STUDY ON AQUACULTURE STRATEGIES TO COPE WITH SUDDEN INCREASE IN SALINITY FOR SUSTAINABLE FISH PRODUCTION

A Thesis

Submitted to the

West Bengal University of Animal and Fishery Sciences

in partial fulfillment of the requirements for the award of the degree Doctor of Philosophy

in

Aquatic Environment Management

By

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Dedicated to

Beloved Parents, sister & farmers...

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CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production"** *submitted by* ***Utpal Kumar Das*** *in partial fulfillment of the requirements for the degree* ***Doctor of Philosophy (Aquatic Environment Management)*** *in the* ***Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences,*** *is the faithful and bonafied research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.*

13.08.2024

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APPROVAL SHEET

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE DOCTOR OF PHILOSOPHY (Aquatic Environment Management)

We, the undersigned, have been satisfied with the performance of Utpal Kumar Das, in the Viva-Voce Examination, conducted today, on the 28th November, 2014, recommended that the thesis be accepted for the award of the Degree Doctor of Philosophy in Aquatic Environment Management.

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Place: Kolkata

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1. Introduction

Fisheries and aquaculture play important roles for food supply, food security and income generation from local to global levels. It is the main source of livelihood and employment for small and marginal farmers living nearby the coastal areas. Fish provides essential nutrition for 3 billion people and at least 50% of animal protein and minerals to 400 million people from the poorest countries (UNEP Report 2009).

Climate change is the most important driver adversely affecting ecosystems, societies and economics, livelihoods and food supplies, including those in the fisheries and aquaculture sector. Increase of mean temperature; increased variability both in temperature and rain patterns; fresh water availability; frequency and intensity of 'extreme events'; sea level rise and salinization, all have profound impacts on agriculture, forestry and fisheries (Gornall, 2010; IPCC, 2007; HLPE, 2012). The Intergovernmental Panel on Climate Change projects that atmospheric temperatures will rise by 1.8-4.0°C globally by 2100 (IPCC 2007). This warming will be accompanied by rising sea temperatures, changing sea levels, increasing ocean acidification, altered rainfall patterns and river flows, and higher incidence of extreme weather events.

The mean global rate of sea level rise during the 20th century was nearly 2 mm/year, which is 10 fold higher than the average of past several millennia (Vivekanandan and Das 2011). The IPCC fourth report has projected that the global annual seawater temperature and sea level would rise by 0.8 to 2.5°C and 8 to 25 cm, respectively by 2050. The sea level rise for Cochin (Southwest cost) is estimated as 2 cm in last one century. However, the rate of increase is accelerating, and it is projected that sea level may rise at the rate of 5 mm per year in the coming decades. Considering this, it is possible that the sea level may rise by 25 to 30 cm in 50 years. An increase in mean water depth will be accompanied by an increase in mean wave height, resulting in a more severe wave attack on the coast and greater wave induced littoral drift. The sea level rise increases the risk of

flooding. An increase in mean sea level will affect waves, currents and bottom pressure in the near-shore region (Vivekanandan and Das 2011).

Sundarban, the estuarine delta of Ganges-Brahmaputra-Meghna, is the largest river-mouth system in the world as well as a UNESCO World Heritage site. The Sundarban ecosystem is characterised by a very dynamic environment due to the effect of tide, flooding, salinity and cyclones. Air temperature over the Bay of Bengal is rising at a rate of 0.019°C per year. If this trend continues, the air temperature in this area is expected to rise by 1°C by 2050 (Hazra *et al.*, 2002). The annual composite sea surface temperature (SST) data during 2003-2009 varied from 28.02 °C in the year 2004 to 29.38°C in the year 2009. During this period the SST showed rising trend at the rate of 0.0453°C/ year (Mitra *et. al.* 2009). Rainfall has been increased over the Bay of Bengal during the last decade. It is also evident that the monsoonal rainfall has significantly increased at the rate of 0.0041 mm/hr along with SST. Over last 120 years, Bay of Bengal registered 26% increase in severe cyclonic storms, intensifying in post monsoon (Singh, 2007). During last part of the decade (2007-2009), northern part of Bay of Bengal has witnessed 4 cyclones *Sird, Nargis, Bijli* and *Aila.* In the year 2013 Odissa coast is affected by the cyclone *Phailyn.* The IPCC Fourth Assessment Report predicts that the climate change will intensify extreme weather events such as cyclones and associated storm surges, especially along the Bay of Bengal. These will seriously affect the agriculture, animal husbandry and fishery (fishing and aquaculture) to great extent. The frequency and intensity of extreme weather events like cyclone and storm surge have increased over the period of time, bringing sudden influx of saline water into the aquaculture areas leading to loss of fish crop and livelihood of the poor people.

Coastal areas are more vulnerable to climate induced risks like sea level rise, rise in temperature, erratic rainfall, increase in frequency and intensity of extreme events like cyclone and storm surges etc. Due of cyclone and storm surges, the low laying areas in the coastal zones are getting increasingly exposed to the threat of saline water flooding and damaging the agricultural lands including freshwater

fishponds. As a result, farmers incur heavy economic loss due to crop failure (both agriculture and fishery). Especially in deltas like Sundarban where the islands in the region are protected by earthen embankments, situations become more precarious as entire ecosystem inside the island is freshwater which more vulnerable to salinity intrusion due to breach of embankment. In such situation, freshwater aquaculture gets adversely affected by putting the fish in sever salinity induced physiological stresses like growth, reproduction, metabolism, osmoregulation, hormonal levels, etc.

To address above problems, under this study an attempt has been made to develop suitable strategies for freshwater aquaculture system to cope with the salinity intrusion. The entire study was comprised of following objectives:

- To study the tolerance limit of certain freshwater fish species towards abiotic climate change stresses like salinity
- To study the natural adaptability capacity of the selected fresh water fish species
- To evaluate different adaptation strategies (Aeration and feed manipulation)
- To suggest strategies against salinity stress caused due to influx of saline water.

REVIEW OF LITERATURE

Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production

The extreme events like cyclone, storm and tidal surge adversely affects the coastal freshwater aquaculture systems through influx of saline water into culture ponds. This lead to either mortality or put the freshwater fishes under salinity stress. There have been some reports on climate change and its impact on extreme events. During last several years the coastal zone of east coast has experienced several extreme event like *Sird, Nargis, Bijli, Aila* and *Phailyn* mainly on northern part of Bay of Bengal and Odissa coast. The adverse impact of saline water and the level of tolerance by fresh water fishes have been studied by the scientists and researchers worldwide and the same being illustrated below.

2.1 Salinity tolerance

Study carried out by Castaneda *et al.* (2010) showed that *Pangasius* juveniles could tolerate moderate salinity. They stated that the fish actively swam in the water and survival was 100% in all the tanks even after 63 days in 3 ppt, 51 days in 13 ppt and 22 days in 18 and 20 ppt. However, 80% of the fish died after three days at 24 ppt and 60% died after nine days at 22 ppt.

Several studies have been made on the salinity tolerance of some fresh water fish species by scientists like Chervinski and Zorn (1974), Whitfield and Blaber (1975), Chervinski (1977), Kilambi and Zdinak, (1980), Maceina and Shireman (1980), Von Oertzen (1985) and Stickney (1986) etc.

Ghosh *et al.* (1973) studied on the salinity tolerance of fry and fingerlings of Indian major carps. The normal salinity tolerance of fry and fingerlings of Indian major carps and their growth performance at different levels of salinities (from traces to 15 ppt), were determined. In the case of early fry (av. size: 17mm) no mortality was observed upto 3.28 ppt. At 4 ppt the mortality was 12.5% and the feeding activity was normal, while at 7.97 ppt the mortality increased to 87.5% and the fry exhibited signs of extreme restlessness and apathy for food. With further rise of salinity up to 10 ppt withering of the scales and ultimate mortality were observed. Advanced fry (av. size: 26mm) exhibited better tolerance up to 10 ppt

(mortality: 50%; feeding moderate). Reluctance of feed and higher mortality (75%) were observed at 11, 11.5, 12 and 12.5 ppt salinity. Immediate total mortality was observed at 13 ppt. and above. Fingerlings of *Catla catla* and *Labeo rohita* could tolerate salinity up to 12.5 ppt with 48% mortality.

Effect of salinity on growth and survival of Rohu, *Labeo rohita* (Ham.) under laboratory and field conditions were assessed by Pillai *et al.* (2003). They found that beyond 8 ppt, the fish showed sign of stress and mortality occurred. There was 100% mortality in 14% salinity within 7-8 days.

Saha *et al.* (1964) studied on the salinity tolerance of Indian Major carps in captivity. They reported a salinity tolerance limit of 14 ppt for the Indian major carps.

Ghosh and Pandit (1976) cited a note on the salinity tolerance of *Cyprinus carpio* under Indian conditions. They found that the lethal limit of salinity by common carp was 12.6 ppt.

An experiment was carried out by Mateen *et al.* (2004) at Fisheries Research Farms, University of Agriculture, Faisalabad to find out the tolerance of Rohu (*Labeo rohita*) and its hybrid (*Labeo rohita* ♂ x *Catla catla* ♀) for different levels of salinity under three temperature gradients. The salinity levels were prepared on the basis of electrical conductivity (E.C.), by adding commercial grade NaCl in the water. The five experimented EC levels were 1.2 mS/cm for control, 5, 10, 15 and 20 mS/cm for treatment T1-T4, respectively. The selected temperature gradients were 14, 21 and 28°C. The 7-days LC_{50} values for rohu were remained as 17.383, 19.649 and 10.158 mS/cm at temperature 14, 21 and 28°C, respectively. Rohu was more resistant against salinity at higher temperature than low temperatures. The important physico-chemical parameters varied significantly but remained favourable during the whole period of study.

Sarma *et al.* (2012) studied the response of a freshwater air-breathing fish, *Clarias batrachus* to salinity stress in order to expedite an experimental case for their farming in brackish water areas in Andaman, India. The LC_{50} of salinity for 96 h exposure to the fingerling (14.5 cm) was 12.52 ppt.

Sahoo *et al.* (2003) studied the effects of different levels of salinity (2, 4, 6, 8 and 10 ppt) on survival, feed intake and growth of freshwater clariid, *Clarias batrachus* in a thirty day laboratory experiment. They observed severe mortality beyond 6 ppt during 7-23 days of rearing.

Wang *et al.* (1997) studied about the influence of salinity on food consumption, growth and energy conversion efficiency of common carp (*Cyprinus carpio*) fingerlings. They reported that the salinity tolerance of common carp fingerlings was 10.5 ppt when the animals were exposed to water in which the salinity was raised at a rate of 0.5-1.0 ppt per day.

A simple test to estimate the salinity resistance of *Oreochromis noloticus* and *Sarotherodon melanotheron* was conducted by Lemarie, *et al.* (2004). Batches of 10 juveniles (5 to 20 g) of two different species *O. niloticus* and *S. melanotheron* reared in freshwater were subjected to gradual increases in salinity. For *S. melanotheron* the mean median lethal salinity (MLS) was 123±3.5 ppt For *O. niloticus* the MLS was 46.3±3.4 ppt for daily increases in salinity ranging from 2 to 8 ppt day^{-1}.

Al-Amoudi (1987) studied about the acclimation of commercially cultured *Oreochromis* species to sea water. He determined the MLS_{96hr} in juveniles of *O. niloticus* and *O. mossambicus* which were 19.5 and 25.4 ppt respectively.

Jamil *et al.* (2004) carried out a study about the salinity tolerance and growth response in juveniles of *Oreochromis mossambicus* at different salinity levels. No mortality was observed at salinity levels 0, 5, 10, and 15 ppt, while the juveniles faced slight mortality at 20 ppt in the same environment conditions, including diet.

Hena *et al.* (2005) studied about the salinity tolerance in superior genotype of tilapia, *Oreochromis niloticus, Oreochromis mossambicus* and their hybrids. Comparisons were made over a range of fixed salinities (0, 7.5, 15, 22.5 and 30ppt) with fish grown for 75 days in cages with concrete tanks with three replicate cages at each salinity. The poor survival in *O. niloticus* at 22.5 and

particularly at 30 ppt reduced resistance to disease. At 30 ppt more than 50% of fish were infected with fin rot and lesions on the body.

2.2 Natural adaptation capacity

A study was carried out by Castaneda *et al.* (2010) on *Pangasius* Juveniles Tolerance moderate salinity in test. They stated that the mean final weight of the fish was similar and not significantly different between 3 and 13 ppt, but tended to decrease at 18 and 20 ppt. Mean final weights at 20 ppt were significantly lower than at 3 and 13 ppt.

Sahoo *et al.* (2003) studied about effects of different levels of salinity (2, 4, 6, 8 and 10 ppt) on survival, feed intake and growth of freshwater clariid, *Clarias batrachus* in a thirty days laboratory experiment. The fingerlings tolerated the salt level in water between 0 and 4 ppt. No significant ($p<0.05$) differences with regard to feed intake and body weight gain were observed when reared in 0 and 2 ppt salinity. The feed intake and specific growth rate were decreased with the increase of salinity. Salinity level above 4 ppt was found to be detrimental to fish and in turn they lost their body weight. It was inferred that *Clarias batrachus* fingerlings could be reared in 2 ppt saline water without any adverse effect on growth, survivability and biomass production.

Brocksen and Cole (1972) studied on physiological responses of three species of fishes to various salinities. Studies on food intake and utilization in some fishes have been done by Raghuraman (1973). Desilva and Perera (1976) studied on the young grey mullet (*Mugil cephalus)*, effects of salinity on food intake, growth and food conversion. They found that salinity was one of the factors that influenced the metabolism of fish, which ultimately affected the survival, growth and feed intake.

Mansuri *et al.* (1979) studied on effects of salinity changes on freshwater murrels, *Channa punctatus*. They observed that *Channa punctatus* could thrive well in 10% seawater for indefinite period and mortality started beyond 30% seawater.

Besra (1997) studied about the effects of salinity on growth. He found that *Anabas testudineus* fingerlings (6-10 g) could withstand 2.5-10% seawater without mortality. Saunders and Henderson (1969) also observed heavy mortality of Atlantic salmon at 30 ppt than at 0, 7 or 15 ppt.

Information is also available on the salinity tolerance of some of the Indian catfishes, *Mystus vittatus* (Arunachalam and Reddy, 1979) and *Heteropneustes fossilis* (Parwez *et al.*, 1979).

Works on *Tilapia aurea* (Chervinski and Yashouv, 1971), *T. nilotica* (Lotan, 1960), *Cyprinus carpio* (Fishelson and Popper, 1968) and *Ctenopharingodon idella* (Routray and Routray, 1997) have already been reported for intensive farming that can survive and grow in brackish as well as seawater systems.

Wang *et al.* (1997) studied about the influence of salinity on food consumption, growth and energy conversion efficiency of common carp (*Cyprinus carpio*) fingerlings. They reported that the growth rate of carp was highest in fresh water and decreased with increasing salinity. According to them food consumption, digestibility, growth rate and feed conversion efficiency were all high for the common carp fingerlings exposed to fresh water and 2.5 ppt salinity, The optimal salinity range for growth of common carp was found to be in the range from fresh water to 2.5 ppt. At 10.5 ppt, the fingerlings had poor growth and become emaciated.

Some fishery scientists found that silver carp (*Hypothalmichthys molitrix*) and common carp had a lower oxygen consumption rate and standard metabolic rate under 3 ppt salinity because they expended less energy on maintaining internal equilibrium (Von Oertzen, 1985; Qui Deyi and Qin Kejing, 1993). Similar hypothesis has also been proposed for other fish (Stauffer *et al.*, 1984; Stauffer, 1986).

Ghosh *et al.* (1973) studied on the salinity tolerance of fry and fingerlings of Indian major carps. They recorded that the salinity level of best growth rate for *Catla catla* and *Labeo rohita* were 5 ppt (14-15mm/week for *L. rohita* and 18-20mm/week for *C. catla*). Beyond this level, reluctance towards feeding was observed and at 11 ppt and above, the fish abstained from feeding. According to them *L. rohita* and *C. catla* fingerlings can grow up to 8 ppt, while for best growth 5 ppt salinity is preferred.

Pillai *et al.* (2003) studied about the effect of salinity on growth and survival of Rohu, *Labeo rohita* (Ham.) under laboratory and field conditions. Salinity range of 0 to 14 ppt was used at 2 ppt interval in the study. Maximum growth was obtained at 0 and 2 ppt, growth was not markedly affected upto 6 ppt salinity. They concluded that there was good potential for culturing the species in low salinity areas.

A study carried out by Routary and Routary (1997) on growth potential of grass carp, *Ctenopharyngodon idella* in saline water with an aquatic weed *Potamogeton pectinatus* as feed. They observed that growth retardation was only beyond 6 ppt salinity.

Suresh and Lin (1992) studied about the tilapia culture in saline waters. They found that some species are known for their ability to acclimate to very different salinity media including extreme environments (> 100 psu). Killifish (Cyprinodontiformes) are particularly interesting because of their capacities to acclimate to such environments and represent nice models in biology.

Sarma *et al.* (2012) studied about response of a freshwater air-breathing fish, *Clarias batrachus* to salinity stress: an experimental case for their farming in brackishwater areas in Andaman, India. Two sublethal salinity levels, viz. 4 and 8 ppt were studied the long-term effects of salinity on *C. batrachus* for a period of 90 days. From the study, they found that growth and survival rate were less in saline water (4 and 8 ppt). Maximum growth and survival were recorded in freshwater (0 ppt salinity) and subsequently at 4 and 8 ppt. From the investigation

they concluded that exposure to higher salinity significantly ($p<0.01$) affects the growth and physiological response of *Clarias batrachus*.

Luz *et al.* (2008) studied about growth, food intake regulation and metabolic adaptation in gold fish (*Carassius auratus*) exposed to different salinities. They investigated the effects of different salinities (0, 2, 4, 6, 8 and 10 ppt) on food consumption, growth, metabolic resources and several stress indicators in goldfish. Salinities up to and including 6 ppt did not affect weight gain, standard growth and feed conversion rates. Higher salinities (8 and 10 ppt) produced significant muscle dehydration, adverse effect on growth, food intake and food conversion rate. *Carassius auratus* exhibits good growth and no signs of stress in saline waters up to 6 ppt salinity. They observed that at 2 ppt salinity, the feeding, growth rate and feed conversion efficiency is similar to fresh water condition. They found that using such salinities to reduce the incidence of disease and mortality and do not produce significant physiological alteration in this species.

A study was conducted on how should salinity influence fish growth by Boeuf and Payan (2001). They reported that physiological functions clearly influenced by water salinity in fish is growth.

Engstrom-Ost *et al.* (2005) studied the growth of pike larvae (*Esox lucius*) under different conditions of food quality and salinity. They reported that physiological functions clearly influenced by water salinity in fish is growth.

The larval culture at low salinities produces higher growth and survival rates than in freshwater conditions in some freshwater species (Britz and Hecht, 1989; Luz *et al.* 2004).

Effects of temperature and salinity on growth and reproduction of the freshwater prawn, *Macrobrachium rosenbergii* (Crustacea-Decapoda) in Egypt was carried out by Habashy and Hassan (2011). The effect of different levels of temperature (24°C, 29°C and 34°C) and salinity (8 and 16 ppt) compared to

dechlorinated tap water (0 ppt) as control group on growth and reproduction performance of female *Macrobrachium rosenbergii* was studied under controlled laboratory conditions. Juvenile prawns of 0.21± 0.021g and 3.1±0.208 cm size were reared at these conditions for 8 months. The results revealed that growth of the prawn was increased as temperature increased from 24 to 29°C but subsequently the growth declined at the highest temperature (34°C). Also as salinity increased from 0 to 16 ppt, growth of females decreased at all temperatures tested. The highest total length (16.2cm) and total weight (40.53g) were obtained at a combination of 29°C–0 ppt. It was clearly found that optimum level of both temperature and salinity for growth, reproduction and hatching success of this species was 29°C-0 ppt and 29°C-8 ppt.

Jamil *et al.* (2004) carried out a study about the salinity tolerance and growth response of juveniles *Oreochromis mossambicus* at different salinity levels. Their result shows that *O. mossambicus* could survive up to 20 ppt salinity. They suggest that the species could grow and could be exploited commercially in brackish waters, rivers and estuarine regions.

Likongwe *et al.* (1996) studied about the combined effects of water temperature and salinity on growth and feed utilization of juvenile nile tilapia (*Oreochromis niloticus*). They studied the combined effects of temperature (24, 28 and 32°C) and salinity (0, 8, 12 and 16 ppt) on growth. Final mean weights were significantly ($p<0.05$) higher at 32 and 28°C than 24°C at 12 ppt salinity, where fish increase their weights seven–fold and four-fold, respectively. In their study growth was lowest at 24°C at 16 and 12 ppt salinity. The highest growth rate was found in fishes exposed to 32°C and 8 ppt salinity. They also suggested that generally, the temperature range of 28 to 32°C and the salinity range of 12 ppt could result in rapid growth of juvenile Nile tilapia, because there were no significant differences ($p>0.05$) in growth of fish in these water temperature-salinity combinations. At all temperature salinity levels higher than 8 ppt depressed growth. They suggested that growth rates of juvenile *O. niloticus* were comparably high at 28 or 32°C in waters of 0 and 8 ppt salinity.

Watanabe *et al.* (1993) conducted an experiment on the effects of temperature and salinity on growth and feed utilization of juvenile, sex-reversed male Florida red tilapia cultured in a recirculating system. They reported that at 0 ppt salinity, feed consumption and growth reached a maximum at 27°C, while at 18 and 36 ppt salinity, consumption and growth were highest at 32°C.

Hena *et al.* (2005) studied about the salinity tolerance in superior genotype of tilapia, *Oreochromis niloticus, Oreochromis mossambicus* and their hybrids. The study were made over a range of fixed salinities (0, 7.5, 15, 22.5 and 30 ppt) with fish grown for 75 days in cages with concrete tanks with three replicate cages at each salinity. Growth was higher at elevated salinities with *O. niloticus,* relatively faster growing at low salinity and *O. mossambicus* at the higher salinity. *O. mossambicus* had better option for pure species culture at salinities above 18-20 ppt.

Philippart and Ruwet (1982) studied about the ecology and distribution of tilapias. According to them tilapias are euryhaline fish that can live and thrive in a wide range of salinity fluctuations from fresh water to full seawater.

Popper and Lichatowich (1975) studied the preliminary success in predator control of *Tilapia mossambica.* The species grow well up to 40 ppt.

Villegas (1990) studied the growth and survival of *Oreochromis niloticus, Oreochromis mossambicus* and their F_1 hybrids at various salinities. He showed that *O. niloticus* grew best at 0-10 ppt and slower at 25-32 ppt.

Payan and Collinson (1983) conducted a comparision of biological characteristics of *Sarotheradon niloticus* (L) with those of *S. aureus* (Steindachner) and other tilapia of the delta and lower Nile. They reported that the salinity range for better growth of *O. niloticus* is 5-10 ppt and *O. mossambicus* performed best intermediate (15 ppt) and high salinites (32 ppt).

Boeuf and Payan (2001) studied about how should salinity influence fish growth. They found that development and growth (continuous in fish) are

controlled by 'internal factors' including CNS, endocrinological and neuroendocrinological systems. Among vertebrates, they also are highly dependent on environmental conditions. Among other factors, many studies have reported an influence of water salinity on fish development and growth. In most species, egg fertilization and incubation, yolk sac resorption, early embryogenesis, swim bladder inflation, larval growths are dependent on salinity. In larger fish, salinity is also a key factor in controlling growth. The changes effect in growth rate that depend on salinity, result from an action on: (1) standard metabolic rate; (2) food intake; (3) food conversion; and/or (4) hormonal stimulation? Better growth at intermediate salinities (8–20 psu) is very often, but not systematically, correlated to a lower standard metabolic rate. Numerous studies have shown that 20 to >50% of the total fish energy budget are dedicated to osmoregulation. However, recent ones indicate that the osmotic cost is not as high (roughly 10%) as this. Data are also available in terms of food intake and stimulation of food conversion, which are both dependent on the environmental salinity. Temperature and salinity have complex interactions. Many hormones are known to be active in both osmoregulation and growth regulation, e.g. in the control of food intake. All of these factors are reviewed. As often, multiple causality is likely to be at work and the interactive effects of salinity on physiology and behavior must also be taken into account.

Boeuf and Payan (2001) reported that fish are dependent on both internal (nervous, endocrinological and neu-roendocrinological) and external (ecological) factors, which control or synchronize many activities or functions, including growth capacity. It is possible to classify such ecological factors into two types: (1) determining factors (temperature, salinity, photoperiod) which act directly through receptors to increase or decrease growth; and (2) limiting factors, which operate above (ammonia) or below (oxygen) a specific threshold or within a tolerance range (pH).

According to Boeuf and Payan (2001) among the ecological factors, salinity is specific to the aquatic environment. Many authors have demonstrated the

influence of external salinity on growth capacities in fish. This is true for a lot of species, including both marine and freshwater (FW) fish. In fact, species not influenced by salinity changes during their development and growth are rare. It is also well known that many juveniles prefer intermediary salinities, as found, e.g. in estuaries, tidal coastal areas or coastal lagoons. In fact, rather than asking: 'does salinity have an influence on fish growth?', a much better question would be: 'how should salinity influence growth?'.

Brett (1979) studied about the environmental factors and growth. He found that growth is continuous, so that fish become larger the longer they live and they are much more dependent on external environmental conditions.

Boeuf and Le Bail (1999) studied about the does light have an influence on fish growth? They found that growth is continuous, so that fish become larger the longer they live and they are much more dependent on external environmental conditions.

Konstantinov and Martynova, (1993) studied about the effect of salinity fluctuations on energetics of juvenile fish. They found that salinity clearly influences growth in fish. In experimenting with fresh water species, *Cyprinus carpio*, *Ctenopharyngodon ide*lla and juvenile of *Acipenser guldenstaedti*, showed that a salinity of 2 psu considerably increased growth rate and food efficiency, by improving the food conversion rate.

2.3 Adaptability study

Griffith (1974) studied about the environment and salinity tolerance in the genus Fundulus. He found that some species are known for their ability to acclimate to very different salinity media including extreme environments (> 100 psu). Killifish (Cyprinodontiformes) are particularly interesting because of their capacities to acclimate to such environments and represent nice models in biology.

Jordan *et al.* (1993) studied Plasma osmotic regulation and routine metabolism in the Eustis pupfish. They found that some species are known for

their ability to acclimate to very different salinity media including extreme environments (> 100 psu). Killifish (Cyprinodontiformes) are particularly interesting because of their capacities to acclimate to such environments and represent nice models in biology.

Sahoo *et al.* (2003) studied about effects of different levels of salinity (2, 4, 6, 8 and 10 ppt) on survival, feed intake and growth of freshwater clariid, *Clarias batrachus* in a thirty days laboratory experiment. Although the fingerlings tolerated the salt level in water between 0 and 4 ppt, severe mortality was observed beyond 6 ppt after 7-23 days of rearing. No significant ($P<0.05$) differences with regard to feed intake and body weight gain were observed when reared in 0 and 2 ppt salinity. The feed intake and specific growth rate were decreased with the increase of salinity. Salinity level above 4 ppt was found to be detrimental to fish and in turn they lost their body weight. It is inferred that *Clarias batrachus* fingerlings can be reared in 2 ppt saline water without any adverse effect on growth, survivability and biomass production.

Several workers have reported the growth potential of different freshwater fishes at certain levels of salinity, beyond which the growth was retarded (Chervinski, 1961; Ghosh *et al.*, 1973; Besra, 1997).

Mckay and Gjerde (1985) observed decreased growth with increase of salinity in rainbow trout. Growth beneficiary effect did not exist in *A. testudineus*, exposed to 7.5 to 10% seawater (Besra, 1997). The growth of freshwater catfish, *M. vittatus* showed a descending trend when exposed to 0 to 10 ppt salinity (Arunachalam and Reddy, 1979).

Smith *et al.* (1999) studied about the salinity effects on early life stages of southern flounder *Paralichthys lethostigma*. They found that the summer flounder (*Paralichthys dentatus*) or Southern flounder (*P. lethostigma*), early development and larval growth were also affected by salinity.

Specker *et al.* (1999) studied about the metamorphosis in summer flounder: effects of acclimation to low and high salinities. They found that the summer flounder (*Paralichthys dentatus*) or Southern flounder (*P. lethostigma*), early development and larval growth were also affected by salinity, optimal conditions being 8 14 and 5 30 psu, respectively.

Luz *et al.* (2008) studied about the growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. The aim of the study was to investigate the effects of different salinities (0, 2, 4, 6, 8 and 10 ppt) on food consumption, growth, metabolic resources, and several stress indicators in goldfish. Possible changes in feeding regulators, brain neuropeptide Y, circulating ghreline, and the hypothalamic monoaminergic transmission were also examined. Salinities up to and including 6 ppt did not affect weight gain, standard growth and feed conversion rates. The goldfish showed good adaptation to these salinities in terms of metabolic resources (lipids and glycogen content in liver and muscle) after 21 days of salinity exposure. Higher salinities (8 and 10‰) produced significant muscle dehydration, significant increases in circulating cortisol, and adverse effects on growth, food intake and food conversion rate. Diurnal locomotor activity was significantly lower in all goldfish exposed to salinity compared to fresh water fish. In conclusion, *Carassius auratus*, a freshwater stenohaline fish exhibits good growth and no signs of stress in saline waters up to 6 ppt salinity. These results demonstrate that using such salinities to reduce the incidence of diseases and mortality does not produce significant physiological alterations in this species.

2.3.1 Aeration as an adaptation strategy

Das *et. al.* (2005) studied about the thermal tolerance, growth and oxygen consumption of *Labeo rohita* fry acclimated to four temperatures. A thirty day feeding trial was conducted using a freshwater fish, *Labeo rohita* (rohu), to determine their thermal tolerance, oxygen consumption and optimum temperature for growth. Four hundred and sixteen *L. rohita* fry (10 days old, 0.385±0.003 g) were equally distributed between four treatments (26, 31, 33 and 36°C) each with

four replicates for 30 days. Highest body weight gain and lowest feed conversion ratio (FCR) was recorded between 31 and 33°C. The highest specific growth rate was recorded at 31°C followed by 33 and 26°C and the lowest was at 36°C. Thermal tolerance and oxygen consumption studies were carried out after completion of growth study to determine tolerance level and metabolic activity at four different acclimation temperatures. Oxygen consumption rate increased significantly with increasing acclimation temperature. Preferred temperature decided from relationship between acclimation temperature and Q_{10} values were between 33 and 36°C, which gives a better understanding of optimum temperature for growth of *L. rohita*. Critical thermal maxima (CTMax) and critical thermal minima (CTMin) were 42.33±0.07, 44.81±0.07, 45.35±0.06, 45.60±0.03 and 12.00±0.08, 12.46±0.04, 13.80±0.10, 14.43±0.06, respectively, and increased significantly with increasing acclimation temperatures (26, 31, 33 and 36°C). Survival (%) was similar in all groups indicating that temperature range of 26-36°C is not fatal to *L. rohita* fry. The optimum temperature range for growth was 31–33°C and for Q_{10} values was 33–36°C.

Das *et al.* (2004) studied the thermal tolerance and oxygen consumption of Indian Major Carps acclimated to four different temperatures.

Kita *et al.* (1996), studied about the temperature preference and tolerance and oxygen consumption of the marbled rock-fish, *Sebastiscus marmoratus*.

Manush *et al.* (2004) studied about the thermal tolerance and oxygen consumption of *Macrobrachium rosenbergii* acclimated to three temperatures.

Merkens and Downing (1957) studied about the effect of tension of dissolved oxygen on the toxicity of unionized ammonia to several species of fish.

Qayyum *et al.* (2005) studied about the effect of aeration on water quality, fish growth and survival in aquaculture ponds. Fingerlings of *Labeo rohita, Cirrhinus mrigala, Hypophthalmichths molitrix* and *Ctenopharyngodon idella* (average weight 32.9 g) were cultured in six brick lined (26'x12'x6') ponds for a

period of six months. The stocking ratio was 7:4:4:3, respectively and the stocking density of each pond was 2508 fish/acre. Three of the six ponds served as aerated ponds and other three as non-aerated ponds. Aeration was done with the help of 1/3 Hp vertical pump. Fish in each pond were fed with 20% protein diet@ 3% body weight. The ponds were fertilized with cattle manure (500 kg/acre) and chemical fertilizers @ 20:2:0 (N:P:K). Significantly high growth and survival of fish ($p< 0.0001$) was observed in aerated ponds as compared to the non-aerated ponds. Pond aeration das resulted in 2095 kg/acre more fish production.

Loyd (1961) studied about the effect of dissolved oxygen concentrations on the toxicity of several poisons to Rainbow trout (*Salmo gairdnerii* Richardson). According to him there may be a common relation between the dissolve oxygen concentration and the toxicity of poisons.

Manush (2004) studied about the thermal tolerance and oxygen consumption of *Macrobrachium rosenbergii* acclimated to three temperatures. They found the rates of oxygen consumption at different acclimation temperatures significantly ($p<0.05$) increased with increasing temperature.

2.3.2 Feed manipulation as an adaptation strategy

Kalla *et al.* (2004) studied the protein requirements of growing Indian major carps under field conditions. The purpose of this study was to determine optimum protein levels required for Indian major carp fry (mean body weight 1.95 g). Fry were stocked @ 10 million ha^{-1} in nursery ponds and fed on one of the five formulated diets (protein content ranged between 33-45%) over a period of 40 days. Growth and specific growth rate (SGR) values remained low when fed on low dietary protein levels (Treatments 1 and 2). Highest growth performance (weight gain, SGR and length) was observed in nursery ponds where the fry were fed on 40% dietary protein irrespective of the protein source (soycake or fish meal, Treatments 3 and 4). An increase in the dietary protein levels (beyond 40%) not only repressed growth performance but also affected survival rate.

Desilva and Perera (1976) studied about the effects of salinity on food intake, growth and food conversion of the young grey mullet, *Mugil cephalus*. They found that salinity is one of the factors that influence the metabolism of fish, which ultimately affects the survival, growth and feed intake.

Watanabe *et al.* (1989) studied about the Salinity during early development influences growth and survival of Florida red tilapia in brackish and seawater. They found that in the red tilapia (hybrid *Oreochromis mossambicus* X *O. urolepis*), early development and growth were optimal at 18 psu (tested range, 4 to 36 psu).

Jonassen *et al.* (1997) studied about the Seawater acclimation of tilapia, *Oreochromis spilurus* spilurus G¨unter, fry and fingerlings. They found that a change from 0 to 36.6 psu SW did not affect growth in the tilapia *O. spilurus*, when the salinity was increased progressively over 120 hour.

Most species grow optimally between 5 and 18 psu, although red tilapia prefer 30, 35 psu. Studies in tilapias, using salinities between 0 and 120 psu, determined that certain species or hybrids have astonishing growth capacities (reviewed in Suresh and Lin, 1992).

In a seminal paper (Watanabe *et al.,* 1988) on red tilapia, food intake, food conversion and growth rates were closely related with increased salinity up to 36 psu. As a consequence, it is extremely important to take this into account, and to clearly separate effects between temperature and salinity. For instance, salmonids can grow better in SW during the winter in net-pen culture, often independently of the higher salinity, since temperature is higher than in rivers during this season.

Boeuf (1993) studied about the salmonid smolting: a pre-adaptation to the oceanic environment. He found that for smoltifying salmonids, where the smolt status profoundly influenced salinity 'receptivity', osmotic capacities and growth.

The physiological functions clearly influenced by water salinity in fish is growth (Boeuf and Payan, 2001; Engstrom-Ost *et al.* 2005). Thus, larval culture at

low salinities produces higher growth and survival rates than in freshwater conditions in some freshwater species (Britz and Hecht, 1989).

The negative effects of salinity on growth performance in juvenile goldfish have been reported by Altinok and Grizzle (2001b), where growth rate and feed conversion ratio were adversely affected by salinity (1, 3 and 9 ppt). They found that the SGR and FCR of juvenile goldfish (mean mass around 2 g) were adversely affected at 3 ppt salinity.

Ghosh *et al.* (1973) studied on the salinity tolerance of fry and fingerlings of Indian major carps. Best growth rate for both species were recorded at the salinity levels of 5 ppt (14-15mm/week for *L. rohita* and 18-20mm/week for *C. catla.* Beyond this level, reluctance towards feeding was observed and at 11 ppt and above, the fish abstained from feeding.

Pillai *et al.* (2003) studied the effect of salinity on growth and survival of *Labeo rohita* under laboratory and field conditions. The maximum growth was obtained at 0 and 2 ppt but was not markedly affected up to 6 ppt salinity. As salinity increased above 8ppt, Rohu showed stress signs. The study indicates that there is good potential for culturing the species in low salinity areas.

Raghuraman (1973) studies on food intake and utilization in some fishes. He found that salinity is one of the factors that influence the metabolism of fish, which ultimately affects the survival, growth and feed intake.

Lin *et al.* (2004) studied about the effect of dilatory probiotics on apparent digestibility coefficients of nutrients of white shrimp *Litopenaeus vannamei.* According to them probiotics can increase digestibility.

Tewary and Patra (2008) studied the use of vitamin C as an immunostimulant, effect on growth, nutrient quality, and immune response of *Labeo rohita* (Ham.). According to their study, teleost fish lack the required enzyme for endogenous synthesis of ascorbic acid (AA), an essential micronutrient for fish. Four groups of *L. rohita* were fed with experimental diets containing no

vitamin C (Control) and another with vitamin C at 500 mg kg^{-1} (Exp-1), 1000 mg kg^{-1} (Exp-2) or 1500 mg kg^{-1} (Exp-3) for 60 days. Growth parameters (NWG, ADG and SGR) were evaluated during the experimental trial. Fish fed with vitamin C supplemented diet showed higher specific growth rate (SGR) up to 1000 mg kg^{-1} compared with control fish.

Burr and Gatlin III (2005) studied about the microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. According to them dietary supplementation of prebiotics, which are classified as non-digestible food ingredients that beneficially affect the host by stimulating growth and/or activity.

Bailey *et. al.* (1991) studied about the effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine. They reported that probiotics can modify the GI tract microbial community to enhance non-specific immune response.

Bongers and van den Heuvel (2003) studied about the prebiotics and the bioavailability of mineral and trace elements. They reported that prebiotics improve mineral uptake.

Gunasekera *et. al.* (2000) studied the effect of dietary protein level on growth and food utilization in juvenile Murray cod *Maccullochella peelii peelii* (Mitchell). Murray cod is a freshwater percichthyid fish having high culture potential. Growth and feed utilization were examined in a 56-day experiment, in which triplicate group of juvenile Murray cod (initial weight 21.5±0.03g) were fed isocalorific diets (gross energy content of about 21 KJg^{-1}) containing 40%, 50%, 55% or 60% protein (designated P40, P45, etc). Final mean weight, percentage increase in weight and specific growth rate (SGR) were highest in fish fed the P50 diet. Food conversion ratio (FCR: 1.05±0.04) and protein efficiency ratio (PER: 1.98±0.11) were also best in fish on the P50 diet, but the differences in these parameters from the corresponding values on diets P55 and P60 were not always significant.

Lin *et. al.* (2012) studied the effects of dietary chitosan oligosaccharides and *Bacillus coagulans* on the growth, innate immunity and resistance of koi (*Cyprinus carpio koi*). They investigated the effects of oral administration of chitosan oligosaccharides (COS) and *Bacillus coagulans*, single or combined, on the growth performance, immunity and disease resistance of *Cyprinus carpio koi.* The fishes (24.9±0.52) were divided into 4 groups and each group was fed with diets supplemented with or without immunostimulant for 8 weeks. After 8 weeks of feeding trial, five fish per tank sampled for immunity determination, ten fish per tank were challenged by *Aeromonas veronii.* The results showed that the fish fed with diets supplemented with a combination of COS and *B. coagulans* has the highest final weight, specific growth rate (SGR), total leukocyte count (WBC), respiratory burst activity, phagocytic activity, lysozyme activity, SOD activity of koi and disease resistance to *A. veronii* ($p< 0.05$), followed by groups fed with diets with *B. coagulans* and COS, and the lowest in koi fed with the control diet. However, there were no significant difference ($p< 0.05$) in these growth index and immune parameters of koi and resistance to *A. veronii* between dietary *B. coagulans* and dietary COS. Under the experimental conditions dietary *B. coagulans* COS had a synergistic effect on enhancing immunity and disease resistance of koi ($p< 0.05$).

Torrecillas *et al.* (2007) studied about the immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Specimens of 35 g at initial density of 3 kg/m^3 were fed during 67 days at 0, 2 and 4 ppt dietary MOS level of inclusion in a commercial sea bass diet. Food conversion rate, specific growth rate, whole body biochemical composition, phagocyctic index of head kidney macrophages, NBT index, lysozyme and alternative complement pathway (ACP) activities as well as gut and liver histological structure were evaluated. Growth significantly increased as both MOS dietary inclusion levels. Histological features of the liver showed lower lipid vacuolization and regular-shaped morphology of hepatocytes around the sinusoidal spaces denoting a better utilization of dietary nutrients. No differences were found

on gut histological evaluation. Statistical differences ($p< 0.05$) on the phagocytic index were denoted with the inclusion of 4 ppt Bio-MOS group. A positive correlation was found between the levels of lysozyme and alternative complement pathway activities in blood and the level of inclusion of MOS in diets. Fish fed MOS supplement diets showed a significant growth improvement.

Yousefian and Amiri (2009) reviewed the use of prebiotic in aquaculture for fish and shrimp. According to them the growth of the fish increased at the using supplementary prebiotic in feed.

Refstie *et al.* (2006) studied about the capacity for digestive hydrolysis and amino acid absorption in Atlantic salmon (*Salmo salar*) fed diets with soybean meal or inulin with or without addition of antibiotics. In their 3-week trial they found that Atlantic salmon fed with a fish meal based diet supplemented with 75 g kg^{-1} inulin had increased relative mass of the gastrointestinal tract, but the absorptive capacity of the fish was not affected.

Wang (2007) studied about the effect of probiotic on growth performance and digestive enzyme activity of the *Penaeus vannamei*. Photosynthetic bacteria and *Bacillus* sp. were added to shrimp basal diets as probiotic at three concentrations: T-1, 2 g kg^{-1} (1 g kg^{-1} lyophilized photosynthetic bacteria cells (PSB) and 1 g kg^{-1} lyophilized *Bacillus* sp. (BS)); T-2, 10g kg^{-1}(5 g kg^{-1} PSB and 5 g kg^{-1} BS); and T-3, 20 g kg^{-1}(10 g kg^{-1} PSB and 10 g kg^{-1} BS). Twelve aquaria with three replicates for each treatment group and control group were used. After 28 days, shrimp receiving the diets supplemented with probiotics showed significantly better growth performance than those fed the basal diet (control). The mean digestive enzyme activity of each treatment groups was significantly different ($p< 0.05$) from that of the control. The protease activity of T-2 and T-3 was significantly higher compared with T-1 and control. There was no significantly difference between T-2 and T-3. The amylase activity of T-2 was highest and significantly different ($p<0.05$) from that of the control and T-1. Both

treatment groups had significantly higher lipaase and celluase activity compared to the control.

Arzel *et al.* (1995) studied the protein requirement of brown trout (*Salmo trutta*) fry. The protein requirement of brown trout of INRA DC 87 strain was studied by feeding diets containing graded levels of protein. These diets were formulated to be isoenergetic on a digestible energy basis and contained protein from 38 to 65%. The fish were 1.15 g triploid fry reared in stream water. Triplicates of 300 fish per replicate were used for each diet. The fish were fed for 52 days a ration which was modified every 2 weeks according to biomass, but the actual overall ration corresponded to excess feeding. Proximate analyses were performed on whole body and epaxial muscle at the end of the trial. No significant growth improvement was obtained over 53% protein, while best feed efficiency was apparently observed with 57% protein. The lower the dietary protein, the better the nitrogen utilization (estimated by protein efficiency ratio or productive protein value). Body protein content was not related to dietary protein but low protein levels resulted in higher body lipid content. Essential free amino acid contents in the muscle were plotted against the dietary protein levels and the values of the protein level corresponding to the slope changes were compared with the estimations of the dietary requirement.

Jesu *et al.* (2007) studied about the effect of dietary lipid levels on survival and growth of the threatened freshwater catfish *Mystus montanus*. *Mystus montanus* fingerlings (0.93±0.03 g) fed on six different formulated diet containing 6% -14% lipid. They were fed to the fish at the ration levels of 5% of their total body weight with three replicates per treatment. After 49 days, final weights were significantly greater ($p<0.05$) in all treatments. Food conversion ratio (FCR) was 1.58 at 7% lipid inclusion fed fishes and 4.28 at 14% lipid inclusion were noticed as minimum and maximum respectively. Fish survival was increased by providing formulated diet, but no further improvement was found after 7% lipid inclusion. This result indicated that the optimal lipid inclusion in the diet for the threatened catfish *M. montanus* fingerling was 7%.

De Silva *et al.* (1991) studied about the interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. They recommended that 10% to 20% lipid in fish diet for optimum growth without any excess fatty carcass.

Singh (1991) studied the nutrition and feed development strategies for aquaculture in India. According to him optimum lipid requirements of Indian major carps and common carp were determined at 4% to 6%.

Bagheri *et al.* (2008) studied about the growth, survival and gut microbial load of Rainbow Trout (*Onchorhynchus mykiss*) fry given diet supplemented with Probiotic during the Two Months of First Feeding. A commercial *Bacillus* spp. probiotic was tested on rainbow trout fry during the two months of first feeding. Probiotic was introduced in diets at five different levels, (T1: 4.8×108, T2: 1.2×109, T3: 2.01×109, T4: 3.8×109, T5: 6.1×109 CFU g^{-1}) and their effects compared with those of control diet containing no probiotic. Survival in treatments was significantly ($p<0.05$) higher than control and a slight increasing mortality rate was observed during the first week of experiment. The counts of bacteria associated with trout intestine in all treatments were significantly ($p<0.05$) higher than controls and *Bacillus* spp. was not detected in controls. Total bacteria counts were significantly different among treatments and controls; it may suggest that the colonization rate of digestive tracts of rainbow trout fry with bacteria was affected by dietary bacteria level. Specific growth rate, condition factor, protein efficiency ratio were slightly but significantly ($p<0.05$) higher and feed conversion ratio was lower in groups received probiotic via diets than controls. It may show that probiotic stimulates digestive development and enzymatic activity in fish. Growth performance in treatment received 3.8×109 CFU g-1 showed the best results. Therefore, it does not appear that higher levels of probiotics improved results and suitable doze of probiotic should be assessed before application in large scale to prevent any undesired effects. The supplementation of trout starter diet with *Bacillus* spp. is probably effective for improving rearing conditions.

Administration of *Bacillus* bacteria to trout fry results in enhanced digestion of food and improved growth.

Narejo *et al.* (2011) studied about the optimum protein requirements for the intensive culture of *Labeo rohita* (Hamilton) in glass aquaria. Three iso-caloric pellet feeds were prepared from locally available feed stuffs (rice protein, rice bran and wheat bran) of different protein levels such as 35%, 38% and 40% (dietary protein levels) of 2 mm diameter. These feed stuffs were tested for proximate (bio-chemical composition) analysis and the amount of protein was found to be 13%, 12% and 40% respectively. Each feed was supplied at a rate of 8% of the body weight of fish twice a day. The results of the various growth parameters like suitability of protein level requirement, specific growth rate, mean total weight gain, percentage weight gain, feed conversion ratio, survival rate and production showed significantly ($p<0.05$) highest growth and production in feed B (38% gross protein) followed by feed C (40% gross protein) while significantly ($p<0.05$) lowest growth and production was recorded for feed A (35% gross protein). The water quality parameters were recorded throughout the study period and were found within the suitable ranges of fish culture. It is therefore concluded that the pellet feed with 38% (gross protein) is the optimum protein level for the better growth and production of major carp, *Labeo rohita*.

Loum *et al.* (2013) studied about the effects of dietary protein level on growth performance, carcass composition and survival rate of fry monosex Nile Tilapia, *Oreochromis niloticus* reared under re-circulating system. Fry monosex Nile tilapia (*Oreochromis niloticus*) were fed in five dietary protein levels (21%, 25%, 32% 37% and 45%) to investigate casual effects on growth performance, carcass composition and survival rate. Tests were carried out in 10 glass tanks (50x25x40 cm), each of 50 l capacity, of reticulating system maintained at 30±1°C. At the beginning of tests, one hundred tilapia fry were randomly divided into five different groups with two replicates. Diets were fed to duplicate groups of ten fry with an initial weight of 1.25±0.25 g/fish during 42 days. The results showed significant effects of dietary protein on growth performance of reared fish.

Weight gain (WG) and Specific Growth Rate (SGR) increased significantly with increasing dietary protein levels between 32.38% and 37.63%. However, 45.5% of crude protein showed less important increase in growth parameters. The best Feed Conversion Rate FCR (1.26) was noticed in diet containing 37.63% of crude protein. Accordingly, higher survival rate (%) was recorded in fish fed on diets containing 32.38 and 37.63%. There was no significant difference in protein body content of tilapia fed on five diets as compared to the initial fish. Lipid body content increased significantly with high dietary protein levels from 21.88% to 45.50%. The carcass crude lipid was recorded as higher (9.4%) in the fry fed on diet containing 45.50% protein, followed by fish fed on diet having protein 21.88%. From results, diet containing 37.63% crude protein appears to be more suitable for mono sex Nile tilapia growth, in related experience conditions.

Jauncy and Ross (1982) studied the tilapia feed and feeding. According to them protein requirement for fish fry is high and ranges from 35% to 56%.

Wang and Lin (2008) studied about the probiotics in aquaculture: challenges and outlook. According to them probiotics supplements in feed improve feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth promoting factors and increase immune response.

Manush *et al.* (2005) studied about the dietary high protein and vitamin C mitigate stress due to chelate claw ablation in *Macrobrachium rosenbergii* males. According to them high protein and vitamin C diet mitigate stress.

Tejpal *et al.* (2009) studied about the Dietary supplementation of L-tryptophan mitigates crowding stress and augments the growth in *Cirrhinus mrigala* fingerlings. They conducted the study for the duration of 60 days for feeding trial of the stress mitigation and hence growth augmenting effects of dietary L-tryptophan during high density group stress in *Cirrhinus mrigala* fingerlings. Four hundred eighty fingerlings were distributed into eight experimental groups. Each group either of low density group (10 fishes/75 L

water) or higher density group (30 fishes/75 L water) was fed with a diet containing either 0, 0.68, 1.36 or 2.72% L-tryptophan in the diet, thus eight experimental groups viz. low density control (LC) (basal feed+0%L-tryptophan); LT1 (basalfeed+0.68% L-tryptophan); LT2 (basalfeed+1.36% L-tryptophan); LT3 (basalfeed+2.72% L-tryptophan) high density control (HC) (basal feed+0%L-tryptophan); HT1 (basalfeed+0.68% L-tryptophan); HT2 (basalfeed+1.36% L-tryptophan); and HT3 (basalfeed+2.72% Ltryptophan) were fed at 3% of the body weight with isonitrogenous (34.33±0.23 to 35.81±0.18 CP%) and isocaloric (423.49 ±1.76 to 425.85±0.31 K.Cal/100 g) purified diets. The possible role of dietary L-tryptophan on stress mitigation was assessed in terms of blood glucose, plasma cortisol, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), alanine amino transferase (ALT), aspartate amino transferase (AST), acetyl choline esterase (AChE) assays, whereas growth was evaluated in terms of weight gain %, specific growth (SGR) and protein efficiency ratio (PER). In both the stocking densities, L-tryptophan supplemented groups found to have higher (pb0.05) growth, SGR and PER. Hence, dietary supplementation of L-tryptophan at a minimum level of 1.36% concomitantly reduced the stress in *C. mrigala* fingerlings. Though 2.72% dietary tryptophan also reduces the stress but 1.36% level appears to be cost effective.

Mustafa *et al.* (2013) studied about the stress modulated physiological responses in Nile Tilapia (*Oreochromis niloticus*), treated with non-ascorbic acid supplemented feed. According to them vitamin C helps to reduce the stress response.

MATERIALS

&

METHODS

Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production

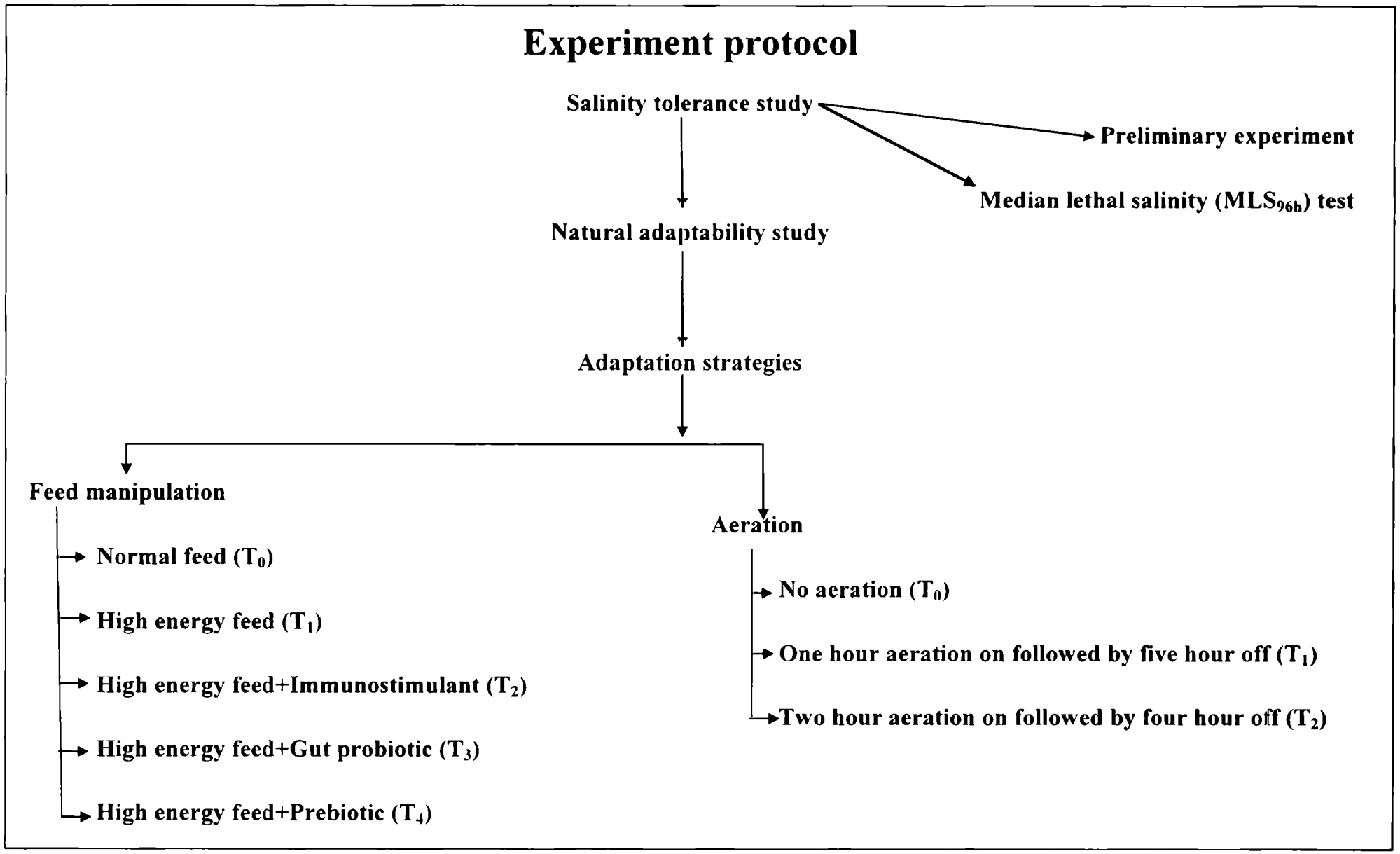
3. Materials and methods

Coastal zones are low-lying. Fishery is main economic activity of these areas and major portion is under fresh water aquaculture as well as shrimp culture. Coastal areas are more vulnerable to cyclone. Due to climate induced risks like sea level rise, rise in temperature, erratic rainfall and increase in frequency of high intensity weather events like cyclone and storm surges these low laying areas become more vulnerable to flooding with saline water. India has 8,181 km coastline experiencing frequent cyclones and storm charge leading to over taping of sea water towards the inland areas, thus adversely affecting the freshwater aquaculture system of the area. Freshwater aquaculture has been adversely affected due to influx of saline water and increase in salinity. Putting the fish in sever salinity induced stress, affect physiological activities like metabolism, osmoregulation, changes in hormonal levels, growth, reproduction etc. This has impacts on the sustainability of fisheries and aquaculture and on the livelihoods of the communities that depend on fisheries. Keeping the above facts in mind, this work was undertaken to study the effects of increase in salinity on fresh water fish species. During the study the survival, tolerance and adaptive capacity of certain commonly cultured freshwater fish were carried out. To combat the salinity stress two adaptation strategies were also evaluated. The work was carried out in the wet laboratory of West Bengal University of Animal and Fishery Sciences (WBUAFS). The following materials and methods have been employed for the work.

Experimental design

The experiment was divided into three phases like salinity tolerance study, natural adaptability study and adaptation strategies. The categories of the experiment are given below.

1. Salinity tolerance
    i. Preliminary experiment (range finding test)
    ii. Median lethal salinity (MLS_{96h}) test


Experiment protocol
Salinity tolerance study
Preliminary experiment
Median lethal salinity (MLS_{96h}) test
Natural adaptability study
Adaptation strategies
Feed manipulation
Normal feed (T_0)
High energy feed (T_1)
High energy feed+Immunostimulant (T_2)
High energy feed+Gut probiotic (T_3)
High energy feed+Prebiotic (T_4)
Aeration
No aeration (T_0)
One hour aeration on followed by five hour off (T_1)
Two hour aeration on followed by four hour off (T_2)

2. Natural adaptability
3. Adaptation strategies tried
    i. Aeration
    ii. Feed manipulations

Table 1: Experimental trial and their objective

Sl no.	Name of trials	Objective
1.	Median lethal salinity (MLS_{96h}) test	To study the tolerance limit of certain freshwater fish species towards salinity.
2.	Natural adaptability study	To study the adaptability capacity of the selected fish species
3.	Adaptation strategies (Experiment against salinity stress)	To evaluate different adaptation strategies to address the adverse impact of salinity stress.
	a) Aeration	
	b) Feed manipulations	

Test solution: The test solution used for the experiment was brine solution. Different concentrations of saline water were prepared by mixing brine with tap water. The brine water was brought from salt pan areas of Digha (the coastal town of West Bengal) in a 2000 l tank and kept.

3.1 Salinity tolerance study

Salinity tolerance study were conducted in two stages and the following protocol was followed

Test organisms: The species were selected based on the culture status of Sundarban areas of West Bengal. The following seven species from three groups like major carps, minor carps, catfish and prawns were selected for the tolerance study. They were *Clarias batrachus, Puntius sarana, Labeo rohita, Cyprinus carpio, Pangasianodon hypothalamus, Oreochromis mossambicus,*

Macrobrachium rosenbergii. In the preliminary experiment the average body weight of the fishes were 9.0, 1.13, 4.9, 0.6, 5.068, 0.45 and 4.05 g for the species *Clarias batrachus. Cyprinus carpio, Labeo rohita, Puntius sarana, Oreochromis mossambicus, Pangasianodon hypothalamus* and *Macrobrachium rosenbergii* respectively. In the median lethal salinity (MLS_{96h}) tests average body weight of the fishes were 2.18, 1.18, 13.42, 1.73, 5.07, 0.45 and 4.50 g for the species *Clarias batrachus. Cyprinus carpio, Labeo rohita, Puntius sarana, Oreochromis mossambicus, Pangasianodon hypothalamus* and *Macrobrachium rosenbergii* respectively.

The fingerlings of fish species were collected from Naihati fish seed market. Pelleted feed was fed twice daily @ 3% of body weight. Fishes were acclimatiesed for two weeks prior to the experiment use. Healthy fishes were selected for the trial.

3.1.1 Preliminary Experiment (Range Finding Test)

The range finding test with respect to salinity were carried out in the laboratory conditions in the rectangular glass aquaria (30 cm X 20 cm X 20 cm: L X W X H) in replicate filled with 3 liters of water. The saline water used in the experiment was obtained by using brine that was diluted with freshwater to get desired salinity. The glass aquaria were covered by net to avoid escaping of fish. Eight numbers of test fish species were kept in each aquarium. A control (without saline water) was also maintained throughout the experiment (Plate 1). All the fishes were acclimatized in the aquariums for 48 hours before experiment. The fish were not fed 24 hours prior to the experiment start. The experiment was carried out for 96 hours. For first eight hours, mortality was recorded at every two hours interval and during of the period, rest it was recorded after every four hours interval. Every day the fecal matters were cleaned with the help of siphoning and same amount with same concentration of saline water were refilled daily. The number of dead fishes from each treatment were counted and removed from the container immediately to avoid any contamination. The salinity levels used in the experiments were 0, 3, 7, 10, 15,

20, 25, 30 and 35 ppt depending on the species. Before starting the experiment the length and weight of the test organisms were recorded.

3.1.2 Median lethal salinity (MLS_{96h}) test

The median lethal salinity (MLS_{96h}) tests were conducted to find the tolerance limit of each species towards increasing salinity. This test was carried out based on the result of Range Finding Test. The median lethal salinity 96 hour (MLS_{96h}) is defined as that salinity at which survival falls to 50% at 96 hour following direct transfer from freshwater to various test salinities (Lemarie, 2004). The number of dead fishes from each treatment were counted and removed from the container immediately to avoid any contamination due to rotting. The species tried for the experiment were the same, used for preliminary experiment. The trials were carried out in the laboratory conditions in the rectangular glass aquaria (30 cm X 20 cm X 20 cm: L X W X H) in replicate filled with 3 liters of water (Plate 2). The saline water used in the experiment was obtained by using brine diluted with tap water. The glass aquaria were covered by net to prevent fish from escape by jumping. Eight numbers of test fish were kept in each aquarium to observe their mortality at different time intervals. A control (without saline water) was also maintained throughout the experiment. All the fishes were acclimatized for 48 hours before the start of the experiment. The fish were not fed 24 hours prior to and during the experimental period. The experiment was carried out for 96 hours. For first eight hours, mortality was recorded at every two hour interval and rest it was recorded after four hours interval. Every day the fecal matters were cleaned with the help of siphoning and same amount and same concentration of saline water was refilled daily. The salinities used in the experiments were 0, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22 and 25 ppt. depending on the species. Before starting the experiment the length and weight of the test organisms were recorded.

Plate 1: Range finding test

Plate 2: Median lethal salinity (MLS_{96h}) trial

Table 2: Salinity tolerance study

Particulates	**Details**
Preliminary Experiment	The experiment were carried out before starting the main experiment
Place of the Experiment:	Laboratory
Test container	Rectangular glass aquarium covered by net to avoid escaping of fish
Aquarium size	(30 cm X 20 cm X 20 cm: L X W X H)
Replicate	Three (3)
Test solution	Prepare desired amount of saline water by mixing brine collected pan areas of Digha with fresh water.
Number of organisms exposed	Eight
Fish species and the salinity level applied	*Puntius sarana* (salinity tested: 0, 3, 7, 10 and 15 ppt.)
	Labeo rohita (salinity tested: 0, 3, 7, 10 and 15 ppt.)
	Clarias batrachus (salinity tested: 0, 3, 7, 10 and 15 ppt.)
	Cyprinus carpio (salinity tested: 0, 3, 7, 10 and 15 ppt.)
	Pangasianodon hypothalamus (salinity tested: 0, 3, 7, 10 and 15 ppt.)
	Macrobrachium rosenbergii (salinity tested: 0, 15, 20, 25 and 30 ppt.)
	Oreochromis mossambicus (salinity tested: 0, 5, 10, 15, 20 and 25 ppt.)
Acclimatization period	48 hours prior to the start of the experiment
Feeding	Not fed 24 hours prior to and during the experimental period
Experiment duration	96 hours
Record keeping	For first eight hours, mortality was recorded at every two hours interval and rest it was recorded after every four hours interval

Median lethal salinity (MLS_{96h}) test (Main experiment)	
Place of the Experiment	Laboratory
Test container	Rectangular glass aquarium covered by net to avoid escaping of fish
Aquarium size	(30 cm X 20 cm X 20 cm: L X W X H)
Replicate	Three (3)
Test solution	Prepare desired amount of saline water by mixing Brine collected pan areas of Digha with fresh water.
Number of organisms exposed	Eight
Fish species and salinity levels applied	*Labeo rohita* (salinity tested: 0, 11, 12, 13 and 14 ppt.)
	Pangasianodon hypothalamus (salinity tested: 0, 11, 12, 13 and 14 ppt)
	Puntius sarana (salinity tested: 0, 3, 7, 10 and 15 ppt.)
	Cyprinus carpio (salinity tested: 0, 11, 12, 13 and 14 ppt.)
	Clarias batrachus (salinity tested: 0, 11, 12, 13 and 14 ppt)
	Oreochromis mossambicus (salinity tested: 0, 5, 10, 15, 20 and 25 ppt.)
	Macrobrachium rosenbergii (salinity tested: 0, 16, 17, 25, 30 and 35 ppt.)
Acclimatization period	48 hours prior to the start of the experiment
Feeding	Not fed 24 hours prior to and during the experimental period
Experiment duration	96 hours
Record keeping	For first eight hours, mortality was recorded at every two hours interval and rest it was recorded after four hours interval

3.2 Natural adaptation capacity study

These experiments were carried out based on the result of Median lethal salinity test (MLS_{96h}) experiment. Natural adaptability studies were carried out in the laboratory at rectangular glass aquarium (60 cm X 30 cm X 30 cm: L X

W X H) in three replicate filled with 45 liters of water. The species tried for the experiment were *Labeo rohita, Cyprinus carpio. Puntius sarana, Oreochromis mossambicus, Macrobrachium rosenbergii*. Ten numbers of test fish were kept in each aquarium to observe their response under static condition for four weeks observation (Plate 3). A control (without saline water) was also maintained throughout the experiment. All the fishes were acclimatized in the test condition for 48 hours before experiment. The fish were not fed 24 hours prior to start of the experiment. During the experiment fishes were with fed pelleted feed twice daily @ 3% of body weight. Every day the fecal matters were cleaned with the help of siphoning and same amount with same concentration of saline water were refilled daily. The salinities used in the experiments were 0, 5, 10, 15 and 18 ppt. Before starting the experiment the length and weight of the test organism were recorded. The glass aquaria were covered to prevent fish from jumping out. Water qualities recorded in the experiment were temperature, pH, DO, ammonia, alkalinity.

3.3 Adaptation strategies experiment against salinity stress

The adaptation strategies experiments were carried out to find the suitable adaptation strategies to be applied against salinity stress to minimize the loss due to saline water inundation.

Two types of adaptation strategies experiment were carried

i. Aeration
ii. Feed manipulations

Plate 3: Natural adaptability test

Plate 4: Experiment on aeration as an adaptation strategy

Table 3: Natural adaptation test

Experiments:	**Median lethal salinity test.**
Place of the experiment	Laboratory
Container	Glass aquarium (60 cm X 30 cm X 30 cm: L X W X H) in replicate
Water volume	45 liters
Species tried for the experiment	*Labeo rohita, Cyprinus carpio. Puntius sarana, Oreochromis mossambicus, Macrobrachium rosenbergii*
Numbers of test fish exposed	Ten
Duration of the experiment	Four weeks
Acclimation period	48 hours prior to the start of the experiment
Feeding	Pelleted feed twice daily @ 3% of body weight
Salinity tested in the experiments	0, 5, 10, 15 and 18 ppt. depending on the species
Growth parameter recorded	Length and weight
Water quality recorded	pH, DO, amonia, alkality

3.3.1 Aeration as an adaptation strategy

The experiment was done in cemented cistern (8' X 4' X 2': L X W X H) with stocking density of 30 juveniles per tank (Plate 4). The experiment was subjected to three levels of salinity (0, 5 and 10 ppt). The experiment tanks were covered by nets to prevent the fish from jumping out. The experiment was done for the species of *Cyprinus carpio* and *Puntius sarana* for six weeks for each species. Three levels of aeration were employed against each salinity level. The trials were set up in triplicates following CRD protocol.The following aeration levels were used.

T_0: No aeration at all salinity levels (Control)

T_1: One hour aeration followed by five hours no aeration, again one hr aeration followed by five hours no aeration. This was followed round the clock all through the experimental period.

T_2: Two hours aeration followed by four hours no aeration, again two hour aeration followed by four hours no aeration. This was followed round the clock all through the experimental period.

Table 4: Aeration as an adaptation strategy

Particulates	T_0	T_1	T_2
Aeration time	0 h (no aeration)	1 h aeration followed by 5 h no aeration, again 1 h aeration followed by 5 h. This was followed round the clock all through the experimental period.	2 h aeration followed by 4 h no aeration, again 2 h aeration followed by 4 h no aeration. This was followed round the clock all through the experimental period.
Experiment place	Wet laboratory	Wet laboratory	Wet laboratory
Experiment tank	cemented cistern (8' X 4' X 2': L X W X H)	cemented cistern (8' X 4' X 2': L X W X H)	cemented cistern (8' X 4' X 2': L X W X H)
Salinitylevels	0, 5 and 10 ppt.	0, 5 and 10 ppt.	0, 5 and 10 ppt.
Species tried	*Cyprinus carpio* and *Puntius sarana*	*Cyprinus carpio* and *Puntius sarana*	*Cyprinus carpio* and *Puntius sarana.*
Stocking density	30 nos.	30 nos.	30 nos.
Feeding	Twice daily @ 3% body weight	Twice daily @ 3% body weight	Twice daily @ 3% body weight
Trials set up	in triplicates following CRD protocol	in triplicates following CRD protocol	in triplicates following CRD protocol..

In this experiment air pressure was 0.2 kg/cm^3 and air volume was 4 litres/m^3/minute. For aeration, stone diffusers were used. Low air pressure

available at larger volume were preferred. Feeding was given twice daily @ 3% of body weight throughout the experimental period. Any food remaining 30 min after feeding was siphoned out.

3.3.2 Feed manipulation as an adaptation strategy

This trial was designed with the hypothesis that even a small amount of feed is taken by the fish under stress, it should be able to maintain the growth and should make fish resilient to salinity stress. Hence fishes under salinity stress were fed with high energy feed and further this was either fortified with immunostimulant or probiotic or prebiotics. The derail of the trial is explained bellow.

T_0: Normal pelleted feed (Control) having 18.14 % protein, 3.8% fat, 10.80% fibre, 10.40% ash, and 11.80% moisture =343.845 Kcal kg^{-1} energy. The main ingredients were de-oiled rice bran and mustard oil cake.

T_1: High energy feed (HE) having 30.26% protein, 6.80% fat, 8.20% fiber 7.10% ash and 10.20% moisture=383.015 Kcal kg^{-1}. The main ingredients were fish meal, soybean meal, wheat bran, coconut meal, rice bran, vitamins and minerals.

T_2: High energy feed with immunostimulant (1 kg high energy feed mixed with 10 ml immunostimulant and 10 ml binder) (Fig 7).

T_3: High energy feed with gut probiotic (1 kg high energy feed mixed with 10 ml gut probiotic and 10 ml binder) (Fig 8) and

T_4: High energy feed with prebiotic (1 kg high energy feed mixed with 10 ml gut prebiotic and 10 ml binder) (Fig 9)

Plate 5: Experiment on feed manipulations as an adaptation strategy

Plate 6: Feed used during the experiment feed manipulations as an adaptation strategy

Table 5: Feed manipulation as an adaptation strategy trial

Particulates	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
Feed	Normal	High Energy Feed (HE)	HE+Immu-nostimulant	HE+Gut-Probiotic	HE+Pre-biotic
Experiment place	Wet laboratory	Wet laboratory	Wet laboratory	Wet laboratory	Wet laboratory
Experiment tank	FRP tanks 6' X 2' X 2': L X W X H	FRP tanks 6' X 2' X 2': L X W X H	FRP tanks 6' X 2' X 2': L X W X H	FRP tank s 6' X 2' X 2': L X W X H	FRP tanks 6' X 2' X 2': L X W X H
Species tried	*Labeo rohita, Cyprinus carpio* and *Oreochromis mossambicus*	*Labeo rohita, Cyprinus carpio* and *Oreochromi smossambicus*	*Labeo rohita, Cyprinus carpio* and *Oreochromis mossambicus*	*Labeo rohita, Cyprinus carpio* and *Oreochromis mossambicus*	*Labeo rohita,*
Stocking density	30 nos.	30 nos.	30 nos.	30 nos.	30 nos.
salinity of water	5, 10 and 15 ppt. depending on species	5, 10 and 15 ppt. depending on species	5, 10 and 15 ppt. depending on species	5, 10 and 15ppt. depending on species	5 and 10 ppt.
Duration of the experiment	Six weeks	Six weeks	Six weeks	Six weeks	Six weeks
Experiment tank	FRP tanks 6' X 2' X 2': L X W X H	FRP tanks 6' X 2' X 2': L X W X H	FRP tanks 6' X 2' X 2': L X W X H	FRP tanks 6' X 2' X 2': L X W X H	FRP tanks 6' X 2' X 2': L X W X H
Feeding	Twice daily @ 3% body weight	Twice daily @ 3% body weight	Twice daily @ 3% body weight	Twice daily @ 3% body weight	Twice daily @ 3% body weight

Immunostimulant (IMMUTRON) **Composition**: Oligomanans, modified amino acids, natural pigments from marine algae, inulin, other immnopopentiators and bioactive ligands.

Gut Probiotic (Gut Act) **Composition**: *Bacillus licheniformis*, *Bacillus pumilus, Bacillus clausils, Bacillus leavalactius* etc.

Prebiotic: Manan-oligo saccharide (MOS).

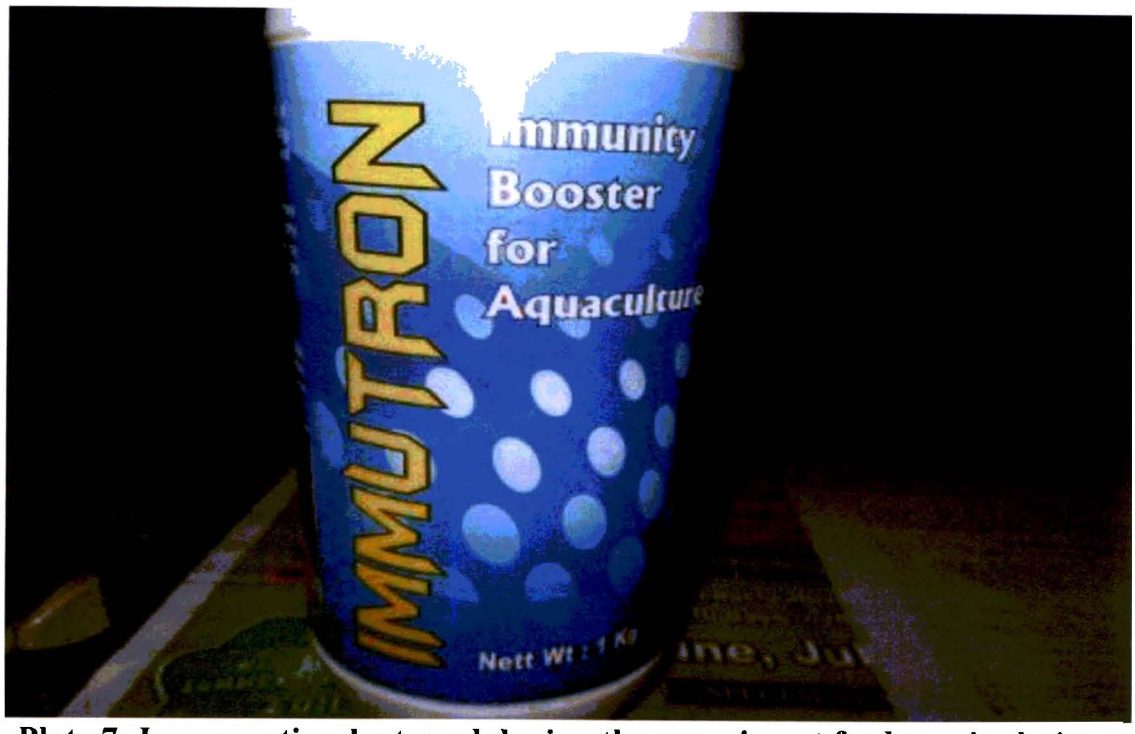


Plate 7: Immunostimulent used during the experiment feed manipulations as an adaptation strategy

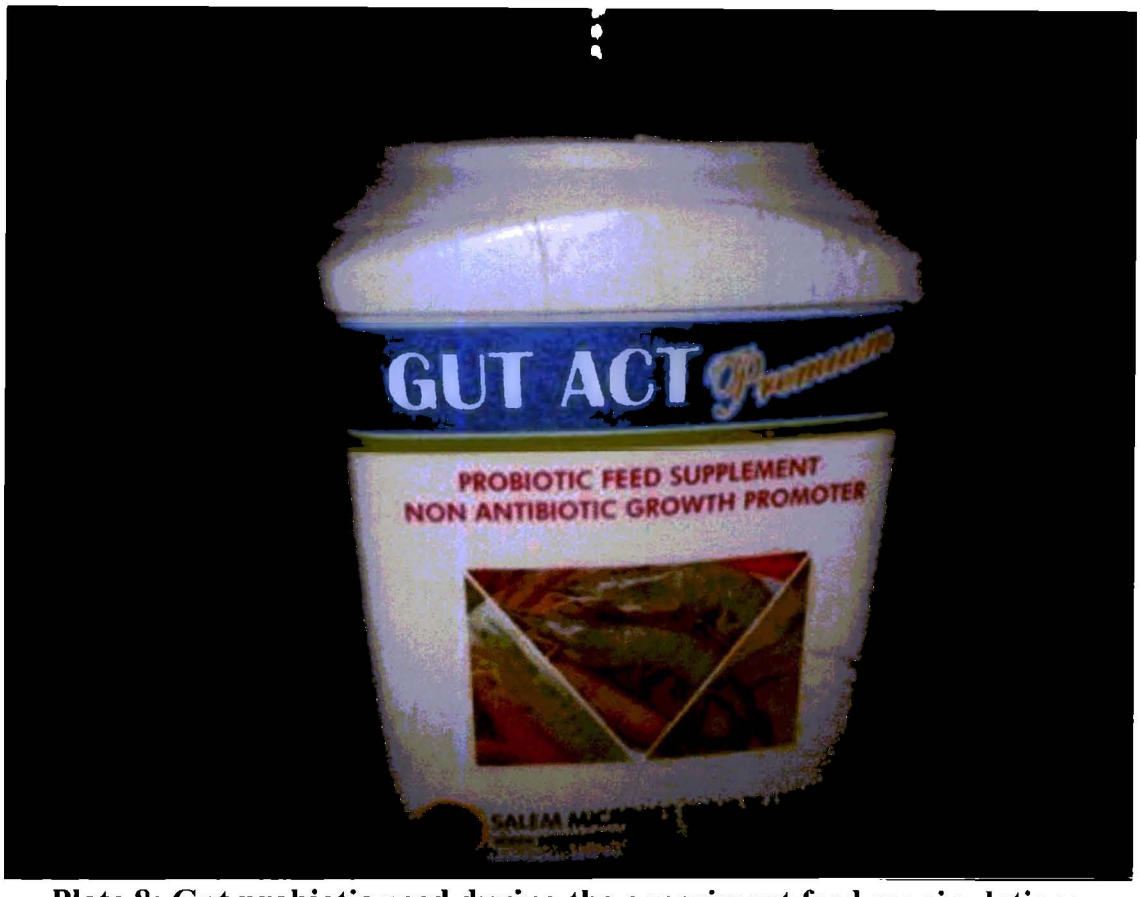


Plate 8: Gut probiotic used during the experiment feed manipulations as an adaptation strategy

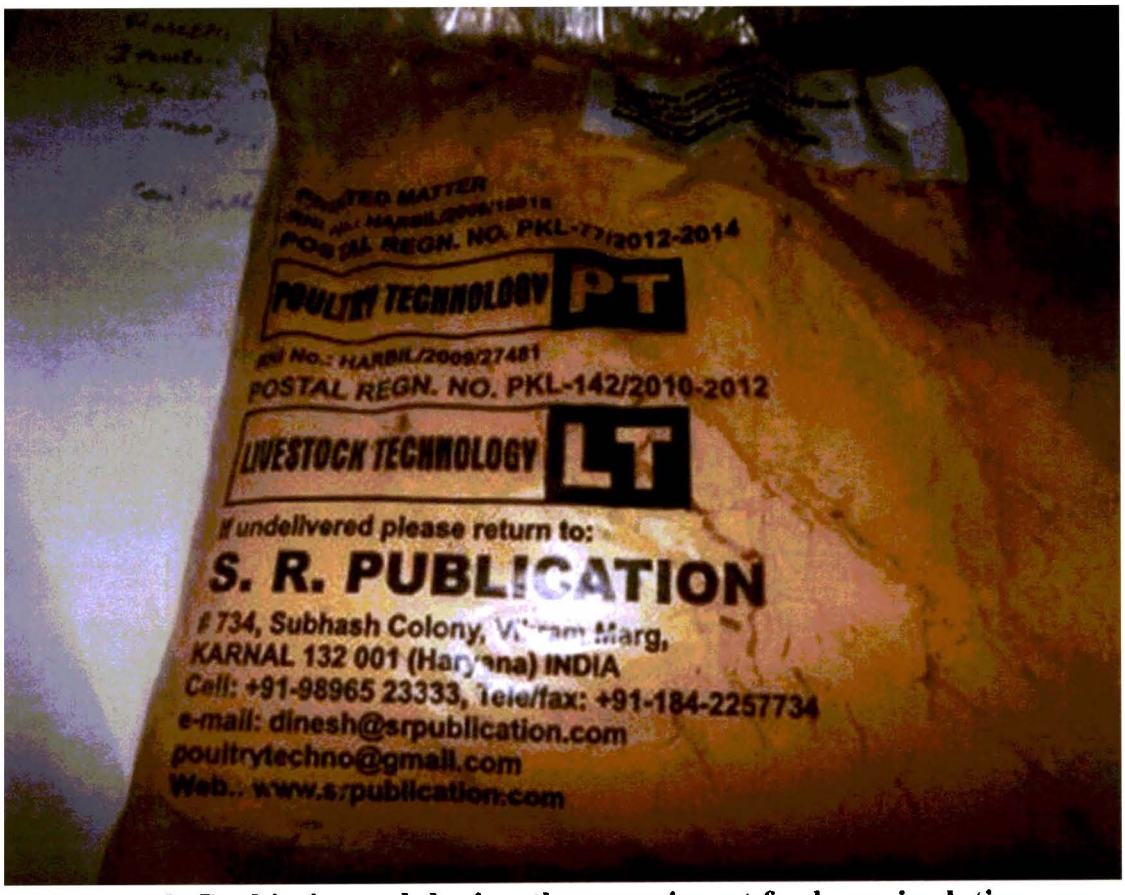


Plate 9: Prebiotic used during the experiment feed manipulations as an adaptation strategy

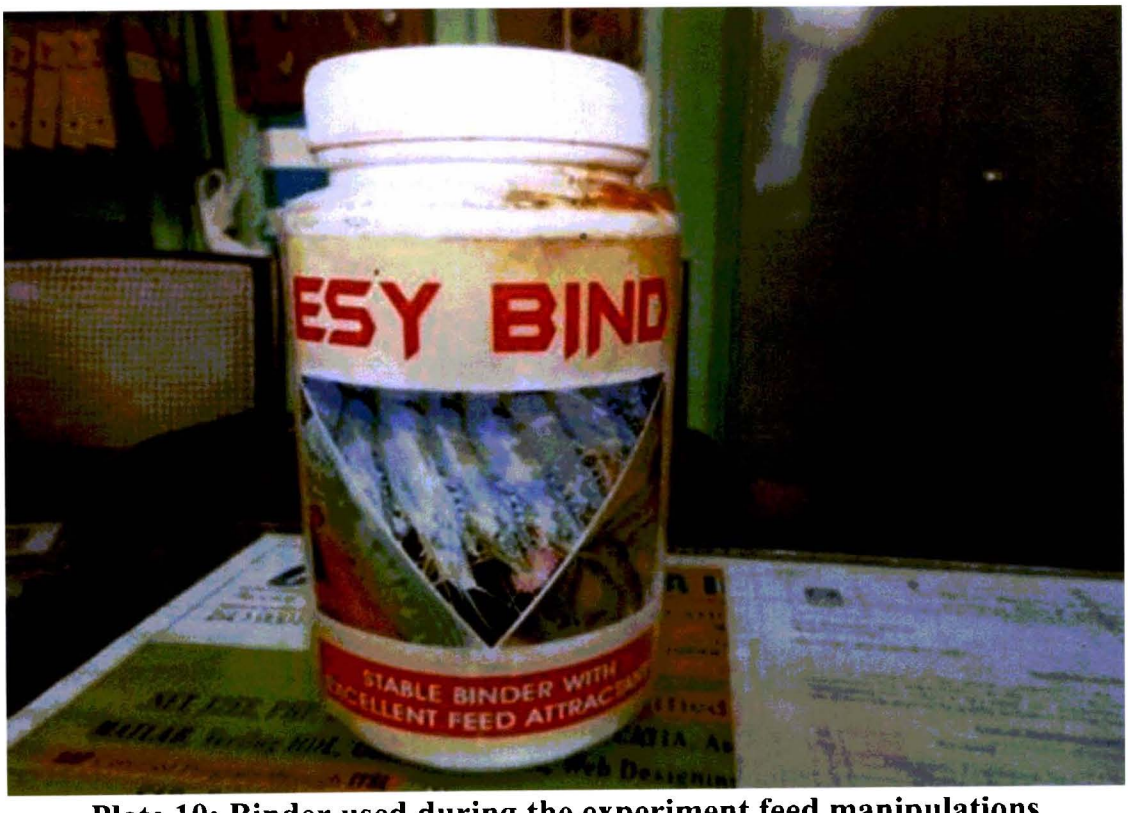


Plate 10: Binder used during the experiment feed manipulations as an adaptation strategy

In this trial fishes *Labeo rohita, Cyprinus carpio* and *Oreochromis mossambicus* were subjected to salinity stress by rearing in 5, 10 and 15 ppt. Six weeks feeding trial was conducted in wet laboratory at FRP tanks of dimension 6' X 2' X 2': L X W X H with stocking density of 30 juveniles per tank (Plate: 5) in triplicate. The experiment tanks were covered by nets to prevent the fish from jumping out. Feeding was done twice daily @3% of body weight. Feeding with high energy feed fortified with immunostimulant (T_2), gut probiotic (T_3) and prebiotic (T_4) stopped after 15 days of experiment. For remaining period of experiment the high energy feed was continued in T_2, T_3 and T_4 set ups to compare the differences in growth with T_1 in which only high energy feed was given throughout the experiment period. The result was also compared with growth obtained in T_0 (control) trial with normal feed.

Live and healthy fingerlings of fishes were collected from a local fish farm at Naihati, West Bengal, India. They were transported in separate oxygenated polythene bags to the laboratory for experiment and kept in glass aquaria filled with clean pond water (Temperature: 24-28°C, pH: 7.9-8.2, DO: 6.5-7.2 mg l^{-1}) separately. All fishes were kept in the laboratory for a minimum period of two weeks and fed @3% body weight commercial pelleted feed. The 20% of the water was replaced daily. Mortality during the period of acclimatization was less than 2%. The tolerances of salinity were conducted in laboratory conditions. Continuous aeration was provided to maintain optimum dissolved oxygen level. The treatments were tested in three replicates. Feeding was given twice daily @ 3% of body weight throughout the experimental period. Any food remaining 30 min after feeding was siphoned out.

The proximate composition (moisture, crude protein, crude fat, crude fiber and ash) of the feed was analysed used AOAC (1980) method. Nitrogen free extract (NEF) was calculated from the difference 100 and the content of protein, fat, fiber and ash. The gross energy content of the feed was calculated based on standard physiological fuel values.

Plate 11: Measurement of DO during the experiment by "WTWOxi3205"

Plate 12: Analysis of physic-chemical property of water

Fig 13: Weighing fish during the experiment

Fig 14: Length measurement during the experiment

Growth parameters

Fish were individually weighted per week (days 1, 7. 14, 21 and 28) throughout the experiment period during natural adaptability study and (days 1, 7. 14, 21, 28, 35 and 42) different adaptation strategy trials. The body weight gain (BWG%) and SGR was calculated as the increase in total biomass at the end of the experiment period.

Total length=the length of a fish measured from the tip of the snout to the tip of the longer lobe of the caudal fin.

$$\text{Body weight gain \% (BWG\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Number of experimental days}} \times 100$$

Estimation of Physico-chemical Parameters

Different physico-chemical parameters like temperature, pH, dissolved oxygen (DO), salinity, alkalinity, ammonia, phosphate-phosphorus of water were estimated during the experiment period. The estimation procedures of the parameters in brief are described below. The temperature and dissolved oxygen were estimated by the digital meter "**WTWOxi3205**" (Plate 11). The pH was measured by digital pH meter. Salinity was measured by the refrectometer "**RHS 10ATC**" (Fig 11). Alkalinity was estimated following the methods of APHA (2002). Ammonia and Phosphate of water were estimated by "**DR 2800**" spectrophotometer. Weight and length of fish measured by digital weight machine "**METTLER TOLEDO**" and 30 cm scale respectively.

Statistical analysis of experimental data

Median Lethal Salinity

The median lethal salinity for 96 hour (MLS_{96h}) was calculated by Probit method (Finney, 1971) by pooling mortality data from replicates within treatments and considered significantly different when the corresponding 95% confidence intervals did not overlap. The statistical calculations were performed with software SPSS 10.0 for Windows (SPSS Inc. Chicago, IL USA).

Growth parameters

One-way Analysis of Variance (ANOVA) was performed to compare different growth parameters like specific growth rate and percentage body weight gain in different salinity and with other treatments. In all cases data were tested normality of variance (homogeneity) before analysis through Levene's Test for Equality of Variances. Duncan's Multiple Range Test (DMRT) was employed to determine the differences among the means with a confidence range of 95%. Significant differences are stated at $p<0.05$ level unless otherwise noted. The statistical calculations were performed with statistical software Medcalc® version 12.7.0 (MedCalc Software bvba, Ostend, Belgium) and statistical software XLSTAT Version 2013.2.07 (© Addinsoft). Results are expressed as mean ± SD.

RESULTS

Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production

4. Results

The present study was conducted in three phases viz: tolerance level of few freshwater species towards increased salinity, natural adaptability (capacity of certain freshwater fishes to increased salinity) and to find the (strategies) to combat the adverse impact of salinity on fishes. The results of median lethal salinity, growth performances in various sub-lethal salinities in terms of specific growth rate and percentage body weight gain, salinity stress mitigation measures of various experimented aquaculture species and water quality during the experiments are briefly described and graphically presented in this chapter. The data obtained during the investigations and from the statistical analysis are presented in Annexure for easy reference.

4.1 Tolerance study

4.1.1 Preliminary experiment (Range Finding Test)

Before commencement of median lethal salinity (MLS_{96h}) experiment, range finding tests were done for certain freshwater species by exposing in different salinities. Species like *Puntius sarana, Labeo rohita, Clarias batrachus, Cyprinus carpio, Pangasianodon hypothalamus, Macrobrachium rosenbergii* and *Oreochromis mossambicus* were exposed to different salinities levels to find out the upper salinity range for determination of Median Lethal Salinity. The result of this experiment is presented in Table 7 and 8.

The fish *Puntius sarana* were exposed to 0, 3, 7, 10 and 15 ppt salinity and the respective percentage of fish dead in 96 hour duration were found to be 0.00, 12.50, 16.67, 20.83 and 100 respectively. In case of *Labeo rohita* 0.00, 0.00, 20.83, 41.67 and 100% mortality was found at 0, 3, 7, 10 and 15 ppt salinity respectively at 96 hour. Percentage mortality of *Clarias batrachus* was found to be 0.00, 8.33, 12.50, 16.67 and 100 at 3, 7, 10 and 15 ppt salinity respectively. The mortality in *Cyprinus carpio* was found to be 0.00, 8.33, 16.67, 25.00 and 100% at 3, 7, 10 and 15 ppt salinity respectively. At 3, 7, 10

Table 6: Water quality during Preliminary trials

Fish / Water quality	*Clarias batrachus*	*Labeo rohita*	*Cyprinus carpio*	*Puntius sarana*	*Oreochromis mossambicuss*	*Pangasianodon hypothalamus*	*Macrobrachium rosenbergii*
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (^{o}C)	21.4-22.3 (21.75)	22.4-22.9 (22.59)	20.3-20.9 (20.58)	21.7-22.7 (22.24)	20.1-20.8 (20.43)	20.3-21.2 (20.73)	29.9-30.7 (30.32)
pH	8.2-8.5 (8.37)	8.3-8.7 (8.47)	8.2-8.6 (8.40)	8.2-8.5 (8.40)	8.3-8.6 (8.47)	8.3-8.6 (8.46)	8.2-8.6 (8.42)
DO (mgl^{-1})	5.9-7.4 (6.71)	5.9-7.7 (6.93)	6.1-7.6 (6.86)	5.9-7.4 (6.70)	6.1-7.5 (6.76)	5.8-7.4 (6.73)	5.8-7.6 (6.76)
Alkalinity (mgl^{-1})	176.00-194.00 (186.48)	179.00-196.00 (187.76)	173.00-197.00 (186.74)	174.00-196.00 (185.28)	163.00-196.00 (182.64)	176.00-195.00 (187.68)	174.00-198.00 (185.59)
Hardness (mgl^{-1})	278.00-286.00 (281.42)	276.00-287.00 (283.56)	278.00-286.00 (282.74)	274.00-285.00 (281.06)	277.00-287.00 (283.68)	275.00-286.00 (281.32)	276.00-287.00 (282.47)
Ammonia (mgl^{-1})	0.14-0.31 (0.22)	0.18-0.37 (0.26)	0.16-0.38 (0.26)	0.14-0.34 (0.21)	0.12-0.37 (0.24)	0.14-0.31 (0.23)	0.16-0.32 (0.24)
Phosphate (mgl^{-1})	0.35-0.49 (0.43)	0.43-0.57 (0.49)	0.34-0.59 (0.45)	0.34-0.54 (0.45)	0.33-0.52 (0.43)	0.37-0.54 (0.46)	0.36-0.52 (0.44)

Note: Trials for all the fishes were conducted during the month of October and November months except *Macrobrachium rosenbergii*, which was conducted in the month of August.

Table 7: Mortality of different fishes at different salinity levels at 96 hours

Salinity	*Puntius sarana*		*Labeo rohita*		*Clarias batrachus*		*Cyprinus carpio*		*Pangasianodon hypothalamus*	
	Average* (no.)	Mortality (%)	Average* (no.)	Mortality (%)	Average* (no.)	Mortality (%)	Average* (no.)	Mortality (%)	Average* (no.)	Mortality (%)
0 ppt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
3 ppt	1.00	12.50	0.00	0.00	0.66	8.33	0.67	8.33	0.67	8.33
7 ppt	1.33	16.67	1.66	20.83	1.00	12.50	1.33	16.67	1.67	20.83
10 ppt	1.66	20.83	3.33	41.67	1.33	16.67	2.00	25.00	2.33	29.17
15 ppt	8.00	100.00	8.00	100.00	8.00	100.00	8.00	100.00	8.00	100.00

*Mean of three replicates

Note: Number of fish exposed = 8

Table 8: Mortality of *Macrobrachium rosenbergii* and *Oreochromis mossambicuss* at different salinity levels at 96 hours

Salinity	*Macrobrachium rosenbergii*		Salinity	*Oreochromis mossambicuss*	
	Average (no.)*	Mortality (%)		Average (no.)*	Mortality (%)
0 ppt	0.00	0.00	**0ppt**	0.00	0.00
15 ppt	0.00	0.00	**5ppt**	0.00	0.00
20 ppt	1.00	16.67	**10ppt**	0.00	0.00
25 ppt	4.00	66.67	**15ppt**	0.33	4.17
30 ppt	6.00	100.00	**20ppt**	3.33	41.67
			25ppt	8.00	100.00

*Mean of three replicates

Note: Number of fish exposed in case of *M. rosenbergii* and *O. mossambicuss* were 6 and 8 respectively

and 15 ppt salinity, the mortality of *Pangasianodon hypothalamus* was found to be 0.00, 8.33, 20.83, 29.17 and 100% respectively. *Macrobrachium rosenbergii* found 0.00, 0.00, 16.67, 66.67 and 100% mortality at 0, 15, 20, 25 and 30 ppt salinity respectively. At 0, 5, 10, 15, 20 and 25 ppt salinity *Oreochromis mossambicus* was found 0.00, 0.00, 0.00, 4.17, 41.67 and 100% mortality respectively.

The range and mean values of physic-chemical characteristics of experimental waters are summarized in Table 6. In general, differences in water quality parameters among different salinity test were very little. Thus considering all test, water temperature was varied between 20.1 to 30.7°C, the minimum and maximum pH were 8.2 and 8.7 respectively. The observed dissolved oxygen levels were fairly suitable for fish and ranged from 5.8 mg l^{-1} to 7.6 mg l^{-1}. The lowest and highest values of alkalinity were found in the aquaria kept for *C. carpio* (173.00 mg l^{-1}) and *O. mossambicus* (202.00 mg l^{-1}) respectively. Further, it would be seen from the table that the hardness of water used for the present investigation was very little (274.00 to 286.00 mg l^{-1}). The ammonia of the experiment were ranged between 0.12 and 0.38 mg l^{-1}.the minimum and maximum Phosphate was found 0.33 mg l^{-1} and 0.59 mg l^{-1} respectively.

4.1.2 Median Lethal Salinity (MLS_{96h}) Test

Median lethal salinity (MLS_{96h}) test was conducted based on the results of preliminary experiment. The species tried for the experiment were the same used during preliminary experiment. The estimated Median Lethal Salinity (MLS_{96h}) and confidence limits computed using Probit for these species is presented in Table 10.

It was found that *Labeo rohita* had the least capacity to tolerate increasing salinity and *Macrobrachium rosenbergii* had the highest capacity to tolerate salinity among the species in this experiment trial. The median lethal salinity (MLS_{96h}) for the species *Labeo rohita, Pangasianodon hypothalamus,*

Table 9: Water quality during Median Lethal Salinity (MLS_{96h}) trials

Fish / Water quality	*Clarias batrachus*	*Labeo rohita*	*Cyprinus carpio*	*Puntius sarana*	*Oreochromis mossambicuss*	*Pangasianodon hypothalamus*	*Macrobrachium rosenbergii*
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (°C)	18.6-19.4 (19.18)	18.5-19.3 (18.94)	19.2-19.8 (19.52)	19.1-19.7 (19.44)	18.2-18.8 18.68	18.4-19.3 18.84	30.2-30.8 30.46
pH	8.2-8.7 (8.38)	8.2-8.7 (8.32)	8.2-8.8 (8.49)	8.2-8.6 (8.33)	8.2-8.5 (8.34)	8.2-8.5 (8.37)	8.2-8.4 (8.29)
DO (mgl^{-1})	5.4-7.6 (6.66)	5.5-7.4 (6.61)	5.9-7.8 (6.92)	5.8-7.6 (6.75)	5.7-7.6 (6.66)	5.7-7.2 (6.47)	5.7-7.3 (6.53)
Alkalinity (mgl^{-1})	173.00-194.00 (186.74)	176.00-196.00 (189.92)	174.00-194.00 (187.45)	172.00-193.00 (185.37)	161.00-191.00 (198.83)	175.00-194.00 (187.54)	171.00-195.00 (186.91)
Hardness (mgl^{-1})	273.00-284.00 (279.97)	272.00-285.00 (281.76)	275.00-287.00 (283.42)	273.00-285.00 (282.07)	274.00-286.00 (282.83)	272.00-287.00 (283.12)	275.00-288.00 (285.34)
Ammonia (mgl^{-1})	0.14-0.31 (0.23)	0.16-0.37 (0.27)	0.15-0.32 (0.24)	0.16-0.31 (0.26)	0.17-0.34 (0.25)	0.14-0.39 0.22	0.12-0.33 (0.22)
Phosphate (mgl^{-1})	0.36-0.64 (0.49)	0.36-0.59 (0.47)	0.32-0.78 (0.47)	0.32-0.59 (0.44)	0.38-0.59 (0.47)	0.32-0.51 (0.42)	0.32-0.49 (0.41)

Note: Trials for all the fishes were conducted during the month of November and December months except *Macrobrachium rosenbergii*, which was conducted in the month of August.

Table 10: Median lethal salinity (MLS_{96h}) (ppt) of certain fresh water fish

Species	MLS_{96h}	95% Confidence limits	
		Lower	Upper
Labeo rohita	10.61	9.93	11.21
Pangasianodon hypothalamus	11.97	10.50	13.32
Puntius sarana	12.17	11.66	12.68
Cyprinus carpio	12.18	11.65	12.79
Clarias batrachus	12.37	11.81	12.95
Oreochromis mossambicuss	20.88	20.03	21.73
Macrobrachium rosenbergii	24.45	23.78	25.25

Puntius sarana, Cyprinus carpio, Clarias batrachus, Oreochromis mossambicus and *Macrobrachium rosenbergii* were 10.61, 11.97, 12.17, 12.18, 12.37, 20.88 and 24.45 ppt respectively.

In the experiment water temperature, pH, DO, alkalinity, hardness, ammonia and phosphate were monitored. The minimum temperature of the experiment was 18.2°C and the maximum was 30.8°C. The pH of the water ranged between 8.2 and 8.8. The DO of the experiment varied from 5.4 mg l^{-1} to 7.8 mg l^{-1}. The minimum and maximum alkalinities of the experiment were 171.00 and 207.00 mg l^{-1} respectively. The hardness level varied between 272.00 and 288.00 mg l^{-1}. The ammonia level during the experiment ranged between 0.12 and 0.39 mg l^{-1}. The minimum and maximum values of phosphate were 0.32 and 0.78 mg l^{-1} respectively. The water quality parameter of the experiment is presented on Table 9.

4.2 Natural adaptation capacity

Based on median lethal salinity data for individual species, the test species were kept in different sub-lethal salinity levels and were allowed to grow for four weeks span to understand their natural adaptability capacity in varying salinity. The natural adaptive capacity to salinity was evaluated in terms of growth responses like specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%). The results of these trials are briefed bellow.

Puntius sarana

The data pertaining of various growth parameters like initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Puntius sarana* recorded in two sub-lethal salinity level, 5 and 10 ppt along with control group (0 ppt), are given in Table 12. In all the salinity treatments, SGR (One-way ANOVA: $F_{2, 6}$=7.94; P=0.02) and BWG% (One-way ANOVA: $F_{2, 6}$=7.82; P=0.02) varied significantly. At the end of

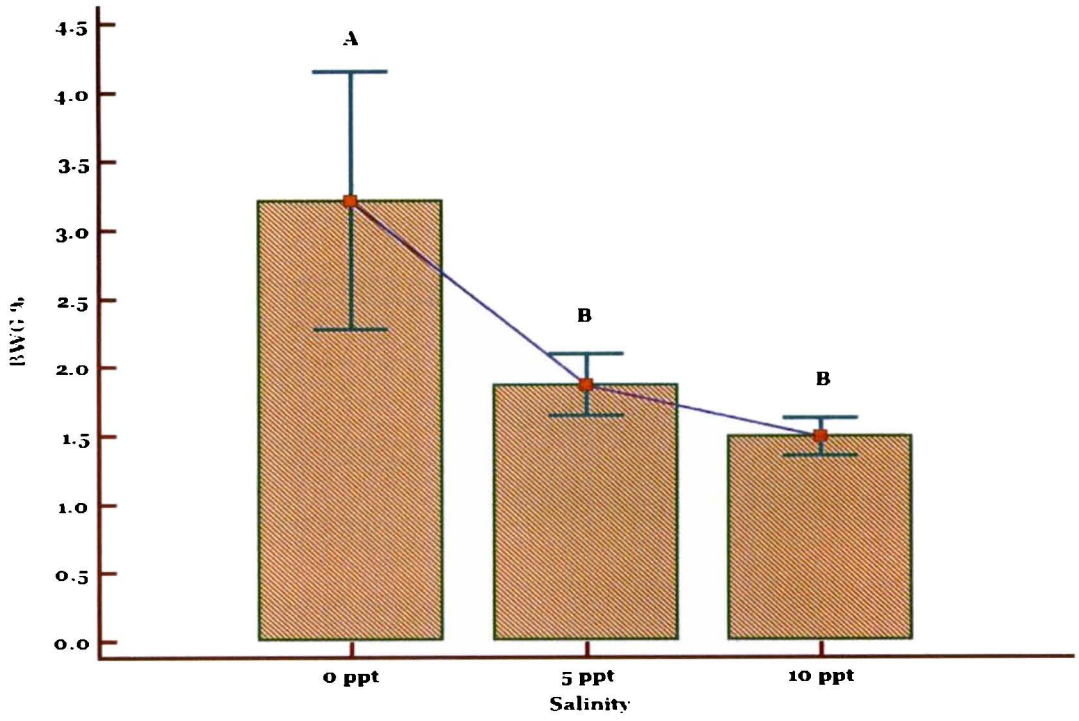


Fig 1: Body weight gain percentage (BWG%) of *Puntius sarana* exposed to different salinity level in natural adaptability trial

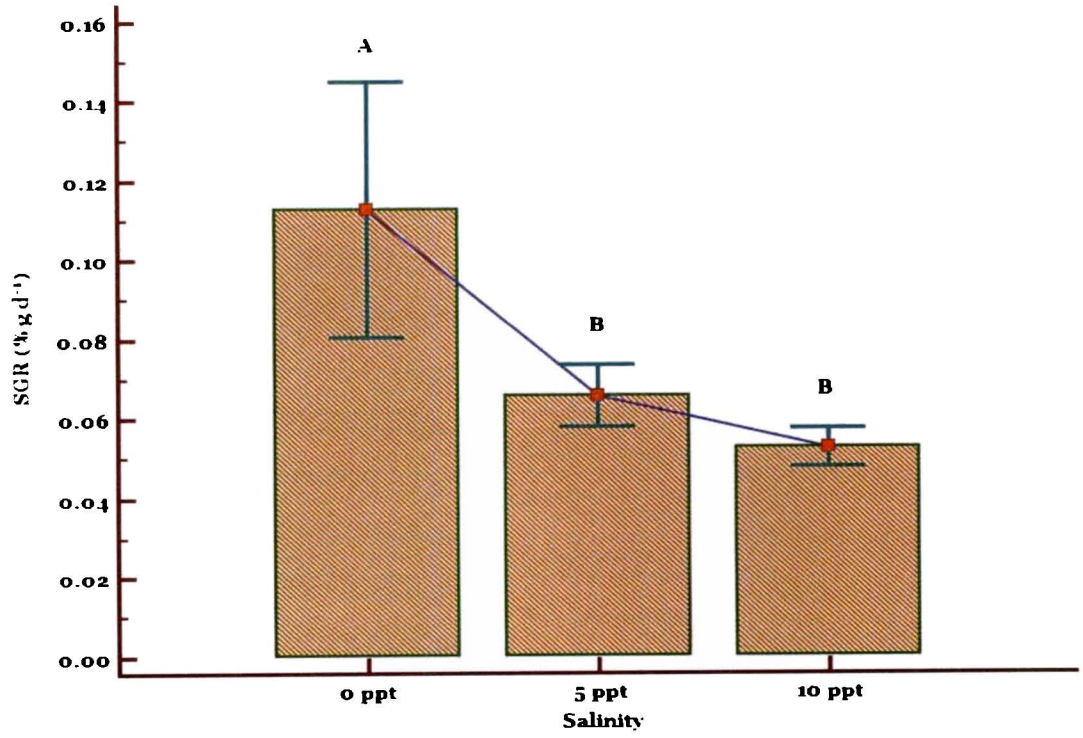


Fig 2: Specific Growth rate (SGR % day^{-1}) of *Puntius sarana* exposed to different salinity level in natural adaptability trial

Table 11: Water quality during natural adaptation trial of *Puntius sarana, Labeo rohita* and *Cyprinus carpeo*

Water quality	*Puntius sarana*			*Labeo rohita*			*Cyprinus carpeo*		
	0 ppt	**5 ppt**	**10 ppt**	**0 ppt**	**5 ppt**	**10 ppt**	**0 ppt**	**5 ppt**	**10 ppt**
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (^{o}C)	19.2-19.8 (19.56)	19.4-19.8 (19.62)	19.3-19.6 (19.47)	19.4-19.8 (19.67)	19.3-19.9 (19.78)	19.3-19.7 (19.52)	19.3-19.7 (19.54)	19.4-19.9 (19.67)	19.3-19.6 (19.48)
pH	7.4-8.9 (8.14)	7.2-8.5 (7.82)	7.1-8.5 (7.98)	7.3-8.8 (8.00)	7.3-8.7 (7.90)	7.5-8.6 (8.10)	7.40-8.80 (8.00)	8.00-8.70 (8.28)	8.00-8.70 (8.26)
DO (mgl^{-1})	6.8-7.8 (7.29)	6.1-7.7 (7.10)	6.0-7.9 (7.06)	5.5-7.7 (6.97)	5.3-7.7 (6.63)	5.1-7.3 (6.08)	6.10-7.50 (6.71)	6.00-7.30 (6.40)	5.30-7.10 (5.91)
Alkalinity (mgl^{-1})	184.0-195.0 (189.47)	173.0-187.0 (178.34)	154.0-165.0 (157.65)	185.0-196.0 (189.73)	166.0-174.0 (169.26)	142.0-156.0 (147.12)	184.0-198.0 (192.59)	163.0-177.0 (168.74)	144.0-156.0 (151.28)
Hardness (mgl^{-1})	262.0-268.0 (264.36)	271.0-279.0 (276.41)	283.0-292.0 (288.12)	252.0-261.0 (257.31)	268.0-272.0 (270.54)	277.0-283.0 (280.27)	252.0-257.0 (254.74)	264.0-268.0 (266.09)	273.0-278.0 (275.62)
Ammonia (mgl^{-1})	0.19-0.39 (0.27)	0.17-0.36 (0.23)	0.16-0.39 (0.28)	0.15-0.39 (0.21)	0.15-0.37 (0.22)	0.17-0.38 (0.24)	0.11-0.31 (0.23)	0.19-0.39 (0.21)	0.11-0.30 (0.18)

Table 12: Variations in length (cm) and weight (g) of *Puntius sarana* during natural adaptability test

Salinity		1st day	1st week	2nd week	3rd week	4th week	BWG%	SGR (% day^{-1})
0 ppt	L	6.84±0.69	6.98±0.47	7.00±0.46	7.01±0.46	7.02±0.45		
	W	2.77±0.38	2.79±0.37	2.80±0.37	2.83±0.37	2.86±0.37	3.13±0.93^{A}	0.11±0.03^{A}
5 ppt	L	6.55±0.12	6.56±0.11	6.57±0.10	6.58±0.10	6.58±0.10		
	W	3.41±0.35	3.43±0.36	3.44±0.35	3.46±0.35	3.48±0.36	1.86±0.22^{B}	0.07±0.01^{B}
10 ppt	L	6.89±0.07	6.94±0.09	6.96±0.11	6.97±0.10	6.98±0.10		
	W	3.79±0.04	3.80±0.05	3.81±0.05	3.82±0.06	3.84±0.06	1.50±0.14^{B}	0.05±0.01^{B}

Note: 1. Values are average±SD, L=Length and W=Weight

2. Values (BWG & SGR) with different subscript indicates significant difference (p>0.05)

Table 13: Variations in length (cm) and weight (g) of *Labeo rohita* during natural adaptability test

Salinity		1st day	1st week	2nd week	3rd week	4th week	BWG%	SGR (% day^{-1})
0 ppt	L	9.76±0.19	9.83±0.10	9.89±0.10	9.94±0.13	10.00±0.15		
	W	9.21±0.40	10.00±21	10.90±0.33	12.39±0.21	13.44±0.29	46.02±3.25^{A}	1.35±0.08^{A}
5 ppt	L	10.44±0.60	10.58±0.73	10.52±0.58	10.55±0.57	10.58±0.58		
	W	10.31±0.09	10.62±0.40	11.62±0.34	12.14±0.10	12.98±0.16	25.90±1.48^{B}	0.82±0.04^{B}
10 ppt	L	10.02±0.50	10.32±0.29	10.36±0.29	10.43±0.30	10.46±0.28		
	W	9.62±0.95	10.46±1.63	10.91±1.65	9.54±1.62	11.73±1.66	21.93±5.59^{B}	0.71±0.16^{B}

Note: 1. Values are average±SD, L=Length and W=Weight

2. Values (BWG & SGR) with different subscript indicates significant difference (p>0.05)

four weeks rearing periods, highest BWG% was observed in 0 ppt (3.13±0.93) which is significantly different ($p<0.05$) from 5 ppt (1.86±0.22) and 10 ppt (1.50±0.14) (Fig 1). However, no significant differences ($p>0.05$) were noticed in BWG% between 5 and 10 ppt. Likewise, maximum SGR were obtained in 0 ppt (0.11±0.031) followed by 5 ppt (0.07±0.01) and 10 ppt (0.05±0.004). SGR of 0 ppt is significantly different ($p<0.05$) from 5 and 10 ppt (Fig 2) and no significant differences of SGR ($p>0.05$) were observed in between 5 and 10 ppt.

The temperature ranged between 19.2 and 19.8°C in this experiment. The pH of the experiment varied from 7.1 to 8.9. Dissolved oxygen (DO) was found in within 6.0 to 7.9 mg l^{-1}. The minimum and maximum alkalinities were 154.0 and 195.0 mg l^{-1} respectively. The hardness was found in the range of 262.0 and 292.0 mg l^{-1}. The lowest and highest values of ammonia were 0.16 and 0.39 mg l^{-1} respectively in the experiment. The results of water quality parameters of this experiment has presented on Table 11.

Labeo rohita

The initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Labeo rohita* recorded in two sub-lethal salinity level, 5 and 10 ppt along with control group (0 ppt) are given in Table 13 and Fig 3 & 4. In all the salinity treatments, growth parameters like SGR (One-way ANOVA: $F_{2,\ 6}=31.84$; $P=0.001$) and BWG% (One-way ANOVA: $F_{2,\ 6}=35.01$; $P=0.0005$) varied significantly. The highest BWG% was observed in 0 ppt (46.02±3.25) which is significantly different ($p<0.05$) from 5 ppt (25.90±1.48) and 10 ppt (21.93±5.59). However, no significant differences ($p>0.05$) were noticed in BWG% between 5 and 10 ppt. Likewise, maximum SGR were obtained in 0 ppt (1.35±0.08 % day^{-1}) followed by 5 ppt (0.82±0.04 % day^{-1}) and 10 ppt (0.71±0.16 % day^{-1}). SGR of 0 ppt is significantly different ($p<0.05$) from 5 and 10 ppt and no

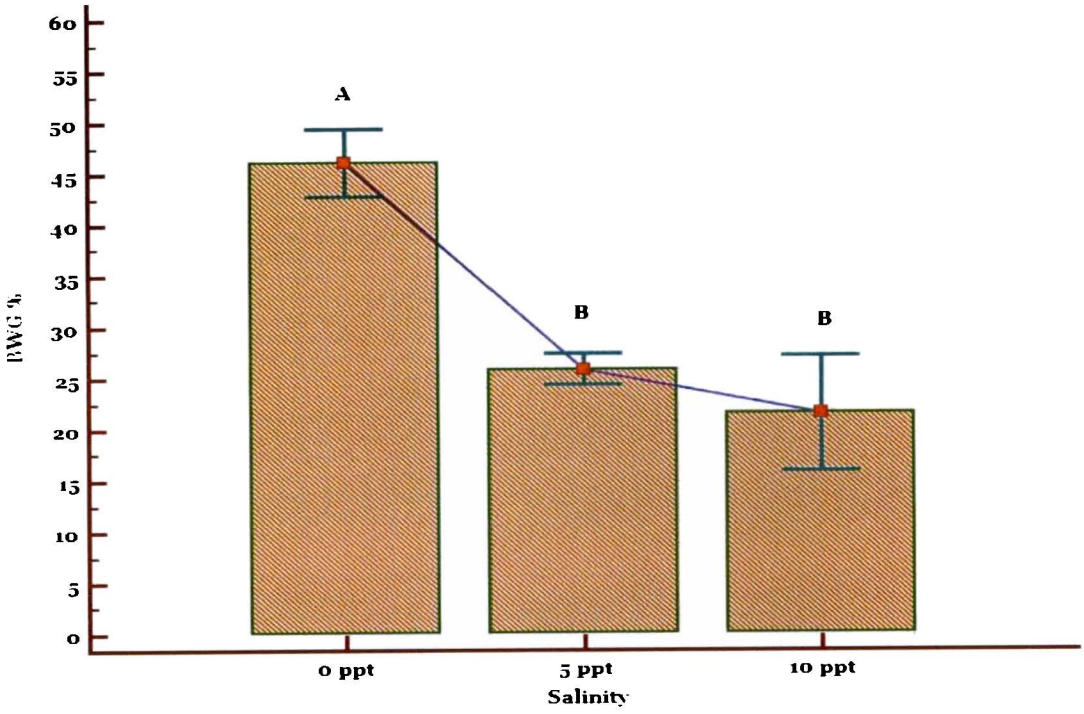


Fig 3: Body weight gain percentage (BWG%) of *Labeo rohita* exposed to different salinity level in natural adaptability trial

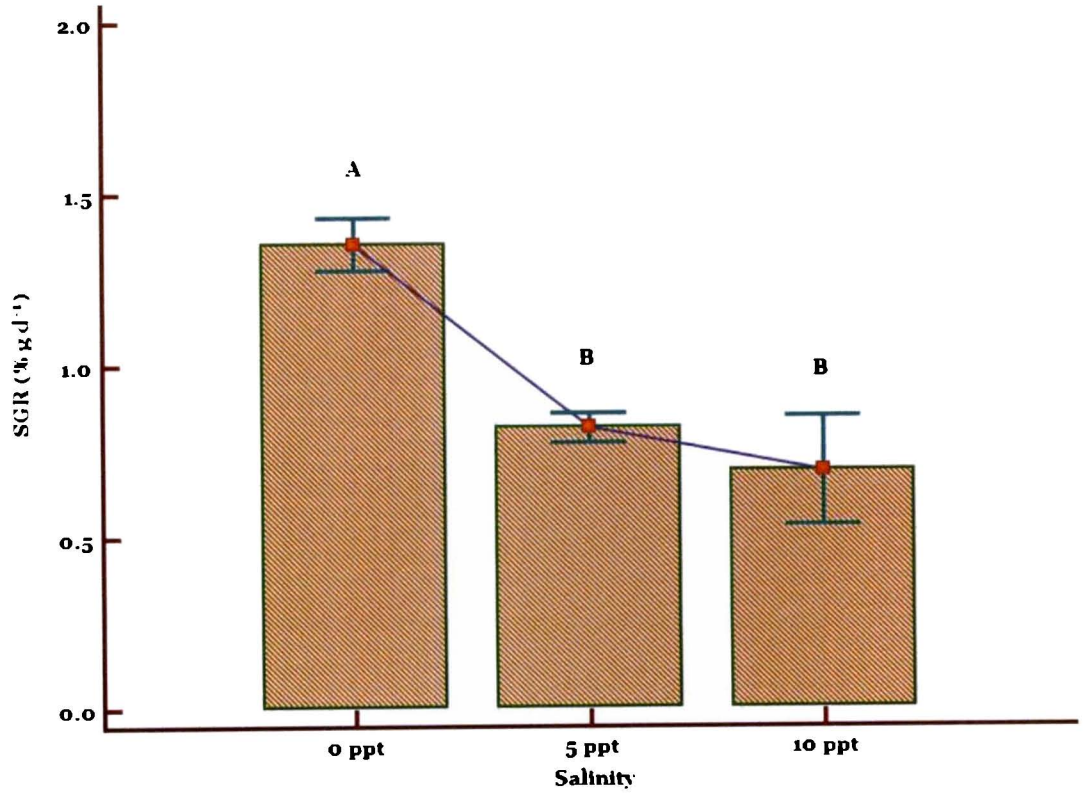


Fig 4: Specific growth rate (SGR % day^{-1}) of *Labeo rohita* exposed to different salinity level in natural adaptability trial

significant differences of SGR ($p>0.05$) were observed in between 5 and 10 ppt.

The minimum and maximum value temperatures were 19.3 and 19.9°C respectively in the experiment. The pH values very between 7.3 to 8.8 and the average DO were found in range of 5.1 to 7.7 mg l^{-1}. The average alkalinity of the experiment was 189.73, 169.26, 147.12 mg l^{-1} and the average hardness of the experiment were 257.31, 270.54, 280.27 mg l^{-1} with respect to 0, 5 and 10 ppt salinity respectively in both the cases. Ammonia was found in the range between 0.21and 0.39mg l^{-1}. The results of this experiment has presented on Table 11.

Cyprinus carpio

The data relate to growth of *Cyprinus carpio* in terms of body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) recorded in two sub-lethal salinity levels, 5 and 10 ppt along with control group (0 ppt), are given in Table 14. In all the salinity treatments, growth parameters like SGR (One-way ANOVA: $F_{2, 6}=40.12$; $P=0.0003$) and BWG% (One-way ANOVA: $F_{2, 6}=39.07$; $P=0.0004$) varied significantly. At the end of four weeks rearing periods. The highest BWG% was observed in 0 ppt (16.89±1.58) followed by 5 ppt (14.67±0.81) and 10 ppt (8.42±1.13). No significant differences (Fig 5) in BWG % ($p>0.05$) was observed between 0 and 5 ppt. Similarly, the maximum SGR were obtained in 0 ppt (0.56±0.05) followed by 5 ppt (0.49±0.03) and 10 ppt (0.29±0.04). No significant differences in SGR ($p>0.05$) were observed between 0 and 5 ppt (Fig 6).

The results of water quality parameters temperatures, pH, DO, alkalinity, hardness are given in Table 11. It indicates that the results of all these parameters were within the normal range.

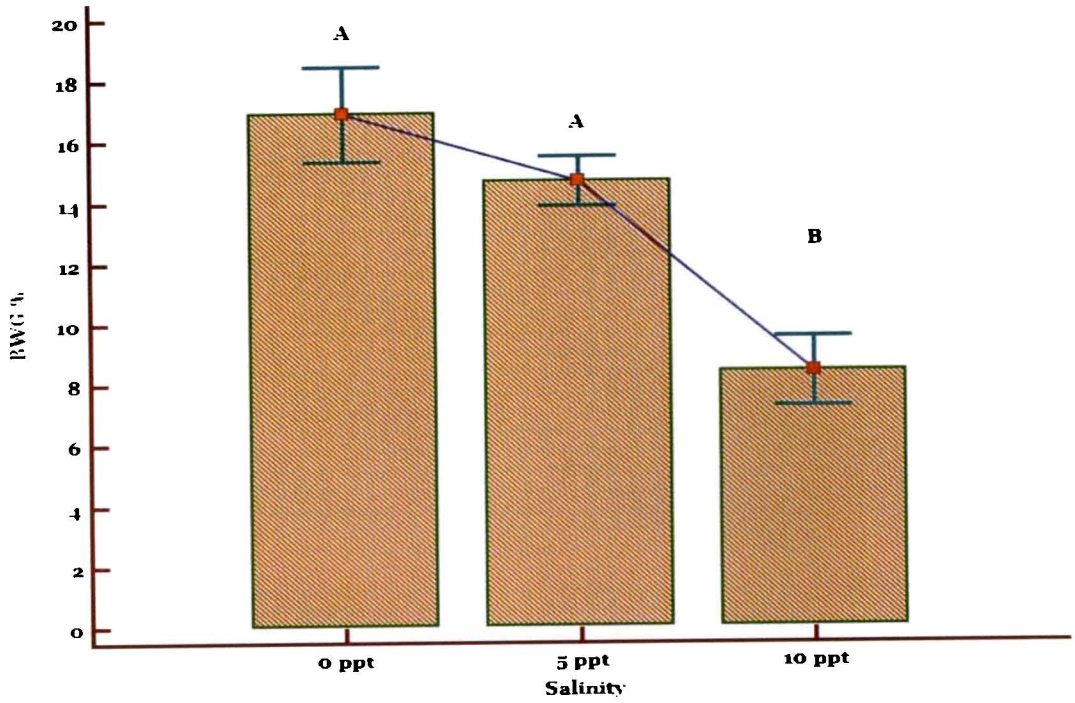


Fig 5: Body weight gain percentage (BWG%) of *Cyprinus carpio* exposed to different salinity level in natural adaptability trial

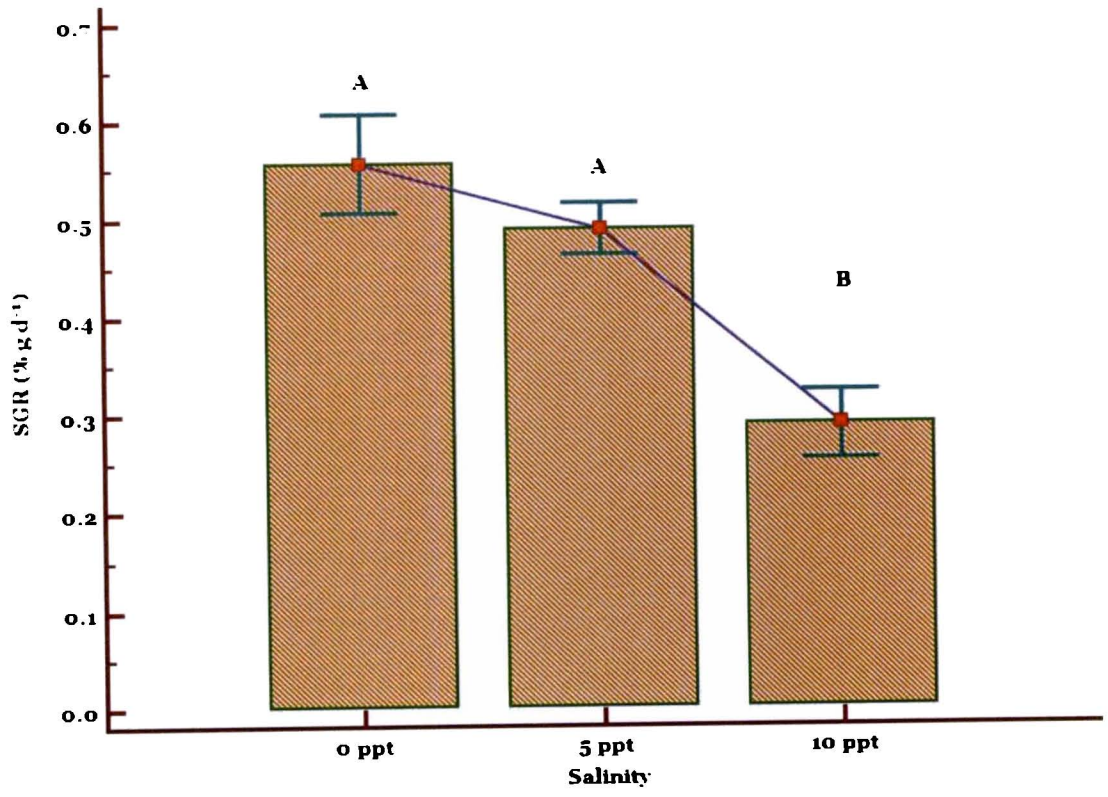


Fig 6: Specific growth rate (SGR % day^{-1}) of *Cyprinus carpio* exposed to different salinity level in natural adaptability trial

Table 14: Variations in length (cm) and weight (g) of *Cyprinus carpio* during natural adaptability test

Salinity		1st day	1st week	2nd week	3rd week	4th week	BWG%	SGR (% day^{-1})
0 ppt	L	5.12±0.03	5.14±0.04	5.17±0.04	5.20±0.05	5.22±0.05		
	W	1.88±0.09	1.97±0.13	2.08±0.15	2.13±0.10	2.20±0.08	16.89±1.58^{A}	0.56±0.05^{A}
5 ppt	L	5.18±0.10	5.31±0.27	5.33±0.27	5.36±0.27	5.38±0.27		
	W	1.94±0.15	1.98±0.17	2.06±0.16	2.14±0.15	2.22±0.16	14.67±0.81^{A}	0.49±0.03^{A}
10 ppt	L	5.94±0.26	6.06±0.24	6.11±0.22	6.24±0.22	6.47±0.20		
	W	2.45±0.06	2.53±0.12	2.57±0.09	2.61±0.11	2.66±0.09	8.42±1.13^{B}	0.29±0.04^{B}

Note: 1. Values are average±SD, L=Length and W=Weight

2. Values (BWG & SGR) with different subscript indicates significant difference (p>0.05)

Table 15: Variations in length (cm) and weight (g) of *Oreochromis mossambicus* during natural adaptability test

Salinity		1st day	1st week	2nd week	3rd week	4th week	BWG%	SGR (% day^{-1})
0 ppt	L	9.33±0.12	10.33±0.12	11.27±0.15	12.17±0.15	13.20±0.10		
	W	12.65±0.09	14.70±0.31	16.28±0.38	17.91±0.57	19.76±0.33	56.14±2.30^{A}	1.59±0.05^{A}
5 ppt	L	9.57±0.21	9.57±0.21	10.07±0.15	10.43±0.15	10.93±0.15		
	W	12.62±0.12	13.89±0.53	14.83±0.71	15.61±0.74	16.93±0.34	34.17±2.76^{B}	1.05±0.07^{B}
10 ppt	L	8.93±0.38	9.13±0.38	9.37±0.42	9.53±0.31	9.77±0.42		
	W	11.39±0.54	11.81±0.75	12.46±0.64	13.23±0.70	14.29±0.44	25.54±2.22^{C}	0.81±0.06^{C}
15 ppt	L	9.60±0.30	9.67±0.20	9.97±0.38	10.30±0.40	10.80±0.26		
	W	12.61±0.25	13.09±0.06	13.79±0.22	14.41±0.25	14.97±0.17	18.74±2.49^{D}	0.61±0.07^{D}
18 ppt	L	8.57±0.55	8.63±0.49	8.73±0.49	8.80±0.61	8.90±0.61		
	W	10.71±0.88	10.94±0.95	11.25±0.96	11.57±0.93	11.93±0.91	11.43±1.40^{E}	0.39±0.04^{E}

Note: 1. Values are average±SD, L=Length and W=Weight

2. Values (BWG & SGR) with different subscript indicates significant difference (p>0.05)

Oreochromis mossambicus

The data pertaining of various growth parameters like initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Oreochromis mossambicus* recorded four sub-lethal salinity level viz., 5, 10, 15 and 18 ppt along with control group (0 ppt) were given in Table 15. In all the salinity treatments, growth parameters like SGR (One-way ANOVA: $F_{4, 10}$=162.28; P=0.001) and BWG% (One-way ANOVA: $F_{4, 10}$=171.31; P=0.0001) varied significantly. BWG% showed decreasing trends in increasing salinity. Significantly highest BWG% ($p<0.05$) was observed in 0 ppt (56.14±2.30) followed by 5 ppt (34.17±2.76), 10 ppt (25.54±2.22), 15 ppt (18.74±2.49) and 18 ppt (11.43±1.40). BWG% in 5 ppt was significantly highest ($p<0.05$) than 15 and 18 ppt. BWG% at 10 ppt was significantly different ($p< 0.05$) from 18 ppt. However, no significant difference has shown in BWG% ($p>0.05$) was found between 0-5 ppt, 5-10 ppt, 10-15 ppt and 15-18 ppt (Fig 7). Similar trends were also observed in case of SGR which showed decreasing trends in increasing salinity. Highest SGR was noticed in 0 ppt (1.53±0.16) which was significantly different ($p<0.05$) than 5 ppt (1.05±0.07), 10 ppt (0.81± 0.06), 15 ppt (0.61±0.07) and 18 ppt (0.39±0.04). SGR in 5 ppt was significantly highest ($p<0.05$) than 15 and 18 ppt. SGR at 10 ppt and 15 ppt was significantly different ($p<0.05$) from 18 ppt. However, no significant difference in SGR ($p>0.05$) was noticed between 5-10 ppt and 10-15 ppt respectively (Fig 8).

The average temperatures were 29.63, 29.82, 29.87, 30.18 and 30.24°C at 0, 5, 10, 15 and 18 ppt salinity in the experiment. The maximum pH was 8.0 and the minimum was 7.1 in the experiment trial. The average DO found in this experiment was 6.07, 5.75, 5.68, 4.72 and 4.29 mg l^{-1} at 0, 5, 10, 15 and 18 ppt salinity respectively. The average alkalinity of the experiment was 197.08, 171.17, 159.74, 152.74 and 143.38 mg l^{-1} with respect to 0, 5, 10, 15 and 18 ppt salinity respectively. The average hardness of found in the range between 252.0

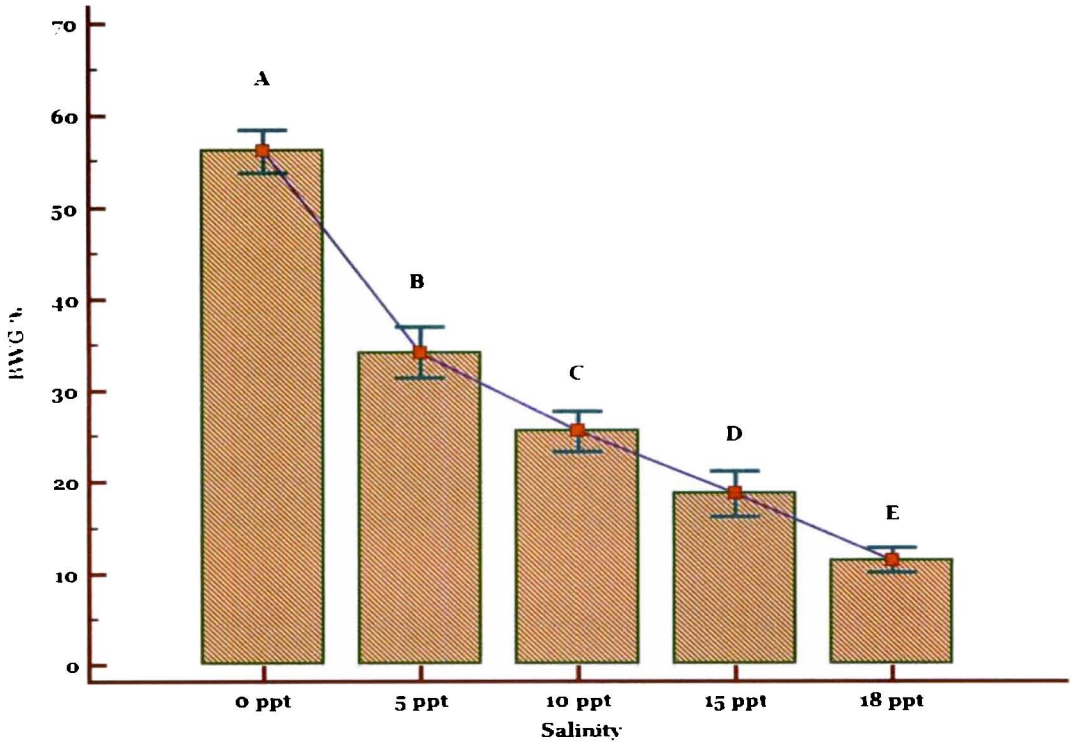


Fig 7: Body weight gain percentage (BWG%) of *Oreochromis mossambicus* exposed to different salinity level in natural adaptability trial

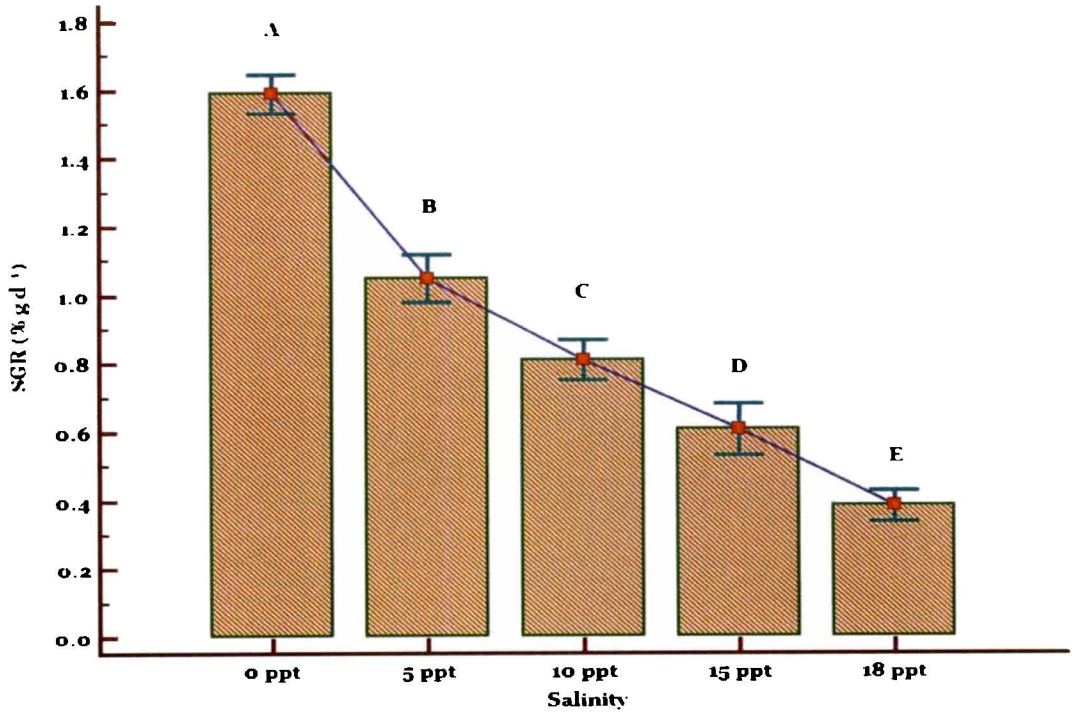


Fig 8: Specific growth rate (SGR % day^{-1}) of *Oreochromis mossambicus* exposed to different salinity level in natural adaptability trial

Table 16: Water quality during natural adaptation trial of *Oreochromis mossambicus* and *Macrobrachium rosenbergii*

Water quality	*Oreochromis mossambicus*					*Macrobrachium rosenbergii*				
	0 ppt	**5 ppt**	**10 ppt**	**15 ppt**	**18 ppt**	**0 ppt**	**5 ppt**	**10 ppt**	**15 ppt**	**20 ppt**
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (°C)	29.4-29.9 (29.63)	29.6-30.2 (29.82)	29.5-30.4 (29.87)	29.7-30.6 (30.18)	29.7-30.7 (30.24)	28.4-29.3 (28.84)	28.6-29.6 (28.87)	28.7-29.8 (29.13)	29.6-30.8 (30.46)	29.5-30.6 (30.52)
pH	7.5-7.9 (7.64)	7.2-7.8 (7.41)	7.1-8.0 (7.43)	7.3-7.9 (7.53)	7.3-7.7 (7.47)	7.4-8.2 (7.77)	7.4-8.1 (7.59)	7.3-7.6 (7.43)	7.3-7.6 (7.44)	7.3-7.7 (7.49)
DO (mgl^{-1})	5.5-6.9 (6.07)	5.2-6.5 (5.75)	5.2-5.9 (5.68)	4.1-5.2 (4.72)	3.3-5.3 (4.29)	5.8-7.7 (6.62)	5.5-7.9 (6.32)	5.1-7.4 (5.89)	4.7-6.9 (5.63)	4.2-5.8 (5.47)
Alkalinity (mgl^{-1})	186.0-197.0 (193.08)	165.0-175.0 (171.17)	153.0-164.0 (159.74)	145.0-157.0 (152.74)	137.0-148.0 (143.38)	184.0-193.0 (187.16)	176.0-184.0 (179.28)	165.0-177.0 (172.46)	154.0-168.0 (163.76)	148.0-159.0 (154.27)
Hardness (mgl^{-1})	252.0-257.0 (255.36)	261.0-266.0 (263.38)	274.0-277.0 (275.12)	283.0-286.0 (285.09)	291.0-295.0 (293.68)	248.0-253.0 (251.27)	263.0-267.0 (265.45)	274.0-277.0 (274.78)	285.0-291.0 (288.74)	289.0-297.0 (293.84)
Ammonia (mgl^{-1})	0.12-0.31 (0.22)	0.15-0.30 (0.21)	0.13-0.30 (0.23)	0.15-0.30 (0.21)	0.17-0.31 (0.24)	0.03-0.29 (0.14)	0.03-0.36 (0.19)	0.02-0.35 (0.22)	0.07-0.37 (0.21)	0.12-0.32 (0.27)

Table 17: Variations in length (cm) and weight (g) of *Macrobrachum rosenbergii* during natural adaptability test

Salinity		1st day	1st week	2nd week	3rd week	4th week	BWG%	SGR (% day^{-1})
	L	7.20±0.10	7.60±0.01	7.67±0.06	7.73±0.06	7.73±0.06		
0 ppt	W	3.34±0.04	3.94±0.03	4.43±0.03	4.63±0.03	4.82±0.03	44.12±1.36^{A}	1.31±0.03^{A}
	L	7.17±0.21	7.33±0.15	7.40±0.10	7.47±0.15	7.50±0.20		
5 ppt	W	3.19±0.05	3.87±0.07	4.37±0.06	4.67±0.06	4.87±0.07	52.51±0.18^{A}	1.51±0.01^{A}
	L	7.17±0.06	7.30±0.10	7.33±0.06	7.43±0.06	7.47±0.06		
10 ppt	W	3.13±0.05	3.63±0.04	3.83±0.04	3.89±0.04	3.92±0.05	17.70±0.24^{B}	0.58±0.01^{B}
	L	7.40±0.10	7.47±0.12	7.53±0.15	7.57±0.12	7.57±0.12		
15 ppt	W	3.38±0.12	3.48±0.13	3.57±0.13	3.60±0.13	3.62±0.13	7.00±0.25^{C}	0.24±0.01^{C}
	L	7.14±0.36	7.17±0.57	7.21±0.24	7.24±0.12	7.28±0.29		
20 ppt	W	3.46±0.09	3.50±0.08	3.54±0.09	3.57±0.08	3.61±0.10	4.33±0.54^{D}	0.15±0.02^{D}

Notc: 1. Values are Average±SD. L=Length and W=Weight

2. Values (BWG & SGR) with different subscript indicates significant difference ($p>0.05$)

and 295.0 mg l^{-1} . At 0, 5, 10, 15 and 18 ppt salinity average ammonia were 0.22, 0.21, 0.23, 21 and 0.24 mg l^{-1} respectively. The results of the water quality parameters of this experiment has presented on Table 16.

Macrobrachium rosenbergii

The data pertaining of various growth parameters like initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Macrobrachium rosenbergii* recorded four sub-lethal salinity level viz., 5, 10, 15 and 20 ppt along with control group (0 ppt) were given in Table 17. In all the salinity treatments, growth parameters like SGR (One-way ANOVA: $F_{4, 10}$=3519.13; P=0.0001) and BWG% (One-way ANOVA: $F_{4, 10}$=3135.70; P=0.0001) varied significantly. BWG% showed decreasing trends in increasing salinity. Significantly highest BWG% ($p<0.05$) was observed in 5 ppt (52.51±0.18) followed by 0 ppt (44.12±1.36), 10 ppt (17.70±0.24), 15 ppt (7.00±0.25) and 20 ppt (4.33±0.54). However, no significant differences of BWG% ($p>0.05$) were observed between 0 and 5 ppt (Fig 9). Similar trends were also observed in case of SGR which showed decreasing trends in increasing salinity. Highest SGR was noticed in 5 ppt (1.51±0.01) followed by 0 ppt (1.31±0.03) which was significantly different ($p<0.05$) than 10 ppt (0.58±0.01), 15 ppt (0.24±0.01) and 20 ppt (0.15±0.02). However, no significant differences of SGR ($p>0.05$) were observed between 0 and 5 ppt. (Fig 10).

The results of water quality parameters of this experiment has presented on Table 7. The temperatures were varied between 28.4 to 30.8°C in this experiment. The average pH was found 7.77, 7.59, 7.43, 7.44 and DO of in this experiment was 6.62, 6.32, 5.89, 5.63 mg l^{-1} at 0, 5, 10 and 13 ppt salinity respectively in both the cases. The average alkalinity value of the experiment at 0, 5, 10 and 13 ppt salinity was 187.16, 179.28, 172.46 and 163.76 mg l^{-1} respectively.

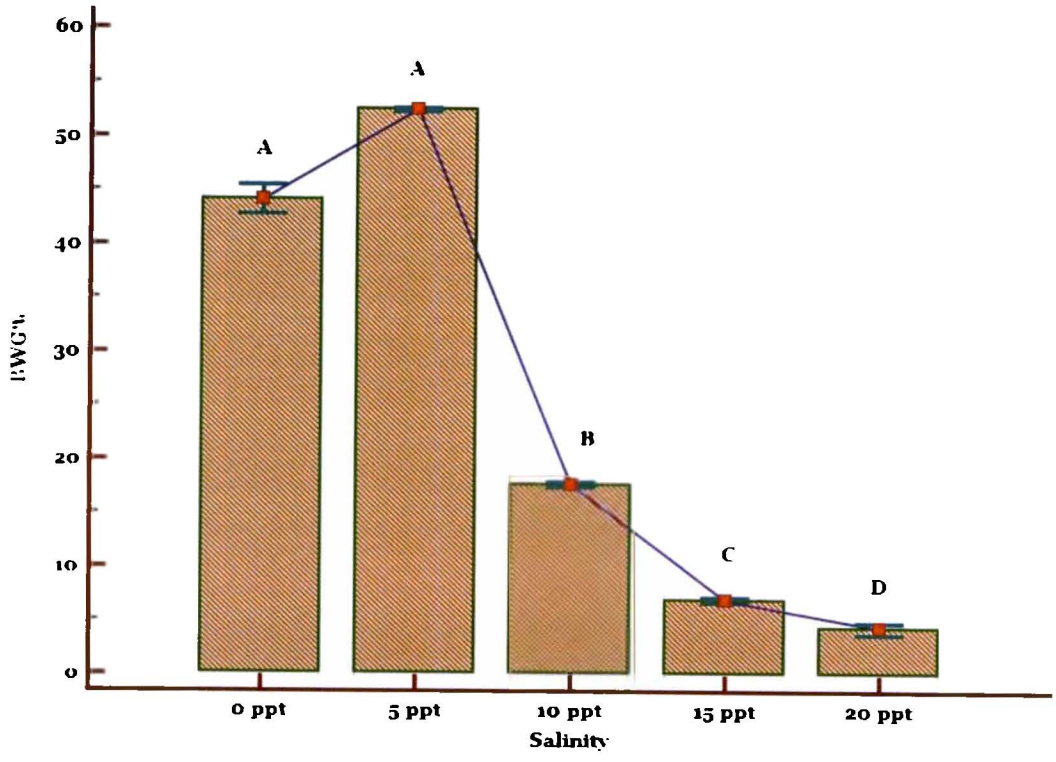


Fig 9: Body weight gain percentage (BWG%) of *Macrobrachium rosenbergii* exposed to different salinity level in natural adaptability trial

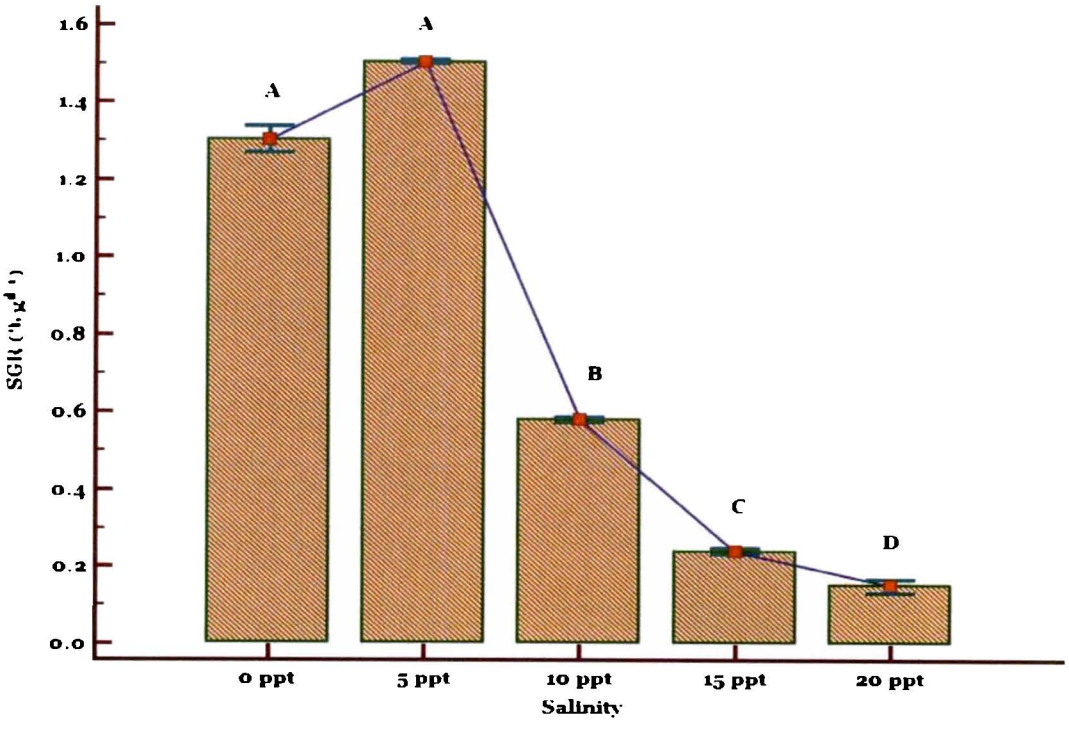


Fig 10: Specific Growth rate (SGR % day^{-1}) of *Macrobrachium rosenbergii* exposed to different salinity level in natural adaptability trial

Average hardness of found 251.27, 265.45, 274.78 and 288.74 mg l^{-1} with respect to 0, 5, 10 and 13 ppt salinity respectively in this experiment. The highest hardness of this study was 291.0 and the lowest was 248.0 mg l^{-1}. The ammonia levels were varied between 0.02 and 0.37 mg l^{-1} in the experiment trial. The results of the water quality parameters of this experiment has presented on Table 12.

4.3 Adaptation strategies against salinity stress

To overcome the adverse impact of salinity stress upon test species, two strategies viz., aeration and high energy feed along with different feed additives at different salinity levels were tried out. The adaptation efficiency for above strategies was evaluated in terms of growth performances. The results obtained from these experiments are summerised bellow.

4.3.1 Aeration as an adaptation strategy

Different levels of aeration were used in different salinity levels like 5 ppt and 10 ppt along with 0 ppt to see its effect on reducing the adverse impact on fish growth. Different aeration treatments were designed as (i) without aeration (T_0) (ii) one hour aeration followed by five hrs without aeration throughout the experiment (T_1) and (iii) Two hours aeration followed by four hours without aeration throughout the experiment (T_2). The results of these trials are given below. The species selected for this experiment were *Puntius sarana* and *Cyprinus carpio*. The duration of this experiment was six weeks.

Puntius sarana

The data pertaining of various growth parameters like initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Puntius sarana* recorded at different aeration levels at various salinity are given in Table 21. In salinity and aeration treatments, growth parameters like SGR (One-way ANOVA: $F_{8, 18}$=251.30; P=0.0001) and BWG% (One-way ANOVA: $F_{8, 18}$ =258.03; P=0.0001) varied significantly.

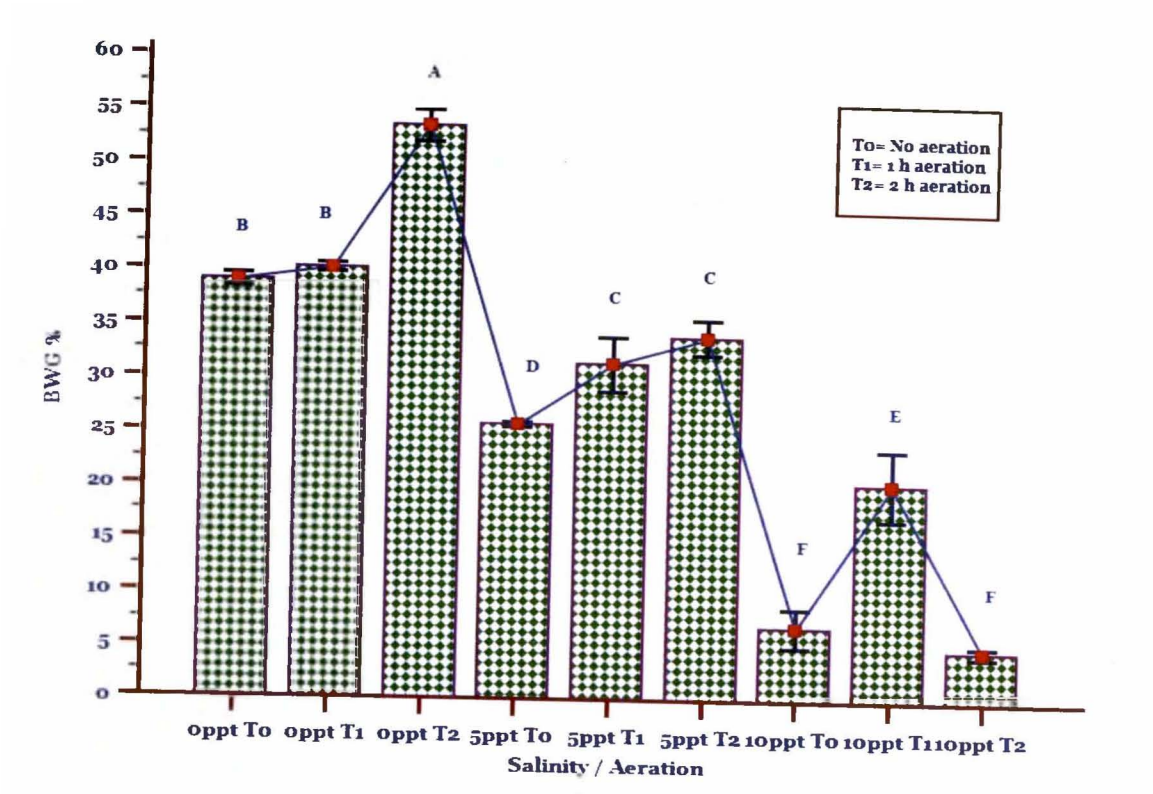


Fig 11: Body weight gain percentage (BWG%) of *Puntius sarana* exposed to different salinity level in aeration as an adaptation strategy trial

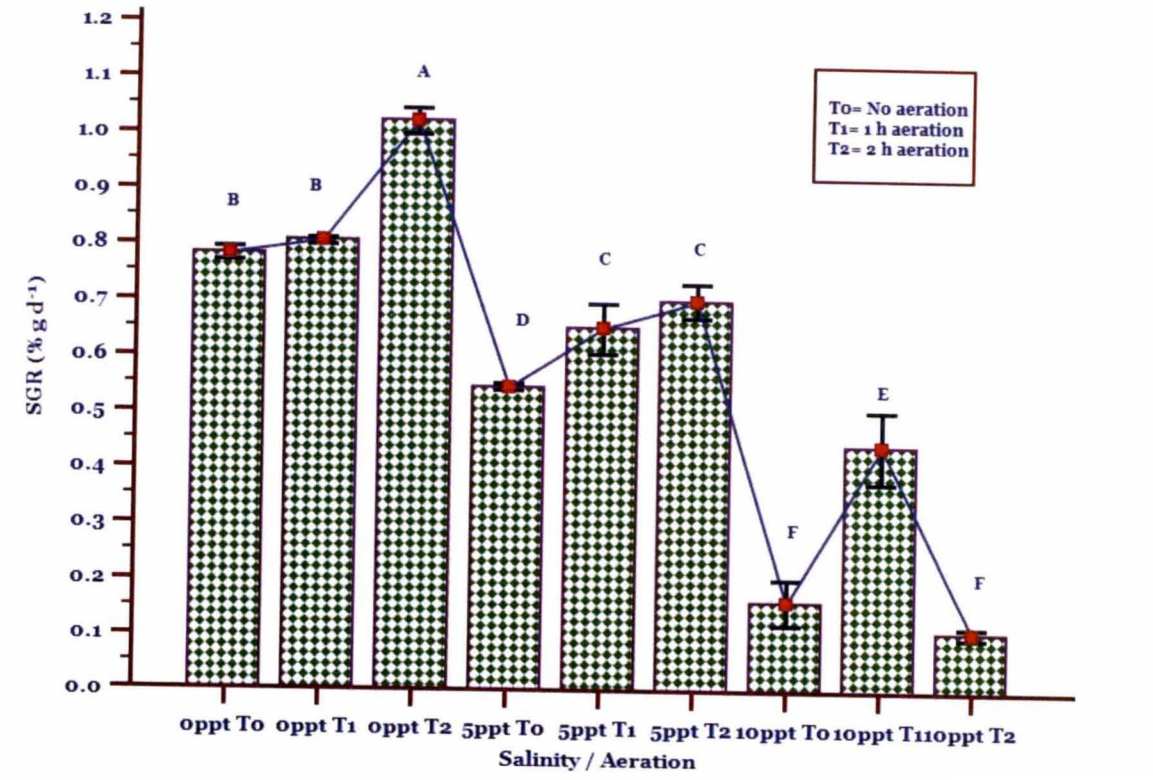


Fig 12: Specific Growth rate (SGR % day^{-1}) of *Puntius sarana* exposed to different salinity level in aeration as an adaptation strategy trial

Table 18: Water quality during aeration trial of *Puntius sarana*

Water quality	0ppt			5ppt			10ppt		
	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (°C)	28.7-34.5 (30.97)	28.7-34.3 (30.90)	28.8-34.3 (30.92)	29.3-33.9 (30.93)	29.0-33.9 (31.08)	29.3-34.4 (31.13)	29.2-34.6 (31.14)	29.3-34.4 (31.15)	29.2-34.2 (31.18)
pH	7.3-7.8 (7.54)	7.3-7.9 (7.58)	7.4-7.9 (7.63)	7.3-8.3 (8.01)	7.6-8.5 (8.21)	8.1-8.6 (8.29)	7.5-8.3 (7.97)	8.1-8.4 (8.26)	8.0-8.4 (8.24)
Alkalinity (mgl^{-1})	181.0-193.0 (186.74)	182.0-192.0 (187.68)	181.0-194.0 (189.98)	143.0-154.0 (148.29)	143.0-154.0 (147.94)	144.0-153.0 (148.18)	137.0-148.0 (142.19)	137.0-148.0 (141.67)	136.0-147.0 (142.97)
Hardness (mgl^{-1})	262.0-267.0 (264.28)	263.0-268.0 (265.39)	263.0-267.0 (264.17)	267.0-277.0 (274.98)	267.0-274.0 (273.54)	266.0-275.0 (273.67)	274.0-284.0 (278.94)	276.0-285.0 (279.17)	275.0-285.0 (279.24)

Note: T_0=No aeration, T_1=One hour aeration five hours off, T_2= Two hours aeration four hours off

Table 19: Water quality during aeration trial of *Cyprinus carpio*

Water quality	0 ppt			5 ppt			10 ppt		
	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (°C)	29.4-34.9 (34.29)	29.7-39.6 (31.86)	29.4-34.2 (31.92)	29.4-38.0 (32.34)	29.5-34.9 (32.23)	29.4-34.9 (32.20)	29.4-34.8 (32.23)	29.6-34.6 (32.08)	29.6-34.4 (32.06)
pH	7.3-7.9 (7.63)	7.4-8.4 (8.10)	7.4-8.5 (8.25)	7.1-7.9 (7.55)	7.9-8.6 (8.35)	7.9-8.5 (7.31)	7.4-8.3 (7.84)	8.1-8.5 (8.32)	8.2-8.5 (8.30)
Alkalinity (mgl^{-1})	185.0-192.0 (188.39)	184.0-193.0 (191.95)	184.0-192.0 (190.57)	165.0-174.0 (169.64)	164.0-175.0 (171.29)	165.0-174.0 (169.47)	147.0-158.0 (153.17)	145.0-157.0 (152.49)	144.0-156.0 (152.28)
Hardness (mgl^{-1})	263.00-271.00 (267.72)	265.00-274.00 (266.63)	263.00-273.00 (267.27)	267.00-277.00 (272.86)	266.00-278.00 (274.37)	268.00-276.00 (273.94)	273.00-282.00 (278.57)	272.00-284.00 (279.65)	273.00-283.00 (279.92)

Note: T_0=No aeration, T_1= One hour aeration five hours off, T_2= Two hours aeration four hours off

Table 20: Dissolved oxygen during aeration as an adaptation strategy of *Puntius sarana*

Date	Time	0 ppt			5 ppt			10 ppt		
		T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
Day 1	6:00 AM	5.27±0.06	6.43±0.06	7.43±0.06	4.53±0.12	6.27±0.06	7.33±0.06	3.50±0.10	6.23±0.06	6.67±0.12
	6:00 PM	5.53±0.06	6.67±0.12	7.63±0.06	4.73±0.12	6.43±0.06	7.43±0.06	3.77±0.06	6.33±0.06	6.77±0.06
Day 2	6:00 AM	5.37±0.06	6.43±0.06	7.47±0.06	4.53±0.06	6.43±0.06	7.33±0.06	3.40±0.10	6.27±0.06	6.57±0.06
	6:00 PM	5.63±0.06	6.67±0.06	7.73±0.06	4.77±0.06	6.57±0.06	7.47±0.06	3.77±0.06	6.37±0.06	6.67±0.06
Day 3	6:00 AM	5.43±0.06	6.67±0.06	7.43±0.06	4.43±0.06	6.40±0.10	7.37±0.06	3.43±0.06	6.23±0.06	6.47±0.06
	6:00 PM	5.73±0.06	6.87±0.06	7.77±0.06	4.73±0.06	6.53±0.06	7.53±0.06	3.67±0.06	6.33±0.06	6.63±0.06
Day 4	6:00 AM	5.27±0.06	6.63±0.06	7.57±0.06	4.37±0.06	6.47±0.06	7.37±0.06	3.37±0.06	6.33±0.06	6.37±0.06
	6:00 PM	5.57±0.06	6.80±0.10	7.73±0.06	4.67±0.06	6.47±0.06	7.43±0.06	3.77±0.06	6.23±0.06	6.67±0.06
Day 5	6:00 AM	5.37±0.06	6.67±0.06	7.47±0.06	4.43±0.06	6.37±0.06	7.43±0.06	3.50±0.10	6.33±0.06	6.40±0.10
	6:00 PM	5.60±0.10	6.83±0.06	7.73±0.06	4.70±0.10	6.33±0.15	7.37±0.06	3.77±0.06	6.43±0.06	6.73±0.06
2nd week	6:00 AM	5.37±0.06	6.63±0.06	7.57±0.06	4.47±0.06	6.37±0.06	7.33±0.06	3.47±0.06	6.37±0.06	6.47±0.06
	6:00 PM	5.70±0.10	6.77±0.06	7.77±0.06	4.73±0.06	6.43±0.06	7.47±0.06	3.77±0.06	6.37±0.12	6.67±0.06
3rd week	6:00 AM	5.43±0.06	6.57±0.06	7.57±0.06	4.47±0.06	6.30±0.10	7.37±0.06	3.57±0.06	6.33±0.12	6.37±0.06
	6:00 PM	5.73±0.06	6.73±0.12	7.73±0.06	4.77±0.06	6.37±0.06	7.53±0.12	3.77±0.06	6.43±0.12	6.73±0.12
4th week	6:00 AM	5.33±0.06	6.63±0.06	7.57±0.06	4.53±0.06	6.37±0.06	7.47±0.06	3.57±0.06	6.27±0.06	6.53±0.06
	6:00 PM	5.67±0.12	6.83±0.12	7.77±0.06	4.77±0.06	6.33±0.06	7.47±0.12	3.83±0.06	6.43±0.06	6.77±0.06
5th week	6:00 AM	5.33±0.12	6.67±0.06	7.67±0.06	4.33±0.06	6.43±0.06	7.47±0.12	3.43±0.06	6.23±0.06	6.57±0.06
	6:00 PM	5.73±0.06	6.77±0.06	7.83±0.06	4.63±0.06	6.50±0.10	7.47±0.06	3.87±0.06	6.43±0.06	6.73±0.12
6th week	6:00 AM	5.37±0.06	6.63±0.06	7.57±0.06	4.43±0.06	6.37±0.06	7.33±0.06	3.53±0.06	6.27±0.06	6.57±0.06
	6:00 PM	5.73±0.12	6.83±0.06	7.70±0.10	4.83±0.06	6.53±0.06	7.53±0.06	3.87±0.06	6.50±0.10	6.73±0.06

Note: T_0=No aeration, T_1=One hour aeration five hours off, T_2= Two hours aeration four hours off

Table 21: Variations in length (cm) and weight (g) of *Puntius sarana* during aeration trials as an adaptation strategy against increased salinity

Salinity	Treatment		1st day	1st week	2nd week	3rd week	4th week	5th week	6th week	BWG%	SGR (% day^{-1})
0 ppt	T_0	L	10.32±0.07	10.34±0.05	10.36±0.05	10.44±0.05	10.56±0.13	10.67±0.15	10.79±0.20		
		W	11.66±0.06	11.76±0.02	12.63±0.77	13.47±0.60	14.37±0.41	15.32±0.16	16.21±0.02	39.03±0.57^{B}	0.78±0.01^{B}
	T_1	L	10.31±0.10	10.31±0.10	10.35±0.05	10.42±0.07	10.46±0.12	10.50±0.10	10.58±0.13		
		W	11.30±0.08	11.39±0.02	12.74±0.65	13.51±0.45	14.28±0.26	15.08±0.05	15.85±0.11	40.29±0.42^{B}	0.81±0.01^{B}
	T_2	L	10.75±0.09	10.81±0.09	10.82±0.07	10.87±0.05	10.38±0.48	11.03±0.06	11.09±0.02		
		W	10.97±0.11	11.35±0.11	11.74±0.20	13.03±0.16	14.38±0.16	15.71±0.19	16.86±0.09	53.67±1.46^{A}	1.02±0.02^{A}
5 ppt	T_0	L	10.81±0.43	10.81±0.42	10.88±0.46	10.95±0.49	11.05±0.59	11.06±0.58	10.85±0.86		
		W	11.37±0.23	12.18±0.11	12.69±0.19	13.14±0.26	13.51±0.21	13.92±0.23	14.32±0.26	25.88±0.26^{D}	0.55±0.01^{D}
	T_1	L	10.71±0.08	10.76±0.12	10.81±0.09	10.78±0.11	10.81±0.08	10.59±0.42	10.34±0.38		
		W	11.37±0.21	12.43±0.02	12.69±0.20	13.24±0.15	13.82±0.11	14.39±0.06	14.96±0.02	31.55±2.55^{C}	0.65±0.05^{C}
	T_2	L	10.59±0.19	10.59±0.18	10.68±0.19	10.50±0.35	10.58±0.44	10.99±0.28	11.08±0.30		
		W	11.12±0.24	11.53±0.62	11.82±0.45	12.59±0.35	13.38±0.25	14.16±0.15	14.92±0.14	34.20±1.66^{C}	0.70±0.03^{C}
10 ppt	T_0	L	10.59±0.12	10.65±0.05	10.67±0.06	10.75±0.05	10.82±0.07	10.86±0.07	10.61±0.44		
		W	11.27±0.26	11.51±0.44	11.77±0.22	11.88±0.16	11.96±0.14	12.02±0.12	12.05±0.12	6.98±1.91^{F}	0.16±0.04^{F}
	T_1	L	10.48±0.23	10.51±0.18	10.59±0.19	10.69±0.20	10.52±0.35	10.59±0.40	10.67±0.48		
		W	10.95±0.34	11.22±0.51	12.07±0.24	12.43±0.44	12.66±0.48	12.95±0.64	13.20±0.77	20.44±3.30^{E}	0.44±0.07^{E}
	T_2	L	10.74±0.49	10.77±0.46	10.82±0.42	10.85±0.39	10.93±0.42	11.05±0.48	11.16±0.48		
		W	11.95±0.34	11.22±0.51	12.07±0.24	12.43±0.44	12.66±0.48	12.95±0.64	13.20±0.77	4.84±0.52^{F}	0.11±0.01^{F}

Note: 1. T_0=No aeration, T_1=One hour aeration five hours off, T_2= Two hours aeration four hours off

2. Values are average±SD, L=Length and W=Weight

3. Values (BWG & SGR) with different subscript indicates significant difference ($p>0.05$)

In 0 ppt the highest SGR ($p<0.05$) was obtained at T_2 (1.02±0.0.02 % day^{-1}) treatment followed by T_1 (0.81±0.01) and lowest were obtained in T_0 (0.78±0.01). Likewise, highest BWG % were obtained at T_2 (53.67±1.46) treatment followed by T_1 (40.29±0.42) and the lowest were obtained in T_0 (39.03±0.57). BWG% and the SGR in T_2 was significantly higher ($p<0.05$) than the SGR in T_0 and T_1. However, BWG% and SGR of in T_0 and T_1 were not significantly ($p>0.05$) different.

In 5 ppt the highest SGR was obtained at T_2 (0.70±0.03) treatment followed by T_1 (0.65±0.05) and the lowest in T_0 (0.55±0.01) and no significantly differences were observed in SGR among the treatment ($p>0.05$) T_1 and T_2 at significant difference between T_0-T_1 and T_0-T_2. Likewise, significantly the highest BWG% ($p>0.05$) were obtained at T_2 (34.20±1.66) treatment followed by T_1 (31.55±2.55) and the lowest T_0 (25.88±0.26). However, significant differences were observed in BWG% among the treatment ($p>0.05$) among T_0-T_1, T_0-T_2 and non significant difference T_1-T_2.

In the 10 ppt the highest SGR were obtained at T_1 (0.44±0.07 % day^{-1}) treatment followed by T_0 (0.16±0.04 % day^{-1}) and the lowest in T_2 (0.11±0.01). However, non significant differences were observed in SGR among the T_0 and T_2 treatment ($p>0.05$). Similar trends were observed in terms of BWG%. Highest BWG% ($p<0.05$) (Fig 11 & 12) were obtained at T_1 (20.44±3.30) treatment followed by T_0 (6.98±1.91) and the lowest in T_2 (4.84±0.52). However, non significant differences were observed in BWG% among the T_0 and T_2 treatments ($p>0.05$).

Dissolved oxygen at different aeration levels

Generally the DO concentration was found to increase with increase in aeration levels. However increase in salinity levels should decrease DO level. At 0 ppt salinity the DO was found to vary from 5.27±0.06 to 5.73±0.06 mg l^{-1} at T_0 (no aeration/control) and the average value was5.51 mg l^{-1}. At T_1 (one hour aeration on and five hours off) in 0 ppt salinity, DO varied from

6.43±0.06 to 6.87±0.06 mg l^{-1} and average DO was found to be 6.69 mg l^{-1}. In T_2 treatment (two hours aeration on and four hours off) at 0 ppt salinity the lowest DO was 7.43±0.06 and the highest was 7.83±0.06 mg l^{-1} and average was7.64 mg l^{-1}.

At 5 ppt salinity the DO ranged from 4.33±0.06 to 4.83±06 mg l^{-1} and average value was 4.59 mg l^{-1} in T_0 treatment. The DO varied from 6.27±0.06 to 6.57±0.06 mg l^{-1} and average was 6.41 mg l^{-1} at 5 ppt salinity at T_1 treatment. At T_2 lowest, highest and average values were 7.33±0.06, 7.53±0.06 mg l^{-1} and 7.43 mg l^{-1} respectively at 5 ppt salinity.

At 10 ppt salinity DO was found in the range between 3.37±0.06 to 3.87±0.06 mg l^{-1} and average was 3.63 mg l^{-1} at T_0 treatment. At T_1 treatment DO varied between 6.23±0.06 to 6.43±0.12 mg l^{-1} and average was 6.34 mg l^{-1} at 10 ppt salinity. At T_2 treatment the minimum and maximum DO were 6.37±0.06 and 6.77±0.06 mg l^{-1} respectively and average was 6.60 mg l^{-1}. The variations in dissolved oxygen (DO) concentration during aeration as an adaptation strategy for the species *Puntius sarana* is presented in Table 20.

The values water quality parameters are presented in Table 18. The average temperature ranged between 28.7 and 34.5°C. The minimum and maximum pH values were 7.3 and 8.6 respectively. The alkalinity varied between 136.0 and 194.0 mg l^{-1} and the hardness ranged between 262.0 to 285.0 mg l^{-1}.

Cyprinus carpio

The initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Cyprinus carpio* recorded in different aeration levels at various salinities are given in Table 23. In salinity and aeration treatments, growth parameters like SGR (One-way ANOVA: $F_{8, 18}$=1793.41; P=0.0001) and BWG% (One-way ANOVA: $F_{8, 18}$=1236.93; P=0.0001) varied significantly.

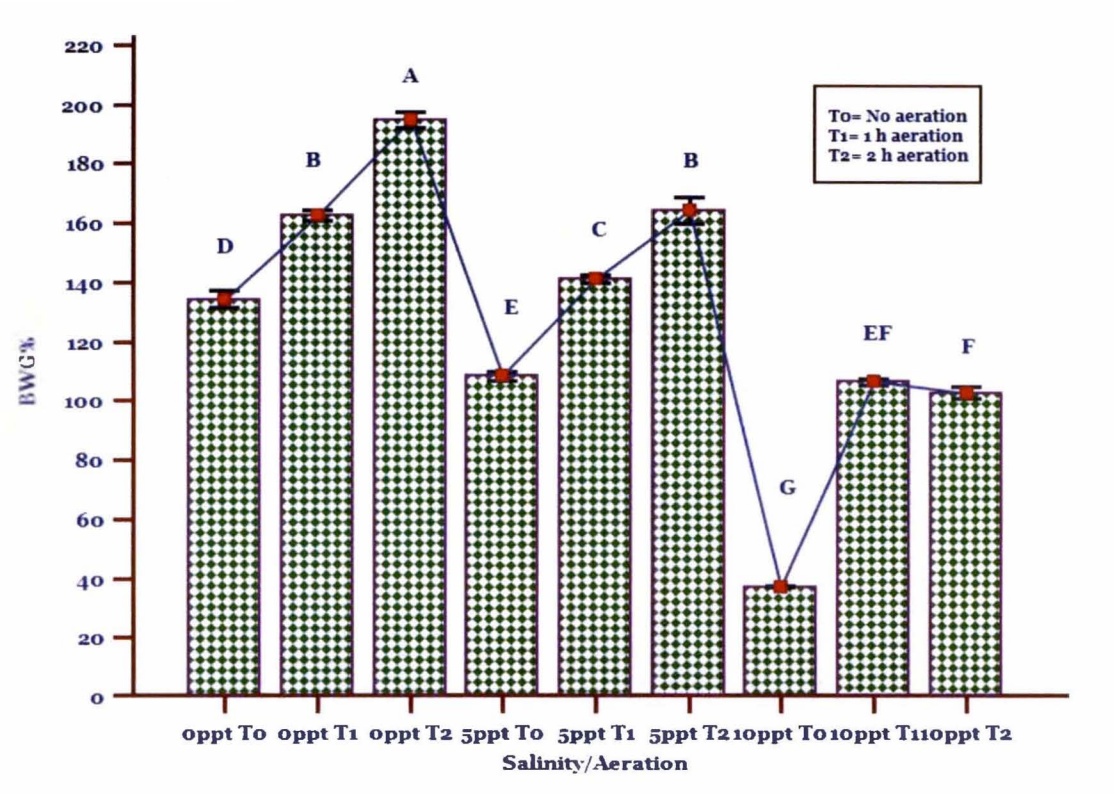


Fig 13: Body weight gain percentage (BWG%) of *Cyprinus carpio* exposed to different salinity level in aeration as an adaptation strategy trial

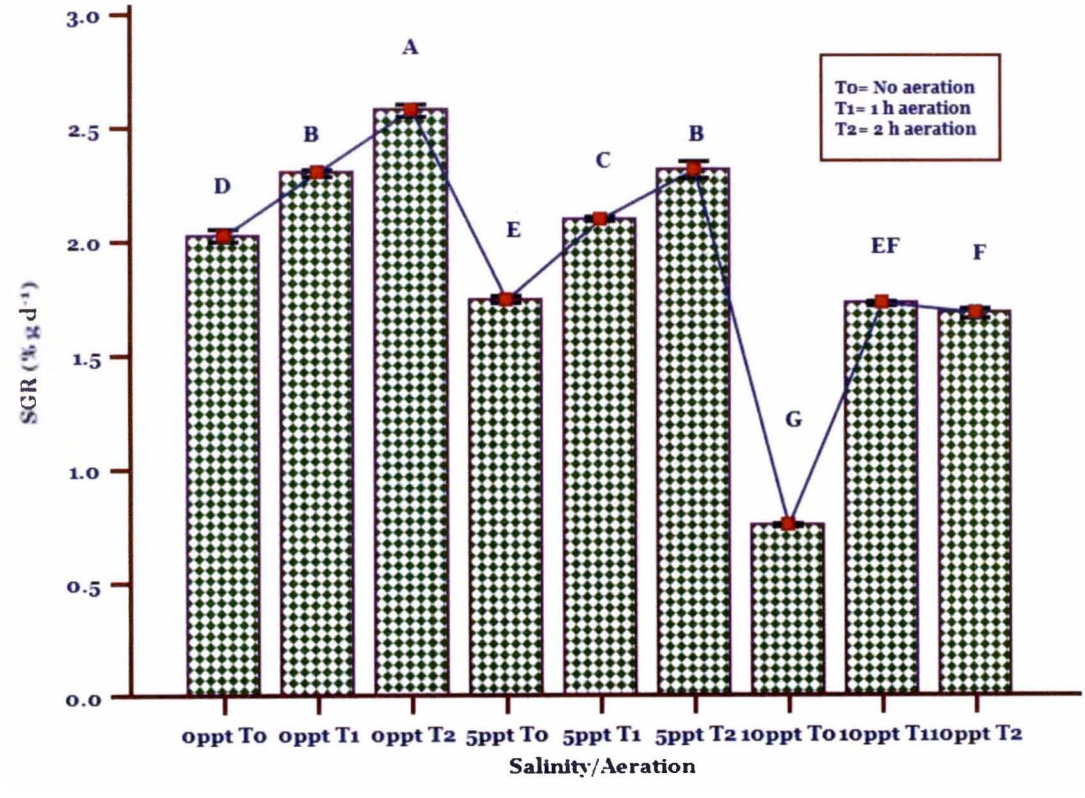


Fig 14: Specific Growth rate (SGR % day^{-1}) of *Cyprinus carpio* exposed to different salinity level in aeration as an adaptation strategy trial

Table 22: Dissolved oxygen during aeration as an adaptation strategy of *Cyprinus carpio*

Day	Time	0 ppt			5 ppt			10 ppt		
		T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
Day 1	6:00 AM	5.47±0.06	6.57±0.15	7.57±0.06	4.67±0.12	6.37±0.06	7.23±0.06	3.47±0.12	6.13±0.06	6.53±0.12
	6:00 PM	5.77±0.06	6.63±0.06	7.70±0.10	4.87±0.12	6.53±0.12	7.33±0.06	3.83±0.06	6.23±0.06	6.70±0.10
Day 2	6:00 AM	5.50±0.10	6.67±0.06	7.50±0.10	4.53±0.06	6.47±0.06	7.20±0.10	3.53±0.06	6.13±0.06	6.53±0.06
	6:00 PM	5.77±0.06	6.77±0.06	7.70±0.10	4.83±0.06	6.57±0.06	7.37±0.12	3.83±0.05	6.27±0.06	6.70±0.10
Day 3	6:00 AM	5.50±0.10	6.70±0.10	7.57±0.12	4.63±0.06	6.37±0.06	7.30±0.06	3.47±0.12	6.17±0.06	6.43±0.06
	6:00 PM	5.80±0.10	6.70±0.10	7.70±0.10	4.83±0.06	6.50±0.10	7.50±0.10	3.83±0.06	6.37±0.06	6.57±0.06
4th day	6:00 AM	5.50±0.10	6.53±0.06	7.63±0.06	4.53±0.06	6.37±0.06	7.43±0.06	3.53±0.06	6.27±0.06	6.43±0.06
	6:00 PM	5.77±0.06	6.67±0.12	7.77±0.06	4.73±0.06	6.53±0.06	7.27±0.12	3.83±0.06	6.33±0.06	6.63±0.06
5th day	6:00 AM	5.53±0.12	6.57±0.15	7.63±0.06	4.43±0.06	6.40±0.10	7.43±0.15	3.43±0.06	6.33±0.06	6.43±0.06
	6:00 PM	5.83±0.06	6.77±0.06	7.73±0.12	4.83±0.06	6.60±0.10	7.27±0.06	3.73±0.06	6.43±0.06	6.70±0.10
2nd week	6:00 AM	5.43±0.15	6.53±0.06	7.53±0.06	4.47±0.12	6.37±0.06	7.47±0.12	3.43±0.06	6.23±0.06	6.50±0.10
	6:00 PM	5.63±0.12	6.73±0.06	7.73±0.06	4.77±0.12	6.57±0.06	7.63±0.06	3.83±0.06	6.43±0.06	6.70±0.10
3rd week	6:00 AM	5.40±0.10	6.53±0.12	7.63±0.06	4.43±0.06	6.43±0.06	7.43±0.06	3.53±0.06	6.23±0.06	6.43±0.06
	6:00 PM	5.70±0.10	6.70±0.10	7.77±0.06	4.83±0.06	6.63±0.06	7.63±0.06	3.83±0.06	6.43±0.06	6.63±0.06
4th week	6:00 AM	5.30±0.10	6.67±0.06	7.63±0.06	4.47±0.12	6.53±0.06	7.33±0.06	3.63±0.06	6.23±0.06	6.43±0.06
	6:00 PM	5.60±0.10	6.73±0.06	7.83±0.06	4.73±0.06	6.67±0.06	7.57±0.06	3.93±0.15	6.43±0.15	6.60±0.10
5th week	6:00 AM	5.33±0.12	6.63±0.06	7.63±0.06	4.43±0.06	6.43±0.06	7.23±0.06	3.57±0.06	6.23±0.06	6.43±0.06
	6:00 PM	5.73±0.12	6.73±0.12	7.73±0.06	4.83±0.06	6.70±0.10	7.47±0.12	3.87±0.06	6.47±0.06	6.53±0.06
6th week	6:00 AM	5.30±0.10	6.53±0.12	7.53±0.06	4.53±0.06	6.40±0.10	7.27±0.06	3.67±0.06	6.33±0.06	6.43±0.06
	6:00 PM	5.70±0.10	6.73±0.06	7.63±0.06	4.93±0.06	6.63±0.21	7.50±0.10	3.93±0.15	6.47±0.06	6.70±0.10

Note: T_0=No aeration, T_1=One hour aeration five hours off, T_2= Two hours aeration four hours off

Table 23: Variations in length (cm) and weight (g) of *Cyprinus carpio* during aeration trials as an adaptation strategy against increased salinity

Salinity	Treatment		1st day	1st week	2nd week	3rd week	4th week	5th week	6th week	BWG%	SGR (% day^{-1})
0 ppt	T_0	L	4.28±0.20	5.19±0.24	5.44±0.36	5.56±0.17	5.62±0.17	5.67±0.19	5.77±0.18		
		W	1.19±0.07	1.42±0.12	1.85±0.11	2.13±0.14	2.40±0.17	2.63±0.15	2.79±0.15	134.29±2.89^{D}	2.03±0.03^{D}
	T_1	L	4.45±0.22	5.09±0.56	5.34±0.38	5.51±0.24	5.71±0.19	5.81±0.20	5.92±0.18		
		W	1.21±0.08	2.17±0.21	2.35±0.31	2.54±0.28	2.73±0.23	2.97±0.23	3.17±0.22	162.96±1.80^{B}	2.30±0.02^{B}
	T_2	L	3.96±0.27	4.95±0.55	5.13±0.42	5.20±0.42	5.32±0.31	5.45±0.35	5.67±0.30		
		W	1.20±	1.95±0.12	2.28±0.10	2.44±0.06	2.57±0.10	2.99±0.07	3.53±0.14	194.92±2.71^{A}	2.58±0.02^{A}
5 ppt	T_0	L	4.37±0.11	4.97±0.28	5.25±0.25	5.33±0.20	5.49±0.14	5.58±0.18	5.74±0.15		
		W	1.28±0.03	2.04±0.01	2.23±0.01	2.37±0.05	2.45±0.06	2.56±0.05	2.66±0.05	108.44±1.38^{E}	1.75±0.02^{E}
	T_1	L	4.20±0.30	4.82±0.40	5.22±0.69	5.24±0.68	5.30±0.68	5.35±0.68	5.48±0.61		
		W	1.20±0.04	1.38±0.09	1.64±0.11	2.00±0.14	2.31±0.09	2.62±0.06	2.90±0.07	141.38±1.23^{C}	2.10±0.01^{C}
	T_2	L	4.17±0.28	4.91±0.50	4.97±0.51	5.06±0.37	5.15±0.25	5.22±0.21	5.31±0.16		
		W	1.22±0.02	2.44±0.13	2.75±0.06	2.79±0.07	2.92±0.05	3.06±0.07	3.24±0.09	164.54±4.28^{B}	2.32±0.01^{B}
10 ppt	T_0	L	4.78±0.09	4.82±0.09	4.98±0.11	5.09±0.10	5.18±0.10	5.25±0.09	5.52±0.29		
		W	1.4±0.03	2.25±0.02	2.32±0.04	2.47±0.09	2.63±0.14	2.73±0.15	2.83±0.14	37.31±0.31^{G}	0.75±0.01^{G}
	T_1	L	4.40±0.16	4.78±0.40	5.18±0.76	5.20±0.76	5.28±0.81	5.36±0.86	5.43±0.92		
		W	1.23±0.18	1.76±0.06	2.28±0.32	2.33±0.30	2.41±0.35	2.46±0.35	2.54±0.37	106.75±1.16^{EF}	1.73±0.01^{EF}
	T_2	L	4.26±0.30	4.89±0.53	4.95±0.60	5.04±0.65	5.11±0.66	5.17±0.68	5.23±0.69		
		W	1.15±0.10	1.42±0.15	1.56±0.11	1.79±0.17	2.00±0.19	2.21±0.18	2.34±0.19	103.06±2.01^{F}	1.69±0.02^{F}

Note: 1. T_0=No aeration, T_1=One hour aeration five hours off, T_2= Two hours aeration four hours off

2. Values are average±SD, L=Length and W=Weight

3. Values (BWG & SGR) with different subscript indicates significant difference ($p>0.05$)

In 0 ppt, significantly ($p<0.05$) the highest SGR was obtained at T_2 (2.68±0.02) followed by T_1 (2.30±0.02) and significantly the lowest ($p<0.05$) in T_0 (2.63±0.03). However, SGR of T_0 and T_2 were significantly ($p>0.05$) different. Likewise, the highest BWG% in the 0 ppt were obtained at T_2 (194.92±2.71) treatment followed by T_1 (162.96±1.80) and significantly lowest ($p<0.05$) were obtained in T_0 (134.29±2.89). However, BWG% in T_0 and T_2 were significantly ($p>0.05$) different.

In the 5 ppt significantly ($p<0.05$) highest SGR were obtained at T_2 (2.32±0.01) treatment followed by T_1 (2.10±0.01) and the lowest in T_0 (1.75±0.02). Significant difference ($p<0.05$) between the SGR of T_2 and T_0 treatment were observed, however, no significantly differences were observed among T_2-T_1 and T_1-T_0 treatments ($p>0.05$). Likewise, highest BWG% were obtained at T_2 (164.54±4.28) treatment followed by T_1 (141.38±1.23) and lowest were obtained in T_0 (108.44±1.38). Significant difference was observed in BWG% ($p<0.05$) between T_2 and T_0 treatment, however, no significantly differences were observed in SGR among T_2-T_1 and T_1-T_0 treatment ($p>0.05$).

In 10 ppt, the highest SGR were obtained at T_1 (1.73±0.01) treatment followed by T_2 (1.69±0.02) and the lowest were obtained in T_0 (0.75±0.01). However, no significantly differences were observed between the three treatments ($p>0.05$). Similar trends were observed in terms of BWG% (Fig 13 & 14) where the highest BWG% were obtained at T_1 (106.75±1.16) treatment followed by T_2 (103.06±2.01) and T_0 (37.31±0.31). No significantly differences were observed in BWG% among the three treatments ($p>0.05$).

Dissolved oxygen at different aeration levels

Generally the DO concentration was found to increase with increase in aeration levels. However increase in salinity levels showed decrease in DO levesl. At 0 ppt salinity in T_0 treatment (no aeration-control) the DO ranged from 5.30±0.10 to 5.83±06 mg l^{-1} and the average DO level was 5.58 mg l^{-1}. The DO varied from 6.53±0.12 to 6.77±0.06 mg l^{-1} and average was 6.66 mg l^{-1}

at T_1 (one hour aeration on five hours off) at 0 ppt salinity. In T_2 (two hour aeration on four hours off) the lowest, highest and average values were 7.50±0.10, 7.83±0.06 mg l^{-1} and 7.66 mg l^{-1} at 0 ppt salinity.

The DO was found to vary from 4.43±0.06 to 4.87±0.12 mg l^{-1} in T_0 with the average value of 4.47 mg l^{-1} at 5 ppt salinity. In T_1 at 5 ppt salinity DO ranged from 6.37±0.06 to 6.70±0.10 mg l^{-1} and average DO was found to be 6.50 mg l^{-1}. In T_2 at 5 ppt salinity, the lowest DO was 7.20±0.10 mg l^{-1} and the highest and average values were 7.63±0.06 and 7.40 mg l^{-1} respectively.

At 10 ppt salinity (T_0 treatment) DO level varied between was found 3.43±0.06 and 3.93±0.15 mg l^{-1} and average was 3.67 mg l^{-1}. In T_1 treatment (10 ppt salinity) DO ranged between 6.13±0.06 and 6.47±0.06 mg l^{-1} with the average value of 6.31 mg l^{-1}. In T_2 treatment DO fluctuated between 6.43±0.06 to 6.70±0.10 mg l^{-1} and average value was 6.55 mg l^{-1}.

The variations in dissolved oxygen (DO) levels during aeration at different salinity levels for the species *Cyprinus carpio* is presented in Table 22 and the range of water quality parameters values during the experiment trial is presented in Table 19.

The temperature ranged between 29.4 and 39.6°C. The pH varied from 7.1 to 8.6 in the experiment. The respective minimum and maximum alkalinity were found to be 144.0 and 193 mg l^{-1}. The lowest and the highest average values of hardness were 263.0 and 284.0 mg l^{-1} respectively.

4.3.2 Feed Manipulation as an adaptation strategy

High energy feed alone and in combination with immunostimulant, probiotic, prebiotic were tried out as adaptation strategies against salinity stress. Three freshwater fish species like *Cyprinus carpio, Labeo rohita* and *Oreochromis mosambicus* were included in the trials as test species. Salinity stress levels of 5 ppt and 10 ppt were used for the species *Labeo rohita* and *Cyprinus carpio* and level 10 and 15 ppt for *Oreochromis mossambicus*. The

efficiency of different combinations of feed regimes for different species at different salinity gradients were judged in terms of growth performances. In this experiment different feed treatments were designed (T_0=Normal feed, T_1=High energy feed, T_2=High energy feed+Immunostimulant, T_3=High energy feed+Gut probiotic and T_4=High energy feed+Gut prebiotic).

Labeo rohita

The data pertaining of various growth parameters like initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Labeo rohita* recorded in different feed regime viz., T_0, T_1, T_2, T_3, and T_4 at 5 and 10 ppt salinity level are presented in Table 25. In all treatments, growth parameters like SGR (One-way ANOVA: $F_{9, 20}$=1208.46; P=0.0001) and BWG% (One-way ANOVA: $F_{9, 20}$=1114.25; P=0.0001) varied significantly.

At 5 ppt, the lowest SGR ($p<0.05$) was found at T_0 (1.29±0.01) and the highest in T_2 treatment (1.82±0.01) followed by T_3 (1.80±0.01), T_4 (1.78±0.02), T_1 (1.75±0.01). There was no significant variation in SGR ($p>0.05$) between T_1-T_4, T_2-T_3, and T_3-T_4. However, significant variation in SGR ($p<0.05$) was observed between T_2-T_0, T_1-T_2, T_2-T_4, T_0-T_4, T_0-T_3, and T_1-T_0

Similar trend was noticed for BWG% also. At 5 ppt, significantly lowest BWG% ($p<0.05$) was obtained at T_0 (72.12±0.62) than other treatments and the highest BWG% in T_2 treatment (114.92±0.87) followed by T_3 (112.74±0.99), T_4 (111.16±1.38), and T_1 (108.52±0.95). There was no significant variation in BWG% ($p>0.05$) between T_1-T_4, T_2-T_3, and T_3-T_4. However, significant variation in BWG% ($p<0.05$) was observed between T_1-T_2, T_1-T_3, and T_2-T_4.

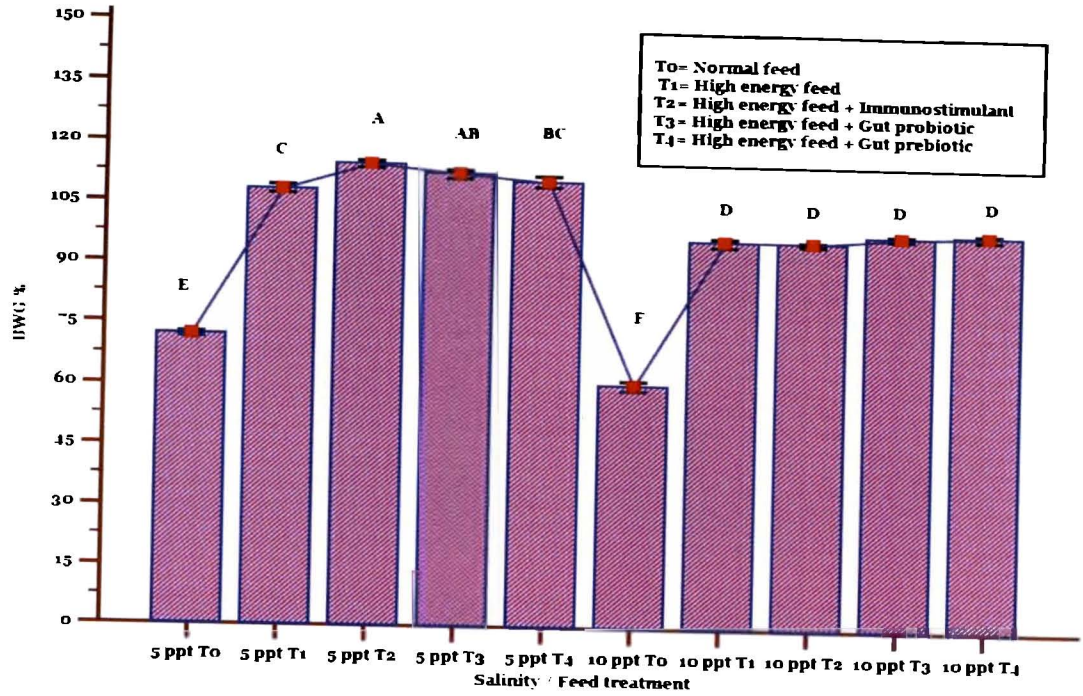


Fig 15: Body weight gain percentage (BWG%) of *Labeo rohita* exposed to different salinity level in feed manipulations an adaptation strategy trial

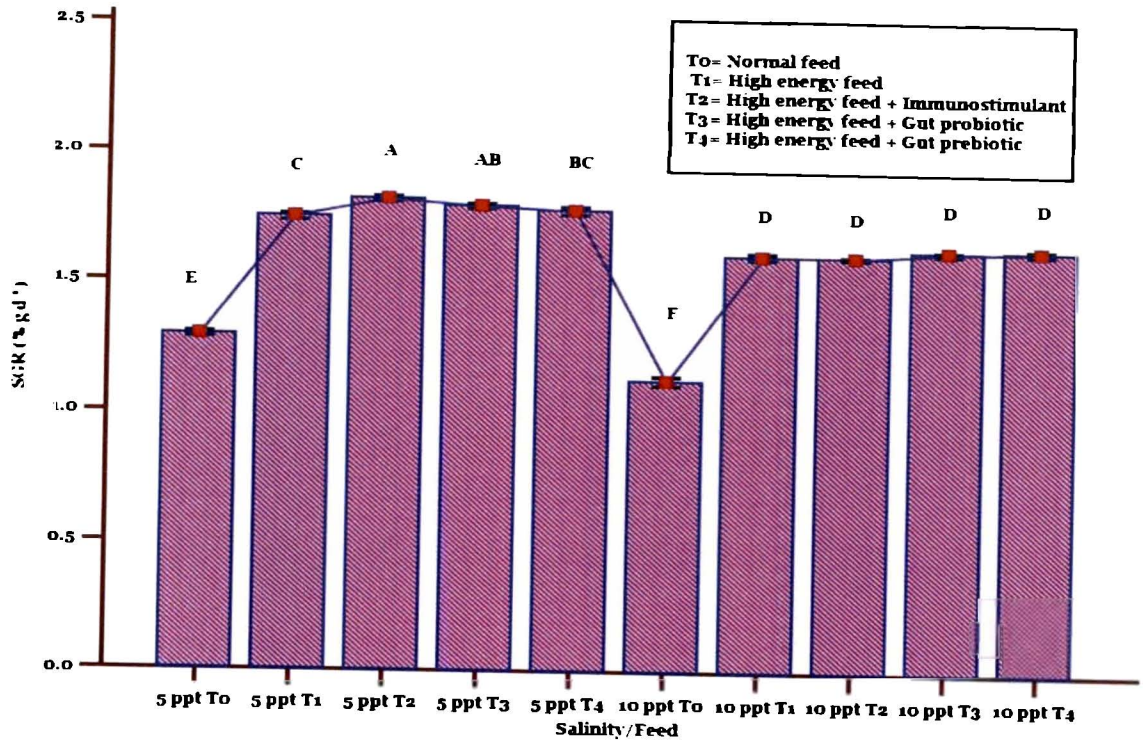


Fig 16: Specific Growth rate (SGR % day^{-1}) of *Labeo rohita* exposed to different salinity level in feed manipulations as an adaptation strategy trial

Table 24: Water quality during feed manipulation as an adaptation strategy against increased salinity in *Labeo rohita*

Salinity	5 ppt					10 ppt				
Water quality	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (°C)	24.1-25.7 (24.43)	24.3-24.7 (24.54)	24.2-24.9 (24.68)	24.2-24.8 (24.63)	24.1-24.8 (24.48)	24.4-25.2 (24.86)	24.6-25.3 (24.94)	24.5-25.4 (24.84)	24.4-25.3 (24.96)	24.6-25.4 (24.89)
pH	7.9-8.4 (8.19)	7.8-8.4 (8.22)	8.1-8.5 (8.32)	8.1-8.5 (8.31)	7.8-8.5 (8.46)	7.9-8.4 (8.12)	8.1-8.6 (8.37)	7.9-8.5 (8.31)	8.0-8.4 (8.23)	7.8-8.3 (8.29)
DO (mgl^{-1})	5.6-6.4 (5.97)	5.8-6.5 (6.17)	5.7-6.5 (6.21)	5.9-6.6 (6.28)	5.7-6.5 (6.34)	5.6-6.6 (6.12)	5.7-6.5 (6.14)	5.7-6.7 (6.09)	5.6-6.9 (6.15)	5.5-6.8 (6.27)
Alkalinity (mgl^{-1})	173.0-183.0 (178.26)	174.0-184.0 (178.54)	173.0-185.0 (178.96)	175.0-182.0 (179.72)	174.0-184.0 (179.38)	142.0-153.0 (147.37)	143.0-154.0 (148.54)	143.0-155.0 (147.68)	144.0-152.0 (149.74)	143.0-154.0 (149.97)
Hardness (mgl^{-1})	273.0-277.0 (275.37)	273.0-278.0 (276.37)	273.0-278.0 (276.94)	275.0-279.0 (277.86)	274.0-279.0 (276.86)	285.0-296.0 (292.21)	285.0-297.0 (292.48)	284.0-297.0 (293.76)	283.0-298.0 (294.54)	284.0-298.0 (294.39)
Ammonia(mgl^{-1})	0.12-0.23 (0.15)	0.13-0.33 (0.24)	0.17-0.34 (0.27)	0.19-0.36 (0.28)	0.14-0.34 (0.26)	0.15-0.34 (0.27)	0.22-0.34 (0.29)	0.21-0.36 (0.30)	0.21-0.36 (0.32)	0.18-0.32 (0.28)

Note: T_0=Normal feed, T_1=High energy feed, T_2=High energy feed+Immunostimulant, T_3=High energy feed+Gut probiotic T_4=High energy feed+Gut prebiotic

Table 25: Variation in length (cm) and weight (g) of *L. rohita* during feed manipulation trial as an adaptation strategy against salinity stress

Salinity	Treatment		1st day	1st week	2nd week	3rd week	4th week	5th week	6th week	BWG%	SGR (% day^{-1})
5 ppt	T_0	L	8.17±0.06	8.27±0.06	8.43±0.12	8.60±0.10	8.90±0.10	9.17±0.06	9.40±0.10		
		W	8.24±0.02	8.29±0.01	10.24±0.04	10.31±0.07	13.07±0.02	14.02±0.02	14.18±0.01	72.12±0.62^{E}	1.29±0.01^{E}
	T_1	L	8.23±0.12	8.30±0.10	8.43±0.12	8.63±0.12	8.77±0.06	9.07±0.15	9.20±0.10		
		W	8.25±0.03	9.08±0.02	11.49±0.03	12.64±0.52	14.59±0.09	16.53±0.02	17.21±0.02	108.52±0.95^{C}	1.75±0.01^{C}
	T_2	L	7.93±0.15	8.20±0.10	8.47±0.11	8.67±0.12	8.80±0.10	9.10±0.20	9.30±0.10		
		W	8.24±0.04	9.34±0.05	11.72±0.03	12.26±0.01	14.94±0.02	16.73±0.02	17.72±0.03	114.92±0.87^{A}	1.82±0.01^{A}
	T_3	L	8.23±0.12	8.40±0.10	8.57±0.06	8.73±0.06	9.00±0.10	9.17±0.06	9.30±0.10		
		W	8.24±0.03	9.23±0.03	11.51±0.02	12.14±0.03	14.66±0.24	16.61±0.02	17.54±0.03	112.74±0.99^{AB}	1.80±0.01^{AB}
	T_4	L	8.23±0.05	8.40±0.10	8.57±0.15	8.67±0.15	8.97±0.21	9.23±0.06	9.40±0.10		
		W	8.21±0.05	9.17±0.12	11.34±0.04	12.16±0.03	14.45±0.02	16.54±0.02	17.34±0.02	111.16±1.38^{BC}	1.78±0.02^{BC}
10 ppt	T_0	L	8.37±0.12	8.46±0.05	8.67±0.06	8.80±0.10	9.00±0.10	9.17±0.06	9.40±0.10		
		W	8.18±0.02	8.25±0.04	10.03±0.02	11.10±0.02	12.06±0.09	12.74±0.08	13.15±0.07	60.72±1.19^{F}	1.13±0.01^{F}
	T_1	L	8.20±0.10	8.33±0.06	8.60±0.10	8.80±0.10	9.00±0.17	9.20±0.10	9.30±0.10		
		W	8.19±0.04	8.94±0.02	11.06±0.04	11.65±0.05	14.40±0.04	15.04±0.02	16.12±0.03	96.86±1.03^{D}	1.61±0.01^{D}
	T_2	L	7.67±0.21	7.97±0.21	8.27±0.21	8.50±0.20	8.70±0.20	8.83±0.12	9.20±0.10		
		W	8.28±0.03	9.05±0.04	11.37±0.02	11.95±0.02	14.83±0.02	15.20±0.07	16.31±0.02	96.90±0.36^{D}	1.61±0.01^{D}
	T_3	L	8.30±0.10	8.47±0.11	8.60±0.10	8.80±0.10	9.13±0.20	9.30±0.10	9.40±0.10		
		W	8.17±0.03	9.02±0.02	11.35±0.03	11.74±0.06	14.54±0.03	15.08±0.01	16.24±0.02	98.74±0.57^{D}	1.63±0.01^{D}
	T_4	L	8.23±0.06	8.37±0.06	8.60±0.10	8.77±0.12	8.97±0.12	9.27±0.15	9.37±0.06		
		W	8.12±0.03	8.99±0.05	11.23±0.03	11.71±0.04	14.51±0.02	15.07±0.02	16.18±0.01	99.26±0.80^{D}	1.64±0.01^{D}

Note: 1. T_0=Normal feed, T_1=High energy feed, T_2=High energy feed+Immunostimulant, T_3=High energy feed+Gut probiotic, T_4=High energy feed+Gut prebiotic

2. Values are average±SD, L=Length and W=Weight

3. Values (BWG & SGR) with different subscript indicates significant difference ($p>0.05$)

At 10 ppt, the lowest SGR ($p<0.05$) was obtained at T_0 (1.13±0.01) than other treatment and the highest SGR was obtained in T_4 treatment (1.64±0.01) followed by T_3 (1.63±0.01), T_2 (1.61±0.02), T_1 (1.61±0.01). No significant (Fig 16) variation was found in SGR ($p>0.05$) between T_1-T2, T_1-T_3, and T_1-T_4. Similar trend was noticed for BWG% also. At 10 ppt, significantly lowest BWG % ($p<0.05$) was obtained at T_0 (60.72±1.19) than other treatment and the highest BWG% was obtained in T_4 treatment (99.26±0.80) followed by T_3 (98.74±0.57), T_2 (96.90±0.36), T_1 (96.86±1.03). There was no significant (Fig 15) variation in BWG% ($p>0.05$) between T_1-T2, T_1-T_3, and T_1-T_4.

The water quality parameters analysed in the experiment for the species *L. rohita* were water temperature, pH, DO, alkalinity, hardness and ammonia. The variation water quality parameters during the experiment trial are presented on Table 24. In this experiment at 5 ppt salinity temperature ranged between 24.1 and 25.7°C. The pH varied 7.8 to 8.5. The minimum and maximum DO of the experiment were 5.6 and 6.6 mg l^{-1}. The lowest and highest alkalinity of the experiment was 173.0 mg l^{-1} and 185.0 mg l^{-1} respectively. The hardness ranged between 273.0 and 279.0 mg l^{-1}. The ammonia varied from 0.12 to 0.36 mg l^{-1} at 5 ppt salinity.

In the experiment at 10 ppt salinity, temperature ranged between 24.4 to 25.4°C. The maximum pH of the experiment was 8.5 and the minimum was 7.8. The DO of the experiment were ranged from 5.5 to 6.9 mg l^{-1}. The alkalinity levels fluctuated between 182.0 and 195.0 mg l^{-1}. The average hardness at T_0, T_1, T_2, T_3, and T_4 treatment were 292.21, 292.48, 293.76, 294.54 and 294.39 mg l^{-1} respectively. The ammonia level ranged from 0.15 to 0.36 mg l^{-1} at 5 ppt salinity.

Cyprinus carpio

The data obtained growth parameters like initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%)

of *Cyprinus carpio* recorded in different feed regime viz., T_0, T_1, T_2, and T_3 at 5 and 10 ppt salinity level were presented at Table 27. The growth parameters like SGR (One-way ANOVA: $F_{7, 16}$=124.76; P=0.0001) and BWG% (One-way ANOVA: $F_{7, 16}$=134.4143; P=0.0001) varied significantly in all treatments.

At 5 ppt, significantly the highest SGR was obtained in T_2 treatment (1.18±0.02) followed by T_3 (1.11±0.02), T_1 (0.89±0.02) and the lowest SGR ($p<0.05$) was obtained at T_0 (0.68±0.02). No significant variation found in SGR ($p>0.05$) was obtained between T_2 and T_3 treatment. However, between T_0-T_1, T_0-T_2, T_0-T_3, T_2-$T_1$and T_3-T_1 significant variation in SGR ($p<0.05$) was observed. Similar trend was noticed in case of BWG% also. At 5 ppt salinity, significantly the lowest BWG% ($p<0.05$) was obtained at T_0 (32.85±1.14) than other treatment and the highest BWG% was obtained in T_2 treatment (64.02±1.10) followed by T_3 (59.36±1.25), and T_1 (45.26±1.13). There was no significant variation in BWG% ($p>0.05$) was obtained between T2 and T3 treatment. However, significant variation in BWG% ($p<0.05$) was observed between T_0-T_1, T_0-T_2, T_0-T_3, T_2-T_1 and T_3-T_1.

At 10 ppt, significantly the lowest SGR ($p<0.05$) was obtained at T_0 (0.40±0.07) than other treatment and the highest SGR was obtained in T_3 treatment (0.90±0.01) followed by T_2 (0.89±0.07), and T_1 (0.81±0.03). There was no significant variation in SGR ($p>0.05$) were obtained between T_1-T_3, T_2-T_3, and T_1-T_2. However, significant variation (Fig 18) in SGR ($p<0.05$) was observed between T_0-T_1, T_0-T_3, and T_0-T_2. BWG% also showed similar trend. At 10 ppt, significantly the highest BWG% was obtained in T_3 treatment (45.86±0.54) followed by T_2 (45.40±4.13), T_1 (40.58±1.57) and

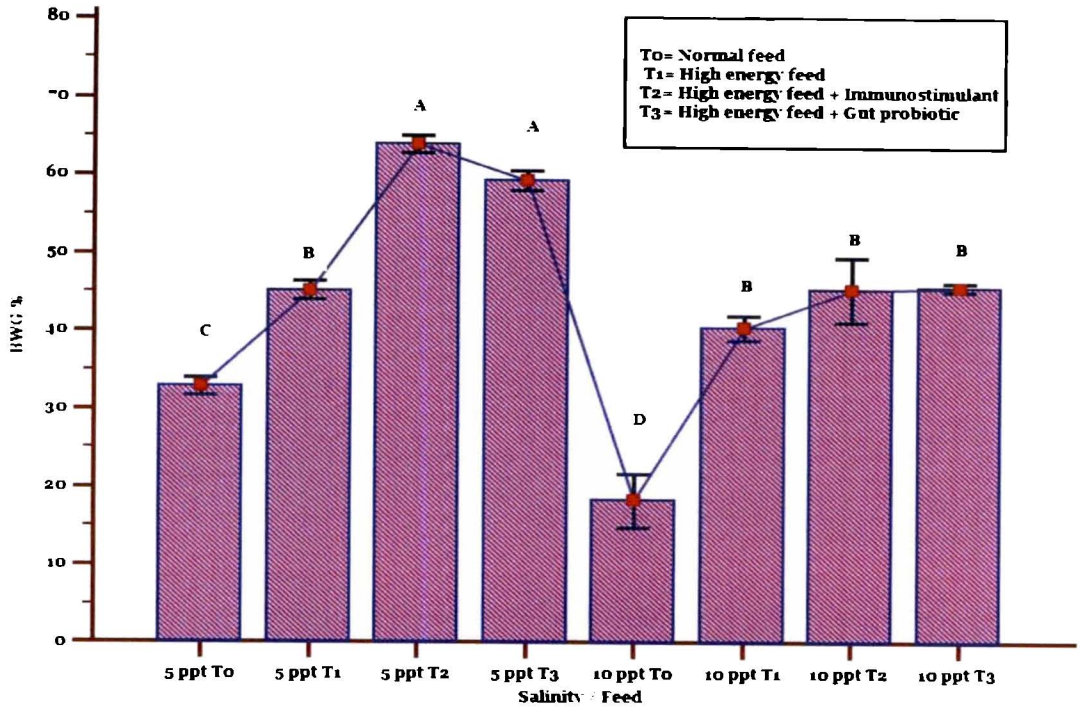


Fig 17: Body weight gain percentage (BWG %) of *Cyprinus carpio* exposed to different salinity level in feed manipulations an adaptation strategy trial

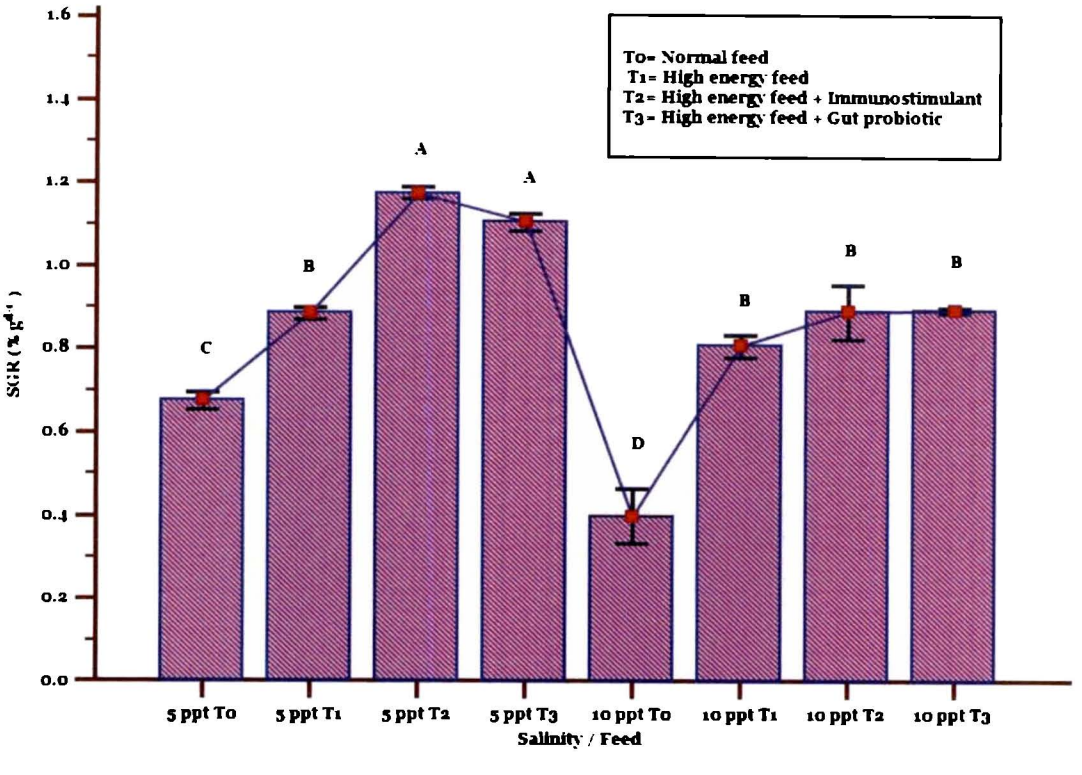


Fig 18: Specific Growth rate (SGR % day^{-1}) of *Cyprinus carpio* exposed to different salinity level in feed manipulations as an adaptation strategy trial

Table 26: Water quality during feed manipulation as an adaptation strategy against increased salinity in *Cyprinus carpio*

Salinity	5 ppt				10 ppt			
Water quality	**T_0**	**T_1**	**T_2**	**T_3**	**T_0**	**T_1**	**T_2**	**T_3**
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (°C)	21.3-21.8 (21.62)	21.4-21.8 (21.68)	21.2-21.7 (21.48)	21.1-21.7 (21.52)	21.4-22.4 (21.84)	21.7-22.6 22.12	21.6-22.7 (22.24)	21.7-22.8 (22.38)
pH	8.2-8.4 (8.26)	8.2-8.5 (8.32)	8.2-8.5 (8.32)	8.2-8.6 (8.31)	8.2-8.5 (8.31)	8.2-8.5 (8.28)	8.2-8.4 (8.26)	8.2-8.5 (8.27)
DO (mgl^{-1})	5.4-5.9 (5.69)	5.5-5.9 (5.64)	5.4-5.8 (5.54)	5.4-5.7 (5.55)	5.4-5.6 (5.53)	5.3-5.8 (5.52)	5.4-5.8 (5.54)	5.4-5.8 (5.55)
Alkalinity (mgl^{-1})	176.0-183.0 (178.28)	176.0-183.0 (178.76)	175.0-182.0 (178.63)	174.0-184.0 (179.56)	136.0-146.0 (141.76)	137.0-145.0 (141.54)	137.0-147.0 (142.48)	136.0-147.0 (141.68)
Hardness (mgl^{-1})	261.0-269.0 (265.54)	262.0-268.0 (266.43)	261.0-268.0 (265.38)	262.0-269.0 (266.67)	284.0-294.0 (287.21)	283.0-294.0 (289..27)	282.0-293.0 (288..49)	283.0-292.0 (287.76)
Ammonia (mgl^{-1})	0.34-0.38 (0.36)	0.34-0.38 (0.36)	0.33-0.38 (0.35)	0.34-0.37 (0.35)	0.33-0.38 (0.35)	0.36-0.38 (0.36)	0.34-0.38 (0.36)	0.32-0.38 (0.35)

Note: T_0=Normal feed, T_1=High energy feed, T_2=High energy feed+Immunostimulant, T_3=High energy feed+Gut probiotic

Table 27: Variation in length (cm) and weight (g) of *C. carpio* during feed manipulation trial as an adaptation strategy against salinity stress

Salinity	Treatment		1st day	1st week	2nd week	3rd week	4th week	5th week	6th week	BWG%	SGR (% day^{-1})
5 ppt	T_0	L	6.23±0.57	6.70±0.10	6.87±0.06	7.00±0.17	7.07±0.15	7.23±0.15	7.27±0.12		
		W	3.38±0.03	3.65±0.03	3.69±0.03	4.02±0.01	4.12±0.01	4.38±0.02	4.49±0.01	32.85±1.14^{C}	0.68±0.02^{C}
	T_1	L	6.37±0.15	6.47±0.15	6.67±0.06	6.73±0.05	6.83±0.06	7.07±0.06	7.20±0.10		
		W	3.26±0.09	3.46±0.08	3.67±0.07	3.93±0.10	4.18±0.02	4.40±0.04	4.73±0.10	45.26±1.13^{B}	9.89±0.02^{B}
	T_2	L	6.83±0.25	6.90±0.26	6.63±0.20	6.73±0.21	6.77±0.15	7.10±0.20	7.20±0.10		
		W	2.85±0.03	3.62±0.03	3.98±0.01	4.21±0.02	4.26±0.01	4.54±0.05	4.68±0.01	64.02±1.10^{A}	1.18±0.02^{A}
	T_3	L	6.17±0.15	6.23±0.12	6.57±0.25	6.63±0.21	6.73±0.21	7.10±0.17	7.20±0.10		
		W	2.90±0.03	3.62±0.03	3.76±0.14	4.13±0.02	4.23±0.04	4.42±0.01	4.63±0.02	59.36±1.25^{A}	1.11±0.02^{A}
10 ppt	T_0	L	6.43±0.15	6.50±0.10	6.60±0.10	6.63±0.15	6.70±0.10	7.13±0.21	7.27±0.15		
		W	3.91±0.12	4.06±0.30	4.07±0.04	4.09±0.04	4.11±0.03	4.42±0.02	4.62±0.01	18.26±3.41^{D}	0.40±0.07^{C}
	T_1	L	6.67±0.15	6.77±0.15	6.93±0.12	6.97±0.06	7.03±0.06	7.17±0.06	7.23±0.12		
		W	3.94±0.19	4.26±0.18	4.60±0.15	4.85±0.12	5.16±0.09	5.35±0.19	5.54±0.21	40.58±1.57^{B}	0.81±0.03^{B}
	T_2	L	6.23±0.15	6.33±0.15	6.43±0.15	6.47±0.12	6.53±0.15	7.00±0.30	7.10±0.20		
		W	4.02±0.10	4.54±0.14	4.97±0.02	5.23±0.03	5.39±0.02	5.68±0.01	5.85±0.02	45.40±4.13^{B}	0.89±0.07^{B}
	T_3	L	6.23±0.15	6.30±0.10	6.47±0.12	6.50±0.17	6.57±0.21	6.87±0.06	7.10±0.10		
		W	3.99±0.01	4.47±0.02	4.69±0.02	5.07±0.02	5.23±0.03	5.62±0.01	5.82±0.01	45.86±0.54^{B}	0.90±0.01^{B}

Note: 1. T_0=Normal feed, T_1=High energy feed, T_2=High energy feed+Immunostimulant, T_3=High energy feed+Gut probiotic

2. Values are average±SD, L=Length and W=Weight

3. Values (BWG & SGR) with different subscript indicates significant difference ($p>0.05$)

T_0 (0.40±0.07). There were no significant variation in BWG% ($p>0.05$) obtained between T_1-T_3, T_2-T_3, and T_1-T_2. However, significant (Fig 17) variation in BWG% ($p<0.05$) were observed between T_0-T_1, and T_0-T_3.

In the experiment at 5 ppt salinity the minimum and maximum temperature were 21.1 and 21.8°C respectively. The highest pH of the experiment was 8.6 and the lowest was 8.2. The average pH of the experiment trial were found 8.26, 8.32, 8.32 and 8.31 at T_0, T_1, T_2, and T_3 respectively. The DO of the experiment ranges between 5.4 and 5.9 mg l^{-1}. The alkalinity of the experiment varied between 174.00 and 184.0 mg l^{-1}. The hardness were ranged between 261.0 and 269.0 mg l^{-1}. The ammonia varied from 0.34 and 0.38 mg l^{-1} for the species *Cyprinus carpio* in the experiment trial.

In this experiment at 10 ppt salinity the highest temperature was found 21.4°C and the lowest was 22.8°C. The pH of the experiment varied between 8.2 and 8.5 at in the experiment. The DO of the experiment ranges from 5.3 to 5.8 mg l^{-1}. The lowest value of alkalinity of the experiment was 186.0 mg l^{-1} and highest was 197.0 mg l^{-1}. The hardness was found on the range between 282.0 and 294.0 mg l^{-1}. The ammonia varied between 0.32 and 0.38 mg l^{-1} at the salinity10 ppt in the experiment trial. The water quality parameters of the species in the experiment trial has presented on Table 26.

Oreochromis mossambicus

The data pertaining of various growth parameters like initial and final body weight, specific growth rate (SGR % day^{-1}) and percentage body weight gain (BWG%) of *Oreochromis mossambicus* recorded in different feed regime viz., T_0, T_1, T_2, and T_3 at 10 and 15 ppt salinity level were presented at Table 29. In all treatments, growth parameters like SGR (One-way ANOVA: $F_{7, 16}$ =160.28; P=0.0001) and BWG% (One-way ANOVA: $F_{7, 16}$=140.873; P=0.0001) varied significantly.

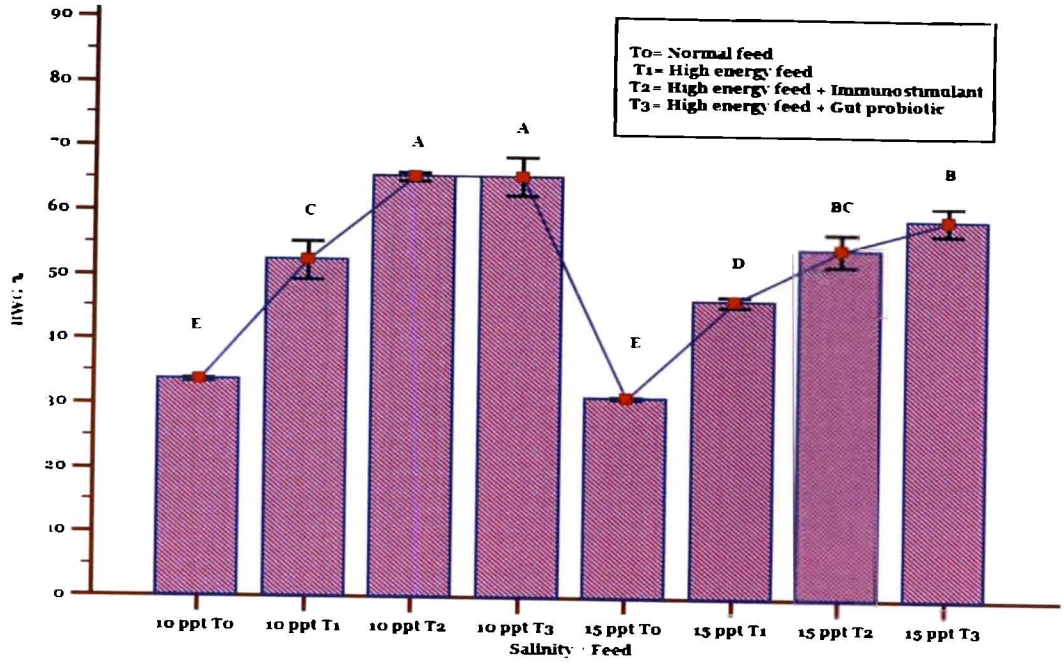


Fig 19: Body weight gain percentage (BWG%) of *Oreochromis mossambicus* exposed to different salinity level in feed manipulations an adaptation strategy trial

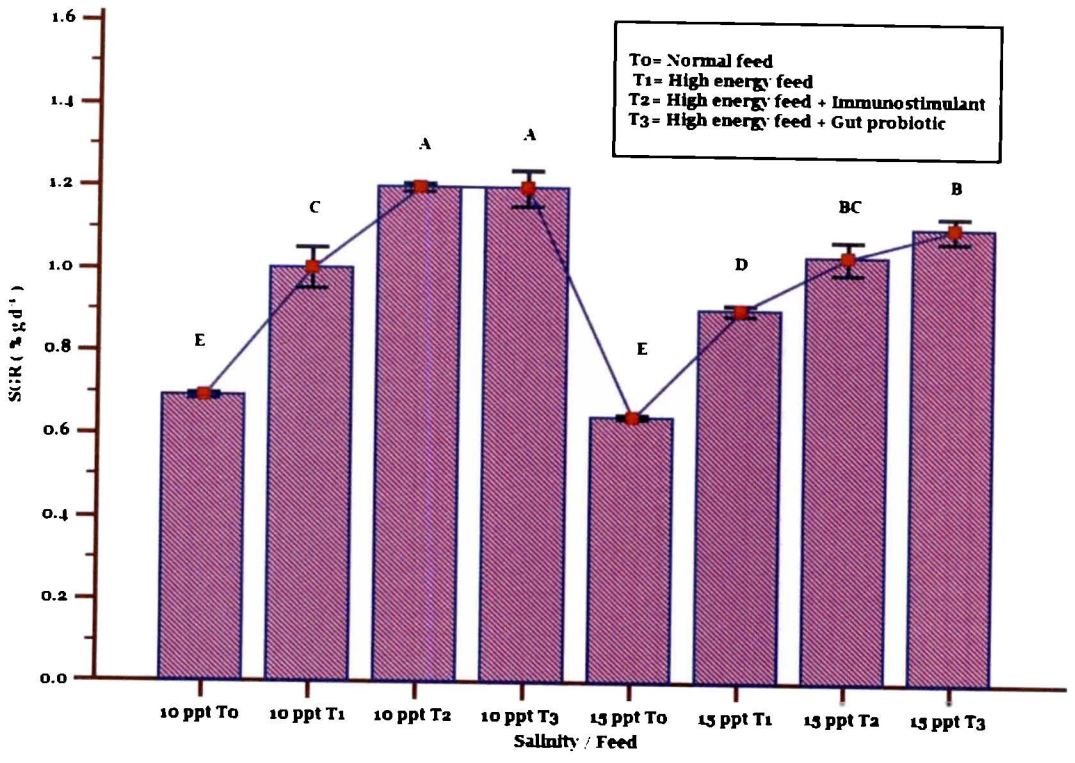


Fig 20: Specific Growth rate (SGR % day^{-1}) of *Oreochromis mossambicus* exposed to different salinity level in feed manipulations as an adaptation strategy trial

Table 28: Water quality during feed manipulation as an adaptation strategy against increased salinity in *Oreochromis mossambicuss*

Salinity	**10 ppt**				**15 ppt**			
Water quality	**T_0**	**T_1**	**T_2**	**T_3**	**T_0**	**T_1**	**T_2**	**T_3**
	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)	Min-Max (Average)
Temperature (°C)	18.1-18.6 (18.48)	18.2-18.7 (18.52)	18.2-18.8 (18.54)	18.3-18.7 (18.56)	18.3-18.8 (18.54)	18.2-18.7 (18.54)	18.1-18.7 (18.53)	18.3-18.9 (18.57)
pH	8.1-8.4 (8.25)	8.2-8.6 (8.39)	8.3-8.5 (8.37)	8.3-8.6 (8.42)	8.1-8.4 (8.29)	8.2-8.5 (8.36)	8.3-8.6 (8.42)	8.3-8.5 (8.38)
DO (mgl^{-1})	5.3-6.3 (5.62)	5.5-6.4 (5.79)	5.5-6.3 (5.85)	5.4-6.3 (5.77)	5.4-6.0 (5.70)	5.4-6.3 (5.68)	5.3-6.3 (5.73)	5.4-5.9 (5.68)
Alkalinity (mgl^{-1})	164.0-172.0 (167.17)	164.0-173.0 (168.21	163.0-174.0 (167.57)	162.0-174.0 (168.38)	144.0-157.0 (152.47)	146.0-158.0 (153.76)	145.0-158.0 (152.73)	146.0-159.0 (154.84)
Hardness (mgl^{-1})	274.0-283.0 (278.54)	274.0-282.0 (279.72)	272.0-283.0 (278.34)	273.0-284.0 (281.56)	286.0-297.0 (292.46)	286.0-298.0 (291.53)	287.0-297.0 (291.37)	287.0-296.0 (294.21)
Ammonia (mgl^{-1})	0.09-0.34 (0.20)	0.07-0.35 (0.23)	0.13-0.34 (0.24)	0.13-0.36 (0.26)	0.13-0.34 (0.25)	0.15-0.33 (0.27)	0.17-0.33 (0.27)	0.16-0.34 (0.26)

Note: T_0=Normal feed, T_1=High energy feed, T_2=High energy feed+Immunostimulant, T_3=High energy feed+Gut probiotic

Table 29: Variation in length (cm) and weight (g) of *O. mossambicus* during feed manipulation trial as an adaptation strategy against salinity stress

Salinity	Treatment		1st day	1st week	2nd week	3rd week	4th week	5th week	6th week	BWG%	SGR (% day^{-1})
10 ppt	T_0	L	6.90±0.36	7.31±0.69	7.34±0.72	7.38±0.71	7.41±0.72	7.44±0.69	7.47±0.68		
		W	6.01±0.03	6.70±0.28	6.89±0.12	7.03±0.17	7.66±0.12	7.91±0.02	8.03±0.02	33.68±0.2^{E}	0.69±0.01^{E}
	T_1	L	6.81±0.52	7.21±0.70	7.39±0.74	7.43±0.73	7.47±0.72	7.56±0.60	7.58±0.57		
		W	5.98±0.10	7.20±0.06	7.46±0.10	7.75±0.15	8.09±0.13	8.31±0.09	9.11±0.02	52.34±3.01^{C}	1.00±0.05^{C}
	T_2	L	7.13±0.45	7.31±0.47	7.62±0.34	7.72±0.22	7.85±0.06	7.90±0.03	7.94±0.02		
		W	6.21±0.02	8.15±0.20	8.91±0.09	9.05±0.17	9.94±0.05	10.02±0.03	10.27±0.02	65.52±0.62^{A}	1.20±0.01^{A}
	T_3	L	7.16±0.62	7.43±0.68	7.38±0.52	7.46±0.48	7.64±0.34	7.76±0.29	8.57±0.63		
		W	5.95±0.13	7.60±0.06	8.03±0.27	8.14±0.30	8.84±0.12	9.28±0.10	9.85±0.33	65.64±2.94^{A}	1.20±0.04^{A}
15 ppt	T_0	L	6.60±0.46	7.27±0.71	7.36±0.59	7.44±0.52	7.49±0.50	7.60±0.32	7.68±0.23		
		W	6.12±0.01	6.62±0.31	6.70±0.22	7.11±0.12	7.54±0.16	7.91±0.01	8.02±0.02	31.23±0.17^{E}	0.65±0.01^{E}
	T_1	L	7.37±0.04	7.57±0.05	7.69±0.02	7.74±0.02	7.84±0.13	7.79±0.03	7.91±0.10		
		W	6.13±0.04	7.47±0.12	7.87±0.02	8.07±0.15	8.30±0.02	8.68±0.16	8.97±0.02	46.52±0.82^{D}	0.91±0.01^{D}
	T_2	L	7.42±0.18	7.70±0.10	7.75±0.07	7.78±0.05	7.81±0.03	7.83±0.04	7.85±0.07		
		W	6.12±0.11	7.04±0.17	7.65±0.16	8.20±0.35	8.54±0.45	9.05±0.38	9.47±0.28	54.69±2.51^{BC}	1.04±0.04^{BC}
	T_3	L	6.77±0.45	7.68±0.23	7.77±0.13	7.81±0.06	7.82±0.04	7.84±0.01	7.87±0.03		
		W	6.00±0.05	7.05±0.18	7.78±0.05	8.22±0.06	8.94±0.11	9.15±0.27	9.56±0.06	59.31±2.16^{B}	1.1±0.03^{B}

Note: 1. T_0=Normal feed, T_1=High energy feed, T_2=High energy feed+Immunostimulant, T_3=High energy feed+Gut probiotic, T_4=High energy feed+Gut prebiotic

2. Values are average±SD. L=Length and W=Weight

3. Values (BWG & SGR) with different subscript indicates significant difference ($p>0.05$)

At 10 ppt salinity, significantly the highest SGR ($p<0.05$) was obtained in T_3 treatment (1.20±0.04) followed by T_2 (1.20±0.01), T_1 (1.00±0.05) and the lowest SGR was obtained at T_0 (0.69±0.001) than other treatment. There was no significant variation in SGR ($p>0.05$) was obtained between T_2-T_3 treatment. However, significant variation in SGR $p<0.05$) was observed between T_0 -T_1, T_0-T_2, T_0-T_3, T_1-T_3, and T_2-T1. Similar trend was noticed for BWG% also. At 10 ppt, significantly the lowest BWG% ($p<0.05$) was obtained at T_0 (33.68 ±0.23) than other treatment and the highest BWG% was obtained in T_3 treatment (65.64±2.94) followed by T_2 (65.52±0.62), and T_1 (52.34±3.01). There was no significant variation in BWG% ($p>0.05$) was obtained between T_2-T_3 treatment. However, significant variation in BWG% ($p<0.05$) was observed between T_0-T_1, T_0-T_2, T_0-T_3, T_1-T_3, and T_2-T_1.

At 15 ppt, significantly the lowest SGR ($p<0.05$) was obtained at T_0 (0.65±0.01) than other treatment and the highest SGR was obtained in T_3 treatment (1.11±0.03) followed by T_2 (1.04±0.04), and T_1 (0.91±0.01). There was no significant variation in SGR ($p>0.05$) was obtained between T_2-T_3 and T_2-T_1 treatment. However, significant variation in SGR ($p<0.05$) was observed between T_0-T_1, T_0-T_2, T_0-T_3, T_1-T_3, and T_3-T_0. Similar trend was noticed for BWG % also. At 15 ppt, significantly lowest BWG% ($p<0.05$) was obtained at T_0 (31.23 ± 0.17) than other treatment and highest BWG% was obtained in T_3 treatment (59.31±2.16) followed by T_2 (54.69±2.51), and T_1 (46.52±0.82). There was no significant (Fig 19) variation in BWG% ($p>0.05$) was obtained between T_2-T_3 treatment. However, significant variation in BWG% ($p<0.05$) were observed between T_0-T_1, T_0-T_2, T_0-T_3, T_1-T_3, T_2-T_1 and T_3-T_0.

The water quality parameters like water temperature, pH, DO, alkalinity, hardness and ammonia has presented in the Table 28. In the experiment at 10 ppt salinity the minimum and maximum temperature were 18.1 and 18.8°C respectively. The pH varied from 8.6 to 8.1 and the DO ranged between 5.3 and 6.4 mg l^{-1} in the experiment. The minimum and maximum alkalinity of the experiment were 162.00 and 174.0 mg l^{-1}. The hardness were varied between

272.0 and 284.0 mg l^{-1}. The ammonia ranged between 0.07 and 0.36 mg l^{-1} at 10 ppt salinity for the species *Oreochromis mossambicus* in the experiment trial.

In this experiment at 15 ppt salinity temperature ranged between 18.1 and 18.9°C. The pH of the experiment varied between 8.1 and 8.6 at in this experiment. The minimum and maximum DO of the experiment were 5.3 and 6.3 mg l^{-1}. The lowest alkalinity of the experiment was 174.0 mg l^{-1} and highest was 189.0 mg l^{-1}. The hardness ranges between 286.0 and 298.0 mg l^{-1} and ammonia ranged from 0.13 to 0.34 mg l^{-1} in the experiment trial.

DISCUSSION

Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production

5. Discussion

Fish are, to a large extent, dependent on ecological factors, which control many activities or functions, including growth. According to Boeuf (1999) growth is continuous process and it depends on external environmental factors. Among the ecological factor salinity is one of the important factor, very specific to aquatic environment (Boef and Payan 2001). In recent years due to the impact of climate change, leading to sea level rise, coupled with extreme events like cyclones and storm surge, have led to flooding of coastal freshwater aquaculture areas with saline water, causing severe detrimental effects on fresh water fish species.

5.1 Tolerance study

The freshwater fish species cultured in coastal areas may fall under stress and may not grow well because of increase in salinity due to influx of saline water during cyclone, storm surges and over taping of sea water. Therefore it is important to determine the threshold or tolerance levels of the fishes. During the present study several commonly cultured freshwater species were evaluated to find out their tolerance (median lethal salinity) levels towards increasing salinity. The results indicated that *Macrobrachum rosenbergii* could tolerate (MLS_{96h}) up to 24.45 ppt salinity followed by *Oreochromis mossambicus* (20.88), *Clarias batrachus* (12.37), *Cyprinus carpio* (12.18), *Puntius sarana* (12.17), *Pangasianodon hypothalamus* (11.97) and *Labeo rohita* (10.61 ppt). In the median lethal salinity (MLS_{96h}) tests average body weight of the fishes were 4.50, 5.07, 2.18, 1.18, 1.73, 13.42, and 0.45 g for the species *Macrobrachium rosenbergii, Oreochromis mossambicus, Clarias batrachus. Cyprinus carpio, Puntius sarana, Labeo rohita, , Pangasianodon hypothalamus* and respectively. The median lethal salinity-96 h (MLS_{96h}) is defined as the salinity at which survival fall to 50% at 96 hr following directly transfer from freshwater to the test salinities. According to Lemarie *et al.* (2004) when salinity increases, large number of chloride cells become activated, allowing fish to excrete Na^+ and Cl^- ions with high efficiency up to a salinity at which mortality appears. This salinity is probably the limit of

salinity tolerance. According to Avella and Douted (1996) and Sardella *et al.* (2004) beyond the tolerance level of salinity, the ion secretion probability become insufficient to maintain the ionic balance that increase the plasma sodium and chloride concentration leading to lethal elevation of blood osmolarity and or cellular necrosis which make fish unable to withstand extreme salinities.

Al-Amoudi (1987) also determined the MLS_{96h} in juveniles of *O. niloticus* and *O. mossambicus* and found 19.5 and 25.4 gl^{-1} respectively. Lemarie *et al.* (2004) calculated MLS value for *O. niloticus* as 21 gl^{-1}. However, Villegas (1990) observed mortality of *O. niloticus* in less than 24 hour at 15gl^{-1}. According to Fishelson (1980), Boeuf and Prunet (1985), Jonassen *et al.* (1997) and Morgon and Iwama (1998) the higher the salinity increment, the lower the MLS as there was likely insufficient time for synthesis of diverse enzymes (ATPases) and cellular structures responsible for the transfer of ions. Jamil (2004) did not observe any mortality at the salinity level of 0, 5, 10 and 15 ppt, while juveniles faced slight mortality at 20 ppt in the same environmental conditions. The result obtained in this study is agreement with the above findings. The results of the study carried out in the laboratory on the adaptability and tolerance of Tilapia fingerlings, *Oreochromis* sp. to different salinities by Hassan *et al.* (2013) indicated that all fish survived in 0 ppt and 5 ppt while 75% died in 20 ppt and 100% mortality was observed in 35 ppt. The mortality rate increased with increase of salinity. According to Hena (2005), *O. mossambicus* could tolerate higher salinity compared to *O. niloticus*. According to them *O. mossambicus* may be a better option for culture at salinity of 18 to 20 ppt. While evaluating the effect of salinity on growth and survival of *L. rohita,* Pillai *et al.* (2003) commented that freshwater region in coastal belt come under the saline water influence posing a threat to culture of freshwater species. During their experiment, *L. rohita* could survive up to 8 ppt salinity and 100% mortality was observed at 14 ppt salinity within 7 to 8 days. During our study the MLS_{96h} value for *L. rohita* was found to be 10.61 ppt. Saha *et al.* (1964) reported a salinity tolerance limit of 14 ppt for the Indian major carps. Ghosh *et al.* (1973) have reported that early and advance fry of Indian major carps of 26 mm length died at salinities of 10 and 13 ppt respectively, while

fingerlings of Indian major carps tolerate salinity up to 12.5 ppt with 48% mortality. The lethal limit of salinity tolerated by common carp was found to be 12.6 ppt (Ghosh and Pandit 1976). According to Hassan *et al.* (2013) direct transfer of Tilapia from fresh water to higher salinity causes the strong respiratory stress. Transfer of fish from freshwater to seawater normally lead to increase in osmotic concentration of blood serum and change ionic contents (Gordon 1959, Miles and Smith 1968). Wang *et al.* (1997) reported that *Cyprinus carpio* can survive at 12.5 ppt for 8 days and at 14.5 ppt for five days. The result obtained in our study is similar to the above findings. Sahoo *et al.* (2003) observed severe mortality beyond 6 ppt after 2 to 23 days of rearing in case of *Clarias batrachus.* Sarma *et al.* (2012) assessed the effects of salinity on growth and biochemical composition of freshwater catfish *Clarias batrachus.* In their study they conducted a static nonrenewable acute bioassay test. The LC_{50} of salinity for 96-h exposure to the fingerling (14.5 cm) was 12.52 ppt. This result is in agreement with the present finding. Pillai *et al.* (2003) studied about the effect of salinity on growth and survival of rohu, *Labeo rohita* under laboratory and field conditions. They found that at 14 ppt, *Labeo rohita* mortality started from the first day and within 7-8 days, 100% mortality was noticed. Similar trend is observed during the present study also.

5.2 Natural adaptation capacity

In order to find out optimum salinity level at which fish can survive and grow, it is desirable to understand the natural adaptability capacity of freshwater fishes towards increased salinity. During this trial five freshwater species were allowed to grow in different salinities (selected based on tolerance limit) for four weeks during which their natural adaptability capacity in terms of growth was observed. *O. mossambicus* not only survived but grew satisfactorily up to 15 ppt salinity. The BWG and SGR at 15 ppt were 18.74±2.49% and 0.61±0.07 % day^{-1} respectively. The BWG at 0, 5, 10 and 18 ppt were 56.14±2.30, 34.17±2.76, 25.54±2.22, 18.74±2.49 and 11.43±1.40% respectively. A review of the work by Suresh and Lin (1992) indicated that most species grow optimally between 5 and

18 psu. According to Lemarie *et al.* (2004) tolerance to salt water is negatively co-related with growth. Tilapias are euryhaline fish and can live and thrive in a wide range of salinity from fresh water to full seawater (Philipart and Ruwet 1982). Villegas (1990) showed that *O. niloticus* grew best at 0 to 10 ppt and slower at 25 to 32 ppt with optimum growth and survival at 7.5 ppt. Payne and Collinson (1983) reported that the salinity range for better growth of *O. niloticus* was 5 to 10 ppt whereas *O. mossambicus* performed best at intermediate (15 ppt) salinities. This is in agreement with the present findings. Hena *et al.* (2005) found that *O. mossambicus* can grow well at 22.5 ppt. The sudden salinity change may impact the physiological condition of the fish and the tolerance limits of the fish will cause stress and lead to decrease in the immune system level (Hassan, 2013).

In case of *C. carpio,* the growth at 5 ppt (BWG%=14.67±0.81) was similar (non significant difference) to the growth observed at 0 ppt (BWG%=16.89±1.58). This indicates that fish is able to adapt up to 5 ppt salinity. However there was sharp decline in BWG (8.42±1.13%) at 10 ppt. The SGR of the species were 0.56±0.05, 0.49±0.03 and 0.29±0.04 at 0, 5, and 10 ppt respectively. The growth rate of the species was the highest in freshwater and decreased with an increase in salinity. At 10.5 ppt, the fingerlings had poor growth and became emaciated (Wang *et al.* 1997). The above findings are similar to the results obtained during present investigation.

In case of *L. rohita* the BWG% at 5 and 10 ppt were 25.90±1.48 and 21.93±5.95% respectively. The SGR of *L. rohita* at 5 and 10 ppt were 0.82±0.04 and 0.71±0.16 % day^{-1} respectively. In case of *P. sarana* the BWG at 5 and 10 ppt were 1.86±0.22 and 1.50±0.14% respectively. The SGR of *P. sarana* at 5 and 10 ppt were 0.07±0.01 and 0.05±0.01 % day^{-1} respectively. The BWG% of *M. rosenbergii* were 52.51±0.18, 17.70±0.24, 7.00±0.25 and 4.33±0.54% at 5, 10, 15 and 20 ppt respectively. The SGR of the species were 1.31±0.03, 1.51±0.01, 0.58±0.01, 0.24±0.01 and 0.15±0.02%day^{-1} at 0, 5, 10, 15 and 20 ppt respectively. The above results indicate that though the growth at higher salinities are comparatively low in the freshwater but fishes are able to adapt to higher salinities

like *P. sarana, L. rohita, C. carpio* upto 10 ppt and *O. mossambicus* and *M. rosenbergii* up to 18 and 20 ppt respectively . According to Pillai *et al.* (2003), maximum growth of *L. rohita* was obtained at 0 and 2 ppt and the growth was not markedly affected up to 6 ppt. Our result also shows similar trend and is in agreement with the above findings. Nair *et al.* (1997) studied about the effects of salinity on the survival and growth of the laboratory reared larvae of *M. rosenbergii*. They reported that within the favorable range of 10 to 20 ppt a sudden increase in salinity does not have any adverse effect on the survival and growth of larvae. Salinities higher than 20 ppt were not found suitable. In the present study similar trend has been found. Singh (1980) demonstrated that prawns were able to grow in salinity up to 17 gl^{-1} with the highest growth achieved at salinity between 0 and 2 gl^{-1}. Smith *et al.* (1982) studied the growth of *M. rosenbergii* and found little difference in growth up to 10 gl^{-1}. Goodwin and Hanson (1975) reported that juveniles of *M. rosenbergii* grew more rapidly in fresh water or slightly brackish water (<5 ppt) when compared to more brackish water of up to 15 ppt. Shailender *et al.* (2012) studied about the effects of temperature and salinity on growth, hatching rate and survival of *M. rosenbergii* under captive condition. In their observation the highest total length and total weight were obtained at 30°C temperature and 6 ppt salinity. This finding is in accordance with our finding.

According to Peterson and Meador (1994) as salinity increases to the point where the blood become isotonic, growth may be reduced in species (relative to growth in freshwater). However the effect of salinity on growth of freshwater fish is not always negative.

Routray and Routray (1997) observed growth retardation beyond 6 ppt salinity in their study on growth potential of grass carp in saline water. Maceina and Shireman (1980) reported that dietary conversion rates of grass carp were less efficient at 3 and 6 ppt salinities than in freshwater. Hence, growth was reduced at lower salinities and greatly at higher salinities. Wang *et al.* (1997) reported that SGR were high in fresh water, reduced with increase in salinity, and reached a negative percentage at 10.5 ppt.

5.3 Adaptation strategies against salinity stress

5.3.1 Aeration as an adaptation strategy

According to Weiss and Botts (1957) the reduction in dissolved oxygen (DO) concentration reduces the oxygen uptake capacity and increases the toxicity of any solution. When there is increase in salt or any other toxicant concentration in water the rate of respiratory flow through the gills may be increased, leading to adverse impact on the survival and growth of the species. Thus, increase in oxygen concentration in water through aeration may help fish to overcome the adverse impact of enhanced salt concentration (salinity) in freshwater environment. Keeping this in mind different levels of aeration were tried as an adaptation strategy to reduce the adverse effect of increase in salinity towards freshwater species. The results of the trial indicated that fish were able to grow normally in the tanks subjected to aeration compared to the growth in tanks without aeration. In case of *Puntius sarana* the BWG at 0 ppt salinity were 39.03±0.57, 40.29±0.42 and 53.67±1.46% at T_0 (no aeration), T_1 (1h aeration) and T_2 (2 h aeration) treatments respectively. The SGR at T_0, T_1 and T_2 treatments were 0.78±0.01, 0.81±0.01 and 1.02±0.2 % day^{-1} respectively. At 5 ppt salinities the BWG were 25.88±0.26, 34.20±1.66 and 31.55±2.55% and SGR were 1.02±0.02, 0.55±0.01 and 0.70±0.03% day^{-1} at T_0, T_1 and T_2 treatment respectively. At 10 ppt salinity the BWG at T_0, T_1 and T_2 treatment were 6.98±1.91, 20.44±3.30 and 4.84±0.52% and the SGR were 0.16±0.04, 0.44±0.07 and 0.11±0.01% day^{-1} respectively. In case of *Cyprinus carpio* at 0 ppt salinity the BWG were 134.29±2.89, 162±1.80 and 194.92±2.71% at T_0, T_1 and T_2 treatment respectively. The SGR were 2.03±0.03, 2.30±0.02 and 2.58±0.02 % day^{-1} at T_0, T_1 and T_2 treatments respectively. At 5 ppt salinity the SGR were 1.75±0.02, 2.10±0.01 and 2.32±0.01% day^{-1}. The BWG% at the respective treatments were 108.44±1.38, 141.38±1.23 and 164.54±4.28%. At 10 ppt salinity the BWG were found to be 37.31±0.31, 106±1.16 and 103.06±2.01% and the SGR were 0.75±0.01, 1.73±0.01 and 1.69±0.02 % day^{-1} at T_0, T_1 and T_2 treatment respectively.

When salinity increases, the availability of dissolved oxygen decreases, and the buoyancy of fish is affected. Salinity also has a direct effect on gaseous exchange. Thus asphyxiation occurs in fishes placed in water of high salinity. The extra energy required for ion and osmoregulation in saline water may raise the rate of standard metabolism and thereby reduce the scope for activity (Holiday, 1971). Aeration may help to reduce this effect. The result of the present trial has to a certain extend proved this. So aeration can be considered to be used at the time of salinity stress so that the fish can survive and its adverse impact on growth can be minimized.

5.3.2 Feed manipulation as an adaptation strategy

During the culture period fish are predisposed to stress due to environmental change resulting in detrimental consequences to aquatic organisms (Reubush and Heath 1996). Abiotic stress like increase in temperature, changes in pH, increase in salinity etc have profound influence on growth and fish production. Worldwide the freshwater aquaculture systems are under stress due to influx of saline water during cyclone, storm surges and over taping of sea water. Thus there is need to develop certain strategies for the coastal fresh water aquaculture farmers to minimise the loss in growth and production. Keeping the above facts in mind the present trial was carried out during which high energy (HE) feed alone and high energy feed fortified with immunostimulant, probiotics and prebiotics were used to overcome the adverse impact of salinity stress and to maintain normal growth and production.

The trial was done in three fresh water fish for six weeks. Fish like *L. rohita* and *C. carpio* were subjected to two levels of salinity stress (5 and 10 ppt) whereas *O. mossambicus* was kept at 10 and 15 ppt salinity. In case of *L. rohita* at 5 ppt salinity in T_0 (control-normal feed), after six weeks of trial the specific growth rate (SGR) was 1.29±0.01% day^{-1}. At T_1 (High energy feed), T_2 (High energy feed with immunostimulant), T_3 (High energy feed with probiotic) and T_4 (High energy feed with prebiotic) specific growth rate (SGR) were 1.75±0.01, 1.82±0.01, 1.80±0.01

and 1.78±0.02 % day^{-1}. In case of 10 ppt salinity in control T_0 (Normal feed), T_1 (high energy feed), T_2 (High energy feed with immunostimulant), T_3 (High energy feed with probiotic), T_4 (High energy feed with prebiotic) the SGR were 1.13±0.01, 1.61±0.01, 1.61±0.01, 1.63±0.0 and 1.64±0.01 % day^{-1} respectively. The results indicate a clear cut positive significant ($p<0.05$) increase in specific growth rate of fish fed with high energy feed, high energy feed with immunostimulant, high energy feed with probiotic and high energy feed with prebiotic compared to the fishes fed with normal feed. Considering the results obtained from all the treatments and from all the three fish experimental set ups, further it can be said that high energy feed fortified with immunostimulant and probiotic had better impact than high energy feed alone. The SGR of *O. mossambicus* at 10 and 15 ppt, when fed with high energy feed fortified with immunostimulant and probiotics, were found to be 1.20 and 1.04 % day^{-1} respectively, which were significantly higher than the SGR 1.00 % day^{-1} (10 ppt) and 0.91 % day^{-1} (15 ppt) when feed with only high energy feed.

Yanbo and Zirong (2006) opined that, in the feeding experiments with probiotic supplemented and non-supplemented control diets, the diet supplemented with probiitics showed significantly better results of growth performance than those with basal diet (control). Probiotics highly increased the growth performance and digestive enzyme activities. Probiotic applications were shown to improve intestinal microbial balance, thus leading to improved food absorption (Parker 1974, Fuller 1989). Similar results of better growth by using probiotics have been reported by Wang (2007). Lin *et al.* (2012) reported that *C. carpio* fed with diets supplemented with combination of COS and *B. coagulans* has the highest final weight and specific growth rate. Increase in growth in aquatic animals fed with probiotics diets may be attributed to the improved digestive activity by enhancing the synthesis of vitamin and enzymatic activity (Liu *et al.* 2009; Ringo *et al.* 2010; Zimer and Gibron 1998) with a consequent improvement of digestibility and weight gain.

Wang (2007) reported that the higher level of enzyme activity obtained with diets containing probiotics improved the digestion of protein, starch, fat and cellulose, which might in tern explain the better growth observed with the probiotic supplemented diets. Similar effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet (Lara-Flores *et al.* 2003; Tovar-Ramigez *et al.* 2004).

The benefits of the supplement probiotics with feed are improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganism antimutagenic and anticarcinogenic activity, growth promoting factor and increase immune response (Mohanty *et al.* 1993; Sharma and Bhukhar, 2000; Verschuere *et al.* 2000; Spanggaard *et al,* 2001; Ziaei-Neiad *et al,* 2006; Wang *et al,* 2005; Wang and Xu 2006; Wang, 2007). Cruz (2012) reported that probiotics can improve the digestibility of nutrients and increase the tolerance to any stress.

Arulvasu *et al.* (2013) demonstrated that diet supplement with Z officinale powder (an immunostimulant) enhanced growth and immunity in *Catla catla.* Similar results were also obtained by Mathivanan *et al.* (2008). According to Pankhurst *et al.* (2008) stress directly reduces feeding activity presumably via its effect on appetite. This might be the region of significantly lower SGR values in case of all the three fish subjected to salinity stress. Stress conditions in all vertebrate including fish bring changes about metabolism and osmoregulation (Chatterjee *et al.* 2006; Wendelaar Bonga 1997). Thus metabolism shifts from anabolism to catabolism is required to supply the extra energy needed to combat stress (Pickering 1992). The outcome of this study also indicates that extra energy provided through high energy feed has resulted in overcoming the stress. The SGR values in T_0 (control/normal feed) for *L. rohita, C. carpio* and *O. mossambicus* at 10 ppt salinity were 1.13±0.01, 0.40±0.07 and 0.68±0.01% day^{-1} respectively where as it was 1.61±0.01, 0.81±0.03 and 1.00±0.05% day^{-1} respectively in case of stressed fishes fed with high energy feed (T_1). Tejpal *et al.* (2009) reported a significant weight gain % and increase in specific growth rate when stressed fish were fed with dietary supplementation of L-tryptophan.

According to Mustafa *et al.* (2013) stress caused by disturbance in normal aquaculture environment hampers growth of fish and makes them susceptible to diseases. They proposed that vitamin-C helps to reduce the stress response. According to Izquiado *et al.* (1989), feed manipulation of dietary composition has shown the effect of larval fish to resist several stressors, hence influencing the survival following stress.

According to Bonga (1997) and Barton (2002) stress cause hormonal and metabolic changes as well as growth, survival and immune depression among fishes. Kanazawa (1997) opined that nutritional manipulation is seen as a promising stress tolerance increment in fish. Prebiotics (Salze *et al.* 2008), vitamin E and C (Chen 2004) and some fatty acids (Kanazawa 1997) were found to enhance stress tolerance in fish. Ashraf *et al.* (2010) worked on the development of salinity stress for larval striped bass, *Morone saxatilis* and Inland silver sides *Menidia beryllina* and found salinity stressed striped bass showing better performance in growth and survival when fed on *artimia naupulii* HUBA (Webster and Lovel 1990). Larval summer flounder, *Paralichthys dentatus* fed on fatty acid enriched rotifers were better able to survive the salinity tolerance test (Willey *et al.* 2004). Jalali *et al.* (2008) observed better growth, survival and stress resistance in those beluga (*Huso huso*) larvae which were fed on HUFA and vitamin E enriched *Artemia umiana.*

The above results indicate that high energy feed and high energy feed fortified with either immunostimulant or probiotics can be prescribed by the farmers for initial 7 days during influx of saline water or salinity stress. Therefore, feeding manipulation can be one of the resilient strategies against flooding of saline water into fresh water culture areas either due to sea level rise or cyclone or storm surges which are the fall out of climate change.

SUMMARY

Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production

6. Summary

The present study was carried out with the objectives of finding strategy to be used during influx of saline water (salinity stress) into coastal freshwater aquaculture areas so that loss to farmers can be minimized. Keeping the above objectives in mind the present experiment was designed and conducted.

As per the results obtained on the medium lethal salinity (MLS) experiment, the MLS_{96h} for the species *Macrobrachium rosenbergii, Oreochromis mossambicuss, Clarias batrachus, Cyprinus carpio, Puntius sarana, Pangasianodon hypothalamus* and *Labeo rohita* were found out to be 24.45, 20.88, 12.37, 12.18, 12.17, 11.97, 10.61 ppt respectively.

Natural adaptation study was conducted for the species *Puntius sarana, Labeo rohita* and *Cyprinus carpio* at various salinities (0, 5, 10 ppt) for four weeks. The species, *Macrobrachium rosenbergii* (Giant Freshwater Prawn) was exposed to 0, 5, 10 and 15 ppt. Similar trials for *Oreochromis mossambicuss* at various salinities (0, 5, 10, 15,18 ppt) were also carried. It was found that *Puntius sarana, Labeo rohita* and *Cyprinus carpio* could adapt upto 5 ppt salinity level. *Macrobrachium rosenbergii* and *Oreochromis mossambicuss* were found to adapt up to 15 ppt salinity.

Aeration as an adaptation strategy against different salinities (0, 5 and 10 ppt) were carried out for *Cyprinus carpio* and *Puntius sarana.* The results of the trial indicated that aeration has positive impact and could reduce the adverse impact of increased salinity and hence can be recommended for use during salinity stress.

The experiment on feed manipulation as an adaptation strategy was carried out for the species, viz. *Labeo rohita, Cyprinus carpio* and *Oreochromis mossambicuss.* High energy feed alone and in combination with immunostimulant or probiotic or prebiotic separately were tried out as adaptation strategies against

salinity stress. High energy feed fortified with immunostimulant showed best growth ($p<0.05$) followed by probiotic and than prebiotic.

From the results obtained it can be summarized that *Macrobrachium rosenbergii* was found to be more tolerant followed by *Oreochromis mossambicuss, Clarias batrachus, Cyprinus carpio, Puntius sarana, Pangasianodon hypothalamus* and *Labeo rohita*. Among the different strategies tried the strategy like Feed manipulation and use of tolerant species can be the recommended options for farmers. The aeration as an strategy against saline water instruction has limited application ie up to 5 ppt salinity only.

CONCLUSION

Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production

7. Conclusion

In this study it was found that fresh water fish species can grow up to 10 ppt salinity but growth shall be less than normal.

From the study it can be concluded that the aeration can be effectively used as an adaptation strategy where the fishes are exposed to low salinity stress (up to 5 ppt). High energy feed fortified with immunostimulant showed best growth under salinity stress condition as compared to normal feed followed by high energy feed fortified with probiotic or prebiotic and high energy feed alone. Hence feed manipulation can also be considered as an adaptation strategy against salinity stress in aquaculture. However before recommending these to the farmers of costal area more field level trials at farmers farms may be conducted.

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ANNEXURE

Study on aquaculture strategies to cope with sudden increase in salinity for sustainable fish production

Table 1: One-way analysis of variance (ANOVA) table: SGR of *P. sarana* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	2	0.006	0.003	7.946	0.021
Error	6	0.002	0.000		
Corrected Total	8	0.008			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 2: One-way analysis of variance (ANOVA) table: BWG of *P. sarana* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	2	4.911	2.456	7.828	0.021
Error	6	1.882	0.314		
Corrected Total	8	6.793			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 3: One-way analysis of variance (ANOVA) table: SGR of *L. rohita* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	2	0.731	0.365	31.845	0.001
Error	6	0.069	0.011		
Corrected Total	8	0.799			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 4: One-way analysis of variance (ANOVA) table: BWG of *L. rohita* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	2	1027.493	513.746	35.016	0.0005
Error	6	88.031	14.672		
Corrected Total	8	1115.523			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 5: One-way analysis of variance (ANOVA) table: SGR of *C. carpio* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	2	0.117	0.058	40.124	0.0003
Error	6	0.009	0.001		
Corrected Total	8	0.126			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 6: One-way analysis of variance (ANOVA) table: BWG of *C. carpio* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	2	115.832	57.916	39.073	0.0004
Error	6	8.893	1.482		
Corrected Total	8	124.725			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 7: One-way analysis of variance (ANOVA) table: SGR of *O. mossambicus* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	2.560	0.640	162.284	< 0.0001
Error	10	0.039	0.004		
Corrected Total	14	2.599			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 8: One-way analysis of variance (ANOVA) table: BWG of *O. mossambicus* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	3566.699	891.675	171.319	< 0.0001
Error	10	52.048	5.205		
Corrected Total	14	3618.747			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 9: One-way analysis of variance (ANOVA) table: SGR of *M. rosenbergii* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	4.580	1.145	3519.136	< 0.0001
Error	10	0.003	0.000		
Corrected Total	14	4.584			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 10: One-way analysis of variance (ANOVA) table: BWG of *M. rosenbergii* of natural adaptability

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	5781.281	1445.320	3135.702	< 0.0001
Error	10	4.609	0.461		
Corrected Total	14	5785.890			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 11: One-way analysis of variance (ANOVA) table: SGR of *P. sarana* in salinity and aeration treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	8	2.170	0.271	251.307	< 0.0001
Error	18	0.019	0.001		
Corrected Total	26	2.190			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 12; One-way analysis of variance (ANOVA) table: BWG% of *P. sarana* in salinity and aeration treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	8	6059.897	757.487	258.034	< 0.0001
Error	18	52.841	2.936		
Corrected Total	26	6112.738			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 13: One-way analysis of variance (ANOVA) table: SGR of *C. carpio* in salinity and aeration treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	8	6.757	0.845	1793.413	< 0.0001
Error	18	0.008	0.000		
Corrected Total	26	6.766			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 14: One-way analysis of variance (ANOVA) table: BWG% of *C. carpio* in salinity and aeration treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	8	50805.383	6350.673	1236.938	< 0.0001
Error	18	92.415	5.134		
Corrected Total	26	50897.798			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 15: One-way analysis of variance (ANOVA) table: SGR of *L. rohita* in salinity and feed treatment

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	9	1.383	0.154	1208.466	< 0.0001
Error	20	0.003	0.000		
Corrected Total	29	1.386			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 16: One-way analysis of variance (ANOVA) table: BWG% of *L rohita* in salinity and feed treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	9	8537.110	948.568	1114.254	< 0.0001
Error	20	17.026	0.851		
Corrected Total	29	8554.136			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 17: One-way analysis of variance (ANOVA) table: SGR of *C. carpio* in salinity and feed treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	7	1.244	0.178	124.76	< 0.0001
Error	16	0.023	0.001		
Corrected Total	23	1.266			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 18: One-way analysis of variance (ANOVA) table: BWG% of *C. carpio* in salinity and feed treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	7	4318.143	616.878	134.143	< 0.0001
Error	16	73.579	4.599		
Corrected Total	23	4391.722			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 19: One-way analysis of variance (ANOVA) table: SGR of *O. mossambicus* in salinity and feed treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	7	0.950	0.136	160.286	< 0.0001
Error	16	0.014	0.001		
Corrected Total	23	0.964			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).

Table 20: One-way analysis of variance (ANOVA) table: BWG% of *O. mossambicus* in salinity and feed treatments

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	7	3660.303	522.900	140.873	< 0.0001
Error	16	59.390	3.712		
Corrected Total	23	3719.693			

Results are expressed as mean±SD. Different superscripts like in same columns were significantly different ($p<0.05$) in Duncan's Multiple Range mean separation test (DMRT).