# An in vitro analysis of the ecological succession of estuarine protozoa and microalgae

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## Background

Astonishingly, 80 percent of our atmospheric oxygen is being produced by microorganisms, commonly called microalgae. Microalgae also known as phytoplankton are the most abundant population on Earth and perform several vital ecological services (4). They serve as tremendous carbon sinks and make up the base of the marine food chain. Although microalgae normally fuel productive ecosystems, some can be threatening to other species. Some microalgae produce toxins under particular circumstances. When their biomass increases, the toxin also increases and affects the health of other organisms in the ecosystem via food chain (2). The recent high mortality of California sea lions related to domoic acid and a recreational camp closure in 2015 due to cyanobacteria toxin are examples of harmful algal blooms (1). To deal with these double-edge –sword like organisms, it is critical to have good understanding of their community structures. Although seasonal variations in microalgae are well documented, there is little research on short-term succession and population changes (5). Moreover, factors regulating species diversity are not well understood for unicellular organisms. This study provides an in vitro analysis of aquatic populations and ecological succession in an estuary over nine weeks.

## **Materials & Methods**

- Water and mud were collected from the west side of San Francisco Bay where Belmont Creek mixes with salt water (Figure 1).
- Collected samples were placed in a microaquarium (Carolina Biological) and incubated at room temperature in east facing window for nine weeks (Figure 2).
- Distilled water was used to refill the evaporated water from the aquarium.
- Daily observations were made using a Leica DME inverted compound microscope. Pictures were taken with an LG G3 smartphone camera.

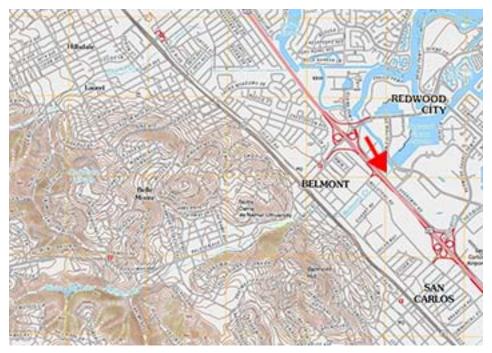


Figure 1: Location of collection site is marked by the red arrow.

#### Results

At the beginning, the most abundant phytoplankton were diatoms, primarily *Diploneis*. In the second week, *Nitzschia* started thriving and dominated until week 7. The *Oscillatoria* population passed *Nitzschia* in week 8 becoming equal to the total population of other diatoms at the end of week 9 (Figure 3).

Ameba and the ciliated protozoan, Colpidium spp., grew in the first week. Colpidium

thrived and dominated the aquarium for about 35 days. When its population reached stationary phase in week 5, predatory protozoa: *Coleps* and testate ameba appeared. Other protozoa grew when *Colpidium* reached stationary growth. Testate ameba became the dominant heterotrophic species on day 66 (Figure 4).

Testate ameba, *Oscillatoria*, and *Nitzschia* were the most abundant species after nine weeks.



Figure 2: The microaquarium is  $75 \times 50 \times 4$ -mm and holds about 5 ml

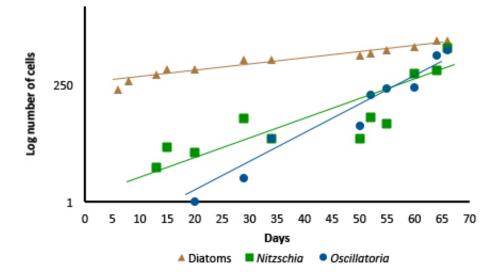


Figure 3: Growth of diatoms, Nitzschia, and Oscillatoria. Oscillatoria passed Nitzschia population on day 55.

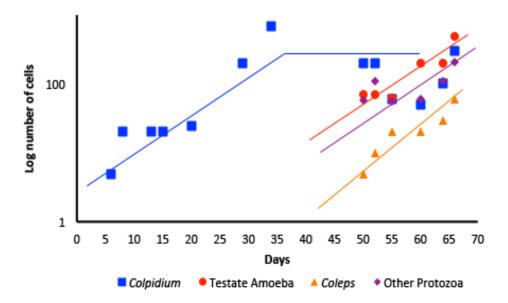


Figure 4: Heterotrophic population changes over 9 weeks. Other protozoa appeared after the Colpidium population entered stationary phase in week 6.

Autotrophs grew slowly (24 min/generation) for the first four weeks compared to the much faster growing heterotrophs (17.3 min/generation) (Figure 5). Testate ameba was favored by the abundance of its primary food, Nitzschia, which grew at 6.5 cells per day (Figure 6).

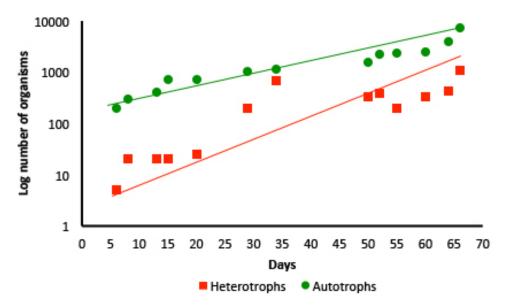


Figure 5: Population growth of heterotrophs and autotrophs in the aquarium

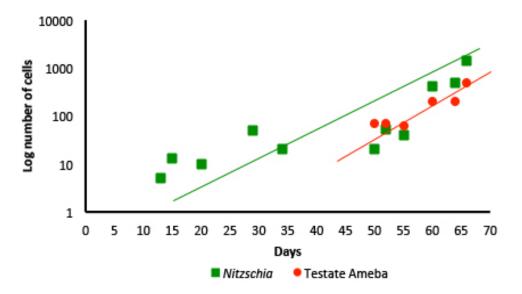


Figure 6: Testate ameba began growing after their food, Nitzschia, grew.

### Discussion

Over the nine-week observation, microalgae grew steadily and their population was continuously higher than protozoa (Figure 5). This suggested that microalgae were the primary producers or base of the food chain in the aquarium (3).

Based on species population changes during nine weeks, ecological succession was concluded to have influence over the community structure in an ecosystem. In the first week, *Diploneis* was pervasive, however, it was taken over by *Nitzschia* diatom in the second week. Possessing a bigger cell size and having no predator, *Nitzschia* thrived until week 7. It was, in

turn, succeeded by the filamentous cyanobacterial species, *Oscillatoria*, at the start of week 8. By forming mats of filaments, *Oscillatoria* had an advantage over single-celled *Nitzschia* in competing for sunlight and nutrition. In addition to the competition by *Oscillatoria*, the rise of predatory testate ameba in week 8 was also a factor in decreasing the *Nitzschia* growth rate (Figure 3). The cyanobacterial population increased at the expense of the diatoms and *Oscillatoria* became the dominant autotroph at the end of week 9 (Figure 3).

A similar pattern of ecological succession was also observed in the protozoan population. At first, *Colpidium* was the dominant species and grew at an increasing rate. In week 5, its growth rate abruptly reached stationary phase once the predator protozoa, testate ameba and *Coleps*, appeared (Figure 4). Both new comers are voracious predators that feed on algae, living and dead protozoa, and plant and animal tissues.

#### Conclusion

This study contributes evidence of how ecological succession and interspecific interaction can shape the community structure of microplankton in an aquatic ecosystem. Moreover, it encourages us to monitor the species population changes in Belmont Slough water given that a large population of *Nitzschia* diatom was spotted in the sample; some species of *Nitzschia* are well known to produce domoic acid neurotoxin. More research should be done on *Nitzschia* species identification, factors regulating its growth, and toxin production.

#### Acknowledgements

I would like to give special thanks to my mentor Dr. Christine Case for all her patience, guidance and immense knowledge making this research possible to be published, my lab partner Sophia Yunanda and BIO 215 classmates for all their contribution of knowledge and finally, our lab technicians in Skyline College for providing all materials needed for my research.

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