

Determining picoplankton community and concentration near Hawaiian Islands with varying nutrient impacts



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Non-Technical Summary

The objective of this study was to assess the difference in the picoplankton community off islands that vary in human impacts. Humans have greatly impacted the Hawaiian Islands with agriculture and sewage outfalls, which generates nutrient runoff that can lead to the ocean. The increased nutrient runoff creates increased primary productivity in otherwise nutrient limited oceans. Picoplankton (plankton smaller than 2 μm) community can be affected by increase nutrients. Picoplankton communities and nutrient concentrations from different islands were compared to determine if there was a change. It is important to determine if human activities cause a shift in plankton communities because plankton are the base of the trophic level. A change in the base of the trophic level will propagate throughout the trophic levels and eventually affect humans and other species higher in the trophic levels. This investigation took place from 27 Dec 2010 - 4 Jan 2011 around the Hawaiian islands onboard the R/V *Thomas G. Thompson* research vessel. The purpose of this research was to determine if there is a change in picoplankton communities or abundances off the coast of different islands. This study used of flow cytometers, to look at picoplankton cell concentrations. Nutrient samples were also collected to determine if inorganic nutrient were present in the waters. This study found a difference in abundance of species of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes off the islands of Molokai and the Big Island. For now, the differences in abundance off the islands cannot be attributed to nutrients or human activities.

Abstract

The Hawaiian islands, once a pristine habitat, are changing due to human impacts from an increase in human population and human activities. Human activities such as agriculture and sewage increase nutrient output into the ocean. Changes in the environment can effect picoplankton population by shifting populations from *Prochlorococcus*, the most dominate type of picoplankton in oligotrophic oceans to a community that does better in areas of high nutrients, such as *Synechococcus*. This research took place around the islands of Hawaii from 27 Dec 2010 - 4 Jan 2011. An in situ flow cytometer; SeaFlow was used aboard the R/V *Thomas G. Thompson* to determine the picoplankton community. Nutrient samples were also taken to determine inorganic inputs possibly from human activities. A difference in plankton community was found nearshore Molokai compared to the Big island. Using a t-test to statistically determine a difference gave small P-values for *Prochlorococcus* (6.45×10^{-5}), *Synechococcus* (1.83×10^{-10}), picoeukaryotes (2.47×10^{-6}). Trace amounts of inorganic nutrient off the islands of Molokai, the Big Island and Oahu were also found. However, observed differences in the populations cannot be statistically connected to nutrient levels.

Introduction

Phytoplankton are small floating cells. There are even smaller types of plankton known as picoplankton. Picoplankton are cells $< 2 \mu\text{m}$ and can make up to 80% of the chlorophyll in the water (Campbell 1993). The waters around Hawaii are known to

contain three types of picoplankton; *Prochlorococcus*, and *Synechococcus*, and picoeukaryotes (Campbell 1993).

Prochlorococcus and *Synechococcus* are cyanobacteria. *Prochlorococcus* are the most abundant photoautotrophs in oligotrophic oceans (Campbell 1993). They are capable of living in waters that vary in concentrations of nutrients, and grow in various levels of light (Rusch 2010). While *Prochlorococcus* usually outnumber *Synechococcus* in oligotrophic environments, *Synechococcus* dominates in nutrient rich waters and prefers well lit surface waters (Partensky 1999). Such nutrient rich areas that *Synechococcus* dominate over *Prochlorococcus* are areas of upwelling or coastal inputs (Partensky 1999). Given iron enrichment *Synechococcus* has a rapid growth rate and high chlorophyll concentrations (Partensky 1999 and Rusch 2010).

The third type of picoplankton are eukaryotes that are smaller than 2 μm . There are many types of picoeukaryotes. Most are found in freshwater and terrestrial habitats (Miller 2004). Classifying picoeukaryotes down to species is difficult, however most belong to the order Chlorococcales and the genus *Chlorococcus* (Miller 2004). Picoeukaryote abundance tends to be higher in areas of higher nutrient levels, such as inner estuaries (Lin 2010). Lin et al in 2010, found that picoeukaryote concentrations positively correlated with nutrient levels at the Pearl River estuaries. When nutrient levels decreased so did the abundance of picoeukaryotes.

The ocean around Hawaii is oligotrophic, especially nitrogen limited (Ringuelet 2005). These conditions make it difficult for larger phytoplankton to take up enough nutrients to thrive. Oligotrophic oceans tend to be dominated by picoplankton because

picoplankton are smaller and have a higher surface to volume ratio allowing them to take up more nutrients (Ringuelet 2010 and Ribalet 2010).

Picoplankton are capable of responding quickly to changes in their environment. Depending on the magnitude and the length of the disruption this can lead to changes in the equilibrium of the ecosystem (Thomas 2010). Such changes can result from increased nutrient runoff of dissolved inorganic nitrogen or soluble reactive phosphorus from fertilizer off large cultivated areas (Boehm 2010). Areas with forested land have lower nutrient loading compared to areas of urbanization and of high agricultural use (Boehm 2010), indicating that islands with a high fraction of urban and cultivated lands can greatly change the picoplankton community with high nutrient loads. Other sources of nutrient loading are sewage outfall plants such as one located on Oahu Island in Mamala Bay. A study by Petrenko (2000) showed sewage plumes can have a vertical distribution which implies excess nutrients from sewage plants are capable of reaching depths that stimulate productivity.

The purpose of this study is to determine if human impacted areas, places of high urbanization, affect the picoplankton community structure and abundance. The hypothesis for this study is that depending on the amount of human impacts on an island there will be differences in the picoplankton community compared with the typical oligotrophic ocean. Human impacts that increase nutrient loading in the ocean would stimulate *Synechococcus* and picoeukaryotes to outcompete *Prochlorococcus*, therefore causing a shift in picoplankton community.

In order to answer this hypothesis, certain questions should be answered. Are the expected types of populations in the waters around Hawaii? The populations that are expected to be found in the water around Hawaii are *Prochlorococcus*, *Synechococcus*, and picoeukaryotes (Campbell 1993). Is there human impacts on the islands? Oahu has a sewage out fall (Petrenko 2000). Molokai's main source of income is from agriculture. The Big Island has irrigated farming at Kona, Waimea, Kohala, and Kau (McCoy 2010). Is there a difference in concentration or species from islands to island? Is there excess amount of nutrients off islands compared to the control site, the open ocean? If so are these nutrients anthropogenic?

Materials and Methods

Field Methods

The investigation took place from 27 Dec 2010 - 4 Jan 2011 onboard the R/V *Thomas G. Thompson*. Sites of interest were off the coast of Oahu, Molokai, the Big Island, and an open ocean station (Fig. 1). Discrete samples were taken for flow cytometry and nutrients analysis. The on board SeaFlow continuous flow cytometer was used to measure variation in picoplankton communities in the surface water..

Two types of flow cytometers were used for this study. The Influx cytometer is a standard lab based instrument used to quantifying picoplankton communities. The Influx was used to process preserved samples, while the SeaFlow was used to measure surface seawater in-situ while onboard the ship. Both flow cytometers can analyze a volume of water using laser scatter as a proxy for size of the particle and fluorescence to determine

chlorophyll pigment (Ribalet 2011). Based off particle size and chlorophyll pigment the species of phytoplankton can be narrowed down and categorized (Ribalet 2010).

Discrete samples taken for the Influx cytometer were taken nearshore, in areas with corals and offshore. The nearshore samples were collected using a one liter Niskin bottle lowered off the side of a zodiac to depths of 5 meters and the surface. Discrete and nutrient samples were also taken from CTD rosette casts for samples offshore. CTD water samples were taken at 0 m, 5 m, 20-50 m and at the chlorophyll max. Discrete samples were fixed with 1% glutaraldehyde in 1.8 ml test tubes and stored at -80 degrees. Nutrient samples nearshore and offshore were taken at the same depths as the discrete samples and filtered with a 0.48 micrometer filter. They were then stored in a refrigerator onboard the R/V *Thomas G. Thompson*.

The SeaFlow was connect to the flow through system onboard the Thomas G. Thompson to sample directly from the ocean. The SeaFlow analyzed the constant flow of water collecting counts of particles containing chlorophyll running through the stream. The SeaFlow was able to run constantly sampling for the length of the cruise. Data was saved every three minutes (Ribalet et al. 2011).

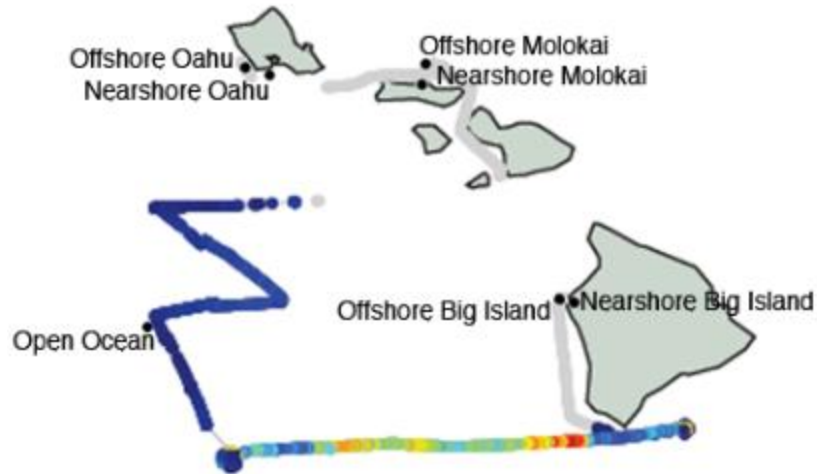


Fig 1. Map of sampling areas, black dots are where the discrete samples were taken. The color lines is the track that the SeaFlow sampled of picoplankton populations.

Analytical Methods

Influx cytometry

Discrete samples analyzed by the Influx cytometer were unfrozen when they were ready to be analyzed. Samples were transferred to 5 ml polypropylene round bottom tubes and 1 μ L of a mixture of 1 μ m beads were added to the sample as a reference for size and to determine if the laser was drifting off focus. Files were set to collect 250,000 ‘events’. The volume taken up by the cytometer, flow rate and running time was recorded. Mass of the samples was measured before and after analysis to determine sample volume.

The files were viewed and picoplankton were categorized through using the FlowJo software package. The first population identified was *Synechococcus* because of its unique pigment Phycoerythrin. This was done by viewing the picoplankton in Phycoerythrin (PE) versus small forward scatter (FSC). A “gate” was drawn around the *Synechococcus* population. With *Synechococcus* identified other populations were

viewed by chlorophyll (CHL) versus FSC. *Prochlorococcus* was assumed to be the group of picoplankton of the smallest size and picoeukaryotes were assumed to be the smaller population with bigger cell size. Counts of the populations from the influx data were exported to an excel spreadsheet to calculate concentration. This was done by dividing counts by the volume of the sample ran.

SeaFlow Cytometry

The SeaFlow is a high-throughput cytometer data (Swalwell 2011). The amount of files that needed to be analyzed was enormous, therefore a software package, *flowPhyto* was used to ‘perform aggregated statistics’ on the files (Ribalet 2011). The data files are subjected to computer analysis of four processing steps; *filter*, *classify*, *census*, and *summarize* (Ribalet 2011). The data is accessible from the SeaFlow interface online. SeaFlow measurements that coincided in areas that matched the discrete samples were compared to the influx to find the Pearson’s coefficient. This was done to determine if the operators of the instrument were able to collect analyzable data while on the cruise. Also a one tailed T-test was performed on SeaFlow data comparing picoplankton populations off of Molokai and the Big Island. Files that were saved around the islands around the time of sampling for the nearshore samples were used in the statistic analysis. The t-test was done for each picoplankton species separately.

Nutrients

Nutrients were analyzed by Kathy Krogslund’s marine chemistry lab at the University of Washington. Water samples were analyzed for phosphate (PO_4), silicate acid ($\text{Si}(\text{OH})_4$), nitrate (NO_3), nitrite (NO_2) and ammonium (NH_4). Analysis of phosphate

was done using a modified procedure of Bernhardt and Wilhelms (1967). Analysis of nitrate, nitrite and silicate were done using a modified or basic method of the Armstrong et al. (1976) procedure. The procedure to analyze ammonium analysis was a modification of Slawyk and MacIsaac (1972) procedure. Nitrogen nutrients (nitrate, nitrite and ammonium) were summed together and graphed against phytoplankton concentrations. *Synechococcus* and picoeukaryotes concentrations were summed together and graphed against nitrogen because their nutrient preferences are similar, preferring higher nutrients. *Pro* concentration was graphed separately against nutrient because it prefers less nutrients.

Results

Influx cytometer

The result of Oahu nearshore have a higher concentration of *Synechococcus* (10.5×10^6 cells/L) compared to the lower concentration of *Prochlorococcus* (2.17×10^6 cells/L) in surface waters (Table 1). Molokai has a higher concentration of *Prochlorococcus* (25.7×10^6 cell/L) nearshore and a lower concentration of *Synechococcus* (10.4×10^6 cell/L). Concentration of *Synechococcus* (13.4×10^6 cell/L) is higher than *Prochlorococcus* (9.49×10^6 cell/L) at the nearshore off the Big Island. However the offshore results from Oahu, Molokai, and the Big Island indicate *Prochlorococcus* has the higher concentration in surface waters compared to *Synechococcus* (Table 1).

For comparing SeaFlow data with the Influx data the Pearson's correlation coefficient was used. The files that were used from the SeaFlow were collected at the same latitude and longitude and the same time that the discrete samples were. To find the Pearson's correlation coefficient concentrations of picoplankton from the Influx samples were compared to concentrations of picoplankton found by the SeaFlow. SeaFlow and influx cytometer was used to find the Pearson's coefficient (0.82) relating all the species concentrations (Fig.2).

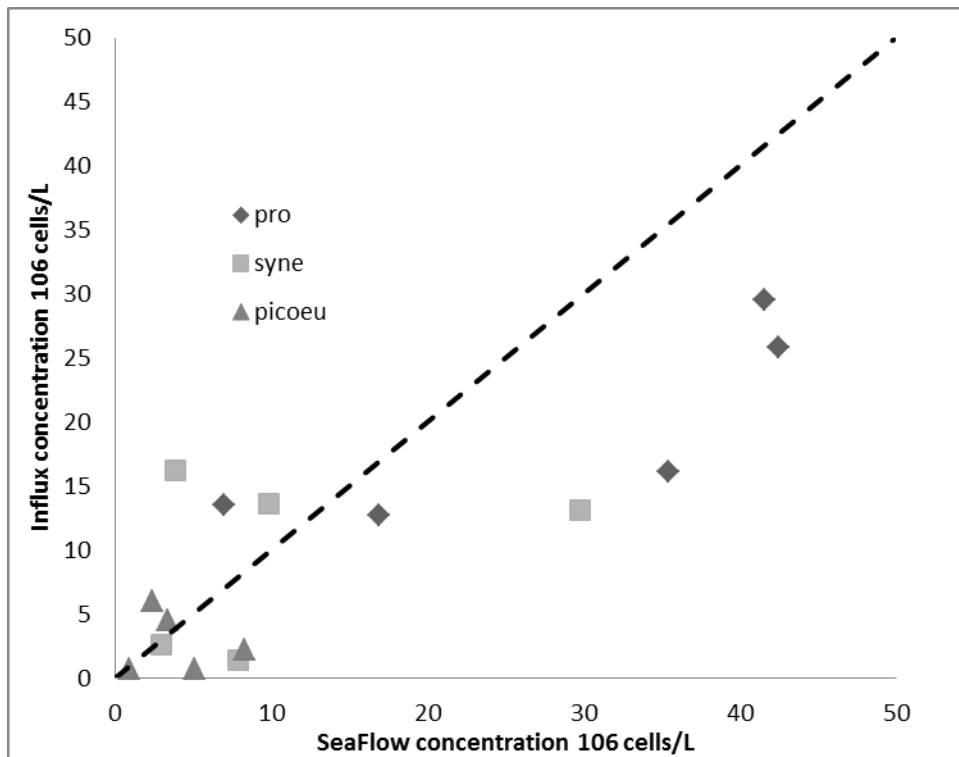


Fig. 2. Correlation between SeaFlow data and Influx data. The overall Pearson's correlation coefficient of the two data sets is 0.82.

SeaFlow data

Figure 3 show the concentrations of picoplankton plotted on maps throughout the course of the cruise. *Prochlorococcus* concentration seems about 220×10^6 cell/L in the

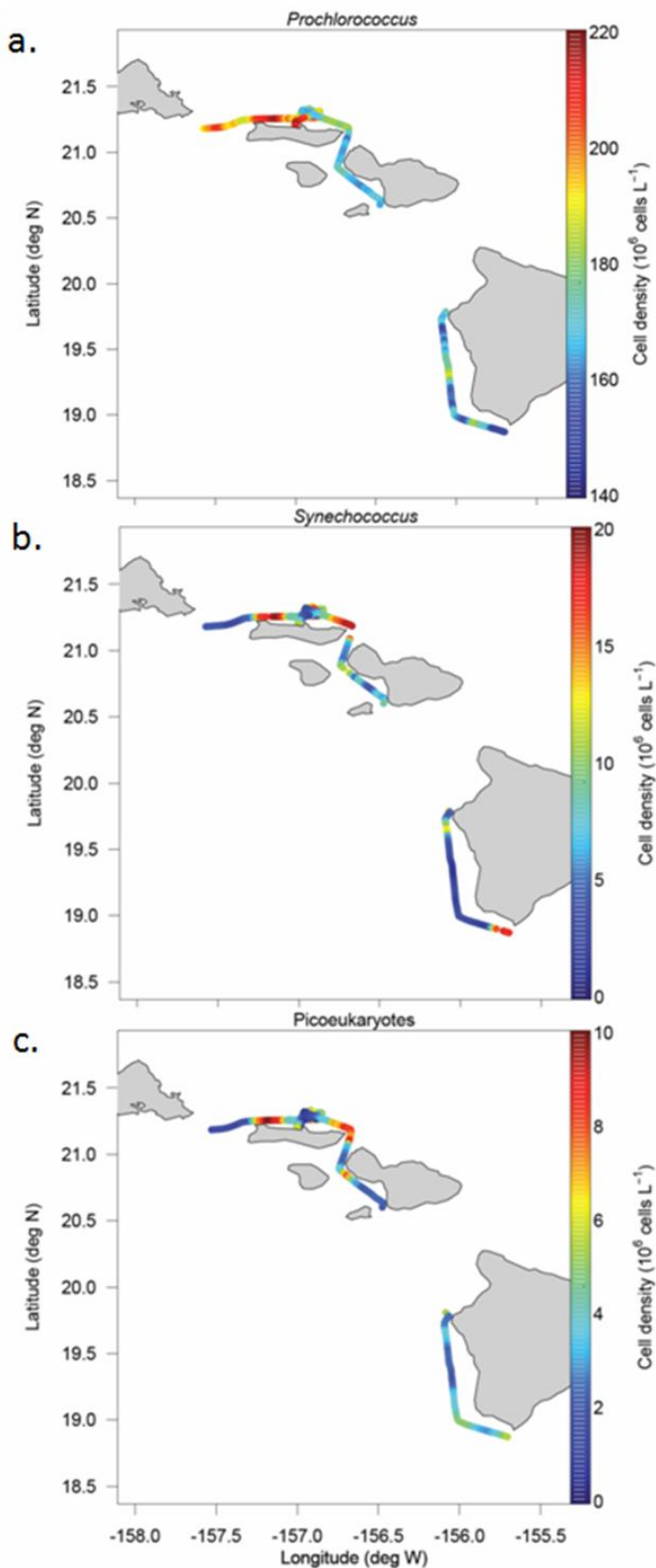


Fig. 3. Concentration of the different picoplankton populations. *Prochlorococcus* (a), *Synechococcus* (b), and picoeukaryotes (c).

open ocean and 190×10^6 cell/L near coastal waters. *Synechococcus* has the highest abundance near coastal waters of northern Molokai with concentrations up to 20×10^6 cells/L and less of a population of the coast of the Big Island. Picoeukaryotes have a low concentration ($\sim 3 \times 10^6$ cells/L) in open ocean waters but seems to have a higher concentration at northern Molokai (10×10^6 cell/L) and at the bottom tip of the Big island (5×10^6 cells/L).

The t-test P-values for *Prochlorococcus* was 6.4×10^{-5} , *Synechococcus* 1.8×10^{-10} , picoeukaryotes 2.5×10^{-6} . The average concentration of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes respectively is 166.6×10^6 cell/L, 16.62×10^6 cell/L, and 166.6×10^6 cell/L.

10^6 cell/L, and 6.61×10^6 cell/L off Molokai. Off the Big Island the average concentration for *Prochlorococcus*, *Synechococcus*, and picoeukaryotes is 176.7×10^6 cell/L, 8.91×10^6 cell/L, and 2.96×10^6 cell/L (Fig. 5).

Nutrients

The graph of *Prochlorococcus* versus nitrogen nutrients does not show a linear regression with nitrogen at the Big Island ($R^2 = 0.1082$) (Fig. 4). Though strangely at Oahu there does seem to be a relationship between *Prochlorococcus* and nitrogen ($R^2 = 0.5843$) (Fig. 4). Though looking at *Synechococcus* and picoeukaryotes concentrations with nitrogen concentrations, there is a relationship between the two at Molokai ($R^2 = 0.805$) and Oahu ($R^2 = 0.552$) (Fig. 4).

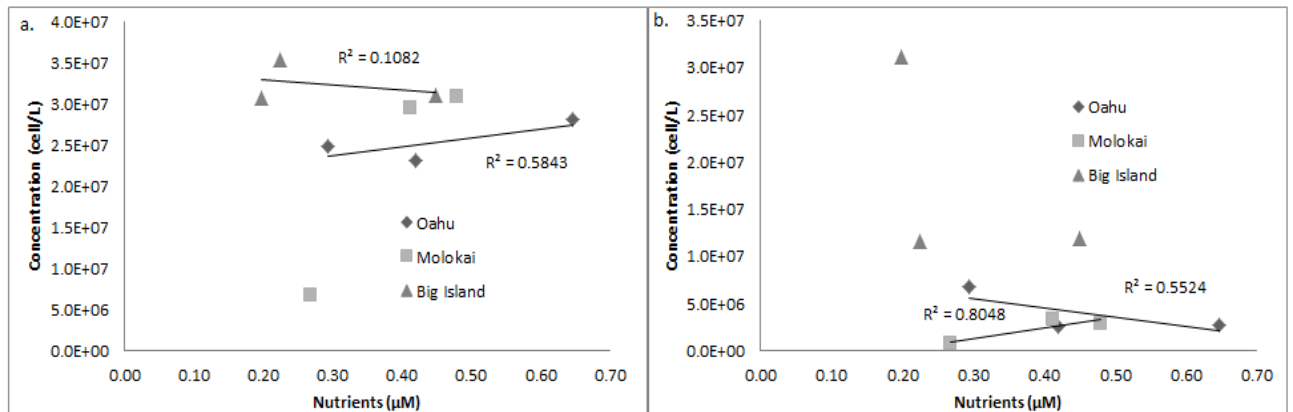


Fig. 4. *Prochlorococcus* concentration (Influx data) of different stations plotted against nitrogen nutrients (a). *Synechococcus* concentration (Influx data) of different stations plotted against nitrogen.

Discussion

The Pearson's coefficient determines if two sets of data are related, the closer the number is to 1 the more the data sets correlate. The Pearson's correlation coefficient was

used because it does not determine one method as a dependent of the other, treating both methods as independent variables. Looking at the correlation for each species gave varying coefficients, however the overall Pearson's coefficient of correlation for Influx data and SeaFlow was 0.82. The differences between the findings of the SeaFlow and Influx can be attributed to the different sample types. SeaFlow analyzes live cells, while the Influx cytometer analyzes preserved cells. It is possible to lose some cells when the preserving the sample, that could account for some of the lower concentrations seen with the Influx cytometer. Also the instruments analyzes the streams of water differently, the Influx aligns individual particles to pass through the laser to provide accurate data. In the SeaFlow raw seawater flow ran through the laser without having the particles aligned. Also multiple particles in the stream can pass through the laser at once. This means that SeaFlow data had to be filtered to use only data of 'optimally position particles' (Ribalet 2011).

Prochlorococcus, *Synechococcus*, and picoeukaryotes were found in the waters of Hawaii during this study as expected from Campbell (1993). The populations were both observed by Influx sampling and SeaFlow. Influx data showed that at Oahu nearshore *Synechococcus* and picoeukaryote concentration was much higher than *Prochlorococcus*. The Big Island also had a higher concentration of *Prochlorococcus* compared to *Synechococcus*. This was expected because both islands had higher nitrogen concentrations (0.67 μM and 0.70 μM) that stimulated *Synechococcus* and picoeukaryote populations (Lin 2010 and Partensky 1999). However, while closer to Molokai *Prochlorococcus* concentrations were much higher than *Synechococcus* and

picoeukaryote, the nitrogen concentration nearshore at Molokai (0.30 μm) was lower than the nitrogen Oahu (0.67 μm) and the Big Island (0.70 μm), this indicates that *Prochlorococcus* does do better in lower nutrient waters (Rusch 2010). The trend of high concentrations of *Prochlorococcus* in lower nitrogen waters is also seen in the open ocean station, the nitrogen concentrations there is 0.50 μm .

	Pro	Syne	PicoE
Oahu	2.17E+06	14.0E+06	14.0E+06
Molokai	25.7E+06	10.4E+06	8.18E+06
Big Island	9.49E+06	13.5E+06	6.24E+06
Open Ocean	13.9E+06	1.35E+06	4.28E+06

Table 1. The nearshore concentrations of the picoplankton species at different islands (Influx data)

The SeaFlow found high concentration of *Prochlorococcus* while sampling at open oceans which matches previous studies (Rusch 2010) (Fig. 5). SeaFlow data was also used to compare offshore populations off of Molokai and the Big Island. The small P-values indicates that there is a difference between the populations found off the coast of the islands. Though the small P-value for *Prochlorococcus* is not clear if it is due to just statistics or if it really is biologically significant of a difference. In biology populations of microbes can reach high densities that the difference between a million cells could actually be insignificant. *Synechococcus* and picoeukaryotes P-values do have significant meaning however, since the populations are small in orders of magnitude.

With a difference of populations offshore of Molokai and the Big Island established, what that difference is should be determined. The averages of the concentrations of the populations Molokai has a higher concentrations of *Synechococcus* and picoeukaryotes compared to the populations offshore of the Big Island (Fig. 5). However, *Prochlorococcus* concentrations off Molokai is less than the Big Island (Fig. 5). This results are different from what was seen nearshore. The changes in concentrations could be attributed to spatial differences. Offshore Oahu (0.65 μm) and the Big Island (0.48 μm) had less nitrogen concentrations than their counterparts near shore. Molokai was a strange in that nitrogen concentrations actually increase (0.48 μm).

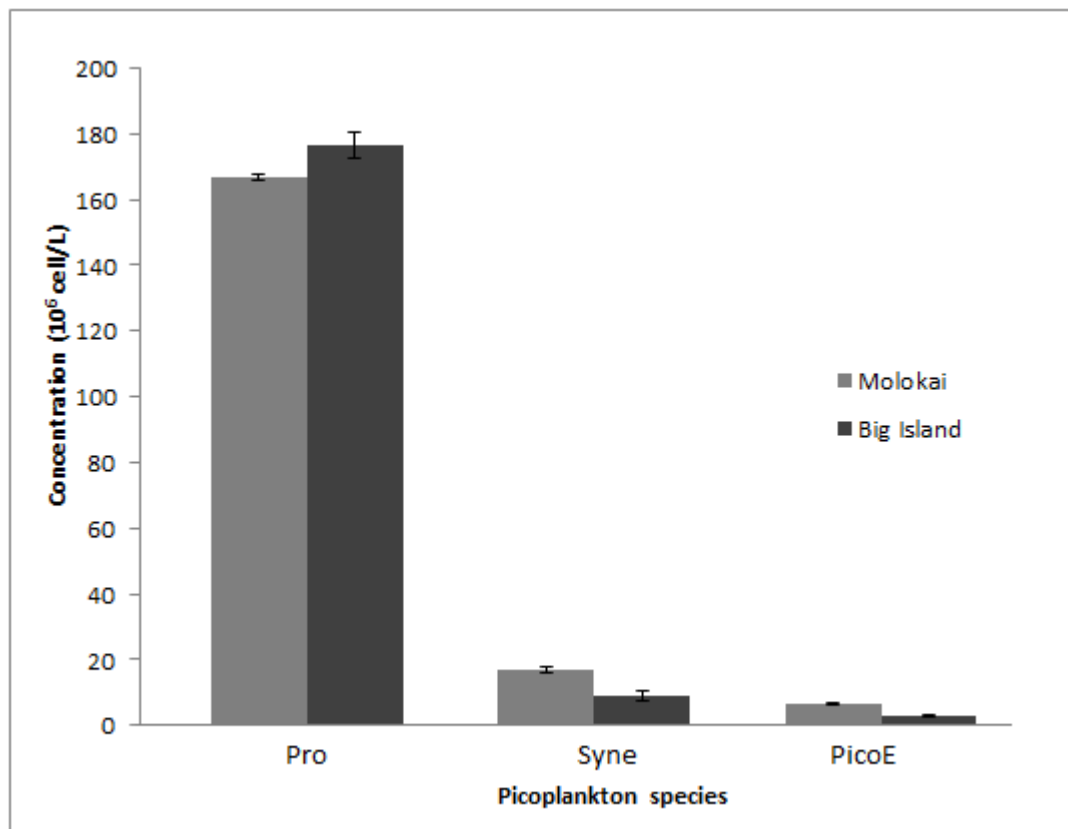


Fig. 5. The average concentration of picoplankton populations offshore of Molokai and the Big Island with the standard deviation bars. SeaFlow data

Nutrient versus picoplankton when originally plotted separately (all nutrients by itself) there was no clear trend or relationship. So all nitrogen nutrients were incorporated together to plot against *Prochlorococcus* separately, then against *Synechococcus* and picoeukaryotes. Nitrogen was chosen because Lin (2010) had found a relationship between picoplankton and DIN. The nitrogen concentrations versus picoplankton graphs showed a picture of a relationship between nitrogen concentrations with *Synechococcus* and picoeukaryotes at two different stations, Oahu and Molokai. Though it was not clear if there was a relationship between *Prochlorococcus* and nitrogen because one station had a high R^2 value while the other had a low value (Fig. 4). Nutrient data did show that there were inorganic nutrients (DIN and SRP) previously found in a study of pollutants (Boehm 2010). Nitrogen concentrations were found in higher concentrations nearshore of Oahu than in coastal areas near the other islands. It is possible that the levels of nitrogen observed can be due to the sewage outfall on Oahu relatively near the station (Petrenko 2000). Nitrogen was also found in high amounts nearshore to the Big Island where sampling for nutrients was near Kona. This part of the Big Island has irrigated farming (McCoy 2010). Irrigated farming diverts water from natural streams through crop fields that would have been fertilized, this is a possible source of DIN from the Big Island.

Even though we did find a statistical difference between populations of Molokai and the Big Island and there is strong potential that it could be attributed to higher nitrogen levels it is important to consider, with the SeaFlow, there is an uncertainty of the findings due to time and space sampling. Phytoplankton are drifting organisms and so go

where currents take them. These populations can vary with time due to currents and could have originated from other sources. This means that the population found at that site is not necessarily the same population that would be there over a longer time scale.

Therefore is not a true indicator of the status of the picoplankton community and concentration over a long time scale. Though the SeaFlow is very good at giving an indication of the type of populations and their concentration for an instance in time. It also good at looking at populations over a distance comparing the populations from one island to another as was used for this study.

If this study were to be repeated or given more time, a longer observation of picoplankton populations should be observed. More research should be put into determining the extent of human impacts on the separate islands. Another change would be to sample more sites and sample at areas of 'pristine' environments and polluted environments. Also more discrete and nutrient samples should be collected at greater depths nearshore and replicates would be taken.

Conclusion

This study found a difference in picoplankton abundance between islands of Molokai and the Big Island. High concentrations of *Prochlorococcus* were found offshore of Molokai an island with less nitrogen concentrations. And high *Synechococcus* and picoeukaryote concentrations offshore the Big Island where higher nitrogen concentrations were found. A tentative connection has been made between the differences in picoplankton abundances and nutrient levels. The differences observed in

picoplankton abundance can tentatively be linked to human activities that have nutrient runoff.

If future studies were to build off the results from this investigation they would know that SeaFlow data is an accurate way to assess picoplankton communities. Also since it has been determined that there are differences in picoplankton abundances from island to island maybe future studies could answer the question of what are cause(s) these variances. They could also study other trophic levels that depend on picoplankton and see if other trophic levels differ from island to island.

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