EVALUATION OF GROWTH, SURVIVAL AND PHYSIOLOGICAL PARAMETERS OF *ANABAS TESTUDINEUS* (BLOCH, 1792) REARED IN INLAND SALINE WATER AT DIFFERENT SALINITIES

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

M.F.Sc. (Aquaculture)

by

SUBAM DEBROY, B.F.Sc.

(AQC-MA8-08)

ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
(Deemed-to-be University Established Under Section 3 of UGC Act 1956)
Panch Marg, Off Yari Road, Versova,
Andheri (W), Mumbai – 400 061

AUGUST, 2020

Debroy, S., 2020. Evaluation of growth, survival and physiological parameters of *Anabas testudineus* (Bloch, 1792) reared in inland saline ground water at different salinities, M.F.Sc. Dissertation, ICAR-Central Institute of Fisheries Education (Deemed-to-be University Established Under Section 3 of UGC Act 1956) Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai – 400 061

DEDICATION

God
"My Parent, My teachers & My friends"
Without whom I could never be who I am today

भा.कृ.अनु.प.- केन्द्रीय मात्स्यिकी शिक्षा संस्थान
ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
(A University Established Under Sec.3 of UGC Act 1956)
Ministry of Agriculture & Farmers Welfere,
Govt. of India.

Dated: 28th August, 2020

CERTIFICATE

Certified that the dissertation entitled "**EVALUATION OF GROWTH, SURVIVAL AND PHYSIOLOGICAL PARAMETERS OF *ANABAS TESTUDINEUS* (BLOCH, 1792) REARED IN INLAND SALINE WATER AT DIFFERENT SALINITIES**" is a record of independent bonafide research work carried out by **Mr. SUBAM DEBROY** during the period of study from September, 2019 to August, 2020 under our supervision and guidance for the degree of **Master of Fisheries Science (Aquaculture)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

Advisory Committee

Major Advisor

(N.K.Chadha)
Principal scientist
Division of Aquaculture
ICAR-CIFE, Mumbai-61

(Satya Prakash)
Scientist
Division of Aquaculture
ICAR-CIFE, Rohtak centre

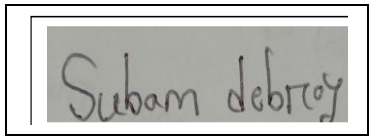
(V. Harikrishna)
Senior scientist
Division of Aquaculture
ICAR-CIFE, Rohtak centre

(Mujahidkhan A Pathan)
Scientist
FGB Division
ICAR-CIFE, Mumbai-61

पंच मार्ग, ऑफ यारी रोड, वरसोवा, अंधेरी (प), मुंबई - ४०० ०६१. (भारत)
Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai - 400 061. (India)
कार्यालय / Office) : 022-26361446/7/8,
Fax : 022-26361573
Website : http ://cife.edu.in

DECLARATION

I hereby declare that the dissertation entitled "**EVALUATION OF GROWTH,SURVIVAL AND PHYSIOLOGICAL PARAMETERS OF ANABAS TESTUDINEUS(BLOCH, 1792) REARED IN INLAND SALINE WATER 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.



(SUBAM DEBROY)

M.F.Sc. Student

ICAR-Central Institute of Fisheries Education

Date: 30th August, 2020

Place: Mumbai

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God for everything I received. I would like to express my heartfelt thanks to my major advisor ***Dr.N.K.Chadha****, principal scientist, Division of Aquaculture, ICAR-CIFE, Mumbai for his continuous guidance and support for making my work more effective and systematic.*

*I also express my sincere thanks to my co-advisor:****Mr.Satya prakash****, scientist, Division of Aquaculture, ICAR-CIFE, and Rohtak centre, for his special interest, great attitude, permanent motivation, academic advice, moral support and great help for my research investigation.*

I would also like to thank all my advisory members **Dr.Mujahidkhan A Pathan***, scientist, FGB Division, ICAR-CIFE, Mumbai, and* ***Mr. V. Harikrishna****, senior scientist, Aquaculture Division, ICAR-CIFE, Rohtak centre, for their valuable assistance and guidance.*

I wish to record my sincere thanks to ***Dr. Gopal Krishnan****, Director and vice-chancellor, ICAR-CIFE, Mumbai, and* ***Dr.Kishore kumar krishnani****. Principal Scientist and Head of Aquaculture Division, ICAR-CIFE, Mumbai, for providing all necessary facilities for conducting the research work.*

I owe a deep sense of gratitude to all my respected teachers of Division of Aquaculture namely, ***Dr.V.K.Tiwari*** *(Principal Scientist),* ***Dr.P.B.Sawant****(Senior Scientist),* ***Dr. Babitha Rani A. M.*** *(Senior Scientist),* ***Dr. A.K. Verma*** *(Senior Scientist),* ***Dr. Upasana Sahoo (****Scientist),* ***Dr. Madhuri Pathak*** *(Scientist),****Mr. Chandrakanth M.H.*** *and* ***Mr. K.D.Raju*** *for rendering their constant help, advice, guidance, support and cooperation throughout my research work.*

I am greatly indebted to all the respected great staff members of the CIFE Rohtak centre for their friendly relationship, friendly gesture and also kindness. Very special thanks to ***Dr.Sreedharan****, Scientist,* ***Dr. Pankaj Kumar****, scientist,****Mr. Satyendra Singh****, technical officer, for their valuable suggestions and help for the improvement of research work.*

I am at loss of words to express my sincere gratitude to ***Mr. N. Aglave****,* ***Mrs. Bharati Ghagare****, and* ***Mrs. Anita Shivekar****for their invaluable guidance, affection, care, help, support and advice. I express my thanks to* ***Mr. Chotu. Mr. Pradeep, Mr. Kalpesh, Mr, Ashis, Mr. Nagesh, Mr Osman, Mr. Namdev*** *and all other staff of ICAR-CIFE, Mumbai for their care, support and help during my research work.*

I also express my thanks to all the respected faculty members of various divisions and sections of CIFE, Mumbai for their kind help, affection and co-operation extended to me during my research period.I am thankful to Librarian and library staff of CIFE for their help in collecting Literature related to my research work.

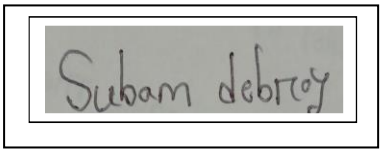
I extend my deepest gratitude to my classmates ***Avinsh Gaikwad, Chigo Basar, C.S. Sanjay, K.V. Ramaraju, Pranab Dihingia, Rida Riyaz, Sumant chandravanshi, SanskrutiPadra, Swaranaprava Mohapatra, Thejaswini, Udipta Roy and John Daudi, Vijay kumar,Tanmoy,Ashraf,Nisha,Chandan,Pradeep,pritam,shiva,somnath,Abi psha,Pragati,Kangkana,Abhilas,Monalisa*** *for their invaluable support and cooperation during my work****.***

I am highly thankful to my seniors ***Mr.Avinash talukdar*** *, sir for his continuous guidance and motivation for conducting my research work. I also thank my senior* ***Mr.Ramjanul hauqe*** *for his continuous assistance and*

suggestions throughout the research period. And I also thank all other seniors ***Mr. Tapas Paul****,* ***Mr. prasanta jena****,* ***Mr. Chittaranjan Raul, Mr. subhas kumar banjare****,* ***Mr. Sandeep Shankar Pattanaik, , Mr. Abhijit mallik, Mr.Suman nama Ms. puja, Ms. Nahida Rasheed, Ms.Hougaina*** *for their support and also extend my heartfelt gratitude to my juniors* ***Sourav, Diganta,*** *for their friendly and kindly support.*

I would convey my heartfelt thanks to Government of India, Indian Council of Agricultural Research, CIFE for providing fellowship during research work.

Finally, my heartfelt thanks to my great father ***Shri Gopal debroy****, my mother* ***Smt. Rinku debroy*** *and my all family members whose selfless love, filial affection, obstinate sacrifices and blessings made my path easier.*



30th August, 2020
Mumbai

*(**SUBAM DEBROY**)*
M.F.Sc. Student
CIFE, Mumbai-61

सारांश

इस अध्ययन का प्रमुख उद्देश्य विभिन्न लवण्ता वाले पानी मे, एनबास टेस्टुडिनस के विकास, अस्तित्व और शारीरिक प्रतिक्रिया पर प्रभाव को जानना है। संखियिकिक अध्य्यन हेतु कम्प्लिट्लि रंडेमिज़ डिजाईन का उपयोग किया गया जहा 6.0 से 6.3 ग्राम के एनाबास टेस्टुडिनस को समान रुप से 5 अलग-अलग लवणता के तहत तीन प्रतिक्रिति (T1, 0 पीपीटी; T2, 3 पीपीटी; T3, 6 पीपीटी, T4, 9 पीपीटी and T5, 12 पीपीटी) मे वितरित किया गया।प्रयोगात्मक अवधि के दौरान विभिन्न उपचार समूह के पानी की भौतिक गुण्वत्ता अधिकतम सिमा के भितर पाया गाया। लवणता का सिधा सह-समबंध विभिन्न मापदण्ड जैसे कि टोटल अलकेलिनिटि, टोटल हार्ड्नेस्स और विभिन्न आयनो ले संद्रता से होता है। पुरे प्रयोग के दौरान एक भी उपचार समूह मे मृत्यु दर्ज कि गई थी। मछली के भार पर 12 पी.पी.टी. तक के पानी का कोई महत्वपुर्ण प्रभाव नही पडा। उच्चतम फीड दक्षता अनुपात और न्युनतम भोजन रूपांतरण अनुपात T3 समूह मे पाया गया। सभी उपचार समूह मे समिपवर्ती रचना का महत्वपुर्ण प्रभाव पडा है जहा सबसे अधिक नमि कि मात्रा T3 समूह के बाद T5, T4, T1 और T2 मे पाया गया। सभी उपचार समूहो मे कच्चे प्रोटिन कि मात्रा एक दुसरे से काफी भिन्न थी जो कि सबसे अधिक T1 मे और सबसे कम T3 मे देखा गया। पाचक एंजाइमो मे प्रोटिएज़ और एमाइलेज़ की मात्रा बढ़ने के साथ बदलते रहती है। एंटीओक्सिडेंट एंजाइम (एसओडी और कैट) और प्रोटिन उपापचय एनजाइम (एएसटी) की गतिविधियों का 6 पीपीटी के उपर की लवणता पर महत्व्पुर्ण से देख़ा गया था। एनबास टेस्टुडिनस के लवणता प्रसारिता, लवणता के बढने के साथ घटते जाता है। सिरम के आइसो- आसमोटिक बिंदु की गणना 292 mOsm/kg तथा आइसोटोनिक लवणता की गणना 9.8 पीपीटी की गई। हेमेटोलाजिकल मापदण्ड (एचबी, एचसीटी, आरबीसी और डब्लुबीसी) लवणत बढ़ने के साथ बदलते रहती है। वर्तामान अधय्यन से यह पता चलता है कि एनबास टेस्टुडिनस के विकास और अस्तित्व पर 12 पीपीटी के लवणता तक कोई महत्वपुर्ण प्रभाव नही पडता, हालांकि, 6 पीपीटी के उपर. फीड इस्तेमाल और विभिन्न शारिरिक मापदण्डो पर महत्वपुर्ण प्रभाव पडता है। **इस प्रयोग के परिणाम स्वरूप यह कह सकते है कि** एनबास टेस्टुडिनस को 6 पीपीटी तक के लवणता वाले पानी मे पालने के लिए एक महत्वपुर्ण प्रजाति माना जा सकता है।

ABSTRACT

The present study aimed to evaluate the growth, survival, and physiological response of *Anabas testudineus* (Climbing perch) in inland saline water of different salinity. 225, *A. testudineus* fingerlings of 6.0 to 6.3 grams were equally distributed under five different salinities (T1, 0 ppt; T2, 3 ppt; T3, 6 ppt, T4, 9 ppt and T5, 12ppt) in triplicates following complete randomized design (CRD). The physicochemical parameters of water of different treatment groups were found within the optimum range throughout the experimental period. The parameters, *viz.* total alkalinity, total hardness, and concentration of different ions found to correlate with the salinity directly. No mortality was recorded in any of the treatments during the experimental period. Salinity up to 12 ppt, as selected for this experiment, had no significant effect on the weight of the fishes. The growth parameters such as specific growth rate (SGR), Protein efficiency ratio (PER), weight gain, and % weight gain were obtained highest in T2, followed by T3, T1, T4, and T5 groups. The highest feed efficiency ratio (FER), along with the lowest food conversion ratio (FCR) was reported in the T3 group. The proximate composition was found to be significantly different among the various treatment groups with the highest moisture content being recorded at T3 followed by T5, T4, T1 and T2 group, respectively. Maximum and minimum crude protein content was noted in T1 and T3, respectively, and they differed significantly ($p < 0.05$) from each other. Among digestive enzymes, protease and amylase activities were significantly ($p < .05$) altered with increasing salinity. A significant alteration of antioxidant enzymes (SOD and CAT) and protein metabolism enzymes (AST) activities were significantly ($p < .05$) was observed at salinity above 6 ppt. Serum osmolality of *A. testudineus* decreased with an increase in the salinity of rearing medium. Iso-osmotic point of serum was calculated to be 292 mOsm/kg and the isotonic salinity was calculated to be 9.8 ppt. Hematological parameters (Hb, HCT, RBCs, and WBCs) were significantly ($p < .05$) altered with increasing salinity. The results of the present study reveal that inland saline water of up to 12 ppt salinity has no significant effect on growth and survival of *A. testudineus,* however, salinity above 6 ppt does have a significant effect on feed utilisation and various physiological parameters related to well being of the fish. Based on the results of this experiment, *A. testudineus* can be considered as an ideal species to promote in the inland saline region up to a salinity of 6 ppt

CONTENT

3. MATERIALS AND METHODS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.0 Introduction

World aquaculture production grew faster as compared to other major food production sectors, with an annual growth rate of 5.5% in 2018 and global production is estimated at 114.5 million tonnes (FAO, 2020).In 2017, fish and seafood accounted for 17% of all animal protein intake and this proportion has been increasing (FAO, 2020). India stands second in the global aquaculture next to china with a production of 5.7 million tonnes (FAO, 2020). The world is faced with the critical challenge of feeding the growing population that is expected to reach 9.6 billion by 2050 (United Nations, 2012). Consequently, the demand for aquaculture produce both in terms of quantity and diversity will also increase in future and is estimated that by 2030 aquaculture will contribute about 62% of fish for direct human consumption (Kobayashi *et al.,* 2015). Aquaculture is world's most diverse farming system in terms of number of farmed species.Inspite of being the diversified farming system, contribution of each species to overall production also called evenness in aquaculture is highly skewed. 90 percent of global aquaculture production is contributed by merely 30 aquatic fish species which is much lesser than two thousand species that contribute to global capture fisheries (FAO 2020). Similarly, significant share of Indian aquaculture production is constituted by limited number of species such as major carps, monosex tilapia, pangasius, and *Penaeus vannamei* (DADF, 2018-19). The two significant factors that play a determining role in the success or failure of aquaculture enterprises are climate change and extreme climate events(FAO,2017). Diversification of species and the culture system in the new farming environment will provide poor livelihood options and would also increase the resilience of farmers to external factors such as climatic, socioeconomic impacts and emerging markets. Aquaculture sector must devise ways and means for its future expansion amid increasing conflict for resources in the freshwater and coastal areas. Utilisation of inland saline areas could be a viable option for the future expansion of the industry.

Salinization is a major problem, and it affects over 380million ha. of land spreading across 20 countries worldwide (Lambers, 2003). In India, nearly 8.62 million ha of the land area is affected by soil salinity out of that, 2.8 million hectares of salt-affected lands are located within the Indo-Gangetic alluvial plain (Allan,

2009). Salinization of soil and water scarcity are the most severe constraints confronting sustainable agriculture production systems in semi-arid and arid regions. Its intensity has expanded in past decades due to poor management of land and water resources, unprecedented regional as well as global climate change and variability (Wong *et al.,* 2010) and led to an increased pressure on freshwater resources. Countries like Australia, China, India, Israel and USA have demonstrated utilization of salt affected land and water resources for commercial aquaculture (Barman *et al.,*2005; Partridge *et al.,*2008). Aquaculture in arid and semi-arid regions has increased economic and social impact through production of food, livelihood security and income generation (Chithambaran, 2016). Although the ionic composition of saline groundwater generally reflects that of seawater, the composition varies with local geology and history. This variability depends on the depth of water aquifer, soil structure, and rainfall or drought (Raizada *et al.,* 2015). The ionic composition of saline groundwater of different countries such as India, Australia and the USA differed in the levels of potassium, calcium and magnesium in comparison to seawater (Fielder *et al.,* 2001; Boyd and Thunjai 2003; Jain *et al.,* 2006). Inland saline water is usually rich in Ca^{+2} and poor in Mg^{+2} and K^{+} (Jain *et al*., 2002). The water hardness index (Ca+Mg): (Na+K) also varies from water to water, for seawater its value is around 0.13, whereas in most ground groundwater types, water hardness index ranges from 0.25 and 0.30(Aklakur, 2017).

ICAR-CIFE Rohtak centre is committed to develop suitable technology for commercial aquaculture in the degraded inland saline areas. In this regard, a number of fish and crustacean species such as Amur carp (Singh *et al.,* 2019), *Pangasianodon hypophthalmus* (Kumar *et al.,* 2016), *Mugil cephalus* (Talukdar *et al.,* 2017), *Chanoschanos* (Raizada *et al.,* 2005), *Trachinotus blochii* (Pathak *et al.,* 2019), GIFT Tilapia (Singh *et al.,* 2020), *Macrobrachium rosenbergii* (Kumar, 2005), *Penaeus monodon* (Purushothaman *et al.,* 2014, Antony *et al.,* 2015) *Penaeus vannamei* (Jahan *et al.,* 2018) *etc*. have been tested for its suitability of culture using ground inland saline water. However, at commecrial scalethe technology for rearing of *L.vannmaei* using amended inland saline water has only been adopted.*L. Vannamei* farming though demonstrated to be highly profitable has the inherent problems such asrequirement of large infastructure and heavy investment making it difficult for the resource poor farmers to adopt the technology. Resource poor

farmers are waiting for new species which can be cultured with low investment and infrastructure. *Anabas testuidneus* could be a suitable option for farmers of the inland saline region.

Anabas testudineus commonly known as Koi or climbing perch is a tasty fish with high consumer demand (Nahar *et al.,* 2015). *Anabas testudineus* most commonly found in open water (streams, lakes, floodplain and beels), paddy fields and swamps and its preferred habitats are heavily-vegetated, stagnant waters. Species can be cultured at high stocking density and has the ability to survive in adverse environmental conditions such as low oxygen due to its air-breathing ability (Rahman *et al.,* 2013). This fish was abundantly available in open water system but due to over exploitation and various ecological changes its natural population has declined in recent times. shorter life cycle, faster growth rate, omnivorous feeding habit, highly nutritious food value, hardy nature, and tolerance of adverse environmental conditions makes this species a good option for aquaculture diversification in inland areas(Bhaskar *et al*., 2015). Species is reported to exhibit faster growth rate in mild saline water compared to freshwater(Widiyati *et al.,*2019). Faizul and Christimus, 2013 reported that the species can tolerate salinity upto 10ppt.There are conflicting reports in the available literature with respect to the performance of this species in saline water of different sainities. Furthemore, there is no information avialble in published literature with respect to the growth, survival and physiological response of this species in ionically imbalanced inland saline water. Keeping the above mentioned aspects into consideration, the present study wasplanned to evaluate the growth, survival, and physiological response of *Anabas testudineus* in inland saline of different salinities water with the following objectives.

Objectives

- To assess the growth and survival of *Anabas testudineus fry reared* in inland ground saline water at different salinities.
- To evaluate the physiological response of *Anabas testudineus fry* reared in inland ground saline water at different salinity levels.

2.0 Review of Literature

2.1. Overview of aquaculture

Aquaculture is the farming of different aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants. Farming implies some forms of intervention in the whole production process, starting from stocking to feeding, protecting the fishes from predators and finally harvesting of thefishes (Tacon, 2003). Aquaculture production mainly signifies the output, which is produced at the end of the rearing period in terms of weight of the fishes for consumption or other purposes (Tacon, 2003). World aquaculture production grew faster as compared to other major food production sectors, with an annual growth rate of 5.5% in 2018 and global production is estimated at 114.5 million tonnes (FAO, 2020).The global aquaculture production is dominated by finfish and provides almost 54.3 million tonnes out of 114.5 million tonnes(FAO, 2020). India stands second in the global aquaculture production next to china with a production of 5.7 million tonnes (FAO, 2020).However, in the face of rapidly increasing population and changing food habits, aquaculture is expected to play a critical role in supplying the much-needed protein requirement. The world is faced with the critical challenge of feeding the growing population that is expected to reach 9.6 billion by 2050 (United Nations, 2012).This increase in population would warrant 60% additional foods (FAO, 2013). The demand for aquaculture produce both in terms of quantity and diversity will also increase in future and is estimated that by 2030 aquaculture will contribute about 62% of fish for direct human consumption (Kobayashi *et al.,* 2015). Per capita fish consumption has increased globally from 9kg in 1961 to 20.5kg in 2018(FAO, 2020).Globally, nearly 2000 species are farmed in different countries, of which 30 species contributes about 90% in the global aquaculture(FAO 2020).Similarly, in India, majorcarps, monosex tilapia, pangasius as well as *Penaeus vannamei* largely shares the major portion in Indian aquaculture(DADF, 2018-19).The issue of feeding the projected population rise becomes even more challenging in the light of dwindling resource base due to urbanization, degradation of land and water resources and climate change. Consequently, research on species better adapted to poor environmental conditions and having a greater ability to tolerate various aquaculture related stressors becomes imperative. Apart from this focus has also to be there on diversification of the culture system and species cultured therein.

Diversification of the species and culture system allows the poor farmers' livelihood option and also opens new market opportunities for the future. Recently, the Ministry of Fisheries, Animal Husbandry and Dairying, Department of Fisheries, Government of India, has launched Pradhan Mantri Matsya Sampda Yojana (PMMSY) which underlines the development of inland saline aquaculture as a focus area for aquaculture expansion in future (DOF, GOI 2020). Diversification of aquaculture in the inland saline and alkaline areas, which is not suitable for agriculture, would create employment and livelihood opportunities for the farmers of the region.

2.2.The extent of saline and sodic soil and water resources

Soil and water salinity and associated problems in the arid and semi-arid regions of the world constitute one of the major abiotic stressors impacting agricultural practices (Minhas *et al.,* 2020). Globally, about 830 million ha land area is estimated to be afflicted by salinity and sodicity (FAO, 2015) and is increasing every year (Qadir *et al.,* 2014). Extent of soil salinisation in south east Asia is estimated to be 103 million ha,of which 47 million ha is strongly saline and 54 million ha is ranked as moderately saline (Massoud, 1974). In India,nearly 8.62 million ha. of the land area is affected by soil salinity out of that, 2.8 million hectares of salt-affected lands are located within the Indo-Gangetic alluvial plain (Allan, 2009).Salinity/sodicity in Indian subcontinent is characterized by high bicarbonates and differs from other places in the world where saline/sodic conditions are dominated by chloride or sulphate (Grattan and Oster 2003). in the absence of proper preventive/ameliorative steps, It is expected that the extent of soil salinisation in India may increase to 16.2 million ha by 2050 accounting for about 11 percent of the net sown area of the country (CSSRI, 2015). Salinization of soil occurs mainly in two ways, such as primary Salinization and secondary Salinization. The majority of Inland saline zones of the world are located in arid, semi-arid, and low-lying poorly drained regions, where high concentrations of salts accumulation occurred within the soil. This type of Salinization is known as primary Salinization and occurs due to insufficient rainfall and poor leaching of soluble salts. Anthropogenic activities like improper agricultural practices cause secondary Salinization in areas with underground saline water (Meera *et al.,* 2019). Though from aquaculture point of view saline and sodic soils are

considered together, it is important to understand the distinction between two classes of soil. Generally, areas with high aridity, shallow water table, water logged areas and in the proximity to coastal regions have a prevalence of saline groundwater. In contrast, sodic waters are prevalent in semi-arid regions with annual rainfall of 500–700 mm. Soil salinity and sodicity differ in terms of their impact, the former being related to salt concentration and the latter to salt composition. The concentration of salts in the irrigation water or soil that is sufficiently high to affect crop yields or crop quality adversely is referred to as salinity. Sodicity, on the other hand, is related to the proportion of sodium in the water, or adsorbed to the soil surface, relative to calcium and magnesium. In India, saline water are defined as water having EC>2 dS m^{-1} (Minhas 1996; Chowdhury *et al.,* 1996). For salinity appraisal of the soils, EC of the saturated extract (ECe) is generally used. An ECe of 4 dS m^{-1} separates a saline soil from a non saline one. The sodicity of soil is characterized by the exchangeable sodium percentage (ESP). An ESP of 15 or more separates a sodic soil from a non-sodic soil. Sodic irrigation water has SAR higher than 10 and RSC higher than 2.5 mmolc L^{-1} (Minhas and Gupta 1992). Agricultural scientists have been trying to devise ways and methods to reclaim/use the saline/sodic land and water resources for agriculture through a number of interventions such as the use of salt-tolerant crops and varieties, chemical amendments such as gypsum beds, use of organic materials such as farmyard manure, fertiliser management and engineering practices such as sub surface drainage.

2.3. Inland saline aquaculture- global and national status

Though the various reclamation/ management measures discussed above have yielded positive results, unfortunately, the rate of land and water degradation through salinisation far exceeds the rate of reclamation. This requires that these land and water resources be put to alternate use. Inland saline water contains different types of salts, which can be used as nutrients for fish production and also offer a lucrative option for sustained utilization of inland saline resources (Sandeep *et al.,* 2013). Aquaculture of suitable fish and shellfish species could be a lucrative option for utilization of these degraded land and water resources. Inland saline aquaculture is a land-based aquaculture system and is currently practised in several countries such as Israel, USA, India, Australia and china (Allan, 2009). Although the

ionic composition of saline groundwater generally reflects that of seawater, the composition varies with local geology and history. The variability of inland saline groundwater is depending upon the depth of the water aquifer, soil structure and rainfall (Raizada *et al.,* 2015). In USA saline groundwater contains excess Ca^{+2} and SO^{-2}_4 but generally deficient in K^+ (Forsberg *et al*., 1996), whereas in Australia, saline groundwater is rich in Ca^{+2} andMg^{+2} but a deficit in K^+ (Fielder *et al*. 2001). Further, in Indian subcontinent ground saline water rich in Ca^{+2} and poor in Mg^{+2} and K^+ (Jain *et al*., 2002). Inland ground saline water (IGSW) contains high level of Ca^{+2} hardness as compared to Mg^{+2}. Moreover, Ca^{+2} to Mg+2 ratios for IGSW is 1:1.60-1.70 is quite different as compared to seawater (1:3.0-3.5) (Lakra *et al*., 2014). The water hardness index (Ca+Mg): (Na+K) also varies from water to water like in case of normal seawater is around 0.13, whereas in most of the IGSW samples, water hardness index ranges from 0.25 and 0.30(Aklakur, 2017).An opportunity exists for manipulating the ionic composition of the ground inland saline water by supplementing deficient ions such as K^+ and Mg^{2+}. Potassium deficiency can be overcome by supplying potassium though murate of potash (KCl) and for overcoming Magnesium deficiency $MgCl_2$ may be used (Roy *et al.,* 2010). Another product, sulphate of potash magnesia ($K_2SO_4.2MgSO_4$) has also been used for supplementing the deficient ions (Boyd, 2003). However, caution must be exercised while supplementing the deficient ions as the requirement for the specific ions and the ionic ratios to be maintained may vary from species to species.

A variety of aquatic crustacean and finfish species have been reared in inland saline water around the world. Countries including Australia, China, India, Israel and the USA have a demonstrated utilization of their affected land and water resources for commercial mariculture (Ron *et al.,*2002; McNevin *et al.,*2004; Zhu *et al.,* 2004; Partridge *et al.,*2004). The western king prawn, *Penaeus latisulcatus*(Melicertus) had been identified as one of the candidate species for culture in inland saline water in Western Australia (Partridge *et al.,* 2004). *P. monodon* is an euryhaline species which can tolerate salinities ranging from 3 ppt (Zhang *et al.,*1989) to 71 ppt (Solis, 1988). Inland culture of penaeid shrimp in low-salinity well water is a growing business in many areas of the world, including southern regions of the USA (Roy *et al.,* 2010). *L. vannamei* is the most commonly cultured shrimp in the western hemisphere and has been grown in inland saline waters ranging in

salinity from 0.5 ppt to 28.3ppt (Smith and Lawrence,1990,Samocha *et al.,*2001). *L. vannamei*, are presently being grown in low-salinity inland waters experimentally and commercially in Alabama, Arizona, Florida, Indiana, Illinois and Texas and in other parts of the world (Davis *et al.,* 2002, Samocha *et al.,*2002, Tantulo *et al.,* 2006). Marine shrimps are also being commercially produced in low salinity waters in other regions of the world such as Ecuador and Thailand. Recent estimates suggest that more than 30% of shrimp culture in Thailand is in inland low-salinity water. Marine shrimp are cultured in inland ponds supplied with saline water in several nations including the United States (Smith and Lawrence, 1990; Limsuwan *et al.,* 2002; Boyd *et al.,* 2002). Some of the fish species on which scientific information for culture in inland saline water is available include, *Lates calcarifer* (Partridge *et al.,* 2008), *Dicentrarchus labrax* (Ercan *et al.,* 2015), *Sparus auratus* (Applebaum *et al.,* 2008), *Argyrosomus japonicus* (Doroudi *et al.,* 2006), Rainbow trout (Starcevich *et al.,* 2003), Silver perch (Doroudi *et al.,* 2007) and Snapper (Fielder *et al.,* 2003).

In India also a number of studies have been carried out in different finfish as well crustacean species to explore the potential for their culture in inland saline water. ICAR-CIFE recognised the need for research in inland saline aquaculture way back in 1980s (Dwivedi, 1984) and research was initiated at the Sultanpur centre of the institute under the Operational research project 1986 (Swivedi and Lingaraju, 1986). Some of the aquatic species for which salinity tolerance and the other aspects such as nutritional requirement in inland saline water has been studied in India include Rohu (Kumar *et al.,* 2018), Catla (Ansal *et al.,* 2016), Mrigal (Rajender, 2000), Amur carp (Singh *et al.,* 2019), Grass carp (Ansal *et al.,* 2016),Common carp(Jahan *et al.,* 2020), GIFT-Tilapia (Singha *et al.,* 2020),Nile tilapia (Garg *et al.,* 2016), Koi carp (Sharma *et al.,* 2017),Pangasius(Kumar *et al.,*2016), Grey-mullet(Barman *et al.,* 2005, Talukdar *et al.,* 2020),Milk fish (Raizada *et al.,*2005),Silver pompano(Pathak *et al.,* 2019),*Macrobrachium rosenbergii* (Jain *et al.,* 2007, Raizada *et al.,* 2015), *Penaeus vannamei* (Jahan *et al.,* 2018), *Penaeus monodon*(Reddy and Harikrishna, 2014, Antony *et al.,* 2015).However, at commercial scale the technology for the rearing of *L.vannmaei* using amended inland saline water has only been adopted. *L. Vannamei* farming, though demonstrated to be highly profitable, has inherent problems such as the

requirement of large infrastructure and massive investment, making it difficult for the resource-poor farmers to adopt the technology. Resource-poor farmers are waiting for new species that can be cultured with low investment and infrastructure. In such a scenario, culture of air-breathing fish *Anabas testudineus,* also known to exhibit salinity tolerance, could be a suitable option for farmers of the inland saline region.

2.4. *Anabas testudineus* biology, distribution and importance

According to the binomial classification of Linnaeus (1758), *Anabas testudineus* belongs to the

Kingdom:Animalia

Phylum:Chordata

Class: Actinopterygii

Order: Perciformes

Family:Anabantidae

Genus:Anabas

Species:*A*. *testudineus.*

Anabas testudineus is commonly known as ‘Kavai’ in Hindi, ‘Kou’ in Orissa, ‘Koi’ in West Bengal, ‘Kai’ in Assam etc. It is a highly preferred fish with a high market price in many states like West Bengal, Tripura, Assam, Bihar, Orissa and even in southern India (Nahar *et al*., 2015; Kuldeep *et al.,* 2012). It is generally found in open water (streams, lakes, floodplain and beels), paddy fields and swamps and its preferred habitats are heavily-vegetated, stagnant waters (Menon, 1999). Climbing perches are common in fresh and slightly brackish water throughout Indian subcontinent and South East Asia (Jayaram, 1981). Koi is one of the most popular fish in West Bengal, Tripura, Assam, Bihar and Orissa, due to its traditional role as a medically prescribed diet for sick and convalescents (Saha, 1971). *A. testudineus* also contain a high amount of iron and copper, which is essentially needed for haemoglobin and haemocyanin synthesis (Chowdhury *et al.,* 2014). Besides, it also contains all the essential amino acids (Saha, 1971). In South-East Asia this fish has attracted great attention as a promising candidate species for

freshwater aquaculture (Zworykin, 2012). In India, as well, attempts have been made to culture this species in weed-infested water bodies having poor water quality where carp culture cannot be undertaken (Kumar *et al.,* 2013). Recently this species has also emerged as species of choice for biofloc based culture.

Historically, *A.testudineus* has been subject of investigation by fish biologists owing to its obligate air-breathing habit (Dubale, 1951; Saxena, 1958; Munshi, 1968) and terrestrial locomotion ability with the help of hard opercular spines (Liem, 1987, Davanport and Martin., 1990). Air breathing in *A. testudineus* is assisted with supra branchial organsThe common name climbing perch for the species derives from the ancient legend that *Anabas* species can climb palm trees and suck their juice. However, the classical experiment of Davanport and Martin., 1990 negated the species to have ability to climb tress based on the inferences drawn from the results of slope climbing experiments and the general results of lateral instability.

A. testudineus appears to be visual feeders, feeding mainly during the day time (Patra, 1993). They are ferocious predators and omnivorous in feeding habit and can tolerate a wide range of environmental fluctuations (Bhaskar *et al*., 2015). They can live without water for six days and also able to survive in a harsh environmental condition such as low oxygen due to the presence of accessory respiratory organ and as well as its air-breathing activity (Rahman and Monir, 2013). IUCN list this species under vulnerable category due to indiscriminate fishing, use of pesticides and fertilizers, habitat modification, obstruction to breeding migration, and management failure (Hossain *et al*., 2015). *A. testudineus* attains a total length of up to 176mm (Rahman, 1989).

Under favourable conditions, *A. testudineus* attains sexual maturity in the first year itself (Chanchal *et al.,* 1978). Based on its relative condition factor, it was concluded that males and females attain their first sexual maturity at 11.3g and12.2g in weight and 8.0 and8.2 cm length, respectively (Chanchal *et al.,* 1978).Sexual dimorphism is seen during the breeding season based on the difference of their colour. During the breeding season, females show a brilliant orange colour with shades of yellow on the ventral side of the abdomen along with the colour variation. Further, female also shows some secondary sexual characters like abdomen are more bulged out in comparison to male. Whereas, males are darker in colour and develop tubercles on the pectoral fin during breeding

(Mookerjoo and Mazumdar, 1946).In India, CIFA has standardised the technology for seed production of the species with synthetic hormones and has also demonstrated the culture potential of this species at various locations (Kumar *et al.,* 2013). Mandal *et al.,* 2016 recommended 0.015 µg/g sGnRHa as the best dosage for breeding of *A. Testudineus* female fish and half the dosage for male fish. They further reported female: male ratio of 1:2 as optimal for enhanced spawning success in the climbing perch. Fecundity of the species ranges from 16,832-46,186, based on body weight (Uddin *et al.,* 2017). In nature, the species breeds only once during its breeding season from April to August. However, multiple spawning of the species can be achieved under captivity with proper management of feeding and water quality parameters (Kuldeep *et al.,* 2012). This fish is also known as a species that has a low survival rate during its early life stage and fry. Its Seed production and stock assessment are still poorly understood due the high mortality at first stage of development (Zalina *et al.,* 2012). Bahera *,* 2013 recommended stocking density of 4 larvae/litre for this species to achieve enhanced survival in early life stages. They also reported that depth of the larval culture tank has a significant effect on the survival of fishes.

A. testudineus is available in different strains which is hard to differentiate based on morphometric characters. Most of the farms in India and Bangladesh are currently culturing, what is popularly referred to as Thai strain. Native strain is not preferred for commercial aquaculture owing to its poor growth in ponds ecosystem *(Kohinoor et al. 1991*). Thai strain of *A. testudineus* was introduced from Thailand to Bangladesh in 2002 and subsequently it entered India in clandestine manner through illegal seed trade. This strain has some special characteristics such as faster growth rate, shorter culture period, higher survival rate etc (Kohinoor *et al.,* 2007).

2.5. Nutritional requirement of *Anabas testudineus*

From the economics point of view, nutrition is one of the most important factors for profitable rearing of different cultivable species. Hossain *et al.,* 2012 studied the dietary protein requirement for Thai strain of climbing perch and reported that species in the size range of 1 to 8 grams grew best at 40% dietary protein level. Incorporation of dietary protein at level higher than 40% negatively affected the growth of the species. Climbing perch was shown to efficiently utilise

the poultry viscera as a protein source and can completely replace fish meal in diet (*Bhaskar et al., 2015)*. Kader *et al.,* 2011 while studying different animal protein sources in the practical diet of *A. testudineus* observed that the combination of fishmeal, protein concentrate, and meat and bone meal is more effective for this species than any of the three protein sources alone. Ali *et al.,* 2012 in their study on estimation of carbohydrate : lipid ratio for *A. testudineus* revealed that climbing perch, can perform equally well on diets containing carbohydrate ranging from 14.43 to 28.81%, lipid ranging from 9.60 to 14.64% and carbohydrate : lipid g/g ratios of 0.99 to 3.00.Further, omnivorous fishes such as mullet, tilapia shows better growth, at 30-32% crude protein diet (Talukdar *et al.,* 2020;De silva *et al.,*1985).There are several research gaps in the nutrient requirement of *A. testudineus* and future studies must focus on estimating the optimal dietary Protein/Energy ratio for the species during various phases of its culture cycle. Dietary protein to energy ratio is important for maintaining proper growth of fishes in aquaculture (Winfree and Stickney, 1981).

2.6. *Anabas testudineus* production

Thakur and Das, 1986 reported that Koi (Anabas testudineus) production was 1,800 kg/ha in India by using supplementary feed such as rice bran, mustard oil cake and fish meal. In this culture technique stocking density was around 60,000 no/ha and culture duration spanned over 170 days. Akhteruzzaman, 1988 also evaluated the production potentials of *Anabas testudineus in* monoculture condition, where the stocking density was 16,000/ha and obtained a production of 450kg/ha in five months. Rahman *et al.,* 2013 reported that production up to 5582.23 kg/ha can be obtained when Thai strain of *A. testudineus* is stocked in ponds at stocking density of 1,00,000/ha. However, FCR was negatively affected as the stocking density was increased and they recommended stocking density of 50,000/ha as the best for getting maximum economic returns.

2.7. Osmoregulatory mechanism in fishes

Salinity is one of the most important parameters that directly or indirectly affects fish growth, survival, reproduction, osmotic regulation and distribution of fishes in different habitats (Boeuf and Payan 2001; Kang'ombe and Brown 2008). Tolerance to wide environmental factors is important for survival of fishes. Salinity tolerance is critical to diversification of species in the new culture environment such as Inland saline region.

There are several mechanisms by which salinity may affect the various parameters related to fish culture. If freshwater and marine water fish reared in inadequate salinity level, it directly effects on fish physiology and leads to reduction of growth, survival, immune response and disease resistance (Semra, 2013). The changes in the salinity in the gastric lumen will alter the different digestive enzyme activities, which directly or indirectly affects feed digestibility along with fish growth performance by increasing energy demand in continuous ionic regulation (Moutou *et al.,* 2004; Tsuzuki *et al.,* 2007; Gheisvandi *et al.,* 2015; Vargas-Chacoff *et al.,* 2015). If salinity is too high or too low in the external environment compared to the fish body fluids then fish need to spend some portions of its gross energy to maintain the osmotic balance (Boeuf and Payan, 2001). Boeuf and Payan, 2001 also reported that fish uses roughly 10% of total energy for osmoregulation. McCormick, 2001 reported that salinity variation also affects the fish hormonal activity as well (gonadotrophin hormone, cortisol, insulin-like growth factor1 and thyroid hormones), which are known to be important because they have the ability to maintain internal body homeostasis compared with the external environment.

Osmoregulation in fishes is mainly done by a group of organs like the intestine, kidney and gill, which are the major organs responsible for balancing ion movement by salt secretion or by salt uptake (Hirose *et al*., 2003). The mitochondria-rich cells (MR cells, *i.e*. chloride cells) located in gill epithelium also known as ionocytes are responsible for ion uptake in case of freshwater and ion secretion in case of seawater fishes (Hirose *et al*., 2003; Hwangand Lee, 2007). Freshwater fish have internal environment which is hyperosmotic compared to the external environment.

So,to maintain the osmotic regulation freshwater fish produced dilute urine via the renal system and ion uptake through specialized branchial cells (Larsen *et al.,* 2014). But in case of seawater fish internal environment is hypo-osmotic in comparison to external environment. So, to maintain the osmotic regulation fish drink lots of water through the gastro-intestinal tract and excrete excess mono-valent ions through gills and divalent ions via the renal system (Larsen *et al.,* 2014). Based on the strategy adopted for dealing with different osmotic conditions fishes can be classified as osmoconformers and osmoregulators. First group comprises of hagfish, and elasmobranches. Osmoconformers do not invest energy in transport mechanisms; thus, the osmolality of their internal medium fluctuates according to the osmolality of the environment and maintain NaCl concentration in their body fluids which is equivalent to habitat. However, most of the teleosts maintain the osmolality of their extracellular body fluids relatively constant at approximately 300 mmosmol kg^{-1}, which is independent of environmental salinity and are referred to as osmoregulators (Kültz., 2015).

Survivality of fish depends upon the ability of fish to maintain the body fluids at least for short time in an abnormal range of internal osmotic and ionic concentrations. The fish has a special ability through which they can regulate the body fluid to restore the level of osmotic pressure to near normal (Holiday *et al.,*1969). The migration or abrupt transfer of fish from freshwater to seawater or vice versa will normally lead to increased osmotic concentration of fish blood serum and change in ionic contents and may finally lead to an osmotic stress (Miles and Smith 1968).Osmolality is defined as the concentration of the substance in 1 L of water divided by its molecular weight (Sanders, 2009*)*.Osmolality is the total concentration of all dissolved inorganic ions and organic compounds such as sugars and amino acids found in the body fluid (Kultz., 2015). The osmolality of the fish body fluids is measured by using freezing-point depression osmometry or cryoscopic osmometer (Sanders, 2009*)*. In cryoscopic osmometer, the sample is cooled below its freezing point after that its temperature is slowly raised and the end point being defined as the temperature of the sample at the time when the last ice crystal melts (Unwin and Willmer,1978).Based on osmolality of body fluid and culture medium, we can refer to the culture medium as hyposmotic, hyperosmotic and isosmotic. A solution is said to be hyperosmotic when the osmolality of the

solution is greater than that of the reference solution and hyposmotic, when the osmolality of the solution is less than that of the reference solution. Further an isosmotic solution has an osmolality identical to that of the reference solution (Sanders, 2009). Boeuf &Payan, 2001 reported that, when fish reared in isosmotic medium fishes doesn't need to spend energy for osmoregulation. So, along with the gross energy this additional energy also fish can utilize for growth. The isotonic salinity level of different hardy stenohaline fish ranges from 9-13g/L (Brett, 1979; Lisboa *et al.,* 2015).

2.8.Salinity tolerance of freshwater fish species

Salinity tolerance of freshwater fishes varies with the species, age, size and acclimating conditions. Attempts were made to culture Indian major carps and common carp, *Cyprinus carpio* in saline ground water of 8-10 ppt salinity at Sultanpur (Haryana) during 1984-87. Indian major carps could not tolerate beyond 5 ppt, whereas, common carp could grow to a size of 550 gm in less than a year and also achieved maturation. Dwivedi and Lingaraju, 1986 could successfully culture *C. mrigala* in saline water of 8-10 ppt salinity at Hisar. He further noted that gonadal maturation was fast in *C. mrigala* in saline water than in fresh water. Ghosh *et al*., 1973studied the salinity tolerance of fry and fingerlings of Indian major carps and their growth properties at different levels of salinity, from 0 to 15 ppt. Best growth rates for both species were recorded at 5 ppt. At salinity above 5 ppt fish exhibited reluctance towards feeding complete cessation of feeding was observed at 11 ppt. European carp from the river Mury survived direct transfer from freshwater to a salinity of 12.5 ppt and with acclimation there was 50 % survival at 15 ppt (Geddes, 1979).In general the culture of Indian major carps could be possible in water of low salinity (5-10 ppt) (Ghosh *et al.,* 1973, Sarma *et al.,* 2020). Recently Jahan *et al.,* 2020 reported the successful breeding and development of early larval stages of common carp in inland saline water of upto 10ppt. Singh *et al.,* 2019 also reported that Amur carp can thrives well upto 5ppt salinity in inland saline water. Islam *et al.,* 2014 reported that rohu fingerlings show normal growth upto 6ppt salinity without any mortality. Furthermore, Kilambi& Zdinak, 1980 shows that grass carp can tolerate 10-16ppt salinity for short period .Apart from that, other carps such as big head carp can tolerate low saline water (2.3-7.6ppt) easily (Garcia *et al.,* 1999).

Sharma *et al.,*2017 shows that koi carp can tolerate salinity upto 8ppt without any mortality in inland saline water.

Upper limits of salinity tolerance of different catfish species was studied by James *et al.,* 1969 and was found to be 11 ppt for channel catfish, 12ppt for blue cat fish ,and 13 ppt for white catfish. Castaneda *et al.,*2010 reported that Asian catfish *P. hypophthalmus*, can be cultured in brackish water with up to 13 ppt salinity and survive excursions up to 20 ppt for at least 22 days. Growth of the fish was hampered at salinities above 13 ppt. Chaturvedi *et al.,* 2012 reported that, successful breeding and seed production of Magur in inland saline water of upto 5ppt.They also shown that the fertilization of eggs (78.10%) and hatching rate(89.65%)of *Clarias batrachus* is high at 5ppt salinity in inland saline water. Ahmmed *et al.,* 2007 also reported that *Heteropneustes fossilis* hows normal growth upto 6ppt salinity without any mortality.

Salinity tolerance limit of Nile tilapia was found to be 0-7ppt (Lawson and Anetekhai, 2011) and also shows good growth in low saline water. Most of the tilapia species breed easily in freshwater as well as in low saline water (Chervinski, 1982). According to Perry and Avault, 1972 *O.aureus* can breed easily in low saline water upto 4.3ppt salinity. The red tilapia shows high salinity tolerance as well as shows high growth rate in brackish water upto 17.5ppt salinity (Stickney, 1986).

Anabas testudineus is reported to exhibit faster growth rate in mild saline water compared to freshwater (Widiyati *et al.,* 2019). Nadirah *et al.,* 2014 reported that *Anabas testudineus* hatching (97.3%) and larval survivality (90%) were high in 3ppt salinity compared to freshwater. According to Dubey*et al.*, 2015*Anabas testudineus* shows satisfactory growth and survival at a salinity range of 5-15ppt.Chotipuntu and Avakul, 2010 also shows that, the successful hatching (90%) and larval rearing Anabas testudineus at3ppt salinity. There are conflicting reports in the available literature regarding the salinity tolerance of this species; some of the literature is mentioned in the table provided below. *Anabas testudineus* shows better specific growth rate at upto 8ppt salinity, without any mortality (Chotipuntu and Avakul, 2010). According to Chowdhury, 2014 *Anabas testudineus* shows better FCR and FCE in 8ppt saline water compared to freshwater.

Table 1.Salinity tolerance of *Anabas testudineus* as per the available literature

Authors	Salinity tolerance (ppt)	weight of the fish used for experiment (g)	Duration of experiment (days)
Bianco and Nordlie, 2008	11ppt	6-7	45
Nahar ,2015	9ppt	1.07 to 7.8 g	60
Chotipuntuand Avakul,2010	10ppt	1.51-1.54	40
Zahari and Christianus,2018	15ppt	1.02	90
Ip *et al.,* 2012	30 ppt	25-45	6 days

Table.2. Salinity tolerance of certain widely cultivated stenohaline freshwater species.

species	Salinity tolerance limit(ppt)	References
Catla	0-8ppt	Ghosh *et al.,* 1973, Sarma *et al.,*2020
Rohu	0-6ppt/0-8ppt	Islam *et al.,*2014
Mrigal	8-10ppt	Dwivedi and Lingaraju, 1986
Common carp	0-6ppt	Mangat and Hundal,2014
Silver carp	0-11.1 ppt	Al-Faiz *et al.,*2011
Grass carp	0-7.5 ppt	Li *et al.,*2007
Magur	0-4 ppt	S.K. Sahoo *et al*.,2003
Pangasius	0-15 ppt	Kumar *et al.,*2016
Singhi	0-6 ppt	Ahmed *et al* .,2017
Channa	0-13 ppt	Dubey *et al.,*2016
A. mola	0-6.20 ppt	Dubey *et al.,*2014
P.ticto	0-6.12 ppt	Dubey *et al.,*2014
Nile tilapia	0-20ppt	Schofield *et al*.,2011
Gold fish	0-10 ppt	Luz *et al.,* 2008

2.9. Effect of salinity on various physiological indicators and haematological parameters

Salinity is one of the most vital environmental parameter that significantly affects on growth, survival and distribution of fishes. Salinity variation causes a series of physiological stress response in aquatic animals, which leads to an imbalance of serum hormone levels, energy metabolism and electrolytes imbalance (Choi *et al.,* 2008). When fish reared in hyperosmotic medium, enhanced cellular metabolic activity leads to the production of reactive oxygen species (ROS) or free radicals (Storey, 1996). However, excessive ROS produced in the body of fish due to salinity stress may lead to oxidative stress and cell malfunction, finally resulting in the apoptosis or necrosis of the cell (Sun *et al.,* 2014). But aquatic animals are known to possess special antioxidant defence mechanism by which they protect themselves from ROS/free radicals. Superoxide dismutase (SOD) is the first stress enzyme which can prevent oxidative stress by catalyzing the dismutation reaction of superoxide anion (O_2^-) into O_2 and H_2O_2 in living organisms (Livingstone,, 2001). Another enzyme which is related to scavenging of oxidative radicals produced under stress conditions such as salinity is Catalase, responsible for the degradation of the reactive oxygen species like hydrogen peroxide (H_2O_2) to produce H_2O and oxygen (Livingstone,, 2001). An increase Catalase activity under salinity stress attributed to adaptive mechanism of tissue to reduce the H_2O_2 and providing protection against oxidative stress (Agarwal and Pandey, 2004).

The ability of an organism to digest the ingested food particles depends upon the presence of an appropriate number of digestive enzymes like Amylase, lipase, protease etc (Smith, 1980). In higher salinities within a range it was observed that digestive enzyme activity was a little bit higher compared to freshwater or low saline water. Fishes under salinity stress has to spend lots of energy for maintaining body homeostasis i.e. osmoregulation so, to regulate other physiological functions i.e. digestive physiology, reproductive physiology etc fishes need more energy for that fishes generally enhance their feeding frequency. This reduces the time required for complete digestion and absorption of nutrients (Ferraris *et al.,* 1986) and finally this

process dramatically increases the digestive enzyme activity for complete digestion and absorption of nutrients(Boeuf and Payan, 2001; Psochiou *et al.,* 2007). Several studies also showed that environmental stresses including temperature fluctuations (Miegel *et al.,* 2010) and starvation (Cara *et al.,* 2007) also increase digestive enzyme activity.

ALTand AST, also known as SGOT and SGPT belong to transaminase family and are known to play an important role in protein metabolism (Fazio *et al.,* 2013).When, liver and myocardial cells are damaged or their permeability increases, ALT and AST will be released into the blood, as a result of that blood transaminase activity also increases. Under conditions of stress the activity of transaminase enzymes increase, which might be directed to speed up the rate of amino acid mobilisation. Amino acids released through transaminase action enter the citric acid cycle leading to production of ATP for various physiological activities **(**Ranjan *et al.,* 2020). The activities of serum ALT and AST can be used to monitor the health status of fish (Wang *et al.,* 2005).ALT/AST ratio is often used in clinical investigations to differentiate between muscular and hepatic damage. Elevation of ALT activity appears to reflect liver disease, and it is more specific for liver disease than AST because of the biological location of these two enzymes. ALT is liver specific, where as AST is produced by hepatocytes as well as other tissues including skeletal muscles (Shahsavani *et al.,* 2010). The magnitude of ALT elevation is usually greater than AST when both are elevated due to hepato- cellular damage because of the longer half-life of ALT and the greater fraction of AST that is bound to the mitochondria unlike AST which is mainly found in cell cytoplasm (Aulbach and Amuzie, 2017). Al-Khashali and Al-Shawi, 2013 reported that ALT and AST activity of goldfish increases when fish transferred from freshwater to saline water. Akhtar *et al.,* 2014 also reported enhanced AST and ALT level in muscle and liver of *Labeo rohita* fingerlings exposed to salinity stress.

The haematological parameters are considered as valuable tools for recognizing the fish health condition and also guide the biologists to interpret the physiological stress which occurs due to changes in fish rearing conditions (Bhaskar & Rao 1984).Changes in haematological parameters with salinity will give an idea on the adaptability of fish with different environmental conditions which further

reflects the immunological potential of fish (Bahmami *et al*., 2001). Different types of blood parameters such as haemoglobin and red blood cells (RBC) are correlated with various environmental factors such as water temperature and salinity (Graham 1997). If fish is transferred from freshwater to saline water, there is a sudden drop of haemoglobin content, RBC and WBC counts (Usha, 2011).Elarabany *et al.,* 2017 also reported that in case of Nile tilapia Hb, HCT and RBC count decreases with increasing salinity. Serum glucose is a stress parameter and gives us idea about the adaptability of fish in different condition. In case of tilapia, serum glucose also showing declining trend in higher salinities, which might be due to higher rate of glycogenolysis to meet high energy requirements for osmoregulation i.e.to mitigate the salinity stress in higher salinities (Kavya *et al.,* 2016).Serum total protein is a nonspecific immune variable and gives a good idea about the immune potential of fish in different environmental conditions (Yılmaz *et al.,*2012).Its concentration in fish is less stable compared to mammals(Satchell ,1991).In higher salinities serum total protein dramatically decreases, it might be due to the metabolisation of protein available in serum for dealing with osmoregulatory stress (Elarabany *et al.,* 2017;Yılmaz*et al.,*2012).

3.0 Material and Methods

3.1. Site of the Experiment

The experiment was conducted in wet laboratory of ICAR-CIFE Rohtak centre, Haryana, India. Subsequently, the analysis part had been carried out both at CIFE, Mumbai and in the laboratory of the CIFE, Rohtak Centre.

3.2. Experimental Animal

The animal used for the experiment was *Anabas testudineus* fingerlings (Bloch, 1792) with an average weight ranging from 6.02±0.02g to 6.22±0.08g.Experimental fishes were collected from wetlands of Assam with the help of local fishermen. Following an acclimation period of 2 days at a local hatchery in Assam; fishes were transported by air route to Delhi in oxygen-filled polyethene bags.On reaching the laboratory, fishes were given mild salt treatment to ameliorate the handling stress and carefully transferred to an aerated circular tank (1000 L) and were left undisturbed. Oxytetracycline was given at the rate of 15 mg/l(APHA, 1981) for the next seven days as a precautionary measure. The fish were acclimatized for a period of 15 days in the same tank with water exchange at every 24 hours and were fed with commercial grade fish feed.

3.3. Experimental design

There were five treatments, and each treatment had three replicates. A completely randomized design (CRD) was followed for this study. A total of 225 fishes were distributed into 15 groups according to treatments and were assigned randomly to 15 different FRP tanks of 300 litres capacity.

3.4. Water preparation

Inland saline groundwater having a salinity of 12-13g/L was obtained from bore well located in the high saline zone of Rohtak Farm. Pumped waster was filtered with 100µm filter bag and was stored in a cemented tank (length 300 cm × width 200 cm × water depth 150 cm). After allowing sedimentation of the collected water for a week, it was diluted to four different test salinities (3, 6, 9 and 12 ppt) by using freshwater from a bore well located in the freshwater section of the farm. Freshwater of 0ppt was used as control.

3.5. Salinity Acclimation Method

The experimental animals were acclimatized to different salinities for 12 days. The fishes were exposed to progressively increasing salinities by adding the appropriate amount of inland saline water at equal intervals. The salinity was increased by 1 ppt every 24 hrs until each treatment had reached an appropriate salinity level.

3.6. Experimental set-up

The experiment was carried out in a wet lab of the Central Institute of Fisheries Education, Rohtak centre, Haryana, India. The fishes were reared in ISGW for a period of 60 days during August-17 to October-16.A total of 15 FRP tanks of 300litre volumes were filled with 150litres water of appropriate salinity and aeration stones were put in each tank. Each treatment and control groups were maintained at three replicates wherein each replication was stocked with 15 fishes (a total of 45 fishes for each treatment). 225 fish with initial length and weight ranging from 6.55±0.08 to7.01±0.09 cm and 6.02±0.02g to 6.22±0.08g respectively was used for the experiment. In order to maintain the optimum water quality, experimental tanks were cleaned manually once in 3 days to remove excess feed and faecal matter and 10 % of water was exchanged on daily basis. During the experiment, water quality parameters such as dissolved oxygen, temperature, pH, alkalinity, hardness, ammonia, nitrite, different ions and salinity were recorded once in a week.

3.7. Physico-chemical Parameters of Water

Water quality parameters viz. temperature, pH, dissolved oxygen, total hardness, total alkalinity, un-ionized ammonia, nitrite and calcium were monitored twice in a 15days interval during the experimental period for all the experimental tanks.

3.7.1. Temperature

The temperature was measured using a handheld thermometer.

3.7.2. pH

pH meter was used to determine the pH of the water.

3.7.3. Dissolved Oxygen (DO)

The dissolved oxygen level of water in which fishes were reared was measured using Winkler's method (APHA, 2005) and calculated using the formula.

$$DO\ (mg\ l^{-1}) = \frac{V \times N \times 1000 \times 8}{\text{The volume of the sample (ml)}}$$

Where, V= Volume of the titrant, N= Normality of the titrant (Sodium thiosulphate)

3.7.4. Total ammonia-N (NH_3 –N)

The ammonia concentration was estimated spectrophotometrically at 635 nm wavelength (APHA, 2005) and compared with the standard graph. The Concentration was expressed as mg/l.

3.7.5. Nitrite-N (NO_2 –N)

The nitrite concentration was estimated spectrophotometrically at 543nm wavelength (APHA, 2005) and compared with the standard graph. The concentration was expressed as mg/l.

3.7.6. Total Hardness

The total hardness was estimated by titrimetric method (APHA, 2005). Here, the sample was titrated against standard EDTA solution using Eriochrome black-T indicator and ammonium chloride and ammonium hydroxide as a buffer and calculated using the following formula.

$$\text{Hardness (EDTA) as mg } CaCO_3/L = \frac{\text{ml EDTA used} \times \text{N of EDTA} \times B \times 1000}{\text{ml of sample}}$$

Where, N= Normality, B=mg $CaCO_3$equivalent to 1.00 ml EDTA titration.

3.7.7. Total alkalinity

The total alkalinity was estimated by titrimetric method (APHA, 2005) by titrating against standard H2SO4 and phenolphthalein and methyl orange as an indicator and calculated using the following formula.

$$\text{Total alkalinity (mg/L) as } CaCO_3 = \frac{\text{Volume of HCl} \times \text{N of HCl} \times 1000 \times 50}{\text{ml of sample}}$$

3.7.8. Calcium

Calcium was estimated by titrimetric method (APHA, 2005) by titrating against 0.01M standard EDTA solution and used Murexide as an indicator and calculated using the following formula.

$$\text{Calcium (mg/l) as } CaCO_3 = \frac{(A \times B \times 1000)}{\text{ml of sample}}$$

B=mg $CaCO_3$equivalent to 1.00 ml EDTA titration; A=ml titrant for sample

3.7.9. Magnesium

Magnesium was estimated by using the following formula (APHA, 2005).Magnesium (mg/L) = [Total hardness (mg CaCO3/L) -Calcium hardness (mgCaCO3/L)] x 0.243

3.7.10. Sodium and potassium

By using the flame photometer (ESICO, 1382).

3.8. Feeding

Feeding was done @4% of the body weight initially and after that based on the observation of feed consumption for 15 days; the feeding rate was adjusted. The daily ration was divided into two equal parts and was fed at 9:00 am in the morning and 5:00pm in the evening. The particle size of the commercial feed (Growel feeds Pvt Ltd) was 2mm and it was found to contain 33.89% crude protein, 4.64%crude lipid.

3.9. Proximate analysis of the diet and carcass tissues

Proximate analysis of the feed and carcass tissues was done by standard methods (AOAC, 2006) at Aquaculture and Fish nutrition Laboratory of CIFE.

3.9.1. Moisture

The moisture analysis was done by oven drying the sample.1 g of sample was taken and heated at a temperature of 110°C until a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula.

$$\text{Moisture (\%)} = \frac{\text{(wet weight of sample – dried weight of sample)} \times 100}{\text{Wet weight of sample}}$$

3.9.2. Crude Protein

The nitrogen content of the feed and carcass tissues was estimated quantitatively by automatic Microkjeldahl unit (Kelpus-Classic DX (VA), Pelican Equipments). 0.5 g of Sample was taken and digested with 1.6 g of digestion mixture (K_2SO_4 and $CuSO_4$ in5:1 ratio) in 20 ml concentrated H_2SO_4. The digestion was carried out in the digestion Unit until the solution became clear. The digested sample was diluted to 100 ml and 5 ml of the digested solution was taken for distillation. 10 ml of 40% NaOH was automatically added to the sample for distillation. The total programmed time was 9 min and the liberated NH_3 was collected with Boric acid in a conical flask containing indicator. The amount of ammonia liberated was determined by titrating with 0.1N H_2SO_4. The crude protein content was obtained by multiplying the total nitrogen content by a conversion factor of 6.25 and expressed as a percentage.

$$\text{Crude protein (\%)} = N_2 (\%) \times 6.25$$

3.9.3. Ether Extract (EE)

Ether extraction was done by Soxhlet apparatus using petroleum ether (Boiling point 40-60 °C) as a solvent. Ether extract is calculated by using the following formula:

$$\text{Ether extract (\%)} = \frac{\text{(Initial weight of the sample – final weight of the sample) x100}}{\text{Weight of initial sample}}$$

3.9.4. Ash

Total ash content of the diet and carcass tissue was estimated by taking a known weight of sample in a pre-weighed and ignited silica crucible and placed in a Muffle furnace at 600°C for 7 hours. Ash content is calculated by the following formula:

$$\text{Ash (\%)} = \frac{\text{(Weight of ash) x100}}{\text{Weight of sample}}$$

3.9.5. Total carbohydrate (TC)

The total carbohydrate (TC) of the experimental diet and carcass tissues was calculated by subtracting the percentage of other nutrients from 100 (Hasting, 1969).

TC (%) = 100 – [Crude protein (%) + Ether extract (%) + Ash (%)]

3.10. Assessment of growth parameters

The growth has been expressed as total length (cm), weight (g), weight gain (g), weight gain (%), specific growth rate (SGR) and Hepato-somatic index (HSI) over a period of 60 days.

3.10.1. Weight gain (g)

The weight gain of the fishes after the end of the experimental period was calculated by using the following formula:

Weight gain (g) = (Final weight-Initial weight)

3.10.2. Weight gain (%)

The weight gain percentage of the fishes after the end of the experimental period was calculated by using the following formula:

$$\text{Weight gain (\%)} = \frac{\text{(Final weight – Initial weight)} \times 100}{\text{Initial weight}}$$

3.10.3. Specific Growth Rate (SGR) (% day-^{1})

The Specific growth rate was calculated by using the following formula:

$$\text{SGR} = \frac{\text{[ln of final weight – ln of initial weight] x100}}{\text{Experimental period (days)}}$$

3.10.4. Hepato-somatic index (HSI)

HSI was calculated by using the following formula:

Hepato-somatic index (HSI) (%) = (wet weigh of liver/whole body wt of fish) ×100

3.11. Assessment of Feeds

3.11.1. Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated by the following formula:

$$\text{Feed conversion ratio} = \frac{\text{Feed given (dry weight) g}}{\text{Body weight gain (wet weight) g}}$$

3.11.2. Feed Efficiency Ratio (FER)

The feed efficiency ratio was calculated by the following formula:

$$\text{Feed Efficiency Ratio} = \frac{\text{Body weight gain (wet weight) g}}{\text{Feed given (dry weight) g}}$$

3.11.3. Protein Efficiency Ratio (PER)

The protein efficiency ratio was calculated by the following formula:

$$\text{Protein Efficiency Ratio} = \frac{\text{Body weight gain (wet weight) g}}{\text{Crude Protein fed (g)}}$$

3.12. Survival Rate

At the end of the experiment, the number of the experimental animals in each tank was counted and the survival rate (%) was calculated by the following formula:

$$\text{Survival Rate (\%)} = \frac{\text{Total number of harvested animal} \times 100}{\text{Total number of stocked animal}}$$

3.13. Collection of Organs

The fish were dissected and gill, intestine, muscle and liver samples were separately and macerated with 0.25M sucrose solution. After centrifugation at 10000 rpm for 10 minutes, the supernatant was collected in sterilized eppendrof tubes and stored at -20 °C for carrying out enzyme assay.

3.14. Sample preparation

The muscle, liver, gill, and intestine of the fish were removed carefully and weighed. They were homogenized with chilled sucrose solution (0.25 M) in a glass tube using tissue homogenizer. The tube was continuously kept in an ice bath while homogenizing. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4˚C in a cooling centrifuge. The supernatant was kept in a deep freezer at -20˚C temperature for further analysis. A 5% homogenate was prepared for all tissues for all analyses.

3.15. Analysis of Digestive Enzymes

3.15.1. Amylase

The reducing sugars produced due to the action of glucoamylase and alpha amylase on carbohydrates was estimated using Dinitro Salicylic Acid (DNS) method (Rick and Stegbauer, 1974). The reaction mixture consists of 1% (w/v) starch solution, phosphate buffer, and the tissue homogenate. The reaction mixtures were incubated at 37˚C for 30 minutes. DNS was added after incubation and kept in boiling water bath for 5 minutes. After cooling, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as mill mole of maltose released from starch per minute at that temperature.

3.15.2. Protease activity

The protease activity was determined by the casein digestion method (Drapeau, 1974). The enzyme reaction mixture consisted of 1% (w/v) casein in 0.05M Tris-phosphate buffer (pH 7.8) and incubated for 5 minutes at 37˚C. Then tissue homogenate was added. Ten minutes later, the reaction was stopped by adding 10%TCA (Trichloro Acetic Acid) and the whole content was filtered. The reagent blank was made by adding tissue homogenate just before stopping the reaction with TCA and with no incubation. One unit of enzyme activity was defined as the amount of enzyme needed to release acid-soluble fragments equivalent to Δ0.001 A280 per minute at 37˚C and pH 7.8.

3.15.3. Lipase activity

The lipase activity was assayed by the method of Cherry and Crandell (1932). Two test tubes labelled as a test (T) and control (C) were taken. Each of the two tubes was added with 3ml of the distilled water and 1ml of the homogenate. One of the tubes (C) was placed in boiling water served to inactivate the lipase in control. Then 0.5ml of buffer solution (phosphate buffer pH 7.0) and 2 ml olive oil emulsion was added to both the tubes, shaken well and incubated at 37˚C for 24 hours. Then 3ml of 95% alcohol and 2 drops of phenolphthalein solution were mixed. Each the tubes were titrated with 0.05 N NaOH up to the appearance of

permanent pink colour. The volume (ml) of N/20 NaOH solution required for 100 mg intestinal or liver tissue in the experimental tube subtract the volume (ml) of N/20 NaOH solution required for the same amount of intestinal or liver tissue in the control tube represented the units of intestinal or liver lipase activity per gram tissue.

3.16. Antioxidant Enzymes

3.16.1. Superoxide dismutase assay (SOD)

According to the method described by Misra and Fridovich (1972), Superoxide dismutase was assayed based on their ability to inhibit auto-oxidation of epinephrine to adrenochrome at pH 10.2. 50 µl of the sample was taken in a cuvette and 1.5 ml 0.1 M carbonate-bicarbonate buffer containing 0.57 mg ml^{-1} EDTA (pH10.2) 30 and 0.5 ml epinephrine (3 mM) was added and mixed well. The change in optical density at 480 nm was measured immediately for 3 min in an UVspectrophotometer. One unit of SOD activity was considered as the amount of protein required to inhibit 50 % auto-oxidation of epinephrine.

3.16.2. Catalase assay (CAT)

Catalase activity was assayed according to the method described by Takahara *et al.,* (1960). The reaction mixture consisted of 2.45 ml of phosphate buffer (50 mM, pH 7.0) and 50 µl enzyme source. The reaction was started by the addition of 1.0 ml of H_2O_2 solution (0.3% in phosphate buffer). The decrease in absorbance was measured at 240 nm at 15-sec intervals for 3 min. The blank was run simultaneously with 1.0 ml distilled water instead of hydrogen peroxide solution. Enzyme activity was expressed as micromoles H_2O_2 decomposed min^{-1} mg^{-1} protein ml^{-1}.

3.17. Collection of blood and serum

At the end of the experimental period of 60 days, three fish per replicate were randomly selected for blood and serum collection .Before going for that fishes were anaesthetized with clove oil (50 µl/L).Blood samples were collected from the caudal vein using a 1 ml hypodermic syringe (without anticoagulant) and transfer the blood immediately into Eppendrof tubes and wait for 2hrs at room temperature.

After this, blood got completely coagulated and serum got separated from rest. Centrifugation at 4850g for 10 minutes enabled the complete separation and collection of serum from the coagulated blood. The straw-coloured supernatant i.e. serum was collected in sterilized eppendrof tubes, marked properly and stored at -20°C for carrying out the further analysis.

3.18. Enzymes for protein metabolism in serum

3.18.1. Aspartate Aminotransferase (AST or SGOT) Activity

SGOT activity was estimated using Aspartate Aminotransferase (ASTor SGOT) Activity Colorimetric Assay Kit (SIGMA-ALDRICH, USA and Catalog Number-MAK055). The assay performance was based on the detection of glutamate which was released during the reversible transamination reaction between α-ketoglutarate and aspartate. The absorbance was measured at 450 nm using ELISA plate reader (Biotek India Pvt. Ltd). The absorbance was taken two times during the reaction to know the activity of aspartate during the transamination reaction.

3.18.2. Alanine Aminotransferase (ALT or SGPT) Activity

SGPT activity was estimated using Aspartate Aminotransferase (ASTor SGOT) Activity Colorimetric Assay Kit (SIGMA-ALDRICH, USA and Catalog number-MAK052). The assay performance was based on the detection of pyruvate which was released during the reversible transamination reaction between α-ketoglutarate and alanine. The absorbance was measured at 570nm using ELISA plate reader (Biotek India Pvt. Ltd). The absorbance was taken two times during the reaction to know the activity of alanine during the transamination reaction.

3.19. OSMOLALITY

The serum and water osmolality (mOsm/kg) were measured using a cryoscopic osmometer (Osmomat® 030; Gonotec GmbH). The osmoregulatory capacity (OC) of the fishes in different treatments was calculated as the difference between the mean osmolality of the fish serum and mean osmolality of their corresponding rearing media (Greenwell *et al.,* 2003).

3.20. Haematological parameters

3.20.1. Total Erythrocyte count (×10^6/ul)

Red blood cells (RBC) count was done using Neubauer's counting chamber of a haemocytometer as already described by Hendricks (1952).In this process 20 µl of blood along with that 3,980 µl of erythrocyte diluting fluid (Qualigens) was taken in a sterilized test tube and mix gently. After that counting was done by putting a drop of this mixture into the counting chamber of a haemocytometer.

3.20.2.White blood cells (WBC) count (×10^3/ul)

White blood cells (WBC) count was done by following the method of Shaw (1930).

3.20.3. Haemoglobin content (mg/dl)

The haemoglobin content of blood was estimated following the cyanmethemoglobin method using Drabkin's Fluid (Qualigens). 20µl of blood was mixed with 5ml of Drabkin's working solution. The absorbance was measured using a spectrophotometer at wavelength of 540nm. The final concentration was calculated by comparing with the standard cyanmethemoglobin (QualigensDiagnostics). The haemoglobin concentration was then calculated by using the following formula-

$$\text{Hemoglobin content(g\%)} = \frac{\text{OD(T)}}{\text{OD(S)}} \text{X} \frac{251}{1000} \text{X60}$$

Where, OD (T) = Absorbance of test, OD (S) = Absorbance of standard

3.20.4. Haematocrit value (%)

Hematocrit value were determined by using a haematocrit centrifuge at 1000g for 10 min (Boon *et al.,*1990).

3.21. Serum parameters

3.21.1. Serum glucose

Serum glucose estimation was done by using Trinder's method using ERBA KIT. Glucose in the sample was oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyzes the oxidative coupling of 4-aminoantipyrine with phenol to yield a coloured quinonemine complex, with absorbance proportional to the concentration of glucose in sample.The absorbance of Standard (S) and Test (T) were measured against the blank (B) in a spectrophotometer at 505 nm. The calculation was done as follows:

$$\text{serum glucose}(\text{mg/dl}) = \frac{\text{Con. of standard} * \text{absorbance of Test(T)}}{\text{absorbance of standard}}$$

Concentration of standard=100mg/dl

3.21.2. Serum protein

Plasma protein estimation was done by biuret method (Reinhold, 1953) using ERBA kit. Proteins present in the plasma binds with copper ions in an alkaline medium of the biuret reagent and produce a purple coloured complex, whose absorbance is proportional to the concentration of protein in sample.The absorbance of Standard (S) and Test (T) were measured against the blank (B) in a spectrophotometer at 546 nm. The calculation was done as follows:

$$\text{serum protein}(\text{g/dl}) = \frac{\text{Con. of standard} * \text{absorbance of Test(T)}}{\text{absorbance of standard}}$$

Concentration of standard=6g/dl

3.22. Statistical analysis

The statistical analysis was done using software Statistical Analysis Software (SAS; version 9.3). One way ANOVA followed by a Tukey's test was employed to compare the significant difference between treatments at a given point at 5% level of significance.

Plate no.1. Experimental setup

Plate no.2.Weighing of fishes

Plate.4.Estimation of water quality parameters

4.0 RESULTS

4.0 RESULTS

4.1. Physico-chemical parameters of water

The physico-chemical parameters of water in all experimental tanks such as temperature (°C), dissolved oxygen (mg l^{-1}), pH, hardness (mg l^{-1}), total alkalinity (mg l^{-1}), ammonia (mg l^{-1}), nitrite (mg l^{-1}) and calcium (mg l^{-1}),Magnesium(mg l^{-1}) and different others ions were periodically recorded and the average values of all the treatments are presented in Table 3.

4.1.1. Temperature

The water temperature of the different treatments ranged between 28.5°C to 30.0 °C throughout the experimental period of 60 days.

4.1.2. Dissolved oxygen

The dissolved oxygen (DO) level of all the experimental tanks was recorded in the range of 5.5 to 6.4mg l^{-1} during experimental period of 60 days.

4.1.3.pH

The water pH of the different treatments ranged between7.39 to 7.84 throughout the experimental period of 60 days.

4.1.4. Total hardness

During the experimental period of 60 days, total hardness of water was found to vary between 178.75 to 3093.75mg l^{-1}.

4.1.5. Total alkalinity

Total alkalinity of all the experimental tanks wasin the range of 126.00 to 213.50 mg l^{-1} during the experimental period of 60 days.

4.1.6. Total ammonia nitrogen (NH_3-N)

Ammonia-N was recorded within the range of 0.50 to 1.52 mgl^{-1} in experimental tanks during the experimental period of 60 days.

4.1.7. Nitrite nitrogen (NO_2-N)

During the experimental period of 60 days, Nitrite-N value varied within the ranges of 0.10 to 0.65mg l^{-1} among the various treatment groups.

4.1.8. Calcium

During the experimental period of 60 days, calcium content of water was found to be 39.75 to 299.00mg l^{-1}.

4.1.9. Magnesium

During the experimental period of 60 days, Magnesium content of water was found to be 8 to 556 mg l^{-1}.

4.1.10. Sodium

During the experimental period of 60 days, sodium content of water was found to be 19.70 to 3540 mg l^{-1}.

4.1.11. Potassium

During the experimental period of 60 days, sodium content of water was found to be 3.72 to 16.73 mg l^{-1}

Table 3. Physico-chemical parameters of water used for rearing of *Anabas testudineus* juveniles in ISW at different salinity level for the period of 60 days

Parameters	Treatments				
	T1(0 ppt)	T2(3 ppt)	T3(6 ppt)	T4(9 ppt)	T5(12 ppt)
Temperature(°c)	28.53 - 29.87	28.50-29.93	28.60-29.73	28.63-29.97	28.67-30.00
DO (mg l^{-1})	5.85-6.30	5.50-6.05	5.75-6.15	6.15-6.40	5.75-6.05
pH	7.61-7.84	7.39-7.55	7.57-7.60	7.57-7.59	7.52-7.72
Hardness (mg l^{-1})	120-140	950-1010	1760-1880	2230-2300	3080-3260
Total alkalinity (mg $CaCO_3$ l^{-1})	126.00-128.25	144.00-146.25	161.50-165.75	177.75-183.75	211.25-213.50
Total Ammonia nitrogen (mg l^{-1})	0.50-0.67	0.83-0.89	0.98-1.20	1.25-1.27	1.20-1.52
NO_2^--N (mg l^{-1})	0.10-0.55	0.28-0.43	0.47-0.65	0.44-0.49	0.47-0.59
Calcium (mg l^{-1})	39.75-51.00	98.00-100.50	157.50-164.00	216.00-220.50	281.50-299.00
Magnesium (mg l^{-1})	8-12	155-170	298-330	391-405	510-556
Sodium (mg l^{-1})	19.70-26	800-860	1580-1670	2740-2800	3480-3540
Potassium (mg l^{-1})	3.72-4.17	6.33-6.44	10.61-10.77	12.65-12.85	15.71-16.73

4.2. Assessment of growth parameters

Growth parameters such as Average Body Weight, Average Length, Food Conversion Ratio (FCR), Food Efficiency Ratio (FER), and Protein Efficiency Ratio (PER), Specific Growth Rate (SGR), Weight Gain (WG), and Percentage Weight Gain (PWG) were measured at the end of experimental period and are represented in Table 4 to 6and Fig. 1 to 6.

4.2.1. Average weight (g)

The initial body weight among the experimental groups varied from 6.02±0.02g to 6.22±0.08g; whereas the final weight varied from 13.50±0.73g to 16.48±0.83g.The highest average weight (g) at the end of the experiment on 60th day was observed in T2 (16.48±0.83g) and the lowest average body weight (g) was observed in T5 (13.50±0.73g). Descriptive statistics for final body weight is provided in table 4. There were no significant difference ($p>0.05$) observed for final average weight in all the treatments as evident from the Tukey's Kramer plot provided in Fig.5.Fig-1 provided below suggests that final weight of fishes in the different treatment was normally distributed.

4.2.2. Average length (cm)

Average length (cm) of *Anabas testudineus* was recorded initially at 0 day and at the end of experiment on 60th day. The initial length of fishes among the experimental groups varied from 6.55 ± 0.08 cm to 7.01 ± 0.09 cm, whereas the final length varied from 8.20±0.15 cm to 9.04±0.17 cm. The highest final length (cm) was found in T3 (9.04±0.17 cm) and the lowest body length (cm) was observed in T5 (8.20±0.15) treatment.As represented in Fig.-6, treatment (T5) occupied a different tukeys kramer grouping to the control (T1) and various treatments (T2,T3 and T4).Final length of the fishes in the different treatment had near to normal distribution as represented in Fig- 2.

Table 4.Descriptive statistic of final length and body weight

Trait	Treatment	N	Mean ± SE	SD	Min	Max	CV
Final length	T1	45	8.82±0.15	1.03	6.90	12.40	11.63
	T2	45	8.91±0.15	1.02	6.80	10.70	11.45
	T3	45	9.04±0.17	1.12	6.60	11.50	12.45
	T4	45	8.58±0.14	0.97	6.60	10.40	11.34
	T5	45	8.20±0.15	0.98	5.90	10.20	11.97
Final body weight	T1	45	15.81±0.72	4.80	7.64	26.12	30.38
	T2	45	16.48±0.83	5.54	7.25	29.48	33.65
	T3	45	16.01±0.94	6.30	6.29	36.36	39.37
	T4	45	13.87±0.68	4.59	5.00	22.57	33.06
	T5	45	13.50±0.73	4.89	5.00	25.85	36.22

Table 5. Different Growth traits of *Anabas testudineus* juveniles reared in ISW at different salinity level for the period of 60 days

Traits/ Treatments	Initial length(cm)	Final length(cm)	Initial body weight(g)	Final body weight(g)
T1(0ppt)	6.78 ± 0.09^{ab}	8.82 ± 0.15^{b}	6.21 ± 0.07^{a}	15.81 ± 0.72^{a}
T2(3ppt)	7.01 ± 0.09^{b}	8.91 ± 0.15^{b}	6.02 ± 0.02^{a}	16.48 ± 0.83^{a}
T3(6ppt)	6.56 ± 0.11^{a}	9.04 ± 0.17^{b}	6.05 ± 0.02^{a}	16.01 ± 0.94^{a}
T4(9ppt)	6.55 ± 0.08^{a}	8.58 ± 0.14^{ab}	6.18 ± 0.10^{a}	13.87 ± 0.68^{a}
T5(12ppt)	6.62 ± 0.10^{a}	8.20 ± 0.15^{a}	6.22 ± 0.08^{a}	13.50 ± 0.73^{a}

Values are expressed as Mean ± SE.

n = 3; Mean values in each column with different superscripts differ significantly ($p < 0.05$).

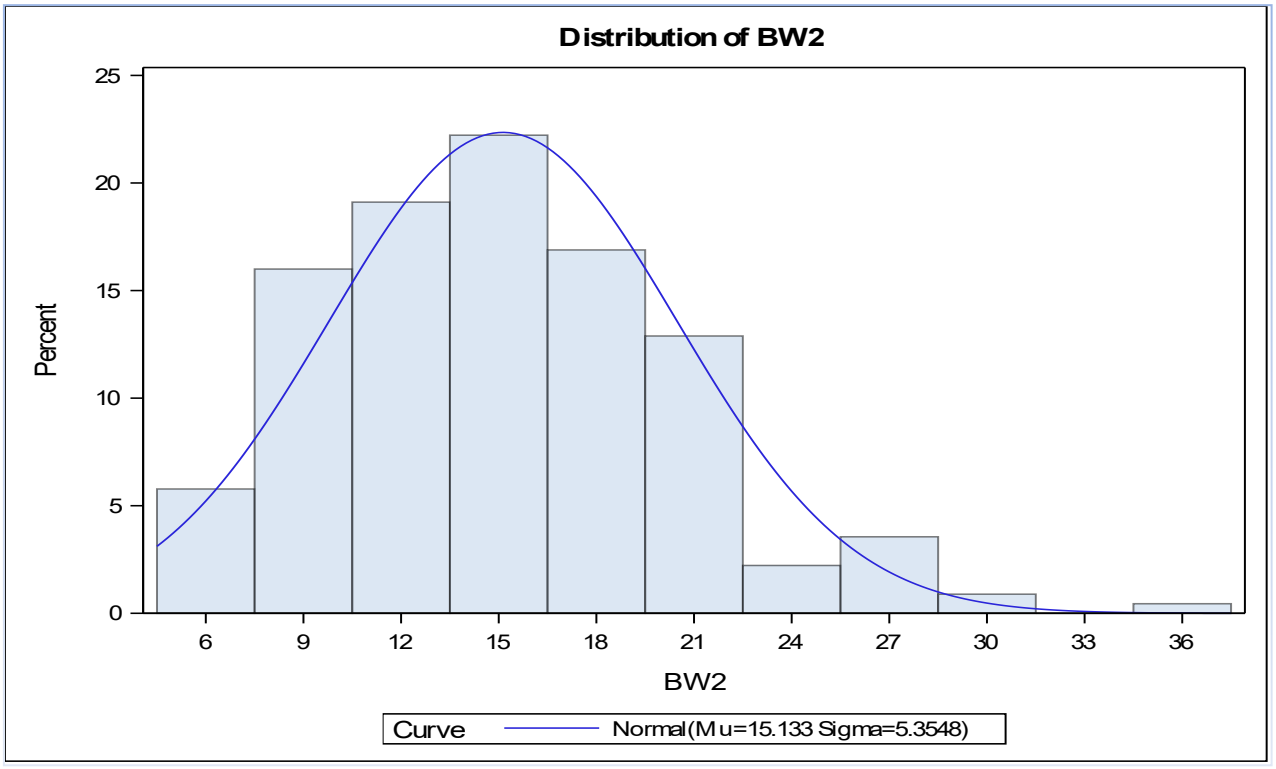


Fig-1; Histogram of final body weight (BW_2)

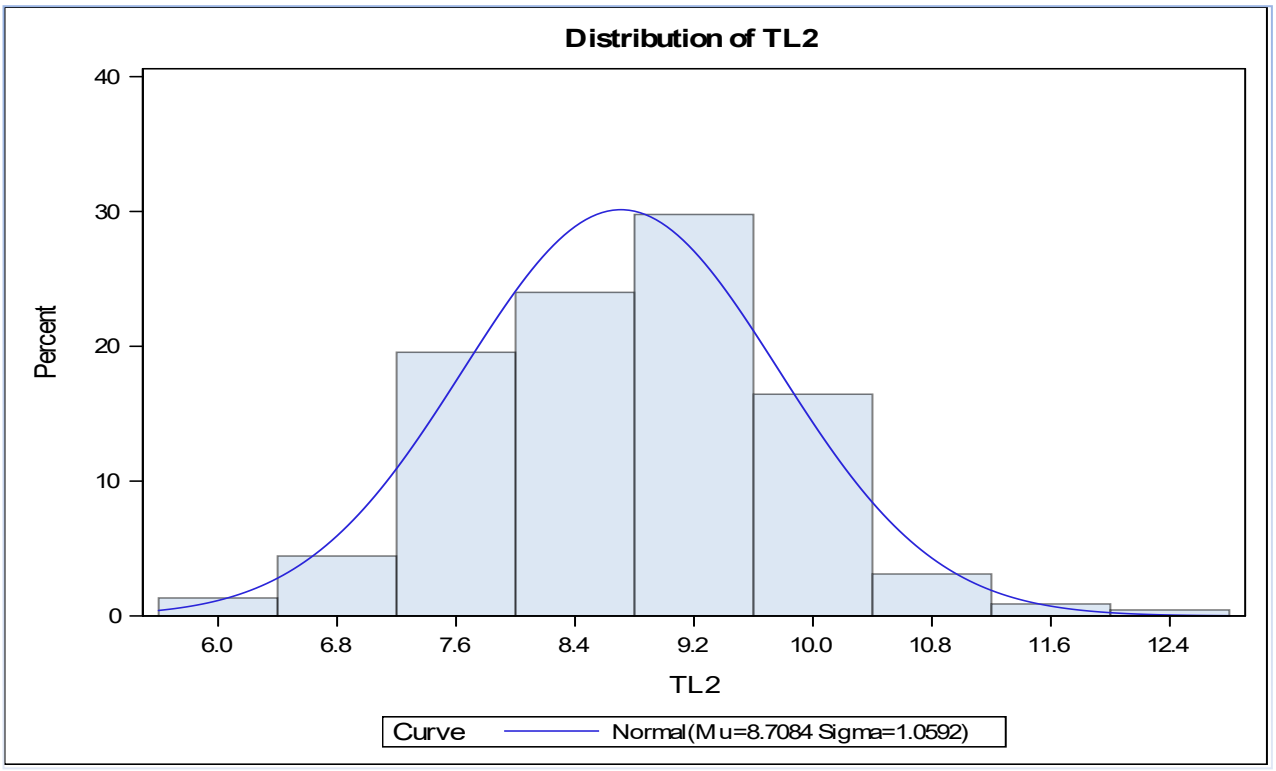


Fig-2; Histogram of final length (TL_2)

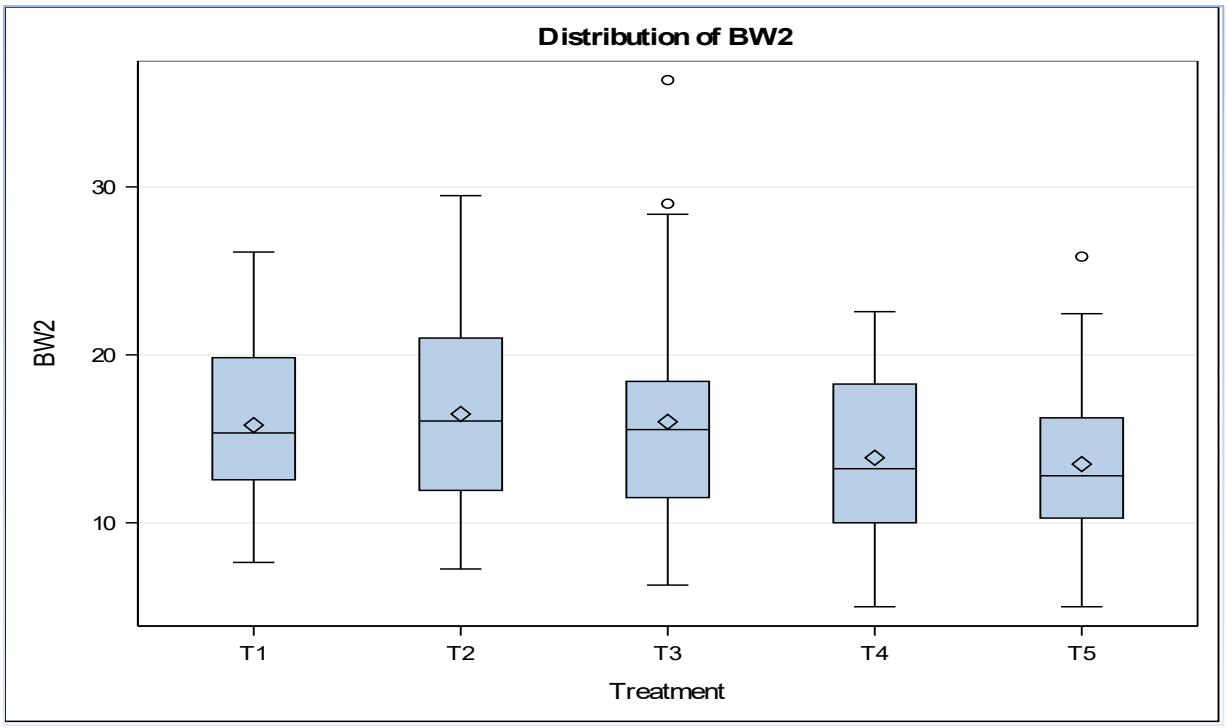


Fig-3; Box plot of final body weight (BW_2)

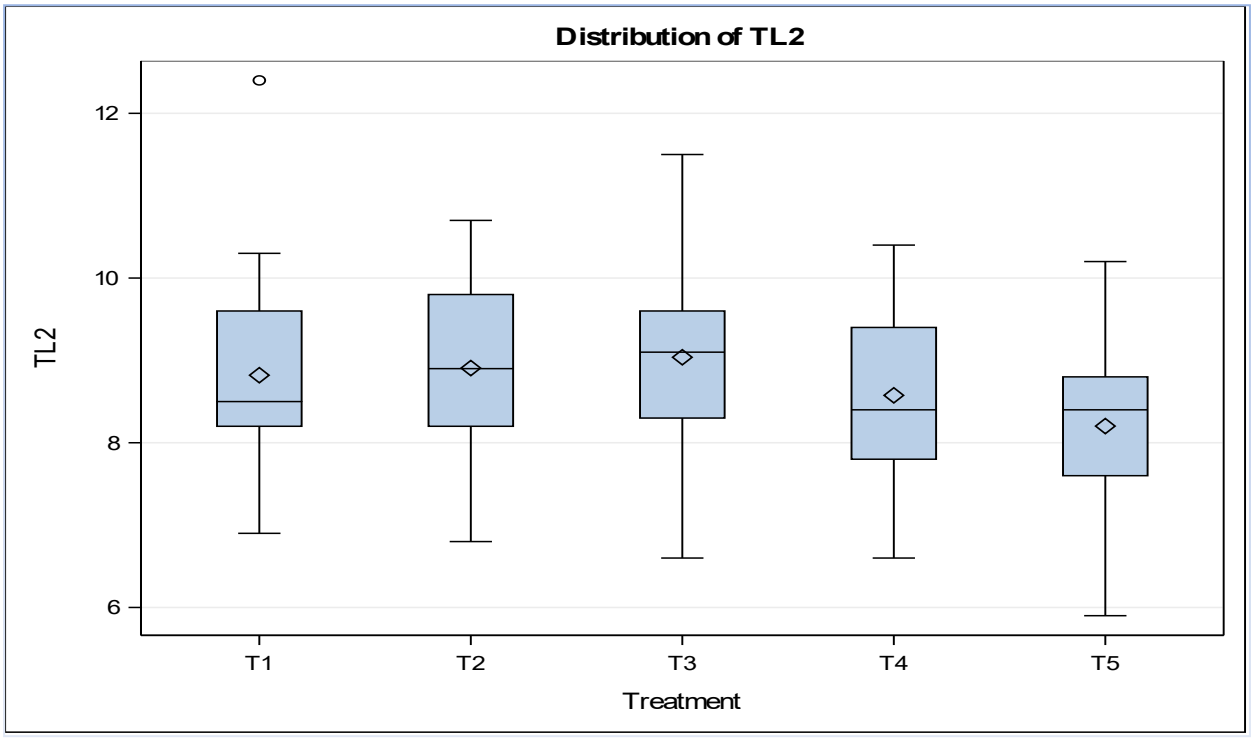


Fig-4; Box plot of final length (TL_2)

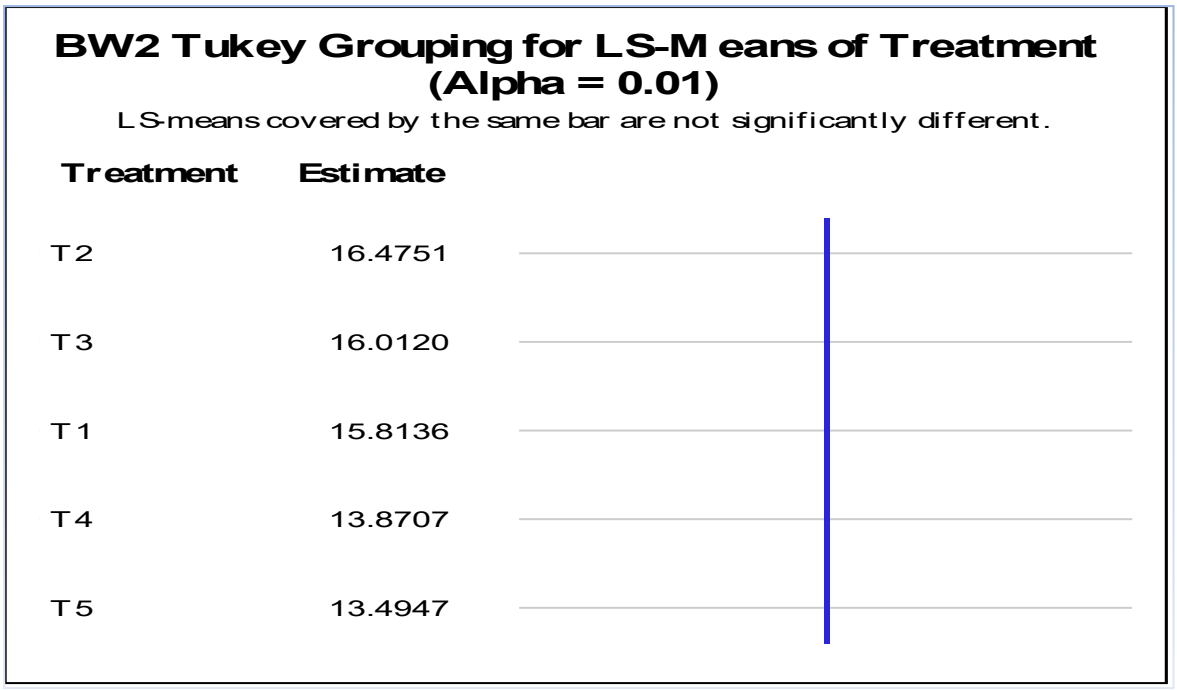


Fig-5; Turkey Kramer grouping plot of Final body weight (BW2)

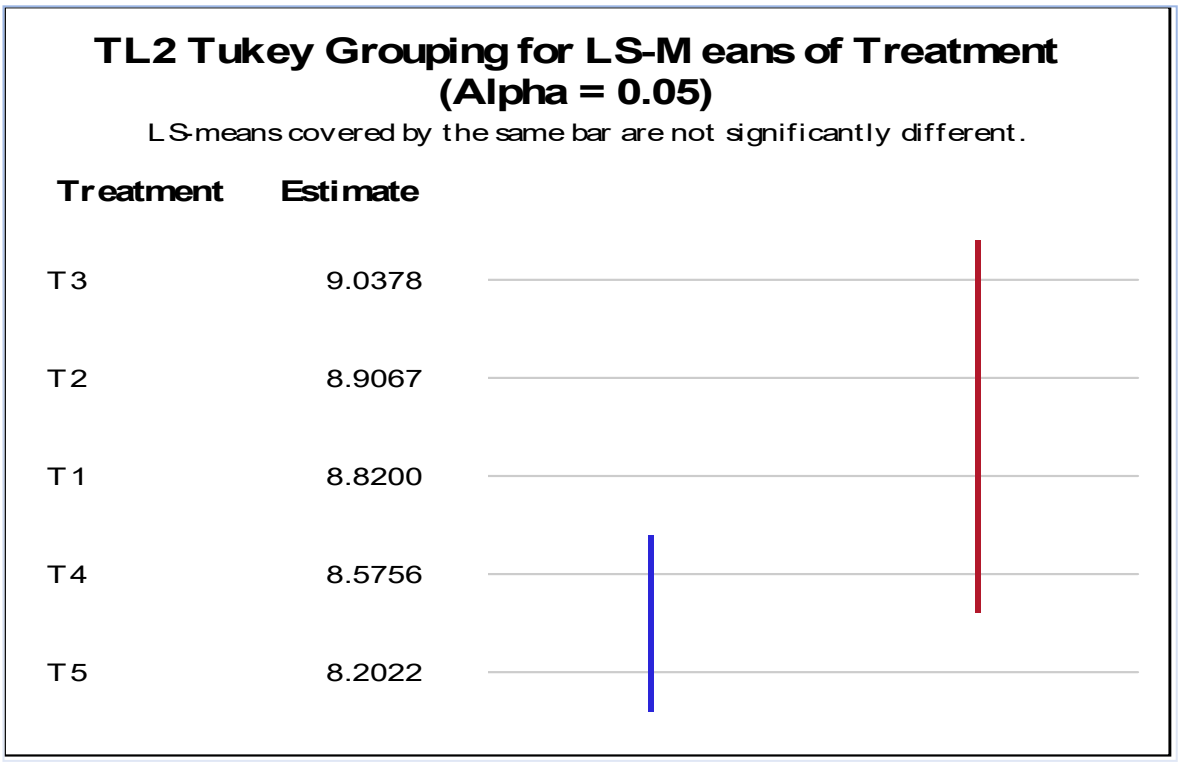


Fig-6; Turkey Kramer grouping plot of final length (TL2)

4.2.3. Weight Gain (g)

Weight gain (g) has been presented in table.6 for all the treatments. The lowest Weight gain was recorded in T5 treatment (109.09±1.38g), which is significantly different (p>0.05) from T1, T2 and T3, but there were no significant differences observed between T4 and T5 (p>0.05).

4.2.4. Percentage Weight Gain (PWG)

Percentage Weight gain (%) has been presented in table.6for all the treatments. Lowest PWG were recorded in T5 treatment (116.88± 0.50), which is significantly different (p>0.05) from T1, T2 and T3, but there were no significant differences observed between T4 and T5 (p>0.05).

4.2.5. Specific Growth Rate (SGR) (%/day)

Specific growth rate has been presented in table.6 for all the treatments. The highest SGR value was recorded in T2 (1.68± 0.03), which is significantly different (p>0.05) from T4 and T5, but there were no significant differences observed between T1,T2 and T3 (p>0.05).

4.2.5. Feed Conversion Ratio (FCR)

The FCR values of the different experimental groups are shown in Table.6.The highest FCR value was recorded in T5 (1.98±0.06), which is significantly different (p>0.05) from T1, T2 and T3, but there were no significant differences observed between T4 and T5 (p>0.05).

4.2.6. Protein Efficiency Ratio (PER)

The PER values of the different experimental groups are shown in Table.6.The lowest protein efficiency ratio was found in T5 (3.22± 0.04), which is significantly different (p>0.05) from T1, T2 and T3,but there were no significant differences observed between T4 and T5 (p>0.05).

4.2.5. Feed Efficiency Ratio (FER)

The feed efficiency ratio (FER) was found to differ significantly (p>0.05) among the various treatment group (Table. 6). The highest FER value was recorded in T3 (0.66±0.01) and the lowest value was recorded inT5 (0.51±0.02).

4.2.6. Survivality

No mortality was found in any of the treatment group during the experimental period.

Table 6.Growth, nutrient utilization and survival of *Anabas testudineus* juveniles reared in ISW at different salinity level for a period of 60 days

Parameters	Treatments				
	T1(0ppt)	T2(3ppt)	T3(6ppt)	T4(9ppt)	T5(12ppt)
WG(g)	144.09± 12.17^{abc}	156.79± 5.02^{b}	149.47± 11.24^{ab}	115.39± 6.27^{ac}	109.09± 1.38^{c}
PWG (%)	154.55± 11.64^{abc}	173.54± 4.90^{c}	164.83± 12.72^{bc}	124.64± 7.57^{ab}	116.88± 0.50^{a}
SGR(%/day)	1.55± 0.06^{ab}	1.68± 0.06^{b}	1.62± 0.06^{b}	1.35± 0.06^{a}	1.29± 0.06^{a}
FCR	1.63± 0.07^{ab}	1.58± 0.03^{a}	1.51± 0.02^{a}	1.81± 0.04^{bc}	1.98± 0.06^{c}
FER	0.62± 0.03^{bc}	0.63± 0.01^{c}	0.66± 0.01^{c}	0.55± 0.01^{ab}	0.5±0.02^{a}
PER	4.25± 0.36^{abc}	4.63± 0.15^{c}	4.41± 0.33^{bc}	3.40± 0.18^{ab}	3.22± 0.04^{a}
Survival (%)	100	100	100	100	100

Values are expressed as Mean ± SE.

n = 3; Mean values in each row with different superscripts differ significantly (p < .05).

4.3. Whole-body carcass composition and body indices

4.3.1. Whole-body carcass composition

The whole body carcass composition of all the experimental fishes in terms of moisture, protein, lipid, total carbohydrate and ash at the end of the experiment are given in Table-7,Fig;7-11. The proximate composition was found to be significantly different ($P<0.05$) among the various treatment groups. The highest moisture content was found in T3 group followed by T5, T4, T1 and T2 groups respectively. The crude protein content was significantly higher ($P<0.05$) in T5as compared to other treatments. The highest crude lipid value was found in T2 and lowest value was recorded inT5. The highest ash content was found in T4and lowest in T3 .Further, the highest total carbohydrate content was recorded in T1 followed by T3, T2, T4 and lowest value was recorded in T5.

4.3.2 Hepato-somatic index (HSI)

Hepato-somatic index of *Anabas testudineus* did not differ significantly ($p>0.05$) among the different treatment groups during the feeding trial, signifying that HSI was not significantly affected with the increase in salinity

Table 7.Whole body proximate composition (on%wetweightbasis) and HSI of *Anabas testudineus* juveniles reared in ISW at different salinity level for the period of 60 days

Trait/ Treatments	Moisture (%)	Crude protein (%)	Ether- extract (%)	Total ash (%)	Total carbohydrate (%)	HSI (%)
T1 (0 ppt)	75.41± 0.61^{ab}	13.32± 0.36^{b}	4.66± 0.06^{a}	3.88± 0.09^{bc}	2.73± 0.13^{b}	2.31±0.06^{a}
T2 (3ppt)	75.21± 0.54^{a}	13.17± 0.29^{b}	6.20± 0.17^{c}	3.58± 0.08^{b}	1.84± 0.10^{a}	2.35±0.04^{a}
T3 (6 ppt)	78.57± 0.40^{c}	11.29± 0.33^{a}	5.49± 0.08^{b}	2.95± 0.05^{a}	1.70± 0.07^{a}	2.25±0.05^{a}
T4 (9 ppt)	77.48± 0.41^{bc}	12.30± 0.22^{ab}	4.51± 0.15^{a}	4.17± 0.10^{c}	1.55± 0.11^{a}	2.24±0.04^{a}
T5 (12 ppt)	77.67±0.06^{c}	12.46± 0.06^{ab}	4.44± 0.07^{a}	4.13± 0.02^{c}	1.55± 0.09^{a}	2.22±0.05^{a}

Values are expressed as Mean ± SE.

n = 3; Mean values in each column with different superscripts differ significantly (p < .05).

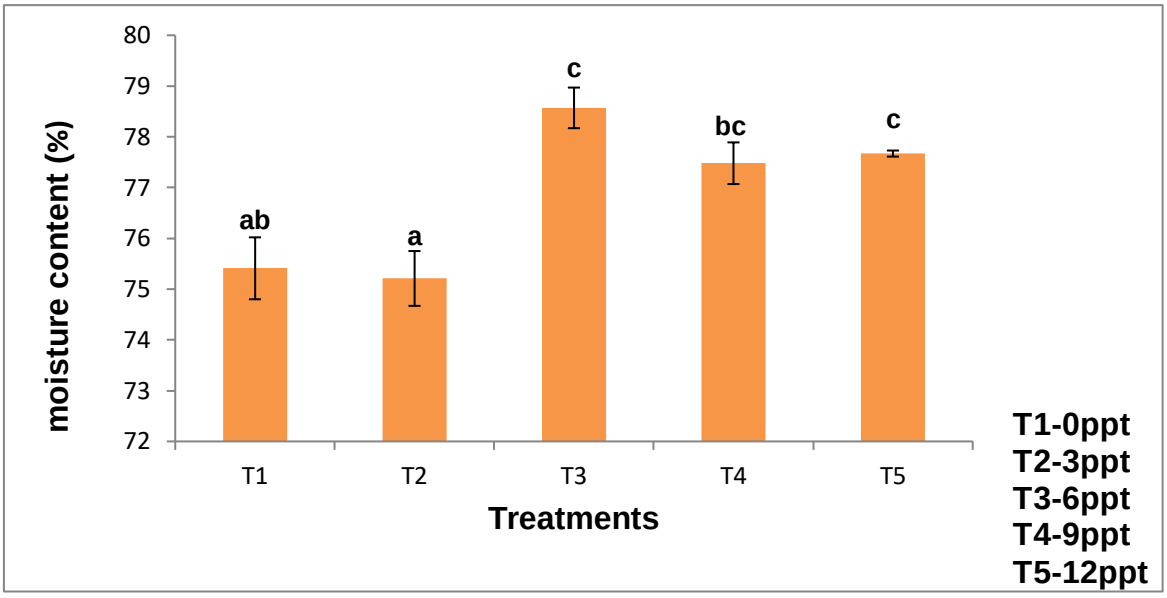


Fig: 7.Moisture contents of *Anabas testudineus* reared in the treatment groups during 60 days of culture period

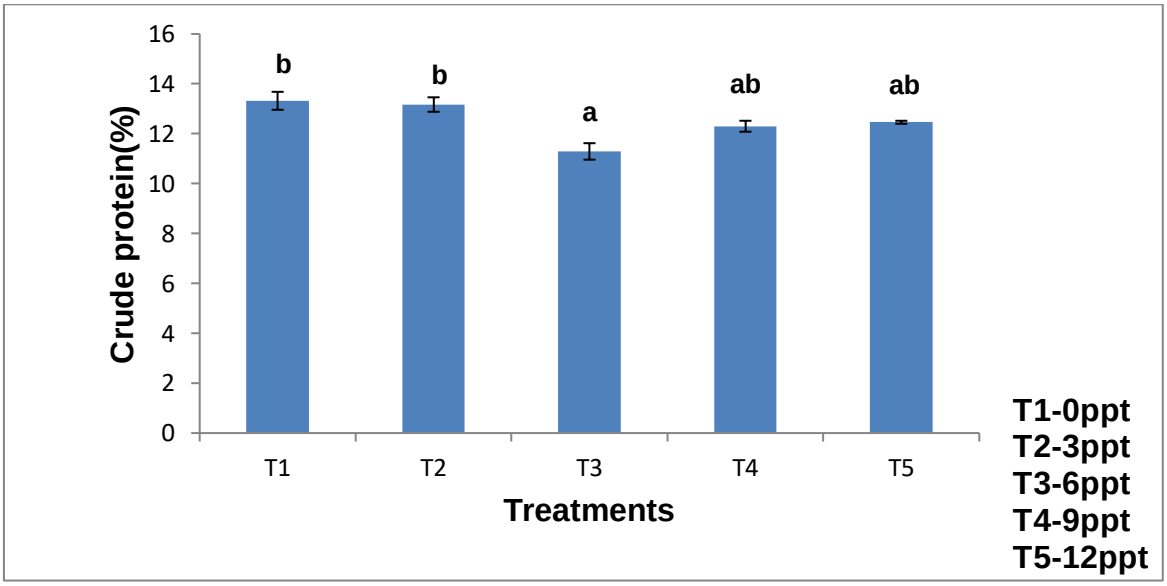


Fig: 8.Crude protein contents of *Anabas testudineus* reared in the treatment groups during 60 days of culture period

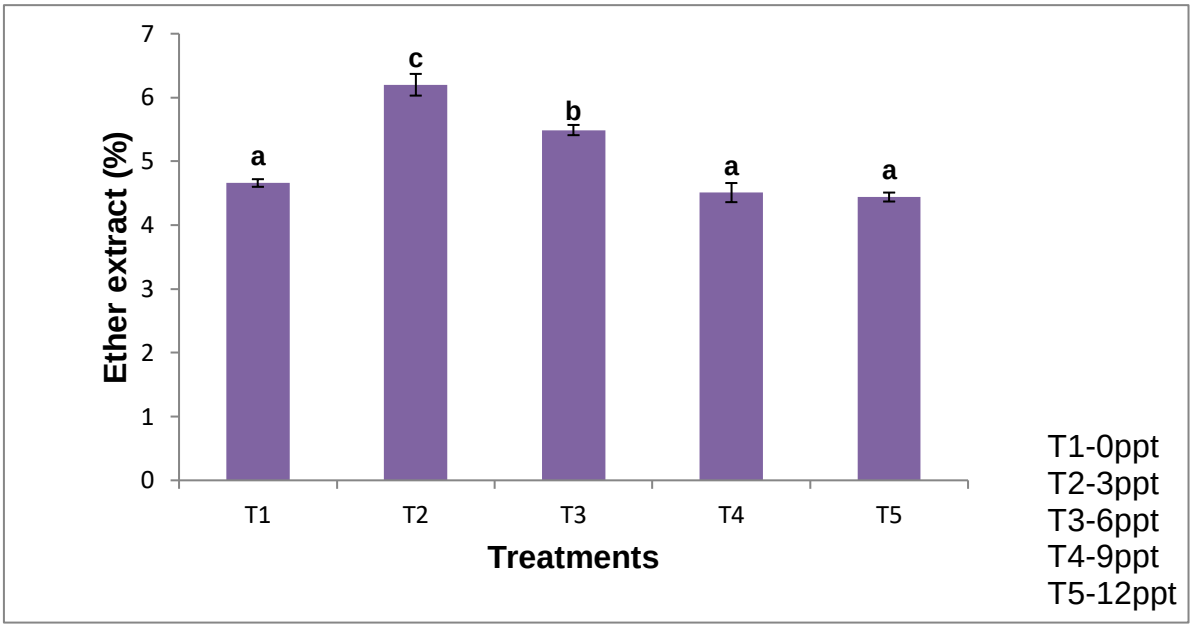


Fig: 9.Ether extract contents of *Anabas testudineus* reared in the treatment groups during 60 days of culture period

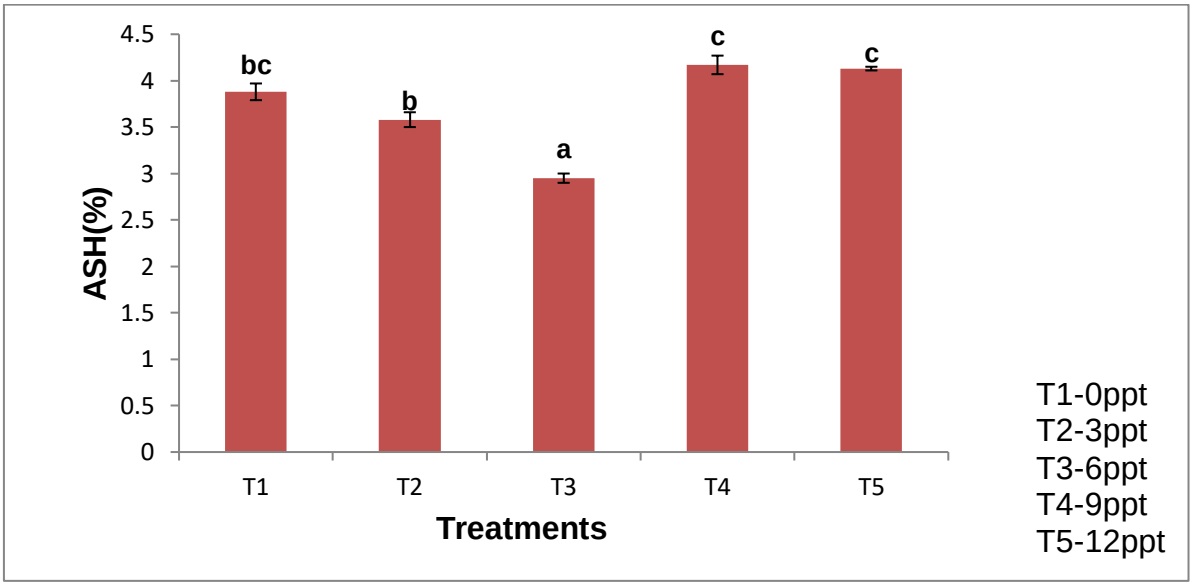


Fig: 10.Total ash contents of *Anabas testudineus* reared in the treatment groups during 60 days of culture period

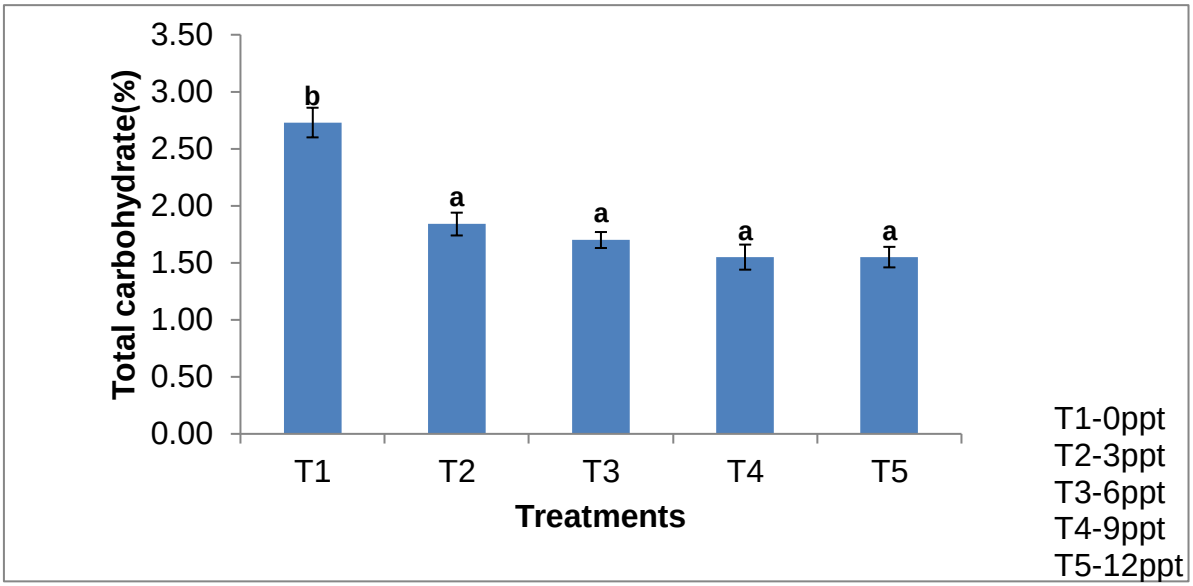


Fig: 11.Total carbohydrate contents of *Anabas testudineus* reared in the treatment groups during 60 days of culture period.

4.4. Digestives enzyme activity

Digestive enzyme activities (amylase, lipase, and protease) were evaluated in the intestine of *Anabas testudineus* at the end of 60 days period (Table 8).Among the treatments, digestive enzyme activities were found to be higher in T4 and T5 groups except lipase.

4.4.1. Amylase

A significantly higher amylase activity was recorded in, T5 ($p<0.05$) in comparison to T1, T2 and T3 group, but there were no significant differences observed between T4 and T5 ($P>0.05$) signifying that, amylase activity increases with the increasing salinity.

4.4.2. Lipase

Lipase activity in intestine was not differed ($p>0.05$) among the different treatment groups during the experiment.

4.4.3. Protease

Protease activity of different treatment groups have been presented in table 5 expressed as U/mg protein/min.A significantly higher protease activity was recorded in, T4 ($p<0.05$) in comparison to T1, T2 and T3 group, but there were no significant differences observed between T4 and T5 ($P>0.05$) signifying that, protease activity also increases with the increasing salinity.

Table 8. Digestive enzyme activities of *Anabas testudineus* juveniles reared in ISW at different salinity level for the period of 60 days

Trait/ Treatments	[1]Amylase	[2]Protease	[3]Lipase
T1 (0 ppt)	1.53±0.01[a]	1.53±0.07[a]	0.230±0.003[a]
T2 (3ppt)	1.54±0.01[a]	1.53±0.02[a]	0.231±0.003[a]
T3 (6 ppt)	1.54±0.17[a]	1.54±0.05[a]	0.226±0.003[a]
T4 (9 ppt)	2.07±0.05[b]	1.89±0.08[b]	0.228±0.003[a]
T5 (12 ppt)	2.09±0.09[b]	1.87±0.03[b]	0.237±0.003[a]

Values are expressed as Mean ± SE

n=3; Mean values in each column with different superscripts differ significantly (p < .05)

[1]Amylase activity is expressed in micromole maltose released/min/mg protein

[2]Protease activity is expressed in mill mole of tyrosine released/min/mg protein

[3]Lipase activity is expressed in Unit/min/mg protein

4.5. Enzymes of Oxidative stress

4.5.1. Superoxide Dismutase (SOD)

The SOD activity in gill and liver tissue of the different experimental groups is shown in Table 9. The highest SOD activity in the gill tissue were recorded in T5 (5.53± 0.14, 50% inhibition of epinephrine auto oxidation/mg protein/min), which is significantly different ($p<0.05$) from T1, T2 and T3, but there were no significant difference observed between T4 and T5 ($p >0.05$). Similarly, in liver, the SOD activity was found to be highest in T5 (4.90± 0.10, 50% inhibition of epinephrine auto oxidation/mg protein/min), which is significantly different ($p<0.05$) from T1, T2 and T3 , but there were no significant difference observed between T4 and T5 ($p >0.05$).

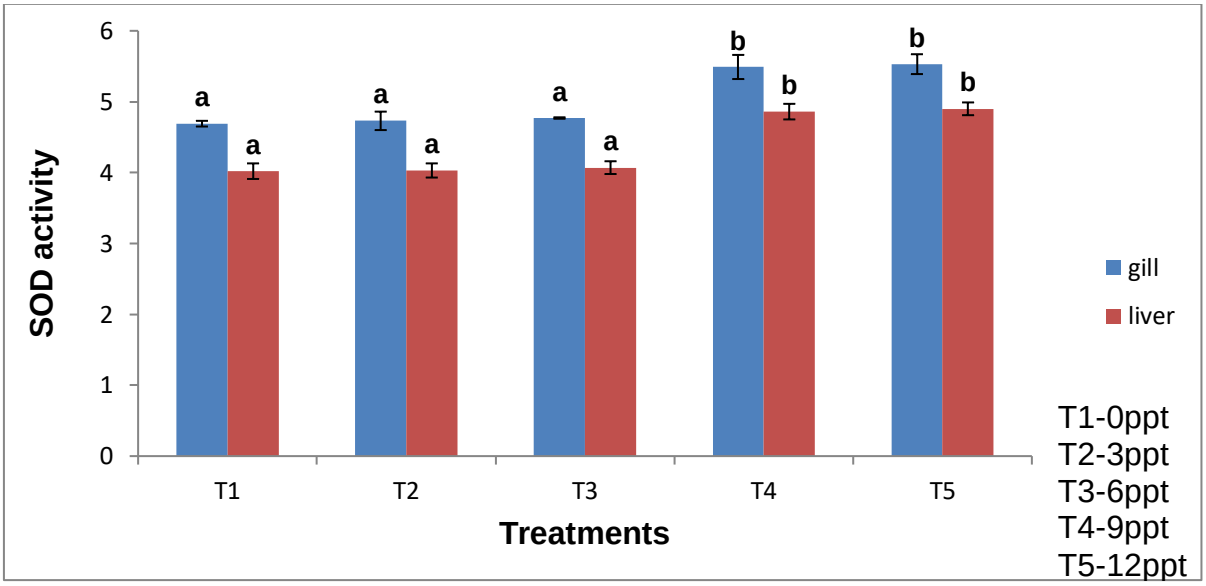


Fig-12: Superoxide Dismutase (SOD) activity (50% inhibition of epinephrine auto oxidation/mg protein/min) in gills and liver of *Anabas testudineus* at the end of 60 days of experimental period

4.5.2. Catalase (CAT)

Catalase activity in gill and liver tissue of the different experimental groups has been presented in Table 9. The highest Catalase activity in the gill tissue were recorded in T5 (2.16±0.06, nanomoles H_2O_2 decomposed/min/mg protein), which is significantly different ($p<0.05$) from T1, T2 and T3, but there were no significant difference observed between T4 and T5 ($P>0.05$). Similarly, in liver, the Catalase activity was found to be highest in T5 (8.15±0.04, nanomoles H_2O_2 decomposed/min/mg protein) ,which is significantly different ($p<0.05$) from T1, T2 and T3 but there were no significant differences observed between T4 and T5 ($P>0.05$) signifying that CAT activity increases with the increasing salinity.

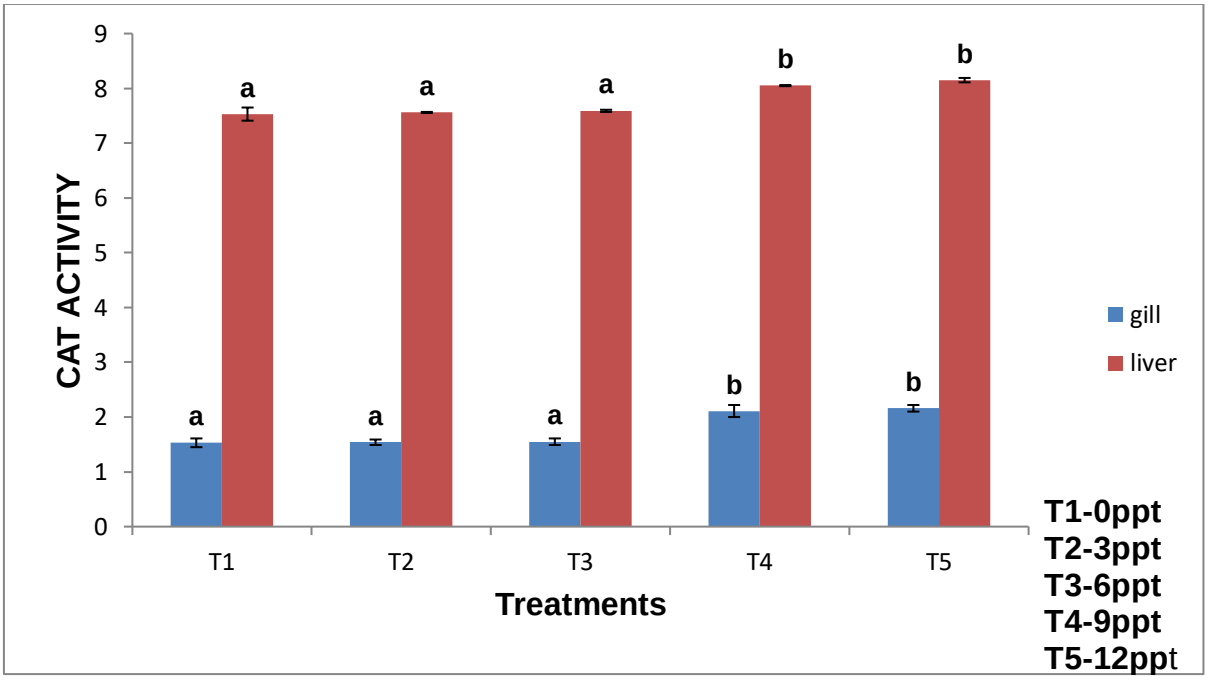


Fig-13: CAT activity (nanomoles H_2O_2 decomposed/min/mg protein) in gills and liver of *Anabas testudineus* at the end of 60 days of experimental period

4.6. Enzymes for protein metabolism in serum

4.6.1. Serum Glutamic Oxaloacetic Transaminase/ Aspartate Transaminase (SGOT/AST)

The SGOT level in serum of the different experimental groups has been presented in Table-9.A significantly higher serum GOT activity was recorded in T5 (38.22±0.46) in comparison to T1, T2 and T3 ($p<0.05$), but there were no significant difference observed between T4 and T5($p>0.05$).

4.6.2. Serum Glutamic Pyruvic Transaminase/ Alanine Transaminase (SGPT/ALT)

The SGPT level in serum of the different experimental groups has been presented in Table-9. Significant differences ($p <0.05$) were recorded among the different treatment groups.Serum GOT level was found highest inT5 (100.31 ±0.59 nmol/min/ml) and lowest in T1 (70.96 ±0.83 nmol/min/ml), signifying that ALT activity increases with the increasing salinity.

Table 9.Oxidative stress and protein metabolism enzymes of *Anabas testudineus* juveniles reared in ISW at different salinity level for the period of 60 days

Trait/ Treatments	SOD liver	SOD gill	CAT liver	CAT gill	ALT	AST
T1 (0 ppt)	4.02±0.11^{a}	4.69±0.04^{a}	7.53±0.12^{a}	1.53±0.08^{a}	70.96±0.83^{a}	28.54± 0.80^{a}
T2 (3ppt)	4.03±0.10^{a}	4.73±0.13^{a}	7.56±0.01^{a}	1.54±0.05^{a}	78.66±0.33^{b}	30.57± 1.41^{a}
T3 (6 ppt)	4.07±0.09^{a}	4.77±0.01^{a}	7.59±0.02^{a}	1.55±0.06^{a}	84.21±0.55^{c}	30.93± 0.96^{a}
T4 (9 ppt)	4.86±0.11^{b}	5.49±0.17^{b}	8.05±0.01^{b}	2.11±0.11^{b}	92.78±2.19^{d}	35.68± 0.28^{b}
T5 (12 ppt)	4.90±0.10^{b}	5.53±0.14^{b}	8.15±0.04^{b}	2.16±0.06^{b}	100.31±0.59^{e}	38.22± 0.46^{b}

Values are expressed as Mean ± SE.

n = 3; Mean values in each column with different superscripts differ significantly (p < .05).

***SOD activity is expressed as 50% inhibition of epinephrine auto oxidation/mg protein/min**

***Catalase (CAT) activity is expressed as nanomoles H2O2 decomposed/min/mg protein**

***ALT and AST activity is expressed as nmol/min/ml**

4.7. Osmolality

4.7.1. Water osmolality

Water osmolality were found to be significantly different ($p<0.05$) among the various treatment groups**.**Water osmolality was found lowest in T1 (36.00±1.53) and increases with increase in salinity having highest water osmolalityat T5 (354.33± 2.03). (Table-10)

4.7.2. Serum osmolality

Highest serum osmolality were recorded in T2 treatment (342.00± 2.31), which is significantly differed ($p < 0.05$) from T4 and T5, but there were no significant difference observed between T1, T2 and T3treatment ($p >0.05$). (Table-10)

Table 10.Osmolality traits of *Anabas testudineus* juveniles reared in ISW at different salinity level for the period of 60 days

Trait/ Treatment	Water osmolality(WO)	Serum osmolality(SO)	Osmoregulatory capacity(OC)
T1 (0 ppt)	36.00±1.53^{a}	336.67± 7.62^{a}	300.67± 6.12^{e}
T2 (3ppt)	106.00±1.73^{b}	342.00± 2.31^{a}	235.00± 2.08^{d}
T3 (6 ppt)	167.67±1.45^{c}	329.33± 3.18^{a}	161.67± 2.85^{c}
T4 (9 ppt)	283.33±1.86^{d}	305.67± 3.53^{b}	22.33± 5.24^{b}
T5 (12 ppt)	354.33±2.03^{e}	264.33±4.37^{c}	-90.00± 2.89^{a}

Values are expressed as Mean ± SE.

n = 3; Mean values in each column with different superscripts differ significantly (p < .05).

Osmolality traits are expressed as mmosmol/kg

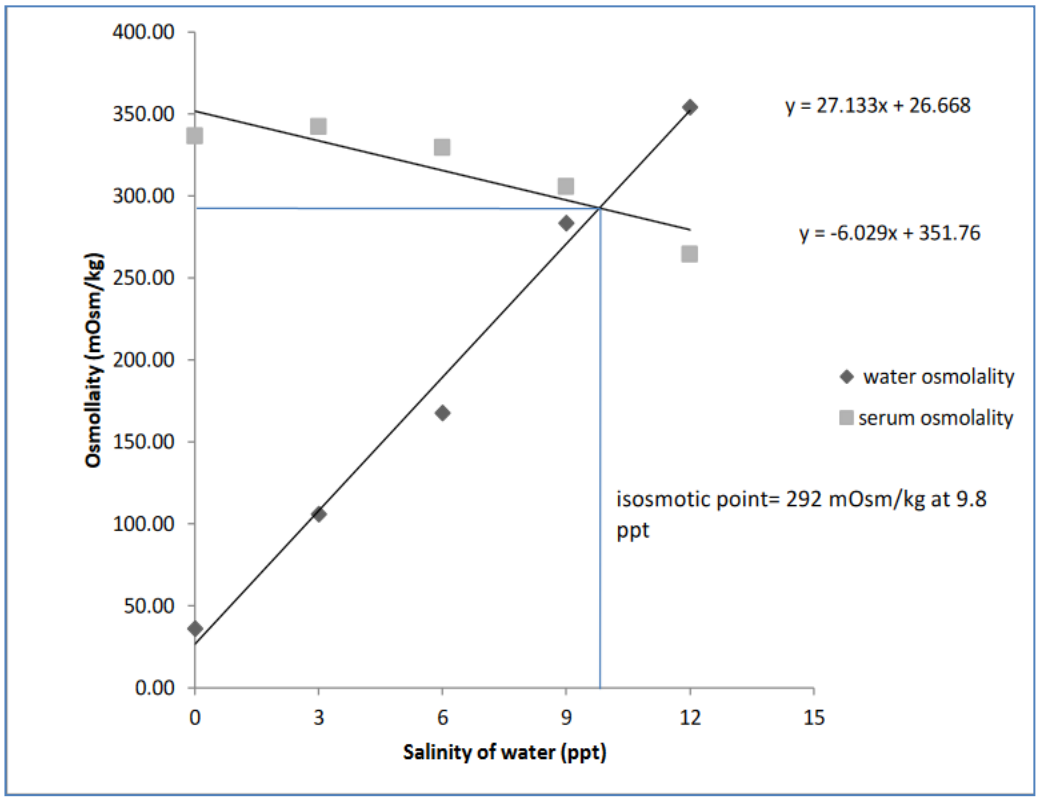


Fig14; Osmolality of *Anabas testudineus* reared in inland saline water of different salinity level for a period of 60 days

4.8. Haematological parameters

Haematological parameters in terms of haemoglobin, haematocrit, RBC and WBC showed declining trend with increasing salinity (T2 to T5) and the difference among the treatments were significant ($p<0.05$). The highest haemoglobin content was found in T2 treatment followed byT3, T1, T4 and T5.The haematocrit value of the fishes were significantly ($p<0.05$) higher in T2 group, compared to T1, T4 and T5, but there were no significant difference observed between T2 and T3 ($p>0.05$).The red blood cells count were significantly higher ($p<0.05$) in T2 group, compared to T4 andT5 and there were no significant difference observed between T2 , T1 and T3($p>0.05$).Further, the highest WBC count was recorded in T2 group followed byT3,T1 and T4 and lowest count was recorded in T5.

4.9. Stress parameter

4.9.1. Serum glucose

Serum glucose level of the different experimental groups is given in Table no. 11. Serum Glucose level was found to be highest in T3 (89.37±1.63 g/dL) group, which is significantly similar ($p>0.05$) to T1, T2 and T4 and lowest serum glucose level were found in T5.

4.9.2. Serum total protein

Serum total protein level of the different experimental groups is given in Table no.11. Serum glucose level was found to be highest in T2 (3.71± 0.06), which is significantly similar ($p>0.05$) to T1 and T3. The lowest value was observed in T5 and significantly similar ($p>0.05$) to T4.

Table 11. Haematological parameters of *Anabas testudineus* juveniles reared in ISW at different salinity level for the period of 60 days

Trait/ Treatments	Haemoglobin (mg/dl)	Hematocrit (%)	RBC (x10^6/µl)	WBC (x10^3/µl)	Serum total protein (g/dl)	Serum glucose (g/dl)
T1 (0 ppt)	10.37± 0.20^{b}	32.43± 1.36^{b}	3.16± 0.10^{d}	146.43± 4.56^{b}	3.66± 0.05^{c}	88.35± 2.39^{b}
T2 (3ppt)	11.00± 0.31^{b}	36.53± 0.68^{c}	3.36± 0.11^{cd}	151.03 ± 3.32^{b}	3.71± 0.06^{c}	87.63± 2.65^{ab}
T3 (6 ppt)	10.40± 0.15^{b}	33.33± 1.10^{bc}	2.92 ± 0.07^{bc}	148.47± 0.72^{b}	3.52± 0.05^{bc}	89.37± 1.63^{ab}
T4 (9 ppt)	8.60± 0.17^{a}	27.60± 0.15^{a}	2.64± 0.06^{ab}	126.20 ± 2.12^{a}	3.38± 0.05^{ab}	84.12± 1.24^{ab}
T5 (12 ppt)	8.27± 0.09^{a}	27.13± 1.01^{a}	2.52± 0.03^{a}	119.27± 4.44^{a}	3.22± 0.04^{a}	80.00± 0.83^{a}

Values are expressed as Mean ± SE.

n = 3; Mean values in each column with different superscripts differ significantly (p < .05).

5.0. DISCUSSION

5. DISCUSSION

5.1. Physico-chemical parameters of Water

The physio-chemical parameters of water are very crucial factor for the aquatic animals as the animals are directly or indirectly dependent on the environmental condition to maintain their physiological homeostasis. In present study, the physiochemical parameters of water such as temperature, pH, dissolve oxygen, ammonia, total hardness, total alkalinity and nitrite-N were found within the optimum range for fish culture at low salinity in inland saline aquaculture system. Among the different physio-chemical parameters of water, temperature plays a vital role to maintain the metabolic activity in poikilothermic fish. The optimum range of temperature for rearing of warm water fish varies from 26-30°C (Boyd, 1990; Widiyati *et al.,* 2019). In the present experiment temperature was maintained within the optimal range of 28.5 to 30.0°C.The optimum range of pH value for koi culture ranges from 6-9 (Widiyati *et al.,* 2019). In this experiment pH value during the trial ranged from 7.39 to 7.84, which is within the prescribed limit. Total alkalinity (mg $CaCO_3$ l^{-1}) value in the experiment ranged from 126.0 to 213.50, an increasing trend with rising salinity. This may be due to rising concentration of bicarbonate anions in the water with increase in salinity of water. Santhose and Singh, 2007 reported that the ideal value of total alkalinity for fish culture is in the range of 50-300mg/l. The total hardness of the water increased from 178.75 mg/l to 3093.75 mg/l as the salinity of the rearing medium was raised. Total hardness can be defined as the concentration of divalent cations in water also expressed as CaCO3. Calcium and magnesium are the most dominant divalent cations in natural waters (Boyd *et al.,* 2016). Higher value of hardness as observed in this experiment is attributable to enhanced concentration of two most vital divalent cations such as calcium and magnesium in water of high salinity. In the present experiment, TAN was found to range from 0.52 to 1.52 mg/l. TAN level in the water was found to be increased at higher salinity. *Anabas testudineus* is capable of active ammonia excretion, hence it is expected that it will also have extraordinarily high tolerance of environmental ammonia (Tay *et al.,* 2006). It has been reported earlier that *A. testudineus,* reduces ammonia excretion and simultaneously accumulated certain amino acids, presumably for cell volume regulation, during a progressive increase in salinity. However, after long term

acclimation to seawater, tissue amino acid concentrations returned to normal, and there was a significant increase in ammonia excretion instead (Chang *et al.,* 2007). Stone and Thomforde, 2004 reported that the desirable limit of nitrite is 0-1 mg/l for fish culture. In the present study, the nitrite value falls within desirable range. The water ions viz Ca^{2+} (39.75-299.00 mg/l), Mg^{2+} (8-556 mg/l), Na^{+} (19.70-3540 mg/l) and K+ (3.72-16.73 mg/l) concentrations during the present experiment were within range of characteristics of ISW as described earlier by various authors (Rahman *et al.,* 2005, Raizada *et al.,* 2005, Jain *et al.,* 2007, Raizada *et al.,* 2015, Reddy and Harikrishna, 2014).

5.2. Growth and nutrient utilization *Anabas testudineus*

Salinity had no significant effect on the final body weight of the fishes, which indicates the ability of *A. testudineus* to tolerate brackish water conditions which is supported by previous finding, where it has been reported to be naturally present in brackish water environment (Jayaram, 1981). However, our findings contrast with the findings of Nahar *et al.,* 2016 who reported a significant effect of salinity on the growth of *A. testudineus* reared for a period of 60 days. The difference in the observation may be explained in the light of differences in the salinity acclimation protocol and the size of the fish selected for experiment.

In the present study, best FCR was recorded at T3 (6 ppt) which differed non significantly from T1, T2 and T3 ($p>0.05$). In higher salinities(9 and 12ppt) FCR is high, which may be due to increased energetic cost for osmoregulation following salinity exposure, inducing fish to consume more feed to derive energy for maintaining other physiological functions (Rahman *et al.,* 2020). Similar findings were also reported by Nahar *et al.,*2016 for *Anabas testudineus*. Several growth traits such as Specific growth rate, weight gain shows maximum value at T2 treatment (3ppt) and there were no significant difference ($p>0.05$) observed between T1 (0ppt), T2 (3ppt) and T3 (6ppt) treatments. Kelly and Woo, 1995 reported that in freshwater condition fish has to spend a certain amount of energy to compensate the salt loss during passive diffusion, so providing mild saline water will reduce the energy expenditure and consequently promotes the growth of fish. Consistent with the present study, Altinok and Grizzle, 2001 also showed that two stenohaline freshwater species, such as Channel catfish and goldfish have the

highest specific growth rates, most efficient feed conversion ratio and energy absorption efficiency in mild saline water compared to freshwater. Dubey *et al.,* 2015 reported that *A. testudineus* shows highest SGR, Weight gain and percentage weight gain at 5ppt salinity and different indexes of growth were not significantly affected up to salinity exposure of 5ppt. Imanpoor *et al.,* 2012 also found that upto 6ppt salinity various biometric indexes of goldfish i.e. SGR, WG, FCR were not significantly affected ($p>0.05$). Highest Feed conversion efficiency was recorded at T3 (6ppt) and there is no significant difference observed between T2, T3 and T4 in comparison to T1 ($p>0.05$). Otto, 1971 reported that *Oncorhynchus kisutch* growth rate, feed intake and feed conversion efficiency shows the highest value at salinity of 5 to 10ppt.

5.3. Survival rate

Stickney 1979, stated that the survival rate of fish against salinity stress completely depends on the quick adaptive ability of fish body fluid ionic concentration with the surrounding environment. When fish are in stress they try to tolerate the changes of plasma osmolality and body fluid ionic concentrations with respect to the surrounding environment and mitigate the stress. In present study 100% survivality was observed up to 12 ppt, which is supported by the findings of Besra, 1997 who reported that *Anabas testudineus* fingerlings (6-10 g) could withstand 2.5 to 10 ppt saltwater without mortality. Zahari *et al.,* 2018 also reported that *Anabas testudineus* can tolerate salinity up to 15 ppt. Chang *et al.,* 2007 in a short term study of 6 days have also reported the ability of *A. testudineus* to tolerate saline water of 30 ppt. Ability to survive under extreme environmental conditions might have been acquired evolutionarily along with their ability to leave without water. Some of the fresh water fishes such as koi carp shows 100% survival rate up to 12 ppt salinity (*Sharma et al., 2017)*, and similar result also found in case of goldfish and crucian carp, up to 16 ppt salinity (Küçük, 2013).

5.4. Whole body composition

The proximate composition of fish is mainly influenced by several factors like seasonal, environmental and biological variations (Desai and Singh, 2009; Tao *et al*, 2012).Several environmental factors like salinity, temperature and pH directly or

indirectly affects the whole body carcass composition of many fishes and the results of the present experiment correlated with some of the previous studies that, salinity truly can affects the proximate composition of fish(Fallah *et al.*, 2013; Jalali *et al.*, 2013; Kumar *et al*., 2016;singh *et al.,*2019).In the present study, moisture content(%) of the raw fish ranges from 75.21 ± 0.54 to 78.57 ± 0.40,which is more or less similar to the findings of Chowdhury *et al.,*2014; where they found the moisture content (%) of *Anabas testudineus* ranged from 65.28 ± 0.002 to 79.29 ± 0.005. On the other hand, in this present experiment highest moisture content was found in T3 (6 ppt) group followed by T5 (12 ppt) ,T4 (9 ppt),T1 (0 ppt) and T2 (3 ppt) group respectively i.e. higher moisture content were found in higher salinities(6, 9 and 12 ppt), which might be correlated with fact that *A. testudineus* starts drinking more water to compensate the body water loss due to dehydration at higher salinities. Marine or seawater acclimatized fish counteract the osmotic loss of water by drinking lots of water and their guts play a vital osmoregulatory role in water absorption. The present findings is also supported by previous research carried on Amur carp (Singh *et al.,* 2019) and *Mugil cephalus* (Barman *et al.*, 2005).The lipid content (%) of the raw fish ranged from 4.44±0.07 to 6.20±0.17, which is more or less similar to the findings of Chowdhury *et al.,* 2014; where they found the lipid content (%) of koi to range from 2.86 ± 0.007 to 6.47 ± 0.009. In the present study, lipid content decreased in higher salinities. It is well known fact that, lipid content in fish is inversely proportional to moisture content (Shearer, 1994). Furthermore, higher moisture content will result in lower lipid deposition in the body muscle or vice versa. Decreasing level of lipid at higher salinities (6, 9 and 12 ppt) indicate that *Anabas testudineus* need more energy for sustaining at higher salinities. Singh *et al.,* 2019 reported that at higher salinities the lipid content of Amur carp showed declining trend which is corroborating with the results of present study. Jarvis and Ballantyne, 2003 also reported that the crude lipid of short nose sturgeon decreased with increasing salinity indicating that at higher salinities fish utilize the lipid as a source of energy for maintaining the internal body homeostasis compared with the surrounding environment.

In this present study, the protein content (%) of the raw fish ranged from 11.29±0.33 to 13.32 ± 0.36, which is more or less similar to the findings of Chowdhury *et al.,* 2014 and Paul *et al.,* 2017, where they found the protein content of *Anabas testudineus* to vary from 12.25 ± 0.001 to 18.62 ± 0.004. At high salinity exposure,

rate of protein degradation far exceeds the rate of amino acid catabolism in *A. testudineus* (Chang *et al.,* 2007). They also reported that salinity exposure had a higher energy demand, and postulated that this was compensated through increased amino acid catabolism, releasing carbon chains that could be channelled into the tri carboxylic acid cycle for ATP production. Amino acid accumulated in the process serves as osmolytes for dealing with hyper-osmotic environment. This explains the low crude protein percentage value observed in this experiment at higher salinities. Bhanu and Deepak, 2015 reported that in higher salinities fish liver suffers damage and hence the rate of synthesis of protein is affected, which supports the results obtained in our study. Further, the ash content (%) of the raw fish ranges from 2.95 ± 0.05 to 4.17 ± 0.10, which is more or less similar to the findings of Paul *et al.,* 2017; Chowdhury *et al.,* 2014. In the present study ash content of *Anabas testudineus* shows higher in higher salinities, which might be attributed to the higher deposition of ions and minerals in the muscle and bones of fishes reared at high salinity. Similar finding was reported by Singh *et al*., 2019 in Amur carp reared at different salinities (0, 5,10 and 15 ppt).

5.5. Digestive enzyme activity

Analysis of intestinal enzyme activity can be used as a way to reflect the process of digestion and nutritional condition of fish in different environmental conditions (Barman *et al.,* 2005; Bolasina *et al.,* 2006).In teleosts, several studies already reported that intestinal enzyme activity changes with the environmental salinity (Bolasina *et al.,* 2007;Gheisvandi *et al.,*2015).In present study, high digestive enzyme activity observed at higher salinities(9 and 12ppt) compared to T1(0 ppt),this increase in digestive enzyme activity at higher salinities may be a sign of activities related to osmotic regulation (Boeuf and Payan, 2001). Furthermore, increased energy requirement for osmotic regulation following stress in the environment causes changes in the physiological process for maintaining energy, such as an increase in the digestive enzyme activity for food digestion and absorption thereof (Boeuf and Payan, 2001; Psochiou et al., 2007). Similar findings were reported by Gheisvandi *et al.,* 2015 for Caspian kutum.

5.6. Enzyme for oxidative stress

Superoxide dismutase and Catalase activity play a significant role to protect cells against H_2O_2 production (Karadag *et al*., 2014). In the present study, the higher SOD and CAT (gill and liver) activity were observed at higher salinities (9 and 12ppt), which indicates that a number of physiological disorders occurred in the *A testudineus*. However, fish at T3 (6ppt) showed no difference in antioxidant enzyme activity relative to the T1 (0ppt), suggesting that *A. testudineus* has the potential to tolerate certain level of salinity (Nahar, 2015). Similar results were also found in case of tilapia*,* where SOD and CAT activities were higher in 16ppt and 24ppt compared to 8 and 0 ppt (Wang *et al.,* 2008; Gan *et al.,* 2016).

5.7. Enzyme for protein metabolism

ALT and AST, mainly found in hepatocytes and cardiomyocytes of fish and they are known play a significant role in protein metabolism (Fazio *et al.,* 2014). In present study Plasma ALT and AST concentrations increased significantly with increase in salinity from 0 ppt to 12 ppt. When faced with liver tissue destruction due to oxidative damage caused by increased metabolic rates under stress, ALT and AST concentrations would also increase because of their role in initial amino acids compensation that the body needs due to changed physiological needs and energy demands (Ebeid *et al.,* 2005). Stress induced by different husbandry processes may elevate the internal oxidizing effort towards membrane permeability that increases the fluxes of ALT and AST enzymes into the blood stream (Bahjat and Shaban, 1985). Mahmoud *et al.,* 2013 reported that ALT and AST activity of goldfish increases when fish transferred from freshwater to saline water. Akhtar et al., 2014 also reported enhanced AST and ALT level in muscle and liver of *Labeo rohita* fingerlings exposed to salinity stress. Transfer of Tilapia from freshwater to seawater for two weeks lead to a significant increase in AST and ALT activities in liver which was explained to be associated with the destruction of liver protein destruction and energy demand (Vijayan *et al.,* 1996). Sultan, 2007 reported that plasma ALT and AST concentration of *Acanthopagrus latus* juveniles increased with increasing salinity.

5.8. Osmolality traits

In the present experiment, Water osmolality was found to be significantly different ($p < 0.05$) among the various treatment groups which is expected as the ion concentration in water will increase, its osmolality would also increase. Osmolality is defined as the concentration of the substance in 1 L of water divided by its molecular weight (*Sanders, 2009).* But, serum osmolality exhibited declining trend with increase in salinity, which is in contrast with all the previous reports where the salinity exposure was reported to increase osmolality of serum. At this point of time we have limited evidence to supports our findings as reported here. However, some of the studies have indicated that osmoregulatory mechanism in *A .testudineus* is quite different than that of other freshwater fishes. For example reduced expression of Aquaporin in gut and increased expression in gill of *A. testudineus* after exposure to saline water is contrary to the observation in other fresh water fishes (Ip *et al.,* 2013). Loong *et al.,* 2012, reported that acclimation of *A. testudineus* to seawater is mediated by the basolateral transport of sodium and chlorides ions down the electrochemical gradient provided by Nka, coupled with the apical export of cl^- via protein channels and the paracellular extrusion of Na^+. They concluded that *A. testudineus* on exposure to high salinity utilise the same mechanism as that of marine teleosts for the exclusion of various ions from entering into body. Furthermore, there are anatomical differences in the gill (primary osmoregulatory organ) of this species when compared to other fresh water fishes. One of the gill arches of *A. testudineus* has lost most of the lamellae and functions mainly as a gill shunt. As for the other gill arches, they are short, and have short primary lamellae with small diameters. Furthermore, the secondary lamellae are small (short/flat) with thick diffusion distances (Graham, 1997). As a result, the gills of *A. testudineus* kept in freshwater have a much smaller gill area (centimeter square): body mass (grams) ratio (0.39) (Hughes *et al.,* 1973) than gills of water breathing fishes (1.96–19.39; Graham, 1997). These differences in the ion exclusion, ion excretion along with anatomical difference in gill structure may account for reduced osmolality of serum as observed in this study for animals

reared at high salinity. However, further study with respect to the concentration of individual inorganic ions and organic osmolytes would be required to get a clear understanding of the processes involved. Future long term experiment with examination of serum osmolytes at short intervals would be helpful in delineating the processes involved. In the present experiment, isosmotic point of *A. testudineus* was 292 mOsm/kg. The isotonic salinity level was found to be 9.8 ppt, which is similar to findings of De Boeck *et al., 2000 for Oreochromis niloticus,* and Farmer and Beamish,*1969 for Cyprinus carpio.*

5.9. Haematological parameters

Haematological parameters can be considered as good indicators for identifying the fish health condition and also guide the fish farmers as well as biologists to interpret the physiological stress, which occur due to sudden changes in the different environmental parameters such as temperature, salinity (Valenzuela *et al.,* 2007; Bhaskar&Rao, 1984).In the present study revealed that haematological parameters such as HCT, Hb, RBCs and WBCs count decreases in higher salinities(9 and 12ppt),these findings are in agreement with other researcher who found a significant effect of salinity on different haematological parameters (HCT, Hb, RBCs) in different species, which may be associated with osmoregulatory dysfunction induced by high salinity levels (Fazio *et al.,*2013; Usha,2011; Soltanian *et al.,*2016; Elarabany *et al.,*2017). Low HCT percentage value in fishes under salinity stress, could be attributed by reduced volume of red blood cells, which is due to osmotic changes caused by ion leakage from the plasma (Alwan *et al.,*2009).White blood cells count in fishes is a good indicator of physiological stress(Svobodová *et al.,*2001). Lower white blood cells count in higher salinities, could be attributed by the weaker defence mechanisms (immuno-suppression) of fishes in new environment. Similar finding were reported by Fazio *et al.,* 2013 for mullet and by Hilali and Al-Khshali, 2016 for common carp.

Blood glucose level in serum or plasma can be used as a stress indicator in fish and gives us idea about the adaptability of fish in different environmental condition (Yin *et al.,* 1995).In present study serum glucose level decreased in higher salinity (9 and 12ppt), which might be due to higher rate of glycogenolysis to meet high energy requirements for osmoregulation (Vijayan *et al.,* 1996; Kavya *et al.,* 2016).

Liver is the main site for glycogen/glucose turnover in case of fishes, during osmotic adaptation in higher salinities liver metabolism may be enhanced to ensure that glucose remains available as fuel for other metabolic and osmoregulatory tissues like gills and kidney (Vijayan *et al.,* 1996). Similar findings were reported by Fazio *et al.,* 2013 for mullet and Imanpoor *et al.,* 2012 for gold fish.

Serum total protein is a nonspecific immune variable and gives a good idea about the immune potential of fish in different environmental conditions (Yılmaz and Ergün, 2012). In the present study, total protein was significantly lower at 9 and 12 ppt salinity group compared to the T1 (0 ppt), it might be due to fish using plasma protein as a source of energy to mitigate the osmoregulatory stress. Chang *et al.,* 2007 reported that *A. testudineus* exposed to progressive increase in salinity adapted to this situation through argininolysis or purine catabolism followed with uricolysis. Carbon chains generated during the process would enter the citric acid cycle leading to production of ATP. Similar findings were reported by Elarabany *et al.,* 2017 for Nile tilapia, by Kelly and Woo,1999 for sea bream.

6.0 SUMMARY

6. SUMMARY

Salinization and water scarcity are the most severe constraints confronting sustainable agriculture production systems in semi-arid and arid regions. A variety of aquatic crustacean and finfish species have been reared in inland saline water around the world as well as in India, to explore the potential of their culture in inland saline water.However in India, at commercial scale the technology for the rearing of *L.vannmaei* using amended inland saline water has only been adopted. *L. Vannamei* farming, though demonstrated to be highly profitable, has inherent problems such as the requirement of large infrastructure and massive investment, making it difficult for the resource-poor farmers to adopt the technology. On the other hand,diversification of aquaculture in the inland saline and alkaline areas, which is not suitable for agriculture, would create employment and livelihood opportunities for the farmers of the region. In such a scenario, culture of air-breathing fish *Anabas testudineus,* also known to exhibit salinity tolerance, could be a suitable option for farmers of the inland saline region. *Anabas testudineus* commonly known as Koi or climbing perch is a tasty fish with high consumer demand. Shorter life cycle, faster growth rate, omnivorous feeding habit, highly nutritious food value, hardy nature, and tolerance of adverse environmental conditions makes this species a good option for aquaculture diversification in inland areas. Information regarding growth, survival and physiological response of this species in ionically imbalanced inland saline water is not available. Keeping this in view, the present study was planned to evaluate the growth, survival, and physiological response of *Anabas testudineus* in inland saline water of varying salinity for the period of 60days.

A. testudineus fingerlings equally distributed under five different salinities (T_1, 0 ppt; T_2, 3 ppt; T_3, 6 ppt, T_4, 9 ppt and T_5,12 ppt) in triplicates following complete randomized design (CRD). A total of 225 fishes were distributed into 15 groups according to treatments and were assigned randomly to 15 different FRP tanks of 300 litres capacity.During the experiment, water quality parameters such as dissolved oxygen, temperature, pH, alkalinity, hardness, ammonia, nitrite and salinity were recorded once in a week.Proximate analysis of the diet and carcass tissues was done by standard methods (AOAC, 2006). The growth parameters were measured for each treatment such as standard length (mm), weight (g), length gain

(cm), weight gain (%) and specific growth rate (SGR) over the time of observation from the start of experiment. At the end of the experiment, fishes in each tank were counted and the survival rate (%) was calculated. Samples for haematological parameters, digestive enzymes such as amylase, protease, lipase activity and metabolizing enzymes such as AST and ALT were collected on the final day of the experiment. The serum and water osmolality (mOsm/kg) were measured using a cryoscopic osmometer. Oxidative stress enzymes such as SOD and Catalase were analysed using standard protocol.

No mortality was observed in any of the treatments during the course of experiment, indicating the excellent salinity tolerance ability of the species. Apart from this we also observed that inland saline water of salinity up to 12 ppt had no significant effect on the growth of *A. testudineus* during the experimental period. However, feed utilisation was affected by the increased salinity level of the culture medium. Highest feed conversion efficiency (FCE) and lowest food conversion ratio (FCR) was found in T3 (6 ppt), which differed significantly from the treatment T4 (9 ppt) and T5 (12 ppt). Salinity also had marked effect on the proximate composition of *A. testudineus*. High moisture content (%) observed in T3 followed by T_5, T_4, T_1 and T_2 group respectively. The highest crude lipid was recorded in T2 and lowest value was recorded at T5. Crude protein content was significantly ($p<0.05$) higher in T5 as compared to treatments T1 and T2. The highest and lowest ash content was recorded at T4 and T3 respectively. Digestive enzymes especially amylase and protease activity was high in higher salinities i.e.T4 and T5 compared to T1. Oxidative stress enzymes like SOD and CAT activities were also found higher at higher salinities i.e. T4 and T5 compared to T1,indicating that animals reared at high salinity suffered significant oxidative damage. Protein metabolic enzymes (ALT and AST) levels were found to be higher at higher salinities (T5 and T4). This may be due to damage to liver cells induced by salinity and its consequent leakage into blood stream. Further, as the salt concentration of the rearing medium increased, as expected its osmolality also increased. However, on contray to all the previous findings in fresh water fishes, the serum osmolality of *A. testudineus* decreased with increase in the salinity of rearing medium. Isoosmotic point of serum was calculated to be 292 mOsm/kg and the isotonic salinity was calculated to be 9.8 ppt. Haematological parameters such as haemoglobin, haematocrit value, RBCs and

WBCs count were significantly affected ($p < 0.05$) at higher salinities (T4 and T5) compared to T1.

Hence, the present study provides an insight that, saline water of up to 12 ppt salinity has no significant effect on growth and survival of A. testudineus, however salinity above 6 ppt does have a significant effect on feed utilisation and various physiological parameters related to well being of the fish. Under such scenario, *A. testudineus* can be considered as an ideal species to promote in inland saline region up to a salinity of 6 ppt. Future studies should focus on supplementation of various osmolytes and anti stressing agents in diet to ameliorate the effect of salinity exposure on various physiological parameters of *A. testudineus*. Future studies should also focus on quantifying the various organic and inorganic osmolytes under different salinity exposure systems such as chronic and acute.

7.0 REFERENCES

7. REFERENCES

Agarwal, S. and Pandey, V., 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. *Biologia Plantarum*, *48*(4), pp.555-560.

Ahmmed, M.K., Ahmmed, F., Kabir, K.A., Faisal, M., Ahmed, S.I. and Ahsan, M.N., 2017. Biochemical impacts of salinity on the catfish, *Heteropneustes fossilis* (Bloch, 1794), and possibility of their farming at low saline water. *Aquaculture research*, *48*(8), pp.4251-4261.

Akhtar, M.S., Pal, A.K., Sahu, N.P., Ciji, A., Gupta, S.K. and Dasgupta, S., 2014. Serum electrolytes, osmolarity and selected enzyme activities of *Labeo rohita* juveniles exposed to temperature and salinity stress: effect of dietary l-tryptophan. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *84*(4), pp.973-980.

Akhteruzzaman, M., 1988. A study on the production of koi fish (*Anabas testudineus*) under semi-intensive culture system. *Bangladesh J. Zool*, *3*, pp.39-43.

Aklakur, M., 2017. Nutritional Intervention for Sustainable Production in Inland Saline Aquaculture A Budding Perspective in India. *J Aquac Mar Biol*, *6*(6), p.00172.

Al-Faiz, N.A., 2011, October. Salinity tolerance of silver carp *Hypophthalmichthys molitrix* fingerlings transferred suddenly and gradually to a different saline. In *The 4th international scientific conference of Salahaddin University-Erbil, October 18* (Vol. 20, p. 2011).

Al-Hilali, H.A. and Al-Khshali, M.S., 2016. Effect of water salinity on some blood parameters of common carp (*Cyprinus carpio*). *International Journal of Applied Agricultural Sciences, 2*(1), pp.17-20.

Ali, M.Z., Zaher, M., Alam, M.J. and Hussain, M.G., 2012. Effect of dietary carbohydrate to lipid ratios on growth, feed conversion, protein utilisation and body composition in climbing perch, *Anabas testudineus*. *International Journal of Fisheries and Aquaculture, 2*(1), pp.1-6.

Al-Khashali, M.S. and Al-Shawi, S.A.S., 2013. Effect of salt stress on ALT and AST enzymes activity and cortisol level in adults of *Carassius auratus*. *Pakistan Journal of Nutrition*, *12*(1), p.97.

Allan, G.L., Fielder, D.S., Fitzsimmons, K.M., Applebaum, S.L. and Raizada, S., 2009. Inland saline aquaculture. In *New technologies in aquaculture* (pp. 1119-1147). Woodhead Publishing.

Altinokand, I. and Grizzle, J.M., 2001. Effects of brackish water on growth, feed conversion and energy absorption efficiency by juvenile euryhaline and freshwater stenohaline fishes. *Journal of fish Biology*, *59*(5), pp.1142-1152.

Alwan, S.F., Hadi, A.A. and Shokr, A.E., 2009. Alterations in haematological parameters of fresh water fish, *Tilapia zillii*, exposed to aluminium. *J Sci Its Appl*, *3*(1), pp.12-9.

American Public Health Association, 2005. APHA (2005) Standard methods for the examination of water and wastewater. *APHA Washington DC, USA*.

Ansal, M.D., Dhawan, A., Singh, G. and Kaur, K., 2016. Species Selection for Enhancing Productivity of Freshwater Carps in Inland Saline Water of Punjab-A Field Study. *Ecological Perspectives*, p.45.

Antony, J., Vungurala, H., Saharan, N., Reddy, A.K., Chadha, N.K., Lakra, W.S. and Roy, L.A., 2015. Effects of salinity and Na+/K+ ratio on osmoregulation and growth performance of black tiger prawn, *Penaeus monodon* Fabricius, 1798, juveniles reared in inland saline water. *Journal of the World Aquaculture Society*, *46*(2), pp.171-182.

Antony, Jose, Harikrishna Vungurala, Neelam Saharan, Appidi K. Reddy, Narinder K. Chadha, Wazir S. Lakra, and Luke A. Roy. "Effects of salinity and Na+/K+ ratio on osmoregulation and growth performance of black tiger prawn, *Penaeus monodon* Fabricius, 1798, juveniles reared in inland saline water." *Journal of the World Aquaculture Society* 46, no. 2 (2015): 171-182.

AOAC, 2006. Official Methods of Analysis, 16th Ed, Association of Official Analytical Chemists. Washington DC, USA.

APHA-AWWA-WPCF., 1981. *Standard methods for the examination of water and wastewater*. APHA American Public Health Association.

American Public Health Association, 2013. APHA. 2005. *Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association, Washington DC, 1220p*.

Appelbaum, S., Raj, A. and Raj, C., 2008. Promoting the culture of gilthead sea bream (*Sparus auratus* L.) in low saline inland water: A novel way to farm saltwater fish in freshwater. Secretariat, Southeast Asian Fisheries Development Center.

Aulbach, A.D. and Amuzie, C.J., 2017. Biomarkers in nonclinical drug development. In *A Comprehensive Guide to Toxicology in Nonclinical Drug Development* (pp. 447-471). Academic Press.

Bahera, S., 2013. Survivability of *Anabas testudineus* larvae In Different Feed, Stocking Density And Water Depth. *Journal of Bio Innovation*, *2*(1), pp.1-7.

Bahjat, E.M. and A.M. Shaban, 1985. Clinical chemistry.Establishment of technical institute, medicalinstitute, *Baghdad, Iraq*. 1st Edn., pp: 256.

Bahmani, M., Kazemi, R. and Donskaya, P., 2001. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and Huso huso). *Fish Physiology and Biochemistry*, *24*(2), pp.135-140.

Barman, U.K., Jana, S.N., Garg, S.K., Bhatnagar, A. and Arasu, A.R.T., 2005. Effect of inland water salinity on growth, feed conversion efficiency and intestinal enzyme activity in growing grey mullet, *Mugil cephalus* (Linn.): Field and laboratory studies. *Aquaculture International*, *13*(3), pp.241-256.
Barman, U.K., Jana, S.N., Garg, S.K., Bhatnagar, A. and Arasu, A.R.T., 2005. Effect of inland water salinity on growth, feed conversion efficiency and intestinal enzyme activity in growing grey mullet, *Mugil cephalus* (Linn.): Field and laboratory studies. *Aquaculture International*, *13*(3), pp.241-256.

Barman, Utpal Kumar, S. N. Jana, S. K. Garg, Anita Bhatnagar, and A. R. T. Arasu. "Effect of inland water salinity on growth, feed conversion efficiency and intestinal enzyme activity in growing grey mullet, *Mugil cephalus* (Linn.): Field and laboratory studies." *Aquaculture International* 13, no. 3 (2005): 241-256.

Besra, S., 1997. Effects of salinity on growth. *Growth and Bioenergetics of Anabas testudineus,* pp.61-68.

Bhanu, A.P. and Deepak, M., 2015. Impact of cypermethrin on biochemical aspects of clinical importance in the blood of freshwater fish *Cyprinus carpio*. *J. Entomol. Zool. Stud*, *3*, pp.126-128.

Bhaskar, B.R. and Rao, K.S., 1984. Influence of environmental variables on haematological ranges of milkfish, *Chanos chanos* (Forskal), in brackish water culture. *Aquaculture*, *83*(1-2), pp.123-136.

Bhaskar, P., Pyne, S.K. and Ray, A.K., 2015. Growth performance study of Koi fish, *Anabas testudineus* (Bloch) by utilization of poultry viscera, as a potential fish feed ingredient, replacing fishmeal. *International Journal of Recycling of Organic Waste in Agriculture*, *4*(1), pp.31-37.

Bhaskar, P., Pyne, S.K. and Ray, A.K., 2015. Growth performance study of Koi fish, *Anabas testudineus* (Bloch) by utilization of poultry viscera, as a potential fish feed ingredient, replacing fishmeal. *International Journal of Recycling of Organic Waste in Agriculture*, *4*(1), pp.31-37.

Bianco, P.G. and Nordlie, F., 2008. The salinity tolerance of *Pseudophoxinus stymphalicus* (Cyprinidae) and *Valencia letourneuxi* (Valenciidae) from western Greece suggests a revision of the ecological categories of freshwater fishes. *Italian Journal of Zoology*, *75*(3), pp.285-293.

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

Bolasina, S., Pérez, A. and Yamashita, Y., 2006. Digestive enzymes activity during ontogenetic development and effect of starvation in Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, *252*(2-4), pp.503-515.

Bolasina, S.N., Tagawa, M. and Yamashita, Y., 2007. Changes on cortisol level and digestive enzyme activity in juveniles of Japanese flounder, *Paralichthys olivaceus,* exposed to different salinity regimes. *Aquaculture*, *266*(1-4), pp.255-261.

Boon J.H.. Cannaerts V.H.M.. Augustijn H.. MachielsM.A.M.. De Charleroi D. & OUevier F. (1990) Effect of different infection levels with *Anguillicola crassus* on the haematological parameters of European eel (*Anguilla anguiilla*). *Aquaculture* 87, 243-253

Boyd, C.E. and Thunjai, T., 2003. Concentrations of major ions in waters of inland shrimp farms in China, Ecuador, Thailand, and the United States. *Journal of the World Aquaculture Society*, *34*(4), pp.524-532.

Boyd, C.E. and Thunjai, T., 2003. Concentrations of major ions in waters of inland shrimp farms in China, Ecuador, Thailand, and the United States. *Journal of the World Aquaculture Society*, *34*(4), pp.524-532.

Boyd, C.E., 1990. Water quality in ponds for aquaculture.

Boyd, C.E., Thunjai, T. and Boonyaratpalin, M., 2002. Dissolved salts in water for inland low-salinity shrimp culture. *Global Aquaculture Advocate*, *5*(3), pp.40-45.

Boyd, C.E., Tucker, C.S. and Somridhivej, B., 2016. Alkalinity and hardness: critical but elusive concepts in aquaculture. *Journal of the World Aquaculture Society*, *47*(1), pp.6-41.

Brett, J.R., 1979. Environmental factors and growth. *Fish physiology, vol. VIII. Bioenergetics and growth*, pp.599-677.

Cara, B., Moyano, F.J., Zambonino, J.L. and Fauvel, C., 2007. Trypsin and chymotrypsin as indicators of nutritional status of post-weaned sea bass larvae. *Journal of Fish Biology*, *70*(6), pp.1798-1808.

Castaneda, R., McGee, M. and Velasco, M., 2010. Pangasius juveniles tolerate moderate salinity in test. *Global Aquaculture Advocate*, pp.27-28.

Chanchal, A.K., Pandey, B.N. and Singh, S.B., 1978. Studies on some aspects of the biology of *Anabas testudineus* (Teleostei: Anabantidae). *Matsya*, *4*, pp.15-19.

Chang, E.W.Y., Loong, A.M., Wong, W.P., Chew, S.F., Wilson, J.M. and Ip, Y.K., 2007. Changes in tissue free amino acid contents, branchial Na+/K+-ATPase activity and bimodal breathing pattern in the freshwater climbing perch, *Anabas testudineus* (Bloch), during seawater acclimation. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *307*(12), pp.708-723.

Chaturvedi, C.S., Raizada, S. and Pandey, A.K., 2012. Breeding of *Clarias batrachus* in low-saline water under controlled condition in Rohtak (Haryana).

Cherry, I. S. and Crandall Jr. L. A., 1932. The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *American Journal of Physiology*, 100(2): 266-273.

Chervinski, J., 1982. Environmental physiology of tilapias. In *The Biology and Culture of Tilapia. Proceedings ofthe 7th ICLARM Conference, Manila, Philippines: International Center for Livin* (pp. 119-128).

Chithambaran, S., 2016. Desert aquaculture & environmental sustainability.

Choi, C.Y., An, K.W. and An, M.I., 2008. Molecular characterization and mRNA expression of glutathione peroxidase and glutathione S-transferase during osmotic stress in olive flounder (*Paralichthys olivaceus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *149*(3), pp.330-337.

Chotipuntu, P. and Avakul, P., 2010. Aquaculture potential of climbing perch*, Anabas testudineus,* in brackish water. *Walailak Journal of Science and Technology (WJST)*, *7*(1), pp.15-21.

Choudhary, O.P., Josan, A.S. and Bajwa, M.S., 1996. Rooting and yield relationships in different barley cultivars grown under increasing soil sodicity stress conditions. *CROP IMPROVEMENT-INDIA*-, *23*, pp.1-11.

Chowdhury, M.I., Mahmud, A.I. and Rahman, A.F.M.A., 2014. Effects of water salinity on feeding efficiencies, growth performances and survival rate of Thai strain koi, *Anabas testudineus* (Bloch, 1792). *World Journal of Fish and Marine Sciences*, *6*(5), pp.479-486.

Chowdhury, M.I., Mahmud, A.I. and Rahman, A.F.M.A., 2014. Effects of water salinity on feeding efficiencies, growth performances and survival rate of Thai strain koi, *Anabas testudineus* (Bloch, 1792). *World Journal of Fish and Marine Sciences*, *6*(5), pp.479-486.

Chowdhury, M.I., Mahmud, A.I. and Rahman, A.F.M.A., 2014. Effects of water salinity on feeding efficiencies, growth performances and survival rate of Thai strain koi, *Anabas testudineus* (Bloch, 1792). *World Journal of Fish and Marine Sciences*, *6*(5), pp.479-486.

CSSRI (2015). Vision 2050, ICAR-Central Soil Salinity Research Institute, Karnal, Haryana, India

Davenport, J. and Matin, A.A., 1990. Terrestrial locomotion in the climbing perch, *Anabas testudineus* (Bloch)(Anabantidea, Pisces). *Journal of Fish Biology*, *37*(1), pp.175-184.

Davis, D.A., Saoud, I.P., McGraw, W.J. and Rouse, D.B., 2002. Considerations for *Litopenaeus vannamei* reared in inland low salinity waters. *Avances en nutrición acuícola*.

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), pp.102-111.

De Silva, S.S. and Perera, M.K., 1985. Effects of dietary protein level on growth, food conversion, and protein use in young *Tilapia nilotica* at four salinities. *Transactions of the American Fisheries Society*, *114*(4), pp.584-589.

Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.2018-19.Basic animal husbandry statistics.NewDelhi.

Desai, A.S. and Singh, R.K., 2009. The effects of water temperature and ration size on growth and body composition of fry of common carp, *Cyprinus carpio*. *Journal of Thermal Biology*, *34*(6), pp.276-280.

DOF, GOI, 2020. Booklet of Pradhan Mantri Matsya Sampada Yojana.

Doroudi, M.S., Fielder, D.S., Allan, G.L. and Webster, G.K., 2006. Combined effects of salinity and potassium concentration on juvenile mulloway (*Argyrosomus japonicus*, Temminck and Schlegel) in inland saline groundwater. *Aquaculture Research*, *37*(10), pp.1034-1039.

Doroudi, M.S., Webster, G.K., Allan, G.L. and Fielder, D.S., 2007. Survival and growth of silver perch, *Bidyanus bidyanus*, a salt-tolerant freshwater species, in inland saline groundwater from southwestern New South Wales, Australia. *Journal of the World Aquaculture Society*, *38*(2), pp.314-317.

Drapeau G (1974) Protease from *Staphylococcus aureus*. In: Methods in enzymology. 45 B, L. Lorand, Academic Press, Newyork, p 469.

Dubale, M.S., 1951. A comparative study of the extent of gill-surface in some representative Indian fishes, and its bearing on the origin of the air-breathing habit. *J. Univ. Bombay*, *19*, pp.90-101.

Dubey, S.K., Trivedi, R.K., Chand, B.K., Mandal, B. and Rout, S.K., 2016. The effect of salinity on survival and growth of the freshwater stenohaline fish spotted snakehead *Channa punctata* (Bloch, 1793). *Zoology and Ecology*, *26*(4), pp.282-291.

.

Dubey, S.K., Trivedi, R.K., Chand, B.K., Rout, S.K. and Mandal, B.A.S.U.D.E.V., 2015. Response of *Anabas testudineus* (Bloch, 1792) to salinity for assessing their culture potentiality in brackish water inundation prone areas of Indian Sundarban. *Journal of the Inland Fisheries Society of India*, *47*(2), pp.59-69.

Dubey, S.K., Trivedi, R.K., Rout, S.K., Chand, B.K. and Choudhury, A., 2014. Median lethal salinity (MLS96 h) of two small indigenous fish species Amblypharyngodon mola and Pethia ticto from Indian Sundarban. *Journal of aquaculture research and development*, *5*(5).

Dwivedi, S. N., 1984. Culture of marine prawns and fishes at Sultanpur, Haryana. Central Institute of Fisheries Education, Mumbai, Publ.no. CIFE-15 (10:84): 4p.

Ebeid, T.A., Eid, Y.Z. and El-Habbak, M.M., 2005, April. Liver and Kidney function parameters in Avian species. In *Review, proceeding of the 3rd International Poultry Conference, 4-7 April, Hughada, Egypt*.

Elarabany, N., Bahnasawy, M., Edrees, G. and Alkazagli, R., 2017. Effects of salinity on some haematological and biochemical parameters in nile tilapia, *Oreochromis niloticus*. *Agri. Forest. Fish*, *6*, pp.200-205.

Ercan, E., Agrali, N. and Tarkan, A.S., 2015. The effects of salinity, temperature and feed ratio on growth performance of European sea bass (*Dicentrarchus labrax* L., 1758) in the water obtained through reverse osmosis system and a natural river. *Pakistan Journal of Zoology*, *47*(3).

Faizul, M.I.M. and Christianus, A., 2013. Salinity and Stocking Density Effect on Growth and Survival of *Barbodes gonionotus* (Bleeker, I860) Fry. *Journal of Fisheries and Aquatic science*, *8*(2), p.419.

Fallah, A.A., Nematollahi, A. and Saei-Dehkordi, S.S., 2013. Proximate composition and fatty acid profile of edible tissues of *Capoeta damascina* (Valenciennes, 1842) reared in freshwater and brackish water. *Journal of Food Composition and Analysis*, *32*(2), pp.150-154.

FAO, 2013. The State of World Fisheries and Aquaculture 2013. Food systems for better nutrition

FAO, 2015. Extent of Salt-affected Soils.

FAO, 2017. The State of World Fisheries and Aquaculture 2017. Sustainability in Action.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action.

FAO (Food and Agriculture Organization of the United Nations)*. 2012. World agriculture towards 2030/2050. ESA Working Paper No.10-30. Rome, Italy: Food and Agriculture Organization of the United Nations.*

Farmer, G.J. and Beamish, F.W.H., 1969. Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. *Journal of the Fisheries Board of Canada*, *26*(11), pp.2807-2821.

Fazio, F., Marafioti, S., Arfuso, F., Piccione, G. and Faggio, C., 2013. Influence of different salinity on haematological and biochemical parameters of the widely cultured mullet*, Mugil cephalus*. *Marine and Freshwater Behaviour and Physiology*, *46*(4), pp.211-218.

Fazio, F., Marafioti, S., Arfuso, F., Piccione, G. and Faggio, C., 2013. Influence of different salinity on haematological and biochemical parameters of the

widely cultured mullet, *Mugil cephalus*. *Marine and Freshwater Behaviour and Physiology*, *46*(4), pp.211-218.

Ferraris, R.P., Catacutan, M.R., Mabelin, R.L. and Jazul, A.P., 1986. Digestibility in milkfish, *Chanos chanos* (Forsskal): effects of protein source, fish size and salinity. *Aquaculture*, *59*(2), pp.93-105.

Fielder, D.S. and Allan, G.L., 2003. *Improving fingerling production and evaluating inland saline water culture of snapper, Pagrus auratus*. Australia: NSW Fisheries, Port Stephens Fisheries Centre.

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), pp.73-90.

Forsberg, J.A., Dorsett, P.W. and Neill, W.H., 1996. Survival and growth of red drum *Sciaenops ocellatus* in saline ground waters of West Texas, USA. *Journal of the World Aquaculture Society*, *27*(4), pp.462-474.

Gan, L., Xu, Z.X., Ma, J.J., Xu, C., Wang, X.D., Chen, K., Chen, L.Q. and Li, E.C., 2016. Effects of salinity on growth, body composition, muscle fatty acid composition, and antioxidant status of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). *Journal of Applied Ichthyology*, *32*(2), pp.372-374.

Garcia, L.M.B., Garcia, C.M.H., Pineda, A.F.S., Gammad, E.A., Canta, J., Simon, S.P.D., Hilomen-Garcia, G.V., Gonzal, A.C. and Santiago, C.B., 1999. Survival and growth of bighead carp fry exposed to low salinities. *Aquaculture International*, *7*(4), pp.241-250.

Garg, Sudhir Krishan, and Shivani Bhatnagar. "Influence of periphyton substrate density on hydrobiological characteristics and growth performance of Nile tilapia, *Oreochromis niloticus* (linnaeus. 1758) stocked in inland saline groundwater ponds." *International Journal of Fisheries and aquatic studies* 4 (2016): 444-452.

Geddes, M.C. and MC, G., 1979. Salinity tolerance and osmotic behaviour of European carp (*Cyprinus carpio* L.) from the River Murray, Australia.

Gheisvandi, N., Hajimoradloo, A., Ghorbani, R. and Hoseinifar, S.H., 2015. The effects of gradual or abrupt changes in salinity on digestive enzymes activity of Caspian kutum*, Rutilus kutum* (Kamensky, 1901) larvae. *Journal of Applied Ichthyology*, *31*(6), pp.1107-1112.

Ghosh, A.N., Ghosh, S.R. and Sarkar, N.N., 1973. On the salinity tolerance of fry and fingerlings of Indian major carps. *J. Inland Fish. Soc. India*, *5*, pp.215-217.

Graham, G.M., Graham, G., Jordan, M.M., Sorbie, K.S., Hill, P., Dyer, S.J. and Little hailes, I., 1997, March. Influence of high temperature and high salinity conditions on the testing and potential application of scale inhibitors under harsh HP/HT reservoir conditions. In *8th NIF International Symposium on Oilfield Chemicals*.

Grattan, S.R. and Oster, J.D., 2003. Use and reuse of saline-sodic waters for irrigation of crops. *Journal of crop production*, *7*(1-2), pp.131-162.

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), pp.169-89.

Hang, Y.J., Min, B.H. and Choi, C.Y., 2007. Black porgy (*Acanthopagrus schlegeli*) prolactin cDNA sequence: mRNA expression and blood physiological responses during freshwater acclimation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *147*(1), pp.122-128.

Hastings, R.E. and Kust, C.A., 1970. Reserve carbohydrate storage and utilization by yellow rocket, white cockle, and hoary alyssum. *Weed Science*, pp.140-148.

Hendricks, L.J., 1952. Erythrocyte counts and hemoglobin determinations for two species of suckers, genus *Catostomus*, from Colorado. *Copeia*, *1952*(4), pp.265-266.

Hirose, S., Kaneko, T., Naito, N. and Takei, Y., 2003. Molecular biology of major components of chloride cells. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *136*(4), pp.593-620.

Holiday, F.G.T., 1969. The effect of salinity on the eggs and larvae of teleost. *Fish Physiology*, *1*, pp.293-309.

Hossain, M., Hossen, M., Pramanik, M., Uddin, N., Ahmed, Z.F., Yahya, K., Rahman, M. and Ohtomi, J., 2015. Threatened fishes of the world: *Anabas testudineus* (Bloch, 1792)(Perciformes: Anabantidae). *Croatian Journal of Fisheries*, *73*(3), pp.128-131.

Hossain, M.Y., Rahman, M.M., Fulanda, B., Jewel, M.A.S., Ahamed, F. and Ohtomi, J., 2012. Length–weight and length–length relationships of five threatened fish species from the Jamuna (Brahmaputra River tributary) River, northern Bangladesh. *Journal of Applied Ichthyology*, *28*(2), pp.275-277.

Hughes, G.M., Dube, S.C. and Munshi, J.D., 1973. Surface area of the respiratory organs of the climbing perch, *Anabas testudineus* (Pisces: Anabantidae). *Journal of Zoology*, *170*(2), pp.227-243.

Hwang, P.P. and Lee, T.H., 2007. New insights into fish ion regulation and mitochondrion-rich cells. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *148*(3), pp.479-497.

Iffat, J., Tiwari, V.K., Verma, A.K. and Pavan-Kumar, A., 2020. Effect of different salinities on breeding and larval development of common carp, *Cyprinus carpio* (Linnaeus, 1758) in inland saline groundwater. *Aquaculture*, *518*, p.734658.

Imanpoor, M.R., Najafi, E. and Kabir, M., 2012. Effects of different salinity and temperatures on the growth, survival, haematocrit and blood biochemistry of Goldfish (*Carassius auratus*). *Aquaculture Research*, *43*(3), pp.332-338.

.

Ip, Y.K., Soh, M.M., Chen, X.L., Ong, J.L., Chng, Y.R., Ching, B., Wong, W.P., Lam, S.H. and Chew, S.F., 2013. Molecular characterization of branchial aquaporin 1aa and effects of seawater acclimation, emersion or ammonia exposure on its mRNA expression in the gills, gut, kidney and skin of the freshwater climbing perch, *Anabas testudineus*. *PLoS One*, *8*(4), p.e61163.

Ip, Yuen K., Ai M. Loong, Jie S. Kuah, Eugene WL Sim, Xiu L. Chen, Wai P. Wong, Siew H. Lam, Inês LS Delgado, Jonathan M. Wilson, and Shit F. Chew. "Roles of three branchial Na+-K+-ATPase α-subunit isoforms in freshwater adaptation, seawater acclimation, and active ammonia excretion in *Anabas American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 303, no. 1 (2012): R112-R125.

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). *J Coast Dev*, *17*, p.379.

Jahan, Iffat, A. K. Reddy, S. Arun Sudhagar, V. Harikrishna, Shashank Singh, Tincy Varghese, and P. P. Srivastava. "The effect of fortification of potassium and magnesium in the diet and culture water on growth, survival and osmoregulation of Pacific White Shrimp, *Litopenaeus vannamei* reared in inland ground saline water." *Turkish Journal of Fisheries and Aquatic Sciences* 18, no. 10 (2018): 1235-1243..

Jain, A.K., Kumar, G. and Raju, K.D., 2007. Culture of freshwater prawns, *Macrobrachium rosenbergii*, in inland saline water. *World aquaculture*.

Jain, A.K., Raju, K.D. and Arasu, A.R.T., 2002. Saline water resources of Rajasthan and their suitability for brackish water aquaculture. *Fishing chimes*, *22*, pp.7-13.

Jain, A.K., Raju, K.D., Kumar, G., Ojha, P.K. and Reddy, A.K., 2007. Strategic manipulation of inland saline groundwater to produce *Macrobrachium rosenbergii* (De Man) postlarvae. *Journal of Biological Research*, *8*, pp.151-157.

Jain, A.K., Reddy, A.K., Kumar, G. and Raju, K.D., 2005. Ionic manipulation of inland saline groundwater for enhancing survival and growth of *Penaeus monodon* (Fabricius). *Aquaculture research*, *36*(12), pp.1149-1156.

Jalali, M., Reza, D., Abdul, A.M. and Sayed, A.M.Z., 2013. A comparative study on body composition of Shyrbot (Barbus grypus) fish reared in different salinities. *Elixir International Journal*, *60*(3), pp.16318-16320.

James, W., Avault, W., Guthrie, P.,and Kenneth.,1969. Cat fish culture in brackish water ponds. *State University and Agricultural and Mechanical College University Station Baton Rouge.*

Jarvis, P.L. and Ballantyne, J.S., 2003. Metabolic responses to salinity acclimation in juvenile shortnose sturgeon Acipenser brevirostrum. *Aquaculture*, *219*(1-4), pp.891-909.

Jayaram, K.C., 1981. Freshwater fishes of India, Pakistan, Bangladesh, Burma and Sri Lanka.

Kader, M.A., Bulbul, M., Ahmed, G.U., Hossain, M.S., Hossain, M.A. and Koshio, S., 2011. Effects of animal proteins in practical diets on growth and economic performance of climbing perch*, Anabas testudineus* (Bloch). *Journal of Applied Aquaculture*, *23*(2), pp.166-176.

Kang'ombe, J. and Brown, J.A., 2008. Effect of salinity on growth, feed utilization, and survival of *Tilapia rendalli* under laboratory conditions. *Journal of Applied Aquaculture*, *20*(4), pp.256-271.

Karadag, H., Fırat, Ö. and Fırat, Ö., 2014. Use of oxidative stress biomarkers in Cyprinus carpio L. for the evaluation of water pollution in Ataturk Dam Lake (Adiyaman, Turkey). *Bulletin of environmental contamination and toxicology*, *92*(3), pp.289-293.

Kavya, K.S., Jadesh, M. and Kulkarni, R.S., 2016. Hematology and serum biochemical changes in response to change in saline concentration in fresh water fish *Notopterus notopterus*. *World Scientific News*, *32*, pp.49-60..

Kelly, S.P. and Woo, N.Y.S., 1999. The response of sea bream following abrupt hyposmotic exposure. *Journal of Fish Biology*, *55*(4), pp.732-750.

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), pp.171-175.

Kobayashi, M., Msangi, S., Batka, M., Vannuccini, S., Dey, M.M. and Anderson, J.L., 2015. Fish to 2030: the role and opportunity for aquaculture. *Aquaculture economics & management*, *19*(3), pp.282-300.

Kohinoor, A.H.M., Akhteruzzaman, M., Hussain, M.G. and Shah, M.S., 1991. Observations on the induced breeding of koi fish, *Anabas testudineus* (Bloch) in Bangladesh. *Bangladesh Journal of Fisheries Resource*, *14*(1), pp.73-77.

Kohinoor, A.H.M., Islam, A.K.M.S., Jahan, D.A., Zaher, M. and Hussain, M.G., 2007. Monoculture of climbing perch, Thai koi, *Anabas testudineus* (Bloch) under different stocking densities at on-farm. *Bangladesh Journal of Fisheries Research*, *11*(2), pp.173-180.

Küçük, S., 2013. The effects of salinity on growth of goldfish*, Carassius auratus* and crucian carp, *Carassius carassius*. *African Journal of Biotechnology*, *12*(16).

Kuldeep, K., Mohanty, U., Kumar, R., Damle, D., Noor, J., Jena, J.K. and Eknath, A.E., 2012. Culture of freshwater climbing perch, *Anabas testudineus*. *Aquaculture Asia Magazine*, *17*(3), pp.27-28.

Kültz, D., 2015. Physiological mechanisms used by fish to cope with salinity stress. *Journal of Experimental Biology*, *218*(12), pp.1907-1914.

Kumar, A., Harikrishna, V., Reddy, A.K., Chadha, N.K. and Babitha, A.M., 2017. Salinity tolerance of *Pangasianodon hypophthalmus* in inland saline water: effect on growth, survival and haematological parameters. *Ecol. Environ. Conserv*, *23*, pp.475-482.

Kumar, A., Krishna, V.H., Reddy, A.K., Chadha, N.K. and Rani, A.B., 2016. Effect of salinity on proximate composition of *Pangasianodon hypophthalmus* reared in inland saline water. *International Journal of Zoology Studies*, *3*, pp.19-21.

.

Kumar, K., Sarma, S., Chakrabarti, P.P., Rajesh, K., Mohanty, U.L. and Sahoo, M., 2013. Anabas (Koi) farming in Sonapur, Assam: A successful demonstration. *Fishing Chimes*, *33*(182), pp.136-137

Kumar, M.U., Ansal, M.D. and Kaur, V.I., 2018. Salinity Tolerance and Survival of Freshwater Carp*, Labeo rohita* Ham.(rohu) in Inland Saline Water. *Indian Journal of Ecology*, *45*(4), pp.872-875.

KUMAR, S. and KUMAR, A., 2005. PRAWN, *MACROBRACHIUM ROSENBERGII* IN INLAND SALINE ECOSYSTEM. *Journal of Aquaculture in the Tropics*.

Lakra, W.S., Reddy, A.K. and Harikrishna, V., 2014. Technology for commercial farming of Pacific white shrimp *Litopenaeus* vannamei in inland saline soils using ground saline water. *CIFE Technical Bulletin-1*, pp.1-28.

Lambers,H.,2003.Introduction: dryland salinity: a key environmental issue in southern Australia. *Plant and Soil*, pp.v-vii.

Larsen, E.H., Deaton, L.E. and Onken, H., 2014. O' Donnell, M. *Grosell, M., Dantzler, WH and Weihrauch, D*, pp.405-573.

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), pp.104-110.

Li, X.Q., Li, X.X., Leng, X.J., Liu, X.M., Wang, X.C. and Li, J.L., 2007. Effect of different salinities on growth and flesh quality of *Ctenopharyngodon idellus*. *Journal of Fisheries of China*, *31*(3), pp.343-348.

Liem, K.F. and Inger, R.F., 1987. Functional design of the air ventilation apparatus and overland excursions of teleosts. *Fieldiana. Zoology (USA)*.

Limsuwan, C., Somsiri, T. and Silarudee, S., 2002. The appropriate salinity level of brine water for raising black tiger prawn under low-salinity conditions. *Aquatic Animal Health Research Institute Newsletter, Department of Fisheries, Bangkok, Thailand*, *11*(1), pp.2-4.

Lisboa, V., Barcarolli, I.F., Sampaio, L.A. and Bianchini, A., 2015. Acclimation of juvenile Mugil liza Valenciennes, 1836 (Mugiliformes: Mugilidae) to different environmental salinities. *Neotropical Ichthyology*, *13*(3), pp.591-598.

Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine pollution bulletin*, *42*(8), pp.656-666.

Loong, A.M., Chew, S.F., Wong, W.P., Lam, S.H. and Ip, Y.K., 2012. Both seawater acclimation and environmental ammonia exposure lead to increases in mRNA expression and protein abundance of Na+: K+: 2Cl− cotransporter in the gills of the climbing perch, *Anabas testudineus*. *Journal of Comparative Physiology B*, *182*(4), pp.491-506.

Luz, R.K., Martínez-Álvarez, R.M., De Pedro, N. and Delgado, M.J., 2008. Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. *Aquaculture*, *276*(1-4), pp.171-178.

Mahmood, S., Ali, M.S. and Anwar-Ul-Haque, M.O.H.A.M.M.A.D., 2013. Effect of different feed on larval/fry rearing of climbing perch, *Anabas testudineus* (Bloch), Bangladesh: II. Growth and survival. *Pakistan Journal of Zoology*, *36*(1), pp.13-20.

Mandal, B., Kumar, R. and Jayasankar, P., 2016. Efficacy of exogenous hormone (GnRHa) for induced breeding of climbing perch *Anabas testudineus* (Bloch, 1792) and influence of operational sex ratio on spawning success. *Animal reproduction science*, *171*, pp.114-120.

Mangat, H.K. and Hundal, S.S., 2014. Salinity tolerance of laboratory reared fingerlings of common carp*, Cyprinus carpio* (Linn.) during different seasons. *Int. J. Adv. Res*, *2*(11), pp.491-496.

Massoud, F.I., 1974. Salinity and alkalinity as soil degradation hazards. *FAO/UNDP expert consultation on soil egradation. June*, pp.10-14.

McCormick, S.D., 2001. Endocrine control of osmoregulation in teleost fish. *American zoologist*, *41*(4), pp.781-794.

McNevin, A.A., Boyd, C.E., Silapajarn, O. and Silapajarn, K., 2004. Ionic supplementation of pond waters for inland culture of marine shrimp. *Journal of the World Aquaculture Society*, *35*(4), pp.460-467.

Meera.,2019.Development of inland saline-water aquaculture in Punjab, India.*Global aquaculture advocate.* May.pp.12-14.

Menon, A.G.K., 1999. *Check list--fresh water fishes of India* (No. 175). Survey.

Miegel, R.P., Pain, S.J., Van Wettere, W.H.E.J., Howarth, G.S. and Stone, D.A.J., 2010. Effect of water temperature on gut transit time, digestive enzyme activity and nutrient digestibility in yellowtail kingfish (*Seriola lalandi*). *Aquaculture*, *308*(3-4), pp.145-151.

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

Minhas, P.S. and Gupta, R.K., 1992. *Quality of irrigation water: assessment and management*. Indian Council of Agricultural Research.

Minhas, P.S., 1996. Saline water management for irrigation in India. *Agricultural water management*, *30*(1), pp.1-24.

Minhas, P.S., Ramos, T.B., Ben-Gal, A. and Pereira, L.S., 2020. Coping with salinity in irrigated agriculture: Crop evapotranspiration and water management issues. *Agricultural Water Management*, *227*, p.105832.

Misra, H. P. and Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry,* 247(10): 3170-3175.

Mookerjee, H.K. and Mazumdar, S.R., 1946. On the life history, breeding and rearing of *Anabas testudineus* (Bloch). *J. Dep. Sci. Cal. Univ*, *2*, pp.101-40.

Moutou, K.A., Panagiotaki, P. and Mamuris, Z., 2004. Effects of salinity on digestive protease activity in the euryhaline sparid *Sparus aurata* L.: a preliminary study. *Aquaculture Research*, *35*(9), pp.912-914.

Munshi, J.D., 1968, January. The accessory respiratory organs of *Anabas testudineus* (Bloch)(Anabantidae, Pisces). In *Proceedings of the Linnean Society of London* (Vol. 179, No. 1, pp. 107-126). Oxford University Press.

Nadirah, M., Abol Munafi, A.B., Anuar, K.K., Raja Mohamad, R.Y. and Najiah, M., 2014. Suitability of water salinity for hatching and survival of newly hatched larvae of climbing perch, *Anabas testudineus*. *Songklanakarin Journal of Science & Technology*, *36*(4).

Nahar, F., 2015. Effects of salinity changes on growth performance and survival of climbing perch, *Anabas testudineus (Bloch, 1795)* (Doctoral dissertation, University of Dhaka).

Nahar, F., Haque, W., Ahsan, D.A. and Mustafa, M.G., 2016. Effects of salinity changes on growth performance and survival of climbing perch, *Anabas testudineus* (Bloch, 1795). *Dhaka University Journal of Biological Sciences*, *25*(1), pp.65-73.

Nations,U., 2012. United Nations Millennium Development Goals Report 2012. *New York, NY: United Nations*.

Otto, R.G., 1971. Effects of salinity on the survival and growth of pre-smolt coho salmon (*Oncorhynchus kisutch*). *Journal of the Fisheries Board of Canada*, *28*(3), pp.343-349.

Partridge, G.J. and Creeper, J., 2004. Skeletal myopathy in juvenile barramundi, *Lates calcarifer* (Bloch),cultured in potassium –deficit saline ground water. *Journal of fish diseases*, *27*(9), pp.523-530.

Partridge, G.J. and Lymbery, A.J., 2008. The effect of salinity on the requirement for potassium by barramundi (*Lates calcarifer*) in saline groundwater. *Aquaculture*, *278*(1-4), pp.164-170.

Partridge, G.J., Lymbery, A.J. and Bourke, D.K., 2008. Larval rearing of barramundi (*Lates calcarifer*) in saline groundwater. *Aquaculture*, *278*(1-4), pp.171-174.

Pathak, M.S., Lakra, W.S., Reddy, A.K., Chadha, N.K., Tiwari, V.K. and Srivastava, P.P., 2019. Growth and survival of silver pompano *Trachinotus blochii* (Lacepede, 1801) at different salinities in inland saline ground water. *Indian Journal of Animal Sciences*, *89*(5), pp.581-587.

.

Patra, B.C., 1993. Satiation time, appetite and daily pattern of feed intake and faeces release by an air-breathing fish, *Anabas testudineus* (Bloch). *Journal of Aquaculture in the Tropics*, *8*(1), pp.41-46.

Perry Jr, W.G. and Avault Jr, J.W., 1972. Comparisons of striped mullet and tilapia for added production in caged catfish studies. *The Progressive Fish-Culturist*, *34*(4), pp.229-232.

Psochiou, E., Mamuris, Z., Panagiotaki, P., Kouretas, D. and Moutou, K.A., 2007. The response of digestive proteases to abrupt salinity decrease in the euryhaline sparid *Sparus aurata* L. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *147*(2), pp.156-163.

Purushothaman, C. S., Sudhir Raizada, V. K. Sharma, V. Harikrishna, G. Venugopal, R. K. Agrahari, M. Rahaman, J. Hasan, and A. Kumar. "Production of tiger shrimp (*Penaeus monodon*) in potassium supplemented inland saline sub-surface water." *Journal of Applied Aquaculture* 26, no. 1 (2014): 84-93.

Qadir, M., Quillérou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R.J., Drechsel, P. and Noble, A.D., 2014, November. Economics of salt-induced land degradation and restoration. *In Natural resources forum* (Vol. 38, No. 4, pp. 282-295).

Rahman, A.K.A., 1989. Freshwater fishes of Bangladesh, Zoological society of Bangladesh. *Department of Zoology, Dhaka University, Bangladesh*.

RAHMAN, R., 2020. Effect of artificial feed enriched with fermented macrophytes on growth and survival rates of Climbing perch (*Anabas testudineus* Bloch). *Asian Journal of Microbiology, Biotechnology & Environmental Sciences Journal Papers*.

Rahman, S., A. K. Jain, A. K. Reddy, K. Girish, and D. R. Koyya. 2005. Ionic manipulation of inland saline groundwater for enhancing survival and growth o f *Penaeus monodon* (Fabricius).*Aquaculture Research*36:1149–1156

Rahman, S., Monir, M.S. and Khan, M.H., 2013. Culture potentials of climbing perch, Thai koi, *Anabas testudineus* (Bloch) under different stocking densities in northern regions of Bangladesh. *Journal of Experimental Biology and Agricultural Sciences*, *1*(3), pp.203-208.

Raizada, S., Chadha, N.K., Ali, M., Kumar, A. and Javed, H., 2005. Length-weight relationship of milkfish, *Chanos chanos* (Forskal*)* reared in inland saline ground water*. Indian Journal of Fisheries, 52(1), pp.115-117*.

Raizada, S., Javed, H., Ayyappan, S., Mukhergee, S.C., Maheshwari, U.K. and Fielder, D.S., 2015. Hatchery seed production of giant freshwater prawn, *Macrobrachium rosenbergii* using inland ground saline water in India. *Aquaculture Research*, *46*(1), pp.49-58.

Raizada, S., Purushothaman, C.S., Sharma, V.K., Harikrishna, V., Rahaman, M., Agrahari, R.K., Hasan, J., Venugopal, G. and Kumar, A., 2015. Survival and growth of tiger shrimp (*Penaeus monodon*) in inland saline water supplemented with potassium. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*(2), pp.491-497.

Raizada, Sudhir, C. S. Purushothaman, V. K. Sharma, V. Harikrishna, M. Rahaman, R. K. Agrahari, J. Hasan, G. Venugopal, and A. Kumar. "Survival and growth of tiger shrimp (*Penaeus monodon*) in inland saline water supplemented with potassium." *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 85, no. 2 (2015): 491-497.

Rajender, K., 2000. Role of inland underground brackish water on survival, growth and reproduction of Mrigal (*Cirrhinus mrigala*). *Animals of Biology*, *16*(2), pp.171-175.

Ranjan, A., SRIVASTAVA, P.P., JAIN, K.K. and Muralidhar, P., 2020. Comparative evaluation of metabolic enzymes activities in different tissues of *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings reared at

higher temperature. *Iranian Journal of Fisheries Sciences*, *19*(2), pp.893-903.

Reddy, A. K. and Harikrishna, V., 2014. Technology for the commercial farming of Tiger shrimp *Penaeus monodon* in saline affected soils using inland ground saline water. In: Training manual on Inland saline water Aquaculture management practices. ICAR-Central Institute of Fisheries Education, Rohtak Centre, Lahli, Rohtak, Haryana, 82p.

Reinhold, J.G., 1953. Total protein, albumin and globulin. *Standard methods of clinical chemistry*, *1*(S 88).

Rick, W. and Stegbauer, H. P., 1974. α-Amylase measurement of reducing groups. In Methods of enzymatic analysis. In: Bergermeyer HU, (eds). *Methods of Enzymatic Analysis*, 2(2): 885-915.

Ron, B., Peduel, A., Bresler, K., Conijesky, D., Eshchar, M., Alon, R. and Mozes, N., 2002. Can gilthead seabream, *Sparus aurata*, be cultured in Ein tamar water? A model for assessing the feasibility of marine fish culture in the Arava's brackish water. *Isr. J. Aquacult. Bamidgeh*, *54*.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. and Boyd, C.E., 2010. Shrimp culture in inland low salinity waters. *Reviews in Aquaculture*, *2*(4), pp.191-208.

Saha, K.C.1971. Fisheries of West Bengal. West Bengal Government Press, Alipore, West Bengal.

Sahoo, S.K., Giri, S.S., Maharathi, C. and Sahu, A.K., 2003. Effect of salinity on survival, feed intake and growth of *Clarias batrachus* (Linn.) fingerlings. *Indian J. Fish, 50*(1), pp.119-123.

Samocha, T.M., Davis, A.D., Lawrence, A.L., Collins, C.R. and Van Wyk, P., 2001. Intensive and super-intensive production of the Pacific white *Litopenaeus vannamei* in greenhouse-enclosed raceway systems. Book of Abstracts. *Aquaculture*, *573*, p.183.

Samocha, T.M., Hamper, L., Emberson, C.R., Davis, A.D., McIntosh, D., Lawrence, A.L. and Van Wyk, P.M., 2002. Review of some recent developments in sustainable shrimp farming practices in Texas, Arizona, and Florida. *Journal of applied Aquaculture*, *12*(1), pp.1-42.

Sandeep, K.P., Shukla, S.P., Harikrishna, V., Muralidhar, A.P., Vennila, A., Purushothaman, C.S. and Ratheesh Kumar, R., 2013. Utilization of inland saline water for Spirulina cultivation. *Journal of Water Reuse and Desalination*, *3*(4), pp.346-356.

Sanders, L.R., 2013. Water metabolism. In *Endocrine Secrets* (pp. 198-218). WB Saunders

Santhosh, B. and Singh, N.P., 2007. Guidelines for water quality management for fish culture in Tripura. *ICAR Research Complex for NEH Region, Tripura Center, Publication*, *29*(10).

Satchell, G.H., 1991. *Physiology and form of fish circulation*. Cambridge University Press.

Saxena, D.B., 1958. Circulation of blood in the respiratory region of *Anabas testudineus*. *Proceedings of the National Academy of Sciences of India B*, *28*, pp.284-292.

Schofield, P.J., Peterson, M.S., Lowe, M.R., Brown-Peterson, N.J. and Slack, W.T., 2011. Survival, growth and reproduction of non-indigenous Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758).Physiological capabilities in various temperatures and salinities. *Marine and Freshwater Research*, *62*(5), pp.439-449.

Semra, K., Karul, A., Yildirim, Ş. and Gamsiz, K., 2013. Effects of salinity on growth and metabolism in blue tilapia (*Oreochromis aureus*). *African Journal of Biotechnology*, *12*(19).

Shahsavani, D., Mohri, M. and Kanani, H.G., 2010. Determination of normal values of some blood serum enzymes in *Acipenser stellatus Pallas. Fish physiology and biochemistry*, *36*(1), pp.39-43.

Sharma, K., Dey, A., Kumar, S.A.N.T.O.S.H., Chaudhary, B.K., Mohanty, S., Kumar, T., Prasad, S.S. and Bhatt, B.P., 2020. Effect of salinity on growth, survival and biochemical alterations in the freshwater fish *Labeo rohita* (Hamilton 1822). *INDIAN JOURNAL OF FISHERIES*, *67*(2), pp.41-47.

Sharma, M., Kaur, V.I. and Ansal, M.D., 2017. Physiological Responses of Freshwater Ornamental Fish Koi Carp*, Cyprinus carpio* (L.) in Inland Saline Water: Growth and Haematological Changes. *Indian Journal of Ecology*, *44*(4), pp.864-868..

Shaw, A.B., 1930. A direct method for counting the leukocytes, thrombocytes and erythrocytes of birds's blood. *The Journal of Pathology and Bacteriology*, *33*(3), pp.833-835.

Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*, *119*(1), pp.63-88.

Singh, S., Reddy, A.K., Srivastava, P.P. and Lakra, W.S., 2019. Influence of different salinity on carcass composition of Amur carp (*Cyprinus carpio haematopterus*) reared in semi-arid region of India. *Journal of Experimental Zoology, India*, *22*(1), pp.633-637.

Singha, K.P., Shamna, N., Sahu, N.P., Sardar, P., HariKrishna, V., Thirunavukkarasar, R., Kumar, M. and Krishna, G., 2020. Feeding graded levels of protein to genetically improved farmed Tilapia (GIFT) juveniles reared in inland saline water: Effects on growth and gene expression of IGFI, IGF-IR and IGF-BPI. *Aquaculture*, p.735306.

Smith, L.L., Lawrence, A.L., 1990. Feasibility of penaeid shrimp culture in inland saline ground-water-fed ponds. *Tex J Sci*, *42*, pp.3-12.

Smith, L.S., 1980. Digestion in teleost fishes. *Fish feeds technology AO/UNDP Aquaculture Development and Coordination Programme. Rome*, pp.3-18.

Solis, N.B., 1988. Brackish water Aquaculture Information System, Biology and Culture of *Penaeus monodon*. Southeast Asian Fisheries Development,Tigbauan, Iloilo, Philippines, pp. 3–36.

SOLTANIAN, S., VAZIRZADEH, A. and FALLAHI, R., 2016. Effects of sudden salinity changes on short-term haematological and biochemical responses in Mudskipper *Periophthalmus waltoni Koumans* 1941 (Gobiidae: Perciformes). *Iranian Journal of Ichthyology*, *3*(1), pp.31-42.

Starcevich, M.R., Lymbery, A.J. and Doupé, R.G., 2003. Potential environmental impacts from farming rainbow trout using inland saline water in Western Australia. *Australasian Journal of Environmental Management*, *10*(1), pp.15-24.

Stickney, R.R., 1979. *Principles of warmwater aquaculture*. John Wiley & Sons..

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

Stone, N.M. and Thomforde, H.K., 2004. *Understanding your fish pond water analysis report* (pp. 1-4). Cooperative Extension Program, University of Arkansas at Pine Bluff, US Department of Agriculture and county governments cooperating.

Storey, K.B., 1996. Oxidative stress: animal adaptations in nature. *Brazilian Journal of Medical and Biological Research*, *29*, pp.1715-1733.

Sultan, F.A., 2007. Effect of salt acclimatization on some nutritional and physiological aspects in juvenile Acanthopagrus latus (Doctoral dissertation, Ph. D. Thesis, College of Agriculture, University of Basrah, Iraq).

Sun, S., Zhu, J., Jiang, X., Li, B. and Ge, X., 2014. Molecular cloning, tissue distribution and expression analysis of a manganese superoxide dismutase in blunt snout bream *Megalobrama amblycephala. Fish & shellfish immunology*, *38*(2), pp.340-347.

Svobodová, Z., Flajšhans, M., Kolářová, J., Modrá, H., Svoboda, M. and Vajcová, V., 2001. Leukocyte profiles of diploid and triploid tench, *Tinca tinca* L. *Aquaculture*, *198*(1-2), pp.159-168.

Swivedi, S.N. and Lingaraju, G.M., 1986. Strategy of prawn and fish culture in saline sub-soil waters of semiarid zone in Haryana. *Mahasagar*, *19*(2), p.97.

Tacon, A.J., 2003. Aquaculture production trends analysis. *Review of the state of world aquaculture. FAO fisheries circular*, *886*(2), pp.5-29.

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y. and Nishimura, E. T., 1960. Hypocatalasemia: a new genetic carrier state. *The Journal of Clinical Investigation,* 39(4): 610-619

Talukdar, A., Deo, A.D., Sahu, N.P., Sardar, P., Aklakur, M., Prakash, S., Shamna, N. and Kumar, S., 2020. Effects of dietary protein on growth performance, nutrient utilization, digestive enzymes and physiological status of grey mullet, *Mugil cephalus* L. fingerlings reared in inland saline water. *Aquaculture Nutrition*, *26*(3), pp.921-935.

Tantulo, U., and R. Fotedar. "Comparison of growth, osmoregulatory capacity, ionic regulation and organosomatic indices of black tiger prawn (*Penaeus monodon* Fabricius, 1798) juveniles reared in potassium fortified inland saline water and ocean water at different salinities." *Aquaculture* 258, no. 1-4 (2006): 594-605.

Tao, N.P., Wang, L.Y., Gong, X. and Liu, Y., 2012. Comparison of nutritional composition of farmed pufferfish muscles among *Fugu obscurus, Fugu flavidus and Fugu rubripes*. *Journal of food composition and analysis*, *28*(1), pp.40-45.

Tay, Y.L., Loong, A.M., Hiong, K.C., Lee, S.J., Tng, Y.Y., Wee, N.L., Lee, S.M., Wong, W.P., Chew, S.F., Wilson, J.M. and Ip, Y.K., 2006. Active ammonia transport and excretory nitrogen metabolism in the climbing perch, *Anabas testudineus*, during 4 days of emersion or 10 minutes of forced exercise on land. *Journal of Experimental Biology*, *209*(22), pp.4475-4489.

Thakur, N.K and Das, P. 1986. Synopsis of biological data on Koi, *Anabas testudineus* (Bloch). Bulletin No. 40, Central Inland Fisheries research Institute, Barrackpore, India. 47p.

Tsuzuki, M.Y., Sugai, J.K., Maciel, J.C., Francisco, C.J. and Cerqueira, V.R., 2007. Survival, growth and digestive enzyme activity of juveniles of the fat snook (*Centropomus parallelus*) reared at different salinities. *Aquaculture*, *271*(1-4), pp.319-325.

Uddin, S., Hasan, M.H., Iqbal, M.M. and Hossain, M.A., 2017. Study on the reproductive biology of Vietnamese climbing perch (*Anabas testudineus*, Bloch). *Punjab University Journal of Zoology*, *32*(1), pp.1-7.

Unwin, D.M. and Willmer, P.G., 1978. A simple field cryoscope-osmometer for freezing-point determination with small fluid samples. *Physiological Entomology*, *3*(4), pp.341-345.

Usha, R., 2011. EFFECT OF SALINITY CHANGES ON HAEMATOLOGICAL PARAMETERS OF THE TIGER SHARK *PANGASIUS HYPOPHTHALMUS*. *Journal of Ecobiology*, *29*(4), p.283.

Valenzuela, A.E., Silva, V.M. and Klempau, A.E., 2007. Some changes in the haematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to three artificial photoperiod regimes. *Fish Physiology and Biochemistry*, *33*(1), pp.35-48.

Vargas-Chacoff, L., Saavedra, E., Oyarzún, R., Martínez-Montaño, E., Pontigo, J.P., Yáñez, A., Ruiz-Jarabo, I., Mancera, J.M., Ortiz, E. and Bertrán, C., 2015. Effects on the metabolism, growth, digestive capacity and osmoregulation of juvenile of Sub-Antarctic Notothenioid fish *Eleginops maclovinus* acclimated at different salinities. *Fish physiology and biochemistry*, *41*(6), pp.1369-1381.

Vijayan, M.M., Mommsen, T.P., Glémet, H.C. and Moon, T.W., 1996. Metabolic effects of cortisol treatment in a marine teleost, the sea raven. *Journal of Experimental Biology*, *199*(7), pp.1509-1514.

Wang, L.N., Liu, W.B., Lu, K.L., Xu, W.N., Cai, D.S., Zhang, C.N. and Qian, Y., 2014. Effects of dietary carbohydrate/lipid ratios on non-specific immune responses, oxidative status and liver histology of juvenile yellow catfish *Pelteobagrus fulvidraco. Aquaculture*, *426*, pp.41-48.

Wang, Y.B., Tian, Z.Q., Yao, J.T. and Li, W.F., 2008. Effect of probiotics, Enteroccus faecium, on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Aquaculture*, *277*(3-4), pp.203-207.

Widiyati, A., Saputra, A. and Setiadi, E., 2019. PRODUCTION PERFORMANCE AND BLOOD PROFILE OF CLIMBING PERCH *Anabas testudineus* Bloch CULTURED IN PEAT POND WITH DIFFERENT STOCKING DENSITIES. *Indonesian Aquaculture Journal*, *14*(2), pp.83-89.

Winfree, R.A. and Stickney, R.R., 1981. Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of *Tilapia aurea*. *The Journal of nutrition*, *111*(6), pp.1001-1012.

Wong, V.N., Greene, R.S.B., Dalal, R.C. and Murphy, B.W., 2010. Soil carbon dynamics in saline and sodic soils: a review. *Soil use and management*, *26*(1), pp.2-11.

Yılmaz, S. and Ergün, S., 2012. Effects of garlic and ginger oils on hematological and biochemical variables of sea bass *Dicentrarchus labrax*. *Journal of aquatic animal health*, *24*(4), pp.219-224.

Yin, Z., Lam, T.J. and Sin, Y.M., 1995. The effects of crowding stress on the non-specific immune response in fancy carp (*Cyprinus carpio L*.). *Fish & Shellfish Immunology*, *5*(7), pp.519-529.

Zahari, Z., Christianus, A. and Ismail, M.F.S., 2018. Effect of stocking density and salinity on the growth and survival of golden Anabas fry. *Survey in Fisheries Sciences*, *4*(2), pp.26-37.

Zahari, Z., Christianus, A. and Ismail, M.F.S., 2018. Effect of stocking density and salinity on the growth and survival of golden Anabas fry. *Survey in Fisheries Sciences*, *4*(2), pp.26-37.

Zalina, I., Saad, C.R., Christianus, A. and Harmin, S.A., 2012. Induced Breeding and Embryonic Development of Climbing Perch (*Anadas testudineus*, Bloch). *Journal of Fisheries and Aquatic Science*, *7*(5), p.291.

Zhang, S., Wang, J. and Huang, N., 1989. The effect of low salinity on growth and survival of juveniles *Penaeus monodon* Fabricius. *Trans. Oceanol. Limnol*, *2*, pp.66-70.

Zhu, C., Dong, S., Wang, F. and Huang, G., 2004. Effects of Na/K ratio in seawater on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture*, *234*(1-4), pp.485-496.

Zworykin, D.D., 2012. Reproduction and spawning behavior of the climbing perch *Anabas testudineus* (Perciformes, Anabantidae) in an aquarium. *Journal of ichthyology*, *52*(6), pp.379-388.

ANNEXTURE I

LIST OF ABBREVIATIONS

%	Percentage
%/day	Percentage per day
%WG	Percentage Weight Gain
<	Less than
±	Plus or minus
° C	Degree Celcius
µl	Micro litre
ABW	Average Body Weight
ALP	Alkaline Phosphatase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
APHA	American Public Health Association
AST	Aspartate aminotransferase
ALT	Alanine aninotransaminase
CF	Crude Fibre
CIFE	Central Institute of Fisheries Education
cm	Centimetre
CO_2	Free Carbon dioxide
CP	Crude Protein
CRD	Completely Randomized Design
DM	Dry Matter
DNPH	2,4- dinitrophenylhydrazine
DNS	Di-nitro salicylic acid
DO	Dissolved Oxygen
EE	Ether Extract

et al.	And others
FAO	Food and Agricultural Organisation
FCR	Feed Conversion Ratio
FER	Feed Efficiency Ratio
FI	Feed Intake
g	Gram
g/kg	Gram per Kilo gram
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
ICAR	Indian Council of Agricultural Research
kg	Kilo gram
$KMnO_4$	Potassium permanganate
L	Litre
mgprotein/min	Per milli gram protein per minute
mg/L or mg L^{-1}	Milli gram per litre
ml/kg	Millilitre per Kilo gram
mm	Millimetre
mM	Milli Mole
MMT	Million Metric Tonnes
Mt	Metric ton
N_2	Nitrogen
NaOH	Sodium Hydroxide
NH_3-N	Ammonia-Nitrogen
nm	nanometre
NO_2-N	Nitrite-Nitrogen
OM	Organic Matter
PER	Protein Efficiency Ratio
PO_4	Phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate

S.E	Standard Error
SGR	Specific Growth Rate
SOD	Superoxide Dismutase
GnRH	Gonadotropin-releasing hormone
T1	Treatment 1 (0ppt)
T2	Treatment 2 (3ppt)
T3	Treatment 3 (6ppt)
T4	Treatment 3 (9ppt)
T5	Treatment 3 (12ppt)
U/mg protein/min	Units per milli gram protein per minute
TCA	Tri carboxylic acid
w/v	Weight by volume
WG	Weight Gain
ppt	Parts per thousand
FAO	Food and Agriculture Organization
TAN	Total Ammonia Nitrogen
NO2-N	Nitrite-Nitrogen
Nm	nano meter
pH	Hydrogen Potential
SPSS	Statistical Package for social Science
T	Treatment
ISW	Inland saline water

ANNEXTURE II

LIST OF USED CHEMICALS

i. **Winkler's A solution (Manganous sulphate):**
Dissolve 480g of $MnSO_4.4H_2O$ or 400g of $MnSO_4.2H_2O$ or 364g of $MnSO_4.H_2O$ in 1L of distilled water.

ii. **Winkler's B solution (Alkaline potassium iodide):**
Dissolve 500g of NaOH; 150g of KI and 20g of sodium azide in 1L of distilled water.

iii. **Standard sodium thiosulphate solution (0.025N or N/40):**
Dissolve 6.205g of $Na_2S_2O_3.5H_2O$ in 1L of distilled water.

iv. **Starch indicator:**
Take 1g of starch in cold water, pour 200ml of boiling water in it and stir, then add 0.1g of salicylic acid of 0.5g of NaCl or 0.5ml of formalin as preservative.

v. **Phenolphthalein indicator:**
Dissolve 5g of phenolphthalein in 50% alcohol and make the volume up to 1L.

vi. **N/44 NaOH:**
Dissolve 0.909g of NaOH pellets in distilled water. Make the volume up to 1L.

vii. **N/50 H_2SO_4:**
Add 1ml of conc. H_2SO_4 in 35ml of distilled water. Take 1ml of the 1N H_2SO_4 solution into 49ml distilled water.

viii. **Methyl orange indicator:**
Dissolve 5g of methyl orange in 1L of distilled water.

ix. **Standard EDTA solution:**
Dissolve 4g of sodium salt of EDTA and 0.1g of $MgCl_2.6H_2O$ in 800ml of distilled water. Dilute the solution to a volume of 1L.

x. **Eriochrome Black T indicator:**
Dissolve 0.1g of Eriochrome black T indicator in 20ml of ethyl alcohol.

xi. **Phenol alcohol solution:**

Dissolve 20g of reagent grade phenol in 200ml of 95% v/v ethyl alcohol.

xii. **Sodium nitropurisside:**

Dissolve 1g of sodium nitropurisside in 200ml of distilled water.

xiii. **Sulfanilamide solution:**

Dissolve 5g of sulfanilamide in a solution of 50ml conc. HCland about 300ml distilled water. Dilute it to 500ml.

xiv. **N-(1-napthyl)-ethylenediamine dihydrochloride (NEDD) solution:**

Dissolve 0.5g of NEDD in 500ml of distilled water.

xv. **Copper sulphate solution:**

Dissolve 100mg of cupric sulphate pentahydrate in distilled water and make the volume to 1L.

xvi. **Hydrazine sulphate solution:**

Dissolve 3.625g of hydrazine sulphate in distilled water and make the volume to 500ml.

xvii. **Ammonium molybdate solution:**

Dissolve 40g of reagent grade ammonium molybdate in 900ml of distilled water and dilute it to 1L.

xviii. **Stannous chloride solution:**

Dissolve 2.5g of stannous chloride in 100ml glycerol.

ANNEXTURE III

LIST OF APPARATUS/INSTRUENTS

- Spectrophotometer
- Hanna Instrument
- Cuvettes (Optil)
- Test tubes (Borosil)
- Flasks (Borosil)
- Micropipettes (Physiocare Concept)
- Burettes (Borosil)
- D.O. bottles
- Refrigerator
- Beaker (Merck)
- Measuring cylinders (Merck)
- Droppers
- Electronic balance
- Digital compact scale (Atom A123)
- Volumetric flask (Borosil)
- Hot air oven
- Muffle furnace
- Automatic Microkjeldahl unit
- Soxhlet apparatus