

RESEARCH NOTE

EFFECTS OF FIXATION AND PRESERVATION METHODS ON THE MORPHOLOGY OF A LOLIGINID SQUID (CEPHALOPODA: MYOPSIDA)

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ABSTRACT

The effects of freezing, fixation and preservation in 70% ethanol or 10% formalin for periods up to 46 months on body morphometry of *Loligo sanpaulensis* Brakoniecki, 1984, were investigated. Significant morphometric changes were observed, mainly between previously frozen and non-frozen specimens. Some forms of long-term preservation produced further, statistically significant changes. Long-term preservation increased variability of individual effects, widening confidence limits of most indices. Fresh squids or material recently fixed in a standard way should be used for population studies if there is no previous knowledge of the effects of fixation techniques on specific measurements.

Body proportions frequently are used as criteria for distinguishing groups of organisms in terms of species or populations. Morphometric indices are calculated from soft part measurements that are more subject to changes due to fixation and preservation in cephalopods, than, for example, in crustaceans and vertebrates. Therefore, care must be taken to recognize real differences as distinct from those caused by processing techniques.

In loliginids with worldwide distributions and closely related species and subspecies, morphometric indices have been used to identify and classify groups in taxonomic studies (Cohen, 1976; Voss, 1977; Juanicó, 1979) as well as to distinguish stocks or subpopulations (Kashiwada and Recksiek, 1978; Juanicó, 1979). Some papers, notably Cohen (1976), compared short-term effects on squid morphometric indices of refrigeration, fixation in 10% formalin, and preservation in isopropyl alcohol. Our paper deals with short and long-term changes on loliginid squids fixed and preserved in 10% formalin and 70% ethanol, with and without previous freezing.

MATERIALS AND METHODS

The effects of fixation and preservation on measurements and consequently on morphometric indices were analysed on samples of *Loligo sanpaulensis* Brakoniecki, 1984, collected in a bottom trawl survey off Rio Grande do Sul, Brazil, in 1983 (see Haimovici and Andriguetto Jr., 1986). *L. brasiliensis* Blainville, 1823, was the name most commonly applied to the common loliginid in Brazilian waters in the majority of papers published in South America (e.g. Castellanos, 1967; Juanicó, 1980; Figueiras and Sicardi, 1980; Vigliano, 1985). Brakoniecki (1984) considered *L. brasiliensis* a *nomen dubium*, since the holotype no longer exists and because the original description was inadequate and could refer to any of the species of Loliginidae of the Southwest Atlantic (see also Voss, 1974).

Eighty-six specimens were measured within two hours of capture. Then, 40 specimens were frozen, 20 fixed in 10% buffered formalin in sea water and 26 fixed in 70% ethanol. The frozen individuals were thawed and measured again.

Table 1. Methods and length of treatments of *Loligo sanpaulensis*

Treatment	Number of animals	Mantle length range (mm)	Length of treatment	
			Short-term (days)	Long-term (months)
10% Formalin	20	36-104	55	46
70% Ethanol	26	39-112	50	46
Freezing	40	52-88	40	46
Freezing; then 10% formalin	20	52-79	40-40	46
Freezing; then 70% ethanol	20	60-88	40-40	46

after 40 days. Half were transferred to 10% formalin and half to 70% ethanol. All measurements were repeated after 46 months of preservation in the corresponding fixatives (Table 1). These procedures can be considered fixation and preservation techniques as defined by Roper and Sweeney (1983).

Measurements taken were: 1, mantle length (ML); 2, fin length (FL), from posterior mantle tip diagonally to the insertion of anterior left border; 3, fin width (FW); 4, arm length (AL), length of third left arm, measured from its tip to the anterior margin of left eye; 5, length of extended left tentacle (TL), measured as for the arm; 6, eye diameter (ED). Measurements 1, 3 and 6 follow Roper and Voss (1983). The corresponding indices were calculated as percentages of the mantle length, e.g. TLI, ALI, FLI, FWI, EDI (Roper and Voss, 1983).

COMPARISONS BEFORE AND AFTER TREATMENTS

Ratios of measurements and indices before and after fixation, and after almost 4 years of preservation are shown in figure 1. Values of 1.0 indicate no change, higher values indicate distension and lower values indicate contraction. Ninety-five-percent confidence intervals were calculated and Student's "t" tests were performed to show significant differences between rate values and the value of one.

Fixation in formalin increased FW, FWI and FLI, and reduced TL and TLI. Formalin preservation reduced FL, TL and TLI. Fixation and preservation in ethanol reduced all measurements and indices, except FL and FLI for fixation, and AL, ALI and FLI for preservation.

Freezing reduced mantle and fin length, and increased the other measurements. Posterior fixation in ethanol or formalin reduced significantly all measurements. The only index reduced was FWI, after freezing/formalin fixation. Further changes occurred for all indices after long-term preservation.

COMPARISONS AMONG TREATMENTS

Growth of *Loligo sanpaulensis* within mantle length ranges of our samples was shown to be allometric by Vigliano (1985) and for other loliginids by Haefner (1964). Indices prior to treatments were observed to be heterogeneous between lots. In order to overcome these constraints, differences between indices before and after each treatment were calculated

and covariance analysis (ANCOVA) was applied to the new sets of variables using ML as covariate. Adjusted means and 95% confidence intervals were determined by the GT2 method, using the modification of Gabriel (Sokal and Rohlf, 1981). The overlap of confidence intervals between any pair of treatments indicated whether or not they operate in significantly different ways (Fig. 2).

Differences were found in FWI and EDI between the groups placed directly into formalin vs. alcohol. Arm length index, TLI and FLI of the lot fixed in ethanol differed from the frozen and ethanol fixed lot. Fin length index, TLI and FWI of the lot directly fixed in formalin differed from the previously frozen one. Only formalin fixation following freezing and ethanol fixation following freezing did not show significant differences in any of the calculated indices.

Except for tentacle and arm indices, most differences between treatments were no more observed after long-term preservation. No differences were detected between animals preserved in formalin and in ethanol, as well as between those previously frozen. However, material fixed and preserved directly in ethanol was different from that preserved following freezing in terms of ALI and TLI, and specimens fixed and preserved directly in formalin differed from those previously frozen in their indices of arm length, tentacle length and eye diameter.

DISCUSSION

Many teuthologists have expressed concern about the validity of comparing measurements and morphometric indices of specimens of Loliginidae subjected to different fixation and preservation procedures (Haefner, 1964; LaRoe, 1967; Cohen, 1976; Hixon *et al.*, 1981). Haefner (1964) found arms and tentacles to shrink more than 5% in *Loligo pealei* Lesueur, 1821 and *Lolliguncula brevis* (Blainville, 1823) preserved in 5% formalin, and showed that growth of those species is allometric, indicating that it is imprudent to compare indices from groups having different sizes. LaRoe (1967) observed a contraction of 1.3% in ML of 15 specimens of *Doryteuthis plei* (Blainville, 1823) fixed in 10% formalin and preserved in 70% ethanol. Hixon *et al.* (1981) point out an approximate 5% shrinkage for *Loligo pealei* fixed in 10% formalin and later transferred to 55% isopropanol.

As far as we know, only Cohen (1976) compared body proportions in specimens submitted to different fixation procedures. She found differences in the adjusted means of ANCOVA performed with ML as covariate for *Loligo pealei*

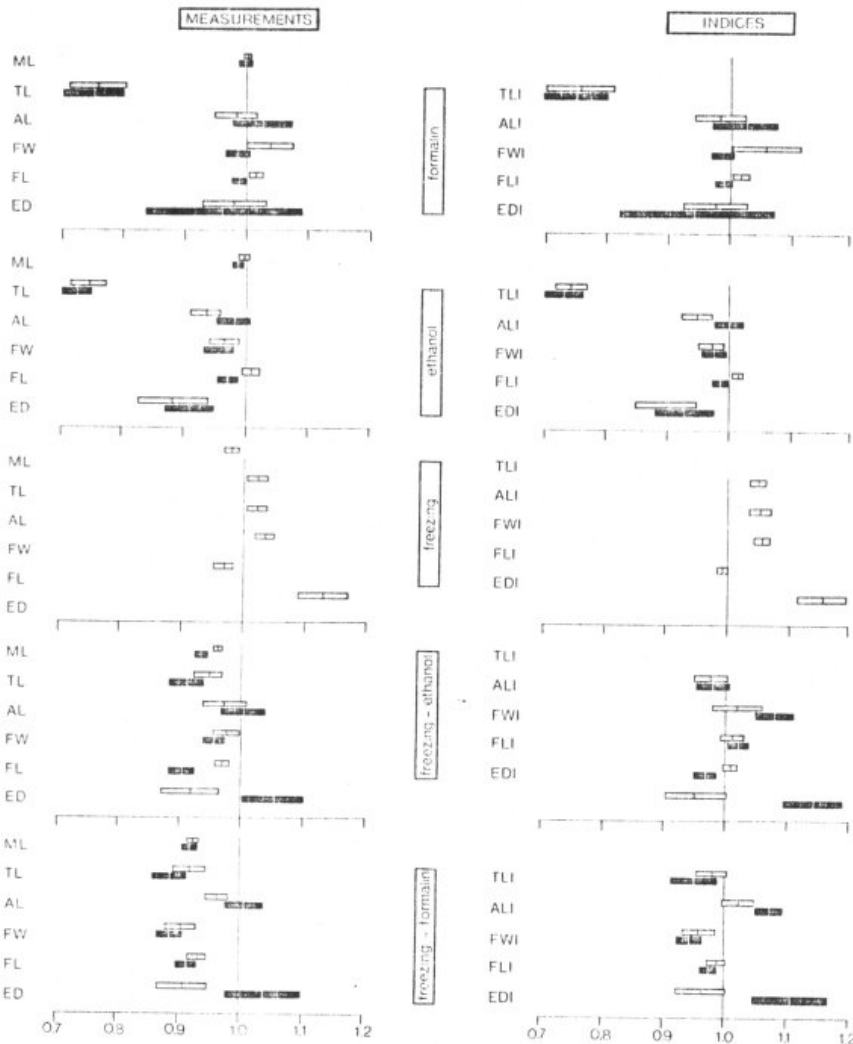


Fig. 1. Means and 95% confidence intervals of the quotients of measurements and indices by treatments. White bars indicate short-term changes; black bars indicate long-term changes. A change is significant when a confidence interval does not include unity.

fixed in 10% formalin and preserved in 40% isopropyl alcohol, when comparing lots of specimens fixed immediately after capture with those previously refrigerated for 48 hours.

Our experiment included other treatments and periods than those tested by Cohen. In addition, we compared changes rather than absolute differences in measurements and indices between lots. This enabled us to compare heterogeneous lots.

Refrigeration and freezing are common methods for stocking squids in commercial fishing, and are useful if fixatives, such as formalin are forbidden on board. The results of Cohen (1976) and those presented here show that some indices in previously refrigerated or frozen specimens are not comparable with those of directly fixed ones, even if the same chemicals were used. The same applies to preservation in ethanol and formalin for several years, although to a lesser degree.

Long-term preservation effects increased the mean variance of indices and consequently the width of confidence limits, making the discrimination of real differences from preservation artifacts more difficult. Despite the small number of indices included in our analysis, the results show that numerical comparisons of populations or species of loliginids based on body dimensions and proportions should consider fixation and preservation induced artifacts, if lots were treated in different ways. The measurement of just caught, fresh specimens, or the comparison of lots fixed and preserved in the same way for similar periods is advisable, unless effects of specific treatments on indices are previously known.

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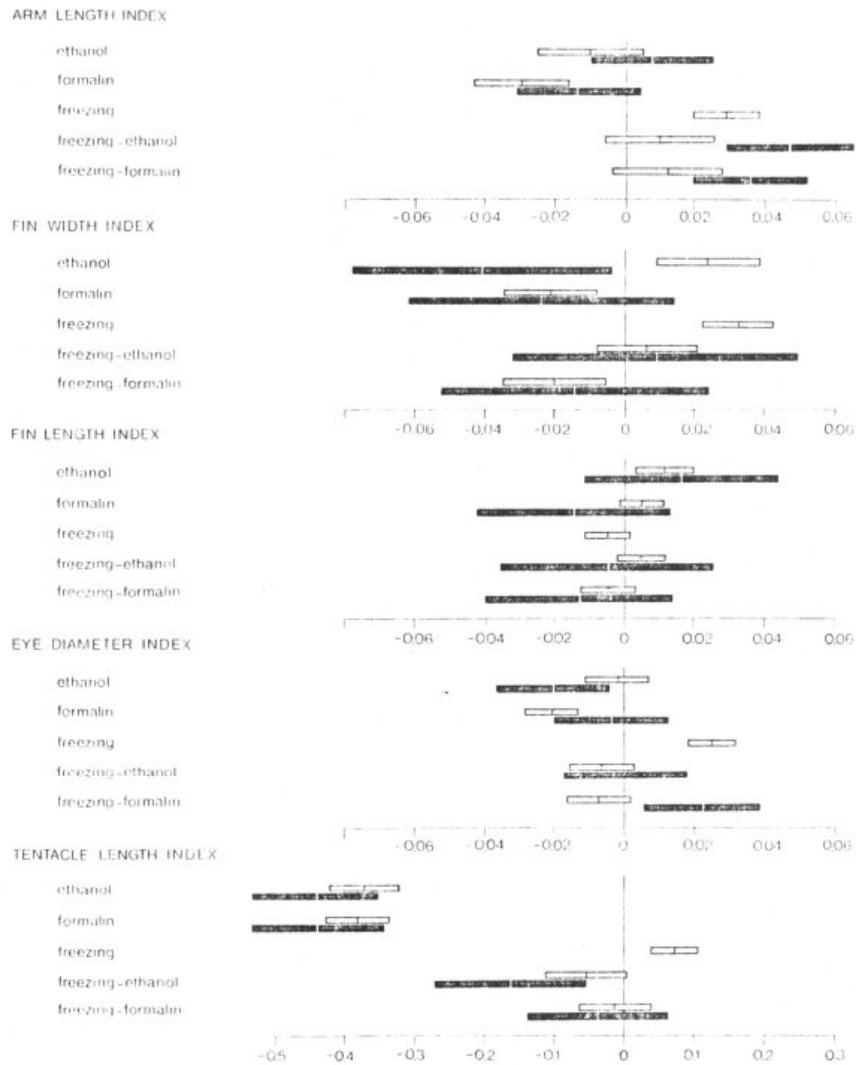


Fig. 2. Adjusted means and 95% confidence intervals of differences between morphometric indices before and after each treatment. White bars indicate short-term differences; black bars indicate long-term differences. Treatments differ significantly when their confidence intervals do not overlap.

LITERATURE CITED

- Brakoniecki, T. F. 1984. A full description of *Loligo sanpaulensis* Brakoniecki, 1984 and a redescription of *Loligo gahi* D'Orbigny, 1835, two species of squid (Cephalopoda, Myopsida) from the Southwest Atlantic. *Bulletin of Marine Science* 34(3):435-448.
- Castellanos, Z. J. A. 1967. Contribución al estudio biológico de *Loligo brasiliensis* Bl. *Boletín del Instituto de Biología Marina* 14:5-35.
- Cohen, A. C. 1976. The systematics and distribution of *Loligo* (Cephalopoda, Myopsida) in the Western North Atlantic with descriptions of two new species. *Malacologia* 15:299-367.
- Figueiras, A. y O. E. Sicardi. 1980. Catálogo de los moluscos marinos del Uruguay. Parte X: Revisión actualizada de los moluscos marinos del Uruguay con descripción de las especies agregadas. Sección II: Gastropoda - Cephalopoda y bibliografía consultada. *Comunicaciones de la Sociedad Malacológica del Uruguay* V(38):179-277.
- Haefner, P. A. 1964. Morphometry of the common Atlantic squid, *Loligo pealei*, and the brief squid, *Lolliguncula brevis*, in Delaware Bay. *Chesapeake Science* 5(3):138-144.
- Haimovici, M. and J. M. Andriquetto Jr. 1986. Cephalopods in bottom trawl fishing off south brazilian coast. *Arquivos de Biologia e Tecnologia do Instituto de Tecnologia do Paraná* 29(3):473-495.
- Hixon, R. F., R. T. Hanlon and W. H. Hulet. 1981. Growth and maximal size of the long-finned squid *Loligo pealei* in the northwestern Gulf of Mexico. *Journal of Shellfish Research* 1(2):181-185.
- Juanicó, M. 1979. Contribuição ao estudo da biologia dos Cephalopoda Loliginidae do Atlantico Sul Ocidental, entre Rio

- de Janeiro e Mar del Plata. Doctoral Dissertation, Instituto Oceanográfico de São Paulo, Brazil. 102 pp.
- Juanicó, M. 1980. Developments in South American squid fisheries. *Marine Fisheries Review* 42(7-8):10-14.
- Kashiwada, J. and C. W. Recksiek. 1978. Possible morphological indicators of population structure in the market squid, *Loligo opalescens*. *California Department of Fish and Game, Fish Bulletin* 169:99-111.
- LaRoe, E. 1967. A contribution to the biology of the Loliginidae (Cephalopoda: Myopsida) of the tropical western Atlantic. Master's Thesis, University of Miami, Florida. 220 pp.
- Roper, C. F. E. and G. L. Voss. 1983. Guidelines for taxonomic descriptions of cephalopod species. *Memoirs of The National Museum of Victoria* 44:48-63.
- Roper, C. F. E. and M. J. Sweeney. 1983. Techniques for fixation and preservation of cephalopods. *Memoirs of The National Museum of Victoria* 44:28-47.
- Sokal, R. R. and F. J. Rohlf. 1981. *Biometry*, 2nd ed. W. H. Freeman, New York. 859 pp.
- Vigliano, P. H. 1985. Contribución al conocimiento de la biología de *Loligo brasiliensis* Blainville, 1823 (Mollusca, Cephalopoda) en aguas argentinas. Doctoral Dissertation, Facultad de Ciencias Naturales y Museo de la Universidad Nacional de La Plata, Argentina. 183 pp.
- Voss, G. L. 1974. *Loligo surinamensis*, a new species of loliginid squid (Cephalopoda, Myopsida) from northeastern South America. *Zoologische Mededelingen* 48(6):43-53.
- Voss, G. L. 1977. Present status and new trends in cephalopod systematics. *Symposium of the Zoological Society of London* 38:49-60.
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