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Differential effects of chemical preservatives and freezing on the length and dry weight of *Daphnia* and *Diaptomus* in an oligotrophic lake

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With 7 figures and 1 table in the text

Abstract: We compare the shrinkage effects of two chemical preservatives and freezing on the length and dry weight of *Daphnia catawba* and *Diaptomus minutus*, zooplankton that commonly occur in the small oligotrophic lakes of south central Ontario, Canada. There was no significant length difference for *Daphnia catawba* preserved in either 4 % sugar-formalin or 70 % ethanol over a 40-d period. *Diaptomus minutus* exhibited a length shrinkage of 6 and 8 % in formalin and ethanol, respectively, over the first 7 days of preservation. Freezing with dry-ice did not affect the length of either zooplankton species. The dry weight of *Daphnia* decreased significantly with time in both chemical preservatives, and weight loss had not stabilized by 21 d. Although there was no significant effect of formalin on the dry weight of *Diaptomus* preserved for up to 21 d, dry weight of animals in the alcohol treatment decreased significantly over the same time period. For a 1-mm *Daphnia*, the dry weight of frozen animals or those preserved in ethanol or formalin (for 14 days) were respectively 73, 47, and 78 % of the dry weight of unpreserved animals. The mean dry weight of *Diaptomus* preserved in ethanol was also significantly lower than that of the control, averaging only 60 % of the dry weight of unpreserved diaptomids, whereas those of formalin-preserved and frozen animals were 76 % and 91 %, respectively of unpreserved animals. We found that the ratio of dry weight to wet weight of *Daphnia* varied with animal size, suggesting that a single wet-weight : dry-weight ratio may be inappropriate.

Introduction

The dry weight and length of individual zooplankters are basic information collected by limnologists on a routine basis (SALONEN & SARVALA 1980, 1985; BIRD & PRAIRIE 1985, BERBEROVIC & PINTO-COELHO 1989 and

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BOERSMA & VIJVERBERG 1994). Because it is rarely practical to take such measurements from unpreserved zooplankton, ecoligists generally have to take measurements from preserved samples. Investigators seldom correct for any effect of preservatives on either the length or the dry weight of zooplankton, even though several studies have shown that zooplankton lose a significant amount of their dry weight in various preservatives (see review by GIGUÈRE et al. 1989), and the possible effects of preservatives on zooplankton length are not yet known. Neglecting to correct for potential effects may produce substantial errors when cross-study comparisons are made in cases where different preservatives have been used, or when length-weight regressions corresponding to preserved animals are subsequently used to estimate the dry weight of unpreserved animals.

Establishing and comparing the relevant effects of preservatives on the length and weight of zooplankton, and evaluating the appropriateness of using a standard wet-dry conversion factor should permit investigators to correct for any differences in length and weight such that a standardized unpreserved weight estimate can be generated regardless of the preservative used. These correction factors should improve both the accuracy and comparability of these basic parameters and facilitate between-study comparisons.

The aim of this study is to compare the effect of common preservatives on the length and dry weight measurement of two herbivorous zooplankton species. *Daphnia catawba* and *Diaptomus minutus*. These species which occur commonly in oligotrophic lakes of south central Ontario, Canada, have disparate body shapes and dry weights. As well, they represent the extremes with respect to calcium content in their carapace (YAN et al. 1989), a variable that may be important if calcium is related to the degree of shrinkage (STEEDMAN 1976). We examined three methods of preservation: freezing, formalin and ethanol. In addition to quantifying the effects of preservatives on dry weights, we also determined the empirical relationship between the dry weight and wet weight of *Daphnia* individuals to investigate the feasibility of predicting wet-weight from dry-weight measurements.

Methods

Description of the study lake and field methods

Ranger lake is an oligotrophic softwater lake located near the town of Dorset in south central Ontario, Canada. It has a surface area of approximately 12 ha, a maximum depth of 13 m and mean Secchi depth transparency of 2.7 m. The herbivorous zooplankton biomass is dominated by *Daphnia catawba*, *Holopedium gibberum*, and *Diaptomus minutus*. Zooplankton samples were always collected by vertical haul

(80 µm mesh square Wisconsin net; 3-m) at an established pelagic station (maximum depth 6 m). Animals were immediately filtered through modified Petri Plates (equipped with mesh), and rinsed with filtered lakewater. Animals were either preserved in the field with dry-ice (transferred to freezer and kept in Ziploc bags within 2 h of collection), 4 % sugar-formalin (PREPAS 1978) or 70 % ethanol.

Effects of preservatives on length over time

Following 30 d of freezing in Ziploc bags, 20 animals of *Daphnia* and *Diaptomus* were selected, rinsed with distilled water and transferred onto depression slides where their individual lengths were measured to the nearest 0.001 mm with an Optimus Image Analysis System (Bioscan Inc., Edmonds, Washington). *Daphnia* were measured from the top of the head to the base of the tail spine. *Diaptomus* were measured from the top of the head to the base of the caudal rami (prosome + urosome) to avoid errors which may result when the angle of the caudal rami relative to the long axis of the body is changed from measurement to measurement (CULVER et al. 1985). Immediately following the initial length measurement, animals were placed into individual 1.5 mL centrifuge capsules with sealing lids and filled to 1.0 mL with one of the chemical preservatives. The length of each animal was measured again after 1,7,14 and 40 d; animals were returned to their respective capsules after each measurement, and these were topped up with the appropriate preservative. To account for any effect of initial freezing on zooplankton length, animals that had been collected in the field and kept in culture were measured unpreserved and then frozen for 8 d or 30 d, after which length measurements were repeated.

We determined the accuracy of all length measurements for this study on each measurement day by randomly measuring a 10 % subsample of animals three times. The repeated measures were always less than 0.5 % of the total body length. As well, the length measurements were well within the practical measurement intervals of 5 % of the shortest body length recommended by BIRD & PRAIRIE (1985).

Effects of preservatives on dry weight over time

To determine the effects of chemical preservatives on dry weight over time, fresh field-caught animals were placed in the two chemical preservatives for 9 or 21 days. To eliminate any confounding effects due to season (CAMPBELL 1994), we conducted all of the experiments with animals collected from a single visit to Ranger L. To avoid the effect of variable clutch size, we used non-ovigerous females in all of the experiments. At the end of the chosen preservation period, random subsamples were placed in distilled water for 20 min to rinse off any excess preservative. *Daphnia* were selected individually (n = 20) and included a range of body lengths so as not to limit the applicability of our results. *Diaptomus* were placed in groups of five similar-sized animals (± 0.01 mm; n = 20 groups). Individuals or groups of animals were transferred to small pre-weighed 7 mm boats that had been punched from aluminum foil. The

weighing boats were placed in a partially closed petri plate, oven-dried at 60 °C for 24 h, cooled and weighed to the nearest 0.5 µg with a Cahn 25 Electrobalance.

Comparison of preservative on length-dry-weight relationship

We compared the effect of the three preservation methods on the relationship between length and dry weight of *Daphnia* with corresponding data obtained for unpreserved animals. Since there is a minimum 2-h delay between field collection of animals and the subsequent sorting and length measurements, and animals may be subjected to an unknown amount of stress that may affect their weight during this time, we decided to use animals that had been maintained in culture for 30-d on a mixture of *Scenedesmus* and *Ankistrodesmus*. In this way, we ensured that animals used in these experiments were all subjected to minimum stress. Four subsamples of the stock culture were drawn in parallel and immediately filtered. Each of three subsamples were preserved immediately in 4 % sugar-formalin, 70 % ethanol or on dry-ice. The last subsample was used as a control to provide measurements of unpreserved animals. To standardize the comparison, we preserved animals in all three treatments for 14 d. Length and weight of animals were measured as previously described.

We also compared the effect of preservatives on the length-dry-weight relationship of *Diaptomus*. Because of the small size of these animals, their dry weight could not be measured individually. Instead, we calculated the mean weight of similar-sized copepods (n = 5 per group). Consequently, there were only sufficient animals to determine the length-weight relationship for unpreserved animals (19 groups with lengths ranging from 0.57 to 0.69 mm). Animals were also preserved for 14 d prior to weighing. To eliminate the confounding effect of size, we only used groups of animals between 0.61 to 0.65 mm to compare the effects of preservatives on dry weights.

Relationship between wet and dry weight of *Daphnia*

Previously frozen animals were thawed and rinsed with distilled water. Individual animals were randomly selected and picked up with fine forceps. Each animal was blotted twice on each side of its carapace with Kimwipe, then transferred with dry forceps onto small pieces of aluminum foil which were pinched closed on the edges to form a half circle with the animal inside. The wet weights were determined with the Cahn 25 Electrobalance. The wet weight was defined as that value which was maintained for 5 sec. Following this, animals were dried for 24 h at 60 °C and weighed again. We assumed that the initial rapid drop in wet weight was indicative of any external water that was present on the animal or weighing pan.

Statistical analyses

We used the ANOVA and Tukey-Kramer HSD test of SAS Jmp (SAS, Cary, N. Carolina) to determine the effects of chemical preservative and preservation duration on lengths and weights of animals. Analysis of covariance (Zar 1984) was used to determine differences between the elevations for the regression equations developed for *Daphnia* in the various preservatives and weight loss over time.

Results and discussion

Effect of preservative on length over time

The length of *Daphnia* did not change significantly over time in either of the chemical preservatives for the duration of the 40 d (ANOVA Repeated Measures; $P > 0.05$; Fig. 1 a and b). By comparison, the length of *Diaptomus* preserved in either ethanol (Fig. 2 a) or formalin (Fig. 2 b) decreased significantly over the first 7 d of observation (Tukey-Kramer HSD test; $P < 0.05$), with an average decrease of 8 and 6 %, respectively. Since none of the measurements taken after 40 d was statistically different from those taken on the 7th day, there was probably no further shrinkage in the size of *Diaptomus* after the first 7 days of preservation.

Effect of freezing on length

We conducted tests to determine the effect of freezing on length measurements because animals in the above experiments had been previously frozen for up to 30 d. For both *Daphnia* and *Diaptomus*, there were no significant differences between length measurements ($n = 15$) for animals that had been frozen for 8 or 30 days (t-tests, $P > 0.20$ in both cases), which suggests that the duration of freezing was not an important factor. Although we could not determine if freezing prior to preserving had a confounding effect, an independent study in which animals from Ranger L. were preserved without any freezing confirmed the extent of shrinkage that we observed within the first 7 days of experimentation (D. McQUEEN, York University, unpubl. data). Therefore, we concluded that freezing prior to the addition of chemical preservatives did not confound the effects of preservatives on length measurements. Further research using parallel experiments with animals frozen prior to preservation and animals preserved without freezing would provide a means of verifying this conclusion.

Effect of preservative on weight over time

We determined the effect of 70 % ethanol and 4 % sugar formalin on the dry weight of *Daphnia* individuals by comparing animals that had been preserved for 9 and 21 days (Fig. 3 a and 3 b). Because of the well-documented allometric relationship between weight and length for *Daphnia* (DOWNING & RIGLER 1984, Chapter 7), it is appropriate to compare the slopes and intercepts of the length-weight regression lines corresponding to the two preservation durations for each of the chemical preservatives. Analysis of covariance indicated that the elevations of 9- and 21-day data were significantly different ($P < 0.02$). For

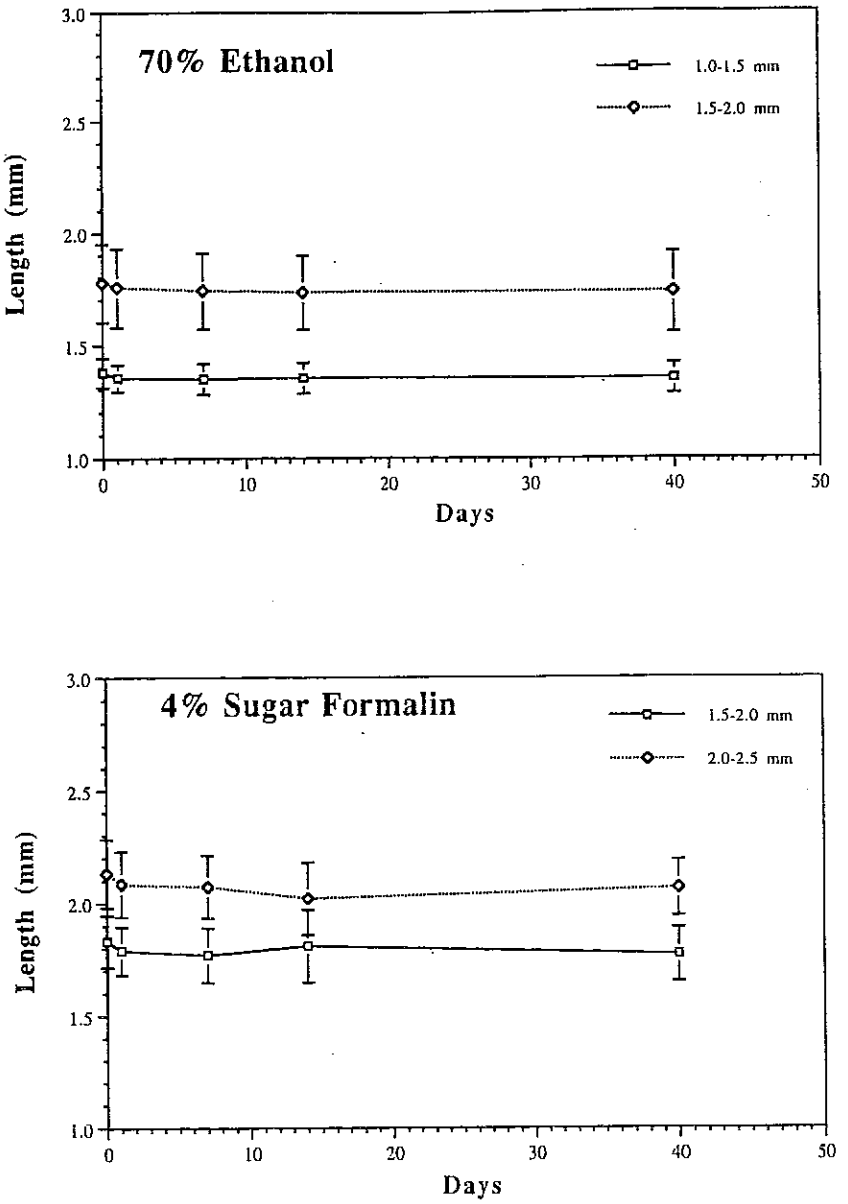

Fig. 1. Effect of preservative type and duration on the mean length (mm; ± 1 S.E.) of *Daphnia catawba* preserved in a) 70 % ethanol and b) 4 % sugar formalin.

a given size of *Daphnia*, the 21-d preservation period resulted in statistically lower dry weight compared with the 9-d preservation in both ethanol ($P < 0.005$) and 4 % formalin ($P < 0.02$), indicating that the length of time in

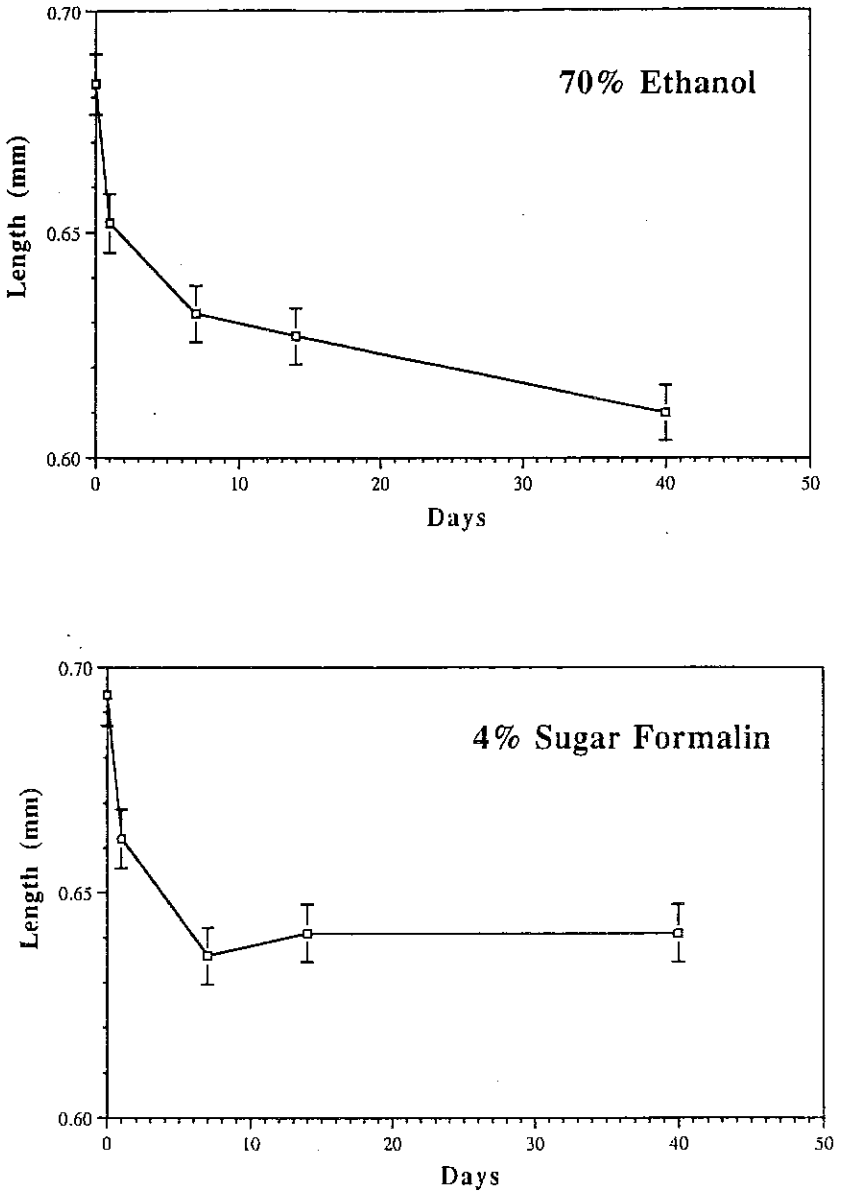

Fig. 2. Effect of preservative type and duration on the mean length (mm; ± 1 S.E.) of *Diaptomus minutus* preserved in a) 70 % ethanol and b) 4 % sugar formalin.

preservative had a significant effect on dry-weight measurements. Further tests should be performed to determine if and when the effect of either preservative will stabilize over time.

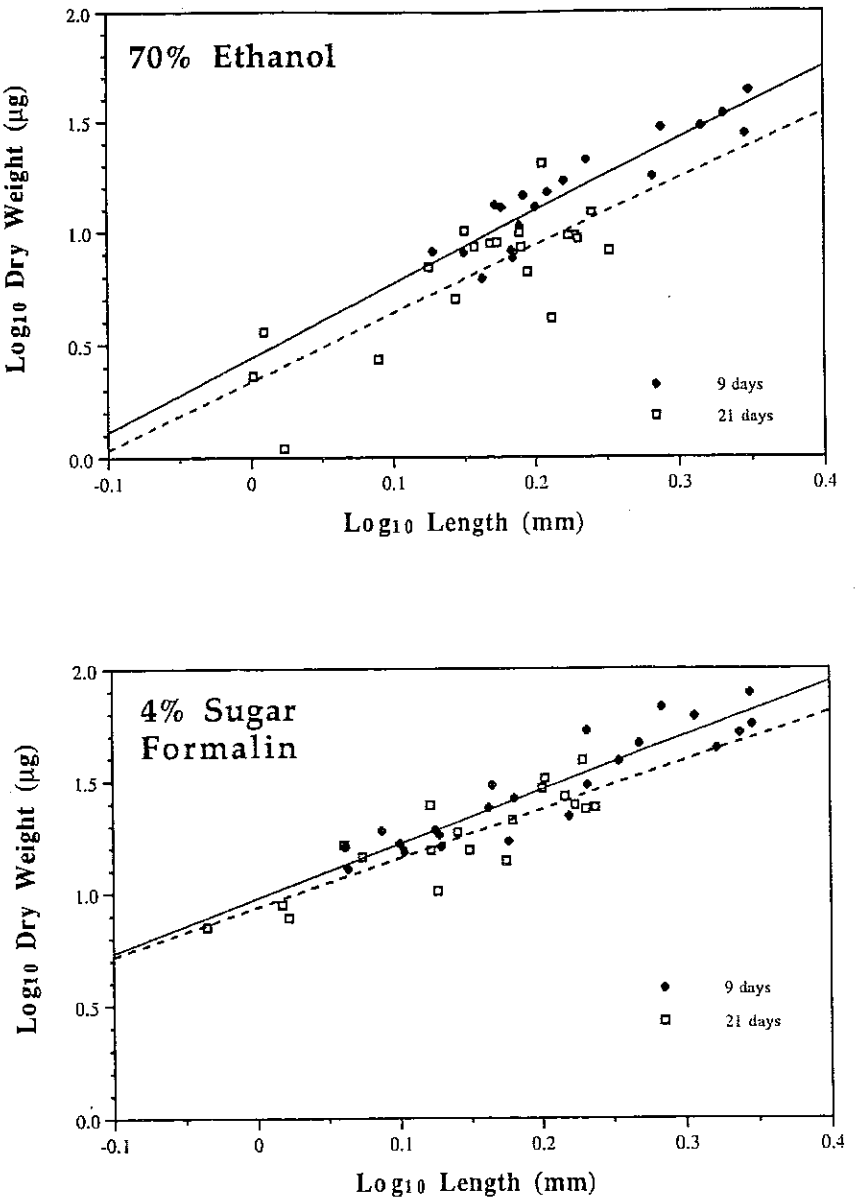

Fig. 3. Effect of preservative type and duration on the length (mm)-dry-weight(µg) relationship for *Daphnia catawba* in a) 70 % ethanol and b) 4 % sugar formalin.

We determined the effect of preservation duration on the dry weight of *Diaptomus* by comparing measurements taken after 2, 9 and 21 d of preservation in ethanol and formalin (Fig. 4). While there were no significant differ-

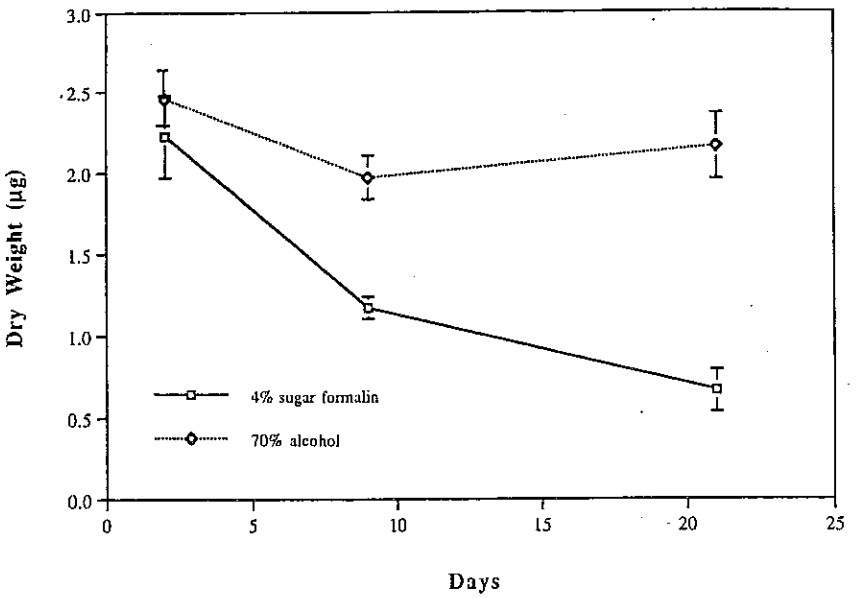

Fig. 4. Mean dry weight (µg; ± 1 S.E.) of *Diaptomus minutus* (0.6–0.7 mm) preserved for different durations in ethanol and formalin.

ences in dry weight among the three preservation durations (ANOVA, $P = 0.06$) for the formalin treatment, there were significant differences for the alcohol treatment ($P < 0.001$). When we compared the effect of the two preservatives, we found a significant effect of preservative type for animals preserved 9 and 21 d ($P < 0.0001$ for both) but none for those preserved 2 d ($P = 0.33$). Therefore, preservation duration did not have a significant effect on dry weights of animals preserved in formalin, but had a significant effect on those preserved in alcohol.

Comparison of dry weights of preserved and unpreserved animals

We determined the length-dry weight relationship of unpreserved *Daphnia* (control) and those preserved in the two chemicals and in dry-ice (Table 1; Fig. 5). We compared the elevations and found significant differences among the three treatments with the control ($P < 0.0005$). Subsequent pairwise comparisons between elevations indicated that for a given length interval, the dry weights of preserved animals were uniformly lower than those of unpreserved animals ($P < 0.05$). Although the effects of freezing and preservation in formalin were similar ($P > 0.50$), there was a substantial residual variation around the best fit line for frozen animals ($r^2 = 0.543$), indicating that freezing may have differentially affected the dry weight. The range of weight loss for eth-

Table 1. Comparison of length (mm)/dry weight (µg) relationships of *Daphnia catawba* preserved for 14 d using three different preservation techniques as compared to fresh animals.

Treatment	Equation	n	r^2	S.E.	P
No preservation	logW = 0.913 + 2.285 logL	26	0.83	0.10	<0.0001
4 % formalin	logW = 0.804 + 2.260 logL	25	0.59	0.16	<0.0001
frozen	logW = 0.776 + 2.576 logL	25	0.54	0.17	<0.0001
70 % ethanol	logW = 0.581 + 3.023 logL	30	0.82	0.12	<0.0001

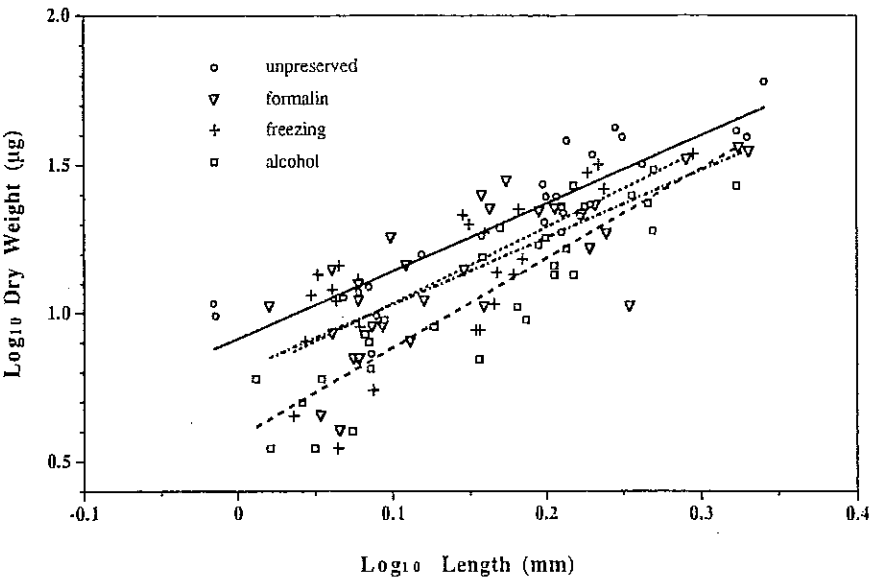

Fig. 5. Length-dry-weight relationships of *Daphnia catawba* corresponding to different preservation treatments.

anol-preserved animals and frozen animals was quite large, ranging from 5 % to 70 % dry weight loss depending on the length of the animal and the preservative used.

We also determined the relationship between the dry weight and length of unpreserved *Diaptomus* (Fig. 6 a). Even though the relationship was significant (P = 0.02), the linear regression only explained 28 % of the residual variation because of the small range in animal size. We compared mean dry weights (n = 10 groups) of preserved diaptomids against similar-sized unpreserved animals and found significant differences among treatments (ANOVA; P = 0.0168). Mean dry weights of animals that were frozen, formalin-preserved or ethanol-preserved were 91 %, 76 % and 60 % of the mean dry weight of unpreserved animals (Fig. 6 b). A Tukey-Kramer HSD test ($\alpha = 0.05$), however

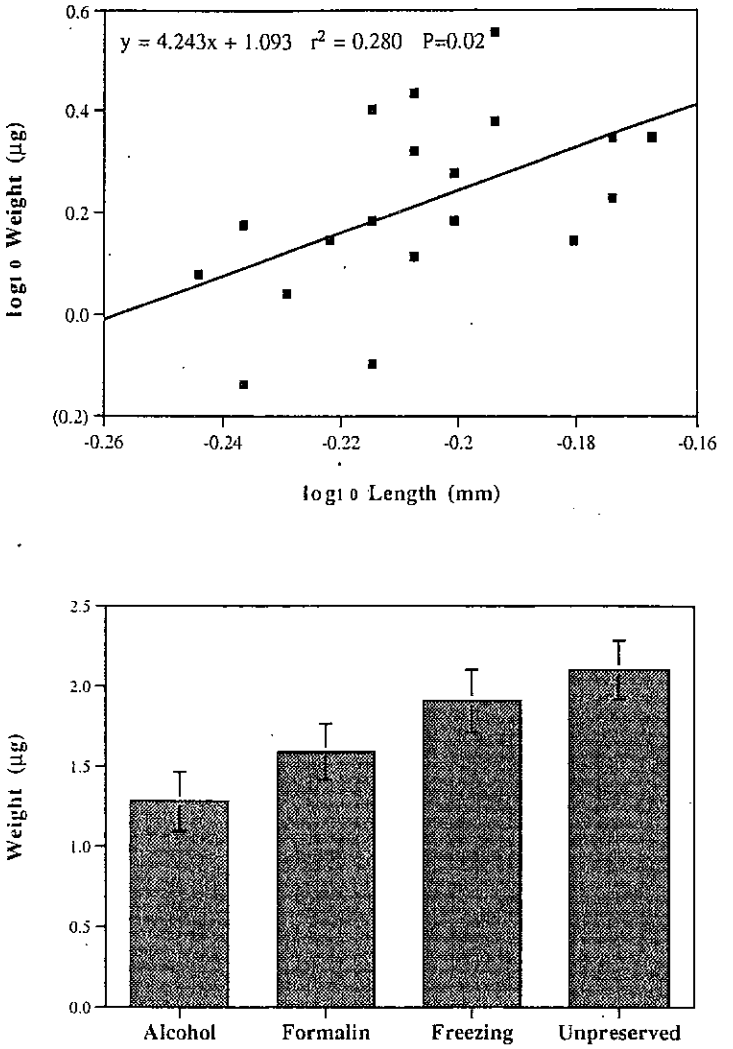

Fig. 6. a) Length-dry-weight relationship of unpreserved *Diaptomus minutus*. Each datum point is the mean of 5 individuals of similar size (± 0.01 mm). b) Mean dry weight (µg; ± 1 S.E.) of alcohol-preserved, formalin-preserved, frozen or unpreserved diaptomids (n = 10 groups of 5 animals in each treatment).

indicated that only alcohol-preserved animals were significantly lower than unpreserved animals.

Relationship between wet weight and dry weight of *Daphnia*

Few investigators have attempted to directly measure the wet weight of animals (GIGUÈRE et al. 1989). Instead, ecologists tend to estimate wet weight

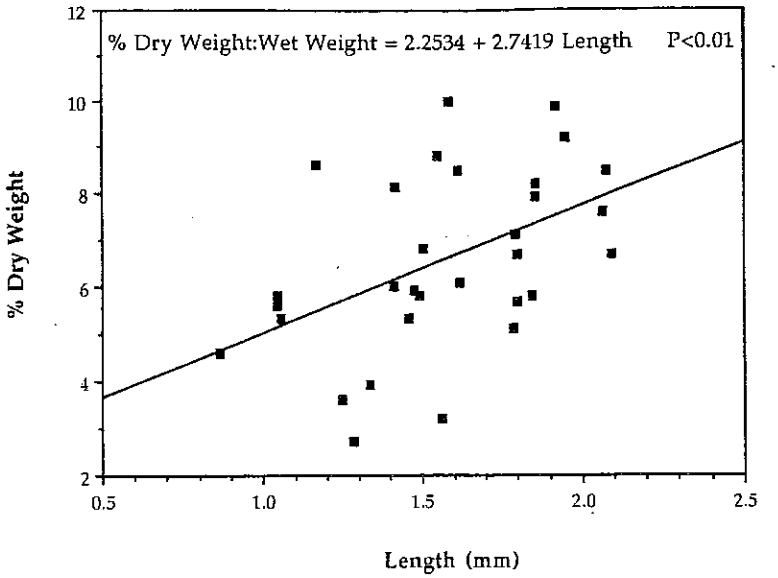

Fig. 7. Percent dry weight of previously frozen *Daphnia catawba* versus carapace length (mm).

from dry weight data by assuming that a single conversion factor can be applied over a range of different taxa. Despite reports that caution against this practice (BOTTRELL et al. 1976), there are too few direct comparisons of dry and wet weights for the assumption to be properly challenged. There was considerable scatter in the relationship between wet weight and dry weight for *Daphnia*. Despite the scatter, there was a significant increase in percent dry weight with size of animal ($P < 0.01$; Fig. 7), and this suggests that a constant conversion factor may not be applicable across the entire size range.

General discussion

Length-weight relationships of zooplankton seem to vary significantly across published studies. This variation could be due to differences in the biochemical composition of the zooplankton and lake specific characteristics, or it may be the result of methodological differences such as type of preservation, length of preservation and weighing methods (GIGUÈRE et al. 1989). Although the former sources of variation are inevitable and interesting, the latter can be controlled and standardized. The fact that chemical preservation of freshwater zooplankton alters the resulting dry weight measurements is an issue that should not be ignored if researchers intend to draw comparisons across studies (BIRD & PRAIRIE 1985, GIGUÈRE et al. 1989, SCHRAM et al. 1981). Acknowl-

edging and accounting for the effects caused by preservation technique will significantly increase the accuracy and allow more meaningful comparisons across studies.

We have shown that the dry weight of *Daphnia catawha* preserved in the most commonly used preservatives differ significantly from unpreserved *Daphnia* of the same size. We have also shown that both *Daphnia* and *Diaptomus* underwent more weight loss in alcohol compared with formalin, and that diaptomids exhibited more shrinkage in alcohol than in formalin. These differences indicate that investigators must be careful when applying length-weight regressions derived from unpreserved animals to length measurements of alcohol-preserved animals, and that the differential effects of preservatives on weight loss must be accounted for when cross-study comparisons are being made.

Our results contrast with those reported by BOTTRELL et al. (1976) and DUMONT et al. (1975) who suggested that formalin preservation did not seriously affect body weight , or that weight loss over a long storage period in 4 % formalin would not likely exceed 5 to 10 %. Our results bracket those of GIGUÈRE et al. (1989) who observed losses in the range of 37 to 43 %. The application of a single factor to correct for all types of preservatives should be used with caution since the dry-weight loss for *D. catawba* varies according to the preservative employed.

At present there is little information available regarding the biochemical basis of how the preservatives actually alter the zooplankton size and weight. Storage in both formalin and alcohol solutions lead to lipid breakdown and dissolution of oil (STEEDMAN 1976); neither is a good preservative for lipids over time. As well, alcohol preservatives have a dehydrating effect (DUMONT et al. 1975) and formalin preservatives have been reported to reduce dry weight (PACE & ORCUTT 1981). These preservative properties may also confound dry weight results because the extent and the rate at which the chemicals leach out the lipids is not known.

The results of this study support the notion that the commonly used preservatives do alter zooplankton dry weight as a function of preservation time. In a study of dry weight loss in *Ceriodaphnia lacustris*, maximum loss was found to occur in the first 30 days of preservation when 3 % formalin was used (SCHRAM et al. 1981). GIGUÈRE et al. (1989) have recommended that for small and large zooplankton, the minimum standard length of time in preservative should be 1 month and 6 months, respectively, to ensure dry weight stabilization. Since the effects were taxa-specific, the length of time required for weight stabilization for each taxon should be determined so that consistent procedures for measuring dry weights of preserved samples can be established.

Further research is necessary to determine the extent to which preservatives affect the length measurements of all freshwater zooplankton, especially since

length measurements are often the only measurements taken when equations borrowed from the literature are employed for dry weight estimates. The results of this study indicated that length shrinkage was significant in *Diaptomus,* although apparently not in *Daphnia.* The change in length may result from decalcification of the carapace from exposure to alcohol (STEEDMAN 1976) and formalin (LESLIE & MOORE 1986), and if *Daphnia* have 50 times more skeletal calcium than copepods (YAN et al. 1989), then *Daphnia* may not be as susceptible to decalcification as are *Diaptomus.* Alternately, shrinkage in copepods may result from dehydration in the inner tissues of the segmented body, especially if muscles contract as a result of preservation.

Our comparison of dry weight to wet weight for *Daphnia* contrast those of BOTTRELL et al. (1976) who suggested that the percent dry weight of taxa such as *Bosmina, Heterocope* and *Leptodora* decreased with body size. Since the proportion of dry weight varied from 0.02 to 0.10, the recommendation of DUMONT et al. (1975) to apply a general dry to fresh-weight ratio of 0.1 may also lead to considerable error. The possible taxa-specific and length-specific relationships indicate that investigators should use reported wet weight-dry weight conversions cautiously, and whenever practical, conduct wet-dry comparisons for each taxon in the study. Without conducting further biochemical analyses, it would be difficult to speculate on the reason for the higher percent dry weight in larger animals.

The differences in dry weight estimates and length-weight relationships across studies may be comparable if the techniques used to determine these estimates and relationships were standardized. Acknowledging and accounting for the effects of preservatives on zooplankton length and weight may establish more accurate and comparable dry weight estimates and potentially reduce the error presently incurred if these factors are ignored.

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References

BERBEROVIC, R. & PINTO-COELHO, R. (1989): Dry first, measure later: a new procedure to preserve and measure zooplankton for ecophysiological studies. – J. Plankton Res. **11:** 1109–1116.

BIRD, D. F. & PRAIRIE, Y. T. (1985): Practical guidelines for the use of zooplankton length-weight regression equations. – J. Plankton Res. **7:** 955–960.

BOERSMA, M. & VIJVERBERG, J. (1994): The effect of preservation methods on the carbon content of *Daphnia*. – Arch. Hydrobiol. **130:** 241–247.

BOTTRELL, H. H., DUNCAN, A., GLIWICZ, Z. M., GRYGIEREK, E., HERZIG, A., HILLBRIGHT-ILKOWSKA, A., LARSSON, P. & WEGLENSKA, T. (1976): A review of some problems in zooplankton production studies. – Norw. J. Zool. **24:** 419–456.

CAMPBELL, L. (1994): Factors contributing to dry weight differences among herbivorous zooplankton in two softwater lakes. – M.Sci. Thesis, McMaster University, Biology Department, Hamilton, Ontario, 116 pp.

CULVER, D. A., BOUCHERLE, M. M., BEAN, D. J. & FLETCHER, J. W. (1985): Biomass of freshwater crustacean zooplankton from length-weight regressions. – Can. Fish. Aquat. Sci. **42:** 1380–1390.

DUMONT, H. J., VAN DE VELDE, I. & DUMONT, S. (1975): The dry weight estimate of biomass in a selection of Cladocera, Copepoda, and Rotifera from the plankton, periphyton, and benthos of continental waters. – Oecologia (Berl.) **19:** 75–97.

GIGUÈRE, L. A., ST. PIERRE, J. F., BERNIER, B., VÉZINA, A. & RONDEAU, J. G. (1989): Can we estimate the true weight of zooplankton samples after chemical preservation? – Can. J. Fish. Aquat. Sci. **46:** 522–527.

LESLIE, J. K. & MOORE, J. E. (1986): Changes in lengths of fixed and preserved young freshwater fish. – Can. J. Fish. Aquat. Sci. **43:** 1079–1081.

PACE, M. L. & ORCUTT, J. D. Jr. (1981): The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. – Limnol. Oceanogr. **26:** 822–830.

PREPAS, E. (1978): Sugar-coated *Daphnia:* an improved fixation technique for Cladocera. – Limnol. Oceanogr. **23:** 557–559.

SALONEN, K. & SARVALA, J. (1980): The effect of different preservation methods on the Carbon content fo Megacyclops gigas. – Hydrobiologia **72:** 281–285.

– – (1985): Combinations of freezing and aldehyde fixation a superior preservation method for biomass determination of aquatic invertebrates. – Arch. Hydrobiol. **103:** 217–230.

SCHRAM, M. D., PLOSKEY, G. R. & SCHMITZ, E. H. (1981): Dry weight loss in *Ceriodaphnia lacustris* (Crustacea, Cladocera) following formalin preservation. – Trans. Amer. Micros. Soc. **100:** 326–329.

STEEDMAN, H. F. (1976): Zooplankton fixation and preservation. – The Unesco Press, Paris.

YAN, N. D., MACKIE, G. L. & BOOMER, D. (1989): Seasonal patterns in metal levels of the net plankton of three Canadian Shield lakes. – Sci. Tot. Environ. **87/88:** 439–461.

ZAR, J. H. (1984): Biostatistical Analysis, 2nd Ed. – Prentice-Hall, Inc., Englewood Cliffs, N. J. (1984).

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