APPLICATION OF TWO-STAGE CHEMOSTATS AND FLOW CYTOMETRY TO STUDY MARINE NANOFLAGELLATE GRAZING AND DIGESTION

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ABSTRACT

Marine heterotrophic nanoflagellates are known to be the main consumers of autotrophic picoplankton and heterotrophic bacteria, as well as playing a role in remineralization of reduced carbon and nitrogen substrates and inorganic phosphorus. In this work, a model nanoflagellate, *Paraphysomonas bandaiensis*, was grown in two–stage continuous culture for periods of 1 week to 1 month. The flagellates' growth, grazing and digestion rates were determined, as were the growth rates of the prey. The flagellates were fed either heterotrophic bacteria or a mixture of heterotrophic bacteria and light-limited *Synechococcus* sp. (WH7803). When limited by reduced nitrogen compounds in the incoming medium, the heterotrophic bacteria grew at the dilution rate of the chemostat. These bacteria were in turn fed to the flagellates, which remineralized reduced nitrogen such that the fed-upon bacteria grew very rapidly, from $1 - 10\ d^{-1}$. *Synechococcus* sp. also showed enhanced growth in the presence of the flagellate, partly from relief of light limitation, but also through heterotrophic utilization of reduced carbon substrates provided by the flagellates. This has not been documented previously for this strain of *Synechococcus*. The gross growth efficiencies of the flagellate when fed heterotrophic bacteria averaged 28% ± 26%. When fed *Synecococcus* sp., the flagellates had a higher gross growth efficiency, averaging 38%. This may have been due to the higher quality of prey offered in the *Synechococcus* experiments, since the heterotrophic bacteria were nitrogen-limited in experiments where they were the sole prey. Potential digestion rates, as measured using the MUF acid lysozyme assay, increased with flagellate growth rate, as well as with bacterivory rates. However, potential digestion rates plateaued at high

bacterivory rates, suggesting a maximal enzyme level per cell and showing that digestion would be limiting at high ingestion rates. In one experiment, the flagellates encysted in response to low food levels, showing this flagellates' survival strategy in a variable food environment. Also, even when high food levels were provided, it took several days until mass excystment occurred. The *Limulus* amoebocyte assay for lipopolysaccharide was not appropriate for quantifying *Synechococcus* WH7803 biomass, because no increased turbidity occurred with this assay.