

LARGE HETEROTROPHIC FLAGELLATES FORM A SIGNIFICANT PART OF PROTOZOOPLANKTON BIOMASS IN LAKES AND RIVERS

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ABSTRACT

Stimulated by studies from marine waters there is now increasing evidence that the components of the microbial food web contribute to the pelagic flux of matter as significantly in freshwater environments as they do in the ocean. Up to now generally only heterotrophic nanoflagellates and ciliates have been quantitatively considered among limnetic protozooplankton. Our investigations using a special live-counting technique showed that large heterotrophic flagellates (LHF, $\geq 15 \mu\text{m}$; mainly chrysomonads and dinoflagellates) have been overlooked in quantitative estimations of limnetic protozooplankton biomass. We present estimations from 19 German lakes of different trophy during different seasons and from 4 rivers as well as detailed seasonal studies of a eutrophic lake and a brook indicating that LHF can form up to 50% (mean about one third) of protozooplankton biomass. LHF reached highest importance in early spring when LHF were one of the most significant herbivore groups.

Large heterotrophic flagellates should be considered as significant components of limnetic food webs. This is in agreement with the present knowledge of marine pelagic ecosystems where LHF (mainly dinoflagellates) have already been recognized as an important part of the pelagic microbial web.

INTRODUCTION

Protozoans are now recognized as important trophic links in marine pelagic ecosystems (Azam et al. 1983, Porter et al. 1985). There is increasing evidence that this is true also for limnetic pelagic ecosystems, but quantitative data are rare (e.g. Berninger & Finlay 1988, Weisse 1990). When protozoans have been considered in the context of the "microbial loop" up to now only heterotrophic and mixotrophic pico- and nanoflagellates and ciliates were viewed as significant components. Recent studies from marine waters indicated that other compo-

nents like large heterotrophic flagellates can be of quantitative importance (Smetacek 1981, Lessard & Swift 1985, Arndt in press a). These large heterotrophic flagellates (mainly dinoflagellates) can be voracious feeders on phytoplankton (e.g. Gaines & Elbrächter 1987, Jacobsen 1988). Recent laboratory studies with large ($\geq 12 \mu\text{m}$) limnetic flagellates indicated that in limnetic waters, too, herbivorous flagellates should be considered (e.g. Suttle et al. 1986). However, quantitative data from the field are lacking. The importance of flagellate algivory under in situ conditions was already addressed by Sherr & Sherr (1989).

When we tried to determine the total protozooplankton biomass in a eutrophic lake, we recognized that large heterotrophic flagellates (dinoflagellates and chrysomonads) and naked rhizopods were insufficiently considered by currently applied methods. During certain periods these components composed up to 50% of protozooplankton biomass in Lake Müggelsee, Berlin (cf. Arndt & Nixdorff 1990). The aim of the present study was to evaluate the general importance of large heterotrophic flagellates ($\geq 15 \mu\text{m}$, LHF) within the protozooplankton in various lakes and running waters of different trophy and during different seasons.

MATERIAL AND METHODS

The quantitative analysis of protozooplankton was carried out in 1988-1990 in subsamples of integrated samples from the mixed surface layer during different seasons (pooled values of sporadic investigations: sp = spring, su = summer, au = autumn, wi = winter) of 3 oligotrophic lakes (Paschensee (in Fig. 4 number 1, sp), Stechlin (2, au), Schaalsee (3, sp; 4, su), 4 mesotrophic lakes (Neustädter See (1, sp; 2, su; 3, au), Pinnower See (4, sp; 5, au), Neumühler See (6, sp; 7, su; 8, wi), Krakower Untersee (9, sp; 10, au)), 7 eutrophic lakes (Krakower Stadtsee (1, sp; 2, au), Klein Pritzer See (3, sp), Rudower See (4, sp; 5, sp su), Schweriner Außensee (9, sp, 10, au), Medeweger See (11, sp; 12, su), Bützower See (13, sp), Schwielochsee (14, su)), 5 hypertrophic lakes (Schönlager See (1, sp), Boissower See (2, sp), Neukirchener See (3, sp), Tempziner See (4, sp), Woezer See (5, sp)) and an estuary (Barther Bodden, 4-7⁰/00 S (1, su; 2, au). All lakes are situated in Mecklenburg (Northern Germany) except for Lake Schwielochsee which lies about 70 km southeast of Berlin.

In addition, four river sites were investigated: Spree (at Neuzittau, 20 km southeast of Berlin), Oder-Spree-Canal (OSC, at Fürstenwalde), Danube (at Göd, 30 km north of Budapest), Elbe (at Wahrenberg, 100 km southeast of Hamburg, near Wittenberge). At all these sampling stations sporadic investigations were carried out and summarized for a season (in Fig. 3 sp, su, au, wi as indicated above). For studies of seasonal changes in the contribution of large heterotrophic flagellates (LHF) to protozooplankton biomass more detailed studies with sampling periods of 1 to 3 weeks were performed at two sampling sites: 1) polymictic

shallow eutrophic Lake Müggelsee (Berlin, area 7.2 km², mean depth 4.9 m), 2) River Warnow which is at the station Rönkenhof (15 km east of Schwerin) still a mesosaprobic brook (flow rate 1-3 m³/sec, mean depth 0.75 m).

Protozoans were counted using a minute live-counting technique under a light microscope (cf. Dale & Burkill 1982, Güde 1986) but on a temperature-controlled microscopic table (slightly below in situ temperature). Unconcentrated samples were analysed immediately after sampling (at least 1-2 hours after sampling, storage in 1l-bottles at in situ temperature) in chambers of different size (5-10 µl, 50 µl, 400 µl, 2-10 ml; several chambers of each type were counted during each investigation). Differentiation regarding autotrophy and heterotrophy of flagellates was done by means of epifluorescence microscopy (cf. Davis & Sieburth 1982) in combination with light microscopy, sometimes uncertainties occurred which were mostly solved using notes in the taxonomic literature. Comparisons were done by DAPI - and Primuline-staining (e.g. Caron 1983) proving that nanoflagellate counts of fixed (2% glutaraldehyd) and live-counts are in the same range, but picoflagellates are underestimated by live-counting. Since heterotrophic picoflagellates accounted for less than 20% of nanoflagellate biomass in tested samples these underestimations seem to be unimportant regarding our estimations of total protozooplankton biomass (though they are important in the matter flux). Biovolumes of all protozoans were calculated from measurements of dimensions of living animals and approximations to simple geometrical forms. Improved investigations of living samples, which are at present generally done only by taxonomists, have considerable advantages for routine investigations especially under eutrophic limnetic conditions: exclusion of biases from fixation, storage and concentration (e.g. Choi & Stoecker 1989), possible registration of behaviour, form and size of organisms as well as counts of protozoans present at very different concentrations. Especially delicate large chrysomonads suffer from fixation and filtration and are difficult to recognize at the low concentrations (less than 1% of nanoflagellates) during routine nanoflagellate counting. The same seems to be true for naked rhizopods (not considered in detail in this paper).

RESULTS AND DISCUSSION

Seasonal changes of LHF in Lake Müggelsee

During investigations of the microbial and metazoan components of Lake Müggelsee (cf. Arndt & Nixdorf 1990) protozooplankters were investigated from April 1988 through July 1990 (Fig. 1). The seasonal cycle of protozooplankton is characterized by a distinct peak in March/April at the end of the first phytoplankton spring bloom, extremely low values in May/June during the clear-water phase with high abundances of cladocerans and moderate values during the other parts of the year.

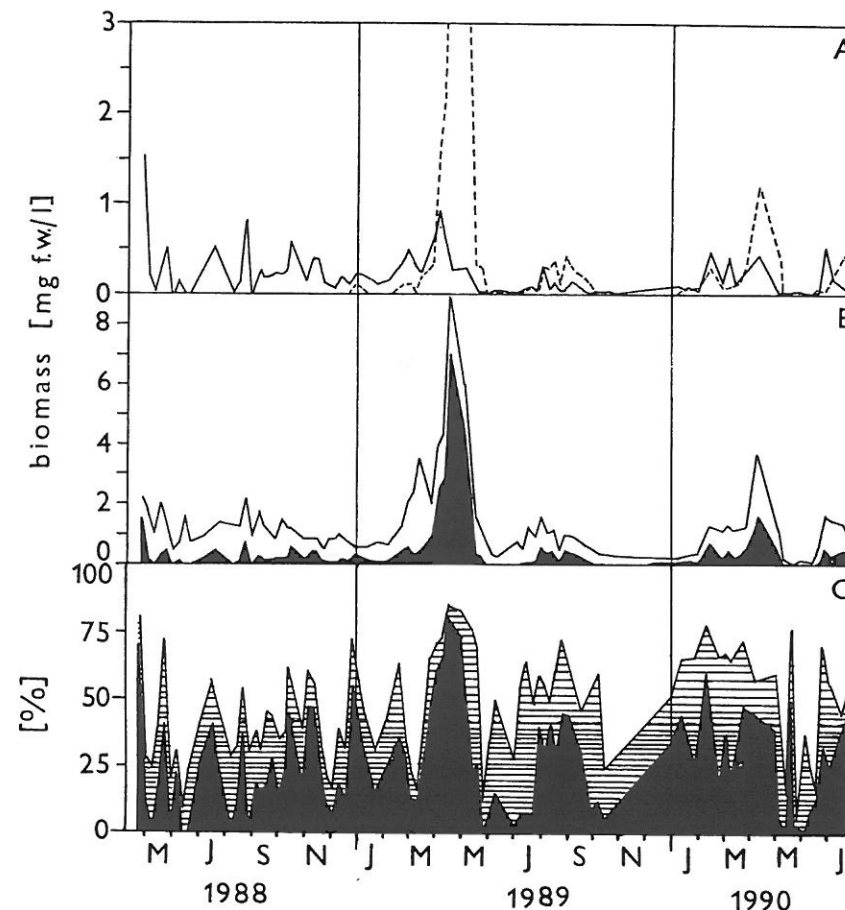


Fig. 1. Seasonal changes of protozooplankton biomass in Lake Müggelsee, Berlin. A: Large heterotrophic flagellates (LHF, — chrysomonads, - - - dinoflagellates), B: Total protozooplankton (black: LHF), C: Percentage of LHF (black), heterotrophic nanoflagellates (hatched) and ciliates and rhizopods (white).

Interestingly, although it has been largely neglected up to the present, the protozooplankton component LHF composes a very significant part of total biomass, on annual average about 30%, and lies in the same range as nanoflagellate biomass. Among LHF chrysomonads (esp. the genera *Spumella* and *Paraphysomonas*) and dinoflagellates (esp. *Diplopsalis acuta* (summer), *Gymnodinium helveticum* (spring peak) and some other gymnodinioids) dominated (see Fig. 1 upper panel). Obviously mixotrophic forms like *Ceratium hirundinella* (abundant during summer) and some peridiniids were not included in the counts. Though it was observed that LHF were able to consume bacteria (Arndt unpubl.) the analysis of

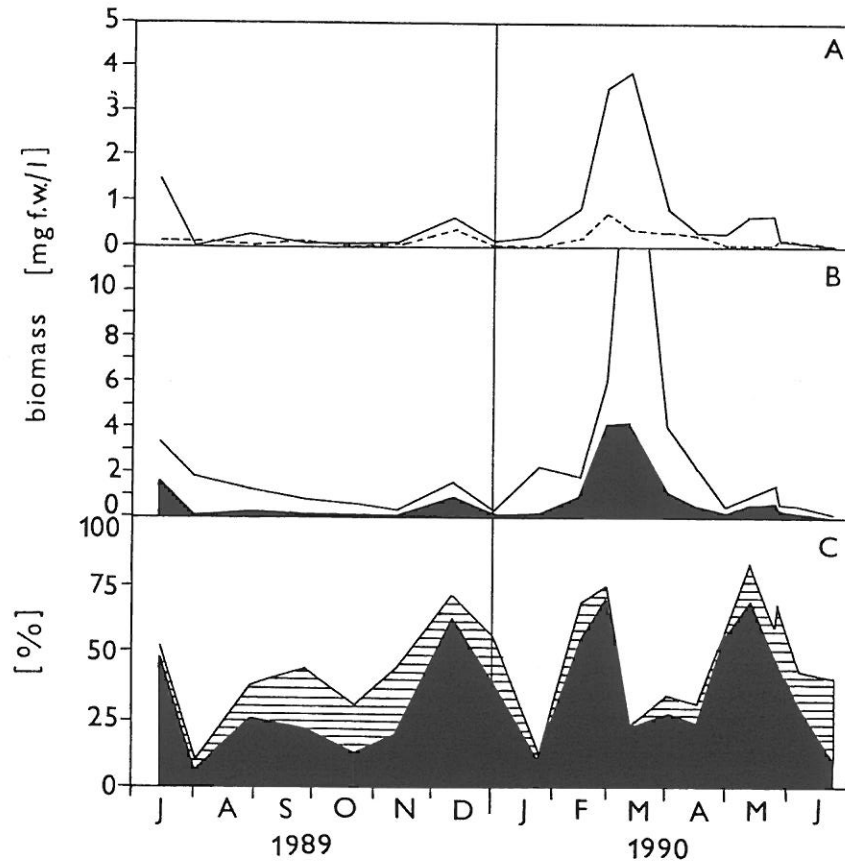


Fig. 2. Seasonal changes of protozooplankton biomass in River Warnow, Mecklenburg (northern Germany) (A-C as in Fig. 1).

food vacuoles led to the conclusion that algivory was the most important feeding type in Lake Müggelsee. Among phytoplankters observed in food vacuoles were representatives of all taxonomic groups from small chlorophytes and blue-greens to small and large diatoms, cryptophytes, chrysophytes and filamentous diatoms and blue-greens up to the individual volume of the LHF itself. Some species seemed to select for special forms: *Gymnodinium helveticum* for centric diatoms (diameter 10-25 μm), *Diplopsalis* for single centric diatoms and *Melosira*, *Spumella* for algae in the size range of 20-40 μm (e.g. filaments of blue-greens, pennate and centric diatoms, cryptophytes). It seems that especially chrysomonads which engulf forms that are sometimes larger than themselves were consumers of the so-called "inedible" part of the phytoplankton. This is in accordance with observations by Suttle et al. (1986) and Smetacek (1981). The thecate dinoflagellates were

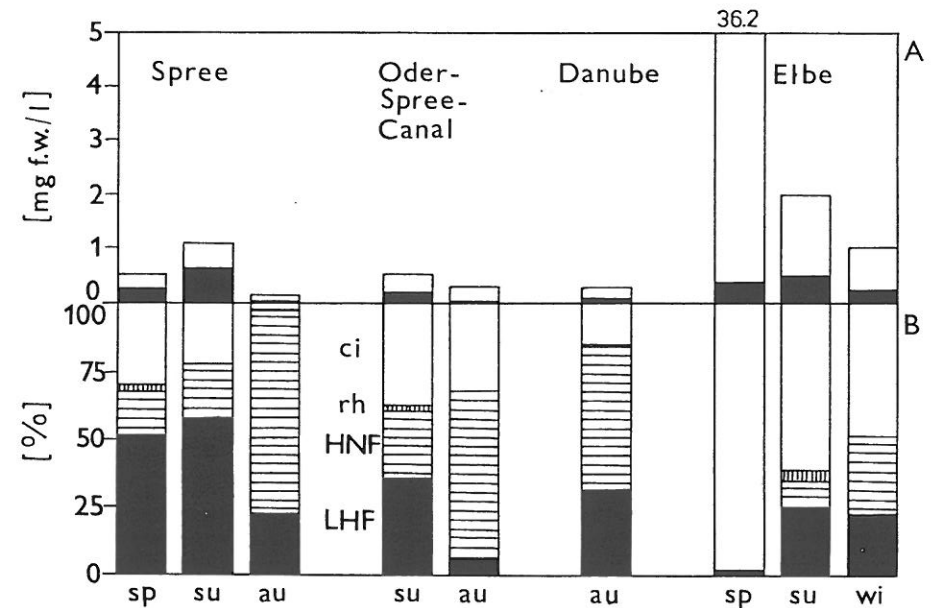


Fig. 3. Biomass and composition of protozooplankton in different rivers. A: Total protozooplankton biomass (black: LHF), B: Percentage of large heterotrophic flagellates (LHF), heterotrophic nanoflagellates (HNF), rhizopods (rh), and ciliates (ci).

often observed to feed by pseudopod formation outside the theca as it is well-known for marine dinoflagellates (e.g. Jacobson 1988). In early spring LHF were by far the most important herbivores in the plankton community of Lake Müggelsee and significantly contributed to phytoplankton mortality (Arndt in press b). In addition LHF were consumers of heterotrophic nanoflagellates, acting as carnivores, and bacteria (Arndt in press b, Arndt & Nixdorf 1990). On the other hand we found that LHF were preyed by metazoans and ciliates (Arndt in press b). The very low abundances of LHF in early summer were the result of a high grazing pressure by cladocerans (cf. Arndt & Nixdorf 1990).

Seasonal changes of LHF in River Warnow

Comparative studies at the eutrophicated brook site revealed that in running waters, too, LHF may be of great significance (cf. Fig. 2). Up to now there are only a few investigations of protozooplankton in rivers (e.g. Nosek & Bereczky 1981, Sorokin 1987). Protozoan dynamics and biomasses were comparable to that of Lake Müggelsee, with the exception that chrysomonads were by far the most important group of LHF. This was also the case in all other investigations of river plankton. The reasons may be that turbulence affects more hard covered bodies

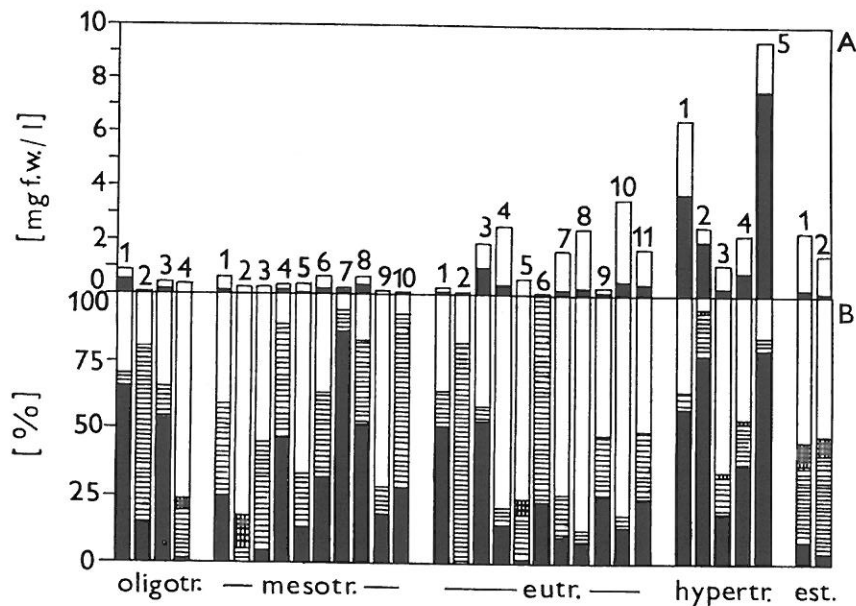


Fig. 4. Biomass and composition of protozooplankton in lakes of different trophicity (A and B as in Fig. 3, numbers refer to samplings in different lakes, cf. Material & methods for explanation).

like that of dinoflagellates, higher growth rates of chrysomonads (estimated in River Spree to be like that in lake water, Arndt unpubl.) promote these forms especially in running waters with high flushing rates and reduced predation pressure by metazooplankton. Since LHF originate from inocula of standing waters it is not surprising that the species composition was similar to that of lake waters.

Occurrence of LHF in different rivers

During our studies of running waters we found that about 75 to 98 per cent of the zooplankton biomass was composed of protozooplankton. This observation may require a re-evaluation regarding the flux of matter in river plankton (see also Sorokin 1987), because up to now only metazoans with their low biomasses in rivers have been considered. In Fig. 3 biomasses and composition of protozooplankton are indicated for four different river sites. As was seen from seasonal changes in River Warnow these values changed significantly from season to season. Except for the spring values of River Elbe, when a mass development of large ciliates on detrital flocs of this highly polluted river occurred, LHF formed an important part of protozooplankton biomass and – with the low relative biomass of metazoans in mind – also of total zooplankton biomass. LHF should be considered as important herbivores in river plankton. From investiga-

tions of sampling following the flowing wave of the River Spree over 150 km we found that *Spumella*-like LHF increased their biomass severalfold in the course of the river indicating their high metabolic activity within potamoplankton. Analysis of food vacuoles of chrysomonads in running waters far from lake water inputs showed their importance as algivores also under turbulent conditions (Mathes & Arndt unpubl.).

Occurrence of LHF in lakes of different trophicity

Since all results mentioned above were from eutrophic waters an important question arose regarding the influence of trophicity on the occurrence of LHF. Fig. 4 presents our results from 20 lakes arranged according to increasing trophicity and a eutrophic estuary of the southern Baltic. It is evident that the biomass of total protozooplankton and that of LHF increased with increasing trophicity, an observation which is in accordance with findings regarding HNANO (Berninger & Finlay 1988) and ciliates (Beaver & Crisman 1989) for waters with a different degree of eutrophication. The dominating taxonomic groups were the same as mentioned above. Regarding the relative contribution of LHF to total protozooplankton biomass there seems to be no general pattern with respect to trophicity, but as already documented for Lake Müggelsee and River Warnow there are strong changes with season. Fig. 5 summarizes the relationships between season and trophicity. The biomasses of LHF during the early phytoplankton spring bloom reached very high values even in oligotrophic waters. Results from the Baltic showed that LHF were also of significance in estuarine waters (cf. Smetacek 1981, Arndt in press a).

Conclusions

As already concluded from laboratory investigations (Suttle et al. 1986) our field investigations revealed that LHF (esp. chrysomonads and dinoflagellates) should be considered a significant component of limnetic protozooplankton. Especially during the early spring bloom LHF form a large part – up to 50 per cent – of total zooplankton biomass and should contribute significantly to zooplankton algiivory during this time when metazoans are generally of reduced importance. In river plankton protozooplankton has up to now only rarely been considered. Our results showed that in the running waters investigated protozoans were by far the most important component of total zooplankton biomass. LHF composed a significant part of it. LHF play a role in limnetic pelagic food webs as important algivores as well as a potential food source for metazoans and also for other protozoans. In early spring LHF seemed to replace metazoans as the dominant herbivores.

These observations are in agreement with our present knowledge concerning the marine pelagic food web, where LHF (especially dinoflagellates) have already

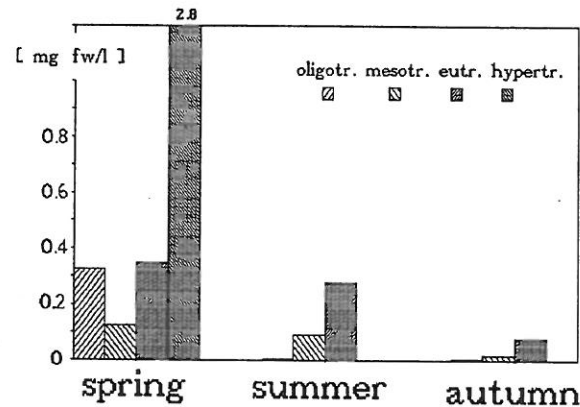


Fig. 5. Biomass of large heterotrophic flagellates summarized for studies at different trophic and season.

been recognized as a significant component (e.g. Lessard & Swift 1985, Sherr & Sherr 1988). However, much more data are necessary to evaluate all the different functions of LHF in limnetic waters. Comparisons using other methods in different waters have to be carried out. An open question is the contribution of heterotrophic nutrition by mixotrophic LHF as well as quantitative data of feeding rates and the energy budget of limnetic LHF.

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