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## Influence of different levels of dissolved oxygen on the success of Greenland halibut (*Reinhardtius hippoglossoides*) egg hatching and embryonic development --Manuscript Draft--

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<b>Abstract:</b>	The aim of this study was to determine the influence of different levels of dissolved oxygen (DO) on embryonic development (ED) and hatching success of Greenland halibut ( <i>Reinhardtius hippoglossoides</i> ) eggs. Fertilized eggs from six females were exposed to five DO levels: severely hypoxic (10 and 20%sat [percent saturation]), moderately hypoxic (35 and 50%sat), and normoxic (100%sat). Greenland halibut eggs were highly tolerant to hypoxia, with hatching occurring at levels as low as 20%sat. In severely hypoxic conditions (10%sat), ED was impaired and no hatching occurred. Lipid composition, during ED, changed as a function of female origin and DO levels. Phospholipids (PLs) were the dominant lipid class in eggs. Although triacylglycerols (TAG) were a minor lipid class in terms of abundance, they were only used under severe hypoxia. The results suggest that severe hypoxia (between 10 and 20%sat) has detrimental effect on the early development of Greenland halibut, and may result in reduced recruitment and lower population abundance if the decreasing trend in the DO levels observed in the bottom waters of the Gulf of St. Lawrence continues in the future. Other species that share similar life histories may also be at risk.

1 **Influence of different levels of dissolved oxygen on the success of Greenland**  
2 **halibut (*Reinhardtius hippoglossoides*) egg hatching and embryonic**  
3 **development**

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5

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14

15 **Abstract**

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17 dissolved oxygen (DO) on embryonic development (ED) and hatching success of  
18 Greenland halibut (*Reinhardtius hippoglossoides*) eggs. Fertilized eggs from six  
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31 future. Other species that share similar life histories may also be at risk.

32

33 **Keywords:** Greenland halibut, eggs, dissolved oxygen, female origin, hatch  
34 success, embryonic development, Lipids composition.

35

36 **Introduction**

37           Oxygen is necessary to sustain the respiration needs of all fishes and  
38 invertebrates (Lim et al. 2006). Over the last 50 years, no other environmental  
39 variable of ecological relevance to estuarine and coastal marine ecosystems has  
40 changed as dramatically—and as quickly—as dissolved oxygen (DO) (Diaz  
41 2001). Low DO levels are responsible for reducing species abundance and  
42 distribution and causing fishery declines (Breitburg et al. 2003). In the Estuary  
43 and Gulf of St. Lawrence (EGSL), oxygen concentrations in the deep waters (>  
44 200 m) have decreased due to anthropogenic effects. Oxygen levels are now <  
45 65%sat (percent saturation) in the gulf and < 35%sat in the estuary (Gilbert et al.  
46 2005). These low DO levels could have a significant impact on deep-dwelling  
47 marine species.

48           Greenland halibut (*Reinhardtius hypoglossoides*) is a commercially  
49 important flatfish species that lives at depths greater than 150 m in the EGSL.  
50 Despite the commercial importance of Greenland halibut, little is known about its  
51 reproductive biology (Gundersen et al. 2001) and knowledge on the factors  
52 influencing reproduction and egg viability is still sparse. Eggs are bathypelagic  
53 (Ådlandsvik et al. 2004), which increases the risk of exposure to low DO levels.  
54 Early development of flatfishes such as the Greenland halibut has not been  
55 extensively documented, the developmental stages for the embryonic period have  
56 been only partially defined (Stene et al. 1999). To the best knowledge, the only  
57 study examining embryonic development (ED) of this species utilized eggs from  
58 one female and only 8 eggs successfully hatched (Stene et al. 1999). Moreover,  
59 there have been no studies relating Greenland halibut ED to abiotic or biotic  
60 factors such as temperature, DO, or egg quality.

61 Lipids are considered to be one of the most important sources of stored  
62 energy in fish eggs. This is especially true for triacylglycerols (TAG), which are  
63 the most common form of energy storage in eggs as well as in the later life stages  
64 of most marine fish (Cowey et al. 1985). The present study was undertaken to  
65 assess the effects of low levels of DO (down to 10%sat) on ED and hatching  
66 success of Greenland halibut eggs. In addition, we describe the changes in egg  
67 lipid composition depending on female origin as well as changes occurring during  
68 ED in eggs exposed to different DO levels. Egg produced by individual females  
69 were divided in different batches and followed from fertilization until hatching to  
70 study the effect of DO on ED. Two hypotheses were tested: 1) there is no effect of  
71 DO levels on ED, and hatching success of Greenland halibut eggs; 2) DO does not  
72 affect lipid composition and their use during ED.

73

#### 74 **Materials and Methods**

75 Greenland halibut broodstock were obtained by longline fishing in the  
76 Gaspé area (48° 59' N; 64° 23' W; Quebec, Canada) in September 2009 at depths  
77 between 252 and 324 m. Fish (average length  $52 \pm 8$  cm, N = 30) were transported  
78 to the Maurice Lamontagne Institute (48° 27' N; 68° 32' W; Mont-Joli, Québec,  
79 Canada) and kept in circular tanks with flow-through seawater at  $\sim 5^{\circ}\text{C}$  and  
80 salinity 32 psu. Fish were fed to satiation twice a week with a diet of capelin  
81 (*Mallotus villosus*) and northern shrimp (*Pandalus borealis*).

#### 82 Fertilization and incubation

83 Eggs from six females and sperm from one male were manually stripped  
84 from ripe fish in February and March 2010 as described by Jelmert and Rabben  
85 (1987). Females were selected by the swelling and redness of the genital pore. For  
86 each female, length, mass, and condition factor (Fulton's K) were estimated.

87 Both female and male fish were anaesthetized with a solution of  
88 metomidate ( $6 \text{ mg L}^{-1}$ ) in a well-oxygenated bath of sea water (32–34 psu) at  $5^\circ\text{C}$   
89 with an added solution of Vidalife™ ( $10^{-4} \text{ mL L}^{-1}$ ) as a water conditioner. Two or  
90 three samples of unfertilized eggs were first taken, counted to estimate the  
91 fecundity of each female by gravimetric method and wet mass was determined. A  
92 wet fertilization method was used: ambient seawater and milt were mixed and  
93 added to the eggs at proportional volumes of 100: 1: 100, respectively.

#### 94 Experimental design

95 Fertilized eggs from each female were divided in 10 equal batches, each  
96 one incubated in a separate cone-shaped incubator (30 cm in diameter and a  
97 volume of 6.3 L) for a total of two replicates of five dissolved oxygen level per  
98 female (~ 800 eggs per incubator). DO level treatments consisted of two  
99 conditions of severe hypoxia (10 and 20%sat; ~0.7 and  $1.4 \text{ mg L}^{-1}$ ), two of  
100 moderate hypoxia (35 and 50%sat; ~2.4 and  $3.5 \text{ mg L}^{-1}$ ) and one of normoxia  
101 (100%sat, ~6.9  $\text{mg L}^{-1}$ ). Incubators ( $n = 60$ ) were placed in 10 circular tanks  
102 (diameter of 1 m) representing replicates of the different DO level treatments.  
103 Water circulation in each incubator was done using circulation pumps immersed  
104 in each tank where seawater was circulated through external chillers to maintain  
105 the temperature at  $5^\circ\text{C}$  and the salinity at 32 psu. The DO level in each tank was  
106 maintained using the experimental set-up developed by Plante et al. (1998). DO  
107 levels were measured by a polarographic  $\text{O}_2$  electrode (OxyGuard, model 420,  
108 Point Four Inc.) and controlled by a computerized system adjusting a bubbling  
109 mixture of air and nitrogen through a degassing column to maintain desired DO  
110 levels. Data from polarographic  $\text{O}_2$  electrodes were validated weekly by the  
111 Winkler titration method (McCormick 1972). Stable DO levels were maintained  
112 for the whole duration of the incubation using this experimental set-up. Kruskal-

113 Wallis test was applied to validate the stability of the different DO treatments and  
114 showed highly significant differences between each treatment ( $H_4 = 37.46$ ,  $p <$   
115  $0.0001$ ).

#### 116 Egg sampling

117 Approximately 200 eggs from each female at 100%sat were randomly  
118 sampled after 24 h to determine fertilization success. Embryonic development was  
119 monitored on 10–25 eggs sampled in each incubator every 2–3 days. Egg samples  
120 were also stored in 1 ml of sterilized seawater at  $-80^{\circ}\text{C}$  for lipid analysis. Dry  
121 mass was determined by drying samples at  $110^{\circ}\text{C}$  for 48 h. The experiment was  
122 conducted until hatching. Hatching success (%) was estimated taking into account  
123 the number of dead and alive eggs, and eggs removed at each sampling.

$$124 \quad \text{Hatching success (\%)} = \frac{N_{\text{HL}}}{(N_{t_0} \times \text{fertilization success}) - T_{\text{ADE}}} \times 100$$

125  $N_{\text{HL}}$  = total number of hatched larvae

126  $N_{t_0}$  =  $N_{\text{HL}}$  + number of dead and live eggs removed + number of eggs sampled

127  $T_{\text{ADE}}$  = total number of live eggs removed and sampled + total number of fertilized  
128 dead eggs

#### 129 Laboratory analysis

130 Digitized images of live eggs were taken immediately after their sampling  
131 (~10 min) using a Leica MZ 75 system (Richmond Hill, Ontario, Canada). These  
132 images were used to identify developmental stages and to measure egg diameter  
133 (mm) with Image ProPlus software 5.1 (Media Cybernetics, Silver Spring, MD,  
134 USA). We followed Shardo's method to define egg developmental stages (Shardo  
135 1995).

136 Lipid extraction from egg samples was done according to Folch et al. (1957).

137 Lipid classes were determined using an Iatroscan Mark-VI analyzer (Iatron

138 Laboratories Inc., Tokyo, Japan). Lipid extracts were developed in a four-solvent  
139 system (Parrish 1987; Parrish 1999). The separated lipid classes in this study were  
140 ketones (KET), triacylglycerols (TAG), sterols (ST), acetone-mobile polar lipids  
141 (AMPL), and phospholipids (PLs).

#### 142 Definition of developmental stages

143 The embryonic development of Greenland halibut has never been fully  
144 described. ED was subdivided into nine periods: fertilization (ED1), cleavage  
145 (ED2–ED8), blastula (ED9–ED11), gastrula (ED12–ED17), cephalization (ED18–  
146 ED21), neurulation (ED22–ED23), cranial regionalization (ED24–ED25), tail lift  
147 (prehatching period; ED PRE-H), and hatching (larva day-0) (Mejri 2011).

#### 148 Statistical analyses

149 Statistical analyses were performed using SPSS 16.0. Normality was tested  
150 using both Kolmogorov-Smirnov and Shapiro-Wilk tests. Homogeneity of  
151 variance was tested with Levene's test. If necessary, data were transformed (log or  
152 arcsine square-root; lipid classes are in %) to achieve homogeneity of variances.  
153 Statistical significance was set at  $\alpha = 0.05$ . A non parametric Kruskal-Wallis test  
154 was applied to test differences in fertilization rates and percentages of normal  
155 blastomeres. A two-way analysis of variance (ANOVA) was used to estimate the  
156 effects of DO ( $97.29 \pm 1.60$ ,  $49.86 \pm 0.65$ ,  $33.65 \pm 1.69$ ,  $17.07 \pm 0.73$ , and  $8.07 \pm$   
157  $2.30\%$ sat) and female origin (4 females) on hatching success. Two females (2 and  
158 6) were not used in this analysis, since no eggs hatched from those females at all  
159 DO levels. A one-way ANOVA was conducted to test the effect of female origin  
160 (6 females) on total lipid content and lipid classes in eggs 7 days post-fertilization  
161 that had been incubated at 100%sat. Finally, a two-way ANOVA was used to test  
162 the effects of DO level ( $97.29 \pm 1.60$ ,  $33.65 \pm 1.69$ ,  $17.07 \pm 0.73$ , and  $8.07 \pm$



163 2.30%sat) and female origin (3 females) on total lipid content and lipid classes for  
164 days 14, 17, and 21.

165 To test the effect of DO and female origin on the early developmental stages,  
166 a three-way contingency table was analyzed by a Multinomial Logistic regression  
167 (MLogit). This model has the same conceptual basis as a log-linear model, where  
168 the conditional relationship between variables is analyzed by taking the natural  
169 logarithm of the cell frequency within the contingency table (Bishop 1969).  
170 Embryonic developmental stage (days 7, 10, 14, 17, and 21) was treated as a  
171 dependent variable and DO and female origin as independent variables. MLogit is  
172 based on the principles of Bayesian statistics and likelihood ratio; it replaces the  
173 familiar classic least-squares linear statistical model. Significance tests proceed  
174 from the likelihood ratio  $G^2$

$$175 \quad G^2 = 2 \sum_{i=1}^n \sum_{j=1}^m O_{ij} \ln \left( \frac{O_{ij}}{T_{ij}} \right)$$

176 where  $O_{ij}$  and  $T_{ij}$  are respectively the observed and theoretical frequencies in each  
177 cell of the contingency table.

178 Decision tree analysis was used in conjunction with MLogit models to  
179 interpret the results. Although decision tree analysis is a powerful predictive  
180 model (Zhao 2007), it was used here for a descriptive purpose. We used a  $X^2$  test  
181 to select discriminate variables ( $p < 0.05$ )

182

## 183 **Results**

184 Females used in the experiment had lengths and wet masses between 48.5  
185 and 65.0 cm and 875 and 2519 g, respectively. Fulton's K varied between 0.73  
186 and 0.92 with the highest value observed for female 3. Relative fecundity was

187 higher in females with lower condition factor while fecundity varied between  
188 10752 and 24194 eggs per fish (Table 1).

### 189 Embryonic development

190 Fertilization rates ranged from 42 to 95% and were significantly different  
191 among females ( $H_5 = 12.71$ ,  $p = 0.026$ ). The proportion of eggs with normal  
192 blastomeres varied between 17 and 47% but no significant differences were  
193 detected (Table 1). Eggs from females 2 and 6, which did not hatch, had the  
194 lowest percentages of fertilization and normal blastomeres. However, eggs from  
195 female 3 had high percentages of fertilization and normal blastomeres.

196 Mlogit analyses showed that in the first 10 days of development,  
197 significant differences in developmental stages were largely explained by female  
198 origin (Table 2). On days 14, 17, and 21, both factors had significant effects on  
199 ED, with increasing importance of DO. On day 24, DO had a larger influence on  
200 ED than female origin.

201 Decision tree analysis showed that on day 10, female origin explained  
202 most of the variation (Fig. 1). ED of eggs from females 1, 2, 5, and 6 were not  
203 significantly different while those from females 3 and 4 differed from the others,  
204 with 84.0% of the eggs at ED19 and 62.1% at ED20, respectively. On day 24, the  
205 DO level explained most of the variation (Fig. 2). At 10%sat, 100% of the eggs  
206 were at ED20, regardless of female origin, indicating a significantly slower  
207 developmental rate than at 20, 35, 50, and 100%sat. For the other DO levels (20,  
208 35, 50, and 100%sat), 59.7% of eggs from female 3 were at the same  
209 developmental stage (ED PRE-H). However, for the other females (1, 2, 4, and 5),  
210 66.0% of the eggs were at ED24 in DO levels corresponding to 20 and 35%sat  
211 while 61.5% of the eggs were at ED25 for both 50 and 100%sat, indicating a  
212 slower development rate at 20 and 35%sat for these females.

213 Hatching success

214 Eggs from two females (2 and 6) did not hatch at any DO level. They were  
215 thus not included in the analysis on hatching success. On average, eggs hatched  
216 28 days after fertilization. The time to hatch was not significantly different  
217 between different DO levels and females. However, hatch success was  
218 significantly affected by the interaction between DO level and female origin ( $F_{(12, 20)} = 12.83$ ,  $p < 0.0001$ ; Fig. 3). Higher hatch success was observed for female  
219 number 3 (around 40%). No eggs hatched at 10%sat for any female.  
220

221

222 Lipid class analyses

223 Total lipid content, KET, AMPL, and TAG differed significantly  
224 according to female origin in eggs incubated at 100%sat on day 7 ( $F_{(5, 17)}^{\text{total lipids}} = 3.22$ ,  $F_{(5, 17)}^{\text{KET}} = 10.93$ ,  $F_{(5, 17)}^{\text{AMPL}} = 3.75$ , and  $F_{(5, 17)}^{\text{TAG}} = 5.11$ ,  $p < 0.05$ ;  
225 Fig. 4). Total lipids in Greenland halibut eggs accounted for  $16.4 \pm 3.2\%$  of the  
226 DM (dry mass), with the highest ( $23.7 \pm 7.0\%$  of DM) and lowest ( $13.4 \pm 3.4\%$  of  
227 DM) levels in the eggs from females 1 and 2, respectively (Fig. 4). PLs was the  
228 major lipid class representing  $77.6 \pm 4.3\%$  of total lipids. The PLs content in eggs  
229 did not differ among females. However, significant differences in TAG, KET, and  
230 AMPL levels were observed among females with the highest TAG level observed  
231 for eggs from female 3 and highest KET and AMPL levels observed in eggs from  
232 female 2 (Fig. 4).  
233

234 Lipid classes in the eggs of females 1, 3, and 5 incubated at the different  
235 DO levels (excluding the 50%sat level) were determined for days 14, 17, and 21.  
236 On day 14, significant differences in total lipids, PLs, and KET percentages were  
237 observed only in relation to female origin ( $F_{(2, 12)}^{\text{total lipids}} = 9.32$ ,  $F_{(2, 12)}^{\text{PLs}} = 4.87$ ,  
238 and  $F_{(2, 12)}^{\text{KET}} = 15.70$ ,  $p < 0.05$ ; Table 3). TAG percentage varied only according

239 to DO ( $F_{(3, 12) \text{ TAG}} = 4.43$ ,  $p = 0.02$ ; Table 3), and was significantly higher in eggs  
240 incubated at 100%sat than in those incubated at 10%sat. On days 17 and 21,  
241 significant interactions between DO levels and female origin were observed in  
242 total lipids, and percentages of PLs, KET, and TAG (day 17:  $F_{(6, 12) \text{ total lipids}} =$   
243  $6.39$ ,  $F_{(6, 12) \text{ PLs}} = 3.53$ ,  $F_{(6, 12) \text{ KET}} = 5.64$ , and  $F_{(6, 12) \text{ TAG}} = 3.50$ ,  $p < 0.05$ ; day 21:  $F_{(6, 11) \text{ total lipids}} = 4.65$ ,  
244  $F_{(6, 11) \text{ PLs}} = 3.09$ ,  $F_{(6, 11) \text{ KET}} = 4.65$ , and  $F_{(6, 11) \text{ TAG}} = 4.29$ ,  $p <$   
245  $0.05$ ; Table 3). The only clear pattern was observed at day 21 for the 10%sat DO  
246 level, where a significant decrease of total lipids was observed.

247

## 248 **Discussion**

249 The lowest concentration of DO currently observed in the bottom waters  
250 of St. Lawrence system is ~20%sat (Gilbert et al. 2005). The large abundance of  
251 Greenland halibut juveniles and adults in these waters suggests a high tolerance of  
252 this species to severe hypoxia (DFO 2006). Greenland halibut eggs are also highly  
253 tolerant to hypoxia, with hatching occurring at levels as low as 20%sat. Similar  
254 tolerance levels have also been observed for Atlantic halibut, *Hippoglossus*  
255 *hippoglossus* (Helvik and Walther 1993). In our experiment, no eggs hatched at  
256 severely hypoxic conditions (10%sat). These results indicate that the threshold  
257 level of environmental oxygen concentration below which no hatching will occur,  
258 is between 10 and 20%sat ( $0.7 \text{ mg L}^{-1}$  and  $1.4 \text{ mg L}^{-1}$ ) for this species. In the black  
259 bream (*Acanthopagrus butcheri*), this threshold level has been observed at 30%sat  
260 ( $2.1 \text{ mg L}^{-1}$ ) (Hassell et al. 2008). Variations in hatch success in relation to both  
261 DO level and female origin, also suggest that hypoxia levels over 10%sat could  
262 reduce hatching success.

263 In some species, hypoxia can act to initiate hatching. For example, eggs of  
264 whitefish (*Coregonus lavaretus*) and vendace (*Coregonus albula*) exposed to

265 hypoxia responded with precocious hatching (Czerkies et al. 2002). The incidence  
266 of early hatching, for those two species in response to low DO levels, increased as  
267 the duration of hypoxia exposure increased. Species experiencing precocious  
268 hatching in response to hypoxic conditions are known to be the less tolerant ones  
269 (Oppen-Berntsen et al. 1990). In fact, precocious hatching is considered as an  
270 extreme reaction that enables embryos to escape from unfavorable oxygen  
271 conditions, and premature hatching of hypoxic embryos may therefore enhance  
272 access to oxygen (Mills and Barnhart 1999). Our results indicate that hypoxic  
273 conditions did not postpone the time of hatching in Greenland halibut eggs.

274           Egg hatching is a complicated process that involves external membrane  
275 components, hatching enzymes, egg origin, and general embryonic development  
276 (Oppen-Berntsen et al. 1990). The assessment of ED in the present study  
277 demonstrated that the timing of developmental stages was more variable and  
278 mainly due to inter-female differences at the beginning of embryogenesis. The  
279 DO effect became important 17 days after fertilization. We suggest that this  
280 phenomenon could be explained by the low oxygen demands of early embryos,  
281 causing the initial limited response to low oxygen concentrations, and to a  
282 cumulative effect of hypoxic conditions on ED through time. Moreover, because  
283 oxygen consumption by the embryo increases during development, embryos may  
284 experience hypoxia in more advanced stages (Oppen-Berntsen et al. 1990; Finn et  
285 al. 1995).

286           Effects of female origin confirmed the important role of egg quality  
287 toward the end of ED. In addition to maternal effects, paternal effects could have  
288 a significant contribution to the survival during embryogenesis (Kamler 2005).  
289 Sperm density and motility have been shown to influence egg quality in some  
290 teleost fishes (Kamler 2005). In the present study, possible paternal effects were

291 controlled by using only the sperm of one male to fertilize the eggs of all females.  
292 Other studies have reported that hypoxia decreases development rate and impairs  
293 egg growth in many other organisms, including fishes (Davenport 1983; Malcolm  
294 et al. 2003) and invertebrates (Chaffee and Strathmann 1984; Lutz et al. 1992).

295 Greenland halibut eggs have high lipid content mainly made up of PLs.  
296 Fish eggs can be classified into different energetic categories according to their  
297 lipid characteristics (Mourente and Vázquez 1996). The presence or lack of an oil  
298 globule corresponds to eggs with high (> 15% of egg DM) or low (< 15% of egg  
299 DM) lipid content. The first type is mainly characterized by high amounts of TAG  
300 or wax esters, and low amounts of PLs while the second category is characterized  
301 by high amounts of PLs (Finn et al. 1995). Thus, Greenland halibut eggs can be  
302 classified, as lipid-poor eggs species mainly constituted of PLs without any oil  
303 globule. In lipid-poor eggs species such as cod (*Gadus morhua*), whiting  
304 (*Merlangus merlangus*), and Atlantic halibut, proportions of PLs tend to be higher  
305 than 60% of total lipids (White and Fletcher 1987; Wiegand 1996).

306 Substantial differences in lipids can exist, not only among the eggs of  
307 individual females in a population, but also among different batches spawned by  
308 the same female (Evans et al. 1996; Wiegand 1996; Rainuzzo et al. 1997). Our  
309 results showed significant differences in total lipids and different lipid classes  
310 among eggs from the six females. Similar results have been obtained by Rainuzzo  
311 et al. (1997) in turbot (*Scophthalmus maximus*) broodstock. Although PLs was the  
312 major lipid class in the Greenland halibut eggs, it did not differ significantly  
313 between egg batches. Minor lipid classes such as TAG, AMPL, and KET showed  
314 more considerable differences and could be related to egg viability. TAG, which  
315 was considerably higher in eggs of female 3, is the primary endogenous energy  
316 reserve fuelling basal metabolism (Sewall and Rodgveller 2008).

317           It has also been suggested that eggs having the best quality are those  
318 coming from batches with higher rates of fertilization and normal blastomeres  
319 (Kjørsvik et al. 1990). This is in agreement with our results, where the best ED,  
320 and hatching success observed for eggs from female 3 were associated with the  
321 highest rates of fertilization success and normal blastomeres.

322           Lipid metabolism during the early life of fish may differ greatly among  
323 species, mainly with regard to the time and level of lipid classes used for either  
324 energy or tissue synthesis, and the environmental factors encountered, such as  
325 temperature and DO (Verreth et al. 1994). It might be speculated that high PLs  
326 contents in Greenland halibut eggs could indicate its utilization as metabolic fuel.  
327 However, in the present study, the percentage of PLs remained constant between  
328 days 7 and 21 post-fertilization and between different DO levels on both days 17  
329 and 21. PLs may be reorganized during embryogenesis and mobilized for  
330 subsequent biomembrane formation during larval ontogeny (Falk-Petersen et al.  
331 1989). Alternatively, ED is characterized by a low consumption of yolk sac lipids  
332 and other endogenous stores, e.g., proteins and free amino acids (FAA) are  
333 probably used (Desvillettes et al. 1997). Moreover, low DO levels did not  
334 stimulate the use of PLs while TAG were highly depleted under severe hypoxia  
335 (10%sat).

336           In conclusion, impaired ED in severe hypoxia affected the viability of  
337 Greenland halibut eggs resulting in the absence of hatching at 10%sat. Moreover,  
338 female origin was a decisive factor in ED and hatching success. Indeed, we found  
339 that females with better condition factor (K) produced eggs of better quality, with  
340 high percentages of normal blastomeres and better fertilization success.  
341 Concerning lipid content, even though Greenland halibut eggs were mainly  
342 constituted of PLs, these levels did not differ significantly between egg batches

343 and they were not used as metabolic fuel during ED or under severe hypoxia. Our  
344 findings show that the lethal threshold level for the early life cycle of this species  
345 is between 10 and 20%sat while slightly higher DO levels (20 and 35%sat) are  
346 still harmful for eggs with lower quality. Oxygen concentrations in the bottom  
347 waters of the St. Lawrence estuary decreased from 37.7%sat in the 1930s to an  
348 average of 20.7%sat for the 1984–2003 period (Gilbert et al. 2005). The actual  
349 westward DO saturation gradient in the EGSL from 50-60%sat in the East to 20-  
350 30%sat in the West could limit the recruitment and the selection of breeding area  
351 for Greenland halibut. This situation may worsen if lower levels of DO ( $\leq$   
352 20%sat) become more widespread in the future.

353

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493 **Figures Legends**

494 **Fig. 1** Classification tree for day 10 of embryonic development (ED). The first  
495 variation is segmented depending on the factor «female origin»

496 **Fig. 2** Classification tree for day 24 of embryonic development (ED). The first  
497 summit is segmented depending on the factor «DO levels»

498 **Fig. 3** Mean hatch success (%) for Greenland halibut embryos obtained from four  
499 females and exposed to five levels of dissolved oxygen (DO) [mean ± SD].  
500 Different letters indicate statistically significant differences between  
501 treatments (female origin × DO levels) ( $p < 0.0001$ ). No data are presented  
502 for 10%sat because no eggs hatched at this level

503 **Fig. 4** Changes in total lipid content and proportions of triacylglycerols (TAG),  
504 ketones (KET), and acetone-mobile polar lipids (AMPL) in Greenland  
505 halibut eggs from different females on day 7 of embryonic development for  
506 eggs incubated at 100%sat in dissolved oxygen [mean ± SD]. Different  
507 letters indicate significant differences among females ( $p < 0.05$ )

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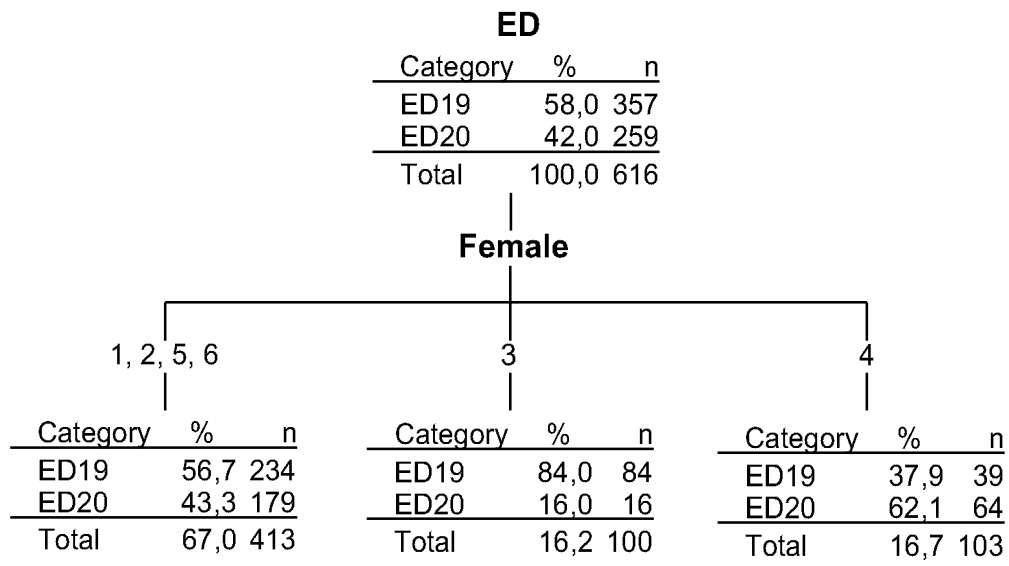
519 **Table Legends**

520 **Table 1** Percentages of fertilization success (mean  $\pm$  SD) and normal blastomeres  
521 (mean  $\pm$  SD), length, mass, condition factor, fecundity, and date of  
522 fertilization for the six Greenland halibut females used in the experiment

523 **Table 2** Summary of Multinomial Logistic regression (MLogit) tests on the  
524 variations in embryonic development of Greenland halibut eggs sampled on  
525 days 7, 10, 14, 17, 21, and 24 as a function of female origin and dissolved  
526 oxygen (DO) levels

527 **Table 3** Total lipid contents and proportions of ketones (KET), triacylglycerols  
528 (TAG), and phospholipids (PL) from Greenland halibut eggs from females  
529 exposed to different dissolved oxygen (DO) levels on days 14, 17, and 21  
530 (mean  $\pm$  SD). Different letters indicate significant differences ( $p < 0.05$ )

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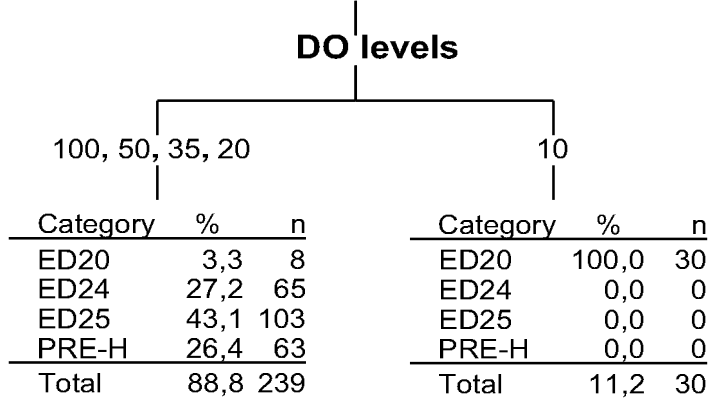




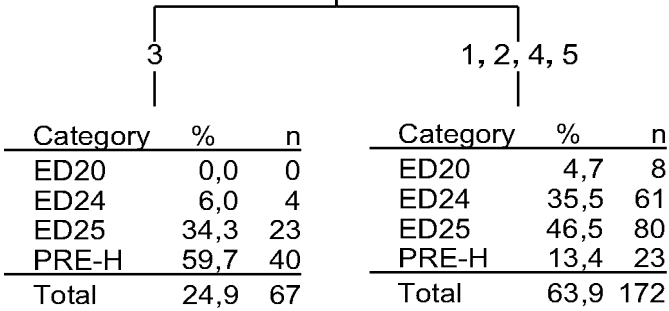
**ED**

Category	%	n
ED20	14,1	38
ED24	24,2	65
ED25	38,3	103
PRE-H	23,4	63
Total	100,0	269

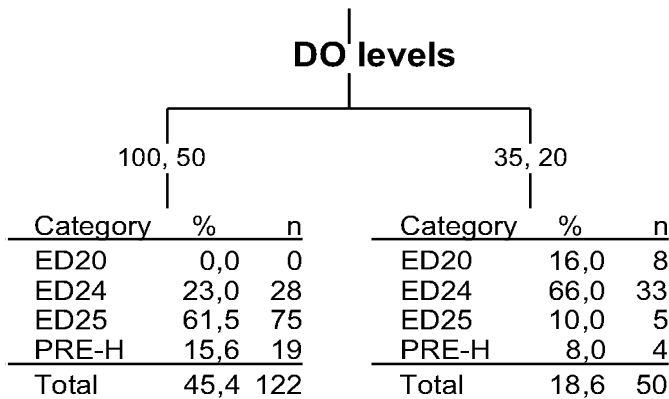
**DO levels**



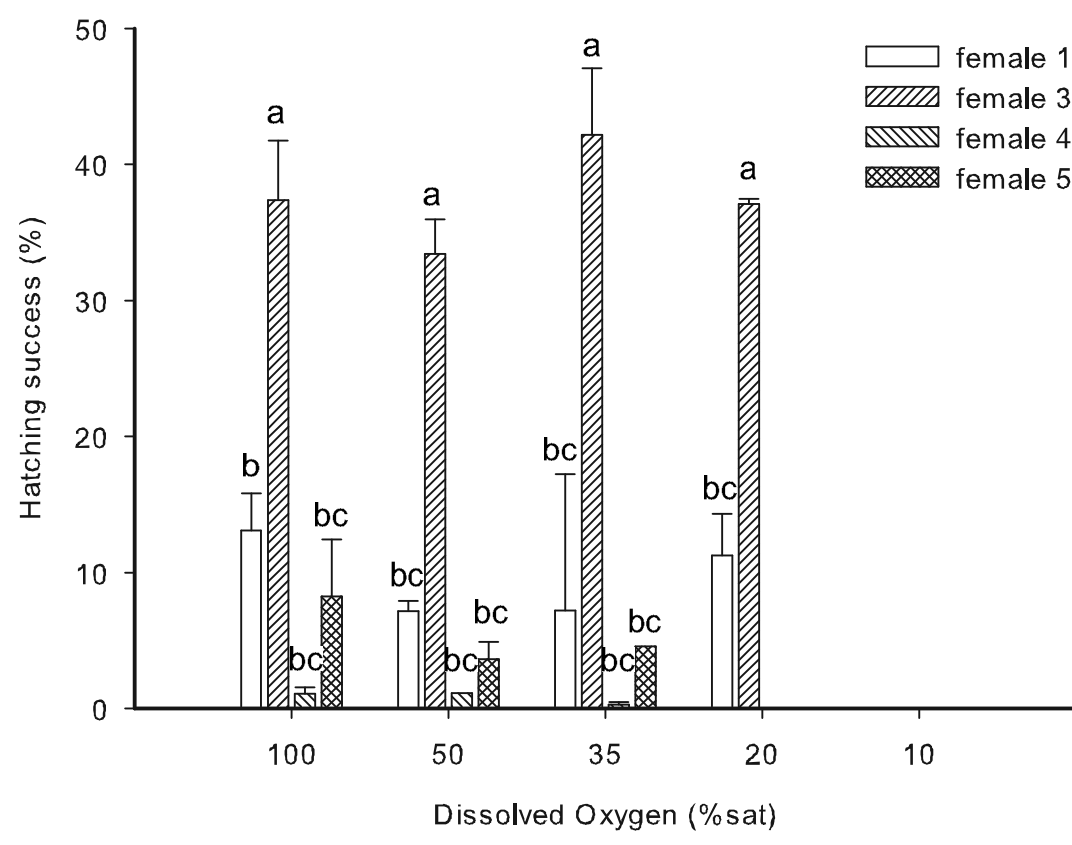
**Female**



**DO levels**



Figure



Figure

