Aquaculture and Fisheries Management 1993, **24**, 365–371

Length reduction and dry weight loss in frozen and formalin-preserved larval walleye, *Stizostedion vitreum* (Mitchill)

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Abstract. We examined the relationships between fresh and preserved measurements of length and dry weight for larval walleye, *Stizostedion vitreum* (Mitchill), subjected to freezing and preservation in formalin. Length reductions for both preservation techniques were <5% and decreased with larval size. Dry weight losses ranged from 32·1 to 54·0% for frozen larvae and from 18·2 to 30·1% for larvae preserved in formalin with larger larvae losing proportionately less weight. Freezing caused significantly greater length shrinkage and dry weight loss than preservation in formalin.

Introduction

Accurate estimates of body length and weight are essential in ecological studies of fishes but time constraints during sampling often necessitate preserving the fish and taking these measurements at a later date. The standard technique for preservation of larval fish is storage in a formalin solution (Ahlstrom 1976; Smith & Richardson 1977; Snyder 1983). However, there are several disadvantages to the use of formalin.

Preservation in formalin has been shown to alter fish length and wet weight (e.g. Parker 1963; Stobo 1972), and dry weight (Hay 1984). Formalin may also cause loss of lipids (Morris 1972; Jones 1976) and decalcification of bone (Taylor 1977). It would therefore not be an appropriate preservative for bioenergetic studies (Dowgiallo 1975) or for studies examining otolith microstructure (Radtke & Waiwood 1980). In addition, formalin fumes are irritating and may pose a human health risk (Anon. 1989).

An alternative to chemical preservation is to freeze fish samples. The effects of freezing on length and wet weight have been examined in juvenile and adult fish (Engel 1974; Treasurer 1990) but rarely in larval fish (Fowler & Smith 1983) and no studies have examined the effects of freezing on fish dry weight. The objective of this study was to examine the relationships between fresh and preserved measurements of body length and dry weight in larval walleye, *Stizostedion vitreum* (Mitchill), subjected to freezing and to compare the results with changes observed in fish preserved in the more commonly used fixative, formalin.

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Materials and methods

Newly-hatched walleye larvae were obtained from the Manitoba Department of Natural Resources, raised in 120-l aquaria at 20°C, and fed a diet of wild zooplankton. Fish were sampled on six dates from 15 May to 6 June 1988. For the 3-week sampling period, walleye fresh length ranged from 8·9 to 21 mm and fresh dry weight ranged from 0·51 to 12 mg.

On each sample date, fish were anaesthetized in a weak solution of MS-222 (ethyl *m*-aminobenzoate methanesulphonate). Measurements were taken to the nearest 0·07 mm using an ocular micrometer and dissecting scope for the first four sample dates and estimated to the nearest 0·1 mm using a scientific ruler mounted under a dissecting scope for the remaining sample dates. Length was measured as total length until a definite fork developed in the tail and as fork length thereafter. Measured fish were sacrificed in a strong solution of MS-222 and immediately transferred to one of three treatments. Fish of the first group were placed on pre-weighed aluminium trays, oven-dried for 24 h at 60°C, moved to a dessicator for 30 min, then weighed to the nearest 0·1 mg. Fish of the second group were placed in vials containing 8 ml of 5% formalin buffered with 2% (by volume) saturated sodium borate solution, the standard fixative for larval fish collections (Smith & Richardson 1977; Snyder 1983). The pH of the buffered formalin was 8·6 but declined to 7·4 when fish were added. Fish of the third group were placed in plastic bags with 15 ml of pond water (pH 8·7–8·8) and placed in a freezer at −18°C. After 180 days of storage, the formalin-preserved fish were rinsed in distilled water, re-measured for length, then oven-dried and weighed. After 1075 days in storage, the frozen fish were thawed at room temperature, re-measured for length, then oven-dried and weighed.

To assess measurement error 5 repeated measures of length and dry weight were taken on five groups of five small larvae (mean length 9·7 mm) and on five individual larger larvae (mean length 14·9 mm). Measurement error was low relative to the treatment effects. Coefficients of variation for length measurements were 0·25% for 9·7-mm larvae measured with the ocular micrometer and 0·56% for 14·9-mm larvae measured with the scientific ruler. Coefficients of variation for dry weight measurements were 1·76% for 0·43-mg larvae and 1·26% for 2·77–mg larvae.

Groups of fish were used for the smaller larvae because of their low weight relative to the sensitivity of the balance. Thus, for the first four sample dates the data consisted of 10 means of five fish each for each of the three treatments. On the last two sample dates the data were derived from ≥20 individual fish for each of the three treatments. Fresh length was regressed against preserved length using standard regression techniques (Draper & Smith 1981). As fresh weight and preserved weight were estimated from different fish, the analysis for weight loss was slightly different. Data were categorized by rounding fresh length to the nearest 0·25 mm for both the fresh and preserved data sets. Mean weights were calculated for each fresh length category and fresh and preserved data sets were match-merged by length category. Mean fresh dry weight was then regressed against mean preserved dry weight for corresponding length categories. The percentage change in length or weight for a given size of larva was calculated as 100 × (Fresh size-Preserved size)/(Fresh size), where preserved size was estimated from the empirical relationships.

Differences in length reduction and dry weight loss between the two preservation techniques were tested by ANCOVA using SAS® software (Freund & Littell 1981). Fresh length was used as the covariate. Natural logarithms of both fresh length and preserved weight were taken prior to testing for differences in weight loss.

Results

Relationships between fresh and preserved measurements are illustrated in Fig. 1. Based on the length relationships of Fig. 1 the predicted mean length reduction for frozen walleye ranged from 4·3% for 9-mm fish to 2·3% for 21-mm fish. At similar sizes, larvae preserved in formalin experienced mean length reductions of 2·3% and 1·8%, respectively. Dry weight loss was much more pronounced. Based on the weight relationships of Fig. 1 the predicted mean dry weight loss for frozen walleye ranged from 54·0% for 0·5-mg fish to 32·1% for 10-mg fish. By comparison, similar sized walleye subjected to formalin preservation would be expected to lose 30·1% and 18·2% of dry weight, respectively.

Slopes of the preserved length *vs* fresh length relationships were not significantly different between freezing and formalin treatments (ANCOVA, $F = 0{\cdot}22$, d.f. = 1,153, $P = 0{\cdot}64$) whereas the intercepts of these relationships were significantly different ($F = 21{\cdot}0$, d.f. = 1,154, $P < 0{\cdot}001$). Thus, frozen larvae showed a significantly greater length reduction than formalin treated larvae. Slopes of the $\log_e$ preserved weight *vs* $\log_e$ fresh length relationships were significantly different between freezing and formalin treatments ($F = 10{\cdot}42$, d.f. = 1,153, $P < 0{\cdot}01$) and further analyses had to be conducted on restricted data sets. When ANCOVA was performed on larvae of 8–10 mm fresh length, the slopes of the $\log_e$ preserved weight *vs* $\log_e$ fresh length relationships were not significantly different between treatments ($F = 0{\cdot}97$, d.f. = 1,17, $P = 0{\cdot}34$) but the intercepts were significantly different ($F = 39{\cdot}9$, d.f. = 1,17, $P < 0{\cdot}001$). The same analysis for larvae of 16–18 mm fresh length also found an insignificant difference in the slopes ($F = 0{\cdot}50$, d.f. = 1,26, $P = 0{\cdot}49$) and a significant difference in the intercepts ($F = 50{\cdot}1$, d.f. = 1,26, $P < 0{\cdot}001$). Thus, frozen larvae lost significantly more dry weight than formalin treated larvae. However, the difference in dry weight loss between freezing and formalin treatments was significantly dependent on larval length.

Discussion

Comparisons between the freezing and formalin treatments in this study were considered justifiable despite differences in storage time. As most of the cellular changes which accompany freezing are associated with the freezing and thawing processes (Farrant 1980), it is unlikely that length and dry weight losses in the larvae were dependent on the duration of storage. In addition, numerous studies have shown that formalin-induced changes in fish occur primarily within the first few days or weeks of preservation (e.g. Heming & Preston 1981; Fowler & Smith 1983; Tucker & Chester 1984). Thus, we assumed that further reductions in length and dry weight would be insignificant beyond 6 months of formalin preservation.

In this study, freezing resulted in greater shrinkage than formalin preservation, but both treatments caused <5% reduction in larval walleye length. With a few exceptions, reported mean length reductions for fish preserved in a similar manner have been ≤5% (Table 1). Most of these studies have also noted that the per cent reduction in length declines with increasing fish length. Weight changes resulting from preservation have been more variable (Table 1). Freezing has been shown to result in a wet weight loss of <3% in juvenile (Engel 1974) and adult (Treasurer 1990) fish. Reported changes in wet weight for fish preserved in formalin range from reductions of 4% (Lockwood & Daly 1975) to increases of 9% (Heming

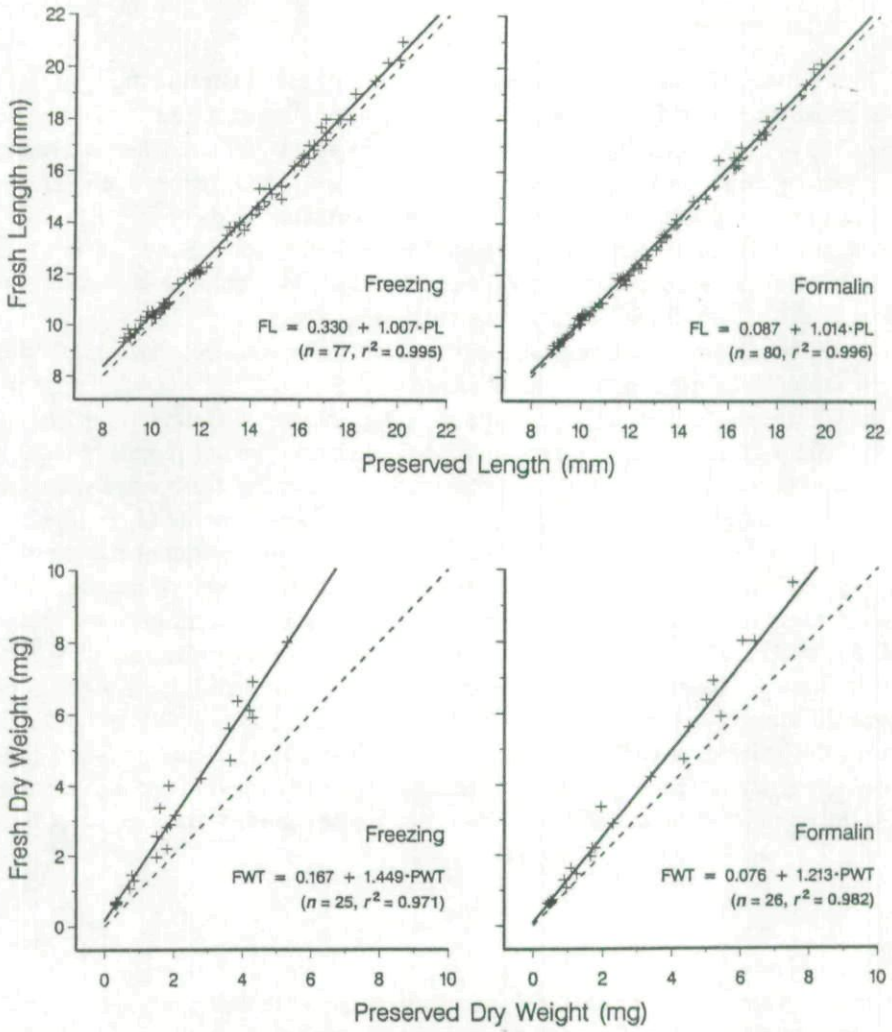

Figure 1. Scatter plots of preserved length versus fresh length (top) and fresh weight versus preserved weight (bottom) for frozen and formalin-preserved larval walleye. Symbols represent individual data values for the length plots and means for the weight plots. Solid line represents the observed relationship. Dashed line represents 1:1 agreement between fresh and preserved measurements.

& Preston 1981). Few studies have examined dry weight loss; however, our results indicated that such losses in preserved larval walleye can be severe, particularly for frozen fish. Dry weight losses in formalin-preserved walleye were slightly higher than those reported by Hay (1984) for formalin-preserved Pacific herring, *Clupea harengus pallasi* Valenciennes.

As with length reduction, dry weight loss declined with increasing fish size. Hay (1984) suggested that weight loss in formalin-preserved herring larvae was a function of the surface area:volume ratio and thus weight loss was proportionately less in larger fish. It is possible that this may be true for both freezing and formalin preservation, and that dry weight loss in juvenile and adult fish may be much less than the relationships of this study would predict.

Table 1. Summary of reported length and weight changes in frozen and formalin-preserved fish. Weight changes refer to wet weight except for the results of Hay (1984) and the results of this study. All formalin mixtures were prepared with fresh water except for Fowler & Smith (1983)(na = not available)

Source	Species	Fresh size	Preservation method	% change	
				Length	Weight
Billy (1982)	*Sarotherodon mossambicus* Trewavas	18–85 mm	5 days in 10% formalin + 65 days in 37·5% isopropyl alcohol	<1	+4
Engel (1974)	*Perca flavescens* (Mitchill)	127–171 mm, 24 g (wet)	72 h frozen at −10 to −15·5°C	<1	−1·7
	Coregonus artedii Lesueur	176–267 mm, 70 g (wet)	72 h frozen at −10 to −15·5°C	−2·1	−2·0
Fowler & Smith (1983)	*Merluccius bilinearis* (Mitchill)	3–15 mm	337 days in 4% neutral formalin–seawater	−4·3	na
			338 days frozen at −15°C	−1·4	na
Glenn & Mathias (1987)	*Stizostedion vitreum* (Mitchill)	9–33 mm	3 days in 5% formalin	−2 to −7	na
			Quick frozen in −70°C ethanol then stored 3 days in freezer	−2 to −15	na
Hay (1982)	*Clupea harengus pallasi* Valenciennes	10–12 mm	6 mo in 5% formalin	−1	na
Hay (1984)	*Clupea harengus pallasi* Valenciennes	0·106 mg (dry)	10 days in 4% formalin	na	−22·2
		0·211 mg (dry)	10 days in 4% formalin	na	−15·5
Heming & Preston (1981)	*Oncorhynchus tshawytscha* Walbaum	26–40 mm, 410–544 mg (wet)	50 days in 5% formalin	−5·3	+2 to +9
Jennings (1991)	*Dicentrarchus labrax* (L.)	5–70 mm	200 days in 4% formaldehyde	−5	na
Kruse & Dalley (1990)	*Mallotus villosus* (Müller)	5–23 mm	168 days in 5% formalin	−5·2	na
Lockwood & Daly (1975)	*Pleuronectes platessa* L.	20–75 mm	1 year in 4% formalin	<1	−4
Parker (1963)	*Oncorhynchus keta, O. gorbuscha* (Walbaum)	34–37 mm, 200–285 mg (wet)	218 days in 3·8% formaldehyde	−4·3	+5
Stobo (1972)	*Perca flavescens* (Mitchill)	68–99 mm	250 days in 10% formalin	−1·4	+7·5

Table 1. Continued

Source	Species	Fresh size	Preservation method	% change	
				Length	Weight
Treasurer (1990)	*Perca fluviatilis* L.	110–340 mm	70–98 days frozen at −25°C	−1·7	−2·7
	Esox lucius L.	190–830 mm	70–98 days frozen at −25°C	−5·4	−2·6
Tucker & Chester (1984)	*Paralichthys lethostigma* Jordan & Gilbert	9–13 mm	1 year in 4% formalin	−2·5	na
This study	*Stizostedion vitreum* (Mitchill)	9–21 mm, 0·5–10 mg (dry)	1075 days frozen at −18°C	−3·3	−32 to −54
			180 days in 5% formalin	−2·1	−18 to −30

Weight loss may be greater in larval walleye because they lack scales. Stobo (1972) hypothesized that heavy scalation in spiny-rayed fishes slowed the osmotic processes involved with formalin preservation. Walleye typically begin to develop scales at a total length of 30 mm (Glenn & Mathias 1985).

Freezing appears to be a suitable preservation method where growth is estimated from length measurements. Length shrinkage was slight and comparable to that seen in formalin preserved fish. However, freezing caused much greater dry weight loss than formalin, particularly in small larvae. Where estimates of larval dry weight are required, such as in energetic studies, suitable correction factors should be applied or other preservation techniques utilized.

Acknowledgements

We wish to thank J. Klapecki for technical assistance with walleye rearing and data recording, and four anonymous referees for reviewing the manuscript. Funding was provided by a Natural Sciences and Engineering Research Council of Canada postgraduate scholarship and a University of Manitoba Fellowship awarded to T.A. Johnston.

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