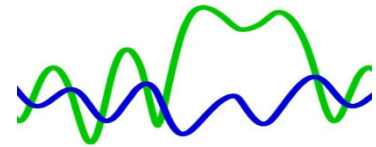


**“Influence of chaotic dynamics on the coexistence of traits:
Experimental studies with aquatic microbes”**



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Summary report

Mechanisms maintaining biodiversity have puzzled biologists since decades, as the number of limiting resources should limit the possible number of coexisting species. This paradox has not been solved yet. In literature, the paradox of plankton is often used as an example to illustrate this problem pointing to the fact that a large number of plankton species with only slightly differing traits is coexisting on only a few limiting resources. Since many years it is known among microbial ecologists that bacterivorous flagellates are species rich and are the major grazers of planktonic bacteria in lakes, however, their functional role in the ecosystems seems to be relatively similar, though not well investigated in detail. We used these bacterivorous flagellates as a model group to investigate what is the potential role of trait variations for the coexistence of the various flagellate species. Planktonic heterotrophic nanoflagellates (HNF) are mostly very similar or even indiscernible and belong to colourless unscaled chrysophytes. We aimed to investigate selected traits which might allow the coexistence of different chryomonad species. So, we isolated many chryomonads from freshwaters. To our surprise, nine out of ten isolates revealed new species indicating the large hidden and not yet described diversity of these flagellates which required an analysis of their taxonomic position (Figure 1). The second surprise was, that there were not so many pronounced differences in traits of the different HNF – we used growth rate and particle-size preference as traits. An exception were those chryomonads which were able to use phototrophy in addition to phagotrophy for their nutrition (Figure 2). Theoretically, an explanation for the coexistence of so many relatively similar species could be nonlinear interactions between species that could lead to oscillating and even chaotic dynamics which may allow for the maintenance of more species/traits than the number of available resources. We could show in our prework to the project (Becks & Arndt 2013 Nat. Communic., Becks & Arndt 2008 Ecology, Becks et al. 2005 Nature) that real-world microbial systems indeed show all possible characteristics of nonlinear dynamics including damped oscillations, stable limit cycles and chaos-like dynamics. In the course of this project, we were able to isolate and cultivate 11 different species/traits, however, only three strains could be cultivated axenically, which hindered our original plan to study the dynamic behavior of a large number of coexisting similar strains. However, we found two other striking phenomena, which were generally overlooked in former studies and might significantly change the understanding of processes allowing the coexistence of closely related species/traits. First, we could show significant individual differences (we used growth rates as a trait) within a single strain (Figure 3), which has the potential to create conditions for nonlinear dynamics and thus allow the coexistence of similar traits. Second, we could show a phenomenon which occurred in our experiments and model analyses that has been ignored in the past: dynamics of even single populations without external forcing may show all the above-mentioned characters of nonlinear dynamics including chaos-like oscillations (Figure 4). In contrast to other single-species studies, we used constant external conditions and no forced cell synchronization for the model and for the experiments (Figure 4). It is the first continuous-time model of a single-species system which can show deterministic chaos without external forcing. The “zooming in” from single-species populations into intracellular processes provided explanations for the appearance of intrinsic nonlinear dynamics and should have an impact on the determination and understanding of population dynamics and cell-cell interactions. The phenomenon has also fundamental consequences for understanding evolutionary processes, when competing species/traits or cell lines can potentially coexist at oscillating abundances, which may be the basis for the high biodiversity on Earth. Maintaining these oscillations is essential for protecting biodiversity and its functions.

Diversity of coexisting traits

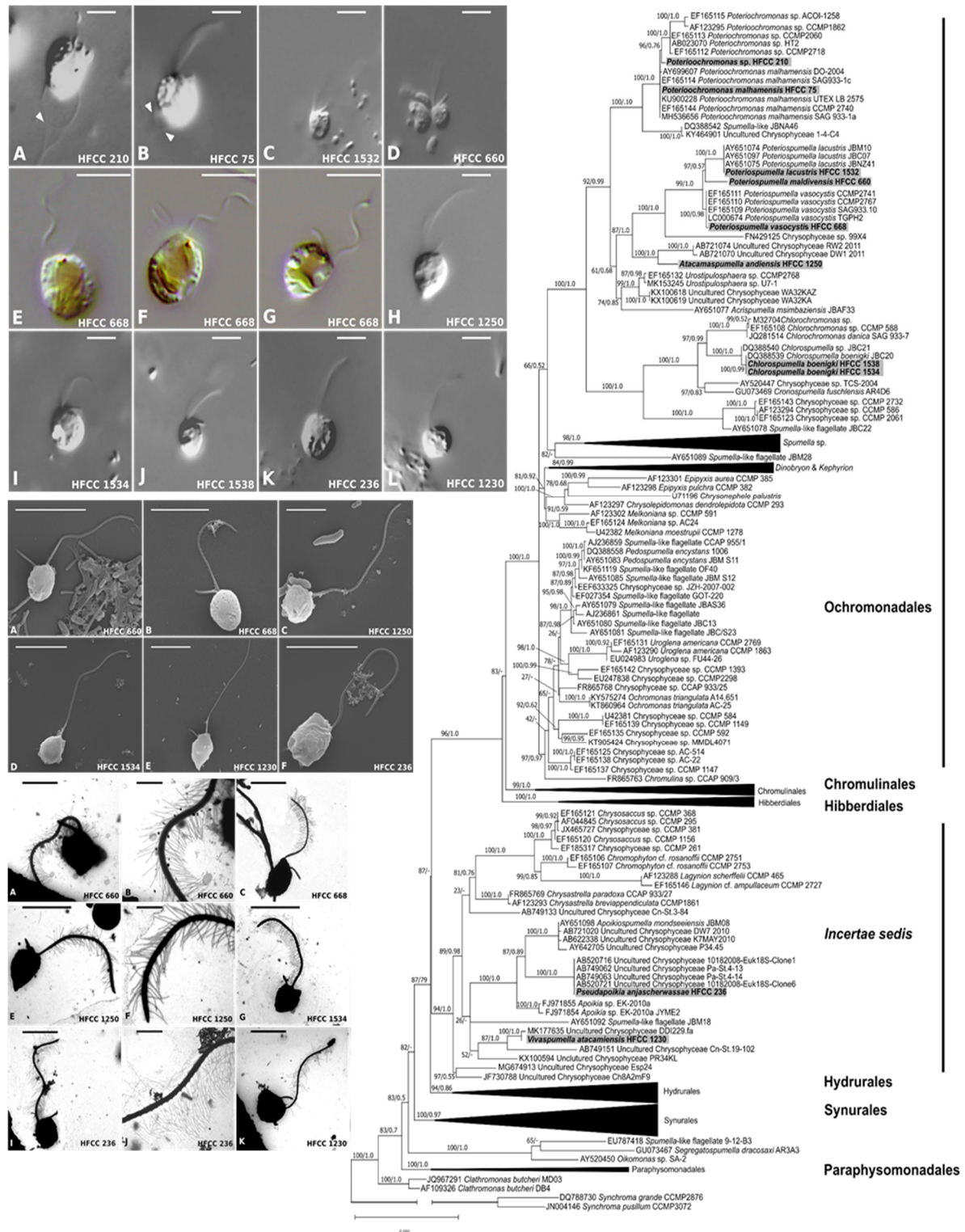


Figure 1 Morphological and genetic characterization of 12 strains of bacterivorous chrysoomonads isolated from the pelagial of lakes. Upper left: Light microscopic images showing vegetative cells of the investigated strains. **A.** HFCC 210 *Poterioochromonas malhamensis* in a broad lorica with visible septum (see arrow). **B.** HFCC 75 *Poterioochromonas malhamensis* in a barely visible lorica without septum (see arrows). **C-E.** HFCC 668 = *Poteriospumella vasocystis* comp. nov. showing a plate-like parietal bilobed chloroplast with a reddish stigma. **F.** HFCC 1532 = *Poteriospumella lacustris* **G.** HFCC 660 = *Poteriospumella maldiviensis* sp. nov. **H.** HFCC 1250 = *Atacamaspumella andiensis* gen. nov. sp. nov. **I-J** HFCC 1534 & HFCC 1538 = *Chlorospumella boenigkii* gen. nov. sp. nov. **K.** HFCC 236 = *Pseudapoklia anjascherwassiae* gen. nov. sp. nov. **L.** HFCC 1230 = *Vivaspumella atacamensis* gen. nov. sp. nov. Scale bar = 5 μ m. **Middle left:** SEM images showing vegetative cells of new chrysophyte species. Scale bar = 5 μ m. **Lower left:** TEM shadow cast images showing vegetative cells and mastigonemes of chrysophyte species shown above. **Right:** Phylogenetic analysis of Chrysophyceae based on a corrected SSU rRNA sequences of 1655 nucleotides. Investigated strains in bold and highlighted in grey. Numbers at nodes indicate bootstrap values of maximum-likelihood and posterior probability of Bayesian analysis (only values > 50 and 0.5 are displayed) (modified after Pietsch et al. 2022, Eur. J. Protistol.).

As mentioned above, we used bacterivorous flagellates (similar or even indiscernible colourless unscaled chrysophytes) as a model group to investigate the potential role trait variations may have for the coexistence of species. The majority of our successful isolates revealed new species which required a thorough analysis of their taxonomic position (Figure 1). Recently, phylogenetic analyses revealed a relatively high taxonomic diversity of “*Spumella*”-like flagellates leading to the description of several new genera and species. Our present work on the functional group of pelagic bacterivorous “*Spumella*”-like flagellates from different water bodies resulted in an extended taxonomic analysis among chrysophytes unveiling yet undescribed genera and species pointing to the very high hidden diversity of bacterivores in the pelagial. On the basis of phylogenetic analyses, we describe four new genera *Atacamaspumella*, *Chlorospumella*, *Pseudapaikia*, and *Vivaspumella* and a new species of the recently described genus *Poteriospumella*. Beside this, we redescribe the species *Ochromonas vasocystis* Doflein, 1923 to *Poteriospumella vasocystis* comb. nov. substantiated on the high sequence similarity with *Poteriospumella lacustris* and the new *Poteriospumella maldiviensis*. We concluded that the diversity of HNF should be much larger than it was thought before (Pietsch (née Romankiewicz) et al., 2022, Eur. J. Protistol.)

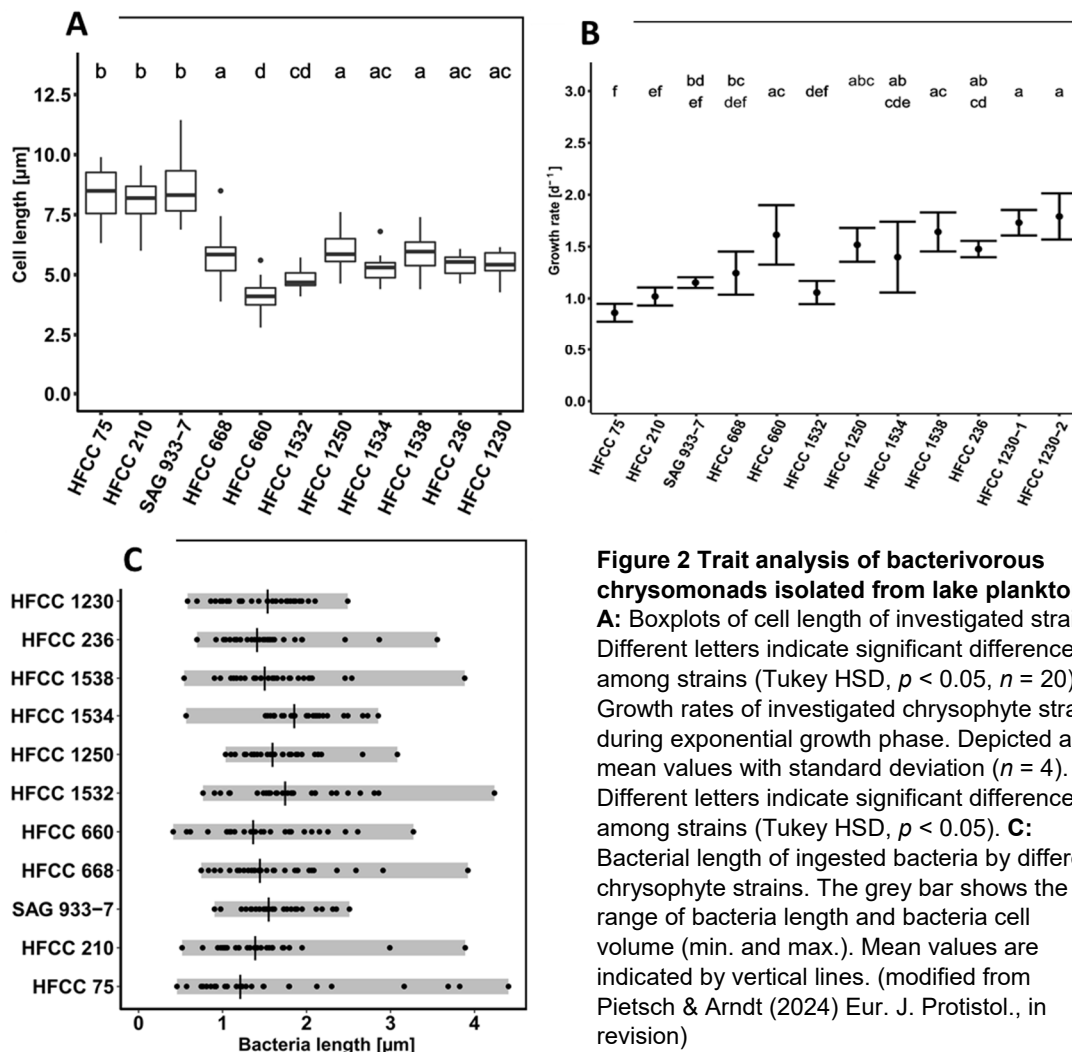


Figure 2 Trait analysis of bacterivorous chryomonads isolated from lake plankton.
A: Boxplots of cell length of investigated strains. Different letters indicate significant differences among strains (Tukey HSD, $p < 0.05$, $n = 20$). **B:** Growth rates of investigated chrysophyte strains during exponential growth phase. Depicted are mean values with standard deviation ($n = 4$). Different letters indicate significant difference among strains (Tukey HSD, $p < 0.05$). **C:** Bacterial length of ingested bacteria by different chrysophyte strains. The grey bar shows the range of bacteria length and bacteria cell volume (min. and max.). Mean values are indicated by vertical lines. (modified from Pietsch & Arndt (2024) Eur. J. Protistol., in revision)

The high diversity of these morphologically almost indistinguishable species leads to the question to which extend their functional role in ecosystems differs and how their ecological traits can be defined. We investigated the prey size and population growth rate of the species with regard to flagellate cell size as important ecological traits across eleven phylogenetically well-defined strains representing seven strains of heterotrophic and four strains of mixotrophic chryomonads. All investigated strains belong to the same functional group of unselective bacterivorous flagellates, feeding on the same bacteria size

range, while population growth depended on nutritional strategy (mixotrophy and heterotrophy) and species-specific differences. Our results show that cell size and size range of ingested bacteria overlap for all investigated strains while growth rate and dynamics differed (Figure 2). Our studies together with recent findings in literature indicated a large number of coexisting genotypes (Figure 1). In addition to seasonal changes and differences in small-scale distribution patterns, our study shows that small changes in growth dynamics might promote the coexistence of chrysoomonad genotypes.

Individuality within populations

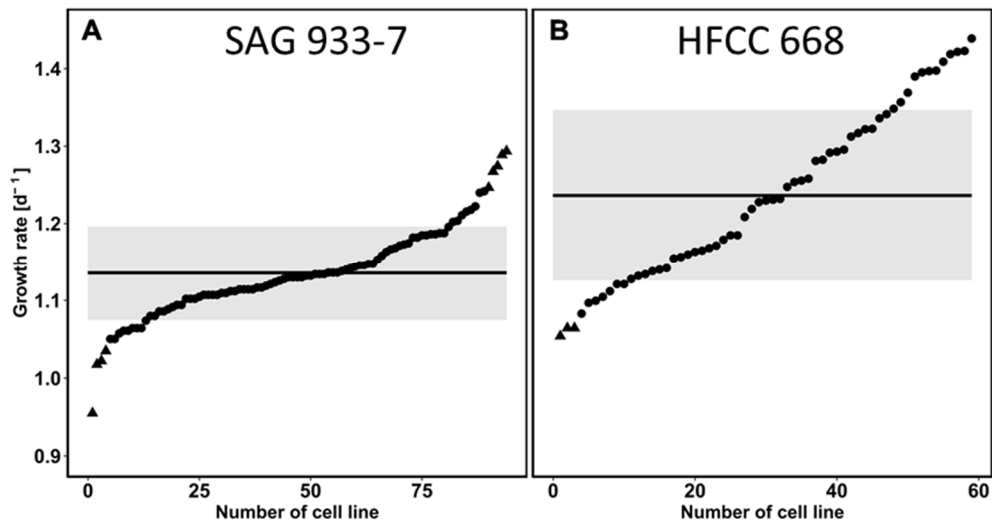


Figure 3 Growth behaviour of individual cells of two different strains HFCC 668 (*Poteriospumella lacustris*, A) and SAG 933-7 (*Chlorochromonas danica*, B). Horizontal lines show mean values calculated from all observed growth rates and grey boxes indicate the corresponding standard deviation. Each symbol represents a single cell. The growth rates of cells marked with triangles differed significantly from mean values (HFCC 668 $n = 94$, SAG 933-7 $n = 59$). Calculated mean growth rates of the two strains differed significantly (Kruskal-Wallis Test, $p < 0,001$) (modified from Pietsch & Arndt, 2024, Eur. J. Protistol., in revision)

Our experiments on individuality indicated significant individual differences (we used growth rates as a trait) within a single strain (Figure 3), which has the potential to create conditions for nonlinear dynamics and thus allow the coexistence of several similar traits. The high variability of individual growth rates leads to the conclusion that even potentially genetically identical individuals can behave differently under well controlled conditions with major impact on population dynamics. According to the theory of deterministic chaos those small differences can have a major impact on population dynamics and thus influence communities within an ecosystem to an unknown extent favouring coexistence of similar species.

Chaos-like dynamics in single species systems

The importance of fluctuations and deterministic chaos in natural biological systems has been debated for several decades and was originally based on discrete-time population growth models by Robert May. Recently, all types of nonlinear dynamics have been demonstrated for experimental communities in which multiple species interact. However, there are no data that demonstrate the range of nonlinear dynamics for single-species systems without trophic interactions. To date, ecological experiments and models have ignored the intracellular dimension, which includes multiple nonlinear processes even within one cell type. In our study, we showed that the dynamics of chrysoomonad populations of different species in continuous experimental chemostat systems and also in corresponding continuous-time models exhibited typical features of nonlinear dynamics and even deterministic chaos. Automatic cell registration enabled a continuous and trouble-free analysis of the dynamic behavior with a very high temporal resolution. Our simple and general model accounting for the flagellate cell cycle showed a remarkable range of dynamic behavior. For the first time, experimental and modeling data demonstrated the importance of nonlinear dynamics even at the level of one single cell type without

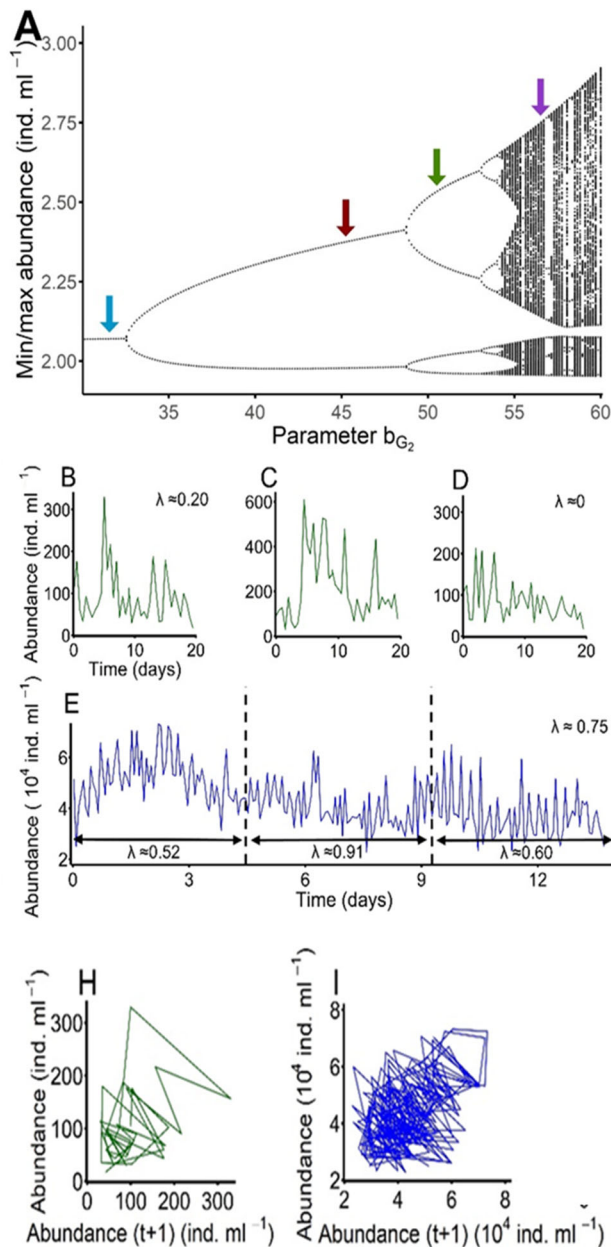


Figure 4. A: Simulations of the single-species chemostat model. Bifurcation diagram dependent on the time cells spend in G2-phase of the cell cycle of theoretical unicell populations. **B-I: Non-linear dynamics of single-species systems.**

Experimental results of abundance dynamics in bacteria-free chemostat systems (well-controlled flow-through systems) with the stramenopile flagellates *Poteriochromonas malhamensis* (dilution rates: 0.2 d⁻¹(B), 0.4 d⁻¹(C), 0.2 d⁻¹(D)). (E) Experimental results of abundance dynamics in bacteria-free chemostats with the stramenopile flagellate *Chlorochromonas danica* (dilution rate 0.2 d⁻¹). Lyapunov exponents are given when they could be estimated. (H-I) Time-delay reconstructions of data sets in B & E, respectively (color-coded correspondingly) (modified from Werner et al., 2022, PNAS)

external forcing. Nonlinearity should be considered an important phenomenon in cell biology and single species dynamics; and also for maintaining a high level of biodiversity in nature, a prerequisite for nature conservation (Werner et al., 2022, PNAS).

Publications of the project

Pietsch, T., Nitsche, F., Arndt, H. (2022) High molecular diversity in the functional group of small bacterivorous non-scaled chrysomonad flagellates. *Eur. J. Protistol.* 86, 125915, <https://doi.org/10.1016/j.ejop.2022.125915>.

Werner, J., Pietsch, T., Hilker, F.M., Arndt, H. (2022) Intrinsic non-linear dynamics drive single-species systems. *PNAS* 119, No. 44 <https://doi.org/10.1073/pnas.2209601119>

Pietsch, T., Arndt, H. (in revision) Overlap and differences in ecological traits of bacterivorous flagellates: Comparison of mixotrophic and heterotrophic chrysomonads. *Eur. J. Protistol.*

Werner, J., Arndt, H. (in revision) Cell biology and spatial ecology on a chip. *Communic. Biol.*