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Effects of fixation and freezing on some morphometric characteristics of Nile tilapia

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ABSTRACT

Fish preservation methods including use of formalin and freezing are widely used to preserve fish specimen in the laboratory to maintain their freshness for future laboratory analysis. This present study aimed to investigate the effects of fixation and freezing on the morphometric characteristics of Nile tilapia, Oreochromis niloticus. Forty (40) samples of a single cohort of O. niloticus were obtained from the Tono reservoir in Navrongo, Ghana. Total length (TL) and body weight (W) of each fish were measured. Twenty (20) samples of O. niloticus were subjected to freezing at -4ºC whilst the remaining twenty (20) were fixed in 4% formaldehyde solution. The study lasted for thirteen (13) days during which the length and weight were determined repeatedly in a sequence during the storage period. Although there was no significant difference ($p > 0.05$) in the change of length and weight measured during the study, all samples showed some degree of shrinkage within the storage period. For samples preserved by freezing, there was a 5.62 % and 19.61 % reduction in length and weight respectively, while those preserved in formalin reduced by 5.24% and 10.72% in length and weight respectively. For condition factor (k), there was no change at the end of the experiment for samples preserved by freezing but a marginal increase of 0.08% was realized for those preserved in formalin. Though shrinkage occurred in both samples preserved in formalin and freezing, the greatest shrinkage was recorded by those preserved by freezing.

Keywords: *Fixation, formalin, freezing, preservation, morphometric, length, weight*

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* is a fish of African origin belonging to the family Cichlidae. It occurs in a wide variety of freshwater habitats like rivers, lakes, canals, and irrigation channels. It is a species of high economic value and it has been widely introduced outside its natural range. *O. niloticus* and its hybrids are the most important cultured fish species, as well as becoming an increasingly important food fish in many parts of the world (Garibaldi, 1996). It is the major species farmed in Ghana, and according to FAO (2007), it constitutes over 80% of aquaculture production in the country and it occurs in several rivers, as well as man-made lakes.

Different preservation methods such as formalin and freezing are used to preserve fish specimen in the laboratory to maintain

their freshness for future laboratory analysis. However, body proportions of fish preserved in this manner may show variable degrees of change after a standard period. Most authors have reported a decrease in length (Al-Hassan and Abdullah, 1992) and some authors report changes in weight and condition factor (Engel, 1974). Studies of fish preservation have been carried out with various fish species such as herring (Schnack and Rosenthal, 1978), marine fish food organisms (Kuhlmann *et al*., 1982), young walleye (Glenn and Mathias (1987), *Stizostedion vitreum* (Treasure, 1990), *Perca fluviatilis* L., and pike *Esox lucius* L., (Stodolnik *et al*., 1992), and sea trout egg (Al-Hassan and Shawafi, 1997). In the reports of all these studies, there were varying changes in the morphometrics of fish after the preservation periods.

Various studies have shown that the use of formalin and freezing have effects on the length, weight and condition factor of fish after a while (Glenn and Mathias, 1987; Al-Hassan and Shawafi, 1997; Al-Hassan and Abdullah, 1992). It is important to find out how these preservation methods will affect the morphometric measurements of an important fish such as Nile tilapia. Such information will help researchers use the appropriate method to preserve their tilapia specimen to meet the needs of their research. It is for this reason that the present study investigated the effects formalin or freezing on the morphometric characteristics of Nile tilapia, *O. niloticus* harvested from the Tono reservoir in Navrongo, Ghana.

MATERIALS AND METHODS

Collection of fish samples

The fish samples were obtained from Tono reservoir in Navrongo in the Upper East Region of Ghana. A total of forty (40) specimens of a single cohort of Nile tilapia, *O. niloticus* were obtained from the Tono reservoir by artisanal fishers who use gillnets of mesh size ranging 2 – 8 cm. The fish samples were packaged in sterile polyethene bags and placed in an ice chest containing ice and immediately transported to the GetFund Laboratory of the University for Development Studies, Navrongo Campus, Ghana, where the experiment was conducted. The identity of the samples was confirmed using the field identification guide by Dankwa *et al*. (1999).

Measurement of length and weight

Immediately the samples got to the laboratory, measurement of length and weight of each fish sample was taken. The total length (TL) of each sample was measured on a measuring board. The measurement was taken from the tip of the snout to the tip of the caudal fin and recorded to the nearest 0.01 cm as the initial length. The body weight was also measured using an electronic balance (XY-2C series electronic balance) after using a tissue to mop off water from the surface of the specimens and recorded to the nearest 0.01g as the initial weight.

Experimental set-up

Twenty (20) specimens of *O. niloticus* were subjected to freezing at -4ºC temperature while the remaining twenty (20) were fixed in 4% formaldehyde treatment after the initial measurement of length and weight. The experiment took place for thirteen (13) days within which the measurement of length and weight were repeated every other day during the storage period (i.e. 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th} and 13^{th} days). Before measurements were taken on a set day, specimens in the formalin were removed and allowed to dry for some time before their measurements were taken. The frozen samples were also allowed to completely thaw before measurements were

taken. The fish samples were placed back into their respective treatments right after the measurements were taken.

Estimation of condition factor (k)

The condition factor (k) was calculated from the relationship:

$k = 100W/L^3$ (Gomiero and Braga, 2005).

Where 'W' and 'L' are the mean body weight and mean total length of the fish, respectively.

Statistical analysis

Data collected from the experiment were presented as the mean ± standard error of the mean (SEM). The data failed normality test and were subjected to Mann Whitney U test to detect the difference in length, weight and condition factor (*k*) before and after treatment.

RESULTS AND DISCUSSION

Although there were no significant differences ($p > 0.05$) between all the values of length and weight measured during the study, the samples showed some degree of shrinkage within the storage period.

Table 1 below shows the effects of freezing and formalin treatments on the length and Figure 1 shows the percentage (%) change in length during the preservation period of *O. niloticus*. A mean reduction in length (from 17.14 - 16.72 cm for freezing and from 17.55 - 17.34 for formalin representing 2.42% and 1.19% change for freezing and formalin preservations respectively) was seen to occur after the first two (2) days of storage. On the final day (13th day), the mean length reduced to 15.90 cm for freezing and 16.63 cm for formalin representing a percentage reduction of 7.22% and 5.24% for freezing and formalin respectively. From this result, it was realized that the greatest change in length was recorded in specimens preserved by freezing.

TABLE 1. **Effect of Fixation and Freezing on the Length (cm) of *O. niloticus***

	Treatment	Initial Length (cm) ± *Se*	Final Length (cm) ± *Se*	Change in Length (cm)	*P*-values
	Freezing	17.14 ± 0.72	17.14 ± 0.72	0.00	-
Day 1	Fixation	17.55 ± 0.76	17.55 ± 0.76	0.00	-
	Freezing	17.14 ± 0.72	16.72 ± 0.69	-0.42	0.5517
Day 3	Fixation	17.55 ± 0.76	17.34 ± 0.75	-0.21	0.5705
	Freezing	17.14 ± 0.72	16.56 ± 0.68	-0.58	0.4651
Day 5	Fixation	17.55 ± 0.76	17.27 ± 0.75	-0.28	0.5703
	Freezing	17.14 ± 0.72	16.4 ± 0.68	-0.74	0.4248
Day 7	Formalin	17.55 ± 0.76	17.08± 0.72	-0.47	0.3638
	Freezing	17.14 ± 0.72	16.3 ± 0.68	-0.83	0.3576
Day 9	Fixation	17.55 ± 0.76	16.91 ± 0.71	-0.64	0.3064
	Freezing	17.14 ± 0.72	16.17 ± 0.68	-0.97	0.2975
Day 11	Fixation	17.55 ± 0.76	16.82 ± 0.70	-0.73	0.2725
	Freezing	17.14 ± 0.72	15.9 ± 0.66	-1.24	0.2034
Day 13	Fixation	17.55 ± 0.76	16.63 ± 0.69	-0.94	0.2561

Note: Fish were frozen at -4ºC and fixed in formaldehyde solution of 4%

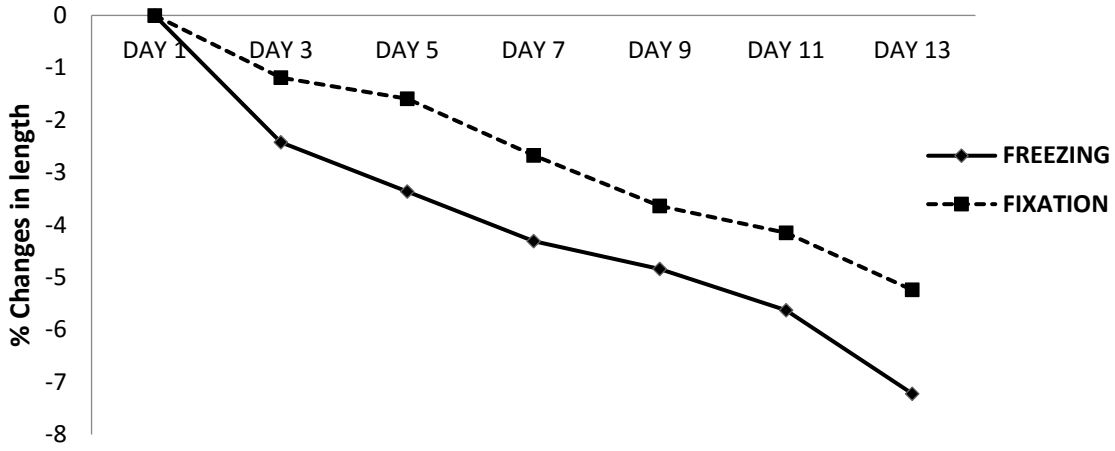

FIGURE 1. **Percentage change in Length during the Preservation Period of *O. niloticus***

Table 2 below shows the effects of freezing and formalin on the weight and Figure 2 shows the percentage (%) change in Weight during the preservation period. A mean reduction in weight (from 102.13 - 96.53g for freezing and from 103.19 - 101.35g for formalin representing 5.47% and 1.74% change for freezing and formalin respectively) was seen to occur after the first two (2) days of storage. On the final day (13th day), the mean weight reduced to 82.1g for freezing and 91.5g for formalin representing a percentage reduction of 19.61% and 10.72% for freezing and formalin respectively. Likewise, the case of length, it was realized that the greatest change in weight was recorded in specimens preserved by freezing.

TABLE 2. **Effect of Fixation and Freezing on the Weight (g) of *O. niloticus***

DAY	Treatment	**Initial Weight (g) ± *Se***	**Final Weight (g) ± *Se***	**Change in Weight (g)**	***P*-values**
	Freezing	102.13 ± 13.24	102.13 ± 13.24	0.00	-
Day 1	Fixation	103.19 ± 9.72	103.19 ± 9.72	0.00	-
	Freezing	102.13 ± 13.24	96.53 ± 12.75	-5.59	0.6554
Day 3	Fixation	103.19 ± 9.72	101.35 ± 9.36	-1.84	0.9097
	Freezing	102.13 ± 13.24	92.89 ± 12.32	-9.21	0.5609
Day 5	Fixation	103.19 ± 9.72	100.39 ± 9.45	-2.80	0.7737
	Freezing	102.13 ± 13.24	90.4 ± 12.11	-11.73	0.4249
Day 7	Formalin	103.19 ± 9.72	96.6 ± 9.29	-6.59	0.5707
	Freezing	102.13 ± 13.24	86.49 ± 11.58	-15.64	0.2977
Day 9	Fixation	103.19 ± 9.72	93.92 ± 8.88	-9.27	0.4274
	Freezing	102.13 ± 13.24	83.75 ± 11.32	-18.38	0.1895
Day 11	Fixation	103.19 ± 9.72	92.53 ± 8.77	-10.66	0.4274
	Freezing	102.13 ± 13.24	82.1 ± 11.31	-20.03	0.1636
Day 13	Fixation	103.19 ± 9.72	91.5 ± 8.75	-11.69	0.3447

Note: Fish were frozen at -4ºC and fixed in formaldehyde solution of 4%

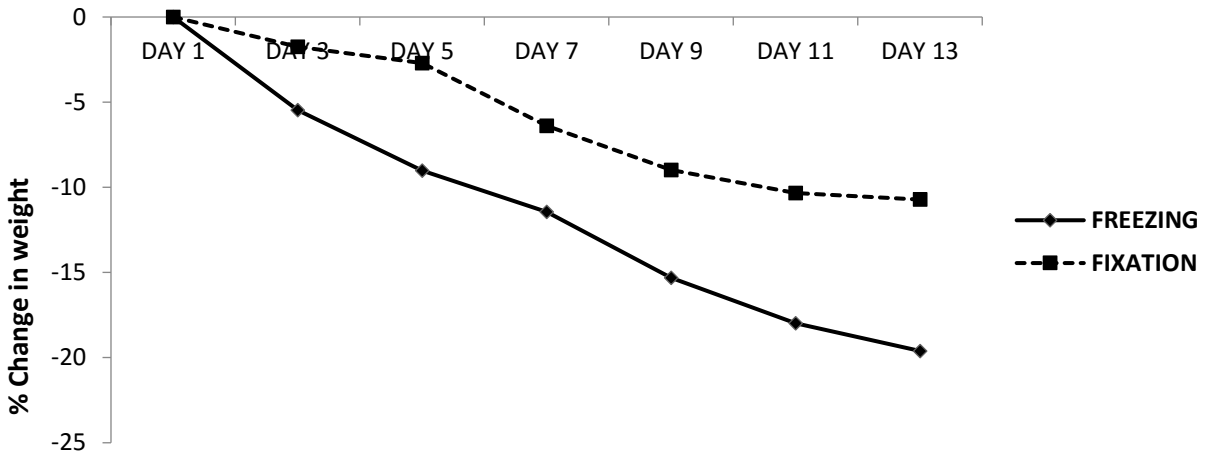

FIGURE 2. **Percentage (%) change in Weight during the Preservation Period of *O. niloticus***

Table 3 below shows the effects of freezing and formalin on the condition factor (*k*) whilst Figure 3 shows percentage (%) change in condition factor (*k*) during the Preservation Period of *O. niloticus*. An increment in condition factor (from 2.03 - 2.07 for freezing, and from 1.91 - 1.98 for formalin were observed representing -0.04 and -0.07changes in condition factor for freezing and formalin respectively) was seen to occur after the first two (2) days. Changes in the condition factor reduced on the 5th, 7th, 9th, and 11th days, while an increment was observed on the 13th day for both freezing and formalin. Likewise, the case of length and weight, it was also realized that the greatest change in condition factor was recorded in specimens preserved by freezing.

TABLE 3. **Effect of Fixation and Freezing on the Condition Factor (*k*) of *O. niloticus***

	Treatment	Initial Mean k	Final Mean k	Change In k
	Freezing	2.03	2.03	0.00
Day 1	Fixation	1.91	1.91	0.00
	Freezing	2.03	2.07	0.04
Day 3	Fixation	1.91	1.98	0.07
	Freezing	2.03	2.05	0.02
Day 5	Fixation	1.91	1.95	0.04
	Freezing	2.03	2.03	0.00
Day 7	Formalin	1.91	1.95	0.04
	Freezing	2.03	1.99	-0.04
Day 9	Fixation	1.91	1.94	0.03
	Freezing	2.03	1.98	-0.05
Day 11	Fixation	1.91	1.94	0.03
	Freezing	2.03	2.03	0.00
Day 13	**Fixation**	1.91	1.99	0.08

Note: Fish were frozen at -4ºC and fixed in formaldehyde solution of 4%

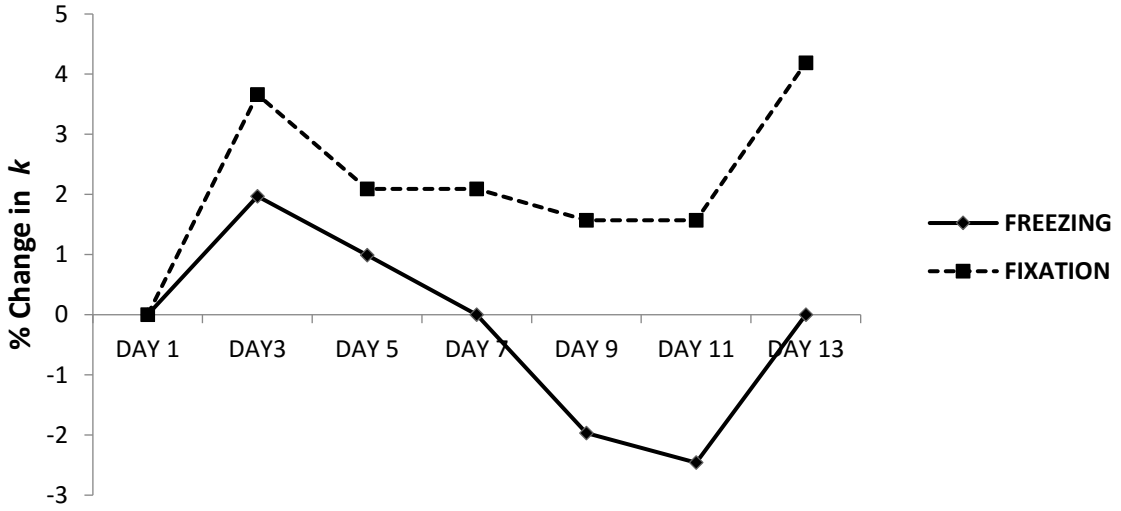

FIGURE 3. **Percentage (%) change in Condition Factor (*k*) during the Preservation Period of *O. niloticus***

The study revealed a reduction in length and weight of the *O. niloticus* samples after preserving them in formalin and freezing. Similar findings have been observed by other researchers using different fish species. For instance, Ajah and Nunoo (2003), observed shrinkage of *Sardinella aurita* after subjecting them to four preservation conditions - freezing, formalin, smoking and salting. *S. aurita* decreased in length, weight and condition factor, except for an increase in condition factor (k) with formalin in this study. They, therefore, proposed that some adjustments in length, weight and condition factor were necessary to equate preserved fish samples to fresh ones. Puigcerver (1999) studied European minnow, *Phoxinus phoxinus* and noted a significant decrease in both length and weight measurements due to fixation and preservation.

Al-Hassan and Shawafi (1997) kept marine *Rastrelliger kanagurta* in different concentrations of formalin and observed that some fishes increased in size, whilst other fishes reduced. This study however, did not show any increment in the morphometric measurement of the *O. niloticus* samples. It rather revealed a faster shrinkage rate especially, in samples preserved by freezing (though not significantly different ($p > 0.05$) from those preserved in formalin), which did not conform to the result of Jawad (2003) who observed a greater shrinkage in fish samples preserved in 5% formalin with tap water than in fishes stored in 70% alcohol with tap water.

However, the result of this study is in accordance with those of Billy (1982) for *Sarotherodon mossambicus* and Al- Hassan and Abdullah (1992) for *Barbus luteus*, in which they both reported slow changes in the fish body proportions due to preservation, though the rate of shrinkage in their study was comparatively higher. According to Theilacker (1980), most literature reports have recorded shrinkage for larvae of various species at all length, when placed in Formaldehyde solution. This discrepancy between studies may be due to different handling of the species before storage which can cause a greater degree of shrinkage than the fixative itself. The shrinkage in this study

might have occurred due to the loss of internal water from the fish. A little change in length and weight or the morphometric characteristics affected the true condition and biology of the fish and that is likely to affect the understanding of existing relationships among various taxonomic categories.

CONCLUSION AND RECOMMENDATION

This study realized a reduction in the morphometric characteristics of *O. niloticus* samples preserved in both formalin and freezing, but the greatest shrinkage was recorded from samples preserved by freezing. However, these changes were not statistically significant. The shrinkage that occurred was attributable to loss of internal water from the fish. As much as possible, samples of fish collected from the field should be worked on immediately on the field or in the laboratory, but if it becomes necessary to preserve, formalin should be used. Further study should be conducted using alcohol as a preservative to see whether its effects will be lower than formalin as reported in some studies.

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Conflict of Interest
The authors declare no conflict of interest.

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