UNIVERSITY OF ALGARVE

FACULTY OF SCIENCES AND TECHNOLOGY

MASTER THESIS IN AQUACULTURE

THE EFFECT OF STRESS ON EGG QUALITY IN FARMED ATLANTIC COD (*Gadus morhua L.)*

SILVESTRE NATÁRIO

APRIL 2011

UNIVERSITY OF ALGARVE

FACULTY OF SCIENCES AND TECHNOLOGY

MASTER THESIS IN AQUACULTURE

THE EFFECT OF STRESS ON EGG QUALITY IN FARMED ATLANTIC COD (Gadus *morhua L.)*

SILVESTRE RAMOS NATÁRIO

APRIL 2011

SUPERVISED BY ***ANDRÉ BOGEVIK . ØRJAN KARLSEN AND BIRGITTA NORBERG***

INSTITUTE OF MARINE RESEARCH **(IMR)**, AUSTEVOLL RESEARCH STATION, NORWAY

CO-SUPERVISED BY ***PROF. DR. ADELINO CANÁRIO***

FACULDADE DE CIÊNCIAS E TECNOLOGIA

UNIVERSIDADE DO ALGARVE

FROM 5/03/10 TO 11/09/10 : **INSTITUTE OF MARINE RESEARCH, AUSTEVOLL RESEARCH STATION**

DEDICATED TO:

MY FAMILY: FATHER, MOTHER, BROTHERS, NEPHEWS AND SISTER-IN-LAW THANK YOU FOR EVERY THING! I LOVE YOU ALL!!

SILVESTRE NATÁRIO

"CONSTRUÍ AMIGOS, ENFRENTEI DERROTAS, VENCI OBSTÁCULOS, BATI NA PORTA DA VIDA E DISSE-LHE: NÃO TENHO MEDO DE VIVÊ-LA"

AUGUSTO CURY

This study was part of the project “Why do Atlantic cod females become eggbound? Studies on possible causes and mechanisms” funded by the Research Council of Norway (RCN; grant no). Firstly I would like to thank the Reproduction and Growth research group of the Institute of Marine Research of Norway to allow me to do my master thesis in their facilities, and extend my thanks to all the staff, scientists and technicians. Thanks to my supervisors Andre Bogevik, Ørjan Karlsen and Birgitta Norberg who gave me a great deal of support and advice throughout this study, which I aspire to be the first of many more studies. I also want to thank Prof. Adelino Canario for being the teacher who made this collaboration FCT-UAlg with IMR possible.

My acknowledgements also go to my colleagues that shared my stay in Norway. My sincere thanks to Yuichi, Cinta, Lene, Karen, David, Steve, Quincy, Jenny, Felicia, Sara Leigo “Cybergirl”, also to my fellow Masters with special attention to those accomplices in my work group and also in classes such as: Sandra Encarnação, Cátia Vera, Debora “Lagos”, Andre “cinema”, Rodrigo Lisboa, Nuno Sales, Diego “Guatemalteco”, Carlos “Venezuelano” Romana Roje, Patricia Silva, Helga Leal “Nenufer”.

My Family: cousin Herrique, Claud et Patricia e la petit Victoria, Stephane Carrilho e seus pais e os meus primaços Pedro, Tiago, Lai, João, Padrinho Veloso, Gilinho, Angela, Ana Isabel, Madrinha, Fatinha, Tias e o meu Adorado e tanto estimado Abuelito Silvestre “Pitche”.

Teachers that direct and indirectly have support my academic life: Maria José Arabolaza, Alfredo Teixeira, Raimundo Maurício, Maria Teresa Dínis, Margarida Castro, Manoel de Andrade, António Cunha, António de Andrade, Roque Takahashi.

At least but not the last to my Charrua comrades Helder Garção, Arménio Ribeiro, Tito Rodas, Hélio Valente, Julio Lopes and wife Telma Lopes, Rui Alvares “Piu”, Hugo Resendes, Marco Sequeira e future wife Ana, Luis Ferreira, Pedro Gonçalves, Nando Varejão, Luis Felisberto, Daniel Veiga, Bruno Veríssimo, Monica Oliveira, Marisa Garção and other comrades who supported me with care and also a sincere thanks to my childhood friends namely: family Ferro, Fernando Cabral “Djodje”, Ercynia Nunes “Xini”, Júlio Cesar, José Rebelo, Carlos Mourinho, Ana Paula Soares, Andrea "Satina", Eugenio Monteiro "Mestre" and of course i can not forget about you, I let you my greatest and most humble kiss of gratitude and thank you "Gata”.

ANOVA Analysis of Variance

ARA Arachidonic acid (20:4n-6)

BPG Brain pituitary-gonadal

DHA Docosahexaenoic acid (22:6n-3)

EPA Eicosapentaenoic acid (20:5n-3)

HF High Fat

HS High Stress

IMR Institute of Marine Research

LF Low Fat

LS Low Stress

The Atlantic cod (*Gadus morhua*), is a prestigious species of fish that belongs to the Gadidae family, and is considered a promising species for future aquaculture in the northern hemisphere. Cod production has grown exponentially since the beginning of the millennium. The but development of cod farming has been hampered by bottlenecks in the production such as deformities, diseases as francisellosis, slow growth and early maturation, and in addition relatively low market prices compared to present production costs.

Farming of cod relies on a stable supply of high quality eggs. Egg quality and production is dependent not only upon the female condition and hence broodstock diet, but can be negatively affected by stress. In addition, farmed females often fail to release their eggs, become eggbound and die. The project "Why do Atlantic cod females become eggbound? Studies on possible causes and mechanisms" (Research Council of Norway, grant no 190187/E40) investigates the physiological events during normal and abnormal (eggbound) spawning, with the aim to determine factors that may cause the problems. Two factors that may affect these are investigated in this project; The first is dietary lipid content as this correlates to gonadosomatic index (GSI, gonad weight as % of total weight). The fecundity of farmed cod is about 50% higher than wild cod, and one could suspect that abnormally large gonads would increase the problems with eggbound females. The second variable is stress, either directly due to distorted spawning behaviour or indirectly through the eicosanoid cascade. Eggs of poor quality can have a negative impact on production resulting in slow growth, high mortality rate and deformities. In this master it has been focused on the egg production from farmed cod fed either a high (20%) or a low (13%) fat diet, and either physically stressed or not.

The stress treatment, imposed randomly once a week, consisted of reducing the water level to a height of 15 cm from the bottom, and for 1 minute the fish were chased with a landing's nets. The low-stress treatment was nothing more than keeping fish in tanks. The amount of eggs spawned in the tanks was measured through the spawning season and used to calculate fecundity, and egg quality assessed by egg diameter, fertilization, deformities, and hatching rate.

The dietary treatment affected final weight; cod fed the low fat diet, had a lower final weight after spawning compared with fish that were fed a high fat diet. The fecundity,

expressed as total production of eggs spawned, was affected by diet. The fishes that were fed with the low fat diet had a lower fecundity than the fish fed with a high fat diet. The duration of the spawning season as like the fecundity was also influenced by the stress; the cod in the high stress treatment prolonged their spawning period compared to the low stress group. Egg diameter decreased during the spawning season in all treatments, The fertilisation rate was directly influenced by the type of treatment during the spawning season, but no significant differences were found when assessed 1 or 2 days after stress. Hatching rate was different between diets, but when combined with stress, only fish fed the low fat diet exhibited different rates of hatching. Finally, the rate of deformities did not differ between treatments. In a general assessment, good egg quality is obtained by finding a balance between diet and type of management. Furthermore, fish fed with high fat levels appear more tolerant to stressful activities. Consequently, egg quality, has to be defined by a combination of several parameters. It is also important not to set aside the inclusion of new parameters that may be decisive in order to minimize misclassification.

Key-words: *Gadus morhua*; Atlantic cod; Stress; Corticosteroids; High fat diet; Low Fat Diet; Egg quality; Fecundity.

O Bacalhau do Atlântico *(Gadus morhua*) espécie de peixe de grande prestígio que pertence a família Gadidae, é considerada uma das espécies mais promissoras para o futuro da aquacultura no hemisfério Norte. O cultivo do bacalhau teve um crescimento exponencial no início do milénio quando as cotas da sua pesca foram limitadas devido a redução de estoques. Actualmente com o crescimento das cotas piscatórias, o seu cultivo tem vindo a sofrer o efeito gargalo por parte dos mercados, onde este efeito sendo a oferta maior que a procura. A produção desta espécie não tem tido a capacidade de combater os preços da pesca extractiva, como também tem tido problemas na qualidade dos ovos e larvas, o que tem limitado o seu potencial de produção.

A indústria das rações tem sido um pilar mestre na sustentabilidade de qualquer cultivo aquícola, nomeadamente para o cultivo do bacalhau. Esta tem a responsabilidade de optimizar o crescimento e engorda dos bacalhaus, uma vez que há uma preocupação em estudar as necessidades alimentares de modo a adequar a correcta dieta para cada fase crescimento. No entanto, apesar de muitos esforços e avanços desta indústria, existem ainda algumas lacunas no que diz respeito a exigências alimentares para esta espécie e de dietas apropriadas a cada fase de crescimento. O objectivo deste trabalho foi estudar a fisiologia reprodutiva do bacalhau no que diz respeito à sua desova e, consequentemente, a análise da qualidade dos seus ovos. As variáveis em estudo foram: diferentes níveis de gordura da dieta e o estresse. Uma das dietas testadas tinha um alto teor de lípidos (20%) e a outra baixo teor de gordura (13%), combinadas com dois tratamentos de stresse, sendo estes, alto e baixo stress. O tratamento de alto estresse consistia em vazar semanalmente em dias aleatórios o tanque dos peixes até uma altura de 15 cm, durante 1 minuto, e nesse período perseguir os peixes com um chalavar, este tratamento ocorreu apenas na fase de desova. O tratamento de baixo stress não foi mais que a manutenção dos peixes nos tanques.

A qualidade de ovos está positivamente correlacionada com a viabilidade dos alevins, minimizando assim os riscos de produção. Ovos de baixa qualidade podem ter um impacto negativo na produção, como consequência um crescimento lento, alta mortalidade e deformidades. A qualidade dos ovos neste estudo foi avaliado através de vários parâmetros: fecundidade, diâmetro dos ovos, fertilização, deformidades, taxa de eclosão. Após análise dos vários parâmetros verificou-se que nem todos respondem da mesma forma às variáveis em estudo. No final do ensaio os reprodutores apresentaram

diferentes pesos, sendo que peixes alimentados com dietas de baixo teor de gordura apresentaram um peso final menor pós desova do que os peixes alimentados com dietas com elevado teor de gordura.

O parâmetro fecundidade, entendido como produção total de ovos desovados, mostrou ser afectado pelo tipo de dieta, tendo os peixes alimentados com a dieta de baixo teor de gordura uma fecundidade menor que os peixes alimentados com a dieta de elevado teor de gordura. Este parâmetro também mostrou ser influenciado pela variável estresse; em que os peixes sujeitos ao tratamento de estresse elevado prolongaram as suas desovas comparativamente ao grupos de baixo estresse. O diâmetro do ovo decresceu ao longo da época de desova em qualquer um dos tratamentos a que foram sujeitos os peixes, a taxa de fertilização foi directamente influenciada pelo tipo de tratamento ao longo da época da desova, mas não se verificaram diferenças significativas quando avaliada 1 ou 2 dias após o efeito de stress. A taxa de eclosão teve diferenças entre as dietas, mas quando combinada com a variável estresse, apenas os peixes alimentados com dietas de baixo teor de gordura apresentaram diferentes taxas de eclosão. Por último, a taxa de deformidades não apresentou diferenças entre tratamentos, o que leva a sugerir que a taxa de deformidades tal como foi avaliada não é o melhor parâmetro preditivo para qualidade de ovos. Numa avaliação generalista, o sucesso para uma boa qualidade de ovos é encontrar um equilíbrio entre a dieta e o tipo de maneio, sendo que peixes alimentados com altos teores de gordura reagem melhor a actividades estressantes. Contudo, a qualidade do ovo, quando medido não pode ser justificada por um único parâmetro, mas terá de ser através de coeficientes de ponderação, onde multiplos parâmetros analisados poderão ter relevância mas não se põe de parte a inclusão de novos parâmetros que podem ser decisivos, por forma a minimizar erros de classificação

Palavra-Chave: *Gadus morhua*; Atlantic cod; Estresse; Corticosteroides; Dieta de alto teor lipídico; Dieta de baixo teor lípidico; Qualidade dos Ovos; Fecundidade.

I. INTRODUCTION

1. ATLANTIC COD

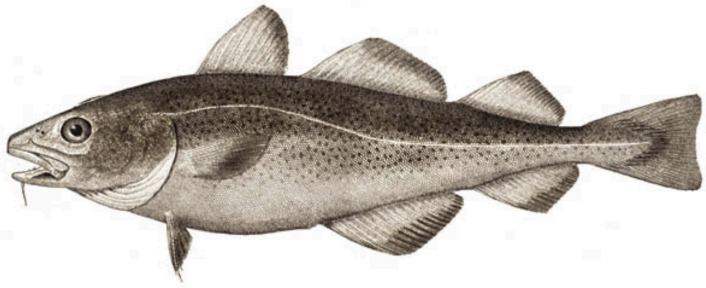

Figure 1 Example of an adult Atlantic cod (*Gadus morhua* L.)

1.1. THE BIOLOGY AND LIFE CYCLE OF WILD ATLANTIC COD

Atlantic cod (Fig.1) is a well-known semi-demersal fish belonging to the family Gadidae. It is a cold water marine finfish that has three dorsal fins and a broad tail. A special characteristic of cod is a long thin organ protruding from the lower jaw known as a barbell (Bigelow & Schroeder, 2002). Their colour can vary from brown to green with spots on the dorsal side, shading to silver ventrally depending on the habitat and diet. They have a well-defined lateral line, which runs along each side of the fish. They can live for 25 years, grow to 2 meters in length and weigh up to 96 kilograms (O'Brien, 1993). In the spawning season females and males have different distributions in the water column, where males are mainly associated with the bottom, whereas females stay closer to the surface (Meager *et al.,* 2009). Brawn (1962) reported that cod are inactive but become restless when they mature and when they are ready to spawn the female swims down to meet the male, and they will swim around close to each other, the male turns on his back with his belly close against that of the female to initiate spawning.

Atlantic cod is a dioecious species with separate sexes, males and females. The median age at maturity is 1.7-2.3 years at lengths between 32 and 41 cm (O'Brien *et al.,* 1993), but can be as late as 8 years in the northeast Arctic (ICES, 2007). Gonad development is affected by temperature which has an impact on the timing of spawning (Howell *et al.,* 2004). The temperature range where cod are found can vary from near freezing to 20ºC but usually they are found below 10ºC, and larger fish prefer colder water (Cohen *et al.,* 1990). Cod are batch spawners and spawn during winter and early spring, usually in water temperatures between 5 and 7°C, with some variation according to location. Normally, the season runs from December to June (Cohen *et al.,* 1990). Generally speaking, larger females produce more eggs (Oosthuizen & Daan, 1974; Kjesbu, 1988). For example, an

average 2 kg female can produce approximately 2.5 million eggs. The maximum production recorded was 9 million eggs from a 34 kg fish (Cohen *et al.*, 1990). The eggs are spawned into the free water masses and fertilized externally. Fertilized eggs have an average size from 1.2 to 1.8 mm and hatch after 2-3 weeks in the upper pelagic layer. Development of larval cod is dependent on water temperature. The yolk sac stage last for 2-5 days before the larvae has to nourish them selves by eating plankton and later larger organisms as crustaceans and fish. As the larvae grow, they change shape to look more like adult fish. After two to four months these larvae settle and become semi-demersal (Fahay *et al.,* 1999).

1.2. BIOGEOGRAPHICAL DISTRIBUTION

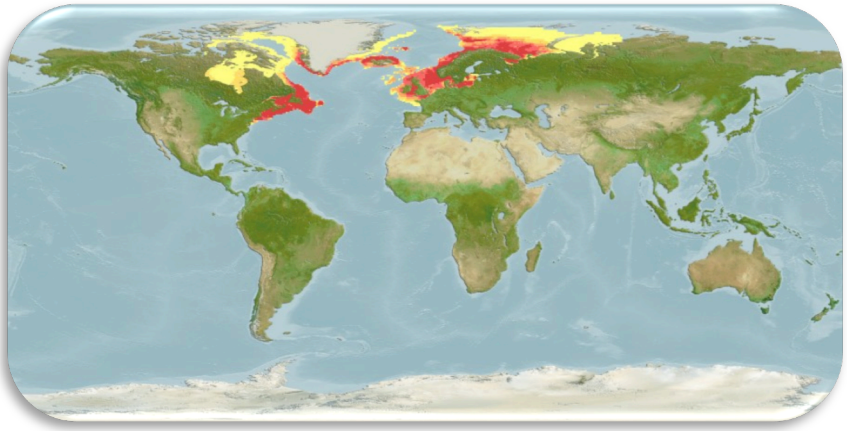

Figure 2 Distribution area of *Gadus morhua* wild populations, red color zone higher concentration of Atlantic cod, yellow color zone possible to find gadus morhua source: Fishbase.org

Atlantic cod can be found from the Bay of Biscay and the Baltic Sea to the Barents Sea, around Iceland, along the southern coast of Greenland and from Newfoundland to North Carolina in North America, as we can see in (Fig.2) painted in red and yellow.

1.3. SYSTEMATIC POSITION OF GADUS MORHUA

Table 1 Classification by Itis

KINGDOM	**ANIMALIA** - ANIMAL,
PHYLUM	**CHORDATA** - CHORDATES
SUBPHYLUM	**VERTEBRATA** - VERTEBRATES
SUPERCLASS	**OSTEICHTHYES** - BONY FISHES, OSTEÍCETO,
CLASS	**ACTINOPTERYGII**- RAY-FINNED FISHES, SPINY RAYED FISHES
SUBCLASS	**NEOPTERYGII** - NEOPTERYGIANS
INFRACLASS	**TELEOSTEI -** TELEOSTS
SUPERORDER	**PARACANTHOPTERYGII -** PARACANTHOPTERYGIANS
ORDER	**GADIFORMES** - CODS, GADIFORMS,
FAMILY	**GADIDAE** RAFINESQUE, 1810 - CODFISHES, CODS, MORUES, TRUE CODS
SUBFAMILY	**GADIDAE** - CODS, HADDOCK
GENUS	**GADUS** LINNAEUS, 1758 - COMMON CODFISHES
SPECIES	***GADUS MORHUA*** LINNAEUS, 1758 - ATLANTIC COD,

1.4. THE COMMERCIAL IMPORTANCE OF COD: EXPLOITATION AND AQUACULTURE

Atlantic cod is considered one of the most promising new species for aquaculture in the northern hemisphere (Rosenlund & Skretting, 2006). Actually, it is one of the most important food fishes in Europe and North America with a good economic value. This species has been excessively fished, and in some cases overfished, in the last decades, which has resulted in red-listing of some stocks. This, among other factors, has lead to a growth of cod aquaculture, in order to answer to the demand of the market (Sawanboonchun, 2008).

In Norway the first attempts at farming cod were made in the late 1800s, where cod were cultivated and released mainly as eggs or yolk-sac larvae in the South of Norway. Farming of cod was first conducted in semi-extensive, enclosed ecosystems called ponds actually known as mesocosm. However, the pond method has limitations as it is based upon natural zooplankton as prey for the cod larvae. Therefore, only one production cycle is normally possible each year, and then during spring. In the 1990s, an intensive production method was developed, where everything is controlled, and by use of photoperiod often 4-6 production cycles are obtained each year. In these intensive systems, larvae are reared in tanks in specialized hatcheries, with green water and live feed (rotifers, later artemia) before they are weaned to a commercially available dry feed. Today the life cycle from egg to juvenile is closed, but problems with malformations and early maturation are still bottlenecks. Farmed cod mature at two years old, before harvest size and one of the possible reasons to this early maturation is the high energy diets.

2. EGG QUALITY AND THEIR DETERMINANTS

2.1. IMPORTANCE OF EGG QUALITY AND FECUNDITY

Egg quality may be a limiting factor for a successful mass production of viable fish fry (Kjørsvik *et al.,* 1990). Problems with egg quality can affect the larvae and lead to later production problems such as slow growth, high mortality and deformities. Poor egg quality will also affect the economy and profitability of production. However it should be noted that poor survival through weaning could also be due to factors relating to environmental and feeding conditions as well as poor egg quality.

Fecundity is a measure of the reproductive potential of a individual fish (Murua *et al.,* 2003), and can be measured as the total number of eggs spawned (total fecundity), or number of eggs spawned per kg fish (relative fecundity). Different species have different fecundities. Some produce many millions of eggs in one spawning whilst others produce only thousands, some spawn in multiple batches over two to three months while others produce only a single batch per year (Bromage & Cumarunatunga, 1988) or all at once, as pacific salmon. Fecundity is affected by factors that influence fish size such as the daily and seasonal rates of feeding (Springate & Bromage 1985; Bromage & Cumarunatunga 1988; Bromage *et al.*, 1992).

2.2. DETERMINANTS OF EGG QUALITY

To determine egg quality is very important for hatcheries since the identification of poor eggs, or batches of poor eggs at an early stage may save time and economical resources. Accurate methods for identification of poor eggs have been developed for hatcheries (Kjørsvik *et al.*, 1990; Fernández-Palacios *et al.,* 1995). The egg quality can be determined by several cues such as fertilization rate, physical and physiological properties, morphology, egg size, chemical content, egg abnormalities, and hatching rate (Kjørsvik *et al.,* 1990). To consider a factor as an indicator of quality implies a linkage with the capacity of the egg to produce viable offspring (Nissling *et al.,* 1998).

2.2.1. FERTILISATION

Fertilisation is a useful parameter to detect poor egg quality (Kjørsvik *et al.,* 1990), but does not always correlate with survival for some species (Kjørsvik *et al.,* 1990). In some species the fertilisation process will activate the cortical reaction, a process that is present in all teleost eggs, and in case of an incomplete process, may result in a smaller perivitelline space, and lack of increase in egg diametre (Kjørsvik *et al.,* 1990).

2.2.2. EGG PHYSICAL COMPOSITION

The hardness of the egg chorion correlates with the ability to sustain mechanical resistance, and good eggs have better chance than poor eggs. This hardening is due to an enzyme reaction during the activation process (Hagenmaier *et al.,* 1976). The egg shape and appearance seem to be a valid way to grade the egg batches in some species (Kjørsvik *et al.,* 1990).

2.2.3. MORPHOLOGY

Initial cell division after fertilization is another cue often used to discriminate between egg batches, since this will influence the development of the embryo, and morphological characters are generally sensitive parameters (Kjørsvik *et al.,* 1990). The symmetry of the early blastomeres seems to be a consistent early indicator of egg viability for some species such Dover sole (Dinis, 1982), red seabream (Sakai *et al.,* 1985), and cod (Kjørsvik and Lønning, 1983). Other morphological characters that are used as indicators is transparency and shape, where good quality eggs are generally described as transparent and perfectly spherical with distinct and symmetrical early stages (Kjørsvik *et al.,* 1990).

2.2.4. EGG SIZE

In general, larger eggs based on egg diameter tend to produce larger larvae, and larger larvae tend to have a higher survival than those hatched from smaller eggs (Bromage *et al.,* 1992). However, egg size is not generally accepted as an indicator of quality in all species, since the hypothesis bigger-is-better may not always hold true: egg size might be related to spawning time, place and broodstock length (Kjørsvik *et al.,* 1990).

2.2.5. MALFORMATION

Malformation or abnormalities is understood as a chromosomal error, which is thought to be lethal when this occurs before the gastrula stage (Longwell, 1977), since there exists a clear correlation between survival and cytogenetic status (Kjørsvik *et al.,* 1990). Chromosome abnormalities at the early embryonic stage seem to be one of the best indicators of sub-lethal damage to the embryo, as they give a very efficient measure of the quality of the egg batch.

2.2.6. CHEMICAL CONTENT

Good eggs should contain all the nutrients needed to sustain the growth and development of the embryo. Essential components differ from organism to organism (Kjørsvik *et al.,* 1990), and the possibility to find a general biochemical formula for fish eggs is questionable because each species have their own requirements.

2.2.7. HATCHING RATE

Hatching rate is perhaps is the most common indicator of egg quality due to the easily available methods. There are two methods used to evaluate hatching rate. In the first, a known number of eggs are incubated and the number of dead eggs removed and counted each day until hatching is complete. The second method uses single eggs cultured in microtitre well plates and the number that hatch recorded (Vallin & Nissling 1998; Panini *et al.,* 2001). The hatching rate is expressed as percentage of eggs hatched in a given batch. However, eggs that hatch in batches with lower hatching rate can be considered good eggs, but the batch is classified with lower overall quality.

2.2.8. BUOYANCY

The easiest and simplest "quality-control" is buoyancy of the eggs (Kjørsvik *et al.,* 1990), where floating eggs is an indicator of good quality. This parameter is questioned in some studies (Mangor-Jensen *et al.,* 1994). However in the past, negative buoyancy was related with poor quality eggs ((Kjørsvik and Lønning, 1983).

2.2.9. OTHER FACTORS THAT AFFECT EGG QUALITY

A good egg is the result of a combination of several factors, where parental factor such as age, spawning, parental stress or genetics may influence the egg quality. Other factors that may also have an influence are the environmental conditions (husbandry practices, temperature, light, water quality, diseases), spawning techniques (stripping) and the nutritional status of the broodstock. Nutritional status is generally considered to be the single most important factor affecting egg quality since unbalanced diet or food restriction generally reduces total fecundity and may delay maturation and decrease the proportion of maturing fish (Watanabe 1985; Kjørsvik *et al.,* 1990; Watanabe & Kiron 1995).

Stress can also induce females to retain the eggs in the lumen of the ovary too long, and as a consequence they may not spawn their eggs in the right moment, when freshness time between ovulation and fertilization and viability are optimal (McEvoy & McEvoy 1992).

3. COD EGGS COMPOSITION

Atlantic cod eggs are composed by several constituents that will be essential for all development of the Atlantic cod. The constitution of the eggs are described in the following table:

Table 2 Typical lipid and fatty acid composition of Atlantic cod eggs. (From Tocher and Sargent 1984). Source : SAWANBOONCHUN (2010)

Egg diameter (mm)	1.35
Moisture content (%)	74
Lipid content (% dry weight)	13.2
Polar lipid (% total lipid)	71.7
Neutral lipid (% total lipid)	28.3
Phosphatidylcholine (% total lipid)	45.6
Triacylglycerol (% total lipid)	12.5
Cholesterol (% total lipid)	6.1
Fatty acids in polar lipid (% total)	
Saturates	28.1
Monounsaturates	20.3
20:4(n-6)	1.9
20:5(n-3)	15.3
22:6 (n-3)	28.6
Fatty acids in triacylgycerols (% total)	
Saturates	21.3
Monounsaturates	41.5
20:4(n-6) 1.2	
20:5(n-3)	10.9
22:6 (n-3)	16.0

4. BROODSTOCK AND NUTRITION

Nutrition is one of the factors that affect the quality of eggs produced by farmed fish, and broodstock nutrition is important for production of a large number of viable fry (Izquierdo *et al.,* 2001). This is because females incorporate essential nutrients into the maturing oocyte for development of normal eggs. Studies in cod have shown fecundity to be affected by feed intake during gonadal growth (Kjesbu, 1991).

The main components of the cod broodstock diet are protein, lipids (including polyunsaturated fatty acids, PUFA), carbohydrates, vitamins, carotenoids and trace elements (Kjørsvik, 1990; Bromage, 1995). A positive relationship between feed quality and egg and larval viability and performance exists in many species (Watanabe, 1985; Kjørsvik *et al.,* 1990). In nature the abundance of food is related with lipid levels with the highest levels found in summer and lowest in winter (Bromage, 1995). During winter, stored lipid will be used to provide energy during periods of low feed supply and some will

be used for gamete production (eggs & sperm). Lipids are used in two ways during gonadal development, to provide energy and as a component of the yolk protein precursor vitellogenin during oocyte growth (Mazorra, 2000).

In general most marine species have limited ability to synthesise HUFA, such as DHA (Docosahexaenoic acid, 22:6n-3), EPA (Eicosapentaenoic acid, 20:5n-3) and ARA (Arachidonic acid, 20:4n-6) (Tocher *et al.,* 1992; Mourente & Tocher, 1993; Sargent *et al.,* 1994; Bromage, 1995; Bell, 1998; Cunnane, 2000) because they lack the enzymes to convert C18 fatty acids into C20 and C22 HUFA due to the absence or very low activity of Δ5-desaturase (Sargent *et al.,* 2002; Tocher, 2003). In cod the major lipid source is the liver, since the muscle contains less than 1% lipids (Kjesbu *et. al.,* 1991). The role of lipids as an energy source during egg and larval development is important because they provide metabolic energy. The eggs use the energy stored in the yolk as a nutrient source for embryo and larvae prior to exogenous feeding (Terner, 1979; Boulekbach, 1981). However, the lipids can be significant important for the development, but previous works argues that is not the single energy source, saying that larvae in the early stages use free amino acids as an energy source (Rønnestad *et al*., 2003, Finn *et al*.,2002).

Eggs must contain enough n-3 and n-6 HUFA (Highly unsaturated fatty acid(s)) in the form of both TAGs (Triacylglycerol) and phospholipids to allow normal development and hatching of the larvae. The level and ratio of the fatty acid composition changes as the embryo develops towards hatching (Bromage, 1995). These fatty acids are also required for normal cellular function and are important for neural tissue development, especially the brain and retina (Tocher & Harvie, 1988; Mourente *et al*., 1991).

5. STRESS IN FISH

Fishes live in an environment where they face constant challenges, from physical-chemical aspects of water, to conflicts with other animals within the school or population (Barton, 1988; Adams, 1990; Wedemeyer, 1996). The constant challenges and their resulting answers can be defined as stress, and understanding this concept in fish has increased immensely in the past few decades. Stress can be defined as nonspecific response of the body to any demand made upon it (Selye, 1973) or alternatively defined in more detail as a condition in which the body's dynamic equilibrium, or homeostasis, is threatened or disturbed as a result of the action of extrinsic stimuli called stressors (Wendelar-Bonga, 1997). The action of stressors is twofold: they produce effects that threaten or disrupt the homeostatic balance and also causes a set of behavioural and physiological responses such as compensatory action and/or adaptive response, enabling the animal to overcome the threats effect. The response to stress is an adaptive mechanism that allows the fish to cope with stressors in order to maintain its homeostatic state. The recovery time depends upon to the severity and duration of the initial stress and on habitat conditions (Billard *et al.,* 1981). Severe stressors may compromise response mechanisms and can become detrimental to the fish's health (Lima *et al.,* 2006).

Stress effects can be caused by a combination of several factors, such stock management, extreme environmental parameters not suitable for cultivation and others. Stress can result in a wide variety of physiological responses (Billard *et al.,* 1981; Barton et al., 1986; Schreck, 1990). The diversification of responses depends on a multitude of factors such as temperature, type of stressor, time of exposure, size and species. Direct comparison between studies is often futile since the cortisol levels in plasma range at least as much as two orders of magnitude among fishes following an identical stressor and can differ significantly between species (Olsen *et al.,* 1991; Bruce and Barton, 2002).

Reproductive physiology in fish is adversely affected by stress, causing changes in plasma cortisol level (Morgan *et al.,* 1999). This hormonal fluctuation may interfere with reproduction (Billard *et al.,* 1981), and result in abnormal larval development and possibly compromise related reproductive processes. Stress does not seem to block spawning completely in cod, but rather has negative effects resulting in irregular spawning cycles (Kjesbu *et al.,* 1990). Handling during maturation can possibly affect egg production negatively, as seen by massive atresia of vitellogenic oocytes or decrease of

ovofertilibility, and may also cause irregular spawning (Kjesbu *et al.,* 1990). Exposure to a stressor, such as drastic changes or aggression, is followed by an elevation of the levels of plasma corticosteroids and catecholamines in the primary stress response. This primary answer involves the activation of brain centres, resulting in massive release of catecholamines and corticosteroids, while the secondary answer is usually defined as the channelling of actions for target cells where the immediate effects of these hormones in blood and tissue levels include increase in heart beat rate and oxygen uptake, mobilisation of energy substrates and also disturbs osmoregulation. The tertiary response is manifested at the level of inhibition of growth, reproduction, immune response, and can be understood as the limitation of the animal's to tolerate subsequent or additional stressors (Lima *et al.,* 2006).

II. OBJECTIVES

As both diet and stress may affect spawning and egg quality, an experiment was performed to assess the effect of dietary lipid levels prior to and during the spawning season, and the effect of physical stress during the spawning season on spawning and egg quality.

The purpose of this study was to impose stress upon cod in the laboratory in an attempt to evaluate the potential stress effect combined with different dietary fat levels (low fat and high fat) on the reproductive physiology and spawning performance of cod and consequently on the egg quality. The egg quality in this work was evaluated based on egg diameter (Bromage *et al*, 1992), fertilisation rate (Kjørsvik *et al.,* 1990), abnormalities rate (Kjørsvik *et al.,* 1990; Thorsen *et al.,* 2003), and hatching rate (Sawanboonchunm, 2008).

III. MATERIALS AND METHODS

1. BROODSTOCK HUSBANDRY CONDITIONS

Figure 3 Picture of Institute of Marine Research, Austevoll Research station showing the facilities.

Atlantic cod were produced in an intensive production system at the Institute of Marine Research (www.imr.no) Austevoll Research Station (IMR, 60°05'N 5°15'E) (Fig.3) in 2008.They were grown in two sea cages located at IMR and fed with a commercial extruded dry diet (Amber Neptun, Skretting AS, Stavanger, Norway) until June 2009 when eighteen hundred fish were randomly transferred into 6 different 5x5x5m netpens (Preview period 2, Fig. 4). Three groups were fed a lean diet (13% fat, LF) and three groups fed a lipid rich diet (20% fat, HF) (Table 1). The fish received natural light conditions and ambient temperature and salinity.

Table 3 Analysed feed composition (g $100g^{-1}$) of lipid, protein, ash and dry matter, and estimated levels of gross energy (kJ g^{-1}), Gross energy calculated using energy contents of 18.0, 33.5 and 12.5 kJ g-1, for protein, fat and carbohydrate, respectively (Brett & Groves, 1979).

DIET	HF	LF
LIPID	19.7	13.2
PROTEIN	55.4	54.1
ASH	9.4	10.0
DRY MATTER	92.1	91.7
GROSS ENERGY		

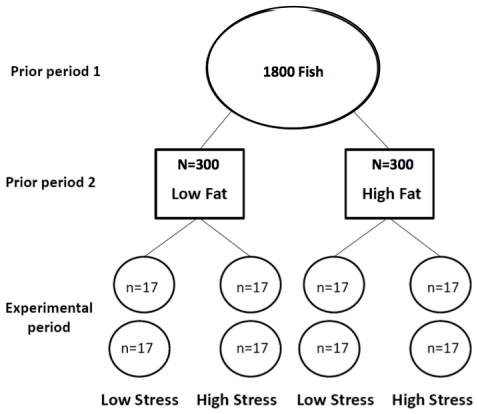

Figure 4 Schematic illustration of the experimental design. In prior period 1 the fish were kept in two 5x5x5 m netpens, and in prior period 2 in 6 netpens (5x5x5m) and fed a lipid rich or poor diet in triplicate netpens, while during the experiment the fish were kept in 3 m tanks. Each tank contained 12 females and 5 males.

In January 2010 (experimental period Fig. 4) 96 females and 40 males were randomly collected from each treatment, measured for weight and length, and females where Pit tagged. The fish were randomly distributed within each sex in 4 outdoor tanks (d=3 m, 7m^3) (Fig. 5) for each dietary treatment, given aerated seawater from 165 m depth with a mean temperature of 8.2±0.4°C and a salinity of 34.5±0.3). The tanks were covered with light reducing nets (Fig.5) and received a natural photoperiod. Each tank contained 12 females and 5 males with an average weight of 1286±263 g and 1132±196 g respectively for female and males in the LF group, and 1426±269 g and 1328±194 g respectively for female and males for the HF group.

Figure 5 Left picture shows the experimental tanks covered; right picture show inside tank

1.1. FEEDING

The fish were fed by hand with the experimental diet once a day to apparent satiation through the experimental period.

1.2. STRESS

Two tanks in each of the dietary groups were left undisturbed (Low Stress; LS) while the other 4 tanks were stressed (High Stress, HS). The fish were stressed by a combination of lowering the water level to 15 cm, combined with chasing the fish for 1 minute (Fig.6). The fish were stressed once a week; day of stress was randomized to prevent the fish from adapting to the stress treatment.

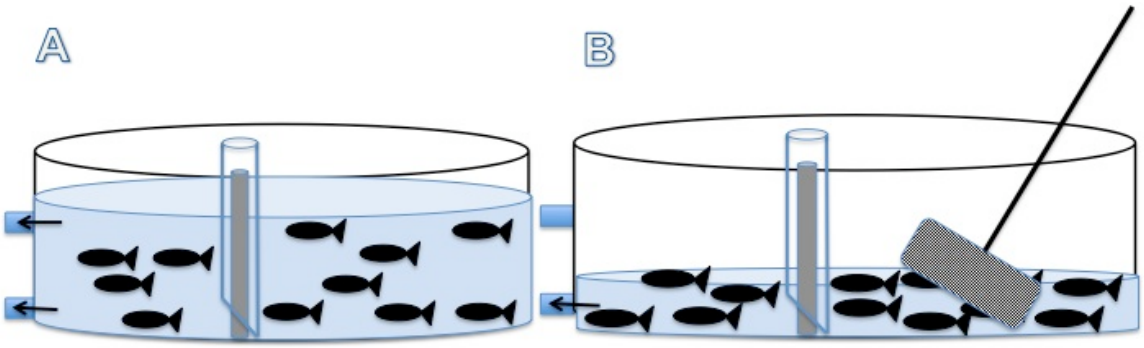

Figure 6 A: Normal harvesting tank ; B: Stress treatment

1.3. EGG COLLECTION

Duplicate tanks in each of the four treatments were supplied with egg collectors (Fig.7). These collected all spawned eggs by sieving the water from both the surface and the water drained through the outlet placed in the centre of the tank. The egg collectors were emptied each morning and the total spawned volume of eggs was measured. This was performed through the spawning season, i.e. from the first appearance of eggs in the egg collectors until all spawning had ceased (February-April).

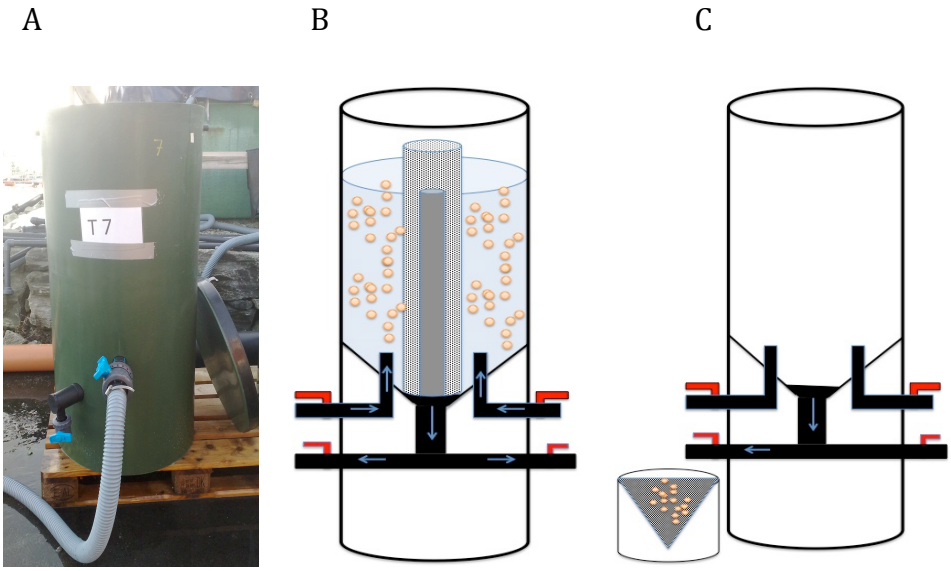

Figure 7 A: Photo of egg collector ;B: Schematic Egg collector full of water; C: Schematic of the cod eggs collection from the egg collector

2. MEASUREMENTS AND FECUNDITY

2.1. EGG PHOTOS

A sample of the eggs spawned in each tank was photographed to be able to measure their diameter and also to determine fertilisation and assess other quality measures (cf below). This was performed by transporting a small, randomized sample of the daily spawning collected in each tank to the lab. Prior to being photographed the eggs were maintained at 7 ºC for 2-4 hours. The photos were from a sub-sample of eggs following a technique improved for this work (Fig.8). The eggs were placed in an O-ring in the centre of a petri dish and digitally photographed under identical conditions each day using an Olympus DP70 camera mounted onto a Leica MS5 microscope. The photos were taken at a magnification of 0.63x. Each photo was saved for later determination of fertilization rate, egg diameter and abnormalities in cell cleavage and development.

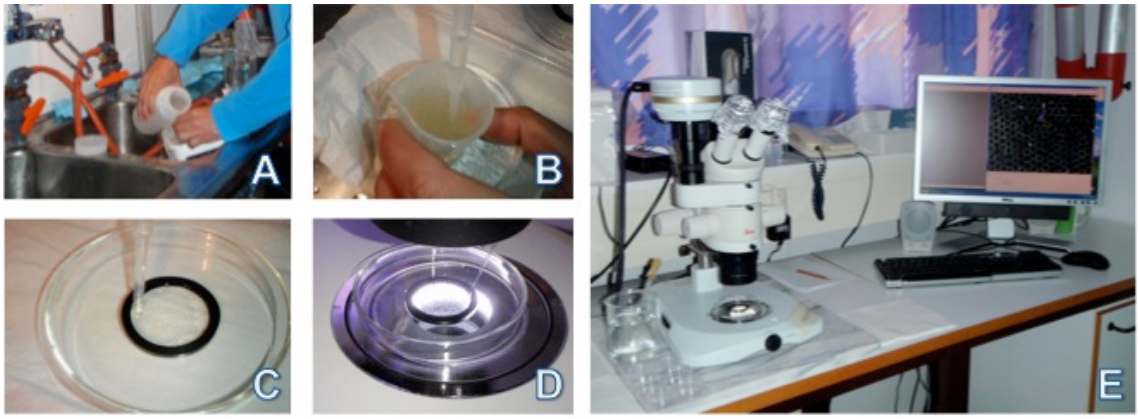

Figure 8 A:transferring eggs from the sub-sample bottle to a filter; B: Collect a sample of eggs with a pipete; C-:placing the in the center of the O-ring; D: O-ring filled with cod eggs; E: Daily Gear used to photograph the daily samples.

2.2. EGG CHARACTERIZATION

The diameters of about 120 eggs in each photograph were measured automatically using the ImageJ 64 software (Fig.9, A.B) (version 1.43, W. Rasband, National Institute of Health, USA) and the plugin ObjectJ (N. Visher & S. Nastase, University of Amsterdam, the Netherlands). The eggs were staged manually using the ObjectJ with 18 different categories (Fig. A): 1-unfertilized, 2-activated, 3-1 cell stage, 4-2 cell stage, 5-4 cell stage, 6-8 cell stage, 7-16 cell stage, 8-32 cell stage, 9-64 cell stage, 10-128 cell stage, 11- more than 128 cell, 12-gastrula, 13-malformations 2 cell to 32 cell, 14-malformations 64 cell to 128 cell, 15-big dead eggs, 16-dead unfertilized eggs, 17-unknown, 18-dead fertilized eggs (Appendix 8).

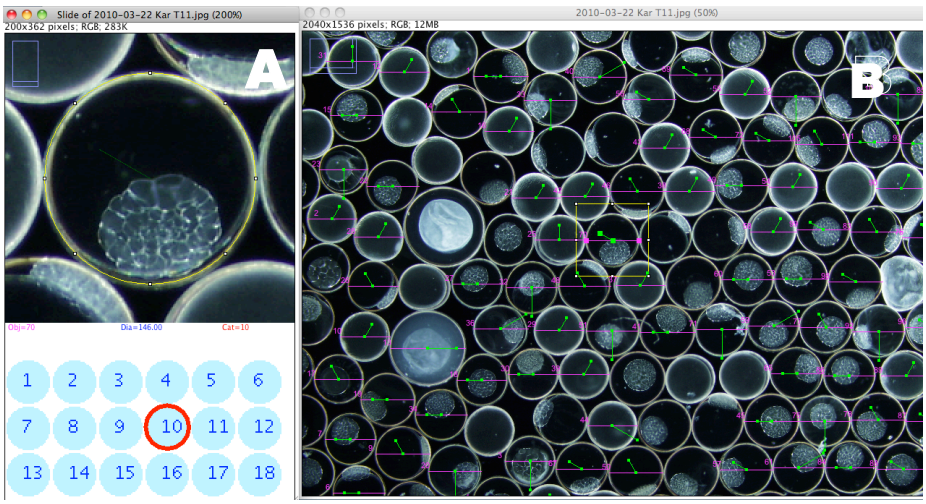

Figure 9 A: Image J menu with 18 categories to qualify the eggs; B: example of a daily egg photo

2.3. INCUBATION EXPERIMENT TO DETERMINE HATCHING RATE

Hatching rates were analysed 3 times during the experiment. This was done by placing one fertilized egg at two cell stage on a individually compartment in a total 24 well multidish tray (Nunc AS, Roskilde, Denmark) by using a sterile pipette. The water used in the trays was filtered and autoclaved (70% salinity). Two trays (48 eggs) were analysed from each tank at the three dates. The trays were maintained in a cold room at 7ºC and were monitored daily for developmental changes, and the numbers of hatched larvae were counted.

3. EXPERIMENTAL CALCULATION

3.1. FECUNDITY

Fecundity was calculated as the total volume of eggs spawned (ml) divided by female biomass (kg) at experimental start at the tank, and corrected for mortality.

Equation 1: $Fecundity\ (\%)$

$$Fecundity\ (\%) = \frac{Volume\ of\ eggs\ (l)}{Female\ biomass\ in\ tank\ (kg)} \times 100$$

3.2. EGG DIAMETER

Total egg diameter (n=41597), was measured automatically using the ObjectJ plugin on ImageJ, after having defined the range of maximum and minimum diameter (max Rad= 50 and min Rad=110 increased Rad=3) for the right magnification (0.63x) used on the photos and then inspected manually. Measurements were inspected and corrected manually. The egg diameter were analysed using the mean per day per treatment.

Equation 2: $Average\ Egg\ Diametre$

$$\boldsymbol{Average\ Egg\ Diametre} = \frac{\boldsymbol{\Sigma\ Egg\ diameter\ per\ treatment}}{\boldsymbol{\sum_{Daily\ total} eggs\ analysed\ per\ treatment}}$$

3.3. FERTILISATION

Fertilisation rate were calculated for 18 days by counting number of unfertilised eggs (cat 1, 2 and 16, Appendix 8) compared to number of fertilised eggs (cat 3-14 and 18, Appendix 8).

Equation 3: $Fertelisation\ rate$

$$Fertilisation\ rate = \frac{Number\ of\ eggs\ with\ cell\ division}{Total\ number\ of\ eggs\ \ in\ a\ sample} \times 100$$

3.4. ABNORMALITY

The parameter abnormality is defined as the inclusion of foreign object(s) or abnormal shape of the cell during cell division in the fertilised eggs. The malformation rates were calculated at 18 occasions of the spawning period, covering all weeks, by counting number of abnormal eggs (cat 13, 14, Appendix 8).

Equation 4: $Malformation\ rate$

$$Malformation\ rate = \frac{Number\ of\ eggs\ with\ malformations}{Total\ number\ of\ eggs\ \ in\ a\ sample} \times 100$$

3.5. HATCHING RATE

The number of hatched larvae was estimated using a duplicate sub-sample of fertilised eggs from the egg collectors.

Equation 5 $Hatching\ rate$

$$Hatching\ rate = \frac{Number\ of\ eggs\ hatched}{Number\ of\ egg\ with\ cell\ division} \times 100$$

Note: Three persons performed independently the egg staging.

4. STATISTICAL ANALYSIS

All calculations were performed with Microsoft Excel for Mac (version 12.0, USA). Calculation and comparison by mean were performed by Excel pivot sheet. Further statistical comparisons were done by using Statistica (version 9.1, Statsoft Inc., Tulsa, OK, USA) with significance values at $P<0.05$ (Zar, 1999). Tanks were used as experimental unit. Differences in mean weight, length and condition factor between treatments were compared using a two-way ANOVA with stress and diet as factors. Differences in egg diameter, fertilization and malformation of the eggs sampled daily was analyzed using a three-way ANOVA with date, stress and diet as factors. Finally the differences in hatching rate were compared using a two-way ANOVA.

IV. RESULTS

1) GROWTH OF FEMALES

The females in all four treatments lost weight during the experimental period (Table 4). Females in the LS LF group lost on average 182 g to a final weight of 1099 g, while the LS HF group lost on average 184 g to 1152 g (Table 4). These differences in weight loss were also seen for the HS group. There were no significant differences in weight loss between the dietary treatments during the experiment (ANOVA, P=0.99), although the final weight was significantly (ANOVA, P=0.03) higher in the HF group (1254 and 1152 g, respectively for HS and LS groups) compared to the LF group (1044 and 1099 g, respectively for HS and LS groups). The weight loss for the HS group were 226 and 224 g, respectively for LF and HF diet, compared to the LS group that was 182 and 184 g, respectively for the LF and HF diet. The HS fish had a higher final weight (1254 g) than the LS fish (1152 g) in the HF group, however this difference was not statistically significant (two-way ANOVA, P=0.26). The liver weight was significantly higher in the HF group compared to the LF group for both stress groups (ANOVA, P=0.00; Table 4).

Table 4 Body weight (mean±SD) of Atlantic cod fed either a low fat (LF) or a high fat (HF) diet and kept undisturbed (low stress) or randomly stressed by handling (high stress) at start (13. January 2010) and body and liver weight in the end (5. May 2010) of the experiment.

	LF group		HF group		*ANOVA*		
	High stress	Low stress	High stress	Low stress	Diet	Stress	D*S
Number surviving	18	15	19	14	-	-	-
Weight start, (g)	1270±273	1282±271	1477±313	1336±199	0.06	0.34	0.26
Weight end, (g)	1044±193	1099±249	1254±304	1152±147	0.03	0.69	0.19
Weight loss,(g)	226	182	224	184	0.99	0.46	0.97
Weight loss, (%)	18	14	15	14	0.89	0.39	0.72
Liver weight end, (g)	77±27	77±33	124±51	112±25	0.00	0.50	0.49
Liver index end, (%)	7.2±1.9	7.0±2.4	9.7±2.1	9.7±1.9	**0.00**	0.85	0.79

2) FECUNDITY

Table 5 Spawning period and relative fecundity, measured as total amount of eggs spawned during the spawning season for cod fed either a low fat (LF) or a high fat (HF) diet, and exposed to low handling stress (LS) or high handling stress (HS).

TREATMENT	SPAWNING PERIOD			TOTAL FECUNDITY	MAX FECUNDITY	
	DATE START	*DATE END*	DAYS (#)	(L EGG/KG FISH)	WEEK NR	ML/KG FISH/WEEK
LF – LS	12.02.10	8.04.10	55	0.86±0.34	9	23.4
LF – HS	12.02.10	25.04.10	71	0.64±0.12	10	15.9
HF – LS	16.02.10	11.04.10	54	0.83±0.16	10	24.4
HF – HS	16.02.10	18.04.10	62	0.86±0.24	13	19.8

The spawning season started on 12 February and the last eggs were collected on 25 April (Table 5). While spawning commenced in February, spawning frequency per week increased during February and March in all treatments fed with HF diet. Females in the LF diet treatment increased the spawning frequency up until April, with all treatments showing a decrease in egg production afterwards (Fig.10). However, there was considerable variation between tanks for the same treatment.

In the HS/HF group, onset of spawning was delayed by 4 days. The spawning period lasted for 71 days in the HS/LF group, with a relative fecundity of 0.64±0.12 L egg/kg fish compared to 62 days and a relative fecundity of 0.86±0.24 L egg/kg fish in the HS/HF group. Both LS groups finished spawning after 54-55 days, with approximately the same relative fecundity as the HS/HF group (Table 5). The HS groups had a more irregular spawning frequency and volume of eggs spawned per week compared to the LS groups. The groups that were not stressed had a more distinct peak spawning in the mid-season (Fig.10). These groups had the highest production of eggs in week 9-10 (early March) with approximately similar weekly egg production at 23-24 ml egg/kg fish/week (Table 5). Fecundity in the HS/LF group had several peaks, reaching the highest in week 10 (16 ml egg/kg fish/week) while the HS/HF group reached a maximum peak by week 13 (20 ml egg/kg fish/week) (Table 5).

Some differences were observed between the tanks in the same treatment. The fishes in the LS/LF group in Tank 4 reached a higher maximum fecundity at 28 ml egg/Kg per week at week 9 compared to a maximum fecundity of 19 ml egg/Kg per week in week 8 and 9 in Tank 18 (Fig.10a). The HS/LF groups in tanks 19 and 20 showed irregular spawning where maximum fecundity was 16.4 ml egg/kg fish/week reached during week 11 in tank 19 and 16.6 ml egg/kg fish/week during week 10 in tank 20. Spawning in tank 20 ended two weeks before that in tank 19 (Fig.10b). Fish in the HS/HF reached high fecundity at two different periods; however, it was more pronounced in tank 11 than in tank 12. Maximum fecundity occurred later in the season compared to the LS groups, with 24 ml egg/Kg per week by week 13 in tank 12 and 16 ml egg/kg per week by week 13 in tank 12 (Fig.10d). The LS/HF groups in tanks 13 and 14 had a more distinct peak in spawning during mid-season, reaching a maximum fecundity of 19 ml egg/kg per week by week 9 in tank 13 and 32 ml egg/Kg per week by week 10 in tank 14 (Fig.10c).

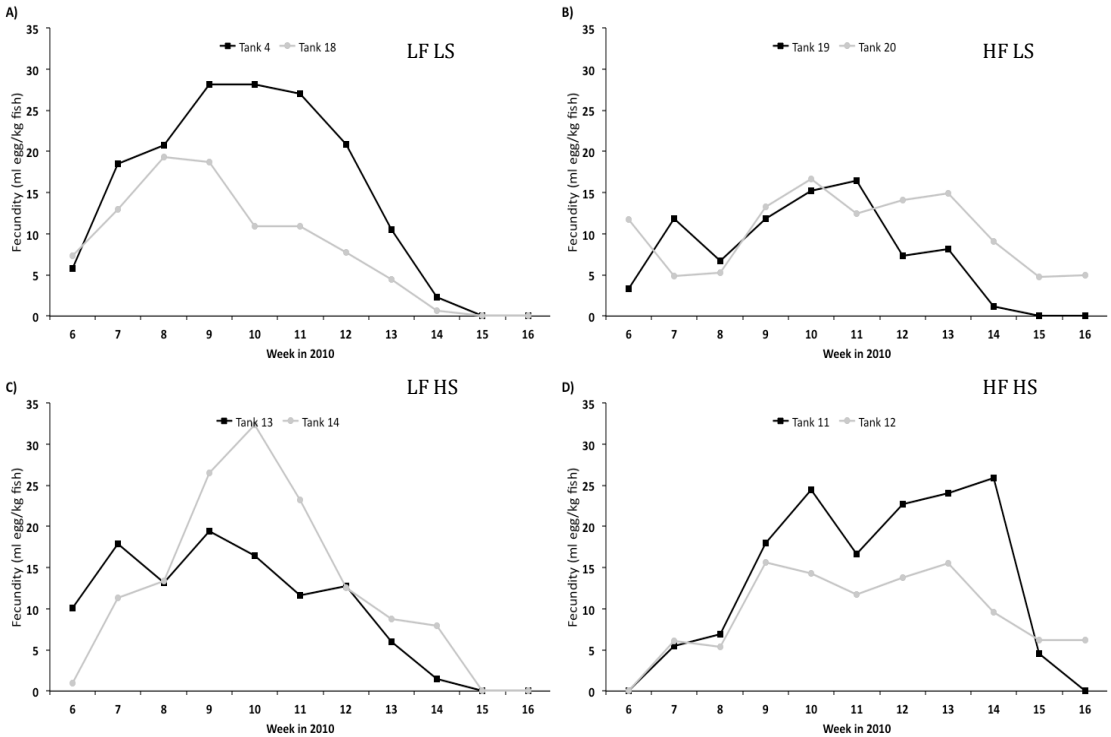

Figure 10 Average fecundity per week in Atlantic cod with different diet and handling stress; A) LF diet and low stress; B) HF diet and low stress; C) LF diet and high stress and D) HF diet and high stress.

Cumulative fecundity varied between the duplicate tanks within the dietary and stress treatments. In the LF/LS treatment rate of egg production was stable during the first ten days. After that the rate changed. On the fourth week, cumulative egg production stabilized until the end of the spawning season. The LF/HS treatment showed a similar increase from the beginning until the middle of the fifth week, after that a different cumulative fecundity was observed in each tank until the end of the season. The HF/LS treatment, varied between tanks during the first ten days, after that both tanks had the same fecundity. From the third week cumulative egg production flattened out until the end of the season. The HF/HS treatment showed in the first two weeks a similar fecundity, after that, the cumulative fecundity showed to be different until the end. In general, cumulative fecundity showed that the LS groups had a higher increase in egg production in the period from the beginning of March to the end of March, while the HS groups had a low egg production, (Fig 12).

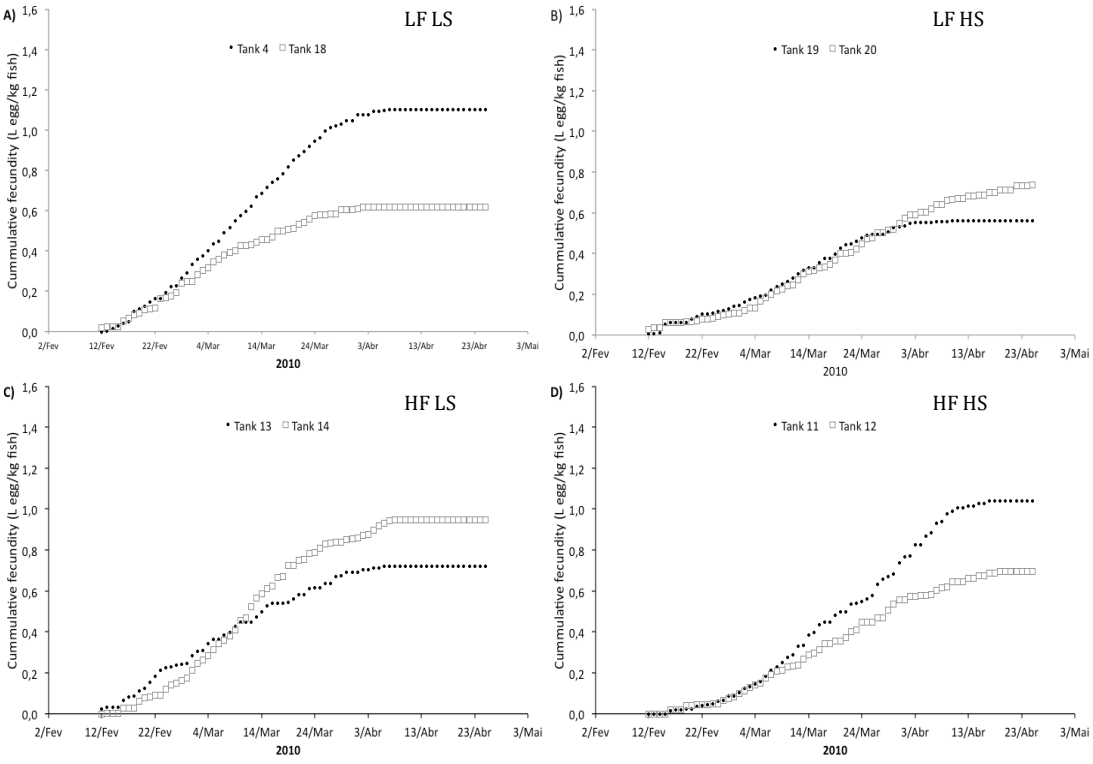

Figure 11 Cumulative fecundity per week in Atlantic cod (Gadus morhua) with different diet and handling stress, A) LF diet and Low Stress; B) LF diet and High Stress; C) HF diet and Low Stress and D) HF diet and High Stress.

3) EGG DIAMETER

In total, diameters of 42.255 eggs were measured from the experiment. All treatments showed a decrease in egg diameter over the spawning season (Fig. 12). Although the differences in egg size were small, there was a significant effect of the treatments due to the high number of eggs analysed. The egg diameter from of the spawning season to mid March ranged between 1.15 to 1.30 mm, with significantly larger eggs from fish fed the HF diet (1.25±0.09) compared to the smaller eggs from fish fed the LF diet (1.23±0.06) (ANOVA, P=0.00). In addition, there was also an effect of stress, with larger eggs in the fish exposed to high stress (ANOVA, P=0.00).

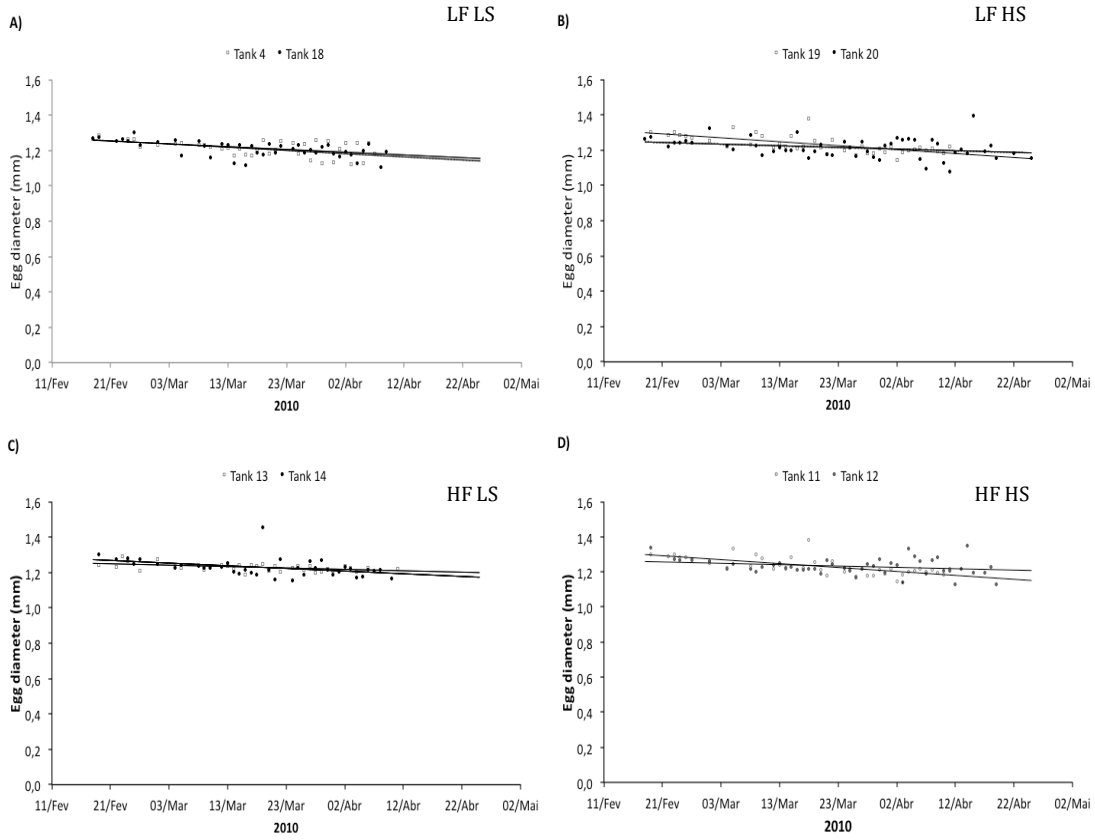

Figure 12 Average of Egg diameter per week Atlantic cod (*Gadus morhua*) with different diet and handling stress, A) LF diet and low stress; B) LF diet and high stress; C) HF diet and low stress and D) HF diet and high stress.

4) FERTILISATION

Significant differences in fertilisation between stress and diet treatments were observed through the season (P<0.05). The LS/LF, HS/LF and LS/HF groups had the highest fertilisation rate late in the spawning season (week 13 and 14), while the HS/HF group had the lowest fertilisation rate late in the season (Fig.13a). The LS/HF treatment showed a gradual increase in fertilisation rate, with an initial fertilisation rate of 74% (week 7 to 9) and maximum fertilisation rate at 84% by the end of the spawning season (Fig.13a). In the HS/HF group, fertilisation rate decreased through the season, from 75% to 55% by the end of spawning, when fecundity was also was lowest (Fig.13a). The HF groups were significantly different at mid (week 10 to 12) and late season, with 80 and 84% in the LS/HF group and 67 and 55% in the HS/HF group, respectively (Fig.13a). Both LF groups had a slight increase in fertilisation rate at the end of spawning (week 13 and 14) compared to the mid-spawning season (Fig.13a). Fertilisation rate for the tanks stressed the day of stress, was not significantly different from the fertilisation the next two days (Fig.13b).

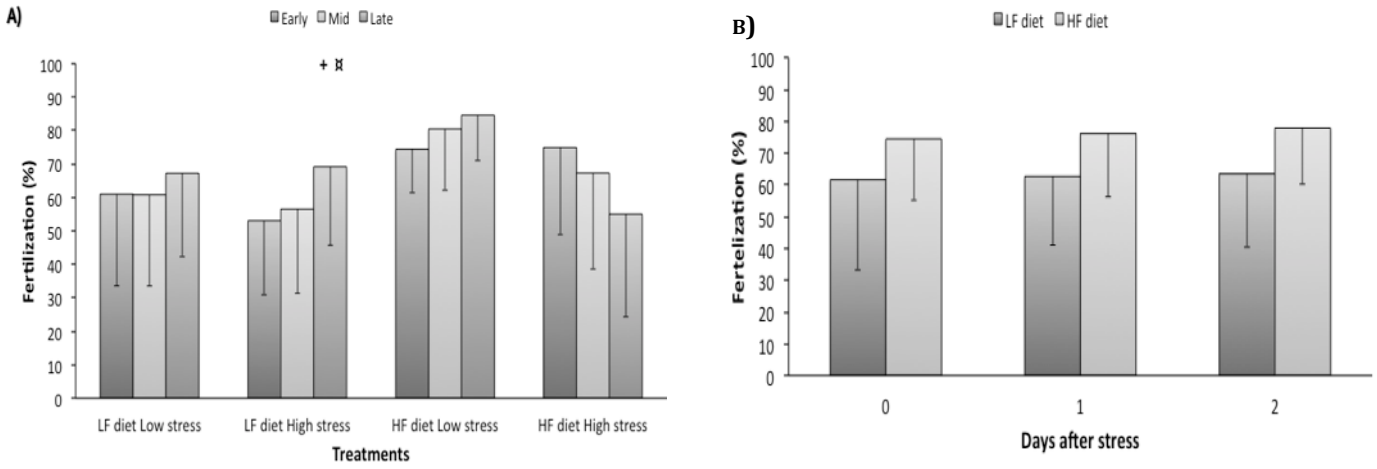

Figure 13 Fertilization rate A) Average ± SD of each treatment long the season B) after 0,1 and 2 days after stress . Value significantly different (P<0.05) with respect to period in the spawning season (*), LF/HF diet (+), LS/HS stress (¤) or interaction of these as determined by three-way ANOVA13.

5) ABNORMALITY

All treatments had approximately the same rate of malformation in the early season (10%). The LS/LF group had the highest tendency to develop malformed cells division, with 10% malformations early in the season followed by an increase to approximately 15% malformations at mid and late spawning season (Fig.14a). In the HS/LF group malformation percentage decreased from 10% to 5% through the spawning season (Fig.14a). The HF treatments showed decreasing malformation rate along the season, showing approximately 10% malformations for both the stressed and unstressed groups. The variation in malformation rate was too high to reveal any significant differences at the end of the season (Fig.14a,14b). There was, however a tendency of increased embryonic malformation rate after stress exposure in both dietary groups, where HF tended to have more malformations than LF.

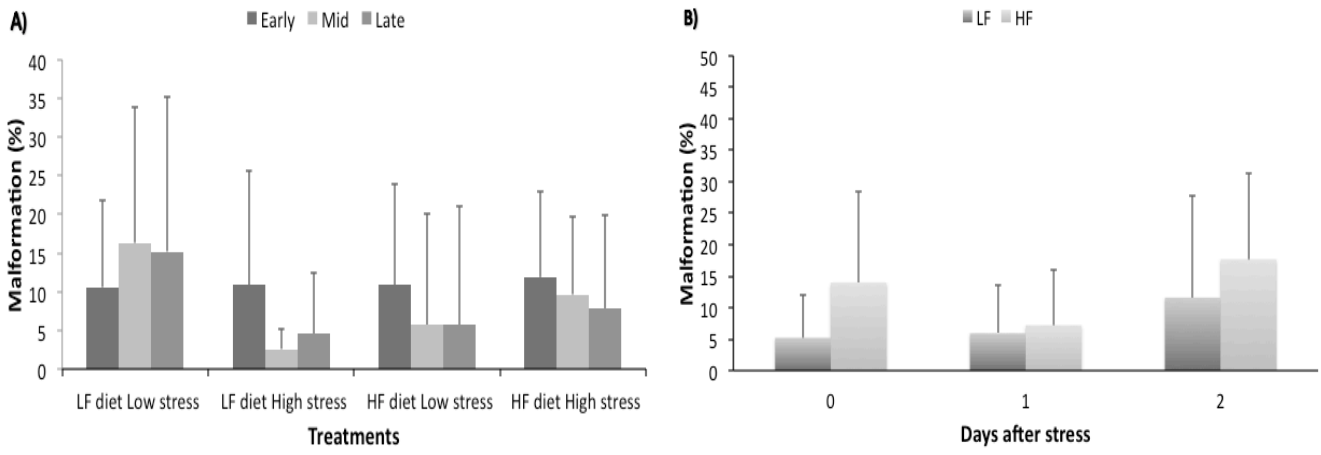

Figure 14 Abnormality rate A) each treatment long the season; B) after 0, 1 and 2 days after stress

6) HATCHING RATE

The HF group showed no significant difference in the hatching rate between the different stress treatments (Fig.15). However, when compared to the HS/LF group, results indicated that the HF diet had a significant positive effect on hatching rate ($P<0.05$) as determined by Duncan post hoc test. The LF groups showed significant differences in hatching rate between the different stress regimes ($P<0.05$) as determined by Duncan post hoc test. The LS/LF group had the highest hatching rate of all treatments, with 80% of fertilized eggs hatching. The lowest hatching rate, 50% was observed in the HS/ LF group (Fig.15).

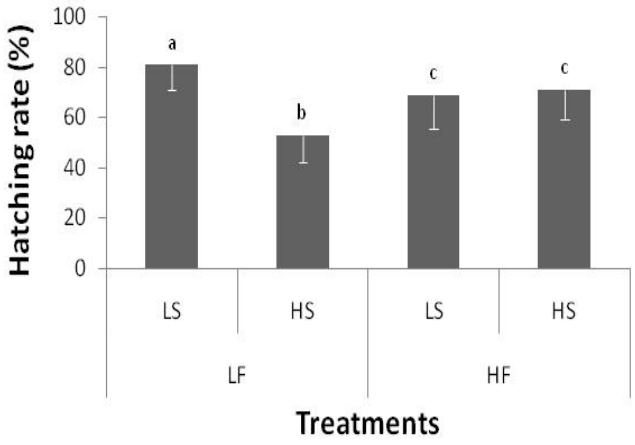

Figure 15 Average hatching rate in sampled eggs from Atlantic cod either fed a low fat (LF) or a high fat (HF) diet and with low handling stress (LS) or high handling stress (HS) in mid spawning season. Bars not sharing common letters are significantly different ($P<0.05$) as determined by Duncan post hoc two-way ANOVA.

V. DISCUSSION

Body weight

Female Atlantic cod lost weight during the spawning season. This was due to a combination of reduced appetite, metabolism and spawning. The cod in our experiment was offered feed to apparent satiation, however, the appetite was very low, as observed in other experiments (Kjesbu *et al.,*1991; Fordham & Trippel, 1999;Skjæraasen *et al.,* 2004). Energy used during the spawning season is therefore mainly drawn from stored reserves. Starved cod mobilise energy firstly from liver lipids, and then muscle proteins (Black and Love,1986). Former studies on cod have reported a reduction in liver size (Krivobok and Tokareva,1973; Karlsen *et al*.,1995; Hansen *et al*.,2001) during the spawning season while an increase in liver size for both genders has been reported during the later stages of gonadal growth (Jangaard *et al.,* 1967; Krivobok and Tokareva, 1973; Karlsen *et al.,* 1995). The relation between spawning and liver weight is still not fully understood. Karlsen *et al.* (1995) observed that males reduce their liver weights prior to spawning, while the liver size in females did not change in this period. However a greater reduction in liver size was observed in females than in males during spawning. The weight loss in the current study can be mainly related to spawning, as female Atlantic cod is a multiple batch spawner that can produce up to 10 batches of eggs at regular intervals during a period of 50-60 days (Kjesbu, 1989;).

The final liver index was lower in the LF group than in the HF group, this difference is explained directly by the different energy content of the diets offered. Assuming that vitellogenesis is a continuous process during the spawning season, females with a higher energetic fat content will have a better support for accomplishment of the spawning season. Kjesbu *et al.(*1991),(1996) reported that cod in poor condition increased the transport of protein from white muscle via the liver to the high number of developing oocytes causing further weight loss in these individuals. However, in the present study the fish were in good condition even after the spawning season, and had only a minor weight loss ranging between 14 to 18 %, with the largest weight loss in the HS/LF group. Overall, weight loss observed in the present study was low compared to what was reported by Karlsen *et al.,* 1995, where the fish were not fed during the spawning season. In the present study, weight loss appeared greater in the HS groups, where it may be assumed that the individuals used more energy for homoeostasis and consequently these groups had a lower egg production compared with LS groups (Table

4). This was particularly true for the HS/LF group. According to Olsen *et al*.,(2008) fish that suffer food deprivation are less resistant to stress than fed cod. Previous works described that Atlantic cod subject to exhaustive exercise elicited the typical stress response including release of cortisol from the interenal gland into plasma (Hemre *et al.,* 1991) and a concomitant release of liver glycogen causing plasma hyperglycaemia (Barton and Iwama, 1991), and like that females with the same stress treatment will have different performance for different diets. This suggests that female body condition is important to minimize negative stress effects on the reproductive system.

Fecundity

In the present study, total fecundity was lowest in cod fed the LF diet and exposed to handling stress. Previous works have related low fecundity to poor body condition or feed deprivation (Lambert and Dutil, 2000; Olsen *et al.,* 2008), while in the present study fish were fed two different diets containing unequal lipid levels. Although none of the individuals tested were in poor condition, fish fed with the LF diet had smaller liver sizes than the HF group. As the liver stores energy to be used for oocyte growth (Kjesbu *et al.,* 1991, Karlsen *et al.* 1995; Dahle *et al.,*2003) the smaller liver size in the LF group could affect accumulation of nutrients into the growing oocytes. Kjesbu *et al.,* (1991) also documented that cod with high condition factors produced more previtellogenic oocytes and used a larger fraction during vitellogenesis. Consequently, fish condition or size of energy stores may affect the reproductive investment. In our study, fecundity was not higher in the HF groups than in the unstressed LF group, which indicates good initial body condition in both dietary groups. The lower fecundity in the LF group exposed to stress can be related to the stress making fish use energy for movement and vital physiological functions (Olsen *et al.,* 2008) rather than oocyte growth (Schreck, 2010). Thus, fish with excess energy, as seen by large liver size, seemed to handle stress better and produce the same amount of eggs as unstressed fish. Another consequence of stress on cod reproduction, that has also been reported by Kjesbu *et al.,* (1990) is the prolonged spawning period with irregular spawning cycles. This prolonged spawning did not increase the total fecundity, as stress reduced the relative fecundity per day during the season. This resulted in a different total fecundity in stressed fish fed different diets, where fishes fed LF had a lower total fecundity (0.64±0.12 L/Kg fish) compared to the HF group (0.86±0.24 L/Kg fish).

Egg quality

In this study egg quality, or egg viability, was measured by several parameters. Egg diameter decreased throughout the season in all treatments. This has previously been seen in gadoid species such as haddock (*Melanogrammus aeglefinus*) (Trippel *et al.,* 1999) and Atlantic cod (Kjesbu, 1988). This decreasing egg size was related to the number of batches shed. However, Kjesbu *et al.,* (1991) observed that eggs sizes from an individual actually increased slightly in the beginning of the spawning period, before decreasing again. Individual observations like this were not possible during our study, as several females spawned in the same tank.

Assuming that larger eggs have better viability (Bromage *et al*., 1992) egg quality would be expected to decrease throughout the season, if we consider only the diameter as the principal characteristic, however, the egg quality concept is a complex concept as already mentioned before and this was not seen in the present study. The fertilization rate had a tendency to increase with decreasing egg diameter in 3 of the 4 treatments, whilst only eggs from HS/HF group showed decreasing fertilization rates through the spawning period. The overall effect was that the LS/HF group had the highest fertilization rate, while the HS/LF group had the lowest. That is, females with larger energy reserves performed slightly better.

Blastomere morphology was used to check abnormalities at the early egg stages. This analysis has already been tested by other researchers in other species, like turbot, *Scopthalmus maximus* (McEvoy, 1984); grey wolffish, *Anarchichas lupus* (Pavlov et al.,1992,1994) and Atlantic halibut ,*Hippoglossus hipoglossus* (Bromage et al., 1994), where some correlation was found between blastomere morphology, egg viability and late larval success. In the present study a large variation was seen in first cell division symmetry between treatments. No significant differences were found between treatments for the malformation rate. This large variation in embryonic malformation rate does not seem to be related to any specific treatment. However, we can find a slightly lower rate in the HF group compared with the LF group. The big variation in malformation can be due collection of eggs from several females in each tank, and not from individual fish. Each female may answer differently to stress, and some of the variation could be explained by the fact that eggs from more than one batch were included in the analyses.

Cod reproduction includes a fairly complex courtship behaviour (Brawn, 1961B), and stress has been shown to alter other forms of behaviour (Paszowski & Olla,1985; Sigismondi & Weber,1988; Olla *et al.,* 1992). The consequence of this change of behaviour might result in a variation in egg fertilisation. This variation cannot only be justified by mating problems (not compatible males or incomplete courtship sequence). However the fertilisation rate showed tendencies to increase as the season progressed in 3 of the 4 treatments, and this increase could be explained at least partly by a mating adaptation.

The fertilisation rate in this study seemed to be related to diet, where the HF group tended to have better rates than the LF group for both stress treatments. In a previous study by Morgan *et al.(*1999) eggs appeared indistinguishable, whether produced by stressed or undisturbed spawners. However in this study the combination Diet x Stress had significant effects, where the HF group had a higher stress tolerance than the LF group, and both dietary groups showed different tendencies along the season. As previously demonstrated by other workers, stress alters other forms of behaviour (Paszowski & Olla,1985; Sigismondi & Weber,1988; Olla *et al.,* 1992), which could result in different fertilisation rates, where fish with a better initial energy supply may have better fertilisation rate.

Hatching rate was the last parameter tested to measure egg quality in the present study. Hatching rates showed no significant differences between diets. However, significant differences between different handlings were present in the LF groups, where LS appeared to have higher hatching rates compared to HS. Yet, this effect was not seen in eggs from females fed with HF diet. The difference in effect on hatching rate may suggest that well-nourished females produce eggs of good quality even when stressed. Kjørsvik *et al.*(2003) found a significant positive correlation between egg quality and hatching rate in turbot (*Scophthalmus maximus L.).* As the hatching rate calculated from fertilized eggs was not directly correlated to the actual calculated fertilization rates, it is difficult to compare these two. However, the overall effect of poor performance of eggs from the HS/LF group was accounted for both in fertilization and in hatching rates. Morgan *et al*. (1999) suggested that low hatching success in eggs from stressed cod can be due to a combination of the decreased success of first time spawners (Trippel, 1998) and the turbulence of the egg collection method. In the present study, 3 of 4 treatments presented the similar results, so the hypothesis presented by

Morgan *et al.(*1999) is not completely valid for our experiment. Another variable that can have importance for the variation in hatching rate is that the eggs used were from the latter part of the spawning season, when eggs were smaller. As already mentioned, larger eggs may result in better larval viability, as they will contain more yolk, however the last batches have eggs with smaller diameter, so they will have less yolk and consequently a lower hatching rate.

VI. CONCLUSIONS

The current study was aimed to assess the effects of different dietary fat content and different stress levels on egg quality in Atlantic cod. The following conclusions were made:

- There was a tendency towards a positive correlation between high dietary fat level, fecundity and fertilisation rate in female Atlantic cod held in a low stress environment.
- Stress effects appeared to impair reproductive performance, since stressed fish had a tendency delayed, desynchronized and prolonged spawning period irrespective of diet.

Some recommendations can be made for future studies on stress effect in Atlantic cod:

- Future stress studies may focus on individual stress responses. DNA microarray analysis may reveal specific genes involved in the stress response.
- In an identical replicate of the current experiment, to set one more tank for each treatment and, and add a third group of fat content between the two fat diets tested.
- Hatching rate may be studied differently, by for example, trying to minimize mechanical shock, and scratching to avoid any cut in the chorion.
-

VII. REFERENCES

ABREHOUCH, A., IDAOMAR,M., ALI,A.A., NHHALA,H., TALBAOUI,E.M. ***BROODSTOCK FEEDING EFFECTS ON SPAWNING PERFORMANCES' (FERTILITY, EGGS AND LARVAE QUALITY) OF THE MEDITERRANEAN RED PORGY (PAGRUS PAGRUS, LINNAEUS 1758) DURING TWO YEARS***. AFRICAN JOURNAL OF FOOD SCIENCE, 2009. 3 (8): P. 193-200.

ADAMS, D.S., KELLER.R., KOEHL, M.A., ***THE MECHANICS OF NOTOCHORD ELONGATION, STRAIGHTENING AND STIFFENING IN THE EMBRYO OF XENOPUS LAEVIS.*** DEVELOPMENT, 1990. 110(1): P. 115-130.

ALMEIDA,F.F.L.,TARANGER,G.L.,NORBERG,B.,KARLSEN,Ø.,BOGERD,J.,SCHULZ,R.W., ***PHOTOPERIOD-MODULATED TESTIS MATURATION IN ATLANTIC COD (GADUS MORHUA, L.)***. BIOL. REPROD, 2009. 80(4): P. 631-640.

ALONSO-FERNÁNDEZ,A., VALLEJOB,A.C., SABORIDO-REYA, F., MURUA, H., TRIPPEL, E.A. ***FECUNDITY ESTIMATION OF ATLANTIC COD (Gadus morhua) AND HADDOCK (Melanogrammus aeglefinus) OF GEORGES BANK: APPLICATION OF THE AUTODIAMETRIC METHOD.*** FISHERIES RESEARCH, 2009. 99(1): P. 47-54.

BARKER, G.A., SMITH, S. N., BROMAGE, N. R., ***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, 1989. 12(4): P. 281-293.

BARKER, G.A., SMITH,S.N., BROMAGE, N. R., ***COMMENSAL BACTERIA AND THEIR POSSIBLE RELATIONSHIP TO THE MORTALITY OF INCUBATING SALMONID EGGS.*** JOURNAL OF FISH DISEASES, 1991. 14(2): P. 199-210.

BARTON, B.A., ***A DIVERSITY OF RESPONSES WITH PARTICULAR REFERENCE TO CHANGES IN CIRCULATING CORTICOSTEROIDS***. INTEGRATIVE AND COMPARATIVE BIOLOGY, 2002. 42: P. 517-525.

BARTON, B.A., IWAMA, G.K., **PHYSIOLOGICAL CHANGES IN ¢SH FROM STRESS IN AQUACULTURE WITH EMPHASIS ON THE RESPONSES AND EFFECTS OF CORTICOSTEROIDS**. ANNUAL REVIEW OF FISH DISEASES.1991, 1: P. 3-26.

BARTON, B.A., RAHN, A.B., FEIST,G., BOLLIG,H., SCHRECK, C.B., ***PHYSIOLOGICAL STRESS RESPONSES OF THE FRESHWATER CHONDROSTEAN PADDLEFISH (Polyodon spathula) TO ACUTE PHYSICAL DISTURBANCES.*** COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY 1988. 120(A): P. 355–363.

BARTON, B.A., SCHRECK, C.B., SIGISMONDI, L.A., ***MULTIPLE ACUTE DISTURBANCES EVOKE CUMULATIVE PHYSIOLOGICAL STRESS RESPONSES IN JUVENILE CHINOOK SALMON.*** TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY, 1986. 115: P. 245–251.

BARTON, B.A., ***STRESS IN FISHES: A DIVERSITY OF RESPONSES WITH PARTICULAR REFERENCE TO CHANGES IN CIRCULATING CORTICOSTEROIDS*** INTEGRATIVE AND COMPARATIVE BIOLOGY, 2002. 42(3): P. 517-525.

BEGG, M.A., ***ESSENTIAL RELATIONSHIPS INCORPORATING THE INFLUENCE OF AGE, SIZE AND CONDITION ON VARIABLES REQUIRED FOR ESTIMATION OF REPRODUCTIVE POTENTIAL IN ATLANTIC COD GADUS MORHUA***. MARINE ECOLOGY PROGRESS SERIES, 2002. 235: P. 235-256.

BELL, J. G., TOCHER, D.R., MACDONALD,F.M., SARGENT,J.R., ***DIETS RICH IN EICOSAPENTAENOIC ACID AND Γ-LINOLENIC ACID AFFECT PHOSPHOLIPID FATTY ACID COMPOSITION AND PRODUCTION OF PROSTAGLANDINS E1, E2 AND E3 IN TURBOT (Scophthalmus maximus), A SPECIES DEFICIENT IN Δ5 FATTY ACID DESATURASE***. PROSTAGLANDINS, LEUKOTRIENES AND ESSENTIAL FATTY ACIDS, 195. 53(4): P. 279-286.

BELL, J.G., ***CURRENT ASPECTS OF LIPID NUTRITION IN FISH FARMING***. IN *BIOLOGY OF FARMED FISH*, A.P. KENNETH BLACK EDITOR 1998, BLACKWELL: SHEFFIELD. P. 114-145.

BELL, J.G.S., J. R., ***ARACHIDONIC ACID IN AQUACULTURE FEEDS: CURRENT STATUS AND FUTURE***

OPPORTUNITIES,. AQUACULTURE, 2003. 218(1-4): P. 491-499.

BERLINSKY, D.L., ***WHOLE-BODY CORTICOSTEROID AND PLASMA CORTISOL CONCENTRATIONS IN LARVAL AND JUVENILE ATLANTIC COD GADUS MORHUA L. FOLLOWING ACUTE STRESS***. AQUACULTURE RESEARCH 2006. 37(13): P. 1282–1289.

BIGELOW, H. B., SCHROEDER, W. C., ED. BRUCE **FISHES OF GULF OF MAINE**, ED. BRUCE COLLETTE AND GRACE KLEIN-MZCPHEE; 2002. EDN, THIRD EDITION. SMITHSONIAN INSTITUTION PRESS.

BILLARD, R.B., GILLET, C., ***STRESS, ENVIRONMENT AND REPRODUCTION IN TELEOST FISH*, IN *STRESS AND FISH***, A.D. PICKERING, EDITOR 1981, ACAD. PRESS: LONDON. P. 185-208.

BISWAS, A., SEOKA, M., INAGAKI, H., TAKII, K., ***REPRODUCTION, GROWTH AND STRESS RESPONSE IN ADULT RED SEA BREAM, PAGRUS MAJOR (TEMMINCK & SCHLEGEL) EXPOSED TO DIFFERENT PHOTOPERIODS AT SPAWNING SEASON***. AQUACULTURE RESEARCH, 2010. 41(4): P. 519-527.

BLACK, D., LOVE, R.M., **THE SEQUENTIAL MOBILISATION AND RESTORATION OF ENERGY RESERVES IN TISSUES OF ATLANTIC COD DURING STARVATION AND REFEEDING**. JOURNAL OF COMPARATIVE PHYSIOLOGY, PART B, 1986.156: P. 469–479.

Bonga ,W, S. E. (1997). The stress response in fish. Physiological Reviews, 1997. 77(3):p. 591-625.

BOULEKBACHE, A., ***ENERGY METABOLISM IN FISH DEVELOPMENT***. AMERICAN ZOOLOGIST, 1981. 21(2): P. 377-389.

BRAWN, V.M., ***REPRODUCTIVE BEHAVIOUR OF THE COD (GADUS CALLARIAS L.).*** BEHAVIOUR, 1961. 18(3): P. 177–198.

BROMAGE, N., BRUCE, M., BASAVARAJA, N., RANA, K., SHIELDS, R., YOUNG, C., DYE, J., SMITH, P., GILLESPIE, M., GAMBLE, J., ***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, 1994. 25(1): P. 13-21.

BROMAGE, N., CUMARUNATUNGA, R., ***EGG PRODUCTION IN THE RAINBOW TROUT***, IN *RECENT ADVANCES IN AQUACULTURE*, (J.F.MUIRE & R.J.ROBERTS) EDN, CROOM HELM/TIMEBER PRESS, LONDON AND SYDNEY/PORTLAND,OREGON., 1988.P. 63-138.

BROMAGE, N., JONES, J., RANDALL, C., THRUSH, M., DAVIES, B., SPRINGATE, J., DUSTON, J., BARKER, G., ***BROODSTOCK MANAGEMENT, FECUNDITY, EGG QUALITY AND THE TIMING OF EGG PRODUCTION IN THE RAINBOW TROUT (ONCORHYNCHUS MYKISS)***. AQUACULTURE, 1992. 100(1-3): P. 141-166.

BROMAGE, N.R., ***BROODSTOCK MANAGEMENT AND SEED QUALITY: GENERAL CONSIDERATIONS*, IN *BROODSTOCK MANAGEMENT AND EGG AND LARVAL QUALITY***, N. R. BROMAGE & R. J. ROBERTS, EDS., 1995.BLACKWELL SCIENCE, LONDON.

BROMAGE, N.R., JONES, J., RANDALL, C., THRUSH, M., SPRINGATE, J., DUSTON, J., AND BARKER, G., ***BROODSTOCK MANAGEMENT, FECUNDITY, EGG QUALITY AND THE TIMING OF EGG PRODUCTION IN THE RAINBOW TROUT (Oncorhynchus mykiss)***. AQUACULTURE, 1992. 100(1-3): P. 141–166.

BROWN, J.A., MINKOFF, G., PUVANENDRAN, V., ***LARVICULTURE OF ATLANTIC COD (Gadus morhua): PROGRESS, PROTOCOLS AND PROBLEMS***. AQUACULTURE 2003.227: P. 357-372.

BUTTS, I.A., LITVAK,M.K., TRIPPEL, E.A. , ***SEASONAL VARIATIONS IN SEMINAL PLASMA AND SPERM CHARACTERISTICS OF WILD-CAUGHT AND CULTIVATED ATLANTIC COD, Gadus morhua***. THERIOGENOLOGY, 2010. 73(7): P. 873-885.

CARDINALE, M., MODIN,J., ***CHANGES IN SIZE-AT-MATURITY OF BALTIC COD (Gadus morhua) DURING A PERIOD OF LARGE VARIATIONS IN STOCK SIZE AND ENVIRONMENTAL CONDITIONS***. FISHERIES

RESEARCH, 1999. 41(3): P. 285-295.

CARRILLO, M., BROMAGE, N., ZANUY, S., SERRANO, R., PRAT, F., ***THE EFFECT OF MODIFICATIONS IN PHOTOPERIOD ON SPAWNING TIME, OVARIAN DEVELOPMENT AND EGG QUALITY IN THE SEA BASS (Dicentrarchus labrax L.).*** AQUACULTURE, 1989. 81(3-4): P. 351-365.

COHEN,D.M., INADA,T., IWAMOTO,T. , SCIALABBA,N., ***GADIFORM FISHES OF THE WORLD (ORDER GADIFORMES). AN ANNOTATED AND ILLUSTRATED CATALOGUE OF CODS, HAKES, GRENADIERS AND OTHER GADIFORM FISHES KNOWN TO DATE***, IN *FAO FISHERIES SYNOPSIS* FAO, EDITOR 1990: ROME. P. 442.

CUNNANE, S.C., ***THE CONDITIONAL NATURE OF THE DIETARY NEED FOR POLYUNSATURATES: A PROPOSAL TO RECLASSIFY 'ESSENTIAL FATTY ACIDS' AS 'CONDITIONALLY-INDISPENSABLE' OR CONDITIONALLY-DISPENSABLE' FATTY ACIDS.*** BRITISH JOURNAL OF NUTRITION, 2000. 84(6): P. 803-812.

DAHLE, R, TARANGER, G.L, KARLSEN, Ø, KJESBU, O.S, NORBERG, B., **"GONADAL DEVELOPMENT AND ASSOCIATED CHANGES IN LIVER SIZE AND SEXUAL STEROIDS DURING THE REPRODUCTIVE CYCLE OF CAPTIVE MALE AND FEMALE ATLANTIC COD (GADUS MORHUA L.)"** COMP. BIOCHEM. PHYSIOL. 2003,A 136: P. 641-653

DAVIE, A.P., MARK J.R., BROMAGE, N.R., MIGAUD, H., ***THE ROLE OF SEASONALLY ALTERING PHOTOPERIOD IN REGULATING PHYSIOLOGY IN ATLANTIC COD (GADUS MORHUA)PART I. SEXUAL MATURATION.*** CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 2007. 64: P. 84-97.

DINIS, M.T., **METHODS OF INCUBATION DOVER SOLE (SOLEA SOLEA L.) EGGS**. RELATORIOS DE ACTIVIDADES DO AQUARIO VASCO DA GAMA, 1982. NO. 12.

EDWARD A. TRIPPEL,E.A. , NEIL,S.R., ***MATERNAL AND SEASONAL DIFFERENCES IN EGG SIZES AND SPAWNING ACTIVITY OF NORTHWEST ATLANTIC HADDOCK (MELANOGRAMMUS AEGLEFINUS) IN RELATION TO BODY SIZE AND CONDITION.*** CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 2004. 61(11): P. 2097-2110.

FAHAY, M.P., BERRIEN, D., JOHNSON, W. M., ***ATLANTIC COD (Gadus morhua) LIFE HISTORY AND HABITAT CHARACTERISTICS***. NATIONAL OCEAN AND ATMOSPHERIC ADMINISTRATION TECHNICAL MEMORANDUM, 1999: P. 122-152.

FERNÁNDEZ-PALACIOS, H., IZQUIERDO, M. S., ROBAINA, L., VALENCIA, A., SALHI, M., VERGARA, J. M. , ***EFFECT OF N-3 HUFA LEVEL IN BROODSTOCK DIETS ON EGG QUALITY OF GILTHEAD SEA BREAM (SPARUS-AURATA L).*** AQUACULTURE, 1995. 132 (3-4): P. 325-337.

FINN, R.N., RØNNESTAD, I., VAN DER MEEREN, T., FYHN, H.J**., FUEL AND METABOLIC SCALING DURING THE EARLY LIFE STAGES OF ATLANTIC COD GADUS MORHUA.** MAR. ECOL. PROG. SER., 2002. 243, 217-234.

FORDHAM, S.E., TRIPPEL, E.A., ***FEEDING BEHAVIOUR OF COD (Gadus morhua) IN RELATION TO SPAWNING.*** JOURNAL OF APPLIED ICHTHYOLOGY, 1999. 15: P. 1-9.

FULTON, T.W., ***ON THE GROWTH AND MATURATION OF THE OVARIAN EGGS OF THE TELEOSTEAN FISHES.*** ANN. REP.FISH.BOARD SCOTL., 1898. 16: P. 88-124.

GALLOWAY, T. F., KJØRSVIK, E., KRYVI,H., ***EFFECT OF TEMPERATURE ON VIABILITY AND AXIAL MUSCLE DEVELOPMENT IN EMBRYOS AND YOLK SAC LARVAE OF THE NORTHEAST ARCTIC COD (GADUS MORHUA)***. MARINE BIOLOGY, 1998. 132(4): P. 559-567.

GIMENEZ, G., ESTEVEZ, A., F. LAHNSTEINER, B. ZECEVIC, J.G. BELL, R.J. HENDERSON, J.A. PINERA, J.A. SANCHEZ-PRADO, ***EGG QUALITY CRITERIA IN COMMON DENTEX (Dentex dentex)***. AQUACULTURE,

2006. 260(1-4): P. 232-243.

GRIER, H.J., ***CELLULAR ORGANIZATION OF THE TESTIS AND SPERMATOGENESIS IN FISHES.*** AMERICAN ZOOLOGY, 1981. 21: P. 345-357.

HAGENMAIER.H.E.,SCHMITZ ,I., FOEHLES ,J.,*HOPPE SEYLER'S* Z. PHYSIOL.CHEM., 1976. 35: P. 1435-143.

HALL T.E., SMITH,P., JOHNSTON I.A., ***STAGES OF EMBRYONIC DEVELOPMENT IN THE ATLANTIC COD GADUS MORHUA***. MORPHOLOGY 2004. 259**:** P. 255-270.

HALL, T.E., JOHNSTON, I., ***TEMPERATURE AND DEVELOPMENTAL PLASTICITY DURING EMBRYOGENESIS IN THE ATLANTIC COD GADUS MORHUA L.***. MARINE BIOLOGY, 2003. 142(5): P. 833-840.

HANSEN, G.M, OLAFSEN, J.A., ***BACTERIAL COLONISATION OF COD GADUS MORHUA AND HALIBUT (HIPPOHLOSSIS HIPPOGLOSSUS) EGGS IN MARINE AQUACULTURE***.APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 1989. 55: P. 1435-1446.

HANSEN, T., KARLSEN, Ø., TARANGER, G.L., HEMRE, G.I., HOLM, J.C., KJESBU, O.S., **GROWTH, GONADAL DEVELOPMENT AND SPAWNING TIME OF ATLANTIC COD (GADUS MORHUA) REARED UNDER DIFFERENT PHOTOPERIODS.** AQUACULTURE, 2001. 203: P. 51–67.

HANSEN,J.O.,BERGE,G.M.,HILLESTAD,M.,KROGDAHL,A., GALLOWAY,T.F., HOLM,H., HOLM,J., RUYTER,B., ***APPARENT DIGESTION AND APPARENT RETENTION OF LIPID AND FATTY ACIDS IN ATLANTIC COD (GADUS MORHUA) FED INCREASING DIETARY LIPID LEVELS***. AQUACULTURE, 2009. 284(1-4): P. 159-166.

HARDY, R.W., ***SALMONID BROODSTOCK NUTRITION***, IN *INTERNATIONAL SYMPOSIUMON SALMONID REPRODUCTION*, R. IWAMOTO, SOWER, S., EDITOR 1983, WASHINGTON SEA GRANT PROGRAM. UNIV. OF WASHINGTON SEATTLE (USA: BELLEVUE, WASHINGTON (USA). P. 98–108.

HEMRE, G.-I., G. LAMBERSEN, AND Ø. LIE. 1991. ***THE EFFECT OF DIETARY CARBOHYDRATE ON THE STRESS RESPONSE IN COD (Gadus morhua)****. AQUACULTURE,1991. 95(3-4): P. 319-328.*

HOWELL, B.R., BAYNES,S.M., OLSEN,Y., ***A BIOTIC FACTORS, IN CULTURE OF COLD- WATER MARINE FISH*****,** IN *CULTURE OF COLD-WATER MARINE FISH*, B.P. LTD, EDITOR 2004. P. 7-26.

ICES, ***ARCTIC FISHERIES WORKING GROUP REPORT*****, IN** ***ANNUAL SCIENCE CONFERENCE*** 2007: HELSINKI.

IZQUIERDO, M. S., FERNANDEZ-PALACIOS, H., TACON, A. G. J., **EFFECT OF BROODSTOCK NUTRITION ON REPRODUCTIVE PERFORMANCE OF FISH**, AQUACULTURE, 2001. VOL. 197, NO. 1-4, P. 25-42.

JANGAARD, P.M., BROCKERHOFF, H., BURGHER, R.D., HOYLE, R.J., **SEASONAL CHANGES IN GENERAL CONDITION AND LIPID CONTENT OF COD FROM INSHORE WATERS.** JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA. 1967. 24: P. 607–612.

JASTREBSKI,C.J.,MORBEY,Y.E.,***EGG SIZE VARIATION IN LAKE TROUT: PHENOTYPE- HABITAT CORRELATIONS SHOW AN EFFECT OF REARING ENVIRONMENT***. TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY, 2009. 138(6): P. 1342-1351.

JESPERSEN, J.A., ***MICRO- AND MACROSCOPIC CHARACTERISTICS TO STAGE GONADAL MATURATION OF FEMALE BALTIC COD***. JOURNAL OF FISH BIOLOGY, 2003: P.1-24.

JOBLING, M., PEDERSEN,T., ***CULTIVATION OF THE ATLANTIC COD***, IN *PRODUCTION OF AQUATIC ANIMALS*, C.E.N. A.J.NOVOTNY, EDITOR 1995, ELSEVIER: AMSTERDAM. P. 347-357.

JØRGENSEN, T., ***LONG-TERM CHANGES IN AGE AT SEXUAL MATURITY OF NORTHEAST ARCTIC COD (GADUS MORHUA L.)***. CONSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER, 1990. 46(3): P. 235-248.

KARLSEN, Ø., HOLM, J.C., KJESBU, O.S., ***EFFECTS OF PERIODIC STARVATION ON THE REPRODUCTIVE***

INVESTMENT IN FIRST TIME SPAWNING ATLANTIC COD. AQUACULTURE, 1995. 133: P. 159–170.

KAWAUCHI, H., S.K., ITOH, H., SWANSON, P., NAITOL, N., NAGAHAMA, Y., NOZAKI, M, NAKAI Y. , ITOH S., ***THE DUALITY OF TELEOST GONADOTROPINS***. FISH PHYSIOLOGY BIOCHEMISTRY, 1989. P.729-38.

KJESBU, O.S., W.P., SOLEMDAL P, GREER WALKER M ***OVULATORY RHYTHM AND A METHOD TO DETERMINE THE STAGE OF SPAWNING IN ATLANTIC COD (GADUS MORHUA L.).*** CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 1990. 47: P. 1185–1193.

KJESBU, O.S., ***A SIMPLE METHOD FOR DETERMINING THE MATURITY STAGES OF NORTHEAST ARCTIC COD (GADUS MORHUA L.) BY IN VITRO EXAMINATION OF OOCYTES***. SARSIA, 1991. 75(4): P. 335–338.

KJESBU, O.S., ***ASPECTS OF THE REPRODUCTION IN COD (GADUS MORHUA L.): OOGENESIS, FECUNDITY,SPAWNING IN CAPTIVITY AND STAGE OF SPAWNING***, IN *ZOOLOGICAL LABORATORY,* 1988, UNIVERSITY OF BERGEN: BERGEN.

KJESBU, O.S., KLUNGSØYR, J., KRYVI, H., WITTHAMES, P.R., AND GREER WALKER, M., ***FECUNDITY, ATRESIA, AND EGG SIZE OF CAPTIVE ATLANTIC COD (GADUS MORHUA) IN RELATION TO PROXIMATE BODY COMPOSITION***. CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 1991. 48(12): P. 2333–2343.

KJESBU, O.S., SOLEMDAL, P., BRATLAND, P., FONN, M. ***VARIATION IN ANNUAL EGG PRODUCTION IN INDIVIDUAL CAPTIVE ATLANTIC COD (GADUS MORHUA).*** CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 1996. **53**: P. 610-620.

KJESBU, O.S., ***THE SPAWNING ACTIVITY OF COD, Gadus-Morhua L***. JOURNAL OF FISH BIOLOGY, 1989. 34(2): P. 195-206.

KJØRSVIK, E. , HOEHNE-REITAN ,K., REITAN,K.I., **EGG AND LARVAL QUALITY CRITERIA AS PREDICTIVE MEASURES FOR JUVENILE PRODUCTION IN TURBOT (*Scophthalmus maximus L.*)**, AQUACULTURE 227 (2003), P. 9–20.

KJØRSVIK, E., ***EGG QUALITY IN WILD AND BROODSTOCK COD GADUS MORHUA L.***JOURNAL OF THE WORLD AQUACULTURE SOCIETY, 1994. 25: P. 22-29.

KJØRSVIK, E., LØNNING, S., **EFFECTS OF EGG QUALITY ON NORMAL FERTILIZATION AND EARLY DEVELOPMENT OF THE COD, GADUS MORHUA** L.. . JOURNAL OF FISH BIOLOGY, 1983. 23: P. 1-12.

KJØRSVIK, E., MANGOR-JENSEN, A., & HOLMEFJORD, I., ***EGG QUALITY IN FISHES***. ADVANCES IN MARINE BIOLOGY, 1990. 26: P. 71-113.

KJØRSVIK, E., ***THE EFFECT OF DIFFERENT INCUBATION CONDITIONS ON THE EGGS OF HALIBUT, HIPPOGLOSSUS HIPPOGLOUSS L.*** FISH BIOLOGY 1990. 37: P. 655-657.

KORTNER, T.M., ROCHA,E., ARUKWE A., ***ANDROGENIC MODULATION OF EARLY GROWTH OF ATLANTIC COD (GADUS MORHUA L.) PREVITELLOGENIC OOCYTES AND ZONA RADIATA-RELATED GENES***. J TOXICOL ENVIRON HEALTH 2009. 72(3-4): P. 184-195.

KORTNER, T.M., ROCHA,E., ARUKWE, A., ***PREVITELLOGENIC OOCYTE GROWTH AND TRANSCRIPTIONAL CHANGES OF STEROIDOGENIC ENZYME GENES IN IMMATURE FEMALE ATLANTIC COD (GADUS MORHUA L.) AFTER EXPOSURE TO THE ANDROGENS 11-KETOTESTOSTERONE AND TESTOSTERONE***. *COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY PART A: MOLECULAR & INTEGRATIVE PHYSIOLOGY* 2009. 152(3): P. 304-313.

KORTNERA, T.M., ROCHA,E.,, ARUKWEA,A., ***PREVITELLOGENIC OOCYTE GROWTH AND TRANSCRIPTIONAL CHANGES OF STEROIDOGENIC ENZYME GENES IN IMMATURE FEMALE ATLANTIC COD (GADUS MORHUA L.) AFTER EXPOSURE TO THE ANDROGENS 11- KETOTESTOSTERONE AND TESTOSTERONE***.

COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY - PART A: MOLECULAR & INTEGRATIVE PHYSIOLOGY, 2009. 152(3): P. 304-313.

KRIVOBOK, M.N., TOKAREVA, G.I., **DYNAMICS OF WEIGHT VARIATIONS OF THE BODY AND INDIVIDUAL ORGANS OF BALTIC COD DURING THE MATURATION OF GONADS**. TRANS. SER. JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA, 1973. 2722, P. 21.

LAGLER, K.F., BARDACH,J.E.,MILLER,R.R., PASSINO,D.R.M. ***ICHTHYOLOGY***. 2ND EDITION ED, 1977: WILEY. 528.

LAMBERT, Y., DUTIL, J.D., ***ENERGETIC CONSEQUENCES OF REPRODUCTION IN ATLANTIC COD (GADUS MORHUA) IN RELATION TO SPAWNING LEVEL OF SOMATIC ENERGY RESERVES***. CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 2000. 57 (4): P. 815-825.

LAPTIKHOVSKY,V., BRICKLE,P., ***ASPECTS OF EMBRYONIC DEVELOPMENT IN TWO SOUTHWEST ATLANTIC GADIFORM FISH: TADPOLE CODLING, SALILOTA AUSTRALIS (MORIDAE), AND SOUTHERN BLUE WHITING, MICROMESISTIUS AUSTRALIS GADIDAE.*** ACTA ICHTHYOLOGICA ET PISCATORIA, 2009. 39(2): P. 127–130.

LIE, LIED, E. AND LAMBERTSEN, G., ***FEED OPTIMIZATION IN ATLANTIC COD (GADUS MORHUA): FAT VERSUS PROTEIN CONTENT IN THE FEED***. AQUACULTURE, 1988. 69: P. 333-341.

LIE,Ø., LIE, LAMBERTSEN,G., ***LIPID DIGESTION AND ABSORPTION IN COD (Gadus morhua), COMPARING TRIACYLGLYCEROLS, WAX ESTERS AND DIACYLALKYLGLYCEROLS.*** COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY PART A: PHYSIOLOGY, 1991. 98(1): P. 159-163.

LIMA L.C., RIBEIRO,L.P., MALISON, J.A., BARRY, T.P., HELD, J.A., ***EFFECTS OF TEMPERATURE ON PERFORMANCE CHARACTERISTICS AND THE CORTISOL STRESS RESPONSE OF SURUBIM PSEUDOPLATYSTOMA SP***. WORLD AQUACULT SOC., 2006. 37: P. 89-95.

LIMA, L.C., R. C. LEITE, D. C. MELO, ***STRESS IN FISHES***. REV BRAS REPROD ANIM, 2006. 30(3-4): P. 113-117.

LONGWELL, A.C., **A GENETIC LOOK AT FISH EGGS AND OIL**. 1977. OCEANUS 20: P. 46-58.

MAELAND, A., RONNESTAD, I., & WAAGBO, R, ***FOLATE IN EGGS AND DEVELOPING LARVAE OF ATLANTIC HALIBUT, HIPPOGLOSSUS HIPPOGLOSSUS L***. AQUACULTURE NUTRITION, 2003. 9(3): P. 185-188.

MAGNADOTTIR,B.,LANGE,S.,STEINARSSON, A., GUDMUNDSDOTTIR,S.,,***THE ONTOGENIC DEVELOPMENT OF INNATE IMMUNE PARAMETERS OF COD (GADUS MORHUA L.).*** COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY, 2004. 139(2): P. 217-224.

MANGOR-JENSEN, A., HOLM, J. C., ROSENLUND, G., LIE, O. , SANDNES, K. ***EFFECTS OF DIETARY VITAMIN C ON MATURATION AND EGG QUALITY OF COD GADUS MORHUA L.*** (1994) *JOURNAL OF THE WORLD AQUACULTURE SOCIETY,* 25: P. 30-40.

MANGOR-JENSEN, A., HOLM, J.C., ROSENLUND,G., LIE, Ø. ,LIE, SANDNES,K., ***EFFECTS OF DIETARY VITAMIN C ON MATURATION AND EGG QUALITY OF COD GADUS MORHUA L.*** JOURNAL OF THE WORLD AQUACULTURE SOCIETY, 1994. 25: P. 30-40.

MARTE A. KJAER, A.V., GERD M. BERGE, TRINA F. GALLOWAY, MARIE HILLESTAD, ASHILD KROGDAHL, HALVOR HOLM, BENTE RUYTER, ***CHARACTERISATION OF LIPID TRANSPORT IN ATLANTIC COD (GADUS MORHUA) WHEN FASTED AND FED HIGH OR LOW FAT DIETS***. AQUACULTURE, 2009. 288(3-4): P. 325-336.

MASUI,Y.,CLARKE,H.J., MAMMALIAN **OOCYTE MATURATION**. IN: G.H. BOURNE AND J.F. DANIELI (ED.)INTERNATIONAL REVIEW OF CYTOLOGY,

MAZORRA DE QUERO, C., ***THE MANAGEMENT OF BROODSTOCK ATLANTIC HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS) AND THE INFLUENCE OF NUTRITION, HOLDING CONDITIONS AND HORMONAL MANIPULATION OF SPAWNING ON GAMETE QUALITY***, 2000, UNIVERSITY OF STIRLING: STIRLING.

MCEVOY, L.A., MCEVOY, J., ***MULTIPLE SPAWNING IN SEVERAL COMMERCIAL FISH SPECIES AND ITS CONSEQUENCES FOR FISHERIES MANAGEMENT, CULTIVATION AND EXPERIMENTATION.*** FISH BIOLOGY, (Suppl. B), 1992. 41: 125-136.

MCEVOY, L.A., ***OVULATORY RHYTHMS AND OVER-RIPENING OF EGGS IN CULTIVATED TURBOT, SCOPHTHALMUS MAXIMUS L.*** JOURNAL OF FISH BIOLOGY 1984. 24: P. 437-448.

MEAGER, J.J., SKAERAASEN,J.E., FERNO, A., KARLSEN, Ø., LØKKEBORG, S., MICHALSEN, K., UTSKOT, S.O.,***VERTICAL DYNAMICS AND REPRODUCTIVE BEHAVIOUR OF FARMED AND WILD COD GADUS MORHUA***. MAR ECOL PROG SER, 2009. 389: P. 233-243.

MEIYU XUA, H.V., ***CLONING, TISSUE DISTRIBUTION AND EFFECTS OF FOOD DEPRIVATION ON PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP)/PACAP- RELATED PEPTIDE (PRP) AND PREPROSOMATOSTATIN 1 (PPSS 1) IN ATLANTIC COD (Gadus morhua).*** PEPTIDES, 2009. 30(4): P. 766-776.

MORGAN, M.J., WILSON, C. E., AND CRIM, L. W, ***THE EFFECT OF STRESS ON REPRODUCTION IN ATLANTIC COD***. JOURNAL OF FISH BIOLOGY, 1999. 54: P. 477-488.

MOURENTE, G., TOCHER, D. R., **THE EFFECTS OF WEANING ON TO A DRY PELLET DIET ON BRAIN LIPID AND FATTY ACID COMPOSITIONS IN POST-LARVAL GILTHEAD SEA BREAM (SPARUS AURATA L.)**, COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY PART A: PHYSIOLOGY, 1993.VOL. 104, NO. 3, P. 605-611.

MOURENTE ,G., TOCHER, D.R., ***THE EFFECTS OF WEANING ON TO A DRY PELLET DIET ON BRAIN LIPID AND FATTY ACID COMPOSITIONS IN POST-LARVAL GILTHEAD SEA BREAM (SPARUS AURATA L.).*** COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY PART A: PHYSIOLOGY, 1993. 104(3): P. 605-611.

MOURENTE, G., TOCHER, D. R., SARGENT, J. R., **SPECIFIC ACCUMULATUON OF DOCOSAHEXAENOIC ACID (22:6N-3) IN BRAIN LIPIDS DURING DEVELPMENT OF JUVENILE TURBOT SCOPHTHALMUS MAXIMUS L.,** LIPIDS,1991. VOL. 26, P. 871-877.

MOURENTE,G., TOCHER,D.R., SARGENT,J.R., ***SPECIFIC ACCUMULATUON OF DOCOSAHEXAENOIC ACID (22:6N-3) IN BRAIN LIPIDS DURING DEVELPMENT OF JUVENILE TURBOT Scophthalmus maximus L.*** **LIPIDS**, 1991. 26: P. 871-877.

MOYLE, P.B., CECH JR.,J.J., ***FISHES. AN INTRODUCTION TO ICHTHYOLOGY.*** PRENTICE HALL, ED. T. EDITION 2004, NEW YORK.

MURUA, H., SABORIDO-REY, F., ***FEMALE REPRODUCTIVE STRATEGIES OF MARINE FISH SPECIES OF THE NORTH ATLANTIC.*** J. NORTHWEST ATL. FISH. SCI., 2003.33: P. 23-31.

NAGAHAMA, Y., ***THE FUNCTIONAL MORPHOLOGY OF TELEOST GONADS.*** **FISHPHYSIOLOGY: ENDOCRINE TISSUES AND HORMONES**. ACADEMIC PRESS, 1983. 9(A):P. 223-75.

NICOLAS, J.-M., ***VITELLOGENESIS IN FISH AND THE EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBON CONTAMINANTS.*** AQUATIC TOXICOLOGY 1999. 45 (2-3): P. 77-90.

NISSLING, A., LARSSON, R., VALLIN, L., FROHLUND, K, ***ASSESSMENT OF EGG AND LARVAL VIABILITY IN COD, GADUS MORHUA: METHODS AND RESULTS FROM AN EXPERIMENTAL STUDY.*** FISHERIES RESEARCH, 1998. 38: P. 169–186.

NÓBREGA, R.H., BATLOUNI, S.R., FRANÇA, L.R., ***AN OVERVIEW OF FUNCTIONAL AND STEREOLOGICAL EVALUATION OF SPERMATOGENESIS AND GERM CELL TRANSPLANTATION IN FISH.*** FISH PHYSIOL. BIOCHEM. , 2009. 35: P. 197–206.

NORBERG, B., BROWN, C.L., HALLDORSSON, O., STENSLAND, K., BJÖRNSSON, B.T.,***PHOTOPERIOD REGULATES THE TIMING OF SEXUAL MATURATION, SPAWNING, SEX STEROID AND THYROID HORMONE PROFILES IN THE ATLANTIC COD (GADUS MORHUA).*** AQUACULTURE, 2004. 229: P. 451–467.

O'BRIEN, L., J. BURNETT, AND R. K. MAYO, *MATURATION OF NINETEEN SPECIES OF FINFISH OFF THE NORTHEAST COAST OF THE UNITED STATES, 1985-1990*, IN. *NOAA TECH. REPORT*. 1993, NMFS. P. 66.

OLLA, B. L., DAVIS, M. W., SCHRECK, C. B. **COMPARISON OF PREDATOR AVOIDANCE CAPABILITIES WITH CORTICOSTEROID LEVELS INDUCED BY STRESS IN JUVENILE COHO SALMON**. TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY.1992, 121: P. 544–547.

OLSEN, E., LILLY, G.R., HEINO, M., MORGAN, M.J., BRATTEY, J., DIECKMANN, U., ***ASSESSING CHANGES IN AGE AND SIZE AT MATURATION IN COLLAPSING POPULATIONS OF ATLANTIC COD (GADUS MORHUA)***. CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 2005. 62: P. 811–823.

OLSEN, R.E., HENDERSON, R. J., PEDERSEN, T., ***THE INFLUENCE OF DIETARY LIPID CLASSES ON THE FATTY ACID COMPOSITION OF SMALL COD GADUS MORHUA L. JUVENILES REARED IN AN ENCLOSURE IN NORTHERN NORWAY***. JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY, 1999. 148(1): P. 59-76.

OLSEN, R.E., SUNDELL, K., RINGØ, E., MYKLEBUST, R., HEMRE, G.I., HANSEN, T., KARLSEN, Ø., ***THE ACUTE STRESS RESPONSE IN FED AND FOOD DEPRIVED ATLANTIC COD, GADUS MORHUA L.*** AQUACULTURE, 2008. 280: P. 232-241.

OOSTHUIZEN, E., DAAN, N., ***EGG FECUNDITY AND MATURITY OF NORTH SEA COD, GADUS MORHUA.*** NETHERLANDS JOURNAL OF SEA RESEARCH, 1974. 8: P. 378-397.

OUELLET, P., LAMBERT, Y., BERUBE ,I, ***COD EGG CHARACTERISTICS AND VIABILITY IN RELATION TO LOW TEMPERATURE AND MATERNAL NUTRITIONAL CONDITION.*** ICES JOURNAL OF MARINE SCIENCE, 2001. 58: P. 672 - 68NC

O'BRIEN, L., ***FACTORS INFLUENCING THE RATE OF SEXUAL MATU- RITY AND THE EFFECT ON SPAWNING STOCK FOR GEORGES BANK AND GULF OF MAINE ATLANTIC COD GADUS MORHUA STOCKS***. J NORTHW ATL FISH SCI 1999. 25: P. 179–203.

PANINI, E.B., MYLONAS, C. C., ZANUY, S., CARRILLO, M., RAMOS, J., & BRUCE, M. P. , ***INCUBATION OF EMBRYOS AND LARVAE OF MARINE FISH USING MICROTITER PLATES.*** AQUACULTURE INTERNATIONAL, 2001. 9(2): P. 189-195.

PAPADAKI,M., PAPADOPOULOU,M., SIGGELAKI,I., MYLONAS,C.C., ***EGG AND SPERM PRODUCTION AND QUALITY OF SHARPSNOUT SEA BREAM (DIPLODUS PUNTAZZO) IN CAPTIVITY.*** AQUACULTURE, 2008. 276(1-4): P. 187-197.

PASZKOWSKI, C. A. , OLLA, B. L. **SOCIAL INTERACTIONS OF COHO SALMON (ONCORHYNCHUS KISUTCH) SMOLTS IN SEA WATER.** CANADIAN JOURNAL OF ZOOLOGY. 1985, 63, 2401–2407.

PAVLOV, D., KJØRSVIK, E., REFSTI, T., ANDERSEN, Ø., ***BROOD STOCK AND EGG PRODUCTION*****. CULTURE OF COLD-WATER MARINE FISH**, ED. E. MOKSNESS, KJØRSVIK, E., OLSEN, Y2004, OXFORD: BLACKWELL PUBLISHING.

PENNEY,R.W.,LUSH, P.L., WADE,A.J., BROWN,J.A.,BURTON,M.P.M., ***EFFECT OF PHOTOPERIOD MANIPULATION ON BROODSTOCK SPAWNING, FERTILIZATION SUCCESS, AND EGG DEVELOPMENTAL ABNORMALITIES IN ATLANTIC COD, GADUS MORHUA***. JOURNAL OF THE WORLD AQUACULTURE, 2006. 37(3): P. 273-281.

PILLING, G. M.,MILNER, R.S., EASEY,M.W., MAXWELL,D.L., TIDD, A. N., ***PHENOLOGY AND NORTH SEA COD Gadus morhua L.: HAS CLIMATE CHANGE AFFECTED OTOLITH ANNULUS FORMATION AND GROWTH?*** JOURNAL OF FISH BIOLOGY, 2007. 70(2): P. 584-599.

RAKITIN, A., FERGUSON,M.M.,TRIPPEL E.A , ***MALE REPRODUCTIVE SUCCESS AND BODY SIZE IN ATLANTIC COD GADUS MORHUA L.*** . . MARINE BIOLOGY, 2001. 138(6): P. 1077-1085.

RØNNESTAD, I., FYHN, H.J., ***METABOLIC ASPECTS OF FREE AMINO ACIDS IN DEVELOPING MARINE FISH EGGS AND LARVAE.*** REVIEWS IN FISHERIES SCIENCE, 1993. 1(3): P. 239 – 259.G.RØNNESTAD, I.,

THORSEN,A., FINN R.N., ***FISH LARVAL NUTRITION: A REVIEW OF RECENT ADVANCES IN THE ROLES OF AMINO ACIDS***. AQUACULTURE, 1999. 177(1-4): P. 201-216.

RØNNESTAD, I., TONHEIM, S.K., FYHN, H.J., ROJAS-GARCIA, C.R., KAMISAKA, Y., KOVEN, W., FINN, R.N., TERJESEN, B.F., BARR, Y., CONCEIÇÃO, L.E.C., **THE SUPPLY OF AMINO ACIDS DURING EARLY FEEDING STAGES OF MARINE FISH LARVAE: A REVIEW OF RECENT FINDINGS**. AQUACULTURE, 2003. 227: P. 147–164.

RØNNESTAD,I.,LIE.Ø.,WAAGBO, R., ***VITAMIN B-6 IN ATLANTIC HALIBUT, HIPPOGLOSSUS HIPPOGLOSSUS - ENDOGENOUS UTILIZATION AND RETENTION IN LARVAE FED NATURAL ZOOPLANKTON***. AQUACULTURE, 1997. 157(3-4): P. 337-345.

ROSENLUND, G. AND SKRETTING, M., **WORLDWIDE STATUS AND PERSPECTIVE ON GADOID CULTURE,** ICES J. MAR. SCI. , 2006. 63: P. 194–197.

ROSENLUND, G., KARLSEN, Ø., TVEIT, K., MANGOR-JENSEN, A., HEMRE, G.I., ***EFFECT OF DIET COMPOSITION AND FEEDING REGIME ON GROWTH, FEED UTILISATION AND NUTRIENT RETENTION ON JUVENILE COD, GADUS MORHUA.*** AQUACULTURE NUTRITION, 2004. 10(6): P. 371-378.

ROSENLUNDA, G., M.S., ***WORLDWIDE STATUS AND PERSPECTIVE ON GADOID CULTURE***. ICES JOUNAL OF MARINE SCIENCE, 2006. 63(2): P. 194-197.

ROUXEL C., SUQUET,M., COSSON J, SEVERE A., QUEMENER, L., FAUVEL, C., ***CHANGES IN ATLANTIC COD (GADUS MORHUA L.) SPERM QUALITY DURING THE SPAWNING SEASON***. AQUACULTURE RESEARCH, 2008. 39(4): P. 434-440.

SABORIDO-REY, MURUA,H., ***FEMALE REPRODUCTIVE STRATEGIES OF MARINE FISH SPECIES OF THE NORTH ATLANTIC***. J. NORTHWEST ATL. FISH. SCI., 2003. 33: P. 23-31.

SAKAI, K., NOMURA, M., TAKASHIMA, F., **CHARACTERISTICS OF NATURALLY SPAWNED EGGS OF RED SEA BREAM.** BULL. JAP. SOC. SCIENT. FISH., 1985.51: P. 1395-1399.

SALZE, G., TOCHER, D. R., ROY, W. J., ROBERTSON, D. A. , ***EGG QUALITY DETERMINANTS IN COD (GADUS MORHUA L.): EGG PERFORMANCE AND LIPIDS IN EGGS FROM FARMED AND WILD BROODSTOCK***. AQUACULTURE RESEARCH, 2005. 36 (15): P. 1488-1499.

SANTOS, J. D., I.C.B., MALCOLM JOBLING, ***PATTERNS OF GROWTH AND LIPID DEPOSITION IN COD (GADUS MORHUA L.) FED NATURAL PREY AND FISH-BASED FEEDS***. AQUACULTURE, 1993. 110(2): P. 173-189.

SARGENT, J., MCEVOY, L., ESTEVEZ, A., BELL, G., BELL, M., HENDERSON, J., & TOCHER, D. , ***LIPID NUTRITION OF MARINE FISH DURING EARLY DEVELOPMENT: CURRENT STATUS AND FUTURE DIRECTIONS***. AQUACULTURE, 1999. 179(1-4): P. 217-229.

SARGENT, J., ***ORIGINS AND FUNCTIONS OF EGG LIPIDS: NUTRITIONAL IMPLICATION*****,** **IN *BROODSTOCK MANAGEMENT AND EGG AND LARVAL QUALITY*** N.R.R. BROMAGE, R.J., EDITOR 1995, BLACKWELL SCIENCES LTD: OXFORD. P. 353-372.

SARGENT, J.R., BELL, G., BELL, M. V., HENDERSON, J., & TOCHER, D. R., ***THE METABOLISM OF PHOSPHOLIPIDS AND POLYUNSATURATED FATTY ACIDS IN FISH AQUACULTURE: FUNDAMENTAL AND APPLIED RESEARCH*****,** **IN *COSTAL AND ESTUARINE- AQUACULTURE: FUNDAMENTAL AND***

APPLIED RESEARCH, A.G. UNION, EDITOR 1994: WASHINGTON, DC. P. 103-113.

SARGENT, J.R., MCEVOY, L. A., & BELL, J. G. , ***REQUIREMENTS, PRESENTATION AND SOURCES OF POLYUNSATURATED FATTY ACIDS IN MARINE FISH LARVAL FEEDS***. AQUACULTURE, 1997. 155(1-4): P. 117-127.

SARGENT, J.R., TOCHER, D.R., BELL, J.G., **THE LIPIDS**. IN: HALVER, J.E., HARDY, R.W. (EDS.), ISH NUTRITION. ACADEMIC PRESS, SAN DIEGO, CA, 2002. P. 181–257.

SARGENTA,J.R., TOCHER,D.R.,BELL,J.G., ***THE LIPIDS***, IN *FISH NUTRITION*, A. PRESS, EDITOR 2003: SAN DIEGO. P. 181-257.

SAWANBOONCHUN, J., ***ATLANTIC COD (GADUS MORHUA L.) BROODSTOCK NUTRITION: THE ROLE OF ARACHIDONIC ACID AND ASTAXANTHIN AS DETERMINANTS OF EGG QUALITY***, IN *SCHOOL OF NATURAL SCIENCES,* 2008, UNIERSITY OF STIRLINGER: STIRLING. P. 1-12.

SAWANBOONCHUN, J., W.R., DA ROBERTSON, JG BELL, ***THE IMPACT OF DIETARY SUPPLEMENTATION WITH ASTAXANTHIN ON EGG QUALITY IN ATLANTIC COD BROODSTOCK (GADUS MORHUA, L.).*** AQUACULTURE, 2008. 283(1-4): P. 97-101.

SCHRECK, C., ***PHYSIOLOGICAL, BEHAVIOURAL, AND PERFORMANCE INDICATORS OF STRESS***. AMERICAN FISHERIES SOCIETY, 1990. 8: P. 29-37.

SCHRECK, C., ***STRESS AND FISH REPRODUCTION: THE ROLES OF ALLOSTASIS AND HORMESIS, GENERAL AND COMPARATIVE ENDOCRINOLOGY***. FISH REPRODUCTION, 2010. 165(3): P. 549-556.

SCHULZ,R.W., DE FRANÇA,L.R., LAREYRE,J.J., LEGAC,F., CHIARINI-GARCIA,H., NOBREGA,R.H., I MIURA,T., ***SPERMATOGENESIS IN FISH***. GENERAL AND COMPARATIVE ENDOCRINOLOGY, 2010. 165(3): P. 390-411.

SELYE, H., ***THE EVOLUTION OF THE STRESS CONCEPT***. AMERICAN SCIENTIST, 1973.61: P. 692-699.

SHERWOOD,G.D., RIDEOUT,M.R., FUDGE,S.B., ROSE,G.A., ***INFLUENCE OF DIET ON GROWTH, CONDITION AND REPRODUCTIVE CAPACITY IN NEWFOUNDLAND AND LABRADOR COD (Gadus morhua): INSIGHTS FROM STABLE CARBON ISOTOPES (Δ13C).*** DEEP SEA RESEARCH PART II: TOPICAL STUDIES IN OCEANOGRAPHY, 2007. 54(23-26): P. 2794-2809.

SIGISMONDI, L. A., WEBER, L. J. **CHANGES IN AVOIDANCE RESPONSE TIME OF JUVENILE COHO SALMON EXPOSED TO MULTIPLE ACUTE HANDLING STRESSES**. TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY, 1988. 117: P. 196–201.

SKJÆRAASEN, J.E., SALVANES, A.G.V., KARLSEN, Ø., DAHLE, R., NILSEN, T.,NORBERG, B., ***THE EFFECT OF PHOTOPERIOD ON SEXUAL MATURATION, APPETITE AND GROWTH IN WILD ATLANTIC COD (GADUS MORHUA L.).*** FISH PHYSIOLOGY AND BIOCHEMISTRY 2004. 30: P. 163-174.

SPRINGATE, J.R.C., .BROMAGE., N. R. , ***EFFECTS OF EGG SIZE ON EARLY GROWTH AND SURVIVAL IN RAINBOW TROUT (SALMO GAIRDNERI RICHARDSON)***. AQUACULTURE, 1985. 47(2-3): P. 163-172.

SWANSON, P., SUZUKI, K., KAWAUCHI, H., W. W. DICKHOFF., WW., ***ISOLATION AND CHARACTERIZATION OF TWO COHO SALMON GONADOTROPINS, GTH I AND GTH II***. BIOLOGY OF REPRODUCTION, 1991. 44: P. 29-38.

TARANGER , G.L., CARRILLO, M., SCHULZ, W., FONTAINE, PASCAL, ZANUY, S., FELIP, A., WELTZIEN, F., DUFOUR, SYLVIE, KARLSEN, Ø., NORBERG, B., ANDERSSON, E., HANSEN, T. , ***CONTROL OF PUBERTY IN FARMED FISH.*** GENERAL AND COMPARATIVE ENDOCRINOLOGY, 2010. 165(3): P. 483-515.

TARANGER , G.L., CARRILLO, M., SCHULZ, W., FONTAINE, PASCAL, ZANUY, S., FELIP, A., WELTZIEN, F., DUFOUR, SYLVIE, KARLSEN, Ø., NORBERG, B., ANDERSSON, E., HANSEN, T. , ***CONTROL OF PUBERTY IN FARMED FISH.*** GENERAL AND COMPARATIVE ENDOCRINOLOGY, 2010. 165(3): P. 483-

515.

TARANGER, G.L., AARDAL, L., HANSEN, T., KJESBU, O.S., ***CONTINUOUS LIGHT DELAYS SEXUAL MATURATION AND INCREASES GROWTH OF ATLANTIC COD (GADUS MORHUA L.) IN SEA CAGES***. ICES JOUNAL OF MARINE SCIENCE, 2006. 63: P. 365-375.

TARANGER, G.L., E.,VIKINGSTAD., U KLENKE, I MAYER, S.O STEFANSSON, B NORBERG, T HANSEN, Y ZOHAR, E ANDERSSON, ***EFFECTS OF PHOTOPERIOD, TEMPERATURE AND GNRHA TREATMENT ON THE REPRODUCTIVE PHYSIOLOGY OF ATLANTIC SALMON (SALMO SALAR L.) BROODSTOCK***. FISH PHYSIOLOGY AND BIOCHEMISTRY, 2000. 28: P. 403–406.

TERNER, C., ***METABOLISM AND ENERGYCONVERSION DURINGH EARLY DEVELOPMENT***.FISH PHYSIOLOGY, 1979. 8: P. 261-278.

THORSEN, A., TRIPPEL, E. A., LAMBERT, Y., ***EXPERIMENTAL METHODS TO MONITOR THE PRODUCTION AND QUALITY OF EGGS OF CAPTIVE MARINE FISH.*** NORTHWEST ATLANTIC FISHERY SCIENCE, 2003. 33: P. 55-70.

TOCHER, D. R., MOURENTE, G., & SARGENT, J. R., **METABOLISM OF (1-C-14) DOCOSAHEXAENOATE (22:6N-3), (1-C-14) EICOSAPENTAENOATE (20:5N-3) AND (1-C-14) LINOLENATE (18:3N-3) IN BRAIN-CELLS FROM JUVENILE TURBOT SCOPHTHALMUS- MAXIMUS**, LIPIDS, 1992. VOL. 27, NO. 7, P. 494-499.

TOCHER, D., ***METABOLISM AND FUNCTIONS OF LIPIDS AND FATTY ACIDS IN TELEOST FISH*** . REVIEWS IN FISHERIES SCIENCE 2003 11 (2): P. 107-184.

TOCHER, D.R., HARVIE, *D.G,*. ***FATTY ACID COMPOSITIONS OF THE MAJOR PHOSPHOGLYCERIDES FROM FISHNEURAL TISSUES; (N-3) AND (N-6) POLYUNSATURATED FATTY ACIDS IN RAINBOW TROUT (SALMO GAIRDNERII) AND COD (GADUS MORHUA) BRAINS AND RETINAS.*** FISH PHYSIOLOGY AND BIOCHEMISTRY, 1988. 5: P. 229-239.

TRIPPEL, E.A., BENFEY, T.J., NEIL, S.R.E., CROSS, N., BLANCHARD, M.J., POWELL, F., ***EFFECTS OF CONTINUOUS LIGHT AND TRIPLOIDY ON GROWTH AND SEXUAL MATURATION IN ATLANTIC COD, GADUS MORHUA***. CYBIUM 2008. 32(2): P. 136-138.

TRIPPEL, E.A., ***ESTIMATION OF STOCK REPRODUCTIVE POTENTIAL: HISTORY AND CHALLENGES FOR CANADIAN ATLANTIC GADOID STOCK ASSESSMENTS.*** NORTHWEST ATLANTIC FISHERY SCIENCE, 1999. 25: P. 61-81.

TVEITEN, H., JOBLING, M., ANDREASSEN, I., ***INFLUENCE OF EGG LIPIDS AND FATTY ACIDS ON EGG VIABILITY, AND THEIR UTILIZATION DURING EMBRYONIC DEVELOPMENT OF SPOTTED WOLF-FISH, ANARHICHAS MINOR OLAFSEN***. AQUACULTURE RESEARCH, 2004. 35(2): P. 152-161.

TYLER, C.R., SUMPTER, J.C., ***OOCYTE GROWTH AND DEVELOPMENT IN TELEOSTS***. FISH BIOLOGY FISHERIES, 1996. 6: P. 287-318.

VALLIN, L., NISSLING, A., **CELL MORPHOLOGY AS AN INDICATOR OF VIABILITY OF COD EGGS RESULTS FROM AN EXPERIMENTAL STUDY**, FISHERIES RESEARCH, 1998. VOL. 38, NO. 3, P. 247- 255.

VALLIN,L., NISSILING,A., ***CELL MORPHOLOGY AS AN INDICATOR OF VIABILITY OF COD EGGS - RESULTS FROM AN EXPERIMENTAL STUDY***. FISHERIES RESEARCH, 1998. 38(3): P. 247-255.

VAN DAMME, C.J.G.,DICKEY-COLLAS, M., RIJNSDORP, A. D., KJESBU, O. S., ***FECUNDITY, ATRESIA, AND SPAWNING STRATEGIES OF ATLANTIC HERRING (CLUPEA HARENGUS).*** CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES, 2009. 66 (12): P. 2130-2141.

VAN DER MEEREN, T., IVANNIKOV, V.P., ***SEASONAL SHIFT IN SPAWNING OF ATLANTIC COD (Gadus morhua L.) BY PHOTOPERIOD MANIPULATION: EGG QUALITY IN RELATION TO TEMPERATURE AND***

INTENSIVE LARVAL REARING. AQUACULTURE RESEARCH 2006. 37: P. 898-913.

VAN LEEUWENA, A., DE ROOS, A.M., PERSSON,L., ***HOW COD SHAPES ITS WORLD***. JOURNAL OF SEA RESEARCH, 2008. 60(1-2): P. 89-104.

VOLKOFF, X.A., ***CLONING, TISSUE DISTRIBUTION AND EFFECTS OF FOOD DEPRIVATION ON PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP)/PACAP-RELATED PEPTIDE (PRP) AND PREPROSOMATOSTATIN 1 (PPSS 1) IN ATLANTIC COD (GADUS MORHUA).*** PEPTIDES, 2009. 30(4): P. 766-776.

WALLACE, R.A., SELMAN, K., ***CELLULAR AND ASPECTS OF OOCYTE GROWTH IN TELEOSTS.*** AMERICAN ZOOLOGIST, 1981. 21: P. 325-343.

WATANABE, T., ***IMPORTANCE OF THE STUDY OF BROODSTOCK NUTRITION FOR FURTHER DEVELOPMENT OF AQUACULTURE***. NUTRITION AND FEEDING OF FISH, 1985: P. 395-414.

WATANABE, T., ***IMPORTANCE OF THE STUDY OF BROODSTOCK NUTRITION FOR FURTHER DEVELOPMENT OF AQUACULTURE*****,** IN *NUTRITION AND FEEDING OF FISH*, A.M.J. B, EDITOR 1985, ACADEMIC PRESS: LONDON. P. 395-414.

WATANABE, T., KIRON, V., ***BROODSTOCK MANAGEMENT AND NUTRITIONAL APPROACHES FOR QUALITY OFFSPRINGS IN THE RED SEA BREAM*****,** **IN** ***BROODSTOCK MANAGEMENT AND EGG AND LARVAL QUALITY***, N.R. BROMAGE, ROBERT, R.J., EDITOR 1995, CAMBRIDGE UNIVERSITY PRESS: CAMBRIDGE.

WEDEMEYER, G., ***PHYSIOLOGY OF FISH IN INTENSIVE CULTURE SYSTEMS***. CHAPMAN & HALL, 1996: P. 232.

WENDELAAR-BONGA, S.E., ***THE STRESS RESPONSE IN FISH.*** PHYS. REV.,BETHESDA,1997.V.77,3 : P. 591-625.

WEST, G., ***METHODS OF ASSESSING OVARIAN DEVELOPMENT IN FISHES: A REVIEW.*** AUSTRALIAN JOURNAL OF MARINE AND FRESHWATER RESEARCH, 1990. 41(2): P. 199 – 222. AFRESHWATER RESEARCH, 1990. 41(2): P. 199 – 222.WESTERNHAGE, H.V., ***SUBLETHAL EFFECTS OF POLLUTANTS ON FISH EGGS AND LARVAE***. FISH PHYSIOLOGY, 1988. 11(1): P. 253-346.

VIII. APPENDIX

APPENDIX 1. WEIGHT AND LENGTH OF ATLANTIC COD A) AVERAGE WEIGHT (G) IN EACH TREATMENT AND B) AVERAGE LENGTH (CM) IN EACH TREATMENT C) AVERAGE WEIGHT (G) IN EACH TANK D) AVERAGE LENGTH (CM) IN EACH TREATMENT.

A)

	JANUARY 2010		MAY 2010			
GENDER	**LF DIET**	**HF DIET**	**LF DIET**		**HF DIET**	
			LOW STRESS	**HIGH STRESS**	**LOW STRESS**	**HIGH STRESS**
F	1276±263	1425±271	1153±268	1036±225	1169±237	1250±320
M	1142±196	1333±197	998±184	1001±167	1157±155	1117±208

B)

	JANUARY 2010		**MAY 2010**			
GENDER	**LF DIET**	**HF DIET**	**LF DIET**		**HF DIET**	
			LOW STRESS	**HIGH STRESS**	**LOW STRESS**	**HIGH STRESS**
F	432±27	439±27	444±30	440±28	445±25	447±33
M	426±21	442±17	434±24	435±23	453±15	449±16

C)

	START OF EXPERIMENT JANUARY 2010			**END OF EXPERIMENT MAY 2010**			
TANK	**GENDER**	**LF**	**HF**	**LF**		**HF**	
				LOW STRESS	**HIGH STRESS**	**LOW STRESS**	**HIGH STRESS**
4	F	1280±314		1127±282			
	M	1113±99		987±188			
18	F	1313±217		1119±322			
	M	1228±314		1067±194			
19	F	1240±206			1018±165		
	M	1144±171			997±158		
20	F	1322±303			1024±253		
	M	1238±227			1094±135		
11	F		1544±329				1273±377
	M		1341±217				1178±195
12	F		1414±253				1149±238
	M		1206±203				1023±192
13	F		1351±219			1024±249	
	M		1217±124			1116±109	
14	F		1399±258			1184±125	
	M		1487±164			1205±156	

D)

		START OF EXPERIMENT JANUARY 2010		END OF EXPERIMENT MAY 2010			
TANK	GENDER	LF	HF	LF		HF	
				LOW STRESS	HIGH STRESS	LOW STRESS	HIGH STRESS
4	F	424±34		437±41			
	M	440±18		441±30			
18	F	439±19		449±20			
	M	431±28		443±29			
19	F	429±25			444±26		
	M	428±24			439±27		
20	F	439±30			446±33		
	M	430±22			446±20		
11	F		443±34				454±38
	M		445±19				450±13
12	F		439±32				450±35
	M		438±20				456±19
13	F		445±20			444±25	
	M		432±10			449±9	
14	F		443±24			452±28	
	M		443±17			453±13	

APPENDIX 2. FECUNDITY A) ML EGG/KG FISH/TANK

	LOW FAT				HIGH FAT			
	LOW STRESS		HIGH STRESS		LOW STRESS		HIGH STRESS	
WEEK/ TANK	4	18	19	20	13	14	11	12
6	6±9	7±10	3±4	12±13	10±13	1±2		
7	18±15	13±10	12±15	5±10	18±14	11±14	5±6	6±9
8	21±14	19±20	7±4	5±3	13±11	13±10	7±7	5±6
9	28±11	19±11	12±8	13±11	19±13	26±7	18±7	16±5
10	28±11	11±9	15±4	17±9	16±12	32±15	24±16	14±10
11	27±7	11±12	16±11	12±13	12±11	23±21	17±14	12±9
12	21±8	8±8	7±7	14±11	13±15	13±12	23±18	14±14
13	10±11	4±7	8±8	15±11	6±7	9±7	24±22	16±15
14	2±2	1±1	1±1	9±9	1±3	8±9	26±16	10±10
15				5±8			5±5	6±7
16				5±8				6

B) MEAN ML EGG/KG FISH/TANK PER DAY

	LOW FAT				HIGH FAT			
	LOW STRESS		HIGH STRESS		LOW STRESS		HIGH STRESS	
DATE/TANK	4	18	19	20	13	14	11	12
12-FEB	0.00	18.75	8.05	26.32	24.07	0.00	0.00	0.00
13-FEB	1.27	3.13	0.00	8.77	6.17	2.96	0.00	0.00
14-FEB	15.92	0.00	2.01	0.00	0.00	0.00	0.00	0.00
15-FEB	10.83	0.00	43.62	26.32	0.00	0.00	0.00	0.00
16-FEB	12.74	31.25	6.71	0.58	37.04	23.67	13.51	17.65
17-FEB	9.55	15.01	0.67	0.00	16.05	0.00	5.41	0.00
18-FEB	50.96	13.65	1.34	1.17	4.32	0.00	0.11	1.18
19-FEB	12.74	8.19	0.67	2.92	24.69	35.50	5.41	20.59
20-FEB	13.38	19.11	16.11	0.00	12.35	17.16	0.00	0.59
21-FEB	19.11	3.41	13.42	2.92	30.86	2.96	13.51	2.94
22-FEB	15.92	4.78	10.07	7.60	27.78	6.80	2.70	0.00
23-FEB	0.00	47.77	2.01	3.51	27.78	0.00	2.70	0.00
24-FEB	31.02	3.41	1.34	2.92	15.43	29.59	4.32	5.88
25-FEB	28.95	8.87	8.72	8.77	3.09	23.67	13.51	0.00
26-FEB	6.89	15.70	4.70	8.77	8.64	6.51	4.32	14.71
27-FEB	37.92	47.77	10.07	4.09	3.70	11.24	20.54	9.41
1-MAR	41.36	1.36	6.71	1.17	37.04	35.50	16.22	17.65
2-MAR	27.57	34.12	15.44	11.70	18.52	35.50	20.54	11.76
3-MAR	14.48	20.47	13.42	14.62	6.17	17.75	11.89	20.59
4-MAR	27.57	13.65	6.71	1.17	33.95	20.71	10.81	8.82
5-MAR	31.02	27.30	10.07	32.16	21.60	26.63	13.51	11.76
6-MAR	13.79	13.65	3.36	14.62	0.06	29.59	22.97	22.06
7-MAR	41.36	20.47	26.85	17.54	18.52	19.53	29.73	16.47
8-MAR	26.89	13.65	16.78	17.25	13.58	20.12	15.68	17.65
9-MAR	32.40	7.78	13.42	9.36	31.48	28.99	22.70	4.12
10-MAR	27.57	27.23	12.08	15.20	17.28	47.34	27.03	17.65
11-MAR	18.93	1.56	14.09	6.25	0.62	11.24	11.35	2.94
12-MAR	26.50	0.78	23.49	25.00	3.09	53.25	40.27	7.65
13-MAR	49.21	14.00	16.78	30.62	21.60	41.42	5.41	29.41
14-MAR	15.14	11.67	10.07	12.50	27.78	23.67	48.65	20.59
15-MAR	29.48	0.00	2.01	0.62	28.40	23.08	16.52	8.82
16-MAR	27.80	14.00	26.17	16.25	12.35	12.89	35.99	12.94
17-MAR	13.48	31.12	19.46	1.25	1.23	44.47	11.80	29.41
18-MAR	25.27	0.78	0.67	12.50	0.62	0.64	0.59	1.18
19-MAR	33.70	7.78	21.48	21.87	1.28	54.79	32.45	11.76
20-MAR	33.70	0.86	28.72	34.37	20.16	0.64	17.70	2.94
21-MAR	25.27	21.60	16.51	0.31	17.48	25.78	1.18	14.71
22-MAR	18.53	9.33	3.59	1.87	3.36	3.22	35.40	29.41
23-MAR	25.27	16.79	11.49	19.77	29.57	32.23	6.49	10.59
24-MAR	25.27	19.58	17.95	21.75	1.34	3.22	8.85	35.29
25-MAR	17.69	0.93	14.36	26.36	0.67	21.91	12.98	0.59
26-MAR	33.70	1.87	1.44	7.20	22.18	19.34	13.57	1.24
27-MAR	16.85	2.80	1.44	21.60	0.07	6.45	57.82	18.67

	LOW FAT				HIGH FAT			
	LOW STRESS		HIGH STRESS		LOW STRESS		HIGH STRESS	
	4	18	19	20	13	14	11	12
28-MAR	8.42	2.80	0.72	0.14	32.18	1.29	23.60	0.62
29-MAR	8.42	18.65	10.77	14.75	3.58	1.29	13.57	37.35
30-MAR	16.85	0.09	20.82	1.55	16.76	12.25	11.80	31.12
31-MAR	1.68	2.80	6.46	32.36	0.08	2.75	54.87	21.79
1-APR	29.48	0.93	2.15	24.27	0.00	6.19	28.32	0.62
2-APR	0.84	8.39	14.36	15.37	13.71	13.77	5.90	15.56
3-APR	0.84	0.09	1.44	0.40	0.00	4.13	53.10	1.24
4-APR	15.16	0.47	0.72	15.37	7.62	20.65	0.59	1.24
5-APR	0.84	0.00	2.15	0.16	0.00	18.58	41.30	0.31
6-APR	4.21	1.87	0.36	14.56	8.38	15.83	15.34	5.60
7-APR	1.68	0.00	0.72	20.23	0.08	12.39	47.20	18.67
8-APR		0.93	2.15	1.62	0.23	0.69	11.21	14.32
9-APR			1.44	20.23	1.52	0.14	37.76	3.11
10-APR			1.44	2.43	0.00	0.07	11.80	24.90
11-APR			0.22	4.05	0.15		16.52	0.31
12-APR				0.00			1.18	1.24
13-APR				16.18			5.90	15.56
14-APR				0.40			2.36	1.24
15-APR				0.40			10.62	12.45
16-APR				0.00				
17-APR								
18-APR				0.08			0.12	0.62
19-APR				9.71				6.22
20-APR				0.00				
21-APR				0.00				
22-APR				21.84				
23-APR				0.00				
24-APR				0.00				
25-APR				3.24				

APPENDIX 3. EGG DIAMETER NUMBER OF EGGS, AVERAGE SIZE PER TANK, DATES

	LOW FAT				HIGH FAT			
	LOW STRESS		HIGH STRESS		LOW STRESS		HIGH STRESS	
DATE/ TANK	4	18	19	20	13	14	11	12
18-FEB	1.26±0.03	1.27±0.05		1.26±0.02				
19-FEB	1.29±0.04	1.27±0.03	1.30±0.02	1.27±0.03	1.24±0.05	1.30±0.02	1.27±0.01	1.34±0.04
22-FEB		1.25±0.04	1.29±0.02	1.22±0.02	1.23±0.04	1.27±0.02	1.25±0.03	
23-FEB		1.27±0.06	1.28±0.03	1.24±0.02	1.25±0.06		1.26±0.02	1.27±0.05
24-FEB	1.26±0.04	1.25±0.02	1.28±0.03	1.24±0.03	1.24±0.03	1.28±0.03	1.33±0.03	1.27±0.05
25-FEB	1.26±0.03	1.30±0.03	1.28±0.02	1.25±0.03	1.24±0.03	1.25±0.03	1.25±0.03	
26-FEB	1.22±0.05	1.23±0.03	1.27±0.03	1.23±0.03	1.20±0.07	1.27±0.03	1.26±0.03	1.27±0.05
01-MAR	1.23±0.03	1.25±0.02	1.25±0.03	1.32±0.03	1.27±0.04	1.25±0.04	1.27±0.03	1.26±0.06
04-MAR	1.24±0.04	1.26±0.02	1.22±0.04	1.21±0.04	1.24±0.03	1.22±0.03	1.23±0.02	1.22±0.04
05-MAR	1.24±0.03	1.17±0.06	1.21±0.06	1.20±0.04	1.23±0.04	1.24±0.03	1.28±0.03	1.25±0.03
08-MAR	1.23±0.05	1.23±0.03	1.22±0.05	1.28±0.05	1.24±0.04	1.24±0.04	1.26±0.02	1.22±0.04
09-MAR	1.23±0.03	1.22±0.03	1.26±0.06	1.22±0.02	1.22±0.03	1.23±0.03	1.25±0.06	1.20±0.07
10-MAR	1.22±0.07	1.16±0.10	1.18±0.04	1.17±0.04	1.23±0.04	1.22±0.03	1.26±0.02	1.23±0.04
12-MAR	1.21±0.05	1.24±0.04	1.21±0.05	1.20±0.04	1.24±0.02	1.23±0.03	1.22±0.06	1.24±0.03
13-MAR	1.22±0.05	1.23±0.04	1.24±0.04	1.21±0.04	1.24±0.02	1.25±0.05	1.24±0.04	1.24±0.04
14-MAR	1.17±0.03	1.13±0.07	1.21±0.04	1.20±0.03	1.22±0.04	1.20±0.04	1.25±0.05	1.22±0.03
15-MAR	1.21±0.06	1.23±0.06	1.23±0.06	1.20±0.03	1.24±0.04	1.19±0.02	1.26±0.03	1.23±0.05
16-MAR	1.18±0.04	1.12±0.08	1.20±0.04	1.30±0.04	1.19±0.07	1.22±0.03	1.21±0.03	1.21±0.04
17-MAR	1.17±0.03	1.23±0.04	1.22±0.06	1.20±0.04	1.24±0.03	1.20±0.04	1.24±0.10	1.21±0.05
18-MAR	1.20±0.07	1.19±0.03	1.25±0.08	1.16±0.03	1.23±0.05	1.19±0.02	1.22±0.03	1.22±0.02
19-MAR	1.26±0.07	1.18±0.07	1.22±0.04	1.19±0.03	1.25±0.05	1.19±0.04	1.26±0.05	1.22±0.03
20-MAR	1.18±0.05	1.24±0.04	1.21±0.04	1.23±0.06	1.21±0.05	1.22±0.05	1.22±0.09	1.19±0.05
21-MAR	1.20±0.08	1.18±0.04	1.18±0.05	1.16±0.06	1.24±0.03	1.16±0.03	1.22±0.05	1.24±0.06
22-MAR	1.25±0.05	1.23±0.04	1.26±0.04	1.17±0.03	1.20±0.06	1.28±0.03	1.30±0.05	1.21±0.05
24-MAR	1.24±0.05	1.21±0.02	1.20±0.05	1.25±0.08	1.23±0.04	1.16±0.03	1.27±0.07	1.22±0.05
25-MAR	1.18±0.05	1.23±0.04	1.21±0.06	1.22±0.04	1.24±0.05	1.23±0.04	1.25±0.04	1.21±0.04
26-MAR	1.24±0.03	1.19±0.02	1.17±0.05	1.16±0.07	1.22±0.03	1.19±0.04	1.20±0.03	1.17±0.04
27-MAR	1.15±0.02	1.20±0.02	1.21±0.06	1.25±0.09	1.23±0.05	1.26±0.02	1.26±0.05	1.22±0.03
28-MAR	1.26±0.05	1.19±0.02	1.18±0.04	1.18±0.07	1.20±0.03	1.23±0.04	1.19±0.04	1.23±0.07
29-MAR	1.13±0.03	1.22±0.03	1.18±0.07	1.16±0.02	1.20±0.03	1.25±0.05	1.31±0.07	1.21±0.05
30-MAR	1.25±0.05	1.23±0.05	1.21±0.06	1.15±0.04	1.22±0.03	1.21±0.05	1.25±0.01	1.23±0.05
31-MAR	1.14±0.06	1.18±0.02	1.19±0.03	1.23±0.06	1.21±0.02	1.19±0.04	1.24±0.05	1.19±0.05
01-APR	1.21±0.04	1.17±0.03	1.21±0.04	1.24±0.06		1.20±0.05	1.19±0.04	1.23±0.06
02-APR	1.24±0.04	1.20±0.02	1.15±0.08	1.27±0.04	1.23±0.01	1.23±0.04	1.21±0.03	1.21±0.05
03-APR	1.12±0.02	1.17±0.02	1.18±0.06	1.26±0.03	1.22±0.03	1.22±0.03	1.23±0.05	1.14±0.04
04-APR	1.24±0.03	1.13±0.02	1.20±0.02	1.27±0.02	1.20±0.01	1.17±0.04	1.20±0.02	1.23±0.06
05-APR	1.13±0.03	1.20±0.05	1.20±0.03	1.26±0.02	1.26±0.02	1.18±0.04	1.24±0.05	1.19±0.08
06-APR	1.24±0.04	1.23±0.06	1.21±0.03	1.15±0.06	1.15±0.06	1.21±0.04	1.21±0.06	1.23±0.05
07-APR	1.18±0.07		1.20±0.03	1.09±0.05	1.09±0.05	1.20±0.03	1.21±0.05	1.18±0.07

	LOW FAT				HIGH FAT			
	LOW STRESS		HIGH STRESS		LOW STRESS		HIGH STRESS	
DATE/ TANK	4	18	19	20	13	14	11	12
08-APR		1.11±0.02	1.21±0.04	1.26±0.03	1.26±0.03	1.21±0.03	1.27±0.03	1.21±0.05
09-APR		1.19±0.02	1.20±0.03	1.24±0.02				1.22±0.04
10-APR			1.18±0.03	1.13±0.06		1.17±0.04	1.19±0.02	1.19±0.06
11-APR				1.08±0.04				
12-APR				1.18±0.09			1.27±0.03	1.10±0.07
13-APR				1.21±0.03			1.18±0.05	1.22±0.02
14-APR				1.18±0.07			1.26±0.03	1.20±0.07
15-APR				1.29±0.11			1.22±0.04	1.19±0.04
16-APR							1.24±0.05	
17-APR				1.19±0.03			1.21±0.03	1.1±90.03
18-APR				1.22±0.04			1.20±0.04	1.19±0.04
19-APR				1.16±0.02				1.13±0.03
20-APR							1.24±0.05	
21-APR								
22-APR				1.17±0.02				
23-APR								
24-APR								
25-APR								

APPENDIX 4. A) FERTILIZATION AVERAGE AND B) MALF, NUMBER FERTILIZED, TOTAL EGG COUNTED, FERTILIZATION RATE

A) FERTILIZATION AVERAGE

	LOW FAT				HIGH FAT			
	HIGH STRESS		HIGH STRESS		HIGH STRESS		HIGH STRESS	
DATE/ TANK	4	18	19	20	13	14	11	12
18-FEB	1.09±0.00	0.46±0.60	0.99±0.22	0.13±0.34	1.01±0.73	1.34±0.53	1.37±0.52	0.96±0.43
23-FEB	0.95±0.25	0.11±0.32	0.71±0.52	0.24±0.43	0.45±0.50	0.85±0.43	0.89±0.34	0.88±0.32
25-FEB	0.54±0.67	0.10±0.36	0.97±0.57	0.14±0.39	0.76±0.74	1.24±0.63	0.24±0.49	1.28±0.56
02-MARS	0.90±0.30	0.49±0.54	0.84±0.37	0.17±0.38	0.75±0.43	0.93±0.25	0.72±0.45	0.80±0.40
03-MARS	0.63±0.59	1.11±0.63	0.88±0.46	0.41±0.61	0.70±0.54	0.75±0.52	0.96±0.23	0.54±0.76
04-MARS	1.05±0.34	0.63±0.70	0.73±0.47	0.55±0.61	0.48±0.60	0.87±0.45	1.05±0.57	1.18±0.51
11-MARS	1.00±0.35	0.54±0.50	0.78±0.46	0.90±0.58	0.97±0.21	1.06±0.58	1.07±0.47	0.37±0.68
13-MARS	0.93±0.26	0.87±0.43	0.54±0.55	0.86±0.55	0.89±0.57	0.97±0.23	0.72±0.45	0.85±0.36
15-MARS	0.93±0.26	0.43±0.63	0.34±0.48	0.11±0.31	0.69±0.52	0.79±0.43	0.99±0.09	0.83±0.38
16-MARS	0.96±0.20	0.73±0.62	0.76±0.54	0.48±0.68	0.98±0.27	0.99±0.09	0.87±0.54	0.89±0.37
17-MARS	0.67±0.66	0.09±0.35	0.86±0.35	0.87±0.33	0.77±0.54	0.88±0.33	0.83±0.38	0.92±0.50
18-MARS	0.88±0.55	0.36±0.67	0.76±0.53	0.28±0.51	0.77±0.43	1.00±0.00	0.81±0.69	1.04±0.54
20-MARS	0.75±0.45	0.36±0.48	0.31±0.47	0.22±0.42	0.87±0.33	0.81±0.42	0.89±0.31	0.15±0.42
21-MARS	0.58±0.73	0.86±0.35	0.96±0.19	0.58±0.61	0.55±0.54	0.84±0.81	0.76±0.43	0.78±0.47
22-MARS	0.94±0.34	0.55±0.50	0.93±0.34	0.87±0.56	1.06±0.41	0.93±0.41	0.94±0.34	0.24±0.63

	LOW FAT				HIGH FAT			
	HIGH STRESS		HIGH STRESS		HIGH STRESS		HIGH STRESS	
DATE/ TANK	4	18	19	20	13	14	11	12
23-MARS	0.67±0.47	0.74±0.61	0.64±0.52	0.47±0.55	0.95±0.28	0.85±0.45	0.97±0.21	0.54±0.54
24-MARS					0.71±0.72			
25-MARS	0.85±0.35	0.95±0.23	0.43±0.51	0.80±0.40	0.97±0.16	0.77±0.48	0.31±0.50	0.86±0.34
26-MARS	0.04±0.19	0.64±0.76						
27-MARS	0.63±0.54	0.93±0.26	0.37±0.49					
28-MARS	0.56±0.73							
29-MARS	0.68±0.52	0.57±0.53	0.64±0.54	0.85±0.47	0.94±0.36	0.72±0.52	0.99±0.09	0.72±0.53
30-MARS	0.87±0.45	0.98±0.13	0.70±0.88	0.66±0.51	0.96±0.19	0.77±0.60	0.80±0.42	0.88±0.59
31-MARS	0.39±0.49	0.48±0.60	0.93±0.25	0.74±0.44		0.86±0.41	0.86±0.35	0.94±0.28
01-APR	0.62±0.49	0.94±0.24	0.40±0.53	0.58±0.50	0.99±0.10	0.93±0.29	0.54±0.50	0.49±0.55
02-APR	0.98±0.19	0.73±0.47	0.89±0.36	0.68±0.47	0.73±0.50	0.95±0.22	0.92±0.30	0.20±0.46
03-APR				1.00±0.00				
04-APR				0.95±0.21				
05-APR				0.90±0.30				
07-APR								0.52±0.54
08-APR								0.36±0.48
09-APR							0.03±0.21	0.17±0.43
10-APR							0.50±0.52	
11-APR							0.34±0.49	