Effects of paternal phenotype on offspring fitness in rohu (*Labeo rohita*)

MD. ASHIKUR RAHMAN

FISHERIES & MARINE RESOURCES TECHNOLOGY DISCIPLINE

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MD. ASHIKUR RAHMAN

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APPROVED AS TO THE STYLE AND CONTENT

BY

G.R.Banu 17.12.18
....

(Signature of the Head)

Professor Dr. Ghausiatur Reza Banu

Head

Fisheries & Marine Resource Technology Discipline

Life science school, Khulna University

Khulna-9208, Bangladesh

NOVEMBER, 2018

DECLARATION

Effects of paternal phenotype on offspring fitness in rohu (*Labeo rohita*)

The results presented in the thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

19.11.18

....
(Signature of the candidate)

MD. ASHIKUR RAHMAN
Student ID: MS-170634
Session: 2016-17

19/11/18
....
(Signature of the supervisor)

DR. MD. MOSHIUR RAHMAN

Professor
Fisheries and Marine Resource Technology Discipline
Khulna University, Khulna

NOVEMBER, 2018

Dedicated

To

My Beloved Parents

Acknowledgement

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Md. Ashikur Rahman
Khulna University
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Abstract

In many species, males influence phenotypic traits in their offspring through non-genetic paternal effects. Such effects can represent a form of paternal investment to maximize offspring fitness. Here, I have conducted an experiment to explore whether bigger males have better reproductive performance and thereby, produce the best quality offspring. In the experiment, I have three male size groups, such as 1) bigger males (3-5kg), 2) medium males (1-2kg), and 3) smaller males (less than 1kg). There were 20 males in each group, and some common females were taken to conduct this study. The experiment was designed in such a way that enabled to measure the variation of some selected reproductive traits and then to assess offspring fitness traits after rearing them up to two weeks under controlled laboratory conditions. The results have revealed that both bigger and medium size males produced significantly higher amount of sperm than smaller ones. In case of hatching rate, bigger males produced significantly higher number of hatchlings than smaller ones, while no variations have been found between big and medium, and also medium and small male groups. No significant difference was observed in hatchlings deformation rate among the experimental male groups. Finally, the study has revealed that bigger fathers offspring had significantly higher survival rate, obtained larger standard length and body area than medium and smaller father groups. These results suggest that parents can be selected to maximize the offspring fitness traits which would be an important message to the associated aquaculture stakeholders.

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CHAPTER ONE

INTRODUCTION

1.0 Introduction

1.1 Background of the study

The fisheries and aquaculture sector is a momentous source of employment and income, corroborating the livelihoods of 12 percent of the world's population. As good as 60 million people are employed in the primary sector alone and a further 140 million are employed along the value chain from harvesting to distribution. Women directly append in primary production account for more than 15 percent of people engaged in fisheries and aquaculture, and 90 percent of those connected in processing activities (FAO, 2016). Besides, world aquaculture production continues to grow and now provides half of all fish for human consumption. Furthermore, the 2030 Agenda for Sustainable Development, the Sustainable Development Goals (SDGs), and the Paris Agreement will have a strong impact on the fisheries and aquaculture sector, and the sector must play its part in order to achieve their narrated societal, economic and environmental aims (FAO, 2016).

Bangladesh is one of the world's leading fish producing countries with a total production of 41.34 lakh MT, where aquaculture contributes 56.44 percent to total production and last 10 years average growth performance of this sector is almost 5.43 percent (DoF, 2017). Government is trying to sustain this growth performance, which eventually ensures to achieve the projected production target of 4.55 million MT by 2020-21 (DoF, 2017). Bangladesh is ranked 6th in world aquaculture production (FAO, 2017). On the other hand, Bangladesh has acquired self-sufficiency in fish production. Even though the target of fish production was 40.50 lakh MT in 2016-17, but it crossed the target by producing 41.34 lakh MT fish and actually it's a huge feat for the country (DoF, 2017). Among, different fish species, carp is the top most species in Bangladesh which was produced around 13.22 lakh MT during 2016-17 (DoF, 2017).

Carp aquaculture is rising sharply in Asia. Carp production from Asia contributed 95% to the world total carp production in 2001. In this region, there are more than 20 main inherent carp species, contributing to about 80% of the total freshwater fish production. The most carp production in Asia is contributed by China, India, Bangladesh and Indonesia. Carps are basically the most important species to aquaculture in Bangladesh. The proportion of freshwater carps in total fish production is about 31.98% in 2016-17 followed by small indigenous species 17.56%, hilsha 12.01%, marine fish 7.47%, shrimp and prawn 5.97%, and cat fish 13.95% (DoF, 2017).

Carp seed production is a profitable business in Bangladesh. As a result, many investors are coming forward to invest in aquaculture. Before making an investment in a carp seed production farm, it is necessary to determine whether the planned investment idea is feasible or not. Considering the importance of carp hatcheries and their productions, and also one of their major problems to produce good quality larvae, the present study has been designed to explore how the stakeholders can recognize the best quality broods to produce the best quality offspring for aquaculture. The carp hatcheries are distributed in most parts of Bangladesh. Jessore, Khulna, Satkhira, Bagarhat, Barishal, Jhalukati, Comilla, Chandpur, Chuadanga, Dhaka, Mymensingh, Borguna, Faridpur, Madaripur, Kushtia, Magura, Narail, Pabna, Bogura, Rangpur, Nator, Rajshahi, are the source district of the carp hatcheries. Most of the hatcheries are seen Jessore, Khulna, Mymensingh and Rajshahi. The leading anxiety in the carp hatcheries in Bangladesh are: Hatcheries without broodstock ponds, main objective is the production of mass seed rather than quality seed, lack of erudition on genetic status of hatchery stocks, lack of experience of hatchery and nursery operators, lack of knowledge on effects of open water stocking on wild stock, degradation in genetic quality of seeds due to inbreeding & negative selection and due to hybrid introgression in some major carp species, genetic drift due to small numbers of parent stocks etc.

Phenotypic traits play as a critical role on gamete quality of fish like other vertebrates (Aliniya et al., 2013; Blukacz et al., 2010; Linhart et al., 2005; Trippel 2003; Ciereszko and Dabrowski, 2000). Among many phenotypic traits, body size is a fundamental trait that can affect many aspects of fish performance, including individuals reproductive output (Uusi-Heikkila et al., 2010; Sogard et al., 2008; Marteinsdottir and Begg, 2002; Marshall et al., 2010). For example, in some species male body size and condition are important during competition for access to females during spawning (Fleming and Gross, 1994; Quinn and Foote, 1994). Some studies have already shown that parents' body condition can also influence the fitness traits of offspring (Evans et al., 2017; Gagliano and McCormick, 2007; Pitcher and Neff, 2007; Bernardo, 1996). Therefore, this study has been carried out having a special breeding design to evaluate the effects of body conditions (e.g. body size, ornaments, etc.) on gamete quality and reproductive success like sperm quality, fertility, larval fitness, etc. using a very commercially important fish species of Bangladesh, rohu (*Labeo rohita*) which is one of the most popular Indian major carps.

It is known that rohu (*L. rohita*) is one of the common freshwater fish of the carp family which is very popular fish in Bangladesh and other parts of the world especially south-east Asia. Locally, this fish is also known as "Rui". This rohu belongs to the family of "Cyprinidae" and genus of "*Labeo*". In alliance with all carp fishes, rohu is popular due to its taste and market value. Besides, farmers can expect strong profits in rohu farming with proper farm management practices. Therefore, this study can significantly enhance empirically and economically rewarding research opportunities for aquaculturists, fish geneticists, population biologists, ecologists and evolutionary biologists to advance our consciousness of selection responses, and rates of evolutionary change in fishes.

1.2 Objectives to the study

The study has the following specific objectives to achieve:

- To assess various phenotypic traits of different sized mature male rohu;
- To find out the reproductive performance of the experimental male rohu; and
- To explore the variation of offspring fitness traits due to fathers' size differences.

CHAPTER TWO

LITERATURE REVIEW

2.0 Literature review

2.1 Fish and fisheries resources of Bangladesh

Bangladesh is considered one of the most suitable regions for fisheries in the world, with the world's largest flooded wetland and the third largest aquatic biodiversity in Asia after China and India. Bangladesh is one of the world's leading fish producing countries with a total production of 41.34 lakh MT in 2016-17, whereas inland open water (capture) contributes 28.14 percent (11.63 lakh MT) and inland closed water (culture) contributes 56.44 percent (23.33 lakh MT) to total production. So, 84.58 percent of total production comes from inland fisheries. On the other hand, Marine fisheries production is 6.37 lakh MT and its contribution to total fish production is 15.42 percent with growth rate 1.75 percent (DoF, 2017).

Table 1: Species/Group-wise annual fish production in inland and marine fisheries 2016-17 (DoF, 2017)

Sl. No.	Species / Group	Inland Fisheries (Metric Ton)	Marine Fisheries (Metric Ton)	Total (Metric Ton)	%
1	Major carp	811588	0	811588	19.63
2	Other carp	100730	0	100730	2.44
3	Exotic carp	409801	0	409801	9.91
4	Pangas (cat fish)	510097	0	510097	12.34
5	Other cat fish	66646	0	66646	1.61
6	Snake head	72991	0	72991	1.77
7	Live fish	127120	0	127120	3.07
8	Tilapia	370017	0	370017	8.95
9	Other inland fish	598923	0	598923	14.49
10	Hilsha	217469	278948	496417	12.01
11	Shrimp/Prawn	197155	49619	246774	5.97
12	crab	14421	0	14421	0.35
13	Sardine	0	48704	48704	1.18
14	Bombay duck	0	69230	69230	1.67
15	Indian salmon	0	775	775	0.02
16	Pomfret	0	10686	10686	0.26
17	Jew fish	0	33768	33768	0.82
18	Sea cat fish	0	8424	8424	0.20
19	Shark/Skate/Ray	0	4495	4495	0.11
20	Other marine fish	0	132827	132827	3.21
TOTAL		**3496958**	**637476**	**4134434**	**100**

2.2 The experimental species (*Labeo rohita*)

2.2.1 Habitat and Ecology

Rohu is the natural inhabitant of freshwater sections of the rivers. Rohu thrives well in all fresh waters below an altitude of approximately 549 m. Rohu is a bottom feeder and prefers to feed on plant matter including decaying vegetation. Rohu attains maturity towards the end of the second year in ponds. The spawning season of rohu generally coincides with the southwest monsoon. Spawning takes place in flooded rivers. The fecundity of rohu varies from 226,000 to 2,794,000, depending upon the length and weight of the fish and weight of the ovary. (Talwar and Jhingran, 1991).

2.2.2 Taxonomy

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Subfamily: Labeoninae

Genus: *Labeo*

Species: *L. rohita*

Figure 1: The experimental species rohu (*Labeo rohita*).

2.2.3 Morphology

Body elongated and highest body length is found at the commencement of dorsa fin. Snout fairly depressed, projecting beyond jaws, without any lateral lobe. Inter orbital space convex. Lips thick, fringed. Post labial grove continuous and cartilaginous part is covering to inside of both lips. A pair of short maxillary barbells is present. Body color is back brownish, silvery on the sides and beneath. Dorsal and abdominal profiles are convex, caudal peduncle is short. Lateral line is complete and scales are moderate (Rahman, 2005; Talwar and jhingran, 2001).

2.2.4 Reproduction

Male and female rohu attain first sexual maturity at two years of age and 50 percent maturity is attained at three years. Complete maturity of male and female is observed at age four and five years respectively (Khan and Jhingran, 1975). The minimum size at first maturity of male is about 46 cm and in the case of female it is about 51 cm. One hundred percent maturity has been found in 65 cm length group in the case of male while in the case of female hundred cent percent maturity has been found in 70 cm group (Chondar, 1999). During the breeding season the pectoral fins of mature males are rough on the dorsal surface, while those of females are smooth. Rohu is highly fecund (109 000 to 535 000 eggs per kg) (Chondar, 1999). The species breeds naturally in rivers, reservoirs, and in large lakes in which fluvial conditions are simulated. The breeding season generally coincides with the southwest monsoon. The fish can be spawned through hypophysation with 2-3 mg pituitary/kg body weight followed by a second dose of 5-8 mg/kg after an interval of 4-6 hours. Males are given only a single dose of 2-3 mg/kg at the time of the second injection given to females. Fish generally spawn within six hours of the second injection.

2.2.5 Feeding behavior

At the fry stage rohu is predominantly a planktivorous surface feeder. From the fingerling stage onwards the feeding habit changes and the fish feed in the water column and on the bottom, mainly on filamentous algae, decomposed vegetation, mud and sand (Chondar, 1999). The mouth with its soft fringed lips and sharp cutting edges also indicate that rohu feed on aquatic vegetation. The modified gill rakers suggest that the species is also capable of filter feeding (Chondar, 1999). Feeding intensity varies with size, the reproductive cycle, season and environment. On average fish in smaller size classes feed more intensively than larger fish and in general, the feeding intensity of juvenile is high throughout the year with a slight drop during the winter months. In adults, the feeding intensity is affected by gonadal recrudescence. Both the males and females feed well when they are immature and there are considerable increase in the feeding intensity as they progress to the maturing stages. In the mature stages, there is reduction in feeding. After spawning, the spent fishes again start feeding actively. This increase and decrease is more prominent in females than males (Khan and Jhingran, 1975).

2.2.6 Maintaining broodstocks and gamete quality

A major issue for the aquaculture industry is control of gamete quality. This is especially true in the context of global environmental changes and the current increase in the number of aquaculture species (Chevassus-au-Louis & Lazard 2009) for which the success of reproduction can be a major issue. The quality of a gamete can be defined as its ability to fertilize or to be fertilized, and subsequently develop into a normal embryo (Bobe & Labbe 2010). The identification of predictive estimators or markers of gamete quality would have major applications in research and industry. Broodstock management involves manipulating environmental factors surrounding the broodstock to ensure maximum survival, enhance gonadal development and increase fecundity. Such conditioning is obligate to ensure the sustainability of aquaculture production, and to enhance the number and quality of eggs produced and control the timing of maturation and spawning (Izquierdo et al., 2001). One of the essential step for aquaculture is management of the technologies for gamete production in captivity that would ensure the growth to this sector. Unfortunately, most fish when reared in captivity condition, exhibit some degree of reproduction dysfunction. Many species of captive fish are able to reach reproduction maturity in aquaculture conditions and gonadal growth occurs normally. However, some of female species often fail final oocyte maturation stage. Hormonal manipulation and acceleration of final oocyte maturation due to the economics of broodstock management is important. For instance, in Salmoniformes, the need to collect the eggs by stripping is a serious limitation, while the time of ovulation must be predicted with accuracy, as over-ripening may take place in minutes or hours after ovulation (Mousavi, 2013). Therefore, control of broodstock management is essential for the sustainability of commercial aquaculture practice.

2.2.7 Paternal phenotypes and offspring fitness

Offspring fitness is a very much important term in case of fish farming. This term defines the health condition of newly hatched fingerlings. The fitness of offsprings depends on a variety of factors as gamete quality, egg quality, sperm quality, parental species quality, environment etc. In order to produce healthy offspring this all factors must be properly maintained by the hatcheries (Sehgal, 1991). It is well established that environmentally moderated changes to an individual's phenotype can have fitness implications that transcend generations. Paternal effects can arise when environmental factors experienced (or imposed) by males influence offspring traits, a phenomenon best described in species exhibiting paternal care (Frazier et al., 2006). However, the possibility that paternal environmental effects can be transmitted to offspring in species lacking paternal care has received considerably less attention. In particular, the possibility that paternal environmental effects are transmitted via sperm (Soubry et al., 2014).

2.3 Research conducted on broodstock quality, paternal phenotypes and offspring fitness

Many researchers have been carried out on broodstock quality and larval fitness, some of them are as follows.

Talwar and Jhingran, (1991) worked on the relationship between fish size and sperm volume, hematocrite index, gonadal characteristics and spermatological parameters in rutilus (*Rutilus caspicus*). Saillant et al., 2001 research on parental influence on early development in the european sea bass (*Dicentrarchus labrax*). Aliniya et al., 2013 worked on influence of age of common carp (*Cyprinus carpio*) broodstock on reproductive traits and fertilization. Blukacz et al., 2010 worked on linking lake white fish (*Coregonus clupeaformis*) condition with male gamete quality and quantity. Bobe, 2010 studied on Egg and sperm quality in fish. Sehgal et al., 1991 studied on Offspring fitness and fecundity of an Indian major carp, *Labeo rohita* in relation to egg size. Izquierdo et al., (2001) worked on effect of broodstock nutrition on reproductive performance of fish. Peixoto et al., (2011) research on broodstock maturation and reproduction of the indigenous pink shrimp (*Farfantepenaeus paulensis*). Fast, (1994) conducted an experiment on ffects of broodstock size and source on ovarian maturation and spawning on *Penaeus monodon*.

2.4 About the present study

The above literature review adverted the various factors that affect hatching of fishes. But phenotypic traits of broodstocks' (e.g. total length, body weight etc.) may affect fertilization success and offspring fitness of fishes. Therefore, a core aim of this present work is to explore any correlation of male phenotypic traits with hatching success using the most popular and commercially demandable carp species, rohu (*L. rohita*).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Materials and Methods

3.1 Source and maintenance of broodstocks

Sexually mature 'rohu' (both male and female) of different sizes were collected from commercial carp hatcheries and fish farms that strictly maintain breeding protocols to avoid associated inbreeding problems. Separately 60 mature males of three size groups (20 individuals of weight below 1kg, 2-3kg and above 3kg) and 20 mature females (weight between 2 to 2.5kg) were collected and then each group of males and females were reared separately under the same environmental conditions providing optimum level of diet up to one week.

Figure 2: A sexually mature male rohu after ejecting sperm.

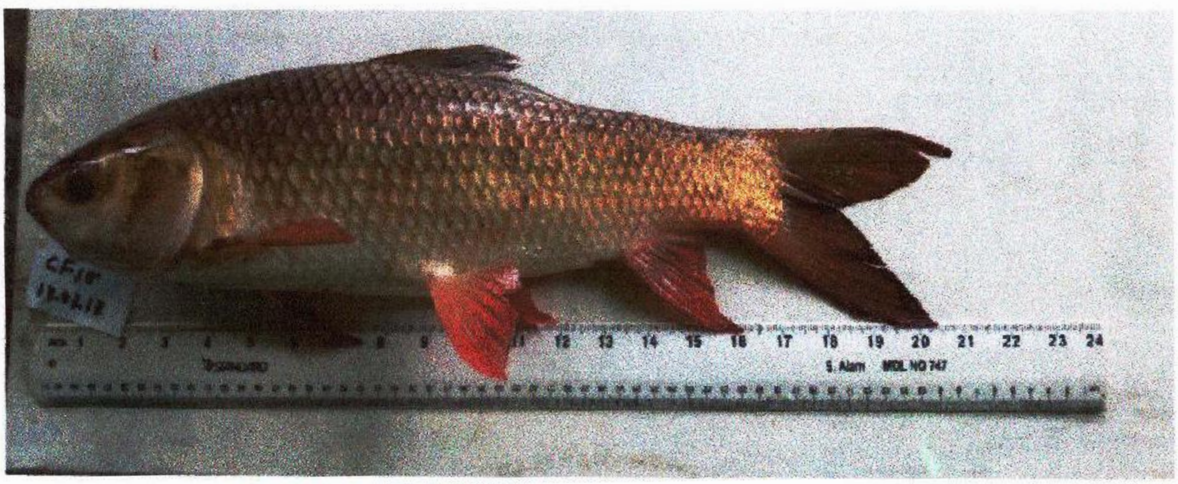

Figure 3: A sexually mature female rohu after collecting eggs.

3.2 Artificial propagation

The artificial propagation technique (also called 'hypophysation') was applied at the base of pectoral fin by using the recommended doses of pituitary glands (PG) for induced spawning (figure 4). The females were administered two times at the rate of 0.5 mg PG/kg body weight for first time and approximately after 6 hours, the second dose was injected at the rate of 6.0 mg PG/kg body weight. The experimental males were administered only a single dose when the females were injected their second dose at the rate of 1 mg PG/kg body weight. Then they were ready for artificial spawning after another 6 hours.

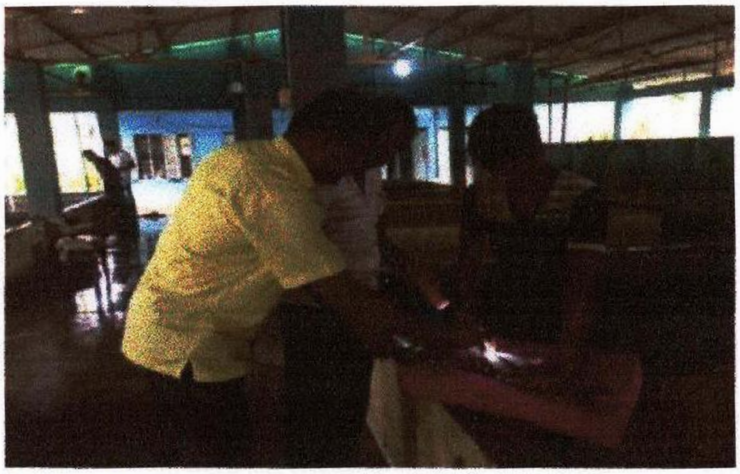

Figure 4: Administering the recommended dose of PG for induced spawning.

3.3 Experimental design

The experiment was designed in a way so that only a limited number of fish could be used to utilize the maximum available facilities for this research. Every week only six males and a common female were used to explore the male size effect on gamete quality (figure 5). In the experiment, first eggs were collected in a clean and dry bowl from a common female by hand stripping (figure 6) and immediately weights of fish and total egg were measured using a digital balance, while photo of fish was taken using a digital camera (Canon DS126621). As described above, there were three treatments (i.e. three sized group) and each treatment had two replications. So, exactly 1 mL of eggs was collected from the bowl contained eggs with a marked syringe for each replication of treatments, and also 1 mL into a marked vial for egg traits measurement. Thus, two bowls were ready for each treatment to put 1 mL of eggs (figure 7). Then sperm were collected from each male by hand stripping (figure 8) and immediately weights of fish and sperm were measured using a digital balance, while photo of individual male was taken using the same digital camera. Exactly 0.5 mL of sperm was collected with a marked syringe from the total sperm pool of an individual male and then added to the bowl of each replication (figure 9). The sperm were properly mixed with the eggs by adding a small amount of clean water and continuously stirring the mixture with a clean chicken's feather (figure 10). The fertilized egg masses were then transferred to previously labeled plastic containers (2 L) filled with sufficient aerated water (figure 11). They were allowed for incubation up to 38-40 hours at ambient temperature (25-30°C) (figure 12). After three days, the hatchlings were transported in plastic bags with sufficient aeration (figure 13) to the Fish Physiology laboratory of FMRT Discipline, Khulna University to count the total number of hatchlings and deformed larvae in each replication using a white plastic spoon (figure 14). Then only 20 good looking larvae from each replication were sorted to rear in individual aquarium up to two weeks (figure 15) to investigate the offspring fitness. Water quality parameters and survival were checked daily and exactly same amount and same quality diet was provided to them. After two weeks, each larva was anaesthetized to take photo using the digital camera for fitness traits assessment.

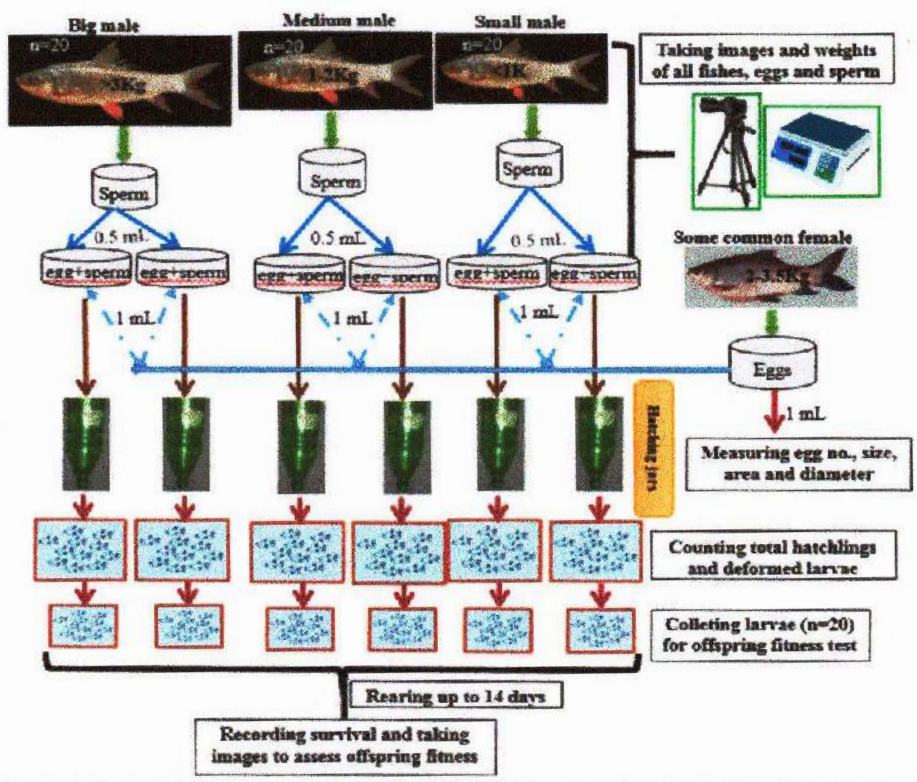

Figure 5: The overall experimental design to explore the male size effect.

Figure 6: Stripping females to collect eggs.

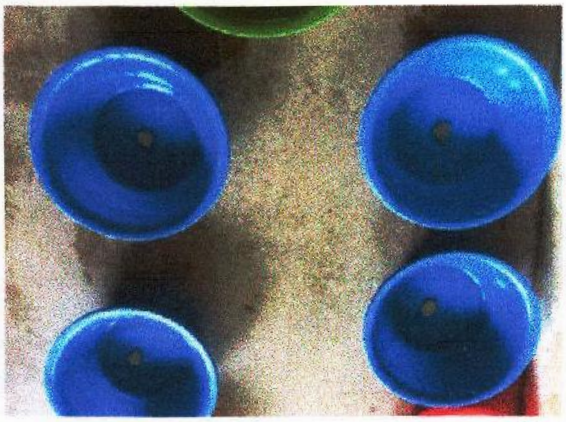

Figure 7: Pouring 1 mL egg to bowl.

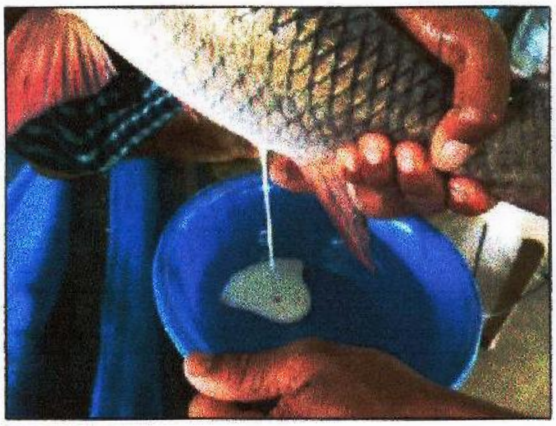

Figure 8: Stripping males to collect sperm.

Figure 9: Adding 0.5 mL sperm with eggs.

Figure 10: Mixing and stirring eggs and sperm for fertilization.

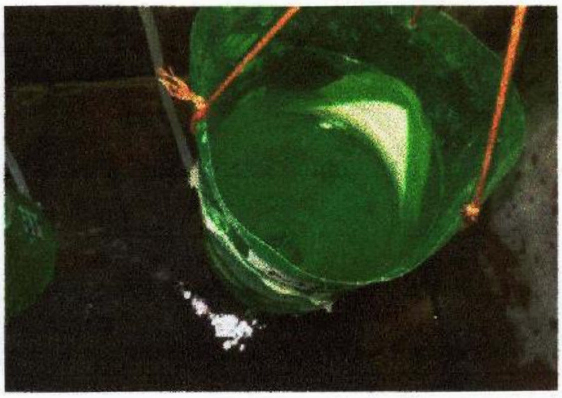

Figure 11: Transferring fertilized eggs in labeled hatching jars.

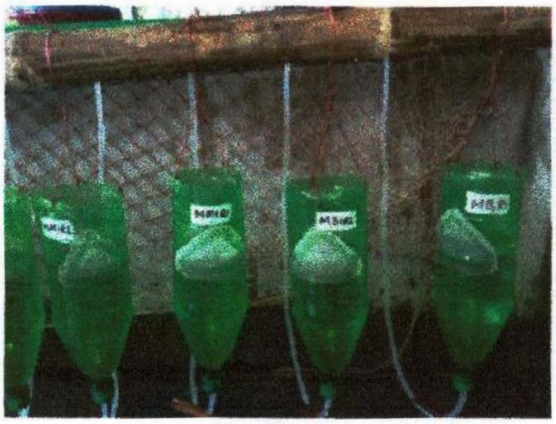

Figure 12: Jars/bottles contained fertilized eggs with sufficient aeration for hatching.

Figure 13: Plastic bags contained hatchlings with sufficient aeration.

Figure 14: Counting normal and deformed hatchlings.

Figure 15: Rearing hatchlings in labeled aquarium.

3.4 Measurements of phenotypic traits

After collecting sperm and eggs from each male and female, individual's photograph along with their ID was taken with a digital camera (Canon DS126621). Then total length (distance in cm from tip of the snout to the posterior tip of the tail, figure 16) and body area (area covered from tip of the snout to the last mark of caudal peduncle, figure 16) were measured from the digital photographs taken of each fish using *ImageJ* software (http://rsbweb.nih.gov/ij/).

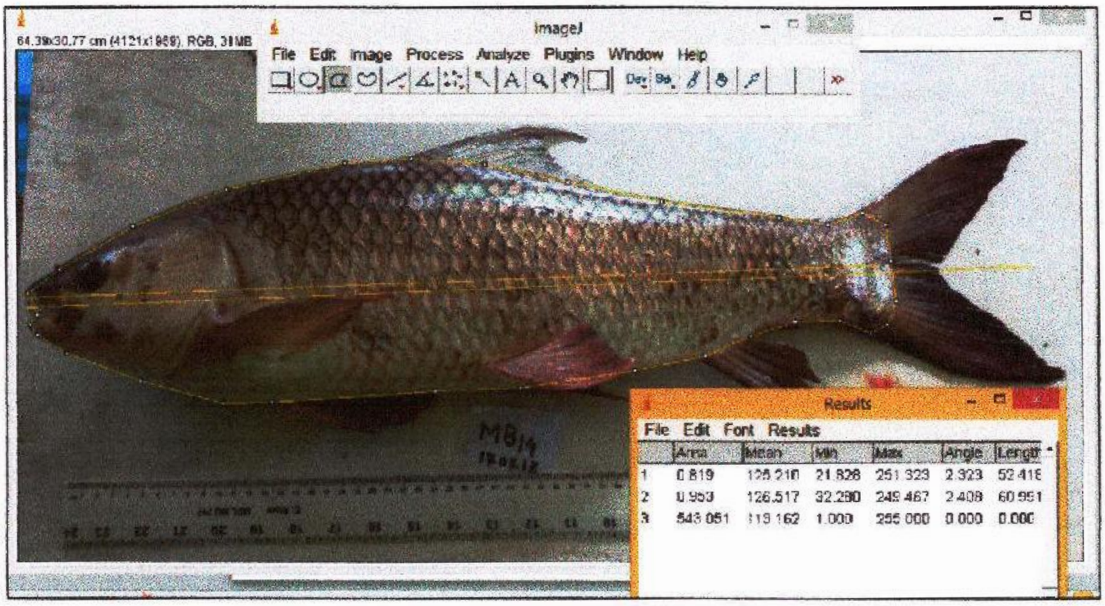

Figure 16: Measuring total length and body area from the taken photograph using ImageJ software.

3.5 Offspring fitness test

After rearing three weeks exactly under same condition, larval survival and deformity were recorded. Then each larva was anaesthetized to take photo using the digital camera to measure total length, body area, etc. using the ImageJ software (figures 17 and 18).

Figure 17: Daily monitoring the reared larval status at the lab.

Figure 18: A photograph of a two-week old larva for ImageJ analysis.

3.6 Statistical analyses

Data analysis was performed using 'RStudio' version 1.0.143 (RStudio Team, 2016). The descriptive statistics (means, SD, SEs, etc.) were calculated using the 'psych' package (Revelle, 2017), normality and homogeneity were tested with the 'car' package (Fox and Weisberg, 2011). Appropriate transformation was applied to yield normal distributions for all interested traits.

A repeated measure anova model was applied using 'car' package (Fox and Weisberg 2011) where target trait was included as a response variable, treatment and the male-by-female interaction as fixed factors and female body weight as a random effect (to account for exact amount of eggs come from the same female). When the target trait was not normally distributed, I used the Kruskal-Wallis test. While the trait was also not homogeneous, I used the Welch's Heteroscedastic F test. Finally, all graphs were made using 'ggplot2' package (Wickham 2009).

CHAPTER FOUR

RESULTS

4.0 Results

4.1 Males phenotypic variations

Since the experiment has been designed keeping the male into three different size groups, the analysis has also revealed that there are significant variations in body weight and standard length among these three male groups used in this study (table 2).

Table 2: Phenotypic variations among different male groups

Male traits	Big male (mean±se)	Medium male (mean±se)	Small male (mean±se)	F-value	*P*
Body weight (kg)	2.99±0.10	1.90±0.05	0.96±0.04	232.89	***<0.001***
Standard length (cm)	49.90±1.18	42.92±0.70	33.38±0.61	98.99	***<0.001***

*Significant level is denoted by bold and italic font along with $P< 0.05$, $P< 0.01$, $P< 0.001$.

4.2 Reproductive performance

4.2.1 Sperm production

The study has shown that both big size (3-5kg) and medium size males (1-2kg) produced significantly higher amount of sperm than smaller ones (less than 1kg), while no variation has been found between big and medium size male groups in sperm production (figure 19).

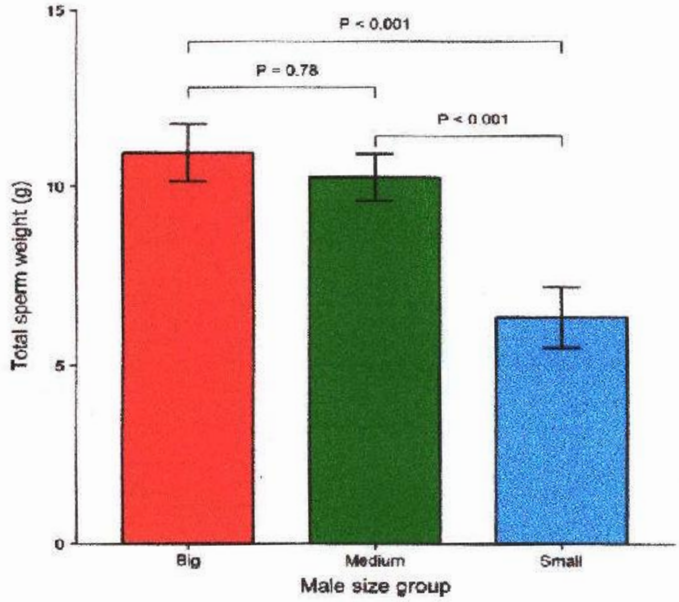

Figure 19: Variation in total sperm weight (mean±se) among different experimental male groups.

4.2.2 Hatching rate

The experiment has revealed that big size produced significantly higher number of hatchlings than smaller ones, while no variations have been found in offspring production between big and medium, and also medium and small male groups (figure 20).

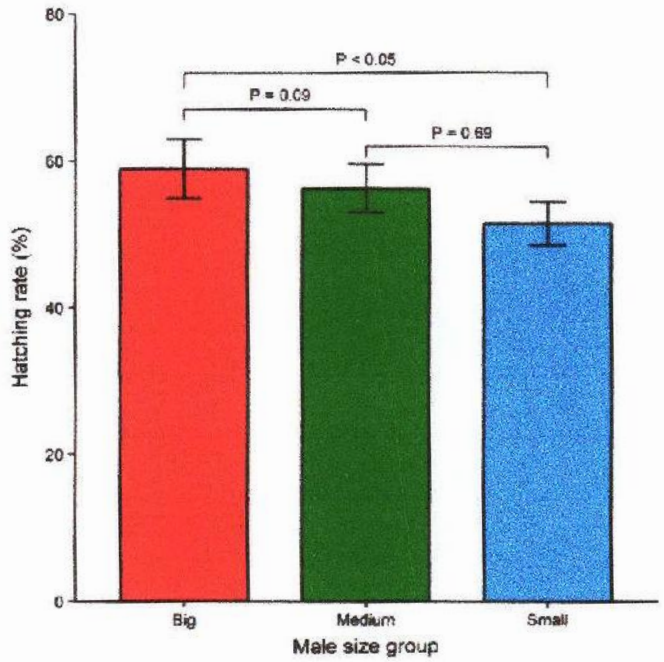

Figure 20: Variation in hatching rate (mean±se) among different experimental male groups.

4.2.3 Hatchlings deformation rate

The study has found no significant variation in hatchlings deformation rate among the different sized experimental male groups ($F_{2,57}$=1.03, P=0.36).

4.3 Offspring fitness

4.3.1 Offspring survival rate

The study has revealed that parents' body size can influence the offspring fitness (e.g. survival, total length and body area) where hatchlings were reared up to 14 days in aquarium under the same controlled conditions. The analysis has shown that the survival percentage of hatchlings sired by big males was significantly higher than medium and small male groups, whereas no significant variation in hatching rate has been found between medium and small male group (figure 21).

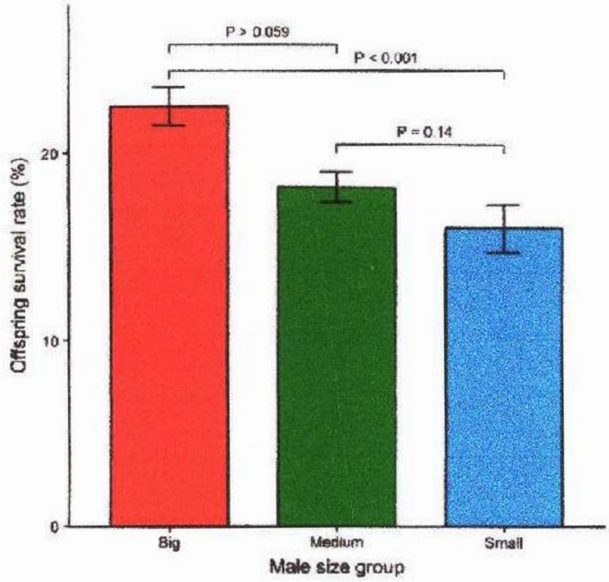

Figure 21: Variation in hatchlings survival (mean±se) among different experimental male groups.

4.3.2 Offspring total length

After two weeks of rearing, it has been found that bigger fathers' offspring became significantly larger than the medium and smaller male groups, and also the medium sized males produced significantly bigger offspring than the smaller male group (figure 22).

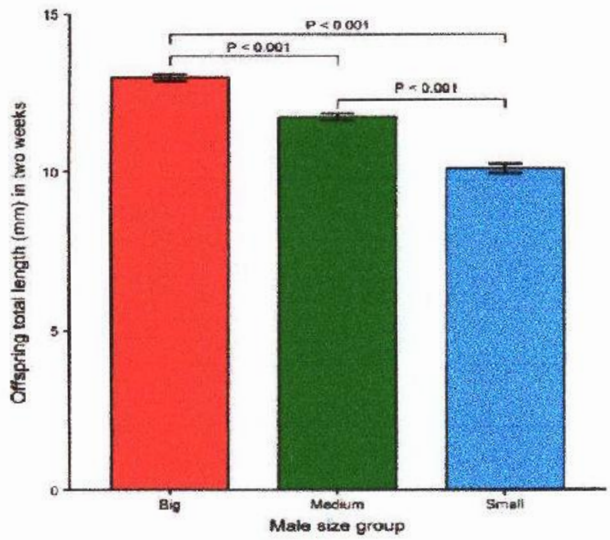

Figure 22: Variation in offspring total length (mean±se) among different experimental male groups.

4.3.3 Offspring body area

Similar to the total length, it has been found that offspring sired by big males became significantly larger during the rearing period (14 days) than the medium and smaller male groups. The result has also revealed that the medium sized males' offspring became significantly larger within two weeks than the smaller male group (figure 23).

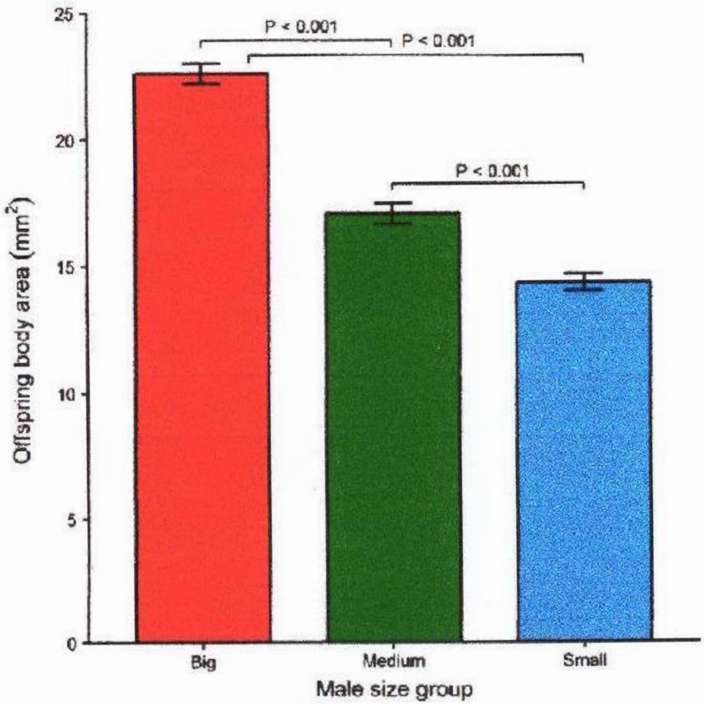

Figure 23: Variation in offspring body area (mean±se) among different experimental male groups.

Chapter Five

Discussion

5.0 Discussion

The overall results of this study have revealed that bigger males produced significantly higher amount of sperm (figure 19) and higher number of hatchlings (figure 20) than the smaller group. The study has also found that bigger fathers' offspring had significantly better survival rate (figure 21), larger total length (figure 22) and bigger body area (figure 22).

5.1 Reproductive traits variation

The study has shown that both big size and medium size males produced significantly higher amount of sperm than smaller ones, while no variation has been found between big and medium size male groups in sperm production (figure 19). This outcome seems to corroborate some other studies. Taku et al. (2008) conducted a research with male maturity, number of sperm, and spermatophore size relationships in the coconut crab (*Birgus latro*) and found that spermatophore volume was increased with increasing male size, and the sperm quantity packed in a spermatophore increased with increasing spermatophore volume. In another study, Imanpour et al. (2009) worked on relationship between fish size and sperm volume in Rutilus (*Rutilus caspicus*) and they noted that increasing in the body length, the gonadal weight significantly increased but sperm volume did not have any significant correlation with fish body length. Ataguba et al. (2013), worked with African catfish (*Clarias gariepinus*), pointed out that weight of male had a strong positive correlation with sperm weight. Huang and Chang (2011) revealed that hatching rates were significantly positively correlated to standard length of parental males of paradise fish (*Macropodus opercularis*)

The study has also revealed that big size males produced significantly higher number of hatchlings than smaller ones, while no variations have been found in offspring production between big and medium, and also medium and small male groups (figure 20). These findings are coherent with the other studies (e.g. Rahman et al., 2018; Pujolar et al., 2012; Baxter, 1991). Recently, Rahman et al. (2018) found that larger males of common carp (*Cyprinus carpio*) had higher hatching rate than that of the smaller one. According to Pujolar et al. (2012), the highest fertilization success of territorial males of grass goby (*Zosterisessor ophiocephalus*) was found with the largest body size. Thus, there is a positive relationship between body size and fertilization success in territorial males of the grass goby (*Z. ophiocephalus*). They discerned that the gonadal weight significantly increased with increasing in the body length, but sperm volume did not have any significant correlation with fish body length. Baxter (1991) worked on size dependent male spawning success in chinnok salmon and found that large, dominant male chinook salmon have a reproductive advantage on the spawning grounds.

In some cases, the result of the present study is somehow different from the observation of Changadeya et al., 2003; Petersen et al., 2001, and Spence and Smith 2006. Changadeya et al. (2003) found that smaller fishes of vendace (*Coregonus albula*) had increased hatchability compared to larger one. There was no relation between male size and reproductive success in zebra fish (*Danio rerio*) while using males ranging between 33.8 and 37.4 mm (Spence and Smith, 2006). Petersen et al. (2001) revealed that there was no correlation between the size of a male and fertilization effectiveness in the bluehead wrasse (*Thalassoma bifasciatum*).

5.2 Offspring fitness traits variation

It has been found that bigger fathers' offspring became significantly larger than the medium and smaller male groups, and also the medium sized males produced significantly bigger offspring than the smaller male group (figure 22). These findings are consistent with the studies of Uusi-Heikkilä et al. (2012) which showed that very large and large males sired significantly larger offspring compared to offspring of medium-sized and small males of zebrafish (*Danio rerio*). Jacob et al. (2007) found significant additive genetic variance on offspring survival in brown trout (*Salmo trutta*), whereas older, heavier and larger males were more successful for offspring survival than smaller one. According to Saillant et al. (2001) and Bang et al. (2006), the evidence for paternal contributions to offspring development is limited compare to the maternal-size effects. Saillant et al. (2001) found that the effect of the male parent on early sea bass development had shown for the first time but most of the parental effects on early performance in this species were due to females. Bang et al. (2006) worked on parental effects on early life history traits of Atlantic herring (*Clupea harengus*) larvae and found that the influence on success of early larval fishes mostly depend upon maternal effects compared to the paternal. Silva et al. (2016) worked on the contribution of father's phenotype on offspring attributes in ringneck blenny (*Parablennius pilicornis*) and indicated that larger adults produced on average smaller larvae. Moreover, father's contribution on larvae characteristics was highly variable between seasons, with larvae average standard length variation mostly explained by father's standard length, body area and body height in end of the reproductive season, while in beginning of the season this was not significant. They suggested that progeny phenotypic traits are highly variable and that mother's influence and environment may play an important role on the determination of larvae phenotype.

CHAPTER SIX

CONCLUSION

6.0 Conclusion

Now-a-days due to the corrosion of ecological systems, natural resources of good quality carp seeds are ruined. Therefore, hatchery is now the key source of carp seed production. Eggs and sperm are the main elements in hatcheries for fish breeding to produce larvae. As egg and sperm quality is significantly important for the production of high quality fish larvae and for economical utilization of hatcheries, good quality eggs and sperm are required to increase fish production and thereby to fulfill the market demand of fish and fish protein. This study has been designed to explore whether some phenotypic traits of male rohu can provide a hint to confirm the fertilization success in this species. The study has revealed that bigger males produced significantly higher amount of sperm as well as higher number of hatchlings than their counter group. The carps are the main species of the aquaculture sector in Bangladesh, and the production of these species completely depends on adequate supply of good quality seeds. Therefore, the production of good quality carp spawns and fingerlings are essential for desired production of fish in Bangladesh. The study has showed that survival percentage and body area of offspring sired by big males was significantly higher than medium and small male groups. These findings can give an important message to the commercial breeders to choose the big sized males for production of good quality seeds and higher fry production. To have more support about this issue, still some research should be performed to look for the use of bigger males and females based on their phenotypic traits for higher fertilization success and thereby, good offspring production in some other commercially important fish species.

CHAPTER SEVEN

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