Environmental variability and growth histories of larval Japanese sardine (*Sardinops melanostictus*) and Japanese anchovy (*Engraulis japonicus*) near the frontal area of the Kuroshio

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ABSTRACT

Environmental variability and growth-rate histories from hatching to capture were investigated for larval Japanese sardine (*Sardinops melanostictus*) and Japanese anchovy (*Engraulis japonicus*). Larvae collected around the front of the Kuroshio Current were examined using otolith microstructure analysis, and their movement was estimated from numerical particle-tracking experiments. Sardine larvae collected inshore of the Kuroshio front originated from a coastal area near the sampling site, while those collected in the offshore area originated from an area 500–800 km west-southwest of the sampling site. Anchovy larvae collected both inshore and offshore had been transported from widely distributed spawning areas located west of the sampling area. At the age of 13–14 days for sardine and 19–20 days for anchovy, the offshore group exhibited significantly higher mean growth rates than did the inshore group. Although the offshore area was generally warmer than the inshore area, temporal variations in growth rate are not attributable solely to fluctuations in environmental temperature. While previous studies have examined the relationship between larval growth rates and environment based solely on data at capture, the methods used in the present study, combining otolith analysis and numerical particle-tracking experiments, utilize data up until hatching. Although the relationship between growth rate and environment was not fully confirmed, this approach will greatly advance our understanding of fish population dynamics.

Key words: anchovy, growth, Kuroshio current, larva, numerical model, otolith, sardine, temperature, transport

INTRODUCTION

Japanese sardine (*Sardinops melanostictus*) and Japanese anchovy (*Engraulis japonicus*) show large-amplitude variations in inter-decadal recruitment, which result in dramatic out-of-phase fluctuations in stock abundance (Yatsu et al., 2005; Takasuka et al., 2007). Analyses of temperature and/or mixed-layer depth suggest that environmental variability underlies the variation in recruitment. For example, previous studies have reported a significant negative (positive) correlation between recruitment rate and temperature (mixed-layer depth) for sardine (Noto and Yasuda, 1999; Yatsu et al., 2005; Nishikawa and Yasuda, 2008; Itoh et al., 2009). For anchovy, recruitment rate is positively correlated with temperature, with an optimum temperature of approximately 22°C (Itoh et al., 2009). Analyses of environmental temperature, based on the numerical tracking of particles from spawning grounds, suggest that correlations between recruitment rate, and linear and quadratic functions of temperature are strongest at the larval age of 5–15 days (Itoh et al., 2009).

The relationship between the environment and variations in recruitment is mediated by larval growth rate, which is thought to be related to survival (Anderson, 1988). Previous studies have estimated the larval growth rate from otolith microstructure and...
have reported that larvae collected offshore showed a significantly lower growth rate than those collected inshore. Such geographical differences in growth rate have been reported in both Japanese sardine (Watanabe and Kuroki, 1997) and Japanese anchovy (Takahashi and Watanabe, 2004b). Takasuka et al. (2007) examined the growth rate of larval samples collected during various seasons and from different locations, and reported optimal temperatures for the larval growth of Japanese sardine (16.2°C) and Japanese anchovy (22.0°C). These temperature optima are consistent with trends in recruitment variations and large-scale fluctuations in sea surface temperatures. Based on intensive field surveys in the Kuroshio–Oyashio transition region, Takahashi et al. (2009) found a difference between Japanese sardine and Japanese anchovy in terms of the response of larval and juvenile growth to a climatic regime shift, caused not only by differences in optimal temperature (16.4–18.9°C for Japanese sardine; >17.3°C for Japanese anchovy) but also by differences in the optimal levels of food concentration (higher for Japanese sardine than for Japanese anchovy).

The effects of environmental factors on larval growth history have yet to be examined in detail. While sagittal otolith microstructure provides age and growth-rate history data in days from birth to capture (Campana, 1990), previous studies have compared growth rates and environment mainly at the time of capture or just before capture (e.g., Takasuka et al., 2007), as reliable environmental information was not available for the period prior to capture. However, given that some larvae are transported from inshore spawning grounds to offshore feeding grounds (Kuroda, 1991), environmental variability during transport might also have a significant influence on larval growth.

To understand the effects of environmental factors on larval growth history, it is necessary to reconstruct the history of the larval environment. This applies not only to Japanese sardine and Japanese anchovy: the important influence of the larval environment on growth and the lack of the environmental information before capture is widely recognized among fisheries oceanographers (e.g., Dower et al., 2002; Pepin et al., 2003). Numerical back-tracking is useful in this regard because it enables environmental factors during transport to be estimated for larval patches collected by field surveys, whose growth histories can also be estimated via otolith microstructure analysis. Although the flow and environmental fields around areas of interest have not previously been determined with sufficient accuracy or resolution to enable back-tracking by observation or hydrodynamic models, we are now able to use state-of-the-art data from a numerical model that assimilates environmental data (Komatsu et al., 2005; see the following section for details). Taking advantage of this model, the present study examined the relationship between larval growth rates and environmental factors.

In the present study, we captured larvae of Japanese sardine and Japanese anchovy (hereafter referred to as sardine and anchovy, respectively) and collected hydrographic data at several inshore and offshore stations. Samples were collected in April in an area off Boso Peninsula, Japan (Fig. 1), near the point where the Kuroshio Current separates from the Japanese Islands. The origins of the larvae and environmental variability were estimated using a numerical particle-tracking technique. The spawning season for sardine is from late autumn to late spring (mainly in early spring), and anchovy spawn from spring to autumn (mainly in early summer). Therefore, larvae of both species were expected to be present in this area during the sampling period.

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Figure 1. Study area: (a) general geography and hydrography around the Japanese archipelago, and (b) stations used for sampling and hydrographic observations. ‘S’ and ‘K’ in (a) denote the islands of Shikoku and Kyushu, respectively.
MATERIALS AND METHODS

Observations of environmental conditions

Observations across the Kuroshio were conducted from onboard the R/V Tansei-maru in April 2007 (Research Cruise KT-07-6 from 10 to 13 April) along two transects, A and B, in the area off Boso Peninsula (Fig. 1). Surveys with a conductivity–temperature–depth profiler (CTD) and water sampling for concentrations of nutrients and chlorophyll-a were carried out using a carousel water sampler (CWS) at Stations 02, 04, 06, 08, 15 and 16. Expendable CTDs (XCTDs) were deployed at Stations 11 and 13, and expendable bathythermographs (XBTs) were deployed at Stations 01, 03, 05, 07, 09, 10, 12 and 14 (Fig. 1 and Table 1).

The water samples collected for nutrient and chlorophyll analyses were immediately frozen at −20°C onboard, and nitrate concentrations were measured in the laboratory using an AutoAnalyzer (AACS II; Bran + Luebbe, Norderstedt, Germany). Chlorophyll-a concentrations were measured in the laboratory using a fluorometer (10AU; Turner Designs, Inc., Sunnyvale, CA, USA) after filtering the samples through Whatman (Maidstone, UK) GF/F filters and extracting them with dimethylformamide on board. While the concentrations of nutrients and chlorophyll-a varied markedly with depth, the mean values within the surface mixed-layer, defined here as the layer with water temperature higher than sea surface temperature +0.5°C, were calculated to represent the environmental conditions for the larvae. At all CTD stations except Station 16, a 45-cm-diameter North Pacific (NORPAC) net (mesh size of 0.11 mm) was towed vertically from 200 m depth to the surface. NORPAC samples were preserved in 10% buffered formalin on board, and the number of copepods (considered the main food of sardine and anchovy larvae; Mitani, 1988; Kuroda, 1991) was counted in the laboratory. Water and plankton samples were not obtained at some of the stations along Transect B because of adverse weather conditions.

Sampling of larvae and analyses

Larvae were collected at night at Stations 02, 11, 13 and 16 using 0–50-m oblique tows of a 1.5 × 1.5 m frame trawl (Matsuda-Oozeki-Hu Trawl, MOHT; Hu et al., 2001) (1 × 2 mm mesh size). At Stations 04, 06, 08 and 15, the MOHT was towed during the daytime. Relatively few samples were obtained during daytime tows, and they were excluded from the study because net avoidance was suspected. The flow volume of the upper 50 m sampled by the trawl was estimated using a flowmeter placed at the opening of the net and a depth meter attached to the frame. Larvae were immediately frozen at −20°C onboard, and the number of each species and standard lengths (SLs) were measured in the laboratory before preserving the samples in 90% ethanol.

Where more than 20 larvae were caught at a station, sagittal otolith microstructure analysis was

<table>
<thead>
<tr>
<th>ST</th>
<th>Species</th>
<th>Total no. (density*)</th>
<th>No. used in analyses</th>
<th>Standard length† (mm)</th>
<th>Daily age† (mm)</th>
<th>Hatching date</th>
<th>Environmental survey‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anchovy</td>
<td>180 (75)</td>
<td>19</td>
<td>16.9 (16.2–17.6)</td>
<td>20.8 (19.3–22.4)</td>
<td>March 12–26</td>
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</tr>
<tr>
<td>4</td>
<td>Sardine</td>
<td>5 (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchovy</td>
<td>13 (3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sardine</td>
<td>5 (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchovy</td>
<td>3 (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Sardine</td>
<td>4 (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchovy</td>
<td>0 (0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Sardine</td>
<td>32 (11)</td>
<td>20</td>
<td>16.8 (15.8–17.7)</td>
<td>14.7 (13.7–15.5)</td>
<td>March 24–April 01</td>
<td>XCTD</td>
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<tr>
<td></td>
<td>Anchovy</td>
<td>217 (74)</td>
<td>20</td>
<td>17.7 (16.2–19.3)</td>
<td>21.0 (19.5–22.6)</td>
<td>March 15–26</td>
<td></td>
</tr>
<tr>
<td>13</td>
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<td>2 (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>XCTD</td>
</tr>
<tr>
<td>15</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchovy</td>
<td>2 (0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Sardine</td>
<td>1 (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchovy</td>
<td>296 (218)</td>
<td>20</td>
<td>16.3 (15.3–17.2)</td>
<td>20.7 (19.4–21.9)</td>
<td>March 17–28</td>
<td></td>
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</table>

*Estimated density (10^{-3} m^{-3}) within upper 50 m.
†Mean (95% confidence interval estimated by the bootstrap method).
‡CTD, conductivity–temperature–depth profiler; CWS, carousel water sampler; XCTD, expendable CTD.

conducted for 19–21 specimens randomly selected from the samples. For these specimens, the number and widths of otolith daily increments were measured using an otolith measurement system (RATOC System Engineering, Tokyo, Japan). Based on the biological intercept method (Campana, 1990), allometric parameters relating otolith radii and SLs were determined for each individual. Fixed SLs were defined at the first ring deposition of 5.0 mm for sardine and 5.6 mm for anchovy at the age of 3 and 4 days, respectively, following Takasuka et al. (2007). Growth-rate histories were obtained by differentiating the back-calculated SL, and mean growth rates from hatching to capture were estimated assuming initial SLs of 3.4 mm for sardine and 2.9 mm for anchovy, following Takasuka et al. (2008).

Larval growth rates are presented in terms of back-calculated age in days instead of calendar date, except for estimates of the most recent growth rates. We calculated the mean values using growth-rate data (averaged over 2-day periods) of individuals from the same stations and within bins of back-calculated age, in days, and their confidence intervals were estimated by the bootstrap method. Because the larval growth rates of sardine and anchovy show ontogenetic changes, compiling the data with respect to the calendar date resulted in wide confidence intervals, which made it difficult to assess differences between the stations.

Particle-tracking experiments

Numerical tracking of particles released at the observational stations was carried out using data from the eddy-resolving data assimilation system, FRA-JCOPE (Komatsu et al., 2005). Based on the Princeton ocean model (Blumberg and Mellor, 1987), FRA-JCOPE is integrated with a data assimilation technique incorporating data from satellites, ARGO floats, and in situ observations from the Global Temperature–Salinity Profile Program (GTSPP; http://www.nodc.noaa.gov/GTSPP/gtsp-home.html) and Japanese prefectural fisheries institutions. In Japanese waters, the horizontal grid interval is set to 1/12° and there are 45 sigma layers. Although the model was constructed primarily for forecasting, a reanalyzed data product is also available that reproduces the observed upper-ocean variability around Japan (Yoshinari et al., 2008).

Figure 2 shows the flow and temperature (depth, 10 m) fields of the study areas on 11 April, as reproduced by FRA-JCOPE. The model assimilated synoptic-scale variability such as the path of the Kuroshio, and meso-scale features along the front of the Kuroshio were expressed realistically. Note that Stations 02 and 16 are located on the inshore side of the Kuroshio temperature front, whereas Stations 11 and 13 are located offshore of the front. Because the Kuroshio temperature front is generally located slightly inshore of the current axis, Station 13 was located almost on the axis itself. Groups of sardine and anchovy larvae collected at each station are hereafter denoted as G-(station number)(initial of species name) (e.g., sardine and anchovy specimens collected at Station 02 are denoted G-02S and G-02A, respectively).

Tracking algorithm

We released 1000 particles in the FRA-JCOPE model with the time and location of release corresponding to the larvae sampling at stations 02, 11, 13 and 16. The putative origins of the sampled larvae were estimated by back-tracking the particles using the horizontal velocity (10 m depth) of currents in the model. Random displacements (random walks) were added to each particle to express the horizontal dispersal of larvae. For simplicity, a fixed depth was employed because sardine and anchovy larvae are distributed near the surface layer. We conducted experiments using other depths (0–50 m), considering the towing depth, but the general pattern of tracked particles was similar in all cases. Although the environmental temperature showed a slight decrease if the fixed depth was deeper than the mixed layer (which was often the case when the depth was...
fixed to 50 m), it basically appeared as an 'offset’ (i.e., the temperature showed a similar decrease for all particles) and did not have a marked effect on the overall results.

Although the above tracking experiments provided a preliminary probability distribution of the occurrence of individuals prior to sampling, it showed low accuracy in the case of the present study, due to significant dispersion during transport (over a period of several weeks) through the frontal area of the Kuroshio. Although the eggs of sardine and anchovy are generally spawned in relatively confined coastal areas and are subsequently widely dispersed throughout the open ocean, a significant portion of back-tracked individuals did not reach their origin, which essentially reflects the irreversibility of the dispersion process. However, the simplicity of back-tracking from the sampling stations remains preferable to forward-tracking, which requires much greater computation from all possible spawning grounds to specify the individuals that reached each station.

In the above context, we considered the probability distribution of the occurrence of eggs, based on abundance data collected during field surveys (Anonymous, 2007; referred to as the ‘egg probability distribution’), in addition to back-tracking from the sampling stations (referred to as the ‘raw probability distribution’). Because the National Research Institute of Fisheries Science, Fisheries Research Agency of Japan (Anonymous 2007) provided logarithmic (e.g., $10^{-11}$–$10^{-12}$, $10^{-12}$–$10^{-13}$ eggs) egg abundance data at a monthly time scale on a $30 \times 30$’grid map, the egg probability distribution in the present study was given simply by weighting these abundances. Then, the modified probability distribution of individuals at the time of spawning was obtained by multiplying the raw probability distribution at the time of spawning (assumed to be 24 h before hatching) by the egg probability distribution. The expected value of the environmental temperature of each larva at each age in days was calculated by multiplying the model temperature (interpolated to the locations of back-tracked individuals) by the modified probability distribution. Although these estimates of temperature were not deterministic, their variances were generally lower than the variance among the estimated temperature of each larva.

The variability in environmental temperature is presented in terms of back-calculated ages of larvae in days, as for the growth rates. The above errors arising from the stochastic calculation were not included in estimation errors of the mean environmental temperature of larval groups, in order to be consistent with the presentation of the data on growth rates.

RESULTS

Environmental profiles and sample statistics

Figure 3 shows environmental profiles along transects A and B. As seen in the vertical cross-sections of temperature, the observational transects were located at the frontal area of the Kuroshio between the relatively cold inshore side and the warm offshore side. The surface temperature front, as indicated by dense outcrops of isotherms, was located roughly around the zone of SST = 19°C, placing Stations 02, 04, 15 and 16 on the colder side, and Stations 06, 08, 11 and 13 on the warmer side. Using the index temperature of the current axis of the Kuroshio (empirically defined in this area as 15°C at 200 m; Kawai, 1969), Stations 02, 04, 15 and 16 were located on the inshore side of the Kuroshio Current axis; Stations 06, 08 and 11 were located on the offshore side; and Station 13 was located on the axis itself. These locations, relative to the thermal front and current axis, are consistent with the model (Fig. 2).

Nitrate concentrations in the mixed layer showed a negative correlation with temperature, with a relatively high concentration of 1.3–4.2 µM on the inshore side (Stations 02, 04, 15 and 16) and 0.0–1.1 µM on the offshore side (Stations 06 and 08). Chlorophyll-a concentrations showed a more complex distribution; however, the lowest concentration was found at Station 8, where the surface temperature (20.7°C) was the local maximum. Copepod abundance was higher on the inshore side than on the offshore side, except for a slight increase in abundance from Stations 04 to 06.

Basic statistics of larvae

Age in days and SL data for the sampled larvae are shown in Table 1. The means and standard deviations of age in days and SLs of all sardine larvae collected at two target stations were 14.6 ± 2.3 days (mean hatching date, 27 March) and 16.5 ± 2.2 mm SL, respectively; the values for anchovy collected at four target stations were 21.1 ± 3.5 days (mean hatching date, 21 March) and 17.3 ± 3.2 mm SL, respectively. For neither sardine or anchovy were there any significant differences among stations in terms of mean age in days or SLs (95% confidence interval overlap; Table 1).

For sardine, the mean values of the average growth rate (rate from hatching to capture) were not significantly different between the groups (G-02S and G-11S); however, the mean latest-stage growth rate (from 3 days before capture) was significantly higher (95% confidence interval, bootstrap method) in
G-11S (sardine larvae collected at the offshore Station 11) than in G-02S (those collected at the inshore Station 2) (Fig. 4c). For anchovy, there were no significant differences in the mean values of the average growth rate and latest-stage growth rate among samples collected at different stations (Fig. 4b,d).

Possible spawning grounds and environmental histories
The locations of spawning grounds were estimated using the back-tracking method based on the FRA-JCOPE reanalyzed data and the actual egg distribution (Figs 5 and 6; the weightings assigned to egg abundance are not reflected in the figures). For sardine, the possible spawning grounds are clearly different among the sampling stations, although hatching dates show no significant difference. The spawning area of larvae collected at G-02S is confined to Sagami Bay (about 150 km west of the sampling site; see Fig. 1), but those collected at G-11S originated from areas south of Shikoku (500–800 km in the upstream direction of the Kuroshio, roughly west-southwest of the sampling site) (Fig. 5). The distribution of the spawning ground for each group shows no relation to hatching date.

Because anchovy eggs were more abundant and more widely distributed in Japanese waters than were sardine larvae in 2007 (Anonymous, 2007), it is possible that anchovy eggs were transported from a wider area (Fig. 6). For anchovy, the origins of the larvae collected at the four target stations were less clear than the origins of sardine larvae. Most of the G-02A larvae, which were collected at an inshore station, originated from inshore areas east of Kii Peninsula (approximately 500 km upstream along the Kuroshio from the sampling site). However, it is possible that the spawning grounds of some of the larvae older than 28 days were located in an area west of Kii Peninsula. The possible spawning grounds of G-16A, a group from another inshore station, were mostly distributed

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Figure 3. Environmental profiles along two observational transects, showing (a,b) temperature cross-sections (°C); (c,d) mixed-layer depth (m); (e,f) mean nitrate concentrations in the mixed layer (μM); (g,h) mean chlorophyll-a concentrations in the mixed layer (μg L⁻¹); (i,j) copepod density in the upper 200 m (N m⁻³). Black circles, nighttime sampling stations; open circles, daytime sampling stations, crosses, XBT stations (as shown in Fig. 1).
west of Kii Peninsula, from as far away as the area south of Kyushu (about 1000 km upstream along the Kuroshio from the sampling site), more similar to the spawning grounds of G-11A and G-13A (groups from relatively offshore stations). Except for the older G-02A larvae, the spawning ground of each group was not related to its hatching date, as found for sardine.

Figure 7 shows the histories of mean environmental temperature based on back-calculated ages in days. While temporal variations were recognized with ranges of about 0.5–1.5°C, the differences between groups are generally larger than the temporal variations for both sardine and anchovy. The environmental temperature of G-02S, from hatching to capture, was about 16.3–17.3°C, significantly lower than that of G-11S (19.5–20.5°C). For anchovy, the environmental temperature of G-02A was 17.1–17.3°C, again significantly lower than that for G-11A (20.0–20.5°C), G-13A (19.3–20.3°C), and G-16A (18.7–20.1°C) for all age bins. Although the larvae of G-16A were sampled on the inshore side of the Kuroshio, the mean temperature did not become significantly lower than that of the offshore groups of G-11A and G-13A until the age of 12 days. The warmer environment of G-16A compared with G-02A reflects the fact that G-16A originated from farther upstream than did G-02A (Fig. 6). Upstream areas are generally warmer than downstream areas, and the warm current of the Kuroshio is expected to have played a major role in transporting larvae to the sampling site.

Growth-rate histories

Figure 8 shows the growth-rate histories of sardine and anchovy based on 2-day increments of back-calculated ages. The growth rates are shown for two stages: average growth rates (from hatching to capture) and latest-stage growth rates (from 3 days before capture).

Figure 4. Mean growth rates of specimens captured at each station, showing average growth rates (from hatching to capture) of (a) sardine larvae and (b) anchovy larvae, and latest-stage growth rates (from 3 days before capture) of (c) sardine larvae and (d) anchovy larvae. Error bars indicate 95% confidence intervals estimated by the bootstrap method.

Figure 5. Possible spawning grounds of sardine, as estimated by particle back-tracking for the groups (a) G-02S and (b) G-11S. Crosses show the locations of sampling stations, and circles indicate the back-tracked positions of particles that reached the observed spawning grounds (based on surveys by the Fisheries Agency of Japan). Colors indicate days of back-tracking (1 day + age in days of the samples, considering hatching time).
ages in days. For both G-02S and G-11S, the growth rates of sardine increased after the age of 5–6 days until the age of 9–10 days, but then decreased until the age of 15–16 days. For anchovy, the increasing trend was not as clear as that for sardine, while the decreasing trend was observed after the age of 13–14 days. After the beginning of the decreasing trend (after the age of 11 days for sardine and 15 days for anchovy), the difference in growth rates of inshore and offshore groups showed a gradual increase. For the age bin of 13–14 days, the growth rate of sardines collected offshore (G-11S) was significantly higher than that of sardines collected inshore (G-02S). Similarly, anchovies collected offshore (G-11A and G-13A) showed significantly higher growth rates than those collected inshore (G-02A and G-16A) for the age bin of 19–20 days. Although significant differences were not observed for other age bins, the growth rates of

Figure 6. As for Fig. 5, but for anchovy samples in groups (a) G-02A, (b) G-11A, (c) G-13A, and (d) G-16A.

Figure 7. Mean temperature histories of the six groups of specimens, based on back-calculated ages in days estimated by particle back-tracking, for (a) sardine and (b) anchovy. Error bars indicate 95% confidence intervals estimated by the bootstrap method.

Figure 8. Growth histories of larvae based on sagittal otolith microstructure analysis, for (a) sardine and (b) anchovy. Error bars indicate 95% confidence intervals estimated by the bootstrap method. For clarity, symbols are shown slightly off-center for each age bin.
offshore groups did not become lower than those of inshore groups for the entire period, after an age of 11 days for sardine and 15 days for anchovy. Figure 9 shows the relationships between the mean environmental temperature (Fig. 7) and the mean growth rates (Fig. 8). Although offshore groups in relatively warm environments showed significantly higher growth rates at the ages of 13–14 days for sardine and 19–20 days for anchovy, no clear relationship was recognized for either sardine or anchovy when considering all the available data from hatching to capture. However, if we focus on the period after an age of 11 days for sardine and 15 days for anchovy, we find significant positive trends between temperature and growth rates for anchovy ($r = 0.64$, $P < 0.01$); sardine also showed a positive trend ($r = 0.57$), although not statistically significant ($P = 0.24$).

**DISCUSSION**

**Errors in back-tracking simulations**

Although numerical back-tracking is a useful tool for examining larval environmental histories, it includes errors related to the following factors: (i) the assumption of habitat depth, (ii) the accuracy of the flow and temperature fields, (iii) the temporal and spatial resolution of the flow and temperature fields, and (iv) the temporal, spatial and data resolution of the egg abundance distribution. As mentioned in Materials and Methods, factor (i) did not affect the overall results. Because the flow and temperature fields of FRA-JCOPE are calculated by assimilating observation data, errors caused by factor (ii) were also insignificant. Although the data from FRA-JCOPE are sufficient to resolve mesoscale eddies, the resolution (i.e., factor iii) is insufficient to specify the trajectories of larval transport deterministically because of subgrid-scale variability (expressed by random walks in the tracking) and the chaotic nature of strong flows. The distribution of tracked individuals from a given station is therefore considered the raw probability distribution of occurrence. The same consideration is applied to factor (iv) – the resolution of the egg abundance distribution. Thus, the estimation of the tracking error was equivalent to the estimation of the modified probability of occurrences, as outlined in the Materials and Methods.

Based on this modified probability distribution, the estimation of spawning grounds had a spatial variance of several hundreds of kilometers (Figs 5 and 6). For the mean environmental temperature, which was compared directly with the mean growth rates, 95% confidence intervals were generally within 0.5°C (Fig. 7), and the mean values were significantly different between the inshore and offshore groups. In conclusion, although the back-tracking did not yield deterministic estimations, errors were smaller than the difference between groups.

**Cause of the difference in growth rate between inshore and offshore stations**

Our results showed significantly higher mean growth rates in groups collected at offshore stations (Stations 11 and 13) than in groups collected at inshore stations (Stations 02 and 16), for ages of 13–14 days for sardine and 19–20 days for anchovy (Fig. 8). For sardine, the mean growth rates just before capture and the average growth rates of larvae at all ages were higher at offshore stations than at inshore stations (Fig. 4). Given that food concentration was lower in offshore areas (Fig. 3), this difference cannot simply be explained by food availability. Therefore, what factor caused this difference among stations?

In the sampling region, temperature is generally higher in offshore areas than in inshore areas during spring. It is possible that higher temperatures result in higher growth rates, as seen in the positive trend of growth rates for anchovy (after an age of 15 days) with...
respect to temperature (Fig. 9). This proposal is consistent with previous rearing experiments, which reported that sardine larvae grow faster at 19 than at 15°C (Zenitani, 1995) and that anchovy larvae grow faster at 21 than at 17 or 13°C (Takahashi and Watanabe, 2004a). However, growth-rate fluctuations in the present study cannot be attributed solely to temperature fluctuations. Despite the significant difference in temperature between inshore and offshore areas, differences in growth rates were less clear, except for the significant difference in the age bin of 13–14 days for sardine and 19–20 days for anchovy (Fig. 8). Moreover, according to the temperature optima and food conditions proposed by previous field studies (Takasuka et al., 2007; Takahashi et al., 2009), sardine should show the opposite trend (i.e., higher growth rate in colder water with abundant food) to that observed in this temperature range.

An alternative reason for the difference in growth rate is suggested by the asymmetry in the frequency distributions of inshore and offshore groups for the periods with significant differences in growth rate (Fig. 10). The offshore group of sardine (G-11S) contained a relatively small number of larvae with low growth rates compared with the inshore group (G-02S). The difference between the first quartile and median, representing the deviation scale of the low growth-rate range, is 0.12 for G-11S, smaller than the value of 0.15 for G-02S. For anchovy, in contrast, the clearest difference is seen in the frequency of larvae with high growth rates. The difference between the third quartile and median is 0.19 for the offshore groups (G-11A and G-13A), greater than the value of 0.08 for the inshore groups (G-02A and G-16A).

It is possible that the relatively small number of larvae with low growth rates for the offshore group of sardine arises in part because the offshore area is less favorable for larval survival. That is, the larvae with a low growth rate are more susceptible to high mortality in the offshore area, possibly because of high predation pressure. Although the frequency distributions of anchovy were also different between inshore and offshore groups, the clearest contrast is in the relative abundance of high growth-rate larvae; therefore, this scenario may not be applicable. Generally, both fast growth and weak predation pressure are considered to be important factors in recruitment success for marine fishes, as recently shown by Robert et al. (2007) for Atlantic mackerel. Therefore, the apparently high mean growth rate of the offshore group of sardine, possibly caused by high predation pressure, does not indicate their contribution to the adult population. However, because the above analysis on the frequency distribution is based on a limited number of specimens (approximately 15 for sardine groups and 30 for anchovy groups), further examinations are needed to validate this interpretation.

**Figure 10.** Frequency distribution of the growth rate of the inshore and offshore groups of sardine and anchovy, for which the periods of significant differences between the inshore and offshore groups are indicated (age bins of 13–14 days for sardine and 19–20 days for anchovy). (a) Inshore group of sardine (G-02S; available number of specimens \( N_0 = 14 \)), (b) inshore group of anchovy (G-02A and G-16A; available number of specimens \( N_0 = 27 \)), (c) offshore group of sardine (G-11S; available number of specimens \( N_0 = 15 \)), and (d) offshore group of anchovy (G-11A and G-13A; available number of specimens \( N_0 = 26 \)). Triangles at the top of each panel indicate quartile points (25th, 50th and 75th percentile). Note that growth-rate data in days were used to draw the distribution.

growth-rate larvae, resulting in a higher mean growth rate among surviving larvae, especially in the case of sardine.

The present study is the first to compare larval growth-rate histories with concurrent environment data derived from an eddy-resolving data assimilation system. Although the present findings do not fully confirm the relationship between growth-rate variability and environment, this method, combining otolith analyses and numerical particle-tracking experiments, is expected to greatly advance our understanding of fish population dynamics.

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