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ABSTRACT

Wastewater management is an essential component of modern society that is necessary for reducing human environmental impact, particularly in aquatic ecosystems. In Humboldt County, California, the Arcata Wastewater Treatment Plant and Wildlife Sanctuary (Arcata Marsh) is a model for other wastewater treatment plants worldwide to treat waste naturally, reducing the use of chemicals. This wastewater is then discharged into the Humboldt Bay. It is well known how treatment helps remove harmful waste materials, but it has not been investigated how phytoplankton diversity is affected before, during, and after treatment. Phytoplankton are primary producers that are responsible for carbon sequestration and production of oxygen, and are an essential food source for higher trophic-level organisms. When nutrient levels are high, such as in human waste water, it can lead to single species proliferation, thus resulting in lower diversity and higher abundance of other species in the community. This can have negative consequences on the higher trophic levels that consume them and also creates "dead-zones" where aerobic species are unable to survive. In this study, pre-treatment water and post-treatment water were sampled and observed for diversity and abundance of diatoms, a quantifiable subphylum of phytoplankton. It was hypothesized that successful treatment would result in higher diversity of diatoms in post-treatment waters than that of pretreatment waters. This study found 30 morphologies across all sample sites, with abundance of 2-4 species being significantly greater in pre-treatment ponds than post-treatment ponds. There were no significant trends in diversity across the ponds.

INTRODUCTION

Aquatic ecosystems are incredibly diverse and have niche microhabitats which are created by inflow of oceans, rivers, and other primary sources of water. The characteristics of different aquatic habitats, such as light availability, nutrient levels, temperature, salinity, and predator presence can strongly influence the composition and abundance of the phytoplankton communities that inhabit them (1, 2). It has been found that areas with higher nutrient contents have a lower diversity and higher abundance of certain species of diatoms (3). This can occur due to factors such as agricultural runoff, sewage discharge, atmospheric deposition, and coastal upwelling, all of which can introduce nutrients into the water. These factors can create conditions that are favorable for the growth and dominance of certain species of phytoplankton, which can outcompete other species and lead to adverse environmental conditions. For example, some species of phytoplankton can produce toxins that can be harmful to other organisms that consume them, resulting in disruptions to the food web and overall decreased productivity (2).

In 2012, a study was conducted to explore whether or not phytoplankton diversity had any effect on the higher trophic levels such as zooplankton, their primary consumer. The study found that phytoplankton diversity directly affects zooplankton growth rate, abundance, and diversity. Daphnia, one of three zooplankton species within the study, saw an increased growth rate, abundance, and diversity with increased phytoplankton species richness. The study concluded that the biodiversity of these primary producers has pervasive consequences throughout the entirety of the food web by affecting the productivity and diversity of the next trophic level (4).

Typically, diatom diversity decreases when nutrient levels increase (6). One way this can occur is through the deposition of wastewater rich in nitrogen and phosphorus (5) into these aquatic ecosystems. A study examining the effect of urban wastewater on diatom communities in receiving alpine rivers found that where the treatment effluents flowed into the river increased abundance of species and decreased diversity. They also found that location specific factors, such as nutrients, have a greater impact on population diversity than seasonal factors like temperature (6). There was no observation of diatom diversity throughout various stages of treatment.

From this, we can hypothesize that wastewater treatment plants, such as the Arcata Marsh, may deposit water with higher nutrient content back into the local environment. This potential high nutrient content could lead to proliferation of individual diatom species and thus affect the productivity of the food web and, in extreme cases, cause the formation of "dead-zones". These zones form when oxygen levels are low due to high oxygen consumption by diatoms that results in hypoxia and consequently, an uninhabitable environment for aerobic organisms (7).

The Arcata Marsh, piloted in 1979, is a multi-step system that uses minimal chemicals to remove waste products. Instead of chemicals, it uses plants, bacteria, and algae to naturally treat the water over time (figure 1). The first part of purification, known as the headworks, is responsible for separating sludge from effluent (8). Not accessible by the public, it is where the sludge is digested by anaerobic bacteria and used for compost. Debris is removed from the effluent, and sent to the clarifiers, which remove more organic solids, and the primary-treated wastewater is sent to the next step known as natural purification. Natural purification begins in the oxidation ponds 1 and 2, which use microorganisms and bacteria to digest waste suspended in the water over time, where oxygen is supplied by aerators and algae (8). After purification via the oxidation ponds, the water travels to treatment marshes to remove the algae and bacteria used in the oxidation ponds for digestion. These are behind fences to prevent destroying of emergent and submergent plants that aid with this process. The second to last part of the purification process is disinfection. Water from the treatment marshes is sent to a chlorine contact basin, which removes pathogens, bacteria, and viruses before flowing into enhancement wetlands (8). The three freshwater enhancement wetlands, Allen, Gearheart, and Hauser, result in reduction of suspended soils and nutrients as the water travels through over 5-10 days. The water flows through the wetlands in series, from Allen, to Gearheart, and finally Hauser. From Hauser, the water is then chlorinated and de-chlorinated before being pumped into Humboldt Bay (8).

In this study, we aim to identify the diversity and abundance of diatom species in the Arcata Marsh. The Arcata Marsh is an established and innovative way to treat wastewater that has been studied by several universities across the country. It is unknown how the wastewater treatment affects diatom diversity, which is important for establishing the risks associated with reintegrating the waste water back into the environment. We hypothesize that the enhancement wetlands Allen, Gearheart, and Hauser will have higher diversity than the oxidation ponds, as treatment is supposed to decrease the nutrient level of the wastewater and create a more balanced ecosystem for all organisms.

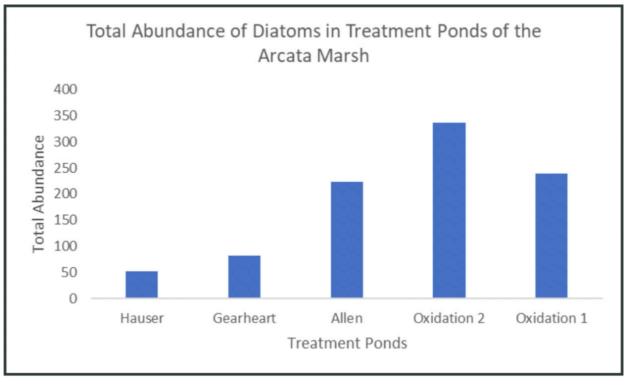
Figure 1.
Aerial view of the Arcata Marsh Wastewater Treatment Plant. Ponds and sampling locations are marked with red.

MATERIALS AND METHODS

Sample site. Samples were collected in 40 ml falcon tubes from the Oxidation ponds 1 and 2, and from enhancement wetlands Allen, Gearheart, and Hauser at the Arcata Marsh. Each location was visited once a day on April 16 and 17 of 2023, around 5:30 PM. The discharge into Humboldt Bay was not collected due to the presence of previous research (6), allowing us to assume similar results. Collection locations were chosen at each site for ease of access, safety of collectors, and resources available. Upon collection, temperature was recorded with a graduated thermometer.

Sample processing. Analysis of the samples took place April 18-23 and between analysis samples were stored in a refrigerator to keep close to the temperature of the ponds. At the

Figure 2.
Comparison of abundance across the treatment ponds of the Arcata Marsh



lab, pH (Fisher Scientific Accumet AE150) and salinity (Fisher Scientific Optical Refractometer) was measured to find the environmental variations. Two samples of 10 µl were collected from the bottom of each falcon tube with a micropipette and wet mounted on slides. Each slide was then analyzed using phase contrast microscopy at 100x and for further identification 400x total magnification. Photos were taken of all possible diatom species, then counted and separated by morphological traits.

Diatoms have a cell wall composed of silica that have two overlapping hypotheca and epitheca (valves) and a cingulum (girdle), which are collectively referred to as frustules. General shape of diatoms can be split into two categories: centric (circular) and pennate (rod) (9). The presence of these frustules was the key indicator that a diatom was present. Gene sequencing technology, the predominant technique for identifying species level, was not available. To quantify biodiversity, diatoms were identified by morphological type for the purpose of recognizing the diversity and abundance within our samples. To analyze the data collected several statistical tests were utilized. A regression analysis comparing total abundance to total diversity across all ponds was run. To compare morphologies across ponds a single sided analysis of variance (ANOVA) test was used.

RESULTS

Across all five treatment ponds, 30 different morphotypes of diatoms were observed at 400x total magnification. The known size range of diatoms is from 2-200 µm (2), but size was not quantified in this study. Environmental factors such as pH, salinity, and temperature had slight variations between days collected, potentially due to the presence of rainfall on the second day of collection. While all five ponds have relatively similar diversity (figure 3), diatom abundance between treatment ponds varied significantly. The pre-treatment ponds, Oxidation pond 1 and Oxidation pond 2, had the lowest diversity and the highest abundance. The post treatment waters,

Figure 3.
Comparison of diversity across the treatment ponds of the Arcata Marsh.

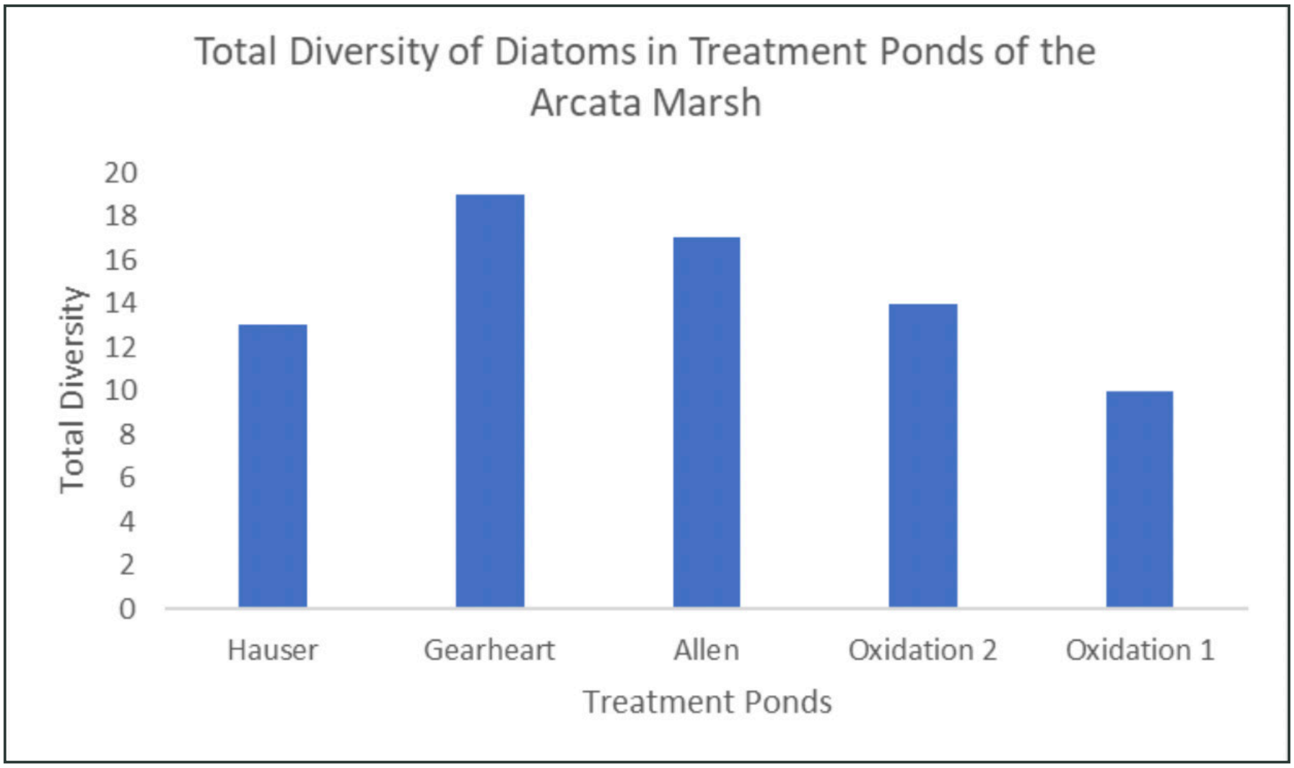
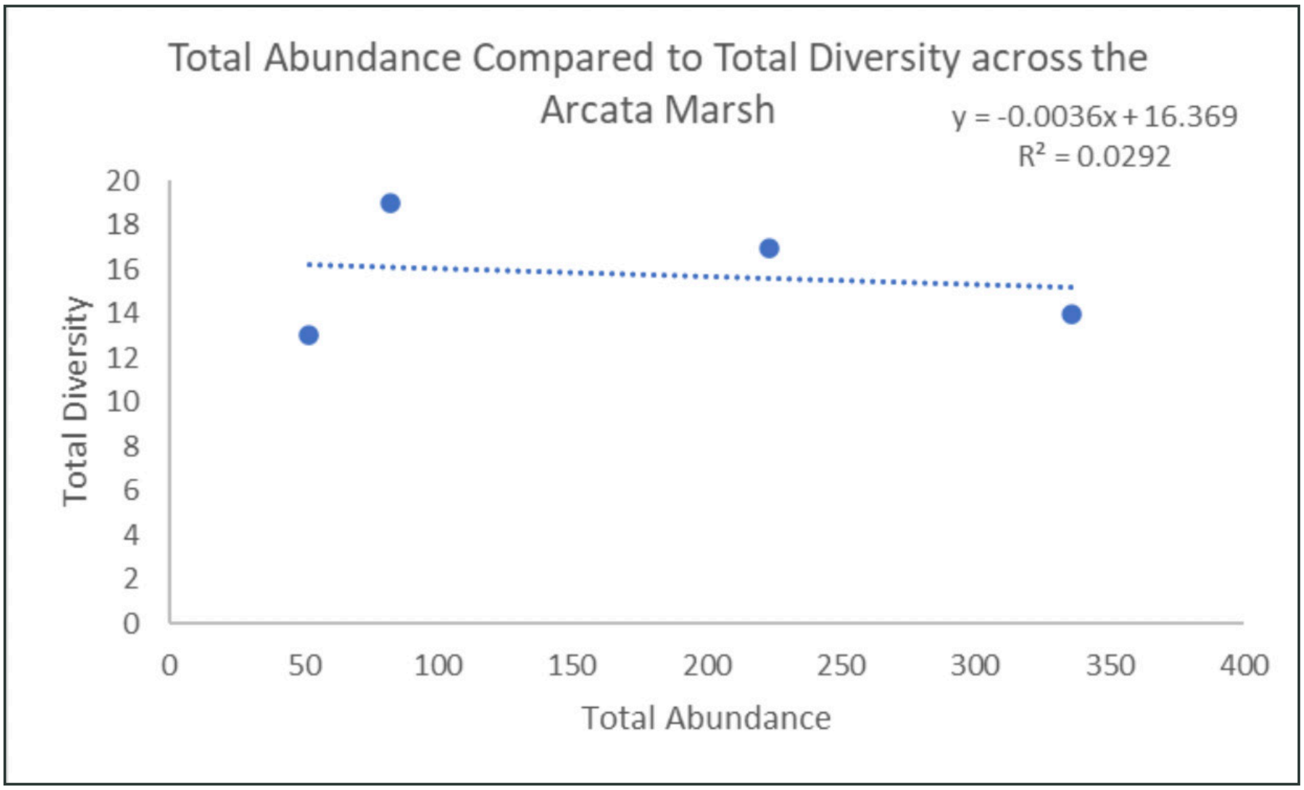


Figure 4.
Regression analysis of abundance and diversity across the Arcata Marsh shows no correlation with the low R2 value of 0.0292



specifically, Hauser and Gearheart, had the highest diversity and lowest abundance.

After receiving an R^2 value of 0.0292 when running a linear regression (figure 4), we concluded that there was no correlation between diversity and abundance across all ponds. The ANOVA revealed a p-value of 0.548, leading us to reject the hypothesis that there would be significant variance of diversity across all treatment ponds with 95% confidence.

DISCUSSION

Nutrient levels throughout the treatment ponds have two effects on diatom ecology. As nutrient levels decreased, we observed no change in diversity, but the abundance of diatoms did decrease. This indicates that diversity and abundance do not influence one another, but are influenced by secondary factors in the environment. Secondary factors such as pH, salinity, and temperature have no effect on diversity and abundance, as no significant difference of these factors was recorded between samples. However, the nutrient levels do affect the abundance of and species evenness of diatoms, which can be an effective indicator of excess nitrogen and phosphorus present in wastewater. Another factor that could have affected our results was the rainfall that occurred on April 17. Rainfall increases water turbidity, which agitates settled diatoms and can affect diatom distribution (10). We cannot conclusively state the effects of wastewater treatment on diatom diversity due to the small sample size collected.

To better study diversity throughout the wastewater treatment plant, a larger sample size would be a more effective way to study diatom ecology. Another study could conduct data collection over a period of time with varying factors, such as weather and seasonality. To correlate nutrient level to diatom diversity and abundance, nutrient concentration should be measured upon collection of other environmental data. In addition, standardization of sample analysis will strengthen the study. A further avenue of study would be to observe diatom nutrient preferences throughout the Arcata Marsh, as Oxidation ponds 1, 2, and enhancement marsh Allen each had a different dominating species. It would be necessary to identify to the species level either using scanning electron microscopy or through DNA analysis before associating preferences to morphotype (11).

In conclusion, this study found that the diversity of diatoms in the Arcata Marsh was not significantly different between pre- and post-treatment ponds. However, the finding that certain diatom species were more abundant in pre-treatment ponds than post-treatment ponds supports the idea that nutrient reduction occurs as wastewater moves through the various stages of treatment, and also reinforces diatom importance as an indicator species. These results can be used to inform future research at the Arcata Marsh and similar treatment plants in other parts of the world.

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