

Functional diversity of heterotrophic flagellates in aquatic ecosystems

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ABSTRACT

There is a lack of taxonomic resolution due to methodological problems in most ecological reports despite the significant contribution of heterotrophic flagellates (HF) to the carbon cycle of most aquatic ecosystems. The determination of HF species on a quantitative level is difficult, especially for most athecate and aloricate taxa. The dominant taxonomic groups among heterotrophic nano- and microflagellate communities within different marine, brackish and limnetic pelagic communities (heterokont taxa, dinoflagellates, choanoflagellates, kathablepharids) and benthic communities (euglenids, bodonids, thaumatomastigids, apusomonads) seems to be surprisingly similar. HF among protista *incertae sedis*, often neglected in ecological studies, were abundant in all investigated habitats. The taxonomic variety of HF reflects the large diversity of functions of HF such as predominant bacterivory, herbivory, carnivory, detritivory and omnivory, respectively. Typical benthic HF can significantly contribute to pelagic HF communities especially in limnetic and marine coastal waters. High tolerances to changes in salinity give rise to the assumption that several species are able to live both in marine as well as in freshwater habitats. The functional diversity of HF is discussed with respect to the feeding ecology, life strategies, tolerances to extreme abiotic and biotic conditions and distribution patterns. Considering the strong predation pressure by metazoans and protists on HF communities many morphological and behavioural features of HF may be explained as predator avoidance mechanisms.

12.1 Introduction

This chapter will concentrate on free-living obligate heterotrophic flagellates (HF) which are abundant or otherwise important components of aquatic ecosystems. For a long time ecologists have dealt with flagellates, as with bacteria, seeing them as bright spots under the epifluorescence microscope. HF were mainly considered as one trophic group - bacteriovores. Nevertheless, treating heterotrophic nanoflagellates as a black box was a very important step in the understanding of heterotrophic flagellates as being one major component in aquatic food webs that is able to transfer a significant amount of bacterial production to higher trophic levels (Fenchel 1982b; Azam et al. 1983; Berninger et al. 1991). A major feature of the microbial loop (Azam et al., 1983) is that a significant portion of phytoplankton

primary production is excreted in the form of dissolved organic matter which then provides a substrate for bacterial growth thereby providing a food source for protozoans (mainly HF). Protozoan production (HF and ciliates) enters the traditional food web via grazing by metazoans (for details see chapter by Johanna Laybourn-Parry). The concept of the microbial loop has subsequently been revised in several important respects for instance: quantitative studies have indicated that herbivory by nano- and microflagellates play an important role within the carbon flux (e.g. Sherr and Sherr 1994); bacterial communities are not only grazed by protozoans but are also structured by protistan grazers (e.g. Güde 1979, Turley et al. 1986; Jürgens et al. 1997a); mixotrophy is of potential importance in many different aquatic ecosystems and among various groups of protists (e.g. Sanders 1991a; Caron and Finlay 1994); predation by metazoans on protists can be very selective (Sanders and Wickham 1993; Arndt 1993a) but predation may also enhance flagellates via indirect effects (e.g. support of bacteria growth, cf. Hahn et al. 1999; Arndt et al. 1992).

In contrast to these recent findings our knowledge of the ecology and quantitative composition of heterotrophic flagellates is still poor. Following the pioneering work of Fenchel (for review see Fenchel 1986a) only about a dozen - mainly bacterivorous - species have been studied in detail regarding their autecology (e.g. Caron et al. 1990; Goldman and Caron 1985; Jürgens 1992; Jones and Rees 1994; Geider and Leadbeater 1988; Eccleston-Parry and Leadbeater 1994a). This is in contrast to the large number of known HF species and to the high species diversity (up to more than 100) recorded by taxonomists from the intensively investigated sites (e.g. Patterson et al. 1989; Vørs 1992; Vørs et al. 1995; Patterson and Simpson 1996; Tong et al. 1998).

Patterson, Larsen and co-workers *The Biology of Free-Living Heterotrophic Flagellates* (Patterson and Larsen, 1991) was a first attempt to combine quantitative data of field work and taxonomic knowledge of specialists. Ten years ago, however, quantitative data were generally restricted to counts of "HNF" (heterotrophic nanoflagellates). Recently a more detailed data base on the quantitative composition of heterotrophic flagellates of several pelagic communities has become available from marine, brackish and fresh waters. The idea of this overview is to summarise the available information and to make a first attempt to compare pelagic and benthic habitats at marine and limnetic sites regarding their major flagellate fauna. Against this background the ecology of different systematic groups and their specific adaptations to environmental conditions will be compared and their functions in different ecosystems will be discussed.

12.2. Methodological problems of quantitative studies

The number of publications presenting quantitative data for pelagic heterotrophic flagellates in aquatic habitats is steadily increasing (see reviews by Sanders et al. 1992; Gasol and Vaqué 1993). One important problem is, however, that most of the quantitative data are based on counts of fixed samples analysed by means of epifluorescence microscopy. One disadvantage of this procedure is that the presence

of one or more flagella on a cell may be difficult to resolve. Thus, epifluorescence nanofauna counts may also include e.g. small naked amoebae, yeasts, zoospores belonging to a range of organisms, nanociliates (though these are mostly distinguishable), disrupted cells from very different eucaryotes and maybe even large bacteria with a large nucleomorph (Patterson and Larsen 1991; Arndt 1993b). Test involving the counting of HF with epifluorescence microscopy gave reproducible results when carried out on laboratory cultures (Caron 1983; Bloem *et al.*, 1986). Diverse HF communities from field samples have, as far as we are aware, never been tested adequately to determine the level of accuracy.

A second problem of the epifluorescence microscopy technique is the significant non-uniform shrinkage of fixed flagellates making estimates of biovolume difficult to calculate. Choi and Stoecker (1989) found that, upon fixation, *Paraphysomonas* could shrink to 38% its original volume. A third fundamental problem is that in general only about 50-70 flagellates are counted per membrane filter. Thus forms that contribute only 1-2% to total abundance are easily overlooked. Though much less abundant than nanoflagellates, the contribution of large flagellates to total flagellate biomass in very different ecosystems can be significant (Sherr and Sherr 1994; Arndt and Mathes 1991). Large heterotrophic flagellates (LHF, >15-20 μ m) are often disrupted by routine fixatives (e.g. some chrysomonads and many representatives of protista incertae sedis) and thus difficult to quantify.

Most of the above mentioned problems also apply to the study of benthic HF which is additionally complex due to the masking of animals by sediment particles. According to the review given by Alongi (1991) nearly all recent estimates of benthic HF densities suffer from methodological problems. Extraction methods like sea ice or fixatives are very selective and underestimate especially nanoflagellates. Even with countings using epifluorescence microscopy (Bak and Nieuwland 1989), and combined density gradient centrifugation (Alongi 1990; Starink *et al.* 1994), the taxonomic composition of the community could not be adequately studied.

As an alternative method, several authors proposed a live-counting technique analysing small droplets by means of light microscopy (e.g. Massana and Güde 1991; Gasol 1993; Arndt and Mathes 1991). One important advantage of this method is that morphological and behavioural features hidden in fixed samples can be used to differentiate HF regarding the major taxonomic groups, sometimes even to species level. Biovolumes can be estimated from video-micrographs. An important disadvantage of live-counting is that samples have to be analysed within short period after sampling and the time consuming procedure. Thus the counting of parallel samples is limited. We have good experiences counting pelagic flagellates (undiluted samples) and benthic flagellates (diluted by a factor of 5- >20 with filtered water) in droplets of 5-20 μ l in a miniaturised version of a Sedgewick-Rafter chamber with a height of about 0.2 mm. Samples are analysed not longer than 1-2 hours after sampling by means of a phase contrast microscope (Zeiss Axioskop) equipped with video-enhancement. 20x, 40x objectives are used for quantitative counts. Concentration of flagellates is adjusted so that counting of a chamber can be completed within a few minutes (this is especially critical for pelagic samples). The use of a 63x LD-objective or water immersion objectives

(63x and 100x) with a long working distance are helpful for a species determination in relatively undisturbed samples.

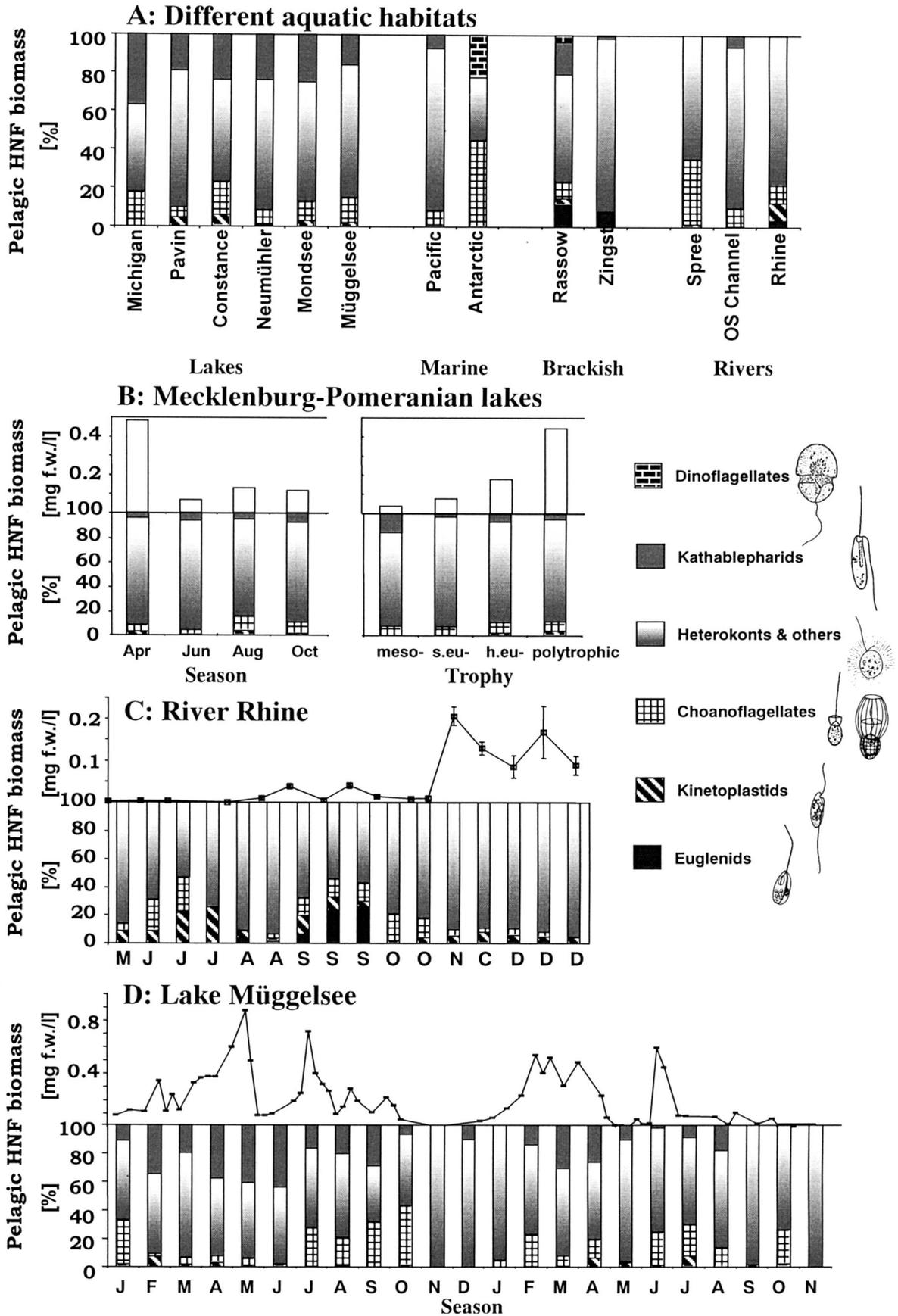
A reasonable compromise should be a combination of live-counts, best in combination with a cultivation of dominant flagellates for a safe determination, with epifluorescence counts. This seems to be an indispensable pre-requisite for determination of the taxonomic composition of heterotrophic flagellates in field samples and for a reliable estimate of HF abundances and biovolumes, respectively. Most of the discussed data sets discussed here have used live-counts, at least as an additional method. Recent advances in molecular biological methodology (e.g. development of specific probes) may serve as elegant methods for future studies to determine field abundances of distinct HF species in fixed samples (e.g. Lim et al. 1999, Tong and Sleigh pers. comm.). Molecular methods may be especially helpful for the determination of common species that are very difficult to distinguish by morphological features (e.g. several chrysomonads, Bruchmüller 1998).

12.3 Composition of flagellate communities

The possible evolutionary relationships between various groups of flagellates are still incompletely understood (Karpov, Chapter Sixteen and Cavalier-Smith, chapter Seventeen, this volume). Major problems for ecologists include: first, that there is no comprehensive key available for the identification of HF species and second, that autecological studies on flagellates have been carried out for only a restricted number of species or strains. In order to characterise the possible functional role of organisms in an ecosystem, ecologists must rely on the autecological data for a few, hopefully, representative species which can be combined with the knowledge on the distribution and quantitative composition of communities.

12.3.1 Pelagic flagellates

The abundances of *nanoflagellates* (HNF) in different pelagic habitats can vary from about 20 to >20,000 HNF per ml (mostly between 100 and 10,000 HNF/ml) and are mostly related to the abundance of bacteria (for review see Sanders et al., 1992). Despite this considerable variability in abundance, the available information on the taxonomic composition of HF from pelagic communities indicates a surprising conformity regarding the dominant taxonomic groups (Fig. 12.1 A, B). On annual average about 20-50% of HNF biomass is formed by heterokont taxa (mainly chrysomonads and bicosoecids). Not only in marine but also in freshwaters, choanoflagellates contribute another significant part of about 5-40% of the biomass. Kathablepharids appear to be a very important group in most lakes accounting for about 10->25% of average HNF biomass. If *Leucocryptos* is included within the kathablepharids, then they may reach a similar importance in marine waters (Vørs et al. 1995). Kinetoplastids always occur in plankton communities but are generally of reduced quantitative importance (1-8% of annual mean HNF biomass). Five other groups - small dinoflagellates, thaumatomastigids, apusomonads, colourless cryptomonads and euglenids - are also generally present in plankton communities, but commonly form only minor parts of the HNF biomass.



There is always a significant part of HNF that cannot be assigned to one of the major groups (*Protista incertae sedis*) or that cannot be identified (for reasons of comparison both of these flagellate groups were included among “heterokonts & others” in Fig. 12.1).

Despite the similarities in the general composition of the investigated plankton communities, there are also several specific features. Several groups of marine flagellates, such as ebridids, silicoflagellates, acanthoecid choanoflagellates, and many genera of dinoflagellates do not occur in lake plankton. Acanthoecid choanoflagellates, with their siliceous loricae are a well-studied component of marine and brackish waters (e.g. Buck and Garrison 1988; Leadbeater 1974; Kuuppo 1994). Aloricate HNF have only recently been studied in more detail. They might generally be much more abundant than choanoflagellates (cf. Kuuppo 1994; Vørs et al. 1995).

In most plankton communities considerable seasonal changes in the abundance, biomass and composition of HNF (e.g. Carrick and Fahnenstiel 1989; Mathes and Arndt 1995; Carrias et al. 1998; Weisse and Müller 1998) have been observed. In samples from River Rhine and Lake Müggelsee (Fig. 12.1C,D) the biomass changed by a factor of 100 in the course of a year. HNF dynamics in temperate regions are generally characterised by maxima in spring, due to increased food supply, and minima in early summer due to intensive grazing pressure by metazoans (e.g. Weisse 1991; Cleven 1995). Flagellate dynamics of River Rhine were more correlated to the water discharge than to seasonal events. High run-offs cause a release of HF from predation pressure by benthic filter-feeders (Weitere *et al.* in prep.).

The biomass of *large heterotrophic flagellates* ($\geq 15\text{-}200\mu\text{m}$, LHF) seems to be as important as that of nanoflagellates (Fig. 12.2.A). This fact was stressed among

(left)

Figure 12.1 Comparison of the composition of heterotrophic nanoflagellate (HNF) communities from very different pelagic environments (percentage of mean biomass in mg fresh weight/litre)

- A Different pelagic habitats (seasonal mean values except for the marine data): Lake Michigan (USA): Carrick and Fahnenstiel (1989) (cryptomonads were considered as katablepharids, Carrick pers. comm.); L. Pavin (France): Carrias (1996); L. Constance (Germany): Springmann (unpubl.); L. Neumühler See (Germany): Mathes and Arndt (1995); L. Mondsee (Austria): Salbrechter and Arndt (1994); L. Müggelsee (Berlin, Germany): Arndt (1994); Equatorial Pacific (Vørs et al. 1995); Antarctica: Hewes et al. (1990; mean of two cruises); Rassower Strom and Zingster Strom (shallow Baltic coastal waters, Germany, 8 and 5 PSU): Arndt (unpubl.); River Spree (Leipsch/Alt-Schadow) and Oder-Spree-Channel (Fürstenwalde, Germany): Arndt (unpubl.); R. Rhine (Cologne, Germany): Weitere (unpubl.)
- B Mecklenburg-Pomeranian lakes (Germany): 55 lakes of different trophy sampled four times per year (1996 and 1997; Auer et al. in prep.). Data are summarized for different seasons and different degrees of trophy.
- C River Rhine at Cologne (Germany), seasonal changes of biomass and composition of HNF (1998; Weitere unpubl.).
- D Lake Müggelsee (Berlin, Germany), seasonal changes of biomass and composition of HNF during two successive years (1989 and 1990; Arndt 1994 and unpubl.)

limnologists already decades ago (e.g. Sandon 1932; Nauwerck 1963). However, its quantitative importance in connection with the microbial food web has only recently been considered (e.g. Sherr and Sherr 1989; Arndt and Mathes 1991). The annual mean size distribution of total flagellate abundance and biovolume (using live-counting technique) from Lake Müggelsee (Fig. 12.2.B) indicates that large HF are much less abundant than small forms, but contribute significantly to HF biomass. With regard to lake communities, the relative contribution of nano- and microflagellate biomass varies depending on the trophic status of lakes with a tendency for microflagellate biomass to dominate under hypertrophic conditions (Fig. 12.2.D, Auer in prep.). Colourless dinoflagellates probably form the major part of the biomass of microflagellates in most marine and freshwaters (Smetacek 1981; Lessard and Swift 1985; Arndt and Mathes 1991). In coastal marine waters ebbriids can occasionally form a substantial part of LHF biomass (e.g. Smetacek 1981). In freshwaters large chrysomonads (e.g. *Spumella*) are, besides dinoflagellates (e.g. *Gymnodinium helveticum*), important LHF seasonally varying in their contribution (Fig. 12.2.C). In some lakes (Mischke 1994) and in brackish waters (Arndt unpubl.) large members of the Protista *incertae sedis* can occur in significant abundances.

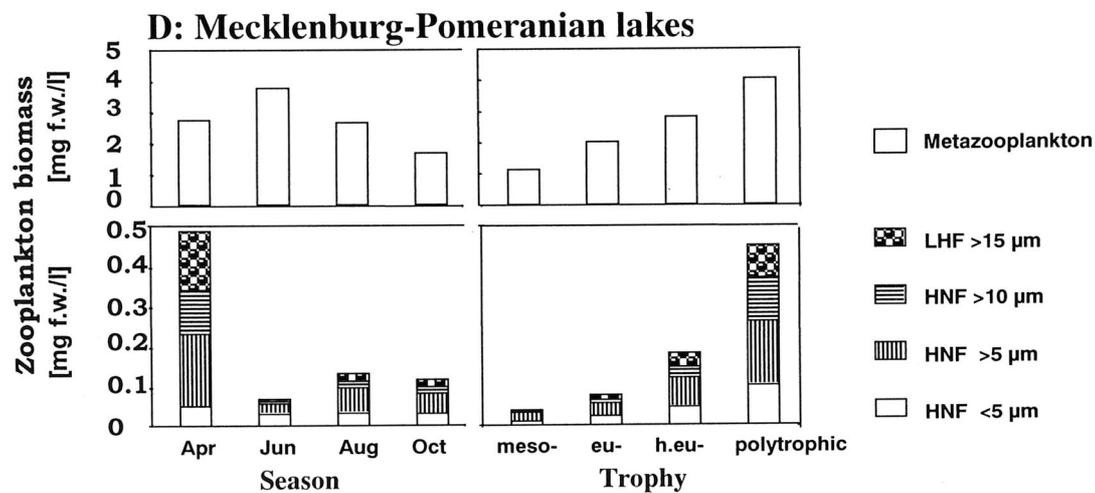
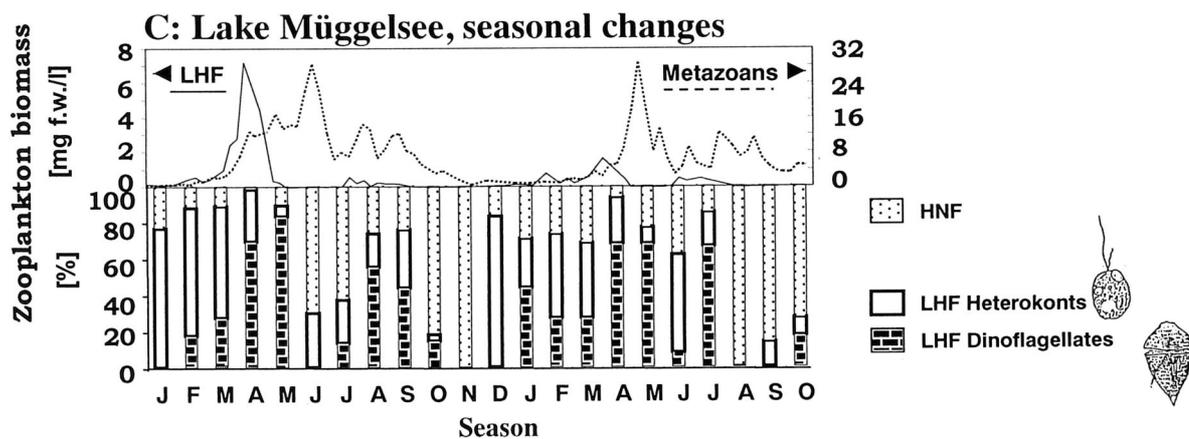
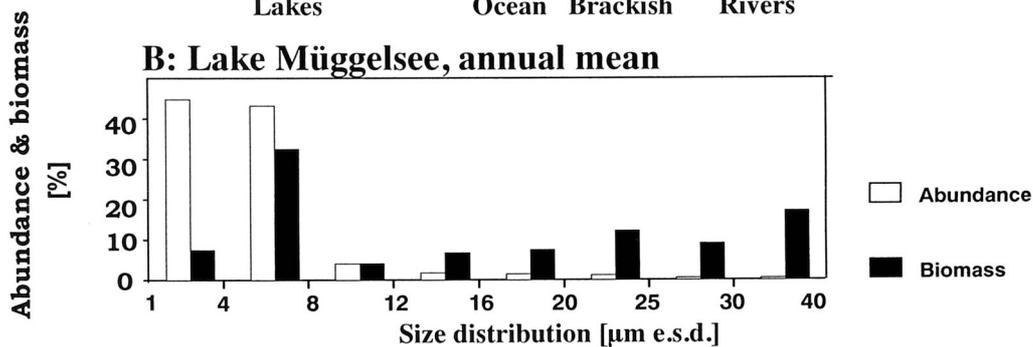
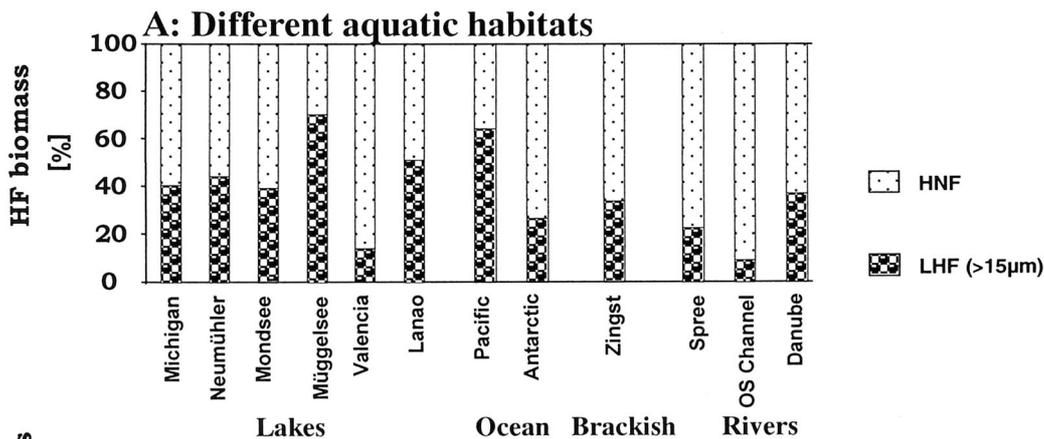
There are major seasonal changes in the contribution of the different size groups of HF in lakes and in coastal waters. Largest HF are generally recorded during the spring bloom of phytoplankton, whereas the smallest forms dominate under conditions of high metazoan grazing pressure in summer or when food concentrations are low during winter (Fig. 12.2.C,D, Smetacek 1981; Weisse 1991). At times of high metazoan grazing pressure LHF often disappear from pelagic communities (Auer et al. in prep.).

Quantitative changes in the vertical distribution of HF are well documented for marine and freshwaters. Generally, maximum numbers occur at sites of highest food concentrations mostly at the surface (Fenchel 1986a). Not much is known about the vertical changes in the taxonomic composition. In the mesotrophic Lake Mondsee chrysomonads and katablepharids did not change significantly regarding their percentage contribution whereas kinetoplastids, choanoflagellates, bicosoecids and dinoflagellates decreased at greater depths (Salbrechter and Arndt 1994).

(right)

Figure 12.2 Comparison of the composition of large heterotrophic flagellate (LHF, >15µm) communities in different pelagic environments (percentage of mean biomass in mg fresh weight/litre).

- A Contribution of LHF to total flagellate biomass. Lake Valencia (Venezuela) and Lake Lanao (Philippines): Lewis (1985); River Danube at Budapest (Hungary): Arndt and Mathes (1991); data from all other sites from references as in Fig. 12.1. A.
- B Size distribution of heterotrophic flagellates regarding biomass and abundance for Lake Müggelsee (1989; Berlin, Germany): Arndt (1994).
- C Seasonal changes of LHF and metazoan biomass and LHF contribution to total flagellate biomass in Lake Müggelsee (1989 and 1990; Arndt et al. 1993 and Arndt and Mathes 1991)
- D Size distribution of HF communities in relation to season and trophy and metazooplankton biomass in 55 Mecklenburg-Pomeranian lakes (Auer et al. in prep. and unpubl.)



12.3.2 Benthic flagellates

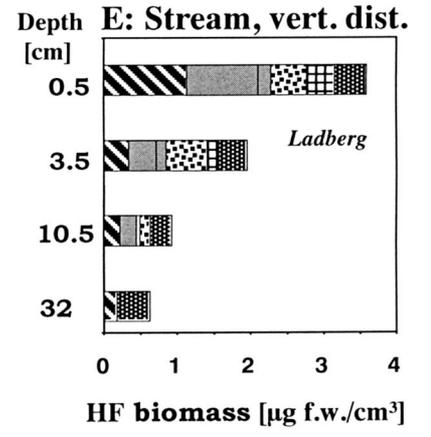
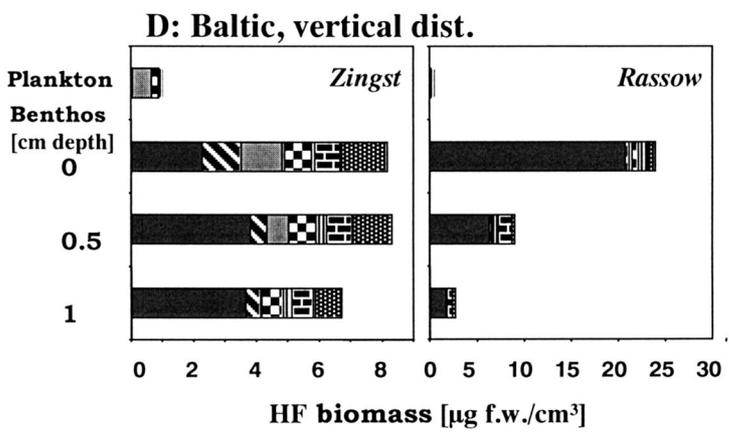
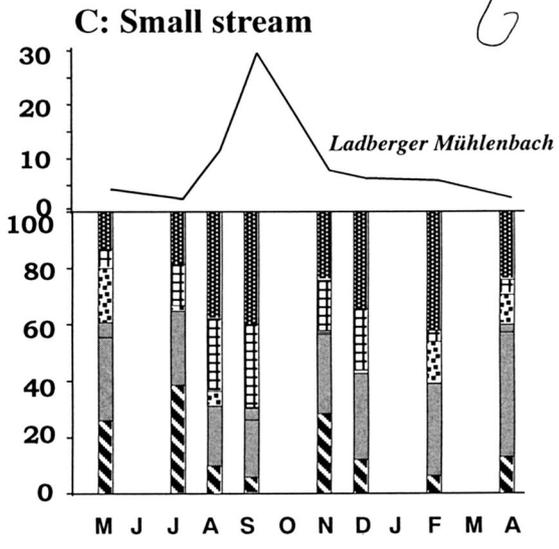
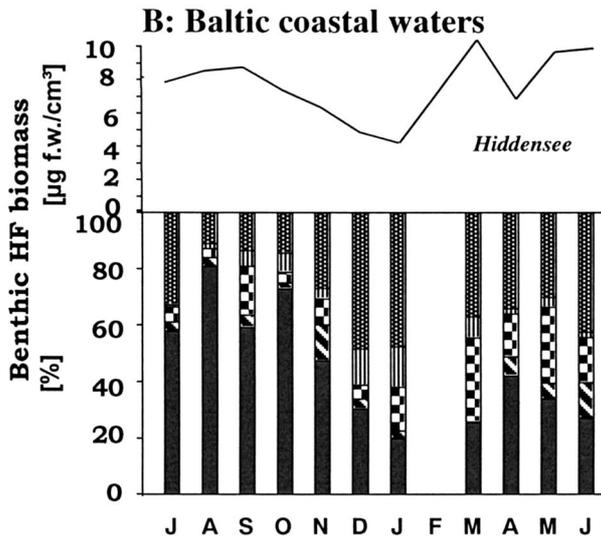
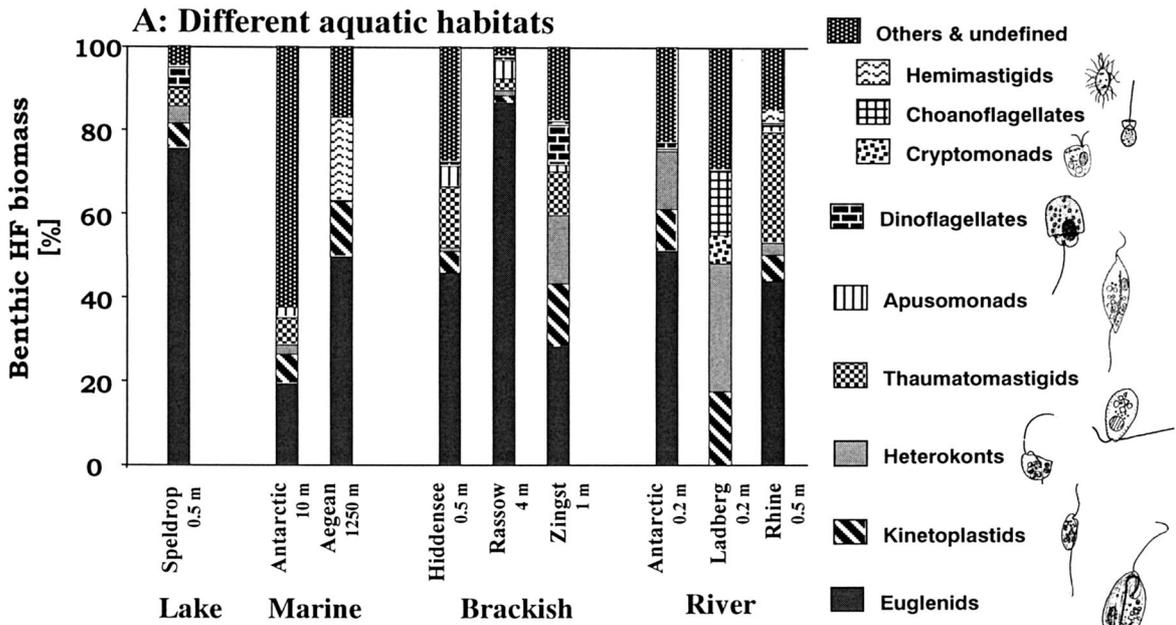
The taxonomic diversity of benthic flagellate communities has been summarised by Patterson et al. (1989). Recently numerous marine sites have been studied and many new species, among them many common ones, have been added to our knowledge (for example Vørs 1992; Patterson and Simpson 1996; Tong et al. 1998). Regarding the quantitative importance of benthic flagellates, due to methodological problems (see section 12.2), we are only just at beginning to be able to compare different habitats. Abundances which have been reported range from below 100 to several millions HF per ml sediment (Gasol 1993). HF abundance seems to be positively correlated to bacteria abundance and grain size, however, available data sets are still contradictory (cf. Alongi 1991; Hondeveld et al. 1994). Quantitative data on the taxonomic composition are very sparse and often restricted to a few taxonomic groups (e.g. Bark 1981, Baldock et al. 1983).

Most of the data on the structure of benthic flagellate communities presented in Figure 12.3 A come from our own recent studies and gives in most cases only a sporadic picture of the community structure in the aerobic surface layer (seasonal averages only from the Baltic at Hiddensee and from Antarctica). Generally, euglenids are most important regarding their biomass contribution (20-85% of HF biomass, for Ladberger Mühlenbach, Fig. 12.3 C, they were included among the "others" category) followed by bodonids (5-20% of HF biomass). Both euglenozoan groups are generally also the most diverse groups (e.g. Patterson *et al.* 1989). Though only recently considered, thaumatomastigids and apusomonads are typical components of benthos communities and contribute together about 1-20% of the HF biomass. Colourless chrysomonads may be abundant at certain sites but contribute in general not more than 30 % to HF biomass, mostly much less. Seawater-ice-extractions reveal a high diversity of benthic dinoflagellates from

(right)

Figure 12.3 Comparison of the composition of benthic heterotrophic flagellate (HF) communities from very different environments (percentage of mean biomass in mg fresh weight/cm³ sediment).

- A Different benthic sites (seasonal mean values except for the lake, the marine sites and River Rhine; the water depth at the sampling sites is indicated on the abscissa): L. Speldrop (Lower Rhine, Germany): Arndt et al. unpubl.; Antarctic site in the Potter Cove, King George Island: Dietrich (unpubl.); Aegean Sea, Sporades Basin at 1250m depth: Arndt and Hausmann (in prep.); Hiddensee (Fährinsel; southern Baltic, Germany): Dietrich (unpubl.); Rassower Strom and Zingster Strom (southern Baltic, Germany; 8 and 5 PSU): Arndt (unpubl.); Antarctic melt water stream, King George Island: Dietrich (unpubl.); Ladberger Mühlenbach (Westfalia, Germany): Cleven (in prep.); River Rhine at Cologne (Germany): Altmann and Arndt (unpubl.).
- B Hiddensee (Fährinsel; southern Baltic, Germany), seasonal changes of biomass and composition of HF during 1996/97 (Dietrich and Arndt submitted).
- C Ladberger Mühlenbach (Westfalia, Germany), seasonal changes of biomass and composition of HF during 1997/98 (Cleven in prep.)
- D Vertical changes of HF biomass and composition at two brackish sites of the southern Baltic (cf. 12.3 A; mean of five samplings): Arndt (unpubl.).
- E Vertical changes of HF biomass and composition in the brook Ladberger Mühlenbach (Westfalia, Germany; May 1997): Cleven (in prep.)



marine sites (Hoppenrath, pers. comm.). It seems from our data that their contribution to total biomass of HF is up to 20 %. Occasionally of importance are colourless cryptomonads, choanoflagellates, cercozoans, bicosoecids, pedinellids and even hemimastigids. Several genera (e.g. *Percolomonas*, *Ancyromonas*) and other members of protista *incertae sedis* are also temporarily abundant, but should not significantly contribute to average HF biomass. There is an obvious variability in the contribution of the major HF groups at different sites, which is certainly related to the quality of sediments and accompanying factors (cf. Patterson et al. 1989; Alongi 1991). Our knowledge regarding the specific requirements of the different taxa and the specific top-down and bottom-up effects, however, is very limited at present.

Two seasonal patterns of HF dynamics from a brackish site and a small stream are presented in Figure 12.3 (B,C) indicating that absolute biomass and the relative contribution of HF groups changes throughout the year. Bark (1981) found a seasonal succession from bodonids and euglenids in early summer to a predominance of diplomonads in autumn in a small highly eutrophic lake. The typical coupling of phytoplankton spring bloom and HF known from the pelagial is not generally recorded from benthic HF communities, where summer but also winter peaks have been reported (Bark 1981; Bak and Nieuwland 1989; Hondeveld et al. 1994; Starink et al. 1996). In benthic systems seasonal changes might be masked by the long-term storage of organic material in the sediment that give rise to a relatively continuous bacterial production.

All the above mentioned insights are restricted to studies of the aerobic surface layer. The vertical distribution of HF abundances is mainly related to the chemical properties of the different sediment layers and highest numbers are generally found in the upper layers of sediment (for review see Alongi 1991). Again, little is known regarding the taxonomic composition of flagellates at different depths. In all three vertical profiles presented in Figure 12.3. (D,E) biomasses decreased with depth and the relative contribution of euglenids (in E included among the "others" category) and bodonids increased. At highly reductive conditions deeper in the sediment the community structure changed significantly towards diplomonads, protista *incertae sedis*, percolozoans, and several undefined forms, however, euglenids were still present (Arndt unpubl.). Amitochondriate flagellates like diplomonads (e.g. *Trepomonas*, *Hexamita*) and archamoebae (e.g. *Pelomyxa*, *Mastigella*) can predominate under anaerobic conditions (for review see Fenchel and Finlay 1995, see also chapter xxx). Even anaerobic communities can be relatively diverse. Mylnikov (1978) recorded about 30 species of archamoebids, trichomonads and diplomonads as well as several species typical for aerobic sites from the sediments of the Ivanjkovski Reservoir in Russia.

12.3.3 BENTHO-PELAGIC COUPLING OF FLAGELLATE COMMUNITIES

From a variety of studies regarding the structure of heterotrophic nanoflagellate communities it seems that several species are well-adapted to be wanderers between the two environments, that of the pelagial and that of the benthic. HF can populate the pelagial as a result of resuspension of the sediment surface layer, by

rafting of biofilms or by active migration. Some forms, such as thaumatomastigids (e.g. Shirkina 1987), dinoflagellates (e.g. Burkholder and Glasgow 1997) and cercozoans (*Massisteria*, Patterson and Fenchel 1990), can rapidly change between amoeboid and flagellated forms. HF are among the smallest organisms which can temporarily find suitable conditions to graze and reproduce even on aggregates (size 50 to > 3000 μm) in the pelagial. Their density is close to that of water, and they can live suspended for days or even longer, enough to reproduce several times before reaching the sediment surface or to form cysts when conditions deteriorate. Aggregates with an organic matrix from different origins suspended in the pelagial have been found to be important constituents of oceanic (Alldredge and Silver 1988), estuarine (Zimmermann and Kausch 1996) and fresh waters (Grossart and Simon 1993) and are known to be "hot spots" of microbial activity. Aggregates can serve as micro-habitats showing ten to thousand fold higher HF concentrations compared to the surrounding water (for review see Caron 1991). Aggregate-dwelling flagellates in the pelagial have been reported especially from estuarine waters (Rogerson and Laybourn-Parry 1992; Zimmermann and Kausch 1996; Garstecki et al. submitted). However, even in the pelagial of the open ocean at least cysts of benthic species occur. Cultures from oceanic sites have revealed typical benthic genera (e.g. *Bodo*, *Amastigomonas*, *Cercomonas*, *Caecitellus*; cf. Patterson et al. 1993; Vørs et al. 1995; Arndt and Hausmann in prep.).

Fig. 12.4 gives a quantitative example for the relative contribution of HNF (data calculated per area) separated for 1) typical pelagic species (free-swimming HF and HF which are only occasionally found in benthic samples), 2) typical benthic species found during the time of sampling in the pelagial (e.g. several bodonids, thaumatomastigids, apusomonads and cercozoans) and 3) for flagellates from the benthos of shallow coastal waters around the Island Rugia (Baltic, Germany). In three out of ten occasions benthic species in the pelagial contributed at least one quarter to all flagellates calculated per area. These flagellates form a benthic-pelagic flagellate community living either in the upper 1-3 millimetres of the sediment (fluff) or on particles in the pelagial. Those flagellates which are typically adapted to move between sand grains, such as large euglenids, flattened dinoflagellates and several amoeboid forms, only rarely occur in the pelagial and avoid probably resuspension by moving deeper into the sediment.

12.4 Comparative ecology of abundant flagellate groups

12.4.1 Feeding ecology

The feeding behaviour of HF can differ between the various groups of flagellates, however, the size spectrum of particles consumed is much larger than previously assumed (for reviews see Sanders 1991b, Radek and Hausmann 1994, and Sleigh in this volume). It can range from 0.2 μm (choanoflagellates) up to >50 μm (some dinoflagellates), thus deviating strongly from the original assumption that HF are primarily bacterivorous. It is known that several nanoflagellates from marine and freshwaters also feed on nanophytoplankton (e.g. Sherr et al. 1991;

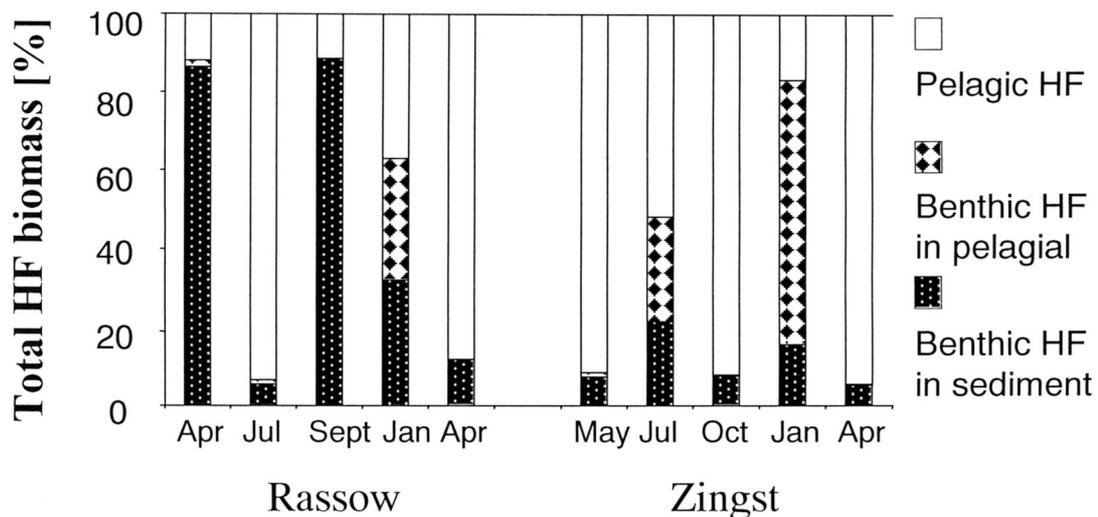


Figure 12.4 Benthic-pelagic coupling of heterotrophic flagellate populations at two brackish water sites of the southern Baltic (Rassower Strom, 4m depth, 8 PSU; Kirr-Bucht at Zingst, 1m depth; 5 PSU; Arndt unpubl.). The relative contribution of HF biomass to total HF (data calculated per area) was separated for typical pelagic species, typical benthic species found during the time of sampling in the pelagial, and for those flagellates which were found in the benthic at the time of sampling. Each column is based on data from four layers (three parallel samples of each of the pelagial (at 0.5m depth) and three sediment layers: upper fluff layer of about 3mm thickness, 0-5mm sediment, 5-10mm sediment). In accordance with our experience in the study area it was assumed that there were no significant vertical differences in the pelagial and that, due to oxygen deficiency, HF biomass in sediment layers below 10mm depth is the same as that for the 5-10mm layer.

Cleven 1995), and may also consume other flagellates, ciliates and even small metazoans (cf. Sanders 1991b). An extreme example of feeding on larger particles is the piscivory by the dinoflagellate *Pfiesteria piscicida* (Burkholder and Glasgow 1997). The knowledge of food vacuole contents was used by several authors to relate abundances of HF to different feeding types or feeding guilds (e.g. Pratt and Cairns 1985). Figure 12.5 indicates the size spectrum of food particles for pelagic and benthic communities. It seems that the contribution of exclusive bacteriovores in pelagic habitats is only about 1/10 in limnetic sites (mainly choanoflagellates, bicosoecids, kinetoplastids), in marine and brackish sites the percentage can be significantly larger. In benthic habitats about one quarter of HF biomass consists of bacteriovores (mainly kinetoplastids, bicosoecids, some protista *incertae sedis*, cryptomonads).

In the *pelagial*, the majority of all HF are most probably omnivores feeding on different trophic levels, except for a few specialists known mainly among dinoflagellates (for review see e.g. Schnepf and Elbrächter 1992). Most dinoflagellates, chrysomonads, some protista *incertae sedis* and kathablepharids are known to feed on bacteria, as well as on algae and most probably also on heterotrophic protists. Figure 12.6 illustrates this important - though often neglected - feature of many HF. Both bacteriovory (feeding on *Aerobacter*) and carnivory (predation on a bacterivorous *Cercomonas*) occurs in the same strain of

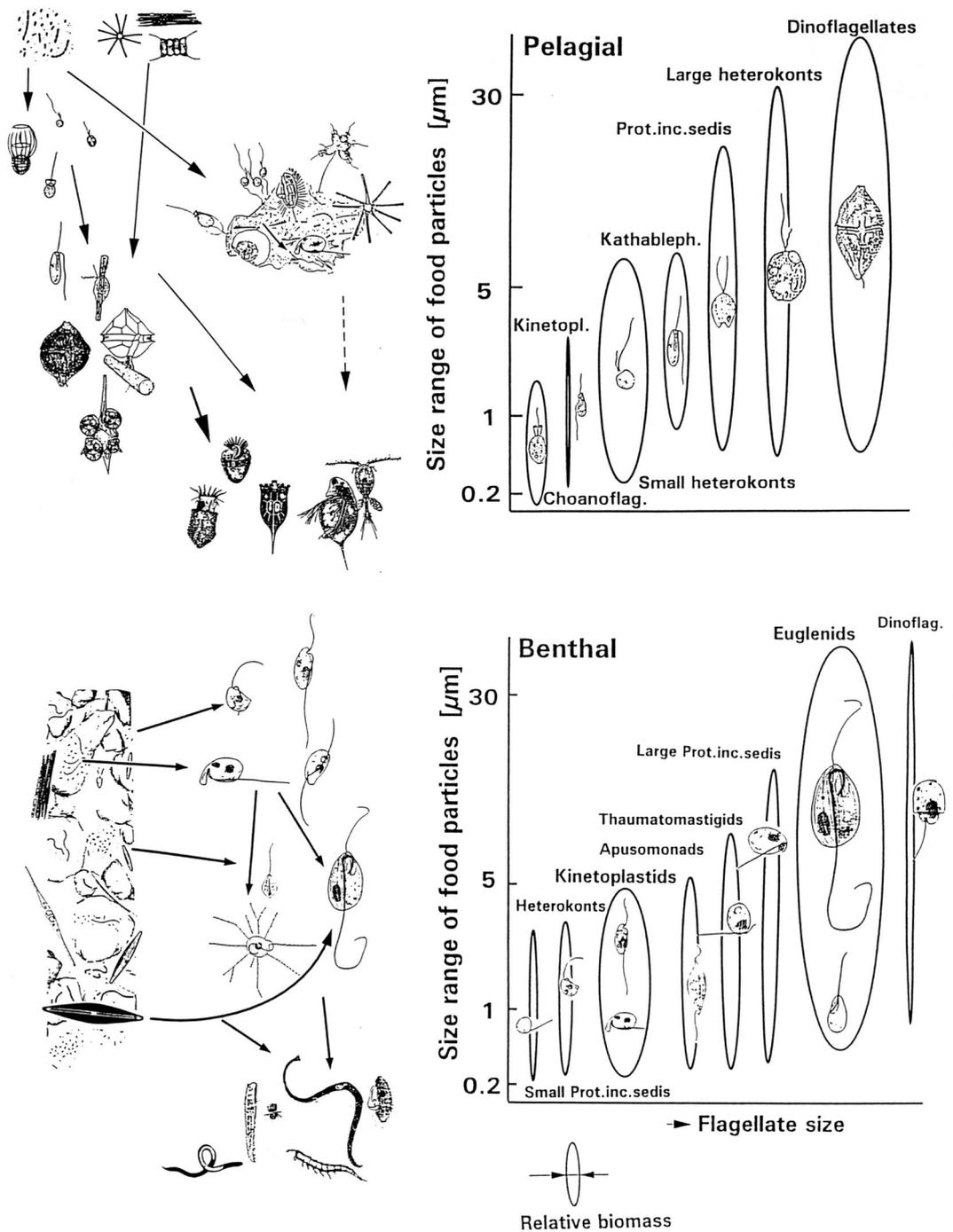


Figure 12.5 Right panels: Food size spectrum of important pelagic and benthic flagellate taxa in relation to their mean body size. The relative importance regarding their biomass contribution is indicated by the width of the symbols. Data summarised in Figs. 12.1-3 served as a data base.

Left panels: Schematic drawing of major fluxes of matter through a typical pelagic and benthic HF community. The species drawn are only representatives of functional groups and might be different in marine, brackish and limnetic sites.

Paraphysomonas feeding on bacteria and flagellates

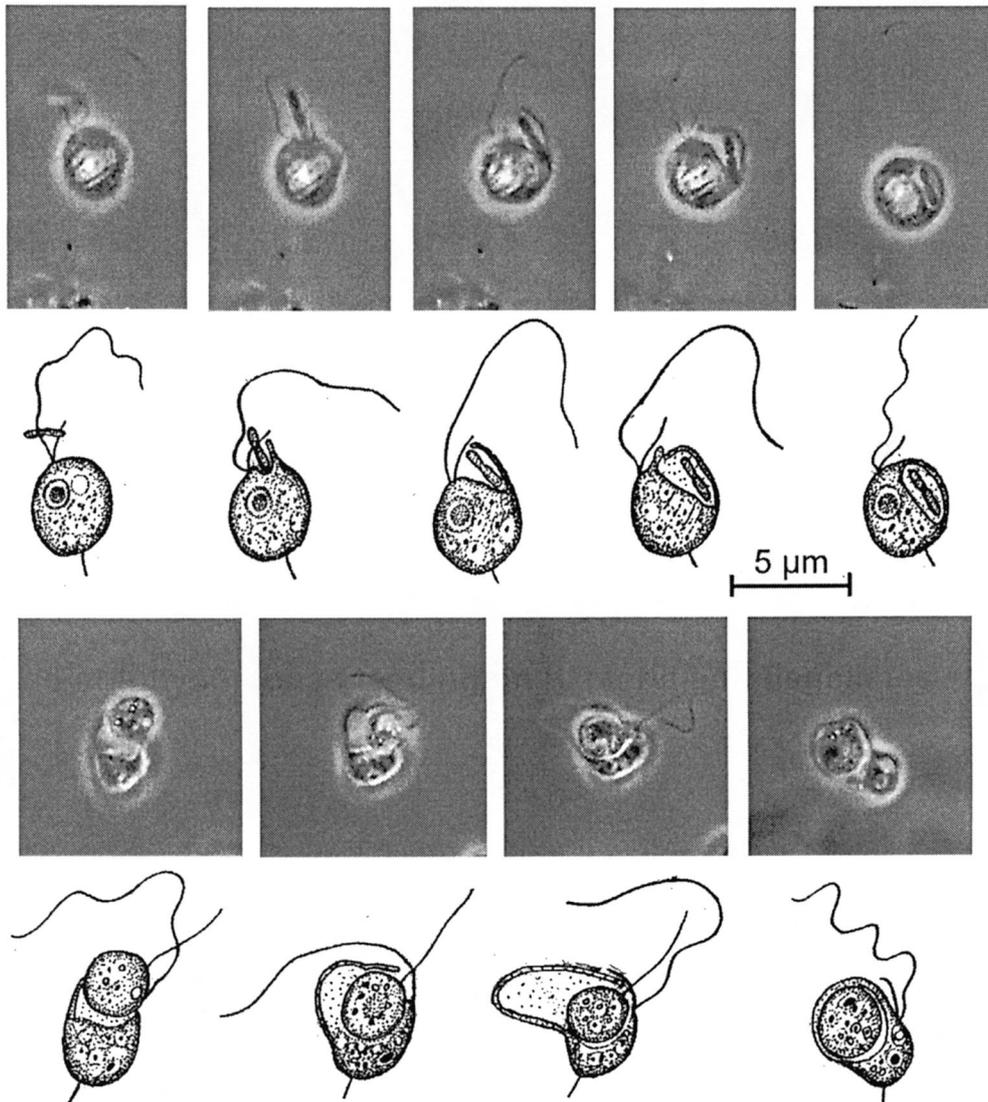


Figure 12.6 Documentation of omnivory by a small *Paraphysomonas* sp. (diameter 3µm) feeding on a bacterium (*Aerobacter*) and another heterotrophic flagellate (*Cercomonas*). Food particles driven by the filter current towards the cell surface are handled by flagella and within 2-4 seconds a long pseudopodium is formed. With the help of the short flagellum even agile flagellates are pushed into the pocket formed by the rapid extending pseudopodium. The food vacuole is closed after a period of about 5-10 (bacteria) up to 30 (flagellates) seconds following the first contact with the prey. Upon closing the food vacuole flagellates are completely immobilised and their membrane is disrupted probably by enzymatic and/or osmotic processes

Source: Arndt and Mylnikov, unpublished

a small *Paraphysomonas* (diameter 3–4 μm). Large flagellates like *Gymnodinium helveticum* and *Ceratium hirundinella* were found to feed on a wide food spectrum such as bacteria, detritus, algae and other protists (Sandon 1932; Arndt unpubl.). The ability of most flagellates to take up particles often larger than their original body volume by engulfment of the whole prey aided by pseudopod-like structures (chrysomonads, some dinoflagellates like *Gymnodinium*), a cytostome (e.g. kathablepharids), or by other mechanisms (e.g. pallium-feeding and myzocytosis outside the theca of dinoflagellates (Schnepf and Elbrächter 1992)) seems to be an important feature to survive at low food concentrations in the pelagial. They seem to have two modes of feeding; grazing of large food particles upon occasional contacts and the uptake of small food items transported towards the cell by a filter current created by their flagella. This feeding behaviour seems to be a similar adaptation to the life in the diluted environment of the pelagial as the feeding behaviour of calanoid copepods which switch between filter feeding of small and grasping of large food particles (e.g. Vanderploeg and Paffenhöfer 1985). Besides bacterivory, HF can reach the same importance as algivores as ciliates can in marine (Lessard and Swift 1985; Sherr and Sherr 1994) and in limnetic pelagic communities (Arndt et al. 1993).

Little is known regarding the relative importance of large food particles (e.g. algae) and bacteria for different large flagellates. But according to energetical considerations (cf. Fenchel 1986b) the importance of interception feeding on small bacteria should decline with the size of the flagellate. This would mean that the flagellate groups included in Figure 12.5 for the pelagial arranged according to their mean size might represent also feeding niches. Choanoflagellates as filter-feeders are known to be able to feed on the smallest prokaryotes up to a size of about 2–3 μm . Bodonids, associated with marine or lake snow, feed on particles not much larger than about 5 μm which they grasp from the surface of particles. Small chrysomonads (generally 3–8 μm) feed on particles from 0.2 μm up to their own size by direct interception feeding aided by pseudopod formation at the base of the flagella (cf. Fig. 12.6). Regarding the bacterivory of nanoflagellates it is well-known that they select for large bacteria up to a certain limit (e.g. Simek and Chrzanowski 1992). Kathablepharids consume bacteria and even cryptomonads (Cleven 1995) with the help of extrusomes and a cytopharynx. Chrysomonads among LHF with an average size between 15 and 25 μm diameter can engulf food items up to 50 μm in length (Arndt unpubl.), the same is known for ebruids and dinoflagellates (Smetacek 1981). But even much larger items such as colonies of centric diatoms can be consumed (e.g. Jacobson and Anderson 1986).

Feeding strategies of pelagic HF are clearly separated by the degree of contact to the substrate: 1) free-swimming forms (e.g. colourless kathablepharids, dinoflagellates, large chrysomonads and some thaumatomastigids), 2) forms which are loosely and temporarily attached by protoplasmic threads (small chrysomonads, some choanoflagellates, pedinellids) or flagellum (e.g. bodonids, thaumatomastigids, apusomonadids, bicosoecids) and 3) attached forms (e.g. some loricate bicosoecids and choanoflagellates). At least half of the biomass of total HF is composed of flagellates living in more or less close contact to sestonic particles, such as algae, colonies of algae, lake or marine snow etc. Most of these

flagellates create filter currents and feed mostly on small food items (0.2-5 μ m). Due to hydrodynamic forces attachment has been considered an important mechanism for an efficient particle concentration (Fenchel 1986b). Another, though generally minor part of pelagic HF biomass, is due to flagellates crawling over surfaces of detrital particles, where bacteria are significantly concentrated compared to the surrounding water. In contrast to these attached HF, free-swimming flagellates seem to feed preferably on relatively large food particles such as algae and other protists.

According to our present knowledge regarding the composition of *benthic* communities (aerobic surface layer) there should be a separation of feeding types similar to pelagic communities (see Fig. 12.5). Among typical bacteriovores are suspension feeders grazing on bacteria of the pore water (bicosoecids, *Bodo saltans*, choanoflagellates, pedinellids) and forms that grasp more or less attached bacteria (most bodonids, small euglenids, several representatives of apusomonads, cercomonads, and protista *incertae sedis*). In the benthic, abundance of bacteria and algae is generally up to three orders of magnitude higher compared to pelagic sites, making grasping on abundant small (e.g. bacteria) as well as on large particles (e.g. diatoms, other protists) an efficient way of nutrition. Benthic flagellates often possess specialised feeding organelles (see 12.5.1). As in pelagic environments, there are many flagellates that feed preferentially on large particles. Many euglenids and dinoflagellates are known to feed on large diatoms and other protists. In addition, there are predatory forms such as *Metopion* and *Metromonas* which seem to feed preferably on other protists. Comparable again to pelagic waters, most forms probably have a relatively wide food spectrum (as indicated in Fig. 12.5, see also Sanders 1991b). It seems that predominant bacteriovores and herbivores are, as in pelagic systems, of similar importance (Epstein 1997; Dietrich and Arndt submitted). There are only a few detailed studies on the feeding of benthic flagellates. The dinoflagellate *Oxyrrhis marina* consumes bacteria, algae and heterotrophic flagellates (e.g. bodonids, bicosoecids, chrysomonads; Premke & Arndt unpubl.) as well as dissolved nutrients (Sanders 1991b). There are differences in the mobility of the HF in the sediment which should affect the feeding niche. The behaviour ranges between fast movement between the sand grains (e.g. some bodonids and euglenids) and ambush predation (e.g. *Massisteria*).

Not only the size but also the concentration of food particles is known as an important factor influencing the co-occurrence of organisms. Data in literature about the incipient limiting concentration of food particles for HF are very variable for similar species and range between 10^4 and 10^7 bacteria per ml (cf. Eccleston-Parry and Leadbeater 1994b). LHF which depend on the frequency of contacts with large food items (e.g. algae) significantly increase in biomass and in their relative contribution to total HF biomass with increases in lake trophy (Mathes and Arndt 1994, Fig. 12.2.D). Choanoflagellates which are known to be very effective filterers should have advantages in comparison to other flagellates when food concentrations are low. The relative contribution of choanoflagellates to HNF biomass increased from about 5% in hypertrophic lakes to about 11% in mesotrophic lakes (Auer unpubl., see also Fig. 12.1.A), bodonids crawling on particles in the pelagial showed the opposite trend, 1% in mesotrophic and about 5% in hypertrophic lakes. From our present knowledge, however, it seems that

generally food concentration has a major influence on absolute numbers but only a minor effect on the composition of HF assemblages regarding the importance of taxonomic groups (Fig. 12.1 A).

Another aspect of feeding niches is the different strategy adopted to survive periods of starvation. Such strategies comprise rapid encystment and excystment, changes in food sources used as well as dramatic changes in metabolic rates and other physiological and cytological changes (for review see Fenchel 1986a). Osmotrophy, though known to occur under conditions of cultivation in a great variety of organisms (Sanders 1991b), seems to play a minor role in pelagic systems where concentrations of easily degradable DOC are very low. In the pore water of benthic systems, however, DOC concentrations can be several orders of magnitude higher compared to the pelagial. Some benthic species seem to be typical osmotrophs (e.g. *Astasia*, *Chilomonas*, cf. Pringsheim 1963). This aspect requires more attention especially for deeper sediment layers.

12.4.2 Tolerances to abiotic factors

The tolerance ranges of flagellate species or taxonomic groups to abiotic conditions is still a neglected topic in protistology. Even regarding major abiotic factors such as salinity, temperature and oxygen there is only anecdotal knowledge available.

A special phenomenon of HF is the observation of several, at least morphologically, identical species both in marine and freshwaters (e.g. Mylnikov and Zhgarev 1984, Larsen and Patterson 1990). This gives rise to the assumption that flagellates have a wide range of tolerance with regard to salinity. This hypothesis is supported by our studies on the tolerances of single clones of ten freshwater and eight marine strains (Fig. 12.7). At least some species occur both under marine and freshwater conditions. Species lists of very different sites often include *Bodo saltans*, *B. designis*, *Rhynchomonas nasuta* as well as *Percolomonas cosmopolitus* which indicates their general tolerance. Our results also indicate that there are several species that have a more or less narrow range of tolerance, and that salinity may well be a limit for the distribution of several freshwater species (see also Mylnikov 1983). Occurrences at extremely high salinities compared to the surrounding water have been reported from sea ice biota (e.g. Ikävalko and Thomsen 1997).

Temperature is another important factor for the distribution of organisms. It is well-known that growth rates of flagellates increases with a rise of temperature (e.g. Fenchel 1986a; Choi and Peters 1992). Most determinations of HF growth rates have been carried out at temperatures of 10-20 °C. However, it seems that HF are well-adapted to live and reproduce also at very low temperatures. For the pelagial, Choi and Peters (1992) determined growth rates of *Paraphysomonas* strains of 0.5-0.8 d⁻¹ at -1.5°C. Recent studies of growth rates at about 0°C of benthic HF revealed values of 0.1 d⁻¹ (total HF community) and 1 d⁻¹ (small heterokonts) for temperate brackish waters and values of 0.3 d⁻¹ (total HF community) and 0.7 d⁻¹ (small heterokonts) for Antarctic freshwaters (Dietrich & Arndt, in prep.). A third important factor for flagellates, especially benthic HF, is the tolerance to low oxygen concentrations. The drastic vertical changes in the

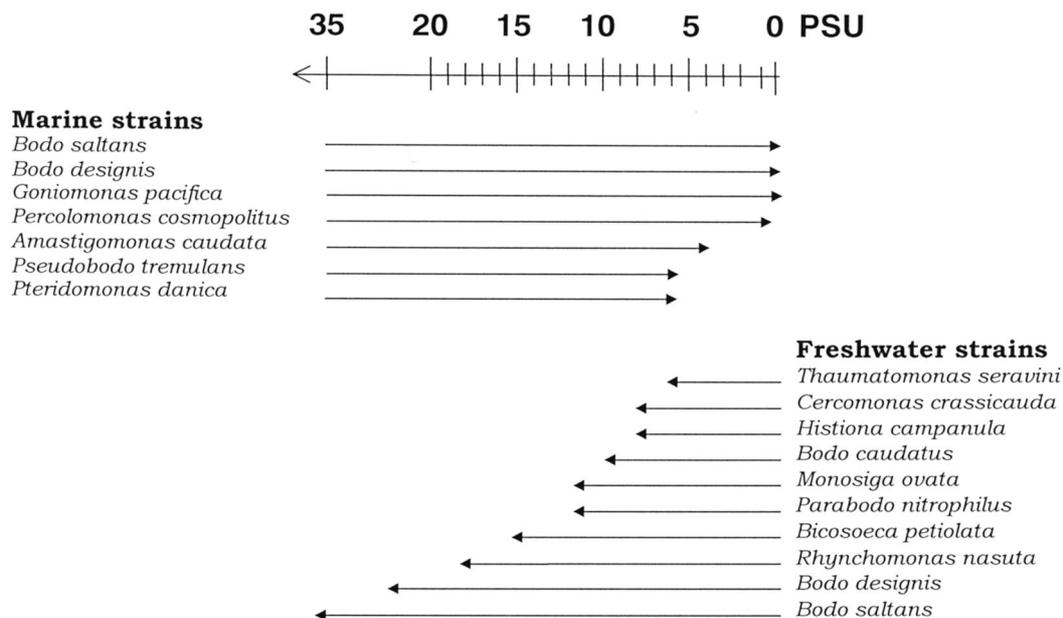


Figure 12.7 Survival of HF strains, isolated from different marine and freshwater sites, at different salinities. Marine strains were transferred in steps from 35, 30, 25, 20, 15, 10, 8, 6, 4, 2, to 0 PSU every day, freshwater strains were transferred in steps of 1 PSU every day, except for *Rhynchomonas* and *B. saltans*, which were transferred in steps of 2 PSU every day. Flagellates were fed by *Aerobacter aerogenes* ad libidum, as they were fed in maintenance cultures. All experiments were done in five parallel petri dishes for both, experimental and control sets

Source: Mylnikov and Gräfenhan unpublished.

structure of HF communities indicate that tolerances strongly differ between different species. Under anoxic conditions typically amitochondriate flagellates appear in field samples, mainly diplomonads, archamoebids, trichomonads. It seems that also several normally aerobic forms can reproduce or at least survive anaerobic conditions by physiological adaptations (for a review see Fenchel and Finlay 1995).

Pressure is assumed to limit the distribution of flagellates in the deep sea (Turley and Carstens 1991). However, high numbers of HF have also been reported from the deep sea sediments (cf. Alongi 1991). Live counts from a depth of 1200m in the Mediterranean revealed comparable compositions of flagellate communities as in shallow waters (Arndt & Hausmann, unpubl., Fig. 12.1.A).

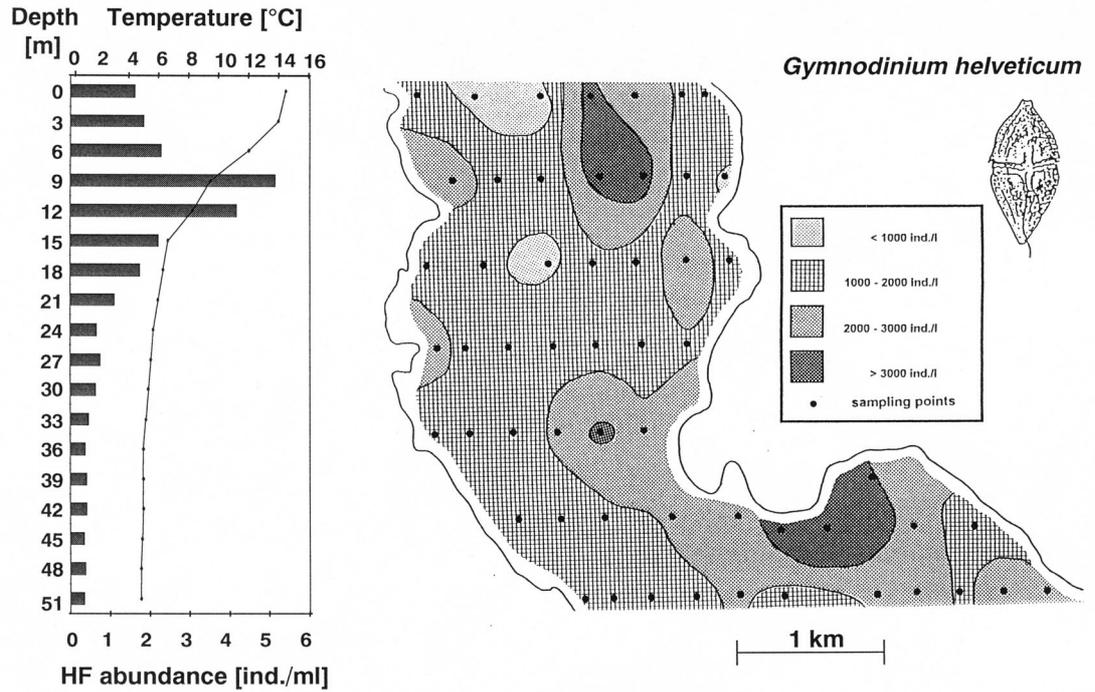
12.4.3 Spatial distribution patterns and life strategies

Horizontal distribution patterns of heterotrophic HF species in the macro- and microscale are not well documented. The examples given in Figure 12.8 indicate a

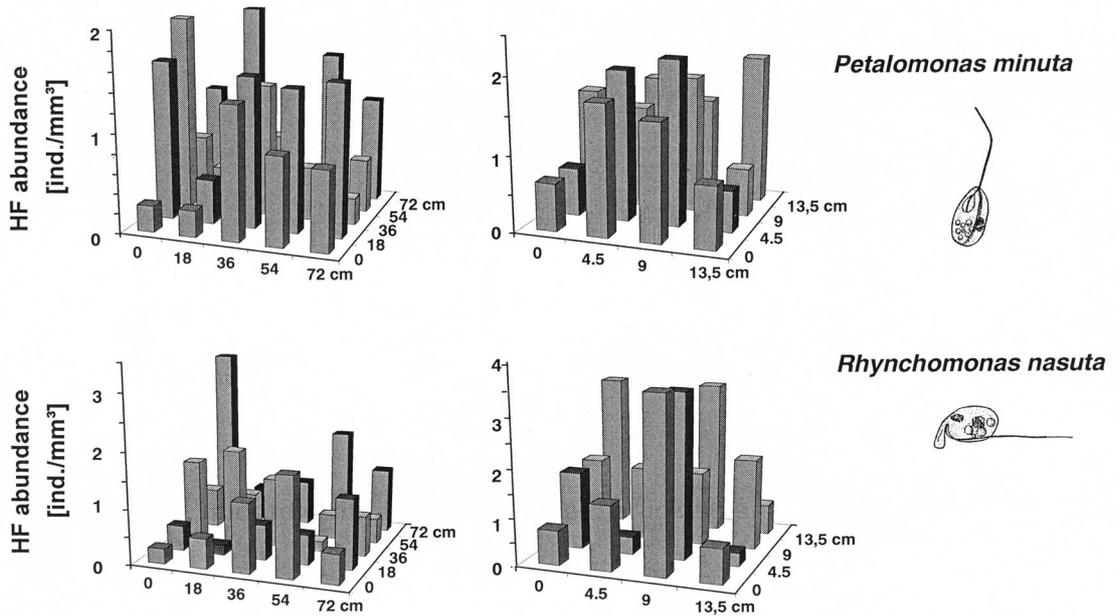
(right)

Figure 12.8 Distribution patterns of heterotrophic flagellates.
A Horizontal (3m depth) and vertical distribution of the heterotrophic dinoflagellate *Gymnodinium helveticum* in the pelagial of Lake Mondsee, Austria, during May 1993 (Salbrechter in Arndt 1994).

A: Pelagial, vertical and horizontal distribution (Lake Mondsee)



B: Benthal, microdistribution (Hiddensee, brackish shallow waters)



B Patterns of the horizontal microdistribution of two benthic nanoflagellates, the euglenid *Petalomonas minuta* and the bodonid *Rhynchomonas nasuta*, in the upper 0-3mm sediment layer at Kloster, Hiddensee (southern Baltic; horizontal distances of samplings 18 and 4.5 cm, resp.; Gräfenhan, Heinrichs and Arndt unpubl.).

several fold change in abundance in the vertical and horizontal distribution of the heterotrophic dinoflagellate *Gymnodinium helveticum*. This pattern for a pelagic HF species is similar to those known for phototrophic protists and metazooplankters. Patchy distribution in the micro-scale also seems to be an important feature of benthic HF populations (Fig. 12.8 B). Variations in abundance were of a similar order of magnitude as is known for benthic ciliates and meiofauna organisms (Arlt 1973). The causes for the horizontal distribution patterns observed for pelagic and benthic HF are not well understood, but are most probably a combined effect of several different factors such as active migration, passive transport, food concentration and reproduction, and predation pressure.

Life conditions in small habitats, e.g. suspended detritus flocs, sand grains, can change very rapidly. An important part of HF life strategies comprises the ability to form cysts. Among the most abundant forms at least chrysomonads, choanoflagellates, bodonids, dinoflagellates, thaumatostigids and cercomonads are able to survive unfavourable conditions in form of cysts. Several species do not form cysts in cultures, but this does not necessarily mean that they are not able to form cysts under field conditions. In many cases the specific environmental cues are probably missing under conditions of cultivation. The speed of encystment and excystment seems to be important for survival and competition of species, but comparative data are rare.

A special property of HF is their ability to grow at relatively high rates, as high as the average growth rate of bacteria in field populations, enabling them to control bacteria dynamics. Specific growth rates are very much dependent on food conditions. Under favourable conditions in cultures many different HNF species can grow with maximum rates (e.g. Fenchel 1982a; Eccleston-Parry and Leadbeater 1994a). Thus growth rates reported from literature are very variable. There are only a few clear trends: doubling times of large flagellates are known to range between less than a day to several days (Carrick et al. 1992; Falkenhayn and Lessard 1992; Hansen et al. 1997) whereas some nanoflagellates may divide within an interval of 3-4 hours. The succession of flagellates in plankton or benthos samples generally starts with a dominance of heterokonts, mainly chrysomonads, in benthos samples also bicosoecids and pedinellids, indicating this group to be an r-strategist compared to other flagellate groups. To survive periods of starvation HF have been reported to significantly lower their metabolic rates (Fenchel 1986a).

12.4.4 Mortality of flagellates due to predation

In pelagic communities predation plays a major role for the regulation of HF abundances. Field studies on the succession of seasonal events in the pelagial revealed that high abundances of metazooplankters mostly correspond with low abundances of flagellates. Laboratory and a few field experiments have shown that ciliates, rotifers, cladocerans and copepods (for reviews see Arndt 1993a; Sanders and Wickham 1993; Cleven 1996; Jürgens et al. 1997b) can act as voracious predators of heterotrophic flagellates. There are only a few quantitative estimates of the different loss factors acting under field conditions (e.g. Weisse 1991; Arndt and Nixdorf 1991). Figure 12.9 gives an example for the seasonal changes regarding the quantitative impact of different predators on nano- and microflagellates in a hypertrophic lake.

Generally the major part of flagellate production is consumed by predators. From early summer until autumn losses were mainly due to mesozooplankton, particularly cladocerans, which caused a drastic decline of HF biomass especially of slowly reproducing large forms (for data of biomass and composition of HF see Fig. 12.1.D and 12.2.C). In winter and spring, however, a major part of HF mortality was due to other heterotrophic protists. Interestingly, spring mortality of HNF was mainly due to other HF in the size fraction 5-28 μm (*Kathablepharis*, large chryomonads, *Gymnodinium*) and to a lesser extent to small oligotrichs and prostomatid ciliates. This result supports the importance of omnivory among HF (see Sections 12.4.1 and 12.4.6). Losses of large flagellates during spring were mainly due to ciliates. As indicated in Figure 12.9 other losses in addition to predation cannot be neglected. Such losses could include encystment or lysis due to starvation or due to other unfavourable conditions, mortality due to viral infections or parasites. According to our estimate these losses can account for more than 50 per cent of daily production at certain times and thus offer an interesting topic for future studies.

Even less information is available on the fate of benthic flagellate production. Ciliates have been shown to be potential predators of benthic flagellates (e.g. Fenchel 1969; Epstein et al. 1992). Recent size-fractionation experiments in the surface layer of a Baltic muddy sand showed that between 0 and 120% of daily HF production can be due to predation by ciliates and meiofauna (Dietrich & Arndt, in prep.). There are at least indications that also in benthic systems predation can be an important factor for the occurrence of flagellates.

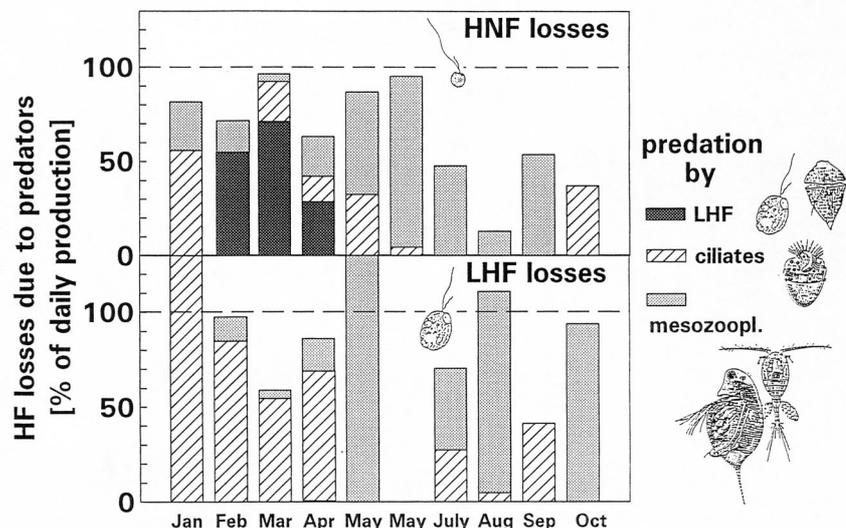


Figure 12.9 Seasonal changes in the losses of the production of heterotrophic nanoflagellates (HNF; $<15\mu\text{m}$) and large flagellates (LHF; $>15\mu\text{m}$) due to the presence of different predators.

Results of size fractionation experiments (Lake Müggelsee, Berlin, cf. Arndt and Nixdorf 1991, Arndt 1994) using two parallel 1-litre-bottles with lake water filtered through sieves of mesh sizes of either 44 μm (without metazoans), 20/28 μm (without large ciliates) or 5/10 μm (without small ciliates and large flagellates) incubated for one day *in situ*. HF production was estimated from population growth rate in the course of the experiment in the fraction 5/10 μm (HNF) or 20/28 μm (LHF).

A consumption of a significant part of HF production by predators would strongly select for avoidance strategies. On the background of the above mentioned data several behavioural and morphological features of HF might well be interpreted today as adaptations to avoid predation: 1) morphological adaptations such as the possession of loricae (e.g. many bicosoecids, choanoflagellates), thecae with appendices (dinoflagellates), scales (chryomonads and thaumatomastigids), the increase of size due to formation of colonies (e.g. several heterokonts, choanoflagellates, spongomonads etc.); 2) chemical defences such as toxin production and extrusomes (e.g. dinoflagellates); 3) behavioural adaptations such as attachment to large particles (e.g. chryomonads, bicosoecids, choanoflagellates), retraction of protoplasmic threads (e.g. pedinellids, chryomonads), vertical migration (e.g. dinoflagellates), swimming patterns (e.g. dinoflagellates, kathablepharids); 4) adaptations of life strategies such as high reproduction rates (e.g. chryomonads, pedinellids) and survival of parts of the population in the sediment in cysts.

Since generally predation by protists is underestimated, several of these defence mechanisms have not been interpreted as such. For instance, scales and loricae may not be effective to avoid predation by metazoans but can probably reduce the impact by other protists. Association or attachment of HF to large particles might reduce their vulnerability to protozoan suspension feeders and to a certain extent also to metazoan filter feeders. In the sediment masking by particles can play a certain role. Maybe that vertical migration into the anoxic zone can protect some species such as euglenids from aerobic predators. Motionless forms (e.g. *Massisteria*) might also be difficult to detect by predators. Species which seems to be not very much protected from predation have often very high reproduction rates (e.g. *Spumella*) or survive periods with strong predation pressure as cysts in the sediment.

Figure 12.5 (lower panels) may serve as a summary to illustrate the diverse functions of HF acting as bacteriovores, algivores, detritivores, carnivores and, probably mainly, as omnivores in pelagic as well as in benthic systems. Since flagellate ecology is a new field many questions require further intensive studies. Detailed autecological studies are available for only very few species (even now the determination of species on a quantitative level is difficult for many athecate and aloricate forms). The systematic position of several ecologically important groups of flagellates is not yet clear. It seems that several important species have to be described yet.

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