EFFECTS OF NUTRIENT ENRICHMENT ON THE GROWTH OF EPIPHYTIC ALGAE AT KEAHOLE POINT

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ABSTRACT

Eutrophication of marine communities has been shown to significantly alter not only the structure and functioning of those ecosystems but also the general esthetic health and economic value of the waterways they occupy (Geertz-Hansen et al. 1993; Hauxwell et al. 1998; Mason 2002; Worm et al. 2000). This eutrophication can affect epiphytic communities as well as the phytoplanktonic.

The effects of nutrient enrichment (NO_3 and PO_4) on the growth of epiphytic algae was examined utilizing the flow through surface sea water systems (SSW) at The Natural Energy Laboratory of Hawaii Authority (NELHA), Keahole Point, HI. Significant differences were found between high orders of magnitude of nitrogen enrichment (500 & 1000 x background) and the background levels of algal growth as indicated by CHL-a and Dry Weight measurements. Phosphorous at levels above the background were found to have an initial effect on CHL-a measurements but over time and with increasing orders of magnitude any significant difference disappeared. The interaction of nitrogen and phosphorous was also tested and results found a similar trend where phosphorous played an initial role but over time and with increasing orders of magnitude had no effect. Dry weight measurements found nitrogen had a very significant effect on algal growth however phosphorous had none. This study suggests that algal growth off Kahole Point is nitrogen, not phosphorous limited. In addition, this study highlights a variety of biological, physical, and anthropogenic influences that are necessary to place water quality data in the proper context for managers.

KEYWORDS

Nutrient Enrichment; Algae; Eutrophication; Nitrogen; Phosphorous; Epiphytic; Keahole Point

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INTRODUCTION

Increases in coastal development of residential, commercial, agricultural, and urban infrastructure significantly increases anthropogenic effects on downstream marine communities (Mason 2002). Pollution from point and non point sources, such as agricultural runoff, residential runoff and waste disposal, and industrial/commercial effluence drain into local watersheds and move downstream into coastal waters (Mason 2002). The destruction of natural filters, such as wetlands and forests, and poor land use methods that remove forests and natural drainage basins with bare dirt for agriculture or pavement for infrastructure increase the runoff and drainage of waste, pollution, effluence, and sedimentation (Reinelt & Horner 1995). These waste streams eventually flow into local water ways and have the potential to disrupt the local marine communities, sometimes in irreversible, catastrophic ways (Diaz & Rosenberg 2008). The drainage basin of the Mississippi delta provides an excellent example of the anthropogenic impacts of upland agricultural and urban effluence. One of the larger dead zones of water on earth, seen easily from space, inhabits the northern Gulf of Mexico where the Mississippi drains into the Gulf of Mexico (Malakoff 1998). The influx of nutrients such as nitrogen and phosphates, called eutrophication, has completely decimated the marine community in the northern gulf (Rabalais et al. 2002).

When marine communities are subject to eutrophication, primary productivity often increases (Rabalais et al. 2002). This can lead to algal blooms, reductions in O_2 content in the water, poor water quality and visibility, and increased toxicity of the water (Diaz & Rosenberg 2008). This can have significant consequences for the marine communities in those waters, including decreased biodiversity, change in species composition, mortality from hypoxia, loss of native species, and increases in invasive species proliferation (WRI 2008). Corresponding social

and economic costs for people include decreased fisheries production, decreased tourist revenue, loss of subsistence fishing sources, and decreased aesthetic value of the water (WRI 2008). The upland topography and ocean geography of an area can also increase the vectors for eutrophication and magnify its effect on marine and coastal communities (Likens & Bormann 1974). In Hawaii, for example, large tracks of land up-slope of the ocean have been significantly altered for agriculture & farm use (Figure 14). Much of the landscape has been cleared for grazing and pastures, drainage basins have been paved over, rain water drainage streams have been altered, and fire regimes have been significantly modified (Hunter & Evans 1995).

The consequences of eutrophication events in Hawaii must be considered within the framework of its unique geology and location. These unique factors may cause the marine community to take longer to recover from harmful events (Kay & Palumbi 1987). The majority of reef life, which forms the basis of the marine community around the islands, rests in a narrow fringing reef around each island (Gulko 1998). This small area extend only a few hundred yards offshore, a characteristic of reefs formed on the steep sloping sides of volcanoes which extend deep and abruptly to the ocean floor. This translates to a small area for reef life to exist and a small reserve to replenish areas stressed by anthropogenic influences. Because Hawaii is outside the major ocean gyres, it does not receive a significant amount of outside larval recruitment and is home to many endemic species (Kay & Palumbi 1987). 18% of Hawaiian reef building corals are endemic, one of the highest endemism rates for reef building corals in the world (Kay & Palumbi 1987).

Loss of species and biodiversity from anthropogenic disturbances such as eutrophication could mean the loss of native endemic species, longer recovery periods, and/or permanent damage to these fragile marine communities (Hobbs & Huenneke 1992) & (Levine & D'Antonio

1999). Invasive species outbreaks such as *Acanthophora spicifera, Eucheuma sp., and Gracillaria salicornia* in Kane`ohe bay on Oahu will be more likely in areas impacted by eutrophication (Diaz & Rosenberg 2008). Currently, Hawaii is spending 8 million dollars/year on invasive species removal and containment, in addition to the tourist dollars and esthetic value lost (HISC 2008). Hawaii also has a strong tradition of subsistence fishing dependant on these narrow reef ecosystems (Friedlander & Brown 2004). Tourism is a mainstay of the Hawaiian economy, accounting for 10 billion dollars of the islands revenue (Darowski et al. 2006). The social and economic costs of eutrophication events must not be underestimated, considering how much of Hawaii's economy, culture, and identity are connected to the coastal marine community. In the Black Sea region of the Bulkans, it is estimated that poor bathing/swimming water from eutrophication has caused 500 million in lost tourist revenue to those economies; this is a situation we must avoid here in Hawaii (BSERP 2006).

The waters located off Keahole Point and the Kailua-Kona coast are mandated Class AA open coastal waters by the Hawaii Department of Health (DOH 2004). Hawaii Administrative Rules - Title 11, Chapter 54, Water Quality Standards - mandate that waters designated as Class AA must remain in their natural pristine state as nearly as possible with an absolute minimum of pollution or alteration of water quality from any human-caused source or actions (DOH 2004). Furthermore, these waters must support the propagation of marine life, the conservation of coral reefs and wilderness areas, and recreational activities. To facilitate these mandates the Department of Health has numerically quantified the physical and chemical water quality parameters indicative of these standards. These levels act as an indicator of acceptable water quality and are outlined in HAR 11-54-06(b)(3) (DOH 2004).

The Natural Energy Laboratory of Hawaii Authority (NELHA) and Hawaii Ocean Science & Technology (HOST) Park are tasked with monitoring the water quality and marine resources off Keahole Point in order to maintain their permits to dump effluence waters into open trenches in the shore side terrain (GK & Associates 1989). Originally created in 1974 by the Hawaii Sate Legislature to provide support facilities for OTEC (ocean thermal-energy conversion) processes, NELHA has since undergone a series of expansions and mergers. It is now the 'landlord' to over 40 commercial enterprises including a variety of aquaculture companies growing abalone, shrimp, microalgae, and many other species; 4 research organizations including University of Hawaii's infrasound laboratory, an underwater autonomous vehicle design and testing company, and a marine algae-to-biofuel production facility; and four educational entities including the West Hawaii Explorations Academy (WHEA – a public charter school), the University of Hawaii's Sea Grant College Program, and The Hawaiian Islands Humpback Whale National Marine Sanctuary headquarters. These are just a sample of the many companies and research organizations currently working at NELHA. NELHA's mission "to develop and diversify the Hawaii economy by providing resources and facilities for energy and ocean-related research, education, and commercial activities in an environmentally sound and culturally sensitive manner" has become known as a fundamental initiative leading sustainable development into the 21st century.

In compliance with state and federal laws, NELHA conducts a Comprehensive Environmental Monitoring Program (CEMP) which includes marine biota and water quality testing (DOH 2004). NELHA monitors ortho-phosphate phosphorus, nitrate + nitrite nitrogen, ammonia nitrogen, dissolved silica, salinity, temperature, total organic carbon, total suspended solids, dissolved oxygen, pH, alkalinity, and microbiology sampling for vibrio and total count

marine agar (Olson 2009). Thirty three benthic marine biota surveys conducted from May 1992 to May 2009 suggests that the total coral cover and coral cover of individual species has gradually increased over the monitoring period (Ziemann & Conquest 2009). Thirty two surveys of fish species, number of individuals and biomass over the same period have also shown no significant change in the resident fish community (Ziemann & Conquest 2009). Anchialine ponds surveys also indicate no effects from anthropogenic influences. Pond waters have remained relatively clear with little macro algal growth. A high abundance of *Halocaridina rubra* (a brackish water shrimp) is reported in ponds without invasive fish species (Ziemann & Conquest 2009). These are encouraging results considering the many clients located on the NELHA grounds. During the same seventeen year period chlorophyll-a, a measure of phytoplankton biomass, has never exceeded the State of Hawaii Department of Health (HDOH) limit (Olson 2009). In addition, the nearshore seawater chemistry has been consistent and the groundwater has also been comparatively stable over the past twenty years (Olson 2009). From 1993 to 2007 water quality measurements were conducted on a quarterly basis following the West Hawaii coastal monitoring program guidelines (WHCMTF 1992). Since July 2007, NELHA has implemented the standard sampling procedure and analytical protocol of the August 31, 2004, HAR Title 11 Chapter 54 aligning their sampling techniques with EPA and HDOH protocols (Olson 2009).

Certain considerations are worth discussing concerning the collection of these water quality data. First, the intrusion of naturally occurring and human enriched groundwater can greatly influence the water quality of the nearshore seawater chemistry depending on the coastal plain geology (Burnett et al. 2001). Groundwater/seawater sampling before most of the NELHA infrastructure was in place occasionally showed levels of nutrients exceeding recommended

standards (Olson 2009). This indicated that there is a complexity of upland factors beyond the immediate shoreline infrastructure that influence coastal nutrient levels. To further add perspective to water quality results found off Keahole Point it is worth considering them within the larger context of the Kailua-Kona coast line. Previous studies off the Kailua Kona coast have shown the importance of underground hydrology and adequate flushing as a critical criterion in maintaining good water quality (Bienfang 1980). As natural occurring Anchialine ponds are only found in a highly porous substrate, the land where NELHA is located demonstrate a porous sub-structure conducive to a high flush rate of seawater with incoming groundwater (Brock 1997). This porous coastal plain below NELHA, and up and down the West Hawaii coast, facilitates the dilution of nutrients from incoming groundwater (Brock 1997). Nutrient concentrations in surface sea water are generally very low with this type of underground dynamic (Brock 1997) (Table 6). The importance of reviewing water quality data with respect to groundwater intrusion is very important to accurately conclude water quality health (EPO 1997).

A conservative mixing plot model, used by most professionals in the field, is used to determine whether groundwater enrichment has occurred (Dollar and Atkinson, 1992). NELHA extensively used the conservative mixing plot model technique to describe their groundwater water quality chemistry (Olson 2009). This is in line with HDOH protocols (DOH 2004). This technique plots salinity versus a given water quality parameter (for instance NO_3 or NO_2) to accurately reflect the concentration of that parameter relative to the amount of groundwater in the sample. A higher percentage of groundwater will contain a higher percentage of a given parameter. This is an important consideration when describing water quality data levels. Second, it can be difficult to accurately measure nutrient concentrations below 5 µg/L with commercially available segmented flow nutrient instrumentation (Olson 2009). The HDOH outlined nutrient

limits, as outlined by HAR Title 11 Chapter 54, August 31, 2004, approach the detection limits of the colorimetric method used for the analysis of ortho-phosphate phosphorus, nitrate + nitrite nitrogen, and ammonia nitrogen (Table 3).

All of these outstanding factors can greatly impact the interpretation of water quality data. The April 2006 "Review of Coastal Monitoring Data for Development in West Hawaii" is a good example of the misinterpretation of the rules, regulations, and methodologies applied to water quality data. It highlighted data that did not employ the use of a conservative mixing plot for sample spots with a salinity of less than 32ppt, it compared sites that did not follow the method of data collection utilizing six transects starting at the shoreline and moving seaward, and it misinterpreted the appropriate levels of Enterococcus bacteria allowed, all of which is either required or outlined by the State of Hawaii, Department of Health (HDOH), Title 11 Chapter 54 West Hawaii Area-Specific rules to determine trends to the nearshore marine environment as the proper way to collect and interpret this type of data (Olson 2009). This emphasizes the importance of understanding and employing the proper techniques, data analysis, and protocols when collecting, analyzing, and interpreting water quality data. It also linked levels of nutrient enrichment to infrastructure that did not exist demonstrating that a solid understanding of temporal considerations is also necessary (Olson 2009).

Another important consideration to the application of water quality data is the mixing process of the seawater and groundwater along the shoreline. The majority of subsurface groundwater intrusion along the west Hawaii coastline is via submarine groundwater discharge (SGD) (Peterson et al. 2009). Furthermore, SGD has been shown to be the primary source of N & P to the coastal areas of west Hawaii (Street et al. 2008). This context is important as the process in which the fresh and salt water mix can significantly impact the amount of nutrients

that reach the epiphytic community (Dollar & Atkinson 1992). The magnitude and mechanisms of SGD are inadequately documented, lack accurate measuring methodologies & tools, and have been shown to be spatially and temporally inconsistent (Hwang et al. 2005; Taniguchi et al. 2008). Therefore, using SGD to quantify nutrient input can be beneficial on a local time/location scale but not as reliable on a regional/long-term scale (Peterson et al. 2009). Although diffuse groundwater seepage happens along the west Hawaii coast, aerial imagery has shown the dominant flow is through distinct point-source SGD based on water temperature fluctuations that outline distinct plumes of colder groundwater that buoyantly flow out and mix with the seawater (Johnson 2008; Johnson et al. 2008) (Figure 11). The amount of nutrients SGD transfer to the adjacent reef communities is likely dependant on local atmospheric and oceanic conditions (Presto et al. 2007). A more in depth understanding of this process locally off Keahole Point and locally in other areas with possible SGD and nutrient input would significantly aid management efforts for those specific areas.

A significant factor not applicable in this experiment is the role of herbivore on algal growth. Understanding the top-down and bottom-up influences upon community dynamics is important when estimating the potential results of nutrient increases on primary production (Boyer et al. 2004). Numerous studies have shown mixed results regarding the predilection of phase shifts from coral to algal dominance based on top down vs. bottom up pressures (Smith et al. 2001, 2002, 2004; Stimson et al. 2001; Thacker et al. 2001; Szmant and Forrester 1996; Lapointe 1997; Miller et al. 1999; Lerman and Biber 2000; Littler and Littler 1984; Littler et al. 1991; Hughes 1994; Hughes and Connell 1999; Aronson and Precht 2000; Williams and Polunin 2001; Hatcher and Larkum 1983; Bell 1992; McCook 1999, 2001). This suggests that community dynamics, such as competitive interactions, nutrient requirements, etc., vary

depending on specific species and adaptations to specific locations (Smith et al. 2004). A more thorough understanding of the species specific interactions and adaptations off Keahole Point may help to interpret the results and implications of this study. For example, the identification of a fast growing opportunistic algal species at higher levels of nutrient enrichment may reveal the presence of a possible indicator species for eutrophication events (Smith et al. 2004); especially if that species were not found in similar abundance out on the reef during periods when the nutrient load did not exceed the background. Also, it would support a more bottom-up effect. Alternatively, many studies in similar reef environments in Hawaii have shown a complex top-down relationship between algae, corals, and herbivorous predation (Vermeij et al. 2010). Increases of turf and crustose corraline algae have been correlated with reefs dominated by sea urchins and low coral cover, whereas macroalgae has been positively associated with reefs dominated by herbivorous fish (Vermeij et al. 2010). Indicating that although many factors influence reefs locally (density-dependant effects, pollution, disease, large-scale oceanographic events, sea urchin harvesting, food subsidies, etc. ; Sala et al. 1998) changes from the top down, such as the removal of herbivorous predation from overfishing, can significantly altered algae species composition by altering the species specific top-down predation effects (Vermeij et al. 2010). This also indicates that turf algae is a better indicator of reef stress, suggesting that the proper identification of algal types, not just algal growth in general, is important in identifying the source of malignant stressors (Vermeij et al. 2010). Although this study does not address the effects of herbivore a more thorough understanding of herbivore on the local reef environment off Keahole Point would complement the results found here.

Herbivorous effects also highlight the role of MPAs in water quality management. Areas of the ocean protected by MPA's in Hawaii have shown significantly higher levels of biomass

and coral health, suggesting that the top-down effect plays a critical role in similar reef environments to the area off Keahole Point (McClanahan et al. 2002; Behrens & Lafferty 2004; Dulvy et al. 2004). Areas with lower levels of herbivorous fish have documented increases in turf algae caused by a trophic level change from an increase in sea urchins that influence the algal community structure from macroalgea to turf (Vermeij et al. 2010). Recognizing the implication of top-down management and its role regardless of bottom-up pressures off Keahole Point would be beneficial. Presently, coral cover has remained constant and steadily increases over the sampling period (1992 – 2009) and fish communities have been constant off Keahole Point (Ziemann & Conquest 2009), suggesting that corals are not being out competed for space from the bottom up nor subject to overfishing from the top-down. However, determining whether the reefs off Keahole Point are more subject to herbivorous effects or nutrient effects would aid managers in determining where best to spend their efforts.

The data collected from this experiment must take into consideration the many factors outlined above concerning water quality monitoring. Understanding nutrient level inputs and their relationship with algal production, monitoring guidelines and their limitations, and geographic/geologic factors influencing nearshore coastal zones is important for managing Hawaiian marine communities around the islands. Data from this experiment can be applied to nutrient monitoring efforts presently in place. Identifying levels of nutrient inputs (nitrogen & phosphorous) that create levels of algal growth significantly above the background can aid managers in predicting and preventing eutrophication events before they create harmful effects. This project can also aid NELHA in the fundamental understanding of potential adverse impacts of nutrients in seawater. In the future, NELHA may be able to better understand potential risks in its tenants operations by extrapolating the biomass data collected from this experiment.

This fundamental understanding of basic nutrient inputs is critical to water quality management. As nitrogen and phosphate move through the environment they regularly cycle in a balance that supports the growth and maintenance of life (Vitousek et al. 1997). It is the disruption of this cycle through humans inputting too much of these nutrients into an ecological community that off-sets the background levels and can create havoc on that system (Mason 2002). Understanding the levels of nutrient input at which primary productivity significantly increases is sequential to managing coastal areas and controlling the acceptable levels of nutrient load into them. To better understand the relationship between nutrient input and epiphytic algal growth in Hawaiian waters, sea water will be sampled at the NELHA laboratories on the Big Island of Hawaii. Experimentation will occur to quantify what levels of eutrophication increase the growth of primary producers. In particular, what levels of nitrate and phosphorous disrupt/increase the background growth of epiphytic algae in Hawaiian waters. Although NELHA monitors many water quality parameters, this experiment will narrow the focus to just nitrogen and phosphorous. This will be achieved by quantifying the growth of epiphytic algae on unglazed ceramic tiles in different tubs with varying concentrations of nitrogen and/or phosphorous. Methodologies used to assess algal growth on ceramic tiles such as from Larned & Santos 2000 will be modified to meet the goals of this experiment.

METHODS AND MATERIALS

Experiments were conducted at NELHA utilizing their wet labs and sea water flow through systems. A series of six-inch un-glazed ceramic tiles set in ‘tile tubs’ were exposed to sea water and some form of nutrient enrichment (see Figures 2 & 3). The amount of growth of epiphytic algae on the tiles was then be measured at set intervals to correlate the effects of

nutrient enrichment with algal growth. The algal growth on the tiles was measured through fluorometric analysis of chlorophyll a. This measurement of chlorophyll was used as an indicator of biomass. Additionally, the Dry Weight of algal mass was used as another indicator of biomass. High Performance Liquid Chromatography (HPLC) was also attempted to assess major taxonomic groups. In addition macroscopic photographic analysis was attempted to identify species. The first phase of experimentation consisted of a two month long calibration trial to set all of the parameters for the main experimental trials and fine tune the methodology. Three main experimental trials each lasting three weeks followed the initial calibration trials.

Experimental Design:

For each trial a total of 18 tile-tubs measuring 22in. x 18in. (28L), each with six tiles, was incubated with sea water flowing through for one week. This was followed by a two week period of nutrient enrichment and seawater dripping into these tubs. All of the tile tubs sat in one of three large temperature baths to keep temperature constant (see Figure 2& 3). 120 liter Rubbermaid 'nutrient source containers' were used to slowly gravity feed surface sea water & nutrients into the tile tubs at 4-8ml /minute using adjustable low-flow irrigation drip valves (see Figure 3 & 6).

The individual tile-tubs were enriched with nitrogen, phosphorous, or a combination of nitrogen and phosphorous during each trial. The concentrations and compositions of nutrients for all of the trials are outlined in Figure 1 and were randomly assigned to the tubs. The concentration of nutrients was selected using the NELHA background concentration of nitrogen 0.05 µm/l (SSW) and phosphorous 0.02 µm/l (SSW) and then increasing this amount by an order of magnitude times the background of 1, 100, 500, & 1000. For each trial, tiles were removed

from the tubs to assess epiphytic algal growth on a weekly interval starting at the end of the one week incubation period and continuing through two weeks.

The design of this experiment includes six (120 L each) nutrient source containers, 18 (28 L each) tile tubs measuring 22x18x6 inches, 18 air stones, 108 (6x6 inch) unglazed ceramic tiles, 3 (4248 L) temperature baths, 18 adjustable irrigation drips (rated between 0-10 GPH) and varying lengths of pvc pipe and brown irrigation tubing (see Figure 2).

Detailed usage of parts/equipment:

1. *Nutrient source containers,* (6) 120 L each (Figure 6)

These held the seawater and nutrient solutions that were gravity fed via irrigation tubes to the tile tubs. They consist of two 60 L large Rubbermaid containers that have been plumbed together. They are positioned above the tile tubs so that gravity pulls the seawater/nutrient solution down into the tile tubs. They were chosen for their opaque color, size, and durability. Air holes have been drilled near the top to allow the intake of air as the water level inside is depleted and to keep the solution from absorbing too much heat. Also, combining two large containers together increased the surface area of the water's head ensuring adequate pressure that remains relatively constant over a day or two without adjustment. Each of the six 120 L containers had some combination of N & P relative to the background concentrations of nitrogen 0.05 µm/l (SSW) and phosphorous 0.02 µm/l (SSW). The amount of each nutrient is either 1x, 100x, 500x, or 1000x the background concentration depending on the trial (see Figure 1). The entire contents of the source containers were emptied in about 1-2 days based upon the flow rate. However, the level was never allowed to go below 25 L. Every 3rd day the level inside the container was replenished with the proper amount of sea water and nutrients to keep the experiment running continuously for the three weeks. At the end of each week for a given trial

the source containers were emptied and cleaned with pressurized water and RODI water. They were then refilled and spiked with the same nutrients for that trial.

2. *Tile Tubs*, (18) 28 L each, 22x18x6 inches (Figure 7)

These each held six tiles within one of the larger temperature bath. They were held above the top of the water line in the temperature baths by concrete blocks. This ensured that the temperature of the water from the large baths was transferred to the water inside the tile tubs without risking contamination of the tile tubs from the temperature bath water. Nutrients from the source containers drip into these tubs and over the tiles. The tubs are designed with a barrier that creates a small area for the nutrients to drip into. Here the nutrients mix with the water in the tub and then flow under a ½ inch opening at the bottom of the barrier. The solution flows through the tub and over the six tiles, exiting the tub via two small holes on the opposite end. The 18 tubs allowed for three replicates of each nutrient concentration being gravity fed from the source containers.

3. *Air Stones*, (18) (Figure 8)

Each tile tub had an air stone placed next to the barrier on the tile side of the tub. This ensured that enough turbulence was created to mix the nutrients with the water in the tub. Also they help slightly with the amount of dissolved oxygen in the tile tubs. The air stones were fed from a central blower mechanism via clear tubing and pvc pipes.

4. *Un-glazed ceramic tiles*, (108) 6x6 inch (Figure 8)

Each trial utilizes 108 un-glazed ceramic tiles. These are the synthetic substrates for algae to grow upon. A 6x6 inch size was chosen based upon the success of numerous other experiments attempting to grow algae in streams, ponds, or artificial waters (Larned & Santos 2000). Each tile tub contained six tiles. Once a week, one tile was removed from each tub for

analysis, except for the third week were three tiles were removed, one for CHL-a, one for Dry Wight analysis, and one for macroscopic photography.

5. *Temperature baths,* (3) 4248 L each, 15x6x3 feet (Figures 2& 3)

All of the tile tubs were contained within one of three large temperature baths. These kept the temperature of the tile tubs at between 78-82 degrees F (recorded every other day) depending on the time of day and weather. They were each fed with surface sea water at 18 L/minute on one end and emptied via a standing drain pipe at the other end. The drain pipe also controled the water level so that it did not rise above the top of the tile tubs resting on concrete blocks within the large baths.

6. *Adjustable irrigation drips,* (18) rated between 0-10 GPH (Figure 9)

Each of these drips controlled the flow rate of the nutrient/sea water solution being fed from the 120 L source containers. They were replaced at the end of each trial. The flow rate was kept between 4 -8 ml/minute. This was checked daily and adjusted as the pressure changed because of the lowering of solution in the source containers.

Nutrient Concentrations:

The concentration of nutrients in each of the six 120 L nutrient source containers was obtained by adding nutrients from stock solutions. Six stock solutions were created (3 for nitrogen & 3 for phosphorous at 100x, 500x, & 1000x the background concentrations) (see Table 1 & 2.). Stock solutions were created by adding the correct amount of $NaNO_3$ or NaH_2PO_4 to 1 Liter bottles with RODI water to obtain the proper orders of magnitude (100, 500, and 1000) above the NELHA background for NO_3 and PO_4. These 1 Liter solutions were mixed so that adding 10ml of any of them to 120 L of sea water would yield the proper order of magnitude

desired in the 120 L nutrient source container. $NaNO_3$ and NaH_2PO_4 were chosen because they are highly dissolvable in water.

Analysis Procedures:

Nutrient Analysis of tile tubs:

Each week, utilizing the *Standard Methods for the Examination of Water and Wastewater 20th Edition* and *EPA test methods for analytical procedures*, the concentration of nitrogen and phosphorous were examined in each of the 18 tile tubs and six source containers to ensure the correct amount of nutrients was flowing though the tile tubs. This was achieved by collecting water samples from the center of each tub as well as from the source containers with a 3ml borosilicate cuvette and analyzing their content using NELHA's Astoria 2 micro-segmented flow nutrient analyzer. Nutrients were sampled at the end of the first week (incubation), after the first nutrients were introduced and mixed (Day 7), and at the end of week two and three. Results were used to verify that the correct amount of nutrients were flowing from the 120 L source containers and later correlated with chl-a results.

HPLC:

To determine the floral composition on the tiles, pigment concentrations were analyzed using high performance liquid chromatography (HPLC). This was intended to obtain an indication of the major taxonomic groups found on the tiles. To obtain these samples, a portion of the acetone extraction used during CHL-a analysis was collected in 2ml amber autosampler vials for use in the Shimadzu HPLC auto-sampler following the standardized method for HPLC identification (Wright et al. 1991).

Macroscopic Photographic Analysis:

Species identification/composition was attempted through the utilization of a high power Leica macroscope at NELHA and the University of Hawaii Hilo's marine science laboratory. At the end of each trial, all 18 tiles were photographed 5 times in different locations on each of the tiles.

Dry Weight:

As a further indicator of biomass, the dry weight of algal mass on the tiles was calculated. This was achieved by scraping one tile from each tile bin every week. Tiles were placed in a custom built holder mechanism that allowed for consistent scraping from tile to tile (Figure 10). Scraped biomass was added to 400ml of RODI water. Under vacuum pressure this solution was passed through a pre-dried and pre-weighed glass fiber filter following Standards Method 2540 D (APHA 1992). Filters were then heated at a constant (103 C-105 C) temperature for 1 hour to obtain a constant weight. After cooling in a desiccators the filters were weighed. Subtracting the pretreated clean filters from the final weight after treatment yielded the dry weight of algal biomass. Tests to verify that constant weights were achieved during the heating and desiccating procedures were done by measuring the weight of blank filters at set intervals after each procedure (oven-after 1hr, 1hr 10min., 1hr 20min., 1hr 30min., desiccator-after 5min., 10min., 15min., 20min., 25min., 30min.).

Procedures to Determine Biomass CHL-a:

Each tile removed from the tubs was analyzed to assess the amount of epiphytic algal biomass growing on the tile. Tiles were removed and placed in individual Whirl-Pak Bags with a 60ml buffered 90% Acetone/ 10% $MgCO_3$ solution using a syringe. This solution was then thoroughly mixed within the Whirlpac bags to ensure adequate removal and even application of acetone to the entire surface of the tile. This was accomplished by rotating the Whirlpac bags in a specially designed rotating device for 10 minutes (see figure 4 & 5). To obtain a homogenous

solution the Whirlpac bags were emptied into 200ml Polyethylene bottles and shaken. 150ml of this solution was removed and centrifuged for 2 minutes. Finally, 4ml was removed for Fluorometric analysis following Standards Method 10200 H (APHA 1998). Results were used to quantify algal biomass and analyzed using SAS PROC mixed statistics.

Tile Preparation:

Each tile was cleaned using 2200 psi of water pressure and rinsed in a mixture of RODI water and diluted Clorox bleach (OIE 2009). Un-glazed ceramic tiles have been shown to be an effective substrate for growing algae (Larned & Santos 2000).

Cleaning Procedures:

To ensure that no nutrients were left on the tiles, 120 L nutrients source tubs, tile tubs, air stones, or irrigation tubes between experiments the following cleaning procedures were followed. All surfaces on 120 L source tubs, tile tubs, air stones, and tiles were subjected to 2200 psi of water pressure. Following this treatment all surfaces were rinsed with a solution of RODI water and 250 ml of diluted bleach (OIE 2009). All adjustable drip heads controlling the flow of nutrients in to the tile tubs were replaced between experiments.

Conditions for growth:

Growth parameters (light, temperature, salinity, photoperiod, pH) for the experiment were set to ideally match the natural conditions in the ocean off Keahole Point as closely as possible. Every attempt was made to re-create the growth parameters in the ocean off the NELHA site staying within the ideal growth parameters for algae (Shiroyama et al. 1971).

1. Light

Appropriate shade cloth was chosen to mimic the Photosynthetic Photon Flux Density (PPFD) measurements of the reef environment off Keahole Point. Light measurements by the

Hawaii Institute of Marine Biology (HIMB) on the Point Reef off Coconut Island in Kaneohe Bay, Oahu, were used as an indicator of proper light intensity at varying depths (Jokiel, 1997). Illumination (Lux) measurements were recorded in the tile tubs (under the shade cloth) and converted to PPFD to verify the desired light intensity and choose appropriate shade cloth that matched as closely as possible the natural light conditions on the reef (Tables 4 & 5) using a Lutron Electronics Lx-101 Lux meter.

2. Temperature

The temperature of the tile tubs was held relatively constant by the flow of seawater through the larger temperature baths that contain the tile tubs (Figure 2). The temperature consistently fluctuated between 24-28.5°C depending on weather conditions and time of day. The retention rate for the 4248 liter temperature baths was 240 minutes (Table 1).

3. Photo period

The photo period was controlled by the regular day/night cycles and weather patterns experienced along the Kailua-Kona coast as the experiment was set up outside.

4. pH

pH was the same as the pH of the surface sea water SSW(~8.3).

5. Salinity:

Salinity was the same as the salinity of the surface seawater off NELHA(~34.7 parts per thousand).

Statistics

The experiment was analyzed using SAS following procedures outlined in Schabenberger and Pierce (2002) for repeated measures in linear mixed models. Nutrient levels were the fixed

effect and periods were a random effect. The three reservoirs were considered a main plot while each tile tub was considered a replicate.

Experimental Design Implementation/Testing/ & Construction:

The implementation of this experiment required a significant amount of planning, engineering of systems, and testing to ensure the experiment functioned properly. This occurred over many months prior to the start of the experiment. For the experiment to function at the NELHA site an initial retrofitting required specialized plumbing, electrical, and drainage additions to be added. This was achieved through the generous help of the technical staff at NELHA working with the PI.

The original design of the system was very similar to Figure 2 except that it utilized a ISMATIC Low Flow High-Accuracy Pump to deliver the nutrients from the source containers to the tile tubs. Testing of this expensive pump showed that not only was it unreliable mechanically, but the specialized teflon micro-tubing that the pump used to control the nutrient flow was not designed to be run continuously. After a day or so the tubing would stretch causing the flow rates to change considerable. This prompted the re-design of a gravity feed system. In addition, the original design, as still indicated in Figure 2, had an independent flow of seawater coming from stand pipes off the main seawater supply lines. The idea was to have a continuous flow through system from the stand pipes, while adding the nutrients from separate, smaller supply lines. Due to the corrosively of salt water the copper flow regulation valves in the seawater supply lines could not consistently control the addition of seawater within the required parameters of the experiment. That is why the gravity flow through system was developed, which integrated the nutrients and seawater into one larger supply line, allowing for precise flow control. The trade off was a hybrid batch system as opposed to a continuous flow though system.

The air flow system was tested and underwent a re-designs to meet the requirements of the experiment. Originally, the compressed air system at NELHA was planned as the air delivery mechanism. However, inconsistent flow and possible mechanical failure made that option less attractive as tests were preformed. An independent blower system was created utilizing a commercial blower and specially built piping to split the flow equally in three separate directions, followed each by another split in six directions.

Within the large temperature baths tests were performed to ensure an optimum flow rate and temperature was achieved for the experiment. The tile tubs were also tested with dye to ensure the design of the mixing chamber and location of airstones adequately met the reuirments of the experiment. In addition, evaporation rates were measured and calculated into the flow rate tables.

Numerous other design challenges emerged as the trials progressed. The custom built scraping mechanism for dry weights analysis was developed to ensure precise scraping from one tile to the next (Figure 10). The limitation of money for acetone was resolved with the custom built holder mechanism for the acetone rinse (Figure 4). The tile holder maximized the surface area in contact with acetone and minimized the amount of acetone needed to extract CHL-a from the tiles. In addition, the custom built manual tile mixer used to rotate the tile holder equally was the second step in obtaining a low cost, low resource treatment of acetone immersion (Figure 5).

In addition, the biological sampling techniques required a significant amount of testing to ensure precise, repeatable measurements of CHL-a, Dry Weights, and nutrient collections before the final methodology was achieved. This involved many smaller checks such as testing filters to ensure a consistent weight was achieved after pre-heating and desiccation, verifying centrifuge time limits to ensure stable readings in the fluorometer, identifying the smallest amount of

acetone needed based on time and rotations in the holder mechanism, testing the nutrient flow delivery system and creating a precise methodologies that ensured the tiny amounts of nutrients in ppb were consistently delivered over weeks, etc. A significant part of this experiment simply came down to creating a high degree of design precision and reliable engineering that was dictated by the means available.

RESULTS

Nutrient Analysis:

Nutrient analysis was successfully completed for all three trials. The nutrient concentrations recorded in the source containers were relatively close to the desired/predicted concentrations of 1(x), 100(x), 500(x), and 1000(x)(x being the background concentration of nitrogen 0.05 µm/l or .7 ppb (SSW) and phosphorous 0.02 µm/l or .62 ppb (SSW)). As the margin for error in delivering the nutrients properly was great considering that the background concentration amounts were measured in ~1 part per billion (ppb), it was not expected to record exact amounts of 1, 100, 500, or 1000 times the background. However, what was expected and achieved as evidence in the results was the delivery of a constant order of magnitude of nutrients close to the desired order of magnitude (Table 7, 8, 9). For example, rather than delivering a consistent 100(x) over the three week period it was considered a success if 75(x), 130(x), etc. was consistently delivered. This was considered close enough to the desired orders of magnitude given the very low amount of nutrients that the experimental design needed to consistently deliver. Throughout the three trials for all nutrient concentrations this trend was consistent and achieved the necessary requirements for the experiment to run successfully.

The nutrient concentrations recorded in the tile bins were consistent after the first week but did show a decreasing trend as the weeks progressed. Although this is not an integral part of the

experiment it is most likely a result of the algae in those bins utilizing the nutrient supply for growth.

Statistical Analysis

Data were analyzed as a repeated measures ANOVA across periods with SAS PROC Mixed procedures (SAS Institute 2003).Within period analysis were performed with PROC GLM procedures (SAS Institute 2003). Comparisons of individual treatment combinations and pooled data were performed by the associated pdiff contrasts of the SAS PROC Mixed and PROC GLM procedures. PROC Mixed for repeated measures were chosen because period effects are not properly modeled by GLM across periods (Littell et al. 1998, Piepho et al. 2003). As periods were correlated, assuming there are 3 or more periods, PROC Mixed account for any collinearity (Littell et al. 1998, Piepho et al. 2003). PROC GLM were chosen for in period effects with the assumption of a completely randomized design and no replication in time (Littell et al. 1998, Piepho et al. 2003).

Before analyzing the CHL-a and Dry Weight results a single factor repeated measures analysis of the controls throughout the three trials was performed to ascertain whether or not the data from the CHL-a and Dry Weights respectively could be pooled from all three trials. The results indicated that there was no significant difference between the CHL-a trial controls ($F_{2,4}$ = 1.8, p =.28) or for trial*period ($F_{4,8}$ = 1.73, p =.24) (Appendix A). Therefore there are no effects with time and the CHL-a data was pooled. For Dry Weight there appeared to be a slight trial effect ($F_{2,6}$ = 4.36, p =.068), however it was only somewhat significant (Appendix B). Trial 1 did differ from trial 2 (p = .05) and from trial 3 (p= .04) but trial 2 and 3 showed no significant difference (p= .84) (Appendix B). Considering the degree of error in measuring Dry weight at such small amounts and so close to the method detection limits (Figure 12) the biological

significance is questionable. Given the small degree of statistical significance and the questionable biological significance the data for dry weight was pooled for analyses over the three trials.

CHL-a

SAS PROC Mixed procedures were used to analyze the CHL-a data. Results indicate that the strongest effect overall was n (nitrogen) ($F_{3,6}$ = 72.19, p =.0001), however there also was an overall p (phosphorous) effect ($F_{3,6}$ = 7.72, p =.02) (Appendix C). There was a significant overall n*p effect ($F_{9,16}$ = 2.56, p =.048) and a very strong period effect ($F_{2,4}$ = 87.27, p =.0005) and n*period effect ($F_{6,12}$ = 19.07, p =.0001) (Appendix C). Due to the strong interaction across periods (period 1 differed significantly from period 2 & 3[p =.004, p =.003.], however 2 & 3 did not differ (p =.55)), PROC GLM procedures were used to analyze the data by period. This allowed the testing of all possible treatment (n*p) interactions by period.

Period 1 showed no overall significance for n ($F_{3,38}$ = .52, p =.67 , p ($F_{3,38}$ = 1.3, p =.29), or n*p ($F_{9,38}$ = 1.91, p =.08) (Appendix D). This is consistent with the experimental design as the first period or week of each trial was an incubation period with no nutrients added. Period 2 showed an overall significant n effect ($F_{3,38}$ = 101.51, p =.0001) , p effect ($F_{3,38}$ = 5.37, p =.004), and n*p effect ($F_{9,38}$ = 4.44, p =.0005) (Appendix G). Period 3 also showed an overall significant n effect ($F_{3,38}$ = 27.29, p =.0001), however the overall p effect ($F_{3,38}$ = 2.63, p =.06) was only marginally significant, and the overall n*p effect ($F_{9,38}$ = 1.41, p =.22) was not significant (Appendix J).

Looking closer at Period 1 there are no clear significant differences between individual treatments n, p, n*p, or within n or p independent of each other (Appendices D, E, & F). This is, once again, consistent with the experimental design as nutrients were not added until the end of

the first period. Figure 16 outlines in detail all combinations of n*p that are significantly different for Period 1. Color bars indicate nutrient combinations (n/p) with no difference between other nutrient combinations with similar color bars (Figure 16).

A closer look at Period 2 does show some clear patterns. Between any treatments of 1n or 100n and 500n or 1000n there is a significant difference (Appendix G) (Figure 17). Within 1n and 100n, p plays no significant role (Appendix G) (Figure 17). Phosphorous does create an effect at 500n. Here, 1 p is significantly different than 100, 500, or 1000 p, however there is no difference between 100, 500, and 1000 p (Appendix G) (Figure 17). The presence of p makes a difference but p is not limiting. At 1000n, 1 p is also significantly different than 100, 500, and 1000 p (Appendix G) (Figure 17). Within these three higher p values there are also some significant differences however no clear pattern emerges. Finally, there are some differences between 500n and 1000n, regardless of p but once again no clear pattern emerges (Appendix G) (Figure 17). Looking at just n or p independently also shows some differences (Appendices H and I). 1n or 100n differed significantly from all other levels of n (Appendix H). 500n and 1000n did not significantly differ from each other but both had an effect from 1n and 100n (Appendix H). 1p is significantly different then 100p, 500p, or 1000p but there are no effects between 100p, 500p, and 1000p (Appendix I). The most obvious pattern in Period 2 is that as nitrogen levels increase there is a significant effect regardless of p, however when n is held constant there is no clear effect of p except at higher levels of n where p creates some effects but no clear pattern emerges (Appendix G) (Figure 17).

For Period 3, as in period 2, between any treatments of 1n or 100n and 500n or 1000n there is a significant difference, except for one discrepancy at 500n/1p where there is no effect for any levels of 1n or 100n (Appendix J) (Figure 18). Within 1n and 100n, p plays no significant

role (Appendix J) (Figure 18). Between 500n and 1000n there are significant effects but no clear pattern emerges in relation to p except to say p can create an effect when n is at 1000 and p is above 1p (Appendix J) (Figure 18). At 500n there are no effects with p except between 1p and 1000 p (Appendix J) (Figure 18). At 1000n there appear to be some differences between p levels but only between high and low levels not among adjacent levels (Appendix J) (Figure 18). Once again, looking at just n or p independently shows some similar differences as in Period 2 (Appendices K and L). Although there is no difference between 1n and 100n, both are significantly different then 500n or 1000n (Appendix K). 500n also shows a significant effect between 1000n (Appendix K). 1p is significantly different then 100p, 500p, or 1000p but there are no effects between 100p, 500p, and 1000p (Appendix L).

Dry Weight

SAS PROC GLM procedures were used to analyze the Dry Weight data. PROC GLM allow for the comparison of all possible contrasts between n and p (Littell et al. 1998, Piepho et al. 2003). Only Period 3 Dry Weights were recorded as Period 1 and Period 2 results for all trial were below the method detection limits (MDL) (Figure 13).

Results indicate that the only significant effect was n ($F_{3,38}$ = 2.77, p =.05) (Appendix M). There was no effect of p ($F_{3,38}$ = .73, p =.54) or n*p ($F_{9,38}$ = 1.09, p =.39) (Appendix M). Looking at n independent of other values shows that 1000n is significantly different than all other levels of n but no difference were found between any other levels (Appendix N).

Macroscopic Photographic Analysis:

No species identification could be made from the over 800 photographs taken of individual slides. Analysis of the photograph with an expert on algae identification could only support the conclusion that diatoms, cyanobacteria, filamentous algae, crustose coralline algae,

and calcified algae were present on the tiles in small quantities. Species identification at this level was not possible. The processing and analysis of the tiles to obtain species identification and abundance was outside the scope of this experiment.

HPLC:

HPLC analysis also yielded no usable results. Technical difficulties plagued the only available HPLC machine. Multiple attempts were made to run samples through the auto-sampler however each run ended with the machine loosing pressure in the columns as well as other mechanical breakdowns. No standards for the machine could be obtained which, even if the HPLC had functioned, would have limited the usefulness of the results.

DISCUSSION

Although many studies have showed varying degrees of bottom up and top down pressures regarding the predilection of phase shifts from coral to algal dominance on Hawaiian coral reefs, few studies have focused on the local environment just off Keahole point (Smith et al. 2001, 2002, 2004; Stimson et al. 2001; Thacker et al. 2001; Szmant and Forrester 1996; Lapointe 1997; Miller et al. 1999; Lerman and Biber 2000; Littler and Littler 1984; Littler et al. 1991; Hughes 1994; Hughes and Connell 1999; Aronson and Precht 2000; Williams and Polunin 2001; Hatcher and Larkum 1983; Bell 1992; McCook 1999, 2001). Results from this study indicate that the algae found in the SSW off Keahole point are nitrogen limited (Appendices C-N). This is a similar characteristic of many marine environments (Ryther and Dunstan 1971). However, local conditions can often vary substantially between nitrogen and phosphorous limitations based on local species, conditions, and ecological processes (Smith 1984). Furthermore, many studies have shown that both N and P can play equal roles (Elser et al. 2007). Identifying this basic dynamic of nitrogen limitation off Keahole point is the first step to making

informed management desicions. Further research also taking into account the effects of herbivore would significantly aid the results from this study.

The CHL-a results display a clear trend of nitrogen limitation for all periods tested (Appendices A-L). There also existed a temporal effect where phosphorous, if it had an effect, lessened as time progressed, whereas nitrogen seemed to create more of an effect over time (Appendices C-L). The interaction with N and P also followed the trend described for P, where an effect was recorded earlier on but ceased to occur over time (Appendix H). The only clear trend for P was that it created an effect if present at a level above the baseline, but the amount over the baseline made no difference (Appendices C-L). This indicates that the algae in the SSW off Keahole Point are not limited by the presence of Phosphorous. Although clearly some phosphorous in the beginning equates to some growth, the input of more phosphorous does not equate to continuous growth and over time the former pattern breaks down, further indicating that more phosphorous does not equal more algal growth. These trends were supported by the Dry Weight measurements which indicate that growth is nitrogen limited (Appendices M and N). Phosphorous showed no effect on Dry Weight. This corresponds to the CHL-a results that recorded phosphorous ceasing to have an effect as time progressed. The Dry Weights were sampled only in the third week of the experiments when the effects of phosphorous were no longer occurring, whereas the CHL-a data were sampled every week during the experiment. The results not only display a clear nitrogen limitation but indicate that orders of magnitude above those tested might produce an even stronger correlation between nitrogen input and algal growth. In both the CHL-a and Dry Weight results the effect of nitrogen became greater at the highest levels, 1000x, and increased over time (Appendices C-N) (Figure 15). Thus testing at higher levels of N and possibly over longer periods could be advantageous. Preliminary tests

were originally conducted over a five week period, but they indicated that growth levels spiked in the third week and dropped in the following weeks, therefore setting the experimental time table at three weeks (Figure 14).

These results also narrow the focus for the identification of both eutrophication indicator species and possible invasives. Further studies determining algal species that proliferate in the presence of nitrogen, particularly nitrate, off Keahole Point would give managers a tool in predicting eutrophication events and save time in reacting to them. Furthermore, this information should aid in the management of invasive species control by identifying those species with nitrogen preference. Particularly at sites where SGD occurs as these are the most likely inputs of N into coastal waters and may act as an early indicator for events (Street et al. 2008).
As Sodium Nitrate ($NaNO_3$), the makeup of the nitrogen additive, is a common chemical found in fertilizers managers can also pay particular attention to the upslope land use and management above Keahole Point. GIS mapping identified significant agricultural land areas upslope of NELHA (Figure 19). Data from this experiment suggests that phosphorous based fertilizers would be less harmful to the local marine community. In addition, any future protocols that intend to manage the dumping of effluence by NELHA tenants could focus more on nitrogen waste streams.

Although the effects of nitrogen were present at all levels, it was the highest (500x and 1000x) that created the most significant growth (Appendices C-N) (Figure 15, 17, & 18). This is perhaps the most useful information from this experiment. Managers should be concerned with any indication of nitrogen levels recorded in the waters off Keahole Point at orders of magnitude 500 times the background or greater. It is at this point where significant algal growth occurs (Appendices C-N). Further experiments at magnitudes above 1000x would provide useful data

that could complement the data from this experiment. Once again, taking into account the effects of herbivore could be significant.

Identification of algal species and interpretation of community dynamics was attempted but not achievable due to technical malfunctions and the overall confines of this study. Future identification of species could significantly aid managers in identifying problematic species.

In conclusion, the effects of nutrient enrichment on algal growth in the waters off Keahole Point indicate that algal growth is greatest at magnitudes 500 and 1000 times the background concentration of nitrogen—specifically nitrate (Appendices A-N). Phosphorous at any order of magnitude tested (100, 500, & 1000) does have a small effect on growth initially but over time does not increase the growth of algae significantly (Appendices A-N). Future research into the effects of herbivore is necessary to place this data into the proper context for managers. In addition, species identification would compliment this but based upon the scale and expertise involved in identifying algae, this would most likely constitute a separate study. A comprehensive study of water quality and the effects of nutrient enrichment in the waters off Keahole Point must take into account not only the biological data, such as that collected from this experiment, but also the physical characteristics and unique features of the coastal terrain, such as its geology and hydrology. Other significant factors include seawater/freshwater mixing dynamics, SGD locations, and temporal fluctuations of environmental conditions. In addition, the coastal terrain and upslope land use must be studied in depth to properly identify the sources of nutrient inputs. Finally, data collection methodologies, interpretation, and comparison must be done precisely to avoid misrepresented assumptions.

Table 1. Nutrient delivery flow computations from gravity feed containers to tile baths. Volumes, flow rates, retention times, and nutrient concentrations were calculated to ensure the correct order of magnitudes based upon NELHA baseline NO_3 and PO_4.

Container / Fluid	Retention Time (min)	Estimated volume (liters)	Flow Rate (liters/min)
Water bath / sea water	240	4248	17.7
Tile containers (tub) / sea water	240	30	

Nutrient Source Containers	Value	Units
Volume	120	liters
Flow rate Nutrients/ SSW	4-8	ml/min
Flow rate Nutrients/ SSW	.004-.008	liters/min

Molecular Weights		
1 mole of $NaNO_3$	85	grams
1 micromole of $NaNO_3$	85	micrograms
1 micromole of $NaNO_3$	14	micrograms N
1 micromole of NO_3	14	micrograms N
1 mole of NaH_2PO_4	120	grams
1 micromole of NaH_2PO_4	120	micrograms
1 micromole of NaH_2PO_4	31	micrograms P
1 micromole PO_4	31	micrograms P

Background concentration		
NO_3 - N	0.05	micromole / liter
NO_3 - N	0.7	micrograms N / liter
PO_4 - P	0.02	micromole / liter
PO_4 - P	0.62	micrograms P / liter

Table 2. Nutrient concentration computation tables describing the correct amount of stock nutrient solution needed to achieve a desired order of magnitude of nutrients based on NELHA baseline NO_3 and PO_4.

Final Concentration (Nitrogen Computations)			Nutrient Addition (Nitrogen Computations)					
(N x background)	micromole/ liter	µg/liter	Amount of N to add to each 120 liter reservoir (µg)	Amount of $NaNO_3$ to add to each 120 liter reservoir (ug)	Amount of $NaNO_3$ 1 liter of 100X reservoir standard (mg)	Number of ml of 1000X standard to add to reservoir	Amount of N to add - Shift decimal place for Column F one space (mg)	Number of ml of 100X standard to add to reservoir
1	0.05	0.7	0.000	0	0	1	0	10
50	2.50	35.0	4116	24990	24990	1	2499	10
100	5.00	70.0	8316	50490	50490	1	5049	10
500	25.00	350.0	41916	254490	254490	1	25449	10
1000	50.00	700.0	83916	509490	509490	1	50949	10

Final Concentration (Phosphorus Computations)			Nutrient Addition (Phosphorus Computations)					
(P x background)	micromole/ liter	µg/liter	Amount of P to add to each 120 liter reservoir (µg)	Amount of NaH_2PO_4 to add to each 120 liter reservoir (ug)	Amount of NaH_2PO_4 1 liter of 100X reservoir standard (mg)	Number of ml of 1000X standard to add to reservoir	Amount of P to add - Shift decimal place for Column F one space (mg)	Number of ml of 100X standard to add to reservoir
1	0.02	0.6	0.00	0	0	1	0	10
50	1.00	31.0	3645.60	14112	14112	1	1411.2	10
100	2.00	62.0	7365.60	28512	28512	1	2851.2	10
500	10.00	310.0	37125.60	143712	143712	1	14371.2	10
1000	20.00	620.0	74325.60	287712	287712	1	28771.2	10

Table 3. Pre and post 2004 HDOH Criteria for the Kona Coast Area as geometric mean µg/L value.

Parameter	1989-2004 criterion	2004 – present criterion
Ammonia Nitrogen (µg NH_3-N/L)	**Wet 3.5 Dry 2.5**	**2.5**
Chlorophyll-a (µg/L)	**Wet 0.3 Dry 0.15**	**0.3**
Turbidity (N.T.U.)	**Wet 0.5 Dry 0.2**	**0.1**
Total Dissolved Nitrogen (µg N/L)	**Wet 150 Dry 110**	**100**
Nitrate and Nitrite Nitrogen (µg NO_3+NO_2-N/L)	**Wet 5 Dry 3.5**	**4.5**
Total Dissolved Phosphorous (µg P/L)	**Wet 20 Dry 16**	**12.5**
Ortho-Phosphate Phosphorus (µg PO_4-P/L)	**n/a**	**5.0**

Table 4. Photosynthetic Photon Flux Density (PPFD) light measurements by the Hawaii Institute of Marine Biology (HIMB) on the Point Reef off Coconut Island in Kaneohe Bay converted to Illuminance (Lux) used as an indicator of light measurements on a similar reef environment.

Water Depth	**.15m**	**1.6m**	**3m**	**4.6m**	**6.1m**	**7.6m**
PPFD Hawaii (umol m-2s-1)	1686	223	175	154	93	86
Lux (lux)	100,357	13,273	10,416	9166	5535	5119

Table 5. Lux measurements under experiment shade cloth and outside of shade cloth at Varying times of day and under varying environmental conditions at Keahole Point to verify light intensity on site.

Date	Time	Lux	PPFD	Location	Weather
3-4-09	7:45 AM	8850 lx	148 umol $m^{-2}s^{-1}$	Lab Parking Lot	Overcast
3-4-09	7:52 AM	1741 lx	29 umol $m^{-2}s^{-1}$	Tile Tub under shade cloth	Overcast
3-4-09	10:17 AM	31400 lx	528 umol $m^{-2}s^{-1}$	Lab Parking Lot	High cloud cover
3-4-09	10:25 AM	1053 lx	18 umol $m^{-2}s^{-1}$	Tile Tub under shade cloth	High cloud cover
3-4-09	1:01 PM	28700 lx	482 umol $m^{-2}s^{-1}$	Lab Parking Lot	High cloud cover
3-4-09	1:03 PM	5810 lx	98 umol $m^{-2}s^{-1}$	Tile Tub under shade cloth	High cloud cover
3-4-09	4:15 PM	13660 lx	229 umol $m^{-2}s^{-1}$	Lab Parking Lot	High cloud cover
3-4-09	4:16 PM	2780 lx	47 umol $m^{-2}s^{-1}$	Tile Tub under shade cloth	High cloud cover
3-12-09	8:51 AM	51200 lx	860 umol $m^{-2}s^{-1}$	Lab Parking Lot	Sunny cloudless
3-12-09	8:51 AM	16430 lx	276 umol $m^{-2}s^{-1}$	Tile Tub under shade cloth	Sunny cloudless
3-12-09	12:27 PM	104200 lx	1751 umol $m^{-2}s^{-1}$	Lab Parking Lot	Sunny cloudless
3-12-09	12:27 PM	33500 lx	562 umol $m^{-2}s^{-1}$	Tile Tub under shade cloth	Sunny cloudless
3-12-09	5:15 PM	24400 lx	409 umol $m^{-2}s^{-1}$	Lab Parking Lot	Sunny cloudless
3-12-09	5:15 PM	2810 lx	47 umol $m^{-2}s^{-1}$	Tile Tub under shade cloth	Sunny cloudless

Table 6. Average values of key parameters in NELHA surface seawater.

Parameter	Surface Seawater (SSW)
Temperature	75 - 83ºF (24 - 28.5ºC)
Salinity (º/oo or parts per thousand)	34.7 º/oo
pH	8.3
Alkalinity (milliequivalents/liter)	2.31 mEq/l
NO_3 / NO_2 (micromoles/liter)	0.24 µm/l
PO_4 (micromoles/liter)	0.15 µm/l
Si (micromoles/liter)	2.64 µm/l
NH_4 (micromoles/liter)	0.20 µm/l
Dissolved Organic Nitrogen (micromoles/liter)	5.39 µm/l
Dissolved Oxygen (milligrams/liter)	6.87 mg/l
Total Organic Carbon (milligrams/liter)	0.68 mg/l
Total Suspended Solids (milligrams/liter)	0.88 mg/l

Table 7. Trial 1 NO_3 and PO_4 nutrient data from nutrient source containers and from individual tile tubs used to verify the correct amount of nutrients were being delivered.

Identifier	Group	NO3 ug/l	Background N (ppb)	PO4 ug/l	Background P (ppb)	Actual Conc. times the N	Actual Conc. Times the P	Desired Conc. Times the N	Desired Conc. Times the P	Chl-a	tub	Week	Tub	Chl-a Trial 3	Source
3-0-13 N 1:1	1/1	15.6	0.7	6.8	0.62	22.3	11.0	1	1	5.10	13	0	1	9.18	6
3-0-15 N 1:1	1/1	6.9	0.7	7.2	0.62	9.9	11.6	1	1	1.02	15	0	2	9.69	4
3-0-17 N 1:1	1/1	0.6	0.7	3.4	0.62	0.9	5.5	1	1	9.18	17	0	3	9.43	6
3-0-13 NA 1:1	1/1	5	0.7	2.9	0.62	7.1	4.7	1	1			0	4	10.20	4
3-0-15 NA 1:1	1/1	3.2	0.7	4.3	0.62	4.6	6.9	1	1			0	5	8.41	6
3-0-17 NA 1:1	1/1	0.4	0.7	0.3	0.62	0.6	0.5	1	1			0	6	13.00	4
3-1-13 1:1	1/1	5.9	0.7	0.1	0.62	8.4	0.2	1	1	11.52	13	0	7	19.12	5
3-1-15 1:1	1/1	0.2	0.7	-2.2	0.62	0.3	-3.5	1	1	3.84	15	0	8	18.10	3
3-1-17 1:1	1/1	1.1	0.7	-0.4	0.62	1.6	-0.6	1	1	6.40	17	0	9	14.27	5
3-2-13 1:1	1/1	0.5	0.7	-2.4	0.62	0.7	-3.9	1	1	28.99	13	0	10	11.98	5
3-2-15 1:1	1/1	0.2	0.7	-0.4	0.62	0.3	-0.6	1	1	10.78	15	0	11	12.49	3
3-2-17 1:1	1/1	0.5	0.7	-1.8	0.62	0.7	-2.9	1	1	10.01	17	0	12	8.67	3
3-1-S1 1:1	1/1	0.3	0.7	1.6	0.62	0.4	2.6	1	1			0	13	5.10	1
3-2-S1 1:1	1/1	0.4	0.7	-0.7	0.62	0.6	-1.1	1	1			0	14	2.04	2
3-0-14 N 1:20	1000/1	14	0.7	14	0.62	20.0	22.6	1	1	2.04	14	0	15	1.02	1
3-0-16 N 1:20	1000/1	16	0.7	8	0.62	22.9	12.9	1	1	6.37	16	0	16	6.37	2
3-0-18 N 1:20	1000/1	6	0.7	16	0.62	8.6	25.8	1	1	6.37	18	0	17	9.18	1
3-0-14 NA 1:20	1000/1	970	700	6	0.62	1385.7	9.7	1000	1			0	18	6.37	2
3-0-16 NA 1:20	1000/1	640	700	18	0.62	914.3	29.0	1000	1			1	1	139.28	6
3-0-18 NA 1:20	1000/1	714	700	12	0.62	1020.0	19.4	1000	1			1	2	123.92	4
3-1-14 1:20	1000/1	574	700	6	0.62	820.0	9.7	1000	1	83.72	14	1	3	158.48	6
3-1-16 1:20	1000/1	674	700	2	0.62	962.9	3.2	1000	1	115.21	16	1	4	148.75	4
3-1-18 1:20	1000/1	672	700	6	0.62	960.0	9.7	1000	1	57.09	18	1	5	119.31	6
3-2-14 1:20	1000/1	496	700	14	0.62	708.6	22.6	1000	1	85.95	14	1	6	88.07	4
3-2-16 1:20	1000/1	470	700	10	0.62	671.4	16.1	1000	1	107.25	16	1	7	229.40	5
3-2-18 1:20	1000/1	558	700	12	0.62	797.1	19.4	1000	1	40.28	18	1	8	12.03	3
3-1-S2 1:20	1000/1	546	700	12	0.62	780.0	19.4	1000	1			1	9	175.89	5
3-2-S2 1:20	1000/1	758	700	14	0.62	1082.9	22.6	1000	1			1	10	159.76	5
3-0-2 N 1:20	1000/100	10	0.7	12	0.62	14.3	19.4	1	1	9.69	8	1	11	19.20	3
3-0-4 N 1:20	1000/100	20	0.7	38	0.62	28.6	61.3	1	1	10.20	11	1	12	16.13	3
3-0-6 N 1:20	1000/100	10	0.7	20	0.62	14.3	32.3	1	1	13.00	12	1	13	11.52	1
3-0-2 NA 1:20	1000/100	790	700	74	62	1128.6	119.4	1000	100			1	14	83.72	2
3-0-4 NA 1:20	1000/100	802	700	78	62	1145.7	125.8	1000	100			1	15	3.84	1
3-0-6 NA 1:20	1000/100	694	700	82	62	991.4	132.3	1000	100			1	16	115.21	2
3-1-2 1:20	1000/100	570	700	46	62	814.3	74.2	1000	100	123.92	8	1	17	6.40	1
3-1-4 1:20	1000/100	580	700	52	62	828.6	83.9	1000	100	148.75	11	1	18	57.09	2
3-1-6 1:20	1000/100	432	700	32	62	617.1	51.6	1000	100	88.07	12	2	1	107.76	6
3-2-2 1:20	1000/100	6	700	10	62	8.6	16.1	1000	100	207.06	8	2	2	207.06	4
3-2-4 1:20	1000/100	50	700	12	62	71.4	19.4	1000	100	389.49	11	2	3	265.30	6
3-2-6 1:20	1000/100	8	700	10	62	11.4	16.1	1000	100	106.48	12	2	4	389.49	4
3-1-S4 1:20	1000/100	834	700	86	62	1191.4	138.7	1000	100			2	5	190.38	6
3-2-S4 1:20	1000/100	548	700	52	62	782.9	83.9	1000	100			2	6	106.48	4
3-0-8 N 1:10	1/1000	3	0.7	19	0.62	4.3	30.6	1	1	18.10		2	7	128.55	5
3-0-11 N 1:10	1/1000	2	0.7	10	0.62	2.9	16.1	1	1	12.49		2	8	20.53	3
3-0-12 N 1:10	1/1000	3	0.7	6	0.62	4.3	9.7	1	1	8.67	2	2	9	98.01	5
3-0-8 NA 1:10	1/1000	3	0.7	580	620	4.3	935.5	1	1000		4	2	10	248.88	5
3-0-11 NA 1:10	1/1000	4	0.7	535	620	5.7	862.9	1	1000		6	2	11	49.26	3
3-0-12 NA 1:10	1/1000	12	0.7	578	620	17.1	932.3	1	1000			2	12	15.14	3
3-1-8 1:10	1/1000	2	0.7	625	620	2.9	1008.1	1	1000	12.03	2	2	13	28.99	1
3-1-11 1:10	1/1000	2	0.7	633	620	2.9	1021.0	1	1000	19.20	4	2	14	85.95	2
3-1-12 1:10	1/1000	3	0.7	592	620	4.3	954.8	1	1000	16.13	6	2	15	10.78	1
3-2-8 1:10	1/1000	4	0.7	512	620	5.7	825.8	1	1000	20.53	2	2	16	107.25	2
3-2-11 1:10	1/1000	0	0.7	586	620	0.0	945.2	1	1000	49.26	4	2	17	10.01	1
3-2-12 1:10	1/1000	2	0.7	488	620	2.9	787.1	1	1000	15.14	6	2	18	40.28	2
3-1-S3 1:10	1/1000	2	0.7	412	620	2.9	664.5	1	1000						
3-2-S3 1:10	1/1000	1	0.7	387	620	1.4	624.2	1	1000						
3-0-7 N 1:10	1000/500	3	0.7	14	0.62	4.3	22.6	1	1	19.12	7				
3-0-9 N 1:10	1000/500	3	0.7	9	0.62	4.3	14.5	1	1	14.27	9				
3-0-10 N 1:10	1000/500	3	0.7	12	0.62	4.3	19.4	1	1	11.98	10				
3-0-7 NA 1:10	1000/500	698	700	90	310	997.1	145.2	1000	500						
3-0-9 NA 1:10	1000/500	479	700	239	310	684.3	385.5	1000	500						
3-0-10 NA 1:10	1000/500	773	700	223	310	1104.3	359.7	1000	500						
3-1-7 1:10	1000/500	5	700	114	310	7.1	183.9	1000	500	229.40	7				
3-1-9 1:10	1000/500	3	700	114	310	4.3	183.9	1000	500	175.89	9				
3-1-10 1:10	1000/500	421	700	256	310	601.4	412.9	1000	500	159.76	10				
3-2-7 1:10	1000/500	3	700	177	310	4.3	285.5	1000	500	128.55	7				
3-2-9 1:10	1000/500	4	700	50	310	5.7	80.6	1000	500	98.01	9				
3-2-10 1:10	1000/500	-1	700	211	310	-1.4	340.3	1000	500	248.88	10				
3-1-S5 1:10	1000/500	810	700	367	310	1157.1	591.9	1000	500						
3-2-S5 1:10	1000/500	319	700	239	310	455.7	385.5	1000	500						
3-0-1 N 1:10	1000/1000	6	0.7	13	0.62	8.6	21.0	1	1	9.18	1				
3-0-3 N 1:10	1000/1000	2	0.7	17	0.62	2.9	27.4	1	1	9.43	3				
3-0-5 N 1:10	1000/1000	7	0.7	8	0.62	10.0	12.9	1	1	8.41	5				
3-0-1 NA 1:10	1000/1000	713	700	517	620	1018.6	833.9	1000	1000						
3-0-3 NA 1:10	1000/1000	769	700	594	620	1098.6	958.1	1000	1000						
3-0-5 NA 1:10	1000/1000	846	700	635	620	1208.6	1024.2	1000	1000						
3-1-1 1:10	1000/1000	3	700	493	620	4.3	795.2	1000	1000	139.28	1				
3-1-3 1:10	1000/1000	487	700	568	620	695.7	916.1	1000	1000	158.48	3				
3-1-5 1:10	1000/1000	565	700	588	620	807.1	948.4	1000	1000	119.31	5				
3-2-1 1:10	1000/1000	7	700	399	620	10.0	643.5	1000	1000	107.76	1				
3-2-3 1:10	1000/1000	103	700	470	620	147.1	758.1	1000	1000	265.30	3				
3-2-5 1:10	1000/1000	157	700	472	620	224.3	761.3	1000	1000	190.38	5				
3-1-S6 1:10	1000/1000	552	700	415	620	788.6	669.4	1000	1000						
3-2-S6 1:10	1000/1000	315	700	386	620	450.0	622.6	1000	1000						

LEGEND

Green	=	Week/Period 1 no nutrients added
Grey	=	Week/Period 1 nutrient added
Yellow	=	Week/Period 2 nutrients added
Blue	=	Week/Preiod 3 nutrients added
Identifier Code		(Trial)-(Period)-(Tile Tub)*
		* S indicates source container vs tile tub

Table 8. Trial 2 NO_3 and PO_4 nutrient data from nutrient source containers and from individual tile tubs used to verify the correct amount of nutrients were being delivered.

	Background		Background		Actual Conc. Times the Background	Desired Conc. Times the Background	Actual Conc. Times the Backgroun	Desired Conc. Times the Background	Chl-a		Week	Tub	Chl-a Trial 4	Source
NO3 ug/l	N (ppb)	PO4 ug/l	P (ppb)	Week	N	N	P	P		tub				
0.6	0.7	-3.2	0.62	0 N	0.42	1	-1.984	1	11.37	13	0	1	2.84	6
19	0.7	-0.3	0.62	0 N	13.3	1	-0.186	1	1.03	15	0	2	21.70	4
11.6	0.7	-2.1	0.62	0 N	8.12	1	-1.302	1	1.55	17	0	3	20.15	6
0.9	0.7	3.1	0.62	0 NA	0.63	1	1.922	1			0	4	12.14	4
4.7	0.7	3.5	0.62	0 NA	3.29	1	2.17	1			0	5	8.78	6
7.5	0.7	0	0.62	0 NA	5.25	1	0	1			0	6	5.94	4
0.3	0.7	-0.4	0.62	1	0.21	1	-0.248	1	10.89	13	0	7	4.65	5
0.4	0.7	-1.3	0.62	1	0.28	1	-0.806	1	14.20	15	0	8	23.25	3
0.2	0.7	-2.6	0.62	1	0.14	1	-1.612	1	30.30	17	0	9	5.42	5
0.5	0.7	-2.5	0.62	2	0.35	1	-1.55	1	12.62	13	0	10	2.58	5
0.6	0.7	-2.8	0.62	2	0.42	1	-1.736	1	19.83	15	0	11	4.39	3
0.7	0.7	-2.2	0.62	2	0.49	1	-1.364	1	25.24	17	0	12	3.87	3
0.2	0.7	-1.9	0.62	0 NA	0.14	1	-1.178	1			0	13	11.37	1
0.1	0.7	0.8	0.62	1	0.07	1	0.496	1			0	14	7.23	2
0	0.7	0.7	0.62	2	0	1	0.434	1			0	15	1.03	1
16.6	0.7	-1.4	0.62	0 N	11.62	1	-0.868	1	7.23	14	0	16	8.01	2
17.8	0.7	-1.4	0.62	0 N	12.46	1	-0.868	1	8.01	16	0	17	1.55	1
19.1	0.7	1.7	0.62	0 N	13.37	1	1.054	1	7.75	18	0	18	7.75	2
364.7	350	1.9	0.62	0 NA	255.29	500	1.178	1			1	1	109.84	6
296.9	350	-0.2	0.62	0 NA	207.83	500	-0.124	1			1	2	211.16	4
274.6	350	1.1	0.62	0 NA	192.22	500	0.682	1			1	3	138.49	6
266.8	350	-2.2	0.62	1	186.76	500	-1.364	1	66.28	14	1	4	171.63	4
136	350	-2.4	0.62	1	95.2	500	-1.488	1	67.94	16	1	5	143.93	6
185.4	350	-2.3	0.62	1	129.78	500	-1.426	1	102.50	18	1	6	99.66	4
177.3	350	-2.9	0.62	2	124.11	500	-1.798	1	102.52	14	1	7	144.40	5
32.6	350	-3.3	0.62	2	22.82	500	-2.046	1	5.41	16	1	8	19.41	3
108.2	350	-3	0.62	2	75.74	500	-1.86	1	109.47	18	1	9	145.59	5
334.3	350	-2.4	0.62	0 NA	234.01	500	-1.488	1			1	10	124.05	5
430.4	350	-0.7	0.62	1	301.28	500	-0.434	1			1	11	17.28	3
301.3	350	-2.5	0.62	2	210.91	500	-1.55	1			1	12	15.15	3
0.2	0.7	0	0.62	0 N	0.42	1	0	1	23.25	8	1	13	10.89	1
6	0.7	1.4	0.62	0 N	12.6	1	2.604	1	4.39	11	1	14	66.28	2
4.7	0.7	0.3	0.62	0 N	9.87	1	0.558	1	3.87	12	1	15	14.20	1
0.2	0.7	96.5	310	0 NA	0.42	1	179.49	500			1	16	67.94	2
-0.1	0.7	101	310	0 NA	-0.21	1	187.86	500			1	17	30.30	1
2.6	0.7	84.6	310	0 NA	5.46	1	157.356	500			1	18	102.50	2
0.3	0.7	102.3	310	1	0.63	1	190.278	500	19.41	8	2	1	87.58	6
0.2	0.7	104.3	310	1	0.42	1	193.998	500	17.28	11	2	2	128.02	4
0.2	0.7	97.7	310	1	0.42	1	181.722	500	15.15	12	2	3	152.23	6
0.2	0.7	111.7	310	2	0.42	1	207.762	500	21.64	8	2	4	123.64	4
4.1	0.7	112.9	310	2	8.61	1	209.994	500	18.03	11	2	5	193.19	6
0.4	0.7	101.2	310	2	0.84	1	188.232	500	10.82	12	2	6	79.85	4
0.1	0.7	99.6	310	0 NA	0.21	1	185.256	500			2	7	135.75	5
-0.1	0.7	82.9	310	1	-0.21	1	154.194	500			2	8	21.64	3
0.1	0.7	88.6	310	2	0.21	1	164.796	500			2	9	69.29	5
0.6	0.7	-2.4	0.62	0 N	0.42	1	-1.488	1	21.70	2	2	10	177.73	5
12.8	0.7	-0.4	0.62	0 N	8.96	1	-0.248	1	12.14	4	2	11	18.03	3
5.9	0.7	-1.2	0.62	0 N	4.13	1	-0.744	1	5.94	6	2	12	10.82	3
264.2	350	51.5	62	0 NA	184.94	500	31.93	100			2	13	12.62	1
342.8	350	58.5	62	0 NA	239.96	500	36.27	100			2	14	102.52	2
322.8	350	52.3	62	0 NA	225.96	500	32.426	100			2	15	19.83	1
133.6	350	28.2	62	1	93.52	500	17.484	100	211.16	2	2	16	5.41	2
140.3	350	30.7	62	1	98.21	500	19.034	100	171.63	4	2	17	25.24	1
85.4	350	21.6	62	1	59.78	500	13.392	100	99.66	6	2	18	109.47	2
0.7	350	3.7	62	2	0.49	500	2.294	100	128.02	2				
1.1	350	9	62	2	0.77	500	5.58	100	123.64	4				
1	350	-0.9	62	2	0.7	500	-0.558	100	79.85	6				
347.7	350	52.8	62	0 NA	243.39	500	32.736	100						
369.6	350	59.8	62	1	258.72	500	37.076	100						
227.4	350	34.7	62	2	159.18	500	21.514	100						
4.6	0.7	0.6	0.62	0 N	9.66	1	1.116	1	4.65	7				
1.6	0.7	-0.6	0.62	0 N	3.36	1	-1.116	1	5.42	9				
7.5	0.7	-0.1	0.62	0 N	15.75	1	-0.186	1	2.58	10				
118.6	350	100.8	310	0 NA	249.06	500	187.488	500						
89.8	350	107.3	310	0 NA	188.58	500	199.578	500						
124.1	350	105.2	310	0 NA	260.61	500	195.672	500						
43.8	350	88.3	310	1	91.98	500	164.238	500	144.40	7				
6.4	350	87.1	310	1	13.44	500	162.006	500	145.59	9				
56.2	350	91.7	310	1	118.02	500	170.562	500	124.05	10				
0.8	350	85.8	310	2	1.68	500	159.588	500	135.75	7				
0.2	350	88.8	310	2	0.42	500	165.168	500	69.29	9				
0.5	350	79.1	310	2	1.05	500	147.126	500	177.73	10				
109.7	350	93.3	310	0 NA	230.37	500	173.538	500						
141.7	350	123.2	310	1	297.57	500	229.152	500						
119.4	350	114.1	310	2	250.74	500	212.226	500						
1	0.7	0.9	0.62	0 N	3.5	1	2.79	1	2.84	1				
0.6	0.7	-0.2	0.62	0 N	2.1	1	-0.62	1	20.15	3				
4.7	0.7	0.1	0.62	0 N	16.45	1	0.31	1	8.78	5				
75.8	350	124.6	620	0 NA	265.3	500	386.26	1000						
62.5	350	122.6	620	0 NA	218.75	500	380.06	1000						
84.7	350	126.4	620	0 NA	296.45	500	391.84	1000						
40.3	350	113.9	620	1	141.05	500	353.09	1000	109.84	1				
40.5	350	120.7	620	1	141.75	500	374.17	1000	138.49	3				
36.1	350	118.1	620	1	126.35	500	366.11	1000	143.93	5				
0.4	350	115.1	620	2	1.4	500	356.81	1000	87.58	1				
0.7	350	116	620	2	2.45	500	359.6	1000	152.23	3				
1.2	350	120	620	2	4.2	500	372	1000	193.19	5				
65	350	108.4	620	0 NA	227.5	500	336.04	1000						
59.4	350	119.1	620	1	207.9	500	369.21	1000						
50.7	350	98	620	2	177.45	500	303.8	1000						

LEGEND

Green	= Week/Period 1 no nutrients added
Grey	= Week/Period 1 nutrient added
Yellow	= Week/Period 2 nutrients added
Blue	= Week/Preiod 3 nutrients added
Identifier Code	(Trial)-(Period)-(Tile Tub)*
	* S indicates source container vs tile tub

Table 9. Trial 3 NO_3 and PO_4 nutrient data from nutrient source containers and from individual tile tubs used to verify the correct amount of nutrients were being delivered.

			Background		Background		Actual Conc. Times the Backgrou	Desired Conc. Times the Backgrou	Actual Conc. Times the Backgrou	Desired Conc. Times the Backgrou			Week	Tub	Chl-a Trial 5	Source
Identifier	**Group**	**NO3 ug/l**	**N (ppb)**	**PO4 ug/l**	**P (ppb)**	**Week**	**N**	**N**	**P**	**P**	**Chl-a**	**tub**				
5-0-13 N 1:1	1/1	1.2	0.7	-3	0.62	0 N	0.84	1	-1.86	1	17.97	13	0	1	8.60	6
5-0-15 N 1:1	1/1	1.6	0.7	-2.1	0.62	0 N	1.12	1	-1.302	1	7.82	15	0	2	5.99	4
5-0-17 N 1:1	1/1	2.1	0.7	2.9	0.62	0 N	1.47	1	1.798	1	3.91	17	0	3	15.11	6
5-0-13 NA 1:1	1/1	1.2	0.7	-3	0.62	0 NA	0.84	1	-1.86	1			0	4	8.34	4
5-0-15 NA 1:1	1/1	1.5	0.7	-3	0.62	0 NA	1.05	1	-1.86	1			0	5	15.11	6
5-0-17 NA 1:1	1/1	1.2	0.7	0.7	0.62	0 NA	0.84	1	0.434	1			0	6	5.73	4
5-1-13 1:1	1/1	2.3	0.7	-0.3	0.62	1	1.61	1	-0.186	1	31.21	13	0	7	9.12	5
5-1-15 1:1	1/1	2	0.7	-0.2	0.62	1	1.4	1	-0.124	1	19.18	15	0	8	5.99	3
5-1-17 1:1	1/1	2	0.7	-1.7	0.62	1	1.4	1	-1.054	1	17.14	17	0	9	7.29	5
5-2-13 1:1	1/1	1.5	0.7	-3.2	0.62	2	1.05	1	-1.984	1	15.92	13	0	10	8.34	5
5-2-15 1:1	1/1	1.7	0.7	-2.6	0.62	2	1.19	1	-1.612	1	12.84	15	0	11	5.47	3
5-2-17 1:1	1/1	1.7	0.7	-3	0.62	2	1.19	1	-1.86	1	12.07	17	0	12	5.73	3
5-0-S1 1:1	1/1	3.4	0.7	2.1	0.62	0 NA	2.38	1	1.302	1			0	13	17.97	1
5-1-S1 1:1	1/1	1.9	0.7	1.2	0.62	1	1.33	1	0.744	1			0	14	9.12	2
5-2-S1 1:1	1/1	1.9	0.7	1	0.62	2	1.33	1	0.62	1			0	15	7.82	1
5-0-14 N 1:1	1/1	1.9	0.7	-2	0.62	0 N	1.33	1	-1.24	1	9.12	14	0	16	13.55	2
5-0-16 N 1:1	1/1	1.7	0.7	-3.2	0.62	0 N	1.19	1	-1.984	1	13.55	16	0	17	3.91	1
5-0-18 N 1:1	1/1	1.9	0.7	-2	0.62	0 N	1.33	1	-1.24	1	15.11	18	0	18	15.11	2
5-0-14 NA 1:1	100/1	1.7	70	-3.4	0.62	0 NA	1.19	100	-2.108	1			1	1	45.28	6
5-0-16 NA 1:1	100/1	1.8	70	-3.3	0.62	0 NA	1.26	100	-2.046	1			1	2	18.93	4
5-0-18 NA 1:1	100/1	1.9	70	-3.2	0.62	0 NA	1.33	100	-1.984	1			1	3	15.60	6
5-1-14 1:1	100/1	2	70	-0.6	0.62	1	1.4	100	-0.372	1	40.67	14	1	4	71.62	4
5-1-16 1:1	100/1	2.4	70	0.5	0.62	1	1.68	100	0.31	1	37.60	16	1	5	59.09	6
5-1-18 1:1	100/1	2.1	70	-0.2	0.62	1	1.47	100	-0.124	1	40.67	18	1	6	25.58	4
5-2-14 1:1	100/1	1.6	70	-3.1	0.62	2	1.12	100	-1.922	1	21.83	14	1	7	13.05	5
5-2-16 1:1	100/1	1.4	70	-1.1	0.62	2	0.98	100	-0.682	1	27.47	16	1	8	19.18	3
5-2-18 1:1	100/1	1.6	70	-1.8	0.62	2	1.12	100	-1.116	1	19.77	18	1	9	28.14	5
5-0-S2 1:1	100/1	107.8	70	2.1	0.62	0 NA	75.46	100	1.302	1			1	10	36.07	5
5-1-S2 1:1	100/1	44.4	70	-2.4	0.62	1	31.08	100	-1.488	1			1	11	17.65	3
5-2-S2 1:1	100/1	27.7	70	-2.5	0.62	2	19.39	100	-1.55	1			1	12	25.58	3
5-0-8 N 1:1	1/1	2	0.7	-1.4	0.62	0 N	1.4	1	-0.868	1	5.99	8	1	13	31.21	1
5-0-11 N 1:1	1/1	1.5	0.7	-1	0.62	0 N	1.05	1	-0.62	1	5.47	11	1	14	40.67	2
5-0-12 N 1:1	1/1	1.7	0.7	-1.6	0.62	0 N	1.19	1	-0.992	1	5.73	12	1	15	19.18	1
5-0-8 NA 1:1	1/100	1.6	0.7	72.3	62	0 NA	1.12	1	44.826	100			1	16	37.60	2
5-0-11 NA 1:1	1/100	2	0.7	68.6	62	0 NA	1.4	1	42.532	100			1	17	17.14	1
5-0-12 NA 1:1	1/100	2	0.7	62.7	62	0 NA	1.4	1	38.874	100			1	18	40.67	2
5-1-8 1:1	1/100	2.3	0.7	50.6	62	1	1.61	1	31.372	100	19.18	8	2	1	56.23	6
5-1-11 1:1	1/100	2.3	0.7	48.5	62	1	1.61	1	30.07	100	17.65	11	2	2	24.65	4
5-1-12 1:1	1/100	2	0.7	48.3	62	1	1.4	1	29.946	100	25.58	12	2	3	45.19	6
5-2-8 1:1	1/100	1.6	0.7	51.5	62	2	1.12	1	31.93	100	17.97	8	2	4	40.06	4
5-2-11 1:1	1/100	1.4	0.7	31.5	62	2	0.98	1	19.53	100	13.87	11	2	5	41.60	6
5-2-12 1:1	1/100	1.7	0.7	47.1	62	2	1.19	1	29.202	100	15.41	12	2	6	21.83	4
5-0-S3 1:1	1/100	6.5	0.7	55	62	0 NA	4.55	1	34.1	100			2	7	37.75	5
5-1-S3 1:1	1/100	3.2	0.7	45.6	62	1	2.24	1	28.272	100			2	8	17.97	3
5-2-S3 1:1	1/100	2.1	0.7	26.6	62	2	1.47	1	16.492	100			2	9	23.88	5
5-0-2 N 1:1	1/1	1.8	0.7	-0.7	0.62	0 N	1.26	1	-0.434	1	5.99	2	2	10	26.19	5
5-0-4 N 1:1	1/1	1.8	0.7	-1.4	0.62	0 N	1.26	1	-0.868	1	8.34	4	2	11	13.87	3
5-0-6 N 1:1	1/1	2	0.7	2.2	0.62	0 N	1.4	1	1.364	1	5.73	6	2	12	15.41	3
5-0-2 NA 1:1	100/100	2	70	60.8	62	0 NA	1.4	100	37.696	100			2	13	15.92	1
5-0-4 NA 1:1	100/100	1.7	70	61.6	62	0 NA	1.19	100	38.192	100			2	14	21.83	2
5-0-6 NA 1:1	100/100	4	70	59.2	62	0 NA	2.8	100	36.704	100			2	15	12.84	1
5-1-2 1:1	100/100	2	70	51.5	62	1	1.4	100	31.93	100	18.93	2	2	16	27.47	2
5-1-4 1:1	100/100	2.5	70	37.8	62	1	1.75	100	23.436	100	71.62	4	2	17	12.07	1
5-1-6 1:1	100/100	2.2	70	41.8	62	1	1.54	100	25.916	100	25.58	6	2	18	19.77	2
5-2-2 1:1	100/100	1.4	70	50.8	62	2	0.98	100	31.496	100	24.65	2				
5-2-4 1:1	100/100	2	70	34.8	62	2	1.4	100	21.576	100	40.06	4				
5-2-6 1:1	100/100	1.6	70	44.8	62	2	1.12	100	27.776	100	21.83	6				
5-0-S4 1:1	100/100	38	70	48.5	62	0 NA	26.6	100	30.07	100						
5-1-S4 1:1	100/100	19	70	48.3	62	1	13.3	100	29.946	100						
5-2-S4 1:1	100/100	16	70	40.5	62	2	11.2	100	25.11	100						
5-0-7 N 1:3	1/1	1.8	0.7	0.2	0.62	0 N	3.78	1	0.372	1	9.12	7				
5-0-9 N 1:3	1/1	1.9	0.7	-0.4	0.62	0 N	3.99	1	-0.744	1	7.29	9				
5-0-10 N 1:3	1/1	1.7	0.7	-0.8	0.62	0 N	3.57	1	-1.488	1	8.34	10				
5-0-7 NA 1:3	100/500	1.8	70	121.3	310	0 NA	3.78	100	225.618	500						
5-0-9 NA 1:3	100/500	1.8	70	113.6	310	0 NA	3.78	100	211.296	500						
5-0-10 NA 1:3	100/500	1.7	70	132.2	310	0 NA	3.57	100	245.892	500						
5-1-7 1:3	100/500	2	70	93.8	310	1	4.2	100	174.468	500	13.05	7				
5-1-9 1:3	100/500	1.9	70	94.4	310	1	3.99	100	175.584	500	28.14	9				
5-1-10 1:3	100/500	1.7	70	96.9	310	1	3.57	100	180.234	500	36.07	10				
5-2-7 1:3	100/500	2.2	70	102	310	2	4.62	100	189.72	500	37.75	7				
5-2-9 1:3	100/500	1.9	70	106.1	310	2	3.99	100	197.346	500	23.88	9				
5-2-10 1:3	100/500	2.2	70	110.6	310	2	4.62	100	205.716	500	26.19	10				
5-0-S5 1:3	100/500	22.7	70	84.7	310	0 NA	47.67	100	157.542	500						
5-1-S5 1:3	100/500	22	70	81.6	310	1	46.2	100	151.776	500						
5-2-S5 1:3	100/500	36	70	87.2	310	2	75.6	100	162.192	500						
5-0-1 N 1:10	1/1	1.9	0.7	1.7	0.62	0 N	13.3	1	10.54	1	8.60	1				
5-0-3 N 1:10	1/1	1.8	0.7	0.3	0.62	0 N	12.6	1	1.86	1	15.11	3				
5-0-5 N 1:10	1/1	1.7	0.7	0.1	0.62	0 N	11.9	1	0.62	1	15.11	5				
5-0-1 NA 1:10	100/1000	1.6	70	57.4	620	0 NA	11.2	100	355.88	1000						
5-0-3 NA 1:10	100/1000	2	70	69.1	620	0 NA	14	100	428.42	1000						
5-0-5 NA 1:10	100/1000	1.8	70	66.7	620	0 NA	12.6	100	413.54	1000						
5-1-1 1:10	100/1000	1.7	70	49.5	620	1	11.9	100	306.9	1000	45.28	1				
5-1-3 1:10	100/1000	2	70	54.1	620	1	14	100	335.42	1000	15.60	3				
5-1-5 1:10	100/1000	1.8	70	56.9	620	1	12.6	100	352.78	1000	59.09	5				
5-2-1 1:10	100/1000	1.8	70	51.4	620	2	12.6	100	318.68	1000	56.23	1				
5-2-3 1:10	100/1000	1.6	70	57.9	620	2	11.2	100	358.98	1000	45.19	3				
5-2-5 1:10	100/1000	1.4	70	56.5	620	2	9.8	100	350.3	1000	41.60	5				
5-0-S6 1:10	100/1000	12.3	70	62.3	620	0 NA	86.1	100	386.26	1000						
5-1-S6 1:10	100/1000	6.5	70	61.6	620	1	45.5	100	381.92	1000						
5-2-S6 1:10	100/1000	7.3	70	61.2	620	2	51.1	100	379.44	1000						

LEGEND

Green	=	Week/Period 1 no nutrients added
Grey	=	Week/Period 1 nutrient added
Yellow	=	Week/Period 2 nutrients added
Blue	=	Week/Preiod 3 nutrients added

Identifier Code	(Trial)-(Period)-(Tile Tub)*
	* S indicates source container vs tile tub

Experimental Design
Note: 15 treatments with 3 replicate trays per trial
Control = Offshore water
x = typical nearshore concentration (NO_3 = .05, PO_4 = .02) micromole / liter

Design for 15 trays + controls - simultaneous incubation & testing, 1 month per trial

Trial	Treatment	Nitrogen addition (x)	Phosphorus addition (x)	Comment
1	1	1(x)	1(x)	Control (3 reps)
1	2	1000(x)	1(x)	3 reps
1	3	1(x)	1000(x)	3 reps
1	4	1000(x)	100(x)	3 reps
1	5	1000(x)	500(x)	3 reps
1	6	1000(x)	1000(x)	3 reps
2	1	1(x)	1(x)	Control (3 reps)
2	2	500(x)	1(x)	3 reps
2	3	1(x)	500(x)	3 reps
2	4	500(x)	100(x)	3 reps
2	5	500(x)	500(x)	3 reps
2	6	500(x)	1000(x)	3 reps
3	1	1(x)	1(x)	Control (3 reps)
3	2	100(x)	1(x)	3 reps
3	3	1(x)	100(x)	3 reps
3	4	100(x)	100(x)	3 reps
3	5	100(x)	500(x)	3 reps
3	6	100(x)	1000(x)	3 reps

Figure 1. The concentrations and composition of nutrients for all of the trials that will be randomly assigned to tubs. Nutrient addition amounts represent magnitudes of order greater than the indicated background concentration for a given nutrient.

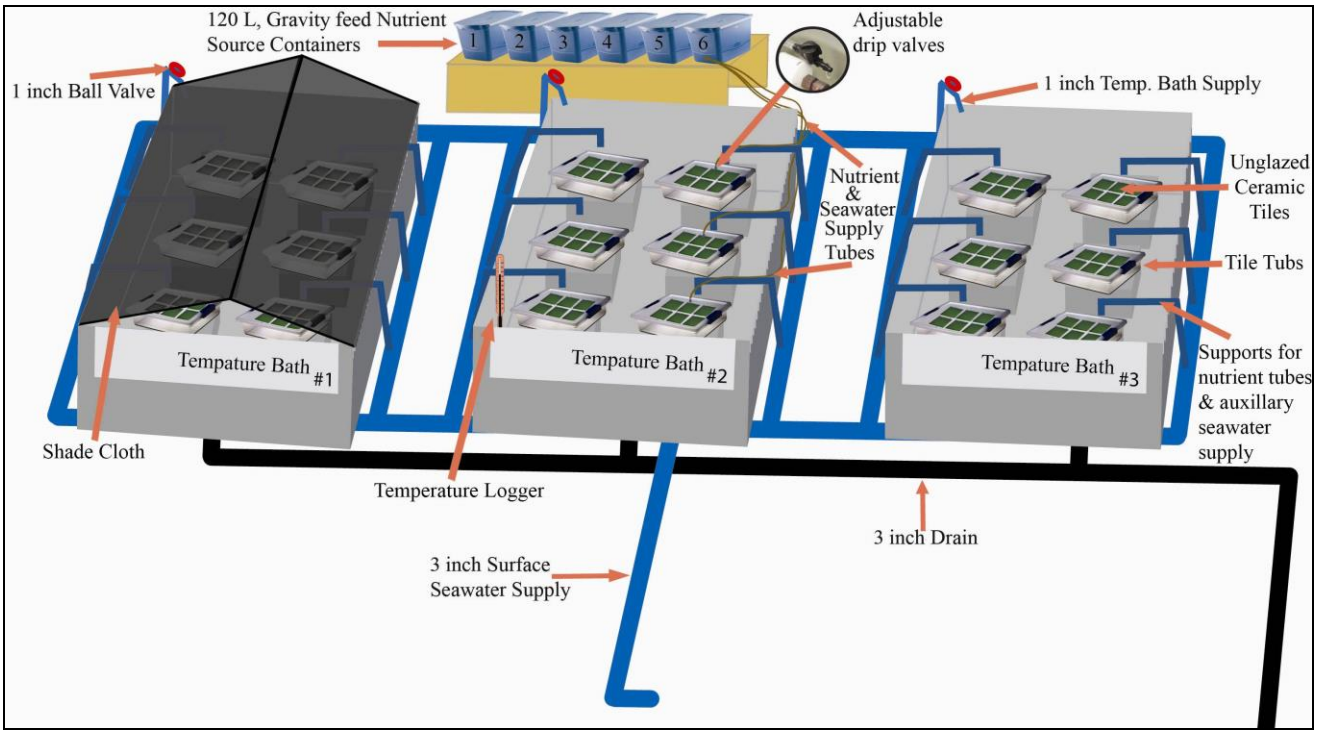

Figure 2. Experimental design layout.

Figure 3. 120 Liter nutrient/SSW source containers & large temperature baths with shade cloth at the NELHA site.

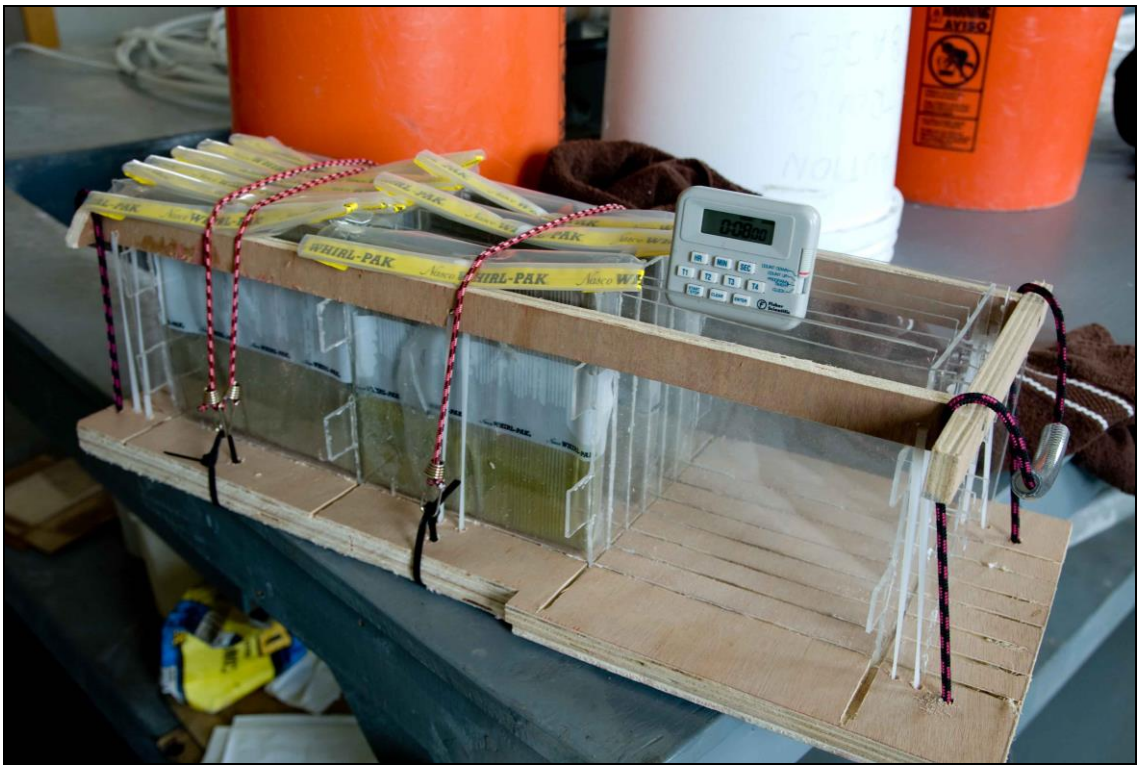

Figure 4. Tile holder used to maximize the surface area and minimize the acetone used during the tiles immersion in acetone to remove chlorophyll sample.


Figure 5. Manual tile mixer used to rotate the tile holder equally so that all tiles receive a uniform treatment of acetone immersion.

Figure 6. Gravity Feed Nutrient Source Containers. Each container feeds three replicate tile tubs.

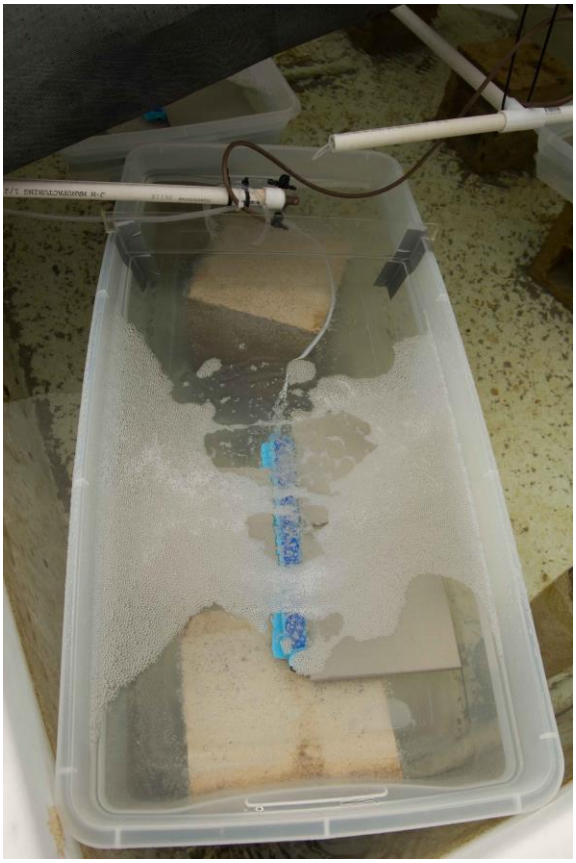

Figure 7. Tile Tubs containing 6 tiles each. A total of 18 tubs were used to provide replicates of each nutrient concentration during each trial.

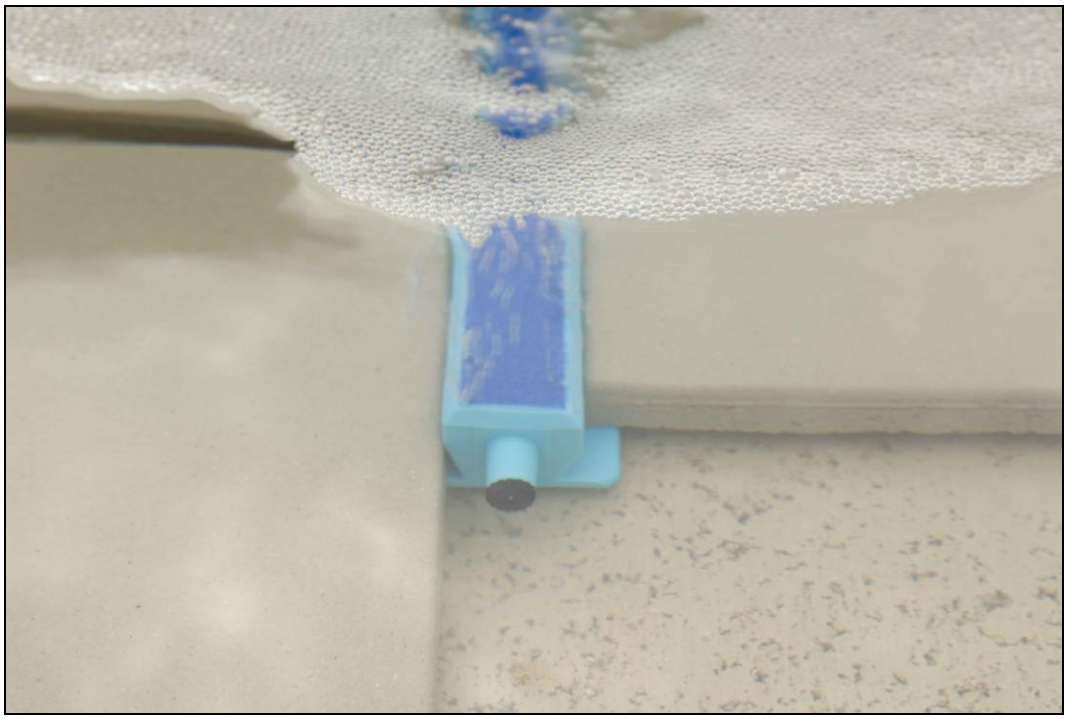

Figure 8. Air Stones used to create turbulence and encourage the mixing of nutrients. All air stones were located in between the unglazed ceramic tiles and the nutrient input reservoir inside of tile tubs.

Figure 9. Adjustable low-flow drip irrigation valves controlling the flow of nutrients into the tile tubs. Drips were adjusted once daily to ensure proper flow rates.

Figure 10. Tile after scraping procedure for dry weight. Each tile was placed in a custom made devise to ensure a precise scrapping from one tile to the next.

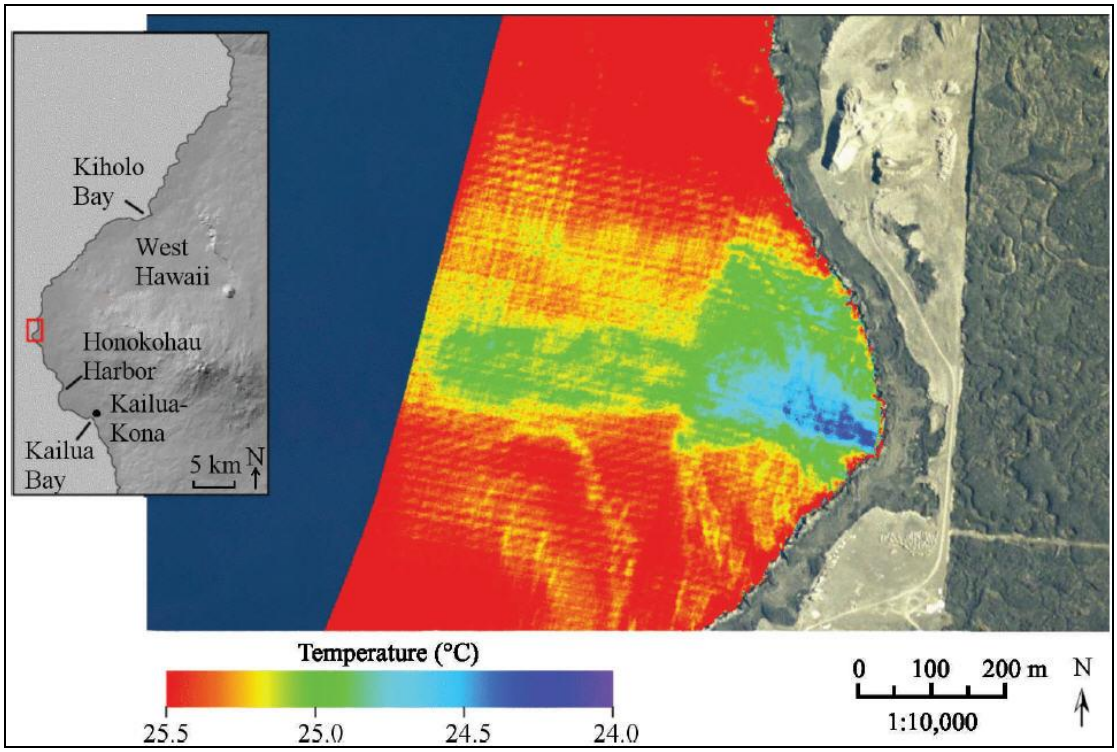

Figure 11. Aerial TIR image of a buoyant SGD West Hawaii SGD plume, located north of Kailua-Kona near Kona International Airport, Makako Bay (Peterson et al. 2009).

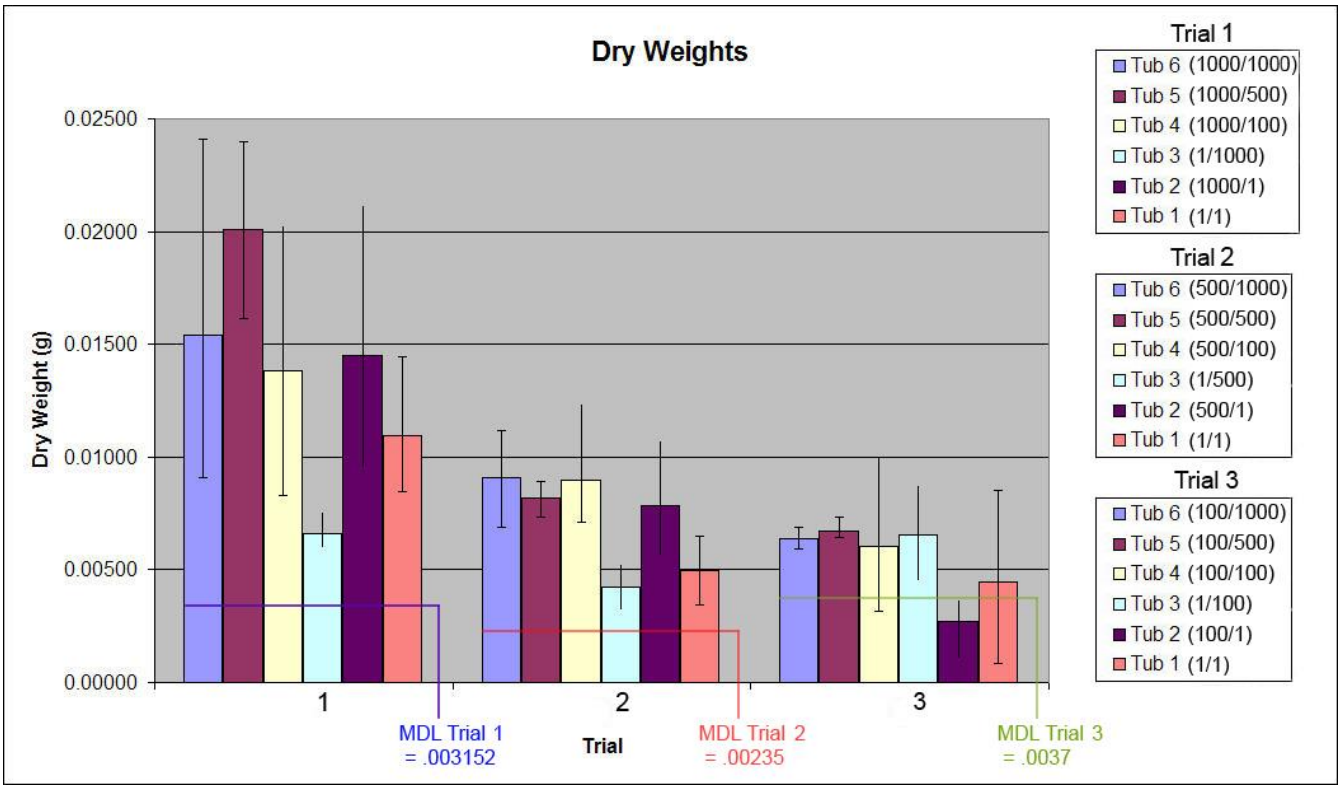

Figure 12. Dry Weight results trials 1, 2, & 3 with Method Detection Limits (MDL).

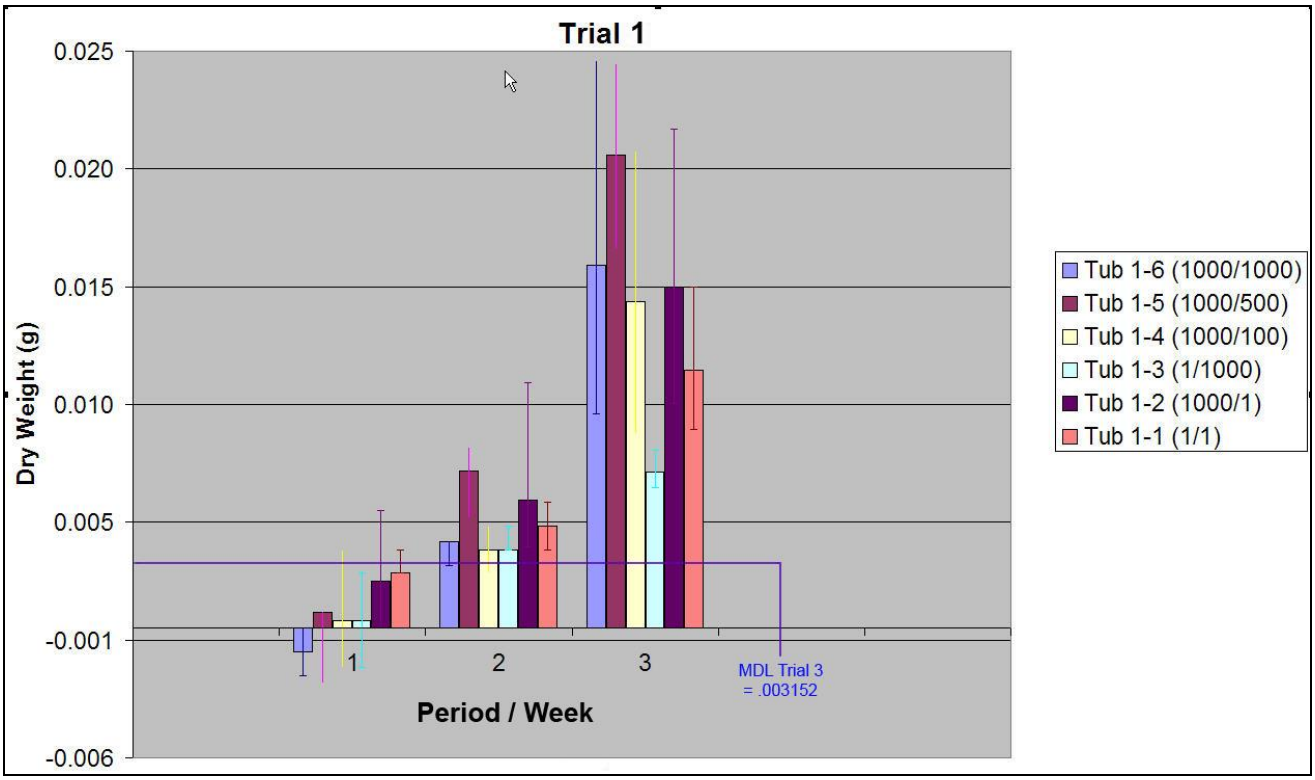

Figure 13. Dry Weight results trial 1- Method Detection Limits (MDL).

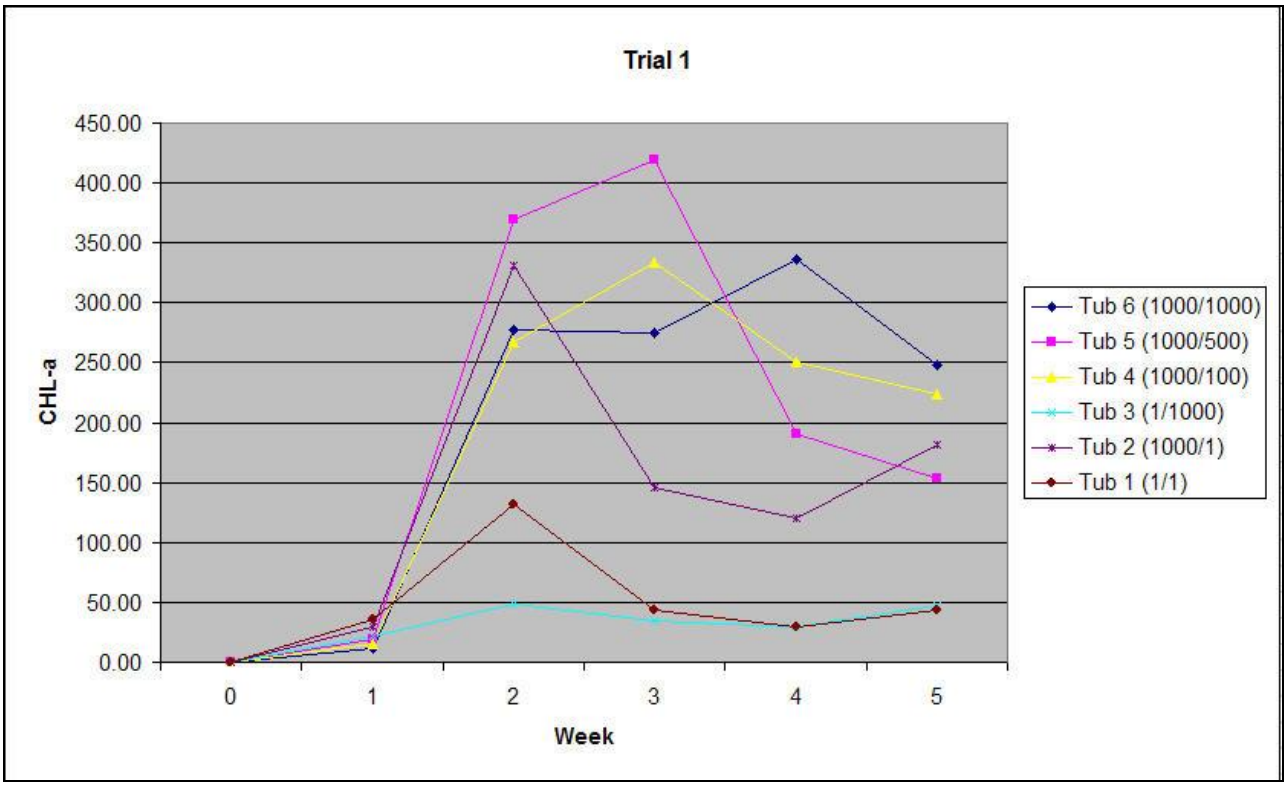

Figure 14. Initial CHL-a trial to determine experimental time scale. Growth drops significantly after the third week.

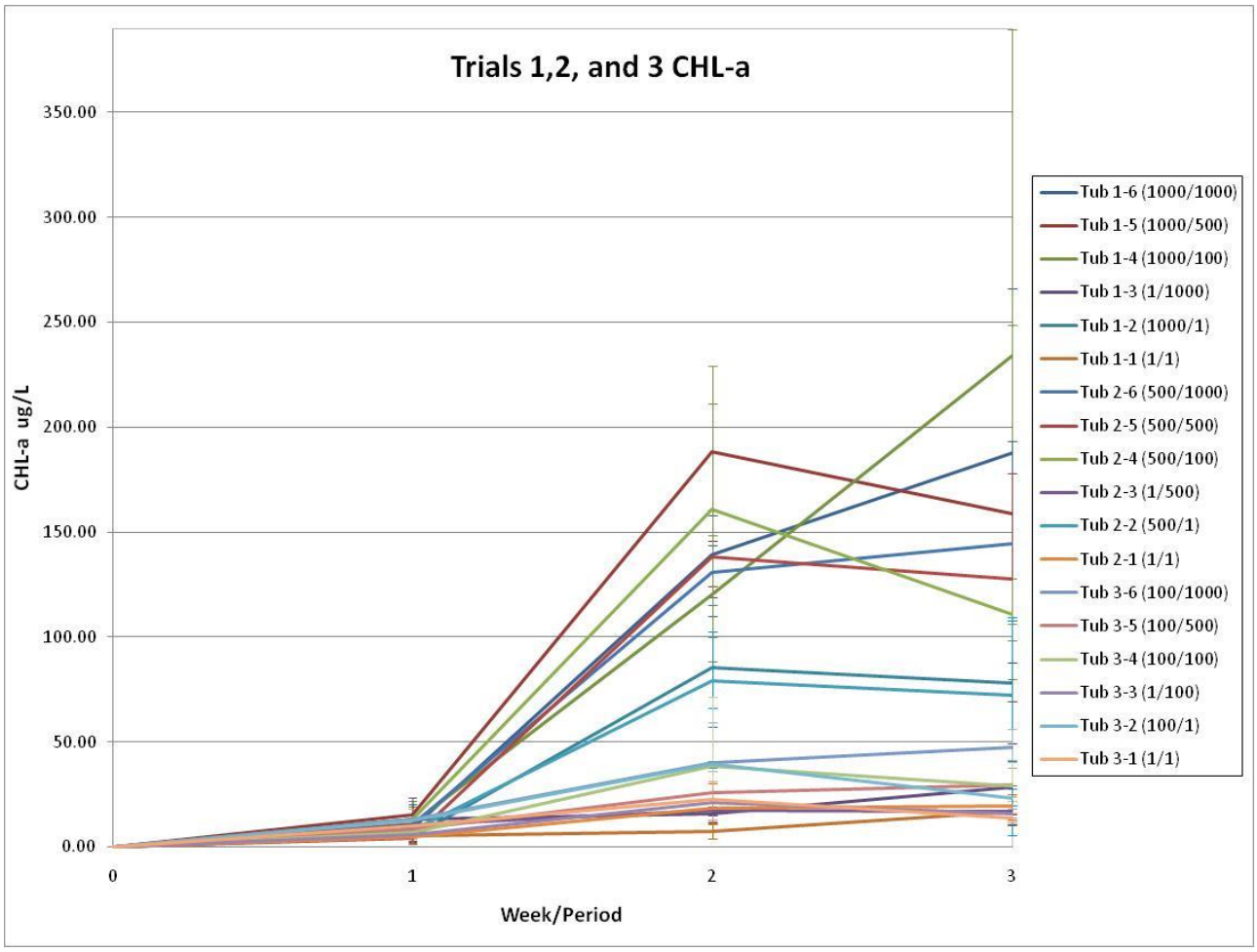

Figure 15. CHL-a results from trials 1, 2, & 3. Legend on the right signifies the nutrient amount in each tile tub (nitrogen/phosphorous).

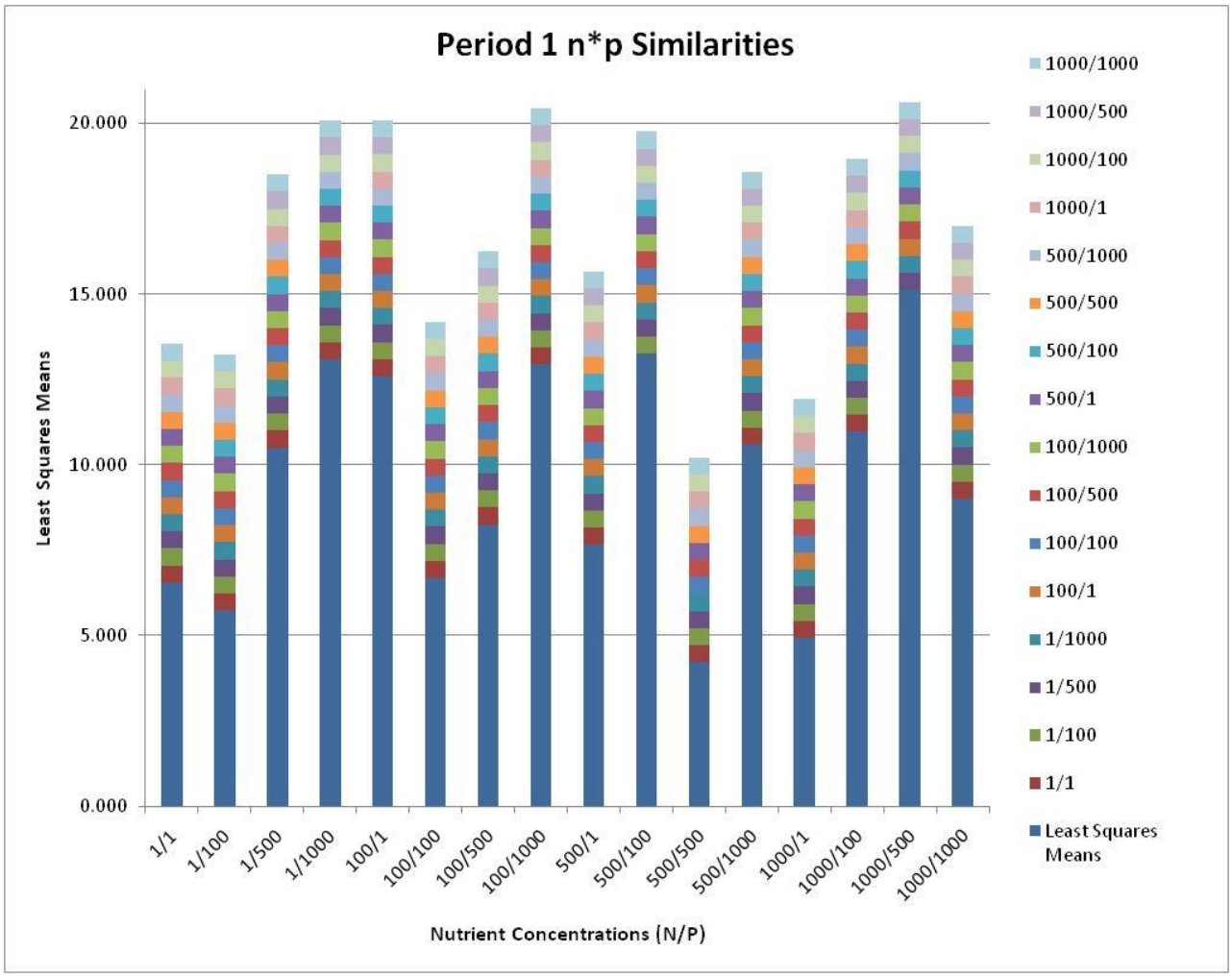

Figure 16. All possible combinations of n*p that are significantly different for Period 1. Color bars indicate nutrient combinations (n/p) with no difference between other nutrient combinations with similar color bars. Period 1, as indicated by the abundance of similar color bars across most of the nutrient concentrations has no significant differences between nutrient levels. This is consistent with the experimental design as nutrients were not added during Period 1.

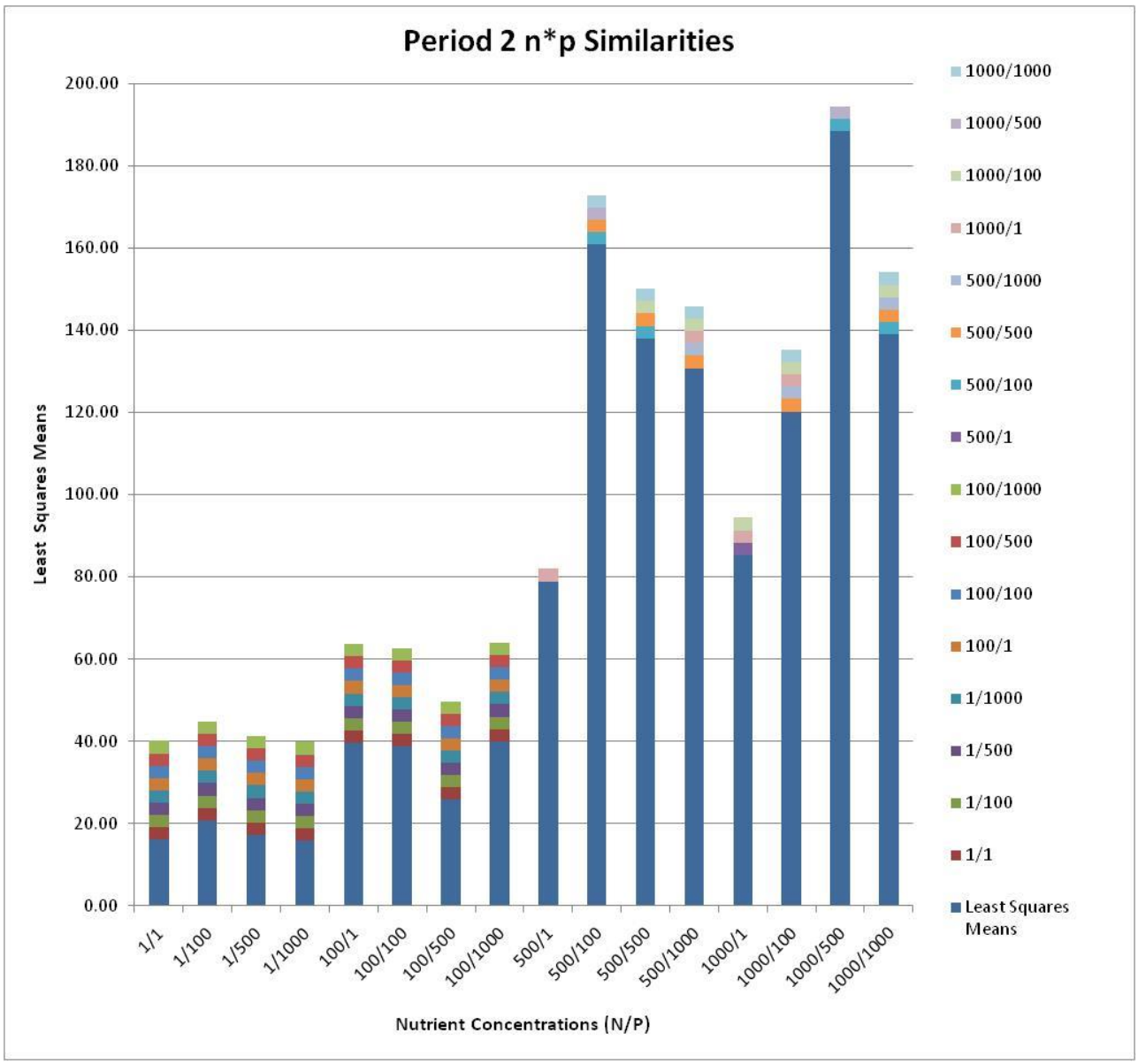

Figure 17. All possible combinations of n*p that are significantly different for Period 2. Color bars indicate nutrient combinations (n/p) with no difference between other nutrient combinations with similar color bars. The most obvious pattern in Period 2 is that as nitrogen levels increase there is a significant effect regardless of p, however when n is held constant there is no clear effect of p except at higher levels of n where p creates some effects.

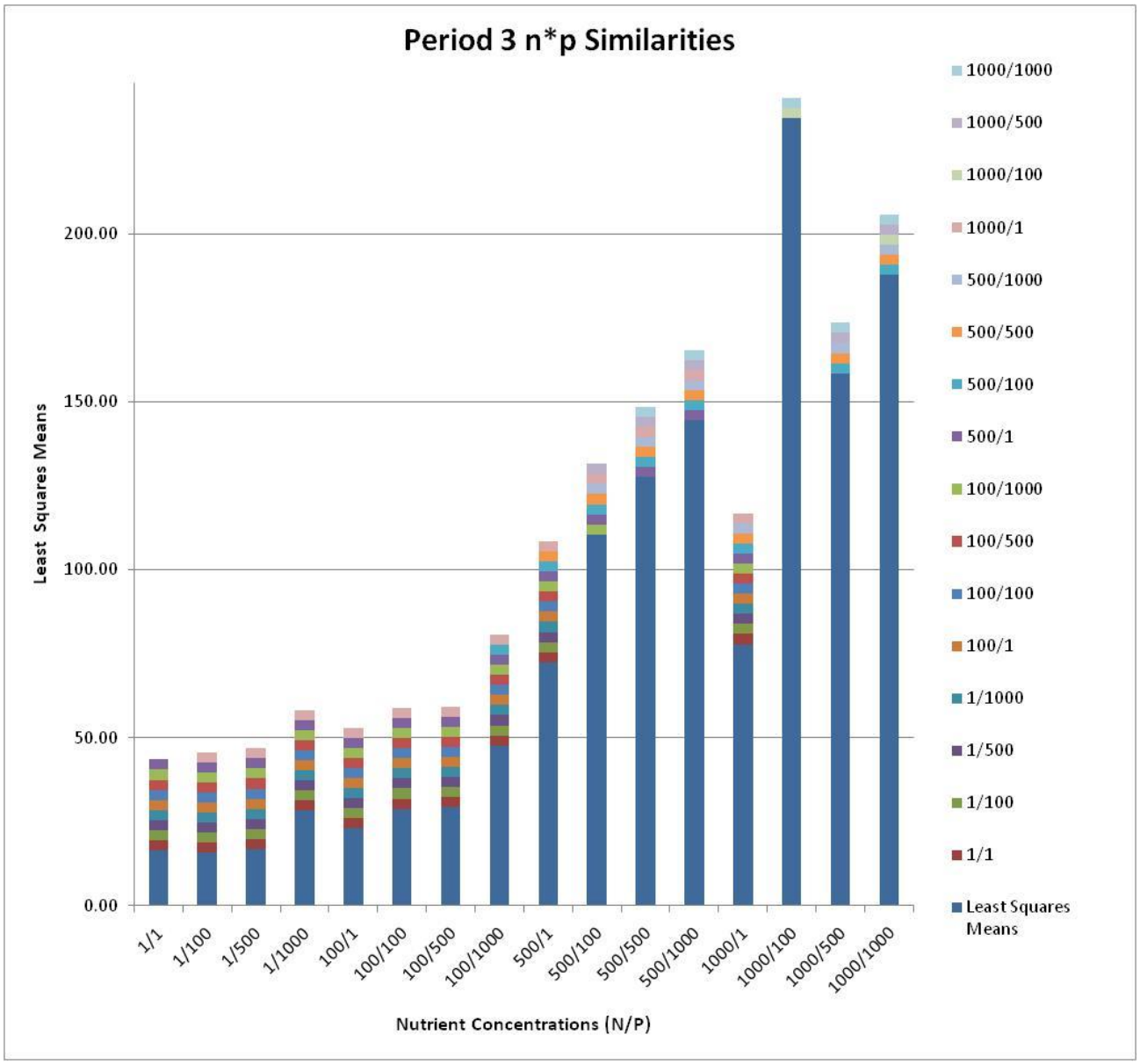

Figure 18. All possible combinations of n*p that are significantly different for Period 2. Color bars indicate nutrient combinations (n/p) with no difference between other nutrient combinations with similar color bars. Between most treatments of 1n or 100n and 500n or 1000n there is a significant difference. Within 1n and 100n, p plays no significant role. Between 500n and 1000n there are significant effects but no clear pattern emerges in relation to p except to say p can create an effect when n is at 1000 and p is above 1p. At 500n there are no effects with p except between 1p and 1000 p. At 1000n there appear to be some differences between p levels but only between high and low levels not among adjacent levels.

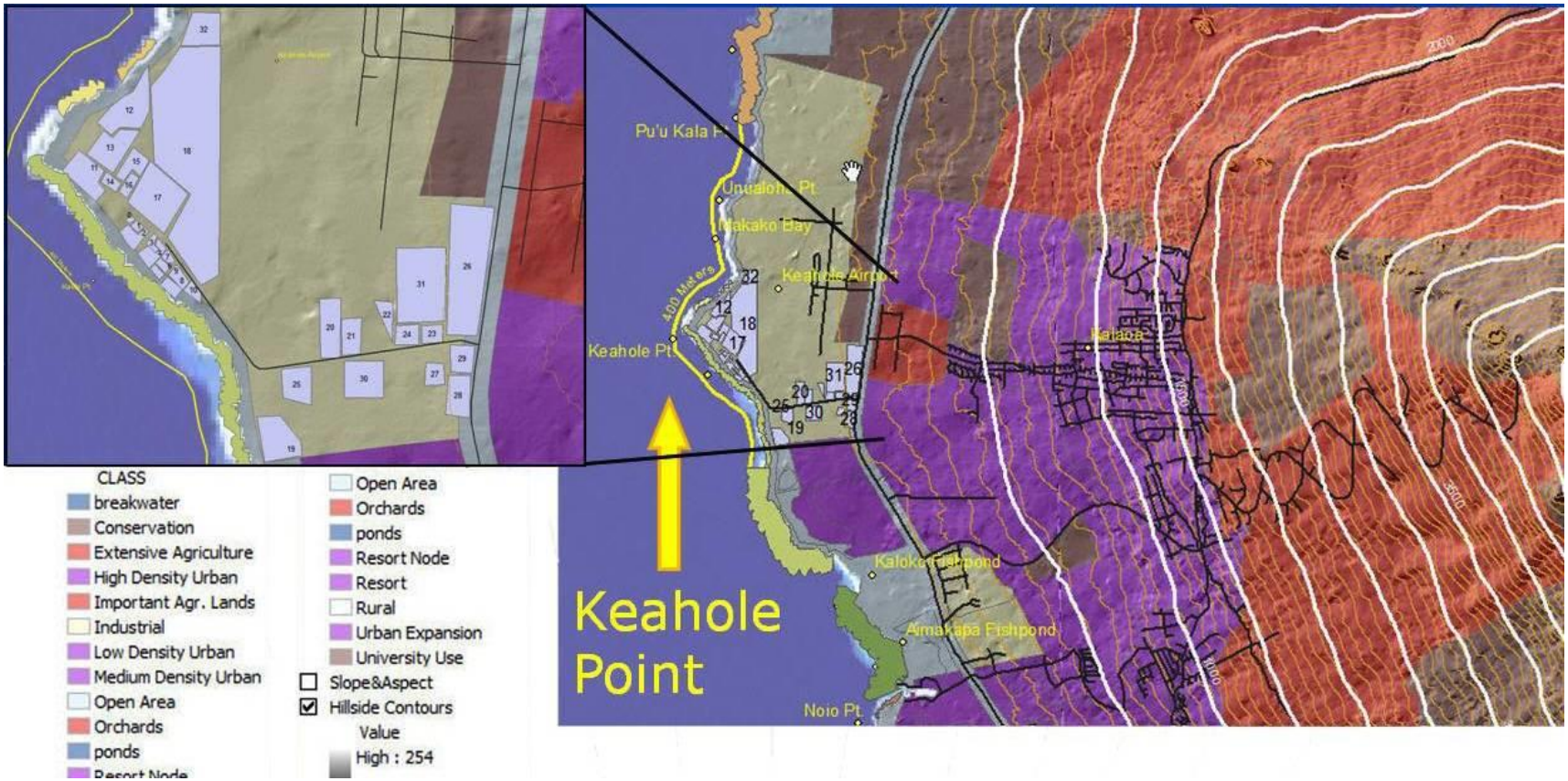

Figure 19. GIS mapping of study site at Keahole point and land immediately upslope including layered description of land use.

Effect	Num DF	Den DF	F Value	Pr > F
trial	2	4	1.80	0.2774
period	2	4	6.33	0.0577
trial*period	4	8	1.73	0.2353

The Mixed Procedure

Least Squares Means

Effect	trial	period	Estimate	Standard Error	DF	t Value	Pr > |t|
period		1	6.5500	2.5403	4	2.58	0.0614
period		2	16.0756	2.5403	4	6.33	0.0032
period		3	16.4778	2.5403	4	6.49	0.0029
trial	1		9.6489	2.5403	4	3.80	0.0191
trial	2		14.1144	2.5403	4	5.56	0.0051
trial	3		15.3400	2.5403	4	6.04	0.0038

Differences of Least Squares Means

Effect	trial	period	_trial	_period	Estimate	Standard Error	DF	tValue
period		1		2	-9.5256	3.1598	4	-3.01
period		1		3	-9.9278	3.1598	4	-3.14
period		2		3	-0.4022	3.1598	4	-0.13
trial	1		2		-4.4656	3.1598	4	-1.41
trial	1		3		-5.6911	3.1598	4	-1.80
trial	2		3		-1.2256	3.1598	4	-0.39

Differences of Least Squares Means

Effect	trial	period	_trial	_period	Pr > |t|
period		1		2	0.0394
period		1		3	0.0348
period		2		3	0.9049
trial	1		2		0.2305
trial	1		3		0.1461
trial	2		3		0.7179

Appendix A. SAS PROC Mixed statistics output for CHL-a controls, trials 1, 2, and 3. The results indicated that there was no significant difference between the CHL-a trial controls ($F_{2,4}$ = 1.8, p =.28) or for trial*period ($F_{4,8}$ = 1.73, p =.24).

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trial	2	0.00007808	0.00003904	4.36	0.0677

Least Squares Means for effect trial
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: weight

i/j	1	2	3
1		0.0499	0.0379
2	0.0499		0.8446
3	0.0379	0.8446	

Appendix B. SAS PROC GLM statistics output for Dry Weight controls, trials 1, 2, and 3. There appeared to be a slight trial effect ($F_{2,6}$ = 4.36, p =.068), however it was only somewhat significant.

Effect	Num DF	Den DF	F Value	Pr > F
n	3	6	72.19	<.0001
p	3	6	7.72	0.0175

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
n*p	9	16	2.56	0.0484
period	2	4	87.27	0.0005
n*period	6	12	19.07	<.0001
p*period	6	12	2.33	0.1005
n*p*period	18	32	1.62	0.1140

Appendix C. SAS PROC Mixed statistics output for CHL-a, trials 1, 2, and 3. The strongest effect overall was n (nitrogen) ($F_{3,6}$ = 72.19, p =.0001), however there also was an overall p (phosphorous) effect ($F_{3,6}$ = 7.72, p =.02). There was a significant overall n*p effect ($F_{9,16}$ = 2.56, p =.048) and a very strong period effect ($F_{2,4}$ = 87.27, p =.0005) and n*period effect ($F_{6,12}$ = 19.07, p =.0001).

---------- period=1 ----------

The GLM Procedure
Dependent Variable: chla

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	554.190617	36.946041	1.51	0.1513
Error	38	931.103267	24.502718		
Corrected Total	53	1485.293883			

R-Square	Coeff Var	Root MSE	chla Mean
0.373118	53.93813	4.950022	9.177222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
n	3	38.1150833	12.7050278	0.52	0.6721
p	3	95.5231744	31.8410581	1.30	0.2887
n*p	9	420.5523589	46.7280399	1.91	0.0806

Source	DF	Type III SS	Mean Square	F Value	Pr > F
n	3	15.5637000	5.1879000	0.21	0.8877
p	3	80.0824730	26.6941577	1.09	0.3653
n*p	9	420.5523589	46.7280399	1.91	0.0806

The GLM Procedure
Least Squares Means

n	p	LSMEAN chla LSMEAN	Number
1	1	6.5500000	1
1	100	5.7300000	2
1	500	10.5033333	3
1	1000	13.0866667	4
100	1	12.5933333	5
100	100	6.6866667	6
100	500	8.2500000	7
100	1000	12.9400000	8
500	1	7.6633333	9
500	100	13.2600000	10
500	500	4.2166667	11
500	1000	10.5900000	12
1000	1	4.9266667	13
1000	100	10.9633333	14
1000	500	15.1233333	15
1000	1000	9.0066667	16

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4	5	6
1		0.8051	0.2383	0.0549	0.0749	0.9672
2	0.8051		0.2449	0.0766	0.0977	0.8142
3	0.2383	0.2449		0.5265	0.6081	0.3510
4	0.0549	0.0766	0.5265		0.9035	0.1216
5	0.0749	0.0977	0.6081	0.9035		0.1521
6	0.9672	0.8142	0.3510	0.1216	0.1521	
7	0.6094	0.5367	0.5804	0.2388	0.2893	0.7011
8	0.0603	0.0824	0.5502	0.9712	0.9321	0.1301
9	0.7377	0.6351	0.4865	0.1876	0.2301	0.8104
10	0.0490	0.0702	0.4993	0.9660	0.8699	0.1121
11	0.4838	0.7102	0.1281	0.0344	0.0450	0.5448
12	0.2284	0.2366	0.9830	0.5404	0.6230	0.3403
13	0.6256	0.8435	0.1757	0.0506	0.0655	0.6657
14	0.1891	0.2032	0.9100	0.6024	0.6890	0.2967
15	0.0133	0.0256	0.2602	0.6172	0.5351	0.0436
16	0.4612	0.4226	0.7132	0.3191	0.3804	0.5693

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	7	8	9	10	11	12
1	0.6094	0.0603	0.7377	0.0490	0.4838	0.2284
2	0.5367	0.0824	0.6351	0.0702	0.7102	0.2366

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	7	8	9	10	11	12
3	0.5804	0.5502	0.4865	0.4993	0.1281	0.9830
4	0.2388	0.9712	0.1876	0.9660	0.0344	0.5404
5	0.2893	0.9321	0.2301	0.8699	0.0450	0.6230
6	0.7011	0.1301	0.8104	0.1121	0.5448	0.3403
7		0.2531	0.8854	0.2227	0.3246	0.5660
8	0.2531		0.1996	0.9373	0.0373	0.5644
9	0.8854	0.1996		0.1742	0.3991	0.4734
10	0.2227	0.9373	0.1742		0.0312	0.5128
11	0.3246	0.0373	0.3991	0.0312		0.1231
12	0.5660	0.5644	0.4734	0.5128	0.1231	
13	0.4161	0.0547	0.5024	0.0461	0.8615	0.1693
14	0.5061	0.6276	0.4193	0.5732	0.1033	0.9269
15	0.0972	0.5922	0.0727	0.6474	0.0103	0.2691
16	0.8525	0.3366	0.7414	0.2993	0.2433	0.6974

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	13	14	15	16
1	0.6256	0.1891	0.0133	0.4612
2	0.8435	0.2032	0.0256	0.4226
3	0.1757	0.9100	0.2602	0.7132
4	0.0506	0.6024	0.6172	0.3191
5	0.0655	0.6890	0.5351	0.3804
6	0.6657	0.2967	0.0436	0.5693
7	0.4161	0.5061	0.0972	0.8525
8	0.0547	0.6276	0.5922	0.3366
9	0.5024	0.4193	0.0727	0.7414
10	0.0461	0.5732	0.6474	0.2993
11	0.8615	0.1033	0.0103	0.2433
12	0.1693	0.9269	0.2691	0.6974
13		0.1435	0.0159	0.3191
14	0.1435		0.3099	0.6311
15	0.0159	0.3099		0.1385
16	0.3191	0.6311	0.1385	

Appendix D. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3 – period 1. There is no overall significance for n ($F_{3,38}$ = .52, p =.67 , p ($F_{3,38}$ = 1.3, p =.29), or n*p ($F_{9,38}$ = 1.91, p =.08).

Least Squares Means for effect n
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4
1		0.5558	0.9857	0.5949
2	0.5558		0.5611	0.9559
3	0.9857	0.5611		0.5987
4	0.5949	0.9559	0.5987	

Appendix E. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3- period 1 (n). There are no clear significant differences between individual treatments of n.

Least Squares Means for effect p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4
1		0.5299	0.4163	0.0807
2	0.5299		0.8583	0.2734
3	0.4163	0.8583		0.3575
4	0.0807	0.2734	0.3575	

Appendix F. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3- period 1 (p). There are no clear significant differences between individual treatments of p.

---------- period=2 ----------

The GLM Procedure
Dependent Variable: chla

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	179260.1676	11950.6778	24.04	<.0001
Error	38	18892.2023	497.1632		
Corrected Total	53	198152.3699			

R-Square	Coeff Var	Root MSE	chla Mean
0.904658	31.16917	22.29716	71.53593

Source	DF	Type I SS	Mean Square	F Value	Pr > F
n	3	151402.8138	50467.6046	101.51	<.0001
p	3	8012.7192	2670.9064	5.37	0.0035
n*p	9	19844.6347	2204.9594	4.44	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
n	3	139148.9174	46382.9725	93.30	<.0001
p	3	10822.5173	3607.5058	7.26	0.0006
n*p	9	19844.6347	2204.9594	4.44	0.0005

T he GLM Procedure
Least Squares Means

n	p	LSMEAN chla LSMEAN	Number
1	1	16.075556	1
1	100	20.803333	2

1	500	17.280000	3
1	1000	15.786667	4
100	1	39.646667	5
100	100	38.710000	6
100	500	25.753333	7
100	1000	39.990000	8
500	1	78.906667	9
500	100	160.816667	10
500	500	138.013333	11
500	1000	130.753333	12
1000	1	85.340000	13
1000	100	120.246667	14
1000	500	188.350000	15
1000	1000	139.023333	16

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4	5	6
1		0.7522	0.9358	0.9846	0.1211	0.1361
2	0.7522		0.8476	0.7844	0.3072	0.3315
3	0.9358	0.8476		0.9351	0.2268	0.2465
4	0.9846	0.7844	0.9351		0.1979	0.2157
5	0.1211	0.3072	0.2268	0.1979		0.9592
6	0.1361	0.3315	0.2465	0.2157	0.9592	
7	0.5189	0.7872	0.6443	0.5873	0.4501	0.4810
8	0.1159	0.2986	0.2199	0.1916	0.9851	0.9443
9	0.0001	0.0028	0.0017	0.0013	0.0374	0.0334
10	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
11	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
12	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
13	<.0001	0.0011	0.0006	0.0005	0.0165	0.0145
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	7	8	9	10	11	12
1	0.5189	0.1159	0.0001	<.0001	<.0001	<.0001
2	0.7872	0.2986	0.0028	<.0001	<.0001	<.0001

The GLM Procedure
Least Squares Means

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	7	8	9	10	11	12
3	0.6443	0.2199	0.0017	<.0001	<.0001	<.0001
4	0.5873	0.1916	0.0013	<.0001	<.0001	<.0001
5	0.4501	0.9851	0.0374	<.0001	<.0001	<.0001
6	0.4810	0.9443	0.0334	<.0001	<.0001	<.0001
7		0.4391	0.0059	<.0001	<.0001	<.0001
8	0.4391		0.0390	<.0001	<.0001	<.0001
9	0.0059	0.0390		<.0001	0.0024	0.0071
10	<.0001	<.0001	<.0001		0.2180	0.1069
11	<.0001	<.0001	0.0024	0.2180		0.6923
12	<.0001	<.0001	0.0071	0.1069	0.6923	
13	0.0023	0.0172	0.7258	0.0002	0.0063	0.0171
14	<.0001	<.0001	0.0289	0.0318	0.3353	0.5673
15	<.0001	<.0001	<.0001	0.1387	0.0087	0.0031
16	<.0001	<.0001	0.0021	0.2387	0.9560	0.6522

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	13	14	15	16
1	<.0001	<.0001	<.0001	<.0001
2	0.0011	<.0001	<.0001	<.0001
3	0.0006	<.0001	<.0001	<.0001
4	0.0005	<.0001	<.0001	<.0001
5	0.0165	<.0001	<.0001	<.0001
6	0.0145	<.0001	<.0001	<.0001
7	0.0023	<.0001	<.0001	<.0001
8	0.0172	<.0001	<.0001	<.0001
9	0.7258	0.0289	<.0001	0.0021
10	0.0002	0.0318	0.1387	0.2387
11	0.0063	0.3353	0.0087	0.9560
12	0.0171	0.5673	0.0031	0.6522
13		0.0627	<.0001	0.0054
14	0.0627		0.0006	0.3089
15	<.0001	0.0006		0.0101
16	0.0054	0.3089	0.0101	

Appendix G. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3 – period 2. There is an overall significant n effect ($F_{3,38}$ = 101.51, p =.0001) , p effect ($F_{3,38}$ = 5.37, p =.004), and n*p effect ($F_{9,38}$ = 4.44, p =.0005).

Least Squares Means for effect n
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4
1		0.0400	<.0001	<.0001
2	0.0400		<.0001	<.0001
3	<.0001	<.0001		0.5056
4	<.0001	<.0001	0.5056	

Appendix H. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3- period 2 (n). 1n(1) or 100n(2) differed significantly from all other levels of n and 500n(3) and 1000n(4) did not significantly differ from each other but both had an effect from 1n and 100n.

Least Squares Means for effect p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4
1		0.0014	0.0001	0.0044
2	0.0014		0.4336	0.6822
3	0.0001	0.4336		0.2360
4	0.0044	0.6822	0.2360	

Appendix I. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3- period 2 (p). 1p(1) is significantly different then 100p(2), 500p(3), or 1000p(4) but there are no effects between 100p, 500p, and 1000p.

---------- period=3 ----------

The GLM Procedure
Dependent Variable: chla

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	242854.8650	16190.3243	6.83	<.0001
Error	38	90047.0724	2369.6598		
Corrected Total	53	332901.9374			

R-Square	Coeff Var	Root MSE	chla Mean
0.729509	64.78572	48.67915	75.13870

Source	DF	Type I SS	Mean Square	F Value	Pr > F
n	3	194032.8098	64677.6033	27.29	<.0001
p	3	18698.5205	6232.8402	2.63	0.0640
n*p	9	30123.5347	3347.0594	1.41	0.2173

Source	DF	Type III SS	Mean Square	F Value	Pr > F
n	3	179947.4683	59982.4894	25.31	<.0001
p	3	24778.6910	8259.5637	3.49	0.0249
n*p	9	30123.5347	3347.0594	1.41	0.2173

The GLM Procedure
Least Squares Means

n	p	LSMEAN chla LSMEAN	Number
1	1	16.477778	1
1	100	15.750000	2
1	500	16.830000	3
1	1000	28.310000	4
100	1	23.023333	5
100	100	28.846667	6
100	500	29.273333	7
100	1000	47.673333	8
500	1	72.466667	9
500	100	110.503333	10
500	500	127.590000	11
500	1000	144.333333	12
1000	1	77.826667	13
1000	100	234.343333	14
1000	500	158.480000	15
1000	1000	187.813333	16

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4	5	6
1		0.9822	0.9914	0.7174	0.8412	0.7052
2	0.9822		0.9785	0.7537	0.8558	0.7436
3	0.9914	0.9785		0.7743	0.8770	0.7640
4	0.7174	0.7537	0.7743		0.8949	0.9893
5	0.8412	0.8558	0.8770	0.8949		0.8843
6	0.7052	0.7436	0.7640	0.9893	0.8843	
7	0.6956	0.7355	0.7559	0.9808	0.8759	0.9915
8	0.3425	0.4269	0.4425	0.6289	0.5388	0.6384
9	0.0926	0.1618	0.1697	0.2736	0.2211	0.2793
10	0.0062	0.0222	0.0237	0.0455	0.0339	0.0469
11	0.0015	0.0077	0.0083	0.0169	0.0122	0.0175
12	0.0003	0.0025	0.0027	0.0059	0.0041	0.0061
13	0.0663	0.1266	0.1332	0.2205	0.1760	0.2254
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15	<.0001	0.0009	0.0010	0.0023	0.0016	0.0023
16	<.0001	0.0001	0.0001	0.0003	0.0002	0.0003

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	7	8	9	10	11	12
1	0.6956	0.3425	0.0926	0.0062	0.0015	0.0003
2	0.7355	0.4269	0.1618	0.0222	0.0077	0.0025

The GLM Procedure
Least Squares Means

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	7	8	9	10	11	12
3	0.7559	0.4425	0.1697	0.0237	0.0083	0.0027
4	0.9808	0.6289	0.2736	0.0455	0.0169	0.0059
5	0.8759	0.5388	0.2211	0.0339	0.0122	0.0041
6	0.9915	0.6384	0.2793	0.0469	0.0175	0.0061
7		0.6461	0.2840	0.0480	0.0180	0.0063
8	0.6461		0.5365	0.1222	0.0515	0.0198
9	0.2840	0.5365		0.3446	0.1736	0.0785
10	0.0480	0.1222	0.3446		0.6697	0.4000
11	0.0180	0.0515	0.1736	0.6697		0.6759
12	0.0063	0.0198	0.0785	0.4000	0.6759	
13	0.2294	0.4527	0.8934	0.4161	0.2182	0.1025
14	<.0001	<.0001	0.0002	0.0035	0.0107	0.0293
15	0.0024	0.0082	0.0368	0.2349	0.4419	0.7239
16	0.0003	0.0011	0.0061	0.0592	0.1380	0.2809

Least Squares Means for effect n*p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	13	14	15	16
1	0.0663	<.0001	<.0001	<.0001
2	0.1266	<.0001	0.0009	0.0001
3	0.1332	<.0001	0.0010	0.0001
4	0.2205	<.0001	0.0023	0.0003
5	0.1760	<.0001	0.0016	0.0002
6	0.2254	<.0001	0.0023	0.0003
7	0.2294	<.0001	0.0024	0.0003
8	0.4527	<.0001	0.0082	0.0011
9	0.8934	0.0002	0.0368	0.0061
10	0.4161	0.0035	0.2349	0.0592
11	0.2182	0.0107	0.4419	0.1380
12	0.1025	0.0293	0.7239	0.2809
13		0.0003	0.0495	0.0087
14	0.0003		0.0639	0.2490
15	0.0495	0.0639		0.4650
16	0.0087	0.2490	0.4650	

Appendix J. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3 – period 3. There is an overall significant n effect ($F_{3,38}$ = 27.29, p =.0001), however the overall p effect ($F_{3,38}$ = 2.63, p =.06) was only marginally significant, and the overall n*p effect ($F_{9,38}$ = 1.41, p =.22) was not significant.

Least Squares Means for effect n
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4
1		0.5031	<.0001	<.0001
2	0.5031		0.0002	<.0001
3	<.0001	0.0002		0.0145
4	<.0001	<.0001	0.0145	

Appendix K. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3- period 3 (n). Although there is no difference between 1n(1) and 100n(2), both are significantly different then 500n(3) or 1000n(4). 500n also shows a significant effect between 1000n.

Least Squares Means for effect p
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: chla

i/j	1	2	3	4
1		0.0125	0.0691	0.0067
2	0.0125		0.4757	0.8154
3	0.0691	0.4757		0.3454
4	0.0067	0.8154	0.3454	

Appendix L. SAS PROC GLM statistics output for CHL-a, trials 1, 2, and 3- period 3 (p). 1p(1) is significantly different then 100p(2), 500p(3), or 1000p(4) but there are no effects between 100p, 500p, and 1000p.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
n	3	0.00596831	0.00198944	2.77	0.0549
p	3	0.00157536	0.00052512	0.73	0.5402
n*p	9	0.00707977	0.00078664	1.09	0.3897

Appendix M. SAS PROC GLM statistics output for Dry Weight, trials 1, 2, and 3. Results indicate that the only significant effect was n ($F_{3,38} = 2.77$, $p = .05$). There was no effect of p ($F_{3,38} = .73$, $p = .54$) or n*p ($F_{9,38} = 1.09$, $p = .39$).

Least Squares Means for effect n
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: weight

i/j	1	2	3	4
1		0.9556	0.8148	0.0184
2	0.9556		0.7814	0.0208
3	0.8148	0.7814		0.0395
4	0.0184	0.0208	0.0395	

Appendix N. SAS PROC GLM statistics output for Dry Weight, trials 1, 2, and 3- period 3 (n). Looking at n independent of other values shows that 1000n(4) is significantly different than all other levels of n (1n(1), 100n(2), 500n(3)) but no difference were found between any other levels.

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