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Effect of collection and acclimation period on grazing rates of limnetic zooplankton

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Abstract

Grazing rates of *Daphnia* sp. and *Diaptomus oregonensis* measured using an in situ method (Haney 1971) were compared with rates measured by collecting animals in vertical townets and placing them in experimental chambers either immediately or after a 24-h acclimation period. Experiments with acclimation yielded grazing rates of *Daphnia* that were statistically higher than in situ rates, whereas experiments conducted without acclimation yielded significantly lower rates. In situ grazing rates of *Diaptomus* were statistically higher than those for both townet techniques; experiments without an acclimation yielded higher rates than those for experiments with a 24-h acclimation period.

Introduction

Methods for measuring the grazing rates of naturally occurring zooplankton include in situ techniques such as those of Haney (1971) and Gliwicz (1969). In these techniques, animals are captured in a plexiglass chamber and experiments are conducted under natural conditions. In techniques that are not performed in situ, animals are first concentrated in vertical townets, and then transferred to experimental beakers either aboard the boat (Bogdan & McNaught, 1975) of in the laboratory (Richman, 1966; Cooley, 1977; Folt & Goldman, 1981; Vanderploeg, 1981). Grazing rates measured with such freshly caught animals are assumed to be more representative of animals in nature than those measured with zooplankton isolated in lakes and maintained in laboratory cultures before experimentation (e.g. Burns & Rigler, 1967).

In situ techniques have an advantage over non-in-situ techniques because collection of animals takes place in the water column (without the use of townets or screens) and animals receive relatively little stress compared with townet techniques. Since stress due to handling can produce hyperactivity or depressed feeding activities (Peters & Downing, 1984), *in situ* techniques should yield results that are more representative of the normal feeding activities of naturally occurring zooplankton, compared with non-*in-situ* techniques.

The disadvantage of in situ techniques, however, are several. They require specially designed grazing chambers that cannot be operated successfully without some skill; I found the performance of duplicate units constructed by the same person at the same place (University of Toronto Work Shop, Erindale College) to be such that one chamber could not be reliably operated (i.e. door of the food chamber does not open to release tracer cells) whereas the other functioned as it should 90% of the time. Experiments are also time-consuming and are inconvenient to carry out on board a small vessel or without adequate field assistance. These drawbacks probably explain why some investigators still use the townet rather than the Haney technique despite the demonstrated advantage of the in situ method (Haney, 1971, 1973).

Since the introduction of the Haney method, no study has been undertaken to compare the grazing rates of naturally occurring zooplankton obtained

by this in situ method with those obtained by methods based on townet collection of animals. Without this, there is very little basis for cross-study comparisons. In this study, I compare grazing rates obtained with townet techniques with those obtained with the Haney method for *Daphnia* and *Diaptomus,* two commonly occurring herbivores in North America. I will also examine the effect of an acclimation period on grazing rates since acclimation is assumed to reduce the stress produced by handling and collection.

Methods and materials

Study sites

The three study lakes are Three-Mile, Picard and Gull Lakes (see Chow-Fraser & Knoechel (1985) and Chow-Fraser (1985) for description of lakes). *Daphnia galeata mendotae* and *D. pulex* were obtained from Three-Mile L. and Picard L., and *Diaptomus oregonensis* were obtained from Gull L. for the laboratory experiments.

Study sites

I labelled three algal taxa with ^{14}C using the forced uptake technique (Holtby and Knoechel 1981). Maximum linear dimensions of *Chlorella vulgaris, Scenedesmus ovalis* and *Pediastrum* sp. were 6–8 μm, 4–6 μm, and 30–40 μm, respectively. Since tracer species ranging in size from 4 to 30 μm did not significantly affect the filtering rate of *Daphnia* (Chow-Fraser, 1985), filtering rate measured with *Chlorella* as tracer is representative of rates for *Daphnia* grazing on a large number of nanoplankton species. By contrast, grazing rate of *Diaptomus* dependend on the size of tracer cell used (Chow-Fraser, 1986); thus, both a small alga, *Scenedesmus,* as well as a large alga *Pediastrum,* were used in the diaptomid experiments.

The specific activity of *Chlorella* and *Scenedesmus* was 1 dpm/cell and the final concentration in the grazing vessels was 1 000 cells/mL. The specific activity of *Pediastrum* was calculated to be 57 dpm/cell and final concentration in the grazing vessels was 35 cells/mL. Amounts of tracer cells added to grazing chambers did not exceed more than 20% of the natural phytoplankton concentration.

Grazing experiments

In situ grazing experiments were performed with 2-L Haney (1971) chambers between 10:00 and 14:00. Feeding experiments were conducted at various depths throughout the epilimnion. Temperatures of the water in the grazing chamber ranged from 18 to 22 °C. Animals were allowed to feed at the depth of sampling for 15 min and were then collected on 64-μm-Nitex screen. The 15 min feeding period did not exceed gut passage time since grazing rates did not usually plateau until after 25 min of feeding (Chow-Fraser, unpub. data). They were subsequently killed with hot water (Chow-Fraser & Knoechel, 1985), rinsed with at least 1 L of filtered lake water and then stored frozen on dry-ice until they were sorted. Three replicate 5-mL aliquots of the labelled water in the grazing chamber were also collected to determine available food radioactivity.

In the second method, I collected zooplankton by hauling townets (150 μm mesh; mouth area 1590 cm^2; 70% efficiency) at approximately 30–35 m/min, vertically from the metalimnion to lake surface at approximately the same time (within 1 h of in situ experimentation) where in situ experiments were conducted. Animals were immediately emptied into a beaker, and transferred with the blunt end of a pasteur pipette to 1L of 64-μm-filtered lakewater that had been collected with a grazing chamber near the depth at which in situ experiments were conducted. This procedure took approximately 3 min. Animals were left in the beaker for approximately 15 min before tracer cells were introduced into the beakers with a 1-mL syringe. Animals were exposed to natural light (slightly overcast to sunny conditions on all sampling dates) during the short acclimation period and experimentation. The grazing period as well as subsequent handling of zooplankton for storage were identical to those reported for in situ experiments. To differentiate this technique from the in situ technique, I will hereon refer to it as the 'boat' method.

The water used in these 'boat' experiments were assumed to be identical to water in the in situ experiments since it was collected in the same locality. This assumption may not be met if the use of the 64-μm-Nitex screen (used to remove zooplankton from the water) differentially retained large net phytoplankton. In Three-Mile L., for example,

removal of filamentous blue-green algae (especially *Anabaena*) from lakewater was highly probable. This removal should favour higher grazing rates relative to those of in situ experiments since blue-green algae interfere with daphniid grazing rates (Burns 1968; Crowley 1973; Porter & McDonough 1984), and this will be considered when interpreting the data.

In the third method, I collected animals as in the 'boat' method, and emptied them into a 20-L plastic carboy containing approximately 10 L of 64-μm-filtered lakewater. Animals were transported back to a field laboratory and placed into 1-L experimental beakers within 4 h of collection. I also collected lakewater from the epilimnion where in situ experiments were conducted, filtered it through 64-μm-Nitex screens and transported it back to the laboratory for use in experiments. The temperature of the water in beakers approximated that of ambient lakewater (20 to 22 °C). Animals were left uncovered in experimental beakers for 24 h prior to experimentation. Since experiments were conducted in the laboratory at approximately 16:00, animals were exposed to normal light levels provided by overhead fluoresent lamps for part of the their acclimation period (16:00 to 22:00), darkness from 22:00 to sunrise and then remained in natural light until 16:00 when experiments were performed. All of the details of the grazing experiments performed in the laboratory were identical to those performed in situ and on the boat. I will refer to this third method as the 'lab' method.

In the 'lab' experiments, only animals that were actively swimming were used. Therefore, the size distribution of *Daphnia* in these experiments does not reflect the lake distribution, but rather that of animals that survived handling and transportation.

Densities of *Daphnia* in the in situ grazing experiments ranged from 6 to $17 \cdot l^{-1}$; that in the 'boat' and 'lab' experiments were 12 to $30 \cdot l^{-1}$, and $13 \cdot l^{-1}$, respectively. Densities of *Diaptomus* in the in situ experiments ranged from 3 to $4 \cdot l^{-1}$, and 7 to $10 \cdot l^{-1}$ in the 'lab' and 'boat' experiments.

Zooplankton processing

Zooplankton were sorted and identified with a dissecting microscope (Mag 25×). *Daphnia* were measured from the base of the tail-spine to the anterior most point of the carapace, along the long axis of the body (referred to as 'carapace length'). Adult female and male *Diaptomus* were used in the grazing experiments (prosome length 0.9 to 1.1 mm).

Individual *Daphnia,* placed in scintillation vials, were digested overnight in 0.25 ml NCS (Amersham) tissue solubilizer at 50 °C. *Diaptomus* were placed into vials in groups of 2 to 4 animals each and similarly digested. Radioactivity of the zooplankton was determined by liquid scintillation counting in OCS counting solution (Amersham). Daily filtering rate of *Daphnia* was calculated as follows:

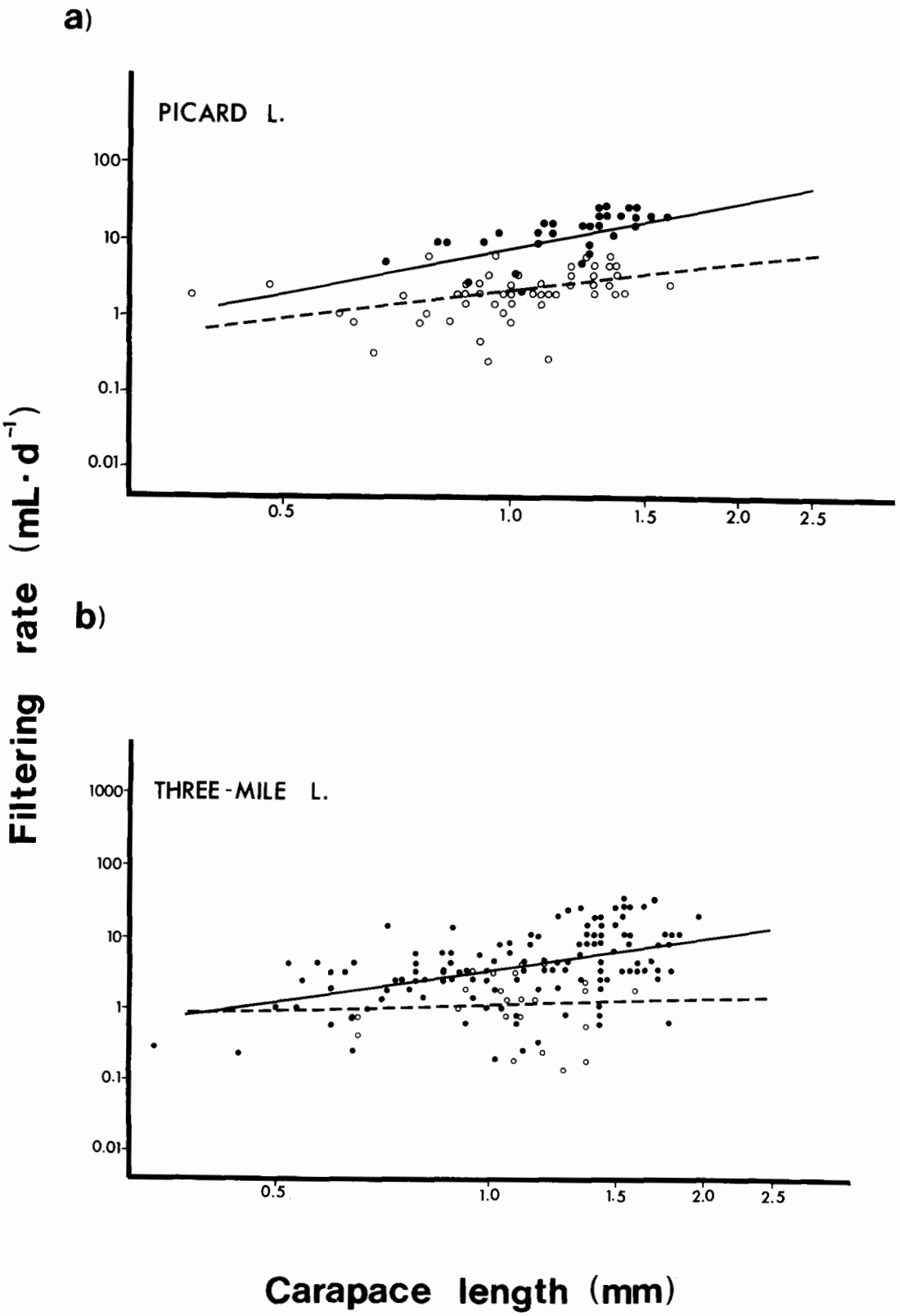
$$FR = \frac{\text{dpm in animal}}{\text{dpm per ml GC water}} \times \frac{1440 \text{ min} \cdot \text{d}^{-1}}{\text{min spent in the GC}}$$

(FR = filtering rate; GC = grazing chamber)

The clearance rate of *Diaptomus* was calculated as above except that mean dpm in animals were used.

Statistical analyses

Model I linear regression analysis (Ray, 1982; SAS) was used to relate filtering rate to carapce length of *Daphnia* for the in situ, 'boat' and 'lab' data for both Picard and Three-Mile L.; the slopes and intercepts of the equations were tested for statistical homogeneity by the Tukey-Kramer method and an analysis of covariance, respectively (Sokal & Rohlf 1981). Although the functional slope of the grazing rate data is most appropriately estimated by Model II regression analysis (see Chow-Fraser & Knoechel 1985), I used Model I regression analysis here because analysis of covariance and test of homogeneity of slopes have not yet been developed for Model II regression statistics (Ricker, 1984). For the comparison between 'lab' and in situ data for Three-Mile L., animals <1.0 mm in the in situ experiments were excluded because they were out of the size range of animals in the 'lab' experiments. I used the Kruskal-Wallis test and a non-parametric multiple comparison (Zar, 1984) to compare the mean grazing rates of *Diaptomus* among the 3 experimental conditions because distribution of the data did not conform to assumptions of parametric statistics.


a)
PICARD L.
100
10
1
0.1
0.01
0.5
1.0
1.5
2.0
2.5
b)
THREE-MILE L.
1000
100
10
1
0.1
0.01
0.5
1.0
1.5
2.0
2.5
Filtering rate (mL·d⁻¹)
Carapace length (mm)

Results

Daphnia

An analysis of covariance of Picard L. data indicates that for a given sized grazer, the filtering rate of *Daphnia* obtained in situ was significantly higher (by almost 4 times) than that for the 'boat' experiments (Fig. 1a; P=0.0001). Three-Mile in situ and 'boat' data were similarly compared; however, the slope for the 'boat' data was not significantly different from zero and no statistical comparisons were made (Fig. 1b). Nevertheless, the 'boat' data were generally lower than in situ data. In both lakes, differences were apparent between 'boat' and in situ rates for any specified size of *Daphnia,* indicating that this townet technique did not yield data comparable to those of the Haney technique.

I found a similar discrepancy between rates obtained with the Haney method and a method similar to my 'boat' method in Cooley (1977). He used freshly caught field animals, concentrated them in a pail, sieved and backwashed them into experimental beakers. Subsequently, he found that this technique underestimated Haney field measurements by up to 50%. He attributed this discrepancy between field and 'beaker' results to differences in seston abundance. By my calculations, however, the difference between his respective seston densities (numbers per litre) was only 500 cells/ml, representing less than 0.01% of the total seston density. I suggest that the extensive handling of *Daphnia* prior to his grazing experiments severely hampered the animal's ability to filter-feed (even after 30 min of acclimation), and resulted in his observed discrepancy between beaker- and Haney- measured grazing rates.

In another study, Bogdan & McNaught (1975) collected animals with a townet and placed them immediately in labelled food, a technique that is also similar to my 'boat' procedure. They reported daily grazing rates for *Daphnia* that ranged from 3.12 to 11.04 ml for animals ranging from 0.91 to 1.29 mm. Since their lake was described as oligotrophic (Bogdan & McNaught 1975), I have assumed that algal concentration in this lake was below the incipient limiting concentration, similar to Picard L. By comparison, animals of equivalent size range in Picard L. have in situ daily filtering rates that range from 7.9 to 17.33 ml, at least 50% higher than their reported rates. I suggest that their lower rates relative to mine may also reflect the stress of their collection and handling method on the filtering rates of *Daphnia.*

Results of the Three-Mile 'lab' experiment were statistically compared with in situ data for animals >1.0 mm (Fig. 2). The slope of the 'lab' regression is statistically homogeneous with that of the in situ data (P=0.6167; F-test). Intercept of the 'lab' regression, 0.75, however, is statistically higher than that of the in situ equation, 0.46 (P=0.001; t-test). Although these results can only be considered preliminary, they suggest that an acclimation of 24 h prior to experimentation will not only restore

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Fig. 1a. Filtering rate (FR) vs carapace length (L) for *Daphnia* in Picard L. experiments. Tracer species used is *Chlorella.* Solid line is the Model I regression line for in situ experiments conducted with Haney chambers (closed circles). Regression equation is:

$$\log FR = 2.25 \log L + 0.99$$

(r=0.4437; P=0.0001). Broken line is the Model I regression line for grazing experiments conducted on the boat with freshly caught zooplankton obtained from vertical net hauls (open circles). Regression equation is:

$$\log FR = 1.09 \log L + 0.27$$

(r=0.1540; P=0.0019).

Fig. 1b. Filtering rate vs carapace length for *Daphnia* in Three-Mile L. experiments. Tracer species used was *Chlorella.* Solid line is the Model I regression line for in situ experiments (closed circles). Regression equation is:

$$\log FR = 1.22 \log L + 0.47$$

(r=0.2000; P=0.0001). Broken line is the Model I regression line for the 'boat' experiments (open circles). Regression equation is:

$$\log FR = -0.02 \log L + 0.03$$

(r=0.0002; P=0.9823).

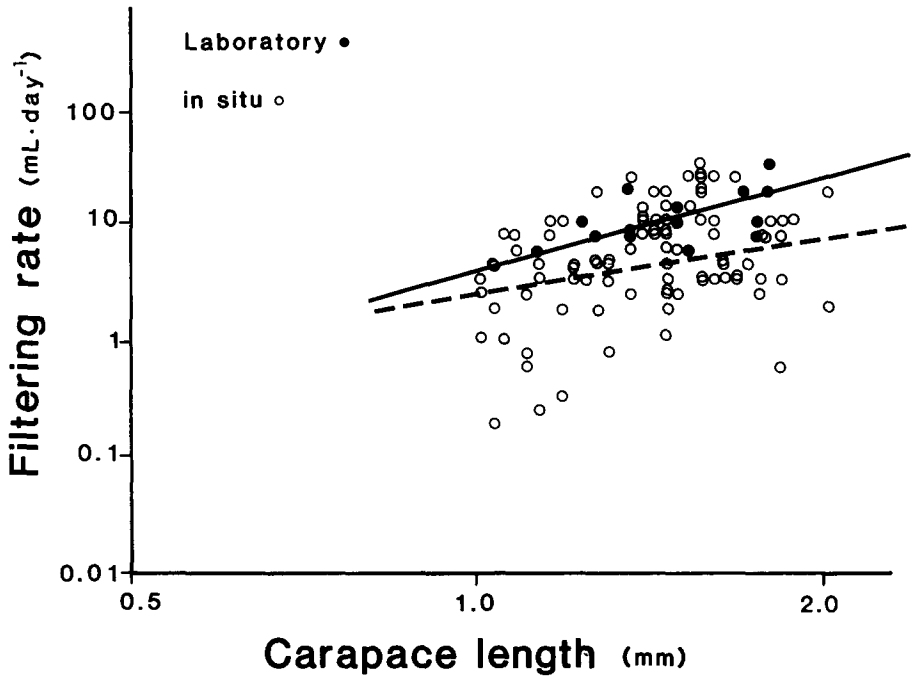

Fig. 2. Comparison of *Daphnia* filtering rates from Three-Mile laboratory and in situ experiments. Tracer species used is *Chlorella.* Broken line is the regression line through in situ data (open circles). The model I regression equation is:

$$\log FR = 1.27 \log L + 0.46$$

($r = 0.04$; $P = 0.04$). Solid line is the regression line through 'lab' data (closed circles). The Model I regression equation is:

$$\log FR = 1.78 \log L + 0.75$$

($r = 0.30$; $P = 0.05$).

feeding activities to the level obtained by the Haney technique, but possibly produce inflated grazing rates. It is possible that during the 24-h acclimation period, animals were able to substantially deplete the algae so that animals were 'starved', and responded with higher than normal filtering activities (Geller, 1975). This compensatory feeding should be considered if investigators plan to incorporate a long acclimation period in their laboratory grazing experiments.

In 'lab' experiments, animals smaller than 1.0 mm did not survive collection, transportation and handling. Most of these small animals were floating on the surface. Burns (1969) also found that early instars of light-bodied *Daphnia* species tended to become trapped in the surface film of the experimental water. Investigators should be aware that handling and collection may severely bias the size distribution in favour of large *Daphnia* in similar types of laboratory experiments.

Diaptomus

I compared the grazing rates of *Diaptomus* for the two tracer species separately. In each case, the 'boat' technique produced grazing rates that were statistically lower than those of the in situ method ($P < 0.01$; Fig. 3). This is consistent with the observations of *Daphnia* (Fig. 1a and b) except that the 'lab' method produced the lowest rates for *Diaptomus* whereas it produced the highest rates for *Daphnia* (Fig. 2).

Discussion

The townet technique without an acclimation period produced grazing rates for naturally occurring *Daphnia* that were statistically lower than those of the Haney technique (Fig. 3). Similarly, grazing rates of *Daphnia* from the townet tech-

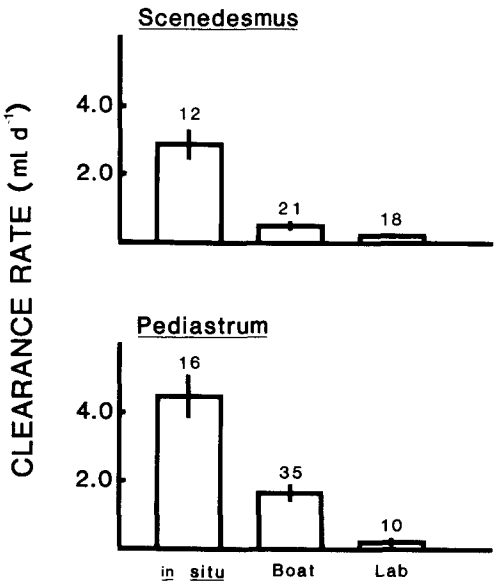

Fig. 3. Mean clearance rate of *Diaptomus oregonensis* from Gull L. Vertical bars are ±1 S.E. of the mean. Numbers above bars are the number of grazing rates measured. Tracer species used in experiments is indicated at the top of each panel.

nique without acclimation were also statistically lower than in situ data (Fig. 1a and b).

Compared with the townet technique, the Haney procedure involved the least amount of shock to animals during collection since they were never handled with filters, pipettes, etc. as in the case of the 'boat' and 'lab' procedure. It is reasonable to interpret the differences between townet and in situ techniques as the effect of handling and collection. Therefore, the lower 'boat' and 'lab' rates for *Daphnia* and *Diaptomus* suggest that animals were negatively affected by handling.

In this study, I tried to control for variables other than handling and acclimation time; however, factors such as ambient light intensity and algal concentration and composition may have been variable between treatments (see Methods and Materials) and may have affected the results. In Three-Mile L, for example, the suspected differential removal of blue-green filaments from lakewater used in the 'boat' experiments should have favoured higher grazing rates relative to in situ data; instead, 'boat' grazing rates were uniformly lower than in situ rates. Thus, even if inequality of food concentrations in experimental treatments had an effect, it did not obscure the effects of handling and acclimation time. It is true that ambient light levels were also variable among treatments, and zooplankton may have responded negatively to increased light intensity in the 'boat' experiments, and this should be further investigated.

Although I used an acclimation period of 24 h, *Daphnia* probably require a much shorter period to recover from handling effects, as suggested by the lack of a consistent effect of acclimation period on the filtering rate of laboratory-cultured *Daphnia.* The grazing rate associated with an acclimation period of 45 min (Burns, 1969) was similar to that associated with 60 min (Geller, 1975) or 3 h (DeMott, 1982). Since differences due to species and growth conditions may obscure any real effect of acclimation period, I suggest that more studies be conducted to examine changes in grazing rates for various acclimation times.

Diaptomus does not seem to recover from the shock of handling since grazing rates after 24 h acclimation were still statistically lower than in situ rates. The fact that 'lab' data were also statistically lower than 'boat' data suggest that an acclimation period had an adverse instead of ameliorating effect. This is consistent with my finding that the grazing rate associated with long acclimation periods (i.e. Richman, 1966; McQueen, 1970) were considerably lower than those associated with no or only a short acclimation period (i.e. Bogdan & McNaught, 1975; Vanderploeg, 1981; Folt & Goldman, 1981). This is also consistent with the observations of Peters & Downing (1984) that experimental duration was negatively correlated with grazing rates of calanoid copepods.

Diaptomus that were subjected to a short acclimation period after handling grazed at higher rates than when subjected to a long acclimation period. This contrasts the observation that *Daphnia* grazed at lower rates after handling when only a short acclimation period was given. This suggests that *Diaptomus* was not as sensitive to handling as it was to containment, whereas *Daphnia* was more sensitive to handling but was unaffected by containment. The difference in response to handling and containment for these two taxa probably reflects differences in their swimming behaviour and modes of food collection.

In conclusion, rates obtained with the townet techniques are different from those of the Haney

method. Investigators working with naturally occurring zooplankton should take into account the effect of collection, handling and acclimation period on the grazing activities of these animals. Since *Daphnia* and *Diaptomus* do not respond similarly to these effects, the townet techniques are inadequate substitutes for the Haney method if grazing rates of both taxa are needed simultaneously.

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