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Distribution and Characteristics of a Photosynthetic Benthic Microbial Community in a Marine Coastal Pond

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Abstract

Distribution and basic characteristics of the photosynthetic benthic community within He'eia Fishpond was determined using chlorophyll-a, High-Performance Liquid Chromatography (HPLC), and Scanning Election Microscopy (SEM). Chlorophyll-a gradients differed across the pond with differing sediment facies. Little to no chlorophyll-a was present in the surface sediments at sampling sites located nearest to salt water inputs. High to moderate concentrations of chlorophyll-a were present at sites nearest to fresh water inputs. HPLC photopigment results indicate that high concentrations of the photopigments, chlorophyll-a, chlorophyll c_1c_2, and fucoxanthin were present at both sites A and B above 0.25-cm, while site A also contained high concentrations of beta-carotene and chlorophyllide-a above 0.25-cm. Photopigment analysis only allowed for organism classification at the phylum level. The possibility that several different phyla could use the same photopigments for photosynthesis made it difficult to distinguish between specific phyla at different depths. The finest classification that could be achieved was that of sub-phyla Bacillariophyceae, which was identified at both sites using both HPLC and SEM analysis.

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Introduction:

To understand the nature and impact of nutrient cycling and remineralization within shallow, coastal water bodies the photosynthetic benthic community must be studied. The metabolic and physical activities of organisms in surficial sediments cause shifts between oxic and anoxic conditions. These shifts may influence redox-sensitive nutrient fluxes at the sediment-water interface.

Over the course of my internship, I studied the microbial community within He'eia Fishpond surficial sediments. Chlorophyll-a profiles were used to define the depth distribution and quantify the biomass of the benthic microbial community. High-Performance Liquid Chromatography (HPLC) and Scanning Electron Microscopy (SEM) analyses was applied to surficial sediment to characterize the benthic photosynthetic microbial organisms into broad taxonomic phyla or classes.

Background

Kathleen Ruttenberg of the Department of Oceanography, at the University of Hawaii at Manoa, along with several graduate and undergraduate students, has been actively studying the role of the bottom sediments within He'eia fishpond since February 2007. According to Ruttenberg's Sea Grant proposal, the "project will actively evaluate the impact of anthropogenically-enhanced erosion on nutrient cycling… and related ecosystem perturbation" in He'eia Fishpond (Ruttenberg 2007). Ruttenberg and her students want to understand how changes in nutrient cycles impact the fishpond's ecology. It is hypothesized that benthic photosynthetic organisms might be changing the chemistry of He'eia Fishpond by either providing or retaining sedimentary nutrients (Ruttenberg, pers. comm.).

Rebecca Briggs, a PhD student currently working under Ruttenberg, is studying the coupled cycling of iron (Fe) and phosphorus (P) in the pond. Both Fe and P are essential, often limiting nutrients for photosynthetic organisms, such as the phytoplankton at the base of the pond's food web. High weathering rates within a tropical drainage basin, such as the He'eia ahupua'a (watershed), supply adjacent coastal regions with an abundance of weathered Fe-oxides as well as other metal-oxides and oxyhydrides (Biscaye 1965). Particulate Fe-oxides in rivers scavenge P from the soil and weathering environment, and transport Fe-bound P to coastal waters. This Fe-bound P settles out of the water column and is deposited in the sediments. Under anoxic conditions, microbial activity reductively dissolves Fe-oxides thus resulting in the release of Fe-bound P and ferrous iron to pore waters (e.g. Rozen et al. 2002; Slomp et al. 1998; McManus et al. 1997; Sundby et al. 1992; Heggie et al. 1990; O'Brien et al. 1990; Krom and Berner 1981). This leads to a build up of pore water phosphate and ferrous iron (Figure 1). The diagenetic processes controlling these sedimentary redox conditions and Fe-P cycling are traditionally believed to occur on a time scale of weeks to months; however, Briggs and Ruttenberg hypothesize that the benthic photosynthetic community is driving diel (day-night) shifts in reactive Fe and P cycling. The process of photosynthesis during the day followed by respiration at night at the sediment-water interface creates alternating oxic and anoxic conditions, respectively (Briggs et al. 2006), and drives the coupled cycling of sediment Fe and P. Briggs'

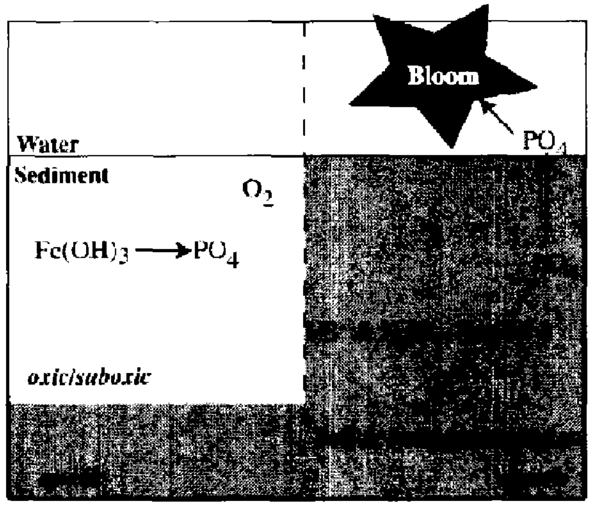


Figure 1. Coupled cycling of Fe-P within oxic/suboxic and anoxic sediment conditions (Ruttenberg 2007).

diel studies will reveal the impact of the benthic photosynthetic communities upon the coupled Fe and P cycling over a 24 hour period (Ruttenberg 2007).

It is important to understand the nature of the surficial sediment microbial communities since it is believed that the photosynthetic benthic communities are driving the redox changes, as just described, and may thus affect sediment fluxes across the sediment-water interface which in turn will impact nutrient cycling within He'eia Fishpond. Of special interest is the potential presence of different microbial groups among the different sediment facies and grain sizes within the pond. The pond is dominated by two distinct substrates: sandy, coarse sediments located near the sites of marine input, and muddy, silty sediments near the sites of freshwater input. With this knowledge, I proposed three hypotheses:

1. Higher concentrations of chlorophyll-a will be found at the sandy sites.

2. Maximum chlorophyll-a concentrations will be found near the surface of all sites sampled.

3. Sediment facies and composition will drive differences in microbial community composition. Calcium carbonate ($CaCO_3$) producing chlorophytes (green algae) will dominate at sandy sites.

Study Site

He'eia Fishpond, located adjacent to Kaneohe Bay on Oahu's windward coast, remains a relic of a ancient Hawaiian civilization. Although the age of the fishpond is unknown, the earliest site map dates back to 1851 (Kelly 1975). Historically, He'eia Fishpond was well maintained and farmed by Hawaiians who used the fishpond as a form of sustenance for the entire ahupua'a (watershed) of He'eia. He'eia Fishpond's 88 acres are completely encompassed by a 5,000 foot man-made wall (Kelly 1975). Along the wall are a total of eight outlets, known as makahas; three control mauka river flows and five control makai oceanic flows. Ancient Hawaiians used these makahas to trap fish in the pond during the changing tides. The herbivorous fish fed on nutritious green algae that grew within the pond. This form of fish farming was so efficient that the Hawaiians increased the fish protein production by 100 times.

Today the pond is cared for by the non-profit organization Paepae O He‘eia (POH). POH's mission is to restore the fishpond to its former non-impacted ecological state and use the pond as a learning venue for elementary and high school students. Currently, POH utilizes a number of sites within the pond to grow several species of fish (Paepae O Heeia 2007). A decline in pond health, maintenance, and ecology began in the mid-1800's due to increased urbanization and subsequent erosion within the ahupua'a (Ruttenberg 2007). The pond's decline in health may be linked to enhanced erosion and bottom sediment emplacement. Before the mid-1800's there was no bottom sediment within the pond; however, today a coat of nutrient enriched sediment covers the entire pond floor. To fully understand the state of the pond, POH seeks to understand the impact of bottom sediments on nutrient cycling and limitation within the pond (Ruttenberg 2007).

He‘eia Fishpond is a uniquely well-suited environment for this type of study since it is an enclosed, coastal water body, allowing better constraints to be placed on nutrient sources and sinks. Further, linking the presence and composition of a benthic, photosynthetic microbial community to coupled Fe-P cycling has not previously been done in a coastal environment of this type (Ruttenberg, pers. comm.)

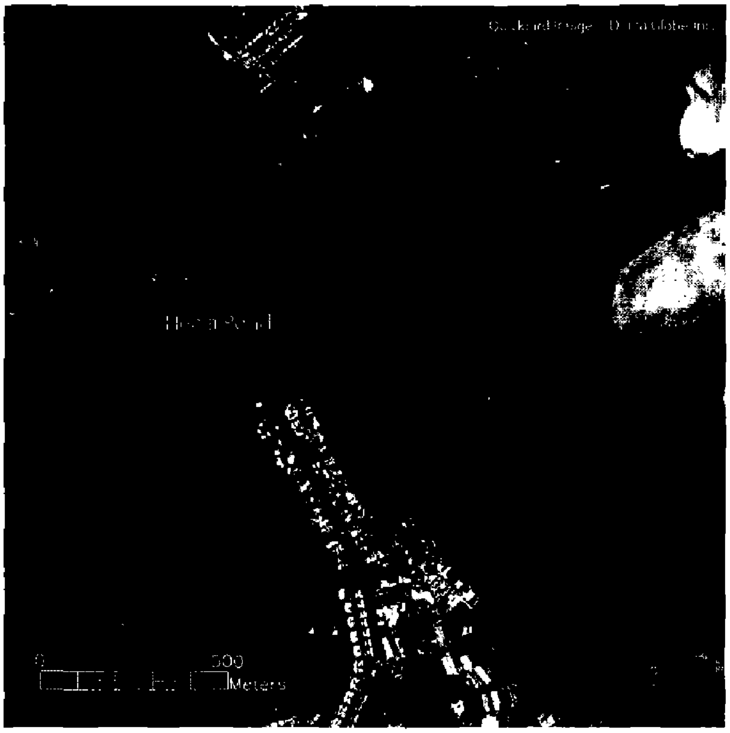


Figure 2. Aerial photograph of He'eia Fishpond

Sampling Methods:

Core Sampling

Microbial community sediment samples were taken at nine different sites within He'eia Fishpond using a push core technique (Fig. 3). Push cores, constructed by removing the tips of 60-ml plastic syringes, were used to collect triplicate sediment samples from each sampling site. The push core samples were taken June 26 thru June 29 at comparable times. At nine sites, designated Pond 1 thru Pond 7 and site A and B (Fig. 4). Sites were chosen to achieve maximum coverage of the fishpond, including differing sediment compositions. Site A and B were chosen because intensive water quality sampling and sediment nutrient analysis will occur at these sites at a later date (Ruttenberg 2007). Three cores from each site were taken, one each for chlorophyll-a concentration, porosity, and HPLC analysis. The first two centimeters of each core were sectioned at 1/8-cm intervals, while the third centimeter was sectioned at 1/4-cm intervals. The samples were then immediately frozen for later analysis.

a) b)

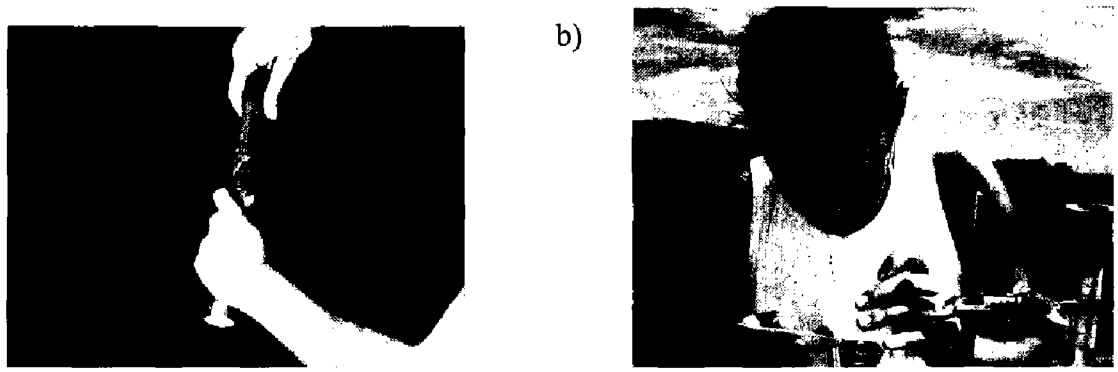


Figure 3. a) Two plungers trap the sediment within the 60-ml push core, b) Sediment samples were sectioned and placed into appropriate vials.

Microbial sampling

At each site an additional 3-cm long push core sample was taken for microbial analysis. These samples were frozen in tact, on site, using liquid propane, following the procedures used in Glazer et al. (2002). Frozen samples were transferred to a –80°C freezer to be stored for analysis at a later time.

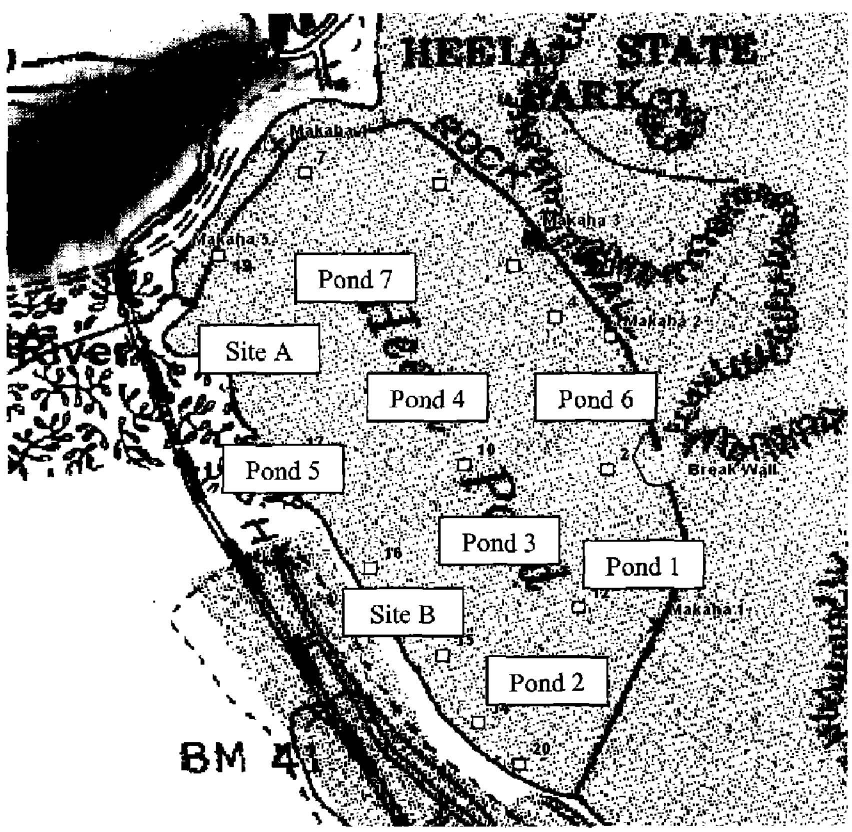


Figure 4. Picture depicting the locations of sampling sites: Pond 1 through Pond 7 and site A and B within He'eia Fishpond, on the island of O'ahu.

Bathymetry map

A bathymetry map of the pond was created by utilizing water depth and a global positioning system (GPS). To conduct field sampling without disturbing the sediment, a wide rubber band was tied around a surfboard, which held down a waterproofed GPS and a clipboard with my record sheets. The surfboard was paddled between a PVC pipe grid in the fishpond, and at approximately 10-m intervals a meter stick with a foam base was used to determine the depth of the pond at that location according to the GPS positioning.

Analytical Methods:

Sediment chlorophyll-a pigments were extracted from approximately 1 g of sediment from each sectioned interval using a sonication/vortex technique in 100% acetone (Sun et al. 1991. Chlorophyll-a fluorescence was determined on a fluorometer and was converted to concentration using previously determined calibration factors (Bienfang, pers. comm.). Further graphical analysis of chlorophyll-a concentration data for all sites was accomplished using Microsoft Excel.

Porosity at each site was determined by weight loss of a known volume of sediment after drying. Once dried, these samples were also used for SEM analysis using a Zeiss DSM 962 SEM. First, the SEM samples were sputter coated with gold palladium, and then observed at a magnification between 1,000 and 10,000X (Daniels, pers. comm.). SEM analysis was employed to visually identify different microbes present within the sediments.

Finally, sediment samples from each site were sent to Dr. James Pinckney at the University of South Carolina (Department of Biological Sciences) to be analyzed for photopigments. Pigments were extracted in acetone by lyophilization and then analyzed using HPLC. Protocols for University of South Carolina methods are available upon request. Photopigment concentration data from the University of South Carolina was analyzed graphically using MatLab.

All GPS points and depth data were analyzed using a combination of MatLab and ARCGIS programs to develop a detailed bathymetric map of the fishpond.

Results:

Porosity

All sites, except site 7, showed porosity maxima at the sediment-water interface (Fig. 5). For all sites, except P2 and P7, porosity decreased gradually with increasing depth. Site P2's high surface percent porosity showed a rapid decrease followed by a rapid increase above 0.5-cm. Below 1.875-cm for P2, porosity stabilized between 65 and 75 percent with no additional decrease. At site P7, porosity stayed between 50 and 70 percent above 2.875-cm, then it increased rapidly until a depth of 3-cm. Sites P1 and P2 had the highest surficial porosity. Most sites displayed average porosities around 60-80 percent except Site B. Site B had a porosity around 54 percent.

Chlorophyll-a

Surface chlorophyll-a maxima were observed at sites P4, P5, and A (Fig. 6). Sites P1, P2, and B had the lowest surface chlorophyll-a concentrations. Site A, closest to the river mouth, had the highest surface chlorophyll-a concentration and was the only site in which chlorophyll-a concentration noticeably decreased with depth. Chlorophyll-a concentration for all other sites either stabilized or increased below 1-cm.

HPLC

Photopigments (chlorophylls and carotenoids) utilized by different photosynthetic phyla are summarized in Table 2. The photopigments observed at sites A and B are summarized in Table 2, while possible phyla present at both sites are displayed in Table 3. Figure 7 depicts the depth profiles of the concentrations of each photopigment, in ng per g, for sites A and B normalized with the chlorophyll-a concentration. Normalizing individual photopigments to chlorophyll-a allows better resolution of individual photopigment depth trends. The chlorophyll-a depth profile is shown for reference.

At site A (Fig. 7a), chlorophyll-c_1c_2 and fucoxanthin were the most concentrated pigments near the surface besides chlorophyll-a. The concentrations of beta-carotene, diatoxanthin (diat), and zeaxanthin increased with depth. Chlorophyll-c_1c_2, and chlorophyllide-a concentrations were diminished to undetectable levels at 0.5-cm depth, while diadinoxanthin (diad) persisted until 1-cm. As seen in Figure 6, nearly all chlorophyll-a was lost below 1.125-cm at site A.

For site B (Fig. 7b), chlorophyll-c_1c_2 and fucoxanthin had the highest concentrations near the surface and reached a maximum at 0.5-cm. Beta-carotene, diat, and zeaxanthin concentrations increased with depth. The concentration of diad reached undetectable levels below 0.5-cm. Maximum concentration peaks for chlorophyll-b, lutein, neoxanthin, and chlorophyll-a are seen at 1.75-cm depth and then they decrease rapidly to zero. At that same depth, beta-carotene, diat, fucoxanthin, and zeaxanthin concentrations completely diminish and then begin to increase below 1.875-cm. A slight increase is seen in chlorophyll-c_1c_2, fucoxanthin, and lutein below 1.75-cm. No chlorophyllide-a was present at site B.

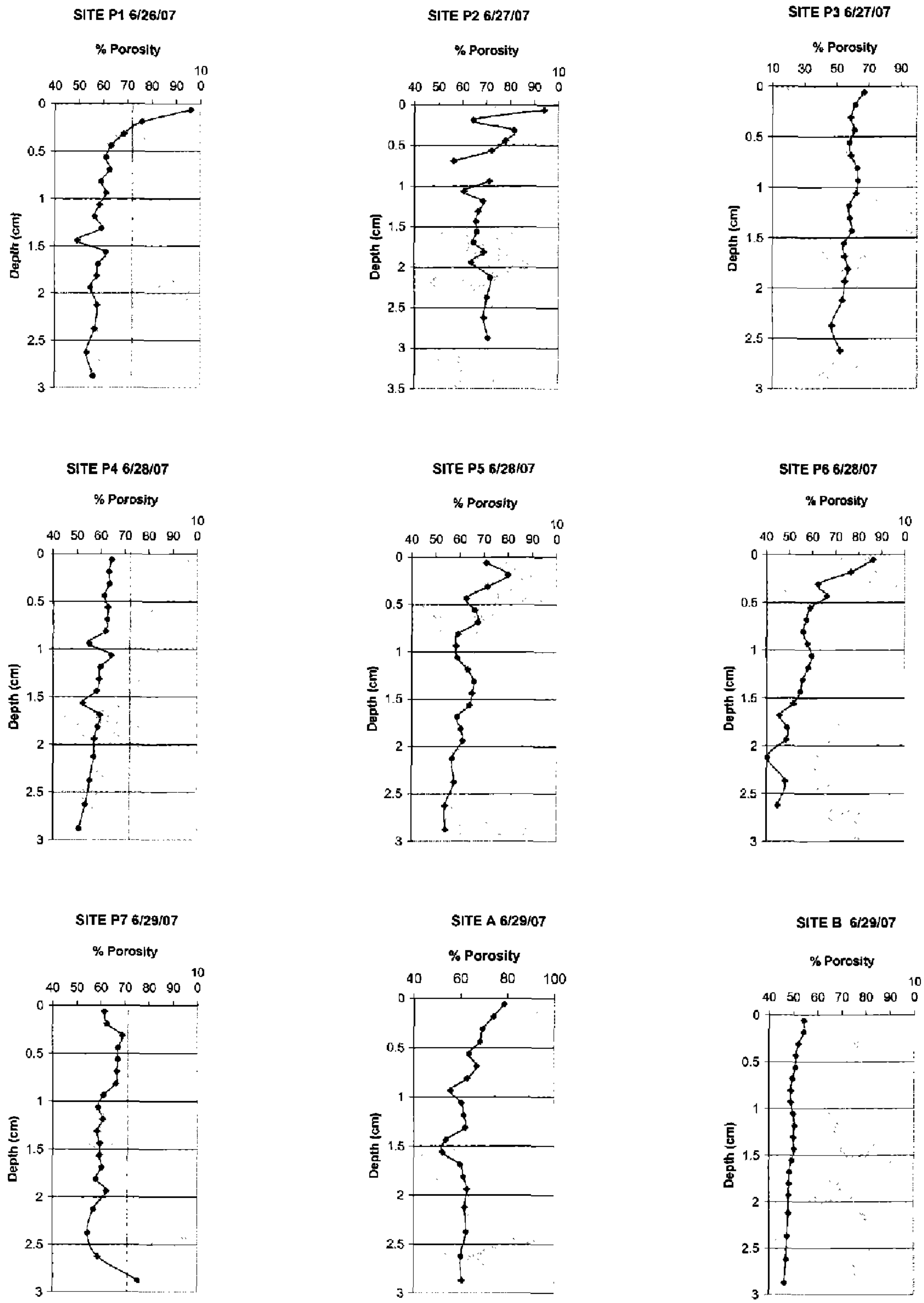


Figure 5. Porosity at the nine sampling sites reported as % porosity with increasing depth below the sediment-water interface, in cm. Note the expanded x-axis range for site P3.

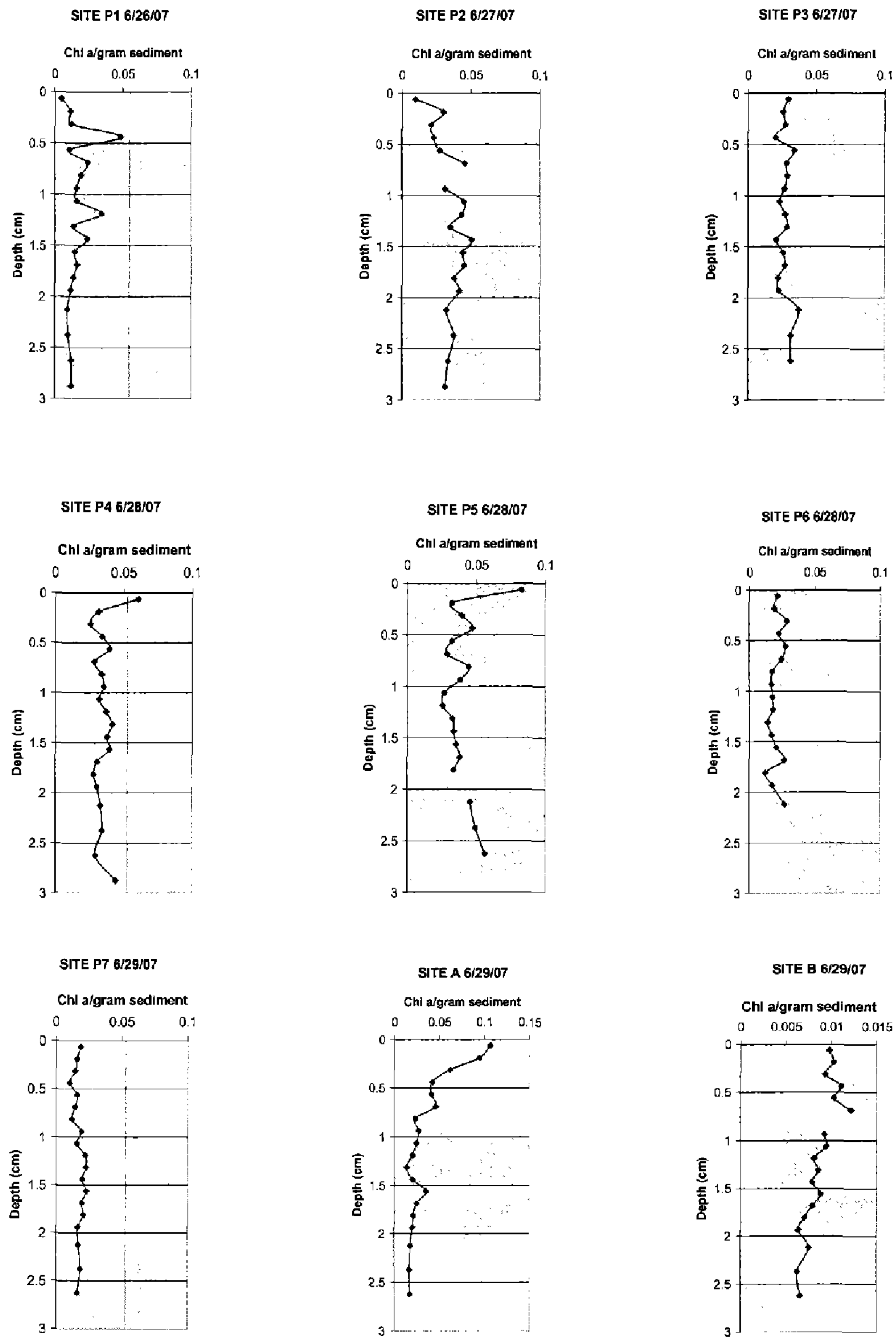


Figure 6. Chlorophyll-a at the nine sampling sites depicting the concentration of chlorophyll-a, in ug, per gram of sediment with increasing depth in cm. Note different concentration scales.

Table 1. Photopigments used by different photosynthetic phyla.

Phylum	**Pigment Name**									
	chl-a	chl-b	chl-c1c2	fucoxanthin (fuco)	zeaxanthin (zeax)	β-carotene	lutein	neoxanthin (neo)	diadinoxanthin (diad)	diatoxanthin (diat)
Chrysophyta (diatoms)	X		X	X		X			X	X
Chlorophyta (green algae)	X	X			X	X	X			
Cyanophyta (blue-green algae)	X				X	X				
Phaeophyta (brown algae)	X		X	X		X				
Vascular Plants	X	X	X			X	X	X		
Pyrrophyta (dinoflagellates)										

Table 2. Pigments extracted during HPLC from sites A and B.

Site	**Pigment Name**									
	chl-a	chl-b	chl-c1c2	fucoxanthin (fuco)	zeaxanthin (zeax)	β-carotene	lutein	neoxanthin (neo)	diadinoxanthin (diad)	diatoxanthin (diat)
A	X		X	X	X	X			X	X
B	X	X	X	X	X	X	X	X	X	X

*Chlorophyllide-a, degraded chlorophyll-a, was also extracted from site A.

Table 3. Possible phyla found at site A and B.

Site	**Phylum**				
	Chrysophyta (diatoms)	Chlorophyta (green algae)	Cyanophyta (blue-green algae)	Phaeophyta (brown algae)	Vascular Plants
A	X		X	X	
B	X	X	X	X	X

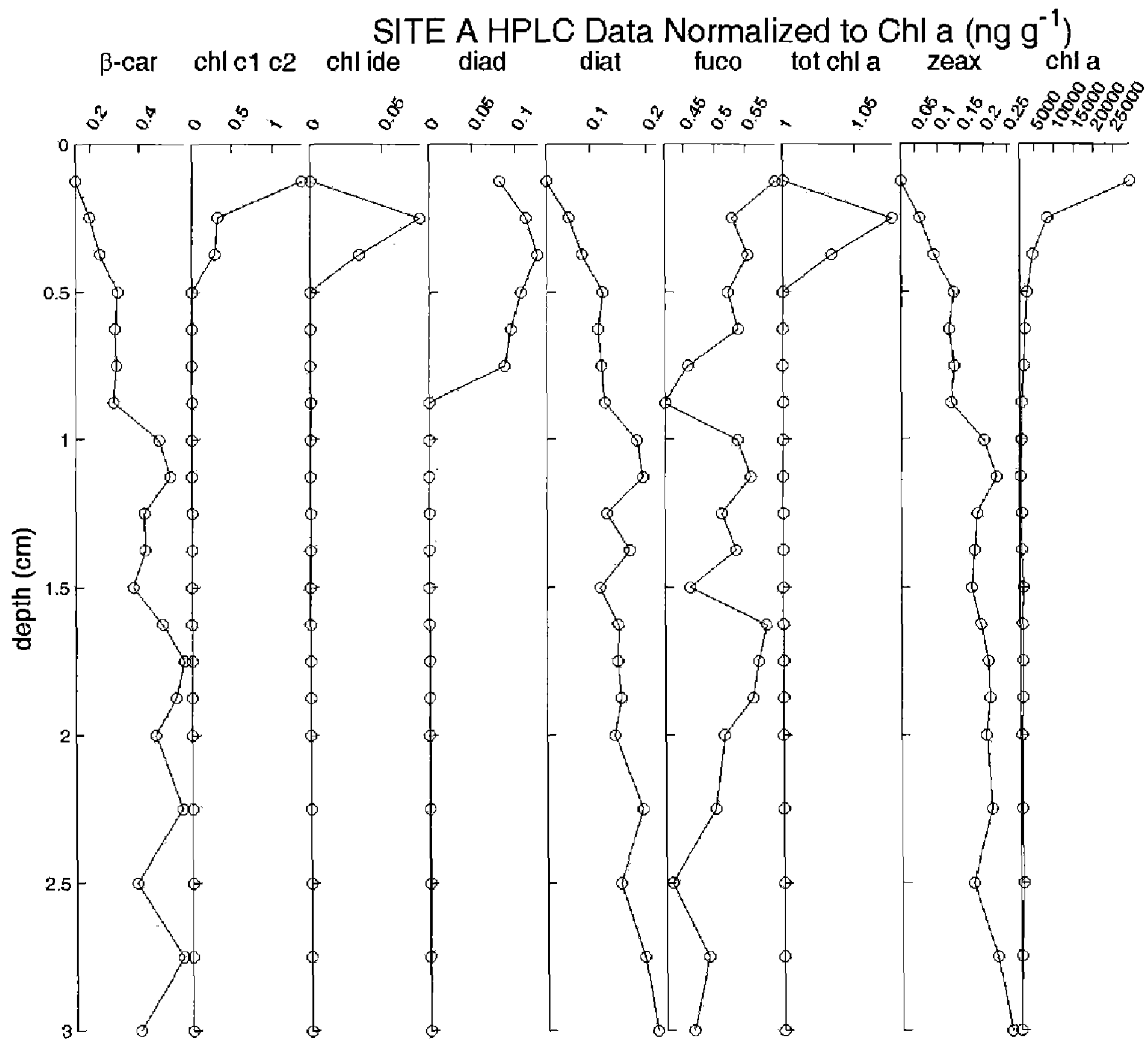


Figure 7a. Site A chlorophyll-a normalized photopigment depth profiles depicting the concentration of each photopigment, in ng per g.

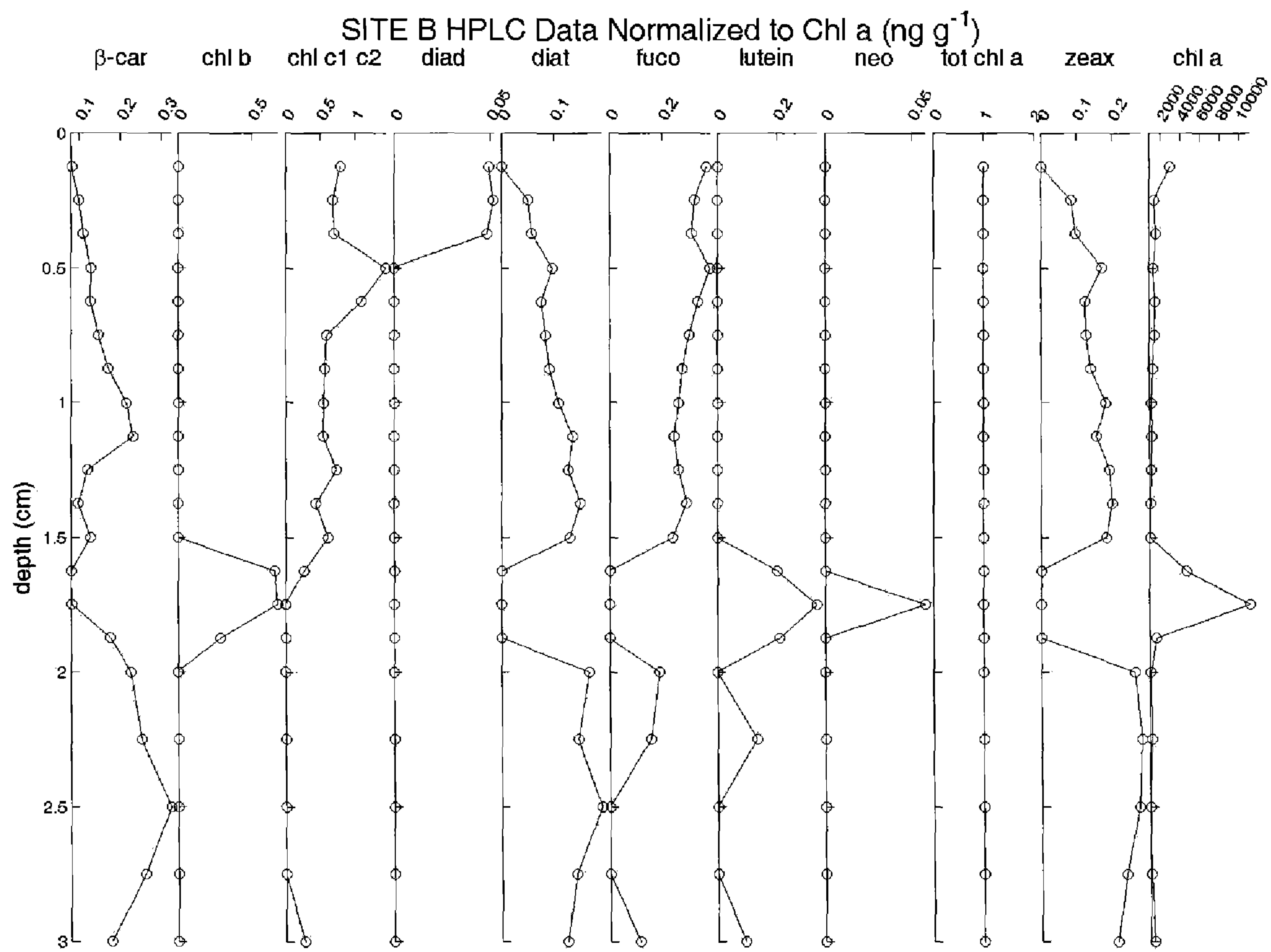
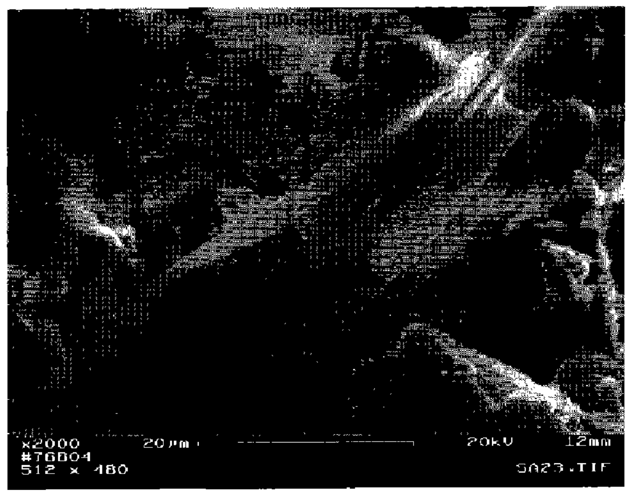


Figure 7b. Site B chlorophyll-a normalized photopigment depth profiles depicting the concentration of each photopigment, in ng per g.

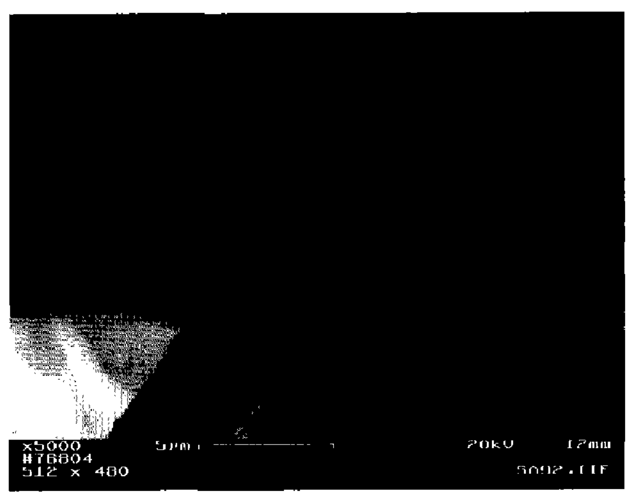
SEM

During SEM several organisms were found. Two were classified taxonomically. The organisms depicted in Figure 8b and 8e have been identified as pennate diatoms, from the sub-phylum Bacillariophyceae (see Fig. 9). All other organisms could not be identified due to rupturing during SEM and inadequate knowledge in terms of identifying phytoplankton based upon morphological characteristics. Below are photographs and descriptions of SEM results.

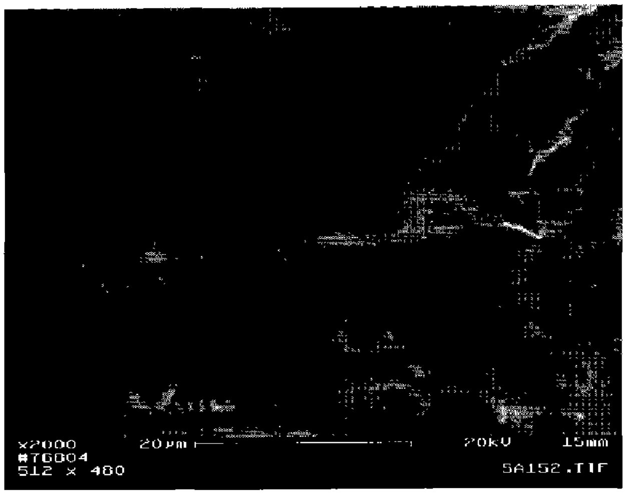
8a) Site A, 0.25-cm depth. Tube-like structures about 60-um in length and 5-um in diameter found in a tight bundle, which were ruptured during SEM analysis. Horizontal striations are visible along the length of each tube.



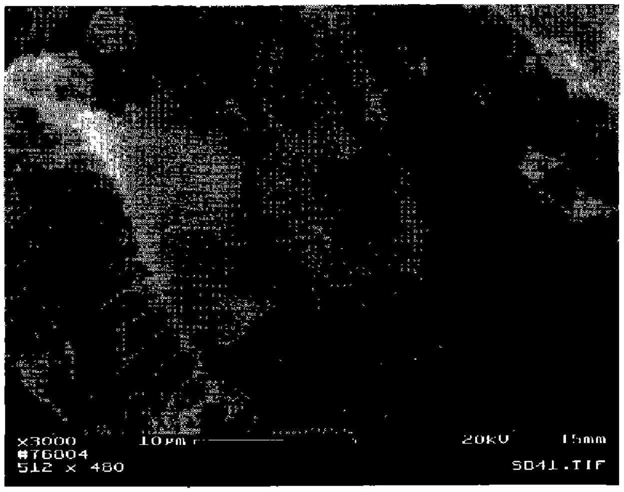
8b) Site A, 1.125-cm depth. Corn-husk shape embedded with rows of 3-4 small holes going down the length of the organism, about 20-um in length and 8-um at its widest point. Holes are located between the outside borders and the middle spine.



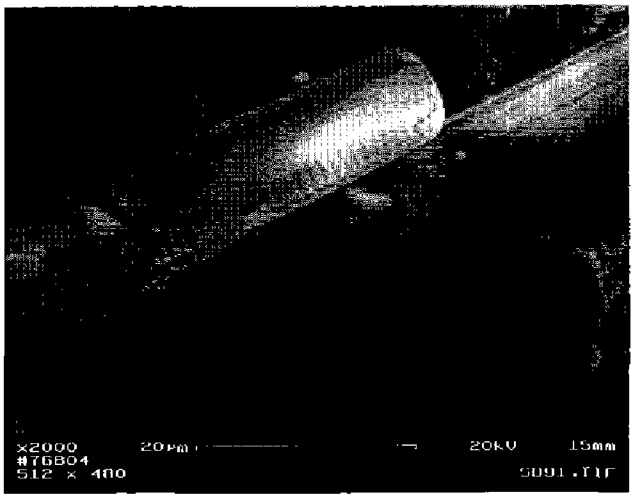
8c) Site A, 1.875-cm depth, the largest organism was about 40-um in length and a little less than 20-um at its widest point. Oval-shape with horizontal slits going down the middle of the organism. Both organisms were ruptured during SEM analysis.



8d) Site B, 0.5-cm depth. Unknown whether the cell, about 25-um in length and 15-um in width, ruptured during SEM analysis. Shield-shape with horizontal spines extending beyond the wall of the organism and that move along the length of the organism.



8e) Site B, 1.125-cm depth. Rectangular-shape with curved edges, about 30-um in length and 10-um in width. Thick spine bordered by horizontal slits extending to outer cell wall. A portion was ruptured during SEM analysis.



f) Site B, 1.75-cm depth. Football-shaped organism, about 15-um in length and 8-um at it widest point, with horizontal slits across the entire width that move down the length of the organism.

Figure 8. SEM photographs of benthic organisms observed at sites A and B in different depth intervals.

Bathymetry Map

Although all data collection was completed, I was not able to create a complete bathymetry map before my internship was finished. Data was given to the Ruttenberg lab and a complete bathymetry map should be completed in the next several weeks after synthesis of all the information. Map will be available upon request.

Discussion:

Overall chlorophyll-a depth and pond distribution

Here I address hypotheses 1 and 2. In hypothesis 1, hypothesized that the highest chlorophyll-a concentrations to would be located at the sandy, coarse sites, P1, P2, P3, P6, and B (Fig. 6), since sand grains are large and do not compact tightly together allowing more sunlight to penetrate deeper into the sediment. However, the muddy sites, P4, P5, and A, displayed the highest chlorophyll-a concentrations. Site A had the highest surface chlorophyll-a concentration of all the sampling sites, while sites P1 and P2, found in the sandy facies, displayed the lowest surface chlorophyll-a concentrations. Sites P4 and P5, located in the facies transition zone, where the sandy sediment mixes with the muddy sediment, showed consistently high chlorophyll-a concentrations with increasing depth. Site B had the lowest chlorophyll-a concentration with depth.

High porosity may have contributed to high chlorophyll-a concentration. According to Berner, the fine, muddy sediments should have a higher surface percent porosity (1980). The finer sediment grains found at the muddy facies repel each other when they are first deposited and create more space for water. Over time, the fine grains become tightly compacted, leaving less room for water (Berner 1980). This was true for sites P4, P5, and A (Fig. 5). Meanwhile, sand grains are larger and do not compact as tightly as mud and silt and the coarser grains have advection that allows overlying water to diffuse to deeper depths. Overall, the porosity of the sandy sites should be in the medium to low percentile and they should stabilize with depth. This was seen at sites P1, P2, P3, P6, and B.

The high surface porosity seen at sites P1, P2, and P6 (Fig. 5) could be due to recent mangrove removal and the deposition of silty clay near these sites. The surface of the sediment at these sites may have been covered by the silty clay material that eroded from the mangrove stands, which explains the high surface porosity. Beneath the silty clay is sand. This is supported by the porosity profiles for sites P1, P2, and P6, which stabilize with depth.

Surface chlorophyll-a concentration for site A (Fig.6) can be attributed to its relative closeness to the river, mangroves. The mud and silt from the mangroves erode from the land and settle on the pond floor. This mud and silt is rich in organic matter and more organisms are able to thrive off this organic rich sediment from the land. The same reasoning goes for sites P5 and P4. Site P7 is located in a high flow rate area, and this may have led to a low chlorophyll-a concentration. The low chlorophyll-a concentrations at the sandy sites are probably due to low organic matter availability, a high flow rate, and lower porosity in those locations.

I anticipated that maximum chlorophyll-a concentrations would be found near the surface since sunlight is unable to penetrate deep within bottom sediments (Hypothesis 2). However, surficial chlorophyll-a maxima were observed only at sites P4, P5, and A (Fig. 5). For these three sites, chlorophyll-a concentration reached its highest concentration at the surface and then diminished rapidly with depth. Below 0.125-cm, chlorophyll-a concentrations for sites P4 and P5 stabilized. A high abundance of photosynthetic organism at these sites may have attributed to relatively high chlorophyll-a concentrations with increasing depth. Both sites A and B showed a steady decrease in chlorophyll-a concentration with increasing depth. For sites A and B, the gradual decrease in chlorophyll-a concentration is due to a decrease in sunlight availability. Mixing or burial of organic matter may have caused the small increases in chlorophyll-a concentration below the surface at sites P1 and P2. The sediment at these sites is very porous (Fig. 5). Since both are located near mangrove stands and much of the pond is covered with invasive macroalgae, wind as well as human and animal disturbances could have mixed vascular plant or macroalgal material to depth causing sub-surface maxima.

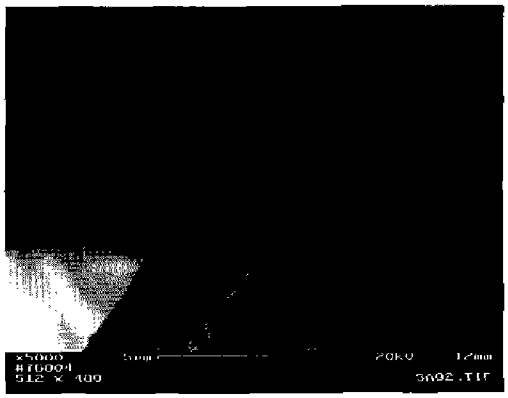
Relationship between algal phylum and substrate facies (sandy vs. muddy)

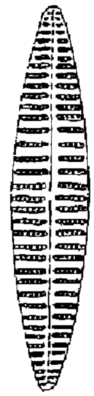
In this section, I address hypothesis 3 and discuss photopigment results. In my third hypothesis, I state that sediment facies and composition will drive differences in microbial community composition. More specifically, I expected to observe calcium carbonate ($CaCO_3$) producing chlorophytes (green algae) at sandy sites. Although identifying photopigment distributions initially seemed an adequate approach for evaluating the presence of specific photosynthetic organisms, in the end, not many decisive conclusions could be drawn. The photopigments extracted at sites A and B are summarized in Table 2, their depth profiles are shown in Figure 7. All photosynthetic benthic organisms harbor different combinations of chlorophylls and carotenoids in order to achieve maximum photosynthetic rates for the depth and sediment facies in which they live. Caratenoids are a class of photosynthetic pigments that differ from chlorophylls (Kirk, 1983). The fact that several different photosynthetic phyla could have nearly the same combination of photopigments makes it difficult to conclusively identify the exact phyla found at each depth (Kirk, 1983). Initially, I anticipated that HPLC analysis would yield enough information to place organisms in taxonomic classes, which would have been more diagnostic for their functionality. The resolution achieved, however, was not sufficient to achieve this goal. For this reason, I will only make broad statements addressing the possible phyla found at each site.

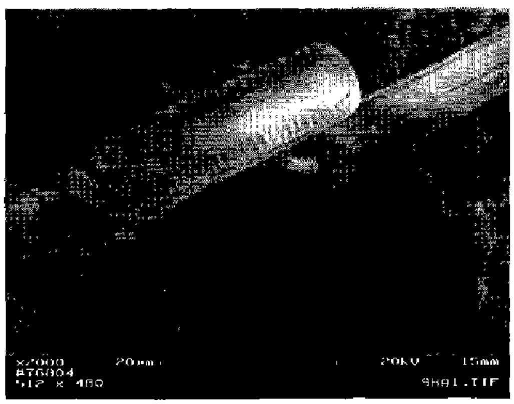
Both sites A and B showed a constant presence of diatoms with depth as indicated by the presence of diadinoxanthin (diad) and diatoxanthin (diat) (Fig. 7a and b). Interestingly enough no dinoflagellates were found at either site. As indicated by Table 1, diatoms are the only organisms that produce the photopigments diadinoxanthin and diatoxanthin. Both diad and diat were detected at sites A and B (Table 2, Figure 7a and b), and thus I can be sure that Chrysophyta is present at both sites. These diatoms are from the sub-phylum Bacillariophyceae and are found in marine and freshwater environments. They are important phytoplankton components that serve as a main food source for zooplankton (Algae Research 2007). Also at both sites, a high concentration of fucoxanthin was extracted. Fucoxanthin is the main photopigment used by phaeophytes, multicellular brown algae and is found mostly in marine environments (e.g., Algae Research 2007; Kirk 1983).

Photosynthetic organisms that belong to the phylum Chlorophyta use chlorophyll-a, chlorophyll-b, beta-carotene, lutein, and zeaxanthin during photosynthesis. Chlorophytes use $CaCO_3$ to construct their cell walls and thus have the ability to produce large quantities of $CaCO_3$. In my third hypothesis I address the fact that chlorophytes would be found at site B, the sandy facies. At site B, chlorophyll-a, chlorophyll-b, lutein, and neoxanthin reach a maximum peak at 1.75-cm depth. Although all of these pigments, except neoxanthin, are characteristic of the photosynthetic benthic phylum, chlorophyta, the rapid increase in these certain pigments, including neoxanthin, could be explained by the presence of a vascular plant leaf. Leaf material could have been mixed into the sediment at site B from the nearby mangrove stand. The high concentrations of these pigments at 1.75-cm swamped other pigments which appeared to drop below detectable limits at 1.75-cm depth.

SEM analysis was intended to confirm photopigment results and it was hoped that these coupled analytical approaches would enable identification and placement of organisms into specific phyla. However, the coupled SEM, photopigment approach was not much more conclusive than the photopigment results alone. Using published morphological descriptions of the specific physical characteristics of the organisms within each phyla that were identified by the photopigment results, I performed visual analysis of SEM photographs in an attempt to place each organism into its own phylum. None of the organisms appeared to be multicellular, which means that no members of Phaeophyta, multicellular brown-algae, were seen during SEM. Photographs in Figure 8b, siteA, and 8e, site B, show characteristics consistent with diatoms from the sub-phylum Bacillariophyceae. They are pennate shape and could possibly belong to the sub-order Bacillariineae, which consists of pennate diatoms (Tomas 1997). Both photographs were taken at a depth of 1.125-cm. For sites A and B, beta-carotene, diatoxanthin, and fucoxanthin, all photopigments found in diatoms, are seen at this same depth. Comparisons of SEM photographs 8b and 8e, to drawings of pennate diatoms from the suborder Bacillariineae (Tomas 1997) are shown in Figure 9. Further visual analysis, beyond the scope of my internship project, could result in additionally positive identification of specific classes and sub-classes of benthic phytoplankton using the coupled SEM-photopigment approach.

a1) 

a2) 

b1) 

b2) 

Figure 9. Comparison of SEM photographs to drawings of diatoms from the Bacillariineae suborder. a1) Site A, b1) Site B (Tomas 1997).

Conclusion:

Contrary to my first hypothesis, chlorophyll-a concentrations were higher within the muddy facies rather that within the sandy facies. While I initially speculated that enhanced sunlight penetration due to higher porosity would promote colonization by benthic algae, my results suggest that porosity and chlorophyll-a concentration is in fact highest within the muddy facies. The small silt grains of the muddy facies have a larger surface area and a higher organic matter content, which better promotes colonization of benthic algae. Sites P4, P5, A, and B showed maximum chlorophyll-a concentration at the surface, which is consistent with hypothesis 2. However, no other sites displayed chlorophyll-a maxima at the sediment-water interface. This was probably due to low organic matter availability, a high flow rate, and lower porosity at these locations. Contrary to my initial expectation (hypothesis 3) that chlorophytes would be present at site B, no chlorophytes were observed. The coupled SEM-photopigment approach was used to successfully identify the sub-phylum Bacillariophyceae at both sites A and B.

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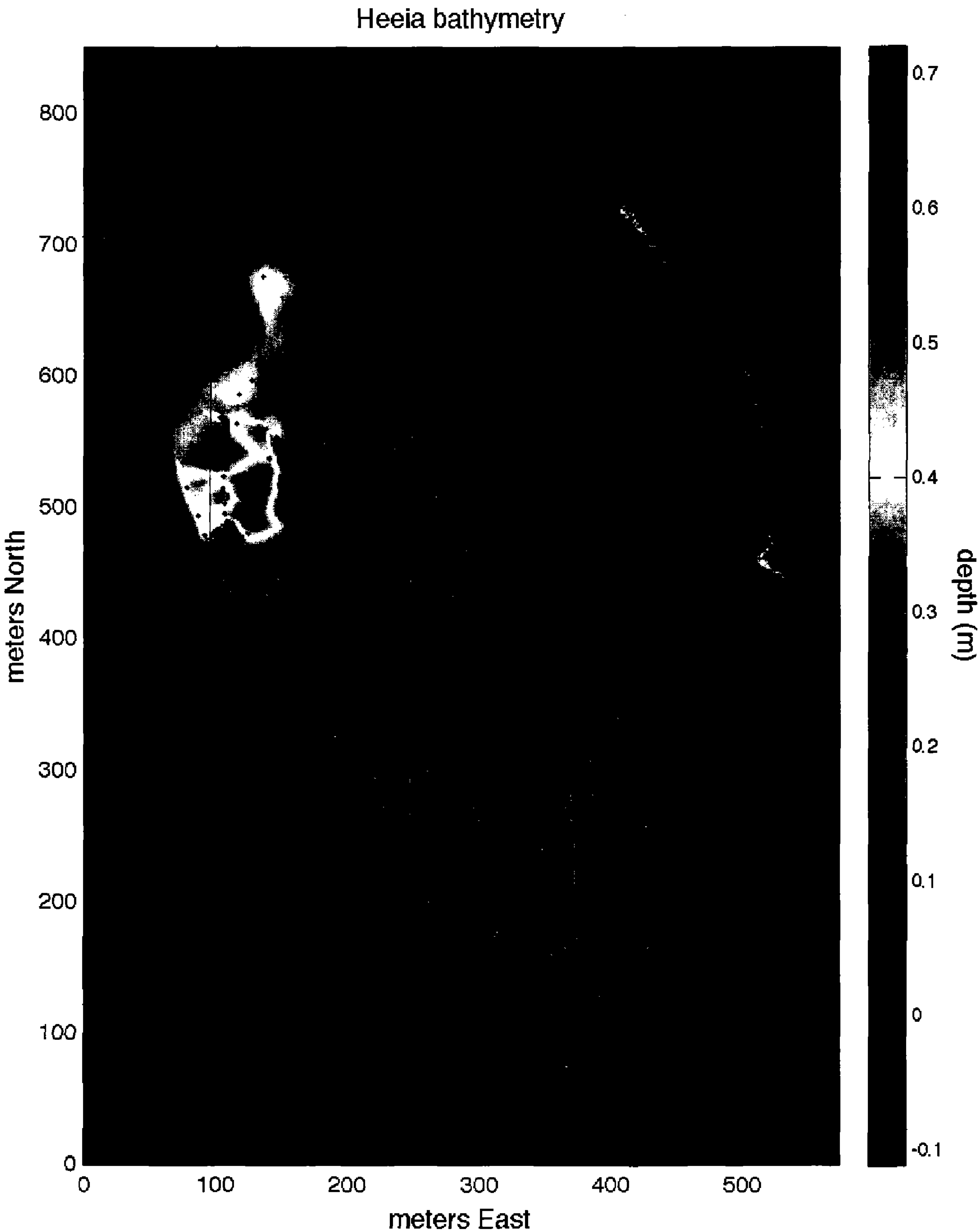
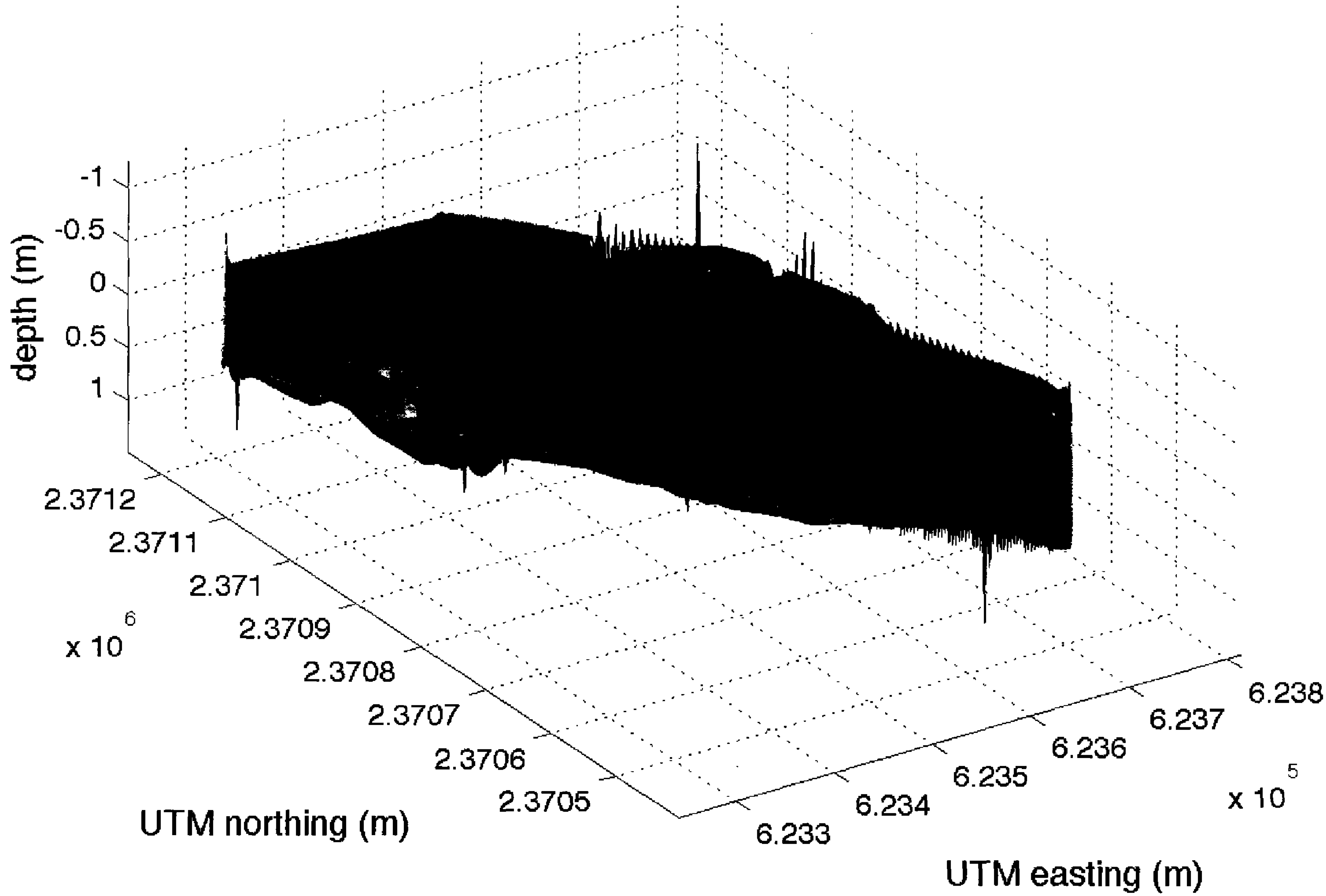

Heeia bathymetry
meters North
meters East
depth (m)
800
700
600
500
400
300
200
100
0
0
100
200
300
400
500
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1


depth (m)
-1
-0.5
0
0.5
1
2.3712
2.3711
2.371
2.3709
2.3708
2.3707
2.3706
2.3705
x 10^6
UTM northing (m)
6.233
6.234
6.235
6.236
6.237
6.238
x 10^5
UTM easting (m)

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