SHRINKAGE EFFECTS ON SARDINE LARVAE *(SARDINA PILCHARDUS)* CONSERVED BY ETHANOL AND LIQUID NITROGEN

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Abstract

Sardine larval shrinkage observed under different conservation agents, liquid nitrogen and ethanol, is assessed. The degree of shrinkage was affected by the type of conserving agent. While shrinkage observed in ethanol was independent of size, liquid nitrogen conserved specimens were size dependent. Highest shrinkage occurred in ethanol. Care is recommended in measurements, because manipulation of larvae can affect enlargement of body size.

Keywords: shrinkage, sardine, ethanol, liquid nitrogen

Introduction

Accurate estimates of larval length are essential in studies on the early life histories of fish. Measurements of larval length are used in order to estimate age, growth and nutritional condition.

Shrinkage of larvae, during fixation, may be a source of error, necessitating the use of adjustments to convert from preserved to fresh measurements. The degree of shrinkage may vary and depend on the type of conservation agent (1; 2; 3), species preserved (3), and larval size and age (2; 4).

Use of ethanol is recommended for the preservation of larvae for age estimation from daily rings in otoliths (5), whereas, for age estimation and biochemical analysis, freezing by liquid nitrogen is recommended (6).

Material and methods

Sardine larvae were collected by means of a 1 m Bongo net (1mm mesh) towed repeatedly at surface during 7-10 minutes at nightime. Sardine larvae were sorted and standard length (SL) measured by an image analysis system. After measurement, each larva was individually conserved in an eppendorf using three conservation methods: (1) stored in liquid nitrogen (LN); (2) 96% ethanol (OH), and (3) in LN and sea-water (LN+SW). The number of larvae analysed for each conservation agent were: (1) LN-conserved: 120 larvae of sizes 9.1-23.8 mm (8% average shrinkage); (2) OH-conserved: 150 larvae of sizes 8.3-24.5 mm (9% average shrinkage); and (3) LN+SW-conserved: 120 larvae of sizes 9.8-34.2 mm (6% average shrinkage).

The conserved larvae were measured after 45 days. Those conserved in LN were defrosted prior to measurement. To avoid bias, as on board, the same measuring device and person did the measurements. To test the effect of larval weight on shrinkage, those conserved in LN were wet weighed, after SL measurement. To assess the effect of manipulation on larval size, the conserved larvae LN + SW were measured twice; the first measurement without manipulation by simply defrosting the ice pellet in a Petri dish, while the second measurement was carried out after transferring the larvae to a slide by means of a paintbrush.

Results and discussion

The results of t-test for dependent samples show that all the conservation agents used have a shrinkage effect on sardine larvae ($p<0.001$). Maximum shrinkage was observed in ethanol-preserved larvae. Linear relationships of conserved size versus fresh size showed that shrinkage is size-dependent in LN, while ethanol-conserved larvae were independent of size. The linear regressions of conserved SL on fresh SL for each conservation method are:

1) LN SL(fresh)= 1.11*SL(conserved)-0.41 ($R^2 = 0.94$, n=120)

2) OH SL(fresh)= 0.96*SL(conserved)+1.94 ($R^2 = 0.94$, n=150)

3) LN+SW SL(fresh)= 0.99*SL(conserved)+0.86 ($R^2 = 0.94$, n=120)

To test the effect of conservation method and shrinkage, a two-way ANOVA was carried out on 5 previously defined size classes (<10, 10-13, 16-19, >19 mm). The results showed that all conservation methods do not cause an equal shrinkage effect, the degree of shrinkage is not the same for all size classes and there is an effect of the conservation method used and the size classes on shrinkage ($p<0.001$). A post-hoc comparison of means (Tukey HSD test) was subsequently done to verify the results of the two-way ANOVA.

The results showed that mean shrinkage was significantly ($p<0.001$) affected by size class in LN-preserved larvae, causing a shrinkage increase with larval size, while those larvae conserved in LN+sea-water, mean shrinkage was not significantly affected by size class ($p>0.05$). For larvae conserved in 96% alcohol, mean shrinkage showed a similar degree of shrinkage in all size classes ($p>0.999$), therefore size-independent (Fig. 1).

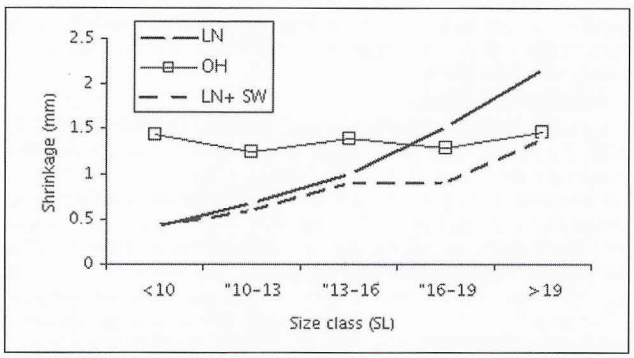

Fig. 1. Mean shrinkage of sardine larvae by conservation agent and size class.

To assess the effect of weight on shrinkage, an ANOVA was applied on the weighed LN conserved larvae. The results indicated that weight significantly ($p<0.001$) affects shrinkage, increasing with weight.

Lastly, to test the effect of manipulation to measure LN-conserved larvae, a t-test was applied to compare measurements of non-manipulated and manipulated larvae. The results showed that manipulation significantly ($p<0.001$) affects size causing body size enlargement.

It is important in larval studies to have accurate measurements at live length. This study contributes to assess the effect of conservation methods, on larval size and weight, as well as, the effect of manipulation on the enlargement of body size.

References

1 - Theilacker, G.H., 1980. Changes in body measurements of larval northern anchovy, *Engraulis mordax* , and other fishes due to handling and preservation. *Fish. Bull.*, 78(3), 685-692.

2 - Hjoerleifsson, E. and Klein-MacPhee, G., 1992. Estimation of live standard length of winter flounder *Pleuronectes americanus* larvae from formalin-preserved, ethanol-preserved and frozen specimens. *Mar. Ecol. Prog. Ser.*, 82(1): 13-19.

3 - Fey, D.P., 1999. Effects of preservation technique on length of larval fish: Methods of correcting estimates and their implication for studying growth rates. *Bull. Sea Fish. Inst. Gdynia*. 155: 47-51.

4 - Hay, D.E., 1982. Fixation shrinkage of herring larvae: Effects of salinity, formalin concentration, and other factors. *Can. J. Fish. Aquat. Sci.*, (39)8: 1138-1143.

5 - Brothers, E.B., 1987. Methodological approaches to the examination of otoliths in aging studies. Pp. 319-330. In: Summerfelt, RC; Hall, GE (eds.) Age and growth in fish. Int. Symp. on Age and Growth in Fish, Des Moines, IA (USA), 9 Jun 1985.

6 - Buckley, L.J., and Bulow, F.J., 1987. Techniques for the estimation of RNA, DNA, and protein in fish. Pp. 345-354. In: Summerfelt, RC; Hall, GE (eds.) Age and growth in fish. Int. Symp. on Age and Growth in Fish, Des Moines, IA (USA), 9 Jun 1985.