



Disentangling the effects of inherent otolith growth and model-simulated ecosystem parameters on the daily growth rate of young anchovies

Eudoxia Schismenou^{1,2,*}, Marianna Giannoulaki¹, Kostas Tsiaras³,
Eugenia Lefkaditou⁴, George Triantafyllou³, Stylianos Somarakis¹

¹Hellenic Centre for Marine Research, Institute of Marine Biological Resources and Inland Waters, Thalassokosmos, 71003 Heraklion, Crete, Greece

²Department of Biology, University of Crete, 71409 Heraklion, Crete, Greece

³Hellenic Centre for Marine Research, Institute of Oceanography, Mavro Lithari, 19013 Anavissos, Greece

⁴Hellenic Centre for Marine Research, Institute of Marine Biological Resources and Inland Waters, Aghios Kosmas, 16610 Helliniko, Greece

ABSTRACT: Otolith increment widths have often been used to infer somatic growth history when studying the influence of temperature and food supply. However, there is no functional relationship between otolith accretion rate ('inherent otolith growth') and somatic growth rate; although both may be mediated by temperature and food, the uncoupling of these rates under changing environmental conditions or during ontogenetic transitions can undermine the sensitivity of increment width analyses. We used otolith microstructure analysis to model the daily growth of anchovy *Engraulis encrasicolus* juveniles collected from the North Aegean Sea in July 2007, December 2007 and February 2009. To assess the environmental causes of seasonal variability in growth we used daily outputs of environmental variables from a coupled 3D hydrodynamic-biogeochemical model (POM-ERSEM) as a proxy for the potential environment that fish experienced during their development. According to the simulation, juveniles that hatched in late autumn-winter developed in unfavorable conditions (low temperature, low productivity); however, they exhibited wider otolith increments before metamorphosis. To specifically study environmental influences on the daily growth rates, we used the simulated environmental variables in generalised additive model (GAM) analysis of otolith increment widths, while also taking into consideration inherent otolith growth, expressed by the explanatory variables 'previous increment width' and 'age'. The inclusion of the interaction term 'age' × 'sampling period', rather than 'age', accounted for the seasonal effects on ontogenetic changes (timing and duration of metamorphosis) and the differences in growth-selective mortality among samples. Results showed that the daily growth rate of anchovy is influenced by temperature, exhibiting an optimum at 24.5°C, and increases significantly with increased mesozooplankton concentration (i.e. food availability). The analysis highlights that, in order to infer the daily somatic growth from fish otolith increment data and understand the environmental influences, inherent otolith growth has to be disentangled and otolith width trajectories standardised for between-sample differences in ontogenetic changes and growth selective mortality.

KEY WORDS: *Engraulis encrasicolus* · Growth · Otolith microstructure · Northeast Aegean Sea · Coupled hydrodynamic-biogeochemical model · Generalised additive model

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INTRODUCTION

Otolith increment widths or the otolith growth rate have often been used together with environmental parameters to assess the factors that affect fish growth rate during early life stages, assuming a high correlation between otolith size and fish size (e.g. Maillet & Checkley 1991, Baumann et al. 2006a, Allain et al. 2007). Recently, Hinrichsen et al. (2010) used generalised additive model (GAM) analysis to compare daily increment widths with environmental parameters and identify the environmental factors that affect the growth rate of larval Baltic sprat *Sprattus sprattus*. In their models, most of the variability in increment width was explained by the variables 'previous increment width' and 'age'. Increment widths are highly autocorrelated (Hinrichsen et al. 2010), i.e. the width between 2 consecutive rings does not change abruptly, and their mean value is affected by age and stage (Pepin et al. 2001). For example, early larvae tend to have smaller increment widths compared to juveniles. The variables 'previous increment width' and 'age' used by Hinrichsen et al. (2010) account for 'inherent otolith growth' (sensu Folkvord et al. 2000), which 'should be explicitly incorporated into models assessing growth or condition in fish larvae' (Folkvord et al. 2000, p. 100).

Inherent otolith growth is associated with the occasional loss of proportionality (uncoupling) between somatic and otolith growth rates. Uncoupling is known to occur, for example, when environmental conditions such as food availability or temperature change, and during transitions between ontogenetic stages (e.g. Molony & Choat 1990, Hare & Cowen 1995, Folkvord et al. 2000, 2004, Otterlei et al. 2002, Fey 2006, Schismenou et al. 2013). In these instances, the speed and magnitude of change in growth can be different between the soma and otolith.

In this study, we used 3 seasonal samples (July, December and February) of juvenile European anchovy *Engraulis encrasicolus* from the North Aegean Sea and a daily dataset of environmental parameters (Schismenou et al. 2013) to study the variability in daily growth based on otolith microstructure analysis. The environmental dataset was derived from the simulation of a coupled hydrodynamic-biogeochemical model (POM-ERSEM) over the period of growth of the sampled fish. The use of simulated ecosystem parameters has been recently proposed as a useful approach to represent the environmental history of growing individuals (Schismenou et al. 2013). We constructed a GAM with daily increment widths as

the response variable (Hinrichsen et al. 2010) and examined the effect of a suite of environmental parameters. In the model, we included the explanatory variables 'previous increment width' and 'age' to account for inherent otolith growth. The results demonstrate that the anchovy daily growth rate in the Mediterranean has a temperature optimum and increases significantly with increasing zooplankton concentration.

MATERIALS AND METHODS

Sample collection and otolith analysis

Anchovy juveniles were collected in a coastal area of the North Aegean Sea in front of the mouth of river Nestos (Fig. 1) during July 2007, December 2007 and February 2009 (Table 1). Sampling took place on board the RV 'Philia' by means of a small pelagic trawl. Fish were frozen immediately after capture at -35°C . In the laboratory, a length-stratified subsample of anchovy juveniles was selected for otolith analysis representing the entire length range of juveniles collected in each survey. Total length (TL) was measured to the nearest millimetre.

The sagittal otoliths were removed and processed following the methodology described in Schismenou et al. (2013) for the treatment of otoliths of large larvae. The right or left otolith was randomly selected from each fish and analysed under a light microscope coupled with an image analysis system (Image-Pro Plus 3.0, Media Cybernetics). The central part (nucleus) of the otolith was read at $\times 1000$ magnification, and the rest of the otolith at $\times 200$ using a mosaic image showing the increments from nucleus to the periphery of post rostrum (Fig. 2). Daily increments were marked along the maximum otolith radius (OR) from nucleus to post-rostrum, following the interpretation criteria proposed by Cermeño et al. (2008). The widths of individual increments (μm) were calculated using a specific algorithm of Image-Pro Plus. All otoliths were read independently by 2 readers, and the readings were only accepted if 100% agreement was reached.

In anchovy, increment deposition starts at hatching, and its daily pattern has been validated for larvae and juveniles (Cermeño et al. 2003, Aldanondo et al. 2008). Thus, individual age from hatching was deduced from the number of increments, and hatch dates (Table 1) were calculated by subtracting the number of increments from the sampling date (Aldanondo et al. 2010).

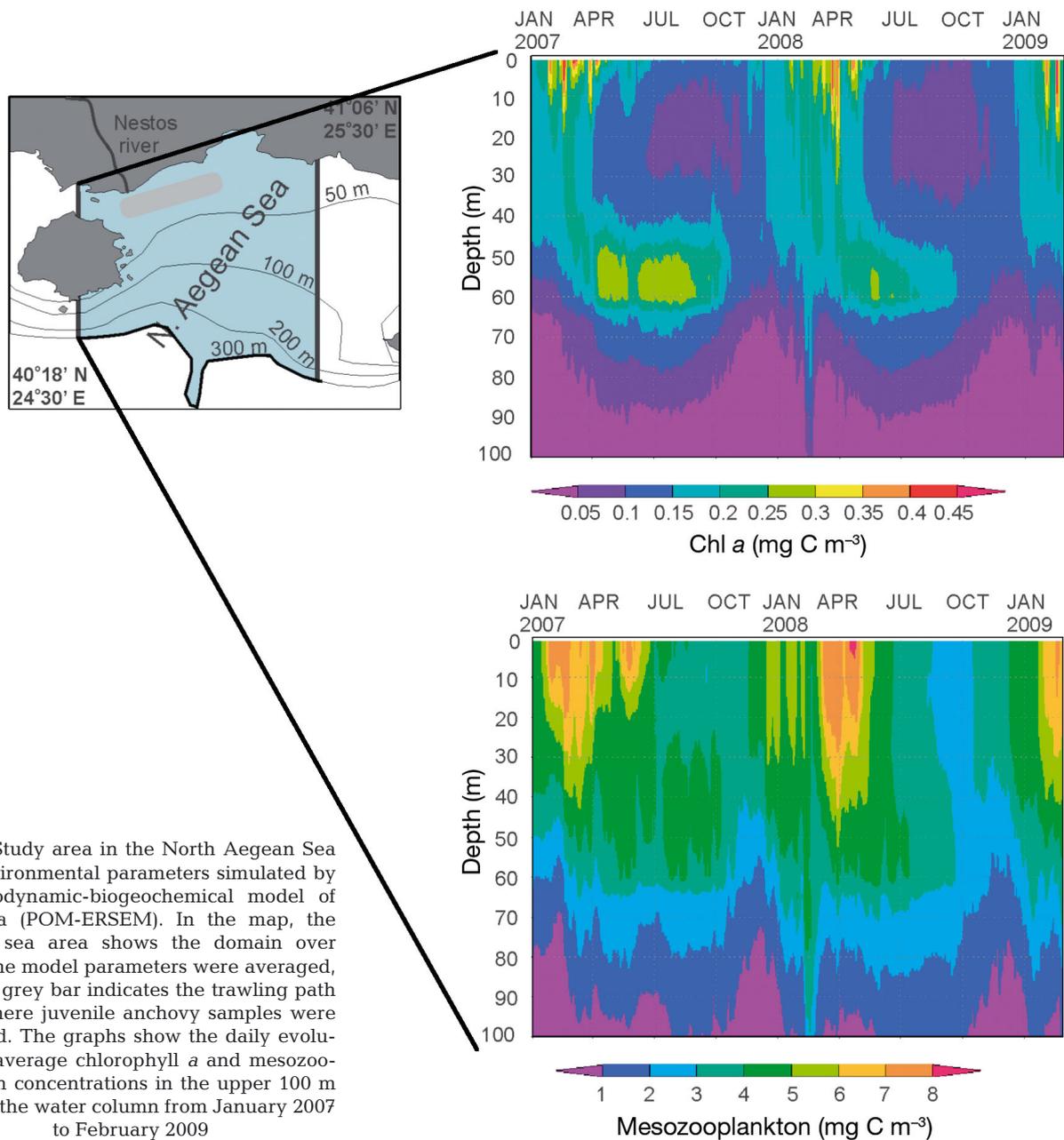


Fig. 1. Study area in the North Aegean Sea and environmental parameters simulated by a hydrodynamic-biogeochemical model of the area (POM-ERSEM). In the map, the shaded sea area shows the domain over which the model parameters were averaged, and the grey bar indicates the trawling path from where juvenile anchovy samples were collected. The graphs show the daily evolution of average chlorophyll *a* and mesozooplankton concentrations in the upper 100 m layer of the water column from January 2007 to February 2009

Table 1. *Engraulis encrasicolus*. Summary information on samples of juvenile anchovy collected from the North Aegean Sea on 27–31 July 2007, 8–12 December 2007 and 10–17 February 2009. N: number of individuals selected for otolith analysis; TL: total length. Dates are dd/mm/yyyy

Sampling date	No. of hauls	N	TL range (mm)	Hatch dates range
July 2007	5	61	43–80	21/04–29/05/2007
December 2007	6	83	44–80	23/08–19/10/2007
February 2009	12	66	60–100	17/10–07/12/2008

Daily biophysical model outputs

Information on the potential daily oceanographic conditions prevailing in the study area during the development of sampled juveniles was obtained by outputs of a 3-dimensional ecosystem model simulation in the North Aegean Sea (POM-ERSEM; Schismenou et al. 2013, Tsiaras et al. 2014). POM-ERSEM includes 2 on-

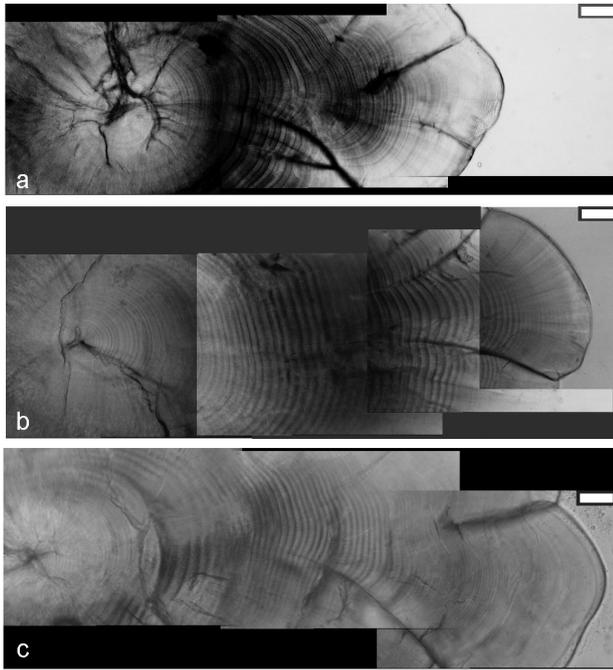


Fig. 2. *Engraulis encrasicolus*. Polished sagittae of anchovy juveniles from (a) July 2007 (TL 50 mm; age 65 d), (b) December 2007 (TL 71 mm; age 93 d) and (c) February 2009 (TL 80 mm; age 102 d). Scale bar (white rectangle): 50 μm

line coupled models, a hydrodynamic and a biogeochemical model, that describe the oceanographic environment through a wide range of physical and biological parameters (for details see Schismenou et al. 2013, Tsiaras et al. 2014).

The dataset of daily averaged oceanographic parameters used in this study was taken from Schismenou et al. (2013), and derived from an inter-annual simulation of POM-ERSEM in the North Aegean Sea from January 2007 to February 2009 in the wider sampling area, from the shore to the 300 m isobath (Fig. 1). The restricted habitats of juvenile anchovies over the continental shelf (up to 150 m depth) (Giannoulaki et al. 2013), the substantial overlap of these habitats with spawning grounds, and water circulation patterns that promote the retention of larvae close to spawning sites (Somarakis & Nikolioudakis 2007, S. Somarakis unpubl. data), all supported the assumption that the spatially averaged simulated parameters used would adequately represent the average conditions that individuals experienced while growing (Schismenou et al. 2013).

To further estimate the average environmental conditions that each group of fish experienced during their development, the model outputs were averaged for the period prior to sampling equal to maximum re-

ported age in the juvenile sample (Schismenou et al. 2013). The model parameters were sea surface temperature (SST, $^{\circ}\text{C}$), and salinity (SSS), both measured at 3 m depth; potential energy deficit (PED, J), a measure of vertical density stratification (Planque et al. 2007); integrated (0–100 m) chl *a* (CHLA, mg m^{-2}); integrated (0–100 m) mesozooplankton (MESOZ, mg C m^{-2}) and integrated (0–100 m) microzooplankton (MICROZ, mg C m^{-2}) biomass. Seasonal differences in the averaged POM-ERSEM parameters were tested with Kruskal-Wallis tests, as ANOVA assumptions were not met. In order to test for significant pairwise differences we performed multiple comparisons with the non-parametric Dunn's test (Zar 1999).

GAM analysis

The influence of biotic and abiotic parameters on juvenile growth was studied with GAMs using the daily increment widths (IW) as the response variable. As shown by Hinrichsen et al. (2010), successive otolith increment widths are autocorrelated, i.e. daily otolith growth is dependent on the growth of the previous day. Hence, we included as an explanatory variable the otolith increment width formed the previous day (IWPREV). Additionally, the pattern of otolith growth changes during the transition period from the larval to the juvenile stage (Baumann et al. 2006a, Allain et al. 2007). The length or age at which ontogenetic transitions occur and the duration of transitional periods in ontogeny decrease with temperature increase (Fuiman et al. 1998, Koumoundouros et al. 2001, Nikolioudakis et al. 2014a). Furthermore, the mean ontogenetic trajectory of otolith increment widths may be affected by growth selective mortality: a sample of individuals that have passed through a mortality bottleneck (e.g. severe overwinter mortality) may be composed of truncated ontogenetic trajectories (corresponding to fast-growing survivors), and such composition would be different if survival opportunities had been better. Thus, in order to account for the effects of seasonal differences in metamorphosis and survival on otolith width ontogenetic series, we introduced an additional explanatory variable, namely the interaction of sampling period (SURVEY) with fish age (AGE).

Further GAM construction for the inclusion of biotic and abiotic parameters (as daily average outputs from the POM-ERSEM simulation) was performed with a forward step-wise approach. The selection of the GAM smoothing predictors was done using the MGCV library in the R statistical software

(R Development Core Team 2009). Each model fit was examined in terms of deviance explained (0 to 100%; the higher the percentage, the more deviance explained), the Akaike's Information Criterion (the lower the better) and the confidence region for the fit (which should not include zero throughout the range of the predictor). The degree of smoothing was chosen based on the observed data and the restricted maximum likelihood estimation that outperforms the generalised cross validation smoothing parameter selection, as suggested by Marra & Wood (2011). To stabilise the variance of IW and IWPREV we used log-transformed values for these parameters (IWlog and IWPREVlog, respectively) (Venables & Ripley 2002). The link function used was Gaussian (family: identity).

RESULTS

Otolith microstructure

The interpretation of the daily increment pattern was easier for juveniles caught in February and more complicated for those caught in July due to the presence of sub-daily increments in the latter group that were difficult to distinguish from the daily rings (Fig. 2). In all 3 groups the first few (7 to 10) increments were the narrowest, up to 2 μm wide (Fig. 2). Sub-daily units first appeared at a short distance from the core; as the distance increased, daily increments widened and rings were gradually replaced by groups of micro-increments. This pattern was in accordance with other observations of anchovy larvae otolith microstructure in the same area (Schismenou et al. 2013). Fish caught in February had persistently wider increment widths for the period from hatching until maximum increment widths were observed. Average daily increment widths reached

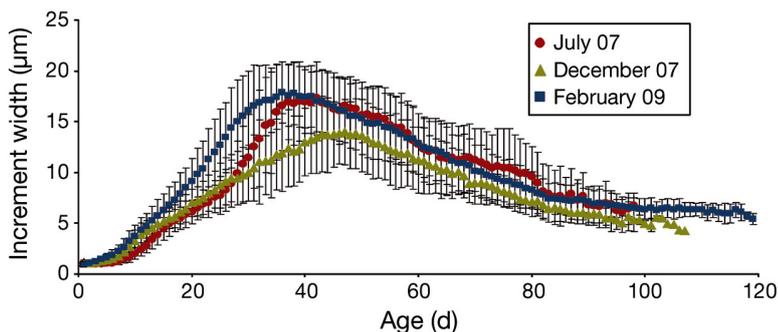


Fig. 3. *Engraulis encrasicolus*. Mean otolith width at age (d) of anchovy juveniles from the 3 sampling periods. Error bars show standard deviations

maximum values at 30 to 55 d old; earlier for the February group and later for the December group. Maximum values were higher for the July and February groups (17 and 18 μm , respectively) and lower for the December group (14 μm). Subsequently, increment widths steadily decreased to a minimum of 4 to 6 μm . In general, juveniles caught in December had the narrowest increments (Fig. 3).

Age and growth

Age readings ranged from 62 to 98 d for the July group, 52 to 107 d for the December group and 72 to 119 d for those caught in February. Hatch date calculation indicated that juveniles in the July group had hatched in late spring, those in the December group in late summer to mid-autumn, and those in the February group in mid-autumn to early winter (Table 1). All 3 groups exhibited high growth rates, as inferred from the slopes of the TL–age relationship, which ranged from 0.685 to 0.781 mm d^{-1} (Fig. 4). The different otolith size–fish size relationships among the 3 groups indicated that otolith and somatic growth were uncoupled ($\log(\text{OR})-\log(\text{TL})$; Fig. 4).

Oceanographic conditions

The simulated oceanographic parameters averaged for the periods of juvenile development indicated that fish from each group grew in different conditions (Table 2). Fish caught in July developed in warm, well-stratified waters of low salinity and high primary and secondary production. On the other hand, fish caught in February experienced unfavorable conditions compared to the July group; according to the POM-ERSEM simulation they grew in cold, saline and mixed waters of low productivity. Fish caught in December developed in intermediate environmental conditions.

GAM analysis

The model best explaining the otolith increment width included IWPREV, the interaction of AGE and SURVEY, and the 2 environmental variables SST and MESOZ. The model was highly significant ($p < 0.001$), explaining 96.2% of the total variance. The model residuals did not show any major bias or obvious pattern (Fig. 5).

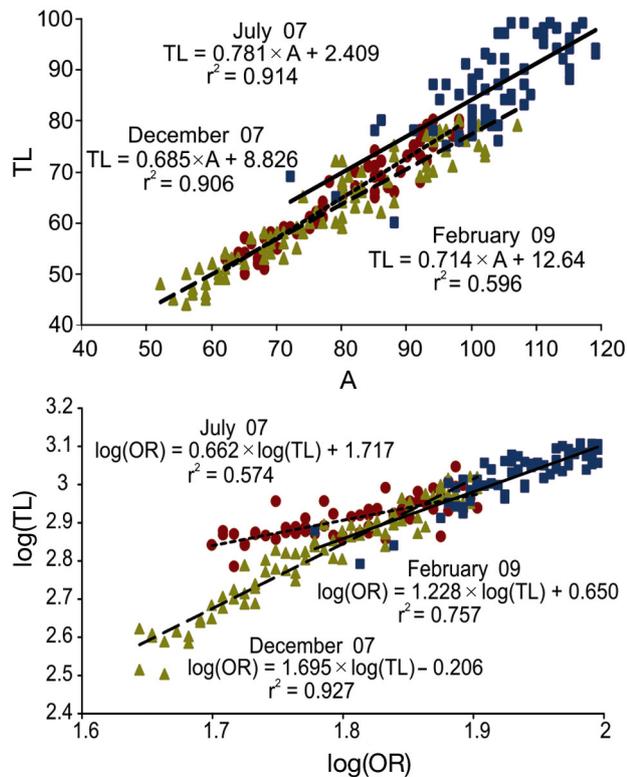


Fig. 4. *Engraulis encrasicolus*. Relationships between growth and age parameters for anchovy juveniles collected in July 2007 (circles, short-dash line), December 2007 (triangles, long-dash line) and February 2009 (squares, solid line). TL: total length (mm); A: age (number of otolith increments); OR: maximum otolith radius (μm)

According to the POM-ERSEM simulation, SST experienced by anchovy juveniles ranged from 13° to 27°C . Temperatures below 17.5°C had a negative effect. Temperatures between 17.5° and 24.5°C positively affected the increment widths, but further increase had a negative effect. MESOZ values

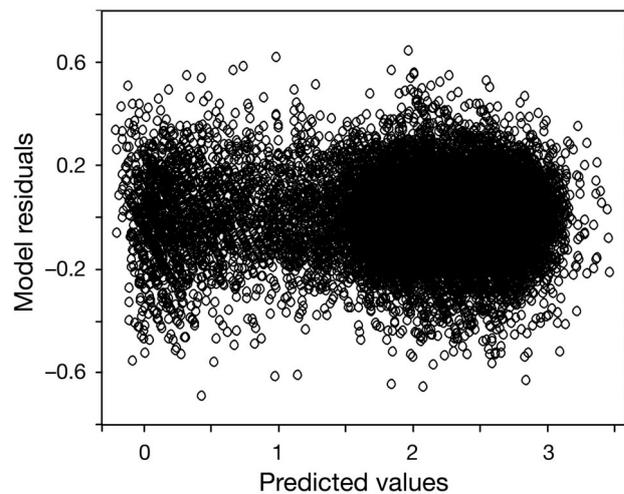


Fig. 5. *Engraulis encrasicolus*. Generalised additive model (GAM) residuals versus predicted values

>300 mg C m^{-2} had a positive effect on the otolith increment width (Fig. 6). The interaction of AGE and SURVEY (Fig. 7) accounted for the seasonal differentiation in the evolution of increment widths, i.e. the different age ranges at which maximum increment widths were observed for the 3 groups (Fig. 3).

According to model results, most of the variance was explained by the variable IWPREV, indicating high autocorrelation between successive increment widths (Fig. 6). An additional GAM analysis was carried out (not shown) using the same explanatory variables but excluding the autocorrelation term and using the difference 'last increment width' minus 'previous increment width' (logged) as the dependent variable. The deviance explained by the latter model was much lower (11.7%) but the effects of SST and MESOZ were significant and similar to the original model (Fig. 6).

Table 2. Output (medians) of environmental variables from a coupled 3D hydrodynamic-biogeochemical model (POM-ERSEM) in the broader area of sampling (Fig. 1) for the periods prior to sampling (equal to the maximum age of juveniles in each sample) in July 2007, December 2007 and February 2009. Values for chlorophyll a (chl a), micro- and mesozooplankton are integrated in the upper 100 m layer of the water column. The final row shows the results of comparative analysis using Kruskal-Wallis tests (H statistic; all values shown were significant at the $p < 0.001$ level). Superscript letters show the results of the pairwise comparisons with the non-parametric Dunn's test ($a > b > c$)

Sampling period	Surface temperature ($^{\circ}\text{C}$)	Surface salinity	Potential energy deficit (J)	Chl a (mg m^{-2})	Microzooplankton (mg C m^{-2})	Mesozooplankton (mg C m^{-2})
July 2007	21.87 ^a	35.68 ^c	248.31 ^a	11.91 ^a	255.00 ^a	320.00 ^a
December 2007	20.08 ^a	36.16 ^b	220.30 ^a	9.125 ^b	177.50 ^b	250.50 ^b
February 2009	16.28 ^b	36.72 ^a	83.70 ^b	8.18 ^b	155.00 ^b	235.00 ^b
H	119.878	215.290	156.875	147.419	167.847	130.398

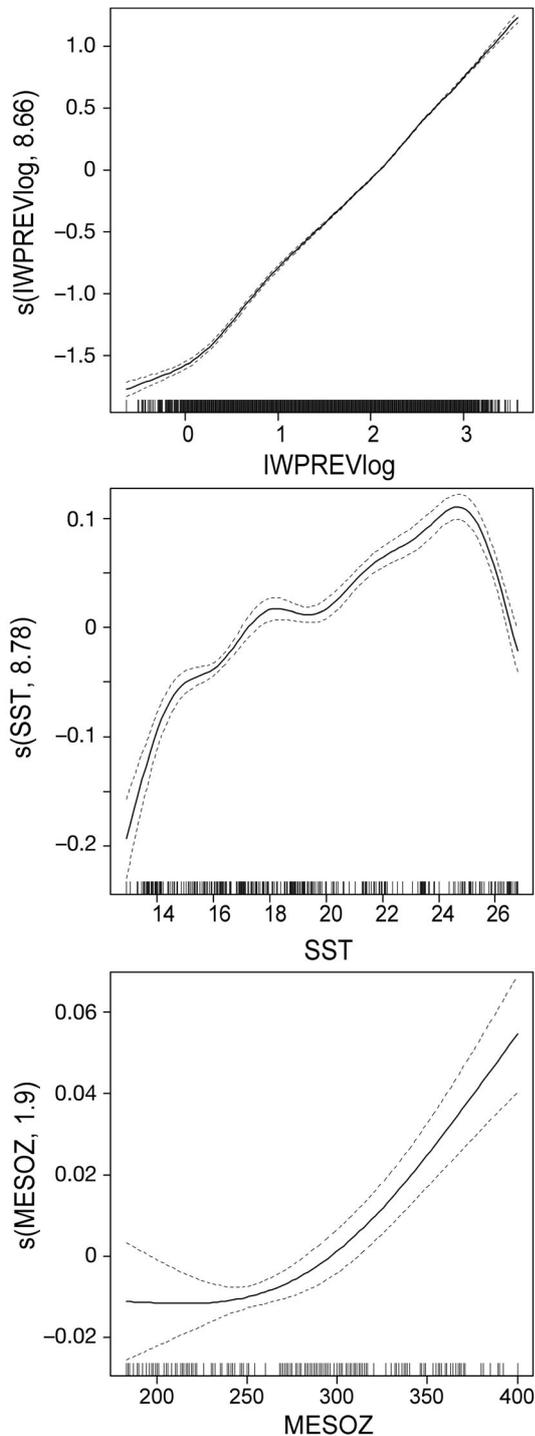


Fig. 6. *Engraulis encrasicolus*. Partial effects of variables in the generalised additive model (GAM) explaining juvenile anchovy growth. Solid lines represent the estimated smooth function and the dashed line the 95% confidence limits. The rug plot along the x-axis shows the values of the covariate; numbers (in parentheses) in y-axis captions are the effective degrees of freedom of each term. SST: sea surface temperature; MESZOZ: integrated mean mesozooplankton concentrations in the upper 100 m layer of the water column; IWPREV-log: width of otolith increment formed the previous day (log transformed values)

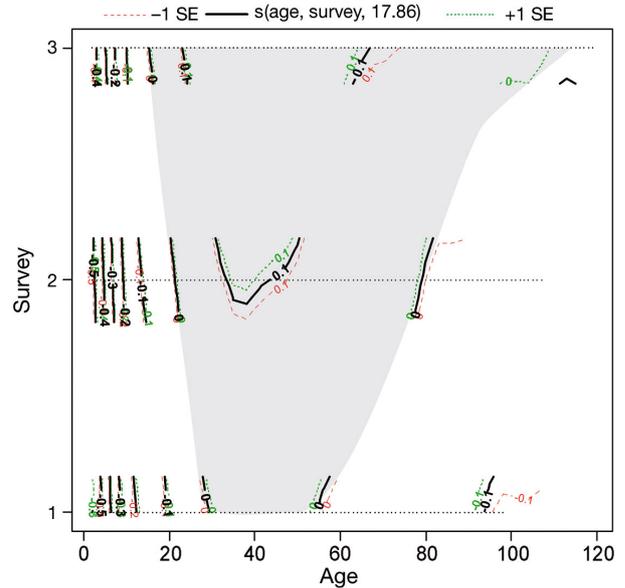


Fig. 7. *Engraulis encrasicolus*. Smooth functions of the interaction of AGE and SURVEY in the generalised additive model (GAM) explaining juvenile anchovy growth. Solid lines represent the estimated smooth function and the dashed red and green lines the -1 and $+1$ standard error limits. The key above the panel shows the interaction term and its effective degrees of freedom. The y-axis values correspond to the 3 surveys undertaken in (1) July 2007, (2) December 2007 and (3) February 2009. The shaded area indicates the duration of the transitional metamorphosing period

DISCUSSION

In this study we examined the seasonal variability in the growth of anchovy juveniles that hatched from April to May 2007, August to October 2007 and October to December 2008. In an attempt to unravel the environmental causes of this variability we used daily outputs of a coupled hydrodynamic-biogeochemical model as a proxy of the potential environment that the fish experienced from hatching to the day of capture. Additionally, for a better understanding of environmental influences on somatic growth, we used the model-derived environmental and ecosystem variables in a GAM analysis with daily otolith increment widths, also taking into consideration the inherent otolith growth (expressed by the explanatory variables IWPREV' and AGE) that is largely responsible for the uncoupling between somatic and otolith growth rates.

Otolith microstructure analysis revealed a dome-shaped pattern for the otolith growth trajectories, common among the 3 seasonal samples. In fish otoliths, transitional zones are generally correlated with life history changes, and similar changes in increment widths have been associated with meta-

morphosis (Rogers et al. 2001, Allain et al. 2003). In Baltic sprat, metamorphosis was related to maximal otolith growth, among other factors, and the metamorphosis point was considered to coincide with the maximum increment width (Günther et al. 2012). In the present study, the change in increment widths occurred at 30 to 55 d of age and varied depending on the season (Fig. 3). Similar observations have been reported for the European anchovy in the Adriatic Sea and the Bay of Biscay, where age at metamorphosis was estimated at 30 to 50 d and 40 to 60 d, respectively (La Mesa et al. 2009, Allain et al. 2003).

Fish caught in February exhibited larger increment widths during the period prior to metamorphosis (from hatching to maximum observed values) and reached the maximum increment widths earlier. As these fish developed in lower temperatures than fish of the other 2 groups (according to the POM-ERSEM simulation, Table 2), this observation seemingly contradicts the well-documented positive effect of temperature on otolith widths in several species (e.g. Otterlei et al. 2002, Folkvord et al. 2004, Takahashi & Watanabe 2004, Fey 2006). In the present study, the larger age-specific increment widths probably indicate that fish caught in February (late-born larvae) were fast growers and survivors from a population that had suffered severe size and/or growth-selective mortality. In general, adverse conditions during early life stages may negatively affect the growth rate and induce high mortality rates. This can result in removal of smaller and/or slower-growing individuals from the population and overestimation of the population growth rate, thus masking the effect of the adverse conditions (Heath & Gallego 1997, La Pape & Bonhommeau 2013). Nishimura et al. (2007) reported similar findings in a study of inter-annual variability in the hatching period and growth of juvenile wall-eye pollock *Theragra chalcogramma*, suggesting that variability in the timing of hatching affects the size/growth-dependent survival and delayed hatching might result in higher size-selective mortality.

We should mention here that the hypothesis of severe size/growth-selective mortality in winter 2008–2009 is largely based on simulated oceanographic data, not actual measurements, which might have presented a different pattern (e.g. zooplankton biomass during the development of the February group could have been higher than estimated from the model). However, comparison of POM-ERSEM simulated surface chlorophyll with remotely-sensed data (MODIS) indicated that the model effectively reproduces the seasonal changes in biological productivity (Schismenou et al. 2013).

Alternatively, increased size-selective mortality could have occurred in 2007, removing larger individuals from the larval population (i.e. specimens that would have wider increment widths than those observed in this study). Takasuka et al. (2004) found that the larger larvae had been selectively removed from an original population of larval Japanese anchovy *Engraulis japonicus*, indicating a higher predation rate on larger specimens. In the present study, the wide increment widths in fish collected in February 2009 could also be explained in terms of differences in fish size and/or condition between the 2007 and 2008 spawning populations, i.e. by females in 2008 being larger or in better condition than in 2007.

The average individual growth rates at the population level as inferred from the TL-AGE relationships were high for all juvenile groups ranging from 0.685 to 0.781 mm d⁻¹ (Fig. 4). Nonetheless, they were in accordance with previous studies on European anchovy late larval and juvenile growth in the same or other areas. In the North Aegean Sea, the average growth rate of anchovy late larvae (standard length range 8 to 55 mm) ranged from 0.63 to 0.80 mm d⁻¹ (Schismenou et al. 2013). In the Adriatic Sea, growth of late larvae and early juveniles was estimated at 0.55 to 0.82 mm d⁻¹ (La Mesa et al. 2009); while in the Bay of Biscay juvenile growth ranged from 0.32 to 0.89 mm d⁻¹ and the estimated maximum somatic growth rate was as high as 1.85 mm d⁻¹ (Aldanondo et al. 2010, 2011).

Slopes of TL-age relationships are often considered as mean individual growth rates at the population level; however, mean growth rates indicated from population data can be biased (Heath & Gallego 1997). In this study, fish in each seasonal sample had hatched over a wide time interval (Table 1), and those spawned earlier in the season may have experienced different oceanographic conditions compared to those born later (i.e. they may belong in the same sample but are not an homogenous group). Hence, for a better understanding of environmental influence on anchovy juvenile growth, we examined the variation of daily otolith increment widths using GAMs (Allain et al. 2007, Hinrichsen et al. 2010).

To account for the uncoupling between otolith and somatic growth rates, we first considered the inherent otolith growth by including in the GAM analysis the effects of ontogenetic changes (changes in the relative otolith growth during metamorphosis) and the autocorrelation of successive increment widths. This approach revealed that daily growth was significantly affected by temperature and food availability.

In the final model, most of the deviance was explained by the variable IWPREV, indicating that the

increment variations within the fish are highly auto-correlated (Fig. 6). Similarly, Hinrichsen et al. (2010) showed for Baltic sprat larvae that successive increment widths are not independent and in an analogous GAM analysis for otolith growth the inclusion of the previous increment width increased the percentage of explained variability from 81.3 to 98%.

Increment width variation was also significantly affected by the interaction of AGE and SURVEY (Fig. 7). The strong dependence of otolith growth on age has been shown in other studies dealing with otolith increment widths in European anchovy and sprat (Baumann et al. 2006a, Allain et al. 2007, Hinrichsen et al. 2010); it has been related with the ontogenetic change occurring during the transition period from larval to juvenile stage (trend of increasing otolith widths during the larval period followed by decreasing widths after metamorphosis). In our model we included the interaction of SURVEY \times AGE rather than AGE solely, to further account for seasonal (primarily temperature) effects on ontogenetic changes. Seasonal changes in temperature regimes may influence ontogenetic transitions, as has been shown for other morphological and morphometric traits; the age at which ontogenetic transitions occur as well as the duration of transitional periods in ontogeny decrease with increasing temperature (Fui-man et al. 1998, Koumoundouros et al. 2001, Nikolioudakis et al. 2014a). In Baltic sprat, Baumann et al. (2006b) demonstrated that the rate and magnitude of the increment-width increase during the larval stage, and the subsequent decrease after metamorphosis are all higher at higher temperatures. This general pattern may be masked by selective mortality as observed in the present study (Fig. 3) and discussed above. According to our GAM results, the age range during which otolith increment widths were affected positively (shaded area in Fig. 7) was different among the 3 groups, wider for the February group (lower temperatures) and narrower for the July group (higher temperature). These differences are actually related to the duration of the transitional metamorphosing period, which is longer at lower temperatures (e.g. Chen et al. 2008).

After accounting for inherent otolith growth, the seasonal (temperature) effect on ontogenetic changes and the level of growth/size-selective mortality experienced by the population from which the sample originated, SST was entered additionally into the GAM, and had a significant effect on daily growth (Fig. 6). In a similar GAM analysis of anchovy larvae and juveniles from the Bay of Biscay, Allain et al. (2007) found that temperature had an increasingly

positive effect on otolith incremental widths, and showed no optimum; however, in their study, temperature ranged from 13 to 20°C. In our case, the response of growth to temperature was positive up to 24.5°C, while further increase had a negative effect, implying the existence of an optimal temperature for anchovy growth. Based on otolith microstructure analysis, Takasuka et al. (2007) and Chiu & Chen (2001) demonstrated a similar dome-shaped temperature effect on Japanese anchovy larval growth. The optimum temperature for growth was estimated at 22°C in Japanese waters and at about 25°C in the East China Sea. In their recent review on life-cycle ecophysiology of small pelagic fish, Peck et al. (2013) identified an optimum temperature for European anchovy larval growth at around 22 to 24°C.

Finally, increased MESOZ (>300 mg C m⁻²) positively affected the daily growth. Hinrichsen et al. (2010), in an analogous GAM analysis, also found that somatic growth was significantly affected by prey abundance in larval Baltic sprat.

Anchovy juveniles feed exclusively on mesozooplankton (Nikolioudakis et al. 2014b), and post-flexion anchovy larvae derive the bulk of their energy from mesozooplankton (Borme et al. 2009). Coupled hydro-biogeochemical models, many of which are currently being developed and validated in several ecosystems around the world, comprise a unique means of simulating prey fields available for planktivorous fish on a daily basis. Their use in bioenergetics modelling (e.g. Megrey et al. 2007, Politikos et al. 2011), as well as in growth studies, as shown in the present study and in Schismenou et al. (2013), is expected to increase our understanding of the relationship between fish growth and pelagic ecosystem productivity. However, one should bear in mind the limitations, assumptions and uncertainty that still characterise these models.

The analysis carried out in the present study and in Schismenou et al. (2013) is based on the assumption that the spatially averaged oceanographic parameters simulated by POM-ERSEM adequately represent the average conditions that fish experienced while growing. Such an assumption requires that anchovy offspring mostly remain close to the spawning sites. Past and recent studies indicate that, in the Mediterranean Sea, anchovy habitats encompass specific continental shelf areas with high productivity, which are spatially restricted and separated by very oligotrophic, deep basins (Somarakis et al. 2004, Giannoulaki et al. 2013). Specifically, in the North Aegean Sea, the spawning, feeding and nursery grounds of anchovy are highly overlapping and restricted to coastal conti-

mental shelf areas with high biological productivity, influenced by fresh water inputs (Somarakis et al. 2012, Giannoulaki et al. 2013). Broad-scale ichthyoplankton studies have shown that the combination of a strong shelf-break front and a series of anticyclonic gyres generated over the continental shelf results in the increased retention of anchovy eggs and larvae close to their spawning sites (Somarakis & Nikoloudakis 2007, Isari et al. 2008).

Summarising, in this study we present a methodology which, through the explicit incorporation of inherent otolith growth in modelling the daily growth rate, allows for the use of otolith incremental width as a corrected proxy of somatic growth. The importance of accounting for seasonal changes in the timing and duration of metamorphosis as well as differences in growth-selective mortality between (pooled) fish samples is also highlighted. Such approaches are biologically justified and are expected to have broad application in future growth studies.

Acknowledgements. Part of this work was supported by the European project SARDONE (Improving assessment and management of small pelagic species in the Mediterranean, FP6-44294). This work was also financially supported by the General Secretariat of Research and Technology, Greece through the project REPRODUCE [(Recruitment Processes Using Coupled Biophysical Models of the Pelagic Ecosystem) — ‘Strengthening the links between European marine fisheries science and fisheries management — MARIFISH’ (ERAC-CT-2006-025989)] within the framework of the EU ERA-Net initiative (6th Framework Program), and the project SEAMAN [(Spatially resolved Ecosystem Models and their Application to Marine Management) — ‘Towards integrated European marine research strategy and programmes — SEAS-ERA’ (ERAC-CT2009-249552)] within the framework of the EU ERA-Net initiative (7th Framework Program). The authors thank Dr. Athanassios Machias, Dr. Konstantinos Tsagkarakis, Dr. Nikolaos Nikolioudakis and the captain and the crew of the RV ‘Philia’ for their help during the sampling cruises. Thanks also to Maria I. Kourepini for her help in the analysis of the fish samples. The SARDONE ‘growth team’ is especially thanked for the preparation of common otolith interpretation protocols. Finally, we thank the 3 anonymous reviewers and the Editor, Ivan Nagelkerken, for their valuable comments.

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