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Early life history of the rockfish *Sebastes inermis* inferred from otolith microstructure and nutritional condition assessment in two temperate bays, central Japan*

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Abstract: In order to identify any factors regulating larval extrusion, growth rates, and settlement patterns of the rockfish *Sebastes inermis*, otolith microstructure analysis was conducted for two samples from two distinct but contiguous fishing sites in the southwest coast of Miura Peninsula in central Japan: Sajima Bay and Aburatsubo Bay. Condition indices (RNA : DNA ratio, total proteins, Fulton's K) were also examined for habitat quality assessment. Four monthly extrusion groups were identified by daily age backcalculation. Growth rate during the planktonic period was positively related with the duration of this life stage and with water temperature. Flexible settlement timings of each group suggested an adaptive strategy to variability in environmental conditions: larvae experiencing unfavourable temperature settle earlier at smaller lengths and grow faster after settlement. Clear differences in traits related to planktonic and post-settlement stages between sites imply the utilization of different habitats during early life stages, and suggest that offshore mixing of larvae might not be occurring. Juveniles in Sajima Bay had higher growth and condition indices, showing a higher habitat quality and importance as a nursery of this site.

Keywords: *growth rate, nutritional condition, otolith, Sebastes inermis*

1. Introduction

The rockfish *Sebastes inermis* inhabits rocky reefs and seagrass (*Zostera* and *Sargassum*) beds, preying upon small fishes and marine invertebrates (NAKABO, 2000). This species is important for both commercial and recreational fishing activities and is a major demersal fish resource in coastal waters of Japan and southern Korea (KAMIMURA *et al*., 2011). In *Sebastes* spp., eggs are fertilized, develop and hatch internally, and soon after larvae are extruded (released) at an advanced stage of development, in which organogenesis is essentially complete before starting a planktonic stage (MOSER and BOEHLERT, 1991, WOURMS, 1991, NAKAGAWA and HIROSE, 2004). Some young-of-the-year migrate into nearshore areas (e.g. seagrass beds) as larvae or very early juveniles, after completing their planktonic stage offshore. This ontogenetic movement has been described as an adaptive strategy to allow nearshore rockfishes to maximize settlement, 1－2 months after extrusion for *S. inermis* (PLAZA *et al*., 2003), and avoiding offshore dispersal.

The survival success during the early life stages depends on both biotic (predation, food

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availability and prevalence of pathogens) and abiotic (temperature, tidal transportation or oxygen depletion) factors (Jenkins *et al.*, 1998, Borges *et al.*, 2007). Fish larvae are highly vulnerable to mortality and larval cohorts tend to experience a rapid loss of individuals (Boehlert and Yamada, 1991). The close relationship between daily growth rate and survival of larvae has been verified for some species and is known as the growth-selective mortality (Anderson, 1988, Takasuka *et al.*, 2007). Defining the timing and duration of the early life stages is crucial to understand the strategies adopted by the species during the transition to benthic habitat (Chambers and Leggett, 1987, Houde, 1987, Moser and Boehlert, 1991). Several studies have presented effective methods by analysing the sequence of daily growth increments of otoliths to describe life traits, in particular the extrusion date and growth rate (Nelson, 2001, Takahashi *et al.*, 2001), and to determine the duration of pelagic larvae (Plaza *et al.*, 2003). There is however a lack of information on the nutritional condition of early life of the rockfish *S. inermis*. Poor overall nutritional condition is frequently associated with poor feeding success and/or unstable environmental factors; therefore, fish with lower condition could have higher mortality rates (Chícharo *et al.*, 1998, Dutil and Lambert, 2000). The RNA : DNA ratio has been used as an index of nutritional condition (e.g. Clemmensen and Doan, 1996). RNA concentration fluctuates in response to food availability and demand for protein synthesis – higher in early life stages due to accelerated somatic growth – while DNA remains relatively constant throughout lifecycle (Caldarone and Buckley, 1991). Hence, in early life stages of fishes, RNA : DNA ratio can be used as a proxy of survival potential via nutritional condition and growth performance (Chícharo *et al.*, 1998).

The aim of the present study was to examine the spatio-temporal variability of the settlement of *S. inermis* in two comparative bays in Miura Peninsula, central Japan, through otolith microstructure analysis. We also aimed to analyze physiological condition indices along with daily growth rates of juveniles to compare habitat quality and suitability for juvenile recruitment. Three hypotheses are tested: H_1- *S. inermis* larval duration, growth rate, and settlement timing along the Miura Peninsula show temporal variability and are temperature related; H_2- Larval transport and settlement into inshore areas occur separately for each bay and in a small geographical range; H_3- Nutritional condition of settled rockfish varies among the study sites and the growth rate is affected by their condition.

2. Materials and methods

2.1 Sampling

Surveys were carried out at two bays in Miura Peninsula, central Japan, separated by 13.5 km from each other, Sajima Bay (Saj) (35°12′40.50"N-139°37′1.12"E) and Aburatsubo Bay (Abt) (35°9′23.38"N−139°36′50.36"E) (Fig. 1). Boat seine net and set net were used for sampling in *Zostera marina* canopy areas in both bays twice per month from May to July of 2010. Juveniles of *Sebastes* spp. were collected and kept at −80℃ Sea surface temperature (SST) data were obtained from Fisheries and Technology Centre of Kanagawa Prefecture database (www.agri-kanagawa.jp/suisoken/kaikyozu). Only juveniles that were correctly identified as *Sebastes inermis* as described by Kai and Nakabo (2008) were used for the study ($N = 550$). Total length (L) and wet weight were measured to the nearest 0.1 mm and 0.1 g, respectively. Both left and right sagittal otoliths were extracted, cleaned, and stored dry.

2.2 Otolith microstructure analysis

Daily growth increment (DGI) readings were carried out using the left otolith, after confirming statistical equality of increment number between each pair of otoliths. All otoliths were mounted in epoxy resin, fixed individually on a glass slide, and polished with waterproof abrasive grit 400. The otoliths on glass slides were observed under an optical microscope system coupled to a camera (Ratoc System Engineering, Japan). DGIs were counted and DGI widths were measured along the longest axis from the nucleus core to the outermost margin. Duplicate readings were conducted and mean age after extrusion was

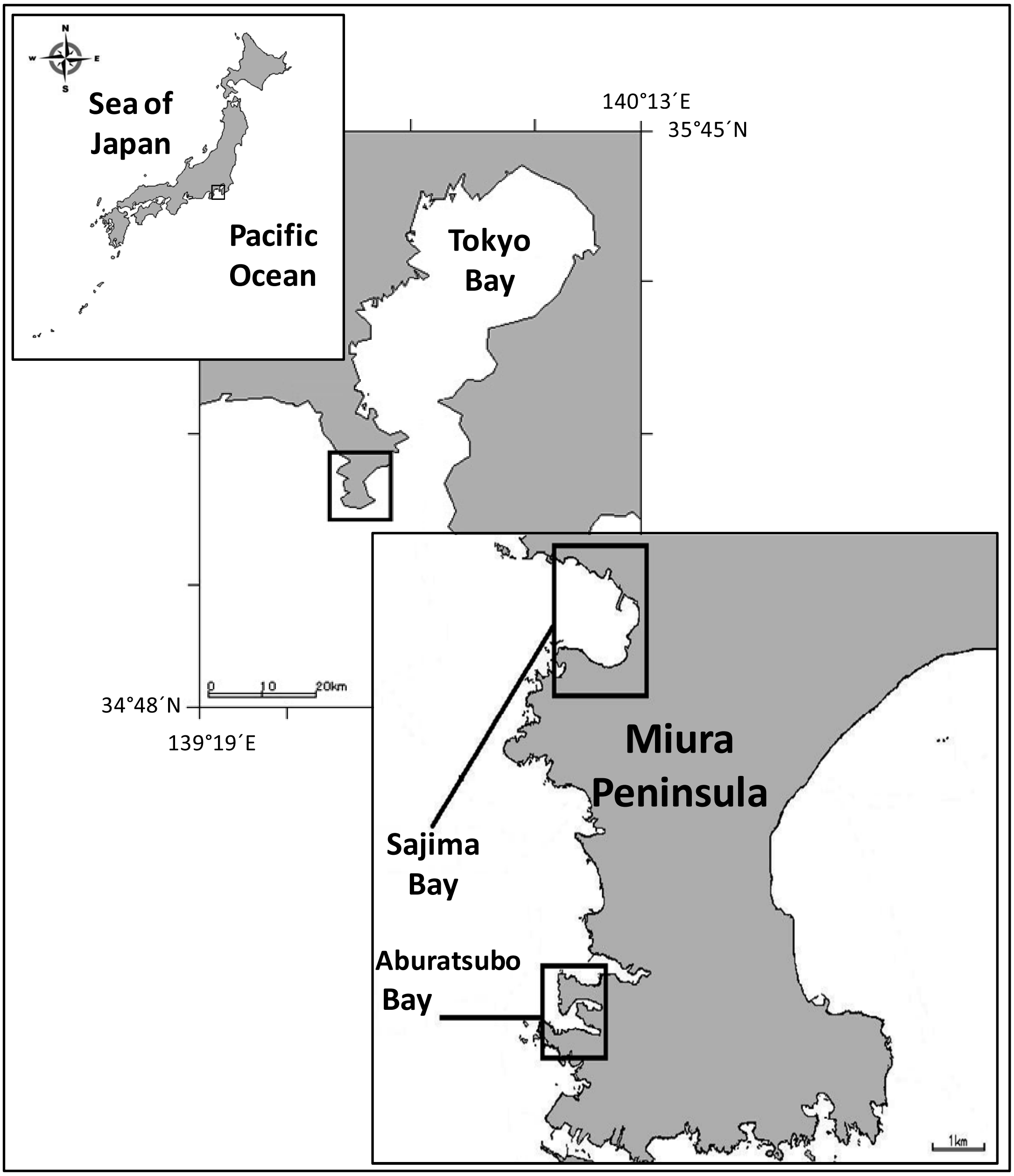

Fig. 1. Location of Aburatsubo Bay (Abt) and Sajima Bay (Saj) on the southwest coast of Miura Peninsula, Japan.

determined. The damaged otoliths without clear distinction of daily increments or clear signs of life stages were excluded from the analysis. Following the validation by Plaza *et al.* (2001), daily periodicity of ring deposition and extrusion check in newly extruded larvae (24.7±1.5 μm from the core, corresponding to 6－10 rings) were adopted in the present study. Plaza *et al.* (2001) also indicated the length at extrusion as 6.68 mm.

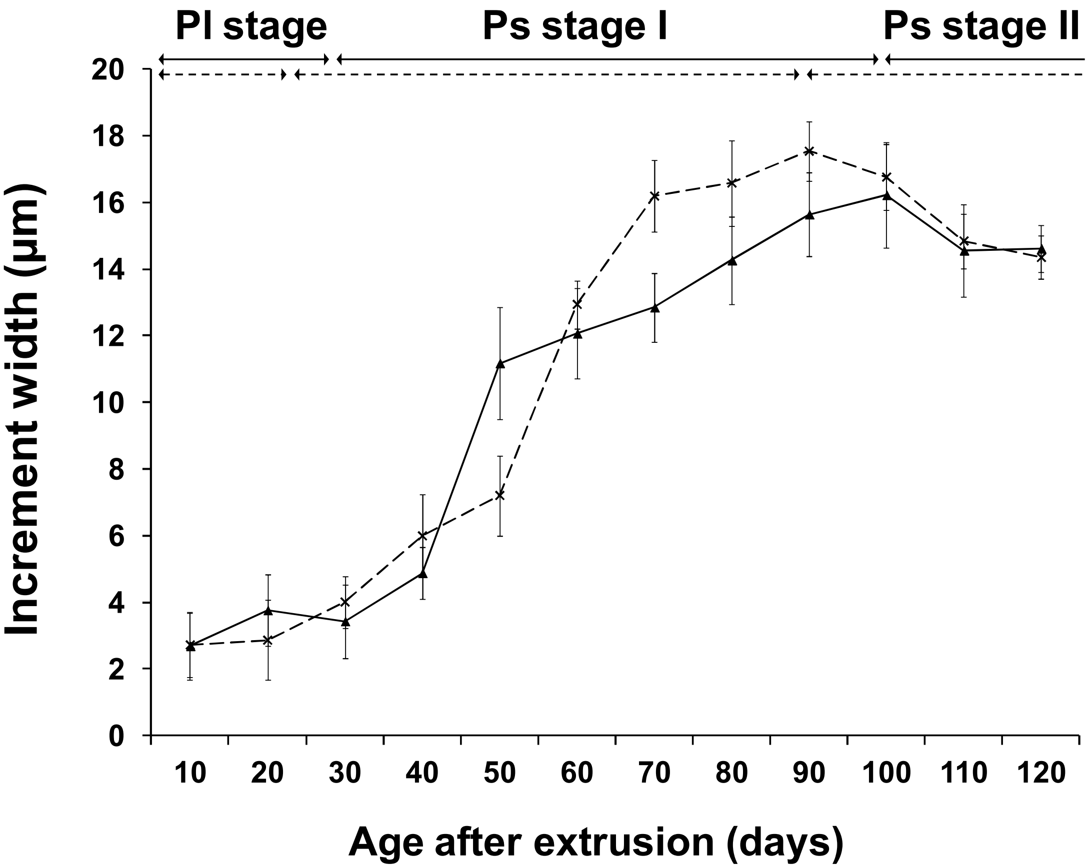

Fig. 2. Mean otolith increment width profile for early stages, planktonic (Pl), and post-settlement (Ps) I and II for Sajima (dashed line) and Aburatsubo (solid line).

Individual age and back-calculated total length of 219 specimens of *S. inermis* were estimated by the biological intercept method (Campana, 1990). Daily growth rates were estimated based on back-calculated sizes using the following equation :

$$Gr_i = L_i - L_{i-1}$$

where Gr_i is the growth rate at daily age of i, L_i is the total length at i and L_{i-1} is the total length at $i-1$ (Campana, 1990, Campana and Jones, 1992). Regression line was fitted to the relationship between total length, otolith radius and age by using least squares method.

Planktonic (Pl) and post-settlement (Ps) stages were defined based on the daily ring width profile (Fig. 2) and observed settlement check formations (Plaza *et al.*, 2001). Pl period starts after extrusion and ends when the increment width shows a sudden increase rate with a settlement check. It was possible to distinguish two growth phases within the Ps period: Ps I showed an increasing trend of growth rate after the start of the Pl period, and Ps II showed a decreasing trend of the growth rate after the Ps I phase. The transition centered method (Wilson and Mccormick, 1999) was used to reduce the age effect on the analysis of growth rates before and after settlement in each group from each site. The parameters extracted from the increment profiles were analyzed and used for definition of each stage, originating several life trait variables (Table 1). Length at 120 days and growth rate until 120 days were assessed to provide average information on early life stages until early Ps II.

Table 1. Summary of the variables extracted from DGI analysis

Otolith radius at extrusion	Time duration of Pl stage
Otolith radius at end of Pl stage	Time duration of Ps I stage
Otolith radius at end of Ps I stage	Pl stage growth rate
Ring number at start of Ps II	Ps I stage growth rate
Length at settlement	Growth rate until 120 days
Length at 120 days	

Abbreviations: Pl – planktonic; Ps I – first period of post-settlement; Ps II – last period of post-settlement; 120 days after extrusion was found in 92% fish aged

Most (92%) of fish examined were aged older than 120 days.

2.3 Condition analysis

White muscle samples from the left dorsal anterior part of the body (100−200 mg) of fish representative of all sampling dates ranging from 65 to 77 mm in total length (n=96) were used for RNA : DNA ratio assay. Nucleic acids were quantified using the method described by GONÇALVES *et al.* (2011). A fluorometer Shimadzu RF-1501 (Shimadzu, Kyoto, Japan) was used and its detection limits and linear range were tested by fluorescence of serial dilutions of DNA and RNA standards (DNA activated from calf thymus, RNA type IV from calf liver, Sigma, St. Louis, MO). The recovery rates of DNA and RNA standard spikes in the rockfish muscle homogenates were 105.4 and 99.0% respectively (n=5). Method precision was tested by replicate muscle homogenate (n=5) and found to be 97.1%. Sample autofluorescence and residual fluorescence were negligible. Muscle homogenates were used to estimate total proteins based on a modified Lowry method kit (Pierce, USA) using bovine serum albumin as standard. Fulton's K, a morphometric condition index was also applied to all individuals, using $K=W/L^3$; where W is the fish eviscerated weight (g) and L is the fish total length (mm).

2.4 Data analysis

Data are shown as mean±standard deviation unless otherwise stated. All variables were screened for normality with Shapiro-Wilk's test and for inequality of variance with Levene's test. Variable of RNA : DNA ratio required a common log $(1+x)$ transformation. DGI variables were tested for differences among extrusion months and sites, while condition variables were tested for differences amongst capture months and sites. The differences were assessed by two-way analysis of variance (ANOVA) followed by Student-Newman-Keuls (SNK) post hoc multiple comparisons test when applicable. Relationship between condition indices and growth rates of otolith marginal increments (10-day mean) for different age classes by 30 days (70−100, 100−130 and 130 − 160 days) was tested with Pearson's correlation coefficient test. The same analysis was also applied to examine the relationship between duration and growth rate during Pl period, and the relationship between early life variables (Table 1) and cumulative water temperature, represented by the thermal sum calculated for each life stage.

In order to further extend and enhance data comprehension, a multivariate approach was performed by means of canonical discriminant analysis (CDA), using DGI extracted data and also condition indices. By analysing these variables in an integrative perspective, CDA provides an estimate for the degree of separation of life history and nutritional traits among different extrusion groups (or different sampling months) and between distinct study sites.

The CDA output is presented in z-1 discriminant functions (DF) scores, where z is the number of groups in the analysis and a DF is a linear combination of the original variables that best separate the groups. Variables loading the DF's were retained when their loading weights were≧0.5 (HAIR *et al.*, 2000) and the efficiency of the discriminatory analysis was assessed with Wilk's lambda test (λ). All analyses were performed with the statistical

Table 2. Sampling data of *Sebastes inermis* juveniles captured in Sajima and Aburatsubo in 2010

	Sampling month	N	Total length (mm)	Range	Weight (g)	Range
Aburatsubo	May	66	62.8±4.5	43.1−69.3	3.8±0.6	1.5−4.6
	June	50	72.5±8.6	51.4−87.2	6.4±2.2	2.1−11.7
	July	74	72.9±3.5	56.6−79.3	6.6±0.8	3.3−7.8
Sajima	May	92	65.0±4.2	44.0−83.5	4.3±2.0	1.4−9.4
	June	256	80.2±9.3	55.6−101.3	9.3±3.3	2.4−16.3
	July	12	87.9±2.8	83.4−92.2	11.5±1.9	10.2−13.2

Total length and weight are shown as mean±SD

software package STATISTICA 8.0 (Statsoft, Inc., OK, USA), and significance level was set to $P<0.05$.

3. Results

3.1 Sampling composition and temperature

Samples from Saj represented 65.2% of the total of captured juveniles ($N=550$) and reached the maximum number (46.3%) during June (Table 2). In Abt, the capture was more homogeneously distributed among sampling months. Total length and weight of sampled *S. inermis* ranged from 43 to 101 mm and from 1.2 to 19.5 g, respectively, with juveniles in Saj presenting higher mean values than those in Abt. In both sites mean total length of samples increased with time (sampling months).

The lowest SST values (Fig. 3) were recorded in late January and early February (11.3 ℃) for Saj, and in mid January and mid February (11.6 ℃) for Abt. Mean monthly SST varied significantly (Two-way ANOVA; SNK test; $P<0.001$) but did not show any statistical differences between sites ($P>0.05$).

3.2 Otolith microstructure

Otolith-based estimates of age indicated that rockfish juveniles ($n=219$) ranged from 88 to 159 (123±18.7) days old after extrusion. Linear regressions fitted to the relationships between total length and otolith radius ($r^2=0.79$), between age and otolith radius ($r^2=0.64$), and between age and fish length ($r^2=0.63$).

From extrusion pattern (Fig. 3a), we observed several monthly extrusion peaks, starting in late December until early March. This distribution was represented by a single peak for all extrusion groups with the exception of January group in Saj which showed three overlapping peaks. For Saj the higher frequency was observed in early groups (Dec and Jan), while for Abt in later groups (Feb and Mar). Although some extrusion peaks seem to be synchronous with new moon phases, this occurrence was not consistent among all extrusion groups: some peaks appeared before and other peaks did after the new moon phases.

Settlement pulses in both sites (Fig. 3b) extended from February to May and were not closely synchronous with the moon cycle. In Saj, the majority of individuals settled in late February and March. In Abt, as observed for the extrusion dates (Fig. 3a), the settlement showed distinct but more indiscernible pulses within time.

Transition centered method (Fig. 4) allowed the reduction of the age effect for each extrusion group regarding growth rates before and after settlement. All groups showed an abrupt increase in increment width during the transition from Pl to Ps stages.

Fish that extruded in January (Table 3) stayed for shorter periods and grew less in Pl stage achieving smaller sizes at settlement. They grew less in Ps I stage achieving smaller size at 120 days. Fish that extruded in March stayed longer and grew more in Pl stage thus achieved bigger sizes at settlement. Despite the shorter period, they grew more in Ps I stage. There was a decreasing trend in the variables (except Pl growth rate) from fish that extruded in December to January, followed by an increase, except in Ps I stage duration of March

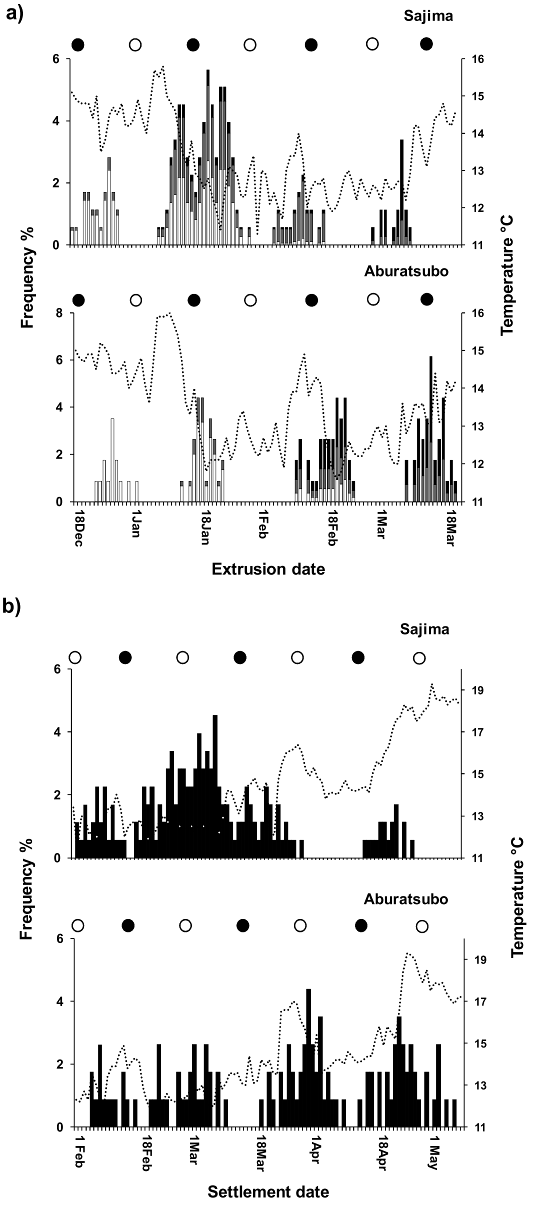

Fig. 3. Frequency of extrusion dates (a) and settlement dates (b) of *Sebastes inermis* in Sajima and Aburatsubo in 2010. Extrusion is shown for respective sampling months: May (open), June (grey), and July (closed). Dotted lines indicate sea surface temperature (SST). Lunar cycles are shown above with full moon (open circle) and new moon (closed circle).

extruded group, although significance was not always true. Individuals from Saj grew more during Pl stage and were bigger at settlement than those from Abt. Although there were no significant differences in Ps I growth rate between the two sites, the individuals from Abt stayed longer period in Ps I stage than those from Saj.

Longer Pl stage reflected higher growth rates (Fig. 5) in both sites (Pearson correlation test; r=0.24, P=0.04 for Saj; r=0.49, P=0.002 for Abt). The relationship was similar within each extrusion group of each site with the exception of February, showing a significant negative relationship in both sites (r=−0.60; P=0.04 for Saj; r=−0.11; P=0.02 for Abt). The DGI variables increased with cumulative temperature in each site with high significant correlations (Table 4).

3.3 Nutritional condition

Juveniles sampled in May had less proteins and lower condition index Fulton's K (Table 5), while in June we observed the opposite. Regardless of sites, the RNA : DNA ratios of individuals sampled in May were the highest (P<0.0001), thereafter it decreased in succeeding months. We found a significant difference in RNA : DNA ratio of juveniles sampled in July: the ratios in Saj were higher than that in Abt (P=0.013). Individuals from Abt had lower RNA : DNA ratios, total proteins, and Fulton's K values than individuals from Saj.

Otolith marginal increment widths of individuals aged 100−130 days after extrusion was positively related with both RNA : DNA ratio (r=0.41, P=0.013) and total proteins (r=0.38, P=0.022), while Fulton's K, although not significant, was negatively related with the otolith growth rate (r=−0.12, P=0.21). The relationship between otolith marginal growth rate and condition indices of other age classes followed the same tendency, although not presenting significant coefficients (age class of 70−100 days: r=0.33, 0.31 and −0.04; age class of 130 − 160 days: r=0.67, 0.25 and −0.82 for RNA : DNA ratio, total proteins, and Fulton′s K, respectively; P> 0.05).

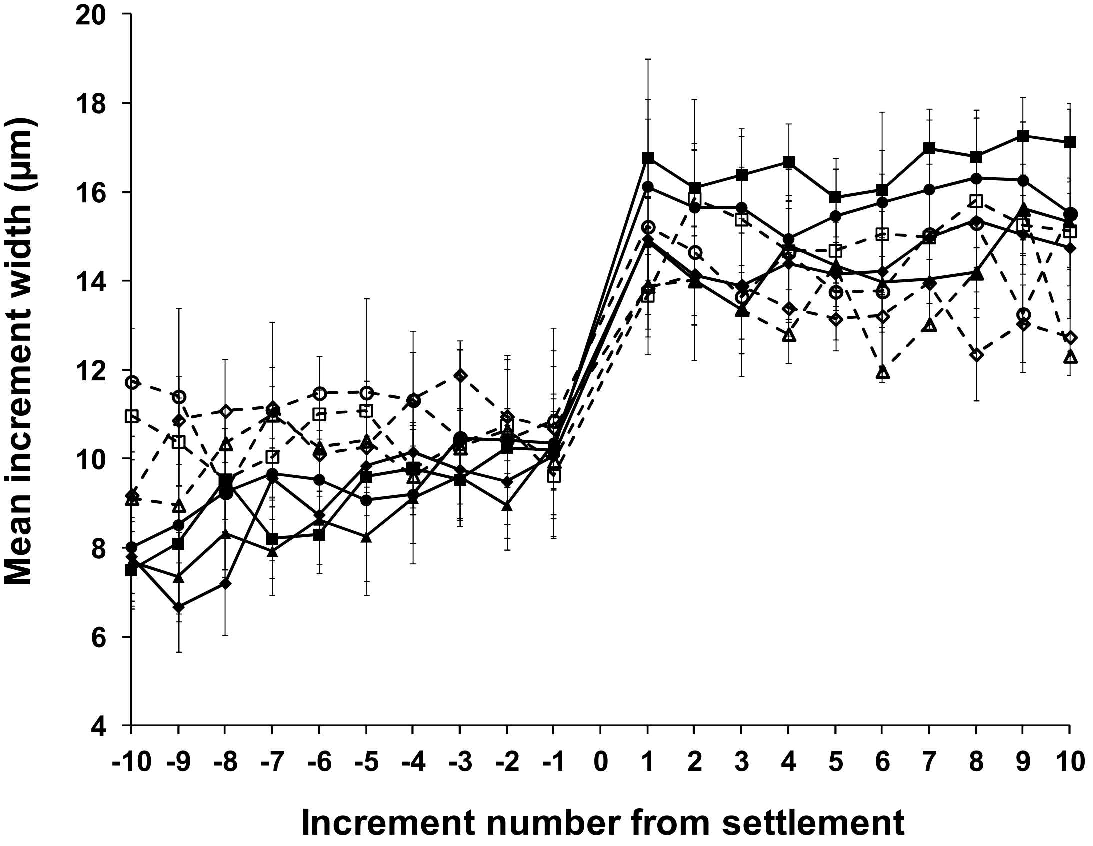

Fig. 4. Otolith increment width before and after settlement based on the transition centered method applied to each extrusion group in Sajima and Aburatsubo. Groups represented using: December (◇), January (△), February (□), and March (○) with open symbols for Sajima (dashed line) and filled symbols for Aburatsubo (solid line).

3.4 Multivariate approach

Based on the canonical correlation patterns of the 11 different DGI variables (Table 1), Saj and Abt were well separated (Table 6). The variables related to Pl and Ps stage, supported this discrimination, which was shown by the great difference between the group centroid scores, and 82% of the individuals were correctly classified. The extrusion groups were highly discriminated (Fig. 6a) with a clear distinction between the early groups (Dec and Jan) and the late (Feb and Mar) groups mostly based on Pl stage related variables (Table 6). This classification was successful in 78% of the cases and the first two discriminant functions explained 90% of the total variance among the extrusion groups.

The CDA based on condition variables showed a strong discrimination between the sites, mainly owing to RNA : DNA ratio, with 84% of correct classification (Table 6). The higher centroid score for Saj showed a higher mean RNA : DNA ratio when compared with Abt. Sampling months separation based on nutritional condition data (Fig. 6b) was also observed (77% of correct classification) and RNA : DNA ratios and total proteins were the variables with higher effects (loads) in the analysis. Hence, when compared with the morphometric condition index (Fulton’s K), the biochemical condition indices (RNA : DNA ratio and total proteins) were more relevant to the temporal discrimination.

Table 3. Mean values of DGI variables for the different extrusion groups for Sajima and Aburatsubo extracted from otoliths of the rockfish *Sebastes inermis*

		Extrusion groups				
		December (n=50)	January (n=67)	February (n=44)	March (n=58)	***Total***
Pl period duration (days)	Abt	47 ± 3^{ab}	41 ± 3^{a}	49 ± 4^{ab}	54 ± 4^{b}	**47 ± 4**
	Saj	50 ± 4^{b}	49 ± 3^{ab}	47 ± 2^{ab}	51 ± 3^{b}	**49 ± 3**
	Total	**$49\pm3^{\dagger\S}$**	**$45\pm3^{\dagger}$**	**$48\pm3^{\dagger\S}$**	**$53\pm4^{\S}$**	
Ps I period duration (days)	Abt	65 ± 4^{a}	57 ± 4^{ab}	66 ± 4^{a}	57 ± 3^{ab}	**$62\pm4^{*}$**
	Saj	60 ± 6^{ab}	55 ± 4^{b}	60 ± 4^{ab}	52 ± 5^{b}	**56 ± 5**
	Total	**$62\pm5^{\dagger}$**	**$56\pm4^{\S}$**	**$63\pm4^{\dagger}$**	**$55\pm4^{\S}$**	
Pl growth rate (mm/day)	Abt	0.33 ± 0.04^{a}	0.35 ± 0.04^{a}	0.38 ± 0.04^{ab}	0.41 ± 0.03^{ab}	**$0.36\pm0.04^{*}$**
	Saj	0.41 ± 0.04^{ab}	0.38 ± 0.05^{ab}	0.42 ± 0.04^{ab}	0.47 ± 0.07^{b}	**0.43 ± 0.05**
	Total	**$0.37\pm0.04^{\dagger}$**	**$0.37\pm0.04^{\dagger}$**	**$0.41\pm0.07^{\dagger\S}$**	**$0.44\pm0.08^{\S}$**	
Ps I growth rate (mm/day)	Abt	0.50 ± 0.05^{b}	0.50 ± 0.05^{b}	0.54 ± 0.04^{b}	0.55 ± 0.02^{ab}	**0.52 ± 0.04**
	Saj	0.55 ± 0.05^{ab}	0.53 ± 0.07^{b}	0.54 ± 0.09^{b}	0.57 ± 0.06^{a}	**0.55 ± 0.07**
	Total	**0.53 ± 0.05**	**0.52 ± 0.06**	**0.54 ± 0.07**	**0.56 ± 0.04**	
Length at settlement (mm)	Abt	22.0 ± 3.4^{b}	20.4 ± 4.0^{b}	23.4 ± 4.1^{b}	27.5 ± 3.3^{b}	**$23.4\pm4.0^{*}$**
	Saj	24.5 ± 5.2^{b}	23.0 ± 3.7^{b}	25.8 ± 2.5^{ab}	30.1 ± 4.7^{a}	**25.9 ± 4.1**
	Total	**$23.4\pm4.3^{\dagger\S}$**	**$21.7\pm3.8^{\dagger}$**	**$24.6\pm3.3^{\dagger\S}$**	**$28.7\pm4.0^{\S}$**	
Length at 120 days (mm)	Abt	61.8 ± 4.5^{a}	59.6 ± 4.0^{a}	61.3 ± 3.2^{a}	63.4 ± 1.8^{ab}	**61.5 ± 3.5**
	Saj	62.2 ± 4.9^{a}	61.4 ± 3.1^{a}	65.4 ± 4.8^{b}	63.3 ± 1.6^{ab}	**62.4 ± 3.6**
	Total	**62.0 ± 4.7**	**61.0 ± 3.4**	**63.4 ± 3.8**	**63.3 ± 1.7**	

Abbreviations: Pl－planktonic; Ps I－first period of post-settlement; Abt－Aburatsubo; Saj－Sajima; 120 days after extrusion was found in 92% fish aged

^{a,b} represents significant differences among extrusion groups in each study area, †,§ indicates significant differences among extrusion groups with both areas combined, and asterisk (＊) represents significant differences between study sites. Data are shown as mean±SD; two-way ANOVA;SNK; $P<0.05$

4. Discussion

The settlement patterns and the nutritional status exhibited by *S. inermis* juveniles in the two bays from Miura Peninsula in central Japan were investigated in the present study. The larvae from the early extrusion groups (Dec and Jan) experienced lower temperatures during the Pl period than the later groups (Feb and Mar), and the larvae from the early groups remained in planktonic habitat for shorter periods with lower growth rates. This phenomenon was confirmed by the correlation between the cumulative SST and DGI data (Table 4) and by a clear seasonal discrimination of early (winter) and late (spring) extrusion groups (Fig. 6a, Table 6). Settlement timings seemed to depend on environmental conditions: the larvae settled earlier at smaller sizes when they experienced unfavorable conditions (e.g. lower temperature) during the Pl period and grew more after settlement (Fig. 4). This pattern could be interpreted as one adaptive strategy of this species to maximize the growth rate and enhance survival during the early life stages in different environmental conditions, and here it was found to be related with temperature as found in other species (LOCKETT and SUTHERS, 1998, ÁLVAREZ *et al.*, 2012). HURST *et al.* (2005) refers to the increasing growth rate in juveniles as a “compensatory growth” mechanism, in response to temperature reduction and growth history of the individuals. The same mechanism might help to explain the early settlement and respective higher growth rates after settlement found in early groups (Dec and Jan).

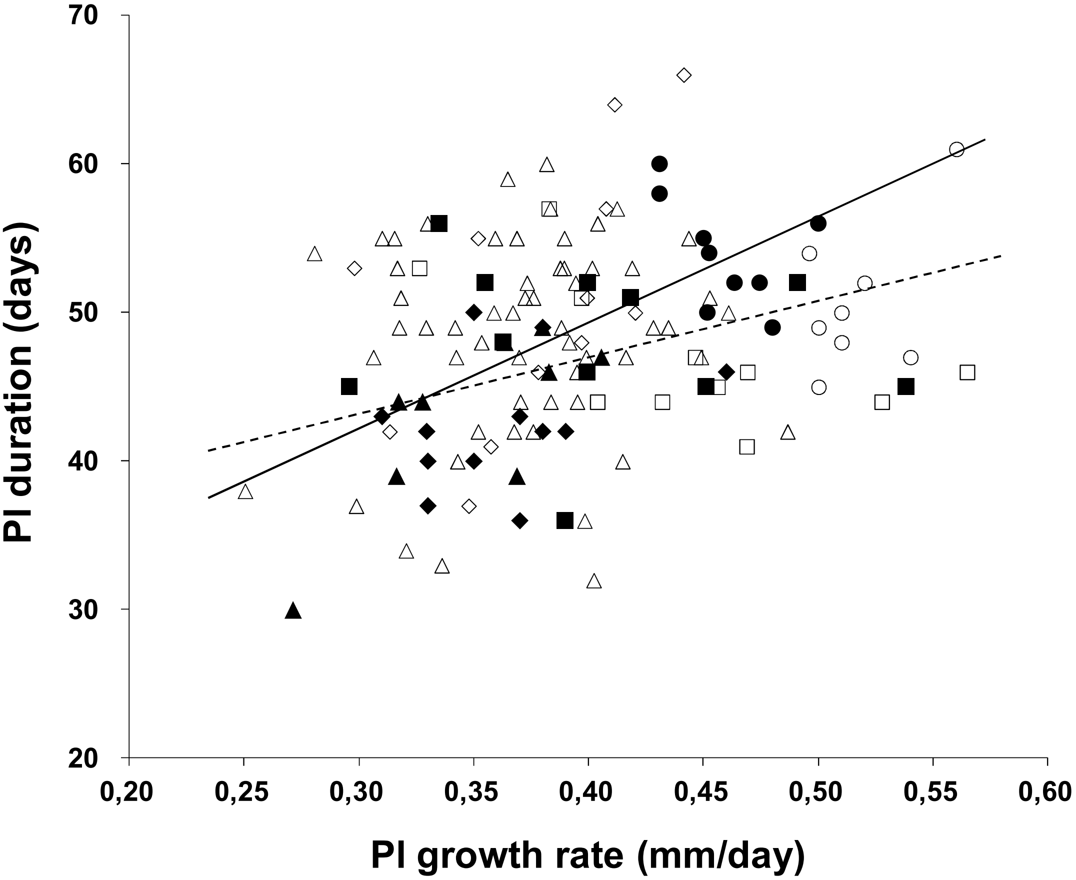

Fig. 5. Relationship between planktonic (Pl) duration and growth rate for distinct extrusion groups of *S. inermis*: December (◇), January (△), February (□), and March (○) with open symbols for Sajima and filled symbols for Aburatsubo. Regression line is shown for Sajima, dashed line ($y=23.0x+38.9$), and Aburatsubo, solid line ($y=51.5x+25.9$).

Plaza *et al.* (2003) found that settlement pulses in *S. inermis* into seagrass areas were induced by selective tidal currents, with governance by semi-lunar cycles crucial for the active migration of these larvae into nearshore areas. However, our results indicated a weak synchronism of extrusion dates and spring tides, and a much weaker relation between tidal variation and the settlement pattern of larvae in both sites (Fig. 3). Although the cause of such differences is not clear, tidal variation does not seem to be the main determinant of habitat shift from planktonic to benthic life in *S. inermis* larvae in our case study. Therefore, further studies will need to examine the effects of abiotic factors in the future.

Duration and growth rate of Pl stage correlated positively in both sites (Fig. 5) in all extrusion groups except in February where this relation was negative. Mizusawa *et al.* (2004) also found a negative relationship between these variables for individuals from two sites in similar geographic area, but those authors examined a shorter extrusion period. We believe that temperature is the main factor responsible for the positive relationship of these variables, as a cue to the reaction norm in their adaptive strategy as discussed above. Nevertheless, we also believe the negative trend found in February group could be explained by the presence of an ontogenetic stabilizing effect. Because size is related to the product of growth rates and

Table 4. Pearson correlation coefficients between DGI variables and the respective cumulative SST (℃) for Sajima and Aburatsubo

	DGI variable	*Correlation coefficient*	*P-value*
Sajima (n=121)	Pl growth rate	0.82	<0.0001
	Pl duration	0.92	<0.0001
	Ps I growth rate	0.43	0.015
	Ps I duration	0.97	0.001
	Length at settlement	0.67	<0.0001
	Length at 120 days	0.51	0.003
Aburatsubo (n=108)	Pl growth rate	0.86	<0.0001
	Pl duration	0.91	<0.0001
	Ps I growth rate	0.58	0.0003
	Ps I duration	0.96	0.041
	Length at settlement	0.79	<0.0001
	Length at 120 days	0.45	0.004

Abbreviations: Pl－planktonic; Ps I－first period of post-settlement; 120 days after extrusion was found in 92% fish aged; Pearson correlation significant for P<0.05

Table 5. Condition indices for the rockfish *Sebastes inermis* in Sajima and Aburatsubo at different sampling dates

		Sampling month			***Total***
		May (n=31)	June (n=33)	July (n=32)	
RNA：DNA ratio	Abt	1.92±0.54^{a}	1.43±0.27^{ab}	1.05±0.19^{b}	**1.32±0.47***
	Saj	1.97±0.42^{a}	1.76±0.51^{ab}	1.99±0.37^{a}	**1.88±0.33**
	Total	**1.95±0.68**†	**1.59±0.41**§	**1.31±0.28**§	
Total proteins (μg/mg tissue)	Abt	37.65±3.81^{a}	50.2±2.21^{c}	47.37±7.83^{bc}	**44.00±4.13***
	Saj	43.45±4.86^{b}	63.4±5.68^{d}	51.65±5.72^{cd}	**47.76±6.28**
	Total	**41.73±5.62**†	**52.90±3.39**§	**48.44±6.74**¶	
Fulton's K	Abt	1.55±0.20^{a}	1.68±0.16^{ab}	1.72±0.11^{b}	**1.66±0.16**
	Saj	1.68±0.22^{ab}	1.86±0.20^{b}	1.75±0.17^{b}	**1.70±0.21**
	Total	**1.64±0.22**	**1.75±0.17**	**1.71±0.12**	

Abbreviations: Abt－Aburatsubo; Saj－Sajima
^{a,b,c,d} represent significant differences among sampling months from each study site, †,§,¶ indicate significant differences among sampling months with both sites combined, and asterisk (＊) represent significant differences between study sites. Data are shown as mean±SD; two-way ANOVA; SNK; P<0.05

period duration, respective settlement sizes will be stabilized if there is a negative relationship between the growth rates and duration of the Pl stage, even if individual variability in growth rates is present within a group. This suggested scenario is schematized in Fig. 7.

Regarding the planktonic stage duration, CHAMBERS and LEGGETT (1987) suggested that a negative relationship between growth rate and duration of larval period is favorable to larval survival: larvae that grow faster stays shorter period in vulnerable stage and enhances survival probabilities (i.e. "stage duration" mechanism). The results for the reaction norm to different temperature observed in the present study were not conformable to such hypothesis so other growth-related mechanism might be occurring in this case. TAKASUKA *et al.* (2004) refers that longer planktonic periods can be advantageous for a successful recruit-

Table 6. Summary of the discriminant analysis (CDA) performed on overall dataset regarding *Sebastes inermis* juveniles in order to identify: sites and extrusion groups based on DGI variables; sites and sampling months based on nutritional condition indices

Discriminant parameters	*DGI*			*Nutritional condition indices*		
	Site	Extrusion group		Site	Sampling month	
Wilk's Lambda (λ)	0.89	0.67		0.67	0.54	
F value	2.56*	2.88**		15.01**	11.04**	
Variability explained(%)	*DF1* 100	*DF1* 59.1	*DF2* 31.4	*DF1* 100	*DF1* 86.3	*DF2* 13.7
Variables loading	Length at settlement Otolith radius at end of Ps I Ps I duration	Pl growth rate Length at settlement Otolith radius at end of Pl	Otolith radius at end of Ps I Ps I duration Ps I growth rate (-)	RNA：DNA ratio	Total proteins (-)	RNA：DNA ratio
Centroids of the groups (*discriminant scores*)	Saj (-0.19) Abt (0.62)	December (-0.39) January (-0.22) February (0.4) March (1.62)	December (0.45) January (-0.21) February (0.56) March (-0.52)	Saj (0.55) Abt (-0.87)	May (0.88) June (-0.78) July (-0.72)	May (0.01) June (0.55) July (-0.35)
Classification success (%)	82.0	78.3		83.9	77.2	

$*P<0.05$, $**\ P<0.001$; (-) indicates variable loading the discriminant function negatively
Abbreviations: DF1,2-discriminant functions 1,2

ment since the probability of larvae to be transported to favorable nursery grounds is higher. It seems that the individuals here studied might be adopting such strategy (i.e. December and January groups in Sajima), yet further investigation should be conducted to clarify this issue.

Regarding the first hypothesis of our study (H_1), settlement of *S. inermis* along Miura Peninsula coast presented temporal differentiation. The larval adaptive strategy as a reaction to different water temperature was the main determinant of the settlement.

Several aspects help to interpret the geographical range of the larval transport and settlement: the clear differences in the pattern of daily extrusion and settlement between the study sites with earlier extrusion in Saj (Fig. 3a), the significant differences of Pl growth rate and Ps I duration between the sites (Table 3, Fig. 5), and the clear differentiation of sites using DGI data, concerning variables related with the Pl and Ps stages (Table 6). We suggest that the groups in both bays are highly separated and larval distribution offshore is unlikely. The differences between the sites as stated above would be eliminated if this offshore mixing had occurred. Concerning the second tested hypothesis (H_2), larval transport and settlement occurred separately for each bay, and migration if it occurs, seems to be confined to a small geographical range around

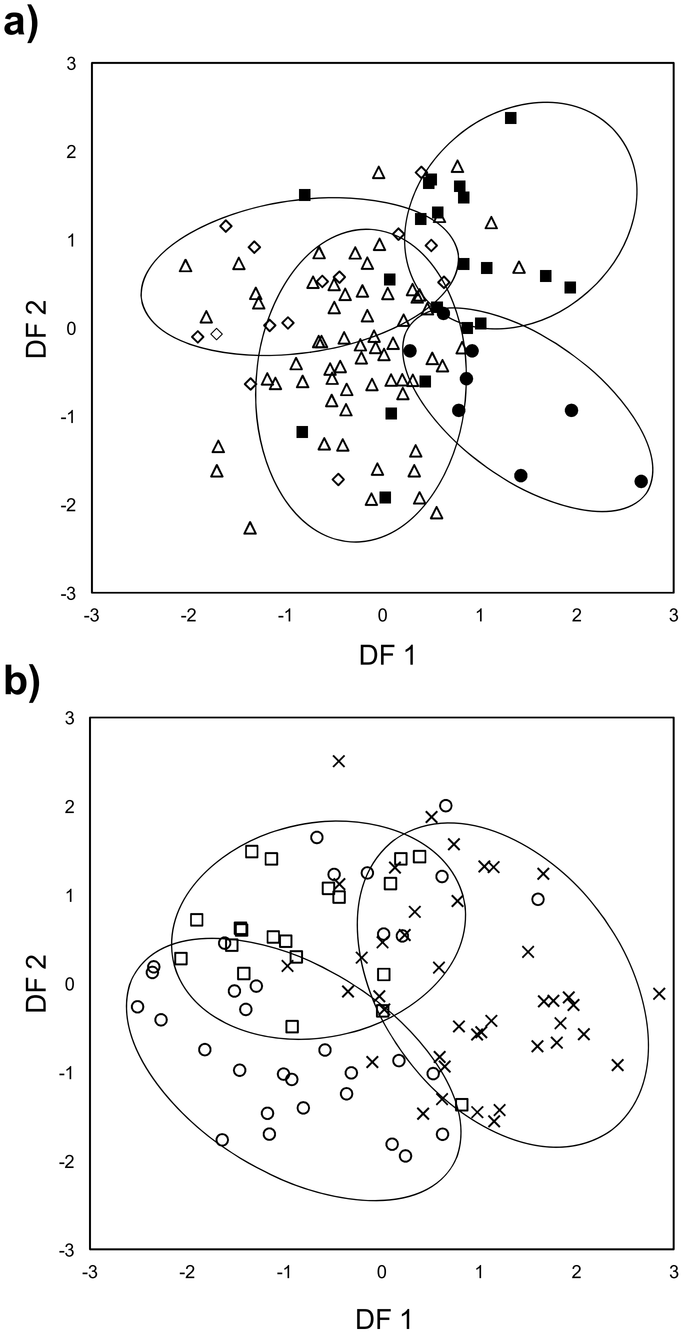

Fig. 6. Canonical discriminant analysis (CDA) based on DGI variables (a) and on nutritional condition indices (b) for rockfish juveniles: a) Canonical discriminant scores of the four extrusion groups from both sites combined, based on the first two discriminant functions (DF1&2) with 78% of individuals successfully classified. Groups represented using: December (◇), January (△), February (■), and March (●), b) Canonical discriminant scores of the three sampling months from both sites combined, based on the first two discriminant functions (DF1&2) with 77% of individuals successfully classified. Months represented as: May (X), June (□) and July (○).

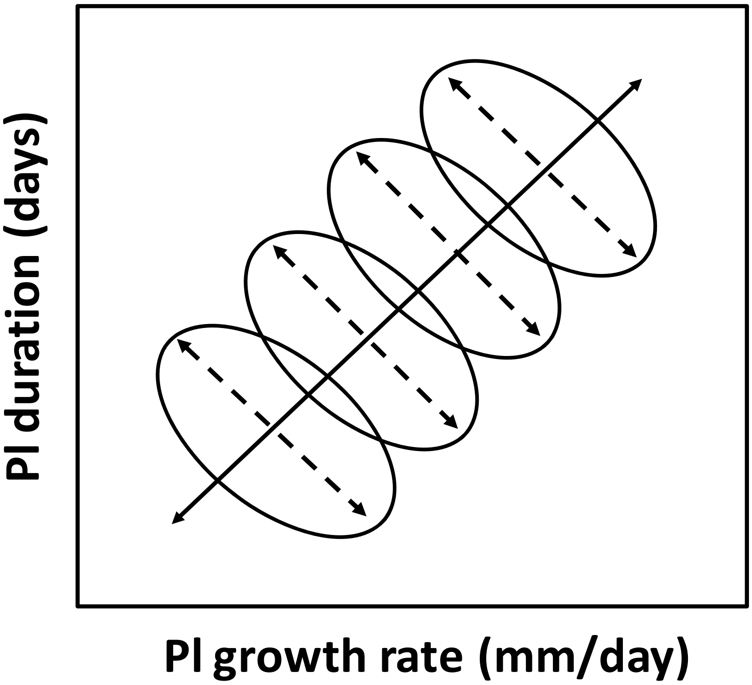

Fig. 7. Diagram of the relationship between Pl duration and Pl growth rate. Ellipses show the range of variability in each extrusion group. Solid arrow shows the direction of reaction norm to different temperature for different extrusion groups. Dashed arrows show the direction of individual's variability within each extrusion group caused by an ontogenic stabilizing effect.

each bay.

Average total length of settled juveniles increased amongst sampling dates in both sites (Table 2). The fact that individuals from early groups (Dec and Jan) were scarce or absent from the later sampling months (Fig. 3a) suggests a possible ontogenic movement of juveniles to adjacent nearshore areas in each site after several months after settlement. Gear selectivity effect was considered negligible partly because adults were also captured and partly because HARADA (1962) also described a short distance movement of post-juveniles to adjacent areas in this species. Such movement could be happening in a possible response to a change in resource requirements and vulnerability to predation (LOVE *et al.*, 1991). From the results of the present study, it is not possible to determine the geographic scale of such movement, therefore, a broader study on the sub adult and adult population is recommended to fully assess this issue.

Differences found in condition indices (Table 5) indicated that juveniles from Saj are in overall better physiological status. This conclusion was supported by CDA (Table 6), where RNA : DNA ratio was determinant. This index is correlated with food availability and is a good indicator of the nutritional condition (GARCIA *et al.*, 1998, GEIGER *et al.*, 2000, ISLAM and TANAKA, 2005). Based on this relationship, the higher values of RNA : DNA ratio found in juveniles from Saj reflect higher energetic reserves after settlement as observed in other species (VASCONCELOS *et al.*, 2009). On the other hand, the lowest indices found in juveniles from Abt might reflect a lower survival potential, as also found in other species (BLACK and LOVE, 1986, PASTOUREAUD, 1991).

Based mainly on the biochemical condition indices rather than the morphometric condition index, *S. inermis* juveniles at the settlement ground were clearly discriminated among sampling months (Fig. 6b). In addition, RNA :

DNA ratio and total proteins correlated positively with the increment widths of the otolith margin. It suggests that there is a positive relationship between fish condition and growth rates as found in other species (MALLOY and TARGETT, 1994, BUCKLEY *et al.*, 1999, RAMIREZ *et al.* 2004). The use of all condition indices (to our knowledge, this is the first time assessment for this species) allows an ecological evaluation of fish habitat (LLORET *et al.*, 2001, ISLAM *et al.*, 2006, VASCONCELOS *et al.*, 2009). Our case showed that Sajima Bay is a higher-quality nursery ground with more suitable characteristics for *S. inermis* early stage development, which supports the third hypothesis (H_3). Saj has larger canopy areas which provide higher protection to the settled juveniles. In addition, the water in this bay may present higher nutrient enrichment since there is an influence of a stream in this area, and this may bring ecological benefits (e.g. LLORET *et al.*, 2001).

The present study provided basic biological information on the early life of *S. inermis*. The results enabled the differentiation of two geographical groups using an integrative analysis of life traits and nutritional condition. Further studies will need to focus on a larger temporal range and on adult populations to enhance the comprehension of inshore/offshore connectivity of populations, for this commercially important species in Japan.

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