HETEROTROPHIC PICO- AND NANO-FLAGELLATES: A FOOD WEB WITHIN PELAGIC FOOD WEBS

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Abstract
We performed a mesocosms experiment to analyze the response of a natural phytoplankton community from the Baltic Sea to ocean acidification and warming. Besides the other important functional groups of the plankton community (phytoplankton, bacteria, microzooplankton, mesozooplankton) we put especial emphasis on the heterotrophic picoflagellates (<3 µm) and nanoflagellates (3-15 µm), including a polyphasic species identification (microscopy and pyrosequencing). Microscopic evidence together with supporting information from the literature revealed a complex food web structure within this functional group which contradicts the widespread assignment of a single trophic role (feeding on bacteria and pico-phytoplankton).

Keywords: Plankton, Baltic Sea

We set up a mesocosms experiment to study the response of plankton communities to the combined impacts of warming and ocean acidification. Two temperature levels (9 and 15 °C) and two CO_2-levels (target values <500 ppm and 1400 ppm CO_2) were combined in a factorial manner. Each treatment combination was replicated three times. Plankton communities were samples 3 times per week. Heterotrophic flagellates were enumerated and size by epifluorescence microscopy. Taxonomic identification was performed by a combination of microscopy and Tag-pyrosequencing of the V4-V6 region of the 18S rRNA gene. Feeding relationships were established on micro-photographs and supported by evidence from the literature.
Flagellate biomass and community composition showed only a weak response to warming and no response to acidification, but strong responses to the temporal succession of phytoplankton (1; Fig. 1). When averaged over time, biomass and abundance were lower in the warm mesocosms (ANOVA: abundance: $F = 47.44$, $p<0.001$; biomass: $F = 180.7$, $p<0.001$), but there was no effect of acidification (ANOVA: $p>0.05$). Succession, warming and acidification effects on taxonomic composition were analyzed by perMANOVA. Successional period had a strong effect ($F = 49.8$, $p<0.001$, $r^2 = 0.615$), temperature a weaker, but still significant effect ($F = 3.65$, $p<0.05$, $r^2 = 0.045$) while there was no effect of CO2 and the pairwise and triple interactions of factors. Before the phytoplankton bloom, feeders of colloidal matter (e.g. *Picomonas*) and bacteria bacterivores (e.g. choanoflagellates) dominated and were followed during the phytoplankton bloom by feeders on the increasingly available algae. There was also an increasing tendency towards intraguild predation within the heterotrophic flagellate community. Several of the larger flagellates fed on all size classes from bacteria up to flagellates only slightly smaller than themselves, giving rise to 5-link food chains like bacteria – *Paraphysomonas* – *Telonema* – *Cryothecomonas* – *Quadricilia.* Thus, our results negate the assignment of a single trophic function (2) and support the idea of trophic complexity in this guild (3). In terms of response to Global Change, the heterotrophic flagellates did not respond significantly to ocean acidification and only slightly to warming, mainly by an acceleration of species succession.

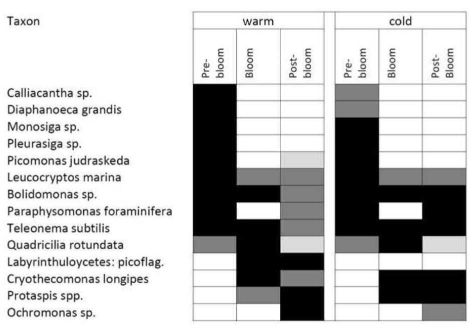

Fig. 1. Schematic representation of heterotrophic flagellate sucession in warm and cold mesocosms indicated by semi-quatitiative biomass-abundance scores, black: dominant, fark grey: common, light grey: rarte, white: not detectable

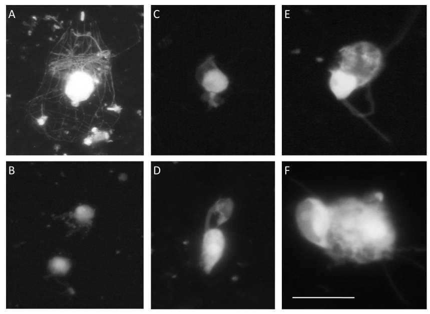

Fig. 2. **Micrographs of feeding.** Heterotrophic flagellates and their prey as seen by epifluorescence microscopy. Epifluorescence micrographs are taken by UV excitation for (A, D, F) DAPI-stained cells or by blue excitation for (B, C, E) Chl a red auto-fluorescence. Flagellates and their prey are (A) *Diaphanoeca*:several bacteria adhering on the cell at the flagellum basis; (B) *Paraphysomonas* in contact with bacterial clumps; (C) *Telonema* digesting a *Chrysochromulina* cell while another *Chrysochromulina* cell is attached at the posterior end of the cell;(D) *Leucocryptos*capturing *Plagioselmis*; (E) *Cryothecomonas*taking pico-chlorophytes; (F) *Quadricilia* beginning ingesting *Chrysochromulina* and a cryptophyte.Scale bar is 10 µm.

References
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