Finfish broodstock nutrition and impacts on reproductive output in Atlantic cod (*Gadus morhua*)

by

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ABSTRACT

In aquaculture, broodstock nutrition is recognized as an influential factor affecting spawning performance and subsequent egg and larval quality. The purpose of this thesis was to examine broodstock dietary effects on the eggs of fish fed different experimental diets. This study also examined broodstock dietary effects on larval growth using RNA:DNA as predictors of growth. Three diets were tested: an OG diet, an experimental BR diet and a BF diet.

The first objective of this work was to categorize the dietary lipid classes and fatty acids in the eggs produced by the broodstock fed these diets. Significant differences were observed between five of the nine dietary lipid classes examined and significant differences were observed in fatty acids (% of total FA) and their ratios among diets for 16 of the 29 dietary fatty acids examined. Of particular importance are, TAG ($p = <0.001$), PL ($p = <0.001$), EPA ($p = <0.001$), DHA:EPA ($p = <0.001$) and EPA:ARA ($p = <0.001$). The BF diet which showed the best reproductive output in terms of fertilization and hatching success had the highest proportions of DHA:EPA and the lowest proportions of TAG and EPA. Overall, the differences present in the dietary lipid classes of this study did not translate into differences in the lipid classes of the eggs. Dietary differences were more evident in the essential FA composition of diets and eggs as the proportions of DHA and EPA in the eggs are reflective of the dietary proportions. However, the proportions observed in the eggs are much higher, and the DHA proportions are larger than the EPA proportions, supporting the idea that DHA is preferentially retained in developing yolk-sac larvae.

The second objective was to determine if the experimental diets led to any growth benefits in the subsequent larvae. No effects of broodstock diet were observed in larval survival, concentration of nucleic acids, protein concentration or SGR of larvae. The slopes of the RNA:DNA ($p = <0.001$) after 7 dph showed a significant difference indicating that the slope of the BR group was significantly lower than the other two groups but this difference did not translate into differences in growth . These results suggest that, although larvae originating from parents fed the BF and OG diets have a higher growth potential (as inferred by the RNA:DNA) when compared to the larvae originating from broodstock fed the BR diet, they grow similarly (up to 49 dph). We found a lack of correlation between the RNA:DNA and growth rates contradicting some of the findings of other authors and questioning the accuracy of RNA:DNA as an indicator of growth in this situation.

Our results suggest that the BR diet may improve reproductive performance, when compared to the OG diet, although the BF diet remains the golden standard for optimizing broodfish reproductive output.

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List of Symbols and Abbreviations

%FA – Percent fatty acids

ALC – Alcohol(s)

AMPL - Acetone mobile polar lipids

ANOVA – Analysis of variance

ARA – Arachidonic acid

BAME – Bacterial acid methyl ester(s)

BCA – Bicinchoninic calorimetric assay

BF – Baitfish diet

BF_3/CH_3OH – Boron trifluoride in methanol

BR – Broodstock diet

BSA – Bovine serum albumin

$CaCl_2$ – Calcium chloride

DFO – Department of Fisheries and Oceans

DHA – Docosahexaenoic acid

DNA – Deoxyribonucleic acid

DNase – Deoxyribonuclease

dph – Days post hatch

EB – Ethidium bromide

EDTA – Ethylenediaminetetraacetic acid

EFA – Essential fatty acid(s)

EPA – Eicosapentaenoic acid

FA – Fatty acid(s)

FAME – Fatty acid methyl ester(s)

FAO – Food and Agriculture Organization

FFA – Free fatty acids

GC – Gas chromatography

G_i d^{-1} – Protein growth coefficient

HC – Hydrocarbon(s)

HUFA – Highly unsaturated fatty acid(s)

KET – Ketone(s)

MFA – Microplate fluorometric assay

$MgCl_2$ – Magnesium chloride

MUFA – Monounsaturated fatty acid(s)

N_2 – Nitrogen gas

OG – On-growing diet

PC – Phosphatidylcholine

PE – Phosphatidylethenolamine

PL - Phospholipids

PUFA – Polyunsaturated fatty acid(s)

Pro:DNA – Protein to DNA ratio

RNA – Ribonucleic acid

RNase – Ribonuclease

rRNA – Ribosomal RNA

SE – Steryl ester(s)

SFA – Saturated fatty acid(s)

SGR – Protein specific growth rate

SQRT – Square root

sRNA:DNA – Standardized RNA:DNA ratio

ST – Sterol(s)

STEB – Sarcosil Tris-EDTA Buffer

TAG – Triacylglycerol(s)

TL – Total lipid(s)

TLC/FID – Thin-layer chromatography with flame ionization detection

WR – Working reagent

Wt - Weight

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Chapter 1: General Introduction

1.1 Finfish Aquaculture

Finfish aquaculture plays a prominent role in the global market with total world production estimated at 45 million tons, approximately two thirds of the world's seafood in 2004 (FOA 2006). Canada has been successful in culturing species of marine and freshwater finfish including Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), Arctic charr (*Salvelinus alpinus*), brook charr (*Salvelinus fontinalis*), Atlantic halibut (*Hippoglossus hippoglossus*), tilapia (*Oreochromis niloticus*), rainbow trout (*Onchorhynchus mykiss*) and has begun the research and development of some new species including spotted wolfish (*Anarhichas minor*) (DFO 2009). The growth of finfish aquaculture carries important ecological, social and economic implications. The aquaculture industry provides many jobs for Canadians in rural, coastal and Aboriginal communities, employing over 8000 Canadians, with two-thirds under the age of 35 (CAIA 2012).

1.2 Atlantic Cod

Atlantic cod, a member of the Gadidae family, is a temperate species that is found on both sides of the North Atlantic (Brown *et al*. 2003). For centuries cod have been fished for both private and commercial purposes. However, overfishing has resulted in a severe drop in population numbers (Myers *et al*. 1996; Lilly *et al*. 1998; Hutchings 2000). Cod aquaculture have developed programs in countries such as Norway, Canada, Scotland, and the US in order to meet market demand. The expansion of the industry has

led to a realization of a need for research into the quantitative nutrient requirements for these marine fish in order to optimize broodstock health and reproductive development (Lall & Nanton 2002). Penney *et al*. (2006) stated that hatchery production could be optimized by starting the larval production cycle with the highest quality eggs available, leading to high egg survival, hatching success and robust larvae with enhanced growth, survival and stress resistance. Currently most cod aquaculture facilities are forced to rely on wild caught broodstock due to issues of egg quality from cultured individuals (Pavlov *et al*. 2004); wild broodfish have been found to produce better quality eggs than their cultured counterparts (Salze *et al*. 2005).

Cod are batch spawners with spawning seasons typically occurring between January and May, but this is dependent on geographical region (Kjesbu 1989; Knickle & Rose 2010). Many studies indicate that wild fish produce higher quality eggs than cultured fish. Studies on cultured Atlantic cod (Salze *et al*. 2005) have reported hatch rates of 17%, while other studies have reported hatch rates of 10 - 15% in cultured European sea bass and gilthead sea bream (Carrillo *et al*. 1989), and less than 1% in cultured Atlantic halibut (Norberg *et al*. 1991). The superior quality of wild fish eggs has been attributed to environmental influences such as broodfish diet and physiochemical conditions of the water during egg incubation (temperature, salinity, pH, etc.) (Brooks *et al*. 1997). However, some environmental influences such as spawning season variation and water pollutants have been found to have negative effects on both wild and cultured fish eggs, reducing egg and larval survival (Westin *et al*. 1985; Kjorsvik *et al*. 1990; Miller 1993).

Variation within and between spawning seasons has been reported by several authors (Kjesbu *et al*. 1996; Trippel 1998; Rose 2007), and Trippel (1998) reported that Atlantic cod produced better eggs in their second spawning season, than in the first. In multiple batch spawners, such as Atlantic cod, considerable variation in egg quality has been observed between batches in one spawning season, even when batches were maintained under identical conditions (West & Mason 1987; Kjesbu *et al*. 1996). Kjesbu *et al*. (1996) reported that egg size and batch size followed a dome-shaped curve throughout the spawning period, indicating that the eggs produced in the mid portion of the spawning period may be of higher quality than earlier and later batches which are often smaller (Bachan *et al*. 2012). However, egg size may also be influenced by age at maturity (Sargent *et al*. 1987), and generally a larger body size results in larger eggs (DeMartini 1991; Rose 2007).

Egg quality in cultured fish may be influenced by poor hatchery husbandry, such as broodstock stress, fertilization procedures, overrippening of eggs and bacterial colonization of fertilized eggs (Springate *et al*. 1984; Barker *et al*. 1989; Hansen & Olafsen 1989; Kjorsvik *et al*. 1990; Brooks *et al*. 1997). Kjesbu (1989) found that approximately 1/3 of cultured cod broodstock are affected by stress, which may have deleterious effects during the spawning period (Campbell *et al*. 1994). Therefore, there is a need to better understand and attempt to eliminate factors that contribute to poor egg quality in marine fish broodstock which are of interest to aquaculture (Roy *et al*. 2007; Daniel *et al*. 1993; Le François *et al*. 2010).

1.3 Broodstock Nutrition

In addition to environmental factors, early life history is also shaped by parent-egg-progeny relationships (Trippel *et al*. 1997). Processes such as embryonic development, yolk sac utilization and survivorship are influenced by both parents, and although both can account for non-genetic sources of variation in offspring, it is the female that is responsible for the nourishment of the egg during its early development (Trippel *et al*. 2005; Green 2008). Due to this, more focus has been placed on the maternal influences affecting reproductive variability, and associated observations in offspring survival. Studies have indicated that maternal influences are important within an evolutionary and ecological context, as variation in offspring fitness can also shape natural selection (Chambers & Leggett 1996; Mousseau & Fox 1998).

The desired traits of cultured broodstock fish differ from those of commercial fish intended for harvest. Several reviews have examined the influences of nutrient availability on reproductive physiology and broodstock performance (Hardy 1985; Watanabe *et al*. 1985; Luquet & Watanabe 1986; Bromage *et al*. 1992, Cerdá *et al*. 1994; Bromage 1995; Bell *et al*. 1997; Navas *et al*. 1997; Bruce *et al*. 1999; Pavlov *et al*. 2004; Roy *et al*. 2007), and concluded that broodstock nutrition, environmental conditions and husbandry practices were all influential in determining the quality of farmed fish eggs (Bromage 1995).

Broodstock diet is recognized as an influential factor because during ovarian development, the dietary and maternal reserves are metabolized and transported to the oocyte (Henderson *et al*. 1984; Henderson & Atlamar 1989). Once present in the oocyte they provide the energy and nutritional requirements for growth and development of the embryo and yolk sac larvae (Marozza *et al*. 2003). The oocyte acts as a repository of all

essential nutrients required for development of the fertilized egg up to the initiation of exogenous feeding (Tocher & Sargent 1984). However, egg investment is not solely influenced by maternal energy stores available for egg production but also by the number of eggs produced. Egg size, fecundity and spawning period of Atlantic cod generally increase with maternal size and age (Kjesbu *et al*. 1996), indicating that larger female broodstock are able to store and invest more energy for reproductive output. However, Hamoutene *et al*. (2009) found that differences between egg batches of a single female are more important than differences between females, and that a consistency exists in batches of an individual females showing higher egg quality with no particular order or time. This suggests that eggs could be collected throughout the spawning season without significantly affecting quality. Dietary differences, especially dietary lipid and fatty acid contents have received the greatest amount of attention with respect to their influences on egg quality, and recent studies indicate that major influences on egg quality are exerted by just a few of the many dietary constituents (Washburn *et al*. 1990; Watanabe *et al*. 1991; Harel *et al*. 1994). However, despite the importance of maternal effects in fish, research is still controversial as some studies have reported direct links associated with maternal effects (Chamber & Leggett 1996; Kamler 2005; Green & McCormick 2005), while others have been unable substantiate these relationships and their adaptive benefits (Kamler 1992; Chambers 1997).

1.4 Egg Quality

Egg quality is a term used to describe the ability of a female to produce viable offspring (Kamler 1992), and is often reflected in egg size and composition (Czesny *et al*.

2005). Egg quality is a major factor affecting the future recruitment of wild and farmed fish populations, particularly in aquaculture where poor egg quality has become a major restraint in expansion (Brooks *et al*. 1997). By developing methods to increase growth and survival rates during the early life stages, total fish production can be maximized (Clarke *et al*. 2005). Typically hatcheries distinguish good eggs from bad by their ability to float in seawater (Carrillo *et al*. 1989; Kjorsvik *et al*. 1990). However, this has proven to be an unreliable method of egg quality assessment in a number of fish species such as Atlantic halibut and Atlantic cod. Instead cell symmetry at early stage cleavage and other biochemical indicators (lipid classes, fatty acids, and amino acids) were found to be better predictors of egg quality (Bromage *et al*. 1994; Clarke *et al*. 2005).

Bromage *et al*. (1992) defined good egg quality as those eggs producing low mortalities at fertilization, hatching and first feeding of the subsequent larvae. However, egg survival and hatch rates are not representative of the factors that influence egg quality. Many factors have been examined as predictors of egg quality, but there has been little agreement on a reliable method for egg quality assessment. A better understanding of the factors that affect egg and larval quality can serve as a means for selecting high quality eggs. Some factors that have been examined as egg quality predictors are the appearance of the zona pellucida (a glycoprotein membrane surrounding the plasma membrane of an oocyte), egg shape, egg transparency, and oil globule distribution (Kjorsvik *et al*. 1990; Bromage *et al*. 1994). Intrinsic properties provided by the female such maternal mRNA transcript, yolk sac nutrients and egg lipids and fatty acids have also been considered to be good predictors of egg quality. However, if an egg does not

contain a particular compound, or contains an inappropriate amount of a compound, it will not be able to sustain the development of a viable embryo (Brooks *et al*. 1997).

1.4.1 Lipid and Fatty Acid Content

Lipids, specifically fatty acids, play a major role in fish as a source of metabolic energy in the form of ATP provided through the β-oxidation of fatty acids (Frøyland *et al*. 2000). Lipids are utilized in marine fish for growth, reproductive development and movement (Tocher 2003), and are known to perform a variety of biological functions including the formation of structural components in cell membranes, precursors for chemical messages and substrates for catabolism (Wiegand 1996; Pickova *et al*. 1997; Tveiten *et al*. 2004). Until the commencement of exogenous feeding of larvae, these roles are fulfilled by the lipids from the yolk sac, after which lipids from environmental sources take over (Tveiten *et al*. 2004). Current knowledge of the relative importance of individual fatty acids in energy provisions is partly based on experience in aquaculture. Aquaculture has played a major role in studying the lipid nutritional requirements of farmed fish, with the objective of optimizing production (Tocher 2003).

Although there is limited research on the quantitative nutrient requirements of most marine fish (Lall & Nanton 2002), some lipid classes and fatty acids have been clearly identified as being important to early development. For example, Pacific Sardines, *Sardinops sagax* (Lasker & Theilacker 1962), Baltic Herring, *Clupea harengus membras*, Roach, *Rutilus rutilus*, Perch, *Perca fluviatilis*, Burbot, *Lota lota*, Rainbow Trout and Atlantic Cod (Kaitaranta & Ackman 1981) eggs have high concentrations of phospholipids (PL) and triacylglycerols (TAG). Tocher & Sargent (1984) suggested that

PL may be more readily mobilized than triacylglycerols in fish eggs and that perhaps they were the preferred energy source in Atlantic Cod, Atlantic Herring, *Clupea harengus*, Haddock, *Melanogrammus aeglefinus*, Saithe, *Pollachius virens*, and Whiting, *Merlangus merlangus*. However, another possibility is that PL in the eggs are destined for use as biomembranes in developing fish and that the initial high concentrations found in eggs may be related to the rapid proliferation of cells and new membranes (Pickova *et al*. 1997). Triacylglycerols and sterols (ST) have been identified as the most abundant neutral lipid classes in fish eggs (Tocher & Sargent 1984), and neutral lipids are considered to be the most important energy reserve in teleost eggs (Jangaard *et al*. 1974; Vetter *et al*. 1983; Fraser *et al*. 1989; Pickova *et al*. 1997; Clarke *et al*. 2005). Lochmann *et al*. (1994) identified TAG as an energy storage product and further questioned whether or not the absolute content of ST could be an adequate predictor of body size since ST are structural lipids.

Particular interest has been given to some essential fatty acids (EFA), particularly docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) and their roles in egg and larval development (Bruce *et al*. 1993, 1999; Ramos *et al*. 1993; Thrush *et al*. 1993; Sargent *et al*. 1995; Bell *et al*. 1997; Czesny & Dabrowski, 1998; Evans *et al*. 1998; Estevez *et al*. 1999; Gunasekara *et al*. 1999). Marine fish contain large amounts of DHA and EPA in the phospholipids of their cellular membranes. As marine fish can neither biosynthesize DHA *de novo* nor form shorter chain precursors such as 18:3ω3 (α-linolenic acid), DHA and EPA are essential dietary requirements for marine fish (Sargent *et al*. 1999; Lall & Nanton 2002). Recent attention has been given to ARA mainly because of its role in eicosanoid production (Bell *et al*. 1997; Abayasekara

& Wathes 1999) and the involvement of eicosanoids in a range of physiological functions, including reproduction and egg development (Abayasekara & Wathes 1999). Most studies on lipid class and fatty acid composition of fish eggs have focused on these important lipid classes and fatty acids.

1.4.2 Larval Quality

Survival rates during the larval stage of many fish are often extremely low and variable and this can have serious consequences on stock recruitment (Kalmer 2005). The early life stages of fish are described as being the most crucial developmental stage as eggs and larvae may succumb to mass mortality under unfavourable environmental conditions, competition, predation and/or starvation (Kalmer 1992; Green & McCormick 2005; Govoni 2005; Green 2008). The biological (prey abundance, size and type) and physical (temperature, salinity and currents) attributes of the environment in which the egg is deposited can influence larval size at hatch, developmental rate, larval condition and larval survival (Pepin & Myers 1991; Blaxter 1992; Jordaan *et al*. 2006). As the larvae exhaust their yolk sac and exogenous feeding commences, such factors as water temperature and prey availability are crucial to subsequent survival (Kalmer 1992; Green & McCormick 2005). Larval quality is generally associated with larval growth, and higher quality larvae are believed to have a higher chance of survival. Several hypotheses have been suggested for explaining recruitment variability in larval fish. The most popular being Hjort's (1914) 'critical period' hypothesis, Cushing's (1972) 'mis-match' hypothesis, Miller *et al*.'s (1988) 'bigger is better' hypothesis and Houde's (1987) 'stage duration' hypothesis.

Hjort's (1914) 'critical period' hypothesis suggests that larval fish must find suitable prey immediately following the switch from endogenous feeding to exogenous feeding. This is because without suitable feeding conditions, larvae experience mass mortalities over a short period of time due to starvation. This hypothesis was one of the first to highlight the effects of larval nutrition and starvation on larval survival, and it paved the way for other hypotheses on larval recruitment. However, Hjort's hypothesis has a major fault, which is that it is narrowly focused on first feeding larvae and fails to account for other environmental variables (Houde 2008).

The 'match/mismatch' hypothesis (Cushing 1972, 1990) is considered to be an extension of Hjort's critical period hypothesis (Houde 2008), and considers larval fish nutrition and prey production within an overlapping period. The hypothesis suggests that top predators must coordinate their reproductive activities with the emergence of prey species (generally zooplankton) (match) or not (mismatch), affecting larval mortality due to starvation. Such environmental factors as water temperature play an important role in this hypothesis because spring zooplankton blooms are often associated with a rise in water temperature. This hypothesis is less relevant in the field of aquaculture as the larval prey source is supplied by hatchery workers and not by zooplankton blooms.

The 'bigger is better' hypothesis (Miller *et al*. 1988) has historically been accepted in aquaculture, and suggests that larger larvae are considered to have a higher chance of survival because they have larger yolk-sacs and may have higher growth rates (Blaxter & Hempel 1963; Moodie *et al*. 1989) and less vulnerability to predation as well as the ability to eat a variety of prey items (Webb & Weihs 1986); however, size is not age specific and most larvae are found to be different sizes at different ages, meaning that

groups of larvae are often heterogeneous mixes of different ages and sizes. In aquaculture cannibalism can be a major source of mortality for larval cod, accounting for 3 – 76% of the total mortality (Ottera & Lie 1991). However, some research suggests that bigger may not be better: Litvak & Leggett (1992) found that larger larvae were preyed upon by predators because they were easier to see and catch, they suggest that smaller/younger larvae may be better suited to avoid predation (Kjesbu *et al*. 1996). This may hold true in the natural environment, but under hatchery conditions continuous grading is necessary to minimize losses due to cannibalism.

The 'stage duration' hypothesis suggests that if growth is poor, a fish will remain in a vulnerable size class for a longer period of time, while fast growing fish require less time to transit the life stages that are vulnerable to predation (Houde 1987; Cushing 1990). Relative size is important for factors such as intracohort cannibalism and for predators making a choice among potential prey. In this hypothesis, rapid growth is thought to be beneficial in terms of survival, while smaller individuals influenced by stage duration are thought to be preferentially removed within cohorts. As mentioned previously, some studies suggest that there may be a benefit to being a small larva (Litvak & Leggett 1992; Kjesbu *et al*. 1996).

However, few attempts have been made to assess and define dietary factors which may underlie and determine larval quality (Nagahama 1994), and even fewer studies have been able to show improvement in larval quality through the implementation of broodstock nutrition (Izquierdo *et al*. 2001). Most studies that have examined broodstock nutritional effects have been conducted on gilthead seabream (*Sparus aurata*). These studies have found that an increase in n-3 highly unsaturated fatty acids (HUFA) levels in

the broodstock diet improved larval growth, survival, and swim bladder inflation (Tandler *et al*. 1995), while excess levels of n-3 HUFA in the broodstock diet (over 2%) caused yolk sac hypertrophy and decreased larval survival (Fernandez-Palacios *et al*. 1995).

More recently larval biochemistry, particularly the concentration of RNA, DNA, protein and their ratios, has been examined as an indicator of larval viability (Buckley *et al*. 1999; Caldarone *et al*. 2003; Tong *et al*. 2010). This is because the concentrations of RNA, DNA and protein have the potential to influence the viability of eggs and larvae in culture, and therefore may be useful as indicators of future performance potential such as fertilization success, egg survival, hatching success, larval size and larval growth rate.

Several studies have been conducted investigating the effects of broodstock diet on egg lipid and fatty acid composition in Atlantic cod (Penney *et al*. 2006), sea bass (*Dicentrarchus labrax*) (Bell *et al*. 1997), Atlantic halibut (Mazorra *et al*. 2003), tropical clownfish (*Amphiprion melanopus*) (Green & McCormick 2005) and a multitude of North Atlantic fish species (Chambers & Leggett 1996). Studying how broodstock diet affects egg lipid and fatty acid composition will help determine which nutrients are transferred to developing embryos and endogenous larvae and whether they are essential to early life traits (Green & McCormick 2005). Moreover, the identification of new biochemical indices for larval quality contributes to the development of better broodstock diets and husbandry protocols. This could in turn allow for optimal quality and survival of cultured cod throughout the early life stages, and the information provided may inform industry on the best means for selecting high quality eggs, which could subsequently lead to better survival and growth during the fish's early life stages.

1.5 Goals

The objectives of this thesis were to determine the effects of broodstock diet on the egg composition of Atlantic cod by assessing the lipid and fatty acid composition of three diets and that of the newly spawned eggs of cod fed these diets. The parental dietary effects on the concentration and ratios of RNA, DNA and protein were also examined. The research was conducted at the Joe Brown Aquatic Research Building (Memorial University of Newfoundland). For the first portion of the study a full range of lipid classes and fatty acids as proportions (%), ratios and concentrations (per mg/g and µg/egg) were obtained for diets and eggs. For the second portion of the study, cod larvae originating from the 3 diet groups were examined at hatch through to approximately 56 days post hatch, and the relationships between RNA concentration, DNA concentration, protein concentration and their ratios were examined.

Chapter 2: Broodstock dietary effects on egg lipid and fatty acid composition of Atlantic cod (*Gadus morhua*)

2.1 Introduction

The nutritional composition and quality of a broodstock diet plays an important role in egg and larval quality (Cerdá *et al*. 1994, 1995; Bell *et al*. 1997; Bruce *et al*. 1999), since dietary and maternal reserves are mobilized into the oocytes during ovarian development (Henderson *et al*. 1984; Henderson & Altamar 1989). The lipid and fatty acid (FA) compositions of broodstock diets have been identified as major dietary contributors that bring about successful reproduction and the survival of offspring (Izquierdo *et al*. 2001). Although the importance of broodstock nutrition in the development of the gonads, egg quality and survival of the larvae is widely acknowledged, few studies have focused on defining the roles of key nutrients in the reproduction of gadoids (Lall & Nanton 2002). Some authors have shown that the fatty acid composition of total lipids in fish eggs reflects that of the maternal diet (Watanabe *et al*. 1984a, 1985a; Mourente & Odriozola 1990; Fernandez-Palacios *et al*. 1995).

There has been particular interest given to essential fatty acids (EFA), specifically docosahexaenoic acid: 22:6ω3 (DHA) and eicosapentaenoic acid: 20:5ω3 (EPA), and their roles in egg and larval development (Bruce *et al*. 1993, 1999; Ramos *et al*. 1993; Thrush *et al*. 1993; Sargent *et al*. 1995a; Bell *et al*. 1997; Czesny & Dabrowski, 1998; Evans *et al*. 1998; Estevez *et al*. 1999; Gunasekara *et al*. 1999). The essential dietary role of omega-3 (ω3) highly unsaturated fatty acids (HUFA), DHA & EPA, for marine fish has been known for quite some time (Sargent *et al*. 1989, 1997, 1999) and the importance

of supplying sufficient amounts of these fatty acids for successful production of marine fish eggs and larvae is well documented (Izquierdo *et al*. 2001). Studies of the biochemical composition of marine fish eggs have found that the major FAs in halibut (*Hippoglossus hippoglossus*) (Falk-Peterson *et al*. 1989), turbot (*Scophthalmus maximus*) (Planas *et al*. 1989), plaice (*Hippoglossoides platessoides*) (Rainuzzo *et al*. 1993), dolphin (*Coryphaena hippurus*) (Ako *et al*. 1991), red sea bream (*Pagrus major*) (Izquierdo *et al*. 1989) and gilthead sea bream (*Sparus aurata*) (Mourente & Odriozola 1990) eggs are DHA, palmitic acid (16:0), EPA, and oleic acid (18:1ω9). However, the relative amounts of each fatty acid differ among species and egg batches (Izquierdo 1996). Lie (1993) found lower levels of DHA and EPA in eggs of cultured cod, than did Tocher & Sargent (1984) and Fraser *et al*. (1988) in wild cod eggs. Marine fish contain large amounts of DHA and EPA in the phospholipids of their cellular membranes. As marine fish can neither biosynthesize DHA *de novo* nor form shorter chain precursors such as 18:3ω3 (α-linolenic acid), DHA and EPA are essential dietary constituents for marine fish (Sargent *et al*. 1999; Lall & Nanton 2002). Insufficient levels of DHA in marine fish diets are likely to impair neural and visual development with serious consequences for a range of physiological and behavioural processes including those dependent on neuroendocrines (Sargent *et al*. 1999), making it particularly important in developing fish. During fish reproduction neuroendocrines play a role in gonadotropin release (Zohar *et al*. 2010). Atlantic cod (*Gadus morhua*) contain high concentrations of highly unsaturated fatty acids (HUFA) (whole body, fillets and liver tissues) (Jobling *et al.* 2008); however, EFA requirements and deficiency signs in gadoids have yet to be reported (Lall & Nanton 2002). Recent attention has shifted to ARA (arachidonic acid:

20:4ω6) mainly because of its role in eicosanoid production (Bell *et al*. 1997; Abayasekara & Wathes 1999) and the involvement of eicosanoids in a range of physiological functions, including reproduction and egg development (Abayasekara & Wathes 1999). Ecosanoids are highly active fatty acid derivatives which include prostaglandins. Prostaglandins play an important role in fish ovulation (Jalabert 1976), and female fish behaviour (Stacey 1976, 1981), and some studies have shown that prostaglandins induce ovulation *in vitro* in several species of teleost fish (Jalabert 1976; Goetz and Theofan 1979).

The efforts to emphasize ω3 HUFA have resulted in the relative neglect of other aspects of marine fish nutrition, namely the role of ARA as a dietary EFA for marine fish and the role of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) as major energy producing nutrients in fish (Sargent *et al*. 1999). In some stocks, hatching success is significantly related to levels of specific fatty acids such as DHA, EPA, ARA and their respective ratios (Pickova *et al*. 1997). Tocher & Sargent (1984) and Zheng *et al*. (1996), suggest phospholipid rich diets containing a DHA:EPA ratio of 2:1 along with the essential fatty acid ARA, may be optimal for marine fish diets. Pickova *et al*. (1997) and Salze *et al*. (2005) found that the DHA:EPA ratio and ARA levels correlated with hatching success and improved egg quality in Atlantic cod (*Gadus morhua*). Mazorra *et al*. (2003) reported that due to competition between DHA and EPA, and EPA and ARA for the same metabolic and enzymatic pathways, the individual amounts and also the optimum ratios of these essential fatty acids should be determined for proper nutritional requirements.

This study examines the lipid composition of three experimental diets, an on-growing pelleted diet (EWOS Marine Grower), a specially manufactured broodstock diet for marine finfish (Skretting Feeds), and the current standard cod broodstock baitfish diet comprised of herring, mackerel, squid and a vitamin/mineral supplement (St. Andrews Biological Station, DFO)(Hamoutene *et al*. 2013). This study also examines the lipid composition of eggs collected from cod broodstock fed these three diets. Lipid classes and fatty acids important in energy storage and formation of membranes were determined in diets and eggs. Studies have shown that by profiling these lipids in early life stages of marine fish, one may be able to predict the nutrient requirements for larval diets (Nocillado *et al*. 2000). This information could also aid in the development of a commercial broodstock diet for Atlantic cod that may lead to higher quality eggs and offspring. This study follows semi-commercial diet trials set up in 2008 where the baitfish diet was found to lead to a superior reproductive output (higher fertilization and hatching success) over the broodstock and on-growing diet (Hamoutene *et al*. 2013). This knowledge is fundamental to understanding how the lipid profile of broodstock diets is reflected in egg composition and will aid in selecting the appropriate nutritional content to be incorporated in broodstock diets.

2.2 Materials & Methods

2.2.1 Experimental Setup & Gamete Sampling

All studies were conducted in accordance with the guidelines published by the Canadian Council on Animal Care, and approved by the Animal Care Committee at Memorial University of Newfoundland (animal care numbers 09-08-KG and 10-08-DH).

All fish were held in seawater tanks at the Joe Brown Aquatic Research Building (Memorial University of Newfoundland). This study was part of a broodstock development project, and the F1 broodstock (representing 13 families) used were hatched in 2006 as part of the Atlantic Cod Genome project selective breeding program (www.codgene.ca). Before trials commenced all fish were fed a commercial on-growing pelleted diet (EWOS Marine Grower). Six 15 m^3 flow-through tanks (2 tanks per diet) were stocked with 20 fish, 10 males and 10 females, for a total of 40 fish per diet, all tanks were exposed to the same environmental conditions with the only difference being diet composition. Diets were randomly assigned and females and males were carefully selected to ensure the 13 families were equally distributed across diets (Hamoutene *et al*. 2013).

Three diets were investigated: 1) An on-growing pelleted diet (OG, EWOS Marine Grower), which is a standard grow out diet designed to optimize fish growth for species such as cod, haddock and halibut, 2) a broodstock diet (BR) specifically manufactured for marine finfish (not commercially available in Canada; Skretting Feeds™©), which has been designed to optimize egg quality and survival to first feeding, and 3) the standard cod broodstock baitfish diet (BF) comprised of herring (*Clupea harengus*), mackerel *(Scomber scombrus)*, squid (*Ilex illecebrosus*) and a vitamin/mineral supplement (St. Andrews Biological Station, DFO), which was designed to emulate the diet of wild cod. On August 26th, 2008 the fish in the BR and BF tanks were weaned onto their new respective diets over a three week period. By September 16th, 2008 all fish were fed the "new" diet exclusively. Throughout the experiment fish in all tanks were fed to satiation and first spawning occurred approximately 13 months later (October-December

2009). The fish spawned three times between May 2008 and October-December 2009, this is due to all fish being 6 month photoperiod advanced prior to the commencement of the study. This was in accordance with industry requests to have a manipulated broodstock that spawned in the fall, thereby introducing young post hatchery cod to sea cages in the spring to optimize growth during warmer water temperatures. All fish were tagged with a Passive Integrated Transponder (PIT) and information on sex and origin (parent) was recorded. Any fish that did not spawn during this period was identified using an AVID PowerTracker, allowing for the determination of the total number of spawners for each diet. During spawning, fish were individually removed by net one tank at a time and placed in 100 L plastic holding containers filled with seawater. Individuals were manually stripped and eggs or sperm were collected in plastic or glass vials (Lush *et al*. 2011). For lipid analysis, eggs were collected from 31 different females, and separated into triplicate samples of 50 eggs each (OG n = 15 (7 females from one tank and 8 from the other), BR n =10 (5 females per tank), BF n = 6 (3 females per tank)). Samples for lipid analysis were collected from the mid portion of the spawning season. All samples were stored in lipid-clean 10 ml capped tubes containing 2 ml of chloroform. Tubes were filled with N_2 gas to prevent sample degradation, sealed with Teflon tape, and stored at -70^{o}C.

2.2.2 Lipid Extraction & Ash Weight

Lipids were extracted from the egg and diet samples, using a modified Folch (1957) method, with 2:1 chloroform:methanol. Each type of baitfish was sampled, homogenized and extracted (n = 3) and an average composition was determined. Lipid

extractions consisted of adding 1 ml of ice cold methanol (EMD Chemicals) to the 10 ml sample tubes and homogenizing the samples manually using a metal pestle with a Teflon® coating. The tubes were then sonicated for 4 minutes in ice water. The organic layer (bottom layer) was removed using a double pipette technique, which involved placing a short (14.6 cm) Pasteur pipette inside the 10 ml tube, then placing a long (22.8 cm) Pasteur pipette inside the short one. The organic layer was pooled in a lipid-clean 2 ml glass vial. This process was repeated three to four times. The pooled extract was flushed gently using a stream of N_2 gas (Air Liquide) and the vials were resealed and stored at -70^{o}C until lipid analysis.

Ash content of the feeds was obtained by processing 5 samples of each feed (n = 5). After weighing 10 to 15 g of thawed baitfish feed, it was homogenized using a Polytron PCU-2-110 tissue homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada), or in the case of pellet food, ground with a mortar and pestle. The ash content was obtained by first drying samples in a drying oven at 80°C overnight, then cooling in a dessicator, and weighing it. The samples were then returned to the drying oven for 1 hr, cooled in a dessicator, and reweighed. This process was repeated at 1 hr intervals until constant weight was achieved. Samples were stored in a dessicator until ashing, this was done by placing the samples in a muffle furnace set at 500°C and leaving them overnight until white ash was obtained (Gaylean 1980).

2.2.3 Lipid Classes

The egg and feed samples were collected in triplicate and the lipid classes were determined using thin-layer chromatography with flame ionization detection (TLC/FID)

with an Iatroscan Mk 6s analyzer (Iatroscan Laboratories, Tokyo, Japan) as described by Parrish (1987). Lipids were separated into classes using silica coated Chromarods (Chromarod-S III, Mitsubishi Kagaku Iatron Inc.) in a four solvent system sequence. First, the samples were focused into a narrow band on the chromarods in 100% acetone (Fisher Scientific), and then twice in hexane:methyl t-butyl ether:formic acid (Fisher Scientific:EMD Omni Solv:J.T. Baker) (98.95:1.0:0.05) (v/v/v). The first development was for 25 minutes followed by a second development for 20 minutes. The second separation consisted of one development in hexane:methyl t-butyl ether:formic acid (Fisher Scientific:EMD Omni Solv:J.T. Baker) (79:20:1) (v/v/v) for 40 minutes. The final separation consisted of two 15 minute developments in 100% acetone (Fisher Scientific) followed by two 10 minute developments in chloroform:methanol:chloroform-extracted water (EMD Omni Solv:EMD Omni Solv) (50:40:10) (v/v/v). After each separation the rods were scanned with the Iatroscan, and the peak areas were quantified in the program PeakSimple version 3.88 (Alan Hetherington©) using calibration curves obtained from scans of standards (Fisher Scientific) as described by Parrish (1987). The 9 components used as standards were: nonadecane (Sigma-Aldrich) at ~25 mg to represent hydrocarbons, cholesteryl stearate (Sigma-Aldrich) at ~25 mg to represent steryl esters, 3-hexadecanone (Sigma-Aldrich) at ~50 mg to represent ketones, tripalmitin (Sigma-Aldrich) at ~50 mg to represent triacyglycerols, palmitic acid (Sigma-Aldrich) at ~25 mg to represent free fatty acids, 1-hexadecanol (Sigma-Aldrich) at ~25 mg to represent alcohols, cholesterol (Sigma-Aldrich) at ~25 mg to represent sterols, 1-mono-palmitoyl-rac-glycerol (Sigma-Aldrich) at ~50 mg to represent acetone mobile polar lipids, and dipalmitoyl phosphatidylcholine (Sigma-Aldrich) at ~50 mg to represent phospholipids.

All data were expressed as mg per g of wet weight for diets and µg per egg for egg samples.

2.2.4 Derivatization and fatty acid analysis

Fatty acids were extracted from the egg and diet samples, according to Parrish (1999). A portion of the lipid extracts (0.25 ml of egg and feed lipids) was transferred into lipid-cleaned 7 ml vials and evaporated under nitrogen to dryness. This was done to evaporate off the chloroform as it will remove phase from the column. The lipids were then dissolved in hexane (0.5 ml) and 1.5 ml of boron trifluoride in methanol (BF_3/CH_3OH) (Fisher Scientific). Boron trifluoride is the catalyst that is used for the formation of fatty acid methyl esters. To keep any lipids from oxidizing during the derivatization process the vial was flushed with N_2 gas (Air Liquide), capped and sealed before being vortexed. The vial was sonicated for 4 minutes to remove any lipids adhering to the sides of the vial. The samples were heated to 85ºC for 1.5 hours, and after 45 minutes the vials were gently shaken. The samples were then cooled and 0.5 ml of chloroform-extracted water was added to stop hydrolysis before vortexing thoroughly. Hexane (Fisher Scientific) (2 ml) was then added to the samples before flushing with N_2 gas (Air Liquide) and once again capping. The samples were vortexed before being sonicated for 4 minutes. The upper organic layer was removed and placed in a hexane-cleaned 2 ml gas chromatography (GC) vial and blown dry to ensure that all the BF_3/CH_3OH had evaporated. Hexane (Fisher Scientific) (1 ml) was then added to the dried sample, prior to flushing with N_2 gas (Air Liquide), capping and sealing with Teflon

tape. The samples were then sonicated for 4 minutes to redissolve the fatty acids, and stored at -20ºC until analysis by GC.

The composition of the fatty acid methyl esters (FAME) of each extract was analysed on an Agilent 7890A GC with a flame ionization detector and a Phenomenex Zebron wax plus column (dimensions: 30 m x 320 µm x 0.25 µm). The GC conditions were as follows, Agilent injector temperature: cool on column injector at 30ºC; column temperature program: 65ºC for 30 secs, 40ºC/minute ramp to 195ºC, hold for 15 mins, 2ºC/min to 220ºC, hold for 2.75 mins; detector temperature: 260ºC; gas flows: air 300 ml/min, H_2 30 ml/min, Helium (He) 30 ml/min. Fatty acid peaks were integrated using Agilent ChemStation software (version 2.0), and identification was made with reference to known standards (PUFA 1, 3 and 37 component FAME mix and Bacterial Acids Methyl Esters (BAME) mix, Supelco, Inc.). Fatty acid values are presented as mass percent of total fatty acids.

2.2.5 Statistical Analysis

All statistical tests were carried out using SigmaPlot Version 11 (Systat Software Inc.). Data for the diets were analyzed for differences among diets using one-way ANOVA. When data did not meet normality, a Kruskal-Wallis one-way ANOVA on ranks was used. When data did not meet normality and significant differences were observed, the Dunn's method (a post hoc pairwise comparison test) was used. When significant differences were observed and normality was met, the Holm-Sidak (a post hoc test) multiple comparison procedure was used to identify significant differences. Data for the eggs were analyzed for tank, diet and interaction effects using a 2-way analysis of

variance (ANOVA). When data did not meet the normality and equality of variance assumptions, the Kruskal-Wallis analysis of variance on ranks (non-parametric test) was used. When significant differences were observed, the Holm-Sidak multiple comparison procedure was used to identify the significant differences for each of the groups. All tests used a confidence interval of 95% ($p = 0.05$).

2.3 Results

No tank effect was detected for any of the measured parameters, except in the concentration of alcohols (ALC) in the cod eggs from the two tanks with the baitfish diet treatment. There were no significant differences observed in the mean ash weight or mean % ash among the three diets (Table 2.1).

2.3.1 Dietary Lipid Classes

Significant differences were observed in total lipids among the diets ($p = <0.001$) (Table 2.1) with the OG diet (8.2 mg g^{-1}) containing lower amounts than both the BR (41.8 mg g^{-1}) and BF (41.3 mg g^{-1}) diets. The statistical analyses indicated that there were significant differences in lipid concentration (µg g^{-1}) in four of the nine dietary lipid classes examined. Differences were observed between the on-growing diet and the broodstock/baitfish diet in KET ($p = 0.004$) and ST ($p = 0.015$), as well as between the on-growing diet and the baitfish diet in acetone mobile polar lipids (AMPL: $p = 0.004$), and among all three diets in phospholipids (PL: $p = \leq 0.001$) (Table 2.1). KET values were higher in the OG diet while ST, AMPL and PL were lower in this diet when compared to BF and/or BR diets. The results indicate that the diets' most abundant lipid classes were TAG and PL. The OG diet had TAG at 62% of the TL content, while PL

comprised 12.8% of the TL content. The BR diet contained 30% of the TL content as TAG and 59% as PL. Finally, the BA diet had 19% of the TL content as TAG and 36% as PL. The baitfish diet contained a similar amount of AMPL as PL (36%), with 33% of the TL content as AMPL. While the AMPL percentages in the on-growing and broodstock diet were found to account for only 0.01% and 2.4% of the TL content respectively (Figure 2.1).

2.3.2 Egg Lipid Classes

Total lipid (TL) made up about 9.0 µg egg^{-1}, 6.2 µg egg^{-1} and 9.4 µg egg^{-1} of the wet weight of eggs, for the OG, BR and BF diet respectively (Table 2.2). No significant differences were observed in the total lipids of the eggs ($p = 0.281$). The average PL concentration for the three diets was 9.4 µg egg^{-1}, the concentration of free fatty acids (FFA) averaged 0.46 µg egg^{-1} and the concentration of ST averaged 0.46 µg egg^{-1} (Table 2.2). No significant differences ($p = 0.295 - 0.878$) were found in the proportions (% total lipid) in the eggs for any of the lipid classes. Phospholipids were found to be the most abundant lipid class present in the eggs accounting for 83.8%, 76% and 83.6% of the TL in the OG, BR and BF diets respectively, while all other lipid classes were found to be less than 10% of TL (Table 2.2). With regards to egg lipid concentrations, no significant differences were found between diet treatments ($p > 0.05$), except for the ALC concentration where there were significant differences between the baitfish and broodstock diets ($p = 0.040$) (Table 2.2).

2.3.3. Dietary fatty acids

The most abundant dietary fatty acid was palmitic acid (16:0) and there were no significant differences in the proportion of palmitic acid among diets (Table 2.3). Palmitic acid accounted for 17% of the fatty acids (FA) in the on-growing diet, 16.9% of FA in the broodstock diet and 17.7% of FA in the baitfish diet. Statistical analysis indicated significant differences in fatty acids (% of total FA) and ratios among diets for 16 of the 29 dietary fatty acids examined (Table 2.3).

In terms of essential fatty acids (EFA), there were no significant differences observed in the DHA and ARA proportions of the diets. However, EPA (20:5ω3) comprised the largest proportion found in the OG and BR diets at 8.5% and 17% of FA, while EPA accounted for the second most abundant EFA in the BF diet at 7.6%. The second most abundant EFA in the OG and BR diet was DHA (22:6ω3) which accounted for 6.5% and 10.5% of FA, while DHA was found to be the most abundant EFA in the BF diet at 16.7%. Finally, ARA (20:4ω6) accounted for the smallest proportion of EFAs in the diets at 0.85% of FA in the OG diet, 1.18% of FA in the BR diet and 0.69% of FA in the BF diet (Figure 2.2).

2.3.4. Egg fatty acids

DHA was found to be the most abundant fatty acid in the eggs (Table 2.4), accounting for 21%, 23% and 29% of FA in the on-growing, broodstock and baitfish diets respectively. The second most abundant fatty acid was palmitic acid (16:0), accounting for 22%, 22%, and 21% of FA in the on-growing, broodstock, and baitfish diets respectively. The third most abundant fatty acid observed in the eggs was EPA (20:5ω3),

which accounted for 14.57%, 16.57%, and 13.79% of FA in the on-growing, broodstock and baitfish diets respectively (Table 2.4).

Significant differences existed among diet treatments for 18 of the 19 egg fatty acids and ratios that were examined (Figure 2.3). Significant differences were found in palmitic acid (16:0) ($p = 0.040$), hexadecenoic acid (16:1ω9) ($p = <0.001$), palmitoleic acid (16:1ω7) ($p = <0.001$), gadoleic acid (20:1ω9) ($p = <0.001$), linoleic acid (18:2ω6) ($p = <0.001$), ARA (20:4ω6) ($p = <0.001$), EPA (20:5ω3) ($p = <0.001$), docosapentaenoic acid (22:5ω3) ($p = <0.001$), DHA (22:6ω3) ($p = <0.001$), ΣSFA ($p = 0.023$), ΣMUFA ($p = <0.001$), ΣPUFA ($p = <0.001$), DHA:EPA ($p = <0.001$), EPA:ARA ($p = <0.001$), Σω3 ($p = <0.001$), Σω3 HUFA ($p = <0.001$), Σω6 HUFA ($p = <0.001$), ΣHUFA ($p = <0.001$). An interaction between diets and tanks was found in stearic acid (18:0) ($p = 0.040$), vaccenic acid (18:1ω7) ($p = 0.047$), oleic acid (18:1ω9) ($p = 0.033$) and a tank effect was found in EPA (20:5ω3) ($p = 0.040$).

Table 2.1. Comparison of dietary lipid concentration (mg g^{-1}), lipid class proportions (%) and ash weight among diets. Values are mean ± Std. Dev. Dissimilar superscript letters indicate significant differences ($p < 0.05$) among diets. Data analyzed using a one-way analysis of variance.

	OG (n=3)	BR (n=3)	BF (n=9)
Lipids	Concentration (mg g^{-1})		
HC	0 ± 0^{a}	$13x10^{-3} \pm 23x10^{-3\,a}$	$43x10^{-3} \pm 27x10^{-3\,a}$
SE	$2.7x10^{-3} \pm 4.6x10^{-3\,a}$	$9.9x10^{-3} \pm 2.0x10^{-3\,a}$	$4.9x10^{-3} \pm 8.7x10^{-3\,a}$
KET	$66x10^{-3} \pm 63x10^{-3\,a}$	0 ± 0^{b}	$1.7x10^{-3} \pm 2.9x10^{-3\,b}$
TAG	5.2 ± 0.89^{a}	13 ± 0.48^{a}	10 ± 9.0^{a}
FFA	$1.2 \pm 58x10^{-3\,a}$	1.2 ± 0.35^{a}	1.4 ± 0.16^{a}
ALC	$49x10^{-3} \pm 16x10^{-3\,a}$	0.42 ± 0.13^{a}	0.29 ± 0.32^{a}
ST	$0.63 \pm 6.6x10^{-3\,a}$	1.8 ± 0.26^{b}	1.4 ± 0.47^{b}
AMPL	$0.8x10^{-3} \pm 1.4x10^{-3\,a}$	1.0 ± 0.45^{ab}	17 ± 16^{b}
PL	1.4 ± 2.2^{a}	25.0 ± 7.4^{b}	12 ± 2.7^{c}
Total Lipids	8.18 ± 3.07^{a}	41.8 ± 7.22^{b}	41.3 ± 26.1^{b}
Lipid Classes	Proportion (%)		
HC	0 ± 0^{a}	0.04 ± 0.06^{a}	0.12 ± 0.07^{a}
SE	0.02 ± 0.04^{a}	0.02 ± 0.01^{a}	0.03 ± 0.06^{a}
KET	0.9 ± 0.9^{a}	0 ± 0^{b}	0.003 ± 0.005^{b}
TAG	62.4 ± 10.3^{a}	30.3 ± 12.7^{b}	18.9 ± 16.8^{b}
FFA	15.4 ± 4.0^{a}	3.3 ± 0.8^{b}	5.2 ± 4.9^{ab}
ALC	0.6 ± 0.04^{a}	0.9 ± 0.3^{a}	0.57 ± 0.36^{a}
ST	7.9 ± 2.4^{a}	4.2 ± 0.1^{a}	5.9 ± 6.4^{a}
AMPL	0.01 ± 0.01^{a}	2.4 ± 0.6^{ab}	33.3 ± 16.9^{b}
PL	12.8 ± 17.2^{a}	58.8 ± 13.4^{ab}	35.9 ± 19.5^{b}
Ash Weight	OG (n=5)	BR (n=5)	BF (n=15)
Mean Ash Weight (g)	0.19 ± 0.001	0.18 ± 0.002	0.04 ± 0.01
Mean % Ash	10.3 ± 0.022	9.8 ± 0.092	7.4 ± 1.9

[Hydrocarbons (HC), steryl esters (SE), ketones (KET), triacylglycerols (TAG), free fatty acids (FFA), alcohols (ALC), sterols (ST), acetone mobile polar lipids (AMPL) and phospholipids (PL)] On-growing (OG), broodstock (BR), baitfish (BF). Baitfish vitamin supplementation (% of Mix): vitamin B12 (0.067), vitamin D3 cholecalciferol (0.075), vitamin A acetate (0.133), folic acid (0.281), vitamin K (0.300), vitamin B6 (0.400), d-biotin (0.400), vitamin B2 riboflavin (0.500), vitamin B1 (1.000), d-calcium pantothenate (1.200), Niacin (1.333), Myo-Inositol (1.333), vitamin E (2.233), vitamin C (6.667), Carophyll Pink 2% CWS (15.000), ethoxyquin (0.889), wheat~~s~~ shorts (68.189).

Table 2.2. Comparison of egg lipid classes in µg egg^{-1} and % total lipids for all three diets. Values are means ± Std. Dev. Dissimilar superscript letters indicate significant differences ($p < 0.05$) among diets. Data analyzed using a two-way analysis of variance.

	OG (n=15)	BR (n=10)	BF (n=6)
Lipids	Concentration (µg egg^{-1})		
HC	0.04 ± 0.05 [a]	0.07 ± 0.10 [a]	0.07 ± 0.05 [a]
SE	0.001 ± 0.002 [a]	0 ± 0 [a]	0 ± 0 [a]
KET	0.0 ± 0.002 [a]	0 ± 0 [a]	0 ± 0 [a]
TAG	0.16 ± 0.29 [a]	0.11 ± 0.14 [a]	0.06 ± 0.06 [a]
FFA	0.48 ± 0.20 [a]	0.44 ± 0.19 [a]	0.45 ± 0.06 [a]
ALC	0.04 ± 0.04 [ab]	0.02 ± 0.03 [a]	0.07 ± 0.07 [b]
ST	0.37 ± 0.41 [a]	0.32 ± 0.38 [a]	0.61 ± 0.41 [a]
AMPL	0.68 ± 0.62 [a]	0.74 ± 0.43 [a]	0.71 ± 0.58 [a]
PL	9.8 ± 3.9 [a]	8.1 ± 3.7 [a]	10.5 ± 2.9 [a]
Total Lipids	9.01 ± 7.99[a]	6.24 ± 3.50[a]	9.38 ± 4.23[a]
Lipid Classes	Proportion (%)		
HC	0.4 ± 0.4 [a]	4.1 ± 10.3 [a]	0.44 ± 0.53 [a]
SE	0.01 ± 0.02 [a]	0 ± 0 [a]	0 ± 0 [a]
KET	0.01 ± 0.03 [a]	0 ± 0 [a]	0 ± 0 [a]
TAG	1.6 ± 2.7 [a]	3.4 ± 8.9 [a]	0.45 ± 0.44 [a]
FFA	4.4 ± 1.7 [a]	4.8 ± 1.6 [a]	3.9 ± 1.4 [a]
ALC	0.34 ± 0.40 [a]	0.59 ± 1.5 [a]	0.54 ± 0.61 [a]
ST	2.9 ± 3.2 [a]	2.5 ± 3.1 [a]	4.4 ± 2.8 [a]
AMPL	6.3 ± 5.7 [a]	8.2 ± 4.9 [a]	6.1 ± 4.8 [a]
PL	83.8 ± 5.1 [a]	76.4 ± 16.4 [a]	83.6 ± 1.7 [a]

[Hydrocarbons (HC), steryl esters (SE), ketones (KET), triacylglycerols (TAG), free fatty acids (FFA), alcohols (ALC), sterols (ST), acetone mobile polar lipids (AMPL) and phospholipids (PL)] On-growing (OG), broodstock (BR), baitfish (BF)

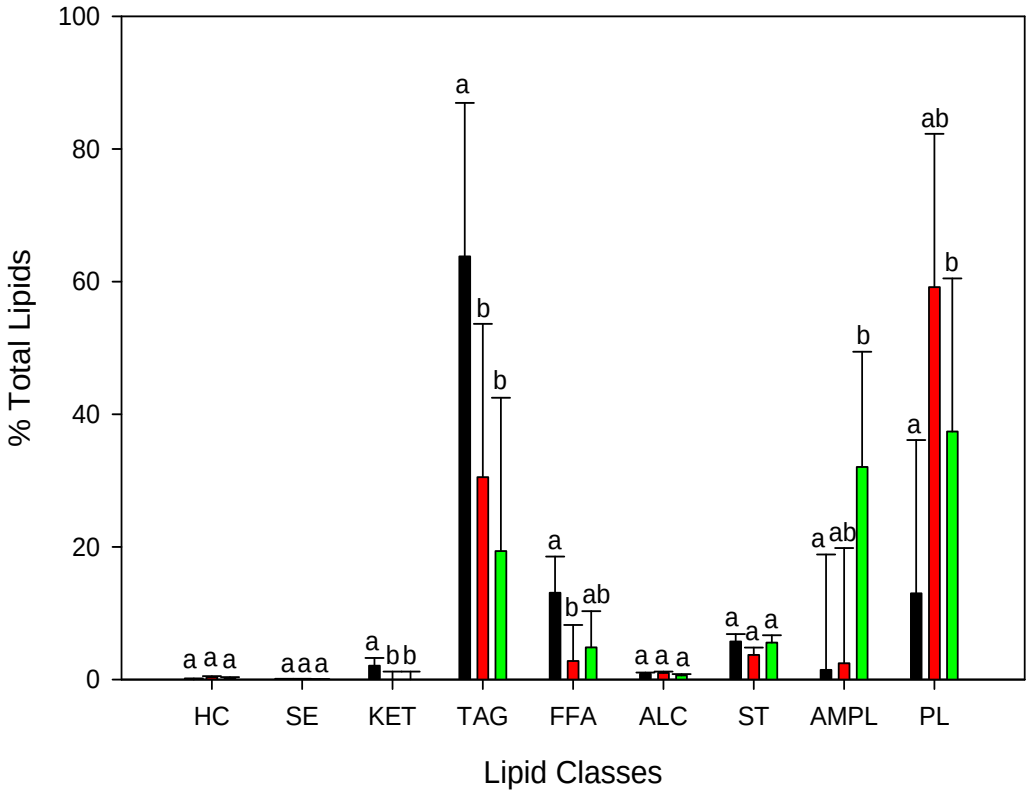

Figure 2.1. Comparison of the dietary lipid class proportions in the three diets presented as mean ± S.D. [Hydrocarbons (HC), steryl esters (SE), ketones (KET), triacylglycerols (TAG), free fatty acids (FFA), alcohols (ALC), sterols (ST), acetone mobile polar lipids (AMPL) and phospholipids (PL)] on-growing (■), broodstock (■), baitfish (■). Dissimilar superscript letters indicate significant differences ($p < 0.05$) among diets.

Table 2.3. Comparison of dietary fatty acid composition (mean % of identifiable FAME) among diets. Values are mean ± Std. Dev. Dissimilar superscript letters indicate significant differences ($p \leq 0.05$) among diets. Data analyzed using one-way analysis of variance.

FAME	OG (n= 3)	BR (n= 3)	BF (n = 9)
14:0	5.07 ± 0.02	6.98 ± 0.07	7.07 ± 4.10
16:0	17.31 ± 0.11	16.94 ± 0.07	17.69 ± 3.28
18:0	4.00 ± 0.04^{a}	3.50 ± 0.09^{b}	2.11 ± 0.25^{c}
Σ SFA[1]	27.71 ± 0.14	28.73 ± 0.17	28.16 ± 6.51
16:1ω7	6.36 ± 0.07^{ab}	7.30 ± 0.03^{a}	3.89 ± 2.24^{b}
18:1ω7	2.99 ± 0.06^{a}	2.87 ± 0.03^{a}	1.89 ± 0.26^{b}
18:1ω9	21.68 ± 0.04^{a}	9.1 ± 0.11^{ab}	7.85 ± 2.79^{b}
20:1ω9	1.36 ± 0.03^{ab}	0.7 ± 0.04^{a}	8.89 ± 3.75^{b}
20:1ω11	0.58 ± 0.01^{ab}	0.11 ± 0.02^{a}	1.14 ± 0.68^{b}
22:1ω9	0.23 ± 0.01^{ab}	0.14 ± 0^{a}	5.75 ± 7.35^{b}
22:1ω11(13)	0.97 ± 0.03	0.47 ± 0.01	5.94 ± 8.22
Σ MUFA[2]	35.85 ± 0.07	22.37 ± 0.17	38.83 ± 17.79
16:2ω4	1.12 ± 0.01^{ab}	1.35 ± 0.04^{a}	0.59 ± 0.42^{b}
16:3ω4	0.84 ± 0.02^{a}	1.48 ± 0.04^{b}	0.09 ± 0.08^{c}
16:4ω1	1.25 ± 0.03^{ab}	2.65 ± 0.01^{a}	0.11 ± 0.16^{b}
18:2ω6	9.49 ± 0.11^{a}	4.92 ± 0.04^{b}	1.16 ± 0.47^{c}
18:3ω3	1.81 ± 0.01^{a}	0.92 ± 0.01^{ab}	0.87 ± 0.52^{b}
18:4ω3	1.23 ± 0.02	1.97 ± 0.05	1.41 ± 0.97
(ARA) 20:4ω6	0.85 ± 0.01	1.18 ± 0.01	0.69 ± 0.61
(EPA) 20:5ω3	8.47 ± 0.09^{ab}	17.33 ± 0.16^{a}	7.55 ± 6.22^{b}
22:5ω3	1.51 ± 0.02^{a}	2.09 ± 0.02^{b}	0.48 ± 0.23^{c}
(DHA) 22:6ω3	6.51 ± 0.03	10.54 ± 0.12	16.67 ± 17.91
Σ PUFA[3]	35.63 ± 0.20	47.73 ± 0.19	31.65 ± 23.57
DHA:EPA	0.77 ± 0.01^{ab}	0.61 ± 0.01^{a}	1.78 ± 0.60^{b}
EPA:ARA	9.93 ± 0.16^{a}	14.73 ± 0.13^{b}	11.95 ± 1.68^{ab}
Σ ω3	20.56 ± 0.18	34.34 ± 0.23	28.22 ± 23.41
Σ ω3 HUFA	17.47 ± 0.15	31.32 ± 0.22	25.84 ± 24.59
Σ ω6 HUFA	1.38 ± 0.06	1.76 ± 0.05	0.93 ± 0.71
Σ HUFA	18.85 ± 0.21	33.08 ± 0.21	26.77 ± 25.29

[1] Includes 15:0, *i*15:0, *ai*15:0, *i*16:0, *ai*16:0, 17:0, *i*17:0, *ai*17:0, 20:0, 21:0, 22:0, 23:0 and 24:0 present at <0.73% in some or all samples
[2] Includes 14:1, 16:1ω5, 16:1ω9, 17:1, 18:1ω5, 20:1ω7, 22:1ω7 and 24:1 present at <0.74% in some or all samples
[3] Includes 16:4ω3, 18:2ω4, 18:3ω6, 18:3ω4, 18:4ω1, 20:2ω6, 20:3ω6, 20:3ω3, 20:4ω3, 21:5ω3, 22:4ω6, 22:4ω3 and 22:5ω6 present at <0.69% in some or all samples
HUFA = fatty acids ≥ 20:3

Table 2.4. Comparison of egg fatty acid composition (mean % of total identifiable FAME) among diets. Values are means ± Std. Dev. Dissimilar superscript letters indicate significant differences ($p \leq 0.05$) among diets. Data analyzed using a two-way analysis of variance.

FAME	OG (n= 22)	BR (n= 16)	BF (n = 9)
14:0	2.08 ± 1.85	2.32 ± 1.18	1.75 ± 0.27
16:0	21.71 ± 1.40^{ab}	22.19 ± 1.40^{a}	20.84 ± 0.84^{b}
18:0**	3.92 ± 0.52	3.36 ± 0.49	2.66 ± 0.72
Σ SFA[1]	28.33 ± 3.13^{a}	28.66 ± 1.64^{a}	25.93 ± 0.69^{b}
16:1ω9	1.47 ± 0.18^{a}	1.05 ± 0.10^{b}	1.61 ± 0.25^{c}
16:1ω7	2.53 ± 0.34^{a}	2.91 ± 0.30^{b}	2.36 ± 0.22^{a}
18:1ω7**	3.46 ± 0.31	3.66 ± 0.17	3.27 ± 0.21
18:1ω9**	14.55 ± 1.08	9.96 ± 0.66	13.21 ± 0.95
20:1ω9	0.57 ± 0.39^{a}	0.36 ± 0.06^{a}	2.43 ± 0.77^{b}
Σ MUFA[2]	23.49 ± 1.24^{a}	18.66 ± 0.82^{b}	25.17 ± 0.93^{c}
18:2ω6	4.62 ± 1.08^{a}	4.99 ± 0.50^{a}	1.34 ± 1.17^{b}
(ARA) 20:4ω6	2.13 ± 0.27^{a}	1.97 ± 0.09^{a}	1.18 ± 0.42^{b}
(EPA) 20:5ω3*	14.57 ± 1.04^{a}	16.57 ± 1.11^{b}	13.79 ± 1.11^{a}
22:5ω3	2.55 ± 0.41^{a}	2.26 ± 0.13^{b}	1.38 ± 0.36^{c}
(DHA) 22:6ω3	21.09 ± 2.55^{a}	23.49 ± 1.07^{b}	28.59 ± 2.64^{c}
Σ PUFA[3]	47.65 ± 2.86^{a}	52.06 ± 1.96^{b}	48.15 ± 1.28^{a}
DHA:EPA	1.45 ± 0.19^{a}	1.42 ± 0.08^{a}	2.09 ± 0.29^{b}
EPA:ARA	6.97 ± 1.38^{a}	8.41 ± 0.55^{b}	12.29 ± 2.03^{c}
Σ ω3	39.56 ± 2.87^{a}	43.63 ± 2.04^{b}	45.01 ± 1.45^{b}
Σ ω3 HUFA	38.60 ± 3.16^{a}	42.85 ± 2.04^{b}	44.29 ± 1.58^{b}
Σ ω6 HUFA	2.49 ± 0.39^{a}	2.20 ± 0.16^{b}	1.29 ± 0.54^{c}
Σ HUFA	41.24 ± 2.85^{a}	45.05 ± 2.11^{b}	45.57 ± 1.35^{b}

[1] Includes 15:0, *i*15:0, *ai*15:0, *i*16:0, *ai*16:0, 17:0, *i*17:0, *ai*17:0, 20:0, 21:0 and 24:0 present at <0.40% in some or all samples
[2] Includes 14:1, 15:1, 16:1ω5, 17:1, 18:1ω5, 20:1ω11, 20:1ω7, 22:1ω11(13), 22:1ω9, 22:1ω7 and 24:1 present at <0.62% in some or all samples
[3] Includes 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:3ω4, 18:3ω3, 18:4ω3, 18:4ω1, 20:2ω6, 20:4ω3, 21:5ω3 and 22:5ω6 present at <0.58% in some or all samples
HUFA = fatty acids ≥ 20:3 (* indicates a tank effect, ** indicates an interaction effect between tank and diet)

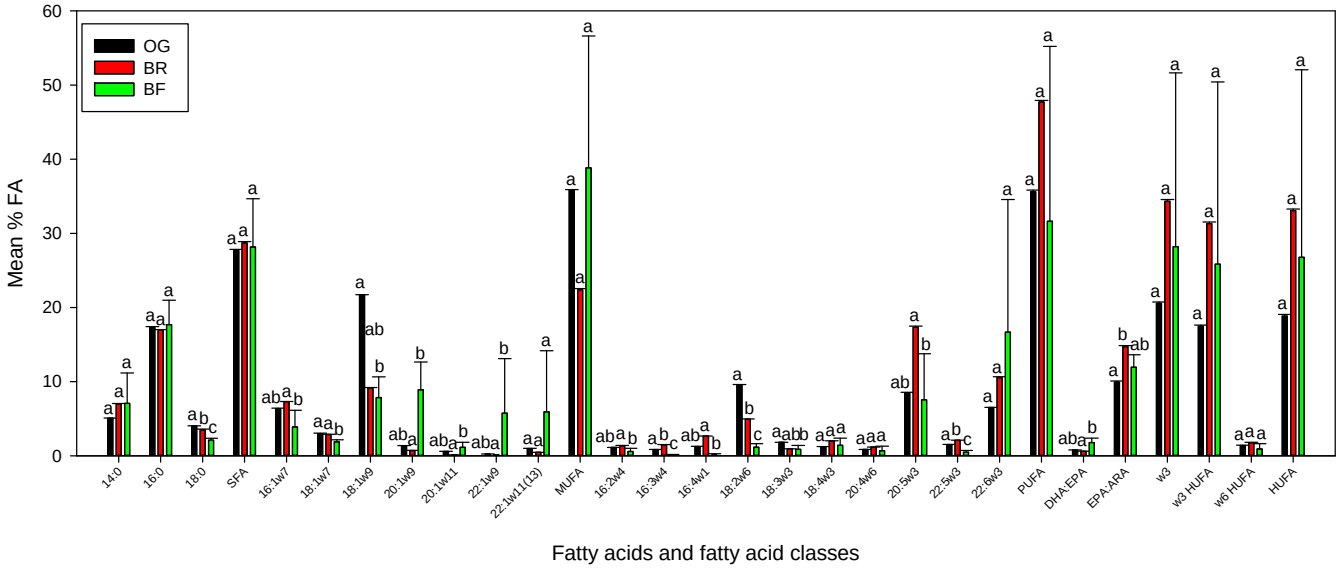

Figure 2.2. Comparison of dietary fatty acid composition (mean % of total identifiable FAME) of the three diet groups. On-growing (■), Broodstock (■), Baitfish (■). Dissimilar superscript letters indicate significant differences ($p \leq 0.05$) among diets.

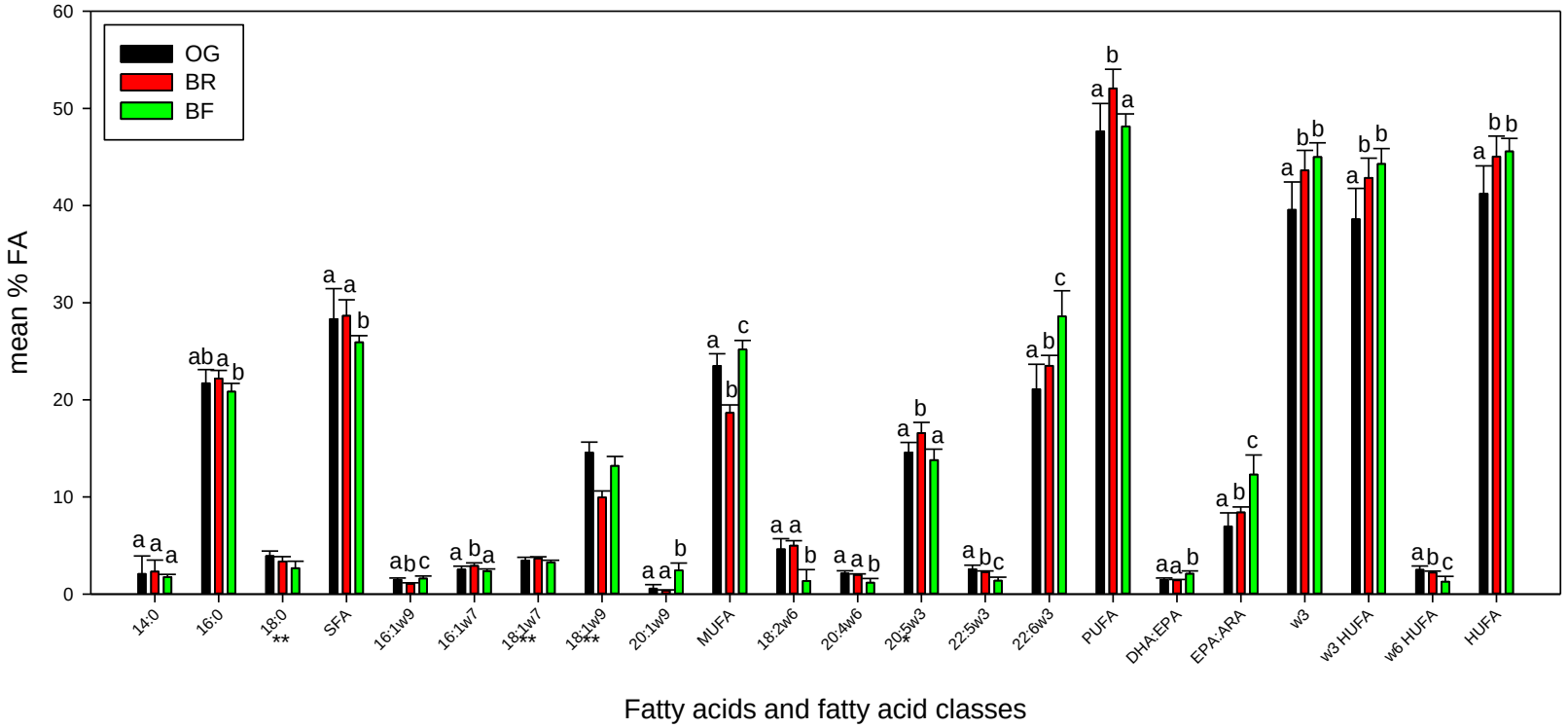

Figure 2.3. Comparison of egg fatty acid composition (mean % of total identifiable FAME) of the three diet groups. On-growing (■), Broodstock (■), Baitfish (■). Dissimilar superscript letters indicate significant differences ($p \leq 0.05$) among diets.

2.4 Discussion

2.4.1 Dietary Ash and lipid content

No significant differences were found in ash weight content of the 3 diets and all diet results are comparable to the results of Fafioye (2005) with the mean ash proportions of the dry weight of the three diets around 9.95%. Ash weight is what remains after the sample is heated at high temperatures and organic matter is burned off leaving only the minerals. These minerals include, but are not limited to calcium, phosphorus, zinc, and iron. Normally the ash content of commercial diets for cold water species average 12 - 13% (*Gadus morhua*: Morais *et al*. 2001; *Hippoglossus hippoglossus*: Brown 2009), with more ash indicating a higher mineral content, especially calcium, phosphorus and magnesium (Toppe *et al*. 2006). The results from the current study found a lower ash content among all three diets, suggesting that the diets contain a lower mineral content than typical commercial fish meals and a higher proportion of protein and lipid.

There were differences in the lipid concentrations of the three diets, with significant differences found for four of the nine lipid classes examined. The on-growing diet contained the highest KET proportion of TL. Ketones are important fuels in many animal tissues, and the brain in particular relies heavily on ketones during times of reduced food intake (Ramos 1978); however, the proportions in this study are so low that the observed differences are not likely to be biologically meaningful. On the other hand, ketones often contribute to flavors and odors and therefore may have affected diet palatability. It was observed that during weaning the broodstock fish took a few weeks to accept the broodstock diet, while the fish fed the on-growing and baitfish diet showed little to no change in feeding habits. All fish were fed to satiation during the course of the

study. Sterols, AMPL and PL were lower in the OG diet when compared to BF and/or BR diets. In particular, substantially higher amounts of AMPL were observed in the baitfish diet in comparison to the on-growing and broodstock diets. Unfortunately little is known about the effects of acetone mobile polar lipids in fish nutrition and development. The broodstock diet yielded the highest PL proportion of TL followed by the baitfish diet and then the on-growing diet. Dietary phospholipids can supply energy as well as components for membrane formation, and high levels in larvae may suggest increased fertilization potential leading to higher reproductive output (Clarke *et al*. 2005).

One main difference in diet content was that both the broodstock and baitfish diet contained a squid component, while the on-growing diet did not (www.skretting.com/internet/SkrettingGlobal). Lall & Nanton (2002) found the stomach contents of wild caught cod to contain 45% fish, 33% crustaceans, 4% echinoderms and 3% molluscs and noted that capelin has been reported as the most important species in the diet of wild cod. However, Izquierdo *et al*. (2001) noted that the protein component of cuttlefish meal and squid meal, as well as their optimal concentrations of highly unsaturated fatty acids (HUFAs), appear to promote higher reproductive performance. Therefore, cuttlefish, squid and krill meal have been recognized as a valuable component of broodstock diets (Izquierdo *et al*. 2001). Squid is also considered to have a superior protein quality and a higher PL and ST content (Watanabe *et al*. 1991). The results from this study both support and contradict these findings, as we found that the broodstock and baitfish diets had higher proportions of PL than the on-growing diet, but had lower proportions of ST.

2.4.2 Egg Lipid Content and Lipid Class Composition

Broodstock nutrition has been shown to affect viability and health of offspring in marine fish, and thus has been recognized as a vital factor in reproductive performance and subsequent egg quality (Luquet & Watanabe 1986; Bromage *et al*. 1992; Cerdá *et al*. 1994; Bell *et al*. 1997; Navas *et al*. 1997; Bruce *et al*. 1999; Pavlov *et al*. 2004). Dietary nutrients are transported to the oocyte to provide the essential energy and nutritional requirements that are needed for the growth and development of the embryonic larvae up until the commencement of exogenous feeding (Marozza *et al*. 2003). In this study, there were no observed similarities between the dietary total lipid and lipid class composition of the eggs. Some studies have suggested that the lipid classes found in fish eggs are reflective of the broodstock diet (*Sparus aurata*: Watanabe *et al*. 1984a, 1985a; Mourente & Odriozola 1990; Fernandez-Palacios *et al*. 1995; *Dicentrarchus labrax*: Bell *et al*. 1997). Other studies report that lipid class composition in fish eggs was not affected by the lipid class composition of the diet (*Oncorhynchus mykiss*: Frémont *et al*. 1984; *Gadus morhua*: Lie 1993). However, few studies have examined nutritional trials on cod broodstock (Salze *et al*. 2005), and Izquierdo *et al*. (2001) speculated that the results of Lie (1993) may be due to a low EFA requirement in cod broodstock when compared to other marine species.

While generally no differences were observed in the lipid classes or total lipids of the eggs from the three diets, some trends could be observed in the proportion of total lipids (TL) of the eggs. Although not significant, the total lipids were highest in the baitfish diet and lowest with the broodstock diet. Finn *et al*. (1995a) found slightly higher amounts of total lipid in cod eggs (14.8 ± 0.50 µg ind^{-1}), compared to total lipid amounts

found in this study (~8.2 µg egg^{-1}). The largest contributor to total lipids in the eggs for all three diets was PL, which was highest in the on-growing diet and baitfish diet. Many other studies have also reported PL to be the predominant lipid class in fish eggs (Lasker & Theilacker 1962; Kaitaranta & Ackman 1981; Tocher & Sargent 1984; Finn 1995a; Penney *et al*. 2006; Palacios *et al*. 2007). Phospholipids were originally thought of as a preferred energy reserve, but have since been identified as important in the proliferation of cells and new biomembranes (Tocher & Sargent 1984). Hakanson (1993) defined eggs with a PL content of less than 80% of TL to be in "poor condition". Though it is difficult to make direct comparisons among studies due to differences in lipid extraction and analysis procedures, our results seem comparable to previous data (Fraser *et al*. 1988; Ulvund & Grahl-Nielsen 1988; Finn *et al*. 1995b; Pickova *et al*. 1997; Penney *et al*. 2006; Clarke *et al*. 2005) which found PLs to account for ~60% to ~80% of the TL in fish eggs. It is important to reiterate that after examination of several lipid parameters as good predictors of hatching success, Penney *et al*. (2006) found that none of the biochemical parameters examined correlated with hatching success when mean hatching success rates were high (>75%). The hatching success in Hamoutene *et al*. (2013) was much lower ~ 14%, and none of the lipid classes examined in this study appeared to hold a relationship with hatching success either. However, Pickova *et al*. (1997) found significant relationships between hatching success and lipid biochemistry in 2 of 4 cod stocks, suggesting that such relationships may be stock specific, or linked to an unmeasured co-varying parameter.

Tocher & Sargent (1984) showed that cold water species such as Atlantic cod generally have about twice as many polar lipids (e.g. PL) than neutral lipids (e.g. TAG).

However, relative levels of TAG observed in our study were much lower (0.45 – 3.4% of TL) and similar to levels reported by Penney *et al*. (2006, 2011) and Clarke *et al*. (2005) for Atlantic cod. Navas *et al*. (1997) examined the lipid composition of European sea bass (*Dicentrarchus labrax*) oocytes and found that the neutral lipids were predominantly TAG, and that levels of TAG were significantly higher during pre-vitellogenesis. Palacios *et al*. (2007) found that TAG concentrations in neutral lipid fractions significantly decreased during early development of the embryo, indicating that TAG is used as an energy source. Tocher & Sargent (1984) found TAG to be the most abundant neutral lipid class in several species of marine fish eggs, including cod, saithe (*Pollachius pollachius*), haddock (*Melanogrammus aeglefinus*), and whiting (*Merlangius merlangus*), followed by sterols (ST). They noted that the polar and neutral lipid fractions were generally similar in all species examined suggesting that the involvement of these lipids is important in basic functions common to all species. This may explain the difference in TAG and ST concentrations of the diets and eggs observed in this study. The dietary proportion of TAG was much higher in the diets than in the eggs, with a similar trend observed in dietary proportion of ST to the eggs, supporting Tocher & Sargent's (1984) claim that neutral lipids (which are generally comprised of TAG and ST) are utilized as energy reserves during embryonic and early larval development. The highest abundance of ST in this study were observed in the baitfish eggs, slightly lower than what Penney *et al*. (2006) reported for ST in wild-caught Atlantic cod (*Gadus morhua*) eggs (6-10% of TL) after being fed similar baitfish diet. Lochmann *et al*. (1994) identified STs as an important structural lipid class in fish eggs, and even hypothesized that ST may be an adequate predictor of larval body size.

Other developmentally important lipid classes in the eggs were acetone mobile polar lipids (AMPL) at 6.3% TL and free fatty acids (FFA) at 4.4% TL. Although not significantly different among diets, acetone mobile polar lipids were found to be the second most abundant lipid class in the eggs, and were observed at their highest proportion in the broodstock eggs. The AMPL proportion observed in this study is comparable to that of Penney *et al*. (2006) who found AMPL in wild caught Atlantic cod broodstock (*Gadus morhua*) eggs to account for ~7% of the TL. Parrish (1987) found that AMPL in solvent extracts of marine dissolved and particulate matter, benthic invertebrates, and net-caught zooplankton, can contain monoacylglycerols (MAG), glycolipid and chlorophyll *a*. Copeman (2004) hypothesized that elevated levels of AMPL in fish eggs may be due to the lipolysis of TAG into diacylglycerol (DAG) and FFA, followed by further change to MAG (or AMPL) and FFA. This process of lipolysis could be significant in the developmental process of the eggs in this study: perhaps the proportion observed in the eggs is a result of the AMPL and FFA produced in the process of lipolysis. This may be the case as the next most abundant lipid class observed in the eggs was free fatty acids (FFA). All treatments showed higher levels than previous published results for FFA in wild caught Atlantic cod (*Gadus morhua*) eggs held in similar conditions and fed similar diets (Penney *et al*. 2006). However, Bligh (1966) found that during cold storage of samples, PL can hydrolyze into FFA. The samples for this study remained in storage at -70°C from November/December 2009 until May/June 2010, and may account for the higher FFA levels. Unfortunately, there has been little research done on the importance of AMPL and FFA in the development of fish embryos.

Overall, the differences present in the dietary lipid classes of this study did not translate into differences in the lipid classes of the eggs. This is likely due to the reorganization of lipids during gonadogenesis and the formation of the oocytes. Lipids are utilized as sources of metabolic energy in the form of ATP provided through the β-oxidation of fatty acids (Frøyland *et al*. 2000). Lipids, specifically fatty acids, are utilized in the fish for growth, reproduction and movement (migration) (Tocher 2003). In the gonadal maturation of female broodfish, lipids are essential materials used in the formation of cell and tissue membranes in developing embryos and larvae (Sargent 1995b).

2.4.3 Dietary Fatty Acids

Significant differences were found among the diets for 16 of the 29 dietary fatty acids and their ratios. In terms of essential fatty acids (EFA), there were no significant differences observed in the DHA and ARA proportions of the diets. ARA accounted for the smallest proportion of EFA's. These results are comparable to those found in the literature, as Watanabe *et al*. (1984b) found a range of 0.6 - 1.8% in soft-dry pelleted feed for red sea bream and Roy *et al*. (2007) found ARA proportions of 0.65% in pelleted diet for Atlantic cod (*Gadus morhua*).

The broodstock diet contained the highest proportion of ω3 HUFA followed by the baitfish diet. Good spawning performance has been observed in gilthead sea bream (*Sparus aurata)* and Japanese flounder (*Paralichthys olivaceus*) when dietary ω3 HUFA levels were 11.27% and 14.25% of fatty acids (Fernandez-Palacios *et al*. 1995; Furuita *et al*. 2002). Lavens *et al*. (1999) also suggested that 22 - 26% of ω3 HUFA in the lipid

fraction may be above optimal levels for gamete quality. Lavens *et al*. (1999) assessed gamete quality by examining fertilization and hatching success, egg and oil droplet diameter, larval biometrics and survival during low food/prey availability. Conversely, it has been observed that low ω3 HUFA levels in broodstock diets decrease egg and larval quality (Watanabe *et al*. 1984ab; Harel *et al*. 1994; Cerdá *et al*. 1995; Abi-ayad *et al*. 1997; Navas *et al*. 1997; Rodriguez *et al*. 1998). Lavens *et al*. (1999) (Turbot; *Scophthalmus maximus*) and Furuita *et al*. (2002) (Japanese flounder; *Paralichthys olivaceus*) suggested that the optimum level of ω3 HUFA required for high egg quality is approximately 11 - 20% of total fatty acids, irrespective of species. This means that only the on-growing diet falls within the range highlighted by Lavens *et al*. (1999) and Furuita *et al.* (2002). However, results found in the 2008 study (Hamoutene *et al*. 2013) suggest that the on-growing diet resulted in the lowest fertilization success, and similarly low hatching success in comparison to the two other diets, thereby contradicting the required ω3 HUFA levels proposed by Lavens *et al*. (1999) and Furuita *et al*. (2002) required to improve egg quality. The differences in results from this study and the previously mentioned studies may be due to differing reproductive requirements of flatfish and roundfish. Perhaps a ω3 HUFA range of 11 – 20 % is optimal for flatfish reproductive output, while roundfish may require a higher ω3 HUFA range.

2.4.4 Egg Fatty Acid Composition

Although the importance of broodstock nutrition in gonadal development, egg quality and the survival of larvae is widely recognized, few studies have been conducted to define the role of key nutrients in reproductive gadoids (Lall & Nanton 2002). The

chemical composition of marine fish eggs is often examined to evaluate egg quality, as eggs must satisfy nutritional needs for embryonic and larval development (Parrish *et al*. 1994; Bell *et al*. 1997; Furuita *et al*. 2002). It has been reported that the composition of EFA in eggs reflects the composition of the broodstock diet (Lasker & Theilacker 1962; Watanabe *et al*. 1978). In this study however, there were few similarities observed between the dietary fatty acid composition and the egg fatty acid composition. Although, clearly some influence of parental diet on egg fatty acid composition had occurred, as mean FA proportions exhibited similar trends between the dietary and egg fatty acids for 18:0, ΣSFA, 16:1ω7, 18:1ω7, 20:1ω9, ΣMUFA, 20:5ω3, 22:6ω3, ΣPUFA and DHA:EPA, as predicted by Tocher & Sargent (1984). It should be noted that other factors can contribute to differences in egg quality, among them biochemical factors such as carbohydrates, enzymes, protein and amino acids, as well as environmental factors such as temperature, water quality, and stock (genetics) and husbandry practices (Clarke *et al*. 2005; Penney *et al*. 2006). However, by comparing the fatty acids in the diets and the eggs, the possible mechanisms and importance of specific fatty acids in broodstock diets and their effect on egg quality can be discussed (Li *et al*. 2005).

Authors have highlighted HUFA such as ARA, EPA, and DHA and their ratios as being important in early finfish development (Bell *et al*. 1986; Watanabe 1993; Sargent 1995b; Pickova *et al*. 1997; Lee 2001; Tveiten *et al*. 2004). However, due to competitive interactions in PUFA metabolism, the requirements of DHA, EPA and ARA should be investigated in more detail (Watanabe & Vassallo-Agius 2003). A sufficient supply of ARA, EPA and DHA, at the appropriate concentrations is generally regarded as important for successful reproduction and embryonic development; this is due to the marine fish's

limited ability to interconvert fatty acids (Sargent *et al*. 2002). The specific role of DHA in the development of neural tissues such as brain and retina has been well documented (Mourente *et al*. 1991; Bell *et al*. 1995). In most studies (Koven *et al*. 1989; Tandler *et al*. 1989; Ako *et al*. 1991; Van der Meeren *et al*. 1991; Rainuzzo *et al*. 1994; Rodríguez 1994) there is a preferential utilization of EPA over DHA. This preferential retention of DHA likely occurs in the phosphatidylethanolamine fraction, as it has been observed in developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*) (Rønnestad *et al*. 1995). The results from the current study show that the highest proportion of DHA was observed in the eggs of the baitfish diet, while it was also found that the eggs from the baitfish diet contained the lowest proportion of EPA followed by the broodstock diet. The proportions of DHA and EPA in the eggs are reflective of the dietary proportions; however, the proportions observed in the eggs are much higher, and the DHA proportions are larger than the EPA proportions, supporting the idea that DHA is preferentially retained in developing yolk-sac larvae. Moreover, the importance of DHA during the developmental stage of the embryo is supported in this study as DHA was found to account for the highest proportion of egg FA across all three diets.

It has been shown that EPA may be converted to DHA in cod tissues such as white muscle, liver, gills and the heart (Lie *et al*. 1992). Some authors have suggested that there are differences between the levels of DHA and EPA in cultured and wild fish (Tocher & Sargent 1984; Fraser *et al*. 1988; Lie 1993) with cultured fish having higher proportions of DHA, EPA and EPA:ARA ratio, and lower proportions of ARA and DHA:EPA ratio than wild fish (Rodríguez Barreto *et al*. 2012). The average portions of DHA, EPA, ARA and DHA:EPA in this study were comparable to the proportions

reported by Penney *et al*. (2006) for cultured Atlantic cod. However, when the fatty acid proportions of this study are compared to that of wild caught Atlantic cod only the proportions of DHA and EPA follow the trend reported by Rodríguez Barreto *et al*. (2012). Fraser *et al*. (1988) found a lower proportion of DHA, EPA, ARA and DHA:EPA, but observed a higher proportion of EPA:ARA than was observed in the current study. Therefore, the FA ratios observed in this study contradict the findings of Rodríguez Barreto *et al*. (2012) and suggest that there may not be as much of a difference between the fatty acid proportions of wild and commercial cod eggs as previously thought, outlining the effect of broodstock nutrition on these proportions.

Competition between DHA/EPA and EPA/ARA for the same metabolic and enzymatic pathways requires that not only the individual amounts but also the optimum ratios of these EFA's be determined (Mazorra *et al*. 2003). There are reports of positive selective incorporation of ARA into eggs of other fish (Thrush *et al*. 1993; Bell *et al*. 1997; Sargent *et al*. 1999, Bruce *et al*. 1999), but there is a competitive relationship between ARA and EPA in eicosanoid production, which is recently receiving more attention and focusing on the importance of EPA:ARA ratios in addition to the individual levels of these PUFAs (Sargent *et al*. 1999). Mazorra *et al*. (2003) suggest that with respect to ARA and EPA levels in eggs and diets, a ratio of 3 - 4:1 EPA:ARA is adequate for Atlantic halibut (*Hippoglossus hippoglossus*) broodstock diets. Bruce *et al*. (1993) found ratios of DHA:EPA of around 2.2:1 in eggs of Atlantic halibut (*Hippoglossus hippoglossus*) fed trash-fish diets, and Sargent *et al*. (1995a) stated that ratios of 2:1 DHA to EPA are found in PL of eggs of most fish species studied. The DHA:EPA ratio observed in this study was slightly lower than 2:1, but the baitfish diet gave the highest

DHA:EPA ratio, at 2.09% and also had the highest fertilization and hatching success (Hamoutene *et al*. 2013). EPA:ARA ratios observed in this study were significantly higher than the recommended range of 3 - 4:1 outlined by Mazorra *et al*. (2003). Salze *et al*. (2005) reported EPA:ARA ratios in wild and wild/fed cod of 5.17 – 5.42 and EPA:ARA ratios in captive cod of 15, and concluded that a lower EPA:ARA ratio in eggs led to better reproductive performance. However, this did not appear to affect the reproductive output of the broodstock in our study, as the baitfish diet which had the highest EPA:ARA ratio seem to yield the highest reproductive success (Hamoutene *et al*. 2013). The high EPA:ARA ratios observed in this study may be due to the fact that all fish were cultured broodstock, as cultured fish have been shown to have EPA:ARA ratios higher than wild cod (Penney *et al*. 2006).

No significant differences were observed in the MUFA composition of the diets, but there were significant differences observed among all three diets in the egg fatty acid composition of MUFA. However, overall levels of MUFA did decrease slightly between the diet and the eggs, supporting the idea that MUFA is preferentially catabolized by developing embryos (Wiegand 1996). Arachidonic acid was present in trace amounts in the diets, but appeared in higher amounts in the eggs, suggesting the possibility that ARA might be synthesized from other fatty acids or shifted from the body reserve of the broodstock (Li *et al*. 2005). The concentrations of ARA in the eggs were comparable to those recorded by Lanes *et al*. (2012) and Tocher & Sargent (1984) and slightly higher than in Penney *et al*. (2011).

In addition to SFA, all organisms including fish, are capable of desaturating palmitic acid (16:0) and stearic acid (18:0) to yield, 16:1ω7 (palmitoleic acid) and 18:1ω9

(oleic acid), respectively (Tocher 2010). It is known that the desaturation of FA in fish takes place in the endoplasmic reticulum of the cells of particular tissues via aerobic processes utilizing CoA-linked substrates and requiring NAD(P)H, cytochrome b_5 reductase, cytochrome b_5 and terminal desaturase enzymes (Brenner 1974). Tocher (2010) noted that irrespective of the special origin of 22:1ω11 and 20:1ω9 FA in fish oils, the extent to which the pathway of SFA and MUFA biosynthesis, including the pathways of further elongation of these FA, occur in fish is still unclear. Tocher (2010) also noted that the extent to which 16:1ω7 and 18:1ω9 are chain elongated to higher homologues in fish, including 18:1ω7, 20:1ω9, 22:1ω9 and 24:1ω9 is not well understood. However, it is known that the 22:1ω11 FA, which can be abundant in fish TAG, is derived from the corresponding fatty alcohol in the wax esters of zooplankton, and the same holds for the 20:1ω9 FA in fish TAG (Tocher 2010). The highest concentration of TAG was observed in the broodstock diet and the on-growing treatment eggs, but 22:1ω11 and 20:1ω9 were highest in the baitfish eggs and diets.

All vertebrates, including fish, lack Δ12 and Δ15 (ω3) desaturases and so cannot form 18:2ω6 and 18:3ω3 from 18:1ω9. Therefore, 18:2ω6 and 18:3ω3 should be considered EFA in the diets of vertebrates (Tocher 2010). Tocher & Sargent (1984) noted that PUFA comprised approximately 50% of the total fatty acids in fish eggs. The underlying reason for the high content of ω3 PUFA in fish eggs is not currently known. One possibility is that phospholipids are more easily mobilized than triacylglycerols in eggs, so that the phospholipids are the preferred energy reserve and a high concentration of ω3 PUFA is an inevitable consequence (Tocher & Sargent 1984). This is seen in our results with PL accounting for the highest percentage of lipids in the eggs, and PUFA

accounting for approximately 50% of the fatty acids found in eggs of each of the respective diets.

2.5 Conclusion

In summary the present study demonstrated that Atlantic cod broodstock eggs with higher fertilization and hatching success (Hamoutene *et al*. 2013), also exhibited higher levels of DHA, DHA:EPA ratio, and EPA:ARA ratio, and lower proportions of ARA and EPA. Overall, the differences present in the dietary lipid classes of this study did not translate into differences in the lipid classes of the eggs. This is likely due to the reorganization of lipids during gonadogenesis and the formation of the eggs. Dietary differences were more evident in the essential FA composition of diets and eggs as the proportions of DHA and EPA in the eggs are reflective of the dietary proportions. However, the proportions observed in the eggs are much higher, and the DHA proportions are larger than the EPA proportions, supporting the idea that DHA is preferentially retained in developing yolk-sac larvae.

Chapter 3: Broodstock dietary effects on larval RNA:DNA ratio and growth rate of Atlantic cod (*Gadus morhua*)

3.1 Introduction

It is widely accepted that effective egg production in marine finfish demands a thorough understanding of the particular nutrient requirements of the broodstock fish, which significantly affects fecundity, fertilization, embryo development, survival, egg size and larval growth (Bromage 1998; Izquierdo *et al*. 2001). It has been well documented that qualitatively and quantitatively inadequate diets can have adverse affects on maternal fecundity and subsequent larvae viability (Watanabe 1982; Castell & Kean 1986; Luquet & Watanabe 1986). A deficiency of nutrients in the broodstock diet can reduce growth rates and affect the health and reproductive performance of the broodstock (Lall & Nanton 2002). Also, it can contribute to poor development of larvae which leads to an increase in mortality rates, including those related to diminished escape response or increased duration of larval stage (Houde 1987; Meekan & Fortier 1996).

In Atlantic Canada, a commonly used cod (*Gadus morhua*) broodstock diet consists of wild baitfish (herring (*Clupea harengus)*, mackerel (*Scomber scombrus)*, squid (*Ilex illecebrosus*)) with vitamin supplementation (Penney *et al*. 2006). Broodstock fed this "natural diet" seem to produce eggs of better quality than those on commercial feeds (Brooks *et al*. 1997). However, there are drawbacks to a baitfish diet, including inconsistent supply, unpredictable quality, prohibitive costs and a high risk of disease transfer; also, the use of unprocessed marine products does not always provide the

appropriate levels of nutrients (Pavlov *et al*. 2004). Formulated diets are desirable because their composition is fully controlled by the producers, they are easier to transport and handle on farms, and the risk of disease transmission from wild fish components are avoided (Mazorra *et al*. 2003). At present, most hatcheries improve the nutrition of their broodstock by feeding them solely marine by-products or in combination with commercial feeds (Izquierdo *et al*. 2001). Marine by-products or 'fish waste', are left over components of the postharvest or industrial preparation processes. These components can account for up to 75% of the catch and can be used to create value-added product (fish feed) that could provide an alternative to low-profit use of silage, fish meal and fish mince (Rustad *et al*. 2011).

The production of hatchery fish can be optimized by starting the cycle with eggs of the highest quality available, which in turn should lead to high egg survival rates as well as high hatching success (Penney *et al*. 2006). When broodstock fish are provided with a plentiful food supply and ample body reserves, the energy expensive process of increasing egg size can be regulated to maximum advantage (Kjesbu *et al*. 1991); further, Kjesbu (1989) found a significantly positive correlation between mean egg diameter and fish length in captive cod. Although, length is not necessarily linked to condition, Rideout *et al*. (2005) noted that condition is a product of prey availability, and Izquierdo *et al*. (2001) found that nutritional condition is known to affect growth, gonadal development, egg number, egg quality, hatching success and larval quality. It is believed that the maternal contribution is more important than the paternal contribution due to nutritional provisioning of the embryo (Bernardo 1996). The embryo and larval characteristics such as egg size, developmental rate, metabolism, growth and variability are affected by the

body condition of the parental (female) fish (Fraser *et al*. 1988; Chamber & Leggett 1996). Although the importance of broodstock nutrition in gonadal development, egg quality, and the survival of larvae is widely recognized, few studies have been conducted to define the importance of broodstock nutrition as it relates to larval success. In 2008, a study was set up in a semi-commercial facility using three different diets to feed hatchery reared cod broodstock: an on-growing pelleted diet, a marine broodstock pelleted diet, and a standard diet of wild baitfish with a marine finfish vitamin and mineral supplement. Results from the diet trials revealed significantly higher fertilization and hatching success in the group fed the baitfish diet but no differences were found in blastomere normality and egg diameters among experimental groups (Hamoutene *et al*. 2013). Larval length was also found to be different at 25 days post hatch (Hamoutene *et al*. 2013), however, measurements were only performed on one occasion. Thus, the question remains whether broodstock nutrition has an effect on the condition and growth of the larvae originating from these eggs.

Traditionally the assessment of growth rates in juvenile and adult fish was done via weight and length measurements, and while larval growth is still assessed in this fashion via image analysis and wet and dry weight, estimation of growth over very short periods of time using morphometric measurements are extremely difficult (Fraser & Sargent 1987; Westerman & Holt 1994). In more recent studies, growth potential of larvae and juvenile fish has been evaluated using nucleic acid-based variables, such as RNA to DNA ratio (RNA:DNA) (Mathers *et al*. 1992; Buckley *et al*. 1999; Fukuda *et al*. 2001; Peck *et al*. 2003; Yamashita *et al*. 2003; Vinagre *et al*. 2008). In both laboratory and field applications RNA:DNA has been related to both long term and recent growth

and many studies have outlined its use as one of the best indicators of growth and quality (Buckley 1979; Buckley & Lough 1987; Robinson & Ware 1988; Folkvard *et al*. 1996; Clemmesen *et al*. 1997; Rooker *et al*. 1997; Chícharo 1998; Heyer *et al*. 2001). Clemmesen (1994), Bailey *et al*. (1995), Folkvard *et al*. (1996) and Buckley (1979, 1980, 1981) demonstrated that RNA:DNA was a particularly useful indicator of quality and growth in winter flounder (*Pseudopleuronectes americanus*), cod (*Gadus morhua*) and sand lance (*Ammodytes dubius*) larvae.

RNA:DNA is an eco-physiological index of the synthetic capacity of the cell which generally correlates with nutritional status (Ferron & Leggett 1994; Buckley *et al*. 1999). The physiological basis for this index is that DNA content, the primary carrier of genetic information, remains stable under varying environmental conditions within the somatic cells of a species and has been used as an indicator of biomass (Holms-Hansen *et al*. 1968; Dortch *et al*. 1983) and cell number (Regnault & Luquet 1974). Whereas RNA content, which consists of ribosomal RNA (75% - 94% of total RNA), messenger RNA and transfer RNA, are essential cellular components in the biosynthesis of proteins (Young 1970; Henshaw *et al*. 1971). The number and activity of the ribosomes is shown to fluctuate in response to food availability and the demand for protein synthesis and growth (Henshaw *et al*. 1971). RNA content has been known to vary with age, developmental stage, size, disease state and varying environmental condition (Bulow 1970; Clemmesen 1987). Larval fish grow rapidly, increasing their body muscle mass through an increase in protein synthesis (Young 1970; Henshaw *et al*. 1971), thus the relationship between RNA and DNA acts as an index of cell metabolic intensity (Bulow 1970). For this reason nucleic acid-based indices for larval growth are considered good

short term measures of condition in young fishes (periods as short as 1 - 4 days) (Buckley 1980; Wright & Martin 1985; Clemmesen 1987; Varnovsky *et al*. 1992; Westerman & Holt 1994; Bisal & Bengston 1995; Gwak & Tanaka 2001; Gwak *et al*. 2003a,b).

In this study, the effect of parental nutrition on progeny performance was assessed for Atlantic cod larvae produced from broodstock fed one of three diets: an on-growing diet, a marine broodstock diet, and a standard marine baitfish diet with a marine finfish vitamin and mineral supplement. Larval quality was evaluated by measuring nucleic acid concentrations and ratios, protein content and growth rates over a period of ~56 days. Building on the Hamoutene *et al*. (2013) research investigating reproductive effects, three different diets for hatchery reared cod broodstock offered a unique opportunity to investigate the effect of broodstock nutrition on the early stages of larval growth in the present study. It is important to state that blastomere normality percentages and egg diameters showed no differences between diets during both of the monitored spawning seasons (Hamoutene *et al*. 2013). Therefore any differences in larval growth could be linked to egg composition as influenced by parental diets.

3.2 Materials & Methods

Phase 1 of the overall project explored the influence of three diets on spawning and growth of first generation photomanipulated cod broodstock. Phase 1 found that the first spawning season (February 2009) revealed no differences in egg quality between diets but higher sperm quality in males fed the baitfish diet. Results from the second spawning season (October 2009) revealed higher fertilization and hatching success in the

baitfish group (Table 3.1). Fertilization success was measured on 100 eggs per batch and recorded as number of eggs showing cell division. Evaluation of sperm performance showed higher quality in both the broodstock and baitfish diets, and an improved synchronicity of spawning between males and females was observed in the broodstock diet group (Hamoutene *et al*. 2013). The current thesis relies on the findings of the previous study and extends the analyses to look at whether the previously observed differences could be linked to dietary-induced differences in the biochemical content of eggs and larvae.

3.2.1 Experimental Setup & Larval Rearing

All studies were conducted in accordance with the guidelines published by the Canadian Council on Animal Care (animal care numbers 09-08-KG and 10-08-DH). All fish in this study were held in seawater tanks at the Joe Brown Aquatic Research Building (Memorial University of Newfoundland). This study was part of a broodstock development project, and the F1 broodstock (representing 13 families) used in this study were hatched in 2006 as part of the Atlantic Cod Genome project selective breeding program (www.codgene.ca). Three diets were investigated: 1) An on-growing pelleted diet (OG, EWOS Marine Grower) which is a standard grow out diet designed to optimize fish growth and is suitable for species such as cod, haddock and halibut, 2) a broodstock diet (BR) specifically manufactured for marine finfish (not commercially available in Canada; Skretting Feeds™©) which has been designed to optimize egg quality and survival to first feeding, and 3) the standard cod broodstock baitfish diet (BF) comprised

of herring (*Clupea harengus*), mackerel *(Scomber scombrus)*, squid (*Ilex illecebrosus*) and a vitamin/mineral supplement (St. Andrews Biological Station, DFO) which was designed to emulate the diet of wild cod. The 3 diets were tested on sexually mature fish (broodstock divided into 6 tanks, 10 males and 10 females $tank^{-1}$) while ensuring that families were almost equally represented in all treatments. For this study, 4 crosses per diet (4 tanks/diet) were monitored for larval growth. Diet trials originated in August 2008 (Hamoutene *et al*. 2013) and then in November 2009, individual fish were manually stripped (Lush *et al*. 2011) to collect gametes and complete crosses as per Lush *et al*. (2005), with particular attention paid to avoid crossing siblings. Only successful egg batches (fertilization > 20%) from the study groups were selected for incubation and rearing.

The fertilized cod eggs were first disinfected with ozone (O_3) (1.5 – 2.0 mg/l of O_3 for 1.5 minutes) in a contact chamber consisting of a 25 l fiberglass tank with a conical bottom. Ozonated seawater was pumped at a constant rate through the base of the chamber throughout treatment. Within the chamber, a round basket consisting of Nitex® mesh held the eggs, allowing ozonated seawater to be pumped in direct contact with the eggs before the O_3 dissipated. After disinfection the eggs were placed in 50 l conical bottom upwelling incubators supplied with ~ 1 l min^{-1} aerated seawater at a temperature of 5 – 6ºC. One incubator tank per diet was used, and the OG tank had 513 122 eggs/tank, the BR tank had 672 859 eggs/tank and the BF tank had 726 052 eggs/tank. Eggs were exposed to a 24 hour photoperiod at a light intensity of 400 lx. Flow and aeration were halted briefly once a day, so any dead eggs could settle out, and be removed from the bottom of the incubators. Once approximately 100% hatch was achieved (0 days post

hatch; (dph)), the larvae were transferred to 500 l experimental rearing tanks supplied with UV treated, filtered, seawater (2 µm active filtration media, Dryden Aquaculture, Edinburgh, Scotland, UK) at an average stocking density of ~19.0 larvae l^{-1} for the on-growing diet, ~14.1 larvae l^{-1} for the broodstock diet and ~ 29.25 larvae l^{-1} for the baitfish diet. The differences in stocking densities were due to differences in numbers of larvae available at hatch.

The tanks were greened with 12.5 ml of algal paste (Reed mariculture, Campbell, CA, USA) twice a day at 9 am and 3 pm. Water temperature was maintained at ~ 10.5ºC and the flow rate of the tanks was set at 0.8 l min^{-1} increasing as needed to a maximum of 4.5 l min^{-1}. Flow was increased to help remove excess materials and improve water quality as larval demands increased due to growth. Cod larvae were fed Origreen (Skretting, Vancouver, BC, Canada) enriched rotifers from ~ 1 dph up to ~ 30 dph, three times daily (1 am, 9 am and 5 pm). At ~ 30 dph the larvae were weaned onto Origreen-enriched *Artemia* four times daily (9 am, 3 pm, 9 pm and 3 am), and co-fed 200 and 300 Gemma wean diamond dry feed (Skretting Canada, Bayside, NB, Canada). The dry feed was added to the tanks 30 - 60 minutes prior to the addition of *Artemia*. At ~ 45 dph the larvae were weaned onto Gemma wean diamond 300 four times daily (9 am, 3 pm, 9 pm and 3 am).

Due to the joint nature of this project as well as constraints in hatchery use and larval access, larval survival was only assessed once at the end of the study. Survival of larvae originating from BF and OG fed broodstock was assessed at 90 dph while BR larvae survival was measured at 60 dph.

3.2.2 Biochemical Parameters

3.2.2.1 RNA:DNA Ratio

For all three diets, larvae were sampled every 7 days including 0 dph. Due to larval size constraints 10 larvae were pooled in triplicate samples from each tank from 0-14 dph, while 10 individual larvae (n=10) were sampled from 21 to 49 dph. The change in the number of larvae per sample is due to the size difference of the larvae and the need to have enough sample material to perform the biochemical analyses. Sampling was completed by carefully netting larvae from the incubators for the 0 dph larvae and from the experimental tanks for older larvae, before transferring them to a beaker containing tank water. The larvae were then strained through a 50 μm mesh, rinsed in distilled water, and dried by gently blotting the bottom of the mesh with Kimwipes® (Kimberly-Clark Professional, Roswell, GA, USA). Then carefully using tweezers, larvae were picked individually and counted. All samples were transferred to RNase and DNase free microcentrifuge tubes. Samples were first frozen on dry ice before being transferred to the -70ºC freezer for storage. All equipment was rinsed with RNA zap (Ambion®, Life Technologies Inc**.**, Burlington, ON, Canada), and RNase and DNase free water between each tank sampling. All larvae were sampled prior to first feeding each morning.

Nucleic acid concentration was measured according to Caldarone *et al*. (2001) using calf liver 18S + 28S rRNA (Bioworld, Dublin, OH) and calf thymus DNA (Sigma-Aldrich, St. Louis, MO) as standards. Briefly, on the day of the analysis, each tube containing larval samples was removed from the freezer, thawed for 15 to 20 mintues on coolracks® (CoolRack, Biocision, Larkspur, CA, USA) and 150 µl of 1% Sarcosil Tris-

EDTA Buffer (STEB) added to dissociate the nucleoproteins. Samples were placed in a multi-head vortexer at high speed (speed of 8-10), for 15 to 60 minutes (until tissue was fully disrupted). After vortexing, 1.35 ml of Tris-EDTA buffer was added to each sample, and the tubes were manually mixed by inversion 40 times. Samples were then centrifuged for 15 minutes at 14,000 rcf (11,180 rpm**)** at room temperature (~21ºC). After centrifugation, the supernatant (containing the nucleic acids) was transferred into a new DNase/RNase free microcentrifuge tube and 75 µl of sample or standard was then added to each well of a microplate.

A working solution of ethidium bromide (EB) (2 µg ml^{-1}) was prepared by adding 32 µl of the 1 mg ml^{-1} EB stock to 16 ml of Tris-EDTA Buffer in a disposable tube. The tube was then capped and inverted 20 times to ensure mixing, and the solution was poured into a reagent reservoir (VWR International, Mississauga, ON, Canada). Using a multi-channel pipette, 75 µl of EB was added to each of the solution wells. The microplate was then gently shaken on the vortexer for ten minutes. An RNase (Sigma Aldrich, Oakville, ON, Canada) working solution was prepared by dissolving 50 mg of solid protein in Tris-EDTA buffer to yield 20 U/ml. Using a disposable plastic pipette and pipette bulb, 3 ml aliquots were transferred into plastic test tubes, capped tightly and stored at -20°C. DNase (Sigma Aldrich, Oakville, ON, Canada) was prepared by diluting 2000 U of DNase powder to 1 U/µl by adding 2 ml of double distilled H_2O and gently but thoroughly mixing, 480 µl aliquots were then transferred to individual microcentrifuge vials and stored at -80°C. On the day of use a DNase working stock was prepared with 1

U/µl DNase, 660 µl of $MgCl_2$, 660 µl of $CaCl_2$, 450 µl of DNase and 1230 µl of Tris-EDTA buffer and stored on ice until use.

The microplate was placed in a Synergy HT multi-detection microplatereader (BIO-TEK, Winooski, VT, USA) and fluorescence (excitation wavelength: 530 nm, emission wavelength: 590 nm) readings were recorded. RNase (20 U/ml) was then poured into a reagent trough and using a multi-channel pipette, 7.5 µl was added to each well. The plate was then placed in the microplatereader for a second fluorescence reading and results were recorded. 7.5 µl of DNase was added to each well; the microplate was then covered and placed in a drying oven for 60 minutes at 37ºC. The drying oven also contained a vortexer, which was set on low speed (1 - 2). Afterwards the plate was left covered on the counter to cool for thirty minutes, and the fluorescence was read and recorded. DNase was only added to the first plate run each day to determine the residual fluorescence. If no significant residual fluorescence was found, then DNase was not added to the rest of the plates for that day.

Serial dilutions, increasing by multiples of two, of commercial preparations of 18S and 28S rRNA (Bioworld, Dublin, OH, USA), ultra-pure highly polymerized calf thymus DNA and 0.1% STEBs were used to construct standard curves. Standard rRNA concentrations ranged from 0.11 to 7.23 µg ml^{-1}, while DNA standards ranged from 0.15 to 2.33 µg ml^{-1}.

3.2.2.2 Protein Analysis

Larval protein content was determined in triplicate with a bicinchoninic acid colorimetric assay kit (ThermoScientific/Pierce, Rockford, IL) using bovine serum albumin as standard (Smith *et al*. 1985).

On the day of analysis, each sample was removed from the freezer and allowed to thaw on coolracks® at 4ºC. 150 µl of 1% Sarcosil Tris-EDTA buffer (STEB) was added to each microcentrifuge tube, and the samples were completely homogenized using a motorized plastic pestle. The samples were placed in a centrifuge for 30 minutes at 9000 rcf. 25 µl of each standard and triplicate sample was then pipetted into each microplate well. Following this, 200 µl of bicinchoninic acid (BCA) working reagent (WR) was added to each well, and the plate placed on a plate shaker for 30 minutes to ensure thorough mixing.

The working reagent was prepared by mixing 50 parts of BCA reagent A (Pierce Biotechnology, Rockford, IL, USA) with 1 part of BCA Reagent B (Pierce Biotechnology, Rockford, IL, USA). After mixing, the plate was covered with aluminum foil and incubated at 37ºC for 30 minutes. The plate was left to cool to room temperature before the absorbance was measured at 562 nm in the plate reader. Standards were done in a dilution series (2000, 1500, 1000, 750, 500, 250, 125, 25 and 0 µg ml^{-1}) of bovine serum albumin (BSA) and were used to construct a standard calibration curve.

3.2.3 Larval Growth

The instantaneous protein growth coefficient (G_i d^{-1}) was calculated over each 7 day sampling interval using the formula:

$$G_i = (\ln P_{t2} - \ln P_{t1}) * (t_2 - t_1)^{-1}$$

Where P_t is the mean protein content (µg) of larvae from 1 triplicate (nominally 1 individual larva) at time t_1 and t_2, with t being age in days. Protein-specific growth rates expressed as % d^{-1} (SGR) were calculated using the formula $SGR = 100 \times (e^{Gi} - 1)$ (“e” is a mathematical constant, approximately equal to 2.71828) (Houde & Schekter 1981).

3.2.4 Statistical Analysis

Prior to statistical analysis, all data were tested for family effects. All statistical analyses were conducted using STATISTICA 10 (StatSoft, Tulsa, OK). Pearson Product Moment correlations were used to examine correlations between each individual biochemical parameter against age (dph). The effect of broodstock diet on larval survival rate was compared for BF and OG using a t-test at 90 dph, while the BR values were not compared due to a difference in days post hatch. Differences in nucleic acids and RNA:DNA (Log_{10} transformed) among experimental diet groups were investigated using analysis of covariance (ANCOVA) with age as covariate. Prior to each ANCOVA the assumption of parallelism between experimental groups was tested, when the assumption of parallelism was not met (DNA & RNA) the separate slope model was used to examine differences among dietary groups. SGR and protein concentration data were analyzed using factorial ANOVAs with broodstock diet and age as factors. Homogeneity of variance was tested using Cochran C-statistic, Bartlett Chi-squares and Hartley F-man statistic. Normal distribution of the residuals was checked using histograms and normal p-

p plots, and correlations (or lack there of) between means and standard deviation was checked by plotting them. All tests used a confidence interval of 95% ($p = 0.05$). Correlations between RNA:DNA and SGR were explored using Pearson product moment correlation, and larvae survival percentages for the OG and BF diet were compared by t-test.

3.3 Results

No cross effect was detected in any of the measured parameters (DNA, RNA, RNA:DNA, protein concentration, and SGR). There were no significant differences in larvae survival (%) from number of larvae hatched to 90 dph (Table 3.2). The daily survival proportions were 0.2% for the on-growing diet, 0.5% for the broodstock diet and 0.3% for the baitfish diet (Table 3.2). Positive correlations were observed between all biochemical parameters measured and age (dph) ($p = <0.001$), with the strongest correlation being observed between protein content and dph ($r = 0.942 - 0.966$, $p = <0.001$), and the weakest correlation being observed between RNA:DNA and dph ($r = 0.571$, $p = <0.001$) (Table 3.3). No statistically significant correlations were found between RNA:DNA and SGR ($p = 0.228$) or Pro:DNA and SGR ($p = 0.353$) (Table 3.4). There were no significant differences detected among experimental groups in nucleic acid concentration; however, ANCOVA analysis of the slopes of the RNA:DNA after 7 dph indicated a significant diet effect with Scheffe post hoc test indicating that the slope of the BR group was significantly lower than the other two groups ($F = 21.55$; $p = <0.001$) (Figure 3.1). With regards to SGR and protein concentration, no significant effect of diet

group was detected. A significant increase in protein concentration from 21 dph onwards was detected for the 3 diet groups ($F = 3.9674$; $p = 0.00127$) (Figure 3.2).

Figure 3.1: Mean (±SE) for fertilization success, hatching success and larval lengths of crosses completed per diet. Means within a column not sharing the same superscript letter are significantly different ($p < 0.05$) after application of two-way ANOVA (diet effect and tank effect) and Holm Sidak method as ad-hoc test.

Diet	Fertilization Success (%)	Hatching Success (%)	Standard Length (mm)
On-Growing	14.7 ± 3.0^{a} (n = 15)	10.37 ± 3.6^{ab} (n = 15)	8.909 ± 0.17^{a} (n = 7)
Broodstock	24.4 ± 4.08^{ab} (n =21)	9.09 ± 2.1^{a} (n = 21)	9.209 ± 0.12^{a} (n = 9)
Baitfish	36.2 ± 5.8^{b} (n = 12)	22.87 ± 5.2^{b} (n = 12)	9.728 ± 0.11^{b} (n = 10)

From: Hamoutene, D., Lush, L., Pérez-Casanova, J.C., Hobbs, K., Burt, K., Walsh, A., and Moir, J. 2013. Effect of diet on growth, survival and reproductive performance of first generation (F1) Atlantic cod, *Gadus morhua* L. broodstock. Can. Tech. Rep. Fish. Aquat. Sci. 3026: v + 21 p.

Table 3.2. Average survival (%) and average daily survival (% day^{-1}) among diets @ 60 dph (OG - on-growing, BR - broodstock, BF - baitfish). Values are means and data was analyzed using one-way analysis of variance. (p = 0.05)

	Survival (%)		
Tank	OG	BR	BF
1	10.2	14.8	36
2	28.4	17.1	6.3
3	2.4	17.3	12.9
4	21.4	67.8	39.4
AVG	15.6	29.3	23.7
Daily AVG	0.2	0.5	0.3
Std Dev	11.6	25.7	16.5
SEM	5.8	12.9	8.3

OG @ 90 dph
BR @ 60 dph
BF @ 90 dph

Table 3.3. Pearson product moment correlations (r) between Atlantic cod larval age (up to 56 dph) and larval DNA, RNA and Protein content (µg larva^{-1}). Parental broodstock were fed 3 different diets (OG - on-growing, BR - broodstock formulated and BF - baitfish) (p = 0.05).

Variable	P	R
DNA (µg larva $^{-1}$)		
OG	<0.001	0.888
BR	<0.001	0.845
BF	<0.001	0.888
RNA (µg larva $^{-1}$)		
OG	<0.001	0.928
BR	<0.001	0.943
BF	<0.001	0.931
Protein (µg larva $^{-1}$)		
OG	<0.001	0.943
BR	<0.001	0.961
BF	<0.001	0.966

Table 3.4. Pearson product moment correlation (r) of RNA:DNA, Pro:DNA and protein-specific growth rate (SGR, % d^{-1}) in Atlantic cod larvae (from 7-42 dph) originating from broodstock fed different diets (OG - on-growing, BR - broodstock formulated and BF - baitfish) ($p = 0.05$)

	SGR			RNA:DNA			Pro:DNA		
	R	P	r^2	R	P	r^2	R	P	r^2
DPH	-0.345	0.00348 (BR, BF)	0.119	0.571	<0.001 (OG, BR, BF)	0.326	0.530	<0.001 (OG, BR, BF)	0.281
SGR	-	-	-	-0.146	0.228	0.021	0.113	0.353	0.013
RNA:DNA	-	-	-	-	-	-	0.500	<0.001 (OG, BF)	0.250
Pro:DNA	-	-	-	-	-	-	-	-	-

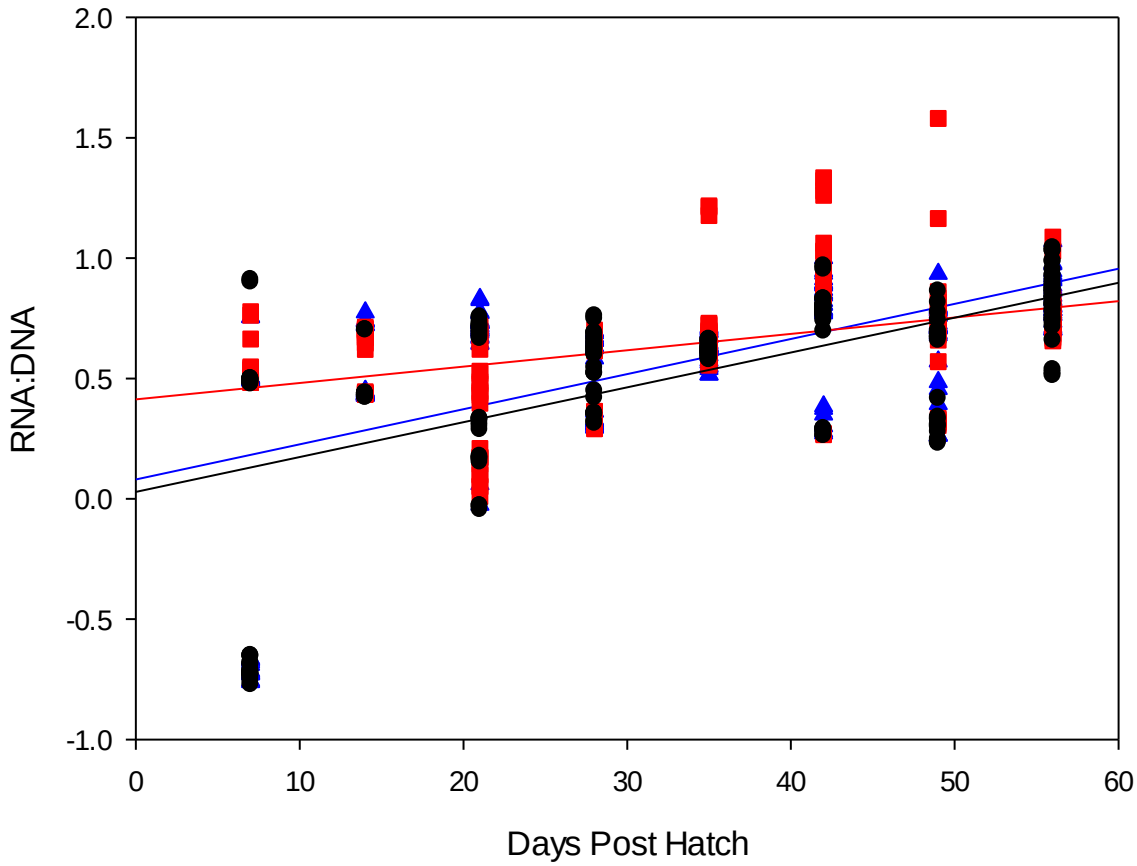

Figure 3.1. RNA:DNA (Log10 transformed) over time (dph) of Atlantic cod (Gadus morhua) larvae originated from broodstock fed three different diets: a) a commercial on-growing pelleted diet (Black, OG), b) a pelleted marine broodstock diet (Red, BR), and c) a standard baitfish diet supplemented with vitamins and minerals (Blue, BF); commencing at 7 dph

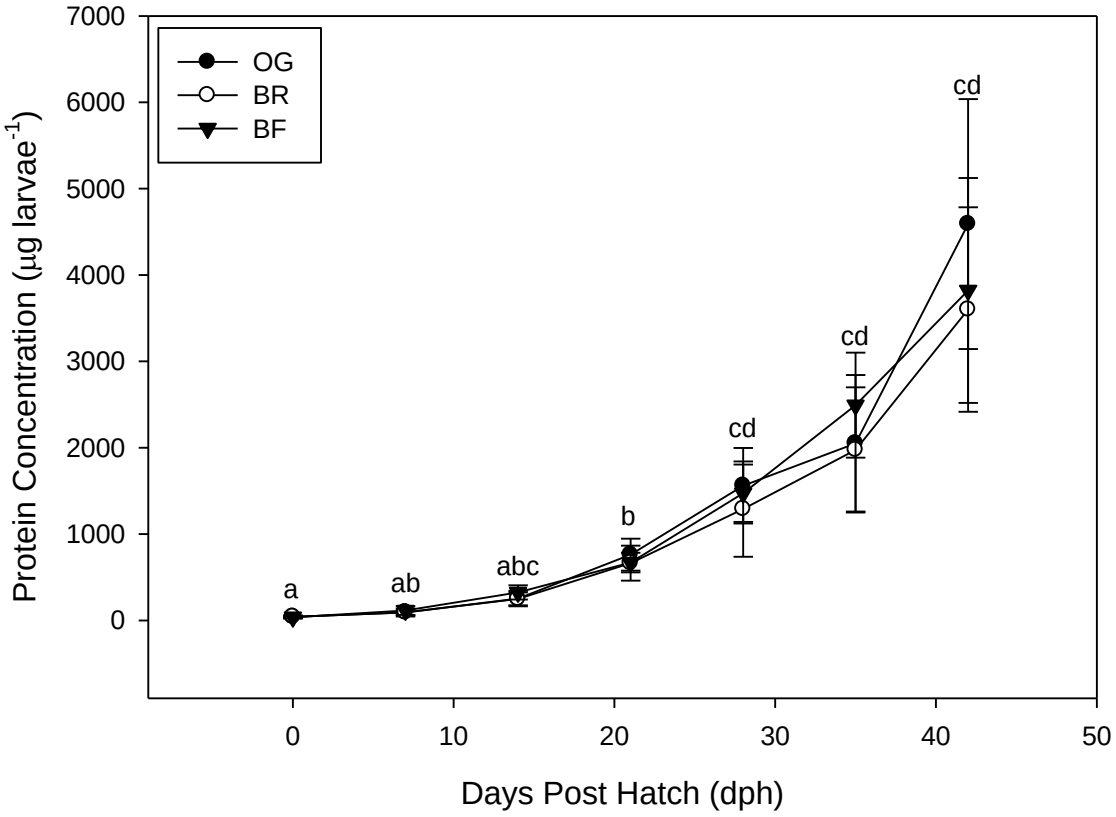

Figure 3.2. Age-specific protein content (µg larvae^{-1}) of cod for each diet. Significant differences among each diet at different dph. Protein values were determined using a bicinchoninic calorimetric assay (BCA).

3.4 Discussion

3.4.1. Nucleic Acids

DNA content has become a standard reference for determining the total number of cells in a given tissue, because the total quantity of DNA per cell is constant in normal tissue within a given species, and the amount per cell is not altered by starvation or other stressors (Park *et al*. 2008). The DNA content has been used as an index for live body weight and cell number. A change in DNA content results in a change in the RNA:DNA, thus this ratio can be utilized to examine the changes in live body weight and cell numbers (Holm-Hansen 1969; Regnault & Luquet 1974; Park *et al*. 2008). It is expected that DNA content will increase with age. In this study, DNA content was positively correlated with age. DNA concentrations ranged from ~1 µg larvae^{-1} to ~40 µg larvae^{-1}, and are comparable to DNA concentrations reported elsewhere for Atlantic cod and haddock larvae (*Melanogrammus aeglefinus*) (Caldarone *et al*. 2003; Caldarone 2005). In this study it was demonstrated that the different diets did not influence larval DNA content. Many studies have outlined the trends in DNA concentrations over time and throughout larval feeding (including times of food deprivation) (Caldarone *et al*. 2003), however there is a lack of information on the influence of parental diet on larval DNA content.

RNA is essential for the biosynthesis of proteins and the quantity can vary depending on nutritional condition (Buckley *et al*. 1999), the RNA content is the most useful way of measuring potential for protein synthesis because it is largely contained in fast growing tissues (Bulow 1970). As expected, our study demonstrated that larval RNA

increases with time from hatch. The RNA concentrations ranged from ~1 µg larvae^{-1} to ~200 µg larvae^{-1} and are comparable to RNA concentrations reported elsewhere for Atlantic cod (1 - 150 µg larvae^{-1}) and haddock larvae (~200 µg larvae^{-1}) (Caldarone *et al*. 2003; Caldarone 2005). Park *et al*. (2008) observed a rapid decrease in RNA concentration in starved slime flounder larvae (*Microstomus achne*), after the yolk was completely absorbed, and found that both the RNA and DNA content remained somewhat constant in fed larvae up until approximately 20 dph before increasing significantly. The results of this study did not demonstrate any rapid decrease in RNA content following the absorption of the yolk sac (which occurs at ~5-7 dph in Atlantic cod), however, the RNA and DNA contents of the larvae in this study remained relatively constant until approximately 21 dph, after which there was a dramatic increase in both concentrations. The rapid decrease in RNA content may not have been observed in this study because the larvae were presented with adequate nutrition once exogenous feeding had commenced.

RNA:DNA ratio is an index of the amount of protein synthetic machinery per cell, and correlations between RNA concentration or RNA:DNA and growth rate have been observed for a variety of organisms (Kennell & Magasanik 1962; Bulow 1970; Sutcliffe 1970). The RNA:DNA explains a large part of growth variability, and is therefore a good indicator of limiting growth factors, primarily food availability (Buckley 1984), as such RNA:DNA should increase with age. This trend was observed in the present study, however, the RNA:DNA yielded the weakest correlation with days post hatch of all the biochemical parameters examined. Caldarone *et al*. (2003) and Caldarone (2005) reported similar results for haddock and Atlantic cod larvae, but the RNA:DNA ratios in their studies were slightly less than those observed in the current study. Ratios are known to

decline following yolk absorption, and have been reported in both fed and starved larval cod (*Gadus morhua*), through 5 to 7 days post hatch (Clemmesen & Doan 1996). Declines in RNA:DNA ratios during early ontogeny have been attributed to the nutritional stress associated with the commencement of exogenous feeding as well as poor prey conditions, but have also been observed throughout the larval period during times of rapid development and tissue reorganization (Westerman & Holt 1988; Bergeron *et al*. 1991). When completing correlations between ratios and age after 7 dph it was found that RNA:DNA was positively correlated with time since hatch. When comparing diets, ANCOVA analyses from 7 dph to 56 dph, revealed significantly different slopes between the BR and both BF and OG diet groups.

3.4.2. Protein and Protein:DNA Ratios

The analysis of the data supported the fact that protein content increases with age; parental diet did not influence this relationship. Protein concentration did not change significantly from hatch to 21 dph, but increased rapidly from 28 dph onwards. It is assumed that the reason for the small variation up to ~20 dph was due to reduced protein synthesis and increased active hydrolysis inside the cells (Park *et al*. 2008). Yolk-sac proteins are used in the growth of the body or as energy sources. The protein concentration begins to increase just after yolk absorption (~5-7 dph), this may be because other nutrients are used to a greater extent as energy sources rather than primarily proteins (Lee 1994). However, larval protein concentrations in this study were 2 to 3 fold higher than the protein concentrations reported by Caldarone *et al*. (2003) for Atlantic cod larvae. This difference could be due to the fact that the larvae in this study were offered

higher prey density levels than those used by Caldarone *et al*. (2003), though these differences did not translate in different nucleic acid or SGR values. Several authors (Lied *et al*. 1983; Foster *et al*. 1991; Foster *et al*. 1992) demonstrated that protein synthesis is a result not only of ribosome number (i.e. RNA content) but also specific ribosomal activity, and that the two variables can vary independently.

The DNA content can be used as an index for cell number and total Pro:DNA can be used as an index for cell weight or cell size, because proteins comprise most of the cell weight (Fukuda *et al*. 1986). The results of this study found that Pro:DNA was positively correlated with days post hatch, representing an increase in cell weight and cell size with age, but no significant differences were observed in the Pro:DNA ratios of the different diet groups. Previous studies have reported that Pro:DNA was highly correlated with RNA:DNA (Westerman & Holt 1994; Caldarone *et al*. 2003), and this was observed in this study in the OG and BF diet group, but not with the BR diet. Caldarone *et al*. (2003) noted that substituting Pro:DNA for RNA:DNA in their growth models results in a lower R^2 value, but had the potential to be used as an index of condition. Both potential growth indicators, RNA:DNA and Pro:DNA, were found not to be statistically correlated with SGR. This contradicts the findings of some other authors (Buckley 1979; Caldarone *et al*. 2003), and raises the question of whether RNA:DNA or Pro:DNA should be used as indicators of growth. One benefit of Pro:DNA is that separate analytical procedures are used to obtain estimates of each parameter (Caldarone *et al*. 2003). This is not the case with RNA:DNA ratios, as current fluorometric techniques employ sequential subtraction steps to obtain the individual nucleic acid concentrations. Therefore, any error in

measurements of RNA may affect the measured concentration of DNA, and could be magnified in the RNA:DNA ratio.

3.4.3. SGR and larval survival

Our results suggest no effect of diet on overall larval survival, though survival at 90 dph for the BF diet would need to be assessed to confirm this affirmation. Survival values described in this study are within the range described by other authors in commercial settings (Kvåle *et al*. 2009). Both field and lab studies of well-fed fish have indicated that, after the initiation of exogenous feeding, growth rate is size dependent. Some studies have found a rapid increase in early growth with larval size, followed by a more gradual increase (Otterlei *et al*. 1999; Buckley *et al*. 2006), and similar trends were also observed in the SGR for this study. Buckley (1984) observed a SGR range of -2.2 (% d^{-1}) in starved larvae up to 9.3 (% d^{-1}) in larvae fed 1000 wild plankton L^{-1}. Results from this study reveal that the average SGR across all three diet groups was around 11.65 (% d^{-1}). These growth rates are higher than those reported by Laurence (1978) and Laurence *et al*. (1981) for laboratory-reared Atlantic cod, and lower than those in Caldarone *et al*. (2003) for laboratory-reared Atlantic cod. Growth rates based on protein or dry weight are comparable since the bulk of larval dry weight is comprised of protein, and the relative proportion of protein to dry weight is fairly constant during the larval period (Buckley & Lough 1987; Caldarone 2005). The observed differences could be due to the quality or quantity of prey provided, as the above mentioned studies used fixed prey densities while this study changed prey density with age, or differences between prey-type/temperature interactions.

3.5. Conclusion

The DNA, RNA and RNA:DNA patterns, as well as the time-averaged SGR observed in this study were within the values reported by Caldarone *et al*. (2003) for Atlantic cod larvae. However, larval protein concentrations were 2 to 3-fold higher in the present study. This difference could be due to the fact that the larvae in this study were offered higher prey density levels than those used by Caldarone *et al*. (2003), though these differences did not translate in different nucleic acid or SGR values. Several authors (Lied *et al*. 1983; Foster *et al*. 1991; Foster *et al*. 1992) demonstrated that protein synthesis is a result not only of ribosome number (i.e. RNA content) but also specific ribosomal activity, and that the two variables can vary independently.

No effect of broodstock diet was observed in larval survival, concentration of nucleic acids, protein concentration or SGR of larvae. ANCOVA of the slopes of the RNA:DNA after 7 dph showed a significant difference with Scheffe post hoc test indicating that the slope of the BR group was significantly lower than the other two groups. This means that the difference in diets is dependent on the level of dph. Our data suggest that, although larvae originated from parents fed the BF and OG diets have a higher growth potential (as inferred by the RNA:DNA) when compared to the larvae originating from broodstock fed the BR diet, they grow similarly (up to 49 dph). The lack of correlation between the RNA:DNA and growth rates (Table 3.4) further supports this result and contradicts some of the findings of other authors (Buckley 1979; Caldarone *et al*. 2003) questioning the accuracy of RNA:DNA as an indicator of growth in this situation. In conclusion, Hamoutene *et al*. (2013) indicated that the broodstock diet affected fertilization and hatching success in Atlantic cod eggs, and the results of this

study found no differences in egg quality or larval growth and survival up to 49 dph between the three diet groups. A significant positive correlation between egg size and larval standard lengths was found to persist for up to 10 weeks by Paulsen et al. (2009). Thus, the absence of differences in egg quality (Hamoutene *et al*. 2013) might explain the fact that larval growth up to 49 dph was similar across diets.

Chapter 4: General Summary

The most commonly examined components of marine fish eggs are lipids, particularly fatty acids. Lipids and fatty acids are of vital importance to fish embryos especially during the endogenous feeding period, and are both a direct result of the maternal reserves. They provide energy, as well as the basic building blocks for growth. The understanding of how these biochemical factors affect egg and larval quality can serve as an index for higher quality eggs and larvae. The levels of total lipids, lipid classes and fatty acids were examined in the diets and the eggs. The lipid classes and fatty acids of the diets and eggs were compared in each dietary group to determine an optimal diet for Atlantic cod (*Gadus morhua*) broodstock, and significant differences were found between some of the lipid classes and fatty acids of the diet and eggs. The results were examined in conjunction with the fertilization and hatching success described in Hamoutene *et al*. (2013). Atlantic cod broodstock fed the baitfish diet produced eggs with the highest fertilization success and hatching success (Hamoutene *et al*. 2013), and also exhibited higher levels of DHA, the DHA:EPA ratio, and the EPA:ARA ratio, and lower proportions of ARA and EPA. With the exception of the above mentioned essential fatty acids (and their ratios), the variability and lack of significant differences in the other lipids of the eggs may mean that only limited aspects of lipid profiles contained in broodstock diets are reflected in egg lipids. It is also important to remember that other factors contribute to differences in egg quality including other biochemical factors such as carbohydrates, enzymes, proteins and amino acid composition, as well as environmental factors such as temperature, water quality, stock differences and husbandry. In this study,

all environmental/culture conditions were similar for all groups and we attempted to minimize potential genetic differences by using limited families.

In order to understand the potential effect of broodstock diets on growth of the progeny, the concentrations of RNA, DNA, protein and their ratios (RNA:DNA, Pro:DNA) were examined in larval cod for the first 49-56 days post hatch. The nucleic acid concentrations of the larvae were compared among dietary groups to determine if one of the diets led to a higher growth rate. No effect of broodstock diet was observed in larval survival, concentration of nucleic acids, protein concentration or SGR of larvae. However, ANCOVA analysis of the slopes of the RNA:DNA after 7 dph showed a significant difference, this means that the difference in diets is dependent on age (dph). The slope of the BR group was significantly lower the other two groups, and the results suggest that although larvae originating from parents fed the BF and OG diets have a higher growth potential (as inferred by the RNA:DNA ratio) when compared to the larvae from broodstock fed the BR diet, they grow similarly, at least up to 49 dph. The lack of correlation between the RNA:DNA and growth rates further supports this result, questioning the accuracy of the RNA:DNA as an indicator of growth in this situation. In conclusion, although broodstock diet affected fertilization and hatching rate of Atlantic cod eggs, there was no difference in egg quality (Hamoutene *et al*. 2013) or larval growth and survival (up to 49 dph) between the three diet groups. The absence of differences in egg quality (Hamoutene *et al*. 2013) might explain the fact that early larval growth was found similar across diets.

Taken together, this study shows that differences in the broodstock diet of Atlantic cod did not translate in a drastic effect on eggs and/or larvae (up to 49 dph). The findings

in chapter 2 support the use of fatty acids (especially DHA, EPA, ARA and their ratios) in the assessment of reproductive output/egg quality in Atlantic cod, while also highlighting the importance of future research in to the roles of other dietary components (protein, vitamins, etc) in egg and larval quality. The results of chapter 3 found no effect in any of the biochemical parameters examined (DNA, RNA, RNA:DNA, protein concentration, SGR). However, ANCOVA analysis of the slopes of RNA:DNA post 7 dph found a significant (age dependent) difference between the BR diet and the other two diet groups. This study also found a lack of correlation between RNA:DNA and SGR, questioning the accuracy of RNA:DNA as an indicator of growth in this situation. Our results suggest that the broodstock experimental diet may improve reproductive performance, compared to the regular on-growing diet, even if the gold standard remains the wild fish diet. Therefore, we recommend that industry broodstock facilities use a baitfish diet to optimize broodfish reproductive output.

References

Chapter 1

Abayasekara, D. R. E. & Wathes, D. C. (1999) Effects of altering dietary acid composition on prostaglandin synthesis and fertility. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **61**, 275-287.

Bachan, M.M., Fleming, I.A. & Trippel, E.A. (2012) Maternal allocation of lipid classes and fatty acids with seasonal egg production in Atlantic cod (*Gadus morhua*) of wild origin. *Marine Biology* **159**, 2281-2297.

Barker, G.A., Smith, S.N. & Bromage, N.R. (1989) The bacterial flora of rainbow trout, *Salmo gairdneri* Richardson and brown trout, *Salmo trutta* L. eggs and its relationship to developmental success. *Journal of Fish Diseases* **12**, 281-293.

Bell, J. G., Farndale, B. M., Bruce, M. P., Navas, J. M. and Carrillo, M. (1997) Effect of broodstock dietary lipid on fatty acid composition of eggs from sea bass (*Dicentrarchus labrax*). *Aquaculture* **149**, pg 107-119.

Blaxter, J.H.S. & Hempel, G. (1963) The influence of egg size on herring larvae (Clupea harengus L.). *Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer* **28**, 211-240.

Blaxter, J.H.S. (1992). The effect of temperature on larval fishes *Netherlands Journal of Zoology* **42**, 336-357.

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G. (1992) Broodstock management, fecundity, egg quality and timing of egg production in the rainbow trout (*Onchorynchus mykiss*). *Aquaculture* **100**, 141-166.

Bromage, N., Bruce, M., Basavaraja, N., Rana, K., Shields, R., Young, C., Dye, J., Smith, P., Gillespie, M. & Gamble, J. (1994) Egg quality determinants in finfish the role of overripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. *Journal of the World Aquaculture Society* **25**, 13-21.

Bromage, N. (1995) Broodstock management and seed quality – general considerations. In: *Broodstock Management and Egg and Larval Quality*. (Bromage, N. R. and Robert, R. J. eds) p 1-24. Blackwell. Oxford.

Brooks, S., Tyler, C. R. & Sumpter, J. P. (1997) Egg quality in fish: what makes a good egg? *Reviews of Fish Biology and Fisheries* **7**, 387-416.

Brown, J.A., Minkoff, G., Puvanendran, V. (2003) Larviculture of Atlantic cod (*Gadus morhua*): progress, protocols and problems. *Aquaculture* **227**, 375-372.

Bruce, M. P., Shields, R. J., Bell, M. V., Bromage, N.R. (1993) Lipid class and fatty acid composition of eggs of Atlantic halibut *Hippoglossus hippoglossus* (L.), in relation to egg quality in captive broodstock. *Aquaculture and Fisheries Management* **24**, 417-422.

Bruce, M., Oyen, F., Bell, G., Asturiano, J.F., Farndale, B., Carrillo, M., Zanuy, S., Ramos, J. and Bromage, N. (1999) Development of broodstock diets for the European sea bass (*Dicentrarchus labrax*) with special emphasis on the importance of n-3 and n-6 highly unsaturated fatty acid to reproductive performance. *Aquaculture* **177**, 85-97.

Buckley, L. J., Caldarone, E. & Ong, T. L. (1999) RNA-DNA ratio and other nucleic acid-base indicators for growth and condition of marine fishes. *Hydrobiology* **401**, 265-277.

CAIA 2012 – Economic benefits (online). http://www.aquaculture.ca/files/economic-benefits.php

Caldarone, E. M., St. Onge-Burns, J. M. & Buckley, L. J. (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series* **262**, 229-240.

Campbell, P.M., Pottinger, T.G. and Sumpter, J.P. (1994) Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. *Aquaculture* **120**, 151-169.

Carrillo, M., Bromage, N., Zanuy, S., Serrano, R. & Prat, F. (1989) The effect of modifications in photoperiod on spawning time, ovarian development and egg quality in the sea bass (*Dicentrarchus labrax* L.). *Aquaculture* **81**, 351-365.

Cerdá, J., Carillo, M., Zanuy, S. and Ramos, J. (1994) Effect of food ratio on estrogen and vitellogenin plasma levels, fecundity, and larval survival in captive sea bass, *Dicentrarchus labrax*: preliminary observations. *Aquatic Living Resources* **7**, 255-266.

Chambers, R. C. & Leggett, W. C. (1996) Maternal influences on variation in egg sizes in temperate marine fishes. *American Zoology* **36**, 180-196.

Chambers, R.C. (1997) Environmental influences on egg and propagule sizes in marine fishes. *Early Life History and Recruitment in Fish Populations Chapman & Hall Fish and Fisheries Series* **21**, 63-102.

Clarke, M. J., Parrish, C. C., Penney, R. W. (2005). Lipids, fatty acids and free amino acids as indicators of egg and larval viability in Atlantic cod (*Gadus morhua*). *Bulletin for Aquaculture Association of Canada*, 1-7.

Cushing, D. H. (1972) The production cycle and the numbers of marine fish. In *Symposium of Zoology Society of London* **29**, 213-232.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in marine biology* **26**, 249-293.

Czesny, S. and Dabrowski, K. (1998) The effect of fatty acid concentration on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). *Aquatic Living Resources* **11**, 371-378.

Czesny, S.J., Graeb, B.D.S. & Dettmers, J.M. (2005) Ecological Consequences of Swim Bladder Noninflation for Larval Yellow Perch. *Transactions of the American Fisheries Society* **134**, 1011-1020.

Daniel, E.S., Parrish, C.C., Somerton, D.C. & Brown, J.A. (1993) Lipids in eggs from first-time and repeat spawning Atlantic Halibut, *Hippoglossus hippoglossus* (L.). *Aquaculture and Fisheries Management* **24**, 187-191.

DeMartini, E.E. (1991) Annual variations in fecundity, egg size and the gonadal and somatic conditions of queenfish *Seriphus politus* (Sciaenidae). *Fishery Bulletin* **89**, 9-18.

DFO 2009 – Aquaculture in Canada (online). http://www.canadainternational.gc.ca/norway-norvege/commerce_canada/aquanor07/aquaculture.aspx?view=d

Estevez, A., McEvoy, L. A., Bell, G., Sargent, J. R. (1999) Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live prey enriched in AA and EPA. *Aquaculture* **180**, 321-343.

Evans, R. P., Parrish, C. C., Zhu, P., Brown, J. A., Davis, P. J. (1998) Changes in phopholipase A(2) activity and lipid content during early development of Atlantic halibut, *Hippoglossus hippoglossus*. *Marine Biology* **130**, 369-376.

Fernandez-Palacios, H., Izquierdo, M. S., Robaina, L., Valencia, A., Salhi, M. and Vergara, J. (1995) Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). *Aquaculture* **132**, 325-337.

FOA 2006 – State of World Fisheries and Aquaculture (SOPHIA) (online). http://www.fao.org/docrep/009/A0699e/A0699e00.htm

Fraser, A.J., Sargent, J.R. & Gamble, J.C. (1989) Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis* Muller from a nutrient-enriched seawater enclosure. *Journal of Experimental Marine Biology and Ecology* **130**, 81-92.

Frøyland, L., Lie, Ø. & Berge, R. K. (2000) Mitochondrial and peroxisomal β-oxidation capacities in various tissues from Atlantic salmon *Salmo salar*. *Aquaculture Nutrition* **6**, 85-89.

Green, B. S. & McCormick, M. I. (2005) Maternal and paternal effects determine size, growth and performance in larvae of tropical reef fish. *Marine Ecology Progress Series* **289**, 263-272.

Green, B.S. (2008) Maternal Effects in Fish Populations. *Advances in Marine Biology* **54**, 1-105.

Govoni, J.J. (2005) Fisheries oceanography and the ecology of early life histories of fishes: a perspective over fifty years. *Scientia Marina* **69**, 125-137.

Gunasekera, R.M., De Silva, S.S. & Ingram, B.A. (1999) The amino acid profiles in developing eggs and larvae of the freshwater Percichthyid fishes, trout cod, *Maccullochella macquariensis* and Murray cod, *Maccullochella peelii peelii*. *Aquatic Living Resources* **12**, 255-261.

Hamoutene, D., Lush L., Drover D., and Walsh A. (2009) Investigation on the temporal effects of spawning season and maternal and paternal differences on egg quality in Atlantic cod, *Gadus morhua* L. broodstock. *Aquaculture Research* **40**, 1668-1669.

Hansen, G.H. & Olafsen, J.A. (1989) Bacterial colonization of cod (*Gadus morhua* L.) and halibut (*Hippoglossus hippoglossus*) eggs in marine aquaculture. *Applied and Environmental Microbiology* **55**, 1435-1446.

Hardy, R. (1985) Salmonid broodstock nutrition. In: Iwanmoto, R., Sower, S. (Eds), *Salmonid Reproduction*, University of Washington, Seattle, 98-108.

Harel, M., Tandler, A., Kissil, G. W. (1994) The kinetics of nutrient incorporation into body tissues of gilthead seasbream (*Sparus aurata*) females and the subsequent effects on egg composition and egg quality. *British Journal of Nutrition* **72**, 45-48.

Hendersen, R. J., Sargent, J. R. and Hopkins, C. C. E. (1984) Changes in the content and fatty acid composition of lipid in an isolated population of the capelin *Mallotus villosus* during sexual maturation and spawning. *Marine Biology* **78**, 255-263.

Hendersen, R. J. and Altamar, S. M. (1989) Seasonal changes in the lipid composition of herring in relation to gonad maturation. *Journal of the Marine Biological Association of the UK* **69**, 307-313.

Hjort, J. (1914) Fluctuations in the great fisheries of northern Europe. *Rapports et Proces-verbaux des Réunions. Conseil International pour l'Éxploration de la Mer* **20**, l-228.

Houde, E.D. (1987). Fish early life dynamics and recruitment variability. *American Fisheries SOC. Symposium* **2**, 17-29.

Houde, E.D. (2008) Emerging from Hjort's Shadow. *Journal of Northwest Atlantic Fisheries Science* **41**, 53–70.

Hutchings, J.A. (2000) Collapse and recovery of marine fishes. *Nature* **406**, 882-885.

Izquierdo, M. S., Fernandez-Palacios, H. and Tacon, AG.J. (2001) Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* **197**, 25-42.

Jangaard, P. M., Regier, L. W., Claggett, F. G., March, B. E. and Biely, J. (1974). Nutrient Composition of Experimentally Produced Meals from whole Argentine, Capelin, Sand Lance and from Flounder and Redfish Filleting Scrap. *Journal of Fisheries Research Board of Canada* **31**, 141-146.

Jordaan, A., Hayhurst, S.E. & Kling, L.J. (2006) The influence of temperature on the stage at hatch of laboratory reared *Gadus morhua* and implications for comparisons of length and morphology. *Journal of Fish Biology* **68**, 7-24.

Kaitaranta, J. K. and Ackman, R. G. (1981) Total lipids and lipid classes of fish roe. *Comprehensive Biochemical Physiology* **69**, 725-729.

Kamler, E. (1992) Early life history of fish: an energetics approach. *Fish and Fisheries Series* **4**. Chapman and Hall, London.

Kamler, E. (2005) Parent–egg–progeny Relationships in Teleost Fishes: An Energetics Perspective. *Reviews in Fish Biology and Fisheries* **15**, 399-421.

Kjesbu, O.S. (1989) The spawning activity of cod, *Gadus morhua* L. *Journal of Fish Biology* **34**, 195–206.

Kjesbu, O.S., Solemdal, P., Bratland, P. & Fonn, M. (1996) Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 610-620.

Kjorsvik, E., Mangorjensen, A. & Holmefjord, I. (1990) Egg quality in fishes. *Advances In Marine Biology* **26**, 71-113.

Knickle, D.C. & Rose, G.A. (2010) Seasonal spawning and wind-regulated retention-dispersal of early life stage Atlantic cod (*Gadus morhua*) in a Newfoundland fjord. *Fisheries Oceanography* **19**, 397-411.

Lall, S. P., and Nanton, D. (2002) Nutrition of Atlantic cod. *Bulletin Aquaculture Association of Canada*, 102-106.

Lasker, R. and Theilacker, H. G. (1962) The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet. *Journal of Lipid Research* **3**, 60-64.

Le François, Tveiten, Halfyard & Foss (2010)myers Chapter 19 in Finfish Aquaculture Diversification CABInternation ed.

Lilly, G. R., Shelton, P. A., Brattey, J., Cadigan, N., Murphy, E. F., Stansbury, D. E., Davis, M. B., and Morgan, M. J. (1998) An assessment of the cod stock in NAFO Divisions 2J+3KL. *DFO Canadian Stock Assessment Section Resource Document* **98**, 46-94.

Litvak, M. K., & Leggett, W. C. (1992) Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. *Marine ecology progress series* **81**, 13-24.

Lochmann, S. E., Maillet, G. L., Frank, K. T., and Taggart, C. T. (1994) Lipid class composition as a measure of nutritional condition in individual larval Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 1294-1306.

Luquet, P. & Watanabe, T. (1986) Interaction "nutrition-reproduction" in fish. *Fish physiology and biochemistry* **2**, 121-129.

Mazorra, C., Bruce, M., Bell, J. G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., and Bromage, N. (2003) Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* **227**, 21-33.

Miller, T.J., Crowder, L.B., Rice, J.A. & Marschall, E.A. (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 1657-1670.

Miller, M.A. (1993) Maternal transfer of organochlorine compounds in salmonines to their eggs. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 1405-1413.

Moodie, G.E.E., Loadman, N.L., Weigand, M.D. & Mathias, J.A. (1989) Influence of egg characteristics on survival, growth and feeding in larval walleye (*Stizotedion vitreum*). *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 516-521.

Mousseau, T.A, & Fox, C.W. (1998) *Maternal effects as adaptations.* In Oxford University Press Oxford, UK:Oxford University Press.

Myers, R.A., Hutchings, J.A., Barrowman, N.J. (1996) Hypotheses for the decline of cod in the North Atlantic. *Marine Ecology Progress Series* **138**, 293-308.

Nagahama, Y. (1994). Endocrine regulation of gametogenesis in fish. *International Journal of Developmental Biology* **38**, 217-217.

Navas, J. M., Bruce, M., Thrush, M., Farndale, B. M., Bromage, N., Zanuy, S., Carrillo, M., Bell, J. G. and Ramos, J. (1997) The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass. *Journal of Fish Biology* **51**, 760-773.

Norberg, B., Valkner, V., Huse, J., Karlsen, I. and Grung, G.L. (1991) Ovulatory rhythms and egg viability in the Atlantic halibut (*Hippoglossus hippoglossus* L). *Aquaculture* **97**, 365-371.

Otterå, H. & Lie, Ø. (1991) Weaning Trials with Cod (*Gadus Morhua* L.) Fry on Formulated diets. *Fiskeridirektoratet*.

Pavlov, D., Kjorsvik, E., Refsti, T. and Andersen, O. (2004) Broodstock and egg production. In: *Culture of cold-water marine fish*. Blackwell Science, Oxford, UK, p 129-203

Penney, R. W., Lush, P. L., Wade, J., Brown, J. A., Parrish, C. C., and Burton, M. P. M. (2006) Comparative utility of egg blastomere morphology and lipid biochemistry for prediction of hatching success in Atlantic cod, *Gadus morhua* L. *Aquaculture Research* **37**, 272-283.

Pepin, P. & Myers, R.A. (1991) Significance of Egg and Larval Size to Recruitment Variability of Temperate Marine Fish. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 1820-1828.

Pickova, J., Dutta, P. C., Larsson, P. O. and Kiessling, A. (1997) Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison

between two cod (*Gadus morhua*) stocks. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 2410-2416.

Ramos, J., Thrush, M., Navas, J. M., Zanuy, S., Carrillo, M., Bromage, N. (1993) Effect of the implementation of lipid enrichment of artificial diets on growth, fecundity and quality of sea bass. *Proceedings of the Fourth National Aquaculture Congress*, p 13-18.

Rose, G.A. (2007) Cod: Ecological History of he North Altantic Fisheries. Pp 1-580 Breakwater Books Ltd, St. John's, Newfoundland, Canada.

Roy, W., Bell, G., Sawanboochun, J., Davie, A., Franco, J., Fermandes, D., Gnassou, J., and Robertson, D. (2007) Cod broodstock nutirion: arachidonic acid and astaxanthin as determinants of egg quality. *The Institute for Scottish Aquaculture Research Forum*, p 1-17.

Salze, G., Tocher, D. R., Roy, W. J. and Robertson, D. A. (2005) Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. *Aquaculture Research* **36**, 1488-1499.

Sargent, R.C., Taylor, P.D. & Gross, M.R. (1987) Parental care and the evolution of egg size in fishes. *American Naturalist* **129**, 32-46.

Sargent, J., Bell, G., Bell, M. V., Henderson, R. J., Tocher, D. R. (1995) Requirement criteria for essential fatty acids. *Journal of Applied Ichthyology* **11**, 183-198.

Sargent, J. R., Bell, J. G., McEvoy, L. A., Tocher, D. R., Estevez, A. (1999) Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* **177**, 191-199.

Springate, J.R.C., Bromage, N.R., Elliot, J.A.K. & Hudson, D.L. (1984) The timing of ovulation and stripping and their effects on the rates of fertilization and survival to eyeing, hatching and swim-up in the rainbow trout (*Salmo gairdneri*). In Cowey, C.B., Mackie, A.M. and Bell, J.A., eds. Nutrition and Feeding in Fish. London: Academic Press, pp. 371-391.

Tandler, A., Anav, F. A., & Choshniak, I. (1995). The effect of salinity on growth rate, survival and swimbladder inflation in gilthead seabream, *Sparus aurata*, larvae. *Aquaculture* **135**, 343-353.

Thrush, M., Navas, J. M., Ramos, J., Bromage, N., Carrillo, M., Zanuy, S. (1993) The effect of artificial diets on lipid class and total fatty acid composition of cultured sea bass (*Dicentrarchus labrax*) eggs. *Proceedings of the Fourth National Aquaculture Congress*, p 37-42.

Tocher, D. R. & Sargent, J. R. (1984) Analyses of lipids and fatty acids in ripe roes of some Northwest European marine fish. *Lipids* **19**, 492-499.

Tocher, D.R. (2003) Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. *Reviews in Fisheries Science* **11**, 107-184.

Tong, X.H., Liu, Q.H., Xu, S.H., Li, J., Xiao, Z.Z. & Ma, D.Y. (2010). Changes in RNA, DNA, protein contents and growth of turbot *Scophthalmus maximus* larvae and juveniles. *Journal of Fish Biology* **77**, 512-525.

Trippel, E.A., Kjesbu, O.S. & Solemdal, P. (1997) Effects of adult age and size structure on reproductive output in marine fishes. *Early Life History and Recruitment in Fish Populations Chapman & Hall Fish and Fisheries Series* **21**, 31-62.

Trippel, E.A. (1998) Egg Size and Viability and Seasonal Offspring Production of Young Atlantic Cod. *Transactions of the American Fisheries Society* **127**, 339-359.

Trippel, E.A., Benfey, T.J., Neil, S.R.E., Cross, N., Blanchard, M.J. & Powell, F. (2005) Effects of continuous light and triploidy on growth and sexual maturation in Atlantic cod, *Gadus morhua. Cybium* **32**, 136-38.

Tveiten, H., Jobling, M. and Andreassen, I. (2004) Influence of egg lipids and fatty acids on egg viablitiy and their utilization during embryonic development of spotted wolf-fish, *Anarhichas minor* Olafsen. *Aquaculture Research* **35**, 152-161

Vetter, R. D., Hodson, R. E. and Arnold, C. (1983) Energy metabolism in a rapidly developing marine fish egg, the red drum (*Sciaenops ocellata*) *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 627-634.

Washburn, B.S., Frye, D.J., Hung, S.S.O., Doroshov, S.I. & Conte, F.S. (1990) Dietary effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **90**, 179-195.

Watanabe, T., Koizumi, T., Suzuki, H., Satoh, S., Takeuchi, T., Yoshida, N., Kitada, T. and Tsukashima, Y. (1985) Improvement of quality of red sea bream eggs by feeding broodstock on a diet containing cuttlefish meal on a raw krill shortly before spawning. *Bulletin of Society and Scientific Fisheries* **51**, 1511-1521.

Watanabe, T., Lee, M. J., Mitzutani, J., Yamada, T., Satoh, S., Takeuchi, T., Yoshida, N., Kitada, T. and Arakawa, T. (1991) Effective components in cuttlefish meal and raw krill for the improvement of quality of red sea bream (*Pagrus major*) eggs. *Nippon Suisan Gakkaishi* **57**, 81-694.

Webb, P.W. & Weihs, D. (1986) Functional locomotor morphology of early life history stages of fish. *Transactions of the American Fisheries Society* **115**, 115-127.

West, C.J. & Mason, J.C. (1987) Evaluation of sockeye salmon (*Oncorhynchus nerka*) production from the Babine Lake development project. In Smith, H.D., Margolis, L. and Wood, C.C., eds. Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. *Canadian Special Publication of Fisheries and Aquatic Sciences* **96**, 176-190.

Westin, D.T., Olney, C.E. & Rogers, B.A. (1985) Effects of parental and dietary organochlorines on survival and body burdens of striped bass larvae. *Transactions of the American Fisheries Society* **114**, 125-136.

Wiegand, M.D. (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries* **6**, 259-286.

Chapter 2

Abayasekara, D. R. E. & Wathes, D. C. (1999) Effects of altering dietary acid composition on prostaglandin synthesis and fertility. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **61**, 275-287.

Abi-Ayad, S., Melard, C., Kestemont, P. (1997) Effects of n-3 fatty acids in Eurasian perch broodstock diet and egg fatty acid composition and larvae stress resistance. *Aquaculture International* **5**, 161-168.

Ako, H., Kraul, S. and Tamaru, C. (1991) Pattern of fatty acid loss in several warm water fish species during early development. In: *Larvi ;91-Fish & Crustacean Larviculture Symposium* (Lavens, P., Sorgeloos, P., Jaspers, E. & Ollevier, F. eds), 23-25.

Bell, M. V., Henderson, R. J., & Sargent, J. R. (1986) The role of polyunsaturated fatty acids in fish. *Comparative Biochemistry and Physiology* **83B**, 911-919.

Bell, M. V., Batty, R. S., Dick, J. R., Fretwell, K., Navarro, J. C., Sargent, J. R. (1995) Dietary deficiency of docosahexaenoic acid impairs vision at low light intensity in juvenile herring (*Clupea harengus* L.). *Lipids* **30**, 443-449.

Bell, J. G., Farndale, B. M., Bruce, M. P., Navas, J. M. and Carrillo, M. (1997) Effect of broodstock dietary lipid on fatty acid composition of eggs from sea bass (*Dicentrarchus labrax*). *Aquaculture* **149**, pg 107-119.

Bligh, E. G., Scott, M. A. (1966) Lipids of cod muscle and the effect of frozen storage. *Journal of Fisheries and Resource Board of Canada* **23**, 1025-1035.

Brenner, R. R. (1974) The oxidative desaturation of unsaturated fatty acids in animals. *Molecular and Cellular Biochemistry* **3**, 41-52.

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G. (1992) Broodstock management, fecundity, egg quality and timing of egg production in the rainbow trout (*Onchorynchus mykiss*). *Aquaculture* **100**, 141-166.

Brown, N. (2009) Development of broodstock diets for the Atlantic halibut, Hippoglossus hippoglossus. Ph.D. Thesis. University of Maine Center for Cooperative Aquaculture Research, Franklin, Maine.

Bruce, M. P., Shields, R. J., Bell, M. V., Bromage, N.R. (1993) Lipid class and fatty acid composition of eggs of Atlantic halibut *Hippoglossus hippoglossus* (L.), in relation to egg quality in captive broodstock. *Aquaculture and Fisheries Management* **24**, 417-422.

Bruce, M., Oyen, F., Bell, G., Asturiano, J. F., Farndale, B., Carrillo, M., Zanuy, S., Ramos, J. and Bromage, N. (1999) Development of broodstock diets for the European sea bass (*Dicentrarchus labrax*) with special emphasis on the importance of n-3 and n-6 highly unsaturated fatty acid to reproductive performance. *Aquaculture* **177**, 85-97.

Cerdá, J., Carillo, M., Zanuy, S. and Ramos, J. (1994) Effect of food ratio on estrogen and vitellogenin plasma levels, fecundity, and larval survival in captive sea bass, *Dicentrarchus labrax*: preliminary observations. *Aquatic Living Resources* **7**, 255-266.

Cerdá, J., Zauny, S., Carrillo, M., Ramos, J., Serrano, R. (1995) Short and long term effects on female sea bass *Dicentrarchus labrax*: seasonal changes in plasma profiles of lipids and sex steroids in relation to reproduction. *Comparative Biochemical Physiology* **111C**, 83-91.

Clarke, M. J., Parrish, C. C., Penney, R. W. (2005). Lipids, fatty acids and free amino acids as indicators of egg and larval viability in Atlantic cod (*Gadus morhua*). *Bulletin for Aquaculture Association of Canada*, 1-7.

Copeman, L. A. & Parrish, C. C. (2004) Lipid classes, fatty acids, and sterols in seafood from Gilbert Bay, Southern Labrador. *Journal of Food Chemistry* **52**, 4872-4881.

Czesny, S. and Dabrowski, K. (1998) The effect of fatty acid concentration on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). *Aquatic Living Resources* **11**, 371-378.

Estevez, A., McEvoy, L. A., Bell, G., Sargent, J. R. (1999) Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live prey enriched in AA and EPA. *Aquaculture* **180**, 321-343.

Evans, R. P., Parrish, C. C., Zhu, P., Brown, J. A., Davis, P. J. (1998) Changes in phopholipase A(2) activity and lipid content during early development of Atlantic halibut, *Hippoglossus hippoglossus*. *Marine Biology* **130**, 369-376.

Fafioye, O. O., Fagade, S. O. and Adebisi, A. A. (2005) Toxicity of *Raphia vinifera,* P. beauv fruit extracts on biochemical composition of Nile Tilapia (*Oreochromis niloticus*, Trewavas). *Biokemistri* **17**, 137-142.

Falk-Petersen, S., Sargent, J. R., Fox, C., Falk-Petersen, I. B., Haug, T. and Kjøsvik, E. (1989) Lipids in Atlantic halibut (*Hippoglossus hippoglossus*) eggs from planktonic samples in northern Norway. *Marine Biology* **101**, 553-556.

Fernandez-Palacios, H., Izquierdo, M. S., Robaina, L., Valencia, A., Salhi, M. and Vergara, J. (1995) Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). *Aquaculture* **132**, 325-337.

Finn, R. N., Henderson, J. R. and Fyhn, H. J. (1995a) Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (Gadus morhua). II. Lipid metabolism and enthalpy balance. *Marine Biology* **124**, 371-379.

Finn, R. N., Fyhn, H. J. and Evjen, M. S. (1995b) Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. *Marine Biology* **99**. 307-313.

Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497-509.

Fraser, A. J., Gamble, J. C. and Sargent, J. R. (1988) Changes in lipid content, lipid class composition of developing eggs of cod (*Gadus morhua*). *Marine Biology* **99**, 307-313.

Frémont, L., Léger, C., Petridou, B., Gozzelino, M. T. (1984) Effects of a (n-3) polyunsaturated fatty acid deficient diet on profiles of serum vitellogenin and lipoprotein of vitellogenic trout (*Salmo gairdneri*). *Lipids* **19**, 522-528.

Frøyland, L., Lie, Ø. & Berge, R. K. (2000) Mitochondrial and peroxisomal β-oxidation capacities in various tissues from Atlantic salmon *Salmo salar*. *Aquaculture Nutrition* **6**, 85-89.

Furuita, H., Tanaka, H., Yamamota, T., Suzuki, N., Takeuchi, T. (2002) Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus. Aquaculture* **210**, 323-333.

Gaylean, M. L. (1980) Laboratory Procedures in Animal Nutrition Research. Department of Animal and Food Sciences, Texas Tech University, Lubbock, pp 193.

Goetz, F.W. & Theofan, G. (1979) In vitro stimulation of germinal vesicle breakdown and ovulation of yellow perch (*Perca flavescens*) oocytes: effects of 17α-hydroxy-20β-

dihydro-progesterone and prostaglandins. *General Comparative Endocrinology* **37**, 273-285.

Gunasekera, R. M., De Silva, S. S., Ingram, B. A. (1999) Early ontogeny-related changes of the fatty acid compositionin the Percichthyid fishes trout cod, *Maccullochella macquariensis* and Murray cod, *M-peelii peelii*. *Aquatic Living Resources* **12**, 219-227.

Hakanson, J. L (1993) Nutritional condition and growth rate of anchovy larvae (*Engraulis mordax*) in the California current: two contrasting years. *Marine Biology* **115**, 309-316.

Hamoutene, D., Lush, L., Pérez-Casanova, J.C., Hobbs, K., Burt, K., Walsh, A. and Moir, J. (2013) Effect of diet on growth, survival and reproductive performance of first generation (F1) Atlantic cod, *Gadus morhua* L. broodstock. *Canadian Technical Report of Fisheries and Aquatic Sciences* **3026**, 1 – 21.

Harel, M., Tandler, A., Kissil, G. W. (1994) The kinetics of nutrient incorporation into body tissues of gilthead seasbream (*Sparus aurata*) females and the subsequent effects on egg composition and egg quality. *British Journal of Nutrition* **72**, 45-48.

Hendersen, R. J., Sargent, J. R. and Hopkins, C. C. E. (1984) Changes in the content and fatty acid composition of lipid in an isolated population of the capelin *Mallotus villosus* during sexual maturation and spawning. *Marine Biology* **78**, 255-263.

Hendersen, R. J. and Altamar, S. M. (1989) Seasonal changes in the lipid composition of herring in relation to gonad maturation. *Journal of the Marine Biological Association of the UK* **69**, 307-313.

Izquierdo, M. S., Watanabe, T., Takeuchi, T., Arakawa, T. and Kitajima, C. (1989) Requirement of larval red sea bream *Pagrus major* for essential fatty acids. *Nippon Suisan Gakkaishi* **55**, 859-867.

Izquierdo, M. S. (1996) Review Article: Essential fatty acid requirements of cultured marine fish larvae. *Aquaculture Nutrition* **2**, 183-191.

Izquierdo, M. S., Fernandez-Palacios, H. and Tacon, AG.J. (2001) Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* **197**, 25-42.

Jalabert, B. (1976) *In vitro* oocyte maturation and ovulation in rainbow trout (*Salvo gairdneri*), northern pike (*Esox luscius*) and goldfish (*Carassius auratus*). *Journal of Fisheries Research Board of Canada* **33**, 974-988.

Jobling, M., Leknes, O., Sæther, B.S. & Bendiksen, E.A. (2008) Lipid and fatty acid dynamics in Atlantic cod, *Gadus morhua*, tissues: Influence of dietary lipid concentrations and feed oil sources. *Aquaculture* **281**, 87-94.

Kaitaranta, J. K. and Ackman, R. G. (1981) Total lipids and lipid classes of fish roe. *Comprehensive Biochemical Physiology* **69**, 725-729.

Koven, W. M., Kissil, G. W. And Tandler, A. (1989) Lipid and n-3 requirements of *Sparus aurata* larvae during starvation and feeding. *Aquaculture* **79**, 185-191.

Lall, S. P., and Nanton, D. (2002) Nutrition of Atlantic cod. *Bulletin Aquaculture Association of Canada*, 102-106.

Lanes, C. F. C., Bizuayehu, T. T., Bolla, S., Martins, C., Fernandes, J. M. O., Bianchini, A., Kiron, V. and Babiak, I. (2012) Biochemical composition and performance of Atlantic cod (*Gadus morhua* L.) eggs and larvae obtained from farmed and wild broostocks. *Aquaculture* **324-325**, 267-275.

Lasker, R. and Theilacker, H. G. (1962) The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet. *Journal of Lipid Research* **3**, 60-64.

Lavens, P., Leregue, E., Jaunet, H., Brunel, A., Dhert, Ph., Sorgeloos, P. (1999) Effect of dietary essential fatty acids and vitamins on egg quality in turbot broodstock. *Aquaculture International* **7**, 225-240.

Lee, S. -M. (2001) Review of lipid and essential fatty acid requirements of rockfish (*Sebastes schlegeli*). *Aquaculture Resources* **32**, 8-17.

Li, Y. Y., Chen, W. Z., Sun, Z. W., Chen, J. H., Wu, K. G. (2005) Effects of n-3 HUFA content in broodstock diet on spawning performance and fatty acid composition of eggs and larvae in *Plectorhynchus cinctus*. *Aquaculture* **245**, 263-272.

Lie, O., Hemre, G. -l. & Lambersten, G. (1992) Influence of dietary fatty acids on the glycerophospholipid composition in organs of cod (*Gadus morhua*). *Lipids* **27,** 770-775.

Lie, O. (1993) Changes in the fatty acid composition of neutral lipids and glycerophospholipids in developing cod eggs. In: *Physiological and Biochemical Aspects of Fish Development* (Walther, B. T. and Fyhn, H. J. eds), 330-337.

Lochmann, S. E., Maillet, G. L., Frank, K. T., and Taggart, C. T. (1994) Lipid class composition as a measure of nutritional condition in individual larval Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 1294-1306.

Luquet, P. & Watanabe, T. (1986) Interaction “nutrition-reproduction” in fish. *Fish physiology and biochemistry* **2**, 121-129.

Lush, L., Hamoutene, D., Drover, D., Walsh, A. & Puvanendran, V. (2011) Gamete collection method and egg quality comparison in Atlantic cod, *Gadus morhua* L. mating pairs and its importance in selective breeding**.** *Aquaculture* **315**, 407-409.

Mazorra, C., Bruce, M., Bell, J. G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., and Bromage, N. (2003) Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* **227**, 21-33.

Morais, S., Bell, J.G., Robertson, D.A., Roy, W.J. & Morris, P.C. (2001) Protein/lipids ratios in extruded diets for Atlantic cod (Gadus morhua L.): effects on growth, feed utilisation, muscle composition and liver hisology. *Aquaculture* **203**, 101-119.

Mourente, G. & Odriozola, J. M. (1990) Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). *Fish physiology and biochemistry* **8**, 93-101.

Mourente, G., Tocher, D. R. and Sargent, J. R. (1991) Specific accumulation of docosahexaenoic acid (22:6ω3) in brain lipids during development of juvenile turbot *Scopthalmus maximus* L. *Lipids* **26**, 871-877.

Navas, J. M., Bruce, M., Thrush, M., Farndale, B. M., Bromage, N., Zanuy, S., Carrillo, M., Bell, J. G. and Ramos, J. (1997) The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass. *Journal of Fish Biology* **51**, 760-773.

Nocillado, J. N., Penaflorida, V. D. and Borlorgan, I. G. (2000) Measures of egg quality in induced spawns of the Asian sea bass, *Lates calcarifer*. *Fish Physiology and Biochemistry* **22**, 1-9.

Palacios, E., Racotta, I. S., Aparicio, B., Arjona, O., Martinez-Palacios, A. (2007) Lipid classes and fatty acids during embryogenesis of captive and wild silverside (*Chirostoma estor estor*) from Pátzuaro Lake. *Fish Physiology and Biochemistry* **22**, 81-91.

Parrish, C. C. (1987) Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Canadian Journal of Fisheries and Aquatic Sciences* **44**, 722-731.

Parrish, C. C., Brown, J. A., Daniel, E. S., Somerton, D. C. (1994) Fatty acid composition of Atlantic halibut eggs in relation to fertilization. *Bulletin of Aquaculture Association Canada* **94**, 36-38.

Parrish, C. C. (1999) Determination of total lipid, lipid classes and fatty acids in aquatic samples. Lipids in freshwater ecosystems, Springer-Verlag, New York, p 4-20.

Pavlov, D., Kjorsvik, E., Refsti, T. and Andersen, O. (2004) Broodstock and egg production. In: *Culture of cold-water marine fish*. Blackwell Science, Oxford, UK, p 129-203

Penney, R. W., Lush, P. L., Wade, J., Brown, J. A., Parrish, C. C., and Burton, M. P. M. (2006) Comparative utility of egg blastomere morphology and lipid biochemistry for prediction of hatching success in Atlantic cod, *Gadus morhua* L. *Aquaculture Research* **37**, 272-283.

Penney, R. W., Hart, M. J., Lush, P. L., and Parrish, C. C. (2011) Effect of photoperiod advancement of Atlantic cod spawning on egg size and biochemistry. *North American Journal of Aquaculture* **71**, 107-115.

Pickova, J., Dutta, P. C., Larsson, P. O. and Kiessling, A. (1997) Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (*Gadus morhua*) stocks. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 2410-2416.

Planas, M., Ferreiro, M. J., Fernandedez-Reiriz, M. J., and Labarta, U. (1989) Evolution of biochemical composition and enzyme activities in eggs of radaballo (*Scophthalmus maximus* L.) during embryogenesis. In: *Intertidal Aquaculture* (Yúfera, M. ed.), p 215-227.

Rainuzzo, J. R., Farestveit, R. and Jorgensen, L. (1993) Fatty acid and amino acid composition during embryonic and larval development in plaice (*Pleuronectes platessa*). In: *Physiological and Biochemical Aspects of Fish Development* (Walther, B. T. & Fyhn, H. J. eds) p 290-295.

Rainuzzo, J. R., Reitan, K. I., Jorgensen, L. & Olsen, Y. (1994) Lipid composition in turbot larvae fed live feed cultured by emulsions of different lipid classes. *Comparative Biochemistry and Physiology* **107**, 699-710.

Ramos, J., Thrush, M., Navas, J. M., Zanuy, S., Carrillo, M., Bromage, N. (1993) Effect of the implementation of lipid enrichment of artificial diets on growth, fecundity and quality of sea bass. *Proceedings of the Fourth National Aquaculture Congress*, p 13-18.

Ramos, F. & Smith, A. C. (1978) Ketone bodies in fish skin mucus as an indicator of starvation: a preliminary report. *Journal Fish Biology* **12**, 105-108

Rodríguez Barreto, D., Jerez Herrera, S., Cejas Pulido, J. R., MartmartNieves, V., GonZana, G. A., & Lorenzo Hern, B. (2012) Assessment of essential fatty acids requirements of greater amberjack *Seriola dumerili* broodstock. *World Aquaculture Society*, Department of Biology University of La Laguna.

Rodríguez, C. (1994) Study of essential fatty acids requirements of European gilthead for the first two weeks of feeding. *PhD thesis, University of La Laguna*, Spain, pp 284.

Rodríguez, C., Cejas, J. R., Martin, M. V., Badia, P., Samper, M. and Lorenzo, A. (1998) Influence of n-3 highly unsaturated fatty acid deficiency on the lipid composition of broodstock gilthead sea bream (*Sparus aurata* L.) and on egg quality. *Fish Physiology and Biochemistry* **18**, 177-187.

Roy, W., Bell, G., Sawanboochun, J., Davie, A., Franco, J., Fermandes, D., Gnassou, J., and Robertson, D. (2007) Cod broodstock nutrition: arachidonic acid and astaxanthin as determinants of egg quality. *The Institute for Scottish Aquaculture Research Forum*, p 1-17.

Rønnestad, I., Finn, R. N., Lein, I. & Lie, Ø. (1995) Compartmental changes in the contents of total lipid, lipid classes and their associated fatty acids in developing yolk-sac larvae of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Aquaculture Nutrition* **1**, 119-130.

Salze, G., Tocher, D. R., Roy, W. J. and Robertson, D. A. (2005) Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. *Aquaculture Research* **36**, 1488-1499.

Sargent, J. R., Henderson, R. J., Tocher, D. R. (1989) The lipids. In: Halver, J. (ED.), Fish nutrition, 2nd Edition, *Academic Press London*, p 153-217.

Sargent, J., Bell, G., Bell, M. V., Henderson, R. J., Tocher, D. R. (1995a) Requirement criteria for essential fatty acids. *Journal of Applied Ichthyology* **11**, 183-198.

Sargent, J. R. (1995b) Origins and functions of egg lipids: nutritional implications. In: *Broodstock management and larval quality* (N. R. Bromage, and R. J. Roberts eds), p 353-372.

Sargent, J. R., McEvoy, L. A., Bell, J. G., (1997) Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* **155**, 117-127.

Sargent, J. R., Bell, J. G., McEvoy, L. A., Tocher, D. R., Estevez, A. (1999) The essential fatty acid nutrition of early developming fish. *Procedures from the 18th European conference for comparative physiology and biochemistry*., Barcelona, August 1997, Aquaculture, In press.

Sargent, J., Tocher, D. R., and Bell, J. G. (2002) The lipids. In: *Fish Nutrition* (third edition) (Halver J. E. and Hardy R. W. eds). Academic Press, San Diego, p 182-308.

Stacey, J.C. (1976) Effects of indomethacin and prostaglandins on spawning behaviour of female goldfish. Prostaglandins 12, 113-126.

Stacey, J.C. (1981) Hormonal regulation of female sexual behaviour in teleosts. *American Zoology* **21**, 305-316.

Tandler, A., Watanabe, T., Satoh, S. & Fukusho, K. (1989) The effect of food deprivation on the fatty acid and lipid profile of red sea bream larvae (*Pagrus major*). *British Journal of Nutrition* **62**, 349-361.

Thrush, M., Navas, J. M., Ramos, J., Bromage, N., Carrillo, M., Zanuy, S. (1993) The effect of artificial diets on lipid class and total fatty acid composition of cultured sea bass (*Dicentrarchus labrax*) eggs. *Proceedings of the Fourth National Aquaculture Congress*, p 37-42.

Tocher, D. R. & Sargent, J. R. (1984) Analyses of lipids and fatty acids in ripe roes of some Northwest European marine fish. *Lipids* **19**, 492-499.

Tocher, D.R. (2003) Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. *Reviews in Fisheries Science* **11**, 107-184.

Tocher, D. R. (2010) Metabolism and Functions of lipids and fatty acids in teleost fish. In: *Reviews in Fisheries Science* **11**, 107-184.

Toppe, J., Aksnes, A., Hope, B. & Albrektsen, S. (2006) Inclusion of fish bone and crab by-products in diets for Atlantic cod, *Gadus morhua. Aquaculture* **253**, 636-645.

Tveiten, H., Jobling, M. and Andreassen, I. (2004) Influence of egg lipids and fatty acids on egg viablitiy and their utilization during embryonic development of spotted wolf-fish, *Anarhichas minor* Olafsen. *Aquaculture Research* **35**, 152-161

Ulvund, K. A. and Grahl-Nielsen, O. (1988) Fatty acid composition of eggs of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 898-901.

Van der Meeren, T., Klungsoyr, J., Wilhelmsen, S. & Kvensenth, P. G. (1991) Fatty acid composition of unfed and growing cod larvae, Gadus morhua L., feeding on natural plankton in large enclosures. In: *Physiological and Biochemical Aspects of Fish Development* (Walther B. T. & Fyhn, H. J. eds), p 34-35. University of Bergen, Bergen, Norway.

Watanabe, T., Takeuchi, T., Shimman, Y. (1978) Influence of SCP feed on lipid contents and fatty acid composition of rainbow trout. *Bulletin of Freshwater Fisheries Resources Laboratory* **28**, 37-46.

Watanabe, T., Takeuchi, T., Saito, M., and Nishimura, K. (1984a) Effect of low protein-high calory or essential fatty acid deficient deit on reproduction of rainbow trout. *Bulletin of Japanese Society and Scientific Fisheries* **50**, 1207-1215.

Watanabe, T., Arakawa, T., Kitajima, C. and Fujita, S. (1984b). Effect of nutritional quality of broodstock diets on reproduction of red sea bream. *Bulletin of Japanese Society and Scientific Fisheries* **50**, 495-501.

Watanabe, T., Koizumi, T., Suzuki, H., Satoh, S., Takeuchi, T., Yoshida, N., Kitada, T. and Tsukashima, Y. (1985a) Improvement of quality of red sea bream eggs by feeding broodstock on a diet containing cuttlefish meal on a raw krill shortly before spawning. *Bulletin of Society and Scientific Fisheries* **51**, 1511-1521.

Watanabe, T., Lee, M. J., Mitzutani, J., Yamada, T., Satoh, S., Takeuchi, T., Yoshida, N., Kitada, T. and Arakawa, T. (1991) Effective components in cuttlefish meal and raw krill for the improvement of quality of red sea bream (*Pagrus major*) eggs. *Nippon Suisan Gakkaishi* **57**, 81-694.

Watanabe, T. (1993) Importance of docosahexaenoic acid in marine larval fish. *Journal of World Aquaculture Society* **24**, 152-161.

Watanabe, T. & Vassallo-Agius, R. (2003) Broodstock nutrition research on marine finfish in Japan. *Aquaculture* **227**, 35-61.

Wiegand, M. D. (1996) Composition accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries* **6**, 259-286.

Zheng, F., Takeuchi, T., Yoseda, K., Kobayashi, M., Hirokawa, J. and Watanabe T. (1996) Requirement of larval cod for arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid using their enriched *Artemia nauplii*. *Nippon Suisan Gakkaishi* **62**, 669-676.

Zohar, Y., Munoz-Cueto, J. A., Elizur, A. & Kah, O. (2010) Neuroendocrinology of reproduction in teleost fish. *General and Comparative Endocrinology* **165**, 438-455.

Chapter 3

Bailey, K. M., Canino, M. F., Napp, J. M., Spring, S. M. & Brown, A. L. (1995) Contrasting years of prey levels, feeding conditions and mortality of larval walleye pollock *Theragra chalcogramma* in the western Gulf of Alaska. *Marine Ecology Progress Series* **119**, 11-23.

Bergeron, J. P., Boulhic, M. & Galois, R. (1991) Effect of the deprivation of food on the content of DNA of the larva of Sole (*Solea solea* L.) *ICES Journal of marine Science* **48**, 127–134.

Bernardo, J. (1996) Maternal effects in animal ecology. *Integrative and Comparative Biology* **36**, 83-105.

Bisal, G. A. & Bengtson, D. A. (1995) Description of the starving condition in summer flounder, *Paralichthys dentatus*, early life history stages. *Fishery Bulletin* **93**, 217-230.

Bromage, N. (1998) Broodstock management and the optimisation of seed supplies. *Suisan Zoshoku* **46**, 395-401.

Brooks, S., Tyler, C. R. & Sumpter, J. P. (1997) Egg quality in fish: what makes a good egg? *Reviews of Fish Biology and Fisheries* **7**, 387-416.

Buckley, L. J. (1979) Relationship between RNA/DNA ratio, prey density and growth rate in Atlantic cod (*Gadus morhua*) larvae. *Journal of the Fisheries and Research board of Canada* **36**, 1497-1502.

Buckley, L. J. (1980) Changes in ribonucleic acid, deoxyribonucleic acid and protein content during ontogenesis in winter flounder, (*Pseudopleuronectes americanus*), and the effects of starvation. *Fisheries Bulletin of the United States* **77**, 703-708.

Buckley, L. J. (1981) Biochemical changes during ontogenesis of cod (Gadus morhua) and winter flounder (Pseudopleuronectes americanus) larvae. Rapports et Proces-verbaux des Réunions. Conseil International pour l'Éxploration de la Mer **178**, 547-552.

Buckley, L. J. (1984) RNA/.DNA ratio: an index for larval growth in the sea. *Marine Biology* **80**, 291-298.

Buckley, L. J. & Lough, R. G. (1987) Recent growth, biochemical composition and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. *Canadian Journal of Fisheries and Aquatic Sciences* **44**, 14-25.

Buckley, L. J., Caldarone, E. & Ong, T. L. (1999) RNA-DNA ratio and other nucleic acid-base indicators for growth and condition of marine fishes. *Hydrobiology* **401**, 265-277.

Buckley, L. J., Caldarone, E. M., Lough, R. G. and Onge-Burns, J. M. S. (2006) Ontogenetic and seasonal trends in recent growth rates of Atlantic cod and haddock larvae on Georges Bank: Effects of photoperiod and temperature. *Marine Ecology Progress Series* **325**, 205-226.

Bulow, F. J. (1970) RNA-DNA ratio as indicator of recent growth rates of a fish. *Journal of the Fisheries Research Board of Canada* **27**, 2343-2349.

Caldarone, E. M., Wagner, M., St. Onge-Burns, J. & Buckley, L. J. (2001) Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. *Northeast Fisheries Science Center Reference Document* **01-11**, 1-22

Caldarone, E. M., St. Onge-Burns, J. M. & Buckley, L. J. (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series* **262**, 229-240.

Caldarone, E. M. (2005) Estimating growth in haddock larvae *Melanogrammus aeglefinus* from RNA:DNA ratios and water temperature. *Marine Ecology Progress Series* **293**, 241-252.

Castell, J. D. & Kean, J. C. (1986) Evaluation of the role of nutrition in lobster recruitment. *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 2320-2327.

Chambers, R. C. & Leggett, W. C. (1996) Maternal influences on variation in egg sizes in temperate marine fishes. *American Zoology* **36**, 180-196.

Chícharo, M. A. (1998) Nutritional condition and starvation in *Sardina pilchardus* (L.) larvae off southern Portugal compared with some environmental factors. *Journal of Exploratory Marine Biology and Ecology* **225**, 123-137.

Clemmesen, C. M. (1987) Laboratory studies on RNA:DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. *Journal du conseil* **43**, 122-128.

Clemmesen, C. (1994) The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae; Laboratory calibration. *Marine Biology* **118**, 377-382.

Clemmesen, C. & Doan, T. (1996) Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. *Marine Ecology Progress Series* **138**, 33-39.

Clemmesen, C., Sanchez, R., Wongtschowski, C. (1997) A regional comparison of the nutritional condition of SW Atlantic anchovy larvae, Engraulis anchoita, based on RNA/DNA ratios. *Archives of Fisheries and Marine Research* **45**, 17-43.

Dortch, Q., Roberts, T. L., Clayton, J. R. Jr. & Ahmed, S. I. (1983) RNA/DNA ratios and DNA concentrations as indicators of growth rates and biomass in planktonic marine organisms. *Marine Ecology Progress Series* **13**, 61-71.

Fraser, A. J. & Sargent, J. R. (1987) Lipid class and fatty acid composition as indicators of the nutritional condition of larval Atlantic herring. *American Fisheries Society Symposium* **2**, 129-143.

Fraser, A.J., Gamble, J.C. and Sargent, J.R. (1988) Changes in lipid content, lipid class composition of developing eggs of cod (*Gadus morhua*). *Marine Biology* **99**, 307-313.

Ferron, A. & Leggett, W. C. (1994) An appraisal of condition measures for marine fish larvae. *Advancements in Marine Biology* **30**, 217-303.

Folkvord, A., Ystanes, L., Johannessen, A. & Moksness, E. (1996) RNA:DNA ratios and growth of herring (*Clupea harengus*) larvae reared in mesocosms. *Marine Biology* **126**, 591-602.

Foster, A. R., Houlihan, D. F., Gray, C., Medale, F., Fauconneau, B., Kaushikj, S. J. & Le Bail, P. Y. (1991) The effects of ovine growth hormone on protein turnover in rainbow trout. *General Comparative Endocrinology* **82**, 111-120.

Foster, A. R., Houlihan, D. F., Hall, S. J. & Burren, L. J. (1992) The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (*Gadus morhua* L.). *Canadian Journal of Zoology* **70**, 2095-2102.

Fukuda, M., Yano, Y., Nakano, H. & Sugiyama, M. (1986) Protein and nucleic acid changes during early development stages of cresthead flounder. *Bulletin of Japanese Society and Scientific Fisheries* **52**, 951-955.

Fukuda, M., Sako, H., Shigeta, T. & Shibata, R. (2001) Relationship between growth and biochemical indices in laboratory-reared juvenile Japanese flounder (*Paralichthys olivaceus*) and its application to wild fish. *Marine Biology* **138**, 47-55.

Gwak, W. S. & Tanaka, M. (2001) Developmental change in RNA:DNA ratios of fed and starved laboratory-reared Japanese flounder larvae and juveniles, and its application to assessment of nutritional condition for wild fish. *Journal of Fish Biology* **59**, 902-915.

Gwak, W. S., Tanaka, Y., Tominaga, O., Tsusaki, T. & Tanaka, M. (2003a) Field evaluation by RNA:DNA ratios on post-release nutritional status of released and wild juvenile Japanese flounder *Paralichthys olivaceus* juveniles. *Journal of Experimental Marine Biology and Ecology* **293**, 107-124.

Gwak, W. S., Tsusaki, T. & Tanaka, M. (2003b) Nutritional condition, as evaluated by RNA:DNA ratios, of hatchery-reared Japanese flounder from hatch to release. *Aquaculture* **219**, 503-514.

Hamoutene, D., Lush, L., Pérez-Casanova, J.C., Hobbs, K., Burt, K., Walsh, A. and Moir, J. (2013) Effect of diet on growth, survival and reproductive performance of first

generation (F1) Atlantic cod, *Gadus morhua* L. broodstock. *Canadian Technical Report of Fisheries and Aquatic Sciences* **3026**, 1 – 21.

Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971) Control of protein synthesis in mammalian tissues through changes in ribosome activity. *Journal of Biological Chemistry* **246**, 436-446.

Heyer, C. J., Miller, T. J., Binkowski, F. P., Caldarone, E. M. & Rice, J. A. (2001) Maternal effects as a recruitment mechanism in Lake Michigan yellow perch (*Perca flavescens*). *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1477-1487.

Holm-Hansen, O., Sutcliffe, W. H. Jr. & Sharp, J. (1968) Measurement of the deoxyribonucleic acid in the ocean and its ecological significance. *Limnology and Oceanography* **13**, 507-514.

Holm-Hansen, O. (1969) Algae: amounts of DNA and organic carbon in single cells. *Science* **163**, 87-88.

Houde, E. D. & Schekter, R. C. (1981) Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. *Rapports et Proces-verbaux des Réunions. Conseil International pour l'Éxploration de la Mer* **178**, 441-453.

Houde, E. D. (1987) Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* **2**, 17-29.

Izquierdo, M. S., Fernandez-Palacios, H. and Tacon, AG.J. (2001) Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* **197**, 25-42.

Kvåle, A., Mangor-Jensen, A., Harboe, T. & Hamre, K. (2009) Effects of protein hydrolysates in weaning diets to Atlantic cod (*Gadus morhua* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture Nutrition* **15**, 218–227.

Kennell, D. & Magasanik, B. (1962) The relation of ribosome content to the rate of enzyme synthesis in Aerobacter aerogenes. *Biochimica and Biophysica Acta* **55**, 139-151.

Kjesbu, O. S. (1989) The spawning activity of cod, *Gadus morhua* L. *Journal of Fish Biology* **34**, 195-206.

Kjesbu, O. S., Klungsøyr, J., Kryvi, H., Witthames, P. R., and Greer Walker, M. (1991) Fecundity, Atresia, and Egg Size of Captive Atlantic Cod (*Gadus morhua*) in relation to Proximate Body Composition. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 2333-2343.

Lall, S.P., and Nanton, D. (2002) Nutrition of Atlantic Cod. *Bulletin Aquaculture Association of Canada*, 102-1.

Laurence, G. C. (1978) Comparative growth, respiration and delayed feeding abilities of larval cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) as influenced by temperature during laboratory studies. *Marine Biology* **50**, 1-7.

Laurence, G.C., Smigielski, A.S., Halavik, T.A. & Burns, B.R. (1981) Implications of direct competition between larval cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in laboratory growth and survival studies at different food densities. *Rapports et Proces-verbaux des Réunions. Conseil International pour l'Éxploration de la Mer* **178**, 304–311.

Lee, B. I. (1994) Morphological and biological studies on the early development of yellowtail, *Seriola quinqueradia*. PhD thesis, Tokyo Fisheries University, Tokyo, Japan, p 1-200.

Lied, E., Rosenlund, G., Lund, B. & von der Decken, A. (1983) Effect of starvation and refeeding on *in vitro* protein synthesis in white trunk muscle of Atlantic cod (*Gadus morhua*). *Comparative Biochemistry and Physiology* **76**, 777-781.

Luquet, P. & Watanabe, T. (1986) Interaction "nutrition-reproduction" in fish. *Fish physiology and biochemistry* **2**, 121-129.

Lush, L., Drover, D., Walsh, A., Puvanendran, V. & Symonds, J. (2005) Gamete collection and egg quality comparison in Atlantic cod (*Gadus morhua*) and its importance to a family-based selective breeding program. *Bulletin of Aquaculture Association of Canada* **105**, 31-38.

Lush, L., Hamoutene, D., Drover, D., Walsh, A. & Puvanendran, V. (2011) Gamete collection method and egg quality comparison in Atlantic cod, *Gadus morhua* L. mating pairs and its importance in selective breeding. *Aquaculture* **315**, 407-709.

Mathers, E. M., Houlihan, D. F. & Cunningham, M. J. (1992) Nucleic acid concentrations and enzyme activities as correlates of growth rate of the saithe *Pollachius virens*: growth-rate estimates of open-sea fish. *Marine Biology* **112**, 363-369.

Mazorra, C., Bruce, M., Bell, J. G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., and Bromage, N. (2003) Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* **227**, 21-33.

Meekan, M. G. & Fortier, L. (1996) Selection for fast growth during the larval life of Atlantic cod Gadus morhua on the Scotian Shelf. *Marine Ecology Progress Service* **137**, 25-37.

Otterlei, E., Nyhammer, G., Folkvord, A. & Stefansson, S. O. (1999) Temperature- and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic cod. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 2099-2111.

Park, S. U., Lim, H. K. & Han, H. S. (2008) Changes in RNA/DNA ratio and growth of slime flounder, *Microstomus achne*, larvae until metamorphosis. *Journal of Applied Ichthyology* **24**, 50-54.

Paulsen, H., Kjesbu, O.S., Buehler, V., Case, R.A.J., Clemmesen, C., Carvalho, G., Hauser, L., Hutchinson, W.F., Moksness, E., Ottera, H., Thorsen, A. & Svasand, T. (2009) Effects of egg size, parental origin and feeding conditions on growth of larval and juvenile cod *Gadus morhua. Journal of Fish Biology* **75**, 516-537.

Pavlov, D., Kjorsvik, E., Refsti, T. & Anderson, O. (2004) Broodstock and egg production. In: *Culture of cold-water marine fish* (ed. by E. Moksness, E. Kjorsvik & Y. Olsen), pp.129-203 Blackwell Science, Oxford, UK.

Peck, M. A, Buckley, L. J., Caldarone, E. M. & Bengston, D. A. (2003) Effects of food consumption and temperature on growth rate and biochemical-based indicators of growth in early juvenile Atlantic cod Gadus morhua and haddock *Melanogrammus aeglefinus*. *Marine Ecology Progress Series* **251**, 233-243.

Penney, R.W., Lush, P.L., Wade, J., Brown, J.A., Parrish, C.C., and Burton, M.P.M. (2006) Comparative utility of egg blastomere morphology and lipid biochemistry for prediction of hatching success in Atlantic cod, *Gadus morhua* L. *Aquaculture Research* **37**, 272-283.

Regnault, M. & Luquet, P. (1974) Study by evolution of nucleic acid content of prepuberal growth in the shrimp, *Crangon vulgaris*. *Marine Biology* **25**, 291-298.

Rideout, R.M., Trippel, E.A. & Litvak, M.K. (2005) Effects of egg size, food supply and spawning time on early life history success of haddock, *Melanogrammus aeglefinus. Marine Ecology Progress Series* **285**, 169-180.

Robinson, S. M. C. & Ware, D. M. (1988) Ontogenetic development of growth rates in larval Pacific herring, *Clupea harengus pallasi*, measured with RNA/DNA ratios in the Strait of Georgia British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 1422-1429.

Rooker, J. R., Holt, G. J. & Holt, S. A. (1997) Condition of larval and juvenile red drum (*Sciaenops ocellatus*) from estuarine nursery habitats. *Marine Biology* **127**, 397-394.

Rustad, T., Storrø, I. & Slizyet, R. (2011) Possibilities for the utilisation of marine by-products. *International Journal of Food Science & Technology* **46**, 2001–2014.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. & Klenk, D. C. (1985) Measurement of protein using bicinchoninic acid. *Analytical Biochemistry* **150**, 76-85.

Sutcliffe, W. H. Jr. (1970) Relationship between growth rate and ribonucleic acid concentration in some invertebrates. *Journal of Fisheries Research Board of Canada* **27**, 606-609.

Varnovsky, V. S., Varnavskaya, N. V., Kalinin, S. V. & Kinas, N. M. (1992) RNA:DNA index as an indicator of growth rate of coho salmon (*Oncorhynchus kisutch*) during early marine life. *Journal of Ichthyology* **32**, 10-19.

Vinagre, B. C., Fonseca, V., Maia, A., Amara, R. & Cabral, H. (2008) Habitat specific growth rates and condition indices for the sympatric soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA-DNA ratio. *Journal of Applied Ichthyology* **24**, 163-169.

Watanabe, T. (1982) Lipid nutrition in fish. *Comparative Biochemistry and Physiology* **73**, 3-15.

Westerman, M. E. & Holt, G. J. (1988). The RNA-DNA ratio: measurement of nucleic acids in larval *Sciaenops ocellatus*. *Contributions in Marine Science* **30**, 117-124.

Westerman, M. E. & Holt, G. J. (1994) RNA:DNA ratio during the critical period and early larval growth of red drum *Sciaenops ocellatus*. *Marine Biology* **121**, 1-9.

Wright, D. A. & Martin, F. D. (1985) The effect of starvation on RNA:DNA ratios and growth of larval striped bass, *Morone saxatalis*. *Journal of Fisheries Biology* **27**, 479-485.

Yamashita, Y., Tominaga, O., Takami, H. & Yamada, H. (2003) Comparison of growth, feeding and cortisol level in *Platichthys bicolaratus* juveniles between estuarine and nearshore grounds. *Journal of Fish Biology* **63**, 617-630.

Young, V. R. (1970) The role of skeletal and cardiac muscle in the regulation of protein metabolism. In: *Mammalian protein metabolism* (Munro H. N. ed). Academic Press, London, p 585-674.

Chapter 4

Hamoutene, D., Lush, L., Pérez-Casanova, J.C., Hobbs, K., Burt, K., Walsh, A. and Moir, J. (2013) Effect of diet on growth, survival and reproductive performance of first generation (F1) Atlantic cod, *Gadus morhua* L. broodstock. *Canadian Technical Report of Fisheries and Aquatic Sciences* **3026**, 1 – 21.

Appendix

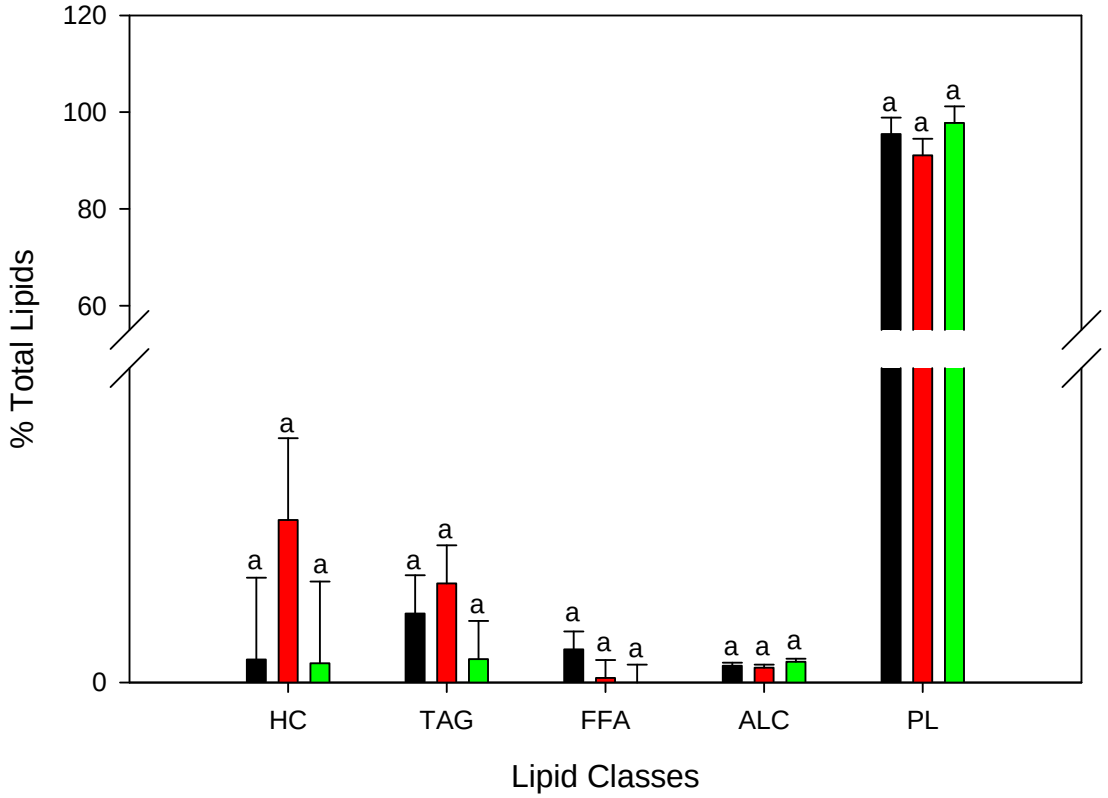

Figure 5.1. Comparison of lipid classes in eggs from cod fed the three diets as mean % ± S.D. of total lipids [hydrocarbons (HC), triacylglycerols (TAG), free fatty acids (FFA), alcohols (ALC), phospholipids (PL)] on-growing ( ), broodstock ( ), baitfish ( )

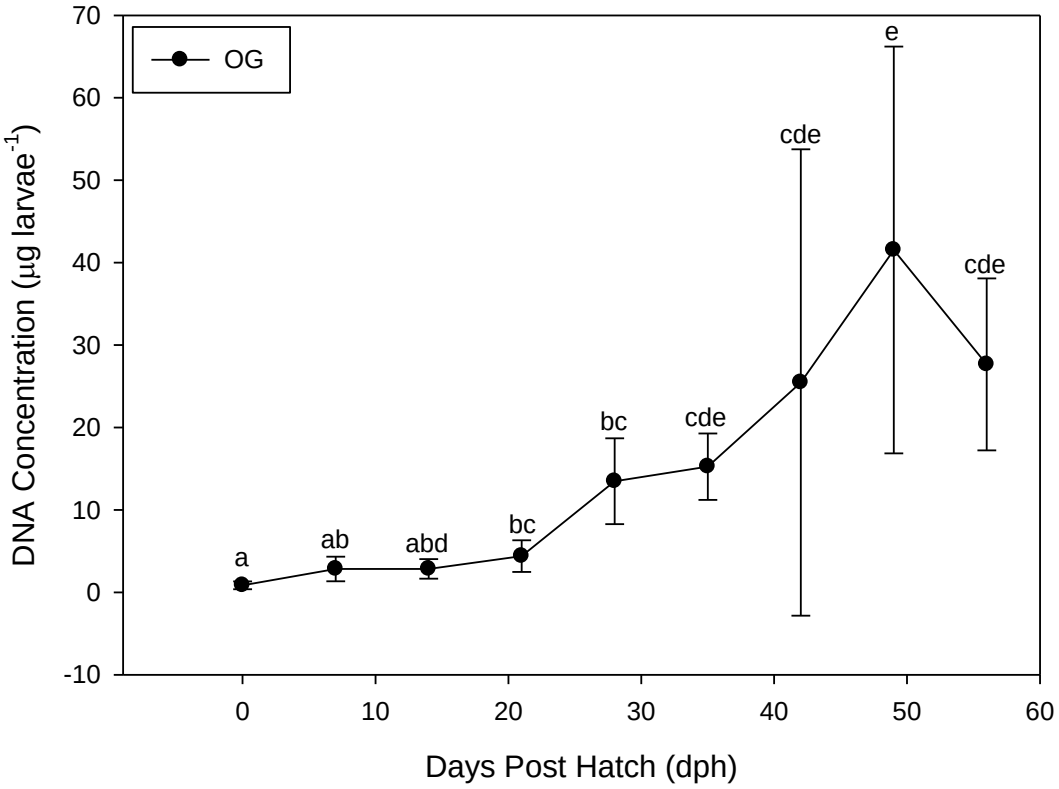

Figure 5.2. Age-specific DNA content (µg larvae^{-1}) of cod larvae within the on-growing diet, significant differences in days post hatch.

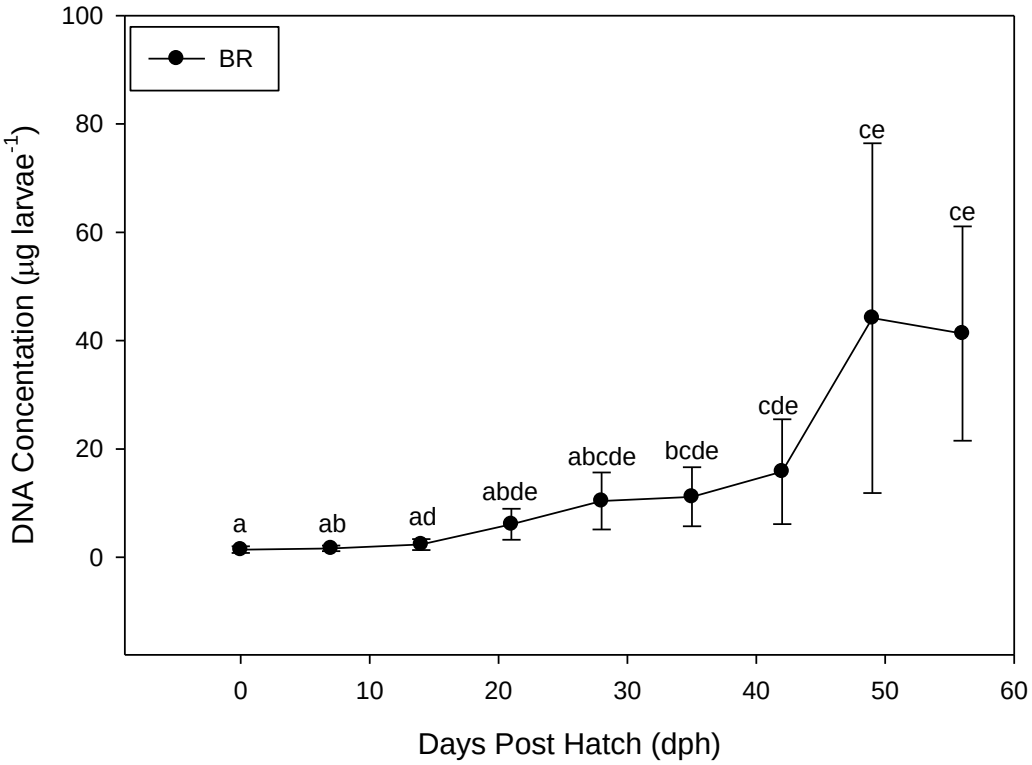

Figure 5.3. Age-specific DNA content (µg larvae^{-1}) of cod larvae within the broodstock diet, significant differences in days post hatch.

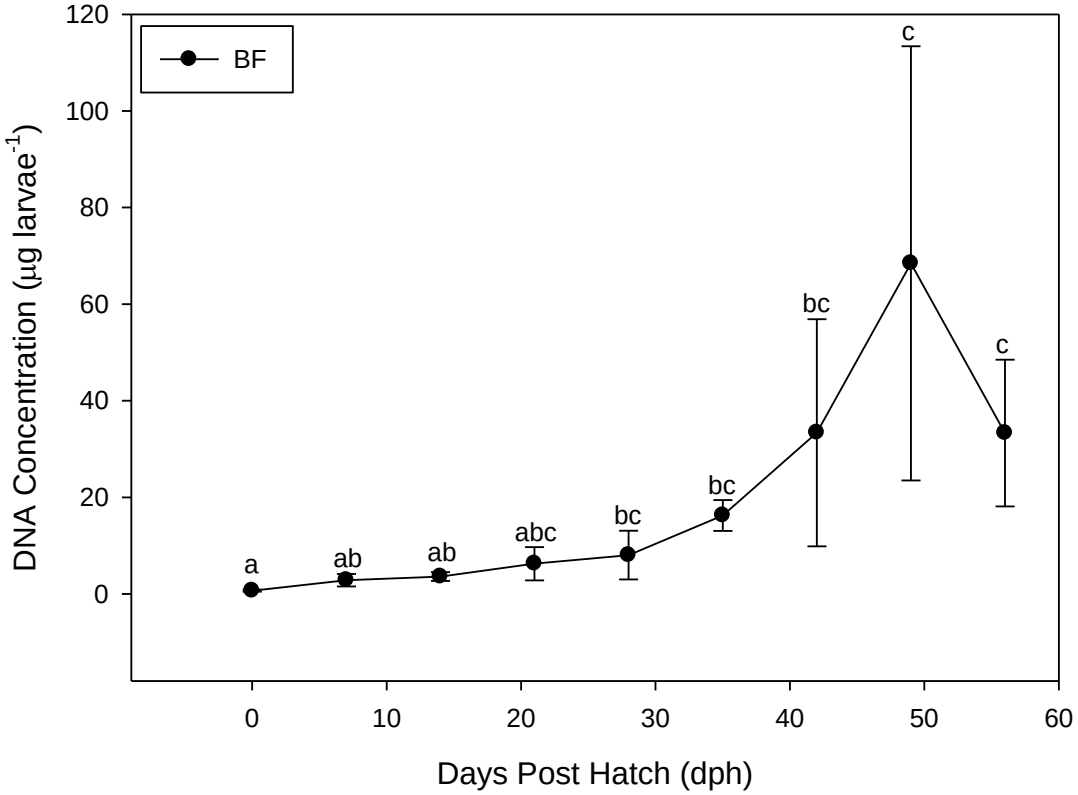

Figure 5.4. Age-specific DNA content (µg larvae-1) of cod larvae within the baitfish diet, significant differences in days post hatch.

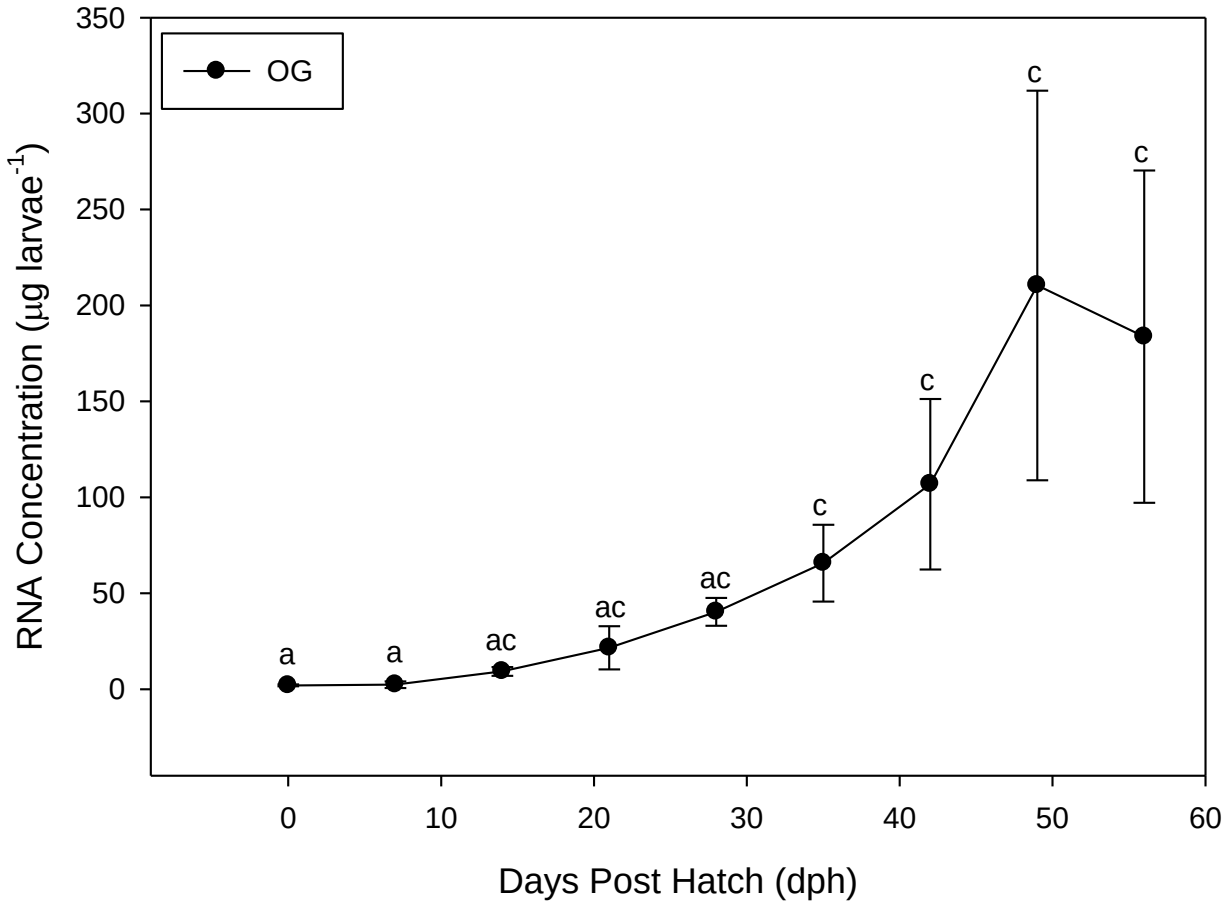

Figure 5.5. Age-specific RNA content (µg larvae^{-1}) of cod within the on-growing diet, significant differences in days post hatch.

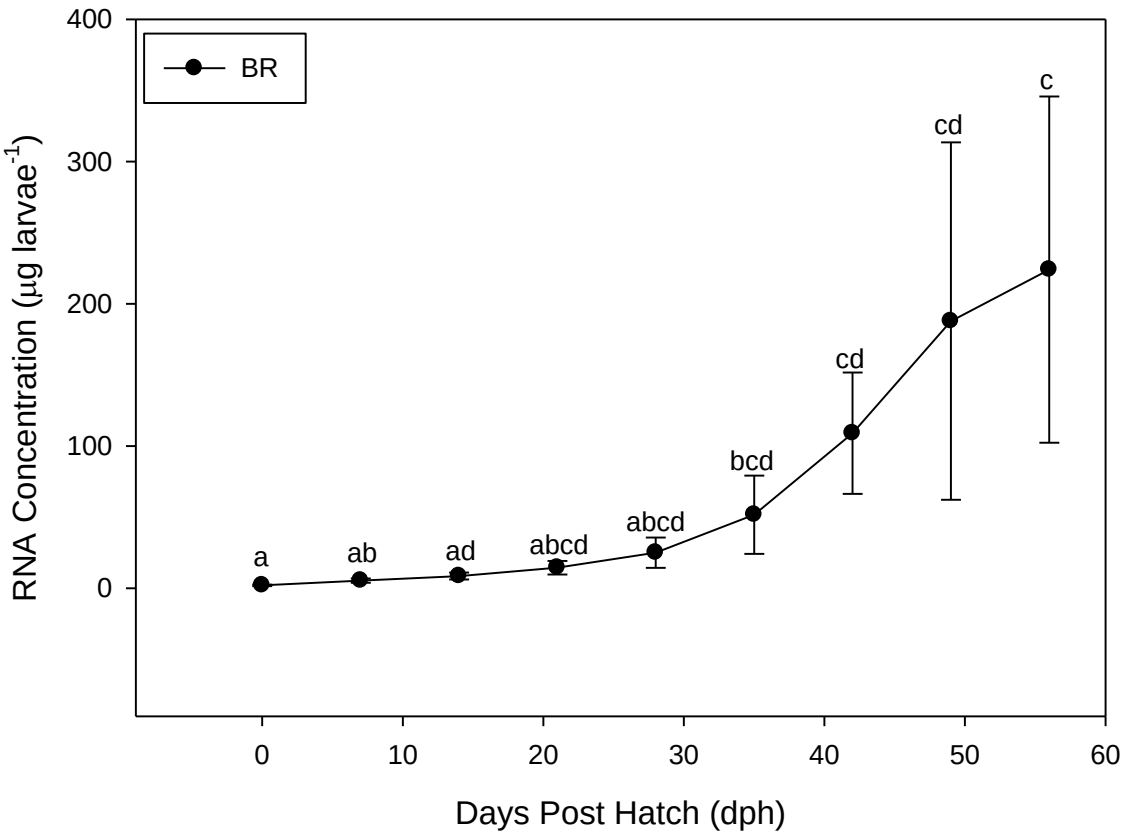

Figure 5.6. Age-specific RNA content (µg larvae-1) of cod within the broodstock diet, significant differences in days post hatch.

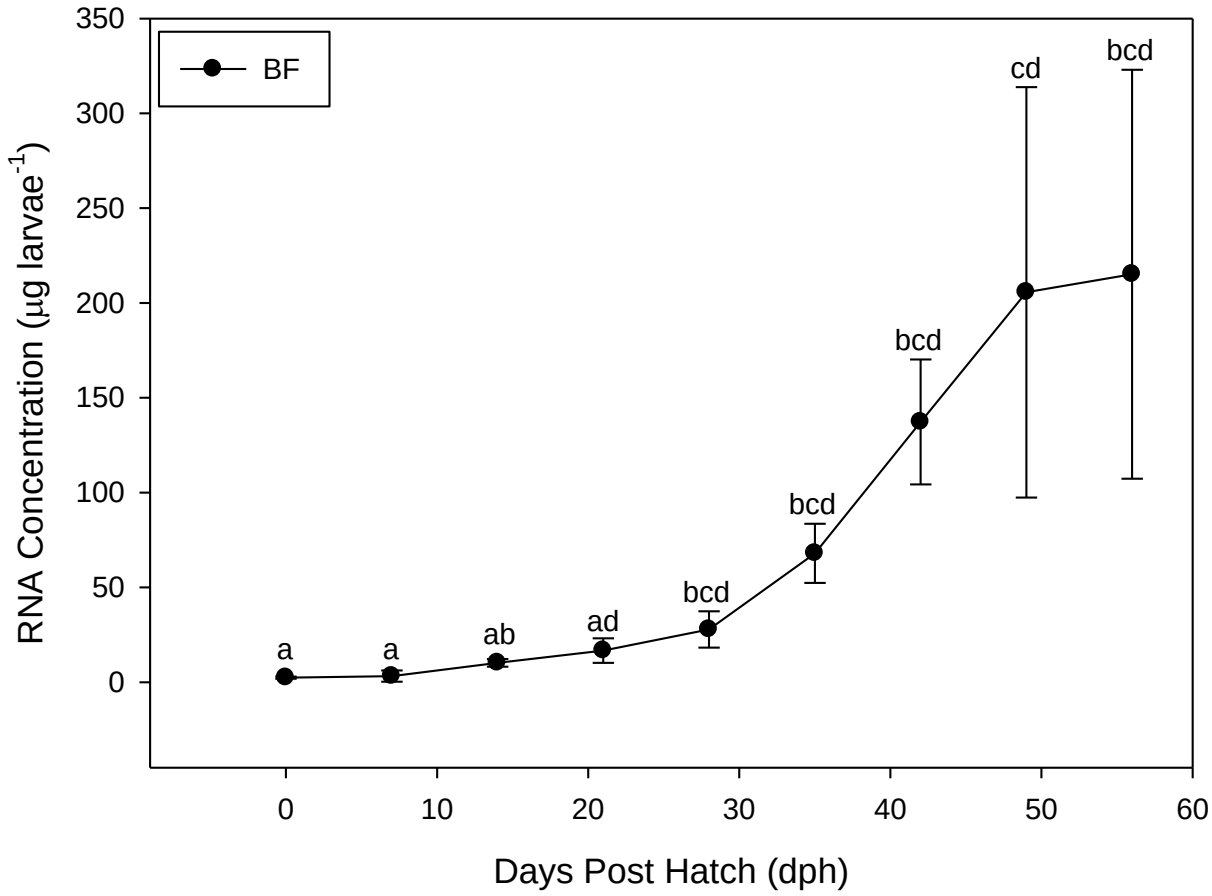

Figure 5.7. Age-specific RNA content (µg larvae-1) of cod within the baitfish diet, significant differences in days post hatch.

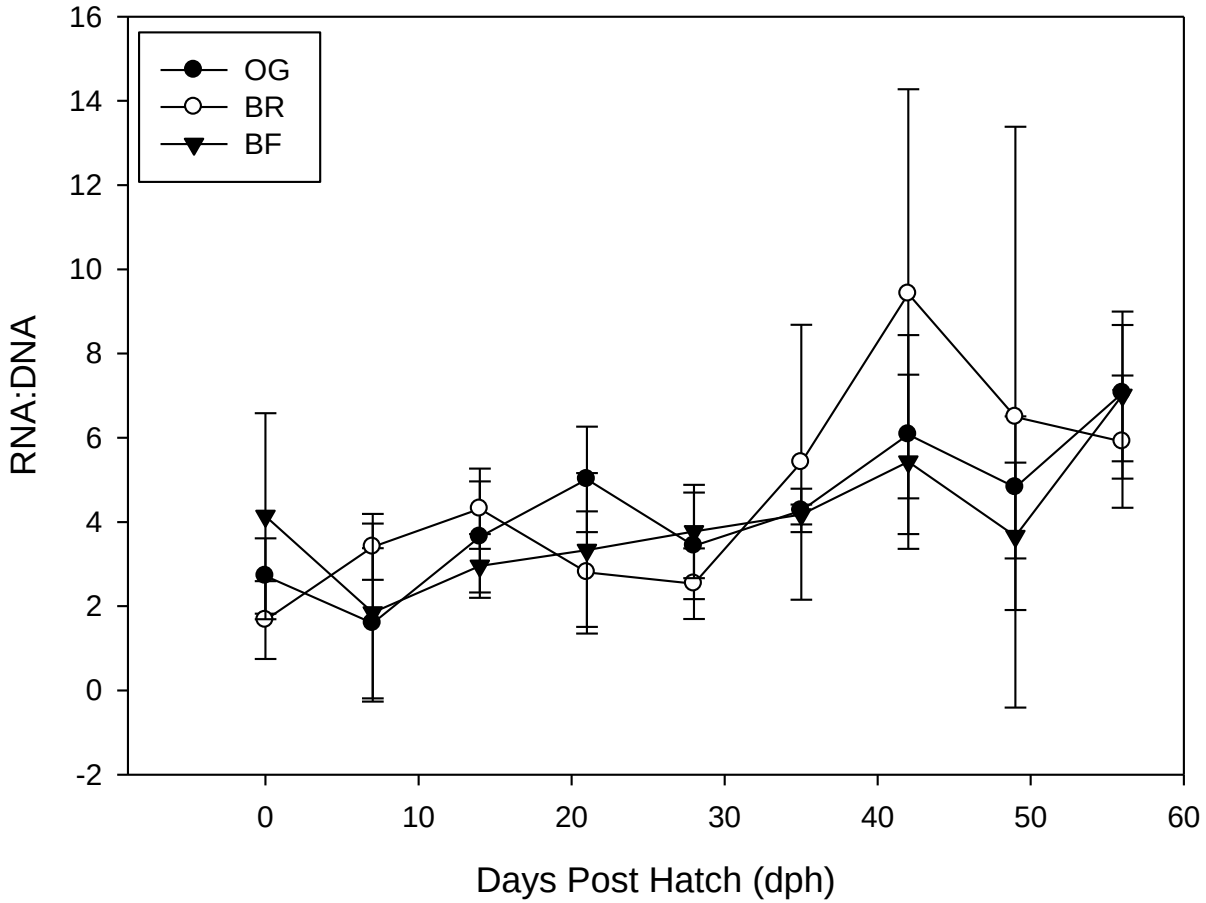

Figure 5.8. Age-specific RNA:DNA content of cod larvae for each diet. Nucleic acid values were determined with the MFA assay. There was no significant differences within or among diets.

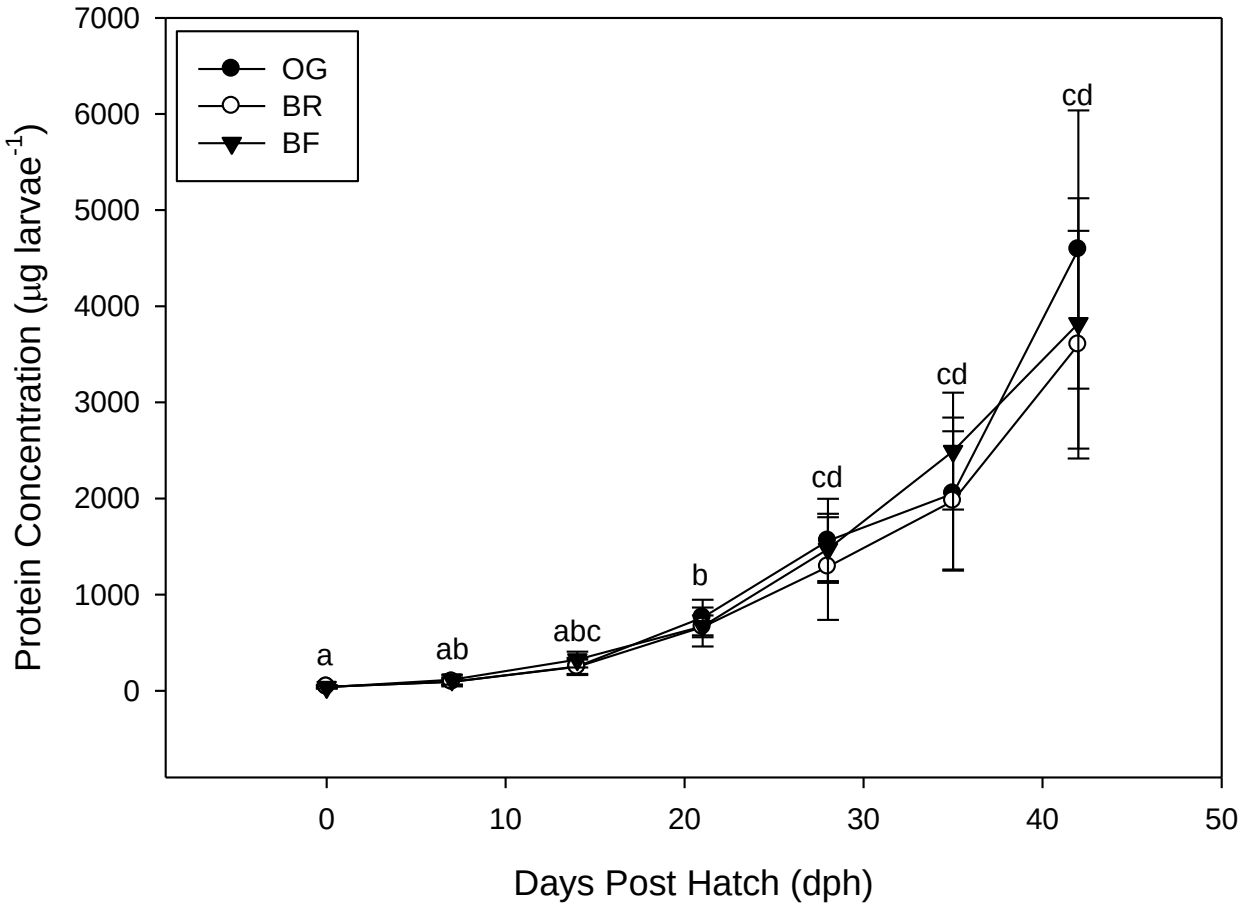

Figure 5.9. Age-specific Pro:DNA content for cod larvae for each diet. Protein values were determined using BCA. There was no significant differences within or among diets.

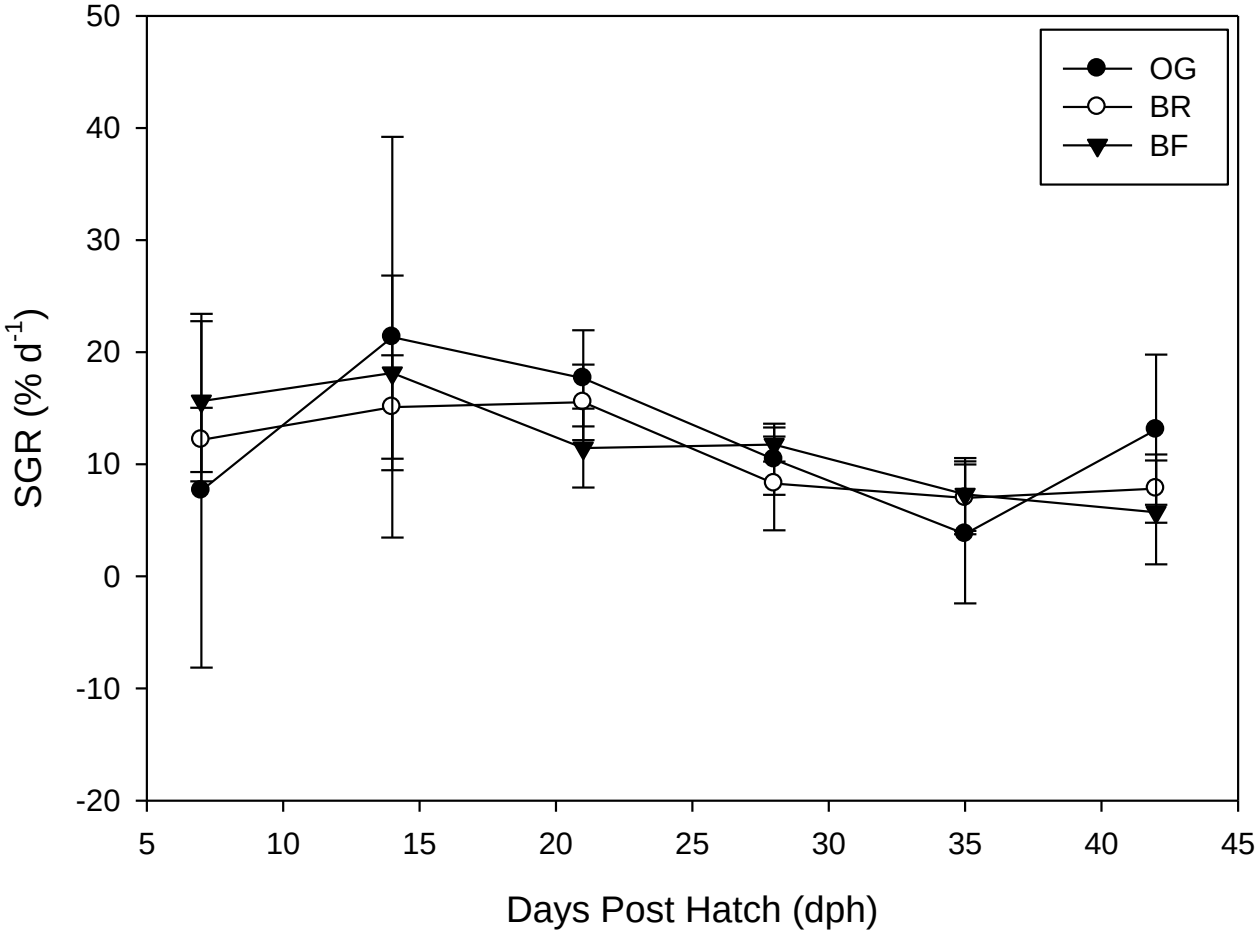

Figure 5.10. Age-specific SGR (% d^{-1}) of cod larvae for each diet. SGR values were calculated using the equation $SGR = 100(e^{Gi} - 1)$, where $G_i = (\ln P_{t2} - \ln P_{t1})/(t_2 - t_1)$. There was no significant differences within or among diets.