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Age and Growth of Black Sea Bream *Acanthopagrus schlegelii* (Bleeker 1854) in Tokyo Bay

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Abstract

The age and growth of male and female black sea bream *Acanthopagrus schlegelii* (Bleeker 1854) was investigated in and around Tokyo Bay, Japan by means of otolith sectioning. Otolith opaque zones form once a year, mainly in June–July, following the spawning season (April–June). Rearing experiments over several years confirmed that the number of otolith opaque zones could be used as an indication of the age of *A. schlegelii*. The maximum ages of males and females were estimated at 20 and 28 years, respectively. The age–length relationships and parameters for the von Bertalanffy growth function were, L_∞ = 407 mm and 439 mm and k = 0.510 yr^{-1} and 0.346 yr^{-1}respectively. Sex ratio by age indicated that males were dominant among small/young fish while females were dominant among old/large fish. This suggested a protandrous life history style, which may be widespread in *Acanthopagrus* species.

Introduction

Black sea bream *Acanthopagrus schlegelii* (Bleeker 1854) is a common coastal fish species of economic importance to recreational and commercial fisheries and represents an important source of food in many Asian countries. The total commercial catch in Tokyo Bay, Chiba Prefecture, has been stable at 30–50 tonnes per year from 1990. A stock enhancement programme of black sea bream was initiated during the early 1990s, with more than 20 million juveniles being released annually between 1991 and 2010 (Gonzalez et al. 2009).

Nevertheless, *A. schlegelii* has recently been regarded as harmful, because of its habit of grazing seed of cultured oysters (Saito et al. 2008), Manila clam (Shigeta 2008), and cultured algae (Kusaka 2007).

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A successful fisheries management and stock enhancement programme requires precise knowledge of life history traits and up-to-date information of recruitment levels and spawning stock biomass. Many *Acanthopagrus* spp., such as *A. schlegelii*, have a protandrous life history characterised by population shifts as the fish increase in size, from an exclusively-male population of small-sized fish to one that is dominated by females (Abol-Munafi and Umeda 1994; Wu and Chang 2009; Wu et al. 2010). Age and growth studies on *Acanthopagrus* spp., based on otolith sectioning methods, have been carried out on *Acanthopagrus latus* (Houttuyn 1782) (Hall et al. 2004), *Acanthopagrus berda* (Forsskål 1775) (James et al. 2003), *Acanthopagrus butcheri* (Munro 1949) (Morison et al. 1998; Sarre and Potter 2000; Protter et al. 2008), and on *Acanthopagrus* hybrid complexes (Ochwada-Doyle et al. 2012).

Similar studies on *A. schlegelii* have not been undertaken, to the best of our knowledge. However, a literature survey by Umino (2010) indicates that *A. schlegelii* achieved a maximum age (based on the scale-reading method) of approximately 19 years at a TL (Total length) of over 500 mm.

The primary aim of the present study was to re-examine and validate the method of age determination in the black sea bream, using the otolith sectioning method that had been used by Hall et al. (2004) and James et al. (2003) in their studies on *Acanthopagrus* species. It should be noted, however, that the first annulus of *Acanthopagrus* species was shown by Tanaka et al. (2008) to be indistinguishable from the other annuli. A re-examination of the otolith structure, in relation to its annuli was thus necessary so as to validate the annual formation of otolith rings as well as the correspondence between the number of annuli and the real age. To achieve this primary aim we conducted fish rearing experiments over several years.

Our secondary aim was to determine the respective growth curves of males and females of *A. schlegelii*. Based on individual age data, we estimated sex ratios of black sea bream for each age group. By comparing the growth pattern and sex ratios of *A. schlegelii* with those of other *Acanthopagrus* species, we were able to examine the life history traits of *A. schlegelii*.

Materials and Methods

Sampling and measurements

Black sea bream *A. schlegelii* are part of the set-net and gill-net fisheries that operate along a section of the east coast in Tokyo Bay (Fig. 1). Black sea bream were collected from catches landed at several fish markets located in the south Chiba Prefecture. Monthly sampling was conducted from 2006 to 2011 (Table 1). We measured the fork length (*FL*), body weight (*BW*) and gonad weight (*GW*) of each fish to the nearest 1 mm and 0.1 g, respectively.

Because sex identification had not been carried out for the fish (*n*=1475) from 2006 to2008, respective growth curves of males and females were analyzed only for the fish (*n*=578) caught during the 2009–2011 period.

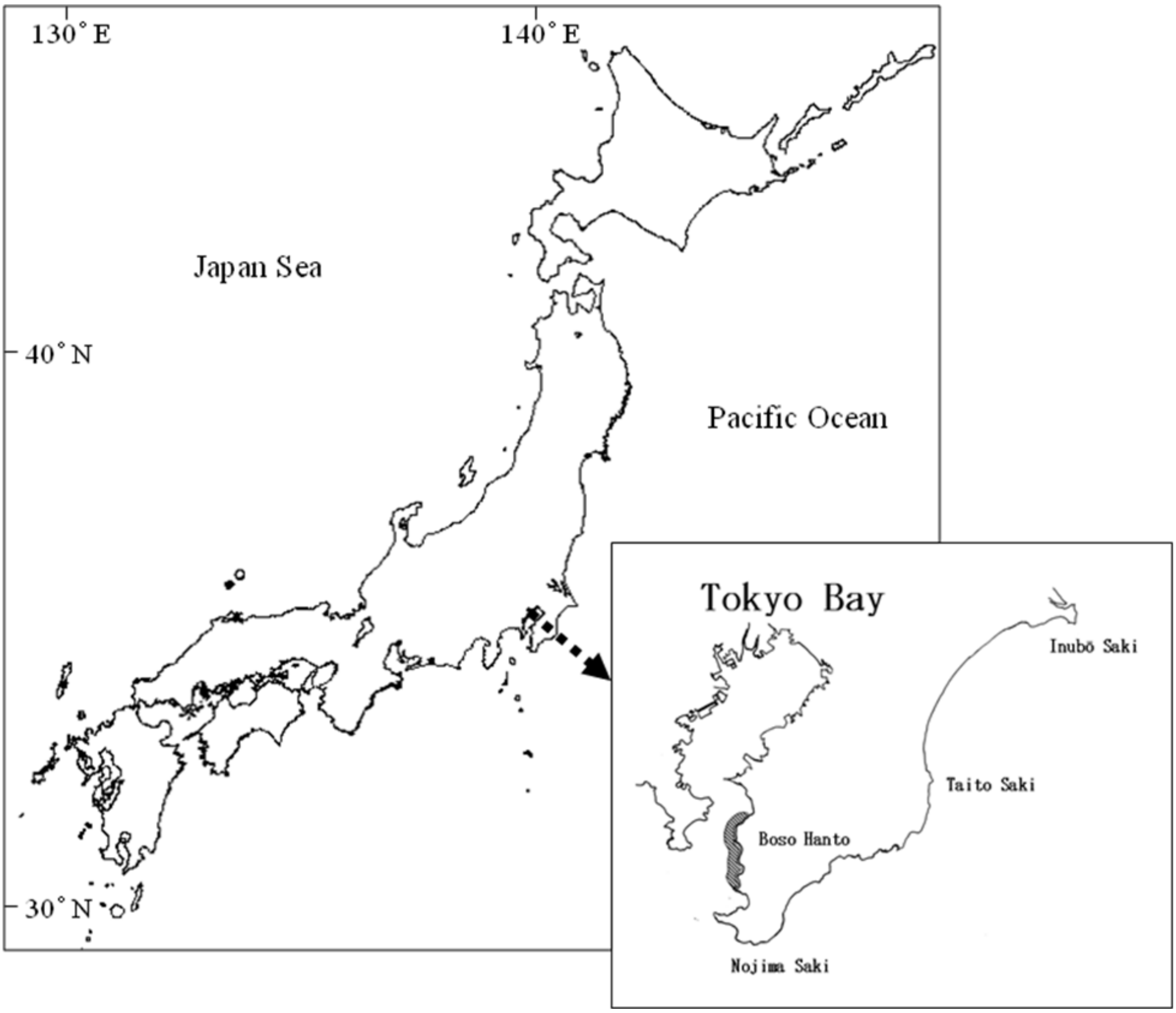

Fig. 1. Map showing Tokyo Bay where samples of black sea bream were collected.

Table 1. Sample size of monthly sampling of black sea bream

year/month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
2006					90	38	10	13	4	7	6	
2007	5			162	93	19	10			10		
2008	16			98	149	156	4	21		6		
2009				173	31	43	7	7	6	30	8	2
2010		19	8		179	22	3	3		3		2
2011	22											
Total	43	19	8	433	542	278	34	44	10	56	14	4

Fish sex was identified by macroscopic examination of gonads. In this context, it should be noted that juvenile sparid fish possess gonads with both immature testicular and ovarian tissue.

As individual growth progresses, the gonad develops testicular tissue (with ovarian rudiments) as a functional male, and later develops ovarian cells and tissues as a functional female (with testicular rudiments) (Buxton and Garratt 1990). Functional males and females could be identified from optical observations, whether the gonad was covered by testicular or ovarian tissue. The maturational condition was determined using the following formula for gonadosomatic index (*GSI*):

$$\boldsymbol{GSI = GW\ (g) * 100 * BW\ (g)^{-1}}$$

Otolith treatment

Sagittal otoliths were removed from each fish, cleaned, dried, and stored in natural conditions until further observation. Otoliths were then mounted and embedded in polyester resin and cut into ≈0.3 mm transverse sections, using a diamond saw (Leica sp1600; Leica Microsystems GmbH, Wetzlar, Germany).

Sections were mounted on glass slides and their surfaces were ground to sequentially finer grades, using carborundum paper of #800–#2000. Sections were examined using a binocular microscope under transmitted light. The range of magnification was 40× or 100×.

Annulus validation

An evaluation of seasonal changes in the frequency of fish with annual rings at the otolith edge was used to validate seasonal periodicity of annulus formation. Fish were also reared to confirm the relationship between the number of annuli and the real age.

Newly hatched larvae, produced in the Chiba Prefecture Center, were reared from 2003 July under conditions of natural temperature and light. The fish were fed dry pellets. A total of 28 individuals were sampled several years later, in 2005–2009. Observations of the annual structure of otolith sections and numbers of annuli were compared with duration, as the years progressed after 2003.

Data analysis

Growth curves were calculated for males and females. Microsoft Excel's solver least squares cosine spectrum analysis was used to fit von Bertalanffy growth curves to the individual FLs of females and males at the age (year) estimated from otoliths. Sample size of young-of-the-year was so small that t_0 (the theoretical age at zero length) of the growth curve would become too large for the purpose of this analysis. For this reason, we utilized the biological intercept method, to avoid an over-estimation of t_0, a parameter which only requires information about body length and the age relationship at the smallest possible size (Katayama and Yamamoto 2012).

Using a cast net, *A. schlegelii* fingerlings were collected, from the sandy shores around the river mouth of the Hota River in May 2008. The FLs ranged from 89-165 mm, while the was 138 mm. Since the main spawning season was in May to June, an assumption was made that the spawning date was June 1st of the previous year. When using this method all growth curves pass through the origin of coordinates, which are t = 0.258 years and TL = 138 mm. AICs (Akaike's Information Criterion), (Akaike 1969) were calculated using "R" statistical package (R Core Team 2013), to examine whether the growth curves differed significantly between sexes.

Results

Fork length (*FL*) distribution without distinction of sexes (Fig. 2a) showed a single normal distribution from 250 mm-500 mm, with a peak of 350-375 mm.

Very few fish smaller than 200 mm *FL* were recorded. Females were relatively larger than males (Fig. 2b). Length classes of less than 300 mm *FL* and over 450 mm were mainly composed of males or females, respectively.

Otolith sections of wild *A. schlegelii* displayed wide translucent zones, and thin opaque zones. Nine opaque zones were easily counted (Fig. 3a). Ages (in years) of the reared fish hatched out in July 2003 were calculated as 2+ (b), 4+ (c), 5+ (d) as time progressed after the assumed date of hatching.

Counts of the opaque zone were two (b), four (c), and five (d). Estimates of ages, by number of opaque zone, were entirely the same as their actual ages (Table 2).

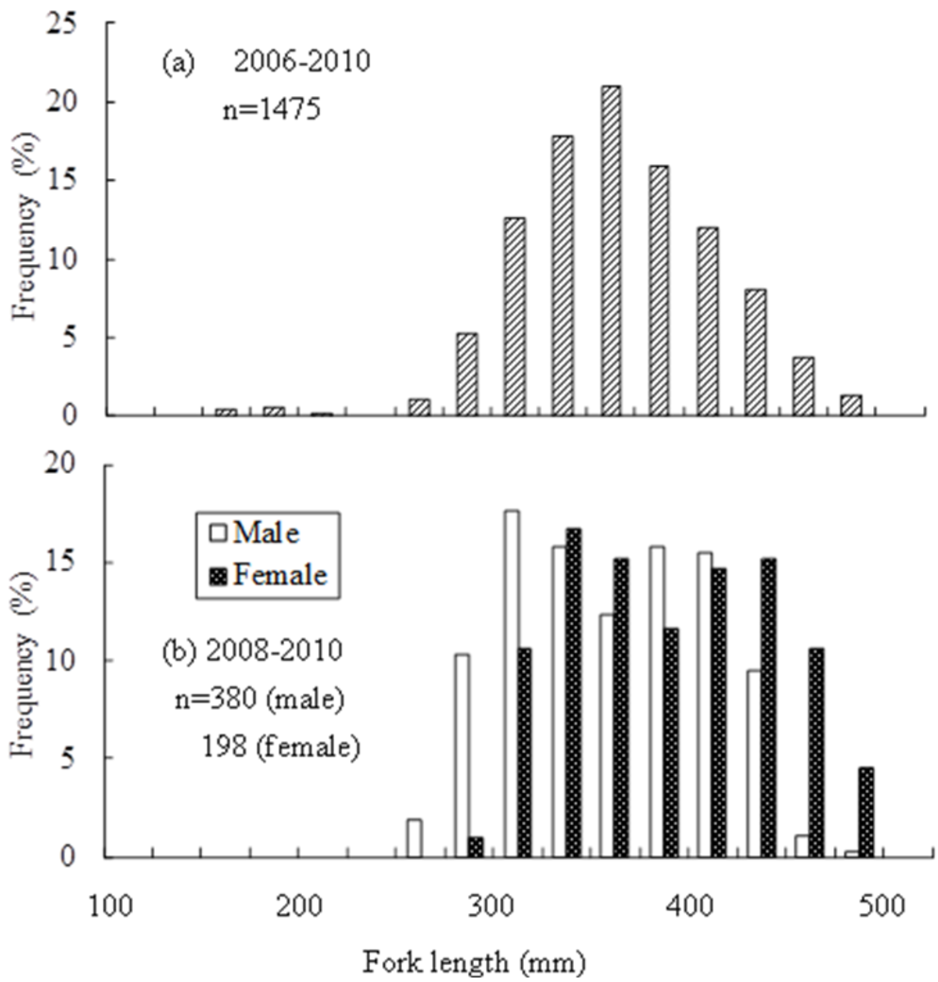

Fig. 2. Fork length distributions of (a) all samples without distinction of sexes in 2006-2010; (b) respective male and female in 2008-2010 of *Acanthopagrus schlegelii* caught from Tokyo Bay.

Table 2. Comparison of number of the otolith opaque zone with actual age of the reared black sea bream. Number in this table indicate sample size.

	Number of opaque zone		
Actual age	2	4	5
2+	13		
4+		3	
5+			12

During the June–July period there was a high proportion of fish (>80%) with the opaque zone at the otolith margin (Fig. 4). The *GSI* of females was stable, at a low level (of under 5), during August-December. Females developed gonads rapidly from February and reached a peak *GSI*, of over 25, in April. The *GSI* had a tendency to decrease towards August. These seasonal patterns suggest that the main spawning period of *A. schlegelii* is from April to June and that the otolith opaque zones form on an annual basis during June-July, following the spawning period.

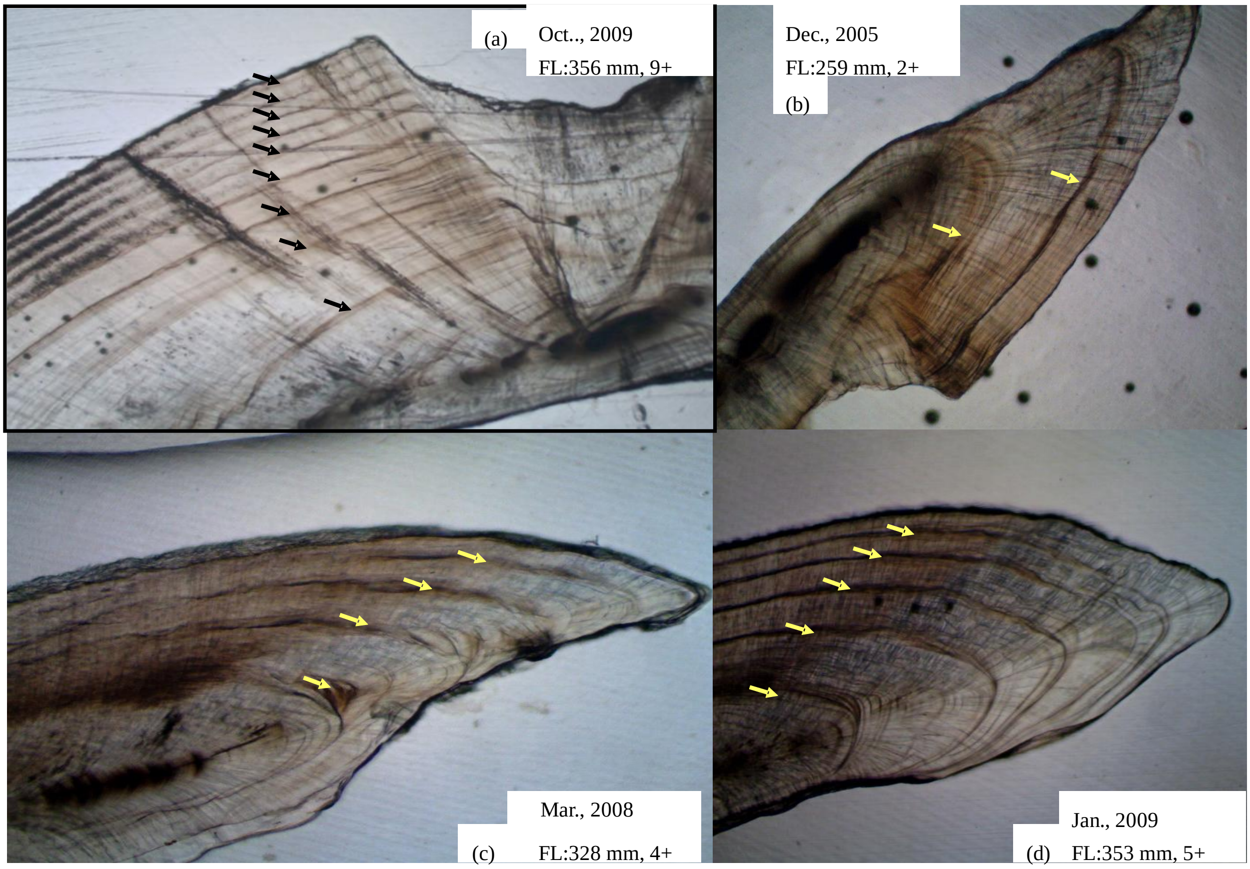

Fig. 3. Photos of otolith sections (Oct., 2009, FL: 356 mm) of wild black sea bream (a). Arrow heads indicate opaque zone. Photos of reared *Acanthopagrus schlegelii* hatched in July 2003 (b-d). Date when the fish were sampled were Dec., 2005, FL: 259 mm, 2+ (b), Mar., 2008, FL: 328 mm, 4+ (c), and Jan., 2009, FL: 353 mm, 5+ (d).

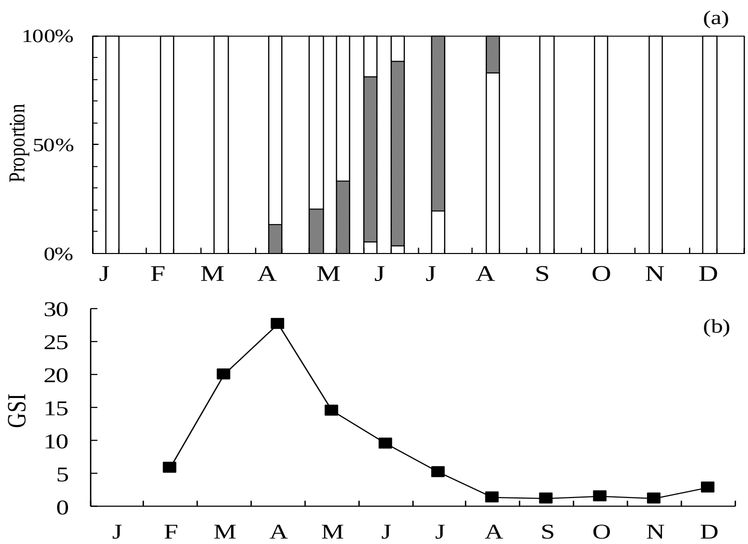

Fig. 4. Seasonal changes in the proportion of each otolith marginal structure of *Acanthopagrus schlegelii* (a) and in the mean GSIs of female (b). Shaded and open bars indicate fish with opaque zone and translucent zones at otolith margins, respectively.

Assuming a hatching date of 1st June, the age of each individual was determined by observing the otolith section. Individual age-length data and von Bertalanffy growth curves are displayed for males and females in Fig. 5. Maximum ages of male and female black sea bream were 20 and 28 years, respectively. Larger specimens (>450 mm *FL*) were almost all females. Estimated growth curves of male and female fish were as follows:

$$\text{Male: } TLt = 407\ \{1 - \exp\ (-0.510\ (t - 0.258))\} + 138$$
$$\text{Female: } TLt = 439\ \{1 - \exp\ (-0.346\ (t - 0.258))\} + 138$$

These formulae differed significantly between sexes (*AIC*=5603 for respective two curves, *AIC*=5640 for pooled curve), indicating that females reach a slightly larger size than males.

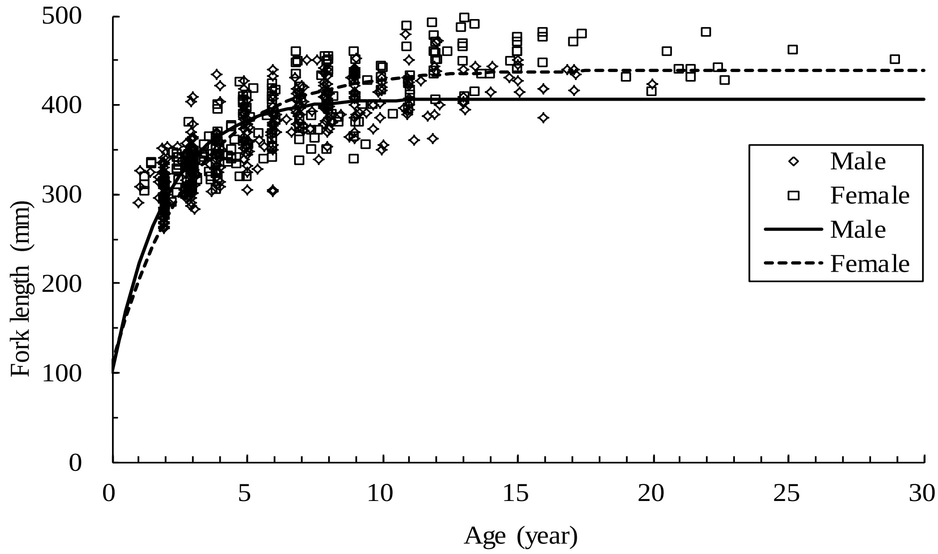

Fig. 5. Age-fork length relationships with Berttalanffy growth curves for male and female of *Acanthopagrus schlegelii*. Sample sizes of male and female are 380 and 198, respectively.

The sex ratio, determined by observation of gonad features, is expressed by age (Fig. 6). A slight male bias exists in the 3-10 year-old group, with males comprising 50-70%, while a slight female bias exists in the 11-20 year-old group, with females comprising 50-70%. In contrast, the sex-ratios of fish younger and older than these age groups were obviously different: almost all fish under 3 years old were males, and almost all fish over 21 years old were females.

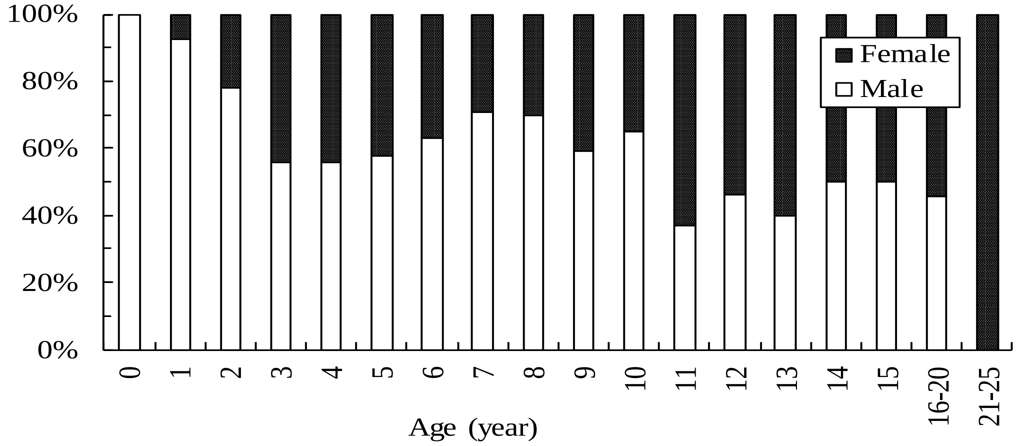

Fig. 6. Sex ratios of each age of *Acanthopagrus schlegelii*.

Discussion

Microscopic examination of otolith sections, which display clear contrasting structures due to seasonal variation in deposition, has been widely used for age determination in fish. Hall et al. (2004) used otoliths to determine the age for *A. latus*. They initially observed the surface of the otolith under reflected light against a black background, but later found that sectioning the otolith improved the resolution of the opaque zones, demonstrating that an otolith, that initially appeared to have five opaque zones, was found to have more than eight opaque zones. Otoliths thicken as age progresses. It is thus advisable to avoid examining the leading hard-to-read otolith annuli when using the surface method, as in studies on a number of species of fish (Hayashi et al. 1995; Sekigawa et al. 2002; Atsuchi et al. 2004; Tanaka et al. 2008; Yamashita et al. 2011). Precise age determination requires the otolith sectioning. Thus in the present study the age and growth was studied, by examining the otolith sections for all specimens of *A. schlegelii*. In our study, *A. schlegelii* was found to be a long-lived fish, attaining a maximum age of 28 years. The otolith sectioning method has been used by a number of workers to determine the maximum age (in years) of a number of species: 24 for *A. latus*; 17 for *A.berda;* up to 21 for *A. butcheri*; 29 and 31 for a hybrid complex (James et al. 2003; Potter et al. 2008; Ochwada-Doyle et al. 2012). In all these studies, however, the male and female fish were grouped together. Although Sarre and Potter (2000) demonstrated that both male and female s were found in populations of older fish of over 20 years, his work was based on single-sample analysis. In contrast, our work on the age composition of male and female *A. schlegelii* indicated that fish of over 20 years were exclusively female. A detailed analysis of our results also indicates that L∞ values of the male and female black sea bream were 407 mm (269+138) and 439 mm (301+138), respectively, and that large-sized *A. schlegelii* fish, of over 400 mm, were commonly found in Tokyo Bay.

Similar relationships between gender and body size have been demonstrated for *A. latus* and *A. butcheri* (Hesp et al. 2004; Potter et al. 2008,). In contrast, fish over 400 mm of *Acanthopagrus* hybrids, *Acanthopagrus bifasciatus* (Forsskål 1775) and *A. berda* were absent regardless of sex (James et al. 2003; Grandcourt et al. 2004; Ochwada-Doyle et al. 2012). These results suggest that a bias towards large and small sizes is species-dependent characteristic of *Acanthopagrus* species.

Hesp et al. (2004), who utilised the growth curve of *A. latus* and determined sex ratio by age, found that the functional male category dominated the 2+, 3+ and 4+ age classes, but the proportion of functional females increased in the 5+ age class and became progressively larger in older age classes. We have similarly demonstrated male dominance in small and young specimens and female dominance in large and older specimens. Similar results have also been reported for *A. berda* (James et al. 2003), so it is possible that this is a common life history pattern in *Acanthopagrus* species.

Acanthopagrus schlegelii is a protandrous hermaphrodite species, as is the case for other *Acanthopagrus* species such as *A. latus*, and also *Acanthopagrus australis* (Gűnther 1859) and *A. berda* (Pollock 1985; Tobin et al. 1997; Abou-Seedo et al. 2003). This characteristic has also been demonstrated in certain other coastal fish in Japanese temperate areas, such as *Cyclothone atraria* Gilbert 1905 (Miya and Nemoto 1985) and lizard flathead *Inegocia japonica* (Cuvier 1829) (Shinomiya et al. 2003). Many of these species are important fisheries resources in the Japanese coastal waters, but little is known of their life history characteristics. We recommend that information on age and growth, in relation to sexual changes should be included in future research.

Acanthopagrus schlegelii in and around Tokyo Bay have been caught by set-net and gill net fisheries, as is the case for a number of other coastal fish species. *Acanthopagrus schlegelii* is also an important component of recreational fishing. Despite the high fishing pressure on this species, our work has indicated that older age classes represent a large component of the black sea bream population.

The stock size has recently increased (Katayama 2011), which suggests that recent oceanographic conditions that affect the habitat have been favourable for this species. Very little information relating to the current population increase is available. To facilitate further understanding of the population dynamics we recommend that more intensive monitoring of oceanographic conditions as well as abundance estimates of year classes, using age determination methods (as presented in this study), should be undertaken.

In this context, it should also be noted that the population dynamics of *A. schlegelii* would have a significant effect on prey organisms (oyster, Manila clam, and algae) and so research into this topic could also prove to be useful in terms of forecasting population sizes of these prey organisms.

Conclusion

Otolith opaque zones form once a year, mainly in June-July, following the spawning season (April-June) for the black sea bream *A. schlegelii* in and around Tokyo Bay. Rearing experiments of fish over several years confirmed that the number of otolith opaque zones can be used as an indication of the fish age. Parameters for the von Bertalanffy growth function of male and female fish *A. schlegelii* were determined as L_∞ = 407 mm and 439 mm and k = 0.510 yr^{-1} and 0.346 yr^{-1}, respectively. Sex ratio by age indicated that males were dominant among small/young fish while females were dominant among old/large fish. This indicates a protandrous life history style, which may be widespread in *Acanthopagrus* species.

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