Comparative study on spermiology and fecundity of *Cyprinus carpio* var. *communis* in farmed and wild conditions

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2016

Comparative study on spermiology and fecundity of *Cyprinus carpio* var. *communis* in farmed and wild conditions

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Thesis

Submitted to

Faculty of Fisheries

Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir
in partial fulfilment of requirement for the award of the degree of

Master of Fisheries Science
(Fisheries Resource Management)

2016

To my

Parents

Sher-e-Kashmir
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Certificate – I

This is to certify that the thesis entitled, **"Comparative study on spermiology and fecundity of *Cyprinus carpio* var. *communis* in farmed and wild conditions"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Fisheries Science (Fisheries Resource Management)**, to the **Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Ishrat Mohd (Regd. No. 2014-F-28-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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ABSTRACT

Keeping the inbreeding depression in view, the present study was conducted to compare the physical and biochemical parameters of seminal plasma and also the female reproductive parameters between the cultured and wild stocks of Scale carp (*Cyprinus carpio* var. *communis*) during the year 2015-16. A total of 120 fish samples were taken for the present study. In wild conditions, the seminal plasma was having 70.64±10.75 mg/dl glucose, 1.99±1.15g/dl total protein, 12.99±5.6 mg/dl triglyceride, 5.98±0.629 mg/dl cholesterol and 25.22±4.047 mg/dl urea, whereas in farmed conditions, the seminal plasma contained 85.06±9.29 mg/dl glucose, 0.917±0.62 g/dl total protein, 12.3±5.22 mg/dl triglyceride, 5.53±1.61 mg/dl cholesterol and 28.4±5.57mg/dl urea. The physical parameters like mean sperm volume, mean sperm motility, mean movement duration, mean sperm density and mean pH in the wild and farmed fishes were recorded as 2.393±1.64 ml, 1.486±0.88 ml, 75.038±10.162%, 68.9±12.46%; 50.367±13.92 sec, 44.66±13.48 sec; 3.534±.272 10^9/ml, 3.84±0.181$10^9$/ml and 8.29±.494, 8.5±0.311 respectively. The result revealed that total protein

concentration were significantly higher in wild males than cultured counterparts (<0.01). Triglyceride and cholesterol were also found higher in wild environs than farmed conditions but difference of these parameters was found to be non-significant (>0.05). The results showed that cultured brooders produced more dense milt than wild individuals. In contrast, the milt volume, percentage and duration of spermatozoa motility were higher in wild brooders than in cultured individuals. However, concentration of glucose and urea were found higher in cultured males than wild ones ($p<0.01$; $p<0.05$, respectively). The higher level of glucose and urea in farmed males may be related to stress condition (confinement, holding or handling) in captivity and higher concentration of urea due to the presence of ammonia in the pond, respectively.

Mean absolute fecundity in farmed females was 44533±28572.59 and in wild fish it was 68864±35950.29. Similar differences were observed in relative fecundity (farmed fishes-115.98±49.25 eggs and wild fishes -185.96±45.7 eggs). These differences can be attributed to the physiological acclimatization of the fish to their environmental conditions and feeding regime (variation in nutrition) which influence the energy metabolism. Short-term preservation of milt in three extenders (E_1, E_2 and E_3) and no extender (control) indicated that the maximum duration and percentage of motility was found with extender I, due to the presence of glucose which serves as the constant supply of energy. The present study showed the significant effect of environment on milt quality in cultured and wild fishes.

Key words: *Cyprinus carpio* var. *communis*; seminal plasma; milt quality; cultured and wild; biochemical parameters; extenders

Signature of Student   Signature of Major Advisor

Dated____________   Dated _________________

ACKNOWLEDGEMENT

In the name of Allah the most gracious and merciful

It is a moment of immense pleasure for me as I reach this point in my journey of quest. And this is surely not an end, but a new beginning with a new vision. The tremendous amount of help and encouragement I received from several people made this work possible. I shall always cherish the support, co-operation, and wholeheartedness on their part. This acknowledgement reflects my very deep sense of gratitude towards all of them.

With deep sense, veneration and obligation from the core of my heart, I considered it as great and proud privilege to ascribe my success in this venture to my venerate Major Advisor Dr. Farooz Ahmad Bhat, Senior Assistant Professor, Fishery Biology, for his valuable advice, splendid supervision and constant patience through which this work was able to take the shape in which it has been presented. It was his valuable discussions and endless endeavors through which I have gained a lot. His constant encouragement and confidence imbibing attitude has always been a moral support for me.

I am indebted and thankful to Prof. M.H. Balkhi Dean, Faculty of Fisheries whose periodical academic interaction and feedback have contributed to the improvement of the Faculty of Fisheries. No words, written and spoken are enough to express my heartfelt gratitude for his tremendous support, insightful comments, suggestions and providing facilities for successful completion of this research work both as a Dean and member of my advisory committee.

I would like to acknowledge and heartily thank to the advisory committee members Dr. A.M. Najar, Professor & Head, Fishery Biology; Dr. Tasaduq H. Shah, Assistant Professor, Fishery Biology; Dr. Ashwani Kumar, Assistant Professor, Fish Nutrition and Biochemistry, Dr. Bilal Ahmad Bhat, Assistant Professor, Statistics, Dr. Feroz Ahmad Shah, Assistant Professor, Fish pathology. I gladly thank them for their valuable guidance, perpetual incitement and timely suggestions in conceptualizing and consummation of this study.

I owe my deepest gratitude to other faculty members of the department viz. Dr. Irfan Khan, Dr. Gowher Bilal, Dr. Rizwana Malik and Dr. Adnan Abubakr for their evaluation, suggestions and recommendations starting from proposal stage until this final thesis.

I feel lacunae of words to express my most heartfelt thanks to my class mates for their continuous support, encouragement and cooperation. They made a significant contribution at every stage in the preparation of this project thesis-lab work, writing and proof reading etc. I express my warm thanks to the members of Fish Pathology Laboratory with special thanks to Dr. Iqbal for being a constant source of support and providing timely help and cooperation during the work.

I am thankful to library staff of Faculty of Fisheries especially Mrs. Asifa Jan for providing all the related books and providing necessary information whenever required

Last but not least, I offer my regards and blessing to my family members whose help made everything possible for me.

Again I am thankful to "Almighty ALLAH' for his blessing bestowed upon me…

Ishrat Mohd

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Chapter-1

INTRODUCTION

Common carp, *Cyprinus carpio* Linneus, 1758) is a freshwater cyprinid fish that is widely distributed in the world (Vostradovisky, 1973; Economids, 1991; Kottelat, 1997). Since the fish is a fast growing and hardy and can withstand adverse environmental conditions, it has been successfully introduced into fresh waters throughout the world (Welcomme, 1988; Seegers *et al.,* 2003). It occurs in shallow ponds, lakes rich in vegetation and slow moving rivers (Vostradovisky, 1973). Common carp dwells in middle and lower reaches of rivers and shallow confined waters. The fish can survive cold winter periods. Carps are omnivorous, with a high tendency towards the consumption of benthic organisms such as water insects, larvae of insects, worms, mollusks, and zooplankton.

Common carp was introduced in Kashmir in 1956 and since then this fish has shown remarkable adaptation in various water bodies of the state and soon began to constitute a major fishery of flat land temperate waters of Kashmir (Fotedar and Qadri 1974; Yousuf, 1996 and Bhat *et al*., 2010). Sunder *et al*. (1978) found *C.c.communis* to be the dominant contributor of the total catch from the Dal lake. *C. carpio* has been reported to be the most dominant fish in the lake both by the number and weight, contributing about 69.13% of the total catch by weight. Of the two varieties of fish which occur in the lake, *C.c.* var.*communis* has been reported to contribute 59.2% while the other *C.c.*var. *specularis* has been reported to form 9.11% of the total catch by weight (Shafi *et al.,* 2005). Feeding and breeding biology of Kashmir fishes has been attempted by a number of workers (Malhotra, 1966; Das and Subla, 1969; Sunder *et al.,* 1984; Sunder and Subla, 1985; Yousuf and Pandit, 1992; Yousuf *et al.,* 2003; Bhat *et al.,* 2005 and Bhat *et al*., 2015). *Cyprinus carpio* has been reported to dominate over the endemic fish of Kashmir in terms of reproductive potential also (Das and

Malhotra, 1964). The fish is found to spawn during spring although the gonads are fully mature at the start of winter, but because of severe winter of Kashmir, the gonads show inactivity or gonadal dispause (Malhotra, 1966 and Jyoti, 1973) which remains up to the middle of February. *Cyprinus carpio* has been freported to be the most fecund fish in the lake and shows adaptability to wide range of habitats (Yousuf and Pandit, 1992).

Jammu and Kashmir is endowed with enough freshwater fishery resources in the form of lakes, rivers, streams and low lying areas. The water bodies of Kashmir valley are blessed with various endemic (*Schizothorax* sp.) and exotic (various carps and trouts) fish species. The endemic fishes of Kashmir have shown tremendous decline in population as well as body size (Yousuf, 1996; Bhat *et al.,* 2013). The decline has been attributed to the factors such as over exploitation of fish, pollution of aquatic resources, habitat destruction, disturbance of breeding & feeding ground and many other factors (Bhat *et al.,* 2013). Common carp benefited from the changing environmental conditions in the Dal Lake and got well established in the lake within a short span of time. But the population of this species also has now declined considerably owing to heavy exploitation and neglected fisheries policies (Bhat *et al.,* 2013). A decrease in fecundity of *Cyprinus carpio* var. *communis* has also been reported from the lake (Shafi *et al.,* 2012) This may be attributed to the declining environmental water quality, which has lead to physiolological stresses in the fish, demanding urgent attention on scientific lines because of its being at present the cheap protein and its well-acceptance by the common man in the Valley (Yousuf, 1996; Bhat *et al.,* 2013). The human population activities in the state has brought these natural water resources under stress, it becomes important to strengthen and further upgrade the culture of fisheries in the State by way of establishing more aquaculture units and strengthening the conservation measures.

Common carp is considered to be a single species with different

populations adapted to local environments forming a range of geographical races. The origin of domestication of Common carp is back to the dawn of aquaculture, 4000 years ago in China. Common carp culture in India was restricted only to a homestead backyard pond activity until late 1950s, with seed from riverine sources as the only input resulting low level of production. Controlled reproduction really started in the 1950s. The importance of fish culture as an economically growing enterprise was gradually realized and it assured the supply of good fish. Asia is the main producing region of the species (China claimed about 70% of the 2005 world production) with the majority of production consumed domestically (FAO 2004). According to the FAO statistics of 2004, production of farmed Common carp was 13% (3,387,918 tonnes) of the total global freshwater aquaculture production. Over 11 million tonnes of Cyprinid are produced by aquaculture worldwide each year and are the most exploited group of farmed fish.

The success of aquaculture is based on good quality eggs and sperm. Poor egg quality and sperm are the major constraints in the expansion of aquaculture of fish. The quality of eggs and larvae produced in hatcheries are considered an important limiting factor in fry production (Kjorsvik *et al.*, 1990) and consequently, in the development of the aquaculture industry (Bromage, 1995). Egg quality has been defined as the potential of eggs to produce viable fry (Kjorsvik *et al.*, 1990; Brooks *et al.*, 1997). Sperm quality is equally important, as it can affect fertilization success and production of viable eggs (Bromage, 1995). The evaluation of milt quality in the reproduction of finfish species is an important tool for establishing its potential fertility. The reproductive potential of a population is a function of selection of the brooders for production purposes (Najar and Qadri, 1999; Najar *et al.*, 2000). Sperm quality is an important variable in aquaculture management as it can ensure percentage of egg fertilization and thus production of viable larvae from given brood stock. Any quantifiable

physical parameter that directly correlates with the fertilization is a measure of sperm quality.

Failure to produce high quality sperm will decrease the possibility to obtain viable offspring with the paternal genes. In the fish farming industry, good quality eggs have been defined as those exhibiting low mortalities at fertilization, hatching and first feeding (Bromage *et al*., 1992). Bobe and Labbe (2010) defined fish egg quality as the ability of the egg to be fertilized and subsequently develop into a normal embryo while Coban *et al*. (2011) define egg quality as the potential of an egg to hatch into a viable larva. Sperm quality has also been defined by Bobe and Labbe (2010) as its ability to successfully fertilise an egg and subsequently allow the development of a normal embryo. Egg and sperm quality is significant for the production of high quality fish larva and for economical utilization of hatcheries (Coban *et al*., 2011). Egg and sperm generally play an important role in the quality of the fish farming, good quality eggs and sperm increases fish production, all year round availability of fish, thereby increasing market demand for fish and fish protein, it also increases the revenue of the country and individuals directly involved in it.

Gamete management is key to achieving high fertilization success in fish farms (Billard *et al*. 1995). Studies on sperm physiology can provide basic knowledge for managing fertilization (Alavi and Cosson, 2005). For controlled and successful production in aquaculture systems, it is necessary to have adequate knowledge of the physical and chemical characteristics of the milt to determine the reproductive ability of cultivated fish. The ionic and organic constituents of the seminal fluid can indicate fish fertilization capacity (Ciereszko *et al*., 2000). Better production in aquaculture depends on fertization process. Testing fertilizing capacity is difficult and time consuming, and it is thus easier for both fish farmers and researchers to use different parameters that allow a more rapid evaluation. Some authors have tried to relate fertilization with other parameters that are more

or less easy to monitor, as has been reviewed by several authors (Bobe and Labbe, 2010; Cabrita *et al*., 2008a; Rurangwa *et al*., 2004). Both intrinsic gamete quality and quantity and environment of gamete fusion determine fertilization success. As a consequence, fertilization is an integrative response to a multiple factors which may hide variations of sperm intrinsic quality. Some studies have shown no sperm quality effect on fertilization in factorial crosses in trout (Nagler *et al*., 2000) and seabass (Saillant *et al*., 2001) where egg quality effect was significant. The latest reviews by Alavi *et al*. (2008) and Cabrita *et al*. (2009) clearly demonstrate that most of the characteristics of sperm contribute to the overall quality but that none of these is sufficiently integrative to describe fully the ability of sperm to fertilize ova. Since studies describing milt characteristics, which can influence fertilisation ability, have shown large individual variations in the different parameters investigated (Dreanno *et al*., 1998), this rendered difficult the use of a single sperm characteristic to define good sperm quality. Other works show that non motile spermatozoa can fertilise eggs (Truscott and Idler, 1969).

All these examples highlight the limitations of using a single trait to define the quality of the sperm. Nevertheless, because quality does not rely on a particular characteristic of the milt, there is not a single predictive parameter. However, the assessment of some parameters together could give us a consistent idea of the sample quality in order to predict its fertilizing ability. Moreover sperm quality is very variable between species and so are the parameters to be used in each species for the quality evaluation. The evaluation of sperm samples is important when testing the maturation level of males, for the identification of males with better reproductive performance, for situations where in vitro fertilization is needed, to monitor the best brood stock rearing conditions in two different environments.

Sperm quality can be assessed by its constituents, like seminal plasma and spermatozoa. Standard analysis may include parameters such as spermatozoa

concentration, motility, sperm volume, seminal plasma osmolarity and pH. Basic studies on seminal plasma constituents and its variation such as enzymes (lytic, oxidative, metabolic), metabolites, sugars, vitamins, amino acids, fatty acids and other inorganic compounds can provide very useful information on sperm status (Rurangwa *et al*., 2004; Cabrita *et al*., 2008). Biomarkers of sperm quality so far documented include spermatocrit, sperm density, osmolarity and pH of seminal plasma, chemical composition of seminal plasma, enzymatic activity, adenosine triphosphate (ATP) concentration, motility, morphology and ultrastructure, fertilising capacity, and several others (Billard and Cosson, 1992; Ciereszko and Dabrowski, 1993; Lahnsteiner *et al*., 1998; Geffen and Evans, 2000).

The seminal plasma has a unique composition; some components support the spermatozoa while others reflect the functions of the reproductive system and the spermatozoa (Ciereszko *et al*., 2000). The main role of seminal plasma is to create an optimal environment for the storage of spermatozoa, very often for a prolonged time (Ciereszko, 2008). During storage, sperm fertilizing ability, sperm motility, and viability must be protected. Damage to any of these functions is likely to result in failure of fertilization. In most of the fish species studied so far, spermatozoa are immotile in the testis and seminal plasma (Stoss, 1983; Billard, 1986), in contrast to the situation in reptiles or mammals (Krasznai *et al*., 1995). The osmolality and composition of the seminal plasma usually prevent sperm motility in the fish sperm duct (Billard, 1986). The seminal fluid not only immobilizes the spermatozoa but also protects them (Cosson *et al*., 1997). In marine species, activation is generally triggered when sperm is diluted in media of high osmotic pressure (Morisawa, 1985). Thus, sperm are normally activated only following release into the marine environment; there are clear relationships between seminal plasma composition and osmolality and the duration of fish sperm motility.

Preservation of fish sperm for short-term duration is generally useful from

the commercial point of view and facilitates various hatchery operations. The short-term storage of sperm at low temperature (4ºC) is mostly applied in short-distance transport of gametes collected in different locations, in synchronizing the timing of obtaining good quality of gamete collection from males and females during artificial insemination, in avoiding the ageing of sperm, in facilitating hatchery operations, also in experimental programs for genetic studies. Asynchrony associated with the maturation of gametes and the time of spawning between the sexes has been documented in species. This problem can become acute when trying to produce hybrids. To ameliorate these problems, researchers have examined procedures such as cryopreservation, short-term, refrigerated storage of milt. Techniques for the short-term, refrigerated storage of milt have been developed for several teleosts, such as walleye (Moore, 1987), Red drum *Sciaenops ocellatus* (Wayman *et al*., 1998), Atlantic sturgeon *Acipenser oryrinchus* (Dilauro *et al*., 1994) Mozambique tilapia *Tilapia mossambica* (Harvey and Kelley, 1984), Paddlefish *Polyodon spathula* (Brown and Mims, 1995).

The fish sperm could be preserved by storage in undiluted and diluted form. Undiluted sperm stored at low temperature has been reported to cause a reduction in fertilization capacity (Lahnsteiner *et al*., 1997). Storage of diluted sperm with extender provides better control compared to undiluted storage (Harvey and Kelley, 1984). For many fish species, the dilution of the sperm in a suitable medium favours the preservation for short time periods, e.g. during transportation from the place of collection to the place of employment, as it helps to keep the physicochemical properties stable (Gwo, 1994; Ohta and Izawa, 1996). These solutions, however, must induce a temporary inactivation of motility, so as to prevent the exhausting energy reserves of the spermatozoa (Gwo, 1993).

Captive condition never entirely mirrors a fish’s natural environment,

however, holding conditions should mimic as closely as possible environmental conditions. Reproduction of fish in captivity can be controlled by environmental manipulations, such as photoperiod, water temperature or spawning substrate. However, the ecobiology of some fishes is not well known, or it is impractical or even impossible to simulate the required environmental parameters for natural reproductive performance (i.e., spawning migration, depth, riverine hydraulics, etc.). Stress imposed on fish under captivity can have negative results on reproductive function and gamete quality (Schreck, 2010). In captivity, confinement and over stocking of fish may affect egg quality. Both chronic confinements experienced during the final stages of reproductive development, and periods of acute stress, have been shown to disrupt the endocrinology underipinnings normal growth and development of the ovary in trout, and may result in significantly lower progeny survival rates (Campbell *et al.*, 1994). Stress can lead to irregular spawning intervals, low fertilization rates and increased occurrence of abnormal embryos in Atlantic cod (Kjesbu, 1989). How stressing brood fish leads to deleterious effects on egg quality has not been established. Some fish, when reared in captivity, do not usually release their eggs; the eggs will then age or over-ripen within the body cavity. Over-ripening of eggs is perhaps the most common reason for poor egg quality in captive brood fish. After fertilization, dying or dead eggs become colonized with bacteria/ fungus, and if these eggs are not removed quickly viable eggs may also be colonized.

The rapid increase in global aquaculture production has raised concerns about the effect of domestication on fish (Naylor *et al.*, 2005). The aquaculture setting provides a very different environment for fish compared to the wild, resulting in changes in the selective pressures that can lead to fundamental genetic changes at the population level (Skaala *et al.*, 2004; Jonsson and Jonsson, 2006). Farming practices often result in a reduction in genetic diversity due to genetic bottlenecks, as well as the divergence of farmed stocks from wild populations as a

result of novel selective pressures associated with domestication (Einum and Fleming 1997; Norris *et al.*, 1999; Skaala *et al.*, 2004). The loss of genetic diversity has been demonstrated by the lower allelic diversity of farmed salmon populations compared to wild salmon (Norris *et al.*, 1999; Skaala *et al.*, 2004), which can result from large numbers of offspring being produced from only a small number of breeding individuals. Small numbers of breeding individuals results in reduced effective population size and can lead to increased incidences of inbreeding (Bentsen and Olesen, 2002).

Captivity reared and naturally produced (wild) adult fish experience sharply different environmental regime. Captivity reared fish are fed artificially formulated diets, and do not undergo extensive migrations. Fish reared in ponds differ morphologically from wild fish, and the degree of divergence appears positively related to the duration of confinement (Fleming *et al.*, 1994). After several centuries of managed and culture spawning, the common carp has been subjected to mass selection so that there are several stocks which are not properly identified in fish culture units of Europe and Asia. Ever since fish for aquaculture are taken from the wild, the characteristics of domestic races have changed progressively because of both brood stock selection and rearing methods (association with other species, type of fertilization, preferred harvest size). So the present study was taken in hand to find out whether the difference lies between farmed and wild common carp in terms of sperm quality and reproductive biology.

Understanding the difference in sperm quality between males in farmed and wild conditions will help in an identification of good quality males in particular environment that would significantly improve brood stock management, discarding non-productive individuals. Better knowledge of semen components is important to understand events leading to production of good quality gametes and to identifying factors that disturb semen function.

The objectives of this study were to examine:

- The physical and biochemical characters of the milt of *Cyprinus carpio* var. *communis* in farmed and wild conditions.
- The reproductive biology in terms of gonadal development and fecundity in farmed and wild conditions.
- Investigate the relationship between the body weight, body length and spermatological parameters of scale carp in wild and farmed conditions

Chapter – 2

REVIEW OF LITERATURE

The use of high quality gametes from fish brood stock is of great importance for ensuring the production of viable larvae for aquaculture (Kjorsvik *et al*., 1990; Bromage and Roberts, 1995). Milt quality is a measure of the ability of sperm to successfully fertilise an egg and such ability mostly depends on qualitative parameters of milt i.e. composition of seminal fluid, milt volume, sperm density and sperm motility (Rurangwa *et al*., 2004). Fish seminal fluid has a unique composition regarding the presence of the organic and inorganic components which support the viability of spermatozoa (Hajirezaee *et al*., 2010a). Semen quality is an important factor that increases the efficiency of artificial fertilization. Techniques for determining semen quality in fish include monitoring semen density, motility and fertilization success (Tekin *et al.,* 2003). Semen consists of seminal plasma and spermatozoa. The main role of seminal plasma is to create an optimal environment for spermatozoa storage. Seminal plasma also benefits external fertilization by creating a favorable micro-environment for sperm movement (Billard, 1986). A lot of work has been carried on the physical and the biochemical parameters of semen (milt) and lot of literature is available worldwide, though negligible in this part of the world. Keeping the voluminous literature available in consideration, literature pertaining to last two decades has been cited here. However, some earlier references, keeping their importance in consideration have also been cited in the review of literature. The review of literature has been categorized into:

2.1 Physical parameters of milt

2.2 Biochemical composition of seminal plasma

2.3 Seasonal variation of milt quality parameter during the reproductive season

2.4 Female reproductive biology

2.1 Physical parameters of milt

2.1.1 Sperm density

Sperm density is an important factor in the determination of sperm quality (Suquet *et al.*, 1992). Its role in the fertilization of spermatozoa is well documented and reported (Aas *et al.*, 1991; Pool and Dillane, 1998). Density of spermatozoa is observed to be highly varying depending on the species as is evident from the reports on carp where it has been reported to be from 1 to 5×10^9 cells/mL, in trout it has been reported from 5 to 15×10^9 cells/mL and in sturgeon from 0.1 to 4×10^9 cells/mL (Cosson *et al.*, 1991; Redondo *et al.*, 1991; Tsvetkova *et al.*, 1996). Babiak *et al.* (1997) estimated the sperm density of *C. carpio* as 20×10^9 numbers per ml. Chutia *et al.* (1998) also mentioned that the sperm density of *C. carpio* var. *communis* as 6.6×10^9 cells per mL of milt. Lahnsteiner *et al.* (2000) found that the sperm density of *C. carpio* was 0.5 to 1.0×10^{11} cells per mL of milt. Akcay *et al.* (2004) reported sperm density of mirror carp to be $17.33\pm1.22\times10^9$ mL/L. Sperm density of grass carp was reported by Bozkurt *et al.* (2008) as $15.43\pm0.72\times10^9$ mL/ L. Similar variations and ranges might be existing in other fish species also. Jaspers (1972) noted that age seemed to be the major factor influencing sperm concentration with 3 years old fish producing more spermatozoa per gram wet testicular tissue than 2 years old fishes of the same species. Sanchez-Rodrique *et al.* (1978) showed that the spermatocrit (packed cell volume/ total semen volume) remained constant throughout the spawning period. In rainbow trout, Munkittrick and Moccia (1987) reported that sperm density declined as the season advanced. In salmonid species, sperm density and milt volume were highest at their peak spawning season and declined as the season progress (Billard, 1983). Whereas Sanchez-Rodriguez *et al.* (1978) and Piironen and Hyvarinen (1983) noted that spermatocrit values increased over the stripping season. The semen characteristic varies from species to species (Verma *et al.,* 2009). A wide variation in the sperm density (spermatocrit) and sperm count was

noticed among the different carp species. These variations were due to the spermatozoa size and species-specific nature of carps.

Based on the observations on various species, it can be said that spermatozoa concentrations in fishes can range from 2×10^6 to 5.3×10^{10} cells per mL (Leung and Jamieson, 1991). A rare phenomenon of increased spermatozoa density to the decreasing volume of milt was observed and reported by Reenaselvi (1991), Nalliappan (1992) and Degraff *et al*. (2004). Rouxel *et al.* (2008) investigated the existence of changes in cod sperm quality during the spawning period. The work aimed at establishing tools to study sperm biology in Cod. Fauvel *et al*. (2010) reported the development of cheaper technical tools and the availability of well established assay kits provide spermatologists with several devices for objective and quantifying assessment of sperm quality. Density of the spermatozoa in the semen can be a factor that will decide the dilution of the milt before cryopreservation. Sarder *et al*. (2009) contended that counting of spermatozoa and determination of the density is essential to standardize the degree of dilution of milt and to determine the density of spermatozoa per straw for maintaining the desired egg and sperm ratio during fertilization.

2.1.2 Motility of spermatozoa

As stated by Terner (1986) motility is a parameter to decide the quality of the spermatozoa. High quality semen (fertilization rate > 80%) has been characterized by high motility rate (>75%) and a high correlation between sperm motility and fertility and between seminal plasma composition and spermatic cell metabolism (Lahnsteiner *et al*., 1998). Sperm quality is usually assessed by the intensity of motility (Sanchez-Rodriguez). Fish spermatozoa are immotile in the seminal fluid (Ciereszko *et al*., 2000). In order to activate the spermatozoa, it has to be transferred into an activating solution which contains solutes that affect the osmotic pressure of the fluid surrounding the spermatozoa. In case of marine species, the osmotic pressure of the activating solution should be higher than the seminal fluid, whereas in freshwater species it should be lower. Linhart *et al.*

(2000) observed that the percentage and velocity of sperm motility at 15 s after activation of frozen/thawed sperm was significantly lower than that of fresh sperm.

The motility duration of fish spermatozoa ranges from 30 to 300 s (Stoss, 1983). Since many fish species have shorter motility period ranging from 30 to 120 seconds (Alavi and Cosson, 2005; 2006), it is necessary to avoid the preactivation of motility by urine contamination during stripping (Perchec *et al.*, 1995a; Dreanno *et al.*, 1998).

Sneed and Clemens (1956) stated that a higher concentration of potassium ions is required for the inhibition of *C. carpio* spermatozoa. It has also been found and reported that the motility of sperm was extended to a longer period in saline and ovarian fluid of matured female than in water (Fredrich, 1984). Percentages of motile spermatozoa decreases significantly with increasing storage period in undiluted than diluted sperm during spawning season (Bozkurt *et al.*, 2009).

Morisawa *et al.* (1983) demonstrated that sperm from Common carp and Crucian carp can be kept immotile in media like NaCl, KCl, mannitol and glucose media having 300 mosmol/kg which is isotonic to the seminal plasma of these species. Salinity of activating medium plays a role in inducing sperm motility; freshwater teleosts' sperm were motile only if salinity was lower than 15 ppt (Billard, 1978). Potassium increased viability and speed of sperm movement at a concentration below that in the seminal plasma, whereas sodium and the non electrolytes were less effective. According to Redondo *et al.* (1991), motility can be initiated by decreasing the osmolality of the seminal plasma with freshwater or in a saline solution (45 mM NaCl, 5 mM KCl, tris 30 mM, pH 8) and it can last for 45 to 90 seconds. Although NaCl has properties similar to that of KCl, the recovery ability for motility is faster with KCl (Perchec *et al.*, 1995b). Activation of carp spermatozoa is not dependent on broad range external pH (6.0-10.0) (Redondo *et al.*, 1991). It has also been reported that external factors, such as pH or ions present, may polarize the cell membrane and stimulate motility of fish

spermatozoa (Morisawa *et al.,* 1999). The depolarization of the membrane potential is an important step in the initiation of sperm motility (Blaber and Hallet, 1988). In carp, osmolality-dependent permeability and structural changes are induced in the sperm membrane by hypo-osmolality and reorganization of lipid structure has been proposed as a possible mechanism (Marian *et al*., 1993).

Osmolalities isotonic to seminal plasma suppress sperm motility in marine and freshwater teleosts. Exposure of sperm to hypertonicity of seawater or hypotonicity of freshwater, induces the initiation of sperm motility at spawning (Takai and Morisawa, 1995). In trout, the inhibition of sperm motility is mainly due to K+ ion concentration (Gatti *et al*., 1990 and Billard and Cosson, 1992). In turbot, anaerobiosis and high CO_2 content within the genital tract contribute to the inhibition of spermatozoa motility (Dreanno *et al.,* 1995). However, most studies suggest that water is not a suitable activation medium (Billard and Cosson, 1992; Cosson *et al*., 2000). In endorsing the above statement, it was found that the sperm motility of Shovelnose sturgeon *Scaphirhynchus platorynchus* has significantly increased by activation in a buffered media instead of activation in distilled water (Cosson *et al*., 2000). The time of motility was prolonged and there were fewer damaged sperm cells in buffered media than in distilled water. At activation, sperm cells are exposed to a hostile environment, i.e., low or high osmolality compared with that of the seminal fluid. Simple physiological solutions and various complicated media are currently used in hatcheries. But it should be admitted that no thorough study has defined the best medium for each species. Comparative studies have been made on the effectiveness of DMSO and Glycerol as cryoprotectants. It has been observed that motility rates are higher (60%) for DMSO than Glycerol (40%) at 15% concentration levels. DMSO has proved better than Glycerol as cryoprotectant for both the sample (Antony *et al.,* 2014).

Cosson *et al*. (1985) observed that in salmonids and cyprinids, temperature affected the sperm beat frequency. In trout, higher temperature increased the beat

frequency and decreased the duration of forward movement (Billard and Cosson, 1992) while the lower temperature that trout experience during natural spawning of trout increases the duration of sperm movement (Van Look, 2001). In African catfish, low temperature (4°C) also prolonged motility and viability of spermatozoa compared to the culture temperature (25°C) (Mansour *et al.*, 2002). Temperature adaptation changes the ion concentrations in spermatozoa and seminal plasma of Common carp without affecting sperm motility. The concentration of spermatozoa in the semen of cold adapted animals was about half that for the warm adapted animals ($0.7\pm0.1\times10^{10}$ vs. $1.4\pm0.2\times10^{10}$ cells/ml). The Na^+ concentration of the seminal plasma of the cold adapted animals (83±12 mM) was higher, while the K^+ concentration in these samples (64±11 mM) was lower than the corresponding data for the warm adapted animals (87±16 mM) (Emri *et al.,* 1998).

In fishes spawning in brackishwater and marine water, motility of spermatozoa is more long lasting than that of freshwater species (Hines and Yashouv, 1971; Ginzburg, 1972). Though there are several ways to detect live spermatozoa, viz., motility (Hodgins and Ridgway, 1964; Sneed and Clemens, 1956) and differential staining (Fribourgh, 1966), the fertility evaluation test is considered more exact and accurate.

It is noted and reported that the motility duration is influenced by the spawning preparedness of the brooders. During the peak spawning season, activated rainbow trout spermatozoa remained motile for 30-55 seconds. But during the end of the spawning season the duration of the motility declined to 15 s (Benau and Terner, 1980). In rainbow trout, the proportion of spermatozoa that are activated may also gradually decrease as the spawning season progresses (Munkittrick and Moccia, 1987). The concentration and the ratio of ions such as K and Na which are implicated in the initiation of sperm motility decreases as the season progresses (Munkittrick and Moccia, 1987). To prolong the duration of motility, minimize osmotic shock during fertilization and disperse the sperm cells

around the ova, inseminating solutions are advocated. The compositions of such solutions are similar to ovarian fluid (Ginsberg, 1963; Scott and Baynes, 1980) or have a salinity of 5 or 20% for fresh and sea water species respectively. Buffered 0.1-0.15 M sodium bicarbonate or chloride is commonly used for salmonids (Stoss, 1983). The addition of compounds such as isobutyl-1-methylxanthine (Benau and Terner, 1980) and theophylline (Scheerer and Thorgaard, 1989; Wheeler and Thorgaard, 1991), to buffered fertilizing medium may also help to reduce osmotic swelling and prolong the duration of sperm motility. Stoss and Holtz (1981) have increased the motility of pink salmon spermatozoa from 30 s to 10 min by activating with a 120 nm $NaHCO_3$ solution to which 1 BMX (3-isobutyl-1-methylxanthium) had been added.

According to Scott and Baynes (1980), the motility and fertility do not reside on the same part of the sperm. Therefore, not all the motile spermatozoa can fertilize the eggs. This has been found true in carp and cod (Mounib *et al.,* 1968). It has been pointed out that fertilization could be obtained with sperm which did not show any sign of motility on dilution.

Dilution is necessary for the observation of the spermatological parameters (density, motility pattern and percentage of motile spermatozoa) due to difficulties in determining the parameters in the raw semen. But dilution of semen itself has an effect on motility. The difference was significant between dilutions 1:10 and 1:1000 in terms of initial motility and duration of movement as observed by Suquet *et al.* (1992).

2.1.3 Milt pH

Hamner (1970) might be one of the first researchers in reporting the pH related finding in semen of higher animals. He reported optimum survival of sperm at a pH of 7 and a progressive decline in motility and metabolism below that optimum pH in higher animals. Similarly the pH of fish milt was also found to affect the motility of spermatozoa and maturation process (Billard *et al*., 1995;

Liley *et al*., 2002). In cyprinids, it has been shown that extracellular and intracellular pH as well as the ionic composition of the seminal plasma influences the initiation and duration of sperm motility (Marian *et al*., 1997). Sahinoz *et al.* (2008) opined that the seminal plasma pH may affect final maturation of spermatozoa in fishes and added that duration of sperm motility in males could be influenced by the changes of semen pH. Chao *et al*. (1987) measured the milt pH of *Oreochromis* species using Bromothymol blue pH test paper and found that the values varied from 6.2 to 8.2. Lahnsteiner *et al.* (1998) noted increased fertility in *Oncorhynchus mykiss* when milt pH was increased from 8.0 to 8.2. Faruk *et al.* (2007) found a milt pH range of 7.70 to 8.40 for *O. mykiss*. They used pH indicator strips to determine the pH of the milt of Rainbow Trout (*O.mykiss*). Bozkurt *et al.* (2009) evaluated pH of grass carp sperm using standard pH papers and mentioned that it was around 7. They also opined that determination of fluctuation in sperm pH could provide necessary information on fertilization capacity of spermatozoa. Verma *et al.* (2009) reported that the pH values of *Catla catla, Labeo rohita*, *Labeo calbasu, Cirrhinus mrigala, Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* ranged from 7.3 to 8.1. There has been an observation on the premature motility of the spermatozoa in salmonid species that has been attributed to the increase in external pH during the passage of spermatozoa from the testis to the spermatic duct (Morisawa and Morisawa 1986, 1988; Billard *et al.,* 1995). This endorses the fact that the seminal fluid pH may also affect the final maturation of spermatozoa as stated by Lahnsteiner *et al.* (1998).

2.2 Biochemical composition of seminal plasma

Semen consists of seminal plasma and spermatozoa. Seminal plasma contains substances that support sperm cells. Some substances reflect the functioning of the reproductive system and spermatozoa (Akcay *et al*., 1995; Ciereszko and Dabrowski, 2000). The main role of seminal plasma is to create an optimal environment for spermatozoa storage. Seminal plasma also benefits

external fertilization by creating a favorable micro-environment for sperm movement (Billard, 1986). Seminal plasma composition in salmonids has been carefully studied particularly in rainbow trout and salmon (Piironen and Hyvarinen, 1983; Munikittrick and Moccia, 1987; Lahnsteiner *et al*., 1998; Glogowski *et al*., 2000). The composition of seminal plasma in other salmonid species has been examined scarcely so far (Cruea, 1969; Morisawa *et al*., 1979; Piironen and Hyvarinen, 1983; Hatef *et al*., 2007). Information on the composition of seminal plasma and other biological fluids can be used to make media for use as a diluent or for gamete storage. Better knowledge of semen components is important to understanding events leading to production of good quality gametes and to identifying factors that disturb semen function. Seminal plasma includes inorganic compounds (ions), organic compounds and enzymes. Inorganic compounds, including sodium, potassium, calcium and magnesium, play the role of preventers or stimulators in spermatozoa (Morisawa, 1985) and organic compounds are important for metabolic activity (Lahnsteiner *et al.,* 1996). Ionic compositions of the sperm may change during reproduction seasons (Alavi and Cosson, 2006). Ions of seminal plasma may polarize the cell membrane and can stimulate sperm mobility (Morisawa *et al.,* 1999). Cations (often bivalent, such as calcium) have antagonistic effects to avoid effects of potassium on sperm mobility (Alavi and Cosson, 2006). K+ plays an essential role in sperm mobility in sturgeons (Alavi and Cosson, 2005). Over-increase of sodium will decrease the duration of sperm movement and the percentage of motile sperms (Morisawa, 1985; Alavi and Cosson, 2006).

Determination of seminal plasma composition can help to understand the design requirements to prepare the appropriate artificial seminal plasma solutions. Such solutions can be used for the dilution of semen for short-term storage or cryopreservation (Billard and Cosson, 1992; Dreanno *et al*., 1998). In addition, seminal plasma is an important constituent of semen that has a vital role in sperm metabolism, function, survival and sperm motility. The ions such as Na^+, K^+ and

Cl^- in the seminal plasma establish osmotic balance. The ranges of serum biochemistry vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish (Jawad *et al.*, 2004). Some energetic substrates such as glucose are found in the seminal plasma and the sperm but in small amounts (Kruger *et al.*, 1984). According to Soengas *et al.* (1993), the presence of glucose in seminal plasma has been connected to high energy demand of testis during spermatogenesis or to lipid synthesis of spermatozoa. The glucose content of seminal plasma is also an important biochemical parameter because it provides membrane protection to spermatozoa and serves an external cryoprotectant as well. Seminal plasma lipids are associated with metabolism in spermatozoa (Piironen, 1994). Lipids and cholesterol might have a protective effect against environmental changes (especially water temperature) when semen was released. Bozkurt *et al.* (2006) ruled out that low triglycerides levels could be indicative of inadequate energy resources, reduced motility sperm rate and fertilization capacity. Seminal plasma proteins prolong the viability of rainbow trout as measured by sperm motility (Lahnsteiner *et al.*, 2004).

The composition of seminal plasma has a great influence on the biological quality of the semen. It is well known that the composition of seminal plasma cans differ even among related species. Seminal plasma also show seasonal variation. Khodadoust (2015) observed the highest glucose levels in spring, the highest cholesterol levels were in autumn and winter and the highest total protein levels were in autumn ($P<0.05$). Strange (1980) also reported that blood glucose level may decrease with increasing water temperature in channel catfish. Blood glucose level may vary according to season and water temperature, and glucose concentration in fish decreases with age and size (Coz-Rakovac *et al.*, 2005). Hrubec *et al.* (2001) stated that protein level in striped bass increased with age. Hajirezaee *et al.* (2010c) reported a decrease in total protein concentration in Caspian brown trout with progress of the spawning season. Jezeh *et al.* (2014)

studied the impact of barley and commercial dry food feeding on biochemical parameters of cultured common carp (*Cyprinus carpio*). The results revealed that the overall mean of total protein, cholesterol, triglycerides and glucose in barley treatment were 4.19±0.549, 253.56±141.303, 320.63±182.172, 49.75±15.356 mg/dl, respectively and in commercial dry food were 4.73±0.306, 302.06±52.488, 187.33±49.082, 64.78±18.164 mg/dl, respectively. Results showed that glucose levels, total protein and triglycerides in treatment were fed with commercial dry food, were significantly higher than the other treatment ($P<0.05$). There was no significant difference between cholesterol levels of two treatments.

2.3 Seasonal variation of milt quality parameters during the reproductive season

Several studies have focused on seasonal aspects of milt quality (Buyukhatipoglu and Holtz, 1984; Kruger *et al*., 1984; Piironen, 1985; Munkittrick and Moccia, 1987; Hajirezaee *et al*., 2010c) and suggested the characteristics which can influence milt quality. In rainbow trout, spermatogenesis is a seasonal event (Billard, 1986) and chemical and physical properties of semen may change since all spermatozoa are eliminated by the end of the reproductive season (Lahnsteiner *et al.,* 1993). The values of milt volume, sperm density and spermatocrit declined in Atlantic salmon (Aas *et al*., 1991) and Caspian brown trout (*Salmo trutta caspius*) (Hajirezaee *et al.,* 2010c) over the course of spawning season, although these parameters increased in landlocked salmon (*Salmo salar*) (Piironen, 1985) and rainbow trout (Sanchez-Rodriguez *et al*., 1978) during spawning season. In addition to these parameters, the concentration of some chemical components of seminal fluid (e.g. Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cl^{-} and total protein) decreased also in Caspian brown trout (Hajirezaee *et al*., 2010c). In most fish species a decline in milt quality throughout the spawning season has been reported (Legendre and Billard, 1980; Piironen, 1985; Munkittrick and Moccia, 1987; Aas *et al*., 1991) due to ageing of spermatozoa (Rana 1995; Suquet *et al.,* 1998; Babiak *et al.,* 2006). As a consequence of ageing, several sperm features may be modified, including cell morphology (Suquet *et al*., 1998), composition of

seminal fluid (Aas *et al*., 1991), spermatozoa concentration (Buyukhatipoglu and Holtz, 1984; Zuromska, 1981), percentage and duration of sperm motility (Benau and Terner, 1980; Buyukhatipoglu and Holtz, 1984; Methven and Crim, 1991; Slominska and Gluchowska, 1994; Suquet *et al.,* 1998).

Spermatocrit, motility and seminal plasma ion concentrations declined as the season progressed, but volume was independent of time (Munkittrick and Moccia, 1987). Alavia *et al*. (2007) studied changes in sperm morphology, volume, density and motility and seminal plasma composition in *Barbus barbus* (Teleostei: Cyprinidae) during the reproductive season. Sperm volume also decreased from 0.42 ml in March to 0.15 ml in May, and density from 18.81 in March to 12.45 × 109 spz ml-1 in May. Osmolality (mOsmol kg-1) was 268±4, 276±2 and 268±2 in March, April and May respectively. Chloride, sodium, calcium and potassium ion concentrations (mM) did not show significant differences between March and April (Cl-: 125.3 vs. 120.5, Na+: 75.7 vs. 69.7, Ca2+: 0.4 vs. 0.3 and K+: 84.7 vs. 84.0). According to Rouxel *et al.* (2008) sperm concentration, sperm velocity and storage capacity at 4 degrees C peaked during the medium period of the spawning season and then decreased to values close to those observed at the beginning of the reproductive period. On the other hand, spermatocrit did not vary with the sampling date. In conclusion, cod sperm quality was modified during the spawning period, the highest-quality samples being collected during the medium part of the spawning season. Alavi *et al.* (2010) reported the semen volume did not change significantly from November to February, but it significantly increased in April. Sperm concentration was higher in November and January than in February and April. The highest and the lowest osmolality of semen was observed in January and April while it decreased in February and April.

Golpour *et al.* (2013) reported the sperm motility parameters (percentage of motile spermatozoa and sperm movement duration) changed significantly (P< 0.05) during the reproductive season, but sperm density, spermatocrit, and sperm

volume did not showed significant differences during spawning migration. Analyses performed at each sampling time (February, March, and April) showed significant differences ($P<0.05$) in calcium, magnesium, potassium, and cholesterol, whereas there were no significant changes in Na^+, pH, total protein, glucose, and cholesterol. There has been substantial variation in volume, concentration and motility among the fortnightly collected sperm samples (Nahiduzzaman *et al.,* 2014). Sahin *et al*. (2014) studied the quantitative characteristics of *O. mykiss* semen throughout the reproductive season and concluded that highest quality milt was collected during medial part of reproductive season.

Bozkurt *et al*. (2009b) reported a positive correlation between cholesterol and sperm motility in Scale carp. The author observed a positive 'r' value of 0.829 between cholesterol and triglycerides. Bozkurt (2006) while studying seminal plasma composition of *Salmo trutta fario* reported a highly significant 'r' value of 0.882 between cholesterol & protein and cholesterol & volume (r=0.667) with mean concentration of 19.2±18.57 $mgdL^{-1}$. Bozkurt (2006) reported a highly significant and positive correlation between protein and cholesterol (0.882) in *Salmo trutta fario* sperm. The author indicated that high protein concentration (3.0±9.42 $mgdL^{-1}$) is necessary for brown trout sperm. Hill (1982) reported that cholesterol concentrations increase as the fish size increased. Bano (1985) observed an increase in the serum cholesterol level after administration of pesticides. A high blood urea concentration recorded in *M. cephalus* is likely to be a sign of stress associated with the increase in the cortisol level (Borges *et al*., 2007). Bozkurt *et al.* (2009a) while studying the seminal plasma composition of *Cyprinus carpio* reported notable urea concentration of 54.72±3.49 $mgdL^{-1}$. While some authors consider urea in relationship with protein metabolism and total protein because it occurs as a result of the digestion of protein which contains N_2, many others report it as an indicator of contamination of milt with urine, faecal matter and unhygienic handling practices during stripping.

Environmental factors that may affect egg quality and sperm quality in fish include the diet of the Brood fish and the physiochemical conditions of the water in which the eggs are incubated (temperature, salinity and pH of the water, etc.). In aquaculture, the photoperiod to which the brood fish have been exposed and the quality of the husbandry-factors such as the level of stress to which the broodstock are exposed, the fertilization procedures adopted, over ripening of eggs in the body cavity and bacterial colonization of fertilized eggs-can all affect egg quality. In both wild and captive fisheries, exposure of maturing females and male, or exposure of the eggs and sperm or developing embryos to environmental pollutants may affect egg, sperm and fry survival (Miller, 1993). More subtle features of the environment may also affect spawning. For example, in the ayu (*Plecoglossus altivelis*, *Plecoglossidae*), sensitivity to hormone signals is affected by the physical features of the spawning environment (Soyano *et al*., 1993).

Few studies have examined how aquaculture impacts on fertility and rarely with regard to the natural mating pattern which can generate sperm competition and/or mechanisms influencing sperm–egg compatibility. In farmed Penaeus prawns, pond-reared males have poor sperm quality and problematically lowered fertility, compared with wild relatives (Leung-Trujillo and Lawrence 1987; Alfaro and Lozano 1993; Pratoomchat *et al*., 1993). In first-generation farmed cod (*Gadus morhua*), males showed reduced sperm quality compared with wild equivalents, especially at the start of the breeding season, and this translated into inferior sperm fertility and competitiveness, possibly mediated by diet (Skjaeraasen *et al*., 2009; Butts *et al*., 2011). In haddock (*Melanogrammus aeglofinus*), however, cultured and wild males showed equivalent sperm motility and concentration (Rideout *et al*., 2004), and in sea trout (*Salmo trutta*), sperm densities between wild and sea-reared males showed differences that were opposite between years (Poole and Dillane, 1998). Papadaki *et al.* (2008) studied egg and sperm production and quality of sharp snout sea bream (*Diplodus puntazzo*) in captivity. A significant drop was observed in mean total annual

relative fecundity from 4.9±0.08 million eggs kg^{-1} female body weight in 2003, to 2.4±0.07 million eggs kg^{-1} in 2004 and 2005. Mean monthly fertilization success also dropped significantly from 81±1% in 2003 to 76±2% in 2004 and 78±2% in 2005. Zhou (2009) revealed that haemoglobin, cholesterol, total protein, creatinine and uric acid levels in the two ecotypes were significantly different (n = 56, df = 54, $P>0.05$). In addition, red blood cell, glucose, triglyceride and urea nitrogen levels were significantly higher in cultured individuals (n = 56, df = 54, $P<0.01$) than in their wild counterparts. In contrast, the white blood cell level in cultured fish was significantly (n = 56, df = 54, $P<0.01$) lower than that in the wild ones. By contrast with the studies in these aquaculture species, however, farm Atlantic salmon have been subjected to almost 50 years of selective domestication since the 1970s (Tilseth *et al.*, 1991) so that genetic influences from relaxed selection, directed domestication and reduced genetic diversity could be influential phenomena. Comparisons between farm and wild Chinook salmon revealed that farm males produce sperm with significantly higher sperm concentration, motility, longevity and velocity compared with wild males (Lehnert *et al.*, 2012), revealing clear potential for fertilization success and therefore introgression after escape.

Exogenous factors, such as management (Svobodova *et al.,* 2006), diseases (Chen *et al.*, 2005) and stress (Cnaani *et al.,* 2004), always induce major changes in blood composition. For example, significant fluctuations were detected in the concentrations of cortisol, glucose, cholesterol and other basic components in response to handling and hypoxic stress (Arends *et al.*, 1999; Skjervold *et al.*, 2001; Svobodova *et al.*, 2006). The levels of cortisol and glucose are considered to be specific indicators of sympathetic activation during stress conditions (Santos and Pacheco 1996, Svoboda *et al.*, 2001). Basic ecological factors, such as feeding regime and stocking density, also have a direct influence on certain biochemistry parameters (Wood *et al.*, 1960; Christofilogiannis, 1993; Coz-Rakovac *et al.*, 2005).

2.4 Female reproductive biology

Knowledge on reproductive biology of fish is essential for evaluating the commercial potentialities of its stock, life history, culture practice and management of its fishery (Islam *et al.*, 2012). Reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect to their gonadal conditions (Akter *et al.*, 2012). In order to achieve success in fish culture, it is important to assess the breeding cycle with fecundity of cultivable fishes. Knowledge on the fecundity of a fish species is important for determining: (a) spawning potential and its success (Das *et al.*, 1989; Karim and Hossain, 1992); (b) fluctuations in the egg production potential of individual stock related to life processes such as age and growth (Shaheena, 2012); (c) effects of environmental factors (Bromage *et al.*, 1992) and (d) formulating the commercial management of fishery (Lagler, 1956). Reddy (1979) mentioned that determination of breeding season is an essential part of biological investigations of fishes.

The 'Fecundity' of a fish is defined as the number of eggs that are likely to be laid during a spawning season (Bagenal, 1957). The reproductive potential, i.e., fecundity is an important biological parameter that plays a significant role in evaluating the commercial potentials of fish stocks (Gomez-Marquez, 2003). Successful fisheries management including practical aquaculture relies on having an accurate assessment of fecundity to understand the recovery ability of fish populations (Lagler, 1956; Nikolskii, 1969; Tracey *et al.*, 2007). The fecundity and its relation to female size make it possible to estimate the potential of egg output (Chondar, 1977) and the potential number of offspring in a season and reproductive capacity of fish stocks (Qasim & Qayyum, 1963). Alikunhi (1966) stated fecundity of the Common carp in the tropics has early maturity and year-round breeding; under temperate conditions there is a specific annual breeding cycle, during which the fish may spawn once or under exceptionally good

conditions, twice or thrice, while in the tropics a mature carp may spawn five or six times in the course of a year under optimum conditions and the absolute fecundity increases with each spawning. As a result, the number of eggs produced per fish per year is significantly higher in the tropics.

Fish species are known to exhibit wide variations in fecundity, even among individuals of the same species, size and range (Bagenal, 1957), which may be due to differential feeding success within the members of population prior to spawning (Bagenal, 1978) and probably due to release of the eggs in batches. Variation in fecundity may also be due to the existence of varied mixture of age classes (Saliu *et al*., 2007).

Nasar (1989) calculated the fecundity ranged from 118 to 687 in *Monopterus cuchia*. Narejo (2003) reported the fecundity of *M. cuchia* ranged from 260 to 5890 and *M. armatus* ranged from 580-10980 respectively. The authors also reported that average number of ova present per g of body weight were 4.61 and per g of ovary weight were 74.27 in case of *M. cuchia* and average number of ova present per g of body weight were 29.38 and per g of ovary weight were 407.57 in case of *M. armatus* respectively. Estimates of fecundity from mean counts of aliquots for an individual American eel (*Anguilla rostrata*) ranged from 1.84 million to 19.92 million eggs in the length range from 45.2 to 113.3 cm (Barbin and McCleave, 1997). Absolute fecundity of *C. carpio* varied from 3173 to 6,29,230 and the relative fecundity varied in the range of 21.00-223.00 with a mean value of 91.17 in Kashmir waters (Shafi *et al.,* 2012). Hailu *et al.* (2013) reported that absolute fecundity (F) of Common carp between 36,955 and 3,18,584 with a Mean±SD of 1,70,937±13,084 for the length group (270-470 mm FL). The total number of eggs in the ovaries of Common carp ranged from 75,645 and 3,56,743 with a mean of 2,10,538 and *C. carpio* (Lemna *et al.,* 2015). It was suggested that perhaps the variation was due to different temperature and feeding regimes, producing variation in fat metabolism (Svedang *et al.,* 1996). The productivity of local waters may thus produce variation in size at maturity and it

may also produce variation in size-fecundity relation. The difference in the fecundity estimation could be due to different environmental conditions in which the populations live. The fecundity also varied with the seasons, climatic conditions and environmental habitat, nutritional status and genetic potential (Bromage *et al*., 1992). Difference in fecundity estimates might be artificial (due to differences in techniques) or natural (due to differences in fecundity with geographic location or time). Fecundity of fishes also varies across species, and within the same species because of differences in age, body length and gonadal weight (Lagler, 1956).

The ovaries of two fish with same body weight, contained different number of eggs. Alam and Pathak (2010) also reported that two same size *L. rohita*, contained different number of eggs. If these two studies are considered carefully, it can be concluded that *L. rohita* can produce eggs at a small size. This variable fecundity may also be associated with genetic diversity in *L. rohita*, indicating that different strains mature and spawn at various body weight and size in its geographical range and is influenced by ecological factors. Lone and Hussain (2009) reported that, in fishes like *L. rohita*, water temperature, photoperiod and rainfall appear to affect growth and development of ovary.

Linear relationship exists between fecundity and fish length, fish weight, ovary length and ovary weight have been reported in different freshwater fish species by various workers (Singh and Srivastava, 1982; Sharaf *et al*., 1997; Somdutt and Kumar, 2004; Joshi, 2008 and Bahuguna and Khatri 2009). Fecundity of *L. rohita* was more closely related to the ovary weight (Alam and Pathak, 2010). Fecundity and ovary weight of *L. rohita* was strongly correlated to weight of fish than the length of fish and length of ova. According to Bhatt *et al.* (1977), fish fecundity is most accurately estimated by two morphometric variables taken together with less accuracy for length than weight variables. Moreover, gonad weight is more accurate for estimating fish fecundity than the two other variables (body length and weight). However, Bhatt *et al.* (1977) reported that it is

not realistic to use gonad weight to estimate fecundity based on live specimens under field conditions. Several studies reported that fecundity has a linear relationship with weight (Bhatt *et al.*, 1977; Bhargava, 1971; Pantulu, 1963; Qasim & qayyum, 1963). This suggests that the number of eggs in the ovaries increases in proportion to the body and gonad weight of the fish. Shafi *et al.* (2013) observed fecundity exhibited a straight line relationship with length and body weight. Fecundity and ovary weight also exhibited linear relationship.

The gonad weight—body weight ratio of the carp shows variations in different countries. In Russia, the ovaries constitute up to 15% of the body weight of the mature fish (Nikolsky, 1963) while in Japan it forms 3.1 to 16.4% of the body weight with an average of 10.4% (Matsui, 1957). The carp in India seems to have better developed ovaries and in healthy ripe females the gonads constitute 26 to 38% of the body weight of the fish. In comparison to the males of Indian and Chinese major carps (where the testes constitute less than 1% of the body weight), the testes in mature healthy carp in India are remarkably highly developed with abdomen bulging as in gravid females and constitute up to 20 to 30% of the body weight and is thus comparable to the proportionate body weight in females. This could be a peculiar feature of the species in tropical environment where, under favourable conditions, after attainment of maturity, the males continue to be in oozing condition (Alikunhi, 1966).

GSI increases with maturation of fish and is highest during spawning season and after spawning it declines (Lone and Hussain, 2009; Alam and Pathak, 2010). Lone and Hussain (2009) reported maximum GSI value (22.73±0.94) in June in *L. rohita*. Alam and Pathak (2010) observed the highest GSI value 7.5 in August in *L. rohita*. Dar *et al.* (2010) studied the spawning season of snow trout, *S. esocinus* from the river Jhelum of Kashmir and observed variation in seasonal fluctuation in GSI and concluded that in case of *S. esocinus* spawning occurred during the month of May and is continued till the month of June. GSI values were maximum during spawning season and declined during post spawning season.

Guha and Mukherjee (1991) studied seasonal changes in gonadosomatic index and gonadal histology of male and female adult *Cyprinus carpio* of the plains of West Bengal and reported 2 clear reproductive cycles in a year. Each reproductive cycle was divisible into 4 stages in both the sexes. In males the stages were growth, maturation, spawning and resting and in females, preparatory, pre spawning, spawning and post spawning. The spawning periods of this species were during January-February in winter cycle and July-August in summer cycle. Parameswaran (1972) while studying maturation, fecundity and breeding of the Chinese stock of the common carp, *Cyprinus carpio* var. *communis* in ponds at Cuttack, reported mature specimen of both the sexes throughout the year, being maximum during late January to March and July to August. Carp is a seasonal spawner in temperate waters and a perennial spawner in tropics (Hickling, 1962; Alikunhi, 1966). The fish may spawn two or three times in ponds, while in the wild condition it may spawn only once (Alikunhi, 1966).

Liao and Chang (2011) studied the reproductive biology of needle fish, *Tylosurus acus melanotus* in waters around Hsio-liu-chi Island of south western Taiwan, Oocyte development was categorized into eight stages based on histological examination of ovaries. Photoperiod and water temperature have been shown to correlate with gonadal weight and gonadosomatic index, and water temperature and long day length influence beginning and conclusion of spawning season in fish like major carps (Day *et al*., 2004, 2005; Bhattacharyya and Maitra, 2006; Mylonas and Zohar, 2007). Mahboob *et al.* (2012) studied the relationship of gonad weight & liver weight with body weight of six fish species viz; *Catla catla, Labeo rohita, Cirrhina mrigala, Hypophthlamichthys molitrix, Ctenopharyngodon idella and Cyprinus carpio* under the influence of artificial feed, broiler manure, buffalo manure, N:P:K (25:25:0). The positive relationship between gonad weight and body weight was significant which showed the dependence of gonadal development on body weight in all the six fish species. The correlation coefficients were higher in female fishes. However, the major

carps had a much smaller proportional gonadal weight as compared with Chinese carps and a common carp due to their faster growth rate. The maximum correlation value was observed with *H. molitrix*. The maximum Gonadosomatic Index (GSI) remained as 32.63 for *C. carpio* followed by *C. idella*

Shinkafi and Ipinjolu (2012) analysed gonadosomatic index, fecundity and egg size of the fish. Linear regression analysis was used to determine the relationship between GSI, fecundity and egg size with total length (TL), total weight (TW), gonad weight (GW) and gonad maturation stages (MS). Hulata *et al.* (1974) studied the relationship of gonads and eggs size to weight and age in the European and Chinese races of the common carp *Cyprinus carpio* L. and observed that (i) Gonad weight in both sexes was correlated with body weight, but proportional gonad weight, i.e. the ratio gonad weight divided by body weight, was independent of body weight, (ii) Proportional gonad weight had reached its maximum value in one year old males, but it more than doubled in two year old females, (iii) Egg size of two year old females was more than twice the size of eggs of one year old females, (iv) The European and Chinese races of carp differed in several aspects of gonad development. In particular, the Chinese carp matured earlier and their relative gonad sizes were considerably larger. Azarin *et al.* (2012) reported correlations between biochemical factors of blood with biological characteristics of gonad and some reproductive indices in Persian sturgeon, *Acipenser persicus*. Relationship between blood glucose and fluid egg rate, relative fecundity as well as gonadosomatic index was reverse ($p<0.05$). The relationship was the same between glucose and the number of eggs/gram of gonad ($p<0.05$). Cholesterol was positively correlated with weight, fork length, the number of larvae/gram and hatching success but these correlations were not significant.

Montchowuii *et al.* (2012) studied the gonad development of *Labeo parvus* and reported the five macroscopic stages of gonad maturation in the ovaries and testes. Oocyte development was subdivided into five stages, based on the presence

of chromatin, the nucleolus oocytes, perinucleolus oocytes, yolk vesicle oocytes, vitellogenic oocytes and mature oocytes. Comparisons of macroscopic and histological observations revealed that the temporal patterns of maturation activity obtained from visual examination closely reflected the seasonal histological trend in gonad development. Ozen (2012) studied the gonadal changes in dusky grouper, *Epinephelus guaza* in its natural environment in relation to sexual maturity. The study revealed four stages in gonadal development.

Sulikowski *et al*. (2005b) studied the reproductive cycle of the Thorny skate, *Amblyraja radiate* and observed a significant increase in GSI during certain months. Narejo *et al.* (2003) studied the reproductive biology of air breathing freshwater mud eel, *Monopterus cuchia* (Hamilton) and observed high GSI values during April-June for both the sexes. Kumar *et al.* (2003) studied the annual reproductive cycle of male rohu*, Labeo rohita* and observed that the testicular maturity and spermiogenesis during spawning phase seem to be correlated with the lowering of water temperature, attributable to rainfall. Yonada *et al.* (1998) examined the reproductive cycle and sexual maturity of the angler fish, *Liphiomus setigerus* and observed that spermatids were released from the germinal cysts into the lumen of the seminal lobules. Micale *et al.* (1987) studied the gonadal cycle of captive white bream, *Diploduss argus* and reported that spawning occurred in winter and the environmental factors have influenced the reproductive activity of the captive fish

Guler *et al.* (2006) observed that in endemic cyprinid species, *Chalcalburnus tarichi*, the granulose cells of postovulatory follicles produced steroid hormone and E_2 Induces spawning. They also reported that the E_2 and P_4 levels were lowest after spawning. Kucharcyzk *et al*. (2008) studied the reproductive parameters of Common carp (*Cyprinus carpio* L) spawners during natural season and out-of-season spawning and reported that there were no differences in the percentage of ovulation, spermatozoa motility and embryo survival to the eyed-egg-stage between the spawning periods. Fish spawned

during the season produced oocytes of higher total weight than those spawned out-of-season.

Kathiravelu *et al.* (2003) reported maturation and reproductive biology of female wild carp, *Cyprinus carpio*, in Victoria, Australi. Mean relative fecundity was 0.163 million eggs kg^{-1} whole weight. Egg size was proportional to maternal size but not age. Seasonal trends in Gonadosomatic indices, together with the changes in the macroscopic and microscopic condition of ovaries, demonstrated that spawning generally peaks during spring–early summer, but also occurs through until autumn and can even start in late winter at some sites. In Victoria, this species is a multiple spawner with asynchronous oocyte development and a protracted spawning season.

Assem *et al.* (2015) investigated some biological characters like oocyte diameter, fecundity, histological and ultra structural features of female *Mugil cephalus* ovaries collected from three different natural habitats: marine (MW), brackish (BW) and fresh water (FW). Monthly Gonadosomatic index (GSI) values clearly showed that the time period of reproductive activity in female *M. cephalus* from marine and brackish water habitats was from early September to late November. No peak value of GSI in females collected from freshwater was observed throughout the year.

Chapter-3

MATERIALS AND METHODS

The research work on the topic "Comparative study on spermiology and fecundity of *Cyprinus carpio* var. *communis* in farmed and wild conditions" was carried out in Fishery Biology Laboratory at Faculty of Fisheries, SKUAST-K, Rangil, Ganderbal. The study involved the following steps for meeting the various set objectives:

3.1 Source of brood stock

Two groups (both male and female) of Scale carp were taken for experiment: Group A, the cultured fish [(n= 60, male = 30, female = 30)]. These brooders were reared under captivity conditions in the hatchery of Faculty of Fisheries and Pandach fish farm (J&K state Govt. owned farm). Group B, the wild fish [(n = 60; male = 30; female = 30)]. These brooders were captured from the Dal Lake during the spawning season (Plate 1, 2).

3.2 Milt collection

Thirty mature wild male brooders (TW =462.5±393.06g; TL=311.1±77.12 mm and 30 farmed scale carp (TW = 293±270.84 g; TL=254.1±64.2 mm) were randomly selected for use as semen donors. Each male was stripped once only and the total amount of expressible milt was collected individually by gently pressing the abdomen. The semen was collected directly into clean 15 ml graduated centrifuge tubes (Plate 3). Care was taken to avoid the contamination of semen with water, urine, blood or faecal matter. The tubes were covered and immediately transported on ice (4°C) to the laboratory for analyses.

3.3 Estimation of physical parameters

3.3.1 Volume and pH

Milt volume was calculated by collecting the milt in 15 ml graduated centrifuge tube and pH was determined by Digital pH meter (pH ep® Hanna instruments, Italy).

Plate 1: Specimen of *Cyprinus carpio* var. *communis*

Site A

Site B

Site C

Plate 2: **Brood collection sites: Site-A: Pandach Fish Farm, Site-B: Dal Lake and Site-C: Faculty Fish Farm at Shuhama**

Plate 3: Milt collection from *Cyprinus carpio* var. *communis*

3.3.2 Sperm motility

Motility was evaluated using a light microscope (Olympus CX31) at 40X magnification and was expressed as percentage of motile spermatozoa. An activating solution of 0.3% NaCl was used to estimate motility. For the evaluation of motility, about 10 µl of semen was placed on a glass microscope slide and 100 µl of activation solution was added (Mims, 1991). Hematoxylin eosin stain was also used to identify the sperms under light microscope at 40X.

3.3.3 Spermatozoa density

Milt was diluted at ratio of 1:1000 with Hayem solution (5g $Na2SO_4$, 1g NaCl, 0.5g $HgCl_2$ and 200ml double distilled water) and mean spermatozoa count was calculated from three replicate samples for each fish at magnification of 40X. A haemocytometer counting chamber (Gem Industrial Corporation, Noida, India) was used to determine the spermatozoa density. A droplet of the diluted milt was placed on a haemocytometer slide (depth 0.1 mm) with a cover slip and counted using light microscopy. After 3-5 min (to allow sperm sedimentation), the number of spermatozoa was counted (Rainis *et al*., 2003).

3.3.4 Short-term storage of sperm

The semen was diluted at ratio of 1:3 with one of three extenders. Extender I contained 300 mM glucose solution as described by Tekin *et al.* (2003). Extender II contained 1% NaCl solution. Extender III contained 75 mM NaCl, 70 mM KCl, 2 mM $CaCl_2$, 1 mM MgSO4, 20 mM Tris (Modified ionic solution) as described by Lahnsteiner *et al.* (1998). The diluted sperm was packaged in small laboratory tubes. Undiluted semen samples were used as control. Experimental and control samples were stored at 4°C for 4 hours in a refrigerator. During refrigerated preservation, motility (%), motility durations (s) of stored sperm were evaluated at 1 hour intervals.

3.4 Estimation of biochemical parameters

Seminal plasma was collected after centrifugation of the semen at 4000 rpm for 10 min at room temperature (20°C) and stored in Eppendorf vials at-20°C until the beginning of analysis. Seminal plasma was centrifuged twice to avoid possible contamination with spermatozoa. Levels of the metabolites (glucose, protein, cholesterol, triglyceride and urea) were determined using spectrophotometer (systronic UV –VIS Spectrophotometer 117) and biochemical Kits procured from Coral clinical system as follows:

3.4.1 Glucose by Glucose Oxidase/Peroxidase (GOD/POD) method

Principle: Glucose is oxisidised to gluconic acid and hydrogen peroxidase in the presence of glucose oxidase. Hydrogen peroxidase further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

Procedure: 1000µl of working glucose reagent was pippetted into three test tubes marked as blank (B), standard (S) and test (T) followed by addition of 10µl of distilled water glucose standard and milt plasma to each of the test tube respectively. After mixing well, the samples were incubated at 37°C for 10 min in an incubator. The absorbance of the standard (Abs.S) and test sample (Abs.T) were measured against blank sample (Abs.B) in a spectrometer at 505nm within 60 min.

Addition sequence	B (ml)	S (ml)	T (ml)
Glucose reagent	1.0	1.0	1.0
Distilled water	0.01	---	--
Glucose Standard	--	0.01	--
Sample	--	--	0.01

Calculations: Total glucose concentration (mg/dl) was calculated using the formula:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

3.4.2 Cholesterol by cholesterol oxidase/phenol + aminophenazone (CHOD/ PAP) method

Principle: Cholesterol esterase hydrolyses esterfied cholesterols to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4-amminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

Procedure: 1000 µl of cholesterol reagent was added by 10 µl of serum, 10 µl of standard cholesterol and 10 µl of purified water to prepare test (T), standard (S) and blank (B) sample respectively. All the tubes were incubated at 37°C for 5 minutes in an incubator. The absorbances of test (Abs. T) and standard (Abs. S) samples were noted against blank sample (Abs. B) at 505nm in a spectrophotometer.

Addition sequence	**B (ml)**	**S (ml)**	**T (ml)**
Working reagent	1.0	1.0	1.0
Distilled water	0.01	--	--
Cholesterol standard	--	0.01	--
Sample	-	--	0.01

Calculations: Cholesterol in mg/dl was measured using the following calculations:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.4.3 Triglycerides by Glycerophosphate Oxidase-Peroxidase (GPO-PAP) method

Principle: Lipase hydrolyses triglycerides sequentially to Di and Monoglycerides and finally to glycerol. Glycerol Kinase (GK) using ATP as PO_4 source converts Glycerol liberated to Glycerol-3-Phosphate (G-3 Phosphate). G-3-Phosphate Oxidase (GPO) oxidises G-3-Phosphate & form dihydroxy acetone phosphate and hydrogen peroxide. Peroxidase (POD) uses the hydrogen peroxide formed, to oxidise 4 Aminoantipyrine and TOOS (N-ethyl-N-Sulphohydroxy propyl-m Toluidine) to a purple colored complex. The absorbance of the colored complex is measured at 546 nm which is proportional to triglyceride concentration.

Procedure: 1000 µl of enzyme reagent was added to three test tubes with 10 µl distilled water, standard reagent and milt serum samples to prepare blank, standard and test samples respectively. After incubation at 37°C for 15 minutes, the absorbance of standard (Abs. S) and milt serum (Abs. T) were measured against blank sample (Abs. B) at 546 nm in a spectrophotometer.

Addition sequence	B (ml)	S (ml)	T (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.01	--	--
Standard reagent	--	0.01	--
Sample	--	--	0.01

Calculations: Triglycerides (mg/dl) were calculated as follows:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.4.4 Urea by Modified Berthelot method

Principle: Urease hydrolyses urea to ammonia and CO_2. The ammonia formed further reacts with a phenotic chromogen and hypochlorite to form a green

colored complex. Intensity of the color formed is directly proportional to the amount of urea in the sample.

Procedure: The buffer reagent (1ml) and the enzyme reagent (0.01ml) were pipetted into three test tubes marked B, S, T for Blank, Standard and Test respectively. Distilled water, urea standard and milt plasma each in 10 µl quantity were added to each test tube respectively. After mixing and incubating at 37°C for 10 minutes, chromogen reagent (200 µl) was added to each test tube. The absorbance was measured in a spectrophotometer for the standard (Abs. S) and test (Abs. T) sample against the blank sample (Abs. B) at 570nm using spectrophotometer.

Addition sequence	**B (ml)**	**S (ml)**	**T (ml)**
Buffer reagent	1.0	1.0	1.0
Enzyme reagent	0.01	0.01	0.01
Distilled water	0.01	--	--
Urea standard	--	0.01	--
Sample	--	--	0.01
Mix well and incubate for 5min at 37°C			
Chromogen reagent	0.2	0.2	0.2

Calculations: Urea (mg/dl) was calculated as follows:

$$\text{Urea (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 40$$

3.4.5 Total protein by Biuret method

Principle: Cupric ions (Cu^{2+}) in the biuret reagent complex with the groups involved in the peptide bond. In the presence of alkaline media i.e. 3% NaOH and at least two peptide bonds a violet colored chelate is formed. Biuret also contains sodium potassium titrate which assists in Cu^{2+} complex formation and further

prevent their precipitation in an alkaline solution. The absorbance of the colored chelate is measured at 550nm. The colour intensifies from pink to reddish violet due to the complexity of the peptide bond in the protein.

Procedure: The biuret reagent was pipetted into three test tubes marked as B, S, T followed by addition of 20 µl distilled water, protein standard and milt plasma to blank, standard and test samples respectively. After mixing and incubating at 37°C for 10 minutes, the absorbance was measured in a spectrophotometer at 550 nm.

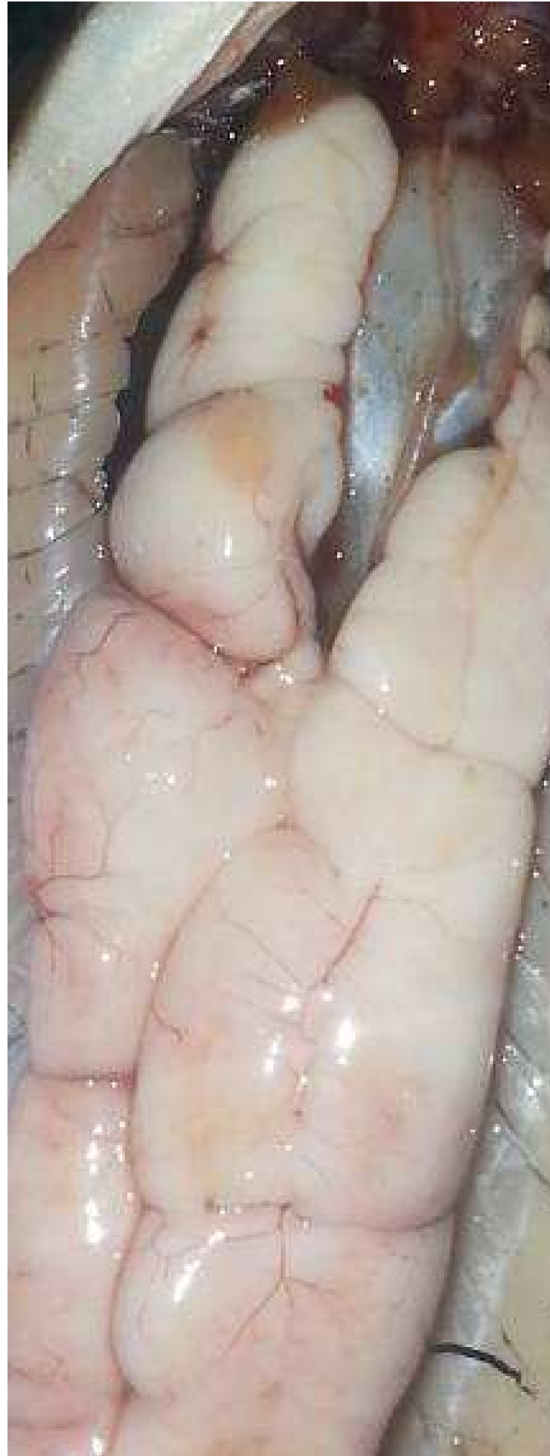
Addition sequence	**B (ml)**	**S (ml)**	**T (ml)**
Working reagent	1.0	1.0	1.0
Distilled water	0.02		
Standard	--	0.02	--
Sample	--	--	0.02

Calculations: Total serum protein (mg/dl) was estimated using the following calculations:

$$\text{Total protein (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 8$$

3.5 Fecundity

For the estimation of fecundity, fishes were sacrificed and both the ovaries were taken out carefully. The moisture was thoroughly wiped out from the ovaries with a blotting paper. The length and weight of ovaries was noted down with complete care (Plate 4). The collected ovaries were placed in 10% formaldehyde for at least 24 hours to bring hardness of eggs, so as to make easy and accurate calculation of sticky eggs. This was followed by drying of eggs on blotting paper for 1-2 hours, three subsamples of one gram each from anterior, middle and posterior region were weighed and then eggs were counted carefully by



(A) (B)

Plate 4 : Mature gonads of *Cyprinus carpio* var. *communis* (A) Ovary (B) Testis

gravimetric method. The mean numbers of eggs were multiplied by gonad parts of ovary weighed on a sensitive mono-pan weighing balance and the total number of eggs per gonad was obtained, i.e fecundity of fish. The absolute fecundity and relative fecundity was calculated as per the formula given by (Bagenal, 1978):

$$\text{Absolute fecundity} = \frac{\text{No. of ova in the subsample} \times \text{total ovary weight}}{\text{Weight of subsample}}$$

$$\text{Relative fecundity} = \frac{\text{Absolute fecundity}}{\text{Weight of fish}}$$

3.6 Gonadosomatic index

Gonadosomatic index (GSI) of the fish was calculated as per the formula given by (Desai, 1970):

$$\text{GSI} = \frac{\text{Weight of gonads (g)}}{\text{Total weight of fish (g)}} \times 100$$

3.7 Statistical analysis

The statistical analysis of the data was analysed out by using Microsoft Excel and SPSS windows.

Chapter – 4

EXPERIMENTAL FINDINGS

The results of the present study entitled “Comparative study on spermiology and fecundity of *Cyprinus carpio* var. *communis* in farmed and wild conditions” carried out during 2015 to 2016 are as under:

4.1 Spermiology of scale carp in wild and farmed conditions

4.1.1 Selection of male brooders

A total of 60 samples of male *Cyprinus carpio* var. *communis* were collected during the breeding season from the wild environment i.e., Dal Lake (N=30) and from the controlled environment i.e., Fisheries Farm, Faculty of Fisheries, Shuhama and from State Fisheries Department owned Fish Farm, Pandach (N=30).

4.1.2 Biometric parameters

The mean values±SD of biometric data recorded in the Scale carp in wild and farmed condition are listed in Table 1. The total length of fishes in wild ranged from 200-422 mm and in farmed condition from 190-420 mm. The Mean±SD of total Length in case of wild condition was 311.1±77.12 mm and in case of farmed conditions it was 254.1±64.2 mm. The fishes from wild ranged from 133-1350 gm in body weight and in farmed condition from 90-1180 g in body weight. The mean±SD of weight (gm) in case of wild condition was 462.5±393.06 and in case of farmed condition was 293±270.84 gm. The results showed statistically there were significant differences in total length between the two groups (wild and farmed) of Scale carp but non-significant difference in weight between the wild and farmed Scale carp. Scale carp in particular, showed the significantly higher length in wild condition than farmed condition. Weight was also found higher in wild condition but the difference was non-significant.

Table 1: Biometric parameters of Scale carp (male) in wild and farmed conditions

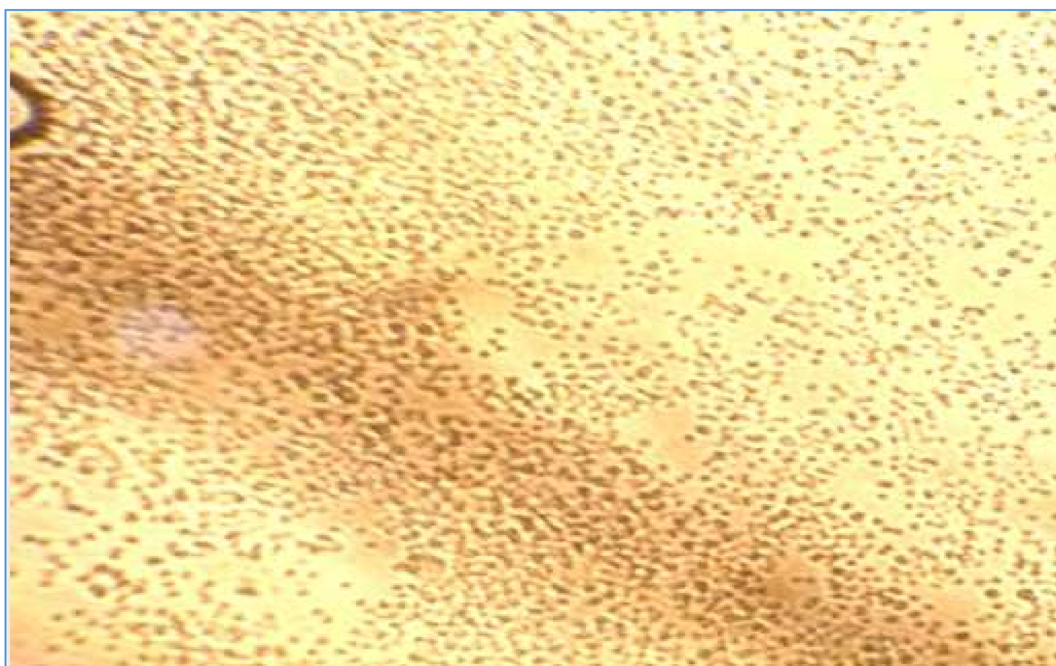
Scale carp	Total length (mm)		Body weight (gm)	
	Range	Mean±SD	Range	Mean±SD
Wild	200-422	311.1±77.12	133-1350	462.5±393.06
Farmed	190-420	254.1±64.2	90-1180	293±270.84
P value	<0.01		<0.05	

4.1.3 Estimation of physical parameters of milt

The collected milt was evaluated for various physical parameters viz., sperm volume, spermatozoa motility percentage, motility duration, spermatozoa density and pH (Plate 5 and 6).

4.1.3.1 Physical analysis

The summary statistics of physical parameters of milt of *Cyprinus carpio* var. *communis* in wild and farmed condition are presented in the Table 2. The range of the length (mm) and weight (gm) in wild condition was 200-422 mm with a mean value of 311.1 mm and 133-1350 gm with a mean value of 462.5 gm respectively while its range in farmed conditions was 190-420 mm with a mean 254±11.72 mm and 90-1180 gm with a mean 293.1±49.44 gm respectively. The range of the sperm volume (ml) in wild condition was 0.3-5.6 ml with a mean value of 2.39±0.29 ml while its range in farmed condition was 0.1-3 ml with a mean value of 1.48±0.17ml. The range of the motility rate in wild condition was 61.2-94% with a mean of 75.03±1.85% while its range in farmed condition was 44-89% with a mean of 68.9±2.27%. The range of the motility duration (sec) in wild condition was 20-85 sec with a mean of 50.36±2.54 sec while its range in farmed condition was 20-69 sec. with a mean of 44.66±2.46 sec. The range of the sperm density in wild condition was 3.165-3.98 $\times 10^9$/ml with a mean of 3.534±0.04 $\times 10^9$/ml while its range in farmed condition was 3.27-4.21$\times 10^9$/ml with a mean of 3.84±0.03$\times 10^9$/ml. The range of the pH in wild condition was 7.2-8.9 with a mean of 8.29±0.09 while its range in farmed condition was 7.9-8.9 with a mean of 8.5±0.05.



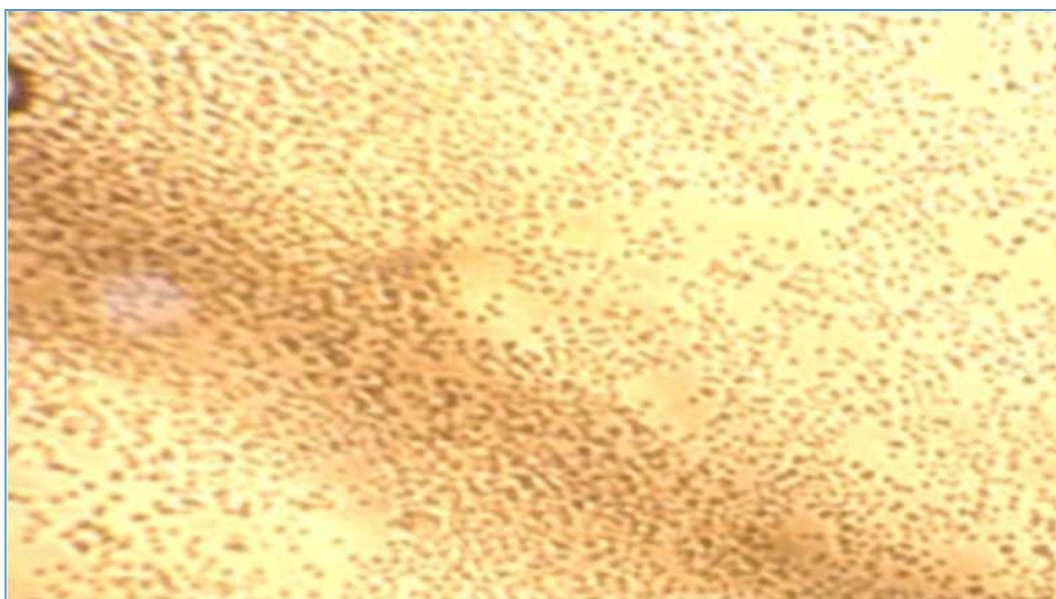
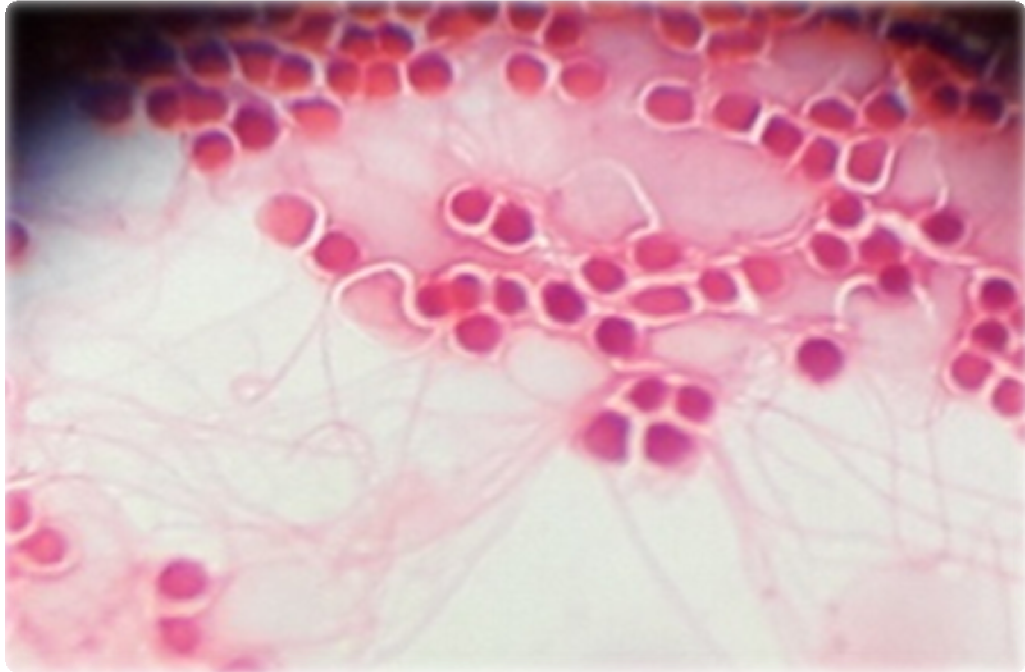
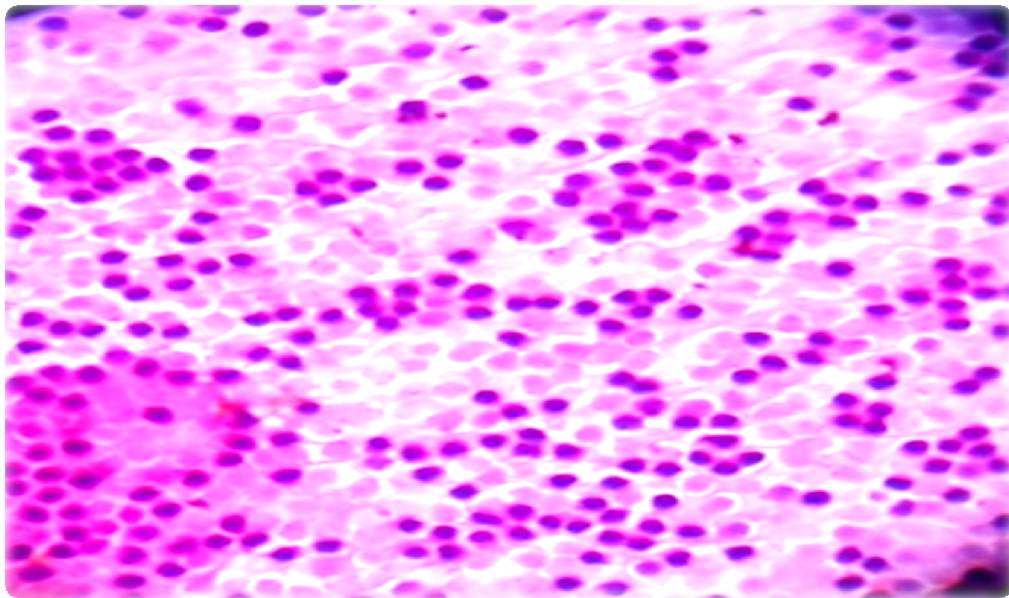


Plate 5 : Spermatozoa of *Cyprinus carpio* var. *communis* under light microscope at 40 X



(A)



(B)

Plate 6: A: Spermatozoa stained with Hematoxylin Eosin under light microscope at 40X. B: Matured spermatozoa with prominent tails

Table-2: Brood size and sperm biology of Scale carp in wild and farmed conditions

Parameter	Habitat	Range	Mean	Standard error
Length (mm)	Wild	200-422	311.1	14.08
	Farmed	190-420	254.1	11.72
Weight (gm)	Wild	133-1350	462.5	71.76
	Farmed	90-1180	293.1	49.44
Sperm volume (ml)	Wild	0.3-5.6	2.39	0.29
	Farmed	0.1-3	1.48	0.17
Motility (%)	Wild	61.2-94	75.03	1.85
	Farmed	44-89	68.9	2.27
Motility duration (seconds)	Wild	20-85	50.36	2.54
	Farmed	20-69	44.66	2.46
Sperm density($\times 10^9$/ml)	Wild	3.16-3.98	3.53	0.04
	Farmed	3.27-4.21	3.84	0.03
pH	Wild	7.2-8.9	8.29	0.09
	Farmed	7.9-8.9	8.5	0.05

The mean values and standard deviation of the physical parameters of the milt of *Cyprinus carpio* var. *communis* in wild and farmed conditions is presented in Table 3 and Figures (1-4). In case of wild conditions, sperm volume, motility rate, duration, sperm density and pH were 2.393±1.64 ml, 75.038±10.162 percent, 50.367±13.92 sec, 3.53±0.272 $\times 10^9$/ml and 8.29±0.494 respectively. In case of farmed condition mean±SD of sperm volume, motility rate, duration, sperm density and pH were 1.486±0.88 ml, 68.9±12.46%, 44.66±13.48 sec, 3.84±0.181$\times 10^9$ and 8.5±0.311 respectively. Statistical analysis revealed non significant difference in motility duration and pH between the two environments. The level of sperm volume, and motility (%) between the two ecotypes of the fish were significantly ($P<0.05$) different. The wild fish was having higher sperm volume and motility percentage than that of farmed fish. In addition, the sperm density in the cultured population was significantly higher ($P<0.01$) than that in the wild type.

Table 3: Statistical analysis of physical parameters of the milt of *Cyprinus carpio* var. *communis* in wild and farmed conditions

Parameters	Mean±SD		t value	P value
	Wild	Farmed		
Sperm volume (ml)	2.393±1.64	1.486±0.88	2.625	<0.05
Motility (%)	75.038±10.162	68.9±12.46	2.0899	<0.05
Duration(sec)	50.367±13.92	44.66±13.48	1.61	>0.05
Sperm density ($\times 10^9$/ml)	3.534±.272	3.84±0.181	-5.148	<0.01
pH	8.29±.494	8.5±0.311	-1.905	>0.05

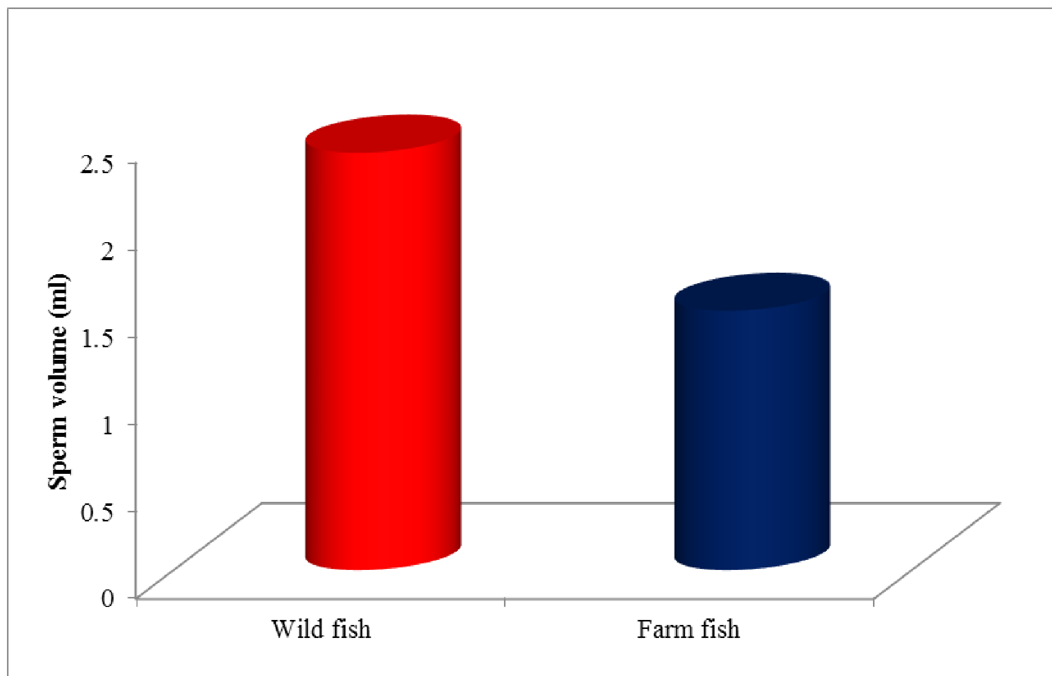


Fig. 1: **Relative (mean) sperm volume of *Cyprinus carpio* var. *communis* in wild and farmed conditions**

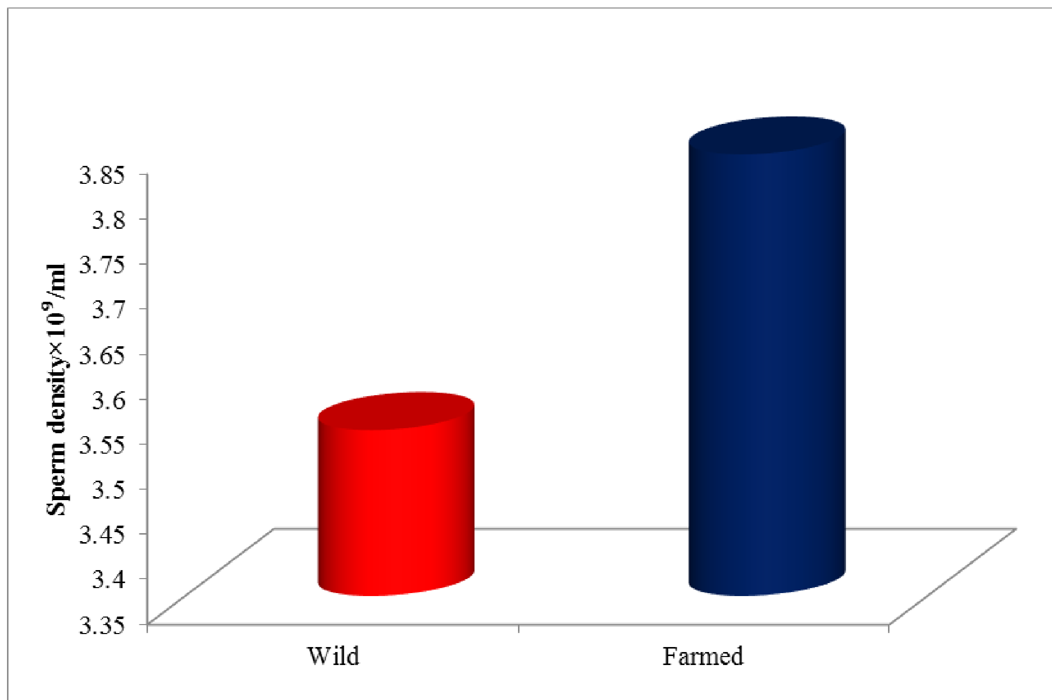


Fig. 2: **Relative (mean) sperm density of *Cyprinus carpio* var. *communis* in wild and farmed conditions**

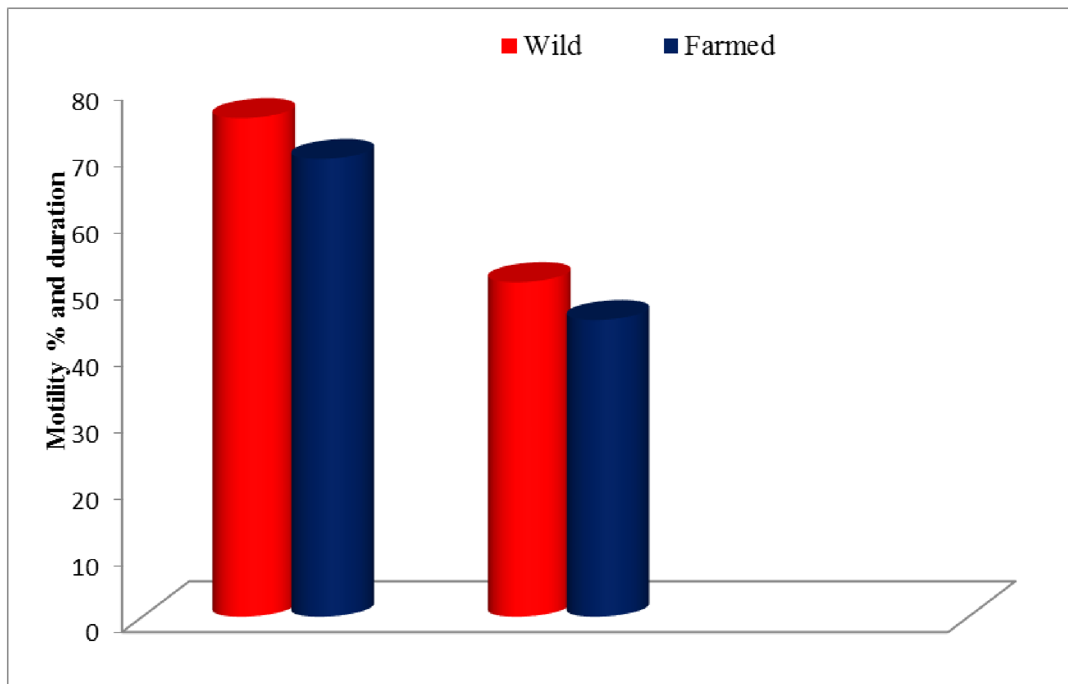


Fig. 3: Relative (mean) motility% and duration of *Cyprinus carpio* var. *communis* in wild and farmed condition

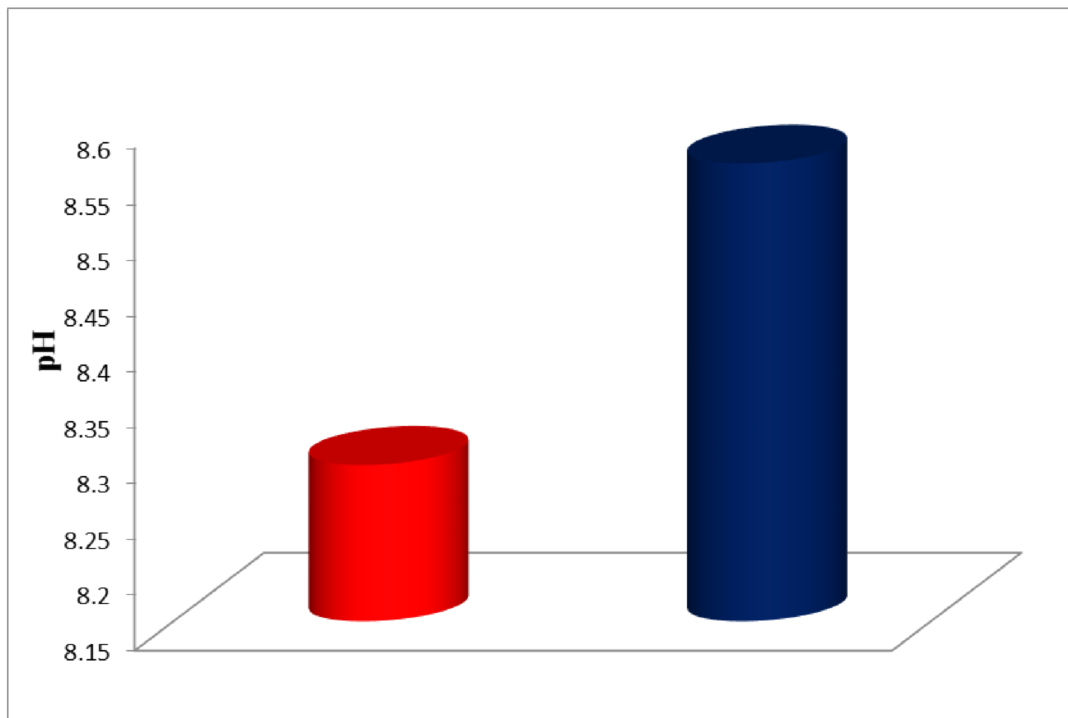


Fig. 4: Relative mean values of seminal pH of *Cyprinus carpio* var. *communis* wild and farmed condition

4.14 Biochemical analysis of milt parameters and the gonad biology.

The mean values, standard errors and ranges of the physical parameters are summarized in Table 4. The range of the glucose (mg/dl) was 35-81 in wild and 71-99 in farmed condition. The Mean±SE value of glucose was 70.64±1.96 mg/dl in wild and 85.06±1.69 mg/dl in farmed condition. The range of the protein (g/dl) was 0.14-4.23 in wild and 0.12-1.9 g/dl in farmed condition. The Mean±SE value of protein was 1.99±0.21 mg/dl in wild and 0.917±0.11 mg/dl in farmed condition. The range of the triglyceride (mg/dl) was 4.3-21 in wild and 4-20 in farmed condition. The Mean±SE value of triglyceride was 12.99±1.023 mg/dl in wild and 12.307±0.95 mg/dl in farmed condition. The range of the cholesterol (mg/dl) was 4.89-6.99 in wild and 3.1-9 in farmed condition. The Mean±SE value of cholesterol was 5.98±0.11 mg/dl in wild and 5.53±0.29 mg/dl in farmed condition. The range of the urea (mg/dl) was 15-38.33 in wild and 21.89-45 in farmed condition. The Mean±SE value of urea was 25.22±0.73 mg/dl in wild and 28.49±1.05 mg/dl in farmed condition.

Comparative statistical analysis of biochemical parameters of milt between the wild and farmed Scale carp are given in Table 5 and Figure 5. In case of wild condition mean±SD of glucose was 70.64±10.75 mg dl^{-1}, protein was 1.99±1.15 g dl^{-1}, triglyceride was 12.99±5.6 mg dl^{-1}, cholesterol was 5.98±.629 mg dl^{-1}, urea was 25.22±4.047 mg dl^{-1}. In case of farmed condition mean±std of glucose was 85.06±9.29 mg dl^{-1}, protein was 0.917±0.62 g dl^{-1}, triglyceride was 12.3±5.22 mg dl^{-1}, cholesterol was 5.53±1.61 mg dl^{-1} and urea was 28.4±5.75 mg dl^{-1}. The levels of glucose, urea in farmed Scale carp were significantly higher ($P<0.05$) than those in the wild scale carp. In contrast, the concentration of total protein in cultured individuals was significantly lower than that in their wild counterpart ($p<0.05$), Cholesterol, triglyceride though higher in wild condition displayed insignificant variation between two environs.

Table 4: **Biochemical parameters of milt in wild and farmed Scale carp**

Parameters	Type	Range	Mean	Standard Error
Glucose (mg/dl)	Wild	36-81	70.64	1.96
	Farmed	71-99	85.06	1.69
Protein (mg/dl)	Wild	0.14-4.23	1.99	0.21
	Farmed	0.12-1.9	0.917	0.11
Triglyceride (mg/dl)	Wild	4.3-21	12.99	1.023
	Farmed	4-20	12.307	0.95
Cholesterol (mg/dl)	Wild	4.89-6.99	5.98	0.114
	Farmed	3.1-9	5.53	0.29
Urea (mg/dl)	Wild	15-38.33	25.22	0.73
	Farmed	21.89-45	28.49	1.05

Table 5: Comparative statistical analysis of the biochemical milt parameters of *Cyprinus carpio* var. *communis* in wild and farmed conditions

Parameters	Mean±SD		t value	P value
	Wild	Farmed		
Glucose (mg dl^{-1})	70.64±10.75	85.06±9.29	-5.55	<0.01
Protein (g dl^{-1})	1.99±1.15	0.917±0.62	4.48	<0.01
Triglyceride (mg dl^{-1})	12.99±5.6	12.3±5.22	0.494	>0.05
Cholesterol (mg dl^{-1})	5.98±0.629	5.53±1.61	1.4207	>0.05
Urea (mg dl^{-1})	25.22±4.047	28.4±5.75	-2.549	<0.05

4.2 Male gonad weight and GSI

In case of wild condition male gonad weight (gm) ranged from 8-74 with a mean±SD values of of 32.33±23.23. In farmed conditions, the male gonad weight (gm) ranged from 3-45 gm with a mean values of 17.21±12.4. The range of the male GSI in wild condition was 4.44-12 with a mean values of 7.403±2.01 while its range in farmed condition was 3.12-9.03 with the mean±SD as 6.37±1.5. The data reveals that male gonad weight showed highly significant difference between the wild and farmed group ($p<0.01$). Also significant difference were found in the male GSI ($p<0.05$) between the two groups (Table 6).

Table 6: Comparison of weight of testis and GSI between the wild and farmed Scale carp

Parameters	Wild		Farmed		t value	P value
	Range	Mean±SD	Range	Mean±SD		
Weight of testis (gm)	8-74	32.33±23.3	3-45	17.21±12.4	3.144	<0.01
GSI	4.44-12	7.403±2.01	3.12-9.03	6.37±1.5	2.2	<0.05

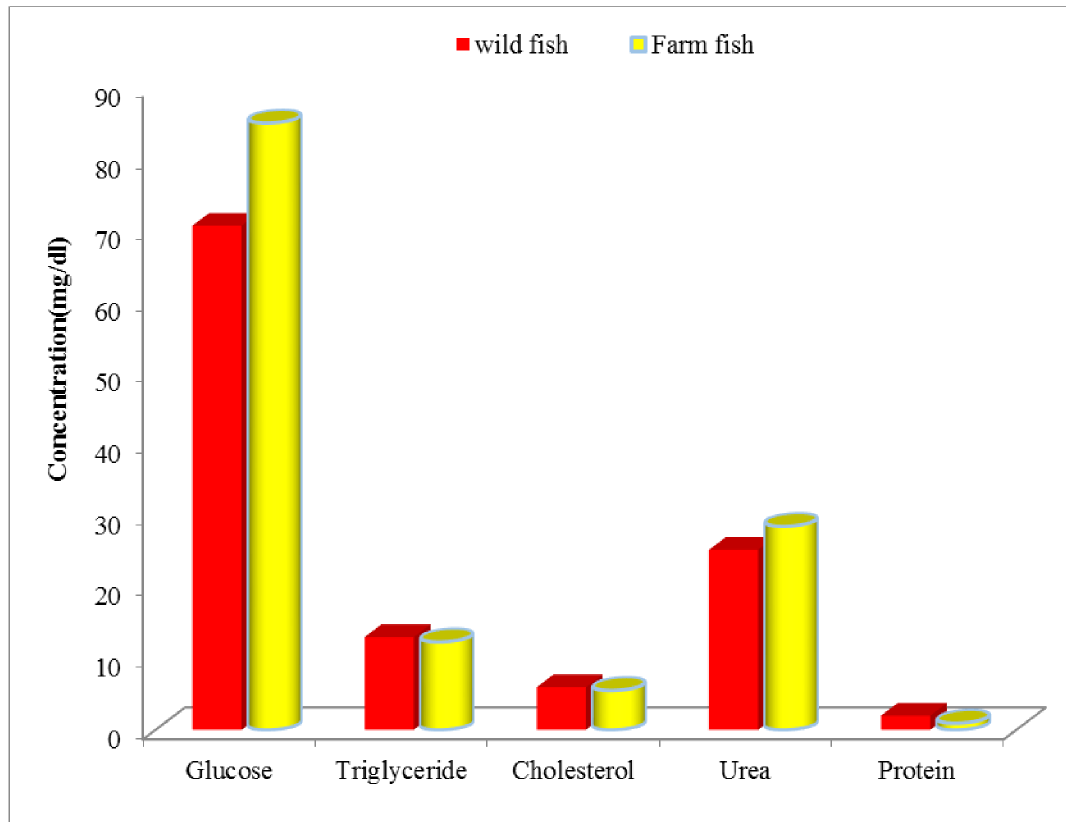


Fig. 5: Mean values of biochemical parameters of wild and farmed *Cyprinus carpio* var. *communis* during the reproductive season

4.3 Short term preservation of Scale carp sperm

4.3.1 Post-activation motility

Data on the effect of extenders and storage periods on motility rate are given in Table 7. Results of the effect of extenders and storage periods on motility rate, motility durations are given in Table 7 and 8. Spermatozoa motility percentage decreased significantly with increasing storage period in diluted and undiluted sperm during spawning season. When the effect of extenders were tested, the best results (74% motility) were achieved with extender I containing glucose solution at the end of 0 hours storage periods. On the contrary, the lowest motility (4.66%) was obtained with control group at 4 hour storage periods. Table 7, shows that statistically there was a significant difference in motility rate in extenders, extender 1, extender 2, extender 3 and no extender at storage time 0, 1, 2, 3 and 4 hr. The maximum value of extender 1 was at 0 hr and minimum value was at 4 hr storage period indicating significant decrease in motility percentage from 0 hr storage to 4 hr storage. The decrease in motility (%) was also found in extender 2, extender 3 and no extender from 0 hr storage to 4 hr storage. At 0 hr storage the maximum value of motility rate (74%) was of extender 1 and minimum value (53.66%) was of extender 3. At 1 hr storage, the maximum value (56.33%) was of extender 1 and minimum value (42.66%) was of extender 2. The maximum value (40%) at 2 hr storage was of extender 1 and minimum value (31%) was that of control (undiluted milt). The maximum value (30%) at 3 hr storage was of extender 1 and minimum value (15%) was of extender control (undiluted milt). The maximum value (14.33%) at 4 hr was of extender 1 and minimum value (4.66%) was of control (undiluted milt). The Extender 1 showed better performance of motility rate at all storage periods with a mean value of 42.9% followed by Extender 2 with a mean value of 35.66%.

Table 7: Effect of extenders and storage periods on motility rates of Scale carp sperm during spawning season

Storage period (hour)	Extender (%)			Control (sec)	Mean (sec)
	1	2	3		
0	74.00	66.33	53.66	69.66	65.91
1	56.33	42.66	44.666	50.33	48.50
2	40.00	35.00	38.66	31.00	36.16
3	30.00	25.33	29.66	15.00	25.00
4	14.33	9.00	10.00	4.66	9.50
Mean	**42.90**	**35.66**	**35.33**	**34.133**	

CD at 5% level of significance; Storage = 2.7435; Extender =2.4538; Storage × Extender = 5.487

4.3.2 Post-activation motility durations

Data on the effect of extenders and storage periods on motility durations are given in Table 8. In case of testing extenders, the highest motility duration (54 sec) was achieved with extender 1 at 0 hours storage periods during spawning season. On the contrary, the lowest motility duration of 5 sec was observed with extender 3 at 4 hour storage period. Decrease in motility duration is significantly important with increasing storage period in diluted and undiluted sperm during spawning season, differences among storage hours and extenders were statistically significant during experiment. The maximum value (54 sec) of extender 1 was at 0 hr and minimum value (16.33 sec) at 4 hr storage indicated significant decrease in motility duration with storage. The decrease in motility duration was also observed in extender 2, extender 3 and control (undiluted milt) from 0 to 4 hr storage. The maximum value (56 sec) of motility duration at 0 hr was that of

extender 2 and minimum value (42 s) was that of extender 3. The maximum value (48 sec) at 1 hr was that of extender 1 and minimum value (33 sec) was that of extender 3. The maximum value (31 sec) at 2 hr was that of extender 1 and minimum value (24 sec) was that of extender 3. The maximum value (21 sec) at 3 hr was that of extender 1 and minimum value (11.66 sec) was that of control (undiluted milt). The maximum value (16.33 s) at 4 hr was that of extender 1 and minimum value (5 sec) was that of extender 3. The extender 1 shows better performance of motility duration at all storage periods with a mean value of 34.067 sec of followed by extender 2 with a mean value 29.22 sec, control (undiluted milt) with a mean value of 26.133 sec and extender 3 with a mean value 24.33 sec.

Table 8: Effect of extenders and storage periods on motility duration of scale carp carp sperm during spawning season

Storage period (hour)	Extender (sec)			Control (sec)	Mean (sec)
	1	2	3		
0	54.00	56.00	42.00	46.00	49.50
1	48.00	42.00	33.00	39.00	40.50
2	31.00	27.00	24.00	25.00	26.75
3	21.00	13.00	19.66	11.66	16.33
4	16.33	8.00	5.00	9.00	9.588
Mean	**34.067**	**29.22**	**24.733**	**26.133**	

CD at 5% level of significance; Storage = 3.3828; Extender =3.0257; Storage X Extender = 6.766

4.4 Relationship between body weight, body length and spermatological properties of Scale carp semen in wild and farmed conditions

Correlation between the body weight, body length and spermatological properties of scale carp semen in case of wild condition are presented in Table 9. The Table reveals that length has significant positive correlation with weight and sperm volume (r=0.857, p<0.01, r=0.663, p<0.01). Significant positive correlation were also found between fish weight and sperm volume (r=0.637, p<0.01), volume and motility (r=0.523, p<0.01), motility and sperm density (r=0.716, p<0.01), motility and protein (r=0.505, p<0.01), motility and triglyceride (r=0.501, p<0.01), motility and pH (r=0.549, p<0.01) motility duration and pH (r=0.436, p<0.05), motility duration and protein (r=0.01, p<0.05). On the other hand sperm density has significant negative correlation with fish length (r=-0.822, p<0.01), fish weight (r=-0.754, p <0.01) sperm volume (r=-0.405, p <0.01). Significant positive correlation was found between the fish weight and testis weight(r=0.986, p<0.01), fish length and testis weight (r=0.870, p<0.01), testis weight and sperm volume (r=0.878, p<0.01).

Correlation between the body weight, body length and spermatological properties of Scale carp semen in case of farmed condition are presented in the Table 10. The Table reveals that significant positive correlation of fish length with fish weight (r=0.965, p<0.01) and sperm volume, (r=0.864, p<0.01). Negative significant correlation was observed between fish length and sperm density (r=-0.593, p<0.01). Fish weight shows significant positive correlation with sperm volume, (r=0.778, p<0.01) and a negative significant correlation was observed between fish weight and sperm density (r=-0.526, p<0.01). Sperm volume was positively correlated with motility (r=0.487, p<0.01) and negatively correlated with sperm density (r=-0.710, p<0.01) and correlation was statistically significant. Significant positive correlation was found between motility and protein (r=0.094, p<0.01), motility and triglyceride (r=0.433, p<0.01). Motility duration also showed significant positive correlation with pH and protein (r=0.430, p<0.05; r=0.051, p<0.05 respectively). Significant positive correlation was found between the fish weight and testis weight (r=0.943, p<0.01) fish length and testis weight (r=0.963, p<0.01) testis weight and sperm volume (r=0.833, p<0.01), testis weight and sperm density(r=0.528, p<0.01).

Table 9: Pearson correlation between body weight, length and sperm physical and biochemical parameters in wild conditions

	Fish Length	Fish Weight	Sperm Volume	Motility	Duration	Sperm Density	pH	Glucose	Protein	Triglycerides	Cholesterol	Urea	Testis weight	GSI
Fish Length	1	.857**	.663**	-.968	-.177	-.822**	.524	.750	.497	.888	.215	.282	.870**	-.193
Fish Weight		1	.637**	-.781	-.202	-.754**	.419	.545	.372	.671	.192	.265	.986**	-.281
Sperm Volume			1	.523**	-.215	-.405**	.530	.727	.443	.856	.153	.229	.878**	-.227
Motility				1	-.161	.716**	.549''	.773	.505**	.501**	.2448*	-.311	.246	-.189
Duration					1	.304	.436*	-.268	.01*	-.193	.013	.106	0.774**	-.193
Sperm Density						1	-.566	-.851	-.455	-.895	-.155	-.236	.421	.087
pH							1	.599	.296	.503	.316	.164	0.583	-.058
Glucose								1	.281	.815*	.037	.174	0.345	-.217
Protein									1	.363	.491	-.061	0.567	..231
Triglycerides								.		1	.163	.246	0.123	-.012
Cholesterol											1	.523	0.298	-.289
Urea					*							1	.178	-.85
Testis weight													1	.38
Gsi														1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 10: Pearson correlation between body weight, length and sperm physical and biochemical parameters in farmend conditions

	Fish Length	Fish Weight	Sperm Volume	Motility	Duration	Sperm Density	pH	Glucose	Protein	Triglycerides	Cholesterol	Urea	Testis weight	Gsi
Fish Length	1	.965**	.864**	-.437	.194	-.593**	-.128	.782	.708	.845	.849	.090	.963**	--.291
Fish Weight		1	.778**	-.398	.151	-.526**	-.060	.715	.657	.756	.797	-.010	.943**	-.352
Sperm Volume			1	.487**	.251	-.710**	-.233	.699	.625	.882	.805	.149	.833**	-.185
Motility				1	.099	-.277	.367**	.380	.094**	.433**	.635	-.361	.396	-.171
Duration					1	-.166	.430*	.186	.051*	.218	.271	.280	.097	-.071
Sperm Density						1	.209	-.568	-.52'5	-.635	-.454	-.170	.528**	.271
pH							1	.131	.041	.412	.012	.111	.052	.096
Glucose								1	.608	.686	.708	.334	.763	-.168*
Protein									1	.691	.475	.018	.701	-.219
Triglycerides										1	.754	.149	.456	-.075
Cholesterol											1	.286	.011	-.145
Urea												1	.232	-.110
Testis weight													1	-0.113
Gsi														1

**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

4.5 Female reproductive characteristics

4.5.1 Selection of female brooders

A total of 60 samples of female *Cyprinus carpio* var. *communis* were collected during the breeding season from the wild environment i.e. Dal Lake (N=30) and from the controlled environment i.e., Fisheries Farm, Faculty of Fisheries, Shuhama and from State Fisheries Department owned Fish Farm, Pandach (N=30).

4.5.2 Body weight, body length and ovary weight of *Cyprinus carpio* var. *communis*

The range, mean value, and standard error of body weight, body length and ovary weight are given in the Table 11**.** The range of the body weight (g) was 120-1300 gm in wild and 126-1236 gm in farmed conditions. The mean±SE value of body weight in wild was 435.1±66.61gm and in farmed it was 375.2±47.71 gm. The range of the total length was 180-430 mm in wild and in 150-420 mm in farmed condition. The mean±SE value of total length in wild was 301.43±13.93 mm and 280.76±13.6 mm in farmed condition. The range of the ovary weight was 15-86gm in wild and 3-75gm in farmed condition. The mean±SE value of ovary weight in wild was 48.96±4.15 gm and 33.68±3.95 gm in farmed condition.

4.5.3 Gonadosomatic index and fecundity of *Cyprinus carpio* var. *communis*

The range, mean value and standard error of Gonadosomatic index and Fecundity are given in the Table 11. The range of the number of ova was 1110-1545 in wild and 987-1590 in farmed condition. The mean±SE value of no. of ova in wild condition was 1362.5±19.38 and 1339±27.03 in farmed condition. The range of the absolute fecundity was 16650-129000 in wild and 4032-105000 in farmed condition. The mean±SE value of absolute fecundity in wild was 68864.07±6563.59 and 44533±5216.6 in farmed condition. The range of the relative fecundity was 99.2-240.8 in wild and 32-186.35 in farmed condition. The mean±SE value of relative fecundity in wild was 185.96±8.34 and 115.98±8.99 in

farmed condition. The range of the GSI was 6.6-18.42 in wild and 2.3-12.9 in farmed condition. The mean±SE value of GSI in wild was 13.77±0.658 and 8.6±0.643 in farmed condition.

Table 11: Reproductive biology of female Scale carp in farmed and wild condition

Parameters	Type	Range	Mean	Standard error
Weight (gm)	Wild	120-1300	435.1	66.61
	Farmed	126-1236	375.2	47.71
Length (mm)	Wild	180-430	301.43	13.93
	Farmed	150-420	280.76	13.6
Ovary weight (gm)	Wild	15-86	48.96	4.15
	Farmed	3-75	33.68	3.95
No. of ova	Wild	1110-1545	1362.5	19.38
	Farmed	987-1590	1339	27.03
Absolute fecundity	Wild	16650-129000	68864.07	6563.59
	Farmed	4032-105000	44533	5216.6
Relative Fecundity	Wild	99.2-240.8	185.96	8.34
	Farmed	32-186.35	115.98	8.99
GSI	Wild	6.6-18.42	13.77	.658
	Farmed	2.3-12.9	8.6	0.643

In case of wild fish the mean Gonadosomatic index was 13.7±3.605 (Table 12; Fig 6-9). The number of eggs/gm ovary was 1362.6±106.18. Absolute fecundity was 68864±35950.29 and relative fecundity was 185.96±45.7. However, in case of farmed condition mean GSI was 8.6±3.52. No. of ova/gm of ovary was 1339±148.28. Absolute fecundity was 44533±28572.59 and relative fecundity was 115.98±49.25. The data reveals that statistically there was a significant difference found in reproductive parameters in terms of absolute fecundity, relative fecundity, ovary weight and GSI ($p<0.01$).

Table 12: Statistical analysis of reproductive parameters of female Scale carp in farmed and wild conditions

Parameters	Habitat (Mean±SD)		t value	P value
	Wild	Farmed		
Weight (gm)	435.1±364.889	375.2±261.32	0.731	>0.05
Length (mm)	301.43±76.32	280.77±74.51	1.0612	>0.05
Ovary weight (gm)	48.96± 22.745	33.68±21.65	2.6	<0.01
No. of ova	1362.6±106.18	1339±148.28	0.707	>0.05
Absolute fecundity	68864±35950.29	44533±28572.59	2.85	<0.01
Relative fecundity	185.96±45.7	115.98±49.25	5.7	<0.01
GSI	13.7±3.605	8.6±3.52	5.5	<0.01

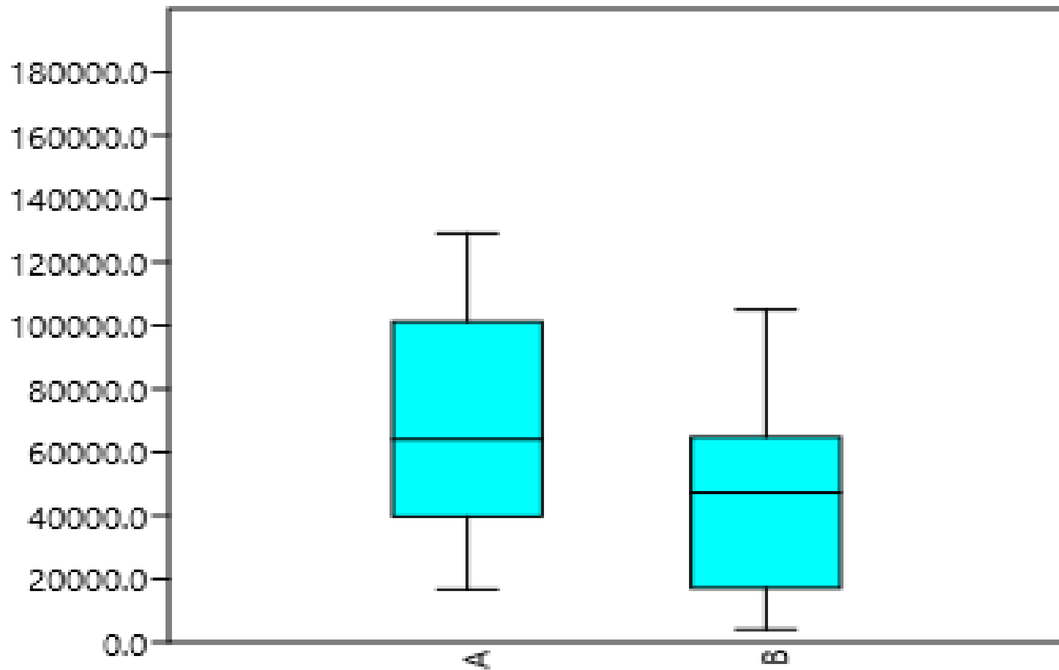


Fig. 6: Box plot depicting the Absolute Fecundity of wild (A) and farmed (B) *Cyprinus carpio* var. *communis*

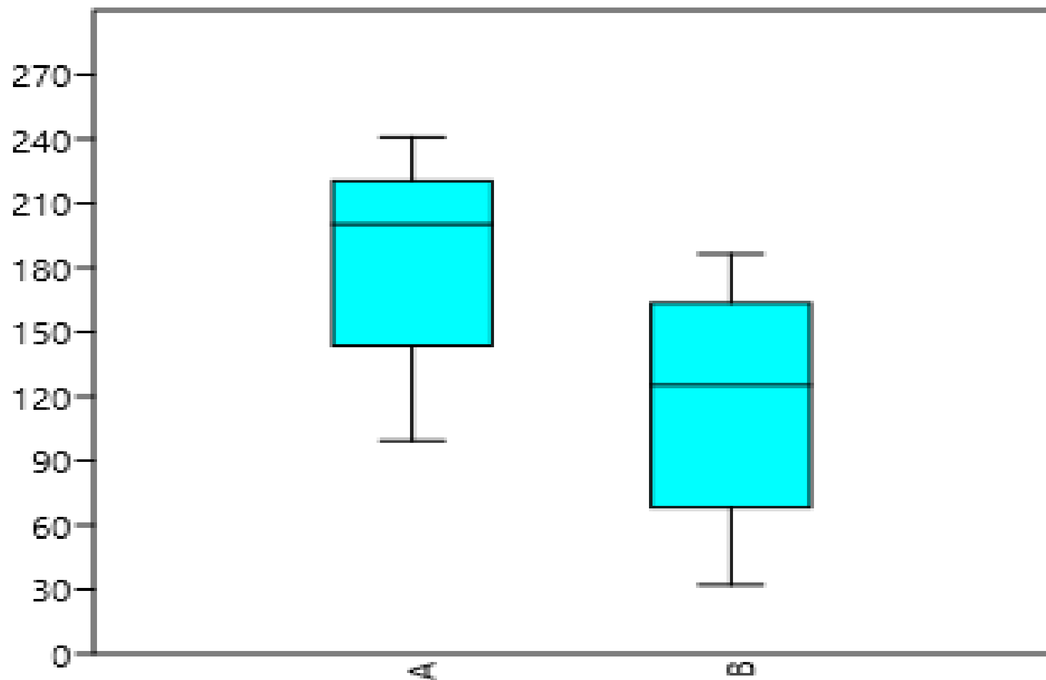


Fig. 7: Box plot depicting the Relative Fecundity of wild (A) and farmed (B) *Cyprinus carpio* var. *communis*

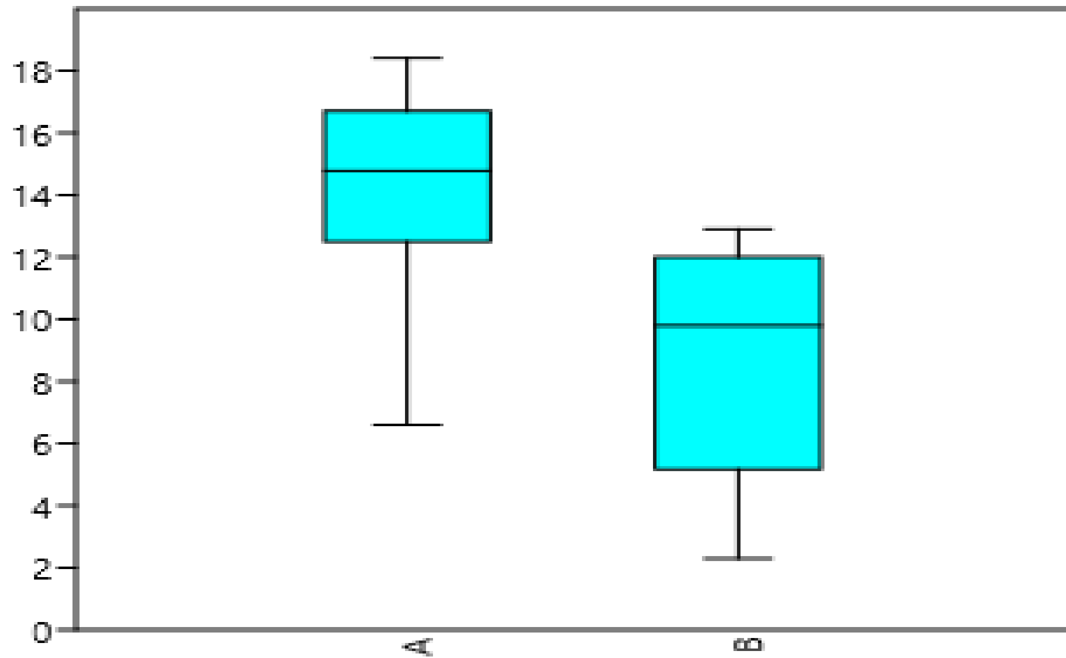


Fig. 8: **Box plot depicting GSI of wild (A) and farmed (B) *Cyprinus carpio* var. *communis* (female)**

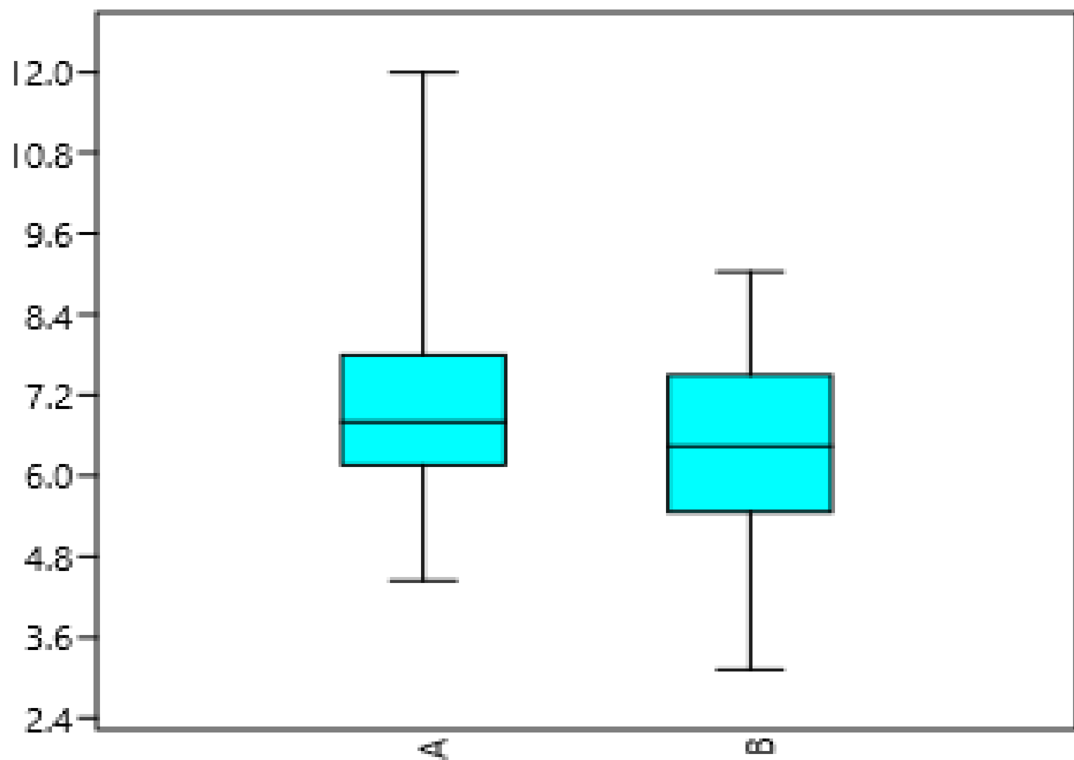


Fig. 9: **Box plot depicting GSI of wild (A) and farmed (B) *Cyprinus carpio* var. *communis* (male)**

4.6 Monthly variation in Gonadosomatic index (GSI) of C*yprinus carpio* var. *communis*

During present study the monthly variation of GSI of male and female specimens of C*yprinus carpio* var. *communis* in both wild and farmed conditions are given in (Table 13 and 14) and (Figures 10 and 11). In wild females, the Gonadosomatic index (Go.S.I) showed a gradual rise from February (6.82±1.008) to May (16.45±0.55). GSI fluctuated from a minimum of 4.124±1.03 in June to a maximum of 16.45±0.55 in the month of May. From May to June the value of GSI showed a decreasing trend. Similar trend was followed by farmed females where the GSI was found to be minimum in the month of June (3.286±1.2) and maximum in the month of May (12.32±1.50). The data reveals that significant difference in female GSI occurred in the month of January, February, March, April and May.

In case of wild males the minimum and maximum values of GSI were 2.36±0.85 and 11.21±1.22 respectively. In case of farmed males the minimum value was 1.99±0.74 and maximum value of GSI was 8.5±2.50. The data reveals that significant difference in male GSI was found in the month of January, March and May.

Table-13: Monthly variation in female Gonadosomatic index (GSI) of *Cyprinus carpio* var. *communis* in wild and farmed conditions

Month	Habitat		P value
	Wild	Farmed	
December	9.314±.64	7.87±1.63	>0.05
January	7.154±2.144	4.36±1.35	<0.05
February	6.82±1.008	2.76±1.14	<0.01
March	15.06±0.98	8.7±2.02	<0.01
April	16.015±1.415	10.86±1.55	<0.01
May	16.45±0.55	12.32±1.50	<0.01
June	4.124±1.03	3.286±1.2	>.05

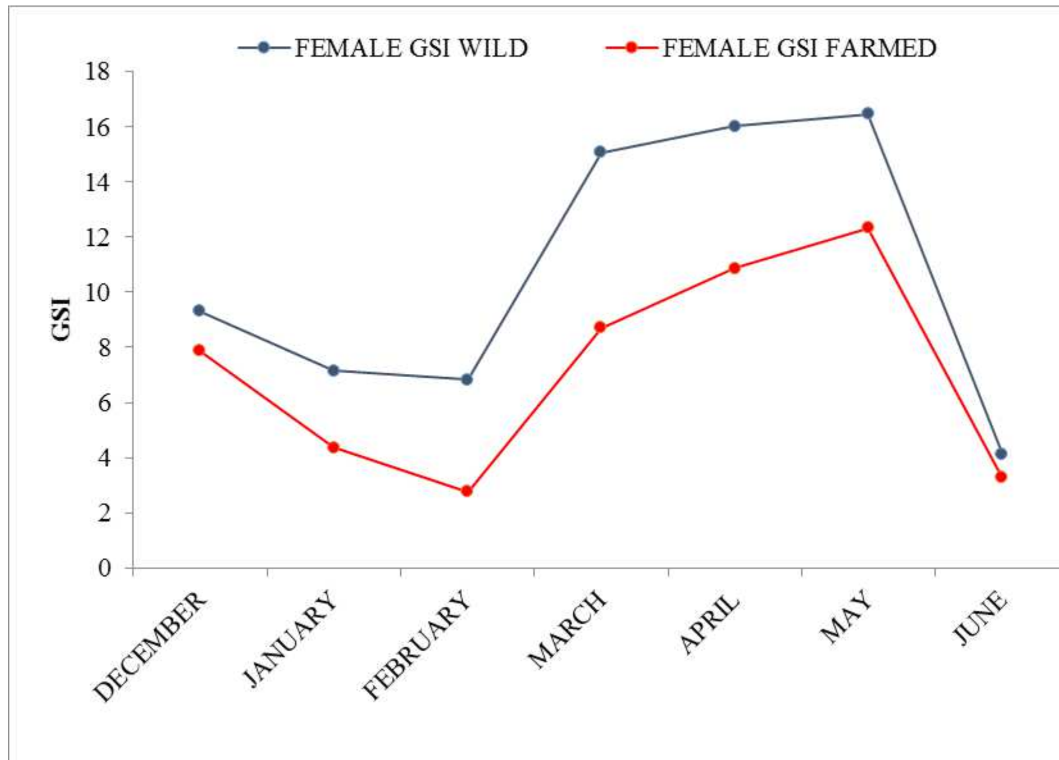


Fig. 10: Monthly variation of GSI of female *Cyprinus carpio* var. *communis* (wild and farmed)

Table 14: Monthly variation in male Gonadosomatic index (GSI) of *Cyprinus carpio* var. *communis* in wild and farmed conditions

Month	Habitat		P value
	Wild	Farmed	
December	7.18±0.96	6.02±1.1	>0.05
January	8.14±.812	3.63±1.51	<0.01
February	4.05±1.12	3.2±1.4	>0.05
March	8.03±1.66	5.84±1.54	<0.05
April	10.036±1.33	6.84±4.6	>0.05
May	11.21±1.22	8.5±2.5	<0.05
June	2.36±0.85	1.99±0.74	>0.05

4.7 Relationship between fish body weight, body length, ovary weight, absolute fecundity and relative fecundity

Table 15 shows the relationship between body weight, body length, ovary weight, absolute fecundity and relative fecundity in case of wild condition. The data reveals that significant positive correlation was found between fish weight and fish length (r=.878, p<0.01), fish weight and ovary weight (r=.888, p<0.01), fish weight and absolute fecundity (r=.907, p<0.01. Furthermore significant positive correlation was formed between fish length and ovary weight (r=0.981, p<0.01), fish length and absolute fecundity (r=.976, p<0.01), Ovary weight and absolute fecundity (r=.998, p<0.01). Relative fecundity showed a significant negative correlation with weight, length and absolute fecundity(r=-0.747, p<0.01; r=-0.419, p<0.05and r=-0.460, p<0.05 respectively).

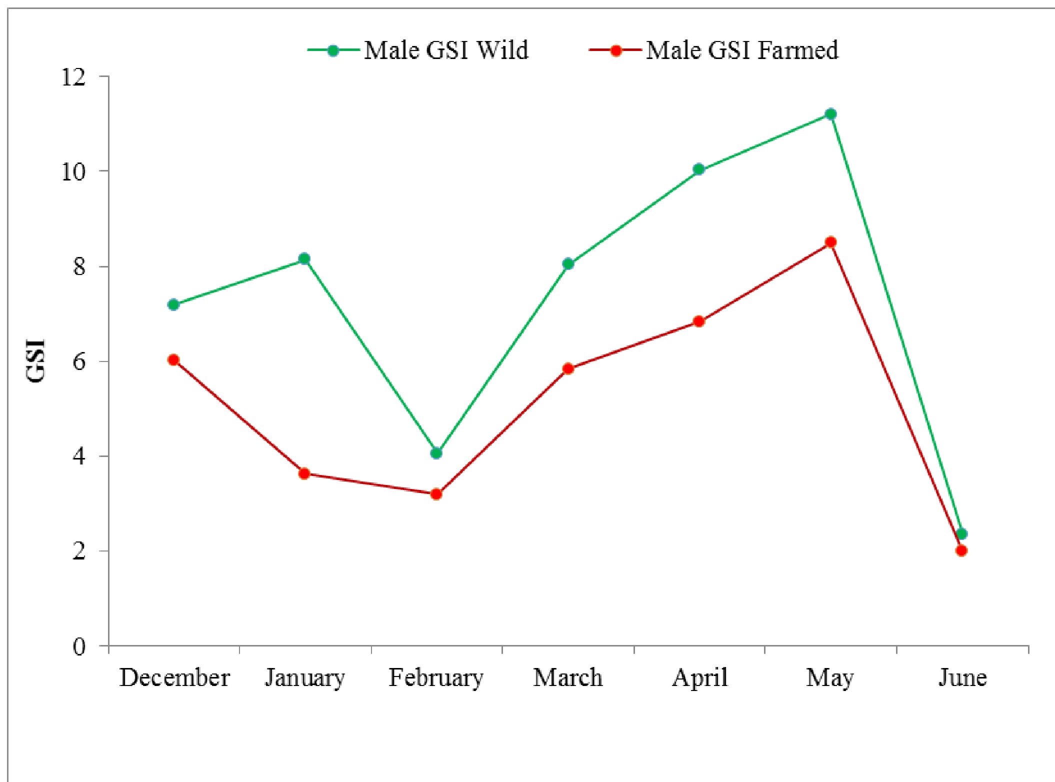


Fig. 11: Monthly variation of GSI of male *Cyprinus carpio* var. *communis* (wild and farmed)

Table 15: Pearson correlation between fish body weight, body length, ovary weight, absolute fecundity and relative fecundity in wild Scale carp

Parameter	Weight	Length	Ovary weight	Absolute fecundity	Relative fecundity
Weight	1	.878**	.888**	.907**	-.747**
Length		1	.981**	.976**	-.419*
Ovary weight			1	.998**	-.419*
Absolute fecundity				1	-.460*
Relative fecundity					1

**correlation is significant at 0.01 level (2 –tailed); * correlation is significant at 0.01 level (2 –tailed)

4.8 Relationship between body weight, body length, ovary weight, absolute fecundity and relative fecundity in farmed Scale carp

Table 16 shows relationship between body weight, body length, ovary weight, absolute fecundity and relative fecundity in case of farmed condition. The data shows a significant positive correlation between fish weight and fish length ($r=.877$, $p<0.01$), fish weight and ovary weight ($r=0.860$, $p<0.05$), fish weight and absolute fecundity ($r=0.813$, $p<0.01$). Also significant positive correlation existed between fish length and ovary weight ($r=0.988$, $p<0.01$), fish length and absolute fecundity ($r=0.959$, $p<0.01$). Ovary weight and absolute fecundity $r=0.974$, $p<0.01$). Relative fecundity showed the significant negative correlation with weight, length and absolute fecundity ($r=-0.081$, $p<0.05$; $r=-0.488$, $p<0.05$; $p<0.05$ and $r=0.610$, $p<0.05$ respectively).

Table 16: Pearson correlation between body weight, body length, ovary weight, absolute fecundity and relative fecundity in farmed Scale carp

Parameter	Weight	Length	Ovary weight	Absolute fecundity	Relative fecundity
Weight	1	.877**	.860**	.813**	-0.081*
Length		1	.988**	.959**	-0.488*
Ovary weight			1	.974**	-0.530
Absolute fecundity				1	-0.610*
Relative fecundity					1

**Correlation is significant at 0.01 level (2 –tailed).

* Correlation is significant at 0.01 level (2 –tailed)

Chapter-5

DISCUSSION

5.1 Physical parameters

Sperm quality is defined as the capacity of the sperm to successfully fertilize oocytes and further develop into a normal embryo. In the present study, the difference in the seminal quality and female reproductive parameters between the farmed and wild scale carp was examined to assess the quality of gamete between the two groups. Following the detailed experimental assessment, the difference was found in major seminal quality parameters and also in the female reproductive parameters.

5.1.1 Sperm volume

Differences in sperm production has been related to age and weight of males, sampling period, sampling method (Suquet *et al.,* 1994), rearing conditions, nutrition, breeding seasonality, method of spawning induction, spawning behavior (Rurangwa *et al*., 2004), feeding conditions and regime, environmental factors, or spawning time (Bozkurt *et al*., 2006). In the present study mean sperm volume was higher in wild brooder (2.393±1.64 ml) than in farmed individual (1.486±0.88 ml). Comparison of wild scale carp with farmed fishes revealed a significant difference in sperm volume ($p<0.05$). Mean sperm volume in farmed and wild fish was found similar with the finding of Belova (1981) who reported the sperm volume in the range of 1-9 ml for scale carp. Mean seminal volume was also similar with the results reported by Nahiduzzman *et al.* (2014), who reported the sperm volume of Scale carp as 2 ml during the spawning season. Bozkurt (2006) also reported the volume of milt in scaly carp as 2.75 ml. Thamizhselvi and Thirumathal (2016) also reported the range of volume of milt in the *Cyprinus carpio* from 1.83 to 1.98 ml.

Higher sperm volume of wild fish than farmed has also been reported by Hajirezaee *et al.* (2011). Morisawa *et al*. (1979) reported that the cultured fish

spent their entire life (fry to adult) in a hypotonic medium contrary to wild males of Caspian brown trout. The hypotonicity of freshwater environment establishes the hydration of testis, possibly causing the dilution of milt and leading to a higher milt volume which is contrary to the results reported by Hajirezaee *et al.* (2011). It is likely that cultured males of Scale carp with the application of an effecient osmoregulation, excrete the excess water of the body in response to hypotonicity of freshwater environment as reported by Hajirezaee *et al.* (2011) in Caspian brown trout while studying the milt quality in the cultured and wild stocks on comparative basis. It is essential to say that the weight of wild males was higher than cultured individuals during the present study. Suquet *et al.* (1994; 1998) have reported that milt volume increases with increase of weight in turbot (*Scophthalmus maximus*). Thus the higher weight of wild fish seems one of the reasons for the higher milt volume of wild males than cultured fishes. The sperm volume has also been found higher in wild European flounder (0.7ml; Sahin *et al.*, 2012) than in cultured ones (0.2 ml; Aydın *et al.*, 2011). Cabrita *et al.* (2006) has found a low sperm quality and volume of stocked animals.

In both the conditions (wild and farmed), sperm volume was found positively correlated with length and weight. The relationship of sperm volume with length and weight was similar with the finding of Nahiduzzman *et al.* (2014) for *Cyprinus carpio.* The relationship between semen volume and fish weight was similar to the relationships found in yellow croaker ($r = 0.975$, $p<0.05$; Le *et al.*, 2011) and cultured European flounder ($r=0.990$, $p<0.01$; Aydin *et al.,* 2011). A positive correlation was also found between body weight and length with semen volume (Tekin *et al.,* 2003). Significant correlation was found between testis weight and sperm volume. Gage *et al.* (1995) also reported that there is a direct relation between the increase in testis weight and the volume of different phenotypes of Atlantic salmon. Therefore, the present study has also confirmed that as the testis weight of fish increases, their sperm volume increases accordingly.

5.1.2 Sperm density

Sperm density is an important factor in the determination of sperm quality (Suquet *et al.*, 1992). Its role in the fertilization of spermatozoa is well documented and reported (Aas *et al.*, 1991; Pool and Dillane, 1998). Density of spermatozoa is observed to be highly varying depending on the species as is evident from the reports on carp where it has been reported to be from 1 to 5×10^9 cells/mL, in trout from 5 to 15×10^9cells/mL and in sturgeon from 0.1 to 4×0^9 cells/mL (Cosson *et al.*, 1991; Redondo *et al.*, 1991; Tsvetkova *et al.*, 1996). In the present study, the average sperm density of 3.534 ± 0.27 x10^9 mL-^{1}in wild and $3.8415\times10^9\pm0.18$ml-^{1} in farm was recorded for the fish which are in conformity with the results of Bozkurt *et al.* (2009) for grass carp (2.87-33.914 x10^9 mL-1in). Chutia *et al.* (1998) have reported the sperm density of 6.6x10^9 sperm cells/ml in *C. carpio*. Thamizhselvi and Thirumathal (2016) recorded the average sperm density of 2.25x10^9 sperm cells/ml in *C. carpio* from January to March, 2013. Lahnsteiner *et al.* (2000) found that the sperm density of *C. carpio* as 0.5 to 1.0×10^{11} cells per mL of milt. Comparison of farmed fish with wild revealed that sperm density of farmed fish was higher than wild individual. Similar results have been reported by Hajirezaee *et al.* (2011).

5.1.3 Motility rate and duration

The spermatozoa motility and its duration have great influence on successful fertilization. However, spermatozoa motility varies in vigor and duration not only among males but also within individual males depending on ripeness (Tekin *et al.*, 2003; Bozkurt *et al.,* 2011). Motility is a parameter to decide the quality of the spermatozoa (Terner, 1986). Sperm motility is a key factor that determines fertilization success (Billard *et al.,* 1995). It has been shown that sperm motility performances (percentage of motile sperm and period of motility) change at spawning season in some teleost fish (Koldras *et al.*, 1996).

In the present study, the value of mean motility percentage and mean

motility duration in case of wild fish was 75.03% and 50.36 sec respectively and in farmed fish the value was 68.9% and 44.6 sec respectively. Significant difference in motility percent was found between the groups but difference in motility duration was found non-significant. Bozkurt *et al*. (2009b) reported the mean sperm motility as 63.18±7.1% and mean motility duration as 56.81±20.3 sec for scale carp. Bozkurt and Ogretman (2012) reported that motility percentage ranges from 70-95% and duration ranges from 35-117 sec in grass carp. These results are in conformity with the present results for both farmed and wild fish. From the present study it is apparent that mean sperm motility and mean motility duration were higher in wild brooder than in farmed individual. Similar results have been reported by Hajirezaee *et al.* (2011). Skjaeraasen *et al.* (2009) reported the wild males of Cod had higher sperm velocity, percentages of motile and progressive cells than their farmed counterparts. However, many workers reported no difference in sperm motility between wild and cultured brooders (Sahin *et al*., 2012; Aydin *et al*., 2011 Rideout *et al.,* 2004). In Striped and White Sea bass, captured from the wild during the spawning season and moved to the captivity produced the milt with non motile sperms (Berlinsky *et al*., 1997).

In the present study higher motility percentage and duration in wild groups seems to help in selecting a wild seed due to its better motility rate and duration than farmed one for culture practice. A positive correlation between the sperm volume and motility was found in both the conditions. In wild conditions, the correlation between the two was found as $r=0.523$, $p<0.01$ and in farmed condition it was recorded as $r=0.487$, $p<0.01$. The relationship between the two parameters (volume and motility rate) agrees with the findings of Bozkurt *et al.* (2009b) and Nahiduzzman *et al.* (2014) for *Cyprinus carpio*. It is likely that with decrease of seminal fluid secretion by spermatic duct epithelium the quantity of materials involved in sperm motility decreases as well. This seems one of the reason during the present study that wild males with higher motility rate may be due to higher secretion of spermatic duct epithelium compared to the farmed ones.

5.1.4 pH

A change in pH of external medium is one of the sperm activating factor in aquatic species (Stoss, 1983). The pH of the milt was recorded alkaline in both the conditions of the fish. There was no significant difference observed in pH between the wild and farmed fish. In the present study, the pH of wild fish was recorded as 8.2±0.49 and in farmed fish as 8.56±0.3.The pH of the milt recorded in the present study is in the conformity with Bozkurt *et al.* (2009b) who reported that pH of the milt of Scale carp from 6.9-9.2. Verma *et al*. (2009) recorded pH of 7.8±0.07 in catla,7.3±0.06 in Rohu, 7.9±in Mrigal, 8.1±0.09 in Kalbasu, 7.8±0.03 in Silver carp and 7.9±0.06 in Grass carp.

As significant positive correlation was found between semen pH and motility in both the conditions. The relationship suggests that this pH may be the most important seminal plasma characters influencing the sperm activation. The findings are similar to the earlier works by Morisawa & Morisawa (1986; 1988) and Billard *et al*. (1995). According to them, during the passage of spermatozoa from the testis to the spermatic duct an increase in external pH is responsible for the acquisition of motility in some salmonid fish and the seminal fluid pH also effects the final maturation of spermatozoa (Lahnesteiner *et al.,* 1998). Morisawa *et al.* (1999) suggested that pH polarize the cell membrane and stimulates the motility of fish spermatozoa.

5.2 Biochemical parameters

Cultured fishes are densely stocked than wild. They are not allowed full exposure to the natural environment and depend upon artificial feeding. In addition, they frequently suffer physical stresses during management. In contrast to the farmed fishes, wild fishes feed on zooplankton and phytoplankton, there is a heterogeneous age structure in populations and the life history is relatively unknown (Coz-Rakovac *et al.,* 2005). Such a gradient in ecological conditions induce significant fluctuations in the level of various biochemical composition of

semen. The composition of seminal plasma has a great influence on the biological quality of the milt and these factors are directly related to the fertilization success (Rurangwa *et al.*, 2004). The knowledge of quantitative characteristics and chemical composition of sperm is a prerequisite for the successful evaluation of the reproductive ability of different fish species. This may also lead to the better understanding of fertilization mechanisms. On the other hand, there are some species-specific characteristics in terms of the mineral and organic compositions of seminal plasma that are needed for artificial insemination or sperm storage.

5.2.1 Protein

Seminal protein protect the spermatozoa against the microbial attack (i.e. transferrin, anti protease), oxidative damage (i.e transferring, superoxide dismutase), and premature activation (i.e. Dietrich *et al.*, 2010). White and Macleod (1963) indicated that protein have a protective role. Lahnsteiner *et al.* (2004) found that seminal plasma proteins prolong the viability of rainbow trout spermatozoa as measured by sperm motility. During the present study, concentrations of total protein were found as 1.99±1.15 g/dl in wild condition and 0.917±0.62 g/dl in farmed condition and are in conformity with the findings of Bozkurt *et al.* (2009b) for scale carp and Faramarzi (2012) for silver carp. Fishes have no accessory glands which contribute to the production of most seminal plasma proteins as in mammals (Billard, 1986), thus the low concentrations of seminal plasma proteins can be related to the absence of accessory glands in fish.

Total protein concentration in the wild condition was found to be significantly higher than farmed conditions. Generally, high protein concentration has been reported as positive characteristic of fish semen. Therefore, the semen of wild common carp can be suggested as better quality semen. The results are in agreement with Butts *et al.* (2011) who worked on the seminal plasma proteins of Cod species and reported the beneficial role towards spermatozoa viability. Percin and Konyalioglu (2008) while studying the serum biochemical profiles of captive and wild northern blue fin tuna (*Thunnus thynnus* L. 1758) in the Eastern

Mediterranean, found the total protein to be higher in wild than in farmed conditions. Phillips *et al.* (1957) also found total protein in wild trout higher than cultured trout. The reason of this difference has been reported due to natural food and higher activity by wild fish (Phillips *et al.,* 1957) for trout species. Significant relationship between the percentage of motility and total protein in seminal plasma of Scale carp in both the environments could be related to the key role of proteins in the motility of sperm cells (Lahnestiner *et al*., 2004).

5.2.2 Glucose

Fish spermatozoa are capable of utilizing extracellular carbohydrates. The glucose level during the present study was estimated in the seminal plasma and its concentration was found to be 70.643±10.75 mg/dl in wild condition and 85±9.29 mg/dl in farmed conditions. Kruger *et al.* (1984) reported that the glucose level in carp species varies from 9-100 mg/l. Bastami *et al*. (2012) reported the glucose concentration in wild common carp as 19.7 mg/dl. The glucose level recorded in the present study is higher than the level reported by Bozkurt *et al.* (2009b) for scale carp (1.43±0.02 mg/dl). The higher level of glucose during the present study can be related to environmental condition, frequency of stripping, hormonal stimulation of spermiation and sampling period and sampling methods (Billard *et al*., 1995; Ciereszko, 2008; Linhart *et al*., 2003). Glucose is the main sugar in the seminal plasma. The presence of this carbohydrate in seminal plasma has been connected to the high energy demand for the testes during spermatogenesis or to lipid synthesis of spermatozoa (Soengas *et al*., 1993).

Higher concentration of glucose in the farmed individuals as compared to wild counterparts is believed to be caused by a wide range of environmental stressors such as hypoxic environment, starvation and captivity (Hardy & Audet, 1990; Torres *et al*., 1991; Cech *et al*., 1996; Santos and Pacheco, 1996 and Svoboda *et al*., 2001). High levels of glucose concentrations in the Scale carp seminal plasma may be related to stress conditions (confinement or holding, handling, etc.) in captivity and subsequently, an increase in the glucose

concentration of body fluids (such as seminal and blood plasma) follows because of the constant activity of glycolysis pathway in liver in response to stress conditions as reported by Portz *et al.* (2006) for Caspian brown trout. Since glucose in serum is a major metabolite of carbohydrate metabolism, the higher glucose concentration detected in cultured strains has also been attributed, in part, to the higher glycogen reserves in artificially cultured fish than in their wild counterparts (Artacho *et al.*, 2007). Kubakowa *et al.* (1999) reported that male Sockeye salmon respond to confinement stress with elevated level of glucose and decreased level of reproductive steroid. Findings of the present study also confirm the results of Coz-Rakovac *et al.* (2005) who reported significantly elevated glucose level in farmed *Dicentrarchus labrax* as compared to the wild ecotypes. The results are also in line with Zhou *et al.* (2009) for Dojo loach.

Glucose had a negative relationship ($r=-0.168$, $p<0.05$) with male Gonadosomatic index in farmed conditions. Negative relationship can be due to physical processes involved in transporting the fish as keeping them in tanks might have caused physical stress and affected hormone and glucose levels in the blood serum. These finding are in agreement with the result obtained by Imanpoor and Bagheri (2011).

5.2.3 Lipids

Seminal plasma lipids are associated with metabolism in spermatozoa (Piironen, 1994). Triglycerides serve as energy sources for sperm motility in fish (Stoss, 1983; Lahnsteiner *et al.*, 1993). During the present work, the triglyceride concentration was recorded as 12.99±5.605 mg/dl in wild and 12.308±5.22 mg/dl in farmed condition. The mean level of triacylglyceride in *Cyprinus carpio* seminal plasma was higher than that in *O. mykiss* (8 mg/dl) (Secer *et al.*, 2004) but it was close to *C. idella* (14.58 mg/dl) (Bozkurt *et al.,* 2008). Bozkurt *et al.* (2009b) reported that the triglyceride level in Scale carp from 5-12 mg/dl. Aramali *et al.* (2014) reported the triglycerides level as 15.2 mg/dl for Persian sturgeon, *Acipenser persicus.* The variation in the level of lipid can be related to

environmental condition, frequency of stripping, hormonal stimulation of spermiation, sampling period and sampling methods (Billard *et al.,* 1995; Ciereszko, 2008 and Linhart *et al.,* 2003) and also to contamination of sperm by urine (Perchec *et al.,* 1995a). The triglyceride level though recorded higher in wild than farmed one but the difference in triglyceride level between the two environments was found to be statistically non-significant. Srivastava and Brown (1991) while studying the biochemical characteristics and hatching performance of cultured and wild Atlantic salmon (*Salmo salar*) eggs, reported that eggs collected from the wild had high lipid content than farmed ones.

Significant correlation of triglyceride with motility percentage was determined in both the environments. The relationship indicated that triglycerides serve as energy resources for energy metabolism of spermatozoa (Stoss, 1983; Lahnesteiner *et al.,* 1993b). Low triglyceride could therefore be indicative of inadequate energy resources, reduced motility rate and fertilization capacity (Lahnestiner *et al.,* 1993; 1998). Generally, high triglyceride concentration is a positive characteristic of fish semen. Therefore, the semen of wild Scale carp was found of better quality.

5.2.4 Cholesterol

The cholesterol level was found to be 5.928±0.629 mg/dl in wild and 5.5±1.610 mg/dl in farmed fish. There is insufficient information about the role of cholesterol in seminal plasma in spite of its identification in the seminal plasma of freshwater fish (Billard *et al.,* 1995). Cholesterol has been reported to have protective effect against environmental changes especially in temperature that occurs when the fish semen is released (Bozkurt *et al*., 2008). The cholesterol content recorded in both the conditions was similar with the finding of Bozkurt *et al.* (2009b) for scale carp. Bozkurt *et al.* (2009b) reported the cholesterol content in Scale carp between 5-11 mg/dl. Bastami *et al.* (2012) reported the cholesterol content in the seminal plasma of wild carp as 106.85 mg/dl. The cholesterol level in the fish during the present study was lower than those reported for

Ctenopharyngodon idella (12.02 mg/dl) by Bozkurt *et al*. (2008) but higher than that observed in *Oncorhynchus mykiss* (2.55 mg/dl) by Secer *et al*. (2004). The function of cholesterol in seminal plasma has been demonstrated in numerous studies with the aim to improve the sperm deep-freezing technique and composition of semen extenders (Beer-Ljubic *et al.* 2009). The difference in the values of the present study with other findings may be due to the difference in age, season, environment, and physiological conditions of fish as stated by Billard *et al.* (1995), Ciereszko (2008) and Linhart *et al.* (2003).

A positive correlation between cholesterol and sperm motility was found during the present study which are similar to the findings of Bozkurt *et al*. (2009b) who determined a positive correlation between cholesterol and sperm motility in Common carp. Diwan and Krishnan (1986) stated a fluctuation of serum cholesterol in males and females of *Etroplus suratensis* due to maturity. Svoboda (2001) found the cholesterol concentration in blood sex and plasma of females lowest when the gonadosomatic index (GSI) was the highest and vice versa, this finding is also in agreement with our results as cholesterol had a negative relationship ($p<0.05$) with male Gonadosomatic index.

5.2.5 Urea

Urea contamination of semen may cause reduced sperm motility and fertilizing ability (Dreanno *et al.,* 1998) influencing the variability of other semen parameters (Glogowski *et al.,* 2000). Notable concentrations of urea were determined in seminal plasma during in the present study, In wild males urea concentration was 25.221±4.047 mg/dl and in farmed conditions was 28.497±5.75 mg/dl. Bozkurt *et al.* (2009b) reported that the urea content in Scale carp as 24.45±7.96 mg/dl, which is in agreement with the present result in both the conditions. Secer *et al*. (2004) reported that the urea content in *O. mykis* as 3.16 mg/dl. The urea content in the mirror carp was reported to be in the range of of 38-97 mg/dl (Bozkurt *et al*., 2009a). Our results show that the mean levels of serum urea in cultured individuals were elevated as compared to those in wild

individuals. It is understandable that the content of ammonia-N tended to be higher in the intensively cultured ponds than in the wild environment. This elevated ammonia-N level along with hypoxic conditions that cause an alternative osmotic pressure seems responsible for the higher values of urea (Shen *et al.*, 1991).

5.3 Preservation of milt

Preservation of fish sperm for short-term duration is generally useful from the commercial point of view and facilitates various hatchery operations. The short-term storage of sperm at low temperature (4ºC) is mostly applied in short-distance transport of gametes collected in different locations, in synchronizing the timing of obtaining good quality of gamete. The fish sperm could be preserved by storage in undiluted and diluted form. Undiluted sperm stored at low temperature has been reported to cause a reduction in fertilization capacity (Lahnsteiner *et al.* 1997). Short-term storage of sperm using different extenders has been reported in *Oncorhynchus mykiss* (Canyurt *et al.,* 2003), *Cyprinus carpio* (Bozkurt and Secer, 2005), *Clarias gariepinus* (Vuthiphandchai *et al.,* 2009), *Salmo trutta abanticus* (Hatipoglu and Akcay, 2010) and *Salmo coruhensis* (Şahin *et al.,* 2013). Post-activation motility is one of the most important indicators of the success of a preservation protocol. Spermatozoa motility has been found to get affected during preservation in the present work. The best motility results were obtained with glucose containing extender. The proportion of motile cells decreased faster with time in undiluted sperm samples than diluted ones. The motility of diluted sperm was always significantly greater than that of undiluted sperm kept under the same conditions, similar results for the motility parameters of chilled stored spermatozoa were reported by different workers (Stoss and Holtz, 1983; Bozkurt and Secer, 2005). Kime *et al.* (1996) also reported that when the spermatozoa are kept in appropriate extender at low temperature (4°C), no significant changes in the quality were found. Harvey and Kelley (1984) observed storage of diluted sperm with extender provides better control compared to undiluted storage.

Canyurt *et al*. (2003) also reported that the best results in fertilization and motility are obtained from the sperm samples of rainbow trout diluted with artificial seminal plasma with the rate of 1:1 stored for 7 days. It is possible to enhance the fertilizing capacity of the fish by using suitable activating mediums that increase the duration of motility. Sperm dilution is a major factor in the induction of sperm motility (Billard and Cosson, 1992). Ginzburg, (1968) and Gallis *et al*. (1991) reported that the duration of sperm motility and intensity of spermatozoa in *A. baeri* increased when the dilution rate increased from 1:6 to 1:100. In the present study, the maximum duration of motility has been determined with extender I containing 300 mM glucose. Therefore the extender I provided longer duration of motility since glucose served as an energy resource for spermatozoa. Similar findings were reported by Hatipoglu and Akçay (2010) who found that glucose based extender is a better preservative than Ringer solution for the short term preservation of Abant trout (*Salmo trutta abanticus*) semen.

5.4 Fecundity

The 'fecundity' of a fish is defined as the number of eggs that are likely to be laid during a spawning season (Bagenal, 1957). The reproductive potential, i.e., fecundity is an important biological parameter that plays a significant role in evaluating the commercial potentials of fish stocks (Gomez-Marquez *et al*., 2003). Successful fisheries management including practical aquaculture relies on having an accurate assessment of fecundity to understand the recovery ability of fish populations (Lagler, 1956; Nikolskii, 1969; Tracey *et al*., 2007). The fecundity and its relation to female size makes it possible to estimate the potential of egg output (Chondar, 1977) and the potential number of offspring in a season and reproductive capacity of fish stocks (Qasim and Qayyum, 1963). Fecundity is known to assess the reproductive potential and to evaluate the commercial potential of a fish stock for the efficient fish culture and effective management (Mian and Dewan, 1984; Das *et al*., 1989). Fecundity has a vital role in the selection of brooders for production purposes (Prasad *et al.,* 2005). Considerable

work has been done on fecundity of fishes in many countries by Clark (1934), Begenal (1967), Chonder (1977), Joshi and Khanna (1980), Singh and Srivastava (1982), Nautiyal (1985), Somdutt and Kumar (2004), Bahuguna and Khatri (2009) and Lone and Hussain (2009). In the present study, the absolute fecundity ranged from 16650 to 129000 having a body weight of 120 to 1300 gm in wild and 4032 to 105000 with body weight of 126 to 1236gm recorded in farmed fish. Significant differences in the absolute fecundity and relative fecundity was found between the wild and farmed groups of Scale carp. Findings of this study are in agreement with those reported by Kristen *et al.* (2012) who also observed the difference in the absolute fecundity between the wild and farmed groups of Eurasian perch. Iqbal and Kauser (2009) also reported that *Cirrhinus mrigala* reared in pond showed lower fecundity than the wild stocks of the same fish size. The fecundity of American plaice in the laboratory was considerably lower than the potential fecundity in the wild (Nagler *et al*., 1999). The significant difference obtained in the absolute and relative fecundity of *Cyprinus carpio* var. *communis* supports the notion that the type of environment has an effect on the reproduction characteristics of female.

The absolute fecundity during the present study in wild fishes ranged from 16650 to 129000 and in farmed fish from 4032 to 105000, which is in conformity with the findings of Shafi *et al.* (2012) who reported the fecundity of *Cyprinus carpio* var. *communis* from 3173 to 629320. The observed fecundity are lower than the ranges of 75,645 to 356,743 and 36955 to 318584 for *C.C* var. *communis* reported by Abera *et al.* (2015) and Hailu (2013) respectively. The fecundity reported by these workers was slightly higher than the present study. Total fecundity of *Cyprinus carpio* (Bankok strain) inhabiting tropical waters; has been reported to have fairly high due to its early maturity and year round breeding behaviour.

The relative fecundity during the present study in wild fishes ranged from 99.2 to 240.8 and in farmed fish from 32 to 186.3, which is in conformity with the

findings of Shafi *et al.* (2012) who reported the relative fecundity of Scale carp in the wild in the range of 21 to 223 with a mean value of 91.17. Fecundity (Absolute) of farmed fish observed here (44533±28572.59) was significantly lower than that of the wild fishes (68864±35950.2). Similar results have been observed by Kouril and Hamackova (2000) and Kristen *et al.* (2012) who reported the relative fecundity in wild condition significantly higher than the farmed conditions. However, Kjesbu *et al.* (1991) found the captive Cod had higher potential fecundity than wild cod. Thorpe *et al.* (1984) also observed that cultured salmon had smaller but higher numbers of eggs per unit weight than wild fish.

Contreas Sanchez *et al.* (1998) reported that when the fishes were subjected to stress in the form of confinement, absolute fecundity were not significantly affected, but significant differences in relative fecundity were found. Common practices in hatcheries such as transportation, handling, cleaning, crowding, use of chemicals, and problems with water quality are stressors that negatively influence the reproduction (Bromage 1995, Billiard *et al.* 1981). Lone and Hussain (2009) reported that when fishes are kept in captivity for culture purpose they show some degree of reproductive dysfunction which may be due to low quality feed given to fish. Randak *et al.* (2006) while studying the effect of culture conditions on reproductive traits of brown trout *Salmo trutta L* reported no difference in the fecundity (absolute, relative) between the farmed and wild fish. According to Simpson (1951) the fecundity of an individual female varies in relation to many factors including age, size, species and environmental conditions, such as food availability, water temperature and salinity. It seems fair to believe that lower fecundity in ponds observed during the present study might be due to the genetic makeup of fish stock; overstocking, or improper and underfeeding of fish in pond which affected the growth of fish and indirectly the gonadal development which are similar to the findings of Billard (1995) who reported that the fish in pond show low fecundity and fail to spawn due to the stress of captivity, insufficient food and higher stocking density.

Absolute fecundity has been usually related to fish or gonad length and weight (Bagenal, 1966). A relationship has been found to exist between fish length and fecundity in different species of fishes. Length has an advantage over other factors in that the fish does not shrink significantly although it can lose weight during the spawning season (Bagenal, 1967). Clark (1934) suggested that the fecundity of a fish increases in proportion to the square of its length. Simpson (1951) pointed out that the fecundity of Plaice was related to the cube of its length. The close relationship between absolute fecundity and fish length demonstrated here is supported by the works of Treasurer (1990); Joshi and Khanna (1980) and Dobriyal (1988) and many others. Fecundity generally increased with total length in several fishes. A positive correlation has been observed between total length of females of *Mugil parsia* (Sarojini, 1957), *Osteogeneiosus militaria* (Pantulu, 1963), *Polynemus paradiscus* (Gupta, 1968), and *Labeo rohita* (Varghese, 1973) and their fecundity. In the mosquito fish *Gambusia affinis*, the maximum average monthly fecundity has reached when the length of the mother was at its highest (Fernandez-Delgado, 1989).

A direct proportional increase in the fecundity with the increase in fish weight has been reported by Dobriyal (1988) and Lehman (1953). During the present study an increase in the number of ova was found with the increase in body weight in both the groups (wild and cultured). Absolute fecundity of *Cyprinus carpio* var. *communis* under both wild and farmed condition had a strong correlation with ovary weight than body weight and Total length. These results are in conformity with the results obtained by Khan *et al.* (1992) for *Mystus tengra* and Nabi *et al.* (2007) for *Glossogobius giuris.* According to Smith (1947), the fecundity has been more related to the fish weight than to the length in *Salvelinus fontinalis.* Same has been reported for *Liza parsia* by Rheman *et al.* (2002). However, the 'r' value in the correlation between fecundity and total weight was higher than that of fecundity and total length, suggesting that total weight is a better predictor of fecundity in the present study than total length.

Similar finding have been reported by Ikomi and Odum (1998) in *Chrysichthys auratus.* Varghese (1961) has found a reduction in the rate of egg production with the increase in ovary weight in *Coilia ramcarati*. But in present study a corresponding increase in the number of eggs with the increase in the weight of ovary has been found, as in *Tilapia nilotica* (Soliman *et al.,* 1986), *Labeo gonius* (Joshi and Khanna, 1980), *Esox lucius* (Treasurer, 1990) and Chinese silver carp (Dobriyal, 1988).

5.5 GSI

Kouril *et al*. (1997a) reported that wild perch showed superior development of gonads under natural conditions. Fontaine *et al.* (2008) has reported the lower quality of reproduction in farmed Perch bred under controlled conditions. Kristen *et al.* (2012) while comparing the GSI of wild and farmed Perch observed the GSI of wild fish of both the sexes higher than the farmed one. The Gonadosomatic index in wild fish of both the sexes was found significantly higher than the farmed one. This differences between farmed and wild Scale carp may be mainly by unsuitable feed for the farmed fish as suggested by Bell *et al*. (1997); Kestemont *et al*. (1999); Izquierdo *et al*. (2001); Kestemont *et al*. (2008); Henrotte *et al*. (2010).

The significant difference in GSI of both sexes between wild and farmed conditions indicated that culture system has an effect on GSI. The GSI of the male fishes ranged from 4.44 to 12 in wild and 3.12 to 9.03 in farmed fishes whereas in the female fishes it ranged from 6.6 to 18.42 in wild and 2.3 to 12.9 in farmed conditions. Parameswaran (1972), Shafi *et al.* (2012), Abera (2015) reported the GSI of Common carp to be in the range of 3.67 to 37.93%, 2.302 to 11.363% and 13.97 to 17.01% respectively. The GSI in the present study was found in the same range. A significant difference in male GSI was found in wild and farmed group.

The GSI value of males was lower than that of females and the GSI values for both the sexes was higher from March to May with the peak values noticed in

May in both the sexes and showed decline from May to June. Shafi *et al.* (2012) have reported the GSI peak in both the sexes in the month of March and reported that the spawning takes place during spring although the gonad were fully mature at the start of winter. Raina (1978) while studying the reproductive biology of Common carp, *Cyprinus carpio* under temperate conditions in Kashmir, observed that the fishes of both the sexes breed from April to June. However, the highest GSI values in the month of May and subsequent decrease after June during the present study indicates that the fish breeds May onwards. Abera *et al.* (2015) have reported that GSI values of females increased from January to April and that of males from December to May, all above finding are in agreement with the present study.

Captive rearing can negatively impact sperm traits in aquatic animals, including an increased percentage of abnormal spermatozoa, reduced number of sperm in spermatophores, reduced percentage of viable sperm (Leung-Trujillo and Lawrence, 1987), and the degeneration of the male reproductive tract (Talbot *et al*., 1989). Therefore the present study showed the higher sperm performance of wild fish than farmed fish in terms of various physical and biochemical parameters, the result are in consent with the finding of Skaeraasen *et al*. (2009) and Butts *et al*. (2011). They reported that wild male cod had greater sperm performance compared to farmed cod. Furthermore, Berejikian *et al.* (1997) found that captive-reared Coho salmon (*O. kisutch*) were reproductively inferior to wild salmon based on observed spawning behavior.

Therefore the analysis of sperm quality is mandatory to help solve the sole reproductive problems and develop better protocols for broodstock husbandry management. The first step in proper broodstock management is the identification of the optimal conditions required for a species to undergo reproductive maturation and produce gametes of good quality. Environmental factors need to be controlled in order to obtain good quality seeds. Reproduction of fish in captivity can be controlled by environmental manipulations, such as photoperiod,

water temperature or spawning substrate. However, the ecobiology of some fishes is not well known, or it is impractical or even impossible to simulate the required environmental parameters for natural reproductive performance (i.e., spawning migration, depth, riverine hydraulics, etc.). In these instances, use of exogenous hormones is an effective way to induce reproductive maturation and produce fertilized eggs. Furthermore, in all cultured fishes, hormonal manipulations may be used as management tools to enhance the efficiency of egg production, increase spermiation and facilitate hatchery operations.

Chapter – 6

SUMMARY AND CONCLUSION

Comparative study on spermiology and fecundity of fish has been carried to assess the seed quality of fish in farmed and wild conditions. The present study provides information regarding the physical and biochemical parameters of milt, fecundity, GSI, short term preservation of milt. The data generated provides the basis to improve the current artificial fertilization procedures that surely will increase the efficiency of brood stock and hatchery management practices.

In the present study, mean sperm volume in the wild fishes was found as 2.393±1.64 ml compared to the farmed ones where it was found as 1.486±0.88 ml. Mean motility was 75.038±10.162 percent in wild and 68.9±12.46 percent in farmed fishes. Mean motility duration was 50.367±13.92 sec in wild and 44.66±13.48sec in farmed fishes; mean sperm density was 3.534±.272 $\times 10^9$/ml in wild fishes and 3.84±0.181$\times 10^9$ in farmed fishes. Mean pH was 8.29±.494 in wild fishes and 8.5±0.311 farmed fishes. The comparison of physical parameters of milt indicates that the Scale carp in wild conditions showed significantly higher sperm volume, motility (%) as compared to the fishes in the farmed conditions. However, the motility duration though higher in wild fishes but the difference was found non-significant. In contrast sperm density was significantly higher in farmed fishes.

Study on the biochemical parameters of milt plasma showed the significant difference of glucose, protein, and urea level and non-significant differences in triglyceride and cholesterol between the two environments. In case of wild condition mean±SD of glucose was 70.64±10.75, protein was 1.99±1.15, triglyceride was 12.99±5.6, cholesterol was 5.98±.629 and urea was 25.22±4.047. Further, in case of farmed condition mean±SD of glucose was 85.06±9.29, protein was 0.917±0.62, triglyceride was 12.3±5.22, cholesterol was 5.53±1.61 and urea was 28.4±5.75. The levels of glucose, urea in farmed Scale carp were significantly

higher ($P<0.05$) than wild fishes. In contrast, the concentration of total protein in farmed individuals was significantly lower than that in their wild counterpart ($p<0.05$). Cholesterol, triglyceride though higher in wild condition displayed insignificant variation between two environs.

The correlation between the body weight and length of the fishes of both the sexes were found very high and statistically significant. Sperm production and quality has been reported to get affected by both fish size and physiolological status (Bozkurt, 2006). On the other hand relationship between the fish size and sperm quality indices has been found low and insignificant in both the conditions which indicated that physical conditions of mature fish has not an influence on sperm quality. However a significant relationship was found between sperm volume and motility, sperm density and motility. Sperm motility also showed significant positive relationship with pH, protein and triglyceride in both the experimental condition.

Short term preservation of milt in three extenders (E_1, E_2 and E_3) and no extender (control) were also tested. The maximum duration and percentage of motility has been determined with extender I containing 300 mM glucose. It can be said that extender I provided longer duration of motility since glucose served as energy resources for spermatozoa. The present study also confirmed that the motility rate decreased with increasing storage time in both diluted and undiluted milt. The storage of sperm in refrigerator (4°C) for short durations enables the separation in time between semen and egg collection. This may facilitate propagation of brood stocks in hatcheries.

The GSI values of both male and female fishes were found low from the month of December to February and reached a peak value in May in both the environmental conditions. Comparison of the female reproductive characters indicated that wild fish has significantly higher absolute fecundity, relative fecundity, ovary weight, GSI than farmed ones. Mean absolute fecundity of 68864±35950.29 was recorded in the wild and 44533±28572.59 in the farmed

conditions. Mean relative fecundity of 185.96±45.7 was recorded in the wild and 115.98±49.25 in the farmed conditions. Mean ovary weight of 48.96±22.745 was recorded in the wild and 33.68±21.65 in the farmed conditions.

Therefore, the present study revealed that absolute fecundity has a strong positive correlation with ovary weight followed by body length and weight. However, the relative fecundity has a negative correlation with ovary weight, body length and weight.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Professor A. R. Yousuf, Jabari Manzil Usmanabad, Malabagh Hazratbal, Srinagar-190006 (Formerly Expert Member National Green Tribunal, New Delhi and Dean Academic Affairs, University of Kashmir-190006) during viva voce examination held on 28-01-2017 have been incorporated in the manuscript entitled **"Comparative study on spermiology and fecundity of *Cyprinus carpio* var. *communis* in farmed and wild conditions"** submitted by **Ms. Ishrat Mohd (Regd. No. 2014-F-28-M).**

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