Doctoral Thesis

Evaluation of function of vegetated habitats as fish nursery: production and growth-survival mechanisms of juvenile black rockfish *Sebastes cheni* in a macroalgal bed

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Abstract

The economic value of ecosystem services of vegetated habitats such as seagrass and macroalgal beds have been estimated to be one of the highest among those of the world's ecosystems. Most of the economic value of ecosystem services of vegetated habitats is composed of supporting services (e.g. nutrient cycling). Provisioning services (e.g. production of fisheries resources) has not been included in the estimated value although vegetated habitats have been recognized as important nursery for a variety of fish species. Estimation of fish production (nursery function) of vegetated habitats based on quantitative samplings is an urgent issue for the future conservation and management of the vegetated habitats. About 100 species of genus *Sebastes* are distributed in the North Pacific and are important fisheries resources in each area. They have strong association with substrates such as seagrass, macroalgae and rocky reef during the juvenile period for a few months. In order to evaluate how the vegetated habitats contribute as fish nursery, production and growth-survival mechanisms of a substrate-associated fish, the black rockfish *Sebastes cheni* (Scorpaenidae), were examined in a macroalgal bed (*Sargassum* spp.) in the central Seto Inland Sea, Japan. *Sebastes cheni* is one of the three species (*S. inermis*, *S. ventricosus* and *S. cheni*) that were recently reclassified from single species (*S. inermis*) by Kai & Nakabo (2008). Ecological information has been rarely accumulated although they are popular species for coastal fishing. Monthly survey was conducted to examine seasonal changes in a fish community structure in the macroalgal bed. The timing of first daily ring deposition (extrusion mark) and daily periodicity of ring formation on otoliths were validated by the use of laboratory-cultured *S. cheni* larvae and juveniles. Otolith microstructures were applied for estimation of their mortality rate, analysis of growth-related survival mechanism and estimation of annual *S. cheni* juvenile production and its economic value in the macroalgal bed. Effect of seasonal change in habitat complexity of the macroalgal bed on cohort-specific mortality rate of post-settlement (20-60 mm in total length: TL) juvenile *S. cheni* was examined. Relationship between the cohort-specific mortality rate and growth-related survival mechanisms of the juvenile was analyzed. Seasonal changes in fish assemblages based on the quantitative fish samplings revealed that juvenile *S. cheni* dominated the fish community in the macroalgal bed from March to May. Otolith daily ring was confirmed to be deposited on both sagitta and lapillus at the birth. Annual juvenile production and its economic value of were estimated to be 13,080-18,360 g ha^{-1} yr^{-1} and 654,000-918,000 JPY ha^{-1} yr^{-1} based on the field survey in 2007 and 2008. Cohort-specific mortality rate of the post-settlement juvenile was significantly higher in the later cohorts that underwent low habitat complexity due to decrease in macroalgal coverage in the later season although cohort-specific growth rate was significantly higher in the later cohorts due to higher temperature. Strong selection for fast-growing juveniles occurred in a cohort with high mortality that underwent low habitat complexity. In the present Ph D thesis, based on the field survey and laboratory experiments, I evaluated annual production and its economic value of juvenile *S. cheni* which dominates the fish community of macroalgal bed in temperate coastal waters of the North Pacific. The variability in habitat complexity affected survival and production mechanisms of juvenile *S. cheni*. The earlier cohorts with low mortality rate were suggested to have highly contributed to *S. cheni* production in this habitat. The total economic value of the ecosystem services of vegetated habitats is suggested to be higher by at least 40-58 % than that estimated in the previous study when the fish production estimated in the present study is included.

General introduction

Ecosystem services (the benefits human populations directly or indirectly derive from ecosystem functions) of vegetated habitats such as seagrass and macroalgal beds were estimated one of the most valuable among world's ecosystem (19,004 USD ha^{-1} yr^{-1}; Costanza et al. 1997). The estimated value of vegetated habitats was mostly composed by supporting services such as nutrient cycling, while provisioning services (e.g. food production) was not included in the value (Costanza et al. 1997). Lack of precise information on production and nursery function based on quantitative investigations on vegetated habitats (Beck et al. 2001) has been a source of difficulty in evaluation of comprehensive ecosystem services although these habitats have been recognized as important nursery for a variety of marine organisms (Fuse 1962*a*, *b*; Kikuchi 1974). Vegetated habitats have been drastically decreasing in coastal areas of the world for the last several decades (Hemminga and Duarte 2000). Quantitative evaluations of fish production and nursery function of vegetated habitats would are indispensable for worldwide recovery from the human disturbance and future conservation and management of coastal habitats (Hemminga and Duarte 2000; Duarte 2002).

Many fish species utilize vegetated habitats as elementary habitat in their early lives (Gillanders 2006). During early life period, fishes experience highest mortality due to a variety of biological and physical sources such as predation, starvation, transportation (Houde 1987). Predation is considered as one of the most important sources of mortality because it prevails all early life stages from egg through juvenile (Houde 1987). Previous studies in vegetated habitats have tested whether habitat complexity reduce vulnerability (Rooker et al. 1998; Anderson 2001; Johnson 2006*b*; Shoji et al. 2007). However, most of these previous studies have focused on a 'snap-shot' phenomenon, fish survival during a relatively short period (in most cases, < 24 hours) during their field and laboratory observations. Information on how fluctuations in environmental conditions affects fish early survival and production is still very limited in natural habitats such as vegetated habitats, mangroves, rocky reef and so on (Valentine-Rose et al. 2007; Faunce and Serafy 2008; Grol et al. 2011; Kamimura et al. 2011).

Rockfishes (genus *Sebastes*) consist of more than 100 species and are mainly distributed in coastal waters in the North Pacific (Love et al. 2002). They are commercially and recreationally important species in many countries and regions of the world. *Sebastes* larvae are extruded from adult female and usually experience a few months of pelagic period. Early juveniles associate with substrates such as vegetated habitats, rocky reef, drifting seaweed, artificial structure and so on for a few months to a year (Kokita and Omori 1998; Love et al. 2002). Clarifying the survival and mortality processes of the substrate-associated period are considered to be essential for forecasting fluctuation and sustainable management of adult stocks since recruitment success is potentially to be determined by the end of the substrate-associated period (Mason 1998; Hobson et al. 2001; Laidig et al. 2007).

Among the black rockfishes, which include several *Sebastes* species such as *S. melanops*, *S. shlegeli* and *S. inermis*, *S. inermis* is widely distributed in the coastal waters of the western North Pacific. Ecological information on the early life history of the black rockfish such as larval seasonal occurrence, distribution, settlement in vegetated habitats and post-settlement growth has been accumulated in previous studies (Harada 1962; Nagasawa et al. 2000; Plaza et al. 2002; Pasten et al. 2003; Guido et al. 2004; Mizusawa et al. 2004; Plaza et al. 2010). However, a recent taxonomic review based on morphological and genetical analyses of *Sebastes* species found the former *S. inermis* included three congeners, *S. inermis*, *S. ventricosus* and *S. cheni* (Kai and Nakabo 2008). Accumulating biological and ecological information on each species is an urgent subject indispensable for understanding the mechanism of recruitment fluctuation and sustainable use of the stock as fisheries resources.

The goal of the present thesis is to quantifying the function of a macroalgal bed as fish nursery based on fish production. In chapter 1, seasonal change of fish community structure was investigated in a mixed vegetated habitat of seagrass

(*Zostera marina*) and macroalgae (*Sargassum* spp.) in the central Seto Inland Sea, Japan, in order to clarify how *Sebastes* fishes utilize vegetated habitats. In chapter 2, validation of daily periodicity of otolith daily ring formation and timing of first increment formation were conducted in order to compare larval and juvenile growth rates between wild and cultured *S. cheni*. Annual fish production of the macroalgal bed and contribution of prey sources to the fish production were evaluated in chapter 3. Effect of seasonal change in habitat complexity of the macroalgal bed on cohort-specific mortality rate of post-settlement (20-60 mm in total length: TL) juvenile *S. cheni* was examined in chapter 4. In chapter 5, relationship between the cohort-specific mortality rate and growth-related survival mechanism of the juvenile was analyzed.

Chapter 1

Seasonal changes in the fish assemblage in a mixed vegetated habitat of seagrass and macroalgae

Introduction

Vegetated habitats such as seagrass (mainly *Zostera* spp.) and macroalgal (mainly *Sargassum* spp.) beds in estuarine and coastal waters have been referred to as nurseries for fish as they serve feeding grounds and refuges from predation for early life stages of fish (Adams 1976; Sogard 1992). Although the role of these coastal habitats as nurseries is an established ecological concept commonly accepted, the nursery-role concept has not been clearly defined. Recently, the ecological processes operating in nursery habitats have been suggested to support greater contributions to adult recruitment as compared with other habitats (Beck et al. 2001). For example, juvenile fish production, but not abundance of juvenile fish, is a good index of the nursery function. However, information on fish production has been restricted while there are many observations on fish abundance and its spatio-temporal variability in the coastal habitats (Houde 1997; Rooker et al. 1999; Shoji and Tanaka 2007; Sano et al. 2008). Quantitative data on vital rates such as fish biomass, growth, mortality and resulting production would strongly contribute to the evaluation of economic value of these vegetated habitats (Costanza et al. 1997).

Many observations on fish assemblages in the field have reported higher abundance and/or diversity of fish assemblages in vegetated habitats than those in adjacent habitats such as sandy shore and mud flat areas (Orth and Heck 1980; Sogard 1992; Mori 1995; Horinouchi and Sano 1999). However, most of these data have been limited to only a list of fish species, and have not included estimations of fish biomass or production. Furthermore, surveys using SCUBA techniques may lack accuracy in data measurements based on visual observations. For example, data on fish size, biomass and growth obtained from the diving observations can be less accurate especially for fish with small size and/or in the turbid waters in coastal and estuarine habitats. Since the growth rate is the highest during young stages in the life (Houde 1987), estimation of larval and juvenile fish production would contribute to accurate evaluations of the importance of vegetated habitats as fish nurseries to the ecosystem.

In many previous surveys, seasonal changes in habitat vegetation have been reported to affect abundance and diversity of fish assemblages. In seagrass beds, fish fauna is more abundant and diverse during summer when seagrass is abundant, while less abundant and diverse in winter when the seagrass disappears (Fuse 1962*a*; Azuma 1981; Mori 1995). In macroalgal beds, on the other hand, there is only limited data available on fish assemblages. Several dominant fish species such as *Sebastes* spp. and *Hexagrammos* spp. have been reported to spawn in winter and inhabit macroalgal beds during early life stages from winter to summer (Fuse 1962*b*). Therefore, fish production in macroalgal beds is expected to be relatively high from winter to summer, in contrast to the seasonal change in seagrass beds where fish production is low during winter.

The present study is part of a series of studies to quantify the role of seagrass and macroalgal beds as fish nurseries based on the fish production. In the present study, seasonal changes in fish assemblages and early growth of the dominant species in a mixed vegetation area (seagrass and macroalgae) are investigated. Since the seasonal peaks in vegetation biomass differ between the seagrass and macroalgal beds, a habitat with mixed vegetation of seagrass and macroalgae may serve as fish nursery for a longer period in a year compared with the habitats covered with either of seagrass or macroalgae. Therefore, annual fish production can be also expected to be higher in the mixed vegetation than areas with either seagrass or macroalgal beds only. Information on seasonal changes in abundance and diversity of fish assemblages in the mixed vegetation area would be helpful for future conservation and optimization of resources usage for coastal habitat. In the present study, 1) seasonal changes in fish abundance, biomass and species composition were investigated, and 2) based on the occurrence and growing season of young stages, dominant fish species were categorized into types.

Materials and Methods

Sampling field

Monthly fish sampling and environmental survey were conducted on a vegetated area (about 50 m in width, 500 m in length) off the southwestern coast of Aba Island, central Seto Inland Sea, Japan (Fig. 1), from August 2006 to July 2007. Aba Island is an uninhabited island with a coast of about 2 km on its southwestern side. The vegetation is dominated by patchness of seagrass *Zostera marina* during summer and macroalgae (mostly, *Sargassum filicinum, S.fusiformis, S. patens, and S. piluliferum*) during winter (Fig. 2). Mean density of seagrass fluctuates between 20-160 shoots m^{-2} and mean biomass (wet weight) of the macroalgae between 100-3500 g m^{-2} (Kamimura and Shoji, unpublished data). The sea bottom of the vegetation area is comprised primarily of sand with occasional small stones (< 100 mm in diameter), on which the macroalgae grow. Fish were collected using a seine net (2 m in height, 30 m in length and 5 mm in mesh aperture: Fig. 3) during a tidal level between 70-130 cm in daytime, when the edge of the vegetated area was close to the shore. Three sides of a square (10 m in side length) were surrounded using the seine net, with another side facing to the shore (around border of the vegetation during the tidal level of 70-130 cm) and this was carried out at four separate locations randomly selected within the vegetated area. In winter, fish were collected together with the macroalgae and their substrates. All fish collected were preserved in 10% seawater formalin solution. Water temperature and salinity were measured at each sampling.

In the laboratory, fish were identified to the lowest possible taxa according to Nakabo (2002) and were measured in total length (TL, mm) and wet weight (g). The black rockfish *Sebastes inermis* was reclassified into three *Sebastes* species (*S. inermis*, *S. ventricosus* and *S. cheni*) by genetical and morphological differences (Kai and Nakabo 2008). In the present study, these species were identified based on morphological differences (Kai and Nakabo 2008). Mean fish abundance and biomass were expressed as number and wet weight of fish 100 m^{-2}. Species composition (% of each species) in number and wet weight was calculated for each month.

Results

Dominant ichthyofauna

A total of 3,363 fish belonging to more than 42 taxa in 26 families were collected during the study period (Table 1). The numerically dominant species (% in number) were *S. cheni* (45.0%), *Favonigobius gymnauchen* (16.9%), *Hypodytes rubripinnis* (16.2%), *Takifugu niphobles* (4.5%), *Rudarius ercodes* (3.7%) and *Sillago japonica* (3.4%) and the weight-based dominant species (% in weight) were *H. rubripinnis* (42.4%), *S. cheni* (22.6%), *Thamnaconus modestus* (6.7%), *F. gymnauchen* (4.9%), *T. niphobles* (4.3%) and *Hexagrammos agrammus* (3.5%). The majority (98.4% in number) of the fish were smaller than 100 mm in total length.

Seasonal change

The water temperature varied between 11.5 (February) and 27.0˚C (September) and salinity between 30.3 (January) and 33.1 (July: Fig. 4a). The mean (± SD) number of species 100 m^{-2} was lowest in February (3.3 ± 1.9) and highest in June (11.5 ± 2.1: Fig. 4b). Sampling month had a significant effect on the mean number of species (Kruskal-Wallis test, P = 0.0003). The mean fish abundance varied between 7.8 ± 4.5 100 m^{-2} (February) and 196.5 ± 145.7 100 m^{-2} (April), and showed a 23.4-fold annual fluctuation (Fig. 4c). Sampling month had a significant effect on the fish abundance (Kruskal-Wallis test, P = 0.003). *Takifugu niphobles* was numerically most dominant in August, *F. gymnauchen* from September to February, *S. cheni* from March to May and in July, and *H. rubripinnis* in June (Fig. 5). Mean of total fish abundance increased by 20.8-fold from 7.8 100 m^{-2} (February) to 162.0 100 m^{-2} (March) greatly according to the increase in abundance of *S. cheni* (Figs. 4c and 5).

The mean fish biomass varied between 19.0 ± 20.3 g 100 m^{-2} (February) and 441.0 ± 222.8 g 100 m^{-2} (June) and showed a 23.2-fold annual fluctuation (Fig. 4d). Sampling month had a significant effect on the fish biomass (Kruskal-Wallis test, $P = 0.0005$). *Hypodytes rubripinnis* was most dominant in weight in August, from October to April, and June, *T. niphobles* in September, and *S. cheni* in May and July (Fig. 5). Mean total fish biomass increased by 7.6-fold from 19.0 g 100 m^{-2} (February) to 143.9 g 100 m^{-2} (March) greatly according to the increases in biomass of *S. cheni*, *H. rubripinnis* and *T. niphobles* (Figs. 4d and 5).

Occurrence and growth of dominant species

Among the numerically dominant species, *S. cheni* and *H. rubripinnis* were abundant during spring, while *T. niphobles* and *S. japonica* were abundant from late summer to autumn (Fig. 5). *Favonigobius gymnauchen* and *R. ercodes* were collected throughout the year (Table 1 and Fig. 6).

Growth of the young-of-the-year *S. cheni* was most prominent in spring with increase in TL from 25.0 ± 2.2 mm in March to 63.4 ± 3.5 mm in July. Thereafter, the growth of *S. cheni* decreased from July to September (64.3 ± 3.1 mm). Frequency distribution of total length of *F. gymnauchen* was separated into two groups with averages of 21.9 ± 3.5 mm and 55.6 ± 7.7 mm in August. Then mean total length of *F. gymnauchen* increased to 34.3 ± 6.6 mm in November while growth from November to April (38.7 ± 9.4 mm) was negligible. Young-of-the-year *H. rubripinnis* (31.3-36.0 mm) which were collected from October to December accounted for only 0.5% of the total number collected through the year. In contrast, older cohorts (> 50 mm) of *H. rubripinnis* occurred throughout the year. Mean total length of young-of-the-year *T. niphobles* and *S. japonica* increased from 31.0 ± 3.4 mm (August) to 40.0 ± 1.2 mm (October) and from 23.0 ± 8.7 mm (August) to 38.0 mm ($n = 1$, December), respectively. Growth of the *R. ercodes* was not detected from the seasonal change of length-frequency distribution.

Discussion

Seasonal changes in the fish assemblages in the vegetated habitat

Vegetated habitats such as seagrass and macroalgal beds have been reported to support larger numbers of fish species and individuals compared to adjacent un- and less-vegetated habitats (Orth and Heck 1980; Sogard 1992; Mori 1995). Habitat complexity produced by vegetation works as feeding ground (Fuse 1962a, b; Kikuchi 1966; Azuma 1981) and predation refuge for young fish (Rooker et al. 1998; Nakamura and Sano 2004; Shoji et al. 2007). Many field studies have demonstrated that fish abundance and species composition fluctuate with the spatio-temporal variations in the shoot density and blade length in seagrass beds (Azuma 1981; Horinouchi and Sano 1999). However, in the majority of these previous studies, fish data were obtained from counting by visual census and from samples collected during operations of commercial fishermen’s boats (seine and trawl), with which quantitative analysis was difficult especially for fish with small sizes.

In the temperate seagrass belts, fish fauna is most diverse and abundant from spring to summer, when seagrass grows, while fish are least abundant in winter (Azuma 1981; Mori 1995). In the present study, sampling by a seine net with a fine mesh size (5 mm) was conducted through a year in order to obtain quantitative data on fish abundance, biomass and species composition in a mixed vegetation area of seagrass and macroalgae in the central Seto Inland Sea. As a result, it was demonstrated that fish abundance and number of fish species increased from late winter to early spring, the season before seagrass grow but macroalgae were still abundant, in addition to early summer. The analysis on fish species composition revealed that the numerically dominant species (*S. cheni*) differed from weight-based dominant species (*H. rubripinnis*). These two species are considered as important components, which can influence the dynamics of fish abundance and trophic flow in the fish assemblages of the surveyed area.

Function of fish habitats as nurseries can be evaluated by how much production is attained in the habitat (Beck et al. 2001). For example, a habitat with a high fish abundance and/or biomass is not always a good nursery. Another habitat with a high juvenile fish production can be considered to be better nursery than the former even though fish abundance and/or biomass are lower in the latter (Beck et al. 2001). In the present study, *H. rubripinnis* occurred throughout the year and dominated in the fish fauna both in number (rank: 3) and in weight (rank: 1). Previous studies also have reported that *H. rubripinnis* was one of the dominant components of the fish fauna in seagrass and macroalgal habitats (Fuse 1962*a*, *b*; Kikuchi 1966; Azuma 1981). However, the majority of the previous information was obtained from the commercial fishing and underwater visual census, with which quantitative analysis was difficult especially for fish with small sizes. In the present study, the majority of the *H. rubripinnis* collected by a seine net with a fine mesh size off the Aba Island (97% in number) was > 50 mm TL (> 1 year: Mori 1995), indicating that early juveniles of *H. rubripinnis* (< 30 mm) mainly inhabit other habitats such as rocky shores and substrates in the surrounding area. We conclude that the surveyed area contributes as a major habitat for *H. rubripinnis* > 1 year but not as a nursery.

The length frequency distribution showed that young-of-the-year *S. japonica and T. niphobles* occurred and grew in the surveyed area from summer to autumn when seagrass dominate the flora. The high abundance of *F. gymnauchen* < 30 mm in September and increase in the total length of this cohort during the following period (September to January) indicate growth of young-of-the-year fish during autumn and winter. Growth of the *R. ercodes* was not detected from the present study due to the high variability in the total length and small sample in each month. According to the previous observations (Kikuchi 1966; Ishida and Tanaka 1980), *R. ercodes* has been categorized as residential species in seagrass and macroalgal habitats since this species occur almost throughout the life in these habitats. On the other hand, young-of-the-year *S. cheni* settled into the surveyed area in March when seagrass were less abundant and macroalgae were still dominant. Mean total length of the *S. cheni* increased by about 40 mm from March to July. Young-of-the-year *S. cheni* are considered to be most dependent on macroalgae during the post-settlement period among the dominant species in the present study.

Occurrence of S. cheni in the vegetated habitat

There seems to be a spatial difference in the seasonal timing of *Sebastes* spp. settlement into coastal vegetated habitats. In the Sendai Bay, northeastern Japan, young-of-the-year *Sebastes* spp. (possibly including three *Sebastes* species) are abundant from April through July, which corresponds to the growing period of the seagrass (Plaza et al. 2002). Seasonal period of birth and juvenile settlement into the seagrass beds of the *Sebastes* spp. in the Sendai Bay is a few months later than those in the Seto Inland Sea (present study). Mizusawa et al (2004) reported that juveniles of *S. ventricosus* and *S. cheni* collected off Miura Peninsula, central Japan, were extruded mainly in January and February and settled in late March and early April. In the surveyed area off Aba Island, *S. cheni* settle into the vegetated habitats in March when macroalgae are still dominant instead of the seagrass. Results from our recent seine surveys and underwater visual censuses at an interval of one week from late February through late May in 2008 have revealed that *S. cheni* is most dominant (> 80 % in number) among *Sebastes* spp. in the mixed vegetation area off Aba Island (Kamimura et al. 2011). Due to the latitudinal difference in the seasonal timing of the settlement, young-of-the-year *Sebastes* spp. seem to be more associated with seagrass in northern area of Japan (e.g. Sendai Bay) while more associated with macroalgae in the southern area (e.g. Seto Inland Sea).

Predation is the most important source of mortality of fish early life stages (Houde 1987). Vegetation reduces vulnerability of larval and juvenile fish to piscivorous fish predators through serving as physical and/or visual barriers and limiting the ability of predators to pursue and capture prey (Rooker et al. 1998; Shoji et al. 2007). In the Seto Inland Sea, it is likely that macroalgae is more important as a predation refuge for the young-of-the-year *S. cheni* during the post-settlement period, when seagrass is not abundant yet. Thereafter, the relative importance of seagrass as predation refuge would increase as the macroalgae become less dominant. We suggest that co-existence of these two different types of vegetation, macroalgae and seagrass, within a small spatial scale can increase the accumulative survival rate of young-of-the-year *S. cheni* from the post-settlement period (March) through summer in the Seto Inland Sea.

Many trials for the development and recovery of vegetated habitats have been conducted in order to improve fish and shellfish production in the coastal waters of Japan (Terawaki et al. 2000). However, seagrass or macroalgal (in most cases, *Sargassum* spp.) beds are individually developed in most of the trials. Results of the present study would give quantitative evidence on the development of a fish habitat with mixed vegetations of seagrass and macroalgae that can retain a higher fish production. Quantitative data on a variety of vegetation conditions (e.g. macroalgae only and no vegetation: sandy beach) are needed for further evaluation and comparative studies of fish production in vegetated habitats.

Chapter 2

Validation of daily periodicity of otolith increment formation and application for growth analysis of wild *Sebastes cheni*

Introduction

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

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

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

Materials and Methods

Validation of periodicity of otolith increments formation of S. cheni

Validation of periodicity of otolith increment formation was conducted by the use of another lot that was naturally extruded in a 200 t tank with several adult fishes on 3 January 2009. Larvae were fed with rotifer and brine shrimp in a 200 t tank at natural temperature (10.5-12.0ºC). Ten fish were sampled from the tank on 10, 20 and 30 days after the extrusion and were preserved in 90% ethanol. The right-side saggita and lapillus were removed from each fish under a dissecting microscope and dried, then embedded in epoxy resin on a slide grass. Each otolith was grounded by 2000-10000 grid lapping films until the nucleus was clearly visible. Otolith rings were counted from the extrusion check (Plaza et al. 2001) to the edge at 400-1000 x magnification under a light microscope. Otolith ring counting was conducted three times and the mean values of the three counts were used as data.

Growth of cultured fish

Rearing experiments using cultured *S. cheni* juveniles (mean total length (TL) = 26.2 mm, *n* = 40) were conducted at Miyako Station, Tohoku National Fisheries Research Institute. Larvae were naturally extruded from an adult female (TL = 275 mm) landed on 18 February 2010 at Miyako Fishermans's Association, Iwate Prefecture. Then larvae were introduced into 1000 *l* black polycarbonate tanks on

6 May 2010 and were maintained at six temperatures (10, 12, 14, 16, 18 and 20 ºC) under a 10: 14 (light: dark) photoperiod at a fish density about 1.0 l^{-1}. Each tank was provided with aeration and fish were fed with rotifer and brine shrimp until satiation four times a day. Twenty fish were sampled from each tank every seven days for three weeks and 40 fish at the end of the experiment (four weeks from the start) and were preserved in 10% seawater formalin solution, then TL was measured.

Field sampling

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

Otolith analysis of wild fish

Growth analysis for wild *S. cheni* was carried out using the otolith daily ring measurement system (Ratoc System Engineering). In the present study, lapillus was used for the growth analysis due to higher visibility of formation of both sagittae and lapilli were validated. A measurement transect was set from the nucleus along the maximum radius. Number of increments on the lapillus was used as age (days after extrusion. Measurement of increment width was conducted from the nucleus to edge. A linear model was fitted to the relationship between otolith radius and TL. In addition, otolith of 30 cultured larvae (7.3-11.7 mm TL) and 46 larvae and small juveniles used for the validation of daily periodicity of increments formation were included in the analysis to establish the regression through the larval and juvenile stages (*n* = 103, including 27 wild juveniles). The biological intercept method (Campana 1990) was applied in order to estimate TL at age of the wild *S. cheni* following the equation:

$$L_a = L_c + (R_a - R_c)(L_c - L_e)(R_c - R_e)^{-1}$$

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

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

Results

Daily periodicity of otolith increments formation

The relationships between the number of increments on sagittae (I_s) or lapilli (I_l) and age (A_c, day) of cultured *S. cheni* were expressed by linear formulas as follows (Fig. 7):

Sagittae: $I_s = 0.975 \times A_c + 0.467$ ($n = 30$, $r^2 = 0.988$, $P < 0.001$)

Lapilli: $I_l = 0.950 \times A_c + 0.6$ ($n = 30$, $r^2 = 0.987$, $P < 0.001$)

The slopes of both regressions were not significantly different from 1.0 and intercept not significant from 0 (ANCOVA, $P < 0.05$).

Growth of wild and cultured S. cheni juveniles

Mean (± SD) and range of TL of wild *S. cheni* used for the growth analysis were 52.2 (± 4.8) mm, 36.1-61.7 mm and those of extrusion date were 2 January

2009 (± 9.1 d) and 20 December 2008-21 January 2009. The relationship between otolith radius (R) and TL (L), which was applied for the back-calculation of wild *S. cheni*, was expressed by a following linear regression:

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

Mean (± SD) back-calculated TL at age of wild fish increased from 26.2 to 36.8 ± 1.3 mm during four weeks, the period for the comparison of growth rate between wild and cultured fishes. Mean (± SD) daily growth rates of fish during the four weeks were 0.38 ± 0.08 mm day^{-1} (Fig. 8). Mean daily temperature which each wild fish were expected to have been experienced during the four weeks, estimated by the use of temperature of the observation buoy, was 10.8 ºC.

Mean (± SD) TLs of the cultured fish increased from 26.1 ± 1.6 to 30.7 ± 1.7 mm (10 ºC), 31.8 ± 2.1 mm (12 ºC), 32.9 ± 3.7 mm (14 ºC), 34.5 ± 4.9 mm (16 ºC), 35.2 ± 5.6 mm (18 ºC) and 36.6 ± 6.2 mm (20 ºC) for four weeks. Mean daily growth rates during the period were 0.16 ± 0.06 (10 ºC), 0.20 ± 0.07 (12 ºC), 0.24 ± 0.13 (14 ºC), 0.30 ± 0.17 (16 ºC), 0.32 ± 0.20 (18 ºC) and 0.37 ± 0.22 mm day^{-1} (20 ºC) (Fig. 8). The effect of temperature on the mean growth rate of cultured fish was significant (ANOVA followed by Tukey's test for multiple comparison, $P < 0.0001$). There was a significant positive correlation between the mean growth rate (G, mm d^{-1}) of cultured fish and temperature (T, ºC) tested in the present study (10-20ºC) as follows:

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

Discussion

Daily periodicity of otolith increments formation and early growth of S. cheni

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

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

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

Growth comparison between wild and cultured S. cheni juveniles

Generally, growth rates obtained from laboratory experiments need to be interpreted carefully since there are a variety of artificial biases in environmental conditions such as tank size, feeding condition and handling, which wild fishes do not experience in nature. In the present study, since the cultured fish were fed with invertebrate zooplankton prey until satiation four times per day, it is plausible that prey availability in the tank was not a restricting factor for their growth rate. Mean daily growth rate of wild fish (0.38 mm day^{-1} at 10.8 ºC) was about double of that of cultured fish under similar temperatures (0.16 and 0.20 mm day^{-1} at 10 and

12ºC). In addition, standard deviation of the mean growth rate of wild fishes (0.38 ± 0.08 mm day^{-1} at 10.8 ºC) was smaller than those of cultured fishes (0.32 ± 0.20 mm day^{-1} at 18 ºC, 0.37 ± 0.22 mm day^{-1} at 20 ºC) which had a similar growth rate as wild fishes. Variability in body size tends to be more prominent under laboratory conditions compared to wild cohorts since there is no size-selective mortality (mostly due to predation: Houde 1987) in captive tanks. In nature, on the other hand, size-selective predation is considered as an important factor which affects length frequency distribution of the survivors of larvae and juveniles in many fish species (Meekan and Fortier 1996, Takasuka et al. 2003; Takahashi and Watanabe 2004; Shoji and Tanaka 2006; Plaza and Ishida 2008; Islam et al. 2010). Although seagrass beds are considered as important refuges from predators, recent field sampling and analysis of stomach contents of predators revealed piscivorous fish predator biomass increased at nighttime (Kinoshita et al. 2012). The higher growth rate at the same temperature and smaller deviation at the same growth rate of wild fish compared to those of cultured fish might reflect size-selective mortality in nature. Further analysis on environmental conditions and size- and growth-selective survival in nature (such as repeated sampling to capture the same cohort coupled with growth back-calculations using otolith daily increments) are needed to understand the mechanisms of survival processes of *S. cheni*.

Chapter 3

Production and food resources of juvenile *Sebastes cheni* in a vegetate habitats: estimation of economic value of a nursery

Introduction

The economic value of ecosystem services of vegetated habitats such as seagrass and macroalgal beds in coastal waters (19,004 USD ha^{-1} yr^{-1}), is approximately the same as that of estuaries (22,832 USD ha^{-1} yr^{-1}), and are estimated to be among the highest of the world's ecosystem services that include marine (open ocean, coral reefs and shelf waters') and terrestrial ecosystems (tropical forest, grasslands and rivers: Costanza et al. 1997). However, there are still many ecosystem services unevaluated although they are important components in relation to the economic value in each ecosystem. Especially, information on the production of fishes, which are important components of the provisioning services (Costanza et al. 1997), has been unavailable mainly because of the difficulty of quantification in the field. Estimation of the value of the provisioning services in highly productive shallow water areas is indispensable for a comprehensive estimation of the ecosystem services and for conservation and management of these ecosystems in the future.

Vegetated habitats (seagrass and macroalgal beds) in estuarine and coastal waters have been considered to highly contribute as fish nurseries since they have a variety of ecological functions such as feeding grounds and predation refuges for fish early life stages (Fuse 1962*a*, *b*; Adams 1976; Azuma 1981). To date, many studies have reported that vegetated habitats support larger numbers of fish compared to adjacent unvegetated habitats (Kikuchi 1966; Sogard 1992). Recent studies have suggested that quantitative analyses on growth, survival and movement (recruitment to the fishery) of young fish, as well as their density, are important components for accurate estimation of fish production in vegetated habitats (e.g. Beck et al. 2001).

Many efforts have been made by researchers on studies of species composition and diet of fish assemblages in vegetated habitats by use of underwater census and quantitative sampling gears such as scoop nets and traps, while information on quantitative fluctuations in number or biomass of fish cohorts and populations is limited (Secor and Houde 1995; Rooker et al. 1999; Shoji and Tanaka 2007; Faunce and Serafy 2008). In coastal and estuarine habitats, underwater census in conditions with much vegetation and high turbidity tend to lead to uncertainty in estimation of fish size, number and biomass measurements. Under such conditions, for example, underestimation is likely for small-sized fish. Since fish growth and production rates are highest during their early life (Fuiman and Werner 2002), accurate information on abundance, biomass and growth are indispensable for quantitative estimation of fish production in the vegetated habitats (Beck et al. 2001).

Rockfishes (genus *Sebastes*: Scorpaenidae) are widely distributed and are one of the major components of fish production in temperate and sub-arctic coastal waters of the North Pacific (Love et al 2002). Black rockfishes, *Sebastes inermis*, *S. ventricosus* and *S. cheni* are abundant and are commercially and recreationally important fishery resources in eastern Asia. Larvae and juveniles of these species dominate seagrass and macroalgal beds from late winter through summer (Fuse 1981; Nagasawa et al. 2000; Plaza et al. 2002). However, these three species had been dealt as one single species (*S. inermis*) in previous studies and fishery statistics before a taxonomic review on these species was made (Kai & Nakabo 2008). Ecological information on each species is indispensable for effective fishery management and conservation of stocks since ecological features and recruitment mechanisms potentially differ among the three species and require independent clarification.

In the central Seto Inland Sea, Japan, *S. cheni* larvae and juveniles dominate seagrass and macroalgal beds of the coastal waters from winter through summer (Kamimura and Shoji 2009). Early juveniles (ca. 20 mm in total length: TL) of *S. cheni* settle from offshore waters into mixed vegetation areas of seagrass (*Zostera* *marina*) and macroalgae (*Sargassum* spp.) from late February to March and grow to about 50 mm TL by May (Kamimura and Shoji 2009). After the vegetation-associated period, they migrate to other habitats with rocky bottoms in deeper waters in summer depending on the water temperature in the shallow waters (Boehlert and Yoklavich 1983; Love et al. 1991). Quantitative information on the abundance, biomass and growth of larval and juvenile *S. cheni* would lead to a better understanding of the processes and determinant factors of juvenile *S. cheni* production in the vegetated habitat. In addition, application of analysis on feeding habits coupled with biochemical analysis of tissues such as stable isotope analysis, which has recently been advanced, would enable us to estimate the relative contribution of prey source to the fish production. Estimation of juvenile *S. cheni* production coupled with trophic flow analysis can contribute to evaluation of two components of the ecosystem services in the vegetated habitat: the provisioning services (production of fisheries resources) and the regulating services (removing organic matter from the ecosystem).

In the present study, cohort-specific dynamics of abundance and biomass of larval and juvenile *S. cheni* were sampled at fine time intervals (one to two weeks) in order to estimate the annual juvenile production in the vegetated habitats by use of otolith microstructure analysis. Cultured juvenile *S. cheni* are sold as seedlings for aquaculture in the Seto Inland Sea and wild juveniles are potentially used for aquaculture (Shoji 2009). Preliminary estimation of the economic value of the annual production of wild *S. cheni* juveniles was conducted by converting the juvenile production and unit price of the cultured *S. cheni* seedlings. Stomach contents analysis coupled with stable isotope analysis were also conducted to detect the prey sources which contribute to the juvenile *S. cheni* production.

Materials and methods

Field sampling

Fish sampling and environmental surveys were conducted on a vegetated area (about 50 m in width, 500 m in length) off the southwestern coast of Aba Island, central Seto Inland Sea, Japan (Fig. 1) and information on the sampling site is detailed in Chapter 1. Fish were collected using a round seine net (2 m in height, 30 m in length and 5 mm in mesh aperture: Kamimura and Shoji 2009) during a tidal level between 50-150 cm in daytime, when the edge of the vegetated area was close to the shore. Three sides of a square (10 m in side length) were surrounded using the seine net, with the other side facing to the shore and this was carried out at four separate locations randomly selected within the vegetated area. Fish samples were preserved in 10% seawater formalin solution and subsamples of *S. cheni* were preserved in 90% ethanol for otolith analysis. Water temperature and salinity were measured at each sampling.

Otolith analysis

In the laboratory, *S. cheni* were sorted and measured for TL and wet weight (g). Mean juvenile abundance and biomass were expressed as the number and wet weight of fish 100 m^{-2}. Right-side lapilus (n = 20 at maximum for each sampling date) was removed from each fish body. Otoliths were embedded in a drop of transparent nail polish on a slide glass and were ground with 2000-10000 grid lapping films until the nucleus was clearly visible. Daily rings (Kamimura et al. 2012) were counted at 400-1000 x magnification using a compound light microscope connected with a monitor. A total of 165 otoliths (2007: n = 72, 2008: n = 93) were analyzed for age estimation. Length-age relationship established for each sampling date was used for estimation of age of the other specimens for which otoliths were not analyzed.

Estimation of annual production and economic value of juvenile S. cheni

Birth dates were used to separate the juveniles into specific cohorts, defined as individuals extruded within a 10-day period following to the previous studies (Secor and Houde 1995; Rooker et al. 1999; Shoji and Tanaka 2007). Each cohort was designated with an alphabetical character from A (12-21 December) to H (20-29 February). Annual production of *S. cheni* juveniles (g 100 m^{-2} yr^{-2}) was calculated by summarizing the maximum biomass of each cohort within a year. In addition, the economic value of the annual juvenile fish production in the vegetated habitat was calculated based on the price of one cultured *S. cheni* juvenile as a seedling for aquaculture (ca. 50-300 JPY g^{-1}: FRA 2008).

Stomach contents and stable isotope analyses

In order to understand the feeding habits during the post-migration period, a total of 30 *S. cheni* juveniles (ten fishes on 24 March and 16 April and five fishes on 1 and 22 May in 2008) were processed for stomach contents analysis. Stomach contents were removed from the fish body and were identified under a dissecting microscope. Composition (% in number) and number of prey organisms per fish was calculated for each juvenile size class of 10 mm TL.

Stable isotope ratios of carbon ($^{12}C/^{13}C$) and nitrogen ($^{15}N/^{14}N$) were measured for 37 juveniles and 1-year-old *S. cheni* (seven, eight, fourteen and eight juveniles for each 10 mm TL class from 20- to 50- mm and an additional two over one-year-old fish from sub-samples: 106.1 and 140.1 mm TL) and their possible prey sources were obtained during the surveys in 2008. Collections of copepods (n = 3), particulate organic matter (POM: n = 4), decapods (n = 6, 11-36 mm in carapace length: CL), and benthic microalgae (n = 2) were carried out. Copepods were collected using a plankton net (250 µm mesh) at 3 separate locations in the survey area. The POM samples were collected from six stations at just below the sea surface prefiltered with a 200 µm mesh to remove macrozooplankton, then filtered onto Whatman GF/C glass-fiber filters. Decapods were collected with the fish samples and benthic microalgae were processed in the same manner as POM. All samples were preserved frozen at -30˚C until further processing for stable isotope analysis; final processing of drying and preparation followed methods obtained by Nagata and Miyajima (2008). Stable isotope ratios of carbon and nitrogen were measured with a mass spectrometer fitted with an elemental analyzer. Isotope ratios are expressed as:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}}-1)\times 1000$$

where δX is the stable isotope ($\delta^{13}C$ or $\delta^{15}N$) in units of ‰, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Atmospheric nitrogen (N_2) and PeeDee belemnite were used as the standards for nitrogen and carbon stable isotopes, respectively. Precision for isotopic analysis was within ± 0.28‰ for both $\delta^{13}C$ and $\delta^{15}N$. Mean stable isotope ratios were estimated for juvenile *S. cheni* by size classes of 10 mm (20-60 mm TL) and > 106 mm TL.

Results

Seasonal changes of environmental conditions and occurrence of S. cheni

Water temperature ranged between 11.4 °C (March 19) and 17.3 °C (May 15) in 2007 and between 10.9 °C (March 11) and 18.2 °C (May 30) in 2008 (Fig. 9a) with higher values almost throughout the sampling season in 2007 than those in 2008. Salinity ranged from 31.9 (April 19) to 33.1 (March 19) in 2007 and from 31.5 (April 3) to 32.8 (February 21, March 11 and May 1) in 2008 (Fig. 9b).

A total of 2,631 and 7,629 juveniles of the three *Sebastes* species (*S. inermis*, *S. ventricosus* and *S. cheni*) were collected in 2007 and 2008, respectively. Among the three species, *S. cheni* was dominant, comprising 77.6% (in number) in 2007 and 80.0% in 2008 of the three species. The mean abundance of *S. cheni* was highest on April 13 (150.8 100 m^{-2}) in 2007 and on March 24 (451.2 100 m^{-2}) in 2008 (Fig. 9c). The maximum mean biomass was 99.2 g 100 m^{-2} (April 13) in 2007 and 115.2 g 100 m^{-2} (April 16) in 2008 (Fig. 9d).

Mean total length of *S. cheni* was between 20 and 30 mm TL in March and reached 50 mm TL in May in both 2007 and 2008 (Fig. 10). Relationships between wet body weight (W, g) and TL (L, mm) of larval and juvenile *S. cheni* were expressed by power regressions (Table 2):

2007: $W = 6.904*10^{-6}*L^{3.164}$ ($n = 85$, $r^2 = 0.991$, $P < 0.0001$)

2008: $W = 3.010*10^{-6}*L^{3.363}$ ($n = 81$, $r^2 = 0.996$, $P < 0.0001$)

Cohort-specific abundance and biomass of S. cheni

Birth date of *S. cheni* ranged between December 18 and February 7 in 2007 and between December 16 and February 23 in 2008 (Fig. 11). The majority (91% in 2007 and 64% in 2008) of fish were extruded in January. The date when the fish biomass was maximum differed among the cohorts and years (Table 3). By summarizing the maximum biomass of each cohort within years, the annual production of juvenile *S. cheni* was estimated as 13,080 g ha^{-1} yr^{-1} in 2007 and 18,360 g ha^{-1} yr^{-1} in 2008. Based on the unit price of cultured *S. cheni* juveniles (50 JPY per juvenile fish = 1.0 g: the lowest value in the literature: FRA 2008), the economic value of annual wild juvenile *S. cheni* production was estimated to be at least 654,000 JPY ha^{-1} yr^{-1} for 2007 and 918,000 JPY ha^{-1} yr^{-1} for 2008.

The relationships between total length (L, mm) and age (A, day) were expressed by a linear regression for each year (Table 2):

2007: $L = 0.440A - 3.401$ ($n = 72$, $r^2 = 0.872$, $P < 0.0001$)

2008: $L = 0.352A + 4.325$ ($n = 93$, $r^2 = 0.895$, $P < 0.0001$)

Stomach contents and stable isotope ratios

Majority of the stomach contents of juvenile *S. cheni* < 60 mm TL was composed of copepods belonging to Calanoida (over 80% was *Paracalanus parvus*), Poecilostomatoida (*Corycaeus affinis*) and Harpacticoida (Fig. 12). Among the copepods identified, calanoid copepods were most dominant through the TL range examined. The mean (± SD) number of food organisms per stomach increased from 38.0 (± 13.0) at 20-30 mm TL class to 689.4 (± 240.5) at 40-50 mm TL class then decreased to 298.4 (± 186.4) at 50-60 mm TL class.

Mean $\delta^{13}C$ of *S. cheni* juveniles at 20-60 mm TL ranged between -21.1‰ and -19.1‰ and $\delta^{15}N$ between 12.2-14.6‰, with $\delta^{13}C$ values close to those of copepods (-20.2‰) and POM (-21.0‰: Fig. 13). Mean $\delta^{13}C$ and $\delta^{15}N$ of over 1-year-old *S. cheni* (> 106 mm TL) was -15.7‰ and -14.5‰, respectively. The mean $\delta^{13}C$ of over 1-year-old *S. cheni* was significantly higher than that of fish <

60 mm TL and was close to that of decapods (-15.1‰).

Discussion

Estimation of the economic value of annual juvenile production

In the present study, seasonal fluctuation in cohort-specific biomass was analyzed in order to estimate the annual production of juvenile *S. cheni*. The annual production of *S. cheni* (13,080-18,360 g in wet weight ha^{-1} yr^{-1}: production of only juveniles) were comparable with the estimates of annual fish production reported for other fish species and ecosystems (12,000-406,820 g in wet weight ha^{-1} yr^{-1}: Valentine-Rose et al. 2007; Faunce and Serafy 2008). The sum of the maximum cohort-specific *S. cheni* biomass was 13,080 g ha^{-1} yr^{-1} for 2007 and 18,360 g ha^{-1} yr^{-1} for 2008. Generally, maximum fish biomass of a single sampling day has been considered as an index of fish production in a habitat. However, such 'snap-shot' information does not represent the fish production since fish samples collected on a single sampling day consist of multiple birth-date cohorts (Beck et al. 2001). Actually, the sum of the cohort-specific-maximum biomasses were 28.1 % (2007) to 37.3% (2008) higher than the maximum of biomass on a single sampling date (9,920 g ha^{-1} yr^{-1}; April 13, 2007 and 11,520 g ha^{-1} yr^{-1}; April 23, 2008, Fig. 9d).

The economic value of the annual fish production (as the provisioning services) in the vegetated habitat off Aba Island would be still higher if values of other fish species were included. Production of the two other *Sebastes* fish (*S. inermis* and *S. ventricosus*) would have added at least 775,800 JPY ha^{-1} yr^{-1} for 2007 and 1,109,400 JPY ha^{-1} yr^{-1} for 2008 to the juvenile *S. cheni* production estimated here. These estimates indicate that the economic value of ecosystem services of vegetated habitats calculated by Costanza et al. (1997), in which the provisioning services of fish production is not included, would increase by 40-58%. Further information on production of other species (e.g. *Hexagrammos* spp. and other *Sebastes* spp.), which are important fishery resources and vegetation-associated would lead to a more comprehensive estimation of the provisioning services of the vegetated area.

Occurrence of juvenile S. cheni in vegetated habitats

S. cheni juveniles were the most dominant among the three *Sebastes* species (*S. inermis*, *S. ventricosus* and *S. cheni*) and their biomass was highest in April in 2007 and 2008 off Aba Island. Both in 2007 and 2008, the majority of *S. cheni* were extruded in January, almost the same period as in Sendai Bay, Miyagi, northern Japan (Plaza et al. 2001) and Miura Peninsula, eastern Japan (Mizusawa et al. 2004), although the three congeners of rockfishes were included altogether for the birth date analyses in these previous papers. Settlement of *S. cheni* into the vegetated habitat was observed at about 20 mm TL in the central Seto Inland Sea (Fig. 10), while it occurred about two weeks later in Sendai Bay (from late March to early April: Plaza et al. 2002). The birth period of *Sebastes* juveniles (peak in January) and size at settlement (ca. 20 mm TL, Plaza et al. 2002) did not differ between the two areas. Mean temperature from December to February in the Seto Inland Sea (11-18 ºC) is approximately 8 ºC higher than that in Sendai Bay (4-9 ºC: http://tnfri.fra.affrc.go.jp/). Recent analysis showed that the larval growth rate of *S. cheni* back-calculated by the use of otolith microstructure of January-birth cohort was about 0.3 mm d^{-1} in the Seto Inland Sea and about 0.2 mm d^{-1} in Sendai Bay and that there was a significant positive correlation between daily growth rates and ambient temperatures (Shoji et al. 2011). The length-age relationships obtained during the larval and early juvenile periods were linear (Shoji et al. 2011; present study). Therefore, it is plausible that the differences in temperature would explain the difference in the seasonal timing of settlement: the higher larval growth rate due to higher temperature would have resulted in the earlier settlement (but at the same TL of 20 mm) of the *S. cheni* juveniles into the vegetated habitat in the Seto Inland Sea.

Feeding habits and prey sources

Analysis of the stomach contents and stable isotopes revealed that juvenile *S. cheni* were highly dependent on copepods during the post-settlement period (20-60 mm TL) in the vegetated habitats. The stable isotope analysis supported the shift in stomach contents composition of young-of-the-year *S. cheni* at about 60 mm TL: mean $\delta^{13}C$ of over 1-year-old *S. cheni* (> 106 mm TL) was higher than those of *S. cheni* at 20-60 mm TL classes and copepods and was close to that of decapods (Fig. 13). A previous study also pointed to the same shift in feeding habits of *Sebastes* fish (Takai et al. 2002) as observed in the present study. However, data from three *Sebastes* species were combined in the previous study (Takai et al. 2002) since the taxonomic review on *Sebastes* fishes (Kai & Nakabo 2008) had not been completed at that time. In the present study, the planktonic trophic pathway originating from phytoplankton is concluded to support the majority of the production of *S. cheni* during the post-settlement period in the seagrass and macroalgal beds through the production of copepod zooplankton off Aba Island, central Seto Inland Sea. Stable isotope analysis demonstrated a shift of feeding habits from the juvenile stage to over 1-year-old *S. cheni*.

Since the developmental changes in feeding habits and prey sources of *S. cheni* was clarified in the present study, further investigation on the daily ration at each developmental stage of *S. cheni* would lead to quantification of the regulating services (e.g. removing organic matter) produced through *S. cheni* production in the vegetated habitats. Successful evaluation of the economic values of the regulating services as well as the provisioning services would show that the economic value of the total ecosystem services of the vegetated habitats would be much higher than those estimated in the previous studies.

Chapter 4

Habitat complexity decreases post-settlement mortality of *Sebastes cheni*

Introduction

Vegetated habitats such as seagrass and macroalgal beds in coastal waters are considered as important ecosystems for many species of fish, molluscs and crustaceans which use them as a habitat (Adams 1976; Heck and Thoman 1984; Haywood et al. 1995). The economic values of ecosystem services provided by these vegetated habitats were estimated to be among one of the highest when both marine (open ocean, coral reefs and shelf waters) and terrestrial ecosystems (tropical forest, grasslands, and rivers) were considered (Costanza et al. 1997). To date, most of the economic values estimated for the vegetated habitats have been composed mostly of the supporting services (e.g. nutrient cycling), while provisioning services (e.g. production of fishes, the major components of the animal community) of them have been hardly quantified because of difficulty in quantification (Costanza et al. 1997). Many fish species migrate from one habitat to another with growth, development and/or change of season. Therefore, it is indispensable to understand the pattern of habitat use of dominant fish species and quantify their production in order to comprehensively evaluate the ecosystem services of coastal habitats (Beck et al. 2001).

Plenty of studies on abundance, growth and survival mechanisms of fish early life stages have been conducted in seagrass beds (e.g. Heck et al. 2003). In macroalgal beds (such as kelp forests *Sargassum* spp. etc.), on the other hand, there has been far less information on the mortality and survival mechanisms (Forrester and Steele 2000; Anderson 2001; Johnson 2006*a*, *b*) compared to that on abundance and growth of fish early life stages (Holbrook et al. 1990; Ornellas and Coutinho 1998; Hixon and Jones 2005; Aburto-Oropeza et al. 2007; Kamimura et al. 2011). In temperate waters of the western North Pacific, seagrass beds grow from spring to summer and macroalgal beds from winter to spring (Mukai 1971; Yoshida 2005) with increase in abundance and/or biomass of dominant species of each habitat (eg. *Zostera marina* for seagrass beds and *Sargassum* spp. for macroalgal beds: Fuse 1962*a*, *b*) according to seasonal changes in water temperature. Since both of the seagrass and macroalgal beds have been referred to serve as important habitats for a variety of fish species in the temperate waters, accumulating information on fish mortality and survival mechanisms are indispensable for comprehensive understanding of the contribution of these habitats.

The black rockfish *Sebastes cheni* is widely distributed in coastal waters of the western North Pacific (Kai and Nakabo 2008). They dominate the fish community of the vegetated habitats (seagrass and macroalgal beds) in coastal waters and are commercially and recreationally important fishery resources (Fuse 1962*a*, *b*; Kamimura and Shoji 2009). Larvae are extruded in December to February in offshore areas and settle into vegetated habitats at about 20 mm in total length, then grow up to 60 mm in late spring (Kamimura et al. 2011). Juvenile *S. cheni* exhibit strong association with macroalgae during the post-settlement period as juveniles of other *Sebastes* species do with substrates such as seagrass beds, kelp and rocky reef until 60 mm (Love et al. 1991, 2002; Plaza et al. 2001). Moreover, recent surveys indicated that larval and juvenile survival during the substrate-associated period has a potential to determine the abundance of regional stocks of yellowtail rockfish *Sebastes flavidus* in the eastern North Pacific (Laidig et al. 2007).

Predation is one of the most dominant factors that control survival of fish early life stages (Houde 1987). In vegetated habitats, habitat complexity potentially affects predation rate of larval and juvenile fishes by piscivorous fish predators through influencing swimming performance, prey catchability of the predators and predator-prey encounter rate (Horinouchi et al. 2009). Previous field and experimental works have examined if the habitat complexity affect vulnerability of larval and juvenile fish to predation (Rooker et al. 1998; Anderson 2001; Johnson

2006*b*; Shoji et al. 2007; Kinoshita et al. 2012). However, most of these previous studies have focused on a ‘snap-shot’ phenomenon, fish survival during a relatively short period (in most cases, < 24 hours). Information on how fluctuations in environmental conditions affects fish early survival is still very limited in natural habitats such as vegetated habitats, mangroves, rocky reef and so on (Valentine-Rose et al. 2007; Faunce and Serafy 2008; Grol et al. 2011; Kamimura et al. 2011).

In the present study, the hypothesis that habitat complexity affects growth, survival and recruitment potential of juvenile *S. cheni* was tested in a macroalgal bed in temperate waters. Seasonal changes in weight-specific growth (*G*) and mortality (*M*) coefficients of each cohort were estimated by the use of otolith daily rings. The relative recruitment potential of individual cohorts was assessed by examining the ratio of G to M, which is commonly used as an index of stage-specific survival of fish early life stages (Houde 1996; Rooker et al. 1999; Shoji and Tanaka 2007).

Materials and Methods

Sampling Field

Biotic and abiotic surveys were conducted at the western shore off Aba Island (Fig. 1), central Seto Inland Sea, Japan and information of the sampling site is detailed in Chapter 1. Fish collection was made using a round seine net (30 m in length, 2 m in height, 5 mm in mesh aperture). The sea floor is comprised of mostly sand with incidental small stones (< 100 mm in diameter), on which the macroalgae flourish and we could conduct fish collections using the net as same as in seagrass beds as detailed in Kamimura and Shoji 2009 and Kamimura et al. 2011. In the macroalgal bed, three sides of a square (10 m in side length) were surrounded using the net, with the other side facing the shoreline (100 m^2). All fish samplings were conducted at four randomly selected separate areas within the macroalgal bed during a tidal level of 50-150 cm on each day.

Day-night comparison

In order to estimate the abundance and cohort-specific mortality of juvenile *S. cheni* during the post-settlement period (20-60 mm) as accurately as possible, differences in catch efficiency by the net was examined by comparing the juvenile abundance at length classes by day-night samplings conducted in March and May 2011. Juvenile *S. cheni* are most abundant in March in the macroalgal bed off Aba Island and dominate the fish community accounting for 68.8-79.8% in number from March to May (Kamimura and Shoji 2009; Kamimura et al. 2011). Fish collection was conducted during 23:00-24:00 h on 16 March 2011 and 15:00-16:00 h on 22 March 2011 and during 14:30-15:30 h on 20 May 2011 and 21:00-22:00 h on 25 May 2011. Juvenile *S. cheni* was fixed in 10% seawater formalin solution and total length was measured in the laboratory. Juvenile abundance was expressed as number of fish 100 m^{-2}. Juvenile abundance at length class of each 10 mm was compared between day and night within the same month.

Seasonal sampling

Fish collection was conducted during daytime (0900-1700 h) at intervals of 5-14 days from 7 Feb to 30 May, 2008. *Sebastes cheni* were preserved in 90% ethanol for otolith analysis and subsamples were preserved in 10% seawater formalin solution for body weight measurement. In the laboratory, rockfish were counted and measured for total length (mm). Mean abundance of *S. cheni* at 20-60 mm (post-settlement period: Plaza et al. 2001; Kamimura and Shoji 2009) on each sampling day was expressed as the number of fish 100 m^{-2}. Water temperature and salinity in the macroalgal bed were measured with a multiple environmental measurement system (Alec Electronics Co. Ltd.). Vegetation index (bulk volume in water column: I_v, %) was estimated by underwater visual census using a 1 m^2 quadrat at four randomly selected separate areas. Mean I_v from the day of settlement into the macroalgal bed (at 25 mm, mean total length at settlement: Kamimura and Shoji 2009) to catch experienced by each *S. cheni* cohort (identified by the use of otolith microstructures: see the results) was calculated based on the relationship between I_v and the Julian date (see the results). Zooplankton sampling was made with a plankton net (30 cm in mouth diameter, 0.1 mm in mesh aperture). The net was towed at a speed of about 0.5 m s^{-1}. Concentration of copepods, the major prey organisms for juveniles of 20-60 mm (Kamimura et al. 2011), was expressed as the number of individuals m^{-3} based on the flow meter count.

Daily sea water temperature measured by the Hiroshima Prefectural Sea-farming Center (HPSC: Fig. 1), located 2 km north from the sampling site, was used for analysis of mean temperature experienced by each rockfish cohort (see the results) in the macroalgal bed. There was a significant, positive correlation between the temperatures measured at the sampling site (T_s, °C) and HPSC (T_h, °C) on the same day ($T_s = 0.974 \times T_h + 0.763$: $n = 15$, $r^2 = 0.92$, $P < 0.001$). T_s on days without the field measurement was estimated from the equation. Mean water temperature experienced by rockfish from the day of settlement into the macroalgal bed (25 mm, mean total length at settlement: Kamimura and Shoji 2009) to catch was calculated for each cohort.

Otolith analysis

In order to estimate the age of juvenile *S. cheni*, 40 fish at maximum from each collection were processed for otolith analysis. Daily periodicity of otolith increment formation and timing of extrusion check formation were validated for both sagittae and lapilli by the use of cultured larvae and juveniles (Kamimura et al. 2012). Right-side lapillus was removed from each fish under a dissecting microscope and was dried, then embedded in epoxy resin on a slide glass. Each otolith was ground using 2000-10000 grid lapping films until the nucleus was clearly visible. Otolith increments were counted from the extrusion check to the edge at 400-1000 x magnification under a light microscope. Increment counting was conducted three times and the mean of the three counts was used as juvenile age. Otoliths with errors > 5% increment counts were excluded from further analysis. A total of 324 otoliths were processed for the estimation of birth date. Age-length key established for the wild rockfish was used to estimate age of fish whose otolith was not processed for the analysis:

$$A = 2.350 \times L + 12.69 \ (n = 324, r^2 = 0.912, P < 0.001)$$

where A and L represent age (days) and total length (mm), respectively.

Estimation of growth and mortality coefficients

Juvenile *S. cheni* were divided into seven specific cohorts with a 7-day period based on their birth date. Each cohort was designated with an alphabetical character: A (25-31 Dec), B (1-7 Jan), C (8-14 Jan), D (15-21 Jan), E (22-28 Jan), F (29 Jan to 4 Feb), G (5-11 Feb). Standardization of juvenile abundance was not made in the present study since the abundance at each length class did not significantly differ between day and night (see the results). In addition, fish < 20 and > 60 mm (before and after settlement: from offshore into macroalgal beds: Plaza et al. 2002; Pasten et al. 2003; Guido et al. 2004; Kamimura and Shoji 2009), which contributed to less than 1.5% of all fishes in number, were excluded from the analysis. Estimation of juvenile abundance requires an assumption that the abundance data from sampling reflects the actual rockfish abundance in the macroalgal bed. We assume that juvenile *S. cheni* at 20-60 mm were retained in the macroalgal bed. Many species of rockfishes (genus *Sebastes*) have been reported to be highly dependent on specific substrates during the early life stages (Plaza et al. 2001; Love et al. 2002). Among the *Sebastes* fishes, species which juveniles inhabit the vegetated habitats in shallow waters tend to stay there during the post-settlement period (Love et al. 1991). Juvenile *S. cheni* settle into the macroalgal bed at 20 mm and then inhabit there until they reach 60 mm (Kamimura and Shoji 2009). In addition, Aba Island is relatively isolated in the sea, surrounded by waters of depths > 50m, without any other vegetated habitats in the area. Stomach contents analysis revealed that the juveniles (20-60 mm) had plenty of zooplankton prey in their stomachs (Kamimura et al.2011). Therefore, it is plausible that rockfish juveniles at 20-60 mm do not positively migrate out of the macroalgal bed. Therefore, we concluded that the results from our field sampling reflect the actual abundance of *S. cheni* at 20-60 mm in the macroalgal bed.

A weight-specific growth coefficient (G, day^{-1}) was estimated for each cohort from the equation:

$$W_a = W_0 \times \exp\,(G \times a)$$

where W_a is the weight (mg) at time a (age, day), W_0 is the weight at birth (age 0), and G is the weight-specific growth coefficient.

Instantaneous mortality coefficients (M, day^{-1}) were estimated for each cohort applying the exponential model of decline (Secor and Houde 1995; Rooker et al. 1999; 2004; Shoji and Tanaka2007).

$$N_t = N_m \times \exp\,(-\,M \times t)$$

where N_t is the juvenile abundance at time t (days after the settlement), N_m is the estimated abundance at day of the maximum abundance of each cohort, and M is the instantaneous daily mortality coefficient. Number of plots (sampling dates) for the estimation varied between six and nine since data from the sampling dates with small numbers of fish were excluded from the analysis. The date at which mean total length of each cohort reached to 25 mm (Kamimura and Shoji 2009) was considered as the day of settlement into the macroalgal bed. In order to detect the effect of vegetation on juvenile survival, correlations between M and mean I_v between the day of settlement and catch date were analyzed. The macroalgal bed covers area over 40,000 m^2 in coastal water around Aba Island. In this study, rockfish collection was carried out on macroalgal bed of 100 m^2 at four separate areas on each day. Therefore, it is plausible that mortality of juvenile rockfish due to the sampling was minimal compared to their natural mortality.

Results

Day-night comparison

There was no significant difference in juvenile *S. cheni* abundance (number of fish 100m^{-2}) at each length class between the day and night samplings in both

March and May 2011 (Mann-Whitney U-test, $P > 0.05$, day; 6.5 ± 13.0 and night; 6.5 ± 7.3 (20-30 mm), day; 1.0 ± 1.2 and night; 2.3 ± 2.6 (30-40 mm) in March and day; 7.0 ± 13.1 and night; 21.1 ± 14.0 (30-40 mm), day; 11.1 ± 22.2 and night; 2.4 ± 3.1 (40-50 mm) and day; 0.7 ± 0.9 and night; 0.7 ± 0.8 (50-60 mm) in May).

Seasonal changes in environmental conditions

Water temperature ranged between 10.9 °C (11 March) and 18.2 °C (30 May) and salinity between 31.5 (3 Apr) and 32.8 (21 Feb and 11 Mar: Fig. 2a). Copepod concentration fluctuated between 1 931 m^{-3} (24 March) and 17 149 m^{-3} (1 May: Fig. 14a), without significant effect of sampling date (Spearman's Correlation Coefficient, $P < 0.05$). The macroalgal vegetation index (I_v, %) ranged between 1.5 (30 May) and 60.0% (11 March). The relationship between I_v and julian date (D, day) was expressed by a quadratic equation:

$I_v = - 0.011 \times D^2 + 1.579 \times D - 3.189$ ($n = 14$, $r^2 = 0.96$, $P < 0.001$: Fig. 14b)

Juvenile abundance and cohort identification

A total of 6,036 rockfish juveniles (19.8-65.2 mm: Fig. 15) were collected during the seasonal sampling in 2008. Juvenile abundance was highest on 24 March (450.6 ± 327.0 100 m^{-2}: Fig. 14c). Birth date of the juveniles ranged between 11 December 2007 and 24 February 2008 (Fig. 16). The majority (79.4% in number) were extruded in January. Of the 11 cohorts identified, seven (cohort A: 25-31 Dec to G: 5-11 Feb: Fig. 16) had a large enough sample size for further analysis of the cohort-specific mortality and growth coefficients. The date of settlement of each cohort was estimated to be 2 March, 16 March, 24 March, 31 March, 8 April, 13 April, 19 April for cohort A, B, C, D, E, F and G, respectively (Fig. 14b).

Growth, mortality and G:M ratio

The G of each *S. cheni* cohort ranged between 0.029 (cohort A) and 0.047

(cohort G: Table 4). There was a significant effect of Julian date on G (Spearman's correlation coefficient, $n = 7$, $P < 0.05$, Fig. 17a) with higher values in later cohorts. Mean daily temperature which juvenile rockfish experienced from the settlement date to catch date ranged between 13.5 °C (cohort A) and 15.5°C (cohort G). There was a significant effect of the mean temperature on G, with higher G in later cohorts ($G = 0.008 \times T - 0.083$, $n = 7$, $r^2 = 0.89$, $P < 0.01$).

The M of each cohort ranged between 0.044 (cohort A) and 0.114 (cohort G: Table 4). The effect of Julian date on M was significant with higher values in later cohorts (Spearman's correlation coefficient, $n = 7$, $P < 0.05$, Fig. 17b). The mean I_v had a significant effect on M with higher M in cohorts which experienced high vegetation coverage between the settlement and catch (Fig. 18). The $G{:}M$ ratio was higher in earlier cohorts (cohorts A to E: 0.646 to 0.793: Fig. 17c) and was lower in the last two cohorts (cohorts F and G: 0.403 to 0.415). There was no significant effect of Julian date on the $G{:}M$ ratio.

Discussion

Factors affecting juvenile survival

The hypothesis that habitat complexity affects survival of fish early life stages has been tested in a variety of species in laboratory and field studies (Adams and Howard 1996; Rooker et al. 1998; Johnson 2006*a*, *b*, 2007; Shoji et al. 2007; Horinouchi et al. 2009). However, the majority of these previous works focused on a 'snap-shot' phenomenon, fish survival during a relatively short time period within several hours or days, or on a year-to-year fluctuation of habitat condition and recruitment of fish population at a broader spatial scale. In the present study, weekly or semi-weekly sampling in an isolated fish habitat at an uninhabited island enabled us to analyze fish survival in relation to the temporal fluctuation in their habitat condition during a longer time period on a fine spatial scale. In addition, application of the otolith daily increments for cohort identification provided evidence that temporal fluctuation of habitat complexity significantly affected juvenile *S. cheni* survival in the field.

Some of the previous studies have focused on temperature and prey availability, other than habitat complexity as possible factors controlling survival of larval and juvenile cohorts. Secor and Houde (1995) estimated cohort-specific mortality of larval striped bass *Morone saxatilis* in upper Chesapeake Bay by the use of larval otolith microstructures and found that larval survival was highly dependent on temperature, with lower mortality rates in cohorts that experienced intermediate temperatures. Rooker et al. (1999) examined mortality rates of post-settlement red drum *Sciaenops ocellatus* in seagrass meadows in the Aransas Estuary. Cohort-specific mortality rate of the red drum was lowest for the mid-season cohort. Shoji and Tanaka (2007) examined the temporal variability of cohort-specific mortality coefficients of larval and early juvenile sea bass *Lateolabrax japonicus* in the Chikugo estuary. Influence of temperature and spring bloom of prey zooplankton (an estuarine copepod *Sinocalanus sinensis*) was suggested to provide the later cohorts with a higher survival probability.

In the present study, in contrast temporal variability in habitat complexity is suggested to have a significant effect on the cohort-specific mortality rate of *S. cheni* through influencing vulnerability to predation in natural habitat. *Sebastes cheni* settle into macroalgal beds at about 20 mm and then feed exclusively on copepods until they reach 60 mm (Kamimura and Shoji 2011). Among the major factors of mortality of fish early life stages (starvation, physical processes and predation: Houde 1987), starvation is considered to be less important for *S. cheni* during the post-settlement period since the juveniles collected in the macroalgal bed off Aba Island had plenty of prey in their stomachs (Kamimura et al. 2011). Effects of physical processes such as temperature effects and transportation also would be minimal since juvenile rockfish are able to swim and capture prey after fin formation at 20 mm TL (Nagasawa et al. 2000). Therefore, it is plausible that predation, rather than starvation and physical processes, is the most important determinant for juvenile *S. cheni* survival during the post-settlement period (20-60 mm). Several experimental studies have showed that high habitat complexity decreases risk of predation of juvenile fishes by piscivorous fish predators (Johnson 2007; Shoji et al. 2007; Horinouchi et al. 2009).

A recent field survey in a seagrass bed in Ikuno Island which is located about 2 km from Aba Island, revealed the fish community differed between day and night with a significant nighttime increase (by ten-fold) in biomass of potential fish predators (piscivorous fish such as conger eel, *Conger myriaster*, and *Sebastes inermis* > 100 mm) (Kinoshita et al. 2012). *Sebastes* spp. juveniles were found in the stomachs of these piscivorous fishes. Instantaneous predation rate of juvenile *Sebastes* spp. estimated from the ratio of the number of juveniles preyed upon (found in the predators' stomachs) to that of population (sum of surviving and preyed upon juveniles) during nighttime was 5-10%. Tethering experiments revealed predation rate of juvenile *Sebastes* spp. was significantly higher in nighttime than in daytime (Kinoshita et al. 2012). Increase in vulnerability of juvenile fishes to piscivorous predators during nighttime in seagrass beds has been supported by underwater observations in Australian waters (Increase in abundance and biomass of piscivorous predators during nighttime: Hindell et al. 2000; Guest et al. 2003). Therefore, it is plausible that these vegetated habitats contribute as 'a nighttime foraging area' of piscivorous fish predators although they serve as a predation refuge for a variety of fish species. In addition, tracking movements of these predators by the use of bio-telemetry combined with day-night fish sampling in Ikuno Island and stomach contents analysis of the predators provided evidence that the piscivorous fishes visit to and feed in the vegetated habitats after sunset (Watanabe et al. unpublished data). Since the piscivorous fish predators were hardly collected during the daytime samplings in the present sampling site, predation on the juvenile rockfish was expected to prevail more often in nighttime. Clarifying the pattern of diel and seasonal movement and feeding of piscivorous fishes would lead to further understanding and quantification of mortality process of juvenile *S. cheni*.

Relationships between growth and survival

According to a previous review by Houde and Zastrow (1993), in which growth and mortality coefficients of 188 species were summarized, the *G*:*M* ratio is less than 1.0 (mean 0.89) in larvae of the most fish species. In estuarine ecosystems, *G*:*M* ratios > 1.0 were reported for striped bass in the Chesapeake Bay (Rutherford and Houde 1995; Secor and Houde 1995), white perch *Morone americana* in the Hudson River (Limburg et al. 1999), red drum in Aransas Estuary (Rooker et al. 1999) and sea bass in Chikugo estuary, Japan (Shoji and Tanaka 2007). In contrast, the *G:M* ratios of juvenile *S. cheni* were lower than 1.0 throughout the present study period (0.403-0.793). The variability in *G:M* ratio among fish species and ecosystems would be partly attributable to the higher variability in *G* induced by ambient temperatures since *M* does not highly fluctuate as *G* does among the species and ecosystems examined to date. For example, the average *G* of juvenile *S. cheni* in the present study (10.9-18.2˚C) was 0.039 (n = 7), which was far lower than that of red drum (0.165, n = 4) in Aransas Estuary (26.4-30.9˚C), while mean *M* was 0.069 in *S. cheni* (present study) and 0.139 in red drum (Rooker et al. 1999).

The seasonal trend of fluctuation in *G* was similar with that of *M* in post-settlement *S. cheni*, showing higher values in later cohorts. The juveniles inhabit macroalgal bed from early spring through early summer when the water temperature increase. Since the dominant macroalgae (*Sargassum* spp.) grow during the low-temperature period, structural complexity of the habitat for juvenile rockfish decrease as season progressed with the increase in temperature. This vegetation phenology would increase predation risk for later cohorts of juvenile rockfish in their habitat. On the other hand, growth rate of juvenile rockfish increases as ambient temperature increases. Consequently, *G:M* ratios as a proxy recruitment potential of last two cohorts were lower than others because of *M* of these cohorts (F and G) abruptly increased under low vegetation.

Chapter 5

Correspondence of strong selection for fast-growing individuals to high mortality in fish early life stages: a comparison among post-settlement *Sebastes cheni* cohorts

Introduction

Growth rates of cohorts and populations of fish early life stages have been considered as indices of survival of fish early life stages. Generally, fast growth shortens the duration of larval stage when the ratio of fish mortality due to starvation, predation and other physical processes is highest (Anderson 1988; Bailey and Houde 1989, Sogard 1997) and can increase probability of faster-growing individuals to survive (bigger is better hypothesis: Miller et al. 1988; stage duration hypothesis: Chambers and Leggett 1987; Houde 1987) compared to those with slower growing individuals. In addition, growth rate itself also correlates with survival probability of fish early life stages regardless of the fish size (growth-selective predation hypothesis: Takasuka et al. 2003, 2004). Consequently, growth rates of cohorts and populations have been studied for predicting recruitment variability in a variety of fishes and ecosystems of the world (Houde 1987).

Previous field studies have reported positive selection for fast growing individuals in a cohort of fish early life stages in which the cohort sampled later (survivors: SV) has fast grow rate compared to those sampled earlier (original population: OP) during the same period showing faster-growing individuals has more chance to survive (Meekan and Fortier 1996; Takahashi and Watanabe 2004; Takasuka et al. 2004; Shoji et al. 2006; Vigliola et al. 2007; Islam et al. 2010; Takahashi and Watanabe 2012). However, how the magnitude of selection (positive or negative) for growth correlates with the mortality rate each cohort has undergone has not been clarified (Robert et al. 2007; Takasuka et al. 2007). Sampling designs which enable quantitative analysis of mortality ratio together with the magnitude of growth selection within/among cohorts are needed in field survey, experimental or mesocosm experiments in order to detect how growth selection relates with mortality rate.

In a previous study, Kamimura and Shoji (in review) reported the effect of habitat complexity on cohort-specific mortality rate of juvenile *S. cheni* in a macroalgal (*Sargassum* spp.) bed by sampling the same cohort repeatedly at a fine time interval (one-two week). The later rockfish cohorts underwent higher mortality rates during the post-settlement period (ca. 20-60 mm in total length: TL) in the macroalgal bed due to decrease in vegetation coverage which has been considered to serve as predation refuge for the juveniles. In the present study, the linkage of cohort-specific mortality rate and magnitude of growth-selective survival are analyzed.

Genus *Sebastes* (Scorpaenidae) is widely distributed in the North Pacific and commercially and recreationally important in each area (Love et al. 2002). Most of *Sebastes* fish have evolved life histories in which juveniles associate with substrates such as plants and rocks for several months after planktonic period (Love et al. 1991, 2002). Recent surveys indicated that survival during the substrate-associated period is an important determinant for recruitment abundance and population dynamics of yellowtail rockfish *Sebastes flavidus* in the eastern North Pacific (Laidig et al. 2007), suggesting that surveys on year-to-year fluctuation in larval and juvenile abundance, growth and survival are indispensable for understanding the mechanism of recruitment variability and population dynamics of *Sebastes* species. *Sebastes cheni* is distributed in temperate coastal waters of the western north pacific and is a commercially and recreationally important fishery resource (Kai and Nakabo 2008). Juveniles settle at vegetated habitats such as seagrass (*Zostera marina*) and macroalgal (*Sargassum* spp.) beds during at 20 mm TL and grow up to about 60 mm in summer (Kamimura et al. 2011). In the present study, the linkage between strong growth-selective survival and high mortality were analyzed in post-settlement juvenile rockfish cohorts which were reported to have experienced different mortality rates in the previous study (Kamimura and Shoji in review). Growth trajectory of juveniles was back-calculated by the use of otolith daily rings. The magnitude of selection for fast growing individuals and cohort-specific mortality rate were compared among three different cohorts

Materials and methods

Sampling field

Field surveys were conducted at coastal waters off Aba Island, the central Seto Inland Sea, Japan from February to May 2008 (Fig. 1) and information of the sampling site is detailed in Chapter 1. The fish assemblage was dominated by juvenile *S. cheni* in the sampling area (68.8-79.8% in number, 18.3-46.0% in weight from March to May; Kamimura and Shoji, 2009).

Biotic and abiotic researches were carried out during daytime (0900-1700 h) with a tidal level of 50-150 cm on each day at intervals of 5-14 days from 7 February to 30 May. Fish collection was made using a round seine net (30 m in length, 2 m in height, 5 mm in mesh aperture: Kamimura et al. 2011). In the macroalgal bed, three sides of a square (10 m in side length) were surrounded using a net, with the other side facing shoreline (100 m^2). All fish samplings were conducted at four separate areas randomly selected within the macroalgal bed. Collected rockfish juveniles were preserved in 10% seawater formalin solution and subsamples were preserved in 90% ethanol for otolith analysis. In the laboratory, *S. cheni* were counted and measured for total length (mm) to the nearest 0.1 mm. Mean abundance of juvenile *S. cheni* on each sampling date was expressed as no. fish 100 m^{-2}. Water temperature and salinity in the macroalgal bed were measured using multiple environmental measurement system (Alec Electronics Co. 144 Ltd.). Vegetation index (bulk volume in water column) was estimated by visual census using a 1 m^2 quadrat at four randomly selected separate areas (Kamimura and Shoji in review). Pelagic copepods, the major prey organisms of juvenile rockfish (Kamimura et al. 2011) was sampled using a plankton net (30 cm in mouth diameter, 0.1 mm in mesh aperture) equipped with a flow-meter. The net was towed for 10 to 20 m at a speed of about 0.5 m s^{-1}. Seasonal change in juvenile abundance, physical and biological environmental properties (temperature, salinity, vegetation index and zooplankton concentration) have been already published in the previous study (Kamimura and Shoji in review).

Otolith analysis

Otolith daily increments were analyzed to estimate birth date of the juvenile *S. cheni*. Daily periodicity of otolith increment and timing of extrusion check formation were validated by the use of cultured larvae and juveniles (Kamimura et al. 2012). Rite-side lapilli of 40 juveniles at maximum for each collection date were removed from the fish body under a dissecting microscope. Each lapillus was embedded in epoxy resin on a slide glass and dried, then grounded with 2000-10000 grid lapping films until the nucleus was clearly visible. Daily rings were counted from extrusion check to the edge at 400-1000 x magnification under a light microscope. Radius of each daily ring was measured by the use of otolith daily ring measurement system (Ratoc System Engineering). The mean of the three counts was used for juvenile age estimate excluding those with errors of > 5% within the three counts.

Mortality rate estimation

A total of 324 otoliths were processed for the estimation of birth date. In the previous study, juvenile *S. cheni* were divided into seven specific cohorts with a 7-day period based on each birth date. Each cohort was designated with an alphabetical character; A (25-31 Dec), B (1-7 Jan), C (8-14 Jan), D (15-21 Jan), E (22-28 Jan), F (29 Jan-4 Feb) and G (5-11 Feb). In the present study, two of these cohorts were combined so that to make three cohorts: I (from B and C), II (from D and E) and III (F and G) due to small sample size for analysis of growth-selective survival. Consequently, each new cohort covers two weeks of birth date period

(Table 4).

Instantaneous mortality coefficients (M, day^{-1}) of the three cohorts were estimated applying exponential model of decline (Secor and Houde 1995; Rooker et al. 1999; Shoji and Tanaka 2007; Kamimura and Shoji, in review) regarding the day of maximum abundance of juveniles in the macroalgal bed (24 Mar for cohort I, 3 Apr for cohort II and 23 Apr for cohort III) as settlement day of each cohort:

$$N_t = N_m \times \exp(- M \times t)$$

where Nt is the juvenile abundance at time t (days after the settlement), Nm is the estimated abundance at day of the maximum abundance of each cohort, and M is the instantaneous daily mortality coefficient. Possible sampling bias due to net avoidance and migration have been considered to be minimal because of the ecological characteristics of genus *Sebastes* (Love et al. 1991, 2002). Estimation of the mortality coefficient was made for the three cohorts in two ways: using the juvenile abundance at all sampling days and using the earliest two sampling days.

Analysis of growth-mortality of juvenile S. cheni

Juveniles collected on the first sampling day (24 Mar for cohort I, 3 Apr for cohort II and 23 Apr for cohort III) were regarded as original population (OP) and those on the second sampling day (3 Apr for cohort I, 16 Apr for cohort II and 1 May for cohort III) as survivors (SV). Juvenile total length and growth rate at age were back-calculated by the use of Biological Intercept method (Campana 1990) and were compared between the OP and SV within each cohort (Table 5). A liner model was fitted to the relationship between otolith radius and TL according previous studies (Plaza et al. 2001; Mizusawa et al. 2004; Kamimura et al. 2012). The total length at age of *S. cheni* was calculated following the equation:

$$L_a = L_c + (R_a - R_c)(L_c - L_e)(R_c - R_e)^{-1}$$

where L_a and L_c is fish size at age a and capture, R_a, R_c and R_e is otolith radius at age a, capture and extrusion, and L_e is mean fish size at extrusion (6.2 mm TL: Kamimura et al. 2012), respectively. Mean growth rate for recent five days at age was calculated and was compared between OP and SV within each cohort to detect growth-selective survival was back-calculated from birth to collection. The relationship between mortality rate and the magnitude of selection for fast-growing fish were compared among the three cohorts with different mortality rates between the two sampling days.

Results

Estimation of cohort-specific mortality rate

A total of 6,036 juvenile *S. cheni* were collected during the sampling period. Juvenile abundance was highest on 24 March (450.6 ± 327.0 100 m^{-2}) and decreased to 15 May (20.1 ± 19.0 100 m^{-2}). Estimation of mortality coefficient showed that the latest cohort (III) underwent highest mortality. Instantaneous mortality coefficient of each cohort was estimated 0.052 (cohort I), 0.055 (II) and 0.106 (III) when the abundance data on all sampling days was used for the estimation and was 0.123 (cohort I), 0.091 (cohort II) and 0.156 (cohort III) when the data only on the two earliest sampling days (OP and SV) was used (Fig. 19). A total of 30-56 fish for each cohort were processed for growth back-calculation (Table 5).

Growth-related survival among the three cohorts

Magnitude of selection for fast-growing individual differed among the three cohorts. In cohort I and II, there were not significant differences between mean daily growth rate of OP and SV while mean daily growth rate of SV was significantly higher than that of OP on day 60, 70 and 80 in cohort III (Student t-test, $P < 0.05$; Fig. 20).

Discussion

Strong selection for fast-growing individuals in the high mortality cohort.

Evidence that strong selection for fast-growing rockfish juveniles occurred in a cohort with high mortality was obtained from the comparison of growth trajectory within the same cohort repeatedly collected in the field. The two earliest cohorts of juvenile *S. cheni* (cohort I and II), which experienced lower mortality than the latest cohort, were exposed to milder selection for fast-growing individuals. On the other hand, the magnitude of selection was higher in the latest cohort (cohort III) which underwent higher mortality.

Relationship between habitat complexity and survival of post-settlement S. cheni

Seasonal change in habitat complexity would explain the difference in the mortality rate and magnitude of selection for fast-growing individuals among the juvenile cohorts. *Sebastes cheni* settle into vegetated habitats such as seagrass and macroalgal beds at ca. 20 mm in TL (Plaza et al. 2002; Kamimura et al. 2011). Contribution of starvation as source of mortality is considered to be minimal during the post-settlement period (20-60 mm TL) since most of the juveniles had plenty of prey organisms in their stomachs (Kamimura et al. 2011). Seasonal change in mortality rate during the post-settlement period was reported in *S. cheni* with higher mortality in later cohorts due to decrease in habitat complexity (Kamimura & Shoji in review). Stomach contents analysis of possible predators and tethering experiments showed mortality of juvenile rockfish due to predation in the vegetated habitat in the central Seto Inland Sea is minimal during the daytime (Kinoshita et al. 2012). Day-night comparison of fish assemblages and stomach contents of piscivorous fishes revealed increase in biomass of piscivorous fishes such as adult rockfish *Sebastes* spp. and conger eel *Conger myriaster* and predation rate by these predators during the nighttime in the vegetated habitats nearby the present sampling field (Kinoshita et al. 2012). However, seasonal changes in predator biomass were not significant from March through May (Kinoshita et al. unpublished data). Therefore, it is plausible that susceptibility of juvenile rockfish to predation was affected by the seasonal change in vegetation coverage under the similar level of predator biomass. Earlier cohort of juvenile rockfish which settled during high habitat complexity period could probably utilize the macroalgae as more effective shelter compared with the later cohort.

Survival probability of juvenile rockfish would not be affected by their growth rate, i.e. physiological condition (such as burst swimming speed) due to the higher habitat complexity (more effective shelter). Contrastingly, in later cohort which settled during low habitat complexity, fast-growing fish with would have more chance to survive since they have better physiological conditions under low vegetation coverage. I conclude that seasonal change in habitat complexity is one of the most important determinants for survival of juvenile rockfish by affecting the process of growth-dependent selection during the post-settlement period.

General discussion

Ecological features of juvenile Sebastes cheni in the vegetated habitat

The monthly fish sampling showed that *S. cheni* juveniles dominated the fish community of the macroalgal bed from March to May. Early juveniles settled at the macroalgal bed at ca. 20 mm TL from March to early April and grew up to 60 mm TL in late May as reported in the previous studies on *S. inermis* juveniles (the three *Sebastes* species were included) in vegetated habitats in other areas of Japan (Harada 1962; Plaza et al. 2001, 2002; Mizusawa et al. 2004). Juvenile *S. cheni* was most dominant among the three species (*S. inermis*, *S. ventricosus* and *S. cheni*) in the vegetated habitats. Recently, day-night comparison of fish assemblages in a seagrass bed around the present study site revealed that adult *S. inermis* dominated the fish community in nighttime through a year (Kinoshita et al. 2012). Stomach contents analysis of adult *S. inermis* showed that juvenile *Sebastes* spp. was preyed on in nighttime. Therefore, juvenile *S. inermis* might settle other habitats to avoid cannibalism. In future study, spatial and temporal fluctuation of juvenile occurrence and growth should be compared between the three species. Moreover, ecological studies (growth, behavior and reproduction) of these adult fishes have to be also investigated and compared among the species in order to manage the stock of each species effectively.

Estimation of annual Sebastes production in a macroalgal bed

In the present study, annual production of *S. cheni* juveniles was estimated to be 13,080 g ha^{-1} yr^{-1} for 2007 and 18,360 g ha^{-1} yr^{-1} for 2008 (Chapter 1). The economic value of annual production was calculated to be at least 654,000 JPY ha^{-1} yr^{-1} for 2007 and 918,000 JPY ha^{-1} yr^{-1} for 2008 based on the unit price of cultured *Sebastes* juveniles. If two other *Sebastes* fish (*S. inermis* and *S. ventricosus*) were included into the value, production would increase at least to 775,800 JPY ha^{-1} yr^{-1} for 2007 and 1,109,400 JPY ha^{-1} yr^{-1} for 2008. These estimates indicate that the economic value of ecosystem services of vegetated habitats calculated by Costanza et al. (1997), in which the provisioning services of fish production is not included, would increase by 40-58%. In addition, the stomach content and stable isotope analysis revealed that juvenile *S. cheni* production was highly dependent on copepods originating from phytoplankton during post-settlement period (Chapter 3). In the Seto Inland Sea, Japan, total area of seagrass beds had been decreased by one third from 1960 (22,635 ha) to 1990 (6,381 ha) by coastal exploitations (Nature Conservation Bureau, Environment Agency and Marine Parks Center of Japan 1994). On the other hand, there is no statistical information available on the loss of area of macroalgal bed in the Seto Inland Sea. Assuming that macroalgal beds (5,511 ha in 1990) also had been decreased by the same ratio as the seagrass bed area depletion during the same period, the loss of economic value which originates from juvenile *Sebastes* production due to the loss of the macroalgal beds could be calculated to be about 10.9-15.5 billion JPY per year in the Seto Inland Sea. The fisheries productivity (fish catch per unit area) of the Seto Inland Sea is among the highest in those of the world's enclosed seas because of its high primary production (Takeoka 1997). Vegetated habitats are important part of the primary production of coastal ecosystems (Costanza et al. 1997). Therefore, loss of the vegetated habitats itself is possible to cause huge loss of the economic values. Moreover, juvenile *Sebastes* spp. was consumed by commercially important fishes such as adult *Sebastes* and conger eel *Conger myriaster* in the vegetated habitats and surrounding areas (Kinoshita et al. 2012). Therefore, juvenile *Sebastes* were considered to support the provisioning services (production of these large fishes) as prey organisms. The total economic value of ecosystem services of vegetated habitats would be higher when the economic values of production of both prey (*Sebastes* juveniles) and their predators are included. Furthermore, other functions such as stabilizing community structures and maintaining biodiversity functions also should be evaluated for comprehensive estimation of ecosystem services of the vegetated habitats, which would contribute to future conservation and management of these habitats.

Survival mechanism of juvenile S. cheni in the macroalgal bed

Estimation of cohort-specific growth and mortality rates of post-settlement *S. cheni* using otolith microstructures in 2008 confirmed that both mean daily growth and mortality rates were significantly higher in the later cohorts that experienced higher temperature and lower habitat complexity. Many studies on fish early life stages indicated that faster growing individuals have higher probability to survive (Anderson 1988; Meekan & Fortier 1996; Sogard 1997). In the present study, habitat complexity seemed to be an important determinant for juvenile *S. cheni* survival. The ratio of G: M (indices of cohort-specific production) was lower in the later cohorts due to higher mortality (Chapter 4). Decrease in larval and juvenile mortality due to predation under high habitat complexity has been supported by other field and laboratory experiments (Rooker et al. 1998; Anderson 2001; Johnson 2006*b*; Shoji et al. 2007; Horinouchi et al. 2009). The contribution of *S. cheni* production in the macroalgal bed is concluded to be greater in earlier cohorts which experienced low mortality under high habitat complexity.

Mean daily growth rates of wild juvenile *S. cheni* (0.38 mm day^{-1} at 10.8 ºC) were double of those of cultured juveniles (0.16 and 0.20 mm day^{-1} at 10 and 12ºC) under similar temperatures, suggesting size- and growth-selective predation (Miller et al. 1988; Takasuka et al. 2003, 2004) in wild juveniles (Chapter 2). Comparison of back-calculated growth trajectories between original population and survivors of *S. cheni* juveniles provided an evidence of selection for fast-growing individuals in the macroalgal bed (Chapter 5). However, the magnitude of selection for fast-growing juveniles varied among cohorts identified by their birth date. During post-settlement period, strong selection for fast-growing juveniles occurred in a cohort with high mortality under low habitat complexity condition. Juvenile survival seemed to be more dependent on individual growth rate, i.e. physiological condition such as burst swimming speed which affects probability to avoid their predators in the low habitat complexity (less effective shelter). Contrastingly, slow-growing juveniles still seemed to have more chance to survive in high habitat complexity due to low vulnerability to predation. As a conclusion, temporal matching between the timing of juvenile settlement in the macroalgal bed and high habitat complexity (more effective shelter) is one of the important determinant for high juvenile production

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References

Aburto-Oropeza O, Sala E, Paredes G., Mendoza A, Ballesteros E (2007) Predictability of reef fish recruitment in a highly variable nursery habitat. Ecology 88:2220-2228

Adams SM (1976) The ecology of eelgrass, *Zostera marina* (L.), fish communities. I. Structural analysis. J Exp Mar Biol Ecol 22:269-291

Adams PB, Howard DF (1996) Natural mortality of blue rockfish, *Sebastes mystinus*, during their first year in nearshore benthic habitats. Fish Bull 94:156-162

Anderson JT (1988) A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. J Northw All Fish Sci 8:55-66

Anderson TW (2001) Predator responses, prey refuges, and density-dependent mortality of a marine fish. Ecology 82:245-257

Azuma M (1981) Importance of *Zostera* beds as nursery ground for juvenile inshore fishes. In “Seaweed beds” (ed. by Japanese Society for Fisheries Science), Koseisha-koseikaku, Tokyo, p 34-56 (in Japanese)

Bailey KM. Houde ED. (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. Adv Mar Biol 25:1-83

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51:633-641

Bohelert GW (1981) The effects of photoperiod and temperature on laboratory growth of juvenile *Sebastes diploproa* and a comparison with growth in the field. Fish Bull 79:789-794

Boehlert GW, Yoklavich MM (1983) Effects of temperature, ration, and fish size on growth of juvenile black rockfish, *Sebastes melanops*. Environ Biol Fish 8:17-28

Campana SE (1990) How reliable are growth back-calculations based on otoliths? Can J Fish Aquat. Sci 47:2219-2227

Campana SE, Neilson JD (1985) Microstructure of fish otoliths. Can J Fish Aquat Sci 42:1014-1032

Chambers RC, Leggett WC (1987) Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can J Fish Aquat Sci 44:1936-1947

Costanza R, D'Arge R, de Groot R, Faber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neil RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The values of the world's ecosystem services and natural capital. Nature 387:253-260

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192-206

Faunce CH, Serafy JE (2008) Growth and secondary production of an eventual reef fish during mangrove residency. Est Coast Shelf Sci 79:93-100

Fisheries Research Agency (2008) Summary of annual production, purchase and release of juveniles for sea-farming in Japan, FY2008. Fisheries Research Agency, Yokohama, p 1-414 (in Japanese)

Forrester GE, Steele MA (2000) Variation in the presence and cause of density-dependent mortality in three species of reef fishes. Ecology 81:2416-2427

Fuiman LA, Werner RG (2002) Fisheries. Science: The Unique Contributions of Early Life Stages. Blackwell Science Ltd, Oxford, UK

Fuse S (1962*a*) The animal community in the *Zostela* belt. Physiol Ecol 11:1-22 (in Japanese with English abstract)

Fuse S (1962*b*) The animal community in the *Sargassum* belt. Physiol Ecol 11:23-45 (in Japanese with English abstract)

Fuse S (1981) Ecology of the black rockfish in *Zostera* and *Sargassum* beds. In: “*Seaweed beds*” (ed by Japanese Society for Fisheries Science), Koseisha-koseikaku, Tokyo, p 24-33

Gillanders BM (2006) Seagrasses, fish, and fisheries. Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: biology, ecology and conservation, Springer, The

Netherlands, p 503-536

Grol MGG, Nagelkerken I, Rypel AL, Layman CA (2011) Simple ecological trade-offs give rise to emergent cross-ecosystem distributions of a coral reef fish. Oecologia 165:79-88

Guest MA, Connolly RM, Loneragan NR (2003) Seine nets and beam trawls compared by day and night for sampling fish and crustaceans in shallow seagrass habitat. Fish Res 64:185-196

Guido P, Katayama S, Omori M (2004) Classification of juvenile rockfish, *Sebastes inermis*, to *Zostera* and *Sargassum* beds, using the macrostructure and chemistry of otoliths. Mar Biol 145:1243-1255

Harada E (1962) A contribution to the biology of the black rockfish: *Sebastes inermis* (Cuvier et Valenciennes). Publ Seto Mar Biol Lab 10:307-361

Haywood MDE, Vance DJ, Loneragan NR (1995) Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semisulcatus* and *P. esculentus*) in a tropical Australian estuary. Mar Biol 122:213-223

Heck KL Jr, Thoman TA (1984) The nursery role of seagrass meadows in the upper and lower reaches of the Chesapeake Bay. Estuaries 7:70-92

Heck KL Jr, Hays G., Orth RJ (2003) Critical evaluation of the nursery role hypothesis for seagrass meadows. Mar Ecol Prog Ser 253:123-136

Hemminga MA, Duarte CM (2000) Seagrass Ecology. Cambridge University Press, Cambridge, UK

Hindell JS, Jenkins GP, Keough MJ (2000) Variability in abundances of fishes associated with seagrass habitats in relation to diets of predatory fishes. Mar Biol 136:725-737

Hixon MA, Jones GP (2005) Competition, predation, and density-dependent mortality in demersal marine fishes. Ecology 86:2847-2859

Hobson ES, Chess JR, Howard DF (2001) Interannual variation in predation on first-year *Sebastes* spp. By three northern California predators. Fish Bull 99:292-302

Holbrook SJ, Carr MH, Schmitt RJ, Cover JA (1990) Effect of giant kelp on local abundance of reef fishes: the importance of ontogenetic resource requirements. Bull Mar Sci 47:104-114

Horinouchi M, Sano M (1999) Effects of changes in seagrass shoot density and leaf height on abundances and distribution patterns of juveniles of three gobiid fishes in a *Zostera marina* bed. Mar Ecol Prog Ser 183: 87-94

Horinouchi M, Mizuno N, Jo Y, Fujita M, Sano M, Suzuki Y (2009) Seagrass habitat complexity does not always decrease foraging efficiencies of piscivorous fishes. Mar Ecol Prog Ser 377:43-49

Houde ED (1987) Fish early life dynamics and recruitment variability. Am Fish Soc Symp Ser 2:17-29

Houde ED (1996) Evaluating stage-specific survival during the early life of fish. In: Watanabe Y, Yamashita Y, Oozeki Y (Eds) Survival Strategies in Early Life Stages of Marine Resources. AA Balkema, Rotterdam, p 51–66

Houde ED (1997) Patterns and consequences of selective processes in teleost early life histories. In: "Early Life History and Recruitment in Fish Populations" (ed by R. C. Chambers and E. A. Trippel) Chapman & Hall, London, p 173-196

Houde ED, Zastraw CE (1993) Ecosystem- and taxon-specific dynamic and energetics properties of larval fish assemblages. Bull Mar Sci 53:290-335

Ishida Y, Tanaka Y (1980) Population fluctuation of the small filefish, *Rudarius ercodes*, in the *Zostera* bed in Odawa Bay. Bull Jap Soc Sci Fish 46:1199-1202

Islam MS, Ueno M, Yamashita Y (2010) Growth-dependent survival mechanisms during the early life of a temperate seabass (*Lateolabrax japonicus*): field test of the 'growth-mortality' hypothesis. Fish Oceanogr 19:230-242

Johnson DW (2006*a*) Density dependence in marine fish populations revealed at small and large spatial scales. Ecology 87:319-325

Johnson DW (2006*b*) Predation, habitat complexity, and variation in density-dependent mortality of temperate reef fishes. Ecology 87:1179-1188

Johnson DW (2007) Habitat complexity modifies post-settlement mortality and recruitment dynamics of a marine fish. Ecology 88:1716-1725

Kai Y, Nakabo T (2008) Taxonomic review of the *Sebastes inermis* species complex (Scorpaeniformes: Scorpaenidae). Ichthyol Res 55:238-259

Kamimura Y, Shoji J (2009) Seasonal changes in the fish assemblages in a mixed vegetation area of seagrass and macroalgae in the central Seto Inland Sea. Aquaculture Sci 57:233-241

Kamimura Y, Kasai A, Shoji J (2011) Production and prey source of juvenile black rockfish *Sebastes cheni* in a seagrass and macroalgal bed in the Seto Inland Sea, Japan: estimation of the economic value of a nursery. Aquat Ecol 45:367-376

Kamimura Y, Mizuno K, Noda T, Hirakawa K, Tamaki H, Shoji J (2012) Validation of daily periodicity of otolith increment formation and application for growth analysis of wild juvenile black rockfish *Sebastes cheni*. Aquaculture Sci 60:413-416

Kikuchi T (1966) An ecological study on animal communities of the *Zostera marina* belt in Tomioka Bay, Amakusa, Kyushu. Publ Amakusa Mar Lab 1:1-106

Kikuchi T (1974) Japanese contributions on consumer ecology in eelgrass (*Zostera marina* L.) beds, with special reference to trophic relationships and resources in inshore fisheries. Aquaculture 4:145-160

Kinoshita H, Kamimura Y, Hirai K, Mizuno K, Iwamoto Y, Shoji J (2012) Vulnerability of juvenile fish to piscivorous fish predators increases during nighttime in a seagrass bed in the central Seto Inland Sea, Japan. Bull Jpn Soc Fish Oceanogr 76:24-30

Kokita T, Omori M (1998) Early life history traits of the gold-eye rockfish, *Sebastes thompsoni* in relation to successful utilization of drifting seaweed. Mar Biol 132:579-589

Laidig TE, Ralston S, Bence JR (1991) Dynamics of growth in the early life history of shortbelly rockfish *Sebastes jordani*. Fish Bull 89:611-621

Laidig TE, Chess JR, Howard DF (2007) Relationship between abundance of juvenile rockfishes (*Sebastes* spp.) and environmental variables documented off northern California and potential mechanisms for the covariation. Fish Bull 105:39-48

Limburg KE, Pace ML, Arend KK (1999) Growth, mortality, and recruitment of larval *Morone* spp. in relation to food availability and temperature in the Hudson River. Fish Bull 97:80-91

Love MS, Carr MH, Haldorson LJ (1991) The ecology of substrate-associated juveniles of the genus *Sebastes*. Environ Biol Fish 30:225-243

Love MS, Yoklavich M, Thorsteinson L (2002) The rockfishes of the northeast Pacific. University of California Press, Berkely, California, USA

Mason JE (1998) Declining rockfish lengths in the Monterey Bay, California, recreational fisheries, 1959-94. Mar Fish Rev 60:15-28

Meekan MG, Fortier L (1996) Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf. Mar Ecol Prog Ser 137:25-37

Miller TJ, Cowder LB, Rice JA, Marschall EA (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can J Fish Aquat Sci 45:1657-1670

Nature Conservation Bureau, Environment Agency and Marine Parks Center of Japan (1994) The report of the marine biotic environment survey in the 4th National Survey on the Natural Environment, vol. 2 algal and sea-grass beds. Ministry of the Environment (in Japanese)

Mizusawa M, Aoki I, Yukami R, Utagawa K, Mitani I (2004) Early life history traits based on otolith microstructure of the black rockfish *Sebastes inermis* off Miura Peninsula, central Japan. Nippon Suisan Gakkaishi 70:138-144 (in Japanese with English abstract)

Mori K (1995) Ecological study on the fishes of Yuya Bay, the Japan Sea. Bull Natl Res Inst Fish Sci 7:277-388 (in Japanese with English abstract)

Mukai H (1971) The phytal animals on the thalli of *Sargassum serratifolium* in the *Sargassum* region, with reference to their seasonal fluctuations. Mar Biol 8:170-182

Nagata T, Miyajima T (2008) Stable Isotopes in Environmental Assessment of

Watersheds –Progress Towards an Integrated Approach–. Kyoto University Press, Kyoto, p 1-476 (in Japanese)

Nagasawa T, Yamashita Y, Yamada H (2000) Early life history of mebaru, *Sebastes inermis* (Scorpaenidae), in Sendai Bay, Japan. Ichthyol Res 47:231-241

Nakabo T (ed) (2002) Fishes of Japan with pictorial keys to the species, Tokai University Press, Tokyo

Nakamura Y, Sano M (2004) Is there really lower predation risk for juvenile fishes in a seagrass bed compared with an adjacent coral area? Bull Mar Sci 74:477-482

Ornellas AB, Coutinho R (1998) Spatial and temporal patterns of distribution and abundance of a tropical fish assemblage in a seasonal *Sargassum* bed, Cabo Frio Island, Brazil. J Fish Biol 53:198-208

Orth RJ, Heck KL Jr (1980) Structural components of the eelgrass *Zostera marina* in the lower Chesapeake Bay fishes. Estuaries 3:278-288

Pannella G (1971) Fish otoliths: daily growth layers and periodical patterns. Science 173:1124-1127

Pasten GP, Katayama S, Omori M (2003) Timing of parturition, planktonic duration, and settlement patterns of the black rockfish, *Sebastes inermis*. Environ Biol Fish 68:229-239

Plaza G, Katayama S, Omori M (2001) Otolith microstructure of the black rockfish, *Sebastes inermis*. Mar Biol 139:797-805

Plaza G, Katayama S, Omori M (2002) Abundance and early life history traits of young-of-the-year *Sebastes inermis* in a *Zostera marina* bed. Fish Sci 68:1254-1264

Plaza G, Ishida M (2008) The growth-mortality relationship in larval cohorts of *Sardinops melanosticus*, revealed by using two new approaches to analyse longitudinal data from otoliths. J Fish Biol 73:1531-1553

Plaza G, Katayama S, Omori M (2010) Daily pattern of settlement and individual growth rate of young-of-the-year of the rockfish *Sebastes inermis* in a *Sargassum* bed. Fish Res 103:48-55

Robert D, Catonguay M, Fortier L (2007) Early growth and recruitment in Atlantic mackerel *Scomber scombrus*: discriminating the effects of fast growth and selection for fast growth. Mar Ecol Prog Ser 337:209-219

Rooker JR, Holt GJ, Holt SA (1998) Vulnerability of newly settled red drum (*Sciaenops ocellatus*) to predatory fish: is early life survival enhanced by seagrass meadows? Mar Biol 131:145-151

Rooker JR, Holt SA, Holt GJ, Fuiman LA (1999) Spatial and temporal variability in growth, mortality, and recruitment potential of postsettlement red drum, *Sciaenops ocellatus*, in a subtropical estuary. Fish Bull 97:581-590

Rooker JR, Landry AM Jr, Geary BW, Harper JA (2004) Assessment of a shell bank and associated substrates as nursery habitat of postsettlement red snapper. Est Coast Shelf Sci 59:653-661

Rutherford ES, Houde ED (1995) The influence of temperature on cohort-specific growth, survival, and recruitment of striped bass, *Morone saxatilis*, larvae in Chesapeake Bay. Fish Bull 93:315-332

Sano M, Nakamura Y, Shibuno Y, Horinouchi M (2008) Are seagrass beds and mangroves in the tropics nursery habitats for many fish species? Nippon Suisan Gakkaishi 74:93-96 (in Japanese)

Secor DH, Houde ED (1995) Temperature effects on the timing of striped bass egg production, larval viability, an recruitment potential in the Patuxent River (Chesapeake Bay). Estuaries 18:527-544

Shoji J (2009) Seagrass and fishes. Koseisha-koseikaku. Tokyo, Japan (in Japanese)

Shoji J, Sakiyama K, Hori M, Yoshida G, Hamaguchi M (2007) Seagrass habitat reduces vulnerability of red sea bream *Pagrus major* juveniles to piscivorous fish predator. Fish Sci 73:1281-1285

Shoji J, Tanaka M (2006) Growth-selective survival in piscivorous larvae of Japanese Spanish mackerel *Scomberomorus niphonius*: early selection and significance of ichthyoplankton prey supply. Mar Ecol Prog Ser 321:245-254

Shoji J, Tanaka M (2007) Growth and mortality of larval and juvenile Japanese seaperch *Lateolabrax japonicus* in relation to seasonal changes in temperature and prey abundance in the Chikugo estuary. Est Coast Shelf Sci 73:423-430

Shoji J, Toshito S, Mizuno K, Kamimura Y, Hori M, Hirakawa K (2011) Possible effects of global warming on fish recruitment: shifts in spawning season and latitudinal distribution can alter growth of fish early life stages through the changes in daytime length. ICES J Mar Sci 68:1165-1169

Sogard SM (1992) Colonization of artificial seagrass by fishes in different estuarine habitats. Mar Ecol Prog Ser 85:35-53

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull Mar Sci 60:1129-1157

Takahashi M, Watanabe Y (2004) Growth rate-dependent recruitment of Japanese anchovy *Engraulis japonicus* in the Kuroshio-Oyashio transitional waters. Mar Ecol Prog Ser 266:227-238

Takahashi M, Watanabe Y (2012) Growth-selective survival of young jack mackerel *Trachurus japonicus* during transition from pelagic to demersal habitats in the East China Sea. Mar Biol (in press)

Takai N, Mishima Y, Yorozu A, Hoshika A (2002) Carbon sources for demersal fish in the western Seto Inland Sea, Japan, examined by $\delta^{13}C$ and $\delta^{15}N$ analyses. Limnol Oceanogr 47:730-741

Takasuka A, Aoki I, Mitani I (2003) Evidence of growth-selective predation on larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. Mar Ecol Prog Ser 252:223-238

Takasuka A, Aoki I, Mitani I (2004) Three synergistic growth-related mechanisms in the short-term survival of larval Japanese anchovy *Engraulis japonicas* in Sagami Bay. Mar Ecol Prog Ser 270:217-228

Takasuka A, Aoki I, Oozeki Y (2007) Predator-specific growth-selective predation on larval Japanease anchovy *Engraulis japonicas*. Mar Ecol Prog Ser 350:99-107

Takeoka H (1997) Comparison of the Seto Inland Sea with other enclosed seas from around the world. In Okaichi T and Yanagi T (eds) Sustainable development in the Seto Inland Sea, Japan-from the viewpoint of fisheries. Terra Sci Publ, Tokyo, p 223-247

Terawaki T, Yoshida G, Yoshikawa K, Arai S, Murase N (2000) “Management-Free Techniques” for the restoration of *Sargassum* beds using subtidal, concrete structures on sandy substratum along the coast of the western Seto Inland Sea, Japan. Environ Sci, 7:165-175

Valentine-Rose L, Layman CA, Arrington DA, Rypel AL (2007) Habitat fragmentation decreases fish secondary production in Bahamian tidal creeks. Bull Mar Sci 80: 863-877

Vigliola L, Doherty P, Meekan MG, Drown DM, Jones ME, Barber PH (2007) Genetic identity determines risk of post-settlement mortality of a marine fish. Ecology 88:1263-1277

Yoklavich MM, Boehlert GW (1987) Daily growth increments in otoliths of juvenile black rockfish, *Sebastes melanops*: an evaluation of autoradiography as a new method of validation. Fish Bull 85:826-832

Yoshida G (2005) Phenological differentiation among populations of *Sargassum horneri* (Fucales, Phaeophyta) in Hiroshima Bay, Seto Inland Sea, Japan. Bull Fish Res Agen 15:27-126 (in Japanese with English abstract)

Table 1. Fish collected in the mixed vegetation area of seagrass and macroalgae off Aba Island, central Seto Inland Sea, Japan, from August 2006 to July 2007. Total length and percentage of individuals in number and wet weight of each taxon to total fish are given with rank of 10 dominant (both in number and weight) taxa.

			N		Weight (g)		Total Length (mm)	
Family	Species	Month of capture	Total	Rank	Total	Rank	Range	Mean (SD)
Clupeidae	*Sardinella zunasi*	Sep	1		9.3			105.0
Plecoglossidae	*Plecoglossus altivelis altivelis*	Apr	2		3.1		63.0-61.7	62.4 (0.9)
Atherinidae	*Hypoatherina valenciennei*	Sep	1		0.0			18.7
Hemiramphidae	*Hyporhamphus sajori*	Sep	1		9.1			170.6
Aulorhynchidae	*Aulichthys japonicus*	Jul	1		0.3			55.6
Syngnathidae	*Urocampus nanus*	Apr-Jul, Nov	10		1.5		51.3-99.4	83.9 (14.1)
	Syngnathus schlegeli	Mar-Jun, Aug-Dec	20		17.6		77.1-235.8	135.5 (44.5)
	Hippocampus coronatus	Mar, Nov	3		2.0		30.6-62.2	49.0 (16.4)
Scorpaenidae	*Sebastiscus marmoratus*	Jan, Apr	3		1.7		35.3-37.2	36.3 (1.3)
	Sebastes cheni	Mar-Sep, Nov	1512	1	1725.2	2	19.3-172.0	32.8 (16.1)
Synanceiidae	*Inimicus japonicus*	Aug	1		34.9			120.3
Tetrarogidae	*Hypodytes rubripinnis*	Jan-Dec	546	3	3232.0	1	31.3-93.4	70.0 (10.0)
Hexagrammidae	*Hexagrammos agrammus*	Jan, Mar-Jun, Aug	15		268.9	6	48.3-158.6	83.4 (29.2)
	H. otakii	Apr-Jun	7		23.6		43.6-92.1	63.8 (18.7)
Cottidae	*Pseudoblennius cottoides*	Feb-Jun	21		16.4		19.1-220.6	46.8 (42.1)
Percichthyidae	*Lateolabrax japonicus*	Jul	1		9.7			99.1
Sillaginidae	*Sillago japonica*	Jul-Dec	116	6	45.1		14.0-123.2	27.3 (18.4)
Carangidae	*Decapterus maruadsi*	Jul-Aug	3		5.9		57.7-65.0	60.6 (3.9)
Sparidae	*Acanthopagrus schlegelii*	Aug	1		0.8			37.0
	A. latus	Dec	7		0.3		15.1-16.8	16.1 (0.7)
	Pagrus major	Aug-Oct	68	7	95.0	10	33.0-90.2	47.0 (16.9)
Embiotocidae	*Ditrema jordani*	Jun-Oct	26	9	238.5	7	35.8-118.2	83.2 (24.2)
Mugilidae	*Mugil cephalus cephalus*	Jul-Sep	29	8	28.2		37.6-48.5	44.2 (3.1)
	Chelon affinis	Jul	3		2.6		39.2-42.2	40.9 (1.5)
Labridae	*Halichoeres poecilopterus*	Mar, Jul, Sep, Oct	4		13.3		31.0-104.0	62.4 (33.5)
	H. tenuispinnis	Jul	1		20.4			118.8
Zoarcidae	*Zoarchias glaber*	Feb, May, Jun	6		9.5		22.5-98.2	74.2 (28.3)
Pholididae	*Pholis nebulosa*	Jun	1		2.4			91.7
Blenniidae	*Petroscirtes breviceps*	Nov	2		0.5		28.1-30.0	29.1 (1.3)
Callionymidae	*Repomucenus beniteguri*	Jun	4		76.4		110.5-185.5	139.8 (32.1)
	R. ornatipinnis	Apr, Jun-Sep	15		154.4	8	45.0-194.6	103.1 (46.1)
Gobiidae	*Pterogobius elapoides*	Apr, Jun	11		36.5		58.1-78.1	70.8 (5.4)
	Favonigobius gymnauchen	Jan-Dec	569	2	357.2	4	16.1-75.2	34.4 (13.1)
	Acentrogobius pflaumii	Mar-Jun, Aug, Oct-Dec	24	10	23.6		26.7-66.3	48.1 (11.0)
	Tridentiger trigonocephalus	Feb, Mar, Dec	4		1.6		32.0-41.6	35.8 (4.9)
	unidentified Gobiidae	Mar	1		0.1			17.7
Soleidae	*Pseudaesopia japonica*	Sep	1		0.6			39.1
Monacanthidae	*Rudarius ercodes*	Jan-Apr, Jun-Dec	126	5	136.8	9	15.0-60.2	34.3 (10.5)
	Thamnaconus modestus	Jul, Oct, Nov	24	10	513.1	3	49.3-226.0	100.4 (38.8)
	Stephanolepis cirrhifer	Sep	1		47.3			131.0
Tetraodontidae	*Takifugu pardalis*	Jun, Jul	2		40.7		85.4-94.9	90.2 (6.7)
	T. poecilonotus	Jan-Mar, Aug, Oct-Dec	18		93.5		28.6-78.0	62.8 (14.4)
	T. niphobles	Feb-Apr, Jun-Dec	151	4	328.4	5	24.0-105.4	43.7 (13.0)
	Total		3363		7627.8			

Table 2. Relationships between body weight (BW: *W*, g) and total length (TL: *L*, mm) and between TL and age (*A*, day) of *Sebastes cheni* by sampling year (2007 and 2008) and for the two years combined

Relationship		Equation	*N*	r^2	*P*
BW-TL	2007	$W = 6.904 \times 10^{-6} \times L^{3.164}$	85	0.991	< 0.0001
	2008	$W = 3.010 \times 10^{-6} \times L^{3.363}$	81	0.996	< 0.0001
	Overall	$W = 4.179 \times 10^{-6} \times L^{3.288}$	166	0.988	< 0.0001
TL-Age	2007	$L = 0.440 \times A - 3.401$	72	0.872	< 0.0001
	2008	$L = 0.352 \times A + 4.325$	93	0.895	< 0.0001
	Overall	$L = 0.369 \times A + 2.718$	165	0.881	< 0.0001

Table 3. Changes of mean biomass (g 100 m^{-2}) of *Sebastes cheni* by the same birth date (BD) cohort in 2007 and 2008. Summary of the maximum biomass of each cohort (asterisk) was used as indices of annual production of juvenile *S. cheni*.

Year & Sampling date		Cohort	A	B	C	D	E	F	G	H	Total
		BD	12-21 Dec	22-31 Dec	1-10 Jan	11-20 Jan	21-30 Jan	31 Jan-9 Feb	10-19 Feb	20-29 Feb	
2007	2 Feb		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6 Mar		1.7*	13.4*	1.6	0.0	0.0	0.0	0.0	0.0	16.7
	19 Mar		0.0	0.5	5.3	1.9	0.2	0.0	0.0	0.0	7.8
	5 Apr		0.0	6.1	17.1	17.3	0.9	0.0	0.0	0.0	41.4
	13 Apr		0.0	2.8	56.8*	38.7	1.0	0.0	0.0	0.0	99.3
	19 Apr		0.0	5.2	27.6	21.4	1.3	0.3	0.0	0.0	55.8
	1 May		0.0	2.3	18.7	44.6*	13.6*	0.7*	0.0	0.0	79.9
	15 May		0.0	0.0	2.7	5.1	0.0	0.2	0.0	0.0	8.0
	Max		1.7	13.4	56.8	44.6	13.6	0.7	0.0	0.0	130.8
2008	6 Mar		0.5	1.6	0.4	0.1	0.0	0.0	0.0	0.0	2.6
	11 Mar		0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.4
	19 Mar		0.0	1.5	6.4	13.7	0.5	0.0	0.0	0.0	22.1
	24 Mar		0.0	0.0	30.8	56.0*	6.8	0.0	0.0	0.0	93.6
	3 Apr		0.0	0.0	7.6	22.6	33.3*	5.2*	0.0	0.0	68.6
	8 Apr		0.0	0.0	4.3	6.6	5.0	1.9	0.3	0.0	18.1
	16 Apr		4.2	6.0	43.8*	40.5	17.4	2.3	1.1	0.0	115.2
	23 Apr		0.0	26.0*	41.7	34.9	7.2	1.0	2.2*	0.0	112.9
	1 May		2.0	5.9	8.3	23.8	9.7	3.3	1.3	0.0	54.3
	8 May		3.2	7.1	32.7	25.8	11.7	1.7	1.1	0.4*	83.7
	15 May		0.0	3.8	6.1	6.6	5.9	3.3	0.0	0.0	25.7
	22 May		16.7*	14.5	21.3	19.3	6.6	1.9	0.0	0.0	80.3
	30 May		2.9	6.9	15.4	13.4	5.6	0.5	0.4	0.3	45.3
	Max		16.7	26.0	43.8	56.0	33.3	5.2	2.2	0.4	183.6

Table 4. Relationships between body weight (*W*, mg) and age (*a*, day) and between abundance (N 100 m^{-2}) and days after settlement of *Sebastes cheni* cohorts (A to G). Each cohort with 7-day birth date period was identified by the use of otolith daily increments.

Cohort	Birth date	W-age equation	N	r^2	P	N-day equation	N	r^2	P
A	25-31 Dec	$W = 0.0287\exp(0.0286a)$	35	0.92	$P < 0.05$	$N = 31.083\exp(-0.0443t)$	9	0.54	$P < 0.001$
B	1-7 Jan	$W = 0.0137\exp(0.0355a)$	98	0.91	$P < 0.01$	$N = 98.442\exp(-0.0475t)$	9	0.73	$P < 0.001$
C	8-14 Jan	$W = 0.0096\exp(0.0395a)$	92	0.93	$P < 0.001$	$N = 184.456\exp(-0.0556t)$	9	0.92	$P < 0.001$
D	15-21 Jan	$W = 0.0091\exp(0.0401a)$	40	0.93	$P < 0.001$	$N = 87.074\exp(-0.0581t)$	8	0.92	$P < 0.001$
E	22-28 Jan	$W = 0.0114\exp(0.0387a)$	20	0.89	$P < 0.001$	$N = 36.249\exp(-0.0488t)$	8	0.87	$P < 0.001$
F	29 Jan-4 Feb	$W = 0.0062\exp(0.0457a)$	15	0.93	$P < 0.05$	$N = 20.599\exp(-0.1134t)$	6	0.74	$P < 0.001$
G	5-11 Feb	$W = 0.0062\exp(0.0471a)$	8	0.95	$P < 0.05$	$N = 6.7924\exp(-0.1136t)$	6	0.79	$P < 0.001$

Table 5. Cohort-specific birth and sampling date, number of individuals (N) and mean and range of total length (TL) of original population (OP) and survivors (SV) processed for analysis of growth trajectory of juvenile *Sebastes cheni* in 2008.

Cohorts	I	II	III
Birth date	Jan 1-14	Jan 15-28	Jan 29-Feb 11
1st sampling date (OP)	24 Mar	3 Apr	23 Apr
N	25	21	14
Mean TL (mm) ± SD	25.8 ± 1.4	27.0 ± 1.6	28.8 ± 1.8
TL range (mm)	23.7-29.1	21.9-29.1	25.9-31.0
2nd sampling date (SV)	3 Apr	16 Apr	1 May
N	31	14	16
Mean TL (mm) ± SD	29.4 ± 2.2	32.0 ± 1.6	32.8 ± 2.1
TL range (mm)	25.4-35.9	29.8-35.7	30.3-36.1

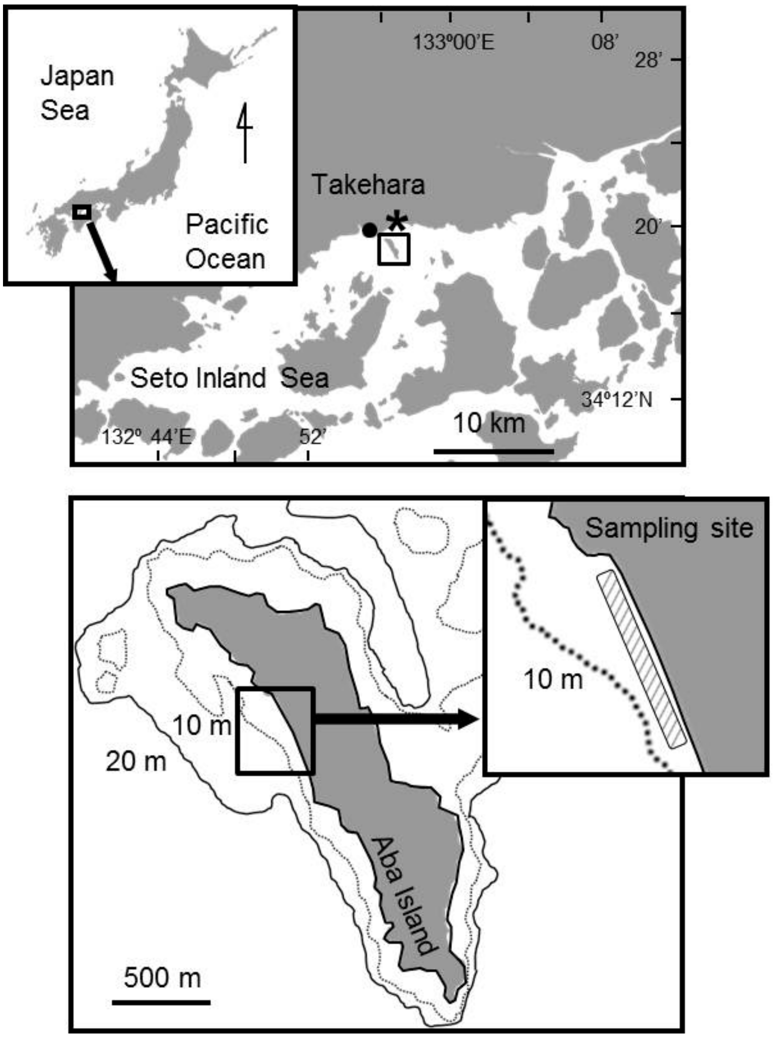

Fig. 1. Map showing the sampling site off western coast of Aba Island, central Seto Inland Sea, Japan. Fish collections and environmental surveys were conducted in the shaded area. Asterisk shows Hiroshima Prefectural Sea-Farming Center where daily water temperature was observed and closed circle show Takehara Marine Science Station, Hiroshima University. Depth contours of 10 (dotted line) and 20 m (solid line) are indicated in the lower panel.

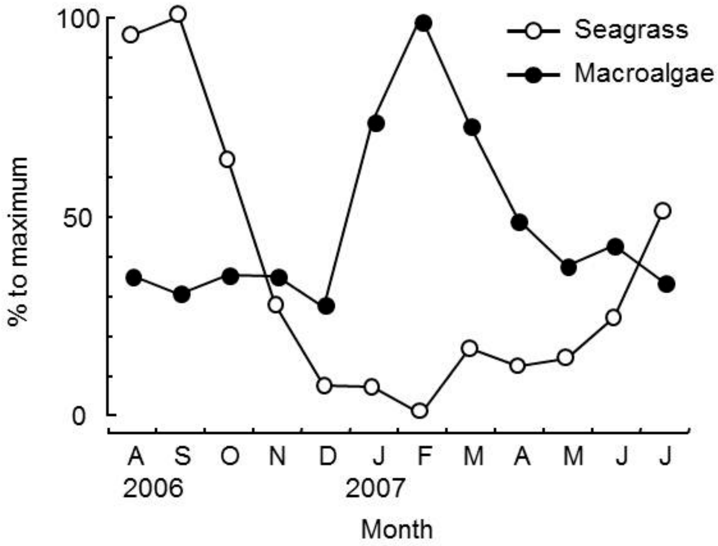

Fig. 2. Seasonal changes in relative abundance of seagrass (open circles) and macroalgae (closed circles) in the mixed vegetation area off Aba Island. Values are shown as the ratio in each month to maximum value. Data was obtained from Kamimura and Shoji (unpublished).

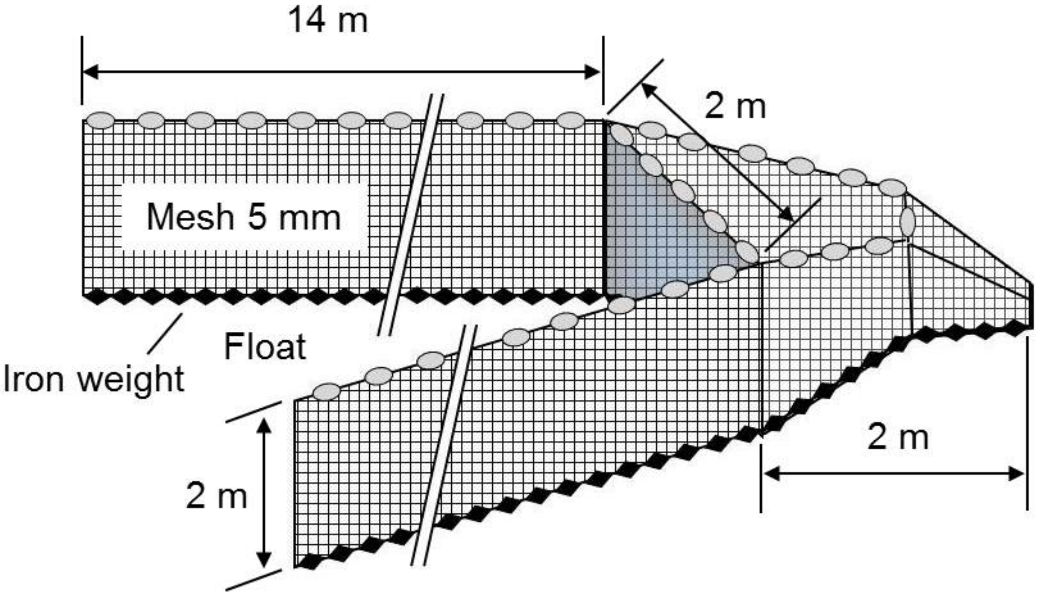

Fig. 3. Seine net used to collect fish in the vegetation area off Aba Island

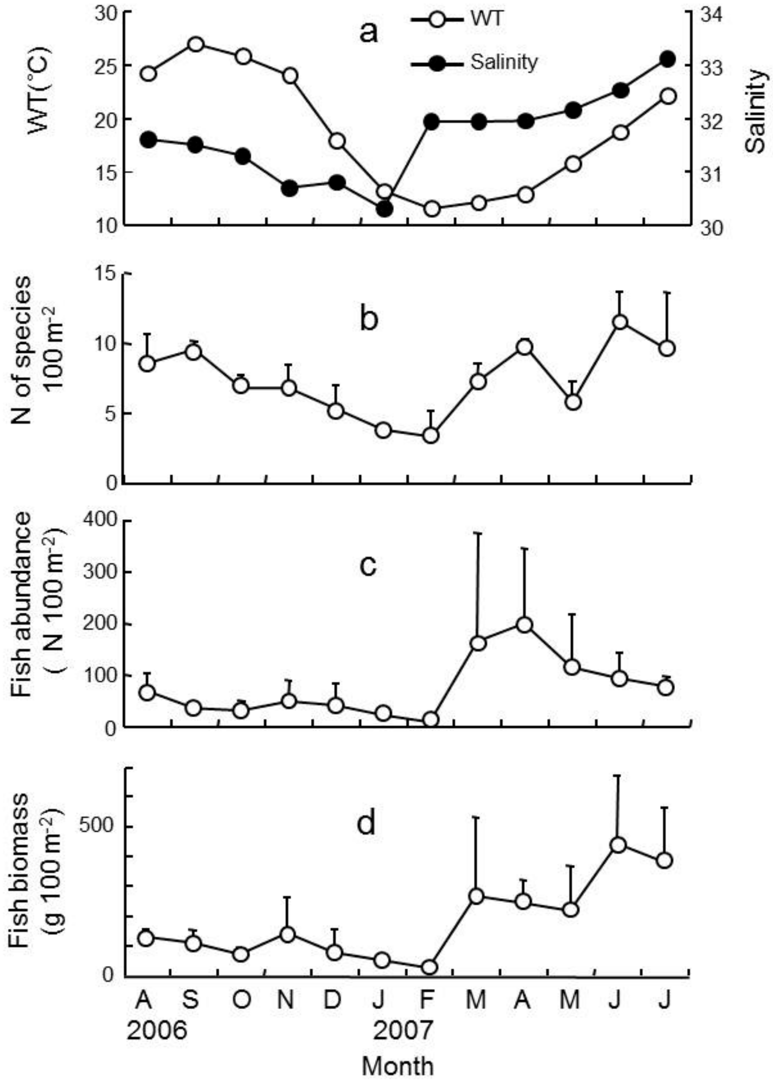

Fig. 4. Seasonal changes in a) water temperature (open circles) and salinity (closed circles), b) mean number of fish species 100 m^{-2}, c) mean fish abundance (N 100 m^{-2}) and d) mean fish biomass (g 100 m^{-2}) from August 2006 to July 2007. Vertical bars indicate standard deviation.

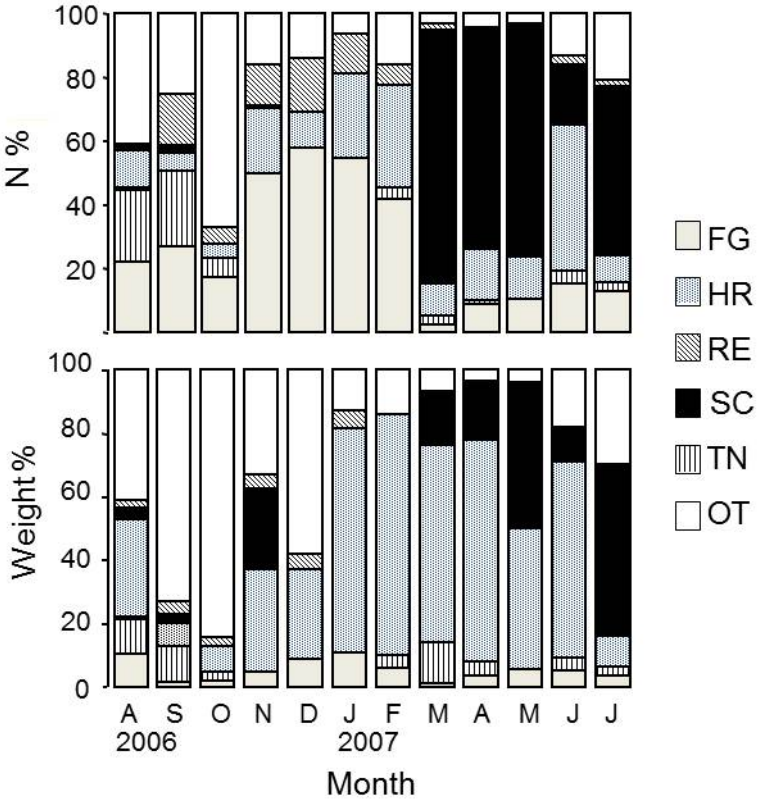

Fig. 5. Seasonal changes in dominant fish species (top: % in number; bottom: % in wet weight) from August 2006 to July 2007. FG: *Favonigobius gymnauchen*; HR: *Hypodytes rubripinnis*; RE: *Rudarius ercodes*; SC: *Sebastes cheni*; TN: *Takifugu niphobles*; OT: others.

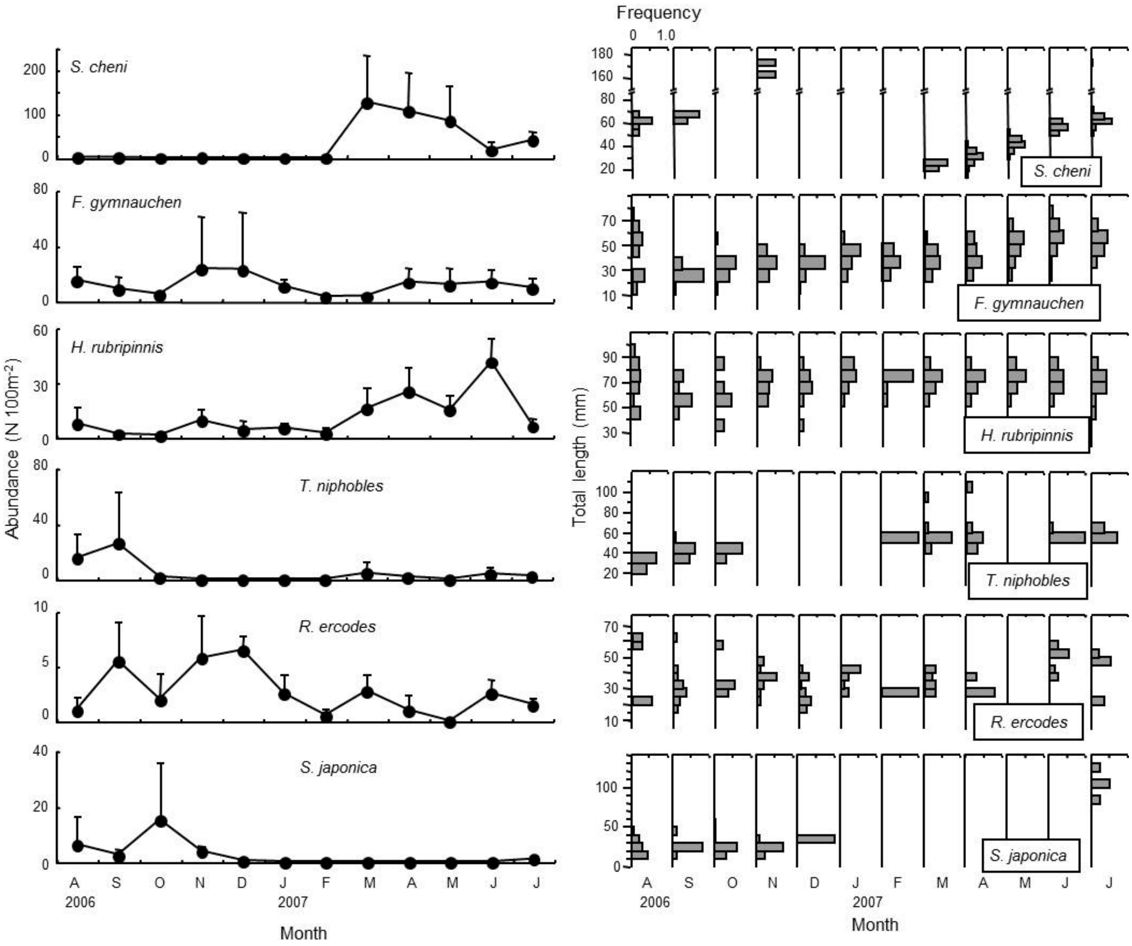

Fig. 6. Seasonal changes in mean abundance (N 100 m^{-2}: left panels) and length frequency distribution (right panels) of the six numerically-dominant species. Vertical bars indicate standard deviation.

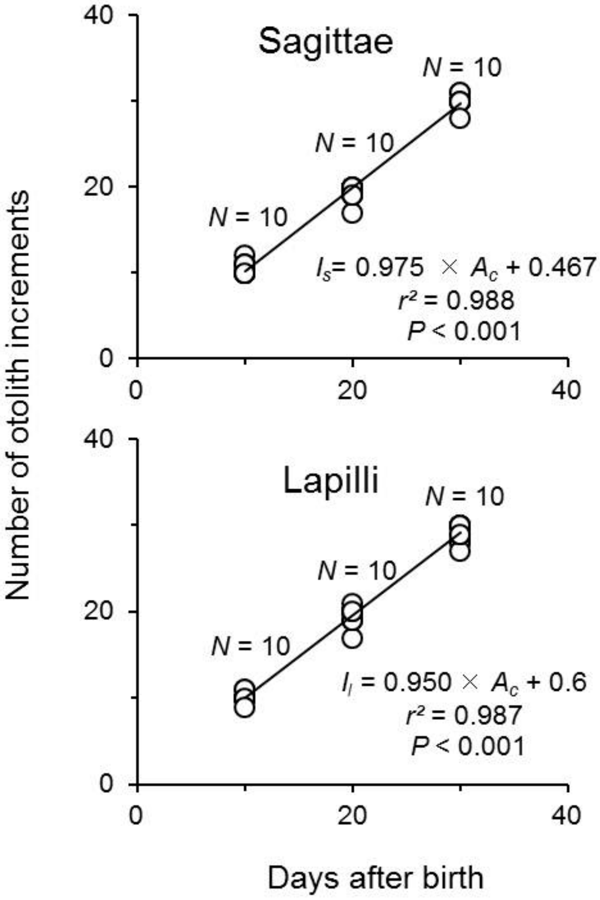

Fig. 7. Relationships between the number of otolith increments (*I*) and days after birth (A_c) of cultured *Sebastes cheni*. A, sagittae ($Is = 0.975 \times A_c + 0.467$, *N* = 30, $r^2 = 0.988$, $P < 0.001$); B, lapilli ($I_l = 0.950 \times A_c + 0.6$, *N* = 30, $r^2 = 0.987$, $P < 0.001$). Ten fish larvae were analyzed for each age.

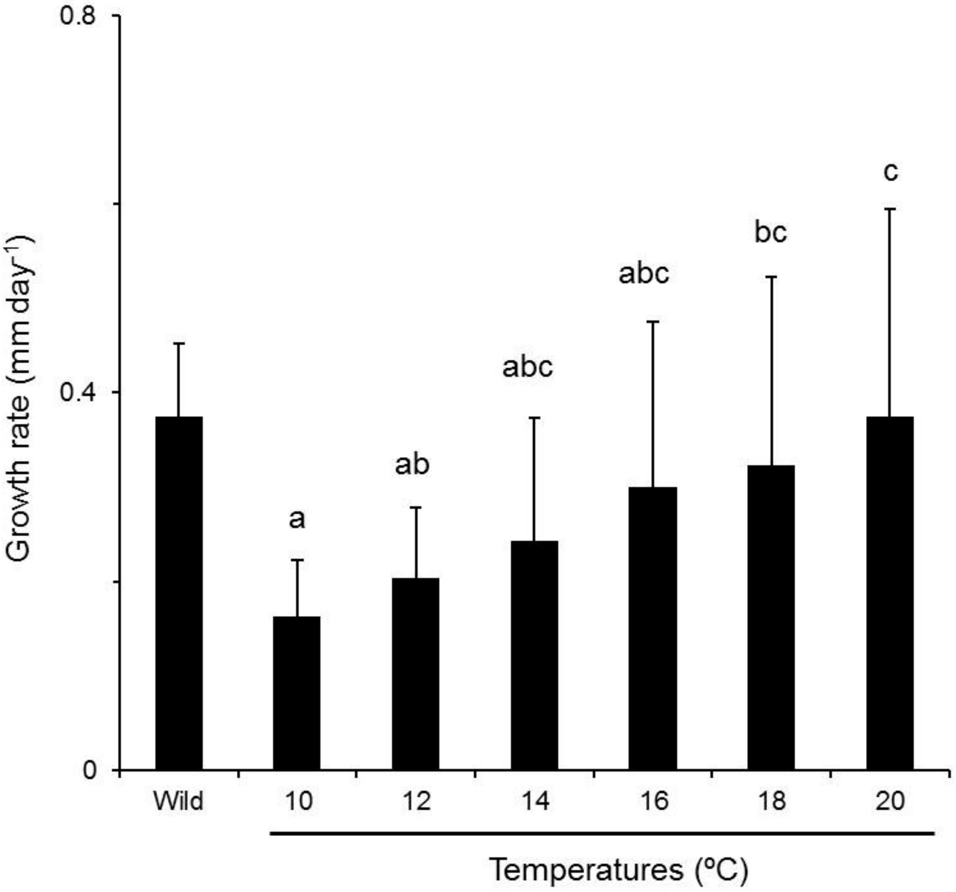

Fig. 8. Mean daily growth rates of wild and cultured *Sebastes cheni* juveniles. Mean growth rate during four weeks from the start of the rearing experiment (26.2 mm TL) were estimated for cultured fish and that for four weeks from the day at which fish reached 26.2 mm TL for wild fish. Different alphabetical characters indicate significant difference among temperatures for cultured fish (ANOVA followed by Tukey's test for multiple comparison, P < 0.0001).

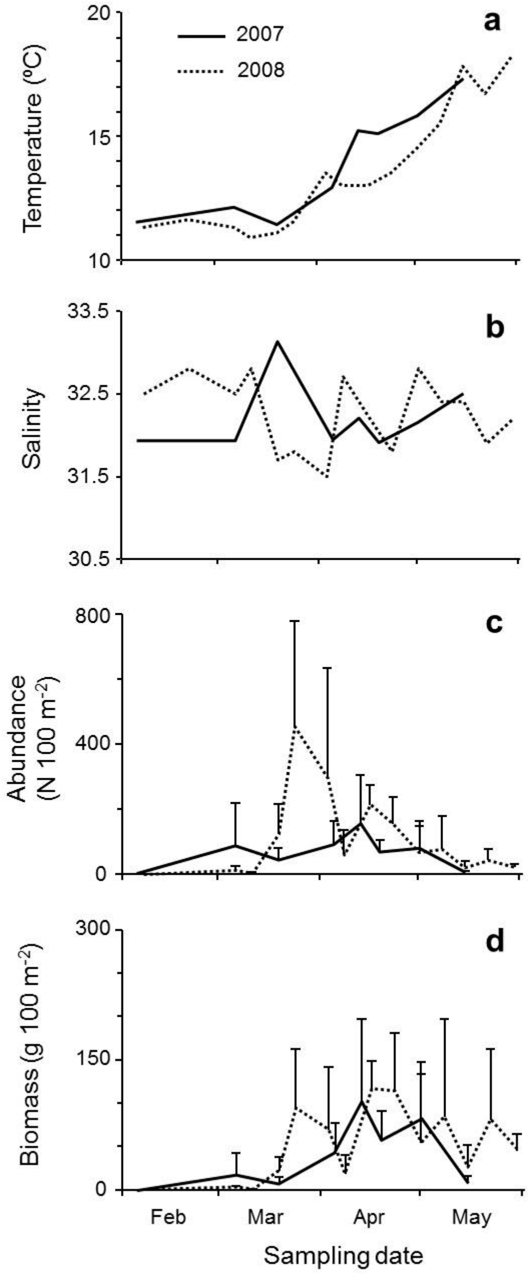

Fig. 9. Seasonal changes in water temperature (a), salinity (b), mean abundance (N 100 m^{-2}: c) and biomass (g 100 m^{-2}: d) *of Sebastes cheni* juveniles from February to May in 2007 and 2008. Standard deviation is indicated for the mean abundance and biomass.

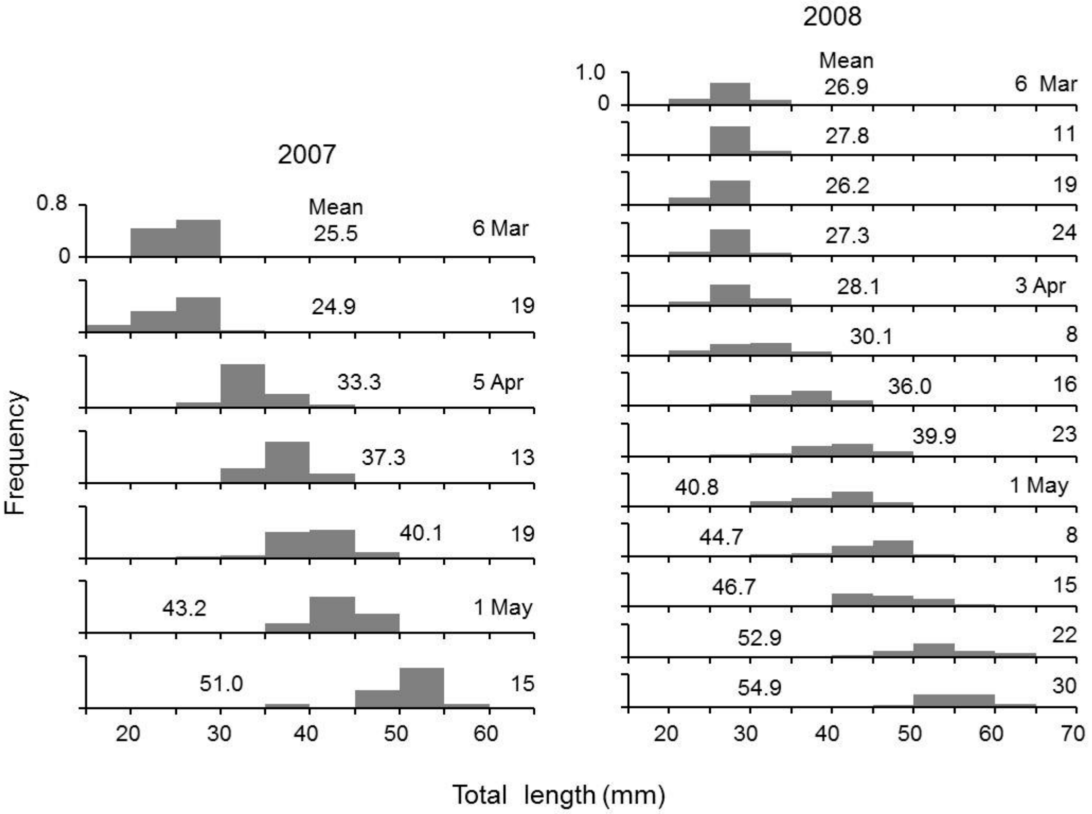

Fig. 10. Length frequency distributions of *Sebastes cheni* by sampling date in 2007 and 2008. Sampling date is indicated in each panel.

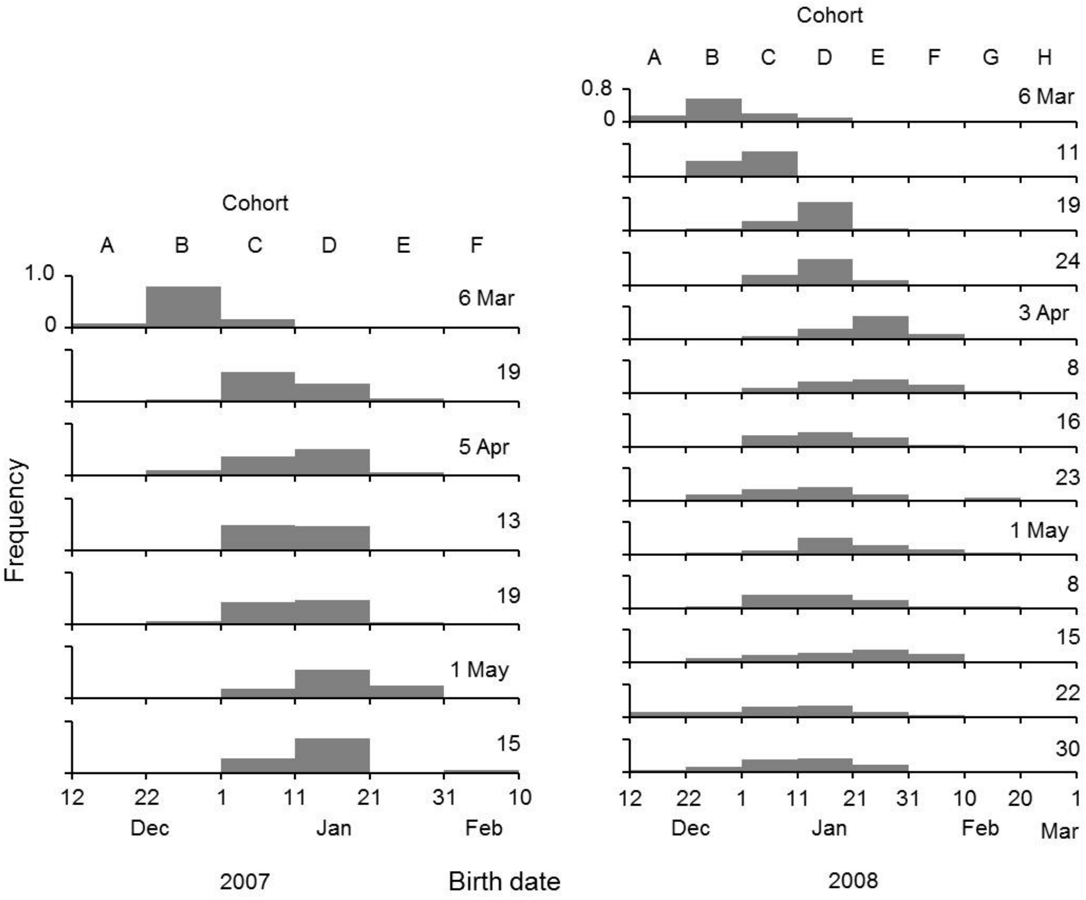

Fig. 11. Birth date frequency distributions of *Sebastes cheni* by sampling date in 2007 and 2008. Fish were divided into the same birth date cohort (A to H), each covering a period of 10 days.

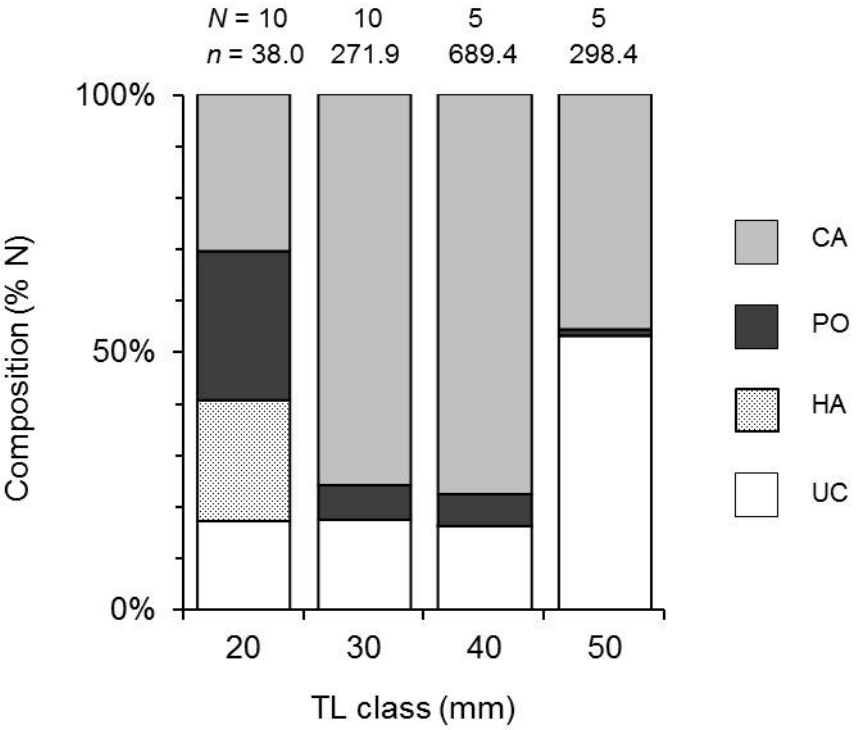

Fig. 12. Stomach contents composition of *Sebastes cheni* juveniles by total length (TL) class of 10 mm. *N* and *n* on the top indicate number of fish examined and mean number of prey organism per fish. CA: Calanoida; PO: Poecilostomatoida; HA: Harpacticoida; UC: unidentified copepod.

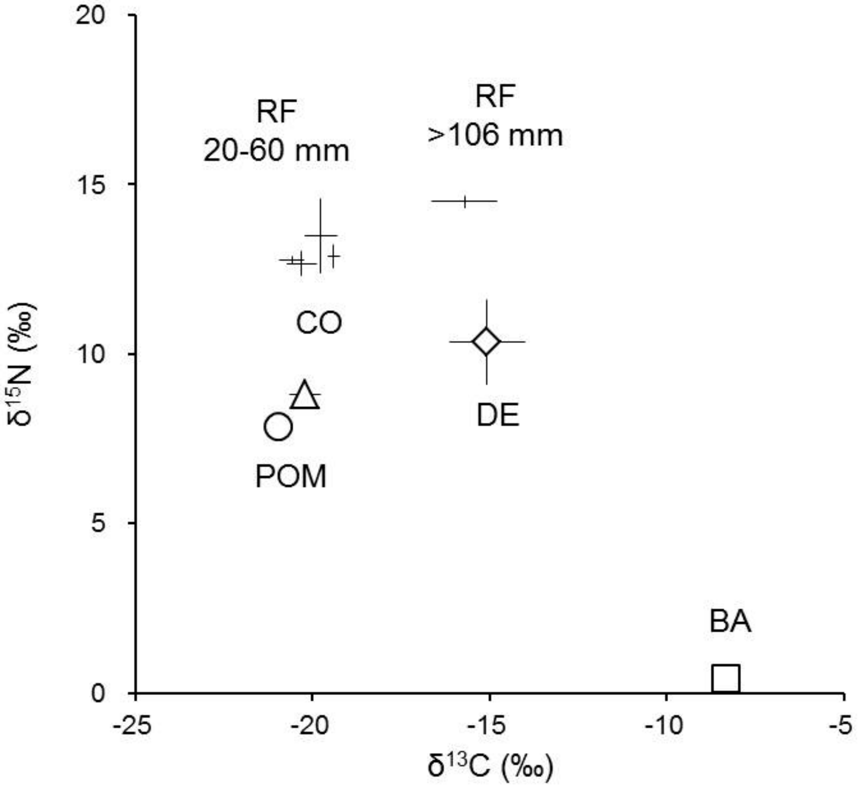

Fig. 13. Plots of mean $\delta^{13}C$ versus $\delta^{15}N$ for samples collected off Aba Island, central Seto Inland Sea. Points are mean values and bars represent standard deviation. BA: benthic microalgae; CO: copepods; DE: decapods; RF20-60: the black rockfish, *Sebastes cheni*, at 20-60 mm TL, RF > 106: the black rockfish > 106 mm TL; POM: particulate organic matter.

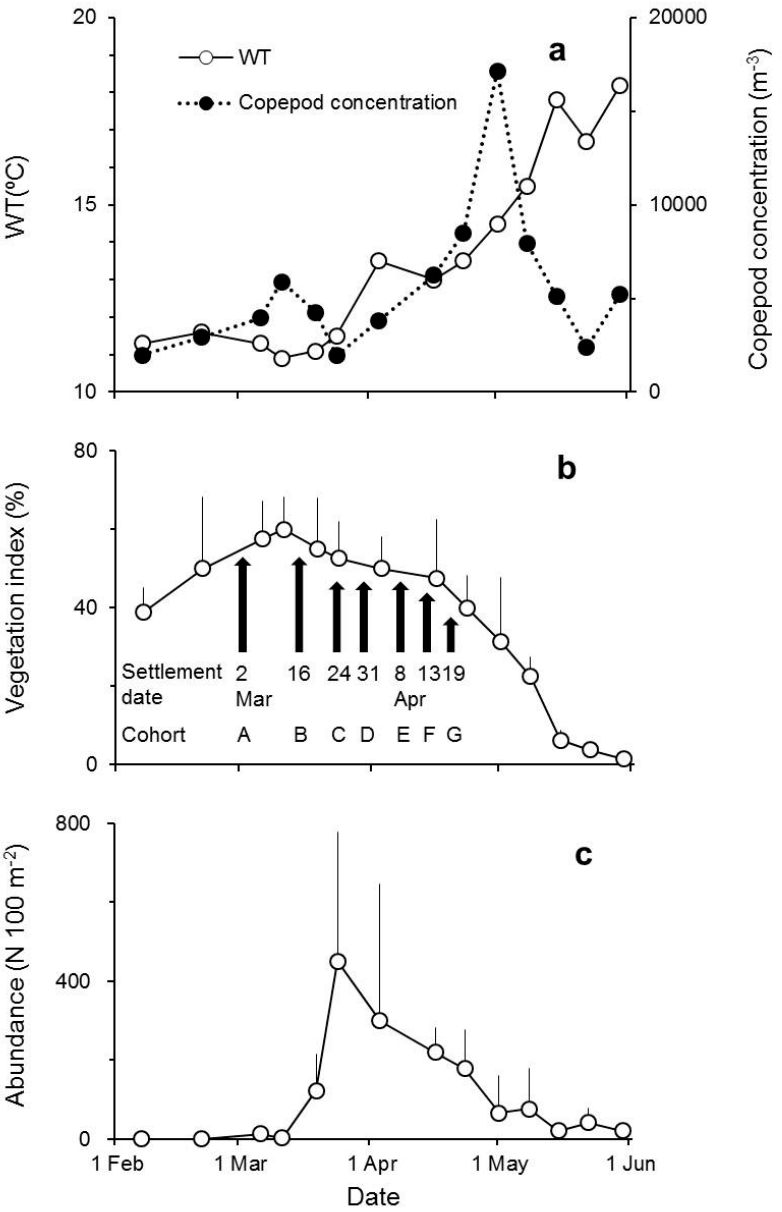

Fig. 14. Seasonal changes in water temperature (WT) and prey density (a), vegetation index of macroalgae (b) and mean abundance of juvenile *Sebastes cheni* collected in the macroalgal bed off Aba Island in 2008. Bars show standard deviations. Arrow indicates settlement date of each *S. cheni* cohort (A to G: see the result) identified using the otolith daily increments.

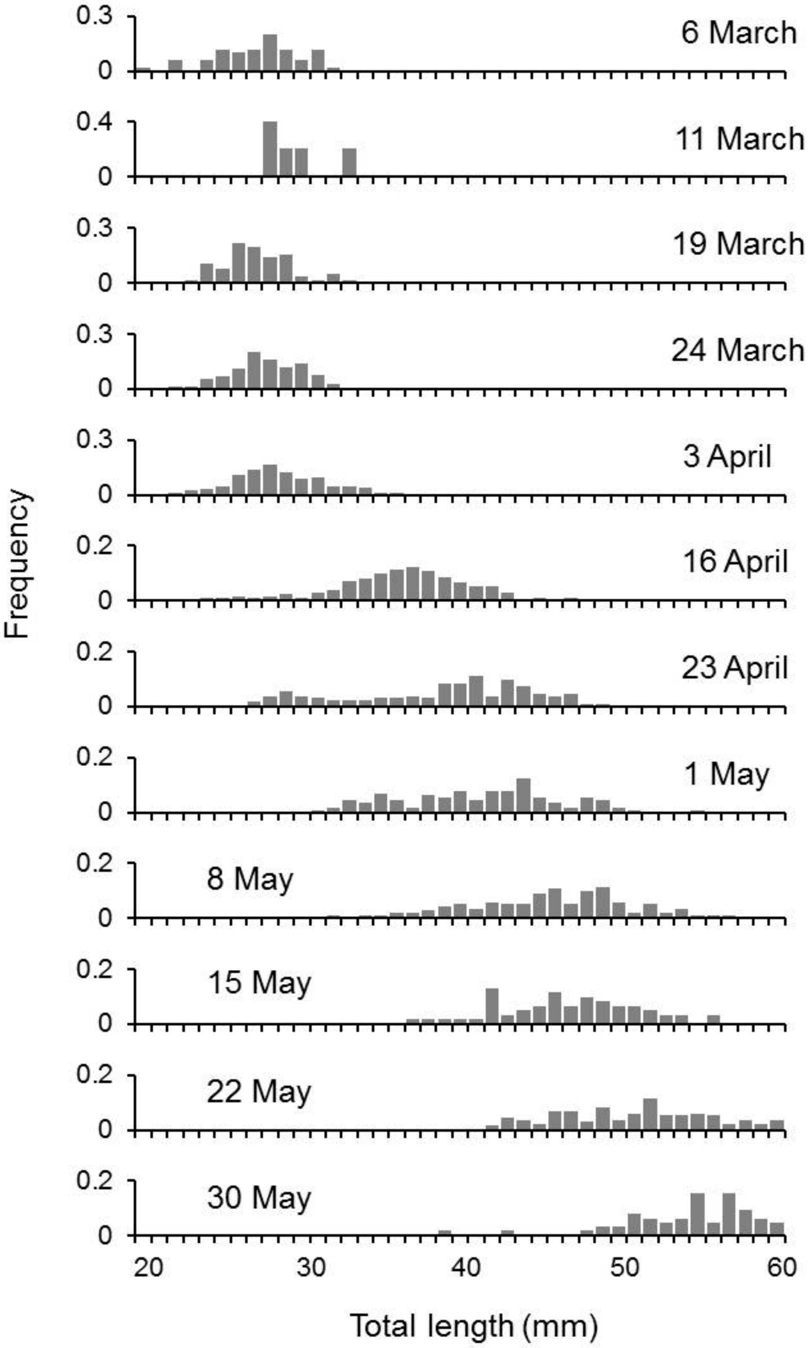

Fig. 15. Length-frequency distributions of juvenile *Sebastes cheni* by sampling date (6 March to 30 May 2008) in the macroalgal bed off Aba Island.

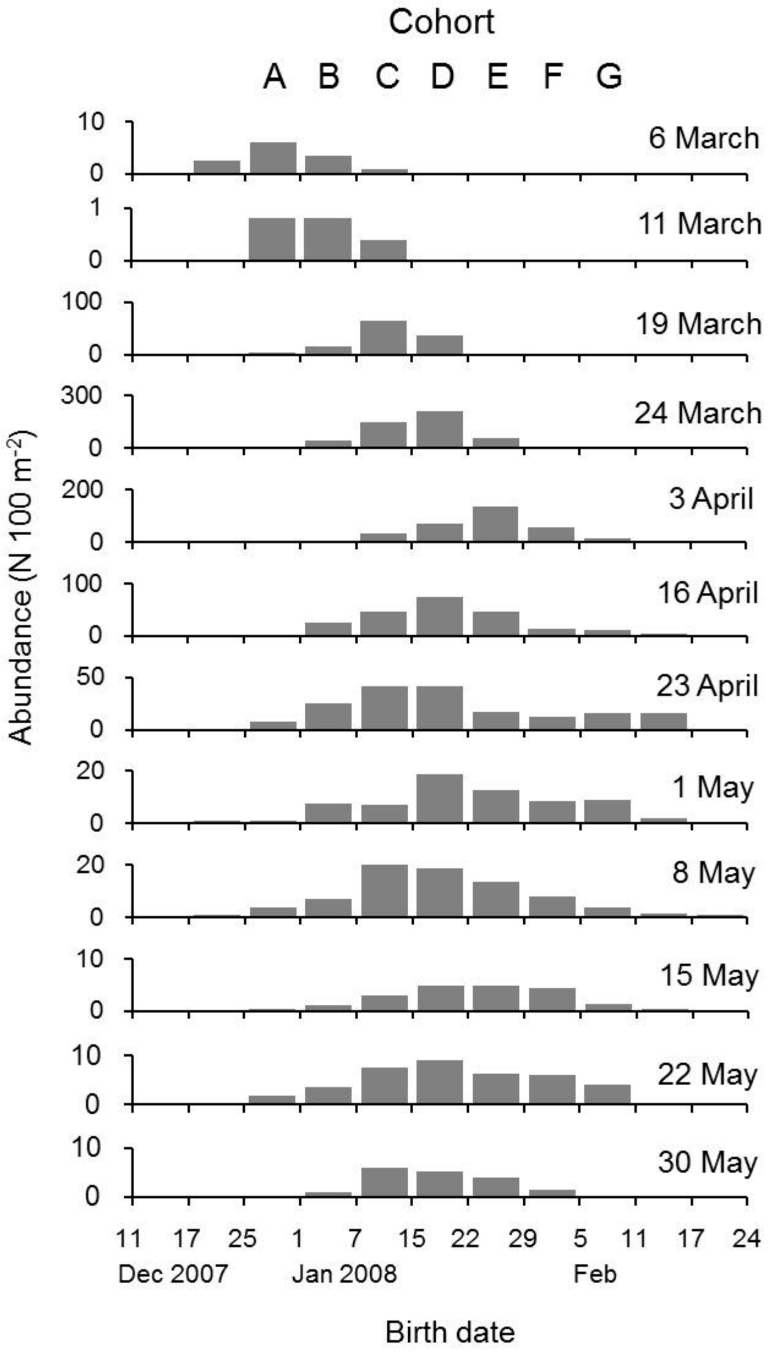

Fig. 16. Birth-date frequency distribution of juvenile *Sebastes cheni* by sampling date (6 March to 30 May 2008) in the macroalgal bed off Aba Island. Fish were divided into specific cohorts (A to G by 7-day birth date period: see the results).

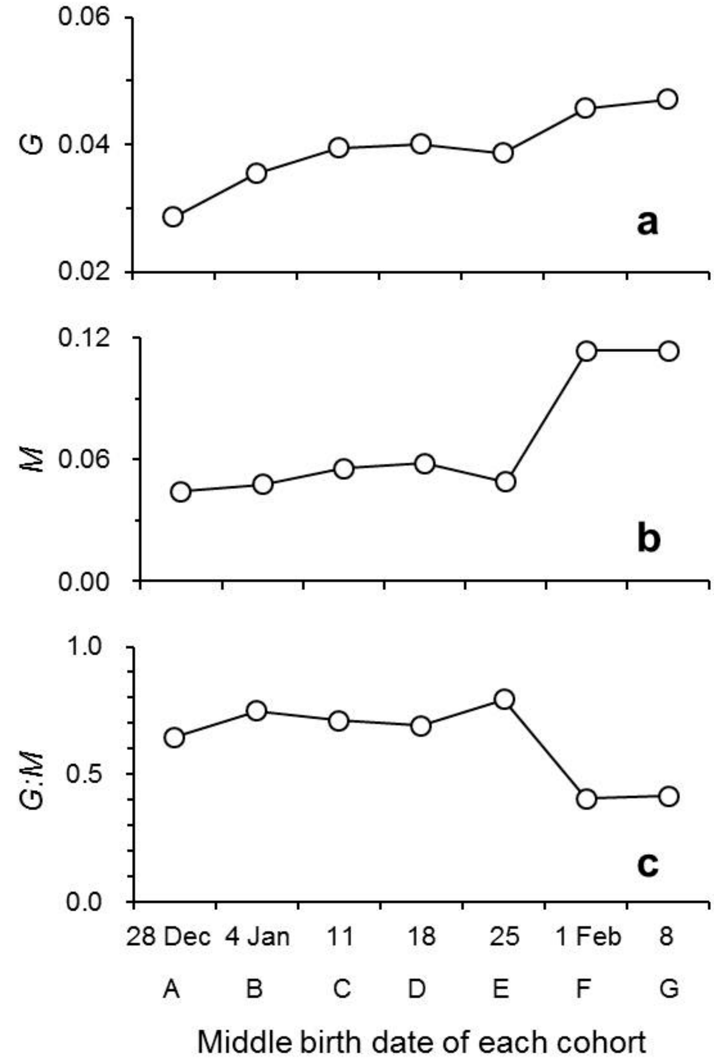

Fig. 17. Weight-specific growth coefficients (*G*: a), mortality coefficients (*M*: b) and the ratio of *G:M* (c) estimated for each *Sebastes cheni* in the macroalgal bed off Aba Island in 2008. The trends of increase in *G* and *M* were significant (Spearman's correlation coefficient, *P* < 0.05).

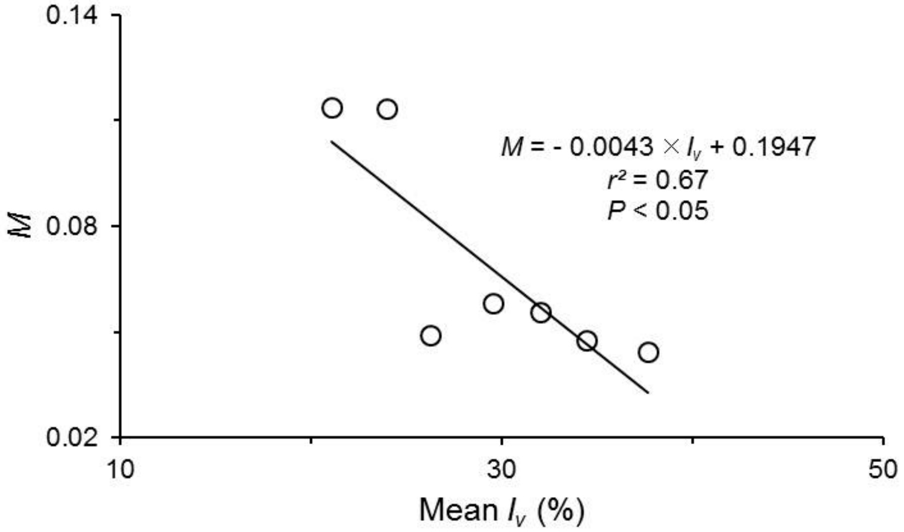

Fig. 18. Plots of mortality coefficients (*M*) of juvenile *Sebastes cheni* cohorts on mean vegetation index (I_v) by each cohort experienced from settlement date to capture.

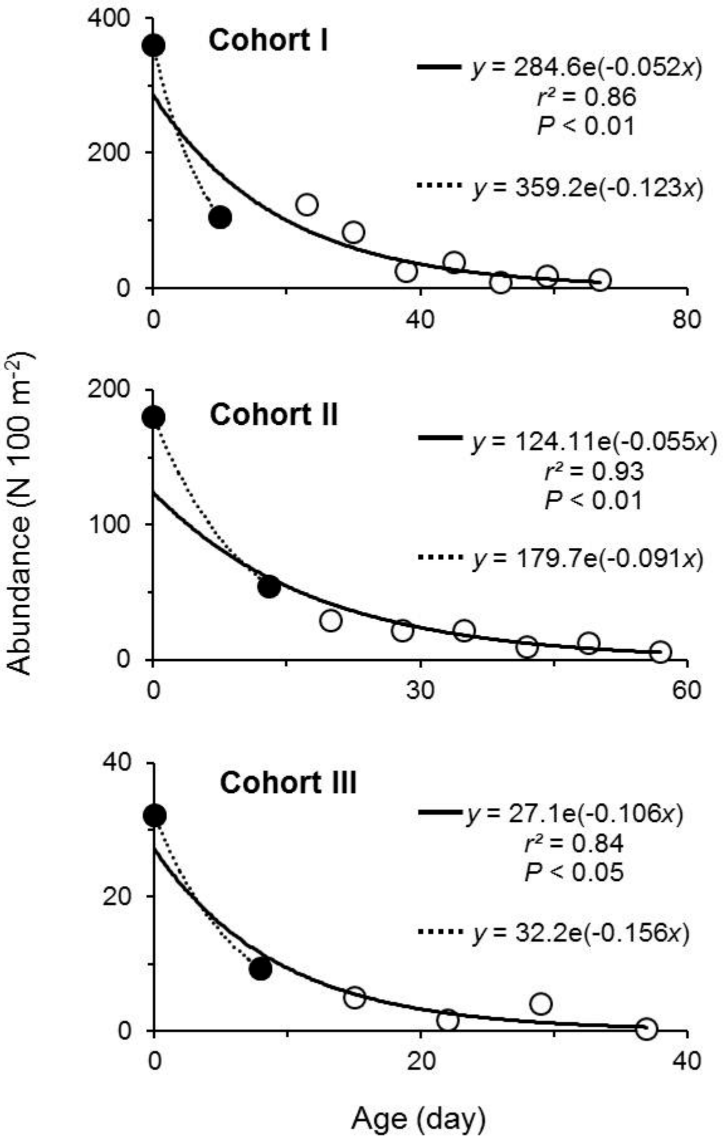

Fig. 19. Relationships between abundance (N 100 m^{-2}) and days after settlement of *Sebastes cheni* cohorts (I to III). Each cohort with 14-day birth date period was identified by the use of otolith daily increments. Mortality coefficient was estimated for each cohort in two ways: using abundance data on all sampling days (solid line) and on two earliest sampling days (dotted line). OP and SV show original population and survivors in each cohort.

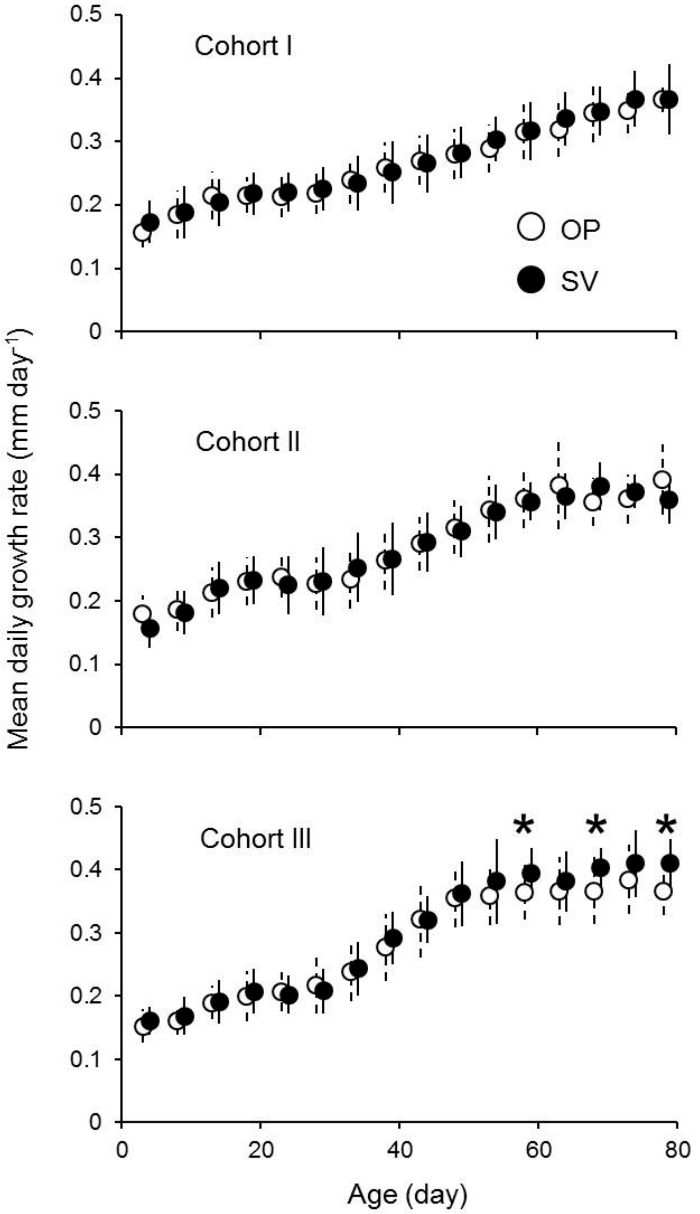

Fig. 20. Mean daily growth rate for recent five days (mm day^{-1}) of *Sebastes cheni* cohorts (I to III) back-calculated by the use of otolith daily ring. OP and SV show original population and survivors in each cohort. Vertical bars show standard deviation and asterisk a significant difference between OP and SV (Student's t-test, $P < 0.05$).