136179

Effects of preservation on dry- and ash-free dry weight biomass of some common aquatic macro-invertebrates*

Rob S. E. W. Leuven[1], Theo C. M. Brock[1] & Hans A. M. van Druten[2]
[1] *Laboratory of Aquatic Ecology,* [2] *Department of Statistical Consultation, Catholic University, Toernooiveld, 6525 ED Nijmegen, The Netherlands*

Keywords: Macro-invertebrates, Preservation methods, Changes in biomass, Dry weight, Ash-free dry weight

Abstract

The effect of preservation methods on dry weight (DW) and ash-free dry weight (AFDW) of *Radix peregra* (Gastropoda), *Asellus aquaticus* (Isopoda), *Erpobdella octoculata* (Hirudinea) and *Glyptotendipes sp.* (Chironomidae) was studied. Ethanol, formaldehyde, and Bouin were used as preservative. In case of preservation of macro-invertebrates in ethanol substancial changes in DW and AFDW biomass were observed. In the four different taxa the loss in DW varied between 7.2–21.9% after a 3 month preservation period in 70% ethanol. A comparatively small range in AFDW loss (16.2–19.7%) was found. Changes in DW and AFDW biomass during preservation were significantly affected by the duration of the preservation, by temperature, light conditions and the volume of the preservative. The changes in AFDW were also significantly affected by the concentration of the preservatives. Preservation in 10% formaldehyde did not cause significant changes in DW and AFDW biomass.

Introduction

A proper understanding of aquatic ecosystems requires an exploration of the structure and functioning of its constituent components (Den Hartog, 1981). Macro-invertebrates play an important role in food-chains in most aquatic systems and therefore it is important to study their biomass and productivity. Sampling, sorting and identification of macro-invertebrates are time-consuming activities. Hence in practice the animals are often preserved before identification, counting and biomass estimations can take place. Preservation of organisms, however, may affect weight estimates.

Geng (1925) already described that preservatives such as alcohol and formalin caused changes in the weight of preserved animals. Hence, estimates of biomass and production of macro-invertebrates in ecosystems can be erroneous if these animals are preserved without compensation being made for changes in weight. Several investigators have studied the effect of preservation of macrofauna on the wet weight biomass (Mackay & Kalff, 1969; Stanford, 1972; Donald & Paterson, 1977; Landahl & Nagell, 1978; Maslin & Pattee, 1981) and dry weight biomass (Howmiller, 1972; Dermott & Paterson, 1974; Wiederholm & Eriksson, 1977). To study organic matter flows in ecosystems measurements of AFDW biomass (= organic weight) should be recommended (Stirn, 1981). Therefore it is striking that the influence of preservation on changes in ash-free dry weight (AFDW) biomass was not investigated in the above mentioned studies. Expressing biomass in AFDW makes comparisons more reliable because the variations in moisture- and ash-content in macro-invertebrates are taken into account. For organisms with calcareous skeletons the AFDW presents a reliable measurement of total organic matter. Wet weights (WW) and dry weights (DW) only approximate organic

* Contribution nr. 42 of the nymphalid project.

Hydrobiologia 127, 151–159 (1985).
© Dr W. Junk Publishers, Dordrecht. Printed in the Netherlands.

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

matter if the weights of calcareous skeletons and/or body moisture are eliminated (Stirn, 1981).

The present paper describes the effects of preservation methods on changes in dry- and ash-free dry weight of common macro-invertebrates. These aspects were studied using *Asellus aquaticus* (L.) (Isopoda), *Radix peregra* (Müller) f. *ovata* (Draparnaud) (Gastropoda), *Erpobdella octoculata* (L.) (Hirudinea) and *Glyptotendipes* spec. (Chironomidae). These animals are representatives of different taxonomic groups, so that species specific susceptibility to weight loss during preservation could be studied.

Materials and methods

In the spring of 1981, large numbers of *Radix peregra* f. *ovata* and *Asellus aquaticus* were collected in the effluent of a sewage treatment plant near Groesbeek (The Netherlands). *Erpobdella octoculata* and *Glyptotendipes* specimens were collected in the Bemmelse Strang, a backwater of the river Waal near Nijmegen, by using litterbags (1 mm mesh size) filled with decaying plant material. Immediately after sorting, the individuals of each species were split up into several replicate subsamples of 2–8 individuals. Of each replicate the fresh weight (FW) was determined by blotting the animals for ca 1 min. on filter paper (Dermott & Paterson, 1974) before weighing on a Mettler electronic balance (type HL 52). At the start of each experiment the ratios DW:FW and AFDW:FW of 6–8 replicates (controls) of each species were determined. By using these ratios the original DW and AFDW of the replicates that were preserved were calculated from their FW. Lappalainen & Kangas (1975) pointed out that prediction of DW and AFDW from FW can accurately be done for fresh material. Power functions describing DW – FW and AFDW – FW relationships show large r^2 and small coefficients of variation. Changes in weight due to preservation can be calculated by subtracting the DW and AFDW of the preserved replicates from their predicted original DW and AFDW. The remaining weights of preserved material were always expressed as a percentage of the predicted weight.

The animals of each replicate were dried by placing them (controls before and other subsamples after preservation) for 16 h in an oven (with forced ventilation) at 105°C. Landahl & Nagell (1978) and Lappalainen & Kangas (1975) described that this drying procedure resulted into constant dry weights. The ash content of the macro-invertebrates was determined by placing them for 3 hours in a muffle furnace at 550°C. Lappalainen & Kangas (1975) reviewed literature concerning incineration of fauna and concluded that the maximum temperature of 550°C can be maintained without an appreciable loss of inorganic components. These authors also pointed out that incineration of 3 hours resulted in a complete combustion of organic matter and resulted into constant ash-free dry weights. Before weighing, the animals were cooled down to room temperature in a desiccator. The AFDW was calculated by subtracting the ash-weight from the DW.

In most experiments 10 ml ethanol (70%) was used as a preservative, but the effects of other amounts and concentrations of this preservative on changes in biomass were also studied. Besides ethanol, 10% buffered formaldehyde, 4% buffered formaldehyde and Bouin were used. Bouin medium was prepared according to Romeis (1948). The pH's of the different preservatives were more or less the same (varying between 5.3–5.6). The preservatives were always diluted with twice-distilled water. Most experiments were preformed in the dark at room temperature (ca. 20°C).

Depending on the aim of the experiment the individuals were selected on body size or divided at random into replicate subsamples.

In experiments in which the effect of the length of the preservation period in 10 ml ethanol (70%) on changes in biomass was studied, replicate subsamples were used which contained animals of different size (randomly chosen). By doing so the field population of macro-invertebrates is more or less simulated.

Replicates with *Radix peregra* f. *ovata* of the same size were used to study the effects of light-dark conditions and temperature as well as type, amount and concentration of preservative on the changes in biomass of the preserved fauna. Effects of body size on weight loss during preservation, as described by Dermott & Paterson (1974), were ruled out in this way.

The effect of body size on changes in weight during preservation was studied with *Radix peregra* f. *ovata*; the relationships between shell-height and ash-free dry weight in fresh and preserved material were described by the power functions $Y =$

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

αX^{β}. Because this model is non-linear the model is transformed to $\ln Y = \ln\alpha + \beta \ln X$. Calling $a = \ln \alpha$, $b = \beta$ and applying the transformed powerfunction to each experiment (fresh material, preserved material in respectively 90%, 70% and 50% ethanol) to describe the relationship, a linear model containing 8 parameters is obtained, i.e.

$\ln Y_{ij} = a_i + b_i \ln X_{ij}$. The $\ln X_{ij}$ and $\ln Y_{ij}$ are transformed measurements in experiment i (i = 1, ..., 4) and a_i and b_i are respectively intercept and slope of the line describing the relationship between the logs of shell height (X_i) and ash-free dry weight (Y_i) in experiment i. To fit the transformed data to the model the GLM (General Linear Model) procedure of SAS (Statistical Analysis System) was applied.

Most experiments were performed in sets of 6–8 replicates. For each sampling day, the mean relative amounts of biomass and standard deviations of the replicates could be calculated in this way. Parameter free tests for comparing group levels (i.e. Kruskal-Wallis, Wilcoxon and the trend test of Terpstra (Hollander & Wolfe, 1973)) were used to detect statistically significant differences and trends. Analysis of covariance was applied to investigate the relationships between shell height and ash-free dry weight for fresh and preserved material of *R. peregra* f. *ovata*.

To describe the results the following abbreviations will be used: FW, fresh weight (of unpreserved material); WW, wet weight (of preserved material); DW, dry weight; AFDW, ash-free dry weight; PDW, predicted dry weight for fresh material; PAFDW, predicted ash-free dry weight for fresh material.

Results

Radix peregra f. *ovata* and *Asellus aquaticus*, represented in the replicates by specimens of different sizes, showed a rapid decrease in DW and AFDW during preservation in 70% ethanol. The Kruskal-Wallis test and Terpstra test for the comparison of group levels and the detection of trend in DW and AFDW are clearly significant in each species ($p < 0.05$). A stabilization in biomass was observed after a period of 25–50 days (Fig. 1a and 1b). After this stabilization period the changes in AFDW biomass of both *Radix* and *Asellus* were of the same magnitude; the remaining AFDW varied between 78.0–82.8% of the original biomass. Particularly in *Radix* the weight of the shell caused clear differences between the remaining DW and AFDW. Table 1 shows the weight ratios of the four macroinvertebrate species at several time points. After a 1 month preservation period in 70% ethanol of *Radix, Asellus, Glyptotendipes* and *Erpobdella*, each of which was represented in the replicates by specimens of different sizes, the remaining DW and AFDW ranged from 75.0–97.8% and 75.7–87.9% respectively of the original biomass. Preservation of the same taxa for three months in 70% ethanol resulted in remaining DW's and AFDW's of 78.1–92.8% and 80.3–83.8% respectively. Hence, after the stabilization period there are still substancial differences in the remaining DW whereas the changes in AFDW of the four different taxa studied are of the same magnitude. The DW as well as the AFDW of all taxa are significantly affected by the length of the preservation period (Kruskal-Wallis test, Terpstra test, each species all tests $p < 0.05$).

Particularly in *Radix* and *Asellus* the DW:FW

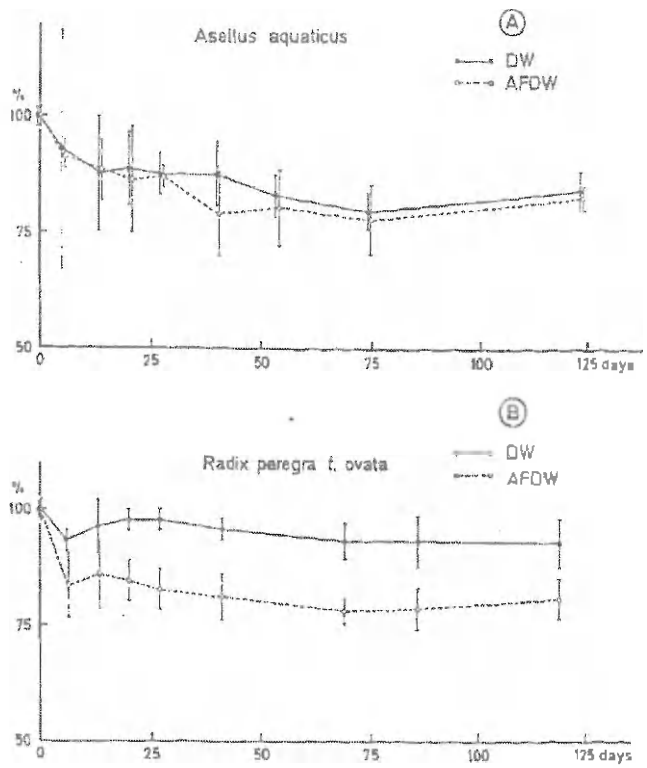

Fig. 1a, b. The effect of the length of the preservation period in 10 ml ethanol (70%) on changes in biomass of *Asellus aquaticus* and *Radix peregra* f. *ovata*. Each sampling point represents the mean remaining weight percentage (± SD) of 6 replicates. Preservation occurred at room temperature in the dark and each replicate contained 8 animals of different sizes.

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

Table 1. Mean weight ratios (± SD) of four macro-invertebrate taxa at several time points after preservation in ethanol (70%) at room temperature in the dark.

Species	N_1	N_2	$\frac{DW}{FW}$ 100% Fresh material	$\frac{DW}{FW}$ 100% After 1 month	$\frac{DW}{FW}$ 100% After 3 months	$\frac{AFDW}{FW}$ 100% Fresh material	$\frac{AFDW}{FW}$ 100% After 1 month	$\frac{AFDW}{FW}$ 100% After 3 months
Radix peregra f. *ovata*	6	8	27.3 ± 1.9	26.7 ± 0.6	25.3 ± 1.4	10.2 ± 0.9	8.4 ± 0.4	8.3 ± 0.4
Asellus aquaticus	6	8	20.3 ± 1.4	17.8 ± 0.9	17.1 ± 0.9	13.0 ± 1.0	11.6 ± 0.4	10.9 ± 0.3
Glyptotendipes spec.	6	4	22.6 ± 1.1	17.0 ± 1.3	17.7 ± 2.8	21.9 ± 0.9	16.6 ± 1.2	17.6 ± 2.8
Erpobdella octoculata	7	2	16.7 ± 0.7	13.6 ± 1.0	13.4 ± 0.4	15.2 ± 0.8	13.2 ± 0.9	13.3 ± 0.5

	$\frac{DW}{PDW}$ 100% After 1 month	$\frac{DW}{PDW}$ 100% After 3 months	$\frac{AFDW}{PAFDW}$ 100% After 1 month	$\frac{AFDW}{PAFDW}$ 100% After 3 months
Radix peregra f. *ovata*	97.8 ± 2.1	92.8 ± 5.4	82.7 ± 4.1	80.9 ± 4.1
Asellus aquaticus	87.8 ± 4.5	84.2 ± 4.3	87.9 ± 2.6	82.8 ± 2.6
Glyptotendipes spec.	75.0 ± 5.9	78.1 ± 12.3	75.7 ± 6.2	80.3 ± 12.8
Erpobdella octoculata	81.5 ± 5.9	80.1 ± 2.4	83.6 ± 5.4	83.8 ± 2.8

N_1, number of replicates; N_2, number of individuals in each replicate.

and AFDW:FW ratios showed remarkable differences due to the ash content of the shell of *Radix* and the exoskeleton of *Asellus*. The weight of these inorganic components comprises a relatively large proportion of the DW in *Radix* and *Asellus* (Table 1).

The effects of different preservation methods on the changes in biomass of *Radix peregra* f. *ovata* are shown in Fig. 2 and Table 2.

Preservation of *Radix* individuals of the same size in 2.5, 5, 10 and 25 ml ethanol (70%) resulted in significantly different group levels for DW and AFDW biomasses (Fig. 2a; Table 2). Trend tests of Terpstra showed that the weight losses increased with an increasing volume of the preservative.

Preservation of *Radix* individuals of the same size in 70% ethanol at different temperatures only resulted in significantly different percentages of the remaining AFDW (Fig. 2b; Table 2). However, the DW as well as the AFDW biomass showed significant negative trends with the preservation temperature, which means that the losses in these weights increase with higher temperatures.

The results of the experiments with *Radix* in which the effects of several ethanol concentrations on the DW and AFDW losses of *Radix* were studied, are presented in Fig. 2c and Table 2. For *Radix* individuals of similar size a significant trend towards an increasing DW and AFDW loss with lower ethanol concentrations was observed.

Transformed power functions for the relationship between shell height and AFDW biomass were used to demonstrate effects of several ethanol concentrations on *Radix* individuals of varying size. 156 data pairs were fitted. The results are presented in Fig. 3. The linear model in $Y_{ij} = a_i + b_i \ln X_{ij}$ (4

Table 2. A statistical comparison, using the Kruskal-Wallis and Terpstra trend test, of data from experiments in which the effects of different preservation methods on changes in biomass of *Radix peregra* f. *ovata* were studied.

Variable during preservation	Data presented in figure	Kruskal-Wallis DW	Kruskal-Wallis AFDW	Trend test Terpstra DW	Trend test Terpstra AFDW
Volume	2a	*	*	*	*
Temperature	2b	–	*	*	*
Concentration	2c	–	*	*	*

* Statistically significant ($p < 0.05$), – statistically not significant ($p \geq 0.05$).

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

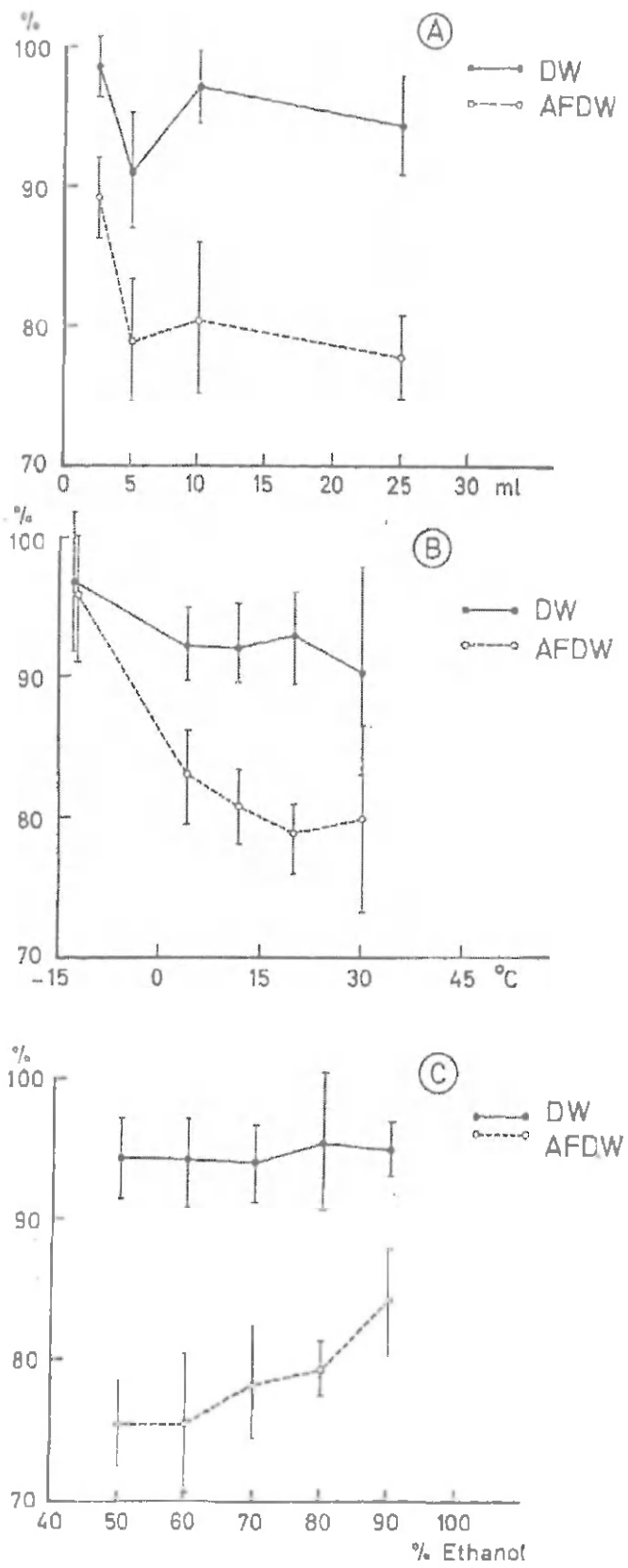

Fig. 2. The effects of different preservation methods on the changes in biomass of *Radix peregra* f. *ovata* (each replicate contained 5 animals of the same size), preserved for 4 months in ethanol under dark circumstances. All sampling points represent the mean remaining weight percentages (± SD) of 8 replicates. a) The influence of different volumes of 70% ethanol (room temperature), b) The influence of temperature (10 ml ethanol 70%), c) The influence of the ethanol concentration (10 ml ethanol, room temperature).

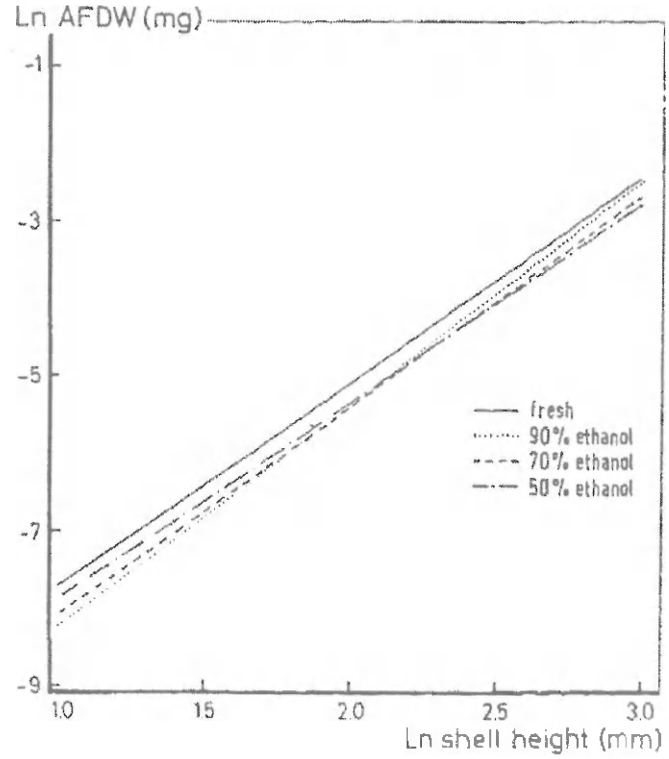

Fig. 3. The relationship between shell height and ash-free dry weight for fresh and preserved material of *Radix peregra* f. *ovata*. The data were fitted using the transformed power function $\ln Y_{ij} = a_i + b_i \ln X_{ij}$. Intercepts ($a_i$) and slopes ($b_i$) of the lines are presented in Table 3.

lines) fits the data well (R-square with respect to the corrected total is 0.952). The estimates of the 8 parameters (with standard errors), are presented in Table 3. To test the homogeneity of slopes, an analysis of covariance was applied ($H_0 : b_1 = b_2 = b_3 = b_4$). Although the overall hypothesis of equal slopes is not rejected ($F_{3,148} = 1.79$, $p = 0.15$) it remains doubtful whether the lines can be considered parallel. All pairwise differences were examined. There are indications that the slopes b_2 (2.88, preserved material 90%) and b_4 (2.55, preserved material 50%) are different (t-test, $p = 0.03$). With respect to the pairwise differences between inter-

Table 3. Parameter estimates of transformed power function $\ln y_{ij} = a_i + b_i \ln X_{ij}$ for relationship between shell height (X_{ij}) and ash-free dry weight (y_{ij}) for fresh and preserved (ethanol) material of *Radix peregra* f. *ovata*.

		Intercept	Slope
Experiment i		$a_i \pm SE$	$b_i \pm SE$
i = 1	fresh material	−10.39 ± 0.22	2.64 ± 0.09
i = 2	90% ethanol	−11.16 ± 0.26	2.88 ± 0.11
i = 3	70% ethanol	−10.84 ± 0.24	2.71 ± 0.10
i = 4	50% ethanol	−10.46 ± 0.21	2.55 ± 0.10

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

Table 4. The influence of different preservatives (10 ml) on remaining DW and AFDW biomass (± standard deviation) for *Radix peregra* f. *ovata*. The replicates were preserved for 4 months under dark circumstances (except treatment 2) and each replicate contained 5 animals of the same size.

Treatment	Preservative	$\frac{DW}{PDW}$ 100% mean ± SD (n = 8)	$\frac{AFDW}{PAFDW}$ 100% mean ± SD (n = 8)
	Fresh material (Control)	100.0 ± 7.0	100.0 ± 8.8
1	70% ethanol (Dark)	94.1 ± 2.9*	79.4 ± 2.9*
2	70% ethanol (Light)	93.4 ± 1.8*	75.5 ± 3.9*
3	Bouin	46.5 ± 8.1*	107.8 ± 18.6
4	10% formaldehyde	104.0 ± 3.4	101.0 ± 4.9
5	4% formaldehyde	98.5 ± 7.3	93.1 ± 7.8*

* An asterisk indicates that the group levels were significantly different from those of the control replicates (Wilcoxon test $p < 0.05$).

cepts: the intercept – 11.16 of the 90% preserved material (a_2) appears to be different from respectively – 10.46 and – 10.39 i.e. the intercepts of 50% preserved material ($p = 0.04$) and the fresh material ($p = 0.02$). Clearly the hypothesis of equal slopes and equal intercepts, that is one line to explain the data pattern, is rejected ($F_{6,148} = 11.48$, $p = 10^{-4}$). On the average the intercept of the line of fresh material is 0.3 à 0.4 units higher than the intercept of the preserved material. Therfore, one should account for the possibility that the AFDW biomass for fresh material is 40 à 50% higher than the AFDW for material preserved in ethanol.

The influence of different preservatives on remaining DW and AFDW biomass are presented in Table 4. Preservation of *Radix peregra* in 10% formaldehyde did not result in significantly different DW and AFDW biomasses when compared with those of the control replicates (Table 4). Preservation of *Radix* in 70% ethanol (light and dark) and Bouin, however, caused a significant decrease in DW biomasses when compared with those of the controls. A significant decrease in AFDW biomass was only found when preservation occurred in 70% ethanol and 4% formaldehyde. These changes in AFDW biomass were significantly larger ($p < 0.05$) when preservation took place in the light as compared to preservation under dark circumstances (Table 4).

Discussion

Recently several investigators have discussed difficulties associated with preservation of macrofauna. A summary of some relevant data concerning the changes caused by preservation in biomass of freshwater macrofauna is presented in Table 5. Depending on the preservation methods used, the WW and DW-biomass of several preserved macrofauna taxa varied between 26–117% and 40–142% respectively when compared with their original biomass (= fresh weight). Howmiller (1972) and Stanford (1972) found very large changes in fauna biomass due to preservation. These investigators used a centrifugation technique to remove surface moisture and this treatment could have caused excessive losses of body fluids and organic matter.

This paper also illustrates that preservation of macrofauna can result in changes in DW and AFDW biomass estimates, particularly when ethanol (70%) is used as a preservative. Nevertheless, a comparatively small range in remaining AFDW (80.3–83.8%) is found in four different macro-invertebrate taxa after a preservation period of 3 months in 70% ethanol.

Changes in DW- and AFDW-biomass also vary with the duration of the preservation. A stabilization of biomass of *Asellus aquaticus* and *Radix peregra* occurred after a preservation period of 25–50 days. Similar patterns in changes in WW and/or DW biomass of preserved macro-invertebrates were observed by Howmiller (1972), Donald & Paterson (1977) and Maslin & Pattee (1981). Different macro-invertebrate taxa, however, showed large differences in stabilization time during preservation (Table 5).

In the present study experiments with *Radix peregra* showed that the changes in AFDW biomass during preservation were not only affected by the preservation time but also by temperature, light conditions, body size, type, volume and concentration of the preservative. Howmiller (1972), Dermott & Paterson (1974), Donald & Paterson (1977) and Landahl & Nagell (1978) already demonstrated the influence of the type of presentative on biomass changes (Table 5). Donald & Paterson (1977) and Dermott & Paterson (1974) illustrated that the diluting water (e.g. distilled, tap or habitat water) of the preservative also affected the biomass of preserved fauna. Stanford (1972) showed that the wet

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

Table 5. Summary of literature data about effects of preservation on biomass determination of aquatic macrofauna.

	Taxa	Preservative	Duration of experiments (Days)	Stabilization period (Days)	Remaining weight percentages		
					WW	DW	AFDW
Oligochaeta	Tubificidae[7]	70% ethanol	67	20	87*	134*	–
	Limnodrilus[3]	70% ethanol	44	21	76	51	–
	Limnodrilus[3]	70% isopropanol	44	44	26	40	–
	Limnodrilus[3]	10% formalin	44	21	44	77	–
Hirudinea	*Glossiphonia*[9]	70% ethanol	120	45	75	–	–
	Erpobdella octoculata[1]	70% ethanol	120	?	–	80	84
Mollusca	*Margaritana*[9]	10% formalin	120	60	76	–	–
	Planorbis[9]	70% ethanol	120	30	73	–	–
	Radix peregra f. *ovata*[1]	70% ethanol	120	25	–	93	81
	Radix peregra f. *ovata*[1]	10% formaldehyde	120	?	–	104	101
	Radix peregra f. *ovata*[1]	4% formaldehyde	120	?	–	99	93
	Radix peregra f. *ovata*[1]	Bouin	120	?	–	47	108
Crustacea	*Pontoporeia affinis*[7]	70% ethanol	67	67	111*	82*	–
	Gammarus[9]	70% ethanol	120	45	85	–	–
	Asellus aquaticus[1]	70% ethanol	120	50	–	84	83
Ephemeroptera	*Baetis*[9]	70% ethanol	120	15	60	–	–
Plecoptera	*Perlodes*[9]	70% ethanol	120	30	74	–	–
	Pteronarcys californica[4]	70% ethanol	38	20	73– 85*	–	–
Coleoptera	*Cybister*[9]	70% ethanol	120	60	90	–	–
Trichoptera	*Brachycentrus*[4]	70% ethanol	38	20	69	–	–
	Hydropsyche occidentalis[4]	70% ethanol	38	15	95	–	–
	Hydropsyche[9]	70% ethanol	120	60	75	–	–
Diptera	*Atherix variegata*[4]	70% ethanol	38	10	55	–	–
	Atherix[9]	70% ethanol	120	30	72	–	–
	Anatopynia[3]	70% ethanol	57	57	40	49	–
	Anatopynia[3]	70% isopropanol	57	21	41	52	–
	Anatopynia[3]	10% formalin	57	57	53	63	–
	Chironomus attenuatus[5]	10% formalin	598	?	–	74–142**	–
	Chironomus attenuatus[5]	70% ethanol	30	?	–	48	–
	Chironomus attenuatus[5]	40% isopropanol	30	?	–	46– 52**	–
	Chironomus attenuatus[6]	10% formalin	370	100	108–117	–	–
	Chironomus attenuatus[6]	70% ethanol	365	365	92–101	–	–
	Chironomus plumosus[8]	70% ethanol	21	?	90– 93**	68– 72**	–
	Chironomus plumosus[8]	4% formalin	21	?	103	90	–
	Chironomus[9]	70% ethanol	120	75	43	–	–
	Chironomus[7]	70% ethanol	67	10	96*	70*	–
	Glyptotendipes[1]	70% ethanol	120	?	–	80	84
	Metriocnemus knabi[6]	70% ethanol	500	?	84*	–	–
	Metriocnemus knabi[5]	70% ethanol	30	?	–	79	–
	Metriocnemus knabi[5]	10% formalin	30	?	–	103	–
	Metriocnemus knabi[5]	40% isopropanol	30	?	–	86	–
	Procladius[6]	70% ethanol	365	?	88*	–	–
	Procladius[7]	70% ethanol	67	20	104*	104*	–
	Tanytarsus barbitarsus[6]	10% formalin	800	60	57	–	–
	Several taxa[2]	70% ethanol	?	?	75	–	–

* Mean percentage over study period; **several weight classes, methods and/or periods were used; [1]present study; [2]Mackay & Kalff (1969); [3]Howmiller (1972); [4]Stanford (1972); [5]Dermott & Paterson (1974); [6]Donald & Paterson (1977); [7]Wiederholm & Eriksson (1977); [8]Landahl & Nagell (1978); [9]Maslin & Pattee (1981).

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

weight losses varied for several size classes of the plecopteran larvae, *Pteronarcys californica* Newport. Landahl & Nagell (1978) found that the biomass changes of preserved *Chironomus plumosus* L. varied for different seasons.

As shown by the data presented, there are many species- and method-specific effects of preservation on the biomass of macro-invertebrates. Therefore, the use of preserved fauna material for the estimations of standing crops and for productivity may result in considerable bias. In order to get reliable biomass data for macro-invertebrates two recommendations can be made:

1) Avoid preservation or minimize changes in biomass during preservation, or

2) Apply procedures to correct biomass data for weight changes due to preservation.

Avoiding preservation is practically feasible if sampling and sorting, as well as identification, of macro-invertebrates are not time-consuming research factors. When identification is the only time-limiting factor, it can be recommended to use different samples for biomass estimations (unpreserved) and for identification (preserved). Nevertheless, in most investigations preservation of macrofauna will be required. In that case, changes in biomass can be minimized if the animals are preserved in 70% ethanol and stored in a freezer at -15°C (see Fig. 2b). Generally the weight changes are smaller too if (buffered) formaldehyde is used in stead of other preservatives (Table 5). The use of formalin, however, may have several disadvantages, e.g., its unpleasant smell and poisonous and carcinogenic character. Furthermore in some cases an increase in animal biomass was observed during preservation in formalin (see e.g. Dermott & Paterson (1974) and Table 4 and 5).

A correction of the measured biomass for weight changes due to preservation is in many cases practically feasible only. Then a strictly standardized preservation method is required. Derivation of suitable correction factors, however, may be impossible because of the numerous factors which affect weight changes during preservation. In publications of studies in which preserved fauna is used for the estimations of standing crops and productivity it is absolutely necessary to describe the preservation circumstances accurately. Only then it can be decided wether it is allowed to compare biomass estimates from different studies.

In the present study the correction factors for DW and AFDW biomass of four different taxa varied between 1.08–1.28 and 1.19–1.24 respectively. The animals, which were represented in the replicates by individuals of different sizes, were preserved for 3 months in 70% ethanol and stored at room temperature in the dark. Whether the range in DW and AFDW loss during preservation under similar circumstances is about the same for most macro-invertebrates is unknown. It may be of practical interest to determine similar factors for other macro-invertebrate species.

Acknowledgements

The authors are greatly indebted to Prof. C. den Hartog, Dr. G. van der Velde and the referees for critical remarks, to Mr. A. M. M. Marques dos Santos and the Illustration Department of the Catholic University for preparing the drawings, and to Mrs. B. H. Aelen-Honer and Mrs. L. Plukker for typing the manuscript.

References

Dermott, R. M. & C. G. Paterson, 1974. Determining dry weight and percentage dry matter of chironomid larvae. Can. J. Zool. 52: 1243–1250.

Donald, G. L. & C. G. Paterson, 1977. Effect of preservation on wet weight biomass of chironomid larvae. Hydrobiologia 53: 75–80.

Geng, H., 1925. Der Futterwelt der natürlichen Fischnahrung. Z. Fisch. 23: 137–165.

Hartog, C. den, 1978. Structural and functional aspects of macrophyte-dominated aquatic systems. Proc. EWRS 5th Symp. aquat. Weeds: 35–41.

Hollander, M. & D. A. Wolfe, 1973. A distribution - free test for ordered alternatives. In Non parametric statistical methods. Wiley & Sons, N.Y.: 120–123.

Howmiller, R. P., 1972. Effects of preservatives on weight of some common macro-benthic invertebrates. Trans. am. Fish. Soc. 101: 743–746.

Landahl, C. C. & B. Nagell, 1978. Influence of the season and of preservation methods on wet- and dry weights of larvae of *Chironomus plumosus* L. Int. Revue ges. Hydrobiol. 63: 405–410.

Lappalainen, A. & P. Kangas, 1975. Littoral benthos of the Northern Baltic Sea, 2. Interrelationships of wet, dry and ash-free dry weights of macro-fauna in the Tvärminne area. Int. Revue ges. Hydrobiol. 10: 297–312.

Mackay, R. J. & J. Kalff, 1969. Seasonal variation in standing crop and species diversity of insect communities in a small Quebec stream. Ecology 50: 101–109.

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)

Maslin, J. L. & E. Pattee, 1981. La production du peuplement benthique d'une petite rivière: Son evaluation par la méthode de Hynes, Coleman et Hamilton. Arch. Hydrobiol. 92: 321–345.

Romeis, B., 1948. Mikroskopische Technik. Leibniz Verlag, München, 696 pp.

Stanford, J. A., 1972. A centrifuge method for determining live weights of aquatic insect larvae, with a note on weight loss in preservative. Ecology 54: 449–451.

Stirn, J., 1981. Manual of methods in aquatic environment research. Part. 8. Ecological assesment of pollution effects. FAO Fish. tech. Pap. 209 (FIRI/T 209), 70 pp.

Wiederholm, T. & L. Eriksson, 1977. Effects of alcohol-preservation on the weight of some benthic invertebrates. Zoon 5: 29–31.

Received 1 March 1984; in revised form 20 March 1985; accepted 23 April 1985.

subito e.V. licensed customer copy supplied and printed for Flanders Marine Institute Library (SLI05X00225E)