

Functional diversity of Heterotrophic Flagellates (Protozoa) in the plankton of the river Danube at Göd (Hungary)

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1 Introduction

The description of an alternative trophic pathway, the microbial loop in the middle of the 1980s (Azam et al. 1983) revealed the crucial trophic role of protozoa in planktonic food webs. This facilitated strongly first the quantitative, later the taxonomic investigation of heterotrophic flagellates. Recent ecosystem studies cannot ignore this trophically important group. In spite of their trophic role, there are few quantitative investigations of heterotrophic flagellates in the river Danube (Vörös et al. 2000), and modern taxonomic studies are completely lacking. However, heterotrophic flagellates have the highest biomass among protozoa in an annual mean in the plankton of the river Danube in Hungary (Kiss et al. 2009), and they are one of the most important consumers in the planktonic microbial food web (Kiss 2007).

Most ecosystem studies have considered heterotrophic flagellates as a functionally uniform, bacterivorous 'black box'. In the past few years information is growing about the functional diversity of heterotrophic flagellates (Arndt et al. 2000). Most classifications establish functional groups according to the morphological and behavioural properties of flagellates. Field studies, which investigate the resource utilization of species in nature are sparse. Many functional data from species are based on investigations of cultures (Boenigk & Arndt 2000). As heterotrophic flagellates are a trophically important group with a huge consumption, it is very important to reveal their functional properties and functional diversity. Besides the functional investigation of the most abundant species, the investigation of local functional diversity would be also important from the viewpoint of the functional diversity - ecosystem stability relationships (Loreau et al. 2001).

The main goal of this study is to reveal the local functional diversity of heterotrophic flagellates in the plankton of the river Danube. Species utilizing the same resources belong to the same niche, and if the method of research utilization is also the same, they belong to the same guild. Applying these definitions in the present work, we reveal the species groups belonging to different realized niches by investigating the utilized resources of species instead of grouping them according to their morphological or behavioural properties. We also investigate the number of guilds by measuring those ecological parameters of species, which influence the method of research utilization. Finally we try to give explanations for the coexistence of species according to the results.

2 Material and methods

To investigate the local diversity, a single plankton sample was collected from the left river bank of the Danube, 10 meters from the shore at Göd, Hungary (1668 riv. km) on 16th March 2008. Fifty litres of water (5 dipping by bucket) was filtered through a 10 µm mesh-sized plankton net. All equipments were sterile or disinfected with ethanol. The 150 ml remnant was transported to laboratory, poured into a wide glass bowl, and let undisturbed for the microbial succession. The sample was monitored for 37 days. After stirring, subsamples were taken and investigated in a microaquarium with an inverted microscope (Olympus IX-70), 100xHI-1.3NA objectives, and strong Nomarski DIC contrast. Videos were taken by an analogous 3CCD camera (JVC KY-F30B), the sign of which was digitalised by an A/D converter (Dazzle Hollywood DV-Bridge), and recorded on computer. Videos were taken from all individuals of species, except of the very abundant ones. Videos were later analysed by additional digital contrast enhancement, in many occasions

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from frame to frame. Seven functional variables were measured/determined from the videos. Four variables, which determine the utilized resources are: 1. Primary source of energy (light, dissolved organic matter or particulate organic matter). 2. The food particle suspended or attached to aggregates. 3. The size of ingested food particle. To parameterize this variable we measured the size of the smallest, the mean and the largest ingested food particles in specimens of every species. We applied the simple approximation that species are non-selective for the quality of food. 4. The presence of a predator avoidance mechanism (lorica) against the predatory pressure of a carnivorous flagellate (*Rhynchobodo* sp.). Three further variables, which influence the method of resource utilization are: 5. For flagellates grazing suspended particles the quotient of the moving speed of the flagellate and the speed of the flow field generated by the flagellum. This quotient is 0 for suspension feeders and almost 1 for free swimming raptors. 6. For gliding flagellates the frequency of wobbling/beating illustrated as the function of swimming speed may be informative from the efficiency of food searching. 7. The relative importance of the motoric forces of beating (mostly the anterior) and gliding (mostly the posterior) flagella, in relation to the moving speed is informative from the efficiency of food searching, and the position and attachment of consumable food particles.

3 Results

Altogether 130 heterotrophic flagellates and other hardly classifiable picoeukaryote species were found in the sample during the investigation period. The distribution of species along the seven variables were the following: According to the primary source of energy (1. variable) two species were obligate osmotrophic, and 128 were able to ingest particulate food. Three from them were facultatively mixotrophic and contained presumably cleptoplasts (*Notosolenus apocamptus*, *Protaspis gemmifera*, *Collodictyon triciliatum*). According to the suspended or attached localisation of the food particles (2. variable), 38 species grazed suspended and 90 species grazed attached particles. The size distribution of food particles (3. variable) among suspended food grazing flagellate species was continuous from 0.1 to 20 μm . Smallest food particles were consumed by choanoflagellates and small chrysomonads, larger sized particles were consumed by free swimming raptors (*Rhynchobodo*, *Kathablepharis*, *Collodictyon*, *Gymnodinium*) and large chrysomonads. The distribution of food size among attached particle grazing flagellate species were continuous between 0.2-48 μm . Smallest particles were ingested by the smallest, 2-3 μm long raptors (spheric undescribed picoeukaryotes, *Kiitoksia ystava*, undescribed *Ancyromonas*, *Heteromita minima*). Most species grazed food particles between 0.4-1.5 μm . Larger-sized particles were consumed by large euglenids, and large *Protaspis* and *Cercomonas* species. Presumably 11 species had an efficient predator avoidance mechanism (lorica or cell wall; 4. variable).

The distribution of suspended particle grazing species along the quotient of the moving speed of the cell and the speed of the flow field (5. variable) showed an interesting transition between the two extremities. At zero value flagellates are attached to the substratum and bring food particles to themselves by the flow field of the beating flagellum. These were suspension feeder choanoflagellates, bicosoecids and pedinellids (12 species). Transitional groups with low quotient value (0.1-0.4) were the swimming forms of small chrysomonads, large *Spumella* species and *Collodictyon* (13 species). Species near value 1 were the free swimming raptors (12 species).

Investigating the attached particle grazing species, many groups can be differentiated according to the speed of the flagellates and the wobbling/beating frequency (6. variable). Zero speed belongs to the non gliding diffusion feeders (7 species). Very slowly moving (0-0.5 $\mu\text{m/s}$), non-wobbling species usually use their filopodia for the movement. These were mostly small spheric radially symmetric undescribed picoeukaryotes (16 species). The beating/wobbling frequency illustrated as a function of the speed for faster gliding species (>0.5 $\mu\text{m/s}$) is illustrated in Fig. 1. Three main groups can be differentiated with continuous transitions. Non-wobbling, smoothly gliding species are located at the abscissa (circle of dashed line in Fig. 1). Most euglenids, *Allantion*, *Thaumatomastix* species and some *Protaspis* and *Cercomonas* species belong to this group (19 species). Gliding and wobbling species are situated along the diagonal; slow species near the origo, fast gliding and wobbling species far from there. Very fast wobbling, but slowly moving species are near the ordinate (continuous circle on Fig. 1). These are *Ancyromonas sigmoides* and undescribed *Heteromita*, *Discocelis* and *Parabodo* species.

The supposed ratio of the motoric forces of the beating and gliding flagella in relation with the swimming speed is illustrated on Fig. 2. (7. variable). Species using only the gliding forces are situated on the left side of the figure with increasing moving speed; 41 species belong here. Considerably high moving speed can be achieved by using only gliding forces (30 $\mu\text{m/s}$). The fastest species were large euglenids (*Heteronema*, *Peranema*).

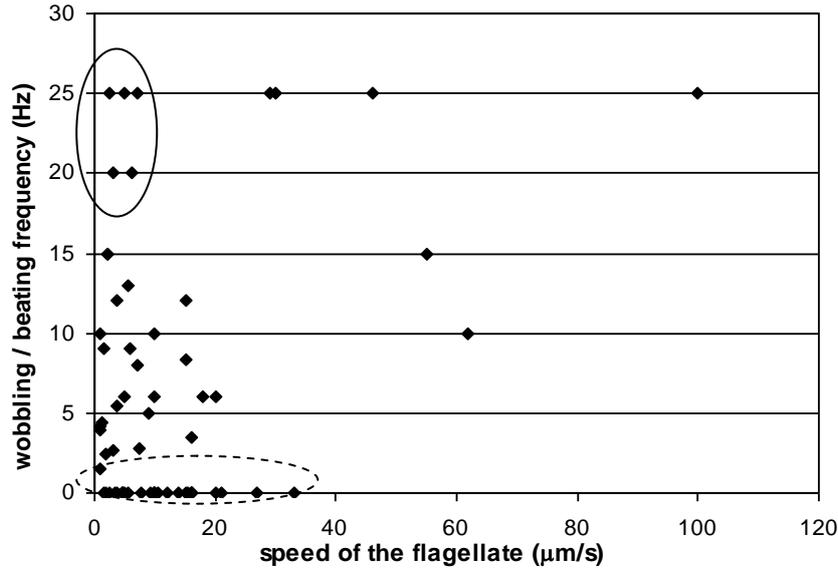


Figure 1. The wobbling/beating frequency of flagellate species illustrated as the function of gliding speed. Beating frequency of euglenids gliding on their anterior flagellum were considered to be zero, as they do not wobble. Non-wobbling species are marked by the circle with dashed line, very fast wobbling, but slowly moving species by the circle with continuous line.

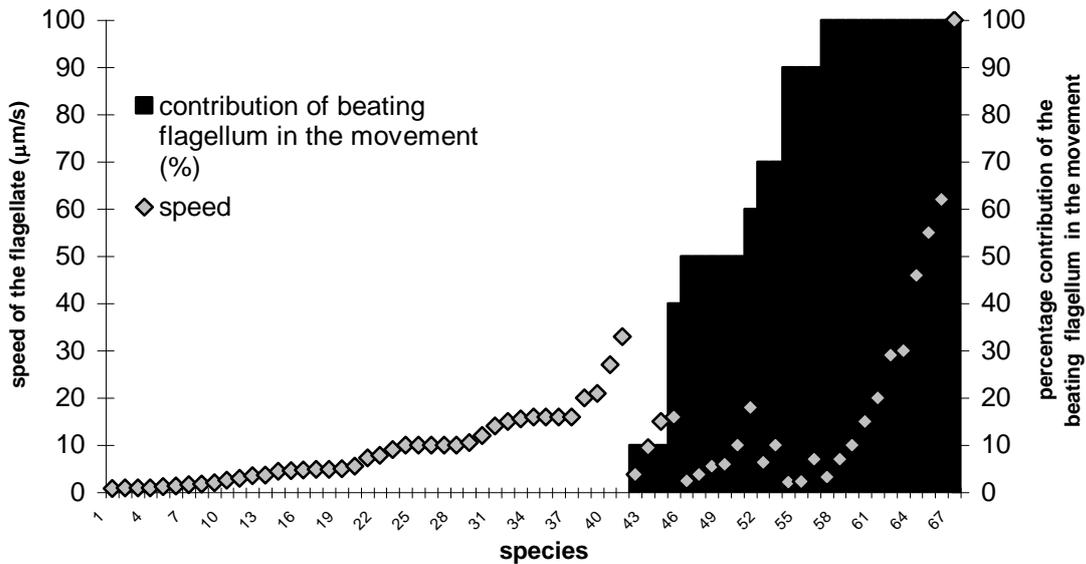


Figure 2. The contribution of the beating flagellum in the movement in relation with the swimming speed.

Species using the pulling force of the beating flagellum are indicated on the right side of the figure (black columns); 26 species belong here. The slowest species beats often with high frequency. Species moving faster than 15 $\mu\text{m/s}$ are attached to the substratum with only the distal part of their gliding flagellum (*Protaspis* species). Species moving faster than 30 $\mu\text{m/s}$ have only temporary contact with the substratum (*Bordnamonas tropicana*, *Goniomonas* spp., *Neobodo designis*, undescribed *Kathablepharis*).

Species belonging to the same niche can be grouped together according to the first 4 variables, which determine the utilized resources. Although the size distribution of the consumed food shows continuous transition, a number of non-overlapping food size ranges can be defined (4 for suspended particle feeders, 5 for attached particle feeders). By this splitting, the functional diversity still remains underestimated.

Accordingly, 15 functional groups can be differentiated, which belong to different realized niches. According to the method of resource utilization (variables 5-7), we can group species further to guilds. By the method of food acquisition we could differentiate diffusion feeders, slow gliders using pseudopodia, suspension feeders, raptors gliding on flagella and raptors swimming over surfaces. Taking these into account, altogether 27 guilds can be differentiated. Guilds comprising the most species among suspended particle feeders were the followings: smallest particle feeder loricate choanoflagellates (6% of total species); suspension feeder small chryomonads and pedinellids that feed particles between 0.6-1.4 μm (6%); raptors (*Colpodella*, kathablepharids, *Rhynchobodo*, 6%). Guilds comprising the most species among attached particle feeders were: smallest particle feeder picoeukaryotes, which move by pseudopodia (12%); gliding raptors that graze particles between 0.3-0.6 μm (18%) and gliding raptors that graze particles between 0.7-1.2 μm (21%).

4 Discussion

This study revealed a huge local species diversity of heterotrophic flagellates in the plankton of the river Danube, which considerably exceeds the species number found by other morphological studies (comparison: Kiss et al. 2008). Besides the general knowledge about the total potential functional diversity of the described heterotrophic flagellate species (eg. Sleight 2000), most field surveys containing functional investigations did not use deep taxonomic resolution, thus only a few coexisting functional groups were detected (Arndt et al. 2000, Boenigk & Arndt 2002). By this detailed study with long observation period, a high number of species could be detected, and by the videomicrography technique many important functional properties of species could be successfully analysed. The huge local functional diversity of heterotrophic flagellates found by this study highly exceeds our previous knowledge, and gives new insights to the possible ecological potential of these organisms. Ecosystem studies should be taxonomically refined at those habitats, where flagellates have large importance. In these cases the simple concept, which considers heterotrophic flagellates as bacterivorous 'black boxes' have to be improved in order to reveal finer trophic relationships.

Among suspended particle grazing flagellates, suspension feeder small chryomonads were the most abundant group in freshwater plankton (Arndt et al. 2000). They were accordingly rich in species in this study. Besides them, loricate choanoflagellates and free swimming raptors also had high species numbers. The first group had a presumably efficient predator avoiding strategy; the second group could exploit the rich food supply (flagellates and other microeukaryotes) with possible sharing of the diverse resources. Among attached particle grazing species, gliding piko-eukaryotes represent a yet unknown, but markedly diverse eukaryotic organization type. Albeit they move very slowly, they have an efficient feeding strategy as indicated by their occasionally high abundance. This group of organisms should clearly call larger attention in future research.

The investigation and quantitative analysis of variables influencing food acquisition methods of flagellates revealed some interesting new relationships. The species rich transitional group between suspension feeders and raptors among suspended particle grazers (5. variable) would call more attention in the plankton, as many abundant species, which channel large carbon fluxes belong here. The interesting group of very fast wobbling but slowly moving species (found in the investigation of the 6. variable) keeps some questions open. This strategy hardly seems to be advantageous for the species, when compared to other gliding raptors: high frequency wobbling requires much energy, and these species move very slow. Diffusion processes are also needed for food capture. The subsistence of this strategy may indicate the lack of strong selection among attached particle feeders. The food searching strategy of the slowest gliding raptors vs. the fast swimming ones (differentiated by the 7. variable) represents two extremities, which may differ in the utilized food resource also. The smallest and slowest species are closely attached to the substratum. They screen and sense the aggregates continuously. They are able to capture the smallest and mostly wedged bacteria in the pores of the aggregates. On the other hand, the almost free swimming raptors sense the substratum only during the short and more random contacts. Chemotaxis may also be an important sensory method for them (Fenchel & Blackburn 1999). They are able to screen a much larger area, and they might therefore find patches very effectively (eg. bacterial colonies).

5 Conclusion

The coexistence of a huge number of flagellate species in the river plankton may be partially explained by the results. The large number of functional groups with different niches, and the lack of competition for

certain resources is an important cause of coexistence. The presence of different utilization methods of the same resource, however, is still an interesting question. Some strategies using the same food source are very different in their efficiencies. The localisation of the species is often separated in these cases. Besides the presumably most efficient, and thus abundant species, a considerable number of other species, which use the same source, but different food acquisition strategies are manifestly coexist in much lower abundances. Many rare species thus do not 'suffer' from competitive exclusion, only their abundance remains low. The coexistence of a large number of attached particle grazing species may be explained by the heterogeneity of their habitat: the surface of riverine aggregates. Heterogeneity may manifest in the random colonisation of aggregates, in the structural, chemical, bacteriofaunistic, etc. diversity of aggregates, and also in the temporal changes of the aggregates, which fall into the generation times of flagellates.

Despite the well visible competition between the most abundant species in growing season, resources are not scant enough and the environment is heterogeneous enough to ensure the survival of a large number of rare species besides the abundant ones. The actual environment does not favour their proliferation, but in a changing environment – and functional diversity have the real importance here – new species can grow up. With the benthos, as a continuous species source, and the possibility of long term planktonic survival, the riverine plankton can maintain a really huge diversity of heterotrophic flagellates.

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