Doctoral Thesis

Ecological study on the Asian sheephead wrasse *Semicossyphus reticulatus* (Labridae) in the western Seto Inland Sea

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Contents

Chapter 1

General introduction

Labridae is one of the largest families of teleost fish, including 71 genera and 519 species mainly distributed in tropical and sub-tropical waters (Westneat and Alfaro 2005; Nelson et al. 2016). Some genera exhibit distribution ranges in temperate waters, e.g., *Parajulis*, *Pseudolabrus*, *Pteragogus*, and *Semicossyphus*. Only three Pacific temperate water species constitute the genus *Semicossyphus*, i.e., the California sheephead wrasse *S. pulcher*, the Galapagos sheephead wrasse *S. darwini*, and the Asian sheephead wrasse *S. reticulatus* (Nelson 2006), and all three species attain close to total length (TL) of 1 m (Allen and Robertson 1994; Nakabo 2002). Their habitats have been geographically separated; *S. pulcher* lives east coast of USA, *S. darwini* lives east coast of South America and Galapagos Islands and *S. reticulatus* lives the north-western Pacific.

The Asian sheephead wrasse *S. reticulatus* occurs widely on rocky reefs of temperate waters around Japan, Korea, and the east coast of China, and is one of the most northern occurring labrids in the western Pacific Ocean (Nakabo 2002). The Seto Inland Sea is connected with the ocean via channels (southward with the Pacific Ocean and northward with the Sea of Japan) and more than 3,000 islands occur in this Sea. The inland sea has a wide tidal height range (>200 cm difference between low and high tides) and rapid and strong tidal currents. Water temperatures are 10.1–25.4°C (monthly mean during 1981–

2010, Hiroshima Prefectural Technology Research Institute 2016), and the lowest water temperature is always recorded in late winter during February–March. This area is known to maintain a fish fauna comprised exclusively of temperate water species (Nakabo 2002), including a few labrid species, i.e., *Halichoeres tenuispinis*, *Parajulis poecilepterus*, *Pseudolabrus sieboldi*, *Pteragogus aurigarius*, and *S. reticulatus* (see Sakai et al. 2010; Shimizu et al. 2010). Adults of this species are well known for their large body size and a developed hump in the forehead. Although giant *S. reticulatus* ≥ 1 m have been reported (e.g., Konishi 1995; Moyer and Nakamura 2003), the longevity and growth rate of this wrasse are unknown. Protogynous sexuality has been suggested for this wrasse (e.g., Moyer and Nakamura 2003), as confirmed in the congeneric species *S. pulcher* (Warner 1975; Cowen 1990). However, no hermaphroditism data have been reported. In addition, it has often been described that the developed humpheads of *S. reticulatus* function to indicate male sexuality (e.g., Inuo 1935). Recently, two types of developmental processes of humpheads in reef fishes have been suggested, namely a sex-associated type and a size-associated type (Liu and Sadovy 2011). In the case of *S. reticulatus*, however, this remains unclear because of the lack of quantitative data clarifying the humphead development pattern in relation to sexuality or body size growth.

Thus, biological studies have been poorly conducted on this wrasse probably because of the considerable difficulty collecting samples. This wrasse is not a main target of commercial fisheries in the western Seto Inland Sea facing Hiroshima, Ehime, and Yamaguchi Prefectures and is occasionally landed on fish markets from set net or gill net bycatch. Fortunately, I captured 287 *S. reticulatus* specimens in the western Seto Inland

Sea and analyzed their growth pattern and reproduction. Herein, I describe the details of the growth pattern and reproductive traits, including sexuality of the Asian sheephead wrasse and compared data with those of other large wrasses. I also described the *S. reticulatus* humphead development pattern.

Chapter 2

Age and Growth

Introduction

Semicossyphus reticulatus is known to their large body size but study on age and growth of this wrasse have ever been conducted probably because of the considerable difficulty in collecting samples. Study on age and growth of fish have well conducted among temperate fishery species with aim to manage those resources. However, reef dependent species were less targeted such studies despite utilized as fishery resources. Particularly, to evaluate age and growth of large reef fishes that affect other animals mainly through their feeding activities is necessary from demands of fishery management also for appropriate conservation of coastal water ecosystems. In this chapter, I described the growth feature of this fish with comparing to other large labroid fishes.

Materials and Methods

This study conducted in the western Seto Inland Sea facing Hiroshima, Ehime and Yamaguchi Prefectures (Fig. 1). Fish samples were collected from fish markets occasionally landed on fish markets as a bycatch of set nets or gill nets, bait fishing from the breakwater and captured by using hand nets with skin diving in the 9-yrs (2005 – 2008 and 2011 – 2015), I obtained a total of 287 specimens. Specimens were measured their standard lengths (SL; range 36.0 mm- 591.0 mm), total length (TL; range 44.5 mm – 711.5 mm) and body weights to the nearest 0.1 g (BW; range 1.2 g – 8490.0 g). Extracted livers and gonads from the fish were weighed to the nearest 0.01g. Gonads and whole of gut were preserved in 10% formalin to use after analyses. Sex was determined histologically by microscopic examination of gonadal sections stained with hematoxylin and eosin. I used the gonadosomatic index (GSI) to analyze the seasonality of reproduction (GSI = GW/BW × 100). All gonads from adult specimens had ovarian or secondary testis structures within the ovarian cavities (see Results). I defined individuals with only ovarian cells occupying the gonads as females (n = 67). Individuals with testicular cells within the ovariform gonads (i.e., secondary testis) were defined as males (n = 24). I used the limited data of adult females > 241.5 mm SL (n = 52) with an increasing GSI (see Results) to compare the sexual characteristics with males. Smaller individuals (< 241.5 mm SL) were treated as immature females (n = 15) in the present study.

All statistical analyses were conducted using R ver. 3.3.1 software (R Development Core Team 2016). Parametric tests were used, except the Mann–Whitney *U*-test was performed for the seasonal comparison of otolith marks due to non-normality of the data and heterogeneous variances. The two sample *t*-test was used to compare the sexes. Pearson's correlation analysis was used to assess associations, and the binominal test was performed to analyze the sex ratio. Data are presented as mean ± standard deviation.

For this age and growth analysis, 91 *S. reticulatus* specimens were used. 70 fishes were obtained at fish markets, and the remaining 21 specimens were taken via hook and line fishing. Sectioned sagittal otoliths were used to determine age. Whole otoliths were not applicable to count annuli because the otolith core was visually indistinguishable (turned dull white), particularly in older specimens. Scales could not be used as an age characteristic because of high rate of regenerated. According to the methods of Masuda and Noro (2004), sagittal otoliths were embedded in polyester resin and the margin on one side was ground transversally to a focus of 0.1 – 0.2 mm using a knife grinder (#180 stone, STD-180E; Shinko, Tokyo, Japan). Then, the sectioned otoliths were polished using a fine stone (#1,000) up to the focus (0.1 – 0.2 mm thickness), mounted on a glass slide with enamel polish, and observed under a microscope (E-100LED MV; Nikon, Tokyo, Japan). The core and opaque bands were dark rings and wider translucent bands, respectively, under transmitted light (Fig. 2). According to the otolith analysis, I supposed that annuli formed during the low-water-temperature season (February – March), namely March 1 in present study.

To assess the annual pattern of deposition of otolith annuli, the appearance of each otolith margin was recorded as opaque or translucent in each monthly sample. The timing of annulus formation was examined by plotting the percent occurrence of otoliths with a peripheral opaque band as a function of sampling month. Apparent ring marks that formed along the outer edge of each opaque zone next to the translucent zone were counted.

The three-parameter von Bertalanffy growth model was applied to the observed length-at-age data for adult and immature female *S. reticulatus* (n = 67) using a non-linear least squares procedure according to Gorie (2001). According to the GSI results (see Results), I supposed that birthdays of all of our *S. reticulatus* specimens occurred in the middle of the spawning season, i.e., June 1 in our age estimation model. For example, a fish sampled in January with three annuli on the otolith was estimated to be 3.58 years of age. Growth was described as: $L_t = L_\infty [1 - e^{-k(t - t_0)}]$, where L_t is SL (mm) at age t, L_∞ is the theoretical maximum SL (mm), k is the growth coefficient value, t is age in years, and t_0 is theoretical age in years of fish at length zero. Growth equation model was not adopted for males (n = 24) because young and small males were lacking in the samples (see Results). Alternatively, simple linear regression analysis was adopted for males.

Results

An opaque otolith margin often occurred during February - March (Fig. 3). The monthly occurrence ratio during February – March (66.7 ± 0%, n = 2) was significantly higher than that of the other months (April – January, 25.3 ± 13.2%, n = 10; Mann–Whitney U-test, U = 20, P = 0.03; Fig. 4), suggesting that a pair of translucent and opaque bands was formed once annually on the otolith as an annual ring around the time of lowest water temperature. According to the GSI analysis (see chapter 3), I supposed that the birthday of specimens was June 1 in the middle of the spawning season for the growth analyses.

Back-calculated lengths at age and the growth patterns of adult and immature female *S. reticulatus* were expressed as $L_t = 489 (1 - e^{-0.12 (t + 1.75)})$ (Fig. 7). Growth of the wrasse seemed to become slower after fish reached 300-400 mm SL at about 10 years of age; the slope value of calculated linear regression for females > 10 years of age [Pearson's correlation, r =0.31, P >0.05, n = 19; SL = 4.3 x t (age) + 360.5] were about 18% of that for younger females [r = 0.842, P < 0.01, n = 48; SL = 24.4 x t (age) + 144.5].

The estimated ages of *S. reticulatus* specimens ranged from 1 to 31 years. The oldest fish had a SL of 467.5 mm (565.1 mm TL), BW of 3.14 kg, and was sexed as a male (Fig. 7). Males were significantly older (19.9 ± 5.2 years, range 11–31 years, n = 24) than that of adult females (9.65 ± 5.9 years, range 2 – 27 years, n = 52; t-test, t = – 7.30, P < 0.01; Fig. 7). The largest fish was also a male with a SL of 591.0 mm (711.6 mm TL), BW of 8.49 kg, and age of 17 years (Fig. 7). Males were significantly larger (481.2 ± 42.6 mm SL, n = 24) than that of adult females (349.3 ± 82.4 mm SL, n = 52; t-test, t = – 7.38, P < 0.01). The liner regression line of males was calculated as follows; SL = -0.9 x t (age) + 498.4 (Pearson's correlation, r = -0.1, P >0.05, n =24; Fig. 7). The SL (432.5 ± 74.1 mm; range, 324.1 – 580.0 mm, n = 16) of females > 11 years where males occurred (Fig. 7) was not significantly different from that of males (t-test, t = 2.65, P > 0.05; Fig. 7).

Discussion

The von Bertalanffy model fit the data of females ≤ 30 years of age well (Fig. 7). Here, I compared the growth rate of *S. reticulatus* with that of other large labrids. Choat et al. (2006) reported that *C. undulatus*, which is one of the largest labrids reaching 2,000 mm TL, 190 kg BW, and 30 years of age (Sadovy et al. 2003), grows rapidly during early life stages, reaching 440 mm TL at 5 years. In contrast, *S. reticulatu*s reached a mere 330 mm TL in 5 years, i.e., 75% of the growth of *C. undulatus*. Furthermore, among the labrids *Achoerodus gouldii* from western Australia (Coulsen et al. 2009), *Choerodon rubescens* (Fairclough 2005) from western Australia, and *Tautoga onitis* from Virginia waters, USA (Hostetter 1993), *Choerodon rubescens* show rapid growth in their early life stages, reaching ca. 400 mm SL at 5 years (Fig. 9). A relatively slower growth pattern has been reported in *T. onitis*, and the growth curve overlaps considerably with that of *S. reticulatu*s, particularly at about 5 years of age (ca, 270 mm SL; Fig. 9). However, *T. onitis* subsequently grows faster than *S. reticulatus*. These examples emphasize the slow growth rate of *S. reticulatus* determined in the present study. Female *S. reticulatu*s after 10 yrs age seemed to slow down the growth further (Fig. 6). The growth patterns in *A. gouldii, C. rubescens* and *T. onitis* commonly have similar tendencies as the growth speeds gradually became slower after 10 yrs ages regardless of some inter-specific difference in growth levels (Fig. 5). The Napoleon fish *C. undulatus* is also reported to exhibit gradual slow growth after 10 yrs age (Choat et al., 2006). Thus, the deceleration of growth speed after 10 yrs may widely occur in large labrid species. Conversely, the growth speed during early life stages (< 10 yrs ages) may largely determine the maximum body size of large-sized labrid species regardless of the length of the longevity.

The California sheephead wrasse *S. pulcher*, which inhabits Santa Catalina Island, CA, USA (Warner 1975), has a similar growth rate to that of *S. reticulatus*. *Semicossyphus reticulatu*s and *S. pulcher* grow to around 250 mm SL after 5 years and subsequently reach 400 mm SL at about 10 years of age (Table 1). These two sheephead wrasses are quite different in their geographical distributions; *S. pulcher* occurs only in the eastern Pacific, whereas *S. reticulatus* only inhabits the northwestern Pacific Ocean. However, these two sheephead wrasses maintain biological similarities as protogynous hermaphrodites (Cowen 1990, see below), in feeding habits by targeting benthic animals (Cowen 1983; Y Ochi unpublished data), and in the humphead development of adults

(Poortvliet et. al. 2013). Our GSI data suggest that female *S. reticulatus* are sexually mature at about 240 mm SL and 3 years of age. Sexual maturity of *S. pulcher* at Santa Catalina Island is 190 – 230 mm SL and 4 years (Warner 1975). Thus, *S. reticulatus* has a similar sexual maturation pattern to that of *S. pulche*r. Moreover, water temperature conditions in their habitats are similar, i.e., 13.9 – 21.1°C for *S. pulcher* (National Oceanic and Atmospheric Administration 2016) and 10.1 – 25.4°C for *S. reticulatus.* These biological and environmental similarities may support maintenance of a similar growth strategy in these closely related *Semicossyphus* sheephead wrasses.

The largest *S. reticulatus* caught by fishing in Japanese waters was 1,160 mm TL from Owase, Mie Prefecture facing the Kuroshio Current (Konishi 1995). In contrast, the L_∞ value from the growth model in the present study was 489 mm (approximately 600 mm TL) for females, which seems to be so small comparing with the size records. Warner (1975) reported that the growth rate of *S. pulcher* tends to increase after sex change to male. I predict that similar growth acceleration may occur even in males of *S. reticulatus*. Though we obtained only male specimens whose SLs were similar to large females in the same age class, there remains a possibility of the presence of larger males within the population. Additional sampling studies including giant male specimens are expected to reveal the growth nature of secondary males of *S. reticulatus*.

The other possibility promoting the size difference between the size record and the L_∞ value in the present study is the population variations of growth speed. As giant-sized *S. reticulatus* have often been recorded from waters facing warm currents and/or the open ocean, habitat conditions may affect the intraspecific growth differences of this wrasse.

For example, waters temperature in the western Seto Inland Sea usually drop to < 10°C in the winter (9.7°C at Kurahashi Island, Hiroshima; Hiroshima Prefectural Technology Research Institute 2016), whereas the lowest water temperatures in the Pacific coastal waters are > 13°C (13.8 at Shimoda on Izu Peninsula and 14.1°C at Katada on Shima Peninsula, Mie Prefecture) (Shizuoka Prefectural Fishery Research Institute 2016; Japan Fisheries Research and Education Agency 2016). I predict that warm water conditions promote rapid growth of *S. reticulatus*. Warner (1975) and Hamilton et al. (2011) reported that water temperature, fish density, prey availability and fisheries activities were factors to promote the regional variations in growth patterns of *S. pulcher*. A comparative growth study is needed to understand the intraspecific growth variations in *S. reticulatus*.

Chapter 3

Reproduction and sexuality

Introduction

Reproduction is one of the important biology in fishes and well studied for management of fisheries resources. For example, it needs accurate information for fishery to configure size limit with respect to their maturation size and prohibition term based on study of spawning seasonality. Meanwhile, as for labroid fishes, rather interested in biological aspects represent as hermaphroditism. Although some types of hermaphroditism are known, protogynous sexuality (change sex from female to male) has been seen in many labrid fishes in relation with their spawning structure namely harem. Also about for this wrasse (e.g., Moyer and Nakamura 2003), as confirmed in the congeneric species *S. pulcher* (Warner 1975; Cowen 1990). However, no hermaphroditism data have been reported. In this chapter, I described details of sexual maturation and spawning seasonality of this fish. Furthermore, discussed about their hermaphroditism based on histological analyses.

Materials and Methods

Sex was determined histologically by microscopic examination of gonadal sections stained with hematoxylin and eosin. I used the gonadosomatic index (GSI) to analyze the seasonality of reproduction (GSI = GW/BW × 100). I defined individuals with only ovarian cells occupying the gonads as females (n = 67). Individuals with testicular cells within the ovariform gonads (i.e., secondary testis) were defined as males (n = 24). I used the limited data of adult females > 241.5 mm SL (n = 52) with an increasing GSI (see Results) to compare the sexual characteristics with males. Smaller individuals (< 241.5 mm SL) were treated as immature females (n = 15) in the present study.

Results

Gonads of females were comprised of only ovigerous tissue containing vitellogenic oocytes surrounding a central lumen (Fig. 5a). The ovariform gonadal structure was confirmed, even in the smallest specimen (194.1 mm SL, 2 years of age).

Males had gonads with a secondary testis structure comprised of spermatogenic tissues including clusters of spermatocytes and sperm inside ovarian lamellae with a retained central gonadal lumen (Fig. 5b). The male gonad included remnants of ovarian cells in the active testis (Fig. 5c), suggesting protogynous sex change. In hermaphroditic gonads, male and female tissues seemed not to be separated by connected tissues but were intermixed (undelimited type 2; sensu Sadovy and Shapiro 1987). No males maintained gonads with a primary testis structure in the present study.

Individuals with high GSI values often occurred during April – June (Fig. 6), indicating the *S. reticulatus* spawning season in the sampled waters. Female GSI was consistently < 2% at times other than spawning (Fig. 6). The smallest female with a GSI > 2% had a SL of 241.5 mm and was 3 years of age, suggesting that females around this size and age are sexually mature. The sex ratio was biased toward females (adult females $n = 52$; males $n = 24$), which was significantly different from 1:1 (binominal test for the proportion, $P < 0.01$).

Discussion

Hermaphroditism among fishes is widespread and takes on many forms, having been reported for >350 species in 34 families in eight orders (Kuwamura and Nakashima 1998). The most common type is protogyny (change sex from female to male). Rarer types are protandry (male to female), simultaneous hermaphroditism and two-way sex change. The coexistence of gonochoristic males that matured directly without passing through any female maturation stages and sex-changed male has often been confirmed in protogynous fish species is called diandry (Warner and Robertson 1978). Meanwhile, only existence of sex changed male is called monandry.

The size advantage model theoretically explains the adaptive significance of protogynous sexuality in polygynous mating systems (i.e. harem) as a life-history strategy (Warner and Robertson 1978; Warner 1988). A very famous male *S. reticulatus* of about 1,000 mm TL named "Benkei" was discovered by recreational divers off the Sado Island reef in the Sea of Japan and was observed spawning with several females within a mating period during daytime (Moyer and Nakamura 2003), suggesting a polygynous mating system. Polygynous mating systems and courtships of congener *S. pulcher* was revealed (Adreani et al. 2004) Therefore, protogyny in *S. reticulatus* suggested from our data are consistent with these finding. In the present study, all males retained some characteristics of ovariform gonads (with secondary testis; Fig. 5), and males maintained large SLs and age in excess of adult females (Fig. 7). In addition, the sex ratio was apparently biased toward females. These results strongly suggest that *S. reticulatus* is a protogynous hermaphrodite.

S. reticulatus is not diandrous because of the lack of small males (Fig. 7) and the lack of primary (non-sex-changed) testis in any male specimens, as already mentioned (Fig. 5). Thus, *S. reticulatus* males are likely derived from females via sex change, i.e., monandric protogyny (sensu Warner and Robertson 1978).

Our GSI data suggest that female *S. reticulatus* are sexually mature at about 240 mm SL and 3 years of age. Sexual maturity of *S. pulcher* at Santa Catalina Island is 190 – 230 mm SL and 4 years (Warner 1975). Thus, *S. reticulatus* has a similar sexual maturation pattern to that of *S. pulche*r.

Chapter 4

Head morphology

Introduction

The most notable external character of *S. reticulatus* may their hump on forehead. Japanese name "Kobudai" may be derived from this feature. Recently, two types of developmental processes of humpheads in reef fishes have been suggested, namely a sex-associated type and a size-associated type (Liu and Sadovy 2011). As for *S. reticulatus*, It has been believed that only mature males have the hump (i.e. sex specific) without any study. In this chapter, I describe the pattern of hump elevation and discuss the possibility of its function on this fish.

Materials and Methods

Humphead elevation was measured and analyzed according to the method of Liu and Sadovy (2011) for the giant labrid *Cheilinus undulates* often called Napoleon fish. I photographed the forehead from the lateral side of 23 individuals chosen randomly (TL, 236 – 712 mm). A straight line was drawn along the image of the forehead, and the maximum angle (MA) of the hump elevation against the forehead was measured using a 180° protractor (Fig. 3). TL was used in accordance with Liu and Sadovy (2011) to allow a comparison with the *C. undulatus* data. *S. reticulatus* TL and SL were significantly correlated [Pearson's correlation, $r = 0.996$, $P < 0.05$, $n = 91$; TL (mm) = 1.21 × SL (mm) – 1.063].

Results

The forehead humps generally grew with the body (Fig. 8); the maximum angle (MA) of the female hump was strongly correlated with TL [Pearson's correlation, $r = 0.86$, $P < 0.05$, $n = 19$; MA = 0.091 × TL (mm) – 12.682]. Hump elevation of males (43.1 ± 10.2º MA, $n = 4$) did not exceed that of large females > 500 mm TL (41.4 ± 7.5º MA, $n = 6$; *t*-test, $t = -0.32$, $P > 0.05$; Fig. 8). Thus, large females, particularly those with TL of around 700 mm, had developed humps similar to those of males. MA of the hump in female *S. reticulatus* > 600 mm TL reached 40°, which is the same as that of male Napoleon fish, *C. undulatus* > 1,200 mm TL (Fig. 8).

Discussion

Some fish species are known to develop obvious humps on their heads, e.g., *Bodianus diplotaenia*, *Coris aygula* and *C. bulbifrons* (Labridae), *Cyphotilapia frontosa* (Cichlidae), *Naso tuberosus* (Acanthuridae), and *Bolbometopon muricatum* and *Scarus perrico* (Scaridae). Liu and Sadovy (2011) reviewed the humpheads of reef fish and categorized the developmental process into a sex-associated type (e.g., *Chlorurus microrhinos* and *Choerodon azurio*) and a size-associated type (e.g., *Bolbometopon muricatum, C. undulatus* and *Naso tuberosus*). The *S. reticulatus* humps were believed to be male-specific (e.g., Inuo 1935). However, the present study revealed that the humphead of *S. reticulatus* gradually enlarges with body growth, and the humps of large females were never inferior to those of males (Fig. 8). Thus, I conclude that the *S. reticulatus* humphead is the size-associated type.

Male *S. pulcher* have been observed underwater acting aggressively toward faces each other during the mating season and defending their mating territories (Adreani et al. 2004). Similar territorial defense, including butting and biting, has been observed between male *S. reticulatus* (Moyer and Nakamura 2003; Y. Ochi personal communications). The developed humps of male *S. reticulatus* may have direct (arms) or indirect (indicators or ornaments) functions that affect the results of the contested battles for mate acquisition. The growth pattern of the humps of female *S. reticulatus* (Fig. 8) strongly suggest considerable time (years) spent developing the humps. Thus, it is plausible that females gradually prepare this male-specific-use character for use after future sex change. Further investigation of the development and functions of humps in males is needed.

Chapter 5

General discussion

Present study shown that *S. reticulatus* have slow growth and relatively long life span. With SL, 280 mm in 5 years, 380 mm in 10 years and 460 mm in 20 years. Male individuals shown overlapping distribution in size and age with female. The smallest male was 399.2mm SL and the age of the youngest one was 11.1 yrs (other individuals). While the largest female was 580.0mm SL and the oldest one was 27.8 yrs (also other individuals). Protogynous hermaphroditic fishes generally show sex-specific size distribution results from social control of polygynous mating system (Kuwamura 1996). In spite of size and age overwrapping among sexes, our whole sample shows female biased sex rate and histological survey strongly suggests their hermaphroditism. It might be caused with captured fish samples from broad area. Social sex control may be worked as homogenous in a population. It needs sampling and/or underwater observation in a certain steady site and study of behavioral area to grasp their social structure.

It is said that family Labriae originaly occur in the tropical water and expand their habitat to sub-tropical and temperate waters. Genus *Semicossyphus* is well adapted to temperate waters with *Parajulis*, *Pseudolabrus* and *Pteragogus*. Many of large labrid species (maximum TL > 700 mm) are belong to Hypsigenyines with *Semicossiphus* species, e.g. *Achoerodus gouldii* and *A. viridis*, *Anchichoerops natalensis*, *Bodianus diplotaenia*, *B. macrognathos* and *B. perditio*, *Choerodon rubescens* and *C. schoenleini*, and *Lachnolaimus maximus* and then many of these species live temperate waters. In addition, *Cheilinus undulatus*, *Coris aygula* and *Tautoga onitis* are large labrid species but not belong to Hypsigenyines. It is interested that almost half of large labrid species lives temperate waters despite small number of inhabitant with marginal distribution of labrids. Many biological similarities with congeners *S. pulcher* are revealed from comparing data of present study to that of former studies. Growth, aging pattern and sexuality resemble *S. pulcher*. It seems genus *Semicossiphus* obtain similar niche on respective temperate Pacific water habitats despite geographical separation.

Considering a slow growth speed, a long life-span, a strongly biased sex ratio and sex-related body size imply their potential weakness against to the fishery activities and environmental change on the habitats. In addition, Topping (2006) monitored the activity area of *S. pulcher* by using acoustic transmitter and described about their limited area utilizing. It shows that passive migration ability of *S. pulcher* and then possibility occurs on *S. reticulatus*. This biology suggests that more negative towards to exploitation because it is not to be expected new recruitment after disappearance of former habitants. I am fortunate to conduct the present study on this large reef fish *S. reticulatus* in this area. This is partly because the fishery pressure for the fish is not so strong in the western Seto Inland Sea, in contrast to most of large reef fishes under severe exploitations by fisheries. I hope our data of *S. reticulatus* would contribute to further scientific understanding of a valued bioresource in Seto Inland Sea and the conservative control of the Sato Umi coastal ecosystem.

Summary

The Asian sheephead wrasse *Semicossyphus reticulatus* is the largest labrid in temperate waters around Japan. However, the growth pattern of this wrasse remains unclear. Age, growth and reproduction of *S. reticulatus* were investigated by sampling in the western Seto Inland Sea, Japan. Opaque ring marks formed on the marginal zone of sectioned otoliths during February – March, suggesting their validity for age estimates. The largest individual was a male, with standard length (SL) of 591 mm, weight of 8.5 kg, and age of 17 years. Males had significantly larger SLs and older ages than females, and male gonads were histologically confirmed to have secondary testis structures retaining central lumens, suggesting monandric protogyny. The oldest *S. reticulatus* individual captured was estimated to be a 31-year-old male. The von Bertalanffy growth equation calculated from estimated age and SL was $L_t = 489\ (1 - e^{-0.12\ (t + 1.75)})$, indicating considerably slow growth, particularly after the age of 10 years, which is similar to that of the California sheephead wrasse *Semicossyphus pulcher*. The humpheads of *S. reticulatus* enlarged gradually as their body grew, often resulting in a conspicuous humphead, even in large females.

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References

Adreani MS, Erisman BE, Warner RR (2004) Courtship and Spawning Behavior in the California Sheephead, Semicossyphus Pulcher (Pisces: Labridae). Environ Biol Fish 71: 13-19

Allen GR, Robertson DR (1994) Fishes of the tropical eastern Pacific. University of Hawaii press, Hawaii, United States of America.

Choat JH, Davies CR, Ackerman JL, Mapstone BD (2006) Age structure and growth in a large teleost, *Cheilinus undulatus*, with a review of size distribution in labrid fishes. Mar Ecol Prog Ser 318: 237-246

Coulson PG, Hesp SA, Hall NG, Potter IC (2009) The western blue groper (*Achoerodus gouldii*), a protogynous hermaphroditic labrid with exceptional longevity, late maturity, slow growth, and both late maturation and sex change. Fish Bull (Wash. D. C.) 107: 57-75

Cowen RK (1983) The effects of sheephead (*Semicossyphus pulcher*) predation on red sea urchin (*Strongylocentrotus franciscanus*) populations: an experimental analysis. Oecologia 58: 249-255

Cowen RK (1990) Sex change and life history patterns of the labrid, *Semicossyphus pulcher*, across an environmental gradient. Copeia 1990: 787-795

Fairclough D (2005) The biology of four tuskfish species (Choerodon: Labridae) in Western Australia. PhD thesis, University of Murdoch University, Perth.

Gorie S (2001) Estimation of the Parameters in von Bertalanffy's Growth Formula by MS-Excel. Suisanzoshoku 49: 519-527

Hamilton SL, Wilson JR, Ben-Horin T, Caselle JE (2011) Utilizing spatial demographic and life history variation to optimize sustainable yield of a temperate sex-changing fish. PLoS ONE 6(9): e24580

Hiroshima Prefectural Technology Research Institute (2016) Official site of Prefectural Government. https://www.pref.hiroshima.lg.jp/uploaded/attachment/224896.pdf. Accessed 21 November 2016

Hostetter EB (1993) Age, growth, and reproduction of tautog *Tautoga onitis* (Labridae: Perciformes) from coastal waters of Virginia. Fish Bull U 91: 45-64

Inuo S (1935) Sexual differences in the labroid fishes of Japan. Botany and Zoology 3: 1621-1626

Japan Fisheries Research and Education Agency (2016) Real-time marine information acquisition and analysis system. http://buoy.nrifs.affrc.go.jp/buoy/buoy_total.php. Accessed 21 November 2016

Konishi H (1995) Fishes – new color guide for sportfishermen. Weekly Sunday Fishing, Osaka, Japan

Kuwamura T (1996) An introduction to reproductive strategies of fishes. In: Kuwamura T, Nakashima Y (eds) Reproductive strategies in fishes, vol 1. Kaiyusha, Tokyo, pp. 1–41

Kuwamura T, Nakashima Y (1998) New aspects of sex change among reef fishes: recent studies in Japan. Environ Biol Fish 52: 125

Liu M, Sadovy Y (2011) Forehead morphology of the humphead wrasse *Cheilinus undulates* (Perciformes: Labridae) in relation to body size. Copeia 2011: 315-318

Masuda Y, Noro T (2004) Recommendation of aging fish with transverse sections of otoliths. Memoirs of Faculty of Fisheries Kagoshima University 52:51-56

Moyer JT, Nakamura K (2003) Communities of Fishes (DVD book). Vol. 1. Tokai University Press, Tokyo, Japan.

Nakabo T (2002) Characteristics of the fish fauna of Japan and adjacent waters. In Nakabo T (ed.) Fishes of Japan with pictorial keys to the species, English edition. Tokai University Press, Tokyo, pp 43–52

National Oceanic and Atmospheric Administration (2016) Water Temperature Table of All Coastal Regions, National Centers for Environmental Information. NOAA. https://www.nodc.noaa.gov/dsdt/cwtg/all_meanT.html Accessed 21 November 2016

Nelson JS, Grande TC, Wilson MVH (2016) Fishes of the world 5th edition. John Wiley & Sons, Inc. New Jersey

Poortvliet M, Longo GC, Selkoe K, Barber PH, White C, Caselle JE, Perez-Matus A, Gaines SD, Bernardi G (2013) Phylogeography of the California sheephead, *Semicossyphus pulcher*: the role of deep reefs as stepping stones and pathways to antitropicality. Ecol Evol, 3 (13), pp. 4558–4571

R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/. Accessed 2016.06.25

Sadovy Y, Kulbicki M, Labrosse P, Letourneur Y, Lokani P, Donaldson TJ (2003) The humphead wrasse, *Cheilinus undulatus*: synopsis of a threatened and poorly known giant coral reef fish. Rev Fish Biol Fish 13: 327–364

Sadovy Y, Shapiro DY (1987) Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1987: 136-156

Sakai Y, Ochi Y, Tsuboi M, Kadota T, Shimizu N, Shoji J, Matsumoto K, Mabuchi K, Kuniyoshi H, Ohtsuka S, Hashimoto H (2010) Fish fauna of shallow waters of Aki Nada, Seto Inland Sea, Japan. J Grad Sch Biosph Sci, Hiroshima Univ 49:7–20

Shimizu N, Kadota T, Tsuboi M, Sakai Y (2010) Fish fauna in the coastal area of Kurahashi island, Seto Inland Sea, Japan. Bulletin of the Hiroshima University Museum 2:43-52

Shizuoka Prefectural Research Institute of Fishery (2016) Water temperature of Shirahama. http://fish-exp.pref.shizuoka.jp/izu/0008/index.html. Accessed 21 November 2016

Topping DT, Lowe CG, Caselle JE (2006) Site fidelity and seasonal movement patterns of adult California sheephead Semicossyphus pulcher (Labridae): an acoustic monitoring study. Mar Ecol Prog Ser Vol. 326: 257–267

Warner RR (1975) The reproductive biology of the protogynous hermaphrodite, *Pimelometopon pulchrum* (Pisces: Labridae). Fish Bull 73: 262-283

Warner RR (1988) Sex change and the size-advantage model. Trend Ecol Evol 3: 133-136

Warner RR, Robertson DR (1978) Sexual patterns in the labroid fishes of the western Caribbean, I: the wrasse (Labridae). Smithsonian Contrib Zool 254: 1–27.

Westneat MW, Alfaro ME (2005) Phylogenetic relationships and evolutionary history of the reef fish family Labridae. Mol Phylogenet Evol 36: 370–390

Table 1 Comparison of calculated standard lengths (mm) at age between two sheephead wrasses, *Semicossyphus reticulatus* and *S. pulcher*. For *S. reticulatus*, length from von Bertalanffy equation model are shown.

	Age (years)										
Species	1	2	3	4	5	6	7	8	10	13	Source
S. reticulatus	142	182	219	250	278	303	324	344	376	411	Present study
S. pulcher	117	150	184	214	242	272	308	343	410	470	Warner 1975

For *S. pulcher*, those from the direct proportionality method are shown, except for 10 year-old (n = 3) and 13 year-old (n = 2), which represent the means of the actual measured data.

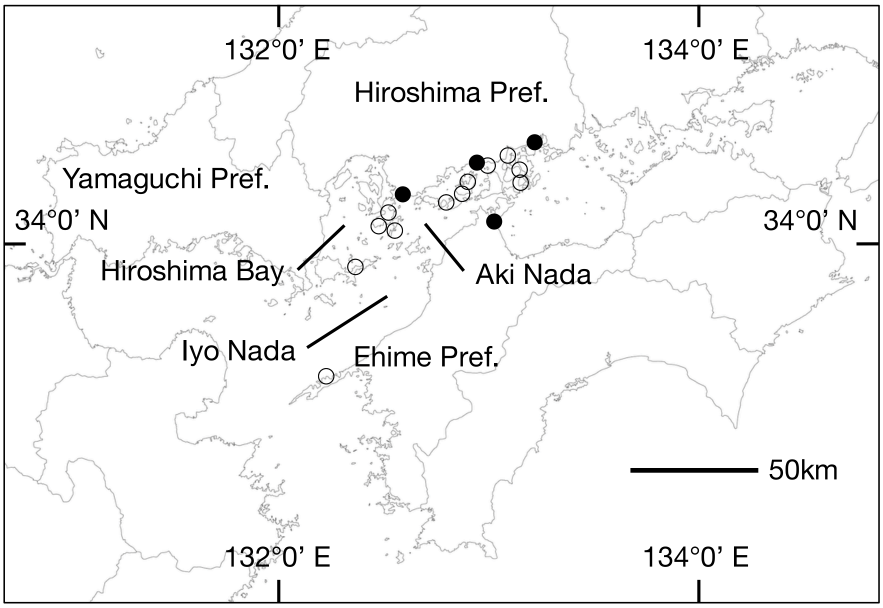


Fig. 1 Sampling locations for *Semicossyphus reticulatus* in the western Seto Inland Sea, Japan (open circles: bait fishing points, solid circles: fish markets)

Fig. 2 Representative sectioned otolith of *Semicossyphus reticulatus* (female with 512 mm SL captured in December). The photograph shows 22 rings (=22.4-years-of-age)

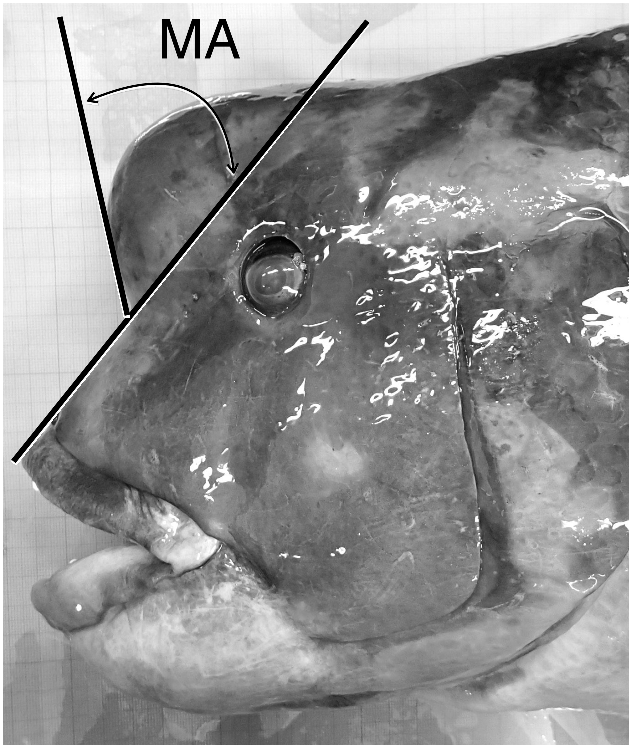


Fig. 3 Measured angle of maximum elevation of the *Semicossyphus reticulatus* forehead hump. This female (706 mm TL) had a hump with a 50.5° maximum angle (MA)

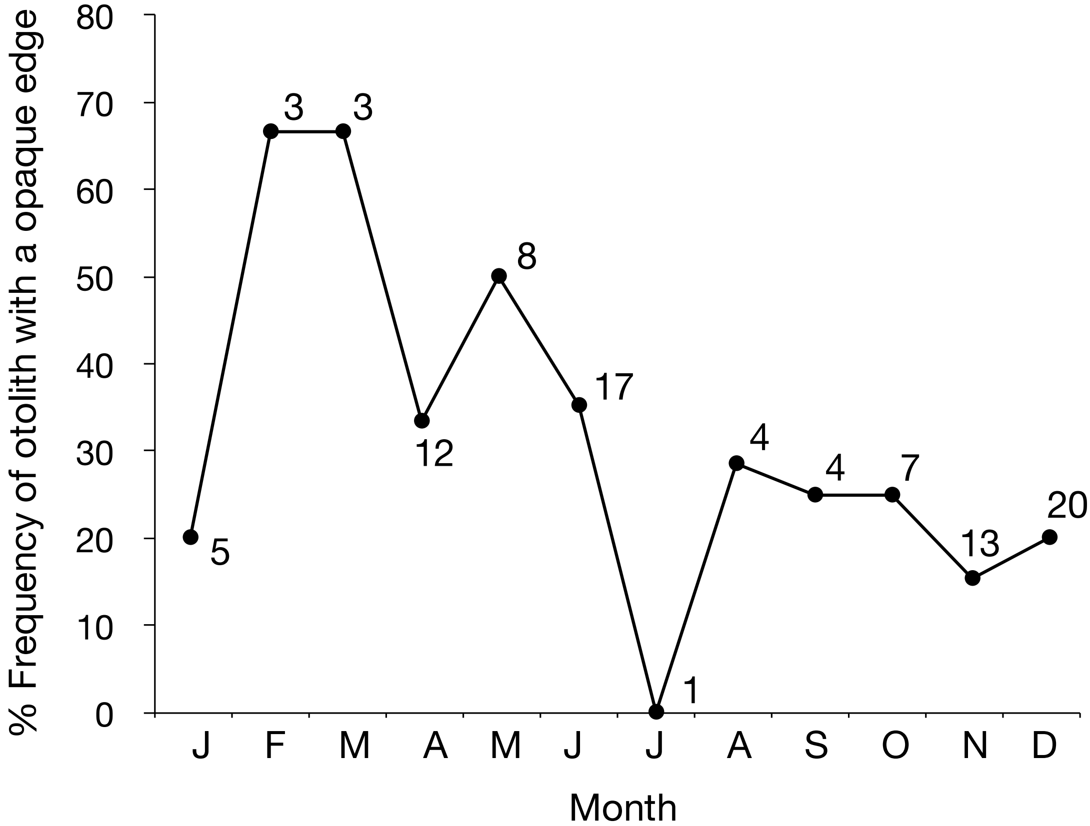


Fig. 4 Occurrence frequency of the opaque zone at the outer margin of a *Semicossyphus reticulatus* otolith (n = 97). Sample size (n) is shown beside each monthly plot

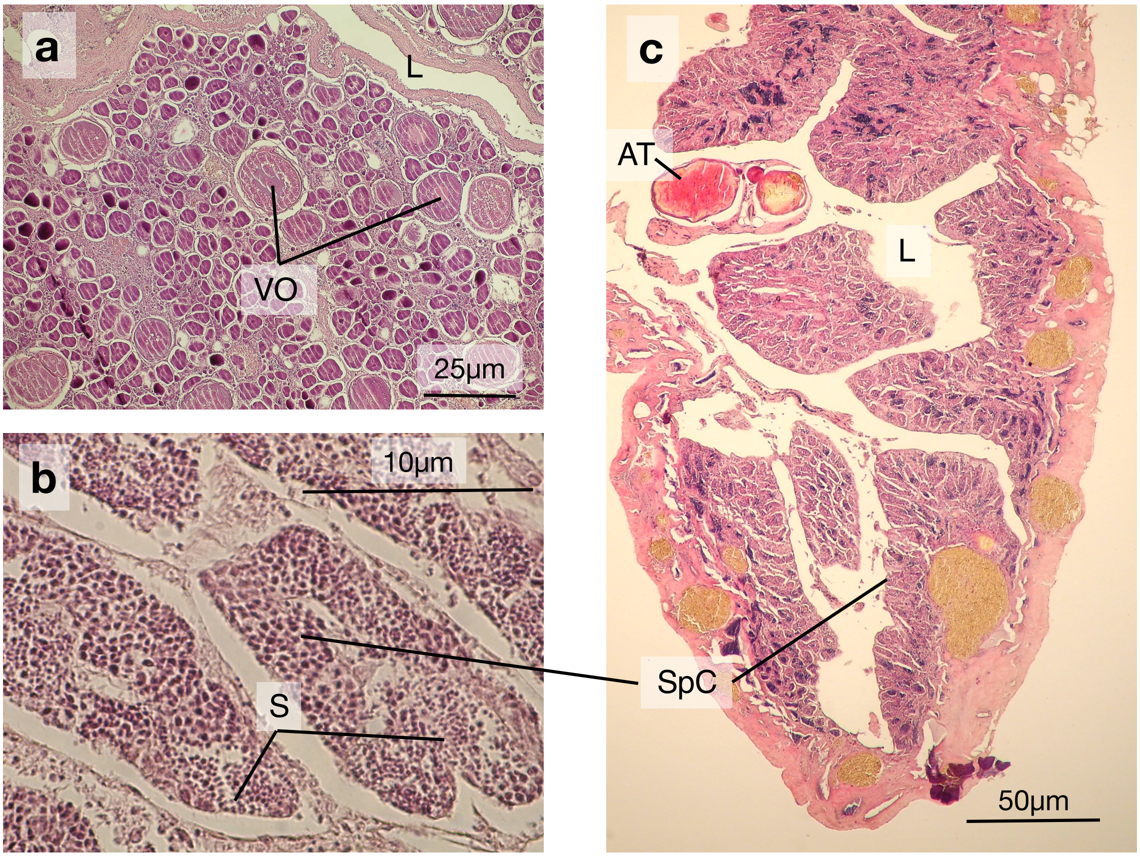


Fig. 5 Histological features of *Semicossyphus reticulatus* gonads. Female-active gonad (**a**) made entirely of ovigerous tissue containing vitellogenic oocytes (*VO*) surrounding a central lumen (*L*). Male-active gonad (**b** and **c**) containing clusters of spermatocytes (*SpC*) and sperm (*S*) interspersed with the ovariform lamellae structure including ovarian atretic follicles (*AT*) and central gonadal lumen (*L*).

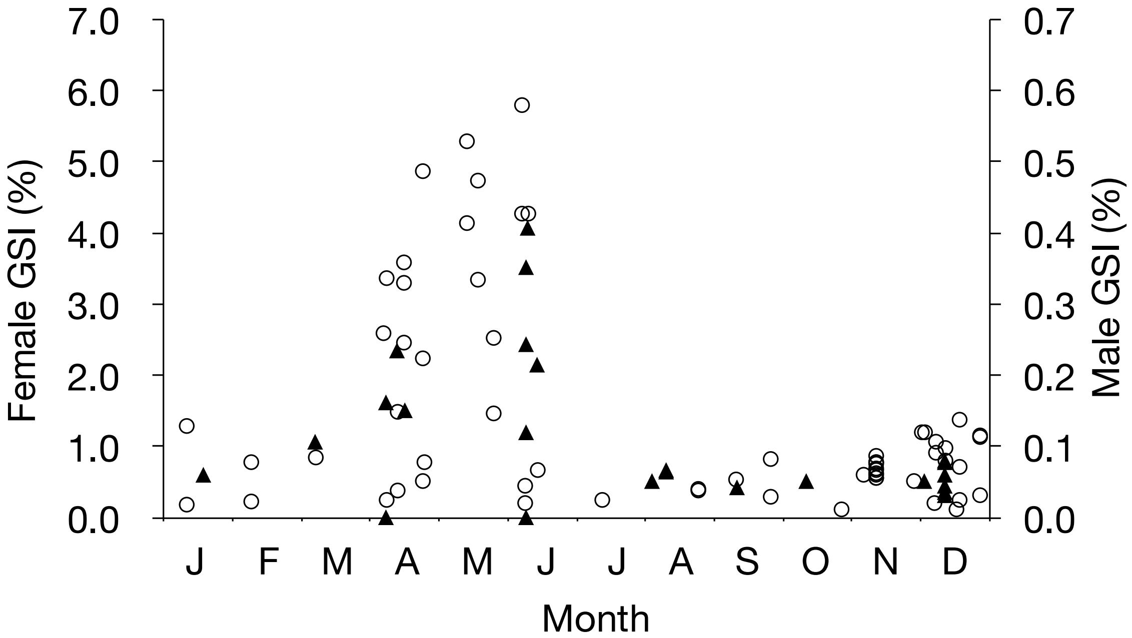


Fig. 6 Monthly changes in the gonadosomatic index (GSI) of *Semicossyphus reticulatus* from the western Seto Inland Sea, Japan. The female values are shown as open circles (n = 67), and the male values are shown as solid triangles (n = 24)

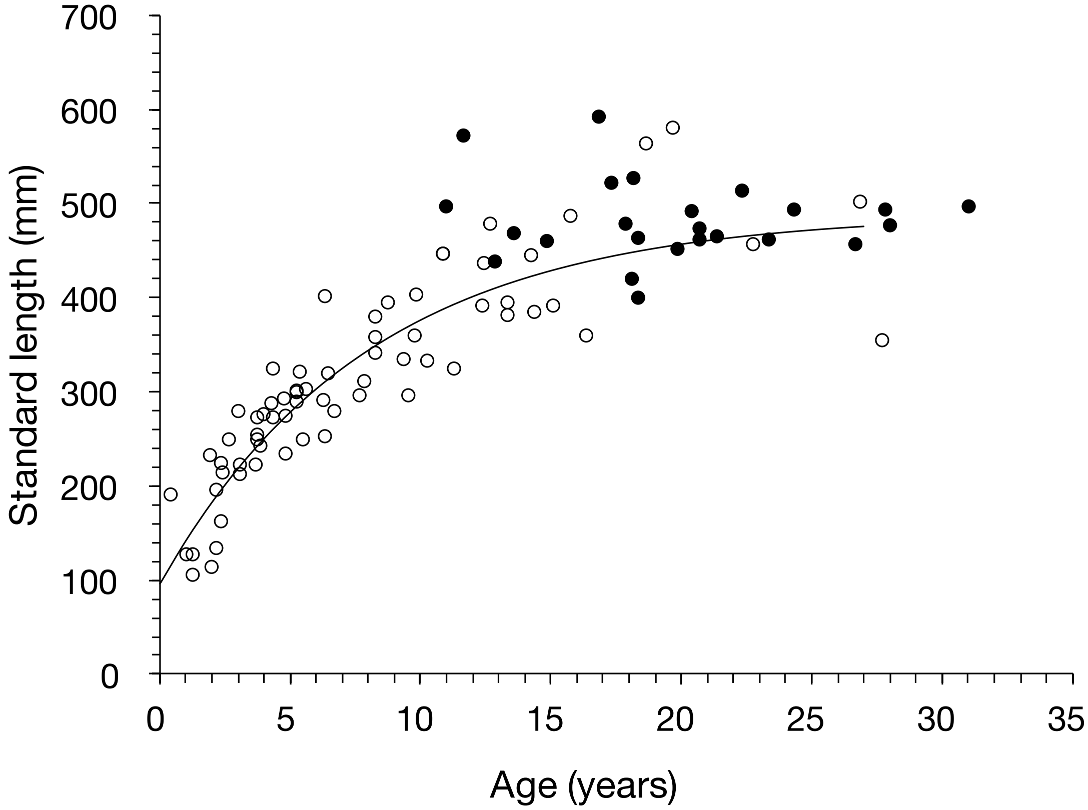


Fig. 7 Growth curves of female *Semicossyphus reticulatus* from the western Seto Inland Sea, Japan using the von Bertalanffy equation model. Individual data are including immature females shown as open circles (*n* = 67). Males that did not fit the growth curve are shown as solid circles (*n* = 24)

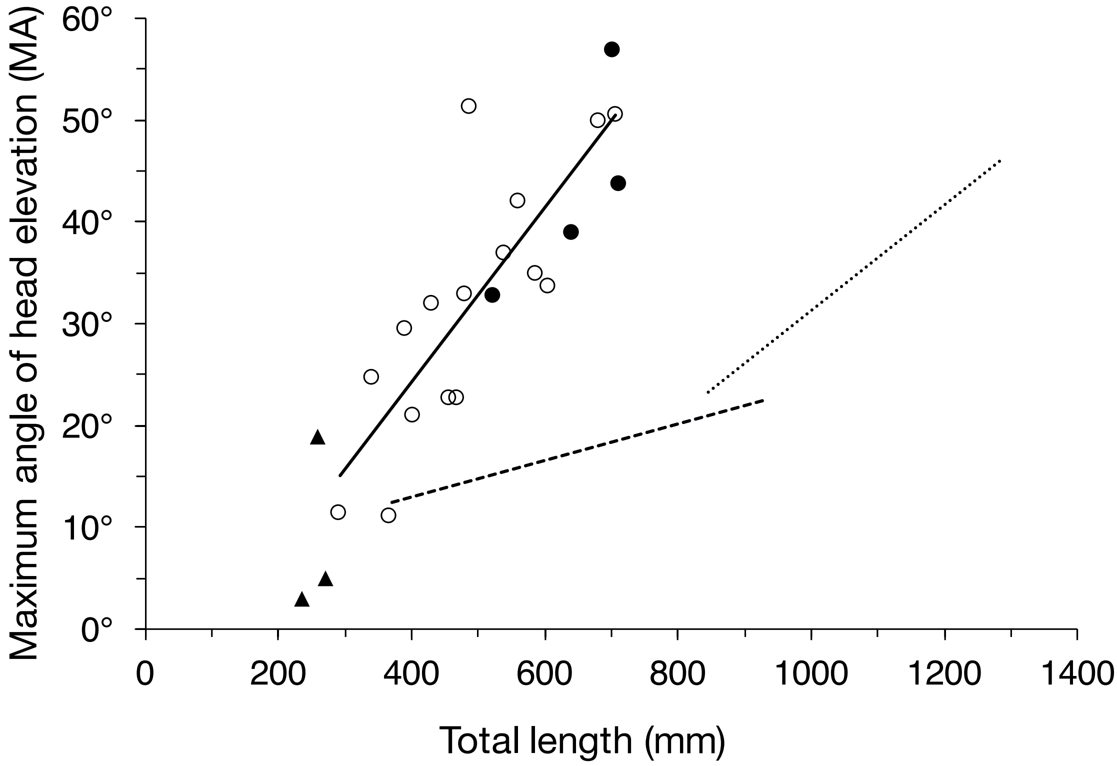


Fig. 8 Elevation of forehead morphology during growth of *Semicossyphus reticulatus*. Individual female data are shown as open circles (n = 16), immature fish are solid triangles (n = 3), and males are solid circles (n = 4). A solid line indicates the linear regression for females (see text). Dashed and dotted lines show the linear regression for female and male *Cheilinus undulatus,* respectively [redrawn from Liu and Sadovy (2011)]

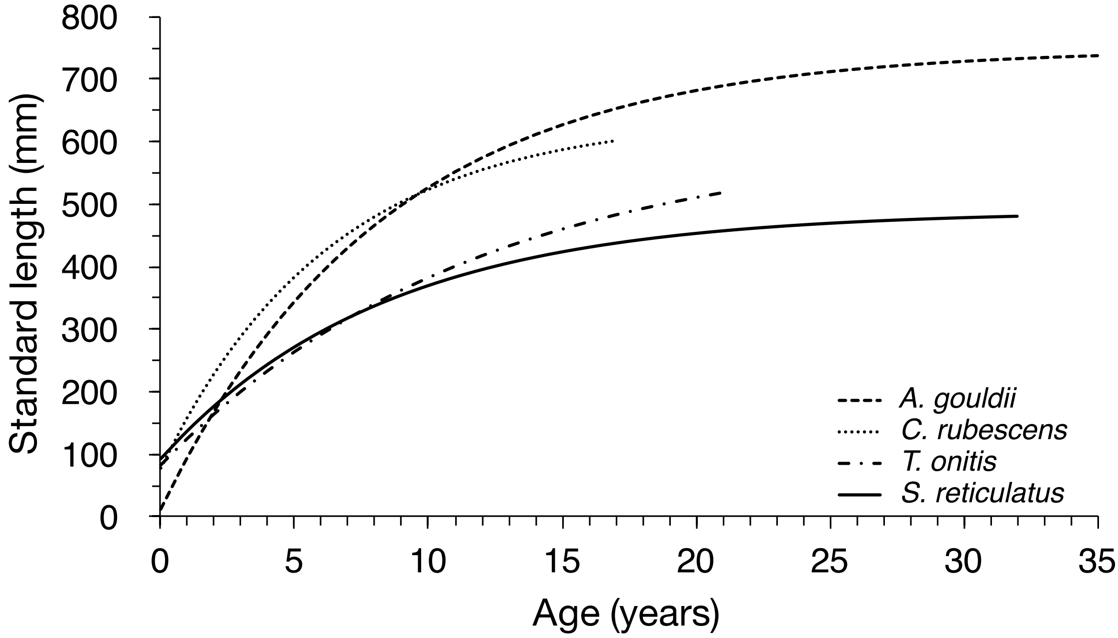
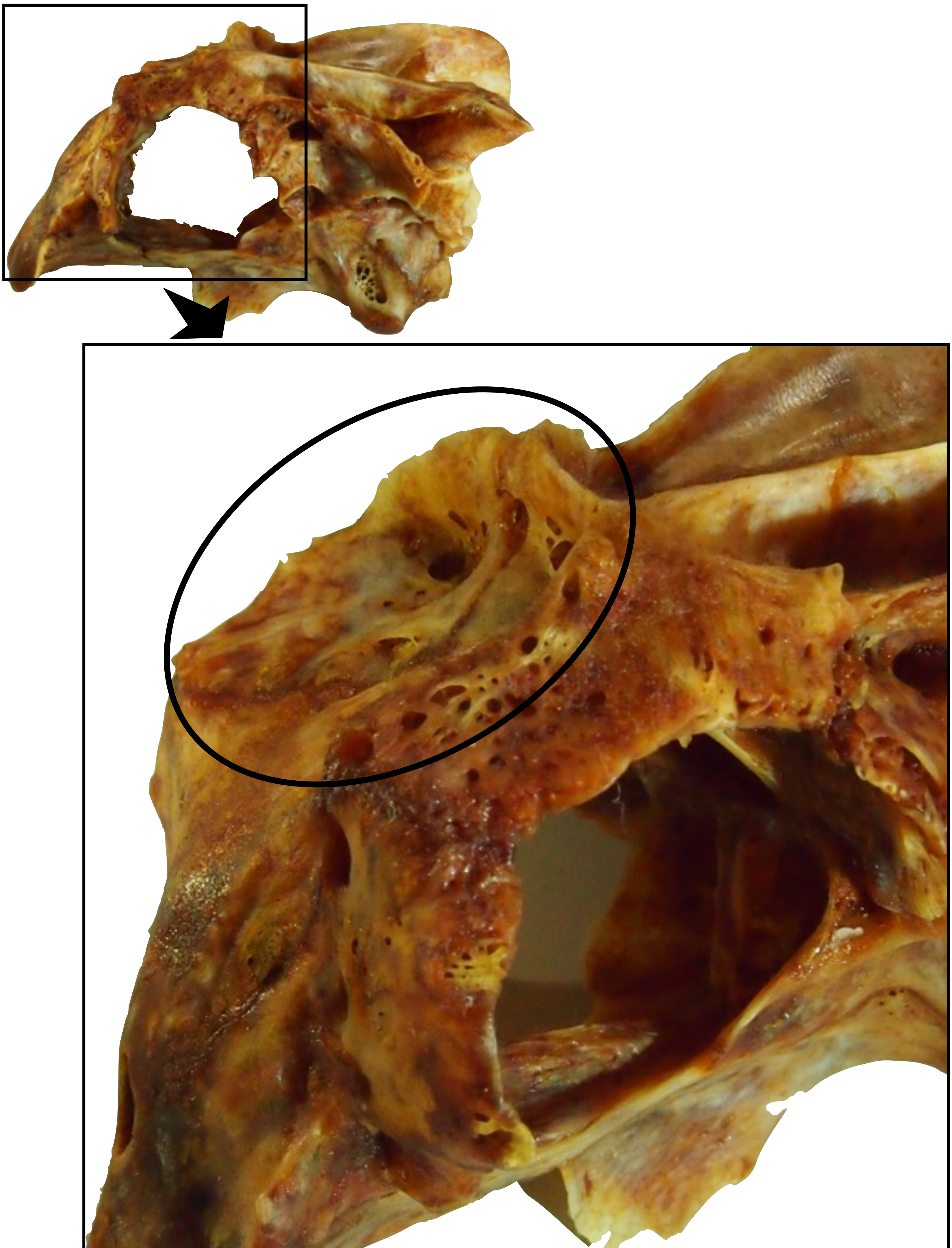


Fig. 9 Growth curves generated from the von Bertalanffy equation model in females of three large wrasse species (*Achoerodus gouldii*, *Choerodon rubscenes*, and *Tautoga onitis*) with the *Semicossyphus reticulatus* growth data in the present study. Each growth curve is redrawn from Coulsen et al. (2009), Fairclough (2005), and Hostetter (1993)

Appendix

Head morphology

Structure of cranial bone

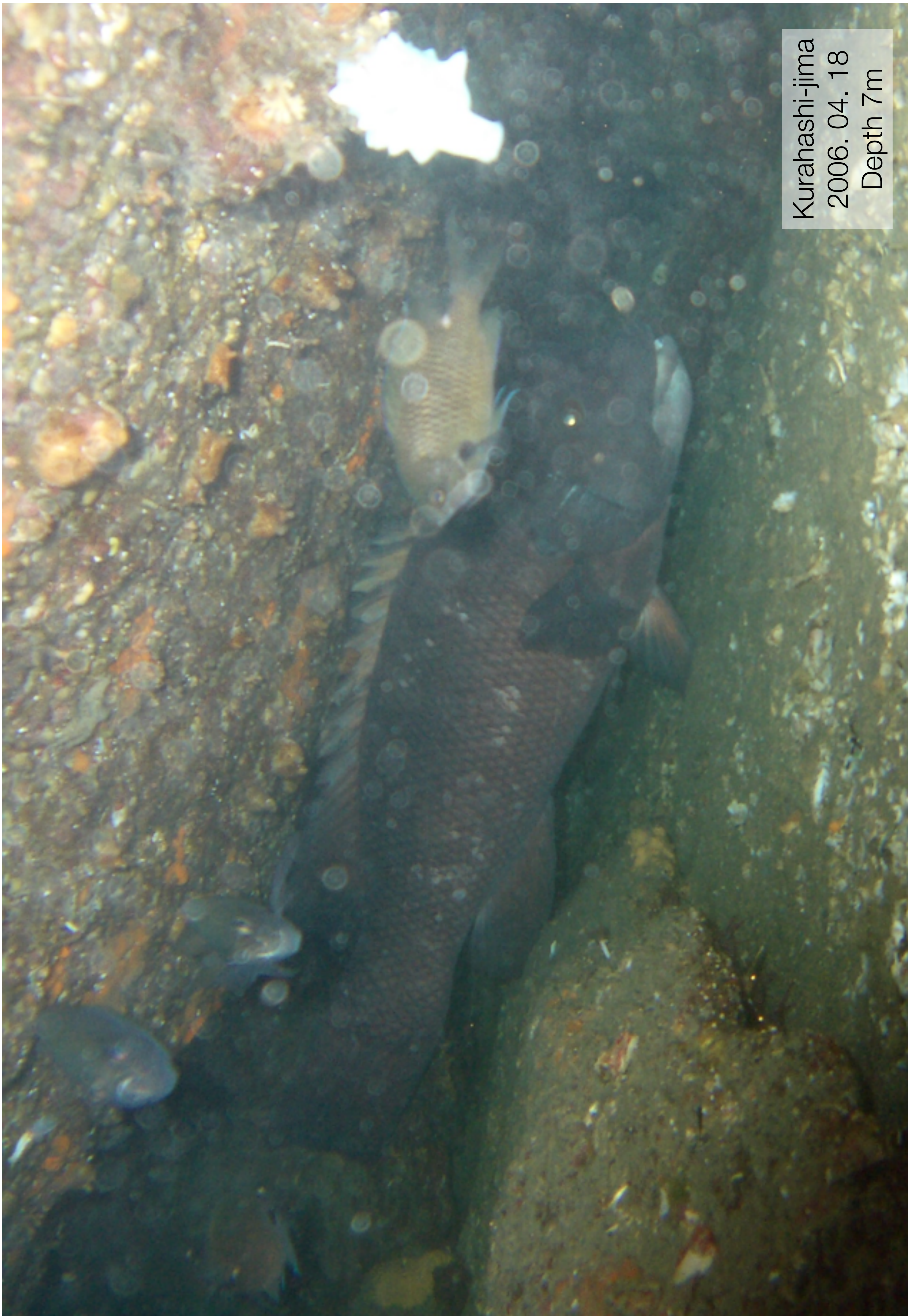


Developed humphead & chin

SL = 591.0 mm
Suou-oh-shima
2005. 06. 16

Pictures of wild
Asian sheephead wrasses

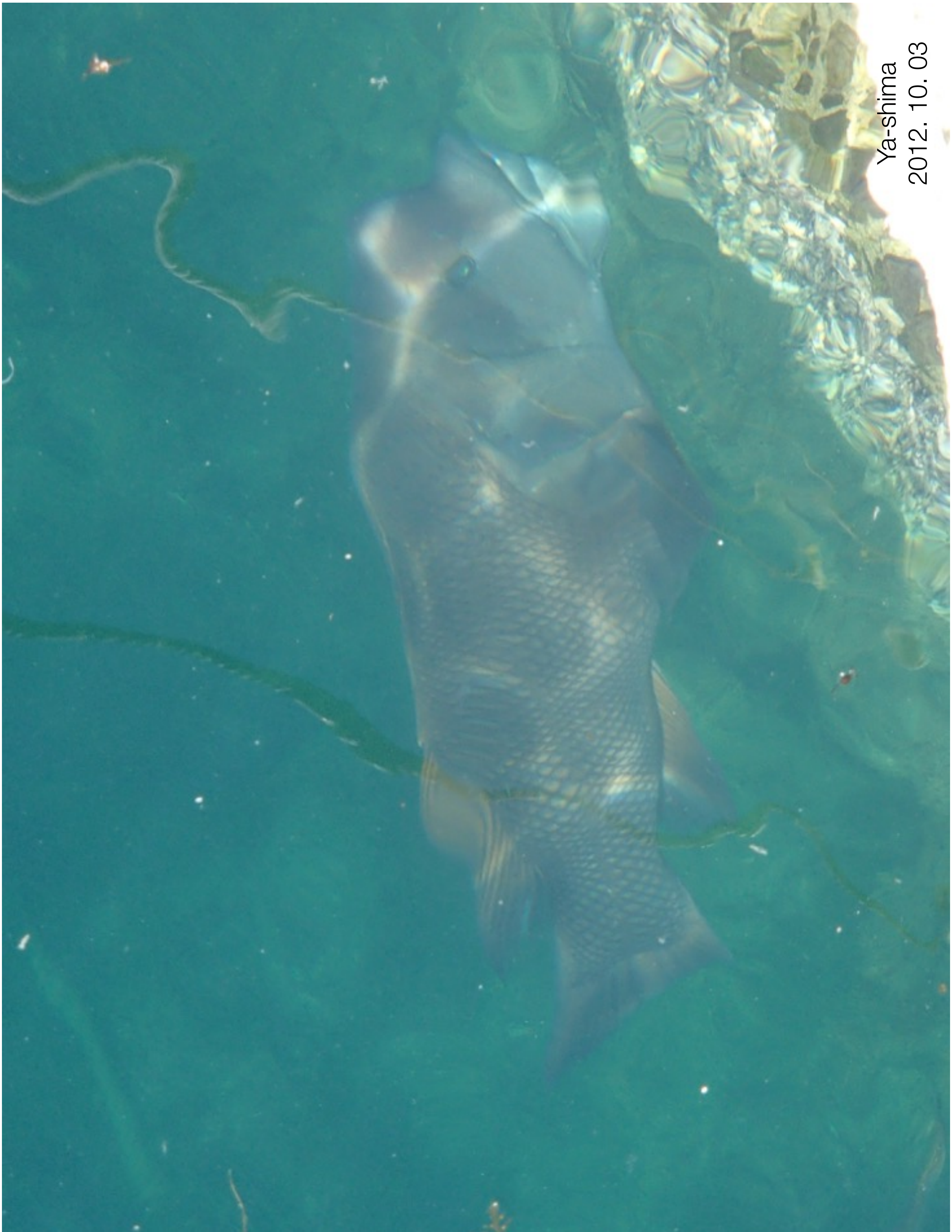

Kurahashi-jima
2007. 04. 20
Depth 7m


Kurahashi-jima
2006. 04. 18
Depth 7m


Ka-shima
2013. 05. 29
Depth 5m


Ka-shima
2012. 06. 14
Depth 5m

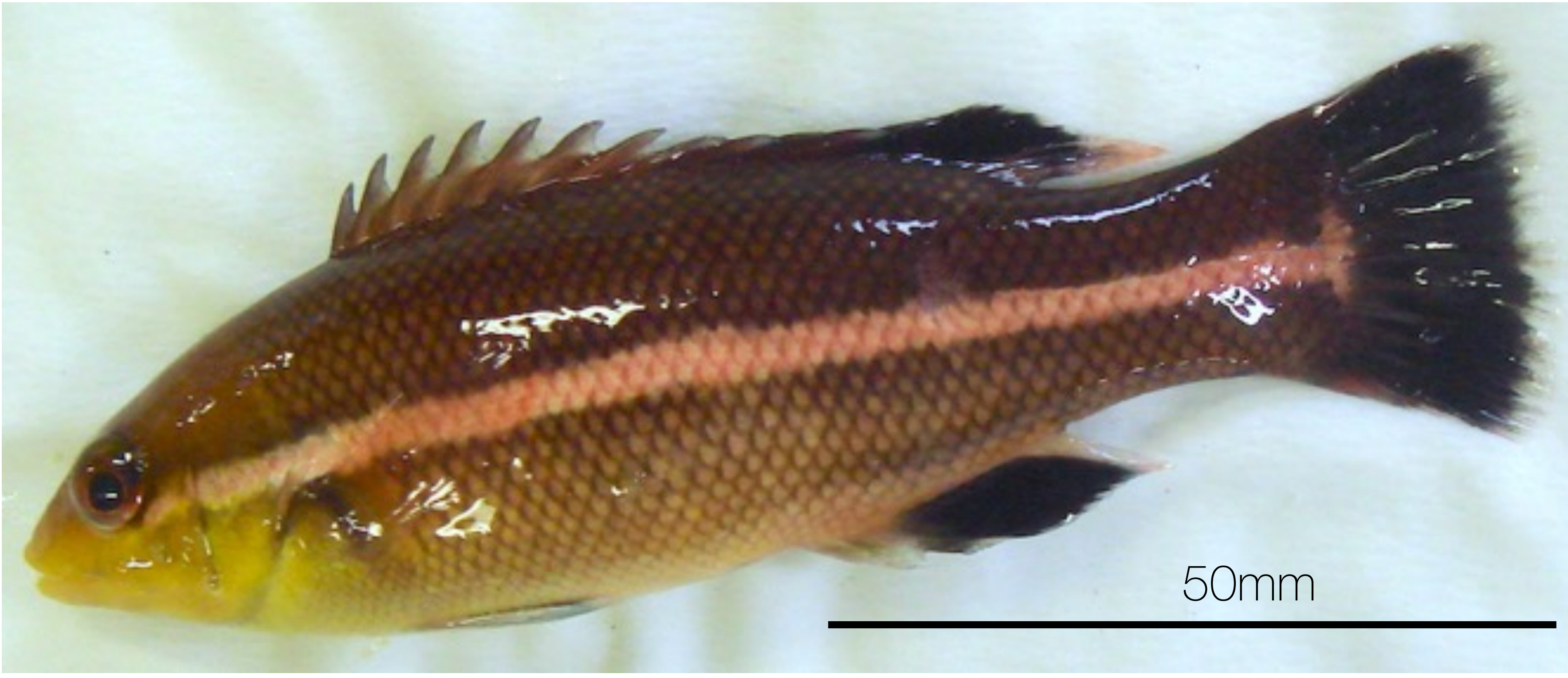

Eta-jima
2015. 07. 25
Depth 2m


Ya-shima
2012. 10. 03

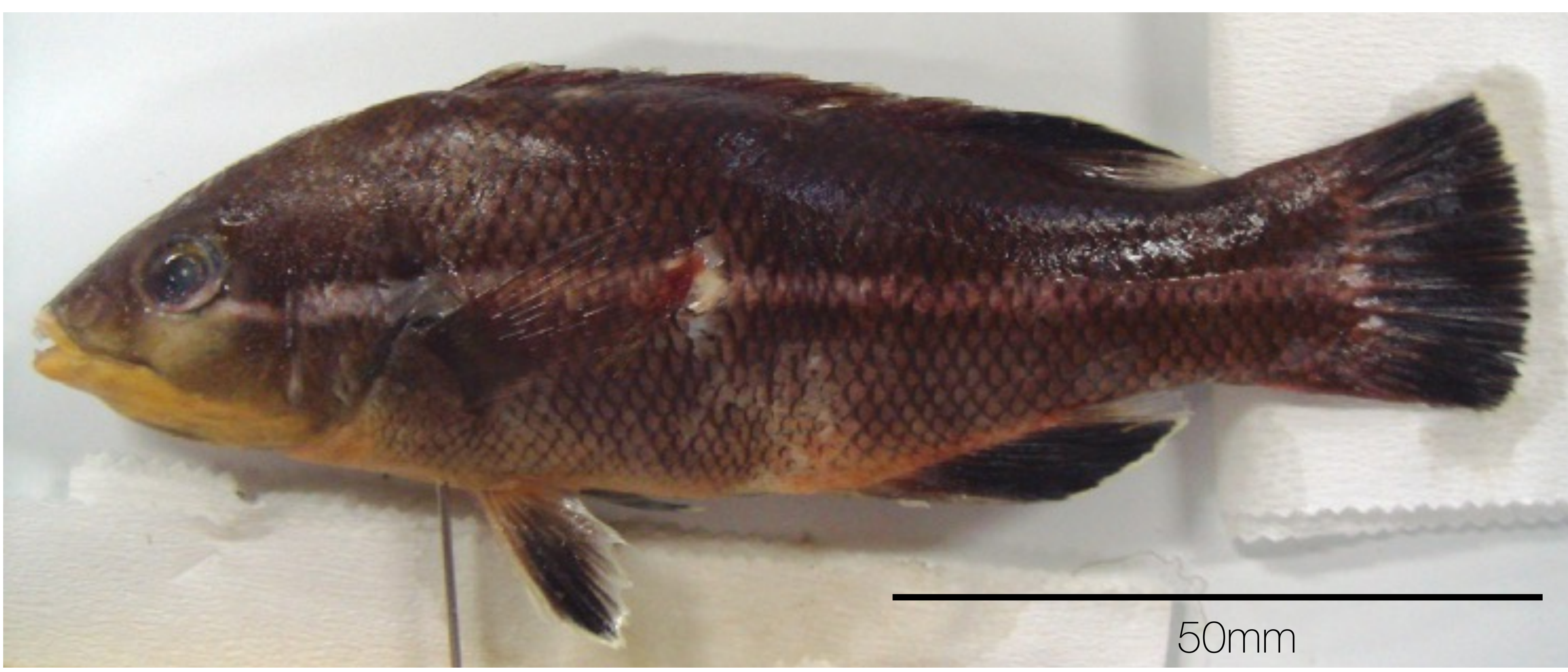
Pictures of sample fishes

SL = 36.0 mm
Juvenile
Ikuno-jima
2007. 08. 13

SL = 37.0 mm
Juvenile
Ikuno-jima
2007. 08. 13



SL = 95.8 mm
Juvenile
Kurahashi-jima
2007. 04. 20



SL = 117.2 mm
Juvenile
Takehara
2006. 08. 27

SL = 477.1.0 mm
Female
Suou-oh-shima
2015. 04. 23

SL = 563.2 mm
Female
Suou-oh-shima
2015. 04. 23

SL = 452.0 mm
Male
Imabari fish market
2005. 04. 14

SL = 580.0 mm
Female
Suou-oh-shima
2015. 04. 23

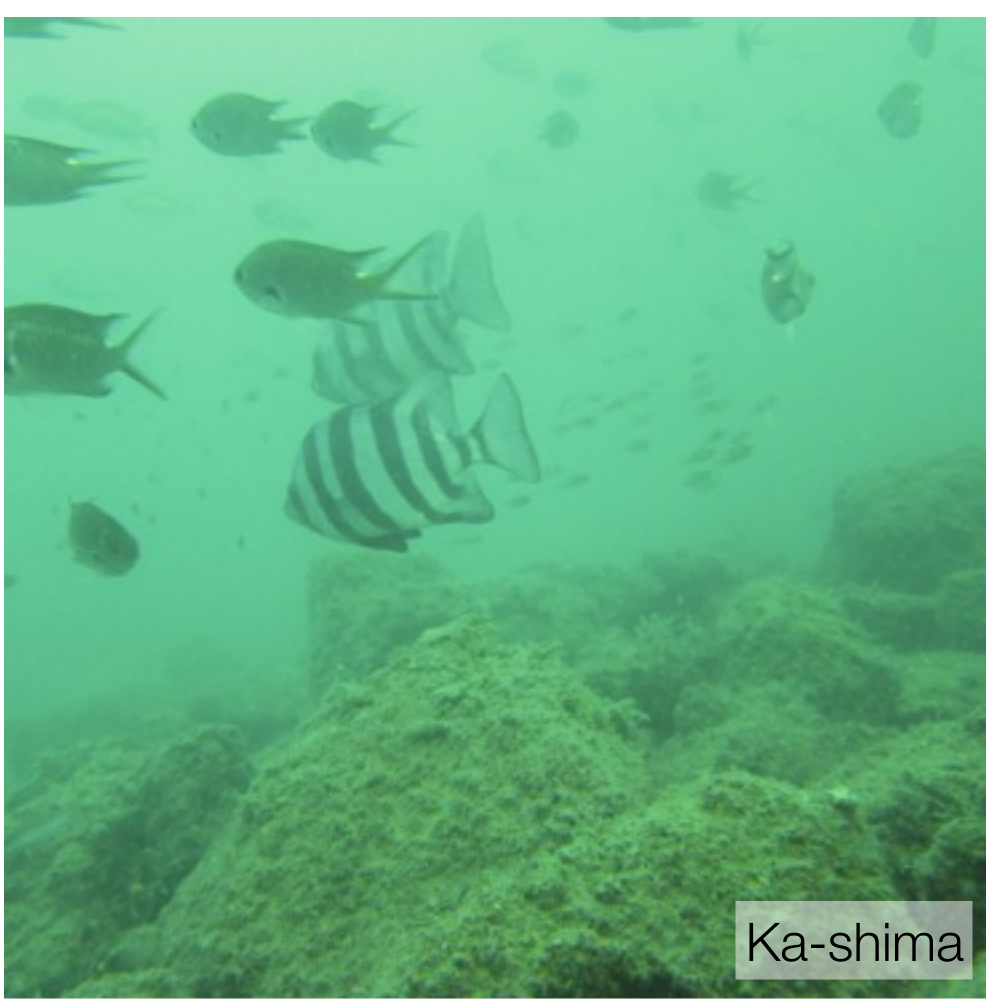
SL = 570.5 mm
Male
Suou-oh-shima
2015. 04. 23

SL = 591.0 mm
Male
Suou-oh-shima
2005. 06. 16

Asian sheephead wrasses landed on fish markets

Pictures of sampling sites

Underwater circumstances



Natural coasts

Natural coasts

Coasts with artificial structures