



# **Effects of Different Ethanol Concentrations on the Morphometric Characteristics of *Clarias gariepinus* and *Oreochromis niloticus***

**H. A. Adedeji <sup>a\*</sup>, P. M. Tadawus <sup>a</sup>, T. A. Idowu <sup>a</sup>  
and R. A. Asiru <sup>b</sup>**

<sup>a</sup> *Department of Fisheries, Modibbo Adama University, Yola, Adamawa State, Nigeria.*

<sup>b</sup> *Department of Biological Sciences, Federal University Gusau, Zamfara State, Nigeria.*

## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/AJFAR/2023/v25i2657

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/103868>

**Original Research Article**

**Received: 08/06/2023**

**Accepted: 14/08/2023**

**Published: 02/09/2023**

## **ABSTRACT**

The effects of ethanol (90% and 95% concentration) preservation on the morphometric characteristics of *Clarias gariepinus* and *Oreochromis niloticus* were investigated. The morphometric characters observed were total length, standard length, head length, body depth, eye diameter, and total weight for the period of sixty-three (63) days. Samples were first euthanized before taking the morphometric characteristics after which the samples were individually preserved in separate bottles filled with respective concentrations (90% and 95%) for nine weeks (63 days). Morphometric characteristics of the samples were later measured at the end of the 63 days to determine any possible changes. The results indicated that varying degree of shrinkage was common in all the observed morphometric characters in both species even though

\*Corresponding author: Email: bowalededeji@mau.edu.ng;

they both reacted differently to different concentration of alcohol. The results also revealed that the percentage shrinkage in all measured morphometric characters was higher in *C. gariepinus* than in *O. niloticus* and higher in 95% concentration than in 90% concentration.

**Keywords:** *Ethanol preservation; morphometric characteristics; shrinkage; Clarias gariepinus; O. niloticus.*

## 1. INTRODUCTION

Preservation methods are generally used to maintain the integrity of biological specimens for long-term storage [1]. Although, these biological samples are generally preserved under varying conditions according to the purpose and further usage [2;3;4]. For the identification of fish specimens, ichthyologists usually use formalin, alcohol, freezing and even chilling as a way to preserve morphological characteristics [4]. However, several fields in fish biology like systematics, growth analyses and fishery stocks management use these morphological data extensively, and any interference that some preservation method may introduce, could directly influence the biological interpretation of the results. Haubrock et al. [5]. reported that storing fish samples in formalin and alcohol as a long-term measure may be reasonable, but the short-term storage of large quantities of such fish samples can be delicate and may not be the most efficient method of fish preservation. However, different short and long-term storage techniques have been shown to affect taxonomically important morphometric characteristics of fish, such as total length, head length, eye diameter, body depth and others [6]. Inaccuracy or error in the morphometric measurement that do not reflect the real length of fish as a results of effects of preservation may considerably affect subsequent analyses [7].

Jawad et al. [2]. Jawad [3] and Neave et al. [8]. have reported the effects of varying preservation methods on pigmentation and morphometric characteristics in different species of fish. These variations in morphology among different preserved species and samples were said to be as a result of several factors such as time elapsing until preservation, preservation method, concentration of preservative, preservation duration and temperature, morphological variability among specimens, and species-specific factors (age, size, state, the osmoregulatory activity of the fish at the point of death) [9]. The present work is aimed to examine

the effect of 90% and 95% concentrations of ethanol on selected morphological characteristics of a scale-less fish, *Clarias gariepinus* and scaled fish, *Oreochromis niloticus*

## 2. MATERIALS AND METHODS

### 2.1 Collection of Samples

Forty (40) individuals of *C. gariepinus* and forty (40) individual of *O. niloticus* of possible similar recruits were obtain from the Upper Benue River, Yola. The *C. gariepinus* ranges between 11.56cm-12.65cm and 12.83g-13.45g in total length and total weight respectively while the *O. niloticus* ranges between 11.95cm-12.25cm and 37.76g-38.27g in total length and total weight respectively.

### 2.2 Experimental Set-up

A set of twenty individuals of *C. gariepinus* and *O. niloticus* were introduced to 90% alcohol preservation while the remaining set of twenty individuals of *C. gariepinus* and *O. niloticus* were introduced to 95% alcohol preservations. Each sample were introduced into individual glass bottles, filled with respective concentration of alcohol after first been euthanized, after which samples were then labelled and preserved. Measurement of the morphometric characteristics were done on the fresh samples prior to preservation and after nine weeks (63 days) of preservation.

### 2.3 Morphometric Measurement

Morphometric characters like body weight (BW), total length (TL), standard length (SL), body depth (BD), head length (HL) and eye diameter (ED) were taken. The body weight was measured by electronic weighing balance to the nearest 0.01g, while the total length, standard length, body depth, head length and eye diameter were measured using digital venier callipar

## 2.4 Data Analysis

The data generated were analyzed using Statistical Package for Social Sciences (SPSS 22.0). A simple descriptive analysis is used to determine the means and one way analysis of variance (ANOVA) was used to separate the means at ( $P= 0.05$ ). The percentage shrinkage was also calculated to determine the decrease in the measured morphometrics characters in the fresh samples compared to the preserved samples.

$$\text{Percentage Shrinkage \%} = \frac{\text{fresh sample} - \text{preserved sample}}{\text{Fresh sample}} \times 100$$

## 3. RESULTS

The effects of ethanol (90%) preservation on *C. gariepinus* and *O. niloticus* for the period of nine weeks (63 days) is presented in Table 1. The results showed varying degree of reduction in the morphometric characters of both studied species. *C. gariepinus* had the lowest percentage shrinkage in the body depth (5.48) and followed by the total length (5.95), while the total weight had the highest percentage shrinkage (16.42) over the preservation period. Similarly, *O. niloticus* had the lowest percentage shrinkage in the total length (2.39) and followed by the standard length (2.89), while the highest percentage shrinkage was observed in the eye diameter (11.54), and followed by the total weight (10.96). The results also revealed that *C. gariepinus* preserved in 90% alcohol showed higher percentage shrinkage in all the parameters measured compared to that of *O. niloticus*. However, there was no significant differences ( $p>0.05$ ) in all the morphometric characters in both *C. gariepinus* and *O. niloticus* before preservation and after preservation except in the mean eye diameter and total weight.

The effects of ethanol (95%) preservation on *C. gariepinus* and *O. niloticus* for the period of nine weeks (63 days) is presented in Table 2. The results also revealed varying degree of reduction in the morphometric characteristics of both fresh and preserved *C. gariepinus* and *O. niloticus*. The results revealed that *C. gariepinus* had lowest percentage shrinkage (7.07) in the head length and followed by the total length (7.31) while the total weight had the highest percentage shrinkage (17.91) and followed by the eye diameter (14.29). *O. niloticus*, had the

lowest percentage shrinkage (3.01) in the head length and followed by the standard length (3.18) while the highest percentage shrinkage was observed in total weight (14.91) and eye diameter (13.25) respectively.

However, there was no significance differences ( $p>0.05$ ) between the measured characters in the fresh and preserved *C. gariepinus* and *O. niloticus* except in the mean eye diameter and total weight. The results also revealed that the *C. gariepinus* preserved in 95% alcohol had higher percentage shrinkage compared to *O. niloticus* that was exposed to the same concentration. The results also revealed significance differences ( $p>0.05$ ) between morphometric characters of *C. gariepinus* and *O. niloticus* preserved in 90% and 95% alcohol. The results also revealed higher percentage shrinkage in all morphometric characters in *C. gariepinus* and *O. niloticus* stored in 95% alcohol and significantly different from that of 90% alcohol

## 4. DISCUSSION

The use of different preservatives have been reported to cause change in the body proportion of the fish. In this research, decrease in length in most of the characters measured was an evident of shrinkage of the morphometric characters and exit of interstitial fluid as reported by Hossaini, et al. [10]. The shrinkage observed over the preservation period is in accordance with the results of Jawad et al. [2]; Jawad [3] and Hossaini, et al. [10] who all revealed that different concentrations of alcohol causes various degrees of shrinkage in total length, standard length and head length. The higher percentage shrinkage observed in total weight, eye diameter and body depth in both species and in both concentration also corroborate with the work of Berbel-Filho et al. [1]. who reported higher percentage shrinkage in body depth and eye diameter. However, the result obtained from this study is not in agreement with the work of Scott et al. [11]. who reported length gain in bluegill larvae preserved in 90% alcohol for the period of twenty-six (26) days while Jawad (3) also reported no effect of alcohol preservations on the head length of *Alepes djeddaba* preserved at 70% concentration. The differences observed in this study and that of Scott et al. [11]. may be attributed to differences in age and sizes of the samples used as Hossaini, et al. [10] already reported them as factors that influence changes in morphometric

characteristics. Differences in this present study and the report of Jawad (3) on *Alepes djeddaba* may be attributed to differences in fish species and concentration of ethanol used. This present study is also in accordance with the study of Sotola et al. [12]. who reported larger changes in eye diameter and body depth of cyprinids preserved for a period of less than 1 year.

Changes in morphometric characters of preserved species has been reported to be influenced by various factors such as method of preservation, concentration and type of chemical preservation agents, length of preservation period, salinity and temperature of the preservative [3;10;13]. The type of species, age, size and developmental state of the

**Table 1. Effects of 90% ethanol preservation on Morphometric characters of *Clarias gariepinus* and *Oreochromis niloticus* for the period of sixty-three days (nine weeks)**

<b>90% alcohol preservation</b>				
<b>Morphological character</b>	<b>Before preservation in alcohol (cm)</b>	<b>After preservation in alcohol</b>	<b>Shrinkage (cm)</b>	<b>Percentage shrinkage</b>
<b><i>C. gariepinus</i></b>				
Total length	11.76±1.24 <sup>a</sup>	11.06±1.23 <sup>a</sup>	0.70	5.95
Standard length	10.39±1.21	9.62±1.09 <sup>a</sup>	0.77	7.41
Head length	3.09±0.31 <sup>a</sup>	2.89±0.32 <sup>a</sup>	0.2	6.47
Body depth	1.64±0.16 <sup>a</sup>	1.55±0.14 <sup>a</sup>	0.09	5.48
Eye diameter	0.23±0.04 <sup>a</sup>	0.20±0.03 <sup>a</sup>	0.03	13.24
Total weight	12.91±3.34 <sup>a</sup>	10.91±2.77 <sup>b</sup>	2.12	16.42
<b><i>O. niloticus</i></b>				
Total length	12.09±1.47	11.81±1.43 <sup>a</sup>	0.28	2.39
Standard length	9.67±1.18 <sup>a</sup>	9.39±1.12 <sup>a</sup>	0.28	2.89
Head length	3.38±0.48 <sup>a</sup>	3.28±0.39 <sup>a</sup>	0.1	2.96
Body depth	4.10±0.33 <sup>a</sup>	3.94±0.31 <sup>a</sup>	0.16	3.90
Eye diameter	0.78±0.09 <sup>a</sup>	0.69±1.00 <sup>a</sup>	0.09	11.54
Total weight	38.04±9.92 <sup>a</sup>	33.87±8.17 <sup>b</sup>	4.17	10.96

\*Mean Values on the same row with the same superscripts are not significantly ( $p=0.05$ ) different

**Table 2. Effects of 95% ethanol preservation on Morphometric characters of *Clarias gariepinus* and *Oreochromis niloticus* for the period of sixty-three days (nine weeks)**

<b>95% alcohol preservation</b>				
<b>Morphological character</b>	<b>Before preservation in alcohol (cm)</b>	<b>After preservation in alcohol</b>	<b>Shrinkage (cm)</b>	<b>Percentage shrinkage</b>
<b><i>C. gariepinus</i></b>				
Total length	11.77±1.29 <sup>a</sup>	10.91±1.06 <sup>a</sup>	0.86	7.31
Standard length	10.46±1.29	9.53±0.96 <sup>a</sup>	0.93	8.89
Head length	3.11±0.32 <sup>a</sup>	2.89±0.28 <sup>a</sup>	0.22	7.07
Body depth	1.55±0.29 <sup>a</sup>	1.41±0.19 <sup>a</sup>	0.14	9.0
Eye diameter	0.21±0.04 <sup>a</sup>	0.18±0.03 <sup>a</sup>	0.03	14.29
Total weight	13.01±4.65 <sup>a</sup>	10.68±3.82 <sup>b</sup>	2.33	17.91
<b><i>O. niloticus</i></b>				
Total length	11.83±1.41 <sup>a</sup>	11.42±1.38 <sup>a</sup>	0.41	3.47
Standard length	9.44±1.05 <sup>a</sup>	9.14±1.03 <sup>a</sup>	0.30	3.18
Head length	3.32±0.43 <sup>a</sup>	3.22±0.40 <sup>a</sup>	0.10	3.01
Body depth	3.89±0.44 <sup>a</sup>	3.71±0.38 <sup>a</sup>	0.18	4.63
Eye diameter	0.83±0.05 <sup>a</sup>	0.72±0.06 <sup>a</sup>	0.11	13.25
Total weight	34.01±10.03 <sup>a</sup>	28.94±8.65 <sup>b</sup>	5.07	14.91

\* Mean Values on the same row with the same superscripts are not significantly ( $p=0.05$ ) different

preserved fish are all factors that determine changes in morphometric characters of a preserved fish. The two studied fish species reacted to the 90% and 95% ethanol concentration differently in which the *C. gareipinus* recorded higher percentage shrinkage compared to that *O. niloticus* may be because of species differentiation, which determines the variation in tissue water content and the ratio of white to red muscle. The presence of scales may also be a factor, which may influence the surface ratio of the studied fish exposed to the preservative agent. The higher percentage shrinkage observed in the 95% alcohol compared to 90% alcohol in both fish species may be influenced by the concentration of the preservatives as already been reported as a factor that determines rate of changes in the morphometric characteristics of fish.

## 5. CONCLUSION

In conclusion, this study has shown that the ethanol preservations can cause dehydration of fish tissues, which may ultimately result to various degree of shrinkage. The study also revealed that 90% and 95% ethanol concentration have different effects but similar pattern on both *C. gareipinus* and *O. niloticus* fish species preserved for the period of sixty-three (63) days. The result also revealed that there was no significant differences in the studied morphometric characteristics in both fresh and preserved *C. gareipinus* and *O. niloticus* except in the eye diameter and total weight. However, significant effects could have been observed had it been the preservation period is increased beyond 63 days, which may considerably affect subsequent and further biological analyses of the examined morphometric characters.

## COMPETING INTERESTS

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

## REFERENCES

1. Berbel-Filho WM, Jacobina UP, Martinez PA. Preservation effects in geometric morphometric approaches: Freezing and alcohol in a freshwater fish. *Ichthyological Research*. 2013;60(3):268–71.
2. Jawad LA, Toumi FFE, Barasi HME, Silini OAE. The effect of preservatives and freezing on the morphological characters of two sparid fishes. *Indian Journal of Fisheries*. 2001;48(4):423-426.
3. Jawad LA. The effect of formalin, alcohol and freezing on some body proportions of *Alepes djeddaba* (Pisces: Carangidae) collected from the Red Sea coast of Yemen. *Revista De Biología Marina Y Oceanografía*. 2003;38(2):77-80.
4. Ghaly AE, Dave D, Budge S, Brooks MS. Fish spoilage mechanisms and preservation techniques. *American Journal of Applied Sciences*. 2010;7(7):859-877.
5. Haubrock PJ, Balzani P, Johović I, Inghilesi AF, Tricarico E. The effects of two different preservation methods on morphological characteristics of the alien channel catfish *Ictalurus punctatus* (Rafinesque, 1818) in European freshwater. *Croatian Journal of Fisheries*. 2018;76: 80-84.
6. Al-Hassan LAJ, Shawafi NA. The effect of preservatives and freezing on the morphological characters of *Rastrelliger kanagurta* (Pisces: Scombridae). *Journal of Animal Morphology and Physiology*. 1997;44:17-20.
7. Porter SM, Brown AL, Bailey KM. Estimating live standard length of net-caught walleye Pollock (*Theragra chalcogramma*) larvae using measurements in addition to standard length. *Fish. Bulletin*. 2001;101:384–404.
8. Neve FB, Mandrak NE, Docker MF, Noakes DL. Effects of preservation on pigmentation and length measurements in larval lampreys. *Journal of Fish Biology*. 2006;68(4):991-1001.
9. Yeh CF, Hodson RG. Effects of formalin on length and weight of bluegill and white crappie from Lake Nasworthy, Texas. *Southwest Naturalist*. 1975;20:315-322.
10. Hossaini SA, Vajargah MF, Sattari M. The Effect of Preservation in Alcohol on The Morphological Characters of The Zagros Tooth-Carp, *Aphanius vladkovi* Coad, 1988. *Journal of Environmental Treatment Techniques*. 2016;4(4):118-120.
11. Scott WM, Dudenhoefter Gregory A, Omara-Alawla, Thomas R. Effects of chemical preservatives on weights and lengths of Bluegill Larvae. *Cooperative Extension Research*; 2016 Publications 1.

12. Sotola VA, Craig CA, Pfaff PJ, Maikoetter JD, Martin NH, Bonner TH. Effect of preservation on fish morphology over time: Implications for morphological studies. PLOS ONE. 2019;14(3): e0213915. Available:<https://doi.org/10.1371/journal.pone.0213915>
13. Macdonald JS, Williamson CJ, Patterson DA, Herunter HE. The shrinkage of Sockeye salmon fry fixed in 10% formalin and preserved in 37.5% isopropanol. Canadian Technical Report of Fisheries and Aquatic Sciences. 1997;2198: 16.

---

© 2023 Adedeji et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*  
*The peer review history for this paper can be accessed here:*  
<https://www.sdiarticle5.com/review-history/103868>