Alelopatia e herbivoria seletiva como mecanismos de promoção de bloom de Microcystis aeruginosa.

Nutrient input in water bodies usually leads to enhancement of primary production, changing the phytoplankton community composition, and harmful algae bloom (HAB) became a common problem. This change usually promotes the appearance of cyanobacteria (REF), mostly toxin producers, causing disruption in trophic interactions.

Since those blooms are nuisance not only to ecosystem health, but also aesthetics, several management efforts are being used to control or prevent such blooms. There are two approaches to control phytoplankton population: 1) stop, control input or nutrients, or trap it in the sediment (bottom-up); or 2) via predation by grazers, as a trophic cascade effect regulation (top-down). The control of nutrient input is a difficult achievement, provided that nutrients come from diffuse pollution, from the whole drainage basin. In the other hand, grazing efforts to control bloom are still a debate in literature.

Grazing by zooplankton is believed to control phytoplankton bloom. In temperate lakes, where the bloom appearance is well defined in seasons, the introduction of large zooplankton in early spring, before the start of phytoplankton growth, can control phytoplankton population by grazing. However, in tropical environments large zooplankton (mostly Daphniids) are encountered very seldom (REF). Further, tropical systems have no seasonal clear change, facing eutrophic states throughout the year (REF).

Cyanobacteria appearance and further dominance in phytoplankton bloom is a result of its ability as great competitors. Some cyanobacteria can get advantage for several reasons, such as buoyance in water column in search for light, N-fixation, toxin production, and allelopathic compounds (B-Beres et al 2012).

Cyanobacteria is well know for overtake the environment after nutrient input, and cause disruption in the trophic interactions. Although the phytoplankton community composition has little cyanobacteria proportion beforehand eutrophic state, they are the first to appear and overcome, establish and form blooms after nutrient input. There are many reasons for that to happen, such as shadow tolerance, N2 fixation, buoyance capacity, and secondary metabolites produced that might be allelopathic/toxic to other phytoplankton groups.

Different groups of zooplankton are known for its own feeding mechanisms, which can shape phyto assemblages. Cladocerans are generalist filters that are believed to suppress algae blooms in temperate environments in early spring, because of its feeding aparatus and the tolerance to some toxins, provided the high rate of ingested particles. However, copepods are selective grazers, and use chemical cues to detect food quality, such as toxicity, and avoid harmful particles or less nutritious particles in detriment of better quality ones.

Even with such mechanisms of allelopathy, an established community might retard or suppress the growth of those cyanobacteria, if the environment is stable, without more nutrient input or grazing pressures that might affect the competition among species. Given that, we want to assess in which initial conditions of *Cryptomonas sp.* that result in a cyanobacteria *Microcystis aeruginosa* (number of strain) to have its growth rate controlled or inhibited, and co-existence between those two algae to be possible.

After that step, we would like to know the process of facilitation generated by the inclusion of a selective grazer, copepod *N. iheringi*, in the system. Copepods, such as *N. iheringi,* are known for being able to avoid bad quality particles due to chemical cues present in the food. That zooplankton can be reject non-nutritious food, such as *Microcystis,* and preffer to graze on good quality algae, i.e. *Cryotomonas sp.* In literature, there is a debate ongoing about the enhancing of eutrophication in copepod dominated water bodies, as a result of this selective grazing behaviour. In order to understand whether facilitation happen, we assessed the competition between the two algal strain (*Cryptomonas sp. and Microcystis aeruginosa*) with and without the presence of the predator (*Notodiaptomus iheringi*).

Allelopathy by Ecology Dictionary (Oxford): The release into the environment by and organism of a chemical substance that acts as a germination or growth inhibitor to another organism. Typical substances include alkaloids, terpenoids, and phenolics. The phenomenon was described originally for heath and scrub communities, notably the Californian chaparral, but is now thought to be a widespread anti-competition mechanism in plants (e.g. barley inhibits competing weeds by means of root secretions). It is, however, extremely difficult to demonstrate in natural ecosystems. Allelopathy is also found in other organims (e.g. antibiotics may be produced by fungi to inhibit competing bacteria, when the term 'antibiosis' may be used). It is a form of interference competition, substituting space for a resource.

Methods

Create two cultures, one of Microcystis and the other Cryptomonas, count their cells everyday and check with spectrophotometer, so then have a standard curve to make the comparisons of my experiment easier. Is it reliable?

Competition experiment

As described in B-Beres (2012) the Microcystis have allelopathic compounds that inhibit Cryptomonas growth when presented in equal ratios or higher Microcystis ratio. Hence, in this experiment we will test in which ratio the Cryptomonas can control the growth rate or the population of Microcystis, to simulate previous condition of a eutrophic cyanobacterial dominated environment.

The inoculated cell number (or concentration in ugC ml-1) of *Microcystis* will be xxxx. - Use a concetration the to avoid long lag phase or early stationary phase (DOES ALI HAVE THIS DATA?). - *Crytomonas sp.* cell number (or concentration) will be 1M:1C ratio, 1M:2C ratio, and 1M:4C ratio. The *Microcystis* inoculation concentration in control cultures (*Microcystis* without *Cryptomonas*) was xxxx. The inoculated concentrations of *Cryptomonas* (*Cryptomonas* without *Microcystis*) were the same as inoculated in mixed cultures.

After finding out the proportion in which *Cryptomonas* will co-exist of overcome *Microcystis*, we will use the same proportions found, and add the Copepod, to assess the algal populations after with the interference of grazing. Since copepods are selective grazers, we expect that he will graze on *Cryptomonas*, neglecting *Microcystis*, and the later will dominate the community after a few days.

According to past experiments, the optimal concentration for *N. iheringi* maximum growth rate (egg production) is around 0.5 ugC mL-1, so we will use this as a total concentration for the food.

Total will be 0.5ugC mL-1, with the following ratios: 1 Microcystis (M): 1 Cryptomonas (C); 1 M : 4 C; and 1 M : 10 C. To test whether the Cryptomonas will control or reduce the concentration (or growth rate) of Microcystis. Previous studies shown that Microcystis win the competition against Cryptomonas when presented in equal or higher ratios, so we will fix the M concentration, and raise C concentration to assess in which proportion M is controlled by C.

In early eutrophic states the phytoplankton dominance is not of cyanobacteria, is green algae species with mixotrophic and other groups.

*Methods*

*Phytoplankton cultures*

*Cryptomonas obovata* culture was initiated through a strain from UFSC (Federal University of São Carlos). The *Microcystis aeruginosa* (NPLJ4) strain was obtained from culture collection at USP (Universidade de São Paulo, Brazil), and it is a microcystin producer strain. Both phytoplankton cultures were maintained in WC medium in 500mL Erlenmeyers, with stoppers that allow gas exchange. The culture conditions were: 23 ºC; 12:12h dark:light cycle. New cultures were started every week to keep the algal growth in exponential conditions.

*Zooplankton culture \*\* (may change if we use the zoo from Alcaçuz)*

Individuals of *Notodiaptomus iheringi* were collected in the upper part of the water column from the Gargalheiras Reservoir (Rio Grande do Norte state, Brazil), using a zooplankton mesh of 68µm diameter. The individuals collected were brought alive to laboratory. Every individual were observed using dissecting scope, to assess if they are in good health or contaminated with parasites, then cleaned several times in water drops and transferred to glass beakers filled with mineral water. The cultures are maintained since 2011, and kept in beakers, and fed with *Cryptomonas obovata* two to three times a week, around 0.5mgC.L-1. The culture conditions were: 23 ºC; 12:12h dark:light cycle; and beakers were cleaned every week to avoid excess of detritus and keep the population in exponential growth.

*Competition experiment*

As previously assessed by Ger et al in press, the optimal concentration for *N. iheringi* reproduction is 0.5mgC.L-1, which is going to be the start concentration of mixed cell cultures. Provided evidence of *Microcystis* (M) allelopathy in mixed cell cultures (B-Beres et al. 2012), we decided to fix M proportion and raise *Cryptomonas* (C) proportion compared to M. The mixed cultures consisted in the following ratios: 1C:1M ratio (0.25mgC.L-1 each); 4C:1M ratio (0.4mgC.L-1:0.1mgC.L-1); and 9C:1M (0.45mgC.L-1:0.05mgC.L-1). The initial *Microcystis* and *Cryptomonas* inoculation concentration in control cultures, solely *Microcystis* (M), or solely *Cryptomonas* (C), were 0.5mgC.L-1 each. The phytoplankton size was measured beforehand the experiment and its biovolume was converted in carbon content following the formulae: pgC cell-1 = 0.1204 x (um3)1.051 (Rocha & Duncan, 1985). The treatments and controls, 3 replicates each, consisted in an inoculation of the ratios in a 250mL Erlenmeyer containing 150mL of WC medium with vitamins, with a lid that allow gas exchange. Aliquots of 20mL from algae suspension were collected every two days to cell count, and assess the algal growth in the different ratios. The samples were preserved with 1% Gluteraldehyde (2mL per sample), and filtered in a 0.4um pore black polycarbonate filter, for further cell count using epi-fluorescent microscopy. Algae cell count was made using transect or field measurement. At least 100 *Cryptomonas* cells and 400 *Microcystis* cells were counted.

*\*\*\*\*\*Interference by predation*

After finding the "golden ratio" where the Cryptomonas and Microcystis would co-exist, or Cryptomonas would suppress / decrease Microcystis growth rate, we would add the zooplankton to see the changes in algal dominance.

*\*\*\*\*\*Statistical tests*

Normality tests were conduced and a repeated measure ANOVA was conducted further on