

SHRINKAGE RATES IN NEWLY HATCHED LARVAE OF *MACROBRACHIUM VOLLENHOVENII* (HERKLOT'S) (DECAPODA, PALAEMONIDAE).

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The effect of formalin/sea-water solution (2% and 4% formalin conc. buffered with borax) on the total lengths of preserved samples of newly hatched *Macrobrachium vollenhovenii* larvae was investigated. The influence of anaesthesia on larvae in 2% and 4% formalin was also studied to determine the combine influence of an aesthesia and formalin on these specimen. Shrinkage was similar in all subgroups studied ($P>0.05$). Prior to shrinkage, specimens showed initial increases in total length, which finally stabilized after 20 days preservation. Larger specimen exhibited lower shrinkage rates than smaller specimens ($P>0.05$). Shrinkage did not take place in larval stages 4 and 5. Specimens above zoea 5 exhibited a drastic size reduction on preservation. Anesthesia influenced shrinkage drastically.

KEY WORDS: Shrinkage, Larvae, *M. vollenhovenii*, Formalin, Anesthesia.

INTRODUCTION

The effect of preservatives on biological samples of aquatic origin has been reported (Parker, 1963; Schnack and Rosenthal, 1978; Rosenthal et al, 1978; Kuhlmann et al 1982). The effects of these preservatives on the life stages of organisms are studied and reported for the newly hatched sea bream larvae (Rosenthal and Westernhagen, 1976), and siganid species (Rosenthal et al, 1978). Also reported in literature is the initial length increases in some marine sea food organism exposed to fixatives over a given period of time (Kuhlmann et al 1982). In these reports, preserved specimens of newly hatched larvae showed initial total lengths increases prior to shrinkage. Other reports have also demonstrated the extent to which various concentrations of preservatives (e.g. formalin /sea water solutions) affects the lengths and weights of preserved fish samples (Howmiller, 1972; Lockwood and Saly, 1975; Rosenthal et al 1978; Kuhlmann et al, 1982). From these studies, it was possible for the authors to determine the so-called "condition factors" (that is the rate of change in size of specimens in relation to the preservation time) for each species.

In this report, the shrinkage rates of *Macrobrachium vollenhovenii* larvae preserved in two concentrations of formalin /seawater solution and on those preserved after anaesthetization are presented. *Macrobrachium vollenhovenii* is a shrimp species of interest to aquaculture in West Africa; its cultural and biological properties are being studied (Udo and Taeye, 1989, 1990). To provide valuable data for future biological studies, samples of the various stages of the larvae were preserved to show the extent to which they are influenced in size (length/weight) over a certain interval of time on exposure to formalin.

MATERIALS AND METHODS

The newly hatched larvae (Zoea I) used in this study were hatched from a single female obtained from the Cross-river estuary. The estuary is located in Eastern

Nigeria between latitudes 4°15' and 4°45'N and longitudes 8°35' and 8°5'E. These larvae were reared in water within the salinity range of 14.0 ppt. and 14.7 ppt., which is the suitable salinity for the rearing of *M. vollenhovenii* larvae (Willfuhr-Nast et al 1993).

The larvae were split into 4 experimental groups consisting of 25 animals each. The first two groups were fixed directly in 2% and 4% formalin /seawater solutions respectively. In the next group, another 25 animals were each anaesthetized with Mss 222 (quinaldine) and fixed in 2% and 4% formalin solution respectively, before the measurement of the life total lengths. The lengths were measured with an inverted microscope, which is equipped with a micrometer. Samples were preserved at $28 \pm 2.0^\circ\text{C}$. Further measurement of preserved samples was carried out after 5, 10, 20, 50, and 90 days of preservation in the various formalin solutions. The student t-test was applied to examine differences in shrinkage rates between the different experimental groups.

To examine the effect of 4% formalin/sea-water solution on newly hatched and advanced larvae (larvae at zoea 5 stage and above); specimens were reared individually each in a 50ml. polystyrol transparent vessel. Twenty-five specimens per larval stage were measured and later on preserved at each molting stage in that formalin concentration. Further measurements of these larvae were carried out after 10, 20, 50, and 70 days of preservation respectively.

RESULTS

A drastic size increase of about 19% and 18% respectively which stabilized after 20 days of storage was measured in specimens preserved in 2% and 4% formalin /sea-water solutions (Table I). This was followed by a uniform reduction in size which stabilized approximately on the 50th day of storage. However, size increase and reduction were hindered in specimen that was anaesthetized with quinaldine before preservation in these formalin concentration (Fig. 1).

Shrinkage in the animals exposed to formalin after anaesthetization were not significant ($P>0.05$) (Table

Table 1: Increases in size of specimens in relation to duration of storage in formalin concentrations and Mss 222. Temperature = $28 \pm 2.0^\circ\text{C}$

Duration of preservation(days)	Increase in size(cm)			
	Formalin concentrations (%)		Mss(Quinaldine) treatment (%)	
	2	4	2	4
0	0	0	0	0
5	-	-	7	8
10	10	7	8	9
20	19 *	7	6 *	5 *
50	11	7	6	5
70	18	7	6	5
90	12	7	6	5
	11	7		

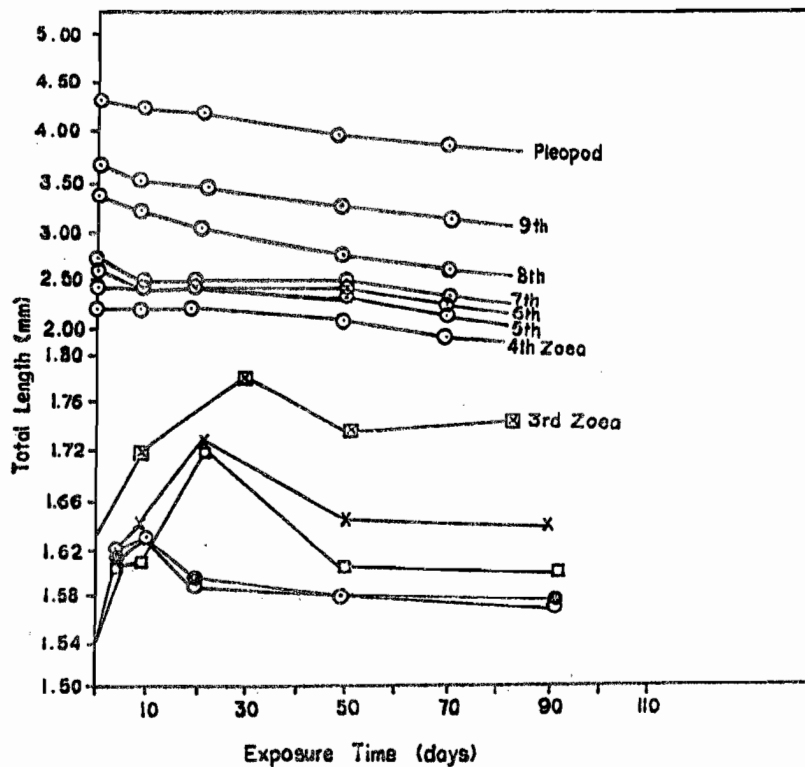


Fig. 1: Changes in total lengths of preserved *M. vollenhovenii* larvae in various concentrations of formalin: 0 represents advanced larvae, X and □ represents specimens preserved in 2% and 4% formalin solutions respectively while ○ and ● are those specimens preserved in 2% and 4% formalin after anaesthetization.

l, Fig. 1). However, older larvae shrink upon preservation, except larval stages 4, which did not respond to formalin. They neither shrink nor swell on preservation. Within approximately 20 days of storage, the specimens without anaesthesia showed great size increases of about 3 to 4 times more than those anaesthetized prior to storage (Table 1).

DISCUSSION

A general size increase was followed by size reduction in all the specimens except in larval stage 4 which neither shrank nor swelled (Fig. 1). Kuhlmann et

al (1982) made similar observations when some marine seafood organisms were preserved in 4% formalin

Rosenthal et al (1978) reported that 2% and 4% formalin influenced the sizes of preserved larvae of red sea bream (*Chrysophrys major*) to some remarkable extent. For the newly hatched larvae of *Macrobrachium vollenhovenii*, no significant differences ($P > 0.05$) were established between the preserved samples in the formalin concentrations of 2% and 4% as reported by Rosenthal et al (1978) for siganid species and the red sea bream. The implication of these results is that 2% and 4% formalin/sea-water solution have the same effects on preserved samples of *M. vollenhovenii* larvae similar to the

findings of Rosenthal et al (1978). Therefore the use of lower concentrations (2%) of formalin for storage of this larvae is recommended since 2% and 4% formalin concentrations seem to show the same influence on the specimens. Anaesthetization with Mss 222 before preservation had great influence on shrinkage / swelling rates of the specimens (Table I, Fig.1). The application of anesthesia before storage reduced swelling, and encouraged size increase in younger specimens. Anesthesia therefore seems to influence formalin penetration into specimen and should be avoided in studies that require the use of total lengths determinations for study and analysis.

Older larvae (zoea 5 and above) shrank when preserved in 4% formalin, while younger ones (zoea 1 to 3) showed initial length increase before shrinkage. Specimens at zoeal stage 4 neither shrank nor swelled in preservatives (Fig 1). The initial swelling of the new larvae in formalin might have been encouraged by its soft and permeable exoskeleton. High magnification/resolution of the exuviate of the younger larvae (zoea 1 to 3) show that the structure is very thin in comparison to those of the advanced larvae. The thin exoskeleton of the young larvae is probably more permeable to fluid than those of the advanced ones. Above stage 4 larvae, a drastic reduction in total length was observed (zoea 5 and above) from commencement of preservation to the end of study. It is assumed that changes in the quality of the exoskeleton as the animal advances in age are probably responsible for the differences in the shrinkage rates of the various larval stages. Younger larvae absorbed more formalin (except those earlier anaesthetized) and swelled and the older specimens either shrank or showed any size change at all in total lengths due to the impermeability of their exoskeleton (Fig.1). The reason for the low permeability of formalin into specimen already anaesthetized before storage is unknown and are subject of further investigation.

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