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FIXATION, PRESERVATION AND FREEZING EFFECTS ON MORPHOMETRICS OF TWO FISH SPECIES COLLECTED FROM LAKE GANVIÉ, BENIN, WEST AFRICA

SUMMARY

The goal of this study was to evaluate the impacts of various preservation methods on disparities in morphometric parameters while also providing correction factors to re-calculate the original body dimensions when sampled fish are measured at a future date. In this study, adult samples of *O. niloticus* and *C. gariepinus* collected from the waters of the Republic of Benin, West Africa was measured directly after capture, then either frozen, preserved in 70% ethanol, or in 10% formalin. Ethanol and formalin solutions were prepared using tap and distilled waters. They were again measured after one week of the initial measurement and thereafter on a weekly basis for 20 weeks. Formalin and ethanol solutions showed to cause a decrease in the three morphometric characters, total, standard and head lengths. In contrast, frozen *O. niloticus* and *C. gariepinus* showed less shrinkage. Of the three preservation procedures, freezing obviously produced the least distortions. Therefore, freezing is recommended as the most desirable preservation method, particularly in multi-disciplinary studies on fish ecology. Equations to correct the effect of fixation, preservation and freezing on fish size are provided so they will assist in the future ecological and physiological studies to pool field and laboratory data.

INTRODUCTION

Morphological variations associated with preservation of adult fishes have broadly reported. Changes have mainly been based on length or proportion measurements (Leslie and Moore, 1986; Lee *et al*., 2012), or length–weight

relations (Al-Hassan *et al.*, 2000). The two cichlids species *O. niloticus* and *C. gariepinus* are important form both the commercial and the ecological points of views. The present study aims to quantify the effect of fixation, preservation and freezing on the morphology of *O. niloticus* and *C. gariepinus* collected from Lake Ganvié, Benin, West Africa and to provide the necessary conversion equations for these species.

MATERIAL AND METHODS

Thirty specimens of each of *O. niloticus* and *C. gariepinus* were obtained directly from fishers operating in Lake Ganvié on 1st December 2015 (Fig. 1). Specimens were divided in five lots of 6 fish to be used for formalin, alcohol and freezing experiments. Fixative and preservative solutions were prepared as follows: two lots of 10% formalin and two lots of 70% ethanol, each pair of lots being mixed with tap and distilled water. The fifth lot was used for the freezing experiment, for which a one cubic metre commercial deep freeze unit was used. Body dimensions chosen were total length (TL), standard length (SL) and head length (HL) (widely used by taxonomists and fisheries biologists), giving a total of 10 experimental variables. Measurements were taken immediately after capture. Specimens in the freezing experiment were directly laid on and covered with ice cubes and were then transported to a standard freezer within one hour and frozen at -20°C. Specimens for fixative and preservative experiments were directly put in jars prepared for this purpose containing either fixative or preservative on site after measurement. Each specimen was measured weekly over a twenty weeks period. In the case of frozen samples, defrosting was achieved at ambient temperature over a period of 2 - 4 hours. Duncan's multiple test was applied to examine the effect of different preservatives and freezing on body proportions (Harraway, 1997). Preservation effects on fish length were examined statistically using paired-sample *t*-tests. Least-squares linear regression was used to examine the effect of preservation time on shrinkage for the specimens of the two species in question. The relationship between fresh and preserved measurements was described using least-squares linear regression. The percentage change in length was calculated as:

100 X (fresh size - preserved size) (fresh size)$^{-1}$

Where fresh size was determined from the linear regression. The range of per cent shrinkage was determined by calculating the per cent change in length for both the smallest and largest fishes used in each equation. For statistical comparison among preservation methods (frozen, 70% ethanol and 10% formalin) was accomplished using ANCOVA with preserved length as the covariate.

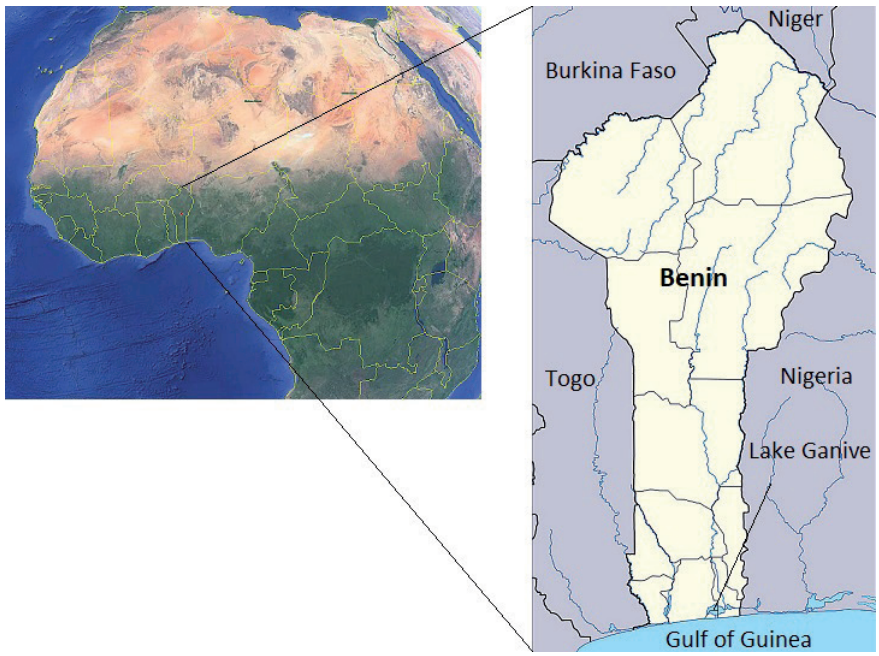

Figure 1. Map showing capture point of *Oreochromis niloticus* and *Clarias gariepinus* in Lake Ganvié, Bénin.

RESULTS

The general trend of the effects of fixatives, preservatives and freezing on the total length , standard length and head length in both *O. niloticus* and *C. gariepinus* respectively is shrinkage with different levels in relation to species, fish body parts and type of fixative, preservatives and freezing (Figs. 2, A-J). The shrinkage of the fish body as result of using formalin, ethanol and freezing range 4-30% and 4.1-17.9% for *O. niloticus* and *C. gariepinus* respectively (Table 1).

Least-squares regression equations also revealed shrinkages in length due to fixation, preservation and freezing fishes of the examined sizes (Table 1). For each species, the slopes of the equations were greater than one indicating that entire loss owing to shrinkage increased with fish size. ANCOVA results between preservatives indicated significant differences length reduction of individuals of the two species in question. Slopes of shrinkages of individuals of *O. niloticus* and *C. gariepinus* put in formalin and ethanol mixed with tap water or distilled water were not significantly different (D.F. = 117, P = 0.483). Similarly, for the slopes of shrinkages in TL, SL and HL due to freezing in *O. niloticus* have shown no significant difference (D.F. = 385, P = 0.524), whereas there was a significant difference between shrinkages of TL, SL and

HL as a result of freezing in *C. gariepinus* (ANCOVA, D.F. = 118, P < 0.483).

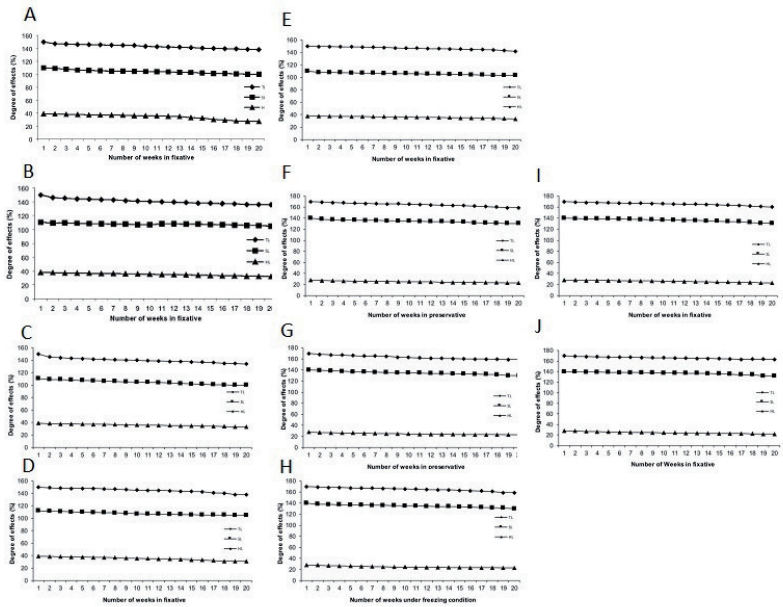

Figure 2. A, Effect of formalin 10% -tap water on different body proportions of *Oreochromis niloticus*. HL, head length; SL, standard length; TL, total length; B, Effect of formalin 10% -distilled water on different body proportions of *Oreochromis niloticus*; *C,* Effect of ethanol 70% -tap water on different body proportions of *Oreochromis niloticus*; D, Effect of ethanol 70% -distilled water on different body proportions of *Oreochromis niloticus*; E, Effect of freezing on different body proportions of *Oreochromis niloticus*; F, Effect of formalin 10% -tap water on different body proportions of *Clarias gariepinus*; G, Effect of formalin 10% -distilled water on different body proportions of *Clarias gariepinus*; H, Effect of ethanol 70% -tap water on different body proportions of *Clarias gariepinus*; I, Effect of ethanol 70% -distilled water on different body proportions of *Clarias gariepinus*; J, Effect of freezing on different body proportions of *Clarias gariepinus*.

Table 1. Least-squares regression equations of L_T, L_S and L_H of *Oreochromis niloticus* and *Clarias gariepinus* collected from Lake Ganvié, Benin, West Africa based on different fixatives, preservatives and freezing. L_{FH}, head length, fresh; L_{FPS}, standard length, preserved in freezing; $L_{FT,}$ total length, fresh; L_{FS}, standard length, fresh; L_{HPF}, head length, preserved in freezing; L_{HPDW}, head length, preserved, formalin-distilled water; L_{HPEDW}, head length, preserved ethanol-distilled water; L_{HPETW}, head length, preserved ethanol-tap water; L_{HPTW}, head length, preserved formalin-tap water; L_{SPDW}, standard length, preserved, formalin-distilled water; L_{SPEDW}, standard length, preserved, ethanol-distilled water; L_{SPETW}, standard length, preserved, ethanol-tap water; $L_{SPTW,}$ standard length, preserved, formalin-tap water; L_{TPF}, total length, preserved in freezing; $L_{TPDW,}$ total length, preserved, formalin-distilled water; L_{TPEDW}, total length, preserved, ethanol-distilled water; $L_{TPETW,}$ total length, preserved, ethanol-tap water; $L_{TPTW,}$ total length, preserved, formalin-tap water.

Species/morphometric character	Fixative, preservative, freezing	Conversion equation
Oreochromis niloticus		
Total length (TL)	10% Formalin + tap water	$L_{FT} = 1.018\ L_{TPTW} + 0.132$
	10% Formalin + distilled water	$L_{FT} = 1.017\ L_{TPDW} + 0.117$
	70% ethanol + tap water	$L_{FT} = 1.013\ L_{TPETW} + 0.211$
	70% ethanol + distilled water	$L_{FT} = 1.011\ L_{TPEDW} + 0.345$
	Freezing	$L_{FT} = 1.036\ L_{TPF} + 0.623$
Standard length (SL)	10% Formalin + tap water	$L_{FS} = 1.013\ L_{SPTW} + 0.286$
	10% Formalin + distilled water	$L_{FS} = 1.012\ L_{SPDW} + 0.562$
	70% ethanol + tap water	$L_{FS} = 1.020\ L_{SPETW} + 0.238$
	70% ethanol + distilled water	$L_{FS} = 1.023\ L_{SPEDW} + 0.362$
	Freezing	$L_{FS} = 1.038\ L_{SPF} + 0.872$
Head length (HL)	10% Formalin + tap water	$L_{FH} = 1.045\ L_{HPTW} + 0.876$
	10% Formalin + distilled water	$L_{FH} = 1.041\ L_{HPDW} + 0.984$
	70% ethanol + tap water	$L_{FH} = 1.082\ L_{HPETW} + 0.298$
	70% ethanol + distilled water	$L_{FH} = 1.083\ L_{HPEDW} + 0.362$
	Freezing	$L_{FH} = 1.039\ L_{HPF} + 0.198$
Clarias gariepinus		
Total length (TL)	10% Formalin + tap water	$L_{FT} = 1.000\ L_{TPTW} + 0.431$
	10% Formalin + distilled water	$L_{FT} = 1.003\ L_{TPDW} + 0.228$
	70% ethanol + tap water	$L_{FT} = 1.016\ L_{TPETW} + 0.298$
	70% ethanol + distilled water	$L_{FT} = 1.014\ L_{TPEDW} + 0.334$
	Freezing	$L_{FT} = 1.076\ L_{TPF} + 0.665$
Standard length (SL)	10% Formalin + tap water	$L_{FS} = 1.010\ L_{SPTW} + 0.292$
	10% Formalin + distilled water	$L_{FS} = 1.009\ L_{SPDW} + 0.671$
	70% ethanol + tap water	$L_{FS} = 1.026\ L_{SPETW} + 0.853$
	70% ethanol + distilled water	$L_{FS} = 1.027\ L_{SPEDW} + 0.532$
	Freezing	$L_{FS} = 1.035\ L_{SPF} + 0.765$
Head length (HL)	10% Formalin + tap water	$L_{FH} = 1.032\ L_{HPTW} + 0.612$
	10% Formalin + distilled water	$L_{FH} = 1.095\ L_{HPDW} + 0.786$
	70% ethanol + tap water	$L_{FH} = 1.093\ L_{HPETW} + 0.261$
	70% ethanol + distilled water	$L_{FH} = 1.092\ L_{HPEDW} + 0.962$
	Freezing	$L_{FH} = 1.099\ L_{HPF} + 0.177$

DISCUSSION

In the present study, it has been confirmed that the fixation, preservation and freezing have an effects on fish body proportions of individuals of *O. niloticus* and *C. gariepinus*. Recognizing that shrinkage occurred with fish preservation supports the results of a number of recent studies (Al-Hassan *et al*., 2000; Lee *et al*., 2012). The results of the present study can affect conclusions of morphometric analysis used in biological research in future studies in the Republic of Benin.

The figures 2-10 showed different levels of dissimilarity intensity between the two species examined, and also different regions on the fish body as showed in other studies (Leslie and Moore, 1986; Al-Hassan *et al*., 2000; Jawad, 2003). The present results showed that the head length shrunk in a range of 4.5-30.8% and 15.6-17.9% for *O. niloticus* and *C. gariepinus* respectively. This is in a contrast with those of Leslie and Moore, (1986), who suggested that the decreased shrinkage in the head length is due to presence of small amount of soft tissue like muscles.

In the present study, the general trend of change was shrinkage. Such results were supported by Sayers (1987). In contrast, BILLY (1982), Al-Hassan and Abdullah (1992), and Al-Hassan *et al*. (1999, 2000) recommended that there was a slight increase in standard length or no shrinkage in specimens preserved in formalin and ethyl alcohol based for other fish species. Our disparate findings on the time and amount of shrinkage using two different species confirm that the response to preservation is species-specific.

The results presented here are the first shrinkage correction equations for the adult individuals of *O. niloticus* and *C. gariepinus* living in the freshwater systems of the Republic of Benin.

Buchheister and Wilson (2005) showed that shrinkage was greater in formalin followed by ethanol and the least shrinkage was in freezing individuals of *O. niloticus* and *C. gariepinus* studied. The results of the present study are in support of Buchheister and Wilson (2005) and justifying freezing as an equally valid means of preserving samples of the two species in question to be used for studies concerning morphometrics.

Freezing can be used for all important analyses such as stomach content, condition factor, otoliths and stable isotopes. Consequently, it should be presented as a typical preservation routine for fish intended for morphometric analyses. Management in the field is easy, cheap, and bears no health risks.

REFERENCES

Al-Hassan L. A. J., Abdullah J. N. 1992- The effect of formalin and freezing on some body proportions of *Barbus luteus*. *Pakistan Journal of Zoology*, 24: 353-354.

Al-Hassan L. A. J., Bujawari J. A., El-Siline O. A. 1999- Additional report on the effect of preservatives and freezing on morphological characters of four sparid fish species collected from Benghazi, Libya. *Journal of Animal Morphology and Physiology*, 46: 57-62.

Al-Hassan L. A. J., Bujawari J. A., El-Siline O. A. 2000- The effect of some preservatives and freezing on certain body dimensions of two species of the family Mullidae collected from Benghazi waters, Libya. *Acta Ichthyologica et Piscatoria*, 30: 127-136.

Billy A. J., 1982- The effects of formalin and isopropyl alcohol on length and weight measurements of *Sarotherodon mossambicus* Trewavas. *Journal of Fish Biology*, 21: 107-112.

Buchheister A., Wilson M. T., 2005- Shrinkage correction and length conversion equations for *Theragra chalcogramma*, *Mallotus villosus* and *Thaleichthys pacificus*. *Journal of Fish Biology*, 67: 541-548.

Fey D. P., 1999- Effect of preservation technique on the length of larval fish: methods of correcting estimates and their implication for studying growth rates. *Archives of Fisheries and Marine Research*, 47: 17-29.

Fey D. P., 2012- Length adjustment of larval and early-juvenile cod (*Gadus morhua*) after up to 3 years of preservation in alcohol. *Journal of Applied Ichthyology*, 1: 2.

Fowler G. M., Smith S. J., 1983- Length changes in silver hake (*Merluccius bilinearis*) larvae – effects of formalin, ethanol, and freezing. *Canadian Journal of Fisheries and Aquatic Science*, 40: 866-870.

Harraway J., 1997- Introductory statistical methods for biological, health and social sciences. University of Otago Press, Dunedin, New Zealand, 342 pp.

Jawad L. A., 2003- 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*, 38: 77-80.

Lee J., Kodama K., Horiguchi T., 2012- Change in body size of juvenile marbled sole *Pseudopleuronectes yokohamae* after preservation in ethanol. *Ichthyological Research*, 59: 49-52.

Leslie J. K., Moore J. E., 198- Changes in lengths of fixed and preserved young freshwater fishes. *Canadian Journal of Fisheries and Aquatic Science*, 43: 1079-1081.

Radtke R. L., Waiwood K. G., 1980- Otolith formation and body shrinkage due to fixation in larval cod (*Gadus morhua*). *Canadian Technical Report on Fisheries and Aquatic Science* 929.

Sayer R. E., 1987- Effects of freezing in and out of water on length and weight of Lake Michigan bloaters. *North American Journal of Fishery Management*, 7: 299-301.

Shields P. A., Carlson S. R., 1996- Effects of formalin and alcohol preservation on lengths and weights of juvenile sockeye salmon. *Alaska Fishery Research Bulletin*, **3**: 81-93.