

Effect of Some Preservative Solutions on Some Morphometric Features of Planiliza abu (Heckel, 1843)

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Abstract

The present study attempts to find out the effect of some fish preservatives in the laboratory, such as alcohol and dilute formalin, on some biological characteristics related to the body measurements of those fish preserved in these materials. The fish used in this study were the local Planiliza abu. The processes of expansion and contraction of the bodies of fish preserved in diluted formalin solution at a concentration of 10% and diluted ethyl alcohol solution at a concentration of 70%. As that the standard length of the specimens of this study, which are separately preserved in formalin 10% and alcohol 70%, in a completely isolated are fluctuating in change. Constant shrinkage in head length in both diluted formalin and alcohol. Most fish bodies preserved in formalin at a concentration of 10% gain significant weight gain, in contrast to alcohol preservation.

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Introduction

Planiliza abu is one of the Iraqi local fish that belongs to the Mugilidae family. Its total length is 26 cm and distributed in the Tigris and Euphrates basins; it does not enter the Arab Gulf. It is found in large quantities in all water bodies in the middle and Southern Iraq. Its small size reduces its economic value; nevertheless, it is a popular and local fish with wide popularity in Iraqi markets. This species is also found in the Euphrates River Basin in Syria (Misra, 1947; Khalaf, 1961; Mahdi, 1962; Al-Daham, 1984). What distinguishes this species of this family is their bright silver color and tapering bodies (Razzaq et al, 2015). It is considered one of the coastal tropical fish that live in a salty and little salty environment as shown by (Thomson, 1997; Nelson, 2006). The most important methods for preserving fishes, in general, are four internationally known methods, which are freezing, preservation in salt, preservation with chemicals represented by diluted formalin and ethyl alcohol. These preservatives may affect some of the biometrics of fish specimens, especially of different lengths (Jawad, 2003). Many standard changes appear on the fish bodies preserved in formalin and alcohol for different standard periods. Evidence come from many researchers as indicated by (Lux, 1960; Parker, 1963; Stobo, 1972; Engel, 1974; Sayers, 1987 and Haubroc et al, 2018). These

substances may cause contractions and shrinkage in the bodies of the fish preserved in them (Billy, 1982; Al-Hassan and Abdullah, 1992; Al-Hassan and Shawafi, 1997; Al-Hassan et al, 1999, 2000).

Some researchers argue that there is a loss in the weight of the specimens preserved in formalin, (Cultter and Whitesel, 1956; Parker, 1963). Billy (1982) says that there is a loss in weight and there is a clear difference between the live weight and the weight of the fish after preserving in both solutions: diluted formalin and alcohol (Banha et al, 2017).

Kelvin 1994 believes that it is possible that the concentrations of some elements, especially heavy elements in fish bodies preserved in formalin, may change over time due to formalin acidity and the interaction of these elements over time. Formalin acidity and the interaction lead to an increase in the concentrations of some of them or a decrease in the concentrations of others, or they even lead to the disappearance of these elements due to the variable interactions caused by the long period of preservation in formalin. The elements also vary in their concentrations over time if the fish specimens are preserved with dilute alcohol to a concentration of 70-75%. The effect of these two solutions, formalin, and alcohol, is clear during the first periods of preservation with the disappearance of fish color if the species have clear coloration

(Dallinger et al, 1987). In this study, we find that these elements are concentrated in the bodies of the fish at a higher rate than those in the water, in their surroundings, or other sediments in this environment, as a result of consuming the organisms in that environment (Olafa et al, 2004). On the other hand, the presence of these heavy elements in different concentrations in the bodies of fish is a sign of the pollution occurring in the environment of these fish (Jassem et al, 2007). The bodies of fish may be preserved in formalin 10% and then stored in alcohol 70%. Great changes in the bodies of fish, preserved according to this method and for a long time in several forms, will appear as indicated by (Wilke et al. 1996; Arrington and Winemille, 2002).

Materials and Methods

Ethical Approval

All applicable national and international guidelines for the care and use of animals were followed.

Experimental Animal

A total of 40 P. abu fish were divided into two groups, each group consisted of 20 fish. The first group was kept in a diluted formalin solution at a concentration of 10%; the second group was kept in a diluted ethyl alcohol solution at a concentration of 70%. The two solutions were diluted with non-ionic distilled water. Some biometrics was measured by using the traditional method to calculate the total, standard length, head length, and weight for both groups. The readings were recorded once a week for an entire month.

Results and Discussion

Figure 1 the local P. abu fish. The general shape and outward appearance of the fish used in this study.



Figure 1. Planiliza abu

The biometric rates of P. abu fish preserved in alcohol were 70% in the four weeks: 20.18cm was the total length, the standard length was 16.5cm, the head length was 4cm and the weight was 22.8g. However, the biometric rates for fish preserved in formalin were 10% for the four weeks: the total length was 19.6cm, standard length was 16.4cm, the head length was 4cm and the weight was 19.8g. From table 1 we can follow up general linear model between-subjects factors. Then it follows with table 2 the descriptive statistics. Table 3 indicates the analysis of variance of specimens preservative and time in general in addition to the interaction between them.

Table 1. general linear model between-subjects factors

		Value Label	N
Preservative_Type	1	Alcohol 70%	80
	2	Formalin 10%	80
Time	1	1st Week	40
	2	2nd Week	40
	3	3rd Week	40
	4	4th Week	40

Table 2. the descriptive statistics of the experiment

	Preservative_Type	Time	Mean	Std. Deviation	N
Total_Length (cm)	Alcohol 70%	1st Week	10.325	.4128	20
		2nd Week	10.255	.4524	20
		3rd Week	10.420	.3901	20
		4th Week	10.370	.4402	20
		Total	10.342	.4209	80
	Formalin 10%	1st Week	9.665	.4258	20
		2nd Week	9.980	.4538	20
		3rd Week	9.920	.4503	20
		4th Week	9.710	.4340	20
		Total	9.819	.4531	80
	Total	1st Week	9.995	.5320	40
		2nd Week	10.118	.4684	40
		3rd Week	10.170	.4869	40

		4th Week	10.040	.5458	40
		Total	10.081	.5090	160
Standard_Length (cm)	Alcohol 70%	1st Week	8.280	.3302	20
		2nd Week	8.415	.3588	20
		3rd Week	8.545	.3410	20
		4th Week	8.310	.3684	20
		Total	8.388	.3588	80
		Formalin 10%	1st Week	8.120	.3901
	2nd Week		8.340	.3169	20
	3rd Week		8.225	.4339	20
	4th Week		8.150	.3763	20
	Total		8.209	.3839	80
	Total	1st Week	8.200	.3658	40
		2nd Week	8.377	.3363	40
		3rd Week	8.385	.4179	40
		4th Week	8.230	.3764	40
		Total	8.298	.3811	160
Head_Length (cm)	Alcohol 70%	1st Week	2.070	.1689	20
		2nd Week	2.060	.0883	20
		3rd Week	2.080	.0894	20
		4th Week	2.050	.1539	20
		Total	2.065	.1284	80
	Formalin 10%	1st Week	1.930	.2296	20
		2nd Week	2.075	.0444	20
		3rd Week	1.985	.0366	20
		4th Week	1.910	.2174	20
		Total	1.975	.1703	80
	Total	1st Week	2.000	.2112	40
		2nd Week	2.068	.0694	40
		3rd Week	2.032	.0829	40
		4th Week	1.980	.1990	40
		Total	2.020	.1569	160
Weight (gm)	Alcohol 70%	1st Week	13.950	1.2763	20
		2nd Week	11.650	1.2258	20
		3rd Week	11.950	1.3945	20
		4th Week	14.000	1.3765	20
		Total	12.888	1.6988	80
	Formalin 10%	1st Week	10.950	1.6376	20
		2nd Week	10.100	1.7741	20
		3rd Week	10.950	1.5720	20
		4th Week	10.900	1.7442	20
		Total	10.725	1.6912	80
	Total	1st Week	12.450	2.0995	40
		2nd Week	10.875	1.6975	40
		3rd Week	11.450	1.5517	40
		4th Week	12.450	2.2066	40
		Total	11.806	2.0078	160

Table 3. Analysis of variance of specimens preservative and time in general in addition to the interaction between them

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Total_Length	12.701 ^a	7	1.814	9.681	.000
	Standard_Length	2.717 ^b	7	.388	2.896	.007
	Head_Length	.661 ^c	7	.094	4.410	.000
	Weight	293.044 ^d	7	41.863	18.288	.000
Intercept	Total_Length	16259.040	1	16259.040	86749.885	.000
	Standard_Length	11017.421	1	11017.421	82201.395	.000
	Head_Length	652.864	1	652.864	30487.044	.000
	Weight	22302.006	1	22302.006	9742.506	.000
Preservative_Type	Total_Length	10.973	1	10.973	58.544	.000
	Standard_Length	1.278	1	1.278	9.536	.002
	Head_Length	.324	1	.324	15.130	.000
	Weight	187.056	1	187.056	81.714	.000
Time	Total_Length	.733	3	.244	1.304	.275
	Standard_Length	1.125	3	.375	2.797	.042
	Head_Length	.177	3	.059	2.747	.045
	Weight	72.919	3	24.306	10.618	.000
Preservative_Type * Time	Total_Length	.996	3	.332	1.771	.155
	Standard_Length	.314	3	.105	.781	.506
	Head_Length	.161	3	.054	2.498	.062
	Weight	33.069	3	11.023	4.815	.003
Error	Total_Length	28.489	152	.187		
	Standard_Length	20.372	152	.134		
	Head_Length	3.255	152	.021		
	Weight	347.950	152	2.289		
Total	Total_Length	16300.230	160			
	Standard_Length	11040.510	160			
	Head_Length	656.780	160			
	Weight	22943.000	160			
Corrected Total	Total_Length	41.190	159			
	Standard_Length	23.089	159			
	Head_Length	3.916	159			
	Weight	640.994	159			
a. R Squared = .308 (Adjusted R Squared = .277)						
b. R Squared = .118 (Adjusted R Squared = .077)						
c. R Squared = .169 (Adjusted R Squared = .131)						
d. R Squared = .457 (Adjusted R Squared = .432)						

Table 4 shows total length of P. abu fish bodies preserved in formalin at the first week began to increase and the expansion occurred in the bodies in the second week. Then the body began to shrink down through the last week of the experiment and the total length regain its initial measurement when first to stored in the dilute formalin solution. We notice, from the same table, that the total length of the fish preserved in diluted alcohol start to decline and the shrinkage of the bodies occurred at the second week. The total length increased and the expansion was at its peak at the third week of the experiment to settle at a total length higher than the initial total length of the fish bodies at the start of the experiment. This indicates the expansion and contraction processes in the fish bodies preserved in these two solutions, which is confirmed by (Jawad, 2003). Most of the biometrics Fish bodies preserved in formalin at a concentration of 5% were stable until the experiment was in the seventh week when changes appeared in these measurements; noting that the formalin concentration was not the same in the two experiments. (Shields and Carlson, 1996) explained that the bodies of salmon fish preserved in alcohol had a loss in total length at day 16 of the experiment; it stabilize on day 70 of the same experiment; the total length of the salmon's bodies increased after this period. The results were identical to the present study when there is shrinkage in the bodies of fish, noting that the two types are different in these two studies. (Shields and Carlson, 1996) studied the bodies of salmon larvae whereas this study included adult P. abu fish. No significant effect was detected in the salmon fish bodies preserved in formalin 5% distilled water. All the studied biometrics appeared to be rather stable as shown by (Shields and Carlson, 1996), but in our study, there was a clear change in most of P. abu studied biological characteristics. It may be attributed to the difference between formalin concentrations in the two experiments, or to the type of water in which these solutions were diluted. (Razzaq et al, 2015) also confirms that there was an increase in the total length of fish preserved in a formalin solution at a concentration of 10%, the length of the experiment, especially in the first days of collecting samples, and this is what happened in this study when there was an increase in the total length of fish preserved for two weeks of experience

Table 4. Total length

Time	N	Subset for alpha = 0.05	
		1	2
1st Week	20	9.665	
4th Week	20	9.710	9.710
3rd Week	20	9.920	9.920
2nd Week	20		9.980
Sig.		.088	.070

Means for groups in homogeneous subsets are displayed.

- a. Preservative_Type = Formalin 10%
- b. Uses Harmonic Mean Sample Size = 20.000.

Table 5. Standard length

Time	N	Subset	
		1	2
1st Week	40	8.200	
4th Week	40	8.230	8.230
2nd Week	40		8.377
3rd Week	40		8.385
Sig.		.715	.075

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .134.

- a. Uses Harmonic Mean Sample Size = 40.000.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
- c. Alpha = .05.

Table 5 shows a continuous increase in the standard length and the bodies expansion of the fish preserved continuously in both solutions. A two-week preservation period (in formalin) gave the highest reading of the standard length measurement. The fish bodies began to shrink again at the end of the experiment; whereas the fish bodies preserved in alcohol reached the highest reading of the standard length at the third week. However; they shrank again at the end of the experiment for both bodies preserved in the two solutions with different compositions. The difference in the chemical composition of these two solutions may be a major impact on the standard length of the fish bodies preserved in them. This is opposite to what was stated by (Jawad, 2003) in his study; the increases in standard length were greater for the bodies of fish preserved in alcohol 70% than those fish preserved in formalin 5%. The cause may be the difference

between the water used to dilute the solutions or maybe the different storage temperatures; besides, the difference in formalin concentration in the two experiments, which gave different durations for the contraction and expansion for the fish bodies in the two experiments. The difference between the studied species in both studies may be also another cause. The bodies may differ according to the type in the speed of response to the preservation solutions and thus the speed of contraction and expansion may be affected.

Some previous studies have shown that standard body lengths are lower when fixed in formalin 10% and then preserved in 70% alcohol for a long time (Shields and Carlson, 1996) and (Greszkiewicz and Fey, 2018). However, in this study the models were preserved, each separately in formalin 10% and alcohol 70% for a month only, and completely isolated, (Hossaini et al., 2016) mentioned that after an initial evaluation on morphological characters, Samples were fixed and preserved in 96% alcohol for 3 months. Results indicated 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

Table 6. Head Length

Time	N	Subset	
		1	2
4th Week	40	1.980	
1st Week	40	2.000	2.000
3rd Week	40	2.032	2.032
2nd Week	40		2.068
Sig.		0.132	0.052

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .021.

- a. Uses Harmonic Mean Sample Size = 40.000.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
- c. Alpha = .05.

On the other hand, the measurement of the head had some changes as well. Table (6) shows the speed of expansion of the heads of fish preserved in formalin 10%. The peak of expansion was in the second week of preservation; then, it quickly shrank

in the third. After these rapid variations in the fourth week. Unlike the measurement of the head length of fish preserved in alcohol 70%, we notice from table 6 that it was slow to stabilize at the second week of the experiment and the measurements of head length fluctuated rapidly to give the highest reading at the third week to stabilize in the fourth week. The measurement of the head length in both solutions in the second week specifically gave the first change. These results were identical to what was stated by (Jawad, 2003), as a constant contraction was recorded in the head length of the fish preserved in 5% of distilled water and formalin; however, there was a sudden shrinkage, although there was a slight fluctuation of water 10% of the fish in the second week studied in these two experiments.

The weight change characteristic of fish preserved in two preservative solutions, formalin 10% and alcohol 70%, were studied. Table 7 shows us that fish shrank sharply in both solutions to record in the second week the highest reading of body shrinkage and weight loss; the average weight of fish preserved in alcohol 70% was 26.5gm, whereas the average weight of fish preserved in formalin 10% was 20.8gm, maintaining a slight increase in the third week, as the bodyweight of fish preserved in alcohol 70% became 26.7gm and in formalin 10% became 20.8gm. Then, in the fourth week, it maintained its initial weights before the experiment. It is noticed that despite the rapid contraction and expansion in the bodies of fish preserved in alcohol 70% and the slow contraction and expansion in formalin 10%, the periods of expansion and weight gain were equal in the second week. There was a slight shrinkage that continued to the third week; the weight, of both bodies preserved in these two solutions, regained its normal level at the beginning of the experiment in the fourth week. (Shields and Carlson, 1996) explained that after 106 days of their experiment there was a significant increase with a high moral value for salmon weight when preserved in formalin 5% distilled water, while there were no significant changes in body weight of fish preserved in alcohol 70% distilled water for the same experiment. (Shields and Carlson, 1996) and (Ajah and Nunoo, 2003) confirm that most fish bodies preserved in formalin at a concentration of 10% gain significant weight after a period of preservation.

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 period is

consistent with the duration of the present study, which also lasted for one month. The bodies shapes undergo significant and clear changes when preserved in solutions of formalin 10% and alcohol 70% (Berbel et al, 2013) and (Martinez et al, 2013). The biometrics of fish bodies preserved for a period of more than ten years may change to be of useless value, due to the long shelf life (Larochelle et al, 2016).

Table 7. Weight

Time	N	Subset	
		1	2
2nd Week	40	10.875	
3rd Week	40	11.450	
1st Week	40		12.450
4th Week	40		12.450
Sig.		.091	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2.289.

a. Uses Harmonic Mean Sample Size = 40.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

There was no curvature in the fish body preserved, for a preservation period that lasted four weeks, in both solutions formalin 10% and alcohol 70%. It was possible that the short preservation period, in the present study, did not allow the fish bodies to curve due to the shrinkage that occurs when preserved for a period of time longer than the preservation period in this study. (Valentin *et al*, 2008) explains that the curvature of fish bodies preserved in formalin and alcohol occurs when the preservation period is long, which gives false and difficult readings of the biometrics of fish bodies preserved in these solutions.

Conclusions

We conclude here that the difference in the response of fish bodies to the states of contraction, expansion, and weight gain, when preserved in alcohol, is due to the difference between the different environments of the studied species, whether they are saltwater fish or freshwater fish. Besides; there is a difference between species

according to their genetic makeup, which is influenced by the environment in which these species are found. The difference in the composition of fish bodies varies according to fish types, having white muscles or red muscles, which lead to apparent differences in the contraction and expansion of fish bodies. Moreover; the size of the body also affects the fish preserved in their response to changes when preserved in formalin and alcohol. The environment is one of the important factors in the formation and the nature of fish types; especially since genetic factors are affected by the environment. Hence, there are differences in the changes occurring on fish bodies, such as the biometrics of some studied characteristics of fish bodies when preserved in solutions preservation or freeze.

Conflict of interests

The author whose name is listed below certifies that he has no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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