Fisheries biology of *Evynnis tumifrons* (Sparidae) off the southwestern coast of Kyushu, Japan

（九州南西部沖におけるチダイ（タイ科）の資源生物学的研究）

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DEDICATION

DEDICATED

TO

MY MOTHER

A strong and gentle soul who taught me to trust in God, believe in hard work and that so much could be done with little

MY FATHER

For earning an honest living for us and for supporting and encouraging me to believe in myself

MY SIBLINGS

For the endless moral supports and words of inspiration to have my eyes fixed not on the challenges of the journey, but on the finishing line

DECLARATION

I declare that work presented in this thesis is entirely my own with all exceptions being clearly indicated or/and properly cited in the context

Lindon Havimana

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Chapter 3

List of Acronyms and Abbreviations

BW	Body weight
CF	Condition factor
EA	Early atretic stage
EYG	Early yolk globule stage
FL	Fork length
GSI	Gonadosomatic index
H	Hydrated stage
LYG	Late yolk globule
LA	Late atretic stage stage
OW	Ovarian weight
MI	Marginal increment
MN	Migratory nucleus stage
PMF	Percentage mature females
PN	Peri-nucleus stage
PV	Pre-vitellogenic stage
POF	Post-ovulatory follicle
RSS	Residual sum of squares

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Abstract

The crimson seabream *Evynnis tumifrons* is endemic to the coastal waters of China, Hong Kong, Japan, South Korea and Taiwan, and is found on the rocky reefs, gravel and sandy bottoms on the continental shelves. *E. tumifrons* is a commercially important fish species and is mainly caught using gillnets, surrounding seine nets and angling. However, its biological aspects have not been sufficiently studied. The present study aims to describe the population biology of *E. tumifrons* off the southwestern coast of Kyushu, Japan.

Fish specimens were mainly collected at Eguchi Fisheries Cooperative, Hioki City, Kagoshima Prefecture, southern Japan. Fishers' belonging to this cooperative caught the fish using gillnets and surrounding nets. After landing, fish were sorted into eight categories according to their weight by the cooperative's staff. Specimens of various sizes from these categories were sampled once a month. For reproductive analysis, samples were collected from April 2012 to June 2014. A total of 801 ovaries were examined by histological observation to estimate the degree of ovarian maturation. For growth analysis, a total of 1599 specimens (794 females and 805 males) of various sizes were collected from all categories once a month from April 2012 to August 2013. 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 in 2004 and with Danish seine and gill net off Ibusuki city from 1999 to 2004 were used for the growth analysis. 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.

The ovarian maturity stages were classified into six categories based on the appearance of the most advanced oocytes, post-ovulatory follicles and atretic oocytes in the ovary as

follows: immature, maturing, mature, spawned, spent and resting. Females with the ovaries at maturing, mature, spawned or spent stages were identified as sexually mature individuals, and the size at sexual maturity was estimated to be 179 mm fork length based on 50% maturity size. Monthly changes in gonadosomatic index and the occurrence of mature or spawned maturity stages showed that spawning season lasts from November to May with an intermission in March 2013. The factor responsible for this intermission was considered to be the low water temperature that occurred in the preceding month.

Results of the monthly changes in marginal increment on the transversely sectioned otoliths revealed that one ring mark was formed per year from late spring to early summer seasons. 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 were not significantly different between the sexes. Maximum age observed was 15 years for females and 16 years for males.

The evidence of regional differences in the reproductive biology and the understanding that previous studies may have underestimated the age and growth could have important implications for proper management. The initial growth at the young age (1 year old) varied in each population distributed across the coastal waters of Japan further exacerbated the concerned implications because it suggests that growth parameters and size at sexual maturity of the populations are likely to vary between each other. Hence, it is paramount that the findings of the present study are taken into consideration and incorporated into management policies targeting this species. This should be done on the determination of the permissible mesh size for the fishing gears and the fishing closure period. Furthermore, fisheries managers could use the estimated growth rate in analytical stock assessments to model the average changes in fish size with age.

CHAPTER 1: Background of the study

1.1 General Introduction

Globally, fish stocks that were once exploited within sustainable levels are decreasing (FAO 2018). In 1974, about 90% of the fish stocks were at sustainable levels, however, they were reduced to 66.9% in 2015. This could be due to the increasing demand for fish, where the per capita consumption has more than doubled in the recent 54 years. It was 9 kg in 1961 and 20.2 kg in 2015. The expansion in consumption has been driven not only by increased production, but also by a combination of many other factors including reduced wastage, better utilization, improved distribution channels and growing demand, linked with population growth, rising income and urbanization, and increasing health consciousness among consumers (FAO 2018). Hence, ensuring proper and well managed fish stocks is important, as demand for fish to sustain the social and economic needs of the world's population is increasing.

In Japan, annual stock assessments for 52 species and 84 stocks by the Fishery Agency revealed that in 2015, 50% of the targeted stocks were considered to be at low level, 31% at medium level and 19% at high level (Martí et al. 2017). This study reported that, although the medium level resources have slightly increased over the decade, the percentage of low-level resources has increased. Therefore, conservation effort is necessary to achieve sustainable use of fishery resources. Fish is of great socio-economic importance to daily survival of the 126,000,000 people inhabiting the archipelago. Their reliance on the resource was estimated to be approximately 40% in the shared percentage of animal protein consumed (Matiya et al. 2006), and the per capita consumption of fish was estimated to be 66.2 kg being the sixth highest in the world (JIFRS 2004). Apart from nutrition, fish is a source of income to the fishers' households. The production from the coastal fisheries constitutes about 45% of the total fishery production and it constitutes about 60% in terms of value of the total fisheries product sales (Matiya et al. 2006). However, in recent years the per capita consumption for fish has been decreasing. In 2014, it was reported to be 27.3 kg per year. Despite the decrease, the trend of the domestic volume and the monetary value of marine fisheries production continued to increase by 0.2% (or about 10,000 tons) per year to 3.74 million tons and 2% (or 21.3 billion yen) to 969.3 billion yen in 2014 (Fisheries Agency of Japan 2016). Therefore, ensuring the long-term sustainability of the fisheries and fisheries products is one of the most important policy objectives for Japan.

The present study examined the fisheries biology of crimson sea bream *Evynnis tumifrons* using the samples collected off the southwestern coast of Kyushu, Japan. *Evynnis tumifrons* (Fig. 1.1) is one of the commercially important coastal fishes in Japan and appropriate stock management is needed. Figure 1.2a shows the catch landings of the *E. tumifrons* in Japan. The landings are decreasing from 4000 tons in 1970 to 1800 tons in 1995; however, data on the recent landings are unknown due to lack of the statistics. Similar trend is also noticed in Kagoshima Prefecture, southwestern coast of Kyushu, Japan (Fig. 1.2b). The present study selects the Eguchi Fisheries Cooperative to represent the fisheries cooperatives on southwestern coast of Kyushu, whose annual catch landing from February 2012 to January 2013 was 28.9 tons (Fig. 1.3). Matiya et al. (2006) reported that in Kaminada Fisheries Cooperative, Futami town in Matsuyama on Shikoku Islands, total fish production has been decreasing over the years, possible due to overexploitation, pollution and climate change. Although recent landing data are unknown in Kagoshima Prefecture, it is likely that the stock is decreasing, possibly due to these factors. Furthermore, previous studies on this species are widely ranged from the northern to southern regions of Japan, but not in the southwestern region, where this species is commercially targeted by a variety of fisheries, e.g. gillnets, surrounding seine nets and angling fishery. For these reasons, I conducted this research to

Fig. 1.1 A specimen of crimson sea bream *Evynnis tumifrons*

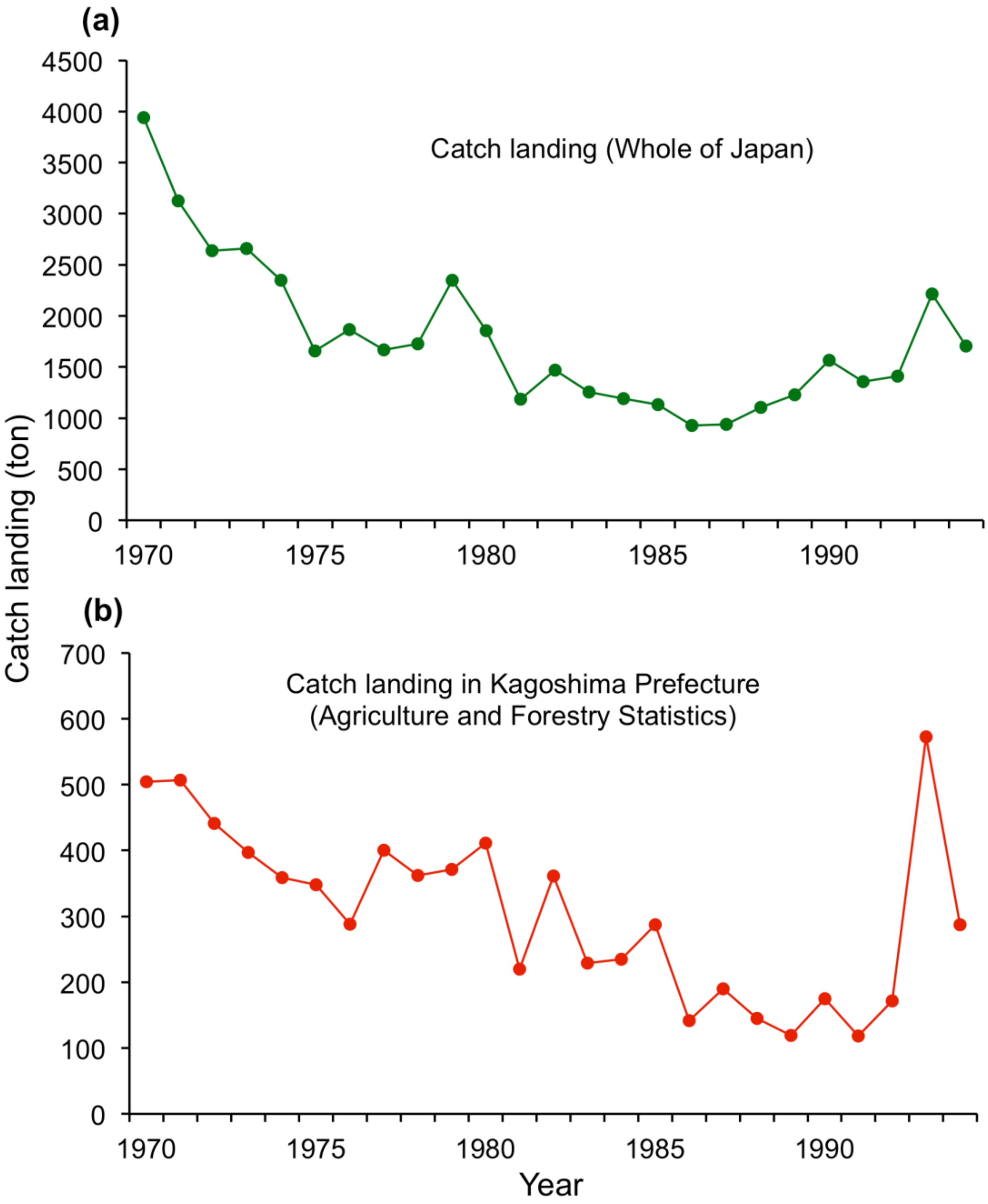

Fig. 1.2 Catch landing of *Evynnis tumifrons* in **(a)** whole of Japan and **(b)** Kagoshima Prefecture. Data were cited from the annual reports of Kagoshima Prefectural Agriculture, Forestry and Fisheries statistics Association

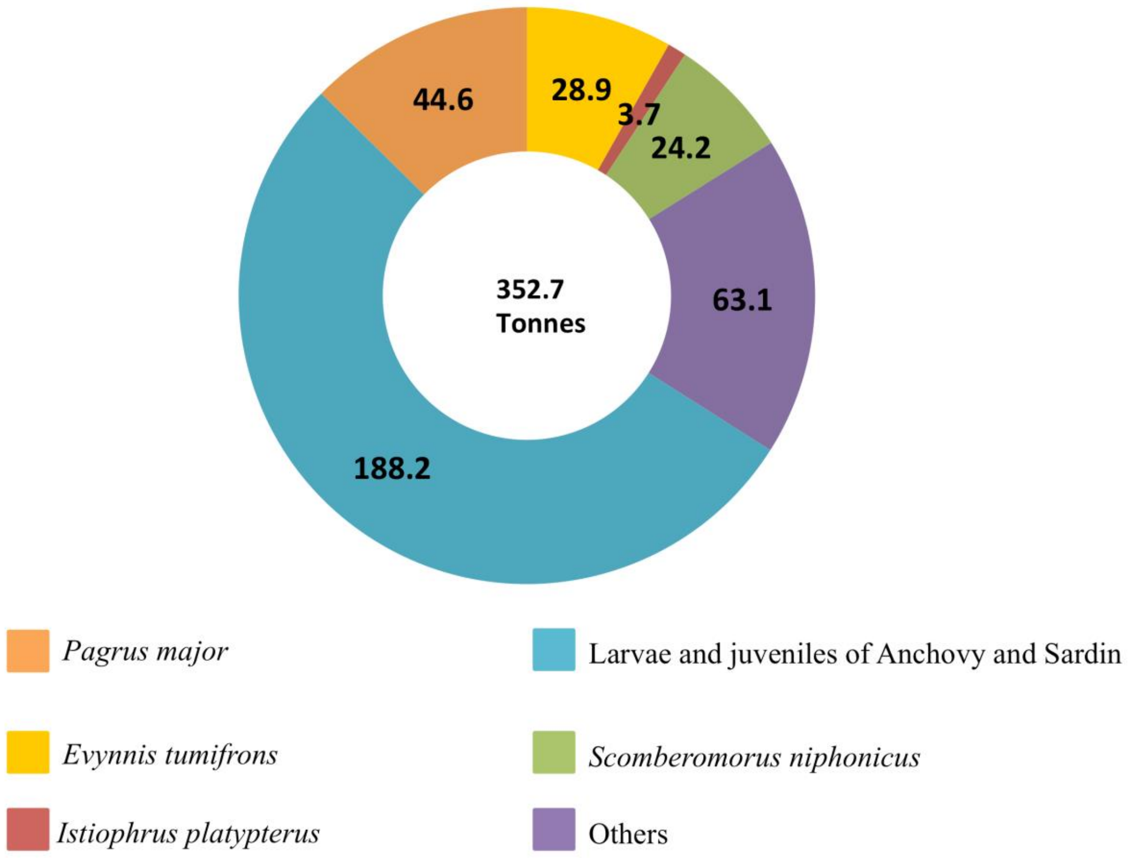

Fig. 1.3 Catch landing at Eguchi Fisheries Cooperative from 1st Feb 2012 to 31st Jan 2013. Data were cited from the annual report of Eguchi Fisheries Cooperative

understand the biology of the species inhabiting the coastal waters off the southwestern coast of Kyushu, Japan because such information is vital towards attainment of sound biological assumptions for stock assessment and management purposes (Schaefer 2001) since it is commercially exploited.

In some previous studies, e.g. Mio (1962), Toriyama et al. (1975), Yamahora (1983) and Anzawa et al. (1987), the spawning seasons of *E. tumifrons* was estimated mainly from the monthly changes in gonadosomatic index (GSI) and the size at sexual maturity was examined only in Niigata Prefecture (Anzawa et al. 1987). Some studies have suggested that the estimation of the reproductive characteristics of fish can be improved through the histological observation of their ovaries (West 1990; Tyler and Sumpter 1996; Blazer 2002; Alejo-Plata et al. 2011). Moreover, age and growth of *E. tumifrons* were previously determined with its 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 otolith has been recommended as age determination character because otolith continues to grow towards the internal (proximal) side as fish age (Beamish and McFarlane 1983; Casselman 1987; Abecasis et al. 2008) and annuli on it are clearly exhibited (Masuda and Noro 2003; Masuda et al. 2003; Lee et al. 2009; Piddocke et al. 2015).

Given that the previous studies estimated the spawning season based on the gonadosomatic index (GSI), and the age and growth were determined from the fish scales, it is apparent that reliable methods should be used to estimate these biological parameters. This research intends to provide the information on the population biology of *E. tumifrons*. It is hoped that results obtained will assist in the development of management measures that take into account the variance in minimum size of the mature females, spawning season, and age and growth of the species between its geographical distributions.

The present study specifically addressed the following:

1. To describe the ovarian maturation, size at sexual maturity and the annual reproductive cycle of the female *E. tumifrons* off the southwestern coast of Kyushu, Japan using the histological approach.

2. To describe the age and growth of *E. tumifrons* with transversely sectioned otoliths using the samples collected off the southwestern coast of Kyushu, Japan.

1.2 Description of the study site

The area where the specimens were caught is off the southwestern coast of Kyushu, Japan and it is the fishing ground permitted to fishers of Eguchi Fisheries cooperative. The area lies adjacently to Fukiage beach on the western side of Kagoshima Prefecture, Japan and is 20 km away from Kagoshima City. Bathymetry of the fishing area consists of sandy and rocky bottoms with portions of patchy reefs where *E. tumifrons* aggregates. Fishing activity concentrates within the 8 km from the shoreline at maximum depth of 30m to 40m (Uda pers.comm).

1.3 Fishing Methods

Specimens used in this study were caught using gillnet and the surrounding net. The descriptions of the two gears are as follows:

1.3.1 Gill Net

It is a long wall net set vertically in the transverse direction of the migrating fish or aquatic animal so that they try to make their way through the net wall and get stuck or entangled in the meshes (Fig. 1.4). The upper end of the wall net is raised by floats and the lower end is weighted by sinkers (Kaneda 2005).

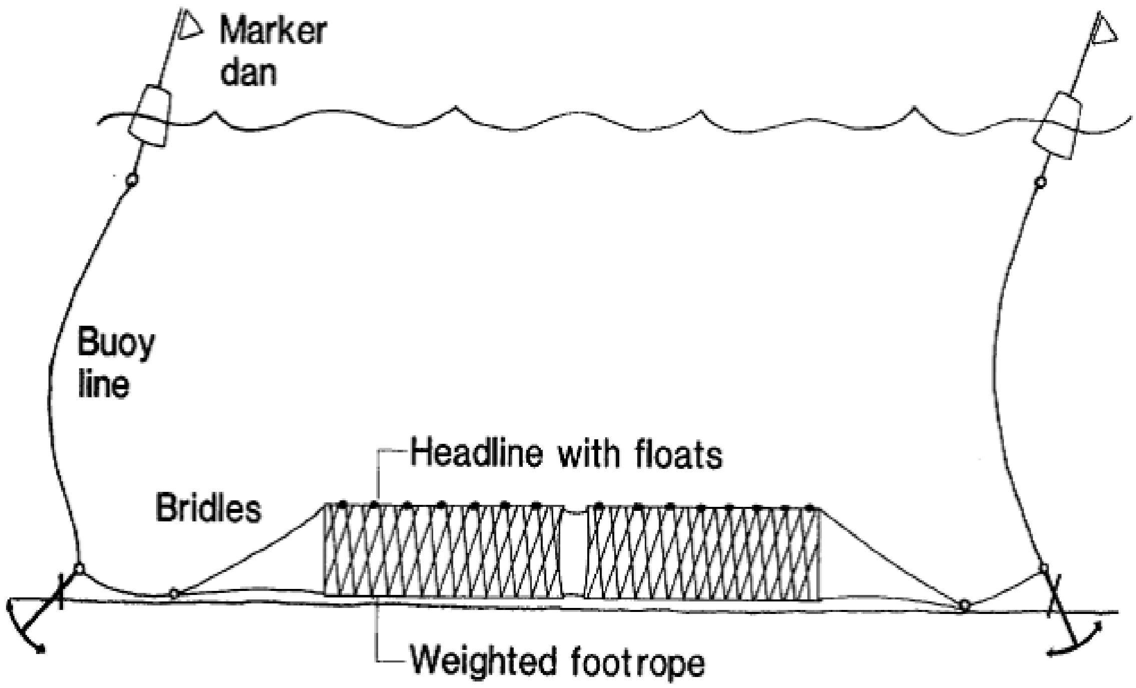

Fig. 1.4 Systematic illustration of the bottom gill net during deployment in the water column (Millner 1985).

1.3.2 Surrounding seine net

The surrounding seine net is constructed of a net bag made of flat oval netting shrinked at the front side and the towlines connecting with both sides of the bag (Fig. 1.5). It threatens the fish schools by tightly encircling the towlines, drives them into the cod end of the net and gets them stuck or entangled in the meshes. The net captures the ground fish such as sea bream, conger eel and so on, but differs in the method either from the trawl fishery which forcibly gathers fish in the net by pulling it on the sea bottom or from the hand trawl fishery which pulls nets by vessels anchored (Kaneda 2005).

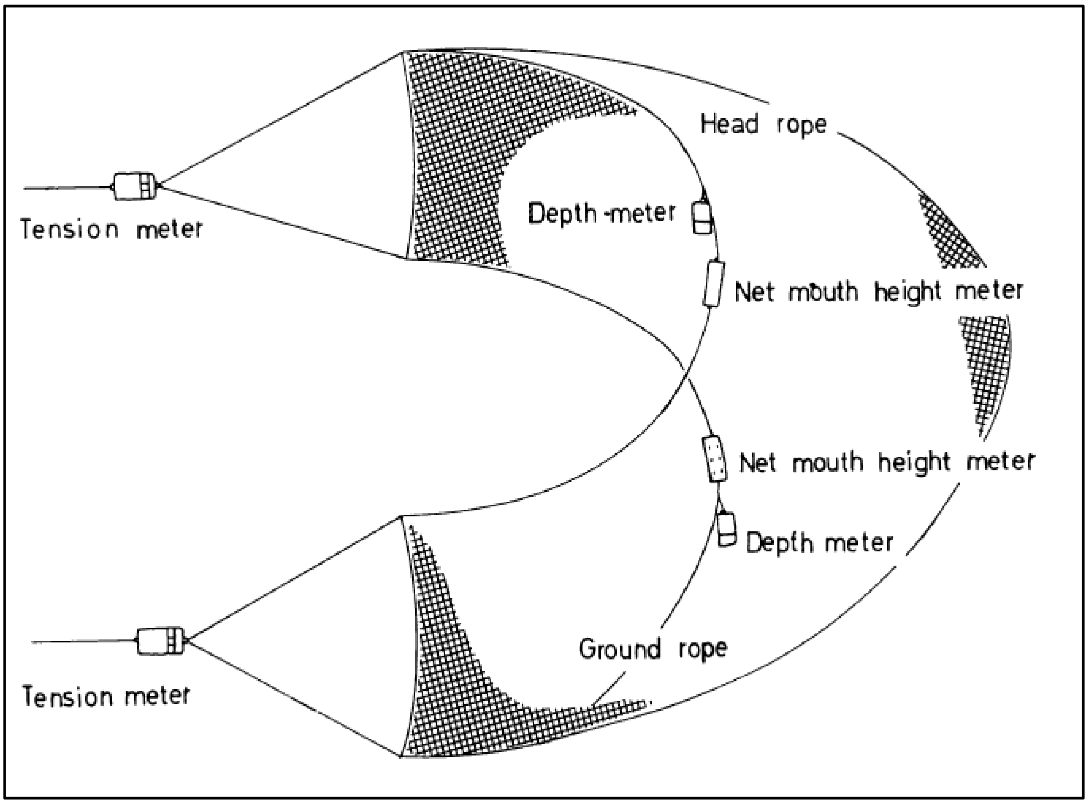

Fig. 1.5 Illustration of the surrounding seine net during deployment in the water column (Higo et al. 1990)

CHAPTER 2: The reproductive biology of female crimson sea bream *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan

2.1 Reproductive biology of female crimson sea bream *Evynnis tumifrons*

2.1.1 Summary

Ovarian maturation, size at sexual maturity and annual reproductive cycle of female crimson sea bream *Evynnis tumifrons* were studied using samples collected from April 2012 to June 2014 off the southwestern coast of Kyushu, Japan. A total of 801 ovaries were examined histologically observation to estimate the degree of ovarian maturation. *E. tumifrons* showed an asynchronous ovary signifying multiple spawning in a single reproductive season. Ovarian maturity stages were classified into six categories based on the appearance of the most advanced oocytes, post-ovulatory follicles and atretic oocytes in the ovary as follows: immature, maturing, mature, spawned, spent and resting. Females with the ovaries at maturing, mature, spawned or spent stages were defined as sexually mature individuals and the size at sexual maturity was estimated to be 179-mm in fork length. Monthly changes in the gonadosomatic index and the occurrence of matured or spawned maturity stages showed that the spawning season lasts from November to May with an intermission in March 2013. The factor responsible for this intermission was considered to be the low water temperature that occurred in the preceding month.

2.1.2 Introduction

The crimson sea bream *Evynnis tumifrons* (Sparidae) is distributed in the coastal waters of China, Hong Kong, Japan, South Korea and Taiwan (Akazaki 1962, 1984; Youn 2002; Iwatsuki et al. 2007, 2014). This species inhabits rocky reefs, gravel and sandy bottoms on the continental shelves (Hayashi 2002). Its bathymetrical distribution is determined by its ontogenetic development where juveniles are commonly found at shallow depths (~10 m) and adult stages mostly in deeper waters (50–100 m) (Iwatsuki et al. 2014).

Evynnis tumifrons is a commercially important fish species in the coastal waters of Japan (Kudoh and Yamaoka 2004), and its population biology has been studied in various localities (prefectures). Mio (1961, 1962) reported the age and growth, and spawning season and fecundity, of this species off Fukuoka. Toriyama et al. (1975) estimated the distribution and spawning season of *E. tumifrons*, and Toriyama and Kudo (1976) determined its age and growth off Kochi and Miyazaki. Yamahora (1983) compared the life history of *E. tumifrons* and *Pagrus major* off Yamagata. In addition, Shibuya (1985) studied the age and growth of *E. tumifrons* off Akita, while Anzawa et al. (1987) examined its spawning season and the age and growth off Niigata.

Among these biological studies, those on the reproduction biology of the species are important for the estimation of reproductive potential of stock and for the construction of appropriate management models for sustainable harvesting (Farley et al. 2015). Reproductive strategies of a fish also play a major role in its geographical distribution and population dynamics. Therefore, reliable information and sufficient understanding of a fish's reproduction are essential. In previous studies, however, the spawning season of *E. tumifrons* was estimated mainly from the monthly changes in its gonadosomatic index (GSI), and the size at sexual maturity has only been examined in Niigata Prefecture (Anzawa et al. 1987). Some studies have suggested that the estimation of the reproductive characteristics of fish can be improved through histological observation of its ovaries (West 1990; Tyler and Sumpter 1996; Blazer 2002; Alejo-Plata et al. 2011). Histological techniques have been used successfully in other related species, such as *Pagrus major*, to determine the diurnal rhythm of oocyte development during spawning season (Matsuyama et al. 1988), and e.g. *Dentex hypselosomus*, to clarify gonadal development and the annual and/or diurnal reproductive cycle (Oki and Tabeta 1998; Tominaga et al. 2005; Yoda and Yoneda 2009), but up to now histological techniques have been infrequently used to determine the fecundity *E. tumifrons* (Mio 1962).

Furthermore, the localities of previous studies range widely from the northern to southern regions of Japan, but did not include in the southwestern region, where *E. tumifrons* is targeted by a variety of commercial fisheries, e.g. gillnets, surrounding seine nets and angling. In addition to these limitations, the duration of spawning season, the percentage of females spawning per day (spawning frequency) and the number of oocytes per spawning (batch fecundity), which are essential for the estimation of the annual fecundity of a multi-spawning fish with indeterminate fecundity (Yoda and Yoneda 2009), should be determined so that appropriate and timely management approaches can be established to protect sexually mature populations *E. tumifrons* and to allow a sufficient proportion of sexually mature fish to spawn.

The focus of the present study is on the elucidation of the spawning season of *E. tumifrons*. Hence, the objectives of this study were to describe the ovarian maturation, size at sexual maturity and the annual reproductive cycle of female *E. tumifrons* off the southwestern coast of Kyushu, Japan using an histological appraoch. The obtained results derived are indispensable for the estimation of the reproductive potential of this species and for the development of appropriate management measures.

2.1.3 Materials and methods

2.1.3.1 Sampling and measurement

Monthly samplings of *E. tumifrons* were conducted at Eguchi Fisheries Cooperative, Hioki City, Kagoshima Prefecture, southern Japan from April 2012 to June 2014. Fishers belonging to this cooperative caught the *E. tumifrons* using gillnets and surrounding seine nets from 31°33' to 31°39'N and from 130°13' to 130°20'E off Hioki City, on the southwestern coast of

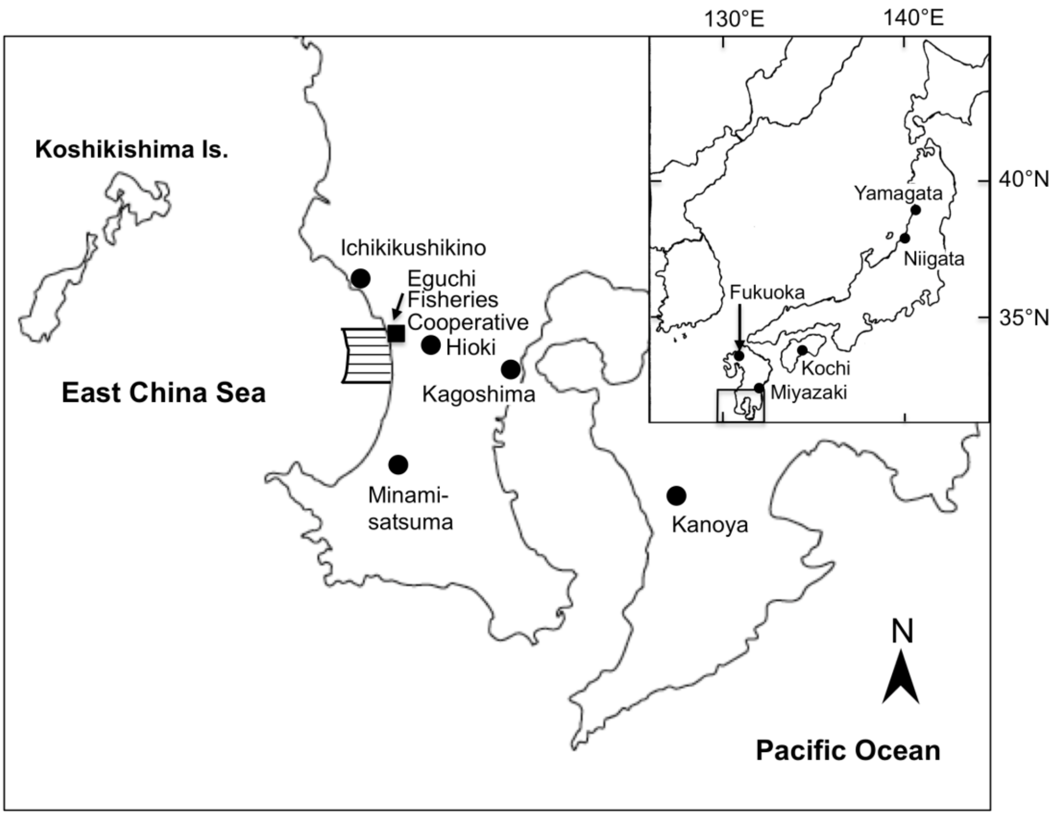

Fig. 2.1 Map of the sampling site (shaded area) of *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan

Kyushu, Japan (Fig. 2.1). After landing, fish were sorted into eight categories according to their weight by the cooperative's staff. I sampled specimens of various size from these categories once a month; a total of 1101 females were collected. Fish specimens were immediately chilled on ice and taken to the Laboratory of Fisheries Biology of Faculty of Fisheries, Kagoshima University.

For each specimen, fork length (FL) was measured on a measuring board to the nearest 1 mm, body weight (BW) on an electronic balance (Shimadzu, UX6200H) to the nearest 0.01 g and ovarian weight (OW) on an electronic balance (Shimadzu, UX620H) to the nearest 0.001 g. Ovaries were fixed in 10% formalin until they were histologically examined. The gonadosomatic index (GSI) was calculated as: $\mathrm{GSI} = 100 \times \mathrm{OW}/(\mathrm{BW} - \mathrm{OW})$.

2.1.3.2 Histological analysis

A total of 801 ovaries were examined histologically to estimate the degree of ovarian maturation. A small piece of tissue was taken from the middle part of a female's gonad, embedded in paraffin, sectioned into 6-μm thickness, and stained with Mayer's haematoxylin and eosin. The developmental stages of oocytes were classified according to Yamamoto (1956) and Granada et al. (2004b) with a few modifications for this species, and the histological classification of atretic oocytes and post-ovulatory follicles followed Hunter and Macewicz (1985). Ovarian maturity stages were estimated from the appearance of the most advanced oocytes, post-ovulatory follicles and atretic oocytes (Granada et al. 2004b; Alejo-Plata et al. 2011; Sun et al. 2015; Okochi et al. 2016).

2.1.3.3 Size at sexual maturity

In the present study, the FL at which 50% of the females are sexually mature (FL_{50}) was estimated as the mean length at sexual maturity (King 2007). Sexually mature individuals were defined as females with ovaries at maturing, mature, spawned or spent stages which had yolked oocytes. The percentage of sexually mature females (PMF) was plotted against FL and size at 50% maturity was estimated by fitting a logistic function based on the least-squares method using the software DeltaGraph 7 (Red Rock Software, Salt Lake City, UT). The logistic equation was expressed as: $\mathrm{PMF} = 100/[1 + \mathrm{Exp}(-r(\mathrm{FL_m} - \mathrm{FL_{50}}))]$,

where r is slope of the curve and $\mathrm{FL_m}$ is the median value of each FL class.

2.1.3.4 Annual reproductive cycle

Annual reproductive cycle was analysed from the monthly changes in the GSI and the occurrence of each ovarian maturity stage. Fish smaller than the minimum size of the sexually mature females were excluded from this analysis.

2.1.3.5 Condition factor

The monthly change in condition factor (CF) was analyzed to examine the seasonal change of the body condition of the fish throughout the year. The formula used was based on Zhu et al. (1989): $\mathrm{CF} = (\mathrm{BW} - \mathrm{OW}) \times 1000/\mathrm{FL}^3$,

where BW is in grams, OW is in grams and FL is in millimetres.

2.1.4 Results

2.1.4.1 Developmental stages of oocytes

The following developmental stages of the oocytes were observed. Peri-nucleolus stage (Fig. 2.2a): the nucleus is relatively large and multiple nucleoli can be seen around its periphery. The cytoplasm was basophilic and stained dark blue by hematoxylin. Oocyte diameter ranges from 60 to 120 µm. Pre-vitellogenic stage (Fig. 2.2b): yolk vesicle- or oil droplet-like granules appeare in the cytoplasm and gradually increase in number and size. The cytoplasm is basophilic and oocyte diameter ranges from 90 to 275 µm. Early yolk globule stage (Fig. 2.2c): small acidophilic yolk globules start to appear in the cytoplasm. Oocyte diameter ranges from 195 to 310 µm. Late yolk globule stage (Fig. 2.2d): yolk globules increase in size and covered the whole cytoplasm. Oil droplets around the nucleus increase in size. Oocyte diameter ranges from 295 to 450 µm. Migratory nucleus stage (Fig. 2.2e): the nucleus migrates towards the animal pole where smaller yolk globules occur and the yolk globules begin to coalesce. Oocyte diameter ranges from 370 to 485 µm. Hydrated stage (Fig. 2.2f): yolk globules completely undergo coalescence and form a single translucent yolk mass. Oocyte diameter ranges from 385 to 650 µm. Early atretic stage (Fig. 2.2g): the nucleus and yolk globules disintegrate and are reabsorbed, leaving only the follicular layers. This stage corresponds with alpha stage atresia described by Hunter and Macewicz (1985). Late atretic stage (Fig. 2.2h): the follicle decreases in size and is characterized by numerous disorganized granulosa cells and intracellular vacuoles. This stage corresponds with beta stage atresia described by Hunter and Macewicz (1985). In addition, post-ovulatory follicle (Fig. 2.2i), which is characterised by a granulosa cell layer, coils up within a less irregular thecal cell layer covering, and is frequently observed during the spawning season.

2.1.4.2 Ovarian maturity stages

Evynnis tumifrons has an asynchronous ovary containing oocytes at various stages of development (Fig. 2.3). Based on the appearance of the most advanced oocytes, post-ovulatory follicles and atretic oocytes, the ovaries were classified into the six ovarian maturity stages: immature (I), maturing (II), mature (III), spawned (IV), spent (V) and resting (VI). The development of oocytes at each stage is shown in Table 2.1.

2.1.4.3 Size at sexual maturity

The size at sexual maturity was estimated from the 177 females collected in the spawning months from November 2013 to May 2014. The minimum size of mature females was 161 mm FL. The estimated logistic function was as follows: $\mathrm{PMF} = 100/[1 + \mathrm{Exp}(-0.119(\mathrm{FL_m} - 179))]$ (n = 18, r^2 = 0.975). FL_{50} was estimated to be 179 mm FL (Fig. 2.4).

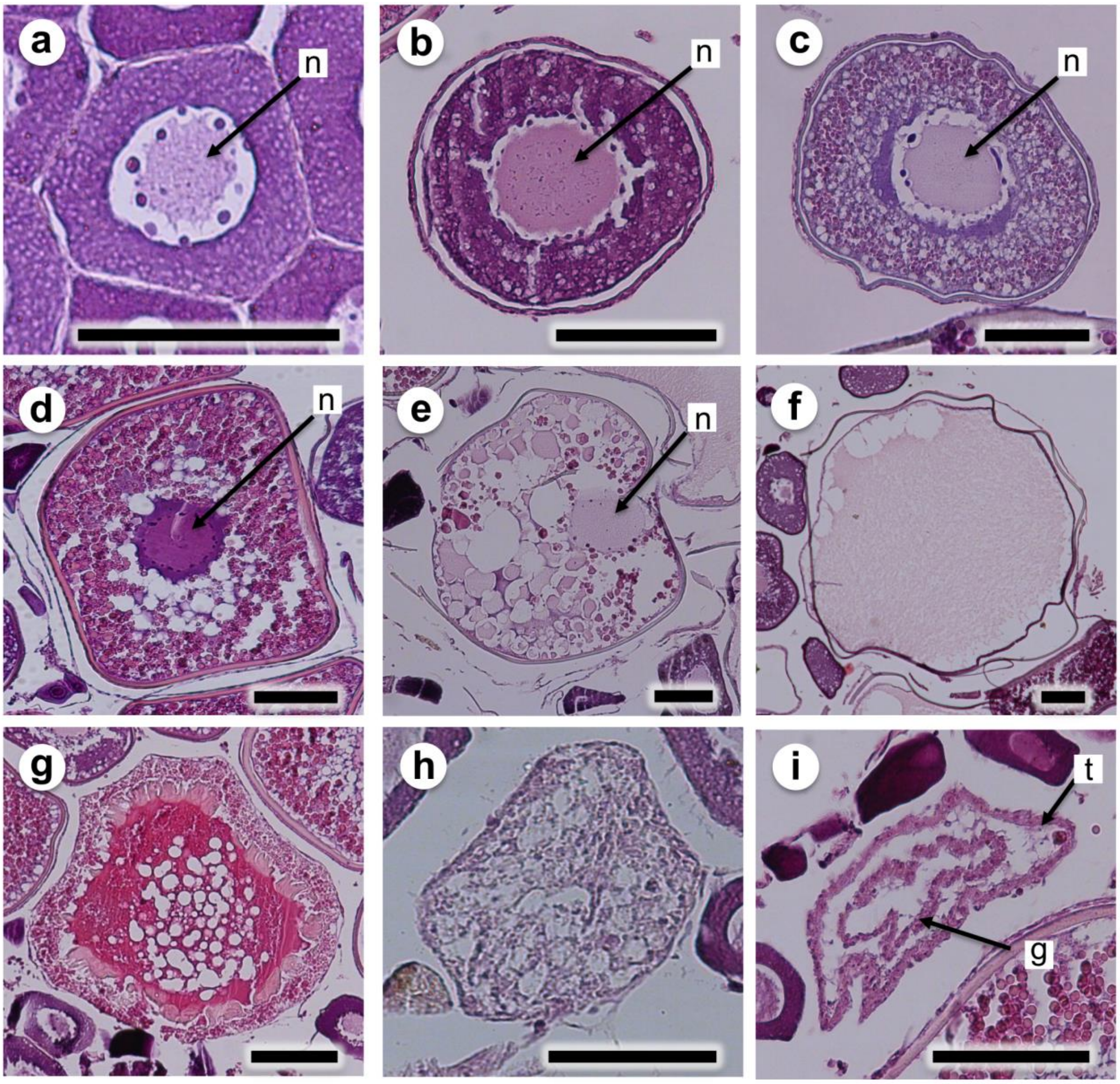

Fig. 2.2 Oocyte developmental stages and post-ovulatory follicle of female *Evynnis tumifrons*: **a** peri-nucleolus, **b** pre-vitellogenic, **c** early yolk globule, **d** late yolk globule, **e** migratory nucleus, **f** hydrated follicle, **g** early atretic follicle, **h** late atretic follicle, and **i** post-ovulatory follicle. *n* Nucleus, *g* granulosa cell layer, *t* thecal cell layer. Scale bars 100 μm

Fig. 2.3 Asynchronous oocyte development in the ovary of female *Evynnis tumifrons*: *PN* Peri-nucleus, *EYG* early yolk globule, *LYG* late yolk globule, *H* hydrated, *POF* post-ovulatory follicle. Scale bar 100 μm1

Table 2.1 Six ovarian maturity stages of female *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan

Ovarian maturity stage	Developmental stage of oocytes
Stage I (immature)	Only unyolked oocytes, such as PN and PV, were present. Oocytes ranged from 60 to 275 µm in diameter
Stage II (maturing)	Most advanced oocyte was in EYG or LYG. Oocytes were 195–450 µm in diameter
Stage III (mature)	Most advanced oocyte was in MN or H. Oocytes were 370–650 µm in diameter
Stage IV (spawned)	Yolked oocytes and POF were present
Stage V (spent)	More than 50% of yolked oocytes were in EA. POF was absent
Stage VI (resting)	Unyolked oocytes and LA oocytes were present

PN peri-nucleolus stage, *PV* pre-vitellogenic stage, *EY* early yolk globule stage, *LYG* late yolk globule stage, *MN* migratory nucleus stage, *H* hydrated stage, *EA* early atretic stage, *LA* late atretic stage, *POF* post-ovulatory follicle

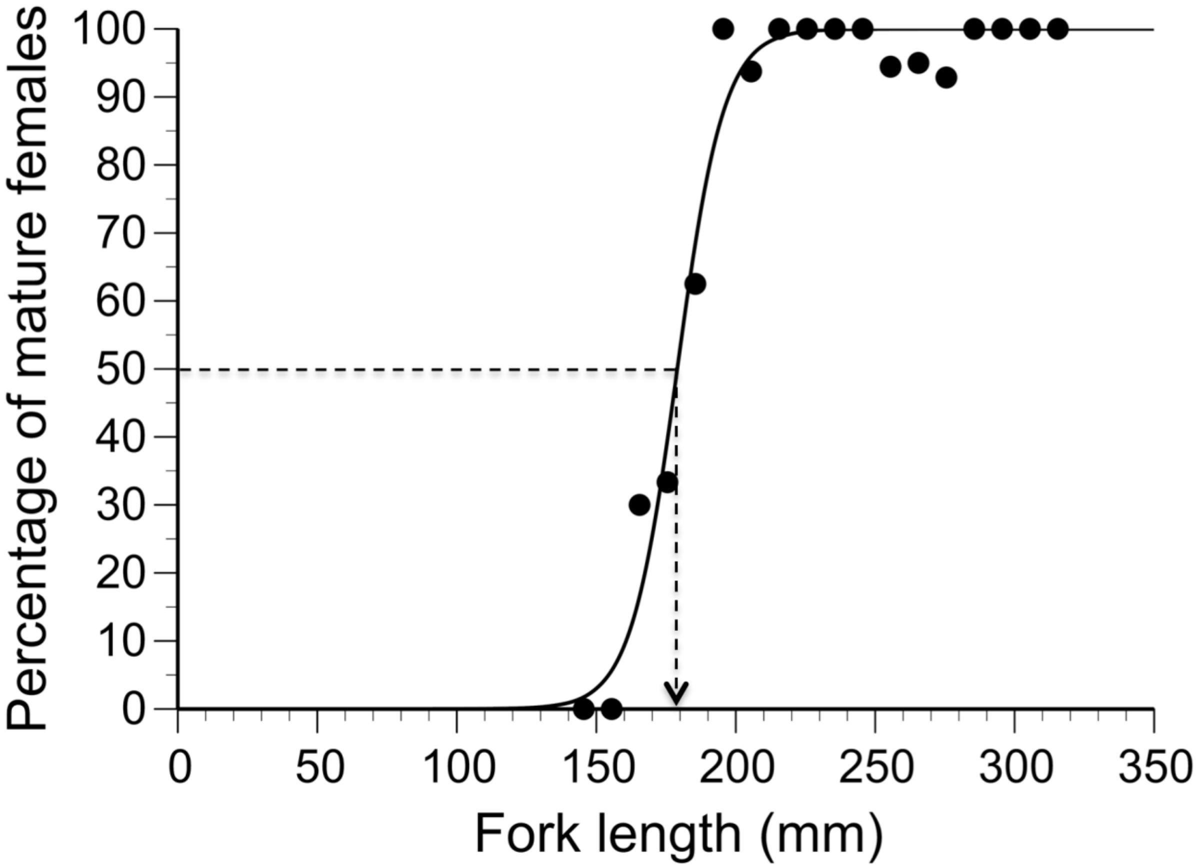

Fig. 2.4 Percentage of sexually mature females at 10-mm fork length (FL) intervals. A logistic curve was fitted to the data and the dotted arrow shows the FL at 50% maturity

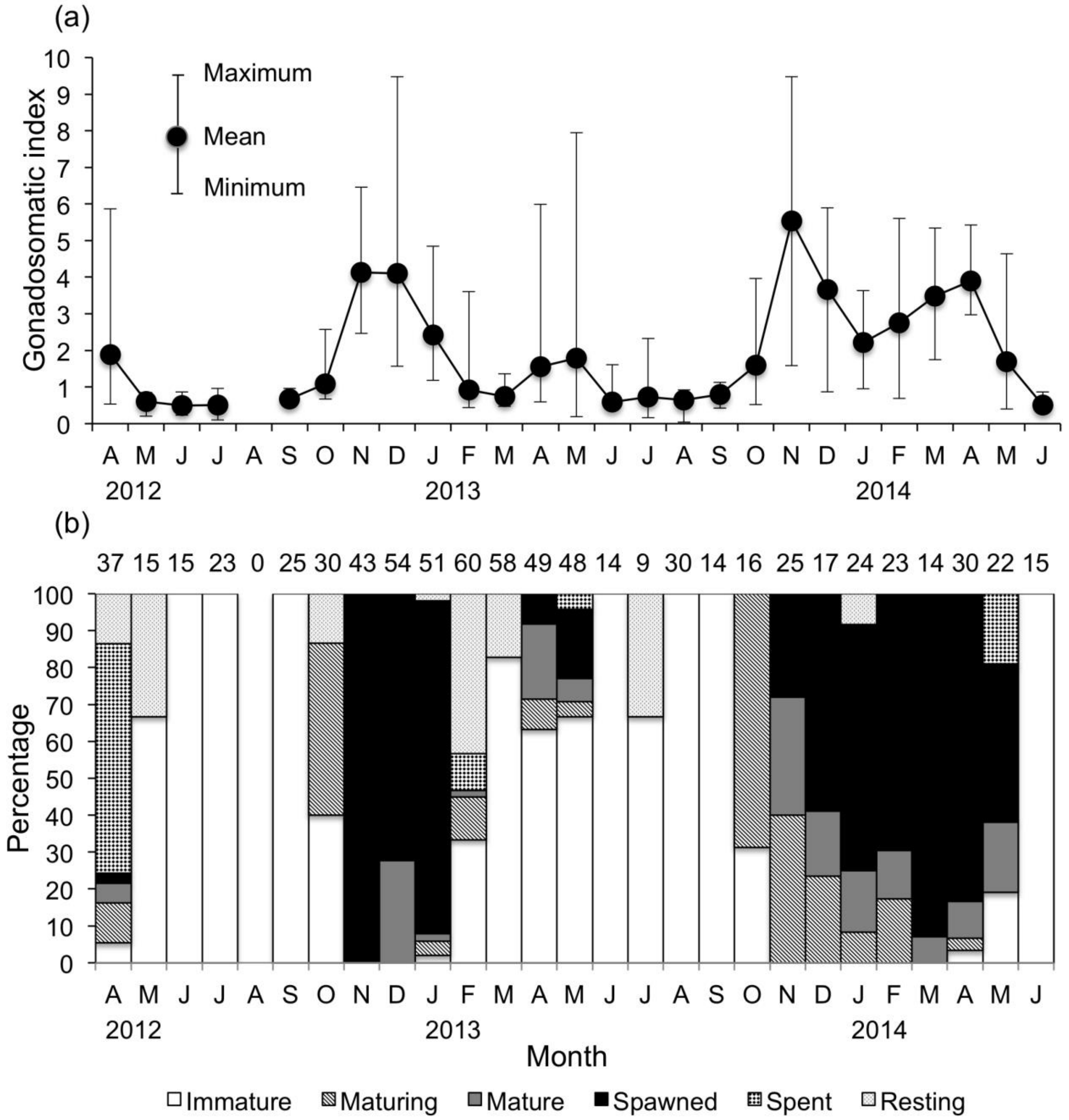

Fig. 2.5 Monthly changes in the gonadosomatic index (**a**) and percentage occurrence of the six ovarian maturity stages (**b**) of female *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan. Samples could not be collected in August 2012. Fish smaller than the minimum size of the mature females (<161 mm) were excluded from this analysis. Numbers of the females examined shown above the bars

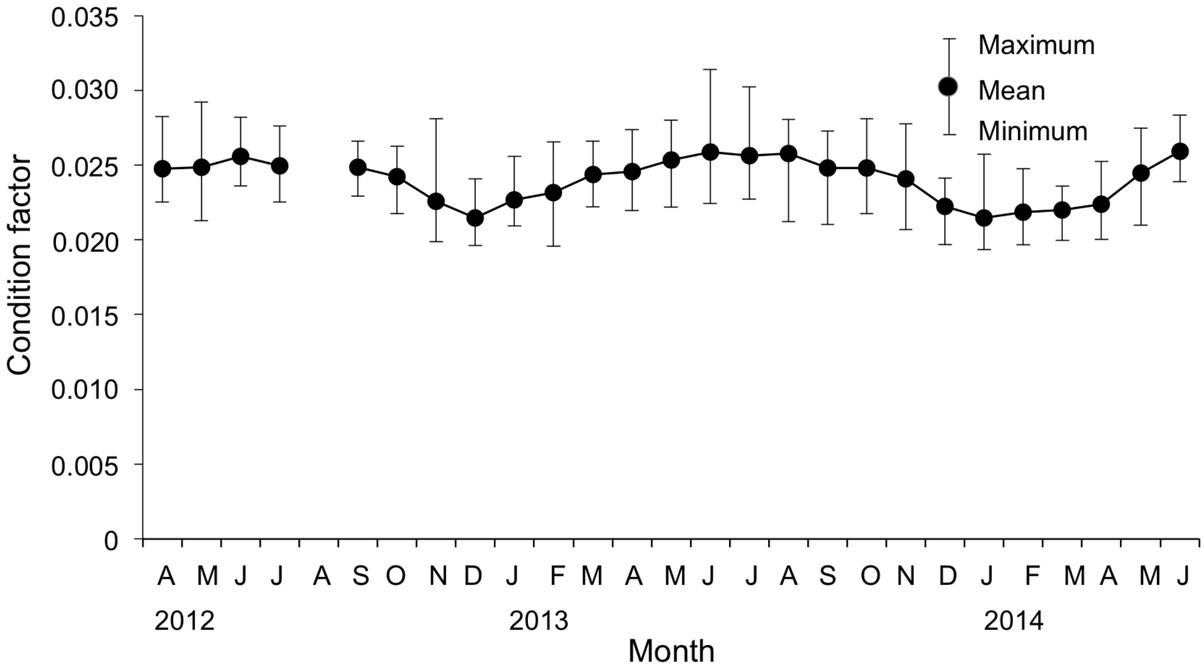

Fig. 2.6 Monthly changes in the condition factor of female *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan. Samples could not be collected in August 2012. Fish smaller than the minimum size of the mature females (<161 mm) were excluded from this analysis

2.1.4.4 Annual reproductive cycle

According to the results shown in Fig. 2.5a, the GSI had two yearly peaks, a higher one in November to December (late autumn–winter 2012, 2013), and a lower one around April (spring 2012, 2013, 2014). The monthly occurrences of the six ovarian maturity stages from April 2012 to June 2014 are shown in Figure 2.5b. Though *E. tumifrons* had an asynchronous ovary, which suggested multiple spawning during its reproductive season (Fig. 2.3), I noticed a general trend in which females tended to be mostly immature from June to September (early summer–early autumn 2012, 2013, 2014), and mature or spawned from November to May (late autumn–late spring 2012–2013, 2013–2014), though in March 2013 mature or spawned females could not be detected within my samples.

2.1.4.5 Annual condition factor cycle

The condition factor of fish tended to be high from June to October (summer–autumn 2012, 2013), decreased in November (late autumn 2012, 2013) and was lowest in December or January (winter 2012, 2014) (Fig. 2.6). However, there seems to be variation in the increase after the periods of lowest condition factor until May (spring 2013, 2014) within the 2 years of study. From January 2013 to May 2013, the increase was sharp compared to that from February 2014 to May 2014.

2.1.5 Discussion

Ovarian maturity stages of female *E. tumifrons* were classified into six categories based on the appearance of the most advanced oocytes, post-ovulatory follicle and atretic oocytes. Stage III (mature) was regarded as mature because migratory nucleus or hydrated oocytes occurred in the ovary. In the present study, the time course of the maturation process was not clarified, but in the related species *Pagrus major* and *Dentex hypselosomus*, nucleus migration starts around 24 h before spawning, and the subsequent hydration of the oocyte starts at around 12 h before spawning (Matsuyama et al. 1988; Yoda and Yoneda 2009). Stage IV (spawned) could be determined from the occurrence of post-ovulatory follicles (evacuated follicles) in the ovary. Post-ovulatory follicles occurring at ovulation are believed to be reabsorbed within 24 h (Matsuyama et al. 1988; Yoda and Yoneda 2009). Thus, stage III (mature) and stage IV (spawned) *E. tumifrons* were assumed to appear within a few days of spawning. It is also important to note that the time course of the maturation process is temperature dependent (Kurita et al. 2011). However, according to Hunter and Macewicz (1985) a migratory nucleus and hydrated oocytes indication imminent spawning, while post-ovulatory follicles are the histological evidence of very recent spawning (Hunter et al. 1992).

Size at sexual maturity is an important reference point for decision-making in fisheries management (Zhu et al. 2011). However, some previous related research (e.g. Mio 1962; Toriyama et al. 1975; Yamahora 1983) did not estimate this vital biological parameter. Anzawa et al. (1987), however, did estimate the minimum size of mature female *E. tumifrons*, i.e. 170 mm FL, from its GSI. In the present study, I estimated the minimum size of mature females as 161 mm FL and their size at FL_{50} as 179 mm FL (Fig. 2.4), based on the histological technique used. Thus the minimum size of mature females differed slightly between the current study and Anzawa et al.'s (1987). According to DeMartini et al. (2000) and Alejo-Plata et al. (2011), a histological approach has been proven to have greater precision for the estimation of size at maturity, so the difference in the minimum size estimated in my and Anzawa et al.'s (1987) might be due to the different methodologies used. On the other hand, the locality and date on which these two studies conducted were very different; this may be another factor contributing to the different estimated minimum size of females at sexual maturity. In future studies, histological techniques should be used to examine samples collected at the same time from different localities.

The simultaneous occurrence of a high GSI and mature or spawned maturity stages, which were considered to show the spawning season, lasted from November to May (7 months). The onset of the spawning season was clearly marked by the sharp increase in GSI and the occurrence of the mature or spawned stage in November. The preceding ovarian maturity stage II (maturing), which also occurred during the spawning season, may subsequently shift to ovarian stages III (mature) and IV (spawned), so that most fish were considered to undergo spawning judging from the transition of the six ovarian maturity stages (Fig. 2.5b). The end of the spawning season around May and June was clearly marked by the subsequent precipitous decline in the monthly change in GSI and the occurrence of fish with spent ovaries at stage V. This spent stage indicates that the cessation of spawning is imminent (Hunter and Macewicz 1985; Yoneda et al. 1998; Granada et al. 2004b). However, in 2013 (Fig. 2.5b), the spent stage occurred in February and in the subsequent month, no mature or spawned females were detected. This suggests that there was an intermission in spawning in March 2013. In some fish species, skipped spawning is observed, which is mostly attributed to a deficient diet and poor nutritional condition (Rideout et al. 2005; Rideout and Tomkiewicz, 2011). However, in the present study, the mean condition factors of the individuals collected in February and March 2013 were higher than those of individuals collected in February and March 2014 (Fig. 2.6). Figure 2.7 shows the monthly size composition during the spawning season (November–May) of the two study years. Small individuals were collected in April and May 2013 and May 2014, but not in March 2013. Thus the intermission in spawning in March 2013 may have not been due to individual status such as poor body condition and small body size. Figure 2.8 shows the water temperature from April 2012 to June 2014 off Ichikikushikino City, which is located near the study area. A similar trend in temperature fluctuations occurred throughout each year, but it should be noted that the minimum temperature in February 2013 was only 15.4 °C and in the following

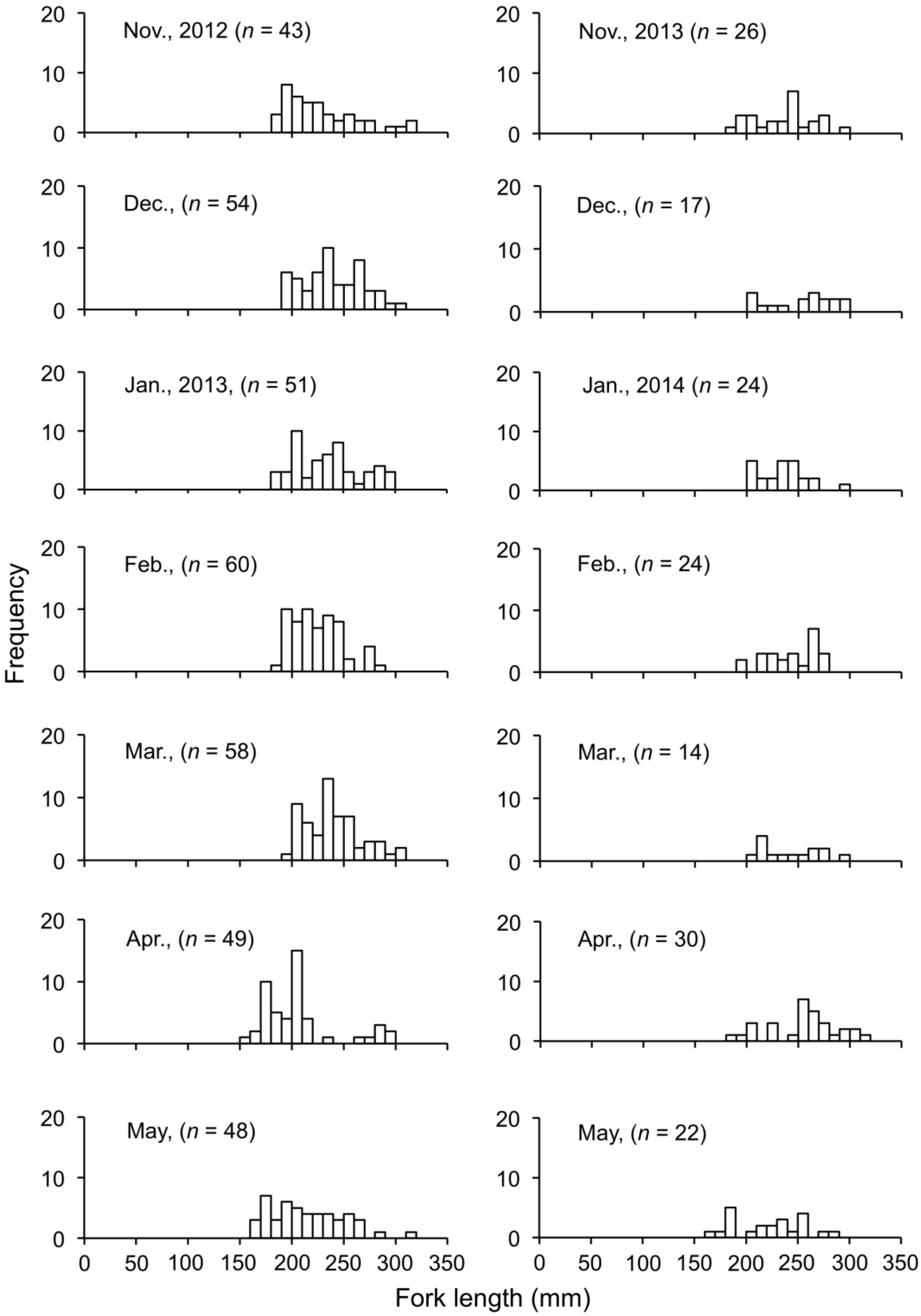

Fig. 2.7 Monthly lengths of female *Evynnis tumifrons* collected during the spawning season of the 2 years of the study. Fish smaller than the minimum size of the mature females (<161 mm) were excluded from this analysis

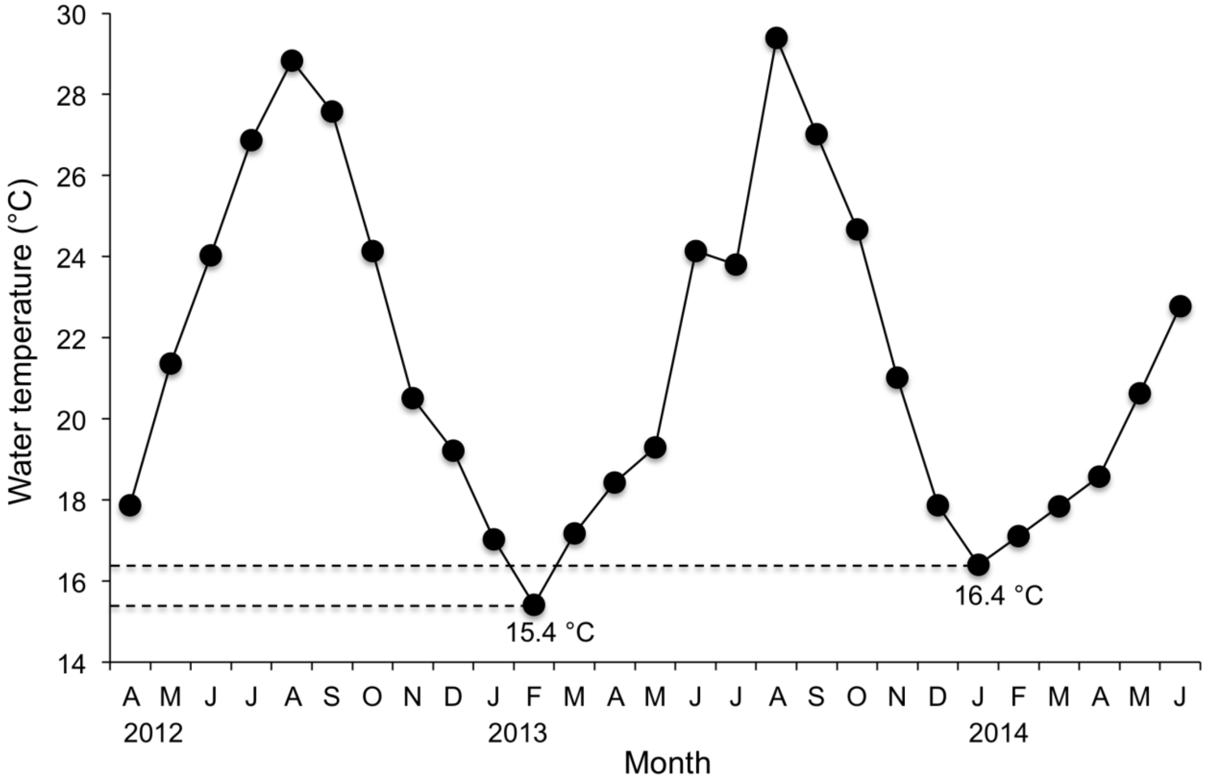

Fig. 2.8 Monthly changes in mean water temperature off the southwestern coast of Kyushu, Japan. Data were recorded daily off Ichikikushikino City near the study area by a temperature sensor at 5 m below the water surface attached to the hull of a ferryboat. Data are cited from the database of Kagoshima Prefectural Fisheries Technology and Development Centre (http://kagoshima.suigi.jp)

month (March) spawning was interrupted. On the other hand, in January 2014 the minimum temperature was 1 °C higher (16.4 °C), and this might have made conditions favourable for continuous spawning until May that year. Although these temperature data were measured at a different depths to those of the species' distribution, it should be noted that 5 m is below the water surface. Moreover, these temperature data were measured at a consistent depth throughout the study. Hence, the monthly temperature change observed should be sufficient to explain the change in spawning behaviour. Furthermore, the co-occurrence of sexually mature and immature individuals in April and May 2013 (Fig. 2.5b) following the intermission in spawning in March 2013 is an interesting phenomenon. According to Lowerre-Barbieri et al. (2011), larger, older individuals exhibited an extended spawning season compared to younger, mature individuals. However, my investigation revealed that nearly half or all larger individuals were immature during this extended spawning season (Fig. 2.9a). There was no significant (P>0.05) in the condition factors of the immature and sexually mature individuals either (Fig. 2.9b). Therefore, I could not determine the reason for the co-occurrence of sexually mature and immature individuals in April and May 2013.

The spawning seasons of *E. tumifrons* in the coastal waters of Japan varied according to locality (see Table 2.2; localities at the top of the table are located further north). In northern Japan (Yamagata and Niigata Prefectures), it tended to occur from mid-summer to mid-autumn, whereas in southern Japan (Fukuoka, Kochi and Miyazaki Prefectures), it occurred from early autumn to early winter. In the present study, which was conducted off the southwestern coast of Kyushu (the southernmost limit of *E. tumifrons'* distribution in Japanase coastal waters), it occurs from late autumn to late spring. This trend shows that in the north *E. tumifrons* spawns during the warmer months of the year, but shifts its spawning season closer to the colder months in southern areas, while at its southernmost limit, it spawns

Table 2.2 Comparison of spawning season of *Evynnis tumifrons* according to latitude in the coastal waters of Japan

Locality	Latitude	Spawning season	Author
Yamagata Prefecture	38º55'N	July–October	Yamahora (1983)
Niigata Prefecture	37º50'N	July–September	Anzawa et al. (1987)
Fukuoka Prefecture	33º50'N	September–November	Mio (1962)
Kochi and Miyazaki Prefectures	33º16'N and 32º10'N	October–December	Toriyama et al. (1975)
Southwestern coast of Kyushu	31º39'~31º33'N	November–May	Present study

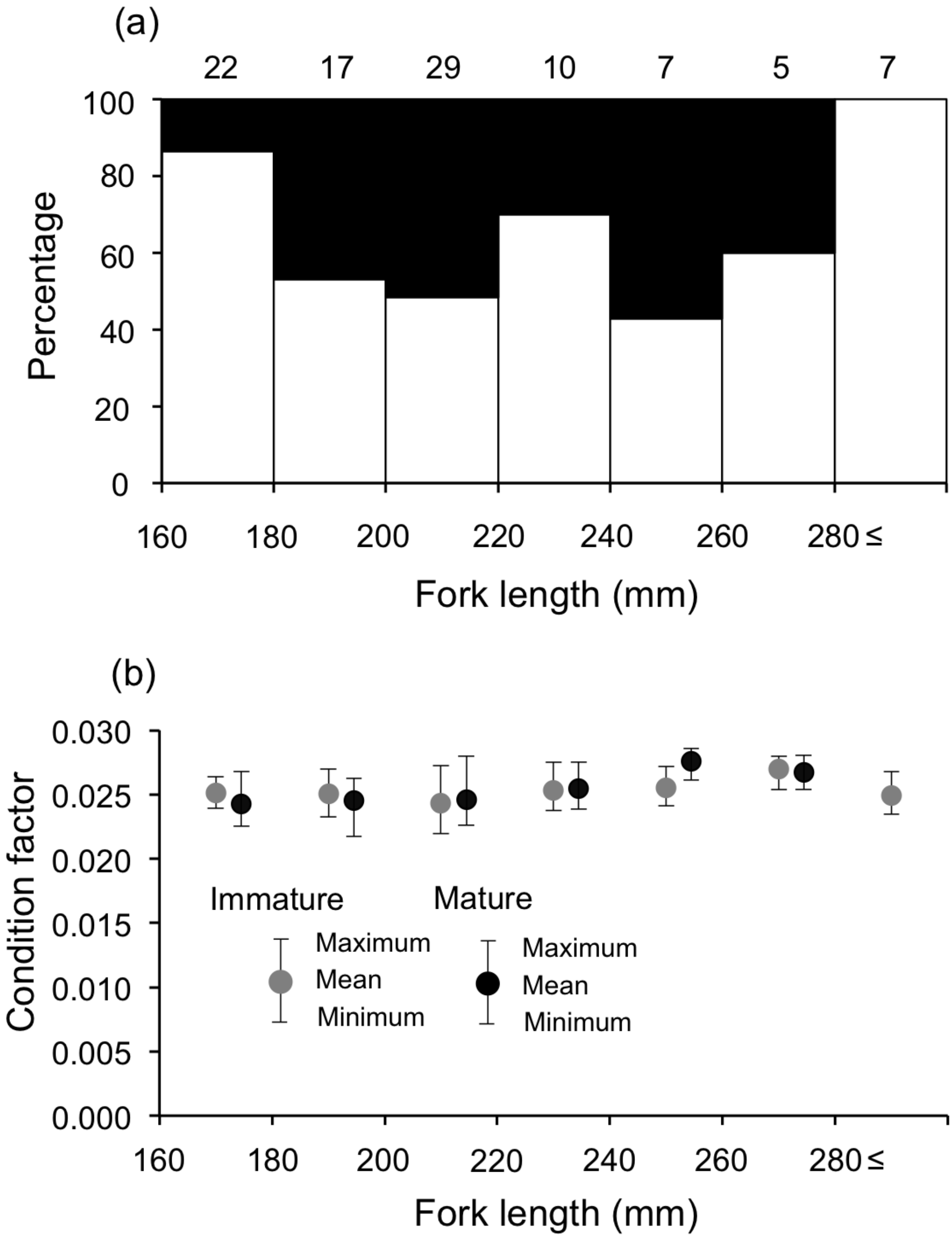

Fig. 2.9 Percentage occurrence (**a**) and condition factor (**b**) at 20-mm FL intervals of immature and mature female *Evynnis tumifrons* collected in April and May 2013 off the southwestern coast of Kyushu, Japan. Fish smaller than the minimum size of the mature females (<161 mm) were excluded from this analysis. Non-shaded bars indicate immature females, and shaded bars indicate mature females. Numbers of females examined shown above the bars

throughout the cold season. In addition, there seems to be a trend in the duration of spawning according to locality: at its southernmost location of *E. tumifrons* (present study), the spawning season lasts longer than at higher latitudes. This phenomenon was also observed in *Plectropomus areolatus* and *Epinephelus fuscoguttatus*, in which spawning season appeared longer nearer to the equator in the northern hemisphere (Sanchez-Cardenas and Arreguin-Sanchez 2012).

To elucidate why the spawning season varied depending on latitude, three localities were selected to investigate the effect of water temperature and day length on spawning season because these two factors are generally thought to be the most important triggers in the timing of gametogenesis and termination of spawning in temperate fish (Lam 1983; Hanyu 1991; Tominaga et al. 2005). In Figure 2.10 I illustrate the mean water temperatures during *E. tumifrons*' spawning season in Niigata (northern location) and Fukuoka (southern location), which had similar high water temperatures at spawning that overlapped. On the other hand, off the southwestern coast of Kyushu (southernmost location, present study), the trigger seemed to change to colder rather than warmer water; spawning in this species probably also has a lower threshold of water temperature beyond which the spawning process may stop.

Regarding the effect of day length (Fig. 2.10), spawning in northern Niigata was favoured when conditions combined warmer water with longer days, while to the south in Fukuoka a combination of warmer water conditions but with shorter days was preferred; finally in the southernmost location off the southwestern coast of Kyushu (present study), day length did not seem to have much effect. Thus, the range as well as the transition of water temperature and day length during the spawning season was differed between these three localities, which might have influenced the timing of gametogenesis and termination of spawning in different ways. Moreover, the following factors should be considered: firstly, each population of *E. tumifrons* distributed in the coastal waters of Japan may have evolved

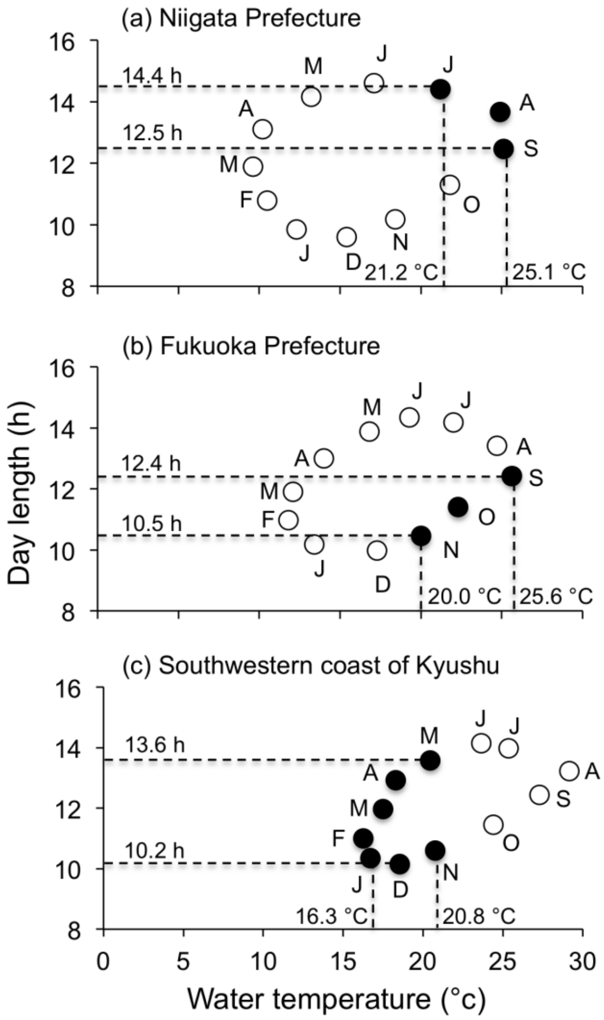

Fig. 2.10 Mean water temperature and day length when spawning occurred: **a** Niigata Prefecture, **b** Fukuoka Prefecture and **c** southwestern coast of Kyushu. Solid circles indicate spawning months and open circles indicate non-spawning months of *Evynnis tumifrons*. Water temperature data in Niigata and Fukuoka Prefectures were obtained from serial station data between 1906 and 2003 at 10-m depth and are cited from the database of the Japan Oceanographic Data Center (http://www.jodc.go.jp/jodcweb/). Water temperature data off the southwestern coast of Kyushu are pooled mean data from Fig. 2.8. Day length data are records for 1987 in Niigata, 1962 in Fukuoka and April 2012–June 2014 off the southwestern coast of Kyushu, and are cited from the database of the National Oceanic and Atmospheric Administration (https://www.esrl.noaa.gov/gmd/grad/solcalc/)

an intrinsical spawning behaviour to ensure a match between emergent larvae and their food source; secondly, each population might have evolved to suit the characteristics of the environment they are distributed in. Because the coastal waters of Niigata (northern Japan) are part of the Sea of Japan, those of Fukuoka (southern Japan) are part of the Tsushima Strait, and those off the southwestern coast of Kyushu (further south) are part of the East China Sea, they differ greatly from one another. Hence, *E. tumifrons* may have evolved over time to contend with the conditions of the habitat they are distributed in. To elucidate this assertion, future experimental studies on each population are needed, as this will help to achieve an understanding of their behavioural characteristics, especially the degree at which genetic variation and/or thermal or light plasticity contribute towards the variation in their spawning seasons.

2.1.6 Conclusion

The present study was conducted to promote an understanding of the reproductive biology of *E. tumifrons* off the southwestern coast of Kyushu, Japan, and is the first to provide detail informations on this species ovarian maturation using an histological technique. The minimum size of the mature females was estimated to be 161 mm FL and the FL_{50} 179 mm FL. The spawning season was estimated to last from November to May. These findings and those of previous studies evidence regional differences in the reproductive biology of *E. tumifrons* that may have important implications for the appropriate management of this species. It is therefore paramount that current management measures targeting this species are scrutinized and updated so that the biological variability of each geographical population is taking into account and incorporated into future management policies.

CHAPTER 3: Age and growth of crimson sea bream *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan

3.1 Age and growth of crimson sea bream *Evynnis tumifrons*

3.1.1 Summary

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. My 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.

3.1.2 Introduction

The seabreams 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 seabream species are distributed in the coastal waters of Japan (Hayashi and Hagiwara 2013); one of these is the crimson seabream *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 on the continental shelves

(Hayashi 2002). It feeds on 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 net, surrounding seine net 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 otolith has been recommended as age determination character because otolith continues 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 determination character has been applied to many fish species such as *Platycephalus indicus* (Masuda et al. 2000), *Rhabdosargus sarb* (Radebe et al. 2002), *Nemipterus bathybius* (Granada et al. 2004a), *Sillago aelus* (Rahman and Tachihara 2005), *Gerres equulus* (Iqbal et al. 2006), *Lutjamus 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.

3.1.3 Materials and methods

3.1.3.1 Sampling and measurement

Samplings of *E. tumifrons* were mainly conducted 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. 3.1a). After the fish were landed, the cooperative’s staff sorted them into eight different weight categories. I 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. 3.1b) in 2004 and with Danish seine and gill net off Ibusuki City (Fig. 3.1c) 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 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 sexed through visual observation of the gonads.

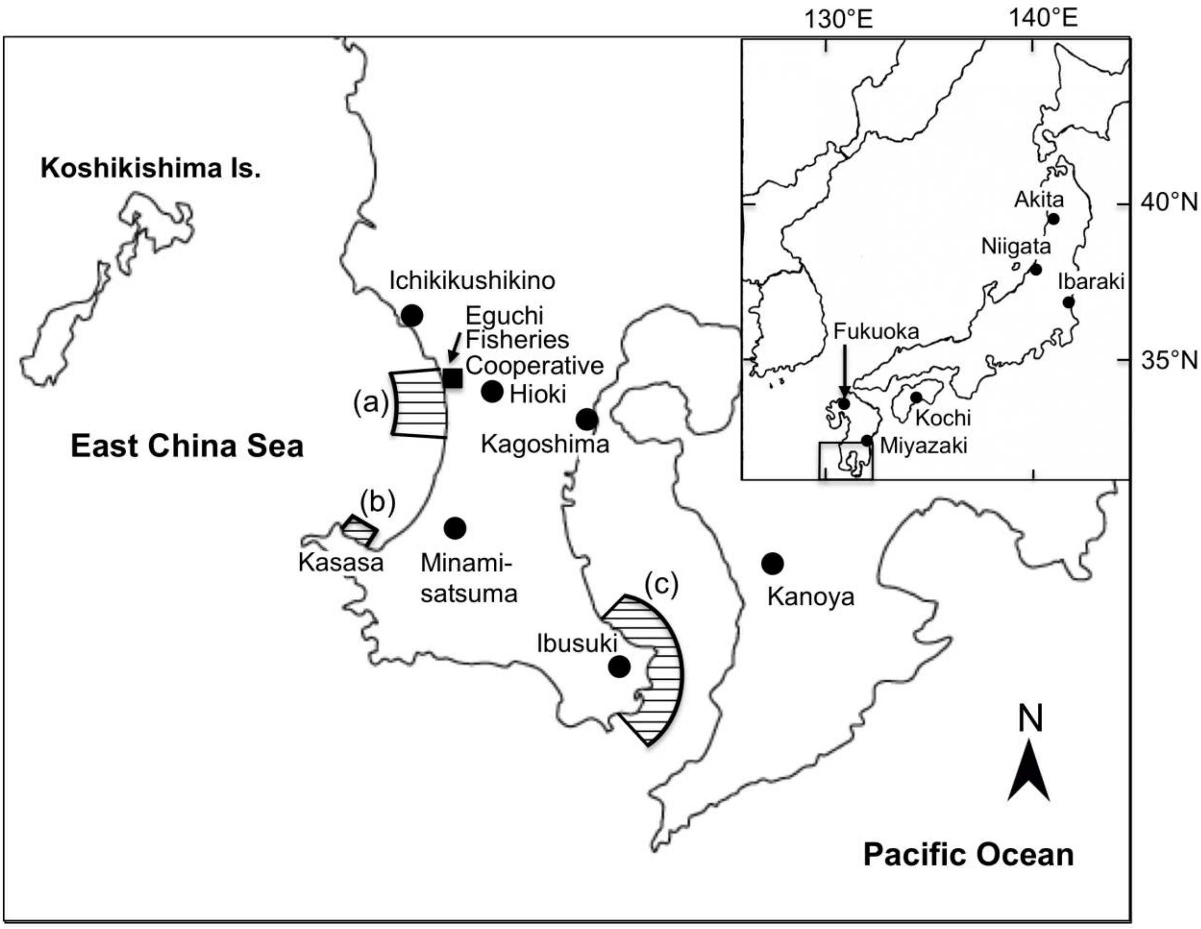

Fig. 3.1 Map of the sampling sites (shaded areas) of *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan. **a** Off Hioki city, **b** off Kasasa town, Minami Satsuma and **c** off Ibusuki city

3.1.3.2 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-thickness 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 mounted on a slide glass with nail enamel (Masuda et al. 2000; Masuda and Noro 2003; Granada et al. 2004a; Iqbal et al. 2006).

3.1.3.3 Counting of ring marks

The number of the ring marks (outer edges of opaque zones) on sectioned otoliths was counted from the focus to the dorsal tip of sulcus acusticus using a microscope (Leica MZ 12.5, Leica Microsystems, Heerbrugg, Switzerland) under a transmitted light at 32 × magnification (Fig. 3.2a). 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.

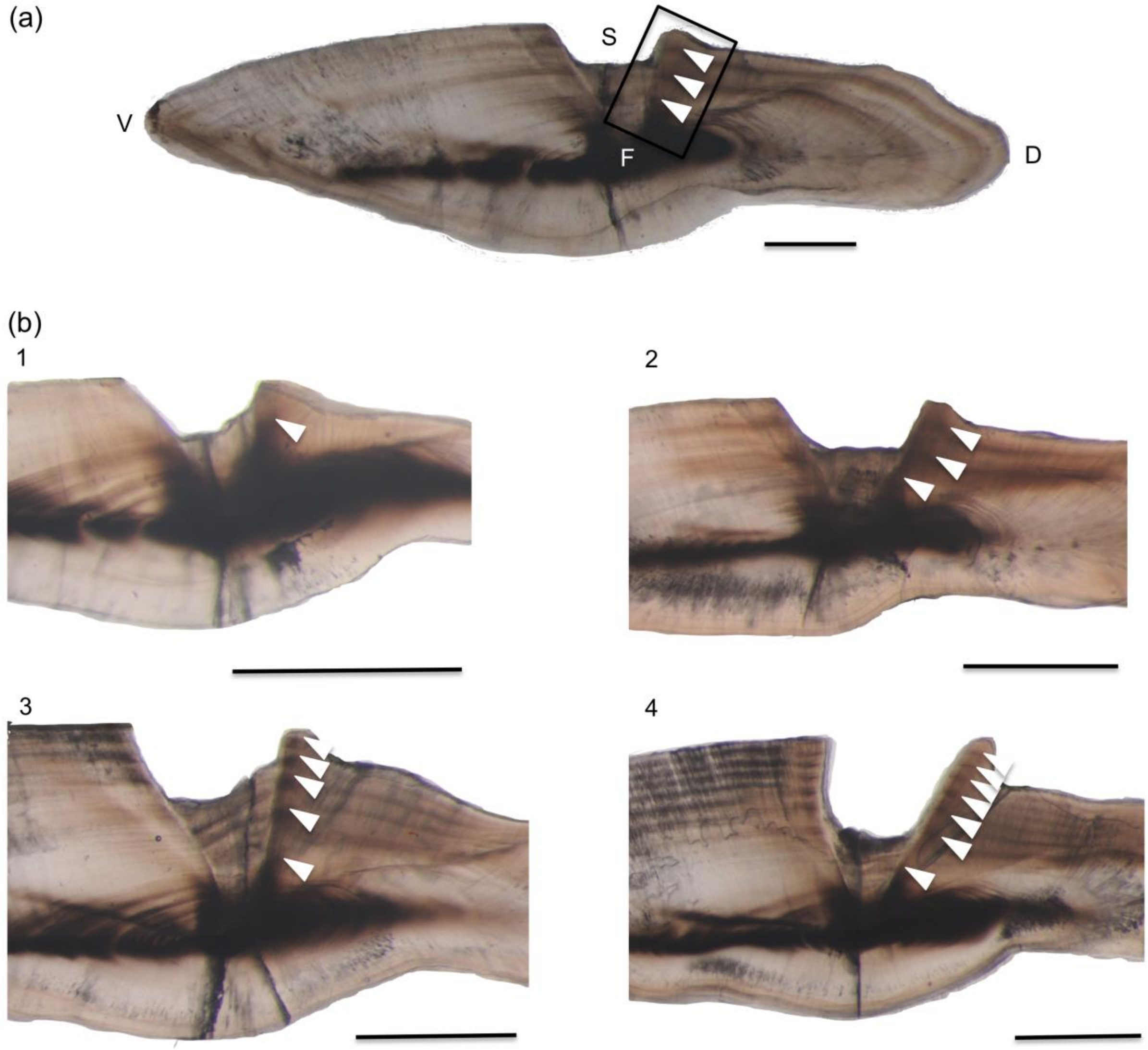

Fig. 3.2 a Counting and measuring area (within a rectangle) of ring marks on sectioned otolith of *Evynnis tumifrons*. *F* focus, *S* sulcus acusticus, *V* ventral side, *D* dorsal side. Open triangles indicate ring marks. **b** Ring marks (open triangles) on transverse sections of otoliths of the four 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

3.1.3.4 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 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 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).

3.1.3.5 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 or weight-at-age data based on the least-squares method using the curve-fitting function of the computer software (Delta Graph 7, Salt Lake City, 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.

F-test was conducted to compare the growth curves between the sexes using the formula provided by Akamine (2010): $F = [(S_p - S_f - S_m)/r] \ / \ [(S_f + S_m) \ / \ (n_f + n_m - 2r)]$, where S_p is the residual sum of squares (RSS) for the 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.

3.1.4 Results

3.1.4.1 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. 3.2b). The opaque zoned 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. 3.3a), 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. 3.3b), a similar trend was also 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 July in the four-ring group. Based on these informations, rings were estimated to form from May to July and were considered as annuli in *E. tumifrons.*

3.1.4.2 Length frequency distribution

The monthly changes in length-frequency distribution by age for both sexes are shown in

Figure 3.4. Assuming that the birth month was December, monthly occurrences of the assigned ages (0 to 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 the 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.

3.1.4.3 Growth

The von Bertalanffy growth function estimated from the length-at-age of the female was $L_t = 272(1 - \exp(-0.599(t + 0.189)))$ (n = 896, r^2 = 0.907) and that of the male 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 female 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. 3.5a) and that of the weight-at-age was $W_t = 519(1 - \exp(-0.484(t + 0.625)))^3$ (n = 1790, r^2 = 0.854) (Fig. 3.5b). The maximum age of female fish was 15 years old (fork length = 310 mm; body weight = 523 g) and that of male fish was 16 years old (fork length = 276 mm; body weight = 445 g).

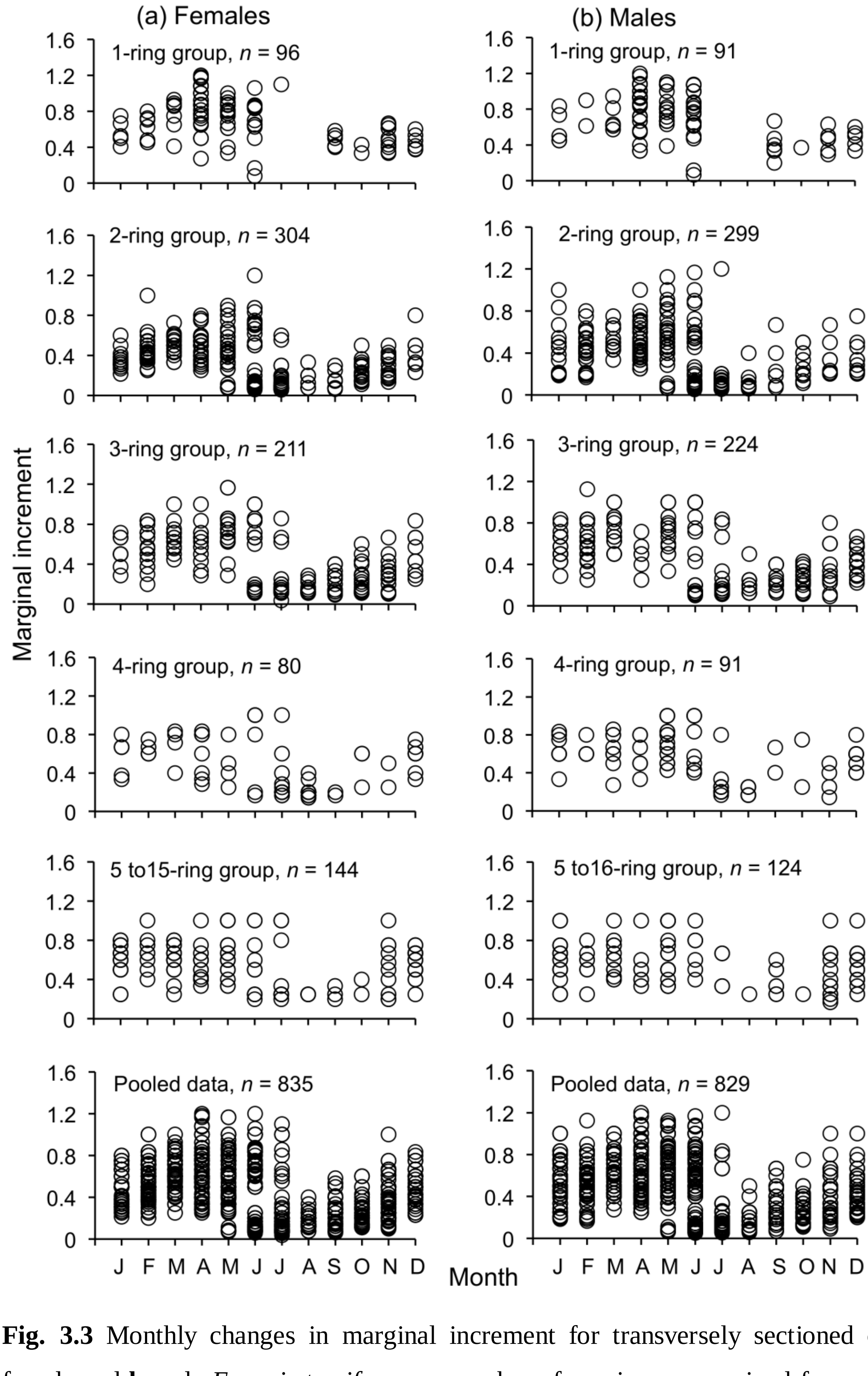

Fig. 3.3 Monthly changes in marginal increment for transversely sectioned otoliths of **a** female and **b** male *Evynnis tumifrons. n*, number of specimens examined for each ring group and pooled data

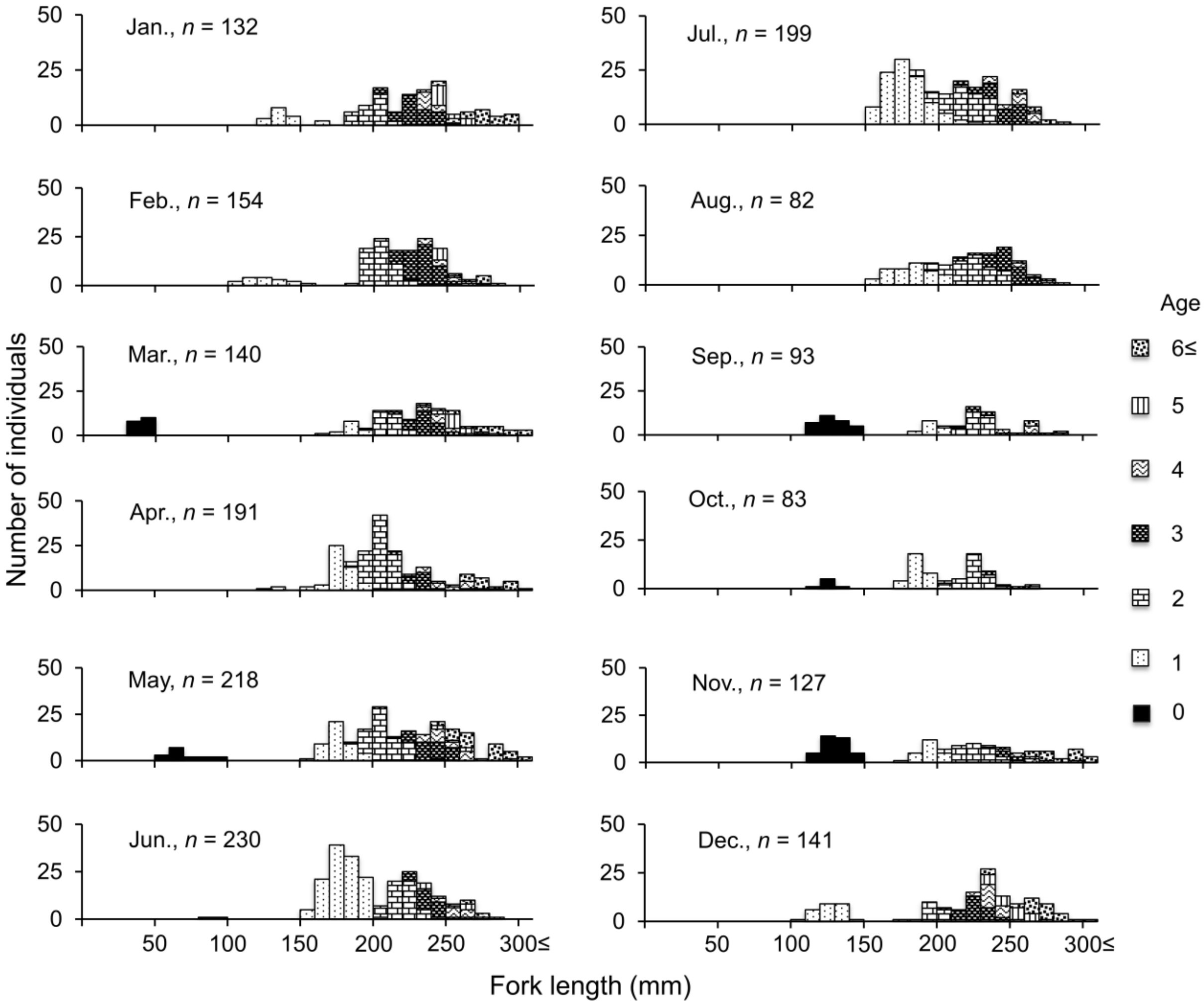

Fig. 3.4 Monthly changes in length-frequency distribution by age for both sexes of *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan. Ages were assigned based on the birth month of December. *n*, number of specimens examined

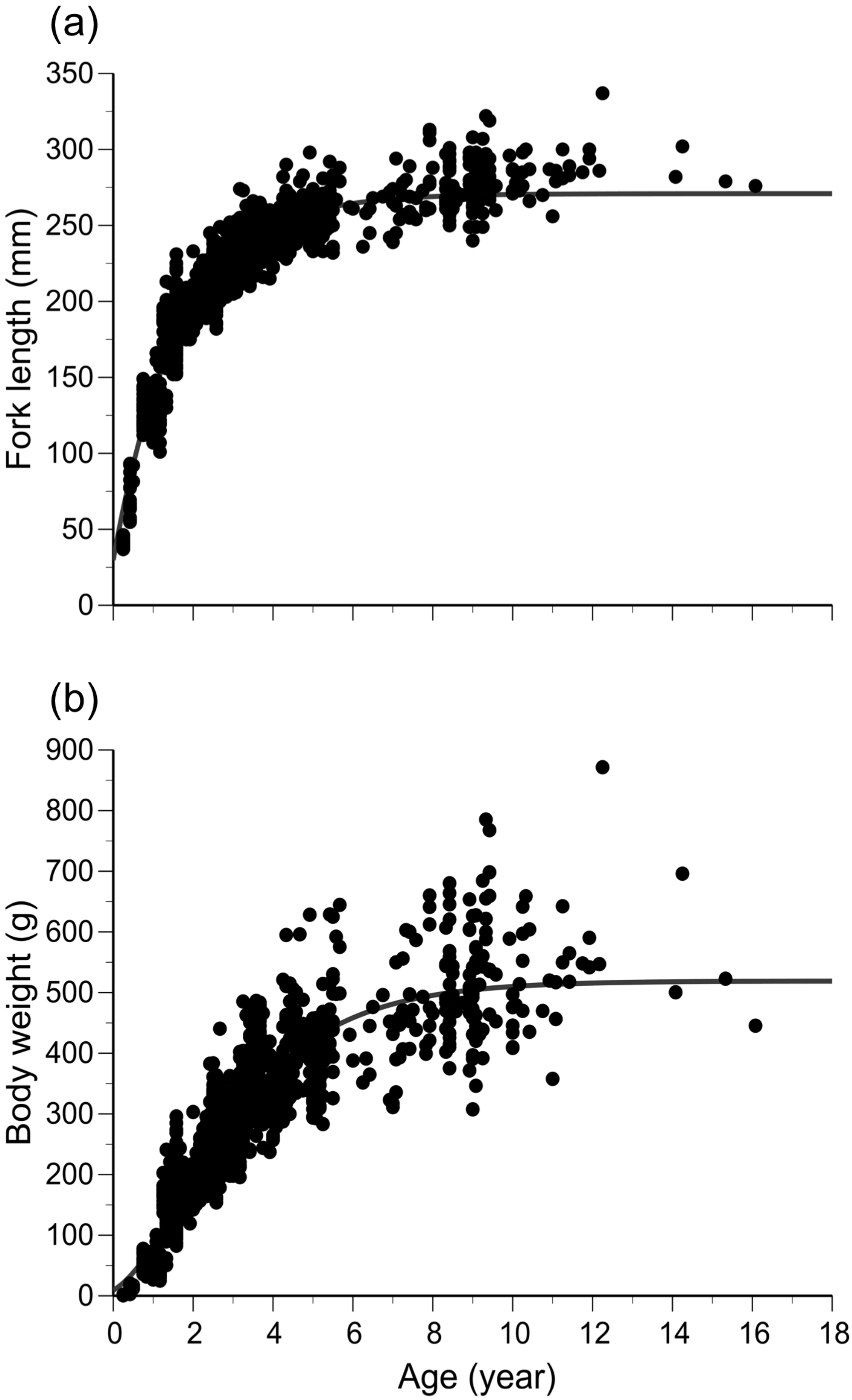

Fig. 3.5 Von Bertalanffy growth curve fitted to **a** length-at-age data and **b** weight-at-age data for both sexes of *Evynnis tumifrons* off the southwestern coast of Kyushu, Japan

3.1.5 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*, 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. My 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 (Figs. 3.3a, b). The accuracy and reliability of an annulus on the 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 Figure 3.6. The length at age 1 year in the present study, conducted in the southernmost location off the southwestern coast of Kyushu, Japan (latitude, 31º33'–31º39'N), was 139 mm FL, and this estimate appears to be the largest when compared with those 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, 33º50'N) and 132 mm FL off Kochi and Miyazaki Prefectures (latitudes, 33º20'N and 32º10'N). In the northernmost areas, off Akita Prefecture (approximate latitude, 39º39'N), it was 52 mm FL, off Niigata Prefecture (latitude, 37º50'N), it was 69 mm FL, and off Ibaraki Prefecure (latitude, 36º20'N) it was 109 mm FL. Based on these observations, the growth of the 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 of 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 the growth was higher in the Hauraki Gulf of northern New Zealand (latitude, 36.3ºS) than in the Stewart Islands of southern New Zealand (latitude, 47ºS). 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

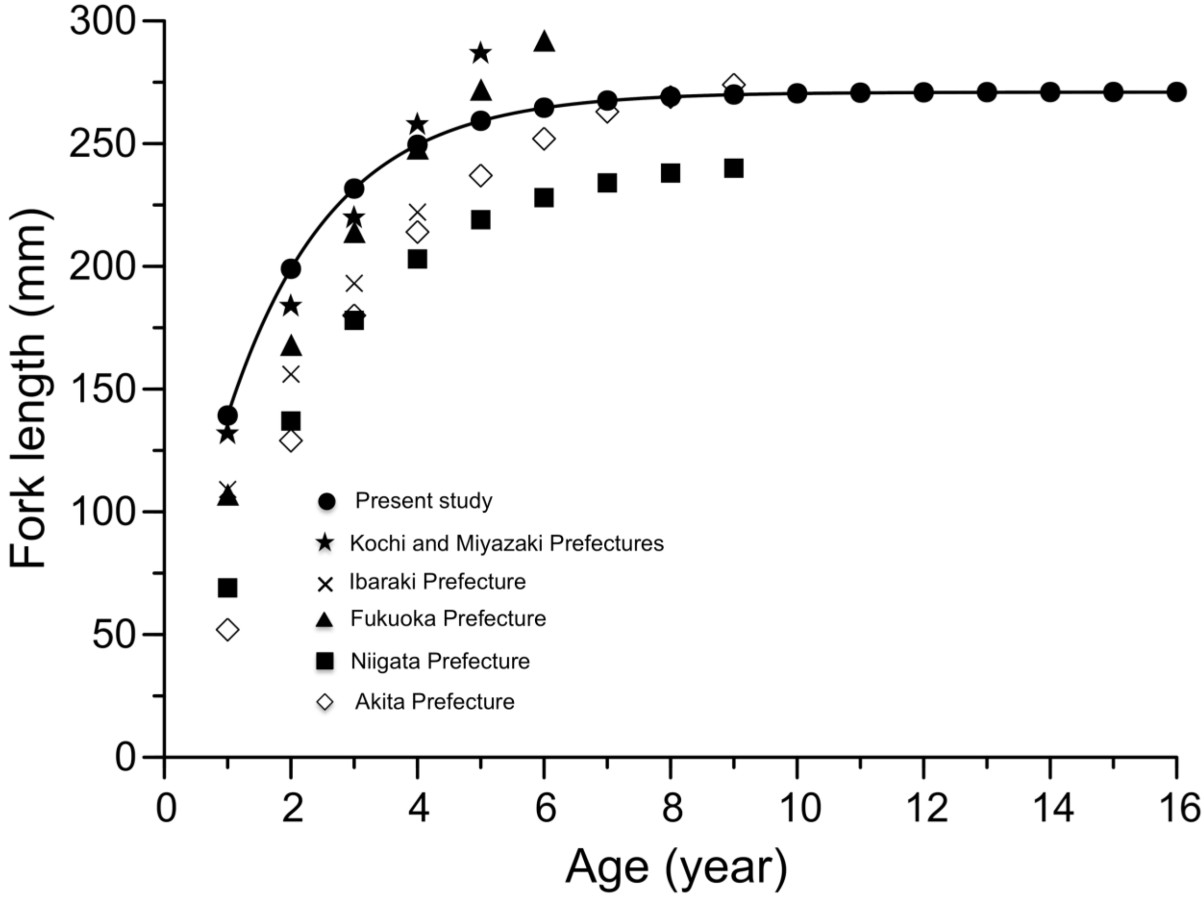

Fig. 3.6 Comparison of age-FL relationship of *Evynnis tumifrons* at six different localities in Japan. Data for the previous studies were cited from Yamada et al. (2007)

of the major factors influencing growth in fish (Conover 1992; Trip et al. 2014). My 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. 3.6). 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 old) is 7 years older than that reported in Akita and Niigata Prefectures (Yamada et al. 2007). In the present study, I 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.

3.1.6 Conclusion

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. My 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 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.

Chapter 4: General Discussion

A total of 1101 females were randomly collected once a month from eight different weight categories adopted by the Eguchi Fisheries Cooperative, and histological technique was used to estimate the degree of ovarian maturation through the observation of the oocytes' developmental stages. The use of histological technique is an improvement to the previous studies, where spawning season was mainly estimated from the monthly changes in gonadosomatic index (GSI) and the minimum size of the mature females was determined only in Niigata prefecture using the GSI (Anzawa et al. 1987). The present study estimated the minimum size of the mature females and the size at which 50% of them are mature through histological observation of the ovaries. The estimated size at 50% maturity was 179 mm FL (Fig. 2.4), which is attained at around 1.5 years old (Fig. 3.5a). All females are capable of spawning at around FL of 200 mm (Fig. 2.4), which is attained at 2 years old (Fig. 3.5a).

The spawning season of *E. tumifrons* in the coastal waters of Japan varied according to locality. In the north *E. tumifrons* spawns during the warmer months of the year, but shifts its spawning season closer to the colder months in the southern areas, while at its southernmost limit, it spawns throughout the cold season. In addition, in the southernmost location (present study), the spawning season lasts longer than in the higher latitudinal areas. The mean water temperature during *E. tumifrons'* spawning season in Niigata prefecture (northern location) and Fukuoka prefecture (southern location) had similar high water temperatures at spawning that overlapped. On the other hand, off the southwestern coast of Kyushu (southernmost location, present study), the trigger seemed to change to colder rather than warmer water. With regards to effect of the day length, I also noticed that spawning in northern Niigata was favored when conditions combined warmer water with longer days, while to the south in Fukuoka a combination of warmer water conditions but with shorter days was preferred;

finally in the southernmost off the southwestern coast of Kyushu (present study), day length did not seem to have much effect.

A study on the spawning season of anchovy in the western Mediterranean revealed that variation in spawning season is associated with the latitudinal differences, possibly due to the regional temperature fluctuations (Garcia and Palomera 1996). They reported that in the northern latitudes, spawning season is more constricted than in the southern latitudes. In the northern region, it occurs at the time of year when water temperature is high and the outflow from the Rhone river is substantial, creating stability and productive environment favorable for spawning which lasts from June to August at the sea surface temperatures ranged from 15°C to 22 °C. In the southern region, spawning season lasted from March-April to September-October with the peak in June-July when the surface temperatures was around 19°C to 23°C. Hence, the variation in spawning season was reported to be associated with latitudinal differences due to regional differences in the water temperature. However, this phenomenon was not observed in *E. tumifrons* off the coastal waters of Japan. It could be due to other factors. A study by Oomen and Hutchings (2015) on the Atlantic cod *Gadus morhua* reveals that five populations distributed in the study area exhibited differences in spawning season and were categorized as follows: winter-spawning, spring-spawning and autumn-spawning populations, respectively. Although the three more northerly populations all spawn in the spring, the two populations with the greatest proximity to one another spawn at different times. Despite of these differences, they revealed that each population of *G. morhua* may have evolved an intrinsically spawning behaviour to ensure a match to the survival requirements of the emergent larvae. It is likely that *E. tumifrons* may have been evolved in the similar way, but this needs to be investigated in laboratory on different populations of this species, as this will help towards understanding the behavioral characteristics of each population, especially their ability to response to the triggers of spawning. The degrees at which thermal adaptation plasticity or genetic variation and/or contributed towards the spawning variation can be better understood through the works in the laboratory. Understanding these factors is essential, as they would help the fisheries managers in the proposal of meaningful management measures that take into account the biology of the species and the condition of their environments.

In the present study, 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. Clear ring marks on the internal (proximal) side of the sectioned otoliths allowed age determination with relative ease. The oldest age in the present study was 16 years, and the phenomenon of stacking of ring marks towards the otolith's margin was not observed. Growth of *E. tumifrons* at an early age differs between to the populations found across the coastal waters of Japan; and this phenomenon was also observed in *Pagrus major*, a related red sea bream in the coastal waters of Japan (Ochiai and Tanaka 1986) and butterfish *Odax pullus* in the coastal waters of New Zealand (Trip et al. 2014). Both studies attributed the factor responsible for the variation in growth to the water temperature differences between the localities.

Furthermore, the steepness of the curve (growth rate) after the age of 1 year appeared to differ between the localities. The least steep curve was obtained in the present study. These differences are attributed to aging characters used. Transversely sectioned otoliths were used in the present study, while scales were used in the previous studies. In some previous studies, the use of scales was criticized 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). Hence, it is likely that an underestimation of maximum age could have happened, as differences in steepness of the growth curves between localities is evident.

In conclusion, the present study is the first to detail the reproductive biology and age and growth of *E. tumifrons*. The evidence of regional differences in the reproductive biology and the understanding that previous studies may have underestimated the age and growth could have important implications for appropriate management. Moreover, the initial growth at the young age (1 year old) varied in each population distributed across the coastal waters of Japan further exacerbated the concerned implications because it suggests that growth parameters and size at sexual maturity of the populations are likely to vary between each other. It is paramount that the findings of the present study are taken into consideration and incorporated into the management policies by the fisheries cooperatives around Japan. Fisheries managers of fisheries cooperatives could use the estimated size at sexual maturity to decide the permissible mesh size for any fishing gear targeting this fish species. Determining a mesh size larger than the estimated size at sexual maturity allows the fish to at least participate in a spawning before being caught by the fishing gear. Moreover, estimated spawning season would help the fisheries managers to decide a fishing closure period for the fishery. This is to allow a sufficient number of mature individuals to spawn. Finally, fisheries managers could use the estimated growth rate in analytical stock assessments to model the average changes in fish size with age. The growth information is central to the yield or YPR models that used to estimate reference points.

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