UNIVERSITÉ DU QUÉBEC À RIMOUSKI

Détermination de l'influence de différentes teneurs en oxygène dissous sur le succès d'éclosion et l'embryogenèse chez le flétan du Groenland (*Reinhardtius hippoglossoides*)

MÉMOIRE PRÉSENTÉ

Comme exigence partielle de la Maîtrise ès Sciences en Océanographie

PAR

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Septembre 2011

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Dépôt initial le 28 juin 2011 Dépôt final le 09 septembre 2011

REMERCIEMENTS

Les remerciements... leur rédaction est le signe qui ne trompe pas que la maîtrise est bel et bien terminée... c'est donc avec beaucoup d'émotion et de plaisir que je les écris mais aussi avec le cœur gros...

Je tiens tout d'abord à remercier M. Philippe Archambault et M. Patrick Ouellet d'avoir accepté d'évaluer ce travail. Je remercie également le gouvernement tunisien, le CRSNG et le RAQ pour leur soutien financier.

Je m'adresse à Monsieur Réjean Tremblay. Réjean, comme j'ai l'habitude de t'appeler, les mots ne pourront jamais traduire toute ma gratitude et ma reconnaissance. Je n'oublierai jamais ce jour de mars 2009 où tu as répondu favorablement à ma candidature spontanée et qui a été le point de départ d'une merveilleuse aventure. Tu as été un «Chef» exemplaire, toujours zen et de bonne humeur, plein de bonnes idées, ouvert à la discussion et d'une pédagogie exemplaire.

C'est avec beaucoup d'émotion que je m'adresse à Madame Céline Audet: Céline, tu as été plus qu'une codirectrice pour moi. C'est incontestablement à toi que je dois mon plongeon dans le domaine de la recherche scientifique. Ton dynamisme, ta patience, ta disponibilité, ta rigueur scientifique, ton perfectionnisme et ton abnégation m'ont toujours fascinée. Un énorme merci!!

C'est avec la même émotion et la même gratitude que je m'adresse à Yvan Lambert. La pertinence de tes remarques et ta clairvoyance ont fait prendre à cette maîtrise un tournant passionnant. Tu n'as jamais lésiné sur les moyens ni sur le temps pour valoriser ce travail. Je n'oublierai jamais ta bonne humeur matinale malgré la journée surchargée qui t'attendait, ni toutes les discussions instructives que j'ai eues avec toi.

Un grand merci à Denis Chabot, qui s'est très vite passionné pour le sujet, de m'avoir fourni le montage pour l'oxygène dissous. Tu n'as pas hésité à te déplacer à la salle des bassins pour régler quelques problèmes techniques et à m'accueillir dans ton bureau pour discuter longuement des améliorations à apporter au montage malgré les nombreuses tâches qui t'accaparent. Je t'en suis vraiment reconnaissante. Un énorme merci à Mario Péloquin, Jean-François et Marie-Claude Lamarche qui m'ont énormément aidée pour la mise en place du dispositif expérimental et pour les nombreux échantillonnages. Je remercie également Jérôme, Tanya, Linda, Rosario Dominguez-Petit et Iften pour leur aide précieuse durant les analyses au laboratoire et la prise de différentes mesures. Je voudrais également remercier tout le personnel de l'ISMER, plus particulièrement Nycole, Jocelyne et Sylvie pour leur aide dans les formalités administratives.

Au tour maintenant des personnes qui ont fait de ces deux ans de maîtrise l'une des périodes de ma vie les plus inoubliables et les plus agréables sur le plan humain.

À Amélie, Isabelle, Lise, Lucie, Claire, Elodie, Cécile et Marie-Pier pour leur présence et leur soutien continus pendant les moments durs. Ce n'était pas facile de me supporter tous les jours !! Je ne te remercierai jamais assez Cécile pour m'avoir initiée au ski alpin. À Elyes et Héla, qui de l'autre côté du Pacifique et de l'Atlantique ont toujours su être des vrais amis. À Yasmine, pour avoir été ma meilleure amie depuis des années, pour les stages inoubliables passés à Tabarka et Sidi Mechreg, pour les sueurs froides que tu m'as données lors du séisme au Japon, pour les bons moments de folies et surtout pour nous consoler mutuellement la veille de chaque départ arrache cœur. Aux deux personnes qui ont eu la lourde tâche de m'avoir pour fille (je sais que cela n'a pas été facile tous les jours), de m'armer pour la vie et de m'avoir donné la meilleure éducation qui soit : bien plus que des remerciements pour Papa et Maman. À ma petite sœur, pas si petite que ça d'ailleurs, pour son soutien et ses bons conseils. Aux jumeaux, qui étaient mon rayon de soleil tous les jours. J'espère être le bon exemple de la grande sœur !! À tous ceux que j'ai pu oublier par mégarde et qui m'ont sûrement épaulée d'une façon ou d'une autre.

RÉSUMÉ

Les eaux profondes (> 150 m) de l'estuaire et du golfe du Saint-Laurent (EGSL) sont hypoxiques avec le plus bas niveau de saturation en oxygène dissous (OD) observé dans l'estuaire (20%sat, [pourcentage de saturation]). La forte abondance du flétan du Groenland (*Reinhardtius hippoglossoides*) dans les chenaux profonds de l'EGSL semble démontrer la tolérance de cette espèce aux conditions d'hypoxie. Cependant le développement et la survie des œufs bathypélagiques chez cette espèce pourraient être affectés par leur exposition à de faibles niveaux d'OD, ces stades de développement étant généralement plus sensibles aux conditions environnementales.

L'objectif de cette étude était de déterminer l'effet des différentes teneurs en OD sur le taux de survie, le développement embryonnaire (DE), le succès d'éclosion et la dynamique des classes de lipides chez les œufs du flétan du Groenland. Pour ce faire, les œufs de six femelles fécondés artificiellement par la laitance d'un seul mâle ont été exposés à cinq niveaux d'OD. Les niveaux d'OD ont été répartis comme suit: deux conditions d'hypoxie sévère (10 et 20%sat ~ 0.7 et 1.4 $mg\,L^{-1}$), deux conditions d'hypoxie modérée (35 et 50%sat ~ 2.4 et 3.5 $mg\,L^{-1}$) et une condition de normoxie (100%sat ~ 6,9 $mg\,L^{-1}$). Le DE a été suivi à tous les 2-3 jours jusqu'à l'éclosion. Les niveaux d'OD et les différentes femelles utilisées ont démontré des effets significatifs sur les taux d'éclosion et le DE. Il n'y a pas eu éclosion et le DE a été bloqué en hypoxie sévère (10%sat). Chez certaines femelles, des résultats similaires furent observés à tous les niveaux d'OD. Nos résultats ont également démontré que la variation des classes de lipides était fonction des niveaux d'OD et des femelles. La majeure partie des lipides présente dans les œufs du flétan du Groenland était constituée de phospholipides (PL). Cependant ils n'ont pas été utilisés ni durant le DE ni sous un stress d'hypoxie sévère. Une utilisation des triacylglycérols (TAG), se reflétant sur les lipides totaux a été observée, sous hypoxie sévère (10%sat) seulement. Un gradient Est-Ouest de saturation en OD au niveau de l'EGSL, où les niveaux d'OD diminuent de 50-60%sat dans le détroit de Cabot à 20-30%sat dans l'estuaire pourrait limiter le recrutement et le choix des aires de reproduction du flétan du Groenland. En hypoxie modérée (20-35%sat), un succès d'éclosion plus faible a été observé chez un certain nombre de femelles. Ceci semble démontrer que ces niveaux d'OD pourraient limiter le DE et le succès d'éclosion chez les femelles dont la qualité des œufs est inférieure.

ABSTRACT

The Estuary and Gulf of St. Lawrence (EGSL) ecosystem is threatened by the extent and severity of hypoxic conditions. The high abundance of Greenland halibut (Reinhardtius hippoglossoides) in deep channels of the EGSL suggests a high tolerance of this species to hypoxia. However, bathypelagic Greenland halibut eggs could be less tolerant to hypoxia since early life stages are usually more sensitive than adult stages to environmental stressors. The aim of this study was to determine the influence of different levels of dissolved oxygen (DO) on survival, embryonic development (ED), and hatching success of Greenland halibut eggs. In a laboratory-based experiment, fertilized eggs from six females were exposed to five DO levels: severely hypoxic (10 and 20%sat [percent saturation]; ~0.7 and 1.4 mg.L-1), moderately hypoxic (35 and 50%sat; ~2.4 and 3.5 mg.L-1), and normoxic (100%sat; ~6.9 mg.L-1). Embryonic development in the different DO conditions was assessed by sampling eggs every 2–3 days until hatching. Changes in lipid composition were also measured to investigate the effects of DO levels and female origin on the biochemical composition of eggs during ED: significant differences ($p<0.05$) were observed in hatch rates and embryonic development as a function of both factors. In severely hypoxic conditions (10%sat) and for eggs from some females, no hatching occurred and ED was impaired. Among developmental stages, phospholipids (PL) were the dominant lipid classes. The effects of DO levels and female origin were visible on lipid class dynamics. Although triacylglycerols (TAG) were a minor lipid class in terms of abundance, they were used under severe hypoxia. The East–West DO saturation gradient in the EGSL, where DO levels fall from 50–60%sat in Cabot Strait to 20–30%sat in the estuary, could limit the breeding area and thus recruitment of Greenland halibut. At moderate hypoxia (20–35%sat), lower hatching occurred for eggs from some females. This shows that these saturation values are critical for ED and hatching success for females with lower eggs quality.

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INTRODUCTION GÉNÉRALE

L'hypoxie en milieu aquatique

L'oxygène dissous (OD) est un des paramètres physico-chimiques important pour la croissance, la reproduction et la survie des organismes aquatiques (Diaz et al. 2009). Dans le milieu aquatique, diverses sources contribuent à l'apport d'oxygène : la diffusion atmosphérique, la photosynthèse phytoplanctonique et le mouvement des masses d'eau, ce dernier permettant surtout un mélange de l'OD vers les couches profondes de la colonne d'eau (Diaz 2001). Dans les environnements marins et côtiers, aucun autre paramètre d'importance écologique n'a changé aussi drastiquement au cours des dernières décennies, ce changement conduisant parfois à l'apparition généralisée d'hypoxie (Diaz 2001).

Globalement, le nombre de zones côtières où l'hypoxie a été signalée s'est accru d'une façon exponentielle avec une moyenne de 5.5 % d'augmentation par année depuis 1916 (Vaquer-Sunyer et al. 2008). De façon générale, l'hypoxie est définie par une gamme de valeurs allant de 0.28 mg $O_2 \cdot L^{-1}$ ~ 3.7 %sat [pourcentage de saturation] (Kamykowski et al. 1990) à 4 mg $O_2 \cdot L^{-1}$ ~ 54.2 %sat (Paerl 2006). La majorité des études se réfère toutefois à un seuil de 2 mg $O_2 \cdot L^{-1}$ ~ 27.1 %sat pour définir l'hypoxie (Turner et al. 2005). Ce seuil correspond à la teneur en OD à partir de laquelle on assiste à un effondrement de l'abondance des espèces exploitées (Chesney et al. 2000). Cependant plusieurs études expérimentales indiquent que des impacts sont possibles à des niveaux supérieurs à ce seuil pour plusieurs organismes aquatiques (Vargo et al. 1977).

Les phénomènes hypoxiques sont principalement observés dans les estuaires et les régions côtières (Diaz 2001). En effet, dans ces milieux, la stratification verticale de la colonne d'eau limite les échanges entre les eaux profondes et celles de surface mieux oxygénées (Diaz et al. 2011). L'hypoxie naturelle est accentuée par l'eutrophisation due en partie aux apports anthropiques de nutriments (phosphore et nitrate principalement) (Gilbert et al. 2005). L'eutrophisation entraine une prolifération excessive du

phytoplancton à la surface. Celui-ci chute éventuellement dans la colonne d'eau, puis se dépose sur le fond. La dégradation de cette matière organique via la décomposition bactérienne réduit la disponibilité en OD dans les eaux profondes. Outre l'eutrophisation, l'augmentation de la température de l'eau provoquée par les changements climatiques accroît la demande en OD des organismes (Harris et al. 2006), réduit la solubilité de l'oxygène (Carpenter 1966) et la ventilation des eaux côtières en affectant les patrons de stratification (Stow et al. 2005). Cet accroissement des activités anthropiques et des changements climatiques globaux amplifie ainsi la fréquence et l'étendue des phénomènes d'hypoxie (Richmond et al. 2006). L'hypoxie est un phénomène commun dans la mer Baltique, la mer Noire, le golfe du Mexique, l'estuaire et le golfe du Saint-Laurent (EGSL) (Plante et al. 1998; Tomkiewicz et al. 1998; Rabalais et al. 2002).

L'hypoxie dans l'estuaire et le golfe du Saint-Laurent (EGSL)

Le système du Saint-Laurent (estuaire et golfe) compte parmi les écosystèmes où le niveau d'OD est inférieur à 100 %sat dans les eaux profondes. Au niveau de l'estuaire et du golfe de Saint-Laurent, les eaux profondes (> 200 m) ont un taux de saturation en OD inférieur à 65 %sat (Gilbert et al. 2005). Les niveaux d'oxygène diminuent dans le détroit de Cabot, avec des teneurs de 50 à 60 %sat, à environ 20 à 30 %sat dans l'estuaire (Thibodeau et al. 2006). Le golfe du Saint-Laurent reçoit à la fois les eaux froides et oxygénées du courant du Labrador et avec une plus grande proportion, celles provenant du *Gulf Stream*, qui sont chaudes et peu oxygénées (Gilbert et al. 2005). Ces eaux progressent dans le chenal Laurentien et le golfe en direction de l'estuaire. Tout au long de leur parcours, elles s'appauvrissent en OD suite à la respiration des organismes et à la décomposition bactérienne de la matière organique (MO) (Gilbert et al. 2005). Ce phénomène s'est accentué entre les années 1930 et 1980 suite aux changements survenus dans la contribution du courant du Labrador au mélange des eaux profondes, celle-ci passant de 72 % en 1930 à 53 % en 1980 (Faucher et al. 2004). De plus, l'augmentation des flux de MO marins et terrigènes ainsi que les différentes provenances des masses d'eau à densités inégales (Océan Atlantique, fleuve Saint-Laurent et bassins versants des Grands

Lacs) ont contribué à la détérioration des conditions d'OD dans les eaux les plus profondes. Les différentes densités des masses d'eau entrainent la stratification de la colonne d'eau en trois couches l'été et deux couches l'hiver (Thibodeau et al. 2006).

Réponse des organismes aquatiques à l'hypoxie

L'hypoxie devient une menace majeure pour les écosystèmes côtiers dans le monde entier et de faibles niveaux d'OD ont déclenché des mortalités d'organismes marins en de nombreux endroits (Chesney et al. 2000). Ces «zones mortes» sont dépourvues de ressources halieutiques incluant poissons, crevettes et crabes (Chesney et al. 2000; Rabalais et al. 2002). L'hypoxie peut entrainer une perte importante de la biodiversité tout en limitant la distribution, la croissance et la reproduction des organismes aquatiques (Vaquer-Sunyer et al. 2008). Le seuil de tolérance à l'hypoxie varie selon les espèces et les écosystèmes (Kramer 1987; Wannamaker et al. 2000). Dans les régions sévèrement touchées, des conséquences comme des migrations forcées, un stress physiologique élevé, une réduction de l'habitat, une augmentation de la vulnérabilité à la prédation et une perturbation des cycles de vie peuvent être observés (Rabalais et al. 2002; Service 2004). Les espèces benthiques sont plus vulnérables que les espèces nectoniques qui sont mobiles et capables de migrer si l'habitat ne leur convient plus (Diaz et al. 1995). Les organismes benthiques qui vivent dans le sédiment appauvri en OD sont éloignés de tout contact avec l'apport de l'oxygène atmosphérique et sont contraints à s'acclimater et/ou s'adapter jusqu'au retour aux conditions normales (Vaquer-Sunyer et al. 2008).

Dans l'EGSL, des espèces de foraminifères tolérantes à l'hypoxie ont été retrouvées dans le fond de l'estuaire (Thibodeau et al. 2006) confirmant la baisse des niveaux d'OD. Malgré l'ampleur du phénomène d'hypoxie dans cette région, ses impacts n'ont été étudiés que pour la morue franche (*Gadus marhua*) dont le seuil létal a été établi à 28 %sat (Plante et al. 1998). Certaines espèces comme la crevette nordique (*Pandalus borealis*) ou le flétan du Groenland (*Reinhardtius hypoglossoides*), encore abondantes dans les chenaux profonds

de l'EGSL, semblent plus tolérantes à l'hypoxie (Gilbert et al. 2005). Cependant aucune étude n'a encore déterminé les effets de l'hypoxie sur celles-ci.

Problématique

Le flétan du Groenland (*Reinhardtius hypoglossoides*) est un poisson plat d'un grand intérêt commercial. En effet cette espèce représente 53 % de la biomasse des captures totales des poissons dans l'EGSL (DFO 2008). Les juvéniles, entre 15 et 30 cm, se concentrent principalement dans l'estuaire et au nord de l'île d'Anticosti alors que les poissons de plus grande taille (> 33 cm) sont répartis dans les différentes régions de l'EGSL (Treble et al. 2008). Ces observations suggèrent d'une part, que la tête du chenal Laurentien est la principale pouponnière pour cette population (DFO 2006) et d'autre part, que le flétan du Groenland tolère la baisse du niveau d'OD avec un seuil létal pouvant se situer sous les 20-25 %sat communément mesuré dans l'estuaire. Les juvéniles semblent donc se retrouver dans des zones bien circonscrites. La principale période de frai du flétan du Groenland est en hiver entre les mois de janvier et de mars, mais peu d'informations sont disponibles sur les zones précises de reproduction dans l'EGSL (DFO 2006).

Malgré le grand intérêt pour la pêche de cette espèce, la biologie de la reproduction de celle-ci est encore peu connue (Gundersen et al. 2001). La connaissance des facteurs influençant la reproduction et la viabilité des œufs du flétan du Groenland est encore clairsemée. Cependant, nous savons que les œufs sont bathypélagiques (Ådlandsvik et al. 2004) ce qui augmenterait l'exposition de ces derniers à de faibles niveaux d'OD pendant leur développement.

Le développement embryonnaire (DE) des œufs de poissons plats comme le flétan du Groenland n'est pas très documenté et les stades de développement embryonnaire ont été partiellement définis (Stene et al. 1999). Toutefois, Stene et al. (1999) ont utilisé un nombre très limité d'échantillons d'œufs provenant d'une seule femelle. De plus, lors de cette expérience, seulement huit œufs ont complété leur DE et ont éclos. Aucune étude n'a encore relié le DE du flétan du Groenland dans l'EGSL aux facteurs environnementaux tels

que la température et l'OD ou des facteurs biotiques, tels que la qualité des œufs. Cependant, des études sur l'impact de l'hypoxie sur l'éclosion et la survie des œufs de poissons ont été effectuées sur diverses espèces comme la morue franche (*Godus morhua*) (Avery et al. 2009), le saumon Atlantique (*Salmo salar*) (Oppen-Berntsen et al. 1990), le flétan de l'Atlantique (*Hippoglossus hippoglossus*) (Helvik et al. 1993), le corégone Lavaret (*Coregonus lavaretus*) (Czerkies et al. 2002) et *Acanthopagrus butcheri* (Hassell et al. 2008).

La qualité des œufs pouvant être définie comme étant le potentiel de l'œuf à produire des alevins viables (Kjørsvik et al. 1990) est liée au développement embryonnaire et au succès d'éclosion (Hansen et al. 2010). Elle peut être affectée par des facteurs génétiques, l'âge des femelles, des facteurs physico-chimiques, le moment de la ponte, les processus de surmaturation ainsi que les propriétés intrinsèques de l'œuf lui-même (Kjørsvik et al. 1990; Bromage et al. 1992; Brooks et al. 1997). Bien que le taux de fécondation semble être un bon indicateur de la qualité des œufs chez les salmonidés, il n'est pas toujours corrélé avec la qualité des œufs chez certains poissons marins (Kjørsvik et al. 1990). Kjørsvik et al. (1990) ont suggéré que l'évaluation de la symétrie des cellules aux premiers stades de clivage (blastomères normaux) peut être un bon indicateur de la qualité de l'œuf se reflétant par des corrélations positives avec le taux d'éclosion chez le flétan de l'Atlantique (Mangor-Jensen et al. 1998). Ce critère morphologique demeure le plus fiable à ce jour.

Les lipides sont considérés comme l'une des plus importantes sources d'énergie dans les œufs de poissons (Rønnestad et al. 1994; Wiegand 1996; Zhu et al. 2003). Ceci est particulièrement vrai pour les triacylglycérols (TAG), qui représentent la forme la plus courante de stockage d'énergie des œufs chez la plupart des poissons marins (Cowey et al. 1985). Toutefois, les œufs du flétan du Groenland sont caractérisés par une teneur en phospholipides (PL) beaucoup plus élevée que celle des TAG. Il a été observé que les espèces contenant moins de TAG dans leurs œufs, les PL peuvent être une importante source d'énergie durant le développement embryonnaire en plus de jouer leur rôle dans le maintien de la structure membranaire (Tocher et al. 1985; Fraser et al. 1988; Falk-Petersen

et al. 1989; Rainuzzo et al. 1997). Cependant, on peut observer au cours du développement embryonnaire une dynamique très variable dans les différentes classes de lipides, comme des variations importantes du contenu en PL. Ceci peut être expliqué par des processus de métabolisme complexes dus en partie aux variations du volume cellulaire et à la prolifération de la membrane cellulaire au cours du DE (Suthers 1992; Norton et al. 2001). Peu d'informations sont disponibles sur le métabolisme et la dynamique des classes de lipides au cours du DE chez les œufs des téléostéens (Suthers 1992).

Objectifs et Hypothèses

Cette étude fait partie d'un projet de recherche plus global qui vise l'étude d'une problématique jugée préoccupante du point de vue des pêcheries commerciales du flétan du Groenland et de la crevette nordique dans l'estuaire et le golfe du Saint-Laurent, soit celle de l'hypoxie. Ma maîtrise visait plus particulièrement l'étude de l'effet des concentrations d'OD sur l'embryogénèse et le succès d'éclosion des œufs du flétan du Groenland. Les objectifs spécifiques étaient (1) évaluer les effets de faibles concentrations en OD sur le taux de survie, le développement embryonnaire et le succès d'éclosion des œufs du flétan du Groenland, (2) décrire les changements dans la composition lipidique des œufs durant le développement embryonnaire en fonction de l'origine des femelles ainsi qu'en fonction des différents niveaux d'OD. À cette fin, des lots d'œufs produits par six femelles ont été suivis à partir de la ponte jusqu'à l'éclosion à des teneurs en OD de 10, 20, 35, 50 et 100 %sat. Au cours de cette étude les hypothèses testées sont (1) les faibles concentrations en OD influencent le taux de survie, le développement embryonnaire et le succès d'éclosion des œufs du flétan du Groenland, (2) les faibles niveaux en OD et l'origine des femelles induisent des changements au niveau de la composition lipidique des œufs du flétan du Groenland durant le développement embryonnaire.

CHAPITRE 1 INFLUENCE OF DIFFERENT LEVELS OF DISSOLVED OXYGEN ON THE SUCCESS OF GREENLAND HALIBUT (*REINHARDTIUS HIPPOGLOSOIDES*) EGG HATCHING AND EMBRYONIC DEVELOPMENT

1.1 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 marine life 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 (Wu 2002; 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 anthropic 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. Fisheries and Oceans Canada (DFO) has reported that these fish are widely distributed throughout the EGSL and spawn in the deep waters of Laurentian channel (DFO 2006). These observations suggest that the Laurentian channel could be a main nursery for this species and that Greenland halibut may tolerate low levels of DO, with a probable lethal threshold level below 20–25%sat.

Despite the commercial importance of Greenland halibut, little is known about its reproductive biology (Gundersen et al. 2001). Knowledge on the factors influencing reproduction and egg viability of Greenland halibut 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). Indeed, Stene et al. (1999) have used a very limited number of eggs samples from a single female. Moreover, in this experiment, only eight eggs achieved their ED and hatched. There have been no studies relating Greenland halibut embryonic development (ED) to abiotic or biotic factors such as temperature, DO, or egg quality.

Fish egg quality can be affected by maternal age and condition factor, timing of the spawning cycle, overripening processes, genetic factors, and intrinsic properties of the egg itself (Kjørsvik et al. 1990; Bromage et al. 1992; Brooks et al. 1997). Kjørsvik et al. (1990) suggested that the assessment of cell symmetry at early cleavage stages (normal blastomeres) might be a possible indicator of egg quality for marine fish and that this criterion may be applicable for rapid egg quality assessment.

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). For Greenland halibut eggs and many other marine species, phospholipids (PL) are the major lipids class. In species with lower TAG levels in the eggs, PL can also be a

source of energy during ED in addition to playing a role in membrane structure (Tocher et al. 1985; Fraser et al. 1988; Falk-Petersen et al. 1989; Rainuzzo et al. 1997).

The present study was undertaken to assess the effects of low levels of DO (down to 10%sat) on the survival rate, 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 batches produced by individual females were followed from fertilization until hatching. We test the hypothesis that the low levels of DO influence negatively the survival, embryonic development, and hatching success of Greenland halibut eggs. We suggest also the hypothesis that the lipid composition of Greenland halibut eggs during the embryonic development is modified not only by the low levels of DO, but also by the female origin.

1.2 MATERIALS AND METHODS

1.2.1 Fish sampling

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

1.2.2 Fertilization and incubation

Eggs from six females and sperm from one male were stripped manually from ripe fish in February and March 2010 as described by Jelmert et al. (1987). Females were selected by the swelling and redness of the genital pore and thickness of the abdomen. For each female, length, mass, and condition factor were estimated. Condition factor (K) is an index of condition, calculated as follows: $K = \left[\frac{Mass}{Lenght^{3}}\right] \times 100$. The better the condition of the fish, the higher the value of K (Fulton 1902). 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 ™ (1 ml per 10 L) as a water conditioner. All manipulations were carried out at low light intensity. Two or three samples of unfertilized eggs were first taken, weighed, and counted to estimate the fecundity of each female by the gravimetric method (extrapolation to the total wet-weight of released eggs). 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.

1.2.3 Experimental design

Fertilized eggs from six females were used for the experiment. Equal proportions of total fertilized eggs from each female were randomly distributed in ten cone-shaped incubators (30 cm in diameter) representing two replicates of five dissolved oxygen level treatments (~ 800 eggs per incubator). DO level treatments consisted of two conditions of severe hypoxia (10 and 20%sat; ~0.7 and 1.4 $mg{\cdot}L^{-1}$), two of moderate hypoxia (35 and 50%sat; ~2.4 and 3.5 $mg{\cdot}L^{-1}$) and one of normoxia (100%sat, ~6.9 $mg{\cdot}L^{-1}$). 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. Seawater was circulated in each tank through external chillers to maintain the temperature at 5°C; salinity was 32.4. 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. Stable DO levels were maintained for the whole incubation period using this experimental set-up. Kruskal-Wallis tests were applied to validate the stability of the different DO treatments and showed highly significant differences between each treatment ($X^2 = 37.46$, d.f. = 4, $p < 0.01$; Figure 1).

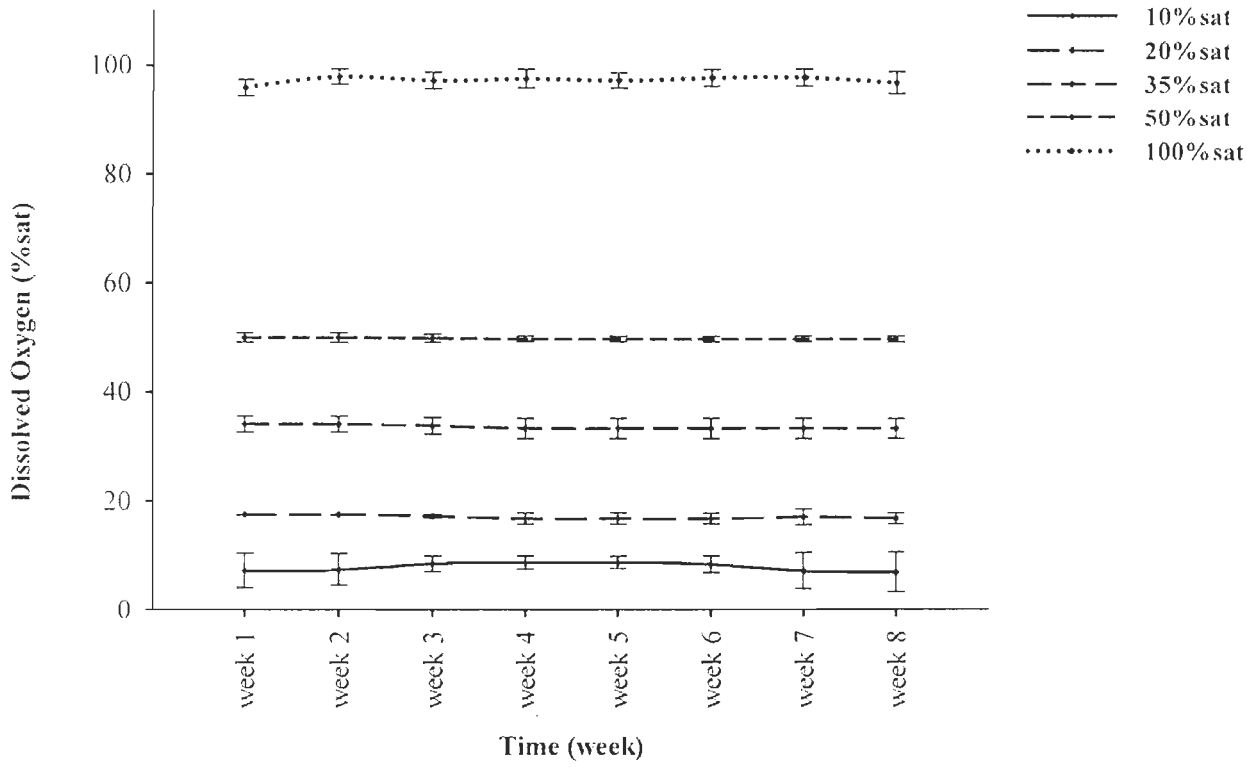


Figure 1. Weekly dissolved oxygen levels (percent saturation) in each treatment tank (mean ± SD) during the 8-week experiment on egg incubation.

1.2.4 Egg samplings

Approximately 150–200 eggs from each female at 100%sat were randomly sampled after 24 h to determine fertilization success. At this time, embryos were at the 4- to 32-cell stage, and any egg observed with cell division was considered as fertilized (Hansen et al. 2010).

Embryonic development was monitored on 10–25 eggs sampled in each incubator every 2–3 days. At each sampling time, egg samples were also stored in 1 mL of sterilized seawater at -80°C for lipid analysis and dry mass determination. Dry mass was determined by drying samples at 110°C for 48 h.

The experiment was conducted until hatching was completed in each incubator. Hatching rate (%) was estimated taking into account the number of dead, live, and eggs removed at each sampling.

$$\text{Hatching success (\%)} = \frac{N_{HL}}{\left(Nt_0 \times \text{fertilization success}\right) - T_{ADE}} \times 100$$

N_{HL} = Number of total hatched larvae

$Nt_0 = N_{HL}$ + number of dead and alive eggs removed + number of sampled eggs

T_{ADE} = number of total alive eggs removed and sampled + number of total fertilized dead eg

1.2.5 Laboratory analysis

Developmental stages

Eggs and larvae were examined and digitized using a Leica MZ 75 system (Richmond Hill, Ontario, Canada). Digitized images were used to identify developmental stages and to measure egg diameter (mm) and larva length (mm) with Image ProPlus software 5.1 (Media Cybernetics, Silver Spring, MD, USA). We followed Shardo's method to define egg developmental stages, in which each stage is recognized on the basis of one or two landmark features (Shardo 1995). We also referred to the Hall et al. (2004) classification developed for Atlantic cod to identify developmental stages.

Lipid extraction and lipid class analyses

Lipid extraction on egg samples was done according to Folch et al. (1957). Lipid classes were determined using an Iatroscan Mark-VI analyzer (Iatron Laboratories Inc., Tokyo, Japan). Lipid extracts were applied to Chromarods-SIII (Iatron Laboratories Inc., Tokyo, Japan) and developed in a four-solvent system (Parrish 1987; Parrish 1999). Chromatograms were analyzed using integration software Peak Simple 3.2 (SRI Inc). The separated lipid classes in this study were ketones (KET), triacylglycerols (TAG), sterols (ST), acetone-mobile polar lipids (AMPL), and phospholipids (PL).

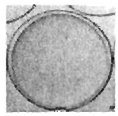
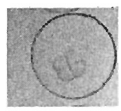
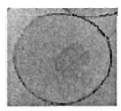
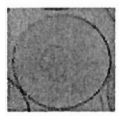
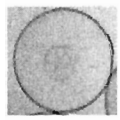
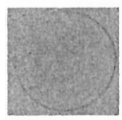
1.2.6 Definition of developmental stages

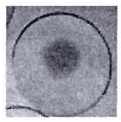
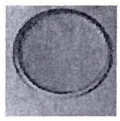
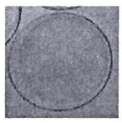
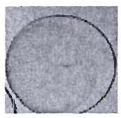
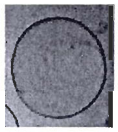
The embryonic development of Greenland halibut has never been fully described. ED was subdivided into nine periods: fertilization (ED1), cleavage (ED2–ED8), blastula (ED9–ED11), gastrula (ED12–ED17), cephalization (ED18–ED21), neurulation (ED22–ED23), cranial regionalization (ED24–ED25), tail lift (prehatching period; ED PRE-H), and hatching (larva day-0) (Table 1). The stages between gastrulation and hatching encompassed the period of organogenesis.

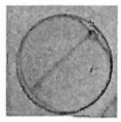
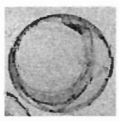
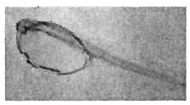
ED proceeded with the formation of a small perivitelline space and subsequent meroblastic cleavage. The cleavage stage was characterized by many mitotic divisions; as long as the cleavage continued, the number of blastomeres increased and their size diminished and became less distinct (ED8). At the blastula stage, cells became bound tightly together and the blastoderm highly flattened with uniform thickness (ED11). The formation of the periblast was initiated at this stage.

The first epiboly movements characterized the end of this stage. At the gastrula stage, epiboly movements were predominant (expansion of the cellular blastoderm over the yolk). Major events during gastrulation included the formation of the thickened germ ring at the periphery of the blastoderm and the appearance of the embryonic shield. During cephalization, the body axis became more clearly defined (ED20). Neurulation occurred simultaneously with cephalization. From ED22 to ED23, the neural keel was present and projected ventrally into the yolk. Cranial regionalization was characterized by the continued

Table 1. Embryonic developmental stages in Greenland halibut.

Embryonic development stages (ED)	Stage		Characteristics
1	Fertilization		Perivitelline space appears; cytoplasm streams toward animal pole
2	Cleavage 1	No image available	2 cells-Meroblastic cleavage
3	Cleavage 2		4 equal cells
4	Cleavage 3		8 cells in 2 parallel rows
5	Cleavage 4		16 cells
6	Cleavage 5		32 cells; cells start to compact
7	Cleavage 6		~ 64 cells
8	Cleavage 7		Cells are no more distinct

9	Blastula (early)		Cells become bound tightly together
10	Blastula (mid)		Blastodisc with the surrounding periblast
11	Blastula (late)		Blastoderm highly flattened with uniform thickness
12	Gastrula (30% Epiboly)		Germ ring and embryonic shield are visible
13	Gastrula (50% Epiboly)		Blastoderm covers 45-50% of the embryo. "Lemon wedge shape"
14	Gastrula (70% Epiboly)	No image available	"Kidney bean" shape
15	Gastrula (80% Epiboly)		The blastoderm covers 80% of the embryo. "Crescent shape"
16	Gastrula (90% Epiboly)		First indication of fold appearing where body axis will lie
17	Gastrula (late)		Body axis forming
18	Cephalization (early)		Body axis formed, blastopore is large.

19	Cephalization (early)		Blastopore closing but not completely
20	Cephalization (mid)		Closed blastopore. First signs of somites. The body axis becomes more clearly defined.
21	Cephalization (late)		Embryo length increases but it is lower than egg diameter
22	Neurulation (early)		Embryo is longer than egg diameter. Beginning of development of optic vessicles
23	Neurulation (late)		The head region is broader and thicker than the tail region. First signs of fins
24	Cranial regionalization		Embryo gets longer. Appears a structure at the back of developing embryo
25	Cranial regionalization		Embryo gets longer. Optic vesicles are more prominent
PRE-H	Tail lift		Embryo gets longer. Sometimes tail is outside the egg
H-0	Larva day-0		Embryo at early developmental stage, without pigmentation

emergence of craniofacial structures; in this stage, optic vesicles were more prominent. Tail lift refers to the separation of the tail from the yolk; it begins when the most posterior portion of the trunk detaches from the yolk at ED PRE-H.

1.2.7 Statistical analyses

Analysis of variance (ANOVA)

Statistical analyses were performed using SPSS 16.0. All percentage data were arcsine-transformed and normality was tested using both Kolmogorov-Smirnov and Shapiro-Wilk tests. Homogeneity of variance was tested with Levene's test. If necessary, data were transformed (log or arcsine square-root; lipid classes are in %) to achieve homogeneity of variances. Statistical significance was set at $\alpha = 0.05$. A two-way analysis of variance (ANOVA) was used to estimate the effects of DO and female origin on hatching success. A one-way ANOVA was conducted to test the effect of female origin on total lipid content and lipid classes in eggs 7 days post-fertilization that had been incubated at 100%sat. Finally, a two-way ANOVA was used to test the effects of DO level and female origin on total lipid content and lipid classes for days 14, 17, and 21.

Multinomial Logistic regression and decision tree analysis

To test the effect of DO and female origin on the early developmental stages of Greenland halibut eggs, a three-way contingency table was analyzed by a Multinomial Logistic regression (MLogit). This model has the same conceptual basis as a log-linear

model, which is an extension of multiway contingency tables, where the conditional relationship between two or more variables is analyzed by taking the natural logarithm of the cell frequency within the contingency table (Bishop 1969; Shelby 1973). The variables investigated by log-linear models are all treated as «response variables», with no distinction between independent and dependent variables (Agresti et al. 1996; Brant 1996). We used the MLogit regression instead: embryonic developmental stage (days 7, 10, 14, 17, and 21) was treated as a dependent variable and DO and female origin as independent variables. MLogit is based on the principles of Bayesian statistics and likelihood ratio; it replaces the familiar classic least-squares linear statistical model. Significance tests proceed from the likelihood ratio G^2

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

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

Decision tree analysis was used in conjunction with MLogit models to interpret the results. Although decision tree analysis is a powerful predictive model (Zhao 2007), it was used here for a descriptive purpose. We used a X^2 test to select discriminate variables ($p < 0.05$) (Singh et al. 2009). In tree structure, leaves represent classifications and branches represent conjunctions of features that lead to those classifications (Rudolfer et al. 1999).

1.3 RESULTS

1.3.1 Physiological variables

Females used in the experiment had lengths and masses between 48.5 and 65 cm and 875 and 2519 g, respectively. Fulton's K varied between 0.73 and 0.92 with the highest value observed for female 3. Relative fecundity was higher in females with lower condition factor while fecundity varied between 10752 and 24194 eggs per fish (Table 2).

Table 2. Length, mass, condition factor, fecundity, relative fecundity, and period of fertilization for the six Greenland halibut females used in the experiment. No data are presented for female 5.

Female	Length (cm)	Mass (g)	Condition factor ($K = \frac{Mass}{Length^3} \times 100$)	Fecundity (no. eggs $fish^{-1}$)	Relative fecundity (no. eggs g^{-1})	Period of fertilization in 2010 (dd–mm)
1	48.5	874.5	0.77	10752.3	12.2	24–02
2	50.7	951.7	0.73	13426.0	14.1	22–02
3	65.0	2519.3	0.92	24194.8	9.6	25–02
4	55.7	1483.6	0.86	15966.8	10.7	02–03
5	-	-	-	-	-	08–03
6	49.1	978.8	0.83	13012.0	13.3	18–03

1.3.2 Hatching rates

On average, eggs hatched 28 days after fertilization. Hatch rate was significantly affected by the interaction between DO level and female origin (F (12, 20) = 12.83, p < 0.0001; Figure 2). The time to hatch was not significantly different between different DO levels and females. Higher hatch rates were observed for female number 3 (around 40%). For this female, hatch rates were not significantly different at 20, 35, 50, and 100%sat. No eggs hatched at 10%sat for any female. Eggs from two females (2 and 6) did not hatch at any DO level (Figure 2).

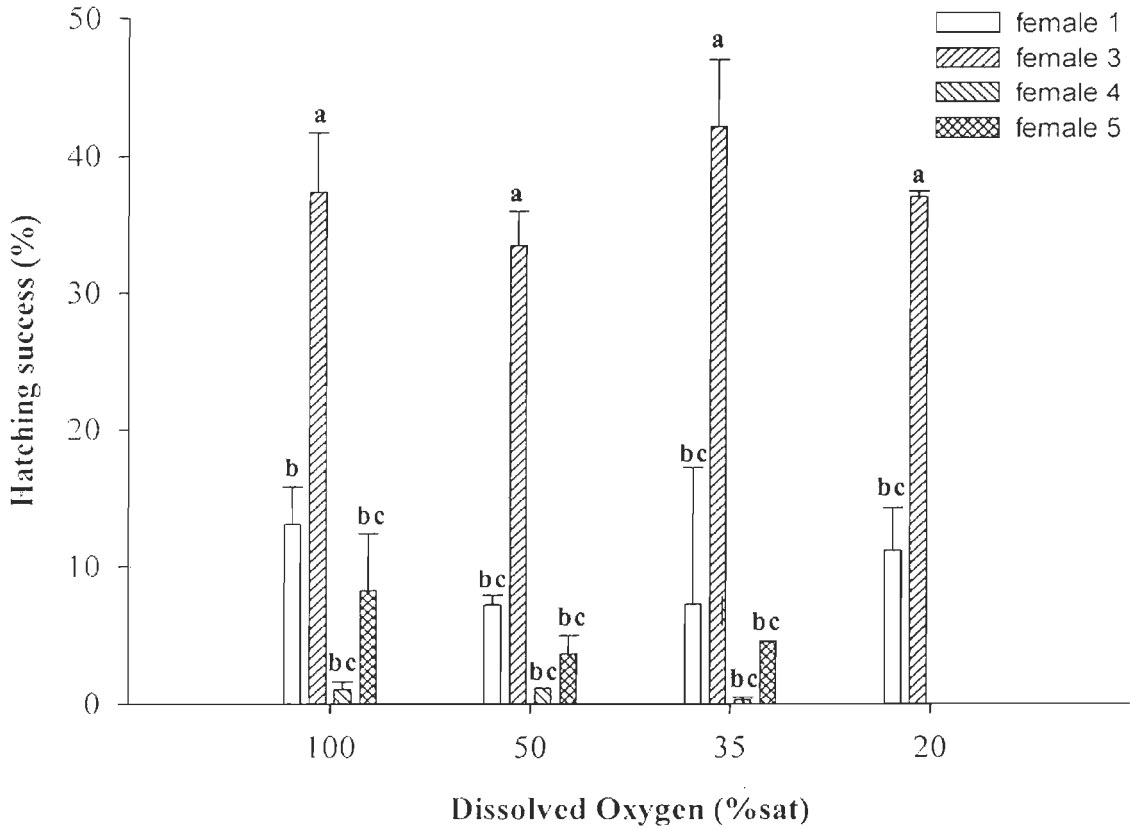


Figure 2. Mean hatch rates for Greenland halibut embryos obtained from four females and exposed to five levels of dissolved oxygen (DO) [mean ± SD]. Different letters indicate statistically significant differences between treatments (female origin × DO levels) ($p < 0.0001$). No data are presented for 10%sat because no eggs hatched at this level.

1.3.3 Early development

Fertilization rates of the different females varied between 42 and 95% and the proportion of eggs with normal blastomeres varied between 17 and 47% (Table 3). Normal blastomeres were regular in size and shape (Figure 3A), while abnormal blastomeres were identified by irregular cell shape (asymmetry) (Figure 3B), unequal cell size (Figure 3C), incomplete cell margins, or incorrect cell numbers (Figure 3D). Eggs from females 2 and 6, which did not hatch, had the lowest percentages of fertilization and normal blastomeres, i.e., 42%–71% fertilization and 17%–18% normal blastomeres. Eggs from female 3, from which we observed the highest hatching rate, had high percentages of fertilization and normal blastomeres.

Table 3. Percentages of fertilization and normal blastomeres obtained for each of the six females used in the experiment.

Female number	% Fertilization	% Normal blastomeres
1	84	26
2	42	17
3	95	47
4	92	27
5	89	33
6	71	18

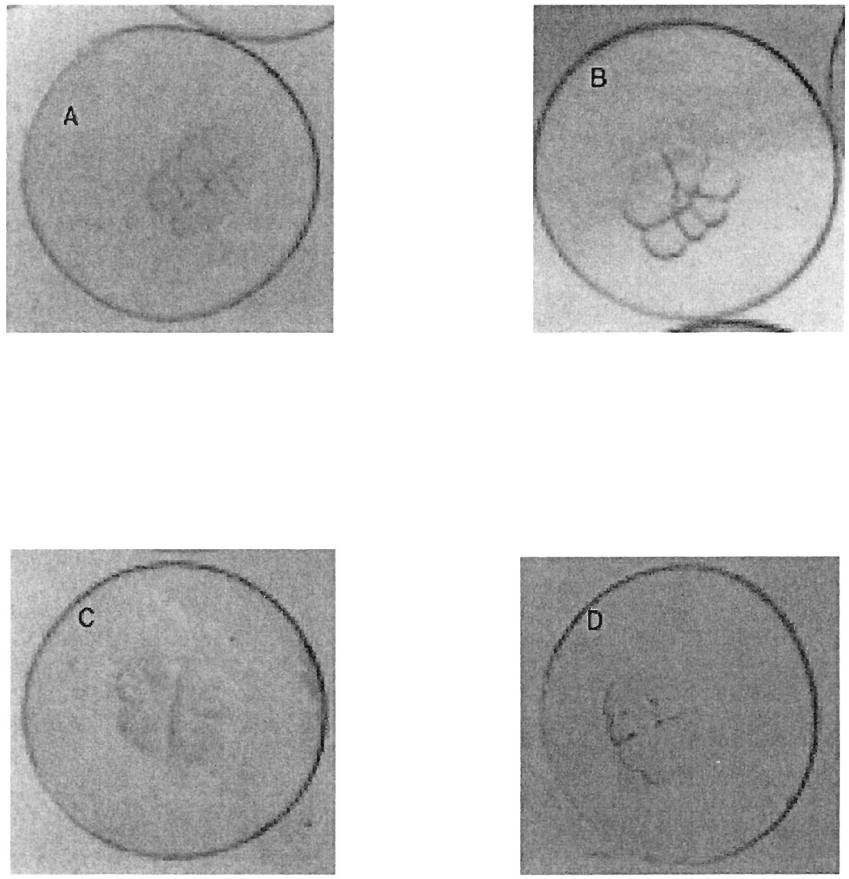


Figure 3. Greenland halibut blastomeres at the 8-cell stage. (**A**) Normal blastomere cleavage; abnormal: (**B**) asymmetrical cell positioning, (**C**) unequal cell sizes, (**D**) cell margins poorly defined.

1.3.4 Variations in embryonic developmental

Multinomial Logistic regression (Mlogit) analyses showed that in the first 10 days of development, significant differences in developmental stages were largely explained by female origin (Table 4). On days 14, 17, and 21, both factors had significant effects on ED, with increasing importance of DO. On day 24, DO had a larger influence on ED than female origin.

In addition to Mlogit analyses, we used decision tree analysis to illustrate the variation in the proportion of eggs at different developmental stages in relation to DO and female origin. On day 10, the first summit was segmented in eggs from some females (Figure 4). ED of eggs from females 1, 2, 5, and 6 were not significantly different while those from females 3 and 4 differed from the others, with 84.0% of the eggs at ED19 and 62.1% at ED20, respectively. On day 24 (toward the end of ED), the first summit was segmented depending on DO levels (Figure 5). At 10%sat, 100% of the eggs were at ED20, regardless of female origin. For the other DO levels (20, 35, 50, and 100%sat), 59.7% of eggs from female 3 were at the same developmental stage (ED PRE-H). However, for the other females (1, 2, 4, and 5), 66.0% of the eggs were at ED24 in DO levels corresponding to 20 and 35%sat while 61.5% of the eggs were at ED25 for both 50 and 100%sat, indicating a slower development rate at 20 and 35%sat for these females.

Table 4. Summary of Multinomial Logistic regression (MLogit) tests on the variations in embryonic developmental of Greenland halibut eggs sampled on days 7, 10, 14, 17, 21, and 24 as a function of female origin and dissolved oxygen (DO) levels.

Days of embryonic development	Effect	d.f.	G^2	*Probability*
7	Female origin	20	904.67	< 0.001
	DO	16	124.66	< 0.001
10	Female origin	5	50.10	< 0.001
	DO	4	5.36	0.252
14	Female origin	10	328.35	< 0.001
	DO	8	210.76	< 0.001
17	Female origin	20	474.76	< 0.001
	DO	16	457.08	< 0.001
21	Female origin	20	396.60	< 0.001
	DO	16	390.79	< 0.001
24	Female origin	12	127.34	< 0.001
	DO	12	252.60	< 0.001

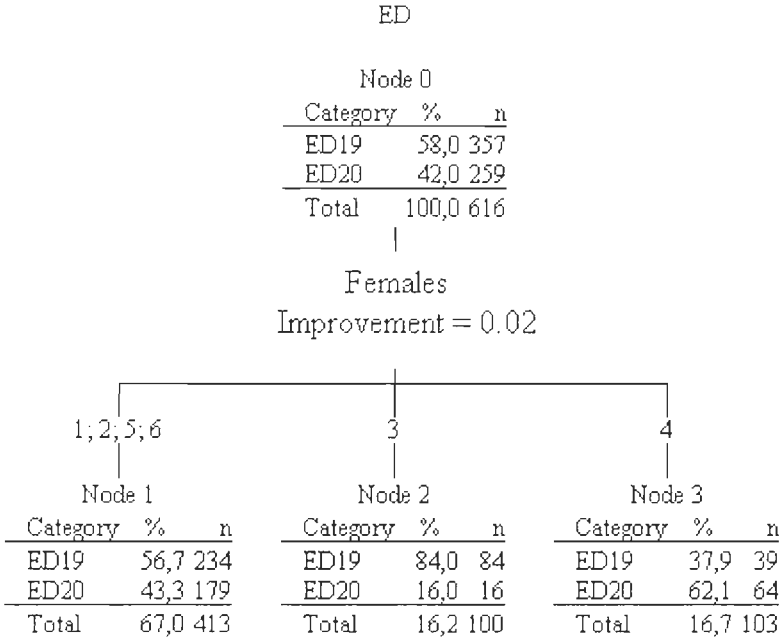


Figure 4. Classification tree for day 10 of embryonic development (ED). The first summit is segmented depending on the factor «female origin».

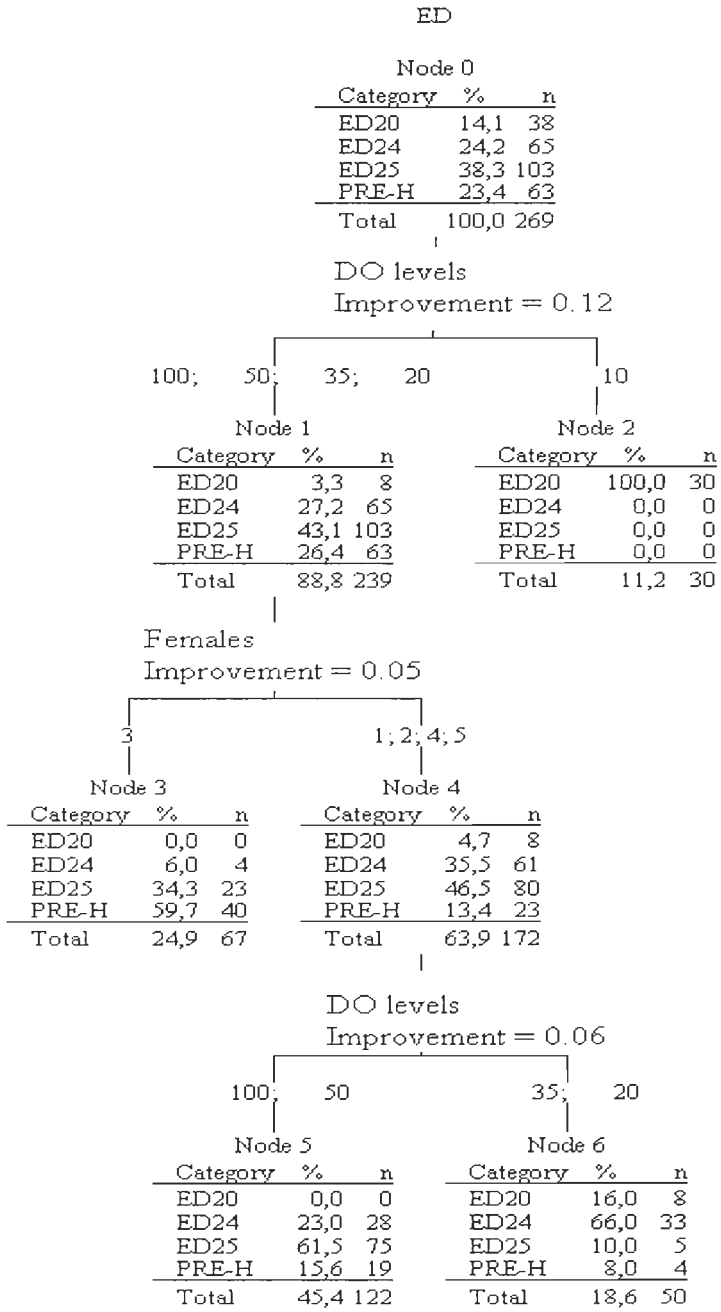


Figure 5. Classification tree for day 24 of embryonic development (ED). The first summit is segmented depending on the factor «DO levels».

1.3.5 Lipid class analyses

Total lipid content and, KET, AMPL, PL, TAG differed significantly according to female origin for 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) $_{\text{AMPL}} = 3.75$, F (5, 17) $_{\text{PL}} = 2.93$, and F (5, 17) $_{\text{TAG}} = 5.11$, $p < 0.05$; Figure 6). The concentration of total lipids in the eggs of female 2 was significantly lower (133.84 ug mg^{-1} DW) than in female 1 (237.34 ug mg^{-1} DW) (Figure 6). Significant differences in the percentage of PL were observed between females. However, pair-wise comparisons did not allow the detection of differences between individual females. PL constituted the major lipid class, with mean values representing 71 to 83% of total lipids. TAG represented 8.7% of total lipids in eggs from female 3, which was significantly different from percentages found in the eggs of females 1, 4, and 5 (< 6%) (Figure 6). The proportions of KET and AMPL in eggs from female 2 represented 5% and 15% of the total lipids, respectively. These proportions were significantly higher than in those from the other females. Lipid classes in the eggs of females 1, 3, and 5 incubated at the different DO levels (excluding the 50%sat level) were determined for days 14, 17, and 21. On day 14, significant differences in total lipids, PL, and KET percentages were observed only in relation to female origin (F (2, 12)$_{\text{total lipids}} = 9.32$, F (2, 12)$_{\text{PL}} = 4.87$, and F (2, 12)$_{\text{KET}} = 15.70$, $p < 0.05$; Table 5). The concentration of total lipids in eggs from female 5 was significantly lower than in those of females 3 and 1 (Table 5). PL and KET were the major lipid classes present in eggs, with mean values of 77% and 5% of total lipids, respectively. Although eggs from female 5 had the lowest proportion of PL compared to females 1 and 3,

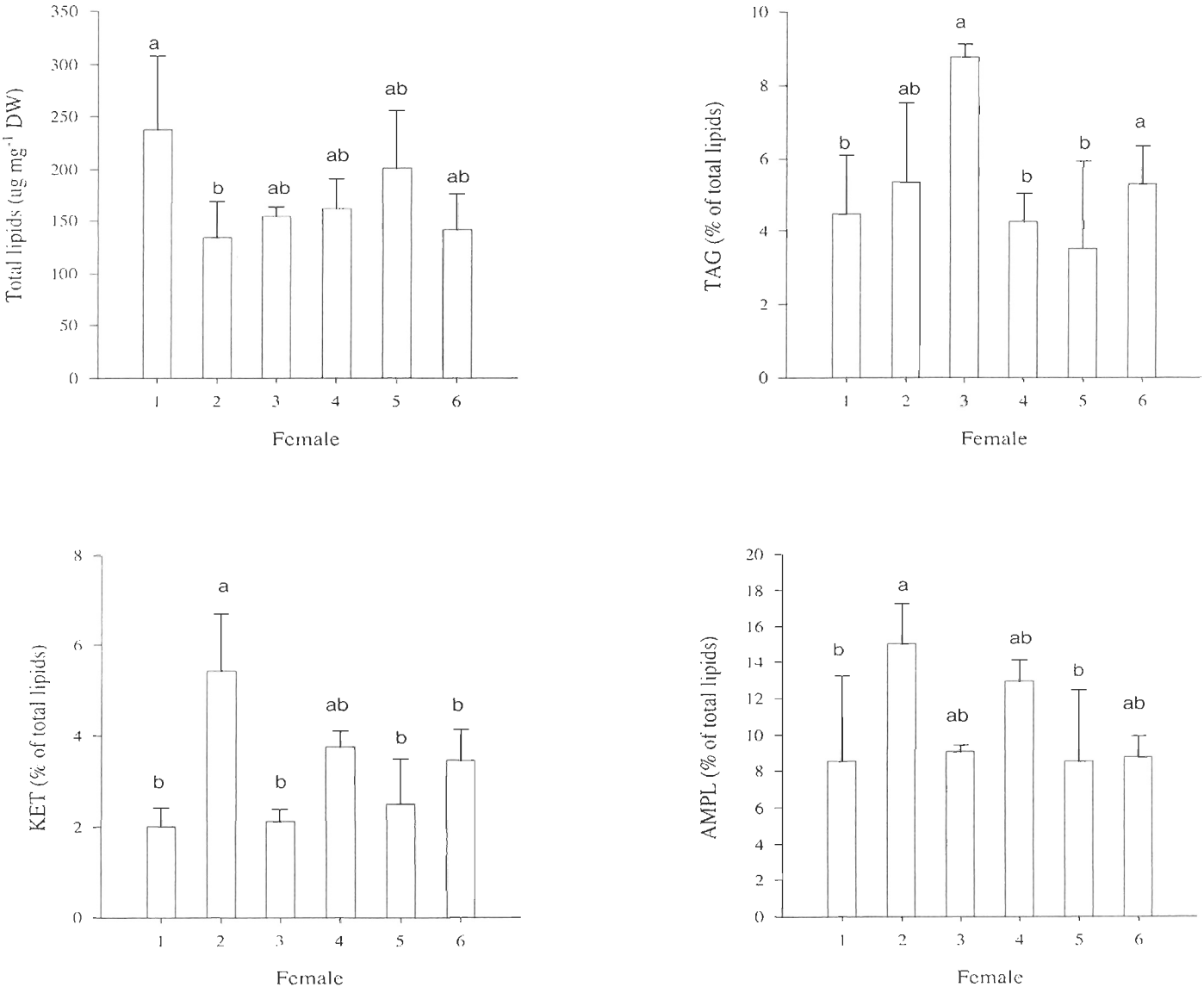


Figure 6. Changes in total lipid content and proportions of triacylglycerols (TAG), ketones (KET), and acetone-mobile polar lipids (AMPL) in Greenland halibut eggs from different females on day 7 of embryonic development for eggs incubated at 100%sat in dissolved oxygen (DO) [mean ± SD]. Different letters indicate significant differences among females ($p < 0.05$).

Table 5. Variations in total lipid contents and proportions of ketones (KET), triacylglycerols (TAG), and phospholipids (PL) from Greenland halibut eggs from females exposed to different dissolved oxygen (DO) levels on days 14, 17, and 21 (mean ± SD). Different letters indicate significant differences ($p < 0.05$).

Female	Day	DO level	Total lipids ($\mu g\ mg^{-1}$ dry weight)	KET (%)	TAG (%)	PL (%)
1	14	100%sat	309.68 ± 135.70^{a}	3.06 ± 0.13^{a}	1.78 ± 0.50^{a}	83.51 ± 2.70^{a}
		35%sat	665.53 ± 58.07^{a}	3.40 ± 1.40^{a}	1.57 ± 0.38^{ab}	85.48 ± 2.42^{a}
		20%sat	378.17 ± 84.94^{a}	2.9 ± 1.41^{a}	2.14 ± 0.82^{ab}	84.13 ± 7.77^{a}
		10%sat	427.99 ± 165.49^{a}	4.79 ± 2.34^{a}	2.33 ± 1.97^{b}	75.99 ± 3.46^{a}
3	14	100%sat	514.72 ± 192.65^{a}	3.42 ± 0.57^{a}	3.32 ± 0.92^{a}	80.92 ± 0.50^{ab}
		35%sat	561.98^{a}	4.75^{a}	1.93^{ab}	52.77^{ab}
		20%sat	497.71^{a}	3.35^{a}	2.08^{ab}	78.32^{ab}
		10%sat	311.33 ± 75.23^{a}	2.52 ± 1.12^{a}	0.71 ± 0.16^{b}	86.66 ± 6.85^{ab}
5	14	100%sat	179.35 ± 102.53^{b}	9.11 ± 3.40^{b}	2.92 ± 1.08^{a}	69.53 ± 14.37^{b}
		35%sat	317.69 ± 153.53^{b}	7.12 ± 2.26^{b}	1.13 ± 0.27^{ab}	78.09 ± 7.22^{b}
		20%sat	240.35 ± 81.19^{b}	7.38 ± 0.63^{b}	1.51 ± 0.09^{ab}	65.95 ± 8.28^{b}
		10%sat	175.58 ± 7.79^{b}	7.61 ± 0.16^{b}	1.93 ± 0.03^{b}	65.67 ± 0.87^{b}
1	17	100%sat	380.27 ± 13.25^{abc}	3.66 ± 0.96^{bc}	2.27 ± 0.94^{a}	84.65 ± 0.19^{ab}
		35%sat	264.77 ± 100.69^{bc}	6.96 ± 4.25^{abc}	2.95 ± 1.19^{a}	74.01 ± 15.42^{abc}
		20%sat	493.76 ± 30.86^{ab}	5.10 ± 0.48^{abc}	1.36 ± 0.03^{a}	82.04 ± 2.42^{abc}
		10%sat	872.42 ± 92.82^{a}	2.35 ± 0.07^{c}	0.69 ± 0.16^{a}	90.94 ± 1.40^{a}
3	17	100%sat	864.81 ± 90.33^{a}	4.65 ± 0.13^{abc}	0.75 ± 0.09^{a}	82.08 ± 0.92^{abc}
		35%sat	502.38 ± 47.46^{ab}	2.90 ± 0.61^{c}	2.11 ± 1.90^{a}	87.04 ± 0.57^{ab}
		20%sat	457.09 ± 100.99^{ab}	5.74 ± 2.15^{abc}	4.07 ± 0.96^{a}	75.05 ± 11.41^{abc}
		10%sat	424.49 ± 91.69^{ab}	4.16 ± 0.26^{abc}	1.36 ± 0.04^{a}	82.69 ± 0.91^{ab}

		100%sat	134.98 ± 10.89^{c}	10.87 ± 1.56^{a}	4.12 ± 1.56^{a}	57.24 ±1.64^{c}
		35%sat	266.73 ± 30.56^{bc}	9.96 ± 1.25^{a}	1.67 ± 0.62^{a}	75.18 ± 0.70^{abc}
5	17	20%sat	258.35 ± 100.07^{bc}	12.03 ± 3.54^{a}	2.39 ± 1.34^{a}	66.54 ± 7.29^{bc}
		10%sat	452.32 ± 69.29^{ab}	2.34 ± 0.19^{abc}	1.02 ± 0.30^{a}	86.21 ± 0.47^{ab}
				Day 21		
		100%sat	682.68 ± 23.99^{abc}	3.18 ± 0.15^{b}	1.12^{bc}	87.11 ± 1.85^{a}
		35%sat	247.09 ± 31.07^{bc}	5.80 ± 0.67^{ab}	3.99 ± 0.39^{abc}	81.68 ± 2.14^{a}
1	21	20%sat	258.66 ± 13.10^{bc}	4.50 ± 0.60^{ab}	2.9 ± 0.42^{abc}	80.02 ± 2.92^{a}
		10%sat	221.52^{bc}	7.85^{abc}	5.38^{ab}	69.08^{a}
		100%sat	915.94 ± 260.70^{a}	5.81 ± 0.60^{ab}	1.31 ± 1.13^{bc}	78.88 ± 7.94^{a}
		35%sat	217.75 ± 97.89^{c}	10.11 ± 5.63^{a}	5.05 ± 1.25^{a}	62.91 ± 20.26^{a}
3	21	20%sat	709.40 ± 71.54^{abc}	4.96 ± 0.58^{ab}	0.8 ± 0.08^{c}	80.53 ± 5.08^{a}
		10%sat	181.12^{abc}	6.12 ± 1.71^{ab}	2.84 ± 2.14^{abc}	76.39 ± 7.68^{a}
		100%sat	477.20 ± 55.67^{abc}	6.65 ± 0.95^{ab}	1.26 ± 0.09^{bc}	74.67 ± 1.27^{a}
		35%sat	1031.66 ± 234.56^{a}	3.65 ± 0.16^{b}	0.60^{c}	88.46 ± 0.32^{a}
5	21	20%sat	717.75 ± 122.28^{abc}	5.29 ± 0.36^{ab}	0.56 ± 0.09^{c}	82.91 ± 0.69^{a}
		10%sat	267.36 ± 28.05^{bc}	4.57 ± 0.60^{ab}	0.94 ± 0.83^{c}	86.71 ± 2.08^{a}

they had the highest proportion of KET (Table 5). TAG varied only according to DO (F (3, 12)$_{TAG}$ = 4.43, p < 0.05; Table 5) and contributed only ~ 2% of the eggs' total lipids. TAG percentage 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 percentages of total lipids, PL, KET, and TAG (day 17: F (6, 12)$_{total\ lipids}$ = 6.39, F (6, 12)$_{PL}$ = 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)$_{PL}$ = 3.09, F (6, 11)$_{KET}$ = 4.65, and F (6, 11)$_{TAG}$ = 4.29, p < 0.05; Table 5). The only clear pattern was observed at day 21 for the 10%sat DO level, where a significant decrease of total lipids was observed.

1.4 DISCUSSION

1.4.1 Hatching and embryonic development

Greenland halibut eggs were highly tolerant to hypoxia, with hatching occurring at levels as low as 20%sat. Similar tolerance levels have also been observed for Atlantic halibut (Helvik et al. 1993). In our experiment, no Greenland halibut 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 $mg{\cdot}L^{-1}$ and 1.4 $mg{\cdot}L^{-1}$) for this species. In the black bream (*Acanthopagrus butcheri*), this threshold level has been observed at 30%sat (2.1 $mg{\cdot}L^{-1}$) (Hassell et al. 2008). However, we found that hatch rates varied according to both DO level and female origin, which could suggest that hypoxia levels over 10%sat could induce selective pressure.

In some species, hypoxia can act to initiate hatching. For example, eggs of whitefish (*Coregonus lavaretus*) and vendace (*C. albula*) exposed to hypoxia responded with precocious hatching (Czerkies et al. 2002). Early hatching has also been observed in two freshwater salmonids (*Coregonus lavaretus* and *Coregonus albula*) in response to low DO levels, and the incidence increased as the duration hypoxia exposure increased (Czerkies et al. 2002). In some species, like rainbow trout (*Oncorhynchus mykiss*), hypoxia seems to affect hatching regulation, resulting in delayed or advanced hatching (Ciuhandu et al. 2005). Species experiencing precocious hatching in response to hypoxic conditions are known to be the less tolerant ones (Oppen-Berntsen et al. 1990). In fact, precocious

hatching is considered as an extreme reaction that enables embryos to escape from unfavorable oxygen conditions, and premature hatching of hypoxic embryos may therefore enhance access to oxygen (Mills et al. 1999; Czerkies et al. 2002). Our results indicate that hypoxic conditions did not postpone the time of hatching in Greenland halibut eggs. No significant differences in time to hatch between severe hypoxic (20%sat), moderate hypoxic (35 and 50%sat), and normoxic conditions (100%sat).

Although hypoxia did not impair survival to hatching for eggs incubated at low DO levels such as 20, 35, and 50%sat, it is quite possible that it may cause high mortality and malformations in post hatch larvae. However, this was not examined in our study and would need further investigations.

Egg hatching is a complicated process that involves eggshell components, hatching enzymes, egg origin, and general embryonic development (Oppen-Berntsen et al. 1990). The assessment of ED in the present study demonstrated that developmental stages varied, mainly depending on female origin at the beginning of embryogenesis. The DO effect started to become important 17 days after fertilization. We suggest that this phenomenon could be explained by the low oxygen demands of early embryos, which limited the response to low oxygen concentrations, and to a cumulative effect of hypoxic conditions on ED through time (Oppen-Berntsen et al. 1990). Moreover, because oxygen consumption by the embryo increases with development, embryos may experience hypoxia in more advanced stages (Hamdorf 1961; Rombough 1988; Oppen-Berntsen et al. 1990). At 10%sat, all Greenland halibut embryos were at the same embryonic stage (ED20) from 17

days post-fertilization regardless of female origin, indicating a significantly slower developmental rate than at 20, 35, 50, and 100%sat. ED was impaired by severe hypoxia (10%sat); embryos were less developed, resulting in a lack of cephalization and an absence of the normal processes that occur later in the gastrula period. At 21 days post-fertilization, ED appeared normal at 20 and 35%sat but it was still slower than at 50 and 100%sat.

Effects of female origin confirmed the important role of egg quality toward the end of ED. Other studies have reported that hypoxia decreases development rate and impairs egg growth in many other organisms, including fishes (Davenport 1983; Malcolm et al. 2003) and invertebrates (Chaffee et al. 1984; Lutz et al. 1992; Strathmann et al. 1995).

1.4.2 Characterization of Greenland halibut eggs and lipid class dynamics

On day 7 following fertilization, total lipids in Greenland halibut eggs accounted for 16.4 ± 3.2% of the dry weight, with the highest (23.7 ± 7.0% of the dry weight) and lowest (13.4 ± 3.4% of the dry weight) levels in females 1 and 2, respectively. Greenland halibut eggs have high lipid content mainly made up of phospholipids, with a mean value of 77.6 ± 4.3% of total lipids. Fish eggs can be classified into different energetic categories according to their lipid characteristics (Mourente et al. 1996). The presence or lack of an oil globule corresponds to eggs with high (> 15% of egg dry weight) or low (< 15% of egg dry weight) lipid content. The first type is characterized by high amounts of neutral lipids, mainly TAG or wax esters, and low amounts of polar lipids; the second category is characterized by high amounts of polar lipids, particularly PL, and low amounts of neutral lipids (Finn et al.

1995; Mourente et al. 1996; Rainuzzo et al. 1997). Thus, Greenland halibut eggs can be classified, as a lipid-poor eggs species mainly constituted of PL without any oil globule. Species such as cod (*Gadus morhua*), saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangus merlangus*), Atlantic halibut (*Hippoglossus hippoglossus*), winter flounder (*Pseudopleuronectes americanus*), and plaice (*Pleuronectes platessa*) produce eggs with high proportions of PL (> 60% of total lipids) (Tocher et al. 1984; White et al. 1987; Falk-Petersen et al. 1989; Wiegand 1996). Conversely, the contribution of PL tends to be lower (< 40% of the total lipids) in more lipid-rich eggs (Finn et al. 1995), like those from red drum (*Sciaenops ocellatus*), Senegal sole (*Solea senegalensis*), Atlantic menhaden (*Brevoortia tyrannus*), gilthead seabream (*Sparus aurata*), and turbot (*Scophthalmus maximus*) (Rainuzzo et al. 1992; Vazquez et al. 1994; Finn et al. 1995) or from freshwater species, such as rainbow trout (*Oncorhynchus mykiss*), burbot (*Lota lota*), and salmon (Boulekbache 1981; Kaitaranta et al. 1981; Cowey et al. 1985). Although the lipid content and lipid class composition vary with species, fish eggs usually contain fairly high proportions of PL (Tveiten et al. 2004), between 50 and 90% of egg lipids in the small, pelagic eggs produced by many marine species (Fraser et al. 1988; Falk-Petersen et al. 1989; Finn et al. 1995; Wiegand 1996).

Substantial differences in lipids can exist, not only among the eggs of individual females in a population, but also among different batches spawned by the same female or even among individual eggs from the same spawned batch (McEvoy et al. 1993; Evans et al. 1996; Wiegand 1996; Rainuzzo et al. 1997). Our results showed significant differences

in total lipids and different lipid classes among eggs from the six females. Similar results have been obtained by Rainuzzo et al. (1997) in turbot broodstock. Although PL was the major lipid class in the six Greenland halibut egg batches, it showed small but significant differences between egg batches ($p < 0.05$). Minor lipid classes such as TAG, AMPL, and KET showed more considerable differences and could be related to egg viability. Thus eggs from female 3, which were characterized by the best hatching rates and the most successful ED, had two times more TAG (8.78% of total lipids) than the other egg batches (around 4%) at 7 days post-fertilization. TAG is the primary endogenous energy reserve fuelling basal metabolism. In agreement with our results, Bruce et al. (1993) revealed that Atlantic halibut egg batches, exhibiting grossly different viability characteristics, were very similar in their lipid composition; cholesterol was the only lipid component that showed a small but significant difference between egg categories.

Fish egg quality can be affected by several other factors (Rainuzzo et al. 1997). It has also been suggested that eggs having the best quality are those coming from batches with higher rates of fertilization and normal blastomeres at cleavage stage (Kjørsvik et al. 1990) and with high concentrations of neutral lipids such as TAG (Sewall et al. 2008). This is in agreement with our results, where we observed the best ED, viability, and hatching success for eggs from female 3, which had the best rates of fertilization and normal blastomeres (95% and 47% respectively) and the highest concentration of TAG.

Lipid metabolism during the early life of fish may differ greatly among species, mainly with regard to the amount and composition of lipids in the yolk, the time and level

of lipid classes used for either energy or tissue synthesis, and the environmental factors encountered, such as temperature and DO (Verreth et al. 1994; Mourente et al. 1996). It might be speculated that high phospholipid contents in Greenland halibut eggs could indicate its higher utilization as metabolic fuel. However, in the present study, the percentage of PL remained constant between days 7 and 21 post-fertilization and between different DO levels on both days 17 and 21. PL may be reorganized during embryogenesis and mobilized for subsequent biomembrane formation during larval ontogeny (Babu 1987; Falk-Petersen et al. 1989). In winter flounder eggs, lipids seemed to accumulate throughout embryogenesis but are heavily drawn upon from hatching to first feeding (Cetta et al. 1982). Alternatively, ED is characterized by a low consumption of yolk sac lipids and other endogenous stores, e.g., proteins and free amino acids (FAA) are probably used (Desvilettes et al. 1997). Recent findings have shown a pool of FAA in marine fish eggs that is available as an additional endogenous resource (Rainuzzo et al. 1997). Moreover, low DO levels did not stimulate the use of PL. However, on day 14 post-fertilization, the amount of TAG was higher at normoxia than at severe hypoxia (10%sat), which could be explained by a depletion in TAG. Similarly, total lipids of eggs incubated at 10%sat decreased by 65%, 24%, and 18%, respectively, for females 1, 3, and 5 from day 17 to day 21 post-fertilization. On day 17, eggs from female 5 seemed to have used KET under severe hypoxia (10%sat). It is known that when DO levels are high enough, some tissues preferentially use free fatty acids or glucose as the substrate for ATP generation; however, during cellular hypoxia, KET can be used for ATP production (Speers-Roesch et al. 2006; Treberg et al. 2006). Further work is needed to confirm such assumptions and to understand

the specific role of KET in fish eggs. Differences in the variation patterns in lipid classes and total lipids appear to be pronounced at severe hypoxia (10%sat), with high depletion of energy reserves.

In conclusion, impaired ED in severe hypoxia affected the viability of Greenland halibut eggs resulting in the absence of hatching at 10%sat. Moreover, female origin was a determinant factor in ED and hatching success. Indeed, we found that females with better condition factor (K) produced eggs of better quality, with high percentages of normal blastomeres and better fertilization success. The lowest concentration of DO currently observed in the bottom waters of St. Lawrence system is 20%sat (Gilbert et al. 2005); Greenland halibut juveniles and adults are abundant in these waters (DFO 2006) suggesting a high tolerance of this species to severe hypoxia (≥ 20%sat). 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. A valid concern: 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 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 a few decades.

DISCUSSION GÉNÉRALE

Le programme de recherche dont fait partie mon projet de maîtrise avait pour objectif général de déterminer l'impact de l'hypoxie sur la productivité des espèces de poissons et d'invertébrés d'intérêt commercial dans l'EGSL. À court terme, l'objectif était d'évaluer la distribution et la tolérance des espèces caractéristiques des eaux profondes de l'estuaire incluant le flétan du Groenland face à différents niveaux d'hypoxie.

À plus long terme, de pouvoir évaluer l'impact d'une diminution encore plus grande des niveaux d'OD sur la productivité des eaux profondes de l'EGSL.

Tel que démontré dans le chapitre I, les œufs du flétan du Groenland sont tolérants à l'hypoxie. On a défini un seuil létal qui se situe entre 10 et 20%sat. Ces valeurs critiques ne sont pas encore observées dans les eaux profondes de l'EGSL et suggère que les bas niveaux en OD actuels ne présentent pas un danger immédiat pour l'abondance de l'espèce. Cependant, ces bas niveaux en OD pourraient affecter de façon considérable le recrutement de l'espèce dans quelques décennies. En effet, les concentrations en OD dans les eaux profondes de l'estuaire du St-Laurent ont diminué de 37.7%sat en 1930 à une moyenne de 20.7%sat pour la période de 1984 à 2003 (Gilbert et al. 2005).

La compréhension de l'écologie et la biologie des espèces de poissons marins au cours des premiers stades de leur vie, en particulier les stades de développement embryonnaire et larvaire, est très importante car la survie des œufs et des larves joue un rôle important dans la détermination de l'abondance des classes d'âge et du recrutement (Houde 1989; Fargo 1994). Les modèles récents qui estiment le recrutement des poissons prennent en compte de plus en plus les facteurs environnementaux (salinité, température et OD) dans l'explication de la variation des stocks de poissons (Gilbert et al. 1997; Daskalov 1999). Le succès de recrutement chez la morue de la Baltique a été affecté par des fluctuations au niveau des conditions de salinité, de température et d'OD à des profondeurs où les œufs étaient déposés (Vallin et al. 1999). En plus de l'importance des facteurs abiotiques, les facteurs biotiques, tel que l'effet maternel sur la qualité des œufs, jouent un

rôle crucial dans la variation du recrutement (Cardinale et al. 2000). En effet des études récentes ont montré que l'effet maternel influence non seulement la taille et la composition biochimique des œufs mais aussi la survie des larves et des juvéniles (Vallin et al. 2000).

Les limites du projet

Une des sources de variabilités majeures rencontrée lors de nos expériences est le décalage de la période de frai pour les six femelles utilisées, échelonnant ainsi la période de fécondation artificielle sur un mois. Cela a empêché d'utiliser la laitance du seul mâle au même moment. En effet, il est possible que les œufs fécondés en dernier par la même laitance aient été de moins bonne qualité que les autres, non en raison de leur provenance mais plutôt en raison de la dégradation de la qualité de la laitance. Ceci n'a pas été validé par notre expérience. En effet, les œufs de la femelle 2, qui ont été fécondés au début de l'expérience étaient de moins bonne qualité et n'ont pas éclos.

Les concentrations théoriques en OD ont été comparables aux niveaux d'OD réels contrôlés par le système informatisé dans les différents bassins. Cependant des fluctuations instantanées ont eu lieu dans certains bassins. Une brève augmentation du niveau d'OD au niveau des œufs d'*Oncorhynchus mykiss* incubés à 5 $mg \cdot L^{-1}$ ~ 72.4%sat a entrainé une reprise du développement qui était très lent et même arrêté en hypoxie (Ciuhandu et al. 2005). Cependant, au niveau de l'EGSL, ce problème ne peut pas se présenter en raison de la stabilité des concentrations en OD qui ne peuvent pas connaître des fluctuations instantanées.

Bien qu'en laboratoire on ne peut simuler les conditions naturelles d'une façon fidèle en raison de la multitude de facteurs et d'interactions entre ces derniers, on peut isoler un effet individuel d'un facteur ce qui peut grandement aider à la compréhension de ce qui se passe dans le milieu naturel.

Perspectives

Des études futures seront indispensables afin de mieux comprendre l'adaptation du flétan du Groenland aux faibles niveaux d'OD pendant les premiers stades de sa vie. Bien qu'on sait, suite à cette étude, que les œufs du flétan du Groenland tolèrent de faibles niveaux en OD (20 et 35%sat) et réussissent à éclore, on méconnait encore la qualité et la survie des juvéniles à ces niveaux en OD. Par exemple, les larves du lompe (*Cyclopterus lumpus*) sont très sensibles au manque d'OD alors que les œufs peuvent survivre à de brèves conditions d'anoxie (Davenport 1983). Il serait donc intéressant d'évaluer la tolérance à l'hypoxie chez les juvéniles du flétan du Groenland puisque les larves sont pélagiques et ne devraient pas se trouver dans des zones hypoxiques.

En termes de réserves énergétiques, on a décrit la composition lipidique dans les œufs du flétan du Groenland, cependant on n'a pas déterminé les réserves en protéines, en glycogène et en acide aminés libres. Chez les œufs de certains poissons marins, comme par exemple chez les œufs de l'ombrine (*Sciaenops ocellata*), les lipides et le glycogène ont été utilisés concurremment plutôt que successivement lors du DE (Vetter et al. 1983). Needham (1931) a été le premier à suggérer une succession dans l'utilisation des réserves énergétiques chez les œufs de certaines espèces de poisson lors du DE en commençant par le glycogène, les lipides puis les protéines. Certaines études ont appuyé ce fait (Terner 1968; Finn et al. 1995; Rainuzzo et al. 1997). Chez les œufs de la morue (*Gadus morhua*), les acides aminés libres peuvent être utilisés comme unique source d'énergie pour le métabolisme énergétique aérobie durant l'embryogenèse (Fraser et al. 1988). L'évaluation des réserves en lipides par rapport aux autres sources d'énergie (glycogène, protéines et acides aminés libres) chez les œufs du flétan du Groenland pourrait mieux nous renseigner sur l'utilisation des réserves énergétiques durant le DE et suite à un stress environnemental.

La présente étude fournit une addition significative à nos connaissances sur l'adaptation des œufs de poissons marins aux différentes conditions environnementales. Elle contribue à la caractérisation de la zone profonde de l'EGSL et à la compréhension de l'adaptation du flétan du Groenland aux bas niveaux d'OD. Toutefois, la caractérisation seule des œufs reste insuffisante pour comprendre la tolérance de l'espèce à l'hypoxie. Des

travaux sont actuellement en cours sur la distribution des juvéniles et des adultes de différentes classes de taille dans l'EGSL afin de mieux comprendre l'impact de l'exposition à l'hypoxie de cette espèce aux différents stades de don développement.

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ANNEXES

Annex 1. Results of two-way ANOVAs, showing effects of dissolved oxygen (DO) level, female origin, and their interactions on hatch rate in Greenland halibut eggs.

Source of variation	df	MS	*F*	P
DO	4	0.029	27.75	<0.0001
Female origin	3	0.193	185.73	<0.0001
DO X Female origin	12	0.013	12.83	<0.0001
Residual	20	0.001		

Annex 2. Results of one-way ANOVA, showing effects of female origin on total lipid contents and proportions of ketones (KET), acetone-mobile polar lipids (AMPL), phospholipids (PL), and triacylglycerols (TAG) in Greenland halibut eggs from different females on day 7 of embryonic development for eggs incubated at 100%sat in dissolved oxygen (DO).

Source of variation	df	Total lipid contents			KET			AMPL			PL			TAG		
		MS	*F*	P	MS	*F*	P	MS	*F*	P	MS	*F*	P	MS	*F*	P
Female origin	5	6330.18	3.22	0.032	6.63	10.93	< 0.001	30.15	3.75	0.018	84.16	2.93	0.043	13.53	5.11	0.00
Residual	17	1966.14			0.60			8.03			28.67			2.64		

Annex 3. Results of two-way ANOVAs, showing effects of female origin, dissolved oxygen (DO) and their interactions on total lipid contents and proportions of ketones (KET), phospholipids (PL), and triacylglycerols (TAG) from Greenland halibut eggs from females exposed to different dissolved oxygen (DO) levels on days 14, 17, and 21.

Day	Source of variation	df	Total lipid contents			KET			PL			TAG		
			MS	*F*	P	MS	*F*	P	MS	*F*	P	MS	*F*	P
14	DO	3	0.57	2.71	0.090	0.05	0.45	0.710	0.005	0.32	0.81	0.00	4.43	0.020
	Female origin	2	1.97	9.32	0.003	1.92	15.71	0.0004	0.08	4.87	0.02	0.00	0.35	0.700
	DO X Female origin	6	0.06	0.31	0.910	0.03	0.31	0.910	0.01	0.99	0.47	0.00	1.41	0.280
	Residual	12	0.21			0.12			0.01			0.00		
17	DO	3	0.33	4.56	0.020	0.91	13.00	< 0.0001	0.05	6.23	0.009	0.00	3.10	0.060
	Female origin	2	1.25	17.17	0.000	0.90	12.86	0.001	0.07	9.41	0.003	0.00	0.47	0.630
	DO X Female origin	6	0.46	6.39	0.003	0.39	5.64	0.005	0.02	3.53	0.02	0.00	3.50	0.030
	Residual	12	0.07			0.07			0.00			0.00		
21	DO	3	0.62	5.20	0.018	0.06	1.24	0.340	0.003	0.29	0.83	0.001	7.98	0.004
	Female origin	2	0.85	7.13	0.010	0.15	3.11	0.080	0.036	3.37	0.07	0.001	15.36	0.001
	DO X Female origin	6	0.56	4.65	0.014	0.22	4.65	0.014	0.033	3.09	0.04	0.000	4.28	0.018
	Residual	11	0.12			0.04			0.011			0.000		