Triploid induction in Atlantic cod (*Gadus morhua* L.) by the use of different pressure levels

Thesis for fulfilment of the degree

Master of Science in Aquaculture biology

Susanne Håvardstun

Department of biology

University of Bergen, Norway

November 2011

Front page photo; Susanne Håvardstun

Takk til

Denne masteroppgaven ble utført på Havforskningsinstituttet på Austevoll, og i den forbindelse er det flere personer jeg ønsker å takke. Jeg vil rette en stor takk til mine tre veiledere Prof. Albert Kjartansson Imsland, Dr. Trine Haugen og Dr. Anders Mangor-Jensen for god veiledning og konstruktive tilbakemeldinger gjennom hele oppgaven min. En takk går også til Dr. Anders Thorsen for god hjelp med blodcelleanalysene og Dr. Geir Dahle for god hjelp med mikrosatellittanalysene.

Jeg vil også takke de ansatte på HI Austevoll for å ha tatt godt imot meg under det praktiske arbeidet med oppgaven.

Videre vil jeg takke mine med-studenter fra lesesalen; Johanna Fall, Sofie Söderström, Christine Elgen, Gaute Jørpeland, Eirik Leknes og Lars Otto Paust som har gjort mastertiden min til en interessant og lærerik tid fylt med mye latter og kaffe.

En stor takk rettes til Kristine Fonnes for gjennomlesing og gode tilbakemeldinger på den engelske delen av oppgaven min.

Sist, men ikke minst, vil jeg rette en stor takk til Tor-Erik og vår datter Thea for all støtte under hele mastergraden min. Den har vært uvurderlig!

Tusen takk!

Bergen, 13.10.2011

Susanne Håvardstun

List of contents

Abstract **6**

1. Introduction **7**

1.1 Historical background 7

1.2 Juvenile production methods 8

1.3 Problems facing the cod on-growing industry – escapes and spawning 9

1.4 Triploidization 12

1.5 Triploid extensiveness 13

1.6 Objectives 15

2. Materials and methods **17**

2.1 Experimental design 17

2.1.1 Incubation 18

2.1.2 First start feeding 19

2.1.3 Daily routines 20

2.2 Sampling 22

2.2.1 Weight and length measurements 23

2.2.2 Blood sampling 24

2.2.3 Microsatellite loci analysis sampling 25

2.3 Blood diameter analyses 25

2.4 Microsatellite loci analyses 25

2.5 Statistical analysis 26

3. Results **27**

3.1 Fertilization and mortality on egg stage 27

3.2 Embryo development 28

3.3 Length and weight 29

3.4 Effects of pressure on blood cell diameter 31

3.5 Microsatellite loci analysis 34

4. Discussion **36**

Conclusions and further perspectives 41

5. Hypothesis evaluation **42**

References **43**

Appendix I **50**

Discussion of Materials & Methods 50

Experimental design – Gamete collection and rearing conditions 50

Mortality 51

Sampling and analyses 52

Appendix II **53**

Descriptive statistics 53

One-Way ANOVA 60

Student-Newman-Keuls multiple comparison (SNK) test 62

ANCOVA 63

Levene's test for homogeneity of variance 63

Appendix III **64**

Appendix IV **65**

Abstract

In the present experiment, different levels of pressure were investigated to see if it was possible to induce triploidy at a lower pressure level than previously used for Atlantic cod (*Gadus morhua*). Newly fertilized eggs (offspring of one male and one female), were exposed to different levels of pressure and accordingly divided into four experimental groups: 400, 500 and 600 bar, and one control group. Each pressure group received the desired pressure for 5 min, beginning at 180 ºC min post-fertilization.

Induction of triploidy occurred at each pressure level used in this experiment. Blood cell diameter analysis showed that pressure treatment had an effect on blood cell diameter; resulting in increased mean blood cell diameter that was correlated with increasing pressure level used. Furthermore, microsatellite loci analysis revealed over 90 % triploid outcome in each experimental group, whereas the remaining part could not be identified as triploids. Overall this study demonstrated successfully triploid induction at both low (400 bar) and medium (500 bar) pressure.

Further studies are recommended in order to reveal the optimal pressure level for triploid induction in Atlantic cod in accordance with less severe deformities, reduced effect of sexual maturation, high growth rate and a high flesh quality. This, in combination with all-female production, will potentially be a promising approach for triploidy in large-scale aquaculture production.

1. Introduction

The Atlantic cod (*Gadus morhua* L.) is a demersal fish belonging to the family gadidae. It is a common species in Norway, and an economically important fish for consumption. It is also processed into fishmeal, cod liver oil an roe (Pethon, 1998). In Norwegian waters, Atlantic cod is usually divided into the stationary Norwegian coastal cod and the migrating Northeast Arctic cod, and these are further divided into several local populations (Moen and Svensen, 2004). The Northeast Arctic cod can reach a size of 1.8 m and 55 kg, but the coastal cod is rarely more than 80 cm long (Pethon, 1998). Atlantic cod is widely distributed across the North Atlantic Sea. On the eastern Atlantic side it is present from Biscaya to the Baltic Sea, around Iceland, Svalbard and Novaya Zemlya. It is also present on both sides of southern Greenland (Moen and Svensen, 2004). Atlantic cod is a batch spawner, and each female can spawn up to 5 million eggs over a period of about two months (Moen and Svensen, 2004).

1.1 Historical background

The marine finfish aquaculture in Norway has focused mainly on Atlantic salmon farming. In order to diversify this industry, Atlantic cod is considered a good candidate species (Feindel et al., 2010). The first attempt of farming cod was done in 1886. Gunder Mathiesen Dannevig tried to farm cod larvae in a land based sea cage in the spring, which resulted in 4000 viable fry by fall (Øiestad, 2005). Based on Dannevig's research from 1886, a large scale spawning system was developed by the Institute of Marine Research at Austevoll and the first farmed cod was slaughtered in 1977 (Havforskningsinstituttet, 2007). In the 1980's, the development of the cod aquaculture industry in Norway was slow due to difficulties in the juvenile production. However, late in the 1990's, improved techniques brought the cod farming industry into a new phase (Moe et al., 2007). In order to succeed in establishing Atlantic cod aquaculture, the industry must be able to produce a high-quality product at an appointed time, regardless of the season. It is also important to keep in mind the higher cost of producing the farmed fish, compared to fish from commercial fisheries (Tilseth, 1990) as well as the quality of the product offered to the marked. In 2008 and 2009, Norway produced 18 052 t (FAO, 2008) and 25 000 t (Jørstad et al., 2010) of farmed cod, respectively. The Norwegian catches of coastal cod in 2009 was 26 000 metric tons (Berg, 2010) and 197 000 metric tons of Northeast Arctic cod in 2008 (Bogstad, 2010).

The successful farming of salmonids, together with the declining fisheries in general, has increased the interest of farming other species. Cod is a popular and a well-known product on

many menus and farming is a sustainable way to meet the increasing demand (Rosenlund and Skretting, 2006). The Atlantic cod is worldwide known for its good quality flesh and a low presence of bones in the fillets. Cod is the basis for a range of products, and have an extensive market including northern and southern Europe and Latin America (Quéméner et al., 2002). In Norway there has been a significant investment into cod farming by private companies, and a strong support from the government in terms of both research and farming (Sogn-Grundvåg et al., 2010).

The advantage of farming cod compared to traditional fishery is the year around availability. Due to the decreasing supply of catches of coastal cod and Northeast Arctic cod in late fall, 60 % of the exported farmed cod in 2008 occurred from September until December (Olsen and Kristiansen, 2009). The total quantum of slaughtered farmed cod has increased steadily from 2003 to 2008 (Lassen, 2009). Total export values from cod in 2008 were 219 million Norwegian kroner, and the largest marked for whole Norwegian farmed cod is Denmark (2 200 tonnes in 2008) followed by France (1 200 tonnes in 2008) (Olsen and Kristiansen, 2009). However, the outbreak of the disease *Francisella philomiragia,* and the financial crisis, contributed to a difficult marked for the cod farming industry in 2008 and 2009. Many cod farmers reduced or stopped the farming while waiting for a proper vaccine, and the market price for fresh cod has decreased significantly since the winter in 2008/2009 (Sogn-Grundvåg et al., 2010).

1.2 Juvenile production methods

The production of juvenile Atlantic cod is a complex process. The success of juvenile production can be measured as growth, survival and the quality of the juveniles (Rosenlund and Halldórsson, 2007). In intensive production of cod, the broodstock normally reach maturity at the age of two years (Kjesbu and Norberg, 2005). Puberty is controlled by an endogenous rhythm and in turn external signals from the environment, i.e. photoperiod, water temperature, food availability and water quality (Taranger et al., 2010). The quality of the spawned eggs is highly dependent on the temperature. Increase in broodstock temperature gives an increase in number of dead eggs and embryos with an uneven cell division (Norberg et al., 2006).

After fertilization the cod eggs are normally incubated in cone-shaped tanks with continuous water flow and intense air bubbling in order to keep the eggs evenly dispersed in the water column until hatching (Brown et al., 2003). Time of hatching is dependent on the water

temperature; higher temperature (8 ºC) results in shorter egg stage compared to lower temperatures (6 ºC) (Brown et al., 2003). The larvae are transferred to start-feeding tanks at 3 – 5 days post hatching (dph) (Thorsen, 2005), and then start-fed on enriched Rotifers for approximately four weeks. Further, they are fed enriched Artemia (Karlsen and Van Der Meeren, 2003) until weaning onto dry feed when the cod are approximately 25 – 30 dph (Mangor-Jensen et al., 2006). To prevent cannibalism, it is important to co-feed with Artemia for some days during the transition onto dry feed (Otterå, 2005).

When the cod is totally adapted to dry feed, one can to some degree compare the farming of Atlantic cod with the farming of Atlantic salmon. In contrast to flatfishes, like turbot (*Scophthalmus maximus* Rafinesque, 1810) and halibut, farming gadoids like Atlantic cod have the benefit of using already established technology developed by the salmon industry. This makes it easier and also more economically sustainable to establish the cultivation of Atlantic cod after adaptation to formulated feed (Rosenlund and Skretting, 2006).

1.3 Problems facing the cod on-growing industry – escapes and spawning

A major disadvantage of using salmon farming cage technologies in the cod production is that cod are more prone to escape from net pens compared to salmon (Svåsand et al., 2011). Moe et al. (2007) estimated that 0 – 6 % of the cultured cod had escaped each year from 2000 to 2005. Moe et al. (2007) suggested that these numbers should be regarded as a minimum estimate, because it is likely that not all escape incidences were reported or even detected. More than 50 % of cod escapees in 2003 – 2005 were through holes in the nets caused by handling, cod or predator biting, and other unknown causes. Based on interviews with cod farmers, Moe et al. (2007) postulated some hypotheses that may explain why there are more episodes of cod escaping than salmon i.e.

i) Cod may be attracted to irregularities in the net pen, like loose threads or existing mechanical damages.
ii) Cod will search the netting wall for holes, and even a small hole can easily lead to escape of many cod. Since Atlantic cod are more proactive in biting the netting and ropes, this will create wear and tear in the net cage.
iii) Cod are popular feed for predators like seals, and several cod farmers have reported that some predators prefer cod over salmon.

Hansen et al. (2008) suggested that Norwegian coastal cod are more willing to escape than Northeast Arctic cod, and the prevention of net damages should be a major goal in order to avoid fish escapes in cod farming.

Early puberty and resulting spawning in on-growing sea cages may affect the genetic composition of local cod population as the fertilized eggs can survive in the environment and potentially contribute to the nearby spawning stock, or escapees that may spawn together with wild Atlantic cod (Skjaeraasen et al., 2009). Recently, there has been a lot of attention of the impact this might have on the genetic pool of the wild stocks of Atlantic cod, and on their ability to survive in their unique local habitat. Research has shown that despite a generally high gene flow in marine species (Conover et al., 2006), there is a certain degree of local adaptation, and Atlantic cod stocks may not be genetically differentiated across larger distances (Nielsen et al., 2009), but also across smaller geographical distances (Gjøsæter et al., 1992; Ruzzante et al., 2000; Sarvas and Fevolden 2005; Jørstad et al., 2008; Nielsen et al., 2009). Large-scale escapes are costly from the farmer's perspective and also from an environmental point of view if fitness costs are incurred on wild populations (Bekkevold et al., 2006). Skjaeraasen et al. (2010) showed that both male and female farmed cod are likely to breed with wild fish. Cod have an elaborate mating behavior, and an influx of large amount of escapees into the natural spawning grounds could interfere with their natural mating system.

Farmed Atlantic cod grow well from juvenile stage to a size of 1.5 – 2 kg. When they reach sexual maturation, approximately 30 % of the body weight is lost during one spawning season. Some mortality is also expected during the spawning season (Kjesbu et al., 2006). In order to reach the desired harvest size after maturation, longer production time is needed. Also, the post spawning period is associated with low food conversion efficiency (Kjesbu et al., 2006). The fillets will become high in water content because of sexual maturation, and this results in a lower market value (Trippel et al., 2008; Taranger et al., 2010). Sexual maturation is therefore an economical bottleneck in the farming of Atlantic cod that needs to be resolved, either by delaying, or preferably, arresting the sexual maturation.

Photoperiod and temperature variations are considered the most important environmental cues concerning sexual maturation (Taranger et al., 2010). The photoperiod influences the endogenous rhythms and results in synchronous spawning within a population at approximately the same time every year (Norberg et al., 2004; Taranger et al., 2010).

Delaying the sexual maturation can to some extent be achieved by using light treatment in both land and sea cages (Kjesbu et al., 2006). Light treatment manipulations has been a success in commercial production of Atlantic salmon, and experiments on Atlantic cod in indoors tanks showed that spawning time can be controlled by photoperiod treatment (Hansen et al., 2001). By postponing sexual maturation, the growth will be continuous and the cod will reach desired market size before first spawning (Karlsen et al., 2006). Davie et al. (2007) developed a model to describe the photoperiodic regulation of reproduction in Atlantic cod. Their results demonstrated that the sexual maturation cycle is entered by the falling photoperiod signal after October. By the application of continuous light they found no reproductive activity and growth was improved up to 60 % at the age of 27 months post hatch. However, full photoperiodic control can be difficult to achieve in commercial farming, and improved lighting technologies are needed (Taranger et al., 2010).

Maturation and growth depends on the same pool of surplus energy, and therefore, sexual maturation takes priority and occurs at the expense of somatic growth. Today's farming industry causes fish to reach puberty earlier than the wild populations (Thorpe, 2004). The farmer wants the fish to convert all of its surplus energy into edible flesh, instead of investment into early maturity and reproduction. The main reason for extensive induction of triploidy for aquaculture purposes is the sterility of the triploid fish. By introducing sterile fish in aquaculture farming, one can avoid the negative effects related to puberty, especially regarding flesh quality (Piferrer et al., 2009) and the negative influence on their wild counterparts (Taranger et al., 2010). An increased concern of farmed Atlantic cod is the high mortality rates of egg bound females which causes economic loss for farmers (Feindel et al., 2011), and is also a welfare problem for the farmed fish. This problem can be avoided using triploid fish in the production.

In triploid females, the ovarian growth during sexual maturation is greatly reduced, but triploid males still develops testes up to normal mature diploid size (Benfey 1999). According to Benfey (1999) this is due to the numbers and size of gametes produced in normal diploids where females produce a small numbers of large oocyte while the male produce a large numbers of small spermatozoa. Recently, Feindel et al. (2011) carried out an experiment comparing the sexual maturation process of diploid and triploid Atlantic cod. Their results were in accordance to the general observations from Benfey (1999) and Piferrer et al. (2009), i.e. triploid female showed vitellogenic and hydrated oocytes, but this was rare and at low densities. Triploid males, on the other hand, followed their diploid counterparts regarding

spermatogenesis and had freely suspended spermatozoa present in testes by onset of spawning (Feindel et al., 2011). Several studies have examined milt characteristics, spawning success and fertility of triploid male cod, and have revealed that mature triploid males produce aneuploid sperm cells and can successfully artificially fertilize diploid egg. However, their offspring will not survive past the yolk sac stage (Peruzzi et al., 2009; Feindel et al., 2010). Peruzzi et al. (2009) displayed that triploid cod milt had a variable spermatozoa concentration like their diploid counterparts, but triploid milt revealed a lower sperm velocity at 20 s after activation. In addition, Feindel et al. (2010) found that although triploid spermatozoa were of a larger size compared to diploids, they still showed potential of *in vitro* fertilization. Escaped triploid males have the ability to fertilized diploid eggs, but the lethal outcome of this combination will result in a reduced fitness of the wild diploid female (Feindel et al., 2011).

1.4 Triploidization

Triploid fish are assumed to be sterile due to irregular meiotic division of chromosomes. This results in reduced gonadal development and aneuploid gametes (Tiwary et al., 2004). The idea is that triploid fish will divert all of their surplus energy into growth instead of sexual maturation (Thorpe, 2004). Polyploid fish can be defined as an organism with one (or more) chromosome set in addition to the number most frequently found in nature (Piferrer et al., 2009). After the female fish has released its eggs, the eggs are arrested at the metaphase stage of meiosis II (Colas and Dubé, 1998). When the spermatozoa enter the egg, meiosis II is resumed, and allows the egg to further develop. Shock induction, e.g. thermal, chemical or pressure shock, during the stage of meiosis II can suppress the natural cell division and prevent the extrusion of the second polar body. This will lock in a third chromosome from the mother; hence the offspring are called maternal triploids. The treated offspring will have two chromosomes of maternal origin and one of paternal origin (Piferrer et al., 2009). This will give offspring which has either XXX or XXY chromosome sets (Figure 1.1).

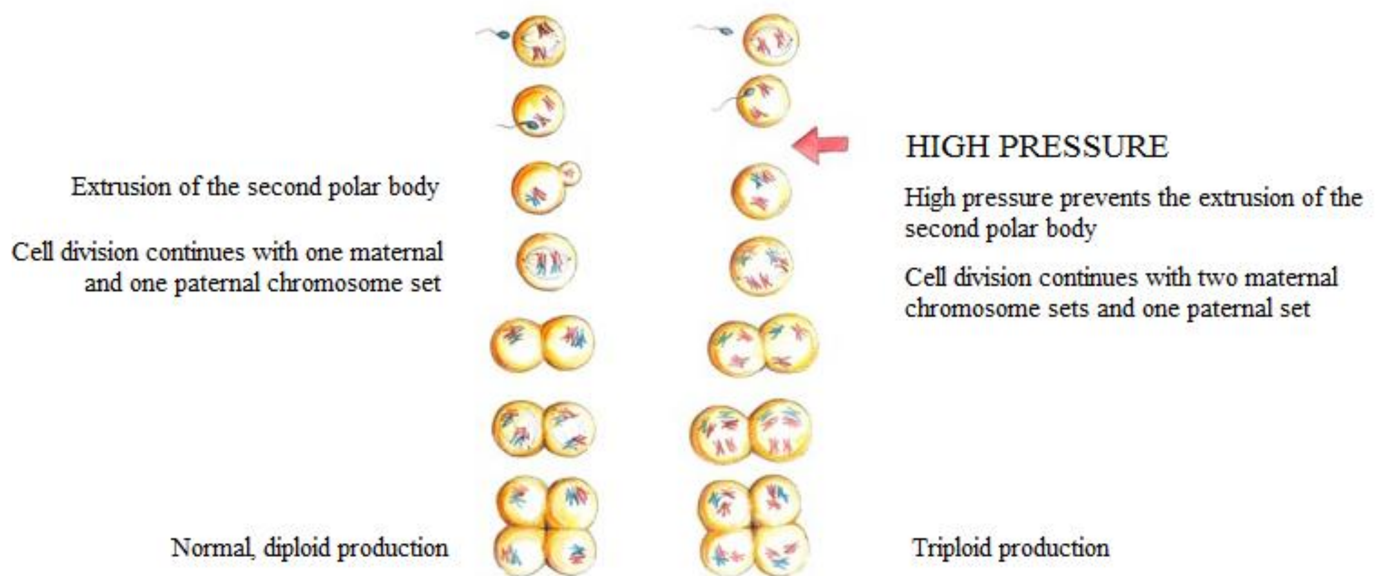

Figure 1.1: Illustration of fertilization and induction of triploidy in fish. Illustration: Stein Mortensen.

Due to the extra set of chromosomes, triploid cell nuclei will in theory contain 50 % more DNA than a diploid cell. Since the nuclear volume is increased in triploid cells, a triploid organism will have larger cells and their gonadal developments are to some extent disrupted. Despite having 50% more DNA content, triploid individuals are not larger than the diploid individuals. This appears, according to Benfey (1999), to be due to a decrease in cell numbers in organs and tissues containing larger cells.

When germ cells enter meiosis I, the triploid gonadal development is disrupted because the presence of a third set of chromosomes will interfere with the homologous chromosome pairing in the meiotic prophase. This will inhibit further gamete development (Feindel et al., 2011).

1.5 Triploid extensiveness

Polyploid plants are associated with greater cell size and disease resistance, and many plants used in modern agriculture are therefore induced polyploids. By inducing polyploids, one can produce seedless fruits from plants with uneven sets of chromosomes. Triploid plants include banana, apple, lemon, orange and sugar beet (Piferrer et al., 2009).

According to Piferrer et al. (2009) triploidy can easily be induced in some vertebrates and in lower vertebrates, but not in higher vertebrates. It is still not known why it is difficult, or impossible, to induce triploidy to higher vertebrates. Niebuhr (1974) reviewed that polyploidy

in mammals seems to have a lethal effect. Most embryos die in the uterus, or only a few hours or days after birth.

Numerous experiments have been carried out to induce triploidy in a variety of species for aquaculture purposes. In 1999/2000, 30 % of commercial production of Pacific oysters farmed on the West Coast of North America was triploid (Nell, 2002). Fertilization between tetraploid males and diploid females produces batches of 100 % triploid offspring (Guo et al., 1996; Nell, 2002). Peruzzi and Chatain (2000) found that 100 % triploidy induction in European sea bass (*Dicentrarchus labrax* L.) was obtained starting from shock intensities of 8.500 psi (586 bar) with a duration of 2 min, 6 min after fertilization. In terms of survival, pressure shock proved to be more effective than cold shocks.

Hydrostatic pressure shock has been used to successfully induce triploidy by preventing the extrusion of the second polar body in a diversity of fish species. Experiments with Atlantic salmon (*Salmo salar* L.) showed that a hydrostatic pressure shock of 3 or 6 min at 700 bar 20 min post fertilization resulted in 100 % triploidy with 70 - 90 % survival (Benfey and Sutterlin, 1984). Fjelldal and Hansen (2010) investigated vertebral deformities in triploid Atlantic salmon underyearling smolts, and found that farmed triploid salmon in freshwater seems to be more vulnerable to develop deformities in the trunk region of the vertebral column than diploids. Further, Leclercq et al. (2011) compared seawater performance and deformity prevalence of diploid and triploid post smolts. They found that triploids smoltified 4 weeks earlier and at a much higher body weight compared to diploids. Further they found that external deformities, such as jaw malformation, were higher in triploids than diploids, and suggested that the difference in heart morphometry may be due to the higher cardiac workload in triploids. Triploids also had a higher rate of cataracts.

Triploid induction in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) has been successfully applied (Lou and Purdom, 1984), although Ojolick et al. (1995) demonstrated that triploid rainbow trout did not survive or grow as well as diploids in chronic high water temperature conditions. Triploid induction has also been applied to Atlantic halibut (*Hippoglossus hippoglossus* L.) (Holmefjord and Refstie, 1997), turbot (*Scophthalmus maximus* Rafinesque, 1810) (Cal et al., 2006) and coho salmon (*Oncorhynchus kisutch* Walbaum, 1792) (Johnson et al., 1986).

Research on thermal shock induction of triploidy in Atlantic cod has been carried out by Peruzzi et al. (2007, 2009). They produced triploid cod by the application of a 20 ºC heat shock for 20 min, 20 min post fertilization. Trippel et al. (2008) focused on using pressure shock treatment and stated that pressure treatment is a more reliable method for triploid induction in Atlantic cod. They used hydrostatic pressure treatment of 8.500 psi (586 bar) for 5 min, and found that triploidy was successfully achieved.

By inducing triploidy, there are three main variables that have to be considered. The first is the time from fertilization until shock treatment (ºC min). The second is the intensity of the shock (bar), and the third variable is the shock duration (min) (Felip et al., 2001).

The ploidity level in manipulated organisms needs to be precisely determined. Today there are several different methods that may be used, including; flow cytometry analysis (Allen Jr, 1983), selective staining of the nucleolar organizer regions (NORs) (Phillips et al., 1986), red blood cell diameter measurement (Benfey, 1999), particle size analysis that measures erythrocyte nuclear volumes (Johnson et al., 1984) and microsatellite loci analyses (Campbell, 2001). Each method has its advantages and disadvantages according to the time required, necessary expertise, costs and chemical hazards (Harrell et al., 1998).

1.6 Objectives

The main objective of the present work was to test if different pressure shock 30 min after fertilization induces different proportions of triploid individuals. To ensure that the genetic interactions were minimised, only one male and one female were used as broodstock. This resulted in sibling offspring among the different treatment groups. The following experiment was carried out with three different pressure levels: 400, 500 and 600 bar, as well as an untreated control.

The experiment was based on the following alternative hypotheses:

H_{A1}: Increasing pressure will result in increasing rate of triploid induction.

H_{A2}: The group exposed to the highest pressure (600 bar) will consist of triploid individuals only.

H_{A3}: Mean blood cell diameter will increase with increasing pressure.

H_{A4}: Mean blood cell diameter and results from microsatellite loci analyses will be positively correlated.

The corresponding null hypothesis (**H_0**) assumes that elevated pressure have no effect on increased ploidy levels.

2. Materials and methods

2.1 Experimental design

The experiment took place at the Institute of Marine Research (IMR) Austevoll from 11 May to 6 October 2010. The cod eggs used for the triploidization trial were the offspring of one female and one male, both two years old, from the broodstock at IMR Austevoll.

The mature cod were netted into a holding tank and sedated by using 0.04 g/l SW MS222 (Tricaine Methanosulfonate, Finquel, Washington). Both the female and male were stripped by a light abdominal pressure from the anterior part of the abdomen directed towards the gonadal opening. Eggs and milt were kept in separate closed containers and was stored in a refrigerator at 6 ºC until fertilization (30 min). Small tissue samples of the anterior dorsal fin was collected from the male and female and stored at – 20 ºC for later microsatellite analysis.

The triploidization experiment consisted of four different experimental groups; pressure treatment at 400 bar, 500 bar and 600 bar, all pressure treated for 5 min, exactly 30 min after fertilization, and one untreated diploid control group. In order to determine possible effect of pressure on the fertilization rate on all experimental groups, two samples (a and b) of eggs (1 ml) were transferred to small beakers. The a) samples were collected before pressure treatment, and b) samples were collected after pressure treatment.

For each experimental group, 50 ml eggs were fertilized with 1 ml of sperm (measured with a pipette). The sperm was first activated in 0.5 l filtrated 6 ºC seawater, and the following addition of the eggs was then considered as time zero (T=0). The eggs were kept at 6 ºC and exactly 30 min post-fertilization the groups were pressurized according to Trippel et al. (2008). Calculation of the exact fertilization time is 180 ºC min/6 ºC = 30 min, where ºC min denotes acquired terminal units calculated as the product of temperature and time in order to account for the fact that development is temperature dependent. Two timers were used (VWR® Large-Digit Digital Desk Timer, VWR International). The first timer was used to measure time zero and the second timer was used to measure the exact treatment time. All the groups were exposed to pressure by using an electrical/hydraulic apparatus for sterilization of fish eggs (TRC-APV™, TRC hydraulics Inc.). The apparatus consists of a cylinder which has a capacity of 2.7 l, and a hydraulic pump working on a piston to increase pressure in the cylinder (Figure 2.1). The closed beaker with fertilized eggs was placed in the cylinder, and the air space filled with 6 ºC water to ensure complete absence of air in the cylinder. The

cylinder pressure was controlled by a pressure gauge, and each group was subjected for the desired pressure for 5 min. The three experimental groups were pressured one by one, each for 5 min. The control group received the same treatment, but without any pressure (Table 2.1).

Table 2.1: Fertilization scheme. Each experimental group was fertilized 10 min after the previous group.

Pressure	Fertilization time (min)	180 ºC min post fertilization	Pressure time (min)	Time until required pressure (sec)
600 bar	0	30	5	49
500 bar	10	40	5	45
400 bar	20	50	5	33
Control	30	60		

Figure 2.1: Electrical and hydraulic apparatus for fish sterilization.

2.1.1 Incubation

After pressure treatment, the four groups were transferred to four incubation tanks, one treatment per tank. The beakers containing the a) and b) samples were left floating in their respective incubation tank. The incubation tanks had a volume of 50 l, with a slight conic bottom and were supplied with a water temperature of 5.7 ± 0.2 °C (mean ± SD). In order to prevent mechanical stress on the eggs, water was supplied with an even, light, flow directly into the walls of the incubation tank. The drain was located in the centre of the tank, and consisted of a perforated plastic tube, covered with a plankton mesh (250 μm). Air was added

to the system by a hose which ended in a ring around the drain to prevent the eggs from being trapped on the sieve mesh. The ring was perforated, allowing the air to make a light current in the tank.

Fertilization rate was calculated from the a) and b) samples approximately 20 hours after fertilization. Eggs were transferred into a counting chamber and examined under a dissecting microscope (Leica Wild M38, Leica Microsystems), and fertilized and unfertilized eggs were counted (Table 3.1). Dead eggs were collected from the incubators on day 1 (12 May 2010) and on day 10 (21 May 2010) post fertilization (dpf). This was done by shutting off both the air and inlet water for approximately 10 min, allowing dead eggs to sink to the bottom, whereas the developing eggs floated near the surface. Dead eggs were removed by opening the drain (flush out). Dead eggs were collected directly into a small net from the drainpipe and measured volumetrically (Table 3.2).

Pictures of developing eggs were taken at IMR Austevoll at 10 dpf (days post fertilization) using a dissecting microscope (Leica Ms5) supplied with an Olympus camera (Model SZX2 – ILLT, Tokyo, Japan). A small number of eggs were placed in a Petri dish and gathered by an o-ring. Diluted seawater (75 % seawater and 25 % freshwater) was added in order to position the eggs at the right angle (Figure 3.1).

2.1.2 First start feeding

The cod eggs reached 50 % hatching (visual observation) at 14 dpf, and were transferred to start-feeding tanks (Figure 2.2) on 28 May 2010 (3 days post hatch (dph)). Each experimental group were divided into three replicate tanks, giving a total of 12 tanks with approximately 2000 larvae in each. The experimental unit consisted of 12 dark green 50 l tanks with a flat bottom. The 50 l tanks had the same design as the incubation tanks in terms of drain, inlet and outlet water and air bubbling. On transfer day, 1 l of seawater was mixed with 15 ml of algae paste (Nanochloropsis) (Reed Aquaculture, USA). Each tank received 80 ml of this green-water as a start, and also 300 000 rotifers, *Brachionus plicatilis*. Rotifers were fortified with Ori-green (Skretting, Norway). The flow of the inlet water was 5 l per hour.

Figure 2.2: Start feeding tanks. Photo: T. Haugen

2.1.3 Daily routines

The larvae were fed rotifers and supplied with green-water according to IMR's standard protocols on start feeding (Table XXIII – Table XXIV, Appendix IV) from 3 dph and up until 38 dph (2 July 2010). Rotifers were added directly to the tank three times per day. 20 ml of algae paste (Nanochloropsis, Reed Aquaculture, USA) was mixed with cold freshwater from the water tap. Green-water was then added from a 60 l header tank to the rearing tanks by separate hoses. The algae were continuously pumped to the header tank from a 10 l stock solution that was refilled daily. The rearing tanks were cleaned by using a custom made squeegee (skimmer) to collect all the detritus, which was then removed by a suction device. Cleaning the tanks did not start before 17 dph, and during the phase of rotifer feeding only occurred on 23 and 29 dph. On 8 June 2010, of unknown causes, all three replicates holding the control group died, and on 23 June 2010 one replicate of the 400 bar group died. In each incident, 100 % mortality occurred over night (see Appendix I).

The seawater (7.3 ± 0.3 ºC) used throughout the entire experiment was collected at 165 m depth, filtrated and aired before use. From 22 dph the water temperature was raised gradually to 11.8 ± 0.5 °C (mean ± SD) over the next two days. The 12 ºC water had the same origin as the 6 ºC water, but was heated by a heat pump and ventilated before entering the tanks.

Feeding with *Artemia salina* started at 35 dph according to IMR's standard protocols on first feeding (Table XXIV, Appendix IV). The Artemia given was fortified with LARVIVA

Multigain Dana feed A/S (BioMar, Norway). The larvae were co-fed with both rotifers and Artemia (Artemia at 10.00 AM and rotifers at 15.00 and 20.00 PM) for four days. Further, Artemia was given directly into each tank at 10.00 AM and 15.00 PM. To ensure sufficient feed at night, 1.2 million Artemia was added to a 10 l bucket filled with 12 ºC seawater with good air bubbling. The bucket was connected to each tank by a dosage pump. At 21.00 PM a timer switched on the dosage pump at full speed (180 min^{-1}), emptying the content of the bucket into each tank over a time period of 30 min. This was considered sufficient Artemia for the larvae during the night. During the Artemia feeding period the tanks were cleaned every third day.

Weaning started on 21 July 2010 (57 dph). All tanks were supplied with an automated conveyer belt feeder (Hølland Teknologi AS, Sandnes, Norway) (Figure 2.3), and the fish were fed 5 ml of formulated feed AgloNorse Extra, larvae feed No. 1 (K/S Tromsø Fiskeindustri A/S & CO, Tromsø, Norway).

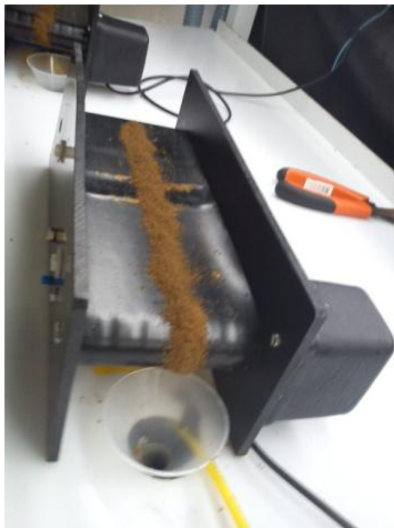

Figure 2.3: Automated feeder.

During 24 h, the feeder had emptied the feed particles evenly through a funnel tube onto the surface of the water in the rearing tanks (Figure 2.4). Co-feeding with 1 million Artemia during the night lasted for five days. When feeding with formulated feed, the rearing tanks were cleaned daily, but only with the suction device. The amount and type of feed given followed IMR's standard feeding protocols (Table XXIV – Table XXVI, Appendix IV).

Figure 2.4: Start feeding tank.

2.2 Sampling

Sampling of pressure treated cod was taken over a period from 27 September 2010 until 6 October 2010 (Table 2.2). Since the entire control group died during the night of 8 June 2010 (see Appendix I), 12 fish (hatched 6 April 2010) from ordinary production at IMR Austevoll were used as a diploid control group. This Diploid group hereby replace the lost Control group. Blood samples of Diploid fish were taken at 204 dph (27 October 2010). Fish from the 500 bar group and the Diploid group (the substitute control group) was sampled during one day each, and the 400 and 600 bar group was sampled over two days each. Weight varied from 0.89 – 14.82 g.

Table 2.2: Overview of sampling dates.

Date	Dph	400 bar (fish sample)	500 bar (fish sample)	600 bar (fish sample)	Diploid (fish sample)
27.09.2010	125	1 – 67			
29.09.2010	127	68 – 95			
30.09.2010	128		1 – 81	1 – 40	
06.10.2010	134			41 – 123	
27.10.2010	204				1 – 12

Fish from one rearing tank at a time were collected and transferred from the rearing facilities to the lab. Here they were transferred to a larger unit (15 l), and filtrated (0.2µm) 10 ºC seawater was added. O_2 level was checked frequently and when lower than 70 %, most of the water was exchanged with fresh seawater until O_2 was approximately 85 %. This was done 1

– 2 times per hour. All the fish were killed by an overdose of MS222. For pressure treated fish, 35 ml MS222 (20 g Tricaine solution/1000 ml seawater) was mixed with 600 ml seawater. The Diploid fish were larger of size and were killed with 70 ml MS222/600 ml SW. To ensure that the overdose was sufficient, one fish was placed in the tricaine and water solution for approximately 3 min. The fish were then placed in saltwater to check for revival. It did not revive and the dose was therefore considered sufficient.

2.2.1 Weight and length measurements

Immediately, post mortem, one fish at a time was wiped dry by paper towels, and photographed with its group-name, number and date on a millimetre paper by a single-lens reflex camera (Canon EOS 550D). This was done for later visual length measurement. Maximum standard length was then measured to the nearest mm from the tip of the snout to the root of the tail fin (Figure 2.5). Each fish was weighed by a digital scale to the nearest mg (milligram) (Sartorius CP 153).

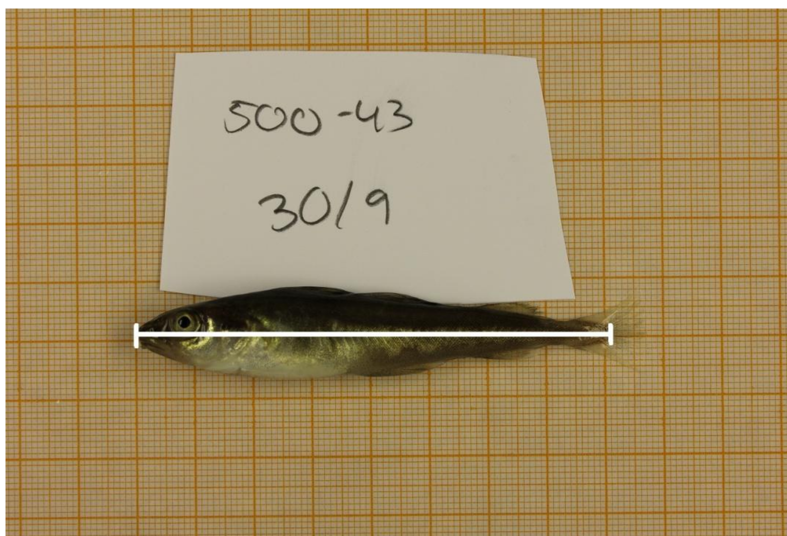

Figure 2.5: Illustration of length measurement.

2.2.2 Blood sampling

In order to take the blood samples, the tail was cut off using a pair of scissors anterior to the posterior dorsal and anal fin (Figure 2.6).

Figure 2.6: Tail-clipping of a 400 bar treated fish.

Figure 2.7: Blood slide.

One drop of blood was added to a microscopic slide (Figure 2.7). With a second clean microscopic slide, the blood was spread out on the first slide by holding the two glasses in a 30º angle. By waving the microscopic slide in the air, the blood dried quickly. Each slide was marked with group-name, fish-number and date. Both the body and tail were kept in zip-lock

plastic bags along with a wet-paper note of the group-name, fish-number and date of the sampling day. All the samples were stored in a – 80 ºC freezer.

2.2.3 Microsatellite loci analysis sampling

10 fish samples in the 600 bar group were pre-tested to ensure that the method used provided reliable results. The pre-test showed good results (see section 2.4) and 240 further samples for microsatellite loci analyses (80 per treatment group) were prepared. Half of the caudal fin was cut off and stored in 0.6 ml eppendorf tubes in a -80 ºC freezer. To ensure no RNase contamination, all equipment and the work bench were cleaned with RNase*Zap*® Wipes, and wiped dry with paper towel. The pair of scissors and the tweezer was cleaned between each fish.

2.3 Blood diameter analyses

Blood cells on slides were photographed at IMR Bergen. The blood samples were observed under a microscope (Nikon Eclipse 80i) at 40x magnification and photographed by using a 5 Mpx digital camera (Micropublisher 5 RTV from QImaging), resulting in a resolution of 8.572 px μm^{-1}. For photography, an area of the blood smear where the blood cells were well enough separated for automatic detection of a large number of cells (usually more than 50) was selected. A specially adapted macro-program was used for measurements based on particle analysis in the open source Image analysis program ImageJ (http://rsb.info.nih.gov/ij/) using the plugin ObjectJ (http://simon.bio.uva.nl/objectj/). Even though blood cells are elliptic in shape, a diameter was assigned for each blood cell. The calculated area for each ellipse was recalculated to a circle with similar area and the diameter for this circle then represented the diameter of the ellipse. In a few cases the blood cells were not well enough separated for automatic detection. In such cases the blood cells were measured manually using a specially adapted ellipse measuring tool in ImageJ/ObjectJ (Anders Thorsen, IMR, 2011, pers. comm.).

Two individuals from the diploid, 400 and 600 bar group were checked to see if blood cell diameters within individuals are normal distributed.

2.4 Microsatellite loci analyses

Microsatellites are short segments of DNA with repeated sequence, e.g. CACACACA. In diploids, each individual will have two copies of any particular microsatellite segment. Over time, a population will recombine their microsatellites during sexual reproduction, and this

will result in a population having a variety of microsatellites that are characteristic for that particular population (Campbell, 2001).

DNA from both of the parents used in this experiment was isolated with the ‘HotSHOT method’ (Appendix III). Both parents where genotyped with a total of 20 microsatellites. Based on the results from this initial analysis, four microsatellites were selected for the possible identification of triploid offspring: Gmo2, Gmo19, GmoG25 and Tch11. In order to increase the possibility of identifying a triploid, four different loci was selected, and one triploid locus in an individual is sufficient for a positive result.

DNA from the offspring was also isolated by the ‘HotSHOT’ method. The four selected primers were mixed in order to run only one PCR reaction per offspring: 2 µl DNA and 8 µl PCR mix (primers, buffer, $MgCl_2$, dNTP’s and dH_2O). The PCR was run for 23 cycles with an annealing temperature of 56 ºC. For identification the forward primer was labelled with fluorescence (each primer gives a different “colour”), and all PCR products were run on an ABI sequencer (ABI3730) for allele separation. The raw data from the sequencer was analysed in GeneMapper, Software v4.0 (Applied Biosystems, Foster City, USA), to determine the size of each allele, and allow identification of any triploids (Geir Dahle, IMR, 2011, pers. comm.).

2.5 Statistical analysis

All data were analysed in Statistica 10 (StatSoft inc. Tulsa, USA). A one way ANOVA was used to test for significant differences in mean weight and length and also for significant differences between sampling days. Both were followed by SNK post hoc test ($p<0.001$). A two way ANCOVA was used to test for significant differences in blood cell diameter between the experimental groups, with weight as a co-variable. In case of significant ANCOVA this was followed by SNK post hoc test ($p<0.001$) to reveal differences between treatments. Data are presented as mean ± SE.

3. Results

3.1 Fertilization and mortality on egg stage

Fertilization rate was calculated both before and after pressure treatment (a) and b) samples), and ranged from 52.0 – 60.0 % for the a) samples and 41.5 – 65 % for the b) samples (Table 3.1). The experimental groups were fertilized in the order 600 bar, 500 bar, 400 bar, and last the Control group. Each group were fertilized 10 min past the previous one, making the last group fertilized 30 min after the first. Although the eggs for the Control group were left for 30 min until fertilization, it had no effect on the fertilization rate. The a) sample of the Control group had a higher fertilization rate compared to the a) sample of both the 400 and 500 bar group. The b) sample of the Control group had a lower fertilization rate compared to the b) sample in the 400 bar group (fertilized 10 min earlier than the Control group), but was higher compared to the b) sample in the 500 bar group, which was fertilized 20 min before the Control group. The 600 bar group had the highest fertilization rate in both the a) and b) sample of all the experimental groups.

Table 3.1: Fertilization rate calculated on day 1 post fertilization.

	Control		**400 bar**		**500 bar**		**600 bar**	
	a)	*b)*	*a)*	*b)*	*a)*	*b)*	*a)*	*b)*
Fertilized (N)	81	85	71	83	64	56	84	93
Unfertilized (N)	60	69	56	62	59	79	56	50
Total (N)	141	154	127	145	123	135	140	143
Fertilization rate (%)	57.5	55.2	55.9	57.0	52.0	41.5	60.0	65.0

The total amount of dead eggs in the incubation stage varied from 62 – 83 %; with the highest mortality rate in the 500 bar group and lowest in the 600 bar group (Table 3.2).

Table 3.2: Volume of dead eggs (ml) measured at day 1 and day 10 post fertilization.

	Control		**400 bar**		**500 bar**		**600 bar**	
	ml dead	% dead	ml dead	% dead	ml dead	% dead	ml dead	% dead
12 May 2010 (1 dpf)	10.0	20.0	5.0	10.0	17.5	35.0	5.0	10.0
21 May 2010 (10 dpf)	30.0	60.0	34.0	68.0	24.0	48.0	26.0	52.0
Total	40.0	80.0	39.0	78.0	41.5	83.0	31.0	62.0

3.2 Embryo development

No visual differences or deformities between the control group (d) and each treatment group (400 (a), 500 (b) and 600 bar (c)) was observed on embryonic stage 4 days pre hatching (Figure 3.1).

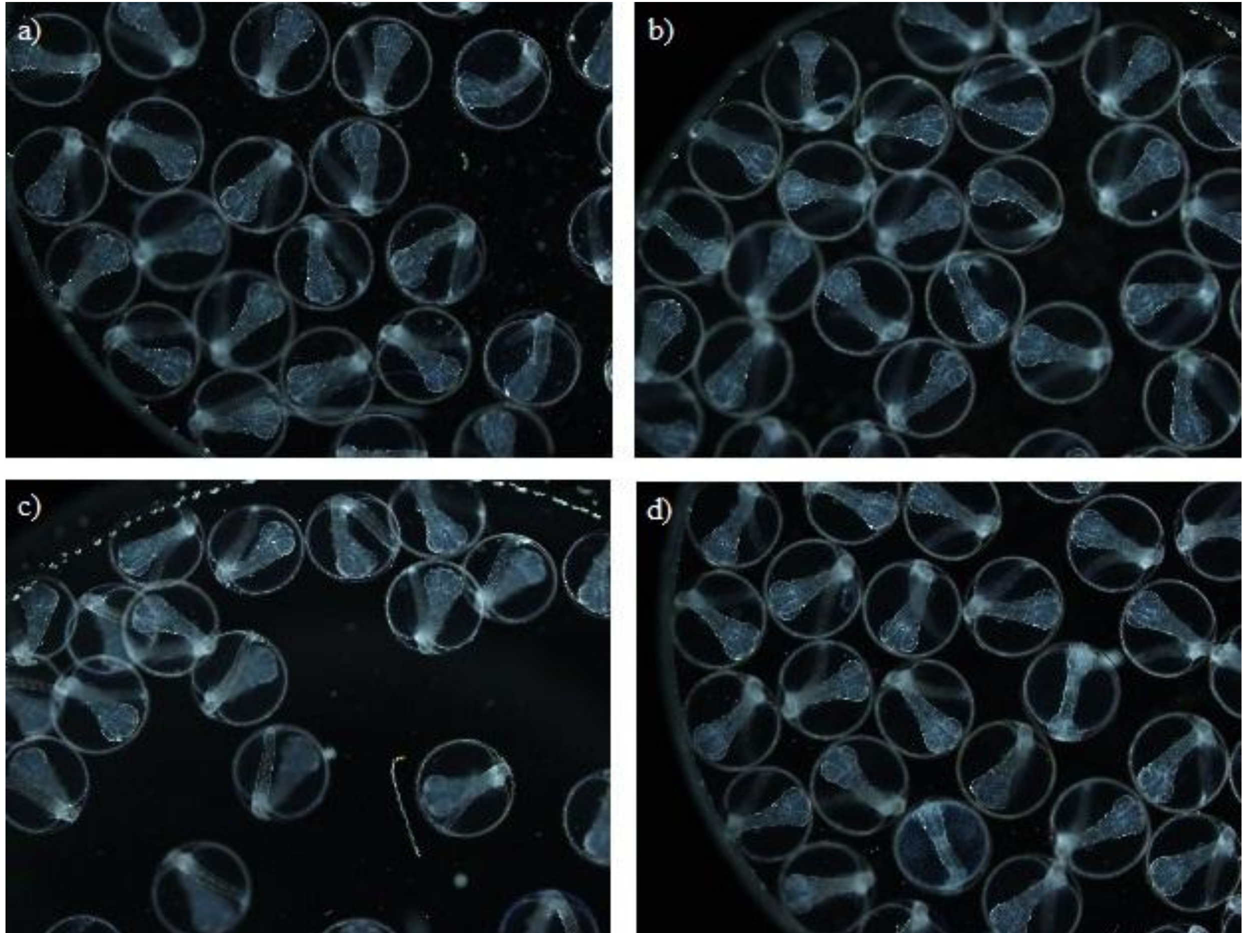

Figure 3.1: Developing embryos 4 days pre hatching. 400 bar (a), 500 bar (b), 600 bar (c) and untreated control group (d).

3.3 Length and weight

Mean length and weight measurements within each group are presented in Figure 3.2. As the Diploid group (see section 2.2) hatched at a different date compared to the other three groups, weight and length data for this group are not shown in Fig. 3.2. The mean length of the 400 bar group was significantly lower compared to the 500 and 600 bar group (SNK test; $p<0.001$, Table XV, Appendix II). Concerning mean weight, the 400 bar group were only significantly different from the 600 bar group (SNK test; $p<0.001$, Table XIV, Appendix II).

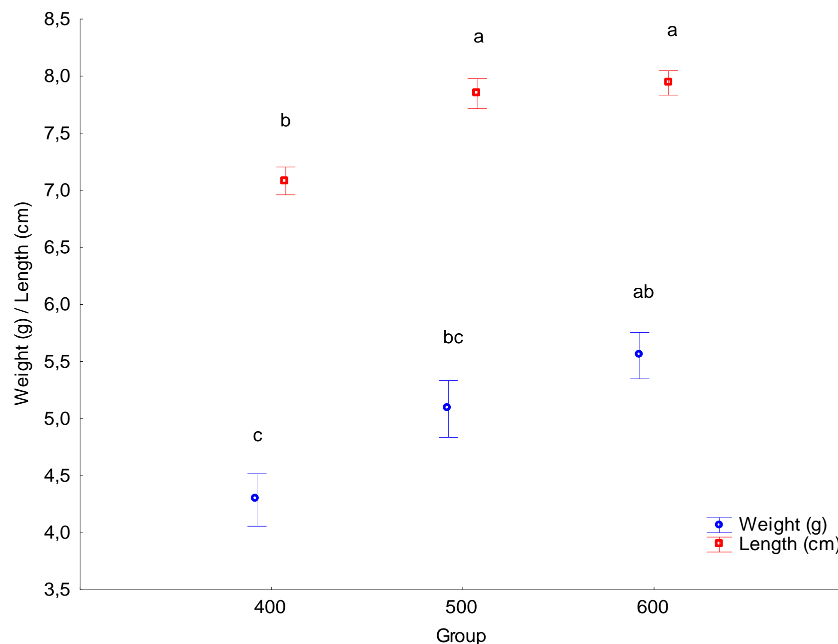

Figure 3.2: Mean weight (g) and length (cm) ± SE of each treatment group at the end of the experiment (125 – 134 dph) (Table I – Table II, Appendix II). Different letters indicate differences between experimental groups (Student-Newman-Keuls multiple comparison (SNK) test: $p<0.001$. Table XIV – XV, Appendix II).

There were significant differences in both length and weight within the 400 (Figure 3.3) and 600 (Figure 3.4) bar group between the different sampling dates (Student-Newman-Keuls multiple comparison (SNK) test; $p<0.001$, Table XVI – Table XIX, Appendix II).

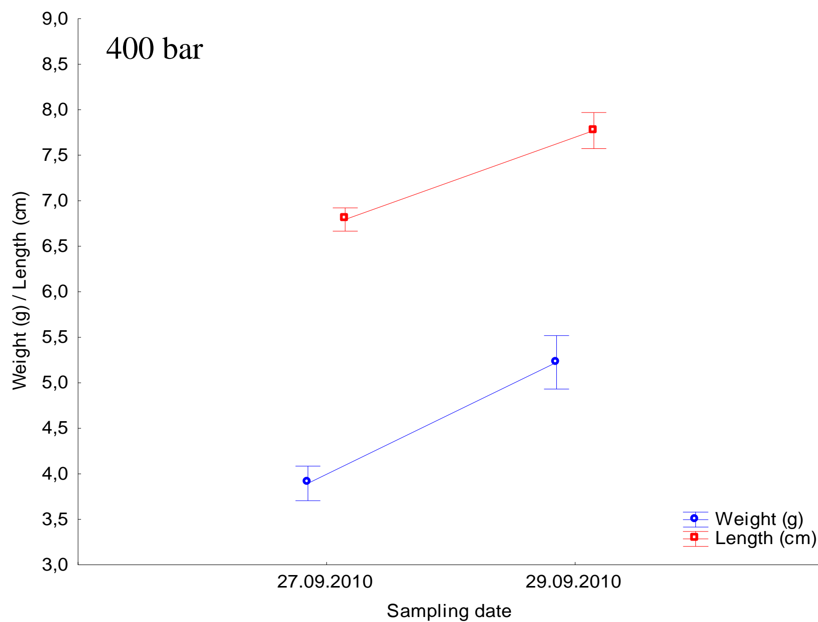

Figure 3.3: Mean weight (g) and length (cm) from two different sampling days of the 400 bar group (Table X – Table XI, Appendix II). The different groups are separated by colour and symbols (presented in figure box).

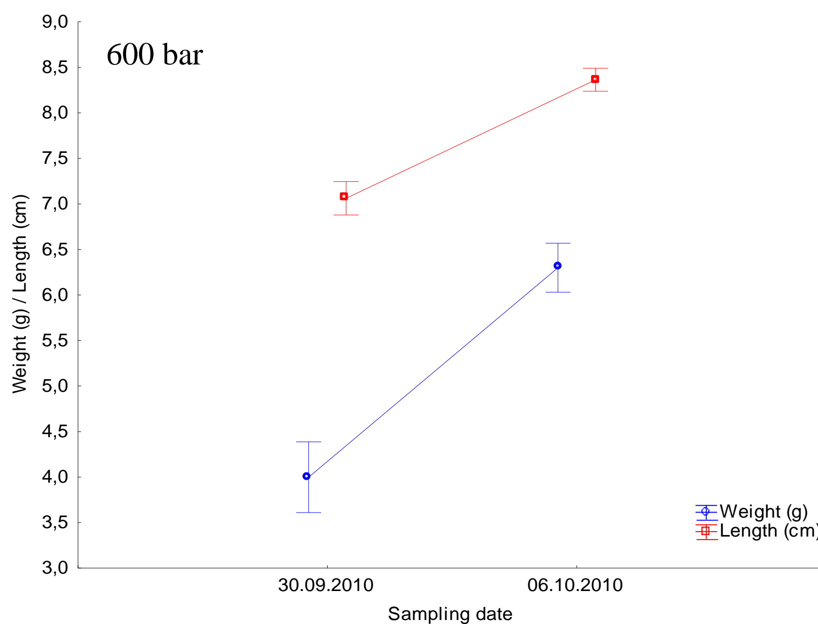

Figure 3.4: Mean weight (g) and length (cm) from two different sampling days of the 600 bar group (Table XII – Table XIII, Appendix II). The different groups are separated by colour and symbols (presented in figure box).

3.4 Effects of pressure on blood cell diameter

Blood cell diameter analysis revealed a positive correlation between mean blood cell diameter and pressure treatment (Figure 3.5), increasing pressure results in increasing mean blood cell diameter (Table III, Appendix II).

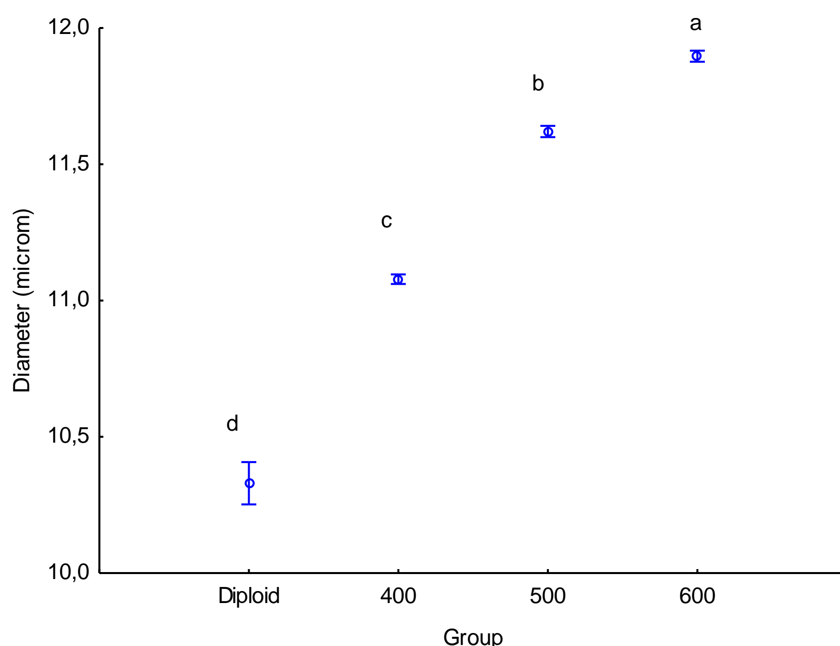

Figure 3.5: Mean blood cell diameter (µm) ±SE of all treatment groups. Different letters indicate differences between experimental groups (Table III and Table XXI, Student-Newman-Keuls multiple comparison (SNK) test; $p<0.001$, Table XX, Appendix II).

The experimental groups had significantly different blood cell diameter (SNK test; $p<0.001$, Table XX, Appendix II).

Figure 3.6 shows blood cells from four individuals, each representing approximately the mean diameter within the respective treatment group (as displayed in Figure 3.5), including the Diploid group. Comparison between total group mean and individual mean blood cell diameter (Fig. 3.6) are shown in Table 3.3.

Table 3.3: Comparison of mean blood cell diameter within the group level and the individuals shown in Fig. 3.6.

Group	Total group mean (µm)	Individual mean (µm)
Diploid	10.33	10.30
400 bar	11.08	12.05
500 bar	11.62	11.67
600 bar	11.90	11.91

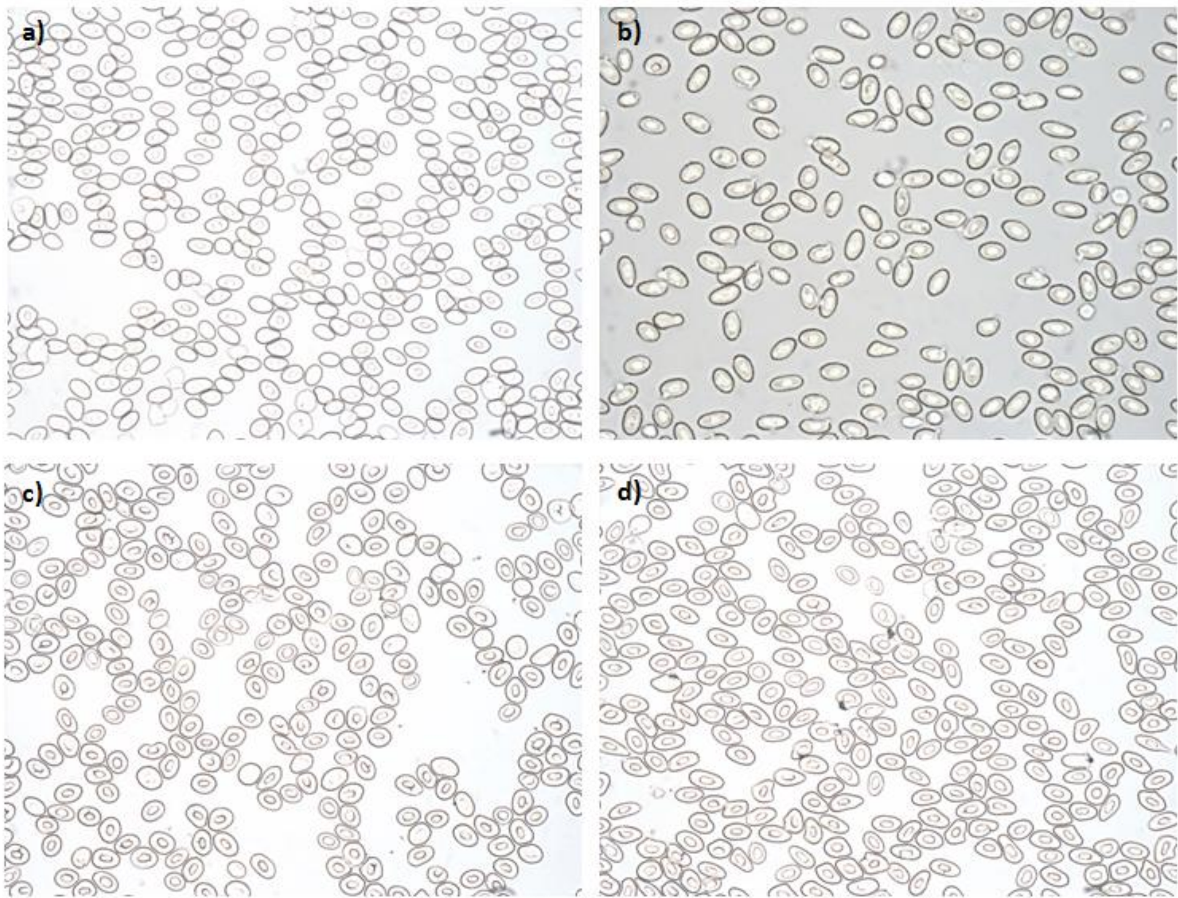

Figure 3.6: Selected area of the blood smear from Diploid (a), 400 bar (b), 500 bar (c) and 600 bar (d) group.

The pressure treated Atlantic cod in the experimental groups were distinguishable from the Diploid control group by the size difference in erythrocyte measurements. Pressure treated samples/Diploid control group had a ratio ranging from 1.07 – 1.15 (Table 3.4), i.e. the mean blood cell diameter of the 400 bar group was 7 % larger compared to the Diploid group, 12 % larger in the 500 bar group compared to the Diploid group, and the 600 bar had a 15 % larger mean blood cell diameter compared to the Diploid group.

Table 3.4: Blood cell diameter measurements in diploid and pressure treated samples. Data are presented as mean ± SE.

Group	Blood cell diameter ± SE	Ratio (triploid : diploid)
Diploid	10.33 ± 0.040	-
400 bar	11.08 ± 0.009	1.07
500 bar	11.62 ± 0.011	1.12
600 bar	11.90 ± 0.010	1.15

Distribution of blood cells within two individuals from the diploid group (a and b), the 400 bar group (c and d), and the 600 bar group (e and f) are shown in Figure 3.7. Each distribution is shown with the same x-axis scale. Two individuals from the 400 bar group had the lowest mean blood cell diameter in the entire 400 bar group, and are individuals not identified as triploids by the microsatellite loci analysis (see section 3.5). The two individuals from the 600 bar group are individuals identified as triploids by the microsatellite loci analysis.

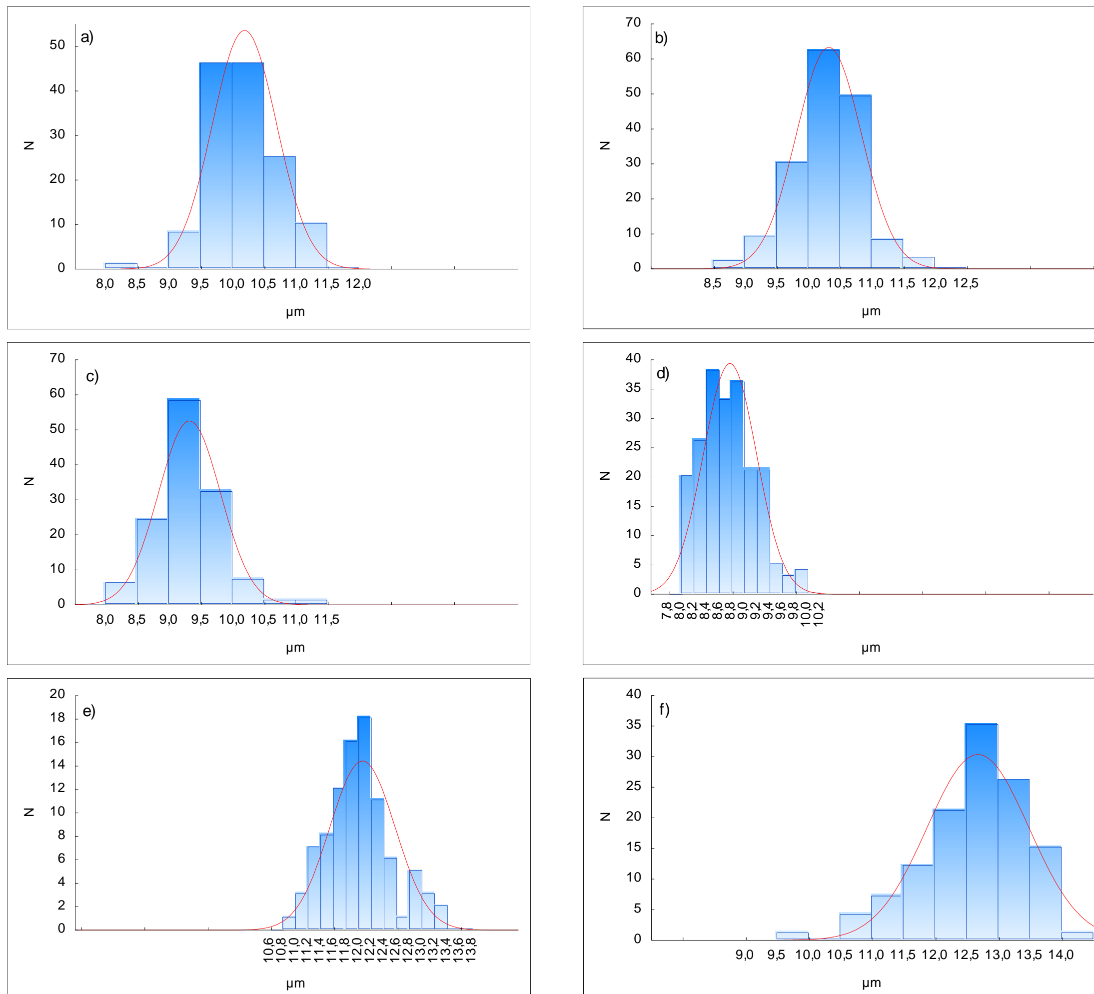

Figure 3.7: Distribution of blood cells from two individuals in the diploid group (a: N=136, b: N=163), two from the 400 bar group (c: N=129, d: N=207), and two individuals from the 600 bar group (e: N=120, f: N=122).

3.5 Microsatellite loci analysis

The PCR reaction was successful for all 250 individuals examined (80 samples in the 400 and 500 bar groups, and 90 samples in the 600 bar group including 10 pre-tested fish). However, three alleles in at least one of the four loci, i.e. positively identified triploids were not found in all individuals. Results within each treatment group are presented in Table 3.5.

Table 3.5: Results from microsatellite loci analysis (including the 10 pre-tested in the 600 bar group).

Treatment	Not identified as triploids (N)	Not identified as triploids (%)
400 bar	7 out of 80	8.8
500 bar	2 out of 80	2.5
600 bar	9 out of 90	10

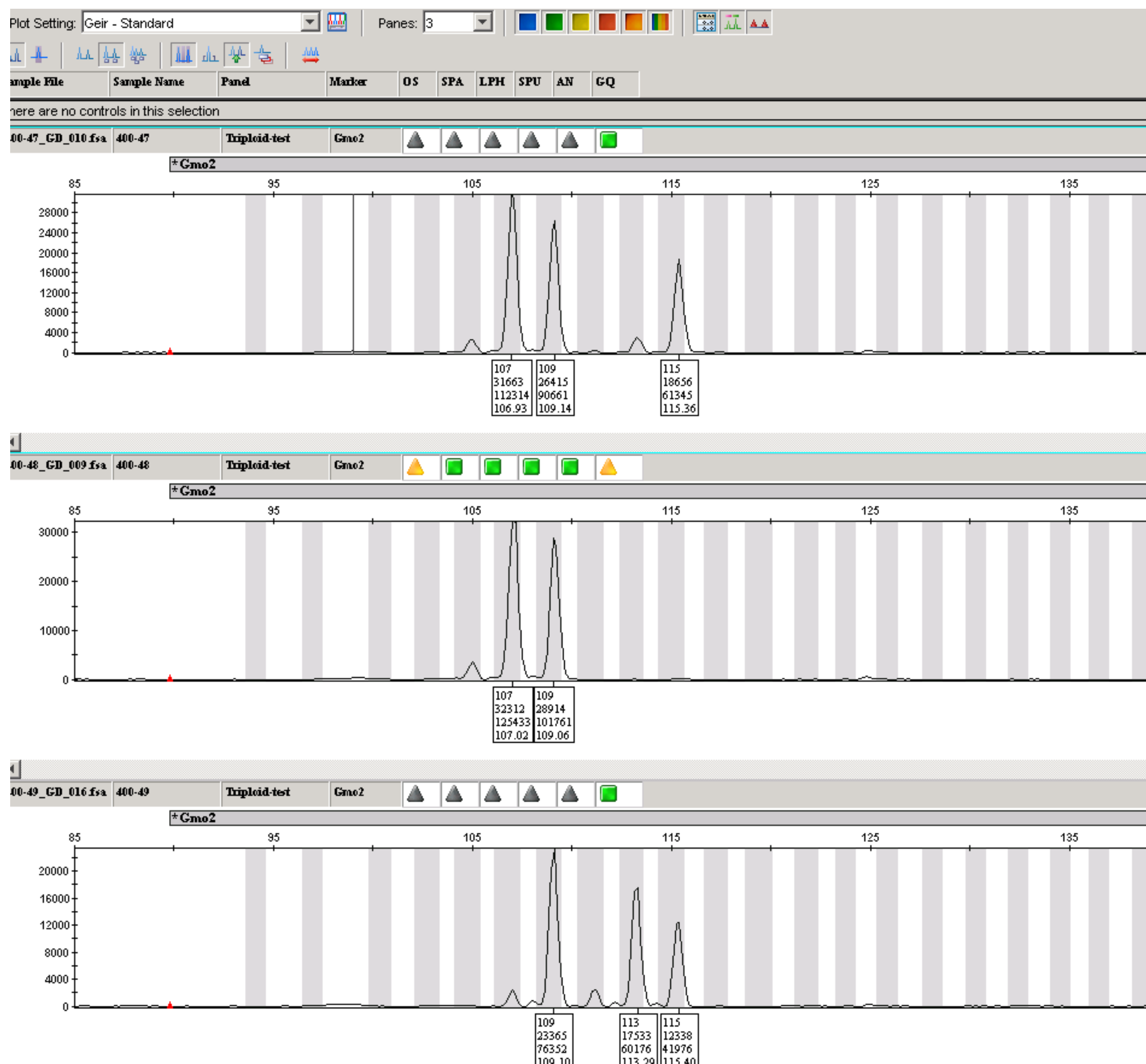

Figure 3.8: Analyse of raw data from the ABI sequencer by GeneMapper. Each peak represents a positive results of the particular loci (Gmo2). Three peaks mean triploid, and two peaks imply diploid for this particular microsatellite locus in these three individuals.

Figure 3.8 shows three individuals analysed by GeneMapper. Top and bottom individuals are triploid while the individual in the middle is diploid for this particular microsatellite loci (Gmo2).

The samples that could not be identified as triploids in GeneMapper are presented with red circle in Figure 3.9. Each spot represent the mean blood cell diameter value of each individual within the respective group. The data indicated a higher variance in mean blood cell diameter between individuals with decreasing pressure and larger variation in mean blood cell diameter between individuals in experimental group of 400 bar (b), compared to the 600 bar group (d) was seen.

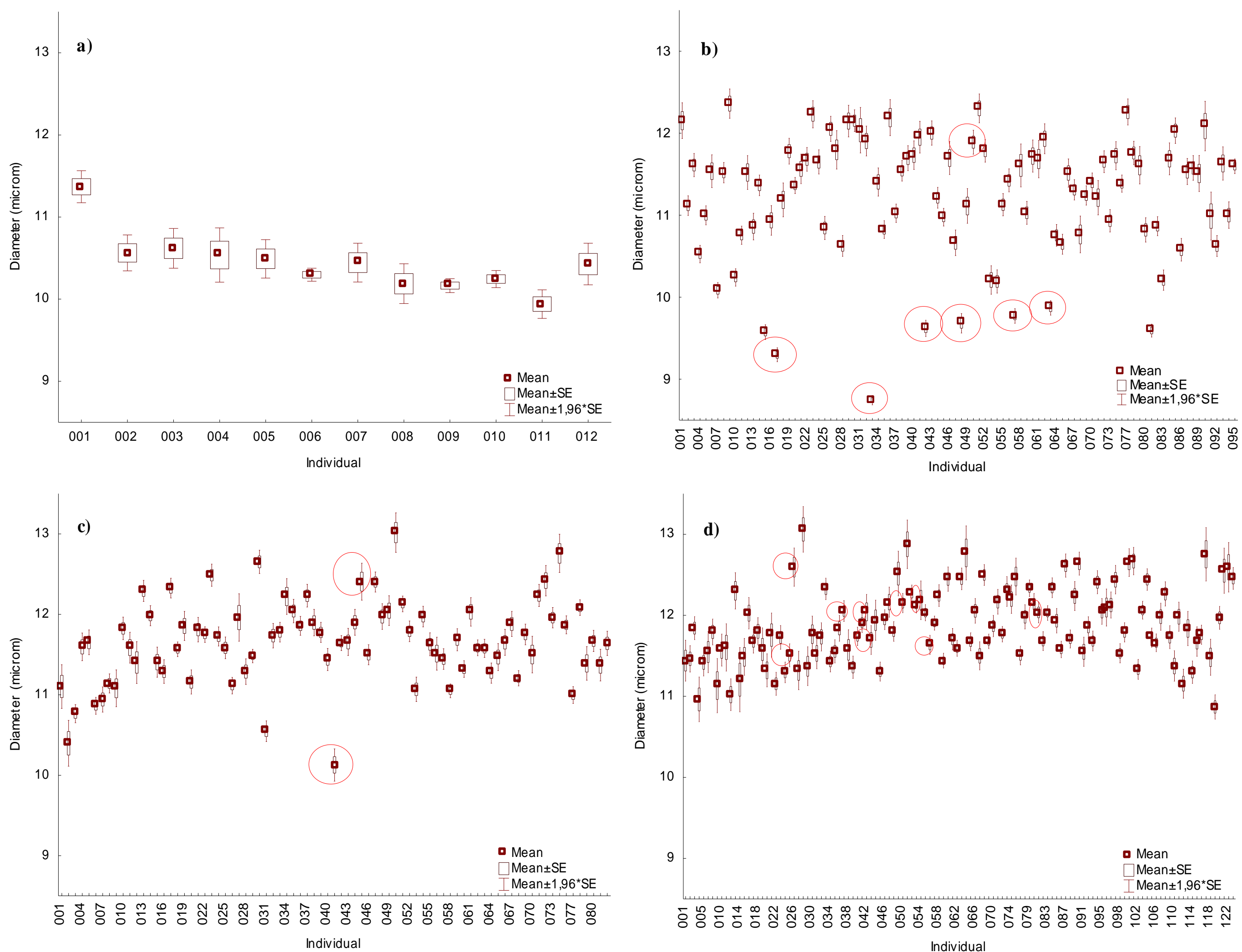

Figure 3.9: Box & Whiskers plot of mean blood cell diameter of each individual fish within the Diploid group(a), 400 bar (b), 500 bar (c) and 600 bar (d) (Table IV – Table VII, Appendix II). Red circle marks individuals not identified as triploids by microsatellite analysis.

4. Discussion

The present study demonstrated that the induction of triploidy occurred on each pressure level used in this experiment. Also, pressure treatment had an effect on blood cell diameter; increasing pressure resulted in increased mean blood cell diameter. Moreover, microsatellite loci analysis revealed over 90 % triploid outcome in each experimental group, whereas the remaining number could not be identified as triploids.

Induced triploidy in Atlantic cod by pressure treatment generally utilizes a hydrostatic pressure of 586 bar (Trippel et al., 2008, Feindel et al., 2010, Feidel et al., 2011). To the author's best knowledge, no one has examined different pressure levels in relation to deformities of triploid Atlantic cod. However, Huergo and Zaniboni-Filho (2006) examined the induction of triploidy in South American catfish (*Rhamdia quelen,* Quoy & Gaimard, 1824), and found that a pressure of 345 bar (3.4473950×10^7 Pa) was efficient enough to induce 100 % triploidy. They also found that a higher pressure of 414 bar (4.1368740×10^7 Pa), resulted in a higher embryonic deformity compared to a pressure of 345 bar.

The present study revealed, that triploid offspring did occur with over 90 % success rate at pressure levels as low as 400 bar. On the other hand, according to Piferrer et al. (2009), the optimal pressure level to prevent the extrusion of the second polar body lies between 580 – 850 bar with the optimal level around 620 bar. Nevertheless, the mechanism of induced triploidy by pressure treatment is still not fully understood. Piferrer et al. (2009) suggest that it probably has an effect on the meiotic spindle, or by the fact that the pressure is acting on the plasma membrane of the oocyte and thereby literally prevents the extrusion of the second polar body.

Given the results from the present study, pressure shock seems to be a reliable method to induce triploidy in Atlantic cod. According to present findings, the pressure itself may not be as important as previously believed in the induction of triploidy. However, due to the unfortunate mortality in the original control group (see Materials and Methods, and Discussion of Materials and Methods, Appendix I), it was not possible to determine the incidence and degree of deformities of triploid fish induced at a lower pressures than today's standard pressure level of 586 bar for Atlantic cod (Trippel et al., 2008; Feindel et al., 2010; Feindel et al., 2011), hence it was not possible to evaluate whether decreasing pressure may affect deformities in either directions. However, pictures of developing eggs 4 days before hatch did not reveal any visual developmental differences between the four experimental

groups. Felip et al. (2001) reviewed triploid induction with emphasis on marine species and suggested that deformities are considered to occur because of the handling during artificial fertilization, abnormal changes in chromosomes during cell division or inbreeding since captive broodstock are typically used in aquaculture. Furthermore, studies suggest that external morphology of triploid marine fish is mainly similar, compared to diploids (Felip et al., 2001). This was supported by Trippel et al. (2008) who stated that deformities found in their study of triploid Atlantic cod were rare and not different between diploids and triploids. The few deformities found were dominant in the head region.

Fertilization rate in this study was calculated both before and after pressure treatment to check if the pressure treatment itself had any effect on the fertilization rate, and also if fertilization rate was influenced by the fact that each group was fertilized at different specified time (each group with a 10 min delayed fertilization from the previous one). No specific trend was found between the experimental groups, nor between samples before and after pressure treatment. Although the control group was fertilized last, it had a higher fertilization rate compared to the 500 bar group who was fertilized secondly. This shows that if newly stripped unfertilized, good quality eggs are stored at proper conditions, a period of up to 1 hour before fertilization has no effect on the fertilization rate (Anders Mangor-Jensen, IMR, 2011, pers. comm.). Mortality during incubation was 80 % in the control group, and 78 and 83 % in the 400 and 500 bar group respectively, and lowest (62 %) in the 600 bar group. Since there were no replicates during the incubation period this could indicate a real pressure effect, or alternatively a tank effect, although more data are needed to verify this. It's important to note that these mortality values also include unfertilized eggs.

The different experimental groups were sampled over a period of 9 days, and the overall mean weight and length between each group differed from each other. Generally, both length and weight showed an increasing trend towards higher pressure treatment, but this could be due to the fact that the experimental groups were sampled in sequential order related to the pressure treatment. Both the 400 and 600 bar group was sampled over two days each. The results revealed significantly differences in both length and weight within the two groups between the different sampling days. Again, due to the unfortunate mortality of the initial control group at 14 days post hatch (dph), it was not possible to determine any influence on growth by triploid induction. Since the Diploid substitute control group was fertilized at a different time and was from different parents than our experimental groups, this group could not be used as a control group in comparing growth rate between pressure treated and non-pressure

treated groups. However, the results from Figure 3.2 showed that the mean length of the 400 bar group was significantly ($p<0.001$) lower compared to the 500 and 600 bar group. In addition, the 400 bar group had significantly lower mean weight than the 600 bar group. Since the time span for sampling was 9 days, these results could indicate a higher growth rate in the 500 and 600 bar groups compared to a lower pressure level of 400 bar, but further research is needed. There are contrasting results of growth of triploid fish compared to diploid fish. In theory, the triploids should grow faster than the diploids since the cell size is larger in triploids than in diploids, hence triploids possess a higher overall heterozygosity. Since the triploids in theory do not develop gonads the energy should be more divert from gonadal growth to somatic growth. However, depending on species and environmental conditions, triploid fishes usually seem to grow equal or less than diploids (Piferrer et al., 2009). Apparently, triploids divert surplus energy into fat deposits rather than into growth of the muscle mass. In these cases, the nutritional regime needs to be altered in order to address the problem (Piferrer et al., 2009).

Due to the extra chromosome in triploid individuals, triploid cells contain, by definition, 50 % more DNA than a diploid cell. As a result of this extra chromosome cell size is increased in triploids (Benfey, 1999), as confirmed in this study. In theory, triploid cells should be 1.5 times the size compared to a diploid cell. The data from present study indicate an increase in blood cell diameter related to the increase in pressure levels, but none of the blood cells measured, regardless of the pressure level, matched a 50 % increase in mean blood cell diameter compared to the Diploid control group. The highest triploid: diploid ratio was found in the 600 bar group, where the ratio was 1.15, i.e. the triploid cells in the 600 bar group was overall 15 % larger compared to the Diploid group. The quantity of blood cells were not measured in this study, but it would presumably be a lower amount of blood cells in the pressure treated groups (triploids) compared to the Diploid group, (see Benfey 1999). Despite less numbers of cells, hematocrit levels are approximately equal in diploids and triploids (Benfey, 1999). Given the high variance in mean blood cell diameter between each individual within the treated groups, especially in the 400 bar group, blood cell diameter measurements alone are inadequate to verify the ploidity level. The mean blood cell diameter of each experimental group shows a positive correlation to increased pressure level, but when looking at mean blood cell diameter of individuals within the respective group levels, some blood cell values are equal and even lower than the mean blood cells value of individuals in the Diploid control group. However, Figure 3.7 shows that the two individuals in the 400 bar group with

the lowest mean blood cell diameter are all shifted towards left, revealing an overall low level of all blood cells measured, whereas the two triploid individuals in the 600 bar group have a blood cell diameter shifted towards right. The reason for this low blood cell diameter in some individuals in the 400 bar group, is unknown. Nevertheless, the fact that increased ploidity levels do increase the erythrocyte size is now so well accepted that some researchers only measure cell size in order to determine the ploidy level (Benfey, 1999). According to the findings in the highest pressure group (600 bar) in this study, triploidy can be confirmed according to blood cell diameter only, but not if pressure levels are decreased down to 400 bar.

Analyses of mean blood cell diameter of individual fish samples within each respective pressure treated group (Figure 3.9), revealed decreased variance between the individuals within the 600 bar group. Further, some individuals that had a mean blood cell diameter that were smaller than what is considered within the normal range, could not be identified as triploids by microsatellite loci analysis. This was the case only in the 400 and 500 bar group, as in the 400 bar pressure group, 6 out of 7 individuals that had a mean blood cell diameter below 10 µm could not be identified as triploids. It is possible that these individuals were diploids, as comparison with the mean blood cell diameter in the Diploid control group was between 10 – 11 µm. On the other hand, individuals in the 400 bar group with a mean blood cell diameter between 10 – 11 µm were confirmed to be triploids by the microsatellite analysis. In the 500 bar group, two individuals could not be identified as triploids by the microsatellite loci analysis. However, the individuals who could not be identified as triploids in the 600 bar treatment group had similar blood cell diameter as other individuals in this group. This could suggest that these individuals can be considered as triploids.

Given the nature of the triploid formation, there are some challenges using genotyping as a method for determining triploid success. The first is the fact that only one chromosome (either paternal or maternal chromosome) from the two chromosomes in a normal cell will take part in the formation of the triploid. In order to be able to identify any triploids in the offspring, an exchange of DNA material from the paternal chromosome to the maternal chromosome (recombination) must occur in the female. This is a very common reaction, but as a result the only conclusion that can be drawn is that the offspring with three alleles in one locus are triploids. The lack of three alleles in the offspring however, might only indicate a missing recombination. The offspring can still be triploid (Geir Dahle, IMR, 2011, pers. comm.).

Deformities have serious welfare implications for cultured fish. If jaw deformities are developed, the fish will have problems ingesting food. On the other hand, from the fish farmers' perspective, the deformed fish cannot be sold as a whole fish, but can still be suitable for marked as cut fillets (Benfey, 2001). If skeletal deformities arises the fish will have trouble to maintain a normal swimming pattern. Deformities are well reported in several triploid species, especially in triploid Atlantic salmon (*Salmo salar*). Fjelldal and Hansen (2010) found that triploid Atlantic salmon smolts appeared to develop more vertebral deformities, and Leclercq et al. (2011) observed a higher occurrence of vertebral deformities, ocular cataracts, external deformities and also differences in heart morphometry in triploid salmon compared to diploids. Deformities found in farmed fish have several negative aspects, including the welfare for the fish itself (Poli, 2009). Piferrer et al. (2009) reviewed several studies regarding deformities in triploid fish, and concluded that the main cause of the high occurrence and the severity of deformities are due to the physical manipulation. However, other studies suggest that the triploid condition alone is the main cause of deformities (Piferrer et al., 2009). In addition, environmental factors (such as dissolved oxygen, temperature, light intensity and salinity) and malnutrition can also be the source of deformities (Imsland et al., 2006; Poli, 2009), and therefore a precise rearing-regime needs to be incorporated to triploid production in order to avoid any unnecessary deformities.

If triploid fish are to be accepted into the consumers marked, proper information about positive outcomes of a triploid fish in production is needed. It is important not to confuse consumers that triploid fish in some way is a transgenic fish. Some people find it ethically debatable to transfer genes from one species to another (Kaiser, 2005). However, the induction of triploidy is not regarded a genetic modification (GM). This is due to the fact that triploidy does not modify DNA sequences, but only alters the chromosome segregation (Triantafyllidis et al., 2007). Furthermore, Triantafyllidis et al. (2007) suggest that consumers' reaction and acceptance to sterile fish will depend on how the information, e.g. the environmental advantages of using triploid fish in aquaculture purposes, is presented. Consumers are already (perhaps unknowingly) consuming polyploid vegetables and fruits (Piferrer et al., 2009). In addition, labeling of polyploidy is not required (Triantafyllidis et al., 2007).

In order to address the problem regarding negative effects of a triploid male cod in commercial production, triploidy can be combined with all-female production (Taranger et al., 2010). This combination is now used in most farmed trout stocks (Piferrer et al., 2009).

Feindel et al. (2011) suggest that triploid all-female production could result in low gonado-somatic index, reduced gamete production and a high gutted head-on carcass yield compared to diploids.

Conclusions and further perspectives

Several studies, including the present study, have demonstrated a great number of successfully induced triploid individuals as a result of high pressure treatment (approximately 600 bar). But, this study additionally demonstrated successfully triploid induction at both low (400 bar) and medium (500 bar) pressure. Further research is needed in order to ascertain the exact triploid induction window, i.e. which exact pressure level, time after fertilization and time of pressure treatment is needed to prevent the extrusion of the second polar body in Atlantic cod. Further, it is important to rear the triploid cod up to harvest size in order to examine essential condition factors, like deformities, growth potential, maturation and flesh quality. After an optimal pressure level is found, the combination of triploid all-female production would be the final step towards a sustainable, sterile farmed cod. The optimal farmed Atlantic cod will meet the demand of all parties involved, resulting in a market fish with high growth rate, high flesh quality, reduced effect of sexual maturation, reduced influence on wild counterparts, and no severe deformities observed.

5. Hypothesis evaluation

H_{A1}: Increasing pressure will result in increasing rate of triploid induction.

- Microsatellite loci analyzes revealed over 90 % triploid outcome in each experimental group. Highest rate of confirmed triploids was found in the 500 bar group, whereas the lowest rate was found in the 600 bar group. Hence, H_{A1} is not accepted.

H_{A2}: The group exposed to the highest pressure (600 bar) will consist of triploid individuals only.

- 9 out of 90 individuals in the 600 bar group were confirmed to be triploids. Still, the rest are not necessarily diploids (could not be confirmed to either diploid or triploid). Therefore, H_{A2} is partly accepted.

H_{A3}: Mean blood cell diameter will increase with increasing pressure.

- Mean blood cell diameter was positively correlated to increasing pressure level. Therefore, H_{A3} is accepted. However, blood cell diameter analyze alone was inadequate to confirm triploid result, especially at lowest pressure level (400 bar).

H_{A4}: Mean blood cell diameter and results from microsatellite loci analyses will be positively correlated.

- Mean blood cell diameter of each group increased with increasing pressure level. However, highest confirmed triploid rate was found in the 500 bar group and lowest in the 600 bar group. Therefore, the two analyze methods do not confirm each other. Hence, H_{A4} is not accepted.

References:

Allen Jr, S.K. (1983) Flow cytometry: Assaying experimental polyploid fish and shellfish. Aquaculture 33, 317-328.

Bekkevold, D., Hansen, M.M. and Nielsen, E.E. (2006) Genetic impact of gadoid culture on wild fish populations: predictions, lessons from salmonids, and possibilities for minimizing adverse effects. ICES Journal of Marine Science: Journal du Conseil 63, 198-208.

Benfey, T.J. (1999) The Physiology and Behavior of Triploid Fishes. Reviews in Fisheries Science 7, 39 - 67.

Benfey, T.J. (2001) Use of sterile triploid Atlantic salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada. ICES Journal of Marine Science: Journal du Conseil 58, 525-529.

Benfey, T.J. and Sutterlin, A.M. (1984) Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). Aquaculture 36, 359-367.

Berg, E. (2010) Torsk - Kysttorsk nord for 62°N. In: Gjøsæter, H., Haug, T., Hauge, M., Karlsen, O., Knutsen, J.A., Røttingen, I., Skilbrei, O. and Hoddevik Sunnset, B. (eds.) *Havforskningsrapporten 2010.* Havforskningsinstituttet, Norway, pp. 153.

Bogstad, B. (2010) Torsk - Nordaustarktisk. In: Gjøsæter, H., Haug, T., Hauge, M., Karlsen, O., Knutsen, J.A., Røttingen, I., Skilbrei, O. and Hoddevik Sunnset, B. (eds.) *Havforskningsrapporten 2010.* Havforskningsinstituttet, Norway, pp. 155.

Brown, J.A., Minkoff, G. and Puvanendran, V. (2003) Larviculture of Atlantic cod (*Gadus morhua*): progress, protocols and problems. Aquaculture 227, 357-372.

Cal, R.M., Vidal, S., Gómez, C., Álvarez-Blázquez, B., Martínez, P. and Piferrer, F. (2006) Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 251, 99-108.

Campbell, M.A. (2001) Microsatellite DNA Methology [Online]. Department of Biology, Davidson College Available: http://www.bio.davidson.edu/courses/genomics/method/microsatellite.html [Accessed 15/08-11 2011].

Colas, P. and Dubé, F. (1998) Meiotic maturation in mollusc oocytes. Seminars in Cell & Developmental Biology 9, 539-548.

Conover, D.O., Clarke, L.M., Munch, S.B. and Wagner, G.N. (2006) Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. Journal of Fish Biology 69, 21-47.

Davie, A., Porter, M.J.R., Bromage, N.R. and Migaud, H. (2007) 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 64, 84-97..

FAO. (2008) Production from aquaculture by country and by species [Online]. FAO. Available: ftp://ftp.fao.org/FI/CDrom/CD_yearbook_2008/navigation/index_content_capture_e.htm [Accessed 23-02-2011].

Feindel, N.J., Benfey, T.J. and Trippel, E.A. (2010) Competitive spawning success and fertility of triploid male Atlantic cod *Gadus morhua*. Aquaculture Environment Interactions 1, 47-55.

Feindel, N.J., Benfey, T.J. and Trippel, E.A. (2011) Gonadal development of triploid Atlantic Cod *Gadus morhua*. Journal of Fish Biology 78, 1900-1912.

Felip, A., Zanuy, S., Carrillo, M. and Piferrer, F. (2001) Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 111, 175-195.

Fjelldal, P.G. and Hansen, T. (2010) Vertebral deformities in triploid Atlantic salmon (*Salmo salar* L.) underyearling smolts. Aquaculture 309, 131-136.

Gjøsæter, J., Jørstad, K., Naevdal, G. and Thorkildsen, S. (1992) Genotype distributions of cod from the Norwegian Skagerrak coast. Sarsia 76, 255-259.

Guo, X., Debrosse, G.A. and Allen, S.K. (1996) All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142, 149-161.

Hansen, L.A., Dale, T., Damsgård, B., Uglem, I., Aas, K. and Bjørn, P.-A. (2008) Escape-related behaviour of Atlantic cod, *Gadus morhua* L., in a simulated farm situation. Aquaculture Research 40, 26-34.

Hansen, T., Karlsen, Ø., Taranger, G.L., Hemre, G.-I., Holm, J.C. and Kjesbu, O.S. (2001) Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. Aquaculture 203, 51-67.

Harrell, R.M., Van Heukelem, W. and Kerby, J.H. (1998) A Comparison of Triploid Induction Validation Techniques. The Progressive Fish-Culturist 60, 221 - 226.

Havforskningsinstituttet 2007. Banebrytende resultater fra havforskningen, Akvakulturforskning 1882-2007, 4-2007. *Havforskningstema.* IMR.

Holmefjord, I. and Refstie, T. (1997) Induction of triploidy in Atlantic halibut by temperature shocks. Aquaculture International, 169-173.

Huergo, G.M. and Zaniboni-Filho, E. (2006) Triploid Induction in Jundià, *Rhamdia quelen,* Through Hydrostatic Pressure Shock. Journal of Applied Aquaculture 18, 45-57.

Imsland, A.K., Foss, A., Koedijk, R., Folkvord, A., Stefansson, S.O. and Jonassen, T.M. (2006) Short- and long-term differences in growth, feed conversion efficiency and deformities in juvenile Atlantic cod (*Gadus morhua*) startfed on rotifers or zooplankton. Aquaculture Research 37, 1015-1027.

Johnson, O.W., Dickhoff, W.W. and Utter, F.M. (1986) Comparative growth and development of diploid and triploid coho salmon, *Oncorhynchus kisutch*. Aquaculture 57, 329-336.

Johnson, O.W., Rabinovich, P.R. and Utter, F.M. (1984) Comparison of the reliability of a Coulter Counter with a flow cytometer in determining ploidy levels in Pacific salmon. Aquaculture 43, 99-103.

Jørstad, K.E., Van Der Meeren, T. and Glover, K.A. (2010) Genene avslører torskerømminger. In*:* Gjøsæter, H., Haug, T., Hauge, M., Karlsen, Ø., Knutsen, J.A., Røttingen, I., Skilbrei, O. and Hoddevik Sunnset, B. (eds.) *Havforskningsrapporten 2010.* Havforskningsinstituttet, Norway, pp. 87-89.

Jørstad, K.E., Van Der Meeren, T., Paulsen, O.I., Thomsen, T., Thorsen, A. and Svåsand, T. (2008) “Escapes” of Eggs from Farmed Cod Spawning in Net Pens: Recruitment to Wild Stocks. Reviews in Fisheries Science 16, 285 - 295.

Kaiser, M. (2005) Assessing ethics and animal welfare in animal biotechnology for farm production. Rev. sci. tech Off. int. Epiz. 24, 75-87.

Karlsen, O. and Van Der Meeren, T. (2003) Oppdrett av torsk [Online]. Institute of Marine Research. Available: http://www.imr.no/temasider/akvakultur/torskeoppdrett/oppdrett_av_torsk/oppdrett_av_torsk3/nb-no [Accessed 05/05 2011].

Karlsen, Ø., Norberg, B., Kjesbu, O.S. and Taranger, G.L. (2006) Effects of photoperiod and exercise on growth, liver size, and age at puberty in farmed Atlantic cod (*Gadus morhua* L.). ICES Journal of Marine Science: Journal du Conseil 63, 355-364.

Kjesbu, O.S. and Norberg, B. (2005) Stamfisk og gyting. In*:* Otterå, H., Taranger, G.L. and Borthen, J. (eds.) *Oppdrett av torsk - en næring med framtid.* Norsk fiskeoppdrett AS, Bergen, Norway, pp. 39-47.

Kjesbu, O.S., Taranger, G.L. and Trippel, E.A. (2006) Gadoid mariculture: development and future challenges. ICES Journal of Marine Science: Journal du Conseil 63, 187-191.

Lassen, T. (2009) Produksjon av torsk og kveite i 2008. In*:* Agnalt, A.-L., Bakketeig, I.E., Haug, T., Knutsen, J.A. and Opstad, I. (eds.) *Kyst og Havbruk 2009.* Havforskningsinstituttet, Norway, pp. 131-132.

Leclercq, E., Taylor, J.F., Fison, D., Fjelldal, P.G., Diez-Padrisa, M., Hansen, T. and Migaud, H. (2011) Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (*Salmo salar*) post-smolts. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 158, 116-125.

Lou, Y.D. and Purdom, C.E. (1984) Polyploidy induced by hydrostatic pressure in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 25, 345-351.

Mangor-Jensen, A., Øiestad, V., Van Der Meeren, T., Lein, I., Harboe, T., Opstad, I., Øie, G., Rainuzzo, J., Evjemo, J.O., Reitan, K.I. and Fyhn Terjesen, B. (2006) Fra ekstensivt til intensivt oppdrett av marin fisk. In*:* Thomassen, M., Gudding, R., Norberg, B. and Jørgensen, L. (eds.) *Havbruksforskning: Fra merd til mat.* Norges forskningsråd, Oslo, Norway, pp. 76-94.

Moe, H., Dempster, T., Sunde, L.M., Winther, U. and Fredheim, A. (2007) Technological solutions and operational measures to prevent escapes of Atlantic cod (*Gadus morhua*) from sea cages. Aquaculture Research 38, 91-99.

Moen, F.E. and Svensen, E. (2004) *Gadus morhua* - Torsk. In*:* Moen, F.E. and Svensen, E. (eds.) *Dyreliv i havet - Nordeuropeisk Marin Fauna.* KOM Forlag a/s, Kristiansund, Norway, pp. 501-502.

Nell, J.A. (2002) Farming triploid oysters. Aquaculture 210, 69-88.

Niebuhr, E. (1974) Triploidy in man. Human Genetics 21, 103-125.

Nielsen, E., Hemmer-Hansen, J., Poulsen, N., Loeschcke, V., Moen, T., Johansen, T., Mittelholzer, C., Taranger, G.-L., Ogden, R. and Carvalho, G. (2009) Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). BMC Evolutionary Biology 9, 276.

Norberg, B., Brown, C.L., Halldorsson, O., Stensland, K. and Björnsson, B.T. (2004) Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). Aquaculture 229, 451-467.

Norberg, B., Taranger, G.L. and Tveiten, T. (2006) Reproduksjonsfysiologi hos marine kaldtvannsarter i oppdrett. In*:* Thomassen, M., Gudding, R., Norberg, B. and Jørgensen, L. (eds.) *Havbruksforskning: Fra merd til mat.* Norges Forskningsråd, Oslo, Norway, pp. 62-75.

Ojolick, E.J., Cusack, R., Benfey, T.J. and Kerr, S.R. (1995) Survival and growth of all-female diploid and triploid rainbow trout (*Oncorhynchus mykiss*) reared at chronic high temperature. Aquaculture 131, 177-187.

Olsen, K. and Kristiansen, L. (2009) Markedssituasjonen for torsk og kveite fra havbruk 2008. In: Agnalt, A.-L., Bakketeig, I.E., Haug, T., Knutsen, J.A. and Opstad, I. (eds.) *Kyst og Havbruk 2009.* Havforskningsinstituttet, Norway, pp. 137-138.

Otterå, H. (2005) Yngelproduksjon - weaning og nursery. In: Otterå, H., Taranger, G.L. and Borthen, J. (eds.) *Oppdrett av torsk - en næring med framtid.* Norsk Fiskerioppdrett, Bergen, Norway, pp. 113-119.

Peruzzi, S. and Chatain, B. (2000) Pressure and cold shock induction of meiotic gynogenesis and triploidy in the European sea bass, *Dicentrarchus labrax* L.: relative efficiency of methods and parental variability. Aquaculture 189, 23-37.

Peruzzi, S., Kettunen, A., Primicerio, R. and Kauri, G. (2007) Thermal shock induction of triploidy in Atlantic cod (*Gadus morhua* L.). Aquaculture Research 38, 926-932.

Peruzzi, S., Rudolfsen, G., Primicerio, R., Frantzen, M. and Kauri, G. (2009) Milt characteristics of diploid and triploid Atlantic cod (*Gadus morhua* L.). Aquaculture Research 40, 1160-1169.

Pethon, P. (1998) Torsk, *Gadus morhua*. In: Pethon, P. (ed.) *Aschehougs store fiskebok, Norges fisker i farger.* 4 ed. H. Aschehougs & Co (W Nygaard), Stockholm, Sweden, pp. 288-290.

Phillips, R.B., Zajicek, K.D., Ihssen, P.E. and Johnson, O. (1986) Application of silver staining to the identification of triploid fish cells. Aquaculture 54, 313-319.

Piferrer, F., Beaumont, A., Falguiere, J.C., Flajshans, M., Haffray, P. and Colombo, L. (2009) Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293, 125-156.

Poli, B.M. (2009) Farmed fish welfare-suffering assessment and impact on product quality. Italian Journal of Animal Science 8, 139-160.

Quéméner, L., Suquet, M., Mero, D. and Gaignon, J.-L. (2002) Selection method of new candidates for finfish aquaculture: the case of the French Atlantic, the Channel and the North Sea coasts. Aquat. Living Resour. 15, 293-302.

Rosenlund, G. and Halldórsson, Ó. (2007) Cod juvenile production: Research and commercial developments. Aquaculture 268, 188-194.

Rosenlund, G. and Skretting, M. (2006) Worldwide status and perspective on gadoid culture. ICES Journal of Marine Science: Journal du Conseil 63, 194-197.

Ruzzante, D.E., Wroblewski, J.S., Taggart, C.T., Smedbol, R.K., Cook, D. and Goddaard, S.V. (2000) Bay-scale population structure in coastal Atlantic cod in Labrador and Newfoundland, Canada. Journal of Fish Biology 56, 431-447.

Sarvas, T.H. and Fevolden, S.-E. (2005) The scnDNA locus Pan I reveals concurrent presence of different populations of Atlantic cod (*Gadus morhua* L.) within a single fjord. Fisheries Research 76, 307-316.

Skjaeraasen, J.E., Mayer, I., Meager, J.J., Rudolfsen, G., Karlsen, O., Haugland, T. and Kleven, O. (2009) Sperm characteristics and competitive ability in farmed and wild cod. Marine Ecology-Progress Series 375, 219-228.

Skjaeraasen, J.E., Meager, J.J., Karlsen, O., Mayer, I., Dahle, G., Rudolfsen, G. and Ferno, A. (2010) Mating competition between farmed and wild cod *Gadus morhua*. Marine Ecology-Progress Series 412, 247-258.

Sogn-Grundvåg, G., Grønhaug, K., Egeness, F.-A. and Trollvik, T. 2010. Kritiske suksessfaktorer for torskeoppdrett: Finnes de? *Økonomisk fiskeriforskning*. Nofima AS.

Svåsand, T., Dahle, G., Van Der Meeren, T., Skilbrei, O. and Skaala, Ø. 2011. Rømming av laks og torsk. *Havforskningsrapporten 2011.* IMR.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.-A., Dufour, S., Karlsen, Ø., Norberg, B., Andersson, E. and Hansen, T. (2010) Control of puberty in farmed fish. General and Comparative Endocrinology 165, 483-515.

Thorpe, J.E. (2004) Life history responses of fishes to culture. Journal of Fish Biology 65, 263-285.

Thorsen, A. (2005) Egg og klekkeridrift. In*:* Otterå, H., Taranger, G.L. and Borthen, J. (eds.) *Oppdrett av torsk - næring med framtid.* Norsk Fiskerioppdrett AS, Bergen, Norway, pp. 63-69.

Tilseth, S. (1990) New marine fish species for cold-water farming. Aquaculture 85, 235-245.

Tiwary, B.K., Kirubagaran, R. and Ray, A.K. (2004) The biology of triploid fish. Reviews in Fish Biology and Fisheries 14, 391-402.

Triantafyllidis, A., Karaiskou, N., Bonhomme, F., Colombo, L., Crosetti, D., Danancher, D., Garcìa-Vàzquez, E., Gilbey, J., Svåsand, T., Verspoor, E. and Triantaphyllidis, C. 2007. Management options to reduce genetic impacts of aquaculture activities. *In:* Svåsand, T., Crosetti, D., Garzìa-Vàzquez, E. and Verspoor, E. (eds.) *Genetic impact of aquaculture activities on native populations.*

Trippel, E.A., Benfey, T.J., Neil, S.R.E., Cross, N., Blanchard, M.J. and Powell, F. (2008) Effects of continuous light and triploidy on growth and sexual maturation in Atlantic cod, *Gadus morhua*. Cybium 32, 136-138.

Øiestad, V. (2005) Torsk i oppdrett - gjennom hundre år. In*:* Otterå, H., Taranger, G.L. and Borthen, J. (eds.) *Oppdrett av torsk - næring med framtid.* Norsk Fiskeoppdrett AS, Bergen, Norway, pp. 27-32.

Appendix I

Discussion of Materials & Methods

Experimental design – Gamete collection and rearing conditions

In the present study, the cod larvae used were offspring of one female and one male, both two years old, from the broodstock at IMR Austevoll. Gamete collection occurred rather late in the spawning season (11 May 2010), and the broodstock used was probably the last mature adults who had any eggs and milt left. The female used for egg collection had a clearly large, distend abdomen at that time, which could indicate that this female was an 'irregular spawner'. Irregular spawners are characterised by distend abdomen due to accumulation of several batches, and offspring from these individuals often have a low fertilization rate (Kjesbu and Nordberg, 2005). Still, a fertilization rate of 52.0 – 60.0 % is considered acceptable. Fertilization in the present study was between 41.5 and 65 %.

Each of the three experimental groups was pressure treated for 5 min, 180 ºC min after fertilization. Although exactly pressure time of 5 min was carefully recorded, the time until required pressure were obtained varied between the groups. It took 33 seconds from 0 – 400 bar, 45 seconds from 0 – 500 bar, and 49 seconds until it reached the required pressure of 600 bar. This extra time spent could potentially influence the pressure treatment, as the real pressure time is different between the experimental groups. In further experiments, this potential problem needs to be accounted for.

After each of the four groups had been transferred from the incubation tanks to the rearing tanks, each treatment group was divided into triplicates with approximately 2000 larvae in each tank (visual amount with the help from Anders-Mangor Jensen). The triplicates were placed next to each other, to ensure no disorganization between the different treatment groups. In hindsight, it is clear that the experimental design should have been done by completely random replicates, to avoid a possible tank effect, or an area of the experimental hall. After placing the triplicates randomly in the experimental unit, each tank should have been marked by a name or a number only known by the authors' to ensure no difference in handling the fish during the experimental period.

To ensure a realistic picture of weight and length measurements, each fish should have been measured at the same day, and then later sampled for blood cell diameter.

Mortality

14 days after hatching (8 June 2010), the three replicates with the control group died. In each tank, 100 % mortality occurred over night. No reasonable cause for this sudden massive mortality was found. Bad water quality and polluted drains was discussed as a possible reason, but since all the 12 rearing tanks received water from the same header tank, there is no reason to believe that only those three groups should have such a mass mortality. The reason for this incident is still not clear, however, there is a possibility that someone cleaned or washed other tanks near the location of the control group with chlorine or some similar form of chemicals, and that this cleaning agent then spattered into the tanks with the control groups. This could represent a logical explanation as to why the control group died, since it was located furthest away from the wall and nearest to the passage way. The curtains protecting the tank were up at that point. Had they been down, this event might not have occurred. Additionally, on the 23 June 2010, 100 % mortality occurred in the middle tank holding the 400 bar group. As with the control group, this also happened over night. There is no logical reason for this, and here the curtains were down.

As a consequence of losing the entire control group, no comparative analyzes of deformities could be performed. 12 diploid fish from ordinary production at IMR Austevoll were sampled as a substitute for the lost control group in order to obtain diploid blood cells for later blood cell diameter comparisons.

Unfortunately, no recording of mortality during the experimental period was done. This should have been done, in order to see if pressure treatment had any effect on survival during the whole period. Still, due to the fact that all the fish in each experimental group were sampled, it seems that the three pressure groups had an approximately equal survival rate. Numbers of individuals sampled were 95, 81 and 123 in the 400, 500 and 600 bar group, respectively. Initial numbers of individuals in each experimental group was approximately the same (~2000 larvae x 3). Still, in order to verify this statement, mortality during the whole experiment should have been recorded.

Sampling and analyses

In order to ensure no specific growth differences between the experimental groups due to the expiration, all sampling should have been carried out in maximum two to three days. Unfortunately, this was not practically possible.

Even though blood cells are elliptic in shape, a diameter was assigned to each blood cell. The calculated area for each ellipse were recalculated to a circle with similar area, and the diameter for this circle then represent the diameter of the elliptical blood cell (Anders Thorsen 2011, pers. comm.). On the other hand, Benfey (1999) reviewed that it has been suggested that erythrocyte cell height is the same for both triploids and diploids, and that blood smears may not reflect the dimensional changes in living blood cells. This could potentially affect the results from blood cell diameter analysis, and further research regarding changes in cell size due to pressure treatment is needed.

Regarding length measurements of individual fish, maximum standard length (as shown in Figure 2.5) was used instead of fork length or total length. This was done because cannibalism had caused damages to the tail fin of a large numbers of individuals.

Appendix II

Descriptive statistics

Table I: Descriptive statistics based on weight measurement of all fish within each treatment group at experimental end (125-134 dph). Numbers of observations, means, standard deviation (SD), standard error (SE), minimum and maximum are included in the table.

Weight (g) - All Treatment Groups						
Group	N	Mean	SD	SE	Min	Max
400	95	4.287	1.664	0.171	1.421	8.34
500	81	5.085	2.131	0.237	1.182	9.603
600	123	5.551	2.672	0.241	0.896	14.822

Table II: Descriptive statistics based on weight measurement of all fish within each treatment group at experimental end (125-134 dph). Numbers of observations, means, standard deviation (SD), standard error (SE), minimum and maximum are included in the table.

Length (cm) - All Treatment Groups						
Group	N	Mean	SD	SE	Min	Max
400	95	7.082	1.136	0.117	4.7	9.2
500	81	7.847	1.033	0.115	5.2	9.6
600	123	7.941	1.305	0.118	4.8	11.1

Table III: Descriptive statistics based on measurement on blood cell diameter of all groups. Means, standard error (SE), ± 95 % confidence interval and numbers of observations (N) for each group are included in the table.

Blood cell diameter – All Groups					
Group	Mean	SE	-95 %	95 %	N
Diploid	10.330	0.040	10.252	10.407	543
400	11.079	0.009	11.061	11.096	10584
500	11.620	0.011	11.600	11.641	7621
600	11.897	0.010	11.876	11.917	7880

Table IV: Descriptive statistics based on measurement on blood cell diameter of each fish sample in group Dip. Numbers of observations (N), means, standard deviation (SD), standard error (SE), minimum, maximum and ± 95 % confidence intervals is included in the table.

Blood cell diameter Diploid								
Fish sample	N	Mean (microm)	SD	SE	Min	Max	-95 %	95 %
1	20	11.369	0.444	0.115	10.467	12.291	11.143	11.596
2	21	10.563	0.512	0.112	9.445	11.770	10.342	10.784
3	20	10.619	0.554	0.115	9.735	11.627	10.393	10.846
4	20	10.538	0.755	0.115	8.976	11.843	10.311	10.764
5	21	10.491	0.547	0.112	9.284	11.169	10.270	10.712
6	163	10.298	0.514	0.040	8.981	11.794	10.218	10.377
7	20	10.445	0.539	0.115	9.687	11.789	10.219	10.671
8	20	10.189	0.553	0.115	9.114	11.173	9.962	10.415
9	136	10.165	0.506	0.044	8.300	11.360	10.078	10.252
10	60	10.245	0.410	0.067	9.369	11.095	10.114	10.376
11	20	9.940	0.396	0.115	9.182	10.640	9.714	10.166
12	22	10.427	0.607	0.110	9.214	11.733	10.212	10.643
Total:	**543**	**10.330**	**0.568**	**0.024**	**8.300**	**12.291**	**10.282**	**10.378**

Table V: Descriptive statistics based on measurement on blood cell diameter of each fish sample in group 400. Numbers of observations (N), means, standard deviation (SD), standard error (SE), minimum, maximum, and ± 95 % confidence intervals are included in the table.

Blood cell diameter 400 bar								
Fish sample	N	Mean (microm)	SD	SE	Min	Max	-95 %	95 %
1	64	12.153	0.890	0.099	8.671	13.730	11.959	12.347
2	244	11.117	0.958	0.051	8.180	13.356	11.018	11.216
3	82	11.611	0.656	0.087	9.707	12.966	11.440	11.783
4	168	10.526	0.701	0.061	8.542	12.471	10.407	10.646
5	199	10.999	0.840	0.056	8.244	13.021	10.889	11.109
6	47	11.537	0.701	0.115	10.081	12.814	11.311	11.763
7	223	10.085	0.716	0.053	8.008	11.403	9.981	10.189
8	127	11.519	0.714	0.070	9.141	12.879	11.381	11.657
9	126	12.361	1.026	0.071	8.467	13.961	12.223	12.499
10	186	10.241	0.737	0.058	8.158	11.721	10.128	10.355
11	181	10.756	0.761	0.059	8.358	12.134	10.641	10.871
12	85	11.521	0.973	0.086	8.985	13.831	11.353	11.689
13	148	10.862	1.010	0.065	8.334	12.812	10.734	10.989
14	168	11.364	0.831	0.061	8.272	13.009	11.245	11.484
15	177	9.574	0.613	0.060	8.017	10.997	9.458	9.691
16	80	10.937	0.825	0.089	8.913	12.346	10.764	11.111
17	129	9.299	0.490	0.070	8.014	11.027	9.162	9.435
18	64	11.187	0.843	0.099	9.155	12.798	10.993	11.381
19	121	11.781	0.885	0.072	9.283	13.332	11.640	11.922
20	187	11.362	0.689	0.058	9.343	12.508	11.249	11.476
21	113	11.565	0.976	0.074	8.115	12.942	11.419	11.711
22	90	11.682	0.700	0.083	8.579	12.790	11.518	11.845
23	66	12.231	0.689	0.097	10.258	13.244	12.040	12.422
24	92	11.650	0.721	0.083	9.229	13.281	11.488	11.812
25	98	10.844	0.710	0.080	8.950	12.388	10.687	11.001
26	82	12.053	0.712	0.087	9.715	13.232	11.881	12.224

27	91	11.797	1.140	0.083	8.591	13.889	11.635	11.960
28	138	10.626	0.766	0.067	8.916	12.464	10.494	10.758
29	52	12.148	0.712	0.110	10.233	13.961	11.933	12.363
30	101	12.153	0.684	0.079	10.199	13.599	11.998	12.307
31	28	12.037	0.749	0.150	10.403	13.119	11.743	12.330
32	44	11.905	0.617	0.119	10.322	13.057	11.671	12.139
33	207	8.745	0.419	0.055	8.007	9.990	8.637	8.853
34	101	11.405	0.881	0.079	8.683	13.233	11.251	11.560
35	206	10.825	0.797	0.055	8.245	12.405	10.717	10.933
36	75	12.190	0.973	0.091	8.342	13.721	12.011	12.369
37	160	11.024	0.724	0.063	8.475	12.517	10.901	11.147
38	224	11.542	0.958	0.053	8.540	13.722	11.438	11.646
39	156	11.696	0.970	0.063	8.197	13.635	11.571	11.820
40	92	11.733	0.853	0.083	9.267	13.144	11.571	11.894
41	44	11.952	0.657	0.119	10.190	13.029	11.718	12.186
42	88	9.620	0.476	0.084	8.151	10.877	9.455	9.785
43	147	12.002	0.915	0.065	9.518	13.545	11.874	12.130
44	157	11.213	0.810	0.063	8.375	13.188	11.089	11.337
45	227	10.976	0.828	0.053	8.176	12.735	10.873	11.079
46	58	11.698	0.785	0.104	9.290	12.941	11.494	11.902
47	76	10.664	0.693	0.091	8.880	12.214	10.486	10.842
48	78	9.683	0.536	0.090	8.223	11.268	9.507	9.858
49	20	11.113	0.484	0.177	10.138	12.140	10.766	11.460
50	142	11.891	0.889	0.066	8.522	13.273	11.761	12.021
51	37	12.303	0.547	0.130	10.711	13.090	12.048	12.558
52	96	11.784	0.682	0.081	9.846	12.953	11.626	11.942
53	100	10.210	0.888	0.079	8.062	12.504	10.055	10.365
54	85	10.193	0.666	0.086	8.731	11.696	10.025	10.361
55	172	11.128	0.909	0.060	8.395	12.670	11.009	11.246
56	89	11.426	0.675	0.084	9.979	13.117	11.261	11.590
57	149	9.771	0.553	0.065	8.507	11.337	9.644	9.898
58	31	11.607	0.746	0.142	9.685	12.595	11.328	11.886
59	89	11.030	0.673	0.084	9.168	12.435	10.865	11.194
60	86	11.721	0.927	0.085	9.230	13.279	11.554	11.889
61	48	11.685	0.810	0.114	9.503	13.182	11.461	11.909
62	97	11.932	0.918	0.080	9.575	13.542	11.775	12.090
63	124	9.870	0.500	0.071	8.076	10.992	9.731	10.009
64	146	10.754	0.816	0.066	8.366	12.747	10.626	10.883
65	125	10.647	0.697	0.071	8.564	12.073	10.508	10.786
66	115	11.515	0.940	0.074	9.181	13.361	11.370	11.659
67	165	11.310	0.814	0.062	8.773	13.115	11.189	11.431
68	60	10.765	0.887	0.102	8.759	12.772	10.565	10.966
69	145	11.241	0.722	0.066	8.930	12.894	11.112	11.370
70	181	11.397	0.817	0.059	8.686	13.003	11.282	11.513
71	100	11.214	1.069	0.079	8.700	13.569	11.059	11.369
72	88	11.653	0.639	0.084	9.874	13.013	11.488	11.818
73	145	10.933	0.843	0.066	8.789	12.633	10.804	11.062
74	104	11.726	0.892	0.078	8.911	13.537	11.574	11.879
75	119	11.385	0.585	0.073	9.366	12.686	11.243	11.527
77	96	12.259	0.796	0.081	10.035	13.563	12.101	12.418
78	114	11.759	0.779	0.074	9.479	13.318	11.614	11.904
79	32	11.609	0.658	0.140	9.752	12.845	11.335	11.884
80	106	10.821	0.778	0.077	8.549	12.195	10.671	10.972
81	124	9.593	0.446	0.071	8.270	10.623	9.454	9.733

82	183	10.866	0.803	0.059	8.299	12.455	10.752	10.981
83	154	10.209	0.784	0.064	8.132	11.880	10.084	10.334
84	75	11.687	0.862	0.091	9.163	13.442	11.508	11.867
85	88	12.031	0.746	0.084	9.790	13.812	11.866	12.196
86	129	10.579	0.799	0.070	8.260	12.520	10.443	10.716
87	84	11.534	0.746	0.086	9.557	12.953	11.364	11.703
88	65	11.577	0.620	0.098	9.833	13.042	11.385	11.770
89	54	11.509	0.826	0.108	9.689	13.595	11.297	11.720
90	53	12.087	1.124	0.109	8.882	14.121	11.874	12.300
91	20	10.990	0.668	0.177	9.590	11.960	10.643	11.337
92	140	10.625	0.772	0.067	8.416	11.876	10.494	10.756
93	64	11.631	0.829	0.099	9.329	13.198	11.438	11.825
94	127	11.002	0.930	0.070	8.812	12.832	10.864	11.139
95	121	11.597	0.502	0.072	10.250	12.813	11.456	11.738
Total:	**10584**	**11.079**	**1.099**	**0.011**	**8.007**	**14.121**	**11.058**	**11.100**

Table VI: Descriptive statistics based on measurement on blood cell diameter of each fish sample in group 500. Numbers of observations (N), means, standard deviation (SD), standard error (SE), minimum, maximum, and ± 95 % confidence intervals are included in the table.

Blood cell diameter 500 bar								
Fish sample	N	Mean (microm)	SD	SE	Min	max	-95 %	95 %
1	30	11.103	0.759	0.128	9.106	12.461	10.975	11.231
2	37	10.397	0.885	0.115	8.284	12.115	10.282	10.512
3	176	10.765	0.770	0.053	8.262	12.567	10.712	10.818
4	62	11.595	0.693	0.089	9.621	12.751	11.506	11.684
5	121	11.652	0.864	0.064	8.843	13.921	11.589	11.716
6	200	10.863	0.756	0.050	8.629	12.749	10.814	10.913
7	140	10.942	0.946	0.059	8.207	13.134	10.882	11.001
8	117	11.142	0.656	0.065	9.337	12.487	11.077	11.206
9	48	11.081	0.810	0.101	8.932	12.800	10.980	11.182
10	107	11.826	0.736	0.068	9.299	13.079	11.758	11.894
11	81	11.586	0.881	0.078	9.580	13.379	11.508	11.664
12	59	11.401	1.021	0.091	8.383	12.943	11.310	11.492
13	97	12.291	0.662	0.071	10.807	14.073	12.220	12.363
14	75	11.983	0.551	0.081	10.262	13.242	11.903	12.064
15	57	11.406	0.721	0.093	9.733	13.177	11.313	11.499
16	99	11.289	0.744	0.070	9.514	13.119	11.219	11.360
17	141	12.326	0.747	0.059	9.763	14.398	12.267	12.385
18	129	11.566	0.533	0.062	9.571	12.827	11.504	11.628
19	92	11.856	0.889	0.073	8.129	13.742	11.783	11.929
20	101	11.160	0.772	0.070	8.963	12.571	11.090	11.229
21	104	11.811	0.793	0.069	9.497	13.459	11.742	11.879
22	180	11.749	0.667	0.052	9.480	13.447	11.697	11.801
23	86	12.486	0.653	0.075	10.391	14.319	12.411	12.562
24	132	11.725	0.697	0.061	9.859	13.312	11.664	11.786
25	100	11.584	0.690	0.070	9.289	13.054	11.514	11.654
26	120	11.113	0.575	0.064	8.905	12.237	11.050	11.177
27	21	11.961	0.692	0.153	10.184	13.114	11.808	12.113
28	90	11.288	0.772	0.074	9.444	13.084	11.214	11.362
29	208	11.482	0.616	0.049	8.901	13.047	11.433	11.531
30	94	12.653	0.714	0.072	10.650	14.596	12.580	12.725

31	121	10.547	0.724	0.064	8.528	11.924	10.483	10.611
32	56	11.735	0.586	0.094	10.634	13.188	11.642	11.829
33	128	11.778	0.708	0.062	9.581	13.261	11.716	11.840
34	57	12.222	0.859	0.093	9.617	13.598	12.129	12.315
35	92	12.041	0.713	0.073	10.263	13.413	11.968	12.114
36	116	11.858	0.647	0.065	9.963	13.530	11.793	11.923
37	84	12.232	0.660	0.076	10.717	14.288	12.156	12.309
38	46	11.898	0.570	0.103	10.637	12.844	11.794	12.001
39	83	11.776	0.582	0.077	10.062	13.337	11.699	11.853
40	82	11.439	0.640	0.077	10.014	13.160	11.361	11.516
41	21	10.128	0.469	0.153	9.467	11.493	9.975	10.281
42	139	11.643	0.560	0.059	9.728	13.169	11.584	11.703
43	28	11.657	0.455	0.132	10.276	12.177	11.525	11.790
44	88	11.901	0.772	0.075	9.393	13.447	11.826	11.975
45	46	12.406	0.797	0.103	9.782	13.855	12.302	12.509
46	114	11.498	0.682	0.066	9.560	12.882	11.432	11.563
47	82	12.403	0.577	0.077	10.219	13.760	12.325	12.480
48	64	11.973	0.653	0.088	10.399	13.601	11.885	12.060
49	51	12.046	0.674	0.098	10.559	13.340	11.948	12.144
50	38	13.016	0.774	0.114	10.817	14.454	12.903	13.130
51	125	12.130	0.555	0.063	10.256	13.637	12.068	12.193
52	139	11.790	0.776	0.059	9.369	13.902	11.730	11.849
53	76	11.068	0.677	0.080	8.581	12.353	10.988	11.148
54	94	11.972	0.634	0.072	10.078	13.106	11.900	12.044
55	77	11.629	0.642	0.080	9.996	13.328	11.549	11.708
56	61	11.512	0.815	0.090	9.540	12.916	11.422	11.601
57	97	11.444	0.647	0.071	9.472	13.029	11.373	11.515
58	227	11.049	0.653	0.046	8.929	12.478	11.003	11.096
59	98	11.684	0.692	0.071	9.142	13.127	11.614	11.755
60	142	11.320	0.630	0.059	9.547	13.218	11.261	11.379
61	72	12.030	0.765	0.083	10.252	13.374	11.948	12.113
62	130	11.584	0.579	0.061	9.349	12.675	11.523	11.646
63	81	11.578	0.522	0.078	10.423	12.899	11.500	11.656
64	109	11.270	0.668	0.067	9.602	13.018	11.203	11.337
65	92	11.469	0.825	0.073	9.599	15.423	11.396	11.542
66	66	11.652	0.673	0.086	9.228	13.352	11.566	11.738
67	83	11.880	0.727	0.077	9.475	13.319	11.803	11.957
68	166	11.205	0.623	0.054	9.157	13.322	11.151	11.259
69	122	11.751	0.727	0.063	9.220	13.487	11.687	11.814
70	28	11.500	0.614	0.132	10.352	12.675	11.368	11.632
71	71	12.234	0.592	0.083	10.914	13.457	12.151	12.317
72	92	12.419	0.923	0.073	9.853	14.588	12.346	12.492
73	92	11.959	0.624	0.073	9.649	13.258	11.886	12.032
74	59	12.758	0.933	0.091	10.959	14.774	12.667	12.849
75	105	11.870	0.587	0.068	10.548	13.367	11.801	11.938
76	68	11.639	0.635	0.085	9.423	12.837	11.554	11.724
77	79	10.997	0.472	0.079	9.665	11.936	10.918	11.076
78	132	12.083	0.569	0.061	10.428	13.521	12.022	12.144
79	53	11.390	0.776	0.096	9.414	12.662	11.294	11.486
80	105	11.670	0.688	0.068	9.568	12.930	11.601	11.738
81	40	11.369	0.635	0.111	10.006	12.463	11.258	11.480
Total:	**7621**	**11.620**	**0.844**	**0.010**	**8.129**	**15.423**	**11.601**	**11.639**

Table VII: Descriptive statistics based on measurement on blood cell diameter of each fish sample in group 500. Numbers of observations (N), means, standard deviation (SD), standard error (SE), minimum, maximum, and ± 95 % confidence intervals are included in the table.

Blood cell diameter 600 bar								
Fish sample	N	Mean (microm)	SD	SE	min	Max	-95 %	95 %
1	35	11.441	0.762	0.129	10.041	12.722	11.233	11.649
2	68	11.463	0.686	0.083	9.837	12.871	11.313	11.612
3	79	11.842	0.646	0.073	10.179	13.365	11.703	11.980
4	20	10.963	0.630	0.141	9.925	11.938	10.687	11.238
5	34	11.420	0.503	0.086	9.956	12.325	11.209	11.632
6	21	11.547	0.594	0.130	10.031	12.556	11.278	11.816
7	88	11.794	0.765	0.082	8.727	13.371	11.663	11.925
8	21	11.132	0.784	0.171	9.366	12.271	10.863	11.401
10	21	11.570	0.463	0.101	10.765	12.435	11.302	11.839
11	23	11.604	0.715	0.149	9.892	13.131	11.348	11.861
12	38	11.020	0.617	0.100	9.551	12.353	10.821	11.220
13	52	12.302	0.828	0.115	10.299	13.941	12.132	12.473
14	21	11.206	0.929	0.203	8.780	13.035	10.937	11.475
15	46	11.487	0.789	0.116	9.615	12.765	11.305	11.669
16	42	12.026	0.641	0.099	10.342	13.425	11.836	12.216
17	33	11.681	0.433	0.075	10.968	12.333	11.467	11.895
18	58	11.803	0.655	0.086	9.192	12.794	11.641	11.964
19	20	11.579	0.609	0.136	10.459	12.513	11.303	11.854
20	52	11.321	0.750	0.104	8.754	12.654	11.150	11.492
21	30	11.756	0.763	0.139	9.330	12.763	11.531	11.981
22	58	11.155	0.553	0.073	10.041	12.416	10.993	11.317
23	75	11.727	0.647	0.075	9.973	13.156	11.585	11.870
24	50	11.295	0.461	0.065	9.587	12.374	11.121	11.469
25	60	11.506	0.560	0.072	10.150	12.664	11.347	11.665
26	49	12.594	0.847	0.121	10.606	14.228	12.418	12.770
27	24	11.319	0.594	0.121	10.205	12.309	11.068	11.570
28	21	13.061	0.660	0.144	11.887	14.083	12.792	13.330
29	22	11.371	0.575	0.123	10.418	12.520	11.109	11.634
30	32	11.767	0.649	0.115	10.389	13.257	11.549	11.984
31	28	11.530	0.514	0.097	9.863	12.360	11.297	11.763
32	58	11.734	0.678	0.089	9.738	12.777	11.572	11.895
33	43	12.325	0.444	0.068	11.512	13.259	12.138	12.513
34	83	11.431	0.595	0.065	10.003	13.268	11.295	11.566
35	80	11.555	0.655	0.073	10.220	13.053	11.418	11.693
36	57	11.840	0.612	0.081	10.437	13.421	11.677	12.003
37	59	12.052	0.504	0.066	10.753	13.067	11.892	12.213
38	51	11.574	0.906	0.127	9.099	13.345	11.402	11.747
39	71	11.360	0.595	0.071	10.059	13.505	11.213	11.506
40	114	11.740	0.621	0.058	9.761	13.265	11.625	11.855
41	90	11.910	0.592	0.062	10.422	13.433	11.780	12.040
42	20	12.042	0.495	0.111	11.341	13.197	11.767	12.318
43	32	11.696	0.516	0.091	10.158	12.640	11.478	11.913
44	20	11.939	0.567	0.127	10.829	12.874	11.664	12.215
45	70	11.306	0.500	0.060	9.039	12.248	11.158	11.453
46	58	11.947	0.479	0.063	10.190	13.082	11.785	12.108
47	55	12.163	0.704	0.095	10.139	13.689	11.997	12.329
48	70	11.801	0.520	0.062	10.362	12.904	11.654	11.948
49	20	12.515	0.630	0.141	11.470	13.940	12.240	12.791

50	120	12.151	0.672	0.061	10.085	14.228	12.039	12.264
51	27	12.877	0.792	0.152	10.667	14.361	12.640	13.114
52	110	12.268	0.566	0.054	10.809	13.938	12.151	12.385
53	106	12.131	0.540	0.052	9.850	13.189	12.012	12.251
54	20	12.186	0.545	0.122	10.657	13.115	11.910	12.461
55	105	12.033	0.526	0.051	10.319	13.516	11.912	12.153
56	117	11.636	0.658	0.061	9.421	13.303	11.522	11.749
57	171	11.904	0.579	0.044	10.469	13.373	11.809	11.998
58	124	12.244	0.764	0.069	9.128	13.806	12.133	12.354
59	113	11.440	0.496	0.047	10.430	12.604	11.324	11.556
60	68	12.447	0.634	0.077	10.003	13.819	12.298	12.597
61	85	11.705	0.625	0.068	10.167	13.201	11.572	11.839
62	131	11.594	0.580	0.051	8.955	12.885	11.486	11.701
63	121	12.449	0.751	0.068	8.571	14.034	12.337	12.561
64	23	12.770	0.817	0.170	10.987	14.388	12.513	13.027
65	91	11.673	0.542	0.057	10.241	12.957	11.544	11.802
66	63	12.055	0.618	0.078	10.833	13.393	11.900	12.210
67	50	11.500	0.629	0.089	9.143	12.804	11.326	11.674
68	65	12.498	0.528	0.065	11.218	13.600	12.345	12.651
69	150	11.679	0.561	0.046	10.310	13.022	11.578	11.779
70	106	11.868	0.699	0.068	10.075	13.815	11.748	11.987
71	86	12.168	0.723	0.078	10.074	13.982	12.035	12.301
72	155	11.767	0.608	0.049	9.243	13.219	11.668	11.866
73	42	12.294	0.614	0.095	10.827	13.288	12.104	12.484
74	55	12.199	0.661	0.089	10.368	13.864	12.033	12.365
75	29	12.478	0.625	0.116	10.991	13.580	12.250	12.707
76	37	12.096	0.711	0.059	10.403	13.310	11.365	11.645
77	77	11.505	0.519	0.076	10.028	12.441	11.810	12.152
78	52	11.981	0.551	0.052	10.792	13.142	12.234	12.437
79	147	12.335	0.630	0.067	10.498	13.545	11.982	12.295
80	62	12.139	0.524	0.066	10.975	13.335	11.868	12.203
81	54	12.036	0.482	0.059	10.988	13.041	11.557	11.806
82	98	11.681	0.588	0.046	10.138	13.210	11.916	12.141
83	120	12.028	0.509	0.063	10.956	13.367	12.209	12.472
84	88	12.341	0.590	0.078	10.019	14.071	11.748	12.119
85	44	11.933	0.515	0.052	10.831	12.830	11.455	11.702
86	99	11.579	0.513	0.074	10.150	12.623	12.465	12.764
87	68	12.614	0.609	0.057	11.414	13.828	11.590	11.826
88	109	11.708	0.594	0.092	10.007	12.934	12.075	12.401
89	57	12.238	0.692	0.073	10.003	13.345	12.526	12.749
90	122	12.638	0.802	0.092	9.817	14.066	11.378	11.723
91	51	11.550	0.660	0.067	10.072	13.212	11.743	11.959
92	131	11.851	0.768	0.062	8.036	13.289	11.578	11.789
93	136	11.683	0.728	0.073	9.089	13.193	12.265	12.551
94	74	12.408	0.629	0.082	10.595	14.398	11.885	12.211
95	57	12.048	0.619	0.117	10.095	13.099	11.894	12.299
96	24	12.127	0.405	0.083	11.265	12.977	11.876	12.379
97	61	12.439	0.703	0.090	10.796	13.915	12.282	12.597
98	67	11.532	0.495	0.061	9.988	12.769	11.382	11.683
99	106	11.790	0.623	0.061	8.751	13.409	11.670	11.910
100	71	12.651	0.738	0.088	11.056	14.262	12.505	12.797
101	72	12.669	0.732	0.086	10.462	14.074	12.524	12.814
102	78	11.333	0.554	0.063	10.167	12.832	11.194	11.473
103	122	12.063	0.620	0.056	9.795	13.296	11.952	12.175

104	91	12.442	0.652	0.068	10.202	13.670	12.313	12.571
105	140	11.725	0.714	0.060	9.635	13.342	11.621	11.829
106	96	11.661	0.553	0.056	10.165	12.906	11.535	11.787
107	49	11.982	0.598	0.085	10.066	13.013	11.806	12.158
108	53	12.265	0.555	0.076	10.818	13.714	12.096	12.435
109	46	11.731	0.490	0.072	10.300	12.594	11.549	11.913
110	44	11.375	0.637	0.096	9.244	12.561	11.189	11.560
111	23	11.983	0.439	0.091	11.339	12.818	11.726	12.239
112	49	11.156	0.640	0.091	9.661	12.940	10.980	11.332
113	20	11.837	0.485	0.108	10.787	12.535	11.562	12.112
114	32	11.298	0.399	0.071	10.240	12.411	11.081	11.516
115	21	11.676	0.425	0.093	10.966	12.302	11.408	11.945
116	94	11.761	0.585	0.060	9.926	13.241	11.634	11.888
117	20	12.753	0.753	0.168	11.528	14.424	12.477	13.028
118	20	11.482	0.509	0.114	10.231	12.324	11.206	11.757
119	23	10.869	0.368	0.077	10.342	11.834	10.613	11.126
120	90	11.969	0.686	0.072	10.595	13.613	11.839	12.099
121	20	12.550	0.636	0.142	11.305	13.601	12.275	12.826
122	21	12.593	0.716	0.156	10.771	14.063	12.324	12.862
123	39	12.456	0.431	0.069	11.534	13.520	12.259	12.653
Total:	**7880**	**11.897**	**0.735**	**0.008**	**8.036**	**14.424**	**11.881**	**11.913**

One-Way ANOVA

Table VIII: Test results from One-Way ANOVA of all groups (Diploid, 400, 500, and 600) regarding weight (g).

Weight - All treatment groups					
One-Way ANOVA					
	SS	DF	MS	F	p
Intercept	7183.200	1	7183.200	1422.496	<0.001
Group	86.065	2	43.033	8.522	<0.001
Error	1494.715	296	5.050		

Table IX: Test results from One-Way ANOVA of all groups (Diploid, 400, 500, and 600) regarding length (cm).

Length - All treatment groups					
One-Way ANOVA					
	SS	DF	MS	F	p
Intercept	16870.54	1	16870.54	12055.10	<0.001
Group	44.15	2	22.08	15.77	<0.001
Error	414.24	296	1.40		

Table X: Test results from One-Way ANOVA of 400 bar group regarding weight (g), between different sampling days (27.09.2010 and 29.09.2010).

Weight – 400 bar					
One-Way ANOVA					
	SS	DF	MS	F	p
Intercept	1642.529	1	1642.529	678.200	<0.001
Sampling date	34.923	1	34.923	14.420	<0.001
Error	225.236	93	2.422		

Table XI: Test results from One-Way ANOVA of 400 bar group regarding length (cm), between different sampling days (27.09.2010 and 29.09.2010).

Length – 400 bar					
One-Way ANOVA					
	SS	DF	MS	F	p
Intercept	4189.455	1	4189.455	3805.071	<0.001
Sampling date	18.865	1	18.865	17.134	<0.001
Error	102.395	93	1.101		

Table XII: Test results from One-Way ANOVA of 600 bar group regarding weight (g), between different sampling days (30.09.2010 and 06.10.2010).

Weight – 600 bar					
One-Way ANOVA					
	SS	DF	MS	F	P
Intercept	2862.318	1	2862.318	475.524	<0.001
Sampling date	142.961	1	142.961	23.751	<0.001
Error	728.334	121	6.019		

Table XIII: Test results from One-Way ANOVA of 600 bar group regarding length (cm), between different sampling days (30.09.2010 and 06.10.2010).

Length – 600 bar					
One-Way ANOVA					
	SS	DF	MS	F	p
Intercept	6423.321	1	6423.321	4800.472	<0.001
Sampling date	45.711	1	45.711	34.162	<0.001
Error	161.905	121	1.338		

Student-Newman-Keuls multiple comparison (SNK) test

Table XIV: p-values from SNK test, testing differences in mean weight (g) between all treatment groups (400, 500 and 600 bar).

Weight - All Treatment Groups			
Group	400	500	600
400		0.014	<0.001
500	0.014		0.149
600	<0.001	0.149	

Table XV: p-values from SNK test, testing differences in mean length (cm) between all treatment groups (400, 500 and 600 bar).

Length - All Treatment Groups			
Group	400	500	600
400		<0.001	<0.001
500	<0.001		0.582
600	<0.001	0.582	

Table XVI: p-values from SNK test, testing differences in mean weight (g) between the two sampling dates (27.09.2010 and 29.09.2010) of the 400 bar group.

Weight – 400 bar		
Sampling date	27.09.2010	29.09.2010
27.09.2010		<0.001
29.09.2010	<0.001	

Table XVII: p-values from SNK test, testing differences in mean length (cm) between the two sampling dates (27.09.2010 and 29.09.2010) of the 400 bar group.

Length – 400 bar		
Sampling date	27.09.2010	29.09.2010
27.09.2010		<0.001
29.09.2010	<0.001	

Table XVIII: p-values from SNK test, testing differences in mean weight (g) between the two sampling dates (30.09.2010 and 06.10.2010) of the 600 bar group.

Weight – 600 bar		
Sampling date	30.09.2010	06.10.2010
30.09.2010		<0.001
06.10.2010	<0.001	

Table XIX: p-values from SNK test, testing differences in mean length (cm) between the two sampling dates (30.09.2010 and 06.10.2010) of the 600 bar group.

Length – 600 bar		
Sampling date	30.09.2010	06.10.2010
30.09.2010		<0.001
06.10.2010	<0.001	

Table XX: p-values from SNK test, testing difference in mean blood cell diameter (μm).

Blood cell diameter – All Groups				
Group	Diploid	400	500	600
Diploid		<0.001	<0.001	<0.001
400	<0.001		<0.001	<0.001
500	<0.001	<0.001		<0.001
600	<0.001	<0.001	<0.001	

ANCOVA

Table XXI: Test results from ANCOVA of all groups (Diploid, 400, 500, and 600) on blood cell diameter (μm). Weight (g) was used as co-variable.

All groups					
ANCOVA					
	SS	DF	MS	F	p
Intercept	138638.939	1	138638.939	165835.905	<0.001
Weight	373.832	1	373.832	447.167	<0.001
Group	4281.508	3	1427.169	1707.139	<0.001
Error	22256.848	26623	0.836		

Levene's test for homogeneity of variance

Table XXII: Test results from Levine's test performed on blood cell diameter (μm) and weight (g) variable, from all groups.

Levene's test for homogenity of variances				
Variable	MS Effect	MS Error	F	p
Diameter	180.259	0.305	591.550	<0.001
Weight	677.489	1.653	409.943	<0.001

Appendix III

HotSHOT* genomic DNA preparation

hot sodium hydroxide and tris

from *Biotechniques. 2000 Jul;29(1):52,54*

obtained from the Camper Lab

Notes before starting:

- DNA is suitable for PCR reactions
    - **TOO MUCH TISSUE WILL NOT WORK FOR PCR.**
    - Use 0.5-5 µl of the reaction product for PCR.
- DNA is NOT suitable for Southerns
    - This is because the protocol yields relatively short DNA segments
- Heating for longer than 30 minutes does not increase DNA concentration
    - Accidentally heating for an entire weekend does not negatively affect DNA concentration
- **Do not worry** about undigested floating tissue – tail snips often won't look like anything has happened to them, but the DNA is still there.
- DNA should be stored at 4ºC or -20ºC.
    - If you are taking tail snips, a good amount is about this size:

Protocol:

1. Obtain tissue and place in a tube.
    a. If you are taking tail snips, a good amount is about this size:
    b. Use a .65 mL tube if you plan on heating in the thermocycler
    c. Can use a 1.7 mL tube if you plan on heating in the sand block.
2. Add 75 µl Alkaline Lysis Reagent.
3. Heat sample to 95ºC for 10 minutes to an hour (30 minutes is optimal)
4. Cool to 4ºC (optional).
5. Add 75 µl Neutralization Buffer.
6. DNA can be used immediately.

Buffers:

Alkaline Lysis Reagent		
Reagent	Final Conc.	Amount for 200 mL
NaOH	25 mM	200 mg
EDTA	0.2 mM	14.88 mg

Neutralization Buffer		
Reagent	Final Conc.	Amount for 200 mL
Tris-HCl	40 mM	1.3 g

Add ddH2O to a final volume of 200 mL. pH of Alkaline Lysis Reagent will be 12. pH of Neutralization Buffer will be 5. There is **no need** to pH these solutions.

Appendix IV

Table XXIII: IMR's standard protocol for start feeding of cod larvae.

TRIPCOD | **Rotifers/Artemia/Weaning** | **Start: 28 May 2010**
Rig 3

						Rotifers			Artemia (number of individuals)			Dry feed (ml)			
Date		**Day**	**Flow**	**Temp.**	**O2.**	**11:00**	**15:00**	**20:00**	**10:00**	**15:00**	**15:00 (bucket)**	**Hand feeding**	**Automat**	**Dead**	**Notes**
Fri	**28 May**	**1**	**5**			-	300 000	300 000							
Sat	**29 May**	**2**	**5**			300 000	300 000	300 000							
Sun	**30 May**	**3**	**5**			300 000	300 000	300 000							
Mon	**31 May**	**4**	**5**	11.6	102.2	400 000	300 000	300 000							
Tue	**1 June**	**5**	**5**	11	101	300 000	300 000	300 000							
Wed	**2 June**	**6**	**5**		97	300 000	300 000	300 000							
Thu	**3 June**	**7**	**5**	10.7	98.8	300 000	300 000	300 000							
Fri	**4 June**	**8**	**5**			300 000	300 000	300 000							No rotifers at night
Sat	**5 June**	**9**	**5**			300 000	300 000	300 000							No rotifers at night
Sun	**6 June**	**10**	**5**		101	300 000	300 000	300 000							No rotifers at night
Mon	**7 June**	**11**	**5**			300 000	300 000	300 000							
Tue	**8 June**	**12**	**5**			300 000	300 000	300 000							Control group died
Wed	**9 June**	**13**	**10**			300 000	300 000	300 000							
Thu	**10 June**	**14**	**10**		99	320 000	300 000	300 000							
Fri	**11 June**	**15**	**10**			320 000	300 000	300 000							
Sat	**12 June**	**16**	**10**			320 000	320 000	400 000							
Sun	**13 June**	**17**	**10**		98	320 000	320 000	400 000							
Mon	**14 June**	**18**	**10**			320 000	320 000	640 000							
Tue	**15 June**	**19**	**10**			400 000	400 000	640 000							Temp increased 6-12 degrees
Wed	**16 June**	**20**	**10**	12.5	101	400 000	400 000	640 000							
Thu	**17 June**	**21**	**10**			400 000	400 000	640 000							
Fri	**18 June**	**22**	**10**			400 000	400 000	800 000							
Sat	**19 June**	**23**	**10**			400 000	400 000	800 000							
Sun	**20 June**	**24**	**10**			800 000	400 000	800 000							
Mon	**21 June**	**25**	**10**			800 000	800 000	1 200 000							
Tue	**22 June**	**26**	**10**			800 000	800 000	1 200 000							
Wed	**23 June**	**27**	**10**			800 000	800 000	1 600 000							One 400-bar tank died
Thu	**24 June**	**28**	**10**			800 000	800 000	1 600 000							
Fri	**25 June**	**29**	**10**			800 000	1 200 000	1 600 000							
Sat	**26 June**	**30**	**10**			800 000	1 200 000	1 600 000							
Sun	**27 June**	**31**	**10**			800 000	1 200 000	1 600 000							
Mon	**28 June**	**32**	**10**			800 000	1 200 000	1 600 000							

Table XXIV: IMR's standard protocol for Artemia feeding and weaning of cod larvae.

TRIPCOD									**Rotifers/Artemia/Weaning**						**Start: 28 mai 2010** **Rig 3**
						Rotifers (N)			**Artemia (number of individuals)**			**Dry feed (ml)**			
Date		**Day**	**Flow**	**Temp.**	**O2.**	**11:00**	**15:00**	**20:00**	**10:00**	**15:00**	**15:00 (bucket)**	**Hand feeding**	**Automat**	**Dead**	**Merknader**
Tue	**29 June**	**33**	**10**			-	1 200	1 600	25000	-	-				Artemia start
Wed	**30 June**	**34**	**10**			-	1 200	1 600	40000	-	-				Middle 600-tank + left 500-tank
Thu	**1 July**	**35**	**10**			-	1 200	1 600	50000	-	-				less Artemia beacause of less
Fri	**2 July**	**36**	**10**			-	1 200	-	50000	-	-				larvae. Appr 20 000 artemia
Sat	**3 July**	**37**	**10**			-	-	-	50000	50000	-				
Sun	**4 July**	**38**	**10**			-	-	-	50000	50000	-				
Mon	**5 July**	**39**	**10**			-	-	-	50000	50000	1200000				
Tue	**6 July**	**40**	**10**			-	-	-	50000	50000	1200000				
Wed	**7 July**	**41**	**10**			-	-	-	50000	50000	1200000				
Thu	**8 July**	**42**	**10**			-	-	-	50000	50000	1200000				
Fri	**9 July**	**43**	**15**						50000	50000	1200000				Change of plankton mesh.
Sat	**10 July**	**44**	**15**						50000	50000	1200000				
Sun	**11 July**	**45**	**15**						50000	50000	1200000				
Mon	**12 July**	**46**	**15**						50000	50000	1200000				
Tue	**13 July**	**47**	**15**						50000	50000	1200000				
Wed	**14 July**	**48**	**15**						50000	50000	1200000				
Thu	**15 July**	**49**	**15**						50000	50000	1200000				
Fri	**16 July**	**50**	**15**						50000	50000	1200000				Only 400 artemia/ml.
Sat	**17 July**	**51**	**15**						50000	50000	1200000				
Sun	**18 July**	**52**	**15**						50000	50000	1200000				
Mon	**19 July**	**53**	**15**						50000	50000	1200000				
Tue	**20 July**	**54**	**15**						50000	50000	1200000				
Wed	**21 July**	**55**	**15**								1000000		5		Dry feed
Thu	**22 July**	**56**	**15**								1000000		5		
Fri	**23 July**	**57**	**15**								1000000		5	~10	
Sat	**24 July**	**58**	**15**								1000000		5		
Sun	**25 July**	**59**	**15**								1000000		5		
Mon	**26 July**	**60**	**15**								-		5		
Tue	**27 July**	**61**	**15**								-		5		
Wed	**28 July**	**62**	**15**								-		10		Increased amount dry feed
Thu	**29 July**	**63**	**15**								-		10		

Table XXV: IMR's standard protocol for dry feed to cod juveniles.

Weaning

TRIPCOD Rig 3.

Date		Day	Flow	Temp	Oxygen (%)	Dry feed (ml)	Dead (number)	Notes
Fri	**30 July**	**64**	15			10		
Sat	**31 July**	**65**	15			10		
Sun	**1 Aug**	**66**	15			10		
Mon	**2 Aug**	**67**	15			10		
Tue	**3 Aug**	**68**	15			10		
Wed	**4 Aug**	**69**	15			10		
Thu	**5 Aug**	**70**	15			10		
Fri	**6 Aug**	**71**	15			10		
Sat	**7 Aug**	**72**	15			10		
Sun	**8 Aug**	**73**	15			10		
Mon	**9 Aug**	**74**	20			10		Increased flow
Tue	**10 Aug**	**75**	20			10		
Wed	**11 Aug**	**76**	20			10		
Thu	**12 Aug**	**77**	20			10		
Fri	**13 Aug**	**78**	20			10		
Sat	**14 Aug**	**79**	20			10		
Sun	**15 Aug**	**80**	20			10		
Mon	**16 Aug**	**81**	25			10		Feed No. 2 Particle size 0.6 - 0.9 mm
Tue	**17 Aug**	**82**	25			10		
Wed	**18 Aug**	**83**	25			10		
Thu	**19 Aug**	**84**	25			10		
Fri	**20 Aug**	**85**	25			10		
Sat	**21 Aug**	**86**	25			10	D:27-2 dead	
Sun	**22 Aug**	**87**	25			10	D:27-2 dead, D:29-1 dead, D:30-2 dead	
Mon	**23 Aug**	**88**	25			10		
Tue	**24 Aug**	**89**	25			10		
Wed	**25 Aug**	**90**	25			10		
Thu	**26 Aug**	**91**	25			10		
Fri	**27 Aug**	**92**	25			10		
Sat	**28 Aug**	**93**	25			10	D:36-1 dead	
Sun	**29 Aug**	**94**	25			10	D:30-1 dead, D:27-2 dead, D:34-3 dead	

Table XXVI: IMR's standard protocol for dry feed to cod juveniles.

TRIPCOD Rig 3.

Date		Day	Flow	Temp	Oxygen (%)	Dry feed (ml)	Dead (number)	Notes
Mon	**30 Aug**	**64**	15			10		
Tue	**31 Aug**	**65**	15			10	D:34-2 dead, D:36-1 dead	
Wed	**1 Sep**	**66**	15			10		
Thu	**2 Sep**	**67**	15			10	D:25-1 dead, D:27-3 dead, D:28-2 dead	
Fri	**3 Sep**	**68**	15			10	D:34-2 dead D36-1 dead	
Sat	**4 Sep**	**69**	15			10	1 dead per tank	
Sun	**5 Sep**	**70**	15		90	10	2 dead in D:29, 2 dead in D:34	
Mon	**6 Sep**	**71**	15	11.8		10		Lower waterline because of jumping fish
Tue	**7 Sep**	**72**	15			10	D:25-4 dead D:27-3 dead D:28-1 dead D:29-1 dead	
Wed	**8 Sep**	**73**	15	12	94	10	D:34-1 dead	
Thu	**9 Sep**	**74**	20			10	D:34-1 dead D:36-1 dead	
Fri	**10 Sep**	**75**	20	11.8	90	10	D:27-2 dead D:30-1 dead	
Sat	**11 Sep**	**76**	20			10	D:29-1 dead D:34-1 dead	
Sun	**12 Sep**	**77**	20			10		
Mon	**13 Sep**	**78**	20	12.2	89	10	D:28-2 dead D:27-1 dead	
Tue	**14 Sep**	**79**	20			15	D:34-2 dead	
Wed	**15 Sep**	**80**	20	12.2	82	15	D:36-1 dead	
Thu	**16 Sep**	**81**	25			15	D:30-1 dead	
Fri	**17 Sep**	**82**	25	11.4	83	15		
Sat	**18 Sep**	**83**	25			15		
Sun	**19 Sep**	**84**	25			15		
Mon	**20 Sep**	**85**	25	11.2	85	15	D:29-2 dead D:27-5 dead D:36-5 dead	
Tue	**21 Sep**	**86**	25			20		
Wed	**22 Sep**	**87**	25	11.3	81	20	D:25-2 dead	
Thu	**23 Sep**	**88**	25			20	D:25-2 dead	
Fri	**24 Sep**	**89**	25	11.3	81	20		
Sat	**25 Sep**	**90**	25			20	D:30-1 dead D:27-1 dead D:34-1 dead D36-1 dead	
Sun	**26 Sep**	**91**	25			20	D:30-1 dead D:35-1 dead	
Mon	**27 Sep**	**92**	25			20	D:35-1 dead D:30-1 dead	Susanne removes fish
Tue	**28 Sep**	**93**	25			20		
Wed	**29 Sep**	**94**	25			20		Susanne removes fish
Thu	**30 Sep**	**95**	25			20		Susanne removes fish
Fri	**1 Oct**	**96**	25			20		
Sat	**2 Oct**	**97**	25			20		
Sun	**3 Oct**	**98**	25			20		
Mon	**4 Oct**	**99**	25			20		Experiment finished