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Marine microorganisms play an important role in aquatic ecosystems as they are an integral part of all major biogeochemical cycles, fluxes and processes that occur in marine systems. Heterotrophic picoplankton (mainly heterotrophic bacteria) and autotrophic picoplankton (Prochlorococcus, Synechococcus and pico-eukaryotic algae) are the main components of the picoplanktonic MFW (Microbial Food Web). These organisms are consumed by heterotrophic nanoflagellates, which in turn are consumed by larger ciliated protozoa, forming a link ('microbial loop') to higher trophic levels. The role of the 'microbial loop' and MFW increases in oligotrophic areas such as the Adriatic Sea.

Heterotrophic bacteria play an important role in aquatic ecosystems by assimilating dissolved organic matter to maintain their metabolism and produce new biomass, and by decomposing organic matter and converting inorganic compounds into forms suitable for primary producers. Autotrophic picoplankton is contributing significantly to carbon production (up to 90%) and energy flow. In the Mediterranean Sea, the contribution of picoautotrophs to primary production varies between 31% and 92%, with important implications for MFW ecology and biogeochemical cycling in marine ecosystems.

In many coastal systems, organisms at the lower levels of a food web are responsible for strong bottom-up processes that control the structure and dynamics of the upper trophic levels. It is possible that even minor changes at the base of the food web in response to human-induced temperature increases could be amplified by trophic chains and consequently significantly affect various aspects of marine ecosystem structure and functioning. Therefore, studying the effects of warming on microbial communities is important for a better understanding of the global carbon cycle in seawater. This is particularly important in oligotrophic environments such as the eastern coast of the middle Adriatic, where MFW is the dominant trophic pathway. Previous studies in the Adriatic showed an increasing effect of temperature on bacterial grazing and the proportion of bacterial production taken up by grazers. However, the relationship with temperatures (below 16 °C) than at high temperatures. Similar on-line linear responses of bacterial growth to temperature, showing a greater positive effect at lower temperatures followed by a plateau or even decline at higher temperatures, have been reported for different marine and freshwater environments.

Experimental studies on the effects of warming on the metabolic processes of all major components of the MFW community, conducted in the ADRIACLIM pilot area in Kaštela Bay, showed a rapid



response of MFW to temperature increase. Temperature is an extremely influential factor on microbiological processes such as production, growth rate and growth efficiency. In addition, temperature affects the complex microbial trophic interactions and alters food web structure and ecosystem functioning.

The response of microbial growth rates to an increase in temperature differed between groups. In the heterotrophic prokaryotes, the cells with lower DNA content (LNA cells) showed a higher growth rate at ambient temperature than cells with higher DNA content (HNA cells). However, the LNA cells were more sensitive to temperature increases. It was suggested that temperature plays a fundamental role in nutrient-rich environmental conditions and has little or no effect on bacterial growth when resources are scarce.

Compared to the heterotrophic prokaryotes (both HNA and LNA), the autotrophic picoplankton (Prochlorococcus and Synechococcus) were much more sensitive to temperature, suggesting that both heterotrophic and autotrophic picoplankton components respond differently to temperature increases. In contrast to the heterotrophic prokaryotes, where the effects of temperature were more pronounced at temperatures below 16 °C and flattened at higher temperatures, this effect on the autotrophic picoplankton was linear over the entire range of temperatures studied (from 10 °C to 26 °C). The response of heterotrophic prokaryote production to temperature could be explained by nutrient-rich conditions during the experiment, which were sufficient to meet the higher energy demand at higher temperatures.

The difference in temperature sensitivity between heterotrophic and autotrophic picoplankton is consistent with the idea that planktonic autotrophs and heterotrophs are characterised by different activation energies. Furthermore, there is general agreement that a rise in temperature increases the dominance of picoplankton in total phytoplankton biomass and production. Therefore, autotrophic picoplankton are more sensitive to temperature increases in source utilisation processes and could be expected to play an extremely important role in microbial carbon flux in the global warming scenario. This is supported by the results of a multi-year study (2008-2015) in the central Adriatic Sea, which found a continuous and significant increase in seawater surface temperature, accompanied by a significant increase in abundances of autotrophic picoplankton and HNF, but a decrease in abundances of heterotrophic picoplankton.

Data analysis showed consistently higher sensitivity of grazing mortality than production to temperature for all microbial groups except Synechococcus. They showed a marked increase in production with increased temperature that was not accompanied by an equivalent increase in grazing pressure. Our results for autotrophic picoplankton are supported by several studies reporting that the mortality rate of autotrophic organisms is more sensitive to warming compared to their growth rate.



A positive effect of temperature on the grazing rate of prokaryotes and consequently an increase in the transfer of their biomass to higher trophic levels was found. This suggests that ciliates are important predators not only of HNF and picoplankton groups with larger cells (SYN and PE), but also of picoplankton groups with smaller cells (HNA, LNA and PROC). Ciliates can strongly influence the picoplanktonic community, both through direct grazing and trophic cascading.

After an experimental temperature increase of 3 °C, the proportion of picoplankton biomass channelled through the microbial food web towards the higher trophic levels increased. Autotrophic organisms showed a higher relative increase in carbon flux towards predators compared to heterotrophic picoplankton. The uptake of carbon biomass from picoplankton into ciliates occurs through two pathways: direct grazing of picoplankton and indirect grazing of HNF. The study showed that although most of the picoplanktonic carbon biomass taken up in ciliates previously passed through HNF, the total amount of picoplanktonic carbon transferred to ciliates was significantly higher at higher temperatures. Therefore, global warming could further increase picoplankton carbon flux towards higher trophic levels in the Adriatic Sea and the role of MFW in carbon flux could become more important.