Influence of Females' Body Size on Reproductive Performance and Offspring Fitness in Rohu (*Labeo rohita*)

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A Thesis

By

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Influence of Females' Body Size on Reproductive Performance and 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.

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Dedicated to

My Beloved Parents

Acknowledgement

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Abstract

Reproductive performance of broodstocks and their produced offspring fitness are crucially important for aquaculture especially in hatcheries. Considering this issue, a study has been carried out to explore whether females' body size can influence their reproductive performance and also offspring fitness traits. In the experiment, there were three female size groups- 1) big (1.5-2.5 kg), 2) medium (>1kg) and 3) small (<1 kg). In each group, there were 13 females which were performed with some common males using a special breeding design. The analysis has revealed that bigger females produced significantly higher number of eggs than the smaller size group, while no variation in egg numbers has been observed between bigger and medium and medium and smaller size groups. The study has also found no significant variation in egg weight, egg diameter and egg area among those female size groups. A significantly higher hatching rate has been found in bigger and medium sized females rather than the smaller group, however no significant variation has been revealed between bigger and medium size group. It has been found a significantly lower number of deformed hatchlings produced by the bigger males than the smaller group, while no significant variation has been observed among bigger and medium and medium and smaller sized groups. The offspring fitness analyses have revealed that bigger mothers larvae became significantly larger (standard length and body area) within two weeks of experimental rearing period than medium and smaller sized groups, while medium sized females' offspring also grew significantly larger than the smaller one. However, no significant variation has been found in offspring survival rate among these female size groups. The overall outcomes provide an important message to the hatchery owners, farmers and other stakeholders about which size of broodstocks they need to use for better production in terms of reproductive outputs as well as larval fitness.

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CHAPTER ONE

INTRODUCTION

1. Introduction

1.1. Background of the study

Bangladesh is endowed with diversified fisheries resources. The fisheries can largely be classified into three groups that are inland capture fisheries, inland aquaculture and marine fisheries (Shamsuzzaman et al, 2017). This sector is contributing significantly in food security through proving safe and quality animal protein. Almost 60 percent animal protein comes from fish. It contributes 3.61 percent to our national GDP and around one-fourth (24.41 percent) to the agricultural GDP (FRSS, 2017). In 2016-17, the total fisheries production is 41.34 lakh MT, 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 (FRSS, 2017). So, 84.58 percent of total production comes from inland fisheries. The growth rates of inland capture and inland culture are 11.01 and 5.89 percent respectively. 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. Overall growth rate of total fish production in 2016-17 is 6.60 percent. The overall growth performance from inland aquaculture shows a moderate increased trend. The fish production has increased more than five times (7.54 MT in 1983-84 to 41.34 lakh MT in 2016-17) over the last three decades. According to FAO statistics 2017, Bangladesh is ranked 3rd in world aquaculture production (FRSS, 2017).

In Bangladesh, inland pond culture represents the mainstay of aquaculture, accounting more than 80% of the total recorded aquaculture production, and is presently dominated by various indigenous and exotic carp species such as catla *(Catla catla)*, rohu (*Labeo rohita)*, mrigal (*Cirrhinus mrigala*), kalibaus (*Labeo calbasu)*, silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*) and common carp (*Cyprinus carpio*). Only carps species contribute 31.98% in total annual production (freshwater and marine water) of Bangladesh in which Rohu (*L. rohita*) production is 370627 MT (10.60%) in 2016-17 (FRSS, 2017). The highly favourable culture conditions, rapid growth, easy culture technique, high market demand and consumers' acceptability in our country results in the rapid expansion of carp production (Dey et al., 2005). Thus, a significant number of traditional, extensive and semi-intensive carp producing farms have been raised in recent years. Carp culture is a direct source of livelihood for poor fishermen families and also provides an indirect employment to about a million of people, including women, engaged in supporting industries or activities. More than 11 percent of total population of Bangladesh is engaged with this sector on full time and part time basis for their livelihoods (FRSS, 2017). Therefore, carp culture now-a-days requires special attention in Bangladesh. This sector also has high potential for the perspective of economic development of the country.

Carp culture mainly depends on several factors such as brood stocks management, seed production, larvae rearing, culture systems, nutrition, disease control, etc. Among these variables, brood stocks collection, rearing and management, and then production of good quality ejaculates, eggs and fry are the primary and prime tasks because without healthy and well fingerlings nothing will be successful. Although many carp hatcheries have been established and successfully producing a huge number of fry, they still experience some embryonic mortality and/or poor quality of offspring due to their less knowledge about the impact of gamete quality in hatching (Rurangwa et al., 2004). Gametes quality especially the females' one has concentrated on relating egg characteristics to fertilization rate and offspring success. For example, egg size is directly related with fertilizing capacity, measured as the percentage of eggs fertilized in a batch (Buckley et al., 1991), and to larval size (Marteinsdottir and Able, 1992; Blaxter and Hempel, 1966). In addition, 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; Edward, 2003). It was found that the larger sized female produces more eggs in the blue gourami (*Trichogaster trichopterus*) (Degani, 1989) and Siamese fighting fish (*Betta splendens*) (Huang et al., 2011).The eggs that exhibit low levels of mortality at fertilization, eying, hatching and first feeding and those which produce the fastest growing and healthiest fry are considered as good quality eggs (Brooks et al., 1997). Therefore, hatcheries are looking for techniques to produce good quality eggs which can ultimately enhance the overall carp production in our country.

Considering the importance of this burning issue, this study has been conducted to investigate whether females' phenotypic traits can influence the egg quality, reproductive performance and offspring fitness using the most popular and commercially important carp species, rohu (*L. rohita*) using a special breeding design.

1.2. Objective of the study

The study has the following specific objectives to achieve:

- To explore the effects of females' body size on egg quality and quantity;
- To determine whether females' body size can influence reproductive performance (e.g. hatching success; hatchling deformation rate, etc.), and
- To expose the variation of offspring fitness (e.g. survival, length, etc.) due to the maternal body size variation.

CHAPTER TWO

LITERATURE REVIEW

2. Literature Review

2.1 Fisheries resources of Bangladesh

Bangladesh being a first line littoral state of the Indian Ocean has a very good source of marine resources in the Bay of Bengal. The country has an exclusive economic zone of 41,000 square miles (110,000 km^2), which is 73% of the country's land area (DOF, 2017). The fisheries resources those are available in Bangladesh shown below:

Table 1: Sector-wise Annual Fish Production in Inland and Marine Fisheries, 2016-17 (FRSS, 2017)

Sector of Fisheries	Water Area (Hectare)	Production (Metric Ton)	% of Production	Productivity
A. Inland Fisheries				
(i) Inland Open Water (Capture)				
1. River and Estuary	853863	271639	6.57	318 kg/ha
2. Sundarbans	177700	18086	0.44	102 kg/ha
3. Beel	114161	98117	2.37	859 kg/ha
4. Kaptai Lake	68800	9982	0.24	145 kg/ha
5. Floodplain	2712618	765782	18.52	282 kg/ha
Capture Total	**3927142**	**1163606**	**28.14**	
(ii)Inland-Closed Water (Culture)				
6. Pond	384700	1833118	44.34	4765 kg/ha
7. Seasonal cultured waterbody	136273	215547	5.21	1581 kg/ha
8. Baor	5488	8002	0.19	1458 kg/ha
9.Shrimp/PrawnFarm	272717	246406	5.96	904 kg/ha
10. Crab	27010	14421	0.35	537 kg/ha
11. Pen Culture	7564	13368	0.32	1767 kg/ha
12. Cage Culture	1.10 lakh cu. meter	2490	0.06	25 kg/cu. m
Culture Total	**833752**	**2333352**	**56.44**	
Inland Fisheries Total	**4760894**	**3496958**	**84.58**	
B. Marine Fisheries		108479	2.62	
13. Industrial (Trawl)		528997	12.79	
14. Artisanal				
MarineFisheries Total		**637476**	**15.42**	
COUNTRY TOTAL		**4134434**	100.00	

2.2 Aquaculture status of Bangladesh

There are many types of fish culture practiced in Bangladesh. These are described below:

2.2.1 Extensive farming

Both carp and catfish are easy-to-culture warm water species which are commonly raised in ponds. Production is achieved from the natural and renewable resources of the pond. Juvenile production is usually carried out in the same ponds where the adult spawning process takes place naturally .Water quality and flow requirements of these species are very modest and. The rearing technique in this type of farming is based on the pond's trophic chain in order to get the maximum fish yield.

Figure 1: Extensive fish farming

2.2.2 Semi-intensive farming

In this type of culture system egg incubation, hatching and larval rearing all are happened in a controlled facilities to produce juvenile fish that are stocked in ponds and also some food are supplied externally and allow higher stocking density in the ponds. These improvements of extensive techniques are also widely used for the production of several freshwater fish species as tench, roach, pike and perch.

Figure 2: Semi intensive fish farming

Intensive farming:

i. **Land-based intensive flow-through farming**

High valued fishes are produced in this technique at higher stocking density. The most important species cultured in these techniques are trout, salmon, sea bass, sea bream, eel and turbot. Apart from eel, artificial reproduction of all these species is possible. Specialized commercial hatcheries are equipped for breeding hatching and the first stages of larval and juvenile development. Year-round supplies of fry are insured by the control of the maturation and breeding processes of the broodstock, the manipulation of light and/or temperature. Marine larvae need live feed for first feeding but when they develop into fry they are fed on fabricated diets, up to market size.

Figure 3: Land-based intensive flow-through farming (Feap, 2017)

ii. Recirculation Aquaculture systems (RAS)

These are land-based systems and here the water is re-used after mechanical and biological treatment. These systems posses several advantages as: water saving, a rigorous control of water quality, high biosecurity levels and an easier control of waste production as compared to other production systems. The main disadvantage of this system is that they have high capital and high operational costs. The main freshwater species produced in RAS are eel, trout and catfish but other species are already being produced using this type of technology.

Figure 4: Recirculation Aquaculture systems (RAS) (Feap, 2017)

iii. Cage farming

In this type of farming different size and types of cages are used to culture various types of fish species. In that case cages tended to be placed at well-protected, largely enclosed and insufficiently flushed sites. Other developments of cage farming concern the formation of submersible cages (which, so far, have not provided the same economic performances as floating cages) and automation of feeding and control by the use of computers. Some cage farming is also practiced in freshwater lakes. (Feap, 2017)

Figure 5: Cage culture (Feap, 2017)

2.3 "Rohu"- the most important aquaculture species in Bangladesh:

Rohu (*L. rohita*) is the most important among the three Indian major carp species used in carp polyculture systems. Carp are generally cultured in a closed system that involves herbivorous species, in which organic materials are used as the principal input sources which makes it a environment-friendly practice. Moreover, the compatibility of rohu in polyculture systems in the case of habitat preference and feeding habits is good. However, the tendency of farmers to increase income per unit area results in an excessive use of fertilizers, proteinaceous feeds and chemicals that may have detrimental effects on the environment. The compatibility of rohu in polyculture systems compared to other carps has already been established. Rohu, as a column feeder, grows well in deeper ponds (2-3 m water depth), which are rather uncommon. Greater growth rates and higher production and also the market preferences make rohu the most important aquaculture species over the others. (FAO, 2018)

Figure 6: *L. rohita*

2.3.1 Classification:

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Subfamily: Labeoninae

Genus: *Labeo*

Species: *L. rohita* (Wikipedia, 2018)

2.3.2 Life-cycle:

Rohu Spawns in middle reaches of rivers, where flood water expands in more or less limpid shallows over fertile flats, well above tidal reaches, in reservoirs and also bundh-type tanks. (Fishbase, 2013).

<u>Life history traits of rohu (averages)</u>

Female sexual maturity	1,073 days
Male sexual maturity	985 days
Adult weight	24,750 g (genomics, 2017)

2.3.3 Habitat and ecology:

The rohu founds in rivers throughout much of northern and central and eastern India, Bangladesh, Nepal and Myanmar, and has been introduced into some of the rivers of Peninsular India and Sri Lanka. This is an omnivore species with specific food preferences at different life stages. When it is in the early stages of its lifecycle eats mainly zooplankton, but as it grows, it eats more and more phytoplankton, and as a juvenile or adult is an herbivorous column feeder, eating mainly phytoplankton and submerged vegetation. It has modified, thin hair-like gill rakers, exposing that it feeds by sieving the water.

Rohu reach sexual maturity between two and five years of age. They usually spawn during the monsoon season, keeping to the middle of flooded rivers above tidal reach. The spawning season of rohu generally coincides with the southwest monsoon. Spawn may be collected from the rivers or reared in tanks and lakes (FishBase, 2013).

2.3.4 Breeding:

Rohu attains maturity towards the end of the second year in ponds. Usually rohu fish reach maturity at the age of 3 years and a female can contains about 3 lakhs eggs. Spawning takes place in flooded rivers. The ideal season for laying egg is from April to July. This species lays eggs in open waters and don't lays eggs in stagnant water (BdFISH, 2017; Agrifarming, 2018)

2.3.5 Maintaining broodstocks and gamete quality:

The aim of broodstock management and breeding plans for hatcheries is to provide good quality (fit and healthy) juveniles suitable for stocking. The quality of juveniles produced by hatcheries rely on a number of factors throughout the different production stages, from broodstock collection to harvesting nursery ponds, and encompass factors related to the nutrition, health and genetics of stock, and good husbandry practices. The most important of these relate to broodstock management. Poorly planned genetic management of broodstock and breeding can result in declines in the quality of stock over a number generations like as reduced fecundity, hatch rates and growth rates, and an increase in the occurrence of abnormalities and susceptibility to diseases. Broodstock management plans should aim to check the loss of genetic diversity and minimize inbreeding within the population. Other relevant factors for broodstock comprise the source, number, size (age) appearance and health of broodstock. It is very necessary to manage broodstock nutrition and pre-spawning conditioning, spawning and the immediate post-hatch stages (egg incubation, larviculture and post-larval husbandry). Broodstock nutrition and husbandry practices can affect gamete quality which can affect seedstock quality. A fish health and biosecurity plan is needed to manage the health of not only broodstock, also larval and juvenile fish, which will eventually be released. Apart from diseases and parasites the hatchery owners should also manage unwanted aquatic species entering the hatchery and being released with stocked fish. These factors are very much important because it determines whether the seedstock produced from hatcheries survive and grow in the waters stocked (Ingram et al., 2014).

2.3.6 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 etc. In order to produce healthy offspring this all factors must be properly maintained by the hatcheries (Sehgal, 1991; Lumley et al, 2016).

2.4 Research already conducted on broodstock quality and larval fitness:

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

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.

2.5 About the present study:

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

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and methods

3.1 Source and maintenance of broodstocks

Sexually mature rohu (both male and female) of different sizes were collected from different hatcheries of Khulna and Jessore districts and then were transported to the rearing facilities at the South Bay (Pvt) Ltd. hatchery, West Siromoni, Khulna. Fish were stocked in 5individulas/m^3 tanks supplied with aerated re circulated water. Water quality and feeding level were kept equal by providing same facilities in all tanks. Fish were fed daily (at approximately 0.7% body weight) with commercial feed to conduct the following experiments:

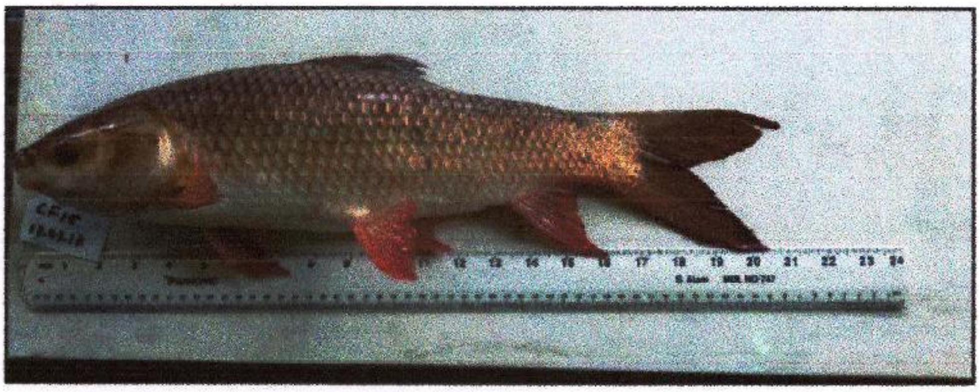


Figure 7: A sexually mature female rohu after collecting eggs.

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 8). 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.

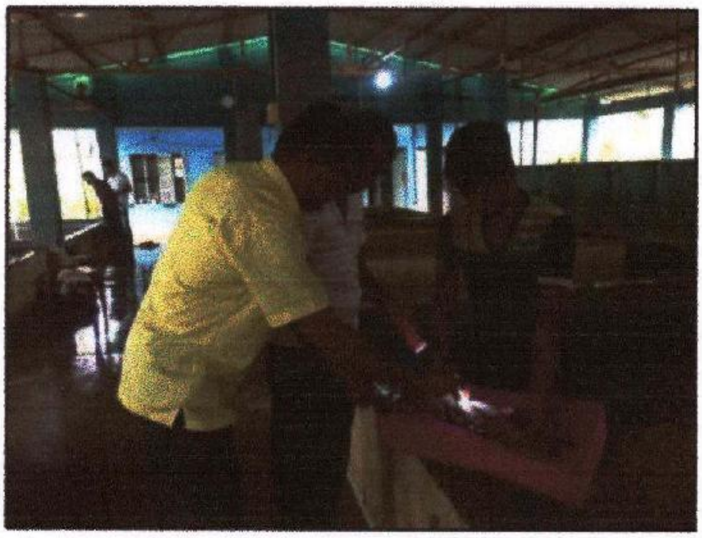


Figure 8: Administering the recommended dose of PG for induced spawning.

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 females and a common male were used to explore the female size effect on gamete quality (figure 9). In the experiment, first eggs were collected in a clean and dry bowl from three sized female by hand stripping (figure 10) 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 groups) 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 11). Then sperm were collected from a common male by hand stripping (figure 12) and immediately weights of fish and sperm were measured using a digital balance. Exactly 0.5 mL of sperm was collected with a marked syringe from the total sperm pool of the common male and then added to the bowl of each replication (figure 13). 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 14).The fertilized egg masses were then transferred to previously labeled plastic containers (2 L) filled with sufficient aerated water (figure 15). They were allowed for incubation up to 38-40 hours at ambient temperature (25-30°C) (figure 16). After three days, the hatchlings were transported in plastic bags with sufficient aeration (figure 17) 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 18). Then only 20 good looking larvae from each replication were sorted to rear in individual aquarium up to two weeks (figure 19) 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.

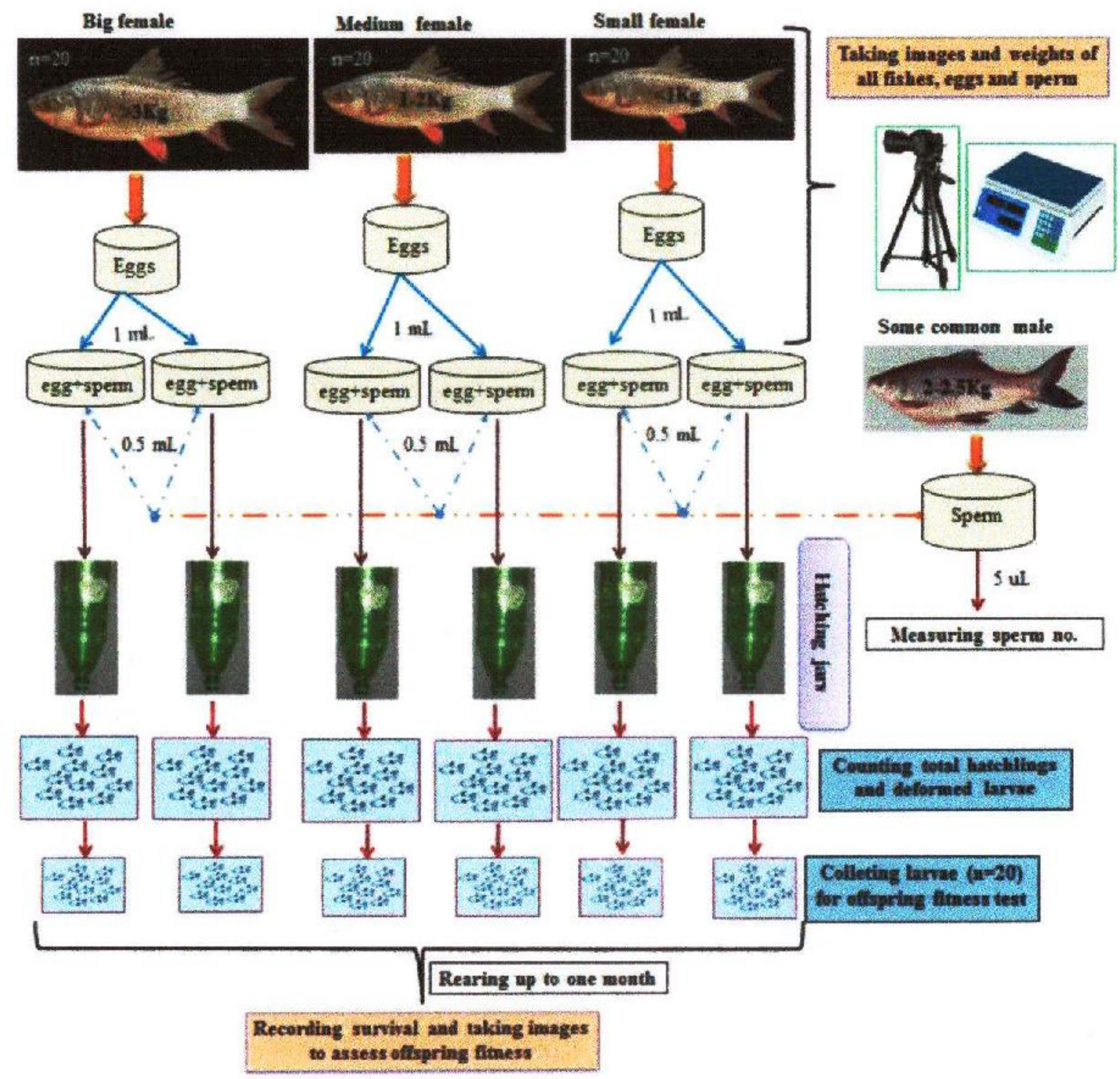


Figure 9: The overall experimental design to explore the female size effect.

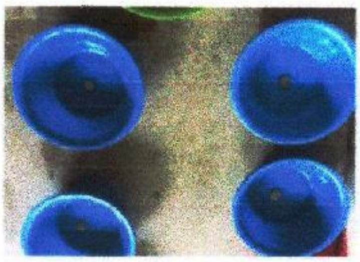
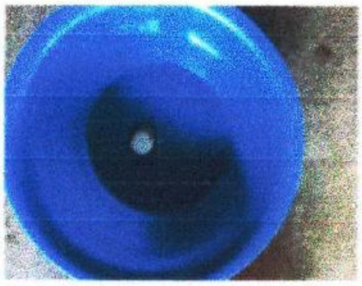


Fig 10: Stripping females to collect eggs

Fig **11:** Pouring 1 mL egg to bowl

Fig 12: Stripping males to collect sperm



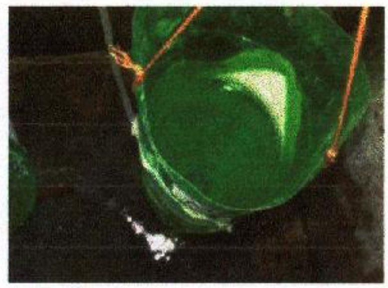


Fig 13: Adding 0.5 mL sperm with eggs

Fig 14: Mixing and stirring eggs and sperm for fertilization.

Fig 15: Transferring fertilized eggs in labeled hatching jars

Fig 16: Jars/bottles contained fertilized eggs with sufficient aeration for hatching.

Figure 17: Plastic bags contained hatchlings with sufficient aeration

Fig 18: Counting normal and deformed hatchlings

Fig 19: 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 20) and body area (area covered from tip of the snout to the last mark of caudal peduncle, figure 20) were measured from the digital photographs taken of each fish using *ImageJ* software (http://rsbweb.nih.gov/ij/).

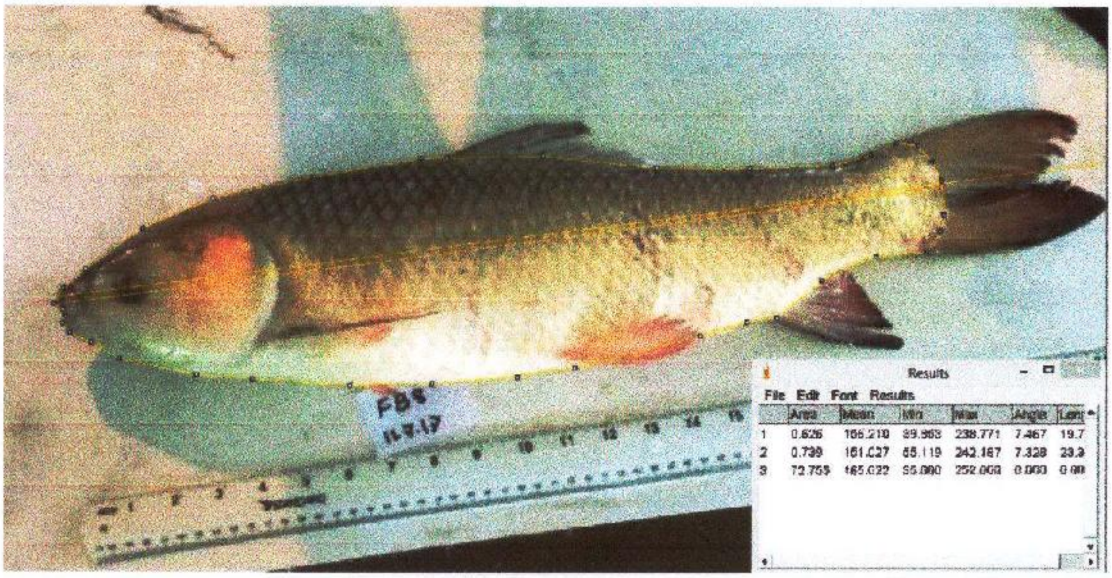


Figure 20: Measuring total length and body area from the taken photograph using ImageJ software.

3.5. Determination of egg traits:

One of the main objectives of this experiment is to investigate how the egg quality can be related to individual female phenotypic traits (e.g. body size). To explore this issue, the eggs were preserved in labeled vials with 2% ethanol at the hatchery and were brought to the lab to assess their quality parameters (e.g. gamete weight, egg size, fecundity, egg area, hatching success, etc.) in order to determine females' gamete quality (figures 21, 22, 23 and 24).

Fig 21: 1 mL eggs preserved in 2% ethanol.

Fig 22: Egg counting.

Fig 23: Egg observation using microscope.

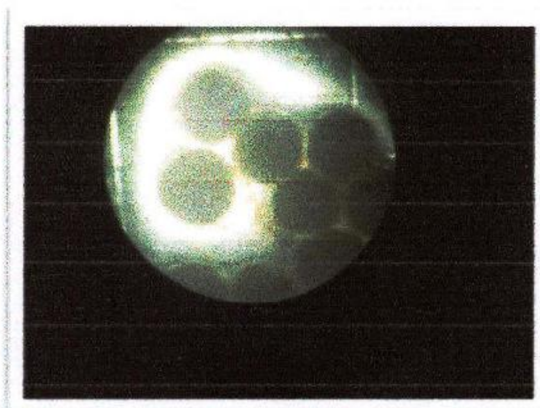


Fig 24: Microscopic images of eggs for ImageJ software.

3.6. 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 25 and 26).

Fig 25: Daily monitoring the reared larval status at the lab.

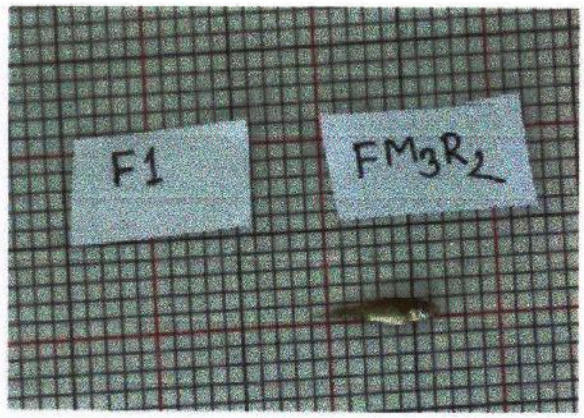


Fig 26: A photograph of a two-week old larva for ImageJ analysis.

3.7. Statistical analyses

All analyses have been conducted using 'RStudio' version 1.0.143(R Core Team 2017). 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 female-by-male interaction as fixed factors and female body weight as a random effect (to account for exact amount of sperm come from the same male). 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. Results

4.1 Females size variation

The analysis has confirmed that there are significant variations in body weight and standard length among the experimental female groups used in this study (table 2). The subsequent post-hoc tests have also revealed that bigger sized females have significantly higher body weight and larger standard length than medium and smaller ones, while medium sized females are also significantly bigger than smaller ones. Thus, these findings have confirmed that there were three distinct female size groups in the experiment who are significantly different from each other in size (body weight and standard length).

Table 2: Size variation of the experimental female groups

Traits	**Big female (mean±se)**	**Medium female (mean±se)**	**Small female (mean±se)**	**F-value**	***P***
Body weight (kg)	1.97±0.08	1.39±0.08	0.82±0.07	61.84	***<0.001***
Standard length (cm)	46.98±2.00	41.42±1.59	32.16±2.15	15.07	***<0.001***

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

4.2 Variations in reproductive performance

4.2.1 Egg weight

The study has found no significant variation in total egg weight during this female size effect experiment ($F_{2,36}$=2.51, P=0.09).

4.2.2 Egg number

The analysis has revealed significant variation in total egg number (figure 27) where bigger females have produced significantly higher number of eggs than the smaller groups($t_{1,24}$=2.81, P=0.02). However, no significantly variations have been found between bigger and medium ($t_{1,24}$=1.90, P=0.15), and medium and smaller groups ($t_{1,24}$=0.91, P=0.64).

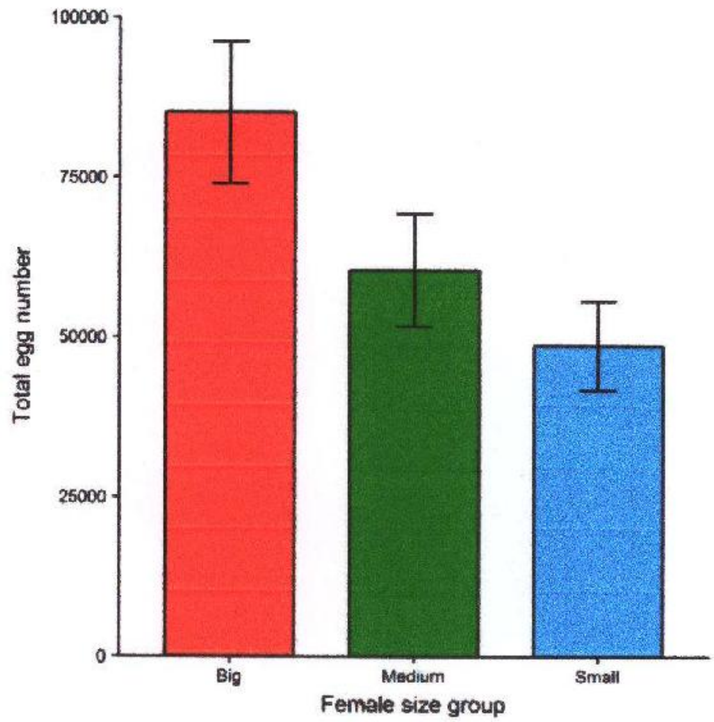


Figure 27: Variation in total egg numbers (mean±se) among different experimental female groups.

4.2.3 Egg diameter

It has been observed that there is no significant variation in egg diameter ($F_{2,36}$=1.48, *P*=0.24) although the experimental females have significant size variations.

4.2.4 Egg area

Similar to the egg diameter, the study has found no significant variation in egg area during this female size effect experiment ($\chi^2_{2,36}$=4.83, *P*=0.09).

4.2.5 Hatching rate

The analysis has revealed significant variation in hatching percentage (figure 28) where bigger ($t_{1,24}$=3.52, *P*=0.003) and medium ($t_{1,24}$=2.62, *P*=0.03) females have produced significantly higher number of eggs than the smaller groups. However, no significantly variations have been found between bigger and medium female size groups ($t_{1,24}$=0.90, *P*=0.64).

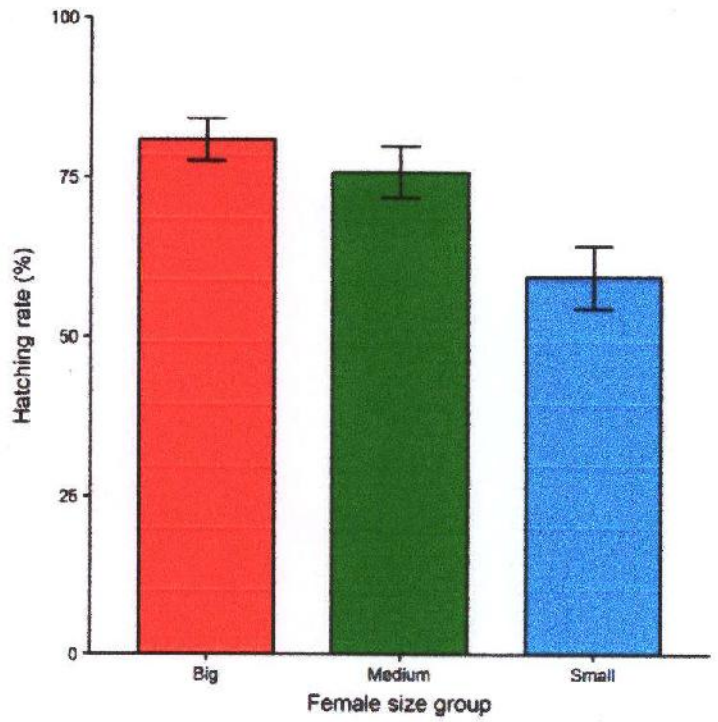


Figure 28: Variation in hatching percentage among different experimental female groups.

4.2.6 Hatchling deformation rate

The study has shown that there is a significant variation in hatchling deformation rate ($F_{2,36}$=8.15, P=0.004 and see figure 29). The subsequent post-hoc tests have revealed that significantly lower number of deformed hatchlings have been produced by the bigger females rather than the smaller groups ($t_{1,24}$=3.52, P=0.04), while no significant variation has been observed in other female size groups.

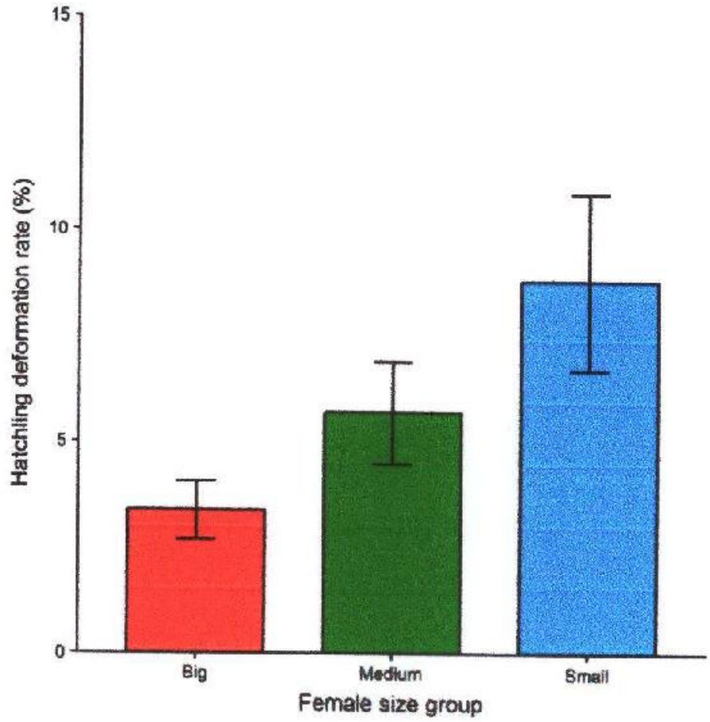


Figure 29: Variation in hatchling deformation rate among different experimental female groups.

4.3 Offspring traits variation

4.3.1 Offspring survival rate

The study has shown that the survival rate of offspring produced by different sized females are not significantly different ($F_{2,345}$=0.61, P=0.55) after rearing up to two weeks (exactly 14 days).

4.3.2 Offspring total length

The experiment has revealed that maternal body size can influence significantly the total length of offspring ($F_{2,345}$=137.80, P<0001 and see figure 30) where hatchings have been reared up to 14 days in aquarium under the same conditions and facilities. It has been found that bigger mothers' offspring have become significantly larger within 14 days than the medium (P<0001) and smaller mothers' babies (P<0001), while the medium sized mothers have also produced significantly larger offspring than the smaller female groups (P<0001).

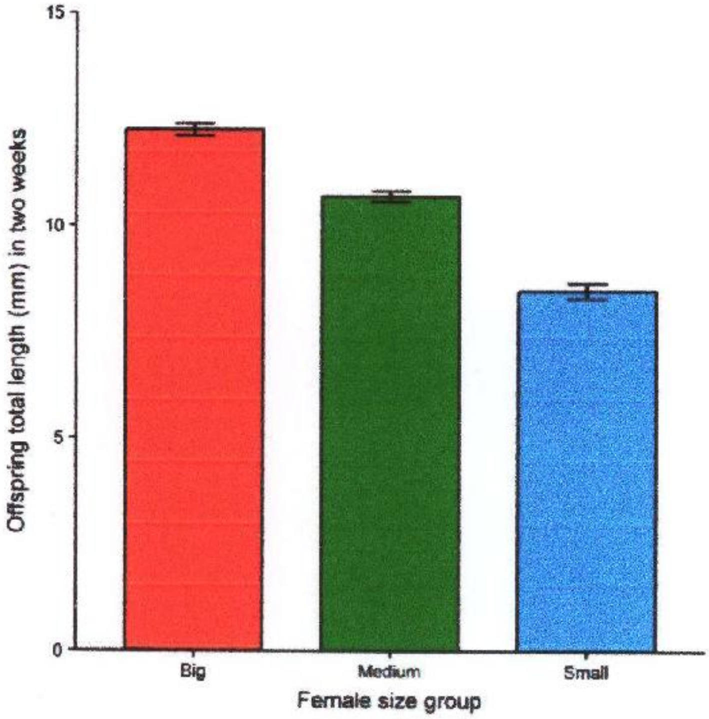


Figure 30: Variation in hatchling total length (mean±se) among different experimental female groups.

4.3.3 Offspring body area

Like total length, the study has shown that maternal body size can also affect significantly the body area of offspring ($F_{2,345}$=98.62, *P*<0001 and see figure 31). It has been revealed that bigger mothers' offspring have become significantly larger within 14 days than the medium (*P*<0001) and smaller mothers' babies (*P*<0001), while the medium sized mothers have also produced significantly larger offspring than the smaller female groups (*P*<0001).

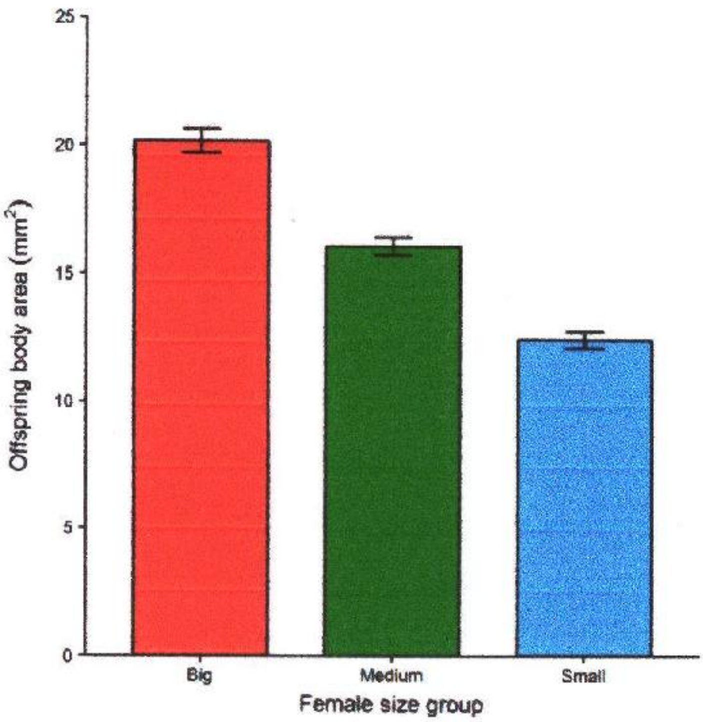


Figure 31: Variation in offspring body area (mean±se) among different experimental female groups.

CHAPTER FIVE

DISCUSSION

5. Discussion

The overall analysis has revealed that bigger females produced significantly higher number of eggs, higher hatching rate and lower deformation rate, offspring having larger total length and body area. However, no significant variations have been observed in weight, diameter and area of eggs and also offspring survival rate.

5.1 Variations in reproductive performance

The present study has shown that bigger females produced significantly higher number of eggs (Fig 27) and higher number of hatchlings than the smaller female group (Fig 28). These findings are consistent with some other studies (Degani 1989; Smalås et al. 2017; Marshall et al. 2007). According to Degani (1989), the larger the parents, the more eggs they produce in the blue gourami (*Trichogaster trichopterus*). Smalås et al. (2017) also found that Fecundity increased with increasing body size in Arctic charr (*Salvelinus alpines*). In another study, Marshall et al. (2007) found that large females spawned more frequently than small females. A very recent study (Rahman et al., 2018) has been carried out which also corroborates this result. Rahman et al. (2018) found that larger males of common carp (*Cyprinus carpio*) have significantly higher hatching rate than that of the smaller one.

On the other hand, some researcher found almost different types of results. Lobon-Cervia et al. (1997) conducted research with salmon species (*Salmon sp.)* and found that the relative fecundity (number of eggs per body weight) is higher in small females than in large ones.

5.2 Variations in offspring fitness

The experiment has revealed that maternal body size can influence significantly the total length (Fig 29) and body area of offspring (Fig 30). It has been found that bigger mothers' offspring have become significantly larger within 14 days than the medium and smaller mothers' babies. The outcome of larger total length and body area of the offspring of bigger female fish corroborates some other studies (Kindsvater et al., 2012 ; Marshall et al., 2010; George SB, 1994; Marshall et al., 2007). According to Kindsvater et al. (2012), there is a positive relationship between offspring size and maternal size. Their study revealed that larger swordtails (*Xiphophorus birchmanni*) produce larger offspring. George SB (1994) also found a positive relationship between offspring size and maternal size in starfish (Echinodermata, Asteroidea).

The results of Marshall et al. (2010) revealed that larger offspring are produced by larger mothers. In another study, Marshall et al. (2007) found that the offspring of the large fish had significantly larger body area. However, some research has not found any positive effect of body size on offsprings body length and body area. Shikano et al. (2005) found negative correlation between maternal brood size and offspring body size in guppy (*Poecilia reticulata*).

These studies also found that smaller female produce higher number of deform offspring than the larger female (Fig 28). Heinimaa et al. (2004) found the similar types of results where they noted that the embryo mortality during the incubation from fertilization to eyed egg stage was positively size dependent and the smaller sized one have more mortality in offspring.

CHAPTER SIX

CONCLUSION

6. Conclusion

Bangladesh is an agrarian country. Fisheries are one of the major components of agricultural activities and play a vital role in nutrition, employment, income generation and foreign exchange earnings. The availability of fish seed is an essential prerequisite for fish culture. The main sources of fish seeds in Bangladesh are spawn produced in government and private hatcheries, and some collected from rivers. The numbers of government fish hatcheries or fry production farms are 136 and the private fish hatcheries are 868 (DoF, 2016). But now many private hatcheries are closed. Nowadays due to the degradation of ecological balance, natural resources of fish seeds are destroyed. So, hatchery is now the main source of fish seed production. Therefore, this study has been conducted to explore whether some phenotypic traits of female rohu can provide an indication to confirm the fertilization success in species. The study found that bigger females have produce higher number of eggs and have larger sized offspring than their counter groups. The study has revealed that bigger females produced significantly higher number of hatchlings than their counter groups. The hatchlings deformation rate is also lower in bigger female group than their counter-groups. The result has shown significant variation in some selected phenotypic traits (total length, body area) among three female groups (i.e. 1.5-2.5 kg, >1kg and <1kg). The overall results provide an indication of direct benefits for males (i.e. reproductive purpose) to prefer the bigger females as their mating partners, and also provide a suggestion to the commercial farmers to choose big sized females for higher fry production. To have more confirmation about this issue, still some research should be carried out to explore 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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