The effects of formalin and freezing on ovaries of albacore, *Thunnus alalunga*

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In almost every biological sampling program, tissue samples are collected and preserved for further examination. In this study, the effects of freezing and 10% buffered formalin on albacore, *Thunnus alalunga,* ovaries are compared to examine how each method of preservation affects ovarian weight and oocyte diameter. Formalin is frequently used to preserve ovaries for histological studies but, due to its toxicity, it may not be a good choice in all cases, and alternative methods, such as freezing, should be investigated. There is limited information on the effects of preservation on tuna gonads and, specifically, on albacore gonads. We investigated the effects caused by freezing and 10% buffered formalin on weight and oocyte diameter, because weight and other measurements (such as oocyte diameter) from fresh samples are not considered interchangeable with those from preserved samples (Lagler, 1968).

The purpose of our investigation was to compare fresh ovarian weights with weights of frozen ovaries and formalin-preserved ovaries from albacore as well as to determine how the diameters from oocytes are affected by differing methods of preservation.

Materials and methods

Albacore are seasonal spawners, spawning mainly during summer months (Otsu and Uchida, 1959; Ramon and Bailey, 1996). Ovaries were collected from albacore caught from 1990 to 1993 (Table 1) with longline gear in the South Pacific (group A) and in waters off Hawaii (group B) and with trolling gear in the North Pacific (group C). In group B, samples were labeled as one of three subgroups (B1, B2, or B3) on the basis of the method of preservation used (Table 1).

In the South Pacific, ovaries from each collected fish were dissected and preserved; a total of 150 pairs were collected. In the North Pacific, sampling took place at two sites: 1) between latitude 42°33'N and 52°02'N and from longitude 129°00'W to 145°52'W and 2) in the waters off Hawaii. Table 1 lists the methods of preservation used for all samples.

To investigate the effects that freezing and 10% buffered formalin have on oocyte diameter, we examined oocyte diameters from 58 immature North Pacific albacore from 70 cm to 89 cm fork length (FL) in group C. Right-side ovaries were preserved in 10% buffered formalin; left-side ovaries were frozen until processed two months later. The diameters of the most developed oocyte for each side of the ovary were measured to the nearest 0.01 mm and compared statistically with Student's *t*-test.

To compare differences in oocyte diameter and weight between the two methods of preservation, we used ovaries from subgroup B, which consisted of large, mature albacore (>95 cm FL). The mean diameter of oocytes in the most developed mode was measured from each side of the ovary and compared statistically with Student's *t*-test. The most developed mode of oocytes was determined with the criteria and method described by Schaefer (1987).

The effect of preservation on ovarian weight was examined for all samples collected in Hawaii (group B). Ovaries in group B were weighed fresh to the nearest 0.1 g and were then either preserved in 10% buffered formalin or were frozen. The samples were then reweighed two to three months later; samples preserved in 10% buffered formalin were placed on a paper towel, and excess moisture was patted off before they were weighed to the nearest 0.1 g on a Mettler PM3000 electronic balance. Frozen samples were thawed before being placed on a paper towel, and excess moisture was patted off before they were weighed.

The preserved weights of ovaries were compared with fresh weights by means of Student's *t*-test.

Results and discussion

Effect of preservation on oocyte diameter

Because measurements of oocyte diameter were not made on fresh oocytes, we assumed that left and right ovaries develop at the same rate. This assumption was tested with preserved specimens. Mean oocyte diameters of oocytes in the most developed mode in the right and left ovaries in group A were compared with mean oocyte diameters of oocytes in subgroups B2 and B3. The results indicated no

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Table 1

Summary of albacore ovaries collected in the Pacific Ocean and preservation treatment used. MFL = Mean fork length.

Group	Location	Date	Number of samples	Collection purpose	Preservation method
A—South Pacific	New Caledonia lat. 21–23°S long. 164–166°E	May 1990–Feb 1992	105	Control group for oocyte diameter MFL = 90 cm (78–103 cm)	Formalin
	Tonga lat. 16–29°S long. 171–177°W	Jan 1990–Feb 1992	45	Control group for oocyte diameter MFL = 88 cm (82–102 cm)	Formalin
B—North Pacific	Hawaii within 200-mi EEZ	Jun 1991–Aug 1992	95	Preservation effects on weight and oocyte diameter MFL = 103 cm (96–116 cm)	
B1			16	Left and right ovary of each pair treated differently	Formalin or Frozen
B2			64		Formalin
B3			15		Frozen
C—North Pacific	U.S. jigboat fishery lat. 42–53°N long. 129–146°W	Aug 1990–Sep 1990	60	Preservation effects on oocyte diameter MFL = 82 cm (78–87 cm) Left and right ovary of each pair treated differently	Formalin or Frozen

Table 2

Mean oocyte diameter (mm) data by ovary weight (g) and preservation method for albacore collected in North Pacific. n = number of fish in sample.

		Mean oocyte diameter (mm)				
		Formalin		Frozen		
Ovary weight (g)	n	$\bar{x}$ +/-SE	Range	$\bar{x}$ +/- SE	Range	Percent difference (formalin vs. frozen)
10–19	19	0.10 ± 0.004	0.07–0.14	0.09 ± 0.003	0.07–0.11	10.0
20–29	27	0.11 ± 0.002	0.09–0.13	0.10 ± 0.002	0.08–0.13	9.1
30–39	11	0.11 ± 0.004	0.08–0.13	0.09 ± 0.032	0.08–0.12	18.1
40–49	2	0.11 ± 0.000	0.11–0.11	0.11 ± 0.002	0.10–0.11	0.0
>50	1	0.11		0.11		0.0
Total	60	0.11 ± 0.002	0.07–0.13	0.10 ± 0.002	0.07–0.13	9.1

significant difference in oocyte diameter (t=0.601, df=225, P>0.05) within method of preservation, and we feel the effects of preservation are largely the source of any differences in measurements.

Ovaries from immature albacore (<88 cm FL) in group C, which had been preserved in 10% formalin, were found to have oocytes in the most developed mode with a significantly larger mean diameter in comparison with those from frozen samples (t=4.614, df=59, P<0.05). The mean diameter of oocytes preserved in 10% buffered formalin was, on average, 9.1% larger than the mean diameter of frozen oocytes (Table 2).

In mature albacore >95 cm FL, the mean diameter of oocytes in the most developed mode within an ovary was measured for each side of the ovary and compared statistically within group B1. Oocytes preserved in 10% buffered formalin were found to have a significantly larger diameter (t=3.581, df=14, P<0.05)—7.1% greater, on average, than the oocyte diameter in frozen ovaries (Table 3).

Thus the mean diameter of oocytes in the most developed mode suggested that mean oocyte diameters of frozen ovaries shrank more than those preserved with 10% formalin. Differential preservation effects were reported by Joseph (1963), who looked at the effects of Gilson's fluid (Simpson, 1951) versus 4% formalin on oocyte diameter in yellowfin and skipjack tuna ovaries. He found that the diameters

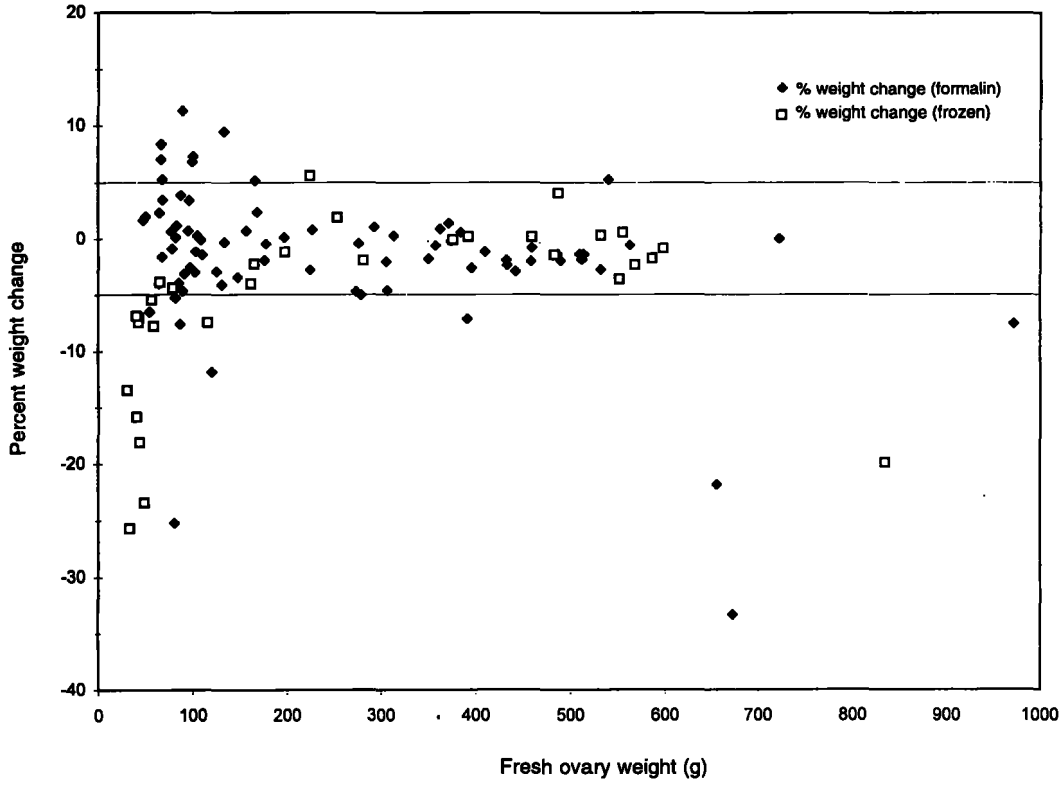

Figure 1

Percent change in ovary weight (fresh versus preserved) for albacore ovaries versus fresh weight in grams for samples collected in Hawaii.

Table 3

Mean oocyte diameter (mm) data by ovary weight (g) and preservation method for albacore collected near Hawaii. n = number of fish in sample.

		Mean oocyte diameter (mm)				
		Formalin		Frozen		
Ovary weight (g)	n	$\bar{x}$ +/-SE	Range	$\bar{x}$ +/- SE	Range	Percent difference (formalin vs. frozen)
<200	11	0.19 ± 0.031	0.12–0.48	0.17 ± 0.027	0.11–0.43	5.3
200–299	1	0.37		0.33		10.8
>300	3	0.59 ± 0.049	0.50–0.67	0.55 ± 0.042	0.48–0.62	6.8
Total	15	0.28 ± 0.049	0.12–0.67	0.26 ± 0.045	0.11–0.62	7.1

of oocytes preserved with Gilson's fluid shrank an average of 24% in comparison with those preserved in 4% formalin. This finding is in contrast with that of Schaefer and Orange (1956) who also examined the effects of Gilson's fluid versus formalin on oocyte diameter. They found that size frequencies for oocytes were similar with both methods for oocytes in the 5–63 μ size range. The strength of formalin used by Schaefer and Orange was greater than that used by Joseph and may be the cause of the different results. Itano[1] examined the effects of brine, refrig-

[1] Itano, D. 1994. Progress report on a large-scale investigation on the reproductive biology of yellowfin tuna in the central and western Pacific region. Fourth meeting of the western Pacific yellowfin tuna research group; Koror, Palau, 9–11 August 1994.

eration, freezing, and 10% formalin on the histological quality of samples of ovary from yellowfin tuna. He reported that histological quality was best for those samples preserved fresh in 10% buffered formalin, whereas samples that had been merely refrigerated could not be used for histology (Itano[1]).

Effect of preservation on weight

The preserved weight of the ovaries from mature fish in group B caught in Hawaii was compared with the fresh weight of ovaries from mature fish in group B (t=2.565, df=94 and P<0.05) and found to have significantly different means at the 95% confidence level. Preserved ovaries averaged a loss of 2% of their fresh weight. Our observations ranged from a loss of 33% to a gain of 11%.

Samples that were frozen (n=31) lost, on average, 6% of their weight and ranged from a loss of 26% to a gain of 6% (Fig. 1). In comparison, those that were preserved in 10% buffered formalin lost, on average, 1% of their weight and ranged from a loss of 33% to a gain of 11%. The greatest difference in albacore ovary weight was observed for ovaries weighing less than 200 g and which had been frozen, as well as for those greater than 600 g and which had been frozen (Fig. 1). The greater percent weight change observed in the smaller ovaries may be a result of processing method. The percentage of weight change for samples greater than 200 g, but less than 600 g, was within 5%. Of the limited number of samples (n=5) greater than 600 g, three of the ovaries that displayed a large loss of weight had oocytes that were hydrating and had mean oocyte diameters near 1 mm.

Conclusion

Freezing was found to have a greater effect on ovarian weight and oocyte diameter than 10% buffered formalin. Consequently, the different methods of preservation used in comparative studies should be evaluated cautiously. Furthermore, a single method of preservation or protocol should be used in a given study.

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