

# Exploring the top-down regulation of freshwater Aerobic Anoxygenic Phototrophic (AAP) bacteria in a large-scale lake mesocosm experiment

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## Introduction & goal

AAP bacteria differ from heterotrophic bacteria in their capacity to use sunlight to supplement their heterotrophic diet of dissolved organic matter. The metabolic flexibility, and cosmopolitan nature of AAP bacteria suggest that this light harvesting capacity may have a significant ecological advantage and therefore an effect on organic carbon fluxes. On the other hand, AAP seldom represent more than 10% of the community across different types of aquatic ecosystems. Experimental evidence from marine environments suggests that AAP may have high intrinsic growth rates, and that their generally low abundance is due to high selective grazing by protists, which may be size-dependent. In freshwaters, there is a larger range of bacterivorous organisms, including metazooplankton such as *Daphnia* and *Holopedium*, but their potential role in regulating AAP has never been investigated. We carried out a lake mesocosm experiment to determine the vulnerability of freshwater AAP bacteria to both protist and metazooplankton predators, and to evaluate how this biological regulation varies under different nutrient regimes.

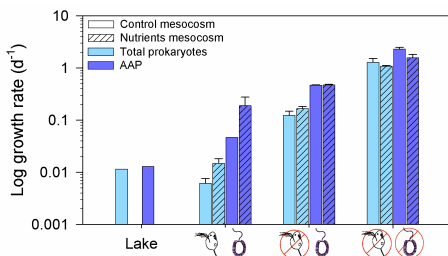


Figure 1. Net growth rates of AAP and total prokaryotes derived from changes in abundance during the two types of incubation experiments

- The removal of metazooplankton led to a rapid growth of AAP and total prokaryotes in both mesocosm treatments.
- The reduction in the protist community resulted in an even higher net growth rate of both groups of bacteria.
- Higher net growth rates were always found for the AAP ( $0.48 \pm 0.013$ ;  $2.3 \pm 0.2 \text{ d}^{-1}$ ) than for the bulk bacterial community ( $0.166 \pm 0.017$ ;  $1.28 \pm 0.24 \text{ d}^{-1}$ ).
- Nutrients did not systematically stimulate AAP growth rates.

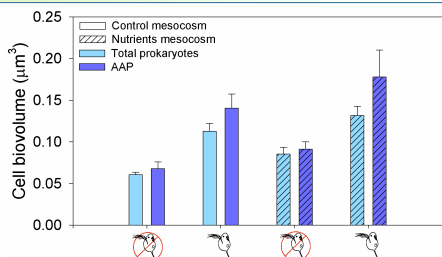


Figure 3. Changes in the average cell size of AAP and total prokaryotes in the metazooplankton removal experiment

- AAP bacterial cells ( $0.07\text{-}0.18 \mu\text{m}^3$ ) were on average larger than the total prokaryotic cells ( $0.06\text{-}0.13 \mu\text{m}^3$ ).
- AAP and total prokaryotes were larger in the nutrient than in the control mesocosm.
- The metazooplankton addition resulted in a two-fold increase in the cell size of AAP, and a smaller increase in total prokaryotes cell size.
- This may be evidence of a size-dependent grazing, or of defense responses especially by AAP bacteria.

## Study site & experimental approach



The experiment was carried out in the oligo-mesotrophic lake Cromwell, located in the temperate region of Québec, Canada ( $45^{\circ}59' \text{ N } 73^{\circ}59' \text{ W}$ ).

### I) Lake mesocosm experimental set-up:

- Experimental treatments were incubated in-situ in polyethylene mesocosms (1 m diameter, 6 m depth, 5000L volume).
- The mesocosms were filled with 54  $\mu\text{m}$ -filtered water from Lake Cromwell, so all metazooplankton were removed.
- Triplicate mesocosms were supplemented with nutrients (N and P), whereas the control treatment reflected the general conditions of Lake Cromwell.
- After the first week, the ambient lake metazooplankton community was added to the mesocosms.
- The evolution of the mesocosms was followed over the course of 47 days, in terms of abundances of zooplankton, total prokaryotes and AAP.

### II) Bottle incubation experimental set-up:

- Triplicate bottles were filled with 1.2  $\mu\text{m}$ -filtered water, thus most of the protist were removed. The control corresponded to unfiltered mesocosm water.
- In the course of the 3 days incubation, samples were taken periodically to determine total prokaryotes and AAP bacteria abundance.



The experiment was carried out in 2.5 L PETG bottles incubated in a water bath at constant temperature and under artificial light conditions (16h-8h light-dark cycles).

- AAP bacteria appear to be selectively grazed relative to total prokaryotes by both protists and metazooplankton.
- Zooplankton accounted for 20% and 12% of the total grazing on AAP and total prokaryotes, respectively.

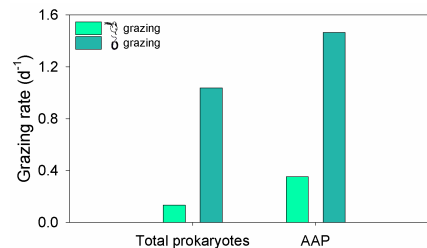


Figure 2. The apparent grazing rates on AAP and total bacteria

## Conclusions

- Our experiments suggest that freshwater AAP bacteria have high potential growth rates but are tightly regulated by grazers.
- The removal of the two types of grazers revealed that AAP bacteria were potentially growing at rates as high as  $2.3 \text{ d}^{-1}$ .
- We provide evidence of a strong top-down regulation on freshwater AAP bacteria, which explains their low net growth (average  $0.013 \text{ d}^{-1}$ ), and their low relative abundances ( $\leq 1\%$ ) in ambient lake waters.
- AAP bacteria appear to be selectively grazed relative to the prokaryotic community, and this selectivity may be size-dependent.
- Although protists account for most of the grazing on AAP bacteria, metazooplankton contribute significantly to freshwater AAP losses (20%).

## Acknowledgments

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