Nitrogen Cycling in Coastal Marine Environments
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CHAPTER 3

Microfauna in Pelagic Food Chains

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3.1 INTRODUCTION

This chapter will consider the phagotrophic pelagic organisms within the size range of about 3 to 200 μm, which corresponds to the heterotrophic nanoplankton (2–20 μm) plus microplankton (20–200 μm) in the terminology of Sieburth (1979). The group so defined is quantitatively and qualitatively dominated by representatives of a variety of protozoan taxa, in particular flagellates and ciliates, but also amoebae, heliozoans, radiolarians, acantharians, and foraminifera. However, the microplankton also includes some metazoa, in particular rotifera and juvenile copepods. The distinction between the phagotrophic and autotrophic components of the micro- and nanoplankton is not always sharp: some flagellate species are at the same time phagotrophs and photosynthetic and some pelagic protozoa (radiolaria, foraminiferans and some ciliates such as *Mesodinium rubrum*) harbour photosynthetic cells which effectively render the symbiotic associations into photoautotrophic organisms.

Recent recognition of the quantitative role of the phagotrophic nano- and microplankton is an integral part of what has been called a 'new paradigm of the planktonic food web (Azam *et al.*, 1983; Ducklow, 1983; Sorokin, 1977; Williams, 1981). This also includes the discovery of the large role of heterotrophic bacteria in the pelagic carbon cycle and the role played by eukaryote nanoplankton and by prokaryote photosynthetic organisms for the primary production of the sea. In this picture of the pelagic food web the role of the phagotrophic nano- and microplankton is to form a link in the food chain between bacteria and nano- and picoplankton primary producers on the one hand and the macroplankton on the other. In fact, the size range covered by the phagotrophic nano- and microplankton means that they themselves constitute two or three links in the food chains. This last observation is particularly relevant when their role as remineralizers of mineral nutrients is under discussion.

3.2 DISTRIBUTION PATTERNS AND QUANTITATIVE ROLE

Representatives of a variety of groups of non-photosynthetic flagellates dominate the phagotrophic nanoplankton although amoebae (see Davis *et al.*, 1978) may

also play a role. During the past 5 years several studies have quantified heterotrophic flagellates in seawater samples from a variety of locations. This has mainly been accomplished by the use of epifluorescence microscopy, a technique which allows the distinction between pigmented and non-pigmented forms and often identification to at least the generic level (Davis and Sieburth, 1982; Fenchel, 1982b; Haas, 1982). These quantitative data are reviewed in Fenchel (1986a). The flagellates measure 3–10 μm and typically occur at densities of round 10^3/ml in surface (0–30 m) waters. As an average number this holds for oligotrophic as well as for eutrophic waters. However, while flagellate (and bacterial) numbers seem rather constant over time in the former type of habitats, oscillating numbers are characteristic of more eutrophic situations. Thus, during the summer in the Limfjord, flagellate numbers vary between 2×10^2 and 1.4×10^4/ml. Peaks in flagellate numbers seem to follow peaks in bacterial numbers with a more or less regular periodicity of 10–20 days.

Several types of heterotrophic flagellates have been isolated into pure cultures, and this has greatly facilitated the quantification of grazing rates and other parameters of ecological bioenergetics (Fenchel, 1982a, 1986a, b; Goldman *et al.*, 1985; Sherr *et al.*, 1983). Such data have also been obtained from incubations of freshly collected seawater samples (Andersen and Fenchel, 1985; Sherr *et al.*, 1984) to approximate *in situ* conditions. The flagellates depend on suspended bacteria (including cyanobacteria) and the most minute nanoplankton organisms for food. The flagellates typically clear a volume of water of about 10^5 times their own cell volume per h or from 5×10^{-6}–5×10^{-5} ml/h (20 °C) depending on size. Most forms, and in particular the often dominating choanoflagellates, are capable of retaining even the smallest prokaryote cells. The flagellates seem to be able to maintain balanced growth at generation times from about 3.5 h and up to about 24 h (20 °C); bacterial densities below around 10^6/ml do not seem to sustain flagellate growth.

These findings together allow the estimation of *in situ* grazing rates. It seems that in most offshore and coastal waters (during summer), zooflagellates on the average clear 20–50% of the water for bacteria per 24 h, although great temporal variation due to varying population sizes occur. Altogether, available evidence shows that phagotrophic flagellates constitute the dominating consumers of bacterial production and control their numbers.

The other dominating group of the planktonic microfauna is constituted by the ciliates. While the presence of the tintinnid oligotrichs has been recognized for a long time, recent studies based on more gentle sampling and fixation techniques have revealed that other ciliates, and in particular non-loricate oligotrichs, often play a substantial role.

Several quantitative studies prior to 1980 are reviewed by Taylor (1982). Other recent studies include Capriulo and Carpenter (1980), Hargraves (1981), Heinbokel and Beers (1979), and Rassoulzadegan (1977).

Ciliates typically number 0.1–10/ml seawater; since they are mostly much

larger than flagellates (15–200 μm for most plankton forms) the two groups on the average represent a similar biomass (0.1–10 mg c/m^3). Like flagellates, the numbers of ciliates fluctuate temporally. In eutrophic areas mass occurrence of ciliates may follow blooms of nanoplankton algae (e.g. > 100/ml have been found in the Limfjord during summer; see Andersen and Sørensen, 1986).

The dominating planktonic ciliates feed on photo- and heterotrophic nanoplankton cells, or on constituents of the microplankton such as dinoglagellates, diatoms or other ciliates. Bacterivorous ciliates play a small role in the plankton (see also Fenchel, 1980, 1984). Exceptions to this are very eutrophic waters (Burkill, 1982) and the microfauna associated with suspended detrital or flocculent material (Caron *et al.*, 1982).

Values of clearance for ciliates which filter larger (> 2 μm) particles are around 10^5 times cell volume per hour (Fenchel, 1986b) and this allows crude estimates of the grazing impact of ciliates. Estimates of ciliate grazing based on *in situ* measurements are still few and not easily compared. Heinbokel and Beers (1979) found that tintinnids consumed from 4 to 20% of the primary production off the California coast; since tintinnids usually constitute less than 50% of planktonic ciliates the total grazing impact of ciliates must have been larger. Capriulo and Carpenter (1980) found that ciliates (mainly tintinnids) consumed up to 41% of the standing crop of chorophyll per day in Long Island Sound. Both these studies ignore the grazing impact on non-photosynthetic cells.

Although still incomplete in some respects, a new picture of the planktonic food web has emerged during the past decade. In this picture the production of small cells (prokaryotes and photosynthetic nanoplankton) makes up for a much larger productivity than previously believed, and this production is largely mineralized through several trophic levels constituted by phagotrophic organisms in the 2 to 200 μm size range.

3.3 MINERALIZATION OF C, N AND P

Johannes (1964, 1965) was among the first to emphasize the role of the microfauna in the regeneration of mineral nutrients, a role traditionally assigned mainly to bacteria in aquatic environments. Several other studies ensued, most of which are reviewed in Taylor (1982). These were mostly carried out with some sort of batch cultures or with microcosms which did not allow balanced growth or steady-state conditions. Consequently the results could not easily be applied to natural conditions. However, the studies did make the point that bacteria often grow on organic substrates poor in mineral nutrients. Consequently, net mineralization of nitrogen and phosphorus is low, or the bacteria may even assimilate mineral nutrients in order to grow and compete with photosynthetic organisms. Grazers of bacteria, on the other hand, feed on particles with C:N and C:P ratios similar to their own cells, and since their growth efficiency is below unity they would regenerate mineral nutrients.

The role of phagotrophs in the cycling of mineral nutrients is, in most respects, much simpler than that of prokaryotes and photosynthetic organisms. This is because phagotrophs do not assimilate dissolved inorganic nutrients, but acquire N and P together with their food particles which have a rather constant composition. Assimilatory or dissimilatory reductions or oxidations of N-compounds do not occur (but see Finlay *et al.*, 1983). In small animals and in protozoa, metabolic rate is closely coupled to growth (Fenchel and Finlay, 1983) so that the regeneration of nutrients by small phagotrophs must be proportional to their metabolic rate, and is thus a simple function of the C:N (or C:P) ratio of the food and of the growth efficiency.

Using the 'IBP terminology' the consumption (on a carbon basis) equals $R + P + F + U$, where R is respiration, P is growth and F and U represent the egested and excreted organic carbon, respectively. Let $A = P + R$ and net growth efficiency becomes $E_n = P/A$. Finally let ρ_f and ρ_p represent the C:N ratio of the food particles and the predator, respectively. If $\rho_f = \rho_p = \rho$, then the excretion of

$$\mathrm{N} = R/\rho = A[1 - E_n]\rho^{-1}$$

If $\rho_f \neq \rho_p$, then regenerated nitrogen will equal the consumed N not egested or excreted as organic N minus that assimilated into cells, or

$$A/\rho_f - P/\rho_p = A[\rho_f^{-1} - E_n\rho^{-1p}]$$

The net growth efficiency of protozoa is 50–60% (Fenchel, 1982a; Fenchel and Finlay, 1983) while gross growth efficiency probably varies much more. Fenchel (1982a) found values of 30–40% of the ingested bacterial carbon was either egested or excreted in two heterotrophic flagellates. We may assume that values of the C:N ratio for bacteria as well as for protozoa are within the range 4–6, while in phytoplankton values between 6 and 10 are found (Finlay and Uhlig, 1981; Wheeler, 1983).

If the above listed values are considered to be correct in general, then it can be seen that in a protozoan food chain around 30–40% of the ingested organic N is excreted as mineral N at each trophic level. An exception may be forms grazing on phytoplankton cells; depending on the C:N ratio of the food particles the figure may be considerably lower. A similar argument, of course, applies to the regeneration of phosphate.

The experiments of Sherr *et al.* (1983) illustrate the considerations given above. These authors measured ammonia excretion in a culture of a heterotrophic flagellate during balanced growth at a known rate. Calculations on their results in conjunction with reasonable assumptions on growth efficiency accord with the simple equations given above (Fenchel, 1986a). The detailed study by Goldman *et al.* (1985) on the nitrogen excretion of another heterotrophic flagellate also accords with the principles outlined above.

The general conclusion of this is that the share of the remineralization of N by the nano- and microplankton must be nearly proportional to their share of the

carbon mineralization. Since probably the entire bacterial production, and a large part of the photosynthetic production, is consumed by the phagotrophic nano- and microplankton organisms, and since this pelagic constituent represents two or more trophic levels, its share in the entire remineralization must be considerable, a point also made recently by Ducklow (1983).

Another approach to the question is to measure remineralization directly in size-fractionated plankton samples. In general these studies suggest that plankton organisms $< 200\,\mu m$ are responsible for the largest part of the NH_4^+ regeneration in the water column. The details of the results, however, differ in some respects. Glibert (1982) found for a number of areas that the $< 10\,\mu m$ fraction generally yielded the largest contribution to N-remineralization only sometimes exceeded by the 10–35 μm or the 35–130 μm fraction. Harrison (1978) found that 39% of the NH_4^+ excretion was due to the $< 1\,\mu m$ (bacterial) fraction, 50% to the 1–35 μm and only 11% to the $> 35\,\mu m$ fraction. In contrast, Paasche and Kristiansen (1982) found that the microplankton (45–200 μm) consisting of heterotrophic dinoflagellates, ciliates, rotifers and copepod nauplii, yielded the largest contribution to the NH_4^+ regeneration. The studies also differ in terms of the degree to which the measured regeneration rate could meet the demands of the phytoplankton; thus Glibert (1982) found a close coupling and balance between remineralization and uptake, whereas Paasche and Kristiansen (1982) found that the regeneration of nutrients could only account for 28% or less of the rate of assimilation.

These discrepancies probably to some extent reflect real differences between the nutrient cycles in different areas. They probably also reflect the complex successional patterns and rapid oscillations in numbers of different functional groups of the plankton maintained by predator–prey interactions. These processes must necessarily also induce fluctuations in nutrient fluxes with similar time scales. While the question of rates of remineralization of N and P of phagotrophs is mainly one of understanding their role in the carbon cycle, the most challenging problem in the ecology of plankton will be to understand the population dynamics of a system which is never in a steady state.

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