Change in Length, Weight and Condition of American eel *Anguilla rostrata* Elvers Preserved in 4% and 10% Formalin

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Table of Contents

Abstract

Replicate samples and individual elvers of the American eel, *Anguilla rostrata*, were measured fresh and after varying periods of preservation from 2 to 312 d in 4% or 10% unbuffered formalin. Preserved elvers shrank in length and gained in weight and condition (weight adjusted to a common length) to a degree that was both statistically significant and of a medium-to-large effect size judged important in biological interpretations of length, weight, and condition data. Preservation may induce high heterogeneity in effect sizes due to the interaction of preservative strength, elver size, and duration of preservation. Such heterogeneity in effect sizes may be an important consideration when comparing preserved elver lengths and weights among studies. Equations for the conversion of individual elver length and weight from preserved values to fresh values after various time periods of preservation in 4% and 10% formalin are provided. However, comparisons and comparative studies (meta-analyses) of effect sizes are best made with elver length, weight, and condition measured fresh rather than after preservation. Studies comparing elvers from different sites should also consider the medium-to-large effect sizes due to factors such as the geographic cline in mean elver lengths, the seasonal decline in mean elver length, weight, and condition during freshwater migration, and the annual variability in mean elver length and weight.

Résumé

Des échantillons répétés et des spécimens individuels de civelles de l'anguille, *Anguilla rostrata*, ont été mesurés à l'état frais et après diverses périodes de conservation allant de 2 à 312 jours dans 4 % ou 10 % de formaline non tamponnée. Les civelles conservées ont rétréci en longueur et ont vu leur poids s'accroître et leur condition (le poids ajusté en fonction d'une longueur commune) modifiée dans une mesure statistiquement significative et dont l'ampleur de l'effet est jugée de moyenne à grande pour les interprétations biologiques des données sur la longueur, le poids et la condition. La conservation pourrait donner lieu à une grande hétérogénéité de l'ampleur de l'effet en raison de l'interaction de la force de l'agent de conservation, de la taille des civelles et de la durée de la conservation. Une telle hétérogénéité de l'ampleur de l'effet pourrait être une question importante à prendre en considération lorsqu'on compare la longueur et le poids de civelles conservées d'une étude à une autre. On trouvera dans le rapport les équations de conversion de la longueur et du poids des civelles de l'état de conservation à l'état frais après divers périodes de conservation dans 4 % et 10 % de formaline. Cependant, il est préférable de faire des comparaisons et des études comparatives (meta-analyses) de l'ampleur de l'effet avec la longueur, le poids et la condition des civelles mesurés à l'état frais plutôt qu'après la conservation. Les études comparant des civelles de divers sites devraient aussi prendre en considération une ampleur de l'effet de moyenne à grande due à des facteurs tels que le gradient géographique de la longueur moyenne des civelles, la baisse saisonnière de la longueur, du poids et de la condition moyens des civelles durant les migrations en eau douce et la variabilité annuelle de la longueur et du poids moyens des civelles.

Introduction

Recent concerns about the status of American eel *Anguilla rostrata* stocks in Canada (Peterson, 1997) and the United States (EPRI 1998; ASMFC 1999) have encouraged increased research into all life stages of this species. Samples may be preserved before measurements for length and weight are made which, in turn, may influence composite measures such as condition factor. Preservation effects can vary depending upon the preservative used, its concentration, the storage time, and the species of fish preserved (Parker 1963; Stobo 1972; Billy 1982; Hay 1982; Fowler and Smith 1983; Morkert and Bergstedt 1990; Anderson and Neumann 1996; Shields and Carlson 1996; Cunningham et al. 2000). The reported effects on fish lengths and weights of preservation in formalin have been inconsistent (shrinkage or gain) but most of these studies concluded that lengths shrank and weights increased. The percent shrinkage in length increases with increasing fish length but the degree of shrinkage may vary among species. For example, the percent shrinkage increased moderately (2.6% for 50 mm larvae to 4.2% for 150 mm larvae in 5% formalin) with increasing fish length for larval sea lamprey *Petromyzon marinus* but was constant for juvenile sockeye salmon *Oncorhynchus nerka* (Morkert and Bergstedt 1990; Shields and Carlson 1996). The shrinkage was greater in 10% formalin than in 5% formalin for larval sea lamprey (4.3% versus 3.8%; Morkert and Bergstedt 1990) and for inland silverside *Menidia beryllina* (3.2% versus 2.2%; Cunningham et al. 2000). The absence of preservation effects directly proportional to length or weight prevents the use of a single correction factor when converting preserved length or weight to fresh values, as does temporal change in the degree of preservation effects.

Preservative effects on a sample of a given species of fish are the cumulative result of effects on individual fish as reflected in the size frequency distribution and modified by the type and strength of preservative and storage time. The effects of preservation on the means and variances of sample lengths and weights may be of more interest than the effects on individual fish when sample statistics from different studies are used in comparative studies. For example, Vladykov (1966; 1970) compared the mean and range of American eel elvers preserved in 4-5% formalin for periods from two weeks to eight years and collected from sites ranging from Florida to Quebec. Haro and Krueger (1988) extended Vladykov's data and compared mean lengths, with confidence intervals, of American eel elvers collected from sites at varying distances from the spawning area, some samples of which were unpreserved and others used different formalin preservation methods. The magnitude of the increase in elver length with increasing latitude or distance from the spawning ground estimated by these studies should be reevaluated with respect to preservation effects. More formal meta-analyses may examine variable means and variances from several studies to determine the magnitude of some effect of interest that cannot be readily examined by a single study (Hedges and Olkin 1985; Osenberg et al. 1999).

The objectives of this study were to (1) evaluate the effects of preservation for various time periods in 4% or 10% formalin on the mean lengths, weights, and condition factor of American eel elvers for both sample-based and individual fish data and (2) to examine the magnitude of preservation effects relative to biological effects such as changes in size due to seasonal, annual, and geographic variability.

Materials and Methods

Three replicate samples of American eel elvers (n = 60 per sample) were drawn non-selectively, after stirring, by dipnet from a container holding the daily catch of elvers collected by Irish-type elver traps (Jessop 1998) from the East River, Chester, Nova Scotia on 7 May 1998 (estimated daily catch of 760 elvers) and again on 12 May 1998 (estimated daily catch of 910 elvers). Elvers were overdosed by MS-222, after which each elver was promptly measured to the nearest 0.1 mm total length (TL) by digital caliper and weighed to 0.01 g by electronic balance after thorough blotting dry by paper towel. Particular attention was given to the gill and buccal area to remove adhering moisture by application of light external pressure. After the initial measurements, each of the May 7 samples was preserved in 4% formalin and the May 12 samples were preserved in 10% formalin. The 4% and 10% solutions were prepared from reagent grade, unbuffered 37% formaldehyde diluted with tap water (drawn from an oligotrophic lake system) on a 1:24 and 1:9 volumetric basis. Each sample was re-measured using the same caliper and

balance for length and weight after 2, 4, 7, 12, 22, 35, 49, 63, 77, and 312 days and then returned to its original preservative.

In 2000, a group of elvers (N = 42) consisting of 10 elvers for each 5 mm interval between 50.0 and 69.9 mm and 2 elvers for the interval 70.0-74.9 mm were collected for preservation in each of 4% and 10% formalin. Killing and initial measurement procedures were similar to those in 1998, after which each elver from a given group was preserved in an individual vial. Elvers from each preservation group were individually re-measured after 2, 6, 18, 55, and 167 (169 d for elvers preserved in 10% formalin) d post preservation.

The 1998 replicate sample data and 2000 individual fish data were analyzed in a similar manner, with regard for their different natures. Differences in mean fresh length and weight among the 1998 samples were evaluated by analysis of variance (ANOVA) and Tukey's Highly Significant Difference (HSD) multiple comparison test (Wilkinson et al. 1996). The temporal effects of preservation on elver length and weight were graphically analyzed by box plots and statistically by a univariate repeated measures design in which preservative concentration was the experimental unit, replicate number (for the sample data) was a categorical variable, the days preserved was the repeated factor, and elver lengths and weights were each dependent variables. Contrasts were used to make multiple paired comparisons of mean elver length and weight on days of preservation. Bonferroni adjustments were made to the significance level to account for the number of comparisons made. The lengths and weights of individual elvers preserved for various time periods were regressed (least squares) on the fresh values. The shrinkage in length and gain in weight were also regressed on fresh values and a lowess smoother was fitted to scatter-plots of the data.

Sample length distributions were near normally distributed while weight distributions were slightly right skewed and leptokurtic. Non-normality of weights was insufficient to require transformation for the use of parametric statistics for subsequent analyses such as the repeated measures analysis or the comparison by analysis of covariance (ANCOVA) of the regressions of preserved length and weight after various times in preservation on fresh length and weight. Heterogeneity of regression slopes for the ANCOVA was examined by the significance of the ANOVA interaction between the treatment mean and covariate (Wilkinson et al. 1996). The range of lengths and weights was similar among samples preserved in 4% formalin and in 10% formalin. Sample variances of both length and weight were homogeneous. However, elver lengths and weights were logarithmically (base 10) transformed for the evaluation of elver mean condition at each remeasurement date because condition was based on the weight-length regression. Condition was the sample mean weight adjusted to the overall mean length of the pooled replicate samples, as determined by an ANCOVA of the sample weight-length relations (Cone 1989). Estimates of mean sample condition and the associated 95% confidence interval (CI) at each remeasurement date were back-transformed from the logarithmic values for presentation following Ricker (1975, p. 275). Statistical significance was accepted at $\alpha \leq 0.05$.

The observed magnitude of preservation change in length and weight over time was estimated by the difference between the fresh, unpreserved (control) sample mean length ($\overline{Y}_C$) and weight and the mean length and weight of the preserved (experimental) samples ($\overline{Y}_E$) after x days in preservation, where $Difference = \overline{Y}_E - \overline{Y}_C$. Confidence intervals (95%) for the difference between two means were calculated following Zar (1984).

The magnitude of the observed preservation effects was evaluated by standardizing the mean differences with Glass's g' statistic of effect size (Hedges and Olkin, 1985): $g' = \left(\frac{\overline{Y}_E - \overline{Y}_C}{S_C} \right) J$, where S_C is the control group standard deviation and J is a correction factor that adjusts for bias due to small sample size. The sample size correction factor J is calculated as $J = \left(\frac{3}{4(N_E + N_C - 2) - 1} \right)$ where N is the sample size for the experimental (E) and control (C) groups. When each sample size N equals or

exceeds 60, the correction factor J exceeds 0.987. The control group standard deviation was believed more appropriate than the pooled sample standard deviation because the preserved sample variance for length may be biased by increased shrinkage in length and gain in weight with increasing fish size (Morkert and Bergstedt 1990; Shields and Carlson 1996). The variance of g' was estimated as

$\hat{\sigma}^2(d) = \frac{n_E + n_C}{n_E n_C} + \frac{d^2}{2(n_E + n_C)}$. The 95% CI for g' was calculated as $g' \pm t_{\alpha/2}\hat{\sigma}(g')$.

Cohen (1988) gives guidelines for the interpretation of the magnitude of the experimental effect size g', where 0.2 is a small effect, 0.5 is a medium effect, and 0.8 is a large effect. The effect size may be positive or negative, depending upon whether the experimental manipulation caused an increase or decrease in the measured variable. The statistical significance of g' was evaluated by the analysis of confidence intervals. Conceptually, the effect size relates statistical significance with sample size, as: effect size = significance-test statistic/size of study where "size of study" may be some function of the sizes of the two samples involved (Rosenthal 1993; Tatsuoka 1993). The biological relevance of an effect size ultimately is determined by the research question asked, the study design, and the researcher's judgement because statistically significant results are not necessarily biologically significant (Snyder and Lawson 1993).

Results and Discussion

Elver Sample Measurements - 1998

The replicate unpreserved samples of American eel elvers (later preserved in 4% formalin) averaged 64.78 mm TL and 0.186 g in weight and did not differ significantly among samples in mean length (F = 1.88, df = 2,177, P = 0.15) or weight (F = 2.75, df = 2,177, P = 0.07) (Table 1). Elvers preserved in 10% formalin averaged 63.11 mm TL and 0.167 g and did not differ among samples in mean length (F = 1.78, df = 2,177, P = 0.17), but did differ in mean weight (F = 8.00, df = 2,177, P = 0.0005), with two samples not differing between themselves but each differing from the third sample according to the Tukey HSD test (Figure 1). The significant difference in mean weights among samples of elvers taken on the same day from a much larger daily elver catch was probably due to chance.

Each sample of elvers preserved in either 4% or 10% formalin showed significant changes in both mean length and weight over the preservation period ($F \geq 16.7$, df = 10,1740, $P < 0.0001$ in 4% formalin; $F \geq 12.6$, df = 10,1770, $P < 0.0001$ in 10% formalin) (Table 2, Figure 1). There was no significant interaction between day and replicate ($F < 1.25$, df = 20,1740 or 20,1770, $P \approx 1.00$) for either length or weight for either formalin concentration. The Huynh-Feldt assumption of homogeneous variances of the differences between all pairs of trials and the Greenhouse-Geiser assumption of equal variances within trials (compound symmetry) were both met for each formalin concentration, as required for the univariate repeated measures analysis (Wilkinson et al. 1996).

Reader effects, as indicated by an inconsistent pattern of relative change among repeated measurements of the samples, appeared minimal (Figures 1, 2, 3). Length measurements may have been more precise and consistent than weight measurements but the blotting procedures helped minimize the variability in weight measurement, which can amount to 2.5% between readers (Parker 1963; Shields and Carlson 1996). Measurement of elvers to 0.01 g provides a potential 5% measurement difference on an elver of 0.20 g with very little change in true weight while measurement to 0.1 mm for a 55 mm elver provides potential for a 0.2% difference. Little difference was observed among replicate samples in the effects over time of preservation in either 4% or 10% formalin (Figures 1, 2, 3) and, as previously noted, the replicates did not differ significantly in length or weight (with one exception). Thus, the replicates were pooled for further analysis.

In 4% formalin, the average shrinkage in mean elver length increased initially from about 3.4 mm (95% CI 2.4-4.4 mm) or 5.2% of unpreserved length after two days of preservation, to 4.1 mm (95% CI 3.1-5.1 mm) or 6.3% of unpreserved length after 312 days (Figures 1, 2). In 10% formalin, the average shrinkage increased from 2.7 mm (95% CI 1.6-3.8 mm) or 4.3% of unpreserved length after two days of preservation, to 3.5 mm (95% CI 2.4-4.6 mm) or 5.4% of unpreserved length after 312 days. Following

the initial shrinkage, mean elver lengths differed little within either 4% or 10% formalin over the 312-day period, but shrinkage increased slightly but non-significantly between day 77 and day 312 (Table 2). Larval (35-160 mm) sea lampreys, *Petromyzon marinus*, preserved in 5% and 10% formalin shrank most in length during the first 2 h of preservation with little further shrinkage after 9 weeks of preservation (Morkert and Bergstedt 1990). Parker (1963) reported shrinkage of 3% in the length of various species of Pacific salmon within 12 h after preservation in 3.8% formalin with little further shrinkage after 40 d. Sockeye salmon smolts varied in results when preserved in 10% formalin, from no significant change in length in one experiment to minor (1%-3%) shrinkage in two other experiments (Shields and Carlson 1996). The average shrinkage in length of 100-mm lamprey larvae was slightly greater (4.3% versus 3.8%) in 10% formalin than in 5% formalin (Morkert and Bergstedt 1990). The shrinkage of inland silverside larvae of 6-23 mm length was also greater in 10% formalin (3.2%) than in 5% formalin (2.2%) (Cunningham et al. 2000). These results contrast with the greater shrinkage of American eel elvers in 4% formalin than in 10% formalin. The rate of initiation of preservation effects may be most rapid in fishes with a high ratio of surface area to volume, such as eel elvers and lamprey larvae, and more rapid in larval fish than in adult, scaled fish. For American eel elvers, 4% formalin may penetrate more slowly and further than 10% formalin before fixation effects stabilize the tissues and prevent further shrinkage (Steedman 1976).

The mean effect size statistic (g') of shrinkage in length was generally greater in 4% formalin, ranging from 1.1 to 1.4 among replicates, than in 10% formalin, where it ranged from 0.7 to 1.1 (Figure 3). Values of g' ranging from 0.7-1.4 were highly statistically significant and are probably biologically significant also since effect sizes of about 0.5 have been commonly found in various studies (Kirk 1996; Arft et al. 1999). Changes in elver size due to preservation are not necessarily of direct biological importance but the interpretation of their effects may have biological meaning depending upon the use of the analysis. The fresh length of elvers preserved in 4% formalin averaged about 1.7 mm larger before preservation in 10% formalin because they were collected earlier in the run (May 7 versus May 12). Elvers from the northern part of their range decline in length and weight during the run (Haro and Krueger 1988; Jessop 1998). Thus, the interaction of formalin concentration with elver length can produce a greater relative shrinkage of longer elvers in 4% formalin than of shorter elvers in 10% formalin. This shrinkage was of "moderate" effect magnitude (difference in g' of about 0.4-0.5). This finding is consistent with the increased shrinkage in length of larval lamprey with increasing fish length (Morkert and Bergstedt 1990) and of American eel elvers (see section on Individual Elver Measurements).

After the initial average weight gain of 0.035 g (95% CI 0.022-0.048 g) in 4% formalin and 0.032 g (95% CI 0.019-0.045 g) in 10% formalin during the first two days of preservation, elver mean weights gradually, but non-significantly, declined over the next 75 days of preservation. After 312 d, the decline in mean weight from the initial gain was significant, resulting in mean weights that were not significantly different from their initial values in five of six samples (Figures 1,2; Table 2). The magnitude of initial gain and then subsequent decline in weight are inversely related to fish size in some species (Stobo 1972; Billy 1982) and may also be for elvers.

The mean effect size statistic (g') for the gain in weight was similar and of high magnitude (range 0.8-1.1) early in the time series and declined through the time series in both 4% and 10% formalin. After 312 d of preservation, the low-to-moderate magnitudes of g' (0.1-0.4) in 4% formalin and in 10% formalin (0.2-0.5) are of little concern, particularly where a lower limit of zero occurs for the 95% CI about g' for all three samples in 4% formalin and for two of three samples in 10% formalin. The effects of preservation on elver weight were clearly sufficient to require consideration for preservation periods between 2 and 77 d, but of little concern after 312 d post-preservation.

Mean condition, adjusted for length, was significantly higher for elver samples preserved in both 4% and 10% formalin than for unpreserved elvers (Figure 4). Within 2 d post-preservation, the mean sample condition of elvers increased 41% in 4% formalin and 37% in 10% formalin. As the preservation period increased from 2 to 312 d, mean condition declined significantly, as is evident in the large degree of non-overlap of the 95% CI for days 4 and 312. The decline in condition was not so great, after 312 d, as to eliminate the significant difference in condition (26% higher in 4% formalin, 27% higher in 10%

formalin) between unpreserved and preserved elvers. The magnitude of effect size for elver condition after 312 d of preservation was greater for the smaller elvers preserved in 4% formalin (g' = 1.1) than for the larger elvers preserved in 10% formalin (g' = 0.5). This result is a consequence of the longer mean length of the elver samples preserved in 10% formalin than in 4% formalin and the interaction of the greater relative shrinkage of longer elvers in 4% formalin than of shorter elvers in 10% formalin and the similar relative gain in weight for both formalin concentrations. Both effect sizes are of sufficient magnitude to be of probable biological importance. The interaction of shrinkage in length and gain, then loss, of weight over an extended period of preservation in formalin is a dynamic process with effects on the estimation of elver condition that may be both statistically significant and of importance to the biological interpretation of a study.

Equations based on linear regressions that converted to fresh length the mean lengths and weights of formalin preserved samples of elvers were evaluated for usefulness (Figure 2, Table 3). The percent shrinkage in length and gain in weight of elvers preserved in formalin decreased with time. The regression slopes were significant for the shrinkage in elver sample lengths over the 312-d preservation period but were non-significant between day 2 and day 77 of preservation in 4% formalin (P = 0.35) and in 10% formalin (P = 0.30). Thus, the correction factor was constant over this intermediate period. The regressions depend largely on the degree of change in length or weight between day 77 and day 312 post-preservation. The regression slopes for the shrinkage in length of elver samples preserved in 4% and 10% formalin were homogeneous (F = 1.05, df = 1,59, P = 0.31) but the intercept was larger for samples preserved in 4% formalin (F = 21.3, df = 1,60, P < 0.0001; Table 2), as expected from the previous analysis of preservation effect size. Consequently, the rate of shrinkage in elver lengths was similar in each preservative strength but the initial degree of shrinkage differed depending on length composition and preservative strength. The slopes of the regressions of the decline over time in the difference (gain) between fresh and preserved (both 4% and 10% formalin) weights of elvers were homogeneous (F = 3.11, df = 1,59, P = 0.08). Thus, the rate of decline in post-preservation weight gain was similar for both 4% and 10% formalin. Elvers preserved in the different formalin concentrations did not differ in their weight gain (F = 2.19, df = 1,60, P = 0.14; Table 3), probably due to the difference in mean size of the elvers preserved in each formalin concentration (smaller size in 10% formalin) and high variability in weight at a given length. Low R^2 values for the conversion equations for length indicate that they should be used cautiously if at all while higher R^2 values for weight indicate greater usefulness. The interactions of preservative strength and elver length and weight composition and their temporal change makes the estimation of fresh length, weight, and, particularly, condition from preserved specimens inadvisable if accuracy is desired. Conversion equations estimated for samples of elvers will be specific to those samples due to their unique length composition. Consequently, there is no general solution to estimating the effects of preservation on the mean length, weight, or condition of samples of elvers where only sample statistics are available. This restraint applies to other species of fish where the percent change in length or weight is not directly proportional to the length or weight, e.g., Hay (1982), Fowler and Smith (1983), Morkert and Bergstedt (1990), Cunningham et al. (2000).

***Individual Elver Measurements* – 2000**

Individual elvers preserved in 4% and 10% formalin decreased in significantly in mean length (P < 0.0001) and gained in weight (P < 0.0001), with most of the initial change occurring within 2 d post-preservation (Table 4, Figure 5). The initial (2 d post preservation) degree of shrinkage in length was higher in 4% formalin (3.8%) than in 10% formalin (3.0%) while the gain in weight was higher in 10% formalin (28.5%) than in 4% formalin (24.8%). Mean elver lengths in both formalin concentrations progressively decreased a small but significant degree as the preservation period increased. Mean elver weights also decreased with increasing preservation period after the initial gain in weight but to a greater degree than did lengths. Consequently, while mean elver lengths continued to shrink, if slowly, mean weights progressively approached the fresh weights. After 168 d in 4% formalin, the elver mean weight remained significantly higher than the fresh weight; after 170 d in 10% formalin, the mean elver weight was not significantly different from the fresh weight. These patterns are consistent with those found for the elver sample data of the previous section.

Regressions of the length and weight of American eel elvers after various periods of preservation in either 4% or 10% formalin on their fresh length and weight varied significantly in parameter values (Table 5, Figure 6). ANCOVAs of these regressions differed in results depending upon the variable and formalin concentration. In 4% formalin, the regression slopes of preserved length on fresh length after various periods of preservation were homogeneous ($F = 0.71$, $df = 4, 200$, $P = 0.58$) and the adjusted (to the overall mean) mean lengths of elvers after various periods in preservation were significantly different ($F = 8.6$, $df = 4, 204$, $P < 0.001$), as would be expected from Figures 6A and 5A. The regression slopes of preserved weight on fresh weight were heterogeneous ($F = 3.14$, $df = 4, 200$, $P = 0.016$) but were homogeneous if days 3 and 168 were excluded ($F = 0.81$, $df = 2, 120$, $P = 0.46$). The adjusted mean weights differed significantly among those regressions with homogeneous slopes ($F = 14.16$, $df = 2,122$, $P < 0.001$). Thus, significant differences among mean elver lengths and weights after various periods of preservation (Figure 5) are reflected in the significant differences among adjusted mean variable values from an ANCOVA (Figure 6). Note that the specific pattern of differences among means generated by each method may differ because of the different natures of a sample mean and a mean adjusted to an overall mean by ANCOVA. Similarly, in 10% formalin, the regression slopes of preserved length on fresh length after various periods of preservation were homogeneous ($F = 0.59$, $df = 4,200$, $P = 0.67$) and the adjusted mean lengths were significantly different ($F = 8.57$, $df = 4, 204$, $P < 0.001$). The regression slopes of preserved weight on fresh weight were heterogeneous ($F = 3.6$, $df = 4, 200$, $P = 0.007$) but again were homogeneous if days 3 and 170 were excluded ($F = 0.60$, $df = 2, 120$, $P = 0.55$). For the weight groups with homogeneous slopes, significant differences occurred in the adjusted mean weights after various times in preservation, for both the 4% and 10% formalin groups. Clearly, the temporal effects of preservation are such that no single conversion equation is appropriate.

The shrinkage in length and gain, then loss, in weight of elvers in both 4% and 10% formalin was neither constant nor linear with increasing fresh length or weight (Figure 7). The form of the relationship varied as the preservation period increased. At longer lengths, the degree of shrinkage decreased but shrinkage increased at longer preservation periods (Figure 7A, C). At higher weights, the gain in weight increased but as the preservation period increased the degree of weight gain decreased, particularly in 4% formalin (Figure 7B, D). The form of the relationship between length shrinkage or weight gain and fresh length or weight changed little when preserved lengths and weights were used as the independent variable. Consequently, a linear regression of shrinkage on preserved length, as used by Morkert and Bergstedt (1990) for lamprey larvae, cannot be used for American eels to estimate the amount of shrinkage to be added to a given preserved length so as to estimate the fresh length. However, regressions of preserved length on fresh length (Table 5, Figure 6) may be used for this purpose. As was previously noted for the sample data, the estimation of condition directly from individually preserved specimens is inappropriate because of the shrinkage in length and gain in weight.

A constant correction factor for converting preserved fish lengths to fresh lengths is inappropriate unless the degree of shrinkage is directly proportional to length, which is often not the case. Three cases are evident in the literature: 1. the percent shrinkage decreases as length increases (Hay 1982; Fowler and Smith 1983), 2. the percent shrinkage changes little with increasing length, i.e., is directly proportional to length (Parker 1963; Shields and Carson 1996; Cunningham et al. 2000), and 3. the percent shrinkage increases with increasing length, either linearly (Morkert and Bergstedt 1990) or non-linearly (this study). Such variability in the observed relations between the percent shrinkage in length after preservation and fresh length may result from the varying length ranges of the fishes examined in each study and the ratios of surface area to length for the different species examined. Lamprey larvae and American eel elver have a low ratio of surface area to length relative to the species of fish used in the other studies. The lengths of the lamprey and eel specimens exceeded those in the studies by Hay (1982), Fowler and Smith (1983), and Cunningham et al. (2000).

A variety of opinions exist about the usefulness of conversion equations for estimating fresh lengths or weights from preserved fish, opinions based on the results of specific studies. Parker (1963) indicated that no standard correction term is appropriate for formalin because of variability in fish size, preservation concentration and preservation time. Billy (1982) suggested abandoning, as unreliable, conversion equations for length or weight because the effects of preservation depend on the species, size composition, preservative, preservative strength and period of preservation, not to mention differences in

measuring methods, particularly the use of blotting before weighing. Morkert and Bergstedt (1990) proposed the use of a correction equation for larval lamprey length following preservation for a specific period but ignored the potential for temporal change. Shields and Carlson (1996) recommended against conversion equations for the lengths of formalin preserved fish because the observed changes in length were minimal and against them for weights because they had not stabilized over the preservation period. They also noted that relatively small changes in length or weight, which might be statistically significant, might be ignored for practical purposes if they are judged not biologically significant. Cunningham et al. (2000) recommended use of a conversion equation for the lengths of formalin-preserved inland silversides but noted that the recommendation was species specific. Clearly, live or fresh lengths and weights should be used whenever possible. Having stated that, this study provides equations (Table 5) for converting to fresh values the preserved lengths and weights of American eel elvers preserved for a variety of time periods. After choosing the equation appropriate for the preservation period, the fresh length or weight of an individual elver can be estimated from the preserved length or weight by inverse prediction. Conversion equations that permit estimation of fresh lengths and weights from preserved specimens are usually applied to individual fish rather than to larger samples of fish. If the constituent data for individual fishes in a sample are available, individual fresh fish lengths and weights might be estimated from preserved values by such conversion equations and then used to estimate sample values. Estimates of fresh length and weight from a sample of individual elvers could be used to estimate mean condition at the sample mean length (Cone 1989). The comparison of condition estimates among several studies would then require similar slopes for all weight-length regressions. The condition of individual elvers from the population of interest could be estimated by the residual from the weight-length regression but residuals are not comparable across populations (Jakob et al. 1996). Any biases from the conversion of preserved length and weight to fresh length and weight will carry through into the estimate of condition.

Although the use of unbuffered tap water may have an effect, depending on its mineral content, on the ultimate pH level of a formalin solution (Steedman 1976), the acidity of the formalin solution was of little concern because the preservation of calcareous structures such as otoliths was not of interest. Buffering changes only the pH of the formalin solution; buffered or unbuffered formalin solutions are very stable for long periods of time, particularly at temperatures between 10 °C and 30 °C (Steedman 1976). If the later collection of otoliths is necessary, freezing or preservation in 95% ethanol is preferable to the use of buffered formalin (Butler 1992).

Among Studies Comparisons of Biological Data

The comparison of elver size and condition among sites along the Atlantic coast of North America is complicated by the existence of a geographic gradient in elver size, by the timing of sample collection, both seasonally and interannually, and by the subsequent treatment of samples (measurement fresh or preserved). Mean elver lengths, at river entrance, increase clinally by as much as 13 mm from south to north along the Atlantic coast (Vladykov 1966, 1970; Haro and Krueger 1988; Dutil et al. 1989; Jessop 1998). Over shorter geographic distances, the decline in elver mean lengths is reduced, e.g., between Florida and Maryland elver mean length increased by about 5 mm. Elver weights presumably increase in a similar manner (assuming a similar weight-length relation) but the available data are insufficient to confirm this although Vladykov (1966; 1970) reported that elvers from Nova Scotia weighed more than those from Chesapeake Bay. Elvers, as previously noted, may also decline in length, by up to 4.5 mm, and weight, by 0.06 g, over the elver run in northern waters (Jessop 1998) although the effect may be less pronounced or absent in mid-continental or southern waters (McCord 1977). In 1998, the effect size (Hedges *g*, using the pooled sample standard deviation; Hedges and Olkin 1985) was 0.60 for the seasonal decline in length of 2.0 mm and was 0.95 for the decline in weight of 0.04 g for elvers entering the East River, Chester. Interannual variability in mean length over several years may be at least 3 mm (Haro and Krueger 1988). For European glass eels, annual variability (37 years) in mean length may range to 11 mm and exceed the difference in length over their geographic range (Dekker 1998). Finally, preservation effects may shrink elvers by 3-4 mm in length and increase weights by 0.035 g. The combination of these geographic, annual, seasonal, and preservation effects increases the difficulty of determining accurate estimates of elver mean size and variability for a particular river stock and of interpreting comparisons of these estimates among studies. Such comparisons may lead to useful

insights about annual and geographic variation in oceanic effects on the growth, and perhaps survival, of American eel elvers. A metric more suitable for comparison among studies than percent change, which varies according to the base value, may be measures of effect size such as g', which enable standardized comparisons (Hedges and Olkin 1985). An effect size measure may also assist evaluation of whether a statistically significant effect might be of biological importance (Cohen 1988; Kirk 1996).

Summary and Conclusions

American eel elvers shrink in length and gain in weight following preservation in either 4% or 10% formalin. Most of the shrinkage in length and gain in weight occurs within the first two days following preservation. A decreasingly slow shrinkage in length continues for over 300 d after preservation. After the initial gain in weight, weight also slowly decreases and, after about 300 d, may approach the fresh weight. The degree of shrinkage in elver length and gain in weight decreases with increasing elver size. The non-linear relation between elver size and preservation effect and the temporal change in preservation effect requires consideration when using equations to convert preserved lengths and weights to fresh values. The preservation effects on length and weight and their interaction in estimates of condition complicates the interpretation of data from preserved elvers both within and among studies and, more so, the comparison of data from preserved and unpreserved samples. Given the magnitude of preservation effects on elver length, weight, and condition, it is recommended that elvers be measured fresh whenever possible and that comparison of unpreserved and preserved elvers be avoided.

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Tables/Figures

Table 1. Parameter values of the fresh lengths and weights of American eel elvers from the East River, Chester, and later preserved in 4% and 10% formalin.

				Length (mm)			Weight (g)		
Year	Formalin %	Sample	*N*	Mean	SD	Range	Mean	SD	Range
1998	4	1	60	64.20	2.785	58.5 – 70.2	0.178	0.0333	0.11 – 0.26
		2	60	65.04	2.888	59.5 – 72.8	0.189	0.0304	0.13 – 0.28
		3	60	65.10	2.817	57.8 – 72.6	0.191	0.0355	0.12 – 0.30
	10	1	60	62.52	3.436	53.3 – 69.7	0.163	0.0324	0.08 – 0.25
		2	60	63.67	3.354	54.8 – 71.7	0.180	0.0359	0.10 – 0.26
		3	60	63.15	3.211	55.0 – 71.9	0.157	0.0298	0.07 – 0.23
2000	4	1	42	61.15	5.554[a]	52.9 – 72.5	0.165	0.0477	0.09 – 0.25
	10	1	42	60.56	5.358[a]	52.9 – 70.6	0.164	0.0510	0.09 – 0.27

[a]The standard deviation is unusually large as a consequence of equal numbers of fish per length interval.

Table 2. Pooled (N = 180[a]) sample mean length (mm) and weight (g) of American eel elvers, by formalin concentration, after various time periods in preservation. Means without a letter in common differ significantly ($P < 0.05$).

Percent Formalin		Days Preserved										
		Fresh	2	4	7	12	22	35	49	63	77	312
4	Length	64.83a	61.37b	61.42b	61.30b	60.93b	61.34b	61.23b	61.17b	61.68b	61.26b	60.72b
	Weight	0.186a	0.221b	0.219b	0.211bc	0.212bc	0.209bc	0.213bc	0.210bc	0.208bc	0.205c	0.192a
10	Length	63.11a	60.41b	60.72b	59.71b	60.16b	60.34b	60.12b	59.69b	60.03b	60.29b	59.65b
	Weight	0.167a	0.199b	0.196b	0.195b	0.195b	0.191b	0.191b	0.190bc	0.189bc	0.187bc	0.177ac

[a]The size for sample 1 of the 4% formalin series declined through loss from 60 elvers to 59 on day 4 and to 58 on day 36 and subsequent days; one elver in sample 3 was not recorded on day 36. Cases with missing values were deleted from the analysis, thus N = 177 for the 4% formalin series.

Table 3. Parameters for the linear regressions of the difference (shrinkage in length and gain in weight) between fresh and preserved lengths (mm) and weights (g) for samples (N = 42) of American eel elvers on preservation time (d) in 4% and 10% formalin. N = 30 is based on 3 replicate samples and 10 re-measurement dates.

Percent Formalin	Variable	N	Intercept	95% CI	Slope	95% CI	Adjusted R^2	P
4	Length	30	-3.4414	-3.5414 – -3.3414	-0.0018	-0.0027 – -0.0008	0.32	<0.001
	Weight	30	0.0291	0.0274 – 0.0308	-0.0001	-0.0001 – -0.0001	0.83	<0.001
10	Length	30	-2.8913	-3.0699 – -2.7126	-0.0019	-0.0036 – -0.0002	0.13	0.027
	Weight	30	0.0280	0.0265 – 0.0295	-0.0001	-0.0001 – -0.0000	0.72	<0.001

Table 4. Mean length (mm) and weight (g) of individually preserved American eel elvers, by formalin concentration, after various time periods in preservation. Means without a letter in common differ significantly ($P < 0.05$).

Percent Formalin		Days Preserved					
		Fresh	2	6	18	55	167[a]
4	Length	61.15a	58.82b	58.49c	58.43c	58.16d	58.10d
	Weight	0.165a	0.206b	0.197c	0.199c	0.185d	0.173e
10	Length	60.56a	58.73b	58.32c	58.57b	58.18cd	57.98d
	Weight	0.164a	0.211b	0.198c	0.186d	0.183d	0.166a

[a]For the 10% formalin concentration, the measurement period was 169 d.

Table 5. Regression parameters for the preserved length (mm) and weight (g) of American eel elvers on fresh length and weight, by formalin concentration, after various time periods in preservation. For all regressions $N = 42$. The adjusted R^2 corrects R^2 to more closely reflect the goodness of fit of the model to the population.

Percent Formalin		Days Preserved	Adj. R^2	Slope	95% CI	Intercept	95% CI
4	Length	3	0.98	0.894	0.856 – 0.932	4.176	1.851 – 6.501
		7	0.98	0.864	0.826 – 0.903	5.648	3.282 – 8.014
		19	0.98	0.858	0.822 – 0.894	5.971	3.768 – 8.174
		56	0.98	0.856	0.820 – 0.891	5.835	3.653 – 8.017
		168	0.98	0.870	0.835 – 0.904	4.926	2.825 – 7.027
	Weight	3	0.96	1.161	1.089 – 1.233	0.014	0.002 – 0.027
		7	0.96	1.151	1.079 – 1.222	0.007	-0.005 – 0.019
		19	0.94	1.091	1.002 – 1.180	0.018	0.002 – 0.033
		56	0.95	1.087	1.011 – 1.164	0.006	-0.007 – 0.019
		168	0.95	0.993	0.922 – 1.063	0.009	-0.003 – 0.021
10	Length	3	0.99	0.929	0.895 – 0.963	2.473	0.389 – 4.557
		7	0.98	0.931	0.890 – 0.972	1.937	-0.570 – 4.444
		19	0.98	0.934	0.895 – 0.974	1.986	-0.418 – 4.390
		56	0.98	0.909	0.866 – 0.953	3.108	0.444 – 5.771
		170	0.98	0.901	0.863 – 0.938	3.439	1.168 – 5.711
	Weight	3	0.97	1.247	1.172 – 1.321	0.006	-0.006 – 0.019
		7	0.97	1.171	1.104 – 1.238	0.006	-0.006 – 0.017
		19	0.97	1.188	1.117 – 1.260	-0.009	-0.021 – 0.003
		56	0.97	1.149	1.090 – 1.208	-0.005	-0.0155 – 0.005
		170	0.97	1.091	1.036 – 1.147	-0.013	-0.022 - -0.003

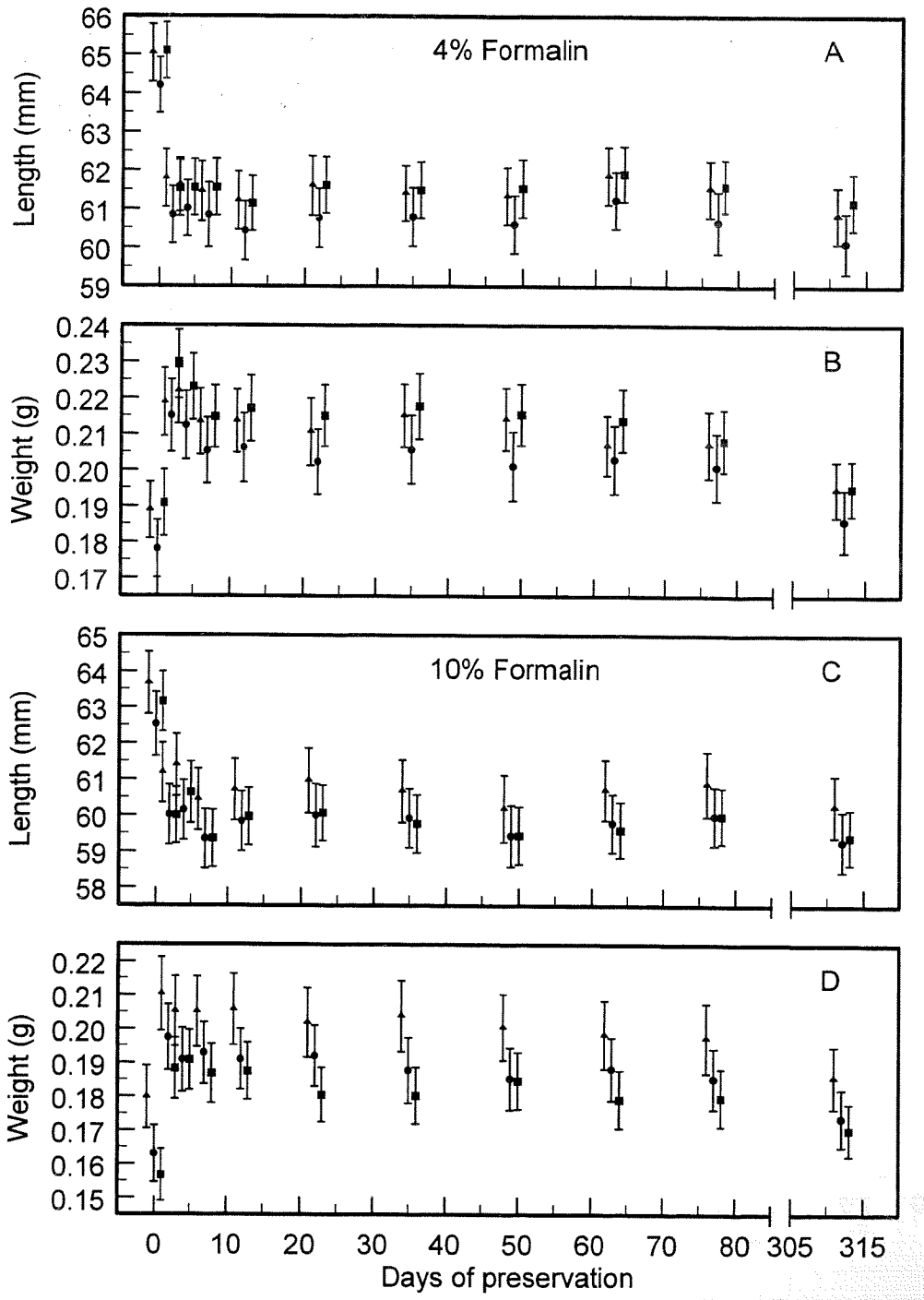

Figure 1. Mean sample lengths (TL) and weights, with 95% confidence intervals, of American eel elvers measured fresh and after varying times of preservation in 4% and 10% formalin. In each sample, $N = 60$ except the 4% formalin trials where sample 1 declined through loss from 60 elvers to 59 on day 7 and to 58 on day 35 and subsequent days and sample 3 was missing data for one elver on day 35. Sample 1 is represented by a circle, sample 2 by a square and sample 3 by a triangle symbol.

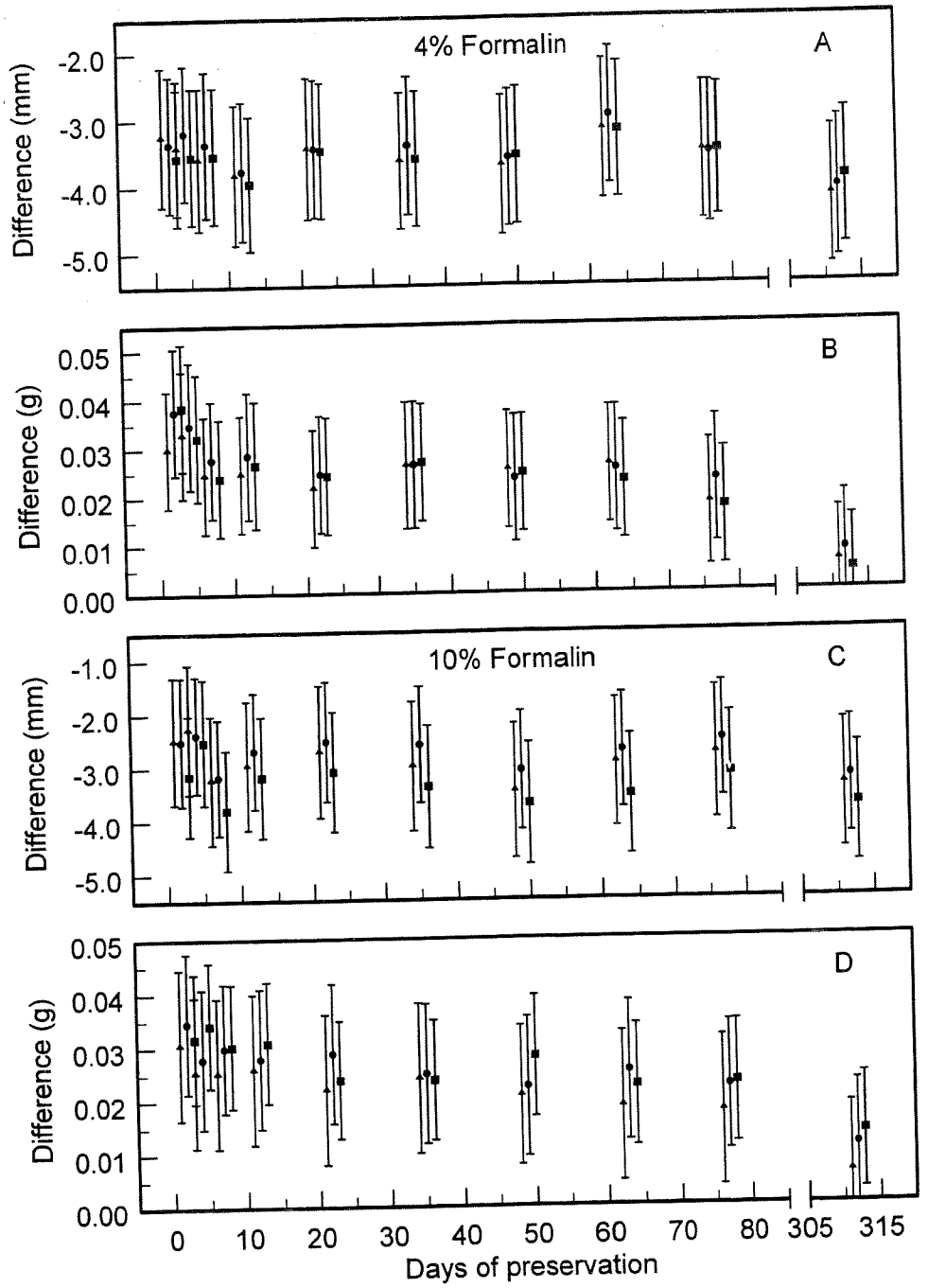

Figure 2. Mean observed change in length (TL) and weight, with 95% confidence intervals, of three samples of American eel elvers measured fresh (control group) and after varying times of preservation (experimental group) in 4% and 10% formalin. In each sample, $N = 60$ except for the 4% formalin trials where sample 1 declined through loss from 60 elvers to 59 on day 7 and to 58 on day 35 and subsequent days and sample 3 was missing data for one elver on day 35. Sample 1 is represented by a circle, sample 2 by a square and sample 3 by a triangle symbol.

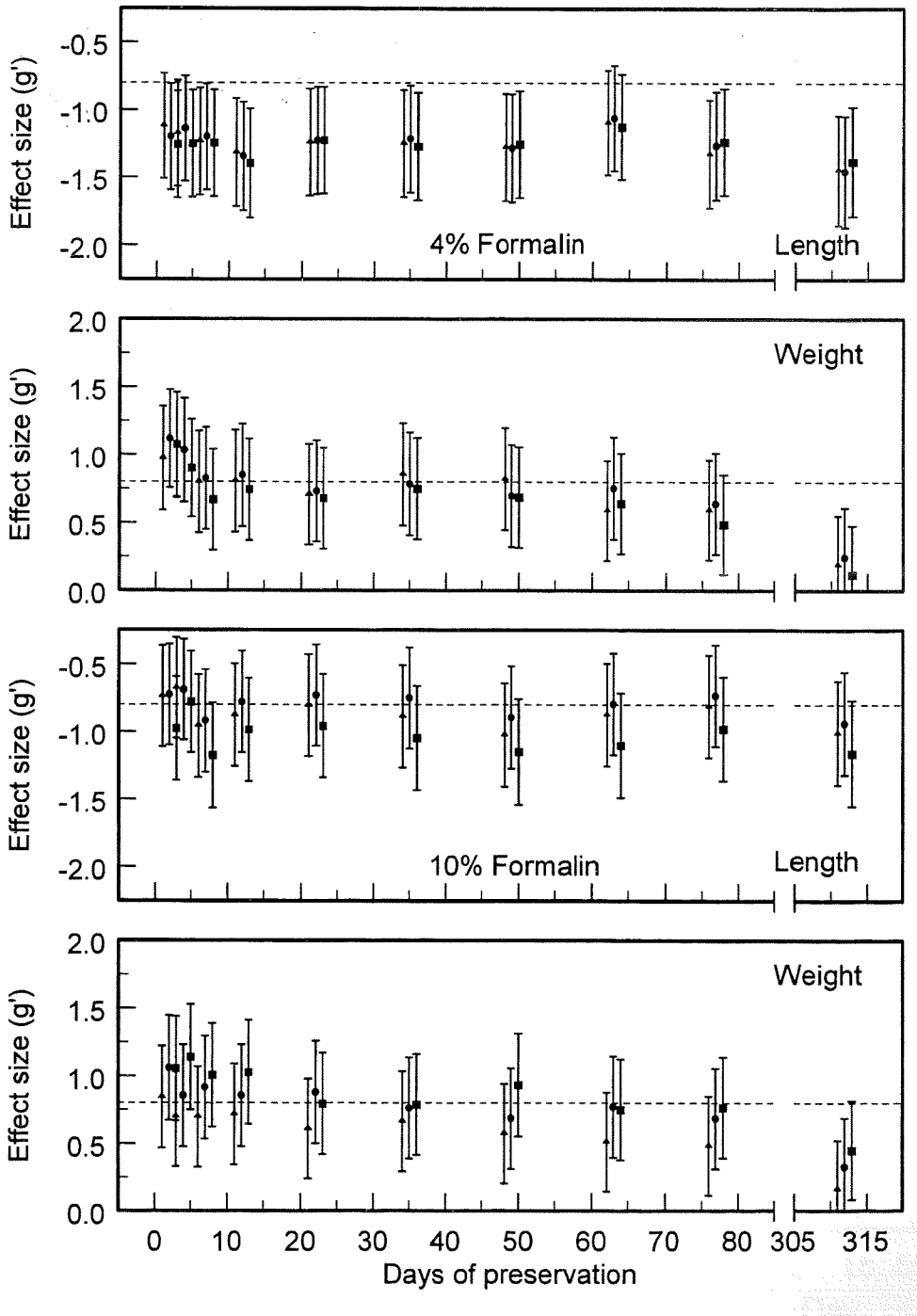

Figure 3. Mean effect sizes (g'), with 95% confidence intervals, of preservation in 4% and 10% formalin on three samples of American eel elvers measured fresh and after varying times of preservation. The dashed line at 0.8 indicates a large effect size, as defined by Cohen (1988). In each sample, $N = 60$ except for the 4% formalin trials where sample 1 declined through loss from 60 elvers to 59 on day 7 and to 58 on day 35 and subsequent days and sample 3 was missing data for one elver on day 35. Sample 1 is represented by a circle, sample 2 by a square and sample 3 by a triangle symbol.

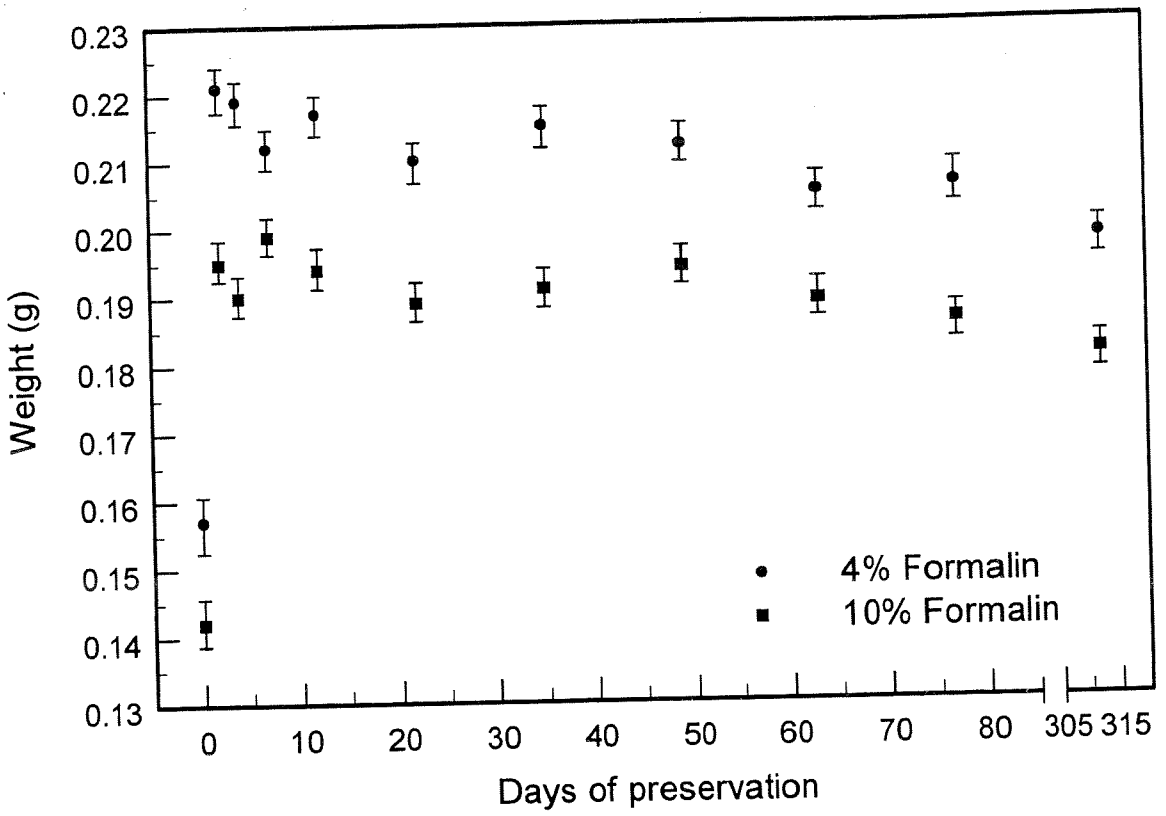

Figure 4. Mean condition of American eel elvers when measured fresh and after varying times of preservation in either 4% or 10% formalin. Condition is the mean weight of elvers adjusted by weight-length regression to the overall mean length of 61.56 mm for elvers preserved in 4% formalin and of 60.38 mm for elvers preserved in 10% formalin. Weights and lengths were logarithmically transformed for analysis then back-transformed for presentation. For elvers preserved in 10% formalin $N = 180$; for elvers preserved in 4% formalin, $N = 180$ for days 2-7, 179 for days 12-22, 177 for day 35, and 178 for days 49-312. Sample 1 is represented by a circle, sample 2 by a square and sample 3 by a triangle symbol.

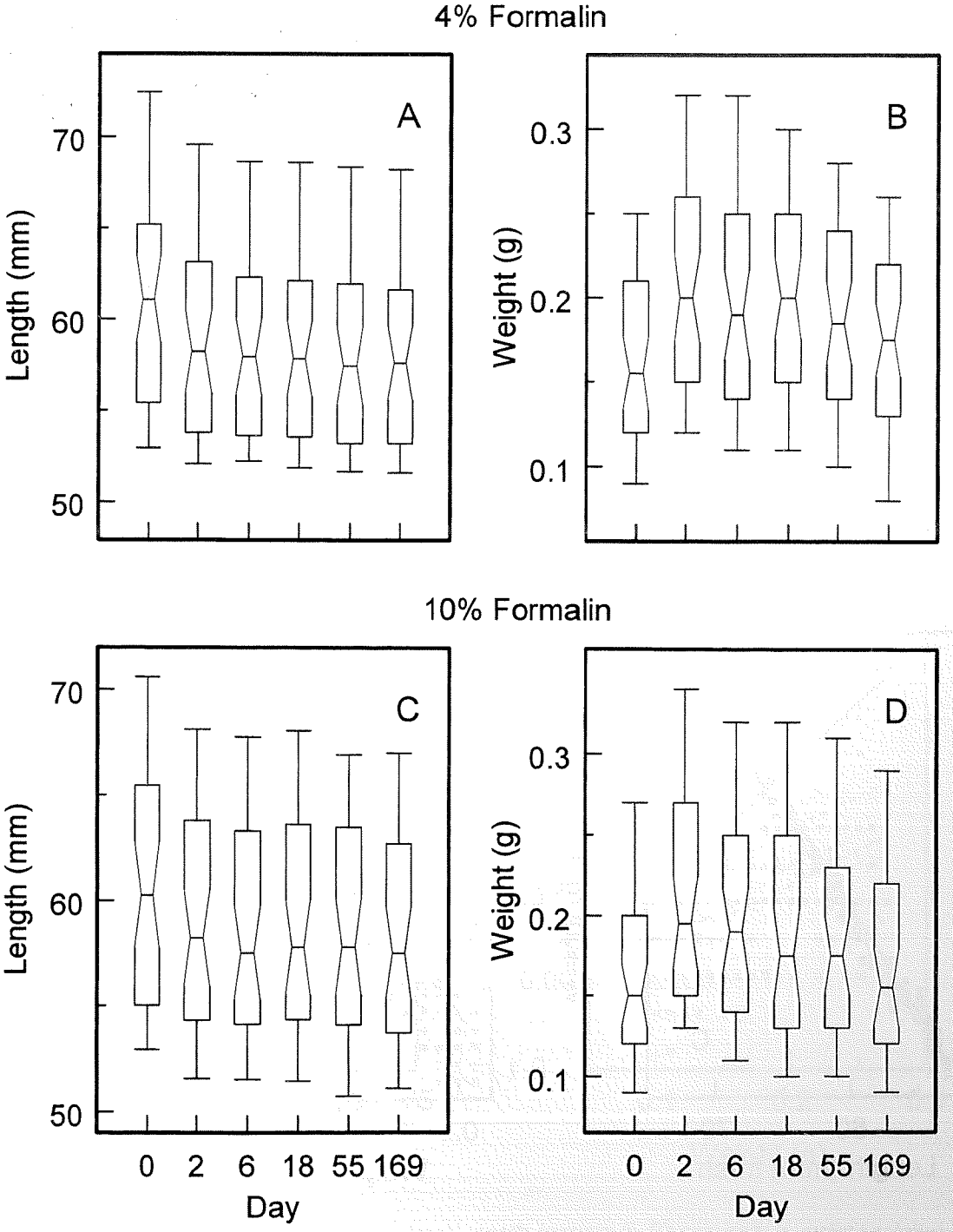

Figure 5. Box plots of the lengths (panels A, C) and weights (panels B, D) of American eel elvers measured fresh (day 0) and after 2, 6, 18, 55 and 169 d (167 d for 4% formalin) of preservation in either 4% or 10% formalin. Notches indicate the 95% confidence interval about the median (medians differed little from the mean), the box encloses the middle 50% of the data, and the whiskers indicate the data range.

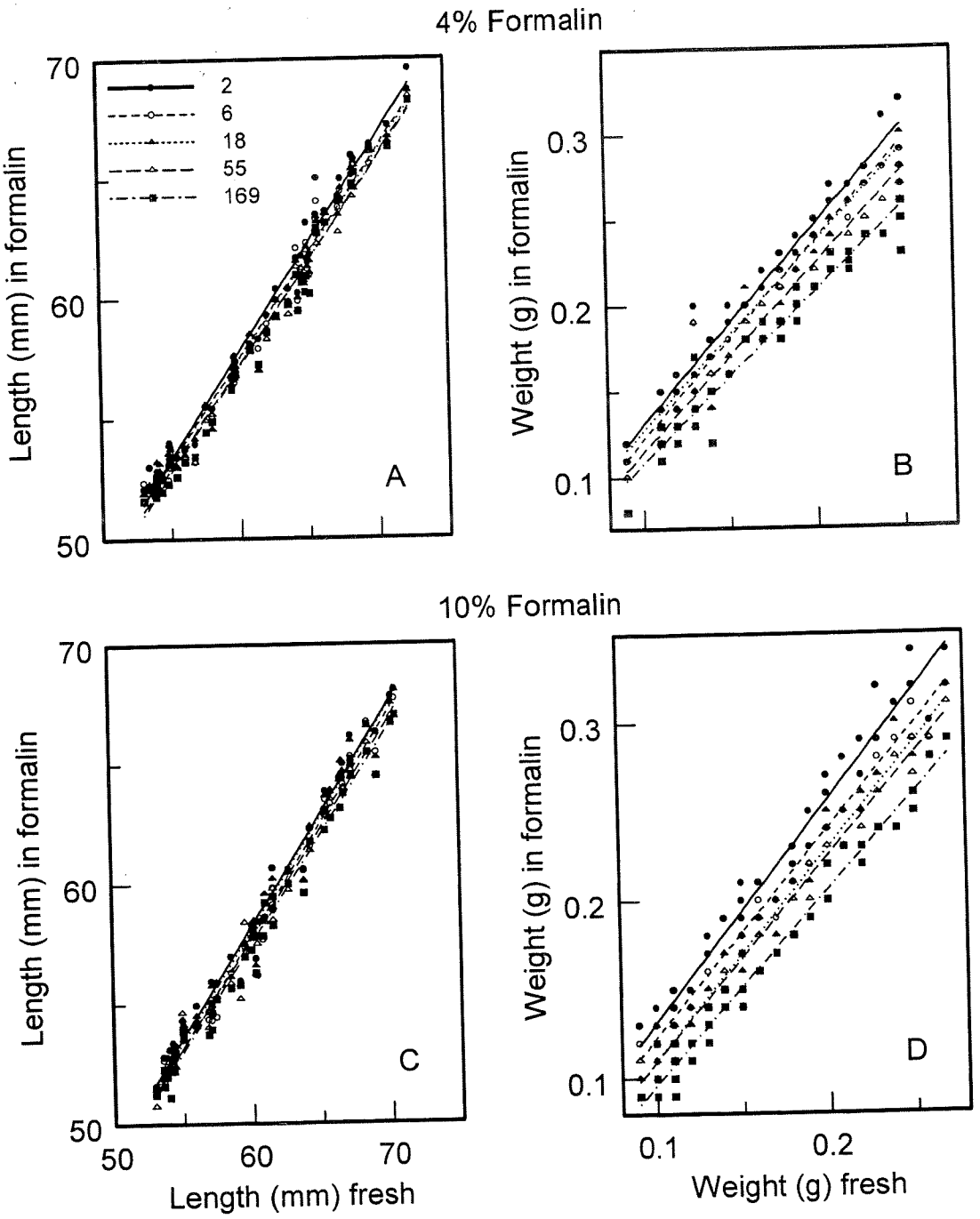

Figure 6. Regressions of the length after preservation on fresh length (panels A, C) and of the weight after preservation on fresh weight (panels B, D) for American eel elvers preserved in either 4% or 10% formalin for 2, 6, 18, 55 and 169 d (167 d for 4% formalin).

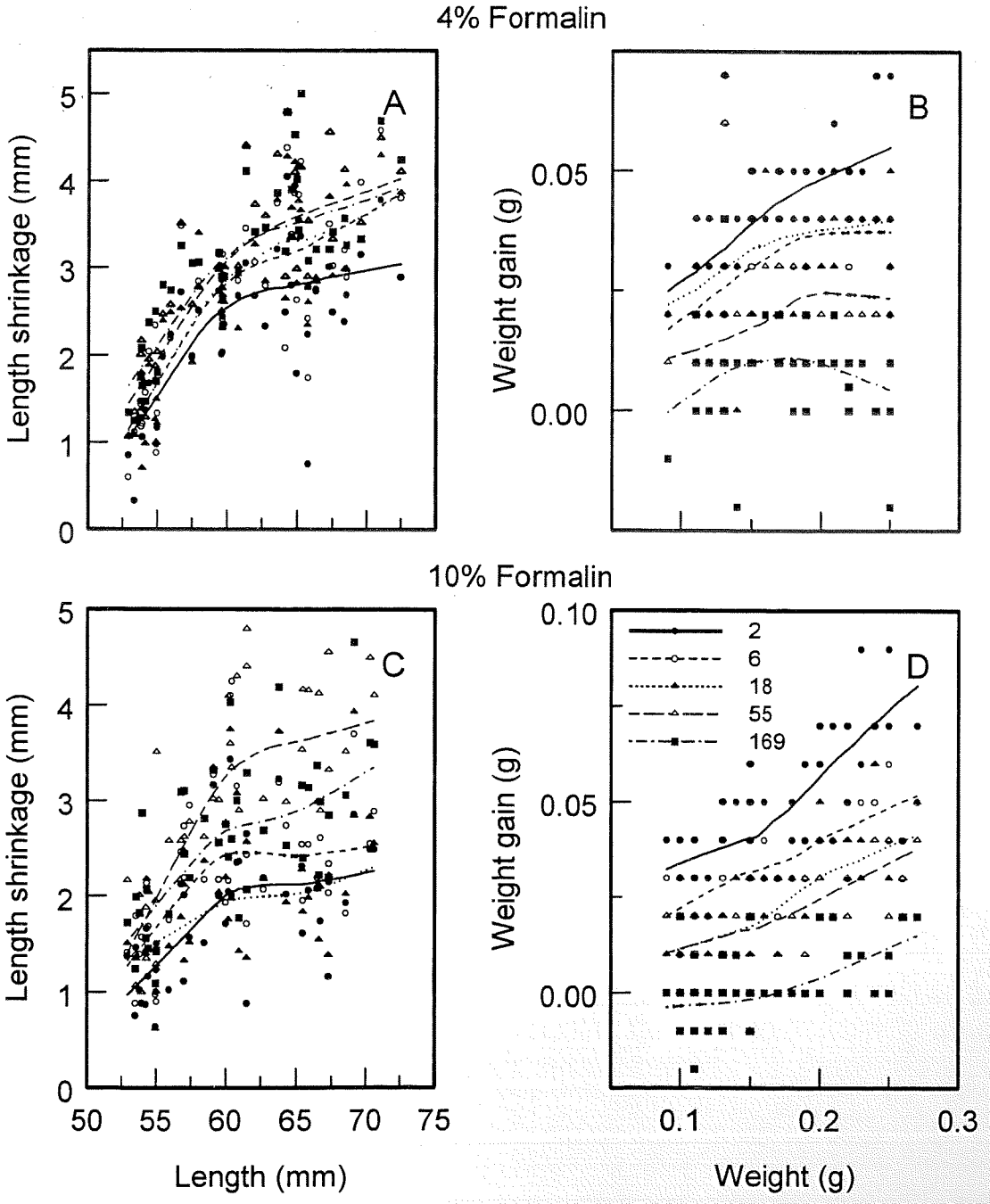

Figure 7. Shrinkage in length relative to fresh length (panels A, C) and gain in weight relative to fresh weight (panels B, D) for American eel elvers preserved in either 4% or 10% formalin for 2, 6, 18, 55 and 169 d (167 d for 4% formalin).