

Temporal patterns of growth in larval cohorts of the Japanese sardine *Sardinops melanostictus* in a coastal nursery area

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Growth patterns of larval sardine *Sardinops melanostictus* were studied in a coastal nursery area, in southern Japan for four monthly hatch cohorts of larvae (November, December, January and February) for the 2003–2004 and 2004–2005 seasons. Laird–Gompertz models were fitted to each cohort using both total length (L_T)-at-age at capture and mean L_T -at-age data derived from backcalculations. In both approaches, the absolute daily growth rates (G_R) and absolute daily growth rates at the inflection point (G_{XO}) were estimated. In parallel, individual growth rates (G_I) were derived from backcalculated L_T (L_B). Growth showed the following general common patterns irrespective of hatch month, season and methods: (1) significant Laird–Gompertz fits, (2) asymptotic growth, (3) a decrease in G_R after the inflexion point, except for February for the 2003–2004 season that showed an apparent constant growth pattern, (4) six in eight cohorts showed G_{XO} ranging from 0.8 to 1.2 mm day⁻¹ and (5) a decreasing tendency of G_I from 1.75 to 0.24 mm day⁻¹, from first feeding through the first month of larval life. The contrasting pattern between the 2003–2004 and the 2004–2005 seasons were: (1) allometric v. logarithmic (ln) L_T and otolith radius relationships, (2) low G_{XO} v. high G_{XO} , (3) high G_R v. low G_R when growth turned asymptotic, (4) low G_{XO} v. high G_{XO} when monthly hatch cohorts were combined and (5) L_B and G_I not differing among monthly hatch cohorts. The differences in growth patterns and growth rates between seasons seemed to be linked to the influx of warmer and oligotrophic waters of the Kuroshio Current that triggered an increase of 3° C in the coastal area for the 2003–2004 seasons. In the overall context, however, the high G_{XO} , within cohorts and seasons reported in the current study, suggests that either sea surface temperature (SST) or food availability, or both are in the optimal range of preferences for *S. melanostictus* larvae. Consequently, nearshore coastal areas seem to be playing an important role as a nursery area for the larval stage of this species.

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Key words: backcalculation; Gompertz; Kuroshio Current; otolith; Tosa Bay.

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INTRODUCTION

An adaptive pattern for a number of clupeoids is the selection of certain geographical areas as spawning and nursery areas. A reason for this selection could be that these areas offer good feeding conditions for adults and their offspring (Blaxter & Hunter, 1982). As a corollary, dispersal of larvae into unfavourable nursery areas would lead to high larval mortality (Blaxter & Hunter, 1982; Nakata *et al.*, 1995). Conversely, unavoidable dispersal mechanisms could transport larvae to highly productive areas and enhance larval survival (Fossum & Moksness, 1993; Olson, 2001). The latter case is true of the Japanese sardine *Sardinops melanostictus* (Temminck & Schlegel), whose larvae are transported northward from the southern spawning areas to a highly productive area (*i.e.* the Kuroshio Extension) by the Kuroshio Current (Heath *et al.*, 1998). A fraction of the spawned sardine larvae, however, are also retained in coastal nursery areas during their larval and juvenile stages (Kishida *et al.*, 1994; Heath *et al.*, 1998; Watanabe & Nakamura, 1998). Larvae transported into the Kuroshio Extension are thought to have a significant implication for the year-class strength of this species. Conversely, larvae transported offshore across the Kuroshio Current experience high mortality and consequently they seem to be less significant for recruitment (Nakata *et al.*, 1995; Zenitani *et al.*, 1996; Heath *et al.*, 1998). The importance to recruitment success of larvae retained in coastal nearshore areas is unknown.

Recent studies have suggested that growth could be a concomitant factor linked to survival, with slow growth having a negative effect on either certain vulnerable stages after first feeding or on the entire first year of fish life. If mortality is size dependent, fast growing larvae have an increased probability of survival due to the shorter time they stay in the potentially vulnerable stages thereby decreasing the risk of predation (Chambers & Leggett, 1987; Anderson, 1988; Leggett & DeBlois, 1994). The concomitant role of growth for survival can be important for *S. melanostictus* because the last recruitment failure of this species at the end of the 1980s has been attributed to cumulative mortality through the larval and juvenile stages rather than instantaneous mortality occurring at the transition of yolk-sac–first-feeding stages (Watanabe *et al.*, 1995). In addition, considering the potential role of growth for survival and the fact that the adult stock had reached its historically lowest level in 2005, information on the patterns of growth of the larvae is critical for future comparisons when the population has recovered again.

Estimation of reliable growth rates during the early larval life can be achieved by otolith microstructure analysis. By measuring the width of daily increments and based on the otolith length and larval length relationship, the somatic length at previous ages can be backcalculated (Campana, 1990; Campana & Jones, 1992). Consequently, the individual growth trajectories of larval cohorts can be reconstructed. The main goal of this study was to make a comprehensive analysis of the growth patterns of larval cohorts of *S. melanostictus* in the unique remaining spawning area in Japan (*i.e.* Tosa Bay; Ishida, 2006) by firstly estimating population growth models for monthly hatch cohorts based on age and length data, and secondly identifying the otolith radius and larval length relationship to estimate the growth history for each fish.

MATERIALS AND METHODS

SAMPLING AND OTOLITH ANALYSIS

Larval and early juvenile *S. melanostictus* were sampled weekly by commercial Shirasu boat seiners in five coastal stations in Tosa Bay: Aki, Akaoka, Haruno, Kinpo, and Tanoura (Fig. 1) from October 2003 to May 2004. The Shirasu fishery corresponds to commercial catches of later stages of three main clupeoid larvae co-occurring in coastal waters, *S. melanostictus*, the Japanese anchovy *Engraulis japonicus* Temminck & Schlegel and the round herring *Etrumeus tere* (DeKay). The seine was used for larval sampling, which consisted of a 70–80 m mouth aperture, 200–300 m in length and a 2 mm mesh cod-end. Monthly sampling was carried out from October 2004 to May 2005 in Aki because the Shirasu fishery moved to this area in this year and logistic difficulties made a more continuous sampling protocol impractical. Nevertheless, a few additional larvae collected by a bongo net, at a station offshore, Haruno, were available for this season to increase the range of total length (L_T)-on-age data (Fig. 1). It is necessary to underline that Shirasu trawlers usually sweep large shoals of clupeoid larvae making sampling representative between sampling dates and years. Because sampling intervals were not consistent between years, however, hatch date rather than catch date was used as the unit of analysis to make growth patterns comparable among monthly hatch larvae and seasons. In addition, the current paper focused on revealing the average growth pattern under the assumption that Tosa Bay can be considered as a spatial unit where differences in sampling locations within the bay did not affect intra-season and inter-season comparison of larval growth. This assumption seems to be justified because at present larvae *S. melanostictus* only come from Tosa Bay (Ishida, 2006).

Random samples of c. 1 kg of larvae were taken and preserved in 90% ethanol for larval identification and otolith analyses. Once larvae were identified, the L_T from random sub-samples of 50 larvae per week (*i.e.* weekly sampling for the 2003–2004 period) and 200 larvae per month (*i.e.* monthly sampling for the 2004–2005 period) were measured to the nearest 0.1 mm without correcting for shrinkage and then used in otolith

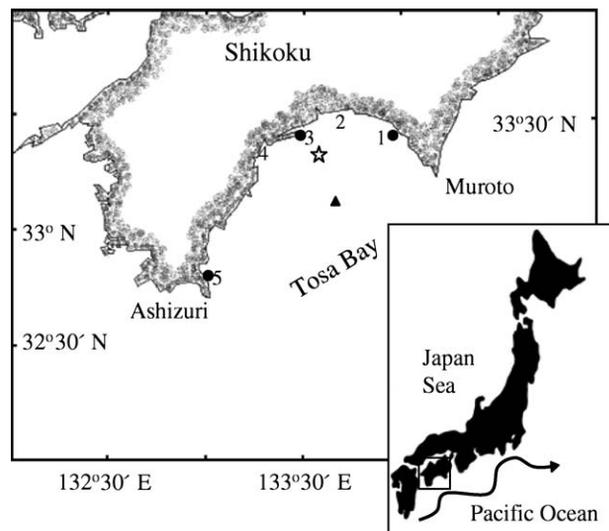


FIG. 1. Coastal locations (1, Aki; 2, Akaoka; 3, Haruno; 4, Kinpo; 5, Tanoura) where larval *Sardinops melanostictus* were collected by Shirasu boat seiners in Tosa Bay, southern Japan. ☆, location of planktonic samples for the 2004–2005 seasons; ●, locations where the sea surface temperature was measured; ~, the path of the Kuroshio Current; ▲, location of the buoy N° 12.

analyses. Each pair of sagittae was removed using fine needles, cleaned from adhering tissue and mounted on glass slides with a drop of nail varnish. Growth increments were clearly discernible; hence, no further polishing was required. Otolith radii (R_O) and increment widths were measured along a linear axis from the core along the post-rostrum of the sagittae, which was consistently the best axis for increment counting and measurement. All counting and measurements were carried out using an image analysis system (LATOC Co. Ltd, Tokyo, Japan) at magnifications of $\times 400$ – 1000 . Otolith increments for *S. melanostictus* are deposited on a daily basis from the third day after hatching, which corresponds to first feeding (Hayashi *et al.*, 1989; L_T range of validation: 5–25 mm). Hence, the total daily age was estimated as the number of increments +3 days. Hatch date distributions of larvae were backcalculated by subtracting the total age in days from the calendar day of sampling. Hatch dates distributions were not corrected for mortality effects because survival rates during the larval stage were not available for the years studied.

For the purpose of the present paper, a total of 302 larvae were aged for the 2003–2004 seasons and 465 larvae for the 2004–2005 seasons, distributed on a monthly hatch date (Table I). Sampling size was lower than the original sub-sample because some larvae showed size erosive features produced by Shirasu seiners and in other cases some otoliths were broken during the extraction. Hence, these examples were excluded.

GROWTH PATTERNS

The Laird–Gompertz curve (equation 1) was used to assess the relation between larval L_T and age (x ; L_{TX}) for monthly hatch larvae. The function has three coefficients, L_∞ is the asymptotic length, X_O is the inflexion point of the curve and α is the instantaneous growth rate at age X_O (Campana & Jones, 1992).

$$L_{TX} = L_\infty e^{\{e^{-\alpha(X-X_O)}\}} \quad (1)$$

In this equation, the absolute growth rate G_R at any given age is estimated as $G_R = \alpha L_{TX} (\ln L_\infty - \ln L_{TX})$ and the absolute growth rate at the inflexion (G_{XO}) point as $G_{XO} = \alpha L_{XO} (\ln L_\infty - \ln L_{XO})$, where L_{XO} is the estimated L_T at X_O . Backcalculation on L_{TX}

TABLE I. Summary statistics of mean \pm s.d. total length (L_T) and age of larval *Sardinops melanostictus* collected monthly in Tosa Bay southern Japan, from autumn to spring, 2003–2004 and 2004–2005 seasons

Season	Hatch		L_T (mm)		Age (days)	
	Month	n	Mean \pm s.d.	Range	Mean \pm s.d.	Range
2003–2004	November	53	21.6 \pm 4.0	14–30	26.6 \pm 8.6	11–51
	December	91	23.6 \pm 4.5	12–30	27.7 \pm 7.5	13–48
	January	92	22.6 \pm 4.0	11–30	26.6 \pm 6.6	9–43
	February	66	21.3 \pm 5.2	12–30	23.9 \pm 7.0	9–40
	Total	302	22.4 \pm 4.5	11–30	26.4 \pm 7.4	9–51
2004–2005	November	160	18.9 \pm 7.2	4–30	20.3 \pm 8.7	3–40
	December	96	19.1 \pm 3.1	12–25	19.8 \pm 3.8	13–36
	January	135	16.1 \pm 7.4	4–28	18.1 \pm 8.8	3–39
	February	74	19.5 \pm 4.6	11–27	19.6 \pm 6.8	8–35
	Total	465	18.2 \pm 6.4	4–30	19.4 \pm 7.7	3–40
Pooled	Overall	767	19.9 \pm 6.1	4–30	22.2 \pm 8.3	3–51

at previous ages can be made using several methods. To be consistent with previous published information on backcalculated growth of this species, backcalculation on L_T at previous ages was made using the biological intercept method (BIM; Campana, 1990; equation 2).

$$L_T = L_c + (R_i - R_c)(L_c - L_T)(R_c - R_h)^{-1} \quad (2)$$

where L_T is the larval L_T at hatching, *i.e.* the biological intercept. R_h is the otolith radius at the hatch check, L_c is the L_T of the fish at capture, R_i is the otolith radius at a previous age and R_c is the otolith radius at the time of capture. The daily individual growth rates (G_1) were estimated by the formula $G_1 = (L_{T+1} - L_T)$. The biological intercept in the current study was set at 5 mm L_T at first feeding, following the criteria described by Watanabe & Kuroki (1997), based on a shrinkage factor of 0.85 and 0.82 after 10 and 20 min net treatments. As ethanol did not cause any additional shrinkage in a related species (*i.e.* *Engraulis mordax* Girard; Theilacker, 1980), further corrections were not needed (Watanabe & Kuroki, 1997).

COMPARISON OF GROWTH CURVES

This study was focused on fitting Laird–Gompertz models to monthly hatch larvae using both L_T -at-age at capture and L_T at age derived from backcalculation. Growth coefficients were estimated using the dual quasi-Newton method implemented in PROC NLMIXED module of SAS statistical software (SAS Institute, Inc., Cary, NC, U.S.A.). The goodness of fit of each model was evaluated by examining residual mean-square error (MSE), coefficient of determination (r^2) and level of significance ($P < 0.05$). The asymptotic length (L_∞) and instantaneous growth rate at the inflexion point (α) were compared by using the ‘contrast statement’ of PROC NLMIXED in which F -tests for each statement use the delta method to approximate the variance–covariance matrix (SAS Institute, Inc.). Comparison of growth coefficients were made among monthly cohorts and between seasons. In addition, to test for significant differences in backcalculated L_T (L_B) and individual growth rates (G_1) among hatched month and season, univariate and multivariate repeated measures ANOVA were used, following the criteria described by Chambers & Miller (1995). L_B and G_1 were estimated for each larva at each day after first feeding, but to reduce the elevated numbers of comparison over age, all RM ANOVAs, were achieved on balanced set of repeated measures (*i.e.* at 3, 6, 9, 12, 15, 18 and 21 days). To address where differences in L_B increased with age, univariate F -tests (Chambers & Miller, 1995) were used. In addition, to better illustrate the differences in growth over age and among cohorts, the data were separated into three groups: (1) 3–7, (2) 8–15 and (3) 15–21 days. Each group was separately analysed by plotting the first and second canonical scores with monthly cohort used as grouping variable and L_B at age used as explanatory variables. Similar procedures were applied for G_1 .

ENVIRONMENTAL DATA

To examine the seasonal variations in surface water temperature (SST) in the study area, weekly data were obtained at three stations from November to April in both seasons (Fig. 1), published by the Japan Meteorological Agency (JMA; <http://www.jma.go.jp/en/menu.html>). In addition, weekly SST were obtained from a monitoring buoy from the Kochi Prefectural Research Center located at 33°07' N; 133°37' E in Tosa Bay (Fig. 1). To evaluate the degree of intrusion of Kuroshio waters into Tosa Bay, weekly reports of the mean distance from the Kuroshio path to the Ashizuri and Muroto's Caves (published by JMA) were compared between the 2003–2004 and 2004–2005 seasons. One-way ANOVA (Sokal & Rohlf, 1995) was used to test for significant

differences in SST between seasons. The Shapiro–Wilks and Bartlett tests were used to test for normality and homogeneity of variance in the ANOVA procedures, and ln transformations were made when it was necessary. The Kruskal–Wallis K-S test was used to test for significant differences in the intrusion of Kuroshio waters into Tosa Bay between seasons because the assumptions of normality and homogeneity of variance were not met.

RESULTS

FREQUENCY DISTRIBUTIONS OF TOTAL LENGTH AND AGE

The L_T and age distributions in almost all cases overlapped within monthly hatch larvae and seasons but showed differences in shape (Fig. 2). For the 2003–2004 seasons, larvae ranged from 11 to 30 mm L_T and from 9 to 51 days, with mean values differing only in *c.* 3 mm, whereas for 2003–2004, larvae ranged from 4 to 30 mm and from 3 to 40 days. Differences in mean values of L_T and age were no >2 mm when smaller larvae collected offshore with planktonic nets were excluded (Fig. 2).

GROWTH PATTERNS

Allometric and logarithmic models fit well to the R_O -on- L_T data for the 2003–2004 [Fig. 3(a)] and 2004–2005 [Fig. 3(b)] seasons; hence, equation 2

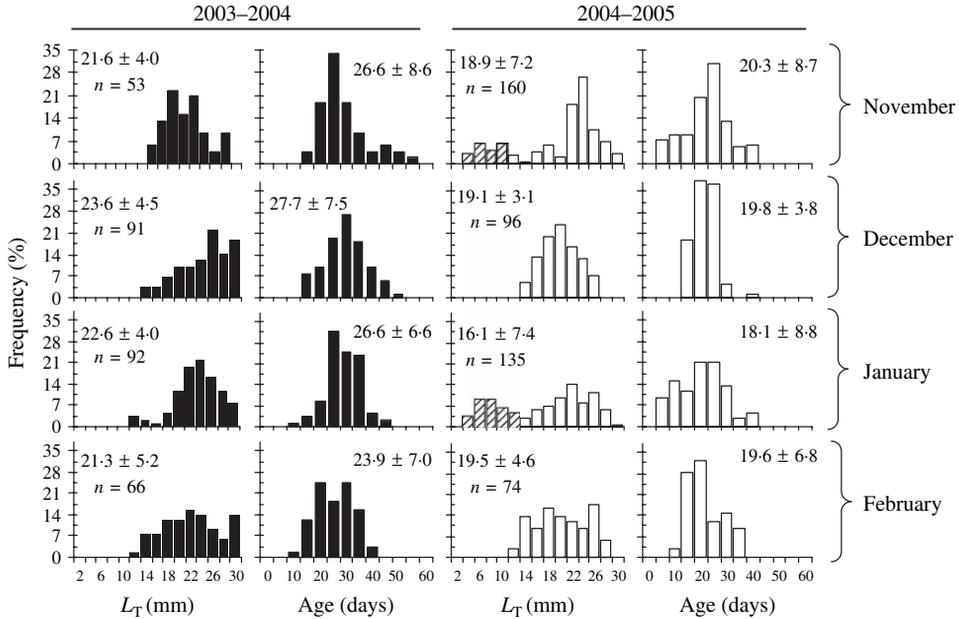


FIG. 2. Age and total length (L_T) frequency distribution [means \pm s.d. and sample size (n) are given] for four monthly cohorts and two seasons of larval *Sardinops melanostictus* collected in the Shirasu fishery ground in Tosa Bay, southern Japan. ▨, planktonic larvae collected from the offshore station ▣, 2003–2004; ▣, 2004–2005.

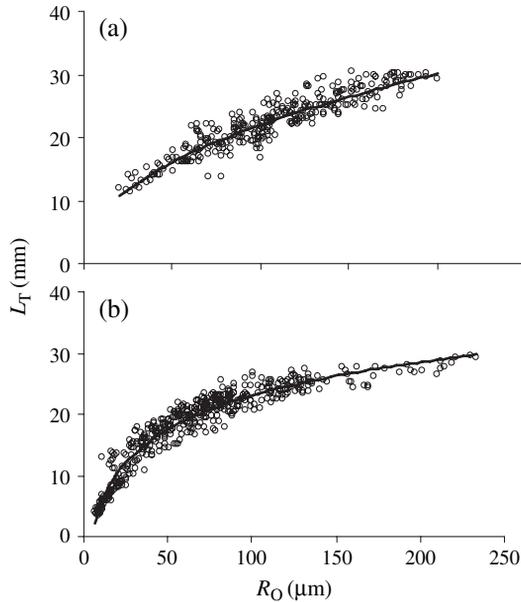


FIG. 3. Relationships of the otolith radius (R_O) and total length (L_T) for the (a) 2003–2003 and (b) 2004–2005 seasons of *Sardinops melanostictus* larvae collected in Tosa Bay, southern Japan. The curves were fitted by: (a) $y = 2.73x^{0.45}$ ($r^2 = 0.86$, $n = 302$) and (b) $y = 7.92 \ln x - 13.44$ ($r^2 = 0.94$, $n = 465$).

was modified accordingly as $L_{T_i} = \ln L_c + (\ln R_i - \ln R_c) (\ln L_c - \ln L_{Th}) (\ln R_c - \ln R_h)^{-1}$ and $L_T = L_c + (\ln R - \ln R_c) (L_c - L_{Th}) (\ln R_c - \ln R_i)^{-1}$, respectively, to estimate L_B and G_I . Both allometric ($r^2 = 0.82$, $F_{1,390}$, $P < 0.001$) and logarithmic ($r^2 = 0.86$, $F_{1,390}$, $P < 0.001$) models fit the R_O -on- L_T data for the 2004–2005 seasons when larvae < 10 mm L_T were excluded from the analysis, although only in the former there was no apparent heterocedasticity in the residuals.

In an overall description, the following general common growth patterns irrespective of hatch month and season were found (Figs 4 and 5): (1) significant Laird–Gompertz fits, (2) 6 of 8 months showed growth rates at the inflection point (G_{XO}) ranging from 0.8 to 1.2 mm day $^{-1}$ and (3) a decreasing tendency of backcalculated individual growth rates from 1.75 to 0.24 mm day $^{-1}$ from first feeding (day 3) through the first 30 days of larval life. The contrasting growth patterns between the 2003–2004 and the 2004–2005 seasons were: (1) allometric v. $\ln L_T$ and R_O relationships, (2) low G_{XO} (range: 0.6–0.9 mm day $^{-1}$) v. high G_{XO} (range: 0.8–1.2 mm day $^{-1}$), (3) high backcalculated individual growth rates (G_I , range: 0.3–0.6 mm day $^{-1}$) v. low G_I , (range: 0.2–0.4 mm day $^{-1}$) when growth turned asymptotic and (4) backcalculated L_T (L_B) and G_I not differing among months.

In a more detailed description, comparison of L_∞ and α through ‘contrast statement’ of PROC NLMIXED derived from backcalculated data showed significant differences in almost all hatched months (Table II), although both coefficients did not differ between seasons (Table II; pooled). Multivariate

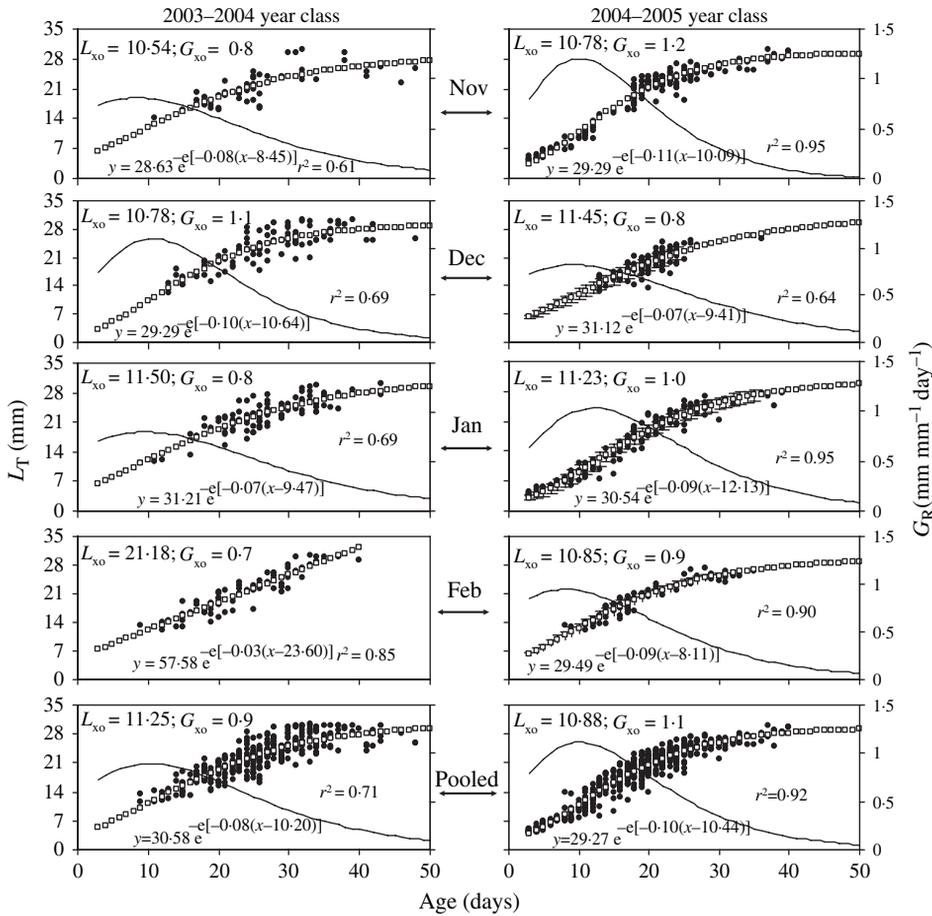


FIG. 4. Monthly and combined analyses of Laird–Gompertz models [—, daily growth rate (G_R); □, fitted Laird–Gompertz equation] of the form $L_T = L_\infty e^{-e^{-\alpha(L_T - X_0)}}$ based on observed total length (L_T)–on-age (x) data (●; cross-sectional) for larval *Sardinops melanostictus* collected in Tosa Bay, southern Japan for the 2003–2004 and 2004–2005 seasons. L_∞ , asymptotic length; α , instantaneous growth rate at the inflexion point (X_0), ($L_{X_0} = L_T$ at X_0); G_{X_0} = absolute growth rate at X_0 $F_{G_{X_0}} = [\alpha L_{X_0} (\ln L_\infty - \ln L_{X_0})]$.

repeated ANOVA also showed differences in individual growth patterns in both L_B and G_I among hatched months over the 3 day interval (Table II), although significant differences in both the multivariate repeated measured ANOVA and the univariate F -tests were only detected for the 2004–2005 seasons (Tables III and IV). Scatter plots of canonical scores for season class showed that inter-monthly differences in L_B and G_I occurred across the entire studied period (*i.e.* 3–21 days), but it was more marked during the first and third week of larval life (Fig. 6). Inter-annual comparison in RM ANOVA showed significant differences in L_B (Wilks $\lambda_{6,492} P < 0.001$) and G_I (Wilks $\lambda_{6,492} P < 0.001$) between seasons, with larvae growing slower for the 2003–2004 seasons than for the 2004–2005 seasons during the onset of larval life, and *vice versa* towards the end of the first month when differences in L_∞ were

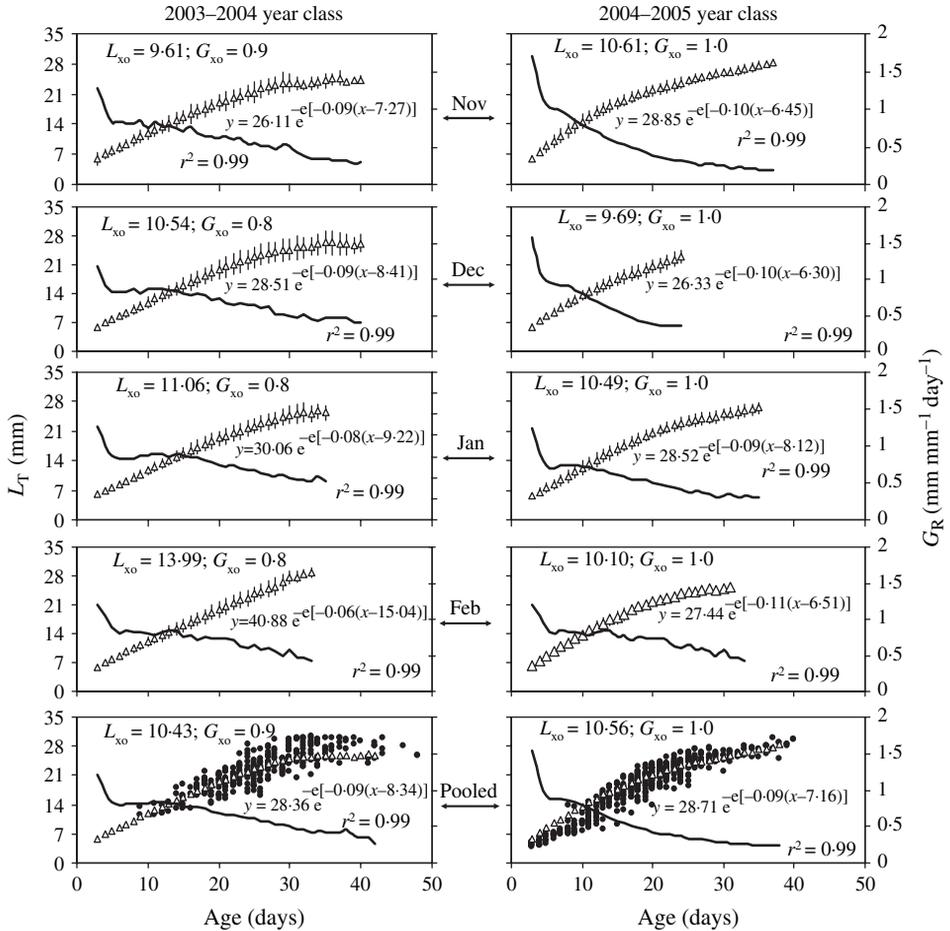


FIG. 5. Monthly and combined analyses of Laird-Gompertz models of the form $L_T = L_\infty e^{-\alpha(x-x_0)}$ based on backcalculated total length (L_T)-at-age (x) data (longitudinal) for larval *Sardinops melanostictus* collected in Tosa Bay, southern Japan for the 2004–2005 and 2004–2005 seasons (—, individual daily growth rates estimated from backcalculation; Δ , mean \pm S.D. back-calculated L_T ; \bullet , observed L_T -at-age data). L_∞ , asymptotic length; α , instantaneous growth rate at the inflexion point (X_0), ($L_{X_0} = L_T$ at X_0); G_{X_0} = absolute growth rate at X_0 [$G_{X_0} = (\alpha L_X / (\ln L_\infty - \ln L_X))$].

not significantly different between seasons (Fig. 5; pooled and Table III). Multi-comparison of L_∞ and α for L_T -on-age data were not achieved because the range of ages and L_T used were not consistent among cohorts. In the combined analysis, however, significant differences were found in α between seasons when the small planktonic larvae were excluded from the analysis (Table II). In a further result, pooled backcalculated L_T -at-age data across hatch months showed that mean L_T -at-age followed the same trajectory as the observed values of L_T and age for 2003–2004. Conversely, an apparent overestimation before the inflexion point was evident for the 2004–2005 seasons (Fig. 5; pooled).

TABLE II. Results from ‘contrast statement’ of PROC NL MIXED module for instantaneous growth rate (α) and asymptotic total length (L_∞) from Laird–Gompertz fit to backcalculated data. (a) Analysis by monthly hatch larvae within seasons (subscripts: 1, November; 2, December; 3, January; 4, February). (b) Interannual comparison for observed total length-at-age data and backcalculated total length-at-age data

	2003–2004 year class		2004–2005 year class	
(a) Contrast	$F_{1,464}$	P	$F_{1,119}$	P
α_1 v. α_2	1.280	>0.05	1.290	>0.05
α_1 v. α_3	25.800	<0.0001	1.960	>0.05
α_1 v. α_4	151.740	<0.0001	4.960	<0.05
α_2 v. α_3	18.220	<0.0001	3.850	>0.05
α_2 v. α_4	141.770	<0.0001	0.120	>0.05
α_3 v. α_4	47.200	<0.0001	11.900	0.001
$L_{\infty 1}$ v. $L_{\infty 2}$	77.370	<0.0001	17.220	<0.0001
$L_{\infty 1}$ v. $L_{\infty 3}$	79.310	<0.0001	1.100	>0.05
$L_{\infty 1}$ v. $L_{\infty 4}$	161.390	<0.0001	18.680	<0.0001
$L_{\infty 2}$ v. $L_{\infty 3}$	11.930	0.001	12.130	0.001
$L_{\infty 2}$ v. $L_{\infty 4}$	112.780	<0.0001	3.110	>0.05
$L_{\infty 3}$ v. $L_{\infty 4}$	79.010	<0.0001	8.810	<0.01
	Observed total length at age*		Backcalculated total length	
(b) Contrast	$F_{1,464}$	P	$F_{1,259}$	P
α_{03-04} v. α_{04-05}	2.242	>0.05	2.57	>0.05
$L_{\infty 03-04}$ v. $L_{\infty 04-05}$	0.713	>0.05	0.41	>0.05

*Small planktonic larvae were excluded from the analyses.
 Subscripts: 04–05, 2003–2004; 04–05, 2004–2005

SEASONAL VARIATION IN SST AND INTRUSION OF KUROSHIO WATER IN COASTAL AREAS

Seasonal variation in SST and intrusion of Kuroshio waters into the coastal area SST from November to April ranged from 13 to 24° C and from 11.8 to 22.5° C for the 2003–2004 and 2004–2005 seasons, respectively; although SSTs were significantly higher in the overall analysis when all coastal stations were

TABLE III. Multivariate repeated measures ANOVA of backcalculated total length (L_B) and individual growth rates (G_I) to test for differences over 3 day intervals and within monthly hatch larvae of *Sardinops melanostictus* in each season

Variable	Season	Source	df _h	df _e	Wilks λ	F	P
L_B	2003–2004	Age	6	240	0.007	5456.779	<0.001
		Age \times cohort	18	679	0.849	2.246	<0.01
	2004–2005	Age	6	240	0.006	6516.663	<0.001
		Age \times cohort	18	682	0.524	9.739	<0.001
G_I	2003–2004	Age	6	240	0.372	67.511	<0.001
		Age \times cohort	18	679	0.871	1.881	<0.05
	2004–2005	Age	6	240	0.090	407.246	<0.001
		Age \times cohort	18	682	0.630	6.737	<0.001

df_h, hypothesis d.f.; df_e, error d.f.; Wilks λ , multivariate test statistics.

TABLE IV. Univariate F -test at 3 day intervals for backcalculated total length (L_B) and individual growth rates (G_I) in monthly hatch larvae *Sardinops melanostictus* in each season

Variable	Season	Source	Age (years)						
			3	6	9	12	15	18	21
L_B	2003–2004	F	0.716	2.325	2.364	1.342	1.407	1.289	2.224
		P	0.543	0.075	0.072	0.261	0.241	0.279	0.086
	2004–2005	F	6.918	21.583	35.018	34.511	28.017	21.726	18.119
		P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
G_I	2003–2004	F	0.772	2.719	2.472	1.346	2.732	2.463	4.086
		P	0.511	0.045	0.062	0.260	0.044	0.063	0.007
	2004–2005	F	6.834	19.878	5.334	5.422	4.629	15.377	17.530
		P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

combined [ANOVA, $F_{1,136}$, $P < 0.01$; Fig. 7(a)] for the 2003–2004 seasons than for the 2004–2005 seasons. Significant differences were also detected in the overall mean SST for the more offshore buoy [ANOVA, $F_{1,136}$, $P = 0.001$; Fig. 7(b)]. There were not significant differences in the distance from the Kuroshio Current to the Ashizuri Cape between seasons (mean \pm s.D._{2003–2004} = 27.09 \pm 8.01; mean \pm s.D._{2004–2005} = 30.45 \pm 8.29; Kruskal–Wallis = 0.64,

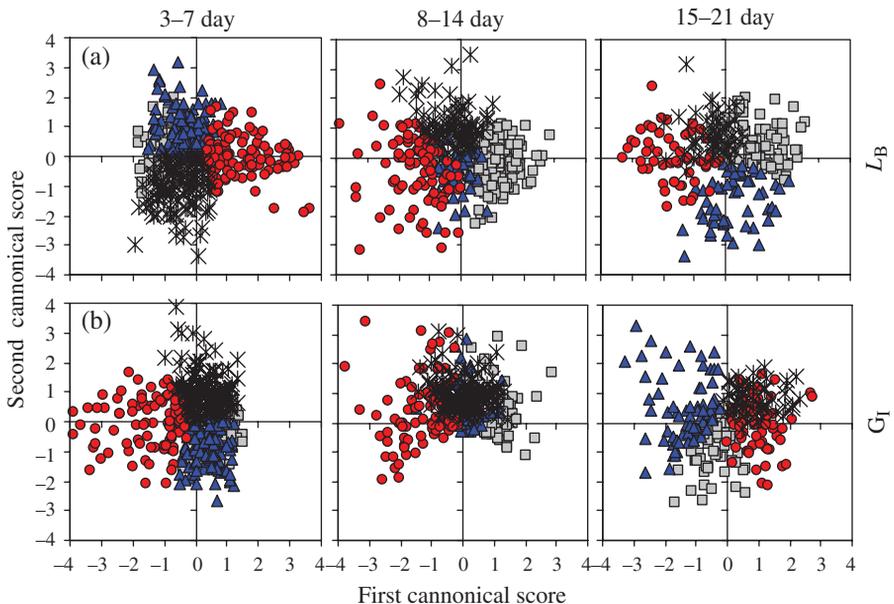


FIG. 6. Scatter plots of the first and second canonical scores derived from classification analysis for (a) backcalculated total length (L_B) and (b) individual growth rates (G_I), illustrating growth differences among cohorts within the first (3–7 days), second (8–14 days) and third week (14–21 days) of larval life of *Sardinops melanostictus* for the 2004–2005 seasons (□, November; ▲, December; ●, January; ✕, February) in Tosa Bay, southern Japan.

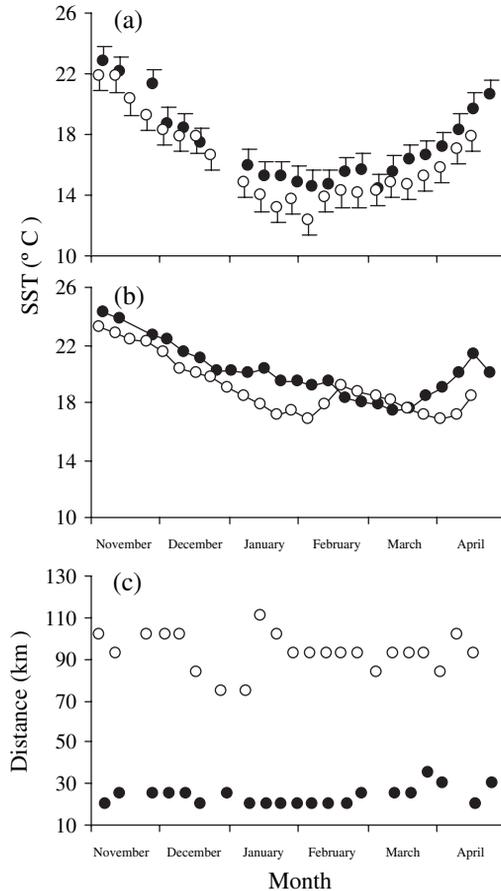


FIG. 7. Scatter plots illustrating the seasonal trend of mean \pm s.d. weekly sea surface temperature (SST) by season from (a) three coastal stations and (b) a monitoring buoy in Tosa Bay, southern Japan, and (c) weekly observed distance from Muroto cape to the main path of the Kuroshio Current for the 2003–2004 (●) and 2004–2005 (○) seasons.

$P > 0.05$), but the opposite occurred for the Muroto's Cave [mean \pm s.d._{2003–2004} = 23.57 ± 4.23 ; mean \pm s.d._{2004–2005} = 50.23 ± 4.98 ; Kruskal–Wallis = 24.7 , $P < 0.001$; Fig. 7(c)].

DISCUSSION

Before evaluating the larval growth patterns found in the current study, it must be underlined that growth coefficients estimated by observed L_T -on-age data at the time of capture are not directly equivalent to the coefficients estimated using L_T -at-age data backcalculated from otoliths (Francis, 1998). Conclusions could probably become more reliable, however, when both approaches are used at the same time to describe growth patterns. Indeed, when information from the otolith reading is retrieved, a minimal additional effort is needed to make a comparative analysis, such as illustrated in the current study.

In this study, the slightly allometric R_O and L_T relationship found for the 2003–2004 seasons contrasted with the strongly curvilinear logarithmic model of the 2004–2005 seasons, and the differences were even evident when the small planktonic larvae were excluded from the analysis (*i.e.* range: 10–30 mm L_T). These results in part agree with previous studies reporting a curvilinear R_O and L_T relationship for larvae of this species over similar ranges of larval L_T . Oozeki & Zenitani (1996) reported logarithmic R_O and L_T relationship in larvae collected in the field (*i.e.* across the path of the Kuroshio Current; range: 9–26 mm L_T) and in rearing experiments (range: 5–25 mm L_T). Likewise, Watanabe & Kuroki (1997) reported allometric R_O and L_T relationships for larvae collected offshore of the Kuroshio Current (range: 6–21 mm L_T) and in coastal waters (range: 18–34 mm L_T). In addition, Uehara *et al.*, (2005) reported slightly allometric R_O and L_T relationships for larval *Sardinops sagax* (Jenyns) in pre-upwelling and upwelling regions in the East Australian Current (range: 6–26 mm L_T). This evidence along with the results of the current study suggests that the otolith size–larval size relationship based on observed age-on-length data become asymptotic at the end of the larval stage. The inter-annual differences found in the current study, however, questions whether the R_O and L_T relationship at the population level indeed can be considered as a mirror of the true R_O and L_T relationship of an individual fish and not merely an artefact of the source of bias affecting cross-sectional data, such as been suggested by a previous study (Schirripa & Trexler, 2000). Even if parameterization of the R_O and L_T relationship is available for a given study, any conclusion derived from backcalculation procedures at the individual level must be interpreted with caution for this clupeid. Nevertheless, a conclusion based on average growth is expected to overcome individual-bias problems, where differences in average growth even among different backcalculation methods seem to be marginal (Secor & Dean, 1992; Sirois *et al.*, 1998).

On the other hand, an unknown influence in both the R_O and the L_T relationship and growth rates derived from backcalculation could be the product of the decoupling between otolith and somatic growth, a process well documented in other species under extreme feeding, temperature and ontogenetic shifts (Marshall & Parker, 1982; Mosegaard *et al.*, 1988; Wright *et al.*, 1990; Baumann *et al.*, 2005). Although, it is reasonable to hypothesize that decoupling between otolith and somatic growth would be less likely in areas with plenty of food such as Tosa Bay, decoupling cannot be ignored because the intrusion into coastal areas of Kuroshio waters brings warmer and oligotrophic conditions for *S. melanostictus* larvae. Therefore, further rearing experiments under simulated feeding and temperature conditions of Kuroshio waters are needed to evaluate these inferences.

The asymptotic growth patterns found in the current study in most monthly hatch larvae are consistent with the allometric growth reported for this species in Japan and elsewhere, where the decrease in growth is thought to be linked to ontogenetic changes occurring at metamorphosis (Blaxter & Hunter, 1982; Watanabe & Kuroki, 1997; Gaughan *et al.*, 2001). The exception was the linear pattern and the biologically unreasonable asymptotic L_T found for the February cohort of the 2003–2004 seasons. Such a pattern seems to be the extreme representation that the population grew more constantly up to the first month of

larval life for the 2003–2004 seasons than for the 2004–2005 seasons. Alternatively, the linear growth could only be a result of either the absence of pre-flexion larvae in the collections or a product of the biological intercept method that tend to linearize ontogenetic transition (Secor & Dean, 1992).

Mean backcalculated L_T passed through the observed values of L_T at age for the 2003–2004 seasons, contrasting with the subsequent season where the Rosa Lee's phenomenon occurred (Ricker, 1979). Backcalculations are based on the assumption that mean backcalculated L_T at any given age is similar to the mean L_T of captured fish at those earlier ages. Violation of this assumption is the so-called Rosa Lee's phenomenon, which can rise due to the following four factors: (1) size-selective mortality, (2) samples are not representative of the entire population, (3) incorrect ageing and (4) contracting of scale (Ricker, 1979; Francis, 1990). Hypothetically, if a random sample free from these biases could be obtained, the expected pattern would be as found for the 2003–2004 seasons (*i.e.* a population model coinciding closely with the individual one). The third and fourth factors are unlikely to occur because this species has high readability of its daily increments and otoliths do not contract. Therefore, it seems that both size-selective mortality and sampling bias triggered the differences found, although the latter could be negligible because the Shirasu fishery usually operates on standard catching protocols on large shoals of clupeid larvae. Indeed, individual growth rates at the end of larval life were higher for the 2003–2004 seasons. These results suggest a major occurrence of faster growing fish due to a major vulnerability of faster growing larvae to the Shirasu fishery, perhaps in response to an environmental cue.

In the current study, the path of the Kuroshio Current was found to be closer to the coastal area of Tosa Bay for the 2003–2004 seasons, which triggered an increase of *c.* 3° C SST in the coastal area in comparison with the 2004–2005 period. Evidently, such a disturbing effect could have triggered the differences found between seasons not only in the R_O and L_T relationship, and in the occurrence or absence of the Rosa Lee's phenomenon but also in the growth rate variations between seasons. Recent studies have suggested that warmer horizontal advection induced by intrusion of Kuroshio Current brings oligotrophic waters decreasing plankton biomass and potentially limiting the conditions for larval growth (Kasai *et al.*, 1993; Yoo & Nakata, 2002; Yoo *et al.*, 2004). The results of the interannual comparison of growth found in the current study agree in part with the results of these studies because larval growth rates were lower when there was a major intrusion of the Kuroshio axes into the coastal area. In general, however, the high growth rate found for all monthly hatch larvae and in both seasons, suggests that either temperature or food availability (*i.e.* the main growth controls of larval growth), or both, were in the range of optimal preferences for *S. melanostictus* larvae.

Intra-season differences in growth patterns evaluated using backcalculated L_T -at-age data showed contrasting results. While growth models showed significant differences in both instantaneous growth rate and asymptotic length for the 2003–2004 seasons, repeated measures ANOVA showed intra-season differences for the 2004–2005 seasons, particularly during the first and third week of larval life. It could be argued that such a contrasting pattern could merely be a result of the functions used for backcalculations (*i.e.* allometric *v.* logarithmic).

Similar results, however, were found when either allometric or logarithmic R_O and L_T relationships were used in both seasons. Consequently, these results are a further proof that growth curve-based analysis are not strictly comparable to individual-based ones, even if the former are built on mean backcalculated length. A further study based on a consistent spatial and temporal scale could provide a way to re-evaluate these findings.

Growth rates in the current study ranged from 0.8 to 1.2 mm day⁻¹ falling beyond the upper range (0.3–0.8 mm day⁻¹) of growth rates reported to date for *S. melanostictus* larvae in the field (Gaughan *et al.*, 2001), and only comparable to the highest growth rates reported to date for winter cohorts of larvae in a coastal nursery area of Miyazaki area at Kyushu (southern Japan) for the 1991 season. In this study, larval growths from coastal areas estimated through a Gompertz model fit to backcalculated L_T were much less variable than from offshore waters. Although in the present study, some degree of variability in growth patterns among monthly cohorts at the individual level was found, differences were minimal when coefficients were estimated from average growth in both cross-sectional and longitudinal analyses. Consequently, from both reports coastal areas seem to be very advantageous for larval growth of this clupeid, at least during the 4 months considered in the current study (*i.e.* from November to February). Hence, annual monitoring of growth patterns could be useful to reveal whether the high growth rates in nearshore coastal areas occur independently of the adult population size. In addition, the low variability in growth is consistent with the idea that larvae collected shared a similar growth history through larval retention in this nursery area. As a corollary, the results suggest that at the current low level of population size, this nursery area can be taken as a large natural mesocosm, in which reliable estimations of larval growth can be obtained.

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References

- Anderson, J. T. (1988). A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. *Journal of Northwest Atlantic Fisheries Science* **8**, 55–66.
- Baumann, H., Peck, M. A. & Herrmann, J. P. (2005). Short-term decoupling of otolith and somatic growth induced by food level changes in post-larval Baltic sprat *Sprattus sprattus*. *Journal of Marine and Freshwater Research* **56**, 539–547.
- Blaxter, J. H. S. & Hunter, J. R. (1982). The biology of the clupeoid fishes. In *Advances in Marine Biology* (Blaxter, J. H. S., Russell, F. S. & Yonge, M., eds), pp. 1–223. New York: Academic Press.
- Campana, S. E. (1990). How reliable are growth back-calculations based on otolith? *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 2219–2227.
- Campana, S. E. & Jones, C. M. (1992). Analysis of otolith microstructure data. In *Otolith Microstructure Examination and Analysis* (Stevenson, D. K. & Campana, S. E., eds). *Canadian Special Publication of Fisheries and Aquatic Sciences* **117**, 73–100.
- Chambers, R. C. & Leggett, W. C. (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. *Canadian Journal of Fisheries and Aquatic Sciences* **44**, 1936–1947.
- Chambers, R. C. & Miller, T. J. (1995). Evaluating fish growth by means of otolith increment analysis: special properties of individual-level longitudinal data. In *Recent Developments in Fish Otolith Research* (Secor, D. H., Dean, J. M. & Campana, S. E., eds), pp. 155–175. Columbia, SC: University of South Carolina Press.
- Fossum, P. & Moksness, E. (1993). A study of spring-and autumn-spawned herring (*Clupea harengus* L.) larvae in the Norwegian Coastal Current during spring 1990. *Fisheries Oceanography* **2**, 73–81. doi: 10.1111/j.1365-2419.1993.tb00122.x
- Francis, R. I. C. C. (1990). Back-calculation of fish length: a critical review. *Journal of Fish Biology* **36**, 883–902. doi: 10.1111/j.1095-8649.1990.tb05636.x
- Francis, R. I. C. C. (1998). Are growth parameters estimated from tagging and age-length data comparable? *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 936–942.
- Gaughan, D. J., Fletcher, W. J. & White, K. V. (2001). Growth rate of larval *Sardinops melanostictus* from ecosystems with different levels of productivity. *Marine Biology* **139**, 831–837.
- Hayashi, A., Yamashita, Y., Kawaguchi, K. & Ishii, T. (1989). Rearing methods and daily otolith rings of Japanese sardine larvae. *Nippon Suisan Gakkai* **55**, 997–1000.
- Heath, M., Zenitani, H., Watanabe, Y., Kimura, R. & Ishida, M. (1998). Modeling the dispersal of larval Japanese sardine, *Sardinops melanostictus*, by the Kuroshio Current in 1993 and 1994. *Fisheries Oceanography* **7**, 335–346. doi: 10.1046/j.1365-2419.1998.00076.x
- Ishida, M. (2006). Rapid decrease of egg production of Pacific stock of Japanese sardine *Sardinops melanostictus*, and characteristics of persistent spawning ground in Tosa Bay, southwestern Japan. *Bulletin of the Japanese Society of Fisheries Oceanography* **70**, 170–175.
- Kasai, A., Kimura, S. & Sugimoto, T. (1993). Warm water intrusion from Kuroshio into coastal areas South of Japan. *Journal of Oceanography* **49**, 607–624.
- Kishida, T., Katsumata, Y., Nakamura, M., Yanagibashi, S. & Funakoshi, S. (1994). An attempt at assessing the relative abundance of Shirasu, larval Japanese sardine, off the Pacific coast of Japan. *Bulletin of the National Research Institute of Fisheries Science* **6**, 57–66.
- Leggett, W. C. & DeBlois, E. (1994). Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stage. *Netherlands Journal of Sea Research* **32**, 119–134.
- Marshall, S. L. & Parker, S. S. (1982). Pattern identification in the microstructure of Sockeye Salmon (*Oncorhynchus nerka*). *Canadian Journal of Fisheries and Aquatic Sciences* **29**, 542–547.
- Mosegaard, H., Svedang, H. & Taberman, K. (1988). Uncoupling of somatic and otolith growth rates in Arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 1514–1524.
- Nakata, K., Zenitani, H. & Inagake, D. (1995). Differences in food availability for Japanese sardine larvae between the frontal region and the offshore waters on the offshore side of the Kuroshio. *Fisheries Oceanography* **4**, 68–79. doi: 10.1111/j.1365-2419.1995.tb00061.x
- Olson, D. B. (2001). Biophysical dynamics of western transition zones: a preliminary synthesis. *Fisheries Oceanography* **10**, 133–150. doi: 10.1046/j.1365-2419.2001.00161.x
- Oozeki, Y. & Zenitani, H. (1996). Factors affecting the recent growth of Japanese sardine *Sardinops melanostictus*. In *Survival Strategies in Early Life Stages of Marine Resource* (Watanabe, Y., Yamashita, Y. & Oozeki, Y., eds), pp. 95–104. Rotterdam: Balkema.
- Ricker, W. E. (1979). Growth rates and models. In *Fish Physiology: Bioenergetics and Growth* (Hoar, W. S., Randall, D. J. & Brett, J. R., eds), pp. 667–743. New York: Academic Press.

- Schirripa, M. J. & Trexler, J. C. (2000). Effects of mortality and gear selectivity on the fish otolith radius–total length relation. *Fishery Research* **46**, 83–89.
- Secor, H. D. & Dean, J. M. (1992). Comparison of otolith-based back-calculation methods to determine individuals growth histories of larval striped bass, *Morone saxatilis*. *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 1439–1454.
- Sirois, P., Lecomte, F. & Dodson, J. J. (1998). An otolith-based back-calculation method to account for time-varying growth rate in rainbow smelt larvae (*Osmerus mordax*). *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 2662–2671.
- Sokal, R. R. & Rohlf, F. J. (1995). *Biometry*, 3rd edn. San Francisco, CA: W.H. Freeman. 887 p.
- Theilacker, G. H. (1980). Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. *Fishery Bulletin* **78**, 685–692.
- Uehara, S., Syahailatua, S. & Suthers, I. M. (2005). Recent growth rate of larval pilchards *Sardinops sagax* in relation to their stable isotope composition, in an upwelling zone of the East Australian Current. *Marine Freshwater Research* **56**, 549–560.
- Watanabe, Y. & Kuroki, T. (1997). Asymptotic growth trajectories of larval sardine (*Sardinops melanostictus*) in the coastal waters off western Japan. *Marine Biology* **127**, 369–378.
- Watanabe, Y. & Nakamura, M. (1998). Growth trajectory of the larval Japanese sardine, *Sardinops melanostictus*, transported into the Pacific coastal waters off central Japan. *Fishery Bulletin* **96**, 900–907.
- Watanabe, Y., Zenitani, H. & Kimura, R. (1995). Population decline of the Japanese sardine *Sardinops melanostictus* owing to recruitment failures. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 1609–1616.
- Wright, P. J., Metcalfe, N. B. & Thorpe, J. E. (1990). Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L: evidence against coupling. *Journal of Fish Biology* **36**, 241–249. doi: 10.1111/j.1095-8649.1990.tb05599.x
- Yoo, J. T. & Nakata, H. (2002). Effect of onshore–offshore shift of the kuroshio axis on the plankton biomass off the pacific coast of Japan. *Bulletin of the Japanese Society of Fisheries Oceanography* **66**, 224–231.
- Yoo, J. T., Nakata, H. & Sugimoto, T. (2004). Effect of horizontal advection induced by intrusion of the Kuroshio water on plankton biomass in the spring fishing period of Shirasu on the Pacific coast of Japan. *Fisheries Science* **70**, 937–944. doi: 10.1111/j.1444-2906.2004.00892.x
- Zenitani, H., Nakata, K. & Inagake, D. (1996). Survival and growth of sardine larvae on the offshore side of the Kuroshio. *Fisheries Oceanography* **5**, 56–62. doi: 10.1111/j.1365-2419.1996.tb00017.x