A STUDY ON ACCLIMATIZATION AND SURVIVAL ANALYSIS OF *CYPRINUS CARPIO* (LINNAEUS, 1758) REARED AT DIFFERENT SALINITIES

Dissertation submitted in partial fulfillment
of the requirement
for the degree of

M. F. Sc. (Fish Genetics and Breeding)

by

RAJANAND S, B. F. Sc.
(FGB-MA6-05)

ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
(University Established under Sec. 3 of UGC Act 1956)
Panch Marg, Off Yari Road, Versova, Andheri (W),
Mumbai – 400 061

JULY, 2019

Rajanand S 2016. A Study on Acclimatization and Survival Analysis of *Cyprinus Carpio* (Linnaeus,1758) Reared at Different Salinities, M.F.Sc. Dissertation, ICAR-Central Institute of Fisheries Education (University Established Under Section 3 of UGC Act 1956) Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai – 400 061

THIS STUDY IS DEDICATED FOR MY ADVISORS,
WHO PROVIDED THE V_E & FOR MY PARENTS,
WHO PROVIDED THE V_G

DECLARATION

I hereby declare that the dissertation entitled "A STUDY ON ACCLIMATIZATION AND SURVIVAL ANALYSIS OF *CYPRINUS CARPIO* (LINNAEUS,1758) REARED AT DIFFERENT SALINITIES" is

an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

Date: 26 July, 2019

Place: Mumbai

(Rajanand S)

M.F.Sc. Student

ICAR-CIFE, Mumbai

ACKNOWLEDGEMENTS

Writing this most difficult but non-taxing chapter is a soulful acknowledgement of gratitude towards people who stood to extend their helping hands in various ways during my academic life and especially during this study. While few names are being mentioned many more are missing, but I hope those names shall be read between the lines, I owe deep seated gratitude to all those missing names.

First and foremost, I express my reverence to ‘God almighty’ for giving me enormous strength to overcome the adversities in my life.

It is exquisitely a jubilating occasion and unique opportunity to express my deepest sense of indebtedness and everlasting gratitude to my esteemed major advisor **Dr. Naresh S Nagpure**, Principal Scientist, Fish Genetics And Biotechnology Division, CIFE, Mumbai for his constructive comments, indefatigable effort, heart-warming encouragement, and especially his friendly gesture at every step of my dissertation work. I also thank him from core of my heart for allowing me to work under his supervision.

My heartfelt gratitude and special thanks to co-advisor **Dr. Shrinivas jahageerdar**, Principal Scientist, Fish Genetics and Biotechnology Division, CIFE, Mumbai, for suggesting this topic and especially for helping me in every sessions of my academic life and this study by treating me same as his own student. Without his help, this work wouldn’t have completed.

My heartfelt gratitude to **Dr.Mujahidkhan A Pathan**, Scientist, Fish Genetics and Biotechnology Division, CIFE, for his unconditional commitment to help me to complete my work.

My sincere thanks to my advisory committee member, **Mr. V Harikrishna**, Scientist, CIFE Rohtak centre for his support, guidance, instinctive attention, candid suggestion and timely support.

It’s my greatest privilege to express sincere gratitude to **Dr. Aparna Chaudhari**, Principal Scientist and HoD, Fish Gentics & Biotechnology Division, CIFE, for her scholarly guidance and constant encouragement throughout the period of the study.

I express my sincere gratitude to **Dr. Gopal Krishna**, Director & Vice-Chancellor, CIFE, for permitting me to do my research work in this esteemed institution. I express my sincere thanks to all my respected teachers, faculty members of FGB Division **Dr. Rupam sharma** and **Mr. Agnom Lenin Singh** for their co-operation, kind support and timely help during the course of my research work.

I am much thankful to all the staffs of CIFE Rohtak Centre, for giving their precious time and who helped me in sampling and the completion of the research possible.

I extend my sincere thanks to my seniors **Dayal, Shyam, Nes, Tushar, Rameez Roshan and Hafeef Roshan** for their kind support. I thank my batch mates **Bibin, Mehroof, Sathya, Dhaya, Kesavan, Rajesh, Gavin, Chittaranjan, Saumya,** and **Ritty** for their constant motivation, support, and for the great moments together and for the friendly environment. It was because of their company and warmth that I was able to accomplish my goal. I am much thankful to my juniors **Ihzan**, **Phibi, Vivek, Alok & Hussain**

I am thankful to my family and friends, who continued to support me throughout my M.F.Sc period and I am gratefully acknowledge the financial help received from CIFE, Mumbai during the entire period of my post-graduation. There are so many others whom I may have inadvertently left out. I sincerely thank all of them for their help.

I sincerely acknowledge the ICAR and CIFE foe providing me fellowship without which I would not have been in a position to complete this work.

Lastly, I bow my head and bend my knees before the Almighty who is most benevolent, beneficent and whose blessings have solely contributed for my success.

26July 2019
CIFE, Mumbai

Rajanand S

सारांश

वर्तमान अध्ययन को सामान्य कार्प, साइप्रिनस कार्पियो, एक उच्च मूल्यवान कार्प प्रजातियों की लवणता सहिष्णुता को समझने के लिए शुरू किया गया था ताकि नर्सरी चरण के पालन की शर्तों को इष्टतम देखकर अनुकूलित किया जा सके वृद्धि और उत्तरजीविता के लिए लवणता। सामान्य कार्प को अध्ययन के लिए चुना जाता है क्योंकि इसमें उच्च लवणता सहनशीलता, अच्छी वृद्धि दर, उच्च बाजार मूल्य प्राप्त होता है । और इसलिए इसे नमक प्रभावित भूमि में अंतर्देशीय खारा जलीय कृषि के लिए एक उम्मीदवार प्रजाति माना जा सकता है। अध्ययन में वर्तमान अध्ययन के परिणाम सामान्य कार्प के पालन के लिए ज्ञान अंतराल को भरने में मदद करेंगे आगे अंतर्देशीय खारा जलीय कृषि के लिए बीज और उन क्षेत्रों में किसानों के लिए आय सृजन में मदद करेगा। खारेपन और तापमान की अलग-अलग डिग्री के लिए जानवरों को उजागर करके आम कार्प फ़िंगरलिंग पर कुल 11 उपचार प्रक्रियाएं आयोजित की गईं। उत्तरजीविता दर के बीच कोई महत्वपूर्ण अंतर नहीं था जब लवणता 10 पीपीटी तक बढ़ गई थी लवणता बढ़ने की दर के बावजूद, 10 पीपीटी लवणता पर औसतन 92% जीवित रहने की दर। हालांकि, उपचार की उत्तरजीविता दर के बीच एक महत्वपूर्ण अंतर देखा गया था जब लवणता में वृद्धि हुई थी अलग-अलग दरों पर 15 पीपीटी तक पहुंचने तक प्रति दिन 1 पीपीटी की दर से लवणता को बेहतर त्वरण प्रोटोकोल माना जा सकता है 15 पीपीटी लवणता पर औसतन 71.7% जीवित रहने की दर देखी गई। जीवित रहने की दर में कोई महत्वपूर्ण अंतर उन उपचारों में दर्ज नहीं किया गया था जहां एक ही दर पर लवणता में वृद्धि हुई थी (T3 के मामले में 70.66% जीवित रहने की दर) जब खारेपन को बढ़ाकर 15 पीपीटी कर दिया गया नियंत्रित और अनियंत्रित तापमान के लिए एक प्रयोगात्मक सेटअप रखकर 25 दिनों की समयावधि, 70% की उत्तरजीविता दर और 68% क्रमशः दर्ज किया गया था। 15 पीपीटी लवणता के ऊपर मृत्यु दर की महत्वपूर्ण दर 20 पीपीटी लवणता पर 100% मृत्यु दर का कारण बनी। आणविक में महत्वपूर्ण परिवर्तन ऊतकों की वास्तुकला अर्थात। गलफड़े, किडनी और लीवर को भी देखा गया था जब फिंगर्स 10 पीपीटी से ऊपर की सलाइनिटीज के संपर्क में थे।

ABSTRACT

The present study was undertaken to understand the salinity tolerance of common carp, *Cyprinus carpio*, a high valued carp species so that the nursery phase rearing conditions can be optimized by observing the optimal salinity for growth and survival. Common carp is selected for the study because it has a high salinity tolerance, good growth rate, fetches a high market value and so it can be a considered as a candidate species for inland saline aquaculture in salt affected lands. The results of the present study will help to fill the knowledge gaps for rearing the common carp seed for inland saline aquaculture and will help in income generation for the farmers in those areas. A total of 11 treatment procedures were conducted on common carp fingerlings by exposing the animals to varying degrees of salinity and temperature. There was no significant difference between the survival rates when salinity was increased up to 10 ppt irrespective of the rate of increasing the salinity, showing an average of 92% survival rate at 10 ppt salinity. However, a significant difference between the survival rate of the treatments was observed when salinity was increased until 15 ppt at different rates. Thus, increasing the salinity at a rate of 1 ppt per day until reaching 15 ppt can be considered the better acclimatization protocol for common carp fingerlings. An average of 71.7% survival rate was observed at 15 ppt salinity. No significant difference in the survival rate was recorded in the treatments where salinity was increased at the same rate (70.66% survival rate in case of T3 & 68% survival rate for T4), while temperature was altered (24^0C in case of T3 and 10-16^0C iin case of T4). When salinity was increased up to 15 ppt within a time period of 25 days by keeping an experimental setup for controlled and uncontrolled temperature, a survival rate of 70% & 68% was recorded respectively. Above 15 ppt salinity significant rate of mortality was observed causing 100% mortality at 20 ppt salinity. Significant changes in molecular architecture of tissues viz. gills, kidney and liver was also observed when the fingerlings exposed to salinities above 10 ppt.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure: 21 Histopathological study of kidney (20 & 25 PSU)

INTRODUCTION

1. INTRODUCTION

In India fisheries, and aquaculture is a fast-developing sector, and, the annual fisheries, and aquaculture production was recorded as 12.6 million metric tonnes during 2017-18 (NFDB, 2019). Of the total aquaculture production, freshwater aquaculture contributes to over 95 percent, which includes the culture of carp fishes, catfishes (air-breathing, and non-air breathing), freshwater prawns, tilapia, etc. The creation of carp in freshwater, and shrimps in brackish water from the bulk of significant areas of aquaculture activity. The three Indian major carps, namely Catla (*Gibelion catla)*, rohu (*Labeo rohita*), and mrigal (*Cirrhinus mrigala*) contribute the bulk of production to the extent of 70 to 75 percent of the total freshwater fish production, followed by silver carp, grass carp, common carp, catfishes forming a second important group contributing the balance of 25 to 30 percent, (FAO, 2014). Common carp contributed 8% to the total world aquaculture production for 2016 (SOFIA, 2016).

The potential of inland saline water for aquaculture in India has been identified as a high-priority research area. Considering this aspect, research, and education facilities have been constructed in Haryana (e.g. Central Institute of Fisheries Education at Rohtak) aimed explicitly to analyse and evaluate the potential for aquaculture using inland saline water, and also to educate farmers in advanced technology.

Cyprinus carpio, commonly known as common carp, is one among the oldest cultured, and domesticated fish. Compared to the global aquaculture production of freshwater fishes, aquaculture production of common carp has also increased accordingly. In 1939, three varieties of the Prussian strain of common carp, *viz.*, the scale carp *(Cyprinus carpio communis)*, the mirror carp *(C. carpio specularis)*, and the leather carp *(C. carpio nudus)* were introduced in India. And in 1950s they were stocked in various higher altitude ponds, and lakes. Later, in 1957, considering the warm water adaptability, natural breeding, omnivorous feeding habits, excellent growth, and hardy nature of the Chinese (Bangkok) strain of the common carp was brought into the country, primarily for aquaculture purposes. Since it is a delicious, and valuable fish species among other major carps Its culture is very prevalent in the Indian sub-continent. It is known to exhibit salinity tolerance, and some studies have revealed that the common carp can tolerate, and survive up to 10 PSU salinity (seawater), its growth is affected beyond six PSU. Similar observations were recorded in a study on Amur carp, a variety of common carp imported from Hungary (Basavaraju, and Reddy, 2013) in inland saline water at CIFE, Rohtak Centre (unpublished data). If salinity tolerance of common carp is increased by genetic selection, then the salinity tolerant strain will have high global demand, and will be of great importance not only in terms of an export commodity (like GIFT tilapia) but also in terms of nutritional security in climate change scenario in the future. According to the various previous studies, it is understood that salinity is one among the significant environmental parameters influencing the growth, and distribution of fish, affecting survival by adversely affecting feeding, and requirement of energy for the regulation of ionic, and osmotic conditions of internal fluids (Boeuf, and Payan, 2001; De Boeck *et al*., 2000).

For optimizing the primary conditions for the cultivation of many freshwater fishes which have the potential for aquaculture in inland saline water, salinity tolerance is one among the crucial factor. Similar to other environmental factors, variations in salinity affect functional traits related to growth, survival, and other physiological functions of freshwater fishes. When environmental salinity changes, osmotic pressure in freshwater fishes is regulated by consuming energy. But rapid changes in salinity exceeding the tolerance limits cause the death of an aquatic animal. Hence, it is vital to understand the impacts of salinity on the tolerance, and survival of common carp when they are subjected to changes in salinity.

Naturally, when compared with those seawaters diluted to the same salinity, inland saline water often have different ionic proportionalities. This is caused by the differential precipitation of salts as water evaporates, and ions get removed by the reactions with other geological material, and soil (Gong *et al.,* 2004). Fielder *et al.,* 2001 studied that the significant anions found in seawater, in order of magnitude, are chloride (Cl^-), sodium (Na^{6+}), sulfate (SO^{2-}), magnesium (Mg), calcium (Ca^{2+}), and potassium (K^+) (Spotte, 1979). In the arid, and semi-arid regions of Rajasthan, Haryana, Punjab, and Gujarat, and to a lesser extent in Uttar Pradesh, Delhi, Madhya Pradesh, Maharashtra, Karnataka, Bihar, and Tamil Nadu, the salinity of the groundwater is prominent. About 2 lakh sq. Km area of land has been estimated to be affected by saline water with an electrical conductivity (EC) over 4000 µS/cm. An EC values of groundwater that are higher than 10000 µS /cm was recorded at several places in Rajasthan, and Southern Haryana where, making water non-potable. Surface water irrigation without consideration of groundwater status also causes inland salinity. Salinity problem in command areas were caused by the gradual rise of groundwater levels with time that resulted in waterlogging, and substantial evaporation in semi-arid regions. According to the recent assessment, about 2.46 million ha of the area under surface water irrigation projects is waterlogged or threatened by waterlogging. The underground waters of Haryana in India are highly saline ranging from 5 to 30 PSU. Inland saline groundwater could pose a serious threat to production in agriculture since it is neither fit for agriculture nor human consumption. However, a potential opportunity for the development of inland aquaculture is possessed by these saline water reserves.

The mechanism of seawater tolerance in between the species (or individuals) is not well understood, it could be related to the physiological adjustments by the gill during salinity acclimation, especially in the regulation of gill Na^+, K^+ ATPase. Teleost fishes inhabiting freshwater and seawater maintain the osmolality of their body fluid at a relatively constant level. Water, and ionic regulations in fish takes place mainly in the gills, kidney, and, intestine, generating ionic, and osmotic gradients between external environments, and the body fluid. In the gill epithelium, and opercular membrane, specialised cells known as chloride cells are present, and these are the important osmoregulatory sites in maintaining ionic balance in fish (Marshall, 1995; McCormick, 1995). The chloride cells are responsible in ion secretion in seawater (Marshall, 1995; Zadunaisky, 1997), and possibly for ion uptake in freshwater (Perry, 1997). The active ion uptake by freshwater teleosts, and active Na^+, and Cl^- secretion by marine teleosts are processed at gill (Evans et al., 2005).

Na^+/K^+-ATPase maintains Na+, and K+ gradients across the basolateral membrane and hence plays a central role in both models. The energy source for the active movement of ions into or out of the fime bas^+/K^+ -ATPase are these gradients and it acts as the sole energy provider in the gill dynamic ion secretion model of marine species (Silva et al., 1977), whereas in the active ion uptake model of freshwater fishes, Na^+/K^+ -ATPase appears to work in tandem with an apical H^+-ATPase to move Na^+ across the gill epithelium (Avella, and Bornancin, 1989).

The understanding on salinity tolerance at different temperatures, and its corresponding complex osmoregulatory mechanism, and the physiological responses of freshwater stenohaline species when exposed to saline environments has recently evoked a greater interest in the scientific community owing to its application in inland saline aquaculture. However, the field-based studies in common carp on aspects such as salinity acclimation, tolerance, metabolism, and, ion-osmoregulation have been minimal. Thus with these priorities, the present study was carried out with the following objectives.

1. To conduct a survival analysis experiment in *Cyprinus carpio* exposed to various salinity levels.
2. To study the level of acclimatization of *Cyprinus carpio* fingerlings to different salinities.

RIVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Inland saline aquaculture may offer income diversification, and potentially productive use of land that can no longer support traditional agriculture in salt-affected parts of inland production, and, investment levels are characteristically low. It needs to develop in a manner that both prevents further degradation of agricultural l, and, and provides opportunities for an alternative, and sustained economic base for dependent rural communities.

2.1. Inland saline waters

Garg. (1996) conducted studies to assess the possibilities of utilizing drainage effluents (salinity range 5.0-12.5 PSU), for fish culture. Experiments on polyculture using cow dung (24,000 Kg ha-1y-l) as pond fertilizer were conducted at five different salinity levels (0.3--8.5 PSU). Studies have revealed that carp perform well in salinities up to 7.5 PSU, and, reasonably high fish production has been obtained. Even though the ponds had a high trophic status, higher salinities (> 7.5 PSU) appear to repress fish growth probably due to low dissolved oxygen (DO), high BOD, and, high NH4-N. Experiments on monoculture of common carp *(Cyprinus carpio)* conducted at two different salinity levels (0.3-0.9, and 6.0-7.0 PSU) using four different organic fertilizers (cow dung at 24,000 Kg, and 20,000 Kg, poultry at 1,500 Kg, duck at 6,000 Kg, and sheep/goat at 1,500 Kg ha-1y-l) have revealed the highest fish growth to be in poultry-treated ponds, followed in decreasing order by duck, and sheep/goat wastes. Similar trends in fish production were observed both in fresh, and saltwater ponds. However, fish production was lower in ponds having higher salinities (> 7.5 PSU). Nevertheless, these studies indicated that inland saline waters could be utilized for fish culture. With -some modifications in the existing technology of fish culture in stagnant freshwater fish ponds, animal wastes could be used to fertilize brackish water fish ponds (Abrol, and Bhumbla, 1971).

Mangat, and Hundal, (2014) explored the possibility of bringing the brackish water area of the state under inland fisheries, the possibility. Laboratory studies have been designed to explore the tolerance of fingerlings at different salinities during different seasons to observe their survival rate. A total of four hundred, and fifty fingerlings were subjected to salinity regimes of 0, 1.5, 3, 6,, and 12 PSU for 60 days during different seasons (summer, autumn, and, winter). Temperature variations were indicative of the seasonal changes in the ambient environment. Hundred percent survivals were detected at 0 PSU to 6 PSU salinity during all the seasons. Mortality recorded was 100% at 12 PSU salinity during summer (28.0^0C-37.0^0C), and autumn (22.5^0C-30.5^0C), while 50% survival was observed during winter (14.50C-19.00C). Fish showed high appetitive behaviour to food between 0 to 6 PSU salinities. Thus the above study suggested that common carp fingerlings can be reared in coastal waters with a salinity of up to 6 PSU with 100% survival rate indicating that the high salinity areas may be explored for fisheries as well as for stocking enhancement programs.

2.2. Biological Studies

To bring more areas under inland fisheries, the possibility of bringing the brackish water area of Punjab is being explored. Laboratory studies have been designed to explore the tolerance of fingerlings at different salinities during different seasons to observe their survival rate (Mangat *et al., 2014)*. Temperature variations were indicative of the seasonal changes in the ambient environment. The 100% percent survivals were observed for fingerlings of common carp at 0 PSU to 6 PSU salinity during all seasons. The fish exhibited high appetitive behaviour to food between 0 to 6 PSU salinities.

Based on the studies of Gudrun de Boeck (2000), it can be concluded that the energy metabolism of common carp was adversely affected by the result of the decreased food intake due to salt exposure. Although food consumption fell, and growth rates dropped, an increase in routine oxygen consumption was observed, which indicated a reallocation of energy use from growth towards stress-related processes. A stress-induced increase in plasma glucose was observed, as a result of low food intake, lower levels of protein were used for fuel. Protein use itself was probably replaced by the use of carbohydrates. These effects were confirmed by the depletion of both muscle, and liver energy stores of glycogen during the experimental period.

2.3. Physiological Studies

Maceina, and Shireman, (1979) exposed the fingerling (100-120 mm total) length of grass carp (*Ctenopharyngodon idella*), were to various hypotonic, and hypertonic waters to determine tolerance, bodyweight loss, and muscle tissue water content. After acclimation at 8.0‰, LD_{50}'s for 24, 48, and 96 hours were of 15.7, 15.1, and 15.1‰ salinity, respectively. No mortalities occurred when fish was exposed at 14.0‰ for 96 h, but a decrement of 8.5 in the total body weight occurred. At lower salinities, the bodyweight got reduced only by 0.8-1.7%, and as the salinity increased, the water content of muscle tissue declined from 80.0% in freshwater to 74.4% (at 14.0‰). Death at salinities higher than 14.0‰ was attributed to the Failure of cellular processes to adjust to dehydration.

2.4. Histopathological Studies

Freshwater fish are under continuous pressure to conserve salts, whereas the reverse is true for marine species, which must preserve water (Greenwell *et al*. 2003). Among fishes in general, the ability to adapt to alterations in salinity varies markedly, and often is indirectly proportional to the pace of the changes. In natural settings, salinity levels can fluctuate with tides, season, or evaporation from surface waters. Few studies have investigated potential morphologic effects of salinity as the sole stressor. An experiment to assess optimal stocking densities for sea bass (*Dicentrarchus labrax*) fingerlings applied hypersalinity as a stressor and temperature modifications (Via *et al*., 1998). But an experiment that specifically evaluated the tolerance of hybrid tilapia (*Oreochromis mossambicus* × *O. urolepis hornorum*) to hypersaline water found that the primary morphologic indicators of hypersaline stress, and the most sensitive of several endpoints tested, were ultrastructural changes in the gills (Sardella *et al*., 2004). In anadromous fish such as salmon, physiological changes associated with smoltification (the metamorphic transformation that occurs in juveniles before their freshwater to marine migration) are consistently stressful, as suggested by changes in plasma cortisol levels (Barton 2002).

Hypersalinity results in a qualitatively different type of negative response. Apoptosis of chloride cells (branchial cells that facilitate ion transport, and have an integral role in acid-base regulation; Perry 1998) occurred in hybrid tilapia exposed experimentally to various concentrations of hypersaline water for a model of salinity tolerance (Sardella *et al.*, 2004).

According to the studies conducted by Galat *et al*.,(1984) regarding the histological changes in the gill, kidney, and liver of Lahontan cutthroat trout, *Salmo clarki henshawi*, living in lakes of different salinity-alkalinity, gill chloride cell hyperplasia, gill lamellar epithelial separation, glomerular kidney, blood congestion in kidneys,, and deposition of hyaline droplets in kidney glomeruli, tubules, and hemopoietic tissues were the histological alterations statistically associated with the change in salinity, and alkalinity of the water.

A study was carried out in the laboratory on the adaptability, and tolerance of the Tilapia fingerlings, *Oreochromis* sp. to different salinities by Hassan *et al*. (2013), possibility of its culture in the marine environment or brackish water. The histopathological changes, and behavioral changes of the fish challenged with four different salinity treatments, including a control (0, 5, 20, and 35 PSU) for 96 hours. The results showed that all fish survive in 0 PSU, and five PSU, while 75% death in 20 PSU, and 100% death in 35 PSU. The mortality rate was increased with an increase of salinity. Fish exposed to different salinities exhibited clinical signs, agitated behavior, respiratory distress, abnormal nervous behavior, and death were recorded. Degeneration, necrosis, hemorrhage, and hyperplasia of kidneys, and gills were observed as significant histopathological changes.

2.5. Causes of stress

Long term exposure to brackish water affected the capacity of common carp *C. carpio* to adjust with hypoxic conditions, and the critical oxygen concentrations for oxygen consumption increased (De Boeck *et al.*, 2000). Besides, the regulation of ammonia excretion was impaired. The cytosolic phosphorylation potential (the index of the energy status of a cell in terms of potential transferable phosphate groups) in the lateral muscle, on the other hand, and remained relatively unaffected, indicating that oxygen transport to the tissues was not severely compromised. It appears that exposure to salty water reduces the capacity of common carp to cope with hypoxic conditions mainly because of the high energetic cost of hyperventilation under conditions where energy stores are depleted, and not because of any impeded oxygen transport mechanisms.

According to Boeck *et al*., 1996, increased salinity caused increased levels of dopamine (DA), and serotonin (5-HT),, and that could be one possible role of these changes in neurotransmitter metabolism could be to control the release of prolactin, and cortisol, two major hormones involved in the regulation of ion homeostasis in teleosts.

Mohamad (1979), conducted studies on salinity tolerance at different temperatures in *Rhinomugil corsula*, *Cirrhinus mrigala, Labeo fimbriatus, Cyprinus carpio*, and *Sarotherodon mossambica*. Among the five species, the fingerling of *R. corsula* tolerated salinity better than the remaining four species at the same temperature (30°C). The saltwater acclimation in *L. fimbriatus* has not enhanced the survival limit of this species to salinity, and it could only increase the resistance to salinity. As in the case of euryhaline *R corsula*, and *S mossambica*, the salinity acclimation did not bring about a marked change in the tolerance limit among the stenohaline carp. *L fimbriatus.* The stenohaline carps have a comparatively low tolerance, and adaptability to salinity.

2.6. Survival and Growth

According to Holiday *et al.* (1969), the survival of the fish depends on the ability of the fish to maintain the body fluids at least for a short time in an abnormal range of internal osmotic, and ionic concentrations. The fish can regulate the body fluid to restore the level of osmotic pressure to near normal. The migration or abrupt transfer of fish from freshwater to seawater will generally lead to increased osmotic concentration of fish blood serum, and change in ionic contents (Miles, and Smith, 1968).

In a study conducted by Sharma *et al.,* (1985), it was observed that the survival, growth, and development of common carp larvae increased with increasing salinity up to 3 PSU. The salinities investigated (0.3, 1.5, and 3 PSU, respectively) also appeared to enhance the viability, and hatchability of the eggs compared with freshwater.

The salinity tolerance of common carp is similar to that of goldfish (Du Jiayin, 1986) higher than that of silver carp (Von Oertzen, 1985), and lower than those of *Tilapia zillii* (Chervinski, and Zorn, 1974, *Tilapia rendalli* (Whitfield, and Blaber, 1981, rainbow trout (Stickney, 1986), and grass carp *(Ctenopharyngodon idella)* (Chervinski, 1977; Kilambi, and Zdinak, 1980).

According to Kumar *et al*. (2017), *P. hypophthalmus* can survive up to 15 PSU in inland saline water. The study indicated that *P. hypophthalmus* could tolerate the salinities up to 15 PSU in inland saline water, whereas 100% mortality has been observed at salinities 20 PSU, and 25 PSU. This is an indication that the fish was entirely able to regulate their body physiology up to 15 PSU, and at further high salinities, the fish could not survive due to osmoregulatory failure. According to Deacon, and Hecht (1999), the mortality observed in the fish when exposed to the higher salinities could be a consequence of the progressive deterioration in the osmotic, and ionic regulatory mechanisms, including excessive water loss to osmoregulatory Failure.

According to Mangat *et al*. (2014), in their studies, the Salinity tolerance of laboratory-reared fingerlings of common carp, *Cyprinus carpio* (Linn.) during different seasons, the fingerlings were able to regulate their body physiology within this salinity-temperature regime, it is supplemented by the findings of Islam *et al*.

(2014), who also recorded 100% survival rate at 0 to 6 PSU salinities in rohu fingerlings. At 12 PSU salinity, mortality was observed from the very first day, and 100% mortality within 3-4 days was recorded during summer, and autumn season temperature indicating stress conditions in fish leading to fatality during these temperature ranges. Salinity stress in freshwater fish primarily affects the gills, as the primary organ involved in osmoregulation, and waste nitrogen excretion (Nikolsky, 1963). Upper tolerance limits have been reported as 6 PSU for young, and 4.5 PSU for eggs (McCrimmon, 1968). While high salinity has been known to display a highly disrupted epithelium with diffuse edema of both the primary, and the secondary lamellae (Holliday, and Jones, 1967), and this could be the reason why 100% mortality of fish fingerlings in 12 PSU salinity during these seasons was observed. Researchers have recorded 71-90% mortality in 24 hours under 15% salinity in grass carp fingerlings (Kilambi, and Zdinak, 1980), Crivelli (1981) has reported that the common carp occurred in brackish-water marshes with salinities up to 14 PSU in southern France.

2.7. Acclimatization

Studies on salinity acclimation, tolerance, metabolism, and ion-osmoregulation in fishes have been made by Armitage, and Olund (1962), Potts, and Parry (1964), Potts, and Evans (1967), Nelson (1968), Rao (1969, 1971), Parvatheswara Rao (1970), Prosser *et al.,* (1970), Feldmeth, and Waggoner (1972), Mackay (1974), and others. Besides acclimation, the knowledge on salinity tolerance at different temperatures, and the complex osmoregulatory mechanism it leads to is essential, mainly because of the present stress being given on the aquaculture in saline, and wastewaters.

Chervinski (1983) conducted experiments to determine the adaptability of mosquitofish to various salt concentrations. Mosquito fish, *Gambusia affinis*, (total length 27-37mm) were subjected to abrupt, and gradual changes from freshwater to different salinities. Low mortality (10%) occurred when the fish were transferred from freshwater (S = 0·5 ‰) to 50% seawater (S = 19·5 ‰). Through gradual adaptation, 65·0% of the fish were able to tolerate 100% seawater for 7 days. After a seven day stay in seawater, fish were readapted to freshwater during a three h period. The results indicated that they were well able to tolerate the abrupt change. Through a gradual adaptation, fish were also able to tolerate salinities ranging from 39·0 ‰ (100 % seawater) to 58·8 ‰.

Hart *et al.*, (1991) reported that in Victoria, Australia, both dryl, and salinity, and salinity in irrigation regions are agricultural severe problems. They studied the lethal, and sub-lethal effects of salinity on microbes (mainly bacteria), macrophytes, and micro-algae, riparian vegetation, invertebrates, fish, amphibians, reptiles, mammals, and birds. Data suggest that direct adverse biological effects are likely to occur if salinity is increased to around 1000 mg L^{-1} in Australian river, stream, and wetland ecosystems.

In the present study, the conduction of survival analysis studies on the fingerlings of common carp can help to develop a better protocol for faster acclimatization of the common carp fingerlings to the salinities of inland saline water for aquaculture production purposes. The study involved exposure of common carp fingerlings to various salinity levels at the inland saline water systems at a different rate of increment in salinities to estimate the survival rate of common carp to each treatment.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Procurement of fish stock

A total of 2100 common carp fingerlings from two stocks (Powerkheda, and Rohtak stock) were acquired from ICAR-CIFE Powerkheda Centre, and ICAR-CIFE Rohtak Centre, and were taken to the wet lab facilities of ICAR-CIFE Rohtak Centre. The average size of the animals was recorded as 3.6 g of total body weight, and 7.2 cm of total body length. The fish were transported in 20 L polyethylene packing (500 fish in each pack) with sufficient oxygen, and fishes were acclimatized in a circular tank (1000 L) of the wet laboratory for 15 days.

3.2 Experimental Setup

For the conduction of the experiment, a total of 2050 animals were used. The fish were stocked into the experimental units with one control, and 11 treatments. Experimental groups were arranged in such a way that, each treatment was availed for both Powerkheda, and the Rohtak stocks. The treatments were designed as described in Table 1.

Table.1. List of treatments performed during the present study

Sl No	**Treatment No**	**Treatment**
1	1	Salinity was raised at a rate of 1PSU/day up to 10 PSU
2	2	Salinity was raised at a rate of 1PSU/day until it reached 5 PSU, & then directly transferred to 10 PSU on the 6th day
3	3	Salinity was raised at a rate of 1PSU/day until 5 PSU & further increased at a rate of 1PSU/ every 2 days up to 15 PSU (controlled temperature)

4	4	Salinity was raised at a rate of 1PSU/day until 5 PSU, & further increased at a rate of 1PSU/ every 2 days up to 15 PSU (uncontrolled temperature)
5	5	Salinity was raised at a rate of 1 PSU/day up to 15 PSU.
Sl No	**Treatment No**	**Treatment**
6	6	Salinity was raised from 0 to 25 PSU at a rate of 5 PSU/ every 3 days
7	7	Salinity was raised from 0 to 25 PSU at a rate of 5 PSU/ every 2 days.
8	8	Salinity was raised from 0 to 25 PSU at a rate of 5 PSU/ Every day.
9	9	Salinity was raised from 0 to 25 PSU at a rate of 5PSU/ every day (controlled temperature 100C)
10	10	Salinity is raised at a rate of 5 PSU/ every day up to 20 PSU (controlled temperature 28^{0}C)
11	11	Salinity is raised from 0 to 20 PSU as a rapid exposure.
12	Control	Salinity was maintained at 0 to 1 PSU

The experiment was conducted in two stages, from 18th October 2017 to 28th January 2018 (90 Days), and from 6th December to 30th December 2018 (23 Days). Inland saline water used in the experiment was obtained from various bore wells located in the high saline, and low saline zones of the ICAR-CIFE Rohtak Centre. The saline water of 20, and 25 PSU have been prepared by evaporating previously obtained 15 PSU water for 4, and 6 days, respectively, for the experiment. The stocking density for T1, T2, T3, T4, and T5 were 100 animals/80 L tanks, and for T6, T7, T8, T9, T10, and T11 were 50 animals/80 L tanks.

3.3 Data Recording

Observations were recorded periodically for each of the treatments, and dead fish were removed from tanks. Water temperature was regularly recorded with 8 h interval (4 am, 12 pm, and 8 pm respectively). Both treatment wise, and stock wise data were recorded for the treatment wise comparison as well as the stock wise comparison.

3.4 Chemicals used

10% Neutral buffered formalin

3.5 Equipment, and Laboratory Ware

Refractometer (Brix, USA), Phase contrast microscope (Olympus FSX 100), FRP tanks (100 L), micropipettes (200 microliters), Scissors, Forceps. The glasswares were sourced from Borosil, India, while plastic wares were sourced from Tarsons, India.

3.6 Sample Collection

Animals from each salinity level (0 PSU, 5 PSU, 10 PSU, 15 PSU, 20 PSU & 25 PSU) were collected to conduct histopathology studies on selected tissues such as gills, kidney, and liver. Tissues were dissected out, and were stored in Neutral buffered formalin (NBF solution) for at least 48 h followed by transferring them to 70% alcohol.

3.7 Histopathological studies

Histological study of gonads had been undertaken round the year from November 2018 to December 2018. (Gupta et al., 2013)

3.7.1 Fixation of tissue

Gills, kidney, and liver were dissected out, and fixed in neutral buffered formalin (NBF) immediately after the dissection. NBF was prepared by mixing of following chemicals:

Formalin : 100 ml

Distilled water : 900 ml

Sodium dihydrogen orthophosphate: 4 g Disodium hydrogen orthophosphate: 6g

3.7.2 Dehydration of tissue

After proper fixation, the tissues were dehydrated using a graded concentration of alcohol in the following steps.

I. The required material was cut into a specific size, and put in 30% alcohol for 30 minutes.
II. The tissue was transferred in 50% alcohol, and kept for 30 minutes. iii. The tissue was transferred in 70% alcohol, and continued for 1 hour.
III. The tissue was transferred into 90% alcohol, and kept for 30 minutes.
IV. The tissue was transferred in absolute alcohol giving 2 changes each of 30 minutes.
V. The material was put in acetone, and alcohol in the ratio of 1:1, and kept for 30 minutes.

3.7.3 Clearing of tissue

The alcohol present in the tissue was cleared by using the clearing agent xylene. For the clearing process, tissue was put in xylene, giving two changes each of 30 minutes.

3.7.4 Wax embedding of material

After proper cleaning of the tissue, the following steps of impregnation were carried out.

I. The tissues were kept in the mixture of xylene, and melted seasoned wax (2:1) for 30 minutes.
II. The tissues were transferred in a mixture of xylene, and melted seasoned wax (1:2), and kept for 60 minutes.

III. The tissues were transferred in melted wax, and kept for 120 minutes maintained at a temperature of 60 ± 2ºC to prevent the solidification of wax.

3.7.5 Block making

The molten wax was kept in an oven at 60ºC overnight before taking it for block preparation. “L” shape molds were used for making the blocks. Before placing the tissue, the base of the mold was filled with wax, and then tissue was pressed into the wax. Afterward, the frames were filled with melted wax. The wax was allowed to cool on a cold plate. After complete solidification, frames were removed, and blocks were ready for sectioning.

3.7.6 Sectioning of tissue block, and spreading of tissue ribbons on the slide

Before sectioning of tissue, the blocks were trimmed into the required shape, and fixed on the chuck of the microtome. The thickness of the section to be cut was set at 5 µ, and divisions in the form of ribbons were obtained using a microtome knife, and the same were collected carefully with the help of a drawing brush, and allowed to float on a water bath maintained at 45-50ºC. The ribbon was raised on a transparent slide, and dried on a hot plate. The slide was kept on the slide warming table, the temperature maintained at 60±2°C. After the proper spreading of the ribbon, excess water on the slide was wiped off with the help of tissue paper.

3.7.7 Hydration of tissue

Coplin jars filled with graded alcohol were used for the hydration. The whole process of hydration was accomplished in the following steps.

I. Very first the slides were put in the xylene, giving 2-3 changes for 5 minutes each for de-waxing.

II. Having cleared the paraffin, the slides were transferred in absolute alcohol for 2 minutes.

III. The slides were then transferred in 90% alcohol for 1 minute.

IV. The slides were transferred in 70% alcohol for 1 minute.
V. The slides were transferred in 50% alcohol for 1 minute.
VI. The slides were washed in running tap water for about 2-3 minutes.

3.7.8 Staining of tissue

The slides were stained in hematoxylin solution for 5-10 minutes. Then slides were soaking in running tap water, for 5 minutes. After washing in tap water the slides were transferred to Scott’s tap water kept for 1 minute. Then the slides were placed in distilled water for 1 minute. After this, the following steps were carried out to get permanent mounts.

I. The slides were put in 30% alcohol for 1 minute.
II. The slides were transferred in 50% alcohol for 1 minute.
III. The slides were transferred in 70% alcohol for 2 minutes.
IV. The slides were transferred in 90% alcohol for 2 minutes.
V. The slides were stained with eosin solution for 2-5 minutes.
VI. The slides were kept in absolute alcohol for 1 minute.
VII. The slides were put in xylene for final clearing giving 2 changes each of 3 to 5 minutes.
VIII. Finally, slides were mounted permanently using D.P.X. Solution.
IX. These permanent mounts prepared as above were observed under a Phase contrast microscope (Olympus FSX 100), and photographs were taken.

3.8 Data Analysis of the survival experiment

Analyses were carried out in SAS using the appropriate SAS procedure on a SAS dataset. The essential SAS procedures for performing survival analysis was PROC LIFETEST. Survival analysis makes an inference about event rates as a function of time. The two primary methods to estimate the correct underlying survival curve are by obtaining the Kaplan–Meier estimator, and Cox proportional hazards regression.

PROC LIFETEST is used to obtain Kaplan Meier survival estimates, and plots. It can also be used to output life table estimates, and plots. It will generate output for the log-rank, and Wilcoxon test statistics if stratifying by a covariate. A new SAS dataset containing survival estimates can be requested. The SAS programming codes for PROC LIFETEST used in this analysis are detailed in Appendix I. (SAS, 2011)

RESULTS

4. RESULTS

For the present study, two stocks were included, and the observations were taken from a total of eleven treatments. The stocking density for each treatment was at 100 animals at 100L of the tank. A total of 4 sets of controls were maintained, with 100 animals each during each of the two stages of the experiment (during the first stage 2 sets of control were retained for 90 days, and during the second stage 2 sets of the control group were maintained for 23 days). A 100% survival has been recorded in all control groups.

4.1.1. Survival Estimates for Treatment 1, and Treatment 2

Table.2. Survival Estimate of Treatment 1 (salinity was increased at a rate of 1 PSU/ day up to 10 PSU)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**2**	0	0	100.0	0	0	1.0000	0	0
2	**4**	0	0	100.0	0	0	1.0000	0	0
4	**6**	0	0	100.0	0	0	1.0000	0	0
6	**8**	0	0	100.0	0	0	1.0000	0	0
8	**10**	1	0	100.0	0.0100	0.00995	1.0000	0	0
10	**12**	4	95	95.0	0.0777	0.0373	0.9900	0.0100	0.00995
12	**.**	0	0	0.0	0	0	0.9131	0.0869	0.0380

In the survival experiment for treatment 1, 100% survival of common carp fingerlings was recorded from 1st to 7th day of the procedure, the lowest survival rate (4% mortality) was recorded during the 10th to 12th day of the procedure (Table 2). The death of fingerlings was evident from the 8th day of the experiment, where the salinity was at 8 PSU.

Table 3. Survival Estimate of Treatment 2 (Salinity was increased at a rate of 1 PSU/ day up to 5 PSU, and then directly transferred to 10 PSU from the 6th day)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**2**	0	0	300.0	0	0	1.0000	0	0
2	**4**	0	0	300.0	0	0	1.0000	0	0
4	**6**	0	0	300.0	0	0	1.0000	0	0
6	**8**	0	0	300.0	0	0	1.0000	0	0
8	**10**	0	0	300.0	0	0	1.0000	0	0
10	**12**	6	0	300.0	0.0200	0.00808	1.0000	0	0
12	**14**	16	0	294.0	0.0544	0.0132	0.9800	0.0200	0.00808
14	**16**	6	0	278.0	0.0216	0.00872	0.9267	0.0733	0.0151
16	**18**	0	0	272.0	0	0	0.9067	0.0933	0.0168
18	**20**	4	0	272.0	0.0147	0.00730	0.9067	0.0933	0.0168
20	**22**	0	268	134.0	0	0	0.8933	0.1067	0.0178
22	**.**	0	0	0.0	0	0	0.8933	0.1067	0.0178

In the survival experiment using treatment 2, the lowest survival rate was recorded during the 1st to 9th day of the procedure (Table 3). However, from the 12th to 14th day onwards, a mortality rate of 5.33% was recorded. In this exposure, the mortality was recorded from the 10th day of the experiment, during which the salinity of the water was 10 PSU.

Table 4. Summary of the Number of Censored, and Uncensored Values for Treatment 1, and Treatment 2

Summary of the Number of Censored, and Uncensored Values					
Stratum	**Treatment**	**Total**	**Failed**	**Censored**	**Percent Censored**
1	T1	100	5	95	95.00
2	T2	300	32	268	89.33
Total		400	37	363	90.75

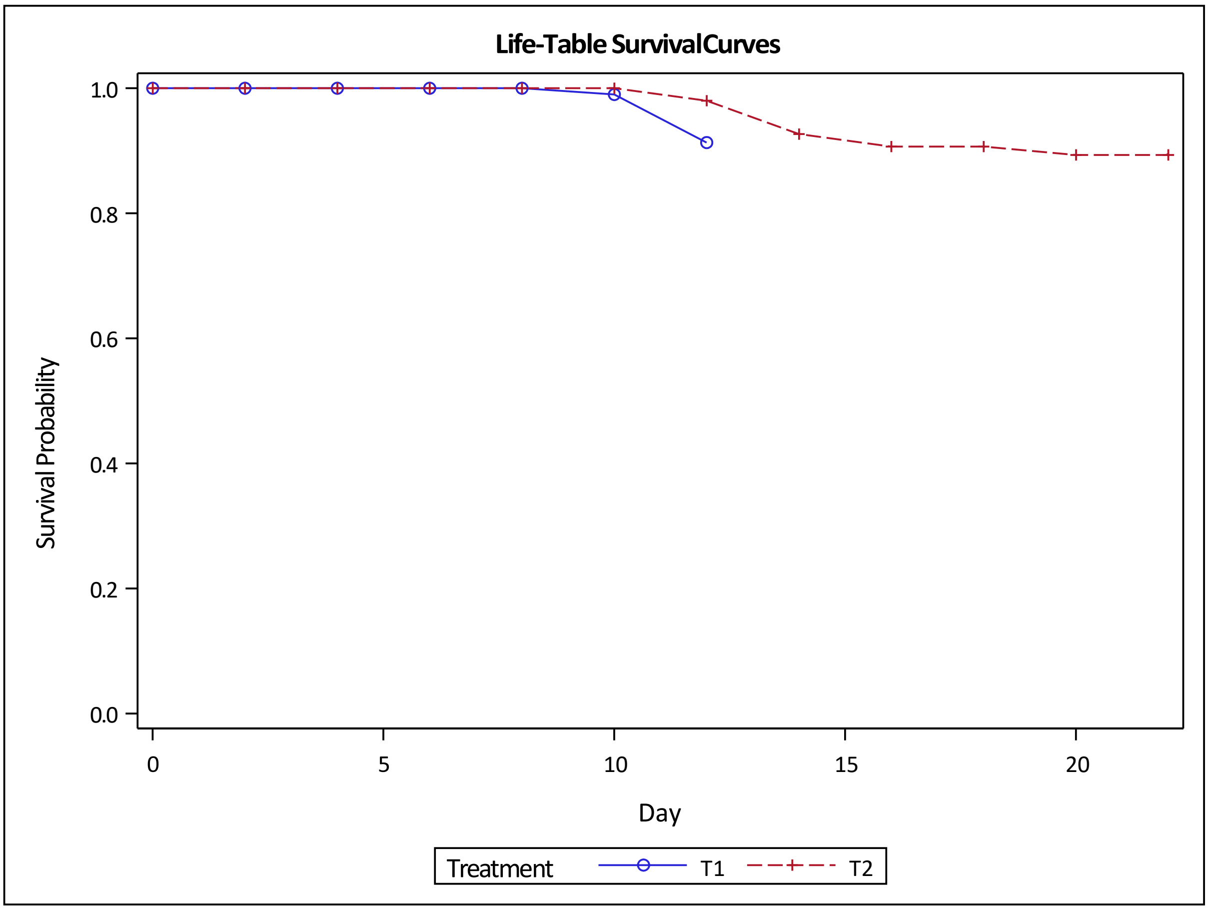
Table 4 depicts the comparison of the two treatments, where a higher survival was observed for T1, 95% survival of the animals was recorded, whereas for T2 89.33% survival of the animals was recorded.

Table 5. Test of Equality over strata for Treatment 1, and Treatment 2

Test of Equality over Strata			
Test	**Chi-Square**	**DF**	**Pr > Chi-Square**
Log-Rank	2.6289	1	0.1049
Wilcoxon	2.6506	1	0.1035

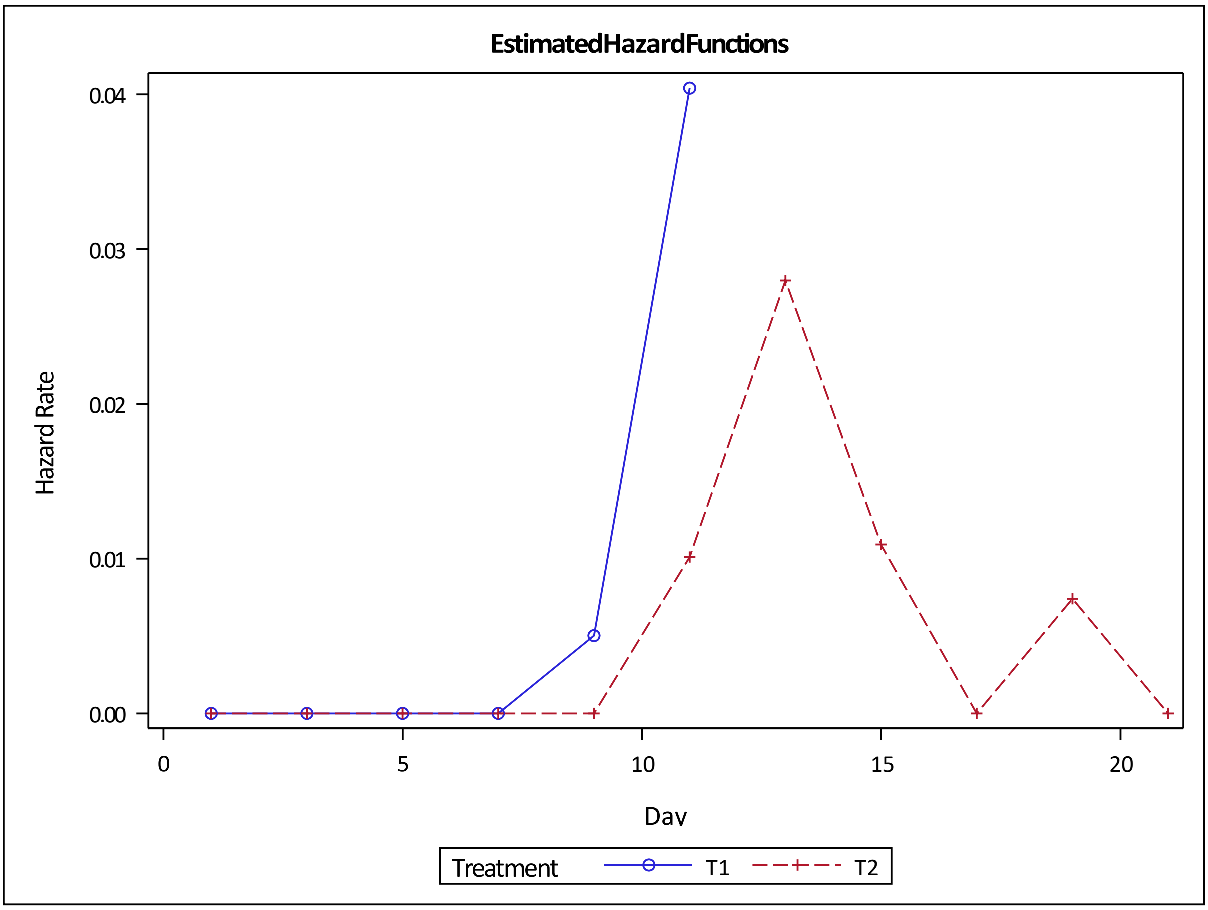
In both the treatments T1, and T2, the salinity was increased up to 10 PSU, from 0 to 10 PSU at different rates, and that the test of equality (Table 5) indicated that there was no significant difference between the treatments, thus there was no effect of the rate at which salinity was increased on the survival rate up to 10 PSU

Figure 1: Life table survival curves for Treatment 1, and Treatment 2



The life table survival curves (Figure 1) for T_1, and T_2, revealed that in the case of T_1 the mortality of the animals had started in the earlier stage of the experiment (8th day) as compared to T_2 (10th day). Also, the fingerlings got acclimatized to the 10 PSU salinity at an earlier stage compared to the T2 (on the 12th day).

Figure 2: Estimated hazard functions for **Treatment 1, and Treatment 2**



As per the estimated hazard function analysis (Figure 2) for T_1, the risk of mortality started increasing 7th day of the treatment, and the fish got stabilized to the 10 PSU salinity from the 11th day of the experiment (as no death was recorded from the 11th day of the experiment. In the case of T_2, the risk of death increased from the 9th day of the experiment, it decreased from the 13th day of the experiment (at 9 PSU salinity), and further stabilized on the 22nd day of the experiment

4.1.2. Survival Estimates for Powerkheda, and Rohtak stocks when salinity was increased up to 10 PSU

Table 6. Survival Estimate for Rohtak stock (up to 10 PSU)

<table>
<tr><th colspan="11">Life Table Survival Estimates</th></tr>
<tr><td colspan="11">Stratum 1: stock = R</td></tr>
<tr><th colspan="2">Interval</th><th rowspan="2">Number Failed</th><th rowspan="2">Number Censored</th><th rowspan="2">Effective Sample Size</th><th rowspan="2">Conditional Probability of Failure</th><th rowspan="2">Conditional Probability Standard Error</th><th rowspan="2">Survival</th><th rowspan="2">Failure</th><th rowspan="2">Survival Standard Error</th></tr>
<tr><th>L</th><th>U</th></tr>
<tr><td>0</td><td>5</td><td>0</td><td>0</td><td>100.0</td><td>0</td><td>0</td><td>1.0000</td><td>0</td><td>0</td></tr>
<tr><td>5</td><td>10</td><td>3</td><td>0</td><td>100.0</td><td>0.0439</td><td>0.00254</td><td>1.0000</td><td>0</td><td>0</td></tr>
<tr><td>10</td><td>15</td><td>5</td><td>0</td><td>97.0</td><td>0.0131</td><td>0.00159</td><td>0.9700</td><td>0.0300</td><td>0.000254</td></tr>
<tr><td>15</td><td>20</td><td>0</td><td>92</td><td>92.0</td><td>0</td><td>0</td><td>0.9200</td><td>0.0800</td><td>0.00161</td></tr>
<tr><td>20</td><td>25</td><td>0</td><td>0</td><td>0.0</td><td>0</td><td>0</td><td>0.9200</td><td>0.0800</td><td>0.00161</td></tr>
<tr><td>25</td><td></td><td>0</td><td>0</td><td>0.0</td><td>0</td><td>0</td><td>0.9200</td><td>0.0800</td><td>0.00161</td></tr>
</table>

By the end of the survival experiment for Rohtak stock, 92% survival of the common carp fingerlings were recorded, where the lowest survival rate was observed from the 10th to 15th day of the exposure. The fishes got acclimatized to the 10 PSU salinity by the 15th day of the disclosure, and no further mortalities were observed.

Table 7. Survival Estimate for Powerkheda stock 1 (up to 10 PSU)

Life Table Survival Estimates									
Stratum 2: stock = P									
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**5**	0	0	100.0	0	0	1.0000	0	0
5	**10**	0	0	100.0	0	0	1.0000	0	0
10	**15**	8	0	100.0	0.0995	0.00130	1.0000	0	0
15	**20**	2	0	92.0	0.0578	0.00373	0.9200	0.0800	0.00132
20	**25**	1	0	90.0	0.1633	0.00835	0.9000	0.1000	0.00390
25		0	89	89.0	0	0	0.8900	0.1100	0.00844

By the end of the survival experiment for Powerkheda stock 1, 89% survival of the common carp fingerlings were recorded, where the lowest survival rate was observed from the 10th to 15th day of the exposure. The fishes got acclimatized to the 10 PSU salinity by the 25th day of the disclosure, and no further mortalities were observed.

Table 8. Survival Estimate for Powerkheda stock 2 (up to 10 PSU)

Life Table Survival Estimates									
Stratum 3: stock = p									
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**5**	0	0	200.0	0	0	1.0000	0	0
5	**10**	0	0	200.0	0	0	1.0000	0	0
10	**15**	11	0	200.0	0.0106	0.00143	1.0000	0.000293	0.000207
15	**20**	5	0	189.0	0.0635	0.00416	0.9450	0.0550	0.00145
20	**25**	0	184	184.0	0	0	0.9200	0.0800	0.00433
25		0	0	184.0	0	0	0.9200	0.0800	0.00433

By the end of the survival experiment for Powerkheda stock 2, 92% survival of the common carp fingerlings were recorded, where the lowest survival rate was observed from the 10th to 15th day of the exposure. The fishes got acclimatized to the 10 PSU salinity by the 20th day of the disclosure, and no further mortalities were observed.

Table 9. Summary of the Number of Censored, and Uncensored Values for Powerkheda, and Rohtak stocks (salinity up to 10 PSU)

Summary of the Number of Censored, and Uncensored Values					
Stratum	**stock**	**Total**	**Failed**	**Censored**	**Percent Censored**
1	**P**	100	11	89	89.00
2	**R**	100	8	92	92.00
3	**P**	200	16	184	92.00
Total		400	35	365	91.00

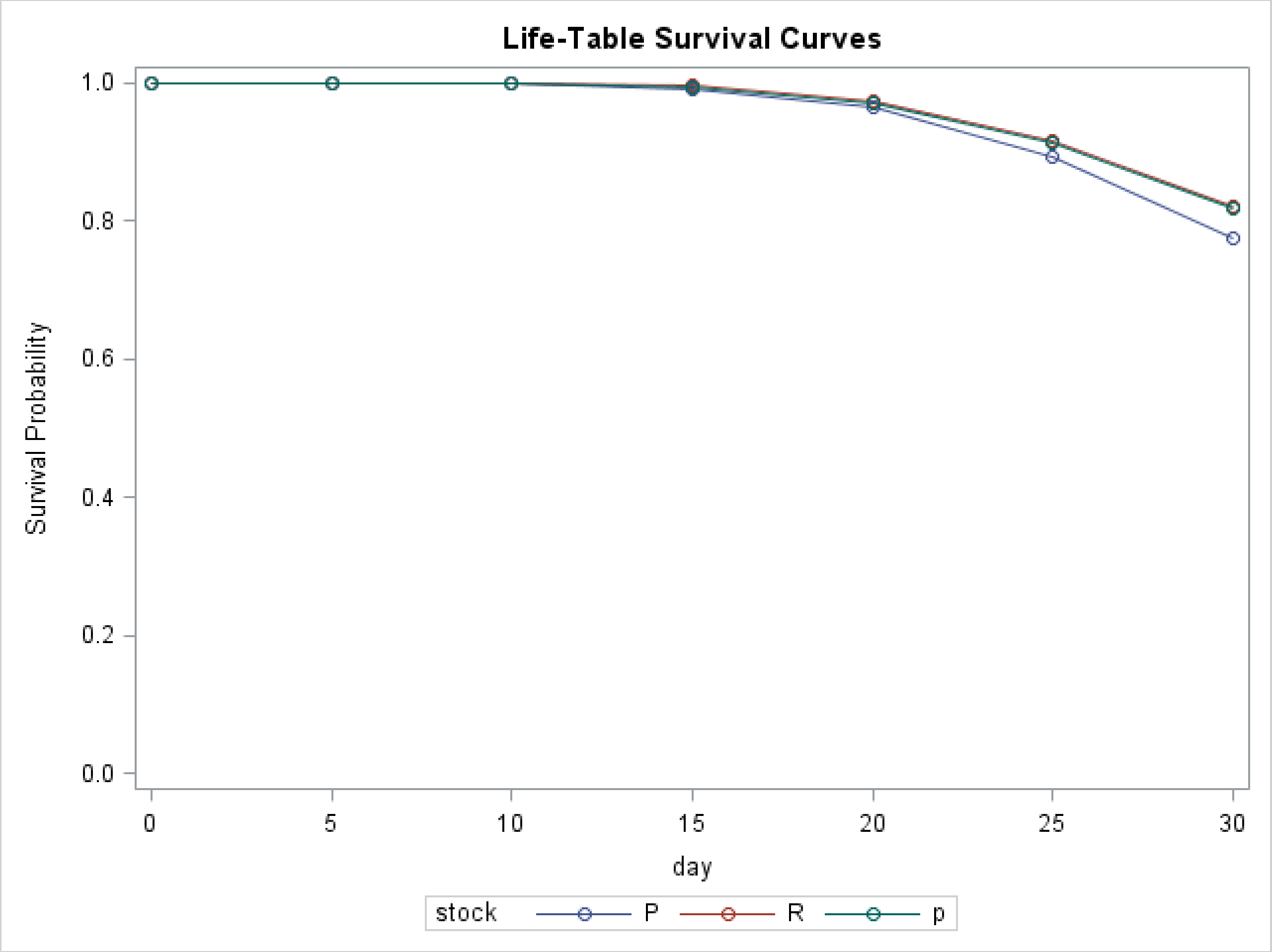
Table 9 depicts the comparison of Powerkheda, and Rohtak stocks, where 95% survival was recorded in the Rohtak, and Powerkheda stock 2, whereas for Powerkheda stock 1, 89% survival of the animals was recorded.

Table 10. Test of Equality over strata for Powerkheda, and Rohtak stocks (salinity up to 10 PSU)

Test of Equality over Strata			
Test	**Chi-Square**	**DF**	**Pr > Chi-Square**
Log-Rank	17.4529	2	0.0002
Wilcoxon	12.6811	2	0.0018

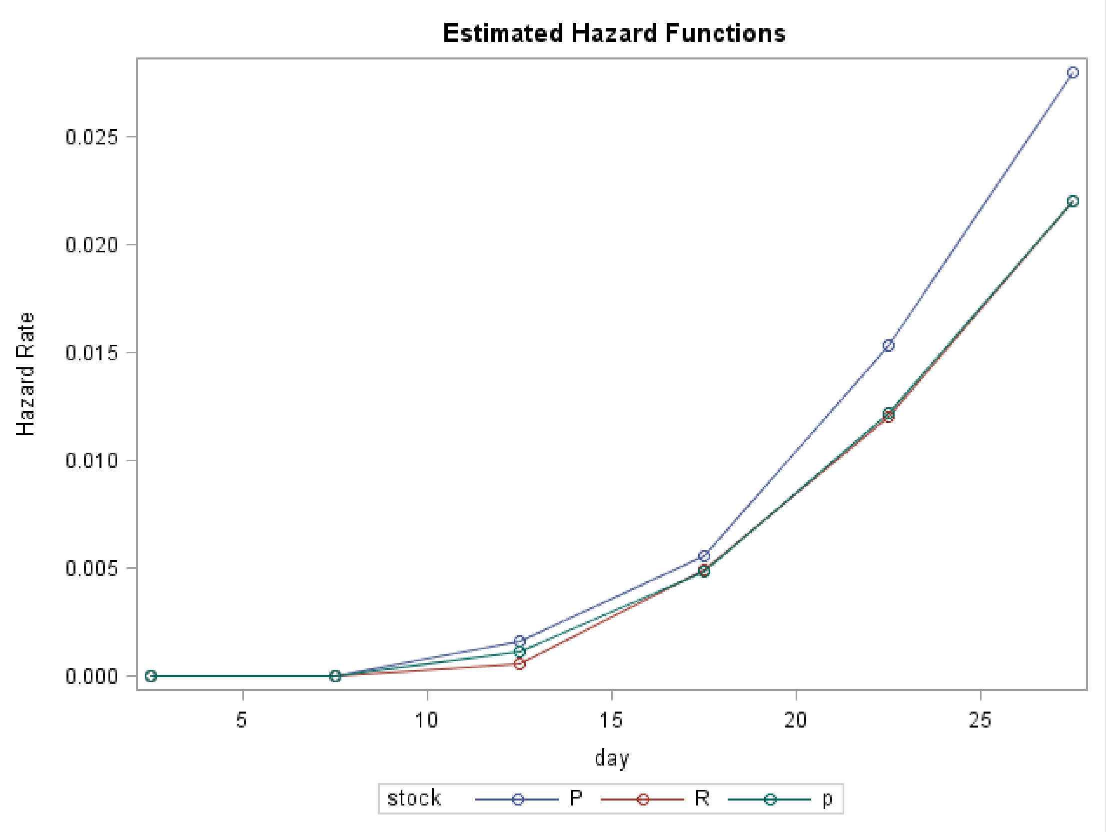
In both the stocks, the salinity was increased up to 10 PSU, and that the test of equality (Table 10) indicated that there was no significant difference between the survival rates from the stocks (Powerkheda, and Rohtak stock), and thus there was no effect of stock on the survival rate when the salinity was increased up to 10 PSU.

Figure 3: Life table survival curves for Powerkheda, and Rohtak stocks (up to 10 PSU)



The life table survival curves (Figure 3) for Powerkheda, and Rohtak stocks, revealed that Rohtak stock, and Powerkheda stock 2 had the same survival probability, in contrast for the Powerkheda stock 1, the survival rate was slightly lower from the other two, and also, for Rohtak stock, and Powerkheda stock 2, the fingerlings got acclimatized to the 10 PSU salinity at an earlier stage compared to the Powerkheda stock 1.

Figure 4: Estimated hazard functions for Powerkheda, and Rohtak stocks (up to 10 PSU)



As per the estimated hazard function analysis, (Figure. 4) for all the three stocks, the risk of mortality started increasing by the 8th day of the exposure., and the risk of death increased at a higher rate in the case of Powerkheda stock 1 compared to the other two. As for the Powerkheda stock 2, and the Rohtak stock, the risk of mortality started increasing from the 12th day of the treatment, and the rate at which hazard rate increment begun falling from the 20th day of the experiment, and the animals got acclimatized to the 10 PSU salinity by 25th day of the experiment.

4.2.1. Survival Estimates for treatments T_3, T_4, and T_5

Table 11. Survival Estimate of Treatment 3 (Salinity increased at a rate of 1 PSU/ day up to 5 PSU, and then increased to 15 PSU at a rate of 1 PSU/ 2 day, and temperature was kept controlled at 24^0C).

Life Table Survival Estimates									
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**5**	0	0	298.0	0	0	1.0000	0	0
5	**10**	0	0	298.0	0	0	1.0000	0	0
10	**15**	18	0	298.0	0.0604	0.0138	1.0000	0	0
15	**20**	29	0	280.0	0.1036	0.0182	0.9396	0.0604	0.0138
20	**25**	37	0	251.0	0.1474	0.0224	0.8423	0.1577	0.0211
25	**30**	5	209	109.5	0.0457	0.0199	0.7181	0.2819	0.0261
30	**.**	0	0	0.0	0	0	0.6853	0.3147	0.0287

Table 12. Survival Estimate of Treatment 4, (Salinity increased at a rate of 1 PSU/day up to 5 PSU, and then increased to 15 PSU at a rate of 1 PSU/2 days, and temperature was kept uncontrolled (10-16^{0}C))

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**5**	0	0	299.0	0	0	1.0000	0	0
5	**10**	0	0	299.0	0	0	1.0000	0	0
10	**15**	21	0	299.0	0.0702	0.0148	1.0000	0	0
15	**20**	25	0	278.0	0.0899	0.0172	0.9298	0.0702	0.0148
20	**25**	41	0	253.0	0.1621	0.0232	0.8462	0.1538	0.0209
25	**30**	9	203	110.5	0.0814	0.0260	0.7090	0.2910	0.0263
30	**.**	0	0	0.0	0	0	0.6513	0.3487	0.0304

Table 13. Survival Estimate of Treatment 5 (Salinity was increased at a rate of 1 PSU/ day up to 15 PSU, and temperature was kept uncontrolled at 10-16^{0}C)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**5**	0	0	301.0	0	0	1.0000	0	0
5	**10**	12	0	301.0	0.0399	0.0113	1.0000	0	0
10	**15**	42	0	289.0	0.1453	0.0207	0.9601	0.0399	0.0113
15	**20**	14	232	131.0	0.1069	0.0270	0.8206	0.1794	0.0221
20	**25**	0	0	1.0	0	0	0.7329	0.2671	0.0297
25	**30**	0	1	0.5	0	0	0.7329	0.2671	0.0297
30	**.**	0	0	0.0	0	0	0.7329	0.2671	0.0297

In the survival experiment for T3, the highest rate of survival (100% survival) was recorded during the 1st to 10th day of the treatment (Table 11). The lowest rate of survival (37 among the 298 animals were found to be dead during the 20th to 25th day of the treatment with a 12.42% mortality) was recorded during 20th to 25th day of the treatment. In this treatment, the mortality was recorded from the 10th day of the experiment, where the salinity was at 8 psu.

In the survival experiment of T_4, 100% survival was recorded during the 1st to 10th day of the experiment (Table 12). The lowest rate of survival (13.71% mortality) was recorded during the 20th to 25th day of the experiment. For this treatment, the mortality occurred from the 10th day of the experiment, where the salinity was at 8 psu.

The survival estimates for T5 showed 100% survival the highest rate of survival during the 1st to 5th day of the experiment (Table 13). The lowest rate of survival (13.95% mortality) was recorded during the 10th to 15th day of the experiment. The mortality for this experiment occurred from the 8th day of the experiment where the salinity was 8 psu.

Table 14. Summary of the number of censored, and uncensored values for Treatment 3, Treatment 4, and Treatment 5

Summary of the Number of Censored, and Uncensored Values					
Stratum	**Treatment**	**Total**	**Failed**	**Censored**	**Percent Censored**
1	T3	298	89	209	70.13
2	T4	299	96	203	67.89
3	T5	301	68	233	77.41
Total		898	253	645	71.83

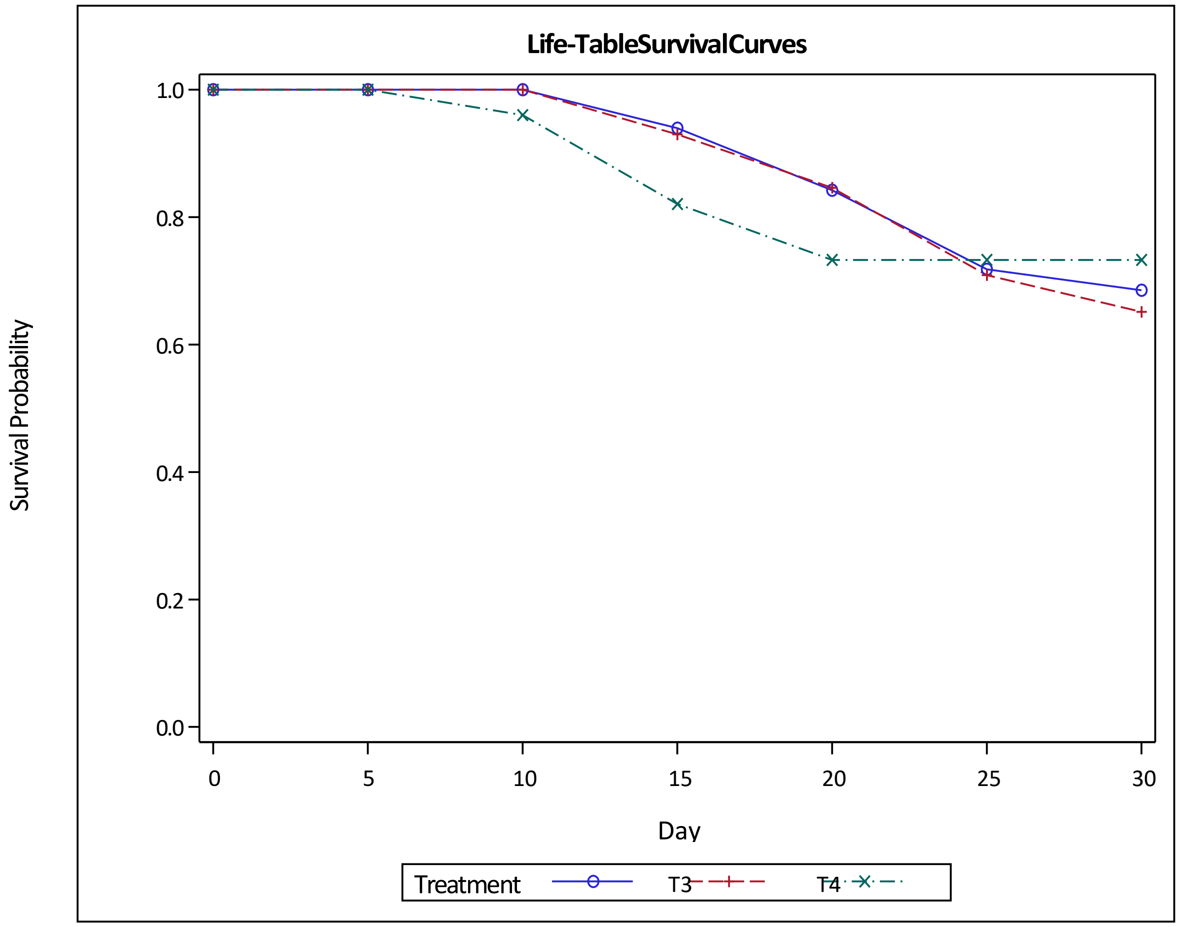
The summary of the survival for the three treatments (Table 14) revealed that for treatment T_3, and T_4, 70.13%, and 67.89% survival of the animals was recorded respectively, whereas, in T_5, 77.41% animals was recorded as survived.

Table 15. Test of Equality over strata for Treatment 3, Treatment 4, and Treatment 5

Test of Equality over Strata			
Test	**Chi-Square**	**DF**	**Pr > Chi-Square**
Log-Rank	36.3462	2	<.0001
Wilcoxon	37.1031	2	<.0001

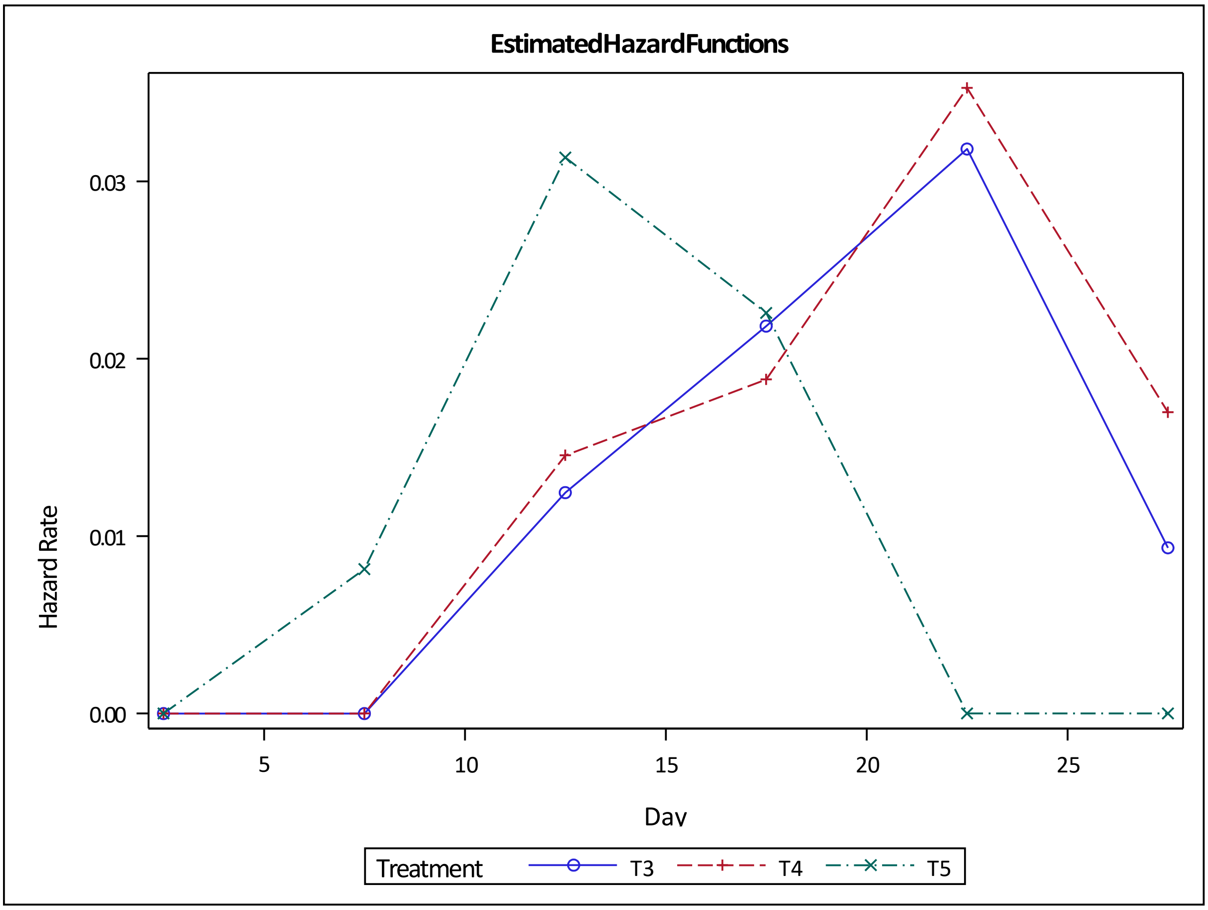
The comparison of treatments T_3, T_4, and T_5 (Table 15), revealed that when salinity was increased from 0 to 15 PSU using different rates, a significant difference between the treatments was recorded concerning the survival rate.

Figure 5: Life table Survival Curves for Treatment 3, Treatment 4, and Treatment 5



The life table survival curves for the three treatments, that is T_3, T_4, and T_5 have been depicted in Figure 5. It can be seen that in the case of T_3, and T4, the probability of survival of the animals started decreasing from the 10th day, and the animals got acclimatized to 15 PSU salinity from the 30th day of the treatments, with a survival rate of 70% for T3, and 68% for T4. Whereas, in the case of T5, the fish got acclimatized to the 15 PSU salinity on the 20th day of the treatment with a survival rate of 77%.

Figure 6: Estimated hazard functions for Treatment 3, Treatment 4, and Treatment 5



The estimated hazard function (Figure 6), revealed that for T_3, and T_4, the risk of mortality increased 8th day of the treatment, and showed a decreasing trend from the 23rd day of the treatment. The fish got stabilized to the 15 PSU salinity on the 30th day of the experiment (because no mortality was recorded from the 30th day of the experiment for T_3, and T_4). There was not much difference in the risk of mortalities for T_3, and T_4. In the case of T_5, the risk of mortality started decreasing from the 12th day of the experiment, and the fish got stabilized from the 22nd day of the experiment. Among these three treatments, the rate of salinity treatment was increased in the same format for T_3 (with controlled temperature), and T_4 (with uncontrolled temperature), but no significant difference in their survival rate was recorded, thus showing that, though there are slight deviations in the rate of survivability, there is not much effect of temperature when its coupled with the estimation of the salinity tolerance in common carps.

4.2.2. Survival Estimates for Powerkheda, and Rohtak stocks when salinity was increased up to 15 PSU

Table 16. Survival Estimate for Powerkheda stock (up to 15 PSU)

Life Table Survival Estimates										
Stratum 1: stock = P										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**
L	**U**								
0	**5**	0	0	600.0	0	0	1.0000	0	0
5	**10**	8	0	600.0	0.0410	0.00205	1.0000	0	0
10	**15**	55	0	592.0	0.0123	0.00129	0.9700	0.0300	0.0205
15	**20**	54	0	537.0	0.0595	0.00338	0.8950	0.1050	0.0131
20	**25**	50	0	483.0	0.1696	0.00757	0. 8050	0.1950	0.0356
25		0	433	433.0	0	0	0.7033	0.2783	0.0762

By the end of the survival experiment for Powerkheda stock, 72.17% survival of the common carp fingerlings were recorded, where the lowest survival rate was observed from the 10th to 15th day of the exposure. The fishes got acclimatized to the 15 PSU salinity by the 25th day of the disclosure, and no further mortalities were observed.

Table 17. Survival Estimate for Rohtak stock (up to 15 PSU)

<table>
<tr><th colspan="10">Life Table Survival Estimates</th></tr>
<tr><td colspan="10">Stratum 2: stock = R</td></tr>
<tr><th colspan="2">Interval</th><th rowspan="2">Number Failed</th><th rowspan="2">Number Censored</th><th rowspan="2">Effective Sample Size</th><th rowspan="2">Conditional Probability of Failure</th><th rowspan="2">Conditional Probability Standard Error</th><th rowspan="2">Survival</th><th rowspan="2">Failure</th><th rowspan="2">Survival Standard Error</th></tr>
<tr><th>L</th><th>U</th></tr>
<tr><th>0</th><th>5</th><td>0</td><td>0</td><td>300.0</td><td>0</td><td>0</td><td>1.0000</td><td>0</td><td>0</td></tr>
<tr><th>5</th><th>10</th><td>4</td><td>0</td><td>300.0</td><td>0.0506</td><td>0.00179</td><td>1.0000</td><td>0</td><td>0</td></tr>
<tr><th>10</th><th>15</th><td>25</td><td>0</td><td>296.0</td><td>0.1048</td><td>0.00286</td><td>0.9866</td><td>0.0134</td><td>0.0183</td></tr>
<tr><th>15</th><th>20</th><td>25</td><td>0</td><td>271.0</td><td>0.1931</td><td>0.00444</td><td>0.9033</td><td>0.0967</td><td>0.0316</td></tr>
<tr><th>20</th><th>25</th><td>27</td><td>0</td><td>246.0</td><td>0.3879</td><td>0.00753</td><td>0.8200</td><td>0.1800</td><td>0.0455</td></tr>
<tr><th>25</th><td></td><td>0</td><td>219</td><td>219.0</td><td>0</td><td>0</td><td>0.7300</td><td>0.2700</td><td>0.0585</td></tr>
</table>

By the end of the survival experiment for Rohtak stock,73% of the common carp fingerlings were recorded, where the lowest survival rate was observed from 20th to 25th day of the exposure. The fishes got acclimatized to the 10 PSU salinity by the 25th day of the disclosure, and no further mortalities were observed.

Table 18. Summary of the Number of Censored, and Uncensored Values for Powerkheda, and Rohtak stocks (up to 15 PSU)

Summary of the Number of Censored, and Uncensored Values					
Stratum	**stock**	**Total**	**Failed**	**Censored**	**Percent Censored**
1	**Powerkheda**	600	167	433	72.17
2	**Rohtak**	300	81	219	73.00
Total		900	248	652	72.59

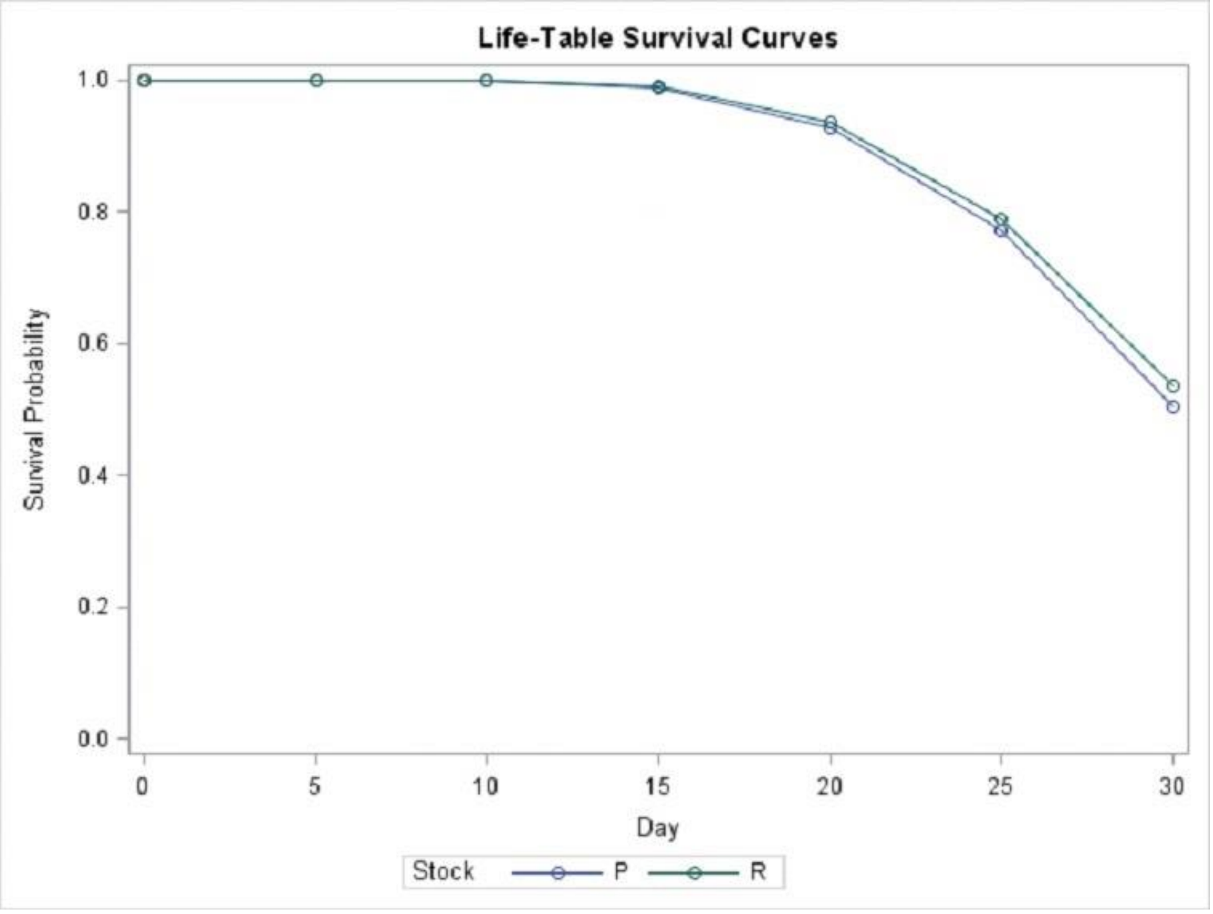
Table 18 depicts the comparison of Powerkheda, and Rohtak stocks, where a higher survival was observed in Rohtak stock, which is 73% survival, whereas in Powerkheda stock a 72.17% survival was recorded.

Table 19. Test of Equality over strata for Powerkheda, and Rohtak stocks (up to 15PSU)

Test of Equality over Strata			
Test	**Chi-Square**	**DF**	**Pr > Chi-Square**
Log-Rank	1954.4837	2	<.0001
Wilcoxon	1832.5339	2	<.0001

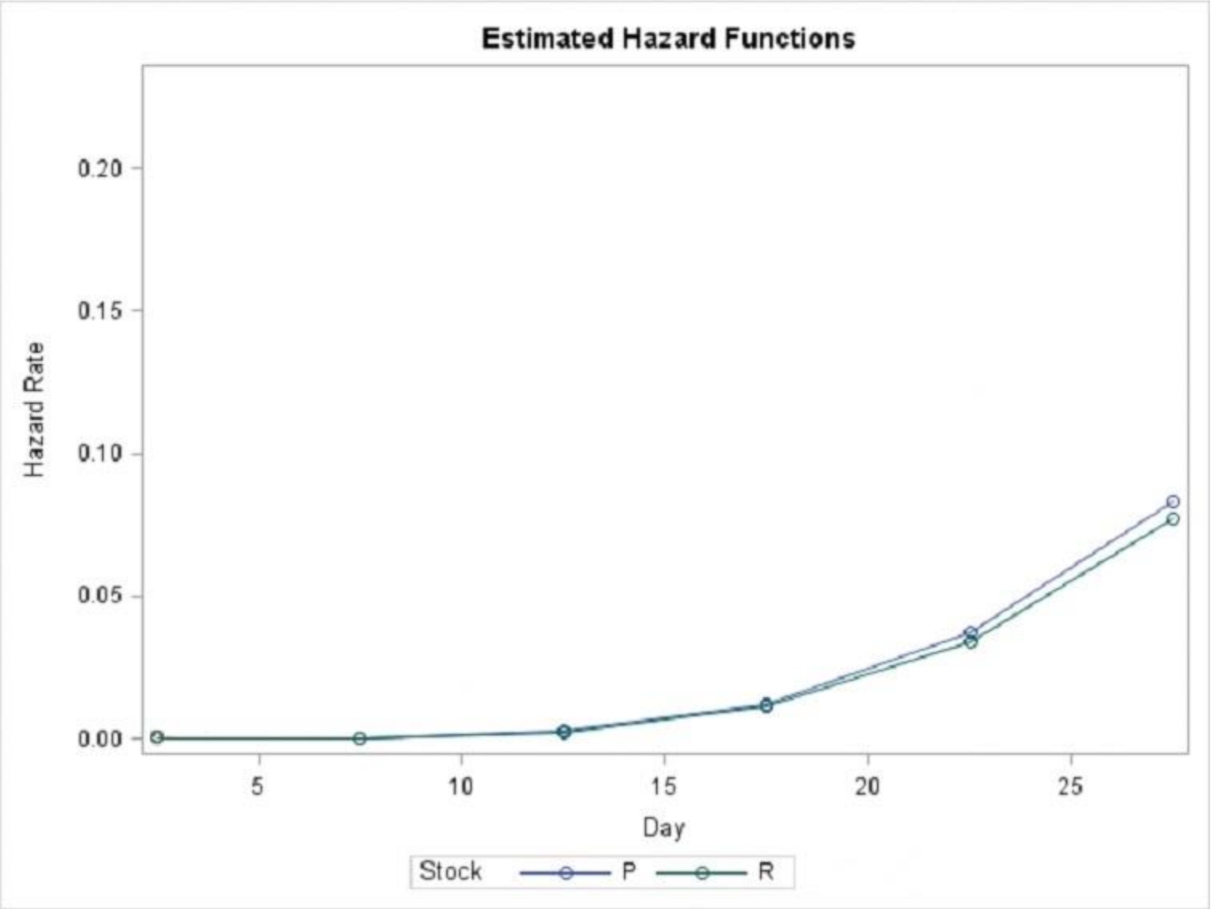
In both the stocks, the salinity was increased up to 15 PSU, and that the test of equality (Table 19) indicated that there was no significant difference between the survival rates from the stocks (Powerkheda, and Rohtak stock), thus there was no effect of stock on the survival rate when the salinity was increased up to 15 PSU.

Figure 7: Life table survival curves for Powerkheda, and Rohtak stocks (up to 15 PSU)



The life table survival curves (Figure 7), revealed that in the case of both Powerkheda, and Rohtak stocks, the mortality of the animals had started from the 10th day of the experiment. The rate of death was observed to be slightly higher than that of its Rohtak counterpart. Also, the fingerlings got acclimatized to the 15 PSU salinity by the 25th day of the experiment.

Figure 8: Estimated hazard functions for Powerkheda, and Rohtak stocks (up to 15 PSU)



As per the estimated hazard function analysis (Figure 8), for both of the powerkheda stocks, and the Rohtak stock, the risk of mortality started increasing from the 8th day of the treatment, and as compared to the Rohtak stock, the risk of death in the case of the Powerkheda stock was slightly higher from the 13th day of the experiment.

4.3.1. Survival Estimates for treatments T_6, T_7, T_8, T_9, T_{10}, and T_{11}

Table 20. Survival Estimate for Treatment 6 (Salinity increased from 0PSU to 25 PSU at a rate of 5 PSU/3 days)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**2**	0	0	100.0	0	0	1.0000	0	0	13.0870
2	**4**	0	0	100.0	0	0	1.0000	0	0	11.0870
4	**6**	0	0	100.0	0	0	1.0000	0	0	9.0870
6	**8**	5	0	100.0	0.0500	0.0218	1.0000	0	0	7.0870
8	**10**	6	0	95.0	0.0632	0.0250	0.9500	0.0500	0.0218	5.1957
10	**12**	14	0	89.0	0.1573	0.0386	0.8900	0.1100	0.0313	3.3261
12	**14**	46	0	75.0	0.6133	0.0562	0.7500	0.2500	0.0433	1.6304
14	**16**	29	0	29.0	1.0000	0	0.2900	0.7100	0.0454	1.0000
16	**.**	0	0	0.0	0	0	0	1.0000	0	.

The survival estimate of T_6, (Table 20) revealed the highest rate of survival during the 1st to 6th day (at 5 PSU salinity) of the experiment (100% survival). The lowest rate of survival (46% mortality) was recorded during the 12th to 14th day of the experiment. In this experiment, the death occurred from the 6th day of the experiment where the salinity was 10 PSU.

Table 21. Survival Estimate for Treatment 7 (Salinity increased from 0 PSU to 25 PSU at a rate of 5 PSU/2 days)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**2**	0	0	100.0	0	0	1.0000	0	0	10.3333
2	**4**	0	0	100.0	0	0	1.0000	0	0	8.3333
4	**6**	7	0	100.0	0.0700	0.0255	1.0000	0	0	6.3333
6	**8**	10	0	93.0	0.1075	0.0321	0.9300	0.0700	0.0255	4.4500
8	**10**	23	0	83.0	0.2771	0.0491	0.8300	0.1700	0.0376	2.6167
10	**12**	60	0	60.0	1.0000	0	0.6000	0.4000	0.0490	1.0000
12	**.**	0	0	0.0	0	0	0	1.0000	0	.

As far in the survival estimate of T_7 is concerned, 100% survival was recorded during the 1st to 4th day (at 5 PSU salinity) (Table 21). The lowest rate of survival of 40% could be recorded during the 10th to 12th day of the experiment. Here,the death started from the 4th day of the experiment where the salinity was 10 PSU.

Table 22. Survival Estimate for Treatment 8 (Salinity increased from 0 PSU to 25 PSU at a rate of 5 PSU/day)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**2**	0	0	100.0	0	0	1.0000	0	0	6.1481
2	**4**	7	0	100.0	0.0700	0.0255	1.0000	0	0	4.1481
4	**6**	39	0	93.0	0.4194	0.0512	0.9300	0.0700	0.0255	2.2778
6	**8**	54	0	54.0	1.0000	0	0.5400	0.4600	0.0498	1.0000
8	**.**	0	0	0.0	0	0	0	1.0000	0	.

As regards the survival estimates for T_8 is concerned (Table 22), 100% survival was recorded during 1st to 2nd day (at 5 PSU salinity) of the experiment, the lowest rate of survival (54% mortality) was recorded during 6th to 8th day of the experiment. In this case, the death happened from the 2nd day of the experiment where the salinity was 10 PSU.

Table 23. Survival Estimate for Treatment 9 (Salinity is increased from 0 PSU to 25 PSU at a rate of 5 PSU/day, and the temperature was kept controlled at 10^{0}C)

Life Table Survival Estimates											
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**2**	0	0	100.0	0	0	1.0000	0	0	6.0769
2	**4**	4	0	100.0	0.0400	0.0196	1.0000	0	0	4.0769
4	**6**	44	0	96.0	0.4583	0.0509	0.9600	0.0400	0.0196	2.1538
6	**8**	52	0	52.0	1.0000	0	0.5200	0.4800	0.0500	1.0000
8	**.**	0	0	0.0	0	0	0	1.0000	0	.

The survival estimates for T_9 showed that there was 100% survival (Table 23) from 1st to 2nd day (at 5 PSU salinity). The lowest rate of survival (52% mortality) was recorded during the 6th to 8th day of the experiment. In this experiment, the death started from the 2nd day of the experiment where the salinity was 10 PSU

Table 24. Survival Estimate for Treatment 10 (Salinity increased from 0 PSU to 25 PSU at a rate of 5PSU/day, and temperature was kept controlled at 28^{0}C)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**2**	0	0	50.0	0	0	1.0000	0	0	4.8636
2	**4**	6	0	50.0	0.1200	0.0460	1.0000	0	0	2.8636
4	**6**	44	0	44.0	1.0000	0	0.8800	0.1200	0.0460	1.0000
6	**.**	0	0	0.0	0	0	0	1.0000	0	.

As regards the survival estimate of T_{10} (Table 24), 100% survival was recorded from 1st to 2nd day (at 5 PSU salinity) of the experiment, in contrast, maximum mortality (88% mortality rate) occurred during the 4th to 6th day of the experiment. In this case, the death started from the 2nd day of the experiment where the salinity was 10 PSU.

Table 25. Survival Estimate for Treatment 11 (Directly transferred from 0 PSU to 20 PSU salinity)

Life Table Survival Estimates										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**2**	0	0	100.0	0	0	1.0000	0	0	3.0000
2	**4**	100	0	100.0	1.0000	0	1.0000	0	0	1.0000
4	**.**	0	0	0.0	0	0	0	1.0000	0	.

The survival estimate for T_{11} has been depicted in table 25. A 100% death had occurred from the 2nd day of exposure of the fish to 20 PSU salinity.

Table 26. Summary of the number of censored, and uncensored Values for Treatment 6 to Treatment 11

Summary of the Number of Censored, and Uncensored Values					
Stratum	**Treatment**	**Total**	**Failed**	**Censored**	**Percent Censored**
1	T10	50	50	0	0.00
2	T11	100	100	0	0.00
3	T6	100	100	0	0.00
4	T7	100	100	0	0.00
5	T8	100	100	0	0.00
6	T9	100	100	0	0.00
Total		550	550	0	0.00

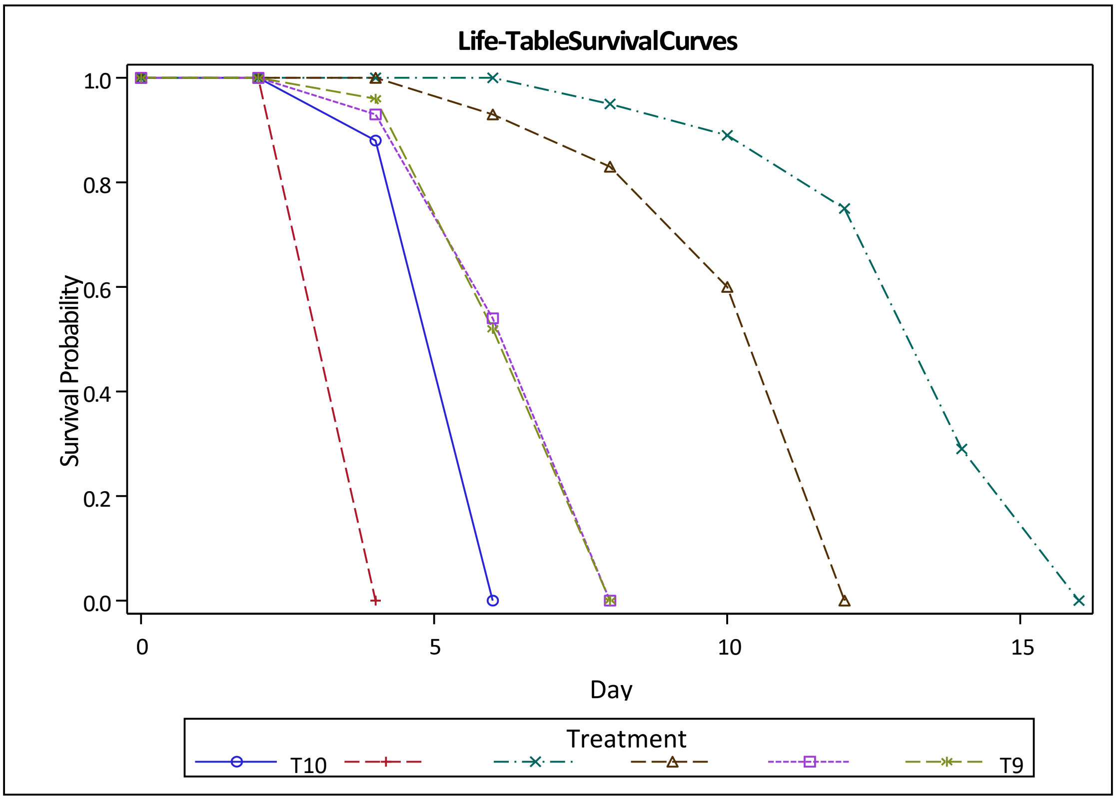
The summary of the mortality when fish were exposed to treatments T_6, T_7, T_8, T_9, T_{10}, and T_{11} has been depicted in Table 26, and it can be seen that 100% death of the animals was recorded in all the treatments.

Table 27. Test of Equality over strata

Test of Equality over Strata			
Test	**Chi-Square**	**DF**	**Pr > Chi-Square**
Log-Rank	948.2221	5	<.0001
Wilcoxon	805.1513	5	<.0001

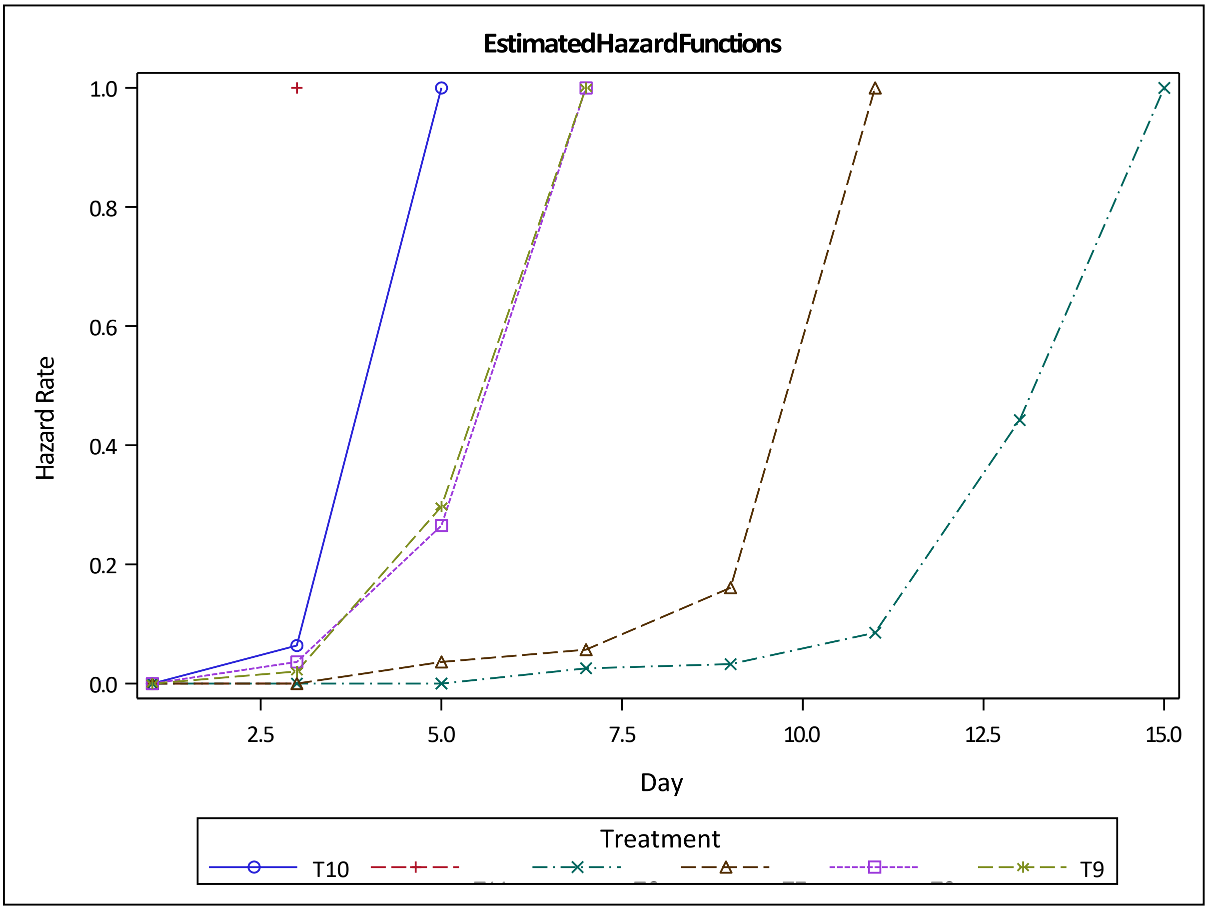
The comparison of the equality of treatments T_6, T_7, T_8, T_9, T_{10}, and T_{11} in which the salinity was increased, from 0 to 25 PSU at different rising rates of salinity, has been shown in Table 27. A significant difference between the treatments was observed concerning the survival rate.

Figure 9: Life table survival curves for Treatment 6 to Treatment 11



The life table survival curves for treatment T_6, T_7, T_8, T_9, T_{10}, and T_{11} have been depicted in Figure 9. For these treatments, the salinities were increased by an increment of 5 PSU at different rates. It was observed that irrespective of the rate at which salinity is increased, none of the animals was survived. Further, for treatments T_8, and T_9, it was noted that there was not much difference in the survival probability between T_8, and T_9, irrespective of the temperature at which treatments were maintained.

Figure10: Estimated Hazard functions for Treatment 6 to Treatment 11



Concerning the estimated hazard function for treatments T_6, T_7, T_8, T_9, T_{10}, and T_{11} (Figure 10), the risk of mortality started increasing from the initial stages of the experiment, and regardless of the treatments conducted, 100% death was recorded at the end of each of the treatments.

4.3.2. Survival Estimates for Powerkheda, and Rohtak stocks when salinity was increased up to 25 PSU

Table 28. Survival Estimate for Rohtak stock (up to 25 PSU)

Life Table Survival Estimates										
Stratum 1: stock = R										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**5**	75	0	250	0.0712	0.00916	0.8850	0.1150	0	19.6675
5	**10**	25	0	175	0.1050	0.00361	0.7500	0.2500	0.000916	14.7467
10	**15**	67	0	150	0.3165	0.00534	0.4150	0.5850	0.00368	10.7389
15	**20**	61	0	83	0.5682	0.00740	0.1000	0.9000	0.00558	7.3630
20	**25**	22	0	22	1.0000	0	0	1.0000	0.00649	1.0000
25		0	0	0	0	0	0	1.0000	0	

Table 29. Survival Estimate for Powerkheda stock (up to 25 PSU)

Life Table Survival Estimates										
Stratum 2: stock = P										
Interval		**Number Failed**	**Number Censored**	**Effective Sample Size**	**Conditional Probability of Failure**	**Conditional Probability Standard Error**	**Survival**	**Failure**	**Survival Standard Error**	**Median Residual Lifetime**
L	**U**									
0	**5**	23	0	200	0.00499	0.00102	0.7000	0.3000	0	17.7412
5	**10**	27	0	177	0.1232	0.00510	0.6000	0.4000	0.00102	12.7874
10	**15**	67	0	150	0.4167	0.00758	0.3320	0.6680	0.00515	8.9223
15	**20**	63	0	83	0.7143	0.0101	0.0980	0.9020	0.00764	6.0000
20	**25**	20	0	20	1.0000	0	0	1.0000	0.00790	1.0000
25		0	0	0	0	0	0	1.0000	0	

In the survival estimate of Rohtak stock (Table 28), the highest rate of mortality was observed from the initial stage of the experiment due to the rapid rate of increment in the salinity levels. And 100% of mortality was recorded by the end of the experiment.

In the survival estimate of Powerkheda stock (Table 29), the highest rate of mortality was observed from the initial stage of the experiment due to the rapid rate of increment in the salinity levels. And a 100% mortality was recorded by the end of the experiment.

Table 30. Summary of the Number of Censored, and Uncensored Values for Powerkheda, and Rohtak stocks (up to 25 PSU)

Summary of the Number of Censored, and Uncensored Values					
Stratum	**stock**	**Total**	**Failed**	**Censored**	**Percent Censored**
1	**Rohtak**	350	350	0	0.00
2	**Powerkheda**	200	200	0	0.00
Total		550	550	0	0.00

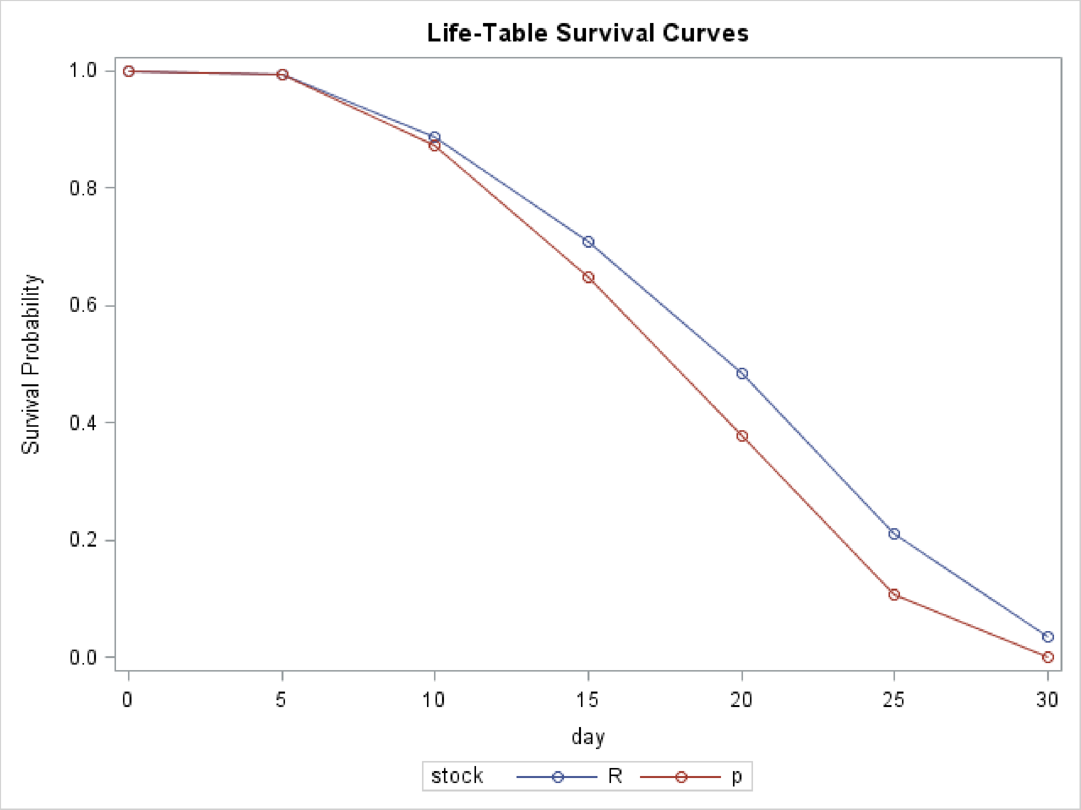
The summary of the mortality when fish were exposed to up to 25 PSU salinity has been depicted in Table 30, and it can be seen that 100% death of the animals was recorded in both of the stocks.

Table 31. Test of Equality over strata for Powerkheda, and Rohtak stocks (up to 25 PSU)

Test of Equality over Strata			
Test	**Chi-Square**	**DF**	**Pr > Chi-Square**
Log-Rank	216.5757	1	<.0001
Wilcoxon	111.8516	1	<.0001

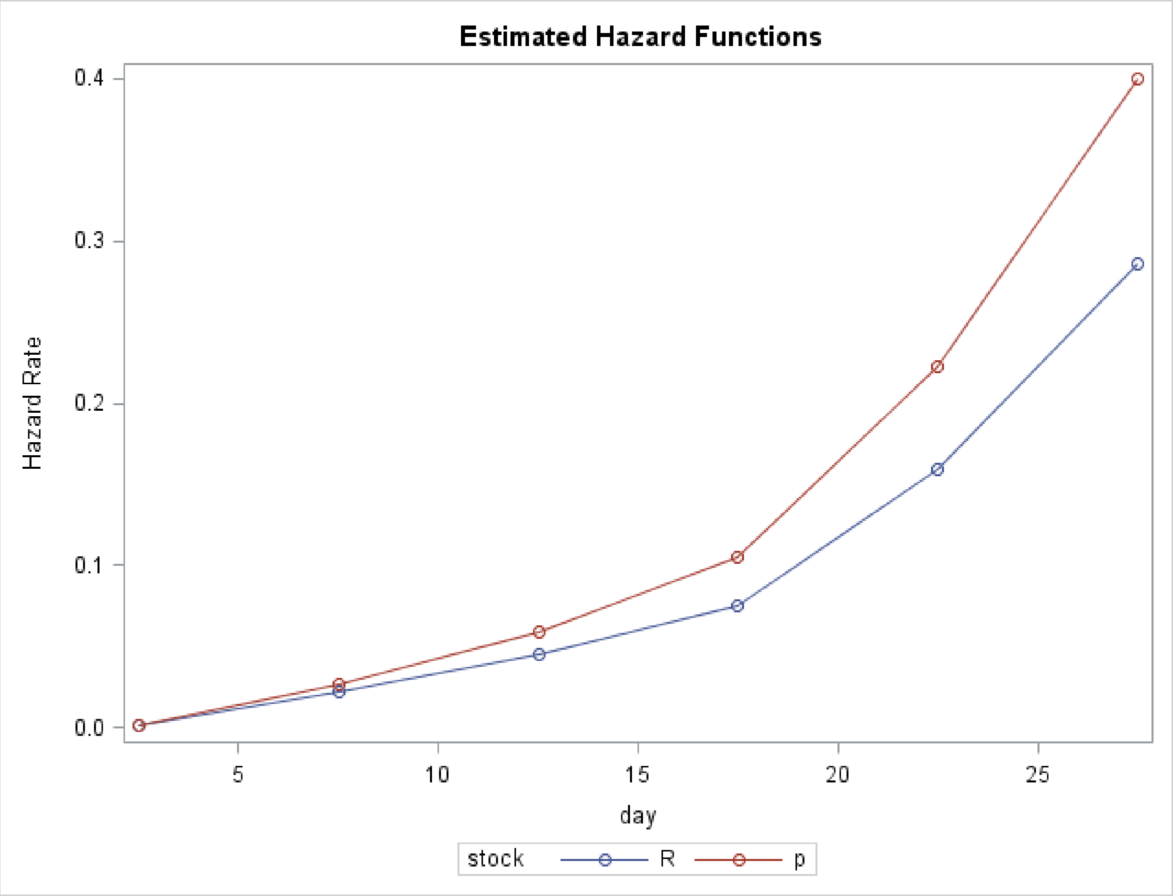
The comparison of the equality of the Powerkheda, and Rohtak stocks, where the salinity was increased, from 0 to 25 PSU at different rising rates of salinity, has been shown in Table 31. No significant difference between the stocks was observed concerning the survival rate.

Figure 11: Life table survival curves for Powerkheda, and Rohtak stocks (up to 25 PSU)



The life table survival curves of Powerkheda, and Rohtak stocks have been depicted in Figure. 11. Here, the salinities were increased by an increment of 5 PSU up to 25 PSU salinity, and 100% mortality was recorded in both stocks. Further, it was observed that that, there was not much difference in the survival probability between the two stocks until the 10th day of the treatment. From the 10th day, the powerkheda stock had lesser survival probability as compared to its Rohtak counterpart.

Figure 12: Estimated hazard functions for Powerkheda, and Rohtak stocks (up to 25 PSU)



In regards to the estimated hazard function Powerkheda, and Rohtak stocks (Figure 12), the risk of death started increasing from the initial stages of the experiment, and conducted, 100% mortality was recorded in both of the stocks., and it was recorded that the risk of mortality was higher in the case of the Powerkheda stock as compared to its Rohtak counterpart.

4.4. Histopathological observations

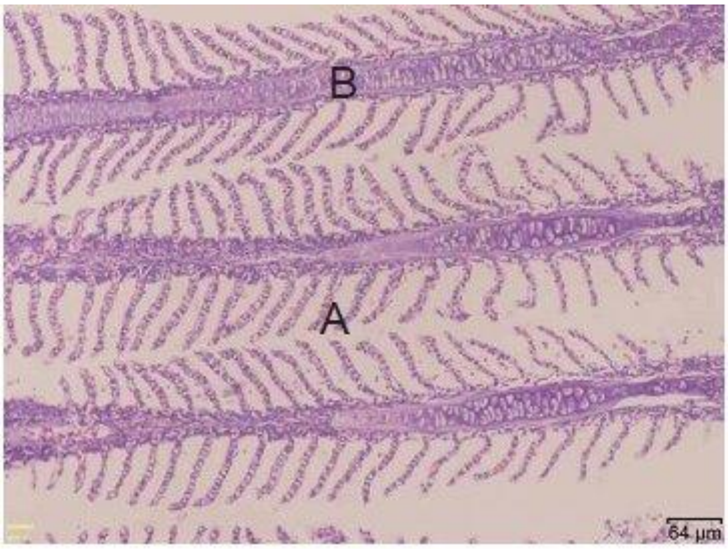
Histology is a powerful tool in assessing the healthiness of various tissues during any disease or disorder, as well as tells about the reproductive status of fish. In the present study, histological observation of gills, liver, and kidney of common carp fingerlings were analyzed at different strata of salinity ranging from 5 to 25 PSU.

4.4.1. Histopathological study of gills

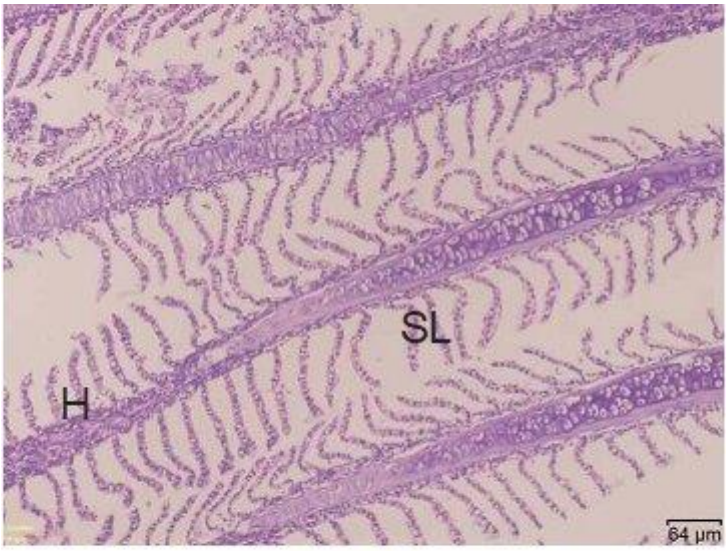
In the control slides (0PSU), typical gill structures with intact gill lamellae were observed. At 5 PSU the secondary lamellae were intact but a mild hyper placidity was found at the base of secondary lamellae. But in both situations, gill lamellae were found to be in a normal case.

At 10 PSU, hyper placidity at the tip of the gill lamellae was seen. Most of the secondary lamellae were normal, and healthy, but the fusion of a few secondary lamellae at specific locations was found.

At 15 PSU, most of the gill lamellae had club formation at the tip due to hyperplasia. Some sections also showed the fusion of secondary lamellae, and hyperplasia at the base of the secondary lamellae. Here enlargement of the primary lamellae was also observed. At 20 PSU, the fusion of secondary lamellae was found, and in some places, complete loss of the secondary lamellae. At 25 PSU, complete disruption of the gill lamellae was kept. Severe hyper placidity was also found in most of the sections. Fusion, and loss of secondary lamellae at several places, and thickening of the primary lamellae were also seen. Fragmentation of the gill lamellae was also observed from 20 PSU, which could be mainly due to the hypersaline effects, and while handling the gill tissues.



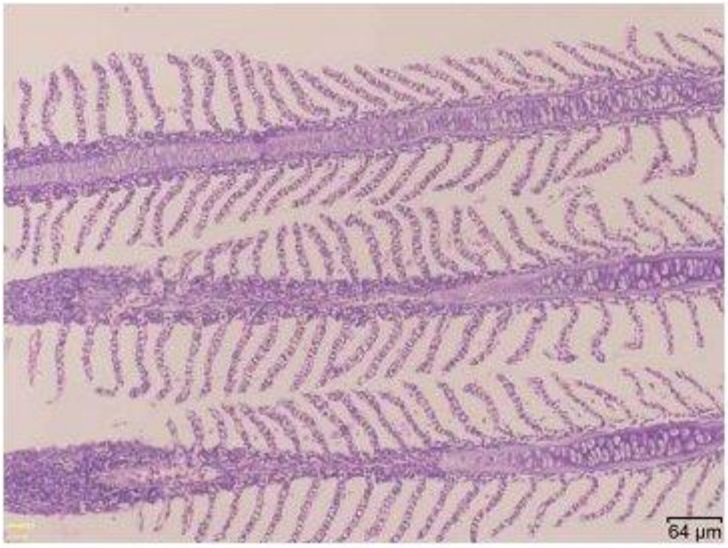
CONTROL GILL



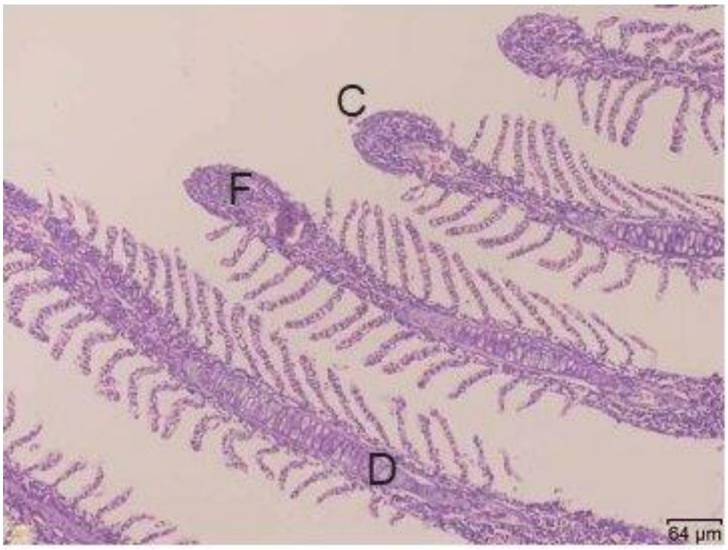
5 PSU GILL

* A, SL - SECONDARY LAMELLAE
C - CLUB FORMATION
E - COMPLETE LOSS OF SL
B - PRIMARY LAMELLAE
D - ENLARGEMENT
G - FUSION OF SL
H - HYPERDLACIA
F - PRIMARY LAMELLAE

Figure 13: Histopathological study of gills (Control, and 5 PSU salinity treatments)



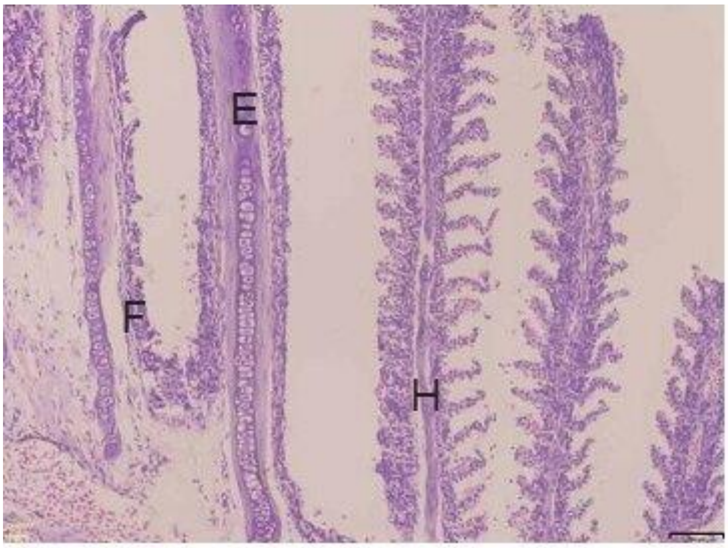
10 PSU GILL



15 PSU GILL

* A, SL - SECONDARY LAMELLAE
C - CLUB FORMATION
E - COMPLETE LOSS OF SL
B - PRIMARY LAMELLAE
D - ENLARGEMENT
G - FUSION OF SL
H - HYPERDLACIA
F - PRIMARY LAMELLAE

Figure 14: Histopathological study of gills (10 PSU, and 15 PSU salinity treatments)



20 PSU GILL

25 PSU GILL

* A, SL - SECONDARY LAMELLAE
C - CLUB FORMATION
E - COMPLETE LOSS OF SL
B - PRIMARY LAMELLAE
D - ENLARGEMENT
G - FUSION OF SL
H - HYPERDLACIA
F - PRIMARY LAMELLAE

Figure 15: Histopathological study of gills (20 PSU, and 25 PSU salinity treatments)

4.4.2. Histopathological study of liver

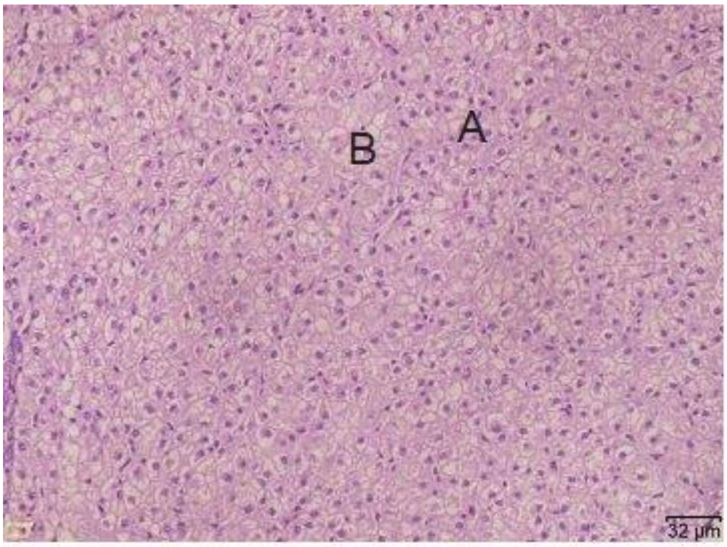
In the control slides (0 PSU), the regular architecture of live parenchyma, and normal hepatocytes were observed. At 5 PSU salinity, no distinct pathological changes were noted; Kauffer cells in the sinusoids were also seen.

At 10 PSU, mild enlargement of the hepatocytes due to intracellular vacuolation was observed. Whereas at 15 PSU salinity, intracellular vacuolation, as well as dilated sinusoids, were found.

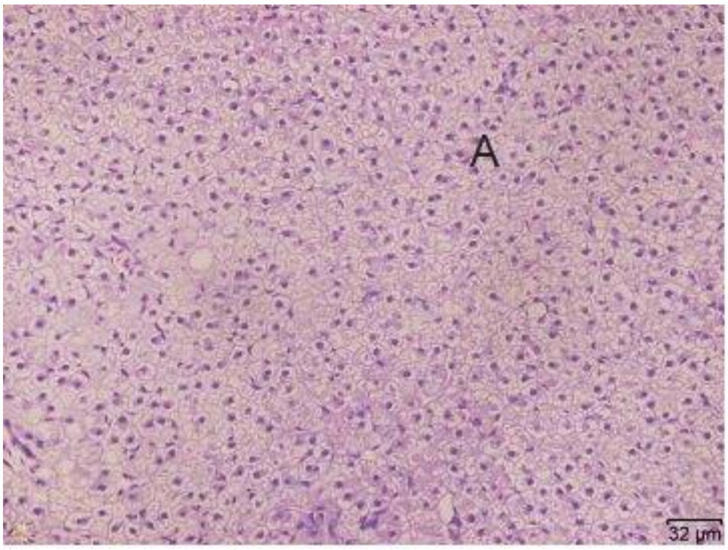
At 20 PSU salinity, a few hepatocytes were observed to have lost their nuclei.

Pyknotic nuclei were also observed in this situation, but no necrosis was found.

At 25 PSU, lipid deposition was seen, which has displaced nuclei into the periphery of the cells. Focal hemorrhage was also observed in these sections.



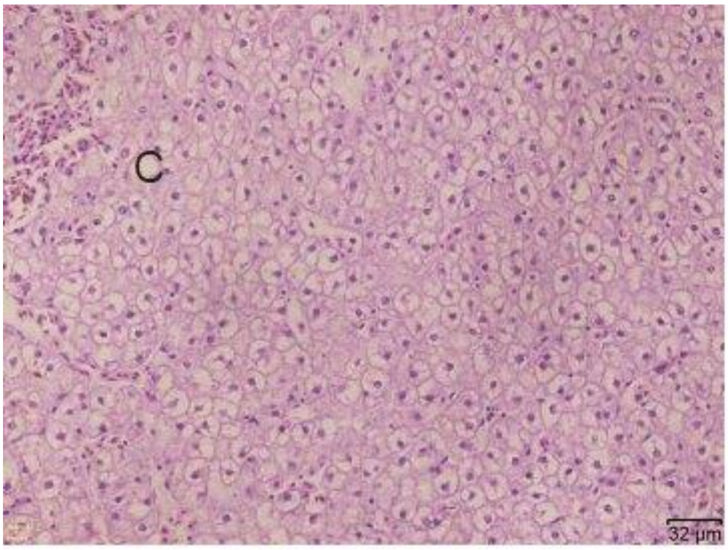
CONTROL LIVER



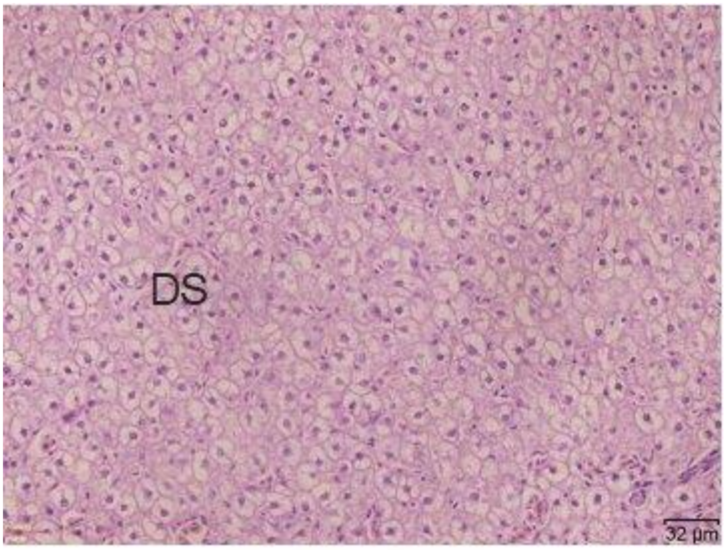
5 PSU LIVER

* A - HEPATOCYTES B - SINUSOIDS C - MILD ENLARGEMENT
DS - DIALATED SINUSOIDS E - LOSS OF NUCLEI

Figure 16: Histopathological study of Liver (Control, and 5 PSU salinity treatments)



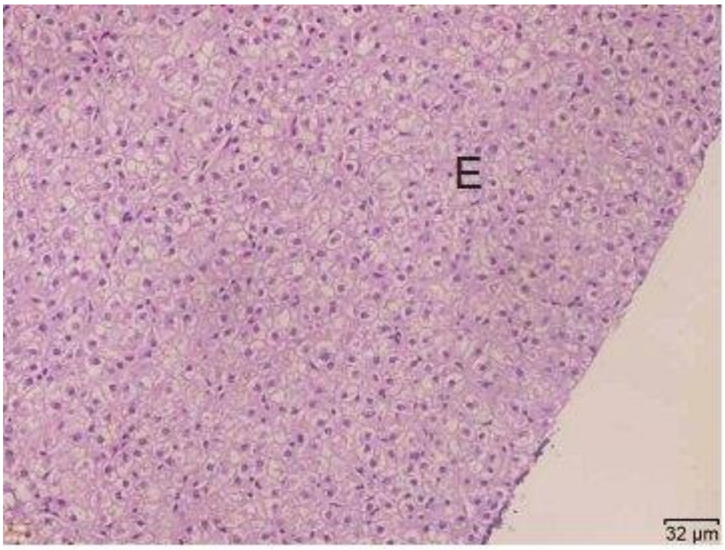
10 PSU LIVER



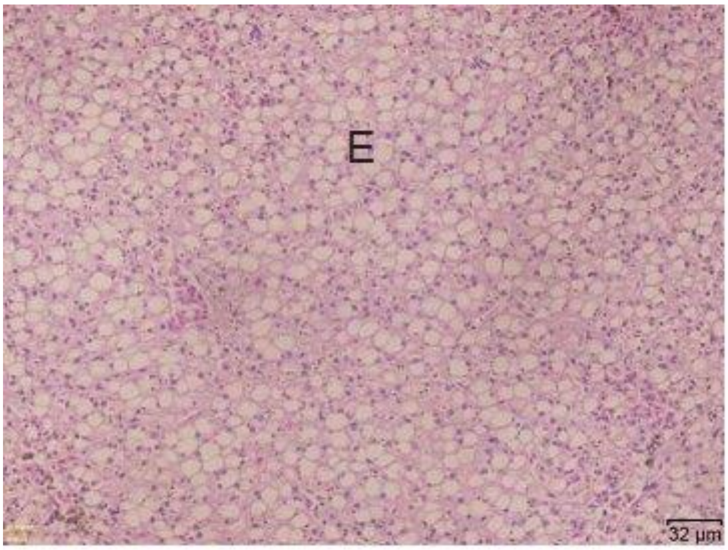
15 PSU LIVER

* A - HEPATOCYTES
B - SINUSOIDS
C - MILD ENLARGEMENT
DS - DIALATED SINUSOIDS
E - LOSS OF NUCLEI

Figure 17: Histopathological study of Liver (10 PSU, and 15 PSU salinity treatments)



20 PSU LIVER



25 PSU LIVER

* A - HEPATOCYTES B - SINUSOIDS C - MILD ENLARGEMENT
DS - DIALATED SINUSOIDS E - LOSS OF NUCLEI

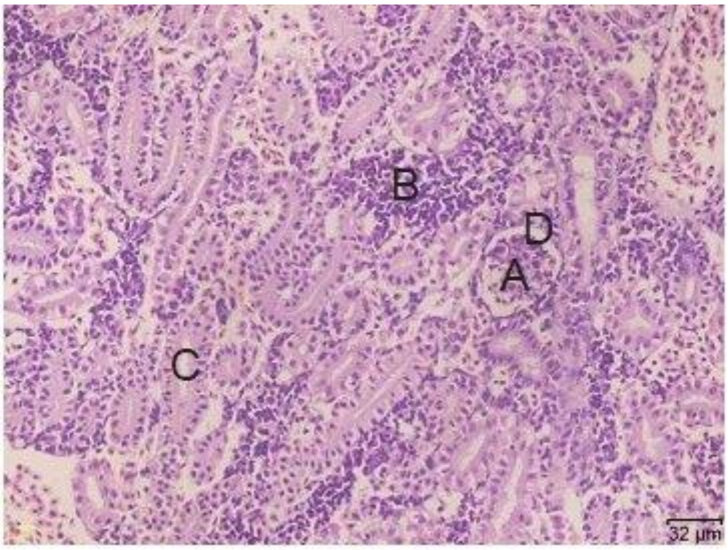
Figure 18: Histopathological study of Liver (20 PSU, and 25 PSU salinity treatments

4.4.3. Histopathological study of Kidney

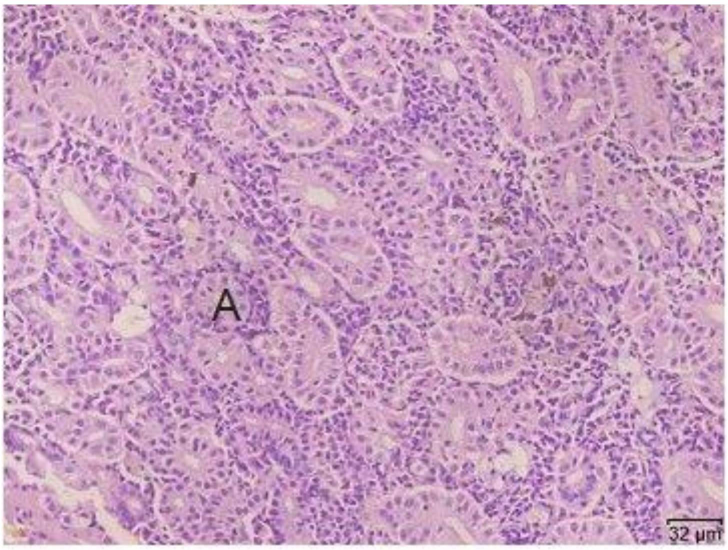
In the control slides (0 PSU), typical renal tubes were seen with regular Bowman’s capsule with glomerulus. Similar situations were found at 5 PSU salinity also.

Increased hematopoietic activities were found in the parts of 10 PSU, and 15 PSU. Signs of high vacuolations were found in individual sections of 15 PSU.

At 20 PSU salinity level, glomerulus is disorganized, and mild hemorrhages at a few places were also found. Whereas at 25 PSU salinity level, sloughed off renal tubules were observed with the disruption of tubules, and a few tubules showing the signs of necrosis. Here the lumens were filled with exudates.



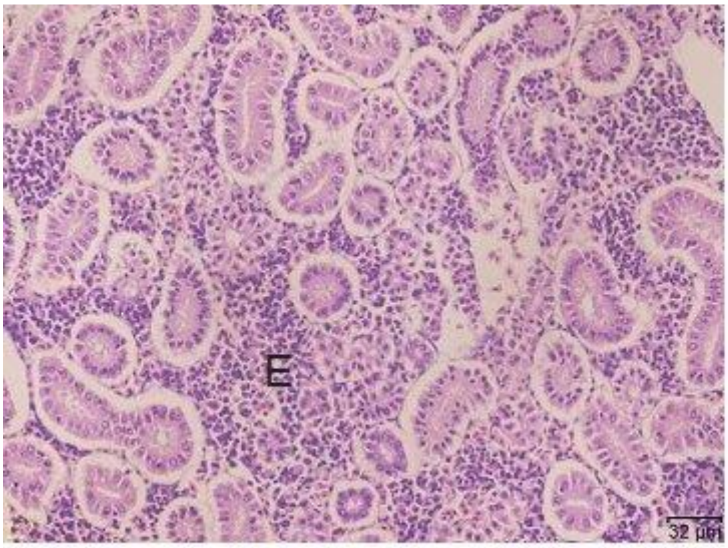
CONTROL KIDNEY



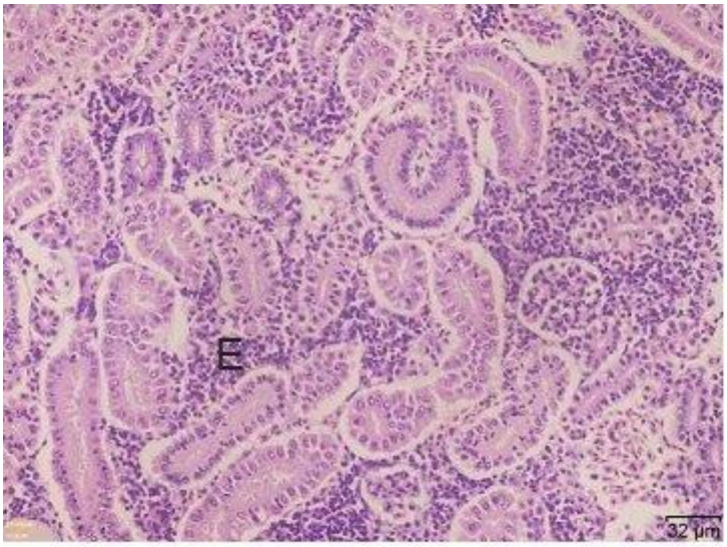
5 PSU KIDNEY

*A - GLOMERULUS
B - HAEMATOPOITIC
C - RENAL TUBULES
D - BOWMANS CAPSULE
E- INCREASED HAEMATOPOITIC ACTIVITY
F - ENLARGEMENT OF RENAL TUBULES
G - VACCULATION
H - NECROSIS

Figure 19: Histopathological study of Kidney (Control, and 5 PSU salinity treatments)



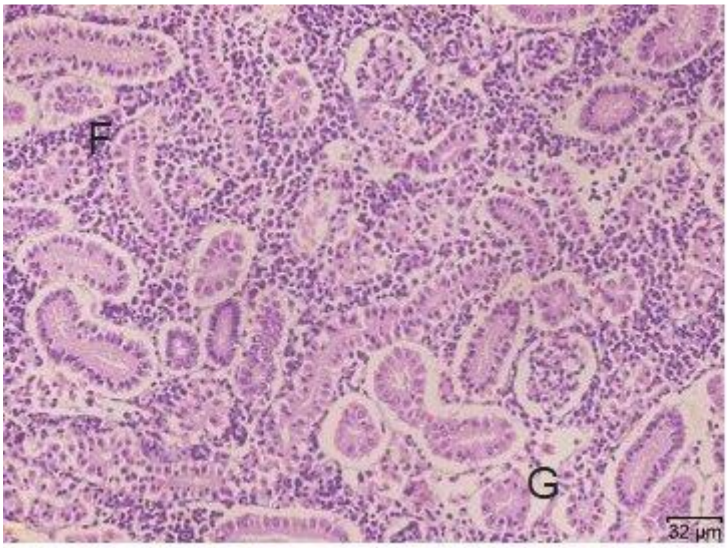
10 PSU KIDNEY



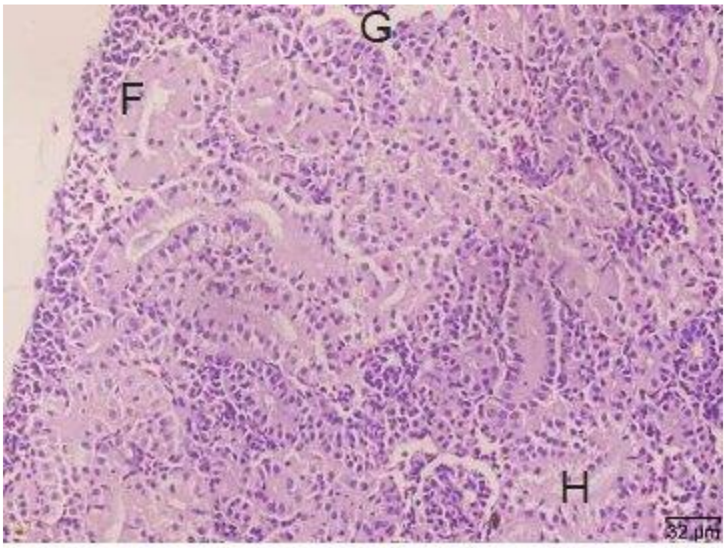
15 PSU KIDNEY

*A - GLOMERULUS B - HAEMATOPOITIC C - RENAL TUBULES
D - BOWMANS CAPSULE E- INCREASED HAEMATOPOITIC ACTIVITY
F - ENLARGEMENT OF RENAL TUBULES G - VACCULATION H - NECROSIS

Figure 20: Histopathological study of Kidney (10 PSU, and 15 PSU salinity treatments)



20 PSU KIDNEY



25 PSU KIDNEY

*A - GLOMERULUS
B - HAEMATOPOITIC
C - RENAL TUBULES
D - BOWMANS CAPSULE
E- INCREASED HAEMATOPOITIC ACTIVITY
F - ENLARGEMENT OF RENAL TUBULES
G - VACCULATION
H - NECROSIS

Figure 21: Histopathological study of Kidney (20 PSU, and 25 PSU salinity treatments)

DISCUSSION

5. DISCUSSION

The result of the present investigation confirmed that common carp fingerlings could survive in water having salinity between 0 PSU, and five PSU. In both salinities of 0 PSU, and five PSU, irrespective of the treatments used, no mortality was recorded. This result indicates that the salinity range was withstood by the fish. As per Kurata (1959), and Holiday, and Jones (1967), the capability of the body fluids to function at least for a short time in an unusual range of atypical internal osmotic, and ionic concentrations influences the survival of the fish. The body fluid could be regulated for restoring the level of osmotic pressure to optimum range could be made possible by the fish. An increase in osmotic concentration of fish blood serum, and change in ionic contents can be caused by the migration or abrupt transfer of fish from freshwater to hypersaline water (Gordon, 1959; Miles, and Smith, 1968)

A 100% mortality was recorded when fingerlings were exposed to 20 PSU salinity that indicated that osmoregulatory failure at this salinity level. Deacon, and Hecht, (1999) while studying the impact of salinity on growth, protein conversion efficiency, and food conversion in juvenile spotted grunter, when the fish were exposed to 20 PSU salinity, it was observed that the mortality occurred after 24 hours, and that could be an outcome of the progressive deterioration in the osmotic, and ionic regulatory mechanisms including the fish inability to control the excessive water loss, leading to osmoregulatory exhaustion, collapse, and finally death. In a similar study regarding the preference of hatchery-reared Nile tilapia, and salinity tolerance, mortality occurred due to stress, duress, and less resistance of the fish to higher salinity level (Lawson, and Anetekhai, 2011).

In the current study, when the fish were exposed in 20 PSU, and 25 PSU salinity, respiratory distress such as gulping of air, increase in opercular activities, excessive mucus, and vertical movement was observed. When exposed to higher salinities, the fish were approaching their tolerance limits, and water loss at a rapid rate to external medium from the fish, erratic behaviour or restlessness were observed (Lawson, and Anetekhai, 2011). This might be caused due to the biochemical body derangement, which includes hepatic compromise (Fadina et al., 1991). As an adaptive mechanism to hypersaline environments, increased opercular frequency activities have been reported (Morgan, and Eiwama., 1991; Iwama et al., 1997; De Boeck et al., 2000). These signs might be caused because the gills reduces respiratory activity in fishes, and unable them to actively carry out the gaseous exchange, which results in the excessive mucus secretions (De Boeck et al., 2000). Reid et al., (2006), lamellar hyperplasia was observed by the increase in the salinity concentration which interrupted the respiratory system in the fish. According to De Boeck et al. (2000), the ability of common carp *Cyprinus carpio* to compact with hypoxic conditions were affected by the long term exposure to hypersaline conditions, and the critical oxygen concentrations for oxygen expenditure increased. Aside from that, it also resulted in disparity in the regulation of ammonia excretion. However, in the lateral muscle, the cytosolic phosphorylation potential (the index of the energy status of a cell in terms of potential transferable phosphate groups) remained relatively uninfluenced, which indicates that the oxygen transport to the tissues was not severely affected. Hence when common carp are exposed to hypersaline conditions, the capacity to cope with hypoxic conditions were reduced, which might be due to the high energetic cost of hyperventilation under conditions where energy stores are depleted, and not because of any impeded oxygen transport mechanisms.

When fish were exposed to 20 PSU, and 25 PSU, severe lesions in gills were observed, such as lamellar fusion, hyperplasia, and necrosis. This is in concordance with the findings of Mallat (1985), who reported that necrosis, hyperplasia, and lamellar fusion are the most common gill lesions induced by toxic substances, and other chemicals. And when exposed to higher salinities, produces lamellar lesions such as necrosis, which would lead to death of the fish. The dysfunction of fish gas exchange ability, which was caused due to the degeneration of gills results in an anoxic internal behaviour (Ajani et al. 2007).

In the present study, when salinities were increased from 0 PSU at any rate, no mortalities were observed to a salinity level of 7 PSU. The mortalities started occurring from a salinity level of 8 PSU, and as the salinity level increased, the rate of mortality was also increased. A survival rate of 95, and 89.33% survival rate was recorded for common carp fingerlings when exposed to 10 PSU salinity, using different scales of salinity treatments. Further, it was observed that irrespective of the increasing rate of salinities, there was no notable difference in the survival rate of the common carp fingerlings when exposed up to 10 PSU salinity level. Whereas, a remarkable difference in the survival rates between the treatments were observed when the common carp fingerlings were exposed to salinity levels of 15 PSU, and 20 PSU. Among the eleven salinity treatments conducted during the present study regarding the survival analysis of common carp fingerlings to different salinity levels, the best acclimatization protocol was treatment number 5, where salinity was increased up to 15 PSU at a rate of 1 PSU per every day, where 77% of survival of the animals were recorded. A 100% mortality was recorded when the animals were exposed to a higher salinity level of 20 PSU.

Variation in tolerance to higher-level salinities was evident from the results obtained in this study, and that may be due to physiological changes during development or possibly due to direct effects of ionic composition on salinity tolerance, differences in experimental procedures, acclimation time, and salinity mediums. Further, the seasonal influences might also cause variation in results of salinity tolerance tests. Among the various stages of stenohaline freshwater fish, salinity tolerances differ among fry, fingerling, and yearling (Allen, and Avault, 1970; Holl, ander, and Avault, 1975; Murai, and, andrews 1977). Common carp, however, have withstood salinities as high as 17 PSU (A1-Hamed, 1971). Because the salinity tolerance of common carp is higher than that of grass carp, the physiological stress on common carp at 12 PSU saltwater was probably not as high as on grass carp in terms of weight loss. Privolnev (1967) reported a 5.4% body weight loss of common carp yearlings at a salinity of 12 PSU.

Mangat, and Hundal (2014), in their study on salinity tolerance of laboratory, reared fingerlings of common carp, which was conducted during different seasons came to a conclusion that in accordance to the salinity-temperature regime, the fingerlings were able to regulate their body physiology. It was supported by the studies of Islam et al. (2014). They reported 100% survival rate for rohu fingerlings at 0 to 6 PSU salinities. When exposed to 12 PSU salinity, mortality was recorded from the very first day, followed by 100% mortality within 3-4 days during summer, and autumn season, which indicates stress conditions leading to fatality during these temperature ranges (Holliday, and Jones, 1967). As the primary organ involved in osmoregulation, primarily the gills get affected, when there is stress in freshwater fish, which also results a waste in nitrogen excretion (Nikolsky, 1963). For young, and eggs, upper tolerance limits have been reported as 6 PSU, and 4.5 PSU respectively (McCrimmon, 1968). A high disruption in the epithelium with diffuse edema of both the primary, and the secondary lamellae was also caused when gills get exposed to higher salinities (Holliday, and Jones, 1967). A 100% mortality of fish fingerlings in 12 PSU salinity during the two seasons might be caused due to this reason. 71-90% mortality in 24 hours under 15% salinity in grass carp fingerlings were also recorded

(Kilambi, and Zdinak, 1980). A report of common carp found in brackish-water marshes with salinities up to 14 PSU in Southern France as also recorded Crivelli (1981).

Substantial respiratory distresses in fish were caused by direct transfer from freshwater to higher salinity conditions. Histopathological studies of fish after exposing them at 25 PSU salinity, which exhibited a severe lesion on the gills such as necrosis, hyperplasia, and lamellar fusion, which indicates that the fish were approaching it's tolerate limits, and developed osmoregulatory failure (Lawson, and Anetekhai, 2011). According to Yahona, and Pablo, (2007) severe lesions on the gills, and kidney, like severe hydropic degeneration, edema, and necrosis which lead to mortality were caused when the fish were exposed to higher salinities. Oti, and Ukpabi (2000) recorded the abnormal nervous behaviours such as sluggish, and swirling movements with different postures, state of motionless, darts, followed by failure of the kidney function, which ultimately resulted in the death of the fish. The lesions of the kidney tissue included hydropic degeneration, edema in Bowman's capsule, hemorrhage, and necrosis.

When the fish were exposed to 15 PSU salinity, the histopathology changes in the kidney indicated mild hydropic degeneration of renal tubules. Severe lesions in the kidney were observed when the common carp fingerlings were exposed 20 PSU salinity. Further, when the fish were exposed to 25 PSU salinity, gross necroses of kidney tubules were found. For the estimation of the maximum bearable concentration of chemicals in the context of fish culture requirements, histopathological changes in fish tissue, as well as the residue levels of test substances in fish are very crucial parameters (Svobodova et al., 1994).

At least under normal circumstances, unlike gills, the liver is protected from environmental exposure to the external environment. However, due to an efficient enterohepatic cycling mechanism it is prone to chemical assault (Gingerich, 1982). Because of the prominent role of liver in energy storage, and metabolism, stress responses may also be evident in it. At 10 PSU, mild enlargement of the hepatocytes due to intracellular vacuolation was observed. Whereas, at 15 PSU salinity, intracellular vacuolation, as well as dilated sinusoids, were also observed. At 20 PSU salinity, a few hepatocytes were observed to have lost their nuclei. Pyknotic nuclei were also observed in this situation. However, necrosis was not found. At 25 PSU, lipid deposition was seen, which has displaced nuclei into the periphery of the cells. Focal bleeding was also observed in these sections. The gill chloride cell hyperplasia, gill lamellar epithelial separation, glomerular swelling, and blood congestion in kidneys, and deposition of hyaline droplets in kidney tubules, glomeruli, and hemopoietic tissues were the most significant of these changes. It was reported that, in response to high external saline concentrations, the number of chloride cells in gill lamellae was increased (Keys, and Willmer, 1932; Parry et al., 1959; Doyle, and Gorecki, 1961).

The genetic structure of the wild populations of common carps is very poorly understood. Most phylogeographic, and population genetics studies were done on farmed stocks, and looked at the difference between two subspecies, the genetic variability within, and among populations, and the genetic difference between them. A combination of forces including geographic locations, adaptation, and accumulation of mutations, and natural as well as human pressures produces the variants of common carp (for example, races, landraces, strains, breeds, and stocks) are developed through (Hulata, 1995). In the studies of Jackson et al. 2001, abiotic elements can have a notable influence on fish populations, often overriding density-dependent controls. Despite the circumpolar distribution of the species, limited information is available describing factors describing the spatial variations in population characteristics. According to Matsuzaki et al., 2009, there were striking differences in morphological, and behavioural traits among the two domesticated strains of common carps to that of the lake Biwa wild strain. Since the fish were reared from eggs under similar environment conditions, these phenotypic differences are considered to have a genetic basis (Johnsson et al., 2001; Metcalfe et al., 2003). However, in the present study on the survival analysis of common carp fingerlings from two different stocks (Rohtak, and Powerkheda stock), which were exposed to different salinity levels ranging from 0 PSU to 20 PSU, no remarkable difference in the survival rates of the two stocks were recorded.

Thus, it can be concluded that common carp fingerlings have a 100% survival rate up to 7 PSU salinity level, and can be survived up to 15 PSU salinity with an average survival rate of above 70%. The salinity of the inland water sources available in Haryana, and Punjab was recorded to be ranging up to a level of 15 PSU. The present results indicate that common carp fingerlings can tolerate salinity up to 7 PSU, and acclimatization of the common carp fingerlings at the rate of 1 PSU per each day may result in higher survivability, and this salinity can be increased up to 15 PSU. Hence, the better protocol for acclimatization of the common carp fingerlings to the salinity levels of inland saline waters (15 PSU) with maximum survival rate could be to increase the salinity at a rate of 1 PSU per every day until 15 PSU (Treatment No.5).

SUMMARY

6. SUMMARY

Salinity tolerance is a crucial factor in optimizing the underlying conditions for the cultivation of many freshwater fishes having the potential for aquaculture in inland saline water. Like other environmental factors, fluctuations in salinity affect functional traits related to survival, growth, and other physiological functions of freshwater fishes. Freshwater fishes regulate osmotic pressure by consuming energy when environmental salinity changes. But sudden changes in salinity exceeding the tolerance limits cause the death of an aquatic animal. Therefore, it is important to understand the effects of salinity on the survival, and tolerance of common carp when subjected to changes in salinity. The knowledge about salinity acclimation, tolerance, metabolism, and ion-osmoregulation could be applied for aquaculture production in inland saline water. The understanding of salinity tolerance at different temperatures, and the complex osmoregulatory mechanism, and the physiological responses of freshwater stenohaline species to saline environments has recently evoked a higher interest. All the above aspects need to be explored before the use of saline water for the optimization of aquaculture practices.

Survival analysis makes an inference about event rates as a function of time. The two primary methods to estimate the actual underlying survival curve are the Kaplan– Meier estimator and Cox proportional hazards regression. The Kaplan–Meier estimator is simple, and supports stratification factors but cannot accommodate covariates. The Cox model does provide a framework for making inferences about covariates, and some versions require proportional hazards, although all versions are quite flexible when used, and interpreted correctly. Independent censoring, either directly in the Kaplan–Meier estimator or given covariates in the Cox model, is a requirement for consistent, unbiased estimates. Survival analysis can handle right censoring, staggered entry, recurrent events, competing risks,, and much more as long as we have available representative risk sets at each time point to allow us to model, and estimate event rates.

In the present study, out of the 3000 fish collected from CIFE Powerkheda Centre, a total of 2050 common carp fingerlings were exposed to various salinity treatments (11 salinity treatments) along with a set of control at 0 PSU salinity. In treatment no. 1, and 2, salinity was increased up to 10 PSU, and in treatment no. 3, 4, and 5, salinity was increased up to 15 PSU to varying speeds of increasing salinities. From treatment no. 6 to 11, salinity was increased up to 25 PSU at different rates of increasing salinities. Observations regarding the survival of fish from each of the treatments were recorded on a daily basis. Tissue samples of gills, kidney, and liver from salinity levels of 0 PSU, 5 PSU, 10 PSU, 15 PSU, 20 PSU & 25 PSU were collected for histopathological studies. The survival analyses were carried out in SAS using the appropriate SAS procedure on a SAS dataset. The essential SAS procedures for performing survival analysis was PROC LIFETEST.

The most significant histopathological changes observed were gill chloride cell hyperplasia, gill lamellar epithelial separation, glomerular swelling, and blood congestion in kidneys, and deposition of hyaline droplets in kidney tubules, glomeruli, and hemopoietic tissues. The number of chloride cells in gill lamellae increased in response to high external saline concentrations.

In general, in common carps salinity up to 5 PSU is tolerated, optimal pH is 6.5 to 9.0, and common carps can survive low oxygen concentration (0.3 - 0.8 mg/L) and supersaturation. In the present study, it's been recorded that, when salinities were increased from 0 PSU at any rate, no mortalities were observed to a salinity level of 7 PSU. The mortalities started occurring from a salinity level of 8 PSU, and as the salinity level was increased, the rate of mortality also increased. A survival rate of 95%, and 89.33% survival rate was recorded for common carp fingerlings when exposed to 10 PSU salinity when exposed to different standards of salinity treatments (T1, and T2, respectively). But it was recorded that, irrespective of the increasing rate of salinities, there is no significant difference in the survival rate of the common carp fingerlings when exposed up to 10 PSU salinity level. In contrast, a considerable difference in the survival rates between the treatments was observed when the common carp fingerlings were exposed to salinity levels of 15 PSU, and 20 PSU. Among the eleven salinity treatments conducted during the present study regarding the survival analysis of common carp fingerlings to different salinity levels, the best acclimatization protocol was treatment number 5, where salinity was increased up to 15 PSU at a rate of 1 PSU per every day, where 77% of survival of the animals were recorded. 100% mortality was recorded when the animals were exposed to salinity levels of 20 PSU. However, in the present study on the survival analysis of common carp fingerlings from two different stocks (Rohtak, and Powerkheda stock), which were exposed to different salinity levels ranging from 0 PSU to 20 PSU, no significant difference in the survival rates of the two stocks were recorded.

It’s been recorded that the salinity range of the inland water sources in the areas of Punjab, and Haryana ranges up to 15 PSU salinity. The primay aquaculture practices adopted by the fish farmers in those areas are of shrimp culture with Litopenaeus vannami, Pangassius, etc. development of a protocol for acclimatization, and culture practices of economically important food fish like as common carp can act as a boost in the total aquaculture production in those areas.

REFFERNCE

7. REFERENCES

Abrol, I.P., and Bhumbla, D.R., 1971. Saline and alkali soils in India-their occurrence and management. *World soil resources report*, 41: 42-51.

Ajani, F., Olukunle, O.A., and Agbede, S.A., 2007. Hormonal and hematological responses of *Clarias gariepinus* (Burchell 1822) to nitrate toxicity. *Journal of Fisheries International,* 2(1): 48 - 53

Avella, M., and Bornancin, M., 1989. A new analysis of ammonia and sodium transport through the gills of the freshwater rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology*, *142*(1): 155-175.

Bártfai, R., Egedi, S., Yue, G. H., Kovács, B., Urbányi, B., Tamás, G., Horváth, L. and Orbán, L., 2003. Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. *Aquaculture*, *219*(1-4): 157-167.

Barton, B. A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and comparative biology*, *42*(3): 517-525.

Basavaraju, Y. and Reddy, A.N., 2013. Growth performance of Amur strain of common carp in southern Karnataka. *Mysore Journal of Agricultural Sciences*, *47*(1): 119-123.

Boeuf, G., and Payan, P., 2001. How should salinity influence fish growth?. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *130*(4): 411-423.

Cary, N.C., 2011. SAS Institute Inc SAS/STAT® 9.3 user's guide: the MIXED procedure (Chapter). *SAS Institute Inc*.

Chervinski, J., and Zorn, M., 1974. Note on the growth of *Tilapia aurea* (Steindachner) and *Tilapia zillii* (Gervais) in sea-water ponds. *Aquaculture*, *4*: 249-255.

Chervinski, J., 1977. Note on the adaptability of silver carp *Hypophthalmichthys* molitrix (Val.) and grass carp *Ctenopharyingodon idella* (Val.) To various saline concentrations. *Aquaculture*, *11*(2): 179-182.

Chervinski, J., 1983. Salinity tolerance of the mosquitofish, *Gambusia affinis* (Baird and Girard). *Journal of Fish Biology*, *22*(1): 9-11.

Crivelli, A.J., 1981. The biology of the common carp, *Cyprinus carpio* L. in the Camargue, southern France. *Journal of Fish Biology*, *18*(3): 271-290.

De Boeck, G., Nilsson, G., Vlaeminck, A., and Blust, R., 1996. Central monoaminergic responses to salinity and temperature rise in common carp. *Journal of experimental biology*, *199*(7): 1605-1611.

De Boeck, G., Vlaeminck, A., Van der Linden, A., and Blust, R., 2000. Salt stress and resistance to hypoxic challenges in the common carp (*Cyprinus carpio L*.). *Journal of Fish Biology*, *57*(3): 761-776.

De Boeck, G., Vlaeminck, A., Van der Linden, A., and Blust, R., 2000. The energy metabolism of common carp (*Cyprinus carpio*) when exposed to salt stress: an increase in energy expenditure or effects of starvation. *Physiological and Biochemical Zoology*, *73*(1): 102-111.

Deacon, N., and Hecht, T., 1999. The effect of reduced salinity on growth, food conversion, and protein efficiency ratio in juvenile spotted grunter, *Pomadasys commersonnii* (Lacépède) (Teleostei: Haemulidae). *Aquaculture Research*, *30*(1): 13-20.

Doyle, W.L., and Gorecki, D., 1961. The so-called chloride cell of the fish gill. *Physiological Zoology*, *34*(2): 81-85.

Evans, D. H., 1967. Sodium, Chloride, and Water Balance of the Intertidal Teleost, *Xiphister atropurpureus*: III. The Roles of Simple Diffusion, Exchange Diffusion, Osmosis and Active Transport. *Journal of Experimental Biology*, *47*(3): 525-534.

FAO, 2014. The state of world fisheries and aquaculture. Rome, FAO.173p.

FAO, 2016. The state of world fisheries and aquaculture. Rome, FAO.197p

.Feldmeth, C. R., and Waggoner, J. P., 1972. Field measurements of tolerance to extreme hypersalinity in the California killifish, *Fundulus parvipinnis*. *Copeia*, *1972*(3): 592-594.

Fielder, D.S., Bardsley, W.J. and Allan, G.L., 2001. Survival and growth of Australian snapper, *Pagrus auratus*, in saline groundwater from inland New South Wales, Australia. *Aquaculture*, *201*(1-2): 73-90.

Galat, D. L., Post, G., Keefe, T. J. and Bouck, G. R., 1985. Histological changes in the gill, kidney and liver of Lahontan cutthroat trout, *Salmo clarki henshawi*, living in lakes of different salinity-alkalinity. *Journal of fish biology*, *27*(5): 533-552.

Garg, S. K., 1996. Brackishwater carp culture in potentially waterlogged areas using animal wastes as pond fertilizers. *Aquaculture International*, *4*(2): 143-155.

Gordon, M. S., 1959. Ionic regulation in the brown trout (*Salmo trutta* L.). *Journal of Experimental Biology*, *36*(2): 227-252.

Greenwell, M. G., Sherrill, J. and Clayton, L. A., 2003. Osmoregulation in fish. Mechanisms and clinical implications. *The veterinary clinics of North America. Exotic animal practice*, *6*(1): 169-89.

Holliday, F. G. T. and Jones, M. P., 1967. Some effects of salinity on the developing eggs and larvae of the plaice (*Pleuronectes platessa*). *Journal of the marine biological Association of the United Kingdom*, *47*(1): 39-48.

Hulata, G., 1995. A review of genetic improvement of the common carp (*Cyprinus carpio L.)* and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture*, *129*(1-4): 143-155.

Islam, M., Ahsan, D. A., Mandal, S. C. and Hossain, A., 2014. Effects of salinity changes on growth performance and survival of rohu fingerlings, *Labeo rohita* (Hamilton, 1822). *Journal of Coastal Development*, *17*: 379.

Iwama, G. K., Takemura, A. and Takano, K., 1997. Oxygen consumption rates of tilapia in freshwater, seawater, and hypersaline seawater. *Journal of Fish Biology*, *51*(5): 886-894.

Jackson, D. A., Peres-Neto, P. R. and Olden, J. D., 2001. What controls who is where in freshwater fish communities the roles of biotic, abiotic, and spatial factors. *Canadian journal of fisheries and aquatic sciences*, *58*(1): 157-170

Keys, A. and Willmer, E.N., 1932. 'Chloride secreting cells' in the gills of fishes, with special reference to the common eel. *The Journal of Physiology*, *76*(3): 368-378.

Kilambi, R. V. and Zdinak, A., 1980. The effects of acclimation on the salinity tolerance of grass carp, *Ctenopharyngodon idella* (Cuv. and Val.). *Journal of Fish Biology*, *16*(2): 171-175.

Kurata, H., 1959. Preliminary report on the rearing of the herring larvae. *Bull. Hokkaido Reg. Fish. Res. Lab*, *20*: 117-138.

Lawson, E. O. and Anetekhai, M. A., 2011. Salinity tolerance and preference of hatchery-reared Nile tilapia, *Oreochromis niloticus* (Linneaus, 1758). *Asian Journal of Agricultural Sciences*, *3*(2): 104-110.

Maceina, M.J. and Shireman, J.V., 1979. Grass carp: effects of salinity on survival, weight loss, and muscle tissue water content. *The Progressive Fish-Culturist*, *41*(2): 69-73.

Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fisheries and Aquatic Sciences*, *42*(4): 630-648.

Mangat, H. K. and Hundal, S. S., 2014. Salinity tolerance of laboratory-reared fingerlings of common carp, *Cyprinus carpio* (Linn.) during different seasons. *International Journal od Advanced Research*, *2*(11): 491-496.

Marshall, W. S., 1995. 1 Transport Processes in Isolated Teleost Epithelia: Opercular Epithelium and Urinary Bladder. In *Fish physiology* (Vol. 14: 1-23). Academic Press

Matsuzaki, S. S., Mabuchi, K., Takamura, N., Nishida, M. and Washitani, I., 2009. Behavioral and morphological differences between feral and domesticated strains of common carp *Cyprinus carpio*. *Journal of Fish Biology*, *75*(6): 1206-1220.

Metcalfe, N. B., Valdimarsson, S. K. and Morgan, I. J., 2003. The relative roles of domestication, rearing environment, prior residence and body size in deciding territorial contests between hatchery and wild juvenile salmon. *Journal of Applied Ecology*, *40*(3): 535-544.

Mccormick, S.D., 1995. 11 hormonal control of gill Na+, K+-ATPase and chloride cell function. In *Fish physiology*,Academic Press, Vol. 14: 285-315

Miles, H.M. and Smith, L.S., 1968. Ionic regulation in migrating juvenile coho salmon, *Oncorhynchus kisutch*. *Comparative Biochemistry and Physiology*, *26*(2): 381-398.

Nelson, J.S., 1968. Salinity tolerance of brook sticklebacks, *Culaea inconstans*, freshwater nine spine sticklebacks, *Pungitius pungitius*, and freshwater four spine sticklebacks, *Apeltes quadracus*. *Canadian Journal of Zoology*, *46*(4): 663-667.

NFDB Annual Report 2017-18: 61-63

Nikolsky, G.V., 1963. *The ecology of fishes* (Trans L. Birkett) Academic Press. London: 352p.

Oti, E.E. and Ukpabi, U.H., 2000. Acute toxicity of milk extract of *Thevetia peruvianna* to African catfish (hybrids). *Journal of Fisheries science and Technologyl*, *1*(1): 132-138.

Paravatheswararao, V., 1970. Adaptation to osmotic stress in fishes. *Indian Biologist*, *2*(1): 16-36.

Perry, S. F., 1998. Relationships between branchial chloride cells and gas transfer in freshwater fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *119*(1): 9-16.

Potts, W. T. W. and Evans, D. H., 1967. Sodium and chloride balance in the killifish *Fundulus heteroclitus*. *The Biological Bulletin*, *133*(2): 411-425.

Privolnev, T. I., 1967. Effects of environmental salinity on water metabolism in freshwater fish. *Metabolism and Biochemistry of Fishes*. *Journal of Fish Biology,* 16(6): 613-619

Rao, G. M. M., 1969. Effect of activity, salinity, and temperature on plasma concentrations of rainbow trout. *Canadian Journal of Zoology*, *47*(1): 131-134.

Rao, G.M.M., 1971. Influence of activity and salinity on the weight-dependent oxygen consumption of the rainbow trout *Salmo gairdneri*. *Marine Biology*, *8*(3): 205-212.

Ried SG, Sundin L, Milson WK (2006) The Physiology of Tropical Fishes*, Elsevier*, Academic Press, Italy, *Environmental Toxicology and Pharmacology* 25(3): 365-72

Sardella, B. A., Matey, V., Cooper, J., Gonzalez, R. J. and Brauner, C. J., 2004. Physiological, biochemical and morphological indicators of osmoregulatory stress inCalifornia'Mozambique tilapia (*Oreochromis mossambicus* × *O. urolepis hornorum*) exposed to hypersaline water. *Journal of Experimental Biology*, 207(8): 1399-1413.

Sarwono and Hidayat, A. 2005. Effect of salinity on osmoregulatory capacity, feed consumption, feed efficiency and growth of juvenile sea bass (*Latescalcarifer Bloch) Graduate School, Kasetsart University* ISBN974-273-031-8.

Schwartz, F. J. (1964): Natural salinity tolerances of some freshwater fishes. *Underwater Naturalist Vol. 2*, No. 2: 13-15.

Singh S. K. and Kanga S., 2017. Mapping and of Salt Affected and Waterlogged Areas using Geospatial Technique. International Journal on Recent and Innovation Trends in Computing and Communication. 5: 1298-1305

Soares M. G M., Menezes N.A., Junk W.J. (2006) Adaptations of fish species to oxygen depletion in a central Amazonian floodplain lake. *Hydrobiologia* 568: 353-367.

Stickney, R.R., 1986. Tilapia tolerance of saline waters: a review. *The Progressive Fish-Culturist*, *48*(3): 161-167.

Svobodova Z., Vykusova B., Machova J. (1994) The Effects of Pollutants on Selected Hematological and Biochemical Parameters in Fish. In: Sublethal and Chronic Effects of Pollutants on Freshwater Fish, Muller R, Lloyd R (Eds.). *Fishing News Books*: 39-52, UN

Svobodova, Z., Vykusova, B. and Machova, J., 1994. The effects of pollutants on selected hematological and biochemical parameters in fish, *Fishing News Books for FAO,* 1994

Via, D. J., Villani, P., Gasteiger, E. and Niederstätter, H., 1998. Oxygen consumption in sea bass fingerling *Dicentrarchus labrax* exposed to acute salinity and temperature changes: metabolic basis for maximum stocking density estimations. *Aquaculture*, *169*(3-4): 303-313.

Von Oertzen, J. A., 1985. Resistance and capacity adaptation of juvenile silver carp, *Hypophthalmichthys molitrix* (Val.), to temperature and salinity. *Aquaculture*, *44*(4): 321-332.

Wang, J. Q., Lui, H., Po, H. and Fan, L., 1997. Influence of salinity on food consumption, growth and energy conversion efficiency of common carp (*Cyprinus carpio*) fingerlings. *Aquaculture*, 148(2-3): 115-124.

Weber, M.J., Brown, M.L. and Willis, D.W., 2010. Spatial variability of common carp populations concerning lake morphology and physicochemical parameters in the upper Midwest United States. *Ecology of Freshwater Fish*, *19*(4): 555-565.

Whitfield, A. K., Blaber, S. J. M. and Cyrus, D. P., 1981. Salinity ranges of some southern African fish species occurring in estuaries. *African Zoology*, *16*(3): 151-155.

Williams, W. D. 2001. Anthropogenic salinization of inland waters. *Hydrobiologia* 466: 329–337.

Yahona M, Pablo E. (2007) Behavioral and gill histopathological effects of acute exposure to sodium chloride in moneda (*Metynnis orinocensis). Journal of Environmental Toxicology & Pharmacology* 25: 365-372

Zadunaisky, J. A., 1997. Gill chloride cell activation by plasma osmolarity. In *Ionic Regulation in Animals: A Tribute to Professor WTW Potts*, Springer, Berlin, Heidelberg: 87-105.

ABBREVIATION

PSU	**Practical Salinity Unit**
ppm	**Parts per million**
BOD	**Biological oxygen demand**
DA	**Dopamine**
5 - HT	**Serotonin**
NH3	**Ammonia**
‰	**Parts per thousand**
µg	**Microgram (10^{-6} gm)**
ATP	**Adinosine Tri Phosphate**
Vol	**Volume**
L	**Litre**
ml	**Milli Litre**
µl	**Microliter (10^{-3} l)**
gm	**Gram**
Mg	**Milligram (10^{-3} g)**

ng	**Nano gram**
µg	**Microgram (10^{-6} gm)**
%	**Percentage**
Conc.	**Concentration**
ppm	**Parts per million**
PSU	**Percentage Saline Unit**
Min	**Minute**
L	**Lower limit (Number of days)**
U	**Upper limit (Number of days)**
DF	**Degree of freedom**
°C	**Degree Centigrade**

APPENDIX 1

1. Life Table Method – Data used is classified for 10 PSU, and involves Treatment 1, and Treatment 2

```
Data
salinity10;
set raja;
if salinity>10 then
delete;
run;
data treat1;/*salinity PSU up to
10)*/ set raja;
if treatment='T3'  then
delete;
if treatment='T4'  then
delete;
if treatment='T5'  then
delete;
if treatment='T6'  then
delete;
if treatment='T7'  then
delete;
if treatment='T8'  then
delete;
if treatment= 'T9' then
delete;
if treatment= 'T10' then
delete;
 if treatment= 'T11' then
delete;
if treatment= 'T12' then
delete;
 if treatment='TC' then
delete;
run;

Proc lifetest data=TREAT1 method=life plots=(s,h)
graphics; time day*event (1);
Strata
treatment;
run;
```

2. Life Table Method. - Data used is classified for up to 15 PSU, and involves Treatment 3, Treatment 4, and Treatment 5

```
data treat2;/*salinity PSU between 10&
15*/ set raja;
if treatment='T1'  then
delete;
if treatment='T2'  then
delete;
if treatment='T6'  then
delete;
if treatment='T7'  then
delete;
if treatment='T8'  then
delete;
if treatment= 'T9' then
delete;
if treatment= 'T10' then
delete;
if treatment= 'T11' then
delete;
if treatment= 'T12' then
delete;
if treatment='TC' then
delete;
run;

Proc lifetest data=TREAT2 method=life plots=(s,h) graphics;
time day*event(1);
strata treatment;
run;
```

3. Life Table Method. - Data used is classified for up to 15 PSU, and involves Treatment 3, Treatment 4, and Treatment for Rohtak, and Powerkheda stock

```
Proc lifetest data=raja2 method=life plots=(s,h) graphics;

time day*event(1);

strata stock;

run;
```

4. Life Table Method - Data used is classified for 10 PSU, and involves Treatment 1, and Treatment 2 for Rohtak, and Powerkheda stock

```
Proc lifetest data=raja1 method=life plots=(s,h) graphics;

 time day*event(1);

 strata stock;

 run
```

5. Life Table Method. - Data used is classified for up to 25 PSU, and involves Treatment 6 to Treatment 11

```
data treat3;/*salinity PSU between
15&20 */ set raja;
if treatment='T1'  then
delete; if treatment='T2'
then delete;
if treatment='T3'  then
delete;
if treatment='T4'  then
delete;
if treatment='T5'  then
delete;
if treatment= 'T12' then
delete;
if treatment='TC' then
delete;
run;

Proc lifetest data=TREAT3 method=life plots=(s,h)
graphics; time day*event (1);
strata
treatment;
run;
```

6. Life Table Method. - Data used is classified for up to 25 PSU, and involves Treatment 6 to Treatment 11 for Rohtak, and Powerkheda stock

```
Proc lifetest data=raja3 method=life plots=(s,h) graphics;
time day*event(1);
strata stock;
run;
```