Unseen worlds: **microscopic lake plankton fuel food chains and befriend algae**

Dr Bettina Sonntag

Scientia

UNSEEN WORLDS: MICROSCOPIC LAKE PLANKTON FUEL FOOD CHAINS AND BEFRIEND ALGAE

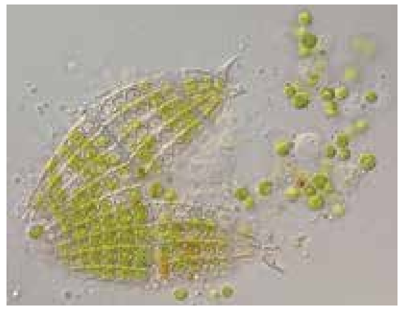
Dr Bettina Sonntag investigates ciliates – microscopic organisms living in freshwater lakes. As a senior scientist at the Research Institute for Limnology in Mondsee, Austria, Dr Sonntag leads a research team exploring how these single-celled protists interact with their environments. Their studies are documenting ciliate diversity and elucidating ciliates' roles in microbial food webs, including their mutually-beneficial partnership with green algae.

Ciliate plankton play essential roles in the environment

Ciliates are tiny unicellular organisms invisible to the naked eye, present in almost all freshwater environments. 'Ciliates are named for their cilia – short hair-like appendages used for movement and eating. Ciliates are not animals, plants, or fungi.' Dr Sonntag explains. 'Unlike single-celled bacteria and archaea, ciliates' organelles, the cellular workhouses, are membrane-bound. With a defined nucleus enveloping genetic material, ciliates belong to the protist kingdom.' Dr Sonntag was drawn to observing these fascinating organisms carry out complex life processes as only a single cell, watching them move, feed, and divide under the microscope. She told us that she is 'again and again fascinated by the fact that these organisms consist of only one cell.'

The investigations carried out by Dr Sonntag and her team provide a glimpse into the secrets into the microscopic world of ciliates. Their work is revealing how ciliates and other freshwater protists interact with other organisms. 'In our studies, we determine the respective food organisms (algal species, bacterial strains, etc.) for a set of planktonic ciliate species, which may then provide an explanatory variable for seasonally changing ciliate diversity patterns.' Dr Sonntag tells us. Her research seeks to elucidate how ciliates survive amidst the shifting environmental stressors in oligotrophic lakes, harsh ecosystems with low nutrient levels. To carry out these investigations, the team 'studies ciliates in lakes all over the world including lakes on islands in the Pacific Oceans, soda lakes in Kenya and of course freshwater lakes in Europe.'

Stokesia



Coleps

Aquatic food webs are a major driving force behind ecosystem functioning. Dr Sonntag and her fellow ecology researchers seek to understand the complex relationships between members of the food chain. She tells us that 'a major component in aquatic food webs are microscopic organisms, including viruses, bacteria, protists and small metazoans.' Ciliates and algae are among these microscopic organisms that make up the base of the food web. Despite their size, ciliates are integral to microbial food webs. By preserving environmental balance, ciliates promote the efficient recycling of chemical substances essential to life. For example, by exchanging carbon and oxygen gases with green algae, they help power essential carbon cycles. Ciliates are prey for microbes in higher positions along the food chain, who in turn feed crustaceans and fish.

Understanding ciliates' contributions to lake ecology must begin at the species level. Dr Sonntag tells us how her work helps advance researchers' understanding of aquatic food webs: 'In the past, "protists" were more or less neglected in such studies or lumped into "black boxes" such as "ciliates" or "heterotrophic flagellates". However, to reveal the complex patterns and interactions in aquatic food webs, the identification of individual species and their ecological traits and specific biotic/abiotic interactions are a prerequisite.' Dr Sonntag and her colleagues are identifying and investigating specific ciliate species, observing their close relationships with green algae, and advancing the use of state of the art technology to genetically categorise plankton species.

Happy together: symbiosis between ciliates and green algae promotes survival

Ciliates and green algae thrive as a pair, and Dr Sonntag's work has investigated how living together benefits both species. Their relationship is most probably not essential, as both ciliates and the algae can survive independently, but symbiosis protects both against mortality amidst harsh lake environments. As part of this work, the team looked at the ciliate species *Paramecium bursaria* and probed its close relationship with green algae. The pair help each other survive in harsh environments with little available food, and protect each other against starvation in nutrient-poor lakes.

'We are building a basic database from morphology up to molecular sequences which can be used by any researcher who investigates lake ecosystems'

Urocentrum

Metopus

When living in symbiosis with algae, ciliates are mixotrophic organisms, meaning that they continue to engulf food as heterotrophs, but also consume photosynthetic energy from autotrophic algae. With extra carbohydrates from photosynthetic algae, symbiotic ciliates need little external food supply. In turn, ciliates give off respiratory CO_2, an essential ingredient for algal photosynthesis. With enough light and ciliate-produced CO_2, algae perform photosynthesis. This cycle produces oxygen by-products that the ciliates can benefit from in turn. As nutrient and sunlight levels are always in flux in oligotrophic lakes as the weather shifts and the seasons change, this exchange between ciliates and green algae increases the survival rates of both species in these harsh habitats.

Stress responses: algae act as ciliate sunscreen

Plankton are highly sensitive to light, and life in lakes is often oriented around avoiding sun exposure. In clear lakes, many plankton travel down deeper at around midday when the sun is at its peak. Although sun exposure may damage ciliates, Dr Sonntag did not observe such midday migration behaviour in ciliate species from a high mountain lake. Instead, some ciliate species may rely instead on the sunscreening properties of their algae friends.

Both shortwave ultraviolet radiation (UVR) and photosynthetically active radiation (PAR) can cause ciliates harm. UVR passes through cell membranes, causing potentially fatal DNA and protein damage, and ciliates exposed to UVR appear deformed under a microscope. Movement and growth slows, with exposure proving fatal for some ciliates. If a freshwater ciliate population is decimated by excessive sun exposure, aquatic food webs can be thrown into disarray. Furthermore, if a particular ciliate species dies out, the bacterial groups they prey on can rise in number, disrupting ecological balance. The imbalance can have massive implications for the entire habitat.

Dr Sonntag and her colleagues investigated how algae act as a ciliate sunscreen by exposing two groups of a ciliate species – those living in symbiosis with green algae and those living independently from their symbionts – to artificial UVR and

PAR. Symbiotic ciliates fared much better under radiation exposure. Strikingly, the ciliates appeared to huddle together under radiation, forming what Dr Sonntag and her colleagues termed a 'collective shield' to protect against sun damage. The green algae living with ciliates migrated to the rear end of cells when light was present. This migration may shield the ciliates' genetic material. Further investigation is needed to fully understand what triggers these rapid protective reorganisations.

Ciliates and other protists offer a model system for scientists interested in studying the responses to environmental stressors, and future investigations will have much to reveal about how ciliate plankton respond. These microscopic single celled organisms provide highly useful models for studying stress response systems in multi-cellular organisms, including investigations of adaptive stress responses and repair mechanisms.

Future directions: building a genetic database for ciliates

The microbial world is incredibly diverse. Lake ecology researchers have historically relied on microscopic observations to identify plankton species and have categorised organisms by structure, size, and movements. This highly variable identification method relies on a trained eye. However, Dr Sonntag and colleagues are pioneering lake ecology by the use of new genetic sequencing technologies. These methods can reveal the DNA code of plankton species in a sample. From these codes, researchers can reliably identify known species, while also discovering unknown species.

Next generation sequencing takes extracts of genetic material and after a series of priming steps, amplifies it to make many copies of DNA so scientists have sufficient amounts of genetic material to analyse. Researchers can then read the sequences of the small DNA fragments generated. Using bioinformatics programs and specialised algorithms, overlapping regions in the small fragments are analysed to re-build complete DNA sequences. Dr Sonntag and her team sought to investigate how well one of the next generation sequencing methods reflected the different types of plankton that were observed via a microscope in a lake sample. Unfortunately, these investigations showed that massive parallel sequencing still has significant shortcomings in the context of protistan research. In a recent study, the team found that nearly two-thirds of morphologically-identified plankton species (observed by looking at the sample under a microscope) failed to be identified by sequencing methods.

There is a missing link in the method: a reliable and thorough database for matching known protist species to their DNA code. Dr Sonntag's team is leading this effort for ciliates species. 'We are building a basic database from morphology up to molecular sequences which can be used by any researcher who investigates lake ecosystems,' Dr Sonntag explains. A complete genetic database will allow for rapid assessment of the species present in a freshwater sample. Dr Sonntag tells us that researchers expect there are around 150 ciliate species in the plankton of one lake, and 'planktonic ciliate species have already been described from morphology,' however 'knowledge on their ecology or molecular sequences are still awaiting to be discovered.' Observing plankton population levels across seasons and in extreme environments would also become easier with a complete genetic database. DNA code matching also will distinguish between species appearing morphologically similar under a microscope. This effort can help researchers identify new species, unearthing more plankton diversity.

'In our studies, we determine the respective food organisms for a set of planktonic ciliate species, which may then provide an explanatory variable for seasonally changing ciliate diversity patterns'

Teuthophrys

Dr Sonntag and colleagues also hope that DNA sequencing can lead to better understanding of rare 'seed bank' plankton. Plankton are highly sensitive to environmental conditions that alter nutrient availability and levels of light. Microscopic lifeforms respond quickly to such changes, reproducing or becoming dormant. The special 'seed bank' species spend most of their time in a dormant state, activating and blooming under certain environmental conditions only. A lake's ciliate diversity is constantly in flux, and these 'seed bank' species provide an important buffer against environmental changes, allowing plankton to replenish population levels after environmental shocks. These rare species are estimated to make up less than 0.1% of the plankton population, making microscopic observation extremely difficult. Next generation sequence methods may hold the key to unlocking the secrets of the 'seed bank' plankton, giving us further insight into how these incredible protists weather the cycles of life in harsh freshwater lakes.

Meet the researcher

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Dr Bettina Sonntag was awarded her PhD from the Leopold-Franzens-University Innsbruck (LFUI) in Austria in 2000. Since then, she worked for several years as a postdoctoral researcher at the Institute of Ecology in Innsbruck and at the Research Institute for Limnology, Mondsee, at the same university. As a senior scientist, she now leads her own research team at LFUI that investigates ciliate species in lakes, which play integral roles in microbial food chains. Her research group has made large strides in understanding freshwater planktonic ciliates and in low-nutrient lake ecosystems. Over the course of her career, Dr Sonntag has been presented with many awards, including the Kanadapreis from the Canadian Studies Center of the LFUI for published work on ciliate-algae symbiosis.

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FUNDING

Austrian Science Fund FWF: projects P21013-B03, I2238-B25 and P28333-B25 (PI B. Sonntag)
Leopold-Franzens-University Innsbruck
German Research Foundation
DFG: project STO414/13-1 (PI T. Stoeck)
Swiss National Science Foundation SNF: D-A-CH project 310030E-160603/1 (PI T. Posch)

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