Prediction of larval viability based on egg quality parameters and early cleavage patterns in the experiments of triploidy induction in Atlantic cod, *Gadus morhua* L.

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M. Sc thesis
2005

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A thesis submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in International Fisheries Management

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ABSTRACT

Abnormal blastomere morphology may be indicative of low egg viability in fish and therefore represent a useful tool for rapid qualitative assessments for commercial and experimental aquaculture purposes. This work reviews the literature on this subject, and compares early cleavage patterns in a series of preliminary experiments of triploidy induction in Atlantic cod, *Gadus morhua*. Data from 4 different egg batches showed that cleavage abnormalities were generally higher in cold-shocked groups than control groups, though significantly only in 2 out of 4 experiments. Cell asymmetry was the most common type of cleavage abnormality in all trials, whereas other patterns such as complete separation of blastomeres or cell asymmetry combined with unequal blastomere size were observed in some of the experiments only. There was no significant difference between control and treated groups in terms of survival at any of the developmental stages in all batches. Proportions of abnormal blastomeres were negatively correlated with survival at day 1 while no correlations were observed at embryonation or hatching. This work shows that cold shock treatments applied shortly after fertilization may significantly increase, in some cases, the number of cleavage abnormalities but without affecting general survival at hatching. This would indicate that cleavage patterns abnormalities may not be considered as a good indicator of egg larval viability in such experiments.

DEDICATION
To my beloved parents

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere appreciation to Dr. Stefano Peruzzi, my supervisor who has been a source of guidance, constant supervision, encouragement and inspiration to me and without whose support this work would not have seen the light of the day. He was never demanded nor pushed but rather guided and advised. He always welcomed questions and enjoyed the ensuing discussions. Dr. Peruzzi, it is very difficult to find the right words to thank you for all your favor.

I am also grateful to Raul Primicerio for his help with data analysis. His advice and comments helped a lot to improve its quality. I thank all the lecturers and the student counsellors of the IFM programme who made these two years academically successful.

I also wish to express my gratitude to Anne Kettunen for the permission to use some of her data. Technical assistance from the staff at the Aquaculture Research Station of Tromsø is greatly acknowledged.

Last but not leas I thank my parents and brothers for their never ending encouragement.

Manira Sultana Rani
Tromsø
May 2005

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ABBREVIATIONS

ACIAR	Australian Center for International Agricultural Research
ADB	Asian development Bank
AFGRP	Aquaculture and Fish Genetic Research Programme
BARC	Bangladesh Agricultural Research Council
BFRI	Bangladesh Fisheries research Institute
CSIRO	Commonwealth Scientific and Industrial Research Organization
DFID	Department for International Development
DOF	Department of Fisheries
FAO	Food and Agricultural Organization
FS	Freshwater Station
GIFT	Genetically Improved Farmed Tilapia
ICLARM	International Center for Living Aquatic Resources Management
IOA	Institute of Aquaculture

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Appendix 1: Country Profile

1. Introduction

Fish hatcheries have a practical requirement to assess the quality of gametes entering the rearing system, for intention of resource allocation (staff time, allocation of incubators and feeding tanks) and to monitor broodstock performance (Shields *et al*., 1997). One of the most important constraints to the growth of aquaculture production is the variable quality of the egg that is available for growing on market sized fish (Kjørsvik *et al*., 1990; Bromage *et al*., 1991). Good quality eggs may be defined as those that exhibit low levels of mortality at fertilization, hatching and up to first feeding, they would also be expected to produce the healthiest and fastest growing fry, although egg quality effects on these characteristics may be masked by a variety of environmental and husbandry factors (Bromage *et al*., 1994). Poor egg quality which can steam from parental genetics, diet stress poor water quality or over ripening (Kjørsvik *et al*., 1990; Brooks *et al*., 1997) can contribute to low survival during the early life history stages for many marine fishes. Attempts to incubate and culture poor quality eggs and embryos are usually futile resulting in inefficient use of time, space and resources. Methods of predicting embryo quality would allow low quality egg batches to be identified and discarded so as to avoid economic loss (Rideout *et al*., 2004). Rapid egg quality assessment procedures are therefore considered an important tool for aquaculture management purposes. Qualitative and early morphological criteria are routinely used in several marine fish species as predictive measures of larval viability under standard commercial or experimental process. One of the major obstacles to the study of the egg quality in different species of fish is the difficulty of establishing parameters of quality that provide reliable prediction of performance. Among marine species, egg quality is more significant problem. Besides the difficulties of providing optimum culture conditions for egg incubation and yolk-sack fry, other reasons for variable egg survival remain unproven.

Preferably the predictive assessments should be simple to perform and not require lengthy or sophisticated laboratory procedures. They should also be capable of being carried out as soon as after ovulation and/or stripping as possible to avoid unnecessary occupation of hatchery staff time and incubation facilities on what may prove to be unproductive batches of eggs (Bromage *et al*., 1994).

In salmonids the percentage rate of fertilization provides a reliable indicator of subsequent performance survivors from batches of eggs with poor fertilization rates generally performing badly at all subsequent stages of development (Springate *et al*., 1984). As a result of the good correlation between eying and fertilization rates on the one hand and performance on the other, many farms use eying rates as indicators of quality (Bromage and Cumaranatunga, 1988). Many hatcheries culturing marine species distinguish “good” from ‘poor” quality eggs by virtue of the eggs ability to float or sink in sea water respectively (McEvoy, 1984; Carrillo *et al*., 1989; Kjørsvik *et al*., 1990). Moreover, other authors have suggested that the appearance of the chorion, the shape of the eggs, their transparency and distribution of lipid globules can be related to quality (Kjørsvik *et al*., 1990). However, for a number of species including the Atlantic halibut (*Hippoglossus hippoglossus*), no such correlation of buoyancy or of any other morphological characteristics with quality has been consistently observed. Supplemental information on chromosome appearance and cell symmetry has proved useful for a number of other species (Kjørsvik *et al*., 1990).

Additional factors that have been suggested as possible determinants of egg quality include the following**:** the nutrition and genetic make-up of the brood fish; the size, chemical composition, microbial colonization and the overripening of the egg (Bromage and Cumaranatunga, 1988; Kjørsvik *et al*., 1990; Bromage *et al*., 1991). However, of these, only bacterial colonization (Barker *et al*., 1989; Hansen and Olafsen, 1989), nutritional status of the brood fish (Watanabe *et al*., 1985; Kjørsvik *et al*., 1990) and overripening, which is the aging process that occurs in an egg in the period following ovulation up to fertilization (Kjørsvik *et al*., 1990; Bromage *et al*., 1991) have been clearly shown to influence egg quality. Results from these studies suggest that indicators of embryo quality are species specific. Also, there are indications that egg size could be used as an indicator of quality too (Kamler *et al*., 1982; Knutsen and Tilseth,. 1985). However studies with salmonids (Thorpe *et al*., 1984; Springate and Bromage, 1985; Jonsson and Svavarsson, 2000) and recent work with other species (Gisbert *et al*., 2000; Ouellet *et al*., 2001; Zaho *et al*., 2001) suggest that egg size is not always a reliable predictor of egg quality in fish.

One test that appears to be effective for a number of species involves examining the cellular divisions that occur shortly after fertilization. Kjørsvik *et al*., (1990) suggested that

assessment of cell symmetry at early stages of cleavage (normal blastomeres) might be a possible general indicator of egg quality for marine fish, including the Atlantic cod (*Gadus morhua*). According to the same authors, this morphological criterion has been the most reliable so far, as significant positive correlation between egg quality in the earliest cleavage stages and subsequent survival has been reported in a number of species. Early embryogenesis is made up of a series of mitotic divisions, producing equally sized, symmetrical cells known as blastomeres. Embryos are transparent in several marine fishes, allowing blastomere cleavage patterns to be observed quite easily. Embryos containing unusual arrangements of blastomeres are referred to as having 'abnormal cleavage'. An abnormality in one or more of these blastomeres has the potential to have a larger impact on development than deformity in one or two cells later in development (Kjørsvik *et al*., 1990). Indeed embryos with abnormal cleavage have been associated with a lower hatching success than those exhibiting normal cleavage. Finally, most studies on cleavage patterns are some way deficient in details. As pointed out by Shields *et al*., (1997), the group 'abnormal can hold lots of diverse types of blastomere deformities, counting asymmetrical cell positioning, unequal cell size, incomplete inter-cell adhesion, poorly defined cell margins and vacuolar inclusions between cells. Therefore, combining these under one category does not always allow the independent effects of each type of abnormality to be evaluated.

The Atlantic cod (*G. morhua)* is a well-known food fish belonging to the family Gadidae, and is one of the most important coldwater marine teleosts for commercial fisheries (Norberg, 2004). The North –East Arctic stock (also known as the Arcto-Norwegian stock), is at present the world's largest stock of Atlantic cod. It is also known as "skrei", a Norwegian name meaning "the wanderer" distinguishing it from the non-migrating coastal cod. The North-East Arctic cod is distributed from the Bay of Biscay North to the Arctic Ocean, including the North Sea, areas around Iceland and the Barents Sea, which is the most important feeding area for this species. Recent assessments report steadily declining stocks throughout the last decade (Nakken *et al*., 2000) and hence reduced fishing quotas. The decline in natural stocks has led to an increased interest in intensive cultivation of cod, both aquaculture and sea ranching (Norberg, 2004).

Before cod can become a substantial aquaculture success, there are still some barriers affecting productivity to break down. In fact, despite the research and commercial efforts, major bottlenecks still remain affecting commercial cod farming activities nowadays. In particular,

intensive production of juvenile cod has been little successful or inconsistent, due to the low survival and quality of the product (Peruzzi, personal communication). As in other important aquaculture species (e.g. salmonids, carps, tilapias, sea bass, …) cod production is also negatively affected by the premature onset of sexual maturation. This usually occurs at the age of one year for early maturing males, and during the second winter of production for the remaining fish resulting growth retardation and flesh quality diminution. Although there could be a market for mature female cod (i.e. roe production) might exists, the general aim is to reach harvesting size before sexual maturation occurs, as well as to postpone maturation under intensive culture conditions.

The induction of triploidy is the most common type of chromosome manipulation in fish and shellfish. It is applied for a variety of aquaculture purposes, but largely relates to its potential association with improved growth and carcass quality for commercial farming purposes. Other interest and practical application of triploidy in aquaculture, fishery management and research areas have been reviewed by Pandian and Koteeswaran, (1998). Triploids have been produced, at least experimentally, in almost all commercially aquacultured fish species, including carps, catfish, tilapia and salmonids, and in most molluscan shellfish groups such as oysters, clams, scallops and abalones (Beaumont and Hoare, 2003). In fish, triploidy is induced by allowing normal fertilization and then forcing retention of the second polar body (Chourrout, 1980, 1984; Lou and Purdom, 1984) (Fig. 1). The latter is commonly retained by applying temperature (hot or cold) or hydrostatic pressure treatments shortly after fertilization (Dunham, 2004 and cited references for detail description). The success of treatments to induce triploidy depends upon the time of initiation of the shock, the magnitude of the shock and its duration. The best time for initiation of the shock varies widely among different species but is related to the development and the timing of the second meiotic division. Naturally within a species, the timing of these cell division events is based on the temperature, so result can vary depending upon fertilization and incubation temperatures (Dunham, 2004). Hydrostatic pressure produces more consistent results, survival of treated eggs and percent triploidy than temperature shocks and other treatments

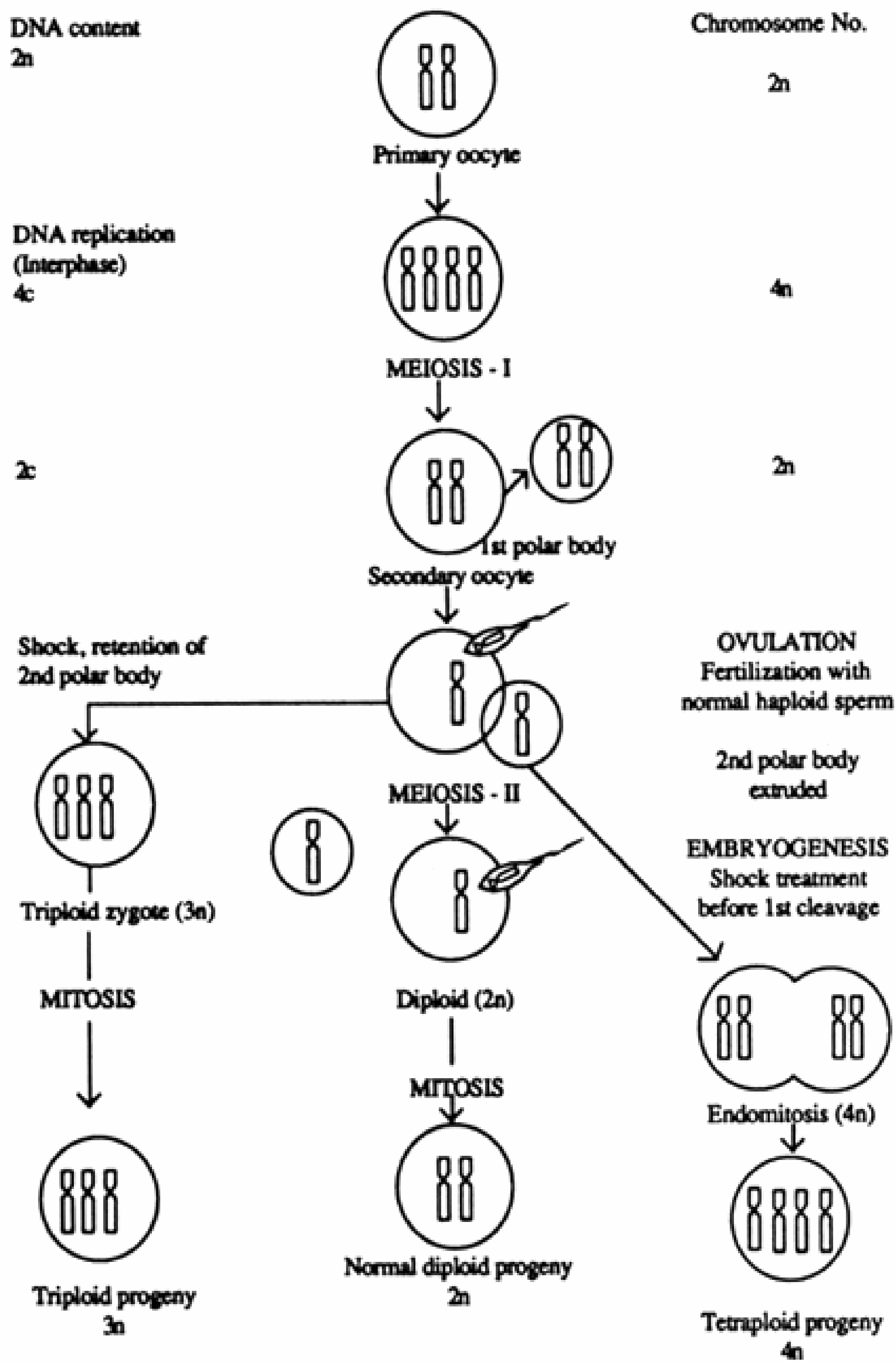


Fig.1: Illustrations of events for inducing polyploidy (triploidy and tetraploidy) in fish. (Reddy *et al.*, 1990)

(Cassani and Canton, 1986; Bury, 1989). Nevertheless, this method requires relatively high capital investment. One important reason for producing triploidy in fish (or shellfish) is that they are at least gametically sterile (Peruzzi *et al*., 2004). Gonad development is much reduced in triploids, particularly in females. Triploid sterility means that, as triploid fish reach maturity, energy that, in diploids, would go to developing gametes is available for somatic growth. As they get older, therefore, triploids may grow faster than diploids. In some species, triploid fish and shellfish demonstrate a clear growth advantage over diploid during annual gametogenesis, but superior growth performance of triploids in many species has only been demonstrated for a small part of their life histories (Beaumont and Hoare, 2003). The use of triploid fish in commercial production would avoid problems related to sexual maturation and potentially increase the market quality by providing a standard product throughout the year (unaffected by the physiological modifications linked to maturity). During the spawning period, carcass quality and visual appeal diminish, making the diploids less appreciated for food. The marketing of triploid fish could partially alleviate these problems (Peruzzi *et al*., 2004). Apart from the actual or potential increase in growth, there are other advantages that occur from the sterility of triploids. Sterility makes feasible the aquaculture of non native species or genetically inbreed or manipulated hatchery produced stock, either of which might otherwise cause adverse environmental impact if they are or their gametes were to escape into the wild (Beaumont and Hoare, 2003). To date, the only published work on triploidy induction in Atlantic cod has been provided by Kettunen *et al*., (2004). These authors have reported the results of a preliminary investigation using cold shocks.

The objective of the present study is to

- Estimate the effect of malformations, at blastula stages (2-8 cells), on further development of cod eggs subjected or not to cold shock treatments.

- Observe the effects of cold shock treatments on early cell morphology and hatching success in control and cold-shocked cod eggs.

- Discuss the relevance of using cytological irregularities at early stages as potential indicators of egg quality/viability in such experiments.

2. Materials and Methods

2.1. Broodstock maintenance and handling

Four experiments were carried out over a one-year period starting from March 2004 until April 2005 (two in 2004 and two in 2005), at the Tromsø Aquaculture Research Station of Kårvik, Norway (UITØ and FiskeriForskning).

The broodstock was Northeast Atlantic cod caught in the Balsfjord area near Tromsø, northern Norway, and transported to the Tromso Aquaculture Research Station (Fig.2)

Fig.2: **Aquaculture Experimental Unit at Karvik**.

Fishes were hold in sea cages (Fig.3) throughout the year and brought indoor one month before the spawning season (February- March).

Fig.3: Sea cages where the brood stocks were kept all the year round.

Brood stock was kept in holding tanks under natural conditions of water temperature and light regime (Fig. 4) and fed on a commercial diet.

Fig.4: Indoor brood stock maintenance

During the experimental trials the seawater temperature ranged around 3-4ºC Mature male and female spawners were kept together in the same tank. Spawners were individually PIT tagged (Passive Integrated Transponder) and equipped with visible tags of different colors for a rapid identification of sex. Mature females were selected by experienced operators and lightly

anaesthetized using MS222 (Tricaine solution %). Eggs and sperm samples were obtained by manually stripping mature fish (Fig.5 and 6)

Fig.5: Stripping of male

Fig.6: Stripping of female.

The gametes were collected in individual beakers and kept at 4ºC in a thermo-insulated container until further use (Fig.7).

Fig.7: Storage of sperm sample on ice

Sperm mobility was rapidly verified under light microscopy (100x) following activation with seawater (approx. 5 µl of sperm/ 10 µl of water). General egg appearance – shape, membrane integrity, and transparency- was evaluated using a standard binocular microscope. Batches containing high proportions of under- or over-mature eggs were discarded

.

2.2. Artificial fertilization

For each experiment, eggs from one female (~ 200 ml) were artificially fertilized with a mixed sperm sample from 3-4 males (0.5 ml/male). Sperm was added to the beakers containing the eggs and after 30 seconds of gentle mixing, sperm activation was initiated using 100ml of seawater at 6ºC. This stage was considered as time=zero in the development of the eggs. After 1 minute, 100ml of water was added and the eggs left undisturbed for approximately 5 minutes before being rinsed twice using a mesh filter (500µm). Approximately 25 ml of eggs were then placed into 50ml individual plastic vials with perforated mesh (500µm), and kept in individual incubators in a thermo-controlled system (6ºC) until treatment (Fig.8).

Fig.8: Artificial Fertilization.

2.3. Cold shocks

Cold-shock induction of triplody in cod was performed according Kettunen and Peruzzi (2004). A cold shock of -1.7±0.1^{0}C was applied at 40 min after fertilization (a.f.) for a duration of 2 hours. Controls groups were kept in their vials at 6^{0}C for the duration of the treatment to apply the same physical handling as treated eggs (sham control). Cold shocks were applied by draining the water in the vials and soaking them in a thermo-insulated seawater bath (salinity 35-37ppt) containing seawater ice stored at -85^{0}C. Temperature was constantly monitored throughout the experiments using a digital thermometer (precision ±0.01^{0}C) (Fig.9).

Fig.9: Cold Shock

2.4. Egg and larval incubation

Immediately after treatment the eggs were gently rinsed and returned to their incubators. Control and treated groups were then divided into three replicate groups of ~400ml eggs/group and hold in 600 ml beakers in a thermo-controlled room at 6^0C. The water was changed every other day until hatching at which point any mortalities were removed. Survival of developing eggs was recorded at 4-8 cell stage (8-10 hours p.f.), morula stage (day 1), embryonation (~50 DD), and hatching (approx. 90-100 DD= 15 days at 5-6^0C) (Fig.10). The experiments were replicated four times using different female and male fish.

Fig.10: Incubation tank

Shortly the method can be described as follows (Fig.11).

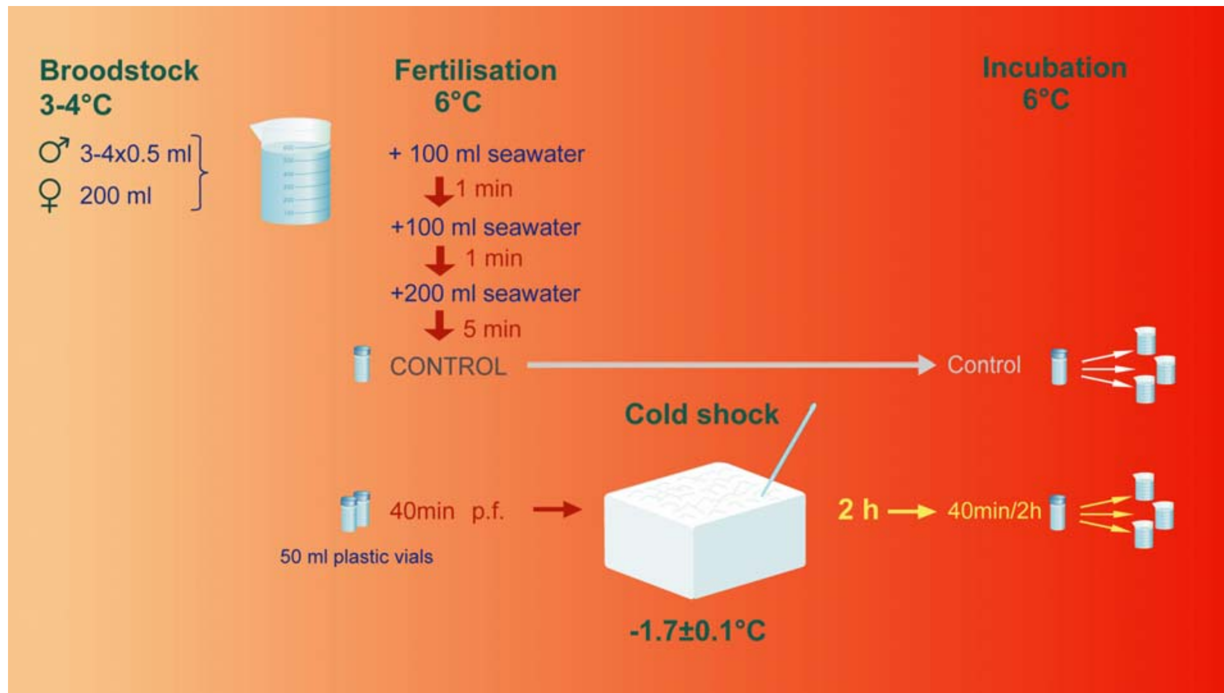


Fig.11: Schematic diagram of the materials and methods. Adapted from Kettunen *et al*., 2004

2.5. Microscopic observation and photography

Samples were taken from the incubator (4 hours after fertilization) and transported to NFH and kept in a temperature controlled room (5-6^{0}C) until the 4-8 cell stage appeared (8-10 hours after fertilization). A small number of eggs was placed in a Petri dish, observed under a light microscope (Leica Wild M10) and photographed by use of a digital camera (Nikon Coolpix 995). Approximately 10 photographs per sample were taken to reach a minimum number of 200 eggs / batch.

2.6. Egg quality parameters

Fertilization rate was calculated from a sample of 200 eggs from each group at 2-4 cell stage. Blastomere morphology was observed as above from a random sample of approximately 200-250 eggs/per group at 2-8 cell stage.

2.8. Statistical Analysis

Proportions of fertilized eggs and cleavage patterns in control and treated groups were compared using λ-square tests using Excel 2003. Percent survival at hatching was analyzed by a Kruskal-Wallis rank test using Systat version 10.2. A Scheirer-Ray-Hare correction for two-way ANOVA design was applied to ranked data. Correlation coefficients were analyzed by bootstrapped nonparametric analysis using the boot package R with 95% Confidence Interval (CI). All means were graphically reported ± 95% CI. Percentage data were arcsine-transformed and the level of significance given as $P<0.05$.

3. Results

Fertilization rates (62-99%) did not vary between control and treated groups except for the experiment II where the rate (99%) was significantly higher ($P < 0.001$) than in control group (62%)

Normal early blastomere shapes in cod eggs were regular in size and shape, as illustrated in Fig 12a-c showing the normal blastomere formation in 2-8 cell stages appearing between 4-8 hours after fertilization.

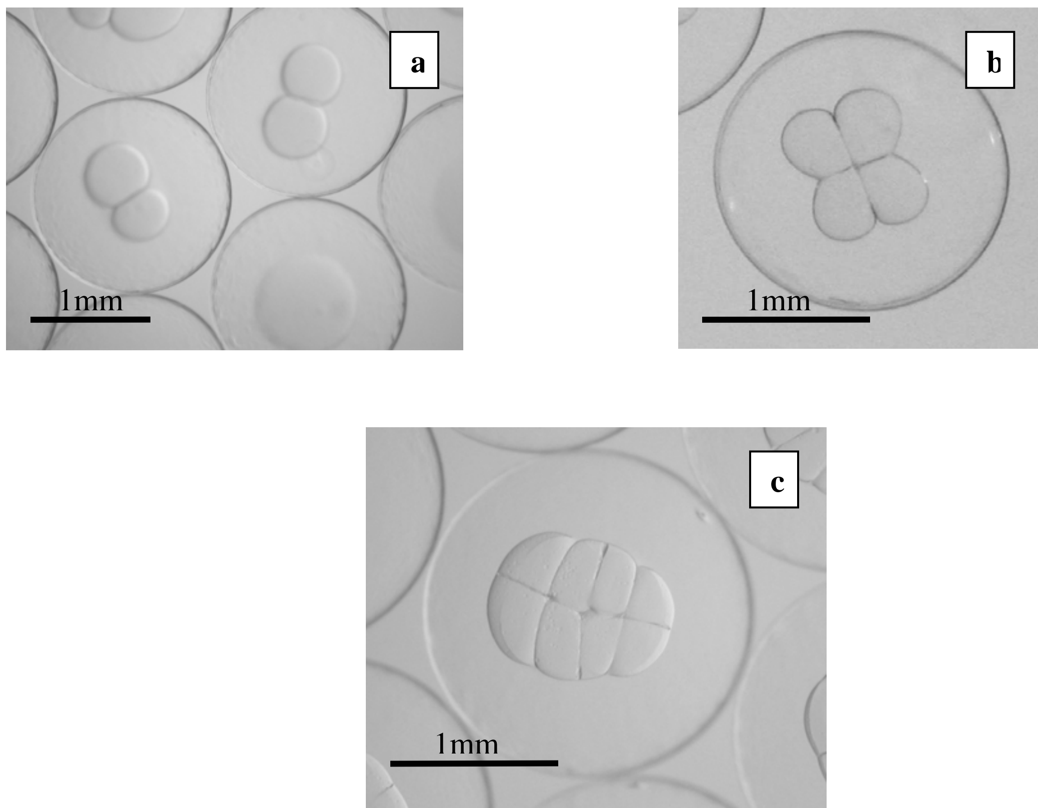


Fig.12a-c: Early stages of cod embryogenesis showing normal blastomere arrangements at the (a)2 cell, (b)4 cell and (c)8 cell stages of developme

Fig.13a-c presents the observed abnormal blastomeres in the different developmental stages.

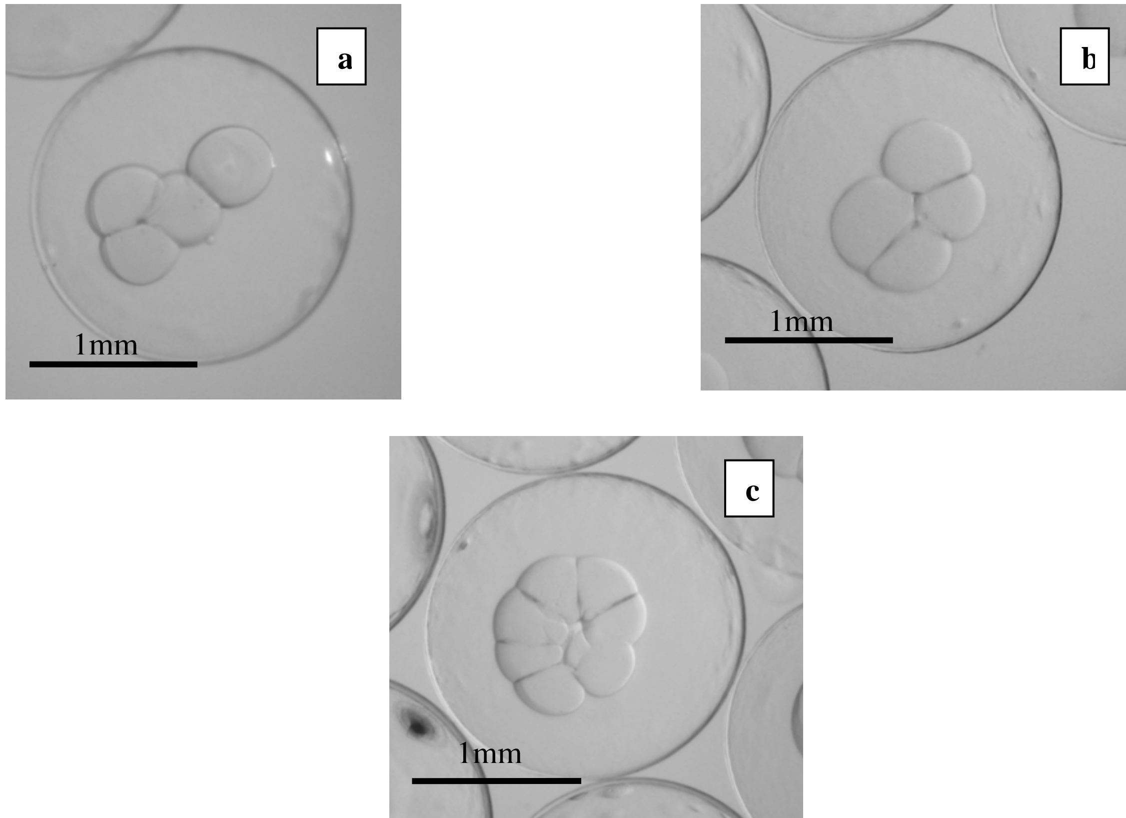


Fig.13 a-c: Types of cleavage abnormalities in cod embryos at the 4-8 blastomere stage of development.(a) asymmetry, (b) unequal blastomere size, (c) asymmetry and unequal blastomeresize. Horizontal bars: 1 mm.

Other unique cleavage abnormalities are also observed in cod. These abnormalities are referred to as 'cellular outcrops' and 'complete blastomere separation' .Embryos with 'cellular outcrops' are those that have one or two blastomeres protruding from the main group of blastomeres (Fig 14).

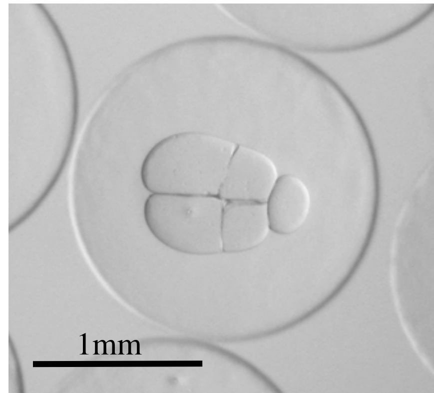


Fig. 14: Blastomere outcrop. Horizontal bars: 1 mm

Embryos classified as having 'complete blastomere separation' have two separate groups of blastomeres (Fig.15). It appeared that the first two blastomeres separated early in development but continued to undergo mitotic division on their own.

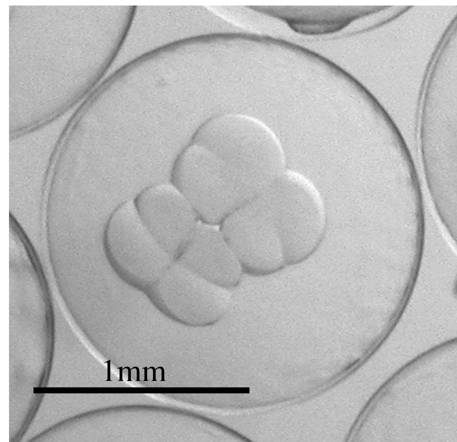


Fig.15: Complete separation of blastomeres. Horizontal bar: 1 mm

In our study we categorized the abnormal blastomeres into different classes which is shown in the table below.

Table 1: Different classes showing different types of abnormalities

Class	Class 1	Class 2	Class 3	Class 4	Class 5
Types of Abnormal Blastomeres	Asymmetry	Unequal cell size	Asymmetry and Unequal cell size	Outcrop	Complete separation of blastomeres

Table2a-d shows the percentages of different classes of abnormal blastomeres, percentages of normal blastomeres and the total number of eggs analyzed in each group of the four females used in the experiment .

Table 2a-d: Percentage of Normal and Abnormal blastomeres a) experiment I (b) experiment II (c) experiment III (d) experiment IV.

a.

EXP.1	Normal Eggs (%)					Abnormal Eggs (%)						Total Eggs
	1 Cell	2 Cell	4 Cell	8 Cell	Total	Class 1	Class 2	Class 3	Class 4	Class 5	Total	
CONTROL	14	0	80	3	97	0	1	1	1	0	3	224
TREATED	7	2	85	0	94	0	0	5	1	0	6	178

b.

EXP.2	Normal Eggs (%)					Abnormal Eggs (%)						Total Eggs
	1 Cell	2 Cell	4 Cell	8 Cell	Total	Class 1	Class 2	Class 3	Class 4	Class 5	Total	
CONTROL	2	0	48	46	96	2	1	1	0	0	4	331
TREATED	1	1	89	1	92	2	0	5	1	0	8	209

c.

EXP.3	Normal Eggs (%)					Abnormal Eggs (%)						Total Eggs
	1 Cell	2 Cell	4 Cell	8 Cell	Total	Class 1	Class 2	Class 3	Class 4	Class 5	Total	
CONTROL	20	5	68	0	93	5	0	0	1	1	7	201
TREATED	15	1	64	0	80	9	2	0	1	8	20	247

d.

EXP.4	Normal Eggs (%)					Abnormal Eggs (%)						Total Eggs
	1 Cell	2 Cell	4 Cell	8 Cell	Total	Class 1	Class 2	Class 3	Class 4	Class 5	Total	
CONTROL	4	12	79	0	95	0	3	0	2	0	5	228
TREATED	5	14	76	0	94	1	0	0	1	4	6	222

Class 1 which represented asymmetry appeared to be the most common type of abnormality observed in all of the 4 experiments. Class 5 (complete separation of blastomeres) was completely absent in the experiment I and II while class 3 (asymmetry and unequal cell size) in the experiment III and IV.

Significant difference was found between the control and treated groups in experiment II (P= 0.04) and in experiment III (P=0.001) only. In experiment II the abnormal blastomeres range from 4 %in the control to 9 % in the treated in whereas experiment III it varies from 7 %to 20 % respectively.

At embryonation, survivals of the control groups in the first two experiments were 32% and 17% respectively. Survivals of the control and treated groups at day 1, embryonation and hatching are reported in the table 3.

Table 3: Survival rate (%) at day 1, embryonation and hatching in the control and treated group

SURVIVALS (%)	EXP I		EXP II		EXP III		EXP IV	
	CTRL	TREATED	CTRL	TREATED	CTRL	TREATED	CTRL	TREATED
Day 1	76	70	77	84	43	47	47	51
Embryonation	32	32	17	32	24	22	33	35
Hatching	14	4	13	8	22	21	31	32

Treated groups perform poorer than the sham control groups at hatching in experiment I, II and III whereas some of the treated groups showed enhanced survival at embryonation and also at day 1.

Survival at day 1, embryonation and hatching are graphically reported in Fig 16a, 17a, 18a and the picture of the typical developmental stages are shown in the Fig 16b, 17b and 18b.

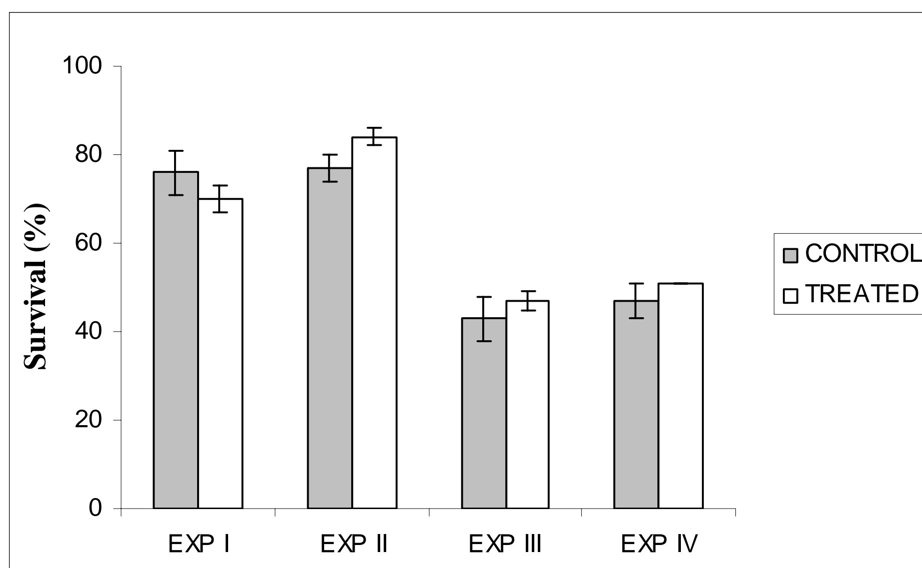


Fig16a: Egg survival at day 1 in control and treated groups. Mean values ± CI

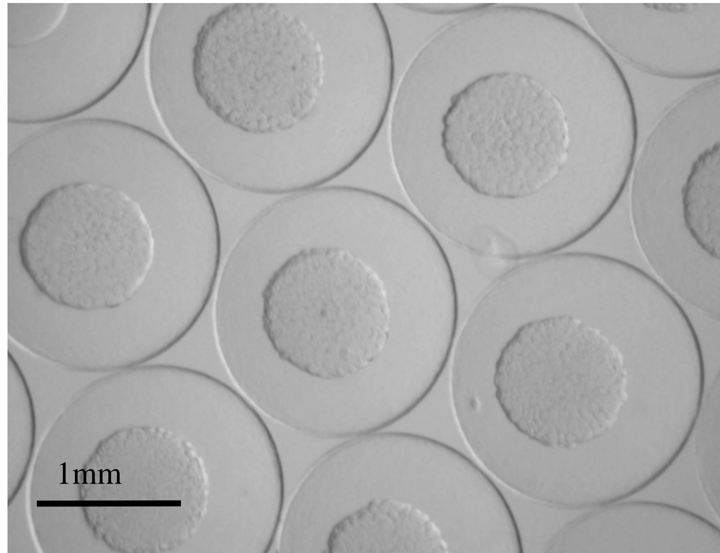


Fig 16b: Day 1 - Mid morula stage. Horizontal bars: 1 mm

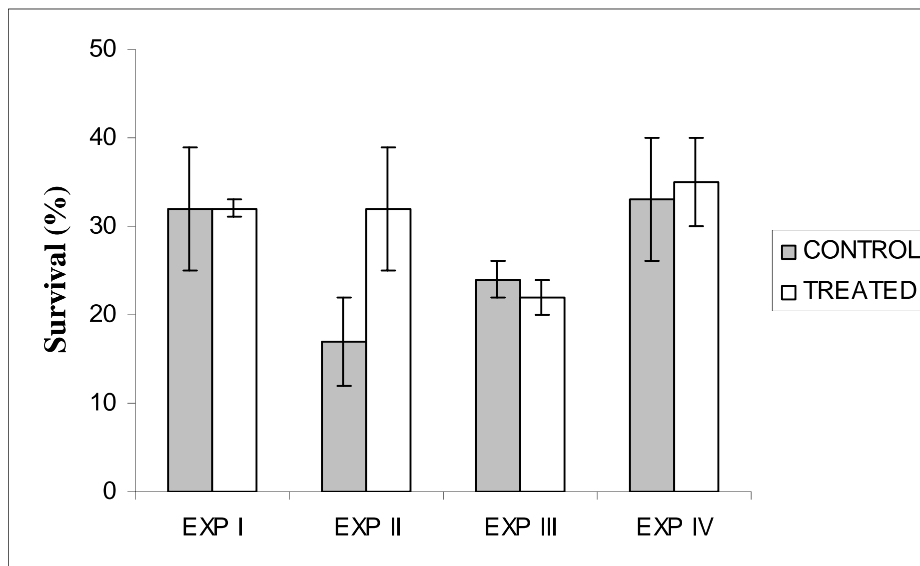


Fig 17a: Egg survival at embryonation in control and treated groups. Mean values ± CI

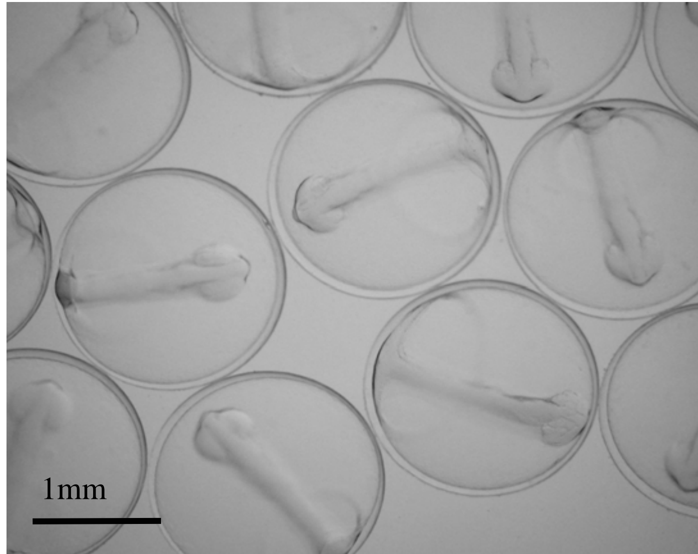


Fig 17b: Day 6 embryonation (pre-organogenesis).Horizontal bars: 1 mm

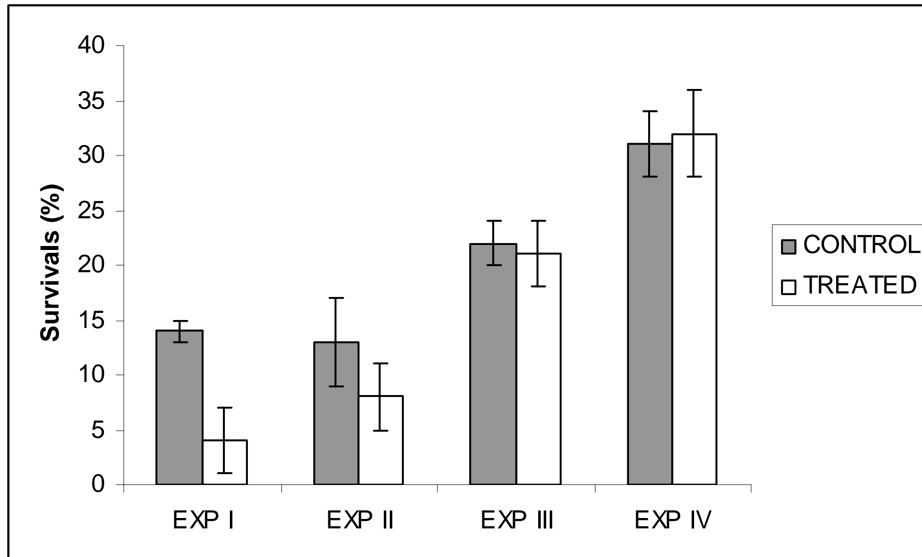


Fig 18a: Larval survival at hatching in control and treated groups. Mean values ± CI

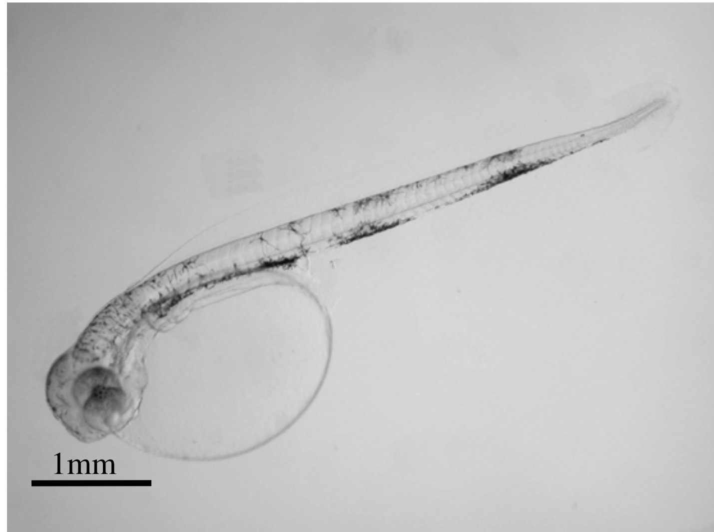
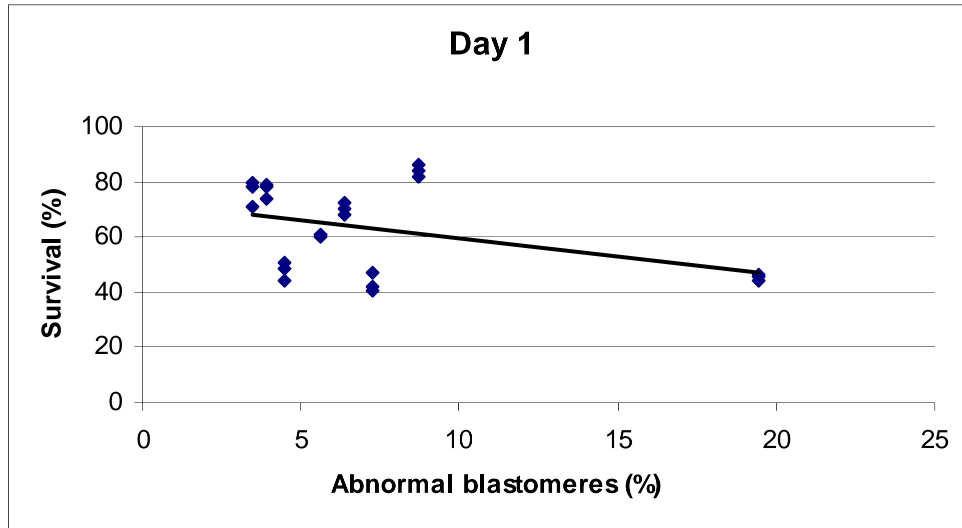


Fig 18b: Day 18 Hatching

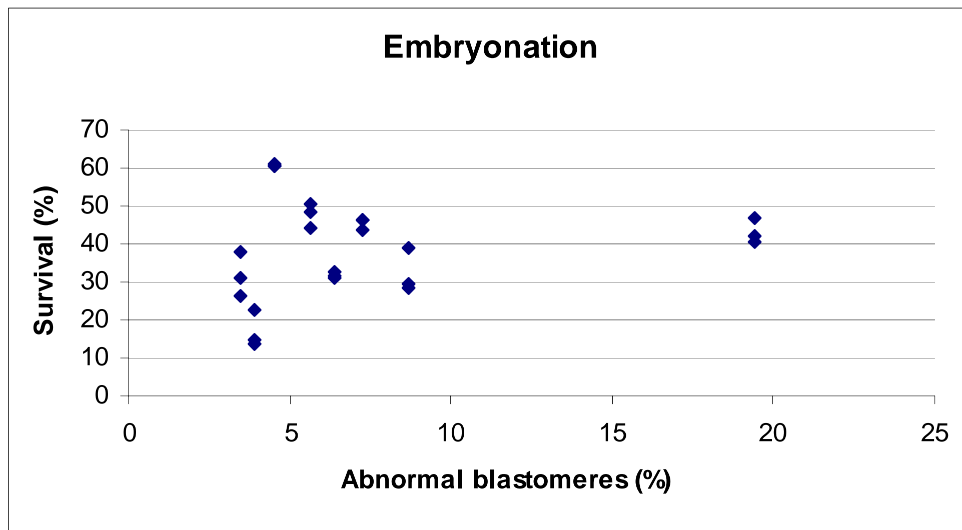
No significant differences were found in terms of survival between control and treated groups at any of the three developmental stages.

A significant negative linear correlation (R= - 0.411) was found between percentages of abnormalities and survival at day 1 in control and treated groups (-0.0445< 95% CI > -0.6842). No correlation was found between abnormal blastomeres and survival percentages at embryonation and hatching. Scattered pair values of these three developmental stages were graphically shown in the Fig .19a-c

a.



b.



c.

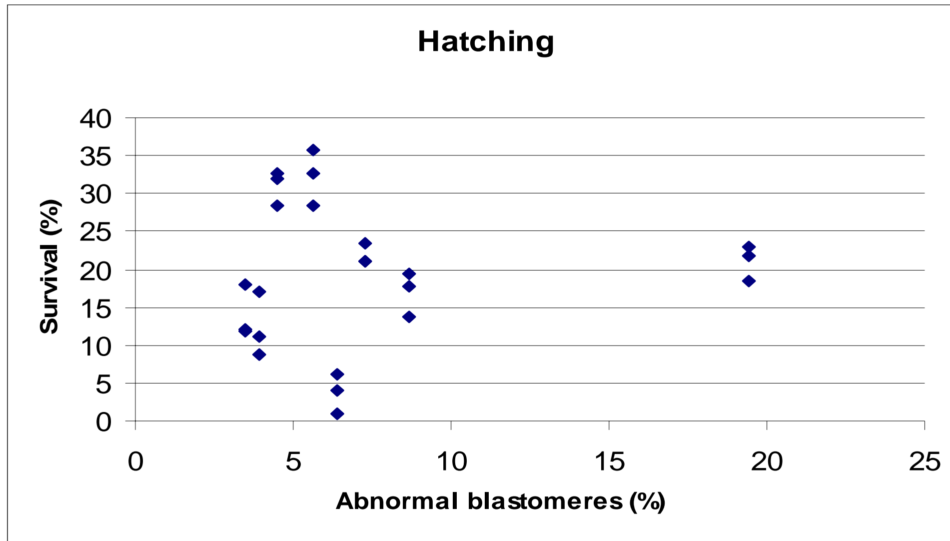


Fig 19a-c: Relationship between the percentages of cod eggs exhibiting abnormal blastomeres cleavage and survival at (a) day 1 (b) embryonation (c) hatching.

5. Discussion

The present experiment demonstrated no significant correlation between observed egg quality characteristics and survivals at embryonation and hatching. That means that the ability of the eggs to be fertilized and to develop further through the stages of embryonation and hatching was not significantly correlated with the abnormal blastomeres. However, there was a negative correlation between the survivals at day 1 and the malformed eggs but this has been lost in the subsequent developmental stages. In terms of number of abnormalities in control and treated groups, we found significant difference in two experiments (experiment II and III) out of four. The number of abnormal blastomeres did not vary following cold shock treatment in experiments I and IV. Cell asymmetry represented the most common cleavage abnormality, probably because of its ease of observation. Kjørsvik *et al*. (1994) pointed out that symmetry of the early blastomeres in developing eggs seemed to be a consistent early indicator of eggs viability in several species, such as Herring (*Clupea harengus*) (Dushkina, 1975), Dover sole (*Solea solea* L.) (Dinis,1982), Red sea bream(*Pagrus major*) (Sakai *et al*.,1985), Turbot (*Scophthalmus maximus* L) (McEvoy, 1984; Devauchelle *et al*,1988), Japanese flounder(*Limanda yokohama*) (Hirose *et al*, 1979), as well as Atlantic cod (Kjorsvik and Lønning, 1983**;** Kjørsvik *et al*, 1984). In contrast with these findings, Rideout and co-workers (2004**)** reported that symmetry in haddock blastomere arrangement did not influence larval viability at hatching. Interestingly, Vallin and Nissling (1998) reported that Baltic cod embryos showing various degrees of deformities in early developmental stages can develop normally and produce viable offspring, but did not distinguish between different types of abnormalities. Hempel (1979) found that during the first cleavages, compensation for the loss of some cells remains possible. According to Vallin and Nissling (1998) abnormalities occurring at later embryonic stages of development are more severe than those at earlier stages because there is less time for recovery before vital tissues and organs begin to develop. This is in contrast to the usual belief that deformities early in developments are more severe because each abnormal cell gives rise to many more identical copies of itself than when the abnormality occurs later in development (Rideout *et al*. 2004). Moreover, several experimental studies have shown that egg mortality seems to occur most frequently during particular developmental stages such as gastrulation (Blaxter, 1969**;** Alderdice and Forrester, 1971**;** Nissling and Westin, 1991**;** de Braak, 1994). Therefore it is of great

importance to define in what stage the deviation occur, when discussing cytological and embryonic abnormalities in fish (Vallin and Nissling, 1998).

In the present study, other types of cleavage abnormalities were observed too. The first of these were cellular outcrops. Along with cell asymmetry, this was one of the most commonly observed abnormality in our experiments. It was also reported as the most common type in haddock (*Melanogrammus aeglefinus*) embryos where it has been shown to have no effect on hatching success (Rideout *et al*., 2004). The presence of cellular outcrops has also been reported for cod (Kjørsvik, 1994). However, like this last author, we were also unable to isolate this type of cleavage deformity and its effect on hatching success could not be determined. Despite some attempts were made, the low number of malformed eggs of this category prevented us to accomplish this task.

The present results document that complete separation of blastomeres were observed in two experiments only (III and IV). This type of cleavage abnormality was completely absent in first two experiments. Again, we did not succeed in isolating and following the fate of this type of deformed eggs. Such observations were performed in haddock embryos and the effect on hatching success was significant (Rideout *et al*, 2004). In this species, fish eggs showing this type of deformity experienced almost no hatching success, suggesting that such deformity severely interferes with normal embryogenesis. Unlike previous work on Atlantic halibut embryos (Shields *et al*., 1997), we did not observe the presence of cellular vacuoles among the blastomeres of any of the observed eggs or embryos. In Atlantic cod, Kjørsvik (1994) and Westernhagen *et al*. (1988) found that malformations rates decreased with progressive developmental and assumed this to be caused by higher losses of eggs with malformations. This could also reflect the fact that aberrations at early stages of development were repaired and that hatching occurred in a normal way. Similar studies on the same species seem to confirm this statement (Vallin and Nissling, 1988). Finally, Kjørsvik (1994) reported that egg viability was highest during peak spawning and became more variable in the later part of the season for Atlantic cod. This suggests that, while embryos collected early or late in the spawning season should not be discarded automatically, they should be carefully analyzed (Rideout *et al*, 2004). In our experiment we analyzed middle batch spawners only.

In terms of overall survival, there was no significant difference between control and treated groups at day 1, embryonation and hatching. That means that cold shock had no

deleterious effect on the survival at any of the observed developmental stages. Also, we did not find any difference in terms of different females. Nevertheless at day 1, the survival percentages in cold shock treated groups were higher, though not significantly, in three out of four experiments (II, III and IV). Also, survivals at embryonation were higher, but not significantly, in the treated groups compared to their controls in experiment II and IV too. This is in good agreement with the results for Atlantic halibut, where cold shocks of 2 or 3h at -1ºC gave significant higher survival than control groups (Holmefjord and Refstie, 1997). Such lower survivability of control groups at day 1 and embryonation strongly contrast with other findings (Chrisman *et al*, 1983**;** Krasznai *et al*, 1984**;** Solar *et al*, 1984). In our work, to make the cold shock more effective we chose to increase the range of the difference between the pre-shock and shock temperature (ΔT or Temperature differential). The eggs were fertilized at 6ºC, although spawning of cod generally takes place at very stable temperature regimes of 3-4ºC. So the cold shocks of -1.7±0.1ºC, well outside the "normal" temperature range for newly fertilized cod eggs, increased the ΔT to approximately -7.7ºC. The importance of the difference between pre-shock and shock temperature was shown by Diaz *et al* (1993), who found increased percentages of triploids in rainbow trout after elevating the difference between pre-shock and heat shock temperatures. Nevertheless, it is possible that fertilization and incubation temperatures of 6ºC may not represent the optimal for cod eggs at very early stages of development and this might explain the apparent and paradoxical decreased survival (though overall not significant) observed in control groups kept at this temperature compared to cold shocked groups kept at lower temperatures.

This work was part of a series of preliminary attempts of triploidy induction in cod (Kettunen *et al*., 2004). According to the results provided by these authors, no triploid larvae were observed in the first experiment, and only low rates of triplody (14%) were found in experiment II. In experiment I, a low number of tetraploid larvae (2-4%) were also detected showing that a small proportion of eggs were blocked during first mitosis too. As a general rule, it is thought that warm water species are more susceptible to cold shock than to heat shock, whereas heat shock may be more effective for cold water species (Pandian and Koteeswaran, 1998). Nevertheless, the same authors pointed out that heat shocks might be more disruptive that cold shocks in terms of survival and that treatment optima may be difficult to identify too. As a preliminary attempt in Atlantic cod, Kettunen *et al*. (2004) chose cold shocks instead of heat shocks as more straightforward method for triploidy induction. Their experiments showed that

Atlantic cod may be little susceptible to cold shock treatments. Nevertheless, these apparent low effectiveness of cold shock in cod contrast with similar experiments carried out on plaice (*Pleuronectes platessa*) (Purdom, 1972) where cold shocks have been highly successful in inducing triploidy (up to 100% triploid larvae). Similarly, Holmefjord and Refstie (1997) induced variable rates of triploidy (11-95%) in Atlantic halibut (*Hippoglossus hippoglossus*) applying 2-3 hour cold shocks at -1.1ºC using similar pre-shock temperatures. In cod, further experiments will be conducted to optimize the use of cold shock treatments possibly using longer treatment durations (3 to 4 hours), as well as comparing such results with those from heat-shocks and hydrostatic pressure experiments (Kettunen *et al*., 2004).

1. Bangladesh a country of deltaic plains dominated by the major river system like Ganges, Brahmaputra and Meghna that flow from the Himalayan mountains into the Bay of Bengal is endowed with unique water resources comprising both inland and marine waters(Table.4). In addition, there is a 710 km long coastal belt, 12 nautical miles of marine territorial waters extending from the coast and 200 nautical miles Exclusive Economic Zone covering much of the Bay of Bengal. A short overview of the country is given in appendix 1 along with a map.

Table.4: **Different types of water bodies in Bangladesh with recent fish production**

SOURCES	*AREA IN HECTARE*	***FISH PRODUCTION IN METRIC TON(99-00)***
A. Inland waters	5,282,157	**1,308,916**
1.Open waters	4,920,316	**659,824**
i)River and estuary	1,031,563	**167,478**
ii)Beel(depression)	114,161	**81,866**
iii)Kaptai lake	68,800	**8,135**
iv)Floodplain	2,832,792	**402,345**
v)Polder/Enclousre	873,000	**unknown**
2.Closed waters	361,841	**649,092**
i)Pond and ditch	215,000	**547,677**
ii)Baor(Oxbow lake	5,488	**4,940**
iii)Shrimp farm	141,353	**96,475**
B. Marine waters	16,607,000	**340,000**
Industrial		**16,450**
Artisanal		**323,550**
Grand total	**21,889,157**	**1,661,151**

Source: Department of Fisheries (2001)

Along with potential water resources, the country is also rich in the diversity of fish species with approximately 300 of fresh and brackish water fish species (Hussain and Mazid, 2001). Enormous fresh water fisheries resources feed millions of people living in the Delta. Fish and fisheries have always been an essential part of the life and culture of the people of Bangladesh. Bengali people were popularly referred to as *Macche-Bhate Bangali* (Fish and rice make Bengali).Unfortunately, over harvesting of fish with an increasing fishing population is likely to

continue and place greater pressure on most small and large size water bodies. Rapid extraction of seed (for stocking) as well as broodfish (for seed production and consumption) from natural waters combined with destructive and unregulated fishing practices that use dynamite ,cyanide ,electro fishing and gillnet has led to the endangerment and possibly extinction of a number of rather valuable native species(Hussain and Mazid, 2001).

Aquaculture productivity has declined in recent years in Bangladesh. Loss of aquatic habitat due to siltation, dam construction (for irrigation, flood control and hydroelectric generation) and other anthropogenic activities has been one of the primary causes. Further stock deterioration in hatchery population due to poor brood stock management and inbreeding depression has been observed in recent years in Bangladesh. Retarded growth, reduction in reproductive performances, morphological deformities, increased incidence of diseases and mortalities of hatchery-produced seeds have been reported. As a result, deterioration of carp and barb seed quality has typically occurred. Introgressed hybrids of carps are being produced intentionally and unintentionally by the private hatchery operators and sold to the farmers and nursery operators. Presumably, large quantity of such seeds are being stocked in grow-out ponds or even in the open water bodies like floodplains, under the Government's massive carp seed stocking programme. There is widespread concern that mass stocking of such genetically poor quality stocks in the floodplains and related open water bodies might cause serious feral gene introgression into the pure wild stocks that could affect the government's planned aquaculture and inland open water fish production (Hussain and Mazid, 2001).

Although the government is making serious efforts to rehabilitate the inland fisheries, it has also focused its attention on aquaculture, which has tremendous opportunity in the country. In consonance with the government objectives, since 1988, the Bangladesh Fisheries Research Institute (BFRI) has developed a fish genetics research programme under its Freshwater Station (FS), Mymensingh to generate better breeds and improved stocks for increasing aquaculture production as well as to minimize genetic stock deterioration in hatchery population. In addition to institute's own programme, a number of international agencies viz. ICLARM, ACIAR/CSIRO and DFID-AFGRP(formerly FGRP) came forward to support some fish genetic research projects. During 1994-2000, a number of projects have been successfully completed. Among these were the ADB funded "Dissemination and Evaluation of Genetically Improved Tilapia in

Asia" (DEGITA) for evaluation of the GIFT tilapia strain, "Genetic Improvement of carp Species in Asia" (both under the auspicious of ICLARM); "Production of all female silver barb" (DFID-FGRP) and "Hilsa Biology and Genetic study" (ACIAR/CSIRO). Presently one more project, entitled "Genetic improvement strategies for production in exotic carps for low input in aquaculture in Asia" is being implemented with the technical assistance of the Institute of Aquaculture (IOA) under DFID-AFGRP funding.

Under BFRI core research and Bangladesh Agricultural Research Council (BARC) contract research funding, some other programmes are in operation

- Selective breeding of rohu, *Labeo rohita*;
- Further genetic selection and development of all male population of GIFT strain;
- Genetic manipulation study of shingi, *Heteropneustes fossilis*;
- Genetic conservation of some endangered carp species, *Tor putitora, Puntius sarana, Cirrhinus ariza, Labeo bata, Labeo gonius* etc.

1.1. Genetics research in progress

Among the fish genetic resources, the different carp species are very important in Bangladesh because they are the food fish most preferred by the people, and contribute nearly 35% to total fish production and 90% to aquaculture production. Combinations of endemic and compatible introduced (exotic) major carps are the main species for increased production in pond polyculture or composite fish culture systems.

1.2. Endemic carp species

There are at least 13 species of carp species under six genera inhabiting Halda and Padma-Brahmaputra River systems which are of interest to aquaculture in Bangladesh. (Table 5).

Table 5: List of endemic carp species of Bangladesh.

FAMILY	SPECIES	COMMON NAME	**LOCAL NAME**
Cyprinidae	*Labeo rohita*	Rohu	Rui
	Catla catla	Catla	Katla
	Cirrhinus cirrhosus	Mrigal	Mrigal
	Cirrhinus ariza	Reba	Laachu
	Labeo calbous	Calbashu	Kalibaush
	Labeo bata	Bata	Bata
	Labeo boga	Boga labeo	Bhangan
	Labeo gonius	Gonius	Gonnia
	Labeo nandina	Nandina labeo	Nandil
	Bengala elonga	Bengala barb	Along
	Puntius sarana	Barb	Sarpunti
	Tor tor	Tor mahaseer	
	Tor putitora	Putitor mahseer	Mahashoal

Source: Hasan (1990), Rahman (1985), Hussain and Mazid (2001)

1.2. Exotic carp species

Although Bangladesh is rich in endemic fish genetic resources, the introduction of exotic fish species (mostly chinese carps) has occurred in 1960. A list of different species of introduced species of carp is shown in Table 6.

Table 6: **List of exotic carp species of Bangladesh.**

SPECIES	*COMMON NAME*	*SOURCE*	*YEAR OF INTRODUCTION*
Ctenopharyngodon idellus	Grass carp	Hong Kong Nepal Japan China	1966 1979 1970 1994
Mylopharyngodon piceus	Black carp	China	1983
Hapophthalmic molitrix	Silver carp	Hong Kong China	1969 1994
Aristichthys nobilis	Bighead carp	Nepal China	1981 1994
Cyprinus carpio var. communis	Common carp	China Vietnam	1960 1995
Cyprinus carpio var. specularis	Mirror carp	Nepal Hungary	1979 1982,1996
Barbodes gonionotus	Silver barb	Thailand Indonesia	1987 1994
Tor putitora	Mahseer	Nepal	1981

Sources: Hasan (1990), Rahman (1985), Hussain and Mazid (2001)

1.4. Genetic conservation of some endangered carp species

Because of natural and man induced phenomena occurring in aquatic ecosystems, the natural breeding and feeding grounds of some of the important floodplain and riverine fishes have been severely degraded. Open water capture fisheries are under great stress and their sustainability is in danger. In addition indiscriminate and destructive fishing practices have caused havoc to the aquatic biodiversity. Recent estimation suggest that worldwide 20% of all fresh water species are extinct, endangered or vulnerable (Moyle and Leidy, 1992). IUCN Bangladesh (2000) has documented about 54 freshwater fish species critically or somewhat endangered carp and barb species (Table.7). There is a need, therefore, for development of artificial breeding and seed production techniques of such carp species for genetic conservation of their "gene pool" and biodiversity.

Table 7: List of endangered carp and barb species of Bangladesh

SCIENTIFIC NAME	LOCAL NAME	CRITICALLY ENDANGERED	ENDANGERED	VULNERABLE
Labeo nandina	Nandina	×		
L.boga	Bhangan	×		
L.gonius	Ghonia		×	
L.bata	Bata			×
L.pangusia	Ghora maach		×	
L.calbasu	Kalbaus		×	
Cirrhinus reba	Laachu			×
Puntius sarana	Sarpunti	×		
P.ticto	Til punti			×
Tor tor	Mahashol	×		
T.putitora	Mahashol	×		

Source: Hasan and Mazid, (2002)

1.5. Genetic Improvement of Farmed Tilapia

In the early 1990s, ICLARM develop a new strain of tilapia by cross-breeding several strains of Nile tilapia (*Oreochromis niloticus*). Neither genetically modified nor transgenic, has the new strain been developed using traditional selective breeding methods under the Genetic Improvement of Farmed Tilapia (GIFT) programme.

Tilapia was introduced to Banglasdesh from Thailand in 1954 (Rahman, 1985). It was expected at that time that tilapia would act as a miracle fish in aquaculture. While tilapias breed several times a year and depend mostly on vegetative food, their distribution and culture among rural farmes has not been as popular as expected. Gradually nilotica (*O. niloticus*) and red tilapia (a mutant of *O. niloticus* × *O .mssambicuss*) were imported to Bangladesh from Thailand (Gupta *et al*., 1992).Genetically Improved Farmed Tilapia strain known as GIFT (Eknath *et al*., 1993) was introduced to Bangladesh by ICLARM and BFRI during 1994. GIFT has now become a popular fish among farmers. GIFT is claim to have better growth and survival rate ,higher meat to bone ratio, economic viability, social acceptability and environmental compatibility in comparison to the conventional strains.

1.6. Present status of genetic research in endemic and exotic carp species

The ongoing research project of the carp species are:

- Stock improvement of silver barb, *Barbodes goninotus*, using selective breeding and line breeding techniques
- Stock improvement of silver barb using chromosome manipulation and sex inversion techniques
- Stock improvement of rohu, *L. rohita* ,using selective breeding techniques
- Stock improvement of rohu, *L. rohita*, through production of mitotic gynogens and genetic clonal lines
- Genetic stock improvement through interspecific hybridization and chromosome manipulation.
- Determination of the extent of genetic introgression (hybrid introgression) in chinese carps using molecular genetic markers.

1.7. Artificial induction of polyploidy in major carps

Some preliminary attempts have been made to induce artificial induction of triploidy and tetraploidy in major carps with varied degrees of success (Reddy *et al*., 1990). Reddy *et al*.(1990) successfully induced triploidy in *L. rohita* and *Catla catla* by using thermal shocks.

Similar successful induction of tetraploid was also reported in the case of *Cirrhinus mrigala* and *L. rohita* (Zhang, 1990).Triploidy was induced in rohu by administering heat shocks to zygotes, seven minutes after fertilization at 42^0C +0.5^0C for duration of 1-2 minutes. But the incidence of triploidy was 12% (Reddy *et al*., 1990). Islam *et al* (1994) reported induction of triploidy in *L. rohita* by applying heat shock at 42^0C for 2 min starting 4 min after fertilization. The rate of triploidy induction was found to be 60%.The survival in the heat shock groups was recorded 15% and 25% in the control groups. The growth rate of triploid individuals was found to be significantly higher than in diploids for weight and length, $P<0.01$ and $P<0.05$ respectively.

In some species, triploids are observed to grow faster than the normal diploids (Kraznai and Marian, 1986). Triploidy is also expected to cause sterility due to the reduced or suppressed development of the gonads. Sterile fish can put to varied use in aquaculture depending on the species. Sterility in species like *Cyprinus carpio* and tilapia may help in controlling unwanted reproduction. In species like Chinese grass carp if made sterile, it can be used effectively to control weeds in the irrigation canal networks, lakes and other open waters without any fear of it's establishment in such waters.

Sterility showing faster growth may be useful in species having shorter maturity cycle. However, there are also reports that triploid sterile fish did not show better growth in species like *Cyprinus carpio* and tilapia (Cherfas *et al*., 1994; Hussain *et al*., 1995).Contrary to this reports *Cyprinus carpio* that triploid sterile individuals have shown better growth than their diploids counter parts (Malison *et al*., 1993).

1.8. Triploidy induction in Stinging catfish *Heteropneuste fossilis* using cold shock

Gheyas (2001) induced triploidy in newly fertilized eggs of *H. fossilis* using cold shock. The eggs were exposed at two temperature- regimes viz. 2^0C and 4^0C.Among the various combinations of temperature and duration tested, shock duration for 10 min at 2^0C applied 3 min after fertilization was the best; it induced triploidy upto 94 to 97% of the eggs and had the best hatching and survival percentage of the triploid larvae. The success of triplody induction was determined by chromosome counting. Erythrocytes measurements from diploid and triploid individuals were compared to results of karyotyping which showed a significant increase (P <

0.05) in the erythrocyte nuclear major and minor axes, area and volume in triploids over those on diploids. This work served as a basis for further work to test other shock protocols and to scale up the most practical method for commercial production of triploid shock. Besides, attempts are, making to produce tetraploid and then and then to produce a hybrid triploid from tetraploid × diploid crosses. In this way, the adverse effects of shock application can be averted.

1.9. Triploidy induction in hybrid Catfish (♀ *Clarius batracus* L. ×♂ *Clarius gariepinus* B) using heat shock

Ezaz (1998) induced triplody in hybrid catfish ♀ ***Clarius batracus* L.** ×♂ ***Clarius gariepinus* B** by heat shocking the recently fertilized eggs at 38^{0}C to 41^{0}C (with 1^{0}C interval)to 1 to 3 minutes (with 0.5 minutes interval) starting 3 to 7 minutes (with 2 minutes interval after fertilization). The triploid status of hybrid catfish was determined by karyological analysis on one day old larvae. The highest rate (70%) triploid (3n=80) was identified in eggs exposed to heat shock at 40^{0}C applied for 1.5 minutes starting 3 minutes after fertilization. Heat shocks longer than one minute and three minutes after fertilization applied at 41^{0}C was found to be lethal and caused 100% mortality. The average survival rate at one week age in triploid group was relatively low (38%) as compared to the diploid control group (88%). The growth of triploid hybrid was significantly ($P< 0.05$) slower than that of the diploid hybrids at the juvenile (18 weeks) age.

1.10. Future Research Plans

Following are future plans for aquaculture genetic research in Bangladesh:

- Genetic characterization of wild land races of *C. catla* and *L. rohita.*
- Initiation of genetic stock improvement of *C .catla* and *L. rohita* using selective breeding and line crossing techniques.
- Evaluation of growth performances of F_4 putative genetically improved *B. gonionotus.*
- Genetic sex determination and production of all female population of *B. gonionotus* through gynogenesis and sex reversal.

- Continuation of selection of GIFT strain *O. niloticus* for further genetic improvement.
- Optimization of sex reversal technique for GIFT strains O. *niloticus.*
- Continuation of genetic manipulation in *H. fossilis*, particularly production of all-female population through inducing homogenetic neomales.
- Continuation of population genetics study of *T. ilisha.*

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Appendix 1: Country map. Project areas for shrimp and carp culture development in Bangladesh - Second Aquaculture Development Project of the Asian Development Bank (ADB). (Source: ADB Project Performance Audit Report, 2002).

Appendix 1: Country Profile	
Country name:	*Conventional long form:* People's Republic of Bangladesh *conventional short form:* Bangladesh *former:* East Pakistan
Background:	Bangladesh came into existence in 1971 when Bengali East Pakistan seceded from its union with West Pakistan. About a third of this extremely poor country floods annually during the monsoon rainy season, hampering economic development.

Location:	Southern Asia, bordering the Bay of Bengal, between Burma and India
Geographic coordinates:	24 00 N, 90 00 E
Area:	*Ttotal:* 144,000 sq km *land:* 133,910 sq km *water:* 10,090 sq km
Area - comparative:	Slightly smaller than Iowa
Land boundaries:	*Total:* 4,246 km *border countries:* Burma 193 km, India 4,053 km
Coastline:	580 km
Maritime claims:	*Territorial sea:* 12 nm *contiguous zone:* 18 nm *exclusive economic zone:* 200 nm *continental shelf:* up to the outer limits of the continental margin
Climate:	Tropical; mild winter (October to March); hot, humid summer (March to June); humid, warm rainy monsoon (June to October)

Natural	Natural gas, arable land, timber, coal

Population:	141,340,476 (July 2004 est.)
Age structure:	*0-14 years:* 33.5% (male 24,359,149; female 23,013,811) *15-64 years:* 63.1% (male 45,557,963; female 43,626,950) *65 years and over:* 3.4% (male 2,575,519; female 2,207,084) (2004 est.)
Median age:	*Total:* 21.5 years *male:* 21.5 years *female:* 21.5 years (2004 est.)
Population growth rate:	2.08% (2004 est.)
Birth rate:	30.03 births/1,000 population (2004 est.)
Death rate:	8.52 deaths/1,000 population (2004 est.)
Net migration rate:	-0.71 migrant(s)/1,000 population (2004 est.)
Sex ratio:	*At birth:* 1.06 male(s)/female *under 15 years:* 1.06 male(s)/female *15-64 years:* 1.04 male(s)/female *65 years and over:* 1.17 male(s)/female *total population:* 1.05 male(s)/female (2004 est.)
Infant mortality rate:	*Total:* 64.32 deaths/1,000 live births *male:* 65.41 deaths/1,000 live births *female:* 63.16 deaths/1,000 live births (2004 est.)
Life expectancy at birth:	*Total population:* 61.71 years *male:* 61.8 years *female:* 61.61 years (2004 est.)
Total fertility rate:	3.15 children born/woman (2004 est.)
HIV/AIDS - adult	Less than 0.1% (2001 est.)

prevalence rate:	
Nationality:	*Noun:* Bangladeshi(s) *adjective:* Bangladeshi
Ethnic groups:	Bengali 98%, tribal groups, non-Bengali Muslims (1998)
Religions:	Muslim 83%, Hindu 16%, other 1% (1998)
Languages:	Bangla (official, also known as Bengali), English
Literacy:	*Definition:* age 15 and over can read and write *total population:* 43.1% *male:* 53.9% *female:* 31.8% (2003 est.)

Government type:	parliamentary democracy
Capital:	Dhaka

GDP:	Purchasing power parity - $258.8 billion (2003 est.)
GDP - real growth rate:	5.3% (2003 est.)
GDP - per capita:	Purchasing power parity - $1,900 (2003 est.)
GDP - composition by sector:	*Agriculture:* 21.7% *industry:* 26.6% *services:* 51.7% (2003 est.)
Investment (gross fixed):	23.2% of GDP (2003)
Population below poverty line:	35.6% (FY95/96 est.)
Inflation rate (consumer	

prices):	5.6% (2003 est.)
Unemployment rate:	40% (includes underemployment) (2002 est.)
Agriculture - products:	Rice, jute, tea, wheat, sugarcane, potatoes, tobacco, pulses, oilseeds, spices, fruit; beef, milk, poultry
Industries:	Cotton textiles, jute, garments, tea processing, paper newsprint, cement, chemical fertilizer, light engineering, sugar
Exports:	$6.713 billion (2003 est.)
Exports - commodities:	garments, jute and jute goods, leather, frozen fish and seafood (2001)
Imports:	$9.459 billion (2003 est.)
Imports - commodities:	Machinery and equipment, chemicals, iron and steel, textiles, foodstuffs, petroleum products, cement (2000)
Reserves of foreign exchange and gold:	$2.624 billion (2003)
Economic aid - recipient:	$1.575 billion (2000 est.)
Currency:	Taka (BDT)
Exchange rates:	Taka per US dollar - 58.15 (2003), 57.888 (2002), 55.8067 (2001), 52.1417 (2000), 49.0854 (1999)
Fiscal year:	1 July - 30 June
Source: The World Fact Book 2005	