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Technical Paper

Effects of Glutaraldehyde Fixation on the Body Size of Red Sea Bream (*Pagrus major*) Larvae

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ABSTRACT

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The use of a buffered 2.5% glutaraldehyde solution as a fixative for red sea bream larvae was investigated. Morphometric data were obtained from live larvae 3, 12, 18, and 25 days old. These were then fixed for a period of 6 months and were remeasured. The only significant changes between live and preserved specimens were in body and muscle depth in 3-day-old specimens. Compared to conventional formalin-fixed specimens, the glutaraldehyde-fixed specimens had negligible shrinkage and a more natural appearance.

INTRODUCTION

Preservation of fish larvae is a common practice during the rearing process. The fixed specimens can be measured when convenient and can serve as a permanent record of a particular trial. Traditionally, a variety of formalin solutions has been employed for this purpose. Formalin fixation, however, usually results in tissue shrinkage. This is particularly acute when used on fish larvae (Blaxter, 1971; Rosenthal and Von Westernhagen, 1976; Schnack and Rosenthal, 1978; Fukuhara, 1979; Hay, 1981; Theilacker, 1980, 1986). Shrinkage of between 11 and 14% has been reported (Blaxter, 1971). In addition, different larval stages exhibit variability in their shrinkage rates (Fukuhara, 1979). There is an obvious need for a more suitable fixative.

This report concerns the use of glutaraldehyde as a fixative for fish larvae. Glutaraldehyde is commonly used to fix specimens for electron microscopy (Hayat, 1970, 1981). We determined the shrinkage rate of cultured red sea bream, *Pagrus major* (Temminck et Schlegel) larvae resulting from fixation in glutaraldehyde.

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TABLE 1

Shrinkage of laboratory-reared red sea bream larvae. Numbers in parentheses represent size of fixed specimen divided by size of fresh specimen (1.00 = no shrinkage). All measurements in millimeters

Days after hatching	Number of specimens	Total length		Head length		Snout to anus		Body depth at anus		Muscle depth at anus	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3	Fresh - 36	3.35	0.14	0.73	0.05	1.25	0.08	0.74	0.07	0.20	0.02
	Fixed - 36	3.35	0.14	0.73	0.04	1.27	0.06	0.69	0.07	0.22	0.02
		(1.00)		(1.00)		(1.01)		(0.93*)		(1.07**)	
12	Fresh - 40	5.25	0.54	1.32	0.18	2.22	0.29	1.17	0.14	0.65	0.13
	Fixed - 39	5.31	0.58	1.32	0.18	2.29	0.32	1.17	0.13	0.70	0.14
		(1.01)		(1.00)		(1.03)		(1.00)		(1.07)	
18	Fresh - 41	7.55	1.01	2.05	0.31	3.50	0.57	1.69	0.22	1.30	0.27
	Fixed - 39	7.58	0.97	2.01	0.30	3.52	0.61	1.67	0.23	1.30	0.31
		(1.00)		(0.98)		(1.01)		(0.99)		(1.00)	
25	Fresh - 36	8.98	0.94	2.52	0.28	4.29	0.52	1.88	0.30	1.67	0.21
	Fixed - 36	8.93	0.70	2.51	0.22	4.12	0.45	1.99	0.23	1.58	0.21
		(0.99)		(1.00)		(0.96)		(1.06)		(0.95)	

*$P < 0.05$; **$P < 0.01$.

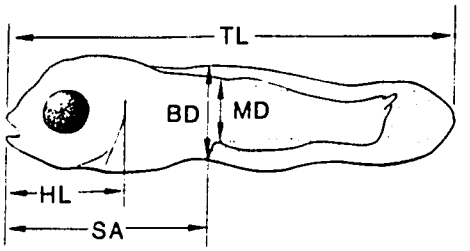

Fig. 1. Illustration of measurements for total length (TL), head length (HL), snout to anus (SA), body depth (BD) and muscle depth (MD).

MATERIALS AND METHODS

Fertilized eggs of red sea bream were obtained from natural spawnings at the Fisheries Laboratory, University of Tokyo. Larvae were reared in 500-l polycarbonate tanks and were fed on rotifers as described by Fukuhara (1978, 1987). Temperature during the rearing trial was 21.6 ± 0.6°C. Measurements of live specimens were made at four different stages; 3, 12, 18, and 25 days after hatching. Live specimens were first anesthetized with MS222, then five separate morphometric determinations were made on each specimen. These were total length (TL), head length (HL), snout to anus distance (SA), body depth at anus (BD), and muscle depth at anus (MD) (Fig. 1). Specimens were then fixed in 2.5% glutaraldehyde (pH 7.2–7.4). The fixative was prepared by first diluting 25% glutarardehyde (Wako Chemical Industrial Corp., Japan) to 5% with distilled water. The final solution was made by mixing equal volumes of the 5% solution with 0.2 *M* phosphate buffer. The specimens were remeasured 6 months after fixation. Data on fresh and fixed material were compared using one-way ANOVA (Sokal and Rohlf, 1969).

RESULTS

The morphometric data for live and fixed specimens, obtained at various larval stages, are presented in Table 1. For the most part, there were no significant differences between measurements of live and fixed specimens after 6 months of fixation. The only significant changes occurred in the body and muscle depth in 3-day-old larvae.

Another advantage to the use of glutaraldehyde as a fixative is that the natural appearance of the larvae is conserved during the fixation process (Fig. 2).

DISCUSSION

Shrinkage rates of red sea bream larvae fixed in 5% formalin for 6 months vary between 7.4 and 15%, depending on the stage of development at which

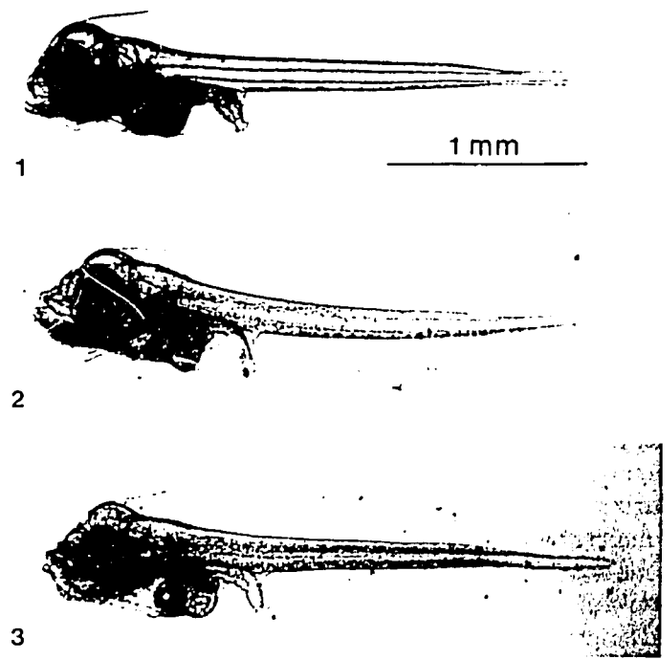

Fig. 2. The appearance of 3-day-old red sea bream larvae: (1) a live specimen; (2) a specimen fixed in buffered 2.5% glutaraldehyde; and (3) a specimen fixed in buffered 5% formalin.

the specimen was fixed (Fukuhara, 1979). The biggest change of body length was reported for larvae fixed just after hatching. Also, the shrinkage rate decreased with the growth of the larvae (Fukuhara, 1979). Similarly, in the current study, the most pronounced changes of body lengths occurred with the youngest larvae. Glutaraldehyde-fixed specimens, however, did not exhibit changes of body length to any significant degree for larvae 12 days old or older. This is in sharp contrast to the formalin-fixed specimens. Another desirable characteristic of glutaraldehyde fixation is the conservation of larval appearance. Both of these traits demonstrate the superiority of this fixation process for use in preserving fish larvae.

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