MATERNAL INFLUENCES ON EGG QUALITY AND LARVAL MORPHOLOGY, SURVIVAL AND GROWTH OF THE BATCH-SPAWNING ATLANTIC COD (GADUS MORHUA)

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By

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science

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St.John's, Newfoundland, Canada

January 2011

ABSTRACT

Recruitment variability in fish populations is considered to be associated with the number and quality of eggs extruded by the female segment of the population, the size of larvae at hatch, and prey type, size and quantity at the start of exogenous feeding. Although a great number of studies have been undertaken to examine these aspects, very little information exists on batch-specific lipid allocation (i.e. lipid classes and fatty acids) in eggs of spawners of wild origin. Moreover, the associations of maternal attributes (e.g., egg size, batch sequence) with early life history traits of larvae under unfed and fed conditions have received little attention though it is perceived to be an important suite of recruitment processes during a critical life period. The purpose of this thesis was to quantify maternal patterns of lipid allocation to egg production and to assess the influence of female attributes on larval morphology, survival, growth and condition in a batch spawning fish, the Atlantic cod (*Gadus morhua*). Eight pairs of Atlantic cod of wild origin (held in captivity four months prior to the onset of the experiment) were allowed to spawn "naturally" in outdoor holding tanks at the St. Andrews Biological Station (St. Andrews, New Brunswick). A total of forty three egg batches were collected from all females and used to address the following two objectives and related experiments.

My first objective was to determine the change in selected lipid classes and fatty acids of each egg batch over the spawning season for the eight females. Phospholipids were the predominant lipid class (40 -86%) within eggs, with polar lipids accounting for 47-87% of total lipids and neutral lipids 15-52% of total lipids. Polyunsaturated fatty acids (PUFAs) made up 16-50% of total fatty acids, where the lower values were representative of samples with lower docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content. It was shown that no common pattern emerged in the deposition of lipids over

the course of the spawning season; three females consistently showed declines in lipid deposition parameters (µg/egg) with both batch number and egg dry weight, while one female showed consistent increases. These disparate trends were interpreted to reflect differences among individual females in relation to environmental foraging history, age, condition and years of reproductive experience.

My second objective was to determine the effects of female and inter-batch differences on larval traits at 0 and 5 days post hatch (dph) and on larval performance under two feeding regimes. Inter-batch and female differences were evident; these differences were manifested in larval traits of unfed larvae at 0 and 5 dph and for larvae exposed to two feeding regimes. Egg size was positively correlated with larval size and other body measurements. Larval survival rates among two feeding regimes (1,500 and 4,000 rotifers/L; 2.7-fold difference) were highly variable ranging from 0 to 60% with mean survivorship near 15% in each treatment and did not differ significantly. Myotome condition index differed significantly between the two regimes and was greater for the better fed group. Both studies detected the presence of female and batch effects, indicating the important role female and batch number play in egg quality as well as larval morphology, growth and survival.

In summary, a number of new findings were made of the reproductive biology and early life history of Atlantic cod that are of relevance to our understanding of recruitment processes of this important demersal species of the Northwest Atlantic.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Edward Trippel for offering me the opportunity to be part of an interesting project and Dr. Ian Fleming for hopping aboard and co-supervising this ambitious thesis. It has been a whirlwind experience filled with many long days, weeks and months and times when I thought statistics and revisions would never end; but it was also filled with the excitement of the ah-ha moments when everything fell into place and the bigger picture became clearer. *Thank you* both for your invaluable input and guidance throughout this process, for allowing me the freedom to work under minimal supervision, as it showed you believed in my ability as a student but more importantly as a researcher.

Special thanks to Pierre Pepin for being part of my supervisory committee and for your valuable comments, suggestions and guidance especially through some of the initial statistical analysis and final thesis revisions.

This research opportunity would not have been possible without grants from Fisheries and Oceans Canada ACRDP Program (Ed Trippel) and a NSERC Discovery grant (Ian Fleming). This opportunity was also made possible in part by a fellowship from the School of Graduate Studies, Memorial University of Newfoundland.

Special thanks to the staff at St. Andrew's Biological station especially Steven Neil, John Reid, Ken Howes and Marc Blanchard for showing me the ropes to get this project off the ground and helping me find my footing around the station. Thanks Susan Fordham for some late night and weekend feeding and Nicola Cross for assisting with the final larval weights.

A thousand thanks to the 'Flemwads'; thank you for your support, input and lab comradeship. Special thanks to Corinne Conway, who was always available to lend a hand when help was needed and to Danny Ings for his time and input for numerous hours spent on statistical analysis. Special thanks to the Parrish lab especially Jeanette Wells, Marsha Clarke and Chris Parrish for providing their time and expertise to help me understand the lipid world a little better. To the Ocean Sciences Centre staff and students, thank you for making the OSC a wonderful place to be by providing opportunities to balance work and play.

Thank you dear friends and family for believing in me and being there for me in your own special way. To my family, *thank you* for allowing me the opportunity to follow my dreams by supporting and believing in me during this journey...at last, it is finally complete ☺ ! These last few years would not have been possible without the wonderful friendships I made and the experiences I had...*a big thank you* to my dear friends both near and afar that always had an ear to lend, a shoulder to lean on and who always provided positive encouraging words. Thanks for the memories and good times in the many forms they took, without them, it would have been a lonely journey. *Love you all.*

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List of Abbreviations and Symbols

PL	Phospholipids
TAG	Triacylglycerols
DHA (22:6ω3)	Docosahexaenoic acid
EPA (20:5ω3)	Eicosapentaenoic acid
AA (20:4ω-6)	Arachondic acid
FFA	Free fatty acids
PUFA	Polyunsaturated fatty acids
MUFA	Monounsaturated Fatty Acid
Saturated	Saturated fatty acids
Other LC	Other lipid classes
Other PUFA	Other polyunsaturated fatty acids
Σ	Sum of the fatty acids which form PUFAs', MUFAs' and saturated fatty acids respectively
MS 222	Tricaine methane sulphonate
°C	Temperature measured in degrees Celcius
mg	milligrams (measure of dry weight)
μg	micrograms (measurement unit)
mm	millimetres (measurement unit of length)
EDW	Egg dry weight
PSS	Phase in the spawning season
Fert	Fertilization rates
PCA	Principal component analysis (statistical analysis)
PC	Principal component
H&B FDR	Benjamini and Hochberg False Discovery Rate

SD	Standard deviation
SE	Standard error
DPH	Days post hatch
DPF	Days post fertilization
LDW	Larval dry weight
SL	Standard length (measured in mm)
MH	Myotome height (measured in mm)
ED	Eye diameter (measured in mm)
JL	Jaw length (measured in mm)
YSA	Yolk sac area
SGR	Specific growth rate (measured as %/day in mm)
CF	Condition Factor
K	Fulton`s condition index
MCI	Myotome-based condition index

All other symbols and abbreviations such as those used in graphs and or equations are explained in the text as they occur.

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Co-Authorship Statement

The research described in this thesis was carried out by Michelle Bachan, with guidance from Edward Trippel, Ian Fleming and Pierre Pepin. Michelle Bachan was responsible for data collection and analysis. Manuscripts resulting from this thesis were prepared by Michelle Bachan, with editing assistance and intellectual input from co-authors as follows:

Authorship for publication arising from *Chapter 2* will be Michelle Bachan, Ian Fleming and Edward Trippel.

Authorship for publications arising from *Chapter 3* will be Michelle Bachan, Ian Fleming and Edward Trippel.

Chapter 1: General Introduction

1.1 Reproductive Strategies

Marine fishes display a vast array of reproductive strategies and tactics which have presumably evolved to maximize lifetime reproductive success (i.e. the ability to produce viable eggs and larvae) in relation to available resources and environmental conditions (Murua & Saborido-Rey, 2003). Most marine species (e.g. cod, mackerel and herring) are iterparous (i.e. spawning more than once during their lifetime), they have separate sexes that do not display sexual dimorphism and fertilization is external with no parental care (Murua & Saborido-Rey, 2003). However, there are exceptions; some species (e.g. capelin, eels and Pacific salmon) are semelparous (i.e. they only spawn once in their life), some species display sexual dimorphism (e.g. salmonids, wrasses, parrotfish) and others are viviparous i.e. fertilization is internal and the embryo develops inside the ovary (e.g. Atlantic redfishes, Pacific rockfishes and some elasmobranchs) (Murua & Saborido-Rey, 2003). Differences in reproductive strategies are often expressed as differences in fecundity among species (Murua et al., 2003), where fecundity is the number of eggs produced by a female.

When considering the reproductive strategy of a species, other factors such as oocyte development, spawning pattern and fecundity must also be taken into account. Oocyte development can be synchronous (all oocytes are developed and ovulated at the same time), asynchronous (oocytes develop at different rates) or group synchronous (a synchronous group of larger oocytes, also known as a clutch, which is spawned during the current breeding event and a heterogenous group of smaller oocytes, which is spawned during future breeding events) (Murua & Saborido-Rey, 2003). The latter is most common in temperate demersal species such as Atlantic cod, haddock and flounder, that have a relatively short spawning season and whose yolk accumulation is dependent on the body reserves of the female (Murua & Saborido-Rey, 2003). Batch spawning occurs in species displaying either asynchronous or group synchronous oocyte development.

Spawning pattern is based on the rhythm that eggs are ovulated. All eggs can be released at the same time (total spawners) or eggs can be released in discrete batches over a protracted spawning period (batch spawners). Batch spawning can be seen as a strategy that may maximize egg production and survival in a stochastic, unpredictable environment, whereby there is an increased chance that at least some eggs will survive when released over a longer period of time (Murua & Saborido-Rey, 2003).

While oocyte development pattern and spawning strategy may place constraints on certain aspects of the reproductive potential for a species, many factors can result in a high degree of reproductive variability within a species. For example, individual reproductive potential can be influenced by female condition, reproductive experience and maternal size which can affect fecundity, egg size and the size of larvae at time of hatch (Kamler, 1992; Kjesbu et al., 1996; Heath & Blouw, 1998; Trippel, 1998; Einum & Fleming, 1999; Murua et al., 2003;). Typically, larger fish produce larger eggs from which larger larvae emerge (Reznick et al., 1996; Murua et al., 2003). Additionally, it has been shown that females in better condition and those who have had more spawning experience are typically more fecund (Kjesbu et al., 1991) and also produce larger eggs (Trippel & Neil, 2004).

1.2 Early Life History

Survival rates during the early life history are often extremely low and variable and this can have immense consequences for recruitment (Kamler, 2005). The early life history stages of fishes are often described as being the most crucial developmental phases as eggs and larvae may undergo massive mortality due to unfavourable environmental conditions (both physical [e.g. temperature, salinity, currents] and biotic [e.g. suitability of prey abundance, type and size]), competition, predation and/or starvation (Kamler, 1992; Green & McCormick, 2005; Govoni, 2005; Green, 2008).

The biological and physical attributes of the environment into which eggs are deposited can influence larval size at hatch, developmental rates, larval condition and larval survival (Pepin, 1991; Blaxter, 1992; Jordaan et al., 2006; reviewed in Rose, 2007). As yolk sac reserves become exhausted at the start of exogenous feeding, factors such as water temperature and prey availability are crucial to subsequent progeny survival (Kamler, 1992; Green & McCormick, 2005).

1.2.1 Recruitment Hypotheses

Several hypotheses have been put forth to explain recruitment variability in fish stocks. The most popular include Hjort's (1914) 'critical period' hypothesis, Cushing's (1972) 'match-mismatch' and Miller et al.'s (1988) 'bigger is better'. Hjort's (1914) critical period is centered on the concept that larvae must find suitable prey (in terms of abundance and type) immediately following the switch from endogenous feeding (i.e. the absorption of its yolk) to exogenous feeding (i.e. obtaining nutrients from its environment). In the absence of suitable feeding conditions, larvae experience mass mortality over a short period of time due to starvation. Hjort was one of the first scientists to highlight the effect that larval nutrition and starvation had on larval survival and his hypothesis laid the foundation for other hypotheses, but its major fault was that it was narrowly focused on first feeding larvae and failed to accommodate other variables (Houde, 2008).

The match-mismatch hypothesis (Cushing, 1972, 1990) is considered to be an extension of Hjort's critical period hypothesis (Houde, 2008); it takes into account larval fish nutrition and prey production within an overlapping time period. The theory behind this concept is that the timing of fish spawning and larval production either matches the bloom usually associated with spring zooplankton production (match) or does not (mismatch), affecting mortality due to starvation. Temperature is an important environmental variable because the spring bloom is often associated with a rise in water temperature.

The 'bigger is better' hypothesis implies larval size is a key fitness component during early life history (Miller et al., 1988). Larger larvae often hatch from larger eggs and they have a survival advantage over their smaller counterparts because they can feed endogenously for longer (due to a larger yolk sac) before the need for exogenous feeding, especially during times of low prey availability (Blaxter & Hempel, 1963; Miller et al., 1988; Rideout et al., 2005). They are also better able to avoid predation and find and capture prey (Blaxter & Hempel, 1963; Reznick et al., 1996, Brooks et al., 1997).

1.3 Maternal Effects

In addition to the environmental processes in the wild, early life history is also shaped by the parent-egg-progeny relationship (Trippel et al., 1997). Processes such as embryonic development, yolk sac utilization and survivorship are shaped by contributions made by both parents i.e. both maternal and paternal effects (Kamler, 2005; Trippel et al., 2005). Although both parents can account for non-genetic sources of variation in the offspring, it is the female who is responsible for nourishing the egg during its early development (the egg contains large amounts of extra-nuclear material in the form of yolk plus the nuclear (i.e. genetic) contribution, whereas the male sperm provides little if any extra-nuclear material) to the progeny and she may have a greater impact on offspring variation (Green, 2008). Accordingly, over the last decade or so, more focus has been placed on maternal effects to explain some of the variability observed in offspring survival as it has been acknowledged that the reproductive population is made up of females of varying sizes and ages and that these individuals can contribute differently to spawning and recruitment (reviewed in Green, 2008).

Generally, it was accepted that an individual's genotype and environment gave rise to its phenotype; however, it is now well documented that an individual's phenotype can be influenced by its mothers' phenotype (not genotype), a phenomenon termed maternal effects (Bernado, 1996; Chambers & Leggett, 1996; Reznick et al., 1996; Marteinsdottir & Steinarsson, 1998; Higashitani et al., 2007). Maternal effects occur when the phenotype of the offspring is influenced during development by the phenotype or environment of its mother (Bernardo, 1996; Heath & Blouw, 1998; Mousseau & Fox, 1998; Wade, 1998) which can affect offspring survival and success (Mousseau & Fox, 1998; Einum & Fleming, 1999). Studies on maternal effects in fishes usually investigate the impact of female age, size (Kjesbu et al., 1996) and condition on egg and offspring size, condition and viability (Chambers & Waiwood, 1996; Trippel & Neil, 2004; Rideout et al., 2005; Higashitani et al., 2007). Such maternal effects may also be manifested in the chemical composition of the eggs, which in turn can determine the size and quality of larvae at hatch (Morley et al., 1999).

Studies have indicated that maternal effects are important within an evolutionary and ecological context, as variation in offspring fitness can shape natural selection

(Chambers & Leggett, 1996; Heath & Blouw, 1998; Mousseau & Fox, 1998). However, despite the importance of maternal effects in fishes, research in this field is still controversial as some studies have found direct links to indicate the presence of maternal effects (e.g. Chambers & Leggett, 1996; Marteinsdottir & Steinarsson, 1998; Einum & Fleming, 2000; Kamler, 2005; Green & McCormick, 2005), while others have not been able substantiate these relationships and their adaptive benefits (e.g. Kamler, 1992; Chambers, 1997; Heath & Blouw, 1998).

1.3.1 Egg Quality

The term "egg quality" is inherent in the ability of a female to produce viable offspring (Kamler, 1992; Nissling et al., 1998) and is often reflected in egg size and composition (Czesny et al., 2005). Differences in egg quality can be manifested as differences in embryonic development, hatching success and larval survival rates (Kjørsvik et al., 1990; Pickova et al., 1997; Mazorra et al., 2003; Czesny et al., 2005). In teleost fishes, lipids are commonly found in the egg yolk or oil goblets, which are energy sources for the developing embryos (Kamler, 1992; Wiegand, 1996). It has been postulated that larger eggs produce larvae of better quality (due in part to their larger yolk sac and longer time to hatch) than their smaller counterparts, thereby providing larvae with a survival advantage as discussed earlier.

It is also argued that there is a direct link between maternal nutrition and egg quality that can influence larval development until the start of exogenous feeding (Fraser et al., 1988; Mazorra et al., 2003; Wiegand et al., 2004; Salze et al., 2005). During ovarian development, both dietary and maternal reserves are collected and transported to the oocytes. These reserves provide the embryo and the yolk sac larva with its energy and nutritional requirements until the start of exogenous feeding (Ohkubo & Matsubara, 2002; Mazorra et al., 2003; Zhu et al., 2003). As such, maternal diet can influence egg viability, fertilization success, larval quality and survivorship (Tveiten et al., 2004; Salze et al., 2005).

Lipids and their associated fatty acids perform a number of biological functions, including the formation of structural components in cell membranes, precursors for chemical messages and substrates for catabolism (Wiegand, 1996; Desvilettes et al., 1997; Pickova et al., 1997; Tveiten et al., 2004). Until the start of exogenous feeding, lipids from the yolk sac fulfill these roles, at which time lipids from environmental sources take over (Tveiten et al., 2004). Additionally, lipids and their associated fatty acids are considered one of the most important sources of stored energy in fish eggs, especially in the form of triacylgycerol ([TAG]; Pickova et al., 1997). Measurements of the essential fatty acids (EFA), docosahexanoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA) and their ratios can also be used as indicators of egg quality (Penney et al., 2006). Inadequate quantities of DHA can result in impaired larval behaviour, while insufficient EPA and AA can influence structural phospholipids (Wiegand et al., 2004). Phospholipids are an important component of cell membranes but they can also be used as an energy source during embryonic development in species with small amounts of TAG (Pickova et al., 1997).

1.4 Cod Biology

Atlantic cod (*Gadus morhua*) is a temperate species with a wide geographic range; it can be found in the Baltic Sea in the east, to Cape Cod in the west and from Cape Cod in the south to above the Arctic Circle in the north (Brown et al., 2003; Geffen et al., 2006).

Across this range, cod are subdivided into different stocks and these stocks display differences in growth and reproductive characteristics (Brander, 1994; van der Meeren, 1994; reviewed in Brown et al., 2003). Additionally, it has been shown that under culture conditions factors such as light and temperature influence larval growth and development (Jordaan & Kling, 2003; Pepin et al., 1997; Puvanendran & Brown, 2002).

Cod is a batch spawner with spawning typically occurring during the winter months (January to May) but it may extend into the summer months (as late as July and August) depending on geographical region (Kjesbu, 1989; Chambers & Waiwood, 1996; Knickle & Rose, 2010). Females are highly fecund, with some females producing upwards of 15-20 egg batches (Kjesbu, 1989) and the spawning season can last on average between 30-90 days per individual female (Kjesbu, 1989; Chambers & Waiwood, 1996; Trippel, 1998). In unexploited stocks, females can have reproductive spans greater than 20 years (Chambers & Waiwood, 1996; Hall et al., 2004). Males and females of similar size tend to breed and adults reach maturity between 3-7 years of age (Rose, 2007). Differences in reproductive output have been reported between captive and wild fishes (Kjesbu, 1989). Differences in spawning patterns have also been noted between first-time and repeat spawners (i.e. fishes who have spawned at least once in their lifetime; Trippel, 1998). Reproductive potential is heavily influenced by age and experience, with older more experienced females being more fecund (Rose, 2007).

Female batch spawning activity can be categorized as 'regular' spawners, which refers to females who produce egg batches between regular intervals (i.e. every 'x' number of days) or as 'irregular' spawners whose spawning pattern is hard to predict as egg batches are not produced in a consistent temporal pattern. Highly fecund cod females can produce egg batches that contain upwards of 400,000+ eggs (Kjesbu, 1989), which are faint yellow in colour and whose size can range from 1.13mm to 1.89mm (Kjesbu, 1989; Evseenko et al., 1996). Egg size generally decreases with batch number (or follows a dome shaped pattern) with a correlation between egg dry weight and egg diameter (Chambers & Waiwood, 1996; Kjesbu, 1989; Kjesbu et al., 1996; Knutsen & Tilseth, 1985; Trippel, 1998).

1.5 Importance of Research

Not all eggs are created equal; eggs from different species and even within species vary in size, quality and quantity. Some species like salmon, spawn once per season and/or lifetime, while other species like cod, spawn multiple times throughout a single season as well as in multiple years. The reproductive mode of cod (i.e. a batch spawner) makes it an ideal study species, as progeny from each batch can be followed over a specified period of time and by maintaining the same sire for a female the genetic influences on seasonal variation in offspring traits is controlled.

Commercially important species are often considered for culture, particularly when their stocks have been overfished, show slow signs of recovery and supply cannot meet the demands of the market (Brown et al., 2003). In Atlantic Canada, cod has a deep rooted history in the groundfish fishery, but with stock declines both regionally and worldwide, alternative ways to meet increasing consumer demand must now be considered (Brown et al., 2003; Rosenlund & Skretting, 2006). Cod culture is one proposed solution, with research and production currently underway in Newfoundland and New Brunswick (Canada), as well as in Norway, Scotland, Iceland and the USA (Rosenlund & Skretting, 2006). However, for both these sectors (wild fisheries and aquaculture) to be successful a thorough understanding of the biology of cod is needed, especially the early life history, which is often associated with mass mortality (Kjesbu, 1989; Kamler, 1992; Chambers & Waiwood, 1996; Brown et al., 2003).

The importance of this study can be considered in the following ways:

1. *Theoretically*: Fishes display a wide range of reproductive strategies and tactics. Some species opt to produce a relatively small number of eggs with each egg having a relatively high nutritional content, while other species opt to produce many batches of eggs with each egg having a lower nutritional content. Why have cod opted to produce many batches of eggs often with a decline in egg size? How does egg quality vary between females? Does each successive egg batch actually have a lower nutritional content than the preceding batch and are these variations associated with egg size?
2. *Aquaculture*: Aquaculture is one proposed solution to meet the increasing demand worldwide for seafood as current wild stocks are not sufficient to sustain this increasing demand (Rosenlund & Skretting, 2006). The first step of aquaculture is to understand the reproductive biology and early life history of the desired species. For batch spawners, like cod, it also means determining the influence batch effects can have on fecundity, egg size, eqq quality and the subsequent progeny. Consequently, aquaculturists can better select females who would produce high quality eggs, which in turn would produce larvae with better growth and survivorship. Additionally, batch effect studies can help aquaculturists determine if selection should be more egg size specific. Knowledge gained on the effects lipid content and fatty acid composition on egg quality could also aid in the development of advanced broodstock diets to improve gamete quality of cultivated fish.

3. *Wild Fisheries*: Do batch differences influence egg quality and the resulting larvae? How are growth, condition and or survivorship affected by seasonal fluctuations in prey availability? In the wild, during periods of favourable conditions, larvae generally survive better than during adverse periods. The 'bigger is better' hypothesis postulates larger eggs give rise to larger larvae and these individuals have a survival advantage over their smaller counterparts during times of unfavourable conditions and predation (Miller et al., 1988; Meekan & Fortier, 1996). A better understanding of egg quality and how and why larval progeny perform the way they do under different prey densities may help us to better predict larval survival in batch spawners.

Most importantly, this research is necessary as there is a lack of information on the maternal effects of batch spawners, especially how egg quality may be affected by batch number and how progeny performance in turn may be affected by egg quality and food availability. The majority of the established literature is based on pooled data and little is known about batches within an individual female. A better understanding of larval morphology and growth from different batches is required. Additionally, it is not known if there are differences in survival with respect to batch number and food supply. This research is unique in that it tries to fill these missing links by determining the change in egg quality with batch number and egg size and by following the progeny from individual females from 0 days post hatch (dph) to 5dph to determine the magnitude of morphological changes that occur and again from 0 dph to 15 dph to determine the impact food supply can have on growth and survival.

1.6 STUDY OBJECTIVES

The aim of this thesis is to evaluate the magnitude of maternal effects in Atlantic cod, using a paired mating system, in which eight male-female pairs were allowed to spawn without human interference throughout the season. This approach attempts to control for paternal effects when examining batch differences and hence, for the purpose of this study, the only male contribution acknowledged is in the form of DNA from the spermatozoa. This study had two main objectives: the first was to determine the egg lipid composition (selected lipid classes and fatty acid composition) of the various egg batches from each of the individual females. The second objective was to follow the larvae from the discrete egg batches of the individual females through an experimental series (i.e. from egg production to hatch (at which point 0, 5 and 15 dph morphometrics was obtained) and thru a feeding experiment

Chapter 2 assesses the seasonal change in lipid allocation by determining the effect that batch number and egg size have on fatty acid composition and lipid classes and examines inter-female differences in these patterns. Chapter 3 evaluates seasonal changes in egg size over the spawning period, the change in larval morphology between 0 dph and 5 dph and it establishes how progeny performance can vary under different prey densities and evaluates the effect of batch on growth, condition and survival.

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Chapter 2: Maternal allocation of lipid classes and fatty acids with seasonal egg production in Atlantic cod (*Gadus morhua*) of wild origin

ABSTRACT

Temperate batch spawning fishes, such as Atlantic cod (*Gadus morhua*), show seasonal variation in maternal allocation to egg production. However, the influence of seasonal variation on within and among female variation in egg size and composition, which may have significant effects on embryo survival and early development, remains poorly understood. To quantify maternal patterns of lipid and fatty acid allocation to egg production, eight breeding pairs of Atlantic cod were followed over a reproductive season. Forty-three eggs batches were collected, with batch fecundity ranging from 33,050 - 519,300 eggs and egg size from 1.34 - 1.73 mm (0.062 – 0.116 mg dry weight). Both parameters tended to decline with each successive batch of a female. Fertilization success between batches within and among females varied from 0-99%, but did not appear to vary with batch fecundity, egg size or composition.

Phospholipids were the predominant lipid class (40 -86%) within eggs, with polar lipids accounting for 47-87% of total lipids and neutral lipids 15-52% of total lipids. PUFAs made up 16-50% of total fatty acids, where the lower values were representative of samples with lower DHA and EPA content. Few trends emerged (after correcting for multiple testing) in the deposition of lipids over the course of the spawning season; three females consistently showed declines in lipid deposition parameters (µg/egg) with both batch number and egg dry weight, while one female showed consistent increases. Additional analysis of the principal component analysis residuals revealed the presence of both maternal effects and batch effects. Variability among females may be a result of multiple factors, including dietary history prior to collection from the wild and factors associated with maternal effects such as age, condition and spawning history.

Key words: Maternal effects, batch effects, egg batch, cod eggs, egg size, fecundity

2.1 Introduction

Although egg composition in marine fishes has been the subject of many studies (Tocher & Sargent, 1984; Almansa et al., 1999; Mazorra et al., 2003; Penney et al., 2006), the majority have pooled egg samples and disregarded variation attributed to egg batch and female effects. Moreover, the plethora of information on egg lipid composition (lipid classes and fatty acids) of batch spawning marine fish is derived mainly from aquaculture studies, which tend to investigate egg quality in relation to broodstock diet (Rainuzzo et al., 1997; Mazorra et al., 2003; Bruce et al., 1993; Penney et al., 2006; Bransden et al., 2007), virgin and repeat spawners (Daniel et al. 1993; Evans et al. 1996), wild and cultured eggs (Salze et al., 2005) and changes in lipid composition during early development (Fraser et al., 1988; Zhu et al., 2003; Tveiten et al., 2004). Relatively few studies have investigated batch effects on lipid composition (Ulvund & Grahl-Nielsen 1988; Pickova et al., 1997) and fewer still have characterized egg lipid profiles of individual females (Ulvund & Grahl-Nielsen, 1988). Temperate batch spawners such as Atlantic cod, Atlantic herring (*Clupea harengus*) and haddock (*Melanogrammus aeglefinus*) display a seasonal decline in egg diameter (Bagenal, 1971; Kjesbu, 1989; Rideout et al., 2005). Moreover, it is speculated that the mother's phenotype (e.g. age, size, condition, etc.) can influence the amount and composition of yolk deposited in each egg, which can have an impact on early life history success. Such maternal effects reflects the influence a mother can have on the phenotype of her offspring that is unrelated to the offspring's own genotype (Bernado, 1996; Reznick et al., 1996; Mousseau & Fox, 1998; Green, 2008).

Egg yolk is the only source of nutrients for the developing embryo prior to the start of exogenous feeding and is reflective of the maternal contribution (Sargent, 1995; Wiegand, 1996). Lipids are the preferred source of metabolic energy for developing embryos, they aid in the formation of cell membranes and usually account for <5% of the egg's wet weight (Rainuzzo, 1993; Sargent, 1995; Evans et al., 1996; Sargent et al., 2002). The biochemical composition of the egg influences available energy reserves for growth (phospholipids (PL) and triacylglycerols (TAG)), egg quality (docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA) and their ratios) and hatching success (individual polyunsaturated fatty acids (PUFA) and their ratios) (reviewed in Sargent et al., 2002; Penney et al., 2006). Egg lipid composition is highly variable among marine species (Sargent et al., 2002).

Triacylglycerol and phospholipids are important energy sources for the developing embryo and larva (Sargent, 1995; Rainuzzo et al., 1992; Evans et al., 1996; Pickova et al., 1997). However, the relative use of these two sources of metabolic energy appears to be somewhat species-dependant (Anderson et al., 1990). Marine fish species with relatively short incubation periods (≤ 20 days; e.g. Atlantic cod and haddock) tend to have higher quantities of polar lipids (predominantly, phospholipids which make up ~ 62-72% of total lipids), while those with longer incubation periods (> 20 days; e.g. capelin (*Mallotus villosus*)) have higher quantities of neutral lipids which are primarily comprised of TAG (~ 77% of total lipids; Tocher et al., 1985).

Eggs with high quantities of phospholipids (e.g. Atlantic cod) also tend to have high PUFA levels, which comprise 40-50% of the total fatty acids (Tocher & Sargent, 1984). PUFAs provide the developing embryo and larva with nutrients until the yolk is absorbed (Tocher & Sargent, 1984). DHA (22:6ω3) and EPA (20:5ω3) are also of interest because of their role in egg and larval development (Mazorra et al., 2003). Similarly, AA (20:4ω-6) has received considerable attention because of its role in eicosanoid production, which is involved with oogenesis and embryogenesis (Mazorra et al., 2003).

Despite the importance of lipids as an energy source for the developing embryo and their contribution to cell formation, only limited attention has been given to their role in egg quality in marine batch spawners (Pickova et al., 1997). This is surprising especially when considering the large proportion of marine fishes that exhibit this reproductive strategy (Murua & Saborido-Rey, 2003).

Here, we quantify the magnitude of seasonal change in lipid composition (lipid classes and fatty acids) of Atlantic cod eggs and hypothesize that as egg size declines over the spawning period egg composition will change correspondingly. Specifically, we test for: (1) within-female changes in the allocation of egg lipid composition among egg batches; (2) relations between egg size and the composition of lipid classes and fatty acids; and (3) changes in egg composition with batch fecundity (i.e., are large egg batches composed of better eggs?). Unlike previous studies, we investigated both female and batch effects on the egg quality of spawners and undertook this study using recently captured wild fish (i.e., 4 months prior to onset of spawning).

2.2 Materials & Methods

2.2.1 Adult Cod Rearing And Egg Collection

Atlantic cod were captured off the coast of southwestern Nova Scotia during August 2005 and transferred to communal holding tanks at the St. Andrews Biological Station, New Brunswick, Canada (45° 4' 56" N, 67° 5' 5" W). Fish were fed three times weekly on a diet consisting of squid (*Ilex illecebrosus*), Atlantic herring (*Clupea harengus*) and mackerel (*Scomber scombrus*), which was discontinued 2 weeks prior to the onset of spawning (Fordham & Trippel, 1999). Pre-spawning, gravid females were identified as individuals with distended abdomens, while males were identified as individuals releasing milt when light pressure was applied to the abdomen.

Eight reproductive females and males were anaesthetized with tricaine methane sulphonate (MS 222), sex assessed using ultrasound (described in Martin-Robichaud et al., 1998), measured for total length (to the nearest 0.1 cm), weighed (to the nearest 0.01 kg) and tagged with a passive integrated transponder (PIT) tag. Fish were presumed to be repeat spawners based on their body size (Trippel, 1998). Attempts were made to pair females and males of relatively similar size (Table 2.1) and each pair was placed in a 3 m^3 tank outfitted with an in-tank egg collector and drain collector (described in Thorsen et al., 2003). Tanks were supplied with a mixture of ambient and heated seawater (water temperature maintained at 4 $\pm$ 1°C) throughout the spawning period.

In-tank egg collectors were positioned such that the inflow of seawater produced a gentle circular current that forced buoyant eggs into the egg collector, while non-buoyant eggs were collected in the drain collector. Collectors were checked twice daily. A spawning event was classified as the interval during which an egg batch was collected (usually a day), followed by a 48 hour period (or more) during which no eggs were produced (Chambers & Waiwood, 1996). Each collected egg batch was transferred into a 3L beaker with chilled seawater (~4°C) and total volume of eggs spawned determined using volumetric displacement. Eggs were stirred and haphazardly chosen samples were used for lipid analysis, and the estimation of fertilization rates, egg diameter and dry weight. Batch fecundity was determined as a function of volume of eggs spawned and mean egg diameter (Thorsen et al. 2003).

Samples for lipid analysis were collected in triplicate for each batch and female (50 eggs per sample). Eggs were rinsed with chilled UV filtered seawater, gently blotted dry with Kimwipes© and placed in a lipid cleaned 10mL vial with approximately 2mL chloroform. Vials were filled with nitrogen gas, capped, sealed with Teflon tape and stored at -20°C until extraction (ca. 1 year).

A haphazard sample of 75-100 eggs per batch was viewed under a stereomicroscope (40X) to determine fertilization success. Mean egg diameter for each batch was determined using image analysis software (*Image-Pro Plus*®) from images captured by a digital camera (*Micropublisher 3.3 RTV Q Imaging*) attached to a stereomicroscope (*Olympus SZH*) prior to egg fixation in 4% formalin. Mean egg dry weight (mg) was determined using samples fixed in formalin for ca. 5 months. Prior to drying, eggs were soaked in weak acetic acid according to Trippel (1998) to remove traces of formalin. Eggs were dried in triplicate (20 eggs/sample/batch/female) at 60°C for 48 hours and cooled in a dissector for an hour before the sample was weighed (± 0.001 mg). Individual egg dry weight was determined by dividing total sample weight by the number of eggs per replicate and the average of these was used to determine mean egg dry weight per batch.

2.2.2 Lipid Extraction

Lipids were extracted using a modified Folch method (Folch et al., 1957) developed by Parrish et al., (1999). Eggs were homogenized using a lipid cleaned glass rod homogenizer in a 2:1 mixture of ice cold chloroform-methanol. Chloroform extracted water was added to bring the ratio of chloroform:methanol:water to 8:4:3. Samples were sonicated in an ice bath for 4 minutes and centrifuged at 5000 rpm for two minutes. The bottom organic layer was removed using the double pipetting technique without disturbing the top aqueous layer. Upon removal of the initial organic layer, chloroform was added again to the extraction tube and the procedure repeated two more times. All organic layers were pooled in a lipid cleaned vial and concentrated down under a stream of nitrogen gas. The concentrated extract was transferred to a lipid cleaned 2mL vial, topped with nitrogen gas, capped and sealed with Teflon tape until it was needed for lipid classes and fatty acid analyses.

2.2.3 IATROSCAN – LIPID CLASSES

Lipid classes were determined using thin layer chromatography (TLC) on a Mark V TLC-FID Iatroscan analyzer using silica coated Chromarods-SIII and a three-step development method according to Parrish (1999). The Chromarods were calibrated using standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Mo., USA) and lipid classes peaks were compared with a 9-component lipid standard. The chromatograms obtained from the three scans were joined together using *T Data Scan 3.0* (RSS Inc. Bennis. Tenn., USA). The absolute amount (μg/egg) and percent of each lipid class was determined. Total lipid was the summation of all lipid classes. Most means represent the average of three samples. However, in some instances the mean represents the mean of two samples (a sample lost or a value out of range). A Q-test was used to determine if one sample was significantly different from the others using the following equation:

$$Q = \frac{Gap}{Range}$$

Where, *Gap* is the absolute difference between the outlier in question and the closest number to it. At a 90% confidence level, the questionable sample was rejected when Q > 0.94 with a sample size of 3.

2.2.4 GAS CHROMATOGRAPHY – FATTY ACID COMPOSITION

Fatty acid methyl ester (FAME) derivatives were obtained by transesterifying the lipid extracted samples using 14% BF_3/MeOH for 1.5 hours at 85°C. The derivatives were analysed using a *HP 6890 Gas Chromatograph (GC) Flame Ionization Detector (FID)* equipped with a 7683 autosampler and a GC column made of ZB wax+ (Phenomenex, U.S.A.) with hydrogen as the carrier gas. Chromatograms were integrated and analyzed using *Galaxie Chromatography Data System*, version 1.9.3.2 (Varian Inc.) and individual fatty acid peaks were identified using retention times from standards purchased from Supelco (product numbers 47885-U, 47080-U, 47033, 47085-U).

2.2.5 DATA ANALYSIS

Principal component analysis (PCA) was used to extract relevant information from the complex data sets, by reducing them into a smaller number of variables that are representative of the original data set (McCure & Grace, 2002). PCA was performed using the correlation matrix (SPSS©16, SPSS Inc.) to help identify the patterns associated with egg size, batch fecundity, phase in spawning season (PSS), fertilization success , lipid classes (phospholipids, sterols, TAG, free fatty acids [FFA], other lipid classes [hydrocarbons, steryl esters/wax esters, ethyl esters, methyl esters, ethyl ketones, methyl ketones, glycerol ethers, alcohols, diacylglycerols and acetone mobile polar lipids]) and selected fatty acids (AA, EPA, DHA, saturated fatty acids, MUFA, and other PUFAs) over the spawning period in Atlantic cod eggs. Phase in the spawning season was determined by dividing the cumulative number of eggs a female had spawned up to and including a particular egg batch by her total fecundity (i.e. total number of eggs of all batches). This allowed us to standardize how far along a female was within her spawning cycle (i.e. seasonal egg production), when comparing the change in lipid composition *among* females, thus avoiding the use of batch number (e.g. the third egg batch of a small female producing few total batches is not necessarily equivalent to that of a large female producing many total batches). However, when we investigated the change in lipid composition *within* a female, we examined both phase in the spawning cycle and batch number and if results were qualitatively similar, reported only those for batch number because of its common use in the literature. PCAs were conducted on *both* absolute (μg/egg) and percent data. Proportional data were arcsine square root transformed prior to analysis. Results of the PCA are based on the rotated component matrix (Varimax rotation with Kaiser Normalization). The effects of female and batch were plotted using the mean residuals from the principal component analysis and one-way ANOVAs with Tukey's post hoc test (SPSS©16) were performed on the residuals to determine if females and batches respectively, were significantly different from each other.

The change in egg lipid composition over the spawning season *within* females was also investigated. Lipid classes (phospholipids, phospholipids + sterols, TAG, FFA, neutral lipids [sum of hydrocarbons, steryl esters/wax esters, ethyl esters, methyl esters, ethyl ketones, methyl ketones, glycerol ethers, triacylglycerols, free fatty acids, sterols, alcohols, diacylglycerols], polar lipids [sum of acetone mobile polar lipids and phospholipids] and total lipids [sum of all lipid classes]) and fatty acids (Σ PUFA, Σ

MUFA, Σ saturated fatty acids, EPA and DHA) were analysed in the context of batch number and phase in the spawning season. However, because the results showed no significant differences between the two parameters, the results are reported in terms of batch number (most common in the literature). Additionally, initial analysis used both the absolute and percent concentration of the aforementioned lipid classes and fatty acids, but since the results obtained from both measures were statistically similar, only the absolute concentrations are reported here when discussing *within* female differences. All residuals were checked for homogeneity and normality. Initially, ANCOVAs (Genmod procedure SAS) were considered for testing whether the lipid composition of eggs by females differed with batch and egg dry weight. However, because interaction terms were significant; individual regressions (Minitab ® 15) were performed for each female. To take into account multiple testing, Benjamini and Hochberg False Discovery Rate (H&B FDR; Benjamini & Hochberg, 1995; reviewed in Verhoeven et al., 2005) was used with the tolerance of type I error set at α= 0.05. Similarly, relations between batch fecundity and the selected lipid classes and fatty acids were examined. Lastly, to simplify the data and identify potential patterns, data was broken into two groups: females with overall low fertilization rates (<50%) and females with overall high fertilization rates (>75%).

2.3 Results

2.3.1 Spawning Activity Summary

Spawning extended from early February until mid-March 2006, during which 43 egg batches were produced by the eight females over an average of 20 days per female, with ca. 3 days between each spawning event (Table 2.1). Fertilization success between batches and among pairs was highly variable ranging from 0% to 99% (Fig. 2.1). Batch fecundity ranged from 33,050 to 519,300 eggs and generally followed a dome shape, with batch fecundity increasing initially and then decreasing with each successive batch (Fig. 2.1). Batch-specific mean egg diameter (1.40 mm ± 0.002 to 1.73 mm ± 0.003) and dry weight (0.062 mg ± 0.0004 to 0.116 mg ± 0.002) also decreased with each successive batch (Fig. 2.2) for *all* eight females (females pooled: log mean egg dry weight [mg] = - 1.45 + 2.12 * log mean egg diameter [mm]; $r^2 = 0.646$, $p < 0.001$).

2.3.2 Lipid Classes and Fatty Acid

Phospholipids were the major lipid class (range 40-86%) within each egg batch followed by sterols, TAG and FFA (Table 2.2). Polar lipids (i.e. sum of phospholipids and acetone mobile polar lipids [AMPL]) accounted for 47-87% of the total lipids, while neutral lipids (i.e. sum of hydrocarbons, steryl esters/wax esters, ethyl esters, methyl esters, ethyl ketones, methyl ketones, glycerol ethers, triacylglycerols, free fatty acids, alcohols and sterols) accounted for 15-52% of the total lipids (see Appendix 1 for complete list of lipid classes per female and batch). Generally, PUFAs accounted for approximately 38-56% of total fatty acids; however, some batches had lower amounts (16-25%) and were associated with lower percentages of DHA and EPA per egg (Table 2.3). Saturated fatty acids varied greatly among females and batches, ranging from 1-35% of total fatty acids, while MUFAs accounted for 15-38% of total fatty acids (Table 2.3). Although AA was often <1% of all fatty acids, it was included in analyses due to its importance in egg development (Wiegand, 1996; see Appendix 2 for complete list of fatty acids >1%).

2.3.2.1 Principal component analysis of absolute (µg/egg) lipids and fatty acids

The first four principal components explained ca. 75% of the cumulative variance of the rotated data matrix (details in Appendix 3). The highest scores (correlations) on the first principal component (PC 1) were DHA (0.963), EPA (0.924), phospholipids (0.840), AA (0.812) and sterols (0.642) (Fig. 2.3; details in Appendix 4). The highest scores on the second principal component (PC 2) were MUFAs (0.883), egg dry weight (0.812), saturated fatty acids (0.780) which were negatively associated with fertilization success (-0.692) (Fig. 2.3). The key variables describing PC 3 were other lipid classes (0.775), TAG (0.745) and FFA (0.636), which had a negative association with batch fecundity (-0.667) (Fig. 2.3). The highest scores on PC 4 belonged to PUFAs (0.831) and phase in the spawning season (-0.642), which were negatively associated with each other (Fig. 2.3).

A one-way ANOVA was performed on the principal component loadings to determine the pattern of batch effects as interpreted from the phase in the spawning cycle. The analysis indicated the presence of significant seasonal changes as interpreted from phase in the spawning (i.e. batch effects) season along PC 2 ($F_{2,40}$ = 7.48, p = 0.002) and PC 3 ($F_{2,40}$ = 3.52, p = 0.039). Post hoc test (Tukey's HSD) indicated significant differences between the first third and second third (p = 0.004) of the spawning season and the second and last third (p = 0.014) along PC 2. Along PC 3, the first and second third of the spawning season were significantly different from each other (p = 0.037).

2.3.2.2 Principal component analysis of percent lipids and fatty acids

The first four principal components of the analysis explained ca. 64% of the cumulative variance, with the first principal component (PC 1) explaining 21% of the cumulative variance (details in Appendix 5). The highest scores on the first principal component (PC 1) were FFA (0.796), TAG (0.769) and other lipid classes (0.861), which was negatively associated with phospholipids (- 0.954) (Fig. 2.4; details in Appendix 6). MUFAs (0.905) and saturated fatty acids (0.895) had the highest scores on the second principal component (PC 2) and were inversely related to fertilization success (-0.498) (Fig. 2.4). The third principal component was mainly a function of EPA (0.883), DHA (0.841) and AA (0.677) (Fig. 2.4), while the forth principal component (PC 4) was associated with an inverse relation between phase in spawning season (0.804) and egg dry weight (-0.702), which was indicative of a decline in egg size through a female's spawning cycle (Fig. 2.4).

A one-way ANOVA was performed on the principal component loadings to determine the pattern of batch effects as interpreted from the phase in the spawning season. Analysis of the principal component loadings based on percent composition of the various lipid classes and fatty acids indicated no significant seasonal changes as interpreted from phase in the spawning season (i.e. batch effects) along PC 1, PC 2 or PC 3 ($p > 0.05$)

2.3.2.3 Seasonal changes in lipid composition within females

Our results for phase in the spawning season and batch number were similar when analyzing *within* female patterns; hence, we opted to use batch number rather than point in the spawning season as batch number is the most common vernacular used when describing *within* female variations in the literature.

The females showing the strongest seasonal trends in lipid composition, were those with high fertilization success (>75%; i.e. females 3, 5, 7 and 8). With regard to neutral, polar, and total lipids, females 5, 7 and 8 exhibited seasonal declines with increasing batch number, while female 3 consistently showed an increase (Fig. 2.5). However, none of these patterns were significant after correcting for multiple testing (B&H False

Discovery Rate), with the exception of neutral lipids of female 3 ($p < 0.0063$ [$P_{critical}$])). Similarly, no significant decrease or increase with batch number was observed for phospholipids, FFA, TAG or phospholipids + sterols (except for female 8; $p < 0.0063$; additional details in Appendix 7). The pattern was again similar in terms of fatty acids (Fig. 2.6), with the exception of a significant increase in EPA with batch number for female 3 ($p < 0.0063$ [$P_{critical}$]; additional details in Appendix 8). Females with relatively low fertilization success (<50%; i.e. females 1, 2, 4 and 6) were not characterized by seasonal changes in lipid classes and fatty acids.

2.3.2.4 Seasonal changes in lipid composition with egg size

While egg size was positively associated with MUFAs and saturated fatty acids and negatively with fertilization success along the second principal component, closer examination revealed considerable variation in trends with egg size among females for all lipid classes and fatty acids examined. Similar to the effects with batch number, females that displayed significant trends were those with relatively high fertilization success (>75% composite fertilization i.e., females 3, 5, 7 and 8). After correcting for multiple testing, significant positive relations remained between polar lipids and egg size for females 7 and 8 ($p < 0.0125$ [$P_{critical}$] and $p < 0.0063$ [$P_{critical}$], respectively) and between total lipids and egg size for female 7 ($p < 0.0063$ [$P_{critical}$]; Fig. 2.7). These two females also showed significant increases in phospholipids and phospholipids + sterols with egg size (in both cases: female 7, $p < 0.0063$ [$P_{critical}$]; female 8, < 0.0125 [$P_{critical}$]; additional details in Appendix 9). In contrast, female 3 showed a significant negative relation between neutral lipids and egg size ($p < 0.0063$; Fig. 2.7). After adjustments for multiple testing, no significant trends were evident between egg size and the concentration of MUFAs, saturated fatty acids and PUFAs (Fig. 2.8), nor between egg size and lipid classes FFA and TAG (additional details in Appendix 9) and fatty acids EPA and DHA (additional details in Appendix 10).

2.3.3 Maternal Effects

2.3.3.1 Female differences in absolute allocation (µg/egg)

To determine *among* female differences, one-way ANOVAs (with Tukey's HSD post hoc tests) were completed on the PCA loadings. One-way ANOVAs indicated the presence of significant maternal effects along PC 1 ($F_{7,35} = 4.67$, $p = 0.001$) and PC 2 ($F_{7,35} = 6.41$, $p < 0.001$; Fig. 2.9 A), but not along PC 3 ($F_{7,35} = 1.52$, $p = 0.193$; Fig. 2.9). Along PC 1, which correlated positively with DHA, EPA, phospholipids and AA, Tukey's HSD post-hoc tests revealed that female 1 differed significantly from females 4, 5 and 6 , and female 7 was significantly different from female 5 ($p < 0.05$). Along PC 2, which was positively correlated with MUFAs, egg dry weight and saturated fatty acids, and negatively correlated with fertilization success, female 4 differed significantly from females 3, 5 and 7, and female 6 differed significantly from female 7 ($p < 0.05$). Not surprisingly, it is this second principal component that appears to separate females with overall high (>75%) and low (<50%) fertilization success.

2.3.3.2 Female differences in percent allocation

Analysis of principal component loadings based on the percent composition of the various lipid classes and fatty acids indicated the presence of significant maternal differences along PC 1 ($F_{7,35} = 2.40$, $p = 0.041$), but not along any of the other principal components (PC 2 $F_{7,35} = 1.45$, $p = 0.217$; PC 3 $F_{7,35} = 2.08$, $p = 0.072$; Fig. 2.10). Post hoc testing (Tukey's HSD), however, did not detect any pair-wise significant differences between females along PC1.

2.3.4 Batch Fecundity and Egg Composition

There was no significant effect of batch fecundity on egg composition across or within females ($p > 0.05$). Noteworthy was that mean egg dry weight tended to increase with batch fecundity, but was not significant ($r = 0.225$, $p = 0.148$, $n = 43$). Similarly, total, neutral and polar lipids did not vary significantly with batch fecundity (Figure 2.11), a pattern also observed with fatty acids such as MUFAs, saturated fatty acids and PUFAs (Fig 2.12). This was also observed when individual lipid classes and fatty acids were analysed (additional details in Appendices 11 and 12, respectively).

2.4 Discussion

The mechanisms by which within and among female variation impacts egg quality as reflected in egg size and composition, still remains poorly understood in batch spawners (Czesny et al., 2005). As such, the main objective of this study was to determine the magnitude of seasonal change in egg lipid composition (absolute and relative amounts) from eight female Atlantic cod of *wild* origin (i.e. captured 4 months prior to the start of the experiment). We found varying patterns, with regard to the allocation of lipids and fatty acids (both absolute and relative amounts) by individual females over the spawning period and with regards to decreasing egg size but no trend was evident with regards to batch fecundity. Additionally, female effects and batch effects (i.e. changes in the spawning period between each successive egg batch as interpreted by the phase in the spawning season) were also evident. The high level of variability observed among females may be the result of multiple factors, including individual variability in their dietary history prior to collection from the wild and factors associated with maternal effects such as age, condition and individual spawning history, factors which were not determined in this study. Unlike other egg lipid studies on marine batch spawners we have considered both female and batch effects from fish recently collected from the wild, and we were principally interested in determining a comprehensive lipid profile of the eggs over the spawning season without considering how other factors such as 'domestication' (e.g., Penney et al., 1996, Salze et al., 2005) , stock differences (e.g., Evans et al., 1996, Pickova et al., 1997) adult diet (e.g., Mazorra et al., 2003) and or embryonic stage (e.g., Fraser et al., 1988; Zhu et al., 2003) might influence the lipid profile.

2.4.1 Changes in Lipid Composition Over The Spawning Season

Egg lipid composition (i.e. lipid classes and fatty acids) was variable among batches and females, but the overall amount of lipid classes and fatty acids deposited in the egg were similar to those of other studies (for example, phospholipids ranged between 40-85%; polar lipids ranged between 47-87% of total lipids (76%, Kaitaranta & Ackman, 1981; 61-77%, Tocher & Sargent, 1984); neutral lipids ranged between 15-52% of the total lipids (12.5%, Tocher & Sargent, 1984); PUFA's ranged 16-50% of fatty acids; lower values attributed to eggs with lower concentration of EPA and DHA (42% Budge et al., 2002; ~50% Penney et al., 2006). It is plausible that the variation in lipid composition values among studies reflect differences due to female effects, batch effects, variability in methodology, stock differences and dietary history.

The change in phospholipid concentration between batches within individual females varied tremendously, with increases as high as 20% to declines as great as 70% between the initial and final egg batches. Eggs with lower concentrations of phospholipids may represent eggs with weaker membranes, as phospholipids are an important structural element in membrane formation in developing embryos (Kaitaranta

& Ackman, 1981; Fraser et al., 1988; Sargent, 1995). Consequently low levels of phospholipids may have an impact on larval survival at the time of hatch (Kaitaranta & Ackman, 1981; Sargent, 1995; Sargent et al., 2002). Furthermore, low levels of phospholipids combined with low levels of TAG may affect embryonic development, growth and survival and these components have been used as predictors of hatching success (Fraser et al., 1988) but may not always be accurate measures of egg quality (Penney et al., 2006). Additionally, eggs with low levels of PUFAs most likely did not survive until hatching or had poor survival rates as DHA, EPA, AA (and their associated ratios) have been shown to be significantly correlated with egg quality, fertilization success, hatching success and larval survival in many fish species (Pickova et al., 1997; Tveiten et al., 2004; Yanes-Roca et al., 2009). A limitation of this study was that we were not able to determine hatching success but it can be postulated that an overall decline in the lipid and fatty acid parameters over the spawning season can have an impact on egg quality and consequently hatching and larval success (Kjørsvik et al., 1990; Pickova et al., 1997; Czesny et al., 2005).

Egg lipid content varies with water content of the egg, egg ripeness and whether or not the egg was measured before or after fertilization (Devauchelle & Coves, 1988; Rainuzzo, 1993). Overripened eggs have been found to have higher lipid content than ripened eggs, however, in this study, egg ripeness was not determined but the observed seasonal decline in egg size in this study was similar to those of other gadoid studies (Kjesbu, 1989; Trippel, 1998; Rideout et al., 2005). This suggests that egg ripeness was not a contributing factor for females whose lipid composition increased over the spawning period. Although fertilization success was determined, eggs used in this analysis contained an assortment of fertilized and unfertilized eggs as the sample was randomly selected. An attempt to differentiate females and patterns based on overall fertilization success indicated females with overall higher fertilization rates (>75%) in this experiment typically showed a decrease in lipid composition over the spawning season and with egg size. Furthermore, variation in fertilization success among females could be attributed to quality of eggs extruded by the female, the interaction between the male-female pair as well as paternal attributes such as milt volume, sperm motility and or sperm concentration (Trippel & Neil, 2004).

In summary, overall individual lipid and fatty acid profiles were highly variable among the eight females investigated, which may reflect natural variation among fish collected from a wild population. Seasonal changes in egg size as well as lipid content for some females were observed and these may be reflective of seasonal changes in egg quality. The implication of this is that over the spawning season, as females' reserves become depleted, they are investing less into each additional egg batch and this reduction in lipid composition in latter batches has been postulated to influence egg quality and offspring viability (Ulvund & Grahl-Nielsen, 1988). Assuming the egg yolk is reflective of the nutritional content invested by the mother, support for a seasonal decline in maternal nutrient investment has been observed by Trippel (1998) and in our own studies (unpublished data, see Chapter 3), which showed a decline in the yolk of newly hatch larvae between the first and final egg batches over the spawning season.

2.4.2 Maternal Effects

The seasonal decline in egg size in this study was similar to that reported in other gadoid studies (Kjesbu, 1989; Trippel, 1998; Rideout et al., 2005), but variation in egg size within wild populations such as those observed in this study, are expected to be greater than those within captive stocks because environmental factors and maternal phenotypes are more diverse in nature (Chambers & Waiwood, 1996). Furthermore, factors such as maternal age (Chambers & Leggett, 1996; Kjesbu et al., 1996; Evans et al., 1996), condition (Kjesbu et al., 1991; Oullet et al., 2001), broodstock origin (Pickova et al., 1997; Czesny et al., 2005) and diet (Almansa et al., 1999; Lavens et al., 1999; Mazorra et al., 2003) can all contribute to inter-female differences in lipid composition. Despite attempts made to minimise confounding effects (using fish captured from the same location, females of similar size and condition and feeding them identical diets while in captivity) associated with maternal studies, we still observed differences in egg composition among females. Furthermore, principal component analysis revealed significant among female differences, especially with regard to the absolute amount of lipid (µg/egg) deposited, though female effects were less evident with regard to the relative (%) composition of lipids in eggs. Maternal effects were also evident in terms of differences in egg size among females, the number of egg batches produced and patterns of lipid deposition over the spawning period.

Although it is known that diet can have a direct impact on egg lipid composition during oogenesis (Almansa et al., 1999; Lavens et al., 1999; reviewed in Rainuzzo et al., 1997 and Sargent et al., 2002), it still remains unclear how the duration of the dietary regime affects egg composition (Yanes-Roca et al., 2009). In this study, it is possible that the food our fish received during their four months in captivity did not have a significant effect on egg composition and that the variation in our results is representative of their dietary composition from the wild. However, Rainuzzo et al. (1997) indicated that factors other than diet (such as those mentioned above) can have an influence on egg lipid composition as they found high variation in the lipid composition of turbot (*Scophthalmus* *maximus*) eggs from females fed identical diets. Studies that explore egg lipid composition of batch spawners (e.g. Daniel et al., 1993; Rainuzzo et al., 1997; Evans et al., 1996; Penney et al., 2006; this study) indicate that there are still many unknowns and variables (for example age, dietary history, spawning history, batch effects) that need to be taken into consideration when determining the factors that affect the lipid profiles of individual females.

2.4.3 Conclusions

Amidst the trends that emerged from this study, it is clear that as the spawning season progresses the trend may be for females to decrease the size of eggs and thus the amount of and type of lipids invested in each successive egg batch. Though the change in lipids was not always statistically significant, it is likely of importance as it illustrates a seasonal reduction in the amount of lipids invested in eggs which may be reflective of a decline in maternal lipid reserves over the spawning period. It can be postulated that seasonal declines in lipid content of eggs may be linked to subsequent energy stores available for catabolism during embryogenesis and early larval growth and survival.

To our knowledge, this is the first study in a temperate batch-spawning fish that quantifies changes in lipid composition over the spawning season. The findings from this study help us to better understand how lipids are allocated to eggs of batch spawners and sheds light on factors that may contribute to inter-female differences and its variability, especially in wild populations. Coupled with other maternal and environmental data, egg lipid composition could be of use to improve our knowledge of contributing factors to annual variability in recruitment of marine fish stocks. Egg lipid composition is known to affect egg and larvae survivorship and can thus affect recruitment within a population. The use of recently captured wild cod in paired mating, coupled with the inability to follow successive egg batches of individuals in the ocean, further highlight the unique significance of the study's results.

ACKNOWLEDGEMENTS

Financial support for this study was provided by the Fisheries and Oceans Canada ACRDP Program (EAT) and an NSERC Discovery grant (IAF). Extra special thanks to Chris Parrish for his valuable suggestions, Jeanette Wells for laboratory assistance in the Parrish lipid lab, Marsha Clarke for guiding me through the initial lipid collection process and Dan Ings for statistical assistance and my supervisory committee for their invaluable contributions.

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Table 2.1: Body size and spawning activity of mated pairs of Atlantic cod.

		Female		Male					Spawning Event	
	Mating Pair	Weight (kg)	Length (cm)	Weight (kg)	Male Length (cm)	No. of Egg Batches	No. of Days Spawning	Mean No. of Days Between Spawnings	First	Last
	1	3.96	68.3	4.54	68.0	5	16	3.0	Feb-06	Feb-21
	2	3.88	67.0	4.09	68.4	3	13	4.0	Feb-10	Feb-22
	3	5.74	75.5	4.53	71.6	5	18	3.4	Feb-03	Feb-20
	4	5.56	76.8	4.21	69.9	6	37	6.2	Feb-05	Mar-13
	5	4.09	66.3	3.09	60.4	6	22	3.5	Feb-03	Feb-24
	6	5.56	74.0	4.13	65.4	5	18	3.4	Feb-20	Mar-09
	7	4.21	68.4	3.42	64.4	6	20	3.2	Feb-06	Feb-25
	8	3.92	64.9	4.08	67.9	7	23	3.1	Feb-06	Feb-28
Mean		**4.62**	**70.2**	**4.0**	**67.0**	**5.4**	**20.9**	**3.7**		

Table 2.2: Mean (± SD and range) egg dry weight and selected lipid classes as a percent of total lipids of egg batches of eight female Atlantic cod.

Female	No. Batches	Egg Dry Weight (mg)	% Phospholipids	% Neutral Lipids	% Polar Lipids	% TAG	% FFA	% Sterols
1	5	0.0951 ± 0.0081 0.0890 - 0.1041	79.00 ± 6.11 75.54 - 85.66	18.39 ± 4.85 13.15 – 22.82	81.65 ± 4.90 77.18 - 86.86	2.80 ± 0.24 2.59 - 3.19	1.57 ± 1.16 0.00 - 3.08	8.37 ± 1.92 5.74 - 11.06
2	3	0.0918 ± 0.0064 0.0862 - 0.0987	83.32 ± 2.25 80.79 - 85.13	15.76 ± 0.81 14.87 – 16.45	84.57 ± 0.89 83.55 - 85.13	2.78 ± 1.03 1.63 - 3.63	1.12 ± 0.98 0.00 - 1.80	8.30 ± 1.11 7.60 - 9.58
3	5	0.0866 ± 0.0072 0.0765 - 0.0939	78.20 ± 6.82 67.72 - 83.81	18.30 ± 5.46 13.41 – 25.15	81.70 ± 5.46 74.85 - 86.59	4.14 ± 1.62 3.02 - 6.69	1.85 ± 0.89 0.81 - 3.25	9.10 ± 2.81 5.36 - 11.61
4	6	0.1042 ± 0.0111 0.0852 - 0.1157	68.23 ± 5.80 60.53 - 74.39	27.49 ± 3.84 22.90 – 33.04	72.51 ± 3.84 66.96 -77.10	5.56 ± 4.08 1.82 - 10.91	4.90 ± 1.43 2.50 - 6.61	12.62 ± 2.33 9.47 - 15.24
5	6	0.0894 ± 0.0102 0.0756 - 0.1032	68.50 ± 5.81 62.39 - 75.22	27.41 ± 3.94 22.27 – 31.91	72.59 ± 3.94 68.09 - 77.73	7.05 ± 4.18 2.86 - 13.17	3.38 ± 1.54 0.66 - 4.67	12.33 ± 6.12 8.90 - 25.26
6	5	0.0999 ± 0.0062 0.0895 - 0.1059	65.73 ± 3.28 61.31 - 69.79	31.26 ± 2.95 26.77 – 33.88	68.74 ± 2.95 66.12 - 73.23	5.41 ± 2.50 3.26 - 9.15	5.14 ± 2.58 2.20 - 7.67	15.58 ± 4.95 12.36 - 24.22
7	6	0.0737 ± 0.0078 0.0618 - 0.0822	70.96 ± 15.63 40.70 - 80.17	25.65 ± 13.71 15.10 – 52.69	74.35 ± 13.71 47.31 - 84.90	4.56 ± 2.16 1.55- 10.64	4.36 ± 2.62 1.65 - 9.02	11.83 ± 6.14 7.90 - 23.11
8	7	0.0913 ± 0.0129 0.0723 - 0.1078	70.31 ± 10.85 59.01 - 82.69	25.73 ± 8.71 15.15 – 34.41	74.27 ± 8.71 65.59 - 84.85	6.77 ± 3.91 1.87 - 10.65	4.62 ± 2.18 0.76 - 7.96	9.40 ± 3.22 4.27 - 11.91

Table 2.3: Mean (± SD and range) percent of selected fatty acids of total fatty acids of egg batches of eight female Atlantic cod.

Female	No. Batches	% AA (20:4ω6)	% EPA (20:5ω3)	% DHA (22:6ω3)	% Σ Saturated	% Σ MUFA	% Σ PUFA
1	5	0.58 ± 0.30 0.28 - 1.04	10.73 ± 4.40 4.86 - 14.22	21.45 ± 11.27 7.75 - 30.77	32.43 ± 8.81 25.92 - 43.58	27.91 ± 7.07 22.28 - 38.22	38.24 ± 15.98 16.52 - 50.42
2	3	1.17 ± 0.06 1.11 - 1.24	15.49 ± 0.90 14.65 - 16.45	30.17 ± 0.99 29.15 - 31.14	26.22 ± 0.55 25.60 - 26.61	20.47 ± 1.74 18.50 - 21.77	52.30 ± 2.45 49.67 - 54.52
3	5	1.19 ± 0.19 0.91 - 1.42	18.13 ± 1.79 15.56 - 19.64	27.08 ± 2.52 24.22 - 29.48	29.71 ± 2.09 26.57 - 31.94	19.19 ± 3.43 16.09 - 23.04	50.78 ± 4.50 45.83 - 55.04
4	6	1.05 ± 0.09 0.91 - 1.11	15.60 ± 1.59 14.00 - 17.97	26.70 ± 5.04 19.24 - 31.01	29.46 ± 3.91 25.84 - 35.13	21.74 ± 2.53 17.93 - 24.96	48.04 ± 6.39 38.77 - 54.63
5	6	1.11 ± 0.18 0.76 - 1.23	16.54 ± 2.04 12.96 - 18.67	29.69 ± 3.92 22.22 - 32.71	27.82 ± 3.37 24.99 - 34.08	20.15 ± 2.77 17.87 - 25.61	51.38 ± 6.13 39.53 - 55.67
6	5	0.78 ± 0.47 0.00 - 1.21	17.13 ± 1.51 15.38 - 19.46	29.09 ± 3.14 25.02 - 33.12	28.54 ± 1.21 27.13 - 30.23	20.31 ± 2.28 16.36 - 22.15	50.11 ± 3.98 45.25 - 56.17
7	6	1.01 ± 0.37 0.41 - 1.35	16.52 ± 3.41 12.18 - 21.34	26.80 ± 5.74 18.63 - 31.06	29.17 ± 5.04 24.47 - 35.72	20.32 ± 3.77 15.88 - 25.08	49.77 ± 8.47 38.71 - 56.09
8	7	0.88 ± 0.28 0.36 - 1.18	17.47 ± 14.31 8.47 - 21.32	26.92 ± 6.86 12.43 - 31.57	28.24 ± 3.79 23.21 - 35.66	20.05 ± 4.22 15.86 - 28.43	49.85 ± 11.18 25.92 - 57.92

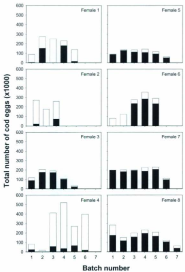


Figure 2.1: Batch-specific fecundity and fertilization rates (■ fertilized, □ unfertilized) over the spawning period of eight mated pairs of Atlantic cod.

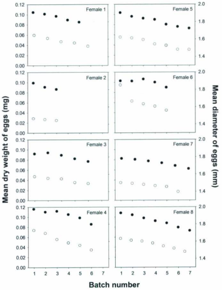


Figure 2.2: Changes in mean egg diameter (O) and dry weight (●) with successive egg batch spawned by eight female Atlantic cod.

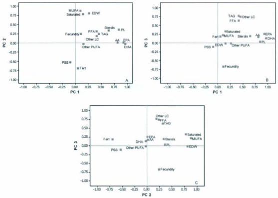
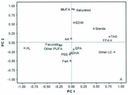
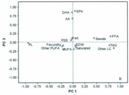


Figure 2.3: Two-factor plots of the rotated principal component data matrix of the absolute amount (µg/egg) of selected lipid classes and fatty acids showing the loadings for: (A) the first two principal components, (B) the principal components one and three and (C) principal components two and three. (PSS = phase in spawning season; EDW = egg dry weight; PL = phospholipids; Other LC = other lipid classes; Fert = fertilization rate).





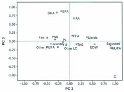


Figure 2.4: Two-factor plots of the rotated principal component data matrix of the relative amount (%) of selected lipid classes and fatty acids showing the loadings for: (A) the first two principal components, (B) the principal components one and three and (C) principal components two and three. (PSS = phase in spawning season; EDW = egg dry weight; PL = phospholipids; Other LC = other lipid classes; Fert = fertilization rate).

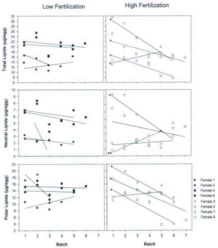


Figure 2.5: Seasonal changes in the absolute amount (µg/egg) of total lipids, neutral lipids and polar lipids in eggs over the spawning cycle as measured by batch number of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left panel) or high fertilization (>75%; right panel) rates. Significant seasonal changes are denoted for individual females prior to (*) and after adjustment (**) for multiple comparisons.

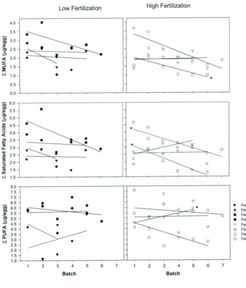


Figure 2.6: Seasonal changes in the absolute amount (µg/egg) of Σ MUFA , Σ saturated fatty acids and Σ PUFA over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.

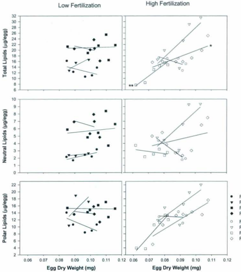


Figure 2.7: Seasonal changes in relation to egg size in the absolute amount (µg/egg) of total lipids, neutral lipids and polar lipids over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.

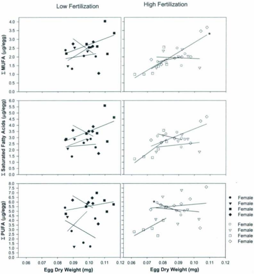


Figure 2.8: Seasonal changes in relation to egg size in the absolute amount (µg/egg) of Σ MUFA, Σ Saturated fatty acids and Σ PUFA over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.

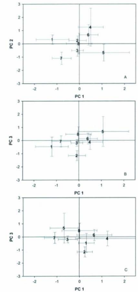


Figure 2.9: Two-factor plots of the mean residual values, including confidence intervals, derived from an analysis of absolute amounts (μg/egg) of selected lipid classes and fatty acids (see Table 2.5) for each of the 8 female Atlantic cod. Along: (A) the first two principal components, (B) principal components one and three and (C) principal components two and three.

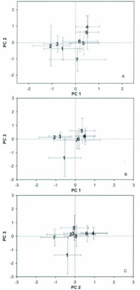


Figure 2.10: Two-factor plots of the mean factor values, including confidence intervals, derived from an analysis of the percentage of selected lipid classes and fatty acids (see Table 2.7) for each of the 8 female Atlantic cod. Along: (A) the first two principal components, (B) principal components one and three and (C) principal components two and three.

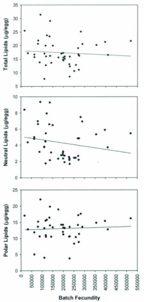


Figure 2.11: Changes in egg composition, as measured by total lipids, neutral lipids and polar lipids, in relation to batch fecundity of eight female Atlantic cod (data pooled).

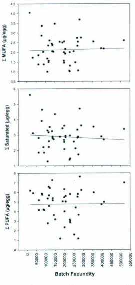


Figure 2.12: Changes in egg composition, as measured by (A) MUFAs, (B) saturated fatty acids and (C) PUFAs, in relation to batch fecundity of eight female Atlantic cod (data pooled).

Table 2.1: Body size and spawning activity of mated pairs of Atlantic cod.

		Female		Male					Spawning Event	
	Mating Pair	Weight (kg)	Length (cm)	Weight (kg)	Male Length (cm)	No. of Egg Batches	No. of Days Spawning	Mean No. of Days Between Spawnings	First	Last
	1	3.96	68.3	4.54	68.0	5	16	3.0	Feb-06	Feb-21
	2	3.88	67.0	4.09	68.4	3	13	4.0	Feb-10	Feb-22
	3	5.74	75.5	4.53	71.6	5	18	3.4	Feb-03	Feb-20
	4	5.56	76.8	4.21	69.9	6	37	6.2	Feb-05	Mar-13
	5	4.09	66.3	3.09	60.4	6	22	3.5	Feb-03	Feb-24
	6	5.56	74.0	4.13	65.4	5	18	3.4	Feb-20	Mar-09
	7	4.21	68.4	3.42	64.4	6	20	3.2	Feb-06	Feb-25
	8	3.92	64.9	4.08	67.9	7	23	3.1	Feb-06	Feb-28
Mean		**4.62**	**70.2**	**4.0**	**67.0**	**5.4**	**20.9**	**3.7**		

Table 2.2: Mean (± SD and range) egg dry weight and selected lipid classes as a percent of total lipids of egg batches of eight female Atlantic cod.

Female	No. Batches	Egg Dry Weight (mg)	% Phospholipids	% Neutral Lipids	% Polar Lipids	% TAG	% FFA	% Sterols
1	5	0.0951 ± 0.0081 0.0890 - 0.1041	79.00 ± 6.11 75.54 - 85.66	18.39 ± 4.85 13.15 – 22.82	81.65 ± 4.90 77.18 - 86.86	2.80 ± 0.24 2.59 - 3.19	1.57 ± 1.16 0.00 - 3.08	8.37 ± 1.92 5.74 - 11.06
2	3	0.0918 ± 0.0064 0.0862 - 0.0987	83.32 ± 2.25 80.79 - 85.13	15.76 ± 0.81 14.87 – 16.45	84.57 ± 0.89 83.55 - 85.13	2.78 ± 1.03 1.63 - 3.63	1.12 ± 0.98 0.00 - 1.80	8.30 ± 1.11 7.60 - 9.58
3	5	0.0866 ± 0.0072 0.0765 - 0.0939	78.20 ± 6.82 67.72 - 83.81	18.30 ± 5.46 13.41 – 25.15	81.70 ± 5.46 74.85 - 86.59	4.14 ± 1.62 3.02 - 6.69	1.85 ± 0.89 0.81 - 3.25	9.10 ± 2.81 5.36 - 11.61
4	6	0.1042 ± 0.0111 0.0852 - 0.1157	68.23 ± 5.80 60.53 - 74.39	27.49 ± 3.84 22.90 – 33.04	72.51 ± 3.84 66.96 -77.10	5.56 ± 4.08 1.82 - 10.91	4.90 ± 1.43 2.50 - 6.61	12.62 ± 2.33 9.47 - 15.24
5	6	0.0894 ± 0.0102 0.0756 - 0.1032	68.50 ± 5.81 62.39 - 75.22	27.41 ± 3.94 22.27 – 31.91	72.59 ± 3.94 68.09 - 77.73	7.05 ± 4.18 2.86 - 13.17	3.38 ± 1.54 0.66 - 4.67	12.33 ± 6.12 8.90 - 25.26
6	5	0.0999 ± 0.0062 0.0895 - 0.1059	65.73 ± 3.28 61.31 - 69.79	31.26 ± 2.95 26.77 – 33.88	68.74 ± 2.95 66.12 - 73.23	5.41 ± 2.50 3.26 - 9.15	5.14 ± 2.58 2.20 - 7.67	15.58 ± 4.95 12.36 - 24.22
7	6	0.0737 ± 0.0078 0.0618 - 0.0822	70.96 ± 15.63 40.70 - 80.17	25.65 ± 13.71 15.10 – 52.69	74.35 ± 13.71 47.31 - 84.90	4.56 ± 2.16 1.55- 10.64	4.36 ± 2.62 1.65 - 9.02	11.83 ± 6.14 7.90 - 23.11
8	7	0.0913 ± 0.0129 0.0723 - 0.1078	70.31 ± 10.85 59.01 - 82.69	25.73 ± 8.71 15.15 – 34.41	74.27 ± 8.71 65.59 - 84.85	6.77 ± 3.91 1.87 - 10.65	4.62 ± 2.18 0.76 - 7.96	9.40 ± 3.22 4.27 - 11.91

Table 2.3: Mean (± SD and range) percent of selected fatty acids of total fatty acids of egg batches of eight female Atlantic cod.

Female	No. Batches	% AA (20:4ω6)	% EPA (20:5ω3)	% DHA (22:6ω3)	% Σ Saturated	% Σ MUFA	% Σ PUFA
1	5	0.58 ± 0.30 0.28 - 1.04	10.73 ± 4.40 4.86 - 14.22	21.45 ± 11.27 7.75 - 30.77	32.43 ± 8.81 25.92 - 43.58	27.91 ± 7.07 22.28 - 38.22	38.24 ± 15.98 16.52 - 50.42
2	3	1.17 ± 0.06 1.11 - 1.24	15.49 ± 0.90 14.65 - 16.45	30.17 ± 0.99 29.15 - 31.14	26.22 ± 0.55 25.60 - 26.61	20.47 ± 1.74 18.50 - 21.77	52.30 ± 2.45 49.67 - 54.52
3	5	1.19 ± 0.19 0.91 - 1.42	18.13 ± 1.79 15.56 - 19.64	27.08 ± 2.52 24.22 - 29.48	29.71 ± 2.09 26.57 - 31.94	19.19 ± 3.43 16.09 - 23.04	50.78 ± 4.50 45.83 - 55.04
4	6	1.05 ± 0.09 0.91 - 1.11	15.60 ± 1.59 14.00 - 17.97	26.70 ± 5.04 19.24 - 31.01	29.46 ± 3.91 25.84 - 35.13	21.74 ± 2.53 17.93 - 24.96	48.04 ± 6.39 38.77 - 54.63
5	6	1.11 ± 0.18 0.76 - 1.23	16.54 ± 2.04 12.96 - 18.67	29.69 ± 3.92 22.22 - 32.71	27.82 ± 3.37 24.99 - 34.08	20.15 ± 2.77 17.87 - 25.61	51.38 ± 6.13 39.53 - 55.67
6	5	0.78 ± 0.47 0.00 - 1.21	17.13 ± 1.51 15.38 - 19.46	29.09 ± 3.14 25.02 - 33.12	28.54 ± 1.21 27.13 - 30.23	20.31 ± 2.28 16.36 - 22.15	50.11 ± 3.98 45.25 - 56.17
7	6	1.01 ± 0.37 0.41 - 1.35	16.52 ± 3.41 12.18 - 21.34	26.80 ± 5.74 18.63 - 31.06	29.17 ± 5.04 24.47 - 35.72	20.32 ± 3.77 15.88 - 25.08	49.77 ± 8.47 38.71 - 56.09
8	7	0.88 ± 0.28 0.36 - 1.18	17.47 ± 14.31 8.47 - 21.32	26.92 ± 6.86 12.43 - 31.57	28.24 ± 3.79 23.21 - 35.66	20.05 ± 4.22 15.86 - 28.43	49.85 ± 11.18 25.92 - 57.92

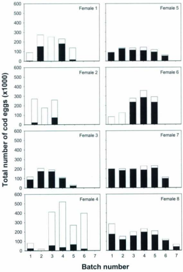


Figure 2.1: Batch-specific fecundity and fertilization rates (■ fertilized, □ unfertilized) over the spawning period of eight mated pairs of Atlantic cod.

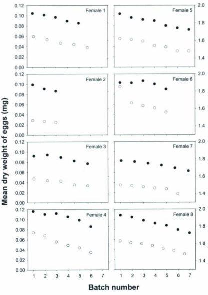


Figure 2.2: Changes in mean egg diameter (O) and dry weight (●) with successive egg batch spawned by eight female Atlantic cod.

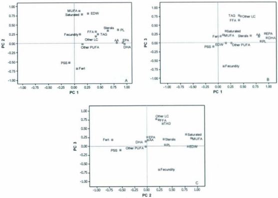


Figure 2.3: Two-factor plots of the rotated principal component data matrix of the absolute amount (µg/egg) of selected lipid classes and fatty acids showing the loadings for: (A) the first two principal components, (B) the principal components one and three and (C) principal components two and three. (PSS = phase in spawning season; EDW = egg dry weight; PL = phospholipids; Other LC = other lipid classes; Fert = fertilization rate).

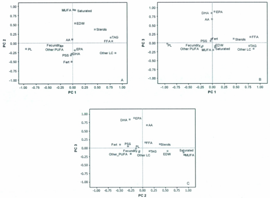


Figure 2.4: Two-factor plots of the rotated principal component data matrix of the relative amount (%) of selected lipid classes and fatty acids showing the loadings for: (A) the first two principal components, (B) the principal components one and three and (C) principal components two and three. (PSS = phase in spawning season; EDW = egg dry weight; PL = phospholipids; Other LC = other lipid classes; Fert = fertilization rate).

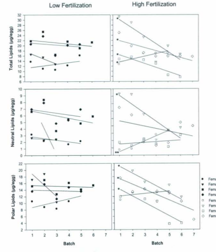


Figure 2.5: Seasonal changes in the absolute amount (µg/egg) of total lipids, neutral lipids and polar lipids in eggs over the spawning cycle as measured by batch number of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left panel) or high fertilization (>75%; right panel) rates. Significant seasonal changes are denoted for individual females prior to (*) and after adjustment (**) for multiple comparisons.

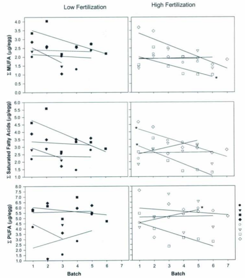


Figure 2.6: Seasonal changes in the absolute amount (µg/egg) of Σ MUFA , Σ saturated fatty acids and Σ PUFA over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.

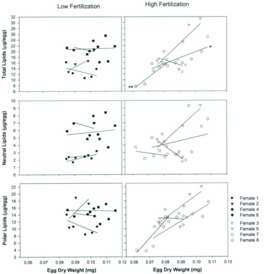


Figure 2.7: Seasonal changes in relation to egg size in the absolute amount (µg/egg) of total lipids, neutral lipids and polar lipids over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.

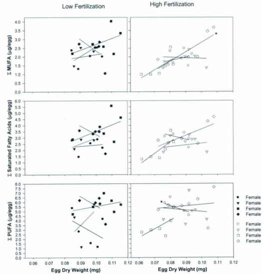


Figure 2.8: Seasonal changes in relation to egg size in the absolute amount (µg/egg) of Σ MUFA, Σ Saturated fatty acids and Σ PUFA over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.

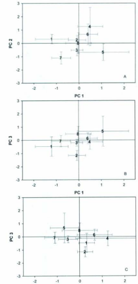


Figure 2.9: Two-factor plots of the mean residual values, including confidence intervals, derived from an analysis of absolute amounts (µg/egg) of selected lipid classes and fatty acids (see Table 2.5) for each of the 8 female Atlantic cod. Along: (A) the first two principal components, (B) principal components one and three and (C) principal components two and three.

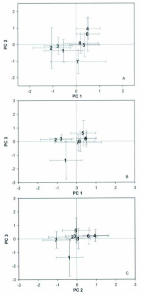


Figure 2.10: Two-factor plots of the mean factor values, including confidence intervals, derived from an analysis of the percentage of selected lipid classes and fatty acids (see Table 2.7) for each of the 8 female Atlantic cod. Along: (A) the first two principal components, (B) principal components one and three and (C) principal components two and three.

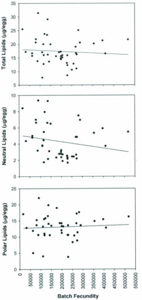


Figure 2.11: Changes in egg composition, as measured by total lipids, neutral lipids and polar lipids, in relation to batch fecundity of eight female Atlantic cod (data pooled).

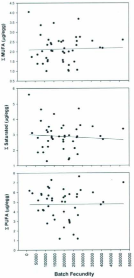


Figure 2.12: Changes in egg composition, as measured by (A) MUFAs, (B) saturated fatty acids and (C) PUFAs, in relation to batch fecundity of eight female Atlantic cod (data pooled).

Chapter 3: Effects of within and among female differences in egg size on the morphology, survival and growth of Atlantic cod (*Gadus morhua*) larvae under differing food regimes

Abstract

The objective of this study was to determine female and seasonal effects on Atlantic cod (*Gadus morhua*) larval traits at 0 and 5 days post hatch (dph) and on larval performance (15 dph) under two feeding regimes. Egg production of eight females was observed from early February to mid March. The number of egg batches produced among females varied between three and seven and in total 43 egg batches were produced over an average of 20 days per female, with approximately three days between each spawning event. Batch fecundity, egg size, larval size and larval dry weight at hatch declined with increasing batch number over the spawning season. Inter-batch and female differences occurred in larval traits of unfed larvae at 0 and 5 dph and for larvae exposed to two feeding regimes. Egg size was positively correlated with larval size and other morphological traits (e.g., eye size, myotome height, jaw length). Larval survival among the two feeding regimes (1,500 and 4,000 rotifers/L; 2.7-fold difference) was highly variable ranging from 0 to 60% with mean survivorship near 15% in each treatment and did not differ significantly. Specific growth rate (SGR) was also highly variable between the two feeding regimes ranging from 0.16 to 3.57 %/day in length with a mean of 2.30%/day for the low food group and 2.40 %/day for the high food group. Myotome condition index (MCI) was the only variable to be significantly different in relation to feeding regime and was slightly greater for the better fed group. Associations between Fulton's condition factor and myotome condition index were explored and their utility for larval fish studies discussed.

Key words: maternal effects, egg batch differences, fecundity, larval size, condition, larval performance, prey concentration

3.1 Introduction

The early life history of marine fishes is a critical period as it represents the ontogenetic phase of greatest growth (Osse, 1995) and the time of highest mortality, primarily as a result of predation (Bailey & Houde, 1989; Letcher et al., 1996) and starvation (Hunter, 1981; Cushing, 1990). The small size of larval fish makes them especially vulnerable to predation, while the transition from endogenous to exogenous feeding represents a critical period during which starvation is possible if prey size, type and abundance are unsuitable and/or insufficient (Hjort, 1914; May, 1974; Lasker, 1981; Govoni, 2005). Feeding success, and thus potentially growth, of larval fish is therefore dependent on a combination of physical (e.g. mouth gape, size at hatch, activity level) and physiological (e.g. ability to acquire, digest and assimilate prey) attributes, as well as the biological and physical characteristics of their environment (Blaxter & Hempel, 1963; Govoni, 2005). The combination of these and other related variables can have immense consequences for recruitment (Houde, 1987, 2008; Govoni, 2005).

Recruitment variability is dependent upon a complex suite of interrelated factors, including but not limited to egg size, size of larvae at hatch, growth rate, time of spawning and food availability for larvae at the start of exogenous feeding (Bagenal, 1971; Chambers, 1997; Govoni, 2005; Wright & Trippel, 2009). Several hypotheses have been proposed to explain recruitment variability including critical period and transport (Hjort, 1914), match-mismatch (Cushing, 1972) and 'bigger is better' (Miller et al., 1988). The latter is based on the premise that larger larvae have an increased resistance to starvation and decreased vulnerability to predation. Larger eggs and subsequently larger larvae have several advantages over their smaller counterparts; a larger size means a larger yolk sac to provide food reserves before and during early phases of exogenous feeding (Blaxter & Hempel, 1963; Brooks et al., 1997), increased likelihood of survival during times of low food availability (Reznick et al., 1996), increased feeding frequency (Puvanendran & Brown, 1999), and increased swimming capabilities to avoid predation and/or to search for prey (Brooks et al., 1997). These factors can have important implications for marine serial spawners, which commonly release eggs of decreasing size over the spawning season (Bagenal, 1971; Blaxter & Hunter, 1982; Kjesbu, 1989; Trippel, 1998; Rideout et al., 2005).

Maternal effects have also been proposed to explain some of the observed variability in offspring survival and refer to a number of different factors that influences the mother's non-genetic contribution to the eggs (reviewed in Bernardo, 1996 and Green, 2008). Such effects occur when the phenotypic development of the offspring (e.g. variability in size, growth, behavior and survival) is influenced by the phenotype or environment of its mother (Bernardo, 1996; Mousseau & Fox, 1998; Einum & Fleming, 2002; Green, 2008). In marine fishes, it has been documented that the age, size and condition of a female can influence the size, condition and viability of her offspring (e.g. Chambers & Leggett, 1996; Solemdal, 1997; Berkeley et al., 2004; Rideout et al., 2005; Higashitani et al., 2007). For Atlantic cod (*Gadus morhua*), positive correlations between egg size and larval size at hatch have been established (Knutsen & Tilseth, 1985; Chambers & Leggett, 1996; Trippel, 1998). Furthermore, the relationship between egg and/or larval size and maternal body size (Marteinsdottir & Steinarsson, 1998), age (Chambers & Leggett, 1996; Kjesbu et al., 1996) and condition (Kjesbu et al., 1991; Oullet et al., 2001) have been well documented. Yet, little is known about inter-batch effects for serial spawners such as the Atlantic cod, where maternal allocation among batches differ (Kjesbu, 1989; Trippel, 1998; Chapter 2) and may thereby influence larval performance.

To better understand how maternal effects can impact larval performance in the batch spawning Atlantic cod, we implemented a paired mating system which allowed us to collect individual egg batches from eight females. The progeny were subjected to a feeding experiment to determine whether larger larvae do have a survival and growth advantage over their smaller counterparts. Therefore, our main objective was to determine how inter-batch differences can impact larval performance, as such differences may be manifested in response to differing food conditions for larvae from the same female, same egg batch or even from consecutive egg batches of the same female. To test for the influences of egg batch and egg size within and among females on progeny traits we: (1) compared the body weight and morphological characteristics (standard length, eye diameter, jaw length, myotome height and yolk sac area) of 0 and 5 day post hatch (dph) larvae of egg batches within and among females; and (2) quantified the effect of feeding regime on survival, specific growth rate and condition within and among egg batches of different females. It is postulated that since larger eggs (produced early in the spawning cycle) give rise to larger larvae, larvae from early egg batches will have higher survival and better growth rates than their smaller counterparts from later egg batches and these differences in larval performance would be more evident at the low food levels.

3.2 Materials & Methods

3.2.1 Mating Pairs of Adult Cod

Atlantic cod captured by longline off the coast of southwestern Nova Scotia during August 2005 were transferred to communal holding tanks at the St. Andrews Biological Station, New Brunswick, Canada (45° 4' 56" N, 67° 5' 5" W). The fish were fed a diet of squid (*Illex illecebrosus*), Atlantic herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) three times weekly until 2 weeks prior to the start of spawning, at which time feeding was discontinued (Fordham & Trippel, 1999). Eight adult cod in pre-spawning condition of each sex (i.e. females with distended abdomens and males releasing milt) were anaesthetized with tricaine methanesulphonate (MS-222), measured for total length, weighed and tagged with spaghetti-type and passive integrated transponder (PIT) tags (Table 3.1). Attempts were made to match breeding males and females of relatively similar body size together (Rakitin et al., 2001), and placed in 3 m^3 tanks outfitted with an in-tank egg collector and drain collector (described in Thorsen et al., 2003). Individuals were presumed to be repeat spawners based on their body size (Trippel, 1998). A mixture of ambient and heated seawater was used to maintain water temperature at ~ 4°C (± 1°C) throughout the spawning period.

3.2.2 Egg Collection and Incubation

A spawning event was classified as the interval during which an egg batch was collected (usually a day), followed by a 48 hour period or more during which no eggs were produced (Chambers & Waiwood, 1996). Collectors were checked twice daily. Eggs from both the in-tank egg collector and drain collector were obtained and combined in a 3 L beaker containing chilled seawater (~4°C) to determine total egg volume for each egg batch using volumetric displacement. Eggs were then gently stirred to homogenize the sample, and a sample of 100 eggs was taken while in suspension in the water column and placed under a stereomicroscope (magnification 40X) to estimate fertilization success for each batch by enumerating number of fertilized and unfertilized eggs. If an egg batch had only unfertilized eggs it was placed in a refrigerator for 6-8 hours and then re-examined to ensure ample time for the first blastodisc cleavage to occur (Rideout et al., 2005).

Mean diameter of 75-100 eggs from each batch was determined using image analysis software (*Image-Pro Plus®*) from images captured by a digital camera (*Micropublisher 3.3 RTV Q Imaging*) attached to a stereomicroscope (*Olympus SZH*). A sample (2- 5 mL) of eggs was fixed in 4% formaldehyde solution, which was used to estimate mean egg dry weight (mg) ~ 5 months post preservation. Prior to drying, eggs were briefly soaked in weak acetic acid to remove traces of formalin residue (Trippel, 1998). Eggs were dried (three samples of 20 eggs per batch) at 60°C for 48 hours and cooled in a desiccator for an hour and then each sample was weighed ($\pm$ 1 mg). Individual dry weight per egg was estimated by dividing total sample weight by the number of eggs in the sample.

Each egg batch was incubated in 3 L plastic containers at 4°C ± 0.5°C until hatch. Dead eggs, which sank to the bottom of the incubator, were removed daily and initially, 75% water changes were performed daily followed by 50% after 3-4 days post fertilization (dpf). Although hatching occurred over a span of several days (larvae were removed daily), the day with the highest number of emerging larvae is referred to as peak or modal hatch (i.e. 0 days post hatch (dph); Rideout et al., 2005) and only larvae from this date were used for morphological measurements of each batch. Hatching success was not estimated.

3.2.3 Larval Morphology (0 & 5 DPH)

Morphological characteristics were measured on unfed larvae at 0 and 5 dph to assess changes in larval morphology over this period for 26 of the egg batches. Although 43 egg batches were collected from the eight spawning pairs, not all batches were fertilized or produced hatchlings. At the time of peak hatch, approximately 40-45 larvae from each batch were randomly removed from each 3 L incubator. Twenty of these larvae (0 dph) were overdosed with MS-222, digital images recorded (to determine morphometric measurements) and each larva individually fixed in a 1.5 mL Eppendorf tube containing 4% buffered formaldehyde. The remaining larvae (20-25; unless fewer were available) from each batch were placed in a 50 mL glass beaker in a controlled temperature room (9 ± 1°C; temperature used for subsequent experiment) for 5 days. After which, larvae were removed, overdosed with MS-222, image recorded and individually fixed in a 1.5 mL Eppendorf tube with 4% buffered formaldehyde. This permitted assessment of morphological development of unfed larvae at the temperature at which feeding trials were conducted. Image analysis software (*Image Pro Plus®*) was used to record standard length (SL), myotome height (MH), eye diameter (ED), yolk sac area (YSA) and jaw length (JL) of 0 and 5 dph larvae from each batch per female (Figure 3.1). Mean larval dry weight was obtained for 0 and 5 dph larvae approximately 5-6 months after preservation. Individual larval dry weight (15 larvae per batch; unless fewer were available) was obtained from the 0 and 5 dph larvae according to Trippel, (1998), as previously described above (see section *3.2.2 Egg Collection and Incubation*).

3.2.4. Feeding Trial

At hatching, larvae were enumerated and placed into 250 ml glass beakers in a temperature controlled room to acclimate the fish to 9°C over a period of 30-45 min. Larvae from 19 of the 26 batches, which were of ample quantity to be used in the feeding trial, were then transferred to 40L glass aquaria maintained at 10 ± 1°C for subsequent rearing. The four external sides of each aquarium were covered with black plastic and aquaria were placed on a light beige surface to allow for upwelling light (Downing & Litvak 1999). Larvae were exposed to a 24-hour light regime with a light intensity of 450 lux and water temperature maintained at 10°C by flow-through delivery (6 l/h) (room temperature 9 ± 1°C). Stocking density varied based on total number of surviving larvae at time of hatch (ranged between 150 and 300 larvae per replicate (duplicate or triplicate) per batch).

Aquaria water was 'greened' with 1 mL of Instant Algae® Nanno 3600TM (*Nannochlorpsis* sp, cell count approximately 68 billion cells/mL) just prior to the addition of the larvae. Larvae were fed 1 mL of algae twice daily for 2 days before the introduction of rotifers *Branchionus plicatilis* (enriched with *Protein Selco® Plus* [INVE]). Each batch of larvae used in the feeding trial was divided in two, with one half receiving a low food treament (1,500 rotifers per L) and the other half a high food treatment (4,000 rotifers per L). The larvae in each treatment were fed three times daily for 15 dph before the experiment was terminated. The termination of the feeding experiment coincided with the time at which *Artemia* would be introduced into the diet (Puvanendran & Brown, 2002). Prey densities were determined based on a study by Puvanendran and Brown (1999), which showed larvae fed 0-1,000 prey/L often displayed poor survival while those fed higher prey densities (≥ 4,000 prey/L) showed greater survival. Aquaria were equipped with a banjo-type filter consisting of a 500 μm mesh screen to filter overflow and an airstone to help circulate larvae and food, but positioned so it did not interfere with larval swimming and or feeding. Aquaria were siphoned daily to remove excess food and dead larvae that may have settled on the bottom.

On the final day of the experiment (15 dph), larvae were fed an hour before they were euthanized to distinguish between feeding and starving larvae. Starving larvae were identified by their thin, emaciated appearance and paucity or absence of food in their gut.

Euthanization was carried out using an overdose of MS-222. Each larva was photographed digitally to assist in identification of nutritional status. Percent survival per replicate was determined based on the number of satiated larvae (i.e., excluding starved larvae) at the end of the experimental period in relation to initial number stocked. Larvae were individually fixed in a 1.5 mL Eppendorf tube with 4% buffered formaldehyde. Digital images of 15 randomly chosen 15 dph larvae (unless fewer were available) were used to assess larval standard length and myotome height using *Image Pro Plus®*. Specific growth rate (SGR) over the experimental period was calculated using:

$$\mathrm{SGR} = \frac{[\ln(L_2) - \ln(L_1)]}{(t_2 - t_1)} \times 100\%$$

where L represents standard length (mm) at time t_1 (0 dph) and t_2 (5 and 15 dph respectively). A modified Fulton's condition factor (K; Wootton, 1990) was also determined using:

$$K = \frac{W}{L^{3.6}} \times 100$$

where W represents larval dry weight (mg) and L represents larval length (mm). Unlike the traditional Fulton's condition factor equation that assumes an allometric slope of 3, we calculated the true slope (3.6) based on a regression analysis of log larval standard length and log larval dry weight. Additionally, myotome based condition index (MCI) was calculated using standard length (SL; mm) and myotome height (MH; mm) (Koslow et al., 1985; Puvanendran & Brown, 1999):

$$\mathrm{MCI} = \frac{\mathrm{MH}}{\mathrm{SL}}$$

Condition indices based on two-dimensional measurements (i.e, myotome and standard lengths) are non-lethal and simple to estimate, but their ability to represent nutritional status like the more traditional measures that use length and body weight have not, to our knowledge, been previously explored. We used this opportunity to examine the relationship between these two condition indices for larvae at three time points; 0, 5 and 15 dph.

Following the methods described previously (see section *3.2.2 Egg Collection and Incubation*), mean dry weight of 15 dph larvae was determined ca. 6-8 months after preservation. Individual dry weights (15 larvae per batch; unless fewer were available) were obtained for the same larvae from which standard length and myotome height were measured.

3.2.5 Statistical Analysis

Two principal component analyses (PCA) were conducted using SPSS statistical software (© SPSS Inc, release 17.0.1). Prior to PCA, percent data were arcsine square root transformed, while all other data were log transformed accordingly to meet the assumptions of the analysis. The first PCA was used to assess the morphological changes between 0 and 5 dph. It was performed using a correlation matrix on the mean log transformed data to identify the patterns associated with female (as measured by condition factor), batch and egg size. The second PCA was used to determine the effects batch and feeding regime had on larval performance over the feeding trial (15 days). It was also performed using the correlation matrix on the mean transformed data to help identify patterns associated with prey availability, batch and female effects on survivorship, specific growth rate and condition factor during the first 15 dph. PCA results were based on the rotated component matrix (Varimax rotation with Kaiser Normalization). Additionally, to determine whether there was a difference in larval survival, growth rate and condition between the two feeding regimes among the females and batches (co-variate; measured as phase in the spawning season [i.e. the cumulative number of eggs a female had spawned up to and including a particular egg batch divided by her total fecundity] *see Chapter 2.2.5* for more details) a series of two-way ANCOVA's were conducted (© SPSS, 17.0.1).

Lastly, to produce predictive relationships between the two condition indices (Fulton's condition and myotome condition index) linear regression equations were determined using the pooled data, as well as separate data at 0, 5 and 15 dph. The purpose of this additional analysis was to determine whether one index was better than the other at determining larval condition at different points during the early life history.

3.3 RESULTS

3.3.1 SPAWNING PERFORMANCE

Egg production was observed from early February to mid March. The number of egg batches produced among females varied between three and seven and in total 43 egg batches were produced by the eight females over an average of 20 days per female, with approximately three days between each spawning event (Table 3.1). Although all females released eggs, not all egg batches were fertilized; fertilization success between batches and among pairs was highly variable ranging from 0 to 99% (see *Chapter 2, Figure 2.1*).

Batch fecundity varied between 33,050 and 519,300 eggs, and generally followed a dome shape for each female, increasing initially before decreasing with each successive batch (see *Chapter 2, Figure 2.1*). Additionally, the investment in spawning resulted in a decrease in body mass over the course of the spawning period; overall, females experienced greater weight loss (25.40% ± 3.31%) than males (16.48% ± 5.70; Table 3.1). Egg diameter (1.73mm ± 0.003 to 1.40mm ± 0.002) and dry weight (0.116mg ± 0.002 to 0.062mg ± 0.0004) also decreased with each successive batch (see *Chapter 2, Figure 2.2*) for the eight females and these egg characteristics were found to be positively correlated ($r^2 = 0.646$, $p < 0.001$; see *Chapter 2*).

3.3.2 Larval Morphometrics: 0 & 5 DPH

Larval morphology differed markedly between 0 and 5 days after hatching (details in Appendix 13). At 0 dph, larvae were characterized by a smaller standard length, dry weight, myotome height and eye diameter compared to 5 dph (Figure 3.2 A-C). Larvae at 0 dph lacked a noticeable jaw, but by 5 dph a well defined jaw line was evident and the yolk sac was partially or almost completely absorbed (Figures 3.1 and 3.2 C, D). Specific growth rate over the first 5 dph averaged 1.84 ± 0.74 %/day in standard length. While Fulton's condition factor of larvae declined between 0 (0.045 ± 0.007) and 5 dph (0.030 ± 0.004) no such pattern was evident in terms of myotome condition index (0 dph: 0.052 ± 0.007; 5 dph: 0.051 ± 0.007). Furthermore, when the relationship between modified Fulton's (K) and myotome condition index (MCI) was examined, it was found to be positive and almost significant at 0 dph (K_{0dph} = - 0.0198 + 1.24 MCI_{0dph}; r^2 = 0.152, p = 0.054) and significant at 5 dph (K_{5dph} = - 0.0245 + 1.0 MCI_{5dph}; r^2 = 0.204, p = 0.020).

The first three principal components explained ca. 57% of the cumulative variance (rotated sums of squared loadings), of which the first component explained 30% of the variance (details in Appendix 14). The first component (PC 1) had high positive loadings with female condition factor (0.574), measures of size, including that of egg dry weight (0.601), 0 dph eye diameter (0.679) and myotome height (0.577) and 5 dph standard length (0.930), eye diameter (0.876), myotome height (0.728) and jaw length (0.895) (details in Appendix 15). This appears to be indicative that females in better condition produced larger eggs and consequently larger sized larvae (Figure 3.3). Myotome condition index at 0 dph (0.802) and 5 dph (0.877) had the highest loadings with the second principal component (PC 2; Figure 3.3), while the third component (PC 3) was explained mainly by strong, positive loadings with yolk sac area at both 0 dph (0.914) and 5 dph (0.917) (Figure 3.3).

3.3.3 Feeding Trial: Larval Survival, Growth & Condition

A PCA of the feeding trial data produced four principal components that explained ca. 78% of the cumulative variance (eigenvalues >1), of which the first component explained 40% (details in Appendix 16). The first principal component (PC 1) had high positive loadings with measures of larval size (dry weight [0.748], standard length [0.948] and myotome height [0.927]), specific growth rate (0.866) and myotome condition index (MCI; 0.924) (Figure 3.4; details in Appendix 17). The second principal component (PC 2) was associated with an inverse relationship between batch (-0.776) and egg size (0.912) (Figure 3.4), whereby egg size declined with each successive egg batch produced. The third principal component (PC 3) was positively associated with food supply (0.541) and modified Fulton's condition index (0.864), which indicates the possibility of feeding regime having a positive influence on larval condition factor as measured by the modified Fulton's condition index (Figure 3.4). The fourth principal component (PC 4) was most associated with female condition factor (0.800) and larval survival (0.663) indicating that the females in better condition produced larvae which had higher survival rates.

Surprisingly, no significant differences were observed in terms of survival, growth rate or condition between the two feeding trials, except for myotome condition index. Survival rates among female and batches between the two feeding regimes were highly variable, ranging from 0.9% to 60% (mean 15.88 ± 12.37%) at low food and from 0% to 45% (mean 15.20 ± 12.09%) at high food (Figure 3.5). Likewise, specific growth rate during the first 15 dph ranged from 1.02 to 3.55 %/day in length (mean 2.30 ± 0.53 %/day) at low food and from 0.16 to 3.57 %/day (mean 2.40 ± 0.80 %/day) at high food (Figure 3.6). Larval condition as measured by modified Fulton's condition factor (K) ranged from 0.0308 to 0.0581 (mean 0.0131 ± 0.002) for the larvae at low food and from 0.007 to 0.058 (mean 0.0136 ± 0.002) at high food (Figure 3.7 A, B), while myotome condition index (MCI) ranged from 0.007 to 0.069 (mean 0.0586 ± 0.004) for the larvae at low food and from 0.045 to 0.0578 (mean 0.0603 ± 0.005) at high food (Figure 3.7 C, D).Furthermore, the relationship between the two condition indices for fed larvae at 15 dph was found to be positive and significant ($K_{15dph} = - 0.0196 + 1.08\ MCI_{15dph}$; $r^2 = 0.691$, $p < 0.001$). Moreover, the amount of variation explained by the relationship was noticeably greater than that at 0 ($r^2 = 0.152$) and 5 dph ($r^2 = 0.204$). The relationship for the pooled data (i.e. from 0, 5 and 15 dph) was: K_{pooled} = - 0.0151 + 1.01 MCl_{pooled} (r^2 = 0.448, p < 0.001).

To further determine if the differences in means for survival, specific growth rate and larval condition differed between the two feeding groups with female and batch (as measured by phase in the spawning season (co-variate)) a series of two-way ANCOVA's were completed. The interaction term between female and food supply on survival ($F_{7,74}$ = 1.079, p = 0.386), specific growth rate ($F_{7,74}$ = 1.263, p = 0.280), modified Fulton's condition ($F_{7,74}$ = 1.205, p = 0.311) and myotome condition factor ($F_{7,74}$ = 2.053, p = 0.059) were all non-significant. When broken into their individual components, significant effects were observed with regards to female for survival ($F_{7,74}$ = 8.208, p < 0.001), specific growth rate ($F_{7,74}$ = 7.286, p < 0.001) and myotome condition index ($F_{7,75}$ = 18.416, p < 0.001), indicating the presence of female effects on these variables. A significant effect of food supply (i.e the low food or high food regime) was only evident on myotome condition index ($F_{1,74}$ = 4.541, p = 0.036). The co-variate batch number, as measured by the phase in the spawning season, was found to have a significant effect on specific growth rate ($F_{1,74}$ = 40.115, p < 0.001) and myotome condition index ($F_{1,74}$ = 13.076, p = 0.001), indicating the presence of batch effects.

3.4 Discussion

With regards to our primary objective, we found that inter-batch differences within females, as expressed in terms of variation in egg size with batch sequence, impacted larval performance through effects on larval morphology, survival, growth and condition. Furthermore, among female effects were detected with regards to the number of egg batches produced, egg size, larval size at hatch and larval survival at 15 dph. Positive relationships were found between female condition and the size of eggs extruded (also Chambers & Waiwood, 1996), as well as between egg size and larval size during the early life history (also Knutsen and Tilseth, 1985; Marteinsdottir and Steinarsson, 1998), indicating the presence of maternal effects. Interestingly, female effects, as measured by female condition, had a much stronger correlation with larval size at 0 and 5 dph than at 15 dph, while larval survival at 15 dph continued to show a strong positive correlation with female condition. These relationships imply that size gains made early in development may not continue long into the larval stage or that differential mortality of the smallest larvae arising predominantly from females with lower condition may reduce size differences associated with female effects as early life history progresses (cf. Einum & Fleming, 2000). Akin to previous cod studies, egg size declined over the spawning season and typically followed a dome shape for each female (Kjesbu, 1989; Chambers & Waiwood, 1996; Trippel, 1998). As a result, a strong inverse relationship between egg size and batch number was observed, as well as a positive relationship between egg size and larval size at hatch.

The positive relationship between egg size and larval size at hatch noted in the present study has important implications. A key premise of many recruitment hypotheses is that larval size plays an important role in survival, in addition to other environmental factors. Larvae of a larger size at hatch typically have larger morphological traits (e.g. eye size, myotome height, jaw length) that may make them more adept at obtaining food and avoiding predation than smaller larvae (Letcher et al., 1996; Reznick et al., 1996; Kamler, 2005; Houde, 2008). Additionally, larger larvae usually have larger yolk reserves, which provide them with prolonged food reserves before the need for exogenous feeding (Trippel, 1998; Rideout et al., 2005; Kennedy et al., 2007). The amount of yolk available to the larvae before the start of exogenous feeding aids in survival, especially during periods of low food availability in the wild, but may not be as critical in larviculture, as food levels are generally high in captive conditions such as those associated with commercial aquaculture. Results from the PCA also indicated a positive relationship between the amount of yolk sac reserves available to the larvae at both 0 dph and 5 dph, which implies that individuals with larger yolk sacs continue to have more food reserves available to them 5 dph than perhaps larvae with smaller yolk sacs. However, no clear relationship can be deduced between yolk sac area and survival to 15 dph from this study. Furthermore, inadequate food supply following hatch can lead to starvation (i.e. little or no food in the gut, very thin with small myotome height and disproportionately large eyes) and increased mortality and/or decreased growth, and moreover prolong the period during which larvae are highly vulnerable to predation (Welker et al., 1994; Puvanendran & Brown, 1999; Smith et al., 2005). During endogenous feeding, the egg yolk lipids are utilized by the developing embryo for energy catabolism during development (Wiegand, 1996) and it has been shown that maternal nutrition can influence egg lipid concentrations (Sargent, 1995; Lavens et al., 1999; Mazorra et al., 2003). However, the effect of different batches (e.g. early versus late in a female's spawning cycle) on egg lipid biocomposition has been rarely explored (Ulvund & Grahl-Nielsen, 1988; Pickova et al., 1997) and results of Chapter 2 indicate there may be little or no change in lipid and fatty acid composition among egg batches of the same female, contrary to what might be expected.

Specific growth rate was strongly positively correlated with measures of larval size and myotome-based condition index at the end of the 15 dph (post yolk sac absorption) feeding trial. While it is not surprising that faster growing larvae were larger at 15 dph than slower growing larvae, their superior condition at the end of the feeding trial may have further compounded effects on subsequent fitness. Furthermore, growth rate increased with dph (i.e. from ca. 1.84 to 2.35%/day between 5 and 15 dph), which may explain why relationships between specific growth rate and larval morphometric measurements strengthened from 5 to 15 dph. In contrast to our findings, Kennedy et al. (2007) studying larval Norwegian plaice (*Pleuronectes platessa*) found specific growth rate to be greatest in larvae which had small standard lengths at hatch and a large yolk sac area. They concluded that the high specific growth rate of the smaller larvae resulted from a lower respiration rate, which provided a longer period of endogenous feeding, compared to larger larvae that presumably required more food in order to meet metabolic needs. Because no significant differences in growth rates were observed between larvae from the low food regime and those from the high food regime and a positive correlation was noted between larval size and specific growth rate, their reasoning does not seem to hold true for our study. A more plausible explanation for our results may be embedded in the notion of the 'bigger is better' hypothesis, which insinuates that larger, faster growing individuals (larger yolk sac for endogenous feeding, a larger size means a larger mouth gape to capture prey and the ability to swim faster) may have a survival advantage over smaller, slow growing counterparts (Houde, 2008; reviewed in Govoni, 2005). Although survival was not strongly correlated with specific growth rate in our study, we did find that larval survival at the end of the feeding trial had a strong maternal influence, as females in better condition produced larvae with higher overall survival rates.

It is not uncommon that feeding experiments of these types are often characterised by high mortality rates, as was observed in this study (ca. 75%). Previous feeding studies on larval growth and survival of gadoids have investigated the effects of delayed feeding (Zhao et al., 2001), the effect on early life history performance (Rideout et al., 2005) and optimal feeding regimes and conditions (Puvanendran & Brown, 1999; Puvanendran & Brown, 2000). This study was most similar to that of Rideout et al. (2005), which investigated the effect of egg size and food supply on early life history performance; however, differences in results between our study and theirs can be attributed to differences in early life history between the two species as haddock larval survival in captivity is often low even under high prey densities (Downing & Litvak, 1999). In the present study, although overall larval performance (i.e. growth, survival) was slightly better under the high food regime , there were no significant differences in larval performance between the two feeding regimes that differed by 2.7-fold in food density. By contrast, previous studies have shown that higher prey densities typically lead to an increase in larval survival and growth (Welker et al., 1994; Puvanendran & Brown, 1999; Zhao et al., 2001; Rideout et al., 2005). On closer examination, larvae from some of the female/batch pairs in our study actually had superior growth and survival at the lower food concentrations (e.g. batches 3 and 4 of female 6). It is possible that the survivors represented the strongest and most 'fit' individuals of the experimental period, as these larvae were best able to maximize the use of the available resources. Additionally, these differences may be attributed to female and batch effects. In hindsight, it is also possible that the range of feeding densities administered was too narrow to detect significant trait differences. Puvanendran & Brown (1999) identified a minimum prey threshold concentration for the survival of cod larvae of 1,000 prey /L and found cod larvae reared at 2,000 prey/L achieved similar growth rates as those reared at 4,000 prey/L by the end of three weeks post hatch. It is also plausible that the duration of our experiment (15 d) did not allow us the opportunity to observe the full scope of effects, as the small, but non-significant, differences in survival, growth and condition at 15 dph between females, batches and feeding regimes may have biological consequences later on.

Also of importance in this study was the observation that the two measures of condition examined, modified Fulton's K and myotome-based condition index (MCI), appeared to provide different measures of body form. As Fulton's K uses the larval weight, declines in K over the first 5 dph might be reflective of a number of processes such as a disproportionate increase in length with only minor loss of mass associated with yolk utilization and metabolism. Consequently, the difference in condition as measured by K, does not necessarily mean larvae are in "bad health" but may rather be representative of physiological processes occurring. As such, under some circumstances, using morphormetric measurements to determine the condition of newly hatched larvae may be more appropriate than using Fulton's. Neilson et al. (1986) suggested that the use of a single morphological index, such as Fulton's K, may inadequately depict the condition of larvae in an early life stage because they are undergoing rapid morphological changes. Our findings appear to support their conclusion; for example, we found that myotome based condition index detected a difference in larval condition in larvae exposed to the feeding trial, though this difference was not detected using Fulton's condition index. Furthermore, our analysis of the two condition indices showed that as time progressed (i.e. from 0 dph (r^2 = 0.152) to 5 dph (r^2 = 0.204) to 15 dph (r^2 = 0.69)), the relation between the two indices became sufficiently strong to where MCI may suffice alone as an indicator of overall condition, avoiding the need to sacrifice larvae to obtain dry weights for calculating K. Additionally, the difference between the two indices suggests they provide different measures of 'nutritional status' and/or shape of post hatch larvae. Although K is usually used to compare larvae within a particular life stage or age group, it might be worth considering using both K and MCI when characterizing nutritional status of early-stage larvae.

In conclusion, the findings of this study could have important implications for understanding recruitment variability in wild fish populations, especially during times of varying food concentrations and in terms of parental attributes that influence egg size. For the aquaculture industry, our findings indicate there can be considerable variability in larval survival between females and even among egg batches within females that can be difficult to predict. It was clear, however, that larval growth was positively associated with egg and larval size, and may thus be a valuable indicator for choosing eggs for culture.

ACKNOWLEDGEMENT

Financial support for this study was provided by Fisheries and Oceans Canada (ACRDP; EAT) and an NSERC Discovery grant (IAF). Special thanks to staff members and technicians of the St. Andrews Biological Station, especially, Steven Neil, John Reid, Ken Howes, Marc Blanchard, Susan Fordham and Nicola Cross for their assistance with various aspects of the project. Additional thanks also go to Corinne Conway for assistance with morphometric measurements at the Ocean Sciences Centre and the graduate students of the Fleming lab for their helpful comments on earlier drafts of the chapter.

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Table 3.1: Spawning activity (% weight loss, number of egg batches produced per female, number of days spawning, the mean number of days between each spawning event per female, and the start and end date of spawning per pair) of the mating pairs of Atlantic cod (weight and length) used in the 2006 experiment.

	Mating Pair	Female Weight (kg)	Female Length (cm)	Female Weight Loss (%)	Male Weight (kg)	Male Length (cm)	Male Weight Loss (%)	No. Egg Batches	No. Days Spawning	Mean No. Days Between Spawning	First Spawning Event	Final Spawning Event
	1	3.96	68.3	21.97	4.54	68.0	8.81	5	16	3.0 ± 1.7	Feb-06	Feb-21
	2	3.88	67.0	37.32	4.09	68.4	16.38	3	13	4.0 ± 2.8	Feb-10	Feb-22
	3	5.74	75.5	25.96	4.53	71.6	22.30	5	18	3.4 ± 0.5	Feb-03	Feb-20
	4	5.56	76.8	25.72	4.21	69.9	13.06	6	37	6.2 ± 1.9	Feb-05	Mar-13
	5	4.09	66.3	17.36	3.09	60.4	11.33	6	22	3.5 ± 0.5	Feb-03	Feb-24
	6	5.56	74.0	22.66	4.13	65.4	24.64	5	18	3.4 ± 1.5	Feb-20	Mar-09
	7	4.21	68.4	25.65	3.42	64.4	21.35	6	20	3.2 ± 0.4	Feb-06	Feb-25
	8	3.92	64.9	26.53	4.08	67.9	13.97	7	23	3.1 ± 0.4	Feb-06	Feb-28
Mean		**4.62**	**70.2**	**25.40**	**4.01**	**67.0**	**16.48**	**5.4**	**20.9**	**3.7 ± 1.0**		

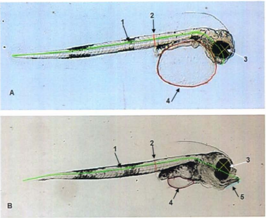

Figure 3.1: Illustration of the morphological traits measured on (A) 0 and (B) 5 days post hatch (dph) larvae: (1) standard length, SL, (2) myotome height, MH, (3) eye diameter, ED, (4) yolk sac area, YSA and (5) jaw length, JL, which is absent in 0 dph larvae but present in 5 dph larvae.

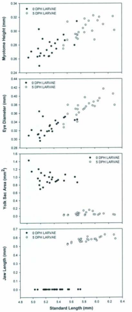

Figure 3.2: Morphological changes in (A) myotome height, (B) eye diameter, (C) yolk sac area and (D) jaw length between 0 and 5 days post hatch Atlantic cod larvae with increasing standard length.

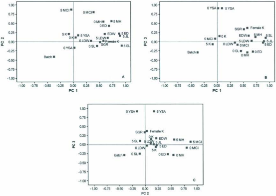

Figure 3.3: Two-factor plots of the rotated principal component data matrix of the 0 and 5 dph morphological traits showing (A) the loadings of the first two principal components and (B) the loadings of principal components one and three of the analysis and (C) the loadings of principal components two and three. (0 = 0 dph; 5 = 5 dph; EDW = egg dry weight; LDW = larval dry weight; SL = standard length; MH = myotome height; ED = eye diameter; YSA = yolk sac area; JL = jaw length; K = Fulton's condition index; MCI = myotome-based condition index).

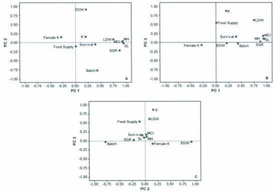

Figure 3.4: Two-factor plots of the rotated principal component data matrix of the feeding trial showing (A) the loading of the first two principal components and (B) the loading of principal components one and three and (C) the loadings of principal components two and three. (EDW = egg dry weight; LDW = larval dry weight; SL = standard length; MH = myotome height; SGR = specific growth rate; K = Fulton's condition index; MCI = myotome-based condition index).

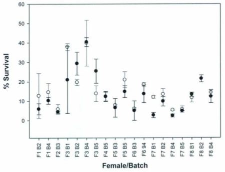

Figure 3.5: Mean (± SE) larval survivourship ([○] low food, [●] high food) per batch per female at the end of the 15 day feeding experiment under low food and high food conditions. (Note: F8 B2 had identical mean (± SE) at both low and high food).

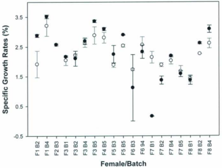

Figure 3.6: Mean (± SE) specific growth rates (%/day in mm) of larvae at the end of the 15 day feeding trial under (○) low food and (●) high food conditions.

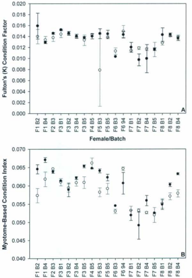

Figure 3.7: Mean (± SE) condition indices of larvae at the end of the 15 day feeding trial for (A) Fulton's condition and (B) myotome-based condition Index under (○) low food and (●) high food conditions.

Chapter 4: General Discussion

4.1 Key Findings

Often studies involving batch spawners tend to neglect the impact batch and/or female effects can have on their results, as eggs are often pooled and/or multiple females used. Chambers & Leggett (1996) indicated that batch spawners deserve "special consideration" due to the high variation associated with egg production, as egg size typically declines over the spawning season. In order to fully consider female effects in these spawners, either all egg batches need to be collected or batch number needs to be considered. As such, the aim of this thesis was to fill a missing gap in the current literature by evaluating the magnitude of various elements of maternal effects in Atlantic cod (*Gadus morhua*), a temperate batch spawner, which is of interest to both the commercial fishery and aquaculture industry. The unique aspect of this study was that we were able to investigate the impact that both *within* and *among* female differences can have on egg composition and larval traits in cod of *wild* origin. A summary of the key findings along with their ramifications are discussed below.

As expected, the duration of the spawning season, fecundity (both batch and total) and the seasonal decline in egg size over the spawning season were similar to that established in the current literature (Kjesbu, 1989; Knutsen & Tilseth, 1985; Chambers & Waiwood, 1996; Trippel, 1998). Likewise, as observed in other gadoids, larval size at hatch and common morphological measurements (i.e. eye diameter, standard length, myotome height, jaw length and yolk sac area) were positively related with egg size (Knutsen & Tilseth, 1985; Chambers & Leggett, 1996; Rideout et al., 2005). However, as I began to explore my original hypotheses, the results were less clear-cut and yielded some exciting results.

4.1.1 Egg Lipid Composition

In the wild, factors that influence early life history (e.g. reproductive strategy, food availability, batch sequence, egg composition, larval size and condition) should be incorporated into models used to forecast recruitment variability (Kamler, 2005). Egg composition is thought to be reflective of egg quality, which in its simplest terms is the ability of an egg to give rise to a viable offspring (Kamler, 1992; Wiegand, 1996; Czesny et al., 2005). Understanding egg composition is essential because it can have a significant influence on embryonic development, hatching success and larval survival (Czesny et al., 2005; Kjørsvik et al., 1990; Mazorra et al. 2003; Pickova et al., 1997). However, maternal reserves in batch spawners may be limited as *some* females fast during the spawning season (Fordham & Trippel, 1999; but see Michalsen et al., 2008). It has been assumed that the decline in maternal reserves as the spawning season progresses is expressed as a decline in the amount of lipid invested in each successive egg batch (i.e. smaller yolk sac area with each subsequent egg batch). However, not many studies have investigated lipid composition with regards to batch sequence as samples are often pooled. Therefore, the primary objective of Chapter 2 was to determine the seasonal change in egg lipid composition over the spawning season within *individual* female cod and to determine whether the change in lipid composition was similar *among* females.

According to my results, over the spawning period, some females showed significant declines between the initial and final egg batch in the investigated lipid classes and fatty acids. However, some females showed little/no change in lipid composition between the initial and final egg batches, but the most unexpected result was that a couple of females showed consistent increases over the spawning season in the deposition of lipid classes and fatty acids. While, I cannot explain why some females would increase the lipid deposition per egg as their overall reserves become depleted, I did infer that the differences in trends among the females could be attributed to their wild origin. Other factors such as maternal age, diet (in the wild) and spawning experience may also explain some of the variation observed among females.

Given that our results demonstrated high levels of variation in lipid deposition in cod eggs of wild origin, it would be worth determining if similar patterns of lipid allocation occur in eggs from captive stocks. It is hypothesised that egg lipid composition among females would be less variable in captive broodstock, as females are maintained on formulated diets. Understanding, how lipids are deposited in eggs can help aquaculturists better select females producing higher quality eggs and this in turn could also aid in formulating broodstock diets composed of preferred lipid content and fatty acid composition.

4.1.2 Effect of Diet on Larval Morphology, Survival, Growth and Condition

Although many studies have investigated the effect of diet on morphology, growth, condition and/or survival, this study was unique as it also took into account both female *and* batch effects. In the wild, prey type, abundance and availability can have huge consequences on the survival of larvae (Hjort, 1914; Cushing, 1972; Kamler, 1992), but with the development of appropriate protocols for administering live planktonic organisms this should not be problematic in an aquaculture setting, where larval food conditions are near optimal.

Surprisingly, in my study no significant differences in terms of survival, growth and/ or condition (except for myotome condition index) between our two feeding trial groups was observed, but perhaps the most important finding was the indication of *both* female and batch effects. Despite the fact that my study did not determine how these batch effects influenced my feeding study, it is reasonable to deduce that under high food conditions (in both the wild and aquaculture) batch number would have very little impact on growth and survival because larvae would have more than enough food available. However, batch number might be more important under low food conditions because food is a limited resource and only those larvae with a survival advantage (i.e. larger sized larvae) can best survive under less than optimal conditions. The 'bigger is better' hypothesis implies that a larger size at hatch infers a survival advantage as larvae typically have larger yolk sacs to sustain them until first feeding (Miller et al., 1988). Furthermore, the findings from this study demonstrated the influence inter-batch and female effects (i.e. variation in egg size, number of egg batches, larval size at hatch) had on survival, growth and condition.

Although it was beyond the scope of this study, one way to improve this part of the study would be to incorporate the lipid analysis study and determine how egg composition could have impacted the results of the feeding trial.

4.2 Future Research

This thesis illustrates the importance of why future research on batch spawners should at least take into account batch and/or female effects. Maternal effects can manifest themselves in terms of the number of egg batches spawned, size of eggs extruded, and differences in egg lipid composition (which could consequently affect hatching success and larval survival) that could affect larval morphology, growth and survival. Further research could include:

1. In this study, we were only interested in selected fatty acids. I think it is vital to establish the complete fatty acid profile for individual females and their egg batches over the spawning season, as the various fatty acids and their ratios can be essential predictors of hatching success and egg quality. This should preferentially be established for wild fish populations as we have shown how variable lipid profiles can be for individual females. However, in aquaculture, broodstock diets are still not optimal and research is ongoing; hence, it would also be worth determining how lipid profiles between egg batches vary with individual females. As a comparison, one could also investigate how lipid composition between egg batches differs between wild and captive stocks of individual females.
2. Replication of the feeding experiment, but using more extreme feeding conditions to determine how low food in particular impacts morphology, growth, condition and survival. Additionally using a longer experimental period to determine if those dietary effects can be manifested beyond a 2 week feeding trial.
3. Larger sample size (i.e. more females and batches) and multiple sample years using the same females if possible to see how the results vary year to year and if a larger sample size can detect additional patterns.

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Appendices

Appendix 1: Lipid class means of mean expressed as a % of total lipids (means <1% excluded) for egg batches collected from eight female Atlantic cod.

Female	Batch	Egg Dry Weight (mg)	% Hydrocarbons	% Phospholipid	% Sterols	% TAG	% FFA	% AMPL§	% Neutral Lipid	% Polar Lipid
1	1	0.1041	6.83	74.54	8.59	3.19	3.08	2.64	22.82	77.18
1	2	0.1009	6.20	75.90	8.70	2.72	1.76	4.28	19.81	80.19
1	3	0.0965	4.59	73.31	11.06	2.85	2.07	3.88	22.81	77.19
1	4	0.0890	4.99	85.66	5.74	2.59	0.00	1.01	13.33	86.86
1	5	0.0848	1.81	85.58	7.74	2.66	0.94	1.27	13.15	86.85
2	1	0.0987	3.18	84.04	7.60	3.63	1.55	7.60	15.96	85.04
2	2	0.0905	2.22	85.13	9.58	3.07	0.00	9.58	14.87	85.13
2	3	0.0862	5.31	80.79	7.71	1.63	1.80	7.71	16.45	83.55
3	1	0.0917	3.00	83.81	6.89	3.02	0.81	2.46	13.73	86.27
3	2	0.0939	2.41	82.15	5.36	3.66	1.98	4.44	13.41	86.59
3	3	0.0889	1.21	82.45	10.23	3.18	1.51	1.41	16.14	83.86
3	4	0.0819	3.11	67.72	11.61	6.69	1.68	9.19	23.09	76.91
3	5	0.0765	4.99	74.85	11.39	5.52	3.25	0.00	25.15	74.85
4	1	0.1157	5.25	62.37	9.44	9.71	6.61	6.61	31.01	68.99
4	2	0.1098	4.34	60.53	11.71	10.91	6.08	6.43	33.04	66.96
4	3	0.1114	4.60	74.39	13.25	2.56	2.50	2.70	22.90	77.10
4	4	0.1049	3.57	73.39	15.13	1.85	4.62	1.44	25.17	74.83
4	5	0.0981	3.53	71.25	15.23	1.82	4.85	3.31	25.44	74.56
4	6	0.0852	5.09	67.44	10.97	6.55	4.74	5.21	27.35	72.65
5	1	0.1032	4.95	65.49	10.35	10.21	4.28	4.72	29.79	70.21

Female	Batch	Egg Dry Weight (mg)	% Hydrocarbons	% Phospholipid	% Sterols	% TAG	% FFA	% AMPL§	% Neutral Lipid	% Polar Lipid
5	2	0.0958	3.47	62.58	10.59	13.17	4.67	5.51	31.91	68.09
5	3	0.0920	4.33	70.62	19.43	3.48	0.66	1.49	27.90	72.10
5	4	0.0896	6.66	74.65	8.90	4.22	3.18	2.40	22.95	77.05
5	5	0.0800	4.15	75.22	12.47	2.86	2.79	2.51	22.27	77.73
5	6	0.0756	4.38	62.39	12.25	8.37	4.66	7.95	29.66	70.34
6	1	0.1024	4.69	61.31	12.37	9.15	7.67	4.81	33.88	66.12
6	2	0.1020	3.38	64.69	24.21	3.26	2.60	1.85	33.45	66.55
6	3	0.1059	6.90	68.00	13.55	3.39	6.04	2.12	29.88	70.12
6	4	0.0996	4.88	69.79	15.12	4.57	2.20	3.43	26.77	73.23
6	5	0.0895	5.79	64.85	12.64	6.69	7.20	2.83	32.32	67.68
7	1	0.0822	2.50	80.17	8.74	3.12	4.20	1.28	18.55	81.45
7	2	0.0803	2.11	83.46	8.09	2.69	2.21	1.44	15.10	84.90
7	3	0.0769	3.36	74.53	14.92	2.32	1.65	3.22	22.25	77.75
7	4	0.0732	6.27	77.85	7.90	1.55	4.02	2.41	19.74	80.26
7	5	0.0677	5.22	69.01	8.21	7.06	5.08	5.41	25.57	74.43
7	6	0.0618	9.92	40.70	23.11	10.64	9.02	6.61	52.69	47.31
8	1	0.1078	3.21	65.12	10.89	10.65	5.41	4.72	30.15	69.85
8	2	0.1039	5.18	62.28	11.91	10.52	4.71	5.40	32.32	67.68
8	3	0.0975	2.83	80.71	10.63	3.26	0.76	1.82	17.47	82.53
8	4	0.0918	3.43	82.69	4.27	3.07	4.39	2.16	15.15	84.85
8	5	0.0873	4.42	81.76	5.25	1.87	5.49	1.21	17.03	82.97
8	6	0.0788	7.58	60.60	10.94	7.93	7.96	4.98	34.41	65.59
8	7	0.0723	7.88	59.01	11.89	10.14	3.65	7.45	33.55	66.45

§ Acetone mobile polar lipids

Appendix 2: Fatty acid means of mean expressed as a % of total fatty acids (means <1% excluded) for egg batches collected from eight female Atlantic cod.

Female	Batch	Egg Dry Weight (mg)	% of Fatty Acids > 1%											
			14:0	16:0	16:1ω9	16:1ω7	18:0	18:1ω9	18:1ω7	20:1ω9	20:5ω3	22:1ω11(13)	22:5ω3	22:6ω3
1	1	0.1041	1.97	20.32	0.71	2.88	2.63	9.35	2.56	3.77	14.22	1.27	1.10	28.59
1	2	0.1009	3.42	34.98	1.61	4.24	3.78	15.04	4.05	6.45	4.86	2.59	0.56	7.75
1	3	0.0965	4.31	30.88	1.83	4.85	3.89	12.72	3.35	4.65	7.14	1.06	0.58	10.60
1	4	0.0890	2.08	20.20	1.11	2.62	2.73	9.30	2.63	3.59	13.65	1.34	1.25	29.52
1	5	0.0848	1.93	21.39	1.12	2.45	2.16	9.12	2.40	3.24	13.78	1.08	1.29	30.77
2	1	0.0987	1.78	20.30	0.96	2.15	2.81	9.18	2.41	3.40	15.37	1.48	1.34	30.23
2	2	0.0905	1.92	20.79	1.14	2.18	2.88	9.06	2.59	3.22	16.55	1.26	0.54	29.15
2	3	0.0862	2.03	21.03	1.26	1.97	2.41	7.81	2.15	2.29	16.45	0.59	1.28	31.14
3	1	0.0917	2.09	20.42	1.14	1.87	2.57	8.02	1.98	2.38	19.06	0.36	1.11	28.99
3	2	0.0939	2.06	23.62	0.70	2.68	2.91	10.10	2.69	3.17	15.56	1.12	1.13	24.51
3	3	0.0889	2.34	24.72	1.36	2.20	2.81	9.30	2.29	2.41	16.95	0.49	0.52	24.22
3	4	0.0819	2.16	24.88	1.11	2.08	2.90	8.73	1.88	2.20	19.46	0.00	0.00	29.48
3	5	0.0765	2.72	22.07	1.31	2.12	2.23	7.86	1.73	1.77	19.64	0.00	0.00	28.22
4	1	0.1157	3.91	25.43	0.80	3.98	2.33	10.20	2.44	3.33	15.11	0.77	0.71	21.78
4	2	0.1098	3.82	26.94	1.42	3.63	2.53	10.46	2.53	3.39	14.00	1.21	0.56	19.24
4	3	0.1114	2.05	21.53	0.90	2.19	2.56	9.09	2.31	3.71	15.27	1.50	1.04	28.95
4	4	0.1049	1.65	20.76	1.15	2.29	2.07	8.68	2.07	2.81	14.04	0.93	1.11	30.67
4	5	0.0981	2.11	22.58	1.08	2.62	2.94	10.34	2.88	3.71	13.50	1.35	1.20	27.91
4	6	0.0852	2.16	22.32	1.12	2.38	2.98	9.91	2.71	3.25	14.19	1.06	1.16	28.83
5	1	0.1032	2.91	21.62	1.02	2.78	2.42	8.21	2.33	2.67	18.26	0.00	0.00	31.95

Female	Batch	Egg Dry Weight (mg)	% of Fatty Acids > 1%											
			14:0	16:0	16:1ω9	16:1ω7	18:0	18:1ω9	18:1ω7	20:1ω9	20:5ω3	22:1ω11(13)	22:5ω3	22:6ω3
5	2	0.0958	2.54	22.68	1.08	2.39	2.86	8.73	2.52	3.12	15.95	0.00	0.81	28.57
5	3	0.0920	2.60	19.61	1.17	2.69	2.14	7.92	2.28	2.55	18.67	0.41	0.94	30.93
5	4	0.0896	3.14	26.71	1.42	3.05	3.16	10.67	3.45	3.60	12.96	0.92	0.71	22.22
5	5	0.0800	2.38	20.43	1.25	2.26	2.43	8.33	2.55	2.31	16.62	0.44	1.04	31.74
5	6	0.0756	2.35	20.66	1.16	2.61	1.83	8.31	2.46	1.94	16.78	0.52	1.12	32.71
6	1	0.1024	2.35	23.27	1.27	2.42	3.11	8.89	2.38	3.11	14.69	1.02	0.82	25.02
6	2	0.1020	2.48	21.61	0.92	3.20	2.00	9.04	2.20	2.66	17.64	0.77	0.68	28.92
6	3	0.1059	2.20	22.84	0.98	2.42	1.54	8.31	2.38	1.37	19.46	0.00	0.49	33.12
6	4	0.0996	1.72	23.49	1.09	2.02	3.28	9.61	2.56	3.53	15.38	1.04	0.36	31.02
6	5	0.0895	2.10	21.87	0.86	2.82	3.63	9.91	2.12	3.29	16.80	0.90	0.08	27.36
7	1	0.0822	3.04	21.76	1.20	2.67	2.63	7.44	1.40	2.05	21.35	0.00	0.00	29.93
7	2	0.0803	3.73	27.64	1.48	3.22	3.31	10.51	2.23	3.29	12.18	0.65	0.38	18.63
7	3	0.0769	3.71	27.50	1.69	3.52	2.55	10.79	2.49	2.75	12.95	0.71	0.81	20.24
7	4	0.0732	1.96	19.55	1.28	2.30	2.07	8.53	1.96	1.81	17.59	0.52	1.05	31.06
7	5	0.0677	2.10	21.48	1.22	1.58	2.36	8.44	1.93	1.38	17.18	0.48	1.07	30.44
7	6	0.0618	2.24	20.19	1.28	2.23	1.94	8.73	2.09	2.13	17.86	0.62	1.13	30.52
8	1	0.1078	2.04	22.88	1.08	2.28	2.66	7.42	2.04	2.73	16.46	1.21	0.80	24.08
8	2	0.1039	2.27	25.18	1.09	2.91	3.08	8.15	2.11	3.57	8.47	1.35	0.38	12.43
8	3	0.0975	0.19	19.83	1.23	2.73	1.79	7.30	1.59	2.17	20.75	0.80	0.91	30.23
8	4	0.0918	1.66	22.30	1.07	2.25	2.46	7.89	1.49	2.12	19.26	0.42	0.39	30.37
8	5	0.0873	2.32	22.28	1.16	2.31	2.48	7.59	1.83	2.06	18.16	0.37	0.79	28.69
8	6	0.0788	2.74	21.68	1.31	2.67	1.00	6.98	1.52	1.19	21.32	0.11	0.57	31.57
8	7	0.0723	2.22	23.29	1.10	2.51	2.35	9.46	2.15	2.20	17.86	0.30	0.64	31.04

Appendix 3: Sums of squared loadings, percent of variance explained and cumulative percent for the first four principal components (Eigenvalues > 1) derived using Varimax rotation with Kaiser normalization to examine patterns in the absolute amounts of selected lipid classes and fatty acids (see Table 2.5).

Components	Rotated Sums of Squared Loadings	% of Variance	Cumulative %
1	4.25	28.33	28.33
2	3.30	21.99	50.31
3	2.36	15.71	66.02
4	1.37	9.14	75.16

Appendix 4: Rotated component matrix (Varimax with Kaiser normalization) for the first four components of the PCA to examine patterns in the absolute amounts of selected lipid classes and fatty acids.

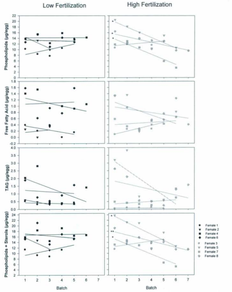
	Component Scores			
	1	2	3	4
Egg Dry Weight	.264	**.812**	-.014	.186
Batch Fecundity	.108	.241	**-.667**	-.200
Phase in the Spawning Season	-.098	-.528	-.111	**-.642**
Fertilization Rate	.051	**-.692**	.184	.146
Phospholipids	**.840**	.358	.031	.203
Sterols	**.642**	.335	.195	-.124
TAG	.451	.242	**.745**	.060
FFA	.412	.315	**.636**	-.057
Other Lipid Classes	.413	.193	**.775**	-.184
Saturated Fatty Acids	.146	**.780**	.327	.104
MUFA	.096	**.883**	.219	.075
AA	**.812**	.014	.173	.192
EPA	**.924**	.006	.253	.146
DHA	**.963**	-.026	.132	.009
Other PUFA	.153	-.027	-.010	**.831**

Appendix 5: Sums of squared loadings, percent of variance explained and cumulative percent for the first six principal components (Eigenvalues > 1) derived using Varimax rotation with Kaiser normalization to examine patterns in the percentage of selected lipid classes and fatty acids.

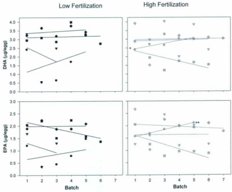
Components	Rotated Sums of Squared Loadings	% of Variance	Cumulative %
1	3.18	21.19	21.19
2	2.66	17.73	38.93
3	2.12	14.14	53.06
4	1.57	10.49	63.55
5	1.29	8.61	72.15
6	1.28	8.55	80.70

Appendix 6: Rotated component matrix (Varimax with Kaiser normalization) focusing on the first four components of the PCA to examine the percentage of selected lipid classes and fatty acids.

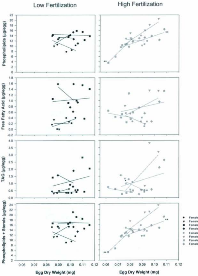
	Component Scores			
	1	**2**	**3**	**4**
Egg Dry Weight	.046	.542	-.091	**-.702**
Batch Fecundity	-.208	-.070	-.086	-.042
Phase in the Spawning Season	-.041	-.324	.059	**.804**
Fertilization Rate	-.029	-.498	.108	.087
Phospholipids	**-.954**	-.146	-.019	-.181
Sterols	.444	.376	.114	.526
TAG	**.769**	.147	-.082	-.109
FFA	**.796**	.054	.170	-.005
Other Lipid Classes	**.861**	-.262	-.166	-.074
Saturated Fatty Acids	.049	**.895**	-.123	-.185
MUFA	.011	**.905**	-.197	-.197
AA	-.012	.098	**.677**	-.015
EPA	.042	-.186	**.883**	.048
DHA	-.022	-.287	**.841**	.141
Other PUFA	-.228	-.081	-.112	-.029



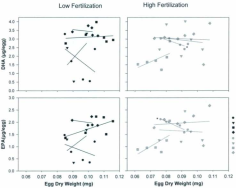
Appendix 7: Seasonal changes in relation to egg size in the absolute amount (µg/egg) of phospholipids, free fatty acids, TAG and phospholipids + sterols over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.



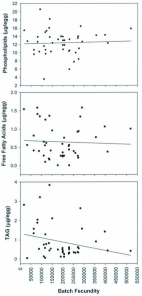
Appendix 8: Seasonal changes in the absolute amount (µg/egg) of the fatty acids DHA and EPA over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.



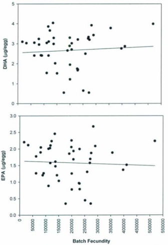
Appendix 9: Seasonal changes in relation to egg size in the absolute amount (µg/egg) of phospholipids, free fatty acids, TAG and phospholipids + sterols over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.



Appendix 10: Seasonal changes in relation to egg size in the absolute amount (µg/egg) of the fatty acids DHA and EPA over the spawning cycles of eight female Atlantic cod. Females were grouped as having low fertilization (<50%; left column) or high fertilization (>75%; right column) rates. Significant seasonal changes are denoted for individual females prior to (*) adjustment and after adjustment (**) for multiple comparisons.



Appendix 11: Changes in egg composition, as measured by phospholipids, free fatty acids and TAG, in relation to batch fecundity of eight female Atlantic cod (data pooled).



Appendix 12: Changes in egg composition, as measured by the fatty acids DHA and EPA in relation to batch fecundity of eight female Atlantic cod (data pooled).

Appendix 13: Size, condition and growth rate of 0 & 5 dph larvae for all females and batches obtained from the experiment. * Data unavailable as the egg batch produced either non-viable eggs or insufficient larvae

		0 days post hatch larvae				5 days post hatch larvae				
Female	Batch	Dry Weight (mg)	Standard Length (mm)	Fulton's Condition Factor	Myotome-Based Condition Index	Dry Weight (mg)	Standard Length (mm)	Fulton's Condition Factor	Myotome-Based Condition Index	Specific Growth Rate (%/day)
1	1	*	*	*	*	*	*	*	*	*
1	2	0.076	5.345	0.050	0.053	0.051	5.971	0.024	0.050	2.22
1	3	*	*	*		*	*	*	*	*
1	4	0.061	5.212	0.043	0.051	0.053	5.770	0.028	0.052	2.03
1	5	*	*	*	*	*	*	*	*	*
2	1	*	*	*	*	*	*	*	*	*
2	2	*	*	*	*	*	*	*	*	*
2	3	0.055	5.240	0.038	0.055	0.063	5.607	0.036	0.056	1.35
3	1	0.064	5.443	0.040	0.050	0.065	5.721	0.035	0.052	1.00
3	2	0.074	5.424	0.046	0.052	0.070	5.986	0.033	0.054	1.97
3	3	0.073	5.338	0.048	0.049	0.047	5.758	0.025	0.050	1.51
3	4	0.054	5.329	0.036	0.049	0.048	5.752	0.025	0.051	1.53
3	5	0.048	5.193	0.034	0.048	0.065	6.003	0.030	0.048	2.90
4	1	0.094	*	*	*	0.085	6.448	0.032	0.051	*
4	2	*	*	*	*	*	*	*	*	*
4	3	*	*	*	*	*	*	*	*	*
4	4	*	*	*	*	*	*	*	*	*
4	5	0.081	5.510	0.048	0.052	0.064	5.876	0.032	0.052	1.28
4	6	0.073	5.225	0.051	0.050	0.058	5.502	0.035	0.052	1.03
5	1	0.077	5.187	0.055	0.058	0.072	6.272	0.029	0.051	3.80

		0 days post hatch larvae				5 days post hatch larvae				
Female	Batch	Dry Weight (mg)	Standard Length (mm)	Fulton's Condition Factor	Myotome-Based Condition Index	Dry Weight (mg)	Standard Length (mm)	Fulton's Condition Factor	Myotome-Based Condition Index	Specific Growth Rate (%/day)
5	3	0.076	5.550	0.045	0.053	0.050	6.049	0.023	0.052	1.72
5	4	0.051	5.463	0.031	0.053	0.073	5.858	0.036	0.054	1.40
5	5	0.074	5.074	0.057	0.053	0.071	5.874	0.035	0.053	2.93
5	6	*	*	*	*	*	*	*	*	*
6	1	*	*	*	*	*	*	*	*	*
6	2	*	*	*	*	*	*	*	*	*
6	3	0.084	5.709	0.045	0.049	0.070	6.294	0.028	0.048	1.95
6	4	0.077	5.368	0.050	0.052	0.059	5.854	0.029	0.051	1.73
6	5	0.075	5.302	0.050	0.051	0.061	5.734	0.032	0.051	1.57
7	1	0.063	5.264	0.043	0.050	0.059	5.746	0.031	0.051	1.75
7	2	0.058	5.379	0.037	0.050	0.041	5.548	0.024	0.050	0.62
7	3	*	*	*	*	*	*	*	*	*
7	4	0.046	5.165	0.033	0.051	0.057	5.605	0.032	0.051	1.63
7	5	0.058	5.198	0.041	0.051	0.049	5.545	0.029	0.049	1.29
7	6	*	*	*	*	*	*	*	*	*
8	1	0.083	5.744	0.044	0.055	0.078	6.072	0.035	0.053	1.11
8	2	0.079	5.175	0.057	0.053	0.052	5.919	0.025	0.053	2.68
8	3	*	*	*	*	*	*	*	*	*
8	4	0.055	5.031	0.043	0.052	0.068	5.835	0.034	0.052	2.97
8	5	*	*	*	*	*	*	*	*	*
8	6	*	*	*	*	*	*	*	*	*
8	7	*	*	*	*	*	*	*	*	*

Appendix 14: The first six principal components (eigenvalues > 1) that show the percent variance explained by the initial solution and the rotated components using egg batches, larval morphometric measurements at 0 and 5 days post hatch (dph), specific growth rate at 5 dph and condition indices.

Components	Rotated Sums of Squared Loadings	% of Variance	Cumulative %
1	5.77	30.34	30.34
2	2.66	13.99	44.33
3	2.34	12.32	56.66
4	2.26	11.90	68.56
5	2.09	11.02	79.58
6	1.74	9.13	88.71

Appendix 15: Rotated component matrix (rotation method: Varimax with Kaiser Normalization) focusing on the first four components of the PCA using 0 & 5 dph morphometrics, condition indices and specific growth rate.

	Component Scores			
	1	2	3	4
Female Condition	**.574**	.027	.379	-.047
Batch	-.378	**-.409**	-.288	.025
Egg Dry Weigh	**.601**	.214	.168	.547
LDW 0DPH	.334	.029	-.027	**.888**
SL 0DPH	.450	-.115	-.252	.103
ED 0DPH	**.679**	.436	-.272	.107
MH 0DPH	**.577**	.548	-.293	.260
YSA 0DPH	.016	-.166	**.914**	.099
Fulton's 0DPH	.041	.115	.153	**.912**
MCI 0DPH	.389	**.802**	-.084	.236
LDW 5DPH	**.656**	.106	.024	.048
SL 5DPH	**.930**	-.103	.102	.243
ED 5DPH	**.876**	.223	-.019	.119
JL 5DPH	**.895**	.164	.010	.170
MH 5DPH	**.728**	.564	.134	.173
YSA 5DPH	.089	.170	**.917**	.064
Fulton's 5DPH	-.093	.205	-.067	-.146
MCI 5DPH	-.045	**.877**	.078	-.046
SGR	.510	-.011	.341	.150

Appendix 16: The first three principal components (eigenvalues > 1) that show the percent variance explained by the initial solution and the rotated components using the feeding trial measurements, survivorship, growth rate and condition indices.

Components	Rotated Sums of Squared Loadings	% of Variance	Cumulative %
1	4.43	40.25	40.25
2	1.55	14.12	54.37
3	1.50	13.64	68.01
4	1.10	10.04	78.04

Appendix 17: Rotated component matrix (rotation method: Varimax with Kaiser Normalization) of the PCA with eigenvalues >1 using the feeding trial morphometrics, survivorship, specific growth rate and condition indices.

	Component Scores			
	1	**2**	**3**	**4**
Female Condition	-.287	.143	-.078	**.800**
Batch	.433	**-.776**	-.030	-.041
Food Supply	.011	-.109	**.541**	.048
Egg Size	.205	**.912**	-.025	.063
Larval Dry Weight	**.748**	.085	.614	-.041
Standard Length	**.948**	-.026	.095	-.044
Myotome Height	**.927**	.030	.108	-.013
Survival	.390	-.052	.156	**.663**
SGR	**.866**	-.222	.026	.106
Fulton's K	.200	.160	**.864**	-.020
MCI	**.924**	.022	.176	-.023