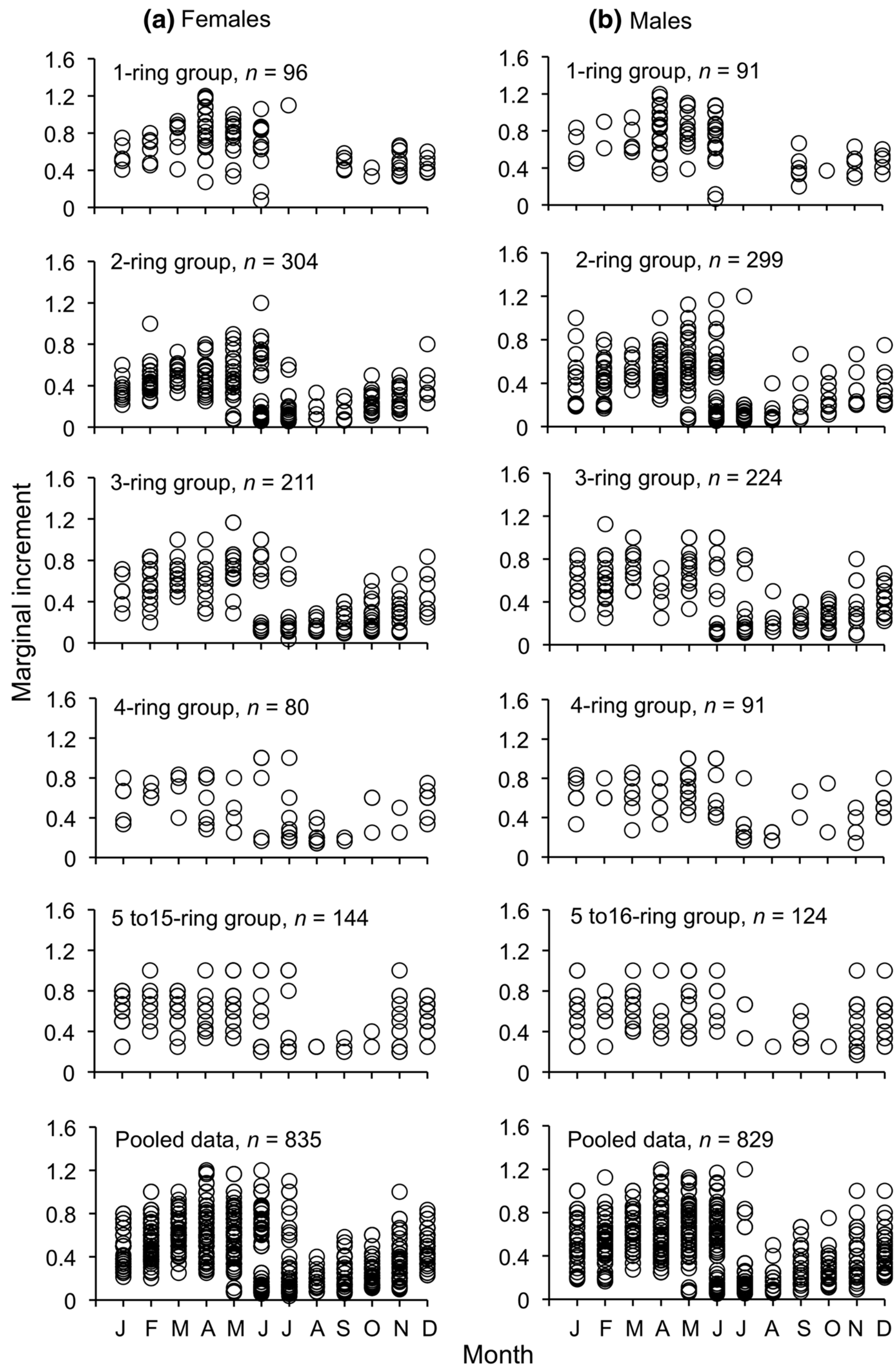

Fig. 3 Monthly changes in marginal increment for transversely sectioned otoliths of **a** female and **b** male *Evynnis tumifrons*. *n*, number of specimens examined for each ring group and pooled data

$W_t = 519(1 - \exp(-0.484(t + 0.625)))^3$ (n=1790, r^2=0.854) (Fig. 5b). The maximum age of female fish was 15 years (fork length = 310 mm; body weight = 523 g) and that of male fish was 16 years (fork length = 276 mm; body weight = 445 g).

Discussion

Age estimation in sparid fish is complicated due to the phenomenon of stacking of ring marks towards the otolith's margin, particularly in older fish (Van der Walt and Beckley 1997; Pajuelo and Lorenzo 2002). However, in *E. tumifrons*,

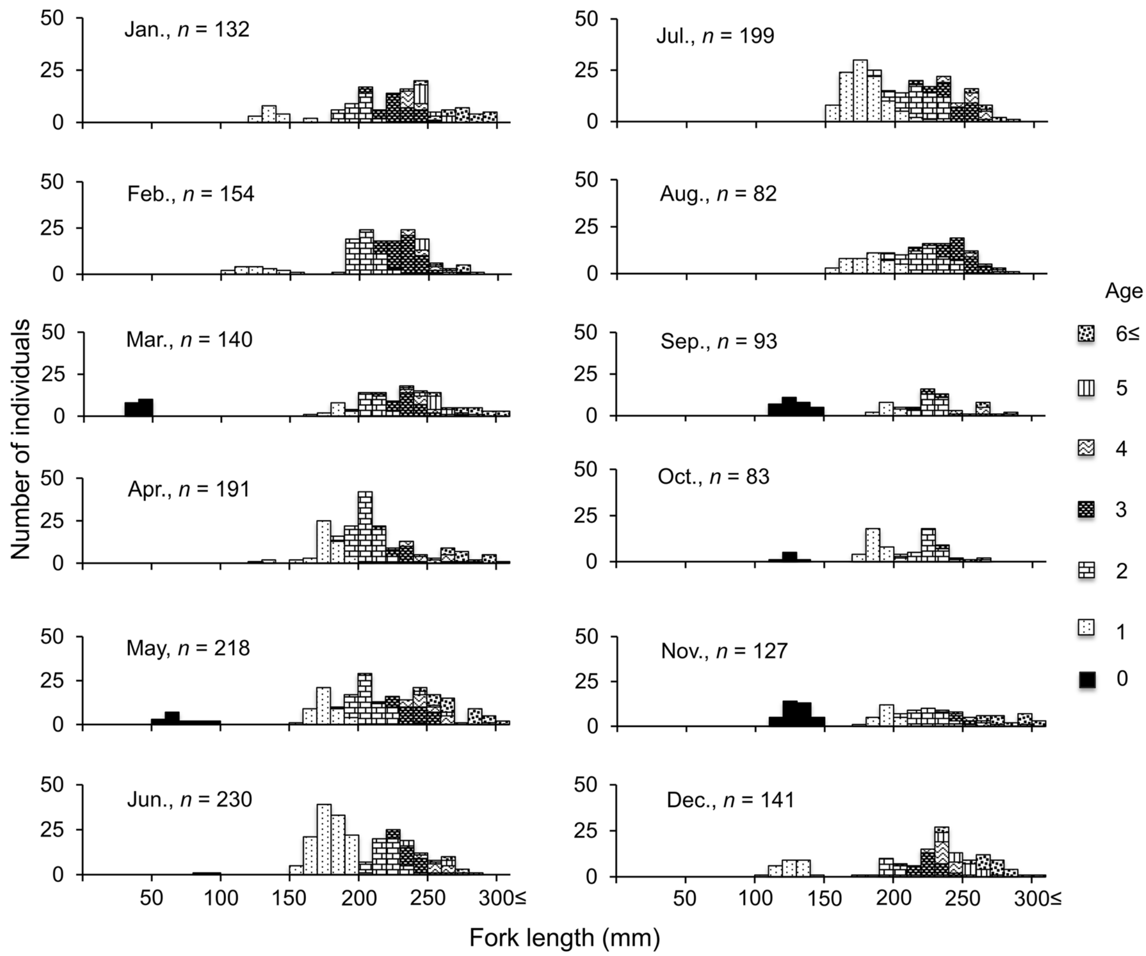

Fig. 4 Monthly changes in length–frequency distribution by age for both sexes of *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan. Ages were assigned based on the birth month of December. *n*, number of specimens examined

clear ring marks on the internal (proximal) side of the sectioned otoliths enabled age determination with relative ease (99.2% of the otoliths were countable). The oldest age in the present study was 16 years, and the stacking phenomenon was not observed.

To confidently assign the age from the count of ring marks (outer edges of opaque zones) on the transversely sectioned otoliths, the periodicity of the increment formation must be determined (Beamish and McFarlane 1983; Campana 2001; Piddocke et al. 2015), and this is typically done through the analysis of monthly changes in the marginal increment. Our results revealed that one ring mark was deposited each year in the sagittal otolith, owing to the alternating period of fast and slow growth (Hou et al. 2008). Therefore, each ring mark was confirmed to be an annulus with a formation period that lasted from late spring to early summer season (Fig. 3a, b). The accuracy and reliability of an annulus on transversely sectioned otoliths has been demonstrated in *Pagrus auratus*, where young individuals captured in the wild were injected with tetracycline and were reared on a natural food in a large pool with flowing seawater under ambient marine conditions (Ferrell et al. 1992). An opaque zone was observed between July and October in the two successive years of the investigation. In *Rhabdosargus holubi,* individuals were captured in the wild, tagged and injected with oxytetracycline, and later released into an artificial saltwater impoundment that contained seawater pumped from the inshore surf zone (Farthing et al. 2016). The study reported that the two individuals recaptured showed one opaque zone deposited annually on the otoliths. In the flathead *Platycephalus indicus*, annuli counts from the cultivated fish were concordant with their known ages (Masuda et al. 2000).

The FLs calculated at each age of *E. tumifrons* in different coastal waters of Japan are shown in Fig. 6. The length at age 1 year in the present study, conducted in the southernmost location off the southwestern coast of Kyushu, Japan (latitude, 31°33′–31°39′ N), was 139 mm FL, and this estimate appears to be the largest when compared with those

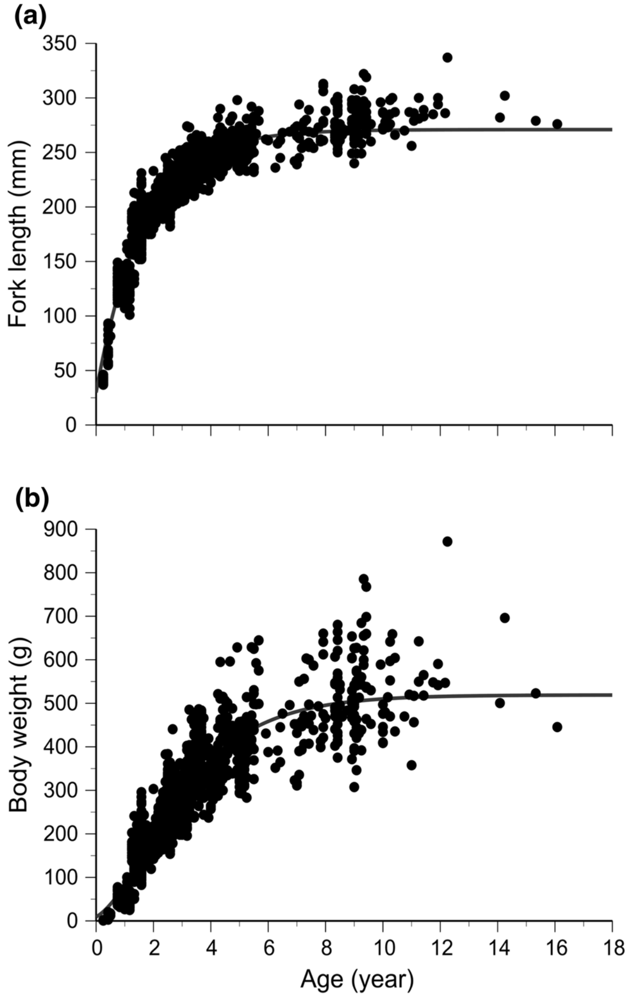

Fig. 5 von Bertalanffy growth curve fitted to **a** length-at-age data and **b** weight-at-age data for both sexes of *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan

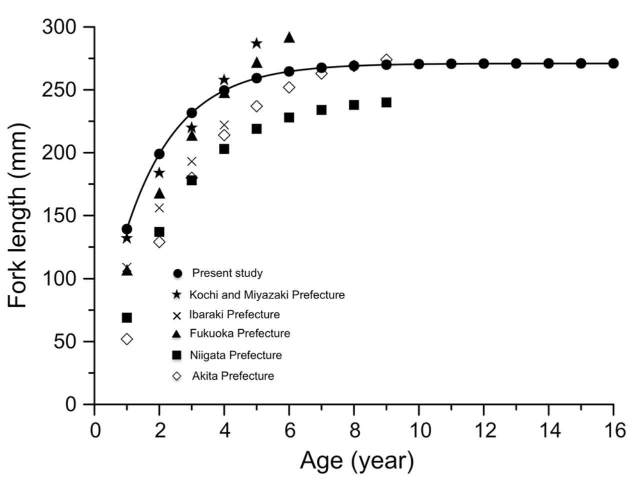

Fig. 6 Comparison of age–FL relationships of *Evynnis tumifrons* at six different localities in Japan. Data for the previous studies were cited from Yamada et al. (2007)

of previous studies. A trend was also realized wherein the size of the young *E. tumifrons* decreased with increasing latitudinal area. In the coastal waters of southern Japan, fish size at age 1 was 107 mm FL off Fukuoka Prefecture (latitude, 33°50′ N) and 132 mm FL off Kochi and Miyazaki Prefectures (latitudes, 33°20′ N and 32°10′N). In the northernmost areas, off Akita Prefecture (approximate latitude, 39°39′N), it was 52 mm FL, off Niigata Prefecture (latitude, 37°50′ N) it was 69 mm FL, and off Ibaraki Prefecture (latitude, 36°20′ N) it was 109 mm FL. Based on these observations, the growth of fish at an early age differs according to the populations found across the coastal waters of Japan. Ochiai and Tanaka (1986) observed a similar phenomenon in *Pagrus major*, a related red sea bream in the coastal waters of Japan, where initial growth was highest in the southern waters of Kagoshima Bay (Kagoshima Prefecture), followed by Kii Channel (Tokushima and Wakayama Prefectures) and Kitakyushu (Fukuoka Prefecture), and that in the Sea of Japan, while that in the Seto Inland Sea was generally low. The authors reported that the factor responsible for this variation is the difference in water temperature among the localities, where warmer temperatures favor a high initial growth. One-year-old butterfish *Odax pullus* in the coastal waters of New Zealand showed a similar phenomenon, where growth was higher in the Hauraki Gulf of northern New Zealand (latitude, 36.3° S) than in the Stewart Islands of southern New Zealand (latitude, 47° S). In *Odax pullus*, the factor responsible for the variation in growth was the water temperature difference between the two localities (Trip et al. 2014). Water temperature differences over latitudinal gradients has been reported as one of the major factors influencing growth in fish (Conover 1992; Trip et al. 2014). Our results revealed that the fork lengths in 1-year-old *E. tumifrons* decreased with an increase in latitude. Hence, the observed variations in the growth of the young fish are likely caused by water temperature differences. However, other factors such as feeding regime and reproductive cycle are possible influences as well, and to elucidate the degrees to which these factors contribute to these changes, future laboratory experimental work in these populations will be needed.

The steepness of the curve (growth rate) after the age of 1 year also appeared to differ among localities (Fig. 6). The least steep curve was obtained in the present study, followed by that of Niigata Prefecture, Ibaraki Prefecture, Akita Prefecture, Fukuoka Prefecture, and Kochi and Miyazaki Prefectures. These differences might be attributed to the estimated maximum ages, i.e., steepness of the curve tended to be inversely proportional to the maximum age. The maximum age in the present study (16 years) is 7 years older than that reported in Akita and Niigata Prefectures (Yamada et al. 2007). In the present study, we used the transversely sectioned otoliths, while scales were used in the previous studies. Some researchers have criticized the use of scales, because they cease to grow as the fish ages (Beamish and McFarlane 1983; Casselman 1987), and the age of old fish is frequently underestimated (Beamish and McFarlane 1987; Carlander 1987). The superiority of transversely sectioned to whole otolith or other aging structures has been demonstrated as well (e.g., Beamish 1979; Erickson 1983; Hyndes et al. 1992; Masuda and Noro 2003; Masuda et al. 2003). Hence, previous studies on *E. tumifrons*, which used scales for age determination, possibly underestimated the maximum age, and this may be one of the factors that caused the differences in steepness of the growth curves between localities.

In the present study, we aimed to promote a better understanding of age and growth of *E. tumifrons* off the southwestern coast of Kyushu, Japan using transversely sectioned otoliths, and to validate the periodicity of ring marks deposited on the otoliths. The accuracy and reliability of age information is important for proper stock assessment and management of any commercially exploited species. Our findings, which revealed the possibility of the underestimation of age by previous studies, may now challenge the authenticity of any existing management measures targeting this species. The finding that initial growth at a young age (1 year) varied in each population distributed across the coastal waters of Japan is interesting from the viewpoint of life strategy, because it suggests that growth parameters and size at sexual maturity are likely to vary among the populations. It is therefore of paramount importance that any existing management measures are updated in pursuit of sustainable management of the resource, taking into account the species biology and the anthropogenic factors impacting each of the different geographical populations.

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