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Effect of food type and concentration on growth and fatty acid composition of early larvae of the anchovy (*Engraulis encrasicolus*) reared under laboratory conditions

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ABSTRACT

Experiments were conducted during the summer of 2008 and 2009 to study the growth of early post yolk-sac European anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) larvae reared under different food regimes. The fatty acid composition was used to assess nutritional condition of the larvae. Prey items used in the experiments were *Gymnodinium sanguineum*, *Brachionus plicatilis* and nauplii of the copepods *Acartia grani* and *Euterpina acutifrons*. Food type and concentration affected the growth of the larvae. Mixed diets composed of rotifers and copepod nauplii at high concentration resulted in higher anchovy larvae growth rates in comparison with single-prey diets using either rotifers or copepod nauplii. The addition of the dinoflagellate *G. sanguineum* (25–50 cells ml^{-1}) to the prey offered did not enhance significantly larval growth. Highest growth rates of anchovy larvae (0.28 mm d^{-1}) were obtained using high concentrations of a mixed diet, particularly the combination of rotifers and *A. grani* nauplii. Fatty acid composition at hatch was similar to the composition observed in the field, but during larvae ontogeny there was a marked decrease in the contribution of polyunsaturated fatty acids such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Such difference reflects the high requirements of these PUFA for larvae development, and suggests that the food offered failed to fulfill the larvae nutritional requirements. The growth rates obtained in our experiments were, overall, in the lower range of those observed in natural conditions. Taking into considerations the fact that larvae in the field are expected to encounter lower prey concentrations, we discuss the reasons for such disagreement.

1. Introduction

Hjort (1926) proposed the hypothesis that year-class strength of marine pelagic fishes is determined within the first year of life, as a consequence of the high vulnerability of the larvae, which are susceptible of experiencing high mortalities. Such mortality seems to be related to inadequate feeding conditions at the time the yolk sac reserves are exhausted. Even if larvae don't die immediately, the slow growers are more susceptible to predation and become too weak to be able to recover, which is called the "point-of-no-return" (Blaxter and Hempel, 1963). Other factors that significantly affect larval mortality are offshore advection and/or disruption of food patches that increase larval mortality due to starvation, past the first feeding phase (Wilhelm et al., 2005).

The European anchovy (*Engraulis encrasicolus*) is a pelagic fish distributed in the Mediterranean Sea and along the eastern Atlantic coast, from Norway to South Africa and represents one of the main targets of purse-seine fisheries for several countries located at Southern Europe and western Africa (Whitehead et al., 1988; ICES, 2011). These fisheries have typically a large variability in population size, which is mostly attributed to recruitment variability. It is thought that variations in the recruitment strength of small pelagic fish are primarily driven by biological and/or physical factors impacting on early life stages (Mullon et al., 2003). In particular for the European anchovy, Aldanondo et al. (2010) demonstrated that larval survival during peak spawning seems to control the annual recruitment of this species in the Bay of Biscay. For this reason, coupled biological–physical models developed to explore the complex interactions between physical oceanography and fish recruitment have mainly

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focused on the early life history stages (review in Gallego et al., 2007). Such models must be parameterized with the vital rates (growth and development) of fish early-life in order to properly mimic the larvae response under different environmental circumstances. Although the growth rates of the European anchovy larvae have been extensively studied through otolith microstructure analysis of individuals collected in the main areas of distribution such as the Bay of Biscay (e.g. Cotano et al., 2008), several Portuguese estuaries (e.g. Ré, 1996; Ribeiro, 1991), the Mediterranean Sea (e.g. Palomera et al., 1988; Catalán et al., 2010) and the Adriatic Sea (e.g. Regner and Dulcic, 1990; Dulcic, 1997), to our knowledge, no laboratory experiments have been conducted to study the relation between prey availability and European anchovy larval growth. Due to this lack of knowledge, up to date models developed to study this species have either described the larvae as inert particles (e.g. Allain et al., 2007) or used the data available for related species (Urtizberea et al., 2008; Politikos et al., 2011). These alternatives may introduce strong biases that can only be solved by using species-specific bioenergetic parameters. European anchovy larvae are mainly diurnal feeders (Ré, 1996; Tudela et al., 2002). The study of its diet in the Mediterranean Sea (Tudela et al., 2002) shows it is mainly composed of copepod eggs, nauplii and copepodites. Nauplii appears to be important mainly for larvae <4 mm, whereas as larvae grow copepodites gain importance, reflecting the broader prey range through ontogeny. There is still, however, some controversy regarding its diet in the natural environment. Morote et al. (2010) found for anchovy larvae in the Mediterranean Sea that besides calanoid copepods, harpacticoids (*Microsetella* and *Euterpina*) and cladocerans were significant contributors to the diet of <9 mm larvae. Contrarily, Catalán et al. (2010) also in the Mediterranean Sea reported that in all size-classes, larvae ingested essentially calanoid copepods and selected negatively against cladocerans. Microzooplankton prey might be an important component of the anchovy larvae diet, however this have not been properly quantified in stomach content analysis due to rapid digestion and absence of hard remains. Rossi et al. (2006) found a high percentage of 18:1(n−9) and 18:4(n−3) in small anchovy larvae apparently reflecting feeding on prymnesiophytes, since those are usually enriched in these fatty acids (Dalsgaard et al., 2003) and their abundance was high in the research area.

There is a dramatic change in the ability to withstand starvation over the larval period, with larvae being usually more vulnerable at first-feeding (Hunter and Sanchez, 1976). Lipid utilization in marine fish mainly occurs after hatching, reflecting the greater energy demand of the free-swimming yolk-sac larvae compared to the egg (Sargent et al., 2002). The large demand of monounsaturated and polyunsaturated fatty acids for the diet of the larvae at these critical early stages makes them crucial for their growth and survival (Tocher, 2003).

The objective of this work is two-fold: firstly, to determine, for the first time for this species, laboratory-derived growth rates of first feeding anchovy larvae in relation to different prey types and concentrations, and secondly, to study the variations of the fatty acid composition of early larvae through ontogeny and in relation to their diet. Finally, we will discuss our results in relation with the available field data on growth and fatty acid composition of anchovy larvae.

2. Material and methods

Two series of anchovy larvae experiments were done; the first during the summer of 2008 and the second during the summer of 2009 (Table 1). For both series, anchovy eggs were obtained from adult fish captured as juveniles by purse-seine fishery in September of 2007 in the Bay of Biscay and maintained in the San Sebastian Aquarium in cylindrical tanks (1300 l). Adult fish started spawning naturally using photoperiod, temperature and increased food concentration stimulus. Each morning the egg collector placed in the overflow of the adult fish tank was inspected, and when present, the eggs were collected, counted and transferred by pipette into the experimental tanks.

Growth experiments were conducted in cylindrical containers wrapped with black plastic, filled with 5-liter filtered seawater and kept in a temperature-controlled, air-conditioned room. 5-l volumes were chosen to conduct the experiments because for the first few weeks of larval life they have proven to offer as good conditions for larvae growth as larger ones (Lasker et al., 1970), and they better allow to study quantitatively early larval feeding. Salinity was maintained at 35.5 (PSS) while temperature was maintained at 19–20 °C, corresponding to the sea temperature at which anchovy present high spawning activity and good larval growth. Photoperiod was kept at 16 h light and 8 h dark. Temperature, oxygen, salinity and water quality parameters (nitrates, ammonia) were measured daily.

Anchovy larvae were provided known concentrations of different food types, which were maintained through the whole experimental period. Experiments lasted until there were no larvae in the tank, as the result of sampling and natural death (Table 1). Prey were introduced in the tanks from day 4 post hatch onwards and comprised the dinoflagellate *Gymnodinium sanguineum*, the rotifer *Brachionus plicatilis* and nauplii of two copepod species: the calanoid *Acartia grani* and the harpacticoid *Euterpina acutifrons*, both cultured for several years at the *Institut de Ciències del Mar* (CSIC, Barcelona). The prey types and concentrations used are within the preferred prey items and range of prey concentrations reported for anchovy larvae in the field, and commonly used in previous experimental studies of the growth and survival of another engraulid species, the northern anchovy *Engraulis mordax* (Lasker et al., 1970; Kramer and Zweifel, 1970; O'Connell and Raymond, 1970; Theilacker and McMaster, 1971; Hunter and Sanchez, 1976; Theilacker, 1987). The microalgae *Isochrisis galbana* was also added to all experimental tanks because although too small to be preyed upon by anchovy larvae, it provided food to the rotifers and copepod nauplii offered to the anchovy larvae. The tanks were supplied with gentle air bubbling, which has been shown not to affect small pelagic fish larvae (Scura and Jerde, 1977), to ensure the microalgae were kept in suspension. Each morning, after quantifying the food remaining in the tank from the previous day, 25% of the tank water was renewed and then new food items were added to the tank to obtain the desired concentrations. Stock cultures of both copepod nauplii were kept in a mixture of *Tetraselmis suecica* and *I. galbana*. The rotifers used as prey were fed *I. galbana*, a microalgae described as a enhancer of the fatty acid content in rotifers, especially 22:6n−3 (Dhert et al., 2001 and references within).

Concentrations of dinoflagellates were counted using a Sedgwick–Rafter counting chamber under an inverse microscope; rotifers and copepod nauplii were counted as 4 replicates of 1 ml subsample under a stereoscope microscope. Prey were measured at the start of the set of experiments and their carbon content estimated using the equations given in Smayda (1978) for dinoflagellates, and van der Lingen (2002) for crustacean nauplii.

The anchovy larvae growth rate was assessed by sampling 5–10 larvae every 3 days and measuring their total length. Length data were fitted to exponential growth curves:

$$SL = l_0 e^{kt}$$

where l_0 is length at hatching, k is the instantaneous growth rate and t is the age.

For the 3 experiments carried out during 2009, the fatty acid content of the zooplankton prey, the anchovy eggs and the anchovy larvae was also analyzed (Table 1). For that purpose, 25–300 anchovy eggs were sampled at the beginning of the experiment, whereas for zooplankton, ca. 30 copepod nauplii and ca. 100,000 rotifers were collected at the first day of larvae feeding; in addition, 10 anchovy larvae

Table 1
Conditions and results of the anchovy larvae growth experiments. Rot. Rotifers (*Brachionus plicatilis*), *A. grani* (*Acartia grani* nauplii), *Gymno* (*Gymnodinium sanguineum*), *Euterp.* (*Euterpina acutifrons* nauplii).

	2008								2009		
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Exp. 9	Exp. 10	Exp. 11
Prey species	Rot.	Rot.	Rot.	Rot.	Rot.	*A. grani*	Rot.		*Gymno*	*Gymno*	*Gymno*
							A. grani	Rot.	Rot.	Rot.	Rot.
								A. grani	*A. grani*	*Euterp.*	*A. grani*
[Prey]	7	4	8	6	10	4	2.5	1	25	50	50
(n ml^{-1})							0.5	1	2.5	5	5
									0.1	0.5	0.5
Initial density of eggs (n ml^{-1})	0.052	0.052	0.052	0.14	0.018	0.21	0.008	0.008	0.11	0.11	0.29
Starting day	3 Jul 08	3 Jul 08	3 Jul 08	20 Jul 08	3 Jul 08	5 Jul 08	17 Jul 08	17 Jul 08	23 Jul 09	23 Jul 09	26 Jul 09
Duration of experiments (dph)	9	12	15	9	24	8	12	12	8	11	14
Growth	x	x	x	x	x	x	x	x	x	x	x
Fatty acids of prey, eggs and larvae									x	x	x

were sampled every 3 days until the end of the experiment for fatty acid analysis. The above-mentioned samples for fatty acid analysis were in all cases rinsed in distilled water and preserved in liquid nitrogen until analysis. After lyophilization, lipid extraction and conversion of acyl groups into methyl ester derivatives (FAMES) were performed after Peters et al. (2007) using modified protocols of Folch et al. (1957) and Kattner and Fricke (1986). FAMES and fatty alcohols were separated by gas chromatography, detected by flame ionization and identified by comparing retention times with those derived from standards of known composition.

All statistical analyses and data manipulations were performed using the open source software R version 2.9.2 (R Development Core Team, 2009).

3. Results

3.1. Anchovy larvae growth

Anchovy larvae hatched approximately 48 h after collection of the eggs, measuring 2.71 ± 0.74 (SD) mm. Instantaneous growth rates varied with the feeding level (Fig. 1 and Table 1), ranging from 0.044 d^{-1} (in one of the experiments where larvae were fed rotifers as single prey) to 0.066 d^{-1} (in one experiment using a mixed diet of rotifers and *A. grani* nauplii). These instantaneous growth rates when computed as length growth for the first 15 days after hatch correspond to rates of 0.17 mm d^{-1} and 0.31 mm d^{-1}, respectively. In general, anchovy larvae growing with a diet that included rotifers and copepod nauplii grew faster than those fed rotifers as single prey, even if provided in higher concentrations. Growth of anchovy larvae fed with a single diet of high concentrations of *A. grani* (4 ind ml^{-1}) was also low and similar to that obtained using rotifers alone. The addition of the dinoflagellate *G. sanguineum* in the diet at concentrations of 25–50 cells ml^{-1} did not enhance significantly first-feeding anchovy larvae growth when compared to a diet of rotifers and copepod nauplii without dinoflagellates. The mixed diet composed of *G. sanguineum*, rotifers and *E. acutifrons* nauplii resulted in lower growth rates (0.046 d^{-1}) than those obtained using the same concentration of the nauplii of the calanoid copepod *A. grani* (0.048 d^{-1}).

The covariance analysis relating larvae size with larval age and experiment (as factors) was significant ($p<0.0001$, $R^2=73\%$) and showed that overall the growth rates obtained among experiments were not significantly different from each other except for experiment #8 ($p=0.05$) and experiment #11 ($p=0.03$), which resulted in significantly higher growth rates (Fig. 2). Those experiments corresponded to larvae fed a mixture of rotifers and *A. grani* nauplii (1 ind ml^{-1}, each), and in case of experiment #11 the dinoflagellate *Gymnodinium* had also been added to the diet.

The instantaneous growth rate was not linearly related to the total prey carbon content of the diet, and this lack of relationship was caused by the effect of prey composition on larval growth rates (Fig. 3). Indeed, experiment #6 had the highest carbon content diet (composed of 4 acartia nauplii ml^{-1}) but resulted in low larval growth rates. Despite all the other experiments had similar total carbon content diets, experiment #8 in which copepod nauplii (1 ind ml^{-1}) were combined with a low concentration of 1 rotifer ml^{-1} resulted in the highest growth rates observed in our study.

3.2. Fatty acid composition of prey, eggs and larvae

In the experiments conducted during 2009, the fatty acid composition of the zooplankton prey, eggs and anchovy larvae was determined (Table 1). The fatty acid composition of the eggs did not vary significantly among eggs spawned at different days of the 2009 spawning season (Fig. 4). The fatty acid 16:0 had the highest contribution to the total fatty acid content of anchovy eggs (30.8 ± 1.16% of total fatty acids (tFA)) followed by 22:6(n−3) or docosahexaenoic acid (DHA, 24.0 ± 1.35% tFA) and the monounsaturated 18:1(n−9) (13.8 ± 0.39% tFA, respectively). The FA 20:5(n−3) or eicosapentaenoic acid (EPA) represented 7.7 ± 0.28% tFA of the total egg fatty acid content (Fig. 4).

Both copepod nauplii species had a higher percentage of polyunsaturated fatty acids than the rotifers, including EPA and DHA (Fig. 5). *A. grani* nauplii had a higher percentage of 20:5(n.3) and 18:1(n−9) whereas *E. acutifrons* nauplii had a higher 22:6(n−3) content. Rotifers, on the other hand, presented higher proportion of monounsaturated fatty acids, particularly of 16:1(n−7) and 18:1(n−9) (Fig. 5).

Regarding the anchovy larvae, of the 17 fatty acids identified the most abundant were the 16:0 and 22:6(n−3), followed by the 18:1(n−9) and 18:0 and finally 20:5(n−3) (Table 2). Overall, polyunsaturated fatty acids were the most relevant fatty acid group in all the larvae analyzed. However the percentage of (n−3) PUFA, particularly EPA and DHA, decreased significantly through ontogeny until day 5, when the yolk sac was being used (Fig. 6). After day 5 the decreasing trend of polyunsaturated fatty acids continued while some uptake occurred in two experiments, after 5 dph for experiment 11 and after 11 dph for experiment 10 (Fig. 6).

4. Discussion

As expected, we observed an effect of prey concentration and prey type on larval anchovy growth. Larvae grew the fastest with a mixed diet of rotifers and copepod nauplii, with concentrations of 1 prey ml^{-1} respectively. Diets using higher concentrations of either rotifers or acartia nauplii as single prey resulted in lower growth rates than the mixed diets tested. Just after the beginning of exogenous feeding,

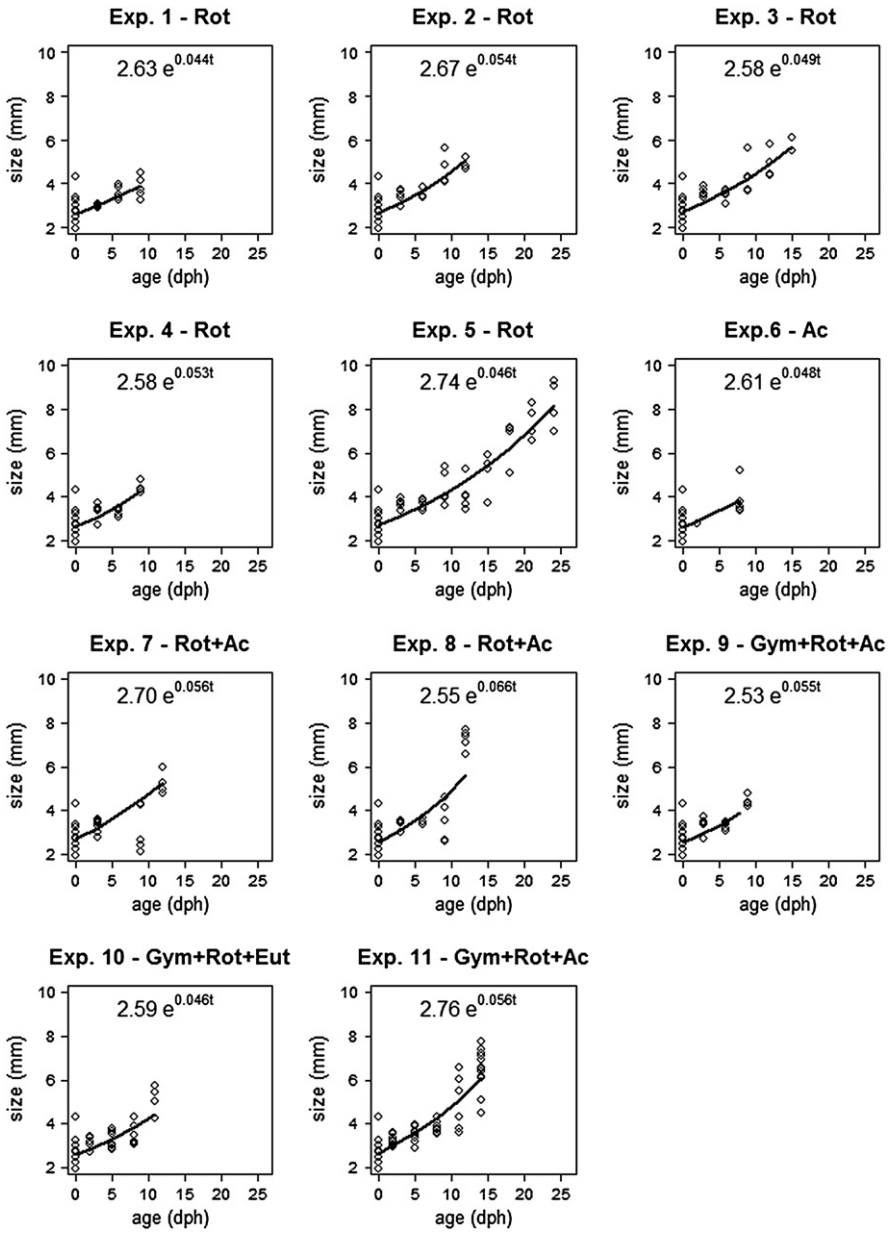

Fig. 1. Scatterplots showing larval size against age for 11 experiments where larvae were fed with different food types and concentrations; *Rot*: rotifers, *Ac*: *Acartia grani* nauplii, *Eut*: *Euterpina acutifrons* nauplii, *Gym*: *Gymnodinium sanguineum*. Details of the experiments are given in Table 1.

which occurred 3–4 days after hatch at 19 °C, anchovy larvae probably depended mostly of prey which were smaller and less motile than copepod nauplii, and only a few days later, when already larger, larvae were able to pursue and capture the copepod nauplii efficiently. Indeed, the inclusion of rotifers in the mixed diet enhanced anchovy growth, whereas high concentrations of *A. grani* nauplii supplied as single prey were not sufficient to sustain high growth rates for first feeding larvae. These observations are in agreement with general patterns of mouth size–prey size relationships in fish larvae (Sabates and Saiz, 2000), in which small mouths (gap size) restrict not only the size of prey but also the range of prey sizes. Specifically for anchovy larvae, Morote et al. (2010) already found this restriction in early stages the European anchovy; similarly, the northern anchovy larvae appeared to fail to capture copepod nauplii (*Tigriopus* sp.) at 1 ml^{-1} until reaching 7–8 dph (Theilacker, 1987).

The comparison of the growth rates of anchovy larvae in those experiments fed, at the same nauplii density, either of both copepod species nauplii shows that *A. grani* nauplii provided higher growth rates than the nauplii of the harpacticoid *E. acutifrons*. Such difference agrees with several field studies showing that calanoid copepods are preferentially selected in comparison with the harpacticoid ones, therefore constituting the bulk of the anchovy larvae's diet (Conway et al., 1998; Catalán et al., 2010).

We expected that the addition of the dinoflagellate *Gymnodinium splendens*, which falls into the same size range as small rotifers but are generally considered more nutritional, especially in terms of fatty acid content (Mansour et al., 1999), to larval diet would enhance anchovy larvae growth, but it did not. This result contrasts with the observations of Lasker et al. (1970), who were able to grow first feeding northern anchovy larvae on a single diet of the dinoflagellates *G. splendens* on slightly higher concentrations. Probably this lack of conspicuous effect in our experiments was due to the use of moderate to high densities (25–50 cells ml^{-1}) of the dinoflagellate. Further efforts have to be devoted to identify the actual small prey that provides suitable food to sustain high growth and survival at those first

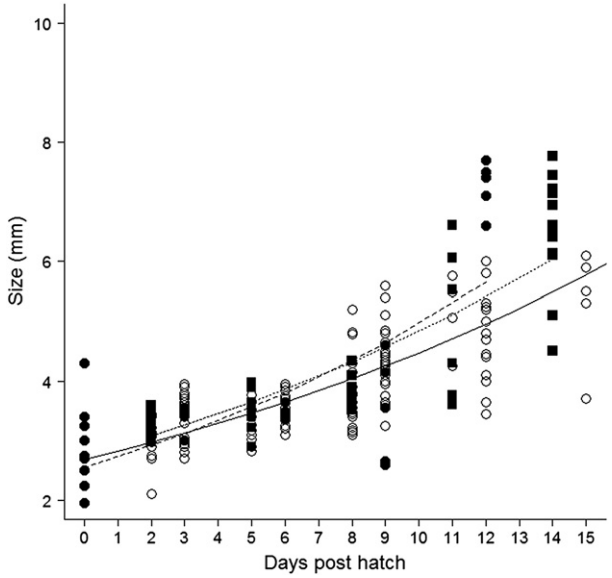

Fig. 2. Growth rates of first feeding anchovy larvae for experiment 8 (dashed line, dark circle); experiment 11 (dashed line, dark square) and for the remaining experiments (solid line, open circles). Details of the experiments are given in Table 1.

days of exogenous feeding, so critical for the larvae survival; ciliates and dinoflagellates are good candidates (Scura and Jerde, 1977; Nagano et al., 2000).

The growth rates we have obtained in the laboratory for the European anchovy larvae are comparable, at high prey concentrations, to the growth rates obtained in the laboratory for the northern anchovy (Theilacker, 1987; 0.31 mm d^{-1} and 0.33 mm d^{-1} at 15 dph, respectively, for the European anchovy and the northern anchovy larvae) and lower than those obtained for first-feeding Japanese anchovy, *Engraulis japonica* (Mito et al., 1973; Fukuhara, 1983; 0.57 mm d^{-1} and 0.43 mm d^{-1}, respectively). This is in agreement with the fact that the prey types and concentrations used in our experiments were similar to those used in previous experiments carried out with the northern anchovy larvae and lower than the feeding concentrations used for Japanese anchovies. The maximum growth rate obtained in the experiments using a combination of rotifers and *A. grani* nauplii is slightly higher than the growth rate determined in previous experiment using rotifers ad libitum (Aldanondo et al., 2008). The growth rates obtained here were also slightly higher than those estimated for first-feeding larvae of the same species (known as *Engraulis capensis* when the work was done), in experiments conducted in South Africa (Brownell, 1983) using unknown concentrations of calanoid and cyclopoid copepods until 15 dph, and rotifers this day onwards. The comparison of our lab-determined growth rates with field data (estimated from otolith microstructure analysis of field caught larvae, Fig. 7) evidences a disagreement, our rates being at the lower range of field values (field data include growth rates obtained in the wild for larvae growing with different food concentrations, temperatures and salinities, explaining their high variability). Growth rates obtained in our experiments ranged between 0.17 and 0.31 mm d^{-1} for larvae growing 15 dph, contrasting with the mean growth rates estimated for the larvae caught in the Bay of Biscay (0.4–0.6 mm d^{-1}, Cotano et al., 2008), several Portuguese estuaries (0.25–0.51 mm d^{-1}, Ré, 1996; Ribeiro, 1991), in the Mediterranean Sea (0.4–0.91 mm d^{-1}, García et al., 1998; Palomera et al., 1988; Sabatés et al., 2007; Somarakis and Nikolioudakis, 2007; Catalán et al., 2010) and the Adriatic Sea (0.9–0.94 mm d^{-1}, Regner and Dulcic, 1990; Dulcic, 1997). The difference between laboratory experiments and field estimates might even be slightly larger because field estimates have assumed that the initial increment deposition in the otoliths of *E. encrasicolus* larvae occurs at the beginning of first feeding or 2 days post hatch but recent laboratory experimentation (Aldanondo et al., 2008) revealed that the first increment occurs on the day after hatch, which means that previous growth rates are slightly underestimated, especially for small larvae. The same discrepancy between laboratory-derived and field estimates of larval growth rates was also observed for the Japanese anchovy, *E. japonica* (Mito et al., 1973; Takasuka, 2008), and other related species such as European sardines (Blaxter, 1969) and Atlantic herring (Checkley, 1984).

The fact that the larval anchovy growth rates obtained in laboratory conditions are in the lower range of the natural variation can probably be explained by growth-limiting prey concentrations, the use of unfitted prey type or size or larval stocking density. For northern anchovy larvae lab-determined growth rates were closer to field values (Methot and Kramer, 1979) than for the European anchovy but no field larvae presented growth rates as slow as the laboratory ones using limited rations (Methot and Kramer, 1979). This fact led the authors to presume the existence of growth-rate dependent selective mortality in anchovy, especially if the mean growth rate is low and the slower growing individuals are near starvation. On the other hand, the absence of slow growers in the field can be explained by the presence of predators. In fact, for the larvae of the Japanese anchovy *Engraulis japonicus*, it was shown that slow growing larvae are more vulnerable to predation (Takasuka et al., 2003). Predation is probably crucial for larvae survival in the field resulting in a growth dependent mortality as opposed to larvae growing in the laboratory in the absence of predators. On the other hand, the larval stocking density of the experiments was higher than the larval densities found in the wild, which might have affected larval foraging behavior and competition and resulted in lower growth rates than the expected from the high concentrations of prey offered.

For the sake of the discussion, one may attempt at calculating the food intake necessary to achieve such larvae growth rates. Using the relation between anchovy larvae size (*SL*, mm) and dry weight (*DW*) estimated by Catalán et al. (2010):

$$\ln DW = 0.867 * 0.5SL$$

the increase in dry weight for a larvae growing in length from 7 to 8 mm is 51.1 μg, which equals 20.45 μg C assuming that carbon

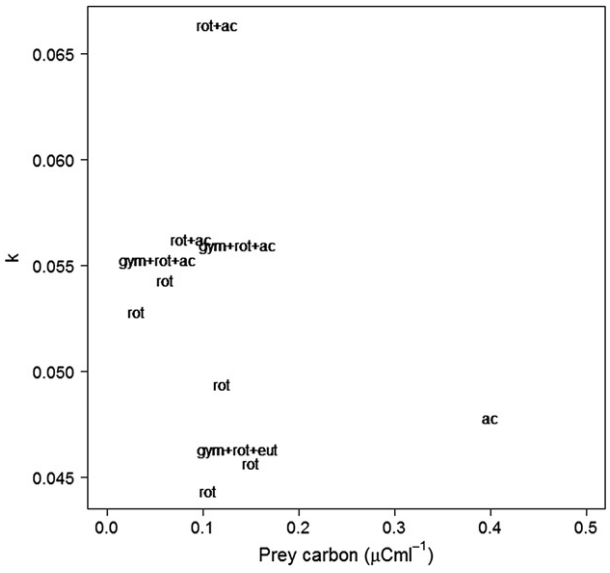

Fig. 3. Relation between the instantaneous growth rate of first feeding anchovy larvae and total prey carbon content (prey μg C ml^{-1}). The different diets used in the experiments are represented: *rot*: *Brachionus plicatilis*; *ac*: *Acartia grani* nauplii, *eut*: *Euterpina acutifrons* nauplii, *gym*: *Gymnodinium sanguineum*.

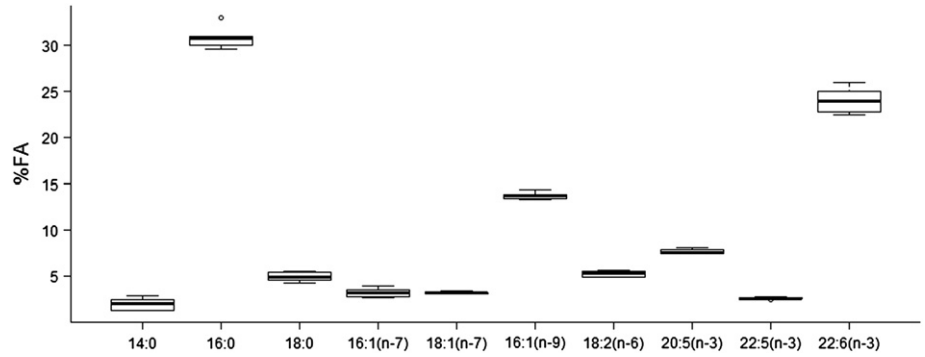

Fig. 4. Fatty acid composition (percentage of total fatty acids) of anchovy eggs spawned in laboratory conditions during 2009. Results correspond to 6 pools of 100–300 eggs spawned during 2009.

content is 40% of dry weight. We further take into consideration that 7–8 mm anchovy larvae feed mainly on calanoid copepod nauplii, with modal prey size of 120 μm (Morote et al., 2010), which in terms of biomass correspond to a prey with carbon content of 0.035 ng C ind^{-1} (Henriksen et al., 2007). Assuming 40% growth efficiency, anchovy larvae should ingest ca. 580 nauplii daily in order to grow equivalently to the average field growth rate estimate, 0.4 mm d^{-1} (25.8% increase of larvae weight). Similarly, to achieve a growth similar to the maximum growth rates found in our laboratory experiments (24.4% increase in dry weight), ca. 200 nauplii should be ingested daily; this value rises up to ca. 1500 nauplii in order to be able to grow 1 mm d^{-1} (34% of larvae dry weight; maximum growth rate found the field, Cotano et al., 2008).

The high daily rations needed to sustain such field-observed growth rates imply that larvae must be feeding in copepod nauplii patches or else they would not have sufficient food to be able to survive. Conway et al. (1998) compared in the Adriatic Sea the abundance of potential prey items in the water column with the gut contents of anchovy larvae, and concluded that the abundance of potential prey coincident to anchovy larvae vertical distribution ranged between 0.018 and 0.2 prey items ml^{-1}, which is far lower than the concentrations used in our experiments. Similarly, Esteves et al. (2000) in an estuary located in the western Portuguese coast reported that the concentrations of copepod nauplii overlapping with the anchovy larvae distribution ranged from 0.05 to 0.6 individuals l^{-1}. The threshold concentrations of food required for survival of marine fish larvae in the laboratory are commonly much higher than the average prey concentrations found at sea, and more than 3 decades ago Hunter and Thomas (1974) argued that this disagreement must be related to the capability of fish larvae to find and remain in patches of food in the water column, with prey densities much higher than the average, integrated value estimated from plankton net catches. At 6 mm anchovy larvae have visible swim bladders (Ré, 1996; Somarakis and Nikolioudakis, 2007) and vertical migrations initiate (Olivar et al., 2001), which has been proposed to be an important mark for the larvae as schooling behavior might begin during that phase (Somarakis and Nikolioudakis, 2010), allowing them, at some extent, to move to areas of high food concentration. Anchovies spawn near or at the river estuaries, which indicates that the first larval stages must be very dependent on the high productivity associated to those systems. In fact, in the Adriatic Sea the peak anchovy larvae concentrations were found in the immediate outflow area of the river flow, coincident with layers with high concentrations of potential prey items, typically reaching >0.5 prey l^{-1} (Coombs et al., 2003).

Fatty acids determined in anchovy larvae collected in the Mediterranean Sea (Rossi et al., 2006) presented similar proportion of fatty acids to the larvae analyzed in the experiments. However, these authors observed an exponential accumulation of polyunsaturated fatty acids (PUFA) through ontogeny, especially in the early stages of the anchovy development. This is contrary to the results of our experiments, in which the PUFA content of the larvae decreased as they grow, probably reflecting the inadequacy of the diet offered, that comprised *G. splendens*, rotifers and copepod nauplii. In our experiments, the n−3 PUFA were the fatty acids that presented the most rapid decline through early larval development, including the essential fatty acids DHA and EPA, which suggests that they are being utilized as energy substrate in the growing larvae. This utilization of n−3 highly unsaturated fatty acids as energy source for the developing larvae has been already reported for other fish species such as the white seabream (Cejas et al., 2004) and haddock (Plante et al., 2007),

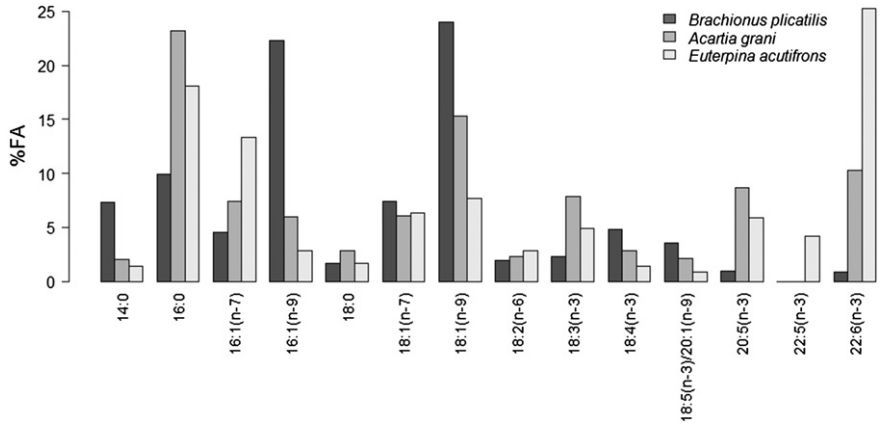

Fig. 5. Fatty acid composition (percentage of total fatty acids) of the prey used to feed anchovy first feeding larvae in the laboratory experiments of 2009.

Table 2
Fatty acid composition (percentage of total fatty acids) of the first feeding anchovy larvae of the growth experiments conducted during 2009. Details of the experiments are given in Table 1.

Experiment		9			10				11				
Larvae age (dph)		2	5	8	2	5	8	11	2	5	8	11	14
%FA	14:0	1.2	2.0	0.0	1.2	1.8	7.9	1.3	1.1	2.7	1.2	2.5	5.2
	16:0	28.6	26.7	25.0	28.6	27.4	37.5	14.3	26.0	29.0	22.1	16.1	15.0
	18:0	8.1	11.3	13.0	8.1	12.1	13.6	12.1	8.9	13.6	14.8	11.1	9.2
	16:1(n−7)	2.2	3.1	2.4	2.2	2.1	2.6	7.5	1.7	2.0	2.8	7.0	11.7
	16:1(n−9)	1.1	4.0	0.0	1.1	2.7	1.4	2.1	0.0	1.8	1.7	2.4	1.9
	18:1(n−7)	3.3	2.7	3.5	3.3	2.7	2.1	10.5	3.6	3.0	3.6	8.4	8.8
	18:1(n−9)	11.6	12.1	10.7	11.6	10.9	9.6	14.5	13.7	10.4	10.5	13.3	14.9
	18:2(n−6)	5.0	3.9	4.0	5.0	3.5	1.1	3.0	4.3	3.2	3.9	3.5	3.0
	18:3(n−3)	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	1.9	2.1
	18:4(n−3)	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	2.5	3.2
	20:5(n−3)	6.5	3.5	4.6	6.5	3.8	1.4	3.9	6.5	3.4	4.5	5.2	3.5
	22:5(n−3)	2.8	2.9	2.3	2.8	2.2	0.0	1.6	2.8	2.0	2.2	2.1	1.4
	22:6(n−3)	27.6	20.0	28.3	27.6	23.2	9.3	12.5	24.8	20.9	22.6	15.1	8.3

in which the decrease in PUFA content might occur naturally and is not necessarily the result of an inadequate, not nutritionally complete diet.

The proportion of 16:1(n−7) and EPA, that are biomarkers of diatoms, were low and similar to those estimated for early larvae (Rossi et al., 2006) and lower than the proportions determined for late larvae (Costalago et al., 2011), both collected off the Mediterranean Sea. When compared to other species, these diatom biomarkers are generally low for anchovy larvae, suggesting that diatoms are not a significant part of anchovy larval diet, which is confirmed by the low preference for diatoms observed at sea (Costalago et al., 2011). However, EPA is an essential fatty acid for the developing larvae and must come from the diet. Fatty acid limitation is likely strongest in laboratory experiments where the zooplankton to serve as fish larvae is fed a monospecific diet (Anderson and Pound, 2000). In future experiments diatoms, although not eaten directly by anchovy larvae, should be certainly supplied to the stock cultures of rotifers and copepods to be used for feeding fish larvae, in order to ensure sufficient amounts of EPA in the fish larvae diet that can eventually be used for larvae development.

In this study we have found that despite offering relatively high food concentrations, the obtained growth rates of first-feeding anchovy larvae were at the lower range of the natural variation. Since no individuals are found in nature with growth rates as low as those found under the restricted feeding conditions in our experiments, we can conclude that growth-dependent mortality at sea must play a major role in determining the recruitment success of the early stages of anchovy larvae. In addition, estimates of food consumption have shown that, in order to sustain the high growth rates found in nature, the early larvae must have very high daily rations. Single prey diets failed to support larvae growth, probably due to the rapidly changing range of preferred prey size through ontogeny. We expect that using higher concentrations of mixed diets including different prey sizes with adequate nutritional condition, particularly those containing high levels of polyunsaturated fatty acids, could enhance anchovy larvae growth to levels similar to those found under natural conditions. Such results would be valuable to parameterize current attempts to model anchovy larvae growth and dispersal. Moreover, the successful rearing of these larvae will enable a wide variety of further experimental studies to examine the impacts of abiotic factors on larval growth and survival.

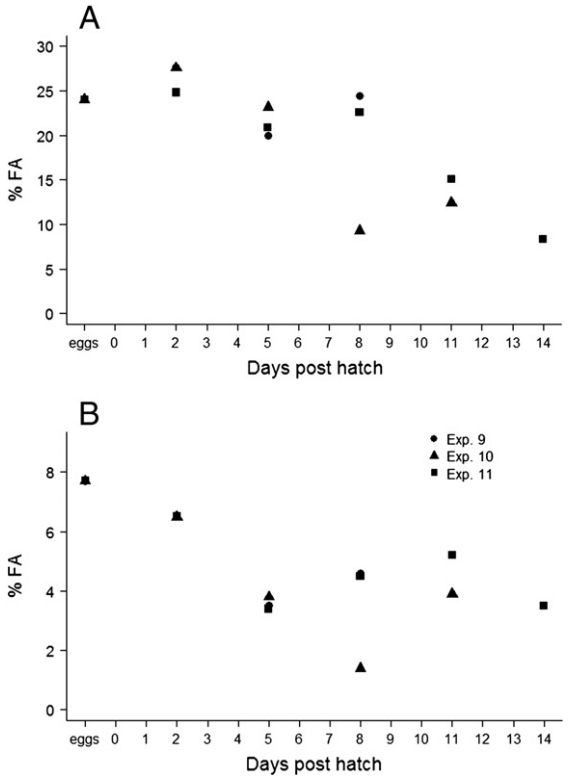

Fig. 6. Percentage of A) DHA and B) EPA contents in relation to total fatty acid content of the first feeding anchovy larvae of the growth experiments 9, 10 and 11 conducted during 2009. Details of the experiments are given in Table 1.

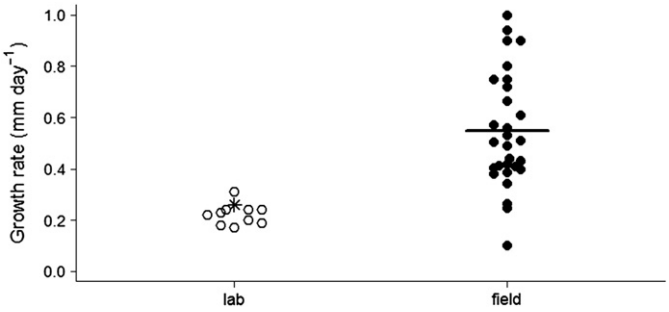

Fig. 7. Growth of first-feeding anchovy larvae estimated from the laboratory experiments (blank circles), conducted in the present work and in the experiments conducted in Aldanondo et al., 2008 (asterisk); and growth rates obtained from field data (solid circles) off the Bay of Biscay, Western Portuguese coast and Mediterranean and Adriatic Seas (Regner and Dulcic, 1990; Ribeiro, 1991; Ré, 1996; Dulcic, 1997; García et al., 1998; Palomera et al., 1988; Sabatés et al., 2007; Somarakis and Nikolioudakis, 2007; Cotano et al., 2008; Catalán et al., 2010).

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