Acta Hydrobiol., **40** (1998), 1, 35–38. Cracow, March 1998

Changes in body length-eye diameter relation of *Coregonus peled* Gmelin (Teleostei, Coregonidae) larvae owing to formalin fixation

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(Received 5 May 1997, Accepted 29 December 1997)

Abstract – A body length-eye diameter relation has been suggested by different authors to predict live fish size before fixation in larval field studies. To estimate the effects of fixation in 4% formalin on body length-eye diameter relationship in larval *Coregonus peled* Gmel., the observations were carried out throughout 6 months following fixation. It was found that the time of fixation in formalin significantly affected the body length-eye diameter relationship in *C. peled* larvae in comparison with this relation in live fish.

Key words: preservation, formaldehyde, morphology, fishes, larvae, *Coregonus peled*.

1. Introduction

Several studies have documented the effect of formalin fixation on live larvae of various fish species (Blaxter 1971, Schnack and Rosenthal 1978, Dabrowski and Bardega 1982, Hay 1982, 1984, McGurk 1985, Glenn and Mathias 1987, Jennings 1991). Fish shrink when fixed and preserved in formalin (Parker 1963, Hay 1981, Heming and Preston 1981). The relation between the lengths of larvae before and after preservation in formalin is important in predicting the live size of field--collected fish. Because the larvae to be measured are sometimes badly damaged, the use of eye diameter for estimating average live size has been proposed (Packard and Wainwright 1974, Theilacker 1980). The aims of present paper were to determine the relationship between the total body length and eye diameter of live and fixed *Coregonus peled* Gmelin larvae and to determine the effect of duration in the fixative on this relationship.

2. Material and methods

Live larvae of *C. peled* from a rearing cage were measured at 4 and 64 d post--hatch to the nearest 0.01 mm from the tip of the upper jaw to the end of the caudal fin (total length, L), and along the horizontal diameter of the eye (D). Both age groups (41 and 49 fish, respectively) were killed by removal for 2–3 minutes

from the water, then immersion in 4% formalin. Each specimen was placed into a separate 50 ml glass jar containing 10 ml of fixative, to ensure equivalent dilution of the fixative for each larva. They were measured after seven days, and then at one month intervals up to six months following fixation. Eye diameter (D) was then regressed on total length (L) for both preserved and live fish (Theilacker 1980): log D = a + b log L.

3. Results and discussion

Analysis of covariance ($P<0.05$) demonstrated that the slopes of these regressions differed (Table I). That for unfixed 4-day-old *C. peled* was less than that for fixed larvae during the first 134 days after fixation, but after 165 days the slopes for fixed and unfixed larvae were similar. The slope for the 64-day-old fish was greater than that for all fixed specimens, except those after 165 days.

Table I. Estimated parameters for linear model (log D = a + b log L; Theilacker 1980) relating eye diameter (D) with total length (L) of *Coregonus peled* Gmelin larvae depending on time of preservation in formalin.

Duration (days)	Equation coefficients			F	$F_{0.05}$
	a	b	r^2		
4d age (n = 41)				3.9620	2.1400
live	0.3221	-0.4339	0.2289		
7	0.3858	-0.4920	0.3827		
37	0.4052	-0.5213	0.2973		
68	0.7545	-0.8585	0.5827		
97	0.8108	-0.9196	0.5831		
134	0.5287	-0.6300	0.4041		
165	0.2312	-0.3099	0.2410		
64d age (n = 49)				6.5182	2.1300
live	0.7575	-0.7251	0.7840		
7	0.6793	-0.6181	0.6837		
36	0.5806	-0.4769	0.5807		
73	0.6364	-0.5653	0.5820		
104	0.6011	-0.4979	0.6003		
130	0.5915	-0.4745	0.6995		
165	0.7459	-0.7137	0.7252		

Several authors (Blaxter 1971, Theilacker 1980) have stressed the role of conditions that damage field-collected fish larvae. To avoid these problems, models have been proposed to estimate average fish length from the size of head or eye. Packard and Wainwright (1974) found that the eye diameter of *Clupea harengus* L. ≤100 mm TL was a useful reference parameter for estimating both size and weight, as did Theilacker (1980) for northern anchovy *Engraulis mordax* Girard larvae. Eye size also may be useful for enumerating fish biomass consumed in predation studies, for the larval fish eye is more resistant to digestion than the larval body.

The use of the shrinkage models seems to be important in the prediction of live size of sea-collected larvae. However, it is of similar importance in relation to freshwater pelagic fish larvae, for example, coregonid fish. Mamcarz (1994) found no significant differences in eye diameter between unfixed and fixed *C. peled* larvae after 165 days of preservation. These results are consistent with those of Theilacker (1980), who reported small changes in the eye diameter size of northern anchovy. However, the results are not consistent with those of Dabrowski and Bardega (1982), who found a 12.3 % increase in the eye diameter of *Coregonus albula* L. by the 13th day after preservation in formalin. This might be due to fish species individuality in shrinkage rate.

The value of b coefficient is very differentiated, what reflects significant differences in reaction of particular specimens preserved in formaldehyde. This phenomenon was observed by several authors (Parker 1963, Mamcarz 1984, Mamcarz 1994). By this fact there is no clear trend in changes of b parameter in relation to time of preservation. It seems to be one of basic problems in finding of precise relationships to correct size of preserved fishes. During six months of study, at each stage of preservation, the observed relationship between body length and eye diameter was different from earlier, and also from initial value of nonpreserved fish. After six months fish size changes closer to initial value, what was reflected by coefficients b and r^2, and confirmed by global tendency to stabilization of fish size after one year of preservation in formadelhyde.

As it was shown in this study, formalin fixation can result in a length-eye diameter relationship, depending upon the duration of fixation. To use eye diameter to predict larval size, samples need to be left in fixative for at least six months before measurements are taken. More work is required for adequate description of these effects on different species for better estimation of live size for field-collected larvae.

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