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Effect of Different Preservatives on the Biomass of some Selected Marine Fauna

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Abstract.- The effect of 70 % ethyl alcohol and formalin (1 % and 10%) was studied on the biomass of crabs, shrimp and fish. Significant weight loss was observed in crabs (4.6 to 33.2%) in shrimps (2.5 to 41.4%) and in fish (26.5 to 59.5%) when preserved in 70% alcohol for ten weeks. The wet and dry weights of crabs also decreased significantly with time when preserved in ethyl alcohol and formalin. There was no significant difference in the weight of shrimps, whereas the fish weight loss was significantly different for both the preservatives. There was no significant difference in the wet and dry biomass of fish.

Key words: Preservative, biomass, crabs, shrimps, fishes, formalin, ethyl alcohol.

INTRODUCTION

Various chemicals and freezing are used to preserve animal specimens for teaching and demonstration purposes. Preserved animals are also kept for longer periods in museums as type specimens. Field samples are also often stored for subsequent biomass estimation. Formalin (1-10%) and ethanol (70%) are the most commonly used preservatives of aquatic and terrestrial vertebrate and invertebrate fauna (AIHA, 1989; Gaston *et al*., 1996).

The fixatives (preservative) primarily arrest the physical and chemical changes that occur upon death, and preserve the gross form and appearance (Stoddard, 1989). They are known to affect the body weight and size of fish and invertebrates (Fox, 1996; Gaston *et al*., 1996; Jawad, 2003; Ajah and Nunoo, 2003). The variations (loss or gain) in body weight when the organism is preserved in different preservatives (Parker, 1963; Yeh and Hodson, 1975; Stobo, 1972) and changes in dry weight of estuarine macrofauna (Gaston *et al*., 1996) have been reported following preservation. Dramatic changes in weight for *Sarotherodon mossambicus* after preservation in both formalin and ethanol have been reported (Billy, 1982). The variation in body proportions of the fish during the period of preservation might be due to tissue water content and the ratio of white to red muscles (Leslie and Moore, 1986).

There have been no studies on the effect of preservative on the biomass of marine fauna from Pakistan. The objectives of present investigation were to evaluate the effects of two commonly used preservatives, ethanol and formalin, on the biomass of invertebrate and vertebrate marine fauna; and to examine the relationship between fresh and preserved weight for biomass estimation.

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MATERIALS AND METHODS

Three species of ocypodid crabs (*Ilyoplex frater, Macrophthalmus bosci, Serenella indica)* were collected from the Korangi creek mangroves, Karachi in March 2002. A total 120 crabs were initially weighed and then divided into ten lots, each of 12 crabs. Each lot was preserved in 70% ethanol, 1% formalin and 10% formalin (four crabs each) for ten weeks. Every week one lot (12 crabs) was scarified and weighed. Crabs were then dried at 70^{o}C for 24 hours in a laboratory oven (WTC, Binder) and dry weights noted.

Penaeid shrimps (*Fenneropenaeus*

merguiensis) were collected from the Sonmiani Bay, Balochistan, Pakistan in March 2002. Likewise, 15 shrimps were weighed and then divided into three lots, each of (5 specimens) and were preserved in 70% ethanol, 1% formalin and 10% formalin for eight weeks. Every week the weights of preserved shrimp were noted.

Two species of family Mugilidae (*Valamugil speigleri and Liza subviridis*) were collected from the Sonmiani Bay, Balochistan in March 2002. A total of 64 fishes were weighed and then divided into sixteen lots, each of four fishes. Eight fish were preserved in 70% ethanol and eight in 10% formalin. Every week wet weight of one lot was noted then this lot was dried at 70°C for 24 hours and dry weight recorded.

RESULTS

Figure 1 shows percent weight loss in the wet weight of crabs with time when preserved in 70% alcohol and 10% and 1% formalin. The ratio of weight loss with time in 70% alcohol (r^2 = 0.697) was greater than that of 10% (r^2=0.486) and 1% formalin (r^2= 0.516) and ranged from 4.56 to 5.0% in first week and 24.8 to 34.3% in ten weeks (Fig. 1). The difference in weight loss in three preservatives was significant for both wet and dry weight (F= 6.7, F=16.98, respectively). Significant weight loss was also observed for wet and dry weight with time (F = 5.9, F=2.13, respectively). The relationships between wet weights of fresh and preserved specimens (70% alcohol, 10% formalin and 1% formalin) were positive (r^2=0.941, r^2=0.952, r^2=0.905, respectively). Similarly there was positive linear relationship between preserved wet weight and dry weight (Fig. 2).

There was significant decrease in percent weight loss of the shrimps with time (Fig. 3) and ranged between 0.54 to 2.52% in first week and 12.8 to 41.14% in sixth week. Highest weight loss was observed in shrimps preserved in 70% alcohol. The percent weight loss in fish in 70% alcohol ranged from 38.7 to 59.5% in first week and 33.3 to 58.9% in eighth week and in 10% formalin it ranged from 7.31 to 11.3% in first week and 12.58 to 17% in eighth week (Fig. 4). The ratio of weight loss was low in 70% alcohol (r^2=0.272) than in 10% formalin (r^2=0.322). The weight loss was significantly different between 70 % alcohol and 10 % formalin F=38.86, F=19.59, respectively, but there was no significant difference with time (weeks) for wet weight as well as for dry weight (F=0.456 and F=0.543, respectively). Fish weight loss was significantly different for both preservatives (F=38.86, 19.59, respectively) but there was no significant difference in the wet and dry biomass with time. The relationships between fresh wet weight and preserved wet weight in 70% alcohol (r^2=0.98) and in 10% formalin (r^2 = 0.995) were positive.

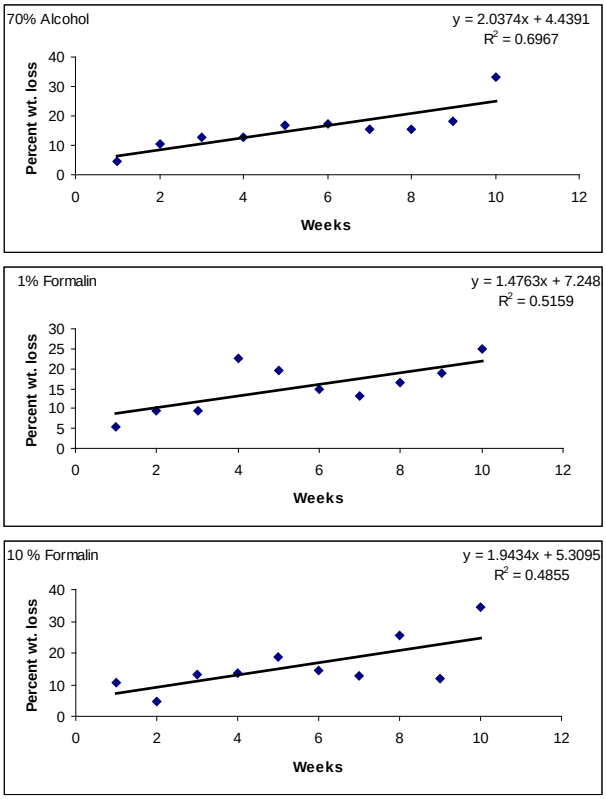

Fig.1. Effect of the three preservatives on biomass of ocypodid crabs expressed as average percent weight loss (n = 40). Data begin a week after preservation.

DISCUSSION

Biomass measurements are important for estimating standing crop and secondary production. It has been demonstrated that concentration of

preservative and exposure can time effect biomass estimation (Kruse and Dalley, 1990; Gaston *et al*.,

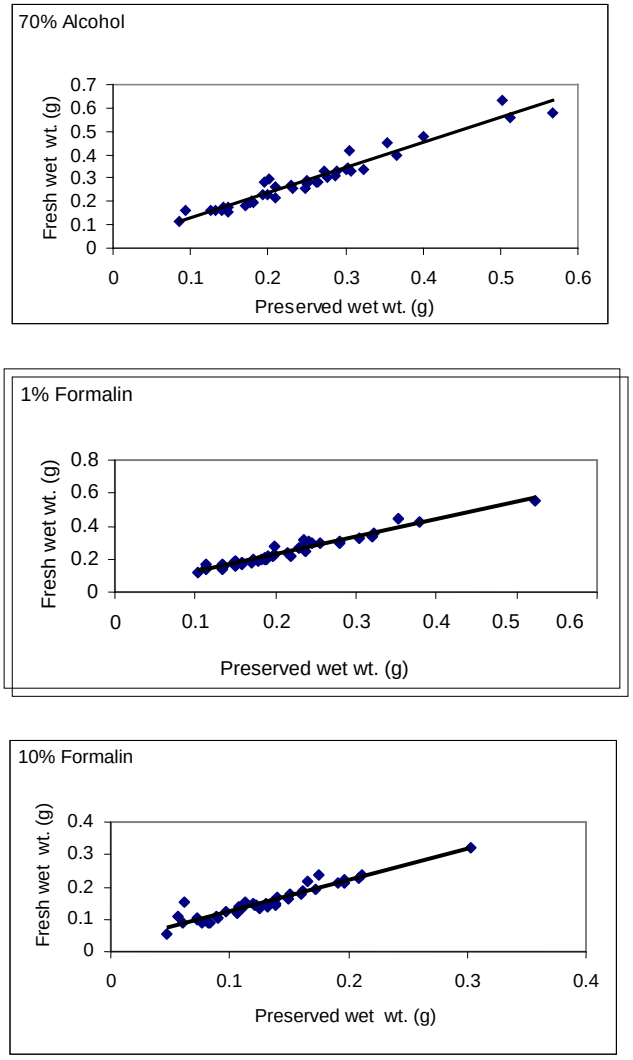

Fig. 2. Relationship between preserved wet weight and fresh wet weight of ocypodid crabs collected from Korangi creek.

1996). Previous studies have demonstrated that ethanol when used to preserve macrobenthos leads to water and lipid loss and effects biomass estimation (Howmiller, 1972; Dermott and Paterson, 1974). In our results, significant difference in the weight loss (wet and dry) were observed in specimens preserved in alcohol (70% alcohol) lost more weight than those fixed in formalin. Especially in the case of ocypodid crabs, significant weight loss was observed with period of preservation. Specimens placed in alcohol became more dehydrated than those kept in formalin. Alcohol is known as a dehydrating agent (Glenn and Mathias, 1987), and crustacean zooplankton become brittle when stored in ethanol (Steedman, 1976). Thus, ethanol may not be the best choice for long term museum storage or situations where fragile anatomical parts may be examined several years after collection.

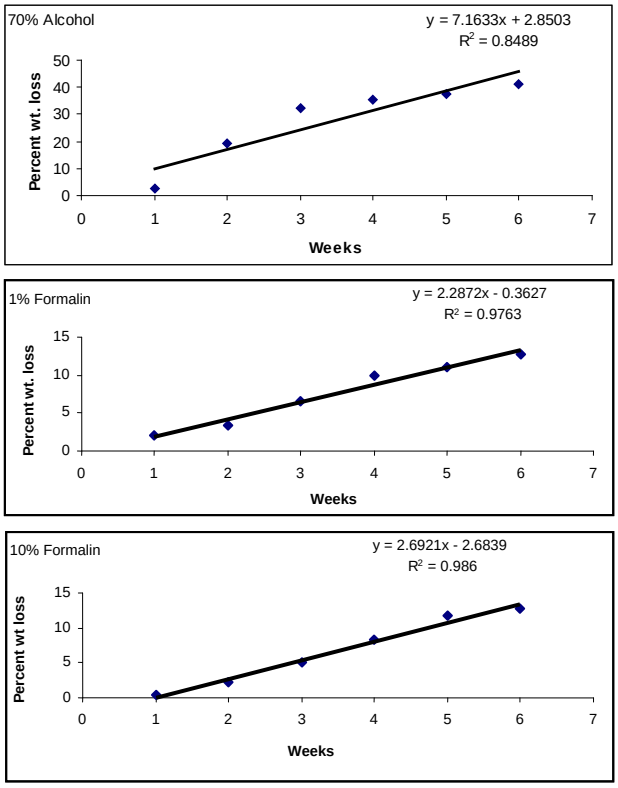

Fig. 3. Effect of the three preservatives expressed as percent weight loss of shrimps, data begin with initial wet weight of shrimp at the time of fixation.

The concentration of formalin also affected the biomass of organisms as in our experiment, greater weight loss occurred in 10% formalin than in 1% formalin. Formalin solutions in sea water are used extensively because of their convenience. Fish lost greater weight in 10% formalin than in 70% ethanol and this observation agrees with and has been reported earlier (Underhill and Cole, 1967;

Mackey and Kalff, 1969; Mason *et al*., 1983). The wet weights of organisms fixed in formalin did not differ among different preservative treatments but slight variability occurred in dry weights (Gaston *et al*., 1996).

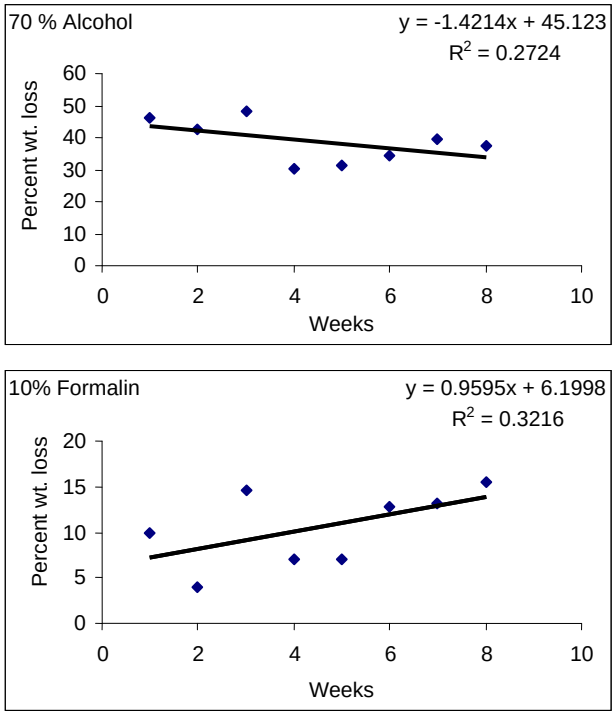

Fig. 4. Average percent weight loss of fish during preservation with time. Data begin a week after preservation.

Marine fish, *Rastrelliger kanagurta* was kept in different concentrations of formalin and some of them showed a slight increase, while the others showed some shrinkage (Al–Hassan and Shawafi, 1997). The bluegill, *Lepomis macrochiris* and white crappie, *Pomoxis annular*, gained weights when preserved in 10% formalin for 69 days (Yeh and Hodson, 1975). For Sockeye salmon, *Onchorynchus nerka*, a gain of 20% of the live weight have been reported after 1 day in 3.8% formalin, but after 225 days weights decreased gradually to the gain of only 10% (Parker, 1963). In present study, significant percent weight loss was observed. In 70% alcohol, the percent weight loss ranged between 4.6 to 33.2% in crabs, 2.5 to 41.4% in shrimps, and 26.5 to 59.5% in fish. Effects of preservatives on length and weight of juvenile Sockeye Salmon was 19.7% percent weight loss after 16 days that stabilized through 77 days (Sheilds and Carlson, 1996), whereas the fish preserved in formalin did not showed significant percent weight loss in one experiment, and in another experiment an increase in percent weight loss by 7.12% in 16 days was observed that stabilized through 77 days (Sheilds and Carlson, 1996). In present study, initial percent weight loss in first week followed by slight increase in weight in eighth week was observed in fish preserved in 10% formalin. The yellow perch (*Perca flavesens*) showed an initially rapid increase in weight, which slowed for a short period, then protracted period of increase followed by a period of decrease (135-557 days) in 10% formalin (Stobo, 1972).

The effect of different preservatives on the morphology of fauna in relation to size has been the subject of several papers, especially for larval, juvenile and adult fishes. However, few studies have described the effect of preservatives on the biomass of different marine fauna. The length of fishes is used to determine growth, examine life history of species, ontogenic shift in habitat, and stock assessment. Changes in length due to formalin and alcohol on the juveniles of herring (*Clupea harenges*) and capelin (*Mallotus villosus*), respectively have been discussed but the effect of these preservatives on the biomass was not studied (Kruse and Dalley, 1990). The differences in the effect of preservatives on the different parts of the fish body might be due to the difference in the chemical composition of each region (Al-Hassan *et al.,* 2000). Such composition differs from region to region of the fish body with respective to the contraction function. The shrinkage due to preservatives and freezing was studied for two mullet fish species, *Mullus barbatus* and *M. surmuletus* (Leslie and Moore, 1986). Both species has its own characters of tissue water content and its specific ratio of white to red muscles that possibly interfere with different preservatives, however, the difference in the effect of 70% alcohol, 10% formalin and 5% formalin on the biomass of fishes was not explained (Leslie and Moore, 1986).

It has been recommended to use regression equation to get good estimation of fresh length from

preserved length (Kruse and Dalley, 1990). It has been also suggested to employ species specific conversion equation and to study large representative of samples from multiple systems (Sheilds and Carlson, 1996). Conversion equations are good for the weight data from ethanol preserved specimens but it is better to use live weights when ever possible. The fresh samples are the best, followed by freezing and formalin treatment (Ajah and Nunoo, 2003). The ethanol preservative methods (70% and 95% treatments) are better compared to using 4% formalin to fix and store samples (Black and Dodson, 2003). In present study we found that formalin and alcohol as well as preservation period affect biomass (wet and dry) of marine organisms and biomass estimation of the preserved specimens can be done by conversion equations developed by using linear regression.

REFERENCES

AIHA [American Industrial Hygiene Association], 1989. *Odor thresholds for chemicals with established occupational health standards*. American Industrial Hygiene Association, Table 5.3.

AJAH, P.O. AND NUNOO, F.K.E., 2003. The effects of preservation methods on length, weight and condition factor of the clupeid *Sardinella auritta*. *J. appl. Ichthyol.*, **19:** 391-393.

AL-HASSAN, L.A.J. AND SHAWAFI, N.A.A., 1997. The effect of preservatives and freezing on morphological characters of *Rastrelliger kanagurta* (Pisces: Scobridae). *J. Anim. Morphol. Physiol*., **44**: 17-22.

AL-HASSAN, BUJAWARI, J.A. AND EL-SILINI, O.A., 2000. The effect of some preservatives and freezing on certain body dimensions of species of the family Mullidae collected from Benghazi waters, Libya. *Acta Ichthyol. Piscat*., **30**: 127-136

BLACK, A.R. AND DODSON, S.I., 2003. Ethanol: a better preservation technique for Daphnia. *Limnol. Oceanogr: Methods*, **1**: 45-50.

BILLY, A.J., 1982. The effects of formalin and isopropyl alcohol on length and weight measurements of *Sarotherrodon mossambicus* Trewavas. *J. Fish Biol*., **21**: 107-112.

DERMOTT, R.M. AND PATERSON, C.G., 1974. Determining dry weight and percentage dry matter of chironomid larvae. *Canadian J. Zool*., **52**: 1243-1250.

FOX, C.J., 1996. Length changes in herring (*Clupea harengus*) larvae: effects of capture and storage in formaldehyde and alcohol. *J. Plankt. Res*., **18**: 483-493.

GASTON, G.R., BARTLETT, J.H.W. MCALLISTER A.P. AND HEARD, R.W., 1996. Biomass variations of estuarine macro benthos preserved in ethanol and formalin. *Estuaries,* **19**: 674-679.

GLENN, C.L. AND MATHIAS, J.A., 1987. Body shrinkage in young walleye, *Stizostedion vitreum*, preserved with AFA, formalin, Ethanol, and quick freezing. *Canad. Field-Natural*., **101**: 408-414.

HOWMILLER, R.P., 1972. Effects of preservatives on weight of some common macro benthic invertebrates. *Trans. Am. Fish. Soc*., **101**: 743-746.

JAWAD L.A., 2003. The effect of formalin, Alcohol and freezing on some body proportions of *Alepes djeddaba* (Pisces: Carangidae) collected from the red sea coast of Yemen. *Rev. Biol. Marina Oceanogr*., **38**: 77-80.

KRUSE, G.H. AND DALLEY, E.L., 1990. Length changes in capelin, *Mallotus villosus* (Muller) larvae due to preservation in formalin and anhydrous alcohol. *J. Fish Biol.*, **36**: 619-621.

MACKAY, R. AND KALFF, J., 1969. Seasonal variation in standing crop and specie diversity of insect communities in a small Quebec stream. *Ecology*, **50**: 101-109.

MASON, W.T. JR., LEWIS, P.A. AND WEBER, C.I., 1983. An evaluation of benthic macro invertebrate methodology. *Environ. Monit. Assess*., **3**: 29-44.

LESLIE, J.K., AND MOORE, J.E., 1986. Changes in length of fixed and preserve young freshwater fish. *Canadian J. fish. Aquat. Sci*., **43**: 1079-1081.

SHIELDS P.A. AND CARLSON, S.R., 1996. Effect of formalin and alcohol preservation on length and weights of juvenile Sockeye Salmon. *Alaska Fish. Res. Bull*., **3**: 81-93.

PARKER, R.R., 1963. Effects of formalin on length and weight of fishes. *J. Fish. Res. Bd. Canada*, **20**: 1441-1455.

STEEDMAN, H.F., 1976. *Zooplankton fixation and preservation. Volume 4: Monographs on Oceanography Mmethodology Series*, UNESCO. 350 pp.

STOBO, W.T., 1972. Effects of formalin on the length and weight of yellow perch. *Trans. Am. Fish. Soc.*, **101**: 362-364.

STODDARD, R. W., 1989. Fixatives and preservatives: their effects on tissue. In: *Conservation of natural history specimens: Spirit collections* (ed. C.V. Horie) Manchester Museum and Department of Environmental Biology, University of Manchester, pp. 1-25.

UNDERHILL, J.C. AND COLE, G.A., 1967. The summer standing crop, growth, and distribution of *Chironomus plumosus* in lake Itasca, Minnesota. *J. Minnesota Acad. Sci.*, **34**: 181-186.

YEH, C.F. AND HODSON, R.G., 1975. Effects of formalin on length and weight of bluegill and white crappie from Lake Newsworthy, Texas. *S. W. Natural*, **20**: 315-322.

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