Estimating the Grazing Impact of Marine Micro-zooplankton *

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Abstract

This paper describes a dilution technique for estimating the micro-zooplankton grazing impact on natural communities of marine phytoplankton. Experiments performed in coastal waters off Washington, USA (October, 1980), yielded estimates of micro-zooplankton impact equivalent to 6 to 24% of phytoplankton standing biomass and 17 to 52% of production per day. Indirect evidence suggests that most of this impact is due to the feeding of copepod nauplii and tintinnids; in contrast, non-loriculate ciliates, comprising 80 to 90% of numerical abundance, appeared to contribute little to phytoplankton mortality.

Introduction

The micro-zooplankton is the component of the marine plankton consisting of Protozoa and Metazoa which pass a 200 μm mesh screen (Dussart, 1965). These organisms are individually inconspicuous, rarely dominate zooplankton biomass, and, consequently, receive less attention than larger zooplankton; even so, their role in marine food webs may be significant since, by virtue of small size, they have disproportionately high specific rates of growth, metabolism, and feeding (Zeuthen, 1947; Johannes, 1964; Fenchel, 1974; Heinbockel, 1978a). Moreover, micro-zooplankton generally feed on the smaller sizes of particulates, which are not utilized efficiently by large consumers; thus, the micro-zooplankton, as trophic intermediates, make the considerable production of nanoplankton accessible to higher order consumers (Beers and Stewart, 1967; Parsons and LeBrasseur, 1970; Berk et al., 1977).

The magnitude of the micro-zooplankton grazing on marine phytoplankton has usually been estimated indirectly from production budgets of phytoplankton (e.g. Riley, 1956) and energetic requirements of organisms based on size (e.g. Beers and Stewart, 1971). Only recently have efforts been made to measure grazing impact more directly. One approach is to extrapolate from laboratory-determined feeding relationships to field situations of known species abundances of micro-zooplankton and size composition of potential prey (e.g. Heinbockel and Beers, 1979). Given the difficulty of individually manipulating tiny organisms in diverse, natural assemblages of plankton, this approach is frequently the only viable alternative for estimating the grazing impact of particular species or groups (e.g. tintinnids) of micro-zooplankton whose feeding rates, behavior, and prey preferences are adequately known from laboratory studies. However, few such experimental data exist for most groups of micro-zooplankton, including the abundant oligotrichs; therefore, the approach, in addition to being laborious, is generally unsuitable for the estimation of total micro-zooplankton impact on phytoplankton.

A more direct technique for estimating micro-zooplankton feeding rates in nature was presented by Capriulo and Carpenter (1980). The natural assemblage of plankton is divided into two size components: one fraction, which passes a 35 μm screen, contains few micro-zooplankton but the majority of their preferred food and serves as a control; the other fraction (prescreened through 202 μm netting to remove macro-zooplankton) is concentrated behind a 35 μm screen to 5–10 times the natural density. Grazing rates are measured, relative to the control, in a mixture of the smaller and larger size fractions. One drawback of this technique is that phytoplankton abundance and size composition differ between experimental and control containers; therefore, interpretation of grazing impact from general measures of phytoplankton biomass (e.g. chlorophyll concentrations) is ambiguous; i.e., one presumes that the measured disappearance of chlorophyll was from the smaller size fraction. An even more important limitation is that the technique measures grazing impact not for the entire micro-zooplankton community but only for the size frac-

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tion retained by a 35 μm screen. For example, non-loricate ciliates, which generally dominate the micro-zooplankton (Beers and Stewart, 1970; Beer et al., 1975), are not retained, even by mesh as small as 20 μm (Smetsacek, 1981). The apparent dominance of tintinnids in samples concentrated by Capriulo and Carpenter (1980) may be the result of this retention problem.

In the present paper, we report on an experimental technique for measuring the natural grazing impact of micro-zooplankton communities, and give the results of initial experiments performed in the coastal waters of Washington state, USA. The relatively simple technique is based on dilution of natural seawater rather than concentration or fractionation and involves minimal handling of the live plankton sample.

Materials and Methods

Theoretical Considerations

We made three assumptions regarding the interactions among nutrients, phytoplankton, and micro-zooplankton in the sea. First, we assumed that growth of individual phytoplankton is not directly affected by the presence or absence of other phytoplankton per se. The implication of this assumption is that a reduction in the density of cells in natural seawater will not, in and of itself, directly cause a change in the growth rate of remaining cells. Second, we assumed that the probability of a phytoplankton cell being consumed is a direct function of the rate of encounter of consumers with prey cells. This implies that consumers are not food-saturated at natural prey densities and that the number of prey ingested by a given consumer is linearly related to prey density. Third, we assumed that change in the density of phytoplankton, P, over some time, t, can be represented appropriately by the exponential equation,

\[ P_t = P_0 e^{(k-g)t}, \]  

where k and g are instantaneous coefficients of population growth and grazing mortality, respectively. This is a common assumption in most feeding studies involving plankton. A constant growth coefficient, k, follows from our first assumption if concentrations of nutrients and other growth factors remain approximately constant (and/or nonlimiting). According to our second assumption, the mortality coefficient g varies directly with the density of consumers but is not affected by changes in phytoplankton concentrations. The coefficients k and g may vary with time of day without affecting our comparisons of growth rates of natural phytoplankton in different dilutions over a fixed period of incubation.

Given our three assumptions, we now consider the implications of diluting natural seawater containing both phytoplankton and small consumers with filtered seawater from the same source. The instantaneous growth rate of individual phytoplankton cells should not change according to our first assumption, provided that nutrient levels do not change appreciably during the incubation. However, the instantaneous rate of phytoplankton mortality should decline in direct proportion to the dilution effect on consumer density. That is, although individual consumers continue to have a constant impact on the phytoplankton population (measured, for example, as clearance rate per consumer), the combined impact of the consumer population is less because there are fewer consumers.

Rates of phytoplankton growth and grazing mortality can be inferred from observed changes in population density following incubations of different dilutions of natural seawater. For example, given a dilution series consisting of unfiltered to filtered seawater in the ratios 1:0 (100% unfiltered seawater); 3:1 (75%); 1:1 (50%); and 1:3 (25%), the appropriate equations describing the changes in phytoplankton over time, t, are:

\[ P_1 = P_0 e^{(k-g)t}, \]  

\[ \ln \left( \frac{P_1}{P_0} \right) = k-0.75g, \]  

\[ \ln \left( \frac{P_2}{P_3} \right) = k-0.25g, \]  

where \( P_0 \) is the initial phytoplankton density, and \( P_1, P_2, P_3 \) are the densities at the end of the incubation period. In this series of equations, the observed rate of change of phytoplankton density at each dilution is linearly related to the dilution factor (decimal fraction of unfiltered seawater). The negative slope of this relationship is the grazing coefficient g; the Y-axis intercept is the phytoplankton growth rate k. It is not necessary to perform experiments at many different dilution levels in order to estimate growth and grazing coefficients; the observed rates of change of phytoplankton density at any two dilution levels will yield two equations with two unknowns which can be solved explicitly for g and k. Linear regression analysis, however, will provide estimates of confidence limits for the coefficients.

Experimental Design and Analysis

The preceding experimental design for estimating microzooplankton grazing was tested on a cruise aboard the R. V. "Wecoma" in coastal waters off Washington during October, 1980. Experiments were performed at 3 stations overlying water column depths of 20, 50, and 200 m and ranging from 7 to 50 km from shore.

A Phoebus bottle was used to collect approximately 80 to 90 liters of seawater from 3 m depth for each experiment. Half of the water was filtered through a 0.45 μm Millipore filter held in a large volume filtration apparatus. This filtered water was then combined with the remaining, unfiltered seawater in ratios of 1:0, 3:1, 1:1, and 1:3 unfiltered to filtered water. Five 2-liter glass reagent bottles were filled with each dilution mixture. To insure that nutrients would be equally available to phytoplankton at all dilution levels, excess nitrate (10 μg-at l⁻¹) and phosphate (1 μg-at l⁻¹) were added to each bottle. In each experiment, several additional bottles of the different dilution levels were prepared without the addition of nutrients. All reagent bottles were sealed without air bubbles and attached to a rotating wheel contained within a large, clear plastic, water-cooled incubator aboard the research vessel. The
bottles were then allowed to incubate at ambient light levels and subsurface water temperatures for 24 h.

In a variation on the basic experimental design, the 4 dilution mixtures in one experiment were also dispensed in triplicate into 1-liter volume dialysis sacs (Spectrapor 2, 54 mm diameter, 12,000 MW cutoff). The sacs were individually placed into nylon mesh bags, tied to a line so that they would be suspended 3 m below a surface buoy, and incubated in situ for 24 h.

At the start of all experiments, dilution mixtures were sampled for nutrients – (nitrate, nitrite, ammonium, phosphate, and silicate), chlorophyll a, and abundances of microzooplankton (preserved in Lugol’s iodine fixative). Individual bottles were sampled for chlorophyll content at the end of experiments; generally, only experimental containers that initially contained 100% unfiltered seawater were sampled for final nutrients and microzooplankton. Nutrients and chlorophyll were analyzed according to methods in Strickland and Parsons (1972) and Lorenzen (1966), respectively. Microzooplankton abundance from settled volumes of 100 ml per sample was measured with an inverted microscope.

The apparent growth rates of phytoplankton in individual reagent bottles were calculated from Eq. (1) using chlorophyll as the measure of phytoplankton standing stock. Instantaneous coefficients of phytoplankton growth (k) and microzooplankton grazing (g) were determined from least-squares and linear regression analysis of the relationship between the rate of change of chlorophyll and the fraction of unfiltered seawater in the various bottles. Clearance rate estimates, expressed as ml animal⁻¹ d⁻¹, were calculated by dividing the estimate of the microzooplankton grazing impact by the density of microzooplankton, determined from microscopical counts.

**Results**

The results of three experiments testing the dilution approach to estimating the grazing impact of microzooplankton communities are presented in Fig. 1. In Experiment 1A, a grazing coefficient of 0.278 d⁻¹ was determined from the regression equation (95% confidence interval for g = 0.189 to 0.367). This level of grazing activity corresponds to a loss of approximately 24% of phytoplankton standing crop per day (17 to 31%). The growth coefficient for phytoplankton was 0.628 d⁻¹, somewhat less than one doubling per day. Generally, because nutrients were added to the experimental containers, we would not expect the calculated growth coefficient from the dilution experiment to reflect accurately the growth rate of phytoplankton in the field. Interestingly, however, the dialysis sac experiment for Experiment 1 (i.e., 1B) gave a similar estimate of phytoplankton growth rate which would suggest that, at the time of this experiment, the field population was not nutrient-limited. Therefore, if we assume an in situ phytoplankton growth rate of 0.628 d⁻¹ and steady state, the microzooplankton grazing effect measured in Experiment 1A accounts for a daily loss of about 52% of production.

Eight of 12 dialysis sacs used in Experiment 1B were recovered undamaged and, of these, 7 yielded apparent growth rates which were in the range observed for similar dilutions in Experiment 1A (Fig. 1). However, the one divergent data point, the only sac recovered with undiluted seawater, contributed substantial variability to the regression analysis. The resulting average of the grazing coefficient, 0.174, has broad confidence limits and, while not statistically different from the estimate of g in Experiment 1A, was only marginally different from zero (0.05 < p < 0.1).

Experiments 2 and 3 yielded estimates of instantaneous mortality for phytoplankton (95% confidence limits) of 0.065 d⁻¹ (−0.042 to 0.172) and 0.123 d⁻¹ (0.045 to 0.203), respectively, corresponding to grazing impacts of 6 and 12% of phytoplankton standing stock per day. Only the estimate for Experiment 3 was significantly non-zero (0.001 < p < 0.01). Assuming (1) steady state and (2) that experimentally determined growth rates were similar to those at ambient nutrient levels (evidence for which is presented below), we calculated that the grazing coefficients from Experiments 2 and 3 account for losses of 17 and 26% of daily phytoplankton production, respectively.
Table 1. Values of variables measured for dilution experiments

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Experiment No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Initial nutrients (µg-at l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>1.20</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.19</td>
</tr>
<tr>
<td>Ammonium</td>
<td>0.47</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.97</td>
</tr>
<tr>
<td>Silicate</td>
<td>15.0</td>
</tr>
<tr>
<td>Initial chlorophyll-a (µg l⁻¹)</td>
<td>3.54</td>
</tr>
<tr>
<td>Micro-zooplankton density (No. l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Copepod nauplii</td>
<td>160</td>
</tr>
<tr>
<td>Tintinnids</td>
<td>2000</td>
</tr>
<tr>
<td>Non-loriculate ciliates</td>
<td>20000</td>
</tr>
</tbody>
</table>

* Excess added to each bottle

Ambient concentrations of nutrients at the times the experiments were run are given in Table 1. Generally, these concentrations appeared sufficient to support the same level of phytoplankton growth observed in the presence of excess nutrients (Fig. 1), but only at the most dilute concentrations of phytoplankton used in the experiments (i.e., 1:3 ratio of unfiltered to filtered seawater). Severely depressed growth rates were observed whenever undiluted concentrations of phytoplankton were incubated in bottles without the addition of excess nutrients. This was probably due to nitrate limitation since, of the 5 nutrients measured, nitrate was the only one decreasing to low levels (0.1 µg-at l⁻¹ or less) when growth was depressed. Nitrate and phosphate concentrations never fell below 7 and 1 µg-at l⁻¹, respectively, in containers where they were initially added in excess; other nutrients changed little during the incubations.

Discussion

Our dilution method for estimating micro-zooplankton grazing impact on phytoplankton gives regression equations with negative slopes, and the resulting estimates of phytoplankton growth and mortality seem reasonable. However, before comparing the results and implications of these experiments with previous estimates of micro-zooplankton grazing, we point out the sources of potential bias in the approach.

The critical assumption in the dilution approach is that microorganisms consume prey in direct proportion to prey density, more specifically, that the natural level of available food is such that ingestion rates of these organisms are not saturated. At very high food levels this would not be true for all or part of the grazing population. In the extreme case, each consumer would eat a constant number of prey regardless of the dilution effect on prey density. This would result in constant, per capita mortality on phytoplankton at all dilutions, and grazing rate would appear to be negligible since its effect would be indistinguishable from constant phytoplankton growth. In the less extreme case of saturated feeding by only a fraction of the consumers or only at the highest prey densities used in experiments, phytoplankton growth and grazing mortality would be underestimated. In laboratory studies with three coastal species of tintinnids, Heinbokel (1978a) found ingestion rates to be approximately linearly related to food concentration up to about 100 µg carbon l⁻¹. Although concentrations of particulate carbon are frequently higher than this level in nature, the feeding of individual species of micro-zooplankton is limited generally, because of preference (e.g. Stoecker et al., 1981), behavior (e.g. Rassoulzadegan and Etienne, 1981), or food handling efficiencies (e.g., Heinbokel, 1978b), to only a fraction of the size range of total particulates.

An opposite bias (i.e., over-estimation of grazing impact), would occur if ambient food levels were so low that micro-zooplankton responded to decreased food levels (i.e., dilutions of prey) by reducing their feeding effort. This behavior, termed threshold feeding response, has been demonstrated for planktonic, filter-feeding copepods where it apparently serves an energy optimizing function (e.g. Frost, 1975; Lam and Frost, 1976). Threshold feeding is not likely to be as significant a problem in the dilution experiments as other possible effects, such as saturated feeding. Heinbokel (1978a), for instance, did not observe threshold behavior in his feeding experiments with tintinnids. Moreover, it is not clear that organisms which depend on random contact with food particles and are characterized by high specific rates of basal metabolism will gain a long-term energetic advantage by reduced foraging activity at low densities of food.

The results of the dilution experiments with and without added nutrients demonstrate the importance of the nutrient treatment in the experimental design and emphasize the weakness of phytoplankton experiments involving appreciable periods of incubation in closed containers. It is clear from the results of Experiment 3, for instance, that markedly different conclusions about phytoplankton growth rates can be reached depending on the composition of the incubation medium. A growth rate of only 0.15 d⁻¹ is obtained when the natural phytoplankton assemblage is incubated without added nutrients. In contrast, incubations of the undiluted phytoplankton with excess nutrients yield a mean estimate of 0.45 d⁻¹. However, the actual growth rate of about 0.628 d⁻¹ is approached only when increasingly more dilute samples of the assemblage are incubated (with or without excess nutrients). Without added nutrients, the relationship between apparent growth rate of phytoplankton and dilution level has an exaggerated negative slope and leads to an erroneous calculation of the magnitude of micro-zooplankton grazing. In Experiment 3, analysis of the results of incubations without added nutrients yields an estimate of 0.585 d⁻¹ for the grazing coefficient, a daily loss of 44% of phytoplankton biomass or 84% of new production. Similarly, the 4 data points from Experiment 2 that involved incubations without additional...
nutrients give a micro-zooplankton grazing impact (0.579 d⁻¹) equivalent to 44% of phytoplankton standing biomass or about 100% of production.

In seawater, any dissolved constituent essential for phytoplankton growth (i.e., macronutrient, micronutrient, or vitamin) may become limiting during the course of a dilution experiment and bias the result. Since the essential component will be depleted more rapidly in the presence of higher densities of phytoplankton, the negative slope of the regression equation (in other words, the estimated coefficient of grazing) will be exaggerated. Nitrate appears to be the primary limiting factor in the experiments reported here, although in another location, perhaps this would not necessarily be the case; more importantly, a second factor may become limiting after nitrate is added in excess. In retrospect, the nutrient addition should include all components necessary for unlimited phytoplankton growth, e.g., the ingredients of a conventional growth media. Alternatively, the experiments may be performed in dialysis containers for a better indication of growth rates of phytoplankton at ambient nutrient levels. This would probably be the best approach when dealing with the delicate organisms from oligotrophic oceans, as these organisms seem to be poisoned by nutrient additions, either from the nutrients themselves or from trace contaminants in the nutrient stocks (own unpublished results). When estimates of the growth rate of phytoplankton are not desired, the incubations may be performed in darkness.

The results presented here indicate a micro-zooplankton grazing impact in the range of 6 to 24% of phytoplankton standing biomass and 17 to 52% of production per day in coastal waters off Washington. These estimates are similar to those reported in other coastal areas. For example, Beers and Stewart (1971) who made assumptions about size-dependent energetic requirements, suggested a micro-zooplankton impact of 7 to 52% (average 23%) of primary production in the Southern California Bight. Heinbockel and Beers (1979) have subsequently indicated that tintinnids from the same area occasionally consume up to 20% of daily production, but more typically account for about 4% of production loss. Riley (1956) suggested that micro-zooplankton grazing in Long Island Sound may account for a loss of as much as 43% of annual production based on the difference between measured production and other measured loss terms in the production budget. Capriulo and Carpenter (1980) measured micro-zooplankton feeding experimentally in Long Island Sound and found that micro-zooplankton consumed up to 41% of phytoplankton standing stock per day.

Although the dilution technique is only appropriate for determining the community grazing impact of micro-zooplankton, a reasonable feeding rate for an individual may be obtained by dividing the measured group impact by the number of grazers in each experiment. If we assume, for example, that all micro-zooplankton contribute equally to the community grazing effect, the per individual clearance rate estimates are 0.6, 0.1, and 1.6 µl h⁻¹ for Experiments 1, 2, and 3, respectively. These estimates are on the low end of feeding rates for tintinnids reported by Heinbockel (1978a) (1 to 5 µl h⁻¹) in laboratory studies and by Heinbockel (1978b) (0.6 to 10 µl h⁻¹) and Capriulo and Carpenter (1980) (1 to 84 µl h⁻¹) in field studies. However, virtually nothing is known regarding the feeding rates and behavior of naked ciliates, which were numerically dominant in the dilution experiment. If we assume that all grazing in the experiments was due to copepod nauplii and tintinnids, we obtain grazing rate estimates per individual of 3.5, 5.4, and 11.6 µl h⁻¹, which agree well with other estimates. Despite comparable numbers of naked ciliates, predominantly oligotrichs, in Experiments 1 and 2, the amount of micro-zooplankton grazing was much greater in Experiment 1. The observed grazing effect was also higher in Experiment 3 than 2, even though naked ciliates were an order of magnitude less abundant in the former. Such results suggest the hypothesis that small, naked ciliates graze little on the dominant phytoplankton types (e.g., diatoms) in coastal areas. Possibly their greatest impact is on bacteria and microflagellates. Detailed studies on the feeding of this most numerous fraction of the marine micro-zooplankton would be an important next step in understanding the feeding role of pelagic micro-zooplankton. Another area needing study is the grazing impact of micro-zooplankton in oligotrophic gyre systems. From energetic considerations, Beers and Stewart (1971) suggested that the micro-zooplankton might consume about 70% of primary production in oligotrophic regions as opposed to 20% in coastal areas. It would be interesting to apply the methods of the present study in regions of the world ocean where micro-zooplankton have been predicted to have their greatest trophodynamic impact.

Acknowledgements. We gratefully acknowledge the efforts of the Captain and crew of the R.V. “Wecoma” in support of this research. We thank M.M. Mullin, R.W. Eppley, and K. Banse for their encouragement and valuable comments. Particular thanks are due to S. Moore of Oregon State University, who provided analyses of our nutrient samples. Our work was supported by Department of Energy Contract DE-AT06-76-EV-75026 (DE-EV-75026-91).

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Date of final manuscript acceptance: January 25, 1982.

Communicated by N.D. Holland, La Jolla

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