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SHORT COMMUNICATION

Dry first, measure later: a new procedure to preserve and measure zooplankton for ecophysiological studies

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Abstract. The processing of freshwater crustaceans for ecophysiological studies can be simplified considerably if species determination, selection and measurements of the specimens are performed after freezing and lyophilization. As the zooplankters do not change shape during this process, reliable taxonomic determination can be performed with dried animals. Besides easy handling, this procedure ensures minimization of metabolic losses due to prolonged exposure to room temperature, and significantly reduces losses of radiotracer material in grazing studies.

The analysis of body constituents and the determination of grazing rates are becoming increasingly common in zooplankton physiological and production studies (Vollenweider, 1985; Downing and Rigler, 1984). Although current preservation techniques are adequate for measurements of length and other conservative properties (Haney and Hall, 1973; Prepas, 1978; de Bernardi, 1984), they may have a deleterious effect on body chemical composition and all related measurements (Giese, 1967; Dowgiallo, 1975). Also, because of their high metabolic rates, the zooplankters should be processed as fast as possible. Lemcke and Lampert (1975) reported weight losses of >25% per day in adult *Daphnia pulex* subjected to starvation at 20°C. Separation of the animals in size classes, measurements of body length, taxonomic determination etc. all result in prolonged exposure to room temperature. This may bias subsequent biochemical and elemental analyses.

In grazing studies with radio-tracer elements, severe losses of ^{14}C and ^{32}P have been reported (Lampert and Taylor, 1985). This leakage may underestimate grazing rates by up to 70%, depending on sample treatment (Peters, 1984). Several fixing procedures, such as the use of lugol or formalin, killing of the animals with boiling water or with dry ice (but with subsequent thawing prior to measurements), have been tested (Sierszen and Watras, 1987). Holtby and Knoechel (1981) estimated losses of radioisotopes in chemically preserved samples and concluded that non-chemical fixation procedures, such as the use of boiling water, minimize ^{14}C losses compared to chemical preservation (formalin, lugol). Nevertheless, all procedures failed to completely prevent losses of radioisotopes and the errors are especially high when grazing rates are determined *in situ*. Lampert and Taylor (1985) showed that isotope leakage can amount to 50% in the first hour following the death of the organisms with formalin. This applies to both ^{14}C and ^{32}P.

Rapid freezing with dry ice and lyophilization have been recommended (Giese, 1967) as a nearly ideal method for drying and preserving animal samples intended for dry wt determination and for analysis of body constituents. Paerl (1984) demonstrated that deep freezing in liquid N_2, followed by lyophilization, is suitable for autoradiography studies with phytoplankton. However, the suitability of this procedure for zooplankton has not yet been evaluated. In this paper we describe a simple way of coping with some of the problems listed above by an unconventional application of this procedure.

The general scheme of the procedure for preservation and measurement of zooplankton samples is presented in Figure 1.

Zooplankton samples for biomass determinations and photomicrographs were collected in Lake Constance by vertical tows of a 140 μm mesh plankton net from 50 m to the surface.

To investigate the influence of deep freezing and lyophilization on body size of the animals, specimens of *Daphnia magna*, *D.galeata* and *D.hyalina* were

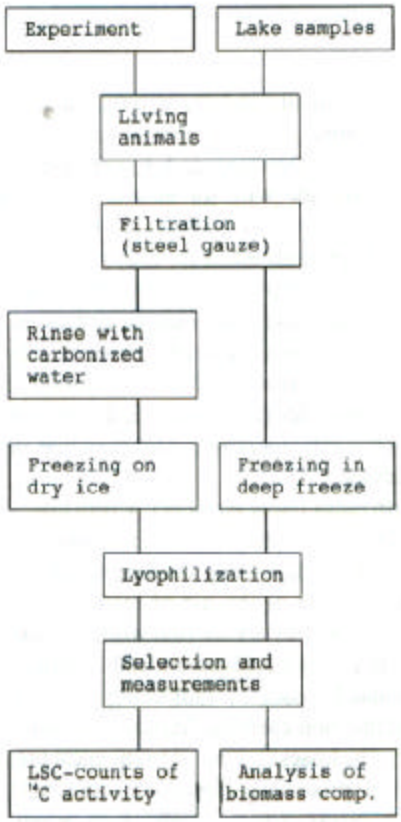

Fig. 1. A flow diagram representing the steps of the procedure used for preparation of samples. (**Left**) The sequence used for the animals from tracer experiments. (**Right**) The sequence used for preparation of samples for analyses of biomass composition.

measured alive and after the procedure was applied. The animals were sorted under a dissecting microscope with thin needles (entomological needle mounted in a glass rod) and curved Dumont tweezers. Usually it is enough to touch the carapace of the animal lightly as the electrostatic forces will hold it to the needle tip. Photomicrographs were taken using a WILD M8 zoom stereomicroscope equipped with photoautomat MPS 11.

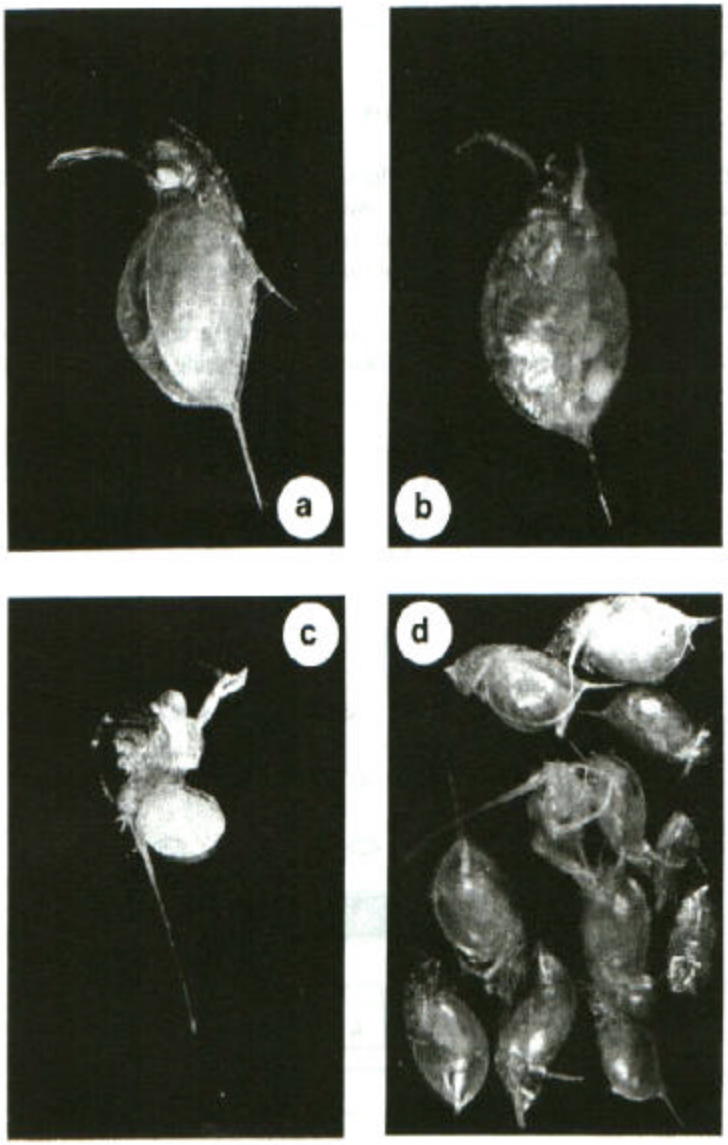

Fig. 2. Microphotographs of freeze-dried zooplankton from Lake Constance. (**a**) *Daphnia galeata*; (**b**) *D. hyalina*; (**c**) *Bythotrephes longimanus*; (**d**) mixed sample.

The procedure does not damage structures important for taxonomic determination (Figure 2). Handled carefully, the animals can be separated successfully into monospecific groups. This holds true even for relatively 'difficult' objects, such as copepods (S.Wölfl, personal communication). Our method makes it possible to avoid the otherwise difficult selection of living, fast-swimming individuals.

Figure 3 compares body length measurements of fresh and of freeze dried individuals. The correlation is highly significant ($r^2 = 0.997$; $n = 62$); the regression equation is

$$L_{dry}\,(\text{mm}) = 0.00 + 0.98880 L_{fresh}\,(\text{mm}) \qquad (1)$$

The values for the intercept and the slope of the regression line show that the lengths of dry individuals are on average 1.12% smaller than those of 'wet' individuals. We presume that this is due to different optical properties of samples in air versus in water, and not to shrinking in the course of freeze-drying.

To assess the suitability of the procedure for grazing studies with radioisotopes, short-term experiments with *D.pulex* using different fixation pro-

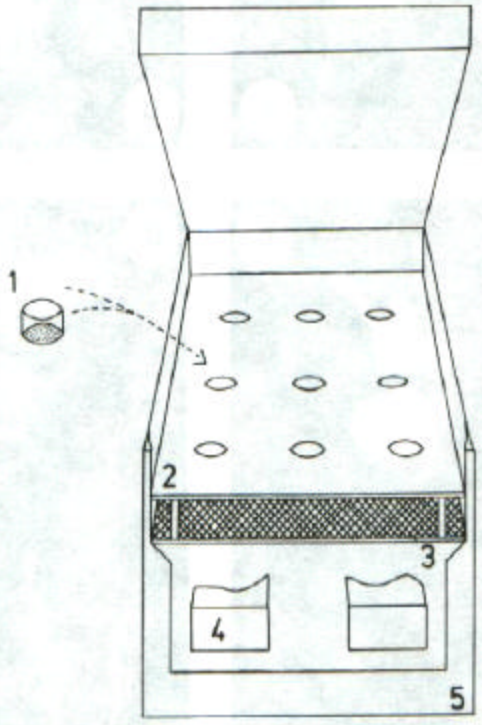

Fig. 3. A scheme of the box used for freezing of the samples on dry ice, and their transportation. (1) 150 μm net; (2) Plexiglas supporting plate with holders for the net-beakers; (3) metal frame; (4) blocks of dry ice; (5) isopore box.

cedures were performed. The grazing experiments were carried out in the laboratory under constant light and temperature conditions (18 $\mu E\ m^{-2}\ s^{-1}$ and 15°C, respectively), and lasted 10 min. The phytoflagellate *Rhodomonas minuta* served as tracer-food and the preparation of radioactive algal suspension followed the method described by Geller (1975). The concentrations of food suspension varied from 1.12 to 1.32 mg $C\ l^{-1}$ and tracer suspension activities ranged from 4500 to 5800 d.p.m. ml^{-1}. Prior to each test the animals were acclimatized to the experimental conditions for 2 h.

After performing the steps illustrated in Figure 1, the animals were treated in three different ways: (i) immediately measured and transferred within 20 min to scintillation vials ('control'); (ii) preserved in sugar–formalin (Haney and Hall, 1973); or (iii) frozen on dry ice (Figure 4), lyophilized and stored in a desiccator. The animals from methods (ii) and (iii) were measured and transferred to scintillation vials after 24 h and 2–3 days respectively. Once the samples were in the vials, they were treated in the same way for all experiments. To digest the animals, 0.3 ml tissue solubilizer Soluene 350 (Packard) was added to each vial and incubated for 24 h at 45°C. As a counting cocktail, 3 ml of Toluol–POPOP–MSB solution was used. Four drops of glacial acetic acid were added to avoid chemoluminescence and the samples were left to stand at room temperature for 2 h before measuring.

Individual filtering rates (FR) were calculated using the equation derived from Nauwerck (1959):

$$\text{FR (ml ind}^{-1}\text{ day}^{-1} = \left(\frac{\text{d.p.m. ind}^{-1}}{\text{d.p.m. ml}^{-1}\text{(food)}}\right) \times \left(\frac{1440}{\text{time(min)}}\right) \qquad (2)$$

Figure 5 and Table I show the results of the grazing experiments. The highest values of measured FR were obtained with the freeze-dry treatment (iii) and the lowest with sugar–formalin fixation (ii). The intermediate values occurred in the

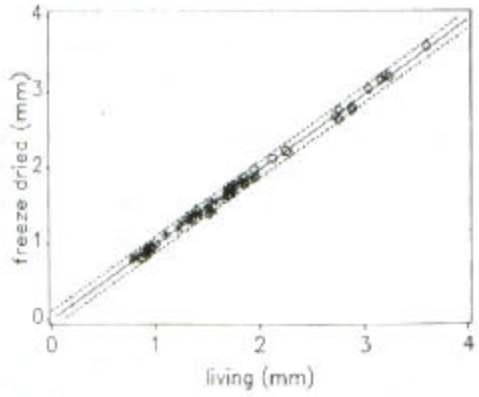

Fig. 4. Linear correlation between length measurements of living and of freeze-dried daphnids. Stars, *D.galeata*; circles, *D.hyalina*; diamonds, *D.magna*; dashed line, 99% confidence limits of regression line.

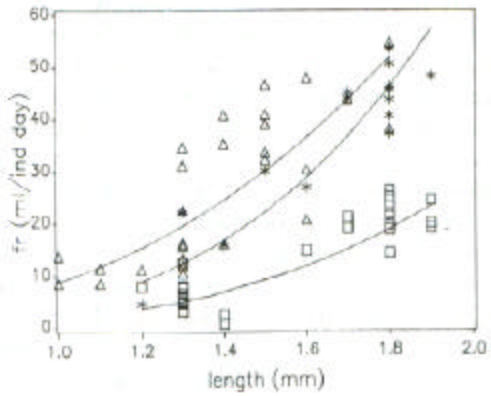

Fig. 5. The measured values and the fitted curves (see Table I) of filtering rates of *D.pulex*, gained with three different methods. Stars, method (i) (control); squares, method (ii) (animals killed with formalin); triangles, method (iii) (freeze-dried animals).

Table I. Regression equations predicting filtering rates (FR) of *D.pulex* from their body length (L): $FR = aL^b$ (mm)

Treatment	n	a	$P > T$	b	$P > T$
(i) None	16	4.24	0.0001	4.06	0.0001
(ii) Formalin	27	1.72	0.1296	4.05	0.0001
(iii) Lyophilization	27	8.84	0.0001	3.01	0.0001

control experiment (i): immediate transfer to the vials without additional treatment. The FR values of small animals in experiment (i) are smaller than those of experiment (iii). This underestimation is probably due to the fact that small animals are more susceptible to losses than larger ones, and even extremely fast handling could not prevent these losses. The significance of differences between treatments could also be confirmed by an ANCOVA using the SAS-Statistical Package (SAS Institute Inc. 1987; Table II). The interpretation of the different F tests was done according to Sokal and Rohlf (1981): the filtering rate differed highly significantly ($P > 0.0001$) among the three treatments when the covariate length (L) was kept constant. Therefore it may be concluded that the procedure of immediate freezing and lyophilization of living animals significantly reduces the loss of tracer. Bias of measured values, due to different susceptibility of different size classes of animals, and thereby the artifact of distorted size-dependent rates/functions, is probably thus minimized as well.

If the determination, selection and measurements of zooplankters are performed after, instead of before, freezing and lyophilization several advantages arise: (i) easy handling of small and otherwise fast swimming animals; (ii) minimization of metabolic losses; (iii) in grazing studies, significant reduction of radio-tracer losses, ensuring more accurate determination of grazing rates; and

Table II. Results of the ANCOVA: analysis of covariance performed on the log-transformed data, with filtering rate as a dependent, length as independent, and treatment as class variable

Source of variation	DF	Type	SS	MS	*F*	PR > *F*
Treatment	2	I	17.35	8.67	38.07	0.0001
Treatment	2	III	21.45	10.74	47.08	0.0001
Length	1	I and III	21.91	21.91	96.18	0.0001
Error	66	–	15.04	0.23	–	–

Sums of squares (SS) of type I are the between treatments sums of squares, and sums of squares of type III are related to the adjusted means of the covariate.

(iv) this treatment allows the storage of dried animals for longer time spans prior to further processing.

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