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Ammonium release by zooplankton in suspensions of heat-killed algae and an evaluation of the flow-cell method

Henry A.Vanderploeg, Gwenyth A.Laird, James R.Liebig and Wayne S.Gardner

National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 2300 Washtenaw Avenue, Ann Arbor, MI 48104, USA

Abstract. The maximum excretion rate of NH_4 (39 nmol mg dry wt $^{-1}$ h^{-1}) was directly measured for *Daphnia pulex* by measuring NH_4 accumulation in bottles containing *D. pulex* and dense, satiating suspensions of heat-killed algae. Ammonium release rates in the algal suspensions were compared to those of individual animals removed from the suspension and placed in flow cells. Ammonium release rate, R (nmol mg dry wt $^{-1}$ h $^{-1}$), in the flow cell decreased very rapidly with time, t (min), after removal according to the relation $R = 26 + 25e^{-0.16t}$. Ammonium excretion obtained by the flow cell method after extrapolation to time zero was not significantly different from that obtained in the bottles. The considerable experiment-to-experiment variation in NH_4 excretion was in large part correlated ($r^2 = 0.73$) with the feeding rate on the algae.

A large percentage of nitrogen excretion in zooplankton and other animals can result from immediate catabolism of ingested proteins (e.g. Brody, 1945; Warren and Davis, 1967; Corner *et al.*, 1972; Lehman, 1980; Gardner and Scavia, 1981; Bidigare, 1983; Roman, 1983). Thus nitrogen excretion should be measured as a function of ingestion rate of food. For herbivorous zooplankton, nitrogen excretion has never been directly measured correctly and unambiguously for zooplankton feeding in suspensions of their food, algae, because of immediate sequestering of excreted nitrogen by the algae (e.g. Takahashi and Ikeda, 1975; Lehman, 1980; Gardner and Scavia, 1981). Two promising approaches to circumvent this problem have been those of Lehman (1980) and Gardner and Scavia (1981). Potential difficulties of the Lehman (1980) method are the great labor and skill requisite for determining both algal and zooplankton nutrient kinetics and the assumptions implicit in the model that describes interactions among nutrients, algae and zooplankton. In the Gardner and Scavia (1980) flow-cell method, a zooplankter is taken from its suspension of food and placed in a small (0.05 ml) flow cell, where nutrient accumulation is measured at 10-min intervals over time. Nutrient release rate in the food suspension is estimated by extrapolation back to time zero because it decreases with time after removal of the organism from food. This method would appear to be ideally suited for determining nitrogen excretion for individuals but not as useful for populations since many individuals' rates must be measured to get a population estimate. Potential artifacts in this method for some zooplankton could be (i) handling and containment effects and (ii) differing release patterns in the presence and absence of food that cannot be corrected by extrapolation back to time zero.

As an alternative to these methods, we proposed that excretion rate could be directly determined by measuring nitrogen accumulation in bottles containing zooplankton and suspensions of heat-killed phytoplankton. In this paper we apply this approach to determine the rate of NH_4 excretion and its relation to feeding rate of *Daphnia pulex* in

dense, satiating concentrations of algae. In addition, we evaluate the assumptions implicit in the Gardner and Scavia (1981) method by comparing NH_4 excretion of zooplankton in suspensions of heat-killed algae with that of zooplankton in the flow cell after their removal from the suspensions.

Methods

Analytical techniques

Ammonium and amino acid concentrations were determined by microfluorometric methods (Gardner, 1978; Gardner and Miller, 1981). The flow-cell method and measurement of NH_4 and amino acid release by zooplankton in the flow cell are described by Gardner and Scavia (1981). To measure NH_4 and amino acid concentrations in algal suspensions, 1-ml subsamples were taken in separate prerinsed disposable syringes and injected into the ammonium analyzer through a microsample-filtering device (Gardner and Vanderploeg, 1982).

Carbon concentrations of algae were determined on an Oceanography International Carbon Analyzer (nondispersive infrared, CO_2-sensitive) following wet oxidation by potassium persulfate and phosphoric acid (Menzel and Vaccaro, 1964) at 95°C for 4 h in sealed precombusted ampoules (Oceanography International Corp., 1978). Algae were concentrated on precombusted (400°C, 4 h) 25 mm Gelman A/E glass fiber filters.

Assumptions of heat-killing method

Proteins and nucleic acids are the major components of cellular nitrogen, and of these, protein accounts for 70–90% (Wheeler, 1983). Ammonium is the major end product of protein and nucleic acid catabolism in zooplankton (e.g. Corner *et al.*, 1972, 1976; Harris, 1973; Gardner and Miller, 1981; Bidigare, 1983). The assumptions of the method are that phytoplankton freshly killed by short exposure to an increase in temperature would: (i) not take up or release NH_4; (ii) have the same chemical composition as live cells; and (iii) be eaten by zooplankton. Moreover, the killed algae should ideally be eaten at the same rate as live algae to facilitate extrapolation of nitrogen excretion from a suspension of killed cells to a suspension of live cells of the same concentration. For filter feeders, like cladocerans, feeding rate depends primarily on the particle-size spectrum of food, assuming it is palatable (e.g. Christensen, 1973; Porter *et al.*, 1983). Thus another requirement of the method is that the particle-size spectrum of algae used as food be unaffected by the heat killing.

Rapid heat-killing of Cyclotella

Cultures of *Cyclotella meneghiniana* (Culture CYOH2 of S.S.Kilham), grown in unbuffered WC medium (Guillard and Lorenzen, 1972) at 15°C at a light intensity of ~70 microeinsteins m^{-2} s^{-1} on a 16:8 L:D cycle, were centrifuged in 300-ml tubes at 2000 revolutions min^{-1} and the supernatant siphoned off to produce ~15 ml of concentrated suspension. Enough hot (85–95°C), double-strength NOPN medium (Lehman, 1980), DNOPN, was added to the concentrate to bring it to 65°C. The suspension was held at that temperature in a water bath for 5 min to ensure that the cells were killed. Then the suspension was quickly cooled to 20°C by adding the appropriate

amount of cold (5 – 10°C) DNOPN medium. To remove any traces of NH_4 or amino acids that may have been in the culture or may have leaked from the cells during heat killing, the cells were centrifuged and washed once or twice in DNOPN medium.

Experiments showed that the implicit assumptions of the method were correct. Heat killing resulted in no release of NH_4 and only insignificant trace amounts of amino acids that were removed by washing. Washed, heat-killed cells suspended in DNOPN medium neither released amino acids nor took up NH_4 over 24 h at 20°C. Carbon concentration after heat killing [$\bar{x} \pm SE(4) = 2.95 \pm 0.23 \times 10^{-7}$ $\mu g\ \mu m^{-3}$] was not significantly different from that of live *Cyclotella* [$\bar{x} \pm SE(4) = 2.89 \pm 0.34 \times 10^{-7}$ $\mu g\ \mu m^{-3}$]. Coulter analyses (Model TA II Coulter counter) showed that the size spectrum of *Cyclotella* was unchanged by heat-killing and centrifugation 24 h after killing. This was not true for the green algae tested (*Scenedesmus*, *Chlamydomonas* and *Chlorella*), which tended to clump. Microscopic examination of *C. meneghiniana* 24 h after heat-killing showed that the cells remained intact; however, they had lost some of their green color and had a slightly brown tint.

Zooplankton culture

Stock cultures of *D. pulex* were grown under continuous light at 20°C in WC medium with phosphate at one-half strength and TES [*N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid] at 80 mg l^{-1} as buffer (P.Rago, personal communication). *Chlamydomonas reinhardti* served as food. For 1 week before the experiments, selected *D. pulex* were well fed by maintaining algal concentrations at ~3 – 10 $mm^3\ l^{-1}$ [the incipient limited concentration was ~1.2 $mm^3\ l^{-1}$ (Geller, 1975)]. This was done to ensure that the animals would be healthy and physiologically adapted to feeding in dense algal concentrations.

Ammonium excretion experiments

Daphnia pulex were preconditioned for 1 day with heat-killed algae prior to the experiment by placing ~15 of them in a 1 l bottle of a suspension of heat-killed *C. meneghinana* at the same initial concentration (5 – 15 $mm^3\ l^{-1}$) to be used in the experiment the next day. The bottles were rotated on a wheel at 0.5 r.p.m. in continuous dim light at 20°C during the preconditioning period and the experiments that followed.

Just prior to the experiments, a suspension (500 ml) of freshly heat-killed *C. meneghiniana* was made in DNOPN. Animals were sorted according to size, egg number and development stage. If possible, females carrying early stage embryos were used. This was done to avoid low feeding rates (Christensen, 1973) and low nutrient release rates (Scavia and McFarland, 1982) in females carrying stage III embryos. It also precluded the elevated nutrient release rates found during ecdysis (Scavia and McFarland, 1982).

The experiments to measure NH_4 release and feeding in bottles were conducted like traditional feeding experiments (e.g. Frost, 1972; Vanderploeg, 1981, Vanderploeg *et al.*, 1984) with added ammonium measurements. The heat-killed algal suspension was poured among seven 28-ml bottles: two served as initial-concentration controls, two served as final-concentration controls and three, each containing 1 – 3 *D. pulex*, served as experimental bottles. Before pipetting the animals into the experimental bot-

tles, they were pipetted first into a 250-ml beaker of DNOPN to minimize carry-over of ammonium from the prefeeding bottle. Final selection of animals for an experiment was done during this rinse stage. Both algal and ammonium concentrations were measured at the beginning and end of the 4-h experiment in a manner appropriate to traditional experimental design. In addition, initial concentration of NH_4 was also determined in the experimental bottles immediately after addition of animals to detect traces of NH_4 pipetted in with the zooplankton and possible spurts associated with handling. A TA-II Coulter counter was used to measure particle concentration (Vanderploeg *et al.*, 1984). Frost's (1972) equations were applied to each channel of the Coulter counter to determine feeding rate as total volume of particles consumed (Vanderploeg, 1984). This measure, net feeding rate (Vanderploeg, 1981), corresponds to assimilation rate [= digestion rate (Christensen, 1973)]—i.e. particle volume ingested minus that egested—for cladocerans since they produce diffuse feces that would be recorded by the Coulter counter.

The time period between making initial and final measurements on the 4-h duration bottle experiments allowed us to make sequential 1-h long flow-cell time histories on three animals. To provide feeding conditions for flow-cell animals identical to those in the bottle experiments, one animal was taken from each of the three bottles filled with the same concentrations of heat-killed algae and animals as in the bottle experiments. Animals in the bottle and flow-cell experiments were paired as much as possible with respect to size and embryo stage and number. In all experiments, algal concentration was above the incipient limited concentration.

To evaluate the physiological conditions of the animals the L-O (lipid-ovary) index (Tessier and Goulden, 1982), a semiquantitative measure of lipid deposition, was recorded at the end of all but one experiment along with embryo number. Both mean number of embryos per female and the L-O index were high: $\bar{x} \pm$ SD for embryos per female was 12.1 ± 9.3 for animals having a mean (±SD) length exclusive of tailspine of 2.6 ± 0.3 mm; the $\bar{x} \pm$ SD for the L-O index was 2.4 ± 1.3, a very high value (Tessier and Goulden, 1982). Variability in the L-O index is expected since fat deposition and ovary development of the female increase with age of the embryos carried. All females carrying older embryos [Stage II or III of Stross *et al.* (1961)] had an L-O index greater than 2. Thus, the L-O index indentified no individuals that were in poor physiological condition.

Predicted feeding rate and weight of animals

The feeding rate-length relation for 20°C found by Geller (1975) for *D. pulex* feeding on small algae at concentrations above the incipient limiting concentration was used to predict feeding rate (μm^3 animal^{-1} h^{-1}) of individual *D. pulex* on live *C. meneghiniana* for comparison with observed feeding rate on heat-killed *C. meneghiniana*.

To normalize NH_4 excretion rates to dry weight, Lampert's carbon content–length relation [given in Geller (1975)] was used to convert length to carbon content. Dry weight was assumed to be 2.5 × carbon content. This method was used instead of direct weight measurement for two reasons. First, it allowed comparison with a few experiments for which dry weights were not obtained. Second, use of this relation smooths out differences in weight caused by varying lipid contents and embryo number. Lipids do not contribute to the animal's NH_4 excretion, and embryos, which do not feed, may

have a lower weight-specific NH_4 excretion rate. Mean dry weight of our animals [0.124 ± 0.008(30) mg] was nearly identical to that predicted by Lampert's relation [0.127 ± 0.010(28) mg].

Results

Flow-cell time trends

Time histories of individual *D. pulex* in the flow cell for each experiment are shown in Figure 1 along with results from the bottle experiments for comparison. Mean results for all experiments, whether with fed or starved animals, are shown in Figure 2. Gardner and Scavia (1981) showed that, for the mean results of many experiments with individual *Daphnia magna*, ammonium release (E_t) in the flow cell followed the empirical relation with time (t)

$$E_t = E_m + E(0)_f \exp(-kt), \tag{1}$$

where E_m = steady-state release due to endogenous ammonium excretion, $E(0)_f$ = release of ammonium from digested and metabolized food at time zero (at time of removal from food) and k = coefficient relating the decrease to time. Moreover, they

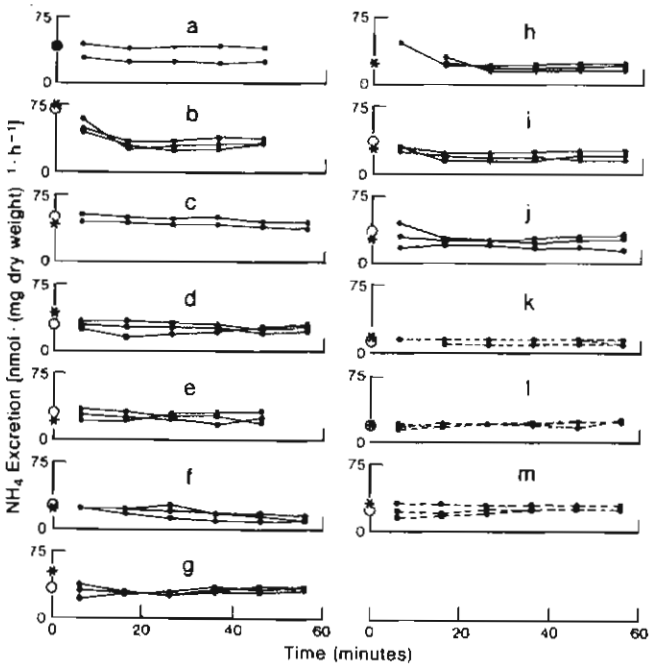

Fig. 1. Time histories of NH_4 excretion by individual *Daphnia pulex* (lines connecting dots) in the flow cell for experiments run on different dates. Each graph (labeled by a different letter, a–m) represents an experiment run on a particular date between March 1982 and August 1983. Solid lines connecting points represent results for animals fed above the incipient limiting concentration; dotted lines indicate results for animals starved for 1–2 days. The open circles on the ordinates are the time-zero extrapolations from the mean values of NH_4 excretion for the first two time points. The asterisks on the ordinates show the corresponding mean values of ammonium excretion determined in bottles containing the heat-killed algae.

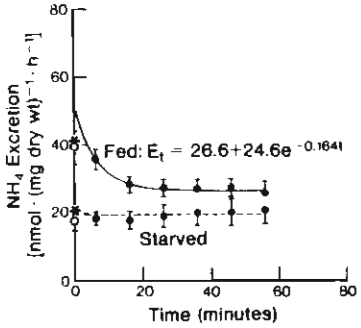

Fig. 2. Upper curve: Mean ($\pm$SE, n = 10) time history of ammonium excretion in the flow cell experiment for *Daphnia pulex* fed above the incipient limiting concentration. The open circle ($\pm$SE) on the ordinate is the mean of the extrapolated estimates of time-zero excretion for individual experiments. The asterisk is the time-zero extrapolation determined from the mean results for the first two time points. The solid line is the fit of equation (1) to the data points (from time $\geq$6 min). **Lower curve:** Time history for experiment ($\pm$SE, n = 3) in which animals were starved for 1 or 2 days. The dotted line shows mean results of all time ($\geq$6 min) points. Symbols are the same as those on the upper curve.

suggested that excretion in the flow cell could be extrapolated back to time zero by using data from the first two time points. Equation (1) was fitted by nonlinear regression to the mean results for fed animals in Figure 2. In addition, exponential extrapolation of results for 6 and 16 min was used to do the time-zero extrapolation for the mean of replicates of each experiment with fed *D. pulex* in Figure 1 and for corresponding overall mean results in Figure 2.

Equation (1) appears to give a reasonable fit to the mean results for fed animals (Figure 2). Flow-cell trends for starved *D. pulex* showed relatively constant values over time (Figures 1 and 2), as expected. However, it is unlikely that the experiments with fed animals were run long enough to provide a good estimate of E_m because results for the starved animals are considerbly lower. The mean of all time points for the starved animals is significantly lower than the mean of the last four time points of the fed animals (Figure 2) at the $P < 0.01$ level. Gardner and Scavia (1981) took the ammonium excretion rate obtained for *D. magna* at 70 min in the flow cell to be a reasonable estimate of E_m since ammonium excretion in starved animals was the same; however, since their animals were starved for only 3–5 h, both estimates may have been too high. Ammonium excretion rate by the marine copepod *Eucalanus pileatus* continued to decrease in the flow cell during the 6-h time interval it was measured (Gardner and Paffenhöfer 1982).

For fed *D. pulex*, many individual flow-cell time histories (Figure 1) show a rapid initial decrease in ammonium excretions followed by a leveling off with time that is consistent with equation (1). However, not all animals exhibited this pattern, and there was considerable variation among experiments run on different dates in both absolute amount of ammonium excretion and shape of the curve (Figure 1). Random-effects analysis of variance (e.g. Scheffé, 1959, pp. 221–227) of ammonium excretion at each time point showed that variance [using the VARCOMP proeedure developed at SAS (SAS Institute Inc., 1982)] among experiments run on different dates was significantly greater ($P < 0.01$) than that for replicates within experiments.

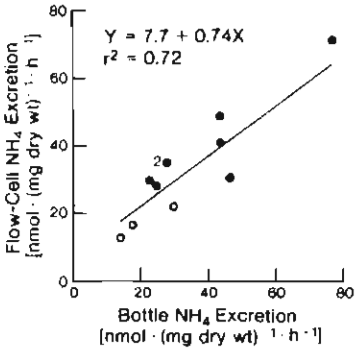

Fig. 3. Linear regression of flow-cell ammonium excretion at time zero versus bottle ammonium excretion The closed circles indicate results for fed animals and the open circles indicate results for starved animals

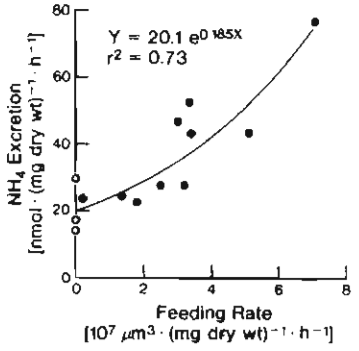

Fig. 4. Fit of exponential equation to excretion rate versus feeding rate data (closed circles) for *Daphnia pulex* fed above the incipient limiting concentration. For comparison, excretion rates of starved animals (open circles) are plotted.

Bottle and flow-cell comparison

Because of the considerable variation in the excretion versus time response of individual animals, which could not be described by equation (1), equation (1) could not be used to make a meaningful estimate of the time-zero excretion rate from the mean results (of two to three replicates) of an experiment. Instead, the mean results of the 6- and 16-min observation times were extrapolated back to time zero by assuming exponential decrease with time. Figure 1 shows that the flow-cell results compared well with that of the bottle results. Respective means [$\pm$SE(N)] for all bottle and flow cell experiments were 38.7 $\pm$ 5.4(10) and 39.2 $\pm$ 4.5(9) for fed animals, and 20.4 $\pm$ 3.5(3) and 19.2 $\pm$ 1.9(3) for starved animals. Because we carefully paired our bottle and flow-cell experiments, and because there was considerble variation among experiments, a paired t test was used to compare flow-cell (F) and bottle (B) excretion rates. The paired t test, done both on the difference $F-B$ and on $(F/B)-1$, indicated no significant differences ($P > 0.05$) between the two methods. The time at which the flow-cell

Table I. Carbon assimilation rates, N assimilation rates (A) and NH_4 excretion rates (E) of well-fed (i.e above incipient limiting concentration) and starved *D. pulex* expressed as percentage of body content of C or N

Experiment	Carbon assimilation rate (% C day $^{-1}$)	A (% day $^{-1}$)	E (% day $^{-1}$)	A/E
Starved	0	0	7.01	0
Fed	57.8	41.6	13.3	3.12

Assumptions: (i) carbon content of zooplankton = 0.4 × dry weight; (ii) C/N mass ratio of diatoms = 5.70 [from mean for species reported by Parsons *et al.* (1961)]; and (iii) C/N mass ratio of zooplankto = 4.1 [from zooplankton of Buzzards Bay (Roman, 1980)].

results first became significantly different ($P < 0.05$) from the bottle results was showr by paired t tests to be 16 min. By 16 min, excretion rate had fallen to 28.19 nmol mg dry wt^{-1} h^{-1}, a value 73 and 72% of the respective values of the bottle results anc the time-zero extrapolations. Linear regression of time-zero extrapolation of flow cel results versus bottle results corroborated results of the paired t tests (Figure 3); the intercept was not significantly ($P > 0.05$) different from zero and the slope was no significantly ($P > 0.05$) different from 1.

Ammonium excretion and feeding rate

We were curious whether the broad range of ammonium excretion values for fed animals in Figure 3 was correlated with feeding rate. Consequently, excretion rate versus feeding rate data were plotted in Figure 4. An exponential model was arbitrarily chosen to fi the pattern of the data. The r^2 value of 0.73 for the fit shows that ammonium excretion rate increases with feeding rate. Although there was considerable variation in feeding rate among experiments, the mean feeding rate for heat-killed algae was nearly identical to that predicted by Geller's (1975) equation for live algae; respective values were $3.10 \pm 0.605(10) \times 10^7$ and $3.00 \pm 0.189(10) \times 10^7$ μm^3 mg dry wt^{-1} h^{-1}. Thus the variability in feeding rate does not appear to be a function of hypothetically varying food quality of different batches of heat-killed algae. As expected, there was no correlation between feeding rate and algal concentration, which was above the incipient limiting concentration.

The data were examined further to determine if either weight-specific feeding rate or weight-specific NH_4 excretion was related to stage of embryos carried (e.g. Christensen, 1973), embryo number (Zánaki, 1983) or animal weight. Neither mean weight-specific feeding rate nor mean weight-specific NH_4 excretion rate varied significantly among females carrying state I, II or III eggs. Linear regression showed no correlation for either weight-specific feeding rate or weight-specific NH_4 excretion rate with egg number per unit weight. This last result is not surprising because the L-O index, which was very high for our animals, is a better monitor of physiological condition in the recent past than is egg number (Tessier and Goulden, 1982). Linear regression showed that both weight-specific feeding rate and weight-specific NH_4 excretion were negatively correlated with animal weight. Although these regressions were significant ($P < 0.05$), respective r^2 values for the feeding-rate and NH_4-excretion regressions were only 0.22 and 0.23. Possibly age rather than weight could be a factor

operating here, since some of the larger animals used in the study were very larg (~3 mm) relative to the expected maximum size of *D. pulex*.

At the satiating algal concentration used in our experiments, assimilation of both C and N was very high relative to their respective body contents (Table I). Assimilation/exeretion of N was 3.12, a value that implies that most of the amino acids assimilated are used for protein synthesis rather than being deaminated for fat synthesis or catabolized. The observation that the NH_4 excretion of the fed animals is less than twice tha of starved animals implies a relatively large endogenous excretion component. A large endogenous excretion component of similar size was also reported for the marine copepod *Eucalanus pileatus* (Gardner and Paffenhöfer, 1982).

Discussion

The excellent agreement between NH_4 excretion for time-zero extrapolations of the flow cell and those in botles validates the flow-cell method, at least for *D. pulex*. Thus the rapid decrease (27% in 16 min) in NH_4 excretion with time subsequent to removal from the algal suspension is not a handling artifact, but represents, as Gardner and Scavia (1981) claimed, a physiological response to removal from food. Apparently this conclusion cannot be extended to all zooplankton. Gardner and Paffenhöfer (1982) reported that release rates in the marine calanoid copepod *E. pileatus* were elevated during the first 10-min interval after removal from the feeding suspension and then decreased slowly during the next four 10-min intervals in the flow cell. Since this drop was also observed for animals removed from filtered seawater, it must have been due partly to handling.

One goal of this study was to determine maximal NH_4 excretion rate by well-fed animals. In Table II, estimates of NH_4 excretion per unit dry weight by *Daphnia* spp. are compared. Variable lipid composition, expected for *Daphnia* of different feeding histories (e.g. Tessier and Goulden, 1982; Tessier *et al.*, 1983), is a major difficulty in comparing NH_4 excretion among the studies. Lipids, although a major contributor to dry weight, are not part of the 'metabolic' weight of the animal in that they are not living cells that respire or excrete N. Vidal and Whitledge (1982) recently underscored this point by showing that the metabolic rate of zooplankton is better normalized to lipid-free dry weight than dry weight for comparing taxa of different lipid contents. Either lipid-free dry weights or N contents would be suitable for comparisons of NH_4 excretion (e.g. Paffenhöfer and Gardner, 1984). To facilitate comparisons in Table II, we have normalized NH_4 to measured dry weight and to dry weight predicted from lengths using Lampert's (Geller, 1975) dry weight versus length relation for well-fed *D. pulex*. Lehman's (1980) NH_4 excretion rate for algae grown under nutrient sufficient conditions is twice our result when excretion is normalized to measured dry weight. However, when these results are normalized to Lampert's predicted dry weights, Lehman's results are lower than ours. Such a comparison is not possible with Gardner and Scavia's (1981) and Scavia and Gardner's (1982) results since they did not measure animal length. Another source of variation may be the number of embryos carried by *Daphnia* spp. The contribution of embryos to NH_4 excretion is not known.

Much (73%) of the considerable experiment-to-experiment variability we observed was correlated with feeding rate. This variability has also been seen for both NH_4 and

Table II. Ammonium excretion by *Daphnia* spp. feeding in suspension of food normalized to measured dry weight and to dry weight predicted by Lampert's length-weight relation (Geller, 1975)

Species	Reference	Food conditions	Temperature (°C)	Ammonium excretion (nmol mg dry wt $^{-1}$ h^{-1}) Using measured dry wt	Using predicted dry wt
D. pulex	Lehman (1980)	live algae, nutrient sufficient	20	82.7 ± 13.6	31.4 ± 5.2[a]
D. pulex	Lehman (1980)	live algae, nutrient deficient	20	35.9 ± 4.7	13.64 ± 1.8[a]
D. pulex	Gardner and Scavia (1981)	bacteria–soy flour mixture	24–26	43 ± 5(6)	–
D. pulex	Present study	heat–killed algae, satiating concentration	20	39.1 ± 4.4(10)	38.7 ± 5.4(10)
D. magna	Gardner and Scavia (1981)	bacteria–soy flour mixture	24–26	41 ± 5(8)	–
D. magna	Scavia and Gardner (1982)	microbe–soy flour mixture	20	80.5 ± 12.4(12)	–

[a]Length of animals was approximated by the mean (1.95 mm) of reported length range (1.7–2.2 mm). The weight predicted was 52 μg, in contrast to the 20 μg measured. Animals were feeding at approximately half the maximum rate predicted by Geller's (1975) equations.

PO_4 excretion by *D. magna* subsequent to their removal from suspensions of live *C. reinhardti* (Scavia, Vanderploeg and Gardner, unpublished data). The challenge of the future will be to determine whether this variability—both that portion correlated with feeding and that portion not—is caused by improper body weight measures, excretion by embryos, age or subtle long-term feeding history effects not controlled for in our experiments.

Measurement of NH_4 accumulation in suspensions of heat-killed algae is an effective method of exploring the relation between NH_4 excretion and food ingestion rate. The heat-killed algae were eaten at the same rate as predicted for live algae, and the algae did not take up or lose NH_4 or significant quantities of amino acids during a 24-h period. It is possible the heat-killed algae could have a different odor quality or strength that could affect their capture rate by certain zooplankton since both marine and freshwater calanoid copepods actively capture large algae in response to the phycosphere of odor around them (e.g. Alcaraz *et al.*, 1980; Koehl and Strickler, 1981; Paffenhöfer *et al.*, 1982; Vanderploeg and Paffenhöfer, 1985). Even if this were the case, NH_4 excretion could still be related to N actually ingested. Our method is not limited to pelagic zooplankton. It should be able to be applied to any consumer of phytoplankton or detritus, including benthic detritivores.

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