



Surveying effects of fixation in formalin on the morphological characteristics of goldfish (*Carassius auratus*)

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Abstract

Formalin is one of the important fixator in fish studies, and has many effects on morphological indices of fishes, so in this study the effect of this fixation was examined. The present study was done to investigate the effects of fixing and preserving in 10% formalin on the morphological characteristics of goldfish (*Carassius auratus*). For this aim, 30 specimens of goldfish were collected from the Researches Center of Aquaculture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, 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% w/v formalin for 11 weeks. 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, *Carassius auratus*, Morphological characteristics, Fixation

1 Introduction

In Ichthyology, there are variety techniques for fixing and preserving of fish specimens such as preserving in formalin for prevent dehydration of samples, preserving in alcohol and freezing that are common than other ways. Identification keys are often based on morphological and pigmental characteristics 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 caused the morphological and pigmental characteristics in live and fresh samples be different with fixed samples.

There are few studies in this field such as: Al- Hassan et al (2000) studied the effects of freezing and preserving in formalin and alcohol on three morphometric characters such as head length (HL), standard length (SL) and total length (TL) in two species of mullidae family and find that fixation has effect on morphology of species. Also, 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 characters of larval Ichthyomyzon lampreys was investigated by Neve et al (2006) and find that fixation have effect of morphological indices.

The goldfish (*Carassius auratus*) is one of the oldest of domesticated fishes and the first aquarium fish that originally from East Asia. It is a freshwater fish from the family Cyprinidae that is similar to common carp (*Cyprinus carpio*) in nutritional and biological aspects (Vesogh&Mostageer, 1995). The goldfish is an important fish aspects of research and economically. In addition, it is the most extensively studied species with respect to hormonal control and reproductive physiology (Bjerselius et al., 1995).

Because of importance of formalin in fixation of fish samples, in this study, the effect of fixation and preservation in formalin was investigated on the morphological characters of goldfish (*Carassius auratus*).

2 Materials and Methods

At the beginning of trial, thirty samples were collected from the Research Center of Aquaculture of BarabadiFazli martyr, Gorgan University of Agricultural Sciences and

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Natural Resources, Gorgan, Iran. To identify fishes, samples were studied by usual methods of morphometric, meristic and descriptive. As first step, the morphological characters measured were such as total length (TL), standard length (SL) (length from head to caudal vein) and head length (HL). Measured was conducted by using a graded caliper with an accuracy of 1 mm. After measuring TL, SL and HL, color pattern of skin and fins in samples were studied. Tagging or marking was done. Then, as second step, dead samples were fixed in 10% formalin. Samples were removed from formalin after 11 weeks and measurement of TL, SL and HL and evaluation of color pattern were performed once again. At the end, the results of two steps were recorded.

3 Results and Discussion

Effects of formalin on morphometric characteristics of goldfish are reported in Table 1. According to the results of present study and observe the average of morphometric characteristics in Table 2, total length (TL) reduced in all the specimens. Also, changes in body color were clearly distinguishable with fresh fish. So that, fresh fish had a color range between orange to red. But after fixed in 10% formalin, color features of samples changed and became pale and opaque.

Table 1. Effect of formalin on morphometric characters of *Carassius auratus* (TL: total length, SL: standard length, HL: head length)

Before preserving in formalin(mm)			After preserving in formalin (mm)			Amount of shrinkage (mm)		
TL	SL	HL	TL	SL	HL	TL	SL	HL
90	75	19	84	71	17	6	4	2
90	70	18	86	67	17	4	2	1
95	75	19	93	74	18	2	1	1
80	60	16	77	58	15	3	2	1
85	70	17	78	66	14	7	4	3
90	75	16	82	70	13	8	5	3
80	60	15	76	58	13	4	2	2
80	65	15	75	62	13	5	3	2
83	70	18	74	65	14	9	5	4
75	65	16	73	65	15	2	1	1
80	65	17	69	58	13	11	7	4
75	63	19	64	55	16	11	8	3
100	60	19	97	58	18	3	2	1
85	70	17	76	65	13	9	5	4
95	65	18	88	61	15	7	4	3
80	60	15	74	56	13	6	4	2
75	70	15	70	67	13	5	3	2
85	60	17	82	58	16	3	2	1
75	60	15	71	58	13	4	2	2
75	60	15	67	55	12	8	5	3
70	70	17	63	66	14	7	4	3
85	55	18	79	51	16	6	4	2
70	55	17	65	52	15	5	3	2
70	65	15	66	63	13	4	2	2
90	78	19	88	77	18	2	1	1
95	75	17	86	69	14	9	6	3
90	80	18	87	78	17	3	2	1
85	60	15	78	54	14	7	6	1
85	60	16	80	57	14	5	3	2
85	60	17	77	54	15	8	6	2

Results of present study showed total length of samples fixed in formalin reduced than fresh fish that probably due to wrinkle and shrinkage of the body and egress of interstitial water during of preservation in 10% formalin. Also, our results are confirmed by the findings of some

researchers such as Lux (1960), Parker (1963), and Engel (1974). In addition, Jawad et al. (2001) 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. (2006) 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. However, Al-Hassan et al. (2000) 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 preserving in formalin

	Before preserving in formalin (mm)	After preserving in formalin(mm)
Average of TL	83.266	77.500
Average of SL	65.866	62.233
Average of HL	16.833	14.700

Different materials with different concentration shave different effects on morphological characters of stored specimens. The mutations in the effect of preservatives and freezing techniques could be describe on the basis that the different body sectors of the fish include different chemical contents; hence, they behave diversely when kept in different preservative concentrations. The differences perceived on the effect of preservation might be due to genetical factors that assess the ratio of white to red muscles and the character of tissue water content (Leslie & Moore, 1986). For example, alcohol (ethanol or methanol) alone expressly coagulates proteins but causes noticeable demolition of the micro-anatomy in animal tissue (Nowacek, 2010).

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 (Macdonald et al., 1997).

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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