*Limnol. Oceanogr.*, 23(3), 1978, 557–559


# Sugar-frosted *Daphnia*: An improved fixation technique for Cladocera[1]

*Abstract*—Egg loss by preserved *Daphnia* was reduced from 35% to 10% by use of a chilled, as opposed to warm, solution of sucrose and Formalin.

In a study of two coexisting species of *Daphnia*, where an estimate of egg number was required, I found that animals fixed with a 40 g·liter$^{-1}$ sucrose and a 4% Formalin solution (Haney and Hall 1973) lost as many as 50% of their eggs. Direct observation of animals being killed with sucrose and a 4% Formalin solution under the microscope showed that the loss was not due to osmotic ballooning after death but to the adults expelling the eggs before death.

Egg loss was not reduced by increasing the concentration of sucrose in solution, as suggested by Haney and Hall (1973); concentrations of sucrose from 60 to 120 g·liter$^{-1}$ were tried without any noticeable change. Formalin concentrations of 2, 6, and 8%, both unbuffered and buffered with Borax, were also tried without any noticeable change. Ethanol (95%) did prevent loss of eggs, but was not satisfactory for the reasons given by Haney and Hall; also, to identify two coexisting species I had to count samples in an open counting chamber, and the convection currents set up by the evaporating alcohol made this impossible. I then transferred animals fixed in ethanol to a sucrose and Formalin solution: this also proved unsatisfactory because many small bubbles formed within the carapace when the animals were transferred, causing them to float. Assuming that bubbles were caused by the lower solubility of gas in water than ethanol, I tried removing the gas before transferring the animals by boiling the water used in the solution and by suction, but the bubbles still formed. I concluded that 95% ethanol was not a satisfactory preservative for my animals.

During the course of this study I observed a considerable reduction in egg loss when the animals were fixed in a cool, rather than a warm solution of sucrose and Formalin, and I decided to test the idea that the fraction of eggs lost from *Daphnia* during preservation was dependent on the temperature of the solution.

The assistance of F. H. Rigler is acknowledged. M. Ewert assisted in the field and did many of the egg counts. A. Nakrossius and J. Martin also assisted in the field. The Halton Region Conservation Authority permitted the use of Crawford Lake.

Zooplankton samples were collected with a 32-liter, Plexiglas, Schindler-Patalas trap (Schindler 1969) from Crawford Lake (42°28′N, 79°57′W), a 2.5-ha meromictic lake in southern Ontario. The animals were concentrated on a 73-$\mu$ Nitex screen, then washed into collecting bottles with the already diluted fixative and preservative—a solution of 60 g·liter$^{-1}$ sucrose and 2% Formalin buffered with

Table 1. Percentage of *Daphnia* eggs released in preservative at three temperatures. First set of data from 13 July 1976, second from 20 July 1976. Total number of eggs in each sample in parentheses. Weighted average is total number of eggs released divided by total number of eggs at each preservative temperature.

| Depth (m) | Preservative temperature % (*n*) | | |
|---|---|---|---|
| | cold | warm | hot |
| 4 | 6 (96) | 44 (146) | 43 (42) |
| 8 | 24 (41) | 20 (30) | 25 (32) |
| 12 | 0 (41) | 28 (47) | 62 (50) |
| 4 | 10 (91) | 25 (72) | 21 (77) |
| 8 | 15 (27) | 21 (33) | 50 (6) |
| 12 | 20 (10) | 37 (79) | 29 (63) |
| Weighted avg | 10 | 34 | 35 |

[1] This research was supported by a research grant to F. H. Rigler from the National Research Council of Canada.

Table 2. Percentage of early and later stage eggs released by *Daphnia* in preservative of various temperatures. Total number of eggs in each cell in parentheses.

| Preservative temp | Early stage % (n) | | Later stage % (n) | |
|---|---|---|---|---|
| | 13 Jul | 20 Jul | 13 Jul | 20 Jul |
| cold | 4 (46) | 8 (48) | 11 (132) | 14 (80) |
| warm | 9 (45) | 10 (51) | 44 (178) | 37 (133) |
| hot | 7 (27) | 4 (25) | 57 (97) | 30 (121) |

Table 3. Analysis of data from 20 July 1976 according to techniques described in Snedecor and Cochran (1967).

| Comparing | Test & result | df | *P* | Significant |
|---|---|---|---|---|
| Early stage eggs | | | | |
| 3 treatments | $\chi^2 = 0.77$ | 2 | >0.5 | no |
| Later stage eggs | | | | |
| 3 treatments | $\chi^2 = 13.10$ | 2 | <0.005 | yes |
| warm and hot | $\chi_c^2 = 1.13$ | 1 | >0.25 | no |
| pooled warm-hot and cold | $Z_c = 3.26$ | – | <0.001 | yes |

Borax. The fixative was kept at a constant temperature in two coolers, one filled with crushed ice and the other with hot water.

On 13 and 20 July 1976, samples were collected in triplicate from three depths (4, 8, and 12 m where the ambient temperatures were 19°, 9°, and 6°C) and treated with preservative at 6°, 20°, and 32°C, which will be referred to as cold, warm, and hot. The animals treated with the cold preservative were left in the cooler filled with ice until sampling was completed. All the eggs of the two *Daphnia* species present (*D. pulex* and *D. rosea*) were counted as released, unreleased, early stage, or later stage eggs (Edmondson 1955). *Daphnia pulex* was the dominant species, accounting for 97% of the eggs in the sample.

Although there is no difference in the average number of eggs released by animals preserved in warm or hot preservative, cold preservative reduces egg loss from that of the warmer treatment by 70% (Table 1). The ratio of the number of eggs retained to total eggs is 275:306 for the cold preservative and 446:677 for the combined data of the warm and hot preservatives. There is a slight positive correlation ($r = 0.47$, df = 16, $P < 0.05$) between the magnitude of the egg loss and the absolute difference between ambient lake and preservative temperatures. However this trend is caused by an experimental artifact—the treatment temperatures chosen—and disappears once the data from the cold treatment are separated from the pooled warm and hot treatment data (cold, $r = -0.31$, df = 4, $P > 0.05$; pooled warm-hot, $r = 0.34$, df = 10, $P > 0.05$). The cold preservative caused fewer eggs to be released regardless of the ambient lake temperature. The decrease in egg loss with cold preservative does not occur uniformly with all the animals, but is confined to eggs in the later stage of development (Table 2).

To find out whether my data were amenable to parametric statistical treatment, I applied a $\chi^2$ test for homogeneity of the binomial distribution (Snedecor and Cochran 1967) to the data in Table 1. These tests showed that the data collected on 20 July, but not on 13 July, conform to the binomial distribution; thus further parametric statistical treatment of these data must be interpreted cautiously. However statistical analyses can be used to give support to the apparent trends in Table 2. This has been done in Table 3 for the data from 20 July 1976 and shows, once again, that the only significant difference between treatments is with the later stage eggs.

It is impossible to compare the results given here with those of Haney and Hall (1973) because they gave no data on egg loss but only on carapace distortion. However animals showing almost no carapace ballooning can have lost eggs. Haney and Hall (1973) also used carbonated water to inactivate *Daphnia*, which may have effected egg loss. However, since they state that when the food habits of the animals are not to be considered, this step is not necessary. Thus I did not use $CO_2$ to inactivate the animals. Finally, one cannot rule out the possibility that

the animals behave differently depending on their nutritional state and on the species involved. However I found that the two species of *Daphnia* from Crawford Lake released fewer eggs and exhibited less carapace distortion, regardless of season, when they were preserved in a cold solution (6°C) of Formalin and sucrose. This technique should prove valuable in any study where fecundity of two or more coexisting species is to be assessed.

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[2] I was supported by a fellowship from the National Research Council of Canada while doing this research.

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*Submitted: 28 October 1976*
*Accepted: 20 September 1977*

*Limnol. Oceanogr.*, 23(3), 1978, 559–563


# Measurement of the vertical flux of particulate organic matter with a free-drifting sediment trap[1]

*Abstract*—Measurements of the vertical flux of particulate organic matter are usually made with moored sediment traps. In situ dye experiments in Buzzards Bay, Massachusetts, suggest that, in a current as low as 15 $cm \cdot s^{-1}$, the turbulent wake established over the entrance of such a device may severely bias estimation of both the quantity and quality of material settling through the water column, but that a free-drifting trap is not hampered by this problem. The free-drifting mode also permits shallow deployments in deep-water columns, without the problems accompanying deep moorings, and tracking of particular water parcels, such as upwelling plumes.

A major energetic link between planktonic and benthic ecosystems is the vertical transport of particulate organic matter synthesized in the plankton. A variety of devices has been used to study this flux; there are almost as many designs as there are investigators. Cylinders (White and Wetzel 1973; Davies 1975), boxes (Heim 1900; Wiebe et al. 1976), and cones (Pennington 1974; Oviatt and Nixon 1975) have all been used, the first being most popular. Cones, although they have the advantage of concentrating material derived from a large surface area into a relatively small collecting vessel, most certainly retain a fraction of the settling particles on their sloped walls.

Since the early studies of Heim (1900), attempts to measure vertical particulate flux in aquatic ecosystems have conventionally been made with moored sediment traps (e.g. Fuhs 1973; Webster et al. 1975; Hartwig 1976; Håkanson 1976). As is generally the case with rain gauges (Middleton 1943), the turbulence set up at the boundaries of fixed sediment traps can be expected to substantially influence both the quantity and quality of the material sampled. Complications arising from the presence of turbulence around the collecting device were recognized by Kleerekoper (1952). Kirchner (1975) evaluated the effect of turbulence on sediment trap performance by setting traps of different aspect ratio (i.e. height:diameter)

[1] Contribution 3835 of the Woods Hole Oceanographic Institution. This research was supported by the Woods Hole Oceanographic Institution/Massachusetts Institute of Technology Joint Program in Biological Oceanography.