

Phytoplankton are small. So small that it took a long time before their importance was recognized. Their combined biomass is less than 0.5% of the biomass of terrestrial plants, but they contribute as much to the global assimilation of carbon dioxide. For a long time they were viewed as more or less passive food particles, but it is now becoming increasingly clear that they interact intensely with their environment, and adjust to changing conditions in adaptive ways. The project LIDPOP was launched in 2009 to bring up new knowledge on the mechanisms and underlying processes controlling phytoplankton community structure and population dynamics. More specifically, the project focused on the importance of grazer induced responses, and how these contribute to phytoplankton population dynamics and ultimately bloom formation. Phytoplankton vision is limited by their small size, and they mainly rely on chemical and hydrodynamical signals to perceive their surroundings. In addition to finding resources and favorable growth conditions, they are also able to perceive grazers. The majority of phytoplankton production is consumed by small crustacean grazers or single celled microzooplankton. Trace amounts of grazer cues triggers complex behavioral, biochemical, and morphological changes in responding phytoplankters. As an example, copepod cues may trigger more than 25 fold increase in toxicity in the harmful algal bloom forming dinoflagellates *Alexandrium minutum* which renders them less susceptible to grazers. In the current project, we have identified additional grazer induced responses and evaluated their role in phytoplankton dynamics. One of the most important findings was that chain forming phytoplankton respond to copepod cues by suppressing chain formation and switch to single celled growth (Fig 1).

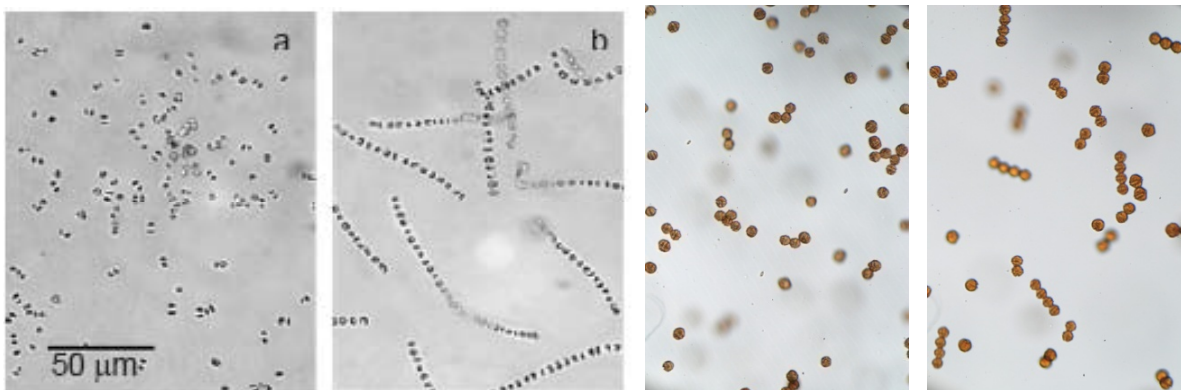


Fig. 1. Examples of grazer induced colony size plasticity. (a,b) *Skeletonema marinoi* with copepod grazers (a) and without (b), followed by (c,d) *Alexandrium tamarense* together with copepods (c) and without (d). Both species suppress chain formation in response to grazer cues (Selander et al 2011, Selander et al in revision and Bergkvist et al in revision).

This was found in both swimming and non swimming phytoplankters, and has large effects on the interactions with grazers for both but for fundamentally different reasons. The risk to encounter grazers can be calculated for rheotactic grazers, that use hydrodynamic signals to detect prey and also for chemosensory grazers, that detect prey by the chemical compounds that leaks from phytoplankton cells. The theoretical models were confirmed in experiments that showed that single cells of *Skeletonema marinoi* was consumed 16 times slower compared to four cell chains. In this sense, it is not who you are, it is what you do that matters in phytoplankton-grazer interactions. The best defended or least detected cells may benefit strongly compared to less adapted cells. Grazing pressure is extremely high in plankton communities, and modeling results suggest that grazer induced responses may structure phytoplankton

community structure by releasing defended or cryptic species from grazers. This is analogous to the success of butter-cups in grazed meadows. Cows do not consume butter cups because of their chemical defense, but instead suppress competing less defended species and thereby release butter-cups from resource competition. Figure 2 is from Selander et al 2011 and shows how swimming dinoflagellates can reduce their encounter rates with grazers by splitting up into single cells.

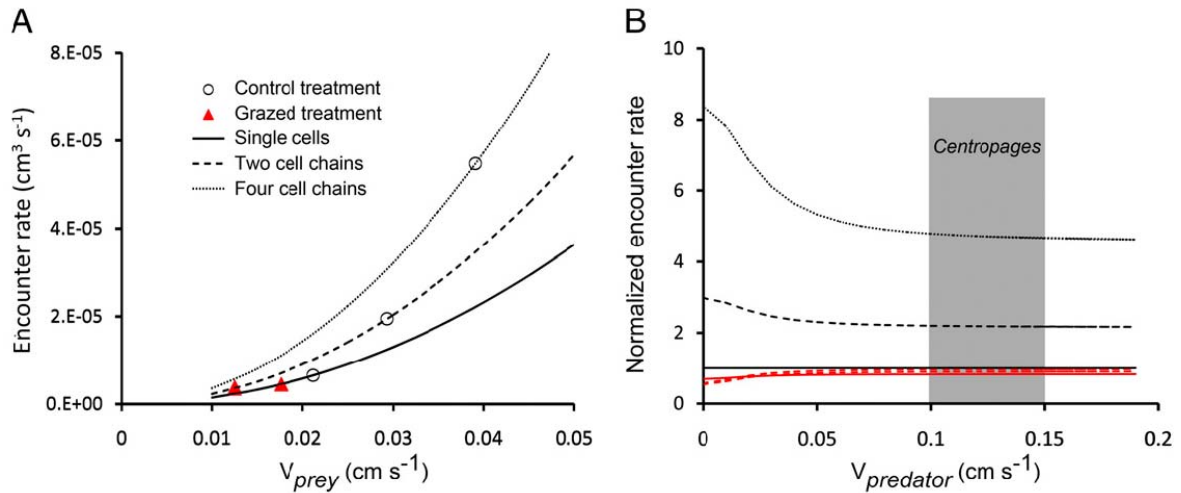


Fig. 2. (A) Effect of chain formation and swimming speed on encounter rate with ambush grazers (risk to get eaten) Values for single cells and chains from the un-grazed control is shown as open circles and grazer induced cells as red triangles (B) The effect of grazer velocity on encounter rates for single cells and chains from grazer free controls (black) and grazer induced (red) treatments. All encounter rates normalized to the encounter rate of single cells in control treatments for comparison. The difference in encounter rate is highest for ambush predators where $v_{\text{predator}}=0$. The shaded box indicate typical swimming speed for the inducing copepod *Centropages*, which results in 2.5 and 5 fold reduced risk to encounter grazers for two and four-cell chains respectively (Selander et al 2011).

Chemical characterization of signal molecules and other bioactive compounds from plankton source organisms has proven hard in aquatic systems. Progress in this area is necessary for the field to progress. We made an effort to implement the recent developments in analytical chemistry to catalyze this development. The methodological framework we used is collectively known as metabolomics, a combination of high resolving analytical instruments like mass spectrometry and nuclear magnetic resonance (NMR) and multivariate statistics. Metabolomics provides a complement and a potential shortcut to the identification of signal substances in aquatic systems. In addition it provides a tool for the cases when the biological activity is caused by a blend of compounds rather than a single molecule. Here we developed a method that use stable carbon isotopes to label alga, high resolving mass spectrometry of both labeled and unlabelled algal exudates makes it possible to filter out specifically compounds of algal origin. This reduces the sample matrix several fold, and provides a perfect starting point for a metabolomic analysis. We use this method in an effort to identify new toxins from dinoflagellates source organisms, and believe that it can become useful for bio-prospecting among aquatic primary producers. Metabolomics certainly has the potential to catalyze and further the field of chemical ecology significantly.