Preservation of Northern Anchovy in Formaldehyde Solution

J. ROE HUNTER
Southwest Fisheries Center,
National Marine Fisheries, Service, NOAA,
P.O. Box 271, La Jolla, CA 92038

ABSTRACT

Preservation and curation techniques used for northern anchovy, *Engraulis mordax*, taken in routine biomass surveys are described, and the effects of formaldehyde solution on length and weight are documented. Preservation in 10% buffered formaldehyde solution caused a 4% increase in anchovy wet weight after 24 h, but only negligible changes occurred over the next 340 d. Preservation also caused a 2% shrinkage in standard length after 24 h which increased to 3% after 340 d.

INTRODUCTION

The fish reproductive parameters used in the egg production biomass estimate (spawning fraction, female weight, batch fecundity, and sex ratio) are based on analysis of formalin-preserved specimens taken in trawl or purse seine collections. Careful attention to preservation and curation techniques is essential for an accurate measurement of these variables.

PRESERVATION

Histological analysis requires special care in preservation. Postovulatory follicles are relatively subtle histological structures, and poor preservation makes it impossible to stage them. Fish should be preserved while alive. Neither frozen specimens nor fish dead for some time can be used. A limited amount of biological material is preserved per collection container. We preserve only five adult anchovies per quart jar, roughly 80-150 g of tissue in 800 ml of formalin (see following). In all specimens, the body cavity is slit open along the side using a long incision to insure adequate preservation of the ovary. Care must be taken not to cut the ovary; this is difficult in females with hydrated eggs. If eggs are lost, they should be added to the jar.

To insure adequate preservation of the ovaries, we use a 10% buffered formaldehyde solution (formalin). Quantities of chemicals required for an 18-L container of buffered 10% formaldehyde preservative are as follows:

16.2 L distilled water
117 g sodium phosphate dibasic (granular, $Na_2H_2PO_4$)
72 g sodium phosphate monobasic (granular, $NaH_2PO_4 \cdot H_2O$)
1.8 L formaldehyde solution (37%).

Dissolve the sodium phosphate diabasic and stir in the distilled water; then mix in the sodium phosphate monobasic until dissolved, and finally add the formaldehyde solution.

Although Bouin's solution (Galigher and Kozloff 1971) might improve the quality of histological sections of ovarian tissue, its use is impractical for production work. Bouin's is more difficult to use on shipboard because represervation is required and because Bouin's preservation makes fecundity estimation difficult. Specimens preserved as described above are kept in the original preservative until curated, which may occur after as much as 2-3 mo of storage.

CURATION

A total of 15-25 females with active ovaries are removed from each collection in random order, blotted, weighed (nearest 0.1 g), and length measured (nearest 1 mm). We curate five more females per collection than needed for the spawning frequency estimate (see Picquelle 1985) in order to allow for females that will be rejected after histological analysis because of immaturity. (It is easier to curate a few additional females than to return to the original collection to process additional fish.) After a female has been weighed, the ovary is removed, blotted dry, and weighed to the nearest mg. Females may be discarded at this point, but the ovary is represerved in fresh buffered formaldehyde solution and stored individually in a vial. Ovaries that appear to be hydrated are noted, as are the presence and quantity of loose eggs in the jar. Each female is assigned a unique identification number (collection number plus fish number).

In female anchovy of 12 g or less, we consider ovaries immature when the ratio of ovary weight/female weight (less ovary) is 0.01. For females of 12 g or less, the probability of maturity is less than 5% when the gonad body weight ratio ≤0.01 (Hunter and Macewicz 1980). These ovaries are stored in vials, but not processed histologically, regardless of the size of the ovary. Histological criteria are used to distinguish between immature and inactive ovaries in females heavier than 12 g (Hunter and Macewicz 1985).

Males are processed along with the females if a separate sample is not taken for sex ratio estimation (Picquelle 1985). If males and females are preserved together, fish must be taken randomly from the jars to maintain a random order by sex. Males are weighed until the combined total of male plus female weight in a single sample equals 500 g (the combined weight needed for sex ratio calculations), and thereafter no additional males are processed in that sample.

Table 1.—Effect of formalin preservation on standard length and wet weight of Northern anchovy preserved alive.

Elapsed time in formalin (Days)	Percent change from live measurement: Length (mean, 2×SE*)	Weight (mean, 2×SE*)
1	−2.26 0.21	4.20 0.54
5	−2.44 0.24	4.20 0.59
10	−2.55 0.24	4.26 0.60
21	−2.81 0.24	5.40 0.74
42	−2.46 0.23	4.35 0.68
84	−2.27 0.21	3.70 0.74
341	−2.93 0.23	3.74 0.72

*Two times the standard error of the mean, where n for each sample = 55, except at elapsed time 21 days where n = 38.

EFFECTS OF PRESERVATION

The reproductive parameters used for biomass estimation are expressed in terms of live wet weight, but as the specimens are preserved in formaldehyde solution, a correction coefficient must be used.

The wet weight and length of 75 northern anchovy (ranging in weight from 2.6 to 27.0 g; mean weight 14.03 g) were determined when alive, 24 h after formaldehyde preservation, and at intervals over 340 d. Fifty-five fish were treated using the standard method described above (preserved alive with body cavity slit), 10 fish were first pithed, body cavity slit, and preserved while still alive, and 10 were pithed, slit, and allowed to die over a 20-min period before preservation.

In nearly all fish, the wet weight increased as a result of preservation. In only one of the 75 fish preserved was the wet weight after 85 d less than the initial live wet weight, and in only four fish was the weight the same. The increase in wet weight was minor. The mean increase in wet weight of the 55 fish preserved using the standard method was about 4%. In these fish, the total gain in wet weight occurred within 24 h after preservation and thereafter it remained about the same (Fig. 1). Pithed anchovy did not struggle in the preservative, and the initial weight gain after preservation was only 1%; but they gained weight steadily, attaining a 3% gain in 20 d and thereafter weight remained about the same. No difference existed between the two groups of pithed fish, indicating that death itself had no effect on subsequent weight in formalin. Struggling of live fish in formaldehyde solution appears to increase the initial rate of fluid uptake, but after 20 or more days the difference between fish preserved alive and those pithed or dead was 1% or less. This could cause a slight error in estimated live weight if some fish were preserved dead and others alive and the collection was curated immediately. For biomass estimation, we use the mean for all data on fish preserved alive (+4.3%). Most fish in our surveys are preserved alive and weighed 30-40 d later.

The length of all fish declined as a result of formalin preservation. Shrinkage after 24 h in preservative was 2% and increased to 3% after 340 d in preservative (Table 1). Shrinkage rates did not vary among the three treatment groups.

Similar effects of formalin preservation (shrinkage in length and gain in wet weight) were documented for salmon by Parker (1963), but plaice lost 9% of their wet weight when preserved in 4% formalin and sea water (Lockwood and Daly 1975), and Rosenthal and Westernhagen (1976) report no significant change in three species of siganids after formalin fixation. These and other studies (see reviews by Parker 1963, and Lockwood and Daly 1975) indicate that considerable variation exists with formalin preservation. Factors that account for this variation include species differences, fish size (life stage), state of the fish when preserved, duration of the fish in formalin, and ratio of formalin and dilutant. Among dilutants, fresh water is clearly preferable to sea water (Lockwood and Daly 1975). Clearly, the effects of formalin preservation are quite specific and calibration is required for any changes in technique, species, or life stage.

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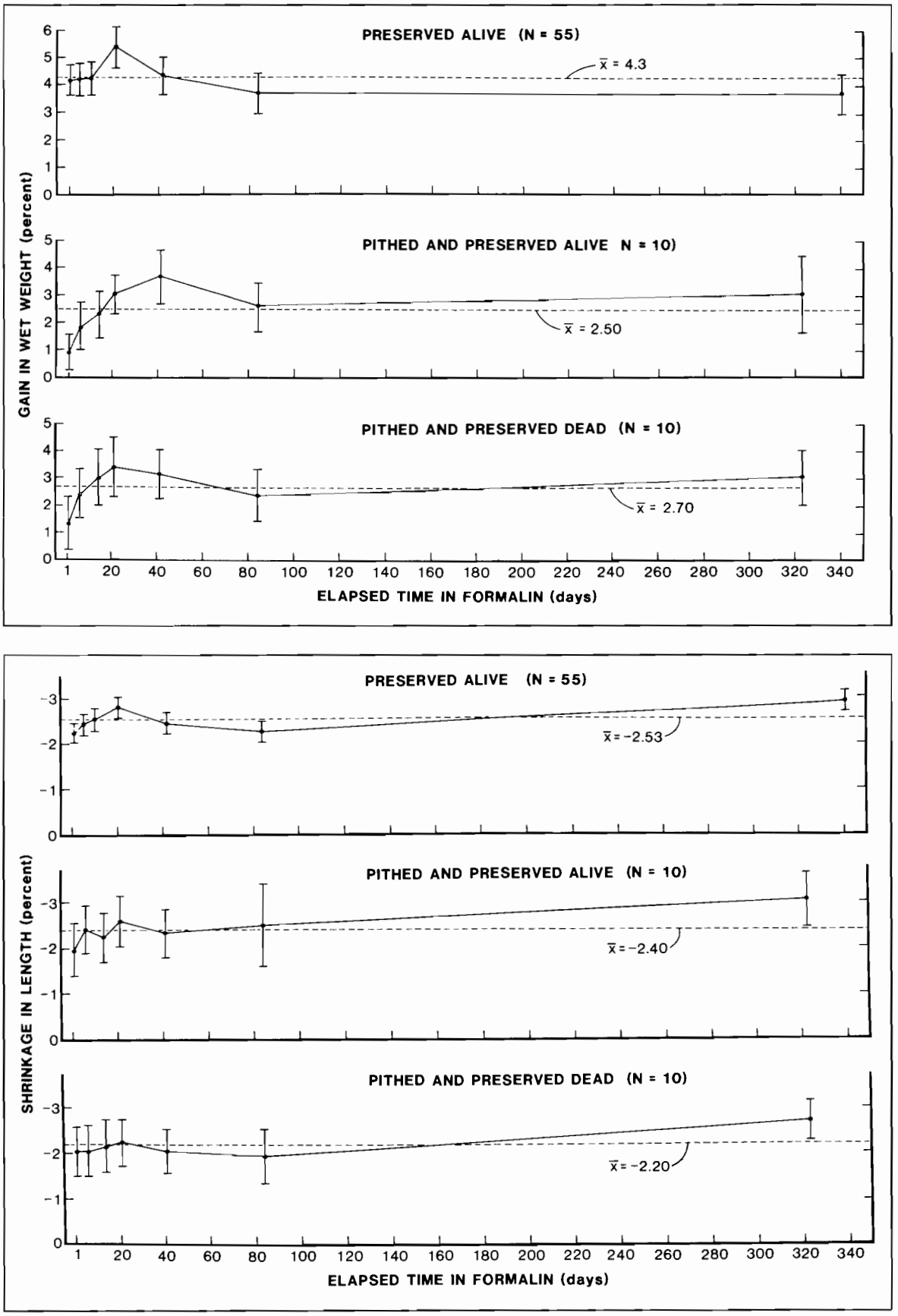

Figure 1.—Effect of 10% formaldehyde preservation on northern anchovy wet weight (upper) and standard length (lower); points are wet weight (upper) or mean loss in standard length (lower) expressed as a percentage of the original live wet weight or live length; bars are ±2×standard error of the mean.

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE

Southwest Fisheries Center
P.O. Box 271
La Jolla, California 92038

April 16, 1987

MEMORANDUM FOR: USERS OF THE EGG PRODUCTION METHOD FOR ESTIMATING SPAWNING BIOMASS OF PELAGIC FISH.

FROM: REUBEN LASKER

SUBJECT: ERRATA FOR NOAA TECHNICAL REPORT NMFS 36; "AN EGG PRODUCTION METHOD FOR ESTIMATING SPAWNING BIOMASS OF PELAGIC FISH: APPLICATION TO THE NORTHERN ANCHOVY".

A number of printing errors have been discovered by Dr. Sachiko Tsuji in the published account of the egg production method. These are important and warrant this memo. Please make these corrections in your copy.

p. 5, Abstract, 4th line should read:
"be estimable and spawning rate constant over the field sampling interval."

p. 12, in equation 8, $\hat{\beta}$ should be β.

p. 17, Table 1. on the January line +3.5 should be -3.5.

p. 20, two lines under the formula in the second column, "sample size" should be "sample scale" and σ_i should read σ_{ij}^2. Five lines under the formula "larger observations" should be "bigger scales."

p. 22, 1st para., No. 3 last line should be simulation, not stimulation.

p. 23. 1st para., line 7. "Table 9" should read "Table 6."

p. 44. Temperature table in second column on the page.

The temperatures read	13.9
	13.5
	16.2
The correct temperatures are	13.9
	15.2
	16.2.

p.45. Second column, $y_{i,t,k}$ should read $y_{i,t}$.

p.46 1st Para., line 7, change the word "spawning" to "tows, $\hat{T}$".

p.49. Table 5d. Strike out the words "within or" in the second line of the heading.

p.55. 9th line from the bottom, x_1 should be x_i.

p.56. First. para. second column, sixth line, 26 should read 25.

p.63. Under "Preservation" $Na_2H_2PO_4$ should be Na_2HPO_4.

p.93. In table 1, atretic state e, change > to <.

p.97. In the! formula after the second para. change < to >.

p.98. In the formula in the first column change -Zt to $-Zt_h$.