Fatty acid remodelling during embryogenesis in Greenland halibut (*Reinhardtius hippoglossoides*) in relation to hypoxia tolerance

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Abstract

Eggs of Greenland halibut (*Reinhardtius hippoglossoides*) are subjected to hypoxic conditions in the deep waters of the Estuary and Gulf of St. Lawrence during their development. The aim of this study was to determine whether there was potential remodelling of fatty acids (FA) during embryogenesis in eggs exposed to several levels of dissolved oxygen (DO). Fertilized eggs were exposed to four DO levels: severely hypoxic (10 and 20% sat [percent saturation]), moderately hypoxic (35% sat), and normoxic (100% sat). No FA remodelling was observed in eggs submitted to the different DO levels. The most abundant FA in the polar lipid fraction were docosahexaenoic (DHA, 22: 6 n-3) and eicosapentaenoic (EPA, 20: 5 n-3) acids (26 and 22%, respectively), the latter being positively correlated with hatching success. Batches with low hatching success were characterized by eggs with decreased levels of polar EPA and DHA at the end of embryogenesis. Whatever the hatching success, the proportion of FA in the neutral fraction was always significantly lower compared to the polar fraction. Oleic acid (18: 1 n-9) (17%) was the main FA found in this fraction; together with gondoic acid (20:1 n-9), they explained most of the variation in hatching success.

Keywords: hypoxia; Greenland halibut; eggs; fatty acids; embryogenesis; hatching success, female

Introduction

In oviparous fish species, fatty acids (FA) are transferred from the female to the eggs prior to their release into the environment. Female nutrition and condition are then

important factors that will determine the FA composition in fish eggs (Grote et al. 2011; Pickova et al. 2007). Fatty acids are important for the successful development of fish embryos and serve multiple purposes, including oxidative fuels, structural components for organogenesis, and precursors of eicosanoids, a group of highly biologically active hormones (De Meester et al. 2013). Some studies have also related hatching success and embryonic survival to the content of essential PUFA, namely arachidonic acid (AA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3) (Fernández-Palacios et al. 2011; Mejri et al. 2014). The DHA and EPA in membrane phospholipids play important roles in the functional integrity of biological membranes and may increase resistance of young stages to potentially extreme environmental conditions.

Once released by females, eggs may be subjected to detrimental environmental conditions such as low dissolved oxygen levels. The occurrence of hypoxia events in water bodies is increasing. The tolerance to hypoxia varies greatly among fish species, and some have developed metabolic pathways and behaviour allowing survival in hypoxic conditions (Van Den Thillart et al. 2002). One of these mechanisms is homeoviscous adaptation, consisting of a remodelling of the phospholipid head groups, fatty acid composition, and/or cholesterol content in cell membranes in order to adjust structure fluidity following environmental changes. The occurrence of homeoviscous adaptation has been extensively demonstrated following changes in temperature conditions in fish species (Hazel 1995; Crocke 1998; Laurel et al. 2012; Barnes et al. 2014). However, for other factors such as pressure and hypoxia, studies are still very scarce (i.e. Sebert 2002; Olufsen et al. 2014). Exposure of salmon (*Salmo salar*) hepatocytes to hypoxia produced significant changes in membrane lipid profile and in biological processes regulating membrane lipid homeostasis

60 (Olufsen et al. 2014). These authors have shown an increased mRNA expression of fatty
61 acid desaturases (FADs) and elongase (FAE); FAD5, FAD6 and FAE, three enzymes of
62 the FA elongation pathway, paralleled with an increase in membrane linoleic acid (18:2 n-
63 6) and DHA levels, following exposure to hypoxia.
64 Membrane bilayers in metabolically active tissues are more polyunsaturated than in those
65 that are less metatolically active (Hulbert and Else 1999; Hulbert 2008). It has been
66 proposed that polyunsaturated membranes facilitate the molecular activity of membrane
67 proteins, so the membrane would then play a pacemaker role in cell metabolism (Hulbert
68 2008). This theory is supported by studies done on bivalves (e.g. Pernet et al. 2007).
69 However, in fishes, contradictory results has been obtained (McKenzie et al. 2000;
70 Chatelier et al. 2006).
71 Greenland halibut (*Reinhardtius hypoglossoides*) is a commercially important
72 flatfish species that lives at depths greater than 150 m in the Estuary and Gulf of St.
73 Lawrence (Ait Youcef et al. 2013), where dissolved oxygen levels can be as low as 18%
74 sat (percent saturation) (Bourgault et al. 2012; Ait Youcef et al. 2013). Greenland halibut
75 eggs are bathypelagic, and embryonic development occurs almost entirely in deep waters
76 (Ådlandsvik et al. 2004; Domínguez-Petit et al. 2013) that may be impacted by hypoxia.
77 Indeed, it has already been shown that the lethal DO threshold level for the early life stages
78 of this species is between 10 and 20% sat, with hatching occurring at DO levels as low as
79 20% sat, suggesting adaptation to hypoxia (Mejri et al. 2012). However, the same authors
80 also showed large differences in egg quality among females. The aim of our study was to
81 test whether there is a remodelling of fatty acids in Greenland halibut eggs in response to
82 hypoxia. We tested the hypothesis that fatty acid remodelling in the polar fraction is linked

to increase embryogenesis success in hypoxic conditions. We also tested the hypothesis that the main sources of energy used by embryos to support their development are the saturated and monounsaturated fatty acids (SFA and MUFA) found in the lipid polar fraction. This hypothesis is based on previous observations indicating that phospholipids are the main lipid class in these eggs and that energy is more efficiently released via β-oxidation of SFA and MUFA than of PUFA. Finally, we verified how FA composition from the neutral and polar fractions might explain differences in hatching success between egg batches produced by different females.

Materials and methods

Experimental design and egg sampling

The egg sampling procedures and the experimental design for Greenland halibut were previously detailed in Mejri et al. (2012). Briefly, mature fish were captured by longline fishing in the Gaspé area (48° 59' N; 64° 23' W; Quebec, Canada) and kept in circular tanks (5°C, salinity 32) with flow-through seawater until we were able to collect the hydrated eggs from three different females, which were fertilized by the sperm of a single male. Fertilized eggs from each female were incubated at four DO levels (severe hypoxia: 10 and 20% sat, ~0.7 and 1.4 mg L^{-1}; moderate hypoxia: 35% sat, ~2.4 mg L^{-1}; normoxia: 100% sat, ~6.9 mg L^{-1}), with two replicates for each DO level. In each incubator, temperature was maintained at 5°C and salinity at 32. DO levels were kept stable using the experimental set-up developed by Plante et al. (1998) and detailed in Mejri et al. (2012). Three replicates (20 embryos each) per egg batch were sampled at 14, 17, and 21 days post fertilization (dpf) and stored at −80°C for fatty acid analysis. Eggs incubated at four DO

106 levels (100, 35, 20, and 10% sat) hatched on average 28 days after fertilization. Hatching
107 success was significantly affected by the interaction between DO levels and females ($p <$
108 0.0001) (see Mejri et al. 2012 for complete statistical analysis). Egg batches from three
109 females (females A, B, and C) characterized by high (38.9 ± 3.9%), medium (12.6 ± 2.5%),
110 and low (4.2 ± 4.1%) hatching successes, respectively, were considered. Highest and
111 lowest hatching successes were observed for females A and C and no eggs hatched at 10
112 % sat for any of the females (Fig. 1, modified from Mejri et al. 2012).
113
114 **Fatty acid analysis**
115 Lipids were extracted using the Folch method (Folch et al. 1957), separated into
116 neutral and polar fractions using silica gel (30 × 5 mm i.d., packed with Kieselgel 60, 70–
117 230 mesh; Merck, Darmstadt, Germany) hydrated with 6% water, and eluted with 10 mL
118 of chloroform:methanol (98:2 v/v) for neutral lipids followed by 20 mL of methanol for
119 polar lipids (Marty et al. 1992). The neutral fraction was further eluted on an activated
120 silica gel with 3 mL of hexane and diethyl ether to eliminate free sterols. All fatty acid
121 methyl esters (FAME) were prepared as described by Lepage and Roy (1984) and analyzed
122 in MSMS scan mode (ionic range: 60–650 m/z) on a Polaris Q ion trap coupled to a Trace
123 GC (Thermo Finnigan, Mississauga, ON, CA) equipped with a Valcobond VB-5 capillary
124 column (VICI Valco Instruments Co. Inc., Broakville, ON, CA). FAME were identified by
125 comparison of retention times with known standards (37 component FAME Mix, PUFA-
126 3, BAME, and menhaden oil; Supelco Bellefonte, PA, USA) and quantified with
127 tricosanoic acid (23:0) and nonadecanoic acid (19:0) as internal standards. Chromatograms

were analyzed using integration Xcalibur 1.3 software (Thermo Scientific, Mississauga, ON, CA).

Statistical analyses

Permutational multivariate analysis of variance (PERMANOVA with 9999 permutations), including a posteriori pair-wise comparisons, was performed on FA profiles and sums of SFA, MUFA, and PUFA from polar and neutral fractions. Each PERMANOVA was tested with three factors: DO levels (100, 35, 20, and 10% sat), females (three females with different hatch successes) and days post fertilization (14, 17, and 21). Assumptions of homoscedasticity were verified with a PERMDISP test, and data were transformed (arcsine square root) when necessary. To analyze the similarity between different females during embryonic development, SIMPER analyses were run using a Bray-Curtis similarity matrix with PRIMER 6 (v. 6.1.12) and PERMANOVA+ (v. 1.0.2). Linear regressions were performed between the hatching success (females) of Greenland halibut embryos and PUFA concentrations in the neutral and polar fractions. Normality was tested using both the Kolmogorov-Smirnov and Shapiro-Wilk tests. These analyses were performed with the JMP 9 package.

Results

Polar fraction of fatty acids

We observed no effect of DO levels on FA composition in polar lipids (*Pseudo* - $F_{\text{polar }(3, 33)} = 1.5$, $p = 0.19$). Percentages of different FA on day 14 post fertilization are presented in Table 1. PUFA percentages made up the larger fraction (> 50% of total polar

151 FA) compared to SFA and MUFA (> 31 and > 17% of total polar FA, respectively) (Table
152 1).

153 There was a significant interaction between female and days post fertilization
154 (*Pseudo* - $F_{(4, 33)} = 14.9$, $p < 0.01$). Similar results were found for the sums of SFA, MUFA,
155 and PUFA, with a significant interaction of female and days post fertilization (*Pseudo* - F
156 $_{(4, 33)} = 16.2$, $p < 0.01$). The highest concentrations of FA were DHA (22:6 n-3) followed
157 by EPA (20:5 n-3) (Table 2). SIMPER analysis showed that DHA and EPA contents
158 explained > 41% of the differences among subgroups and PUFA explained > 42% (Table
159 3). Both DHA and EPA percentages were significantly lower in eggs from female C (the
160 low hatching success batch), particularly at 21 dpf (≤ 6%; Table 2).

161 **Neutral fraction of fatty acids**

162 The same tendency was observed in the neutral fraction as in polar fraction: there
163 was no effect of DO levels on FA composition (*Pseudo* – $F_{\text{neutral } (3, 33)} = 0.8$, $p = 0.56$). Here
164 SFA and MUFA made up the larger fractions (> 31 and > 35% of total neutral FA)
165 compared to PUFA (around 20% of total neutral FA) (Table 1). Moreover, a significant
166 interaction was noted between female and days post fertilization (*Pseudo* - $F_{(4, 33)} = 11.1$,
167 $p < 0.01$), but not with the same FA. In this case, oleic (18:1 n-9) and gondoic (20:1 n-9)
168 FA explained up to 13% of the differences (Table 3). These FA were significantly lower in
169 eggs from female C (the batch with low hatching success) at 21 dpf (≤ 3.5%; Table 4).
170 Sums of SFA, MUFA, and PUFA varied significantly according to the interaction between
171 female origin and days post fertilization as well (*Pseudo* - $F_{(4, 33)} = 15.2$, $p < 0.01$), with
172 MUFA explaining > 43% of the variation (Table 3).

173 .

174 **Relation between hatching success and relative fatty acid content**

175 Positive correlations between hatching success and polar lipid DHA (22:6 n-3) and
176 EPA (20:5 n-3) concentrations were found at 21 dpf (DHA: $F_{(1, 19)} = 4.62$, $r^2 = 0.19$, $p =$
177 0.04; EPA: $F_{(1, 19)} = 17.68$, $r^2 = 0.48$, $p < 0.001$; Fig. 2), but no correlation was found at 14
178 dpf (DHA and EPA: $r^2 \leq 0.03$, $p \geq 0.42$) or 17 dpf (DHA and EPA: $r^2 \leq 0.28$, $p \geq 0.06$). No
179 other significant correlations were found for other fatty acids. In neutral lipids, significant
180 correlations between hatching success and relative contents of DHA and EPA were
181 observed on days 17 and 21, respectively (DHA: $F_{(1, 22)} = 17.15$, $r^2 = 0.44$, $p < 0.01$; EPA:
182 $F_{(1, 19)} = 9.06$, $r^2 = 0.34$, $p < 0.01$; Fig. 3). Again, no significant correlation was found at
183 14 dpf (DHA and EPA: $r^2 \leq 0.03$, $p \geq 0.42$) or when other fatty acids were considered. For
184 both neutral and polar lipids, regressions were calculated using data from 24 sets obtained
185 from each batch of eggs (four DO levels × two tanks per DO level × eggs from each female
186 in each tank: 4 × 2 × 3).

187

188 **Discussion**

189 **Remodelling fatty acids in low DO**

190 We hypothesized that the presence of a homeoviscous mechanism, including the
191 selective retention of PUFA to increase membrane cell fluidity, would be present in
192 Greenland halibut eggs exposed to hypoxic conditions. The results do not support this
193 hypothesis: we found no specific effect on any essential fatty acids related to hypoxia
194 exposure in Greenland halibut eggs during embryogenesis. Some studies have been
195 conducted on the role of EFA in hypoxia tolerance, but these were mainly on fish larvae
196 and juveniles. For instance, McKenzie et al. (2008) showed that enriching live feed with

197 AA, DHA, and EPA led to a significant accumulation of these EFA in the tissues of early
198 life stages, with a significant effect on respiratory metabolism and tolerance to hypoxia in
199 Dover sole (*Solea solea*) larvae and juveniles. Deprivation of EFA in Dover sole larvae
200 increased their mortality during acute hypoxic stress (Logue et al. 2000). However, none
201 of these studies looked at the mechanisms underlying these effects. However, using in vitro
202 experiment on salmon hepatocytes, Olufsen et al. (2014) were able to show an
203 overexpression of enzymes involved in the the elongation of fatty acids in response to
204 hypoxia. In our study, even though we did not detect any effect of hypoxia on FA
205 remodelling during embryogenesis, this does not rule out the possibility of such response
206 in later developmental stages.
207
208 **Fuel for egg development**
209 We also tested the hypothesis that egg development in Greenland halibut is mainly
210 supported through the use of saturated and monounsaturated fatty acids from the polar
211 fraction. The results obtained on the use of SFA and MUFA from the polar fraction during
212 embryogenesis do not support this hypothesis. Indeed, MUFA from the neutral fraction
213 seemed to have been used during development. The fatty acid profile of Greenland halibut
214 eggs, which were rich in PUFA, was comparable to those from other fish species that
215 produce eggs with no oil globule (Sargent et al. 1997). In Greenland halibut, there were
216 proportionally more MUFA and less PUFA in neutral lipids than in polar lipids. Similar
217 results have been reported in other marine fish eggs (e.g., gilthead bream [*Sparus aurata*];
218 Fountoulaki et al. 2003). The higher levels of PUFA in polar lipids most likely reflect the
219 important role of these FA in maintaining appropriate cell membrane structure (Hazel and

220 Williams 1990). The use of MUFA, mainly oleic and gondoic FA, to support energetic
221 demands for embryonic development has also been demonstrated in other fish species,
222 including common snook (*Centropomus undecimalis*) and spotted wolffish (*Anarhichas*
223 *minor* Olafsen) (Tveiten et al. 2004). In accordance with our results, oleic acid (18:1 n-9)
224 in spotted wolffish eggs was found to be preferentially catabolized during embryogenesis
225 (Tveiten et al. 2004). Although DHA and EPA are known to be incorporated into cell
226 membranes during embryonic development, we observed that they also appear to serve as
227 an energy source during embryogenesis in Greenland halibut. In contrast to some other
228 studies, PUFA, such as DHA and EPA, were retained by devil stinger (*Inimicus japonicus*)
229 and Pacific cod (*Gadus macrocephalus*) eggs during development (Laurel et al. 2012; Wen
230 et al. 2013).
231
232 **FA composition in relation to hatching success**
233 We were interested in how FA composition from the neutral and polar fractions
234 might explain differences in hatching success between egg batches produced by different
235 females. Hatching was positively correlated with the relative profiles of DHA and EPA in
236 eggs at 21 dpf in both the neutral and polar fractions. Other studies have shown
237 relationships between egg viability and concentrations of DHA, EPA, and AA in different
238 marine fish eggs and larvae (Salze et al. 2005; Mansour et al. 2011; Lanes et al. 2012).
239 Eggs that had high and medium hatching success (females A and B, respectively) contained
240 higher proportions of DHA and EPA during embryonic development.
241 The maternal effect (i.e., female origin) was important in our study. The use of fatty
242 acids was greater in eggs from the female that had the lowest hatching success (female C),

243 resulting in varying FA allocation patterns between the three categories (high, medium,
244 and low hatching success) during embryonic development. Fatty acid composition in
245 embryos and larvae during development may vary both in quality and quantity within the
246 same species depending on environmental conditions (Dantagnan et al. 2007),
247 physiological events, or energy demands (Sargent et al. 1997). Fish egg quality, determined
248 by its biochemical composition, reflects the maternal contribution and is considered as a
249 key maternal factor for successful embryonic development and hatching (Murua and
250 Saborido-Rey 2003; Bachan et al. 2012). In our study, despite attempts made to minimize
251 confounding effects (using fish captured from the same location and feeding them identical
252 diets while in captivity), we still observed noticeable differences in egg composition among
253 females that resulted in large differences in hatching success. Although our fish were
254 maintained in captivity for one year, it is possible that the food they received in captivity
255 did not have a significant effect on egg composition and that the variations we observed
256 are representative of their previous wild diet. It remains unclear how the duration of the
257 dietary regime can affect egg composition (Yanes-Roca et al. 2009), even though it is
258 known that diet can have a direct impact on egg lipid composition during oogenesis
259 (Rainuzzo et al. 1997). Greater variation is expected in general in wild stocks because
260 environmental factors and maternal phenotypes are likely to be more diverse in nature than
261 in captivity. Females used in this study could have had different ages as well as dietary,
262 spawning, and distribution histories. Factors such as maternal age of spawners (Izquierdo
263 et al. 2001), condition (Ouellet et al. 2001), broodstock origin (Czesny et al. 2005), diet
264 (Mazorra et al. 2003), and environmental factors (Ayers 2006) can all contribute to

differences in FA composition among individual females that could result in variable egg quality and thus hatching success.

Intraspecific variation is also possible between eggs from the same batch. For example, some clutches of zebrafish (*Danio rerio*) eggs were found to be more affected by hypoxia than others, thus contributing to the high variation in developmental rate and also suggesting that genetics plays a significant role in the hypoxic response (Bagatto 2005). The change in egg phospholipids concentrations between batches of cod varied tremendously, with increases as high as 20% to declines as great as 70% between the initial and final egg batches (Bachan et al. 2012). Thus, the strong variation in FA profiles of Greenland halibut eggs could be related to both the different hatching percentages and days post fertilization.

Conclusion

In conclusion, during exposure to hypoxia, Greenland halibut eggs did not show a specific adaptive mechanism in relation to fatty acids, such as homeoviscous adaptation or selective retention of certain essential fatty acids. Moreover, Greenland halibut egg development is mainly supported by PUFA and MUFA from the polar and neutral fractions, respectively, and DHA and EPA were positively correlated with hatching success, suggesting the importance of these FA in the early life stages of this species. Our study shows the importance of fatty acids as a biochemical tool to investigate the effects of different biological and environmental factors on egg quality and hatching success.

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431 **Figures Legends**

432 Fig. 1. Mean hatching success (%) for Greenland halibut embryos obtained from three
433 females exposed to four levels of dissolved oxygen (mean ± SD) (modified from Mejri et
434 al. 2012).

435 Fig. 2. Content of docosahexaenoic acid (left) and eicosapentaenoic acid (right) in egg
436 polar lipids in relation to hatching success. Regressions were calculated using data from 24
437 sets obtained from each batch of eggs (four DO levels × two tanks per DO level × eggs
438 from each female in each tank: 4 × 2 × 3). The dashed lines represent data at 21 days post

439 fertilization (dpf).

440 Fig. 3. Content of docosahexaenoic acid (left) and eicosapentaenoic (right) in egg neutral
441 lipids in relation to hatching success. Regressions were calculated using data from 24 sets
442 obtained from each batch of eggs (four DO levels × two tanks per DO level × eggs from
443 each female in each tank: 4 × 2 × 3). The dashed lines represent data at 17 days post
444 fertilization (dpf) (left) and at 21 dpf (right).

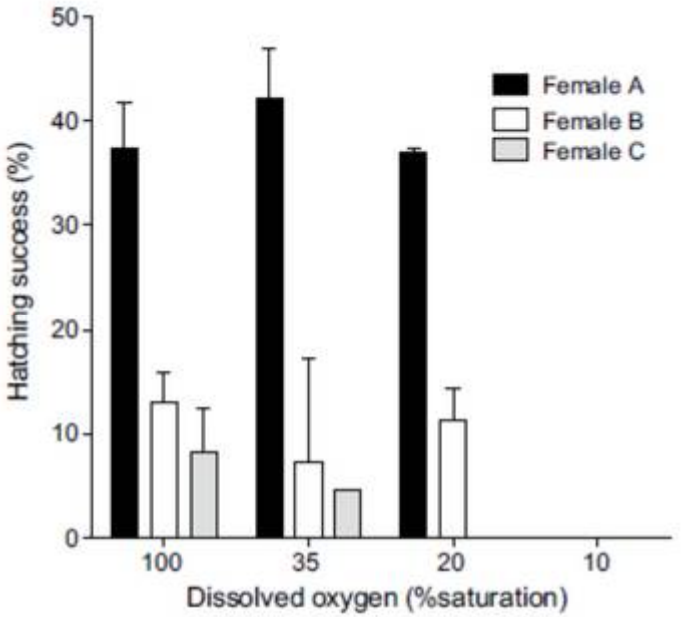
445 Table 1. Fatty acid composition (mean ± SD, expressed as percentage of total polar and
446 neutral lipids detected) in Greenland halibut eggs exposed to different dissolved oxygen
447 (DO) levels (100, 35, 20, and 10% sat) at 14 days post fertilization.

448 Table 2. Fatty acid composition of polar lipid fraction in Greenland halibut eggs (% weight
449 of total polar lipids ± SD) from females A, B and C.

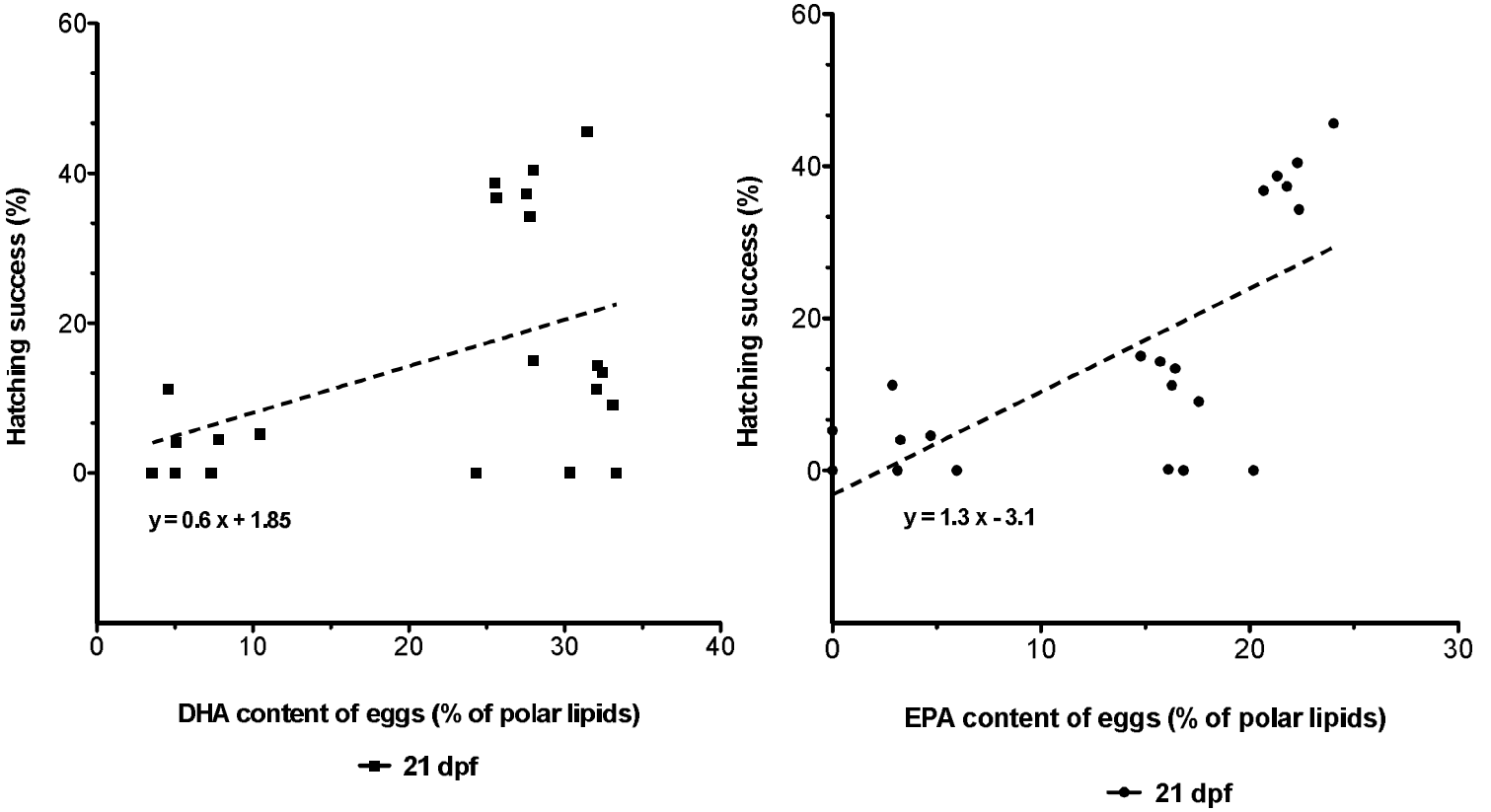
450 Table 3. SIMPER analysis results for fatty acids from polar and neutral lipid fractions
451 explaining most of the differences between 14, 17 and 21 days post fertilization (dpf) and
452 female Monounsaturated fatty acids (MUFA) include 16:1 n-7, 18:1 n-9, 20:1 n-9, 22:1 n-
453 9, 17:1, and 24:1 n-9; polyunsaturated fatty acids (PUFA) include 18:2 n-6, 20:3 n-6, 20:4
454 n-6, 18:3 n-3, 18:4 n-3, 20:3 n-3, 20:5 n-3, and 22:6 n-3.

455 Table 4. Fatty acid composition of the neutral lipid fraction (% weight of total neutral lipids
456 ± SD) in Greenland halibut eggs obtained from three different females.

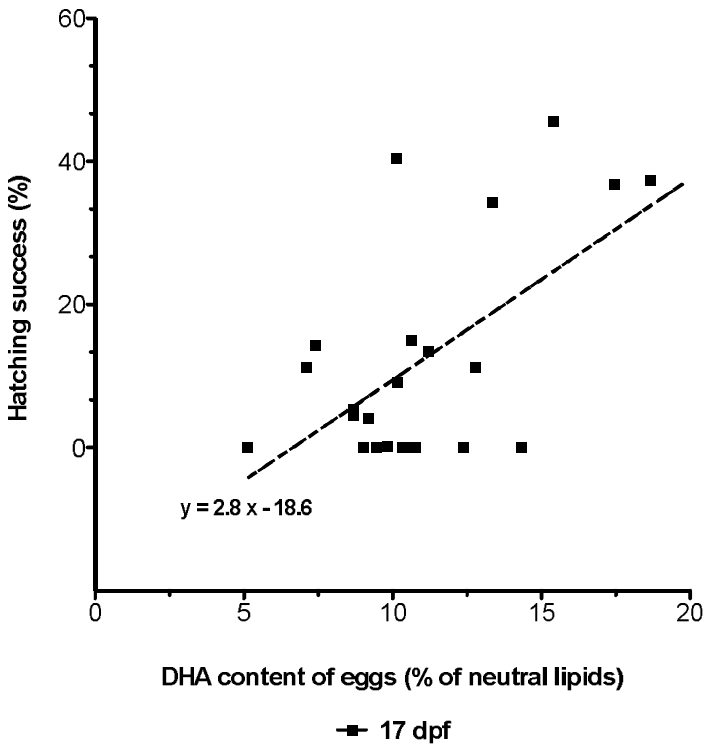
457

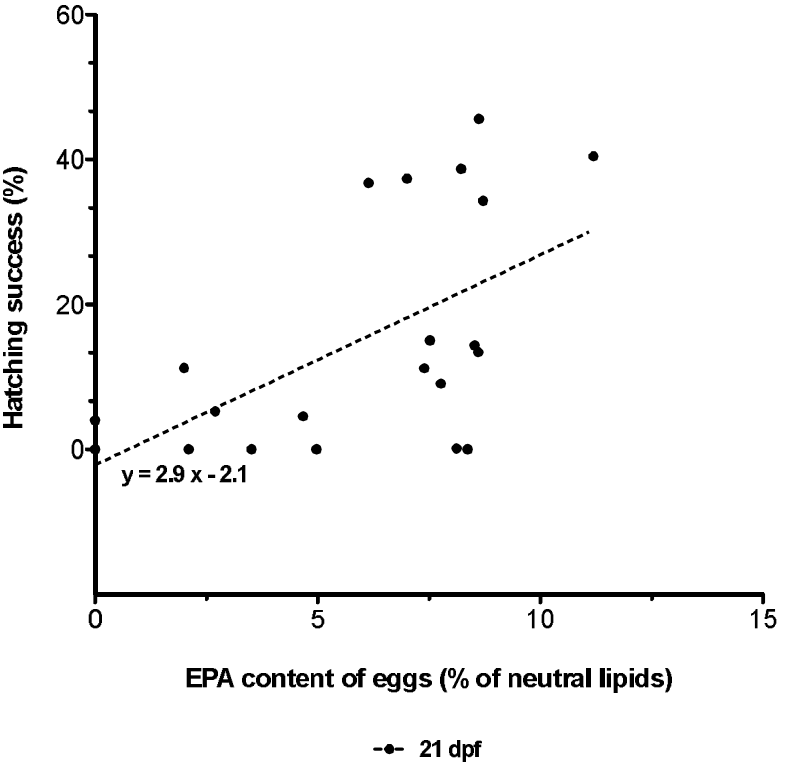

Female A
Female B
Female C
Hatching success (%)
0
10
20
30
40
50
100
35
20
10
Dissolved oxygen (%saturation)

Figure


Hatching success (%)
60
40
20
0
y = 0.6 x + 1.85
0
10
20
30
40
DHA content of eggs (% of polar lipids)
21 dpf
Hatching success (%)
60
40
20
0
y = 1.3 x - 3.1
0
10
20
30
EPA content of eggs (% of polar lipids)
21 dpf

Figure


60
40
20
0
Hatching success (%)
y = 2.8 x - 18.6
0
5
10
15
20
DHA content of eggs (% of neutral lipids)
17 dpf


60
40
20
0
Hatching success (%)
y = 2.9 x - 2.1
0
5
10
15
EPA content of eggs (% of neutral lipids)
21 dpf

Table

Table 1. Fatty acid composition (mean ± SD, expressed as percentage of total polar and neutral lipids detected) in Greenland halibut eggs exposed to different dissolved oxygen (DO) levels (100, 35, 20, and 10% sat) at 14 days post fertilization.

Fatty acid	Polar fatty acids				Neutral fatty acids			
	Dissolved oxygen levels (%sat)							
	100	35	20	10	100	35	20	10
C14:0	1.5 ± 0.4	1.6 ± 0.5	1.2 ± 0.0	1.3 ± 0.1	2.9 ± 0.4	2.4 ± 0.4	2.9 ± 0.8	2.8 ± 0.7
C16:0	24.3 ± 6.8	23.9 ± 11.0	18.9 ± 1.5	19.2 ± 1.2	11.9 ± 0.8	10.1 ± 1.5	12.8 ± 2.0	12.7 ± 2.5
C18:0	7.4 ± 2.0	8.6 ± 3.3	6.1 ± 0.5	6.0 ± 0.4	3.2 ± 1.1	3.3 ± 0.5	3.1 ± 0.7	2.9 ± 0.8
C22:0	0.16 ± 0.05	0.59 ± 0.33	0.27 ± 0.16	0.14 ± 0.08	1.1 ± 0.9	1.6 ± 0.6	0.5 ± 0.3	0.6 ± 0.2
C24:0	0.28 ± 0.09	1.01 ± 0.57	0.47 ± 0.28	0.41 ± 0.27	1.8 ± 1.3	2.7 ± 0.8	1.3 ± 0.8	1.1 ± 0.3
∑SFA†	37.5 ± 10.6	45.4 ± 21.3	31.7 ± 5.9	31.1 ± 3.5	33.2 ± 9.0	40.4 ± 13.7	33.8 ± 13.3	31.5 ± 8.5
C 16:1 n-7	3.43 ± 0.83	2.8 ± 0.5	2.8 ± 0.5	2.9 ± 0.7	7.7 ± 2.5	5.8 ± 2.1	7.7 ± 0.9	8.4 ± 1.4
C18:1 n-9	9.26 ± 1.83	8.7 ± 1.6	7.9 ± 1.2	8.3 ± 1.1	20.3 ± 5.6	16.6 ± 4.1	20.4 ± 2.5	22.1 ± 2.2
C20:1 n-9	6.31 ± 1.66	5.5 ± 1.6	5.5 ± 0.9	5.4 ± 1.0	8.5 ± 2.1	6.8 ± 1.7	9.4 ± 3.5	9.4 ± 2.0
C22:1 n-9	0.53 ± 0.48	0.7 ± 0.3	0.5 ± 0.2	0.5 ± 0.3	5.3 ± 2.4	4.5 ± 2.0	6.1 ± 3.2	6.2 ± 3.1
C24:1 n-9	0.20 ± 0.26	0.51 ± 0.47	0.22 ± 0.20	0.29 ± 0.21	2.3 ± 2.3	1.6 ± 1.5	1.3 ± 1.1	1.3 ± 1.0
∑MUFA‡	19.7 ± 5.1	18.3 ± 4.6	17.0 ± 3.2	17.6 ± 3.4	44.2 ± 15.1	35.6 ± 11.5	45.1 ± 11.5	47.5 ± 9.9
C18:2 n-6	0.49 ± 0.10	1.0 ± 0.4	0.7 ± 0.2	0.6 ± 0.2	1.8 ± 1.2	2.4 ± 0.8	1.3 ± 0.4	1.2 ± 0.3
C20:4 n-6	1.25 ± 0.36	1.2 ± 0.2	1.2 ± 0.6	1.4 ± 0.1	0.9 ± 1.2	0.8 ± 1.2	0.3 ± 0.3	0.5 ± 0.5
C20:5 n-3	15.6 ± 5.9	12.9 ± 7.7	18.7 ± 3.8	18.8 ± 3.5	6.6 ± 1.0	5.6 ± 0.9	6.0 ± 1.6	6.5 ± 1.0
C22:6 n-3	24.2 ± 7.9	18.1 ± 9.2	28.8 ± 2.3	28.6 ± 2.2	8.2 ± 1.3	7.5 ± 1.6	8.5 ± 3.5	8.7 ± 2.2
∑PUFAƀ	42.4± 14.8	35.5 ± 19.9	50.7 ± 8.0	50.6 ± 6.8	20.9 ± 7.9	22.1 ± 8.8	20.0 ± 9.0	19.8 ± 5.9
∑n-3	40.2 ± 14.1	32.0 ± 18.2	48.2 ± 6.6	48.0 ± 6.1	16.7 ± 3.9	16.4 ± 4.6	16.7 ± 6.6	16.7 ± 4.1
∑n-6	2.1 ± 0.6	3.5 ± 1.7	2.4 ± 1.3	2.6 ± 0.7	4.1 ± 3.9	5.6 ± 4.2	3.2 ± 2.3	3.0 ± 1.7

† Includes 15:0, 17:0, 20:0; ‡ includes 17:1; ƀ includes 20:2, 18:3 n-3, 18: 4 n-3, 18:3 n-6, 20:3 n-3, 20:3 n-6

Table 2. Fatty acid composition of the polar lipid fraction (% weight of total polar lipids ± SD) in Greenland halibut eggs with different hatching successes: high (38.9 ± 3.9%), medium (12.6 ± 2.5%), and low (4.2 ± 4.1%).

Polar fatty acids	Hatching success								
	High			Medium			Low		
	Days post fertilization								
	14	17	21	14	17	21	14	17	21
14:0	1.3 ± 0.3	1.4 ± 0.2	1.4 ± 0.1	1.6 ± 0.6	1.2 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	4.9 ± 1.1
16:0	19.9 ± 5.4	19.9 ± 2.4	18.3 ± 1.0	26.4 ± 8.4	19.1 ± 1.2	19.8 ± 0.9	18.5 ± 2.5	18.3 ± 0.7	23.5 ± 13.4
18:0	6.5 ± 1.6	6.2 ± 0.6	6.2 ± 0.3	8.4 ± 2.9	6.3 ± 0.3	6.4 ± 0.3	6.3 ± 0.7	5.7 ± 0.2	5.9 ± 2.3
∑SFA†	33.6 ± 10.5	30.1 ± 3.7	30.9 ± 4.3	42.0 ± 15.0	30.9 ± 2.8	30.5 ± 2.4	33.8 ± 9.6	29.2 ± 1.6	61.4 ± 34.7
16:1 n-7	2.5 ± 0.4	2.6 ± 0.2	2.6 ± 0.2	2.9 ± 0.6	2.6 ± 0.2	2.6 ± 0.2	3.6 ± 0.6	3.6 ± 0.1	7.6 ± 4.4
18:1 n-9	7.4 ± 1.3	6.9 ± 0.5	6.8 ± 0.4	9.8 ± 1.3	8.8 ± 0.2	8.9 ± 0.5	8.6 ± 0.8	8.0 ± 0.3	6.9 ± 9.9
20:1 n-9	6.8 ± 1.0	6.5 ± 0.5	6.1 ± 0.4	6.0 ± 0.8	5.3 ± 0.0	5.6 ± 0.4	4.3 ± 0.7	4.6 ± 0.7	1.6 ± 3.5
MUFA‡	17.9 ± 3.2	17.1 ± 1.6	16.6 ± 1.2	19.9 ± 3.3	17.9 ± 0.9	18.1 ± 1.3	16.8 ± 2.3	16.5 ± 1.2	17.9 ± 18.9
18:2 n-6	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.4	0.5 ± 0.0	3.1 ± 3.1
20:4 n-6	1.3 ± 0.2	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.3	1.9 ± 0.1	1.8 ± 0.1	1.1 ± 0.5	1.3 ± 0.1	0.0 ± 0.0
20:5 n-3	20.1 ± 4.7^{ab}	22.3 ± 2.0^{a}	21.8 ± 1.3^{a}	11.2 ± 5.3^{c}	15.9 ± 1.0^{b}	16.2 ± 0.9^{b}	18.3 ± 2.8^{ab}	19.9 ± 0.9^{ab}	2.8 ± 2.2^{d}
22:6 n-3	25.1 ± 5.6^{ab}	27.5 ± 2.6^{ab}	27.1 ± 2.0^{ab}	22.9 ± 10.9^{b}	30.9 ± 1.8^{a}	31.6 ± 1.9^{a}	26.8 ± 4.5^{ab}	30.9 ± 1.4^{a}	6.2 ± 2.4^{c}
∑PUFAƀ	48.2 ± 11.5^{a}	52.5 ± 4.9^{a}	52.2 ± 4.4^{a}	37.4 ± 15.6^{b}	50.6 ± 3.2^{a}	50.9 ± 3.2^{a}	48.9 ± 9.9^{a}	53.8 ± 2.6^{a}	20.0 ± 14.9^{c}

Different letters indicate significant differences among subgroups (hatching success × days post fertilization)
† Includes 15:0, 17:0, 20:0, 22:0, and 24:0
‡ Includes 17:1 and 22:1 n-9
ƀ Includes 20:3 n-6, 18:4 n-3, 18:3 n-3, and 20:3 n-3

Table 3. SIMPER analysis results for fatty acids from polar and neutral lipid fractions explaining most of the differences between 14, 17, and 21 days post fertilization (dpf) and egg batches presenting different hatching successes: high (38.9 ± 3.9%), medium (12.6 ± 2.5%), and low (4.2 ± 4.1%). Monounsaturated fatty acids (MUFA) include 16:1 n-7, 18:1 n-9, 20:1 n-9, 22:1 n-9, 17:1, and 24:1 n-9; polyunsaturated fatty acids (PUFA) include 18:2 n-6, 20:3 n-6, 20:4 n-6, 18:3 n-3, 18:4 n-3, 20:3 n-3, 20:5 n-3, and 22:6 n-3.

Polar fraction	Average dissimilarity	Contribution (%)	**Neutral fraction**	Average dissimilarity	Contribution (%)
Days post fertilization (dpf)					
		14 and 17 dpf			
DHA	3.1	29.4	20: 1 n - 9	2.0	8.5
EPA	2.2	21.2	18: 1 n - 9	1.6	6.8
PUFA	2.1	46.7	MUFA	4.7	46.4
		14 and 21 dpf			
DHA	6.1	24.6	18: 1 n - 9	2.1	10.5
EPA	4.2	17.1	20: 1 n - 9	1.6	8.1
PUFA	4.3	44.6	MUFA	5.1	50.2
		17 and 21 dpf			
DHA	5.3	25.4	18: 1 n - 9	2.0	7.3
EPA	3.6	17.1	20: 1 n - 9	1.9	7.2
PUFA	3.42	42.9	MUFA	5.0	43.7
Hatching success					
		Medium and high			
DHA	3.8	28.1	20: 1 n - 9	1.9	7.0
EPA	3.0	22.5	18: 1 n - 9	1.7	6.2
PUFA	1.9	45.8	MUFA	4.9	47.1
		Medium and low			
DHA	5.8	24.1	20: 1 n - 9	2.6	10.2
EPA	4.1	17.1	18: 1 n - 9	2.2	8.7
PUFA	3.8	44.1	MUFA	5.9	49.3
		High and low			
DHA	4.8	21.8	18: 1 n - 9	2.9	9.2
EPA	4.2	19.3	20: 1 n - 9	2.3	7.5
PUFA	3.2	42.9	MUFA	5.4	45.8

Table 4. Fatty acid composition of the neutral lipid fraction (% weight of total neutral lipids ± SD) in Greenland halibut eggs with different hatching successes: high (38.9 ± 3.9%), medium (12.6 ± 2.5%), and low (4.2 ± 4.1%).

Neutral fatty acids	Hatching success								
	High			Medium			Low		
	Days post fertilization								
	14	17	21	14	17	21	14	17	21
14:0	3.2 ± 0.5	5.1 ± 1.3	3.5 ± 1.3	2.2 ± 0.4	2.6 ± 0.3	2.4 ± 0.1	2.9 ± 0.5	4.1 ± 0.7	5.6 ± 1.7
16:0	11.8 ± 1.6	3.2 ± 5.9	10.7 ± 4.9	10.9 ± 0.9	10.8 ± 0.7	11.3 ± 0.6	12.9 ± 2.8	16.7 ± 2.7	31.7 ±14.6
18:0	2.5 ± 0.5	4.4 ± 4.1	3.0 ± 1.4	3.5 ± 0.8	2.8 ± 0.2	3.1 ± 0.2	3.4 ± 0.8	4.1 ± 1.1	7.4 ± 2.3
∑SFA†	34.5 ± 8.7^{b}	50.3 ± 30.8^{a}	38.9 ± 33.3^{b}	30.1 ±10.1^{b}	27.8 ± 5.1^{b}	25.2 ± 2.9^{b}	39.7 ± 14.8^{b}	46.8 ± 11.6^{b}	69.2 ± 37.8^{a}
16:1 n-7	7.8 ± 1.4	1.0 ± 2.9	6.5 ± 3.1	6.0 ± 1.7	6.5 ± 0.7	49.1 ± 0.5	8.5 ± 2.1	12.6 ± 2.9	6.2 ± 4.4
18:1 n-9	19.7 ± 2.3^{ab}	11.6 ± 7.7^{bc}	16.7 ± 7.8^{bc}	17.6 ± 3.6^{ab}	19.5 ± 0.9^{ab}	21.3 ± 0.9^{a}	22.4 ± 5.0^{ab}	14.1 ± 10.1^{ab}	3.5 ± 3.0^{c}
20:1 n-9	9.0 ± 1.3^{ab}	1.7 ± 3.9^{c}	7.6 ± 3.8^{ab}	9.9 ± 2.9^{ab}	10.6 ± 0.4^{a}	11.5 ± 0.6^{a}	6.7 ± 2.2^{b}	1.9 ± 2.5^{c}	0.2 ± 0.5^{d}
22:1 n-9	7.5 ± 1.3	3.9 ± 1.9	5.2 ± 2.2	6.8 ± 1.9	7.7 ± 0.5	6.9 ± 0.5	2.4 ± 0.8	0.7 ± 0.3	1.3 ± 0.6
∑MUFA‡	45.2 ± 6.7^{ab}	18.2 ± 16.7^{c}	36.4 ± 17.5^{ab}	43.8 ±11.5^{ab}	47.7 ± 3.2^{a}	49.1 ± 2.9^{a}	40.3 ± 10.5^{ab}	24.9 ± 16.1^{bc}	11.1 ± 8.6^{c}
18:2 n-6	1.3 ± 0.5	1.3 ± 3.3	1.0 ± 0.6	2.3 ± 1.2	2.1 ± 0.4	1.1 ± 0.2	1.6 ± 0.6	1.9 ± 0.7	3.7 ± 1.7
20:3 n-6	0.7 ± 0.3	3.4 ± 3.6	0.5 ± 0.4	1.1 ± 0.9	0.9 ± 0.4	0.4 ± 0.2	0.6 ± 0.4	0.7 ± 0.3	1.3 ± 1.4
20:4 n-6	0.1 ± 0.1	0.1 ± 0.4	0.2 ± 0.1	1.6 ± 0.9	1.5 ± 0.3	0.4 ± 0.0	0.1 ± 0.2	0.3 ± 0.1	0.0 ± 0.0
18:4 n-3	0.8 ± 0.3	0.0 ± 0.1	0.6 ± 0.4	1.2 ± 0.8	1.1 ± 0.3	0.5 ± 0.1	0.9 ± 0.4	1.0 ± 0.5	1.7 ± 1.0
20:5 n-3	6.7 ± 0.9	3.7 ± 5.2	7.8 ± 2.0	7.0 ± 0.7	6.6 ± 0.7	8.0 ± 0.5	4.9 ± 0.7	6.3 ± 2.7	2.1 ± 1.7
22:6 n-3	7.4 ± 0.9	15.3 ± 3.6	9.9 ± 4.4	10.8 ± 1.9	10.2 ± 1.6	12.9 ± 0.9	6.6 ± 0.8	8.7 ± 1.8	5.1 ± 2.3
∑PUFAƀ	19.3 ± 4.2^{bc}	31.3 ± 22.0^{a}	23.8 ± 12.3^{bc}	24.3 ± 7.1^{ab}	22.8 ± 4.2^{b}	24.8 ± 2.4^{ab}	18.7 ± 5.1^{bc}	22.6 ± 7.7^{bc}	17.4 ± 11.1^{c}

Different letters indicate significant differences among subgroups (hatching success × days post fertilization)
† Includes 12:0, 15:0, and 17:0
‡ Includes 17:1 and 24:1 n-9
ƀ Includes 18:3 n-6, 18:3 n-3, and 20:3 n-3