

1 **EFFECT OF FOOD TYPE AND CONCENTRATION ON GROWTH AND**
2 **FATTY ACID COMPOSITION OF EARLY LARVAE OF THE ANCHOVY**
3 **(*Engraulis encrasicolus*) REARED UNDER LABORATORY CONDITIONS**

4

5 S. Garrido^{1,2*}, E. Saiz², J. Peters³, P. Ré¹, P. Alvarez⁴, U. Cotano⁴, D.L Herrero⁵, A.
6 Martínez de Murguía⁵, X. Irigoien^{4#}

7

8 ¹ University of Lisbon, Centre of Oceanography – Guia Marine Laboratory (CO-LMG), Faculty
9 of Sciences, Avenida Nossa Senhora do Cabo, nº939, 2750-374 Cascais - Portugal

10 *corresponding author: garridosus@gmail.com

11 ² Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta 37-49, E-08003
12 Barcelona.

13 ³ Hamburg University Institute for Hydrobiology and Fisheries Science, Grosse Elbstrasse 133,
14 22765 Hamburg, Germany.

15 ⁴ Marine Research Unit – AZTI Foundation, Herrera Kaia Portualdea z/g 20110 Pasaia, Basque
16 Country, Spain.

17 ⁵ Aquarium of San Sebastian, Plaza de Carlos Blasco de Imaz 1, 20003 San Sebastián, Spain

18 # Present address: Red Sea Research Center, King Abdullah University for Science and
19 Technology, KAUST, 23955-6900 Thuwal, Saudi Arabia

20

21

22

23

24 **ABSTRACT**

25

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

47

48 **Keywords:** Anchovy, *Engraulis encrasicolus*, fatty acids, larval growth

49

50 INTRODUCTION

51

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

61 The European anchovy (*Engraulis encrasicolus*) is a pelagic fish distributed in the
62 Mediterranean Sea and along the eastern Atlantic coast, from Norway to South Africa
63 and represents one of the main targets of purse-seine fisheries for several countries
64 located at Southern Europe and western Africa (Whitehead *et al.* 1988; ICES 2008).
65 These fisheries have typically a large variability in population size, which is mostly
66 attributed to recruitment variability. It is thought that variations in the recruitment
67 strength of small pelagic fish are primarily driven by biological and/or physical factors
68 impacting on early life stages (Mullon *et al.* 2003). In particular for the European
69 anchovy, Aldanondo *et al.* (2010) demonstrated that larval survival during peak
70 spawning seems to control the annual recruitment of this species in the Bay of Biscay.
71 For this reason, coupled biological-physical models developed to explore the complex
72 interactions between physical oceanography and fish recruitment have mainly focused
73 on the early life history stages (review in Gallego *et al.* 2007). Such models must be
74 parameterized with the vital rates (growth and development) of fish early-life in order to

75 properly mimic the larvae response under different environmental circumstances.
76 Although the growth rates of the European anchovy larvae have been extensively
77 studied through otolith microstructure analysis of individuals collected in the main areas
78 of distribution such as the Bay of Biscay (e.g. Cotano *et al.* 2008), several Portuguese
79 estuaries (e.g. Ré 1996; Ribeiro 1991), the Mediterranean Sea (e.g. Palomera *et al.*
80 1988; Catalan *et al.* 2010) and the Adriatic Sea (e.g. Regner and Dulcic 1990; Dulcic
81 1997), to our knowledge, no laboratory experiments have been conducted to study the
82 relation between prey availability and European anchovy larval growth. Due to this lack
83 of knowledge, up to date models developed to study this species have either described
84 the larvae as inert particles (e.g. Allain *et al.* 2007) or used the data available for related
85 species (Urtizbera *et al.* 2008; Politikos *et al.* 2011). These alternatives may introduce
86 strong biases that can only be solved by using species-specific bioenergetic parameters.
87 European anchovy larvae are mainly diurnal feeders (Ré 1996; Tudela *et al.* 2002). The
88 study of its diet in the Mediterranean Sea (Tudela *et al.* 2002) shows it is mainly
89 composed of copepod eggs, nauplii and copepodites. Nauplii appears to be important
90 mainly for larvae <4mm, whereas as larvae grow copepodites gain importance,
91 reflecting the broader prey range through ontogeny. There is still, however, some
92 controversy regarding its diet in the natural environment. Morote *et al.* (2010) found for
93 anchovy larvae in the Mediterranean Sea that besides calanoid copepods, harpacticoids
94 (*Microsetella* and *Euterpina*) and cladocerans were significant contributors to the diet of
95 <9 mm larvae. Contrarily, Catalan *et al.* (2010) also in the Mediterranean Sea reported
96 that in all size-classes, larvae ingested essentially calanoid copepods and selected
97 negatively against cladocerans. Microzooplankton prey might be an important
98 component of the anchovy larvae diet, however this have not been properly quantified
99 in stomach content analysis due to rapid digestion and absence of hard remains. Rossi *et*

100 *al.* (2006) found a high percentage of 18:1(n-9) and 18:4(n-3) in small anchovy larvae
101 apparently reflecting feeding on prymnesiophytes, since those are usually enriched in
102 these fatty acids (Dalsgaard *et al.* 2003) and their abundance was high in the research
103 area.

104 There is a dramatic change in the ability to withstand starvation over the larval period,
105 with larvae being usually more vulnerable at first-feeding (Hunter 1976). Lipid
106 utilization in marine fish mainly occurs after hatching, reflecting the greater energy
107 demand of the free-swimming yolk-sac larvae compared to the egg (Sargent *et al.*
108 2002). The large demand of monounsaturated and polyunsaturated fatty acids for the
109 diet of the larvae at these critical early stages makes them crucial for their growth and
110 survival (Tocher 2003).

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

117

118 **MATERIAL AND METHODS**

119

120 Two series of anchovy larvae experiments were done; the first during the summer of
121 2008 and the second during the summer of 2009 (Table I). For both series, anchovy
122 eggs were obtained from adult fish captured as juveniles by purse-seine fishery in
123 September of 2007 in the Bay of Biscay and maintained in the San Sebastian Aquarium
124 in cylindrical tanks (1300 L). Adult fish started spawning naturally using photoperiod,

125 temperature and increased food concentration stimulus. Each morning the egg collector
126 placed in the overflow of the adult fish tank was inspected, and when present, the eggs
127 were collected, counted and transferred by pipette into the experimental tanks.

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

138 Anchovy larvae were provided known concentrations of different food types, which
139 were maintained through the whole experimental period. Experiments lasted until there
140 were no larvae in the tank, as the result of sampling and natural death (Table I). Prey
141 were introduced in the tanks from day 4 post hatch onwards and comprised the
142 dinoflagellate *Gymnodinium sanguineum*, the rotifer *Brachionus plicatilis* and nauplii of
143 two copepod species: the calanoid *Acartia grani* and the harpacticoid *Euterpina*
144 *acutifrons*, both cultured for several years at the *Institut de Ciències del Mar* (CSIC,
145 Barcelona). The prey types and concentrations used are within the preferred prey items
146 and range of prey concentrations reported for anchovy larvae in the field, and
147 commonly used in previous experimental studies of the growth and survival of another
148 engraulid species, the northern anchovy *Engraulis mordax* (Lasker *et al.* 1970, Kramer
149 and Zweifel 1970, O'Connell and Raymond 1970, Theilacker and MacMaster 1971,

150 Hunter 1976, Theilacker 1987). The microalgae *Isochrysis galbana* was also added to
151 all experimental tanks because although too small to be preyed upon by anchovy larvae,
152 it provided food to the rotifers and copepod nauplii offered to the anchovy larvae. The
153 tanks were supplied with gentle air bubbling, which has been shown not to affect small
154 pelagic fish larvae (Soura and Jerde 1977), to ensure the microalgae were kept in
155 suspension. Each morning, after quantifying the food remaining in the tank from the
156 previous day, 25% of the tank water was renewed and then new food items were added
157 to the tank to obtain the desired concentrations. Stock cultures of both copepod nauplii
158 were kept in a mixture of *Tetraselmis suecica* and *Isochrysis galbana*. The rotifers used
159 as prey were fed *Isochrysis galbana*, a microalgae described as a enhancer of the fatty
160 acid content in rotifers, especially 22:6n-3 (Dhert et al. 2001 and references within).
161 Concentrations of dinoflagellates were counted using a Sedgwick-Rafter counting
162 chamber under an inverse microscope; rotifers and copepod nauplii were counted as 4
163 replicates of 1 ml subsample under a stereoscope microscope. Prey were measured at
164 the start of the set of experiments and their carbon content estimated using equations
165 given in Smayda (1978) for dinoflagellates, and van der Lingen (2002) for crustacean
166 nauplii.

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

169

$$170 \quad SL = l_0 e^{kt}$$

171

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

173 For the 3 experiments carried out during 2009 the fatty acid content of the zooplankton
174 prey, the anchovy eggs and the anchovy larvae were also analyzed (Table I). For that

175 purpose, 25-300 anchovy eggs were sampled at the beginning of the experiment,
176 whereas for zooplankton, ca. 30 copepod nauplii and ca. 100000 rotifers were collected
177 at the first day of larvae feeding; in addition, 10 anchovy larvae were sampled every 3
178 days until the end of the experiment for fatty acid analysis. The above-mentioned
179 samples for fatty acid analysis were in all cases rinsed in distilled water and preserved
180 in liquid nitrogen until analysis. After lyophilisation, lipid extraction and conversion of
181 acyl groups into methyl ester derivatives (FAMES) was performed after Peters et al.
182 (2007) using modified protocols of Folch et al. (1957) and Kattner and Fricke (1986).
183 FAMES and fatty alcohols were separated by gas chromatography, detected by flame
184 ionisation and identified by comparing retention times with those derived from
185 standards of known composition.

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

188

189 **RESULTS**

190

191 **1 - Anchovy larvae growth**

192 Anchovy larvae hatched approximately 48 h after collection of the eggs, measuring
193 2.71 ± 0.74 (SD) mm. Instantaneous growth rates varied with the feeding level (Fig. 1
194 and Table I), ranging from 0.044 d^{-1} (in one of the experiments where larvae were fed
195 rotifers as single prey) to 0.066 d^{-1} (in one experiment using a mixed diet of rotifers and
196 *Acartia grani* nauplii). These instantaneous growth rates when computed as length
197 growth for the first 15 days after hatch correspond to rates of 0.17 mm d^{-1} and 0.31 mm
198 d^{-1} , respectively. In general, anchovy larvae growing with a diet that included rotifers
199 and copepod nauplii grew faster than those fed rotifers as single prey, even if provided

200 in higher concentrations. Growth of anchovy larvae fed with a single diet of high
201 concentrations of *A. grani* (4 ind ml⁻¹) was also low and similar to that obtained using
202 rotifers alone. The addition of the dinoflagellate *Gymnodinium sanguineum* in the diet at
203 concentrations of 25-50 cells ml⁻¹ did not enhance significantly first-feeding anchovy
204 larvae growth when compared to a diet of rotifers and copepod nauplii without
205 dinoflagellates. The mixed diet composed of *G. sanguineum*, rotifers and *Euterpina*
206 *acutifrons* nauplii resulted in lower growth rates (0.046 d⁻¹) than those obtained using
207 the same concentration of the nauplii of the calanoid copepod *A. grani* (0.048 d⁻¹).
208 The covariance analysis relating larvae size with larval age and experiment (as factors)
209 was significant (p<0.0001, R²=73%) and showed that overall the growth rates obtained
210 among experiments were not significantly different from each other except for
211 experiment #8 (p=0.05) and experiment #11 (p=0.03), which resulted in significantly
212 higher growth rates (Fig. 2). Those experiments corresponded to larvae fed a mixture of
213 rotifers and *A. grani* nauplii (1 ind ml⁻¹, each), and in case of experiment #11 the
214 dinoflagellate *Gymnodinium* had also been added to the diet.
215 The instantaneous growth rate was not linearly related to the total prey carbon content
216 of the diet, and this lack of relationship was caused by the effect of prey composition on
217 larval growth rates (Fig. 3). Indeed, experiment #6 had the highest carbon content diet
218 (composed of 4 acartia nauplii ml⁻¹) but resulted in low larval growth rates. Despite all
219 the other experiments had similar total carbon content diets, experiment #8 in which
220 copepod nauplii (1 ind ml⁻¹) were combined with a low concentration of 1 rotifer ml⁻¹
221 resulted in the highest growth rates observed in our study.

222

223 **2 – Fatty acid composition of prey, eggs and larvae**

224

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

234 Both copepod nauplii species had a higher percentage of polyunsaturated fatty acids
235 than the rotifers, including EPA and DHA (Fig. 5). *A. grani* nauplii had a higher
236 percentage of 20:5(n-3) and 18:1(n-9) whereas *E. acutifrons* nauplii had a higher
237 22:6(n-3) content. Rotifers, on the other hand, presented higher proportion of
238 monounsaturated fatty acids, particularly of 16:1(n-7) and 18:1(n-9) (Fig. 5).

239 Regarding the anchovy larvae, of the 17 fatty acids identified the most abundant were
240 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
241 II). Overall, polyunsaturated fatty acids were the most relevant fatty acid group in all the
242 larvae analyzed. However the percentage of (n-3) PUFA, particularly EPA and DHA,
243 decreased significantly through ontogeny until day 5, when the yolk sac was being used
244 (Fig. 6). After day 5 the decreasing trend of polyunsaturated fatty acids continued while
245 some uptake occurred in two experiments, after 5dph for experiment 11 and after 11
246 dph for experiment 10 (Fig. 6).

247

248 **DISCUSSION**

249

250 As expected, we observed an effect of prey concentration and prey type on larval
251 anchovy growth. Larvae grew the fastest with a mixed diet of rotifers and copepod
252 nauplii, with concentrations of 1 prey ml⁻¹ respectively. Diets using higher
253 concentrations of either rotifers or acartia nauplii as single prey resulted in lower growth
254 rates than the mixed diets tested. Just after the beginning of exogenous feeding, which
255 occurred 3-4 days after hatch at 19°C, anchovy larvae probably depended mostly of prey
256 which were smaller and less motile than copepod nauplii, and only a few days later,
257 when already larger, larvae were able to pursue and capture the copepod nauplii
258 efficiently. Indeed, the inclusion of rotifers in the mixed diet enhanced anchovy growth,
259 whereas high concentrations of *Acartia grani* nauplii supplied as single prey were not
260 sufficient to sustain high growth rates for first feeding larvae. These observations are in
261 agreement with general patterns of mouth size - prey size relationships in fish larvae
262 (Sabates & Saiz 2000), in which small mouths (gap size) restrict not only the size of
263 prey but also the range of prey sizes. Specifically for anchovy larvae, Morote *et al.*
264 (2010) already found this restriction in early stages the European anchovy; similarly, the
265 northern anchovy larvae appeared to fail to capture copepod nauplii (*Tigriopus* sp.) at 1
266 ml⁻¹ until reaching 7-8 dph (Theilacker 1987).

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

273 We expected that the addition of the dinoflagellate *Gymnodinium splendens*, which falls
274 into the same size range as small rotifers but are generally considered more nutritional,

275 especially in terms of fatty acid content (Mansour *et al.* 1997), to larval diet would
276 enhance anchovy larvae growth, but it did not. This result contrasts with the
277 observations of Lasker *et al.* (1970), who were able to grow first feeding northern
278 anchovy larvae on a single diet of the dinoflagellates *Gymnodinium splendens* on
279 slightly higher concentrations. Probably this lack of conspicuous effect in our
280 experiments was due to the use of moderate to high densities (25-50 cells ml⁻¹) of the
281 dinoflagellate. Further efforts have to be devoted to identify the actual small prey that
282 provides suitable food to sustain high growth and survival at those first days of
283 exogenous feeding, so critical for the larvae survival; ciliates and dinoflagellates are
284 good candidates (Scura and Jerde 1977; Nagano *et al.* 2000).

285 The growth rates we have obtained in the laboratory for the European anchovy larvae
286 are comparable, at high prey concentrations, to the growth rates obtained in the
287 laboratory for the northern anchovy (Theilacker, 1987; 0.31 mm d⁻¹ and 0.33 mm d⁻¹ at
288 15 dph, respectively, for the European anchovy and the northern anchovy larvae) and
289 lower than those obtained for first-feeding japanese anchovy, *E. japonica* (Mito *et al.*,
290 1973, Fukuhara, 1983; 0.57 mm d⁻¹ and 0.43 mm d⁻¹, respectively). This is in agreement
291 with the fact that the prey types and concentrations used in our experiments were similar
292 to those used in previous experiments carried out with the northern anchovy larvae and
293 lower than the feeding concentrations used for Japanese anchovies. The maximum
294 growth rate obtained in the experiments using a combination of rotifers and *A. grani*
295 nauplii is slightly higher than the growth rate determined in previous experiment using
296 rotifers *ad libitum* (Aldanondo *et al.* 2008). The growth rates obtained here were also
297 slightly higher than those estimated for first-feeding larvae of the same species (known
298 as *Engraulis capensis* when the work was done), in experiments conducted in South
299 Africa (Brownell, 1983) using unknown concentrations of calanoid and cyclopoid

300 copepods until 15 dph, and rotifers this day onwards. The comparison of our lab-
301 determined growth rates with field data (estimated from otolith microstructure analysis
302 of field caught larvae, Fig. 7) evidences a disagreement, our rates being at the lower
303 range of field values (field data include growth rates obtained in the wild for larvae
304 growing with different food concentrations, temperatures and salinities, explaining their
305 high variability). Growth rates obtained in our experiments ranged between 0.17 and
306 0.31 mm d⁻¹ for larvae growing 15 dph, contrasting with the mean growth rates
307 estimated for the larvae caught in the Bay of Biscay (0.4-0.6 mm d⁻¹, Cotano *et al.*
308 2008), several Portuguese estuaries (0.25-0.51 mm d⁻¹, Ré 1996; Ribeiro 1991), in the
309 Mediterranean Sea (0.4 - 0.91 mm d⁻¹, Garcia *et al.* 1998; Palomera *et al.* 1988; Sabates
310 *et al.* 2007; Somarakis *et al.* 2007; Catalan *et al.* 2010) and the Adriatic Sea (0.9-0.94
311 mm d⁻¹, Regner and Dulcic 1990; Dulcic 1997). The difference between laboratory
312 experiments and field estimates might even be slightly larger because field estimates
313 have assumed that the initial increment deposition in the otoliths of *E. encrasicolus*
314 larvae occurs at the beginning of first feeding or 2 days pos hatch but recent laboratory
315 experimentation (Aldanondo *et al.* 2008) revealed that the first increment occurs on the
316 day after hatch, which means that previous growth rates are slightly underestimated,
317 especially for small larvae. The same discrepancy between laboratory-derived and field
318 estimates of larval growth rates were also observed for the Japanese anchovy, *Engraulis*
319 *japonica* (Mito *et al.*, 1973, Takasuka *et al.* 2009), and other related species such as
320 European sardines (Blaxter, 1969) and Atlantic herring (Checkley Jr., 1984).
321 The fact that the larval anchovy growth rates obtained in laboratory conditions are in the
322 lower range of the natural variation can probably be explained by growth-limiting prey
323 concentrations, the use of unfitted prey type or size or larval stocking density. For
324 northern anchovy larvae lab-determined growth rates were closer to field values

325 (Methot and Krammer 1979) than for the European anchovy but no field larvae
326 presented growth rates as slow as the laboratory ones using limited rations (Methot and
327 Krammer 1979). This fact led the authors to presume the existence of growth-rate
328 dependent selective mortality in anchovy, especially if the mean growth rate is low and
329 the slower growing individuals are near starvation. On the other hand, the absence of
330 slow growers in the field can be explained by the presence of predators. In fact, for the
331 larvae of the Japanese anchovy *Engraulis japonicus*, it was shown that slow growing
332 larvae are more vulnerable to predation (Takasuka et al. 2003). Predation is probably
333 crucial for larvae survival in the field resulting in a growth dependent mortality as
334 opposed to larvae growing in the laboratory in the absence of predators. On the other
335 hand, the larval stocking density of the experiments was higher than the larval densities
336 found in the wild, which might have affected larval foraging behavior and competition
337 and resulted in lower growth rates than the expected from the high concentrations of
338 prey offered.

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

$$342 \ln DW = 0.867 + 0.5 SL$$

343 the increase in dry weight for a larvae growing in length from 7 to 8 mm is 51.1 μg ,
344 which equals 20.45 μg C assuming that carbon content is 40% of dry weight. We
345 further take into consideration that 7-8 mm anchovy larvae feed mainly on calanoid
346 copepod nauplii, with modal prey size of 120 μm (Morote *et al.* 2010), which in terms
347 of biomass correspond to a prey with carbon content of 0.035 ng C ind⁻¹ (Henriksen *et*
348 *al.* 2007). Assuming 40% growth efficiency, anchovy larvae should ingest ca. 580
349 nauplii daily in order to grow equivalently to the average field growth rate estimate, 0.4

350 mm d⁻¹ (25.8% increase of larvae weight). Similarly, to achieve a growth similar to the
351 maximum growth rates found in our laboratory experiments (24.4% increase in dry
352 weight), ca. 200 nauplii should be ingested daily; this value rises up to ca. 1500 nauplii
353 in order to be able to grow 1 mm d⁻¹ (34% of larvae dry weight; maximum growth rate
354 found the field, Cotano *et al.* 2008).

355 The high daily rations needed to sustain such field-observed growth rates imply that
356 larvae must be feeding in copepod nauplii patches or else they would not have sufficient
357 food to be able to survive. Conway *et al.* (1998) compared in the Adriatic Sea the
358 abundance of potential prey items in the water column with the gut contents of anchovy
359 larvae, and concluded that the abundance of potential prey coincident to anchovy larvae
360 vertical distribution ranged between 0.018 and 0.2 prey items ml⁻¹, which is far lower
361 than the concentrations used in our experiments. Similarly, Esteves *et al.* (2000) in an
362 estuary located in the western Portuguese coast reported that the concentrations of
363 copepod nauplii overlapping with the anchovy larvae distribution ranged from 0.05 to
364 0.6 individuals l⁻¹. The threshold concentrations of food required for survival of marine
365 fish larvae in the laboratory are commonly much higher than the average prey
366 concentrations found at sea, and more than 3 decades ago Hunter and Thomas (1974)
367 argued that this disagreement must be related to the capability of fish larvae to find and
368 remain in patches of food in the water column, with prey densities much higher than the
369 average, integrated valued estimated from plankton net catches. At 6 mm anchovy
370 larvae have visible swim bladders (Re' 1996; Somarakis 2007) and vertical migrations
371 initiate (Olivar *et al.* 2001), which has been proposed to be an important mark for the
372 larvae as schooling behavior might begin during that phase (Somarakis *et al.* 2010),
373 allowing them, at some extent, to move to areas of high food concentration. Anchovies
374 spawn near or at the river estuaries, which indicates that the first larval stages must be

375 very dependent on the high productivity associated to those systems. In fact, in the
376 Adriatic Sea the peak anchovy larvae concentrations were found in the immediate
377 outflow area of the river flow, coincident with layers with high concentrations of
378 potential prey items, typically reaching >0.5 prey l^{-1} (Coombs *et al.* 2003).

379 Fatty acids determined in anchovy larvae collected in the Mediterranean Sea (Rossi *et*
380 *al.* 2006) presented similar proportion of fatty acids to the larvae analyzed in the
381 experiments. However, these authors observed an exponential accumulation of
382 polyunsaturated fatty acids (PUFA) through ontogeny, especially in the early stages of
383 the anchovy development. This is contrary to the results of our experiments, in which
384 the PUFA content of the larvae decreased as they grow, probably reflecting the
385 inadequacy of the diet offered, that comprised *G. splendens*, rotifers and copepod
386 nauplii. In our experiments, the n-3 PUFA were the fatty acids that presented the most
387 rapid decline through early larval development, including the essential fatty acids DHA
388 and EPA, which suggests that they are being utilized as energy substrate in the growing
389 larvae. This utilization of n-3 highly unsaturated fatty acids as energy source for the
390 developing larvae has been already reported for other fish species such as the white
391 seabream (Cejas *et al.* 2004) and haddock (Plante *et al.* 2007), in which the decrease in
392 PUFA content might occur naturally and is not necessarily the result of an inadequate,
393 not nutritionally complete diet.

394 The proportion of 16:1(n-7) and EPA, that are biomarkers of diatoms, were low and
395 similar to those estimated for early larvae (Rossi *et al.* 2006) and lower than the
396 proportions determined for late larvae (Costalago *et al.* 2011), both collected off the
397 Mediterranean Sea. When compared to other species, these diatom biomarkers are
398 generally low for anchovy larvae, suggesting that diatoms are not a significant part of
399 anchovy larval diet, which is confirmed by the low preference for diatoms observed at

400 sea (Costalago *et al.* 2011). However, EPA is an essential fatty acid for the developing
401 larvae and must come from the diet. Fatty acid limitation is likely strongest in
402 laboratory experiments where the zooplankton to serve as fish larvae is fed a
403 monospecific diet (Anderson and Pond 2000). In future experiments diatoms, although
404 not eaten directly by anchovy larvae, should be certainly supplied to the stock cultures
405 of rotifers and copepods to be used for feeding fish larvae, in order to ensure sufficient
406 amounts of EPA in the fish larvae diet that can eventually used for larvae development.
407 In this study we have found that despite offering relatively high food concentrations, the
408 obtained growth rates of first-feeding anchovy larvae were at the lower range of the
409 natural variation. Since no individuals are found in nature with growth rates as low as
410 those found under the restricted feeding conditions in our experiments, we can conclude
411 that growth-dependent mortality at sea must play a major role in determining the
412 recruitment success of the early stages of anchovy larvae. In addition, estimates of food
413 consumption have shown that, in order to sustain the high growth rates found in nature,
414 the early larvae must have very high daily rations. Single prey diets failed to support
415 larvae growth, probably due to the rapidly changing range of preferred prey size through
416 ontogeny. We expect that using higher concentrations of mixed diets including different
417 prey sizes with adequate nutritional condition, particularly those containing high levels
418 of polyunsaturated fatty acids, could enhance anchovy larvae growth to levels similar to
419 those found under natural conditions. Such results would be valuable to parameterize
420 current attempts to model anchovy larvae growth and dispersal. Moreover, the
421 successful rearing of these larvae will enable a wide variety of further experimental
422 studies to examine the impacts of abiotic factors on larval growth and survival.

423

424 **ACKNOWLEDGEMENTS**

425 S.G. is supported by the Portuguese Foundation for Science and Technology (FCT)
426 through a Post-Doctoral Fellowship (SFRH/BPD/38332/2007). This work was partially
427 supported by project VITAL (FCT - PTDC/MAR/111304/2009). E.S. was supported by
428 the project CTM2010-10036-E from the Spanish Ministerio de Ciencia e Innovación.
429 The authors wish to thank the team of the San Sebastian Aquarium and Udane Martinez,
430 Inma Mikelarena, Ion Laucirica of the AZTI Foundation. SG also wishes to thank M.C.
431 and L. Freijido. We are grateful to C.D. van der Lingen for his comments on the
432 manuscript.

433

434 **REFERENCES**

435

436 Aldanondo, N., Cotano, U., Etxebeste, E., Irigoien, X., Alvarez, P., Martínez de
437 Murguía, A., Herrero, D.L., 2008. Validation of daily increments deposition in the
438 otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different
439 temperature conditions. Fish. Res. 93, 257-264.

440

441 Aldanondo, N., Cotano, U., Tiepolo, M., Boyra, G., Irigoien, X., 2010. Growth and
442 movement patterns of early juvenile European anchovy (*Engraulis encrasicolus* L.) in
443 the Bay of Biscay based on otolith microstructure and chemistry. Fish. Oceanogr. 19
444 (3), 196–208.

445

446 Allain, G., Petitgas, P., Lazure, P., 2007. The influence of environment and spawning
447 distribution on the survival of anchovy (*Engraulis encrasicolus*) larvae in the Bay of
448 Biscay (NE Atlantic) investigated by biophysical simulations. Fish. Oceanogr. 16:6,
449 506–514.

450

451 Anderson, A.T., Pound, D.W., 2000. Stoichiometric theory extended to micronutrients:
452 Comparison of the roles of essential fatty acids, carbon, and nitrogen in the nutrition of
453 marine copepods. *Limnol. Oceanogr.* 45(5), 1162-1167.

454

455 Blaxter, J.H.S., Hempel, G., 1963. The influence of egg size on herring larvae. *J. Cons.*
456 *Perm. Int. Explor. Mer* 28, 211-240.

457

458 Blaxter J.H.S., 1969. Experimental rearing of pilchard larvae, *Sardina pilchardus*. *J.*
459 *Mar. Biol. Assoc. U.K.*,49, 557-575.

460

461 Brownell, C.L., 1983. Laboratory rearing of Cape anchovy *Engraulis capensis* and
462 South African pilchard *Sardinops ocellata* through metamorphosis. *S. Afr. J. mar. Sci.*
463 1, 181-188.

464

465 Catalán, I., Folkvord, A., Palomera, I., Quílez-Badía, G., Kallianoti, F., Tselepides, A.,
466 Kallianotis, A., 2010. Growth and feeding patterns of European anchovy (*Engraulis*
467 *encrasicolus*) early life stages in the Aegean sea (NE, Mediterranean). *Est. Coast. Shelf*
468 *Sci.* 86, 299-312.

469

470 Cejas, J.R., Almansa, E., Jérez, S., Bolaños, A., Felipea, B., Lorenzo, A., 2004. Changes
471 in lipid class and fatty acid composition during development in white seabream
472 (*Diplodus sargus*) eggs and larvae. *Comp. Biochem. Physiol. B* 139, 209–216.

473

474 Checkley Jr. D.M., 1984. Relation of growth to ingestion for larvae of Atlantic herring
475 *Clupea harengus* and other fish. Mar. Ecol. Progr. Ser. 18, 215-224.
476

477 Conway, D.V.P., Coombs, S.H., Smith, C., 1998. Feeding of anchovy *Engraulis*
478 *encrasicolus* larvae in the northwestern Adriatic Sea in response to changing
479 hydrobiological conditions. Mar. Ecol. Progr. Ser., 175, 35-49.
480

481 Coombs, S.H., Giovanardi, O., Halliday, N.C., Franceschini, G., Conway, D.V.P.,
482 Manzueto, L., Barrett, C.D., McFadzen, I.R.B., 2003. Wind mixing, food availability
483 and mortality of anchovy larvae *Engraulis encrasicolus* in the northern Adriatic Sea.
484 Mar. Ecol. Progr. Ser. 248, 221–235.
485

486 Cotano, U., Irigoien, X., Etxebeste, E., Álvarez. P., Zarauz, L., Mader, J., Ferrer, L.,
487 2008. Distribution, growth and survival of anchovy larvae (*Engraulis encrasicolus* L.)
488 in relation to hydrodynamic and trophic environment in the Bay of Biscay. J. Plankton
489 Res. 30, 467–481.
490

491 Costalago, D., Tecchio, S., Palomera, I., Álvarez-Calleja, I., Ospina-Álvarez, A.,
492 Raicevich, S., 2011. Ecological understanding for fishery management: Condition and
493 growth of anchovy late larvae during different seasons in the Northwestern
494 Mediterranean. Est. Coast. Shelf Sci. 93, 350-358 .
495

496 Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., and Hagen, W., 2003. Fatty
497 acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46, 225-340.
498

499 Dhert, P., Rombaut, G., Suantika, G., and Sorgeloos, P., 2001. Advancement of rotifer
500 culture and manipulation techniques in Europe. *Aquac.* 200, 129-149.
501

502 Dulcic, J., 1997. Growth of anchovy, *Engraulis encrasicolus* (L.), larvae in the Northern
503 Adriatic Sea. *Fish. Res.* 31, 189–195.
504

505 Esteves, E., Pina, T., Chícharo, M.A., Andrade, J.P., 2000. The distribution of estuarine
506 fish larvae: Nutritional condition and co-occurrence with predators and prey. *Acta*
507 *Oecol.* 21 (3), 161–173.
508

509 Fukuhara, O., 1983. Development and growth of laboratory reared *Engraulis japonica*
510 (Houttuyn) larvae. *J. Fish Biol.* 23(6), 641-652.
511

512 Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for isolation and
513 purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497-509.
514

515 García, A., Cortes, D., Ramirez, T., 1998. Daily larval growth and RNA and DNA
516 content of the NW Mediterranean anchovy *Engraulis encrasicolus* and their relations to
517 the environment. *Mar. Ecol. Progr. Ser.* 166, 237–245.
518

519 Gallego, A., North, E.W., Petitgas, P., 2007. Introduction: status and future of
520 modelling physical-biological interactions during the early life of fishes. *Mar. Ecol.*
521 *Progr. Ser.* 347, 121-126.
522

523 Hjort, J., 1926. Fluctuations in the year classes of important food fishes. J. Cons. Int.
524 Explor. Mer. 1, 5–38.
525

526 Henriksen, C.I., Saiz, E., Calbet, A., Hansen, B.W., 2007. Feeding activity and
527 swimming patterns of *Acartia grani* and *Oithona davisae* nauplii in the presence of
528 motile and non-motile prey. Mar. Ecol. Progr. Ser. 331, 119-129.
529

530 Hunter, J.R., Sanchez, C., 1976. Diel changes in swim bladder inflation of the larvae
531 of the northern anchovy, *Engraulis mordax*. U.S. Fish. Bull. 74, 847-855.
532

533 Hunter, J.R., Thomas, G.L., 1974. Effect of prey distribution and density on the
534 searching and feeding behavior of larval anchovy *Engraulis mordax* Girard. In The
535 Early Life History of Fish, ed. J.H.S. Blaxter, Springer-Verlag, Berlin, 559-574.
536

537 ICES, 2011. Report of the Working Group on Anchovy and Sardine (WGANSA). ICES
538 CM 2011/ACOM:16, pp 470.
539

540 Kattner, G., Fricke, H.S.G., 1986. Simple gas-liquid chromatography method for
541 simultaneous determination of fatty acids and alcohols in wax esters of marine
542 organisms. J. Chromatogr. 361, 263-268.
543

544 Kramer, D., Zweifel, J.R., 1970. Growth of anchovy larvae (*Engraulis mordax* Girard)
545 in the laboratory as influenced by temperature. Calif. Mar. Res. Comm., CalCOFI Rep.,
546 14, 1-9.
547

548 Lasker, R., Feder, H.M., Theilacker, G.H., May, R.C., 1970. Feeding, growth and
549 survival of *Engraulis mordax*: larvae reared in the laboratory. Mar. Biol. 5,345-353.
550

551 Mansour, M.P., Volkman, J.K., Jackson, A.E. Blackburn, S.I., 1999. The fatty acid and
552 sterol composition of five marine dinoflagellates. J. Phycol. 35, 710-720.
553

554 Morote, E., Olivar, M.P., Villate, F., Uriarte, I., 2010. A comparison of anchovy
555 (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) larvae feeding in the
556 Northwest Mediterranean: influence of prey availability and ontogeny. ICES J. Mar.
557 Sci. 67, 897-908.
558

559 Methot, R.D., Kramer, D., 1979. Growth of northern anchovy, *Engraulis mordax*, larvae
560 in the sea. Fish. Bull. 2, 413-423.
561

562 Mito, S., Ukawa, M., Higuchi, M., 1973. Growth of some marine fish larvae hatched
563 out from pelagic eggs. J. mar. biol. Ass. India, 15 (2), 490-495.
564

565 Mullon, C., Fréon, P., Parada, C., van der Lingen, C.D., Huggett, J., 2003. From
566 particles to individuals: modelling the early stages of anchovy (*Engraulis*
567 *capensis/encrasicolus*) in the southern Benguela. Fish. Oceanogr. 12 (4/5), 396–406.
568

569 Nagano, N., Iwatsuki, Y., Kamiyama, T., Shimzu, H., and Nakata, H., 2000. Ciliated
570 protozoans as food for first-feeding larval grouper, *Epinephelus septemfasciatus*:
571 Laboratory experiment. Plankton Biol. Ecol. 47, 93-99.
572

573 O'Connell, C.P., Raymond, L.P., 1970. The effect of food density on survival and
574 growth of early post yolk-sac larvae of the northern anchovy (*Engraulis mordax*,
575 Girard) in the laboratory. J. Exp. Mar. Biol. Ecol. 5, 187-197.
576

577 Olivar, M.P., Salat, J., Palomera, I., 2001. A comparative study of the spatial
578 distribution patterns of the early stages of anchovy and pilchard in the NW
579 Mediterranean Sea. Mar. Ecol. Progr. Ser. 217,111–120.
580

581 Palomera, I., Morales-Nin, B., Lleonart, J., 1988. Larval growth of anchovy, *Engraulis*
582 *encrasicolus*, in the Western Mediterranean Sea. Mar. Biol. 99, 283-291.
583

584 Peters. J., Dutz. J., Hagen. W., 2007. Role of essential fatty acids on the reproductive
585 success of the copepod *Temora longicornis* in the North Sea. Mar. Ecol. Progr. Ser.
586 341, 153-163.
587

588 Plante, S., Pernet, F., Haché, R., Ritchie, R., Ji, B., McIntosh, D., 2007. Ontogenetic
589 variations in lipid class and fatty acid composition of haddock larvae *Melanogrammus*
590 *aeglefinus* in relation to changes in diet and microbial environment. Aquac. 263 (1-4),
591 107-121.
592

593 Politikos, D.V., Triantafyllou, G., Petihakis, G., Tsiaras, K., Somarakis, S., Ito, S.I.,
594 Megrey, B.A., 2011. Application of a bioenergetics growth model for European
595 anchovy (*Engraulis encrasicolus*) linked with a lower trophic level ecosystem model.
596 Hydrobiol. 670,141–163.
597

598 Ré, P., 1996. Anchovy spawning in the Mira Estuary (southwestern Portugal). *Scientia*
599 *Marina*, The European Anchovy and its Environment I. Palomera and P. Rubiés (eds),
600 60 (Supl. 2), 141-153.
601
602 R Development Core Team., 2009. *R: A language and environment for statistical*
603 *computing*. R Foundation for Statistical Computing, available from [http://www.R-](http://www.R-project.org)
604 [project.org](http://www.R-project.org).
605
606 Ribeiro, R.J., 1991. Ecologia do ictioplâncton e reprodução da anchova *Engraulis*
607 *encrasicolus* (L.) (Pisces, Engraulidae) no estuário do Mondego. PhD thesis, Univ.
608 Coimbra, Portugal.
609
610 Regner, S., Dulcic, J., 1990. Growth parameters of anchovy postlarvae in the Adriatic
611 estimated from otolith growth rings. *Biljeske-Notes, Inst. Oceanogr. Fish. Split.* 76, 1–8.
612
613 Rossi, S., Sabatés, A., Latasa, M., Reyes, E., 2006. Lipid biomarkers and trophic
614 linkages between phytoplankton, zooplankton and anchovy (*Engraulis encrasicolus*)
615 larvae in the NW Mediterranean. *J. Plankton Res.* 28, 551-562.
616
617 Sabates, A., Saiz, E., 2000. Intra- and interspecific variability in prey size and niche
618 breadth of myctophiform fish larvae. *Mar. Ecol. Progr. Ser.* 201, 261-271.
619
620 Sabatés, A., Olivar, M.P., Salat, J., Palomera, I., Alemany, F., 2007. Physical and
621 biological processes controlling the distribution of fish larvae in the NW Mediterranean.
622 *Progr. Oceanogr.* 74, 355–376.

623

624 Sargent, J.R., Henderson, R.J., Tocher, D.R., 2002. The Lipids. In Fish Nutrition (ed. JR
625 Halver) pp 154-219. San Diego: Academic Press.

626

627 Scura, E.D., Jerde, C.W., 1977. Various species of phytoplankton as food for larval
628 northern anchovy, *Engraulis mordax*, and relative nutritional value of the
629 dinoflagellates *Gymnodiniurn splendens* and *Gonyaulux polyedrum*. U.S. Fish. Bull. 75,
630 577-583.

631

632 Smayda, T.J., 1978. From phytoplankters to biomass. In: Sournia A (ed) Phytoplankton
633 manual. UNESCO, New York: 273–279.

634

635 Somarakis, S., Nikolioudakis, N., 2007. Oceanographic habitat, growth and mortality
636 of larval anchovy (*Engraulis encrasicolus*) in the northern Aegean Sea (eastern
637 Mediterranean). Mar. Biol. 152, 1143-1158.

638

639 Somarakis, S., Nikolioudakis, N., 2010. What makes a late anchovy larva? The
640 development of the caudal fin seen as a milestone in fish ontogeny. J. Plankton Res.
641 32(3), 317–326.

642

643 Takasuka, A., Aoki, I., Mitani I., 2003. Evidence of growth-selective predation on larval
644 Japanese anchovy *Engraulis japonicus* in Sagami Bay. Mar. Ecol. Progr. Ser. 252, 223-
645 238.

646

647 Takasuka, A., 2008. Growth effect on the otolith and somatic size relationship in
648 Japanese anchovy and sardine larvae. *Fish. Sci.* 74, 308-313.
649
650 Theilacker, G.H., McMaster, M.F., 1971. Mass culture of the rotifer *Brachionus*
651 *plicatilis* and its evaluation as a food for larval anchovies. *Int. J. Life Oceans Coast.*
652 *Wat.*, 10 (2), 183-188.
653
654 Theilacker, G.H., 1987. Feeding ecology and growth energetics of larval northern
655 anchovy, *Engraulis mordax*. U.S. National Marine Fisheries Service. *Fish. Bull.* 85,
656 213-228.
657
658 Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish.
659 *Rev. Fish. Sci.*, 11 (2), 107-184.
660
661 Tudela, S., Palomera, I., Quílez, G., 2002. Feeding of anchovy *Engraulis encrasicolus*
662 larvae in the north-west Mediterranean. *J. Mar. Biol. Assoc. U.K.*, 82, 349–350.
663
664 Urtizberea, A., Fiksen, O., Folkvord, A., Irigoien, X., 2008. Modelling growth of larval
665 anchovies including diel feeding patterns, temperature and body size. *J. Plankton Res.*
666 30 (12), 1369-1383.
667
668 van der Lingen, C.D., 2002. Diet of the sardine *Sardinops sagax* in the southern
669 Benguela upwelling ecosystem. *South African J. of Mar. Sci.* 24 (1), 301-313.
670

671 Wilhelm, M.R., Painting, S.J., Field, J.G., Kerstan, M., Durholtz, M.D., 2005. Impact of
672 environmental factors on survival of larval and juvenile Cape anchovy *Engraulis*
673 *encrasicolus* (G.) in the southern Benguela upwelling region, determined from
674 hatchdate distributions: implications for recruitment. Mar. Fresh. Res. 56 (5), 461-572.
675
676 Whitehead, P. J. P., Nelson, G. J., Wongratana, T., 1988. Clupeoid Fishes of the world.
677 Engraulidae. Fao Fisheries Synopsis 125, 7 (part 2), 305-579.
678
679

680
681
682

Figure Captions

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

687

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

691

692 **Fig. 3** – Relation between the Instantaneous growth rate of first feeding anchovy larvae
693 and total prey carbon content (prey $\mu\text{gC ml}^{-1}$). The different diets used in the
694 experiments are represented: *rot*: *Brachionus plicatilis*; *ac*: *Acartia grani* naupli, *eut*:
695 *Euterpina acutifrons* nauplii, *gym*: *Gymnodinium sanguineum*.

696

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

700

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

703

704

705 **Figure 6** – Percentage of A) DHA and B) EPA content in relation to total fatty acid
706 content of the first feeding anchovy larvae of the growth experiments 9, 10 and 11
707 conducted during 2009. Details of the experiments are given in Table I.

708

709 **Figure 7** – Growth of first-feeding anchovy larvae estimated from the laboratory
710 experiments (blank circles), conducted in the present work and in the experiments
711 conducted in Aldanondo *et al.*, 2008 (asterisk); and growth rates obtained from field
712 data (solid circles) off the Bay of Biscay, Western Portuguese coast and Mediterranean
713 and Adriatic Seas (Regner and Dulcic 1990, Ribeiro 1991, Ré 1996, Dulcic 1997,
714 Garcia *et al.* 1998, Palomera *et al.* 1988, Sabates *et al.* 2007, Somarakis *et al.* 2007,
715 Cotano *et al.* 2008, Catalan *et al.* 2010).

716

717 **Table I** - Conditions and results of the anchovy larvae growth experiments. *Rot.* Rotifers (*Brachionus plicatilis*), *A. grani* (*Acartia grani* nauplii),
 718 *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. A. grani | Rot. A. grani | Gymno. Rot. A. grani | Gymno. Rot. Euterp. | Gymno. Rot. A. grani |
| [Prey] (n ml ⁻¹) | 7 | 4 | 8 | 6 | 10 | 4 | 2.5 0.5 | 1 1 | 25 2.5 0.1 | 50 5 0.5 | 50 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 |

719

720

722 **Table II** – Fatty acid composition (percentage of total fatty acids) of the first feeding
 723 anchovy larvae of the growth experiments conducted during 2009. Details of the
 724 experiments are given in Table I.
 725

| 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 |

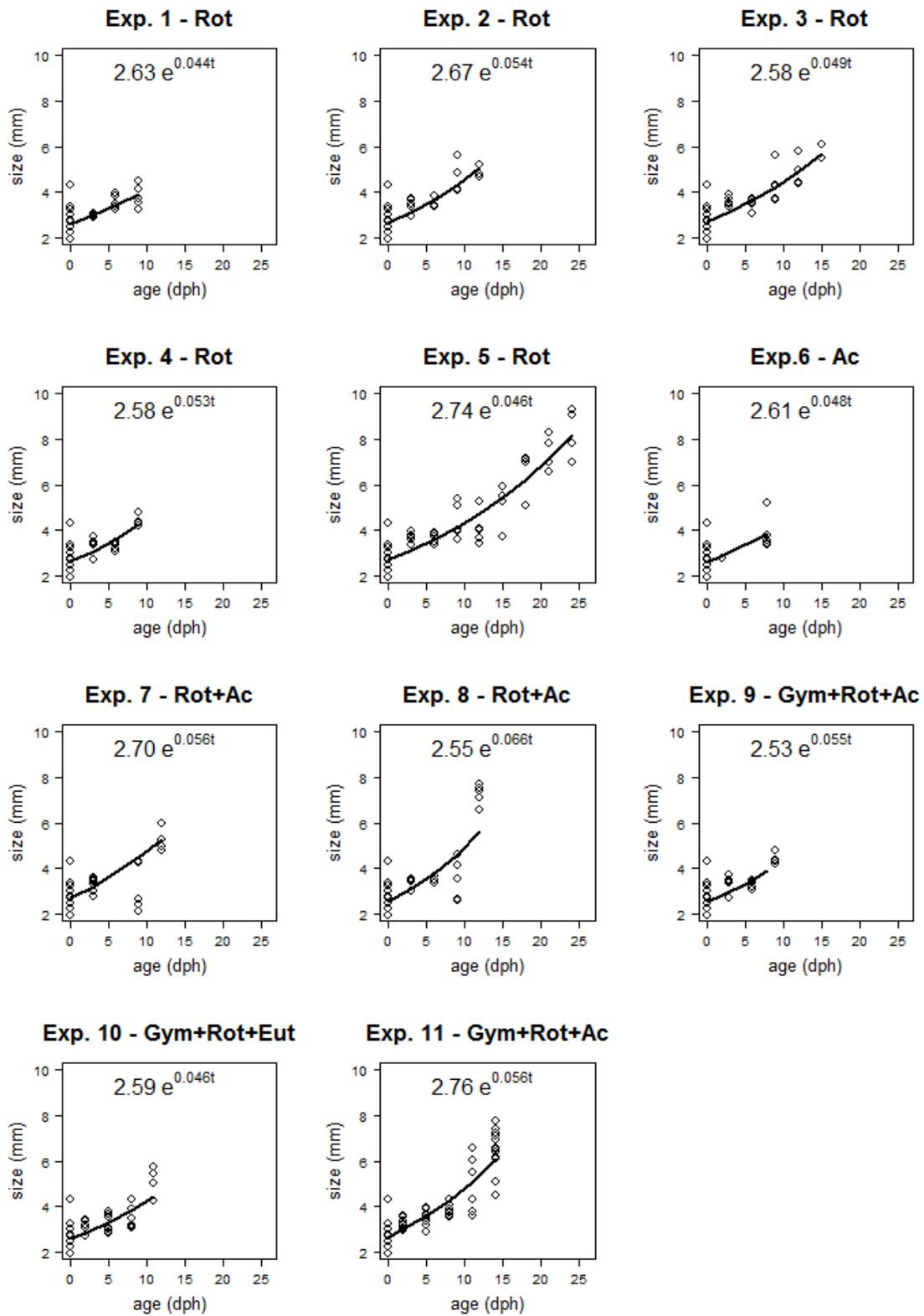
726

727

728

729

FIGURE 1



731

732

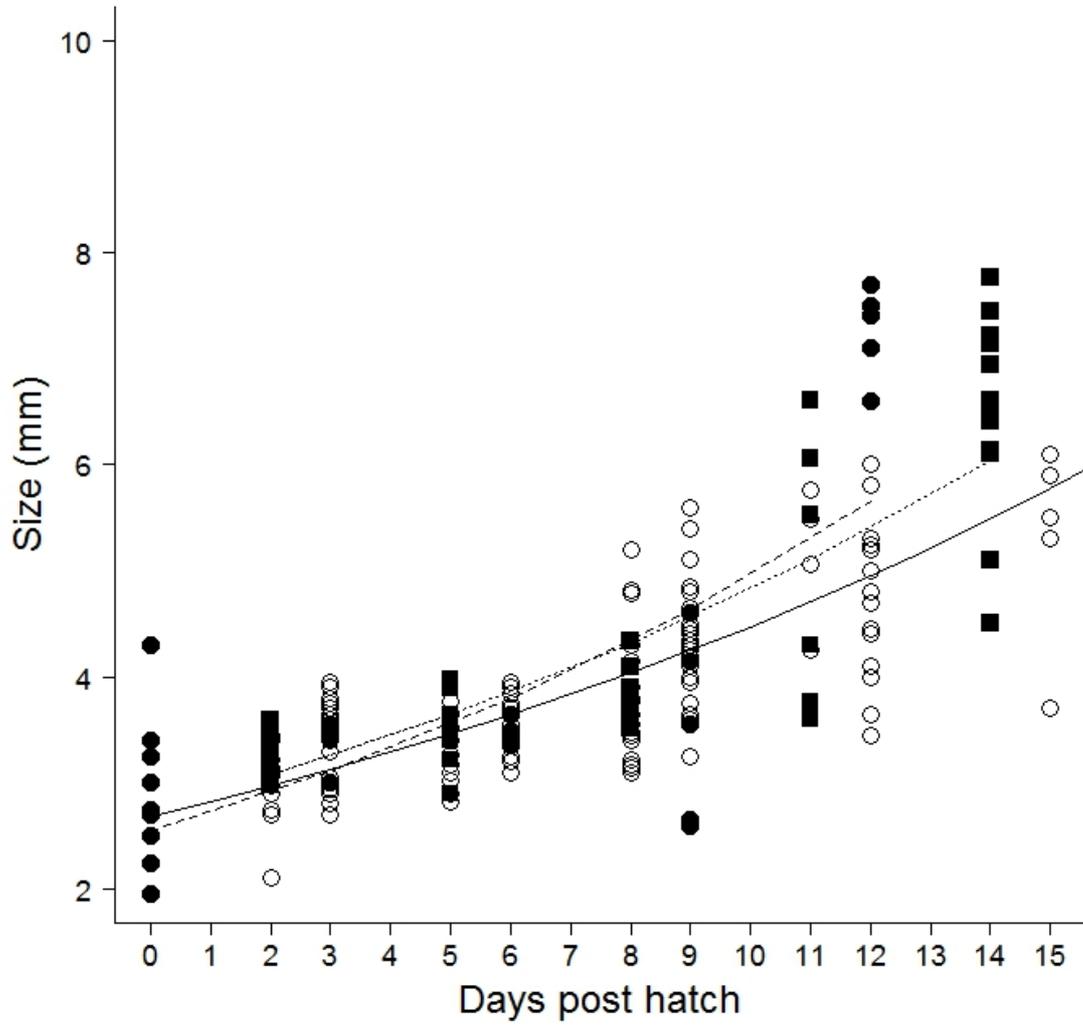
733

734

FIGURE 2

735

736



737

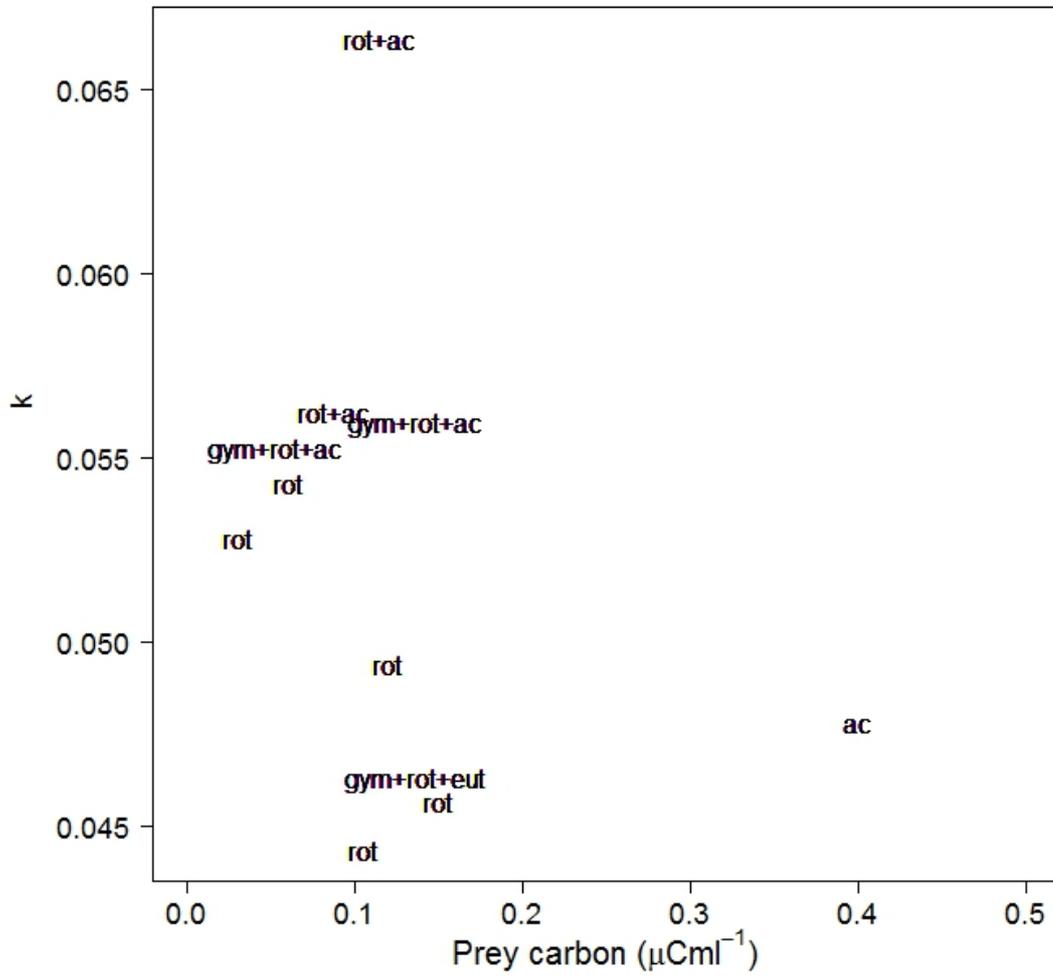
738

739

740

FIGURE 3

741



742

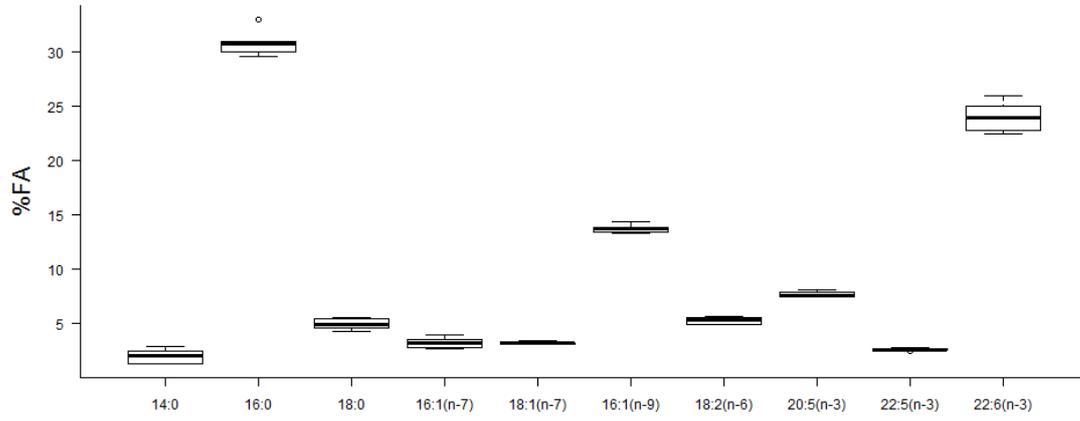
743

744

745

FIGURE 4

746



747

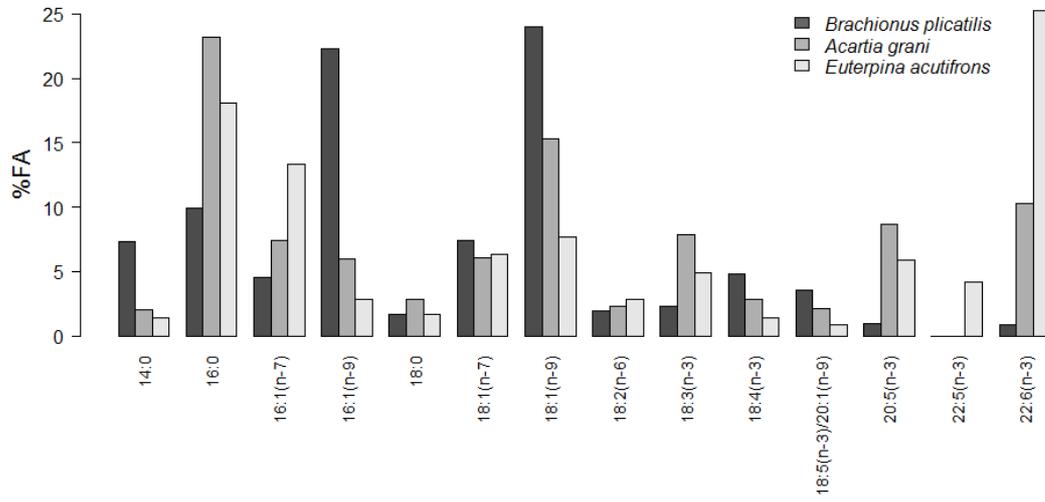
748

749

750

FIGURE 5

751

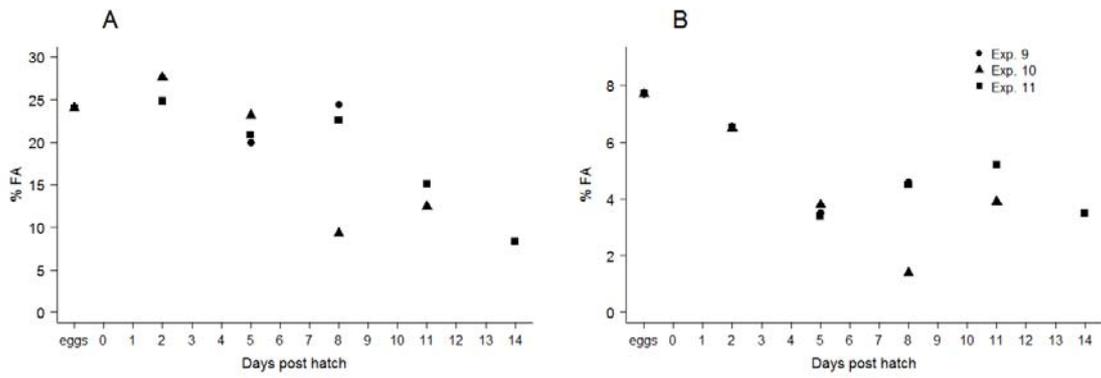


752

753

754

FIGURE 6

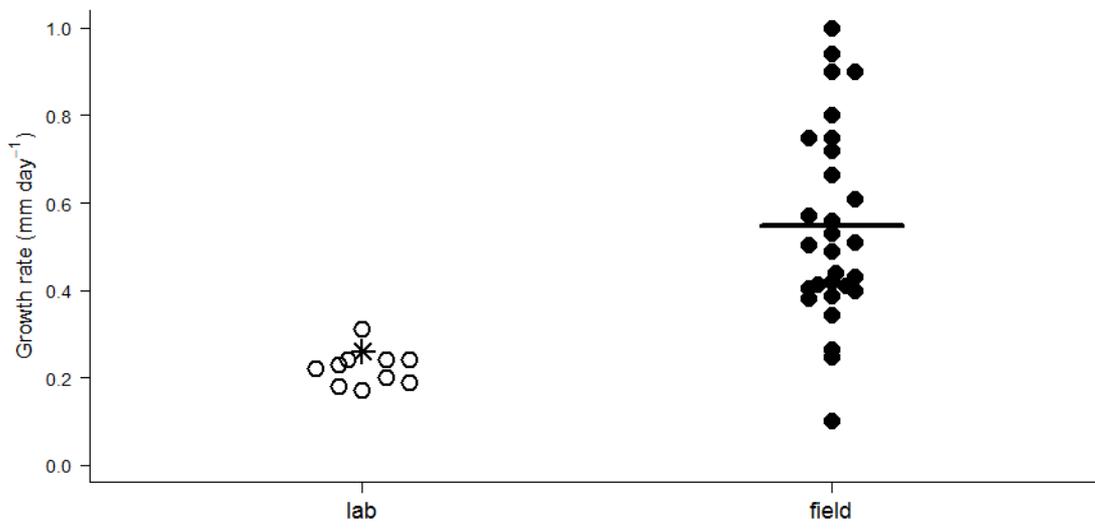


755

756

757

FIGURE 7



758

759

760

761