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Effect of thermal and nutritional conditions on fatty acid metabolism and oxidative stress response in juvenile European sea bass (Dicentrarchus labrax)

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Abstract :

Coastal nursery areas are subjected to a wide range of natural and anthropogenic stressors, including global warming, which indirectly influence trophic food webs. A global rarefaction of n-3 polyunsaturated fatty acids (PUFA) in trophic networks is in progress. The aim of this study was to assess the effect of a reduction in the dietary availability of n-3 PUFA on some molecular and biochemical parameters related to lipid metabolism and oxidative stress response in juvenile European sea bass (Dicentrarchus labrax) raised at two temperatures (15 °C and 20 °C). Fish were fed for five months with 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 is plentiful, and a lower n-3 PUFA diet (LD; 0.73% n-3 PUFA DM), designed to mimic a decrease in n-3 PUFA resulting from global changes (the n-3 PUFA levels tested remained above the nutritional minimum required for this species). Results showed that diet did not affect the hepatic expression of some mRNA coding for transcriptional factors involved in regulating the metabolic pathways related to fatty acid bioconversion. Although our molecular analysis was limited to transcript expression, these data suggest the presence of a threshold in the nutritional supply of PUFA above which the activation of these molecular pathways does not occur. However, the expression for most of the transcripts tested was up-regulated at 20 °C. Despite the high peroxidation index in fish fed RD, very few modifications of the oxidative stress response were associated with diet. At 20 °C, an increase of the enzymatic antioxidant response was observed, but there was no correlation with the peroxidation index or malondialdehyde products.

Introduction

Many fish species have life history strategies in which successive ontogenetic stages occupy different habitats. In those characterized by the production of large amounts of small pelagic eggs and the lack of parental care, the survival of eggs, larvae, and juveniles strongly depends on abiotic and biotic environmental factors acting on their habitats (Cushing, 1995; Sogard, 1997; Juanes, 2007; Houde, 2008). Coastal zones and estuaries are productive nursery grounds for a large number of marine species because they provide refuge from predators together with high food availability and enhanced survival, development, and growth of early stages (Beck et al., 2001; Able et al., 2013). However, coastal and estuarine nursery areas are subjected to a wide range of anthropogenic and natural stressors. Coastal habitats in particular face anthropogenic activities, including global warming, which indirectly influence trophic food webs (Harley et al., 2006; Lehodey et al., 2006; Brander, 2007; Rijnsdorp et al., 2009; Gattuso et al., 2015; Hixson and Arts, 2016).

Lipids represent an important energy supply and play a crucial role as major components of cell membranes (Sargent et al., 2002). In marine trophic networks, polyunsaturated fatty acids (PUFA) are mainly supplied by the dominant phytoplankton species, such as diatoms and dinoflagellates (Hu et al., 2008; Lang et al., 2011). The n-3 PUFA (also known as omega 3), particularly eicosapentenoic acid (EPA) and docosahexaenoic acid (DHA), are naturally abundant for primary consumers. However, it has been shown that phytoplankton growth and biochemical composition, including fatty acids (FA), are affected by temperature (e.g., Arts et al., 2009). An increase in water temperature results in a decrease in the omega 3 content of phytoplankton species (Ackman and Tocher, 1968; Thompson et al., 1992; Renaud et al., 2002; Guschina and Harwood, 2006). At least two recent studies have shown a global decline of n-3 PUFA in trophic networks while n-6 tends to be more abundant (Colombo et al., 2016; Hixson and Arts, 2016). The high availability of n-3 PUFA in phytoplankton may explain the lack of

selection pressure on its biosynthesis pathway in several marine fish species and thus why piscivorous fish have lost the capacity for de novo synthesis of n-3 PUFA (Tocher et al., 2006). In most other fish species, it has been shown that marine species have low capacities to convert 18-carbon fatty acids into n-3 PUFA (Owen et al., 1975; Mourente and Tocher, 1994; Sargent et al., 2002; Geay et al., 2010, 2012) due to apparent deficiencies in one or more steps of the biosynthesis pathway (Ghioni et al., 1999; Tocher and Ghioni, 1999; Santigosa et al., 2010). This makes these fishes highly dependent upon dietary sources of n-3 PUFA (Ghioni et al., 1999; Tocher and Ghioni, 1999). To what extent the n-3 PUFA decline induced by global climate change may impact species that depend on phytoplankton to fulfill their needs whether as primary or secondary consumers is not yet known. However, it has also been reported that most of the genes coding for the main components of this biosynthesis pathway are present **and that their transcription** could be up/down regulated by some nutritional or environmental factors (Dias et al., 1998; Gomez-Requeni et al, 2003; Figueiredo-Silva et al., 2009). Thus it is possible to study the regulation of lipid metabolism and the FA synthesis pathway that directly or indirectly result from climate change.

Environmental and nutritional factors may alter the amount of functional protein involved in lipid metabolism through a range of transcriptional (e.g., activity of transcription factors), post-transcriptional (e.g., mRNA stability), **translational,** and post-translational (e.g., phosphorylation) mechanisms. The transcriptional regulation of genes involved in lipogenesis by PUFA is well documented. PUFA are known to bind and directly influence the activity of a variety of transcription factors including peroxisome proliferator activated receptors (**PPAR**), which in turn have been shown to **regulate the transcription of** many genes involved in lipid homeostatic processes (Jump, 2002). There appears to be several ***ppar*** variants expressed in fish tissues: ***pparα*** is mainly expressed in the liver, ***pparγ*** is mainly expressed in adipose tissue, and ***pparβ*** has been found in most tissues (Boukouvala et al., 2004). **Sterol regulatory element**

binding protein-1 (***srebp-1***) **is another key transcription factor known to be involved in fatty acid synthesis assembly (Horton et al., 2002). It is well documented that expression of the *ppar* and *srebp-1* genes are regulated at the transcriptional level by dietary FA in mammals** (Lindi et al., 2003; Juliano et al., 2004; MacLaren et al., 2006). Similar results have been reported in fish (Leaver et al., 2005, 2007; Jordal et al., 2007; Dong et al., 2015), including the European sea bass (Geay et al. 2010, 2012).

In mammals, *ppar* and *srebp-1* are the main transcription factors regulating the **mRNA** expression of elongase and desaturase (Nakamura and Nara, 2002, 2003)**, which are key enzymes involved in PUFA biosynthesis. The first step of the bioconversion pathway requires the delta 6-desaturase gene (*fads2*; Sprecher, 2000).** Another important enzyme system is the fatty acid synthetase complex (FAS), which catalyzes the key lipogenesis pathway in fishes (Sargent et al., 2002). The main products are saturated 16:0 (palmitic acid) and 18:0 (stearic acid) FA. In the carnivorous fishes blackspot seabream (*Pagellus bogaraveo*; Figueiredo-Silva et al., 2009), European sea bass (*Dicentrarchus labrax*; Dias et al., 1998), and gilthead sea bream (*Sparus aurata*; Gomez-Requeni et al., 2003), dietary fat intake has been shown to inhibit FAS activity.

Biological membranes rich in PUFA are highly sensitive to oxidation due to their high level of unsaturation (Pamplona et al., 2000; Hulbert, 2005); this oxidation can lead to the production of reactive oxygen species (ROS). ROS are generated as by-products of cellular metabolism, and mitochondria are a primary site for ROS production (Barja, 2007). Higher temperatures may promote ROS production, especially in organisms rich in membrane PUFA, and this could lead to various cellular and tissular dysfunctions (Kawatsu, 1969; Watanabe et al., 1970; Murai and Andrews, 1974; Sakai et al., 1998). The relative rates of peroxidation of different acyl chains have been used to convert the fatty acid composition of a particular membrane into the peroxidation index (PI; see Hulbert et al., 2007, for details). The PI

115 represents the theoretical susceptibility of membrane lipid composition to lipid peroxidation,
116 and it appears to be homeostatically regulated with respect to dietary PI. In practice,
117 malondialdehyde (MDA) is one lipid peroxidation product that can be measured (Frankel,
118 2005). Under most physiological states, ROS production is closely matched by the antioxidant
119 response. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and
120 peroxidases, form an important part of the antioxidant response (Lesser, 2006). In fish species,
121 COX and CS activity have been monitored because they are known to be adjusted to the
122 maximum aerobic metabolism capacity in fish (Childress and Somero, 1979; Thuesen and
123 Childress, 1993, 1994).

124 European seabass migrate offshore to spawn, and the developing larvae drift to sheltered
125 coastal or estuarine nurseries where they spend their first few years of life (Holden and
126 Williams, 1974; Kelley, 1988; Vinagre et al., 2009). During that period, juvenile sea bass
127 exhibit rapid growth and are faced with a broad spectrum of environmental constraints and
128 feeding conditions, making it an attractive model organism to examine the regulatory response
129 of the PUFA biosynthesis pathway. The aim of this study was to test the effects of two levels
130 of n-3 PUFA dietary contents on different molecular (**transcript** expression) and biochemical
131 (MDA, enzymatic activity) variables related to FA metabolism and antioxidant response,
132 respectively, in European sea bass juveniles raised at two different temperatures. To obtain
133 realistic responses in terms of a global change scenario, we exposed European sea bass to
134 temperatures of 15°C (current average temperature to which the Atlantic sea bass population is
135 exposed) and 20°C (to mimic the temperature rise that is expected over the next 80 years;
136 Gattuso et al. 2015). We hypothesized that a diet poor in n-3 PUFA would activate lipid
137 biosynthesis while a diet richer in n-3 PUFA would lead to oxidative stress and would worsen
138 under higher temperature conditions.

139 Materials and methods

Feeding experiment

The protocols for fish maintenance, diet composition, and nutritional conditioning are detailed in Gourtay et al. (2018). They are summarized here to facilitate understanding of the experimental protocol. 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, a batch of four females and 10 males were bred in the facility. At day 2 post hatching (dph-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 10,000 larvae tank-^{1}). Water temperature in the tanks was progressively increased from 14°C to 20°C over six days. Larvae were fed with *Artemia* from mouth opening (dph-8) to dph-39 and with microparticulate feed (Marinstart, Le Gouessant, Lamballe, France) until dph-74. After dph-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.

The two diets tested were identical except for the FA source. The low n-3 PUFA diet (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]; EPA+DHA = 0.73% DM), while the reference diet (RD) contained 50% colza oil and 50% fish oil, the latter being richer in EPA and DHA (20:5n-3, 22:6n-3; EPA+DHA = 1.65% DM). Diets were isoenergetic and contained the same percentages of proteins and lipids (Table 1).

At d-93, juveniles (0.75 ± 0.02 g; 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 the two experimental diets. Feeding took place for 7 h during daytime (08:00 to 15:00) using an

automatic distributor (treadmill, 2 cm h^{-1}); daily visual control was done to ensure that the food distributed had been consumed and that fish were fed *ad libitum*. Each diet × temperature combination was replicated in three tanks.

Tissue sampling

To **account for** the impact of temperature alone on fish growth, sampling dates were calculated in degree-days **(dd)** relative to the first day of the experiment **(D0) at dph-93 and 0 dd.** At 720 **dd** after the beginning of the experiment (**dph-**129, **D36,** and d**ph-**141, **D48,** at 20°C and 15°C, respectively) and at 1660 dd (d**ph-**176, **D83,** and d**ph-**204**, D111,** at 20°C and 15°C, respectively), 32 fish were randomly sampled from each tank (three tanks per treatment, N = 96 per treatment), anaesthetized (lethal concentration of MS-222, 200 mg L^{-1}), and their livers excised. Because livers were small, the livers of eight juveniles were pooled for a total of four pools per tank and 12 per experimental condition. Pools were stored at –80°C. Two pools per tank were used for molecular biology analysis (N = 6 per experimental treatment for a total of 24), one pool was used for enzyme activity, and the fourth was used for malondialdehyde (MDA) analysis (N = 3 per experimental condition).

Peroxidation index, enzyme activity, and malondialdehyde assay

The peroxidation index (PI) was calculated for total dietary lipids and the polar fraction of muscle lipids (see Gourtay et al. [2018] for details of fatty acid content) following the equation provided by Hulbert et al. (2007):

PI = (0.025 × % monoenoics) + (1 × % dienoics) + (2 × % trienoics) + (4 × % tetraenoics) + (6 × % pentaenoics) + (8 × % hexaenoics).

Liver samples were reduced to powder using a mixer mill (MM 400, Retsch, 30 Hz, 30 s) with liquid nitrogen. To extract total proteins, 100 mg of liver powder was diluted in 1 mL lysis

buffer (150 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X100, 0.5% Igepal, 1% phosphatase inhibitor cocktail II, 2% NaPPi, and 1 tablet EDTA-free protease inhibitor cocktail, pH adjusted to 7). Samples were then homogenized on ice using a pro polytron® (BioBlock Scientific; Illkirch, Bas-Rhin, France). For protein extraction, samples were left on ice for 40 min and then centrifuged (3-30K Sigma, 6000 g, 1 h, 4°C). The middle phase containing the protein extract was recovered with a Pasteur pipette and centrifuged (3-30K Sigma, 10000 g, 45 min, 4°C). The resulting middle phase containing the protein extract was recovered with a Pasteur pipette, separated into aliquots, and stored at –80°C for the enzyme activity assay.

Total protein concentrations of the liver homogenates were assayed in triplicate according to the Bio-Rad DC Protein Assay (ref: 5000116, Bio-Rad), which is based on the Lowry assay (Lowry et al., 1951).

Specific CAT activity was measured in triplicate according to Aebi (1984) by observing the decomposition of H_2O_2 into oxygen and water. The reaction mixture consisted of 20 mM phosphate (pH 7) and 10 mM H_2O_2. We used a Multiskan GO (Thermo Scientific) plate reader with GREINER UV-Star 96 (ref: M3812, Sigma). The kinetics of the colouring solutions were monitored at 22°C by reading absorbance at 240 nm every 10 s for 4 min.

The specific enzyme activity of superoxide dismutase (SOD, combined Cu, Zn-SOD, and Mn-SOD) was determined in triplicate using a commercial kit (ref: 19160, Sigma), with a NUNC F-bottom 96-well (Thermo Scientific) and Multiskan GO (Thermo Scientific) plate reader. Final absorbances were read at 450 nm after 20 min incubations at 37°C.

The specific enzyme activity of cytochrome c oxidase (COX) was measured in triplicate using a commercial kit (ref: CYTOCOX1, Sigma) combined with an Evolution 21 UV-Visible Spectrometer (Thermo Scientific). The kinetics of colouring solutions were monitored at 25°C by reading absorbance at 550 nm every 0.25 s for 1 min.

The specific enzyme activity of citrate synthase (CS) was measured in triplicate according to Srere (1969) and Bergmeyer et al. (1974). The assay medium contained 100 mM Tris-HCl (pH 8), 10 mM DTNB, and 2 mM acetyl-CoA. Liver homogenates and assay media were protected from light and incubated for 5 min in NUNC F-bottom 96-well plates (Thermo Scientific); 5 mM oxaloacetate solution was added to initiate the reaction. The kinetics of colouring solutions were monitored at 25°C by reading absorbance at 412 nm every 10 s for 4 min.

The lipid peroxide assay was adapted from the thiobarbituric acid reactive substances (TBARS) protocol (Uchiyama and Mihara, 1978). Liver samples were ground on ice using a POLYTRON PT-MR 2100 with distilled water containing 1% BHT in methanol, with 50 mg of liver for 1 mL of grinding solution. A total of 500 µL of 1% TBA and 1.5 mL of 1% phosphoric acid were added to 500 µL of liver homogenate. This mixture was heated for 30 min at 100°C and cooled for 30 min on ice, at which point 2 mL of butanol were added and the mixture vortexed. Protein precipitates were removed by centrifugation at 800 g. The absorbance of the supernatant was read in NUNC F-bottom 96-well plates (Thermo Scientific) with a Multiskan GO spectrophotometer (Thermo Scientific) at 532 nm. The results were calculated using an MDA standard curve with butanol as the solvent.

mRNA expression analysis

RNA extraction

Liver samples were reduced to powder using a mixer mill (MM 400, Retsch, 30 Hz, 30 s) with liquid nitrogen. Samples (50–100 mg) were placed in 1 mL of Extractall reagent (Eurobio; Courtaboeuf, Essone, France) and homogenized using a pro polytron® (BioBlock Scientific; Illkirch, Bas-Rhin, France) tissue disruptor for 30 s. Potential contaminating DNA

was removed using an RTS DNase™ Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's recommendations. Total RNA was extracted following the manufacturer's instructions. The quantity and purity of RNA were assessed using an ND-1000 NanoDrop® spectrophotometer (Thermo Scientific Inc.; Waltham, MA, USA). RNA integrity was determined by electrophoresis using an Agilent Bioanalyzer 2100 (Agilent Technologies Inc.; Santa Clara, CA, USA). All samples had an RNA integrity number (RIN) higher than seven and could thus be used for real-time quantitative polymerase chain reaction (qPCR) analysis. RNA samples were stored at −80°C until use.

cDNA synthesis

After extraction, total RNA was reverse transcribed into cDNA using an iScript™ cDNA Synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA, USA) following the manufacturer's instructions. Briefly, 500 ng total RNA was reverse transcribed into cDNA in a volume of 20 µL that was composed of 15 µL of sample; 4 µL 5 × iScript™ Reaction Mix containing oligo (dT), random primers, and RNaseA inhibitor; and 1 µL of iScript™ reverse transcriptase. Reverse transcription (RT) negative controls were also performed on each sample using the same reaction mix except for the reverse transcriptase (substituted by water).The cDNA synthesis reaction was incubated for 5 min at 25°C followed by 30 min at 42°C and terminated by incubation for 5 min at 85°C to inactivate the enzyme. RT was performed using a T 100 Thermal-cycler (Bio-Rad Laboratories Inc.). cDNA samples were stored at −20°C until use.

Quantitative real-time RT-PCR analysis

The analysis of **mRNA** expression in liver tissue of European sea bass was carried out by qPCR using the primers listed in Table 2. Primers were designed using Primer3plus (http://primer3plus.com/) based on sequences available from the Genbank database

(https://www.ncbi.nlm.nih.gov/genbank/). **mRNA** expression was quantified using a C1000 touch thermal cycler (CFX96 system, Bio-Rad Laboratories Inc.). For each primer pair, qPCR efficiencies were estimated by standard curves resulting from serial dilutions (from 1/10 to 1/270) of a pool of cDNA. In the present study, qPCR efficiencies for each primer pair ranged from 95 to 100%, with $R^2 > 0.99$. Samples were then analyzed in triplicate. The final well volume was 15 µL, containing 5 µL cDNA (1/30 dilution) and 10 µL of reaction mix (0.5 µL of each primer [10 µM]), 1.5 µL RNase/DNase-free water, 7.5 µL iQ™ SYBR® Green Supermix (Bio-Rad Laboratories Inc.) containing antibody-mediated hot-started iTaq DNA polymerase, dNTPs, MgCl2, SYBR® Green I dye, fluorescein, stabilizers, and enhancers. In each plate, negative controls (RT negative controls) were systematically included to ensure the absence of residual genomic DNA contamination. Non-template controls (H_2O) were also analyzed. Each plate contained an inter-run calibrator in triplicate (dilutions of a pool of all samples). The qPCR thermal cycling included an initial activation step at 95°C for 2 min followed by 39 cycles of 5 s at 95°C and 20 s at 60°C. A melting curve was performed at the end of the amplification phase to confirm the amplification of a single product in each reaction. The corresponding Cq (quantification cycle) value was determined automatically for each sample using the Gene Expression Module of the CFX Manager software (Bio-Rad Laboratories Inc.). Cq is the number of cycles required to yield a detectable fluorescence signal. The CFX Manager Software was used to normalize the relative quantity of messenger with the ΔΔCt method. The **expression level of** elongation factor 1 (***ef1a***) and ribosomal (***28S***) **mRNA** were tested to correct for loading differences or other sampling variations present in each sample. The *ef1a* **mRNA** was used **for normalization** in the liver tissue of juvenile fish since it did not show any significant variation of expression among experimental conditions ($P > 0.05$).

Statistical and data analysis

Relative **mRNA** expressions and enzyme activity data were log10 transformed to obtain normal distributions. The effects of time, temperature, and diet were tested for peroxidation index, relative **mRNA** expression (*pparα*, *pparβ*, *pparγ*, *fas*, *srebp-1*, *fads2*), enzyme activity (CAT, SOD, CS, COX), and MDA content using three-way ANOVAs. Normality and homoscedasticity of data were tested using Shapiro-Wilk and Levene tests, respectively. When appropriate, Tukey mean comparison tests were done because homoscedasticity 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

Liver mass and protein content

Results showing the effect of diet, temperature, and time on liver mass and hepatic protein content are shown in Table 3A. After 720 dd of feeding, liver mass was significantly higher (+48%) in juveniles reared at 20°C than at 15°C, and diet had no significant effect (Table 3A). At 1660 dd, the increase in liver mass was higher for juveniles reared at 20°C (+67%) than for those reared at 15°C (+59%**), while liver mass was still higher (+58%) in juveniles reared at 20°C than at 15°C.** Again, no diet effect was detected. Liver protein concentration decreased significantly over time (0.12 ± 0.01 mg protein mg liver^{-1} at 720 dd vs. 0.10 ± 0.00 mg protein mg liver^{-1} at 1660 dd), and no significant effect of diet or of temperature was observed.

Peroxidation index and MDA

The PI of the RD diet was 34% higher than that of the LD diet (15.86 ± 1.78 vs. 10.52 ± 0.50). Accordingly, the PI in the polar lipids of juveniles fed RD was significantly higher (+18%) compared to fish fed LD (45.36 ± 6.96 vs. 38.50 ± 5.45; Table 3A). The temperature × diet interaction was significant although no difference was detected with the post-hoc test

309 (Table 3A). Surprisingly, MDA content was not affected by diet or temperature; however, MDA
310 content increased with time (2.06 ± 0.48 vs. 2.77 ± 0.45 nmol mg protein^{-1}; Table 3A).

311 ## Oxidative metabolism and antioxidant response

312 Results showing the effect of diet, temperature, and time on oxidative stress indicators
313 are also shown in Table 3A. CS activity remained stable regardless of the experimental
314 treatment, while COX activity varied depending on diet × temperature and degree-days ×
315 temperature interactions. There was no temperature effect on COX activity at 720 dd, but fish
316 raised at 20°C exhibited a higher COX activity at 1660 dd than fish reared at 15°C. COX activity
317 was significantly lower in fish fed RD at 720 dd, although this diet effect was no longer present
318 at 1660 dd. The COX/CS ratio was higher (+15%) in juveniles fed LD than in those fed RD
319 (6.01 ± 1.21 vs. 5.09 ± 1.80**)**. In addition, the COX/CS ratio remained stable over time for fish
320 raised at 15°C but increased in fish raised at 20°C. CAT activity increased slightly but
321 significantly over time at both 15°C and 20°C (70 ± 7.93 vs. 105 ± 27.01**)**. At 20°C, however,
322 juveniles fed the RD diet displayed CAT activity that was more than twice the activity measured
323 in LD-fed fish. At 1660 dd, the diet effect was no longer present. SOD activity was significantly
324 lower (–8%) in juveniles fed RD compared to those fed LD (3.94 ± 0.36 U mg protein^{-1} vs. 4.25
325 ± 0.43 U mg protein^{-1}**)**. SOD activity significantly decreased (–8%) with time (4.25 ± 0.31 U
326 mg protein^{-1} at 1660 dd vs. 3.95 ± 0.47 U mg protein^{-1} at 720 dd). There was also a significant
327 increase in SOD activity (+10%) at the higher temperature (3.91 ± 0.43 U mg protein^{-1} at 15 °C
328 vs. 4.29 ± 0.33 U mg protein^{-1} at 20°C).

329 ## Relative **mRNA** expressions

330 The relative **mRNA** expression of *fads2* increased with time. At 720 dd, the relative
331 **level** of *fads2* **mRNA** was highest at 20°C in juveniles fed LD and lowest at 15°C in juveniles
332 fed RD (Table 3, Fig. 1A). After 1660 dd of feeding, there was no significant increase of the

fads2 **mRNA level** in juveniles fed LD at 15°C or RD at 20°C; however, the **level** was higher in juveniles fed RD at 15°C and LD at 20°C (Table 3B, Fig. 1A). The expressions of other **mRNA** showed no differences according to diet: expressions of *fas*, *pparγ*, and *srebp-1* **mRNA** were significantly lower (158%, 29%, and 24%, respectively) for fish raised at 15°C than at 20°C (Table 3B, Fig. 1B). These expressions were respectively 630%, 91%, and 118% lower at 720 dd than at 1660 dd (Table 3B, Fig. 1B). The highest relative **mRNA** expression for *pparα* was observed at 1600 dd for fish raised at 20°C (Table 3B, Fig. 1B); no treatment effect was observed for the expression of *pparβ* **mRNA** (Table 3B).

Discussion

In a previous study, Gourtay et al. (2018) showed that the depletion of n-3 PUFA and a lower temperature (15°C) contributed to a decreased growth rate in sea bass: the depleted n-3 PUFA diet resulted in lower contents of essential fatty acids (EFA, ARA, DHA, EPA) in muscle even though a higher retention of those FA was noted in fish fed this diet (Gourtay et al., 2018). The aim of the present work was to examine to what extent these diets and temperatures (15°C and 20°C) can affect the expression of mRNA coding for enzymes/transcription factors involved in lipid metabolism and biochemical variables related to antioxidant response in liver. We hypothesized that a diet poor in n-3 PUFA would activate lipid biosynthesis while a diet richer in n-3 PUFA would lead to oxidative stress.

We are aware that our quantitative analyses of transcripts coding for actors involved in lipid metabolism do not necessarily reflect the activities of the proteins and thus cannot be used to infer metabolic activities. However, the relative levels of transcripts reflect the transcriptional regulation of metabolic constituents, which may reflect the sensitivity of the associated metabolic pathway to environmental or dietary factors.

mRNA expression, lipid metabolism, and antioxidant enzymes

Our results based on *fads2* mRNA level quantification only partly support the hypothesis of an activation of the pathway involved in lipid biosynthesis. Indeed, the relative expression of *fads2* **mRNA** was affected by diet, but even though the highest **mRNA level** was observed in LD-fed fish at 20°C on the first sampling date, the situation was completely reversed at the end of the study, making the results difficult to interpret. We observed no effect of diet on *fas, pparα, pparβ, pparγ,* or *srebp-1* mRNA expression. Regulation at translational and post-translational levels certainly cannot be excluded, but data on mRNA expression suggest no diet effects on these indicators at the dietary PUFA levels tested here. This indicates that LD was probably not low enough in PUFA to exert negative effects **at the transcriptional level on these constituents**, even though it did result in a lower growth rate in both length and mass (Gourtay et al., 2018). Comparable conclusions were drawn by Yilmaz et al. (2016) for juvenile sea bass and by Tocher et al. (2006) for cod. Our results contrast with those obtained for sea bass by Vagner et al. (2007a, b, 2009), who showed that the *fads2* **mRNA level** was higher in larvae fed a low PUFA diet (0.5% or 0.7% EPA+DHA, % DM) compared to those fed a high PUFA diet (1.7% or 3.7% EPA+ DHA, % DM). González-Rovira et al. (2009) also showed a significantly higher *fads2* mRNA level in the liver of sea bass fed linseed and rapeseed oils compared to those fed fish oil, while it remained stable in individuals fed a diet based on olive oil. Because all these sea bass studies were based on different dietary formulations and different developmental stages, it is difficult to determine why our results would differ from theirs. As mentioned above, previous studies reporting modulation of the elongation and desaturation pathways used diets that were highly deficient in EPA + DHA (Vagner et al. 2007a, b, 2009). We could have used a diet that would have been more deficient. However, LD was already sufficiently low in n-3 PUFA to significantly slow the growth rate, and an effect on *fads2* mRNA level in the liver was expected. Obviously, the level of depletion

we used does not seem to dramatically affect regulation of the lipid metabolism pathway because the requirements are still met in this species (Skalli et al., 2006), even though this was not tested at all the possible steps from mRNA to the number of proteins.

There is growing evidence that inclusion of high levels of PUFA in fish diet, because of their high degree of unsaturation, can induce oxidative lipid damage (Álvarez et al., 1998; Luo et al., 2012). Thus, we were expecting lower response indicators of oxidative stress in fish fed LD. **The CS enzyme catalyzes the first step of the tricarboxylic acid cycle, while COX has a specific role as terminal electron acceptor in the electron transport system. They are both key enzymes involved in** aerobic metabolism and can be used as indicators of aerobic metabolic capacity (Marie et al. 2006). **The tricarboxylic acid cycle in liver also supports biosynthetic processes such as lipid biosynthesis or gluconeogenesis from malate (Owen et al., 2002; Windisch et al., 2011). Accordingly, the COX to CS ratio can be used to reflect preferred metabolic pathways and relative metabolic adjustments in response to warming in a tissue. Furthermore, changes in COX activity may be related to alterations in mitochondrial membrane structure (Wodtke, 1981; O'Brien and Mueller, 2010) and those in CS activity to changes in mitochondrial matrix volume (e.g., Hardewig et al., 1999; Guderley and St-Pierre, 2002; Guderley, 2004). In our study, COX activity was the lowest in fish fed RD at 720 dd; low COX activity can suggest decreased oxidative capacities, which may support lower metabolic costs with the RD diet.** The COX/CS ratio was higher in LD-fed fish, suggesting higher aerobic metabolism and possibly indicating a functional adaptation of mitochondria to adjust energy demand and metabolic fuel availability (i.e., lipids **biosynthesis**). PI is a theoretical index that is calculated with *in vitro* oxidation rate values (Holman, 1954; Cosgrove et al., 1987; Hulbert et al., 2007), and it does not account for all the *in vivo* processes that occur to deal with ROS production. CAT activity (degradation of hydrogen peroxide into water and oxygen) was twice as high in RD-fed juveniles compared to

those fed LD, and they showed the highest PI—which is consistent with our expectations—but only at 20°C. On the other hand, MDA content remained stable regardless of diet, but we are aware that the method we used was not the most reliable one, even though it is commonly used in fish studies (Mourente et al., 2002; Simonato et al., 2015; Sobjak et al., 2017). However, the activity of SOD (catalyzing the dismutation of superoxide radicals into oxygen or hydrogen peroxide) was higher in juveniles fed LD than in those fed RD, suggesting the presence of more substrate to be catalyzed.

Temperature and diet effects

Higher temperatures in combination with poor n-3 PUFA could affect the lipid metabolism pathway. The relative mRNA expressions of *fas*, *pparγ*, and *srebp-1* increased over time and with acclimation temperature, but the temperature effect was independent of the diet effect, indicating that **a positive transcriptional regulation of** lipid metabolism may occur at high temperatures. The *fads2* **mRNA** expression tended to increase over time, but not significantly due to the high inter-individual variability within experimental treatments at 1660 dd. Such a variability in relative **mRNA** expression has been observed in this species (Geay et al., 2010), and more investigation is required to determine the reasons. Gourtay et al. (2018) showed that higher growth was observed in juveniles fed the same experimental diets at 20°C compared to 15°C, accompanied with higher polar DHA and ARA contents and neutral DHA content (only at 1660 dd for this parameter). **Again, it must be remembered that the quantity of transcripts measured by qPCR does not necessarily reflect the amount of proteins, especially since temperature may affect the transcription–translation relationship. Nevertheless, our results** support the hypothesis that acclimation temperature enhanced the expression of mRNA associated with regulation of the lipid metabolism.

In several organisms including fish, it has been shown that COX and CS activities reflect maximum aerobic metabolic capacity (Childress and Somero, 1979; Thuesen and Childress, 1993, 1994). Elevated temperature stimulates metabolic processes in ectotherms, increasing ATP demand and, therefore, electron transfers along the mitochondrial respiratory chain, possibly explaining the higher COX activity and COX/CS ratio recorded at 20°C. **These results corroborate those of Seebacher et al. (2005) in *Pagothenia borchgrevinki*, which responds to warming by increasing muscle COX activity. The authors suggest that high COX activity comes along with increased oxidative capacities, and these may support elevated metabolic costs at warmer temperatures.** Such circumstances are also likely to enhance ROS production as a side product**, which can imply higher likelihood of oxidative stress, but only if the rates of ROS production exceed the system's capacity to eliminate those ROS that** result in oxidative stress. Increased environmental temperature has been associated with oxidative stress in fish (Parihar and Dubey, 1995; Heise et al., 2006a, b; Lushchak and Bagnyukova, 2006; Bagnyukova et al., 2007). Vinagre et al. (2012) studied the impact of temperature on oxidative stress in sea bass juveniles and found that lipid peroxidation and catalase activity were very sensitive to environmental temperature. Accordingly, SOD activity was higher at 20°C than at 15°C, and the highest CAT activity was observed at 720 dd in fish fed LD at 20°C.

Liver mass increased with time and temperature, but proteins remained relatively stable. This means that total enzymatic activity (per mg of tissue) was related to liver mass only. At 20°C, total enzymatic activity should be higher and thus enable fish to cope with oxidative stress. The absence of change with temperature in the MDA content, which is one of the products of lipid peroxidation (Frankel, 2005), again did not match the original hypothesis. **Some of the variables that we measured showed differences during the experiment without interaction with either diet or temperature. Thus, liver protein concentration and**

liver SOD activity decreased over time in juveniles, while the MDA content and relative mRNA expression of *fads2* increased with time. Whether these modifications related to development or with acclimation to physical experimental conditions is hard to say, but obviously they were independent of diet or temperature conditions.

Conclusion

Our objective was to use long-term experimental conditions with treatment levels that were realistic in terms of a global change scenario to test the hypothesis that a diet poor in n-3 PUFA would activate lipid biosynthesis while a diet richer in n-3 PUFA would lead to oxidative stress and would worsen under higher temperature conditions. We were unable to confirm this working hypothesis. We conclude that both levels of n-3 PUFA tested in this study were high enough so as not to impair lipid metabolism in sea bass juveniles. The combination of a decrease in dietary n-3 PUFA along with an increase in temperature conditions will modify the oxidative stress response, but at a level that juveniles can cope with.

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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).

List of symbols and abbreviations

ARA:	arachidonic acid
CAT:	catalase enzyme
COX:	cytochrome c oxidase enzyme
CS:	citrate synthase enzyme
d-:	days post hatch
dd:	degree-days
DHA:	docosahexaenoic acid
DM:	dry matter
ef1a:	elongation factor 1
EFA:	essential fatty acid
EPA:	eicosapentaenoic acid
FA:	fatty acid
fads2:	delta 6 desaturase gene
fas:	fatty acid synthase gene
LD:	low n-3 polyunsaturated diet
MDA:	malondialdehyde
MUFA:	monounsaturated fatty acid
n-3:	omega 3
n-6:	omega 6
PI:	peroxidation index
ppar:	peroxisome proliferator activated receptors
PUFA:	polyunsaturated fatty acid
qPCR:	quantitative polymerase chain reaction
RD:	reference diet
RIN:	RNA integrity number
ROS:	reactive oxygen species
RT:	reverse transcription
SOD:	superoxide dismutase enzyme
TBARS:	thiobarbituric acid reactive substances

References

Able KW, Wuenschel MJ, Grothues J, Vasslides TM, Rowe P (2013) Do surf zones in New Jersey provide "nursery" habitat for southern fishes? Environ. Biol. Fishes 96, 661-675.

Ackman RG, Tocher CS (1968) Marine phytoplankter fatty acids. J. Fish. Res. Board Can. 25, 1603-1620.

Aebi H (1984) Catalase in vitro. Methods Enzymol. 105, 121-126.

Álvarez MJ, López-Bote CJ, Diez A, Corraze G, Ariel J, Dias J, Kaushik SJ, Bautista JM (1998) Dietary fish oil and digestible protein modify susceptibility to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*). Br. J. Nutr. 80, 281-289.

Arts M, Brett M, Kainz M (2009) Lipids in aquatic ecosystems. New York, USA: Springer.

Bagnyukova TV, Luzhna L.I., Pogribny I.P., Lushchak V.I. (2007) Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. Environ. Mol. Mutagen. 48, 658-665.

Barja G (2007) Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. Rejuvenation Res. 10, 215-223.

Beck MW, Heck KL, Able KW, Childers DL, Egglston DB, Gillanders BM, Halpern BS, Hayes CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The role of nearshore ecosystems as fish and shellfish nurseries. BioScience 51, 633-641.

Bergmeyer HU, Gawehn K, Grassi M (1974) Enzymes as biochemical reagents. In Methods of Enzymatic Analysis. (ed H.U. Bergmeyer), pp 425-522. New York, USA: Academic Press.

Boukouvala E, Antonpoulou E, Favre-Krey L, Diez A, Bautista JM, Leaver MJ, Tocher DR, Krey G (2004) Molecular characterisation of three peroxisome proliferator-activated receptors from the sea bass (*Dicentrarchus labrax*). Lipids 39, 1085-1092.

Brander KM (2007) Global fish production and climate change. PNAS 104, 19709-19714.

514 Childress JJ, Somero GN (1979) Depth-related enzymatic activities in muscle, brain and heart
515 of deep-living pelagic marine teleosts. Mar. Biol. 52, 273-283.

516 Colombo SM, Wacker A, Parrish CC, Kainz MJ, Arts MT (2016) A fundamental dichotomy in
517 long-chain polyunsaturated fatty acid abundance between and within marine and
518 terrestrial ecosystems. Environ. Rev. 25, 163-174.

519 Cosgrove JP, Church DF, Pryor WA (1987) The kinetics of the autoxidation of polyunsaturated
520 fatty acids. Lipids 22, 299-304.

521 Cushing D (1995) Population production and regulation in the sea: A fishery perspective.
522 Cambridge, UK: Cambridge University Press.

523 Dias J, Alvarez MJ, Diez A, Arzel J, Corraze G, Bautista JM, Kaushik SJ (1998) Regulation of
524 hepatic lipogenesis by dietary protein/energy in juvenile European seabass
525 (*Dicentrarchus labrax*). Aquaculture 161, 169-186.

526 **Dong X, Xu H, Mai K, Xu W, Zhang Y, Ai Q (2015) Cloning and characterization of**
527 **SREBP-1 and PPAR-α in Japanese seabass *Lateolabrax japonicus*, and their gene**
528 **expressions in response to different dietary fatty acid profiles. Comp. Biochem.**
529 **Physiol. Part B 180, 48-56.**

530 Figueiredo-Silva AC, Corraze G, Rema P, Sanchez-Gurmaches J, Gutiérrez J, Valente LMP
531 (2009) Blackspot seabream (*Pagellus bogaraveo*) lipogenic and glycolytic pathways
532 appear to be more related to dietary protein level than dietary starch type. Aquaculture
533 291, 101-110.

534 Frankel EN (2005) Lipid oxidation. Bridgewater, UK: Elsevier.

535 Gattuso JP, Magnan A, Billé R, Cheung WWL, Howes EL, Joos F, Allemand D, Bopp L,
536 Cooley SR, Eakin CM, Hoegh-Guldberg O, Kelly RP, Pörtner HO, Rogers AD, Baxter
537 JM, Laffoley D, Osborn D, Rankovic A, Rochette J, Sumaila UR, Treyer S, Turley C
538 (2015) Contrasting futures for ocean and society from different anthropogenic CO2
539 emissions scenarios. Science 349, aac4722.

540 Geay F, Culi ESI, Corporeau C, Boudry P, Dreano Y, Corcos L, Bodin N, Vandeputte M,
541 Zambonino-Infante J-L, Mazurais D, Cahu CL (2010) Regulation of FADS2
542 expression and activity in European sea bass (*Dicentrarchus labrax*, L.) fed a
543 vegetable diet. Comp. Biochem. Physiol. B 156, 237-243.

544 Geay F, Zambonino-Infante J, Reinhardt R, Kuhl H, Santigosa E, Cahu C, Mazurais D (2012)
545 Characteristics of fads2 gene expression and putative promoter in European sea bass
546 (*Dicentrarchus labrax*): Comparison with salmonid species and analysis of CpG
547 methylation. Mar. Genomics 5, 7-13.

548 Ghioni C, Tocher DR, Bell MV, Dick JR, Sargent JR (1999) Low C18 to C20 fatty acid
549 elongase activity and limited conversion of stearidonic acid, 18:4n-3, to
550 eicosapentaenoic acid, 20:5n-3, in a cell line from the turbot, *Scophthalmus maximus*.
551 Biochim. Biophys. Acta 1437, 170-181.

552 Gomez-Requeni P, Mingarro M, Kirchner S, Calduch-Giner JA, Medale F, Corraze G, Panserat
553 S, Martin SAM, Houlihan DF, Kaushik SJ, Pérez-Sáncheza J (2003) Effects of dietary
554 amino acid profile on growth performance, key metabolic enzymes and somatotropic
555 axis responsiveness of gilthead sea bream (*Sparus aurata*). Aquaculture 220, 749-767.

556 González-Rovira A, Mourente G, Zheng X, Tocher DR, Pendón C (2009) Molecular and
557 functional characterization and expression analysis of a Δ6 fatty acyl desaturase cDNA
558 of European sea bass (*Dicentrarchus labrax* L.). Aquaculture 298, 90-100.

559 Gourtay C, Chabot D, Audet C, Le Delliou H, Quazuguel P, Claireaux G, Zambonino-Infante
560 J-L (2018) Will global warming affect the functional need for essential fatty acids in
561 juvenile sea bass (*Dicentrarchus labrax*)? A first overview of the consequences of
562 lower availability of nutritional fatty acids on growth performance. Mar. Biol. 165,
563 https://doi.org/10.1007/s00227–018–3402–3.

564 **Guderley H (2004) Metabolic responses to low temperature in fish muscle. Biol. Rev. 79,**
565 **409-427.**

566 **Guderley H, St-Pierre J (2002) Going with the flow or life in the fast lane: contrasting**
567 **mitochondrial responses to thermal change. J. Exp. Biol. 205, 2237-2249.**

568 Guschina IA, Harwood JL (2006) Mechanisms of temperature adaptation in poikilotherms.
569 FEBS Letters 580, 5477-5483.

570 **Hardewig I, Van Dijk PLM, Moyes CD, Portner HO (1999) Temperature-dependent**
571 **expression of cytochrome-c oxidase in Antarctic and temperate fish. Am. J.**
572 **Physiol.-Reg 277, R508-R516.**

573 Harley CD, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJ, Thornber CS, Rodriguez
574 LF, Williams SL (2006) The impacts of climate change in coastal marine
575 systems. Ecol. Lett. 9, 228-241.

576 Heise K, Puntarulo S, Nikinmaa M, Abele D, Pörtner HO (2006a) Oxidative stress during
577 stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparous*. L.
578 J. Exp. Biol. 209, 353-363.

579 Heise K, Puntarulo S, Nikinmaa M, Lucassen M, Pörtner HO, Abele D (2006b) Oxidative stress
580 and HIF-1 DNA binding during stressful cold exposure and recovery in the North Sea
581 eelpout (*Zoarces viviparus*). Comp. Biochem. Physiol. A 143, 494-503.

582 Hixson SM, Arts MT (2016) Climate warming is predicted to reduce omega-3, long-chain,
583 polyunsaturated fatty acid production in phytoplankton. Global Change Biol. 22, 2744-
584 2755.

585 Holden MJ, Williams T (1974) The biology, movements and populations dynamics of bass,
586 *Dicentrarchus labrax,* in English waters. J. Mar. Biol. Assoc. UK 54, 91-107.

587 Holman RT (1954) Autoxidation of fats and related substances. Prog. Chem. Fats Other Lipids
588 2, 51-98.

589 **Horton JD, Goldstein JL, Brown MS (2002) SREBPs: activators of the complete program**
590 **of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. 109, 1125-1131.**

591 Houde E (2008) Emerging from Hjort's shadow. J. Northwest Atl. Fish. Sci. 41, 53-70.

592 Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008)
593 Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and
594 advances. Plant J. 54, 621-639.

595 Hulbert AJ (2005) On the importance of fatty acid composition of membranes for aging. J.
596 Theor. Biol. 234, 277-288.

597 Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA (2007) Life and death: metabolic rate,
598 membrane composition, and life span of animals. Physiol. Rev. 87, 1175-1213.

599 Jordal AEO, Lie O, Torstensen BE (2007) Complete replacement of dietary fish oil with a
600 vegetable oil blend affects liver lipid and plasma lipoprotein levels in Atlantic salmon
601 (*Salmo salar* L.). Aquaculture Nutr. 13, 114-130.

602 Juanes F (2007) Role of habitat in mediating mortality during the post-settlement transition
603 phase of temperate marine fishes. J. Fish Biol. 70, 661-677.

604 Juliano RA, Deckelbaum RJ, Worgall TS (2004) Fatty acids, cholesterol, and sphingolipids:
605 interactive regulators and targets for SREBP processing. Int. Congr. 1262, 55-58.

606 Jump DB (2002) The biochemistry of n-3 polyunsaturated fatty acids. J. Biol. Chem. 277, 8755-
607 8758.

608 Kawatsu H (1969) Studies on the anemia of fish - III. An example of macrocytic anemia found
609 in brook trout, *Salvelinus fontinalis*. Bull. Freshwater Res. Lab. 19, 161-167.

610 Kelley DF (1988) The importance of estuaries for sea bass *Dicentrarchus labrax* (L.). J. Fish
611 Biol. 33, 25-33.

612 Lang I, Hodac L, Friedl T, Feussner I (2011) Fatty acid profiles and their distribution patterns
613 in microalgae: A comprehensive analysis of more than 2000 strains from the SAG
614 culture collection. BMC Plant Biol. 11, 1-16.

615 Leaver MJ, Boukouvala E, Antonopoulou E, Diez A, Favre-Krey L, Ezaz MT, Bautista JM,
616 Tocher DR, Krey G (2005) Three peroxisome proliferator-activated receptor isotypes
617 from each of two species of marine fish. Endocrin.146, 3150-3162.

618 Leaver MJ, Ezaz MT, Fontagne S, Tocher DR, Boukouvala E, Krey G (2007) Multiple
619 peroxisome proliferator-activated receptor beta subtypes from Atlantic salmon (*Salmo*
620 *salar*). J. Mol. Endocrin. 38, 391-400.

621 Lehodey P, Alheit J, Barange M, Baumgartner T, Beaugrand G, Drinkwater K, Fromentin JM,
622 Hare SR, Ottersen G, Perry RI, Royj C, van der Lingenk CD, Wernerl F (2006) Climate
623 variability, fish, and fisheries. J. Climate 19, 5009-5030.

624 Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological
625 ecology. Annu. Rev. Physiol. 68, 253-278.

626 Lindi V, Schwab U, Louheranta A, Laakso M, Vessby B, Hermansen K, Storlien L, Riccardi
627 G, Rivellese AA, KANWU Study Group (2003) Impact of the Pro12Ala
628 polymorphism of the PPAR-c2 gene on serum triacylglycerol response to n23 fatty
629 acid supplementation. Mol. Genet. Metab. 79, 52-60.

630 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin
631 phenol reagent. J. Biol. Chem. 193, 265-275.

632 Luo Z, Tan XY, Li XD, Yin GJ (2012) Effect of dietary arachidonic acid levels on growth
633 performance, hepatic fatty acid profile, intermediary metabolism and antioxidant
634 responses for juvenile *Synechogobius hasta*. Aquaculture Nutr. 18, 340-348.

635 Lushchak VI, Bagnyukova TV (2006) Effects of different environmental oxygen levels on free
636 radical processes in fish. Comp. Biochem. Physiol. B 144, 283-289.

637 MacLaren LA, Guzeloglu A, Michel F, Thatcher WW (2006) Peroxisome proliferator-activated
638 receptor (PPAR) expression in cultured bovine endometrial cells and response to
639 omega-3 fatty acid, growth hormone and agonist stimulation in relation to series 2
640 prostaglandin production. Domest. Anim. Endocrin. 30, 155-169.

641 Marie B, Genard B, Rees J F, Zal F (2006) Effect of ambient oxygen concentration on activities
642 of enzymatic antioxidant defences and aerobic metabolism in the hydrothermal vent
643 worm, *Paralvinella grasslei*. Mar. Biol. 150, 273-284.

644 Mourente G, Dıaz-Salvago E, Bell JG, Tocher DR (2002) Increased activities of hepatic
645 antioxidant defence enzymes in juvenile gilthead sea bream (*Sparus aurata* L.) fed
646 dietary oxidised oil: attenuation by dietary vitamin E. Aquaculture 214, 343-361.

647 Mourente G, Tocher DR (1994) In vivo metabolism of [1-14C] linolenic acid (18:3n-3) and [1-
648 14C] eicosapentaenoic acid (20:5n-3) in marine fish: time-course of the desaturation /
649 elongation pathway. Biochim. Biophys. Acta 1212, 109-118.

650 Murai T, Andrews JW (1974) Interaction of dietary α-tocopherol, oxidized menhaden oil and
651 ethoxyquin on channel catfish (*Ictalurus punctatus*). J. Nutr. 104, 1416-1431.

652 Nakamura M, Nara T (2002) Gene regulation of mammalian desaturases. Biochem. Soc. Trans.
653 30, 1076-1079.

654 Nakamura M, Nara T (2003) Essential fatty acid synthesis and its regulation in mammals.
655 Prostag. Leukotr. Ess. 68, 145-150.

656 **O'Brien KM, Mueller IA (2010) The unique mitochondrial form and function of Antarctic**
657 ***channichthyid* icefishes. Int. Comp. Biol. 50, 993-1008.**

658 Owen JM, Adron JW, Middleton C, Cowey CB (1975) Elongation and desaturation of dietary
659 fatty acids in turbot (*Scophthalmus maximus*) and rainbow trout (*Salmo gairdneri*).
660 Lipids 10, 528-531.

661 **Owen OE, Kalhan SC, Hanson RW (2002) The key role of anaplerosis and cataplerosis**
662 **for citric acid cycle function. J. Biol. Chem. 277, 30409-30412.**

663 Pamplona R, Portero-Otin M, Ruiz C, Gredilla R, Herrero A, Barja G (2000) Double bond
664 content of phospholipids and lipid peroxidation negatively correlate with maximum
665 longevity in the heart of mammals. Mech. Ageing Dev. 112, 169-183.

666 Parihar MS, Dubey AK (1995) Lipid peroxidation and ascorbic acid status in respiratory organs
667 of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature
668 increase. Comp. Biochem. Physiol. C 112, 309-313.

669 Renaud SM, Thinh LV, Lambrinidis G, Parry DL (2002) Effect of temperature on growth,
670 chemical composition and fatty acid composition of tropical Australian microalgae
671 grown in batch cultures. Aquaculture 211, 195-214.

672 Rijnsdorp AD, Peck MA, Engelhard GH, Möllmann C, Pinnegar JK (2009) Resolving the effect
673 of climate change on fish populations. ICES J. Mar. Sci. 66, 1570-1583.

674 Sakai T, Murata H, Endo M, Shimomura T, Yamauchi K, Ito T, Yamaguchi T, Nakajima H,
675 Fukudome M (1998) Severe oxidative stress is thought to be a principal cause of
676 jaundice of yellowtail *Seriola quinqueradiata*. Aquaculture 160, 205-214.

677 Santigosa E, Saénz de Rodrigáñez MÁ, Rodiles A, Barroso FG, Alarcón FJ (2010) Effect of
678 diets containing a purified soybean trypsin inhibitor on growth performance, digestive
679 proteases and intestinal histology in juvenile sea bream (*Sparus aurata* L.).
680 Aquaculture Res. 41, e187-e198.

681 Sargent JR, Tocher DR, Bell JG (2002) The lipids. In Fish Nutrition 3rd ed. (ed. J.E. Halver,
682 R.W. Hardy), pp 181-257. San Diego, California: Elsevier.

683 **Seebacher F, Davison W, Lowe CJ, Franklin CE (2005) A falsification of the thermal**
684 **specialization paradigm: compensation for elevated temperatures in Antarctic**
685 **fishes. Biol. Lett. 1, 151-154.**

686 Simonato JD, Mela M, Doria HB, Guiloski IC, Randi MA, Carvalho PS, Meletti PC, Silva de
687 Assis HC, Bianchini A, Martinez CB (2015). Biomarkers of waterborne copper
688 exposure in the neotropical fish *Prochilodus lineatus*. Aquatic Tox. 170, 31-41.

689 Skalli A, Robin JH, Le Bayon N, Le Delliou H, Person-Le Ruyet J (2006) Impact of essential
690 fatty acid deficiency and temperature on tissues' fatty acid composition of European
691 sea bass (*Dicentrarchus labrax*). Aquaculture 255, 223-232.

692 Sobjak, TM, Romão S, do Nascimento CZ, dos Santos AFP, Vogel L, Guimarães ATB (2017).
693 Assessment of the oxidative and neurotoxic effects of glyphosate pesticide on the
694 larvae of *Rhamdia quelen* fish. Chemosphere 182, 267-275.

695 Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull.
696 Mar. Sci. 60, 1129-1157.

697 Sprecher H (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. BBA-Mol. Cell
698 Biol. Lett. 1486, 219-231.

699 Srere PA (1969) Citrate synthase. Methods Enzymol. 13, 3-11.

700 Thompson PA, Guo MX, Harrison PJ, Whyte JNC (1992) Effects of variation in temperature.
701 II. On the fatty acid composition of eight species of marine phytoplankton. J. Phycol.
702 28, 488-497.

703 Thuesen EV, Childress JJ (1993) Metabolic rates, enzyme activities and chemical compositions
704 of some deep-sea pelagic worms, particularly *Nectonemertes mirabilis* (Nemertea;
705 Hoplonemerinea) and *Poeobius meseres* (Annelida; Polychaeta). Deep-Sea Res. I 40,
706 937-951.

707 Thuesen EV, Childress JJ (1994) Oxygen consumption rates and metabolic enzyme activities
708 of oceanic California medusae in relation to body size and habitat depth. Biol. Bull.
709 187, 84-98.

710 Tocher DR, Ghioni C (1999) Fatty acid metabolism in marine fish: low activity of Δ5
711 desaturation in gilthead sea bream (*Sparus aurata*) cells. Lipids 34, 433-440.

712 Tocher DR, Zheng X, Christian S, Hastings N, Dick JR, Teale AJ (2006) Highly unsaturated
713 fatty acid synthesis in marine fish: Cloning, functional characterization, and nutritional
714 regulation of fatty acyl Δ6 desaturase of Atlantic cod (*Gadus morhua* L.). Lipids 41,
715 1003-1016.

716 Uchiyama M, Mihara M (1978) Determination of malonaldehyde in tissues by thiobarbituric
717 acid test. Anal. Biochem. 86, 271-278.

718 Vagner M, Robin JH, Zambonino-Infante J-L, Person-Le Ryuet J (2007a) Combined effect of
719 dietary HUFA level and temperature on sea bass (*Dicentrarchus labrax*) larvae
720 development. Aquaculture 266, 179-190.

721 Vagner M, Robin JH, Zambonino-Infante J-L, Tocher DR, Person-Le Ruyet J (2009) Ontogenic
722 effects of early feeding of sea bass (*Dicentrarchus labrax*) larvae with a range of
723 dietary n-3 highly unsaturated fatty acid levels on the functioning of polyunsaturated
724 fatty acid desaturation pathways. Br. J. Nutr. 101, 1452-1462.

725 Vagner M, Zambonino-Infante J-L, Robin JH, Person-Le Ruyet J (2007b) Is it possible to
726 influence European sea bass (*Dicentrarchus labrax*) juvenile metabolism by a
727 nutritional conditioning during larval stage? Aquaculture, 267, 165-174.

728 Vinagre C, Ferreira T, Matos L, Costa MJ, Cabral HN (2009) Latitudinal gradients in growth
729 and spawning of sea bass *Dicentrarchus labrax* (L.) and their relationship with
730 temperature and photoperiod. Estuar. Coast. Shelf Sci. 81, 375-380.

731 Vinagre C, Madeira D, Narciso L, Cabral H, Diniz M (2012) Impact of climate change on
732 coastal versus estuarine nursery areas: cellular and whole-animal indicators in juvenile
733 seabass *Dicentrarchus labrax*. Mar. Ecol. Prog. Ser. 464, 237-243.

734 Watanabe T, Takashima F, Ogino C, Hibiya T (1970) Effect of α-tocopherol on carp. Bull.
735 Japan. Soc. Sci. Fish 36, 623-630.

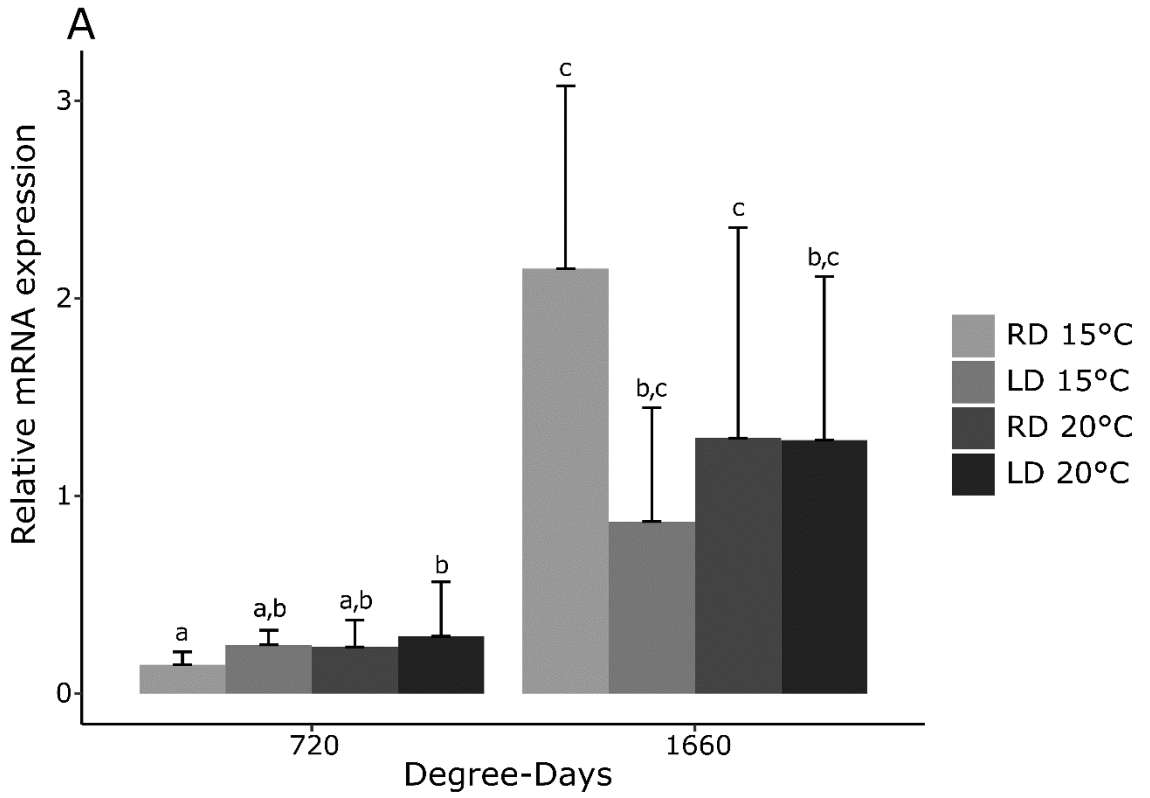
736 **Windisch HS, Kathöver R, Pörtner HO, Frickenhaus S, Lucassen M (2011) Thermal**
737 **acclimation in Antarctic fish: transcriptomic profiling of metabolic pathways.**
738 **American J. Physiol.-Reg. 5, R1453-R1466.**

739 **Wodtke E (1981) Temperature adaptation of biological membranes. Compensation of the**
740 **molar activity of cytochrome c oxidase in the mitochondrial energy-transducing**
741 **membrane during thermal acclimation of the carp (*Cyprinus carpio* L.). Bioch.**
742 **Bioph. Acta, 640, 710-720.**

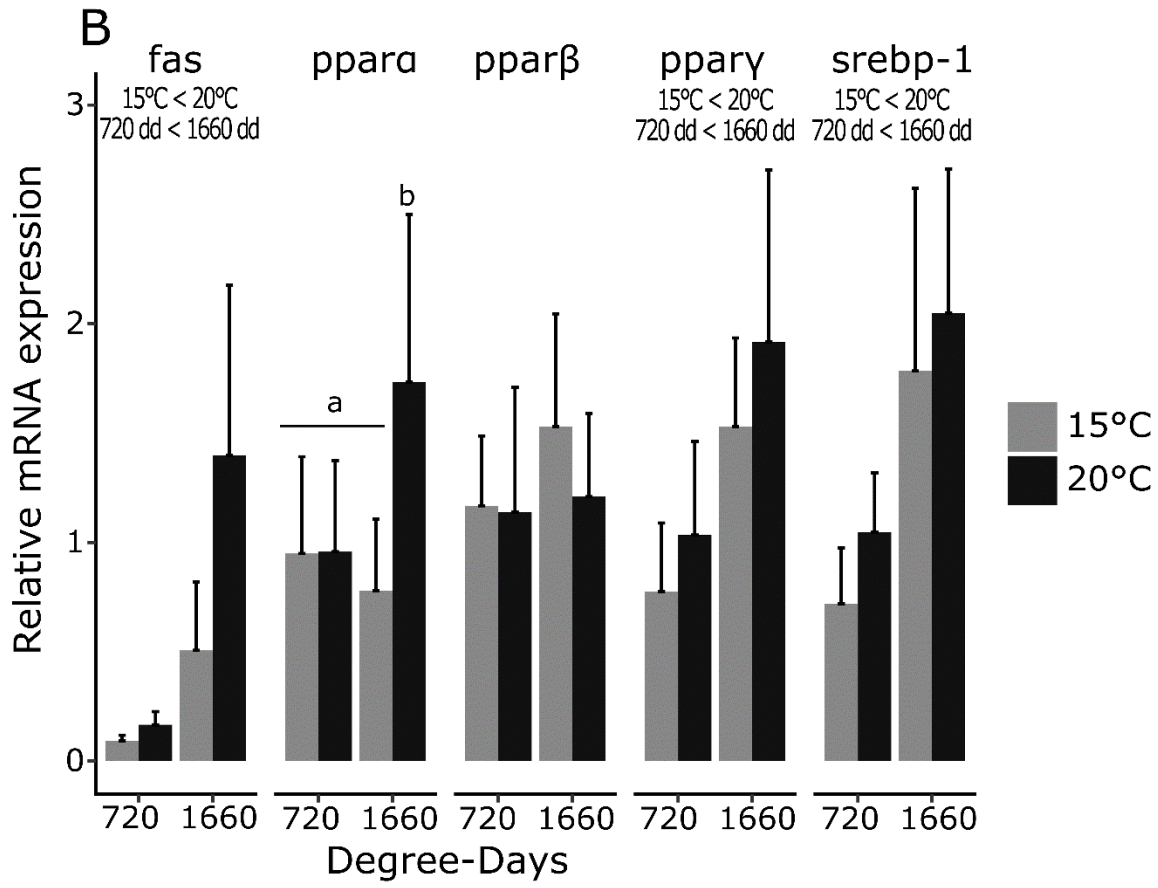
743 Yilmaz HA, Corraze G, Panserat S, Eroldoğan OT (2016) Effects of alternate feeding with
744 different lipid sources on fatty acid composition and bioconversion in European sea
745 bass (*Dicentrarchus labrax*). Aquaculture 464, 28-36.

Figures

Figure 1: Results of three-way ANOVAs on lipid metabolism. A) Interaction between diet, time (in degree-days [dd]), and temperature **on *fads2* mRNA expression level**. B) Effect of time (dd) and temperature on relative **mRNA** expression for *fas*, *pparα*, *pparβ*, *pparγ*, and *srebp-1*. When factor interactions were significant, groups were compared with Tukey post-hoc tests ($\alpha = 0.05$); significantly different groups were assigned different letters. Bars represent means and error bars are standard deviations; N = 6.


A
Relative mRNA expression
0
1
2
3
a
a,b
a,b
b
c
b,c
c
b,c
720
1660
Degree-Days
RD 15°C
LD 15°C
RD 20°C
LD 20°C

753


B
fas
pparα
pparβ
pparγ
srebp-1
15°C < 20°C
720 dd < 1660 dd
Relative mRNA expression
0
1
2
3
a
b
720
1660
Degree-Days
15°C
20°C

754

Tables

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

	Low n-3 PUFA diet	Reference n-3 PUFA diet
	LD	RD
	Proximal composition (%DM)	
Dry matter	94.84	95.12
Ash	5.16	4.88
Proteins	50.48	50.23
Nitrogen Free Extract (NFE)[a]	22.38	23.26
Total lipids	21.98	21.63
Triglycerides	16.99	17.05
Phospholipids	4.70	4.71
Energy (kJ)[b]	2045.41	2042.73
	% of total lipids in diet	
SFA	2.18	2.97
Monosaturated FA	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	0.03	0.07
20:5n-3	0.28	0.94
22:5n-3	0.03	0.07

22:6n-3	0.45	0.71

761 [a] Calculated as 100 – (%proteins + %total lipids + %ash)

762 [b] Calculated as %proteins × 16.7 kgJ g^{-1} + %total lipids × 37.7 kgJ g^{-1} + NFE × 16.7 kgJ g^{-1}

763 Each diet (LD and RD) was manufactured in a single batch of 50 kg to supply the entire
764 experiment. Since diets were only produced once, there was no variation during the experiment.

765

766 Table 2: Specific primers used for quantitative PCR with Genbank accession numbers

Gene name (abbreviation)	**Accession number**	**Forward primer sequence (5′-3′)**	**Reverse primer sequence (5′-3′)**
28S ribosomal RNA (*28S*)	CBXY010007006	TTTCCCATGAGAGAGCAGGT	TCAGATGCGCTTCTTAGGATGT
Elongation factor 1 a (*ef1a*) *	AJ866727.1	GCTTCGAGGAAATCACCAAG	CAACCTTCCATCCCTTGAAC
Fatty acid synthase (*fas*)	MF566098.1	CCTCTGAACCTGGTCTGGTG	ATTGGAGAGAGCCTCCACGA
Delta-6-desaturase (*fads2*)	EU439924.1	AGCATCACGCTAAACCCAAC	CAAGCCAGATCCACCCAGTC
Peroxisome proliferator activated receptors alpha (*pparα*)	AJ880087.1	ACCTCAGCGATCAGGTGACT	AACTTCGGCTCCATCATGTC
Peroxisome proliferator activated receptors beta (*pparβ*)	AJ880088.1	GCTCGGATCTGAAGACCTTG	TGGCTCCATACCAAACCACT
Peroxisome proliferator activated receptors gamma (*pparγ*)	AY590303	CAGATCTGAGGGCTCTGTCC	CCTGGGTGGGTATCTGCTTA
Sterol regulatory element binding protein 1 (*srebp-1*)	FN677951	CTGGAGCCAAAACAGAGGAG	GACAGGAAGGAGGGAGGAAG

767 *Used as housekeeping gene

Table 3: A) Effect of diet, temperature, and time on liver oxidative stress indicators (N=3) and B) lipid metabolism (N=6). When factor interactions were significant, groups were compared with Tukey post-hoc tests ($\alpha = 0.05$); significantly different groups were assigned different letters. RD: reference diet, LD: low n-3 polyunsaturated fatty acid diet, dd: degree-days, Temp.: temperature.

A	720 Degree-days				1660 Degree-days				Three-way ANOVA			
	15 °C		20°C		15 °C		20°C					
	RD	LD	RD	LD	RD	LD	RD	LD	Time	Temperature	Diet	Interaction
Liver mass (g)	0.3 ± 0.02^{a}	0.31 ± 0.01^{a}	0.62 ± 0.07^{bc}	0.55 ± 0.14^{b}	0.86 ± 0.07^{c}	0.61 ± 0.06^{bc}	1.77 ± 0.4^{d}	1.74 ± 0.19^{d}	**n.s.**	**n.s.**	**n.s.**	Time × Temp. × Diet $P < 0.05$ $F(1,16) = 4.89$
Protein (mg prot mg liver $^{-1}$)	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0	0.11 ± 0.02	0.11 ± 0	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	$P < 0.001$ $F(1,16) = 24.71$ 1600 dd < 720 dd	**n.s.**	**n.s.**	**n.s.**
CAT (mU mg prot^{-1})	60.05 ± 2.9^{a}	69.62 ± 4.59^{ab}	78.28 ± 1.94^{bc}	143.37 ± 22.97^{e}	78.12 ± 5.82^{bc}	73.62 ± 4.04^{ab}	103.75 ± 7.3^{d}	97.68 ± 8.5^{cd}	**n.s.**	**n.s.**	**n.s.**	Time × Temp. × Diet $P < 0.001$ $F(1,16) = 11.67$
SOD (U mg prot^{-1})	3.98 ± 0.36	4.35 ± 0.17	4.09 ± 0.08	4.56 ± 0.23	3.47 ± 0.22	3.84 ± 0.45	4.22 ± 0.18	4.27 ± 0.57	$P < 0.05$ $F(1,16) = 5.83$ 1600 dd < 720 dd	$P < 0.01$ $F(1,16) = 8.86$ 15°C < 20°C	$P < 0.05$ $F(1.16) = 5.66$ RD < LD	**n.s.**
COX (mU mg prot^{-1})	46.5 ± 13.16	65.3 ± 3.97	39.12 ± 8.78	71.04 ± 6.38	67.9 ± 9.52	61.84 ± 11.06	98.39 ± 2.04	88.16 ± 6.61	**n.s.**	**n.s.**	**n.s.**	Time × Temp. $P < 0.01$ $F(1,16) = 16.63$ 15°C 20°C 720 dd ≤ 15°C 1660 dd ≤ 20°C 1660 dd
CS (mU mg prot^{-1})	9.75 ± 1.06	10.22 ± 0.64	12.84 ± 1.29	13.53 ± 5.24	13.6 ± 0.8	11.99 ± 1.16	13.12 ± 0.43	12.38 ± 1.41	**n.s.**	**n.s.**	**n.s**	Time × Diet $P < 0.001$ $F(1,16) = 8.26$ RD 720 dd < other groups **n.s.**
COX/CS	4.72 ± 0.98	6.4 ± 0.48	3.11 ± 1.02	5.61 ± 1.4	5.03 ± 0.96	5.14 ± 0.64	7.51 ± 0.39	7.22 ± 1.3	**n.s.**	**n.s.**	**P < 0.05 F(1.16) = 7.56 RD < LD**	Time × Temp. $P < 0.001$ $F(1,16) = 16.63$ 20°C 720 dd ≤ 15°C 720 dd 1660 dd ≤ 20°C 1660 dd
MDA	0.27 ± 0.04	0.3 ± 0.08	0.23 ± 0.08	0.21 ± 0.02	0.28 ± 0.06	0.28 ± 0.03	0.32 ± 0.05	0.25 ± 0.03	**n.s.**	**n.s.**	**n.s.**	**n.s.**
PI	41.88 ± 3.07	37.90 ± 1.83	46.33 ± 3.31	40.73 ± 1.05	54.07 ± 2.07	38.24 ± 10.44	39.15 ± 7.12	37.13 ± 6.25	**n.s.**	**n.s.**	$P < 0.01$ $F(1,16) = 9.90$ LD < RD	Time × Temp. $P < 0.05$ $F(1,16) = 7.16$ Tukey test not significant

773

774

B	720 Degree-days				1660 Degree-days				Three-way ANOVA*		
	15°C		20°C		15°C		20°C				
	RD	LD	RD	LD	RD	LD	RD	LD	Temperature	Time	Interactions
fads2	0.15 ± 0.07^{a}	0.25 ± 0.07^{ab}	0.24 ± 0.14^{ab}	0.29 ± 0.28^{b}	2.15 ± 0.93^{c}	0.87 ± 0.58^{bc}	1.29 ± 1.07^{bc}	1.28 ± 0.83^{c}	n.s.	n.s.	Time × Temp. × Diet $P < 0.05$ $F(1,38) = 4.47$
fas	0.1 ± 0.02	0.09 ± 0.03	0.15 ± 0.02	0.18 ± 0.08	0.7 ± 0.31	0.35 ± 0.23	1.36 ± 0.86	1.43 ± 0.79	$P < 0.001$ $F(1,38) = 30.37$ 15°C < 20°C	$P < 0.001$ $F(1,38) = 153.77$ 720 dd < 1660 dd	n.s.
pparα	0.9 ± 0.42	1 ± 0.5	0.93 ± 0.28	0.99 ± 0.55	0.74 ± 0.22	0.82 ± 0.42	1.89 ± 1.05	1.6 ± 0.5	n.s.	n.s.	Time × Temp. $P < 0.01$ $F(1,38) = 9.91$ Other groups < 20°C 1660 dd
pparβ	1.09 ± 0.31	1.25 ± 0.33	1.22 ± 0.55	1.06 ± 0.63	1.82 ± 0.66	1.29 ± 0.2	1.28 ± 0.47	1.15 ± 0.32	n.s.	n.s.	n.s.
pparγ	0.83 ± 0.37	0.72 ± 0.27	1.22 ± 0.46	0.85 ± 0.33	1.68 ± 0.33	1.4 ± 0.45	2.13 ± 0.95	1.74 ± 0.66	$P < 0.05$ $F(1,38) = 4.68$ 15°C < 20°C	$P < 0.001$ $F(1,38) = 38.46$ 720 dd < 1660 dd	n.s.
Srebp-1	0.77 ± 0.32	0.67 ± 0.18	1.12 ± 0.28	0.98 ± 0.27	2.13 ± 0.89	1.49 ± 0.74	2.12 ± 0.66	1.99 ± 0.71	$P < 0.05$ $F(1,38) = 6.95$ 15°C < 20°C	$P < 0.001$ $F(1,38) = 43.41$ 720 dd < 1660 dd	n.s.

775 *No significant effect of diet was observed; diet column has been removed from the table.

Figure1A

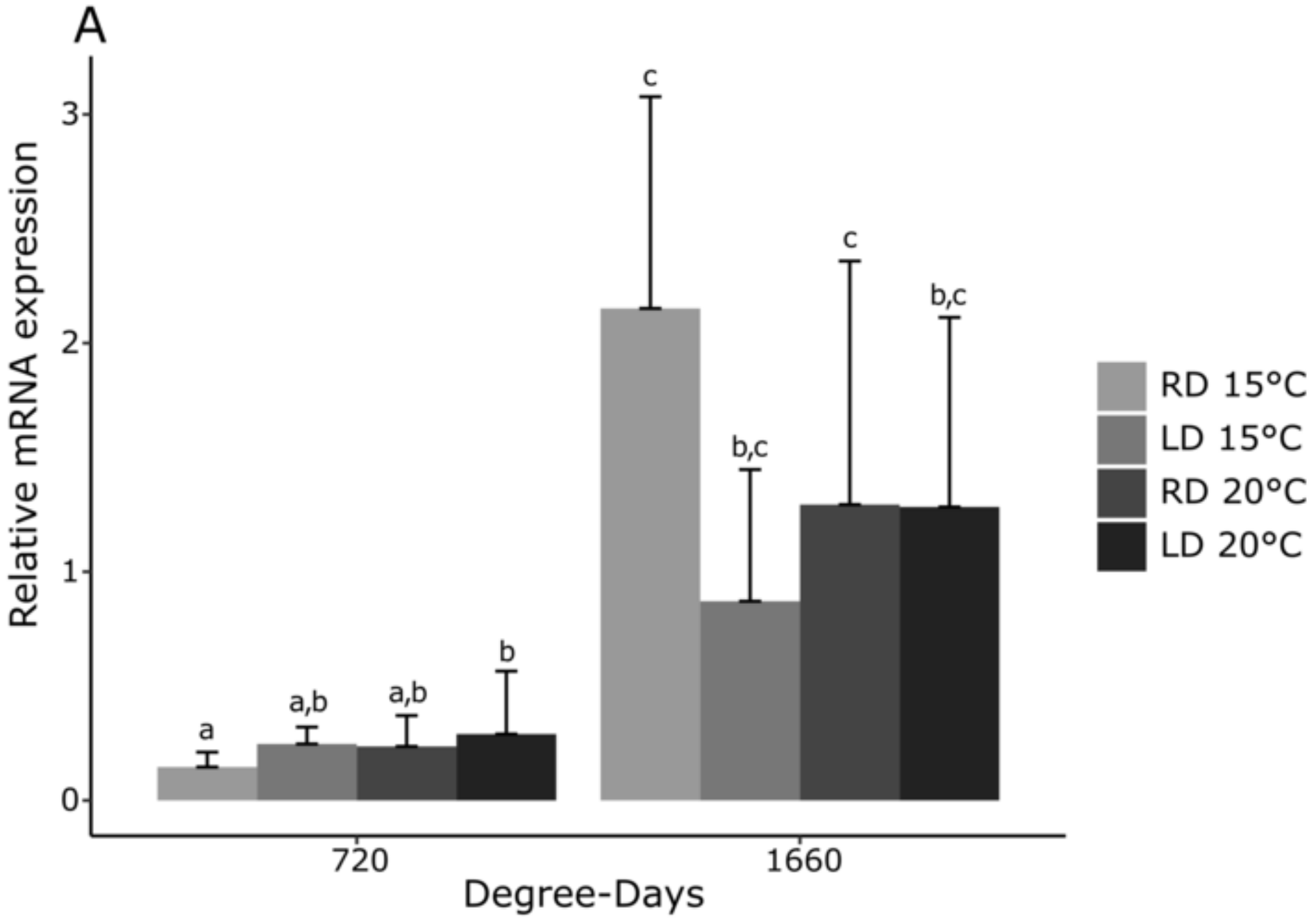


Figure1B

Click here to access/download;Figure;fig B mRNA.png