



The Effect of Preservation in Alcohol on The Morphological Characters of The Zagros Tooth-Carp, *Aphanius vladykovi* Coad, 1988

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Abstract

The present research was performed to investigate the effects of fixed and preserved in 96% alcohol on the morphological characteristics of the Zagros pubfish, *Aphanius vladykovi* Coad, 1988. For this purpose, 30 specimens of *A. vladykovi* were collected from rivers in Chaharmahal-o-Bakhtiyari Province, Iran. After an initial evaluation on morphological characters (TL, SL and HL), Samples were fixed and preserved in 96% alcohol for 3 months. After this period, samples were removed from the alcohol and then measurement and evaluation of color features were performed once again. The 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.

Keywords: Alcohol, *Aphanius vladykovi*, Morphological characters, Fixation

1 Introduction

The Zagros tooth-carp, *Aphanius vladykovi*, is an endemic species found in Chaharmahal-o-Bakhtiyari Province, Iran and is an appealing species for holding in aquaria. It also has sexually dimorphism and is a valuable biological species to deal with malaria. Researches precedent on this fish are studies on morphology, biology, parasitology, karyology and genetic diversity (1; 2; 3; 4; 5). To prevent the degradation of biological samples, often they are fixed.

Ichthyologists often have employed freezing, formalin or alcohol for preserving fish specimens. Identification keys are often based on morphological characteristics and pigmentation of fixed samples. Because of the variability of properties in the process of fixing and maintaining and also the effects of different fixation methods on the color pattern of fixed samples, the morphological characters in live and fresh samples tend to be different from fixed samples.

There are few studies in this field. Neave et al. (6) 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. (7) studied the effect

of preservatives and freezing on the morphological characters of two sparid fishes. Vajargah and Hedayati (8) studied morphological variations of common carp, *Cyprinus carpio* due to fixing and preserving in %10 formalin. Also the effect of some preservatives and freezing on two species of the family Mullidae was investigated by Al-Hassan et al. (9). In the present study, the effect of preservation in alcohol was investigated on the morphological characters of Zagros tooth-carp.

2 Materials and Methods

To perform this experiment, 30 specimens of *A. vladykovi* were collected from Chaharmahal-o-Bakhtiyari Province, Iran. They were measured during the day of capture. The morphological characters measured were total length (TL), standard length (SL) and head length (HL). Measurements were carried out using a graded caliper with an accuracy of 0.02 mm. After measuring the TL, SL and HL, color patterns of skin and fins were recorded. Then each sample was placed into a graded tube filled with 96% alcohol to fix the samples.

Samples were removed from the alcohol after three months to perform measurements and evaluation of color features once again.

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3 Results and Discussion

Effects of alcohol on morphometric characters of *A. vladykovi* are listed in Table 1. According to the averaged morphometric characters shown in Table 2, decreased length was evident in all the specimens. Changes in body color

were also clearly distinguishable from fresh fish. So that the body and fin colors were opaque. There were color differences between the samples due to changing fish color before placing in alcohol, such a way that fresh fish with light brown tint, became pale and opaque, while fish with green color tones, became darker and opaque.

Table 1: Effect of alcohol on morphometric characters of *A. vladykovi* (TL: total length, SL: standard length, EL: head length)

Before preserved in alcohol (mm)			After preserved in alcohol (mm)			Amount of shrinkage (mm)		
TL	SL	HL	TL	SL	HL	TL	SL	HL
31.21	30.57	2.47	30.60	30.00	2.23	0.61	0.57	0.24
33.25	27.10	9.83	32.00	26.00	9.18	1.25	1.10	0.65
32.57	27.51	9.19	32.00	27.00	9.00	0.57	0.51	0.19
33.94	26.00	8.70	30.00	24.00	7.70	3.94	2.00	1.00
34.16	29.09	9.51	31.60	27.00	8.62	2.56	2.09	0.89
32.09	27.24	9.54	31.10	26.50	9.26	0.99	0.74	0.28
33.27	25.95	8.36	32.00	24.80	7.93	1.27	1.14	0.43
29.35	24.15	7.95	28.00	23.00	7.50	1.35	1.15	0.45
29.51	22.96	7.33	26.30	22.10	7.00	3.21	0.86	0.33
27.92	18.05	5.61	26.00	17.30	5.30	1.92	0.75	0.31
20.95	15.61	5.25	17.20	14.60	4.66	3.75	1.01	0.59
20.30	16.72	5.85	17.20	14.80	4.83	3.10	1.92	1.02
24.70	19.13	10.28	20.70	17.10	9.20	4.00	2.03	1.08
25.65	18.19	6.72	23.00	16.60	5.73	2.65	1.59	0.99
25.33	19.10	7.13	22.00	17.00	6.10	3.33	2.10	1.03
45.92	36.91	10.84	43.00	35.60	10.00	2.92	1.31	0.84
36.39	33.35	11.22	35.80	32.00	10.80	0.59	1.35	0.42
35.34	26.09	9.15	34.60	25.80	9.00	0.74	0.29	0.15
35.11	28.74	9.20	34.30	28.00	8.76	0.81	0.74	0.24
35.54	29.79	9.08	32.40	28.70	8.42	3.14	1.09	0.66
32.93	27.08	9.18	31.70	26.20	8.60	1.23	0.88	0.58
35.07	37.71	9.61	34.30	37.00	9.22	0.77	0.71	0.39
34.44	29.08	9.25	31.50	27.30	8.25	2.94	1.78	1.00
35.15	28.81	9.32	32.50	27.60	8.36	2.65	1.21	0.96
33.43	27.53	8.03	32.00	26.60	7.85	1.43	0.93	0.18
34.05	26.45	8.09	30.50	25.60	7.88	3.55	0.85	0.21
34.46	28.54	8.84	30.50	26.30	7.80	3.96	2.24	1.04
28.00	22.28	7.71	24.00	20.00	6.61	4.00	2.28	1.10
25.71	21.36	12.13	22.50	19.60	11.30	3.21	1.76	0.83
23.62	18.79	6.19	20.00	16.50	5.00	3.62	2.29	1.19

Table 2: Averaged TL, SL and HL before and after preserving in alcohol

	Before preserved in alcohol(mm)	After preserved in alcohol(mm)
Averaged TL	31.312	28.976
Averaged SL	25.662	24.353
Averaged HL	8.385	7.736

The uses of different preservatives have caused change in the body proportion of the fish. In this research, decrease in length was evident in all the specimens probably due to the shrinkage of the body and egress of interstitial fluid. Our results are also in accordance with the results of some researchers such as Lvix (10), Parker (11), Engel (12), As well as results of Jawad et al. (7) who revealed that the different concentrations of formalin and alcohol on one hand and freezing on the other, caused variable degrees of shrinkage in the three characters studied (SL, TL, HL), whereas Al-Hassan and Abdullah (13) have reported a slight length increase or no shrinkage in preserved *Sarotherodon mosambicus* and *Barbus luteus*. Also Al-hassan et al. (9)

stated that the three methods including freezing, storage in alcohol and 10% formalin increase the TL, SL and HL.

Materials with different concentrations have various effects on morphological properties of preserved samples. The variations concerning the effects of preservatives and freezing techniques on fish could be explained such that the different body regions of the fish contain different chemical contents, thus they behave differently when kept in different preservative concentrations. The differences with respect to the effects of preservation might be due to genetic characteristics determining the ratio of white to red muscles and the character of tissue fluid content (14). For instance, alcohol (ethanol or methanol) alone instantly coagulates

proteins but causes considerable distortion of the micro-anatomy in animal tissue (15). So that, formalin can obviously reduce fish colors, however, color pattern is visible (16; 17).

The nature of the morphological change is influenced by many factors including: method of preservation such as fixation and freezing, concentration and type of chemical preservation agents, length of preservation period, salinity and temperature of the preservative. Factors specific to the fish being preserved are also important including: species, age, size and developmental state, the presence of rigor mortis, and the osmoregulatory state of the fish at death (18).

In the present study, changes in body color were also clearly distinguishable compared with fresh fish, but this color loss, does not mean complete loss of color pattern, i.e. colors are distinguished and often color intensity are changed. Body length and color of preserved fish showed diverse degrees of change after a standard period of preservation in different preservatives, so noteworthy, reduce in color intensity of fixed samples in alcohol, does not interdict with color identification keys.

4- Conclusion

Result of the present study showed that change in body color of fish maintained in alcohol had significant differences with the fresh fish, but this color loss, does not mean complete loss of color pattern. Colors were distinguishable, while often color intensity were changed. Body length and color of preserved fish showed diverse degrees of change after a standard period of preservation in different preservatives. So reducing in color intensity of samples fixed in alcohol, does not interdict with color identification keys.

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