FLAGELLATES IN THE MARINE MICROBIAL FOOD WEB: THE ECOLOGY OF A MIXOTROPHIC NANOFIAGELLATE, Ochromonas sp.
ABSTRACT

FLAGELLATES IN THE MARINE MICROBIAL FOOD WEB: THE ECOLOGY OF A MIXOTROPHIC NANOFLAGELLATE, Ochromonas sp.

Agneta Andersson-Nordström, Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden.

Nanoflagellates were found to be abundant in a coastal area of the northern Bothnian Sea. The maximum concentration of nanoflagellates, approximately 8000 cells ml\(^{-1}\), was observed in July, coinciding with a decrease in the abundance of cyanobacteria. Pigmented and non-pigmented nanoflagellates were approximately equally distributed throughout the year. Most of the identified genera are known as being phagotrophic, independent if autotrophic or not.

A non-cyst-forming pigmented flagellate, Ochromonas sp., was isolated and nutritionally characterized. This chrysophycean flagellate was shown to be a mainly heterotrophic organism: Photosynthesis was too poor to support multiplication of the cells, whereas when feeding on bacteria, high growth rates were obtained. The biological function of the photosynthetic apparatus is suggested to be a survival mechanism during poor bacterial conditions.

The flagellate grazed bacteria selectively, preferring cyanobacteria and large cells of heterotrophic bacteria, presumably depending on size-selective grazing. Despite higher growth rates of the bacteria in the sea during summer (July) than spring (May), heterotrophic bacteria in the sea was observed to be smaller in the summer. Nanoflagellates showed a maximum in July, and by selective grazing of large bacteria they might have caused the decrease in the average size of the bacteria and the decrease in the abundance of cyanobacteria.

During the consumption of bacteria the flagellate was shown to remineralize nutrients at high rates and excrete dissolved free amino acids. Assuming the existence of a protozoan predator-prey chain of several trophic levels, it seems likely that a significant part of the nutrients fixed by primary producers is remineralized in the euphotic zone. Furthermore, data from this work indicate that flagellate activity may be a significant source of dissolved free amino acids, utilizable for the heterotrophic bacteria.

Keywords: Microbial food web / flagellates / Ochromonas sp./ mixotrophy / size-selective grazing / remineralisation / excretion of DFAA

ISBN 91-7174-417-7
FLAGELLATES IN THE MARINE MICROBIAL FOOD WEB: THE ECOLOGY OF A MIXOTROPHIC NANOFLEGELLATE, *Ochromonas* sp.

Agneta Andersson-Nordström

Dept. of Microbiology
University of Umeå
Umeå 1989
A. FOREWORD
B. LIST OF PAPERS
C. ABSTRACT
D. INTRODUCTION
   1. THE "CLASSICAL" VIEW OF THE MARINE FOOD WEB............7
   2. THE CHANGING PARADIGM......................................................8
      2.1. Evidence for bacterial activity in the sea
      2.2. Significance of bacterial growth
      2.3. The environment for marine bacteria
   3. ADDITION OF A "MICROBIAL LOOP" INTO THE "CLASSICAL"
      FOOD WEB.............................................................................11
   4. FLAGELLATES.................................................................................13
      4.1. Purely autotrophic forms
      4.2. Mixotrophic forms
      4.3. Saprotrophic/osmotrophic forms
      4.4. Phagotrophic forms
   5. BACTERIVORES IN THE SEA.....................................................15
      5.1. Feeding mechanisms of the bacterivores
      5.2. Organisms
E. AIM OF THE THESIS........................................................................18
F. RESULTS AND DISCUSSION
   1. NANOFLAGELLATES IN THE SEA................................................19
      1.1. Annual and diurnal dynamics
      1.2. Nutrition of the identified nanoflagellates
   2. OCHROMONAS sp.................................................................26
      2.1. Autotrophy and heterotrophy
      2.2. Clearance rate
      2.3. Heterotrophic feeding on DOM
      2.4. Temperature optimum
      2.5. Remineralization
      2.6. Excretion of amino acids
      2.7. Size-selective grazing
      2.7.1. Impact on the bacterial community in the sea
G. CONCLUSIONS
H. ACKNOWLEDGEMENTS
I. REFERENCES
J. PAPERS I-IV
A. FOREWORD

Throughout this thesis work I have been crossing the borders of different scientific fields; Biology, Microbial Physiology and Marine Ecology. This was not my intention from the beginning, but I soon realized that ecological studies have a tendency to branch out. During this excursion in natural science I have come to realize that nature is as largest in its smallest compartments...
B. LIST OF PAPERS

This thesis is based on the four published papers listed below, and subsequently referred to by their roman numerals.


C. ABSTRACT

Nanoflagellates were found to be abundant in a coastal area of the northern Bothnian Sea. The maximum concentration of nanoflagellates, approximately 8000 cells ml\(^{-1}\), was observed in July, coinciding with a decrease in the abundance of cyanobacteria. Pigmented and non-pigmented nanoflagellates were approximately equally distributed throughout the year. Most of the identified genera are known as being phagotrophic, independent if autotrophic or not.

A non-cyst-forming pigmented flagellate, *Ochromonas* sp., was isolated and nutritionally characterized. This chrysophycean flagellate was shown to be a mainly heterotrophic organism: Photosynthesis was too poor to support multiplication of the cells, whereas when feeding on bacteria, high growth rates were obtained. The biological function of the photosynthetic apparatus is suggested to be a survival mechanism during poor bacterial conditions.

The flagellate grazed bacteria selectively, preferring cyanobacteria and large cells of heterotrophic bacteria, presumably depending on size-selective grazing. Despite higher growth rates of the bacteria in the sea during summer (July) than spring (May), heterotrophic bacteria in the sea was observed to be smaller in the summer. Nanoflagellates showed a maximum in July, and by selective grazing of large bacteria they might have caused the decrease in the average size of the bacteria and the decrease in the abundance of cyanobacteria.

During the consumption of bacteria the flagellate was shown to remineralize nutrients at high rates and excrete dissolved free amino acids. Assuming the existence of a protozoan predator-prey chain of several trophic levels, it seems likely that a significant part of the nutrients fixed by primary producers is remineralized in the euphotic zone. Furthermore, data from this work indicate that flagellate activity may be a significant source of dissolved free amino acids, utilizable for the heterotrophic bacteria.
D. INTRODUCTION

Flagellates represent a diverse group of unicellular or colonial eucaryotic organisms which taxonomically have been shared by both zoologists and botanists, as both pigmented and non-pigmented forms occur. By the botanists flagellates are placed among the Chlorophyta, Euglenophyta, Chrysophyta, Cryptophyta, Pyrrhophyta, Xanthophyta and Eustigmatophyta (21), whereas zoologists place them in the subphylum Sarcomastigophorea (91). Since single species can be both autotrophic (photosynthetically active) and saprotrophic or osmotrophic (feeding on dissolved organic material) as well as phagotrophic (ingestion of particulate material) nutritional versatility is common. The present phylogenetic view of the origin of the eucaryotic algae, is the theory of endosymbiosis between zooflagellates and ingested autotrophic procaryotic organisms (94). According to this theory, many phytoflagellates should be regarded as "primitive", as they are motile photosynthetic predators. An example of a transitory stage is the *Cyanophora paradoxa* which has "cyanelles" that is an intermediate form of cyanobacteria and chloroplasts (21).

Flagellates, both pigmented and non-pigmented, first became identified and cultured approximately 100 years ago (cf. 81). These organisms were found to multiply in sugar solutions and were therefore sometimes called "sugar flagellates". However, these cultures were not axenic, i.e. bacteria were not excluded, and since bacteria are more efficient in using dissolved substances, the bacteria in the culture might have been used as food by the flagellates. In the early 20th century extensive taxonomic studies were made by Skuja and others (e.g. 89, 90). Many species of flagellates with versatile nutrition were described in these studies. However, most of the data was derived from observations of "field samples". One of the first studies of pure (axenic) cultures of flagellates was made by Pringsheim (81). He found an *Ochromonas* to be nutritionally versatile, i.e. photo-sapro-phagotrophic. This organism needed dissolved or enzymatically soluble organic carbon for growth. Organic particles, e.g. bacteria, were taken up and digested, but did not support growth of the flagellate. The flagellate also carried one single chloroplast, which nutritionally seemed to be of minor importance since the organism did not multiply photosynthetically. As saprotnrophy was regarded the most important mode of nutrition, studies were made on minimal nutritional requirements (35, 45, 50). The bacteria-produced vitamin B_{12} was thought to be of special importance, since it was suggested to be a limiting factor for the growth of the planktonic community of the oceans (35).

The role of marine protozoa as remineralizers of important nutrients for the phytoplankton was studied by Johannes (57). He found that considerable amounts of
inorganic nutrients were excreted by protozoa while consuming bacteria. In an early study of the nanoplankton in the waters around the British Isles (61) it was observed that a minute (1.5 μm) pigmented chrysomonad was frequently abundant (1000 ml⁻¹). It was speculated that this kind of organisms might be included in the food webs of the oceans, since it was possible to feed oyster-larvae with a *Monas* sp, an apochlorotic (non-pigmented) flagellate (51). Furthermore it was noted that this chrysomonad was abundant in a bay with a dense shell-fish population (52). However, at the time, these findings were too scattered to be recognized as ecologically important and bacteria in the sea were considered to be scarce and non-productive (see below).

1. THE "CLASSICAL" VIEW OF THE MARINE FOOD WEB

For a long time, more than half a century, the marine food web was described as a simple predator-prey chain starting with photosynthetically produced organic matter in the form of phytoplankton. The phytoplankton were believed to be consumed by herbivorous zooplankton with a high transfer-efficiency (92) (Fig 1).

The traditional techniques used for enumerating pelagic bacteria were based on plate counts, serial dilutions or phase-contrast microscopy. By these techniques only about 0.1% of the actual bacterial numbers were counted. Suspended pelagic bacteria were therefore regarded as being scarce and non-growing (56) and were thus not included in

![Food Web Diagram](image)

**Figure 1.** A food web consisting of ten hypothetical species (Redrawn from Pianka (78)).
the outlined food web of the oceans. Pelagic bacteria in the sea were believed to be attached to particles, and their major ecological role was hypothesized as being that of decomposers of particulate matter, e.g. dead algae (cf. 92). Remineralization was thought to occur partly in the euphotic zone by bacteria attached to particles, and partly in the sediments by benthic bacteria.

Early investigations showed significant amounts of dissolved organic material (DOM) in both sea and fresh water (e.g. 76, 92). It was speculated that the source of DOM was either through degradation of formerly living material and / or excretion by algae. However, it was believed that less than 10 % of the fixed carbon was released by algae and that this material could not be converted into fresh living matter by processes with higher efficiency than 30 %. Therefore, the generalized view was that at the most only a few percent of the initially fixed energy was deflected from the main pathway and used elsewhere in the food chain. It was hypothesized that most of the derived organic macromolecules adhered to detritus particles by physico-chemical processes. Hence, DOM would be channeled into the food chains via bacterial activity on surfaces of detritus particles, and utilization of detritus by filter feeding organisms (59).

2. THE CHANGING PARADIGM

2.1. Evidence for bacterial activity in the sea

In 1962, Strickland and Parsons (95) presented a technique to measure the in situ metabolic activity of marine microheterotrophs. This technique was a parallel to the $^{14}$C-uptake-technique to measure primary production, and made use of a radioactive organic substrate added directly to a sea water sample and the subsequent measurement of the uptake of radioactivity into organisms. By the use of this technique significant heterotrophic activity was shown both in fresh water and in the sea (97, 104, 105). Evidence of a dominating heterotrophic activity in organisms of less than 3 μm was given by Azam & Hodson (7).

During the 70s, a technique was developed for the precise enumeration of pelagic bacteria (36, 47). The procedure is basically as follows: staining of the bacteria with fluoresceins which adhere to protein or DNA; filtering a suitable amount of sample onto polycarbonate filters and analysis of the sample using an epifluorescence microscope. Studies of the abundance and sizes of the pelagic bacteria led to the conclusion that most of the bacteria in the sea are free-living small spherical or rod-like cells, about 0.4 μm in diameter, with volumes of about 0.08-1.5 μm$^3$ (2, 7, 18). Larger cells are mostly found
in eutrophicated waters and in estuaries with a strong physical mixing (99). Williams (102) estimated that the free-living bacteria accounted for about 5% of the biomass, and, what might be more important, 70% of the reactive surface area of the organisms present. Bacterial numbers in sea water fluctuate somewhat on a yearly, daily and hourly basis. However, in oceanic and off-shore waters the span is $2 \times 10^5$-$10^6$ cells ml$^{-1}$, and in more productive estuarine waters the bacterial numbers vary from 1 to $4 \times 10^6$ cells ml$^{-1}$ (33, 41, 42, 43, 68). The small variation in bacterial numbers suggests a tight control of bacteria in the sea.

2.2. Significance of bacterial growth

One explanation as to why marine bacteria are small is that they grow under poor nutrient conditions. At decreasing growth rates the bacterial volume decreases (e.g. 65), and this is accompanied by an increasing surface to volume ratio. The latter generally means higher efficiency in the scavenging of substrate. Some scientists interpreted the dominating small round cells as being in an inactive or dormant stage due to starvation (74, 93). This opinion was basically supported by experiments with pure cultures of bacteria, which when starved produced small cells identical in size to those found in nature. Another hypothesis of the occurrence of "small" bacterial cells was that it could be caused by a predation pressure (41, 99). If the grazers prefer large bacteria, the occurrence of small cells could be a consequence of size-selective grazing.

In the 70s several independent methods for the quantification of bacterial growth were developed. Of these the tritiated thymidine method (TTI-method) and the frequency of dividing cell method (FDC-method) are used most frequently. The TTI-method is based on the uptake of radioactive thymidine by bacteria in a sea water sample. Thymidine is directly incorporated by growing bacterial deoxyribonucleic acid (DNA) (37). With the use of a conversion factor, the growth of bacterial biomass can be determined. The FDC-method (44) is based on the fact that the frequency of dividing cells is positively correlated with the growth rate of the bacteria. These two methods have independently given evidence that the bacteria in the sea are actively growing, with estimated generation times of less then half a day to a few days. Furthermore, studies of substrate-uptake kinetics of marine bacteria have shown that bacteria exhibit high affinity and low capacity (low flow) as well as low affinity and high capacity (high flow) transport systems (8). This physiological feature makes them efficient organisms in both low and rich nutrient environments.
2.3. The environment for marine bacteria

Heterotrophic bacteria have an osmotrophic feeding behaviour, i.e. utilizable dissolved organic compounds are transported through the cell membrane into the cell where they are metabolized. Dissolved organic material is taken up either directly (e.g. simple sugars and amino acids) or after hydrolyzation (e.g. proteins, polysaccharides and nucleic acids) into monomers or oligomers by exoenzymes in close connection to the cell surface. The amount of DOM in the sea is at least a hundred-fold higher than the amount of particulate organic material (POM) (16, 88). Although the DOM-pool in sea water is substantial (1-3 mg carbon per litre in surface waters), bacteria are only able to utilize about 5% of it (2). Sea water bacteria, grown in batch cultures on unsupplemented filtered sea water, multiply once or twice before the amount of nutrient is too reduced for bacterial growth. Several different sources of utilizable DOM have been identified:

1. **Phytoplankton exudation** may represent an overflow of photoassimilated carbon during periods when carbon fixation exceeds biomass production, e.g. under nutrient limitation. However, in a study of an axenic culture of an alga continuous exudate release during the dark period was observed (66), indicating that this is not the only explanation. Measured exudation rates have been shown to be highly variable. This may be partly attributed to methodological problems, e.g. lysis of fragile algal cells due to filtration. The present theory is that 5 - 50% of the primary production is channeled into the bacteria partly through exudation (e.g. 6, 63, 102). Larsson & Hagström (63) made an attempt to estimate the fraction of the labile DOM-pool which is exudate derived. They found that only about 0.1% of the total dissolved organic carbon (DOC) originated from exudates from phytoplankton. Compared to published values of what is normally considered to be the pool of easily utilized DOC, their exudate data were 1 to 2 orders of magnitude smaller. If only a minor part of the DOM-pool is exudate-derived, something else must contribute to this pool.

2. There is evidence that a significant loss of algal cell contents can occur during grazing by herbivores (e.g. 62), a phenomenon often referred to as "sloppy feeding". The quantitative significance of "sloppy feeding" as a mechanism for DOM-production probably depends on the types of algae and herbivores involved. For instance, fragile phytoflagellates may burst during handling much easier than more robust diatoms. Leakage of DOM from faecal pellets might also occur. Copping & Lorenzen (20) fed a zooplankton species on a $^{14}$C-labeled diatom and found that 18% of the ingested carbon was lost as DOM. Protozoa may also produce DOM when they empty their food vacuoles.
3. **Autolysis** may be another source of DOM. Nutrient limitation and other physico-chemical stresses may cause the death and autolysis of photoautotrophs (cf. 5). However, if so and to what extent this occurs in nature is not known. It can be speculated that cells lacking cell walls, e.g. flagellates and ciliates, lyse easier than cells with a cell wall, e.g. bacteria. Lysis may also be caused by other factors, such as viral attacks.

4. **POM** in the euphotic zone is a mixture of living plankton and detritus, in approximately equal proportions. The concentration of POM is highly variable, but normally 0.1 mg l\(^{-1}\) is found in the euphotic zone. The detrital part of the POM consists of dead cells, organelles, vesicles, faecal pellets and possibly aggregations of molecules arising from DOM. Bacteria are known to colonize faecal pellets (55) and algal cells (53). The attached bacteria feed on particles by producing exohydrolytic enzymes which convert POM into DOM, after which it can be taken up by the bacteria (17). However, it can be speculated that a significant amount of the produced food substrate (DOM) is wasted due to diffusion. Jacobsen & Azam (55) tested this hypothesis in an experimental system with \(^{14}\text{C}\)-labeled faecal pellets of a zooplankton species and colonizing bacteria. They found that the amount of \(^{14}\text{C}\) released into the sea water was two to three-fold of that assimilated by the bacteria.

3. **ADDITION OF A "MICROBIAL LOOP" INTO THE "CLASSICAL" FOOD WEB**

   In the classical view of the marine food web, bacteria were believed to be the main remineralizers in the sea, but this paradigm has been changed. Growth yields (growth efficiencies) of natural communities of marine heterotrophic bacteria have been determined to be as high as 70 - 80 % for the uptake of glucose and amino acids (77). Therefore, it might be argued that sea water is a good growth-medium, with substrates present in the "right proportions", and hence the bacteria capable of scavenging substrates at low concentrations would not be the main remineralizers in the sea.

   Furthermore, marine bacteria are not dormant, but are in fact the most important secondary producers in the sea. For instance, in the Norrby archipelago in the northern Bothnian Sea it has been shown that more than 50 % of the primary production is reassimilated into bacterial biomass (42). Since bacterial numbers in natural sea water usually remain quite stable (1 - 2 x 10\(^{6}\) ml\(^{-1}\)), there must be a sink for bacteria, e.g. lysis of or predation on the bacteria. Predation on the bacteria was hypothesized by Azam et al. (6) to channel some of the photosynthetically fixed carbon into the
conventional planktonic food chain, by the formation of a "microbial loop" (Fig. 2). Several methods for measuring predation rates on pelagic bacteria have been developed during the 80s. The methods used are based on the addition of trace amounts of labeled bacteria or bacteria like particles into a sea water sample, and an analysis of the decrease in the tracer bacteria after a period of incubation. One of these methods was developed by Wikner et al. (101), and it was found that the most important bacterial grazers have a size of 1-3 μm (100), i.e. small flagellates.

Figure 2. Flagellates and bacteria in sea water, illustrating compartments of the "microbial loop".
4. FLAGELLATES

Flagellates are ubiquitous in aquatic environments; fresh water, estuaries and marine environments. Flagellates in the sea are nutritionally versatile organisms. Purely autotrophic forms are found as well as mixotrophic, phagotrophic and saprotrophic / osmotrophic forms:

4.1. Purely autotrophic forms

According to the endosymbiotic theory of the origin of eucaryotic algae, the "primitive" forms of photosynthetic flagellates should be "zooflagellate-like", i.e. have an asymmetrical cell form, including asymmetrical inserted flagella, lack a cell wall and be phagotrophic (94). The more "advanced" forms ought to be purely autotrophic and have a symmetrical cell form, since that would seem to be the most efficient arrangement if the cell's shape only has to be concerned with motility and streamlining.

Most groups of phytoflagellates have an asymmetrical cell form and their cells are naked or scaly. One exception is the chlorophycean flagellates which have a cell shape approaching symmetry and some forms have a true wall (94). Consequently, many of the green algae are also purely autotrophic flagellates. The green algae are abundant in fresh water, while they are scarce in marine environments (cf. 11). Pure autotrophy has also been shown in the chrysophycean genera Synura and Mallomonas (cf. 10).

4.2. Mixotrophic forms

Documentation of phagotrophy can be found among the Prymnesiophyceae, Chrysophyceae, Cryptophyceae, Euglenophyceae and Dinophyceae (94). During the early 80's the dominating interest for bacterivores in the sea was focused on the heterotrophic flagellates, which were often identified as the non-pigmented forms of flagellates (e.g. 6, 86, 87). However, in 1985, Porter et al. (80) suggested that the pigmented forms of flagellates are able to utilize particulate food sources as well. This assumption was based on the fact that some pigmented and non-pigmented forms are structurally similar (e.g. asymmetric cell form) and hence systematically close. Few studies have been concerned with the relative importance of the phagotrophy and photosynthesis of the mixotrophic organisms.
Estep et al. (26) investigated the possibility of heterotrophy in isolated pigmented chrysophycean clones. These flagellates grew in light with the addition of bacteria and transmission electron microscope studies revealed the presence of bacteria within their food vacuoles. Contrary to the light incubated culture, dark incubated flagellates did not grow. Autotrophy therefore seems to be the main source of nutrition in these forms.

Bird & Kalff (9) found that six common species of fresh water algae, of the genera *Dinobryon* and *Uroglena*, ingest bacteria-sized fluorescent latex beads. Phagotrophy was found to be of more importance than autotrophy in *Dinobryon* (10). As much as 30% of the phytoplankton in the oligotrophic lake Bowker, were observed to ingest small particles actively. The phytoplankton and not the less numerous zooplankton were responsible for most of the bacterial grazing in the lake (10).

4.3. *Saprotrophic / osmotrophic forms*

Some chlorophytan flagellates, e.g. *Polytoma* and *Polytomella*, have lost their chloroplast and thus turned to heterotrophy, presumably feeding on DOM (cf. 94). Nevertheless, it has been shown that they still possess starch-containing plastids (21). Feeding on dissolved organic matter seems to be of minor quantitative importance in nature (e.g. 27, 40).

4.4. *Phagotrophic forms*

Many non-pigmented flagellates of different species have been successfully cultivated on bacteria. These have been shown to have bacteria as well as degrading bacteria within their cells (27, 40). Non-pigmented phagotrophic flagellates are also ubiquitous in aquatic environments, and have been suggested to be the most important bacterial grazers (30). Peaks of growth by the bacterial communities have been observed to be followed by peaks of heterotrophic flagellates some 3 to 5 days later (4, 30). Choanoflagellates, chrysomonads and bicoesids are examples of frequently occurring forms of heterotrophic flagellates found in marine environments (4).
5. BACTERIVORES IN THE SEA

5.1. *Feeding mechanisms of the bacterivores*

Common to many bacterivores is that they are suspension-feeding organisms with adaptations for gathering food particles. The capturing mechanisms cannot be based on inertial forces since the Reynolds number (inertial force / viscous force) is \(<1\) (31). Therefore, the predator has to produce a water current directed towards its cell body in order to capture the suspended bacteria. Particle capture is often quantified as the volume of water an organism can clear from particles per unit time ("clearance rate"), i.e., uptake rate divided by food concentration. Different organisms have developed different mechanisms for particle-capture. The capture-mechanisms available can be grouped into sieving (filter feeding), direct interception and diffusion (Fig. 3).

---

**Figure 3.** Feeding mechanisms in bacterivorous organisms: A) Filter feeding, B) Direct interception C) Diffusion feeding.
Some flagellates are filter feeders, e.g. choanoflagellates and helioflagellates. In choanoflagellates the filters are "collars" consisting of 20 - 50 pseudopodia, each about 0.1 μm thick. The distance between each tentacle is 0.1 - 0.3 μm (32). In the centre of the base of the collar there is a smooth flagellum, which drives a water current from the cell so that bacterial particles are trapped on the outside of the collar (Fig. 3 A). The helioflagellates have a filter consisting of 10 - 12 pseudopodia, which are also arranged in a collar. The diameter of the pseudopodia is about 0.2 μm and the distance between them about 1 μm at the base and 3 μm at the top (27). Similarly a flagellum is situated in the middle of the collar, but in comparison to the choanoflagellates the helioflagellates sweep the water toward the cell and hence bacteria are trapped inside the collar. Another example of a group of organisms which are filter feeders is the ciliates. In the ciliates water currents are produced by membranelles which are situated close to the mouth region and bacteria are trapped on motile or non-motile cilia (32).

The feeding mechanism of the type "direct interception", is found in many small flagellates. In the chrysomonads, water is swept towards the cell by a hairy flagellum. Particles which are carried by the water-flow and touch the mouth region are caught and ingested (Fig. 3 B). Interception feeding is less efficient than e.g. sieving, since the food particles are handled one by one. Due to the increasing surface to volume ratios with decreasing size of the organisms and vice versa, it is only the smallest bacterivores which possess "direct interception" as a feeding mechanism.

Diffusion feeding is found among amoebae, foraminifers and some ciliates. In this type of feeding the food particles, motile or non-motile, are caught and intercepted by a motionless grazer (Fig. 3 C). As an example, in the foraminifera the prey organisms are attached to the pseudopodia and drawn toward the predator by contraction or cytoplasmatic streaming.

5.2. Organisms

Possible bacterivores in the sea are the metazoans and the protozoans. Some metazoan organisms, such as the cladoceran *Daphnia*, are capable of feeding on bacteria (e.g. 46, 72), although the efficiency as a bacterivore is low (Table 1). One exception is the appendicularian *Oikopleura*, which has such a high specific clearance rate (clearance rate / biovolume) that it can possibly base its nutrition on the ingestion of bacteria.
Table 1. Maximum specific clearance rate in some protozoa and metazoan. From Fenchel (31) unless indicated by *, ** or ***.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Approximate volume (ml)</th>
<th>Specific clearance (cell vol. h⁻¹)</th>
<th>Mechanism</th>
<th>Minimum particle size retained (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagellates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosiga</td>
<td>2 x 10⁻¹¹</td>
<td>9.8 x 10⁴</td>
<td>sieving</td>
<td>0.2</td>
</tr>
<tr>
<td>Actinomonas</td>
<td>7.5 x 10⁻¹¹</td>
<td>1.1 x 10⁶</td>
<td>sieving</td>
<td>1 - 2</td>
</tr>
<tr>
<td>Paraphysomonas</td>
<td>1.9 x 10⁻¹⁰</td>
<td>9.1 x 10⁴</td>
<td>interception</td>
<td>no sharp limit</td>
</tr>
<tr>
<td>Pseudobodo</td>
<td>9 x 10⁻¹¹</td>
<td>1.1 x 10⁵</td>
<td>interception</td>
<td>no sharp limit</td>
</tr>
<tr>
<td>Monas*</td>
<td>3 x 10⁻¹¹</td>
<td>3.2 x 10⁴</td>
<td>interception</td>
<td>no sharp limit</td>
</tr>
<tr>
<td>Ochromonas**</td>
<td>2 x 10⁻¹⁰</td>
<td>5.2 x 10⁴</td>
<td>interception</td>
<td>no sharp limit</td>
</tr>
<tr>
<td>Ciliates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>small pelagic ciliates;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. Cyclidium</td>
<td>5 x 10⁻¹⁰</td>
<td>6 x 10³</td>
<td>sieving</td>
<td>0.3</td>
</tr>
<tr>
<td>plankt. oligotrichous;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. Strombidium ***</td>
<td>0.9-4 x 10⁻⁸</td>
<td>6-9 x 10⁴</td>
<td>sieving</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Metazoa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cladocera;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia appendicularia;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oikopleura</td>
<td>10⁻⁶ - 10⁻⁴</td>
<td>2.2 x 10⁴</td>
<td>sieving</td>
<td>0.2 - 0.5</td>
</tr>
</tbody>
</table>

* = Data from Sherr et al. (84)
** = Data from Fenchel (28).
*** = Data from Jonsson (57)

Protozoans feeding on suspended marine bacteria are the ciliates, the amoebas and the flagellates. The quantitatively dominating groups of marine planktonic ciliates are the tintinnids and oligotrichous ciliates, which are specialized for larger particles than bacteria (Table 1); yet there are a restricted number of ciliates, mainly the oligohymenophorans, which specialize on smaller food particles (Table 1, cf. 31). However, these ciliates require higher concentrations of bacteria than is normally found in the pelagic environment. Bacterial concentrations are high enough for sustenance of these bacterivorous ciliates only in sediments and in early successional stages of decomposing of organic material. Amoebas have a gliding movement and are hence specialized for living on surfaces. They have been found in pelagic systems (e.g. 30), but their quantitative importance is not well-known. They are believed to feed on bacteria attached to detritus particles or water films. Empirical data show that among the bacterivores, it is the flagellates which have the highest specific clearance rates (Table 1).
E. AIM OF THE THESIS

When starting the work on my thesis I was inspired by the comprehensive protozoan studies of Prof. Tom Fenchel, who proposed that nanoflagellates are the most important bacterivores in the sea. I choose to have an autoecological approach in my work using an isolated flagellate as a model organism for flagellates in the sea. The flagellate, *Ochromonas* sp, is commonly occurring in fresh waters and marine waters as well as in brackish waters. *Ochromonas* is nutritionally versatile as it can feed on DOM, bacteria and is able to photosynthesize as well. The main area of investigation was the Norrby archipelago in the northern Bothnian Sea, with a salinity of 3 - 7 °/oo.

The questions to be answered were:

# How common are nanoflagellates in the sea? What forms occur and how are their annual and diurnal dynamics?

# What is the relative importance of phagotrophy and photosynthesis of the isolated flagellate? What is the selective advantage which permits the extra cost of using different modes of nutrition?

# What is the contribution of flagellates to the nutrient remineralization when consuming bacteria?

# Do the flagellates contribute to the DOM-pool by excretion of free amino acids?

# What impact do flagellates have on the size-distribution of the bacterial community in sea water?
F. RESULTS AND DISCUSSION

1. NANOFLAGELLATES IN THE SEA

1.1. Annual and diurnal dynamics

The seasonal succession of pigmented and non-pigmented nanoflagellates was determined in the euphotic zone (0-14 m) in a coastal area in the northern Bothnian Sea (Fig. 4). During the winter the flagellate concentration was low, about 100 - 200 cells ml$^{-1}$. When the ice cover was decimated and the productive season started in late April - middle of May (42), the flagellates became more abundant. The maximum concentration of flagellates, about 8000 cells ml$^{-1}$ occurred in late July. The average temperature in the euphotic zone during this period was 15 °C, and noticed as the maximum temperature for the year. The distribution of pigmented and non-pigmented flagellates was similar throughout the year. On an average, 54 % of the flagellates were pigmented and 46 % non-pigmented.

![Graph showing abundance of pigmented and non-pigmented nanoflagellates](image)

**Figure 4.** Abundance of pigmented and non-pigmented nanoflagellates in the euphotic zone in the Norrby Archipelago in the northern Bothnian Sea, 1987. Total numbers of flagellates were quantified using epifluorescence microscopy of formaldehyde fixed and primulin-stained samples (13).
During the winter season, the number of unicellular cyanobacteria was lowest, about 3000-6000 cells ml\(^{-1}\) (Fig. 5). In June a drastic increase in numbers was observed, followed by a decrease in late July and early August. This decrease in abundance of cyanobacteria in July have been observed to be a yearly recurrent event. A second maximum of cyanobacteria was observed in September, after which the numbers decreased as winter approached. We have shown that the flagellate *Ochromonas* sp., isolated from the waters of the northern Bothnian Sea, prefers to feed on cyanobacteria rather than heterotrophic bacteria (paper IV). Therefore, it is possible that the decrease observed during summer was an effect of flagellate predation on cyanobacteria. In a study of an oligotrophic marine environment we calculated that flagellate predation on cyanobacteria accounted for 57\% of the primary production, and hence was an important pathway in the energy-flow (paper IV). Grazing experiments, *in situ*, have also shown that cyanobacteria are actively consumed and metabolized by a variety of micrograzers, such as the protozoa (54).

The species composition of any group of organisms in estuarine habitats is characterized by relatively low number of species, compared to marine and fresh water habitats (82). The community of nanoflagellates in this study was also characterized by relatively few species (Table 2). Species >20 \(\mu\)m such as the dinoflagellate *Gonyaulax catenata* (26 \(\mu\)m), occurring during early spring under the ice (71), are excluded from this study. The non-pigmented nanoflagellates were, on an average, smaller in size.
Table 2. Identified nanoflagellates in the Norrby archipelago in the northern Bothnian Sea.

<table>
<thead>
<tr>
<th>CHRYSOPHYCEAE</th>
<th>pigmentation</th>
<th>size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysomonadales</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ochromonadaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ochromonas spp.</td>
<td>pigmented</td>
<td>3.0</td>
</tr>
<tr>
<td>Monas spp.</td>
<td>apochlorotic</td>
<td>2.4</td>
</tr>
<tr>
<td>Chromulina sp.</td>
<td>pigmented</td>
<td>3.0</td>
</tr>
<tr>
<td>Prymnesiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysochromulina sp.</td>
<td>pigmented</td>
<td>3.3</td>
</tr>
<tr>
<td>Prymnesium sp.</td>
<td>pigmented</td>
<td>2.6</td>
</tr>
<tr>
<td>Bodonaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodo sp.</td>
<td>apochlorotic</td>
<td>3.0</td>
</tr>
</tbody>
</table>

| CRYPTOPHYCEAE                  |              |           |
| Cryptomonadales               |              |           |
| Cryptomonadaceae              |              |           |
| Rhodomonas spp.               | pigmented    | 8.8       |
| Katablepharis ovalis          | apochlorotic | 7.0       |
| Phyllophitus sp.              | apochlorotic | 13.0      |
| Peridinates                   |              |           |
| Gymnodiniaceae                |              |           |
| Amphidinium sp.               | pigmented    | 10.0      |

| CHLOROPHYCEAE                  |              |           |
| Volvocales                     |              |           |
| Chlamydomonadaceae             |              |           |
| Polytopa sp.                   | apochlorotic | 7.0       |
| Carteria sp.                   | pigmented    | 5.9       |

Flagellates were identified in fixed samples, by using 1) epifluorescence microscopy, 2) the Uthermöhl technique of Lugol stained samples, or 3) alive in enrichment cultures.

(3 µm) than the pigmented flagellates (6 µm). Among the non-pigmented flagellates, the genus Monas was the most dominating group throughout the year, causing the relatively low average size. The pigmented flagellates demonstrate a higher taxonomic variation during the year, and in August-September there was a bloom of Carteria and Rhodomonas (Table 3). During other seasons the chrysophyceans were the most abundant flagellates. Species belonging to the genus Ochromonas were found all through the year. On an average they consisted of about 14 % of the total nanoflagellates. As stated by Throndsen (96), most naked flagellates should be identified alive in fresh concentrated water samples. The genera Bodo and Ochromonas were identified in this way but the other groups were identified in fixed (Lugols solution or formaldehyde) samples.
Table 3. Relative abundance of different groups of nanoflagellates in the Norrby Archipelago in the northern Bothnian Sea observed in the middle of August, 1987.

<table>
<thead>
<tr>
<th>Taxonomic group</th>
<th>relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ochromonas sp.</td>
<td>16</td>
</tr>
<tr>
<td>Monas spp.</td>
<td>37</td>
</tr>
<tr>
<td>Chromulina sp.</td>
<td>1</td>
</tr>
<tr>
<td>Chrysochromulina sp.</td>
<td>4</td>
</tr>
<tr>
<td>Prymnesium sp.</td>
<td>2</td>
</tr>
<tr>
<td>Carteria sp.</td>
<td>22</td>
</tr>
<tr>
<td>Rhodomonas spp.</td>
<td>17.5</td>
</tr>
<tr>
<td>Amphidinium sp.</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Few studies of the dynamics of flagellates in the Bothnian Sea are found in literature. Niemi & Åström (73) studied the vernal dynamics of phytoplankton at the entrance to the Gulf of Finland (Tvärminne). In agreement with our results (Fig. 4) they found that non-pigmented flagellates increased noticeable after the phytoplankton spring-bloom. Andersen & Sørensen (4) studied the population dynamics of the microorganisms in the Limfjord and found that in autumn, winter and early spring the heterotrophic nanoflagellates were dominated by loricate choanoflagellates. During the summer the heterotrophic nanoflagellates were dominated by choanoflagellates, the chrysomonad Paraphysomonas and a non-loricate bicoesid (Pseudobodo tremulans). Pigmented flagellates of quantitative importance were Ochromonas and Cryptomonas. Heterotrophic nanoflagellates showed several peaks in abundance during the summer months which were assigned predator-prey fluctuations including ciliates, flagellates and bacteria. At the maxima in flagellate abundance, concentrations of 5000 to 14000 cells ml⁻¹ was observed. Their investigation area in the Limfjord is a more marine habitat (salinity 25 %/oo) than our station in the northern Bothnian Sea (salinity 3 - 5 %/oo) so the species-composition can not be compared strictly. Yet, it is interesting to note that many genera are found at both locations and that the total concentration of nanoflagellates is in the same order of magnitude as in the northern Bothnian Sea.

In a study of the nanoplankton in a marine environment, in the subarctic Pacific, it was observed that among the most abundant forms were 2 species of Cryptomonas and 4 species of choanoflagellates (12). Studies of marine nanoflagellates have shown that species with and without chloroplasts are about equally common (13, 23), which is in agreement with our data.
Figure 6. Vertical distribution of flagellates during four different seasonal occasions in the Norrby Archipelago in the northern Bothnian Sea, 1987.

The vertical distribution of flagellates in this study showed a positive relationship to temperature (Fig. 6). Almost no vertical variation in abundance was observed under non-stratified conditions, which was the dominant hydrographic state of the water this year. Continuous mixing of the water mass and thus the flagellates, due to non-stratified conditions, might be the explanation for the equal distribution in the depth profiles. A clear vertical distribution of flagellates was observed in July, when the water-mass was stratified. The highest concentration was observed in the surface water,
and decreasing abundances was observed with depth. The reason for this distribution must be that there are more favourable conditions in the euphotic zone than below: Flagellates themselves may assimilate the solar radiation and/or they are indirectly favoured through their prey, which are also directly or indirectly dependent on the sun.

In mesocosm experiments we have shown that flagellates have a diel variation: The maximum concentration of nanoflagellates was observed in the early morning, whereas during the day the flagellates decreased in abundance until around midnight when they increased in abundance (paper IV). Our results indicate a synchronous division of flagellate cells. In agreement with our findings, Elbrächter (25) showed that species of the genus *Ceratium*, in the coastal waters of the Kiel Bay, divide synchronously during the early hours of the morning. These pigmented flagellates may be phagotrophic, since bacteria have been observed in the food vacuoles of at least one *Ceratium* species (cf. 10). The observed diel pattern might be a response to starvation during the night, causing a duplication of the cells (29). A reason for the observed decrease in the flagellate abundance may be predation on the flagellates, presumably by ciliates (83).

### 1.2. Nutrition of the identified nanoflagellates

An interesting question is: what types of nutrition do the identified groups of flagellates utilize? The abundantly occurring non-pigmented flagellates, *Monas* spp., are in literature described as bacterivorous (e.g. 84). The pigmented chrysophyceans and cryptophyceans can be expected to be mixotrophs (10, 21). The pigmented chlorophycean *Carteria* is presumably a purely autotrophic organism, since the cells seem to have a high pigment content (they are highly autofluorescing) and have a cell wall (21), which phagotrophs usually do not have. The non-pigmented chlorophycean *Polytoma* has a cell wall and is described as being saprotrophic in literature (94). The non-pigmented cryptophyceans *Katablepharis* and *Phyllomitus* are regarded as being phagotrophic, since phagotrophy is common in this group. In conclusion, most of the identified nanoflagellates are interception feeding phagotrophs, some of them are also photosynthetic (mixotrophic) and only one group, the genus *Carteria*, is purely photosynthetic.

Wikner and Hagström (100) studied the predation on bacteria and found indications of a predator-prey chain of four trophic levels in the sea. Organisms in the size range 1-3 μm were found to be the most important bacterivores. Using their data together with the flagellate data derived in this study it is possible to outline a food chain (Fig 7). The most important bacterivorous organisms are found in the size range 1-3 μm. These
flagellates, as well as the purely autotrophic *Carteria* are food for the larger mixotrophic flagellates of the genus *Rhodomonas*. Dinoflagellates (*Amphidinium*) and presumably ciliates, feed on *Rhodomonas*. Ciliates <20 μm have been reported to compose 4 - 57% of heterotrophic nanoplanckton in marine ecosystems (85). Ciliates should therefore be included, if the outlined food web was continued to higher trophic levels.

**Figure 7.** Outlining of a food web valid for August-September in the Norrby archipelago in the northern Bothnian Sea.
2. OCHROMONAS sp.

2.1. Autotrophy and phagotrophy

In light of the scarcity of published information on the relative importance of phagotrophy to photosynthesis among mixotrophic flagellates, a nutritional study of the non-cyst-forming Ochromonas was carried out (paper I). The flagellate was found to grow rapidly on bacterial food (at $\mu_{\text{max}}$ the generation time was 5.1 hours), and light did not influence the heterotrophic growth. Nevertheless, the flagellate seems to have a functional photosynthetic apparatus with reference to the pattern of light saturation curves and changes in the chlorophyll $a$ content, with varying light conditions. The flagellate showed a high light acclimation compared to many other algae. However, Porter (79) reported that a related flagellate, Poterioochromonas malhamensis, also has a high light acclimation. Their result indicated that the flagellate was not photoinhibited at extremely high light intensities (1500 $\mu$E m$^{-2}$ sec$^{-1}$). The reason why this type of algae is high light saturated might be their low chlorophyll content (paper I). The efficiency of the photosynthesis, i.e. photosynthetic rate divided by chlorophyll content, was found to be at least one order of magnitude lower than for other marine algae.

The Ochromonas did not multiply in an inorganic "autotrophic" medium. Nevertheless, the cells remained viable when incubated in light, while an increased mortality was observed for the dark-incubated cultures. Hence it can be speculated that photosynthesis is a survival mechanism for Ochromonas during starvation periods. This idea was confirmed by a starvation experiment where Ochromonas were first grown on bacteria. The flagellate became starved when the bacteria were sufficiently reduced in numbers. Light incubated cells survived starvation while dark incubated cells died.

Bird & Kalff (10) determined the importance of phagotrophy to photosynthesis in Dinobryon by in situ measurements. They found that these chrysophycean algae depend more strongly on ingested bacteria than on photosynthesis. Furthermore, the clearance rate per cell was found to be similar during both day and night, i.e. independent of light. The clearance rate was, however, strongly correlated to temperature. The nutritional characteristics of Dinobryon was similar to that in Ochromonas. Another chrysophycean alga, Poterioochromonas malhamensis, was also shown to share some nutritional characteristics with Ochromonas (79). The algae was found to be phagotrophic, but contrary to our results phagotrophy could be regulated by environmental factors: The ingestion rate of bacteria was shown to decrease when treated under high light conditions. High concentrations of DOM, above 4 mg C l$^{-1}$, were also found to have an inhibiting effect on the ingestion of particles. To my knowledge there is not many
studies performed on the relative importance of phagotrophy to autotrophy in the chrysophycean algae, but available data shows that at least two forms are mainly heterotrophic organisms.

Due to the extra cost to produce components for and to utilize different types of nutrition it may be hypothesized that mixotrophy must serve a designated purpose for the organism. For a mainly autotrophic organism it could be to ensure survival during poor autotrophic conditions, e.g. low light levels or nutrient depletion. Alternatively it may be argued that mainly heterotrophic organisms would benefit from a photosynthetic apparatus in order to survive poor heterotrophic conditions, e.g. depletion of food particles. Situations with poor autotrophic or heterotrophic conditions occur at both short and long time scales, due to diel as well as seasonal variations. Thus a mixotrophic life-strategy could be successful in a number of different environments.

2.2. Clearance rate

A direct measure of the competitive ability with scarce resources is the maximum clearance rate (F_m) of an organism. The F_m of the isolated Ochromonas was calculated from the growth response curve presented in paper I. The growth response curve can be fitted to a hyperbolic function (when plotted on a linear scale), which is analogous to the Michaelis-Menthen equation for describing enzyme kinetics. The maximum growth rate (\mu_{max}) and the half saturation constant (K) were calculated to 0.135 h^{-1} and 1.79 \times 10^6 bacteria ml^{-1} respectively. The maximal production of new flagellate biomass on a per cell basis would equal 9.8 \mu m^3 h^{-1}, as the average cell volume in the this experiment was found to be 50 \mu m^3. At temperatures around 20 - 23 °C gross growth- efficiencies (growth / ingestion) varying from 30 to 60 % have been reported (14, 28, 84), while the average growth yield seems to be about 45 %. Assuming a growth yield of 45 %, the corresponding maximal ingestion rate (U_m) of bacterial biomass would thus equal 21.8 \mu m^3 h^{-1}, or 218 bacteria (Escherichia coli minicells) flagellate^{-1} hour^{-1} (minicell volume 0.1 \mu m^3 (101)). Using the equation F_m = U_m K^{-1} (32), the maximum clearance rate was calculated to be 1.22 \times 10^{-4} ml hour^{-1}. The corresponding specific clearance rate (clearance rate divided by biovolume) is 2.44 \times 10^6 hour^{-1}. The maximum specific clearance rate calculated for our isolated Ochromonas is higher than what is reported for another isolate of Ochromonas (28), possibly due to the smaller size of our isolate. Also, when comparing this with the clearance rates of other flagellated protozoa (Table 1), it is clear that this species must be regarded as a highly efficient / competitive bacterivore. However, maximum specific clearance data for several different flagellates presented by Davis (22) range from 1.3 \times 10^6 to 2.8 \times 10^6, which is similar to the rate.
obtained for our isolate of *Ochromonas*. Since the bacteria that were used in this experiment are similar in size to those found in nature, the half saturation constant (K) in the growth response experiment gives an indication of the need of bacterial abundance in the sea for sustenance of *Ochromonas* and other similar-sized flagellates. In agreement with this, K (1.79 x 10^6 bacteria ml\(^{-1}\)) was found to be similar to the bacterial concentrations found in nature (42).

2.3. Heterotrophic feeding on DOM

*Ochromonas* was shown to grow slowly, with a generation time of 30 hours, in a liquid organic medium containing approximately 6 g C l\(^{-1}\) (paper I). A bacterial concentration of 1.76 x 10^{11} cells ml\(^{-1}\) would be needed to achieve the same carbon content. However, when grown on bacteria the flagellate reaches \(\mu_{\text{max}}\), a generation time of 5.3 hours, already at a concentration of about 5 x 10^8 bacteria ml\(^{-1}\). In the Pacific Ocean the utilizable DOM (UDOM) was determined to be about 5 % of total DOM (2). In the northern Bothnian Sea the DOM-pool is high, about 5.5 mg C l\(^{-1}\) (Sehlstam, unpublished), whereas the concentration of humic substances is high due to a large run-off from rivers. Humic substances are generally considered to be refractory substances, not easily utilizable compounds and therefore the percentage of UDOM can not be expected to be higher than in the Pacific Ocean. Five percent of the UDOM corresponds to 0.275 mg C l\(^{-1}\) which is far less than the concentration in the artificial DOM-medium, which gave rise to a generation time of 30 hours. It could be argued that the compounds in the mixture of the artificial DOM-medium is not as good for growth as is the sea water. However, when the flagellate was incubated in filtered and autoclaved sea water (supplemented with vitamins) the cell numbers did not increase neither in the light nor in the dark ( paper I).

The efficiency by which organisms can utilize DOM increases with the square when the cell size decreases (60):  

\[ E = 3 \times D \times R^{-2} \]

\(D = \text{diffusion constant}, R = \text{radius of the cell}\)

Heterotrophic bacteria in the Norrby archipelago in the northern Bothnian Sea have an average volume of 0.139 \(\mu\)m\(^3\) during spring- and summer-time (paper III) and if they were coccoid they would have a median diameter of 0.64 \(\mu\)m. Since the nanoflagellates on an average have a diameter of 4.4 \(\mu\)m, they would be 40 - 50 times less competitive for growth on DOM in the sea than the sea water bacteria. Therefore, saprotrophic /
osmotrophic feeding of the natural community of nanoflagellates can not be of any major nutritional importance.

2.4. Temperature optimum

The growth rate of the *Ochromonas* increased sharply from 5 to about 15 °C with a relatively broad temperature optimum around 20 °C. The corresponding $Q_{10}$-values (the increase in the growth rate for a 10 °C change in temperature) for each 5 °C increment was calculated using the equation (98):

$$Q_{10} = \left(\frac{\mu_1}{\mu_2}\right)^{\frac{10}{(t_1-t_2)}}$$

$\mu =$ growth rate, $t =$ temperature

The growth rate is highly temperature dependent within the temperature range 5 - 15 °C as shown by the high $Q_{10}$ values (Table 4). Generally, biological rates have $Q_{10}$ values between 2 and 3 (98). Between 15 and 25 °C a slow increase in the growth rate was found giving rise to values close to 1. Above 25 °C the $Q_{10}$ values for the temperatures are <1, indicating a decrease of the growth rates. Relatively few data on temperature effect on the growth of flagellated protozoa are available. In Table 4 the $Q_{10}$ values between the studied *Ochromonas*, a fresh water *Monas* and a marine *Paraphysomonas* are compared. There is a striking similarity in the pattern of $Q_{10}$ values between the *Ochromonas* in this study and the freshwater *Monas*. The marine *Paraphysomonas* obviously have a higher temperature optimum than the others.

Table 4. $Q_{10}$ values for growth rates of 3 different nanoflagellates.

<table>
<thead>
<tr>
<th>Temp. range (°C)</th>
<th><em>Ochromonas</em></th>
<th><em>Monas</em></th>
<th><em>Paraphysomonas</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5 - 10</td>
<td>3.4</td>
<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td>10 - 15</td>
<td>3.7</td>
<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td>15 - 20</td>
<td>1.2</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td>20 - 25</td>
<td>1.4</td>
<td>1.6</td>
<td>2.6</td>
</tr>
<tr>
<td>25 - 30</td>
<td>0.6</td>
<td>0.6</td>
<td>ND</td>
</tr>
<tr>
<td>30 - 35</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* calculated from data from Sherr et al. (84)
** calculated from data from Caron et al. (15).
ND = not detected
One possible interpretation of these data is that the growth rates of flagellates in the sea are highly temperature-dependent within temperatures ranging from 0 to 15 - 20 °C. In the northern Bothnian Sea the temperatures only exceed 15 °C in July and August (42), coinciding with the observed maximum in flagellate abundance (Fig. 4). Other possible factors controlling the density of the flagellate community are access of food (bacteria or other particles), abundance and activity of predators on the flagellates, and solar radiation and nutrient supply for the mainly photosynthetic forms.

2.5. Remineralization

Despite, some exohydrolytic enzymes (e.g. alkaline phosphatase), phytoplankton are dependent of remineralization of nutrients by other organisms. Heterotrophic bacteria have been excluded from the group of possible remineralizers since they have been shown to have high growth efficiencies (77). Other heterotrophic organisms, which may remineralize nutrients in the sea, are the protozoa and the metazoa.

We focused our interest on flagellates as being remineralizers, and used the Ochromonas as a model organism. The flagellate was fed bacteria, pregrown under non-limiting conditions, and the amount of remineralized nitrogen and phosphorus quantified (paper II) Thirteen percent of the ingested nitrogen and 30 % of the ingested phosphorus were excreted in the form of ammonia and phosphate, respectively. No excretion of NO_3 was observed.

Similar remineralization rates of nitrogen and phosphorus have been shown in both Monas and Paraphysomonas imperforata (3, 15, 39, 84). Furthermore, P. imperforata was shown to remineralize varying amounts of both nitrogen and phosphorus depending on if the bacterial prey are pregrown under nutrient-limited conditions or not. This phenomenon is probably due to the bacterial ability to produce storage products, e.g. phosphorus in the form of polyphosphate when accessive amounts are available (34).

A question that arises from the previous discussion is whether heterotrophic bacteria, an important food source for the nanoflagellates in the sea, grow under nitrogen- or phosphorus-limited conditions. Ammerman et al. (2) showed that sea water bacteria were able to grow in unsupplemented particle-free sea water. Nevertheless, it is possible that a nutrient, e.g. phosphorus or nitrogen, limits the rate of growth and final cell yield. To investigate if heterotrophic sea water bacteria, from the studied coastal area of the northern Bothnian Sea, grow under nitrogen or phosphorus limiting conditions a
Table 5. Growth rates and final cell yields from experiments of sea water bacteria grown in particle free sea water +/- supplement of nutrients.

<table>
<thead>
<tr>
<th>Date</th>
<th>NO₃ (100 μM)</th>
<th>PO₄ (2 μM)</th>
<th>Glycerol (2 mg C/l)</th>
<th>Control (---)</th>
</tr>
</thead>
<tbody>
<tr>
<td>88-04-24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth rate (h⁻¹)</td>
<td>0.13</td>
<td>0.13</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Yield (No ml⁻¹)</td>
<td>1.0 x 10⁶</td>
<td>1.1 x 10⁶</td>
<td>1.0 x 10⁶</td>
<td>9.0 x 10⁵</td>
</tr>
<tr>
<td>88-05-25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth rate (h⁻¹)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Yield (No ml⁻¹)</td>
<td>1.1 x 10⁶</td>
<td>1.0 x 10⁶</td>
<td>1.0 x 10⁶</td>
<td>1.0 x 10⁶</td>
</tr>
</tbody>
</table>

Selective filtration (0.6 μm Nuclepore, filtration by gravity) was used to obtain the heterotrophic bacteria fraction and the sea water medium was obtained by vacuum filtration (<200 mm Hg) of sea water through a 0.22 Millipore filter. Batch cultures (final volume 200 ml) of bacteria were grown in polycarbonate bottles, bacteria were inoculated by 10%. The cultures were supplemented with either NO₃ (100 μM), PO₄ (2 μM) or glycerol (2 mg C l⁻¹). The cultures were allowed to grow for 1.5 weeks in the dark at 15 °C. Samples were taken twice per day and cell abundance determined with the use of epifluorescence microscopy. Growth rates (μ) were calculated as μ=ln2 g⁻¹ (g= generation time) and yield was determined as the cell abundance when the culture reached stationary phase.

similar experimental system to Ammerman et al. (2) was used; Sea water was filtered free from bacterivores and the bacteria allowed to grow in particle-free sea water +/- supplement of nutrients. Neither the obtained growth rates nor the final yield of cells indicated that bacteria were exposed to a nitrogen, phosphorus or carbon limitation (Table 5). Since combinations of supplements were not tried it might be possible that the nutrients in the sea are balanced whereas they are limiting. It is also possible that nitrogen or phosphorus limitation occurs in patchiness or during other parts of the year than what was studied. Horrigan et al. (49) investigated the possibility of bacterial nutrient limitation in the Pacific Ocean using a two stage continuous culture system. They found a possible nitrogen limitation, since the cell yield was higher in the nitrogen supplemented culture than in the control. The northern Bothnian sea have a higher concentration of DOM than the Pacific ocean (Sehlstam, unpublished, 103), possibly causing the different results.

An implication of the results from the bacterial growth experiment is that nanoflagellates in the northern Bothnian Sea feed on bacteria which grow under non-limiting or possibly substrate balanced conditions. Consequently, moderate to high rates of ammonia and phosphate release can be expected. It seems likely that a significant part of the primary production is recycled in the water mass, considering several trophic levels in the water mass (83, 100).
2.6. Excretion of amino acids

Excretion of dissolved organic molecules by flagellates might be an important source of DOM, because of the relatively high abundance and activity of these organisms. Furthermore most of them lack a cell wall, giving rise to fragile cells that easily lyse. The flagellate *Paraphysomonas imperforata* has been shown to excrete 10% of the ingested particulate organic carbon (POC) as DOC and approximately 10% of the ingested POC as POC, presumably ejected food vacuoles (14). The flagellate has also been shown to excrete about 10 - 14% of the ingested phosphorus as dissolved organic phosphorus (DOP), when grown on organisms that are precultured with excess nutrients (3). If the prey organisms were phosphorus limited then virtually no DOP was excreted. When the flagellate was fed prey pregrown on excess nitrogen, and the culture reached stationary phase, then approximately 7% of the ingested nitrogen was excreted as urea (39).

Concentrations of about 50 - 100 nM of dissolved free amino acids (DFAA) are found in sea water (70, paper II), and rapid turnover of this pool have been demonstrated (48). To bacteria this serves as high qualitative food. The origin of DFAA is far from clear. In a diel study off Scripps Pier, the DFAA concentration tended to vary with the flagellate abundance (paper II). In growth experiments of *Ochromonas* the concentration of DFAA was shown to increase during the consumption of bacteria. Approximately 0.02% of the ingested nitrogen reappeared as DFAA. However, bacterial uptake of DFAA were not inhibited during the experiment. Hence, a considerably higher value of excretion of DFAA, 7.4%, was calculated after correction for the simultaneous bacterial uptake of DFAA. Excretion of certain individual amino acids may function as chemical signals, possible attractants for bacteria. We observed increased concentrations of aspartic acid, glutamic acid, serine and ornithine. The conclusion to be drawn from these experiments is that the flagellate excrete free amino acids to some extent, possibly to attract bacteria.

Assuming that nearly all bacterial production is consumed by flagellates and that DFAA are excreted to the same extent as in the laboratory experiment, 45 nM per day of DFAA would be released in the diel study off Scripps Pier. Considering several trophic levels in the water mass and that each trophic level release DFAA to some extent during feeding, then significant amounts of DFAA would be released by grazing.
2.7. Size-selective grazing

The relatively small sizes of marine bacteria compared to laboratory cultures, was suggested by some scientists to be due to either starvation or slow growth of the cells (74, 93). Others proposed that the size structure of the bacterial community might be influenced by a predation pressure (41, 99). Fenchel (27) presented a model where the clearance of a specific collector was assumed to be proportional to the square of the radius of the prey. In this model it is assumed that the radius of the predator is much larger than the radius of the prey and that bacteria are intercepted along the equator of the cell. These assumptions might not be correct since prey almost as large as the flagellate have been observed to be ingested (38, 81) and the attachment and ingestion mechanisms are virtually unknown.

The isolated *Ochromonas* has a similar size as the most important bacterial grazers (1-3 μm) in the sea (100). It may therefore be a good model organism for studies of the bacterivorous impact on the size-structure of bacterial communities. A two-stage continuous culture experiment was set up to find out the possibility of an altered size distribution of bacteria due to grazing (paper III). A mixed sea water culture of bacteria was grown in the first stage, and sterilized sea water was used as a medium. The growth rates of the continuous culture were similar to rates which can be found in nature (paper III), namely 0.03 h⁻¹ and 0.016 h⁻¹. The size of the bacteria decreased significantly when grazed by flagellates, from 0.245 μm³ (average volume in the control) to 0.130 μm³ in the chemostat culture with flagellates. The reduction in volume was mostly due to a reduction in the cell length. Bacteria less than approximately 0.6 μm in length presumably escaped ingestion, since they increased in abundance in the flagellate culture in comparison to the bacterial control, thus representing refugee bacteria. On the contrary, a 70 % reduction of bacteria larger than approximately 0.78 μm was found in the flagellate culture. In a separate experiment of similar design, *Ochromonas* was fed on both mixed sea water bacteria and cyanobacteria (paper IV). The flagellate was shown to prefer cyanobacteria to heterotrophic bacteria, since 59 % of the cyanobacteria present were grazed, compared to 11 % of the present heterotrophic bacteria: In the inflow the cyanobacteria accounted for 0.5 % of the total number of bacteria, while of the total grazed bacteria, the cyanobacteria accounted for 2.6 %. These experiments were not designed in such a way that makes it possible to test the model of size-selection, as presented by Fenchel (27, 31). Nevertheless, it is clear that the flagellate select large bacteria, due to either size or quality.

Size selectivity was tested on a *Dinobryon*, which was given fluorescent latex beads with diameters of 0.99 μm, 0.57 μm and 0.28 μm (10). Beads with diameters of 0.28
were almost totally discriminated for ingestion, while beads of the size of 0.99 \( \mu m \) and 0.57 \( \mu m \) were ingested equally. Their results are in agreement with our results for \textit{Ochromonas}. The model of size selective grazing presented by Fenchel would predict uptakes ratios of 1 to 4.1 to 12.8, whereas the observed uptake ratios were 1 to 76 to 76 indicating that the model of interception-feeding presented by Fenchel (31) is not valid in this case.

Feeding selectivity may not only be attributed to the size of the prey but also to the quality of the prey. Qualitative selectivity has been shown in some ciliates (cf. 31). The flagellate \textit{Paraphysomonas imperforata} has shown some selectivity for quality of the food, since in feeding experiments it did not feed on certain algae. However, several studies show that flagellates are rather versatile in their nutrition on particles (28, 39, 81). Inert particles such as latex beads are also taken up, although at lower rates than bacteria, and have been used in studies of trophic interactions in the sea (e.g. 67, 75). It can be hypothesized that high qualitative particles, e.g. bacteria, initiate phagocytosis and inert particles will follow passively, e.g. the phagocytising region is occupied by more than one particle. On the other hand, an \textit{Ochromonas} grown in an axenic culture, without any qualitative particulate food present, has been shown to ingest inert polystyrene latex beads (24), which make this hypothesis unlikely. The amoeba, \textit{Entamoeba histolytica} has shown selectivity in its feeding on bacteria, since only those bacteria which have the appropriate recognition mechanisms will become attached and ingested. Furthermore, the attachment of bacteria onto the surface of the amoeba has been shown to be mediated by both bacterial and amoebic lectins (cf. 69). It is known that flagellates excrete a "sticky" material which acts aggregating on particles and possibly DOM (1). Flocculation may be a mechanism for external particle feeding, with digestion occurring outside the cell. Alternatively it may be a way to concentrate prey organisms.

2.7.1 \textit{Impact on the bacterial community in the sea}

In the Norrby Archipelago of the northern Bothnian Sea, the bacterial community was studied for three consecutive years (paper III). It was found that the median bacterial volume was lower during the summer (0.11 \( \mu m^3 \)) than during spring (0.17 \( \mu m^3 \)). Factors that may alter the cell volumes of the bacteria are temperature, growth rate and predation. The growth rate of the bacteria increased from approximately 0.019 h\(^{-1}\) to 0.039 h\(^{-1}\) from spring to summer. It is well known that enteric bacteria increase their cell volume when they are shifted from low to high growth rates (65), a feature valid also for sea water bacteria (64). Assuming the relation between growth rate and cell
volume derived from experimental data of Larsson & Hagström (64) to be valid also in nature, the corresponding increase in the growth rate of the bacteria would be followed by an increase in the cell volume by 26%. From the spring situation to the summer situation the temperature increased approximately by 8 °C. Hagström & Larsson (43) showed that sea water bacteria, forced to grow at the same growth rate at different temperatures, increase their cell sizes at decreasing temperatures. Translating this into the temperature effect on bacteria in nature, a 27% decrease would be expected. Thus, the effects of changed temperatures and growth rates on the bacterial cell sizes should approximately equal each other. The remaining factor, predation, thus has to be taken into account. The number of nanoflagellates increased 8-fold, from about 800 cells ml\(^{-1}\) in May to 6200 cells ml\(^{-1}\) in July (Fig. 4). The *Ochromonas* was found to reduce the average size of sea water bacteria to 0.13 \(\mu\text{m}^3\), similar to the *in situ* size of the bacteria in summer (average for three years: 0.11 \(\mu\text{m}^3\)). It is therefore likely that a significant part of the decrease is caused by size-selective (either by size or by higher food quality of large bacteria) predation on the bacteria.

Chrzanowski (18) and Chrzanowski et al. (19) studied the seasonal variation of bacterial volumes in a monomictic lake. They found the bacterial volumes to be negatively correlated to the temperature: In winter the maximum bacterial volumes were observed to be 0.217 \(\mu\text{m}^3\), and in the summer the minimum bacterial volumes were observed to be 0.127 \(\mu\text{m}^3\). Approximately 38% of the variation in bacterial cell volume could be accounted for by changes in temperature. They suggested that the decrease in size during the summer was a result of an intrinsic property of the bacteria at increasing temperatures. Still, only a part of the decrease in cell volume of the bacteria could be attributed to temperature changes, so the effect of a predation-pressure could not be excluded.
G. CONCLUSIONS

In the Norrby Archipelago in the northern Bothnian Sea, nanoflagellates have a characteristic seasonal pattern, with lower abundance in the winter (100-200 cells ml\(^{-1}\)) and higher abundance during the summer (reaching 8000 cells ml\(^{-1}\)). Throughout the year, pigmented and non-pigmented forms are about equally common. Most of the identified flagellates are phagotrophic organisms.

A pigmented form was isolated, *Ochromonas* sp., and shown to be a mainly heterotrophic organism: Autotrophic growth was poor, whereas phagotrophic feeding on bacteria gave high growth rates. However, photosynthesis seems to be an important survival mechanism during poor heterotrophic conditions.

In laboratory experiments, the flagellate has been shown to select large sea water bacteria, leaving small bacteria as a "refugee-community". In the Norrby Archipelago a maximum of the nanoflagellates coincides with a decrease of the sizes of the heterotrophic bacteria and in the abundance of cyanobacteria.

The flagellate remineralize nitrogen and phosphorus, and excrete amino acids during consumption of bacteria. Hence, flagellates both graze and nurture planktonic heterotrophic and autotrophic bacteria and can be regarded as "microbial gardeners"
H. ACKNOWLEDGEMENTS

The first time I visited this research group Åke Hagström took me on a snow scooter on very weak ice covered with half a meter of water. Other people that were present around us fell through the ice, but surprisingly enough we managed to carry out a sampling at the field station "Systrarna" without any complications. Despite this attempt to drown me, I want to thank him for introducing me to the marine science and for enthusiastically supervising me in this study.

I am also grateful to the other members of this group; Sven-Erik Hall who has always wished me "good luck" in whatever I have done, Eva-Lena Hörnfeldt for technical assistance and who seems to be synchronized with me in the respect of given birth to children. Johan Wikner who also had the extra work of taking care of his two children during his thesis work, Stina Bäckman for technical assistance, Bosse Norrman for new inspiration to this group and Fia Rehnstam who sings joyfully and works at the "DNA-level".

Many thanks to the people at the department of Microbiology and the Norrby laboratory for their support and for providing a pleasant atmosphere.

I want to thank Bosse Norrman, Peter Blomqvist, Agnes Müller-Haeckel, Göran Lundberg and Sif Johansson for their comments on drafts of this manuscript.

Finally, I want to thank my husband, Åke, who has given me support and has taken care of the family; our son Einar who wanted me to bring the computer home and sometimes said: "Mamma inte gå labet", and our daughter Ylva who eats and flourishes.

This work is a contribution from the Center for Marine Research at the Norrby laboratory, University of Umeå. It was financially supported by grants from the Swedish Natural Science Research Council and the Swedish National Environmental Protection Board.
I. REFERENCES


51. Imai T, Hatanaka M (1950) Studies on marine non-colored flagellates, Monas sp., favourite food of larvae of various marine animals. I. Preliminary research on cultural requirements. *Science Rept Tohoku Imp Univ* 18: 304-315


