



The Effect of Preservation in Formalin on the Morphological Characters of Spiralin (*Alburnoides Eichwaldii*)

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Abstract

The present study was done to investigate the effects of fixing and preserving in 10% formalin on the morphological characteristics of Spiralin (*Alburnoides eichwaldii*). For this aim, 35 specimens of Spiralin were collected from Vajargah River, Gilan, Iran. After initial evaluation on morphological characters such as Total Length (TL), Standard Length (ST) and Head Length (HL), samples were fixed and preserved in 10% formalin for 6 months. After this period, samples were removed from formalin and measurement and evaluation of color features were done once again. The results showed shrinkage was common in all of the specimens and changes in body color were clearly distinguishable with fresh fish such a way that the body and fins color were opaque but color pattern is acceptable but the intensity is reduced.

Key words: Formalin, Spiralin, Morphological characteristics, Fixation

1 Introduction

Alburnoides eichwaldii inhabit streams and rivers in foothills with well oxygenated, fast-flowing water. All age classes occur in open water of streams and small rivers [1]. Found also in rivers with very calm waters. Feeds on insect larvae and dead insects as well as on crustaceans and diatoms [2]. *Alburnoides eichwaldii* was selected for the bioassay experiments because of it is widespread in most part of Europe and Asia and It can be found in Marine-Neritic, Marine-Oceanic, Brackish water and Freshwater [3].

Ichthyologists often have used freezing, formalin or alcohol for preserving fish specimens. Morphological characters are essential for taxonomic and population studies [4; 5; 6; 7; 8]. Identification keys are often based on morphological characteristics and pigment of fixed samples. Because of the variability of properties in the process of fixing and maintaining and also the different effects of different fixation methods on the color pattern of fixed samples, the morphological characters in live and fresh samples are different with fixed samples [9]. There are few studies in this field such as: Neve et al [10]. Investigated the effects of two methods of preservation (fixation and storage in 10% formalin, and fixation in 10% formalin followed by storage in 95% alcohol) on pigmentation and morphometric features of larval Ichthyomyzon lampreys. Jawad et al [11] studied the effect of preservatives and freezing on the morphological

characters of two sparid fishes. Also the effect of some preservatives and freezing on two species of mullidae family was investigated by Al-Hassan et al [12].

In this study, the effect of fixation and preservation in formalin was investigated on the morphological characters of Spiralin (*Alburnoides eichwaldii*).

2 Materials and Methods

To performing this test, 35 specimens of *Spiralin* (*Alburnoides eichwaldii*) were collected from Vajargah River, Gilan, Iran. They were measured within one day of capture. The morphological characters measured were total length (TL), standard length (SL) and head length (HL). Measured was conducted by using a graded caliper with an accuracy of 0.02 mm. After measuring the TL, SL and HL, color pattern of skin and fins were investigated. Then each sample was placed into a Tube with a certain number filled with 10% Formalin for fixed samples.

Samples were removed from the 10% Formalin after Six months and measurement and evaluation of color features were performed once again.

3 Results and Discussion

Effect of Formalin on morphometric characters of *Alburnoides eichwaldii* are listed in Table 1 According to the present results and observe the average of morphometric characters in Table 2, length reduction was common in all the specimens. Also, changes in body color were clear. Fresh fish had a silver and black color but after removing from 10% formalin, body color became opaque (see Fig.1).

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Table 1: Effect of formalin on morphometric characters of *Alburnoides eichwaldii* (TL: total length, SL: standard length, EL: head length)

Before preserved in Formalin (mm)			After preserved in Formalin (mm)			Amount of Shrinkage (mm)		
TL	SL	HL	TL	SL	HL	TL	SL	HL
85.00	71.00	13.00	80.69	65.63	14.92	-4.31	-5.37	1.92
82.00	68.00	15.00	81.44	67.34	16.74	-0.56	-0.66	1.74
67.00	55.00	12.00	65.94	54.78	13.74	-1.06	-0.22	1.74
81.00	65.00	14.00	78.01	63.61	15.77	-2.99	-1.39	1.77
91.00	75.00	18.00	88.43	73.66	16.37	-2.57	-1.34	-1.63
70.00	57.00	14.00	67.80	56.86	13.55	-2.20	-0.14	-0.45
88.00	72.00	15.00	82.19	63.67	16.30	-5.81	-8.33	1.30
75.00	62.00	15.00	75.74	62.67	15.35	0.74	0.67	0.35
73.00	60.00	14.00	67.06	56.45	14.71	-5.94	-3.55	0.71
67.00	54.00	13.00	64.89	53.81	13.91	-2.11	-0.19	0.91
72.00	59.00	14.00	71.23	57.40	15.04	-0.77	-1.60	1.04
73.00	61.00	15.00	71.14	58.17	14.42	-1.86	-2.83	-0.58
94.00	78.00	18.00	73.89	59.29	18.97	-20.11	-18.71	0.97
69.00	55.00	13.00	65.82	52.73	13.24	-3.18	-2.27	0.24
66.00	54.00	12.00	65.37	54.48	14.47	-0.63	0.48	2.47
85.00	70.00	15.00	83.28	68.15	15.50	-1.72	-1.85	0.50
72.00	59.00	13.00	77.55	60.07	15.27	5.55	1.07	2.27
75.00	62.00	14.00	71.64	60.25	14.15	-3.36	-1.75	0.15
88.00	73.00	17.00	84.90	70.71	15.96	-3.10	-1.29	-1.04
90.00	72.00	17.00	88.63	69.30	16.45	-1.37	-2.70	-0.55
72.00	59.00	14.00	70.32	57.30	13.44	-1.68	-1.70	-0.56
70.00	56.00	13.00	67.81	55.39	13.89	-2.19	-0.61	0.89
77.00	62.00	13.00	77.04	59.48	14.66	0.04	-2.52	1.66
69.00	57.00	13.00	68.15	56.87	14.36	-0.85	-0.13	1.36
78.00	66.00	14.00	70.09	64.30	15.40	-7.91	-1.70	1.40
77.00	64.00	14.00	75.44	61.75	15.74	-1.56	-2.25	1.74
70.00	57.00	13.00	68.79	56.12	14.97	-1.21	-0.88	1.97
97.00	81.00	17.00	95.69	79.33	20.04	-1.31	-1.67	3.04
80.00	68.00	16.00	79.81	66.75	15.62	-0.19	-1.25	-0.38
89.00	72.00	16.00	87.51	71.39	17.13	-1.49	-0.61	1.13
78.00	68.00	14.00	77.76	64.72	15.43	-0.24	-3.28	1.43
81.00	67.00	15.00	78.21	64.03	16.40	-2.79	-2.97	1.40
77.00	65.00	14.00	77.85	64.29	16.41	0.85	-0.71	2.41
67.00	54.00	16.00	67.49	50.85	15.75	0.49	-3.15	-0.25
80.00	67.00	15.00	81.70	68.23	15.37	1.70	1.23	0.37

Our results show that during fixation, total and standard length were decreased due to tissue shrinkage, but head length was increased that it is probably because of water and formalin absorbent in gill tissue. Fish sample number 13 had the most change in total length with 20.11 mm decrease. Also most change in standard length was in number 13 with 18.71 decreases. Head length had the most change in sample number 28 with 3.04 decreases. Al-Hassan et al. [12] studied on effects of freezing, preserving in alcohol and formalin on morphometric characters of two species of the family mullidae were different with our results and gained different results with our findings. They observed increasing of TL, SL and HL of specimens after 10 weeks preserving in 10% formalin that probably was due to period and method of preserving in 10% formalin.

Table 2: Average of TL, SL and HL before and after preserved in formalin

	Before preserved in formalin (mm)	After preserved in Formalin (mm)
Average of TL	77.857	75.694
Average of SL	64.142	62.023
Average of HL	14.514	15.412

Before fixation

After fixation



Figure 1: Picture Spiralin before and after preserved in formalin

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Jawad et al [11] revealed that different concentrations of formalin and alcohol on one hand and freezing on the other caused variable degrees of shrinkage of the three characters studied (SL, TL, HL). Also, Neve et al [10] investigated the effects of fixing in ethanol and formalin on pigments and total length in lampreys larval and found color and total length of body decreased after 3 weeks preserving in formalin.

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, particular factors to the fish being preserved are serious including: age, size, species and developmental state, the presence of rigor mortis, and the osmoregulatory state of the fish at death [13].

4 Conclusions

In the present study, changes in body color of samples after preserving in formalin were clearly distinguishable with fresh fish, but this color loss, does not mean complete loss of color pattern. Body length and color of fixed fish

displayed diverse degrees of change after a standard period of preservation in different preservatives, thus, it should be noted that reduction of color intensity in fixed samples in formalin, does not have interdiction in color identification keys.

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