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Characterizing diatom biofilms and their influence
on the sand biogeochemistry of high energy beaches

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Marine Science

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Abstract

High energy beaches are among the most dynamic ecological settings on Earth. Compared to mudflats, diatoms of high energy beaches have been more neglected in the literature, particularly true of the intertidal biofilms that form and dissipate with nutrient cycling and light intensity over the tidal cycle. Although short lived, the productivity of these biofilms may be critical to the organic-poor sand. Through sediment coring and subsequent analyses of the uppermost sand of three suspected biofilm stations—non-runnel, runnel crest, and runnel trough—along with seemingly bare sand as a control, this study sought to verify the presence of diatom biofilms and characterize their influence on the sand biogeochemistry of the uppermost sand at Waties Island, South Carolina. Diatom abundance was determined using H2O2 digestion followed by enumeration via microscopy. Four parameters related to biological productivity were analyzed: chlorophyll α concentration using fluorometry, total carbohydrate concentration using phenol-sulfuric acid extraction, organic carbon concentration using loss on ignition (LOI), and total phosphorus concentration using HCl extraction of ignited subsamples followed by the phosphomolybdate blue method. Water column diatom concentration and chlorophyll α concentration were also determined for additional comparisons. Each parameter was plotted against depth to produce profiles to visualize the influence of biofilms on the underlying sand. Regression analyses were used to examine correlations between parameters, which were compared to time series data for Waties Island. Our findings suggest diatom biofilms significantly contribute to overall productivity on high energy beaches.
Introduction

High energy beaches have great economic and ecological importance. In the United States alone in 2017, Houston (2018) estimated beaches produced $285 billion in direct spending and supported 4.4 million jobs directly and indirectly. These same beaches also provide storm buffering, nutrient cycling, water filtration/purification, nursery habitats, and feeding and/or breeding habitats, including for endangered species like sea turtles and shorebirds (Nel et al., 2014). These economic impacts and ecological importance are threatened by climate change however, perhaps most seriously by the global sea level rise of roughly 2.0mm per year (Engelhart et al., 2009).

Although once known as “marine deserts,” the challenging environment provided by high energy beaches features thousands of adapted species (Riedl and McMahan, 1974). These include thriving microbial communities, even at the surface that is submerged and exposed over the tidal cycle (Novitsky and MacSweeney, 1989). Perhaps most important of these are the epipelic, primarily pennate diatoms whose extensive extracellular polysaccharides, often called extracellular polymeric secretions (EPS), affect sediment texture and erodibility to form biofilms (Paterson, 2001; Paterson, 1989). This EPS binds and concentrates organic matter and ions near diatoms as well as provides surface area to localize bacterial activity, both of which contribute to the efficient biomineralization of organic matter and nutrients (Decho, 2000). The productivity of these biofilms has been shown to contribute these beaches’ ability to support much larger species like shorebirds (Schlacher et al., 2017).

Compared to similar biofilms that form in mudflats, the literature surrounding the biofilms of high energy beaches is relatively poor despite their importance. It is critical that understanding of these biofilms improve to predict how productivity on high energy beaches
may change with beach development and restoration, as well as climate change. In freshwater streams, similar epipelic diatom biofilms can be used with environmental parameters to more accurately judge the health of a system (Kivrak and Uygun, 2012). Better understating these biofilms may be particularly relevant to endangered species conservation, since these biofilms have been shown to effectively concentrate and transfer contaminants into food webs (Decho, 2000).

To promote the understanding of these biofilms as found on high energy beaches, this study investigated diatom biofilms and their influence on surface sand biogeochemistry using syringe coring to determine four parameters related to biological activity: chlorophyll $\alpha$ concentration, loss on ignition (LOI), total carbohydrate concentration, and total phosphorus concentration. Chlorophyll $\alpha$ concentration is directly related to the presence of photosynthetic cells, with the pheophytin $\alpha$ to chlorophyll $\alpha$ ratio inversely proportional to the relative proportion of chlorophyll $\alpha$ found in living cells (Yentsch and Menzel, 1963). LOI is a quantitative method to determine organic carbon content, which will increase as the number of cells present increases (Santisteban et al., 2004). Total carbohydrate concentration similarly increases with the presence of cells, with the EPS of the diatom biofilm contributing to this concentration (Smith and Underwood, 1998). Total phosphorus concentration is also similar, as organic forms of phosphorus are incorporated into the macromolecules in cells (Libes, 2009). Since each parameter is related to biological activity, regression analysis was used to investigate correlations between the parameters that should intrinsically exist between them. Observations of these biofilms and their distributions over multiple days of varying cloud cover are also presented.
**Hypotheses**

1. Across any of the four biological parameters sampled, there will be notable enrichment at the surface associated with the presence of a biofilm.

2. These enrichments will be most significant in the non-runnel biofilms because this area has been exposed by the tide for the longest time.

3. The runnel trough enrichments will be lower than those of the runnel crest because the morphology of the crest may fuel biofilm development.

4. The pheophytin α to chlorophyll α ratio will be lowest in the biofilm stations, since more chlorophyll α will be present in living cells, and increase with depth as chlorophyll α becomes degraded in non-living cells.

5. Significant positive correlations between the four parameters will be detected through regression analysis, since each is related to biological activity.

**Methods**

*Study Site and Sampling Design*

Sampling was conducted on 10-20-18 during low tide at Waties Island, South Carolina (Figure 1). Waties Island is an undeveloped, private barrier island complex along the Grand Strand of South Carolina, an otherwise developed and highly economically developed region (Schwab et al., 2009). Prior to sampling, observations were made on 7-29-18 and 8-25-18 during low tide to determine what stations to sample and better characterize the biofilms at Waties Island.
Figure 1. Satellite image of Waties Island, South Carolina. Sampling and observations occurred within the region shaded in green. Historic and time series sampling by Hannides et al. (in prep.) also occurred in this region.

Sampling consisted of four types of stations: bare sand, non-runnel biofilm, runnel crest, and runnel trough. The bare sand station included a section of the beach with no observable biofilms, and served as a baseline when comparing parameters to the biofilm stations. The non-runnel biofilm station included suspected biofilm activity that occurred between the wrack line and runnel (Figure 2A). As for the two runnel biofilm stations, the crest and trough of a ripple with suspected biofilm activity were sampled (Figure 2B). The unique morphology of runnel ripples may create mixing conditions conducive of biofilm formation in the crest, as demonstrated in the past for Waties Island with respect to chlorophyll $a$ (Figure 3).
Figure 2. A The non-runnel biofilm station being sampled on 10-20-18 at Waties Island, South Carolina, with bare sand like the bare sand station present in the background. B The runnel stations being sampled together on 10-20-18 at Waties Island, South Carolina.

Figure 3. Integrated chlorophyll α values over 0-5cm as sampled and analyzed by Hannides et al. (in prep.) between 11/16 and 3/18. On many sampling dates, runnel crest cores had a higher integrated chlorophyll α value than those of the runnel troughs. Runnel points represent dates with no ripple formation and low-tide swash points represent coring in the swash zone.
For each individual station, two 5-cm syringe cores were collected and divided into 0.5-cm intervals until 2 cm deep from the surface, after which cores were sliced at 1-cm intervals. This produced 7 intervals per core, which were placed on ice until they were frozen. Just prior to freezing, a small (~15 mg) aliquot was taken from the surface intervals (0.0-0.5 cm) of all stations and dried to use for diatom verification under a light microscope. One core from each station was randomly selected for chlorophyll α and total carbohydrate analyses. The remaining core from each station was subjected to combustion for loss-on-ignition (LOI) and subsequently phosphorus analysis. Water samples were also taken from the overlying runnel water and the swash zone to help determine cumulative variation for the chlorophyll α analysis.

Chlorophyll α Analysis

The chlorophyll α concentration for each interval was determined using the acetone extraction and fluorometry as described by Hannides et al. (2014), which was based on the method for water samples by Arar and Collins (1997) that was applied to the water samples. For each interval, weighed wet sediment samples (which represented the entire interval minus the carbohydrate aliquot) and filters from water samples vacuum filtered according to Parsons et al. (1984) were combined with 10mL acetone, then extracted while frozen for 24 hours. Using a Turner Designs Trilogy fluorometer, the raw fluorescence of all extractions were measured as extracted, then acidified with concentrated HCl to determine chlorophyll α and pheophytin α concentrations. The dry weight of sediment samples was determined by decanting off the extraction solution and allowing samples to dry completely.

The concentrations of chlorophyll α and pheophytin α were determined in μg pigment per cm³ sediment as follows:

\[
[\text{Chl } \alpha] = \frac{(F_S) \left( \frac{r}{F - 1} \right) (R_{\text{Int}} - R_{\text{Acid}})(V_E)}{(D)(F_C)(V_{\text{sed}})}
\]
\[ [\text{Phe } \alpha] = \frac{(F_s)\left(\frac{r}{r - 1}\right)(rR_{\text{Acid}} - R_{\text{Int}})(V_E)}{(D)(F_C)(V_{\text{sed}})} \]

\( F_s, r, \) and \( F_c \) are values related to the fluorometer itself \( (F_s) \) and reflect the corrections needed over the lifetime of a fluorometer \( (r \) and \( F_c) \) as shown by variance in solid standards. \( R_{\text{Int}} \) and \( R_{\text{Acid}} \) represent the raw fluorescence of the initial and acidified extractions respectively. \( V_E \) represents the volume of the extraction in liters \( (\text{in this study, } V_E = 0.010 \text{ L}). \) \( D \) represents the dilution factor of the extraction:

\[ D = \frac{V_E}{(W_{\text{wet}} - W_{\text{dry}} + V_E)} \]

\( W_{\text{wet}} \) and \( W_{\text{dry}} \) represent the wet and dry weights of a sample respectively. The last variable in the concentration equations, \( V_{\text{sed}} \), represents the sample’s sediment volume calculated from the mass of dry sediment, \( M_{\text{drysed}} \), corrected for the mass of sea salt in the sample \( (M_{\text{salt}}): \)

\[ M_{\text{salt}} = (W_{\text{wet}} - W_{\text{dry}})(\rho_w - 1) \]

\[ M_{\text{drysed}} = W_{\text{dry}} - W_{\text{vial}} - M_{\text{salt}} \]

\[ V_{\text{sed}} = (W_{\text{wet}} - W_{\text{dry}}) + \frac{M_{\text{drysed}}}{\rho_{\text{sed}}} \]

\( W_{\text{vial}} \) is simply the weight of the empty sampling vial, \( \rho_w \) reflects the density of pore water in g per cm\(^3\) as measured in the field using a YSI Pro 2030, and \( \rho_{\text{sed}} \) reflects the density of the sediment, typically cited as 2.65 g per cm\(^3\) for marine sediment \( (\text{Breitzke, 2006}). \)

For the water samples, the concentrations of chlorophyll \( \alpha \) and pheophytin \( \alpha \) were determined in \( \mu g \) pigment per L as follows:

\[ [\text{Chl } \alpha] = \frac{(F_s)\left(\frac{r}{r - 1}\right)(R_{\text{Int}} - R_{\text{Acid}})(V_E)}{(F_C)(V_S)} \]
Total Carbohydrate Analysis

The total carbohydrate concentration was determined using the phenol-H$_2$SO$_4$ assay for sediment as outlined by Underwood et al. (1995), except in a miniaturized version with a total reaction volume of 1 mL being developed by the Sand Biogeochemistry Group of Coastal Carolina University. For each interval, a weighed aliquot of ~50 mg wet sediment (collected prior to the addition of acetone for chlorophyll $\alpha$ analysis) was subjected to this miniaturized reaction between aqueous phenol and concentrated H$_2$SO$_4$. This reaction was repeated for a triplicate set of glucose standards. Each reaction was allowed to stand for 10 minutes, then placed in a water bath at 25°C with shaker for several hours (Dubois et al., 1956). Following the completion of these reactions, absorbance was measured at 485 nm using a Thermo Scientific Genesys 30 spectrometer for each interval and compared against the standard curve generated using the glucose standards to produce a total carbohydrate concentration in μmol glucose equivalents per liter, or a μM concentration.

This concentration was expressed in units of μg glucose equivalents per cm$^3$ of sediment as follows:

$$[\text{Glucose Equivalents}] = \frac{(E_s)(\text{molar mass of glucose})(V_E)}{V_{sed}}$$

$E_s$ represents the μM glucose equivalents concentration from the standard curve for a sample. The molar mass of glucose is in μg per μmol to cancel the μmol of $E_s$. $V_\text{E}$ is again in liters as in the chlorophyll $\alpha$ analysis, leaving only μg in the numerator of this equation. $V_{sed}$ was calculated as before, although $W_{dry}$ was not measured directly for these aliquots. Instead, $W_{dry}$
was determined from the subsampling ratio, $R_{sub}$, between the carbohydrate aliquot and chlorophyll $\alpha$ sample, since the ratio of water to sediment within the overall interval should be conserved within an aliquot:

$$R_{sub} = \frac{(W_{wet} - W_{tube}) \text{ for carbohydrate aliquot}}{(W_{wet} - W_{vial}) \text{ for chlorophyll } \alpha \text{ sample}}$$

$$W_{dry} \text{ for carbohydrate aliquot} = (R_{sub})(W_{dry} \text{ for chlorophyll } \alpha \text{ sample})$$

Similar to $W_{vial}$ for a chlorophyll $\alpha$ samples, $W_{tube}$ is simply the weight of the reaction tube and takes the place of $W_{vial}$ when calculating $M_{salt}$ for a carbohydrate aliquots. Because the ratio of water to sediment is conserved, $M_{salt}$ for a carbohydrate aliquot can also be expressed as:

$$M_{salt} \text{ for carbohydrate aliquot} = (R_{sub})(M_{salt} \text{ for chlorophyll } \alpha \text{ sample})$$

**Loss-on-ignition (LOI)**

Organic carbon content was determined using loss-on-ignition (LOI) as in Santisteban et al. (2004). For each interval, the wet weight was measured, then intervals were dried in an oven at 65 °C for several hours (this represents the drying process for any dried sample across all analyses). The dried intervals were then combusted at 550 °C in a muffle furnace for 4 hours to combust their organic carbon content. As is standard, LOI was calculated as a percent using the weight of each combusted interval and correcting for salt content as follows:

$$\text{LOI (\%)} = \frac{W_{dry} - W_{comb}}{W_{dry} - M_{salt}} \times 100$$

$W_{comb}$ represents the weight of the combusted sediment. $M_{salt}$ for these samples was calculated exactly as with the chlorophyll $\alpha$ samples.

**Total Phosphorus Analysis**

Total phosphorus concentration was determined from combusted (sometimes described as ashed) sediment through a modification of the SEDEX scheme (Ruttenberg, 1992) and the
phosphomolybdate blue method (Murphy and Riley, 1962). The SEDEX scheme is a sequential method to extract different types of phosphorus in each step for a given sediment sample, with the final step being combustion at 550 °C and HCl extraction to extract organic phosphorus. If this final step was performed first, total phosphorus should be extracted, since any phosphorus in the sample following combustion should exist as phosphate. For each LOI interval, this assumption was explored by taking a ~250 mg aliquot of combusted sediment and extracting with 10 mL 1 M HCl over 16 hours in a 25°C water bath with shaker. Following this incubation, the entire extraction solution was transferred to a separate tube and centrifuged at 2500 rpm for 10 minutes, both steps attempting to remove any sediment. Triplicate blanks also underwent these steps.

The uppermost 5mL of supernatant were separated, but only 1.25 mL of this supernatant were used for the phosphomolybdate blue method, which generates phosphomolybdate with phosphate using a mixed reagent of ammonium heptamolybdate and potassium antimonyl tartrate with an ascorbic acid reagent containing ascorbic acid and H2SO4. The absorbance of the generated pigment was measured at 880nm, again using the Thermo Scientific Genesys 30 spectrometer. This complexion method requires a pH of 1 in the extraction prior to being applied however. Because all HCl extractions were slightly too acidic (pH = ~0), 1 mL of 4 M NaOH was added to the uppermost 5 mL of separated supernatant to raise them to a pH of 1 prior to analysis with the phosphomolybdate blue method. Triplicate standards were generated from stock phosphate and underwent the phosphomolybdate blue method also to create a standard curve to compare the absorbances of samples to, but an undetermined problem that resulted in no reaction in the standards resulted in reliance on a historic standard curve instead. These
phosphorus concentrations were in \(\mu\)M concentrations, and converted to \(\mu\)g phosphorus per cm\(^3\) of sediment as follows:

\[
[P] = \frac{(P_s) (\text{molar mass of P})(V_E)}{V_{sed}}
\]

This equation is principally the same as that used to determine the \(\mu\)g glucose equivalents per cm\(^3\) from a \(\mu\)M concentration. \(P_s\) is this \(\mu\)M concentration from the absorbance, with the molar mass of phosphorus and \(V_E\) using the same units as the aforementioned equation. Like with the carbohydrate aliquots, \(V_{sed}\) for the phosphorus aliquots requires the calculation of the subsampling ratio \((R_{sub})\) from the larger samples whose weights are known:

\[
R_{sub} = \frac{(W_{comb}) \text{ for phosphorus aliquot}}{(W_{comb}) \text{ for LOI sample}}
\]

\[
W_{dry} \text{ for phosphorus aliquot} = (R_{sub})(W_{dry} \text{ for LOI sample})
\]

\[
W_{wet} \text{ for phosphorus aliquot} = (R_{sub})(W_{wet} \text{ for LOI sample})
\]

\[
M_{salt} \text{ for phosphorus aliquot} = (R_{sub})(M_{salt} \text{ for LOI sample})
\]

**Parameter Regression Analyses**

Least-squares regression tests were performed between all parameters analyzed by calculating the integrated sum over the entire core to generate integrated values in [unit mass] per m\(^2\) sediment.

For chlorophyll \(\alpha\), this integration was performed as in Hannides et al. (2014) using the chlorophyll \(\alpha\) concentration calculated for each interval to produce an integrated value in mg chlorophyll \(\alpha\) per m\(^2\) sediment:

\[
\Sigma[\text{Chl a}] (0 - 5\text{cm}) = \frac{([\text{Chl a}]_{0\text{cm}} + \cdots [\text{Chl a}]_{5\text{cm}})(5\text{cm} - 0\text{cm})(1\text{mg})(100^2\text{cm}^2)}{(1000\mu\text{g})(1\text{m}^2)}
\]
For total carbohydrate, this integration was performed using the glucose equivalents (GE) concentrations calculated for each interval to produce an integrated value in g glucose equivalents per m\(^2\) sediment:

\[
\Sigma[\text{GE}] (0 - 5\text{cm}) = \frac{([\text{GE}]_{0\text{cm}} + \cdots + [\text{GE}]_{5\text{cm}})(5\text{cm} - 0\text{cm})(1\text{g})(100^2\text{cm}^2)}{(10^6\mu\text{g})(1\text{m}^2)}
\]

For total phosphorus, this integration was performed using the phosphorus concentrations calculated for each interval to produce an integrated value in g phosphorus per m\(^2\) sediment:

\[
\Sigma[\text{P}] (0 - 5\text{cm}) = \frac{([\text{P}]_{0\text{cm}} + \cdots + [\text{P}]_{5\text{cm}})(5\text{cm} - 0\text{cm})(1\text{g})(100^2\text{cm}^2)}{(10^6\mu\text{g})(1\text{m}^2)}
\]

For organic carbon, LOI had to be expressed in SI units rather than percent LOI for this integration. For each interval, this was done using the equation derived by Vess and Hannides (2018) to express the organic carbon concentration in mg organic carbon (OC) per cm\(^3\) of sediment accounting for salt content and the volume of seawater, \(V_w\):

\[
[\text{OC}] = \frac{M_{\text{drysed}} - (W_{\text{dry}} - W_{\text{comb}})}{V_w + V_{\text{sed}}}
\]

\[
V_w = \frac{(W_{\text{wet}} - W_{\text{dry}})(\rho_w - 1)}{\rho_w}
\]

The integrated value for organic carbon in kg organic carbon per m\(^2\) sediment is then:

\[
\Sigma[\text{OC}] (0 - 5\text{cm}) = \frac{([\text{OC}]_{0\text{cm}} + \cdots + [\text{OC}]_{5\text{cm}})(5\text{cm} - 0\text{cm})(1\text{kg})(100^2\text{cm}^2)}{(10^9\mu\text{g})(1\text{m}^2)}
\]

Results

Observations

Over all dates, biofilms were often strikingly green, somewhat inconsistent with their traditional brown-gold descriptions (Callow, 2014). However, biofilms that fit this description were also common. The non-runnel biofilms tended to closely follow slight depressions created
by the receding tide. Outside of runnels, biofilms often developed at or seaward of large debris, such as driftwood. Rarely, biofilms may have been associated with infaunal burrows (Figure 4).

Figure 4. Biofilm amongst several infaunal burrows on 7-29-18 at Waties Island, South Carolina. Note the brown-gold coloration of the biofilm.

The distribution of biofilms varied widely with relative cloud cover. On dates that were especially cloudy or overcast like 7-29-18 and 10-20-18, significant biofilms covered the beach profile between the wrack and runnel or area just beyond the swash zone if no runnel was present (Figure 5A, Figure 6). On the date with little cloud cover, 8-25-18, biofilm development was much more limited (Figure 5B). Referencing a driftwood marker just seaward of the wrack line, this difference between 7-29-18 and 8-25-18 was particularly pronounced (Figure 7A and B). Notably, on the sunny date that was 8-25-18, biofilms formed around the parameter of sampling equipment not related to this study in roughly 10 minutes after the equipment was set down, and dissipated totally within 10 minutes when the equipment was moved (Figure 8A). Similar
biofilms were observed forming around the parameters of natural objects on the beach (Figure 8B).

**Figure 5.** A Abundant biofilms characteristic of the beach profile of Waties Island, South Carolina on the cloudy 7-29-18. Note the directional streaking of the biofilms and their relatively green coloration.

B Lack of biofilms characteristic of the beach profile of Waties Island, South Carolina on the sunny 8-25-18. While the beach was not totally barren, biofilm distribution was much more limited than on 7-29-18 or 10-20-18.
Figure 6. Beach profile of Waties Island, South Carolina on the 10-20-18 sampling date looking leeward. Note overcast and significant brown-gold biofilm development behind the runnel, but absent in front of it. Indentations around the margins of the runnel are horse tracks from recreational riders.

A

B

Figure 7. A The driftwood marker at Waties Island, South Carolina on the cloudy 7-29-18, which featured some biofilm development. B The driftwood marker at Waties Island, South Carolina on the sunny 8-25-18, which featured no biofilm development.
Figure 8. A Biofilms that rapidly formed around the parameter of a storage cooler and sandals on 8-25-18 at Waties Island, South Carolina. Biofilms dissipated even more rapidly than they formed. B Biofilm found under a discarded crab claw on 8-25-18 at Waties Island, South Carolina.

Under ×400 magnification, the bare sand station featured some small pennate diatoms associated with sediment grains that were difficult to differentiate from quartz crystals, diatoms which were also present at the biofilm stations only in greater number (Figure 9A). These small pennate diatoms could not be identified at this magnification. Each biofilm station featured larger pennate diatoms not present in the bare sand station, especially the non-runnel biofilm station, but obscuring sediment in the unpurified samples made identification difficult (Figure 9B and C). Of the biofilm stations, the runnel trough featured the fewest of these larger pennate diatoms. The biofilm stations also featured a number of cocci as well as cellular debris, both of which were most common in the non-runnel biofilm station (Figure 9D). These cocci appeared as lone cells or in small and large clusters, the latter of which may be due to the EPS of the diatoms. The
non-runnel biofilm station also featured a single small centric diatom not identifiable easily at ×400 magnification.

Figure 9. A Unidentifiable small pennate diatom from the surface of the runnel trough station. B Large pennate diatom from the surface of the non-runnel biofilm station suspected to be of Stauroneis or perhaps Diploneis (Kociolek and Spaulding, 2003). C Large pennate diatom too obscured to identify from
the surface of the runnel crest station. D Large cluster of cocci from the surface of the non-runnel biofilm station.

*Chlorophyll α*

As predicted, all biofilm stations had enriched chlorophyll α concentrations at the surface and less at depth, a pattern that persisted even at the bare sand station that had no visible activity (Figure 10A and B). Chlorophyll α was particularly enriched at the surface of the non-runnel biofilm. Interestingly, the runnel trough, compared to the bare sand, was less enriched. Across all stations, chlorophyll α was not depleted to zero at any measured depth. At depth, the runnel stations were more similar to one another than were the bare sand and non-runnel biofilm stations, There may have been some slight enrichment at depth at non-runnel biofilm station. These finding were consistent with historic sampling of the bare sand and non-runnel biofilm stations (Figure 10C) and runnel stations (Figure 10D) conducted and analyzed by Hannides et al. (in prep). The pheophytin α to chlorophyll α ratios were lower in the biofilm stations than bare sand as predicted, although these ratios only increased with depth just beyond the surface interval (Figure 11A and B). Similar to the chlorophyll α concentrations, the runnel stations’ ratios were strikingly similar.

*Total Carbohydrate*

Unlike what was predicted, the bare sand station had higher total carbohydrate concentration at the surface than all stations except the runnel crest station (Figure 12A and B). The runnel stations both contained a similar peak around mid-depth followed by depletion. However, each profile was fairly jagged, limiting the ability to detect relationships with depth and to compare stations.
Figure 10. A Chlorophyll α concentration depth profiles for the bare sand and non-runnel biofilm stations sampled on 10-20-18. B Chlorophyll α concentration depth profiles for the runnel crest and trough stations sampled on 10-20-18. C Chlorophyll α concentration depth profiles for the bare sand and non-runnel biofilm stations sampled on 8-15-17 by Hannides et al. (in prep) with sampling photograph consistent with sampling on 10-20-18. D Chlorophyll α concentration depth profiles for the runnel crest and trough stations sampled on 11-21-16 by Hannides et al. (in prep) with sampling photograph consistent with sampling on 10-20-18. All error bars represent cumulative variation about single samples (Sokal and Rohlf, 1994).
Figure 11. A Pheophytin α to chlorophyll α ratio depth profiles for bare sand and non-runnel biofilm stations sampled on 10-20-18. B Pheophytin α to chlorophyll α ratio depth profiles for runnel crest and trough stations sampled on 10-20-18. All error bars represent cumulative variation about single samples (Sokal and Rohlf, 1994).

Figure 12. A Total carbohydrate concentration depth profiles for bare sand and non-runnel biofilm stations sampled on 10-20-18. B Total carbohydrate concentration depth profiles for runnel crest and trough stations sampled on 10-20-18. All error bars represent cumulative variation about single samples (Sokal and Rohlf, 1994).
Unlike predictions, the non-runnel biofilm station did not show a higher percent LOI at the surface compared to the bare sand station, although the non-runnel biofilm station did show higher percent LOI at depth comparative to the bare sand station (Figure 13A). Surprisingly, both runnel stations featured higher percent LOI at the surface than the non-runnel biofilm station (Figure 13B). While the runnel trough station showed a decline in percent LOI with depth, the runnel crest station showed a maximum around mid-depth nearly twice as high as any station’s surface value that was depleted slowly at depth.

**Figure 13.** A Percent LOI depth profiles for bare sand and non-runnel biofilm stations sampled on 10-20-18. Note that the 0.5-1.0 cm interval for the bare sand station was removed as an outlier (~1.2% LOI) that likely resulted from a procedural error. B Percent LOI depth profiles for runnel crest and trough stations sampled on 10-20-18. Note that the 0.0-0.5 cm and 0.5 cm-1.0 cm intervals for the runnel crest station were combined following a procedural error prior to combustion. All error bars represent cumulative variation about single samples (Sokal and Rohlf, 1994).
Total Phosphorus

The total phosphorus concentrations of the bare sand station exceeded the non-runnel biofilm station almost over the entire depth, opposite of what was predicted (Figure 14A). Similarly defying expectations, the total phosphorus concentrations of the runnel trough station exceeded that of the runnel crest station expect at the deepest interval (Figure 14B).

**Figure 14.** A Total phosphorus concentration depth profiles bare sand and non-runnel biofilm stations sampled on 10-20-18. B Total phosphorus concentration depth profiles for runnel crest and trough stations sampled on 10-20-18. Note that the 0.0-0.5cm and 0.5cm-1.0cm intervals for the runnel crest station were combined following a procedural error prior to combustion. All error bars represent cumulative variation about single samples (Sokal and Rohlf, 1994).

Parameter Regression Analyses

Although the relationship between no two parameters was significant, the bizarre negative relationship between integrated chlorophyll α and integrated total phosphorus came much closer than any other (Figure 15). Though not significant, this relationship was opposite of the expected positive relationship that should live between parameters related to biological activity.
Figure 15. Integrated chlorophyll α plotted against integrated total phosphorus for each station sampled on 10-20-18. The negative relationship was not significant (P = 0.1138).

Discussion

Only the chlorophyll α concentrations were largely as predicted, with the exception of the runnel trough station that had a lower surface concentration than the bare sand station. Still, the pheophytin α to chlorophyll α ratios of the biofilm stations compared to the bare sand station were indicative of a larger portion of living cells, which was qualitatively supported by the microscope verification of diatoms. The integrated chlorophyll α for the runnel stations also fit well within what time series sampling data for Waties Island would predict for the month of the sampling date (Figure 16).

Nothing like predicted, the jaggedness of the carbohydrate profiles may have been the result of a methodical error relating to mixing of reagents during the extraction. During the incubation of this reaction in the water bath with shaker, the narrow reaction tubes were upright or vertical, minimizing the mixing of the reagents. Mixing could have been improved had the vials been shaken while horizontal, although it was unclear if mixing impacted the results given
the similarity between the runnel stations somewhat consistent with the similarity between the chlorophyll α profiles of the runnel stations.

Figure 16. Integrated chlorophyll α values over 0-5cm as sampled and analyzed by Hannides et al. (in prep.) between 11/16 and 3/18 including the runnel crest and runnel trough stations’ integrated values sampled during 10/18 in black and white.

The unexpected high percent LOI at mid-depth in the runnel crest station may have been the result some kind of infaunal burrow breached by the syringe core, but may also have been related to the mixing of material in the runnel ripple itself, which also could explain why the runnel trough station was depleted at depth more than any other station. While it was tempting to correlate the mid-depth peak in the runnel crest station’s total carbohydrate concentration profile to this peak in percent LOI, the same mid-depth peak in the runnel trough station’s total carbohydrate concentration profile had no corresponding peak in percent LOI. The seemingly
low percent LOI at the surface of the non-runnel biofilm station could not easily be explained nor could the apparent enrichment at depth, although the enrichment at depth was consistent with the enrichment present in the chlorophyll α profile for this station. The latter may be related to the movement of epipelic diatoms from the surface biofilm back into the sediment, but this was unclear.

Representing a total inversion of expectations, the total phosphorus concentration profiles and the decently strong yet not significant negative correlation between integrated chlorophyll α and integrated total phosphorus were likely the result of interference during the HCl extraction by an unknown agent. When verifying the amount of 4M NaOH needed to raise the pH of the separated supernatant using the extra supernatant left in the centrifugation tube of one of the deeper non-runnel biofilm station intervals, an orange precipitant formed in a resulting solution that was much more basic (pH = 8 to 14) than the amount of 4M NaOH added should have produced (Figure 17). Repeatable with the runnel crest station intervals, this effect seemed to be most pronounced if the centrifugation tube was shaken especially vigorously. Based on the appearance of the precipitant and knowing Fe is assimilated by marine diatoms (Goldberg, 1952), this precipitant was assumed to be FeP, or a closely related Fe species. Undetectable by the phosphomolybdate blue method that requires phosphate to be in solution, these species are removed prior to combustion in the SEDEX scheme. A method able to extract FeP and related species could be used rather than the phosphomolybdate blue method to better quantify total phosphorus than in this study.
Figure 17. Example of the orange precipitant that formed upon addition and mixing of 4M NaOH to the centrifugation tube containing extra supernatant from a deeper (3-4 cm) non-runnel biofilm station interval. This precipitant was assumed to be FeP or a related Fe species.

Though total carbohydrate and total phosphorus profiles were not as predicted and largely inconclusive, the chlorophyll $\alpha$ and LOI profiles suggest these biofilms are sites of biological activity that likely contribute significantly to the overall productivity of high energy beaches, especially considering the widespread distribution on cloudy or overcast days. Analyses similar to this study on a sunny day could quantify the observed differences in biofilm development based on cloud cover, which are most likely the result of photoinhibition. In the water column, marine phytoplankton, including diatoms, have been shown to suffer DNA damage from too much exposure to UV radiation (Helbling et al., 2001). An experiment could also be designed to use filters of varying light absorbance on a sunny day to generate varying amounts of artificial shadow or protection from UV radiation for biofilm development. On sunny days, biofilm development may be more defined in the runnels, since the overlying water offers diatoms protection from UV radiation. The opposite is likely true for cloudy days, as the overlying water attenuates light the diatoms need for photosynthesis, which would be consistent with the chlorophyll $\alpha$ profiles in this study (though these profiles may just be related to their overall
exposure time at the time of sampling as hypothesized originally). Noting the seasonal variability in the runnel time series data for Waties Island (Hannides et al., in prep.), these biofilms appear to vary over larger temporal scales than simply the tidal cycle and this variance warrants further investigation. Beyond qualitative verification of raw samples, diatom enumeration using purified samples via microscopy or molecular methods, like qPCR (Bourlat et al., 2013), could be used to generate enumeration profiles comparable to biological parameters. LOI profiles prompt further study into the role of ripple-associated mixing of organic matter and nutrients in runnels. Though not explored in this study, the in situ production of these biofilms can also be measured with radioactive $^{13}$C to directly quantify production beyond simply analyses of parameters related to biological activity (Middelburg et al., 2000).

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Literature Cited


