

How complex are aquatic microbial food webs? Unveiling the trophic role of middle-sized heterotrophic flagellates in freshwater and brackish habitats

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The presently observed global warming has been caused by the human-induced imbalance in the carbon cycle. Comprehending the carbon flow through food webs, from CO₂ fixed by primary producers to top predators, is crucial for understanding ecological processes. Such knowledge is essential for calculating global carbon budgets and forecasting future climate scenarios by the IPCC panel. **Microorganisms are the key component in the carbon cycle**, whose response to rising temperatures will drive climate change. They include photoautotrophic algae and cyanobacteria, and heterotrophic protists and prokaryotes. **Approximately half of the global primary production is driven by aquatic phototrophic microorganisms**. Moreover, about **50% of CO₂ fixed during photosynthesis is consumed by bacteria, entering microbial food webs**. It is subsequently transferred to the higher trophic levels through diverse protists, forming a link of immense complexity at the base of the aquatic food webs.

The current view of aquatic microbial food webs assumes a relatively simple transfer of energy from prokaryotes via heterotrophic flagellated protists (HNF, cell size 2.5-20 µm) to ciliates, and finally to zooplankton. It is assumed that the vast majority of HNF are **bacterivores**, preying predominantly on prokaryotes, even though it is widely accepted that major bacterivores actually are flagellates smaller than 5 µm. Moreover, recent studies have suggested that middle-sized HNF in the size range 5-20 µm are **omnivores**, feeding not only on prokaryotes, but also on bacterivorous HNF and algae, or **predators**, preying primarily on bacterivorous HNF and algae. In the light of these findings, it is thus probable that the current model of aquatic microbial food webs is simplistic and requires a considerable revision. **In this study, we propose a novel model with a more complex view on the trophic roles of recently discovered HNF groups with different life strategies and feeding modes.**

The newly proposed model will be verified experimentally. We will perform food web manipulation experiments that will cause the trophic cascades, allowing us to determine the trophic roles of diverse HNF in microbial food webs. We will apply **cutting-edge molecular methods to identify the taxonomic identity of HNF**. Moreover, we will use state-of-the-art single-cell techniques to estimate the abundances and growth rates of the key HNF taxa, their food preferences, and grazing rates, to provide direct evidence for the trophic relationships in microbial food webs. **This unique methodology will allow us also to verify the existence of the newly discovered trophic links in natural environments.** To confirm that the proposed model is relevant for most aquatic ecosystems, we will work in two contrasting environments: a freshwater Římov reservoir and the brackish Baltic Sea.

The main outcome of our project will be a novel, more complex, and thus more realistic model of microbial food webs, which will considerably modulate the current understanding of rates and efficiencies of carbon and energy transfer in aquatic habitats. It will have a major impact on the present view of the carbon cycle, and in consequence on the diverse science fields, such as microbial ecology, aquatic (marine and freshwater) ecology or biogeochemistry. The proposed innovative methodology that combines cutting-edge molecular and single-cell techniques will enable to reveal yet unknown trophic links between the newly discovered HNF groups.

