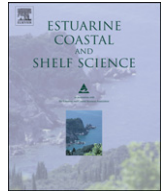




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# Ecological understanding for fishery management: Condition and growth of anchovy late larvae during different seasons in the Northwestern Mediterranean

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## ABSTRACT

The fishery of the European anchovy *Engraulis encrasicolus* in the Mediterranean needs several ecological approaches to be properly managed. As such, several surveys were carried out to study the ecology of larvae and juveniles of this species, which reproduces during the warmest period of the year (May through September) in the Gulf of Lions. In particular, we studied the late larvae (15 mm total length until metamorphosis), especially as other authors have focused on larvae below that size. Unexpectedly, we also collected anchovy late larvae during the December 2007 survey, whose range in size corresponded to a later spawning period than previously reported. Differences in the nutritional condition of these larvae were assessed by comparing indices of lipid composition and estimating growth rates from otolith measurements to provide information on the probability of survival between the two groups. The analysis of fatty acids, used as tracers of trophic relationships, indicates that these larvae fed mainly on zooplankton. Nutritional conditions of summer and late autumn larvae were very similar. In contrast, growth rates were higher for August larvae, probably due to the different temperatures in the two seasons. Our results are especially relevant in an ecological context where the increasing mean water temperatures in the Western Mediterranean could favor the extension of the anchovy spawning period up to late-Autumn months.

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## 1. Introduction

The European anchovy *Engraulis encrasicolus* is a highly valued fishery resource in the Western Mediterranean Sea (García and Palomera, 1996; Pertierra and Leonart, 1996; Barange et al., 2009). Several studies have been carried out regarding diverse aspects of the biology and the ecology of the species in this area to improve the available tools for its fishery management (Palomera et al., 2007). The Northwestern Mediterranean is one of the most productive areas in this sea due to the cyclonic current that flows southwards over the slope of the Gulf of Lions, carrying a significant nutrient load from the Rhône River (Salat, 1996). The Gulf of Lions also displays notable environmental differences between seasons, which directly influence low trophic level species (Calbet et al., 2001). The anchovy population could then be easily compromised by any sort of alteration that additionally impinges on these organisms, especially during the early development stages (i.e. eggs, larvae and juveniles) when they are particularly sensitive to any change (Palomera et al., 2007). The anchovy spawning period in the Gulf of Lions extends from April to late September, with a peak

in late June (Palomera, 1992). Therefore late larvae are expected to be found mainly during the summer, while juveniles should emerge in the autumn and winter.

During the SARDONE project, devoted to improve the management strategies of the European anchovy, two cruises were undertaken in August and December of 2007 in the Gulf of Lions in order to collect anchovy late larvae and juveniles, respectively. Unexpectedly, and in spite of the allegedly unfavorable conditions, a notable amount of anchovy late larvae were caught in December (a mean of 26.75 larvae/tow, against 34.20 larvae/tow in summer), well after the reported end of the spawning period.

An understanding of the growth and feeding ecology of the species is important given that this knowledge is essential to understand how the population temporally and spatially develops in the environment. The relationship between this population and the plankton community is not straightforward but some approaches have been attempted (Isari et al., 2008; Morais et al., 2010), especially those concerning how the zooplankton affects the population strength. Indeed, the growth rates and the nutritional condition of a population of several species, including European anchovy, have been key subjects for the study of the recruitment strength (Butler, 1991; Ward et al., 2006; Palomera et al., 2007; Hidalgo et al., 2008; Islam and Tanaka, 2009; La Mesa et al., 2009).

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In the present case we analyze for the first time in the Mediterranean the nutritional condition of anchovy late larvae (19–35 mm) in the field during both summer and late-Autumn seasons via lipid composition studies, and we also study their diet through the fatty acids found both in larvae and in zooplankton, which is the basic prey of anchovy at all the development stages (Plounevez and Champalbert, 1999; Pasquaud et al., 2008; Bacha and Amara, 2009; Borne et al., 2009; Catalán et al., 2010; Morote et al., 2010). Growth rates were also studied for both larvae populations to determine whether the different environmental conditions affect the early stage development of the anchovy. This work will thus help to determine to what extent the general conditions of an unexpected December late larvae population differs from the August late larvae and whether these two populations have similar viability.

## 2. Materials and methods

### 2.1. Sample collection

Samples were collected during two cruises carried out in the Gulf of Lions (Northwestern Mediterranean sea; Fig. 1) in 2007 on board the *R/V L'Europe* (IFREMER, France). The first cruise (PELMED07) was conducted in the summer from the 28th of July to the 9th of August 2007 and the second cruise (JUVALION07) was carried out in late autumn, from the 8th to the 21st of December 2007. Temperature and salinity of the water column from sea surface to 50 m depth were measured via a Seabird 19 CTD at each station. Also data of sea surface temperature in September to December since 1982 to early 2011 of Gulf of Lions area from NOAA were acquired to study possible trends.

Zooplankton samples for an analysis of biomass and plankton composition were collected using a standard WP2 net with a mesh size of 200  $\mu\text{m}$  and sieved through a 3000  $\mu\text{m}$  plankton mesh to obtain the 200–3000  $\mu\text{m}$  mesozooplankton fraction, and by means of a scaled-down version WP2 net, with a mesh size of 53  $\mu\text{m}$  and a mouth diameter of 25 cm, and sieved through a 200  $\mu\text{m}$  plankton mesh to obtain the 53–200  $\mu\text{m}$  fraction of microplankton. All zooplankton samples were split with a Motoda plankton splitter (Motoda, 1959) and one-half was preserved in formalin to carry out subsequent qualitative analysis, while the other half was frozen on board for biomass measurements and lipid analysis. As the need to comparatively assess the nutritional condition of the two different temporal larvae populations only arose during the December cruise, plankton samples for lipids analysis were not collected during the first cruise.

Late larvae of anchovy were caught with a pelagic trawling net, towed at an average speed of 3.6 knots over a 30–40 min time span. This trawling time might seem too long to obtain larvae in proper conditions for biochemical analysis; however, the alteration of lipid composition in muscular tissue of fish larvae is small within a time up to 3 h after death (Lochmann et al., 1996).

Samples were immediately frozen in liquid nitrogen after sorting on board and transferred into a  $-80\text{ }^{\circ}\text{C}$  freezer just after the arrival in the laboratory.

### 2.2. Lipid and fatty acid analysis

Wet weight and standard length of each larva were measured in the laboratory to the nearest 10  $\mu\text{g}$  and 0.1 mm, respectively, before removing the head for otoliths analysis and the gut, as

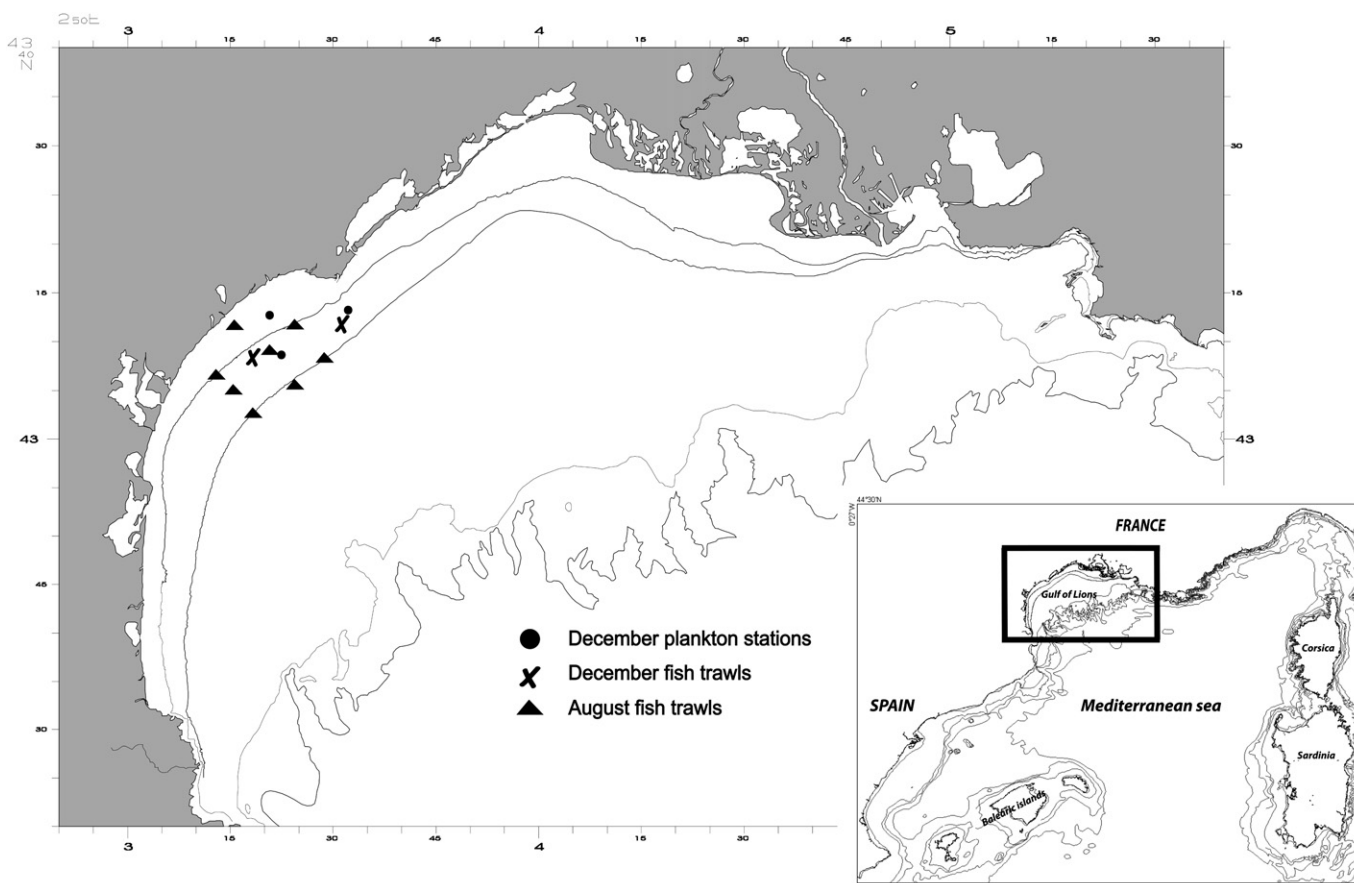


Fig. 1. Map of the study area, with the positions of plankton stations and trawls in August and December 2007.

recommended by Lochmann et al. (1996). The empirical relationship between larval wet and dry weights was calculated via linear regression from other larvae of the same cruises and size ranges. Microplankton and mesozooplankton samples from each station were pooled together before proceeding with the fatty acid extraction.

Lipid extraction was performed according to the method of Folch et al. (1957). Lipid content was measured following the protocol of Olsen and Henderson (1989) via high-performance thin-layer chromatography (HPTLC), which was followed by quantitative densitometry in visible light with a Bio-Rad Gel Doc XR densitometer, using Quantity One 4.6.2 software.

The nutritional condition of the *Engraulis encrasicolus* late larvae was evaluated by comparing the triacylglycerol/cholesterol (TAG/CHOL) index (Håkanson, 1993) and the ratio between the percentage of total lipids and the dry mass (Norton et al., 2001). Fulton's condition index (FCI) was calculated with wet weight (W, in g) and standard length (SL, in mm) data, following the equation:

$$FCI = W \times 100/SL^3$$

Fatty acids extraction and trans-methylation was accomplished following the protocol of Christie (1989) as modified by Li and Watkins (2001). Four out of seven samples of larvae from August 2007, together with five larvae samples and three zooplankton samples from December 2007, were suitable for analysis by gas-chromatography.

Gas chromatographic (GC) analysis of fatty acid methyl esters (FAMES) was then performed using a Thermofisher Scientific GC8060 gas-chromatograph coupled with a MD800 mass-spectrometer. The apparatus was fitted with a BPX-70 capillary column (30 m × 0.25 mm i.d. × 0.25 μm). Helium was used as carrier gas, with a speed of 1 ml/min. The programmed oven temperature was 60 °C (1 min) to 260 °C (10 min) with an increment of 8 °C min<sup>-1</sup>. The injector temperature was set at 270 °C and the injector split was set at 35 ml min<sup>-1</sup>. Mass-spectrometry was conducted with an ion source temperature of 200 °C and an interphase temperature at 260 °C. Ionization was performed by electron impact at 70 eV, and the weight range analyzed was 50–550 Da.

FAMES were identified by comparing their retention times with those of the standard mixture, Supelco 37 Component FAME mix. The quantification of the identified FAMES was calculated through GC peak areas integration.

The diet of late larvae was evaluated according to the indices based on fatty acid relations 16:1(ω-7)/16:0, 18:1(ω-9)/18:1(ω-7) and EPA/DHA, and on PUFA/SFA relation (St. John and Lund, 1996; Auel et al., 2002; Rossi et al., 2006).

### 2.3. Growth analysis

A total of 61 larvae from August and 44 larvae from December, ranging in size from 19 to 31 mm (SL) and from 20 to 27 mm (SL), respectively, were used for a growth analysis. Both sagittal otoliths were extracted from the head of the anchovy larvae under a Leica dissection microscope (Wild M12) equipped with polarizing filters and mounted in Crystalbond 509 Amber on labeled glass slides. The otolith growth analysis was undertaken at 100× magnification under transmitted light with a microscope (Zeiss Axiospoh) coupled to a digital video recorder, while the otolith nucleus was analyzed at 1000× magnification. Otolith radius (OR) and increment width (IW) (μm) were measured to the nearest 0.1 μm using Image-Pro Plus 5.0. The increments were measured along the longest radius, from the middle of one D-zone to the middle of the next D-zone. Following the results of Aldanondo et al. (2008), for the same species in the Bay of Biscay and under experimental

conditions, increments were assumed to be daily (DI) being the first increment laid down at hatching. Prior to Aldanondo et al. (2008), studies on European anchovy growth had assumed that the first increment deposition took place at the beginning of exogenous feeding, i.e. two days after hatching, as proposed by Palomera et al. (1988) in the first paper on anchovy larval otoliths. All otoliths were read twice by two different persons, and only if the DI differed by 1 daily increment were they accepted.

Taking into account the narrow range of lengths that we are analyzing, we have assumed linear growth in agreement with the results of La Mesa et al. (2009), the first study of anchovy that analyzes the growth at the metamorphic period, as is the case of our samples. Accordingly, the individual growth rate (IGR, mm d<sup>-1</sup>) from the time of hatching until the time of capture was then calculated by using the equation proposed by Takahashi and Watanabe (2005).

$$IGR = (SL - SL_0)/Age$$

where SL is the measured larvae standard length corrected by using the method of Theilacker (1980), SL<sub>0</sub> is the larvae standard length at hatching, estimated to be 2.5 mm according to laboratory studies on the studied species (Regner, 1985), and Age = DI.

### 2.4. Data analysis

Seasonal differences between oceanographic parameters as well as Fulton's condition index, lipid total content, lipid classes and proportion of fatty acids in larvae and plankton were assessed by means of Mann–Whitney non-parametric tests for independent samples (Dytham, 2003), and for oceanographic data ANOVA tests were performed.

Fatty acid percentage compositions were pairwise compared between larvae of both cruises, and between larvae and zooplankton collected during the December 2007 cruise using the former test. Similarities in the fatty acid composition between samples were measured by Euclidean distances (Legendre and Legendre, 1998). A non-metric multi-dimensional scaling (nMDS) was carried out on the samples similarity matrix to visually describe overall patterns. Statistical analyses were carried out using STATISTICA 6.0 by Statsoft, Inc., and PRIMER-E 6 software. Significance level for all tests was adopted at  $p < 0.05$ .

## 3. Results

### 3.1. Oceanographic data and zooplankton composition

The mean temperature within the water column (0–50 m) and the mean surface temperature (0–5 m) were significantly higher ( $p < 0.0001$ ) in August (mean ± standard deviation values of 19.14 °C ± 1.32 for surface temperature and 16.75 °C ± 1.02 for average temperature) compared to December (mean ± standard deviation values of 12.64 °C ± 0.91 for surface temperature and 13.04 °C ± 0.66 for average temperature), while no significant differences were observed for salinity between the two cruises (mean ± standard deviation values of 37.74 ± 0.20 and 37.35 ± 1.48 for surface salinity and 37.94 ± 0.06 and 37.93 ± 0.40 for average salinity in August and December, respectively).

Data of temperature acquired from NOAA (Reynolds et al., 2002) were monthly averaged in the Gulf of Lions (area comprised between 2.5° W to 6.5° W and 41.3° N to 45.5° N), from September 1981 to December 2010, showing a positive trend in sea surface temperature during the last 4 months of every year (16.5° in September 1981 to 17.2 °C in December 2010; SST = 14.97 + 0.54 × 10<sup>-4</sup> \* Serial\_date; where Serial\_date is the number of days since the January 1st of 1900).

There were significant differences for microplankton biomass between August and December cruises (non-parametric Mann–Whitney  $U$  test,  $p < 0.05$ ), with higher values recorded during the summer (mean  $\pm$  standard deviation values of  $201.59 \pm 283.25 \text{ mg m}^{-3}$  and  $22.76 \pm 24.18 \text{ mg m}^{-3}$ , respectively). The same pattern was observed for the mesozooplankton, where pairwise multiple comparisons ( $t$ -test) revealed that August 2007 samples had significantly higher biomass ( $p < 0.05$ ) compared to those collected in December 2007 (mean  $\pm$  standard deviation values of  $33.65 \pm 10.34 \text{ mg m}^{-3}$  and  $14.07 \pm 10.65 \text{ mg m}^{-3}$ , respectively). The analysis of both microplankton and mesozooplankton composition revealed a dominance of copepods, especially calanoids and cyclopoids, within the community during the two seasons (Fig. 2). Nevertheless, in August cladocerans were also important, while they were not recorded in December cruise (Fig. 3).

### 3.2. Growth rate

Otolith growth was significantly different between the two periods with the otoliths of the December larvae being smaller compared to those of the August larvae of the same age ( $t$ -test,  $p < 0.001$ ) (Fig. 4). For both groups, increment width increased continuously, although for larvae caught in December, that ranged on age from 33 to 54 days, the largest increment width reached was half that of the maximum increment width in the August samples, that ranged from 22 to 44 days (4 vs.  $8 \mu\text{m day}^{-1}$ , respectively). This pattern was matched by mean individual growth rates (IGR) from hatching until the time of capture, that ranged between  $0.50$  and  $0.93 \text{ mm d}^{-1}$

(mean  $\pm$  standard deviation values of  $0.74 \pm 0.09 \text{ mm d}^{-1}$ ) for August larvae and  $0.43\text{--}0.74 \text{ mm d}^{-1}$  (mean  $\pm$  standard deviation values of  $0.59 \pm 0.07 \text{ mm d}^{-1}$ ) for December larvae, indicating that IGR was significantly higher in the warmer period ( $t$ -test,  $p < 0.001$ ).

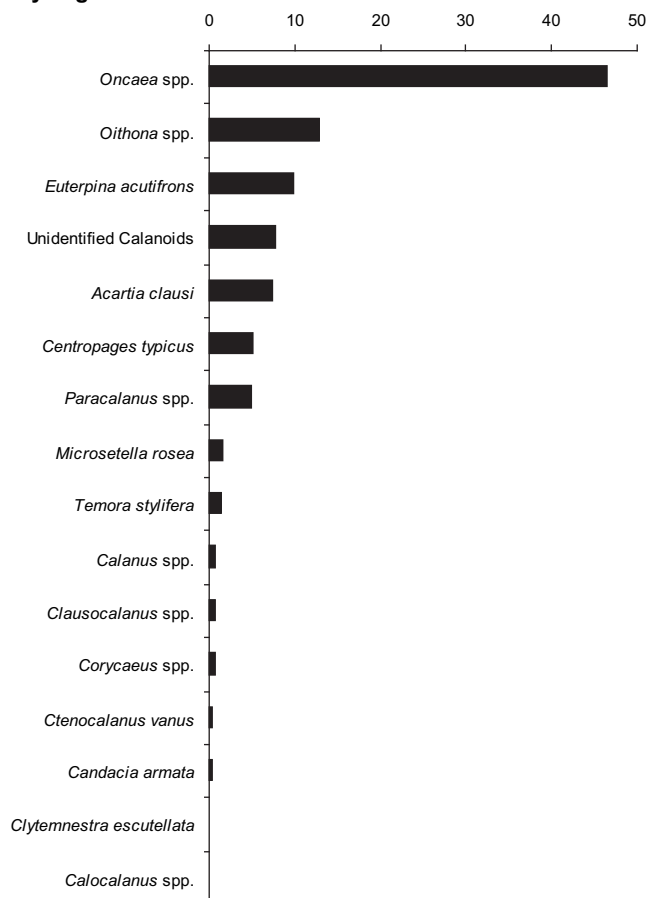
### 3.3. Nutritional condition

A single linear regression between larvae dry mass and wet weight was estimated for both cruises (Fig. 5) as the one-way ANCOVA did not show significant differences between August and December ( $p > 0.05$ ), considering wet weight as covariant. Subsequently, the empirical equation obtained from that relationship was applied to estimate the larvae dry mass (mean  $\pm$  standard deviation values of  $23.0 \pm 7.0$  and  $18.0 \pm 7.0 \text{ mg/larva}$  for August and December late larvae, respectively) as well as in further analysis regarding the dry mass of lipid contents in the samples.

Mean standard lengths did not significantly differ between the two cruises (mean  $\pm$  standard deviation values of  $27.3 \pm 3.0 \text{ mm}$  and  $27.0 \pm 3.4 \text{ mm}$  for August 2007 and December 2007, respectively). On the contrary, Fulton's condition index of larvae was significantly higher ( $p < 0.05$ ) in the August individuals (mean  $\pm$  standard deviation values of  $0.598 \pm 0.131$  and  $0.489 \pm 0.153$  in August and December, respectively).

Pools of 4–6 larvae each from August and December cruises were processed for lipid extraction. Lipid content in larvae did not show any statistical differences between the two seasons ( $p = 0.123$ ), and no significance was found regarding triacylglycerol ( $p = 1.000$ ), cholesterol ( $p = 0.705$ ), free fatty acid ( $p = 0.570$ ) or polar lipid content ( $p = 1.000$ ) within the anchovy samples

#### July/August 2007



#### December 2007

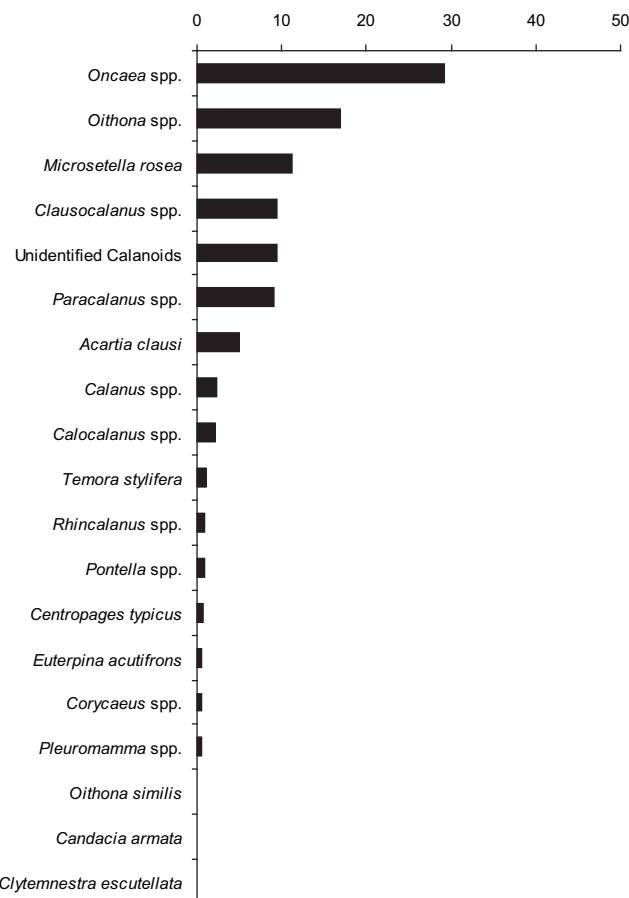


Fig. 2. Relative abundances of the main copepods species within micro and mesozooplankton samples of both August and December 2007 campaigns.

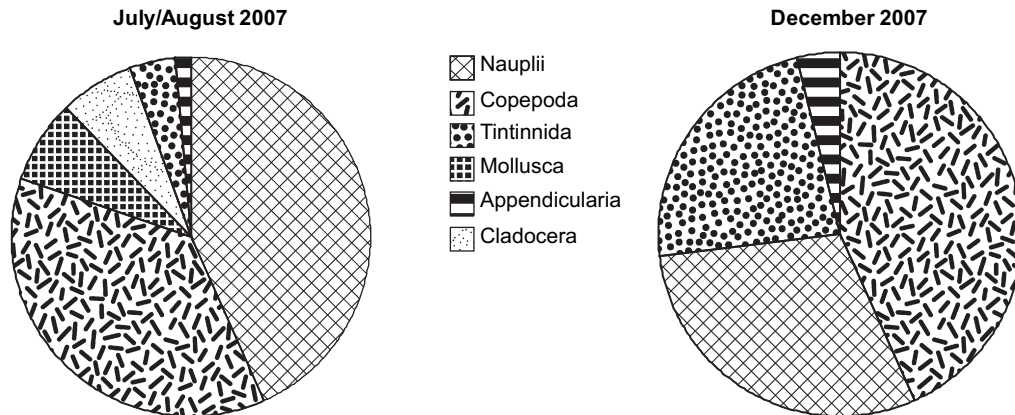


Fig. 3. Pie charts illustrating the mean percentage composition of the main plankton groups in August and December 2007 cruises.

(Table 1). The TAG/CHOL ratio was determined to range between 0.53 and 0.72 for August larvae and 0.60–0.82 for December larvae, and it also did not exhibit significant differences between cruises ( $p = 0.186$ ).

### 3.4. Fatty acids analysis

Of the 23 fatty acids identified, 16:0, eicosapentaenoic acid or EPA (20:5( $\omega$ -3)) and docosahexanoic acid or DHA (22:6( $\omega$ -3)) made up 58–75% of total fatty acids in the zooplankton and larvae samples (Table 2), with DHA and 16:0 being the most common fatty acids found in both August and December larvae and within the zooplankton. The other abundant fatty acids found were 14:0, 18:0, 16:1( $\omega$ -7), 18:1( $\omega$ -9) and 18:1( $\omega$ -7). The proportion of PUFA was higher than any other type of FFAA among larvae of both cruises, while SFA were the most abundant in the zooplankton.

A multi-dimensional scaling (MDS) ordination, with a stress coefficient  $<0.01$ , shows the similarity in fatty acid composition among the larvae of August and December and the zooplankton of December. Three groups can be differentiated on the plot, specifically a group comprised of 4 out of 5 samples of the December anchovy larvae, the 4 samples of August larvae, and the 3 samples of zooplankton (Fig. 6). The ANOSIM test confirmed the presence of significant differences in the multivariate fatty acid composition between all three groups ( $R^2 = 0.83$  at  $p < 0.0001$ ).

Table 3 shows the mean values of the fatty acid indices estimated from the data obtained in this work, apart from those related to the summer zooplankton, which were acquired from (Rossi et al.,

2006). It is of note that the composition of the zooplankton during the summer of 2007 was equal to that described by Rossi et al. (2006).

The indices 18:1( $\omega$ -9)/18:1( $\omega$ -7) and PUFA/SFA indicate the degree of carnivory in late anchovy larvae and zooplankton. No statistically significant difference was observed among the larvae of the two cruises or among the December larvae and the December zooplankton, although the overall values of these indices were relatively high (Auel et al., 2002).

The indices 16:1( $\omega$ -7)/16:0 and EPA/DHA, which estimate the importance of diatoms in the diet of larvae, show significant differences between August and December larvae, with both indices being higher in August. There is also a statistically significant difference between December larvae and zooplankton, with the larvae having a lower ratio than the zooplankton.

## 4. Discussion

*Engraulis encrasicolus* in the Northwestern Mediterranean has been intensively exploited (Palomera et al., 2007) and so alterations of any factor (e. g. temperature, salinity, currents, predation, food availability and overexploitation) affecting early stages (i.e. eggs, larvae and juveniles) of engraulids could be important for the strength of recruitment and thus for the future of the population, due to their high larval growth and mortality rates (Houde, 1989; Takahashi and Watanabe, 2005; Ruiz et al., 2006).

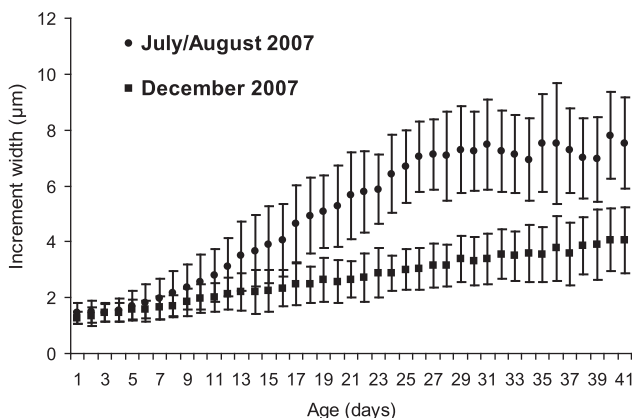


Fig. 4. Anchovy larvae mean increment width by estimated age (days) for larvae caught in August and December 2007. (Error bars: standard deviation).

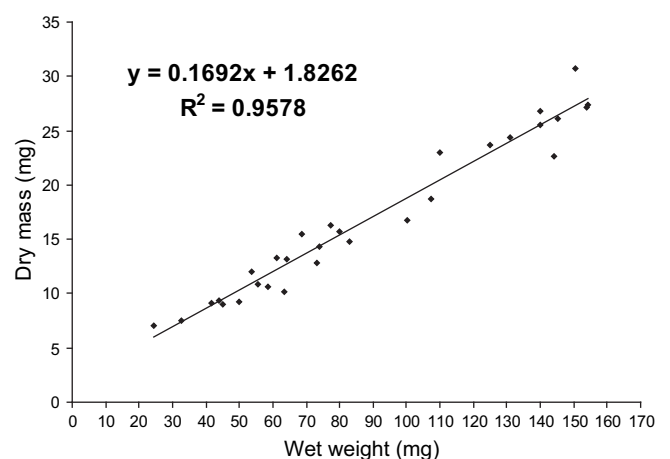


Fig. 5. Relationship between dry mass (DM) and wet weight (WW) of anchovy late larvae. Linear regression fitted by  $DM = 0.1692 \cdot WW + 1.8262$  ( $r^2 = 0.9578$ ).

**Table 1**  
Total lipid content, lipid class values and TAG/CHOL index ( $\mu\text{g} \cdot \text{larva}^{-1}$ ), presented as Mean  $\pm$  SD.

	August 2007 (N = 7 pools of 4–6 larvae each)	December 2007 (N = 5 pools of 4–5 larvae each)
Total lipid content	770.4 $\pm$ 275.1	664.5 $\pm$ 92.7
% lipid/dry weight	4.2 $\pm$ 0.8	4.4 $\pm$ 0.2
<i>Neutral lipids</i>		
Triacylglycerol (TAG)	922.2 $\pm$ 379.4	873.8 $\pm$ 242.2
Cholesterol	1414.4 $\pm$ 476.6	1227.8 $\pm$ 249.9
Free fatty acid	1406.0 $\pm$ 425.6	1228.2 $\pm$ 221.7
Steryl ester	–	–
Polar lipids	230.6 $\pm$ 65.9	223.1 $\pm$ 37.8
TAG/CHOL index	0.64 $\pm$ 0.06	0.70 $\pm$ 0.09

Evidence showing that European anchovy larvae feed on plankton (Conway et al., 1999; Tudela et al., 2002; Bacha and Amara, 2009; Morote et al., 2010) lead us to assume that changes in the plankton community affect the feeding habits of larvae, thus influencing their nutritional condition and, possibly, their survival (Fuiman and Cowan, 2003).

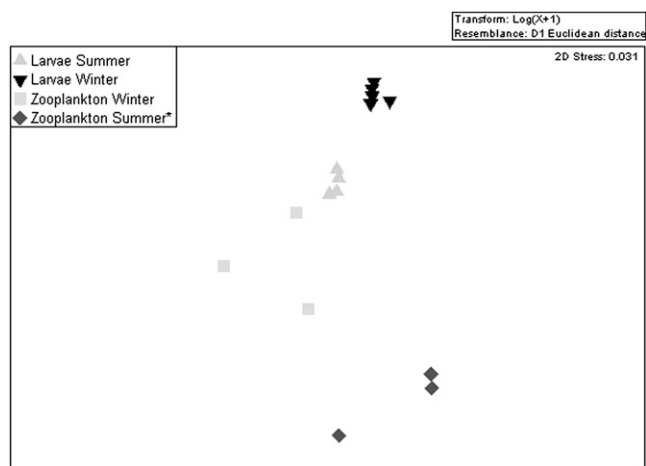
Zooplankton biomass and taxonomic composition showed clear differences between August and December 2007, with the summer being the period when a higher presence of these organisms was recorded. Nevertheless, neither the TAG/CHOL index (Fraser et al., 1987) nor the lipid percentage or the polar lipids content (Norton et al., 2001) indicate differences between seasons regarding the nutritional condition of anchovy late larvae. Håkanson (1989, 1993) pointed out that TAG/CHOL values below 0.2–0.3 indicate a poor nutritional condition, thus according to our results that showed TAG/CHOL average values around 0.64–0.70 in both seasons, it can be stated that both larvae in August and December presented a satisfactory nutritional condition. For this reason the observed

**Table 2**  
Fatty acids composition of anchovy late larvae and zooplankton, presented as mean  $\pm$  SD.

	Engraulis encrasicolus		Zooplankton
	August 2007 (N = 4)	December 2007 (N = 5)	December 2007 (N = 3)
<i>Fatty acids</i>			
14:0 <sup>a, b</sup>	5.06 $\pm$ 0.39	1.71 $\pm$ 0.32	8.94 $\pm$ 2.10
15:0 <sup>a, b</sup>	0.61 $\pm$ 0.02	0.49 $\pm$ 0.06	0.88 $\pm$ 0.37
16:0	23.39 $\pm$ 1.47	25.63 $\pm$ 7.17	24.31 $\pm$ 4.83
17:0 <sup>a</sup>	0.51 $\pm$ 0.02	0.69 $\pm$ 0.03	0.67 $\pm$ 0.19
18:0 <sup>b</sup>	4.22 $\pm$ 0.52	4.32 $\pm$ 0.12	5.73 $\pm$ 0.48
20:0 <sup>a</sup>	0.06 $\pm$ 0.01	0.04 $\pm$ 0.003	0.11 $\pm$ 0.02
22:0	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	–
24:0 <sup>a</sup>	0.14 $\pm$ 0.03	0.08 $\pm$ 0.02	0.07 $\pm$ 0.00
<i>Total saturated</i>			
15:1	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.11 $\pm$ 0.00
16:1 ( $\omega$ -7) <sup>a, b</sup>	3.66 $\pm$ 0.38	1.42 $\pm$ 0.09	6.47 $\pm$ 1.74
18:1 ( $\omega$ -9) <sup>a, b</sup>	5.16 $\pm$ 0.33	5.57 $\pm$ 0.13	6.82 $\pm$ 1.48
18:1 ( $\omega$ -7)	1.91 $\pm$ 0.28	1.92 $\pm$ 0.16	1.84 $\pm$ 0.36
20:1 ( $\omega$ -9)	0.20 $\pm$ 0.15	0.13 $\pm$ 0.01	0.42 $\pm$ 0.10
22:1 ( $\omega$ -9)	–	–	0.08 $\pm$ 0.00
24:1 ( $\omega$ -9) <sup>a</sup>	0.12 $\pm$ 0.03	0.27 $\pm$ 0.05	0.08 $\pm$ 0.00
<i>Total monounsaturated</i>			
18:2 ( $\omega$ -6) <sup>a</sup>	1.19 $\pm$ 0.11	0.62 $\pm$ 0.12	0.83 $\pm$ 0.29
18:3 ( $\omega$ -6)	0.55 $\pm$ 0.09	0.40 $\pm$ 0.05	0.64 $\pm$ 0.08
18:3 ( $\omega$ -3) <sup>a, b</sup>	0.85 $\pm$ 0.15	1.18 $\pm$ 0.15	1.89 $\pm$ 0.05
20:3 ( $\omega$ -6)	0.04 $\pm$ 0.00	0.03 $\pm$ 0.01	–
20:3 ( $\omega$ -3)	0.05 $\pm$ 0.01	0.07 $\pm$ 0.01	–
20:4 ( $\omega$ -6)	0.48 $\pm$ 0.06	0.55 $\pm$ 0.09	0.31 $\pm$ 0.05
20:5 ( $\omega$ -3)	11.29 $\pm$ 0.64	10.79 $\pm$ 0.85	16.57 $\pm$ 6.34
22:6 ( $\omega$ -3) <sup>b</sup>	34.25 $\pm$ 2.96	39.28 $\pm$ 4.47	17.35 $\pm$ 6.01
<i>Total polyunsaturated</i>			
Others	6.2	4.8	5.9
Total	100	100	100

<sup>a</sup> Statistical difference between August and December 2007 larvae,  $p < 0.05$ .

<sup>b</sup> Statistical difference between December 2007 larvae and zooplankton,  $p < 0.05$ .



**Fig. 6.** Non-metric multi-dimensional scaling (nMDS) plot of similarities in the fatty acid composition of anchovy late larvae and zooplankton. \*Data of zooplankton summer from Rossi et al. (2006).

differences in food availability cannot be assumed to affect the biochemical condition of both larvae populations.

Fulton's index showed statistically significant differences between August and December larvae concerning physical condition, showing that weight at size is higher in summer larvae compared to those collected in late autumn. The apparent discrepancy between biochemical nutritional condition and physical condition data can be explained by considering that morphometric condition indices take longer to show the effects of food intake (Catalán et al., 2007). In addition, taking into account that information from both FCI and growth rates show effects of a previous period compared to biochemical indices, it is not surprising that results of FCI agree with those of growth analysis. As the condition of our larvae needs to be evaluated for a short period of time, we consider that Fulton's index should not be taken into account to assess the nutritional condition in this study. The absence of differences between biochemical conditions of anchovy larvae observed in our study could be explained to a certain extent by the observed seasonal differences in sea water temperature. Moreover temperature is recognized to substantially influence the metabolic rates in marine organisms (McLaren, 1963; Ikeda, 1985) and in the specific zooplankton composition. Indeed, during the summer, larvae must compensate for the higher metabolic rate imposed by the higher temperature, with an energetically richer diet. Conversely, larvae could simply eat more. However, this assumption could be insufficient to compensate for the energy expense since higher prey capture requires a higher energetic cost.

In summer, despite the higher zooplankton biomass, a significant presence of cladocerans was recorded (Fig. 3). Cladocerans are considered of less energetic value than copepods (Boldt and Halderson, 2002), in agreement with previous findings of

**Table 3**  
Fatty acids trophic indices in August and December 2007 anchovies larvae and February 2007 zooplankton, presented as mean  $\pm$  SD.

	Engraulis encrasicolus		Zooplankton	
	August 2007	December 2007	June 2000 <sup>a</sup>	December 2007
16:1( $\omega$ -7)/16:0	0.16 $\pm$ 0.01	0.06 $\pm$ 0.01	0.96	0.27 $\pm$ 0.08
20:5 ( $\omega$ -3)/22:6 ( $\omega$ -3)	0.33 $\pm$ 0.01	0.27 $\pm$ 0.02	0.53	0.96 $\pm$ 0.42
C16/C18	1.79 $\pm$ 0.38	1.94 $\pm$ 0.55	1.70	1.76 $\pm$ 0.30
18:1( $\omega$ -9)/18:1 ( $\omega$ -7)	2.70 $\pm$ 0.30	2.89 $\pm$ 0.23	3.15	3.71 $\pm$ 0.62
PUFA/SFA	1.43 $\pm$ 0.21	1.60 $\pm$ 0.42	0.65	0.92 $\pm$ 0.42

<sup>a</sup> June 2000 zooplankton taken as reference level from Rossi et al. (2006).

Champalbert (1996) and Calbet et al. (2001). Moreover, stomach contents analysis carried out on juvenile stages of anchovy of the investigated area show a high preference for cladocerans in summer (SARDONE project, 2010). Although we cannot be sure that the studied late larvae feed on the exact same prey as juveniles, feeding preferences of larvae are based on zooplankton rather than on any other group (Tudela et al., 2002; Borme et al., 2009; Catalán et al., 2010; Morote et al., 2010) due to their inability to catch smaller prey without a proper development of gill rakers (van der Lingen et al., 2006). Therefore it is very likely that anchovy late larvae are also frequently feeding on cladocerans during the summer, as occurs in the Adriatic Sea (Borme et al., 2009). On the other hand, the December larvae population is supposed to feed predominantly on copepods, which are higher energetic zooplankters. The differences in metabolic rates as well as in the energy content of diet could explain the similar nutritional condition observed in the seasonal pools of late larvae.

Masuda (2003) demonstrated that a lack of docosahexanoic acid could produce an ineffective feeding behavior, and even a higher mortality rate within a population. The finding that DHA was present and showed similar concentrations in both populations further confirm the occurrence of similar nutritional conditions in anchovy late larvae in both cruises. This may also be explained by the fact that, in summer, lower DHA concentration was expected because a high UVB radiation can negatively affect DHA production by algae and August is the month with the highest solar radiation in the Western Mediterranean. In addition, Kainz et al. (2004) stated that the cladoceran community shows lower DHA concentrations. The higher plankton biomass available during August would help to compensate for the DHA deficit of larvae and result in equal conditions compared to winter.

Several studies regarding the effect of essential fatty acids (EFAs) on fish development have been devised for reared larvae (Morais et al., 2007), but only a few examine natural populations at sea (Reuss and Poulsen, 2002). The present study intended to ascertain the role that FFAA play as trophic markers between anchovy late larvae and plankton in the Gulf of Lions, and to determine the main planktonic groups that are part of the diet of the larvae. Anchovy fatty acid composition suffers seasonal variation (Zlatanov and Laskaridis, 2007). In particular, saturated fatty acids (SFAs) 16:0, 20:0, 22:0 and 24:0 can be more easily synthesized by all aquatic organisms (Dalsgaard et al., 2003), while PUFAs, which are the first preference for fish lipases (Lie and Lambertsen, 1985) and must be obtained from the diet, are present in low concentration in oligotrophic seas (Fahl and Kattner, 1993). In addition, PUFAs are essential for the survival of marine organisms (Brett and Müller-Navarra, 1997). For these reasons, trophic indices used in this study were based basically on unsaturated fatty acids.

The higher proportion of PUFAs compared to other types obtained in this study can be easily explained when taking into account the accumulation of these FFAA with age in anchovy larvae (Rossi et al., 2006). The absolute values of the indices 16:1( $\omega$ -7)/16:0 and EPA/DHA are comparatively low (Auel et al., 2002), and confirm a clear tendency towards a non-diatom diet (Table 3). The low value of the C16/C18 index corroborates the inferred low preference for diatoms.

Although limited, diatom consumption appears significantly higher during summer. This should be expected because there is a spring bloom of diatoms in this area (Leblanc et al., 2003). As shown by the low overall values of the above mentioned indices and by other works (e.g. Conway et al., 1999; Tudela et al., 2002; Islam and Tanaka, 2009; Morote et al., 2010) anchovy larvae normally do not feed on phytoplankton. Thus, it is likely that any trace of phytoplankton in the larvae comes either indirectly from the consumed zooplankton or from accidentally ingested phytoplankton. On the

contrary, our find of moderately high levels of 18:1( $\omega$ -9) and long-chain monounsaturated fatty acids points out that calanoid copepods are important prey for anchovy late larvae (Werner and Auel, 2005), as shown in other studies with the carnivory indices 18:1( $\omega$ -9)/18-1( $\omega$ -7) and PUFA/SFA (Cripps and Atkinson, 2000; Garrido et al., 2008).

To date, the knowledge of European anchovy reproductive behavior in the western Mediterranean indicates that the spawning period begins in the spring, when the water starts to heat up and reaches 14 to 15 °C, and finishes in late-September, when water temperature starts to decrease (Palomera, 1992). However, the growth rates estimated in this work, together with otoliths analysis and age determination, indicate that anchovy larvae gathered in December 2007 were hatched approximately between the end of October and mid-November, which is well after the end of the spawning period previously known for this species (Palomera, 1992; Palomera et al., 2007). Thus, a prolongation of the spawning period took place that year, likely brought about by a process of sea surface heating. During the last decades, mean temperatures in the Western Mediterranean have been rising (Salat and Pascual, 2002; Reynolds et al., 2002). If the autumn–winter anchovy larvae population is becoming a norm in the biology of the species, we may assume that the spawning period of *Engraulis encrasicolus* is being extended, favoring a wider spawning period and perhaps the survival of these late larvae of European anchovy in colder months.

Our analysis regarding the growth of both August and December cohorts shows that growth rates were significantly different among cohorts, being higher in August. This difference could be due to water temperature or to food availability (Takahashi and Watanabe, 2005; Aldanondo et al., 2008). Mean temperature during August 2007 was 19 °C, while during December cruise it was 12 °C, a difference that could cause statistically different growth rates, yet still in agreement with the similar nutritional condition recorded in this study, as explained above in terms of different energetic expenditure.

Feeding activities have a positive correlation to water temperature (Houde, 1989), so lower growth rates in December may be due to a reduction in food intake and metabolic rates, rather than to food shortage (Takahashi and Watanabe, 2005), as the good nutritional condition observed in this period seems to confirm. Moreover, the Gulf of Lions is a rich environment in terms of food availability, favoring an adequate nutritional condition in anchovy larvae, as exposed by García et al. (1998).

The noted lack of significant differences both in the TAG/CHOL index and in the polar lipids content between seasons in the anchovy larvae would imply a major relevance of temperature in the differences found in growth rates. These differences are also confirmed by Takasuka and Aoki (2006), who found a direct relationship between temperature and growth rate in Japanese anchovy larvae.

The present study does not indicate that either of the populations of larvae has a greater probability of survival than the other. Further research concerning the recruitment success of these late larvae populations is needed. Some studies suggest that mortality of anchovy larvae of the Mediterranean is inversely related to growth rates (Allain et al., 2003; Palomera et al., 2007; La Mesa et al., 2009), supported by the idea that larvae with slower growth rate remain as larvae longer (“stage-duration” mechanism, see Chambers and Leggett (1987)) and extends the exposure to predation. In this sense, we think that the “bigger is better” hypothesis, as described by Leggett and DeBlois (1994), is a paradigm that fits our results.

Takasuka et al. (2003) also found that larvae of Japanese anchovy with lower growth rates were proportionally more abundant within predators' stomachs than in the sea, inferring that they were more

vulnerable to predation. If this holds true, anchovy larvae found in December 2007 would be more vulnerable to predation and have less probability to reach the adult stage. However, further studies regarding the biology of predators that feed on anchovy larvae should be carried out, in order to estimate how their feeding activity could affect the probability of mortality of anchovy larvae.

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