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Research article

Growth of larval anchovy (Clupeiformes: *Engraulis encrasicolus* L.) in İskenderun Bay, the Northeastern Mediterranean

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Abstract: In this study, growth patterns of larval anchovy (*Engraulis encrasicolus* L., 1758) were investigated based on otolith microstructure analyses and a meta-analysis of previously recorded growth rate estimates. For this purpose, samples were collected from Iskenderun Bay, the northeastern corner of Levant Basin, between 21 and 22 April 2017 and stored at -20 °C. The average temperature weighted by the abundance of larval anchovy was 18.0 ±0.24 °C (± standard deviation). In context of the study, a total of 76 larvae were investigated. Standard lengths (SL) ranged between 2.78 and 18.61 mm with an average value of 8.24 ±3.35 mm and the number of increments on the sagittal otoliths (A) ranged between 4 and 30. No significant difference was found between the morphometrics of the left and right sagitta. Positive allometric relationships were detected between otolith radius (OR) - SL ($OR=0.33SL^{1.87}$; $R^2=0.87$) and OR - A ($OR=0.95A^{1.24}$; $R^2=0.75$). The length at age relationship was SL=2.87+0.47A ($R^2=0.85$). In the meta-analysis, larval anchovy growth rates varied in a wide range without a clear spatial pattern regardless of coupling temperature conditions.

Keywords: Engraulidae, Levant Basin, micro-increments, otolith morphometry, otolith growth, somatic growth.

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Introduction

Small pelagic fishes are important elements of marine food webs constituting the connection between lower and higher trophic levels (Palomera et al., 2007). Opportunistic life history strategy is prevalent among small pelagic fishes, most of which have a short life span, fast growth, and early maturity (Pecuchet et al., 2017). Because of these life history traits, their populations can quickly respond to changes in environmental conditions. Therefore, fluctuations of small pelagic fishes constitute a good indicator of disturbances in marine ecosystem such as climate change, eutrophication, pollution and overfishing (Gücü et al., 2017; Oguz, 2007; Peck et al., 2013; Takasuka et al., 2007). Along with their ecological importance, small pelagic fishes are the major component of marine capture fishery throughout the world as being an important resource for direct human consumption and raw material for fish oil and fish meal industry (FAO, 2020).

Anchovy, *Engraulis encrasicolus* (L. 1758), is the most important commercial fish species for the Turkish fisheries, particularly in the Black Sea and the Marmara Sea. Although it is mostly discarded by the fishers operating in the Mediterranean (Mavruk & Avşar, 2010), anchovy constitute an important part of diet of many commercial fish species such as bluefin tuna (Karakulak et al., 2009) and lizardfish (Özyurt et al., 2017) in this area.

Early life stages of anchovy is among the most dominant elements of ichthyoplankton assemblages in the

Northeastern Mediterranean (Ak, 2004; Avsar & Mavruk, 2011; Mavruk et al., 2018). Its eggs and larvae can be encountered throughout the year, however its abundance is highest in spring and summer months from April to October (Mavruk et al., 2018). Although extensive research has been carried out on the growth of larval anchovy distributed in the Eastern Atlantic (Cotano et al., 2008; Ré, 1987), the Western Mediterranean (García et al., 2005; Palomera et al., 1988; Palomera et al., 2007), the Adriatic Sea (Coombs et al., 1997; Dulcic, 1997; Regner, 1996) and the Aegean Sea (Catalán et al., 2010; Somarakis & Nikolioudakis, 2007), only one study have produced estimates of larval growth rate in the Levant Basin (Walline, 1987).

Levant Basin is the warmest and saltiest edge of the Mediterranean (Miller, 1983). Although the basin is characteristic with narrow continental shelf and highly oligotrophic waters, Iskenderun Bay, its northeastern corner (Figure 1), constitutes an exception to this generalization. The primary production of the bay is 2 to 4 fold higher than offshore waters because of the shallow topography and vast amount of freshwater inputs (Avsar, 1999; Yilmaz et al., 1992). The bay is a very important spawning area for small pelagic fishes including anchovy, particularly in April when spring bloom occurs (Mavruk et al., 2018).

So far, no previous study exists on the biology of larval anchovy in the northern coasts of the Levant Basin. The purpose of this study is therefore to estimate growth parameters of larval anchovies distributed in Iskenderun Bay via otolith microstructure analyses and to investigate regional and temperature dependent variations of growth rates by performing a meta-analyze.

Material and Methods

Ichthyoplankton samples were collected at 30 stations during 21-22 April 2017 in İskenderun Bay, the Northeastern Mediterranean, Turkey. Eight stations were located at 0-20m, 20 stations at 20-50 m, five stations at 50-100 m and three stations at >100 m depth contours (Figure 1). Double oblique tows were performed from surface to the depth layer 5 m above the bottom or 100 m at the deeper stations using a Bongo-60 Net equipped with 300 µm meshes, a mechanical flowmeter (Hydrobios 438-110) and a depth gauge with maximum depth indicator. Plankton samples were filtered to 200 ml volume bottles and kept at -20 °C until laboratory procedures. Temperature and salinity profiles were recorded at each stations using a YSI 6600 CTD.

The mixed layer depth was calculated according to 1°C deviation from the surface water temperature. Then, temperature and salinity values were averaged for above and below the mixed layer depth, representing the surface and bottom layers. To analyze chlorophyll-a concentrations, water samples were taken from the surface at ten of the stations. Chlorophyll-a analyzes were performed by the spectrophotometric method of Parsons (2013).

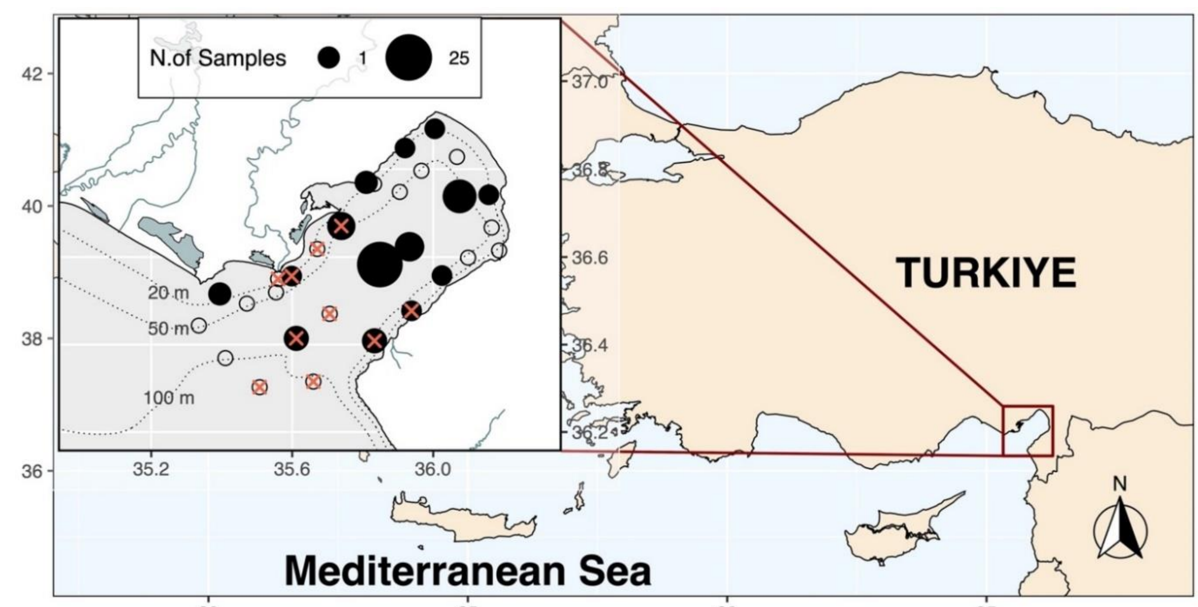

Figure 1. Study area, locations of sampling stations and number of investigated larvae in each station. Filled circles and empty circles are the stations with or without anchovy larva included to otolith microstructure analyses, respectively. x shows the stations where surface water samples were taken for Chlorophyll-a analyses.

After samples were thawed at the laboratory, undamaged anchovy larvae were identified based on the morphological criteria given by Lo-Bianco (1956). Then, each individual was photographed under a stereo microscope (Leica M125) equipped with a DSLR camera (Nikon D7100). Standard length (SL) of each larvae were then measured using Fiji extension of ImageJ software (Schindelin et al., 2012). Afterwards, head of each larva was dissected, and sagittal otoliths were removed using microdissection needles under polarized light. Sagittal otoliths were then mounted on microscope slides using Crystalbond 509 epoxy resin. Depending on the size, otoliths were photographed under 40x or 100x magnification using a compound microscope (Olympus BX50) equipped with an imaging system. Oil immersion was used in 100x magnification. Multiple photographs were taken from each otolith, slightly changing focus of objective to distinguish daily increments from sub-daily ones (Campana, 1992).

In larval fishes, sagittal otoliths usually compose of concentric translucent and transparent zones which appear as dark and light rings under the transmitted illumination. Light rings are low protein content areas, which are assumed to be representing daytime depositions when larvae grow faster, whereas dark rings are protein rich units assumed to be nighttime depositions (Campana & Neilson, 1985). Hatching and first feeding leave two well defined marks on the otolith of larval anchovies (Aldanondo et al., 2008; Palomera et al., 1988). Aldanondo et al. (2008) have shown that increment deposition occurs in daily basis in larval anchovies under rearing temperatures higher than 17.6 °C. Sub-daily units can also be observed among regular daily rings, but, the structure of daily rings is continuous along the whole otolith and daily rings are persistent when focus of microscope is slightly readjusted (Palomera et al., 1988).

In context of this study, the maximum otolith radius (OR) and surface area (OA) were measured from the photographs using ImageJ. Multiple photographs of the same otolith were then aligned and stacked to distinguish daily formations from sub-daily ones. Daily increments were counted twice from the longest axis of otoliths by the same person. Counting started from the second check which assumingly represented the transformation to exogenous feeding (shown in Figure 2b). The readings were performed three months apart using ObjectJ and TreeRings macros of ImageJ.

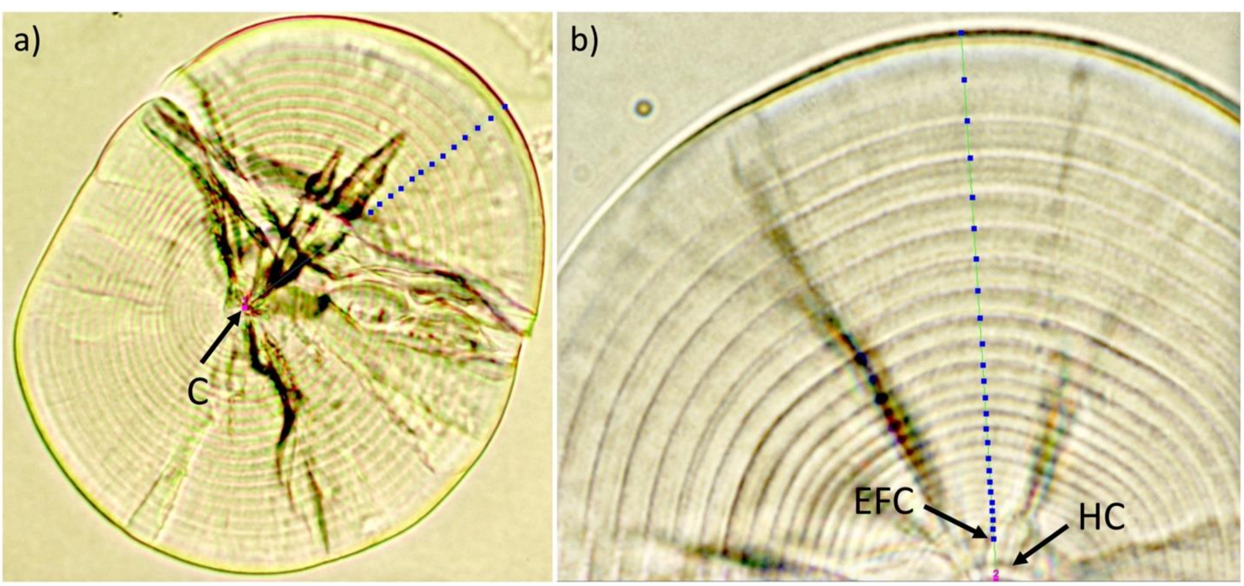

Figure 2. Enhanced photographs of sagittal otoliths of 12 (a) and 24 (b) days old larvae. Daily increments are marked with blue dots. C represents the core, HC is the hatch check and EFC is the exogenous feeding check.

Percentage agreement tests were applied to investigate precision of age readings. The otoliths were excluded from the further analyses if difference between two independent age readings was higher than 10% of the average age (Jones, 2002; Walline, 1987). Difference between the morphometrics of left and right sagittal otoliths were tested using paired samples Welch t-tests. SL - OR, and number of increments (A) - OR relationships were fitted to an allometric equation using non-linear least squares method. To model length at age relationship of larval anchovy; linear, allometric, exponential, logistic, von-Bertalanffy and Gompertz growth functions were fitted using least squares or non-linear least squares methods. Determination coefficient (R^2), Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) values were calculated and investigated to select which functional form provided the best fit.

For the larval anchovies, Urtizberea et al. (2008) and Peck et al. (2013) are listed available growth rate estimates coupled with temperature of ambient environment. Both lists and the estimate of present study were combined excluding the growth rates derived from mesocosm experiments and estuarine samples. Regional changes of daily growth rates were then analyzed with Welch's Heteroscedastic F Test using "onewaytest" package (Dag et al., 2018). Temperature dependence in daily growth rates were also analyzed using a linear regression. Throughout the text, values were reported as average ± standard deviation unless otherwise stated. All statistical analyses and modelling procedures were applied in R Language and Environment for Statistical Computing (R Core Team, 2021).

Results

The average values of environmental conditions measured at the stations were given in Table 1. Water column temperature ranged between 17.6 and 18.7 °C at the stations where anchovy larvae were sampled. The average temperature weighted by the abundance of anchovy larvae was calculated as 18.0 ±0.24 °C. A weak vertical stratification was observed in most of the stations and the average difference between the surface and bottom layer temperature was 1.73 ±0.42 °C.

Table 1. Summary of environmental conditions measured during the study (* No vertical stratification)

Parameters	range	mean ± sd
Sea Surface Temperature (°C)	17.7 - 20.5	18.6 ±0.5
Sea Bottom Temperature (°C)	17.2 - 19.4	17.7 ±0.3
Sea Surface Salinity (psu)	36.0 - 39.7	39.4 ±0.3
Sea Bottom Salinity (psu)	37.9 - 39.9	39.6 ±0.1
Secchi Depth (m)	2 - 14	9 ±4
Mixed Layer Depth (m)	* - 39	11 ±8
Sea Surface Chlorophyll-a (mg/m^3)	0.12 – 2.49	0.76 ±0.36

Sagittal otoliths of a total of 76 undamaged anchovy postlarvae were successfully extracted and prepared. Spatial distribution of investigated larvae was given in Figure 1. In 57 of investigated larvae (75%), the difference of two independent age readings was lesser than 10% of the average of two readings. These were considered as passed the percentage agreement test providing sufficient precision for further analyzes. SL of investigated postlarvae ranged between 2.78 and 18.61 mm with an average value of 8.24 ±3.35 mm. The number of increments ranged from 4 to 30 with an average value of 13 ±7 (Figure 3). No statistically significant difference was detected between the number of increment (t=0.22, p=0.83), otolith radius (t=0.19, p=0.86) and otolith surface area (t=0.01, p=0.99) of the left and right sagitta. The maximum radius of larval otoliths was found to be between 6.9 and 79.6 μm with an average value of 22.7 ±16.2 μm. The distance from the center to the first increment deposition ranged from 4.3 to 11.8 μm with an average value of 7.1 ±1.4 μm (±0.4 μm, 95% confidence intervals).

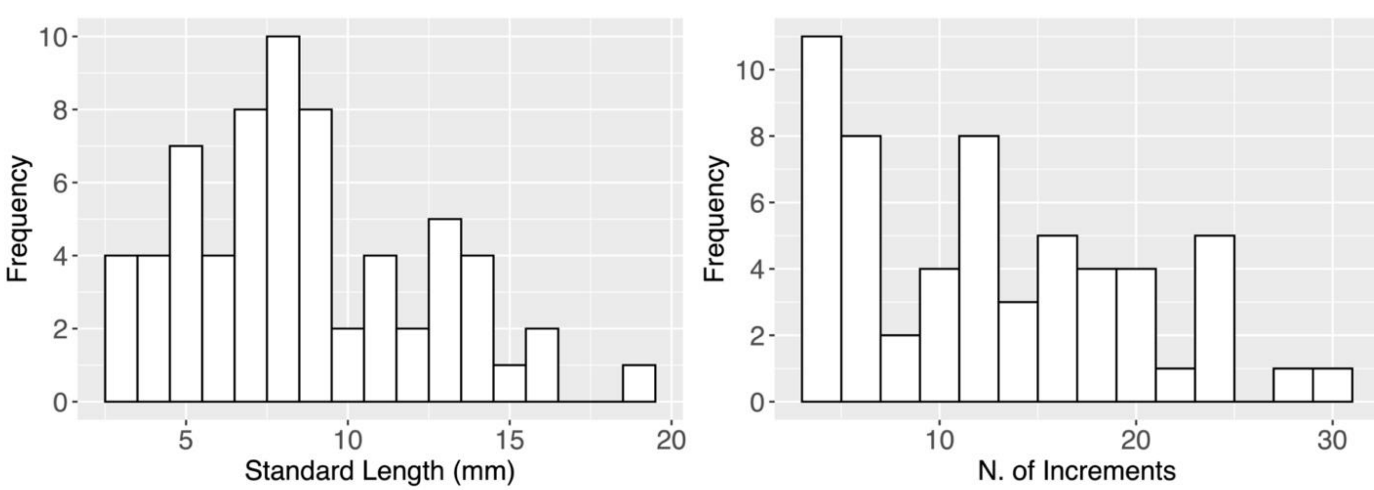

Figure 3. Length (a) and age (b) frequency distributions of investigated anchovy larvae.

Parameters of allometric regressions between SL-OR, and A-OR were given in Table 2. Length and age specific growth of larval anchovy otoliths were found to be positive allometric with b values significantly higher than one ($p<0.05$). Goodness of fit statistics and parameters of different growth models used to fit somatic growth of larval anchovies were given in Table 3. Linear model provided the best fit for the somatic growth with the lowest AIC and BIC values and the highest determination coefficient. Therefore, larval growth was found to be isometric within the investigated length range of anchovy. The intercept was detected as 2.9 ±0.8 mm (±95% confidence interval) and daily somatic growth rate was 0.47 ±0.05 mm/day (±95% confidence interval) (Figure 4).

Table 2. Parameters of allometric regressions between standard length (SL) - otolith radius (OR) and number of increment (A) - otolith radius (OR).

		Parameter	±95% CI	Std. Error	t value	Pr(>|t|)
$OR=a*SL^b$	a	0.33	0.13	0.06	5.30	<0.001
	b	1.87	0.15	0.07	25.57	<0.001
	RSE= 3.65; df= 41; R^2_{adj}= 0.87					
$OR=a*A^b$	a	0.95	0.60	0.27	3.49	0.0013
	b	1.24	0.20	0.09	13.17	<0.001
	RSE= 6.05; df= 34; R^2_{adj} = 0.75					

* RSE: Residual standard error, df: degrees of freedom, R^2_{adj}: adjusted determination coefficient

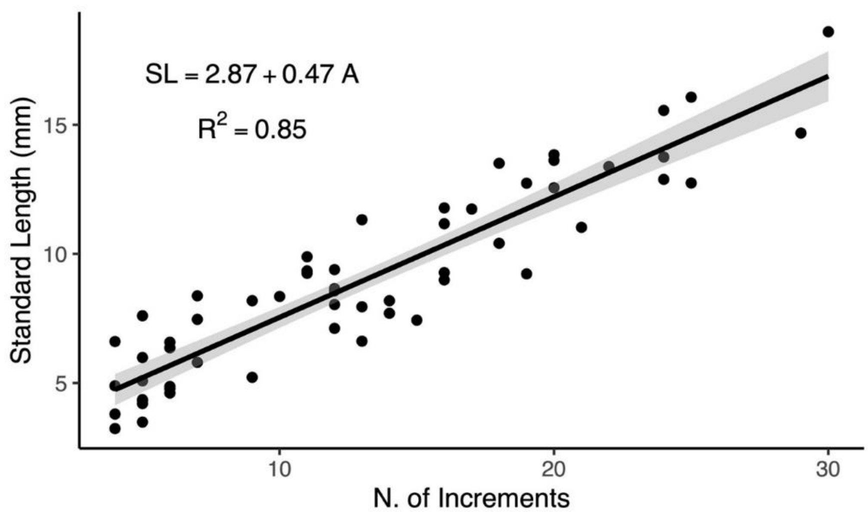

Figure 4. Linear growth model of larval anchovy (SL: standard length, A: number of increments, shade shows 95% confidence band of prediction).

Table 3. Parameters and goodness of fit statistics of different models fitted for somatic growth of larval anchovies.

	Linear	Allometric	Exponential	Logistic	von-Bertalanffy	Gompertz
a	2.874 (p<0.001)	1.759 (p<0.001)	4.673 (p<0.001)	21.483 (p<0.001)	35.012 (p=0.066)	28.405 (p<0.01)
b	0.467 (p<0.001)	0.642 (p<0.001)	0.046 (p<0.001)	4.850 (p<0.001)	0.547 (p<0.001)	2.126 (p<0.001)
c	-	-	-	0.094 (p<0.001)	0.031 (p=0.066)	0.046 (p<0.01)
R^2	**0.851**	0.820	0.826	0.848	0.850	0.849
R^2_{adj}	**0.848**	0.816	0.823	0.842	0.844	0.844
AIC	**204.849**	206.987	213.481	207.784	207.128	207.282
BIC	**210.978**	213.116	219.611	215.957	215.300	215.455

Results of meta-analysis showed that the length specific somatic growth rates estimated for the various parts of the Mediterranean marine environment ranged between 0.41 and 0.96 mm/day with an average value of 0.59 ±0.15 mm/day. Since only one estimate was available in the Adriatic, it was excluded from the analyze of regional changes. The average growth rate of the Eastern Mediterranean and the Aegean Sea was found to be similar as 0.51 mm/day. Although the average growth rate was higher in the Western Mediterranean (0.65 mm/day), the difference was not significant (p=0.14) (Figure 5a). The slope of growth rate – temperature regression was not found to be significant (b=0.001, p=0.92) (Figure 5b).

Discussion

In this study, length specific somatic growth rate of larval anchovy was estimated as 0.47 ±0.05 mm/day (±ci) in Iskenderun Bay. A considerable amount of literature is available on the growth of larval anchovies sampled from estuary (Ré, 1987) and marine environment (Walline, 1987, Catalán et al., 2010; Dulcic, 1997; Palomera et al., 1988; Peck et al., 2013 and references therein) as well as reared in the laboratory conditions (Aldanondo et al., 2008; Regner, 1980). Based on the lists of previous studies provided by Urtizberea et al. (2008) and Peck et al. (2013), length specific growth rate estimates vary in a wide range from 0.41 to 0.96 mm per day with an average value of 0.59 mm per day in the marine environment. Although our estimate is within the range of previous studies, it is lesser than the overall average. The only previous estimate from the Levant Basin is given by Walline (1987) as 0.55 mm per day from the Israeli coasts and this value is also significantly higher than our results. On the other hand, the growth rate values reported from the northern Aegean Sea are close with our results (Catalán et al., 2010; Somarakis & Nikolioudakis, 2007). Although, mostly higher growth rates were reported from the western Mediterranean (García et al., 2005; Palomera et al., 1988; Palomera et al., 2007), no significant difference was found between the areas.

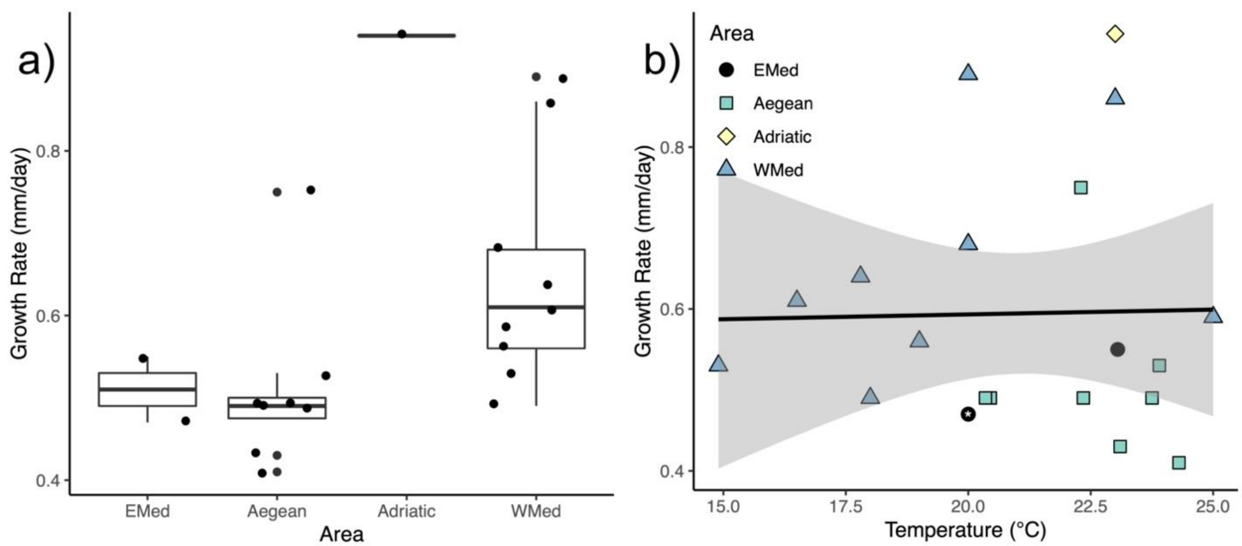

Figure 5. Regional changes of daily somatic growth rates (a) and growth rate – temperature relationship (b) (G=0.57+0.001T, p>0.05). * Estimate of the present study. Growth rates and coupling temperature values were taken from Urtizberea et al. (2008) and Peck et al. (2013) representing the studies performed in the natural marine environment.

Variation in larval growth rates is attributable to many endogenous and exogenous conditions such as the genetic factors (Hutchings et al., 2007), maternal effects (Berkeley et al., 2004), influence of temperature (Klimogianni et al., 2004; Pörtner & Peck, 2010; Takasuka et al., 2007), food density (Gleiber et al., 2020; Paulsen et al., 2016), food quality (Paulsen et al., 2014), presence of chemical compounds (Westernhagen, 1988) or other pollutants such as microplastics (Xia et al., 2020). Influence of temperature has had particular attention in the previous studies. In the Sea of Japan, Takasuka et al. (2007) revealed quadratic relationships between temperature and the recent growth of larval anchovy as well as sardines. In another study, recent 5-day growth of larval Japanese anchovy is linearly increased with temperature at multiple locations in Kii Channel (Yasue & Takasuka, 2009). In laboratory conditions, Aldanondo et al. (2008) have reported that the growth rates of European anchovy significantly change in different rearing temperatures, and the relationship is accordingly quadratic with a maximum growth rate of 0.33 mm/day at 20.8 °C. The maximum growth rate of European anchovy is found as 0.8 mm/day at 21.3 °C in rearing experiments performed by Regner (1980). On the other hand, for European anchovy, these results are not supported by the studies performed in the marine environment. In the northern Aegean Sea, Somarakis & Nikolioudakis (2007) have reported that the somatic growth rate of larval European anchovy is 0.49 mm per day, regardless of different sampling temperatures ranging from 19.3 to 25.5 °C. In the same area, Catalán et al. (2010) reported highly variable somatic growth rates ranging from 0.41 to 0.75 mm/day regardless of temperature conditions. Consistently, in this study, no significant relationship was detected between the daily growth rates and temperature for larval European anchovy collected from the various parts of the Mediterranean.

This study was performed during the spring bloom conditions which assumingly provided a rich feeding field for larval fishes (Cushing, 1990). Previously, Takahashi et al. (2012) revealed a positive relationship between the recent growth of larval anchovy and chlorophyll-a values in an upwelling area in the California current system, although conflicting observations are available as well. For instance, no positive correlation was found between the chlorophyll-a and growth rate in the northern Aegean Sea (Catalán et al., 2010). Also, our results revealed no evidence on food limitation in the growth of larval anchovies. In comparison with the northern Aegean Sea, remarkably higher surface chlorophyll-a values were observed in our study, although no significant difference was found between the growth rates (Catalán et al., 2010). The diet of larval anchovy primarily consists of Copepod nauplii and copepodite stages, and the chlorophyll-a values are not necessarily correlated with the intensity of its main food items (Catalán et al., 2010; Conway et al., 1998; Horstman & Fives, 1994). Therefore, our study was not considered to be sufficient to investigate the influence of feeding conditions on the larval growth since no further data was available on the potential food items.

Although anchovy is a yearlong spawner in the Mediterranean, our sampling was limited with April when the abundance of larval anchovy starts to increase in the area (Mavruk et al. 2018). On the other hand, growth of larval anchovy may significantly differ amongst seasons as shown by Leonarduzzi et al. (2010) in the southwest Atlantic waters. Further studies, which take seasonal effects into account, will therefore need to be undertaken.

Palomera et al. (1988) and Dulcic (1997) used Gompertz function to model length at age relationship in larval European anchovies revealing that the instantaneous growth rate is decreasing after 11^{th} and 14^{th} days, respectively. Also, García et al. (2005) have determined that the growth of larval European anchovy is negative allometric. Although the performance of both Gompertz and allometric functions was investigated also in this study, linear model provided the best fit indicating the growth of larval European anchovy was isometric within the range our samples (2.78-18.61 mm; 4-30 increments). This also accords with the majority of previous studies (Catalán et al., 2010; Palomera et al., 2007; Somarakis & Nikolioudakis, 2007; Walline, 1987).

For the anchovy reared in laboratory conditions, Aldanondo et al. (2008) revealed that the radius of otoliths ranges between 3.13 and 4.48 µm at hatching. Slightly lower (2.93-4.05 µm; Aldanondo et al., 2008) and higher values (4.3-4.7 µm; García et al., 2005) are also reported by the studies performed in the marine environment. Although first regular increment deposition is reported to be formed after the hatching (Aldanondo et al., 2008), these early depositions are mostly invisible in large otoliths because of thickening (Palomera et al., 1988). Therefore, in this study, increments were counted after the second well defined mark on the otolith which potentially represented the transformation from endogenous to exogenous feeding (Aldanondo et al., 2008; Palomera et al., 1988). In accordance with this, distance from center to the first increment deposition was found to be 7.11 µm which was significantly higher than the reported otolith radius at hatching (Aldanondo et al., 2008; García et al., 2005). Additionally, the intercept of somatic growth model was found as 2.9 ±0.8 mm. In an adjacent area, Mersin Bay, Ak (2004) have reported that the size of anchovy prelarvae ranges from 2.17 to 3.82 mm, and the smallest postlarva is at 2.45 mm, suggesting that our intercept value corresponds to the length at first feeding. These arguments therefore indicates that the number of increments given in this study represents the age of larva in days after the first feeding started.

In accordance with previous studies (Dulcic, 1997; García et al., 2005; Palomera et al., 1988), length and age specific growth of larval European anchovy otoliths was determined as positive allometric in the present study. There was a high correlation between the otolith radius and standard length indicating that the widths of daily increments could potentially be employed in reconstruction of growth history (Jones, 2002; Palomera et al., 1988) in further studies.

In conclusion, this study is first to determine larval growth parameters of European anchovy in the northern coasts of the Levant Basin. Analyzing our results together with previous studies, a great variation was detected in the growth rates reported from various parts of the Mediterranean. No regional pattern was observed and the variation in growth rates was not attributable to temperature differences as well. Future studies on the reasons of observed changes in growth parameters are therefore recommended.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Ethical Approval

All applicable national guidelines for the care and use of animals were followed.

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