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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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Introduction

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

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

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

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

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

Materials and methods

Fish origin and maintenance

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

Environmental and nutritional conditioning

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

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

Experimental diets

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

Experimental time line

Biometry

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

Mass gain (Δmass), specific growth rate (SGR), and thermal growth coefficient (TGC, which is a thermal unit approach; for reviews see Dumas et al. 2010) were calculated as follows:

$$\Delta\text{mass (g)} = BM_{final} - BM_{initial}$$

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

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

Samplings

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

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

Fatty acids

Lipid extraction

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

Separation of polar and neutral lipids

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

Transesterification

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

Gas chromatography analysis

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

Statistical and data analysis

Data normality and homoscedasticity were tested using Shapiro-Wilk and Levene tests, respectively. Growth rate in BM and SL was regressed against time. The effects of diet and temperature were tested by comparing regression slopes; when slopes were homogeneous, an ANCOVA was run to compare intercepts. Two-way ANOVAs were used to test for significant differences among diets and temperatures for Δmass, SGR, and TGC. Scatter plots between organ mass and BM for each temperature did not overlap due to a large difference in BM because of the temperature differences. For this reason, the effect of diet on organ allometry was tested separately for each temperature by comparing slopes and using ANCOVA if required. In order to meet normality, BM, SL, and organ mass were log10 transformed. Because the response at 1660 dd could be considered as dependent on the

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

Results

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

Growth performance and organ allometry

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

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

RD-fed juveniles (Fig. 2C; ANCOVA: $F(1,54) = 8.73$, $P < 0.01$), while the quantity of mesenteric fat relative to BM increased more in juveniles fed RD (Fig. 2D; slopes significantly different, log10 [Body Mass] × diet: $F(1,54) = 9.90$, $P < 0.01$). There were no differences for heart or gastrointestinal mass. Allometric regression parameters are available in Supplementary Table 2.

Fatty acids

At 720 dd, significant effects of both temperature and diet were observed in the white muscle FA composition. Both temperature and diet affected polar ΣSFA levels and proportions, which were significantly higher at 20°C than at 15°C and with RD compared to LD (Table 3A). Temperature (20°C > 15°C) but not diet significantly affected white muscle polar ΣMUFA (monounsaturated FA) and ΣPUFA. The ΣPUFA to ΣSFA ratio was significantly higher in fish fed LD and raised at 15°C. Polar Σn-3 and Σn-6 both significantly increased with temperature. However, independently of the temperature conditions, Σn-3 was higher in juveniles fed RD while Σn-6 dominated in juveniles fed LD in the polar fraction (Table 3A). Consequently, polar Σn-3/Σn-6 ratios were higher in RD-fed juveniles. In LD-fed juveniles, the ratio was higher at 15°C than in those reared at 20°C (Table 3A). At both temperatures, the percent content of 18:2n-6 was significantly higher in juveniles fed LD, but the difference between the two diets was larger at 20°C than at 15°C. On the contrary, the 18:3n-3 content was higher in juveniles fed RD, although juveniles contained overall more 18:3n-3 at 20°C than at 15°C. The ARA and DHA levels were significantly higher in juveniles reared at 20°C, while there was no effect of temperature on DHA present in the polar fraction. A significant diet effect was only present for ARA and EPA, with higher levels in juveniles fed RD than in those fed LD. The lowest DHA/EPA ratios were observed in fish fed RD at both temperatures. Juveniles reared at 20°C and fed LD had a higher DHA/EPA ratio than those raised at 15°C (Table 3A). Changes in the neutral fraction were clearly less

pronounced at 720 dd. No effect of either temperature or diet was observed for Σn-3, Σn-6, Σn-3/Σn-6, ΣSFA, ΣMUFA, ΣPUFA, or the percent content of 18:2n-6 and 18:3n-3. The ARA and EPA contents were significantly lower in juveniles fed LD, while the DHA/EPA ratio was significantly lower in juveniles fed RD. The ratio was also generally lower at 15°C than at 20°C.

At 1660 dd, polar ΣSFA, ΣMUFA, ΣPUFA, Σn-3, and Σn-6 percentages were similar regardless of the rearing conditions (Table 3B). However, the Σn-3/Σn-6 ratio was 8.4% lower when temperature increased and 51.2% lower in juveniles fed LD compared to those fed RD (Table 3B). As observed at 720 dd, the 18:2n-6 content was the highest in juveniles fed LD, but only at 20°C. A similar response was observed for the 18:3n-3 content. The EPA content was significantly higher in juveniles fed RD, whereas DHA and the DHA/EPA ratio were similar among treatments. However, the ARA content was lowest in juveniles fed LD at both temperatures, while those fed RD at 20°C had the highest proportion (Table 3B). In contrast to what was observed at 720 dd, more pronounced effects of diet and temperature were observed in the neutral fraction at 1600 dd. Indeed, the ΣSFA content was 26.2% higher at 20°C than at 15°C, while the ΣMUFA was 34.1% higher in juveniles fed LD than in those fed RD (Table 3B). The ΣPUFA to ΣSFA ratio was significantly higher in fish fed LD and raised at 15°C, while the ΣPUFA to ΣMUFA ratio was higher at 20°C. The Σn-6 was significantly higher in juveniles fed LD and, consequently, the Σn-3/Σn-6 ratio was significantly higher in juveniles fed RD compared to those fed LD (Table 3A, B). Both 18:2n-6 and 18:3n-3 contents were lower in juveniles fed RD. Interestingly, the ARA content was extremely low in juveniles fed LD at both 15°C and 20°C. EPA was significantly higher in juveniles fed RD at 20°C compared to the three other treatments, while DHA was overall significantly lower at 15°C and in juveniles fed LD. At 20°C, RD-fed fish had the lowest DHA/EPA ratios, while LD-fed fish had the highest.

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

Discussion

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

Growth performance

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

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

Organ allometry

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

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

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

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

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

Fatty acids

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

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

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

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

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

Conclusions

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

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

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

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

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Table index

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

700 Table 2: Effect of temperature and diet on growth indices (Δmass: 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 ± standard deviations

	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
Δmass (g)	2.5 ± 0.51	1.9 ± 0.07	5.6 ± 0.11	4.7 ± 0.49	$P < 0.001$	$P < 0.01$	—
TGC (g degree-days^{-1})	0.36 ± 0.06	0.30 ± 0.01	0.61 ± 0.02	0.55 ± 0.04	$P < 0.001$	$P < 0.01$	—
SGR (%BM d^{-1})	1.3 ± 0.18	1.1 ± 0.02	1.9 ± 0.07	1.8 ± 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 × diet interactions, significantly different groups were assigned
708 different letters. Σn-3 includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n3;
709 Σn-6 includes 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6; ΣSFA (saturated FA) includes
710 20:0s, 22:0s, 24:0s; ΣMUFA (monounsaturated FA) includes 14:1n-9, 18:1n-11, 18:1n-7,
711 20:1n-7, 22:1n-9, 24:1n-9; ΣPUFA includes 18:3n-6, 18:4n-3, 20:2n-6, 20:3n-3, 20:3n-6,
712 20:4n-3. Values are means ± standard deviations

A	POLAR						
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	$1.29^{a} \pm 0.06$	$1.82^{b} \pm 0.06$	$1.44^{a} \pm 0.09$	$2.19^{c} \pm 0.05$	—	—	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	$1.61^{a} \pm 0.05$	$2.12^{b} \pm 0.11$	$1.75^{a} \pm 0.09$	$2.57^{c} \pm 0.1$	—	—	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	$3.42^{c} \pm 0.06$	$2.45^{b} \pm 0.05$	$3.34^{c} \pm 0$	$2.18^{a} \pm 0.03$	—	—	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	—	—	—

713

B	POLAR						
FA % DM	15°C		20°C		Two-way ANOVA		
	RD	LD	RD	LD	Temperature	Diet	Interaction
18:2n-6	1.61 ^{ab} ± 0.02	1.83 ^{ab} ± 0.51	1.22 ^{a} ± 0.1	2.27 ^{b} ± 0.31	—	—	P < 0.05
ARA	0.38 ^{b} ± 0.01	0.18 ^{a} ± 0.05	0.3 ^{b} ± 0.03	0.19 ^{a} ± 0.03	—	—	P < 0.05
18:3n-3	0.43 ^{a} ± 0	0.5 ^{ab} ± 0.12	0.34 ^{a} ± 0.01	0.62 ^{b} ± 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 *LD < RD*	—
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 *20°C < 15°C*	P < 0.001 *LD < RD*	—
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 *20°C < 15°C*	P < 0.01 *RD <LRD*	—
ΣPUFA/ΣMUFA	2.20 ±0.08	1.85 ± 0.14	1.91 ± 0.20	1.60 ± 0.09	P < 0.05 *20°C < 15°C*	—	—
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 *RD < LD*	—
ARA	0.07 ^{b} ± 0.01	0.04 ^{a} ± 0.02	0.11 ^{c} ± 0.01	0.0 ^{a} ± 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 *RD < LD*	—
EPA	0.68 ^{a} ± 0.14	0.42 ^{a} ± 0.2	1.14 ^{b} ± 0.08	0.48 ^{a} ± 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	—

					15°C < 20°C	*LD < RD*	
DHA/EPA	20.09 [b] ± 3.56	32.41 [b] ± 14.36	29.87 [a] ± 1.72	35.95 [c] ± 5.53	—	—	P < 0.001
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 *RD < LD*	—
Σn-3/Σn-6	0.97 ± 0.01	0.57 ± 0.01	1.01 ± 0.11	0.66 ± 0.02	—	P < 0.001 *LD < RD*	—
ΣSFA	4.28 ± 0.82	5.9 ± 1.7	6.96 ± 0.03	6.83 ± 1.14	P < 0.05 *15°C < 20°C*	—	—
ΣMUFA	9.95 ± 1.74	17.7 ± 8.29	14.41 ± 1.03	19.29 ± 2.88	—	P < 0.05 *RD < LD*	—
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 *20°C < 15°C*	P < 0.01 *RD < LD*	—
ΣPUFA/ΣMUFA	0.59 ± 0.01	0.49 ± 0.02	0.59 ±0.01	0.51 ± 0.02	P < 0.05 *15°C < 20°C*	—	—
ΣTotal	2.88 ± 0.51	3.19 ± 1.56	4.25 ± 0.12	3.92 ± 0.69	—	—	—

Figure captions

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

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

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

Figure 1

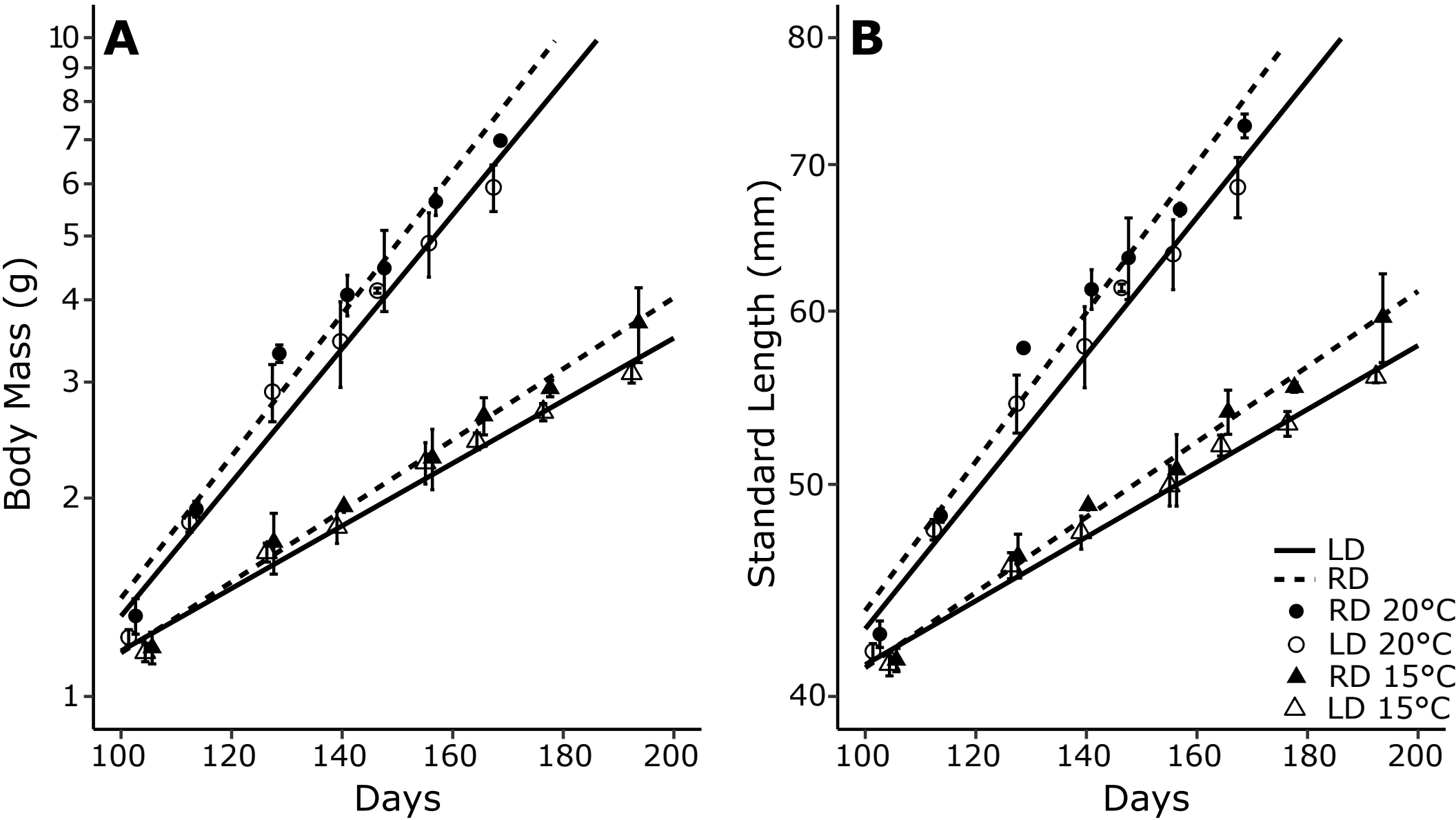
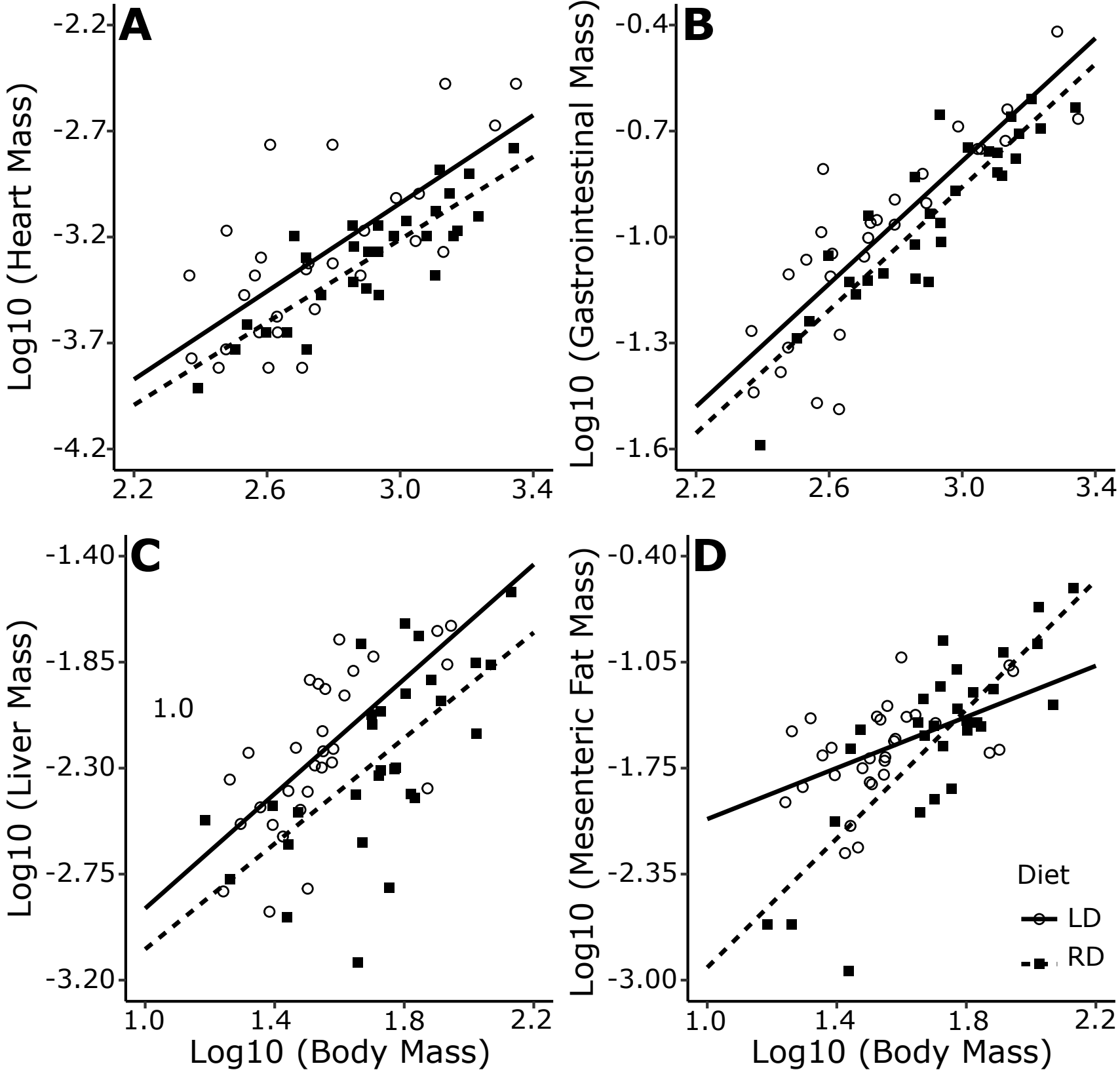

A
B
Body Mass (g)
Standard Length (mm)
Days
Days
10
9
8
7
6
5
4
3
2
1
80
70
60
50
40
100
120
140
160
180
200
LD
RD
RD 20°C
LD 20°C
RD 15°C
LD 15°C

Figure 2


A
B
C
D
Log10 (Heart Mass)
Log10 (Gastrointestinal Mass)
Log10 (Liver Mass)
Log10 (Mesenteric Fat Mass)
Log10 (Body Mass)
Log10 (Body Mass)
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-2.7
-3.2
-3.7
-4.2
-0.4
-0.7
-1.0
-1.3
-1.6
-1.40
-1.85
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-2.75
-3.20
-0.40
-1.05
-1.75
-2.35
-3.00
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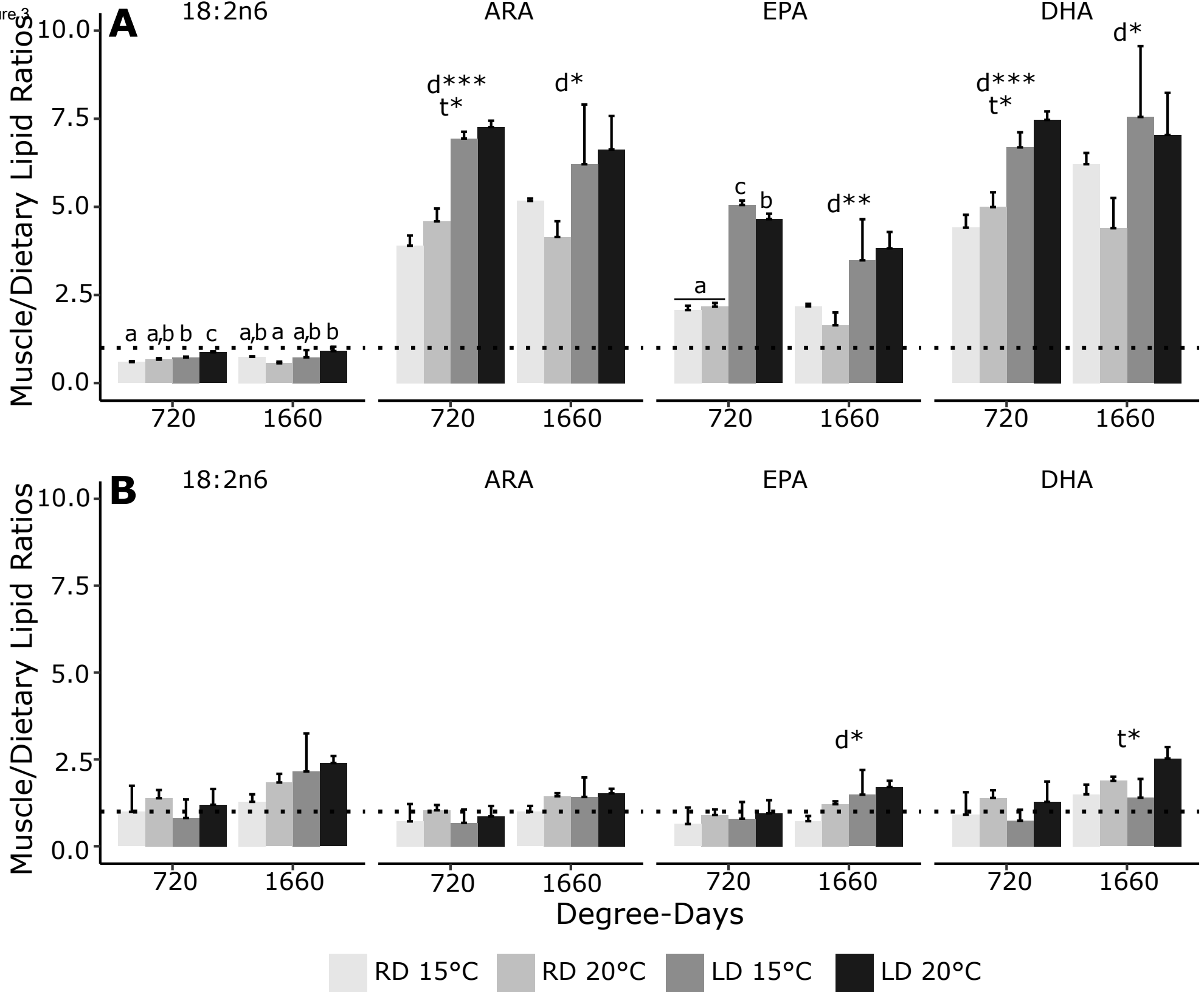
3.0
3.4
1.0
1.4
1.8
2.2
Diet
LD
RD

Figure 3


A
B
18:2n6
ARA
EPA
DHA
Muscle/Dietary Lipid Ratios
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7.5
5.0
2.5
0.0
a
a,b
b
c
a,b
a
a,b
b
d***
t*
d*
a
c
b
d**
d***
t*
d*
720
1660
Degree-Days
RD 15°C
RD 20°C
LD 15°C
LD 20°C

Electronic Supplementary Material (Tables, Figures, Video, Movie, Audio, etc.)