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Influence of different levels of dissolved oxygen on the success of Greenland halibut (Reinhardtius hippoglosoides) egg hatching and embryonic development

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Corresponding Author:	Rejean Tremblay, PhD Universite du Quebec a Rimouski Rimouski, Quebec CANADA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universite du Quebec a Rimouski
Corresponding Author's Secondary Institution:	
First Author:	Sahar Mejri, MSc
First Author Secondary Information:	
Order of Authors:	Sahar Mejri, MSc
	Rejean Tremblay, PhD
	Yvan Lambert, PhD
	Céline Audet
Order of Authors Secondary Information:	
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Influence of different levels of dissolved oxygen on the success of Greenland halibut (*Reinhardtius hippoglossoides*) egg hatching and embryonic development

Sahar Mejri[1], Réjean Tremblay[1*], Yvan Lambert[2], and Céline Audet[1]

[1] *Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski (ISMER, UQAR), 310 allée des Ursulines, Rimouski, QC, G5L 3A1*

[2] *Fisheries and Oceans Canada, Institut Maurice-Lamontagne, Mont-Joli, QC, G5H 3Z4*

***Corresponding author,** rejean_tremblay@uqar.qc.ca, Phone: 418-723-1986 ext1705, Fax: 418-724-1842

Abstract

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 (*Reinhardtius hippoglossoides*) 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.

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

Introduction

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

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

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

Materials and Methods

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

Fertilization and incubation

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

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

Experimental design

Fertilized eggs from each female were divided in 10 equal batches, each one incubated in a separate cone-shaped incubator (30 cm in diameter and a volume of 6.3 L) for a total of two replicates of five dissolved oxygen level per female (~ 800 eggs per incubator). DO level treatments consisted of two conditions of severe hypoxia (10 and 20%sat; ~0.7 and 1.4 mg L^{-1}), two of moderate hypoxia (35 and 50%sat; ~2.4 and 3.5 mg L^{-1}) and one of normoxia (100%sat, ~6.9 mg L^{-1}). Incubators (n = 60) were placed in 10 circular tanks (diameter of 1 m) representing replicates of the different DO level treatments. Water circulation in each incubator was done using circulation pumps immersed in each tank where seawater was circulated through external chillers to maintain the temperature at 5°C and the salinity at 32 psu. The DO level in each tank was maintained using the experimental set-up developed by Plante et al. (1998). DO levels were measured by a polarographic O_2 electrode (OxyGuard, model 420, Point Four Inc.) and controlled by a computerized system adjusting a bubbling mixture of air and nitrogen through a degassing column to maintain desired DO levels. Data from polarographic O_2 electrodes were validated weekly by the Winkler titration method (McCormick 1972). Stable DO levels were maintained 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°C for lipid analysis. Dry
121 mass was determined by drying samples at 110°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 $$\text{Hatching success (\%)} = \frac{N_{HL}}{\left(Nt_0 \times \text{fertilization success}\right) - T_{ADE}} \times 100$$

125 N_{HL} = total number of hatched larvae

126 Nt_0 = N_{HL}+ number of dead and live eggs removed+ number of eggs sampled

127 T_{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 ± 1.60, 49.86 ± 0.65, 33.65 ± 1.69, 17.07 ± 0.73, and 8.07 ±
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 ± 1.60, 33.65 ± 1.69, 17.07 ± 0.73, and 8.07 ±

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

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 ($F_{(12,}$
219 $_{20)} = 12.83$, $p < 0.0001$; Fig. 3). Higher hatch success was observed for female
220 number 3 (around 40%). No eggs hatched at 10%sat for any female.

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

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 ($F_{(2, 12)\ total\ lipids} = 9.32$, $F_{(2, 12)\ PLs} = 4.87$,
238 and $F_{(2, 12)\ KET} = 15.70$, $p < 0.05$; Table 3). TAG percentage varied only according

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

Discussion

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

In some species, hypoxia can act to initiate hatching. For example, eggs of 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 (Desvilettes 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

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

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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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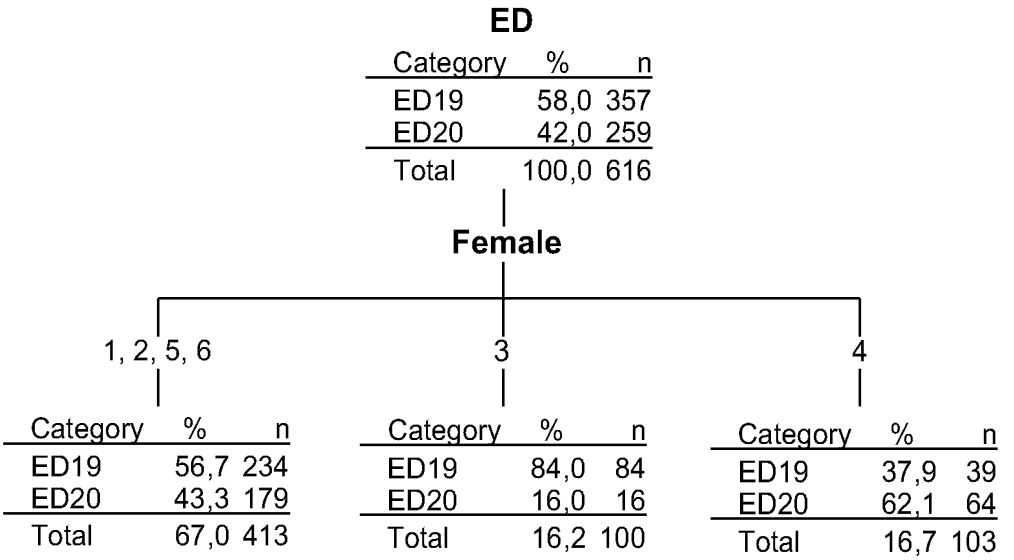
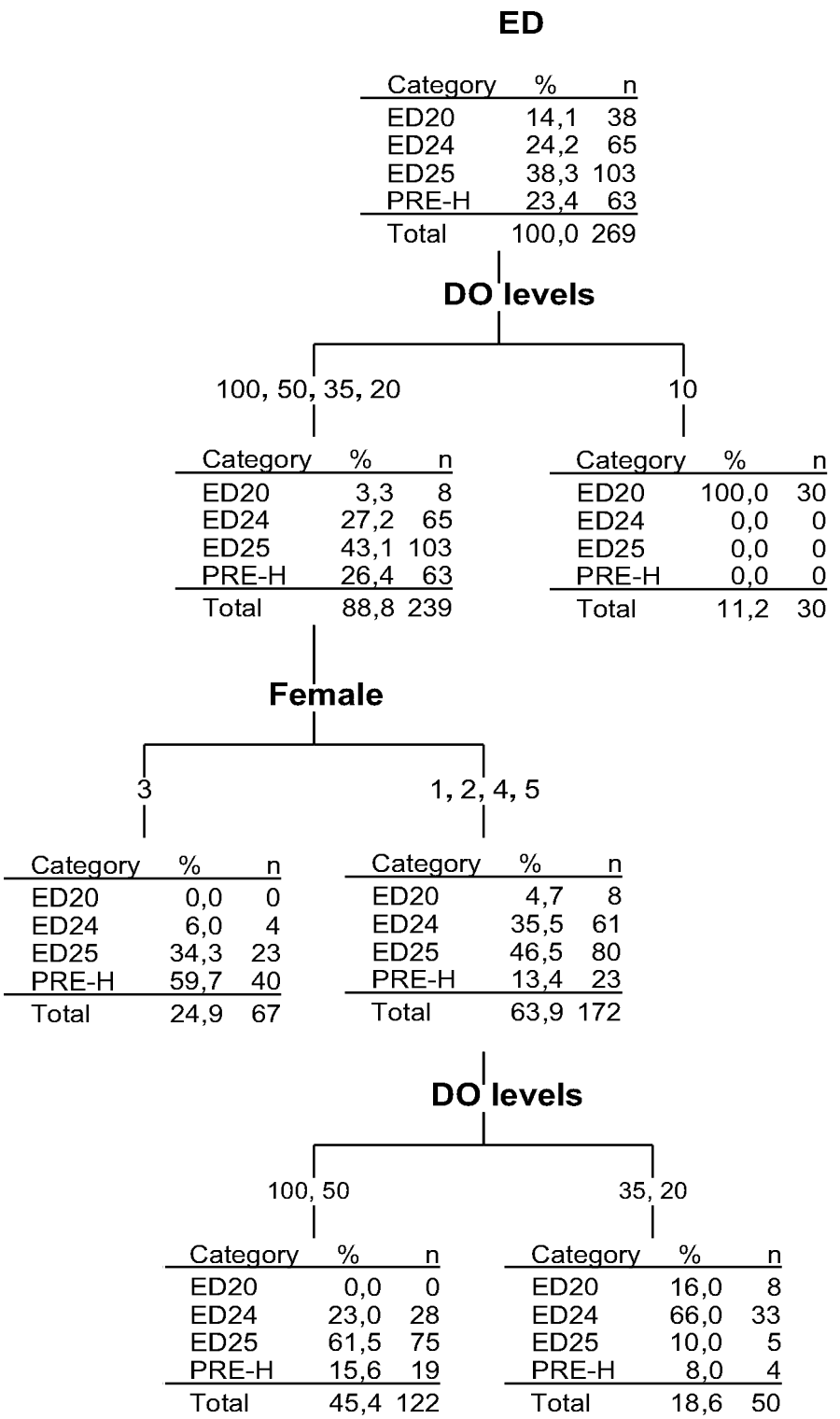
519 **Table Legends**

520 **Table 1** Percentages of fertilization success (mean ± SD) and normal blastomeres
521 (mean ± 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 ± SD). Different letters indicate significant differences ($p < 0.05$)
531

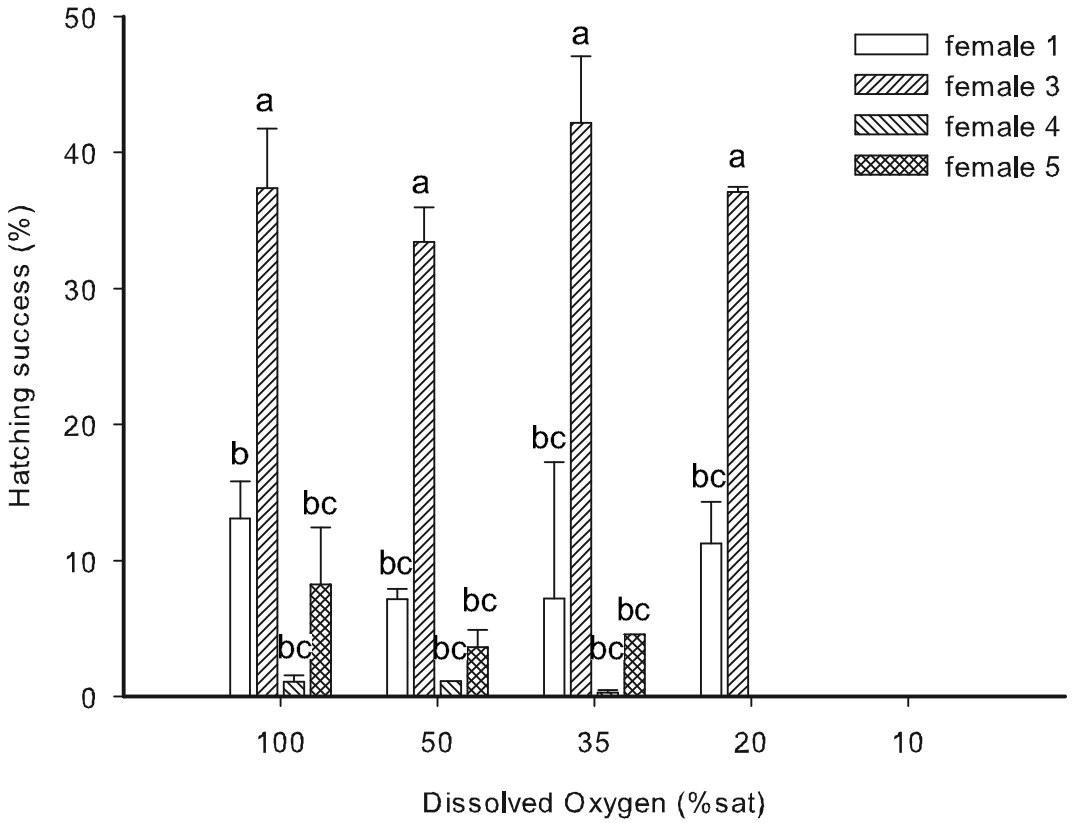
Figure


ED
Category % n
ED19 58,0 357
ED20 42,0 259
Total 100,0 616
Female
1, 2, 5, 6
Category % n
ED19 56,7 234
ED20 43,3 179
Total 67,0 413
3
Category % n
ED19 84,0 84
ED20 16,0 16
Total 16,2 100
4
Category % n
ED19 37,9 39
ED20 62,1 64
Total 16,7 103

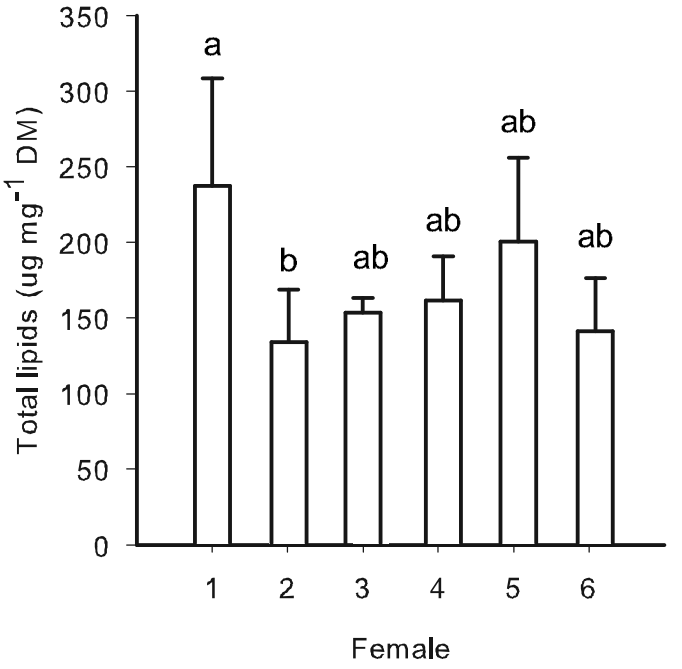
Figure

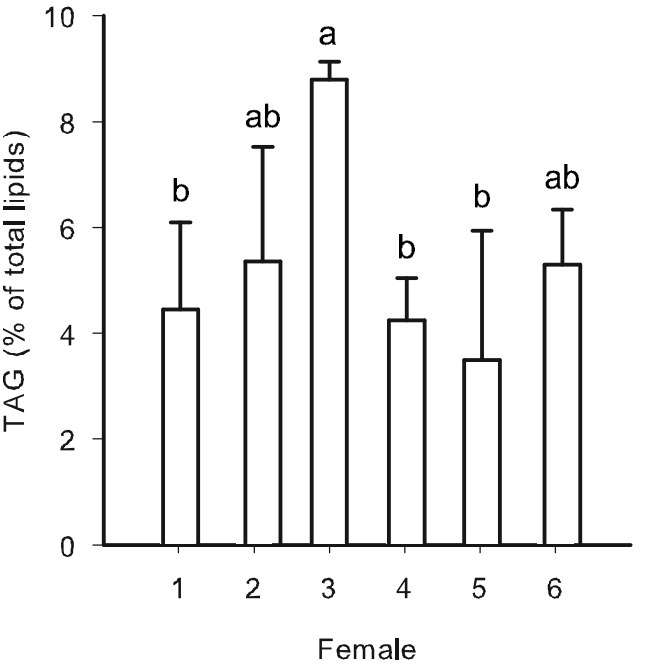

ED
Category % n
ED20 14,1 38
ED24 24,2 65
ED25 38,3 103
PRE-H 23,4 63
Total 100,0 269
DO levels
100, 50, 35, 20
10
Category % n
ED20 3,3 8
ED24 27,2 65
ED25 43,1 103
PRE-H 26,4 63
Total 88,8 239
Category % n
ED20 100,0 30
ED24 0,0 0
ED25 0,0 0
PRE-H 0,0 0
Total 11,2 30
Female
3
1, 2, 4, 5
Category % n
ED20 0,0 0
ED24 6,0 4
ED25 34,3 23
PRE-H 59,7 40
Total 24,9 67
Category % n
ED20 4,7 8
ED24 35,5 61
ED25 46,5 80
PRE-H 13,4 23
Total 63,9 172
DO levels
100, 50
35, 20
Category % n
ED20 0,0 0
ED24 23,0 28
ED25 61,5 75
PRE-H 15,6 19
Total 45,4 122
Category % n
ED20 16,0 8
ED24 66,0 33
ED25 10,0 5
PRE-H 8,0 4
Total 18,6 50

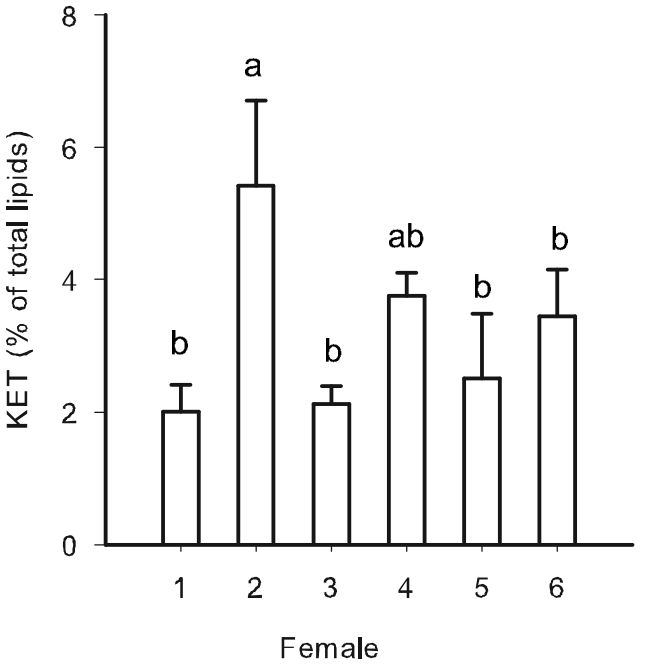
Figure

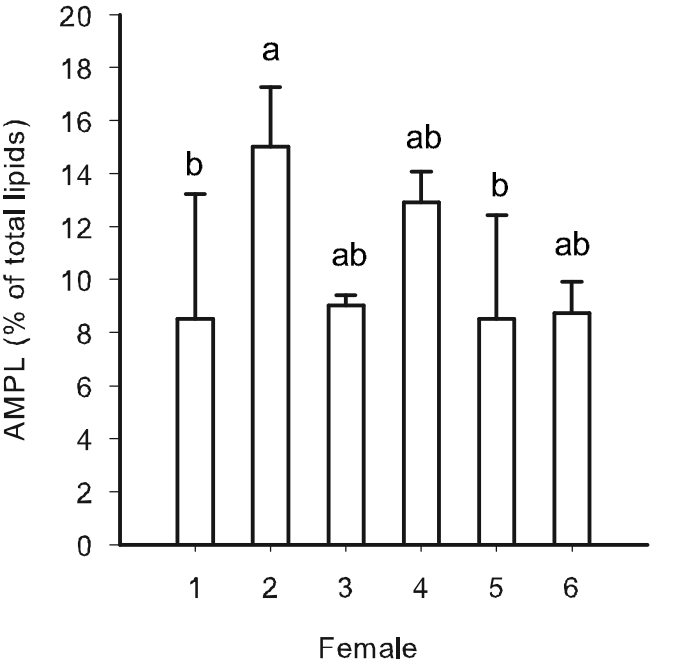

Hatching success (%)
50
40
30
20
10
0
a
a
a
a
b
bc
bc
bc
bc
bc
bc
bc
100
50
35
20
10
Dissolved Oxygen (%sat)
female 1
female 3
female 4
female 5

Figure


Total lipids (ug mg-1 DM)
350
300
250
200
150
100
50
0
a
b
ab
ab
ab
ab
1
2
3
4
5
6
Female


TAG (% of total lipids)
10
8
6
4
2
0
b
ab
a
b
b
ab
1
2
3
4
5
6
Female


KET (% of total lipids)
8
6
4
2
0
b
a
b
ab
b
b
1
2
3
4
5
6
Female


AMPL (% of total lipids)
20
18
16
14
12
10
8
6
4
2
0
b
a
ab
ab
b
ab
1
2
3
4
5
6
Female