The effect of preservation on the morphology of selected schizothoracids.

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(MSFy-2019-104)

Faculty of Fisheries

Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

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Thesis

Submitted to

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in partial fulfilment of requirement for the award of the degree of

Master of Fisheries Science
(Fisheries Resource Management)

2022

Dedicate My Thesis

Lovingly to my beloved parents

Mumma and Abu Ji

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Fisheries, Rangil, Ganderbal

Certificate – I

This is to certify that the thesis entitled, **"The effect of preservation on the morphology of selected schizothoracids"**submitted in partial fulfilment of therequirements for the award of the degree of **Master of Fisheries Science (Fisheries Resource Management)**, to the **Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research workcarried out by **Ms. Mir Iqra Farooq (Reg. No. MSFy-2019-104)** under mysupervision and guidance. No part of the thesis has been submitted forany other degree or diploma.

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

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We, the members of the Advisory Committee of **Ms. Mir Iqra Farooq (Reg. No. MSFy-2019-104)** a candidate for the degree of **Master of Fisheries Science (Fisheries Resource Management)** have gone through the manuscript of the thesis entitled, **"The effect of preservation on the morphology of selected schizothoracids"**and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

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Certificate – III

This is to certify that the thesis entitled, **"The effect of preservation on the morphology of selected schizothoracids"** submitted by **Ms. Mir Iqra Farooq (Reg. No. MSFy-2019-104)** to the **Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Master of Fisheries Science (Fisheries Resource Management)** was examined and approved by the Advisory Committee and External Examiner on……..

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Title of the Thesis : **"The effect of preservation on the morphology of selected schizothoracids "**

ABSTRACT

In biological studies of fish, the use of preserved samples is a common practice. Under the framework of morphometrics, the effect of preservation on morphology is rarely taken into account. Changes during preservation can cause errors in the results which may eventually lead to wrong conclusions. The present research was conducted to examine the effects of formalin and alcohol preservation on the morphological characters of two congeneric species, *Schizothorax niger* and *Schizothorax esocinus* over time: from fresh specimen to 14 weeks of preservation.To accomplish this, conventional morphometry and morphometric characteristics based on landmark based truss network analysis was used. 45 specimens, each of *S. niger* and *S. esocinus* were collected from commercial catches of Dal lake, Kashmir.Thirteen conventional morphometric traits and a 12-point truss network analysis of the experimental specimens was carried out in the present study. Conventional morphometric measurements and images for truss analysis were taken from fresh specimens and subsequently after two weeks of formalin preservation and six, ten and fourteen weeks of alcohol preservation to assess changes in morphology.

The conventional morphometry revealed significant differences ($p<0.05$) in somemorphometric traits like head length, pre-orbital length and post-orbital length in all the readings relative to fresh specimens in both the species. In *S. niger,* characters like pre-pelvic, pre-dorsal and pre-pectoral lengths displayed significant differences ($p<0.05$) after ten weeks of preservation relative to fresh measurements while in *S. esocinus* significant difference ($p<0.05$) in characters of pre-dorsal and pre-pectorallengths were observed after ten weeks of preservation relative to fresh measurements. Significant difference ($P<0.05$) in the total length in both the species was observed after 14 weeks of preservation. Principal component analysis of 30 truss measurements extracted from three components explained 64.23% of the total variance in *S. niger* and 66.18% of the total variance in *S. esocinus*. The first and second principal components (PC1) and (PC2) showed clear separation among the fresh and preserved specimens in both the species studied, depicting the preservation effect on shape of the fish. Conversion equations that allow for back calculation to original live lengths were developed for both formalin and alcohol preserved fish.

Keywords: *S. niger*, *S. esocinus*, Formalin, Ethyl alcohol, Morphology, Morphometry, preservation.

Signature of Student

Dated_____

Signature of Major Advisor

Dated _____

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Mir Iqra Farooq

Place : Rangil, Ganderbal

Dated :

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Chapter -1

INTRODUCTION

India is one of the rich biodiversity hotspots in the world and in terms of freshwater mega biodiversity occupies the 9th position (Mittermeier and Mittermeier, 1997). The country has varied and vast cold water/hill fishery resources which are spread over the peninsular and Himalayan regions as, high and low altitude natural lakes, streams, upland rivers and reservoirs. There are around 20,500 ha of natural lakes, 8,243 km long streams and rivers, 50,000 ha of reservoirs and 2500 ha of brackish water lakes in the high altitude (Mahanta and Sarma, 2010). The cold-water fisheries harbours 258 species belonging to 21 families and 76 genera. Out of these, 255 species are recorded from North-east Himalaya, 203 from the west and central Himalaya and 91 from the Deccan Plateau. The commercially important Indian cold water species are mahseers (*Tor tor, T. putitora, T. mosal, T. khudree, Neolissochielus hexagonolepis* etc.), schizothoracids *(S. esocinus*, *S. richardsonii*, *S. plagiostomus, S. curvifrons, S. progastus, S. kumaoensis,* etc.), catfishes (*Barilius bendelisis*, *B. bola, Garra gotyla,* etc.) and exotics (*Oncorhynchus mykiss, Salmo trutta fario, Ctenopharyngodon idella, Cyprinus carpio, Hypopthalmichthys molitrix, Carrasius carrasius*) (Sehgal, 1999; Sunder *et al.,*1999; Jena and Gopalakrishnan, 2012; Sehgal,2012).

Indian fisheries are increasingly contributing to the nutritional security of the country. Fishing in India employs about 14.5 million people. Fish production has increased more than tenfold since independence in 1947. The sector has shown a rise in production from only 0.8 million tons in 1950 to 14.1 million metric tons in 2020. India is one of the largest fish producing nation in the world. India is also a prime producer of fish through aquaculture and ranks second in the world after China. The total fish production in 2019-2020 was estimated at 14.16

million metric tons (MMT) with contributions of 10.43 MMT from inland sector and 3.72 MMT from marine sector (Anon., 2020).

The population of the world is growing sharply and thus, the demand for food has been increasing likewise. In the modern era, the awareness on the benefits of consuming nutritious food has made the demand for certain nutritious food stuffs, such as fish, to rank at the top of the highly demanded food stuffs. Global fish consumption has increased dramatically from 5.2 kg per capita in the 1961 to 19.4 kg in 2017. In addition to this, the exports of fishery products increased from USD 7.8 billion in 1976 to USD 164 billion in 2018 (FAO, 2020). Schizothoracids, the indigenous cyprinids are dominant in mountain streams of the Himalaya and Central Asia and inhabit both lotic as well as lentic water bodies of Kashmir. These fish are commonly called snow trouts and are confined to cold regions and to the localities possessing snow fed streams/ rivers. They are highly valued fishes and serve as important food item of the local populace and are believed to have migrated into lakes and streams of Kashmir from Central Asian watersheds bordered by inner and southern slopes of Hindu Kush, Karakoram and inner ends of north western Himalayan and Suleiman Ranges (Sehgal, 1999).

Schizopyge niger, locally known as 'Ale gad' (Kashmiri) inhabit both lentic as well as lotic water bodies of Kashmir and is a prized indigenous fish belonging to the family Cyprinidae and order Cypriniformes. *S. niger*has been found to be herbivorous fish, feeding mainly on green algae, plant fragments, diatoms, detritus and unrecognizable matter (Das and Subla, 1969). Mouth is inferior with upper jaw little projecting beyond the lower and is horse shoe-shaped. Barbels are shorter than eyes, origin of dorsal fin a little nearer to the root of the caudal fin than to the end of snout. Anal fin is small, shorter than pectoral fin. Length of snout is much more than the diameter of the eye which is $1/5^{th}$ of the head length and sides with small blackish dots (Jhingran, 1991). The fish has been recently placed among prioritized list of fish for special attention by Directorate of Coldwater Fisheries Research, Indian Council of Agricultural Research, Bhimtal,India (Anon., 2013).

Schizothorax esocinus locally known as "Chhurru" (Kashmiri) is a species of cyprinid fish found in the Himalayas in Pakistan, India, Afghanistan, Nepal and China. They are moderately sized native demersal fish that are found in Indus river system. This river system includes

Indus and it's main tributaries namely Chenab, Jhelum, Ravi, Beas and Sutlej. They are mostly found in mountain streams, rivers and gravel-bottomed rivers. They are herbivorous, feeding on detritus. Mature adults undertake spawning migration to incoming streams where they breed amidst gravel and sandy beds. Fry occur in quiet parts of the streams (Menon, 1999). Body elongated with minute scales. Mouth slightly ascending forward, the upper jaw being little longer than the lower. Head depressed with long snout. Barbels longer than eye and origin of dorsal fin somewhat nearer to the root of caudal than to the end of snout. Anal fin is narrow reaching to base of caudal fin when laid backward. Anal scales are moderately developed. Body with numerous black spots.(Jhingran, 1991).

Various preservatives and preservation techniques are used to preserve fish specimens so that they are available for further studies. When preserving fish for later analysis, a number of storage methods like freezing, preservation in formalin and ethanol is employed. Formalin and alcohol are the standard preservatives used in ichthyological studies. Different preservation methods lead to different changes in size and weight of fishes (Kristoffersen & Salvanes, 1998). Ichthyologists regularly preserve samples of study animals for later examination and evaluation. However, preservatives can cause dehydration, brittleness and excessive softening of the specimen tissues, loss of colour and other problems (Robert *et al.,* 1994). Study results and elucidations may be influenced by alterations in physical tone that affect morphometric measurements and thus hampers taxonomic identification and effect the biological interpretation of the results. Several morphological studies make use of animals coming from biological collections which usually pass through a preservation treatment of 10% formalin fixation and

then ethanol preservation. Fixation by formalin is accomplished to prevent changes in tissues immediately subsequent to death and ethanol preservation is done to keep the wholeness of fixed specimen for long term storage (Leslie and Moore, 1986). Both substances, formalin and ethanol have similar effects on body water and leave the specimen stiff. Moreover, the oxidation caused by the formalin generate formic acid which lowers the degree of calcium in bones and in time subjects turn into soft tissues (Sturgess and Nicola, 1975). Alterations in size and shape caused by formalin and ethanol preservation have been manifested using conventional morphometric analysis and the potency of these can be different according to species (Treasurer 1992; Fey 1999). Earlier studies have found that the standard body length are shorter after formalin fixation and ethanol preservation although fish specimens treated in a 10% formalin solution tend to increase in weight. The most common effect of preservation is shrinkage, potentially altering morphometric configurations (Al-Hassan *et al.,* 2000, Cunningham *et al*., 2000, Lee *et al.*, 2011). Shrinkage occurs when water is lost or replaced with preservative (Al-Hassan *et al*., 2000), albeit not all preservatives alter size and shape similarly. For example, ethanol causes a greater degree of shrinkage (Cunningham *et al*., 2000) when compared to buffered formalin (Moku *et al.,* 2004). Further, freezing will also cause shrinkage due to evaporative loss, but no fluids are replaced (Jawad, 2003). Moreover, length measurements of preserved fishes are necessary in many types of fish surveys and logistics often do not allow for immediate measurement of fish after catch. Fixation and preservation techniques often cause changes in length, weight and quality in most of fish species and in different ways (Parker, 1963; Billy, 1982; Fowler and Smith, 1983; Oozeki and Hirano, 1988; Cunningham *et al*., 2000). If the shrinkage caused by fixative is significant then the preserved lengths cannot be used to indicate accurate live lengths. Most preservatives replace water in the tissues due to which the specimen becomes rigid which often leaves sample bent or twisted and may affect difference in length measurements between readers because returning the fish back into a flat plane to make the measurement can be a

source of error. Therefore, preservation affects fish lengths and weight, as does the length of time that fish are stored in preservative. Compounding the discrepancies observed in length and weight data is the need to evaluate potential inter and intra reader measurement differences that may bias result and further mask real variation.

Morphometric analysis is a fundamental tool used to understand the development of organisms, growth, systematic variation, structure of fish populations. Among vertebrates, fish display greater differences in morphological traits between populations and within species than any other vertebrates (Turan*et al.* 2004). As a result of morphometric analysis, one can easily identify the genetic and environmental characteristics of given fish population. This is necessary to determine how different populations are affected by natural factors. The changes in the aquatic environment due to human activities, such as use of fertilizers and pesticides, is expected to cause morphological changes within species. Morphometric characters respond to changes in the environmental factors. In conventional morphometrics, linear distances (such as height, weight, length) are measured and described by multivariate statistical methods to examine patterns of shape variation within and among groups which is partly what helps to describe allometric patterns in body shapes.

Fish morphometrics has been the hot-spot of ichthyological studies for several decades, but the initial steps date back to the time of Galileo Galilei (Froese, 2006). Yet the scientific basis for morphometry in fishes, particularly the mathematical way that weight relates to length, was set by Fulton(1906), who for the first time introduced fisheries science into “allometry” (Froese 2006). Morphometric characters are continuous characters describing aspects of shape of body. It refers to the quantative analysis of form, a concept that encompasses shape and size. Morphological characters are subjected to environmental modifications and have been used commonly in fisheries biology to measure discreteness between different stocks of fish.

Morphology, refers to the phenotype of an organism. It is a direct and primary means by which organisms interact with environment. In population biology, it is important to know if two populations of organisms have the same typical body form to indicate shape changes, size allometry, characterization of the difference between sexes and responses to environmental variation, accompanying size increase over the life span.

Morphometry, the study of geometrical form of organisms, indicate differences in growth and maturity patterns which are sensitive to environmental fluctuations and show little variation in the gene pool. The study of the morphometric traits is one of the most frequently employed and cost-effective methods for stock identification (Sajina *et al.,* 2011).

Taxonomic and evolutionary studies have traditionally been based principally on the morphology of fishes. There are numerous characters like upper jaw length, standard length, body depth at dorsal fin origin, mandibular length, body depth at dorsal fin origin, fleshy orbit head length, pre-dorsal length, pelvic fin length, pre-anal length, pre-pelvic length, dorsal fin base, peduncle depth, anal fin base, peduncle length, snout length, pre-maxillary teeth and head width available for morphological study. Most of these measurements are parallel and horizontal along the vertebral column of the fish. Many measurements have a common landmark i.e., the tip of snout. Morphometric systems have a drawback of being highly correlated with total length and their dependency on the fish size. Morphometric analysis is most commonly used method for identifying and discriminating the populations because of its practicability and low cost (Francisco *et al.,* 2008). It is generally based on multiple measurements of various body parts that are assessed across several individuals (Rattanawannee *et al.,* 2012). For measuring discreteness and relationships among stocks, meristic and morphometric characters are considered as powerful tools (Ihssen *et al.,* 1981; Melvin *et al.,* 1992). The description of each morphometric character was previously performed independently and, consequently, did not always yield efficient and accurate results (Surre *et al.,* 1986). By incorporating multivariate

techniques such as factor analysis, cluster analysis, and discriminant analysis, into morphometric analysis it has become a more accurate, precise and practical aaproach to discriminate the fishes at population level and have been greatly adopted by several authors in the study of population structure of fishes (Hedgecock *et al.,* 1989; Mamuris *et al.,*1998; Trapani, 2003).

Several methods are used for species identification. Of these, morphometric methods are the simplest. Morphometric analysis are useful in analyzing the fossil record, the impact of mutations on shape, developmental changes in form, and shape as well as for estimating quantitative-genetic parameters of shape of an organism. Shape, size and morphological measurements provide data useful for taxonomic status (Ihssen *et al*., 1981). Amongst the various techniques available for identification like biochemical or molecular genetic variation, the conventional methods of morphometry have an important role in stock identification (Swain & Foote, 1999). Morphometric method is the most direct way among the methods of species identification (Samaradivakra *et al*., 2012). Morphometric characters are very much popular to identify different fish races or populations as well as to identify pure stocks of fish (Parvej *et al*., 2014). For taxonomic and evolutionary studies, the morphology of the fishes is the main source of information (Brraich and Akhter, 2015).

Truss Network System is a landmark-based technique using geometric morphometrics and imposes no restrictions on the direction of variation or localization of shape changes. The truss network system is highly effective in capturing information about the shape of an organism (Cavalcanti *et al.,* 1999). Truss network measurements are a series of distances calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form (Strauss and Bookstein, 1982). One major advantage of deriving morphometric data from digital images is the ability to store the image and the potential for reprocessing each individual to confirm anomalous measurements or derive alternative sets of characteristics. The truss network analysis overcomes the disadvantages of conventional data sets and this method produces a more

systematic geometric characterization of fish shape and has demonstrated increasing resolving power for describing inter specific shape differences (Humphries *et al*., 1982). Storage of images also allows detailed inspection of extreme variants or outliers, as well as more flexible characteristic selection (Cadrin and Friedland, 1999). Landmarks are the anatomical points on the organism's body form. In case of truss system, homologous landmarks are grouped into two tiers and paired. The homogeneity in landmarks allows us to appropriately archive the body form and these anatomical landmarks should identify the same developmental feature among specimens and they should be easy to locate. Ideally the landmarks should represent the intersection of different tissues, such as the insertion points of tissues and anal pores.

The present research work entitled "The effect of preservation on the morphology of selected schizothoracids" was conducted with the following objectives**:**

1. To assess changes in body shape among and between two species of schizothoracids (*S. niger*and *S. esocinus)* due to preservation.
2. To quantify morphological changes in the two selected species of schizothoracids (*S. niger*and *S. esocinus)*.

Chapter –2

REVIEW OF LITERATURE

Stobo (1972) while studying the effects of formalin on length and weight of yellow perch reported immediate shrinkage in the smaller length groups and increase in length in larger ones. Weight of all perches exhibited four stages during the study. a) initial rapid increase. b) short period of decreased rate of gain. c) protracted period of increase, and d) finally a period of gradual decrease.

Engel (1974) while studying the effects of formalin (10%) and freezing on length, weight and condition factor of cisco (*Coregonus artedii*) and yellow perch (*Perca flavescens*) reported reduction in median total length by 0.7- 2.1% for both the species and increased median condition factor by 6.3-9.4% for cisco and 4.8-8.8% for yellow perch and increase in weight by 1.8% in formalin for Cisco and 5% for yellow perch and loss of weight by 1.7- 2.0% by freezing and thawing for each species.

Johnson and Swanson (1974) while studying the length and weight changes of preserved black crappie and yellow perch in 10 and 20% formalin observed increase in weight and decrease in length in both the concentrations. The general rate of shrinkage decreased with time with one half occurred within first six days.

Yeh and Hodson (1975) while studying the effects of formalin on length and weight of bluegill and white crappie from lake Nasworthy Texas reported slight shrinkage in length and significant increase in weight for both species.

Lockwood (1975) studied the effect of preservation on the length and weight of 0-group flatfish in 4% neutral formalin and observations were made on the post-mortem weight/length changes in one group and the preserved weight/length changes in the other group over 12 months. The author reported that the relationship between preserved length (l) and live length (L) was L= l +

0.85mm. Fresh gutted weight exceeded the preserved weight (w) by ΔW when ΔW =32.22 + 0.20 w mg.

Donald and Paterson (1977) conducted a study on the effects of preservation (10% formalin; 70% ethanol) on wet weight biomass of chironomid larvae and reported significant changes in preserved wet weight. However, the magnitude of change varied with species, preservative, preservation time and the nature of water used for preservative dilution.

Theilacker (1980) while studying the changes in body measurement of larval northern anchovy, *Engraulismordax*and other fishes due to preservation and handling observed that the larvae were damaged by net abrasion and those netted before preservation shrank more than those that were laboratory preserved (i.e. larvae pipetted directly into preservative). It was also found that shrinkage of net treated individuals decreased with age and increased with handling time but in laboratory preserved larvae shrinkage was constant for the size class studied.

Hay (1981) studied the effect of fixation and capture on body size and gut content of Pacific herring larvae and observed that towing resulted in considerable loss in gut contents in 10-day-old larvae, of 73 larvae recovered from the tows, 46 (63%) had empty guts while of the 37 control larvae only one had an empty gut. Shrinkage in total body length in larvae fixed immediately after a tow averaged 12% for the shortest, 1-min tow, to 18% for the longest 10-min tow.

Schram*et al*. (1981) while studying the dry weight loss in *Ceriodaphnialacustris*(crustacean, cladocera) following formalin preservation observed that specimens of *Ceriodaphnialacustris*demonstrated a 47% dry weight loss in 3% formalin after 45 days of preservation.

Billy (1982) conducted a study on the effects of formalin (10%) and isopropyl alcohol (37.5%) on length and weight measurements of *Sarotherodonmossambicustrewavas*. Fishes were first fixed in formalin for 5 days and then transferred to isopropyl alcohol for 65 days and results showed greatest increase in length and weight after five days in formalin and decrease in the length in isopropyl alcohol after 65 days.

Fowler and Smith (1983) while studying the effects of formalin, ethanol and freezing on the larvae of silver hake (*Merluccius bilinearis*) reported reduction in length over time. Greater shrinkage was achieved in the first 15 days and the variability and magnitude of the length reduction was larger for smaller larvae. Rate and magnitude of shrinkage for ethanol preserved larvae exceeded those of the formalin preserved.

Hay (1984) conducted a study on weight loss and change of condition factor during fixation of eggs and larvae of Pacific herring, *Clupeaharenguspallasi*, for 10 days in fixatives varying in salinity (0, 15, 28%) and formalin concentration (4, 10, 20%) and reported that weight loss varied inversely with salinity and formalin concentration and that weight loss was greater in larvae than in eggs, greatest in recently fertilized eggs and decreased in older eggs.

Tucker and Chester (1984) investigated the effects of formalin concentration (4%, 7%, 10%), salinity (0%, 35%) and buffer (none, sodium borate) on quality of preservation on southern flounder (*Paralichthyslethostigma*) larvae and reported that unbuffered 4% formalin in fresh water, buffered and unbuffered 10% formalin in salt water caused least shrinkage after fixation while salt water caused significant shrinkage. Unbuffered formalin in fresh water preserved pigments the best but decalcified the skeleton while buffered formalin in salt water preserved the skeleton but bleached pigments.

Leuvan *et al*. (1985) while studying the effects of preservation (ethanol) on dry and ash free dry weight biomass of some aquatic macro-invertebrates (*Radix peregrax, Asellusaquaticus, Erpobdellaoctoculata* and *Glyptotendipes*) reported substantial changes in dry weight and ash free dry weight biomass. Loss in dry weight varied between 7.2 to 21.9% in the four different taxa after three months of preservation and comparatively small range in ash free dry weight loss was observed (16.2 to 19.7%).

Leslie and Moore (1986) while studying the changes in lengths of fixed and preserved young freshwater fish reported shrinkage of upto 4.9% and increase in

length. Trunk and snout to vent length indicated most susceptibility to change; head length was least affected.

Kruse and Dalley (1990) conducted a study on length changes in Capelin, *Mallotusvillosus* larvae due to preservation in formalin and anhydrous alcohol and observed greater rate and extent of shrinkage occurred in alcohol than in formalin. Significant ($P<0.05$) reduction in mean total length of the fish occurred in alcohol through the 24 week preservation period.

Morkert and Bergstedt (1990) while studying shrinkage of sea lamprey larvae preserved in formalin observed significant shrinkage in length after fixation and preservation in 5 and 10% formalin. Most shrinkage was within 2 hours and average shrinkage for 100 mm larvae was 3.8% in 5% formalin and 4.3% in 10% formalin.

Jennings (1991) while studying the effect of capture, net retention and preservation upon the lengths of larval and juvenile bass, *Dicentrarchuslabrax*reported shrinkage in both larvae and juvenile. Preserved lengths of larvae decreased significantly when increased period of net retention preceded preservation.

Al-Hassan and Abdullah (1992) while studying the effects of formalin and freezing on some body proportions of *Barbusluteus* observed slight increase in length during 1st week of preservation in different formalin concentrations and shrinkage in those specimens kept under freezing.

Hjorleifsson and Macphee (1992) while studying the effects of formalin, ethanol and ice preservation on standard length of winter flounder, *Pleuronectesamericanus* larvae reported significant shrinkage in all media except for larvae > 5mm preserved in ethanol. Shrinkage in longer larvae was proportionally less than shorter larvae and was highest in 2% formalin, intermediate in -70ºC seawater and lowest in 95% ethanol.

Johnston and Mathias (1993) conducted a study on length reduction and dry weight loss in frozen and formalin preserved larval walleye, *Stizostedionvitreum*and reported 5% reduction in length for both preservation

techniques. Dry weight loss ranged from 32.1 to 54.0% for frozen larvae and 18.2 to 30% for formalin preserved larvae. Greater length shrinkage and dry weight loss was observed in freezing than formalin.

Robert *et al.* (1994) while studying the relative performance of four preservatives (formalin, isopropyl alcohol, ethanol and freezing) on fish and crayfish reported that all treatments caused reduction in total length of fish (less than 3% after 180 days), formalin increased weight (12%: 180 d), freezing had the least effect and in crayfish all treatments except formalin decreased weight (4-16%: 180 d).

Gaston *et al.* (1996) conducted a study on biomass variations of estuarine macrobenthos preserved in ethanol and formalin. Specimens were fixed in 10% formalin for two weeks then transferred to 70% ethanol. The authors reported no significant change in weight over time while minor variations were recorded in dry weight biomass.

Armstrong and Stewart (1997) while studying the effects of initial length and body curvature on shrinkage of juvenile Atlantic salmon during freezing observed shrinkage in all fishes. Reduction in length was significantly greater in curved fish than in straight fish.

Peppin *et al*. (1997) while studying the changes in the probability density function of larval fish body length due to preservation observed significant increase in body length for individuals of 3-6mm (Total length) and significant decrease in body length for individuals greater than 7mm.

Kristoffersen and Salvanes (1998) examined effects of preservatives on otoliths, fish size and weight for 200 days in 4% seawater formaldehyde solution and 80% ethanol for two small mesoplagic fishes, *Benthosema glaciale* and *Maurolicus muelleri* and reported that loss in body weight was much higher in ethanol (37-39%) than in formaldehyde (13-16%). Reduction in standard length was small in both preservatives and for both species (0.8-3%). The weight of the otoliths of *B. glaciale* decreased by approximately 3% in both formaldehyde and

ethanol while as no significant change was reported in the otholiths of *M. muelleri* in any of the preservatives.

Shannon *et al.* (1998) studied the total larvae length reduction in yellow perch subjected to six preservative treatments (100%, 95%, 80% and 50% ethyl alcohol and 50% and 10% formalin). Total lengths measurements were recorded on 1, 7, 14, & 21 days after storage in each of six preservatives. The authors reported significant reductions in total length (11.5-14.3%) during first 24 hours in all ethyl alcohol treatments and total length reduction of upto 2.5% during first 24 hours in each formalin concentrations.

Fey (1999) while studying the effect of preservation technique (formalin and alcohol)) on larval fish length reported significant reduction in length after first week of preservation. Formalin caused greater shrinkage than alcohol and smaller larvae shrank more than the larger ones.

Al- Hassan *et al.*(2000) studied the effect of some preservatives (formalin and alcohol) and freezing on certain body dimensions of *Mullusbarbatus* and *Mullussurmuletus* and observed a variable effect of preservatives used and the freezing techniques. Shrinkage was the most common effect observed in the preservation tests although some cases of expansion in body dimensions were obtained as well.

Fey (2002) while studying length correction of larval and early juvenile smelt (*Osmeruseperlanus* L.) and herring (*Clupeaharengus* L.) after preservation in formalin (4%) and alcohol (96%) reported significant shrinkage in length of larval and juvenile smelt and herring in 4% formaldehyde seawater solution after two days of preservation and additional shrinkage in 96% alcohol over the next eight days. No significant shrinkage was observed over the subsequent 90 days of preservation. Smaller specimens shrunk more than the larger ones.

Jawad (2003)examined the effect of different concentrations of formalin, alcohol and freezing on selected morphological characters of the fish *Alepes djedaba*.The different concentrations of formalin and alcohol besides freezing showed different degrees of effects on the three dimensions of *A. djedaba*. Three

types of effect were observed: shrinkage, increase in body length and no effect. The greatest shrinkage was noticed in specimens preserved in 5% formalin-tap water (6.27%) while the least shrinkage was in fishes stored in 70% alcohol-tap water.

Smith and Walker (2003) studied shrinkage of 0+ carp *Cyprinuscarpio* after preservation in ethanol (70% and 95%) for 180 days and reported that shrinkage varied with initial (pre-preservation) size and ethanol concentration. Length shrinkage peaked at about 14% and weight shrinkage peaked at about 75% and weight loss was almost twice in 95% ethanol than in 70% ethanol.

Mokku *et al*. (2004) while studying the shrinkage in the body length of myctophid fish larvae with various preservatives (5 and 10% formalin, 70 and 90% ethanol and 70% isopropyl alcohol solutions) reported significant shrinkage in the body length of the fish. Shrinkage was greatest in 70% isopropyl alcohol followed by 90% ethyl alcohol and 70% ethyl alcohol and the formalin solutions. No significant difference in shrinkage was observed between the 5% and 10% formalin solutions.

Rufino *et al*. (2004) while studying the effects of alcohol and freezing on size and shape of carapace in *Liocarcinusdepurtor*reported significant reduction in carapace width due to preservation.

Buchheister and Wilson (2005) while studying shrinkage corrections and length conversion equations for *Theragrachalcogramma, Mallotusvillosus* and *Thaleichthyspacificus* caused due to preservation and freezing reported reduced fork length and mass. Shrinkage in standard length was more in 90% ethanol than in 5% formalin.

Fey and Hare (2005) studied length correction for late larval and early juvenile Atlantic menhaden (*Brevoortiatyrannus*) after preservation in 95% alcohol for 90 days and reported significant shrinkage in length. Length decreased by 3.62% during first three days of preservation, 0.22% during the following 17 days and 0.073% during the remaining 70 days.

Wetzel *et al*. (2005) conducted a study on preservation effects on wet weight, dry weight and ash free dry weight biomass estimates of four common estuarine macro-invertebrates (*Heteromastusfiliformis, Corophium* spp., *Gammarus* spp. and *Hediste diversicolor*) for 90 days and reported loss in wet weight, dry weight and ash free dry weight in all four species which was most pronounced within first 10 days and an additional weight loss was observed between 10 and 21 days. However, no further loss in weight for samples kept for as long as 90 days in preservative was observed.

Neave *et al.* (2006) conducted a study on the effects of preservation and length measurements in larval lampreys for a period of six months using 10% formalin and 90% alcohol and reported that both the preservatives reduced pigmentation levels and caused shrinkage to varying degrees and observed that formalin had less of detrimental effects than ethanol.

Polopuu (2007) conducted studies on adults and nauplii of *Acartiabifilosa* to observe the effect of preservation(4% formalin) on total body length. The length of the same individualwas measured before, immediately, one week, and two months after formalin fixation and results showed no statistically significant difference in live and preserved copepod body length at any time ofmeasurement either in the case of adults or nauplii.

Thorstad *et al*. (2007) studied the effect of ethanol and freezing on shrinkage of juvenile Atlantic salmon and European minnow and reported mean shrinkage in the body mass of Atlantic salmon preserved in ethanol at 23% (maximum 48%) and in the length at 2% (maximum 11%). Estimated fresh body mass deviated from the observed by on average 5% (maximum 57%) while fresh body length deviated on average 0.9% (maximum 9%). In European minnow, mean shrinkage in body mass preserved in ethanol was 30% (maximum 66%) and in body length at 4% (maximum 12%). The estimated fresh body mass deviated from the observed by on average 5% (maximum 48%) and estimated fresh body length deviated by on average 2% (maximum 8%).

Xiong *et al*. (2007) conducted experiments to study effect of formalin preservation on standard length of larval forms of *Gobiocyprisrarus*, *Procyprisrabaudi*and*Sinilabeorendahli*. Fishes were measured(to nearest 0.01 mm) and individually fixed in the appropriateformalin solution (2.5% or 5.0%), then re-measuredat 0.5, 1, 3, 7, 14, 30, 45 and 75 days after preservation tofollow the time course of shrinkage. Results showed shrinkageoccurred within the first half day after preservation. The 5.0%formalin solution caused a higher relative shrinkage rate than 2.5% formalin solution. However, the difference observed was not statisticallysignificant. In *G. rarus*, initial shrinkage of newly hatchedlarvae was higher than that of 24-day-old larvae.

Naureen *et al*. (2008) while studying the effect of different preservatives on the biomass of some selected marine fauna (crabs, shrimps and fishes) reported a significant weight loss in crabs (4.6-33.2%), in shrimps (2.5-41.4%) and in fishes (26.5-59.5%) preserved in 70% ethanol for ten weeks.

Santos *et al.* (2009) while studying length corrections for early juvenile Brazilian herring after preservation in ethanol (70% and 95%), formalin (2.5% and 5%) and freezing (-20ºC) reported significant reduction in length and body mass during first five days after preservation. The specimens continued to contract significantly through thirty days though at a lesser rate in most methods. However, the body mass in 5% formalin and freezing reduced greatly after thirty days of preservation.

Maleky*et al.* (2011) conducted studies to determine the effect of freezing and preservation on *Barbusluteus*fora period of 11 weeksby using10% formalin, 70% alcohol and freezing.The results showed three types of effects on morphometric characters: Shrinkage, increase and no effect, the greatest shrinkage was noticed in body depth of specimens preserved in 10% formalin, the greatest increase was in head length of specimens preserved in freezing and no effect was noticed in snout length and eye diameter preserved in 10% formalin and total length preserved in freezing.

Lee *et al*. (2011) while studying the change in body size of juvenile marbled sole *Pseudopleuronectesyohohamae* after preservation in ethanol reported decrease in standard length by 5.6% after 12hr of preservation. Body weight was found to decrease by 27.8% after 24hr preservation in ethanol (70%). However, there was no further decrease in standard length and body weight after 12 and 24hr of preservation

Fey (2012) while studying the length adjustment of larval and early juvenile cod (*Gadusmorhua*) after upto 3 years of preservation in alcohol (95%) reported average shrinkage of 0.70 mm for the entire size range of 4-40 mm standard length. Relative shrinkage varied from 2-20% of the fresh length and was dependent on size while the absolute shrinkage values were not size dependent and did not change during observed preservation period.

Frimpong and Henebry (2012) studied short term effects of formalin (10%) and ethanol (70%) fixation and preservation techniques on size and weight of fish eggs of *Catostomuscommersonii* and fantail darters *(Etheostomaflabellare*) and recorded significant gain (36%) in egg dry weight during the first 12 h of treatment with formalin, while no detectable changes were recorded on the egg dry weight after 9 days of fixation and preservation in ethanol. There was a reduction in egg diameter (approximately 17% after drying) between ethanol and formalin treatments.

.Martinez *et al*. (2012) analyzed the effect of preservation on the shape of two fish species: *Eucinostomusargenteus* and *Pomadasyscorvinaerformis* preserved in 10% formalin for 1 week, and 70% ethanol for 83 days. Significant difference between treatments was observed on both species with Procrustes ANOVA and Discriminant Analysis. In addition, Principal Component Analysis showed a separation between groups of treatment on both species.

Niazie *et al.* (2013) investigated the effects of fixation and preservation in10% formalin on the morphological characteristics of goldfish (*Carassiusauratus*). Samples were fixed and preserved in 10% formalin for 11

weeks. It was reported that shrinkage wascommon in all of the specimens and changes in body color were clearly distinguishable.

Waldir *et al.* (2013) analysed the effect of freezing and alcohol preservation on geometric morphometric data of peacock fish *Cichla kelberi.* Significantdifference between before and after treatments were reported in the study.

Vajargah and Hedayati (2014) studied the effects of fixation and preservation in 10% formalin on the morphologicalcharacteristics of Spirlin (*Alburnoideseichwaldii*). Samples were fixed and preserved in 10% formalin for 6 months. After this period, samples were removed fromformalin and measurement and evaluation of color features were done once again. The results showed shrinkage was common inall of the specimens and changes in body color were clearly distinguishable.

Rakka and Ganias (2015) assessed the effect of 10% neutral buffered formalin and of three ethanol solutions of different concentration on Mediterranean sardine and European anchovy oocytes over several temporal scales (days, weeks, months) and recorded that the effect of preservatives on oocyte size was stage specific while different preservation periods of ovarian material might lead to discrepancies among studies.

Vajargah and Hedayati (2015) investigated the effect of fixation and preservation in formalin on the morphological characters of common carp (*C. carpio*) and reported that shrinkage was usual in all the specimens and changes in body and fin color were clear.

Hossaini *et al*. (2016) investigated the effects of fixation and preservation in 96% alcohol on the morphological characteristics of the Zagros pubfish,*Aphaniusvladykovi* for a period of 3 months and reported that shrinkage was common in all the specimens and changes in body color were clearly distinguishable compared with fresh fish such a way that the body and fin colors were opaque, while color pattern was detectable, although the intensity was reduced.

Haubrock*et al.*(2018) studied two comparably easy applicable methods, the effects of freezing and moderate cool storage on morphological traits of the scaleless North American channel catfish, *Ictaluruspunctatus*. Results revealed that freezing, like preservation with alcohol or formalin, affected the generally considered characteristics (colour, standard length, fork length, total length, shape), while cool storage was shown to preserve most traits without notable alteration.

Sotola*et al.* (2019) examined the effects of formalin and ethanol preservationon the body shape of 10 freshwater fish species over time and found significant changes in body shape among freshand formalin fixed specimens. Furthermore, changes in body shape continued to occur aftersubsequent ethanol preservation. Two fish species collected at multiple localities showed significantmorphological differences for a limited number of morphometric characters. However,the significance, or lack thereof, often changed inconsistently from one stage ofpreservation to another.

Jawad *et al*. (2020) conducted studies on fixation, preservation and freezing effects on morphometrics of *O. niloticus* and *C. gariepinus* collected from lake Gannie, Benin West Africa and reported a decrease in three morphometric characters, namely total length, standard length and head length. In contrast, frozen *O. niloticus* and *C. gariepinus* showed less shrinkage.

Emmanuel*et al.* (2020) investigated the effects of fixation and freezing on some morphometric characteristics of Nile tilapia and reported shrinkage in all the specimens. Freezing caused 5.62% and 19.61% reduction in length and weight respectively while in formalin 5.24% and 10.72% reduction in length and weight was observed respectively. There was no change in the condition factor due to freezing but a marginal increase of 0.08% in formalin.

Ghazwan (2021) studied the effect of formalin (10%) and alcohol (70%) on some morphological characters of *Planiliza abu* and observed fluctuations in standard length (SL), shrinkage in head length (HL) in both the preservatives and a significant gain in weight in formalin in contrast to alcohol.

Chapter-3

MATERIALS AND METHODS

3.1 Collection of Fish samples

A total of 90 fish samples, 45 each of *Schizopyge niger* and *Schizothorax esocinus* were collected from commercial landings and were transported in insulated boxes containing ice packs to Fisheries Resource Management Laboratory at Faculty of Fisheries, Rangil. SKUAST-K (Plate 1). The total length and total weight of the fish samples ranged from 172 mm- 267 mm and 44g -134 g in case of *S. niger* and 194 mm-329 mm and 55g- 335g in case of *S. esocinus.*

3.2 Morphometry

3.2.1 Conventional morphometry

For conventional morphometry, 13 characters of each sample were measured to the nearest 0.01mm with the help of digital Vernier callipers (Tru size) using standard methods as described by Lagler *et al.,* (1962), Laevastu (1965), Lowe- McConnel (1971), Dwivedi and Menezes (1974) and Grant and Spain (1977). All measurements were taken on the left side of the fish by the same person in order to mimimize the measurement bias. The morphometric characters (Figure 1, plate 2) measured in the present study included:

- **Total Length (TL):** The distance from the tip of the snout to the tip of the caudal fin.
- **Standard Length (SL):** The distance from the tip of the snout to the base of caudal fin.
- **Pre-Dorsal Length (PDL):** Distance from the tip of the snout to the anterior margin of the base of the dorsal fin.
- **Caudal Fin Length (CFL):** Distance from the origin of caudal fin to its maximum length.

- **Pre-Anal Length (PAL):** Distance from the tip of the snout to the origin of anal fin.
- **Pre-Ventral Length (PPvL):** Distance from tip of the snout to the origin of pelvic fin.
- **Pre-Pectoral Length (PPcL):** Distance from the tip of the snout to the anterior margin of the base of the pectoral fin.
- **Head Length (HL):** The distance from the tip of the snout to the posterior margin of operculum.
- **Body Depth (BD):** Maximum vertical length of body (deepest part of body).
- **Snout Length (SL):** The distance from the tip of the snout to the anterior margin of the fleshy orbit.
- **Eye Diameter (ED):** The distance from the anterior margin to the posterior margin of the eye.
- **Pre-Orbital Length (PrOL):** Distance from the tip of the snout to the anterior margin of eye.
- **Post-Orbital Length (PoOL):** Distance from the posterior margin of the eye to the end of operculum.

3.3 Truss Morphometry

3.3.1 Digitization of samples

Digital images of individual fish samples were taken immediately after collecting them from landing centers. In order to digitize the samples, they were first cleaned in running water, drained and placed on a flat platform with graph paper background, for calibrating the coordinates of digital images. The fins were erected and placed on the platform so that the origin and insertion points are made visible. Each individual was labeled with a specific code for identification. A Cybershot digital camera (Nikon, D5600), mounted on a leveling tripod was used in the study (Plate 3).

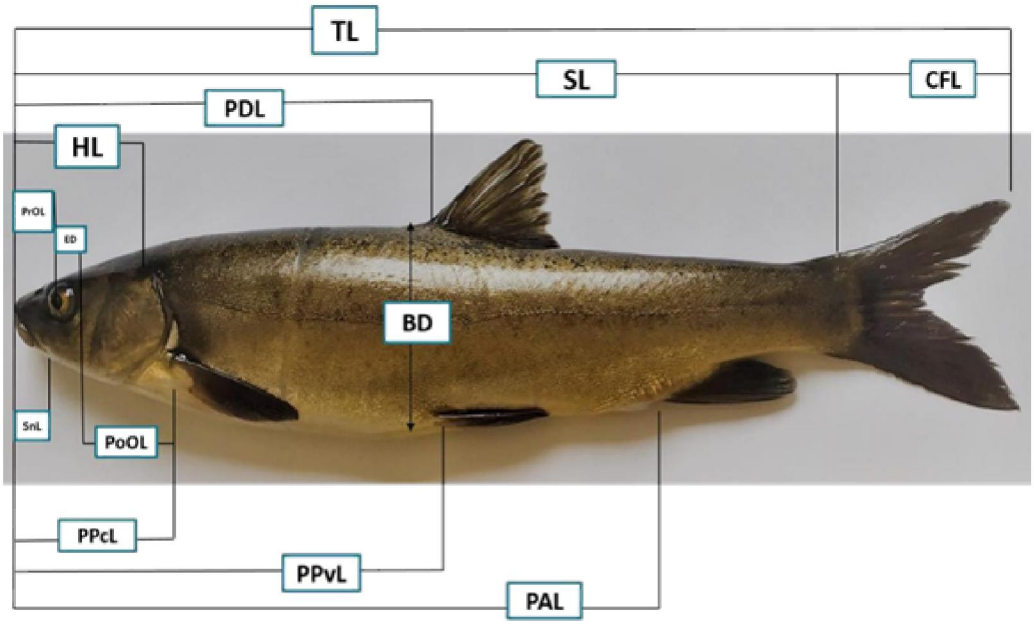

Fig. 1 Morphometric characters measured in the study.

TL = Total length	PPcL = Pre Pectoral Length	ED = Eye Diameter
SL = Standard Length	PDL = Pre Dorsal Length	SnL = Snout Length
CFL = Caudal fin Length	HL = Head Length	BD = Body Depth
PAL = Pre Anal Length	PrOL = Pre Orbital Length	
PPvL = Pre Pelvic Length	PoOL = Post Orbital Length	

Plate 1. Image showing fish samples at FRM laboratory

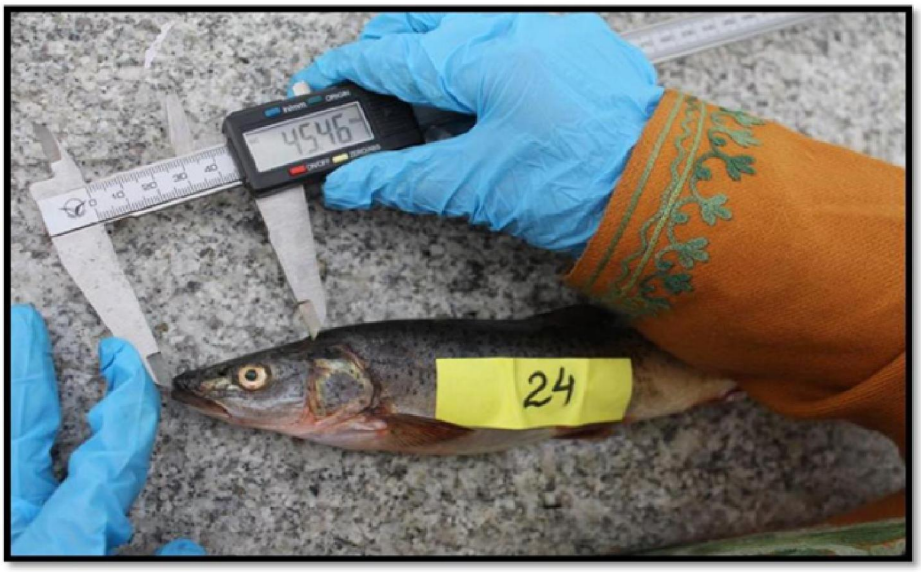

Plate 2. Morphometric measurements of fish using Vernier Callipers.

Plate 3.Capturing images by digital camera (Nikon D5600) for truss network analysis.

3.3.2 Measurement of truss distances

The truss protocol of *S. niger* and *S. esocinus* in the present study was based on twelve landmarks(Plate 4). A truss network was constructed by interconnecting the twelve landmarks to form a total of 30 truss measurements (plate 5, Table 1).

The extraction of truss distances from the digital images of specimens was done using a combination of two softwares, tpsDig2 V 2.1 (Rohlf, 2006a) and Paleontological Statistics (PAST) (Hammer *et al*., 2001). All the images were first converted from JPEG (*.jpeg) to TPS (*.tps) format by using a utility program called tpsUtil V1.38 (Rohlf, 2006b) (Plate 6). Input of the image as tps format is a prerequisite for the tpsDig2 program to analyze and extract the morphometric data. The landmarks were digitized on the image using the 'Digitize landmarks' mode of the software and the landmark data were encrypted into the tps files as X-Y coordinates. The data encrypted tps format image files were used as input source in thePAST and the data on distances between the landmarks were extracted using the 'all distances from landmarks' and '2 dimensional' options of the 'Geomet' menu.

In the present study, there were significant correlations between body length and truss measurements. Therefore, transformation of absolute measurements to sizeindependent shape variables was the first step of the analyses and it was done by modifying a formula originally given by Ihssen *et al.* (1981) and Hurlbut and Clay (1998). Size-dependent variation for truss variables was removed using the formula:

$$D_{trans} = D \times (SLmean/SL)^{b}$$

Where, D_{trans}: transformed truss measurement

D: original truss measurement

SL: standard length of fish

SL mean : overall mean standard length b: within group slope of the geometric mean regression calculated with logtransformed variables, D and SL

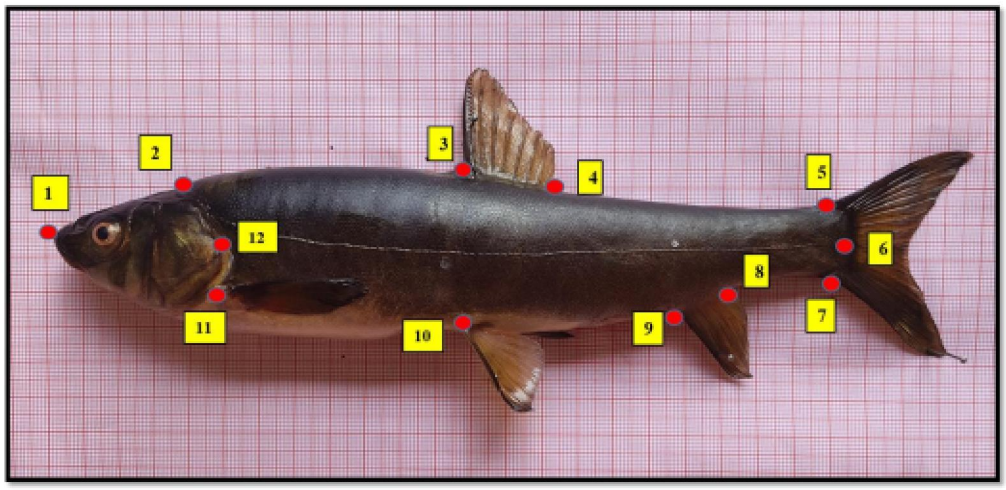

Plate 4. Locations of the twelve landmarks on *S. niger* and *S. esocinus*.

1 = tip of snout 2 =frontal bone end; 3 = dorsal fin origin; 4 = dorsal fin end; 5 = caudal peduncle dorsal border; 6 = lateral line end; 7 = caudal peduncle ventral border; 8 = anal fin origin; 9 =anal fin end;10= pelvic fin origin; 11 = pectoral fin (ventral origin); 12= opercular posterior edge

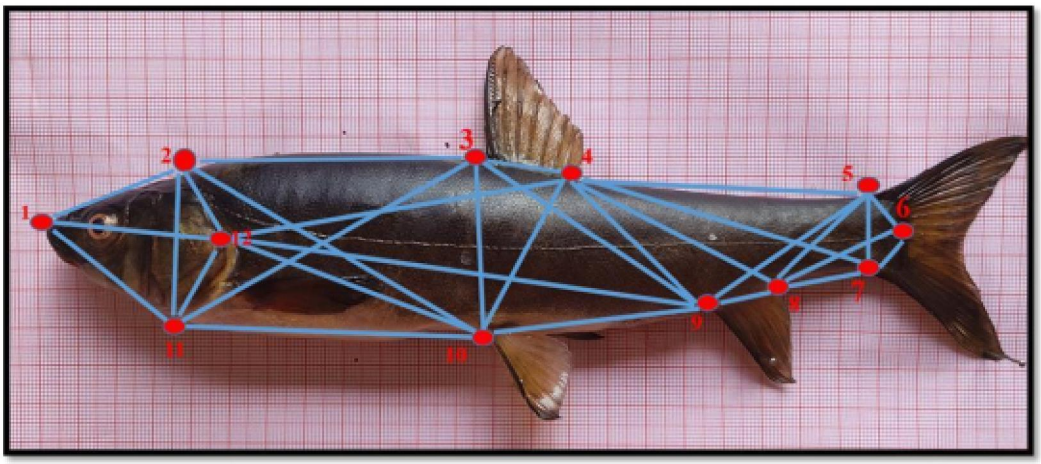

Plate 5. Truss network on *S. niger* and *S. esocinus* depicting the truss distances.

Table 1. Truss distances obtained from the selected twelve anatomical landmarks

S.No	Landmark Distances	Description
1	1-2	Tip of the snout to frontal bone end
2	1-12	Tip of the snout to opercular posterior edge
3	1-11	Tip of the snout to pectoral fin origin
4	11-12	Pectoral fin origin to opercular posterior edge
5	2-12	Frontal bone end to opercular posterior edge
6	2-11	Frontal bone to pectoral fin origin
7	2-3	Frontal bone end to dorsal fin origin
8	3-10	Dorsal fin origin to pelvic fin origin
9	10-11	Pelvic fin origin to pectoral fin origin
10	2-10	Frontal bone end to pelvic fin origin
11	3-11	Dorsal fin origin to pectoral fin (ventral origin)
12	3-4	Dorsal fin origin to dorsal fin end
13	4-9	Dorsal fin end to anal fin origin
14	9-10	Anal fin origin to pelvic fin origin
15	3-9	Dorsal fin origin to anal fin origin
16	4-10	Dorsal fin end to pelvic fin origin
17	4-5	Dorsal fin end to caudal peduncle dorsal border
18	5-8	Caudal peduncle dorsal border to anal fin end
19	8-9	Anal fin end to anal fin origin
20	4-8	Dorsal fin end to anal fin end
21	5-9	Caudal peduncle dorsal border to anal fin origin
22	5-6	Caudal peduncle dorsal border to lateral line end
23	7-8	Caudal peduncle ventral border to anal fin end
24	6-7	Lateral line end to caudal peduncle ventral border
25	6-8	Lateral line end to anal fin end
26	5-7	Caudal peduncle dorsal border to caudal peduncle ventral border
27	4-12	Dorsal fin end to opercular posterior edge
28	10-12	Pelvic fin origin to opercular posterior edge
29	9-12	Anal fin origin to opercular posterior edge
30	4-7	Dorsal fin end to caudal peduncle ventral border

3.4 Fixation and preservation of samples

After the initial conventional morphometric measurements and capturing of fish images for truss morphometry, the specimens were individually tagged with a specific number code (Plate 7) and preserved in 10% formalin solution (Plate 8). Fishes were stored in 10% formalin for 2 weeks at the end of which 2nd readings for both conventional and truss analysis were taken. The fishes were then transferred to 70% ethanol solution (Plate 9), conventional and truss analysis readings were taken every 4 weeks upto 14 weeks for a total of five time periods. The time period were referred to as Fresh, Two weeks of formalin preservation (2WF), Six weeks of alcohol preservation (6WA), ten weeks of alcohol preservation (10WA) and fourteen weeks of alcohol preservation (14WA).

3.5 Statistical analysis

All statistical analysis was carried out by using SPSS (version20) and PAST-3 software.

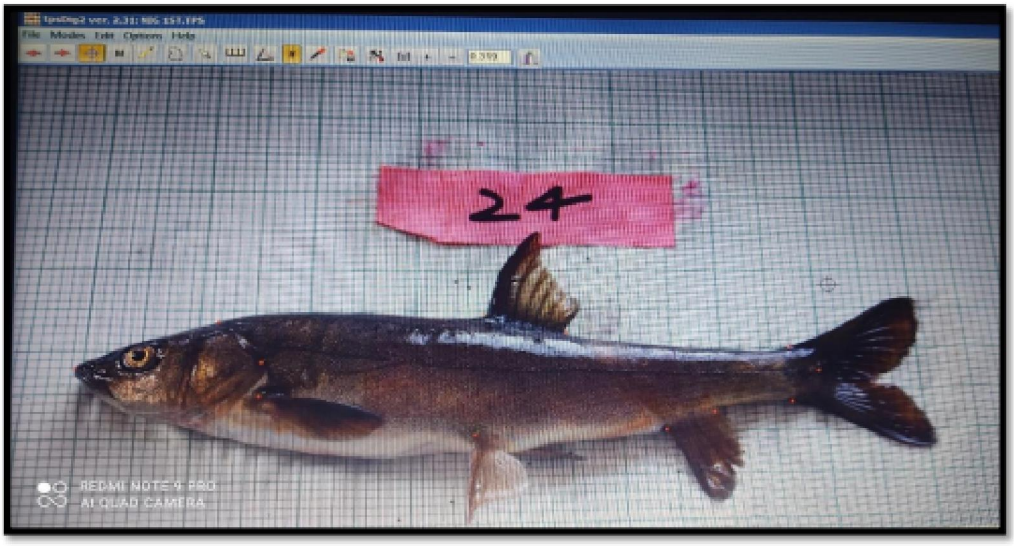

Plate 6. Screengrab of tpsDig2 software used for extraction of truss distances from digital image

Plate 7. Image showing tagged fishes in aquarium tank prior to formalin fixation.

Plate 8. Image showing tagged fishes stored in formalin.

Plate 9. Images showing fish specimens preserved in ethanol.

Chapter-4

EXPERIMENTAL FINDINGS

4.1 Conventional morphometry

4.1.1 Conventional morphometry of *S. niger*

4.1.1.1 Descriptive statistics

The descriptive statistics of 13 morphometric characters viz., minimum, maximum, mean, median, standard error, standard deviation and coefficient of variance of the five measurements (Fresh, 2WF, 6WA,10WA and 14WA) of *S. niger* are presented in Table 2, Table 3, Table 4, Table 5 and Table 6. In Fresh, the total length of the fish ranged from 172.30 to 267.08 mm with a coefficient of variation of 9.22%. The total length ranged from 171.67 to 259.33 mm in 2WFindicating a coefficient of variation of 8.92%. In 6WA, the total length ranged from 174.96 to 258.71mm with 9.02% coefficient of variation. In case of 10WA, the total length ranged from 171.48 to 263.75mm and 9.51% of coefficient of variation was observed and in 14WA the total length ranged from 168.22to 259.33mm with a coefficient of variation of 9.56%. The maximum coefficient of variation at 20.90% in Fresh was found in body depth and minimum at 7.92% was found in eye diameter. 12.48%of maximum coefficient of variation was reported in snout lengthand minimum at 7.58% was reported in eye diameter in 2WF. In 6WA, maximum coefficient of variation was found in body depth at 14.24% and minimum was found in eye diameter at 4.10%. The maximum coefficient of variation in 10WA was observed in snout length at 13.53% and minimum was observed in eye diameter at 5.54% and in case of 14WA the maximum coefficient of variation at14.00% in snout length and minimum at 4.9% in eye diameter was recorded. The line graphs and box plots of 13 morphometric traits of individuals from the five measurements (Fresh, 2WF, 6WA, 10WA and 14WA) are presented in figures 2-14 and figures 15-27.

Table 2: Statistical estimates of various morphometric characteristics of *S. niger*(Fresh)

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	172.3	267.08	211.58	211.35	19.51	2.90	9.22
Standard Length (SL)	142.2	225.31	172.51	171.58	16.32	2.43	9.46
Pre-Anal Length (PAL)	114.33	175.64	139.51	139.49	12.72	1.89	9.12
Pre-Pelvic Length (PPvL)	77.71	119.56	96.93	96.97	8.20	1.22	8.46
Pre-Dorsal Length (PDL)	76.60	116.78	92.25	92.31	8.36	1.24	9.07
Pre-Pectoral Length (PPcL)	33.55	51.01	43.86	43.98	3.68	0.54	8.39
Head Length (HL)	27.15	44.17	35.07	34.95	2.97	0.44	8.46
Pre-Orbital Length (PrOL)	10.48	16.87	13.85	13.98	1.32	0.19	9.53
Post-Orbital Length (PoOL)	12.98	26.19	21.33	21.92	2.39	0.35	11.21
Snout Length (SnL	6.32	13.88	10.68	10.47	1.52	0.22	14.29
Eye Diameter (ED	6.06	8.29	7.39	7.50	0.58	0.08	7.92
Body Depth (BD)	30.08	87.45	38.74	37.17	8.10	1.20	20.90
Caudal Fin Length (CFL)	31.88	52.39	41.05	40.98	4.09	0.61	9.97

Table 3: Statistical estimates of various morphometric characteristics of *S. niger*(2WF).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	171.67	259.33	210.02	209.91	18.74	2.79	8.92
Standard Length (SL)	141.38	210.39	170.11	170.93	14.69	2.19	8.63
Pre-Anal Length (PAL)	111.19	173.64	137.55	138.16	13.03	1.94	9.47
Pre-Pelvic Length (PPvL)	75.06	117.22	94.98	95.15	8.40	1.25	8.84
Pre-Dorsal Length (PDL)	73.18	115.28	90.11	89.72	8.25	1.23	9.15
Pre-Pectoral Length (PPcL)	32.23	50.70	42.59	42.62	3.78	0.56	8.88
Head Length (HL)	26.89	38.81	32.93	33.16	2.64	0.39	8.03
Pre-Orbital Length (PrOL)	9.43	15.59	12.73	12.68	1.29	0.19	10.17
Post-Orbital Length (PoOL)	11.19	23.98	19.84	20.07	2.37	0.35	11.96
Snout Length (SnL)	6.41	13.28	10.01	10.11	1.28	0.19	12.84
Eye Diameter (ED)	6.03	8.96	7.51	7.56	0.56	0.08	7.58
Body Depth (BD)	28.68	44.40	36.52	35.72	3.46	0.51	9.49
Caudal Fin Length (CFL)	32.22	48.61	40.20	40.20	3.87	0.57	9.64

Table 4: Statistical estimates of various morphometric characteristics of *S. niger*(6WA).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	174.96	258.71	209.73	210.29	18.93	2.82	9.02
Standard Length (SL)	138.20	214.72	168.89	169.63	15.81	2.35	9.36
Pre-Anal Length (PAL)	107.90	169.53	134.44	135.77	12.90	1.92	9.59
Pre-Pelvic Length (PPvL)	71.17	114.81	91.86	91.98	8.50	1.26	9.26
Pre-Dorsal Length (PDL)	71.01	114.97	87.45	86.84	8.42	1.25	9.63
Pre-Pectoral Length (PPcL)	30.38	48.12	41.15	41.85	3.86	0.57	9.39
Head Length (HL)	26.43	37.82	32.45	32.65	2.61	0.39	8.06
Pre-Orbital Length (PrOL)	9.04	14.99	12.31	12.34	1.20	0.17	9.78
Post-Orbital Length (PoOL)	10.92	23.07	19.41	19.67	2.36	0.35	12.20
Snout Length (SNL)	7.44	15.32	10.79	10.72	1.53	0.22	14.24
Eye Diameter (ED)	6.48	7.96	7.55	7.61	0.31	0.04	4.10
Body Depth (BD)	28.38	46.43	37.05	36.34	3.57	0.53	9.64
Caudal-Fin Length (CFL)	33.04	54.27	40.52	41.15	4.53	0.67	11.19

Table 5: Statistical estimates of various morphometric characteristics of*S. niger*(10WA).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	171.48	263.25	207.59	207.87	19.74	2.94	9.51
Standard Length (SL)	136.86	213.63	166.23	167.41	15.74	2.34	9.47
Pre-Anal Length (PAL)	103.78	155.54	130.56	132.44	12.51	1.86	9.58
Pre-Pelvic Length (PPvL)	67.63	109.83	87.88	88.23	8.57	1.27	9.76
Pre-DorsaL Length (PDL)	68.32	110.18	83.77	82.84	8.48	1.26	10.12
Pre-Pectoral Length (PPcL)	28.10	46.63	39.62	40.24	3.91	0.58	9.87
Head Length (HL)	25.46	37.29	32.16	32.38	2.64	0.39	8.22
Pre-Orbital Length (PrOL)	8.59	14.95	11.96	11.92	1.21	0.18	10.13
Post-Orbital Length (PoOL)	10.32	22.49	19.02	19.43	2.39	0.35	12.58
Snout Length (SNL)	8.16	15.00	11.06	10.90	1.49	0.22	13.53
Eye Diameter (ED)	6.89	9.35	7.79	7.74	0.43	0.06	5.54
Body Depth (BD)	28.69	45.94	37.15	36.19	3.43	0.51	9.23
Caudal Fin Length (CFL)	30.98	50.02	40.19	40.05	4.02	0.59	10.00

Table 6: Statistical estimates of various morphometric characteristics of *S. niger*(14WA).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	168.22	259.33	205.13	205.78	19.62	2.92	9.56
Standard Length (SL)	135.04	210.49	164.22	165.04	15.64	2.33	9.52
Pre-Anal Length (PAL)	100.83	150.23	126.95	128.61	12.61	1.88	9.93
Pre-Pelvic Length (PPvL)	64.16	105.52	84.16	85.18	8.32	1.24	9.88
Pre-Dorsa Length (PDL)	63.42	106.71	79.97	78.86	8.53	1.27	10.67
Pre-Pectoral Length (PPcL)	27.16	45.11	38.18	38.05	4.02	0.60	10.54
Head Length (HL)	25.23	37.40	31.97	32.41	2.70	0.40	8.47
Pre-Orbital Length (PrOL)	8.21	14.95	11.77	11.86	1.25	0.18	10.61
Post-Orbital Length (PoOL)	10.01	22.51	18.71	19.13	2.46	0.36	13.17
Snout Length (SNL)	7.66	13.27	10.20	10.20	1.42	0.21	14.00
Eye Diameter (ED)	6.44	7.96	7.25	7.19	0.35	0.05	4.93
Body Depth (BD)	29.54	44.31	36.57	35.78	3.27	0.48	8.96
Caudal-Fin Length (CFL)	29.76	54.47	40.94	41.05	4.40	0.65	10.76

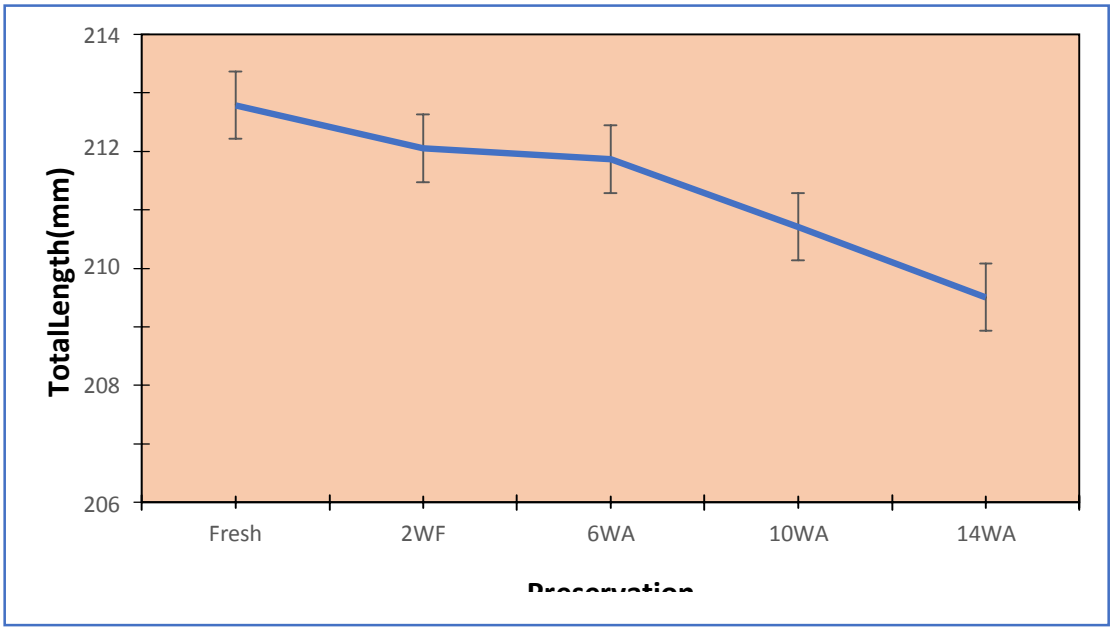

Fig2: Mean (±SE)changein Total length(TL)of *S.niger*dueto preservation.

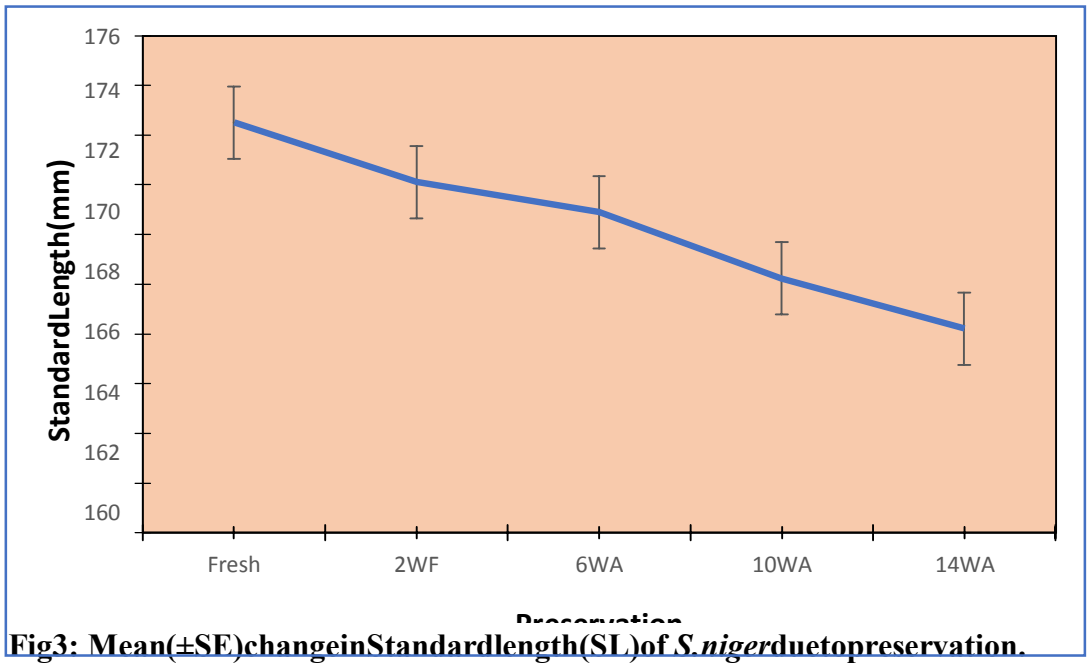

Fig3: Mean(±SE)changeinStandardlength(SL)of *S.niger*duetopreservation.

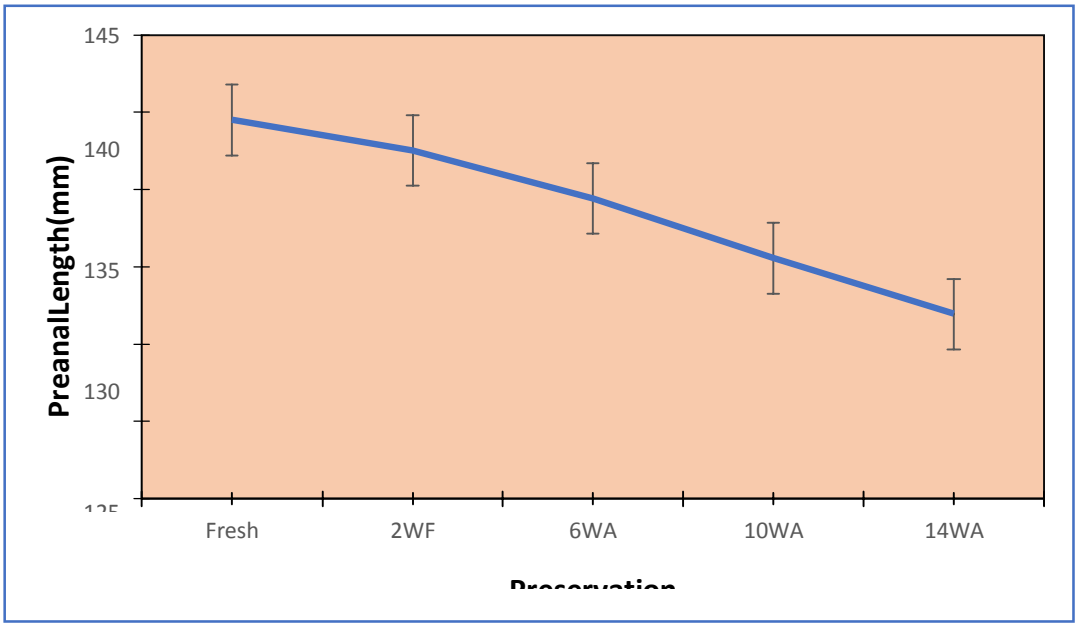

Fig4: Mean(±SE)changeinPre-anallength (PAL)of *S.niger*duetopreservation.

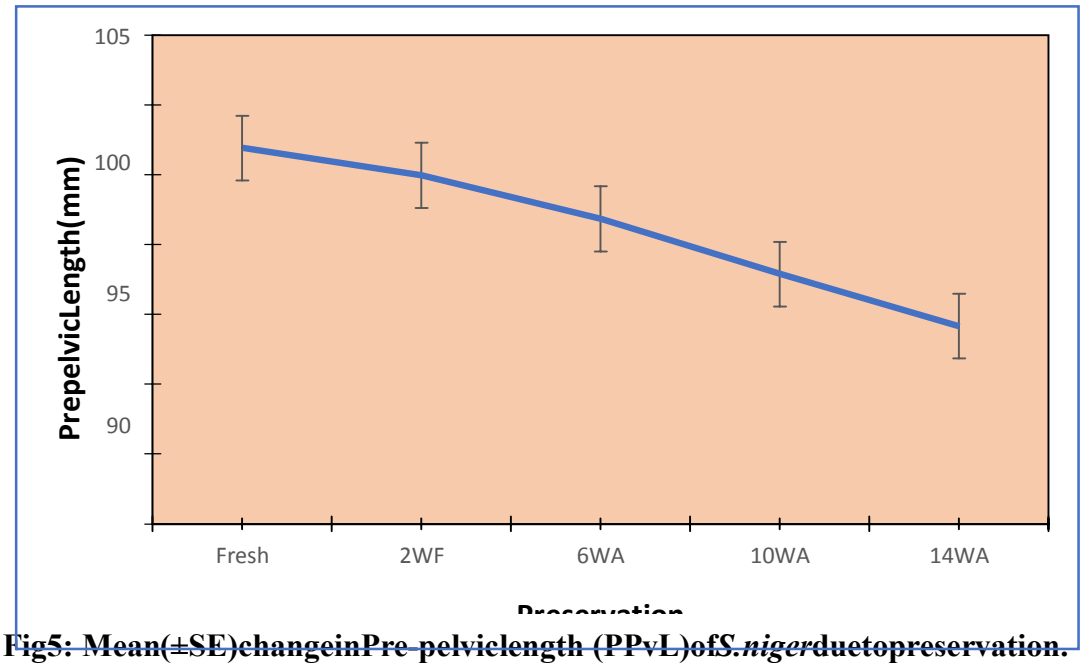

Fig5: Mean(±SE)changeinPre-pelviclength (PPvL)of*S.niger*duetopreservation.

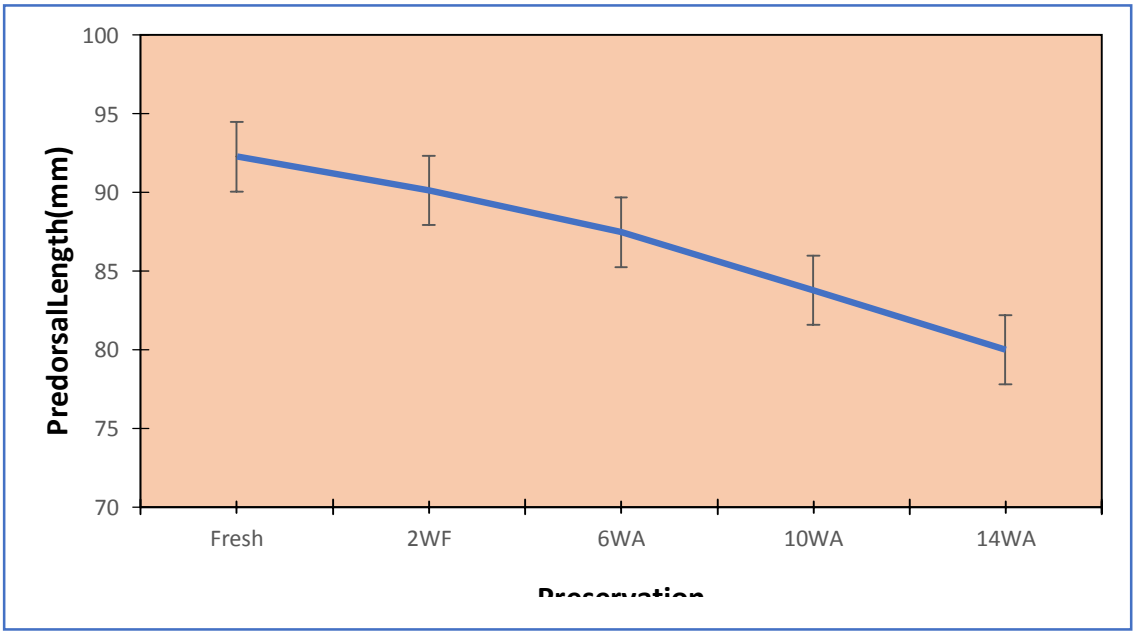

Fig6: Mean(±SE)changeinPre-dorsal length(PDL)of *S.niger*duetopreservation.

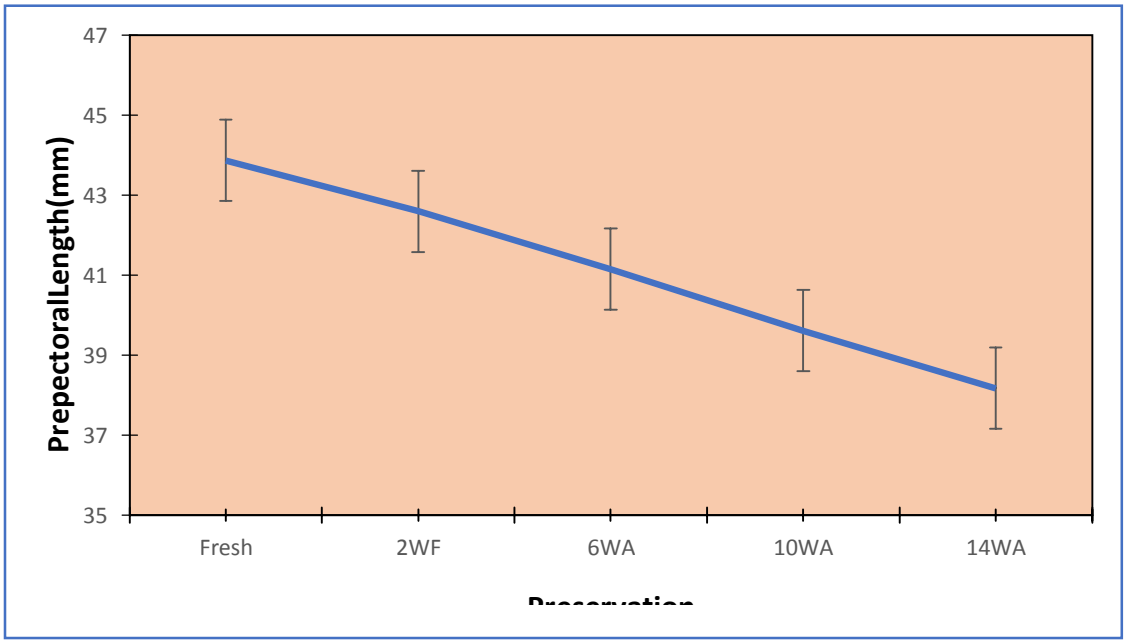

Fig7: Mean(±SE)changeinPre-pectoral length (PPcl)of*S.niger*duetopreservation.

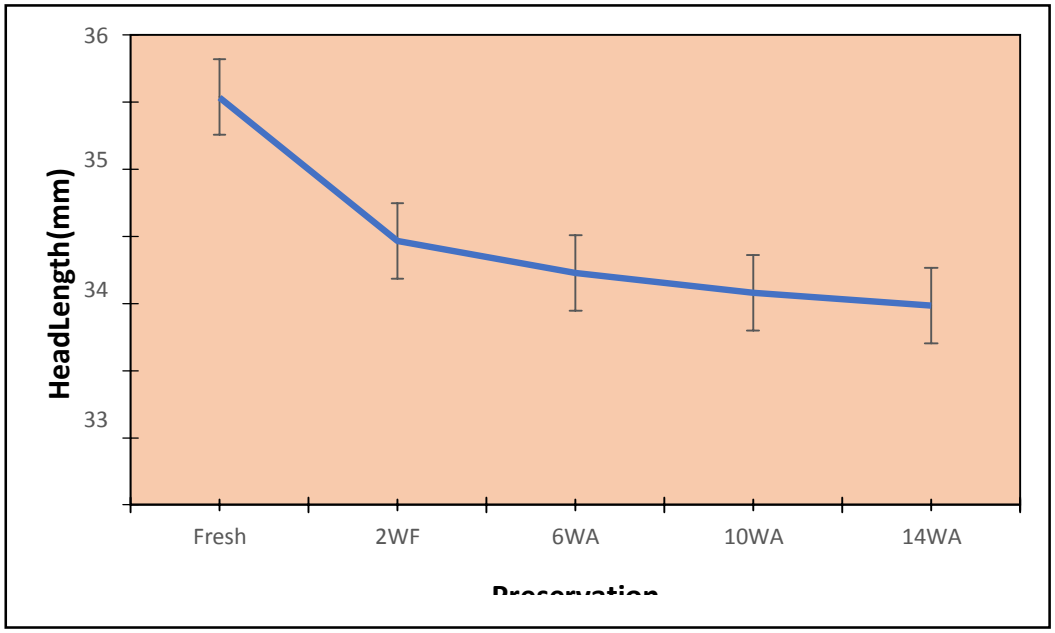

Fig8: Mean(±SE)changeinHeadlength (HL)of *S.niger*duetopreservation.

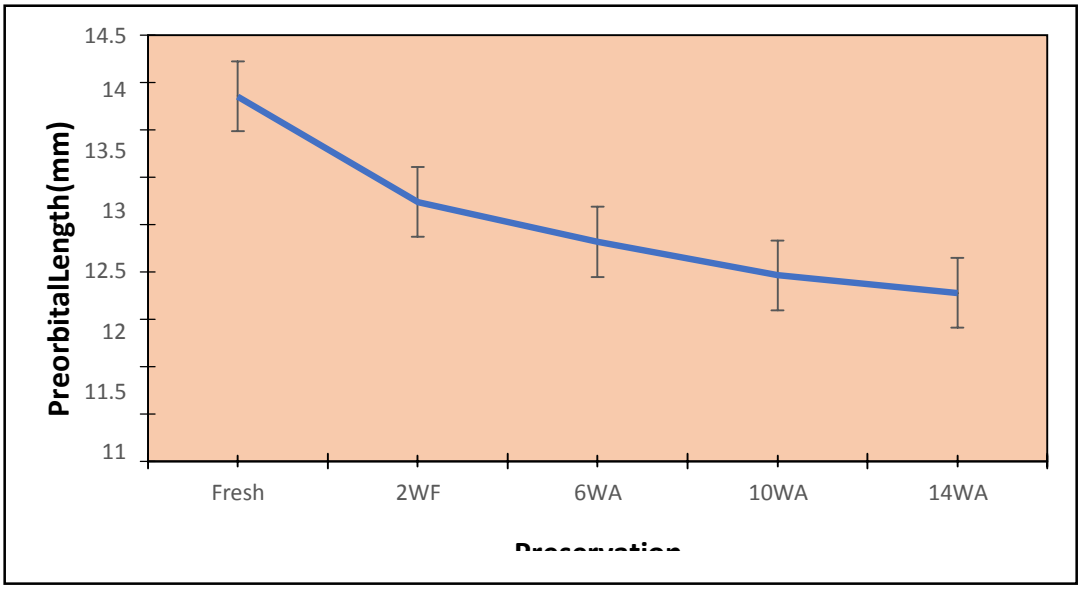

Fig9: Mean(±SE)changeinPre-orbitallength(PrOL)of *S.niger*duetopreservation.

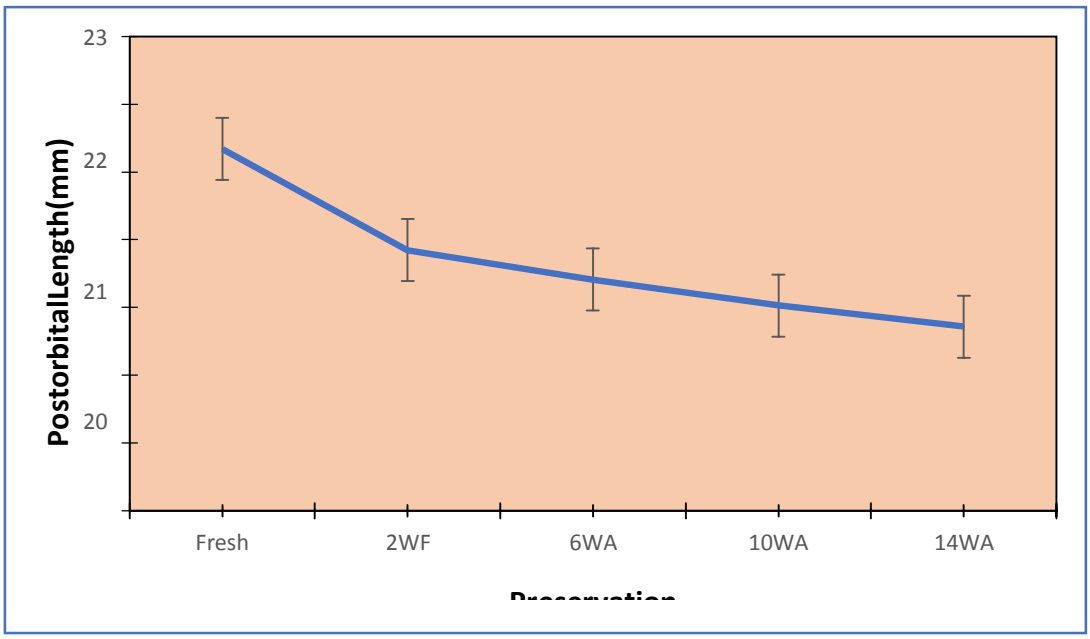

Fig10:Mean(±SE)change inPost-orbitallength (PoOL)of *S. niger*duetopreservation.

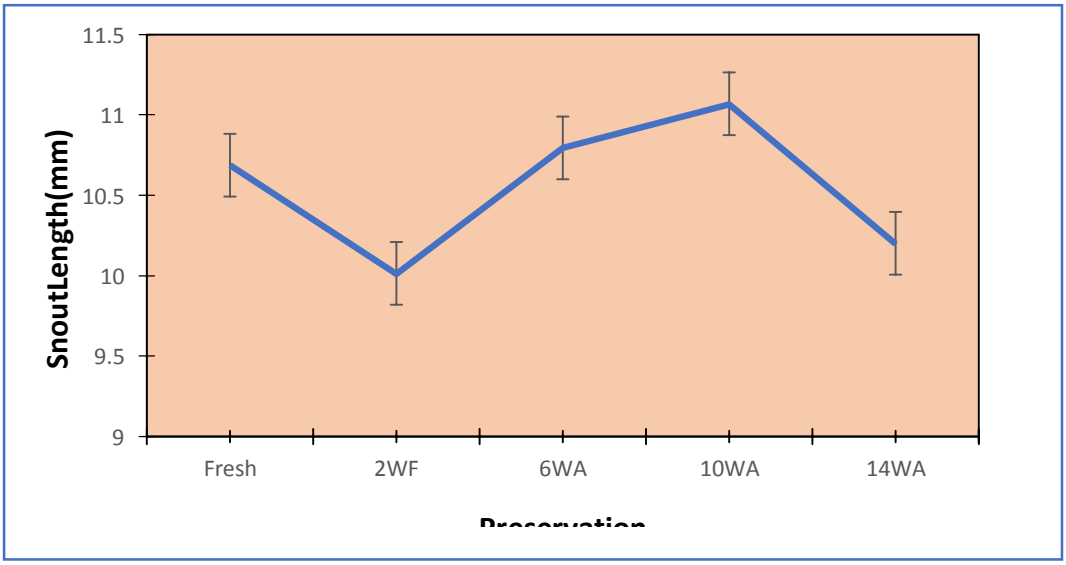

Fig11: Mean(±SE)changeinSnoutlength (SnL)of*S.niger*duetopreservation.

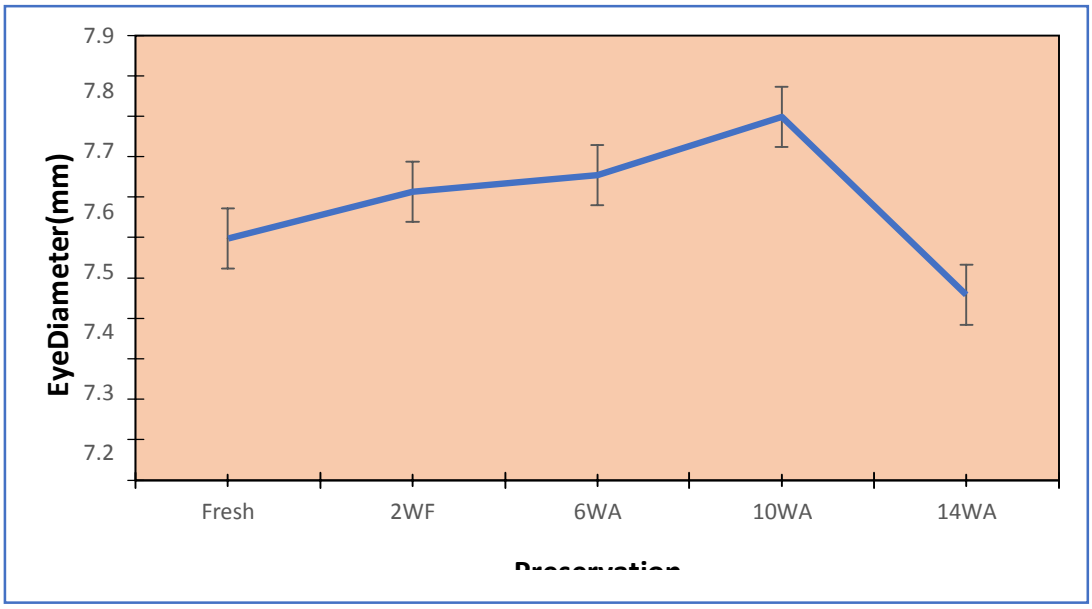

Fig12: Mean(±SE)changeinEyediameter(ED)of *S.niger*duetopreservation.

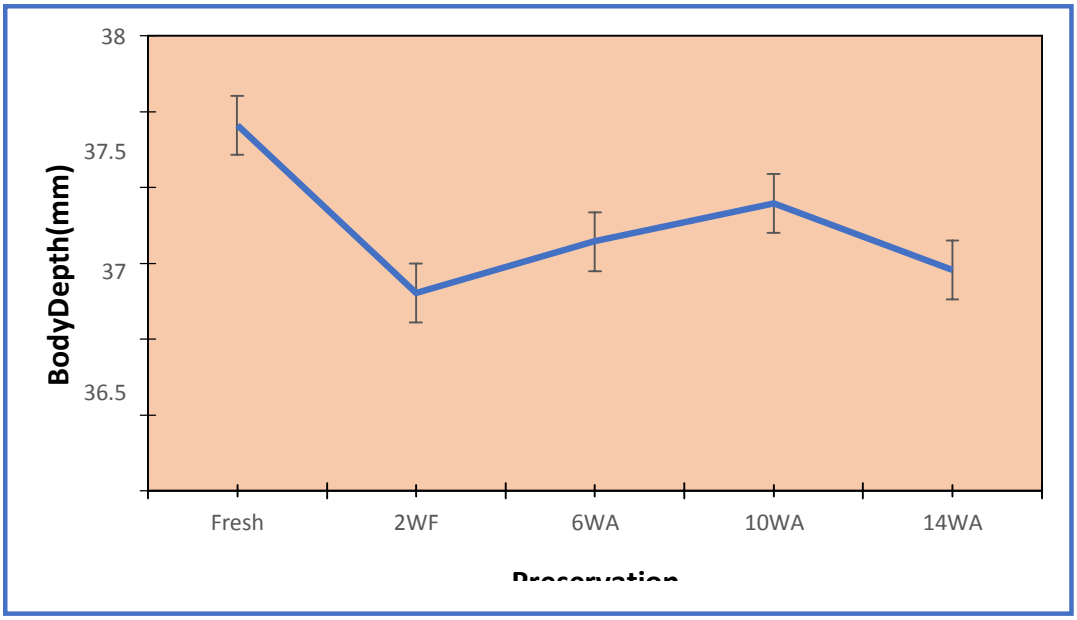

Fig13: Mean(±SE)changeinBodydepth (BD)of *S.niger*duetopreservation.

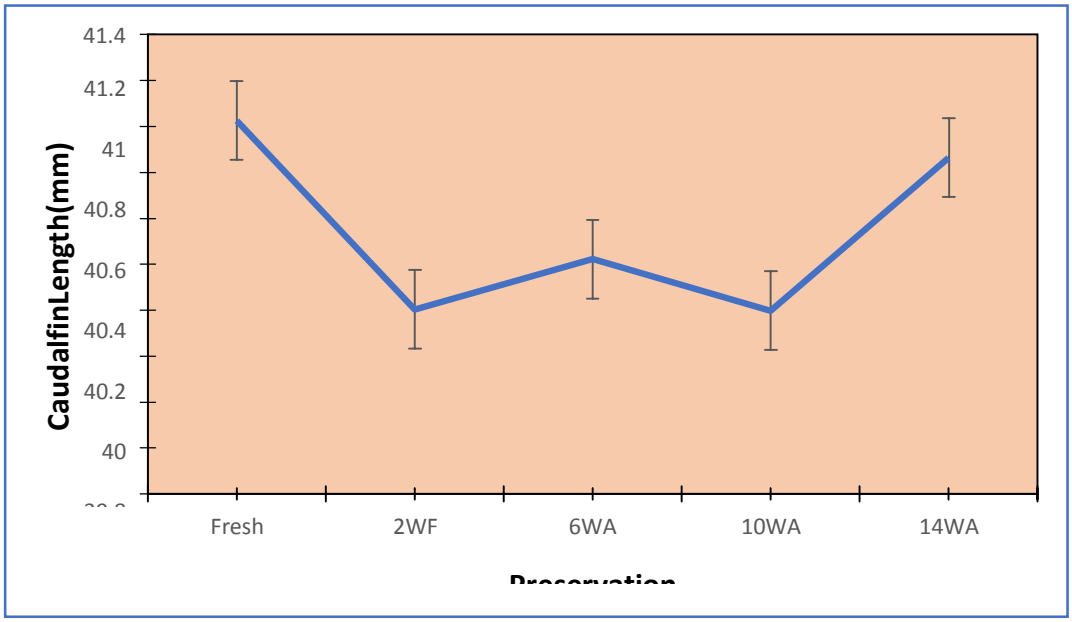

Fig14: Mean(±SE)changeinCaudalfin length (CFL)of *S.niger*duetopreservation.

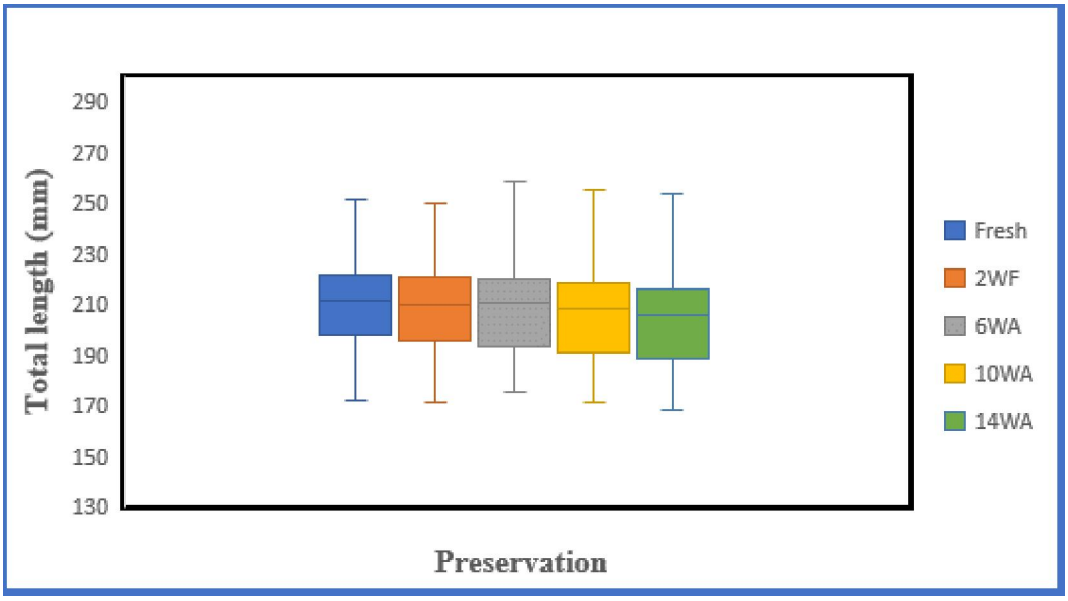

Fig15:BoxplotsofTotallength(TL) of*S.niger*(Fresh,2WF, 6WA,10WAand14WA).

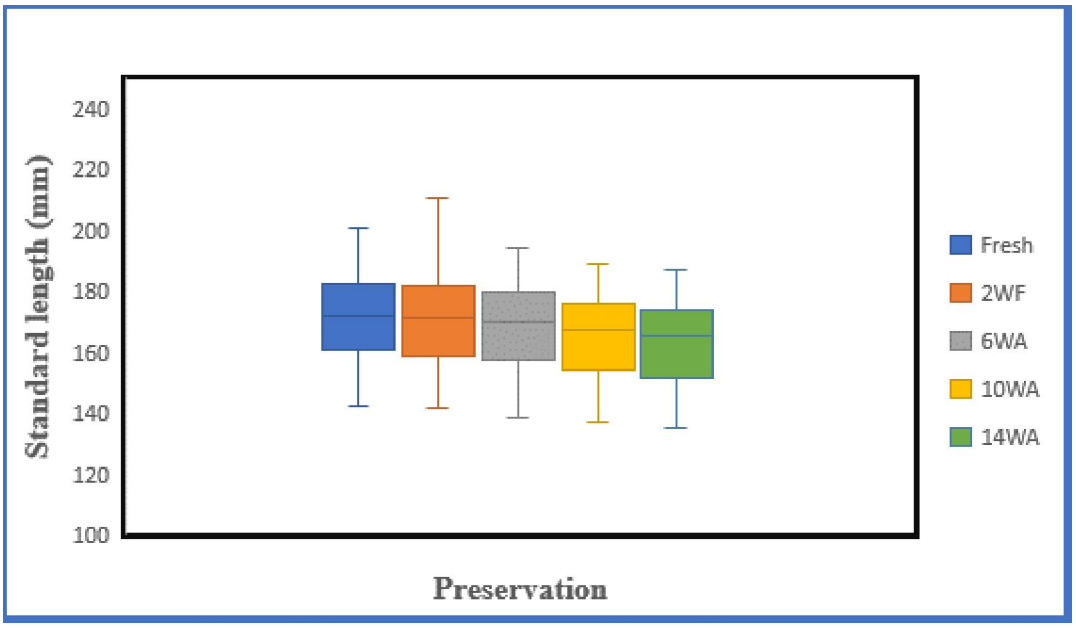

Fig 16: Box plots of Standard length (SL) of *S. niger*(Fresh, 2WF, 6WA, 10WAand14WA).

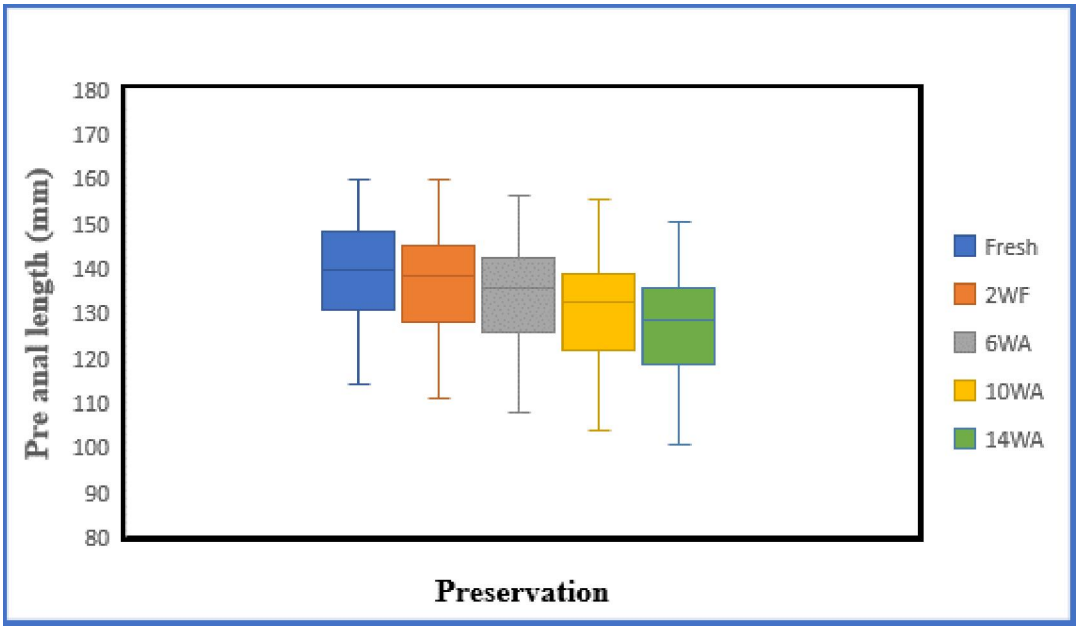

Fig17:Boxplotsof Pre-anallength (PAL)of *S. niger*(Fresh, 2WF,6WA, 10WAand14WA).

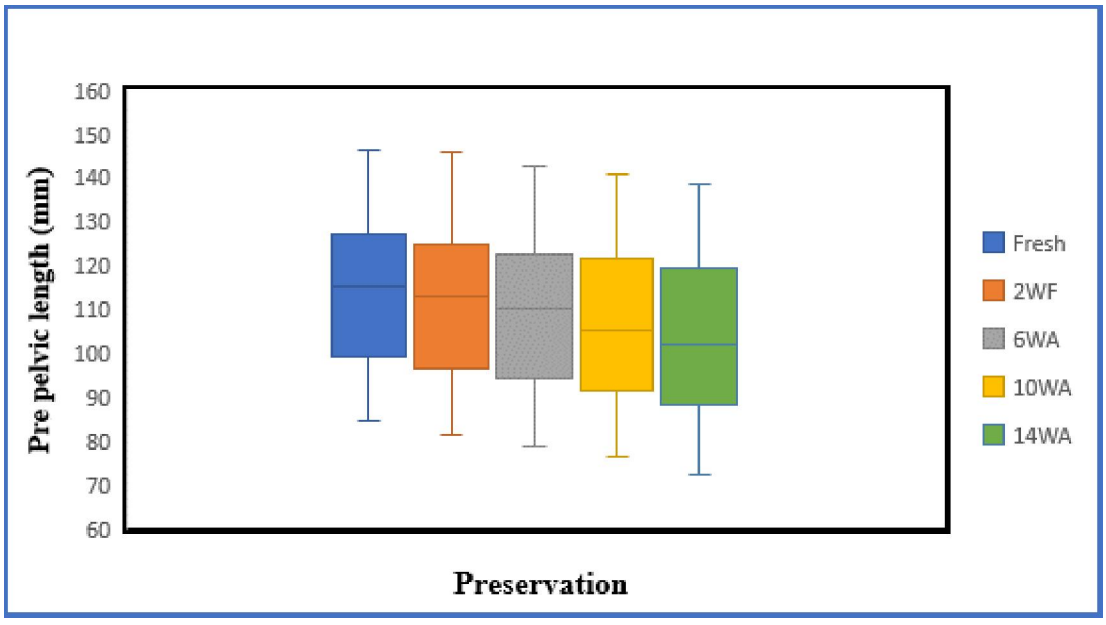

Fig18:BoxplotsofPre-pelviclength (PPvL)of*S.niger*(Fresh,2WF, 6WA,10WAand14WA).

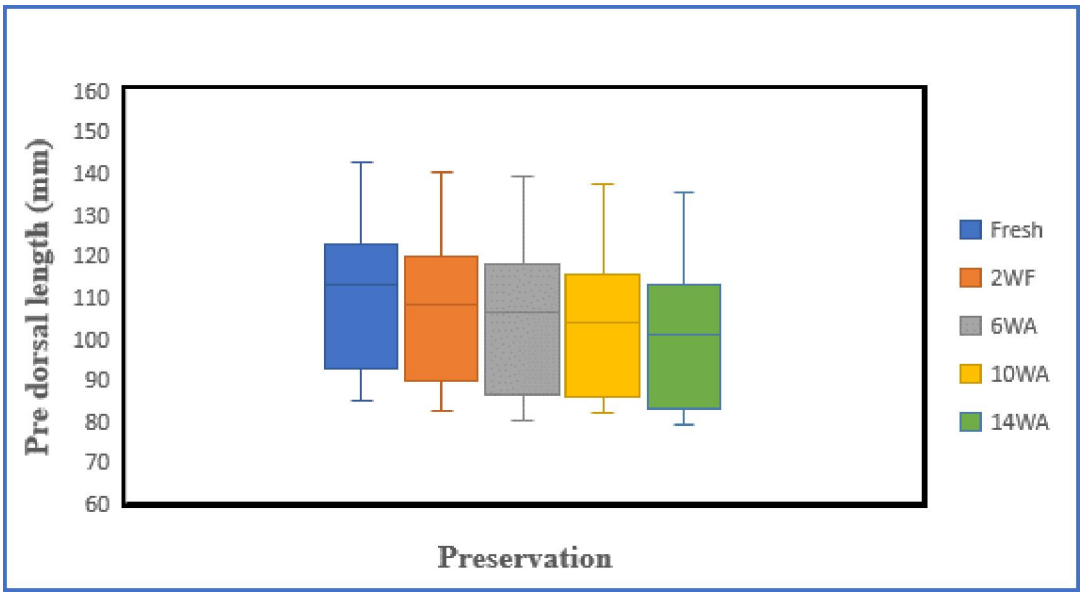

Fig19:Boxplots ofPre-dorsal length(PDL) of*S. niger*(Fresh, 2WF,6WA,10WAand 14WA).

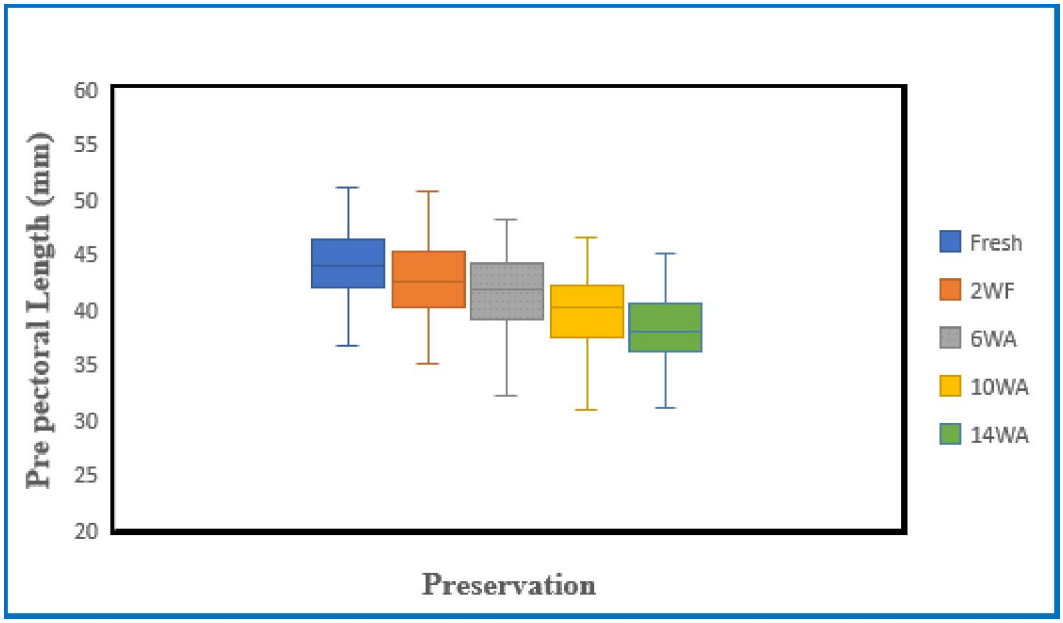

Fig20:Boxplots ofPre-pectorallength(PPcL) of*S. niger*(Fresh,2WF,6WA, 10WAand 14WA).

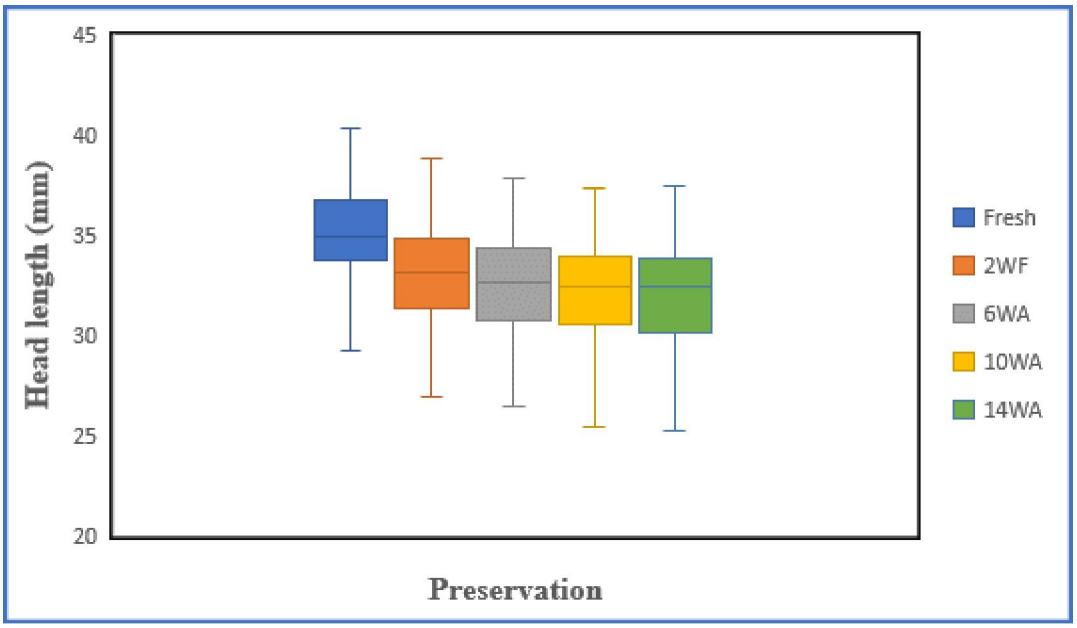

Fig21:Boxplots ofHead length(HL) of*S.niger*(Fresh,2WF, 6WA,10WAand14WA).

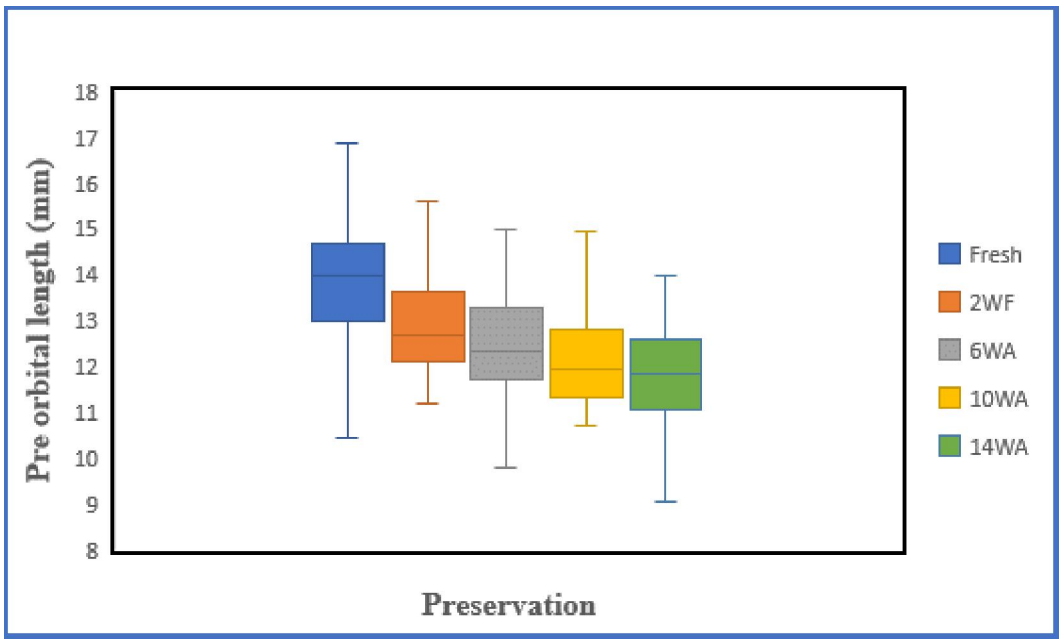

Fig 22: Box plots of Pre-orbital length (PrOL) of *S. niger*(Fresh, 2WF, 6WA, 10WA and14WA).

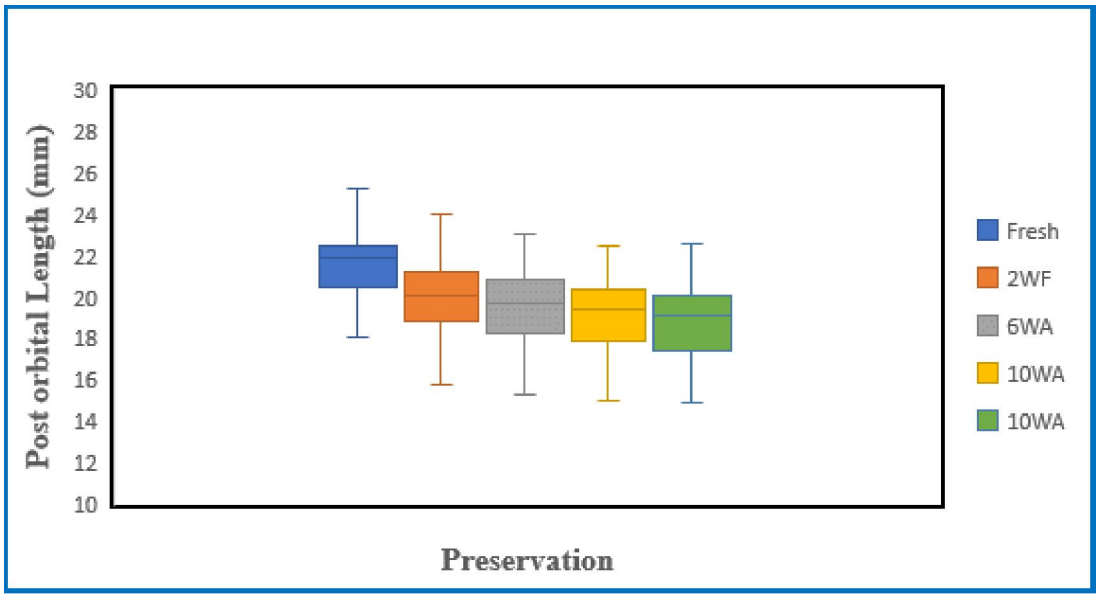

Fig23:BoxplotsofPost-orbitallength(PoOL)of *S.niger*(Fresh,2WF,6WA,10WA and14WA).

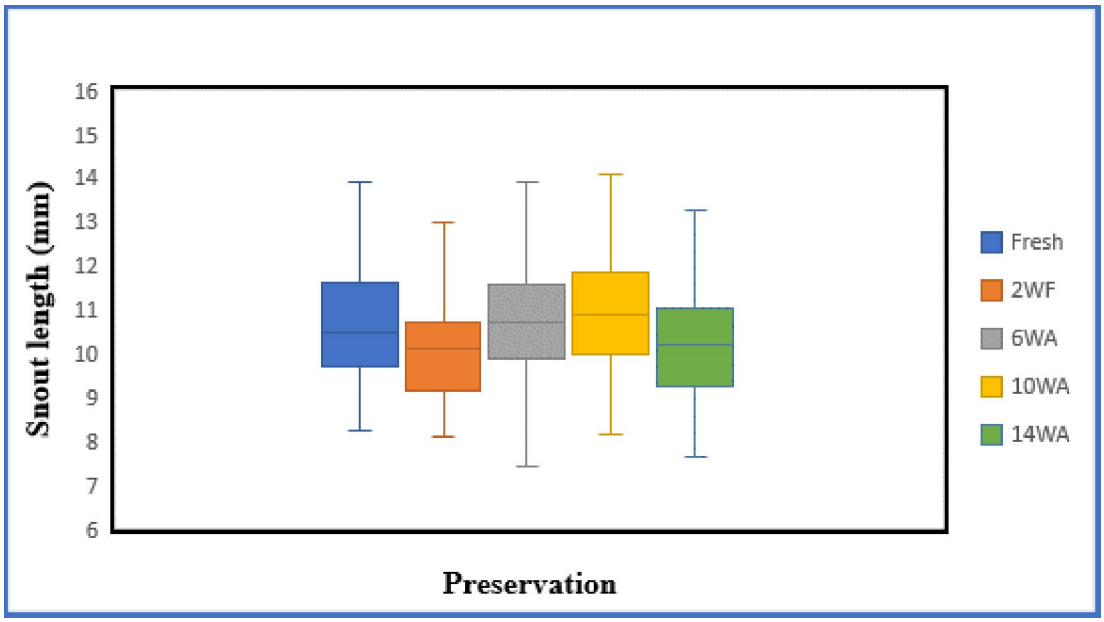

Fig24:Boxplots of Snoutlength(SnL) of *S. niger*(Fresh,2WF, 6WA,10WAand14WA).

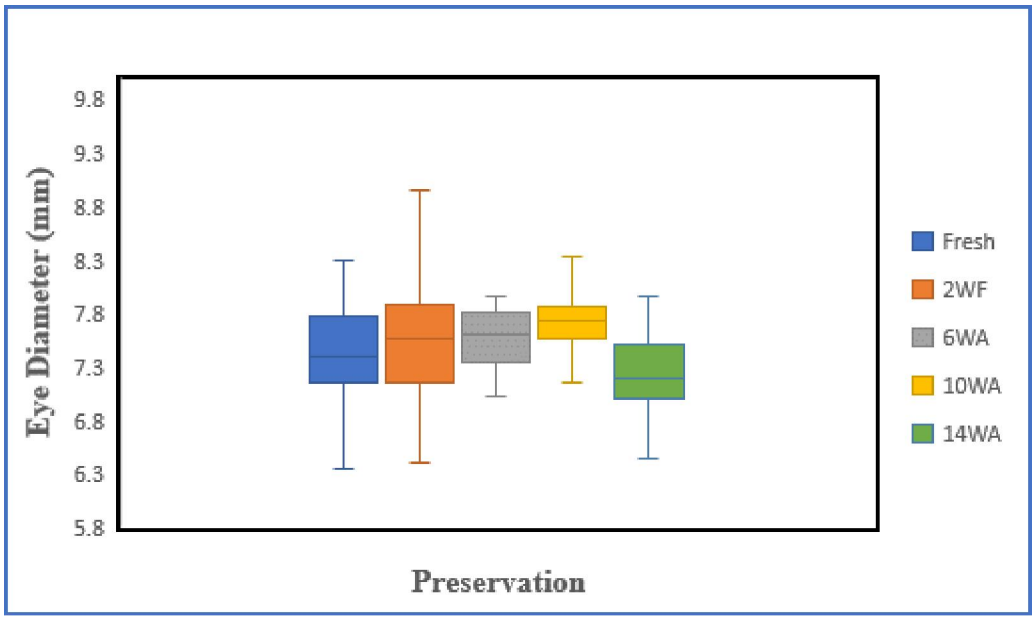

Fig25:Boxplots ofEye diameter(ED) of*S. niger*(Fresh, 2WF,6WA, 10WAand14WA).

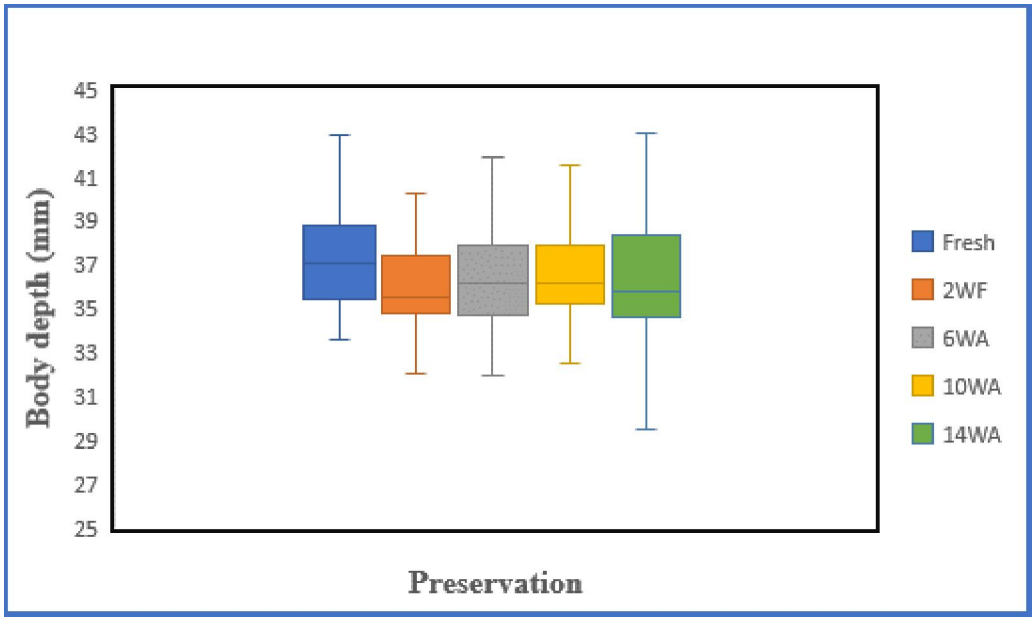

Fig26:Boxplots ofBody depth(BD) of*S. niger*(Fresh, 2WF, 6WA, 10WAand14WA).

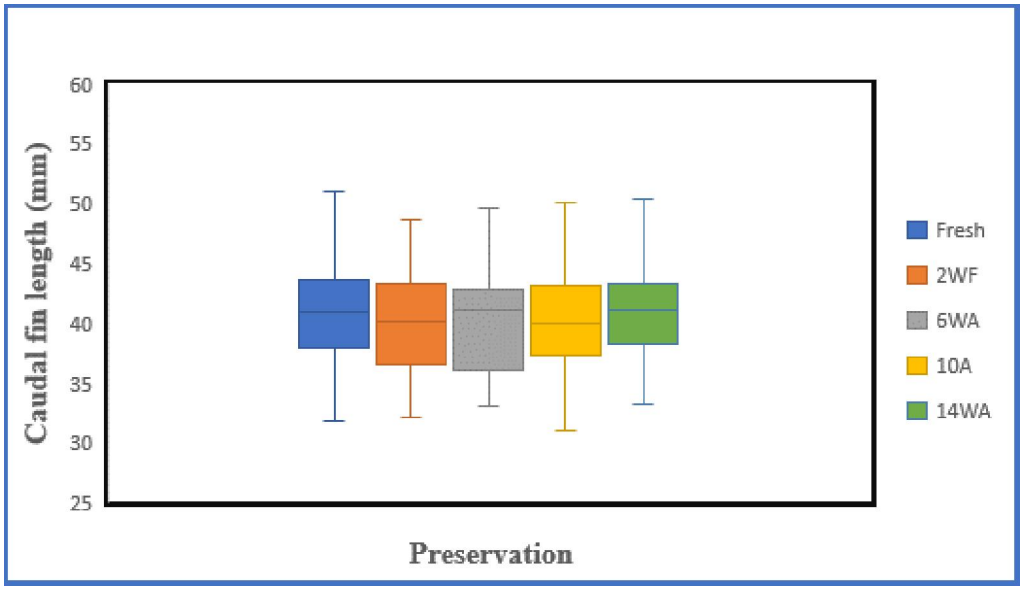

Fig 27: Box plots of Caudal fin length (CFL) of *S. niger*(Fresh, 2WF, 6WA, 10WA and14WA).

4.1.1.2 Comparisons between morphometric traits of *S. niger.*

Comparison of morphometric traits between five measurements (Fresh, 2WF, 6WA,10WA and 14WA) of *S. niger* is presented in Table 7. In *S. niger,* out of thirteen morphometric characters, three characters namely, Head length, pre-orbital length and post-orbital length showed significant difference between Fresh & 2WF, and onwards upto 14WA ($p<0.05$). The characters of pre-dorsal length, pre-pelvic length and pre-pectoral length showed significant difference between Fresh & 6WA, and onwards upto 14WA ($p<0.05$). Insignificant difference ($p>0.05$) in values was observed between fresh and 2WF. Total length, standard length, and pre-anal length displayed significant difference only between Fresh & 14WA ($p<0.05$) and insignificant difference in Fresh against 2WF, 6WA and 10WA. Non-significant difference was observed in four characters namely, body depth, caudal fin length, eye diameter and snout length ($p>0.05$) for Fresh and all subsequent readings.

Parameters	Fresh	2WF	t-value	p-value	Fresh	6WA	t-value	p-value	Fresh	10WA	t-value	p-value	Fresh	14WA	t-value	p-value
TL vs TL	211.58± 19.51	210.02 ±2.79	0.38	>0.05	211.58± 19.51	209.73± 2.82	0.45	>0.05	211.58± 19.51	207.59± 2.94	0.96	>0.05	211.58± 19.51	205.13± 2.92	0.12	**<0.05**
SL vs SL	172.51± 2.43	170.11 ±2.19	0.73	>0.05	172.51± 2.43	168.89± 2.35	1.06	>0.05	172.51± 2.43	166.23± 2.34	1.85	>0.05	172.51± 2.43	164.22± 2.33	2.45	**<0.05**
PAL vs PAL	139.51± 1.89	137.55 ±1.94	0.72	>0.05	139.51± 1.89	134.44± 1.92	1.87	>0.05	139.51± 1.89	130.56± 1.86	3.36	>0.05	139.51± 1.89	126.95± 1.88	4.69	**<0.05**
PPVL vs PPVL	96.93±1 .22	94.98± 1.25	1.13	>0.05	96.93±1 .22	91.86±1 .26	2.87	**<0.05**	96.93±1 .22	87.88±1 .27	5.10	**<0.05**	96.93±1 .22	84.16±1 .24	7.3	**<0.05**
PDL vs PDL	92.25±1 .24	90.11± 1.23	1.22	>0.05	92.25±1 .24	87.45±1 .25	2.71	**<0.05**	92.25±1 .24	83.77±1 .26	4.77	**<0.05**	92.25±1 .24	79.97±1 .27	6.89	**<0.05**
PPCL vs PPCL	43.86±0 .54	42.59± 0.56	1.61	**>0.05**	43.86±0 .54	41.15±0 .57	3.41	**<0.05**	43.86±0 .54	39.62±0 .58	5.30	**<0.05**	43.86±0 .54	38.18±0 .60	6.99	**<0.05**
HL vs HL	35.07±0 .44	32.93± 0.39	3.61	**<0.05**	35.07±0 .44	32.45±0 .39	4.42	**<0.05**	35.07±0 .44	32.16±0 .39	4.90	**<0.05**	35.07±0 .44	31.97±0 .40	5.17	**<0.05**
PrOL vs PrOL	13.85±0 .19	12.73± 0.19	4.07	**<0.05**	13.85±0 .19	12.31±0 .17	5.77	**<0.05**	13.85±0 .19	11.96±0 .18	7.07	**<0.05**	13.85±0 .19	11.77±0 .18	7.66	**<0.05**
PoOL vs PoOL	21.33±0 .35	19.84± 0.35	2.97	**<0.05**	21.33±0 .35	19.41±0 .35	3.84	**<0.05**	21.33±0 .35	19.02±0 .35	4.58	**<0.05**	21.33±0 .35	18.71±0 .36	5.11	**<0.05**
SnL vs SnL	10.68±0 .22	10.01± 0.19	2.26	>0.05	10.68±0 .22	10.79±0 .22	0.32	>0.05	10.68±0 .22	11.06±0 .22	-1.90	>0.05	10.68±0 .22	10.20±0 .21	1.56	>0.05
ED vs ED	7.39±0. 08	7.51±0. 08	-0.94	>0.05	7.39±0. 08	7.55±0. 04	-1.58	>0.05	7.39±0. 08	7.79±0. 06	-3.68	>0.05	7.39±0. 08	7.25±0. 05	1.35	>0.05
BD vs BD	38.74±1 .20	36.52± 0.51	1.69	>0.05	38.74±1 .20	37.05±0 .53	1.28	>0.05	38.74±1 .20	37.15±0 .51	1.21	>0.05	38.74±1 .20	36.57±0 .48	1.66	>0.05
CFL vs CFL	41.05±0 .61	40.20±	1.01	>0.05	41.05±0 .61	40.52±0 .67	0.58	>0.05	41.05±0 .61	40.19±0 .59	1.00	>0.05	41.05±0 .61	40.94±0 .65	0.11	>0.05

Data are mean ± SE (n=45 for Fresh, 2WF, 6WA, 10WA and 14WA. The p-value with $p < 0.05$ are significantly different

4.1.1.3 Percentage change due to preservation in *S. niger*.

The percentage change between morphometric characters of *S. niger*for the Fresh and 2WF and Fresh and 14WA is given in Table 8. Out of thirteen morphometric characters, pre orbital length showed highest percentage change in formalin (8.06%) and total length showed lowest percentage change (0.70%). Whereas in alcohol pre dorsal length displayed highest percentage change (-13.31%) and caudal fin length displayed lowest percentage change (0.39%).

4.1.1.4 Conversion equations for formalin (2WF) and alcohol (14WA) preserved *S. niger*.

Least-squares regression equations of total length, standard length, pre-anal length, pre-pelvic length, pre-dorsal length, pre-pectoral length, head length, pre-orbital length, post-orbital length, eye diameter, snout length, body depth and caudal fin length of *S. niger* in formalin (2WF) and alcohol (14WA) are given in a Table 9.

Table 8: Percentage change of *S. niger* in formalin and alcohol

S. NO	Morphometric characters	Percentage change in formalin	Percentage change in alcohol
1	Total Length (TL)	-0.70	-3.20%
2	Standard Length (SL)	-1.39%	-4.81%
3	Pre-Anal Length (PAL	-1.40%	-9.00%
4	Pre-Pelvic Length (PPvL)	-2.01	-13.17%
5	Pre-Dorsal Length (PDL)	-2.32%	-13.31%
6	Pre-Pectoral Length (PPcL)	-2.90%	-12.96%
7	Head Length (HL)	-6.11%	-8.85%
8	Pre-Orbital Length (PrOL)	-8.06%	-14.99%
9	Post-Orbital Length (PoOL)	-7.00%	-12.28%
10	Snout Length (SNL)	-6.31%	-4.56%
11	Eye Diameter (ED)	1.56%	-1.88%
12	Body Depth (BD)	-2.95%	-2.55%
13	Caudal Fin Length (CFL)	-2.00	-0.39%

Table 9: Conversions equations for formalin and alcohol preserved *S. niger*.

S. NO	Morphometric characters	For Formalin (2WF)	For Alcohol (14WA)
1	**Total Length (TL)**	$TL_{Fresh}=1.11+0.98(L_{FP})$	$T_{Fresh}= 23.36+0.85(L_{AP})$
2	**Standard Length (SL)**	$SL_{Fresh}=3.11+1.00(L_{FP})$	$SL_{Fresh}=27.30+0.79(L_{AP})$
3	**Pre-Anal Length (PAL)**	$PAL_{Fresh}=4.88+1.02(L_{FP})$	$PAL_{Fresh}=8.35+0.96(L_{AP})$
4	**Pre-Pelvic Length (PPvL)**	$PPvL_{Fresh}=3.50+1.01(L_{FP})$	$PpVL_{Fresh}=12.54+0.99(L_{AP})$
5	**Pre-Dorsal Length (PDL)**	$PDL_{Fresh}=0.09+0.97(L_{FP})$	$PDL_{Fresh}=11.60+0.99(L_{AP})$
6	**Pre-Pectoral Length (PpcL)**	$PPcL_{Fresh}=1.51+1.00(L_{FP})$	$PPcL_{Fresh}=3.11+0.94(L_{AP})$
7	**Head Length (HL)**	$HL_{Fresh}=3.56+0.83(L_{FP})$	$HL_{Fresh}=2.75+0.83(L_{AP})$
8	**Pre-Orbital Length (PrOL)**	$PrOL_{Fresh}=0.29+0.89(L_{FP})$	$PrOL_{Fresh}=0.70+0.79(L_{AP})$
9	**Post-Orbital Length (PoOL)**	$PoOL_{Fresh}=0.22+0.94(L_{FP})$	$PoOL_{Fresh}=1.99+0.97(L_{AP})$
10	**Snout Length (SnL)**	$SnL_{Fresh}=5.55+0.41(L_{FP})$	$SNL_{Fresh}=5.30+0.45(L_{AP})$
11	**Eye Diameter (ED)**	$ED_{Fresh}=5.43+0.28(L_{FP})$	$ED_{Fresh}=6.43+0.11(L_{AP})$
12	**Body Depth (BD)**	$BD_{Fresh}=4.97+0.83(L_{FP})$	$BD_{Fresh}=13.25+0.62(L_{AP})$
13	**Caudal Fin Length (CFL)**	$CFL_{Fresh}=11.44=0.70(L_{FP})$	$CFL_{Fresh}=14.03+0.65(L_{AP})$

4.1.2 Conventional morphometry of *S. esocinus.*

4.1.2.1 Descriptive statistics

The descriptive statistics of 13 morphometric characters viz., minimum, maximum, mean, median, standard error, standard deviation and coefficient of variance of the five measurements (Fresh, 2WF, 6WA,10WA and 14WA) of *S. esocinus* are presented in Table 10, Table 11, Table 12, Table 13 and Table 14. In Fresh, the total length of the fish ranged from 194.79 to 329.21mm with a coefficient of variation of 13.79%. The total length ranged from 191.75 to 325.00mm in 2WF and displayed a coefficient of variation of 13.79%. In 6WA, the total length ranged from 186.67 to 322.17mm and a coefficient of variation of 13.85% was observed. The total length ranged from 181.09 to 318.67mm in 10WA with coefficient of variation of 14.16%. In 14WA, coefficient of variation in total length was recorded at 14.46% when length of fish ranged from 176.00 to 315.55mm. The box plots and line graphs of 13 morphometric traits of individuals from the five experimental measurements (Fresh, 2WF, 6WA, 10WA and 14WA) are presented in figures. 28-40 and figures 41-53.

Table 10: Statistical estimates of various morphometric characteristics of *S. esocinus* (Fresh).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	194.79	329.21	246.62	250.06	34.01	5.07	13.79
Standard Length (SL)	159.78	270.49	200.41	200.93	27.81	4.14	13.87
Pre-Anal Length (PAL)	128.78	216.23	163.45	163.82	23.57	3.51	14.42
Pre-Pelvic Length (PPvL)	84.62	146.41	113.07	115.47	16.36	2.43	14.47
Pre-Dorsal Length (PDL)	85.09	142.66	109.08	112.82	115.48	2.30	14.19
Pre-Pectoral Length (PPcL)	42.36	73.97	54.43	55.09	8.18	1.22	15.03
Head Length (HL)	30.77	50.82	39.49	40.17	5.31	0.79	13.45
Pre-Orbital Length (PrOL)	11.02	24.42	17.76	17.69	2.96	0.44	16.69
Post-Orbital Length (PoOL)	19.34	35.54	25.89	25.69	3.97	0.59	15.35
Snout Length (SNL	10.23	19.51	15.31	15.02	2.53	0.37	16.54
Eye Diameter (ED	7.2	10.43	8.55	8.50	0.77	0.11	9.01
Body Depth (BD)	31.36	63.81	43.46	43.35	7.73	1.15	17.79
Caudal Fin Length (CFL)	32.00	63.78	48.14	46.89	7.19	1.07	14.93

Table 11: Statistical estimates of various morphometric characteristics of *S. esocinus*(2WF).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	191.75	325.00	245.19	246.15	33.81	5.04	13.79
Standard Length (SL)	156.76	269.01	197.75	199.18	28.10	4.18	14.21
Pre-Anal Length (PAL)	126.07	216.00	161.38	162.39	23.76	3.54	14.72
Pre-Pelvic Length (PPvL)	81.53	145.85	110.65	113.17	16.84	2.51	15.22
Pre-Dorsal Length (PDL)	82.66	139.94	106.59	108.30	15.38	2.29	14.42
Pre-Pectoral Length (PPcL)	38.44	72.75	52.36	54.17	8.58	1.28	16.39
Head Length (HL)	32.20	54.22	41.93	42.24	5.74	0.85	13.70
Pre-Orbital Length (PrOL)	12.78	26.98	19.41	19.51	3.00	0.44	15.47
Post-Orbital Length (PoOL)	20.48	36.53	27.93	27.87	4.02	0.60	14.42
Snout Length (SNL)	10.60	20.58	16.16	15.95	2.49	0.37	15.40
Eye Diameter (ED)	7.62	9.90	8.69	8.71	0.50	0.25	5.77
Body Depth (BD)	30.63	62.72	42.53	41.55	7.31	1.09	17.19
Caudal Fin Length (CFL)	31.85	62.99	46.14	44.85	8.06	1.20	17.48

Table 12: Statistical estimates of various morphometric characteristics of *S. esocinus* (6WA).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	186.67	322.17	240.95	241.00	33.38	4.97	13.85
Standard Length (SL)	154.09	267.94	195.72	196.27	28.25	4.21	14.43
Pre-Anal Length (PAL)	124.09	215.08	159.63	160.90	23.75	3.54	14.87
Pre-Pelvic Length (PPvL)	78.79	142.76	108.90	110.32	17.11	2.55	15.71
Pre-Dorsal Length (PDL)	80.19	138.91	104.08	105.99	15.55	2.31	14.94
Pre-Pectoral Length (PPcL)	38.44	72.75	52.36	54.17	8.58	1.28	16.39
Head Length (HL)	32.20	54.22	41.93	42.24	5.74	0.85	13.70
Pre-Orbital Length (PrOL)	10.48	24.77	17.28	16.75	3.13	0.46	18.15
Post-Orbital Length (PoOL)	10.93	34.98	25.14	24.73	3.98	0.59	15.85
Snout Length (SNL	10.02	21.91	16.25	16.24	2.80	0.41	17.22
Eye Diameter (ED	7.04	9.87	8.61	8.70	0.61	0.09	7.08
Body Depth (BD)	31.08	61.81	42.50	42.13	7.34	1.09	17.28
Caudal Fin Length (CFL)	30.84	60.19	47.77	48.19	7.24	1.07	15.15

Table 13: Statistical estimates of various morphometric characteristics of *S. esocinus*(10WA).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	181.09	318.67	236.59	236.91	33.52	4.99	14.16
Standard Length (SL)	151.76	264.06	193.83	194.42	28.08	4.18	14.48
Pre-Anal Length (PAL)	121.25	215.97	157.91	159.99	24.00	3.57	15.19
Pre-Pelvic Length (PPvL)	76.42	140.88	106.49	105.05	17.42	2.59	16.35
Pre-Dorsal Length (PDL)	10.16	24.68	16.66	16.22	3.18	0.47	19.11
Pre-Pectoral Length (PPcL)	34.76	69.32	48.81	48.72	8.65	1.29	17.73
Head Length (HL)	30.17	51.45	39.53	39.55	6.04	0.90	15.29
Pre-Orbital Length (PrOL)	10.24	24.92	17.01	16.82	3.16	0.47	18.57
Post-Orbital Length (PoOL)	17.61	34.59	24.75	24.37	3.95	0.58	15.96
Snout Length (SNL	9.25	22.36	15.84	15.88	2.76	0.41	17.43
Eye Diameter (ED	7.56	9.80	8.56	8.51	0.49	0.07	5.80
Body Depth (BD)	31.24	62.12	41.75	41.59	7.29	1.08	17.46
Caudal Fin Length (CFL)	32.35	58.35	45.22	44.24	6.53	0.97	14.46

Table 14: Statistical estimates of various morphometric characteristics of *S. esocinus* (14WA).

Statistical estimates	Min (mm)	Max (mm)	Mean	Median	SD	SE	CV%
Total Length (TL)	176.00	315.55	32.15	231.97	33.57	5.00	14.46
Standard Length (SL)	148.85	260.64	191.78	193.79	27.81	4.14	14.50
Pre-Anal Length (PAL)	118.18	214.31	155.79	157.52	24.14	3.59	15.49
Pre-Pelvic Length (PPvL)	72.32	138.63	104.11	102.25	17.87	2.66	17.17
Pre-Dorsal Length (PDL)	79.23	135.20	99.74	100.72	15.72	2.34	15.76
Pre-Pectoral Length (PPcL)	33.01	68.26	47.08	47.02	8.83	1.31	18.76
Head Length (HL)	30.00	51.12	39.10	39.39	5.89	0.87	15.06
Pre-Orbital Length (PrOL)	10.16	24.68	16.66	16.22	3.18	0.47	19.11
Post-Orbital Length (PoOL)	17.26	34.13	24.49	24.18	3.95	0.59	16.16
Snout Length (SNL	9.85	19.52	15.79	15.92	2.44	0.36	15.48
Eye Diameter (ED	7.27	9.65	8.61	8.61	0.55	0.08	6.49
Body Depth (BD)	31.13	62.20	41.86	40.67	7.76	1.15	18.55
Caudal Fin Length (CFL)	33.26	60.45	47.26	46.99	6.88	1.02	14.55

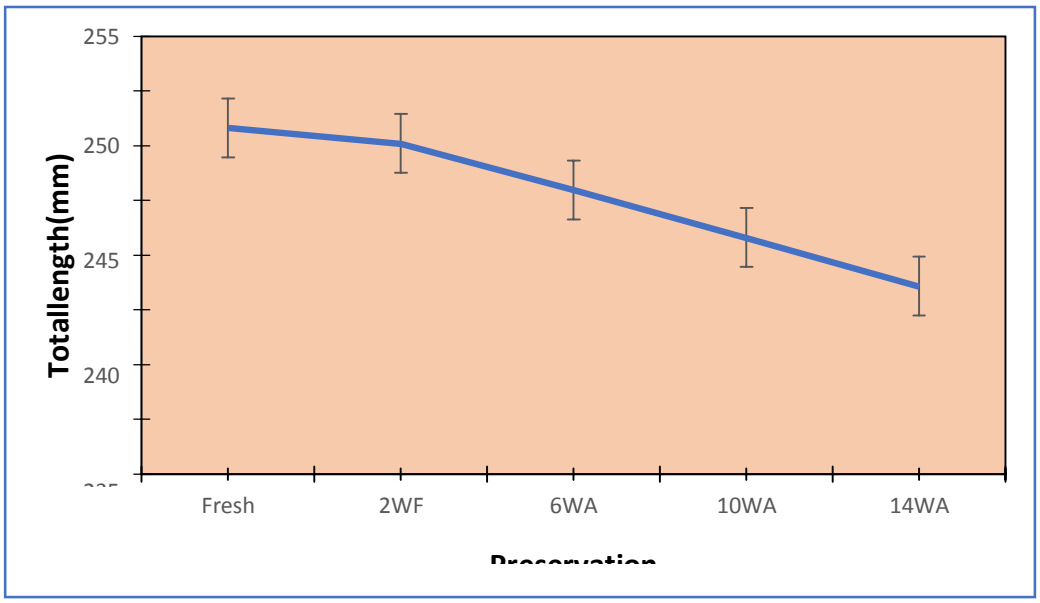

Fig28: Mean(±SE)changein Totallength(TL)of *S.esocinus*duetopreservation.

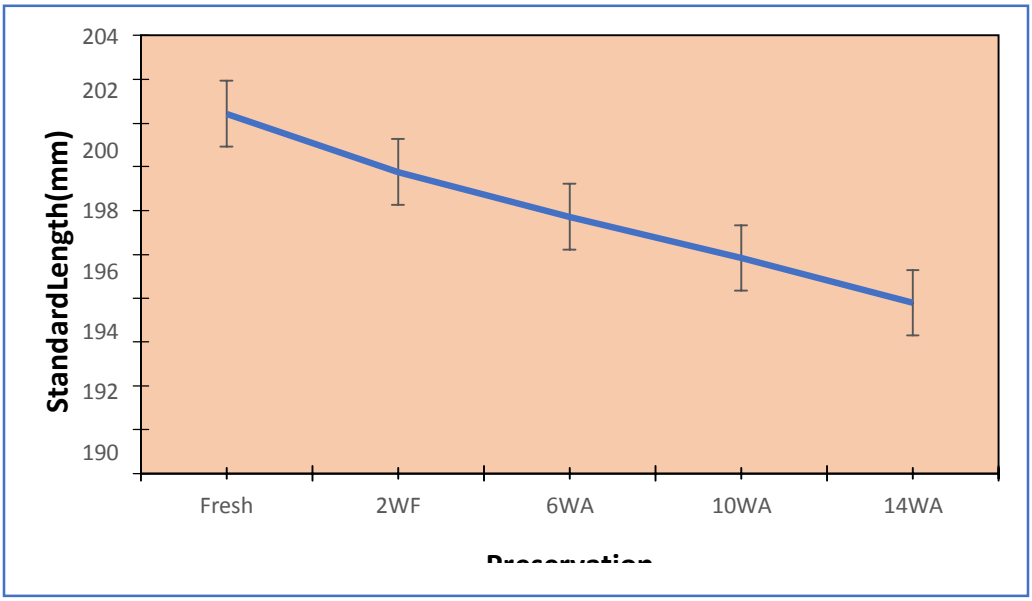

Fig29: Mean(±SE)changeinStandardlength(SL)of *S.esocinus*dueto preservation.

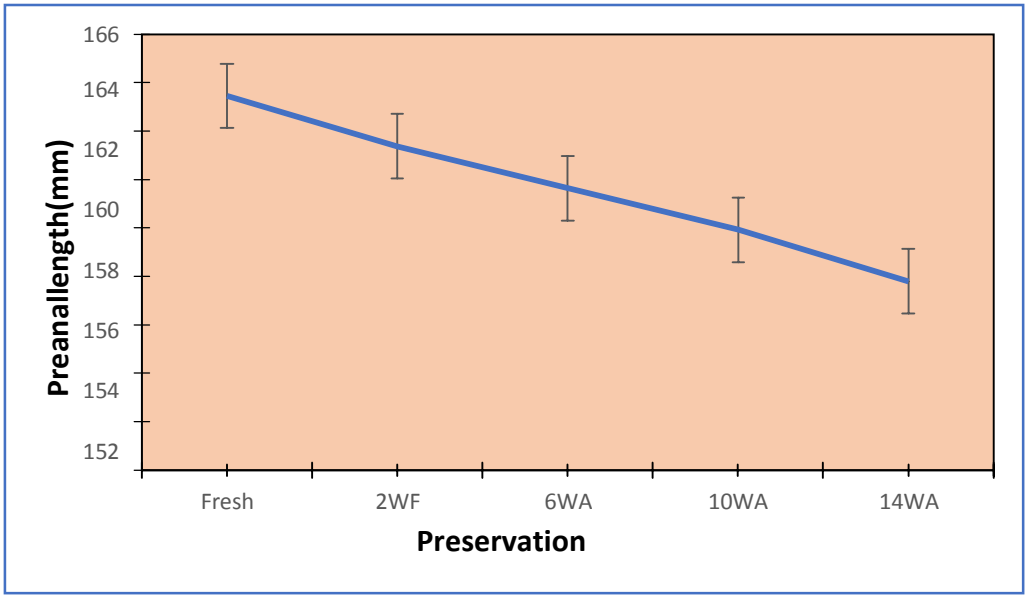

Fig30: Mean(±SE)changeinPre-anallength(PAL)of *S.esocinus*duetopreservation.

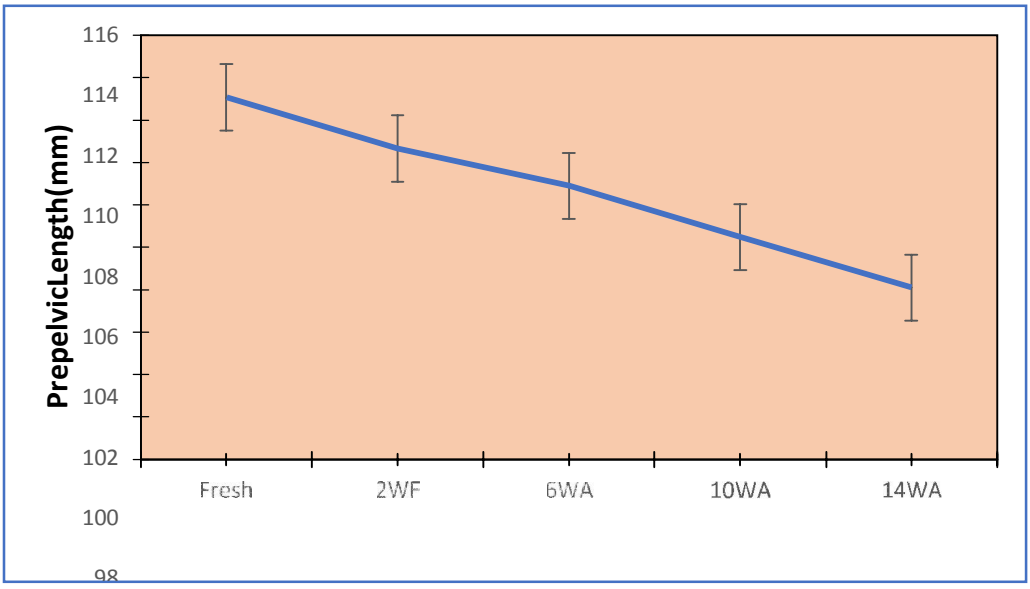

Fig31: Mean(±SE)changeinPre-pelviclength(PPvL)of*S.esocinus*duetopreservation.

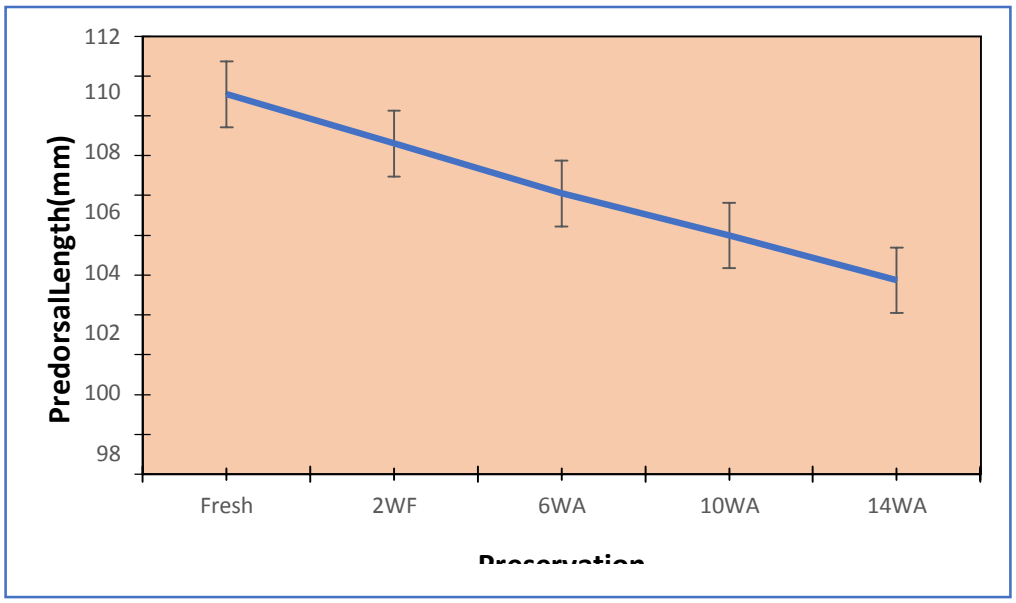

Fig 32: Mean (±SE) change in Pre-dorsal length (PDL) length of *S. esocinus* due topreservation.

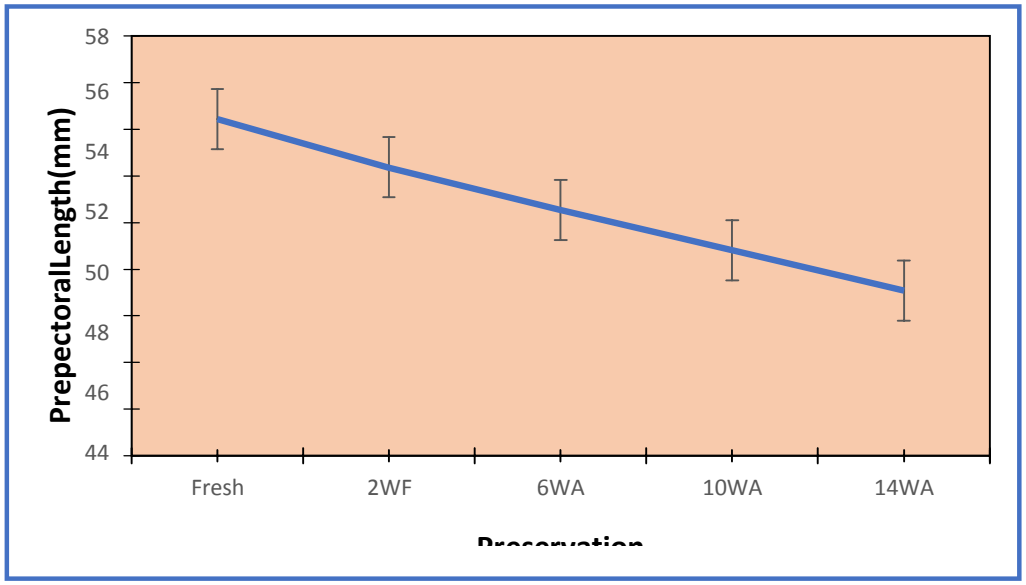

Fig 33: Mean (±SE) change in Pre-pectoral length (PPcL) length of *S. esocinus* due topreservation.

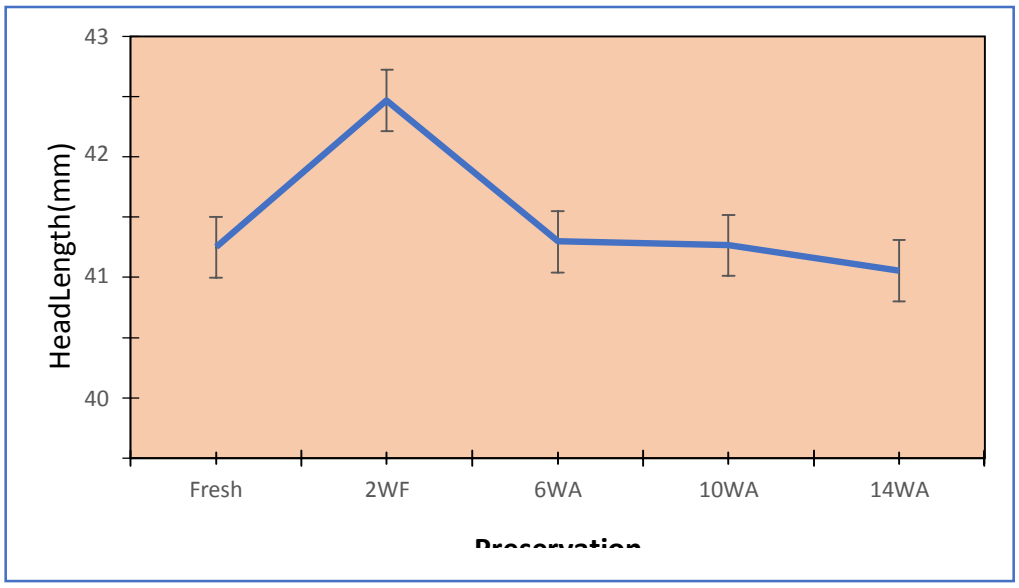

Fig34: Mean(±SE)changeinhead length(HL)of *S.esocinus*duetopreservation.

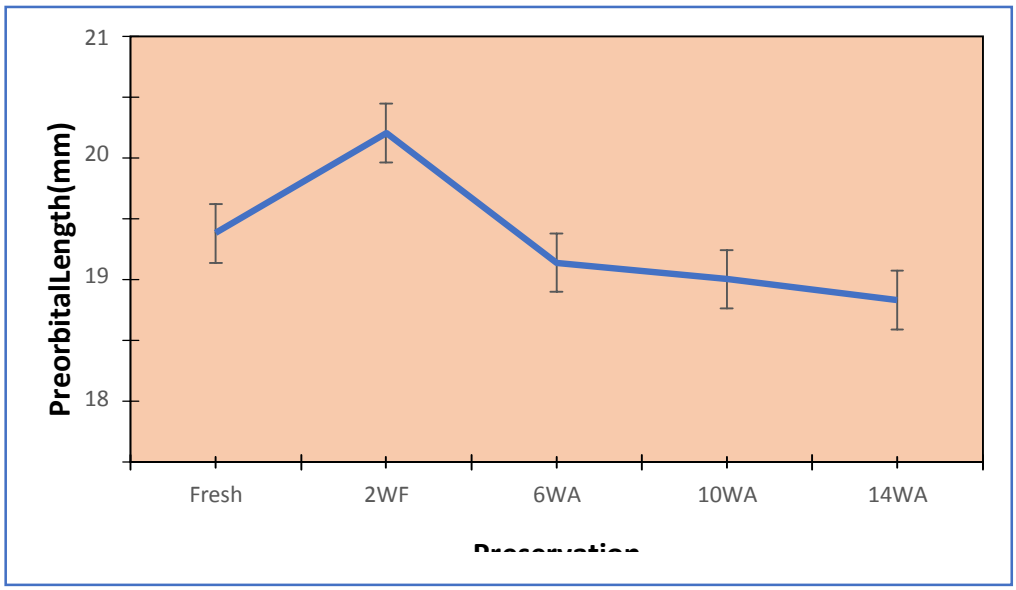

Fig35: Mean(±SD) changeinPre-orbitallength (Pool)of *S.esocinus*dueto preservation.

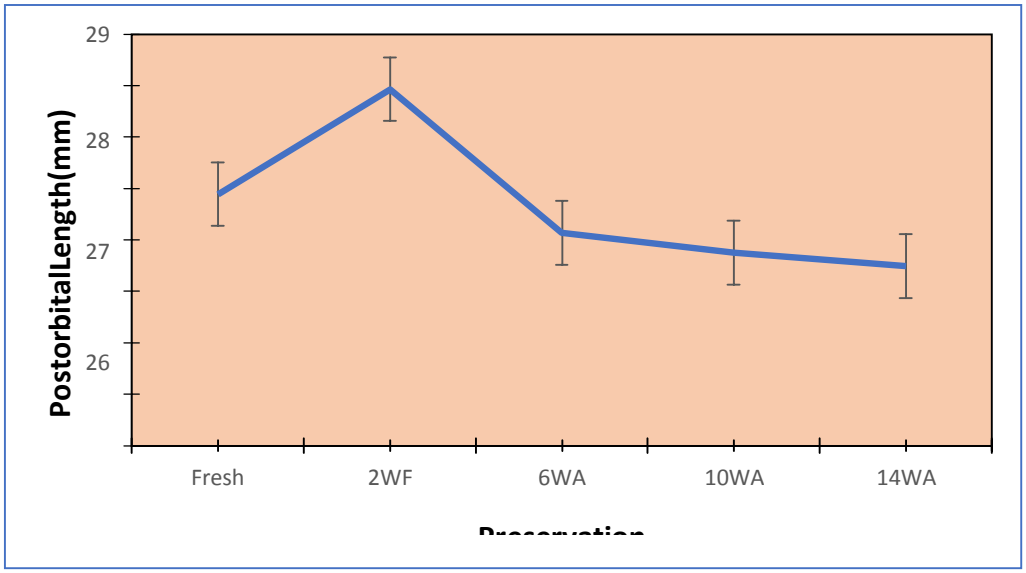

Fig 36: Mean (±SE) change in Post-orbital length (POoL) of *S. esocinus* due topreservation.

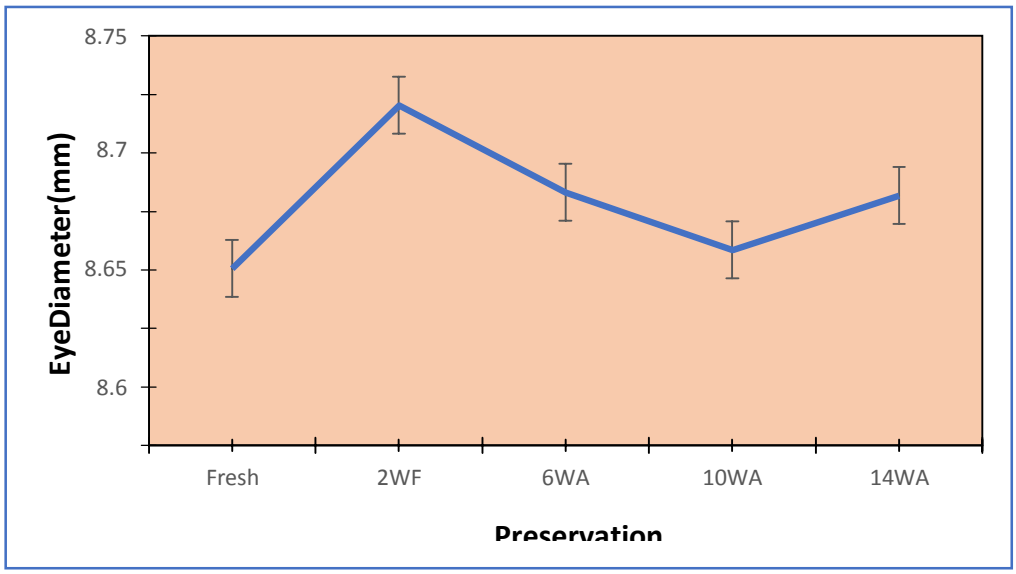

Fig37: Mean(±SE)changeinEyediameter(ED)of *S.esocinus*duetopreservation.

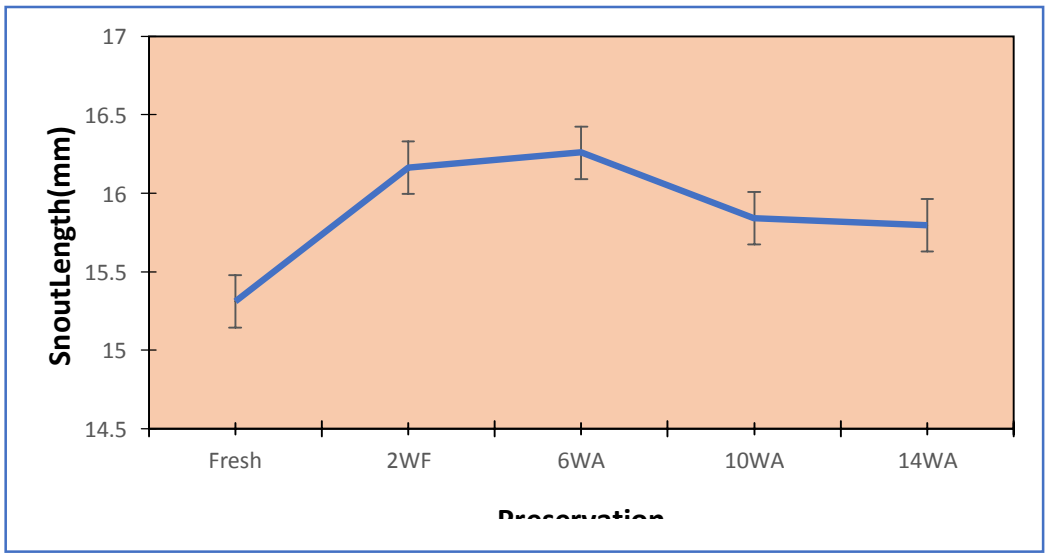

Fig38: Mean(±SE)changeinSnoutlength(SnL)of*S.esocinus* duetopreservation.

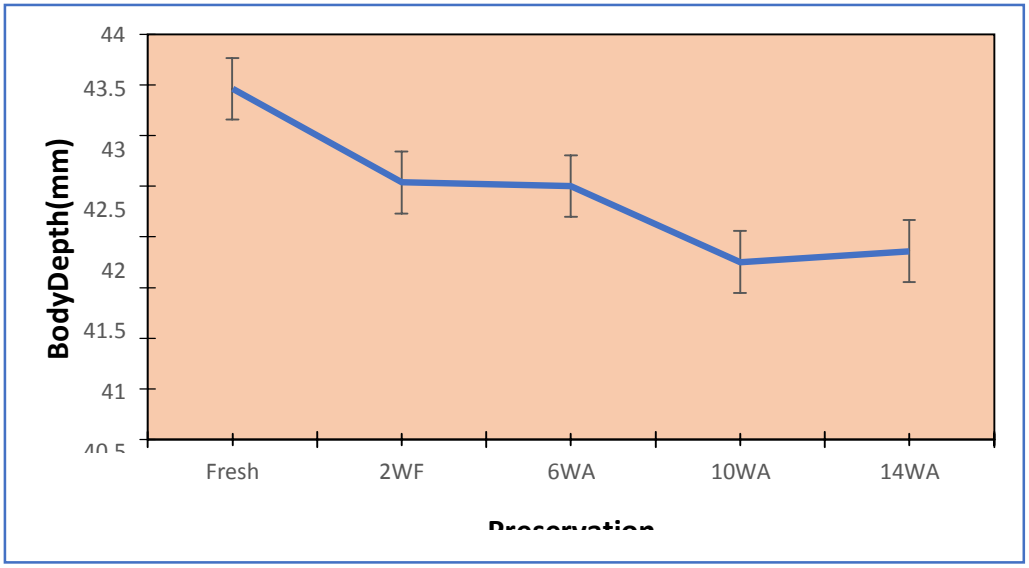

Fig39: Mean(±SE)changeinBodydepth(BD)of *S.esocinus*duetopreservation.

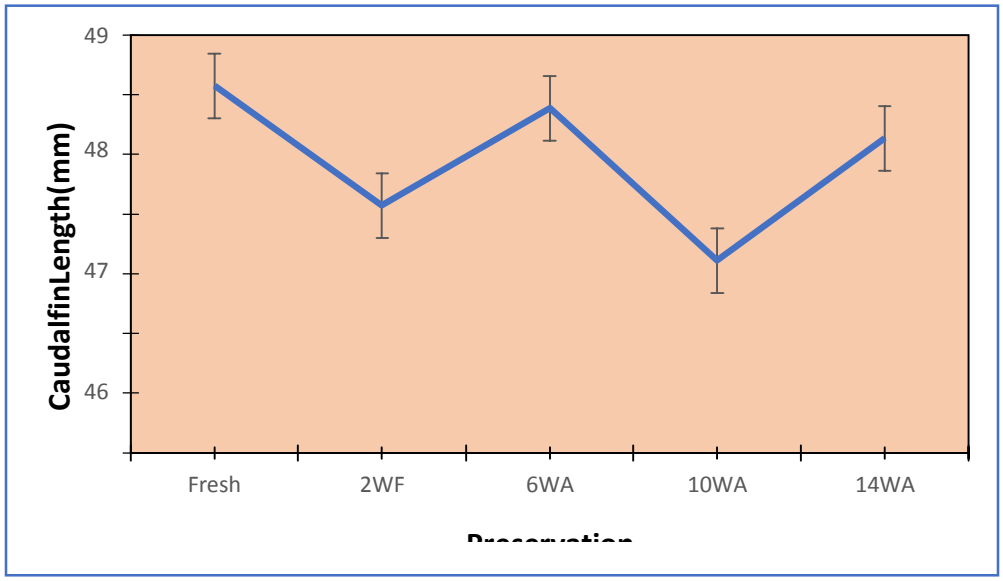

Fig40: Mean(±SE)changein Caudalfinlength (CFL)of*S.esocinus*duetopreservation.

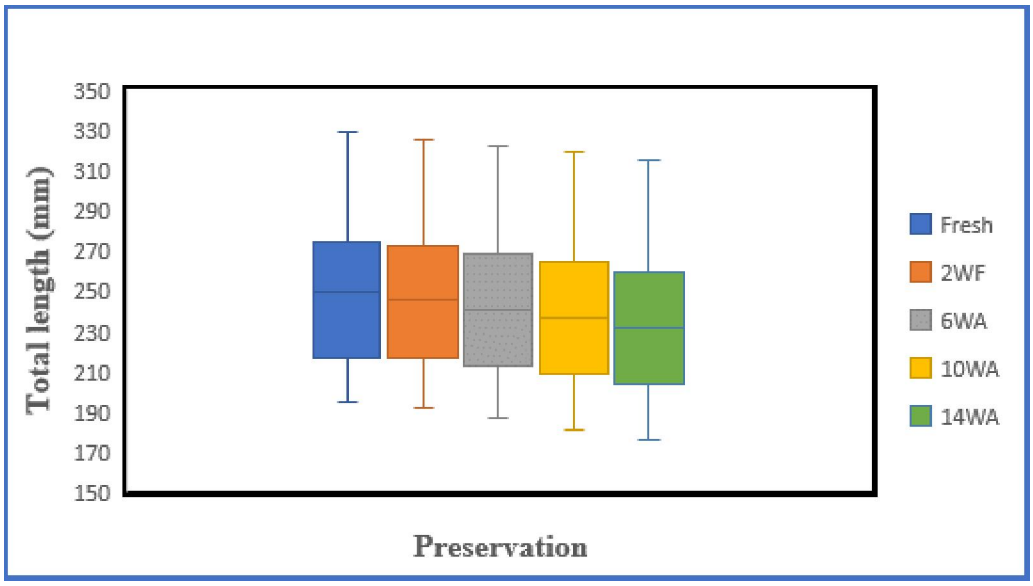

Fig41:BoxplotsofTotallength (TL) of*S.esocinus*(Fresh, 2WF, 6WA,10WA, and14WA).

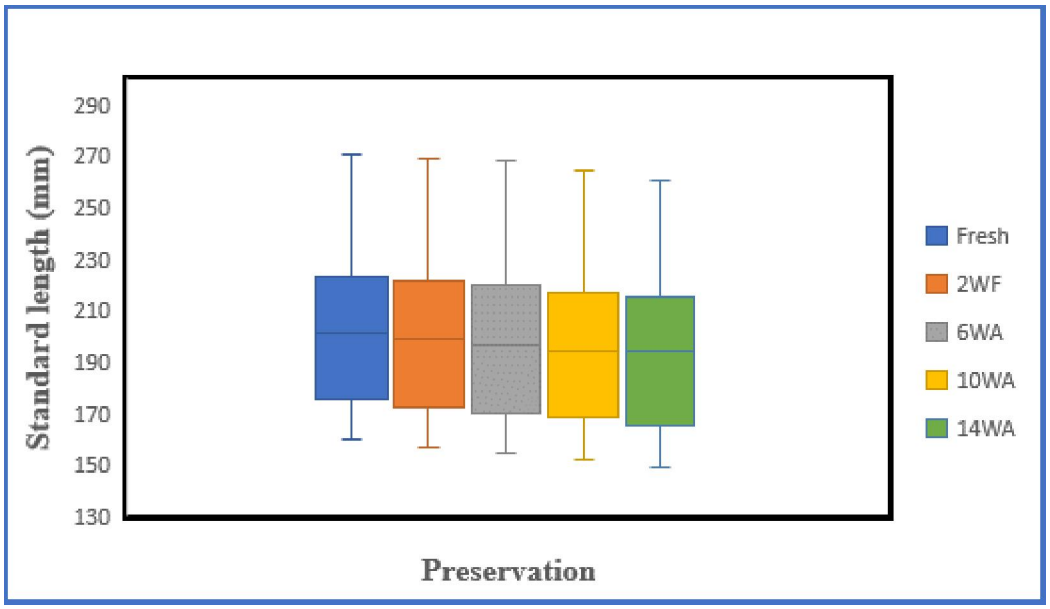

Fig42:BoxplotsofStandardlength(SL)of*S.esocinus* (Fresh,2WF,6WA,10WA,and14WA).

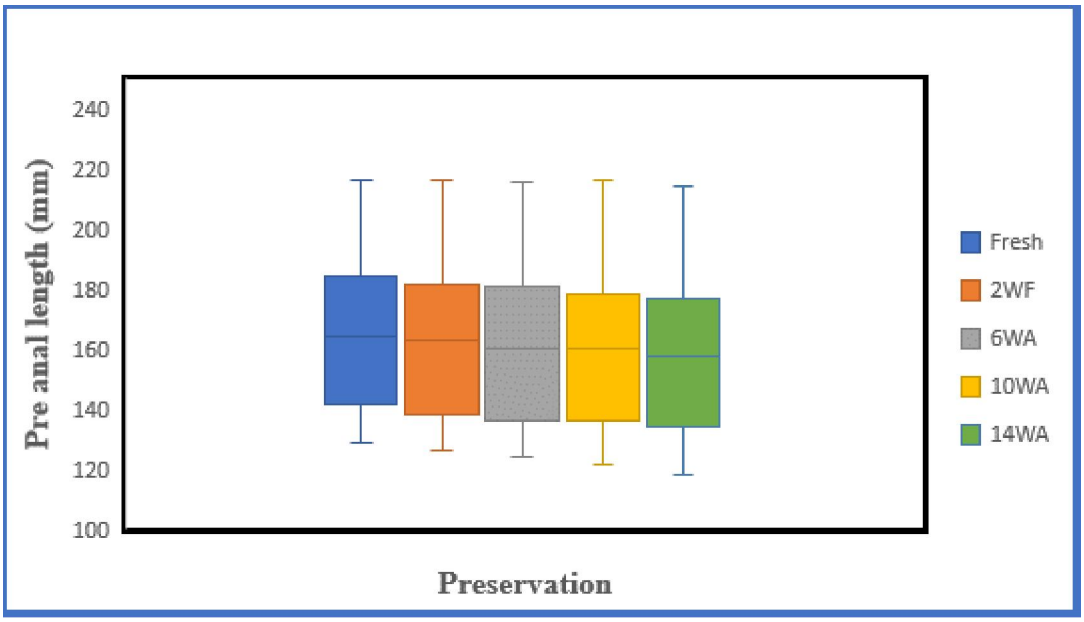

Fig 43:Boxplotsof Pre-anal length(PAL) of*S. esocinus*(Fresh, 2WF,6WA,10WAand14WA).

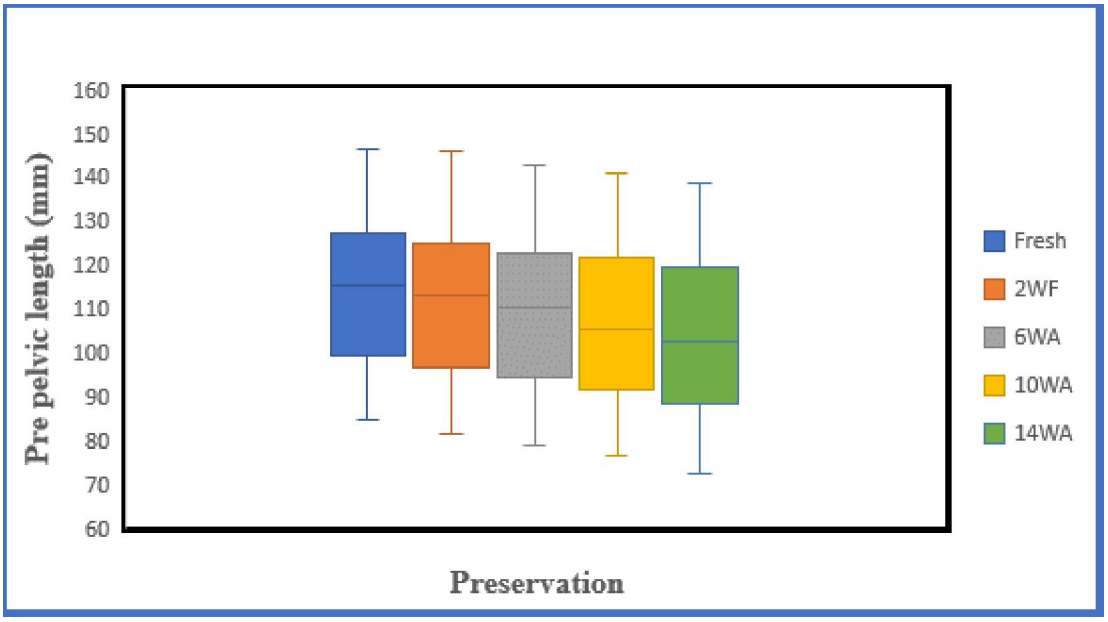

Fig44:BoxplotsofPre-pelviclength(PPvL)of*S.esocinus*(Fresh,2WF,6WA, 10WAand14WA).

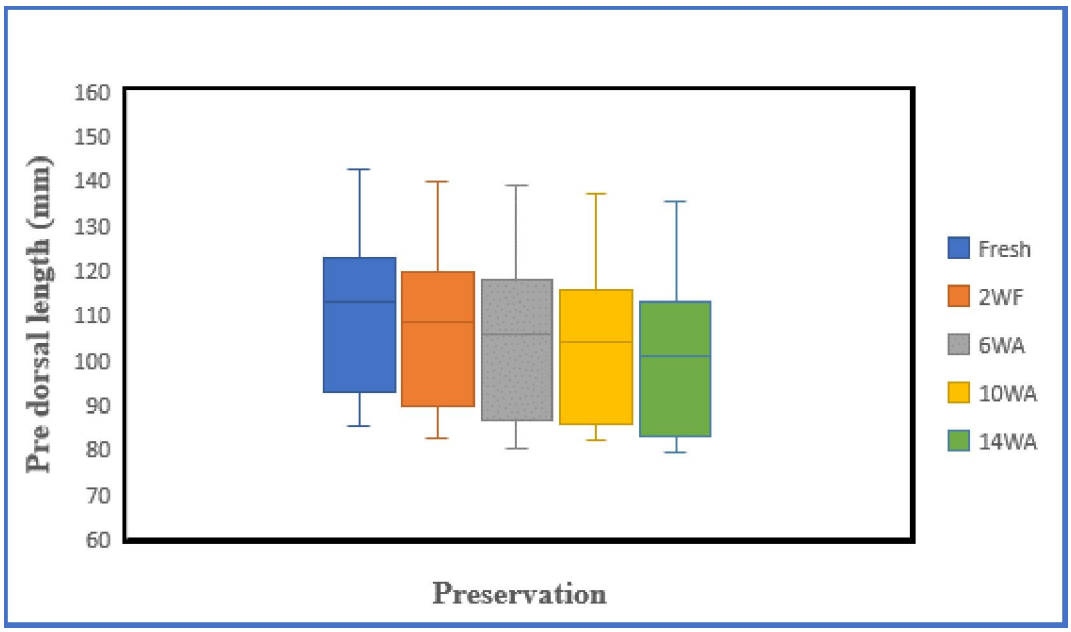

Fig45:BoxplotsofPre-dorsallength(PDL)of*S. esocinus* (Fresh,2WF,6WA, 10WAand14WA).

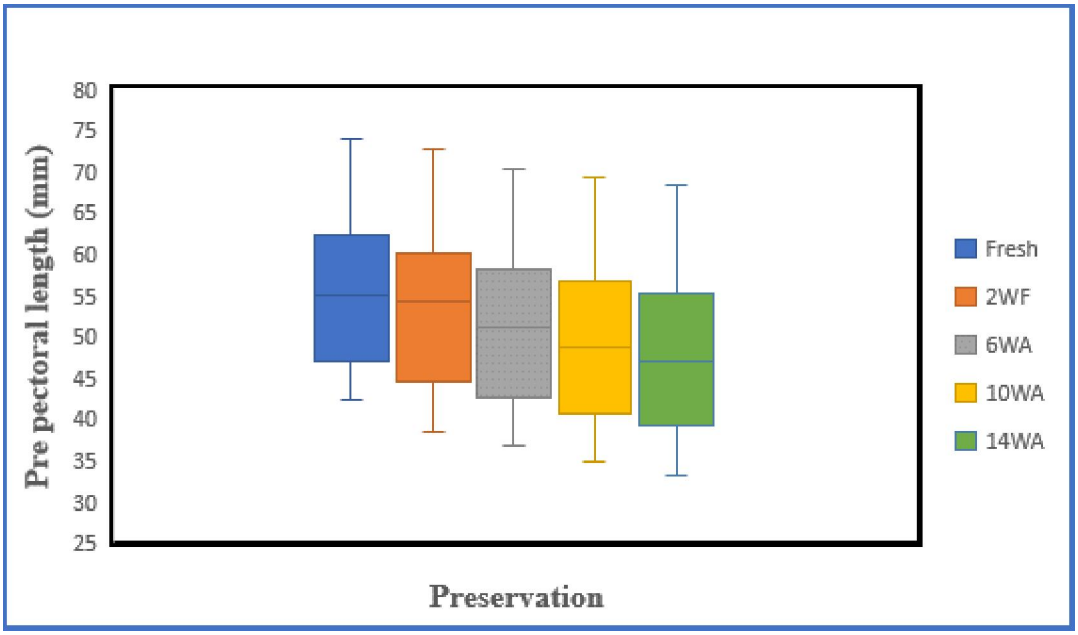

Fig 46: Box plots of Pre-pectoral length (PPcL) of *S. esocinus* (Fresh, 2WF, 6WA, 10WAand14WA).

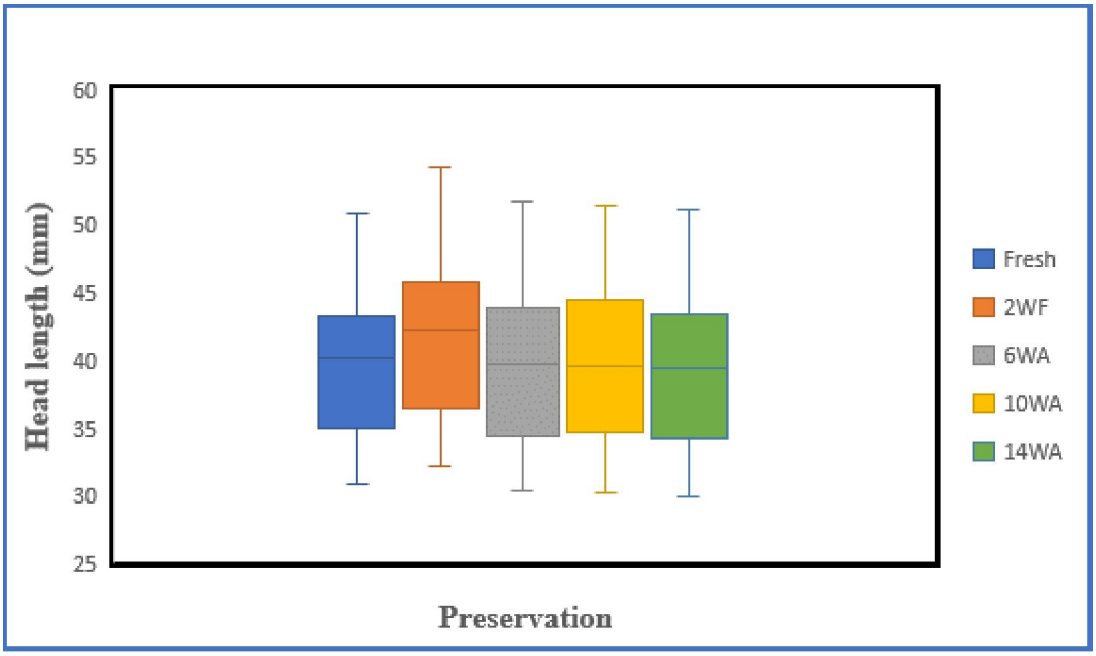

Fig47:Boxplotsofhead length(HL)of*S.esocinus* (Fresh,2WF,6WA,10WAand14WA).

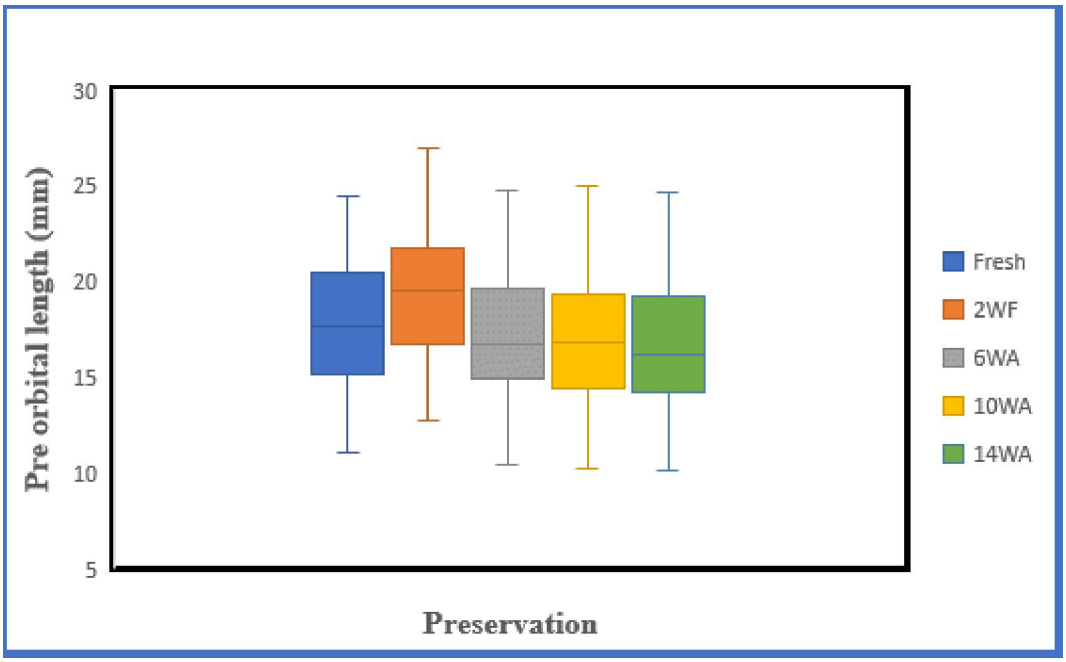

Fig48:BoxplotsofPre-orbitallength(PrOL)of *S.esocinus* (Fresh,2WF,6WA,10WAand 14WA).

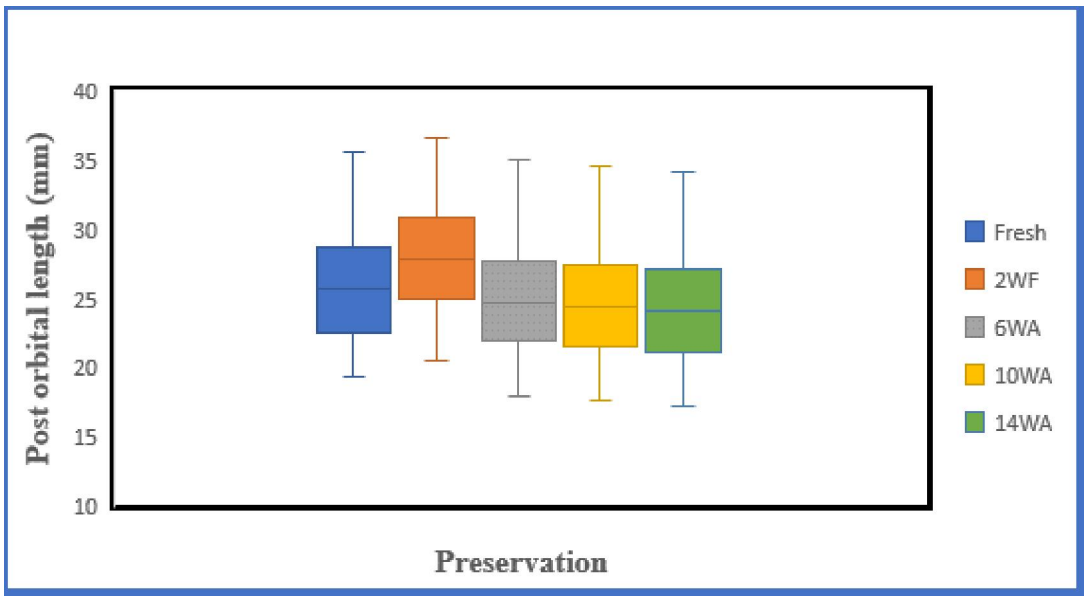

Fig49:BoxplotsofPost-orbitallength(POoL)of*S.esocinus* (Fresh,2WF,6WA,10WA and14WA).

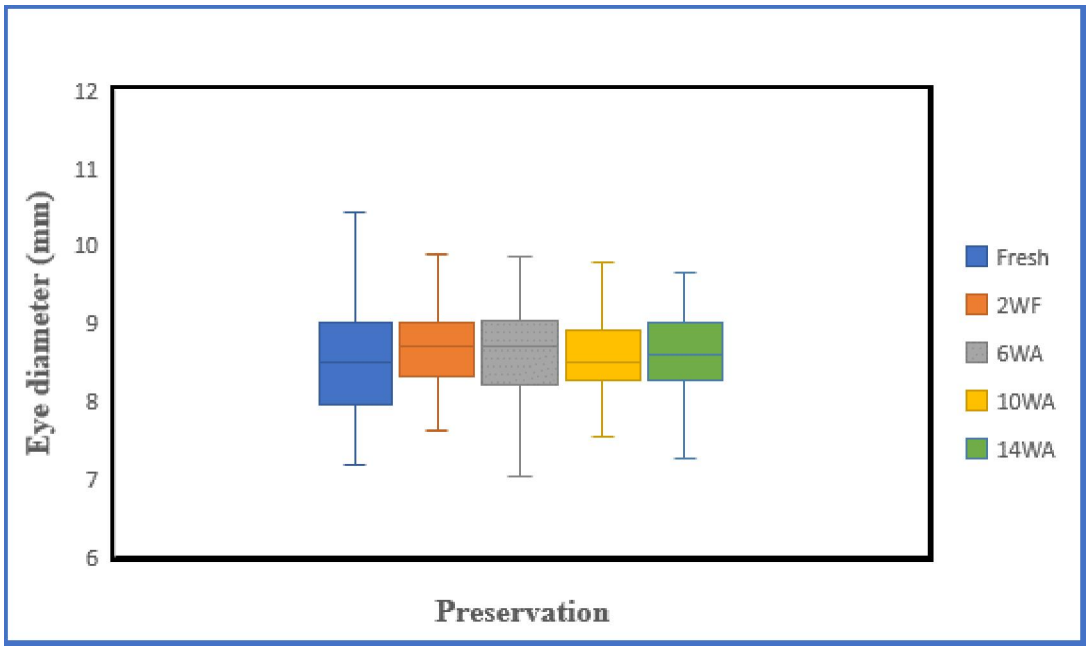

Fig50:BoxplotsofEyediameter(ED)of *S.esocinus* (Fresh, 2WF,6WA,10WAand 14WA).

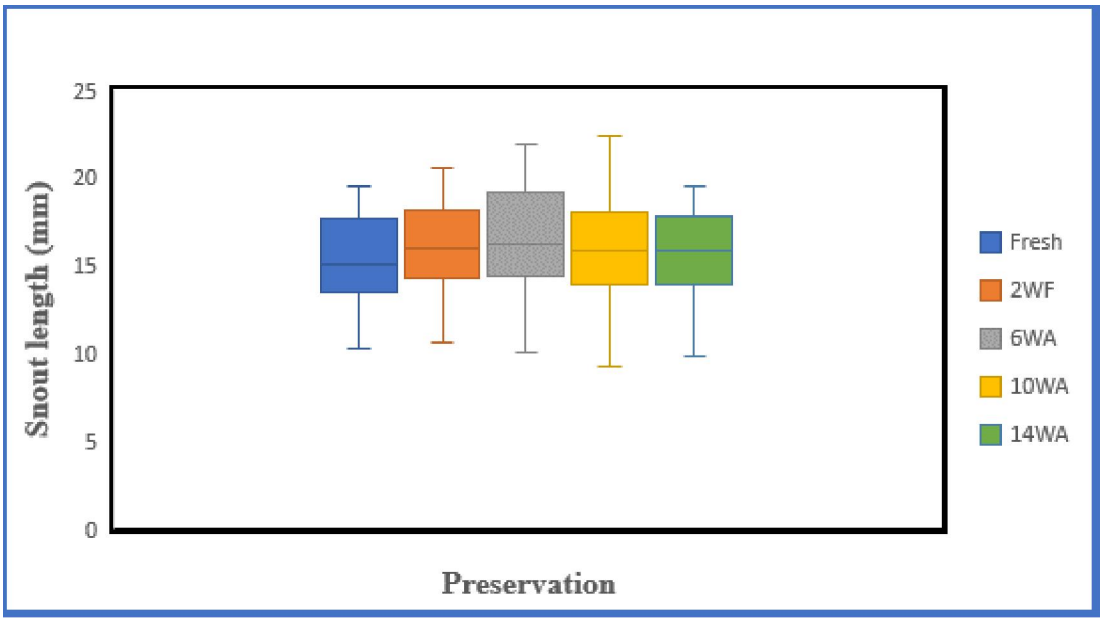

Fig51:BoxplotsofSnoutlength(SnL)of*S.esocinus* (Fresh,2WF,6WA,10WAand 14WA).

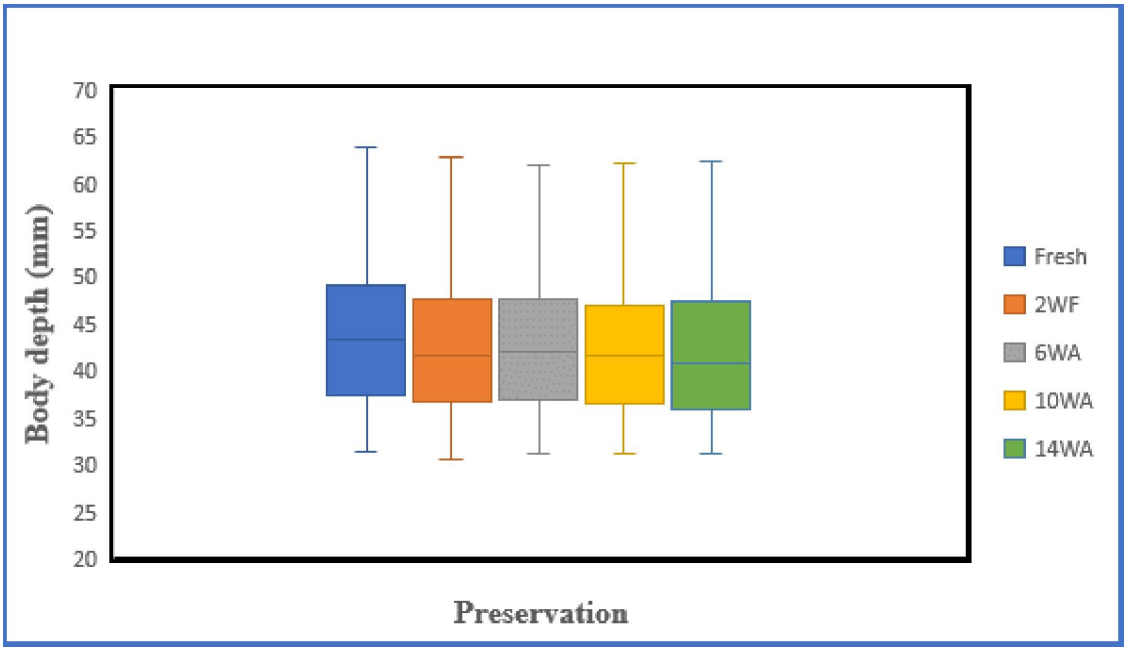

Fig52:BoxplotsofBodydepth (BD)of *S.esocinus* (Fresh,2WF,6WA,10WAand14WA).

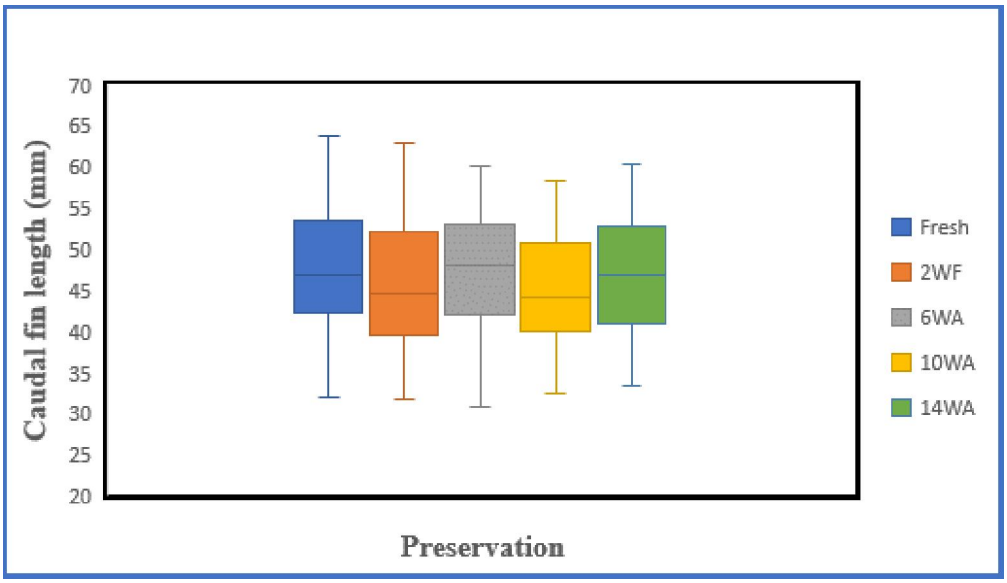

Fig53:BoxplotsofCaudalfin length(CFL)of *S.esocinus*Fresh,F,6WA,10WAand14WA).

4.1.2.2 Comparisons between morphometric traits of *S. esocinus.*

Table 15 shows comparison of morphometric traits between five measurements (Fresh, 2WF, 6WA, 10WA and 14WA) of*S. esocinus*.Out of thirteen morphometric characters, in three characters namely, Head length, pre-orbital length and post-orbital lengthsignificant difference was observed between Fresh and 2WF, and onwards upto 14WA ($p<0.05$). Pre dorsal and pre pectoral lengths displayed significant difference between Fresh and 10WA and onwards upto 14WA. In Characters like total length, standard length and pre-anal length significant difference was observed only between Fresh and 14WA ($p<0.05$) and non-significant difference in Fresh against 2WF, 6WA, 10WA. Non-significant difference was observed in characters of body depth, caudal fin length, eye diameter and snout length ($p>0.05$) for Fresh and all subsequent readings.

Parameters	Fresh	2WF	t-value	p-value	Fresh	6WA	t-value	p-value	Fresh	10WA	t-value	p-value	Fresh	14WA	t-value	p-value
TL vs TL	246.62±5.07	245.19±5.04	0.20	>0.05	246.62±5.07	240.95±4.97	0.79	>0.05	246.62±5.07	236.59±4.99	1.48	>0.05	246.62±5.07	32.15±5.00	2.03	**<0.05**
SL vs SL	200.41±4.14	197.75±4.18	0.45	>0.05	200.41±4.14	195.72±4.21	0.79	>0.05	200.41±4.14	193.83±4.18	1.11	>0.05	200.41±4.14	191.78±4.14	1.47	**<0.05**
PAL vs PAL	163.45±3.51	161.38±3.54	0.42	>0.05	163.45±3.51	159.63±3.54	0.78	>0.05	163.45±3.51	157.91±3.57	1.28	>0.05	163.45±3.51	155.79±3.59	1.55	**<0.05**
PPVL vs PPVL	113.07±2.43	110.65±2.51	0.70	>0.05	113.07±2.43	108.90±2.55	1.20	>0.05	113.07±2.43	106.49±2.59	1.88	>0.05	113.07±2.43	104.11±2.66	2.53	**<0.05**
PDL vs PDL	109.08±2.30	106.59±2.29	0.78	>0.05	109.08±2.30	104.08±2.31	1.56	>0.05	109.08±2.30	16.66±0.47	2.22	**<0.05**	109.08±2.30	99.74±2.34	2.90	**<0.05**
PPCL vs PPCL	54.43±1.22	52.36±1.28	1.93	>0.05	54.43±1.22	52.36±1.28	2.25	<0.05	54.43±1.22	48.81±1.29	3.23	**<0.05**	54.43±1.22	47.08±1.31	4.18	**<0.05**
HL vs HL	39.49±0.79	41.93±0.85	-2.13	**<0.05**	39.49±0.79	41.93±0.85	-0.08	**<0.05**	39.49±0.79	39.53±0.90	-0.03	**<0.05**	39.49±0.79	39.10±0.87	0.33	**<0.05**
PrOL vs PrOL	17.76±0.44	19.41±0.44	-2.67	**<0.05**	17.76±0.44	17.28±0.46	0.77	**<0.05**	17.76±0.44	17.01±0.47	1.19	**<0.05**	17.76±0.44	16.66±0.47	1.73	**<0.05**
PoOL vs PoOL	25.89±0.59	27.93±0.60	-2.47	**<0.05**	25.89±0.59	25.14±0.59	0.91	**<0.05**	25.89±0.59	24.75±0.58	1.39	**<0.05**	25.89±0.59	24.49±0.59	1.71	**<0.05**
SnL vs SnL	15.31±0.37	16.16±0.37	-1.64	>0.05	15.31±0.37	16.25±0.41	-1.71	>0.05	15.31±0.37	15.84±0.41	-0.97	>0.05	15.31±0.37	15.79±0.36	-0.94	>0.05
ED vs ED	8.55±0.11	8.69±0.25	-1.04	>0.05	8.55±0.11	8.61±0.09	-0.43	>0.05	8.55±0.11	8.56±0.07	-0.13	>0.05	8.55±0.11	8.61±0.08	-0.44	>0.05
BD vs BD	43.46±1.15	42.53±1.09	0.58	>0.05	43.46±1.15	42.50±1.09	0.60	>0.05	43.46±1.15	41.75±1.08	1.08	>0.05	43.46±1.15	41.86±1.15	0.98	>0.05
CFL vs CFL	48.14±1.07	46.14±1.20	1.24	>0.05	48.14±1.07	47.77±1.07	0.24	>0.05	48.14±1.07	45.22±0.97	2.01	>0.05	48.14±1.07	47.26±1.02	0.59	>0.05

4.1.2.3 Percentage change due to preservation in *S. esocinus.*

The percentage change between morphometric characters of *S.* esocinus for the Fresh and 2WF and Fresh and 14WA is given in Table 16. Out of thirteen morphometric characters, pre-orbital length displayed highest percentage change in formalin (9.25%) and total length displayed the lowest percentage change (-0,58%). Whereas in alcohol, pre-pectoral length showed highest percentage change (-13.50%) and eye diameter showed lowest percentage change (0.73%).

4.1.2.4 Conversion equations for formalin (2WF) and alcohol (14WA) preserved *S. esocinus.*

Table 17 represents the Least-squares regression equations of total length, standard length, pre- anal length, pre-pelvic length, pre-dorsal length, pre-pectoral length, head length, pre-orbital length, post-orbital length, eye diameter, snout length, body depth and caudal fin length of *S. esocinus* in formalin (2WF) and alcohol (14WA).

Table 16: percentage change of *S. esocinus* in formalin and alcohol

S. N0	Morphometric characters	Percentage change in formalin	Percentage change in alcohol
1	**Total Length (TL)**	-0.58%	-5.78
2	**Standard Length (SL)**	-1.33%	-4.30%
3	**Pre-Anal Length (PAL**	-1.27%	-4.69%
4	**Pre-Pelvic Length (PPvL)**	-2.14%	-7.92
5	**Pre-Dorsal Length (PDL)**	-2.28%	-8.56%
6	**Pre-Pectoral Length (PPcL)**	-3.79%	-13.50%
7	**Head Length (HL)**	6.17%	-0.98%
8	**Pre-Orbital Length (PrOL)**	9.25%	-6.19%
9	**Post-Orbital Length (PoOL)**	7.87%	-5.41%
10	**Snout Length (SNL)**	5.56%	3.16%
11	**Eye Diameter (ED)**	1.63%	0.73%
12	**Body Depth (BD)**	-2.13%	-3.69%
13	**Caudal Fin Length (CFL)**	-4.13%	-1.83%

Table 17: Conversion equations for formalin and alcohol preserved *S. esocinus.*

S. NO	Morphometric characters	For formalin (2WF)	For alcohol (14WA)
1	**Total Length (TL)**	$TL_{Fresh}=1.11+0.98(L_{FP})$	$TL_{Fresh}= 10.08+0.98(L_{AP})$
2	**Standard Length (SL)**	$SL_{Fresh}=3.11+1.00(L_{FP})$	$SL_{Fresh}=2.31+1.01(L_{AP})$
3	**Pre-Anal Length (PAL**	$PAL_{Fresh}=4.53+1.00(L_{FP})$	$PAL_{Fresh}=0.05+0.95(L_{AP})$
4	**Pre-Pelvic Length (PPvL)**	$PPvL_{Fresh}=5.55+1.02(L_{FP})$	$PPvL_{Fresh}=17.45+1.07(L_{AP})$
5	**Pre-Dorsal Length (PDL)**	$PDL_{Fresh}=1.58+0.99(L_{FP})$	$PDL_{Fresh}=9.39+1.00(L_{AP})$
6	**Pre-Pectoral Length (PpcL)**	$PPcL_{Fresh}=4.14+1.03(L_{FP})$	$PPcL_{Fresh}=10.09+1.05(L_{AP})$
7	**Head Length (HL)**	$HL_{Fresh}=0.49+1.04(L_{FP})$	$HL_{Fresh}=2.06+1.04(L_{AP})$
8	**Pre-Orbital Length (PrOL)**	$PrOL_{Fresh}=1.76+0.99(L_{FP})$	$PrOL_{Fresh}=1.28+1.01(L_{AP})$
9	**Post-Orbital Length (PoOL)**	$PoOL_{Fresh}=2.27+0.99(L_{FP})$	$PoOL_{Fresh}=0.64+0.97(L_{AP})$
10	**Snout Length (SnL)**	$SnL_{Fresh}=3.46=0.82(L_{FP})$	$SNL_{Fresh}=3.80+0.78(L_{AP})$
11	**Eye Diameter (ED)**	$ED_{Fresh}=6.09+0.30(L_{FP})$	$ED_{Fresh}=6.30+0.26(L_{AP})$
12	**Body Depth (BD)**	$BD_{Fresh}=5.42+0.85(L_{FP})$	$BD_{Fresh}=4.80+0.85(L_{AP})$

4.2 Truss morphometry

4.2.1 Truss morphometry of *S. niger*

The effects of preservation on*S. niger* showed different degrees of effects on the thirty truss distances for all preservation times examined.There was some shrinkage but also corresponding enlargementbetween landmarks. Trends in shape change progressing from fresh to 2WF displayed adecrease inall the 30 truss distances, then a dramatic increase in alcohol (6WA) followed by decrease after 10WA and an increase after 14WA. However, the proportion by which every truss dimension changed due to preservation in formalin and alcohol was different.Comparison of truss dimensions between five measurements (Fresh, 2WF, 6WA,10WA and 14WA) of*S. niger*is presented in Table 18. All the thirty truss dimensions varied significantly (<0.01) between Fresh and 2WF, Fresh and 6WA, Fresh and 10WA and Fresh and 14WA. The box plots of 30 truss dimensions obtained from 12 landmarks of *S. niger* (Fresh, 2WF, 6WA,10WA and 14WA) are presented in figures 54 -83.

Table 18: Comparison between the Truss dimensions of*S. niger* for Fresh and 2WF, Fresh and 6WA, Fresh and 10WA and Fresh and 14WA.

S. No	Dimension	Fresh	2WF	P-value	Fresh	6WA	P-value	Fresh	10WA	P-value	Fresh	14WA	P-value
1	**1-2**	3.91±0.05	3.06±0.05	**<0.01**	3.91±0.05	7.73±0.09	**<0.01**	3.91±0.05	6.59±0.07	**<0.01**	3.91±0.05	7.33±0.07	**<0.01**
2	**1-11**	4.46±0.07	3.26±0.05	**<0.01**	4.46±0.07	8.68±0.11	**<0.01**	4.46±0.07	7.97±0.1	**<0.01**	4.46±0.07	8.56±0.1	**<0.01**
3	**1-12**	4.55±0.07	3.5±0.17	**<0.01**	4.55±0.07	8.77±0.12	**<0.01**	4.55±0.07	7.91±0.1	**<0.01**	4.55±0.07	8.64±0.1	**<0.01**
4	**2-3**	6.61±0.09	5.1±0.08	**<0.01**	6.61±0.09	13.56±0.18	**<0.01**	6.61±0.09	12.17±0.11	**<0.01**	6.61±0.09	13.02±0.13	**<0.01**
5	**2-10**	7.5±0.11	5.7±0.13	**<0.01**	7.5±0.11	15.88±0.22	**<0.01**	7.5±0.11	12.32±0.12	**<0.01**	7.5±0.11	15.37±0.14	**<0.01**
6	**2-11**	2.13±0.05	1.42±0.04	**<0.01**	2.13±0.05	4.6±0.09	**<0.01**	2.13±0.05	4.49±0.06	**<0.01**	2.13±0.05	4.58±0.07	**<0.01**
7	**2-12**	1.53±0.05	0.91±0.03	**<0.01**	1.53±0.05	3.03±0.07	**<0.01**	1.53±0.05	3.14±0.05	**<0.01**	1.53±0.05	3.2±0.06	**<0.01**
8	**3-4**	2.17±0.03	2.1±0.04	**<0.01**	2.17±0.03	5.23±0.1	**<0.01**	2.17±0.03	4.29±0.06	**<0.01**	2.17±0.03	4.85±0.07	**<0.01**
9	**3-9**	6.14±0.08	4.81±0.1	**<0.01**	6.14±0.08	12.93±0.1	**<0.01**	6.14±0.08	11.25±0.1	**<0.01**	6.14±0.08	12.36±0.12	**<0.01**
10	**3-10**	3.63±0.06	2.62±0.08	**<0.01**	3.63±0.06	7.83±0.14	**<0.01**	3.63±0.0	7.15±0.11	**<0.01**	3.63±0.06	7.47±0.11	**<0.01**

Data are mean ± SE (n=45 for Fresh, 2WF, 6WA, 10WA and 14WA. The p-value with p < 0.01are significantly different

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Dimension	Fresh	2WF	P-value	Fresh	6WA	P-value	Fresh	10WA	P-value	Fresh	14WA	P-value
3-11	6.22±0.09	4.96±0.09	**<0.01**	6.22±0.09	13.27±0.19	**<0.01**	6.22±0.09	11.63±0.1	**<0.01**	6.22±0.09	12.49±0.13	**<0.01**
4-5	6.4±0.1	4.44±0.13	**<0.01**	6.4±0.1	12.29±0.21	**<0.01**	6.4±0.1	10.9±0.13	**<0.01**	6.4±0.1	11.51±0.14	**<0.01**
4-7	7.04±0.11	4.94±0.12	**<0.01**	7.04±0.11	13.46±0.2	**<0.01**	7.04±0.11	11.85±0.12	**<0.01**	7.04±0.11	12.63±0.14	**<0.01**
4-8	4.97±0.07	3.5±0.07	**<0.01**	4.97±0.07	9.48±0.13	**<0.01**	4.97±0.07	8.4±0.09	**<0.01**	4.97±0.07	9.1±0.1	**<0.01**
4-9	4.21±0.06	3.04±0.06	**<0.01**	4.21±0.06	8.31±0.12	**<0.01**	4.21±0.06	7.42±0.08	**<0.01**	4.21±0.06	8.05±0.09	**<0.01**
4-10	3.77±0.06	2.89±0.05	**<0.01**	3.77±0.06	8.2±0.14	**<0.01**	3.77±0.06	7.2±0.09	**<0.01**	3.77±0.06	7.66±0.1	**<0.01**
4-12	7.88±0.11	6.66±0.13	**<0.01**	7.88±0.11	17.29±0.24	**<0.01**	7.88±0.11	14.8±0.12	**<0.01**	7.88±0.11	16.15±0.17	**<0.01**
5-6	1.22±0.02	0.83±0.02	**<0.01**	1.22±0.02	2.38±0.04	**<0.01**	1.22±0.02	2.09±0.03	**<0.01**	1.22±0.02	2.26±0.04	**<0.01**
5-7	1.75±0.03	1.33±0.04	**<0.01**	1.75±0.03	3.65±0.05	**<0.01**	1.75±0.03	3.22±0.03	**<0.01**	1.75±0.03	3.45±0.08	**<0.01**

Data are mean ± SE (n=45 for Fresh, 2WF, 6WA, 10WA and 14WA. The p-value with p < 0.01are significantly different

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S. No	Dimension	Fresh	2WF	P-value	Fresh	6WA	P-value	Fresh	10WA	P-value	Fresh	14WA	P-value
20	**5-8**	2.86±0.05	2.04±0.06	**<0.01**	2.86±0.05	5.63±0.11	**<0.01**	2.86±0.05	5.01±0.06	**<0.01**	2.86±0.05	5.24±0.1	**<0.01**
21	**5-9**	4.11±0.06	2.96±0.06	**<0.01**	4.11±0.06	8.24±0.14	**<0.01**	4.11±0.06	7.28±0.08	**<0.01**	4.11±0.06	7.58±0.03	**<0.01**
22	**6-7**	1.06±0.02	0.84±0.26	**<0.01**	1.06±0.02	2.3±0.03	**<0.01**	1.06±0.02	1.97±0.03	**<0.01**	1.06±0.02	2.13±0.08	**<0.01**
23	**6-8**	3.17±0.06	2.24±0.06	**<0.01**	3.17±0.06	6.14±0.12	**<0.01**	3.17±0.06	5.35±0.08	**<0.01**	3.17±0.06	5.62±0.07	**<0.01**
24	**7-8**	2.39±0.05	1.66±0.05	**<0.01**	2.39±0.05	4.52±0.11	**<0.01**	2.39±0.05	3.92±0.07	**<0.01**	2.39±0.05	4.07±0.04	**<0.01**
25	**8-9**	1.35±0.03	1.02±0.03	**<0.01**	1.35±0.03	2.7±0.05	**<0.01**	1.35±0.03	2.35±0.04	**<0.01**	1.35±0.03	2.44±0.14	**<0.01**
26	**9-10**	5.01±0.08	4.02±0.11	**<0.01**	5.01±0.08	10.35±0.16	**<0.01**	5.01±0.08	8.87±0.12	**<0.01**	5.01±0.08	9.7±0.22	**<0.01**
27	**9-12**	10.96±0.15	8.81±0.21	**<0.01**	10.96±0.15	23.23±0.31	**<0.01**	10.96±0.15	20.05±0.17	**<0.01**	10.96±0.15	21.9±0.14	**<0.01**
28	**10-11**	6.07±0.09	4.91±0.12	**<0.01**	6.07±0.09	13.19±0.2	**<0.01**	6.07±0.09	11.43±0.11	**<0.01**	6.07±0.09	12.51±0.14	**<0.01**
29	**10-12**	6.18±0.09	5.14±0.1	**<0.01**	6.18±0.09	13.44±0.2	**<0.01**	6.18±0.09	11.7±0.11	**<0.01**	6.18±0.09	12.7±0.13	**<0.01**
30	**11-12**	0.77±0.02	0.61±0.02	**<0.01**	0.77±0.02	1.86±0.05	**<0.01**	0.77±0.02	1.62±0.03	**<0.01**	0.77±0.02	1.7±0.04	**<0.01**

Data are mean ± SE (n=45 for Fresh, 2WF, 6WA, 10WA and 14WA. The p-value with p < 0.01are significantly different

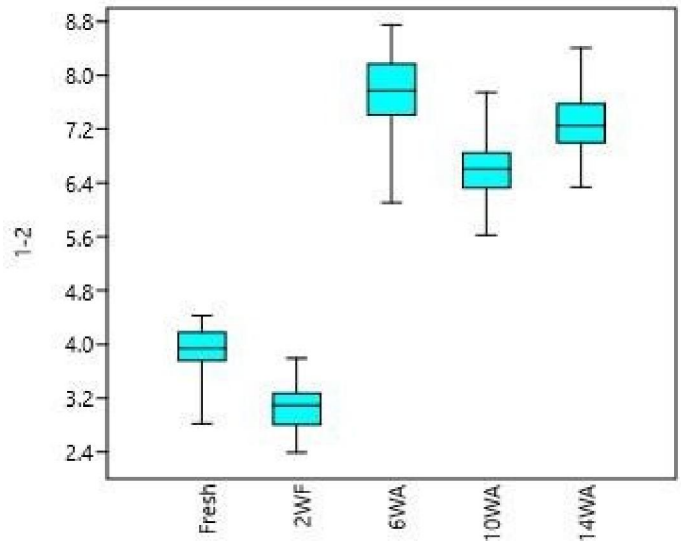

Fig54.Boxplots of trussdimension 1-2(Tipofsnout tofrontalbone end)for*S.niger* (Fresh,2WF,6WA,10WA,14WA).

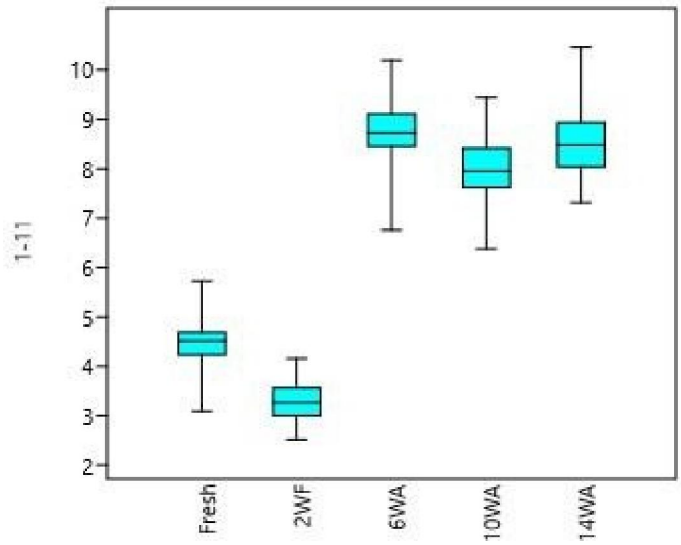

Fig 55. Box plots of truss dimension 1-11 (Tip of snout to pectoral fin origin) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

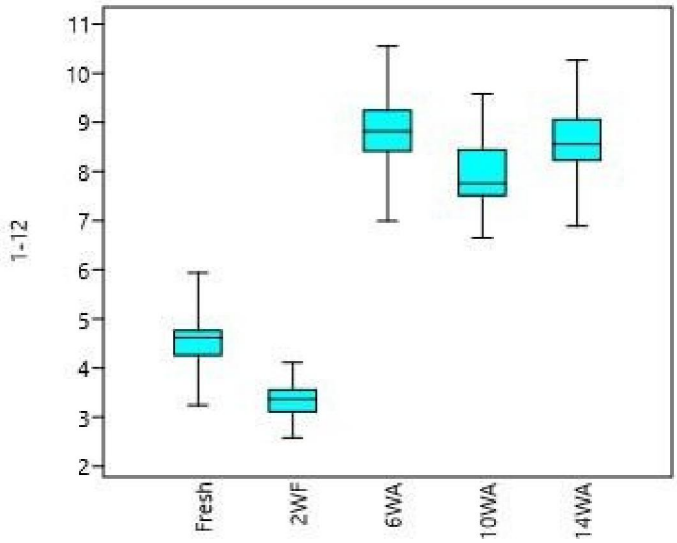

Fig 56: Box plots of truss dimension 1-12 (Tip of snout to opercular posterior edge) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

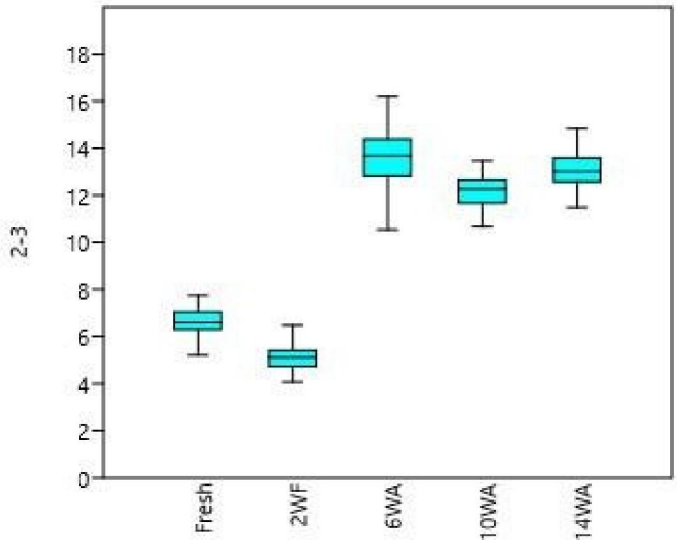

Fig 57: Box plots of truss dimension 2-3 (Frontal bone end to dorsal fin origin) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

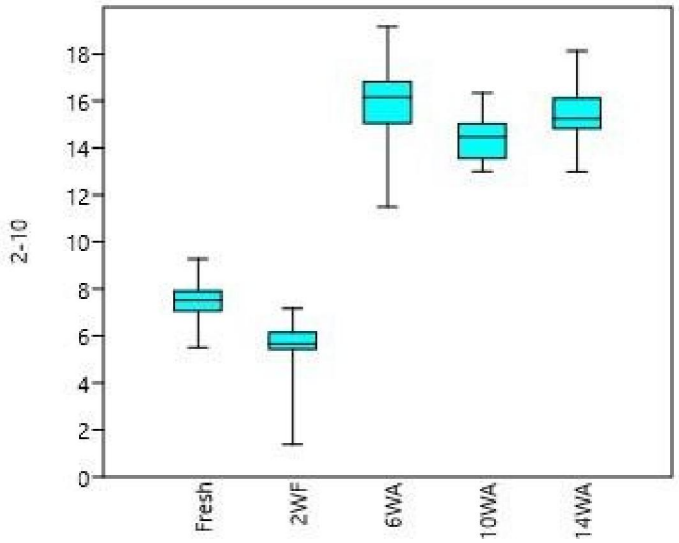

Fig 58: Box plots of truss dimension 2-10 (Frontal bone end to pelvic fin origin) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

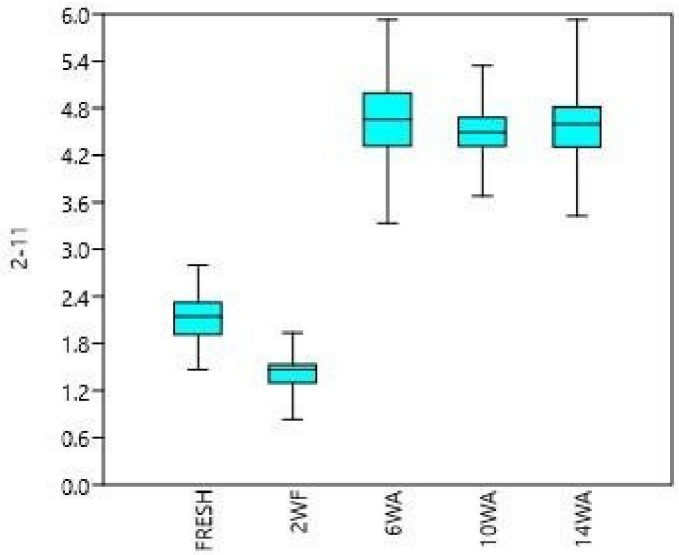

Fig59:Boxplotsof trussdimension 2-11 (Frontal bone end topectoralfin origin)for*S. niger* (Fresh,2WF,6WA,10WA,14WA).

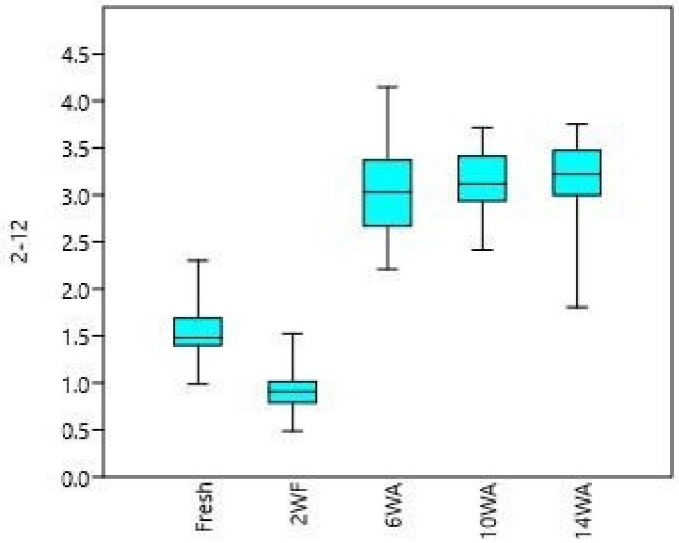

Fig 60: Box plots of truss dimension 2-12 (Frontal bone end to opercular posterior edge)for*S. niger*(Fresh, 2WF,6WA, 10WA, 14WA).

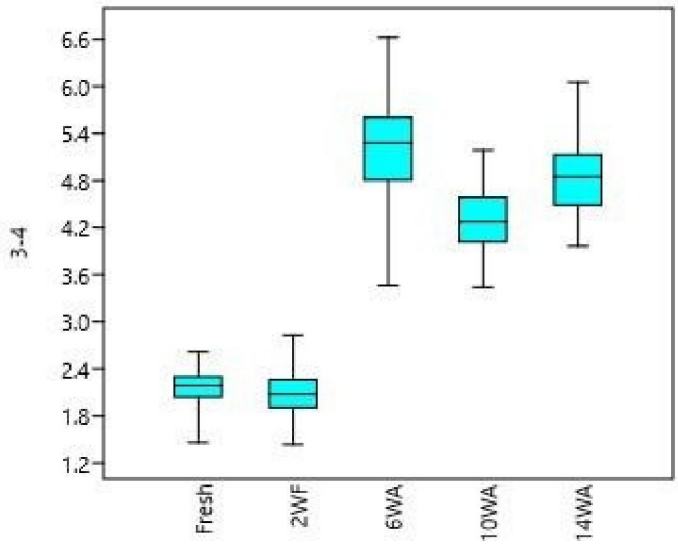

Fig 61:Boxplotsoftrussdimension 3-4(Dorsal finorigin todorsalfinend)for*S.niger* (Fresh,2WF,6WA,10WA,14WA).

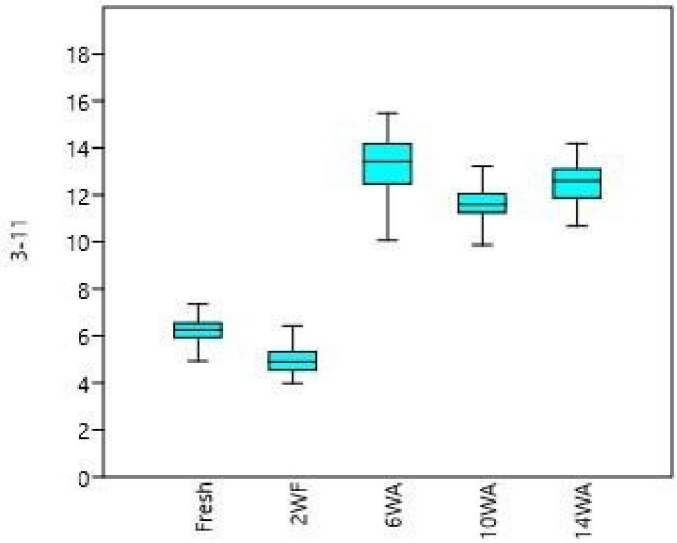

Fig 62: Box plots of truss dimension 3-11 (Dorsal fin origin to pectoral fin (ventralorigin)for*S. niger*(Fresh,2WF, 6WA, 10WA, 14WA).

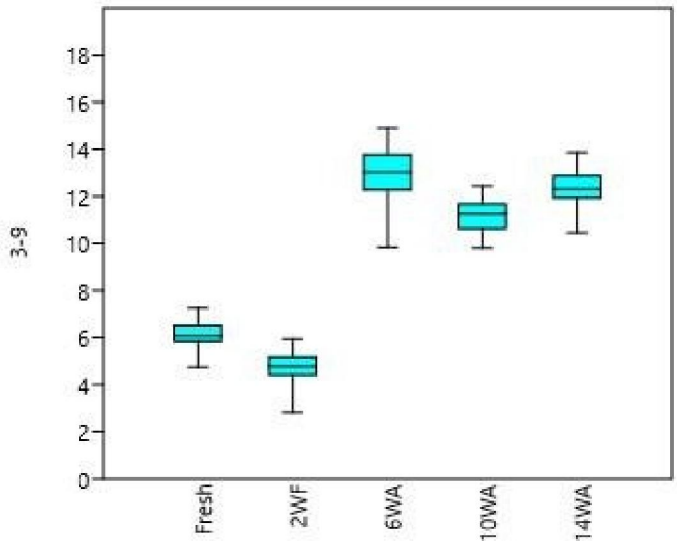

Fig 63: Box plots of truss dimension 3-9 (Dorsal fin origin to anal fin origin) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

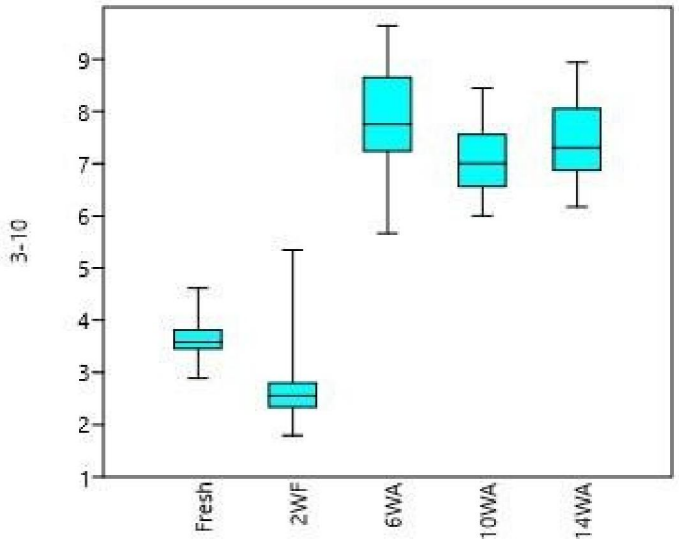

Fig 64: Box plots of truss dimension 3-10 (Dorsal fin origin to pelvic fin origin) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

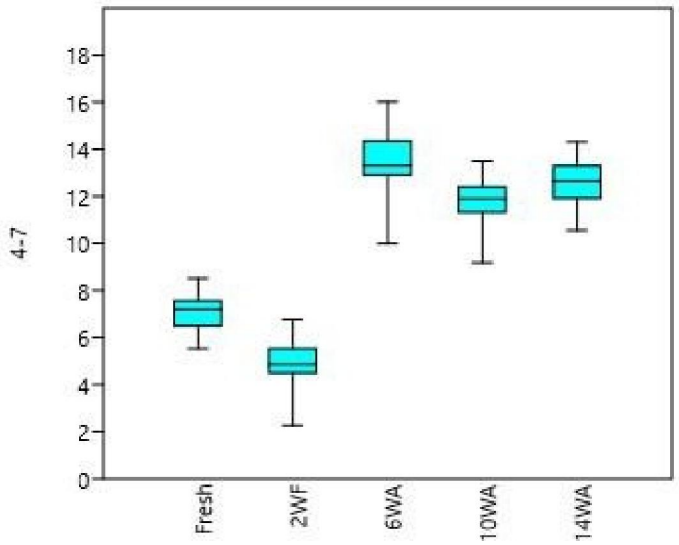

Fig 65: Box plots of truss dimension 4-7 (Dorsal fin end to caudal peduncle ventralborder)for*S. niger*(Fresh, 2WF, 6WA, 10WA,14WA).

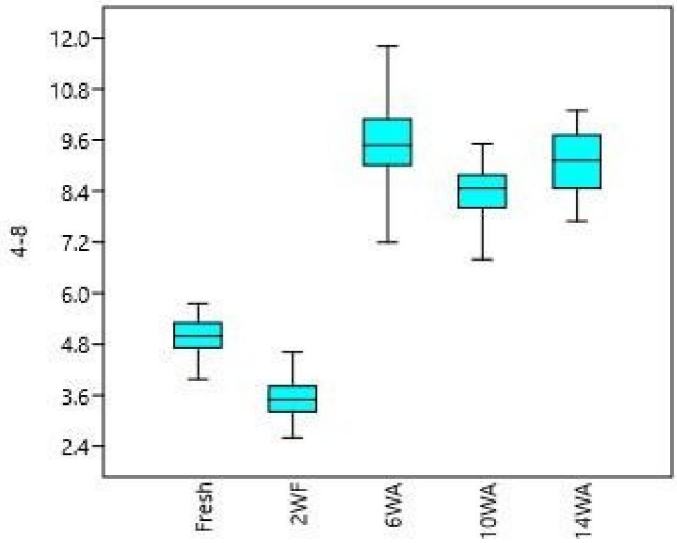

Fig 66:Boxplotsoftrussdimension4-8(Dorsal finendtoanalfinend)for*S.niger* (Fresh,2WF,6WA,10WA,14WA).

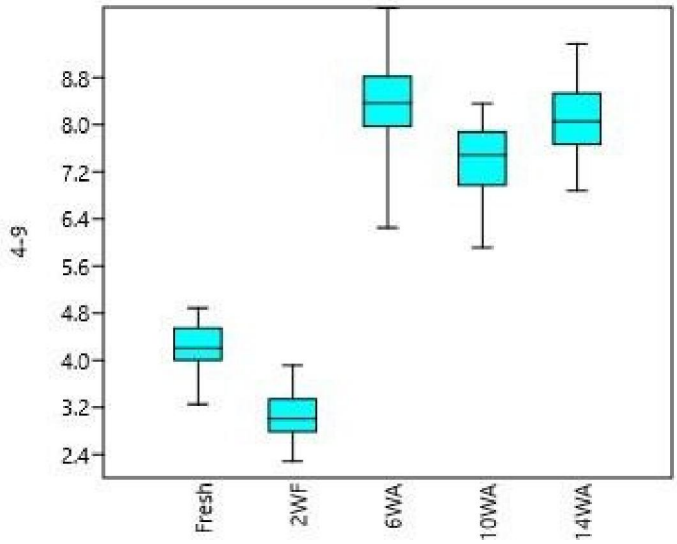

Fig 67:Boxplotsoftrussdimension 4-9(Dorsalfinorigin toanalfinorigin)for*S. niger* (Fresh,2WF,6WA,10WA,14WA).

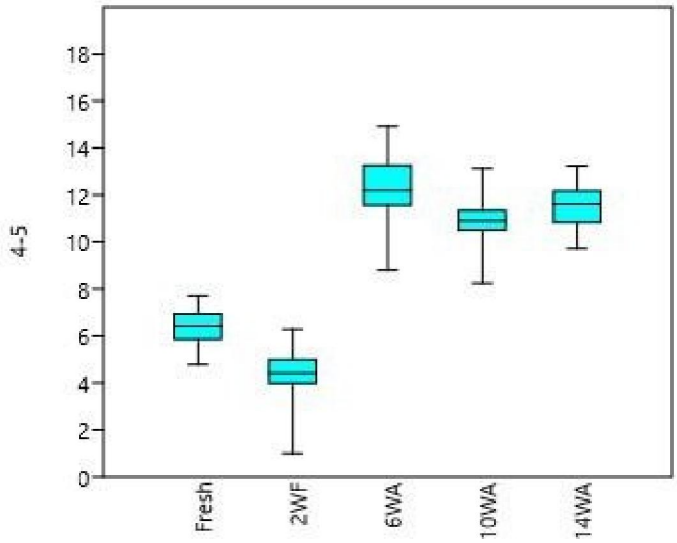

Fig 68: Box plots of truss dimension 4-5 (Dorsal fin end to caudal peduncle dorsalborder)for*S. niger*(Fresh, 2WF, 6WA, 10WA,14WA).

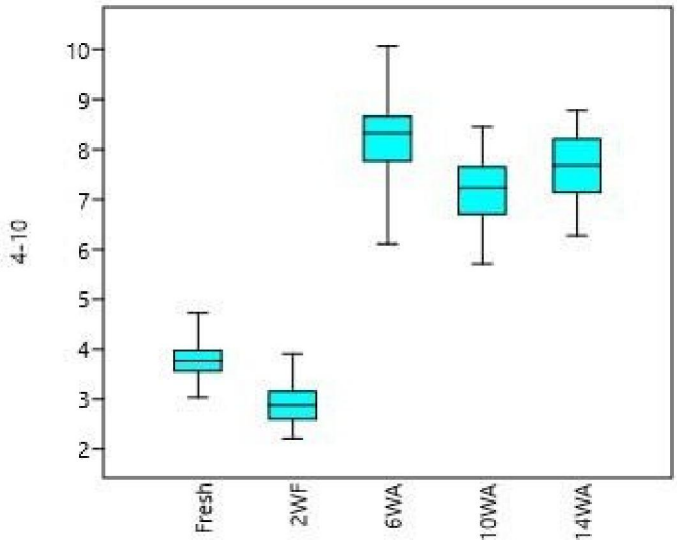

Fig 69: Box plots of truss dimension 4-10 (Dorsal fin end to pelvic fin origin) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

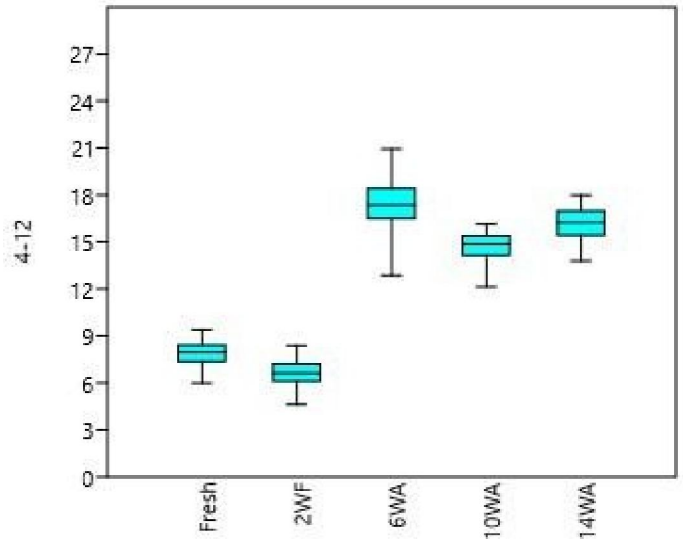

Fig70:Boxplotsoftrussdimension4-12 (Dorsalfin endto opercular posterioredge)for *S.niger*(Fresh,2WF,6WA,10WA,14WA).

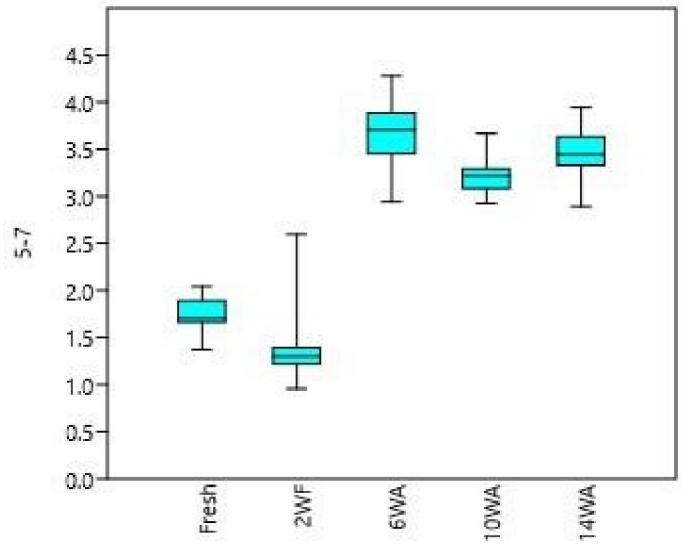

Fig 71: Box plots of truss dimension 5-7 (Caudal peduncle dorsal border to Caudalpeduncleventralborder)for*S.niger* (Fresh,2WF,6WA, 10WA,14WA).

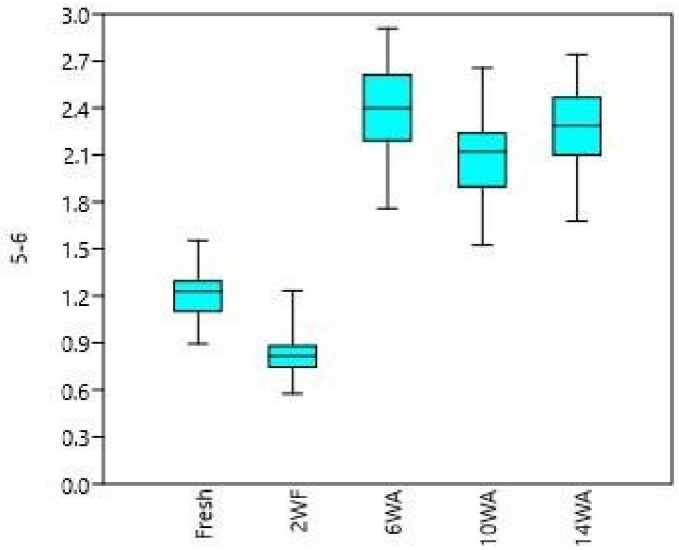

Fig 72: Box plots of truss dimension 5-6 (Caudal peduncle dorsal border to lateral line end)for*S. niger*(Fresh, 2WF,6WA, 10WA, 14WA).

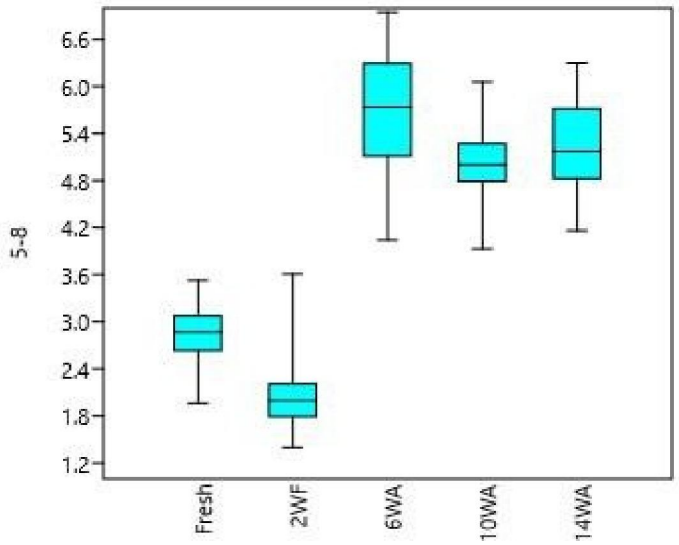

Fig 73: Box plots of truss dimension 5-8 (Caudal peduncle dorsal border to anal fin end) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

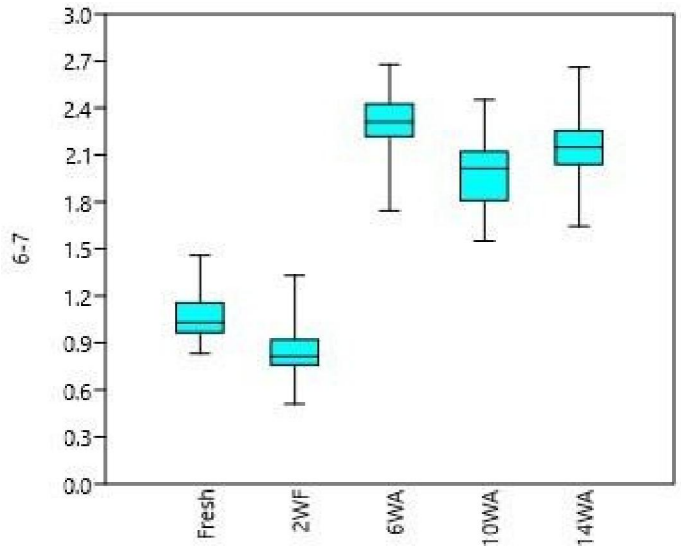

Fig 74: Box plots of truss dimension 6-7 (Lateral line end to caudal peduncle ventralborder)for*S. niger*(Fresh, 2WF, 6WA, 10WA, 14WA).

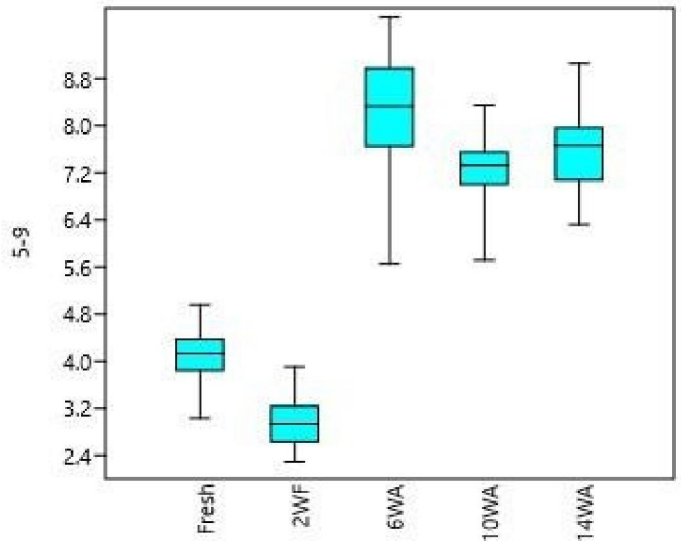

Fig 75: Box plots of truss dimension 5-9 (Caudal peduncle dorsal border to anal finend)for *S. niger*(Fresh, 2WF,6WA, 10WA, 14WA).

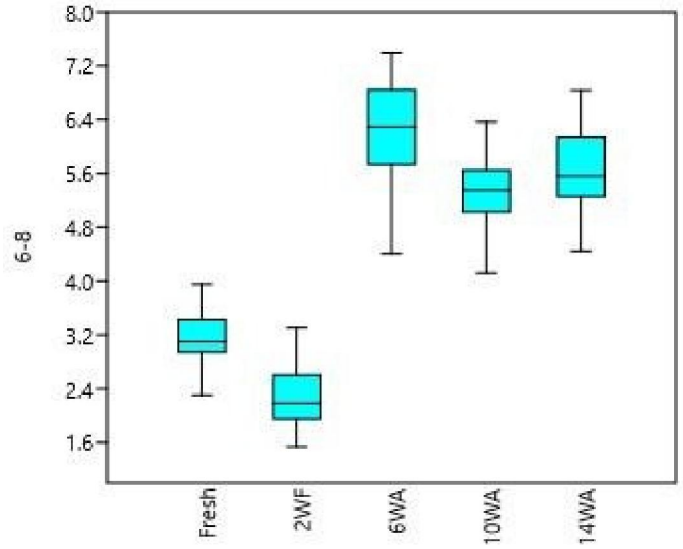

Fig 76:Boxplotsof trussdimension 6-8(Lateralline end toanalfin end)for*S. niger* (Fresh,2WF,6WA,10WA,14WA).

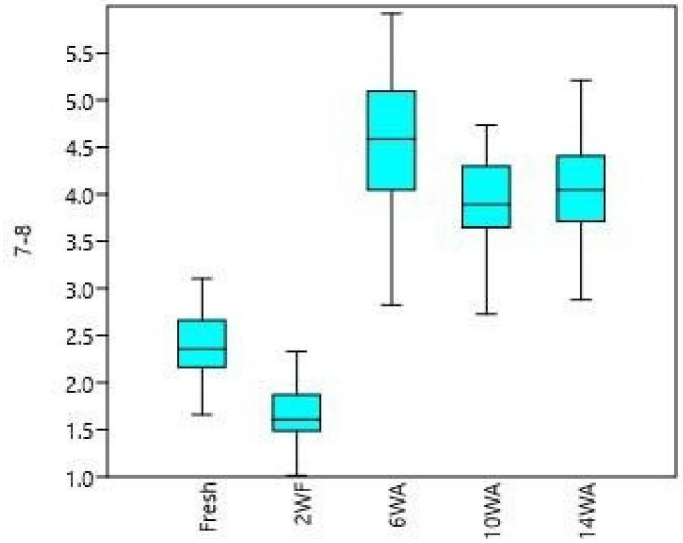

Fig 77:Boxplotsof trussdimension 7-8(Caudalpeduncle ventralbordertoanalfin end)for *S.niger*(Fresh,2WF,6WA,10WA,14WA).

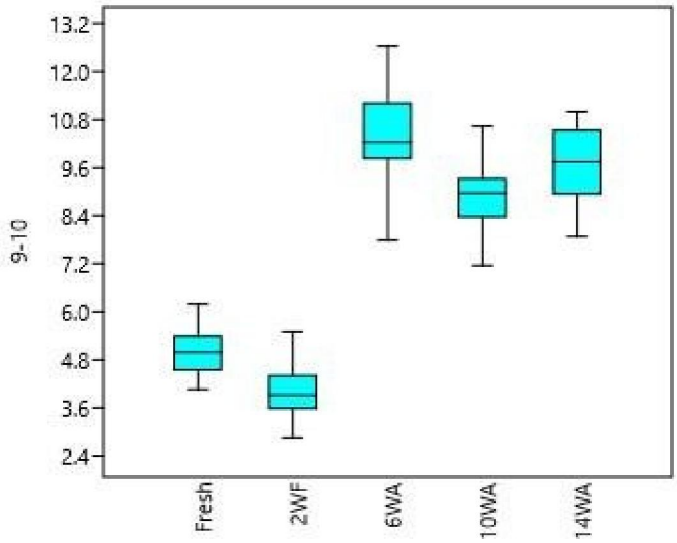

Fig 78:Boxplotsof trussdimension 9-10 (Analfinorigin topelvicfinorigin)for*S. niger* (Fresh,2WF,6WA,10WA,14WA).

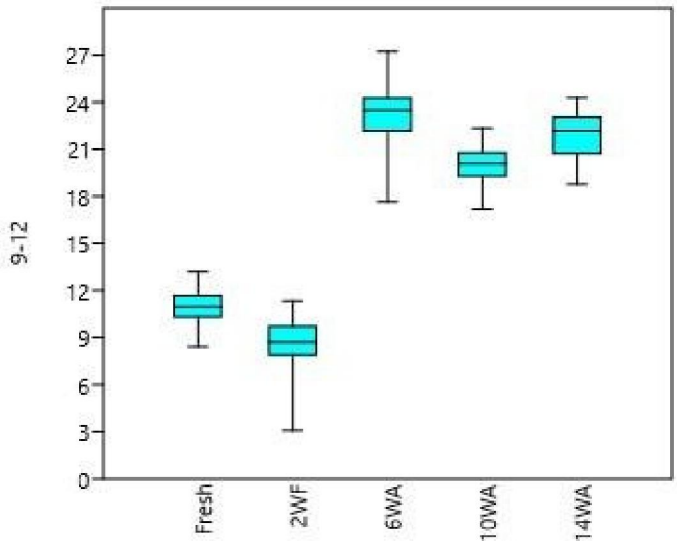

Fig 79: Box plots of truss dimension 9-12 (Anal fin origin to opercular posterior edge) for *S.niger*(Fresh, 2WF, 6WA,10WA,14WA).

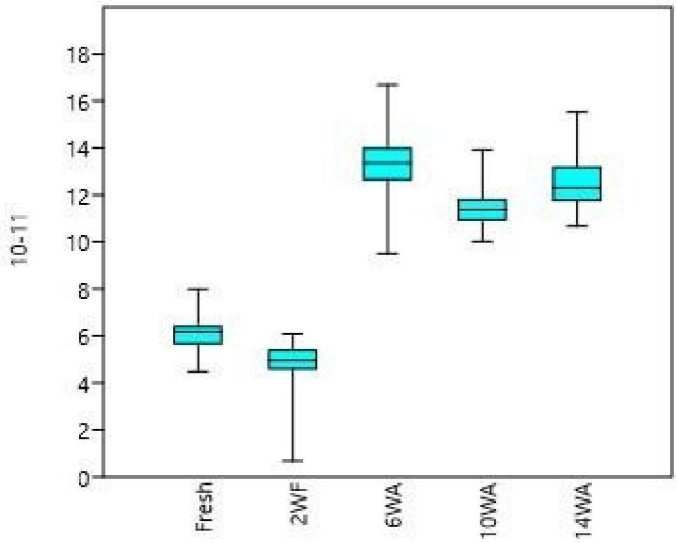

Fig80:Boxplotsoftrussdimension10-11 (Pelvicfin origin topectoralfinorigin)for*S. niger* (Fresh,2WF,6WA,10WA,14WA).

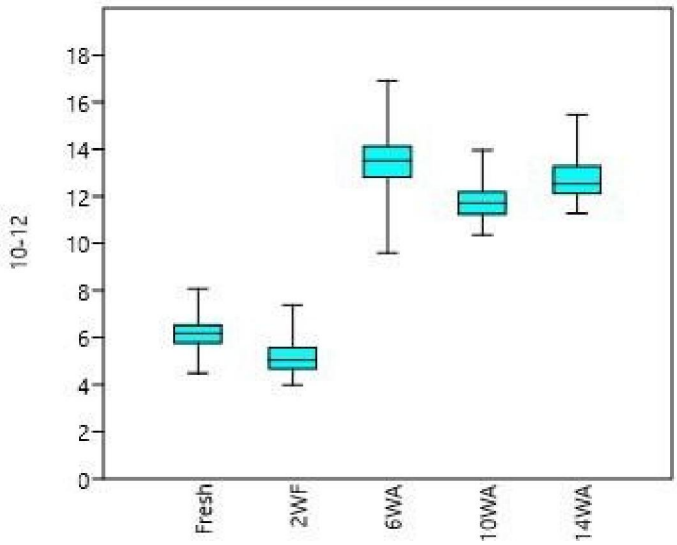

Fig81:Boxplotsoftrussdimension10-12(Pelvicfinoriginto opercularposterioredge)for*S.niger* (Fresh,2WF,6WA,10WA,14WA).

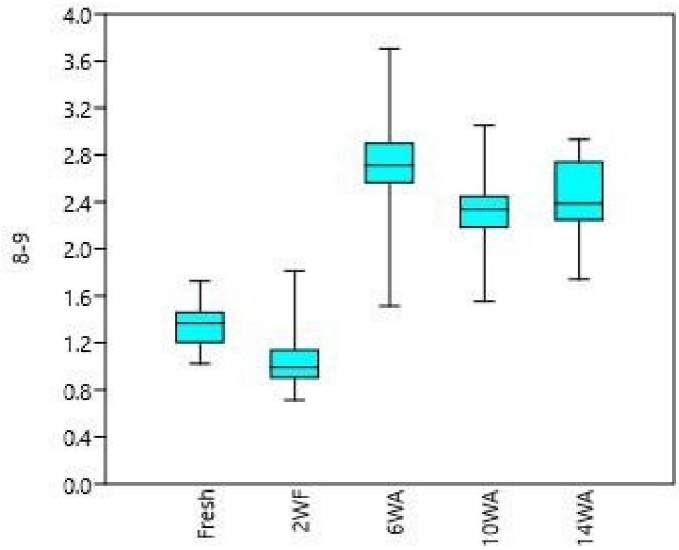

Fig 82: Box plots of truss dimension 8-9 (Anal fin end to anal fin origin) for *S. niger*(Fresh,2WF,6WA, 10WA, 14WA).

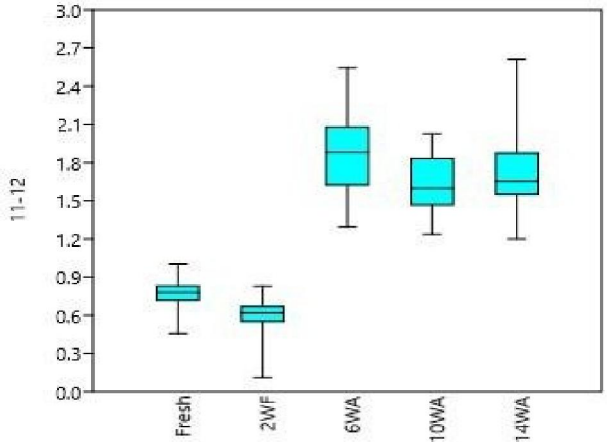

Fig 83: Box plots of truss dimension 11-12 (Pectoral fin origin to opercular posterior edge) for *S.niger*(Fresh, 2WF, 6WA,10WA, 14WA).

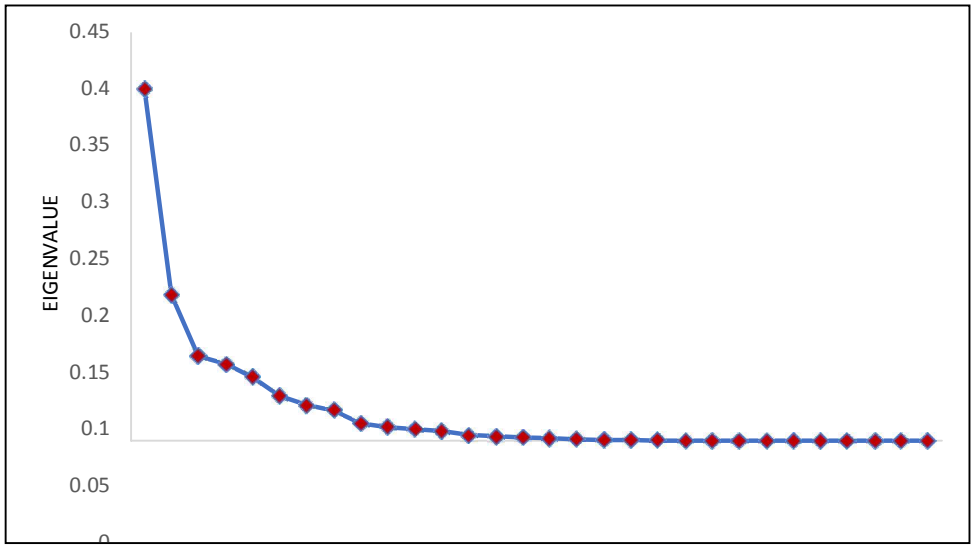

Figure84:Screeplot of eigenvaluesof*S.niger*fortrussmeasurements

4.2.1.2Principal Component Analysisof *S. niger*

The correlation coefficients between truss measurements and standard length of *S. niger* were very close to 1 before the transformation for size correction. After transformation of truss measurements, none of the standardized truss measurements showed significant correlation with standard length. It indicates that the effect of body length had been successfully removed with the allometric transformation. In the Principal Component Analysis, the first three Principal Components together explained 64.23% of total variation, with eigen values of 0.10, 0.04 and 0.02 respectively (Table 19). Eigen values shows variance explained by that particular component out of the total variance. The proportion of variance explained by each Principal Component is depicted as scree plot (Fig. 84). The loadings of the 30 truss distances (variables) are listed in Table 20 and it showed the variance explained by the variable on that particular principal component.

The truss distances with meaningful loading on first Principal Component (PC1) were 3-10, 3-9, 3-4, 4-8, 4-7, 4-5, 5-7, 6-7and 11-12 which explained 38.78% of total variance (Table 20, Plate 10). Out of these nine distances, six distances characterize the straight and oblique depth measurements of middle and posterior region of fish body.

Though the second Principal Component (PC2) explained 16.11% of total variation, four variables showed significant loadings on it and those distances were 2-11, 2-12, 1-12 and 1-11 (Table 20, Plate 11). It indicates that a significant amount of variation explained by PC2 can be accounted solely by differences in shape of head region.

Four distances i.e. 5-8, 5-9, 6-8 and 7-8 loaded heavily on the third Principal Component (PC3), which explained 9.34 percent of total variance. These distances belonged entirely to the caudal region of the fish body (Table 20, Plate 12).

Table 19: Eigen values and proportion of variance contribution to the total variance of truss distances.

Eigen values of the Covariance matrix				
PC	**Eigen value**	**Difference**	**Proportion (%)**	**Cumulative (%)**
1	0.10420477	0.06090336	38.78%	38.78%
2	0.04330141	0.01821573	16.11%	54.89%
3	0.02508568		9.34%	64.23%

Table 20: Variable loadings of truss data.

Truss distances	**PC1**	**PC2**	**PC3**
1-2	0.154069	-0.104746	-0.026119
1-12	0.151342	**-0.298161**	-0.019817
1-11	0.127654	**-0.307466**	-0.029452
11-12	**0.372426**	0.000176	-0.164706
2-12	0.081612	**-0.627976**	0.154876
2-11	0.165164	**-0.367106**	0.0411
2-3	0.082292	0.035596	-0.035188
3-10	**0.23487**	0.002674	0.084036
10-11	0.044176	0.107919	-0.182167
2-10	0.068678	-0.047169	-0.106818
3-11	0.108348	0.158865	-0.051347
3-4	**0.347855**	0.111611	-0.053607
4-9	-0.192323	0.016647	-0.104271
9-10	0.012291	0.17092	-0.081742

3-9	-0.021411	0.139918	0.086191
4-10	**0.31405**	-0.018479	0.065518
4-5	-0.331748	0.054837	**0.433296**
5-8	0.03939	0.176815	-0.043273
8-9	0.036145	0.176815	-0.043273
4-8	-0.261417	0.004537	-0.096833
5-9	0.026458	0.091836	**0.281994**
5-6	0.186466	0.10339	-0.024174
7-8	-0.039716	0.1307	**0.56939**
6-7	**0.2587**	0.123832	0.090219
6-8	0.020644	0.117452	**0.402373**
5-7	**0.249182**	0.071922	0.136035
4-12	0.150478	0.182276	-0.068364
10-12	0.049233	0.118827	-0.17049
9-12	0.008114	0.138864	-0.131421
4-7	-0.232321	0.032456	0.077013

Values given in bold represent variables with meaningful loadings.

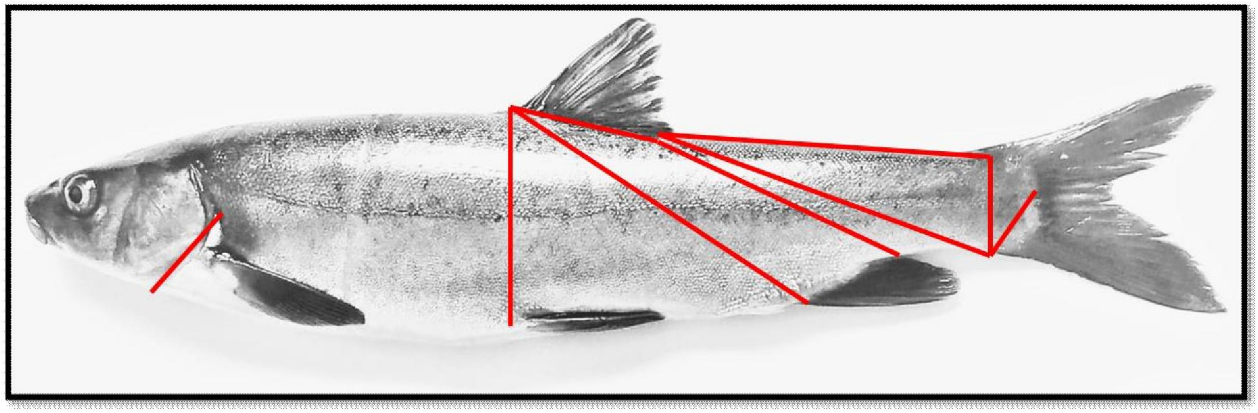

Plate10:Distanceswith meaningfulloadingsonfirstPrincipalComponent(PC1)intrussnetworkanalysisof*S.niger*

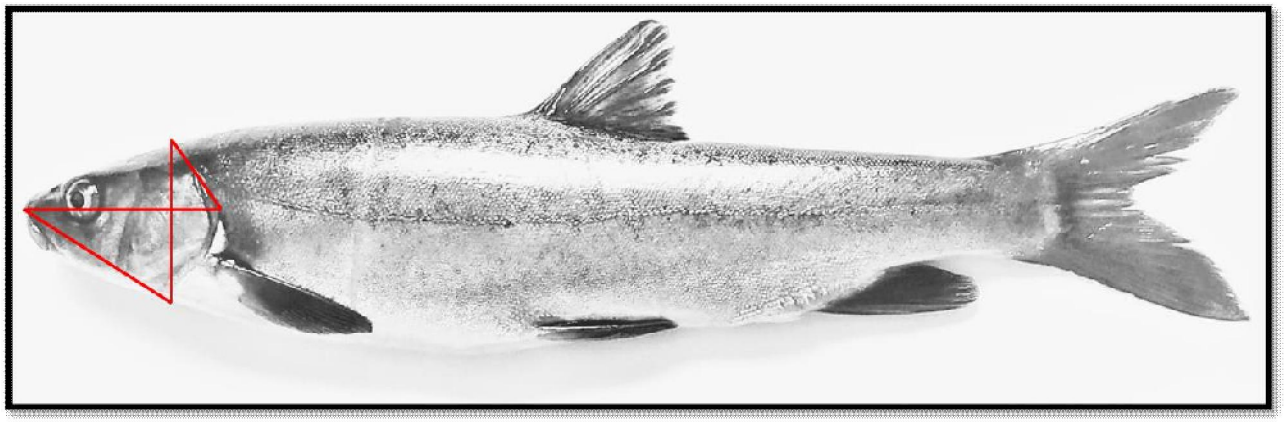

Plate11:Distanceswith meaningfulloadingsonsecondPrincipalComponent (PC2)intrussnetworkanalysisof*S.niger*

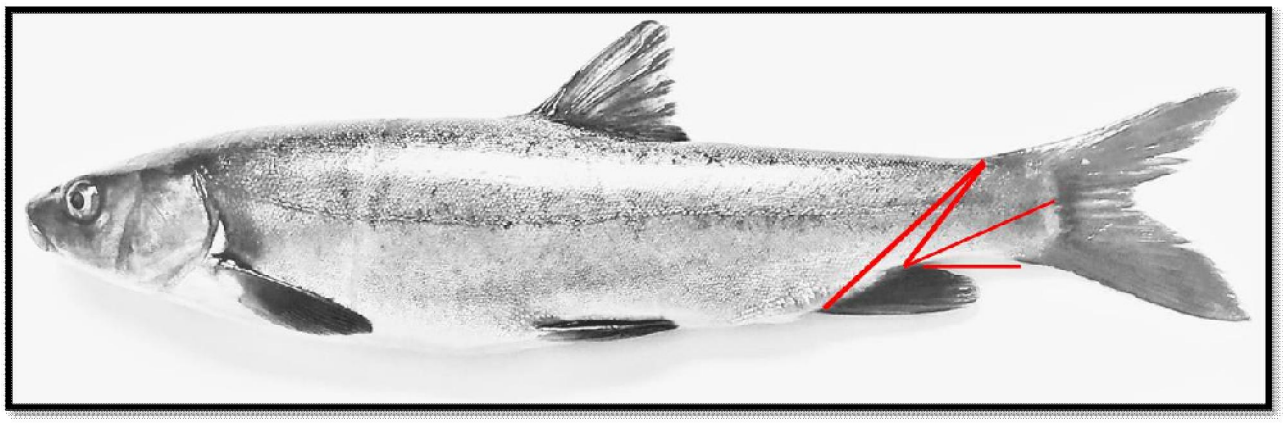

Plate12:Distanceswithmeaningfulloadingson thirdPrincipalComponent(PC3)intrussnetworkanalysisof *S.niger*

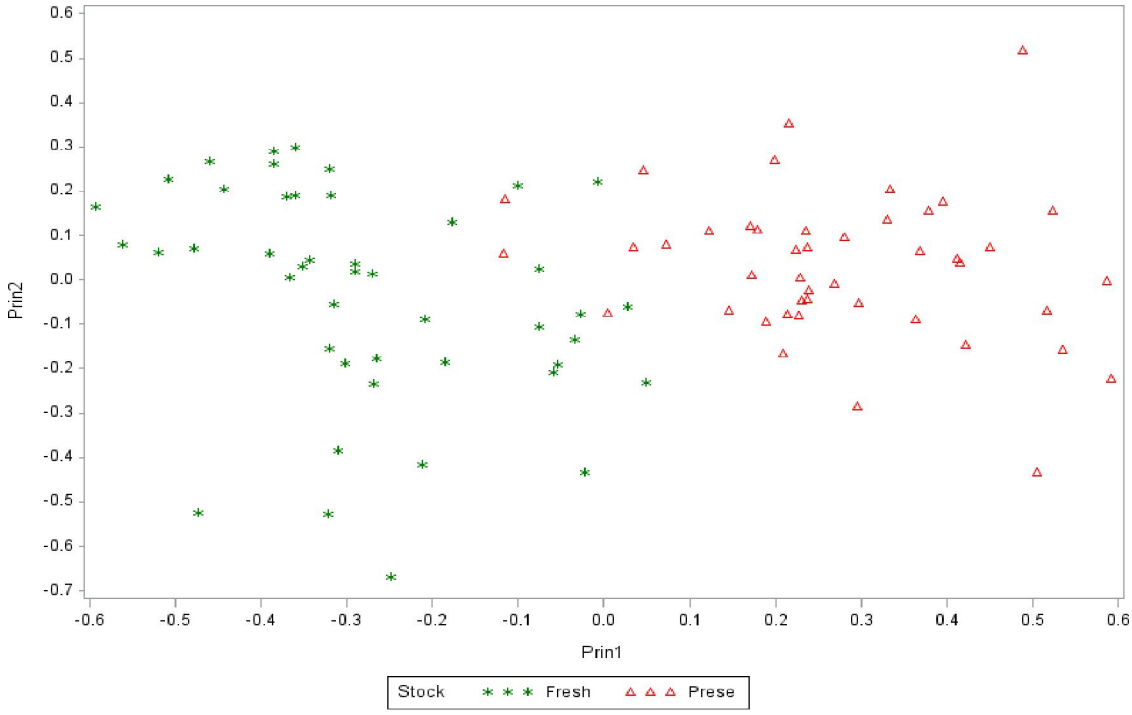

Fig 85: Bivariate plot of scores on the two Principal Components (PC1and PC2)extractedfrom12pointtrussmeasurementsof*S.niger*(freshandpreserved).

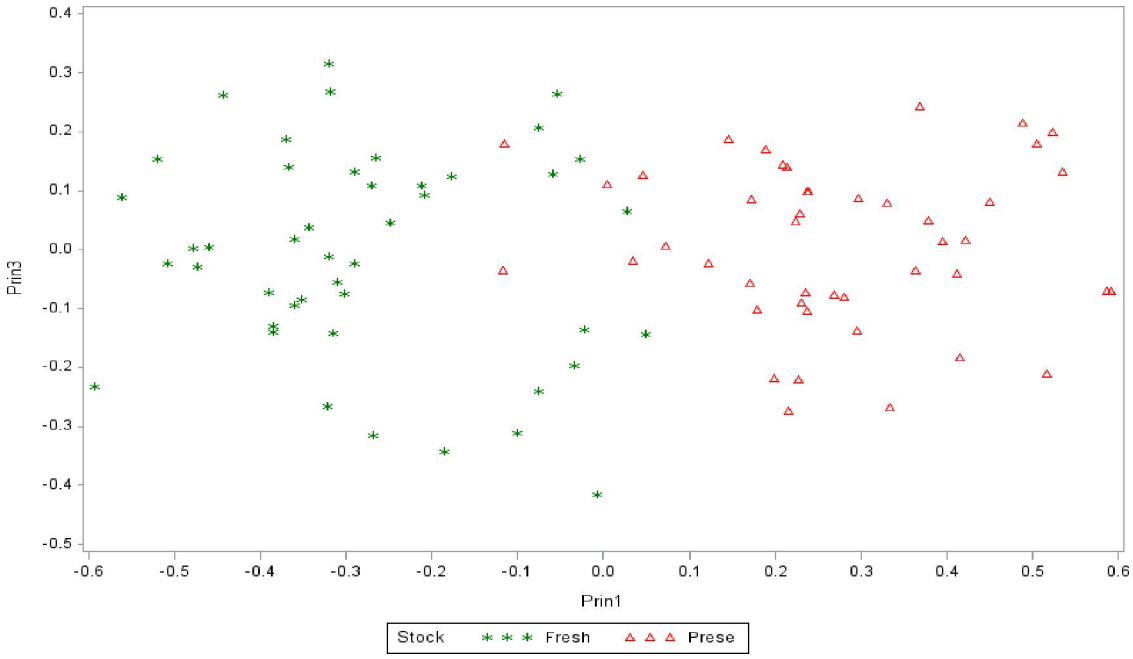

Fig 86: Bivariate plot of scores on the two Principal Components (PC1 and PC3)extractedfrom12pointtrussmeasurementsof*S.niger*(freshandpreserved).

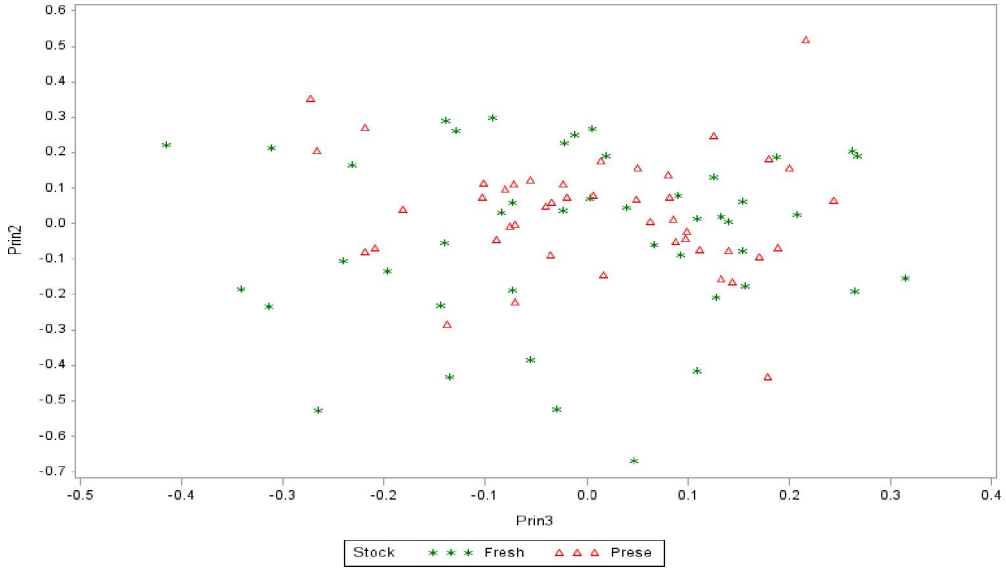

Fig 87: Bivariate plot of scores on the two Principal omponents (PC3 and PC2)extractedfrom12pointtrussmeasurementsof*S.niger*(fr eshandpreserved).

The bivariate plot of the PC1 & PC2 and PC1 & PC3 extracted from the PrincipalComponentAnalysisofthetrussnetworkof*S.niger*forthefres handpreservedspecimens indicates separation along the X-axis, i.e. PC1, but there happened to be aslight mixing along Y-axis, i.e. PC2 (Fig. 85, Fig. 86). The bivariate plot of the PC3 andPC2 revealed there is no separation along both axes, indicating mixing of the fresh andpreservedspecimens(Fig. 87).

4.2.2 Truss morphometry of *S. esocinus*.

Throughout the preservation period there were varying effects on the 30 truss dimensions of *S. esocinus*. Shrinkage as well as corresponding enlargement between landmarks due to preservation was observed. Shape change progressing from Fresh to 2WF were an increase in all the 30 truss distances, followed bycontinuous decrease in 6WA, 10WA & 14WA.However, there was an increase after 6WA in some dimensions like 2-12, 2-11, 3-10, 4-5, 4-7, 4-9, 5-7 5-8, 6-7, 6-8, 7-8, 4-8 and 11-12 with the values (3.37±0.06), (4.63±0.06), (7.67±0.08), (12.42±0.11), (13.67±0.11), (8.09±0.09), (3.85±0.04), (6.04±0.07), (2.53±0.04), (6.77±0.08), (4.86±0.07), (9.47±0.09), and (1.74±0.04).

Comparison of truss dimensions between five measurements (Fresh, 2WF, 6WA,10WA and 14WA) of*S. esocinus* is presented in Table 21. All the thirty truss dimensions displayed significant difference (<0.01) between Fresh and 2WF, Fresh and 6WA, Fresh and 10WA and Fresh and 14WA. The box plots of 30 truss dimensions obtained from 12 landmarks of *S. esocinus* (Fresh, 2WF, 6WA,10WA and 14WA) are presented in figures 85-117.

S. No	Dimension	Fresh	2WF	P-value	Fresh	6WA	P-value	Fresh	10WA	P-value	Fresh	14WA	P-value
1	**1-2**	3.52±0.08	8.17±0.13	**<0.01**	3.52±0.08	7.85±0.09	**<0.01**	3.52±0.08	7.01±0.07	**<0.01**	3.52±0.08	6.62±0.08	**<0.01**
2	**1-11**	3.7±0.1	9.14±0.15	**<0.01**	3.7±0.1	9.21±0.12	**<0.01**	3.7±0.1	7.93±0.11	**<0.01**	3.7±0.1	7.81±0.13	**<0.01**
3	**1-12**	3.92±0.1	9.5±0.16	**<0.01**	3.92±0.1	9.46±0.13	**<0.01**	3.92±0.1	8.21±0.12	**<0.01**	3.92±0.1	8±0.13	**<0.01**
4	**2-3**	5.24±0.13	13.09±0.17	**<0.01**	5.24±0.13	13.11±0.12	**<0.01**	5.24±0.13	11.8±0.11	**<0.01**	5.24±0.13	11.09±0.11	**<0.01**
5	**2-10**	5.81±0.16	15.06±0.19	**<0.01**	5.81±0.16	15.36±0.11	**<0.01**	5.81±0.16	13.37±0.11	**<0.01**	5.81±0.16	12.68±0.14	**<0.01**
6	**2-11**	1.4±0.05	4.28±0.07	**<0.01**	1.4±0.05	4.63±0.06	**<0.01**	1.4±0.05	3.83±0.06	**<0.01**	1.4±0.05	3.66±0.06	**<0.01**
7	**2-12**	0.99±0.04	3.11±0.07	**<0.01**	0.99±0.04	3.37±0.06	**<0.01**	0.99±0.04	2.69±0.05	**<0.01**	0.99±0.04	2.7±0.06	**<0.01**
8	**3-4**	1.9±0.05	5.3±0.1	**<0.01**	1.9±0.05	4.82±0.08	**<0.01**	1.9±0.05	4.48±0.06	**<0.01**	1.9±0.05	4.33±0.07	**<0.01**
9	**3-9**	5±0.13	12.32±0.15	**<0.01**	5±0.13	12.32±0.09	**<0.01**	5±0.13	11.04±0.09	**<0.01**	5±0.13	10.28±0.13	**<0.01**
10	**3-10**	2.67±0.08	7.33±0.11	**<0.01**	2.67±0.08	7.67±0.08	**<0.01**	2.67±0.08	6.59±0.09	**<0.01**	2.67±0.08	6.11±0.09	**<0.01**

Data are mean ± SE n=45 for Fresh, 2WF, 6WA, 10WA and 14WA. The p-value with p < 0.01are significantly different

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S. No	Dimension	Fresh	2WF	P-value	Fresh	6WA	P-value	Fresh	10WA	P-value	Fresh	14WA	P-value
11	**3-11**	5.11±0.12	12.7±0.17	**<0.01**	5.11±0.12	12.57±0.12	**<0.01**	5.11±0.12	11.42±0.11	**<0.01**	5.11±0.12	10.43±0.11	**<0.01**
12	**4-5**	5.53±0.13	11.8±0.18	**<0.01**	5.53±0.13	12.42±0.11	**<0.01**	5.53±0.13	11.22±0.13	**<0.01**	5.53±0.13	10.25±0.18	**<0.01**
13	**4-7**	6.02±0.15	13.14±0.19	**<0.01**	6.02±0.15	13.67±0.11	**<0.01**	6.02±0.15	12.31±0.11	**<0.01**	6.02±0.15	11.19±0.17	**<0.01**
14	**4-8**	4.12±0.1	9.11±0.14	**<0.01**	4.12±0.1	9.47±0.09	**<0.01**	4.12±0.1	8.42±0.1	**<0.01**	4.12±0.1	7.68±0.12	**<0.01**
15	**4-9**	3.35±0.09	7.76±0.11	**<0.01**	3.35±0.09	8.09±0.09	**<0.01**	3.35±0.09	7.15±0.09	**<0.01**	3.35±0.09	6.6±0.1	**<0.01**
16	**4-10**	3.07±0.08	7.97±0.13	**<0.01**	3.07±0.08	7.92±0.1	**<0.01**	3.07±0.08	7.14±0.08	**<0.01**	3.07±0.08	6.53±0.09	**<0.01**
17	**4-12**	6.6±0.15	16.67±0.23	**<0.01**	6.6±0.15	16±0.15	**<0.01**	6.6±0.15	14.72±0.12	**<0.01**	6.6±0.15	13.63±0.14	**<0.01**
18	**5-6**	1.11±0.04	2.69±0.04	**<0.01**	1.11±0.04	2.66±0.05	**<0.01**	1.11±0.04	2.29±0.04	**<0.01**	1.11±0.04	2.01±0.04	**<0.01**
19	**5-7**	1.49±0.04	3.81±0.05	**<0.01**	1.49±0.04	3.85±0.04	**<0.01**	1.49±0.04	3.42±0.03	**<0.01**	1.49±0.04	3.18±0.04	**<0.01**
20	**5-8**	2.52±0.06	5.81±0.1	**<0.01**	2.52±0.06	6.04±0.07	**<0.01**	2.52±0.06	5.51±0.07	**<0.01**	2.52±0.06	5.11±0.08	**<0.01**

Data are mean ± SE (n=45 for Fresh, 2WF, 6WA, 10WA and 14WA. The p-value with p < 0.01are significantly different

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S. No	Dimension	Fresh	2WF	P-value	Fresh	6WA	P-value	Fresh	10WA	P-value	Fresh	14WA	P-value
21	5-9	3.62±0.08	8.44±0.12	**<0.01**	3.62±0.08	8.61±0.08	**<0.01**	3.62±0.08	7.88±0.07	**<0.01**	3.62±0.08	7.19±0.1	**<0.01**
22	6-7	1.04±0.03	2.43±0.04	**<0.01**	1.04±0.03	2.53±0.04	**<0.01**	1.04±0.03	2.25±0.04	**<0.01**	1.04±0.03	2.03±0.03	**<0.01**
23	6-8	2.95±0.07	6.5±0.11	**<0.01**	2.95±0.07	6.77±0.08	**<0.01**	2.95±0.07	6.15±0.07	**<0.01**	2.95±0.07	5.51±0.08	**<0.01**
24	7-8	2.14±0.05	4.68±0.1	**<0.01**	2.14±0.05	4.86±0.07	**<0.01**	2.14±0.05	4.49±0.07	**<0.01**	2.14±0.05	4.07±0.07	**<0.01**
25	8-9	1.19±0.03	2.81±0.05	**<0.01**	1.19±0.03	2.74±0.04	**<0.01**	1.19±0.03	2.52±0.03	**<0.01**	1.19±0.03	2.2±0.04	**<0.01**
26	9-10	4.25±0.1	9.83±0.15	**<0.01**	4.25±0.1	9.72±0.11	**<0.01**	4.25±0.1	8.99±0.1	**<0.01**	4.25±0.1	8.21±0.12	**<0.01**
27	9-12	9.09±0.22	21.85±0.29	**<0.01**	9.09±0.22	21.62±0.19	**<0.01**	9.09±0.22	19.64±0.17	**<0.01**	9.09±0.22	18.15±0.21	**<0.01**
28	10-11	5.05±0.13	12.49±0.19	**<0.01**	5.05±0.13	12.4±0.13	**<0.01**	5.05±0.13	11.04±0.12	**<0.01**	5.05±0.13	10.24±0.13	**<0.01**
29	10-12	4.99±0.13	12.44±0.19	**<0.01**	4.99±0.13	12.45±0.13	**<0.01**	4.99±0.13	11.04±0.12	**<0.01**	4.99±0.13	10.24±0.13	**<0.01**
30	11-12	0.6±0.02	1.62±0.04	**<0.01**	0.6±0.02	1.74±0.04	**<0.01**	0.6±0.02	1.54±0.04	**<0.01**	0.6±0.02	1.34±0.03	**<0.01**

Data are mean ± SE (n=45 for Fresh, 2WF, 6WA, 10WA, 14WA. The p-value with p < 0.01are significantly different

4.2.2.1 Principal Component Analysis of *S. esocinus*.

A high correlation coefficient existed between the truss measurements and standard length beforethe transformation for size correction. None of the standardized truss measurements showed significant correlation with standard length after transformation. It depicts that byallometric transformation, the effect of body length had been successfully removed. In the Principal Component Analysis, the first three Principal Components together explained 66.18% of total variation, with eigen values 0.11, 0.03 and 0.02, respectively (Table 22). Eigenvalues showed variance explained by that particular component out of the total variance. The proportion of variance explained by each Principal Component is depicted as scree plot (Fig. 118). The loadings of the 30 truss distances (variables) are listed in Table 23 and it shows the variance explained by the variable on that particular principal component.

The first Principal Component (PC1) included six truss distances with meaningful loadings which explained 42.01% of total variance(Table 22, Plate 13). These distances were2-12, 2-11, 3-4, 4-5,4-8 and 4-7.Out of these six distances, four distances belonged to the caudal region and two distances belong to the head region of fish body.

Out of thirty distances, four distances showed significant loadings on second Principal Component(PC2) which explained a total variance of 13.90%.These distances were 2-11, 2-12, 1-12 and 1-11 (Table 22, Plate 14). Two of these distances belonged to the trunk region and other two distances comprise the head region of fish body.

Two distances i.e. 11-12 and 5-6 loaded on third Principal Component (PC3), explained 10.27% of total variance. These distances correspond to the caudal and head region of the fish body (Table 22, Plate15).

Table 22: Eigen values and proportion of variance contribution to the total variance of truss distance distances.

Eigen values of the Covariance matrix				
PC	**Eigen value**	**Difference**	**Proportion (%)**	**Cumulative (%)**
1	0.11036333	0.07385248	42.01%	42.01%
2	0.03651086	0.00953941	13.9%	55.91%
3	0.02697144		10.27%	66.18%

Table 23: Variable loadings of truss data.

Truss distances	**PC1**	**PC2**	**PC3**
1-2	0.034077	-0.065399	0.09573
1-12	0.070123	**-0.209101**	0.152227
1-11	0.12546	**-0.216439**	0.104016
11-12	0.043016	-0.006393	**0.851529**
2-12	**0.493494**	-0.541797	-0.121307
2-11	**0.43958**	-0.278358	0.08864
2-3	0.150367	0.094401	-0.04604
3-10	0.182834	0.154049	0.054606
10-11	0.048541	0.167229	0.083493
2-10	0.133517	0.048881	0.037782
3-11	0.11871	0.191305	0.020046
3-4	**0.276563**	0.304089	-0.113169
4-9	-0.154244	-0.059511	0.091192
9-10	-0.027082	0.083399	0.066935
3-9	-0.010184	0.041147	0.029149

4-10	0.194946	**0.264983**	0.028225
4-5	**-0.306758**	-0.18438	0.038511
5-8	0.082098	0.046062	-0.075403
8-9	-0.094711	0.135097	-0.03603
4-8	**-0.257082**	-0.125323	0.107285
5-9	0.022741	0.0731	-0.078188
5-6	0.00604	0.115528	**0.249536**
7-8	-0.020679	-0.004616	-0.167599
6-7	0.124842	0.155478	0.191982
6-8	0.002375	0.031529	-0.025226
5-7	0.175802	0.141941	0.075489
4-12	0.166778	**0.249484**	-0.067577
10-12	0.075119	0.181214	0.045543
9-12	0.025193	0.128983	0.030923
4-7	**-0.229474**	-0.117458	0.027947

Values in bold represent variables with meaningful loadings.

Chapter 5

DISCUSSION

5.1 Conventional Morphometry

External morphological measurement is still one of the main research approaches used in ichthyology and aquaculture, most likely due to its long tradition and simplicity. In many cases, it is a sufficient method for many purposes for which it is required. Conventional morphometric methods involve measuring of linear distances such as length, width, and height and then analysing the patterns of shape variation within and among the groups with multivariate statistical tools which further assist in describing allometric patterns in body shapes.Morphometrics allows one to describe complex shapes more rigorously and to make quantitative comparisons between different forms. In fish, morphometric characters represent one of the major keys determining their systematics, growth variability, ontogenetic trajectories (Kovac and Copp, 1999), and/or population parameters (Verep*et al.,* 2006). Preserved samples are often used for morphological studies by ichthyologists due to constraints of time, space, etc. Despite the wide usage of preserved specimens, the effects of preservation are often not discussed as a limitation or a potential source of error in morphometric studies.

In the present study, it was found that preservation in both formalin and ethanol change the fish body proportions of individuals of *S. niger*and *S. esocinus*. It was observed that the general trend of change progressing from Fresh to 14WA was that of a continuous reduction in the majority of the morphometric characters studied in both the species.This progressive change was also observed in the two preservatives across the preservation time (Fresh, 2WF, 6WA,10WA and 14WA).Our results are consistent with Shields and Carlson(1996) and Greszkiewicz and Fey (2018) who reported reduction in standard length due to formalin and alcohol preservation in juvenile sockeye salmon and larval northern pike respectively.Hossaini*et al*. (2016) also reported shrinkage in Zargos tooth-carp, *Aphaniusvladykovi* due to preservation in alcohol for 3 months. However, in

S. esocinus, increase in the head measurements namely, head length, pre-orbital length and post-orbital length was observed after two weeks of preservation in formalin. The possible explanation for this could be absorption of formalin in the head area. Similar results have been reported by earlier researchers (Lux, 1960; Parker, 1963; Engel,1974). In addition, Jawad*et al*. (2001) revealed that different concentrations of formalin and alcohol caused variable degrees of shrinkage in the three characters of total length, standard length and head length. Also, Neave*et al*. (2006) investigated the effects of fixing in ethanol and formalin on total length in larvallampreys and found that total length decreased after 3 weeks of preservation in formalin due to high amount of water present in larvae as formalin extracts water from the tissues which results in the shrinkage of specimens. However, Al-Hassan *et al*. (2000) reported contrasting results of increase in total length, standard length and head length in two species of family Mullidae preserved in different concentrations of alcohol and formalin. The greatest length increment was recorded in total length of *M. barbatus* in 10% formalin and the least length increase was also observed in the same species but in 5% formalin which the authors opined was due to difference in the concentration of the preservative.The nature of the morphological variation is influenced by many factors including style of preservation such as fixation and freezing, concentration and kind of chemical preservation agents, length of preservation period, salinity and temperature of the preservative. Also, a number of factors have been reported to effect morphology of preserved fish including age, size, species and developmental state, the presence of rigor mortis, and the osmoregulatory state of the fish at death (Macdonald *et al.,* 1997). The effect of preservation on the morphology of fishes in relation to size has been the subject of several research workssuch as for *Gadusmorhua* (Radtke andWaiwood, 1980), *Clupeaharengus*(Hay, 1982),*Merlucciusbilinearis* (Fowler and Smith, 1983), *Pleuronectesamericanus*(Hjorleifsson and Klein-MacPhee, 1992),*Stizostedionvitreum*(Johnston and Mathias, 1993),*Sprattussprattus* (Fey, 1999), *Enchelyopuscimbrius* (Fey, 1999) and *B. tyrannus* (Fey & Hare, 2005) who

reported that there was a reduction in the shrinkage as body size increased. Johnston and Mathias (1993) also suggested that percentage change in length due to preservation is inversely proportional to fish size. Species specific effect of preservation has also been widely reported in the literature. Sotola*et al*. (2019) investigated the effect of preservation in ten different species of fish and observed varied effects. Al- Hassan *et al.* (2000), while working on the effect of formalin and alcohol preservation on *M.barbatus*and *M.surmuletus* observed a variable effect on morphological characters of these two species.According to Theilacker (1980), the internal osmolar concentration at the time of death effects the amount of change caused by the preservative.(Shetter, 1936) estimated that brook trout,*Salvelinusfontalis* within the total length range of 2.75-10.25 inches shrank approximately 2.6 % from rigor mortis alone.

In the present study, three morphometric measurements of head length, pre-orbital length and post-orbital length revealed significant difference ($P<0.05$) due to preservation in all the readings (2WF, 6WA, 10WA and 14WA) relative to fresh specimens in both the species. However, the nature and magnitude of the change was not the same.In *S. niger,* significant differences were observed in the characters of pre-pelvic, pre-dorsal and pre-pectoral lengths after six weeks of preservation relative to fresh measurements, while in *S. esocinus,* significant difference in characters of pre-dorsal and pre-pectoral lengths were observed after ten weeks of preservation relative to fresh measurements. This can be attributed to the difference in the fat concentration in different species, among tissue types and with body size (Vizza*et al*., 2013). As alcohol extracts the lipids present in the specimens (Glauert, 1974), this produces gross morphological changes in preserved specimens. Thus, extraction of the lipids which are more in *S*. *niger*than *S. esocinus*(Ganai, 2012) may account for the early change in some of the characters in *S. niger*.

Significant decrease ($P<0.05$) in the total length in both the species was observed after 14 weeks of preservation in our study. Our results are consistent with the studies of Jawad(2003) who reported significant decrease in total length

of *A. djeddaba*preserved in 70% alcohol. Since the amount of water present in fishes is generally greater than 70% although it varies in different species and as a result of ethanol being less dense than water, there is a reduction in overall muscle density because of the cellular fluid exchange between water and ethanol in each muscle tissue (Klemm, 1990, 1998). This may be the reason for the reduction in total length of *S. niger* and *S. esocinus* in 70% ethanol observed in the present study.

In the present study, non significant($P>0.05$) difference was observed in four characters namely, eye diameter, snout length, body depth and caudal fin length in both the species throughout the preservation period. Thus, it may be possible that change in these characters has occurred at a very slow rate and a much longer period of immersion in formalin and alcohol may be required before significant change can be detected in these characters.

In the present study, among the selectedmorphometric parameters, pre-orbital length showed the highest percentage change in formalin (-8.06%in *S. niger* and 9.25% in *S. esocinus*) while total length displayed the lowest percentage change (-0.70% in *S. niger* and -0.58% in*S. esocinus*). In alcohol preservation, pre-dorsal length displayed highest percentage change (-13.31%) and caudal fin length displayed lowest percentage change (0.39%) for*S. niger*. On the other hand, in *S. esocinus,* pre-pectoral length showed highest percentage change (-13.50%) and eye diameter showed lowest percentage change (-0.73%) during alcohol preservation. Different preservatives with different concentrations have different effects on morphological characters of specimens. The changes in the effect of preservatives could be described on the basis that the different body sections of the fish include different chemical contents; hence, they behave diversely when kept in different preservatives. The differences perceived on the effect of preservation might be due to genetical factors that assess the ratio of white to red muscles and the character of tissue water content (Leslie & Moore, 1986). It has been reported that alcohol alone expressly coagulates proteins and causes noticeable demolition of the micro-anatomy in animal tissue (Nowacek, 2010).

Discrepancies, however, have been demonstrated in species specific measurement changes due to preservation. Engel (1974), Sayers (1987)and Hunter (1985)described a decrease in the total length inlake cisco, *Coregonusartedi*, yellow perch, *Percaflavescens,*Californian anchovy, *Engraulismordax*, and Lake Michigan bloaters, *Coregonushoyi*respectively due to preservation. In contrast, Billy (1982) in Mosambique tilapia, *Oreochromismossambicus*, Al-Hassan and Abdullah (1992) in *Barbusluteus* and Al-Hassan and Shawafi (1997) inIndian mackerel, *Rastrelligerkanagurta* suggested that there was a slight increase in standard length due to preservation. While no shrinkage in the specimens of *Mullusbarbatus* was observed by Al-Hassan *et al.*(2000) preserved in formalin and alcohol.Our findings on the time and amount of change using two congeneric species confirm that the response to preservation is species-specific.This can be due to the fact that each species has its own characters of tissue water content and its specific ratio of white to red muscles which might interfere with the different preservatives (Leslie and Moore, 1986).Thus, whenintroducing any alternative preservation method, it may not be possible to predict the morphological changes to the specimen.

Until recently, it was a common practice to use standardized correction factors to calculate live measurements from preserved fish. For example, Bailey (1952) used a correction factor to determine live weights from preserved sculpins, *Cottusbairdipunctulatus.* Similarly, Sigler (1949a,b) determined the correction factors required to obtain live weights and lengths from preserved white bass, *Roccuschrysops,* in one study and used the same correction factor in another study involving the same species. Hile (1936) also determined a correction factor for preserved ciscos, *Coregonusartedii,* from one area and applied it to preserved fish from a different area. Oosten (1929) also used a correction factor in a series of studies using lake herring, *C.artedii,* as did Shetter**(**1936) while working on brook trout, *Salvelinusfontinalis,* and brown trout, *Salmotrutta.* In the present study, conversion equations to back calculate the fresh length from the preserved samples were developed for all the conventional parameters studied for both the

species and in both the preservatives. These conversion equations provided here are the first conversion equations for the adult individuals of *S. niger*and *S. esocinus* and may be used by other researchers to arrive at fresh morphological measurements from measurements that have been taken on preserved specimens.

5.2 Truss Morphometry

Trust Network System' has emerged as a new tool with more effective strategies. Recent developments made in the discipline of morphometric differentiation in body shape among fish populations showed that the truss based techniques is more effective than manual distance measurement for the management of fishery resources throughout the world. When combined with multivariate statistical methods(Principal Component Analysis, Cluster Analysis, etc.) they offer powerful tool for testing and displaying differences in shape.Itentails whole fish body in a uniform network which could possibly help in extracting morphometric differences within and between species (Turan, 2000). According to Dwivedi and Dubey, (2013) the truss network is more useful and an effective strategy for the descriptions of shape; it has better data collection and diversified analytical tools in comparison to traditional morphometric method. Thus, it is able to discriminate phenotypic stock because the configuration of the constructed landmarks covers the entire fish body with no loss of information, and it is more sensitive to change. Several authors (Cheng *et al*., 2010; Hossain *et al.,* 2010; Nahar*et al*., 2013; Gul *et al*., 2019;Neizaria, 2019; Khan*et al*.,2021) emphasized on the validity of the truss network of morphometric characters which enforces systematic coverage of the form and also exhaustively and redundantly archives the landmark configuration.

Preserved specimens are often included in morphometric studies. Formalin-fixation and ethanol preservation has been the most common whole-organism preservation practice. Despite being used for over 350 years, the effects of ethanol as a fluid preservative are not fully understood and even modern practices remain imperfect in specimen maintenance (Simmons, 2014). Ethanol exposure produces significant structural changes across the entirety of cell

membranes (Patra *et al*., 2006) and their internal structures (Klemm, 1990, 1998). These changes are caused by the hydrophilic properties and fluidity potential of ethanol in water, which results in dehydration of several cell regions and their structures (Klemm, 1990, 1998). This dehydration produces gross morphological changes in preserved specimens (Oosten, 1929; Shetter, 1936;Hile, 1936; Burgner,1962;Shields and Carlson,1996;Valentin,*et al.*, 2008;Vervust,*et al.,* 2009; Berbel-Filho*et al.,* 2013; Gaston *et al*., 2013; Greszkiewicz and Fey, 2018.;Martinez *et al,* 2012;Hossaini*et al.,*2016; Sotola *et al*., 2019).

In our study,it was demonstrated that the preservation in formalin and ethanol has a significant effect in the shape variation between fresh and preserved fish. According to the objectives of the study different statistical approaches were used in order to explore the effect of preservation on the two congeneric species (*S. niger* and *S. esocinus*). The effect of preservation could havean important implications on studies that seek to differentiate groups (sexual dimorphism, populations, ecotypes, species, etc.) as well as on those of shape variation analysis (allometry, relations between shape and environmental or bio-geographical variables, reconstructions of evolutionary exchange) (Martinez *et al.,* 2012). Our study introduces the first evidence that this kind of preservation can affect the results and conclusions of geometric morphometric analysis used in biological research.

Among experimental groups (Fresh, 2WF, 6WA, 10WA and 14WA) of *S. niger*and *S. esocinus*, the landmark configurations reconstructed from the measured distances without loss of information provide an advantage over classical morphometric characteristics. Our findings illustrate that using truss network as a character set has a more classical coverage over the body form and enhance the discrimination among the fresh and preserved specimens.Using a criss-cross pattern on the body shape of fish to uniformly measure distances, Humphries *et al*. (1982) proposed a truss dimension system for covering a fish's shape. Strauss and Bond (1990) also observed the same thing. Our findings

demonstrate that this truss dimension character set allows the detection of shape differences in oblique, vertical and longitudinal directions.

In the present study, the box plots showed different levels of variation intensity through the different landmarks for both the species, and also different regions of the body shape varied with different intensity. These differentiated variations forsome species of fish had already been demonstrated (Leslie and Moore, 1986; Al-Hassan *et al*., 2000; Jawad*et al.,* 2001). This could be explained by the differentiated genetic component present in these species, which influences the proportions of composition of white to red muscles in the tissues. Another possibility is that this change is due to the variable amount of water in each tissue, making it respond differently to different methods of preservation (Leslie and Moore, 1986).

Our findings obtained in Truss Network Analysis support that preservation alters the body shape of fishes, and through time in varying ways. We found significant differences ($P<0.01$) in morphology across preservation time (Fresh & 2WF, Fresh & 6WA, Fresh & 10WA and Fresh & 14WA) in both the species examined. Our results are similar to previous geometric morphometric preservation studies which reported that the body shapes undergo significant and clear changes when preserved in solutions of 10% formalin and 70% alcohol(Berbel-Filho*et a*l., 2013; Martinez *et al*., 2012).Sotola*et al*. (2019) argues that most of the biometrics of fish bodies fixed in formalin 10% and then preserved in 70% alcohol show clear changes at the fourth week of preservation. This result is very important in the biological context, since there are several studies that compare groups (sex, population, species, ecotypes, etc.) using individuals from biological collections, mainly to fill some locations, sites, or specimens that are difficult to obtain. In these cases, when geometric morphometric study is conducted on organisms that have been through chemical preservation, the researcher could obtain wrong results (false positive) and this could subsequently lead to wrong conclusions as the morphological differences

have been caused by preservation and not by biological factors (Martinez *et al.,* 2012).

Results from current study provide a more complete understanding of specific morphometric changes that occur over preservation time in the two fish species studied.Since our fishes were stored in formalin for two weeks, then subsequently stored in ethanol we assessed changes in both the formalin and ethanol stages of preservation for different time periods. Our results of *S. niger* are consistent with the studies of Shields and Carlson(1996), Ajah and Nunoo(2003)andGreszkiewicz and Fey (2018) who worked on *Sockeye salmon,Sardinellaaurita* and Northern pike respectively and found that formalin preservation tends to decrease the overall size of specimens.

In *S. niger*, the size of specimens decreased after two weeks of formalin preservation indicating an overall reduction in specimen sizes during formalin preservation. However, in *S. esocinus* there was an increase in size of specimens after formalin preservation (2 weeks). The contradicting results in truss dimension of the two species due to formalin preservation may be due to the variation in tissue water content and the ratio of white to red muscles, which differs in different species. After six weeks of preservation, a dramatic increase in all truss dimensions was observed in *S. niger* followed by a continuous decline thereafter, while in *S. esocinus* continuous reduction was observed in alcohol at all the time periods. Some of the dimensions showed increase after ten weeks of preservation.The varying changes that occur at different stages in the time periods could be related to factors like difference in the chemical composition of each region. Changes in the first two weeks of preservation may indicate that the formalin stage of preservation tends to expand muscular body areas which after subsequent ethanol preservation, may become dehydrated and consequently decrease in size (Fox *et al.,* 1985). However, initial expansion and subsequent contraction was observed only in *S. esocinus* and not in *S. niger* which may be due tothe difference in the composition of fish bodies, having white muscles or red muscles, which lead to apparent differences in the contraction and expansion

of fish muscles.Moreover,muscle and visceral organization of each species, including swim bladder presence may be differentially affected by formalin-ethanol preservation and could lead to inconsistent measurements (Sotola*et al.,* 2019) that were observed across preservation time periods in our morphometric analyses. Additionally, high variability in water retention in the muscle tissue of species could also affect morphological changes during the preservation process (Leslie and Moore, 1986).

In the present study, the truss dimensions of *S. niger*and *S. esocinus* were investigated under preserved conditions and at different time periods of preservation. All the dimensions showed high correlation coefficients before the transformation for size correction. In order to get size-independent shape variables, the absolute measurements were transformed to remove the effect of size in the data matrix. In order to get comprehensive view of the results and to define the most important variables involved in the preservation effect, PCA (Principal Component Analysis) was performed. All the truss measurements were reduced to get important information in the form of Eigen values, loadings and scores (Chen *et al*, 2015). The PCA was done to explore a larger number of variables, summing the total variance in some variables, called principal components. In our data, the three principal components separated clearly and partly the shape variance before and after preservation. These results indicated that biological conclusions came from PCA results must be taken with caution, as this analysis is often used to link variation with other variables such as phylogenic distance and climatic variables, and establishing possible connections among them. Our results emphasize the idea that fixation or preservation methods could change the PCA values.

In the present study, in case of *S. niger,*principal component analysis of 30 morphometric measurements extracted from three components explained 64.23%of total variance and in *S. esocinus* the first three principal components were responsible for 66.18% of total shape variance. In both the species, Principal components were highly relevant towards the separation of the fresh and

preserved specimens. Martinez *et al*. (2012) also documented 56% of the shape variance in *E. argentus* and 51.84% in *P. corvinaeformis.*This can be attributed to the variation in the amount of water in the muscles in different species.

Bivariate plot of two components in principal component analysis of truss measurements of both *S. niger* and *S. esocinus* indicated separation of fresh and preserved specimens, though some extent of mixing was also observed.

In *S. niger,*out of nine distances that showed meaningful loadings on the first principal component (PC1), seven were straight and oblique depth measurements of fish body. In case of *S. esocinus,*out of six distances that showed meaningful loadings on Principal Component (PC1), four were straight and oblique depth measurements. This may be because of difference in water concentration present in tissues of different body regions of *S. niger*and *S. esocinus*(Ganai, 2012).All the four distances loaded on Principal Component second (PC2) of*S. niger*and twodistances out of four of *S. esocinus*belonged to the head region of the fish body and this could be probably because of the absorption of preservative in gill tissues. Three distances that loaded heavily on the third Principal Component (PC3) in *S.niger*entirely comprised the caudal region of the fish body and in *S. esocinus*two distances that loaded on third Principal Component correspond to the caudal and head region of the fish body. The difference in the effect of preservatives on the different parts of the fish body obtained, might be due to the difference in the chemical composition of each region. Such composition differs from region to region of the fish body respectively to the contraction and expansionfunction (Al-Hassan *et al*., 2000).

Chapter-6

SUMMARYAND CONCLUSION

The present study was conducted to evaluate the effect of preservation (formalin and ethanol) on morphology of *S. niger* and *S. esocinus*. To accomplish this, conventional morphometry and morphometric characteristics based on landmark based truss network analysis was used. Samples of *S. niger* and *S. esocinus* were collected from commercial landings of Dal lake, Kashmir.

Thirteen conventional morphometric measurements were analysed and studied. For truss Morphometry, digitization of samples was done to obtain veritable body shape for morphometric analysis and the morphometric variables and truss distances were extracted from the images using a combination of three software's viz. tpsUtil V1.38, tpsDig2 V 2.1 and Paleontological Statistics (PAST v.3.0). Both univariate and multivariate statistical analysis was carried out to obtain the results. Principal component analysis was applied to size-corrected truss variables to identify influential variables. The results can be summarized as follows:

- The conventional morphometry revealed significant differences in the morphometric characters studied between the fresh and the preserved specimens in both the preservatives at different levels of dissimilarity intensity between the two species examined and also different regions of the fish body at different stages of preservation time.
- In *S. niger,* out of thirteen conventional morphometric characters, three characters namely, head length, pre-orbital length and post-orbital length showed significant difference between fresh and preserved specimens. The characters of pre-dorsal length, pre-pelvic length and pre-pectoral length showed significant difference after 6 weeks of preservation. Total length, standard length, and pre-anal length displayed significant difference only after 14 weeks of preservation while as non-significant difference was observed in

four characters namely, body depth, caudal fin length, eye diameter and snout length ($p>0.05$).

- In *S. esocinus,* among the selected thirteen characters, in three characters namely, head length, pre-orbital length and post-orbital length significant difference was observed after 2 weeks of preservation. Total length, standard length, pre-anal and pre pectoral length displayed significant difference after 14 weeks of preservation. However, non-significant difference was observed in characters of body depth, caudal fin length, eye diameter and snout length ($p>0.05$).
- In case of *S. niger*, pre-orbital length showed highest percentage change in formalin (8.06%) and total length displayed lowest percentage change (0.70%) in formalin. Whereas in alcohol, pre-dorsal length showed highest percentage change (-13.31%) and caudal fin length showed lowest percentage change (0.39%). In case of *S. esocinus,* out of thirteen morphometric characters, pre orbital length showed highest percentage change in formalin (9.25%) and total length showed lowest percentage change (-0,58%) in formalin. Whereas in alcohol, pre-pectoral length was found to display highest percentage change (-13.50%) and eye diameter showed lowest percentage change (0.73%).
- Least –squares regression equations were developed for both the species and in both the preservatives for the thirteen morphometric characters studied.
- In Principal Component Analysis, the first three Principal Components (PC1, PC2 and PC3) were responsible for 64.23 % of the shape variance in *S. niger*.
- The bivariate plot of *S. niger* between PC1 & PC 2 and PC1 & PC3 extracted from the Principal Component Analysis of the truss network of *S. niger* for fresh and preserved samples indicated a clear separation along the X-axis and the graph between PC2 and PC3 clearly demonstrated the segregation of fresh and preserved samples.

- In *S. esocinus* using Principal Component Analysis the first three Principal Components were responsible for 66.18% of the shape variance in *S. esocinus*.
- The bivariate plot of *S. esocinus* between PC1 & PC 2 and PC1 & PC3 extracted from the Principal Component Analysis of the truss network of *S. esocinus* for fresh and preserved samples indicated a clear separation along the X-axis and the graph between PC2 and PC3 clearly demonstrated the segregation of fresh and preserved samples.
- In case of *S. niger*, in Principal Component 1(PC1)out of nine distances that showed meaningful loadings on first Principal Component(PC1), seven were straight and oblique depth measurements of fish body. In case of *S. esocinus,*out of six distances that showed meaningful loadings on first Principal Component (PC1), four were straight and oblique depth measurements.
- All the 4 distances loaded on Principal Component 2 (PC2) of *S. niger* and 4 distances out of 7 of *S. esocinus* belonged to the head region of the fish body

Conclusion

The value of the preserved fishes cannot be understated as these preserved specimens make an ongoing contribution towards our understanding and advancement of science. Although the preservation process alter morphology, efforts to define and detail the limitations that preservation places on specimens are necessary. Our findings will advance the abilities to study and evaluate morphology of preserved *S. niger* and *S. esocinus*. The results of this study contribute significantly towards a fundamental yet often overlooked aspect of preservation effects on fishes that is their body size changes during preservation. Overall this study demonstrates fish body shapes vary within the different species over preservation time. Significant morphological changes occurred incrementally throughout preservation, it would be advantageous to know the timing and degree to which such changes occur such that morphometric measurements are taken at similar time-periods during preservation. Using truss network as a character set has amplified the classical coverage over the body form and enhance the

discrimination among fresh and preserved specimens. Our study introduces the first evidence that the effects of formalin and ethanol on the adults of *S. niger*and *S. esocinus* can effect the results and conclusions of geometric morphometric analysis used in biological research. For truss morphometry, future studies comparing differences in body shapes should be aware of the potential effects that preservation has on morphology. We showed that body shapes can change in inconsistent, varying, and complicating ways after preservation. We would recommend the use of fresh specimens for morphometric measurements, wherever possible. The conversion equations provided here are the first conversion equations for the adult individuals of *S. niger*and *S. esocinus*. We recommend our conversion equations to be used for conventional morphometry of formalin or ethanol preserved *S. niger* and *S. esocinus*where fresh specimens are not available.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Hidaytullah Tak, Associate Professor, Department of Zoology, University of Kashimir** during viva-voce examination held on **24-02-2022**have been incorporated in the manuscript entitled **"The effect of preservation on the morphology of selected schizothoracids"**submitted by**Ms. Mir Iqra Farooq**

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