QUANTITATIVE ASPECTS AND DIVERSITY RELATIONS OF THE SMALL SIZED PROTOZOAN COMMUNITY IN THE PLANKTON OF THE RIVER DANUBE WITH SPECIAL EMPHASIS ON HETEROTROPHIC FLAGELLATES

Abstract of Ph.D. Thesis

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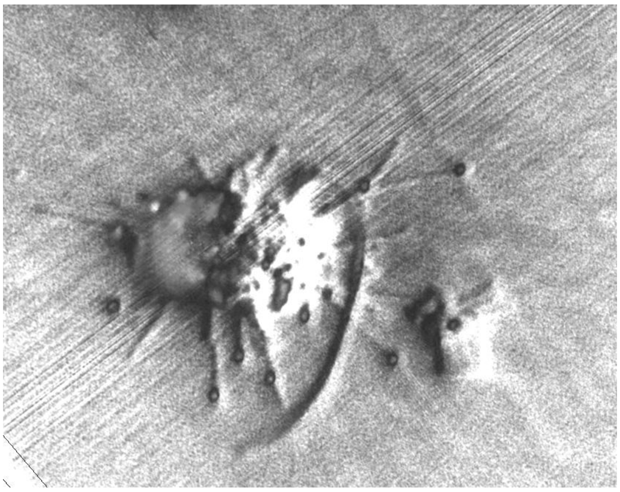

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2009

I. Introduction, aims of the study

The study of heterotrophic flagellates has significantly developed and become popular in the past three decades. The description of the microbial loop was the key element in understanding the role of heterotrophic flagellates in the carbon flow of ecosystems. The microbial loop is an alternative pathway besides the classical herbivorous food chain. It channels the production of bacteria, grown on phytoplankton exudates, via heterotrophic flagellates, towards ciliates, rotifers, and higher trophic levels. Quantitative studies of heterotrophic flagellates became widespread after this recognition. Recently, these organisms must be included in every serious microbial food web researches. In the past decade it has become obvious that heterotrophic flagellates do not constitute a uniform functional group. Besides their huge species diversity they are characterised by large functional diversity also. Light microscopic taxonomic and diversity researches have become popular in the past two decades by the pioneer work of a few taxonomists. Since the spread of molecular phylogenetics, the most complete recent species descriptions contain the ultrastructural description of the flagellar rootlet system and the sequence of the 18S rDNA. The phylogenetic species revealed by molecular techniques often do not correspond with the light microscopic morpospecies. Molecular ecological methods have become a new and effective tool in diversity researches of microbial eukaryotes. Some main questions has come to the centre of interest in this strongly diversifying scientific field.

Parallel to the recent spread of diversity researches in biological sciences, the study of protistan diversity and biogeography has also become a popular field. The two main questions in different spatial scales are the local species richness and the distribution pattern and biogeography of protists. The extent of local species diversity is in close connection to practical problems of conservation biology and the question of functional diversity – ecosystem stability relations. Understanding the biogeography of protists helps us to estimate the global species number of the biosphere and to reveal the processes of speciation. Both questions fundamentally influence the methodology of practical ecological studies as well as the accessible or necessary taxonomic resolution, and the universality of the conclusions. One current biogeographical model is available for heterotrophic flagellates, the model of the cosmopolitan distribution.

The role of eukaryotic microbial communities is essential in the material cycles of waters and in the shaping of the water quality. Heterotrophic flagellates take part significantly in these processes. The water quality of large rivers is a practical key issue for the society, because large rivers serve as a fundamental drinking water source for millions of people, besides their important touristic, shipping, waste water channelling, etc. roles. Despite, very few data are available about the heterotrophic flagellate communities of large rivers. Except of a few quantitative studies and some old faunistic data, there are very few modern researches, and almost nothing is known about the local diversity of heterotrophic flagellates in large rivers.

In the present work we investigate the dynamics of heterotrophic flagellates in the plankton of the River Danube, during a one-year long period. We search for correlation between the quantity of heterotrophic flagellates and their food organisms, and we reveal their role in the carbon flow in comparison with other protists. We work out a method to reveal the diversity of small sized heterotrophic protists, by which we determine the local species richness and functional diversity of small sized protozoa, with emphasis on heterotrophic flagellates.

Our main questions are the followings:

1. How does the quantity of main heterotrophic flagellate groups change during the seasons?
2. Are there any correlation between the quantity of heterotrophic flagellates and their food organisms?
3. What is the role of heterotrophic flagellates in the matter flow of the plankton?
4. What kind of heterotrophic flagellate species constitute the fauna of the River Danube?
5. What is the extent of local species richness of small sized heterotrophic protists (<30μm) and heterotrophic flagellates in the plankton?
6. How can be the functional diversity of heterotrophic flagellates characterised?

II. Material and methods

For quantitative study of main heterotrophic flagellate groups samples were collected biweekly between November 2004 and November 2005 from the main arm of the River Danube at Göd (1668. riv. km), 10 m far from the shore of the left bank. The dipped heterotrophic flagellate samples were killed by poring into saturated aqueous mercuric chloride. For larger flagellates and other protozoa 5 l water were filtered through a 10 μm mesh sized plankton net, and were fixed by the same way. Dipped and glutaraldehyde-fixed samples were collected for bacteria. The counting of protozoa were performed in Utermöhl-chambers by an inverted microscope, bacteria were counted after fluorescent staining by epifluorescent microscope.

In the carbon-flow model, the biomass values were calculated from individual cell measurements, assuming a shrinkage to 70% because of the fixative. The growth rates and the gross growth efficiency were estimated using literature data. Potential production was calculated as the product of the biomass and the growth rate, while potential consumption as the quotient of the potential production and the gross growth efficiency. We calculated the consumption of every species from every feeding compartment according to the feeding preferences and the ingestible food size. The intercompartmental carbon fluxes were calculated as the sum of the consumption of every species. The consumption ratios from the different compartments were generally proportional to the available biomass of the food groups. We used correlation analysis to investigate the relationships between the temporal dynamics of some flagellate groups and their food organisms (program Statisctica).

The local diversity was revealed by the following new methodology: A single plankton sample was collected near the left bank of the River Danube at Göd in March 2008. Fifty litres of water (5 dipping by a bucket) was filtered through a 10 μm mesh sized plankton net. The 150 ml concentrated plankton was poured into a flat glass with 15 cm diameter, and left alone for microbial succession. We investigated the sample with some smaller interruptions along 37 days, in 8 working hour per day. Subsamples were taken after stirring and were investigated in microaquariums by a high resolution inverted microscope with strong Nomarski-DIC contrast. Video records were made from every specimen, except the most abundant species. The signal of the analogous CCD camera was recorded on PC hard disc after digitalization. To reveal the functional diversity, the research utilization of the species was measured for four resource variables. We established the functional groups with different realized niches according to this. Further guilds can be differentiated by taking into account three additional variables, which were related to the method of resource utilization. We performed an extended diversity research in June 2008, including all microbial eukaryotic groups. The algal and micrometazoan samples were fixed during this investigation, for the protozoa we used the methods described above.

III. New scientific results

1. The dynamics of heterotrophic flagellate abundance and biomass showed peaks in January, June, and a double peak in October-November. The abundance varied between 0.27-7.8 10^6 ind. l^{-1}, the biomass between 0.019-0.58 mm^3 l^{-1}. Nanoflagellates constituted the 99.7% of the abundance on average. Microflagellates (mainly large chrysomonads and *Collodictyon triciliatum*) composed an important fraction of the biomass in summer and autumn, with a maximal biomass contribution of 53%. Chrysomonads were the most important group, they dominated the abundance during the whole year. Kinetoplastids were also present throughout the year, but never reached a significant abundance contribution. Choanoflagellates and bicosoecids formed summer and autumnal peaks.

2. The biomass of bacteria and nanoflagellates did not have a significant correlation. The biomass of nanoflagellates correlated negatively with the water discharge. Significant correlation was found between the number of centric diatoms in the family Thalassiosiraceae and the abundance of attached choanoflagellates and bicosoecids. The biomass of large chrysomonads and *Collodictyon* correlated positively with the biomass of their food organisms. High phytoplankton production appeared in the warm, lower discharged early summer period, with abundant, rapidly growing and disappearing consumer populations. The number of algae decreased at the end of the period, while the abundance and size of bacteria increased after the collapse of the tintinnid population.

3. Except in June, heterotrophic flagellates had the highest biomass among protozoan groups during the year. The biomass of the phytoplankton was usually one order of magnitude higher than the biomass of protozoa. The only exception was in winter, when the biomass of nanoflagellates exceeded the biomass of phytoplankton. A winter period and a growth season can be differentiated according to the seasonal distribution of the different organisms. The winter period lasted from November to April in the investigated year. It was characterised by low phytoplankton, low ciliate and high heterotrophic flagellate biomass. The growth season from May to November was characterised by high phytoplankton biomass, and high ciliate and flagellate biomass. The role of heterotrophic flagellates in the microbial food web can be described as the followings: Nanoflagellates utilised other carbon sources besides free floating bacteria. In winter time nanoflagellates were the most productive group among the investigated compartments. The role of the herbivor food web was negligible compared to the microbial loop. In the growth season the phytoplankton was the most productive group. The herbivor food web had an important role in carbon flow besides the microbial loop. Usually only a minor, but sometimes a larger (56%) fraction of the primary production was consumed in the microbial food web. The most productive consumer group in the microbial loop was nanoflagellates during the growth season, while algivorous ciliates and microflagellates were the most productive consumers in the herbivor food web.

4. We found altogether 213 new protozoan species for the Hungarian fauna during our investigations, from which 163 are heterotrophic flagellates. We found 417 protozoan species in the River Danube, including 211 flagellates. Presumably 82 flagellate species, 3 naked amoebae, 8 testate amoebae, 1 pseudoheliozoa and 3 ciliate species are new for science. Among the identified species there are 13 new freshwater records, regarding to all flagellate species 118 new riverine records and 166 new species for the fauna of the Danube have been found.

5. We found 130 heterotrophic flagellates and other nano/pikoeukaryotic species, 28 naked amoebae, 11 minute testate amoebae, and 14 heliozoa, altogether 183 heterotrophic protistan species under 30 μm in the plankton sample in March 2008. To our knowledge, 44% of the flagellates, altogether 57 species are not described yet. In addition we found 6 undescribed testaceans, 2 naked amoebae, 1 pseudoheliozoa and one ciliate species. The cumulative curve of new species for the sample during the observational days is not saturated; we found new species for the sample even on the last investigation day. The highest diversity was found in the first week, especially in the first four days, according to the number of species found during one day, the Shannon diversity, and the distribution of those species that were found only in one or two specimen during the whole period. The rank-abundance curve that includes all occurrences during the investigation period has a very long, extended tail; the proportion of the rare species is very large. During the whole investigation period 48 species occurred in only one and 23 species in only two specimens. By combining the results of the research in June 2008, we found 139 algae, 4 fungi, 183 small sized protozoa, 7 larger testate amoebae, 25 ciliates and 21 micrometazoa species, altogether 379 eukaryotes in one plankton sample. If the species number – occurrence curves are extrapolated to the ordinate, the estimated species number in one sample is 765.

6. Among the flagellate species in the sample March 2008 two species were osmotrophic, 38 fed from the water and 91 fed from surfaces. The food size range of the species feeding from the water is between 0.01-20 μm, the food size range of those feeding from surfaces is between 0.2-48 μm. Continuous transition is present between the size ranges. According to the quotient of the swimming speed and the speed of the flow field for those species that feed from the water, between the two extremities (attached suspension feeders at zero and swimming raptors near one), there is a transition type with small chrysomonads, large *Spumella* species and *Collodictyon*. By investigating the wobbling / flagellar beating frequency as a function of the speed of movement for the species feeding from surfaces, three categories can be differentiated: One is the group of the non-wobbling gliding species, in an other group the frequency of wobbling increases with the moving speed, and a third group is constituted of very fast wobbling, but slowly moving species. The adaptive character of the latter behaviour is questionable, diffusion processes are needed for interception. With the investigation of the ratio between the motoric force of the beating flagellum and the motoric force of the gliding flagellum in relation to the speed of movement, the following statements can be made. Two thirds of the species feeding from surfaces are driven almost exclusively by the gliding flagellum. Among the species that are driven by the beating flagellum, those that are faster than 15 μm/s touch the surface only by the distal part of the gliding flagellum, and those that are faster than 30 μm/s touch the surface only temporarily. The smallest and slowest species are capable to find the smallest and mostly wedged bacteria by the thorough screening of the surface. Although the contact sensation of the swimming species occurs only when running into the surface, they presumably find the nutrient rich patches (e.g. bacterial colonies) the most effectively. According to the different resource utilization, species belong to at least 15 different realized niche-groups. By further classification according to the different methods of research utilization, 27 guilds can be differentiated.

IV. Conclusions

1. Minimal and maximal abundance and biomass values in the Danube fall into the middle ranges of those in other rivers. The seasonal dynamics of heterotrophic flagellates differ considerably in the different rivers. Our results correspond well with the hypothesis, that the average ratio of larger heterotrophic flagellate taxonomic groups is similar in different freshwater bodies. When compared to earlier data, it can be assumed that the abundance of heterotrophic flagellates in the Danube decreased in the past one and a half decade. The late spring and autumnal peaks found in the present and the former investigations presumably correspond to each other. Both peaks coincide with the phytoplankton maximums in the investigated years, and this may be a general phenomenon in the River Danube.

2. The lack of significant correlation between the biomass of bacteria and nanoflagellates corresponds with the lack of coupling hypothesis of GASOL and VAQUÉ. Besides our data, negative correlation was found between the abundance of algae and ciliates and the water discharge in the Danube. Contrary, in the River Rhine the correlation was positive. Water discharge is presumably a fundamental factor in controlling the abundance of heterotrophic flagellates. In the low discharged late spring – early summer periods the abundance of planktonic populations were regulated primarily by biotic, trophic relations. Competition was found among the consumers on the same trophic level, the collapse of a population enables the growth of an other population using the same resource. Lower levels are regulated by strong top-down control. The collapse of some populations is not explainable with trophic relationships at the present temporal resolution. The late spring and the autumnal phytoplankton and consumer peaks in the Danube correspond to the peaks in temperate lakes. The winter nanoflagellate consuming peritricha-hymenostomatida communities and the late winter Centrales blooms in the Danube have no correspondence with lakes. The timing of the steps and the type of the abundant organisms in the seasonal succession processes in the Danube are presumably more variable compared to the lakes. Seasonal dynamics are significantly influenced by the water discharge.

3. Heterotrophic flagellates are surpassingly important consumers in the plankton of the River Danube. In winter time, among the investigated ones, heterotrophic flagellates are the most productive organisms in the Danube. In growth season, besides algivorous ciliates and microflagellates, nanoflagellates is one of the most productive consumer group. In some periods microflagellates are the most important algivors. According to our result and the available literature, heterotrophic flagellates are the most important planktonic consumer organisms in the investigated rivers, regardless of the peaks of some abundant ciliate and metazooplankter populations in the growth season. The taxonomic relations of the large chrysomonads, which are sometimes responsible for the greatest algivory, are unknown. In some periods, the larger fraction of the primary production is consumed directly in the Danube, which emphasises the important regulatory role of the consumers. The production of nanoflagellates exceeds the production of the investigated bacteria. Besides free floating bacteria, nanoflagellates could feed on ultramicrobacteria, floc-forming bacteria, aggregate associated bacteria, bacterial polysaccharide, detritus, dissolved organic matter and eukaryotes (algae, fungi, flagellates).

4. The total number of heterotrophic flagellate species found in the Danube and other rivers most likely does not reflects the total species diversity of the rivers. The high number of first riverine occurrences and new species for the Danube emphasizes the lack of modern heterotrophic flagellate researches in freshwater habitats.

5. The 131 species found in the single sample from March 2008 significantly exceed the species number found in previous heterotrophic flagellate studies. As neither the sampling site, nor the sampling date was extraordinary, it is assumed that this huge diversity is a typical phenomenon, it only has been missed by our previous methods. We could decrease the under-reporting (the species is in the sample, but is overlooked) considerably because of the followings: 1. Close to nature circumstances, lack of enrichment. 2. The good optical settings allowed the study of electronmicroscopic structures. 3. We made video records from every interesting specimen, these could be investigated later. 4. The investigation period was long. The most important factors decreasing the under-reporting were the videomicrography and the long observation period. The ratio of rare heterotrophic flagellate species exceeds the ratio of rare algal species in the 2008 June sample, and the rare eukaryotes in molecular clone libraries from the literature. The revealed ’rare protistan biosphere’ and the long tail of the rank-abundance curves opens a new perspective to our knowledge of the microbial eukariotic diversity. The proportion of new species in the sample (44%) is large when compared to present flagellate or ciliate taxonomical studies. The following important conclusions can be made about the diversity of flagellates: 1. The local species number of heterotrophic flagellates is much larger, than previously reported. 2. The large proportion of rare species does not support the ubiquity hypothesis. 3. The global number of morphospecies may be much larger than previously thought, a high number of morphospecies are still undescribed in freshwater habitats. 4. In large rivers, besides abundant species, a huge background diversity consisted of rare species is present. The species number found by our morphological method exceeds our present knowledge about local eukaryotic diversity revealed by molecular clone libraries. The almost 400 species found in a single sample opens new perspectives of microbial eukaryotic diversity. According to this new paradigm, the presence of many undescribed morphospecies should be accepted, new species can be found during any investigations, and the diversity of heterotrophic flagellates is much larger, than the one-two dozen abundant species found in enrichment cultures.

6. The revealed huge functional diversity may mean a significant diversity pool in changing conditions, from which the most important functional components of the ecosystem could be re-established by replacing species to others with similar function. By this way, the biogeochemical processes can be continued undisturbedly. The large diversity of flagellates can be explained by the presence of many different functional groups without competition, and for the species feeding from surface, the heterogeneity of the habitat. The latter can be manifested in the random colonisation of aggregates, the diversity of the aggregates, and the temporal changing of the aggregates. Because of the lack of strictly limiting constraints and the heterogeneity of the environment, besides the abundant ones, many rare species may survive in the plankton. With the benthos, as a continual source of species, and the possibility of long term survival, the riverine plankton can maintain an enormous diversity of microbial eukaryotes.

V. Publications in the theme of the thesis (papers, abstracts)

Kiss, Á. K., Ács, É., Bolla, B., Tóth, A., Tóth, B., Kiss, K. T. (2008): Diversity of eukaryotic microorganisms (algae, protozoa, rotifers and microcrustacea) in the River Danube at Göd (Hungary). Limnological Reports. Proceedings of the 37th International Conference of IAD, Chisinau, Moldova. 35: 113-117

Áron Keve Kiss, Éva Ács, Keve Tihamér Kiss, Júlia Katalin Török (2009): Structure and seasonal dynamics of the protozoan community (heterotrophic flagellates, ciliates, amoeboid protozoa) in the plankton of a large river (River Danube, Hungary). – European Journal of Protistology 46 (in press) doi:10.1016/j.ejop.2008.08.002

Áron Keve Kiss, Júlia Katalin Török, Éva Ács, Keve Tihamér Kiss (2009): *Pseudodifflugia klarae* nov. spec., *Bereczkya minuta* nov. gen. nov. spec. and *Paramphitrema muelleri* nov. spec.: three new filose testate amoebae from the plankton of the river Danube. – Acta Protozoologica 28/2 (in press)

Kiss Áron Keve (2007): Annual changes of protozoan community and their role in microbial food web in plankton of River Danube. – Hidrológiai Közlöny 87:159-162 (in Hungarian with English summary)

Kiss Áron Keve (2008): Feeding strategies of heterotrophic flagellate community in River Danube: functional diversity and niche segregation. – Hidrológiai Közlöny 88: 87-90 (in Hungarian with English summary)

Kiss Áron Keve, Ács Éva, Kiss Keve Tihamér (2009): Unexpected heterotrophic nanoeukaryote diversity in the plankton of a large river (River Danube, Hungary). – Hidrológiai Közlöny (submitted, in Hungarian with English summary)

A. K. Kiss, J. K. Torok, K. T. Kiss, E. Acs (2007): Protozoan community in plankton of River Danube: temporal dynamics, trophic interactions and role in microbial food web. – V. European Congress of Protistology, St-Petersburg, Russia, Protistology 5: 42

A. K. Kiss, K. T. Kiss, E. Acs (2007): Food selection and feeding strategies of Protozoa in plankton of River Danube. – V. European Congress of Protistology, St-Petersburg, Russia, Protistology 5: 41

Áron Keve Kiss, Éva Ács, Keve Tihamér Kiss (2008): Rare and likely new heterotrophic flagellate species in plankton of River Danube. – 27. Jahrestagung der Deutsche Gesellschaft für Protozoologie, Tagungsprogramm p. 58

Kiss, Áron Keve; Ács, Éva; Kiss, Keve Tihamér (2009): The Local Diversity of Heterotrophic Nanoeukaryotes has been Underestimated: A Case Study in the River Danube with Emphasis on Heterotrophic Flagellates. – 28. Wissenschaftliche Tagung der Deutsche Gesellschaft für Protozoologie, Universität Leipzig p. 43

Kiss, Áron Keve; Török, Júlia Katalin; Ács, Éva; Kiss, Keve Tihamér (2009): New Testate Amoeba Species from the Plankton of the River Danube. – 28. Wissenschaftliche Tagung der Deutsche Gesellschaft für Protozoologie Universität Leipzig p. 21