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## Will global warming affect the functional need for essential fatty acids in juvenile sea bass (*Dicentrarchus labrax*)? A first overview of the consequences of lower availability of nutritional fatty acids on growth performance

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### Abstract :

Global climate changes have led to a depletion in omega-3 polyunsaturated fatty acids (n-3 PUFA) in marine phytoplankton that—with food web transfers—could negatively impact fish performance. The aim of this study was to assess the effect of a reduction in the dietary availability of n-3 PUFA on growth performance, organ allometry, and fatty acid composition in juvenile European sea bass (*Dicentrarchus labrax*) raised at two different temperatures: 15 °C (natural conditions) and 20 °C (global warming scenario). Fish were fed for 5 months with two isoenergetic and isoproteic diets: a reference diet (RD; 1.65% n-3 PUFA on a dry matter basis, DM) used as a proxy of trophic networks where n-3 PUFA were plentiful, and a lower n-3 PUFA diet (LD; 0.73% n-3 PUFA on DM) designed to mimic the expected decrease in n-3 PUFA sources resulting from global climate changes. Results showed decreasing growth rates and slight changes in the muscle polar lipid profile in LD-fed sea bass juveniles, whereas neutral lipids were more affected over the long term. The relative masses of the heart and gastrointestinal system were higher at 20 °C, while liver mass was higher at 15 °C in LD-fed juveniles. However, the mesenteric fat of RD-fed juveniles was higher at 15 °C. Altogether the results suggest that sea bass juveniles are able to implement physiological mechanisms to cope with a decrease in dietary n-3 PUFA and are able to improve growth at the higher temperature, even with a decreased availability of n-3 PUFA. The temperature-driven increase in growth is also observed under the restricted n-3 PUFA diet, and this is accompanied by significant effects on organ allometry and FA profiles. This may indicate the presence of some metabolic costs that remain to be evaluated, but which illustrate that the combination of warming temperatures and n-3 PUFA depletion has significant effects on life history traits.

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## 32 **Introduction**

33 Oceans, which cover 71% of Earth's surface, play a major role in regulating the global  
34 climate (Reid et al. 2009). Over the past 30 years, rising atmospheric greenhouse gas  
35 concentrations have increased global average temperatures by  $\sim 0.2^{\circ}\text{C}$  per decade (Hansen et  
36 al. 2006), with most of this added energy being absorbed by the oceans. The resulting global  
37 climate changes have already had a large impact on ecosystems, and especially on marine  
38 ecosystems (Harley et al. 2006; Lehodey et al. 2006; Brander 2007; Rijnsdorp et al. 2009;  
39 Gattuso et al. 2015) through increasing temperatures (Genner et al. 2010; Johansen et al.  
40 2014), acidification (Wittman and Pörtner 2013; Gaylord et al. 2015), and eutrophication  
41 (Rijnsdorp and Van Leeuwen 1996; Wasmund et al. 1998).

42 Phytoplankton form the base of most marine food webs. They are a major source of  
43 lipids, including fatty acids (FA) that represent an important source of energy for higher  
44 trophic levels (e.g. Sargent et al. 2002). FA are crucial constituents of biological membranes  
45 (e.g. Sargent et al. 2002). The lipid composition of cell membranes is critical for the structure  
46 and function of cells and tissues, and thus has important effects at different  
47 biological/ecological levels (Arts et al. 2009; Parrish 2013). It has been shown that water  
48 temperature affects FA composition in phytoplankton species and that the omega-3  
49 polyunsaturated fatty acid (n-3 PUFA) content generally decreases with warming (Ackman  
50 and Tocher 1968; Thompson et al. 1992; Renaud et al. 2002; Guschina and Harwood 2006).  
51 This conclusion has been confirmed for six major groups of marine and freshwater  
52 phytoplankton (Hixson and Arts 2016): temperature is positively correlated with the relative  
53 contents of omega-6 FA (n-6; notably arachidonic acid, 20:4n-6, ARA) and saturated fatty

54 acids (SFA), but inversely correlated with the relative contents of eicosapentaenoic acid  
55 (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). The same trends were observed  
56 by Colombo et al. (2016) in a more comprehensive study, where more than 3000 FA profiles  
57 from marine and terrestrial organisms were analyzed. The relationship between water  
58 temperature and cell membrane FA content is generally explained through the concept of  
59 homeoviscous adaptation: ectotherms are said to adjust membrane lipid and FA composition  
60 to preserve its viscosity, fluidity, and function when faced with changes in ambient  
61 temperature.

62 DHA, EPA, and ARA are identified as essential fatty acids (EFA) in species that are  
63 not able to synthesize them and depend on food intake to fulfill their needs (Sargent 1976).  
64 Interestingly, phytoplankton produce most of the DHA and EPA present in the biosphere  
65 since land plants are unable to synthesize these molecules (Arts et al. 2009). These two n-3  
66 PUFA are of particular interest because they are biochemically important but scarce in nature.  
67 They are largely produced by diatoms, cryptophytes, and dinoflagellates (Brett and Muller-  
68 Navarra 1997) and are consumed and selectively retained while moving to higher trophic  
69 levels (Kainz et al. 2004; Hixson and Arts 2016). Piscivorous marine fish consume prey likely  
70 to be situated at lower trophic levels and, therefore, potentially richer in EPA and DHA; this  
71 may explain why they have lost the capacity for *de novo* synthesis of n-3 PUFA (Tocher et al.  
72 2006). In most other fish species, pathways of *de novo* EFA synthesis are present but their  
73 efficiency is reduced, making these species also highly dependent upon dietary sources of n-3  
74 PUFA (Ghioni et al. 1999; Tocher and Ghioni 1999). Despite their scarcity in marine food  
75 webs, n-3 PUFA are key compounds involved in fish growth, reproduction, behaviour, vision,  
76 osmoregulation, cell membrane structure (thermal adaptation), and immune function (Higgs  
77 and Dong 2000; for reviews see Sargent et al. 2002; Glencross 2009; Tocher 2010; Kiron et  
78 al. 2011; Tian et al. 2014).

79           The European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) is commercially  
80 important along European coasts. It is typically a marine species that spends most of its life in  
81 coastal and estuarine areas, although it is occasionally observed in rivers, particularly at early  
82 life stages. Until now, the consequences of nutritional n-3 PUFA depletion have been studied  
83 under aquaculture conditions and rarely in the field, with an ecological perspective. For  
84 example, the substitution of dietary fish oil with vegetable oils (which lack n-3 PUFA) has  
85 been extensively investigated in farmed fish. Most studies have concentrated on salmonids  
86 (McKenzie et al. 1998; Tocher et al. 2000; Torstensen 2000; Caballero et al. 2002),  
87 freshwater fishes (Martino et al. 2002; Ng et al. 2003), and some marine fish species such as  
88 European sea bass (Yildiz and Sener 1997; Montero et al. 2005; Chatelier et al. 2006), turbot  
89 (*Psetta maxima*) (Regost et al. 2003), gilthead sea bream (*Sparus aurata*) (Kalogeropoulos et  
90 al. 1992; Caballero et al. 2003; Montero et al. 2003), and red sea bream (*Pagrus auratus*)  
91 (Glencross et al. 2003). In European sea bass juveniles, Skalli and Robin (2004) showed that  
92 low dietary n-3 PUFA (0.2% of the diet on a dry matter [DM] basis) significantly lowered  
93 growth compared to diets with at least 0.7% n-3 PUFA. Moreover, the level of dietary n-3  
94 PUFA modified FA composition in muscle neutral lipids, while muscle polar lipid  
95 composition was less affected. Skalli et al. (2006) tested a crossed factorial design combining  
96 two diets (0.4 and 2.2% DM n-3 PUFA) and two temperatures (22°C and 29°C). One of the  
97 main outcomes of this study was that 0.7% DM was found to be the minimal n-3 PUFA level  
98 necessary to sustain juvenile sea bass growth.

99           Studies that combine the effects of n-3 PUFA and temperature on fish growth and  
100 physiology are scarce. The aim of this study, therefore, was to test the effect of a reduction in  
101 n-3 PUFA dietary content on growth performance, organ allometry, and the FA profile in  
102 juvenile European sea bass raised at two different temperatures, 15°C and 20°C. Two  
103 experimental diets were tested: a reference diet (RD) that mimicked a trophic network where

104 n-3 PUFA are plentiful, and a lower n-3 PUFA diet (LD) that simulated the expected decrease  
105 in phytoplankton sources resulting from ocean warming (Colombo et al. 2016; Hixson and  
106 Arts 2016). We hypothesized that (1) juveniles fed the depleted diet will show higher EFA  
107 tissue retention than those fed RD, and (2) that fish raised at the higher temperature will retain  
108 a lower amount of EFA in tissues in accordance with the homeoviscous adaptation concept  
109 and also because of higher energetic needs.

## 110 **Materials and methods**

### 111 **Fish origin and maintenance**

112         Adult European sea bass were captured in winter 2013 by fishermen in the Gulf of  
113 Morbihan (Plomeur, France) and brought to the Aquastream hatchery (Lorient, France). After  
114 three years in captivity, four females and 10 males were bred in the facility. At day 2 post  
115 hatching (d-2), sea bass larvae were transferred to the Ifremer rearing facility in Brest  
116 (France), where experiments were conducted. Larvae were divided among three conical tanks  
117 (230 L, 10 µm filtered seawater, UV, salinity 35‰, initial density 10000 larvae tank<sup>-1</sup>). Water  
118 temperature in the tanks was progressively increased from 14°C to 20°C within six days.  
119 Larvae were fed with *Artemia* from mouth opening (d-8) to d-39. To condition the larvae to  
120 more readily accept the manufactured diet at the end of the live-feed period, they were co-fed  
121 with both *Artemia* and a microparticulate diet (Marinstart, Le Gouessant, Lamballe, France)  
122 for four days starting at d-40. Larvae were then fed exclusively with the microparticulate diet  
123 until d-74. After d-74, juveniles were fed with larger pellets for ornamental fish (EPA + DHA  
124 = 1.5%; Le Gouessant, Lamballe, France) until the beginning of the experiment at d-93.

### 125 **Environmental and nutritional conditioning**

126         At d-93, juveniles (mass = 0.75 ± 0.02 g; standard length = 3.57 ± 0.02 cm; mean ±  
127 SD) were divided among 12 indoor 500 L tanks supplied with filtered and aerated natural  
128 seawater, six of which were maintained at 15°C and the other six at 20°C. Each tank



129 contained 300 fish, representing a mean biomass of  $263.93 \pm 0.28$  g. During the following 150  
130 days, fish were fed one of two experimental diets: a reference n-3 PUFA diet (RD;  
131 EPA+DHA = 1.65% DM) and a low n-3 PUFA diet (LD; EPA+ DHA = 0.73% DM). Feeding  
132 took place for 7 h during daytime (08:00 to 15:00) using an automatic distributor ( $2 \text{ cm h}^{-1}$ ).  
133 Each diet  $\times$  temperature combination was replicated in three tanks.

## 134 Experimental diets

135 The two diets tested were identical except for the FA source. LD contained only colza  
136 oil as a source of FA (essentially oleic acid [18:1n-9], linoleic acid [18:2n-6], and linolenic  
137 acid [18:3n-3]), while RD contained 50% colza oil and 50% fish oil, the latter being richer in  
138 EPA and DHA (20:5n-3, 22:6n-3). Diets were isoenergetic and contained the same  
139 percentages of proteins and lipids (Table 1).

## 140 Experimental time line

### 141 Biometry

142 For the growth survey, seven samplings were done at intervals of 200 degree-days (dd,  
143 day  $\times$  exposed temperature). Fish were not fed 24 h prior to sampling. A total of 30 fish,  
144 randomly sampled in each tank ( $n = 90$  per diet–temperature treatment), were lightly  
145 anaesthetized with tricaine methanesulfonate (MS-222; dose adapted to water temperature and  
146 fish body mass [BM]), weighed, and their standard length (SL) measured. After recovery,  
147 individuals were returned to their original tanks.

148 Mass gain ( $\Delta\text{mass}$ ), specific growth rate (SGR), and thermal growth coefficient (TGC, which  
149 is a thermal unit approach; for reviews see Dumas et al. 2010) were calculated as follows:

$$150 \Delta\text{mass (g)} = \text{BM}_{\text{final}} - \text{BM}_{\text{initial}}$$

$$151 \text{SGR (\%BM d}^{-1}\text{)} = 100 (\ln[\text{BM}_{\text{final}}] - \ln[\text{BM}_{\text{initial}}]) \text{ days of feeding}^{-1}$$

$$152 \text{TGC (g degree-days}^{-1}\text{)} = 1000 ([\text{BM}_{\text{final}}^{0.33}] - [\text{BM}_{\text{initial}}^{0.33}]) \Sigma(\text{degree-days})$$

### 153 Samplings

154 At 720 dd (d-129 and d-141 at 20°C and 15°C, respectively) and 1660 dd (d-176 and  
155 d-204 at 20°C and 15°C, respectively), eight fish per tank were randomly sampled, euthanized  
156 with an overdose of MS-222 (1 mL L<sup>-1</sup>), and a piece of white epaxial muscle located under  
157 the first dorsal fin was dissected out. Muscle samples obtained from the same tank were  
158 pooled and stored at -80°C until analyses (n = 3 per experimental treatment).

159 At the end of the experiment (d-243), 10 fish per tank (30 per diet-temperature combination)  
160 were euthanized as described above. After fish were weighed and measured, heart, liver,  
161 gastrointestinal system, and mesenteric fat were sampled and weighed for the organ allometry  
162 study.

## 163 Fatty acids

### 164 Lipid extraction

165 White muscle samples were ground in liquid nitrogen using a mixer (MM 400,  
166 Retsch). Lipids were then extracted following a procedure derived from that of Folch et al.  
167 (1957). Muscle powder was homogenized in Folch solution and stored at -20°C until polar  
168 and neutral lipids were separated.

### 169 Separation of polar and neutral lipids

170 Samples were sonicated for 5 min at 4°C then centrifuged for 2 min (4°C, 1482 g).  
171 Lipids were fractionated into neutral lipids (including triglycerides, free FA, and sterols) and  
172 polar lipids (including phospholipids and glycolipids). An aliquot of total lipid extract (1 mL)  
173 was evaporated to dryness under nitrogen, recovered with three (0.5 mL) washings of  
174 chloroform:methanol (98:2, v:v), and deposited at the top of a silica gel micro-column  
175 (40 mm × 5 mm i.d. Pasteur pipette plugged with glass wool and filled with silica gel 60 that  
176 had been heated for 6 h at 450°C and deactivated with 6% water by weight). Neutral lipids  
177 were eluted with 10 mL of chloroform:methanol (98:2, v:v), and polar lipids were eluted with  
178 15 mL of methanol. Tricosanoic acid (23:0, 200 µg) was added as an internal standard.

## 179 Transesterification

180 Each lipid fraction was vacuum dried and directly transesterified under nitrogen using  
181 0.5 mL of 2M methanolic potash (KOH-MeOH) for 3 min at 90°C. A total of 0.5 mL of 6N  
182 hydrochloric acid was added and vortex mixed. Before gas chromatography analysis, 2 mL of  
183 hexane was added and centrifuged for 10 min at 630 g to collect the organic phase containing  
184 fatty acid methyl esters (FAMES). FAMES were washed three times with 1 mL of hexane. The  
185 organic phase was finally transferred to tapered vials and stored at -20°C.

## 186 Gas chromatography analysis

187 FAMES were analyzed in a CLARUS 500 gas chromatograph (Perkin-Elmer)  
188 equipped with a split/splitless injector and a flame ionization detector. FAMES were identified  
189 using two different capillary columns (BPX70 30 m × 0.25 mm i.d., 0.25 µm thickness; SGE  
190 Analytical Science) using a standard 37 component FAME mix (Sigma) and other known  
191 standard mixtures (i.e. 18919-1AMP FAME Mix, C4-C24 and 18918-1AMP FAME Mix, C8-  
192 C24; Sigma). The FA were expressed as the molar percentage of the total FA content.

## 193 Statistical and data analysis

194 Data normality and homoscedasticity were tested using Shapiro-Wilk and Levene  
195 tests, respectively. Growth rate in BM and SL was regressed against time. The effects of diet  
196 and temperature were tested by comparing regression slopes; when slopes were  
197 homogeneous, an ANCOVA was run to compare intercepts. Two-way ANOVAs were used to  
198 test for significant differences among diets and temperatures for  $\Delta$ mass, SGR, and TGC.  
199 Scatter plots between organ mass and BM for each temperature did not overlap due to a large  
200 difference in BM because of the temperature differences. For this reason, the effect of diet on  
201 organ allometry was tested separately for each temperature by comparing slopes and using  
202 ANCOVA if required. In order to meet normality, BM, SL, and organ mass were log<sub>10</sub>  
203 transformed. Because the response at 1660 dd could be considered as dependent on the

204 response at 720 dd, two-way ANOVA were performed for each sampling period to assess the  
205 effects of diet and temperature on muscle FA content and the muscle/dietary FA ratio. When  
206 required, pairwise comparisons (Tukey tests) were performed (the homoscedasticity condition  
207 was respected). Differences were considered significant at  $\alpha = 0.05$ . Statistical analyses were  
208 conducted in R (ver.3.3.3; R Development Core Team).

## 209 **Results**

210 Only a few mortalities occurred during the experiment (less than 0.2%), therefore this  
211 effect was not considered.

### 212 **Growth performance and organ allometry**

213 BM and SL differed according to time and temperature (higher slopes at 20°C than at  
214 15°C, Fig. 1; BM:  $F(1,76) = 222.23$ ,  $P < 0.001$ ; SL:  $F(1,76) = 211.46$ ,  $P < 0.001$ ;  
215 Supplementary Table 1). Diet composition also had a significant effect on growth, which was  
216 similar at both temperatures: growth was faster in RD-fed fish than in LD-fed fish (BM:  
217  $F(1,76) = 19.40$ ,  $P < 0.001$ ; SL  $F(1,76) = 27.48$ ,  $P < 0.001$ ). In addition, the  $\Delta_{\text{mass}}$  was about  
218 20% higher in the juveniles fed RD than in those fed LD independent of temperature, while  
219  $\Delta_{\text{mass}}$  was about twice as high at 20°C than at 15°C (Table 2). The same pattern was  
220 observed for TGC, whereas only temperature affected SGR, which was 35% higher at 20°C  
221 (Table 2). Linear regressions showed that fish fed RD at 20°C were 14.5% heavier (Fig. 1A)  
222 and 34.1% longer (Fig. 1B) at the end of the experiment compared to fish fed LD. The  
223 difference was less pronounced at 15°C, with RD-fed fish being 12.8% heavier (Fig. 1A) and  
224 5.7% longer (Fig. 1B) than LD-fed fish.

225 Differences in the relationship between organ masses and BM were examined at each  
226 rearing temperature. At 20°C, RD-fed fish had lower heart (Fig. 2A; ANCOVA:  $F(1,54) =$   
227  $6.82$ ,  $P < 0.05$ ) and gastrointestinal (Fig. 2B; ANCOVA:  $F(1,54) = 4.52$ ,  $P < 0.05$ ) masses,  
228 but no differences for liver or mesenteric fat were observed. At 15°C, liver mass was lower in

229 RD-fed juveniles (Fig. 2C; ANCOVA:  $F(1,54) = 8.73, P < 0.01$ ), while the quantity of  
230 mesenteric fat relative to BM increased more in juveniles fed RD (Fig. 2D; slopes  
231 significantly different,  $\log_{10} [\text{Body Mass}] \times \text{diet}: F(1,54) = 9.90, P < 0.01$ ). There were no  
232 differences for heart or gastrointestinal mass. Allometric regression parameters are available  
233 in Supplementary Table 2.

## 234 Fatty acids

235 At 720 dd, significant effects of both temperature and diet were observed in the white  
236 muscle FA composition. Both temperature and diet affected polar  $\Sigma\text{SFA}$  levels and  
237 proportions, which were significantly higher at 20°C than at 15°C and with RD compared to  
238 LD (Table 3A). Temperature (20°C > 15°C) but not diet significantly affected white muscle  
239 polar  $\Sigma\text{MUFA}$  (monounsaturated FA) and  $\Sigma\text{PUFA}$ . The  $\Sigma\text{PUFA}$  to  $\Sigma\text{SFA}$  ratio was  
240 significantly higher in fish fed LD and raised at 15°C. Polar  $\Sigma n-3$  and  $\Sigma n-6$  both significantly  
241 increased with temperature. However, independently of the temperature conditions,  $\Sigma n-3$  was  
242 higher in juveniles fed RD while  $\Sigma n-6$  dominated in juveniles fed LD in the polar fraction  
243 (Table 3A). Consequently, polar  $\Sigma n-3/\Sigma n-6$  ratios were higher in RD-fed juveniles. In LD-fed  
244 juveniles, the ratio was higher at 15°C than in those reared at 20°C (Table 3A). At both  
245 temperatures, the percent content of 18:2n-6 was significantly higher in juveniles fed LD, but  
246 the difference between the two diets was larger at 20°C than at 15°C. On the contrary, the  
247 18:3n-3 content was higher in juveniles fed RD, although juveniles contained overall more  
248 18:3n-3 at 20°C than at 15°C. The ARA and DHA levels were significantly higher in  
249 juveniles reared at 20°C, while there was no effect of temperature on DHA present in the  
250 polar fraction. A significant diet effect was only present for ARA and EPA, with higher levels  
251 in juveniles fed RD than in those fed LD. The lowest DHA/EPA ratios were observed in fish  
252 fed RD at both temperatures. Juveniles reared at 20°C and fed LD had a higher DHA/EPA  
253 ratio than those raised at 15°C (Table 3A). Changes in the neutral fraction were clearly less

254 pronounced at 720 dd. No effect of either temperature or diet was observed for  $\Sigma n-3$ ,  $\Sigma n-6$ ,  
255  $\Sigma n-3/\Sigma n-6$ ,  $\Sigma SFA$ ,  $\Sigma MUFA$ ,  $\Sigma PUFA$ , or the percent content of 18:2n-6 and 18:3n-3. The ARA  
256 and EPA contents were significantly lower in juveniles fed LD, while the DHA/EPA ratio was  
257 significantly lower in juveniles fed RD. The ratio was also generally lower at 15°C than at  
258 20°C.

259 At 1660 dd, polar  $\Sigma SFA$ ,  $\Sigma MUFA$ ,  $\Sigma PUFA$ ,  $\Sigma n-3$ , and  $\Sigma n-6$  percentages were similar  
260 regardless of the rearing conditions (Table 3B). However, the  $\Sigma n-3/\Sigma n-6$  ratio was 8.4% lower  
261 when temperature increased and 51.2% lower in juveniles fed LD compared to those fed RD  
262 (Table 3B). As observed at 720 dd, the 18:2n-6 content was the highest in juveniles fed LD,  
263 but only at 20°C. A similar response was observed for the 18:3n-3 content. The EPA content  
264 was significantly higher in juveniles fed RD, whereas DHA and the DHA/EPA ratio were  
265 similar among treatments. However, the ARA content was lowest in juveniles fed LD at both  
266 temperatures, while those fed RD at 20°C had the highest proportion (Table 3B). In contrast  
267 to what was observed at 720 dd, more pronounced effects of diet and temperature were  
268 observed in the neutral fraction at 1600 dd. Indeed, the  $\Sigma SFA$  content was 26.2% higher at  
269 20°C than at 15°C, while the  $\Sigma MUFA$  was 34.1% higher in juveniles fed LD than in those fed  
270 RD (Table 3B). The  $\Sigma PUFA$  to  $\Sigma SFA$  ratio was significantly higher in fish fed LD and raised  
271 at 15°C, while the  $\Sigma PUFA$  to  $\Sigma MUFA$  ratio was higher at 20°C. The  $\Sigma n-6$  was significantly  
272 higher in juveniles fed LD and, consequently, the  $\Sigma n-3/\Sigma n-6$  ratio was significantly higher in  
273 juveniles fed RD compared to those fed LD (Table 3A, B). Both 18:2n-6 and 18:3n-3 contents  
274 were lower in juveniles fed RD. Interestingly, the ARA content was extremely low in  
275 juveniles fed LD at both 15°C and 20°C. EPA was significantly higher in juveniles fed RD at  
276 20°C compared to the three other treatments, while DHA was overall significantly lower at  
277 15°C and in juveniles fed LD. At 20°C, RD-fed fish had the lowest DHA/EPA ratios, while  
278 LD-fed fish had the highest.

279           The muscle/dietary FA ratios suggest that the retention of EFA was more pronounced  
280 in the polar fraction than in the neutral fraction (Fig. 3A, B). A stronger selective retention of  
281 polar ARA, DHA, and EPA was observed in juveniles fed LD compared to those fed RD.  
282 Retention of ARA, DHA, and EPA was significantly higher at 20°C than at 15°C, but only at  
283 720 dd (Fig. 3A). At 1660 dd, the temperature effect was no longer evident. It should be noted  
284 that even though the muscle/dietary 18:2n-6 ratio was higher than one in the neutral fraction,  
285 it decreased below one in polar lipids (Fig. 3B). No effect of diet, except for DHA at 1660 dd,  
286 and no effect of diet, except for EPA at 1600 dd, was observed in neutral fraction (Fig. 3B).

## 287 **Discussion**

288           Diet and temperature both induced different growth trajectories. After 720 dd, diet  
289 clearly modified the FA composition of muscle; this effect was modulated by temperature for  
290 ARA, linolenic acid, and DHA, whereas the FA profiles were more alike at 1660 dd. Diet also  
291 influenced organ allometry at both 20°C and 15°C, although with some variations.

### 292 **Growth performance**

293           As expected, increased water temperature significantly improved the growth of  
294 juvenile seabass fed both diets, with the differences in body length and mass increasing with  
295 time. Both  $\Delta$ mass and TGC were higher at 20°C than at 15°C, and higher in RD-fed than in  
296 LD-fed fish, which is consistent with the growth trajectories obtained by a linear model.  
297 Several indices have been developed to determine the daily growth increment in fish. The  
298 most commonly used is SGR (Ricker 1979). SGR may be affected by both the fish's body  
299 size (Jobling 1983; Iwama et al. 1997) and environmental temperature (Tidwell et al. 1999;  
300 Person-Le Ruyet et al. 2004); to avoid such bias, the thermal unit growth coefficient was  
301 proposed by Iwama and Tautz (1981). Even though this estimate is thought to be less  
302 sensitive to body size and temperature (Azevedo et al. 1998; Cho and Bureau 1998; Kaushik

303 1998), TGC and SGR of sea bass were affected by temperature in a similar way in our study.  
304 Such a result was also reported for the Eurasian perch (*Perca fluviatilis*; Strand et al. 2011).  
305 Skalli and Robin (2004) defined the minimal n-3 PUFA levels necessary to sustain juvenile  
306 sea bass growth at 0.7% DM, and they observed no improvement with higher n-3 PUFA  
307 content. However, we observed better growth with 1.65% DM (RD), indicating that n-3  
308 PUFA in excess of 0.7% DM may increase growth further under certain circumstances. This  
309 different requirement may also result from the fact that our fish were younger than those used  
310 by Skalli and Robin (2004). RD allowed better growth than LD, and this diet-related effect  
311 was observed at both temperatures. Consequently, the low dietary levels of n-3 PUFA did not  
312 impair the temperature-related growth-promoting effect.

### 313 Organ allometry

314 From the perspective of evolutionary biology, the functional capacity of an organ  
315 should match the demands imposed upon it (Starck 1999). Therefore, individuals ought to  
316 respond to changes in actual demands by adjusting their functional capacities (Diamond and  
317 Hammond 1992; Elia 1992; Diamond 1998; Weibel 1998), including organ size. In  
318 ectotherms, temperature has a direct effect on metabolism. In the sea bass, Claireaux and  
319 Lagardère (1999) showed that when temperature was increased from 15°C to 20°C, standard  
320 metabolism and active metabolism increased by 37% and 125%, respectively. This implies  
321 that more oxygen and nutrients are needed to cover the energetic demand. In sea bass, energy  
322 costs related to digestion mobilize a great proportion of the cardiac output (Farrell et al. 2001,  
323 Axelsson et al. 2002; Altimiras et al. 2008). In our study, the LD-fed fish reared at 20°C had  
324 higher heart and gastrointestinal masses. The change in fatty acids may have induced a  
325 thickening or an elongation of the gastrointestinal tract. Our hypothesis, is that this increase in  
326 heart mass relative to BM may result from a greater energetic demand due to the simultaneous  
327 increase in gastrointestinal mass. It has also been established in vitro that tissues and organs



328 have mass-specific metabolism (Krebs 1950; Schmidt-Nielsen 1984). An evaluation of the  
329 heart's working capacity—and, for instance, of the stroke volume—would be necessary to  
330 know if the increased heart mass also implied better performance.

331         Why did the gastrointestinal mass increase more in juveniles fed LD? One explanation  
332 could be that offering a diet that minimally meets nutritional needs of juveniles may require  
333 an optimization of the nutrient assimilation processes, thus increasing the overall energy  
334 demand. Indeed, previous studies have shown that size and activity of the gastrointestinal tract  
335 are phenotypically plastic and respond strongly to consumption and food availability (Starck  
336 1999; Armstrong and Bond 2013). Here, we showed that the type of FA provided in the diet  
337 affects the gastrointestinal mass. It seems reasonable to speculate that the higher values of  
338 gastrointestinal mass could be explained either by an elongation of the gut or by modifications  
339 of the brush border epithelium of the small intestine induced by the necessity to improve FA  
340 assimilation. In addition, Torrecillas et al. (2017) showed that fish oil replacement by  
341 vegetable oil increases the lipid deposition in anterior gut *lamina propria* in sea bass, which  
342 could also explain the largest viscera mass obtained with LD.

343         Knowing that the optimum temperature for European sea bass growth was reported to  
344 be about 22°C to 25°C (Barnabé, 1991), colder temperatures represent an additional constraint  
345 on juvenile metabolism. The liver has a major role in energy storage, and it is the first site for  
346 lipid storage in a number of benthic and demersal species (Drazen 2002; Hoffmayer et al.  
347 2006; Lloret et al. 2008). Another important storage site is the mesenteric fat that surrounds  
348 the gastrointestinal tract. It is much more labile than other fat stores, such as muscular fat, and  
349 therefore mesenteric fat is likely to be the first fat store to be mobilized. In our study, liver  
350 mass was higher in fish fed LD than RD at 15°C. This result corroborates the findings of  
351 Mourente and Bell (2006), who found that the liver mass of juvenile sea bass fed vegetable oil

352 was higher than that of fish fed fish oil. As n-3 PUFA were scarcer in LD, further experiments  
353 will be needed to assess if the higher liver mass could be explained by greater FA storage.

## 354 Fatty acids

355 Muscle/diet ratios greater than 1 mean that the muscle is richer in FA than the diet is,  
356 suggesting that retention occurred. After 720 dd, fish were already showing the effects of their  
357 diet, with muscle/dietary ratios  $> 1$ . At 720 dd, both temperature and diet had significant  
358 effects on most of the FA in polar lipids. At 1660 dd, however, the differences in the FA  
359 profiles among dietary treatments were smaller in the polar than in the neutral lipid fraction.  
360 This suggests that, over time, fish regulated their phospholipid composition, possibly  
361 membrane phospholipids, in order to maintain tissue functionality; this agrees with previous  
362 work (Sargent 1976; Skalli and Robin 2004). It should be noted that a high retention rate,  
363 approximately six times higher than the diet content, was observed for polar EFA (EPA,  
364 DHA, and ARA) in LD-fed fish, but this high retention rate did not compensate for the low  
365 EFA contents induced by this diet.

366 The main representatives of the n-3 and n-6 FA in the dietary lipids were linolenic  
367 (18:3n-3) and linoleic (18:2n-6) acids, respectively. In fish, these FA are accumulated without  
368 transformation due to the reduced capacity of these species for chain elongation and  
369 desaturation (Bell et al. 1986, 1994). However, n-3 intermediates in the desaturation  
370 elongation pathway such as 20:5n-3 (EPA), 22:5n-3, and 22:6n-3 (DHA) were found in both  
371 lipid fractions at higher values than those present in the diets. This may indicate a certain  
372 biochemical capacity to elongate or to selectively preserve specific EFA, even though the  
373 conversion rates are probably extremely low (Mourente and Dick 2002; Mourente et al.  
374 2005). It should be noted that polar EPA and DHA were high in juveniles fed RD; these two  
375 FA are eicosanoid precursors involved in several physiological functions such as stress  
376 response or osmoregulation (Sargent et al. 2002). One could then wonder whether low dietary

377 EPA and DHA levels could impair stress response capacity, and this would justify  
378 examination of fish response to specific challenge tests. As previously reported, a significant  
379 decrease in n-3 PUFA content in fish tissues was observed when fish oil was replaced by  
380 vegetable oil (Bell et al. 2001; Mourente et al. 2005; Torstensen et al. 2005; Pettersson et al.  
381 2009; Sanden et al. 2011). This was confirmed in the present study, where we observed the  
382 same overall tendencies.

383 Fish, as ectothermic species, do not control their body temperature. The relationship  
384 between water temperature and cell membrane FA content is generally explained through the  
385 concept of homeoviscous adaptation (Sinensky 1974). For instance, the proportion of  
386 unsaturated acyl chains in membrane lipids is generally increased under cold conditions to  
387 maintain membrane fluidity (Los and Murata 2004). Changes in the proportions of polar  
388  $\Sigma$ PUFA to  $\Sigma$ SFA and/or  $\Sigma$ MUFA were already present at 720 dd but were more pronounced  
389 at 1660 dd. At 720 dd,  $\Sigma$ SFA,  $\Sigma$ PUFA, and  $\Sigma$ MUFA polar contents were higher at 20°C than  
390 at 15°C. However, the  $\Sigma$ PUFA/ $\Sigma$ SFA ratio was similar at both 15°C and 20°C (respectively  
391 2.31 and 2.22), while a higher proportion of  $\Sigma$ PUFA to  $\Sigma$ MUFA was observed at 15°C than at  
392 20°C (respectively 2.10 and 1.83). Such a change in the  $\Sigma$ PUFA/ $\Sigma$ MUFA ratio would be  
393 consistent with adjustments related to homeoviscous adaptation. At 1660 dd, both polar  
394  $\Sigma$ PUFA/ $\Sigma$ SFA and  $\Sigma$ PUFA/ $\Sigma$ MUFA ratios were higher at 15°C than at 20°C. Ratios remained  
395 very stable in the neutral lipid fraction over time and between temperature conditions. The  
396  $\Sigma$ PUFA/ $\Sigma$ SFA ratio was about 1.34, while the  $\Sigma$ PUFA/ $\Sigma$ MUFA ratio was around 0.56. These  
397 results clearly indicate remodelling in polar lipids (mostly represented by membrane  
398 phospholipids) with temperature changes, while storage lipids remained stable regardless of  
399 temperature or time. In marine fishes, n-3 PUFA (EPA + DHA) tend to decrease with  
400 increasing temperature, while n-6 PUFA (ARA and linolenic acid) and SFA increase (Hixson  
401 and Arts 2016). In salmonids, a classical thermal response is a higher proportion of PUFA at

402 low temperatures (Hazel et al. 1992; Calabretti et al. 2003). Similar effects of temperature  
403 were observed in European sea bass, with lower SFA and conversely higher n-3 PUFA  
404 contents at 22°C than at 29°C (Skalli et al. 2006). In our study, temperature affected both  
405 relative FA contents and the muscle/dietary FA ratio of most polar FA at 720 dd, whereas  
406 temperature only affected polar  $\Sigma n-3/\Sigma n-6$  at 1660 dd. The fact that n-3 and n-6 PUFA  
407 showed an inverse relationship with regard to temperature is coherent from a biosynthesis  
408 perspective, since synthesis depends on the activities of the same enzymes (desaturases and  
409 elongases). Competition for enzymes, in the context of increasing ambient water temperature,  
410 tends to favour n-6 over n-3 production (Hixson and Arts 2016). This hypothesis remains to  
411 be tested.

## 412 **Conclusions**

413 Depletion of n-3 PUFA and a decreased temperature contributed to the decrease in sea  
414 bass growth rate while only slightly altering the muscle polar lipid profile. Neutral lipid  
415 profiles were more affected than polar ones. However, regarding the PUFA/SFA and  
416 PUFA/MUFA ratios, a higher proportion of PUFA at low temperature was present in polar  
417 lipids. This effect increased with time. These results are consistent with the homeoviscious  
418 adaptation theory. Higher contents of SFA and MUFA compared to PUFA were present in the  
419 storage lipids, but ratios remained stable regardless temperature and time. A depleted n-3  
420 PUFA diet induced low EFA contents in muscle even though a higher retention of EFA was  
421 noted in fish fed this diet. For the first time in fish, the allometry of several organs has been  
422 shown to respond to the type of dietary FA acid provided. This topic has been little  
423 investigated in fish even though it is easily achievable and inexpensive, and can reveal  
424 valuable information on key organs like the heart, liver, and gastrointestinal system. We also  
425 showed that the allometric organ response depends on temperature conditions. Dietary n-3  
426 PUFA affected organ allometry of the heart and gastrointestinal system at the higher

427 temperature (20°C), while liver and mesenteric fat were affected at the lower temperature  
428 (15°C). Juvenile sea bass are able to implement rapid phenotypic change in response to  
429 dietary FA. This is interesting from the point of view of global warming, where fish species  
430 are faced with rapid changes in mean temperature. More broadly, these results open up new  
431 perspectives in the study of seasonal adaptations. Altogether, these results suggest that  
432 juvenile sea bass are able to implement compensatory mechanisms to cope with a reduced  
433 availability of dietary n-3 PUFA. Because of this, the temperature-driven increase in growth is  
434 still observed under a restricted diet. However, the fact that this was accompanied by  
435 significant effects on organ allometry and FA profiles may indicate the presence of some  
436 metabolic cost, although this physiological adjustment remains to be evaluated. Further  
437 studies should be conducted to assess the impacts of these modifications of FA profiles on sea  
438 bass life history traits.

## 439 **Compliance with ethical standards**

### 440 **Conflict of interest**

441 The authors declare that they have no conflicts of interest.

### 442 **Ethical approval**

443 All applicable international, national, and/or institutional guidelines for the care and  
444 use of animals were followed. Experiments were performed under French national regulations  
445 and approved by the Comité d'Éthique Finistérien en Expérimentation Animale (CEFEA,  
446 registration code C2EA-74) (Authorization APAFIS 3056# 20151207173873100).

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691 fish oil on the growth of seabass (*Dicentrarchus labrax* L. 1758). *Cah Options Mediterr*  
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693 **Table index**

694 Table 1: Composition of experimental diets. For dry matter, proteins, total lipids,  
 695 triglycerides, and phospholipids, data are presented as % of dry mass. Data for specific fatty  
 696 acid (FA) categories are presented as % of total lipids. LD: low n-3 polyunsaturated fatty acid  
 697 (PUFA) diet, RD: reference n-3 PUFA diet; SFA: saturated FA; MUFA: monounsaturated  
 698 FA; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

	LD Mean	RD Mean
	% of dry mass	
Dry matter	94.84	95.12
Proteins	50.48	50.23
Total lipids	21.98	21.63
Triglycerides	16.99	17.05
Phospholipids	4.70	4.71
	% of total lipids in diet	
SFA	2.18	2.97
MUFA	7.32	6.48
n-3	1.93	2.68
n-6	2.65	2.28
n-9	6.56	5.46
EPA+DHA	0.73	1.65
18:1n-9	5.69	4.65
18:2n-6	2.50	2.16
18:3n-3	0.97	0.77
18:3n-6	0.00	0.01
18:4n-3	0.08	0.14
20:4n-6 (ARA)	0.03	0.07
20:5n-3 (EPA)	0.28	0.94
22:5n-3	0.03	0.07
22:6n-3 (DHA)	0.45	0.71

699



700 Table 2: Effect of temperature and diet on growth indices ( $\Delta$ mass: mass gain; TGC: thermal growth coefficient; SGR: specific growth rate). LD:  
 701 low n-3 polyunsaturated fatty acid (PUFA) diet, RD: reference n-3 PUFA diet; BM: body mass; d: day. Values are means  $\pm$  standard deviations

	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
$\Delta$ mass (g)	2.5 $\pm$ 0.51	1.9 $\pm$ 0.07	5.6 $\pm$ 0.11	4.7 $\pm$ 0.49	P < 0.001	P < 0.01	—
TGC (g degree-days <sup>-1</sup> )	0.36 $\pm$ 0.06	0.30 $\pm$ 0.01	0.61 $\pm$ 0.02	0.55 $\pm$ 0.04	P < 0.001	P < 0.01	—
SGR (%BM d <sup>-1</sup> )	1.3 $\pm$ 0.18	1.1 $\pm$ 0.02	1.9 $\pm$ 0.07	1.8 $\pm$ 0.10	P < 0.001	—	—

702

703 Table 3: Effect of temperature and diet on muscle fatty acid (FA) profiles. Values are given as  
704 % of dry matter (DM) in the neutral and polar lipid fractions at A) 720 degree-days and B)  
705 1660 degree-days. LD: low n-3 polyunsaturated FA (PUFA) diet, RD: reference n-3 PUFA  
706 diet. When factor interactions were significant, groups were compared with a posteriori tests  
707 ( $\alpha = 0.05$ ). For temperature  $\times$  diet interactions, significantly different groups were assigned  
708 different letters.  $\Sigma n-3$  includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n3;  
709  $\Sigma n-6$  includes 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6;  $\Sigma SFA$  (saturated FA) includes  
710 20:0s, 22:0s, 24:0s;  $\Sigma MUFA$  (monounsaturated FA) includes 14:1n-9, 18:1n-11, 18:1n-7,  
711 20:1n-7, 22:1n-9, 24:1n-9;  $\Sigma PUFA$  includes 18:3n-6, 18:4n-3, 20:2n-6, 20:3n-3, 20:3n-6,  
712 20:4n-3. Values are means  $\pm$  standard deviations

A		POLAR					
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	1.29 <sup>a</sup> ± 0.06	1.82 <sup>b</sup> ± 0.06	1.44 <sup>a</sup> ± 0.09	2.19 <sup>c</sup> ± 0.05	—	—	P < 0.05
ARA	0.29 ± 0.02	0.2 ± 0.01	0.34 ± 0.03	0.21 ± 0.01	P < 0.05 15°C < 20°C	P < 0.001 LD < RD	—
18:3n-3	0.33 ± 0.01	0.49 ± 0.02	0.38 ± 0.02	0.6 ± 0.02	P < 0.001 15°C < 20°C	P < 0.001 RD < LD	—
EPA	1.94 ± 0.12	1.41 ± 0.04	2.03 ± 0.1	1.3 ± 0.04	—	P < 0.001 LD < RD	—
DHA	3.13 ± 0.26	2.99 ± 0.19	3.55 ± 0.3	3.34 ± 0.11	P < 0.05 15°C < 20°C	—	—
DHA/EPA	1.61 <sup>a</sup> ± 0.05	2.12 <sup>b</sup> ± 0.11	1.75 <sup>a</sup> ± 0.09	2.57 <sup>c</sup> ± 0.1	—	—	P < 0.05
Σn-3	5.76 ± 0.41	5.22 ± 0.24	6.34 ± 0.44	5.56 ± 0.14	P < 0.05 15°C < 20°C	P < 0.01 LD < RD	—
Σn-6	1.68 ± 0.09	2.13 ± 0.06	1.89 ± 0.13	2.55 ± 0.06	P < 0.001 15°C < 20°C	P < 0.001 RD < LD	—
Σn-3/Σn-6	3.42 <sup>c</sup> ± 0.06	2.45 <sup>b</sup> ± 0.05	3.34 <sup>c</sup> ± 0	2.18 <sup>a</sup> ± 0.03	—	—	P < 0.01
ΣSFA	3.33 ± 0.16	3.07 ± 0.09	3.73 ± 0.27	3.62 ± 0.11	P < 0.001 15°C < 20°C	P < 0.001 LD < RD	—
ΣMUFA	3.33 ± 0.21	3.7 ± 0.02	4.12 ± 0.28	4.88 ± 0.05	P < 0.05 15°C < 20°C	—	—
ΣPUFA	7.44 ± 0.5	7.34 ± 0.29	8.23 ± 0.56	8.11 ± 0.2	P < 0.01 15°C < 20°C	—	—
ΣPUFA/ΣSFA	2.23 ± 0.12	2.39 ± 0.11	2.21 ± 0.01	2.24 ± 0.02	P < 0.001 20°C < 15°C	P < 0.001 RD < LD	—
ΣPUFA/ΣMUFA	2.23 ± 0.03	1.98 ± 0.07	2.00 ± 0.03	1.66 ± 0.02	—	—	—
ΣTotal	14.1 ± 0.82	14.12 ± 0.34	16.07 ± 1.11	16.62 ± 0.36	P < 0.001 15°C < 20°C	—	—

		NEUTRAL					
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n6	2.14 ± 1.62	2.03 ± 1.34	2.99 ± 0.51	2.99 ± 1.14	—	—	—
ARA	0.05 ± 0.04	0.02 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	—	P < 0.01 LD < RD	—
18:3n3	0.69 ± 0.52	0.7 ± 0.47	0.97 ± 0.17	1.06 ± 0.42	—	—	—
EPA	0.6 ± 0.44	0.22 ± 0.13	0.84 ± 0.15	0.27 ± 0.11	—	P < 0.05 LD < RD	—
DHA	0.65 ± 0.46	0.33 ± 0.14	0.99 ± 0.16	0.57 ± 0.26	—	—	—
DHA/EPA	1.09 ± 0.04	1.6 ± 0.31	1.18 ± 0.04	2.12 ± 0.16	P < 0.05 15°C < 20°C	P < 0.001 RD < LD	—
Σn-3	2.16 ± 1.56	1.39 ± 0.81	3.1 ± 0.53	2.08 ± 0.85	—	—	—
Σn-6	2.3 ± 1.75	2.12 ± 1.4	3.2 ± 0.55	3.11 ± 1.18	—	—	—
Σn-3/Σn-6	0.96 ± 0.04	0.68 ± 0.05	0.97 ± 0.01	0.66 ± 0.02	—	P < 0.001 LD < RD	—
ΣSFA	3.58 ± 2.6	2.4 ± 1.39	5.16 ± 0.77	3.7 ± 1.71	—	—	—
ΣMUFA	7.43 ± 5.57	6.53 ± 4.18	10.56 ± 1.52	9.85 ± 4.16	—	—	—
ΣPUFA	4.46 ± 3.31	3.52 ± 2.21	6.3 ± 1.07	5.18 ± 2.03	—	—	—
ΣPUFA/ΣSFA	1.23 ± 0.06	1.44 ± 0.08	1.22 ± 0.05	1.43 ± 0.10	P < 0.001 20°C < 15°C	P < 0.001 RD < LD	—
ΣPUFA/ΣMUFA	0.60 ± 0.02	0.54 ± 0.00	0.66 ± 0.03	0.53 ± 0.00	—	—	—
ΣTotal	15.47 ± 11.47	12.44 ± 7.77	22.02 ± 3.35	18.73 ± 7.9	—	—	—

B		POLAR					
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	1.61 <sup>ab</sup> ± 0.02	1.83 <sup>ab</sup> ± 0.51	1.22 <sup>a</sup> ± 0.1	2.27 <sup>b</sup> ± 0.31	—	—	P < 0.05
ARA	0.38 <sup>b</sup> ± 0.01	0.18 <sup>a</sup> ± 0.05	0.3 <sup>b</sup> ± 0.03	0.19 <sup>a</sup> ± 0.03	—	—	P < 0.05
18:3n-3	0.43 <sup>a</sup> ± 0	0.5 <sup>ab</sup> ± 0.12	0.34 <sup>a</sup> ± 0.01	0.62 <sup>b</sup> ± 0.08	—	—	P < 0.05
EPA	2.04 ± 0.07	0.97 ± 0.33	1.54 ± 0.34	1.07 ± 0.13	—	P < 0.001 <i>LD &lt; RD</i>	—
DHA	4.41 ± 0.23	3.38 ± 0.9	3.12 ± 0.61	3.15 ± 0.53	—	—	—
DHA/EPA	2.17 ± 0.12	3.55 ± 0.41	2.05 ± 0.26	3.05 ± 0.28	—	—	—
Σn-3	7.3 ± 0.27	5.14 ± 1.4	5.31 ± 0.95	5.05 ± 0.84	—	—	—
Σn-6	2.14 ± 0.03	2.16 ± 0.58	1.61 ± 0.14	2.46 ± 0.49	—	—	—
Σn-3/Σn-6	3.42 ± 0.07	2.37 ± 0.02	3.28 ± 0.31	2.06 ± 0.13	P < 0.05 <i>20°C &lt; 15°C</i>	P < 0.001 <i>LD &lt; RD</i>	—
ΣSFA	4.6 ± 0.29	3.23 ± 0.92	3.45 ± 0.4	3.77 ± 0.75	—	—	—
ΣMUFA	4.29 ± 0.02	3.91 ± 0.8	3.62 ± 0.2	4.7 ± 0.85	—	—	—
ΣPUFA	9.44 ± 0.3	7.3 ± 1.98	6.92 ± 1.09	7.51 ± 1.32	—	—	—
ΣPUFA/ΣSFA	2.06 ± 0.17	2.27 ± 0.07	2.00 ± 0.08	2.00 ± 0.12	P < 0.01 <i>20°C &lt; 15°C</i>	P < 0.01 <i>RD &lt; LRD</i>	—
ΣPUFA/ΣMUFA	2.20 ± 0.08	1.85 ± 0.14	1.91 ± 0.20	1.60 ± 0.09	P < 0.05 <i>20°C &lt; 15°C</i>	—	—
ΣTotal	18.33 ± 0.26	14.43 ± 3.69	13.99 ± 1.68	15.98 ± 2.89	—	—	—

		NEUTRAL					
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	2.76 ± 0.47	5.38 ± 2.74	3.97 ± 0.54	5.99 ± 0.5	—	P < 0.05 <i>RD &lt; LD</i>	—
ARA	0.07 <sup>b</sup> ± 0.01	0.04 <sup>a</sup> ± 0.02	0.11 <sup>c</sup> ± 0.01	0.0 <sup>a</sup> ± 0	—	—	P < 0.05
18:3n-3	0.91 ± 0.16	1.87 ± 1	1.44 ± 0.07	2.18 ± 0.17	—	P < 0.05 <i>RD &lt; LD</i>	—
EPA	0.68 <sup>a</sup> ± 0.14	0.42 <sup>a</sup> ± 0.2	1.14 <sup>b</sup> ± 0.08	0.48 <sup>a</sup> ± 0.05	—	—	P < 0.05
DHA	1.06 ± 0.2	0.63 ± 0.24	1.34 ± 0.09	1.13 ± 0.15	P < 0.01	P < 0.05	—

714						<i>15°C &lt; 20°C</i>	<i>LD &lt; RD</i>	
	DHA/EPA	20.09 <sup>b</sup> ± 3.56	32.41 <sup>b</sup> ± 14.36	29.87 <sup>a</sup> ± 1.72	35.95 <sup>c</sup> ± 5.53	—	—	P < 0.001
715	Σn-3	2.88 ± 0.51	3.19 ± 1.56	4.25 ± 0.12	3.92 ± 0.69	—	—	—
	Σn-6	2.97 ± 0.5	5.62 ± 2.81	4.25 ± 0.56	5.91 ± 0.88	—	P < 0.05 <i>RD &lt; LD</i>	—
	Σn-3/Σn-6	0.97 ± 0.01	0.57 ± 0.01	1.01 ± 0.11	0.66 ± 0.02	—	P < 0.001 <i>LD &lt; RD</i>	—
	ΣSFA	4.28 ± 0.82	5.9 ± 1.7	6.96 ± 0.03	6.83 ± 1.14	P < 0.05 <i>15°C &lt; 20°C</i>	—	—
	ΣMUFA	9.95 ± 1.74	17.7 ± 8.29	14.41 ± 1.03	19.29 ± 2.88	—	P < 0.05 <i>RD &lt; LD</i>	—
	ΣPUFA	5.86 ± 1.02	8.81 ± 4.37	8.5 ± 0.67	9.82 ± 1.57	—	—	—
	ΣPUFA/ΣSFA	1.37 ± 0.03	1.42 ± 0.40	1.22 ± 0.09	1.44 ± 0.09	P < 0.01 <i>20°C &lt; 15°C</i>	P < 0.01 <i>RD &lt; LD</i>	—
	ΣPUFA/ΣMUFA	0.59 ± 0.01	0.49 ± 0.02	0.59 ± 0.01	0.51 ± 0.02	P < 0.05 <i>15°C &lt; 20°C</i>	—	—
	ΣTotal	2.88 ± 0.51	3.19 ± 1.56	4.25 ± 0.12	3.92 ± 0.69	—	—	—

716 **Figure captions**

717 **Fig. 1** Effect of diet and temperature on A) body mass (g) and B) standard length (mm) in  
718 logarithmic scale. LD: low omega-3 polyunsaturated fatty acid (n-3 PUFA) diet; RD:  
719 reference n-3 PUFA diet. Values are means  $\pm$  standard deviations

720 **Fig. 2** Effect of diet (LD, low omega-3 polyunsaturated fatty acid [n-3 PUFA] diet; RD,  
721 reference n-3 PUFA diet) on organ allometry of fish raised at 20°C: A) heart; B)  
722 gastrointestinal system; and for fish raised at 15°C: C) liver; D) mesenteric fat

723 **Fig. 3** Effect of diet on muscle/dietary lipid ratios for linoleic acid (18:2n-6), arachidonic acid  
724 (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosapentenoic acid (DHA,  
725 22:6n-3) in A) the polar fraction and B) the neutral fraction. When factor interactions were  
726 significant, groups were compared with a posteriori tests ( $\alpha = 0.05$ ). For temperature  $\times$  diet  
727 interactions, significantly different groups were assigned different letters. \*:  $P < 0.05$ ; \*\*:  $P <$   
728  $0.01$ ; \*\*\*:  $P < 0.001$ . LD: low omega-3 polyunsaturated fatty acid (n-3 PUFA) diet; RD:  
729 reference n-3 PUFA diet; d: diet; t: temperature. Values are means  $\pm$  standard deviations

730

Figure 1

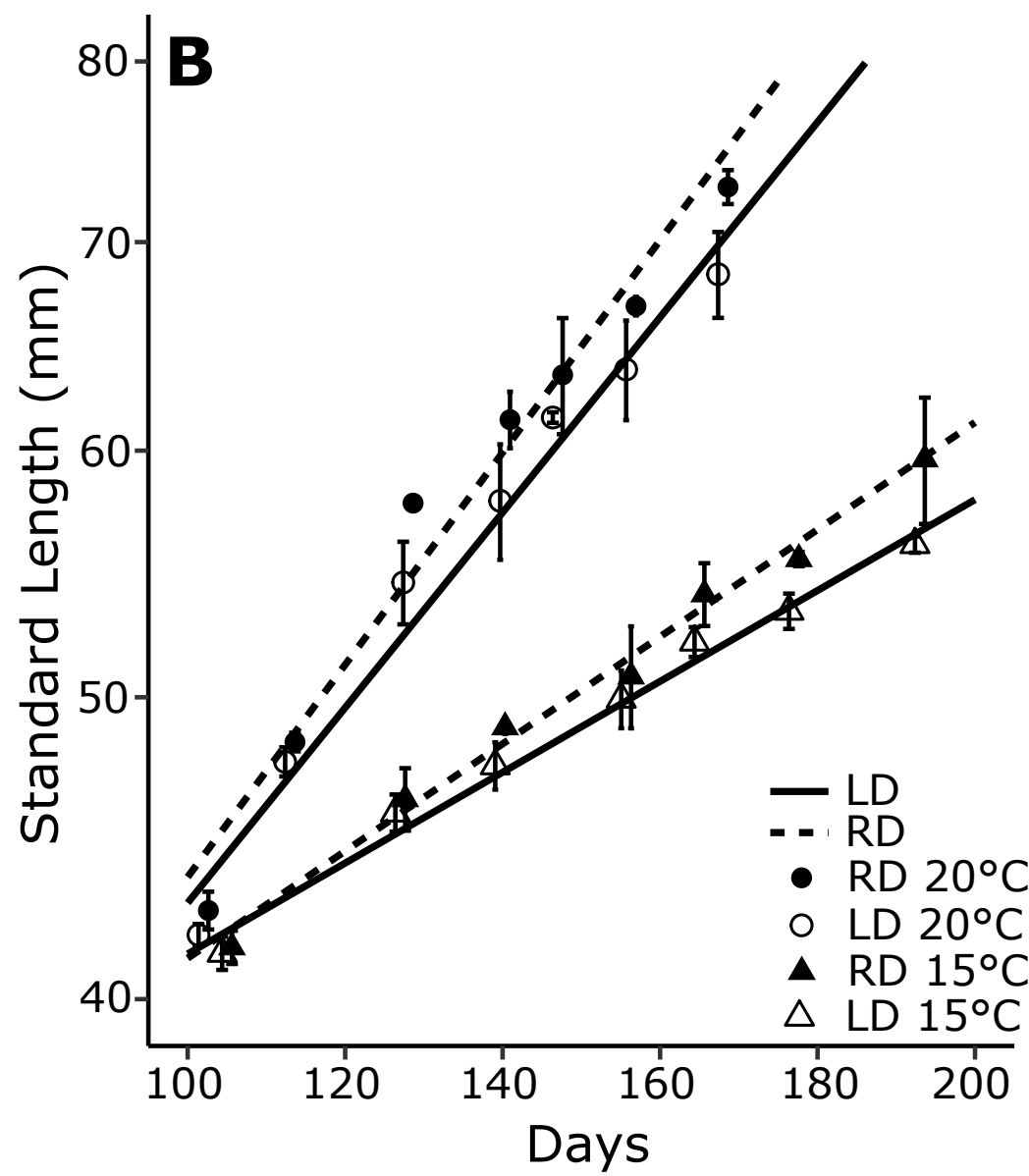
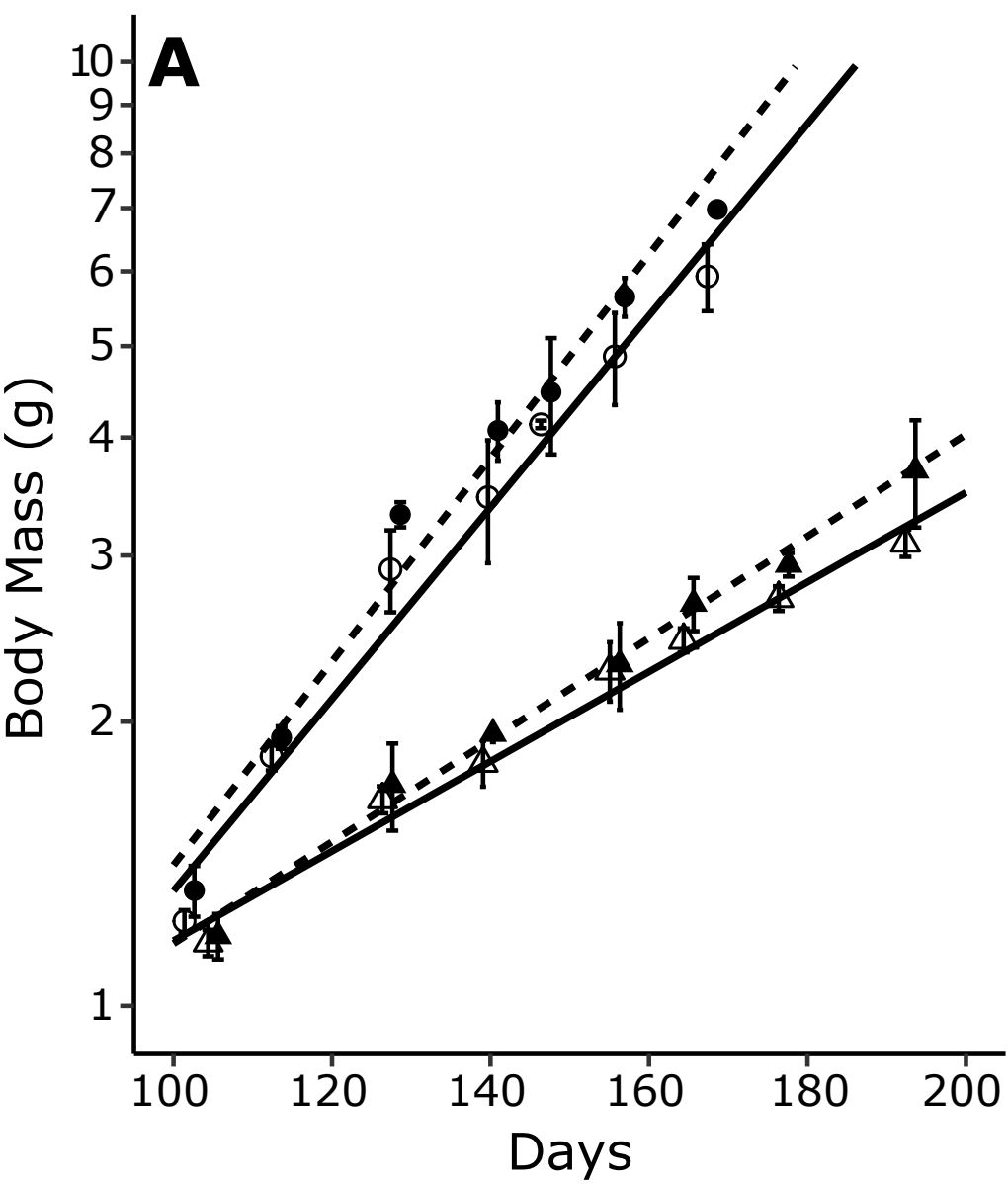
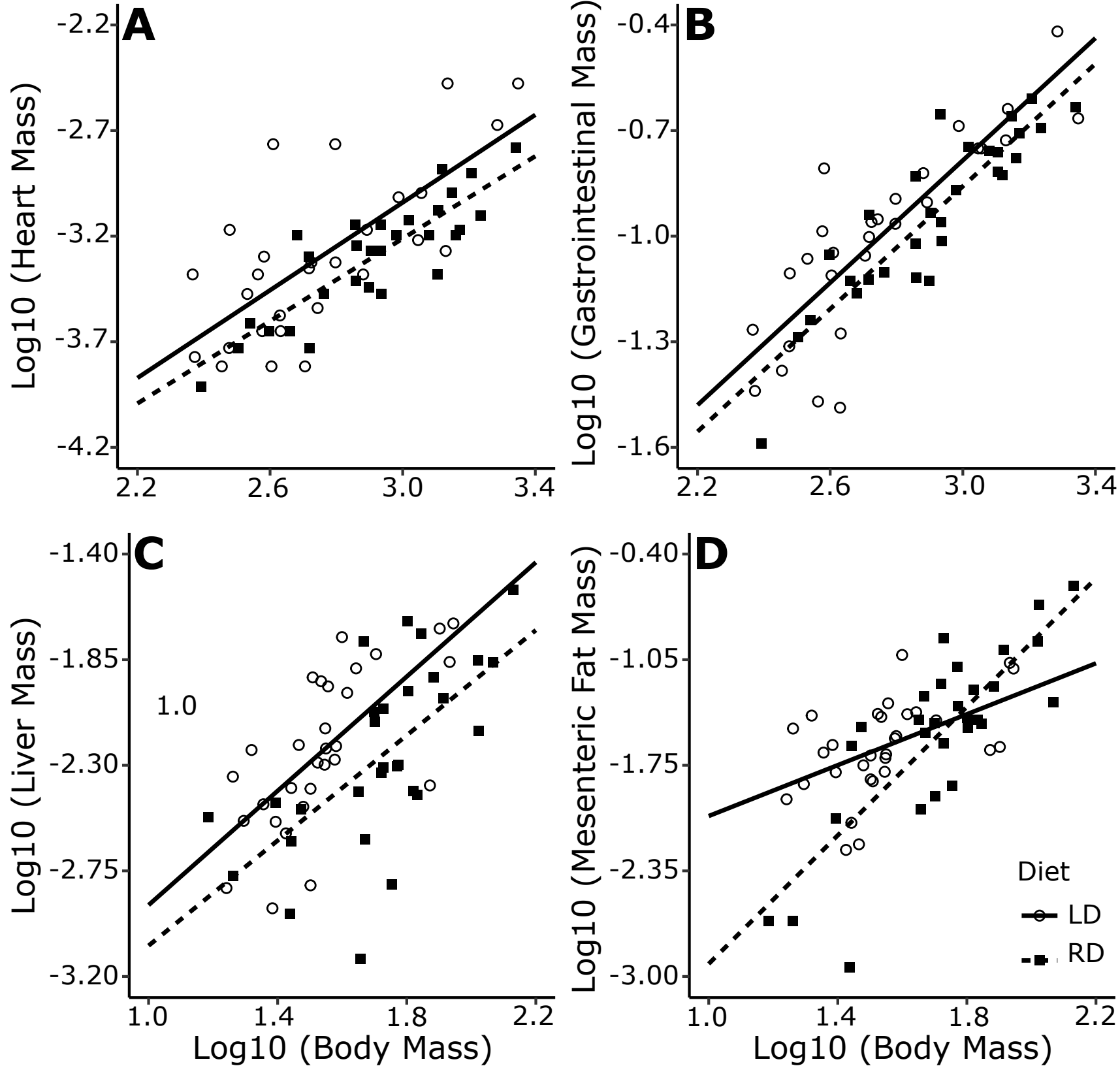
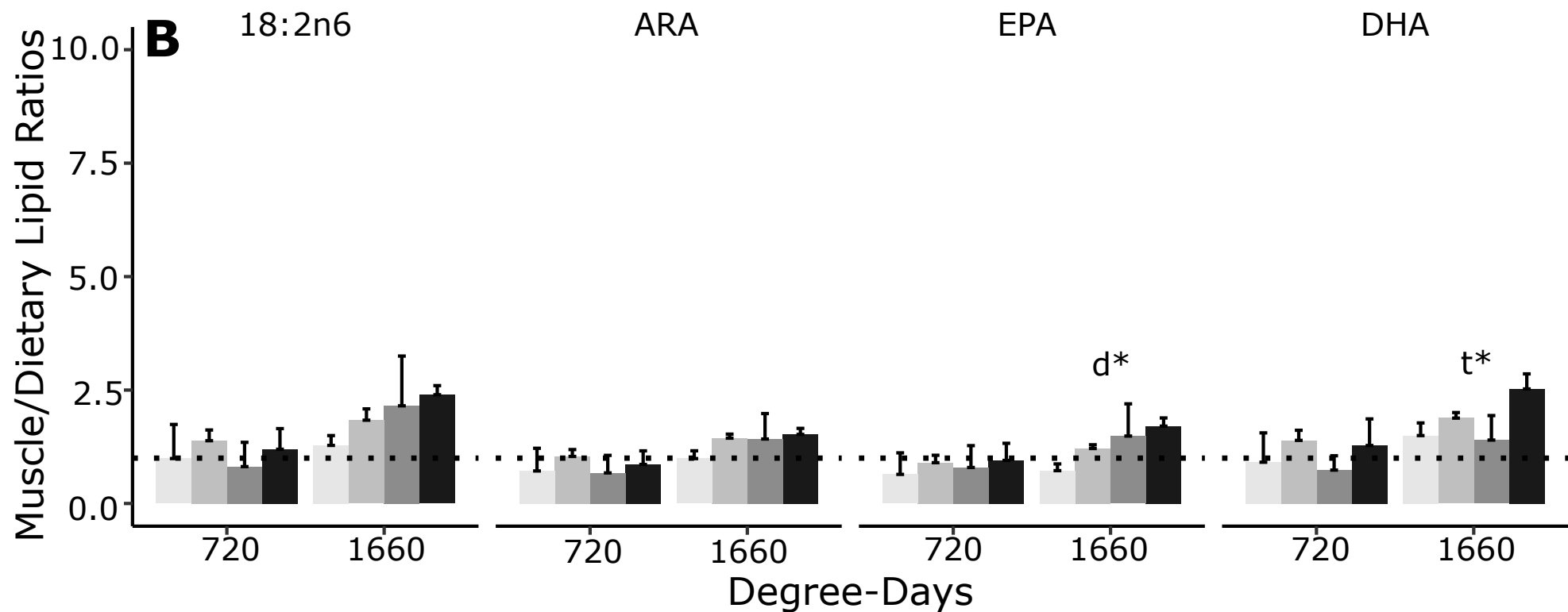
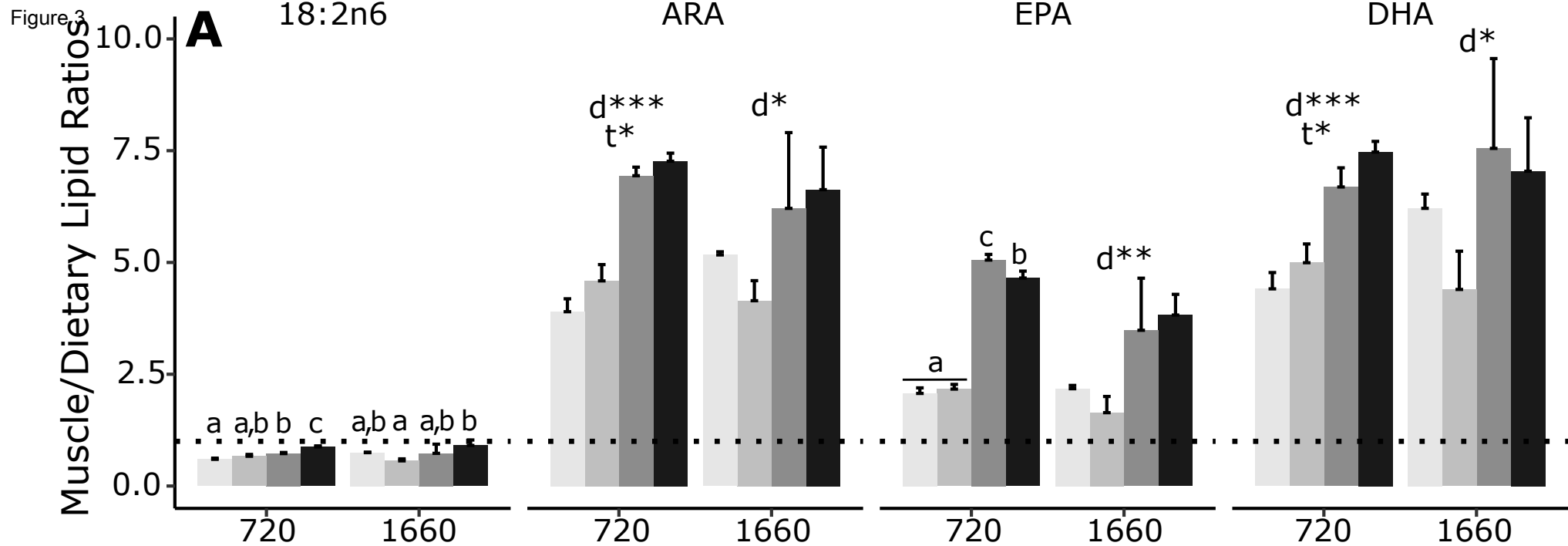


Figure 2







RD 15°C RD 20°C LD 15°C LD 20°C



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