



HAL
open science

The relationship between membrane fatty acid content and mitochondrial efficiency differs within- and between-omega-3 dietary treatments

Karine Salin, Margaux Mathieu-Resuge, Nicolas Graziano, Emmanuel Dubillot, Fabienne Le Grand, Philippe Soudant, Marie Vagner

► To cite this version:

Karine Salin, Margaux Mathieu-Resuge, Nicolas Graziano, Emmanuel Dubillot, Fabienne Le Grand, et al.. The relationship between membrane fatty acid content and mitochondrial efficiency differs within- and between- omega-3 dietary treatments. *Marine Environmental Research*, 2021, pp.105205. 10.1016/j.marenvres.2020.105205 . hal-02999004

HAL Id: hal-02999004

<https://hal.science/hal-02999004v1>

Submitted on 5 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **TITLE:** The relationship between membrane fatty acid content and mitochondrial efficiency differs
2 within- and between- omega-3 dietary treatments

3

4 **AUTHORS:**

5 Karine Salin^{1*}, Margaux Mathieu-Resuge², Nicolas Graziano^{1,3}, Emmanuel Dubillot³, Fabienne Le
6 Grand¹, Philippe Soudant¹, Marie Vagner^{1,3}

7

8 **AFFILIATIONS:**

9 ¹ Univ Brest, Ifremer, CNRS, IRD, LEMAR, F-29280, Plouzané, France

10 ² WasserCluster Lunz — Inter-University Centre for Aquatic Ecosystem Research, Dr. Carl Kupelwieser
11 Promenade 5 A-3293 Lunz am See, Austria

12 ³ UMR 7266 LIENSs, 2 rue Olympe de Gouges 17000 La Rochelle, France

13

14 ***CORRESPONDING AUTHOR:** Karine Salin; Email: Karine.salin@ifremer.fr

15

16 **RUNNING TITLE:** n-3 HUFA levels alter mitochondrial efficiency

17

18 **KEYWORDS:** ATP/O ratio, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), food quality,
19 *Chelon auratus*, global change.

20 **ABSTRACT**

21 An important, but underappreciated, consequence of climate change is the reduction in
22 crucial nutrient production at the base of the marine food chain: the long-chain omega-3 highly
23 unsaturated fatty acids (n-3 HUFA). This can have dramatic consequences on consumers, such as fish
24 as they have limited capacity to synthesise n-3 HUFA *de novo*. The n-3 HUFA, such as
25 docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), are critical for the
26 structure and function of all biological membranes. There is increasing evidence that fish will be
27 badly affected by reductions in n-3 HUFA dietary availability, however the underlying mechanisms
28 remain obscure. Hypotheses for how mitochondrial function should change with dietary n-3 HUFA
29 availability have generally ignored ATP production, despite its importance to a cell's total energetics
30 capacity, and in turn, whole-animal performance. Here we (i) quantified individual variation in
31 mitochondrial efficiency (ATP/O ratio) of muscle and (ii) examined its relationship with content in
32 EPA and DHA in muscle membrane of a primary consumer fish, the golden grey mullet *Chelon*
33 *auratus*, receiving either a high or low n-3 HUFA diet. Mitochondria of fish fed on the low n-3 HUFA
34 diet had higher ATP/O ratio than those of fish maintained on the high n-3 HUFA diet. Yet,
35 mitochondrial efficiency varied up about 2-fold among individuals on the same dietary treatment,
36 resulting in some fish consuming half the oxygen and energy substrate to produce the similar amount
37 of ATP than conspecific on similar diet. This variation in mitochondrial efficiency among individuals
38 from the same diet treatment was related to individual differences in fatty acid composition of the
39 membranes: a high ATP/O ratio was associated with a high content in EPA and DHA in biological
40 membranes. Our results highlight the existence of interindividual differences in mitochondrial
41 efficiency and its potential importance in explaining intraspecific variation in response to food chain
42 changes.

43 1. Introduction

44 Fish provide critical sustenance for millions of people worldwide and have far reaching impacts on
45 the productivity of ecosystems (McIntyre et al., 2016). Yet, ongoing and future climate change
46 threatens the persistence of fish populations globally (Pörtner and Knust, 2007). An important, but
47 underappreciated, consequence of climate change is the reduction in production at the base of the
48 food chain of essential nutrient: the long-chain omega-3 highly unsaturated fatty acids (n-3 HUFA)
49 (da Motta Pacheco et al., 2014; Galloway and Winder, 2015; Hixson and Arts, 2016). **Water warming**
50 **(Hixson and Arts, 2016), acidification (Bermudez et al., 2015), or UV irradiation (Kang, 2011), all affect**
51 **n-3 HUFA primary producers physiology and community assemblages, leading to a dominance of n-3**
52 **HUFA-impooverished taxa (Galloway and Winder, 2015).** The n-3 HUFA such as eicosapentaenoic acid
53 (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are essential to the structure and function
54 of all biological membranes and are thus considered to be important drivers of organism
55 performance (Hulbert et al., 2005; Ishizaki et al., 2001; Mazorra et al., 2003; Vagner et al., 2015).
56 Endogenous biosynthesis of n-3 HUFA from precursors is limited in most vertebrates, including
57 marine fish (Alimuddin et al., 2005; Arts and Kohler, 2009; Oboh et al., 2017). Changes in the n-3
58 HUFA availability in the fish diet causes strongly correlated changes in the fatty acid composition of
59 their biological membranes (Guderley et al., 2008; Nogueira et al., 2001; Ramsey et al., 2005). Fish on
60 n-3 HUFA-deficient diets can perform badly: reductions in EPA and DHA dietary content **reduced**
61 **growth (Norambuena et al., 2015; Vagner et al., 2015) and thermal tolerance (Vagner et al., 2014),**
62 **and altered whole-organism metabolic traits (Vagner et al., 2015; Vagner et al., 2014).** Determining
63 what causes animal performance to vary with n-3 HUFA diet constitutes a fundamental step in
64 predicting the impacts of projected oceanic changes on fish resilience (Kang, 2011).

65 Consideration of mitochondrial capacity to make ATP may improve our understanding of the
66 links between dietary n-3 HUFA availability and whole-animal performance. Mitochondrial ATP is
67 produced via oxidative phosphorylation, a process through which energy substrates are oxidized to
68 generate a protonmotive force that drives the phosphorylation of ADP to ATP. Hypotheses for how
69 mitochondrial function should change with dietary n-3 HUFA availability have focused on variation in
70 respiratory capacities and have generally ignored variation in ATP production (Kraffe et al., 2007;
71 Ramsey et al., 2005; but see Herbst, 2014; Vagner et al., 2015; Vagner et al., 2014). Although ATP
72 production depends on the rate of substrate oxidation, the number of ATP molecules produced for
73 each atom of oxygen consumed by the mitochondria during substrate oxidation (ATP/O ratio) can
74 vary (Brand, 2005; Salin et al., 2015). A fraction of the protonmotive force that is generated from
75 substrate oxidation is dissipated through proton leak across mitochondrial inner membranes and this
76 leakage can decrease the protonmotive force available to produce ATP (Brand, 2005; Kadenbach,
77 2003). Thus, the **greater** the mitochondria leak, the less efficiently an animal converts its metabolic

78 substrates into ATP, and the **lower** the ATP/O ratio. The proportion of energy dissipated in the
79 proton leak and the efficiency to make ATP vary among conspecific (Bottje and Carstens, 2009;
80 Robert and Bronikowski, 2010; Salin et al., 2016b) and can be influenced by environmental factors
81 including diet (Fontaine et al., 1996; Salin et al., 2018). A number of studies have found positive links
82 between intraspecific heterogeneity in efficiency to produce mitochondrial ATP and the whole-
83 organism performance, such as locomotory performance (Coen et al., 2012; Distefano et al., 2018;
84 but see Jahn and Seebacher, 2019), developmental rate (Salin et al., 2012), growth efficiency (Bottje
85 and Carstens, 2009; Salin et al., 2019) and reproductive output (Robert and Bronikowski, 2010),
86 suggesting that it might be a trait of ecological relevance.

87 Recent research has recognized the importance of accounting for individual heterogeneity in
88 predicting responses to global changes (Hamel et al., 2018). Because some individuals perform much
89 better than others **within** the same environment, individual heterogeneity is likely to directly
90 influence the potential for species to evolve adaptations for a reduced n-3 HUFA availability. A
91 number of studies have found positive links between n-3 HUFA content in membranes and
92 mitochondrial proton leak when comparing among species (Brand et al., 1994; Brookes et al., 1998),
93 but contradictory results were found when comparing treatment groups with studies reporting
94 positive (Martin et al., 2013), negative (Fontaine et al., 1996; Guderley et al., 2008) or no
95 relationships (Guderley et al., 2008) between content in n-3 HUFA in mitochondrial membranes and
96 leak respiration. N-3 HUFA are thought to have important effects on the mitochondrial capacity to
97 make ATP; but until now, there has, to our knowledge, been no assessment of whether membrane n-
98 3 HUFA content could explain variation in mitochondrial metabolism among individuals.

99 The present experiment integrates measurements of mitochondrial efficiency and
100 mitochondrial proton leak to determine whether reductions in EPA and DHA availability in food led to
101 changes in energy metabolism of a primary consumer fish. We first examined the effect of dietary n-
102 3 HUFA content on mitochondrial function, in particular on the efficiency to produce ATP (ATP/O
103 ratio) and the respiratory capacities to offset the proton leak (LEAK respiration). Secondly, we tested
104 whether differences between individuals in mitochondrial efficiency and mitochondrial LEAK
105 respiration vary with membrane n-3 HUFA content. To address this, we experimentally manipulated
106 the quantity of n-3 HUFA in food for wild-caught juvenile golden grey mullet (*Chelon auratus*). Fish
107 were fed either a high n-3 HUFA or low n-3 HUFA **diet**, and their membrane fatty acid composition
108 and mitochondrial functioning were determined in skeletal muscle. We choose juvenile golden grey
109 mullet as our study organism because they are likely to be the first levels of the food chain to face a
110 decline in availability of dietary n-3 HUFA, as this fish fed mainly on primary producers (Lebreton et
111 al., 2011; Mourente and Tocher, 1993). We analysed mitochondrial properties in the skeletal muscle
112 because the mitochondrial function of this tissue is known to influence whole-animal performance

113 (Coen et al., 2012; Salin et al., 2016a), and that fatty acid dietary content influences muscle
114 membrane fatty acid composition (McKenzie et al., 1998; Vagner et al., 2015).

115

116 **2. Materiel and methods**

117 *2.1. Fish origin and care*

118 Wild juvenile golden grey mullets (n = 23) were netted from the marshes of L'Houmeau France
119 (46°12'14.4''N 1°11'43.7''W) in November 2017 and transported to the laboratory Littoral
120 Environment Society (LIENSs), France, where all the experiments were conducted. Fish were kept in a
121 common thermoregulated 300 L-tank supplied with aerated recirculated sand-filtered natural
122 seawater and equipped with an external biological filter (Eheim, Deizisau, Germany). Fish were
123 maintained under a 12 L : 12 D photoperiod, and fed daily with commercial pellets (Le Gouessant®,
124 Lamballe, France). Temperature ($13.8 \pm 0.2^\circ\text{C}$) and salinity (28.7 ± 0.1) were monitored daily
125 (TetraCon® 325, Laboratoires Humeau, La Chapelle-sur-Erdre, France) and were kept similar to that
126 of the sampling site. Oxygen ($87.9 \pm 2.6\%$ air saturation) was monitored once a week. Fish were
127 acclimated for seven weeks to these conditions. The collection and handling of the animals were
128 carried out under the jurisdiction of the Departmental Service of Fisheries and the Animal Care
129 Committee of France (# 12886), respectively.

130 In January 2018, fish were anesthetized in 0.1 g L^{-1} MS-222 (Ethyl 3-aminobenzoate
131 methanesulfonate) in seawater, measured for body mass ($34.3 \pm 6.5 \text{ g}$), individually pit-tagged
132 (Biolog-id, Bernay, France), and randomly assigned to one of the four replicate 300-L holding tanks
133 (n=5-6 fish per tank). All fish were fasted for 24h beforehand to ensure their guts were empty. Fish
134 were acclimated for 4 weeks in their new tanks, during which time they were fed commercial pellets
135 twice daily to a total of 2% of their biomass (Le Gouessant®, Lamballe, France).

136 In February 2018, fish were gradually acclimated to 20°C by means of a sequence of 3 step
137 increases of 2°C over a two-week period. This temperature was chosen because it is the temperature
138 at which whole-organism performance declined in mullet fed on a low n-3 HUFA diet, but is within
139 their natural thermal range as 20°C reflects the mean summer temperature in mullet natural
140 environment (Vagner et al., 2015; Vagner et al., 2014). Fish were left another 2 weeks period of
141 acclimation to initiate physiological adjustment to the change in temperature (Bouchard and
142 Guderley, 2003). Temperature ($19.9 \pm 1^\circ\text{C}$) and salinity (29.6 ± 1.1) were daily measured in the four
143 experimental tanks.

144

145 *2.2. Diet treatment and tissue sampling*

146 Differences in dietary n-3 HUFA content were achieved by replacing fish oil (rich in n-3 HUFA) of the
147 High n-3 HUFA diet with rapeseed oil (poor in n-3 HUFA) in the Low n-3 HUFA diet (See ingredients in

148 Table S1 (a)). Experimental diets were isocaloric and isolipidic. High and Low n-3 HUFA diets
149 contained 17.5 % and 1.2 % EPA+DHA, respectively, per total fatty acid mass of the diets, which
150 represents about 15-fold difference in EPA and DHA content between diets (See Table S1 (a) for fatty
151 acid composition of the diets). The EPA and DHA content in High n-3 HUFA diet cover the needs of
152 several fish species (Robin and Skalli, 2007), **although** the needs of the golden grey mullet are not
153 known. The Low n-3 HUFA diet was estimated to significantly reduce n-3 HUFA content in biological
154 membranes and has been shown to impair aerobic performance of golden grey mullet at 20°C
155 (Vagner et al., 2015).

156 Following the period of acclimation to water temperature of 20°C, fish were switched to the
157 experimental diets for about two months (mean \pm SEM = 61 ± 2 days, range: 45 – 73 days). This
158 duration was chosen because it is sufficient to detect differences in the membrane fatty acid
159 composition between diet treatments (Robin and Skalli, 2007). Because only two fish per day could
160 be analysed for their mitochondrial function at the end of the experiment, the duration of the diet
161 treatment differed between processing batches. Fish were randomly allocated to the treatment: fish
162 had their food progressively switched to either the High n-3 HUFA diet (n = 11) or the Low n-3 HUFA
163 diet (n = 12). The experimental diet to commercial diet ratio was increased every second day from
164 25%:75% to 50%:50%, 75%:25% and finally to 100%:0%. Body mass did not differ between fish
165 groups subsequently assigned to the two food treatments (High n-3 HUFA diet: 31.3 ± 1.8 g; Low n-3
166 HUFA diet: 34.9 ± 1.5 g, *t*-test: $t_{21} = -1.510$, $p = 0.146$). Body mass was re-measured (as above) every
167 2-3 weeks and rations were recalculated to adjust for growth.

168 At the end of the food treatment period, fish were fasted for 24 h before being anesthetized
169 and culled. Fish were weighed, measured and two samples of skeletal muscle were immediately
170 dissected, taken dorsally from the lateral line (to have both red and white muscle) and just behind
171 the head. One aliquot was collected from one side of the fish and kept in ice-cold isolation buffer
172 (sucrose 250 mM, EGTA 1mM, Tris-HCl 20mM, pH 7.4 at 4°C) for immediate mitochondrial assay,
173 while the other aliquot (≈ 1 g) was collected from the other side and immediately flash-frozen for
174 subsequent fatty acid analysis.

175

176 2.3. Determination of fatty acid composition

177 2.3.1. Lipid extraction

178 Lipids from muscle of fish were extracted following the method used in Mathieu-Resuge et al. (2019).
179 Muscle aliquots were ground into a fine homogeneous powder in liquid nitrogen. Lipids from 200-
180 250 mg of muscle powder were then extracted in 6 ml of mixture chloroform/methanol (2:1; v/v). To
181 ensure complete lipid extraction, samples were sonicated at 4 °C during 2 x 5 min. Lipids from
182 experimental diets were extracted following the same method. Pellets were ground in a mortar and

183 powder was transferred in chloroform/methanol (2:1; v/v). All lipid extracts were then stored at -20
184 °C under nitrogen atmosphere until further analysis.

185

186 *2.3.2. Purification of polar lipids*

187 Fatty acids of biological membranes are connected to a polar head group, altogether named polar
188 lipid (PL), unlike fatty acids of energy stores that constitute triglyceride, a neutral lipid. To analyse the
189 FA composition of biological membrane, PL were separated from neutral FA and FA from only PL
190 were considered. PL were separated from neutral one by solid-phase extraction following the
191 method described in (Martin et al., 2013). Briefly, an aliquot of muscle total lipid extract (1/6) was
192 evaporated to dryness under nitrogen, recovered with 3 washings of 0.5 ml of chloroform/methanol
193 (98:2; v/v) and deposited at the top of a silica gel column (40 mm × 4 mm, silica gel 60A 63–200 µm)
194 previously heated at 450°C and deactivated with 6 weight % H₂O. After elution of neutral lipids with
195 10 mL of chloroform/methanol (98:2; v/v), PL were eluted with 20 mL of methanol, transferred into a
196 vial containing 2.3 µg of internal standard (tricosanoic acid C23:0) and stored at -20°C under nitrogen
197 atmosphere.

198

199 *2.3.3. Polar lipid transesterification*

200 Polar lipid transesterification was realized as described in Mathieu-Resuge et al. (2019). PL fractions
201 were evaporated to dryness under nitrogen. Fatty acid methyl esters (FAME) were obtained by
202 addition of 800 µL of H₂SO₄ / methanol (3.4 %; v/v) and heating at 100°C for 10 min. FAME were then
203 extracted in 800 µL of hexane and washed three times with 1.5 ml of distilled water saturated in
204 hexane.

205

206 *2.3.4. Gas chromatography analysis of FAME*

207 FA composition was analysed by GC coupled with Flame-Ionization Detector (GC-FID) as described by
208 Mathieu-Resuge et al. (2019) with a Varian CP8400 gas chromatograph. After splitless-mode
209 injection, FAME were separated in parallel on two different columns (DBWAX 30 m × 0.25 mm ID x
210 0.2 µm and DB5 30 m × 0.25 mm ID x 0.2 µm, Agilent). FAME were identified by comparisons of their
211 retention time with those of commercial standards (Supelco 37 Component FAME Mix, PUFA No.1
212 and No.3, and Bacterial Acid Methyl Ester Mix, Sigma) and lab-made standard mixtures. The internal
213 standard (C23:0) allowed to calculate the FA content (µg mg⁻¹ muscle wet mass). Fatty acid content
214 was then determined as the percentage of fatty acid mass per total mass of fatty acids in membrane
215 lipids from the polar lipids fraction. The sum of EPA and DHA content in membrane lipids was
216 calculated as:

217
$$\Sigma \text{ EPA + DHA content} = \frac{\text{mass of EPA} + \text{mass of DHA}}{\text{total mass of fatty acids}} \times 100$$

218

219 *2.4. Mitochondrial function*

220 *2.4.1. Isolation of mitochondria*

221 Isolation of muscle mitochondria and measurement of mitochondrial efficiency were adjusted from
222 published protocols (Ghanizadeh Kazerouni et al., 2016; Salin et al., 2016c). Briefly, mitochondria
223 were isolated from 1 g (mean \pm SEM: 1.07 \pm 0.03 g) of muscle. Tissue was finely chopped on ice and
224 homogenized in 10 mL of isolation buffer by four gentle passes in a Potter-Elvehjem homogenizer
225 and centrifuged at 500 g for 5 min at 4°C. The supernatant was transferred in a clean tube and
226 centrifuged at 1000 g for 5 min at 4°C and then centrifuged at 9000 g for 10 min at 4°C. The pellet
227 containing mitochondria was gently resuspended in 500 μ L assay buffer at 4°C (20mM Taurine,
228 10mM KH₂PO₄, 20 mM HEPES, 110 mM D-sucrose, 60 mM K-lactobionate, 1g L⁻¹ BSA fatty acid free,
229 pH 7.2 at 20°C).

230

231 *2.4.2 Mitochondrial ATP/O ratio and LEAK respiration measurements*

232 Mitochondrial efficiency has been quantified through the measurement of the ATP/O ratio, which is
233 the amount of ATP generated per unit of oxygen consumed. Mitochondrial LEAK respiration was
234 determined as the rate of oxygen consumption when ATP synthesis was inhibited. We used a
235 protocol that simultaneously measures both mitochondrial ATP production and the oxygen
236 consumption related to that ATP production, as in Salin et al. (2016c). Assays were **conducted** at
237 20°C.

238 Oxygen and magnesium green fluorescence signals were detected simultaneously using two
239 respirometry chambers equipped with fluorescent sensors and recorded using DatLab software
240 (Oroboros Instruments, Innsbruck Austria). To estimate ATP production we used the magnesium-
241 sensitive fluorescent probe, Magnesium Green, to determine changes in [Mg²⁺] (Szmackinski and
242 Lakowicz, 1996). ATP production was then calculated from the rate of change in [Mg²⁺] and is based
243 on the unequal affinities of ATP and ADP for Mg²⁺ (Chinopoulos et al., 2009).

244 The oxygen electrodes were calibrated at two points: air-saturated **assay** buffer (daily) and
245 zero oxygen after sodium dithionite addition (fortnightly). Stepwise additions of MgCl₂ at each run
246 were performed for calibration of the fluorescent signal into Mg²⁺. The two binding affinity (K_d)
247 values of ATP and ADP for Mg²⁺ were determined in presence of isolated mitochondria and calculated
248 as in (Chinopoulos et al., 2014) ; the values were K_{d-ATP} = 0.197 mM and K_{d-ADP} = 1.609 mM.

249 Isolated mitochondria from each fish were added to one of the two measurement chambers
250 of the oxygraph immediately following isolation; fish from a processing pair (i.e. a Low and a High n-3
251 HUFA) were measured in parallel. The remaining part of the isolated mitochondria was preserved on

252 ice for use in a replicate trial. Assays contained 200 μ l of isolated mitochondria with 1.8 mL of assay
253 buffer in the presence of complex I substrates (5 mM pyruvate and 0.5 mM malate) and complex II
254 substrate (10 mM succinate). Magnesium green (2.2 μ M), EGTA (0.1 mM), EDTA (5 μ M) and $MgCl_2$ (1
255 mM) were added to determine changes in $[Mg^{2+}]$ and so to calculate the rate of ATP production as in
256 (Chinopoulos et al., 2014).

257 The rate of oxygen consumption to support ATP production was assessed by adding a
258 saturating concentration of ADP (2 mM, Mg^{2+} free) to the chamber. The raw rate of ATP production
259 was also measured in this condition. The rate of oxygen consumption to offset the leakage of proton
260 – LEAK respiration - was then measured after inhibition of mitochondrial ATP synthesis (with 4 μ M
261 carboxyatractyloside). The rate of ATP hydrolysis was also measured in this condition. The rate of
262 ATP hydrolysis was then added to the raw rate of ATP production to obtain the corrected rate of ATP
263 production. Addition of complex I inhibitor (0.5 μ M rotenone) and complex III inhibitor (2.5 μ M
264 antimycin A) allowed determination of residual oxygen consumption, which was then subtracted
265 from all other oxygen consumption values. The second replicated trial was used to control for
266 repeatability of the assay. It was identical to the first one, but started an hour and half later, using
267 the remaining isolated mitochondrial and the same measurement chamber. Every second day, the
268 measurement chamber associated with a treatment group was reversed to control for any inter-
269 respirometry chamber difference in readings. No effect of the choice of measurement chamber on
270 mitochondrial function was detected.

271

272 2.4.3. Analysis of Mitochondrial ATP/O ratio and LEAK respiration

273 We expressed respiration rate as pmoles of $O_2 \text{ sec}^{-1} \text{ ml}^{-1}$ of assay buffer and ATP production
274 as pmoles of $ATP \text{ sec}^{-1} \text{ ml}^{-1}$ of assay buffer for each replicate. Finally, the ATP/O ratio was calculated
275 as the ratio of corrected ATP production to two-fold respiration that supported ATP production; the
276 rate of respiration is doubled since each molecule of oxygen is comprised of two oxygen atoms.
277 Replicated ATP/O ratios were highly correlated (Pearson's $r = 0.749$, $P < 0.001$). However, we found a
278 consistent shift in the values of the measurements between the first and the second trials of ATP/O
279 ratio (drift between trials: Paired $t = -6.194$, $P < 0.001$). The data from the second trial of muscle
280 assay were excluded because the mitochondrial integrity may have been impaired with time post-
281 isolation, as previously found in liver mitochondria (Salin et al., 2016c). Only data of the first LEAK
282 respiration and ATP/O ratio trial were kept for the main analyses. Protein concentration was
283 measured at 595 nm using the Bradford method with bovine serum albumin as a standard. We
284 expressed LEAK respiration as pmoles of $O_2 \text{ sec}^{-1} \text{ mg}^{-1}$ of mitochondrial protein. See descriptive
285 statistics of the mitochondrial function in Table S2.

286

287 2.5. Statistical analysis

288 We first examined the effect of the treatments (High and Low dietary n-3 HUFA content) on
289 the membrane fatty acid composition (Σ EPA + DHA content). Normality (Shapiro-Wilk test) and
290 homoscedasticity (Bartlett-test) were tested. When normality and homoscedasticity were not
291 satisfied, non-parametric analyses were carried out. We used Wilcoxon tests to determine whether
292 muscle membrane content of EPA and DHA, and their sum (Σ EPA + DHA content) differed between
293 dietary treatment groups. To examine potential differences in final body mass between treatments
294 that could explain differences in mitochondrial function, a *t*-test was employed. The effect of the
295 dietary treatment on mitochondrial function (ATP/O ratio and LEAK respiration) were also analysed
296 using *t*-tests.

297 We then used linear model **analyses** to test whether ATP/O ratio and LEAK respiration were
298 correlated with membrane lipid content in Σ EPA + DHA. The models included mitochondrial function
299 (ATP/O or LEAK respiration) as the dependant variable, dietary treatments as a categorical effect, and
300 Σ EPA + DHA content as continuous predictor. The normality of the linear model residuals was
301 validated for all models using Shapiro–Wilk tests. We also tested the interactions between dietary
302 treatment and membrane lipid content in Σ EPA + DHA. All interactions were non-significant, so they
303 were removed from the models.

304 The duration of the dietary treatment **was included as potential covariate**, the processing
305 batch and fish tanks were included as potential **random factor** in **initial analysis**. **Each of these**
306 **variable** were not significant, so were removed from all the **final models**. All statistical analyses were
307 performed with the free software R (R Core Team, 2017), with R Version 3.6.1, with significance level
308 set to $p < 0.05$. Data are presented as means \pm standard error of the mean (SEM).

309

310 3. Results

311 3.1. Effect of the dietary treatment on membrane fatty acid composition

312 Muscle of mullets fed on Low n-3 HUFA diet showed significant differences in their membrane
313 fatty acid composition compared to those of fish fed on High n-3 HUFA diet (Table S3). EPA and DHA
314 content in muscle membranes were significantly lower in Low n-3 HUFA fish compared to High n-3
315 HUFA fish (EPA: $W = 119$, $p < 0.001$; DHA: $W = 132$, $p < 0.001$; Table 1). Not surprisingly, fish on
316 average had a lower content of Σ EPA+ DHA in their muscle when fed the Low n-3 HUFA diet ($W =$
317 130 , $p < 0.001$, Table 1). Final body mass did not differ between High and Low n-3 HUFA fish ($t = -$
318 1.668 ; $p = 0.112$), excluding body mass as confounding sources of differences between dietary
319 treatments.

320

321 3.2. Effect of the dietary treatment on mitochondrial function

322 ATP/O ratio was significantly higher in the mitochondria of Low compared to High n-3 HUFA
323 mullet ($t = -3.107$, $p = 0.005$; Fig. 1A). However, there was no effect of dietary n-3 HUFA content on
324 LEAK respiration in muscle mitochondria ($t = -0.423$, $p = 0.677$; Fig. 1B).

325

326 *3.3. Relationships across individuals between mitochondrial function and membrane fatty acid* 327 *composition*

328 The ATP/O ratio ranged from 1.46 to 3.33 for mitochondria of mullet eating the High n-3
329 HUFA diet and from 2.05 to 3.67 for mitochondria of fish on Low n-3 HUFA diet (Fig. 2A). **Variation in**
330 **mitochondrial function between individuals was mainly explained by differences in n-3 HUFA content**
331 **in muscle (Figure S1)**. Regardless of the food treatment, the muscle ATP/O ratio of a fish was strongly
332 and positively related to its Σ EPA + DHA content: individuals that had the higher mitochondrial
333 efficiency under either diet had the highest content in EPA and DHA in their membranes ($t = 2.513$, p
334 $= 0.021$; Fig. 2A and Table 2). There was no relationship between a fish's mitochondrial LEAK
335 respiration and its Σ EPA + DHA content among individuals with the same dietary treatment ($t = -$
336 0.104 , $p = 0.919$, Fig. 2B and Table 2). We found no correlation between ATP/O ratio and LEAK
337 respiration from the same mitochondria (High n-3 HUFA diet: Pearson's $r = 0.229$, $p = 0.499$; Low n-3
338 HUFA diet: Pearson's $r = -0.157$, $p = 0.627$).

339

340 **4. Discussion**

341 We asked whether a decline in dietary n-3 HUFA content leads to changes in mitochondrial
342 metabolic phenotype for a model of primary consumer fish, the golden grey mullet. We manipulated
343 dietary content in n-3 HUFA and assessed membrane FA composition and mitochondrial function in
344 mullet. We found that diet strongly influenced membrane fatty acid composition: mullets on a Low
345 n-3 HUFA diet had lower levels of n-3 HUFA in muscle biological membranes, which suggested that
346 the mitochondrial membranes contained less n-3 HUFA. Previous studies have indeed demonstrated
347 that modification of the fatty acid composition of the diet causes strongly correlated changes in the
348 membrane fatty acid composition of mitochondria (Herbst et al., 2014; Jeromson and Hunter, 2014;
349 Ramsey et al., 2005), including those of fish species (Guderley et al., 2008; Morash et al., 2009). Our
350 findings reveal that ATP/O ratio increased significantly in mitochondria of fish fed a low n-3 HUFA
351 diet, so that a decline in dietary content in n-3 HUFA elicits greater mitochondrial efficiency to
352 produce ATP. **Surprisingly, the differences in membrane n-3 HUFA content between individuals had**
353 **an opposite effect on mitochondrial efficiency**: under the same dietary treatment, **individuals** that
354 displayed higher EPA and DHA **membrane content** had mitochondria with the highest ATP/O ratio.

355 Aerobic performance of marine fish will not only be challenged in a near future by increased
356 oxygen needs and low oxygen availability at high temperatures but probably also by a shift in ocean

357 productivity (Behrenfeld et al., 2006), where EPA and DHA availability might decline considerably
358 (Hixson and Arts, 2016). Mitochondrial aerobic capacity has traditionally been determined in terms
359 of oxygen consumption, enzymatic activities or density of mitochondria (Brookes et al., 1998;
360 Guderley et al., 2008; Martin et al., 2013; Morash et al., 2009; Ramsey et al., 2005; Yu et al., 2014).
361 However, oxygen consumption provides only an indirect measure of ATP production, and the
362 relationship between oxygen consumption and ATP production can vary both among and within
363 individuals. Our results clearly show that when evaluating mitochondrial adjustment in response to a
364 reduction in n-3 HUFA availability in diet, variation in the amount of ATP generated per molecule of
365 oxygen consumed by mitochondria can have major importance in explaining associated changes in
366 whole-animal performance. Past work on mullets found that fish fed on a low n-3 HUFA diet
367 consumed less oxygen compared to the fish fed a high n-3 diet to reach a similar swimming speed
368 (Vagner et al., 2015; Vagner et al., 2014). Our results suggest that higher mitochondrial efficiency
369 may result in lower oxygen requirement for fish to produce ATP and sustain muscle contraction for
370 locomotory performance, as previously demonstrated in human (Coen et al., 2012). Mitochondria
371 with high efficiency can be beneficial for energy-demanding cellular processes (Salin et al., 2015), yet
372 there could also be a cost. Mitochondria are a major producer of reactive oxygen species (ROS) and
373 mitochondrial efficiency can be positively related to ROS production (Brand, 2000; Salin et al., 2015),
374 an area which would require further study.

375 Here, we found no significant differences in LEAK respiration of mullet muscle across dietary
376 treatments. In contrast, LEAK respiration in trout muscle were higher for mitochondria from fish fed
377 a low n-3 HUFA diet than for mitochondria from fish fed a high n-3 HUFA diet (Guderley et al., 2008).
378 Another experiment in trout has shown the opposite effect: animals fed a lower n-3 HUFA diet
379 displayed lower LEAK respiration in muscle mitochondria (Martin et al., 2013). We stress that it is
380 entirely possible for the mitochondrial LEAK response to dietary content in n-3 HUFA to vary among
381 individuals, species and other environmental factors, but also that the consequence on the proton
382 leakage might depend on the magnitude and duration of the dietary treatment. Equivocal support
383 may indicate that the responses of LEAK to changes in membrane fatty acid composition does not fall
384 along a linear response curve, as typically assumed. Instead, the effect of dietary content in n-3 HUFA
385 on membrane composition, and in turn proton permeability might be biphasic (Abbott et al., 2010)
386 and might promote non-linear response of LEAK. While testing a large range of n-3 HUFA dietary
387 content was beyond the scope of the present study, it may only be possible to explain contrasted
388 pattern of LEAK response if many dietary levels of n-3 HUFA are considered.

389 In our study, mullets from the same food treatment displayed important individual variation
390 in mitochondrial efficiency that covary with membrane fatty acid composition: individual that had
391 higher EPA and DHA content in their membranes had mitochondria that synthesized ATP more

392 efficiently than those of individuals with lower EPA and DHA content. Previous studies suggested that
393 variation in FA composition of the mitochondrial inner membrane can influence the mitochondrial
394 membrane conductance of protons, and in turn the proportion of oxygen used to offset the proton
395 leakage (Hulbert et al., 2007), leading to variation in the efficiency of ATP production (Brand, 2005).
396 However, this does not appear to be the case in the present study, as LEAK did not covary with
397 ATP/O ratio and membrane fatty acid composition. An explanation for this discrepancy might lie in
398 the fact that mitochondrial efficiency also depends on protein abundance and activity of the
399 mitochondrial inner membrane-bound complexes involved in oxidative phosphorylation (i.e. electron
400 transport complexes and ATP synthase). This alternative explanation is based on the fact that fatty
401 acid composition of membrane lipids may affect lipid–protein interactions and therefore the function
402 of embedded proteins (Brenner, 1984). As well as varying with membrane FA composition and
403 protein function, mitochondrial ATP/O ratio can shift in response to energy substrate use (Brand,
404 2005). Previous work in Atlantic salmon has demonstrated that individual variation in DHA and EPA in
405 muscle content was associated with differences in the expression of genes involved in lipid
406 catabolism and carbohydrate metabolism (Horn et al., 2019). However, further research is needed to
407 determine whether differences in energy substrates utilization determine individual variation in
408 mitochondrial efficiency in mullet.

409 Some mullets on the low n-3 HUFA dietary treatment had actually higher EPA and DHA
410 contents than others on the high n-3 HUFA dietary treatment that were eating almost 15 times as
411 much EPA and DHA. Individual variation in membrane fatty acid composition is likely to be a
412 complex, integrative characteristics influenced by several metabolic pathways. If individual
413 differences in fatty acid membrane composition covary with the rates of lipid assimilation,
414 biosynthesis and degradation, it is important to recognize that fatty acid composition may vary with
415 pathways that are generally neglected. For example, ability to biosynthesis EPA and DHA in marine
416 fish is very limited, as it is generally insufficient to compensate dietary deficiency (Tocher, 2003), but
417 the link between individual variation in n-3 HUFA content and their rate of biosynthesis might only
418 appear across individuals eating the same amount of n-3 HUFA. Another explanation might be an
419 individual heterogeneity in the rate of degradation of EPA and DHA, while also considered minor in
420 fish (McKenzie, 2001). Individual variation in the rate of assimilation may also be significant for n-3
421 HUFA content in membrane. Regardless of their food intake, individual fish that have higher levels of
422 EPA and DHA in membrane lipids may have preferentially retained these fatty acids from their diet.
423 Our observations illustrate that an understanding of individual variability in membrane fatty acid
424 composition can be gained only through consideration of differential metabolic pathways of fatty
425 acids.

426 The individual covariation between mitochondrial efficiency and membrane fatty acid
427 composition we observed might be because higher EPA and DHA contents in the membrane promote
428 higher efficiency of the mitochondria, as explained above. However, given that our study is
429 correlative, it is perhaps high mitochondrial efficiency that may promote membranes with relatively
430 high levels of EPA and DHA, because more ATP is available to fuel metabolic pathways that retain
431 dietary n-3 HUFA. Assimilation of fatty acids in the intestine can be energetically costly (Mansbach
432 and Gorelick, 2007), and for example, fish that have higher efficiency to make ATP may actually have
433 higher assimilation rate of EPA and DHA compared with fish at lower mitochondrial efficiency. The
434 variation in mitochondrial efficiency of muscle tissue studied here might be representative of the
435 mitochondrial efficiency in other tissues, including intestine, although previous studies looking at
436 correlation of mitochondrial efficiency across tissues in the same individual have shown equivocal
437 results (Salin et al., 2019). Further study looking at mitochondrial metabolism in different tissues will
438 be necessary to determine if golden grey mullets are able to improve their mitochondrial efficiency
439 across multiple tissues.

440 Flexibility in mitochondrial efficiency may be particularly important since the capacity of the
441 mitochondria to produce ATP can set limits on the capacity of an organism to respond to
442 environmental changes (Blier et al., 2013; Sokolova et al., 2012). Our data imply that the greater
443 mitochondrial efficiency induced by the Low n-3 HUFA diet might benefit for the ability of the
444 mitochondrial to make ATP for energy-demanding cellular processes when reduction in EPA and DHA
445 availability in food web. Information on the consequences of individual heterogeneity in
446 mitochondrial efficiency on fish performance would allow a better understanding of the effect of
447 decline in EPA and DHA availability in marine food web. Further research should focus on identifying
448 the individual variation in metabolic pathway of EPA and DHA. This type of variation may be very
449 important in an evolutionary context as well, not only generating phenotypic variation among
450 individuals, but also allowing animals to reduce the energy costs of making ATP by increasing the
451 mitochondrial efficiency.

452
453 ACKNOWLEDGEMENTS: We thank Salomé Ducos for help with fish handling and José-Luis Zambonino
454 Infante for designing the diets. We thank the three referees for helpful comments that improved the
455 manuscript.

456
457 [CRediT author statement](#): KS, MMR, FLG, PS and MV conceived the ideas and designed methodology.
458 KS, MMR, NG, ED, FLG and MV collected the data. KS, MMR, NG, FLG, PS and MV analysed the data.
459 KS led the writing of the manuscript except for section 2.3 written by FLG; MMR, FLG, PS and MV
460 revised the manuscript and added comments. All authors gave final approval for publication.

461

462 DECLARATION OF INTEREST: The authors declare that they have no known competing financial
463 interests or personal relationships that could have appeared to influence the work reported in this
464 paper.

465

466 DATA ACCESSIBILITY: The dataset supporting this article will be made available by archiving it in the
467 Dryad Digital Repository.

468

469 FUNDING: This work is a contribution to the project ECONAT Axe 1 - Ressources Marines Littorales:
470 qualité et éco-valorisation, funded by the Contrat de Plan Etat-Région and the CNRS and the
471 European Regional Development Fund.

472

473 References

- 474 Abbott, S.K., Else, P.L., Hulbert, A.J., 2010. Membrane fatty acid composition of rat skeletal muscle is
475 most responsive to the balance of dietary n-3 and n-6 PUFA. *British Journal of Nutrition* 103, 522-
476 529.
- 477 Alimuddin, Yoshizaki, G., Kiron, V., Satoh, S., Takeuchi, T., 2005. Enhancement of EPA and DHA
478 biosynthesis by over-expression of masu salmon Delta 6-desaturase-like gene in zebrafish. *Transgenic*
479 *Res.* 14, 159-165.
- 480 Arts, M.T., Kohler, C.C., 2009. Health and condition in fish: the influence of lipids on membrane
481 competency and immune response, in: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), *Lipids in Aquatic*
482 *Ecosystems*. Springer New York, New York, NY, pp. 237-256.
- 483 Behrenfeld, M.J., O'Malley, R.T., Siegel, D.A., McClain, C.R., Sarmiento, J.L., Feldman, G.C., Milligan,
484 A.J., Falkowski, P.G., Letelier, R.M., Boss, E.S., 2006. Climate-driven trends in contemporary ocean
485 productivity. *Nature* 444, 752-755.
- 486 Bermudez, R., Feng, Y., Roleda, M.Y., Tatters, A.O., Hutchins, D.A., Larsen, T., Boyd, P.W., Hurd, C.L.,
487 Riebesell, U., Winder, M., 2015. Long-term conditioning to elevated pCO₂ and warming influences the
488 fatty and amino acid composition of the diatom *Cylindrotheca fusiformis*. *Plos One* 10.
- 489 Blier, P.U., Lemieux, H., Pichaud, N., 2013. Holding our breath in our modern world: will mitochondria
490 keep the pace with climate changes? *Canadian Journal of Zoology* 92, 591-601.
- 491 Bottje, W.G., Carstens, G.E., 2009. Association of mitochondrial function and feed efficiency in
492 poultry and livestock species. *Journal of Animal Science* 87, E48-E63.
- 493 Bouchard, P., Guderley, H., 2003. Time course of the response of mitochondria from oxidative muscle
494 during thermal acclimation of rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Biol.* 206, 3455-3465.
- 495 Brand, M.D., 2000. Uncoupling to survive? The role of mitochondrial inefficiency in ageing.
496 *Experimental Gerontology* 35, 811-820.
- 497 Brand, M.D., 2005. The efficiency and plasticity of mitochondrial energy transduction. *Biochemical*
498 *Society Transactions* 33, 897-904.
- 499 Brand, M.D., Couture, P., Hulbert, A.J., 1994. Liposomes from mammalian liver mitochondria are
500 more polyunsaturated and leakier to protons than those from reptiles. *Comparative Biochemistry*
501 *and Physiology Part B: Comparative Biochemistry* 108, 181-188.
- 502 Brand, M.D., Nicholls, D.G., 2011. Assessing mitochondrial dysfunction in cells. *Biochemical Journal*
503 435, 297-312.
- 504 Brenner, R.R., 1984. Effect of unsaturated-acids on membrane-structure and enzyme-kinetics.
505 *Progress in Lipid Research* 23, 69-96.

506 Brookes, P.S., Buckingham, J.A., Tenreiro, A.M., Hulbert, A.J., Brand, M.D., 1998. The proton
507 permeability of the inner membrane of liver mitochondria from ectothermic and endothermic
508 vertebrates and from obese rats: correlations with standard metabolic rate and phospholipid fatty
509 acid composition. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*
510 *119*, 325-334.

511 Chinopoulos, C., Kiss, G., Kawamata, H., Starkov, A.A., 2014. Chapter seventeen - Measurement of
512 ADP-ATP exchange in relation to mitochondrial transmembrane potential and oxygen consumption,
513 in: Lorenzo, G., Guido, K. (Eds.), *Methods in Enzymology*, Elsevier academic press inc, San Diego, pp.
514 333-348.

515 Chinopoulos, C., Vajda, S., Csanády, L., Mándi, M., Mathe, K., Adam-Vizi, V., 2009. A novel kinetic
516 assay of mitochondrial ATP-ADP exchange rate mediated by the ANT. *Biophysical Journal* *96*, 2490-
517 2504.

518 Coen, P.M., Jubrias, S.A., Distefano, G., Amati, F., Mackey, D.C., Glynn, N.W., Manini, T.M.,
519 Wohlgemuth, S.E., Leeuwenburgh, C., Cummings, S.R., Newman, A.B., Ferrucci, L., Toledo, F.G.S.,
520 Shankland, E., Conley, K.E., Goodpaster, B.H., 2012. Skeletal muscle mitochondrial energetics are
521 associated with maximal aerobic capacity and walking speed in older adults. *The Journals of*
522 *Gerontology Series A: Biological Sciences and Medical Sciences* *68*, 447-555.

523 da Motta Pacheco, L.F.C., Uribe, E., Pino, J., Troncoso, J., Quiróz, A., 2014. The effect of UV light and
524 CO₂ in the production of polyunsaturated aldehydes in *Skeletonema costatum* (Bacillariophyceae).
525 *American Journal of Plant Sciences* *5*, 3632.

526 Distefano, G., Standley, R.A., Zhang, X.L., Carnero, E.A., Yi, F., Cornnell, H.H., Coen, P.M., 2018.
527 Physical activity unveils the relationship between mitochondrial energetics, muscle quality, and
528 physical function in older adults. *J. Cachexia Sarcopenia Muscle* *9*, 279-294.

529 Fontaine, E.M., Moussa, M., Devin, A., Garcia, J., Ghisolfi, J., Rigoulet, M., Leverve, X.M., 1996. Effect
530 of polyunsaturated fatty acids deficiency on oxidative phosphorylation in rat liver mitochondria.
531 *Biochim. Biophys. Acta-Bioenerg.* *1276*, 181-187.

532 Galloway, A.W.E., Winder, M., 2015. Partitioning the Relative Importance of Phylogeny and
533 Environmental Conditions on Phytoplankton Fatty Acids. *PLOS ONE* *10*, e0130053.

534 Ghanizadeh Kazerouni, E., Franklin, C.E., Seebacher, F., 2016. UV-B exposure reduces locomotor
535 performance by impairing muscle function but not mitochondrial ATP production. *J. Exp. Biol.* *219*,
536 96-102.

537 Guderley, H., Kraffe, E., Bureau, W., Bureau, D.P., 2008. Dietary fatty acid composition changes
538 mitochondrial phospholipids and oxidative capacities in rainbow trout red muscle. *Journal of*
539 *Comparative Physiology B-Biochemical Systemic and Environmental Physiology* *178*, 385-399.

540 Hamel, S., Gaillard, J.-M., Yoccoz, N.G., 2018. Introduction to: Individual heterogeneity – the causes
541 and consequences of a fundamental biological process. *Oikos* *127*, 643-647.

542 Herbst, E.A.F., Paglialunga, S., Gerling, C., Whitfield, J., Mukai, K., Chabowski, A., Heigenhauser, G.J.F.,
543 Spriet, L.L., Holloway, G.P., 2014. Omega-3 supplementation alters mitochondrial membrane
544 composition and respiration kinetics in human skeletal muscle. *The Journal of Physiology* *592*, 1341-
545 1352.

546 Hixson, S.M., Arts, M.T., 2016. Climate warming is predicted to reduce omega-3, long-chain,
547 polyunsaturated fatty acid production in phytoplankton. *Global Change Biology* *22*, 2744-2755.

548 Horn, S.S., Sonesson, A.K., Krasnov, A., Moghadam, H., Hillestad, B., Meuwissen, T.H.E., Ruyter, B.,
549 2019. Individual differences in EPA and DHA content of Atlantic salmon are associated with gene
550 expression of key metabolic processes. *Scientific Reports* *9*.

551 Hulbert, A.J., Pamplona, R., Buffenstein, R., Buttemer, W.A., 2007. Life and death: metabolic rate,
552 membrane composition, and life span of animals. *Physiol Rev* *87*, 1175-1213.

553 Hulbert, A.J., Turner, N., Storlien, L.H., Else, P.L., 2005. Dietary fats and membrane function:
554 implications for metabolism and disease. *Biological Reviews* *80*, 155-169.

555 Ishizaki, Y., Masuda, R., Uematsu, K., Shimizu, K., Arimoto, M., Takeuchi, T., 2001. The effect of
556 dietary docosahexaenoic acid on schooling behaviour and brain development in larval yellowtail.
557 *Journal of Fish Biology* *58*, 1691-1703.

558 Jahn, M., Seebacher, F., 2019. Cost of transport is a repeatable trait but is not determined by
559 mitochondrial efficiency in zebrafish (*Danio rerio*). The Journal of Experimental Biology 222,
560 jeb201400.

561 Jeromson, S., Hunter, D.J., 2014. Influencing mitochondrial membrane composition and
562 bioenergetics through omega-3 supplementation. The Journal of Physiology 592, 1913-1914.

563 Kadenbach, B., 2003. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. Biochim.
564 Biophys. Acta-Bioenerg. 1604, 77-94.

565 Kang, J.X., 2011. Omega-3: A link between global climate change and human health. Biotechnology
566 Advances 29, 388-390.

567 Kraffe, E., Marty, Y., Guderley, H., 2007. Changes in mitochondrial oxidative capacities during thermal
568 acclimation of rainbow trout *Oncorhynchus mykiss*: roles of membrane proteins, phospholipids and
569 their fatty acid compositions. J. Exp. Biol. 210, 149-165.

570 Lebreton, B., Richard, P., Parlier, E.P., Guillou, G., Blanchard, G.F., 2011. Trophic ecology of mullets
571 during their spring migration in a European saltmarsh: A stable isotope study. Estuarine Coastal and
572 Shelf Science 91, 502-510.

573 Mansbach, C.M., II, Gorelick, F., 2007. Development and Physiological Regulation of Intestinal Lipid
574 Absorption. II. Dietary lipid absorption, complex lipid synthesis, and the intracellular packaging and
575 secretion of chylomicrons. American Journal of Physiology-Gastrointestinal and Liver Physiology 293,
576 G645-G650.

577 Martin, N., Bureau, D.P., Marty, Y., Kraffe, E., Guderley, H., 2013. Dietary lipid quality and
578 mitochondrial membrane composition in trout: responses of membrane enzymes and oxidative
579 capacities. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology
580 183, 393-408.

581 Mathieu-Resuge, M., Kraffe, E., Le Grand, F., Boens, A., Bideau, A., Lluch-Cota, S.E., Racotta, I.S.,
582 Schaal, G., 2019. Trophic ecology of suspension-feeding bivalves inhabiting a north-eastern Pacific
583 coastal lagoon: Comparison of different biomarkers. Marine Environmental Research 145, 155-163.

584 Mazon, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M.,
585 Bromage, N., 2003. Dietary lipid enhancement of broodstock reproductive performance and egg and
586 larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 227, 21-33.

587 McIntyre, P.B., Reidy Liermann, C.A., Revenga, C., 2016. Linking freshwater fishery management to
588 global food security and biodiversity conservation. Proceedings of the National Academy of Sciences
589 113, 12880-12885.

590 McKenzie, D.J., 2001. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of
591 fish. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 128, 607-621.

592 McKenzie, D.J., Higgs, D.A., Dosanjh, B.S., Deacon, G., Randall, D.J., 1998. Dietary fatty acid
593 composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. Fish
594 Physiol. Biochem. 19, 111-122.

595 Morash, A.J., Bureau, D.P., McClelland, G.B., 2009. Effects of dietary fatty acid composition on the
596 regulation of carnitine palmitoyltransferase (CPT) I in rainbow trout (*Oncorhynchus mykiss*).
597 Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 152, 85-93.

598 Mourente, G., Tocher, D.R., 1993. Incorporation and metabolism of C-14-labeled polyunsaturated
599 fatty-acids in wild-caught juveniles of golden gray mullet, *liza-aurata*, *in vivo*. Fish Physiol. Biochem.
600 12, 119-130.

601 Nogueira, V., Piquet, M.A., Devin, A., Fiore, C., Fontaine, E., Brandolin, G., Rigoulet, M., Leverve, X.M.,
602 2001. Mitochondrial adaptation to *in vivo* polyunsaturated fatty acid deficiency: Increase in
603 phosphorylation efficiency. Journal of Bioenergetics and Biomembranes 33, 53-61.

604 Norambuena, F., Morais, S., Emery, J.A., Turchini, G.M., 2015. Arachidonic acid and eicosapentaenoic
605 acid metabolism in juvenile *Atlantic salmon* as affected by water temperature. Plos One 10, 25.

606 Oboh, A., Kabeya, N., Carmona-Antoñanzas, G., Castro, L.F.C., Dick, J.R., Tocher, D.R., Monroig, O.,
607 2017. Two alternative pathways for docosahexaenoic acid (DHA, 22:6n-3) biosynthesis are
608 widespread among teleost fish. Scientific Reports 7, 3889.

609 Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of
610 thermal tolerance. Science 315, 95-97.

611 Ramsey, J.J., Harper, M.E., Humble, S.J., Koomson, E.K., Ram, J.J., Bevilacqua, L., Hagopian, K., 2005.
612 Influence of mitochondrial membrane fatty acid composition on proton leak and H₂O₂ production in
613 liver. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 140, 99-108.

614 Robert, K.A., Bronikowski, A.M., 2010. Evolution of senescence in nature: Physiological evolution in
615 populations of garter snake with divergent life histories. *American Naturalist* 175, E47-159.

616 Robin, J.H., Skalli, A., 2007. Incorporation of dietary fatty acid in European sea bass (*Dicentrarchus*
617 *labrax*) - A methodological approach evidencing losses of highly unsaturated fatty acids. *Aquaculture*
618 263, 227-237.

619 Salin, K., Auer, S.K., Anderson, G.J., Selman, C., Metcalfe, N.B., 2016a. Inadequate food intake at high
620 temperatures is related to depressed mitochondrial respiratory capacity. *J. Exp. Biol.* 219, 1356-1362.

621 Salin, K., Auer, S.K., Rey, B., Selman, C., Metcalfe, N.B., 2015. Variation in the link between oxygen
622 consumption and ATP production, and its relevance for animal performance. *Proc. R. Soc. B-Biol. Sci.*
623 282, 20151028.

624 Salin, K., Auer, S.K., Rudolf, A.M., Anderson, G.J., Selman, C., Metcalfe, N.B., 2016b. Variation in
625 metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak
626 respiration. *Physiological and Biochemical Zoology* 89, 511-523.

627 Salin, K., Luquet, E., Rey, B., Roussel, D., Voituron, Y., 2012. Alteration of mitochondrial efficiency
628 affects oxidative balance, development and growth in frog (*Rana temporaria*) tadpoles. *J. Exp. Biol.*
629 215, 863-869.

630 Salin, K., Villasevil, E.M., Anderson, G.J., Auer, S.K., Selman, C., Hartley, R.C., Mullen, W., Chinopoulos,
631 C., Metcalfe, N.B., 2018. Decreased mitochondrial metabolic requirements in fasting animals carry an
632 oxidative cost. *Functional Ecology* 32, 2149-2157.

633 Salin, K., Villasevil, E.M., Auer, S.K., Anderson, G.J., Selman, C., Metcalfe, N.B., Chinopoulos, C., 2016c.
634 Simultaneous measurement of mitochondrial respiration and ATP production in tissue homogenates
635 and calculation of effective P/O ratios. *Physiological Reports* 4, e13007.

636 Salin, K., Villasevil Eugenia, M., Anderson Graeme, J., Lamarre Simon, G., Melanson Chloé, A.,
637 McCarthy, I., Selman, C., Metcalfe Neil, B., 2019. Differences in mitochondrial efficiency explain
638 individual variation in growth performance. *Proceedings of the Royal Society B: Biological Sciences*
639 286, 20191466.

640 Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an
641 integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates.
642 *Marine Environmental Research* 79, 1-15.

643 Szmajcinski, H., Lakowicz, J.R., 1996. Fluorescence lifetime characterization of magnesium probes:
644 Improvement of Mg²⁺ dynamic range and sensitivity using phase-modulation fluorometry. *Journal of*
645 *Fluorescence* 6, 83-95.

646 Team, R.C., 2017. R: A language and Environment for Statistical, 3.6.1. ed. R Foundation for Statistical
647 Computing, Vienna, Austria.

648 Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in*
649 *Fisheries Science* 11, 107-184.

650 Vagner, M., Lacoue-Labarthe, T., Zambonino-Infante, J.-L., Mazurais, D., Dubillot, E., Le Delliou, H.,
651 Quazuguel, P., Lefrançois, C., 2015. Depletion of essential fatty acids in the food source affects
652 aerobic capacities of the golden grey mullet *Liza aurata* in a warming seawater context. *Plos One* 10,
653 19.

654 Vagner, M., Zambonino-Infante, J.-L., Mazurais, D., Imbert-Auvray, N., Ouillon, N., Dubillot, E., Le
655 Delliou, H., Akbar, D., Lefrançois, C., 2014. Reduced n-3 highly unsaturated fatty acids dietary content
656 expected with global change reduces the metabolic capacity of the golden grey mullet. *Marine*
657 *Biology* 161, 2547-2562.

658 Yu, L., Fink, B., Herlein, J., Oltman, C., Lamping, K., Sivitz, W., 2014. Dietary fat, fatty acid saturation
659 and mitochondrial bioenergetics. *Journal of Bioenergetics and Biomembranes* 46, 33-44.

660

661 **Table 1:** Mean \pm SEM eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) content and sum of
 662 EPA and DHA content (Σ EPA + DHA) in muscle membrane fatty acids (percentage of the EPA and DHA
 663 mass per total fatty acids mass in membrane, %), of juvenile golden grey mullet given High (n=11) or
 664 Low (n=12) omega-3 highly unsaturated fatty acids (n-3 HUFA) diet. Different letters within a row
 665 indicate significant differences between diet treatments (Wilcoxon tests, at the significant level $p <$
 666 0.05). More details on FA composition of muscle membranes are provided Table S3.

667

<i>Muscle membrane FA composition (%)</i>	High n-3 HUFA fish	Low n-3 HUFA fish
<i>EPA</i>	9.8 \pm 0.3 ^a	5.7 \pm 1.0 ^b
<i>DHA</i>	20.8 \pm 0.7 ^a	10.1 \pm 2.0 ^b
Σ EPA + DHA	30.6 \pm 0.8 ^a	15.8 \pm 3.0 ^b

668

669

670 **Table 2:** Results from linear model analyses of muscle mitochondrial efficiency (ATP/O ratio) and
 671 respiratory capacities to offset the proton leak (LEAK respiration) in juvenile golden grey mullet as a
 672 function of the sum of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) content - Σ EPA +
 673 DHA in muscle (percentage of the EPA and DHA mass per total fatty acid mass in membrane). Bold
 674 denotes significant results.

675

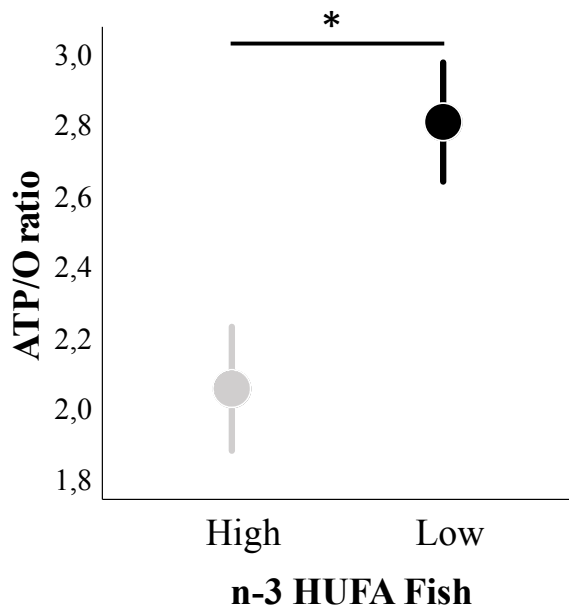
<i>Dependant variable</i>	<i>Source of variation</i>	<i>Parameter estimate \pm SEM</i>	<i>t-value</i>	<i>p-value</i>
ATP/O ratio	Intercept	2.21 \pm 0.23	7.978	< 0.001
	Σ EPA + DHA	0.04 \pm 0.01	2.513	0.021
	diet	1.30 \pm 0.31	4.232	< 0.001
LEAK respiration	Intercept	56.59 \pm 6.45	8.769	<0.001
	Σ EPA + DHA	0.04 \pm 0.34	-0.104	0.919
	diet	2.61 \pm 7.17	0.364	0.720

676

677 FIGURES

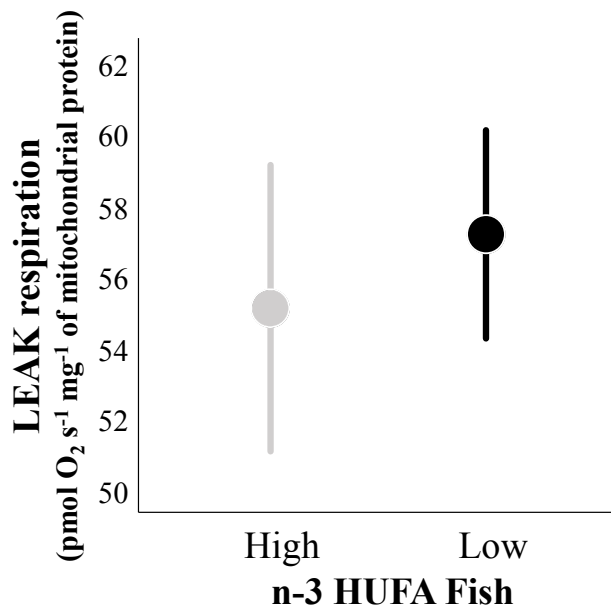
678 **Figure 1.** Effect of omega-3 highly unsaturated fatty acids (n-3 HUFA) dietary content on (a)
679 mitochondrial efficiency estimated as ATP/O ratio ($t_{21} = -3.107$, $p = 0.005$) and (b) mitochondrial LEAK
680 respiration ($t_{21} = -0.423$, $p = 0.677$) of muscle of golden grey mullet that were kept either on High
681 (n=11) or Low (n=12) n-3 HUFA diet. Data are plotted as mean \pm SEM. * denotes significant effect.

682 (a)



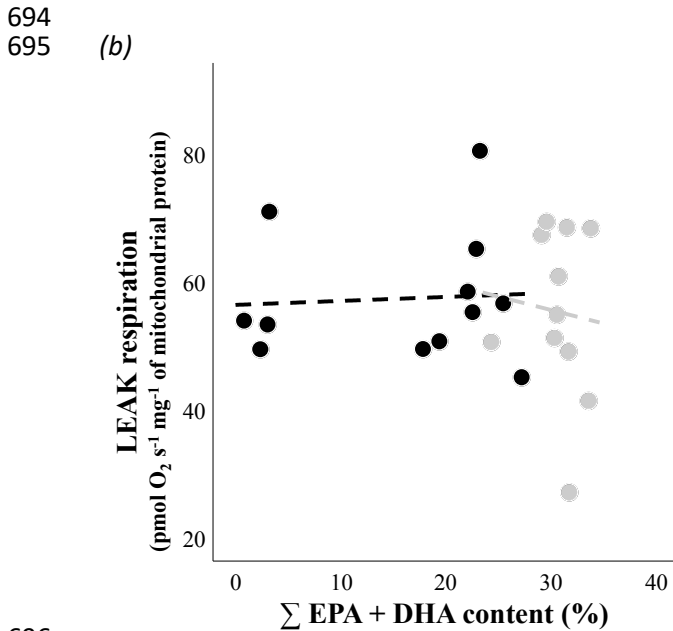
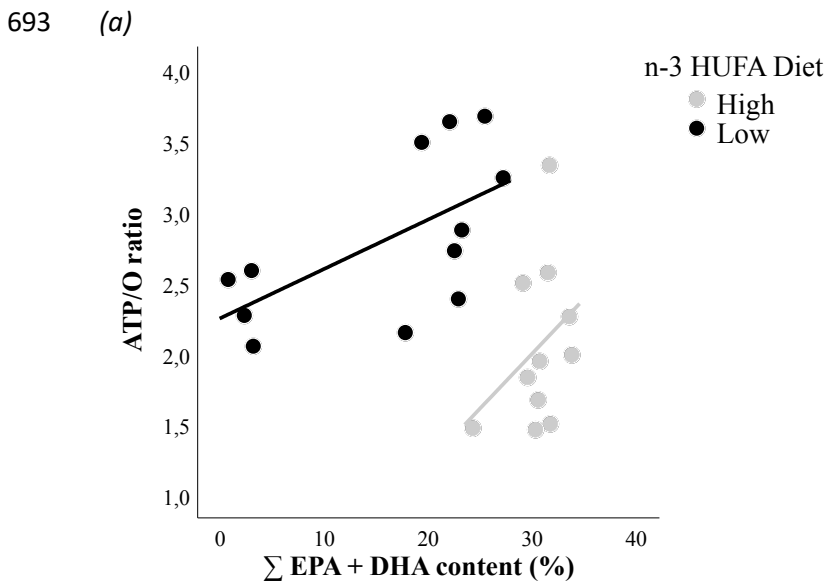
683

684 (b)



685

686 **Figure 2.** Relationships between the mitochondrial function and membrane lipid content in
 687 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content (expressed as Σ EPA + DHA
 688 content, percentage of the sum of EPA and DHA mass per total fatty acid mass in membranes) of
 689 muscle of juvenile golden grey mullet fed on a Low (n=12) versus High (n=11) omega-3 highly
 690 unsaturated fatty acid (n-3 HUFA) diet. Mitochondrial efficiency (ATP/O ratio) in relation to (a) Σ EPA
 691 + DHA content, (b) LEAK respiration in relation to Σ EPA + DHA content. Continuous lines show
 692 significant effects, dashed lines show non-significant effects (see Table 2 for details).



696
697

698 **ELECTRONIC SUPPLEMENTARY MATERIAL**

699 **Table S1:** (a) Ingredient (in g 100 g⁻¹) and (b) fatty acid (FA) composition expressed as a percentage
 700 of FA mass per total FA mass (% mean ± standard error of the mean; n = 3) in the High and Low
 701 omega-3 highly unsaturated fatty acid (n-3 HUFA) diets (made at INRA, Donzag, France). Only FA that
 702 occur above 1 % in at least a treatment group are represented. Bold denotes significant results of
 703 one-way ANOVA. Assumptions of normal distribution and homoscedasticity of residuals were met.

704 (a)

Ingredients ^a	High n-3 HUFA diet	Low n-3 HUFA diet
Fish meal LT 94	17	17
Casein	30	30
Rapeseed oil	2	10
Fish oil	8	0
Precooked starch	30	30
Vitamin mixture ^b	8	8
Mineral mixture ^c	4	4
Betaine	1	1

705

706 ^a Sources: fish meal LT 94: Norse (Fyllingsdalen, Norway); casein: Sigma-Aldrich (Germany); rapeseed oil:
 707 Système U (Créteil, France); fish oil: pure cod oil Cooper (Melun, France); precooked starch: Prégéflo Roquette
 708 Frères (Lestrem, France); vitamin mixture (INRA Jouy-en-Josas, France)

709 ^b Vitamin mixture (g kg⁻¹ vitamin mix): retinyl acetate 1; cholecalciferol 2.5; D α -tocopheryl acetate 5;
 710 menadione 1; thiamine-HCl 0.1; riboflavin 0.4; D-calcium pantothenate 2; pyridoxine-HCl 0.3; cyanocobalamin
 711 1; niacin 1; choline 200; ascorbic acid (ascorbyl polyphosphate) 5; folic acid 0.1; D-biotin 1; mesoinositol 30.

712 ^c Mineral mixture (g kg⁻¹ mineral mix): KCl 90; KI 0,04; CaHPO₄·2H₂O 500; NaCl 40; CuSO₄·5H₂O 3; ZnSO₄·7H₂O 4;
 713 CoSO₄ 0.02; FeSO₄·7H₂O 20; MnSO₄·H₂O 3; CaCo₃ 215; MgOH 124; Na₂SeO₃ 0 03; NaF 1.

714

715 (b)

Diet FA composition (%)	High n-3 HUFA	Low n-3 HUFA	F	Df	p-value
14 :0	4.30 ± 0.20	0.41 ± 0.02	362.2	1	< 0.001
16 :0	18.67 ± 0.80	6.10 ± 0.26	223.6	1	< 0.001
18 :0	4.59 ± 0.21	1.91 ± 0.09	142.7	1	< 0.001
16 :1n-7	4.83 ± 0.11	0.44 ± 0.01	1630	1	< 0.001
18 :1n-9	25.04 ± 0.23	56.74 ± 0.30	7004	1	< 0.001
18 :1n-7	2.56 ± 0.36	0.05 ± 0.05	46.2	1	< 0.01
20 :1n-9	1.82 ± 0.08	4.94 ± 2.09	2.2	1	0.21
18 :2n-6	5.06 ± 0.36	15.13 ± 1.82	29.6	1	< 0.01
18 :3n-3	2.42 ± 0.25	6.49 ± 1.03	14.7	1	0.02
20 :4n-6	1.38 ± 0.07	0.27 ± 0.04	205.1	1	< 0.001
20 :5n-3	6.63 ± 0.60	0.39 ± 0.07	106.3	1	< 0.001
22 :5n-3	1.64 ± 0.09	0.88 ± 0.31	5.5	1	0.07
22 :6n-3	10.95 ± 0.63	0.78 ± 0.14	246.3	1	< 0.001

716

717 **Table S2:** Mean \pm SEM mitochondrial function of muscle of golden grey mullet fed with High (n=11)
 718 and Low omega-3 highly unsaturated fatty acid (n-3 HUFA) diet. Rate of ATP production, rate of oxygen
 719 consumption to support ATP production (OXPHOS respiration), and to offset the proton leakage (LEAK
 720 respiration). Fluxes are expressed in pmol ATP produced and oxygen consumed $s^{-1} mg^{-1}$ of
 721 mitochondrial protein. The respiratory control ratio (RCR), an index of mitochondrial coupling (Brand
 722 and Nicholls, 2011) was calculated as the ratio of OXPHOS respiration to the LEAK respiration.

Muscle mitochondrial function **High n-3 HUFA fish** **Low n-3 HUFA fish**

<i>ATP production</i>	3267.6 \pm 445.8	3869.7 \pm 237.4
<i>OXPHOS respiration</i>	836.8 \pm 112.0	733.6 \pm 60.8
<i>LEAK respiration</i>	55.1 \pm 4.0	57.1 \pm 2.9
<i>RCR</i>	14.6 \pm 1.5	13.0 \pm 1.0

723

724 **Table S3:** Results from Kruskal-Wallis tests comparing fatty acid contents (percent of FA mass per total
 725 FA mass) in membrane lipids of the muscle of golden grey mullet (*Chelon auratus*) that were either fed
 726 a High or Low omega-3 highly unsaturated fatty acid (n-3 HUFA) diet. Only FA that occur above 1 % in
 727 at least a treatment group are represented. Means are expressed \pm standard error of the mean. Bold
 728 denotes significant results. N = 11-12 per treatment group.

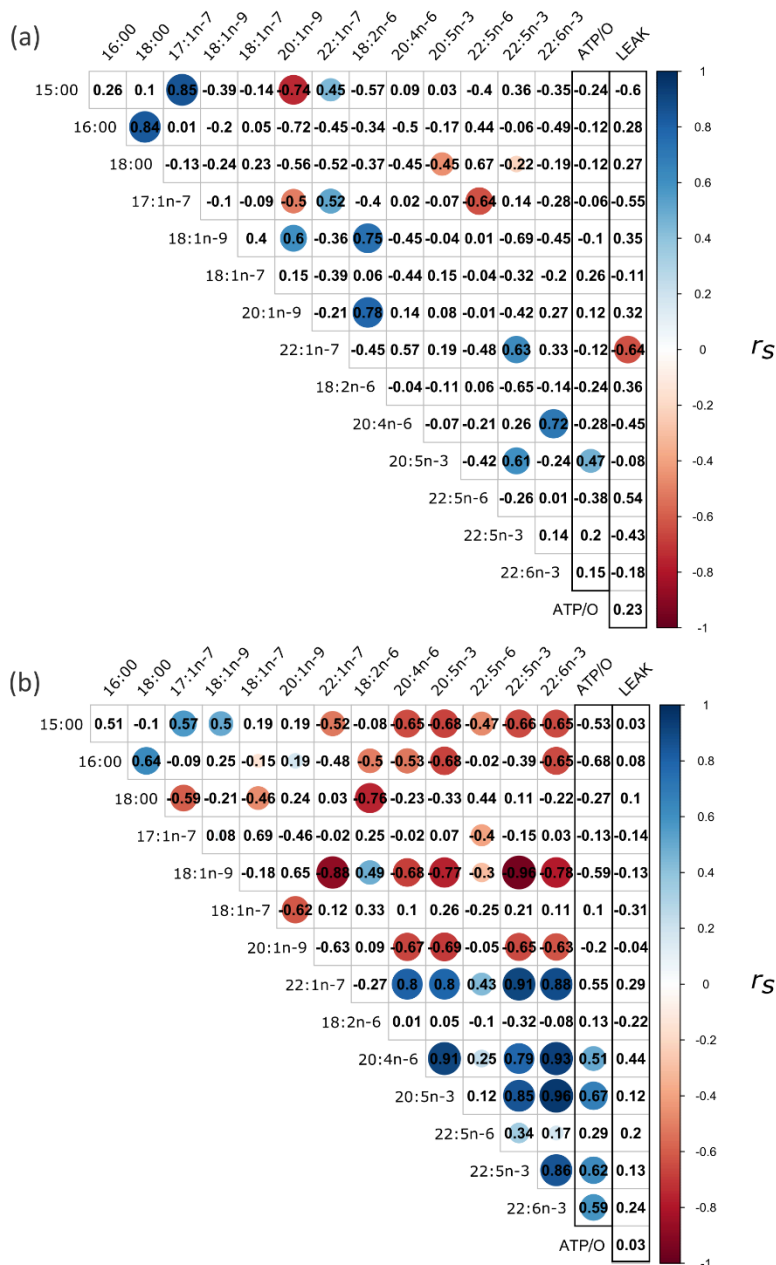
729

<i>Muscle membrane FA composition (%)</i>	<i>High n-3 HUFA</i>	<i>Low n-3 HUFA</i>	<i>Chi²</i>	<i>p-value</i>
15 :0	0.97 \pm 0.08	1.68 \pm 0.29	4.125	0.04
16 :0	18.49 \pm 0.75	21.15 \pm 2.20	0.015	0.902
18 :0	9.78 \pm 0.39	9.91 \pm 1.44	0.379	0.54
17 :1n-7	1.29 \pm 0.09	1.66 \pm 0.17	1.83	0.18
18 :1n-9	11.42 \pm 0.46	19.32 \pm 1.98	15.51	< 0.001
18 :1n-7	2.95 \pm 0.07	2.81 \pm 0.08	1.67	0.20
20 :1n-9	1.03 \pm 0.06	1.41 \pm 0.09	8.72	0.003
22 :1n-7	1.42 \pm 0.10	1.40 \pm 0.19	0.034	0.85
18 :2n-6	1.70 \pm 0.24	4.95 \pm 0.72	11.05	< 0.001
20 :4n-6	4.16 \pm 0.08	2.31 \pm 0.42	16.5	< 0.001
20 :5n-3	9.82 \pm 0.32	5.70 \pm 1.03	10.64	< 0.001
22 :5n-6	1.35 \pm 0.08	0.89 \pm 0.14	8.37	< 0.01
22 :5n-3	5.58 \pm 0.15	3.97 \pm 0.62	2.76	0.09
22 :6n-3	20.78 \pm 0.66	10.08 \pm 1.96	16.5	< 0.001

730

731

732 **Figure S1:** Spearman's correlation analyses between the membrane lipid fatty acids and the
 733 mitochondrial function of muscle of golden grey mullet fed with (a) High (n=11) and (b) Low omega-3
 734 highly unsaturated fatty acid (n-3 HUFA) diet. FA composition were calculated as percent of FA mass
 735 per total FA mass. Only FA that occur above 1 % in at least a treatment group are represented.
 736 Mitochondrial function were expressed as the mitochondrial efficiency (ATP/O) and the LEAK
 737 respiration. N = 11-12 per treatment group. The coloured circles represent significant correlations
 738 between two variables ($p < 0.05$). The numbers inside cells are the associated Spearman's correlation
 739 (r_s).



740