POND CULTURE AND REPRODUCTIVE PERFORMANCE OF SEENGHAREE, *SPERATA SEENGHALA* (SYKES)

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by

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CERTIFICATION

I hereby undertake that this research is an original one and no part of this thesis falls under plagiarism. If found otherwise, at any stage, I will be responsible for the consequences

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DEDICATED

TO

MY THE LOVING

MOTHER

WHO IS NO LONGER WITH ME

BUT

WITHIN ME

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LIST OF ABBREVIATION

AFP	Aquaculture and Fisheries Program
AMD	Age-related Macular Degeneration
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CF	Condition Factor
CMC	Corboxy Methyl Cellulose
CP	Crude Protein
CRD	Complete Randomize Design
DMRT	Duncan's Multiple Range Test
DO	Dissolved Oxygen
EC	Electrical Conductivity
EE	Ether Extract
FCR	Food Conversion Ratio
FER	Feed Efficiency Ratio
FM	Fish meal
G	grams
Hrs	Hours
L	Liters
M	Meter
MT	Metric Tons
NARC	National Agriculture Research Center
NFE	Nitrogen Free Extract

NPU	Net Protein Unit
PER	Protein Efficiency Ratio
PPS	Plant Protein Source
PUFAs	Polyunsaturated fatty acids
PWG	Percent Weight Gain
Raceways	Outdoor concrete raceways
SBM	Soybean Meal
SGR	Specific Growth Rate
SRAC	Southern Regional Aquaculture Center
Tanks	Indoor circular fiberglass tanks
TDS	Total dissolved solids
USA	United States of America
WG	Weight Gain

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Chapter 1

INTRODUCTION

The population of the world is increasing at a fabulous rate. As a consequence of rapid population explosion, the problem of food shortage, particularly good quality protein is critical. Therefore without a corresponding increase in food production; this situation may lead to a risk of severe malnutrition in developing countries including Pakistan. It is unfortunate to mention that one or more nutrient is deficit in the food of a considerable population of the world (FAO, 2012) and this situation is adversely affecting the health and vitality of the people. Aquaculture provides aquatic animals including fish that are rich source of vitamins, minerals, protein, and essential fatty acids (FAO, 2012) and can play an important role in eliminating hunger and malnutrition.

Aquaculture and capture fisheries supplied the world with about 154 million tones of fish in 2011, of which about 131 million tones was utilized as food for people. World fish food supply has grown with an average growth rate of 3.2 percent per year in the last five decades, leaving behind the increase of 1.7 percent per year in the world's population. In the 1960s, world per capita average food fish supply was 9 kg (live weight equivalent), which have increased to 18.6 kg in 2010. Worldwide production of farmed food fish was 59.9 million tons in 2010 (FAO, 2012). According to the latest estimates by FAO and OECD, 60% more food is needed in 2050 and there will be a need to produce animal protein at least three times by 2050. The meats (poultry/swine/beef) and dairy will double while aquaculture production will grow tenfold (FAO, 2012).

Fish is one of the best aquatic animals that can help in compensating protein supply (Barlas, 1986). Fish oil, especially from marine species, is rich in omega 3 polyunsaturated fatty acids (PUFAs) which are associated with health benefits; preventing cardio-vascular diseases in the adults, dementia, age-related macular degeneration (AMD), attention deficit hyperactive disorder and asthma in pediatric population. Fish is also an important source of micronutrients. In addition, fish flesh can be converted into body tissues more efficiently and is the tastiest than all other meat varieties, such as beef and mutton. It is easily digestible; its digestibility is 85-95% (Rudolf, 1971).

Pakistan, despite of being an agricultural country and maintaining millions of people on its land, is facing an acute shortage of proteins. It is therefore the need of the time to increase the animal protein production to solve the problem of protein shortage. Aquatic resources are among the major alternatives for the production of animal protein. Pakistan has vast inland fisheries resources including rivers, streams, lakes and ponds, which support a wide variety of economically important fish and posses a great potential of fish industry (Akhtar, 2001). These water resources offer opportunities for sustainable fisheries/aquaculture development through exploitation of various varieties of fish (Niaz, 2001).

The fisheries sector contributed around 1% of the GDP of Pakistan during the year 2010-11; the total fish production was 925,755 metric tons (MT), out of which, 667,782 MT came from marine and 257,973 MT was contributed by inland waters. Aquaculture production including culture based fisheries was 51594 MT in year 2010-11(GOP, 2012). It has been estimated that 20% of the total fish produced from inland waters is coming from aquaculture (fish farming) which is mainly used to meet the domestic meat requirements (GOP, 2012).

The culture system in Pakistan mainly gyrates around carp fishes including both indigenous major carps and exotic Chinese carps (Basavaraga *et al*., 1999). Indigenous major carps namely *Catla catla, Labeo rohita,* and *Cirrhinus mrigala* are the fish for culture. In order to improve aquaculture system Chinese carps i.e. *Hypophthalmichthys molitrix* (Silver carp), *Ctenopharyngodon idella* (Grass carp), *Aristichthys nobilis* (Bighead carp) and *Cyprinus carpio* (Common carp) were introduced to the culture system of Pakistan. These fishes have gained popularity in fish farming due to their number of traits, such as culture suitability in captive conditions and good growth in ponds (Mirza and Bhatti, 1999).

In Pakistan, total area of fish ponds is consisting of about 60,500 ha according to the latest estimates. The number of fish farms established till now is more than 12,000 (Jarwar, 2008). The fish farming is operating on extensive or limited level of semi intensive system using low stocking density ranging from 800-1000 fish/acre with low inputs. This resulted low fish productivity ranging 1000-1200 kg/acre compared to other Asian countries which are producing up to 3000-3500 kg/acre through semi-intensive fish culture and utilization of artificial diet (FAO, 2012). Pakistan has about 198 freshwater fish species. Among the species at least 31 species are economically important. Bringing more indigenous species in aquaculture will boost the fish production in the country. The potential candidates are catfish like *Sperata seenghala, Wallago attu, Rita rita*, and *Channa marulius* (Rafique and Khan, 2012).

Different species of catfishes have been tested for their suitability in freshwater culture and among them only few species have promising culture characteristics like Channel catfish, African catfish and Pangasius (Mingkang, 2005). The Channel catfish production in pond has been practiced for about 50 years and is the largest component of fresh water aquaculture and most successful aquaculture business in the United States of America (NASS, 2001; Engle, 2003). In sub-Saharan Africa the major fish species cultured is African catfish. Another import catfish is Pangasius, *Pangasius hypophthalmus.* The largest producer of world's *Pangasius hypophthalmus* is Vietnam. The production of *Pangasius* was more than 1.10 million tones in 2008 (Posma, 2009). Beside Vietnam major producers of *Pangasius* are Cambodia, Thailand, Lao People's Democratic Republic, Bangladesh, China and Myanmar.

Many commercially important catfish i.e. Seengharee (*Sperata seenghala*), Khaga (*Rita rita*) Malli (*Wallago attu*) and *Clupisoma naziri* are available in the natural water bodies of Pakistan (Murugesan, 1978). Among these catfishes Seengharee (*S. seenghala*) is of great importance. This is a common giant catfish in the Indian region and is of considerable fishery value. This species have wide range of salinity tolerance, high consumer preference due to good quality of meat and fewer spines (Agbayani, 2004), and fetches the higher price than carps. The flesh of Seengharee is firm and delicious. This fish is very common in the market and makes excellent food (Pethiyagoda, 2005). It has been considered as one of the most admired edible fish among indigenous catfish species due to good taste and high market demand.

Sperata seenghala, Seengharee is the most common catfish species in South

Asia (Talwar and Jhingran, 1991; Jayaram, 2002). Seengharee, (*Sperata seenghala*) is found in Indus drainage system of Pakistan and India (family: Bagridae) (Mirza *et al.,* 1992). Naturally the Seengharee subsists on various types of organisms such as fish, frogs, snakes, insects, earthworms, tadpoles, crustaceans and debris (Rahman *et al.*, 2005a). Being carnivorous in habit (Nawaz *et al.*, 1994; Sandhu and Lone, 2003), it comes on dead bait and provides a good sport. This fish usually spawns twice a year from May to July and from September to November in natural conditions (Talwar and Jhingran, 1991; Rahman *et al*., 2005a).

Recently, natural stocks of *S. seenghala* have been drastically reduced due to natural and man-made catastrophes. Due to these factors there is a serious threat to the genetic resources of this fish and the fish has become gradually endangered (IUCN, 1998; Rahman *et al*., 2005a). In order to maintain the population this fish as well as to conserve its biodiversity, development of suitable techniques for the rearing and culture of *S. seenghala* is very essential. No systematic information is available on the culture techniques of this important fish. Aquaculture potential of *Sperata seenghala* has not yet been explored.

By observing the importance of Seengharee, the present study was planned to evaluate its viability in fish culture system. It was expected that current study will provide some basic information regarding its transportation, acclimatization, feeding and culture in captivity. By the culture of Seengharee not only per unit fish production would increase but would also improve the farmers’ income. Hopefully in near future this fish may find its way in the inland fish farming system.

The scope of the present study was restricted to transportation, acclimatization, feed formulation and performance of Seengharee in captivity. The purpose of study was "Culture of Seengharee, *Sperata seenghala* in captive conditions" with the specific objectives:

- Identification of potential sites for the collection of Seengharee and its transportation from natural habitat
- Systematic study and acclimatization of Seengharee under captive conditions
- Diet development for Seengharee from locally available feed ingredients
- Performance of Seengharee in earthen ponds

Chapter 2

REVIEW OF LITRATURE

The giant river catfish (*Sperata seenghala*), locally known as "Seengharee" is found in rivers, floodplains, inundated swamp fields, ditches, canals and other freshwater areas. The geographical distribution of Seengharee is in Pakistan, Afghanistan, India, Nepal and Bangladesh (Jayaram, 1977; Jhingran, 1991; Talwar and Jhingran, 1991; Rahman, 1989). The largest specimen measuring 112.3 cm in length and 10.0 kg in weight was recorded from the Kuliarchar Fish Landing Centre in Kishorganj district of Bangladesh (Rahman, 1989). It is considered to be the best fish as far as consumers are concerned because of it taste. The Seengharee has huge cannibalistic habit because it is carnivorous in nature (Rahman, 1989). The fish spawn twice in a year; from May to July and from September to November in natural conditions. Aquaculture potential of this species is very low because it shows poor reproductive performance in pond culture and captive conditions (Talwar and Jhingran, 1991; Rahman *et al*., 2005a).

Seengharee is widely distributed in the Ganges and Indus river drainages, and also occurs in several major rivers in peninsular India at least as far south as the Krishna River (Ferraris and Runge, 1999). Records of this species from south of the Krishna River drainage (e.g. the Cauvery River) may represent introductions (Jayaram *et al*., 1982). The fish was once found in abundance in the Punjab, Sindh, Balochistan, NWFP and Azad Kashmir. The trend of decline in the population of Seengharee was found in Pakistan due over harvesting and pollution.

Recently, natural stocks of *S. seenghala* have drastically reduced due to natural and man-made catastrophes. These factors have created a serious problem to the genetic resources and thus, the fish has become gradually endangered (IUCN, 1998; Rahman *et al*., 2005a). In order to maintain this fish population as well as to conserve their biodiversity, development of suitable techniques for the rearing and culture of S. seenghala is very essential but no systematic information is available on the culture techniques of this important fish (Jhingran, 1991; Rahman *et al*., 2005a).

2.1 TRANSPORTATION OF SEENGHAREE

No standard procedure for transportation for Seengharee is available in published literature. There are two general basic transport systems that have been adapted all over the world for live fish, the closed system and the open system. (Orlov *et al*., 1973, 1974; Lusk and Krcal, 1974; Orlov *et al*., 1974; Popov, 1975) All the requirements for survival of fish are self contained in the closed system of transportation, which is a sealed container. The sealed plastic bag containing oxygen and water is the simplest of close system. (Woynarowich and Horvath, 1980, Hamman, 1981). The most simple of open system is a container with an air pump and stone. The methods of fish transport in sealed medium are described in detail in several studies (Ioshev, 1980; Amend *et al*., 1982; Garadi and Tarnai, 1983; Pecha-Berka and Kouril, 1983).

The effects of loading density with time scale under different water medium; temperature with relation to fry size and oxygen consumption; and the effect of saline water bath before transportation were evaluated by Islam and Hossain (2013). Mortality rate was significantly increased with time scale and loading density under different water medium. Higher mortality was observed at 400 g L^{-1} loading density (river water 82%, pond water 70% and tube well water 67.5%) than at 200 g L-1 and 300 g L-1 loading density after 24h. Oxygen consumption rate increased significantly with increasing temperature and decreased significantly with increasing fry body weight. Therefore, mortality rate could decrease (22%) if fish fry are bathed in saline water before journey and tube well water can be used as a medium for long distance transportation.

The deterioration of the quality of transport water owing to secretion of metabolic wastes is major limiting factor to raise the fish loading density in a live fish transport system. To control the quality of transport water during transport many techniques have been made. These comprise fish starvation before packaging, addition of anesthetics, lowering the temperature of transport water and ion exchange resin. A sufficient level of dissolved oxygen is the most critical factor in transportation of fish. However, an abundance of oxygen within a tank does not necessarily indicate that the fish are in good condition. Water temperature, tolerance to stress, pH, metabolic products such as ammonia and concentrations of carbon dioxide directly affect the ability of fish to use oxygen (Phillips and Brockway, 1954; Nemato, 1957; Norris *et al*., 1960; Amend *et al*., 1982; Takashima *et al*., 1983; Teo *et al*., 1989; Lim and Chua, 1993; Teo and Chen, 1993; Guo *et al*., 1995a b).

Disturbance of fish by handling increases oxygen requirements three to five times, for example, after the end of the transport, the normal level of oxygen metabolism in salmon fry require numerous hours. The oxygen content in water usually is not a limiting factor during fish transport in closed systems because it contained pressurized oxygen atmosphere. In few cases when transport is longer than the fish can stand or loading density of fish is high the oxygen deficiency may occur (Lusk and Krcál, 1974).

According to Berka (1986) several factors including temperature of the water, the duration of transportation, water quality, density and size of the fish, duration of the depuration period before fish transportation and physical condition of the fish are major factors which contribute toward the transportation success. The dissolved oxygen availability in the water directly affects the juvenile survival. However, the mortality during transportation of fish may occur due to high ammonia and carbon dioxide levels, since they accumulate in the water and may reach toxic levels (Wedemeyer, 1996).

To reduce stress during juvenile fish transportation, salts and anaesthetics are widely used (Guest and Prentice, 1982; Ross and Ross, 1999). The sodium chloride (salt), gypsum and bezocaine, which are relatively cheap and easy to use are recommended products. The gypsum and salt helps the fish to maintain their homeostasis by reducing the osmotic gradient between fish and the water. For juvenile tambaqui, an efficient and safe anaesthetic is Benzocaine (Wedemeyer, 1997),

Sealed plastic bags containing small quantities of water and pure oxygen are often used for transportation of fish, shellfish, and plants. From the bag, surplus air is removed and filled with pure oxygen. The bag placed in a Styrofoam box and finally into a cardboard shipping box and shipped. The shipment in bag is the best choice for the shipper due to quite a lot of causes. First, if fry and small size fish are being transported in large tanks, it may not be damaged. Second, the bag shipment have economic advantages over standard tank transportation where the great distances are involved (Jadhav, 2009).

2.2 LENGTH WEIGHT/MORPHOMETRY

The south Asian countries are inhabited by over 930 fish species (Talwar and Jhingran, 1991) of which Siluroidei (catfish) comprise about 142 species belonging to 13 families and 46 genera (Jayaram, 1977). The potentially culturable catfish species in South Asia are represented by the family Bagridae. One of the most common species of Bagridae found in South Asia is the giant river catfish *Sperata seenghala*. This species is found throughout Pakistan, India, Bangladesh, Nepal and Afghanistan (Talwar and Jhingran, 1991; Jayaram, 2002). It is easily identifiable by its silvery flanks, broad spatulate snout with smooth upper surface, brownish-gray back, and belly and a dark well-defined spot on the adipose dorsal fin. This species is mostly found in rivers, while, other freshwater habitats also inhabit this species (Talwar and Jhingran, 1991). The giant river catfish is a very imperative commercial species, as it contribute significantly to the total inland fish production in South Asia. Due to the low number of intramuscular bones and its tasty flesh it is the most favorite fish species in Pakistan. Among the catfishes it is also a popular species for capture as its price is also higher than carp (Tripathi, 1996).

In domestic markets of Pakistan, Giant river catfish are of immense demand; however, the pond culture of this fish has not yet been explored in Pakistan. In the domestic market of Pakistan the whole demand for this fish is met through capture from natural bodies. Therefore, to conserve the wild stock of this fish effective management is important (Tripathi, 1996).

The fundamental information about the biology of the species, including knowledge of population structure is necessary for the management of fish resources. For the development of management strategies, this type of information is useful for the protection of the biodiversity associated with different stocks, species, sub-species, and races (Turan *et al*., 2005). For sound management and successful commercial fishing of this species, comprehensive understanding on the population structure of the species is needed.

By measuring weight and length of a particular fish all over their life, the relationship between weight and length of a fish in a given population can be analyzed. Weight-length relationship is usually used for two different objectives. Firstly, the weight and length mathematical model is described, so as to derive one from the other (Wootton, 1998). Secondly, to compute the departure from the predictable weight for length of the individual fish or a group of fishes, the weight length relationship is used; it is a sign of fatness or degree of well being (Condition factor) of fish. Condition factor helps to evaluate the improvements in experiments in a situation for an existing fish and for the purpose of new stocking. For assessments of fish population on commercial scales, weight-length relationship is used (Steeby *et al*., 1991; Ali *et al*., 2000).

The cube of linear dimension (Length) is related to weight, which represents the growth in terms of increase in volume. The relationship between length (L) and weight (W) usually is the allometric form: W = aLb. This logarithmical transformation expression is suggested by Le Cren (1951), Log W = Log a + b Log L Where L stands for total length, W weight, ‘b’ is the exponent or growth coefficient, and ‘a’ is constant. The fishery biologists mostly used these relationships, if growth coefficient “b” has the value b = 3.0 then growth is isometrical, means that fish retains the same shape (Ali, 1999). An allometric growth is represented if the value “b” is significantly smaller or larger than b = 3.0 (Bagenal and Tesch, 1978). The well being of fish is determined by an index known as condition factor (K) which is calculated by weight- length relationship. Fish with a low “K” value are lighter while fish with high value of K are heavy for its length, (Bagenal and Tesch, 1978; Wootton, 1998; Zafar *et al*., 2003).

For the identification of different fish stocks and for separation of species, populations and races, the morphometric analyses have been very useful (Turan *et al.,* 2004, 2005). Further, to evaluate the interactive effect of heredity, selection and environment on the body shapes and sizes within species the morphometric studies of fish populations are important (Cadrin, 2000). Numerous studies have been conducted on the comparative morphometric of different fish populations (Nakamura, 2003; Turan *et al*., 2005; Ibanez- Aguirre *et al*., 2006). The individuals with the identical morphometric characteristics are often understood to comprise of a stock and this rule has been used generally in fishery stock differentiation studies (Avsar, 1994).

2.3 ACCLIMATIZATION

The cannibalism is a common ecological interaction and has been reported for more than 1500 species (Elgar and Crespi, 1992). Cannibalism is not only restricted to carnivorous species, but is commonly found in herbivores and detrivores. Cannibalism has been classified into seven types, depending on life-history stage, age difference between cannibal and prey, and whether or not they are related. The main constraint for commercial culture of catfishes is the non-availability of stocking material. The survival during its rearing period is greatly reduced due to high cannibalism, which is noticed even in the day old larvae in some catfishes. Some of the factors like fish and food density, size difference, space for activity, delayed feeding, suitable feeds etc. are known causes which influence aggression among the fishes. Provision of an acceptable feed during this critical period is the most important factor affecting the survival of catfish fry. Loses due to cannibalism can be minimized by providing cover (shade) and adequate amounts of high quality feed.

Survival and cannibalism of *Wallago attu* larvae has been investigated by Sahoo *et al.* (2002) at different stocking densities during in-door larval rearing. The larvae of average weight 2.64 ± 0.21 mg and length 5.80 ± 0.36 mm were stocked at a density of 2,4,6,8 and 10 nos/l and reared by feeding live zooplankton, for a period of 10 days. The percent survival of larvae decreased significantly with the increase of stocking density and the rate of survival was similar among 6nos/I-10 nos/I groups. The loss of larvae was observed due to cannibalism during rearing. The lowest stocking density resulted in least cannibalism. Systematic approach and some anatomical features of *W. attu* larvae are believed to be helpful for predation during rearing.

Kasi *et al.* (2011) investigated the effect of different feeding rates on the survival and cannibalism of African catfish, *Clarias gariepinus* fingerlings (initial weight; 1.629 ± 0.016 g). Four feeding rates were evaluated (2%, 5%, 8% and 12%), as a percentage of fish body weigh. Fish survival did not increase by providing more feed. Cannibalism was not reduced by providing commercial feed. Folkvord and Ottera (1993) opined that strict size-grading reduced cannibalism in Atlantic cod. It was reported that cannibalism among the larvae led to size hierarchy and the cannibals got spurt of growth after each predation and become more efficient than the smaller ones. The difference in growth between cannibals and non-cannibals has been reported in other fish species (Hecht and Appelbaum, 1988; Folkvord and Ottera, 1993).

The effect of segregation on the performance of *W. attu* larvae were studied by *Sahoo et al*. (2002). The higher rate of survival was reported in segregated tanks in comparison to non-segregated tanks. This might be due to the absence of bigger cannibals in the segregated tanks decreasing the chance of predation. Fujiya (1976) and Parazo *et al*. (1991) also observed the beneficial effect of segregation in different fish species. They reported that suitable management practice and appropriate feed could significantly reduce cannibalism among *W. attu* larvae.

Seengharee, *Sperata (S) seenghala* is an Indus catfish (family Bagridae) present in Pakistan and Indus drainage system in India. It is a carnivorous fish, feeds mainly on animal food (Nawaz *et al*., 1994). Ahmad (1943) pointed out that there was only one species of the singhari *(Mystus aor)* in the fish market of Lahore. However, subsequent authors have recorded two species viz., *Aorichthys aor* (Hamiliton) and *Aorichthys seenghala* (Sykes) from Pakistan (Ahmad, 1963; Qureshi, 1965). It was further pointed out by Mirza (1990) that there was only one species, *Aorichthys aor* present in Pakistan. This necessitated a thorough survey of the singhari in Pakistan. As a result of this survey, it was found that the population of singhari in Pakistan resembles to *Aorichthys aor* in its structure of snout and the number of rays in the caudal fin. It differs from the typical form in the length of maxillary barbels. The maxillary barbels reach at the most to anal fin and not to the caudal fin base as in typical form from the Ganges (Hamilton, 1822). Based on the shape of the snout and the number of caudal fin rays, Mirza *et al.* (1992) concluded that the Indus River specimens were similar to *Aorichthys aor* but differed sufficiently in the length of the maxillary barbels. It also differs from *Aorichthys seenghala* in the structure of snout and the number of caudal fin rays. The Indus population was therefore described as a new subspecies of *Aorichthys aor;* it was named as *Aorichthys aor sarwari (*Mirza *et al.,* 1992).

As pointed out by Ferraris and Runge (1999), the Indus *seenghala* is more closely related to *S. seenghala* than *S. aor*. It can however be differentiated from *S.*

seenghala in the relative length of the nasal barbels and the anterior margin of the snout. *S. sarwari* agreed with *S. seenghala* in most of the characters, but it differs from S. seenghala in the length of the nasal barbels and the shape of the snout. In *S. sarwari* the snout is round as compared to the snout of *S. seenghala*, which is truncated.

The seengharee was once found in abundance in the Punjab, Sindh, Balochistan, NWFP and Azad Kashmir. Few years back, Punjab fisheries department used to catch seengharee from river Ravi, river Sutlej, river Chenab and other related drains. Recently industrial effluents have started entering in the drain water that fall into these rivers. Pollution, over-fishing and depletion of natural breeding grounds have caused irreparable damage to its existence. The status of *S. sarwari* is more or less "endangered" due to poor knowledge of its biology, but also due to the declining stocks of this fish in natural waters due to over fishing and pollution. In Pakistan, limited information is available on the meristic and morphometric characteristics of this important fish. In view of the importance of this fish, there is an urgent need to study its biological performance in natural water bodies of Pakistan. The present work is planned to study the morphometric and meristic characters of Mangla Lake inhabiting fish specimen of *S. sarwari* for their identification in relation to the species/varieties.

2.4 DIETS DEVELOPMENT

Nutrition is one of the most important factors influencing the capability of cultured fish to show its genetic potential for growth and reproduction. Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. Since Seengharee is carnivorous in nature and feeds on small fishes and other aquatic worms, it requires a sufficient supply of protein in its feeds (Ferraris, 2007). The major variable operational cost for catfish production is cost of feed, and high quality commercial diets include a considerable amount of high-quality, expensive protein.

Catfish must be fed with a feed that is 100 percent nutritionally complete. The most costly component of fish feed is protein, therefore, the accurate determination the protein requirements for each species and size of cultured fish is important. Research has shown that catfish feed is nutritionally complete if it contains 28 percent protein content. But in case of fingerlings and juvenile catfish, the percentage increases to 32 percent as they are growing much faster than larger fish (Wurts, 2005). In the catfish feeds, the optimal level and quality of dietary protein depends upon a number of factors; including protein in the diet, the balance between energy and protein, feeding rate and the amino acid composition of the feed. The average protein levels in aqua feeds is normally 28-32% for catfish, 18-20% for marine shrimp, 38-42% for hybrid striped bass and 32-38% for tilapia. Protein requirements are usually higher for carnivorous (flesh-eating) followed by omnivorous fish (plant-animal eaters) and lower for herbivorous fish (plant eating). The fish reared in high density (recirculation aquaculture) have higher protein requirements than low density (pond aquaculture) aquaculture (Houlihan *et al.*, 2001).

Ingredients like soybean meal, canola seed meal and sunflower meal can be used in fish feed because of relatively high protein content and well balanced amino acid profile to fulfill the requirements of fish nutrition (Storebekkan *et al*., 2000). Several experiments conducted with channel catfish in ponds have revealed that diets containing 28-32% crude protein primarily from soybean meal provided the growth equivalent to diets containing some animal protein such as fish meal, meat and bone meal (Robinson and Li, 1999; Li *et al.,* 2000).

Fish farming and particularly carnivorous fish farming depends on high protein feeds. The fishmeal is traditional source of protein, produced from fish caught from wild, while source of the lipid is the fish oil from the same source. As the area of fish farming is continually increasing, the assessment of alternative protein ingredients and lipid feed ingredients is needed. The alternate sources of protein are of plant origin that are relatively of low cost and are abundant. However, generally, growth performance seem to be reduced by the replacement of the fishmeal with plant protein ingredients in Atlantic salmon (Olli *et al*., 1995; Refstie *et al*., 1998; 2000; Storebakken *et al*., 1998; Carter and Hauler, 2000; Krogdahl *et al*., 2003; Opstvedt *et al*., 2003) as well as in other fish species of commercial interest such as Atlantic cod and *Gadus morhua* (Von der Decken and Lied, 1993; Hussain *et al*., 2007).

Supplementary feed directly increases the fish production. Two types of artificial feeds are in common use, which are rice polish and gluten. Rice polish is a by-product of rice industry. It is finely powdered material obtained by polishing the rice kernel after the removal of hulls and bran. It contains 12.7% fiber, 3.6% fat 12.4% protein and is rich in thiamin and riboflavin. It becomes rancid during storage, so should be fed as fresh as possible. It is easily available at very low cost and it is widely used in fish culture as well as in poultry. Gluten is a by-product of maize. The protein content of gluten is 50-60% but in pure form it contains 60% proteins. It is an important source of nutrients in its own i.e. amino acids, vitamins, minerals and energy. It contains 28% protein, 2% fat and 10% fiber. Aziz (1983) studied the effect of artificial feed (95% rice bran and 5% fish meal) and fertilizers on the growth performance of *Labeo rohita* and reported a considerable increase in fish production. According to Sheri and Ahmad (1983), artificial feeds comprising of different ingredients increased fish production.

The response of *Catla catla* toward different levels of supplementary feeding (rice polish), indicates that maximum weight gain in fish was observed in that pond in which supplementary feeding was done at the rate of 4 % of body weight of fish per day, followed by 6, 2 and 0% respectively (Akram *et al*., 1994). Ghosh *et al*. (1984) studied the effect of artificial feed (rice bran) and mustard oil meal 1:1 on the production of common carp (*Cyprinus carpio*) and water quality in paddy-cum-fish culture. The feeding rates were 2, 4 and 6% of total body weight. The growth of an individual fish and total fish yield increased with increasing feeding rates till 4% body weight .They reported that feeding beyond 4% was wasteful and accumulation of feed caused deterioration of water quality.

Omer (1986) provided 12 test diets containing 18, 25, 33 and 40% crude protein from animal or plant sources or both for 98 days to carp fingerlings in 24 circular, 35 litter plastic tanks. Carps were fed 6 days a week at a rate of 3% of body weight and daily feed allowances were increased weekly on the basis of weight gain. The diet containing 33% crude protein from animal sources produced greatest growth and feed utilization. The mixture of animal and plant protein in the diets increased growth and feed utilization more than did the plant protein when given alone.

Ahmad (1991) reported the best growth performance of *Channa marulius* where the pond was treated with organic and inorganic fertilizers, artificial feed (rice bran) and Tilapia as forage fish was available. This was followed by the treatment where fertilizers and Tilapia were available, and then treatment where fertilizer and artificial feed were added and minimum growth was observed in treatment where only fertilizers were used for *Channa marulius*. Das and Ray (1991) examined the carp (*Cirrhinus mrigala*) fingerlings (5.7g $\pm$ 1.79g), kept in 50 liter tanks at 29.0-30.5 ^{o}C and fed on pelleted diets containing 5, 15, 25, 35, 45% casein at 5% body weight twice daily. Maximum net weight gain was recorded with feed containing 35% dietary protein.

Silva and Gunasekera (1991) studied the growth of *Catla catla*, *Lebeo rohita*, *Cirrhinus mrigala*, *Ctenopharyngodon idella* and *Cyprinus carpio* in relation to the dietary protein content. The dietary protein content at which maximal growth occurred was 45% and economically optimal dietary protein content was 31%. Khan and Jafri (1991) studied two sized classes, about 0.134 $\pm$ 0.004 and 5.12 $\pm$ 0.46 g body weight of *Catla catla* fed on a series of energetic (307 Kcal/g) purified diets containing 25, 35, 40 and 45% protein twice daily for 6 weeks. They reported a weight gain for both classes and indicted a requirement for 40% protein in small class and 35% protein in the large class.

Hasan and Das (1993) fed fingerling carp (394 ± 0.12g body weight) on diets having 0, 25, 50, 75 and 100% poultry offal meal (POM) replacing fish meal. All diets contained about 30% protein; no significant variation was reported in growth response and feed conversion ratio among diets containing 50, 75 and 100% POM but all these diets gave better ($P<0.05$) performance than 0 and 25% POM diet. Protein efficiency ratio values for control and POM diets ranged between 1.21 and 1.36. Apparent net control and POM diets ranged between 14.64 and 23.43%, the highest value being with 50% POM diet. Increasing POM in the diet increased carcass, moisture and lipid contents. In a study by Belogu *et al*. (1993) channel catfish of initial body weight 13-15 gm was given diets containing 0, 5, 10, 15 and 20% whole sunflower seed at 5% fish body weight until fish attained 30gm body weight, then feeding rate was reduced to 3.5% body weight. Final live weight of fish was significantly higher for the fish that consumed 15 and 20% whole sunflower seed compared to those that consumed other diets.

Javed *et al*. (1993) reported the response of artificial feed (30% crude protein) in the major carps reared in ponds. They reported that the artificial feed increased the productivity in two ways i.e. (i) direct utilization of feed and (ii.) indirect response of left over feed in terms of planktonic productivity. The planktonic biomass accounted for about 42% of variations in the fish yield. Correlation coefficients between increase in fish yield and planktonic productivity in both treated and control ponds were significant. Added nitrogen in the form of crude protein resulted in significant increase in the wet weight, fork length and total length of the fish. The maximum benefit from artificial feed was derived by *Cirrhinus mrigala* followed by *Labeo rohita* and *Catla catla*.

Parveen and Sheri (1994) determined the effect of supplementation of three protein mixtures containing 40, 45 and 50% crude protein on the growth performance of major carps. The result showed significant differences among weight of fishes raised under different protein levels of supplemental mixtures. The higher growth rate of *Calta calta*, *Labeo rohita* and *Cirrhinus mrigala* was obtained at 40% crude protein followed by 45 and 50% crude protein. In another study (Hassan and Jafri, 1994), the optimum feed requirement of cat fish, *Calarius batrachus,* fed on purified diet (40% crude protein) at 0 to 80% body weight per day was reported. A linear (r = 0.985) increase was reported in daily average growth increment up to a feeding level of 4% body weight per day. The optimum feeding rate for this species in terms of specific growth rate and conversion efficiencies seem to be 3% of body weight per day at 30 $\pm$ 2^{o}C.

Batool (1999) evaluated the effect of supplementary feed containing maize gluten (20% crude protein) on growth performance of major carps in fertilized ponds. A significant difference among weight of fish raised in two ponds was recorded, the treated pond have 2.72 times greater net fish production as compared to that of control pond. Similarly, the artificial feeds containing rice polish was evaluated for the growth performance of major carps. The average body weight gains were 179.5, 74.5 and 201.4 g for *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* respectively in ponds without artificial feed. However, in treated ponds, the gained average body weight were 429.0, 449.8 and 345.6 g for *Labeo rohita, Catla catla* and *Cirrhinus mrigala* , respectively (Jahangir, 1999).

The animal origin feedstuffs are usually considered best protein sources because of higher protein content and their indispensable amino acids profile that is superior to those of plant origin (Robinson and Li, 1998). The fishmeal has been replaced by other animal protein sources such as black soldier fly pupae meal (St-Hilaire *et al*., 2007), maggot meal (Adewolu, 2010), poultry by-product meal (Turker *et al*., 2005), feather meal (Hasan *et al*., 1997) and poultry viscera meal (Usman *et al*., 2007). Complete replacement of fishmeal was not possible with most of the single animal protein sources (Tacon and Jackson, 1985).

A large variety of alternative fish feed ingredients together with combinations of ingredients from animal origin will find their way in future fish feeds (Glencross *et al*., 2007). The high cost of fish feed is one of the major problems facing catfish production, which is caused by the increasing price of fishmeal, the main protein source in fish diets. Reduction in the fishmeal content of aqua feeds by plant protein sources of excellent nutritional quality that are readily available and more cost effective is necessary for the sustainability of the catfish culture industry.

The catfish production cost and the profitability of catfish farming is significantly affected by the feeding practices. During the feeding season (May through October), channel catfish are normally fed to satiation once daily, in the southeastern U.S. (Tucker and Robinson, 1990), while during the winter season when low water temperatures repress voluntary feed intake, the fish were fed occasionally or not at all (Hawke *et al.*, 1998). To maximize the growth rates during the production season, catfish are fed to apparent satiation (i.e., as much as they appear to be willing to eat without feed wastage; typically up to 100 kg ha^{-1} day^{-1}) (Reigh *et al.,* 2006). With feed restriction in channel catfish, the decline in growth has been reported (Gaylord and Gatlin, 2000; Peterson and Small, 2004), however, changes in gene expression and the fundamental physiological responses associated with the growth depression due to feed restriction are not well known (Weber and Bosworth, 2005).

For growth and maintenance, fish require amino acids. As these are naturally found in proteins, the amount of protein required by fish varies with the amino acid composition of the protein and the species. Protein requirements of trout are 28% CP as reported by Phillips et al. (1957). De-Long *et al.* (1958) reported that within the temperature range of 8.3-12.8°C, salmon require 40-55% protein. For optimum growth of channel catfish approximately 25% protein is needed (Nail, 1962). For the grow-out of catfish presently, 28-32% protein feeds are recommended (Robinson *et al.,* 1994).

When channel catfish were fed with high protein and high energy diets, the growth rate was increased (Gaylord and Gatlin, 2001). Channel catfish fry have higher protein requirements than those of fingerling or adult fish. The feed of 52 % and 40 % protein are necessary for catfish fry and fingerling weighing 0.02 g and 20 g, respectively (Robinson, 1994). Further, the availability of dietary protein can differ considerably among different sources. Plant and animal sources of feed stuff have 65 % to 92 % apparent crude protein digestibility for catfish (Brown and Strange, 1985; Gaylord and Gatlin, 1976; Hossain *et al*., 1997).

Davis *et al*. (1993) studied the commercial-type diets containing 24, 28, 32, 36 or 40 percent protein in production ponds and reported that catfish fed the lowest-protein diet had more body fat than those fed higher-protein diets. The lower protein diets may increase the fat content of catfish. When catfish were fed to satiation daily in ponds with diets containing protein levels varying from 16% to 32%, weight gain of catfish fed 24 % and 28 % protein was not significantly ($p<0.05$) different and higher than that of fish fed 16 %, 20 % or 32 % protein (Robinson and Li, 1997). When fish were fed to satiation, feed conversion is less efficient (Andrews, 1979; Li and Lovell, 1992; Munsiri and Lovell, 1993). This is partially due to more feed being wasted when fish are fed to satiation. Therefore, feeding a relatively high percentage of protein on a restricted basis may be more efficient than feeding a lower percentage of protein to satiation (Cole and Boyd, 1986; Cho and Lovell, 2002).

Ali *et al.* (2003) evaluated the optimum feeding level for carp's species. The supplementary feed was added daily at the rate of 2%, 4%, 6% and 8% of wet body weight of fish in earthen ponds. By increasing the feeding levels, the total fish production was increased. However, statistically the difference between 6% and 8% feeding level was non significant and there was an increase in weight gain up to 6% of feeding rate. Food conversion ratio (FCR) had increasing trends, while with increase in feeding levels, feed conversion efficiency (FCE) was decreased. There was a highly positive correlation between feed added and weight gain in all treatments. Specific growth rate was maximum in *Cirrhinus mrigala* followed by *Labeo rohita* and *Catla catla*. Optimum feeding level for major carps in earthen ponds came out to be 6% of wet body weight per day.

2.5 WATER QUALITY

Khan and Siddiqui (1974) investigated the seasonal changes in physical, chemical and biological characteristics in fish pond and reported wide seasonal fluctuations in transparency, turbidity and phytoplankton crop. They also reported fluctuations in the carbonate, alkalinity along with pH values mainly due to photosynthesis. According to them, pond understanding remained saturated with dissolved oxygen throughout the period of study. According to Keesen *et al*. (1981), the concentration of dissolved oxygen fluctuated over the whole year and there was a positive correlation between the increase in dissolved oxygen concentration and growth of fish which increased in relation to oxygen supply. Bosserman (1983) studied the dynamics of physico-chemical parameters in Oekefenokee Swamp, USA and reported that temperature, electric conductivity, dissolved oxygen, free CO_2, K^+, Na^+, Mg^{++} and Ca^{++} were affected by physical and biotic factors.

Dobriyal *et al*. (1983) reported positive correlation between total alkalinity and pH values and also between diurnal temperature variation and dissolved oxygen contents. Free CO_2 amount decreased during the day and increased during night. The planktonic peak was observed during noon and minimum in the morning. According to Saleem (1985), the maximum values of carbonates (alkalinity) in water were recorded during January, in the range of 20-60 mg/L and from 20-120 mg/L in the control and treated ponds respectively. The bicarbonate alkalinity ranged from 120-420 mg/L in the treated pond and from 300-400 mg/L in the control pond.

Khatri (1985) studied the seasonal variations in ecosystem of the Lokhotia Lake in Rajastan (India). He noted that conditions of ecosystem is reserved in monsoon due to effect of dilution by rains on chemical and biotic factors. In winter, the ecosystem comes back to balance state. Mahboob (1986) studied the seasonal changes in Planktonic life and water chemistry of a commercial fish farm in Faisalabad (Pakistan) and found an increase in chloride concentrations due to stagnation and evaporation of water. The occurrence and distribution of both phytoplankton and zooplankton were affected by season. Productivity of a farm was based on the dry weight of planktonic biomass which ranged from 32 to 126 mg/L. They also reported high values of pH during blooming periods due to phytoplankton blooms (Mahboob *et al.*, 1993; Mahboob *et al*., 1988).

High feed inputs and stocking densities cause the accumulation of large quantities of nutrients waste in the ponds. These nutrients may cause the production for algal propagation, resulting in incidence of toxic algal blooms (Tucker, 1996, Zimba *et al*., 2001) and delayed harvesting from off-flavor compounds produced by algae (Zimba *et al*., 2002). In spite of a fairly wide range of stocking densities (6000-10000/acre), the losses from infectious diseases continue to plague catfish producers. Some of infectious diseases are due to of high stress related with culture conditions such as higher stocking densities and resulting oxygen depletion, (Hargreaves and Tucker, 2003).

In the aquaculture industry, fish diseases are one of the most important problems. A single factor is not responsible for fish diseases but there are the result of the interactions of environment, the fish (host) and the pathogen(s) (Wedemeyer, 1996). If the fish's immune system is not suppressed by a stressor, fish germ interactions are generally harmless. However, if fish are subjected to stressful conditions, fish diseases frequently occur (Pickering, 1998; Plumb, 1999; Wedemeyer, 1996).

Hassan (1989) while working on the physico-chemical aspects of fish pond, noticed that the concentration of magnesium and sodium vary with changes in season. The magnesium was found to vary from 35.98 mg/L (March) to 52.77 (November) and 39.97 (May) to 64.5 mg/L (October) in two different ponds. Similarly sodium varied from 276.0 (April) 492.0 (August) and 280.5 mg/L (March) to 435.6 mg/L (January) in control and treated ponds respectively. Javed *et al*. (1992) while working on growth of 3 fish species namely *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* in fertilized ponds, observed curvilinear relation between the water temperature and increase in fish weight. Okpokwasili and Obah (1991) indicated that ammonia, dissolved CO_2, nitrates, nitrites, sulphates concentration and temperature were higher during day in pond water. They also reported that dissolved oxygen concentration was increased during rainy season but pH showed no seasonal effects.

Mahboob *et al.* (1993) studied the physico-chemical factors like water temperature, light penetration, pH, electric conductivity, dissolved oxygen, carbonates, bicarbonates, total alkalinity, calcium, magnesium, total hardness, chlorides, sodium, potassium, nitrates, phosphates, total solids and total dissolved solids for a period of one year to establish the standards for the contributions of these parameters to the dry weight of planktonic biomass of the physical factors.

They reported a significant interaction (56.98%) between light penetration, electrical conductivity and biomass .The correlation of biomass with magnesium, chlorides, sodium and nitrates were reported to be significant. The overall contribution of physico-chemical factors towards dry weight of planktonic biomass was 78.9%.

Oxygen concentration and photo period are potent influencers on feed consumption, metabolic rate and energy expenditure, and thus, on growth of cold blooded vertebrates, including fish. Therefore, the effects of these environmental factors on fish growth and metabolism warrant thorough investigation (Buentello *et al*., 2000). Maintaining good water quality in production ponds is absolutely essential. Failure to do so will minimally result in poor growth and high feed conversions or maximally a total loss of all fish in the pond. To achieve better production, the farmer must ensure good water quality, maintained 24 hours a day, 365 days a year (Durburow, 2000).

Catfish requires dissolved oxygen at least 4 parts per million (ppm) for routine maintenance and undergoes stress at 3 ppm and will die at 1-2 ppm. The ranges for total alkalinity, total hardness, pH, unionized ammonia, carbon dioxide and dissolved oxygen are 20-400 ppm, 6-9 pH, less than 0.05 ppm and less than 20 ppm, respectively (Morris, 1993). Evidence suggests that for a conventional pond management, most of water quality factors are sustained within tolerance limits of fish especially through aeration. There are few examples of water quality causing acute mortality in catfish, with the exception of dissolved oxygen depletion and temporary increase of nitrite (Hargareaves and Tucker, 2003).

Electric paddlewheel aeration is standard technology in commercial catfish ponds, which are aerated at 1-2 hp/acre (1.8-3.7 kW/ha). In large commercial catfish ponds, paddlewheel aeration cannot affect dissolved oxygen concentration throughout the pond. Rather, paddlewheel aeration produces zones of sufficient dissolved oxygen to maintain the fish standing crop. Paddlewheel aeration is not designed to meet the overall pond respiratory demand, but rather provides sufficient supplemental oxygen to satisfy fish respiratory demand when pond oxygen concentration declines to some critical threshold, usually 2-3 mg/L (Hargareaves and Tucker, 2003).

Water temperature is the most important variable related to dissolve oxygen concentration at dawn. Water temperature affects dissolved oxygen concentration in two ways: (1) the solubility of dissolved oxygen is inversely related to water temperature and (2) respiration rates are directly related to water temperature. Therefore, solubility is slow and respiratory oxygen demand is high during summer (Hargreaves and Kucuk, 2001; Hargreaves and Tucker, 2003).

2.6 REPRODUCTIVE PERFORMANCE

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 and Qayyum, 1963).

Artificial propagation of fish is the most promising and reliable way of ensuring availability of good quality fish seed all year round and sustainability of the aquaculture industry. It involves the use of natural (hypothecation) or synthetic hormones to induce ovulation and spawning in farmed fishes (Viveen *et al*., 1985). As artificial propagation of catfish seems promising, one of the major constraints to fish breeders is the cost of procurement of these hormones. Ovaprim, for instance cost about, Rs. 500 per vial, Ovatide ,Rs. 200 while the cost of a donor cat fish brood stock is approximately Rs. 500 (Nwokoye, 2007). Other synthetic hormones in use include Human Chorionic Gonadotrophin (HCG), Decorticosterone Acetate (DOCA), and Leutenizing Hormone Releasing Hormone (Shepherd and Bromage, 1988).

Induced breeding performance of *Clarias gariepinus* was evaluated by Olumuji and Mustapha (2012) using five different doses of normal saline diluted ovaprim at 0%, 25%, 75% and 100% while undiluted ovaprim served as the control. The ovaprim was administered at the rate of 0.5 ml for each treatment per kg body weight of the fish, represented as treatments A, B, C, D and E respectively. Mean weight of stripped eggs collected were 18.45 g, 17.50 g and 17.25 g in treatments A, B and C respectively with no significant difference ($P<0.05$) in the values. Spawning did not occur in D and E, thus no egg was collected. Percentage fertilization of the stripped eggs in treatments A, B and C were 88.70%, 87.50% and 77.38% respectively with treatment A showing significant difference ($P<0.05$) from B and C. Percentage hatchability from the stripped eggs were 56.58%, 54.07% and 57.75% for treatments A, B and C respectively with no significant difference ($P<0.05$) among the three treatments, while percentage survival of the fry were observed to be 40.27%, 40.87% and 42.52% in treatment A, B and C. There was no significant difference ($P<0.05$) in the survival rate among the treatments. Comparative cost benefit analysis between the control (undiluted ovaprim) and the different doses of normal saline diluted ovaprim shows that normal saline diluted ovaprim at 50% is the most cost effective.

Adebayo *et al.* (2012) evaluated the reproductive performance of male Clarias gariepinus broodstock. Testes from C. gariepinus broodstock were surgically removed; 25% of the testes, 50% of the testes, 75% of the testes, and sperm were removed using syringe after the abdominal cavity is cut opened. They reported no significant difference in sperm production, percentage fertilization, hatchability and survival of the larvae using sperm derived from regenerated testes of the partially gonadectomized *C. gariepinus* and nongonadectomized *C. gariepinus*. It also reveals that partial gonadectomy could not alter the quality of sperm production of *C. gariepinus*. Sperm derived from regenerated testes performed effectively for fertilization of eggs. Based on the results of this study, the removal of 75% of testes during partial gonadectomy proved to be the best as the total number of spermatozoa was more than that of other methods and the sperm were able to fertilize more eggs.

The effects of different levels of ascorbic acid supplementation on growth, reproductive performance and larval quality of *Heterobranchus longifilis* female broodstock fishes were determined by Adeebayo and Fawole (2012). Five feeds were prepared having the levels of ascorbic acid as 0 (control), 50, 100, 150 and 200 mg kg^{-1}. Fish with average weight of 700g were fed with the experimental feeds for a period of 8 weeks. There was a significant difference in percentage fertilization and hatchability in all the treatments. The fish fed with the feed having 200 mg kg-1 ascorbic acid diet showed best percentage fertilization and hatchability. The performance in terms of weight of eggs and fecundity was best by fish supplemented with 150 mg kg-1 ascorbic acid. The progeny percentage survival was maximum in broodstock fed with diet supplemented with 150 mg kg-1 ascorbic acid. They recommend that ascorbic acid requires to be supplemented at 150-200 mg kg-1 in the diet of female *Heterobranchus longifilis* broodstock (Adeebayo and Fawole, 2012).

Economically important fish species have highly variable reproductive success. Usual spawning rates in brood fish ponds can vary from 8 to 80% and average may be as low as 30% (Silverstein and Small, 2004). The investigations to improve reproductive success proposed that the final oocyte maturation is often inhibited (Silverstein *et al*., 1999) in captive silurids, e.g. *Clarias gariepinus* (Burchell) (De-Leeuw *et al*., 1985), *Clarias batrachus* (L.) (Manickam and Joy, 1989) and *Heteropneustes fossilis* (Bloch) (Tharakan and Joy, 1996). Many researchers have confirmed that stress inhibits the reproductive performance (Schreck *et al*., 2001), as exhibited in pike *Esox lucius L.* (De-Montalembert *et al*., 1978), white sucker *Catostomus commersonii* (Stacey *et al*., 1984) and rainbow trout *Oncorhynchus mykiss* (Walbaum) (Campbell *et al*., 1994).

Santiago and Gonzal (1997) investigated the effect of the different diets on growth and reproductive performance of catfish. To calculate the relative performance of the catfish fed alternative broodstock diets, the feed different in protein sources but similar in nutrient composition (crude protein = 42-44%; P/E ratio = 115-120 mg/kcal), were formulated. These feeds were fed to hatchery reared catfish and a significant difference ($P < 0.05$) was reported among treatments (hatching rate).

The information about the quantity of eggs produced by fishes is of immense importance in culture of fish, as based on this information, the rearing facilities requirements and the amount of various kinds of equipment needed is decided. According to Corbin (1948, 1952), if survival is to be estimated, the egg's number produced by fishes should be well-known. The data regarding the fecundity are also valuable for separating different stocks of fish from the same population (Farran, 1938), in knowing the density-dependent factor affecting population size (Simpson, 195I). However, the research done on the number of eggs of Indian freshwater fishes is restricted to a few species.

Bhatt *et al.,* 1977 studied the fecundity of four species of catfishes, namely, *Mystus cavasius* (Ham), *Mystus seenghala* (Sykes), *Heteropneustes fossilis* (Bloch) and *Wallago attu* (Bloch). The fecundity of *Mystus cavasius* and *Mystus seenghala* ranged between 3314 to 63135 and 20064 to 46443, respectively. In

Heteropneustes fossilis and *Wallago attu*, it ranged between 1375 to 46737 and 66070 to 453148, respectively. There was linear relationship between number of eggs and body weight and weight of gonad in all the fish species and a curvilinear relation with length in three fish species excluding *Sperata seenghala*, where it is linear. By taking two variables together, the fecundity of fish species can be most precisely calculated as indicated by multiple regression analysis. The relative fecundity (Egg/body wt.) does not change significantly either with body weight or length in all the fishes studied.

Chapter 3

MATERIALS AND METHODS

3.1 STUDY SITE

The research was carried out at Aquaculture and Fisheries Program (AFP), National Agriculture Research center (NARC), Islamabad, Pakistan. Fish farm unit at AFP, NARC have nursery ponds (0.02 ha each), growing ponds (0.5 ha), concrete raceways, fiber glass circular tanks, fish hatchery, wet Lab with aquaria, tube well (10000 liters cap./hour) and three functional research laboratories (Fish Nutrition Lab, Water Quality Lab and Fish Disease Lab). Program also has a small pelleted fish feed processing unit. The fish farm area has an altitude of 518 m, is located in Pothohar region and lies diagonally from the northeast to southwest, at 33^{0}-42^{0} N latitude and $73^{0-}08^{0}$ E longitude. The climate of Islamabad is humid subtropical, with five seasons Autmn (September, October), Spring (March, April), Winters (Nov to Feb), Summer (May, June) and Rainy Moon Soon (July, August). June is the hottest month, where average heights routinely exceed 38°C. July is wettest month, with heavy rainfalls. January is coolest month with different temperature at different locations. The temperatures in Islamabad drop below zero some time. The minimum weather range is 3.9°C in January and maximum 46°C in June (Pakistan Meteorological Department, 2013).

3.2 SITES IDENTIFICATION, COLLECTION OF *S. SEENGHALA* FROM NATURAL HABITAT AND ITS RANSPORTATION

3.2.1 Sites Selection and Collection of *S. seenghala*

Sperata seenghala (Giant river catfish locally known as Seengharee) is an imperative commercial species, contributing significantly to the total inland fish production in Pakistan. The potential of giant river catfish for aquaculture has not yet been explored. To identify the potential sites for the availability of *S. seenghala,* survey of different water bodies in Punjab, Azad Jammu & Kashmir and Sindh was conducted. The information regarding availability of this fish was collected from local fishermen and provincial fisheries departments. On basis of this information the sites were selected from where fingerlings, juvenile and adults were easily collected and transported to AFP, NARC, Islamabad. Drag net, cast nets and gill nets were used to collect Seengharee.

3.2.2 Transportation of *S. seenghala*

The spinous fins of most of catfishes are the major constraint for transportation which not only damage the containers but also injure other fish during transport. The cannibalism behavior of many fishes, especially when young, is another great threat during transport. According to available information, the techniques for transport of *S. seenghala* were not reported yet. Therefore, the methods described for transport of other fish species were modified and standardized before adoption.

After collection, fish (*Sperata seenghala)* were transported to Aquaculture and Fisheries Program, NARC using two systems of transportation depending on size of fish and the distance of collection site.

i. The open system of transport for live fish

ii. The closed system of transport for live fish

3.2.2.1 Transportation of large size Seengharee using hauling tanks

The live *S. seenghala* (weight 400-500 g) collected from Head Rasul and Mangla Dam, Province of Punjab, Pakistan were transported by using Open System of Transport in hauling tanks. To avoid the damage to the containers and injures during transport, fish were sedated by the two kind tranquilizers.

3.2.2.1.1 Experimental design

The experiment was designed to evaluate the effect of two tranquilizers (tricaine methane sulfonate (MS-222) and quinaldine) with two loading densities (25 and 50 fish per tanks) on survival of *S. seenghala* during transportation. Effective concentrations of MS-222, 20 mg L^{-1} as reported by Dupree and Huner (1984) and that for quinaldine, 25 mg L^{-1} (Woynarowich and Horváth, 1980) were used. The six treatments with three replications were as follows.

Treatment-I	Loading density 25 without tranquilizer
Treatment-II	Loading density 50 without tranquilizer
Treatment-III	Loading density 25 with MS222
Treatment-IV	Loading density 50 with MS222
Treatment-V	Loading density 25 with Quinaldine
Treatment-VI	Loading density 50 with Quinaldine

3.2.2.1.2 Fish loading and transportation

The fish collected from Mangla and Head Rasul was loaded in water (22^{o}C) filled hauling tanks of 270 cm diameter with capacity of 2000L water with fresh air supply system. The fish were transported to NARC, Islamabad (transportation time 4 hours), percent mortality was studied after every two hours interval. Mortalities were recorded during transportation and after stocking the fish in tanks. The dead fish were immediately isolated. The fish were transferred to indoor circular fiberglass tanks (1000 L) under flow throw system.

3.2.2.2 Transportation of fingerlings of *S. seenghala*

3.2.2.2.1 Experimental design

To avoid damage to the containers and fish from the spinous fins of *S. seenghala* during transportation, fish were sedated using Tricaine methane sulphonate. An experiment was performed to evaluate the effective concentration of MS222 and optimum loading density for transportation of Giant river catfish fingerlings. The experiment design was 3x2 factorial, having three sedation doses of MS222 (20, 30 and 40 mg L-^{1}) and two loading densities (50 and 75 fish per bag). Sedation was carried out in two steps, rapid anesthetic doze (200 mgL-^{1} MS222) followed by sedation doze to the fingerlings of giant river catfish collected from Sajawal Sindh, with the average weight of 9.0 g and length of 11.2 cm.

T1 Loading density 50 with 20 mgL-1 MS222
T2 Loading density 50 with 30 mgL-1 MS222
T3 Loading density 50 with 40 mgL-^{1} MS222

T4 Loading density 75 with 20 mgL^{-1} MS222

T5 Loading density 75 with 30 mgL_{-1} MS222

T6 Loading density 75 with 40 mgL^{-1} MS222

3.2.2.2.2 Experimental procedure for transportation

After collection, the fish were starved for 24 hours in circular tanks prior to transport so as to reduce oxygen consumption and ammonia excretion into the water. Fingerlings were then weighed and transferred to plastic bags filled with 5L water and oxygen. The plastic bags were packed in Styrofoam boxes. Transportation proceeded by roads for 3h from Sajawal to Karachi. From Karachi the transportation boxes were air lifted to Islamabad (about 3h time). After arrival at AFP, NARC, the fingerlings were transferred to circular tanks with water flow system. Fingerlings from each bag were kept in separate circular tanks subsequent monitoring (Cavero *et al.*, 2003).

3.2.2.3 Water quality analysis

Important parameters of water quality like pH, temperature, dissolved oxygen were monitored before and after transportation with help of Limnology field meter (Consort Model C6030, Belgium).

3.2.2.4 Data analysis

The data on water quality, mortality rate and survival during transportation using different systems were analyzed through analysis of variance (ANOVA) by using Ms Excel. When F value was found significant, treatment means were compared using Duncan's Multiple Range test (DMRT) by using MSTAT-C Statistical Software

3.3 ACCLIMATIZATION OF SEENGHAREE UNDER CAPTIVE CONDITIONS AND TAXONOMIC STUDY

3.3.1 Treatment of Injured Fish

After transportation of fish to AFP, NARC, the fish which got injuries during collection and transportation were given the following treatment.

- Injured fish were bath treated with $KMnO_4$ at first step
- In the second step, fish were treated (bath) with antibiotics, E.C.M 350 (oxy tetracycline 150gm; Neomycin sulphate 60gm; Furaltadone HCl 150gm in every 1000gm of E.C.M).

3.3. 2 Acclimatization in Captivity

For the acclimatization of wild Seengharee in captivity, indoor fiberglass circular tanks and outdoor concrete raceways were used. The indoor circular tanks had 1000-liter water capacity, running water system with controlled temperature that was maintained at 25 $^{\circ}$C with the help of water heaters. Aerators were used for continuous air supply. Outdoor concrete raceways were having 5000 liter water capacity with flow through water system from tube well.

3.3.2.1 Fish stocking and feeding the fish

After the recovery from injury, the treated fish along with healthy fish were transferred to fiberglass circular tanks and out door concrete raceways. Fifty fingerlings of average initial weight 9.0g were kept in each of five circular tanks, while 100 fingerlings having average initial weight of 8.9g were kept in each of the five raceways. Initial body weight and total length were recorded at the time of stocking.

The fingerlings were offered live trash fish in start, but later on it was replaced with the freshly minced fish meat. After one week, the meat was gradually replaced with artificial diet containing 40% crude protein. Artificial diet was offered in the form of pellets and dough along with minced meat. After complete shifting on artificial diet, the fish were fed twice daily till satiation (at morning and afternoon) seven days a week. The water in the tanks was renewed every morning. Each time, the bottom of the tanks was cleaned thoroughly before offering the feed. Fish (N=10) were collected randomly using hand nets on fortnightly basis and the wet body weight and the total length were recorded. After recording the data on growth parameters, fish were released back into their respective system.

3.3.2.2 Cannibalism study

During the acclimatization and experimentation it was observed that the number of live fish in cemented raceways was decreasing without any mortality. An experiment was designed to study this phenomenon. All the experimental conditions adopted in Experiment-I was followed in Experiment-II. Fingerlings of Seengharee were segregated on the basis of difference in their body weight (15 in each group)

i) Group1: size 5-10 g

ii) Group2: size 11-15 g

iii) Group3: size 16-20 g

iv) Group4: size 21-25 g

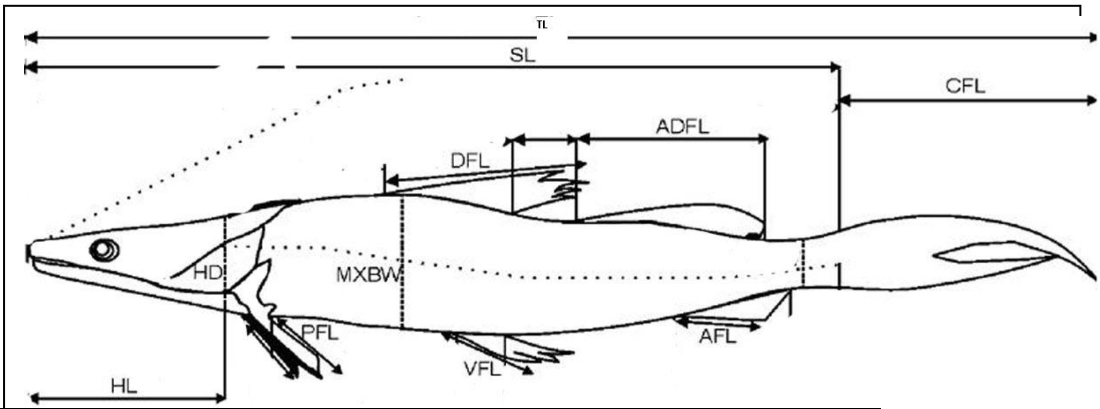
v) Group5: mixed size fish

Circular tanks having water capacity of 1000 L and running water system with controlled temperature were selected. Artificial feed containing 40% crude protein (CP) was fed at 5% of the body weight of fish twice a day. Experiment was conducted for a period of two weeks. The observations were made daily morning to record the survival and disappearance of larvae due to cannibalism. All fish were collected from each tank using hand net, counted at 7-days intervals in order to quantify cannibalism. In each tank the numbers of dead fish were recorded daily and were not replaced. Calculation of cannibalism was done based on difference in numbers of fish in each count; natural mortality was not included (Qin and Fast, 1996). At the end of the experiment, final body weight was recorded and survival, percent weight gain and specific growth rate (SGR) were calculated.

3.3.2.3 Water quality

The temperature of the indoor tanks and outdoor raceways were recorded daily during the acclimatization. Water quality parameters viz., pH, electrical conductivity (EC), dissolved oxygen (DO) salinity, aalkalinity, hardness andtTotal dissolved solids (TDS) were determined with the help of limnology field meter (Consort Model C6030, Belgium).

Figure 1: Diagram of external morphometric characteristics of *S. seenghala*



TL, total length; SL, standard length; CFL, caudal fin length; ADFL, adipose fin length; DFL, dorsal fin length; AFL, anal fin length; VFL, ventral fin length; PFL, pectoral fin length; PSL, pectoral spine length; MXBW, maximum body width; HD, head depth; HL, head length.

3.3.3 Meristic and Morphometric Study of *S. seenghala*

S. seenghala fish transported from Sajawal, Sindh and Mangla Dam, Pakistan, were used for this study.

3.3.3.1 Meristic study

Meristic characters were studied as described by Hubbs and Lagler (1958). Total 20 fishes of mixed sex were used for this study. A magnifying glass was used to count the ray numbers precisely. Meristic counts included pelvic fin rays, anal fin rays, dorsal fin rays, caudal fin rays and pectoral fin rays. In the head region, gill rackers were also counted.

3.3.2.2 Morphometric study

Morphometric characters of Seengharee were measured for 21 (Fig. 1) selected parameters as reported by Turan *et al.* (2005) and Jayaram (2002). Total weight, total length, standard length, fork length, head length, dorsal fin length, head depth, length of anal fin, length of pectoral fin, length of pelvic fin, length of adipose fin, length of caudal fin, longest pectoral fin, longest dorsal fin, longest anal fin, longest pelvic fin, height of adipose fin, longest caudal fin, nasal barbel length, mandibular barbel length and maxillary barbel length were measured for morphometric study of *S. seenghala*.

3.3.4 Length Weight Relationship of Seengharee

To monitor the growth of *S. seenghala* in captivity, length and weight was recorded after the transport. The total length of the Seengharee was measured from the caudal fin farthest tip to the most anterior part of the head with mouth. The fish weight was recorded with electronic balance (model: BL 150 S, Sartorious AG Gottingen Germany). The condition factor was calculated (Weatherly and Gill, 1987; Wootton, 1998) as follows

$$K = W / L^3 \times 100$$

	K	=	$W / L^3 x$ 100
Where:	K	=	Condition factor
	L	=	Total length of fish
	W	=	Body weight

The length-weight relationship was calculated using the regression analysis and was $W = a \pm bL$ where, L is the length of the fish measured in centimeters and W is the weight of the fish in grams. The proportionality constant "a" and the method of least square regression was used to determine coefficient of regression "b" of the length weight relationship (Zar, 1984).

3.4 DIET DEVELOPMENT FOR *S. SEENGHALA* FROM LOCALLY AVAILABLE FEED INGREDIENTS

3.4.1 Growth Performance of Seengharee Fed on Diets Having Different Crude Protein Levels

3.4.1.1 Experimental design

The experiment was conducted for 60 days at Aquaculture and Fisheries Programme (AFP), National Agriculture Research Center (NARC), Islamabad, Pakistan. Twelve glass aquaria of length 60 cm, width 35 cm and height of 30 cm were used. Four types of feeds having 25, 30, 35 and 40% CP were prepared from locally available ingredients. All aquaria were filled with about 42 liters of water and this level was maintained throughout the experiment. Experimental design was CRD with 4 Treatments having 3 replications.

3.4.1.2 Experimental diets

To determine the optimum level of balanced diet for *S. seenghala,* four kinds of artificial diet having crude protein level 25 %, 30 % 35 % and 40% were formulated from locally available feed ingredients viz., soybean meal, fish meal, sunflower meal, gluten, rice polish, wheat bran, oil and vitamin mineral premix (Ng *et al.*, 2001; NCR, 1993). To prepare diets, the dry ingredients were thoroughly mixed with oil in ribbon type mixture and the moist mixtures were pelleted. The feed were then dried in sun light. Proximate analysis of diets was done to determine percent crude protein (Kjeldahl methods), fat (Continuous extraction), fiber (Ether extract method), moisture (Air oven at 105°C), and total ash content (Prosky method, AOAC, 2003). The diets composition and the proximate analysis are shown in Table 1.

3.4.1.3 Experimental procedure

Fifteen fry (*S. seenghala*) with average weight 9.0 g were placed in each aquarium. Body weight was measured and recorded at the time of stocking. Fish were offered feed at the rate of 7% of wet body weight with pelleted feed formulated to contain different levels of protein, twice daily. Aquaria were cleaned daily before giving the feed in the morning. Fish were sampled on fortnightly basis to record wet body weight and the total length. After recording the required data, fish were released back into their respective aquarium. Daily feed ration was adjusted according to total biomass of fish under experiment.

All fish were collected to record the final weight, weight gain, percent weight gain, food conversion ratio (feed offered/wt gain) feed efficiency [(wt. gain/feed offered)100] and specific growth rate {[(ln Wf – ln Wi) × 100]/days} at the end of the experiment. For whole body proximate analysis, four fish from each aquarium were randomly captured with hand net. Carcasses of fish were mixed, dried and crushed. The dry matter, moisture, lipid, crude protein, ash contents and crude fiber of fish were analyzed by standard methods (AOAC, 2003).

Water quality parameters Viz., temperature, salinity, EC, pH, dissolved oxygen (DO), TDS were measured daily with the help of limnology field meter (Consort Model C6030, Belgium) during the experimental period. Alkalinity and hardness were estimated by titration method on weekly basis.

3.4.1.4 Data analysis

The growth performance of fish was calculated through weight gain, feed consumed, FCR and Specific growth rate. All the data were analyzed through analysis of variance (ANOVA) by using Ms Excel. Significant difference among treatment means was tested through a Duncan's Multiple Rang Test (DMRT) by using statistical software MSTAT C (Steel and Torrie, 1986).

Table 1: Percentage and proximate analysis of feed having different protein levels formulated from locally available feed ingredients

Feed ingredients	**F1** (25% CP)	**F2** (30% CP)	**F3** (35% CP)	**F4** (40% CP)
Fish meal	30	30	30	30
Soybean meal	4	10	10	10
Sunflower meal	4	5	10	10
Canola seed meal	4	5	10	5
Rice polishing	25	22	10	6
Gluten 30%	9	19	6	0
Gluten 60%	0	0	10	25
Wheat bran	20	5	10	10
Vitamin –C	0.5	0.5	0.5	0.5
Vitamin premixes	1.5	1.5	1.5	1.5
Soybean oil	2	2	2	2
Total	**100**	**100**	**100**	**100**
Proximate Composition (%)				
Dry matter	89.7	90.0	89.9	90.1
Crude fat *	16.6	15.0	15.3	14.6
Crude protein *	25.2	30.34	34.96	40.1
Total ash *	8.5	11.0	10.0	9.5
Crude fiber *	8.14	7.24	6.54	5.23

*Percentage of dry matter

3.4.2 Replacement of Animal Protein with Plant Protein in The Diet of Seengharee

3.4.2.1 Experimental design

The experiment was conducted in nine circular fiberglass tanks of 1000L water capacity under flow through system (8L/min) Experimental design was CRD with 3 treatments (Three feeds with animal protein 30, 20 and 10 % replaced with plant protein) having 3 replications.

3.4.2.2 Feed preparation

To replace the animal protein in feed of seengharee, three feeds (crude protein 30%) were formulated having different ratios of animal and plant protein (Table 2). Animal protein was replaced with plant protein source (PPS) formulated by a blend of soybean meal and corn gluten 60% in ratio of 1:1. All dietary ingredients were weighed using top loading balance, mixed to homogeneous mass and corboxy methyl cellulose (CMC) was used as binder. The mixture was then pushed with 4 mm die attached to the pellating machine. Diets were immediately sun-dried at ambient temperature (40^{o}C) for two days. After drying, the proximate composition of diets was estimated to determine the moisture content, fat, fiber, crude protein and ash (AOAC, 2003). The diets were stored at room temperature in air tight polyethylene bags prior to feeding.

Table 2: Composition and proximate analysis of different diets containing varying concentration of plant and animal protein sources

Feed ingredients	F1 (Animal CP 30%)	F2 (Animal CP 20%)	F3 (Animal CP 10%)
Fish meal	30.0	20.0	10.0
PPS (Soybean meal + Gluten 60%) **	10.0	20.0	30.0
Sunflower meal	10.0	10.0	10.0
Canola seed meal	6.0	6.0	6.0
Rice polishing	15.0	15.0	15.0
Gluten 30%	10.0	10.0	10.0
Wheat bran	15.0	15.0	15.0
Vitamin-C	0.5	0.5	0.5
Vitamin premixes	1.5	1.5	1.5
Soybean oil	2.0	2.0	2.0
Proximate composition (% dry matter)			
Moisture	7.80	7.20	7.50
Crude protein*	30.34	30.18	30.50
Crude lipid*	15.23	15.18	15.12
Crude fiber	7.2804	7.7273	8.05
Ash*	9.12	9.14	9.17
Nitrogen free extract (NFE)	24.54	24.55	24.57

*Percentage of dry matter
** PPS: Plant Protein Source

3.4.2.3 Experimental procedure

The experimental system consisted of nine 1000 L circular fiberglass tanks under flow through system (8L/min). Fifty juveniles of Seengharee having average body weight 9.11, 9.22 and 9.44 g were placed in each tank. At the time of stocking, body weight was recorded. Fish were fed twice daily at the rate of 5% body weight with experimental pelleted feed. Fish were sampled on fortnightly basis to record wet body weight and the total length. After obtaining the required data, fish were released back into their respective tank. Amount of feed was adjusted according to total biomass of fish under experiment.

3.4.2.4 Data analysis

Data on growth parameters of fish fed on different feed were analysed using ANOVA and comparison of different means were determined by DMRT where applicable. Correlation and regression analysis was performed to find out relationship among different parameters of growth.

3.4.3 Effect of Variable Feeding Rates on Growth Performance of *S. Seenghala* in Captivity

3.4.3.1 Experimental design

To evaluate the feeding allowance for the optimum growth of Seengharee an experiment was conducted in twelve fiber glass circular tanks of capacity 1000 L with flow through system (8 L/m). Experimental design was CRD with 4 feeding rates (2%, 4%, 6% and 8% of wet fish body weight daily) having 3 replications. Duration of experiment was eight weeks.

3.4.3.2 Experimental procedure

Before the start of experiment, all tanks were dried and disinfected with $CaCO_3$. Tanks were filled with tube well water up to 0.9 m height. For 24 hrs the fish were deprived of feed before the start of feeding experiment. The fish having initial mean weight of 11.7± 0.12g were divided into 12 groups each having 50 fish and were randomly stocked into 12 circular tanks. Artificial feed having 30% CP (Feed 2 of previous experiment, Table 2) was prepared by the extruder machine at AFP, NARC. The fish were fed four rates of feeding (2%, 4%, 6% and 8% of wet fish body weight) having three replication in each treatment. The fish were offered feed two times per day at dawn and dusk.

Duration of experiment was 8 weeks. From each tank all the fish were collected on fortnightly basis, measured individually, counted and weighed for two months. The amount of feed offered was calculated according to total weight of fish in each tank.

3.4.3.3 Sample collection and analysis

All fish were collected to record the final weight, weight gain, percent weight gain, feed efficiency, food conversion ratio, protein efficiency ratio (PER), specific growth rate (SGR), and net protein utilization at the end of the experiment. For whole body proximate analysis, four fish from each aquarium were randomly captured with hand net. Carcasses of fish were mixed, dried and crushed. The dry matter, moisture, lipid, crude protein, ash contents and crude fiber of fish were analyzed by standard methods (AOAC, 2003).

Parameters of water quality viz., temperature pH, dissolved oxygen (DO), electrical conductivity (EC), salinity and TDS were measured daily with the help of limnology field meter (Consort Model C6030, Belgium) during the experimental period. Alkalinity and hardness were estimated by titration method on weekly basis.

3.4.3.4 Data analysis

Comparison of various growth parameters of fish fed on different feed was carried out using one-way analysis of variance (ANOVA) and to test significant differences, Duncan's Multiple Range Test was used where applicable. Correlation and simple regression was performed to find out relationship among different parameters of growth.

3.5 PERFORMANCE OF *S. SEENGHALA*

Under this objective two types of performance of *S. seenghala* i.e. growth performance in cemented raceways and earthen ponds and reproductive performance in earthen ponds were evaluated.

3.5.1 Growth Performance of Seengharee in Cemented Raceways and Earthen Ponds

3.5.1.1 Experimental design

An experiment was conducted for period of six months (May to October 2009) to evaluate growth performance and production of Seengharee in out door cemented raceways and earthen ponds. Three outdoor cemented raceways and three earthen ponds were selected for the experiment. The size of Raceways was 7.0 m length, 1.6 m width and with a depth of 1.0 m. Dimensions of nursery pond selected for the experiment were 15×11m with a depth of 1.5m, area 0.017ha.

3.5.1.2 Fish stocking and pond management

Before stocking, the ponds and raceways were dried and disinfected with $CaCo_3$. Water capacity of cemented raceways was 5000 L with running water 15.0 L/min and the source of water was tube well. One hundred Seengharee Juveniles caught from Mangla dam in month of December, having mean initial weight of 100 g were stocked in each pond, while fifty fish with mean initial weight of 104 g were stocked in each raceway. All the ponds were filled with tube well water up to the level of 5ft and this level was sustained all over the experimental period. Body weight was measured and recorded at the time of stocking.

Fish in both raceways and ponds were fed two times daily at the rate of 5% of wet fish body weight with pelleted feed formulated to contain 30% CP diet. The ten fish were randomly captured every month with the help of nylon drag nets. The wet body weight was measured and fish were released back into their respective ponds and race ways. Daily ration was adjusted according to total biomass of fish under experiment.

3.5.1.3 Water quality analysis

During the study period, water temperature was monitored daily in the morning (8 am) and evening (4 pm) using common laboratory thermometer. Electrical conductivity (EC), dissolved oxygen (DO), pH, salinity and TDS were recorded daily by using Limnology field meter (Consort Model C6030, Belgium). Alkalinity and hardness were measured by titration method in the laboratory. No artificial aeration was provided to the ponds during the whole study.

3.5.1.4 Data analysis

The growth performance of the fish was calculated through weight gain, feed consumed, FCR and cost of fish production. All the data were tested by using analysis of variance (ANOVA) and Ms Excel. Means having significant differences were compared through a Duncan's Multiple Rang Test (DMRT) by using statistical software MSTAT-C (Steel and Torrie, 1986).

3.5.2 Reproductive Performance of Seengharee

3.5.2.1 Brood fish development

The fish (size 100-1000 g) were captured (December 2008) from Mangala reservoir, Mirpur, AJK using drag nets, and transported by hauling tanks with fresh air system to Aquaculture and Fisheries Program, NARC, Islamabad. The captured fish were released into circular tanks with water flow through system. Brood fish did not adapt to formulated feed immediately as they are extremely predatory. For two days, the fish were offered fish fingerlings. Then, minced meat was offered as feed for a week. This fish did not accept pelleted feed, therefore feed was offered in the form of dough (a ball of feed) for two weeks. Then, the fish were released in three 0.04 ha earthen ponds. In ponds, fish were fed with 45% CP diet at satiation. Male and female fish were kept separately in different ponds for a period of 1.5 years. During study, water quality parameters were monitored regularly on fortnightly basis

3.5.2.2 Gonadosomatic Index (GSI)

For the study of gonadosomatic index, the healthy at least three fish were collected from ponds on monthly basis for the period of one year. These fish were brought to the laboratory and weighed using electrical balance. To study the gonadosomatic index, the fish were dissected to remove the gonads. The weight of gonads of individual fish was recorded and gonadosomatic index was calculated by using the formula

$$GSI = \frac{\text{Weight of gonad}}{\text{Weight of the fish}} \text{X } 100$$

3.5.2.3 Fecundity and relative fecundity

In order to analyze the reproductive performance of *S. seenghala*, fecundity, and relative fecundity were calculated as described by Qasim and Qayyum (1963). From mature *S. seenghala,* ovaries were collected during late March to early April). Three fish were cleaned with a dry towel, cut along the belly from the cloacal hole to the pelvic fins with anatomical scissors. The lateral muscles were opened and the ovaries were removed carefully. The fish (N=3) were weighed without ovaries and internal organs (Recorded the weight as Wb). Weight of the ovary was recorded (Wo). A small piece of ovary (Size 2cm approximately) was cut, weighed (Ws), and put it in a small beaker, the number of eggs were counted (Recorded the number as S).

Absolute Fecundity = (S/Ws)*Wo

Relative Fecundity = Absolute Fecundity/Wb

3.5.2.4 Data analysis

Data obtained were subjected to statistical analysis, following Steel and Torrie, (1986). Correlation and Regression analysis were performed to find out relationship between different parameters by using Microsoft Excel Program. Descriptive Statistics of data on fecundity and other parameters were calculated.

Chapter 4

RESULTS AND DISCUSSION

4.1 IDENTIFICATION OF POTENTIAL SITES, COLLECTION OF SEENGHAREE AND ITS TRANSPORTATION

For the potential sites identification, collection and transportation of Seengharee, two studies were performed.

4.1.1 Site Selection and Collection of *S. seenghala*

S. seenghala is the most abundant species found in South Asia that belongs to Bagridae family and contributing significantly in economy (Talwar and Jhingran, 1991; Jayaram, 2002). *S. seenghala* has also a great consumer demand in the Pakistan (Mirza and Omer, 1984). The potential of Seengharee for aquaculture has not been explored. Availably of this fish depend on capture fisheries from rivers, dam and reservoirs. Mirza and Omer (1984) reported presence of Seengharee in River Jhelum and other natural water bodies of Punjab, AJK, Baluchistan and Sindh, Pakistan. It is usually found in rivers, canals, ditches, flooded fields and other wild freshwater areas. Seengharee has also been reported from Indus river system by Sahini *et al.* (2008). Two distant populations of Seengharee were reported from the Beas and Sutlej rivers. The present study reported the availability of *S. seenghala* and its collection from natural habitat (Table 3 and Figure 2). Sites visited were, Balloki Headworks, Qadirabad Baraj,

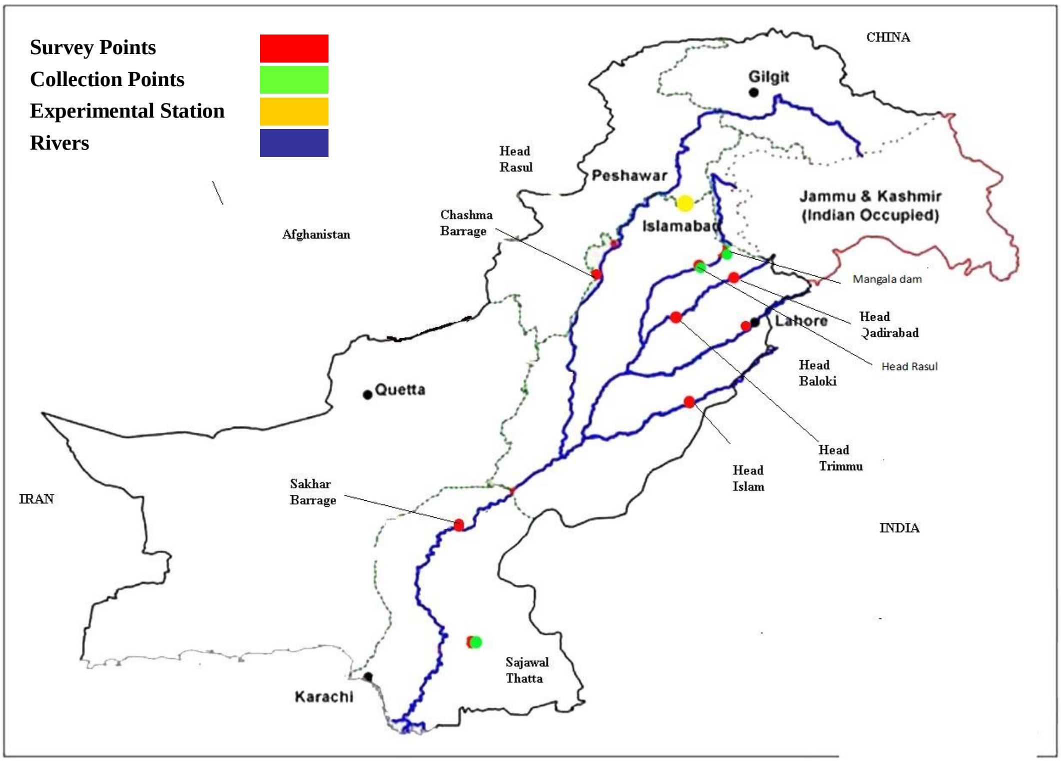


Figure 2: Map of Pakistan showing the rivers, survey sites and collection sites of Seengharee from natural habitat

Table 3: Numbers of fish (*S. seenghala*) captured from river, dam and ponds using different nets

Water body	**Gill nets**	**Drag nets**	**Cast nets**
River running water	2	45	-
River pouches	40	65	1
Dam main water	82	-	-
Dam pouches	-	35	2
Ponds (fingerlings)	Not used	300	5

Rasul Baraj, Chashma Baraj, Trimmu Headworks, Sakhar Baraj, Islam Headworks, Mangla Dam and Pouches of river Indus at Thatta. These water bodies are present on River Jhelum, River Ravi, River Satlej and River Indus. Seengharee was available at all these sites. Sites selected for collection of adult Seengharee were Rasul Headworks and Mangala Dam (River Jhelum). Fingerlings of Seengharee were only available and collected from Sajawal, Thatta, Sindh. The non availability of fingerlings from rest of the sites may be attributed to destruction of breeding grounds in natural water bodies due discharge effluents from industries (Shakir, 2008).

For the collection of Seengharee, drag net was more effective in running water while, gill nets were effective in stagnant waters of Dams and Head works. Drag nets were also useful for the collection of Seengharee from pouches of river and ponds. Seengharee could not be collected at any place with cast net, while the fish caught using gill net sustained injuries. The wild source of fish and collection method was identified in this study which provided base for further studies.

4.1.2 Transportation of *S. seenghala*

The spinous fins of *S. seenghala* were major constraint for transportation which not only damaged the containers but also injured other fish during transport. The cannibalism behavior of this species, especially when young, was another great threat during transport. No technology was yet reported for transport of' this fish, therefore, the available methods for other species were modified and adapted for Seengharee. Based on size of fish and the distance of collection site, two systems of transportation were used to transport the fish to Aquaculture and Fisheries, NARC.

i) The open system -hauling tanks

ii) The closed system oxygen filled polyethylene bags

4.1.2.1 Transportation of large size Seengharee using hauling tanks

To minimize stress and to reduce activity in fish, anaesthetics are useful (Iversen *et al*., 2003). The sedation reduces general activity, metabolic rate and hence oxygen demand and ultimately mitigate the stress response and increase ease of handling (Cooke *et al.,* 2004). For the fish transport, deep sedation is referred the ideal level of sedation, including decrease in metabolic rate and loss of reactivity to external stimuli but with maintenance of homeostasis (McFarland, 1959). Generally the choice of anaesthetics depends on cost-effectiveness, ease of use, safety for the user, availability and nature of the study (Cho and Heath, 2000).

In present study, Seengharee was transported to Aquaculture and Fisheries Program, NARC using water filled hauling tanks with fresh air supply using anaesthetics, MS222 (loading density of 25 and 50) and Quinaldine (loading density of 25 and 50)**.** After two hours of transportation period, the mortality of Seengharee was maximum at in T_1 and T_2 (fish without any tranquilizer at both loading densities) followed by T_6 (with Quinaldine at higher loading density); While there were no mortality in $T_{3,}$ T_4 (tranquilizer MS222 at both loading densities) and T_5 (Quinaldine at lower loading density). After four hours transportation period there were no mortality in $T_{3,}$ (tranquilizer MS222 at lower loading densities) (Figure 3). Accumulated mortality after 6 h of transportation were maximum in the hauling tanks without any tranquilizer followed by Quinaldine, while minimum mortality was observed in Seengharee sedated with MS222.

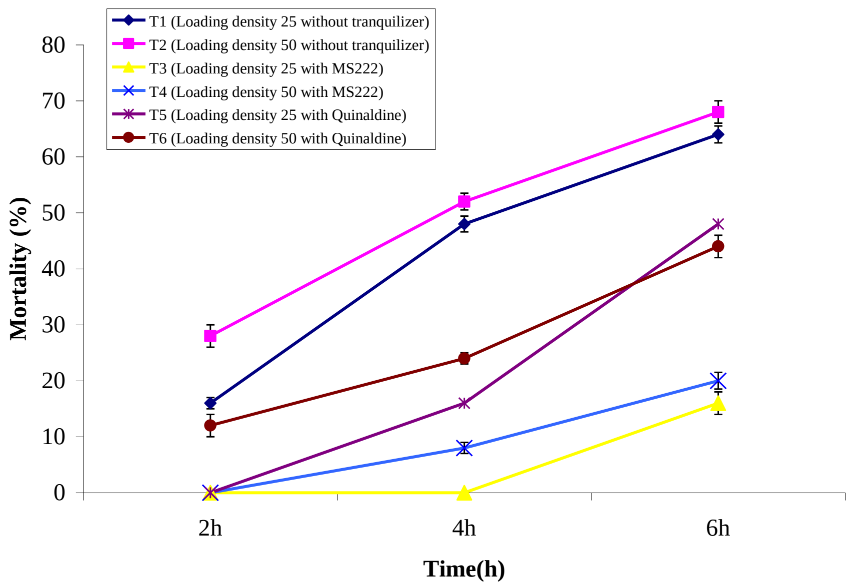


Figure 3: Mortality (%) of Seengharee during transportation in hauling tanks

Table 4: Survival (%) of large sized Seengharee during transportation in hauling tanks having different anesthesia and loading density

	Without tranquiller	**MS222**	**Quinaldine**	**Mean**
Loading density 1 (25fish per Tank)	36e	84.0a	52.0d	**57.5A**
Loading density 2 (50 fish per Tank)	32f	80.0b	56.0c	**56.0A**
Mean	**34.0C**	**82.0A**	**54.0B**	

The values with different alphabets are significantly different ($p<0.05$)

The interaction between tranquilizers and loading densities during transportation of Seengharee in hauling tanks from Mangla Dam and Head Rasul was highly significant (Table 4). In present study anesthesia proved much effective for transportation of *S. seenghala*; it insured not only the safety of containers but also the safety of fish from hard spines. Use of anesthesia during transportation of fish was effective both for closed and open transportation system.

Fish transported at the lowest density (25 per tank) could withstand the entire procedure without any measurable change. The fish held at high densities are usually under stress (Procarione *et al*., 1999). Our study supported the work of Gomes *et al* (2003) who reported lower mortality, less stress and acceptable range of water quality parameters during transportation of juvenile tambaqui at lower densities Gomes *et al.* (2003).

With the increase in time of transport of fish in plastic bags, increased mortality has been reported (Gomes *et al.,* 2003). When the time of transport increases, oxygen level starts depleting and a rise in temperature during transportation is unavoidable. Even though catfish can tolerate temperature extremes in their natural environment (Ali and Ahmad, 1988), the water temperature in the plastic bag must be allowed to equilibrate slowly with the water temperature of the place to be stocked to avoid shock that might kill the fry (Sampson, 1987).

4.1.2.2 Transportation of Seengharee fingerlings in oxygen filled polyethylene bags

Data on survival of fingerlings of Seengharee during transportation from

Sajawal Sindh to AFP, NARC, Islamabad in air filled polyethylene bags (Gomes *et al.,* 2003) having different doses of anesthesia and loading density are given in Table 5. The survival of fingerlings of Seengharee was maximum in T2 (30 mg/L MS222 with loading density 50 fish) flowed by T5 (30 mg/L MS222 with loading density 70 fish) and T3 (40 mg/L MS222 with loading density 50 fish), while minimum survival was observed in T4 (20 mg/L MS222 with loading density 75 fish) and T1 (20 mg/L MS222 with loading density 50 fish).

In present study, MS222 at 30 mg/L was found best for the transportation of fingerlings of Seengharee in plastic bags. The results were in agreement with Shawn *et al*. (2004) who reported 25 to 50 mgL^{-1} of MS222 for sedation of *Ictalurus punctatus* (Channel catfish). To sedate fish prior to transport, anaesthetics should be added to the water at low doses; if heavily sedated, the fish cease swimming, loose equilibrium, and if they all are settled to the tank bottom fish will die from suffocation. Some species, including tilapia required up to 100 mg/L for sedation. The effective dose of MS222 for trout is 40 mgL^{-1} and the maximum safe dose is 63 mgL^{-1}. As temperature raises the safety dose margin become less and for fish of small size its value is smaller (Ross and Ross, 1999).

The data on important water parameters before and after transportation are shown in Figure 4. There was an increase in water temperature in all treatments. The dissolved oxygen (DO) concentration was very low in T_1 and T_4 while in all other treatments, DO level was high; the pH value was decreased in all treatments. The minimum survival observed in T_4 (20 mg/L MS222 with loading density 75 fish) and T_1 (20 mg/L MS222 with loading density 50 fish) might be leakage of oxygen from some polyethylene bags.

Table 5: Survival of fingerlings of Seengharee in oxygen filled polyethylene bags during transportation from Sajawal, Sindh to NARC, Islamabad.

	Sedation Dose 1 (20 mgL^{-1})	**Sedation Dose 2 (30 mgL^{-1})**	**Sedation Dose 3 (40 mgL^{-1})**	**Mean**
Loading density 1 (50 fish per bag)	29.67e (T_1)	90.66a (T_2)	68.33c (T_3)	62.88A
Loading density 2 (75 fish per bag)	24.67f (T_4)	80.00b (T_5)	59.00d (T_6)p	58.72B
Mean	27.16C	85.33A	63.67B	

The values with different alphabets are significantly different ($p<0.05$)

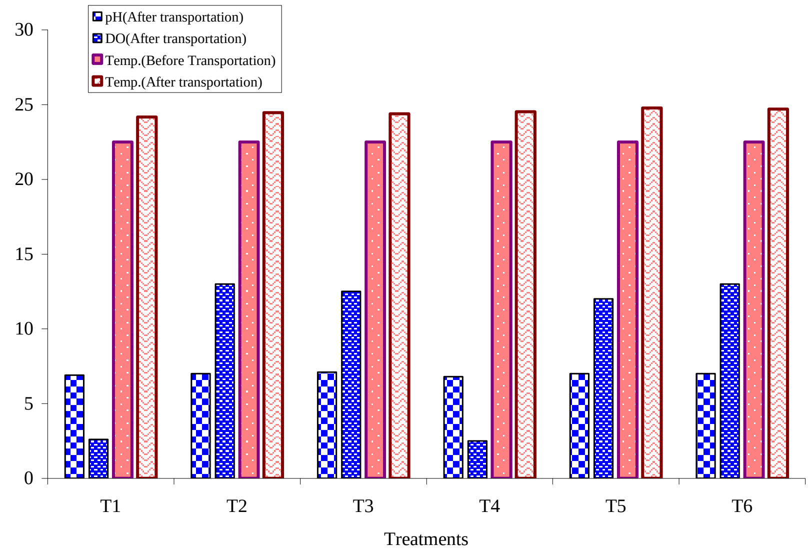


Figure 4: The important water parameters during transportation in oxygen filled polyethylene bags from Sajawal, Sindh to NARC Islamabad.

Conclusion: From this study it was concluded that the live transportation of large size *S. seenghala* in hauling tanks with the use of anesthesia (MS222) was very successful when loading density was at lower side. Oxygen filled polyethene bags packed in styrofoam boxes were useful for the transportation of fingerlings of *S. seenghala* when fish were sedated with MS222. While sedation dose of 30 mgL^{-1} of MS222 and loading density of 50 fingerling per bag in oxygen filled polyethene bags gave maximum survival.

4.2 ACCLIMATIZATION OF SEENGHAREE UNDER CAPTIVE CONDITIONS AND TAXONOMIC STUDY

4.2.1 Acclimatization of Seengharee in Circular Tanks and Raceways

After transportation, fish were transferred to circular tanks and raceways for recovery. Major mortality was recorded during first day of acclimatization. It was 15% within 24 hrs of stocking the fish in circular tanks and raceways. The mortality was recorded in only those fish which were injured due to hard sharp spines present on their body with fin rays or stressed with leakage of oxygen. The injured fish treated with $KMnO_4$ showed no improvement, while after treatment with antibiotics (E.C.M 350 oxytetracycline 150gm +Neomycin sulphate 60gm + Furaltadone HCl 150gm in every 1000gm) twice, fish showed recovery from injuries after two weeks. The results of this study are in conformity with Gillmartin *et al*. (1976), who described that bath treatments with antibiotics have been used almost exclusively for control of superficial infections. The same author suggested the Kanamycin as an effective systemic therapy in a bath treatment procedure against susceptible bacteria. Terramycin was selected for disease treatment because it is commercially available in market and extensively used in poultry industry along with other antibiotics. Further, government of Pakistan has already established free veterinary centers, from where fish farmers can get antibiotics.

Performance of Seengharee during acclimatization in circular tanks and raceways is shown in Table 6. Comparison of growth performance of Seengharee fingerlings during the acclimatization period in tanks and raceways showed gradual increases in weight gain. The initial weight of fingerlings of Seengharee kept in fiber glass tanks and outdoor concrete raceways was 9.0 g and 8.9 g, respectively. The fingerlings of Seengharee kept in outdoor concrete raceways had more final average weight (13.3g) as compared with fish stocked in fiber glass tanks (12.9g). The weight gained by the fingerlings stocked in outdoor concrete raceways (4.4g) was significantly higher ($p<0.05$) than that of fiber glass tanks (3.9g). The specific growth rate and percent weight gain followed the same pattern. The survival rate of fish during acclimatization was not better; although raceways had higher survival rate (42.31%) than circular tanks (31.54%). The significantly ($P<0.05$) better growth achieved in the raceways compared to the circular tanks might be due to the difference in water volume; rate of water circulation and daylight exposure. This may also be due to the difference of temperature in two treatments which was relatively higher in raceways compared to tanks (Jensen and Crew, 1997). All the physico-chemical parameters of water recorded during the experimental period were under suitable range (data not shown) as reported by Wellborn (1988) and

Table 6: Comparison of growth performance of Seengharee in indoor fiber glass tanks and out door concrete raceways during acclimatization

Parameters	**Fiber glass tanks**	**Outdoor concrete raceways**
Initial average weight (g)	9.0a	8.9a
Final average weight (g)	12.9b	13.3a
Weight gain (g)	3.9b	4.4a
Specific Growth Rate	0.32b	0.36a
Percent weight gain	43.33b	49.44a
Survival rate (%)	31.54b	42.31a

The means with different letters are significantly different ($p<0.05$)

Weight gain (WG): Weight final – Weight initial

Percent weight gain (PWG): Wf – Wi) × 100/ initial weight

Specific growth rate (SGR) :[(ln Wf – ln Wi) × 100]/days

Morris (1993). The average temperature during acclimatization period was 24.8°C in tanks and 27.9°C in raceways.

During acclimatization in Circular tanks and Raceways, no mortality was observed but survival rate of Seengharee decreased with time (Figure 5). The observation was that numbers of small sized fish decreased with the passage of time without any mortality. It was supposed that this decrease in number may be due to cannibalism behavior of this fish (Plate 1). The cannibalistic behavior of Seengharee was confirmed by segregating of fish on the basis of their body size. The size grading decreased the cannibalism among the fry/fingerlings of Seengharee.

The difference of survival rate among the fish of different segregated groups was non significant but highly significant with non segregated group. The rate of survival was high in segregated tanks in comparison to non-segregated tanks (Figure 6). The survival was 80.0, 86.0, 84.0 and 86.0 % in segregated tanks, while in non-segregated tanks survival rate was only 14.0% at the end of five weeks rearing period (Table 7). This might be due to the absence of bigger cannibals in the segregated tanks decreasing the chance of predation. Fujiya (1976) and Parazo *et al*. (1991) also observed the beneficial effect of segregation in different fish species. The results showed that suitable management practice could significantly reduce cannibalism among *S. seenghala*. Sahoo *et al*. (2006) suggested that the size grading decreased the cannibalism among the larvae of catfish. Segregation of larvae during rearing enhanced their

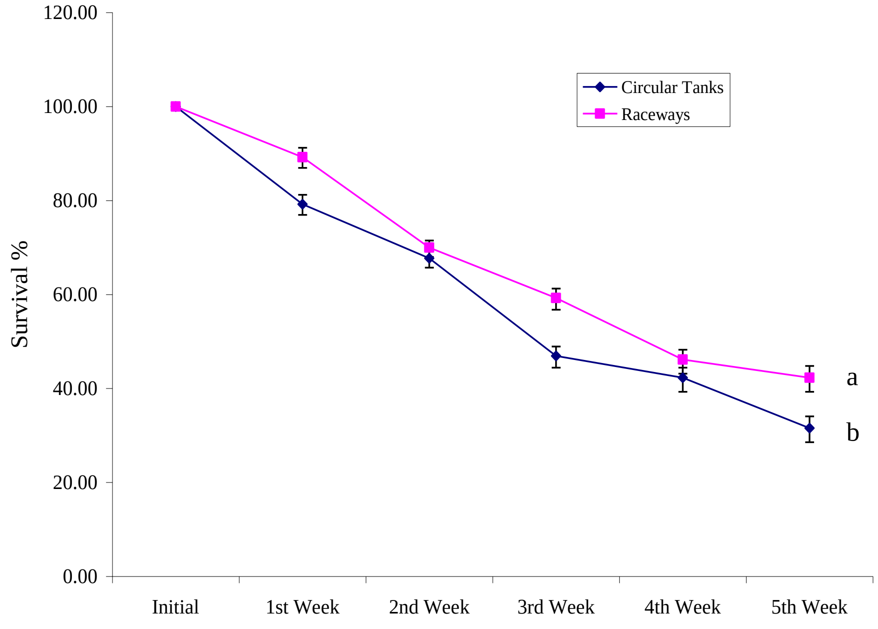


Figure 5: Survival rate (%) of Seengharee during acclimatization in circular tanks and raceways

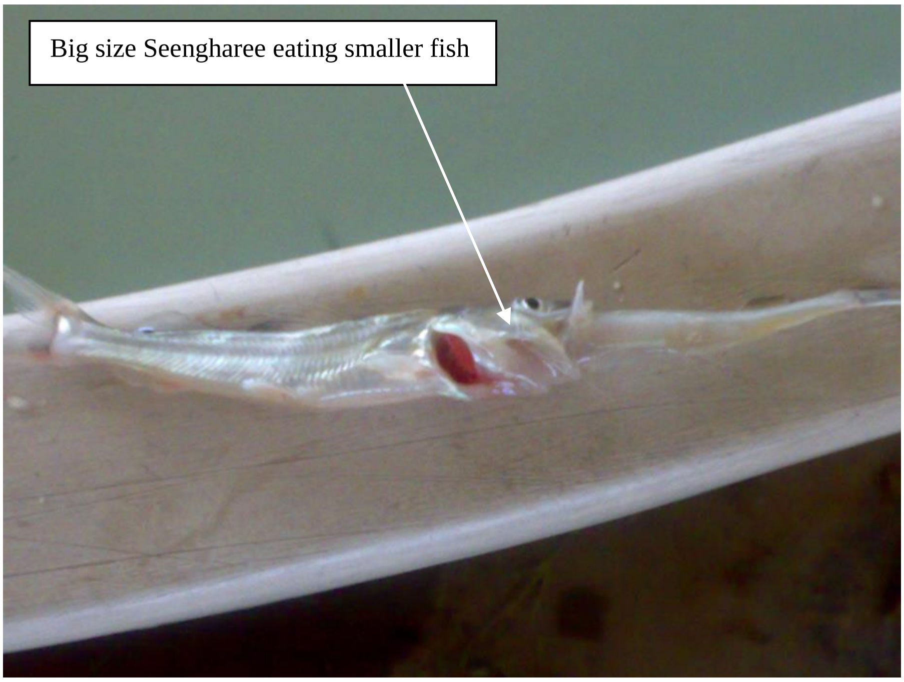


Plate 1: Cannibalism behavior of *S. seenghala*

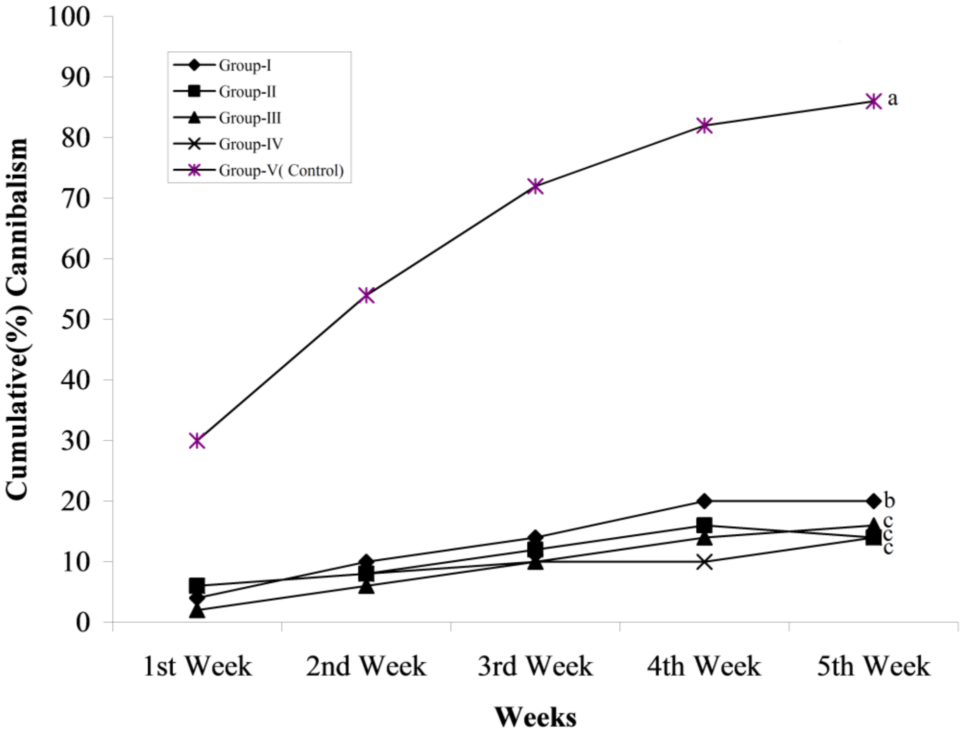


Figure 6: Effect of segregation on cumulative mean percent cannibalism of *S. seenghala* kept in circular tanks over a time of 5 weeks

Table 7: Effect of segregation on cumulative mean percent survival of Seengharee kept in circular tanks over a time of five weeks

Time in Weeks	**Group-I (Wt. 5-10 g)**		**Group-II (Wt. 11-15 g)**		**Group-III (Wt. 16-20 g)**		**Group-IV (Wt. 16-20 g)**		**Control group (mixed size fishes)**	
	No.	**Survival rate (%)**	**No**	**Survival rate (%)**	**No**	**Survival rate (%)**	**No**	**Survival rate (%)**	**No**	**Survival rate (%)**
Start	50	100	50	100	50	100	50	100	50	100
1st Week	48	96	47	94	49	98	47	94	35	70
2nd Week	45	90	46	92	47	94	46	92	23	46
3rd Week	43	86	44	88	45	90	45	90	14	28
4th Week	40	80	42	84	43	86	45	90	9	18
5th Week	40	80	43	86	42	84	43	86	7	14
Mean		88.67a		90.67a		92.00a		92.00a		46.00b

The means with different letters are significantly different ($P<0.05$)

rate of survival. Folkvord and Ottera (1993) opinioned that strict size-grading reduced cannibalism in Atlantic cod. The cannibalism increased with variation in size of the stocked snakehead (*C. striatus*) and due to injuries with hard spine, mortality rate was also increased (Qin and Fast, 1996).

The cumulative cannibalistic pattern in *S. seenghala* per week revealed that segregation according to size resulted in lowest cannibalism (Figure 6). Cannibalism was noticed first either from head or tail. Size variation in fishes have been found to be a main reason of cannibalism in fish, that may be caused by either inadequate food supply or genotype differences (Hecht and Appelbaum, 1988). For controlling cannibalism, decreasing the size difference could be further effective than the food availability as reported by Deangelis *et al*. (1979).

4.2.2 Taxonomic Study of *S. seenghala*

Meristic counts of Seengharee are summarized in Table 9. Meristic counts were 8 (1-7) for rays of dorsal fin, 12 (1-11) for rays of anal, 17 for rays of caudal fin, 10 (1-9) for pectoral fin rays, 6 (1-5) for pelvic fin rays, 103 for lateral line scales, 14 for gill rackers, 2 for nasal barbels, 4 for mandibular barbels, and 2 for maxillary barbels. In the present study, the fin ray count for dorsal, pectoral, pelvic and caudal fin remained constant in all specimens having different length of body. It indicates that the meristic counts remained unchanged with increase in body length and meristic counts are independent of body size. Apart from present study, many workers have reported the same results of meristic counts in different fish species Nakamura (2005).

Data on selected morphometric characteristics of Seengharee (*S. seenghala*) viz., total weight, body measurements, characteristic of fins and other parameters are given in Table 8. These results showed that the most of values of morphometric characters gradually increased with increasing size. With increase in the total length there was an increase in standard length and lengths of pectoral, dorsal, pelvic, and anal fins. Pelvic fin base, pectoral fin base, dorsal fin base and anal fin base were increased with body length (Table-8). While the head length has correlation with eye diameter, interorbital width, pre orbital length of head and post orbital length of head. The mean total length of *S. seenghala* was measured as 58.0 cm, while the total wet body weight was recorded as 958.0 g. The standard length of sampled fish, Seengharee was 42.0 cm.

Body color of Seengharee was brownish-gray on back, silvery on flanks and belly. A black colored spot was found at the end potion adipose fin. The body was elongated and compressed, having depressed head and spatulate snout. Dorsal spine was serrated on posterior side and comparatively weak than pectoral fin spine. Caudal fin was forked, the upper lobe being longer than lower. Lateral line was complete. There were four pairs of barbels**,** maxillary pair reached at the end of pelvic fin. Maxillary barbel was without medial membrane, slender and long as reported by Ferraris and Runge (1999). The length of the supraoccipital spine was less than that of the interneural shield that is only found in *Sperata* species (Ferraris and Runge, 1999). Meristic studies of *S. seenghala* found in Pakistan confirmed that it's belonging to the class *Actinopterygii* (ray-finned fishes), order; *Siluriformes* (catfish) and family; *Bagridae* (Bagrid catfishes). Based on meristic and morphometric characteristics, the fish was identified as Seengharee.

Table 8: Selected Morphometric Features of Seengharee, *S. seenghala*

Sr. No.	Characters	Measurement
1	Total weight(g)	958.0
2	Total length(cm)	58.0
3	Standard length	42.0
4	Fork length	47.0
5	Head length	11.5
6	Dorsal fin length	9.4
7	Head depth	4.5
8	Length of anal fin	6.1
9	Length of pectoral fin	6.2
10	Length of pelvic fin	5.6
11	Length of adipose fin	6.2
12	Length of caudal fin	13.8
13	Longest pectoral fin	7.0
14	Longest dorsal fin	9.2
15	Longest anal fin	6.7
16	Longest pelvic fin	5.7
17	Height of adipose fin	1.9
18	Longest caudal fin	13.8
19	Nasal barbel’s length	2.1
20	Mandibular barbel length	6.8
21	Maxillary barbel length	19.0

Table 9: Meristic counts of Seengharee from Mangla Lake

Sr. No	Characters	Numbers
1	Gill rakers	14
2	Nasal barbels	2
3	Mandibular barbels	4
4	Maxillary barbels	2
5	Lateral line scales	103
6	Pectoral fin rays	10(1-9)
7	Pelvic fin rays	6(1-5)
8	Anal fin rays	12(1-11)
9	Dorsal fin rays	8(1-7)
10	Caudal fin rays	17(0-17)

Values in the parenthesis indicate hard-soft rays

Based on meristic counts, following is the fin formula for this species:

Fin formula: D. I/7; A.11-12; P1.I/9; V.5; C 17

Many authors had described the Seengharee present in Indus system as separate species (Mirza *et al,* 1992). Ferraris (2007) reported it as *Aorichthys aor sarwari*, while Hamilton (1822) described it as synonym of *Aorichthys aor*. Mirza *et al,* (1992); Rafique *et al,* (2003) and Mirza, (2003) reported Seengharee as new species *Sperata sarwari*. However Sykes (1839) Ferraris and Runge (1999) Jayaram (2006) and Ferraris (2007) declared it as synonym of *S. seenghala*. The current status of this is that it is synonym of *S. seenghala* (Sykes, 1839). (Fish base, Catalog of Fishes and Fishwise, 2013)

Further studies at gene or molecular level is necessary to decide either Seengharee present in Pakistan and Indus drainage system is genetic variety of the genus or may be the same species.

4.2.3 Length Weight Relationship of Seengharee

For good management and successful commercial fishing of *S. seenghala*, the understanding on the population structure is needed. Length-Weight relationship parameters (a, b) are useful for the stock assessment studies (Moutopoulos and Stergiou, 2002); for condition of the fish (Barros *et al*., 2001), for evaluation of growth of fish species between different ecological parts (Petrakis and Stergiou, 1995), and estimates to provide some measurements of biomass (Froese, 1998). An index called condition factor (K) or "well being" of a fish is calculated from the weight- length relationship. Fish with a greater K value are heavier, while fish with a less "K" value are not in good conditions (Zafar *et al*., 2003; Wootton, 1998).

The total 264 specimens of *S. seenghala* fingerlings collected from Sajawal, Thatta, Sindh during the year 2009 were studied for length –weight relationship (Table 10). The average length of Seengharee ranged from 11.3 cm to 21.0 cm, while average weight ranged from 5.3 g to 35.44 g. The relationship between total length and weight and regression equation for Seengharee is presented in Figure 7. There was a positive and linear relationship between length and weight of fish. The intercept "a" value (-37.54) and coefficient of growth "b" (3.53) were calculated. There was a higher correlation coefficient value in the length-weight of *S. seenghala*. The Variations in the "b" value was observed for the *S. seenghala*. The value of growth coefficient of *S. seenghala* was near 3.53; this showed that fish was growing isometrically. Ali (1999) stated that if fish grows isometrically it retains its shape and growth coefficient "b" has the value 3.

In a similar study (Ogbe and Ataguba, 2008) an isometric growth pattern for *Malapterurus electricus* was also reported. The value greater or lesser than 3.0 indicates the allometric growth (Ricker, 1975). The value of growth coefficient "b" significantly greater than 3.0 (b: 3.53) showed that the growth of fish is positive. Findings of this study correlate with results of Shakir *et al*. (2008), who reported the growth coefficient (b:3.57) for this species.

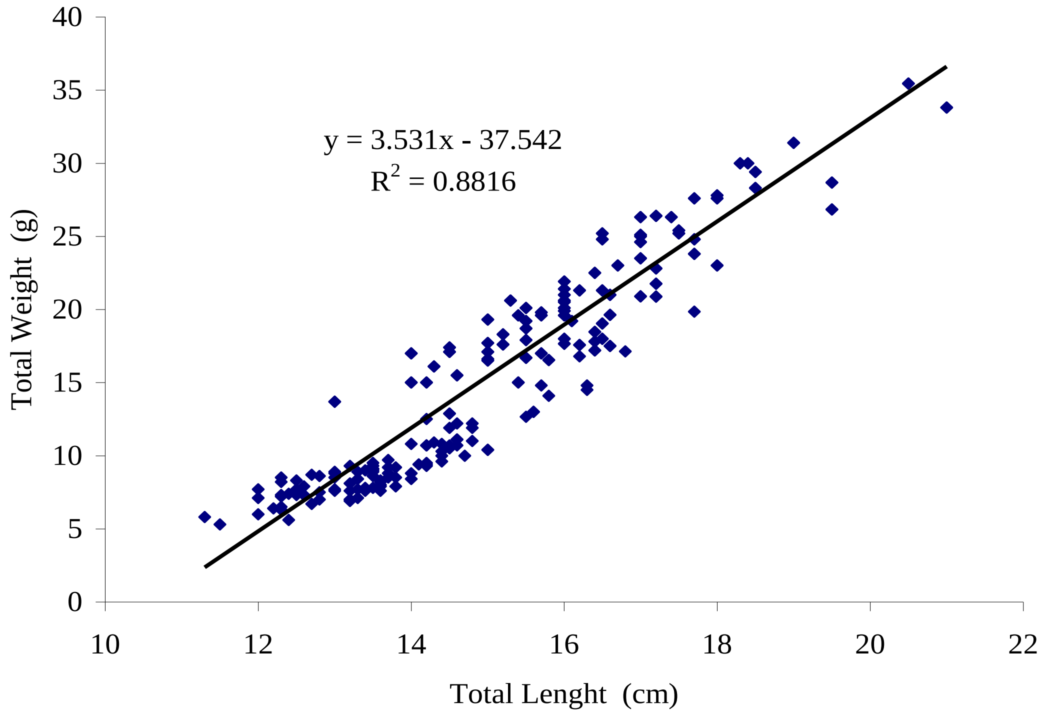


Figure 7: Length weight relationship of *S. seenghala*

Table 10: The parameters of regression of total length (TL) on body weight (W) for Seengharee

Fish	n	Length	Growth Coefficient (b)	95% CL of b	Correlation (r)	Condition factor (K)
S. seenghala	264	11.3-21.0	3.53	3.37-3.69	0.94	0.43±0.07 (0.29-0.62)

The mean condition factor (k) of *S. seenghala* is presented in Table 10. The mean condition factor for the fish was 0.43±0.07. Condition factor "K" (0.43) when examined against body weight and total length, it increased with increasing weight or length. The value of K may differ when increase in fish average weight is not directly proportional to the cube of its length (Wootten, 1998; Abbas, 2000). As "K" value increases length would increase, if the increase in weight is rapid than the cube of length. Condition factor would have a tendency to reduce with the fish growth, when the weights increase less than the cube of length (Javaid and Akram, 1972).

Conclusion: The *S seenghala* accepted artificial feed in form of dough of feed. During the acclimatization period, fish showed better growth in out door concrete raceways. The size grading decreased the cannibalism among the fry/fingerlings of Seengharee. On the basis of morphometric and meristic studies, the fish was identified as *S. seenghala*. The growth coefficient (b: 3.53) was significantly greater than 3 showing positive growth coeffient. Condition factor also increased with increasing length which shows that fish gained more weight than the cube of its length.

4.3 DIET DEVELOPMENT FOR SEENGHAREE FROM LOCALLY AVAILABLE FEED INGREDIENTS

The most important factors influencing the capacity of cultured fish to show its genetic potential for growth and reproduction is the nutrition. To produce economically, high quality product, good nutrition in animal production systems is indispensable. Since Seengharee is carnivorous in nature and feeds on small fish and other aquatic worms, it requires a sufficient supply of protein in its feed (Ferraris, 2007). Artificial feeding plays an important role to increase fish yield in culture systems. The carrying capacity of culture systems is increased by artificial feeding and fish production may be enhanced by many folds. By use of fish feed the production time in the ponds can also be reduced.

4.3.1 Growth Performance of Seengharee Fed on Diets Having Different Crude Protein Levels

For any aquaculture initiatives, precise knowledge on the protein requirement of fish is vital due to high cost ingredients that are usually required at greater levels for the majority of fishes (NRC 1993; Wurts, 2005). When thes intensive culture of a new fish species is taken into account, the first experiments to be conducted is studies of protein requirement. In present study, four kinds of artificial diet having crude protein level 40%, 35%, 30% and 25% were formulated from locally available feed ingredients to evaluate the balanced diet for *S. seenghala*. The data on growth pattern of fingerlings of *S. seenghala* fed the experimental feeds are given in Figure 8. The average final body weight of *S. seenghala* fed F_4 (40% CP) was higher followed by those fed on F_3 (35% CP) and F_2 (30% CP) which remained similar to each other within six weeks of the trial. The lowest average body weight was found in the fish fed F_1 (25% CP).

The data on average gain in weight, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio and feed efficiency (%) of

Seengharee fed the diet with different protein levels are given in Table 11. The average weight gain of *S. seenghala* fed the test diet increased with the increase in CP level. The feed F_4 (40% CP) was significantly better ($p<0.05$) in terms of weight gain than F_3 (35% CP) F_2 (30% CP) and F_1 (25%CP) within six weeks of the trial. Weight gain of fish fed with diet containing 35, 30% CP and those having 35 and 40 % CP did not vary ($p>0.05$) with each other. Specific growth rate (SGR) and food conversion ratio (FCR) values indicated that best feed was F_3 (35% CP) followed by F_4 (40%CP), F_2 (30%CP) and F_1 (25%CP). Feed efficiency (FE) of Seengharee fed 35% CP feed was considerably greater than that of fish fed diets containing 40, 30 and 25% CP.

For Seengharee fingerlings, the optimum feed protein level found out in present experiment is parallel with the feed requirements of protein reported for some other catfish species like bagrid catfish, *Pseudobagrus fulvidraco* (Kim and Lee, 2005), *Mystus nemurus*, Malaysian freshwater catfish (Khan *et al.,* 1993; Ng *et al*., 2001) *Clarias nieuhofii* (Kiriratnikom and Kiriratnikom, 2012) and other catfish, especially channel catfish, *Ictalurus punctatus*, (Rab *et al.,* 2007) but was lesser than the protein requirements of Malaysian freshwater catfish, *Mystus nemurus* (42–44%) (Khan *et al*., 1993; Ng *et al*., 2001).

Protein efficiency ratio (PER) of fish fed with feed of lower protein level (25% CP) was maximum while PER of fish fed 40% CP feed remained minimum. Excess dietary protein, did not enhance the growth of the fish, the reason may be that excess amino acids are metabolized by oxidative deamination and are used to generate energy (Cho *et al.,* 1985; Shiau and Huang, 1989; Vergara *et al.*, 1996;

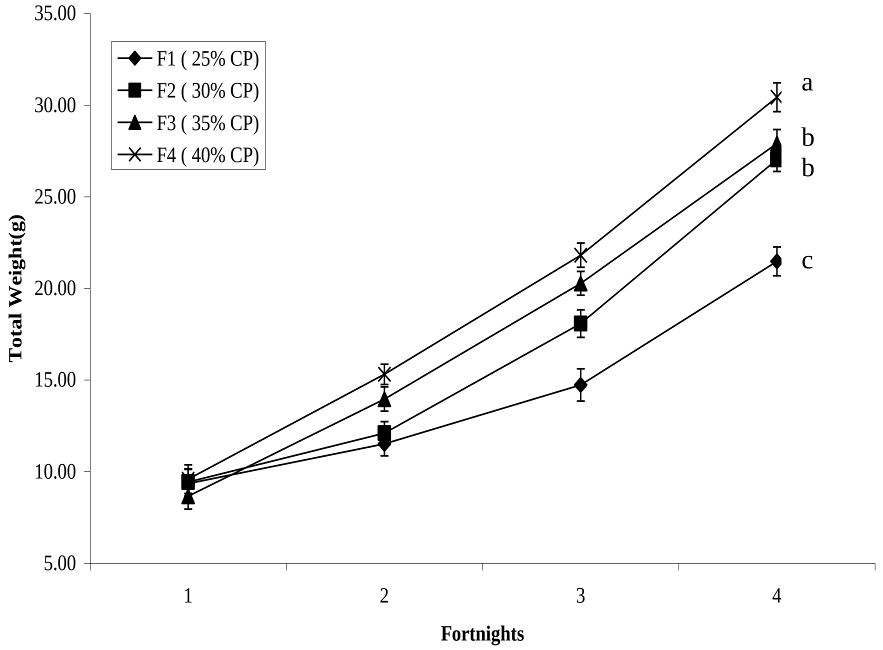


Figure 8: Effect of different diets containing different levels of crude protein on the weight increment of *S. seenghala* fingerlings

Table 11: Growth performance of Seengharee fingerlings fed on diet containing 25, 30, 35 and 40 % CP

Parameters	**F_1 (25% CP)**	**F_2 (30% CP)**	**F_3 (35% CP)**	**F_4 (40% CP)**
Initial weight (g/fish)	9.35±0.02a	9.43±0.01a	8.65±0.02b	9.59±0.03a
Final body weight (g/fish)	21.47±0.77c	27.03±0.55b	27.90±66b	30.43±0.78a
Weight gain (g/fish)	12.13±0.78c	17.61±0.55b	19.25±0.66ab	20.84±0.79a
FCR	3.06±0.03a	2.91±0.02b	2.71±0.01c	2.87±0.02b
SGR	1.38±0.05c	1.76±.03b	1.95±0.04a	1.92±0.04a
PER	1.30±0.64a	1.15±0.60b	1.06±0.61c	0.87±0.92d
FE (%)	32.61±0.36c	34.33±0.28b	36.95±0.24a	34.76±0.22b

Means (±SD) with different letters differ significantly (P<0.05)

FCR: Food conversion ratio; SGR: Specific growth rate; PER: Protein efficiency ratio; FE: Feed efficiency

Kim and Lee, 2009). Excess protein levels in the feed increased the amino acid catabolism in the fish body, and this resulted in higher ammonia excretion and accumulation of nitrogen waste in the culture system (Yang *et al.*, 2002; Webb and Gatlin-III, 2003). Moreover, increasing excess protein in a practical diet resulted in higher feed costs, which is the major variable cost in the aquaculture production system (Goddard, 1996). On the other hand, insufficient dietary protein levels resulted in poor growth performance in many fish species (Yang *et al.*, 2002; Giri *et al.*, 2003; Kim and Lee, 2005) due to insufficiency of amino acids supplied to maintain the body composition (Halver and Hardy, 2002).

The data on water quality parameters of aquaria where feed having different levels of CP were fed to Seengharee are given in Table 12. Dissolved oxygen, water temperature, alkalinity, pH, electrical conductivity, and hardness were not affected by experimental feeds having 40 %, 35%, 30 % and 25% CP levels and remained under suitable range of catfish culture throughout the study period. (Buentello *et al*., 2000).

To approximate the requirements of protein of a number of fish species second order polynomial regression analysis have been used (De-Silva and Perera, 1985; Tacon and Cowey, 1985; Santiago and Reyes, 1991). As specific growth rate was curvilinear, the second order polynomial regression analysis appeared to be more suitable in the present experiment. The regression analysis (Second order polynomial) explained the maximum growth rate at 35% dietary CP level. The best SGR (1.95–1.92%/day) was obtained with feed having 35% and the 40% CP level (Figure-9).

Table 12: Water quality characteristics during culture of seengharee fed different CP levels

Parameters	**F_1** (25% CP)	**F_2** (30% CP)	**F_3** (35% CP)	**F_4** (40% CP)
Temperature (°C)	26.3±1.7	26.2±1.6	26.3±1.6	26.0±1.7
Dissolved oxygen (mgL^{-1})	6.7±0.57	6.3±0.36	6.5±0.14	6.0±0.97
pH	7.9±0.00	7.9±0.01	8.0±0.06	7.9±0.02
Electrical conductivity (µs/cm)	156.0±2.53	135.0±3.13	160.0±2.7	160.0±4.9
Alkalinity (mgL^{-1})	174.5±11.8	135.0±20.2	145.0±19.2	187.0±16.9
Hardness (mgL^{-1})	170.0±8.75	169.0±16.2	150.0±13.2	200.0±9.5

Values are mean ± S.D.

Table 13: Carcass composition of *S. seenghala* fingerlings fed with diets containing different protein levels

Parameters	**F_1 (25% CP)**	**F_2 (30% CP)**	**F_3 (35% CP)**	**F_4 (40% CP)**
Dry matter (%)	20.70b	20.90b	21.60a	21.65a
Moisture (%)	79.30a	79.10a	78.40b	78.35b
Crude protein*	56.25d	56.90c	57.90b	58.10a
Crude lipid*	9.10a	8.95ab	8.33b	8.10c
Ash*	14.24b	14.21b	15.27a	15.12a

* Percentage of dry matter
Means with different letters differ significantly ($P<0.05$)

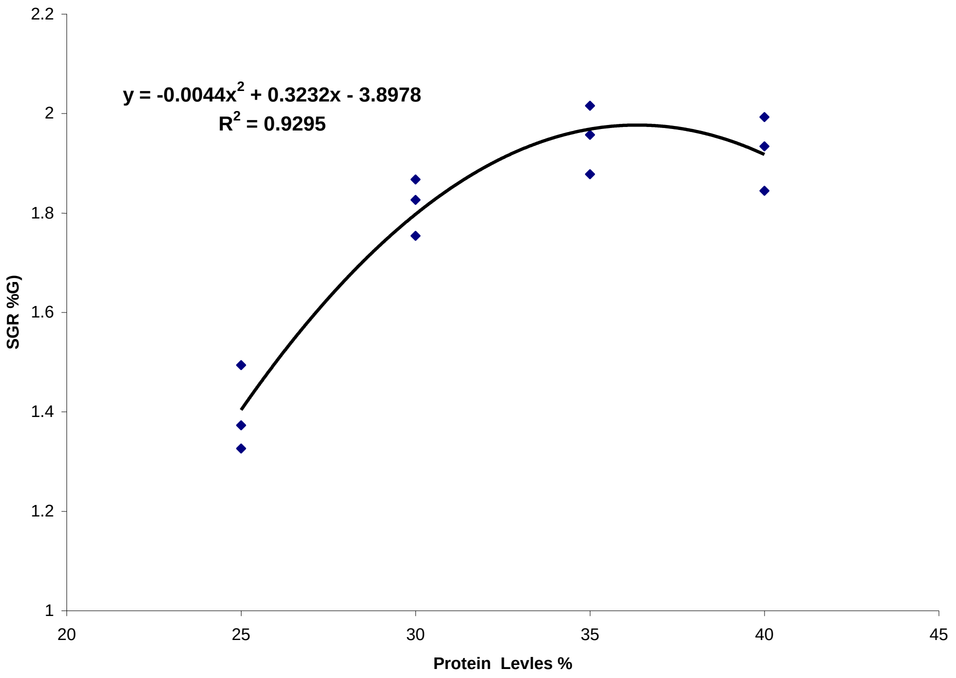


Figure 9: Relationship of SGR and dietary protein levels for *S. seenghala* fingerlings fed feeds of various protein levels

These results pointed that a feed containing 35% protein was optimal for fingerlings of Seengharee.

The proximate composition of carcass of Seengharee, fed diet containing different levels of protein are presented in Table 13. There was a positive effect of dietary protein levels on fish body's crude protein. Crude protein in the Seengharee fed the diets containing 40% protein were higher ($P<0.05$) followed by the fish fed diets with 35, 30 and 25% protein, respectively. Kiriratnikom and Kiriratnikom (2012) reported body's crude protein of *Clarias nieuhofii* fed diets containing 40% to 44% protein were higher than the catfish fed diets with 32% to 36% protein. The body's lipid content decreased with increasing protein in feed; the lower protein diets may increase the fat content of catfish. Davis *et al*. (1993) found that the catfish fed the low protein diet had more body fat than those fed higher-protein diets. Body moisture contents tended to decrease with increasing protein in diet. The ash contents were grater in fish fed at high protein levels, whereas contradictory results were reported by Ali *et al*. (2003); who found that ash content in the fish fed diets containing varying levels of protein did not vary significantly.

4.3.2 Replacement of Animal Protein with Plant Protein in the Diet of *S. seenghala*

Animal protein is the major protein source in fish feed the indispensable amino acid profile of which reflects that of the ideal protein pattern for fish (Anderson *et al*., 1995). The cost of animal protein is high that is one of the major constraints for catfish production. The sustainability of the catfish culture industry, therefore, depends on reducing the animal protein content of fish feeds by finding alternative protein sources that are good in nutritional quality, readily available and more cost effective. The search for economically viable diets (Jones *et al.*, 1996) has lead to the replacement animal protein with cheaper plant sources such as soybean meal and gluten (Lovell, 1988; Lim *et al.*, 2004). Fish meal had been successfully replaced by mixtures of different plant protein sources even in intensively-reared fish, thus leading to significant economy as well as addressing sustainability issues (Kaushik, 2004; 2008). Fish meal is high in lysine and methionine amino acids which is deficient in most of plant sources. In present study a blend of corn gluten and soybean was used as corn gluten is high in methionine and soybean in lysine amino acids.

Keeping in view these concerns, present experiment was conducted to formulate a diet in which dependence on animal protein could be minimized to an extent, without affecting the growth of catfish. To replace the animal protein in feed of seengharee, three feeds (crude protein 30%) in which animal protein was replaced with plant protein source (PPS) was formulated by blend of soybean meal and corn gluten 60 % in ratio of 1:1. Three feeds with animal protein 30 % (no plant protein), 20 % (10 % PPS) and 10 % (20 % PPS) were evaluated for culture of Seengharee in nine circular fiberglass tanks (3 replicates) of 1000 L water capacity under flow through system (8L/min)

The data on growth rate of fingerlings of *S. seenghala* fed the experimental diets having 30% crude protein containing different ratio of animal and plant protein sources for the period of eight weeks are given in Figure 10. The average final body weight of *S. seenghala* fed the F_2 (containing 20% animal and 20% plant protein) was significantly higher ($P<0.05$) followed by F_1 (containing 30% animal and 10% plant protein). The lowest average body weight was found in the fish fed F_3 (containing 10% animal and 30% plant protein).

The data on final weight gain, FCR, SGR, PFR and FE (%) of Seengharee fed on different diet with different ratio of animal and plant proteins are shown in Table 14. Weight gain and SGR of Seengharee were significantly different with diets containing different ratios of animal protein. The numerically F_2 (containing 20% animal and 20% plant protein) showed best growth results (weight gain and specific growth rate), although it varied non significantly from those of fish fed the F_1 (containing 30% animal and 10% plant protein). Fish fed the F_3 (containing 10% animal and 30% plant protein) had lower weight gain and SGR. The lowest FCR was recorded for Seengharee fed F_2 and F_1, while maximum value of FCR was recorded with F_3. AS regards to feed efficiency (%) and protein efficiency (%) of *S. seenghala* fed F_2 and F_1 was considerably higher than that of fish fed F_3.

In present study, replacement of fish meal up to 50 % levels does not effect the growth of fish, but at 75% it reduced the growth (Figure 11). Previous researchers had also reported reduced growth performance of catfish by replacement of the animal protein with plant protein ingredients (Rab *et al*., 2008; Krogdahl *et al*., 2003; Opstvedt *et al*., 2003, Khan *et al*., 2003).

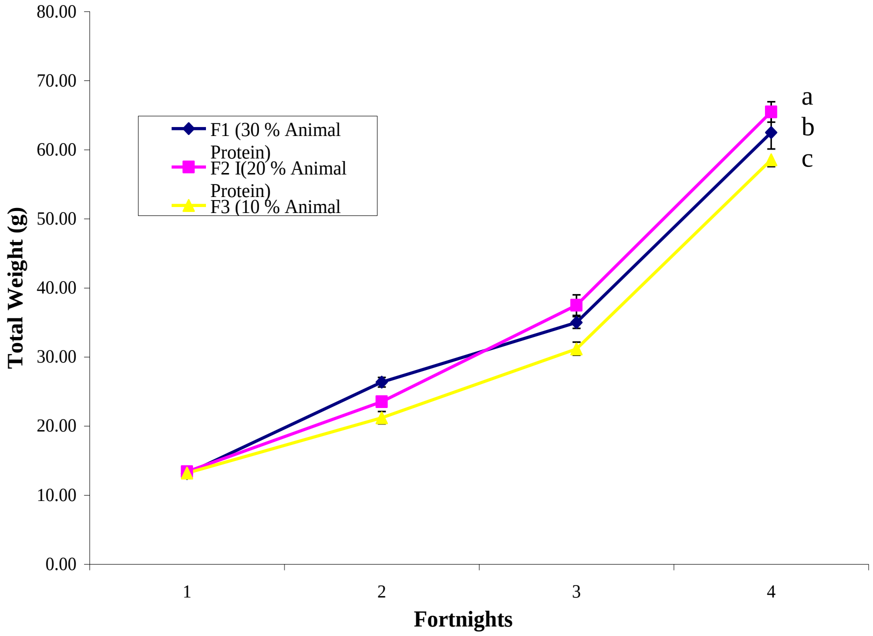


Figure 10: Weight increment of *S. seenghala* fingerlings fed on diets containing different ratio of animal and plant proteins

Table 14: Growth performance of *S. seenghala* fed on diets containing different ratio of animal and plant proteins

Parameters	**F_1 (Animal CP 30%)**	**F_2 (Animal CP 20%)**	**F_3 (Animal CP 10%)**
Initial weight(g)	9.44±0.02a	9.22±0.01a	9.11±0.02a
Final weight(g)	58.83±1.23ab	61.29±2.32a	54.43±1.21c
Weight gain(g)	49.39±2.40ab	52.07±1.47a	45.32±0.80b
Feed offered(g)	117.22±1.87b	124.44±2.02a	113.61±2.12c
FCR	2.37±0.04b	2.39±0.04b	2.51±0.02a
SGR	1.73±0.01ab	1.76±0.02a	1.65±0.01b

Means (±SD) with different letters differ significantly ($p<0.05$)

FCR: Food conversion ratio; SGR: Specific growth rate

CP: Crude protein

Table 15: Water quality parameters during replacement of animal protein in feed of Seengharee with plant protein in fiberglass circular tanks

Parameters	**F1 (Animal CP 30%)**	**F2 (Animal CP 20%)**	**F3 (Animal CP 10%)**
Temperature (°C)	24.2±1.6	22.3±1.6	22.0±1.7
Dissolved oxygen (mgL^{-1})	6.3±0.40	6.4±0.20	6.8±0.90
pH	7.8±0.21	8.0±0.16	7.9±0.02
Electrical conductivity (µs/cm)	140.0±4.14	160.0±3.71	150.0±3.80
Alkalinity (mgL^{-1})	145.0±21.20	155.0±20.20	153.0±14.90
Hardness (mgL^{-1})	159.0±12.20	160.0±11.20	170.0±9.40

Values are mean ± S.D.

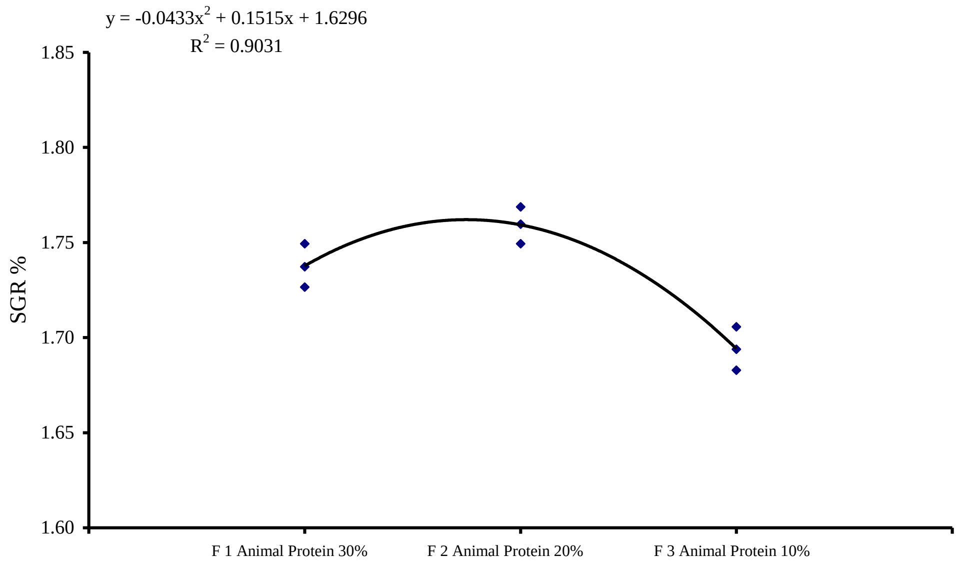


Figure 11: Second-order polynomial relation of SGR and animal protein levels in diet for *S. seenghala* fingerlings

The reduction in growth is due to the fact that protein synthesis requires amino acids. The synthesis of protein cannot occur, if one amino acid is present in lesser concentrations than necessary or absent. Amino acids thus available will be used as fuel and metabolized due to the fact that free amino acids are never stored in tissues (Geiger, 1947). These amino acids are stored in the muscle (Houlihan *et al.,* 1995), but these amino acids are not available for protein synthesis.

Our results indicate that a blend of plant protein (corn gluten and soybean meal) can compensate fish meal in diets for Seengharee, however, the total replacement of fish meal showed less growth and higher FCR is not successful. Previously young catfish have shown some growth reduction by a complete replacement of animal protein with plant protein (Hendricks, 2002; Andrews and Page, 1974). In a similar study, the fish fed the all plant diet (30% CP) had a significantly ($p<0.05$) higher FCR than fish fed animal protein (30% CP) as reported by Li *et al*. (2000).

In present study, water temperature, dissolved oxygen, pH, electrical conductivity, alkalinity and hardness (Table15) were more or less consistent among different treatments and remained under suitable range for catfish culture as reported by Tucker (2001).

4.3.3 Effect of Variable Feeding Rates on Growth Performance of *S. seenghala* in Captivity

The optimum fish feeding rate is essential for management for fish culture. To decrease unnecessary expenses, several experiments had been conducted on the rate of feeding of many fish species (Qin and Fast, 1996; Dong-Fang *et al*., 2003). Waste food and overfeeding deteriorate the quality of water (Ng *et al*., 2000) while insufficient supply of food has direct impact on cost of production（Mihelakakis *et al.*, 2002). The present study evaluated the feeding allowance of Seengharee for the optimum growth in fiber glasses circular tanks of capacity 2000L with flow through system (8 L/m). The feeding rates; 2%, 4%, 6% and 8% of wet fish body weight daily were evaluated during eight weeks culture period.

The data on growth curve of Seengharee fingerlings offered feed with different feeding rates are presented in Figure 12. The fish fed on T_4 (feeding rate 8%) had higher weight followed by T_3 (6% feeding rate), T_2 (4% feeding rate) and T_1 (2% feeding rate) respectively. Data on mean values of total weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and percent weight gain (PWG) of Seengharee fingerlings fed at four feeding rates are given in Table 16. Higher ($P<0.05$) weight gain was achieved by T_4 followed by the T_3, T_2 and T_1. Specific growth rate and percent weight gain of Seengharee were significantly ($P<0.05$) higher in the T_4 compared to T_3 and T_2 which did not differ with each other. The values for SGR and PWG remained lowest in T4. The best FCR was recorded in T_1 followed by T_2, T_3, and T_4, respectively. By increase in feeding level, FCR increased while FE decreased which shows that when feed is given at satiation or over satiation, FCR increases.

In present study, the growth performance and specific growth rates increased by increasing the feeding rate. Similarly, increase in body weight with the increase in feeding levels have been reported previously (Omer., 1986; Hasan

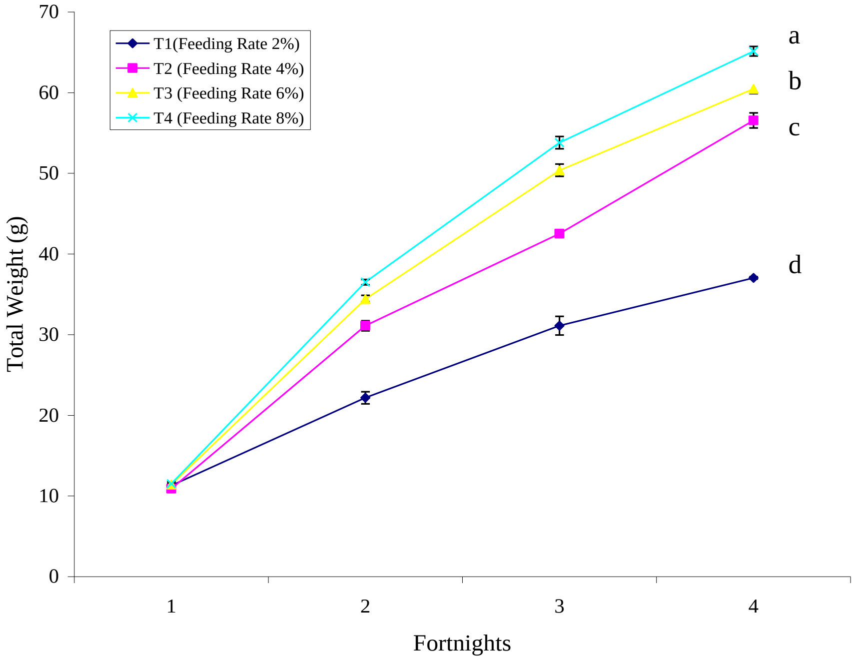


Figure 12: Weight increment of *S. seenghala* fingerlings at 2, 4, 6 and 8% of wet fish body weight daily

Table 16: Growth performance of *S. seenghala* fingerlings at 2, 4, 6 and 8% of wet fish body weight daily

	T_1 **(Feeding rate 2%)**	**T_2** **(Feeding rate 4%)**	**T_3** **(Feeding rate 6%)**	**T_4** **(Feeding rate 8%)**
Initial weight (g/fish)	11.29±0.02	10.9±0.04	11.4±0.03	11.5±.03
Final body weight (g/fish)	37.03±0.14d	56.55±0.94c	60.47±0.79b	65.13±0.59a
Weight Gain (g/fish)	25.75±0.15d	45.65±0.94c	49.07±0.78b	53.63±0.59a
FCR	0.70±0.00d	1.04±0.03c	1.61±0.02b	2.13±0.03a
SGR	1.98±0.01c	2.74±0.03b	2.78±0.02b	2.89±0.02a
PWG (%)	228.08±1.29c	418.78±8.58b	430.41±6.90b	466.38±4.15a
FE (%)	142.47±1.55a	96.50±2.45b	61.97±2.09c	47.05±1.16d

Means (±SD) with different letters in each row differ significantly (P<0.05)
FCR: Food conversion ratio; SGR: Specific growth rate; PWG: percent weight gain;
FE: Food conversion efficiency

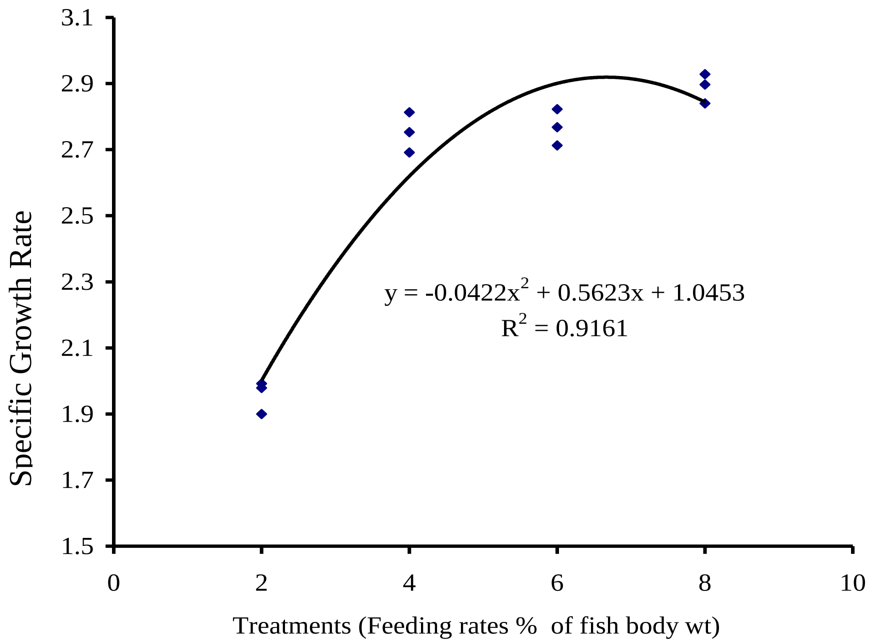


Figure 13: Second order polynomial relation of specific growth rate (SGR) and feeding rates for *S. seenghala* fingerlings

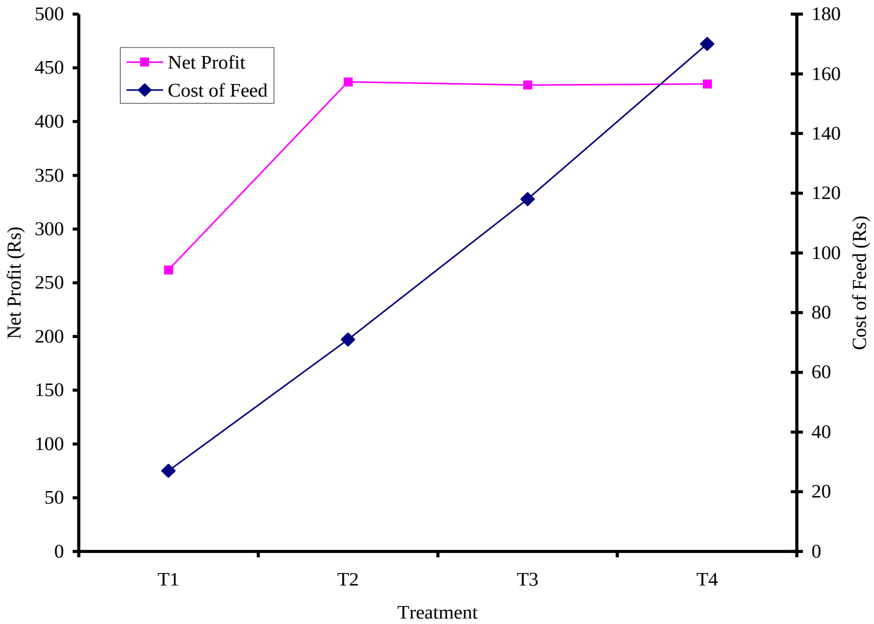


Figure 14: Relationship between cost and profit at different feeding rates of *S. seenghala*

and Macintosh, 1991; Ali *et al*., 2003). Other catfish species like Channel catfish (*Ictalurus punctatus*) and bagrid catfish (*Mystus nemurus*) when offered feed with higher rates showed more growth (Borghetti and Canzi, 1993; Robinson and Li, 1999; Ng *et al*., 2000; Eroldogan *et al.,* 2004). In present study, the relationship between feed added and weight gained (Fig. 4.13) indicated that feeding is definitely the most important factor in the growth of fishes. Same results were also reported by Cui-YiBo *et al.* (1996), Seenappa and Devareak (1991) and Sen *et al.* (1980).

The restriction feeding methods that limit the amount of food provided, reducing maximum daily feeding rates have potential to lower production cost, however, decreasing the quantity of food used may affect pond fish production (Bosworth and Wolters, 2005). In present study, significant differences were observed in feed to weight gain ratio (FCR) at different levels of feeding. The best FCR was observed at 2% feeding level, indicating that increasing the feeding rate decreases the ability of fish to convert feed into biomass. When fish is fed at satiation or over satiation, digestibility of feed ingredients is decreased and FCR increased, while FCE decreased (Mazur *et al*., 1993; Ali *et al*., 2003).

From economical point of view, net profit was increased with the increase in feeding level up to 4% but above 4% feeding level there was non significant change in net profit (Figure 14). These findings are in accordance with the findings of Ghosh (1984); Akram *et al*. (1994) and Janjua (1996) who reported that increase in feeding rates till 4% body weight increased the growth of the fish, while feeding beyond 4% was wasteful.

Conclusion: From the above experiments, performed to develop artificial feed for *S. seenghala* it was concluded that feed with crude protein level of 35% was optimum for growth of this fish. Reduction of animal protein up to 20 % does not effect the growth of fish, but below this level it reduced the growth. The feeding of 4% fish wet body weight daily was optimum for growth of this fish.

4.4 PERFORMANCE OF SEENGHAREE IN EARTHEN PONDS

Under this objective, growth performance of *S. seenghala* in cemented raceways and earthen ponds and reproductive performance in earthen ponds were evaluated.

4.4.1 Growth Performance of *S. seenghala* in Cemented Raceways and Earthen Ponds

The catfish are mainly cultured in earthen ponds using high density culture systems, such as raceways, cages, pens, tanks. To evaluate the growth performance and production of Seengharee in earthen ponds and out door cemented raceways an experiment was conducted for a period of six months (May to October 2009). One hundred Seengharee Juvenile having mean initial weight of 100 g were stocked in each pond, while fifty fish having mean initial weight of 104 g were stocked in each of the raceways. Fish in both raceways and ponds were fed twice daily at the rate of 5% body weight with pelleted feed formulated to contain 30% CP diet.

The data on growth pattern of *S. seenghala* juveniles stocked in ponds and raceways are given in the Figure15. Total weight of fish showed a trend of increased growth in ponds and raceways throughout experimental period.

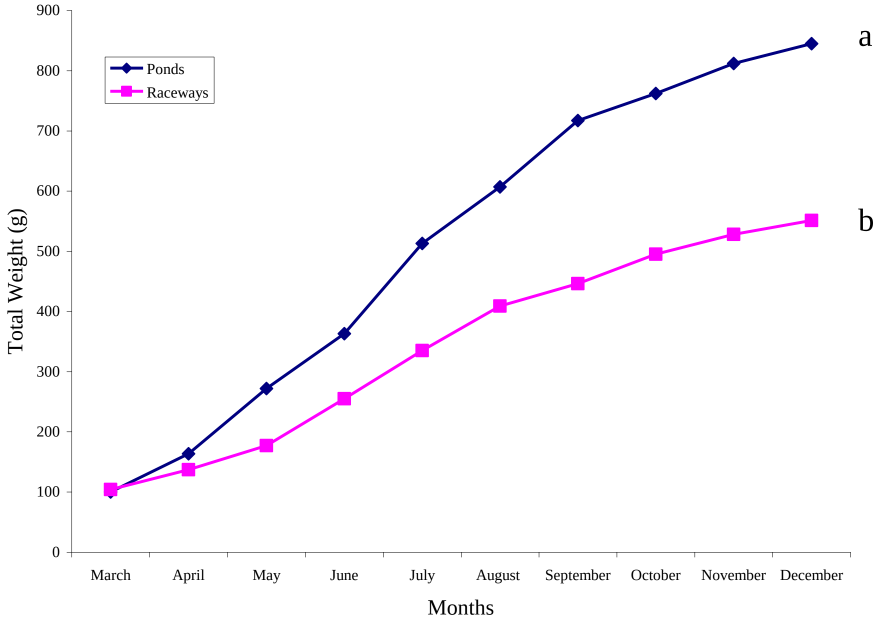


Figure 15: Growth curve of *S. seenghala* cultured in cemented race ways and earthen ponds

Table 17: Growth performance of *S. seenghala* juvenile cultured in ponds and race ways

Parameters	**Ponds**	**Raceways**
Initial weight(g)	100±1.32a	104±2.12a
Final weight(g)	845±3.12a	551±2.41b
Weight gain(g)	745.00±2.54a	447±2.12b
Av. feed offered(g)	1481±3.21a	951.6±2.12b
FCR	1.97±0.03b	2.6±0.01a
SGR	3.57±0.01a	2.51±0.02b

Means (±SD) with different letters in each row differ significantly ($p<0.05$)
FCR: Food conversion ratio; SGR: Specific growth rate

Relatively slow growth was observed in raceways compared to ponds. Relationship between water temperature and weight gain of *S. seenghala* in two different culture systems throughout the study period is shown in Figure 16. In earthen ponds better weight gain was recorded during the months of May to August when the temperature was maximum. In raceways the monthly temperature was almost constant but lower than that of ponds during the months of October-December (Table 18). Weight gain of *S. seenghala* in earthen ponds was higher compared to raceways and this variation in weight gain may be attributed to the difference in water volume and difference of temperature in both culture systems. The water temperature of raceways was lower than that of earthen ponds and catfish generally do not feed consistently when water temperature drops below 21°C (Andrews and Stickney, 1972). It has been reported that the fish activities including growth depend highly on temperature (Alliots *et al*., 1983). The low temperature ($<22.6^{o}$C) resulted in decreased food intake and activity (Villaluz and Unggui, 1983). Hargreaves and Tucker (2003) reported markedly depressed growth in channel catfish when temperature decreased from optimum. Furthermore, lower temperature also decreases the fish appetite thus reducing the growth (Wellborn, 1988).

Data on growth performance of *S. seenghala* in earthen ponds and raceways are given in Table 17. There was a significant difference ($p<0.05$) in average total fish weight, weight gain, SGR, and FCR among fish stocked in raceways and ponds. All growth performance parameters of Seengharee were recorded better in the earthen ponds as compared with cemented raceways.

Cost of fish Production (Per kg) was less for the fish produced from ponds compared to raceways (Table 19).

In the present study, the better growth performance *S. seenghala* in ponds compared to raceways may be due to presence of natural food in ponds. These results are in accordance with Rab *et al*. (2007) who reported considerable growth of fishes in fertilized earthen ponds during summer, when there is availability of natural food (Robinson and Rushing, 1994; Li *et al*., 2004). It has been reported that almost 8-9% of the growth of food-sized catfish raised in earthen ponds was derived by consuming natural food organisms (Bosworth and Wolters, 2005). In present study, the better growth performance of Seengharee may be due to the availability of higher number of tadpole larvae in ponds due to their breeding season in monsoon as reported by Wellborn (1988).

The data on water quality parameters recorded during the experimental period are given in the Table 19. The highest water temperature was recorded during July to September, while the lowest was recorded in the month of December. Water temperature, dissolved oxygen, pH, electrical conductivity, alkalinity and hardness were almost consistent among different treatments and remained under suitable range for catfish culture in ponds.

Overall range of water temperature in ponds was 20 to 32^{o}C, and in raceways it was 21.0 to 24.4^{o}C. All the physico-chemical parameters of water recorded during the experimental period were under suitable range as reported previously (Wellborn, 1988; Morris, 1993).

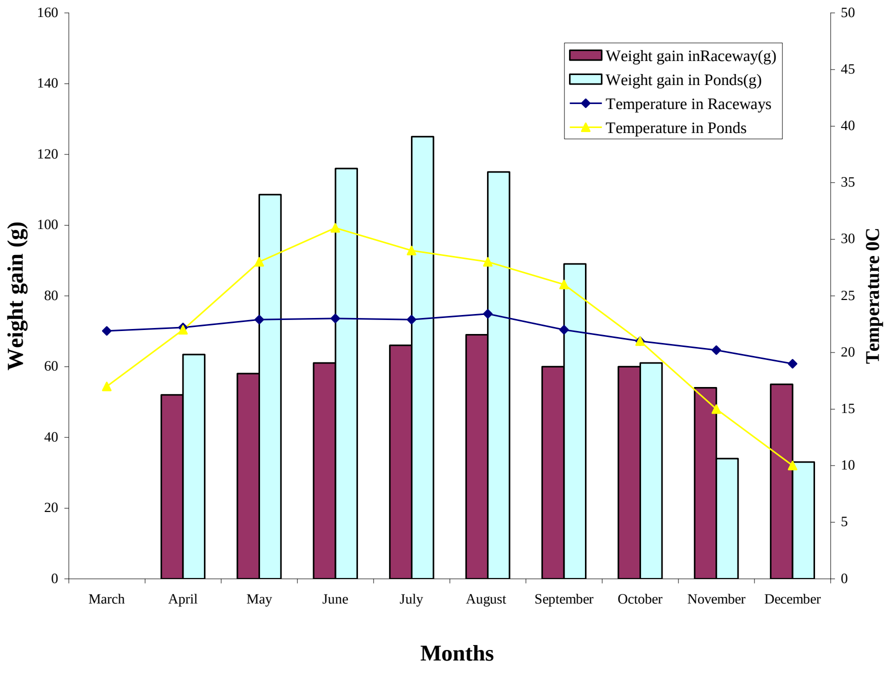


Figure 16: Monthly weight gain of *S. seenghala* stocked in ponds and raceways during the study period

Table 18: Production characteristics of *S. seenghala* juvenile cultured in ponds and raceways

Treatments	Av. weight gain (g)	FCR	Cost of feed/kg (Rs)	Cost of fish production (Rs) per kg
Ponds	752.00	1.97	60	118.2
Race ways	366.00	2.60	60	156.0

Table 19: Physico-chemical parameters of water recorded during study period (March-December) in ponds and raceways

Months	Temp °C		DO (mg/L)		pH		Alk (mg/L)		Hard (mg/L)	
	Ponds	Race ways	Pond s	Race ways	Ponds	Race ways	Ponds	Race ways	Ponds	Race ways
March	17.0	21.9	6.8	8.4	7.5	8.0	103.0	140.0	140.0	150.0
April	22.0	22.2	6.0	8.0	8.0	8.0	170.0	140.0	130.0	140.0
May	28.0	22.9	6.4	7.9	8.0	7.9	165.0	150.0	140.0	155.0
June	31.0	23.0	5.7	8.2	8.5	8.1	160.0	145.0	140.0	145.0
July	29.0	22.9	5.0	8.0	8.4	8.0	170.0	150.0	130.0	165.0
August	28.0	23.4	4.7	7.8	8.0	8.0	175.0	140.0	120.0	155.0
September	26.0	22.0	5.6	8.1	7.9	8.4	165.0	150.0	140.0	140.0
October	21.0	21.0	6.8	8.4	8.5	8.0	190.0	155.0	150.0	150.0
November	15.0	20.2	7.0	8.4	8.0	7.5	170.0	145.0	160.0	140.0
December	10.0	19.0	7.2	8.5	8.0	8.0	165.0	150.0	140.0	130.0

Temp (ºC) = Temperature

Alk (mg/L) = Alkalinity

Hard (mg/L) = Hardness

EC ųs/cm = Electrical conductivity

DO (mg/L) = Dissolved oxygen

4.4.2 Brood Stock Development and Reproductive Performance of *S. seenghala*

Gonads, particularly the ovaries undergo regular seasonal changes in weight (Dadzie *et al*., 2000) therefore the study of gonadal weight in relation to body weight, referred as gonadosomatic index is used as a technique for studying the spawning season (Ahirrao, 2002). Gonadosomatic index gives the detailed idea regarding the reproductive status of the species and help in determining breeding period of fish (Shankar and Kulkarni, 2005). The number of eggs that are laid during a spawning season is termed as 'fecundity' of a fish (Bagenal, 1957). 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). Fecundity of fish is species specific that varies depending upon the age, weight, length, habitat and ecological conditions including climatic factors of the locality. In a single population, the fecundity may also vary in relation to the availability of food in the natural system.

In present study, the fish that were captured (December 2008) from Mangala reservoir, Mirpur, AJK and kept in ponds were studied for a period of 1.5 years. Fish ovaries collected from mature Seengharee during late March to early April were studied to calculate the fecundity, and relative fecundity.

The information on gonadosomatic index is helpful to determine the pre spawning, spawning and post spawning periods in any of the fish that may help in management of fishes. The data on average value of GSI of Seengharee collected on monthly basis for one year are shown in Figure 17. Values for GSI increased gradually from November and in March it reached on peak. In March and April this peak is maintained, which declined in May to some extent and thereafter dropped to become least in October. The maximum GSI value in the month of March pointed out the spawning period of *S. seenghala*. It shows that the fish spawned once in a year mainly during March to April. In a similar study, the highest GSI value of *Mystusgulio* indicated the peak spawning season of the fish in the month of July (Sarkar *et al.*, 2002). Similar findings revealed that most of the Indian freshwater teleosts attained maturity and breed during monsoon season (Encina and Lorencio, 1997). The lowest values of gonadosomatic index in winter season indicate depletion of gonadal products. Kumar *et al.*, (2003) determined the maturity and spawning periods in *Tetrodon cuticutia* by gonadosomatic index and measurement of mean egg diameter. The gonadosomatic index of Cyprinid fish *Cirrihinus reba* reported to be directly proportional to spawning while inversely proportional to post spawning season (Shengde and Mane, 2006)

The correlation among various parameters i.e. fecundity, total length (L), total body weight (W) and gonad weight (G) is given in Table 21 while descriptive statistics of data are presented in Table 4.20. The mean fecundity of *S. seenghala* ranged from 21500 to 31000 with an average value of 2584. The minimum fecundity (21500) was found in the fish having body weight 965g, a total length of 54cm, and gonad weight 9.6g; while the maximum fecundity (31000) was observed in the fish with a body weight 2000g, total length of 76cm, and gonad weight of 19.0g.

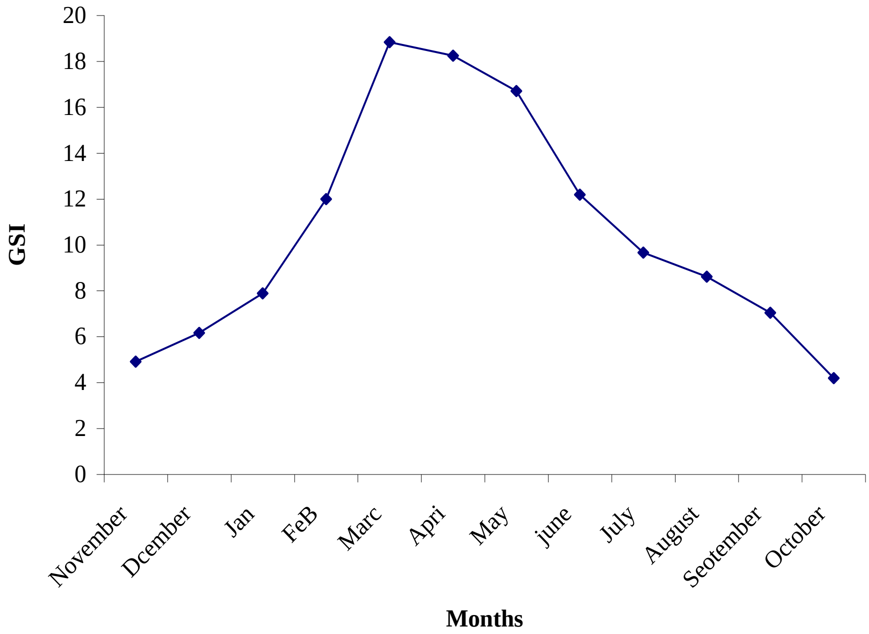


Figure 17: Monthly changes in Gonadosomatic Index of *S. seenghala* kept in captivity

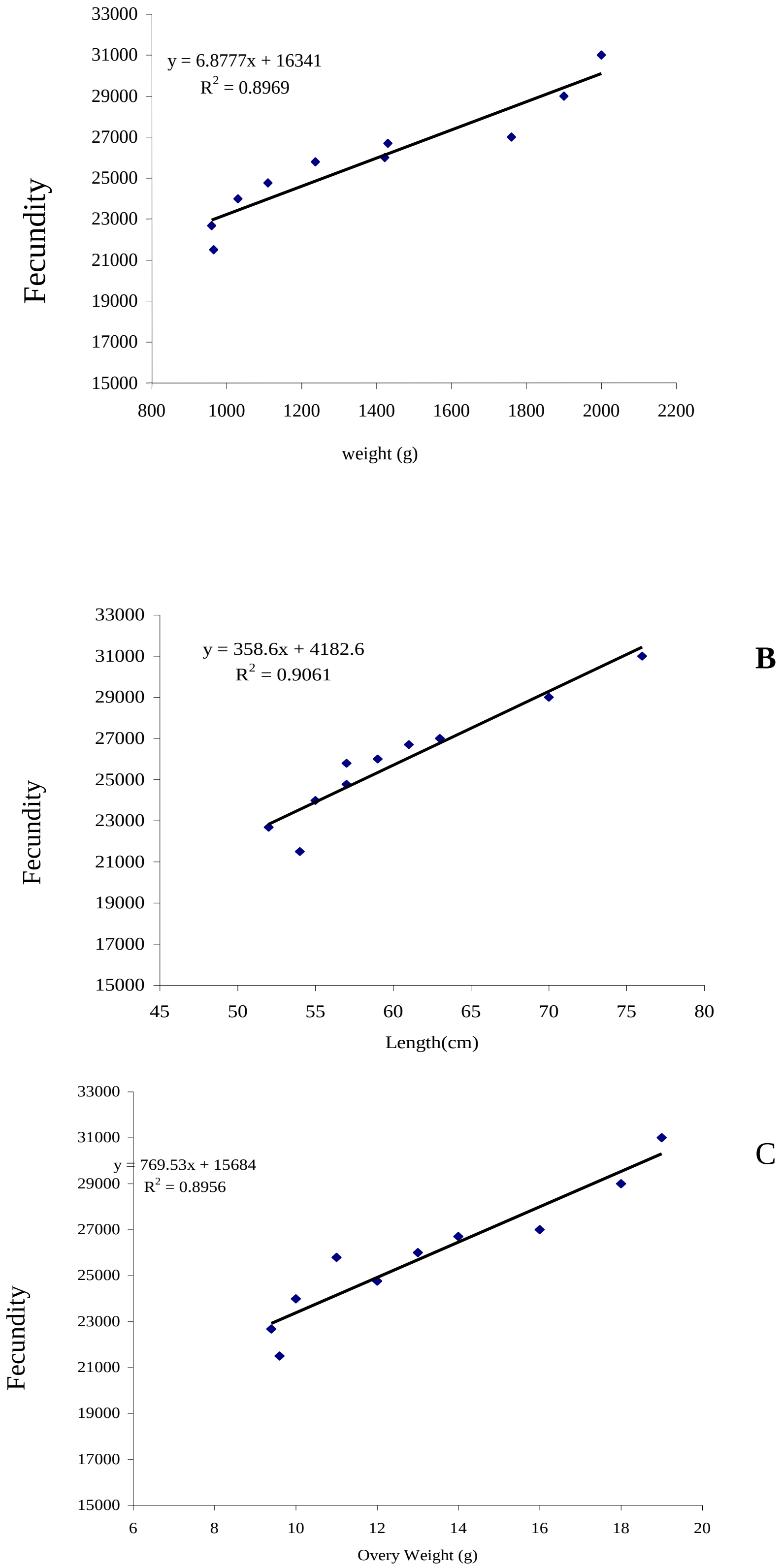


Figure 18: Relationship between fecundity and length, body weight and ovary weight of *S. seenghala*

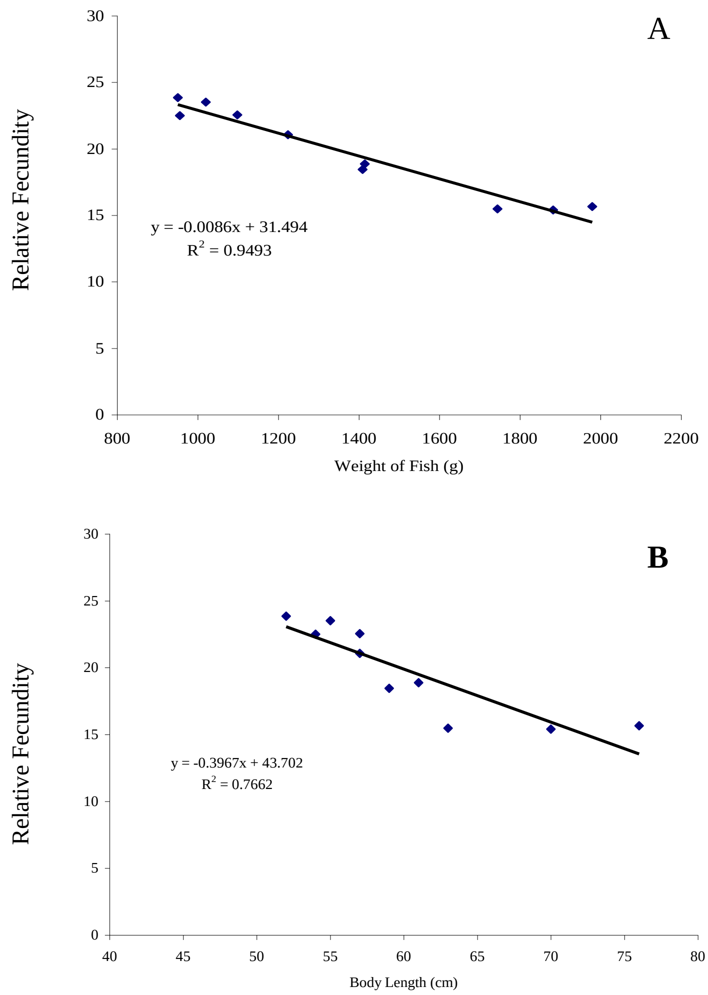


Figure 19: Relationship between relative fecundity and body weight and body length of *S. seenghala*

Table 20: Descriptive statistics of data (three replications) of *S. Seenghala* fecundity and other parameters

Parameters	**Minimum**	**Maximum**	**Mean**	**Standard error**	**Standard deviation**	**Confidence level (95.0%)**
Body weight (g)	960.00	2000.00	1381.40	123.28	389.86	278.89
Body length (cm)	52.00	76.00	60.40	2.38	7.52	5.38
Fecundity	21500.00	31000.00	25841.90	895.34	2831.33	2025.41
Weight of ovary (g)	9.40	19.00	13.20	1.10	3.48	2.49
GSI	0.89	1.08	0.96	0.02	0.05	0.04
Relative fecundity	15.41	23.86	19.74	1.08	3.41	2.44

Table 21: Correlation coefficient "r" between body weight, length, gonad weight, GSI and relative fecundity of *S. seenghala*

Parameters	BW (g)	B L (cm)	Fec.	WO (g)	GSI	RF
Body weight (g)	1.00					
Body length (cm)	0.95	1.00				
Fecundity	0.95	0.95	1.00			
Weight of ovary (g)	0.99	0.97	0.95	1.00		
Gonadosomatic index	-0.43	-0.26	-0.36	-0.27	1.00	
Relative fecundity	-0.97	-0.88	-0.87	-0.94	0.49	1.00

BW: Body weight (g); B L: Body length (cm); Fec.: Fecundity; WO: Weight of ovary (g); GSI: Gonadosomatic index; RF: Relative fecundity

Relationship of fecundity with total weight, total length and weight of ovaries were linear and positive (Figure 18). Many workers have reported similar findings in other fishes (Hoque and Hossain, 1993; Bhuiyan *et al*., 2006; Roy and Hossain 2006; Musa and Bhuiyan, 2007).

To evaluate egg production by all individuals regardless of their size or age, studies on relative fecundity, or number of eggs per gram of body weight, have been reported to be useful (Martinez and Houde, 1975). The mean relative fecundity of Seengharee, were estimated to be 19.74 (Table 20). Figure 19 showed the relationship between relative fecundity, body weight and body length of *S. seenghala*. In present study, the relationship of relative fecundity with its body weight and length was linear and negative. In *M. seenghala* (Synonym of *S. seenghala*) and *W. attu* the relative fecundity decreases with the increase in body length (Bhat *et al.*, 1977). Similar trends were also reported for *Alburnus chalcoides* (Springate, 1990; Heinimaa and Heinimaa, 2004; Shamekhi Ranjbar *et al*., 2012; Rahbar, 2013).

Conclusion: Growth performance of Seengharee was better in earthen ponds compared to cemented raceways. Reproductive season for Seengharee was March to May, and fecundity showed linear relationship with length, body weight and gonad weight.

SUMMARY

Research on freshwater aquaculture in Asia has mainly been focused on propagating carp culture and their breeding techniques that has been standardized or transferred to fish farmers. The catfish production mainly depends on catches from wild and, due to high demand and over exploitation of natural stock; the catfish population is decreasing in natural environment. Despite their great demand, catfish culture systems are not yet established in many countries of Asia including Pakistan. Catfish culture has advantages over carp culture in terms of greater survival in oxygen depleted waters, tolerance to crowding and high stocking rates on artificial feeds. Among some candidate catfish species found in the natural waters of Pakistan, the Seengharee, *S. seenghala* is highly preferred one owing to its meat quality and fewer spines. The present study on local catfish (*S. seenghala)* was also conducted to evaluate its culture potential in captive conditions, with the specific objectives: Identification of potential sites, collection, transportation, systematic study, acclimatization, diets development and performance of Seengharee under captive conditions.

To assess the availability of *S. seenghala,* the survey of the different water bodies in provinces of Punjab, Sindh and Azad Jammu & Kashmir (AJK) was conducted. The identified potentials sites for the collection of this fish were Head Rasul in District Jhelum, Punjab; Mangla reservoir, Mirpur, AJK and Sajawal, District Thatta, Sindh. Collection of Seengharee was carried out with the help of drag nets, gillnets and cast nets. Dragnets and gillnets were successful for the collection of adult *S. seenghala*. The live fish having average weight ranged from

400 to 500g were collected from Head Rasul and Mangla reservoir during December 2008 and January 2009 and transported live to Aquaculture and Fisheries Program (AFP), NARC, Islamabad, in fish hauling tanks having adequate supply of fresh air supply. The levels of tranquilizers, tricaine methane sulfonates (MS-222) and quinaldine at two loading densities (25 and 50 fish per tank) during transportation were evaluated. For the transportation of Seengharee in hauling tanks use of anesthesia MS-2222 showed maximum survival rate. Fingerlings collected from Sajawal Sindh, with the average weight of 9.0g and length of 11.2 cm were transported in oxygen filled polyethylene bags. To avoid damage to the bags and fish from the spinous fins of *S. seenghala* fish were sedated using Tricaine methane sulphonate. Three sedation doses (20, 30 and 40 mgL^{-1}) at two loading densities (50 and 75 fish per bag) were evaluated. The sedation dose of 30 mgL^{-1} of MS222 and loading density of 50 fingerling per bag in air filled polyethylene bags had good results in term of survival of Seengharee.

After the transportation, the fish were shifted for acclimatized in indoor fiberglass tanks (1000-liter water capacity) and outdoor concrete raceways (5000 liter water capacity) at AFP, NARC, Islamabad. Fifty fingerlings of average initial weight 9.0g were kept in each of the circular tanks, while 100 fingerlings having average initial weight of 8.9g were kept in each of the five raceways. The fingerlings were offered live trash fish in start, but later on it was replaced with artificial diet containing 40% crude protein. During acclimatization, cannibalism behavior was observed in fry and fingerlings of Seengharee which was tried to control by segregation of fish on the basis of their size. The size grading decreased the cannibalism among the fry/fingerlings of *S. seenghala*.

During the acclimatization period, the growth performance of Seengharee in cemented raceways and ponds was also compared. Fish showed better growth in ponds compared to cemented raceways. In the present study, *S. seenghala* have value of growth coefficient “b” 3.53. This value is significantly greater than 3 showing positive growth coeffient. Condition factor also increased with increasing length which that fish gained more weight than the cube of its length. Morphometric and meristic studies were conducted and as the result, the Seengharee present in Pakistan, was identified as *Sperata seenghala*.

As fish feed plays a major role in determining the fish growth, different experiment were conducted to formulate complete diet for Seengharee. To determine the optimum level of protein in the diet for Seengharee, four kinds of artificial diet having different crude protein levels (25, 30, 35 and 40% CP) were formulated from locally available feed ingredients. Twelve glass aquaria of length 60 cm, width 5 cm and height of 30 cm were used for this experiment. It was concluded that a feed of 35% CP is best for the optimum growth of Seengharee. Second important factor for diet development is determination of animal and plant protein ratio in feed of catfish. To replace the animal protein in feed of *S. seenghala*, three feeds (crude protein 30%) were formulated; animal protein was replaced with Plant Protein Source (PPS) formulated by blend of soybean meal and corn gluten 60% in ratio of 1:1. The experiment was performed in fiberglass circular tanks for 8 weeks. Replacement of animal protein by plant protein up to 20 % does not affect ($p>0.05$) the growth of fish, but below this level it reduced the growth. The effect of feeding rates on the growth performance of Seengharee in cemented raceways was also studied. The fish were fed four feeding rates (2%, 4%, 6% and 8% of wet body weight. By increasing the feeding rate from 2% up to 8% of fish body weight, there was a significant effect on the growth performance and specific growth rates of Seengharee. From economical point of view, net profit was increased with the increase in feeding level up to 4% but above 4% feeding level there was significant decrease in net profit

To evaluate the growth performance and production of *S. seenghala* in cemented raceways and earthen ponds, an experiment was conducted for a period of six months (May to October 2009). A total of 100 Seengharee juveniles having mean initial weight of 100 g were stocked in each pond, while fifty fish with mean initial weight of 104 g were stocked in each of the raceways. Fish in both raceways and ponds were fed twice daily at the rate of 5% body weight with artificial feed having 30% CP diet. Fish showed better growth in terms of weight gain in earthen ponds. To study the reproductive performance of *S. seenghala* in captivity the adult fish were released in three 0.04 ha earthen ponds and were fed with 45% CP diet, at satiation. Male and female fish were kept separately in different ponds for a period 1.5 years. The fish were collected on monthly basis for the period of one year and gonadosomatic index (GSI) was determined. Fecundity and relative fecundity of *S. seenghala* were also calculated. This species breed from March to May. Fecundity and relative fecundity of Seengharee had a linear relationship with length, body weight and gonad weight.

From this study we may extract following conclusions

i) Use of anesthesia at the rate of 30 mg L^{-1} and loading density of 50 fish per bag showed better results for the transportation of *S. seenghala*

ii) Fingerling and fry of *S. seenghala* was successfully acclimatized in captivity by segregation of fry and fingerling on the basis of size

iii) Dietary requirements of Seengharee was 35% CP having 20% of animal protein offered at rate 4% of wet fish body weight daily

iv) Growth performance of *S. seenghala* was better in earthen ponds compared to cemented raceways

v) Reproductive season for *S. seenghala* was March to May, and fecundity showed linear relationship with length, body weight and gonad weight.

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