Relationships between microbial enzymatic activity and environmental parameters in the coastal Gulf of Maine and the deep Pacific Ocean

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Relationships between microbial enzymatic activity and environmental parameters in the coastal Gulf of Maine and the deep Pacific Ocean

By

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THESIS

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Abstract

Microorganisms contribute to the cycling of organic matter and inorganic nutrients through extracellular enzymatic depolymerization of high molecular weight compounds, providing essential nutrients to the aquatic environment. Since microorganisms adapt relatively quickly to their environment, their metabolic rates reflect biological responses to changing environmental conditions, such as those predicted as a result of climate change. Yet, before it is possible to predict how microorganisms will respond to future change, it is important to understand how current environmental conditions influence microbial activity. To capture a range of environmental conditions, the response of microbial enzymatic activities was studied seasonally at three Eastern US coastal sites in the Gulf of Maine, and comparatively with a one-time sampling at an offshore deep ocean site in the northeast Pacific Ocean. The study hypothesizes that patterns in enzymatic activities will be influenced by environmental parameters such as sea temperature, salinity, chlorophyll-a (chl-a), oxygen, and bacterial cell abundance. To evaluate this hypothesis, variations of environmental parameters were compared to the rates and patterns of β-glucosidase (BG), N-Acetyl-β-D-Glucosaminidase (NAG), xylosidase (XYL), leucine aminopeptidase (LAP), and alkaline phosphatase (AP), enzymes which catalyze the hydrolysis of carbohydrates, peptides, and phosphorous compounds, respectively. The Gulf of Maine is a highly productive and rapidly warming body of water, but unlike what has been observed in warmer settings, water temperature was not a strong influencing factor on seasonal patterns of enzyme activities there. The lack of correlation might be due to these sites in situ temperature being further from the temperature optima of the enzymes. Moderately strong relationships were found between chl-a and AP activities at two of the three coastal sites, and at the deep ocean site, enzymatic activities correlated to temperature, oxygen, and bacterial cell abundance. Enzymatic activities were closely coupled with oxygen concentration, highlighting the influence of oxygen minimum zones on microbial activity. AP activity was decoupled from bacterial cell abundance, yet high activity was maintained throughout the column indicating activity by cell-free AP. Patterns in enzymatic activity at all four sites were not attributed to any one environmental factor, but rather, a combination of environmental influences that varied temporally and spatially. Results from this study further our understanding of the current relationships between enzymatic activities and environmental parameters, insights necessary for accurately predicting ecosystem responses to climate change.
1. Introduction

Marine microorganisms (bacteria, archaea, viruses, protists, and fungi) are the most abundant lifeforms in the ocean, and although individually minute, they are essential to the biogeochemical processes of the aquatic ecosystem (Zaccone & Caruso, 2019). Heterotrophic bacteria (hereafter referred to as heterotrophic microbes) are responsible for most of the organic matter recycling, nutrient remineralization, decomposition, and carbon flow through the marine food web. Heterotrophic microbes process up to an estimated 50% of the organic matter produced through primary productivity (Arnosti et al., 2005). Much of this organic matter pool is dominated by particulate and high molecular weight (>1 kDa) organic matter, which are too large to pass through the cell membranes of heterotrophic microorganisms (Cunha & Almeida, 2010). In order to meet cellular nutrient requirements, microbes produce substrate-specific extracellular enzymes to breakdown high molecular weight organic matter outside the cell prior to uptake, cleaving off essential nutrients from complex matter for assimilation into the cell. Extracellular enzymes can exist attached to the outer cell wall, within the outer membranes of the cell (periplasm), and/or as dissolved enzymes which are released into the environment to perform hydrolysis outside the cell (Chróst, 1991).

Extracellular hydrolytic transformation of complex organic matter within the dissolved organic matter pool recycles essential nutrients back to the aquatic environment, a process which has been coined the microbial loop (Pomeroy, 1974). The microbial loop is closely linked to the biological carbon pump, a process responsible for fixing atmospheric carbon and exporting it to the deep sea where it can be sequestered on timescales of up to >1000 years (C. Zhang et al., 2018). Biological processes, such as heterotrophic degradation of organic matter, ultimately determine the net CO₂ removal from the atmosphere (Arndt et al., 2013). The extent to which
Microorganisms process carbon in the ocean is dependent upon several factors, one of which is environmental conditions. Given that climate change is altering ocean conditions, it is important to understand how environmental factors such as temperature, salinity, chl-a concentration, oxygen concentration, and bacterial cell abundance influence microbial activities. Variations in patterns of activity are critical indicators of changes occurring in the marine environments.

Microbial activities in coastal zones and the open ocean are increasingly affected by environmental changes. Predicted effects of climate change include rising sea surface temperatures, increased storm intensities, eutrophication (and more), all of which disrupt the ecological balance in the ocean, and consequentially, affect the rates of organic matter cycling performed by microbial communities. Some studies have shown that higher sea temperatures have a strong effect on microbial enzymatic activities, where at warmer temperatures higher rates of hydrolysis occur (Caruso et al., 2013). More intense tropical cyclones and hurricanes cause vigorous vertical ocean mixing which alter upper-ocean temperature structure; warm surface waters are forced into deeper ocean layers where they persist past seasonal changes, ultimately resulting in a net heating of ocean temperature (Hu & Meehl, 2009; Li & Sriver, 2018). In coastal water, increased storm intensity and frequency affect salinity and change the overall seawater chemistry. More land runoff, amplified wave action, and increased erosion, add greater volumes of not just organic material to coastal areas, but also potentially toxic contaminants from anthropogenic sources (McCarthy et al., 2008). The rapid increase in the supply of organic matter (eutrophication) triggers excess algal growth disrupting the dynamics between production and metabolism (a consequence of which is oxygen reduction to the point of hypoxia) (Cloern, 2001; Nixon, 1995; Smith, 2003). Though such environmental changes have made observable differences to the ecosystem, the direct impact on microbial enzymatic activities remains unclear.
Before it is possible to accurately predict the future effects of climate change on microbial activities, it is necessary to discern how environmental factors influence enzyme activities under current climate conditions. To address this goal, this study investigates relationships between rates of hydrolytic enzymes and selected environmental variables in the coastal Gulf of Maine and the deep Pacific Ocean. Three coastal sites were sampled throughout the year: Coastal Marine Laboratory (CML) on the New Hampshire coast, Jackson Estuarine Laboratory (JEL) in an estuarine system along the New Hampshire coast, and Boothbay Harbor (BBH) along the coast of Maine. The deep ocean site was accessed for a one-time sampling event located offshore in the Northeast Pacific Ocean. The two contrasting environments (coastal and offshore) are characterized by distinct chemical and physical water properties and both regions are affected by climate change. Coastal sites are subject to a greater diversity of temperature, salinity, oxygen concentration, organic matter flux, chl-a concentration, and bacterial cell abundances compared to the open ocean, resulting in more dynamic patterns of microbial activity. However, although relatively few studies measure extracellular enzyme activity in the deep sea, findings in the deep ocean display distinct patterns compared to surface ocean studies. With no light in the deep sea to fuel autotrophic production, deep sea microbial communities rely on sinking organic matter from the upper ocean to survive (Baltar et al. 2010). Contrast to the fresh organic matter in coastal surface waters, the organic matter that reaches the deep sea is biologically and chemically altered as it travels through the water column, resulting in changed energetic value and matter composition (Alldredge & Silver, 1988). The specific enzymes released by deep sea microbes can reflect the types of organic substrates the organisms are exposed to at depth.

The environmental parameters tested as potential drivers of enzymatic activities were temperature, salinity, chl-a, bacterial cell abundance, and additionally at the deep ocean site,
oxygen concentration. Temperature is a relevant variable to evaluate given the current climate crisis, as global ocean temperatures have been rising by +0.01°C per year (Pershing et al., 2015). The Gulf of Maine waters however, have experienced even greater temperature variance over the past decade, increasing as much as +0.03°C per year (Pershing et al., 2015). In the Gulf of Maine, the extreme variability and increased water temperatures are expected to cause changes to the activities of the microbial community. In isolated enzyme assays over a range of temperatures, an optimum temperature is observed where higher temperature enables greater enzymatic activity, until a temperature maxima is reached (Daniel et al., 2008). In areas with warmer and higher ranges of water temperature, such as in the Mediterranean Sea, temperature is closely correlated to enzymatic activity (Caruso et al., 2013). The higher temperatures allow for more efficient recycling of organic matter through enzymatic hydrolysis.

Salinity is a variable parameter in estuaries and coastal zones as freshwater inputs change the water chemical composition. Salinity also alters the microbial community composition, and therefore the functions performed in biogeochemical cycling (Bachmann et al., 2018). Freshwater inflows carry nutrients and organic debris off the land, supplying an array of organic matter to the ecosystem. Microbes that can respond with the appropriate enzymes capable of breaking down such material will succeed over others, resulting in changes in microbial community composition when compared to marine communities. Salinity is considered a main environmental driver of community structure, however, there are conflicting results regarding its correlation with microbial metabolic activity (Bouvier & Giorgio, 2002; Cunha & Almeida, 2010). Some studies demonstrate a weak correlation between salinity and microbial metabolism, while others show the opposite effect (Cunha & Almeida, 2010).
Chlorophyll-a (chl-a) is the predominant photosynthetic pigment found in phytoplankton, and the concentration of chl-a is a commonly used proxy of phytoplankton biomass abundance in the sea. Blooms of phytoplankton fuel the oceanic carbon cycle by fixing atmospheric carbon into organic compounds (Allison et al., 2012; Jiao & Zheng, 2011). Phytoplankton are a primary source of organic matter to marine systems (Romankevich, 2013). Microbial enzymatic activity is related to phytoplankton biomass because phytoplankton are responsible for producing 10 – 50% of the carbon utilized by bacteria (Azam et al., 1983). Interactions between phytoplankton biomass and microbial activity shape the energy flow through the marine food web.

As a result of climate change, accelerated oxygen loss is occurring in both coastal and open ocean systems (Levin & Breitburg, 2015). Fundamental for all aerobic life, dissolved oxygen in seawater is a critical component for metabolic and geochemical processes (Keeling et al., 2010). Under low levels of oxygen, major changes in biogeochemical cycles occur, as the microbes that mediate such processes become limited by oxygen availability (Keeling et al., 2010). Eutrophication induces increased rates of primary production and organic matter accumulation that results in greater microbial consumption of oxygen (Rabalais et al., 2014). In addition to biological processes, higher water temperature reduces oxygen solubility in seawater (Rabalais et al., 2014). Increasing temperatures also strengthen upper-ocean stratification of the water column, thus reducing the transport of oxygen to the ocean interior (Keeling & Garcia, 2002; Rabalais et al., 2014). Ocean currents are weakened under warmer conditions further reducing the distribution of oxygen throughout the ocean (Caesar et al., 2018). Intermediate-depth water masses with poor dissolved oxygen concentrations, known as oxygen minimum zones (OMZ), have been expanding due to the overall decline of ocean mixing and warming temperatures (Levin & Breitburg, 2015;
Many microbes are reliant on oxygen for respiration; therefore, a reduction of dissolved oxygen is predicted to slow the biogeochemical cycles mediated by microbes.

Heterotrophic microorganisms play an important role in the cycling of carbon (C), nitrogen (N), and phosphorous (P) via extracellular enzymatic hydrolysis of carbohydrates, peptides and proteins, and phosphate compounds, respectively. For the coastal sites, the enzymes β-glucosidase (BG), leucine aminopeptidase (LAP), and alkaline phosphatase (AP) were selected as they represent the hydrolysis of these major components of organic matter in natural seawater, organic carbon, nitrogen, and phosphorous, respectively. BG and LAP are mainly associated with the activity of heterotrophic bacteria, whereas AP is produced by other marine organisms including phytoplankton and marine protozoa (flagellates and ciliates) (Hoppe, 2003). Nitrogen and phosphorus are often limiting nutrients for primary productivity, being utilized by organisms faster than they are supplied in the environment (Labry, 2002). In contrast, microbial growth in the deep ocean is often limited by carbon that is exported from the surface through sinking particles (Arístegui et al., 2009). To account for potential carbon limitation, a broader range of carbohydrate hydrolyzing enzymes were included at the deep ocean site including N-Acetyl-β-D-Glucosaminidase (NAG), and xylosidase (XYL). To study extracellular enzymatic activity, the fluorogenic substrate enzyme assay is an extensively used method, as established by Hoppe (Hoppe, 1983). Fluorophore-labelled substrate analogues are specifically synthesized to release a fluorescent molecule when the internal bonds are cleaved by an enzyme. The change in fluorescence over time allowed for the hydrolysis rate to be calculated.

In the Gulf of Maine, seasonal variation in sea surface temperature is predicted to become more extreme, and because enzyme activity can be affected by temperature this work tests the hypothesis that, under current climate conditions:
1. Enzyme activities in coastal Gulf of Maine waters will follow seasonal variations in sea surface temperatures. (i.e., activities will be higher under warmer conditions).

Salinity is a relevant factor to monitor when evaluating chemical changes within the aquatic environment. Storm events may increase in frequency and intensity in the future, changing seawater salinity with added precipitation, land runoff, riverine input, and vertical mixing. Due to this, at the estuarine site (JEL), tidal currents drive changes in salinity making this site viable for comparing enzymatic activities across a range of salinities. Variations in salinity alter the microbial community, and therefore the hydrolytic functions performed. To assess the extent to which variations in salinity affect microbial activity the following hypothesis was developed:

2. Enzyme activities at the estuarine site (JEL) will correlate with tidally driven ranges in water salinity. (i.e., activities will be higher under higher salinity conditions).

Chl-a concentration, as a proxy of phytoplankton biomass, is interconnected with microbial enzymatic activity as the process of primary production provides organic substrates for hydrolytic breakdown by microbial communities. As seasonal blooms of phytoplankton affect the patterns of microbial activity, the following hypothesis was tested:

3. Enzyme activities in coastal Gulf of Maine waters will follow seasonal variations of chl-a concentrations (as a proxy of phytoplankton biomass). (i.e., an increase in the presence of chl-a corresponds to an increase in enzymatic activity).

Dissolved oxygen in seawater is essential to most marine organisms, however, increased oxygen loss and expanding oxygen minimum zones are expected consequences of climate change. Increased ocean temperatures not only reduce the solubility of oxygen in seawater but enhance stratification and slow ocean circulation both of which reduce oxygen ventilation throughout the ocean. The oxygen concentration profile was recorded at the deep ocean site, which revealed an
oxygen minimum zone within the water column. Because many microbes rely on oxygen in order to carry out essential biogeochemical cycling, it is hypothesized that:

4. Enzyme activities at the deep ocean site will correlate with oxygen concentrations throughout the water column. (i.e., activities will be higher under higher oxygen concentrations).

When enzyme activity and bacterial cell abundance patterns coincide, this indicates bacterial expression of enzymes, as opposed to activity by other enzyme producing marine organisms. Out of the enzymes measured in this study, AP is the only enzyme known to be primarily produced by other marine organisms (microeukaryotes), not only heterotrophic bacteria (prokaryotes). Additionally, AP can remain active outside of the cell and accumulate at depth as a cell-free enzyme. In such instances, AP activity is not associated with bacterial cell abundance. In order to gain insight on the source of enzymatic activity the following hypothesis was tested:

5. Activities of BG, LAP, NAG, and XYL will follow patterns of bacterial cell abundances while AP activities are disconnected from bacterial cell numbers.

2. Materials and Methods

2.1 Sampling Sites

Seawater samples were collected from three coastal sites in the Gulf of Maine: Coastal Marine Laboratory, Jackson Estuarine Laboratory, and Boothbay Harbor (Figure 1A). An additional sampling event occurred in the Northeast Pacific Ocean near the Juan de Fuca Ridge Flank where deep ocean water column samples were collected (Figure 1B).
Figure 1: Sampling Sites. A: Coastal sampling sites on east coast of U.S., includes BoothBay Harbor (BBH) at northernmost point, Jackson Estuarine Laboratory (JEL) located in the Great Bay west of Portsmouth, and Coastal Marine Laboratory (CML) along the Portsmouth coastline. B: Deep Ocean Site. Expedition to the Juan de Fuca Ridge Flank located offshore from Washington coastline along the west coast of the U.S.
2.1.1 Coastal Sites

Coastal Marine Laboratory (CML) is a marine research facility associated with University of New Hampshire located in Portsmouth Harbor, New Hampshire. CML is situated at the mouth of the Piscataqua River along the border of New Hampshire and Maine. Surface water samples were collected from the laboratory’s pier. The Piscataqua River water is exposed to both industrial and residential development along its shores as it is in close proximity to the city of Portsmouth (New Hampshire), the Portsmouth Naval Shipyard, and downtown Kittery (Maine) (Short, 1992). CML is located where the river joins the open ocean, therefore, it experiences tides and oceanic water conditions. Water temperatures and salinities measured here are comparable to typical marine conditions rather than freshwater conditions.

Jackson Estuarine Laboratory (JEL) is located on the coastline of the Great Bay, an estuary system with high tidal energy in southeastern New Hampshire. This site hosts a research center for the University of New Hampshire and provides access to a dock where water samples were taken. This estuarine ecosystem is characterized by its diverse habitats including eelgrass meadows, mudflats, salt marshes, oyster reefs, deep channels, and rocky intertidal zones (Short 1992). The Great Bay Estuary has seven major rivers that contribute to the input of freshwater and connects to the Gulf of Maine via the Piscataqua River. Though there are many freshwater sources, the freshwater input typically only represents 2% or less of the tidal volume. Tidal currents are essential to the productivity of the Great Bay Estuary (Short, 1992).

Sampling of Booth Bay in West Boothbay Harbor (BBH), Maine, took place on a dock at the Maine Department of Marine Resources, near the former location of the Bigelow Laboratory for Ocean Sciences. This site is located in one of Maine’s many rocky inlets, about an hour north
of Portland, Maine. It has no direct major river input, but instead is subject to semi-diurnal tidal mixing with offshore Gulf of Maine coastal waters (Cormier et al., 2013).

2.1.3 Deep Ocean Site

The Juan de Fuca Ridge is an undersea mountain chain created by a divergent plate boundary between the Juan de Fuca Plate and the Pacific Plate. It lies about 200 miles offshore parallel to the Washington and Oregon coastline. The sampling site was not directly on the ridge itself but was located on the eastern flank of the ridge, several dozen miles away from the ridge axis. This region of the Northeast Pacific Ocean along the U.S. Pacific Northwest Coast (PNW) differs substantially from that of the U.S. East Coast. Productivity in the PNW region is driven by eastern coast boundary upwelling events which bring ample nutrients from deep waters to the surface (Hickey & Banas, 2003).

2.2 Sampling procedure and frequency

Samples were collected manually at each coastal site by first rinsing a clean 500 mL plastic bottle with seawater three times then filling the bottle with seawater. Samples were taken in sync with high tide at each coastal site. Samples were brought in a cooler to the lab to initiate the enzyme assays close to the time of sampling. Salinity and temperature measurements were taken using a YSI EcoSense EC300 meter at the time of sampling for most dates; otherwise this data was obtained from nearby buoy measurements.

At CML, sampling included a total of 12 timepoints, with 7 sampling dates in the spring months of March and April 2019, and 5 during the summer which included the months of May, June, and July 2019. At JEL, 10 samples were taken during spring months (March and April), 9 samples were taken during summer (May, June, July) and one in fall (September), for a total of 20
samples. Enzyme assays from 2018 only included the activities of LAP and AP, whereas BG was added to the enzyme assays in 2019. At BBH, 7 timepoints were taken in total spanning from April 2019 to January 2020. At BBH, there was one sample taken during spring (April), three during summer the months (July and August), two during fall (October and November), and one during winter (January).

Sampling at the deep ocean site was conducted during a 2-week expedition AT42-11 on R/V Atlantis in May 2019 to the Juan de Fuca Ridge Flank in the Pacific Ocean (47°45’39.7” N 127°45’40.4” W). A CTD, an acronym for a tool that measures conductivity, temperature, and depth, with a rosette of niskin bottles was deployed to collect seawater and measure parameters at varied depths of the water column. The first CTD rosette collected samples at 5 m (surface), 500 m (mid-mesopelagic), and 2000 m (bathypelagic). The following CTD casts captured additional water depths after additional sensors revealed a deep chlorophyll max at 65 m, an oxygen minimum zone (OMZ) at 950 m, and a bottom nepheloid layer at 2650 m. Water column properties associated with this sampling are publicly available on R2R and BCO-DMO. Seawater was subsampled from the rosette of niskin bottles for immediate use in the lab onboard. All assays were stored in dark, temperature-controlled refrigerators, and conducted under in situ temperatures.

2.3 Analytical Methods

2.3.1 Hydrolytic Enzyme Activities

At the coastal sites, the activities of leucine aminopeptidase (LAP), alkaline phosphatase (AP), and β-glucosidase (BG) were selected as they represent enzymes that hydrolyze commonly found substrates in natural surface seawater (Hoppe, 1983). For the deep ocean site, two additional enzymes were included to capture a wider array of potential carbohydrate hydrolyzing enzyme activities: N-Acetyl-β-D-Glucosaminidase (NAG), and xylosidase (XYL).
The fluorescent substrate L-leucine-7-amido-4-methylcoumarin hydrochloride (Leu-MCA) is used to assess the activity of LAP, an enzyme that hydrolyses a large number of peptides and amino acid amides (Cunha & Almeida, 2010). Though typically related to nitrogen cycling, LAP is also important to the carbon cycle (Zaccone & Caruso, 2019). LAP increases the availability of nitrogen as a result of protein hydrolysis; however, protein particles are also constituted of carbon which can also be released through hydrolysis (Zaccone & Caruso, 2019). High levels of LAP activity are expected in surface waters and coastal waters where there is an ample supply of freshly produced organic matter (Caruso, 2010).

The fluorescent substrate analogue 4-methylumbelliferyl-phosphate (MUF-P) is used to assess the activity of AP. AP is an enzyme capable of hydrolyzing dissolved organic phosphates and regenerating inorganic phosphate in the marine environment (Hoppe, 2003). AP is a widely spread enzyme in aquatic environments, as it can be produced not only by bacteria, but by eukaryotes such as phytoplankton and zooplankton (Vidal et al., 2003). AP is generally known as an inducible enzyme, being produced in response to low levels of PO₄, indicating a P-deficiency (Caruso, 2010; Zaccone & Caruso, 2019).

The activity of β-glucosidase is assessed with the fluorescently labelled analogue substrate 4-methylumbelliferyl-β-D-glucopyranoside (MUF-β-glucose). BG cleaves beta-linked glucose polymers to release carbon and is generally used as an indicator of carbohydrate and polysaccharide hydrolysis. In addition to BG, MUF-labelled analog substrates for NAG, and XYL were also assayed for the deep ocean samples, as they are also responsible for hydrolysis of carbohydrates. NAG is primarily associated with the hydrolysis of glycosidic bonds in chitin, a structural material found in many marine invertebrates, fungi, and algae (including diatoms), organisms which are predominately found in the upper layers of the ocean (Riemann & Azam,
XYL hydrolyzes complex matter such as bio celluloses, and the hemicellulose structures within the cell walls of marine algae (Shen et al., 2019).

The substrates used in these assays were chosen to denote the most abundant substrates present in the marine dissolved organic matter pool; however, the concentration at which these substrates exist in the sea is generally unknown (Arnosti et al., 2019; Zaccone & Caruso, 2019). As a result, the laboratory assay cannot exactly replicate in situ conditions. The concentration of a substrate used in an assay must be high enough so that the rate of reaction reaches its maximum potential velocity ($V_{max}$). When the rate of reaction is at $V_{max}$, it is assumed that all enzymes in the sample are active and are not being inhibited by an insufficient supply of substrate. It is essential to ensure that the limiting factor is the rate of reaction and not the amount of substrate available (Roskoski, 2015). The concentrations used in an assay will be much higher than that of the natural environment, and because of this, the hydrolysis rates measured represent ‘potential’ enzymatic activity. For the assays, substrates were added in concentrations predetermined to achieve $V_{max}$ according to Michaelis-Menten Kinetics. The ‘potential’ enzyme activity nonetheless provides insights into the hydrolytic capabilities of the naturally occurring enzymes at the time of sampling.

Stock solutions of each analog substrate stored at -20°C were thawed and added individually to acrylic cuvettes in amounts to achieve a saturating final concentration in 4 mL of seawater. According to Michaelis-Menten kinetics, the $V_{max}$ for the enzymes used in these assays was achieved with a substrate concentration of 200 µM in coastal water, and 150 µM in the deep ocean samples.

For each substrate, three replicates were incubated with whole seawater, and one control cuvette was incubated with autoclaved artificial seawater. For coastal samples taken from 2019 onward, enzyme assays were conducted with size-fractionated water. In those experiments, three
replicates were incubated with 1.2 µm mesh GF/C filtered seawater. The assay of the <1.2 µm size fraction primarily consists of bacteria and extracellular enzymes, whereas whole water samples include activity by all bacteria and all plankton. The activity by the larger size class (>1.2 µm) was determined by calculating the difference between whole water hydrolysis rates and the <1.2 µm rates. Each assay was incubated at in situ temperature. Ultraviolet (UV) fluorescence was measured with a TBS-380 Mini-Fluorometer. After the first addition of substrate to each of the cuvettes, the fluorescence was measured to establish a T0 initial measurement. Fluorescence measurements were taken at 24-hour increments for the coastal samples, for a total incubation time of 72 hours and at 12-hour increments for 48 hours for the deep ocean samples. Fluorescence measurements increased linearly over time and were used to calculate hydrolysis rates. To establish units of fluorescence, a calibration was performed by measuring the fluorescence of 4-methylumbelliferone (MUF) and 4-methylcoumarin (MCA) substrate standards (Sigma-Aldrich) at known concentrations in whole water samples from each of the sampling sites. The mean of three triplicate hydrolysis rates were scaled by the fluorescence calibration values and were subtracted from the autoclaved control rates to obtain a final average hydrolysis rate for each enzyme.

Hydrolysis rates were calculated both volumetrically (nM / h) and normalized to cell abundance (amol / cell h). Volumetric rates represent the potential hydrolysis performed within the microbial community including cell-free enzymes. When normalized by cell abundance, the rates represent potential hydrolysis per cell. Cell-specific rates, however, do not always accurately represent the potential hydrolysis rate because an assumption is made that every cell is active, and every cell is producing the specific enzyme being studied. Volumetric rates consider that not all enzymatic activity is associated with a single cell; that instead, hydrolysis rates are in part
attributed to dissolved enzymes which are active outside the cell. Dissolved enzymes can contribute significantly to total hydrolysis both in surface water and in the deep ocean where extracellular enzyme production is a successful mode of nutrient acquisition from high molecular weight substrates (Baltar et al., 2010). For the purpose of this study, enzymatic activities are expressed volumetrically in order to include activity by extracellular enzymes. Cell-specific rates are reported in the appendix (Tables A - 1, A - 2, and A - 3).

2.3.2 Bacterial cell counts

Water samples from CML, JEL, and the deep ocean site for bacteria counts (~1 mL) were preserved with 0.1% glutaraldehyde and stored at liquid nitrogen until analysis on a Guava flow cytometer (Millipore, Billerica MA), equipped with a blue laser (excitation at 488 nm), emission detectors at 525, 575 and 695 nm, and side and forward scatter detectors. The instrument was calibrated with fluorescent beads (Millipore) according to the manufacturer’s instructions. Samples were vortexed, and 200 μL of sample were pipetted into a 96-well plate and dyed using 2 μL SYBR Green I (1 x, final concentration, Invitogen) for 30 min in the dark. Filtered seawater and 0.2 μm-filtered samples were run as blanks. Data were acquired and analyzed using the GuavaSoft InCyte software (v. 3.1.1). Cell counts were determined by plots of green (525 nm) versus side scatter fluorescence (Gasol & Giorgio, 2000).

Cell counts for samples from BBH as well as from the deep ocean site were obtained from Bigelow Laboratory for Ocean Sciences. Samples were stained using PicoGreen and 180 μL was run on a BioRad ZE5 flow cytometer using the 488 nm laser. Bacterial populations are gated in FlowJo 10.0 using SSC vs. 525/35nm (green fluorescence).
Note that cell counts were not available for samples taken during 2018 at JEL, and for two timepoints at BBH (November 2019 and January 2020).

2.4 Ancillary abiotic and biotic data

For the coastal sites CML and JEL, nearby buoys were able to provide temperature, salinity, and chl-a concentration data. Utilizing the NERCOOS Gulf of Maine buoy system, any data that was not manually taken onsite for temperature and salinity were filled in using the hourly buoy data, and chl-a data was obtained for all corresponding sampling dates. Data was accessed through the NERACOOS online graphing and download widget (http://www.neracoos.org/datatools/historical/graphing_download). For measurements at CML, the UNH Coastal Marine Lab Field Station Buoy data was used, however, for the several dates in which this buoy was out of service, the Appledore Island CO2 buoy was accessed instead. Given the Appledore Island buoy’s relative proximity to CML (9.5 km), it is assumed that the water conditions during high tide at the CML station are analogous. At JEL, the Great Bay buoy provided temperature, salinity, chl-a and nitrate concentration data during spring and summer months (see supplementary Figure A-1).

At BBH, chl-a and FlowCAM data were obtained in coordination with Bigelow Laboratory for Ocean Sciences (Nicole Poulton, Laura Lubelczyk, pers. comm). FlowCAM analyses supplied data regarding phytoplankton biomass separated by functional group (diatoms, dinoflagellates, ciliates, and others in µgC/L) which characterized the communities present in Boothbay Harbor during spring, summer, and fall of 2019.
2.5 Statistical Analysis

Hydrolysis rates were calculated for each enzyme assay performed and statistically analyzed for patterns. Linear correlations and analysis of variances (ANOVA) were generated using the JMP 15 Pro software to evaluate the effect of environmental parameters on enzymatic hydrolysis rates. Differences in hydrolysis rates were tested at the 5% significance level. JMP 15 Pro was used to conduct Tukey Honest Significant Difference (HSD) tests at a 5% significance level. Tukey HSD is used to test the statistical difference between means of enzyme hydrolysis rates. The Tukey HSD letters report groups statistically similar values by assigning a letter. Enzymatic activities in the coastal ocean were correlated to sea surface temperature, salinity chl-a concentration, and bacterial cell abundance (Pearson correlation). At the deep ocean site, enzymatic activities were compared to temperature, oxygen, and bacterial cell counts. Chlorophyll fluorescence measurements rely upon light, and therefore most accurately detect fluorescence in the top layers of the ocean. Deeper into the ocean, where there is no light penetration, chlorophyll fluorescence becomes less detectable. Therefore, the enzymatic activities of water column samples were not compared to fluorescence.

3. Results

3.1 Coastal Sites

3.1.1 Temporal variations in SST, salinity, chl-a, bacterial cell abundance, and nitrate

Sea surface temperature (SST) ranged from 1.7°C to 23.5°C throughout the study (Tables 1, 2 and 3, and Figure A - 2). The minimum temperature was recorded in early spring at CML, and the maximum temperature was recorded during July at JEL. Overall, the highest temperatures were measured during July at all three sites. A salinity range of 12.8 - 32.5 ppt was measured in this
study. JEL experienced the greatest variance in salinity compared to the other sites with measurements ranging from 12.8 - 28.6 ppt (Table 2). At CML, salinity ranged from 24.4 - 32.1 ppt (Table 1), and at BBH salinity ranged from 30.5 - 32.5 ppt (Table 3). Fluvial input and tidal mixing substantially influenced salinities at JEL and CML but was not as prominent at BBH.

Including measurements from all sites, chl-a concentration ranged from 0.7-52.9 µg/L (Tables 1, 2, and 3, and Figure A - 2). Phytoplankton blooms were detected at CML during spring, at JEL during summer, and BBH during fall. Historic data from CML provided temporal variations in phytoplankton community structures from 2004-2007 (Figure A - 3). At BBH, FlowCAM data was used to describe the phytoplankton communities present during the 2019 sampling period (Figure A - 4).

Bacterial cell abundances are reported in site specific environmental parameter Tables 1, 2, and 3. Cell abundances at CML and JEL were on the order of hundred million per liter ($10^8$ L$^-1$) whereas cell abundances at BBH were on the order of one billion per liter ($10^9$ L$^-1$). At all sites, the greatest cell abundances were recorded during summer. At CML and JEL, cell abundance remained near $3.0 \times 10^8$ L$^{-1}$ (CML: Mean, $M = 2.7 \times 10^8$, standard deviation, $SD = 0.4 \times 10^8$, JEL: $M = 3.2 \times 10^8$, $SD =1.6 \times 10^8$), until the last timepoint on July 11 when cell abundance more than doubled at both sites. At BBH, sampling dates extended past July, providing a wider scope of bacterial cell abundances throughout the year. The maximum cell count at BBH was recorded in July, nearly doubling the next greatest cell abundance recorded in August. Overall, bacterial cell counts were low during spring and fall, and high during summer.

Nitrate data was obtained via the Great Bay buoy deployed in waters near the JEL site. The available data coincided with JEL sampling dates from April 15, 2019 through July 11, 2019
During April, nitrate concentrations were ~5 µM, followed by an increase to 10 µM during the end of April and early May. A significant decrease in nitrogen concentration occurred from Mid-May to mid-June, dropping to concentrations near 0. Coordinating nitrate data was only available for a short period at one site (JEL), and for lack of comparison to other sites, nitrate was not pursued as one of the main potential environmental drivers during this study.

Table 1: Environmental parameters at CML. SST, sea surface temperature; Chl-a, chlorophyll-a.

<table>
<thead>
<tr>
<th>Sampling Date (n=12)</th>
<th>SST (°C) (n=12)</th>
<th>Salinity (ppt) (n=12)</th>
<th>Chl-a (µg/L) (n=6)</th>
<th>Bacterial Cells (L⁻¹)</th>
</tr>
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<tbody>
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<td>5-Mar-19</td>
<td>1.7</td>
<td>28.9</td>
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</tr>
<tr>
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<td>32.0</td>
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<td>2.6 x 10⁸</td>
</tr>
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<td>-</td>
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<td>-</td>
<td>2.6 x 10⁸</td>
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<td>1.1</td>
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<td>-</td>
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Range: 1.7 - 15.4, 25.4 - 32.1, 0.7 - 12.7, 2.0 x 10⁸ - 4.8 x 10⁸
Table 2: Environmental parameters at JEL. SST, sea surface temperature; Chl-a, chlorophyll-a.

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<th>Sampling Date</th>
<th>SST (°C)</th>
<th>Salinity (ppt)</th>
<th>Chl-a (µg/L)</th>
<th>Bacterial Cells (L⁻¹)</th>
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<td>2.9 x 10⁸</td>
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<td>5.4</td>
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Range: 6.1 - 23.5  12.8 - 28.6  2.2-52.9  2.3 x 10⁸– 8.1 x 10⁸
### Table 3: Environmental parameters at BBH. SST, sea surface temperature; Chl-a, chlorophyll-a.

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<th>SST (°C) (n=7)</th>
<th>Salinity (ppt) (n=7)</th>
<th>Chl-a (µg/L) (n=6)</th>
<th>Bacterial Cells (L⁻¹)</th>
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</thead>
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</tr>
<tr>
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<td>2.9 x 10⁹</td>
</tr>
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<td>8.6 x 10⁹</td>
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<td>-</td>
<td>-</td>
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<td><strong>30.5 - 32.5</strong></td>
<td><strong>1.4 – 5.4</strong></td>
<td><strong>1.6 x 10⁹ – 8.6 x 10⁹</strong></td>
</tr>
</tbody>
</table>

3.1.2 Temporal variations of enzyme activities

A total of 39 enzyme assays were performed using seawater samples from CML, JEL, and BBH between March 2018 and January 2020. Combined data from all three sampling sites display a temperature range of 1.7°C to 23.5°C, salinity range of 12.8 ppt to 32.5 ppt, and chlorophyll-a range from <1 ug/L to 53 ug/L (Tables 1, 2, and 3). The magnitude of enzymatic activity at each site was in the order LAP>AP>BG, matching the general order of magnitude for these enzymes as stated in Cunha et al. (Cunha & Almeida, 2010). To compare the statistical similarities between enzymatic rates of activity among sampling dates, Tukey HSD letters report was used to assign letter groupings to similar mean values (when statistically different, mean values are grouped into different letter categories).
Figure 2: Time series of Leucine Aminopeptidase (LAP) activity. Hydrolysis rates (nM/h) in surface water at A: Coastal Marine Lab (CML), B: Jackson Estuarine Laboratory (JEL), and C: Boothbay Harbor (BBH). Bars represent means of triplicate rate measurements and include standard deviation error bars. Tukey HSD connecting letters report compares mean hydrolysis rates by grouping statistically similar values.

Leucine Aminopeptidase (LAP)

LAP activity showed peaks in spring and summer, but the seasonal timing of the maximum activity was not consistent between sites or years (Figure 2). At CML (Figure 2A), two peaks of activity were recorded: one moderately high on April 16 and a maximum rate on July 11. All other samples from CML were statistically similar to one another, except for a distinct low rate of hydrolysis on March 5 (M = 138.5 nM/h SD = 27.5). At JEL (Figure 2B), the maximum hydrolysis rate occurred during the summer of 2018 on July 9. Overall, the hydrolysis rates recorded in the
summer of 2018 were greater than the rates recorded at JEL the following year. In 2019 at JEL, high rates were observed in early spring (Mar 23 & Apr 1) and mid-summer (July 11). The activity of LAP at BBH (Figure 2C) was highest in early spring, with a maximum rate of hydrolysis on April 2. The following summer timepoints during July and August were considerably lower than the maximum rate, though still moderate compared to the activity during November and January.

**Figure 3: Time series of Alkaline Phosphatase (AP) activity.** Hydrolysis rates (nM/h) in surface water at A: Coastal Marine Lab (CML), B: Jackson Estuarine Laboratory (JEL) (note: y-axis is on different scale to accommodate 2018 data), and C: Boothbay Harbor (BBH). Bars represent means of triplicate rate measurements and include standard deviation error bars. Tukey HSD connecting letters report compares mean hydrolysis rates by grouping statistically similar values. AP activity peaks at various times of the year at each site.
**Alkaline Phosphatase (AP)**

AP activity reached several peaks at different times of the year at each site (Figure 3). At CML (Figure 3A), two peaks of activity were recorded, a moderate level of activity during spring (Apr 9), and a maximum rate on July 11. At JEL (Figure 3B), the rates of hydrolysis performed by AP were higher than the other two sites. The maximum rate of hydrolysis at JEL was recorded during spring of 2018 on March 26, followed by high levels of activity until June 14. The following year at JEL, the rates during early spring were high compared to the rest of 2019, however, after the first two timepoints of 2019 the activity remained below 100 nM/h until June 6. On June 6, activity increased to nearly 150 nM/h. At BBH (Figure 3C), the activity of AP reached two high peaks near 250 nM/h, one during July and the next during October. In the summer months between July and October, rates of hydrolysis by AP were under 100 nM/h, whereas all other timepoints were under 50 nM/h.

**β-Glucosidase (BG)**

Across all three sites, the activity of BG generally remained below 20 nM/h for most of the year and only exceeded this hydrolysis rate in summer months (Figure 4). At CML, the highest rate of hydrolysis was recorded on July 11. Two smaller peaks of activity occurred in early spring and mid-spring (26 Mar & 16 Apr). At JEL, hydrolysis by BG reached a maximum rate on July 11. The activity of BG at JEL was ~15 nM/h at the start of spring, then remained under 10 nM/h for the following months until summertime. At BBH, the activity of BG reached a greater peak than recorded at the other two coastal sites. The maximum hydrolysis rate of 160 nM/h was recorded during mid-summer on July 9. The following summer timepoints were the next highest rates of hydrolysis by BG at BBH. The early spring, fall, and winter hydrolysis rates all remained at a similarly low activity level, below 20 nM/h.
Figure 4: Time series of Beta-glucosidase (BG) activity. Hydrolysis rates (nM/h) in surface water at A: Coastal Marine Lab (CML), B: Jackson Estuarine Laboratory (JEL), and C: Boothbay Harbor (BBH). Bars represent means of triplicate rate measurements and include standard deviation error bars. Tukey HSD connecting letters report compares mean hydrolysis rates by grouping statistically similar values. Activity of BG was greatest in the month of July at all sites.
Table 5: Enzymatic hydrolysis rates and ratios. Mean enzymatic hydrolysis rates at CML, JEL, and BBH from sampling dates in 2019. Enzyme ratios were rounded to the nearest whole value. (Ratio bold when <1).

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>BG (nM h⁻¹)</th>
<th>LAP (nM h⁻¹)</th>
<th>AP (nM h⁻¹)</th>
<th>LAP/BG Ratio</th>
<th>LAP/BG Ratio Quotient</th>
<th>LAP/AP Ratio</th>
<th>LAP/AP Ratio Quotient</th>
</tr>
</thead>
<tbody>
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<td>101:17</td>
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<tr>
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<tr>
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<td>29-Apr</td>
<td>2.7</td>
<td>44.5</td>
<td>38.1</td>
<td>44:3</td>
<td>16.3</td>
<td>22:19</td>
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</tr>
<tr>
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<td>6-May</td>
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<td>132.0</td>
<td>58.3</td>
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<td>66:29</td>
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</tr>
<tr>
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<td>170.9</td>
<td>56.9</td>
<td>57:1</td>
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</tr>
<tr>
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</tr>
<tr>
<td></td>
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<td>105.1</td>
<td>37.2</td>
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<td>378.4</td>
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<td>6.1</td>
<td>126:37</td>
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<tr>
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<td>18.9</td>
<td>57:2</td>
<td>5.9</td>
</tr>
<tr>
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<td>13:34</td>
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<tr>
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<td>169:85</td>
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<td>146.6</td>
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<td>8.6</td>
<td>21:34</td>
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<tr>
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<td>2.2</td>
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<td>9.0</td>
<td>12:1</td>
<td>10.8</td>
<td>8:3</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>9-Jan</td>
<td>1.2</td>
<td>13.8</td>
<td>16.6</td>
<td>14:1</td>
<td>11.4</td>
<td>14:17</td>
<td>0.8</td>
</tr>
</tbody>
</table>
LAP/BG ratios indicate the relative substrate preferences and functions of microbial communities. High LAP/BG ratios (when the ratio quotient is >1), indicate microbial communities preferentially hydrolyzed peptides and proteins over polysaccharides (Zaccone & Caruso, 2019). Low ratios, and quotients >1, indicate a preferential flux of organic matter through polysaccharides (Zaccone & Caruso, 2019). The LAP/BG ratio was greater than 1 across all sites throughout the duration of sampling except for July 9 at BBH (Table 5).

LAP/AP ratios evaluate nutrient limitation of microbial communities. Sala et al. demonstrated with enrichment experiments that the ratio of LAP/AP decreased in P-deficient treatments and increased after inorganic P addition (Sala et al., 2001). Low LAP/AP ratios, when the ratio quotient is less than 1, indicate P-limitation. The LAP/AP ratios observed at CML were all greater than 1, indicating that nitrogen was the limiting nutrient over phosphorous (Table 5). At JEL, one occurrence of P-limitation was observed on June 6, with a LAP/AP ratio less than 1. BBH was more frequently limited by P than the other two sites, with three timepoints exhibiting LAP/AP ratios less than 1 in July, October, and January.
Figure 5: Percent of relative contribution of hydrolysis by each size fraction. Dark colored bars represent percent hydrolysis by <1.2 µm size fraction, lighter colored diagonal stripes represent percent of hydrolysis performed by >1.2 µm size fraction at each site. A: At CML, the smaller size fraction of the microbial community accounts for most enzymatic activity. B: At JEL, the large size fraction (>1.2 µm) is responsible for the majority of AP activity. C: At BBH, hydrolysis rates of BG are attributed to the large size fraction. D: Hydrolysis rates from all three sites summed by each enzyme; overall, most hydrolysis is performed by the small size fraction.

### 3.1.3 Relative contribution to total hydrolysis by size classes (<1.2µm)

The activity by each size fraction varied in their relative contribution to hydrolysis rates (Figure 5). At CML, the small fraction accounts for the majority of enzymatic activity by BG,
LAP, and AP. At JEL, more than 60% of the enzymatic activity of BG and LAP is due to the smaller fraction, but for AP most of the hydrolysis was performed by the larger fraction. At BBH, the small size class contributed to most of the LAP and AP hydrolysis rates, whereas BG hydrolysis rates were determined mainly by the large size class. Except for AP activity at JEL and BG activity at BBH, the smaller size fraction contributed the most. Overall, the <1.2 µm size class is responsible for at least 50% of more of the enzymatic activity of BG, LAP, and AP.

Table 6: Correlation analyses between enzymatic activity rates and environmental parameters. Linear regression coefficients (R²) describe strength of relationship between activities of BG, LAP, and AP, with sea surface temperature (SST), salinity, chlorophyll-a (chl-a) and bacterial cell count at three coastal sites (CML, JEL, BBH). Values in bold represent significant P-value (P<0.05).

<table>
<thead>
<tr>
<th>Coastal Sites</th>
<th>SST (°C)</th>
<th>Salinity (ppt)</th>
<th>Chl-a (µg/L)</th>
<th>Bacterial Cells (L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>P-Value</td>
<td>R²</td>
<td>P-Value</td>
</tr>
<tr>
<td>CML</td>
<td>BG 0.25</td>
<td>0.10</td>
<td>0.03</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>LAP 0.21</td>
<td>0.14</td>
<td>0.04</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>AP 0.15</td>
<td>0.22</td>
<td>0.05</td>
<td>0.48</td>
</tr>
<tr>
<td>JEL</td>
<td>BG 0.29</td>
<td>0.09</td>
<td>0.41</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>LAP 0.52</td>
<td>3 x 10⁻³</td>
<td>0.65</td>
<td>2 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>AP -0.10</td>
<td>0.17</td>
<td>0.01</td>
<td>0.76</td>
</tr>
<tr>
<td>BBH</td>
<td>BG 0.16</td>
<td>0.38</td>
<td>-0.33</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>LAP -0.07</td>
<td>0.58</td>
<td>0.00</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>AP 0.13</td>
<td>0.43</td>
<td>-0.11</td>
<td>0.47</td>
</tr>
</tbody>
</table>
3.1.4 Correlations between enzyme activities and selected environmental parameters

Linear correlation analyses revealed the strength of relationships between enzymatic activity and environmental parameters at the time of sampling. Correlation values are reported in Table 6. Nine out of thirty-six correlations reported statistically significant values.

Enzymatic activities and bacterial cell abundances had the most statistically significant correlations, although only at the CML and JEL sites. At CML, bacterial cell abundance explained 70% of the variance in the activity of BG, 60% of the variance in AP activity, and more than 50% of the variance in LAP activity. At JEL, the activities of BG and LAP correlated with bacterial cell abundance. Up to 93% of the variance in the activity of BG correlated to bacterial cell abundance. LAP had a moderately strong relationship with bacterial cell abundance supported by a significant P-value.

Chl-a had negative relationships to the enzymatic activities at CML and JEL, and a positive relation to the activities at BBH. Of all the enzymatic activities, chl-a had the greatest effect on AP activity, correlating with AP at two of the three sites. At CML, chl-a had a strong negative correlation to AP. At BBH however, the coefficient of determination shows that chl-a had a positive correlation with AP activity, yet the corresponding P-value indicates that the variability among samples was too high to fully explain a relationship; potentially, this uncertainty could be related to the small number of chl-a measurements included in the analysis.

Microbial enzymatic activity is not always directly correlated to co-located chl-a measurements due to a lag in response time by the microbial loop to phytoplankton blooms. At JEL, the activities of LAP and BG both had the highest peaks of activity on the sampling on 11-July, whereas the maximum chl-a measurement was observed 30 days prior on 11-June (Figure A
When a 30-day lag is assumed, strong correlations arise between enzymatic activity and chl-a (With delay, LAP: \( R^2 = 0.978, p = 0.094 \ N=3 \), BG: \( R^2 = 0.975, p = 0.099, \ N=3 \)). Due to the period during which the Great Bay Buoy is deployed at JEL, only three corresponding chl-a measurements existed that were 30d prior to sampling timepoints.

SST had little effect on enzymatic activity for any enzyme at any site, except for the activity of LAP at JEL. The rates of hydrolysis by LAP at JEL were moderately affected by sea surface temperature.

Salinity had a limited effect on any enzymatic activity at CML and small negative relationships with the activities at BBH. However, at JEL, where the greatest salinity range was experienced, salinity had a medium to large effect on the enzymatic activities of BG and LAP.

<table>
<thead>
<tr>
<th>Coastal Sites: Combined by Enzyme</th>
<th>SST (^{\circ}C)</th>
<th>Salinity (ppt)</th>
<th>Chl-a ((\mu g/L))</th>
<th>Bacterial Cells (L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(R^2)</td>
<td>P-Value</td>
<td>(R^2)</td>
<td>P-Value</td>
</tr>
<tr>
<td>BG</td>
<td>0.14</td>
<td>0.04</td>
<td>0.09</td>
<td>0.12</td>
</tr>
<tr>
<td>LAP</td>
<td>0.29 (4 \times 10^{-4})</td>
<td>0.01</td>
<td>0.51</td>
<td>0.00</td>
</tr>
<tr>
<td>AP</td>
<td>0.00</td>
<td>0.91</td>
<td>-0.03</td>
<td>0.34</td>
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</tbody>
</table>

Enzymatic activity data for each enzyme was pooled to isolate each enzyme’s overall activity, regardless of site. Reported in Table 7, three relationships arose with significant P-values, but each had small coefficients of determination. For the enzymatic activity of BG and LAP, SST
had a small but significant effect. For BG, 14% of the activity can be explained by SST, and for LAP, 29% of the activity is positively correlated to SST. Bacterial cell abundance was partially correlated to the activity of BG. A significant P-value supported that 16% of the overall variance in BG activity is related to the variance in bacterial cell abundance.

Correlation analyses between environmental parameters and the activity by each size class (<1.2 µm) is presented in Table 8. Three significant correlations were identified: two between enzymatic activity and salinity, and one between activity and chl-a. At JEL, the <1.2 µm size class was responsible for most of the activity by BG, and 43% of the variance in activity can be explained by variance in salinity. Salinity had a small, yet significant, effect on the activity of LAP in the <1.2 µm fraction. One additional significant correlation arose in the <1.2 µm size fraction between chl-a and AP at BBH. At BBH, most AP hydrolysis was performed by the smaller size class overall. The maximum hydrolysis by AP occurred on October 9 and was entirely driven by the small size fraction. Correlation analyses show that chl-a had a strong effect on the <1.2 µm size class AP hydrolysis rates.
Table 8: Coastal Sites: Fractionated Assays: Linear correlation analyses for size fractionated enzyme assays. Regression coefficients ($R^2$) reported, followed by P-value in parenthesis if significant (P<0.05). The smaller size class (<1.2 μm) passes through a 1.2 μm GF/C filter, whereas the difference between the small fraction and the whole water assay reveals the activity by the large size class (>1.2 μm). SST, sea surface temperature; Chl-a, chlorophyll-a.

<table>
<thead>
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<th>Small Size Class (&lt;1.2 μm)</th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>SST (°C)</td>
<td>Salinity (ppt)</td>
<td>Chl-a (µg/L)</td>
<td></td>
<td>R²</td>
<td>P-Value</td>
<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG</td>
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<td><strong>0.43</strong></td>
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<td>-0.04</td>
<td>0.69</td>
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<tr>
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<td>0.56</td>
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<tr>
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<td>0.22</td>
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<td>0.00</td>
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<td><strong>0.81</strong></td>
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<td>Salinity (ppt)</td>
<td>Chl-a (µg/L)</td>
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<td>P-Value</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG</td>
<td>0.15</td>
<td>0.21</td>
<td>0.09</td>
<td>0.35</td>
<td>-0.17</td>
<td>0.42</td>
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<tr>
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<td>0.40</td>
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<td>0.32</td>
<td>-0.22</td>
<td>0.34</td>
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<tr>
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<td>-0.14</td>
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<td>0.22</td>
<td>0.12</td>
<td>-0.18</td>
<td>0.40</td>
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<td>-0.09</td>
<td>0.09</td>
<td>0.09</td>
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<td>-0.03</td>
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</tr>
<tr>
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<td>-0.06</td>
<td>0.48</td>
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<td>0.19</td>
<td>0.03</td>
<td>0.76</td>
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<tr>
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<td>0.31</td>
<td>0.04</td>
<td>0.69</td>
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<tr>
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<td>0.28</td>
<td>0.06</td>
<td>0.65</td>
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</tbody>
</table>
Figure 6: Seasonal Enzymatic Activities. Seasonal average hydrolysis rates by each enzyme (BG, LAP, AP), categorized by season based on sampling date and in-situ temperature. BG experienced the greatest seasonal average during summer. Overall, the seasonal average hydrolysis rate by LAP was greatest during spring, yet this is driven by high activity at BBH. The seasonal average hydrolysis rate by AP was greatest during spring, closely followed by high activity during summer and fall.
To evaluate seasonal differences, enzymatic activities were grouped by sample date and with regard to sea surface temperature into either spring, summer, fall, or winter. Seasonal means of activity by each enzyme are represented in Figure 6. Samples taken in March and April were considered spring; samples collected in May, June, July, and August were grouped as summer; September, October, and November were categorized as fall, and January was considered winter. The seasonal average rates of activity by LAP and AP were greatest during spring, whereas BG activity had maximum rates in the summer. The magnitude of seasonal average activities by LAP was in the order of spring>summer>fall>winter, however, ANOVA tests did not detect any one seasonal activity to be significantly different from the hydrolysis rates during another season ($p = 0.46$, $N=39$). The seasonal average activity rates by AP were in the order of spring>fall>summer>winter. Comparison of means (ANOVA) reports did not support a significant seasonal difference of AP activity ($p = 0.51$, $N=39$). Overall, the magnitude order for seasonal BG activity was summer>winter>fall>spring, however, means comparisons of the seasonal average rates did not indicate that any one season was significantly different from another ($p = 0.18$, $N=30$).

Comparing seasonal means revealed site specific differences among enzymatic activities. One significant report arose during spring when the activity of LAP was significantly different at each site ($p < .001$, $N=18$). During spring at BBH, the activity by LAP was much greater than at the other two sites and deemed significantly different according to Tukey HSD connecting letters report. At CML and JEL, the seasons with the greatest LAP activity were summer and fall, respectively.
Additionally, the mean activity by AP during spring varied at each site, although ANOVA reports do not support a statistically strong variance. During spring, the mean activity of AP was near 300 nM/h at JEL, whereas the mean activities at CML and BBH were 48 nM/h and 38 nM/h respectively. The variance within each mean, however, was too high to report a significant difference of means at each site. During summer and fall, the activities of each enzyme were similar across sites, according to Tukey HSD statistical comparison of means.

### Table 9: Seasonal average enzyme hydrolysis rates and ratios.
Mean enzymatic hydrolysis rates grouped by season from CML, JEL, and BBH. Enzyme ratios were rounded to the nearest whole value. (Ratio bold when <1).

<table>
<thead>
<tr>
<th>Site</th>
<th>Season</th>
<th>BG (nM h(^{-1}))</th>
<th>LAP (nM h(^{-1}))</th>
<th>AP (nM h(^{-1}))</th>
<th>LAP/BG ratio</th>
<th>LAP/GP ratio quotient</th>
<th>LAP/AP ratio</th>
<th>LAP/AP ratio quotient</th>
</tr>
</thead>
<tbody>
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<td>8.8</td>
<td>161.2</td>
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<td>18.3</td>
<td>161:48</td>
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</tr>
<tr>
<td></td>
<td>Summer</td>
<td>24.7</td>
<td>245.1</td>
<td>67.1</td>
<td>49:5</td>
<td>9.9</td>
<td>245:67</td>
<td>3.6</td>
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<tr>
<td>JEL</td>
<td>Spring</td>
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<td>229.9</td>
<td>299.6</td>
<td>115:4</td>
<td>29.5</td>
<td>23:30</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>17.3</td>
<td>549.0</td>
<td>120.9</td>
<td>549:17</td>
<td>31.7</td>
<td>549:121</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>-</td>
<td>808.3</td>
<td>163.7</td>
<td>-</td>
<td>-</td>
<td>202:41</td>
<td>4.9</td>
</tr>
<tr>
<td>BBH</td>
<td>Winter</td>
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<td>13.8</td>
<td>16.6</td>
<td>7:19</td>
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<td>0.8</td>
</tr>
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<td></td>
<td>Spring</td>
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<td>859.7</td>
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<td>172.9</td>
<td>131.9</td>
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<td>173:132</td>
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<td>0.7</td>
</tr>
</tbody>
</table>

Across all three sites, LAP/BG ratios were high throughout spring and summer, which indicates a greater occurrence of microbes hydrolyzing proteins rather than polysaccharides (Table 9). The LAP/BG ratio was less than 1 only during winter at BBH, when BG activity prevailed over LAP activity, suggesting that polysaccharides were more efficiently recycled than proteins. The low LAP/AP ratios suggest that microbial communities at JEL had greater need for P during spring, and the communities at BBH experienced P-limitation during winter and fall.
3.2 Deep Ocean Site

3.2.1 Water column properties (CTD profile)

Data on the temperature, oxygen concentration, and chlorophyll fluorescence were collected throughout the water column at the Juan de Fuca site (Table 4, and Figure A - 6). Temperature ranged from 1.7 – 11.1°C from the surface layer (5 m) to the bottom nepheloid layer (2560 m). In the top layers of the ocean, temperatures remained within 8.8 – 11.1°C, then rapidly decreased between 250 m and 500 m to 4.6°C. From the mesopelagic (500 m) to the seafloor, temperature gradually decreased to 1.7°C.

The oxygen concentration profile revealed an oxygen minimum zone around 950 m. Oxygen decreased from a maximum concentration of 6.5 mg/L in the top ocean layers to a minimum of 0.2 mg/L at 950 m (OMZ). Below the OMZ, oxygen concentration increased to >1 mg/L in the bathypelagic (2000 m) and continued to rise in the bottom nepheloid layer to a concentration of 1.8 mg/L.

A deep chlorophyll maximum zone was observed in the chlorophyll fluorescence profile near 65 m. In the surface layer, chlorophyll concentration was 0.2 mg/m³, whereas deeper into the water column the chlorophyll fluorescence increased to a maximum of 1.3 mg/m³. Below the deep chlorophyll maximum, fluorescence readings significantly decreased as light dissipated in the deep layers of the ocean.

Bacterial cell abundance was greatest in the top layers of the ocean and decreased with increasing depth. As reported in table 4, the bacterial cell abundance decreased from the order of one billion per liter to one hundred million per liter between the surface layer and the deep chlorophyll maximum and decreased by another order of magnitude between the deep chlorophyll
maximum and the mesopelagic. From the mesopelagic to the bottom nepheloid layer, the bacterial cell abundance remained on the order of ten million. Although cell count generally decreased with increasing depth, the cell count in the bottom nepheloid layer was slightly higher than the previous ocean layer (bathypelagic).

<table>
<thead>
<tr>
<th>Sampling Depth, description</th>
<th>Temp (°C)</th>
<th>Oxygen (mg/L)</th>
<th>Fluorescence (mg/m³)</th>
<th>Bacterial Cells (L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 m, Surface</td>
<td>11.1</td>
<td>6.4</td>
<td>0.2</td>
<td>1.06E + 09</td>
</tr>
<tr>
<td>65 m, DCM</td>
<td>8.8</td>
<td>6.5</td>
<td>1.3</td>
<td>4.80E + 08</td>
</tr>
<tr>
<td>500 m, Mesopelagic</td>
<td>4.6</td>
<td>1.0</td>
<td>0.0</td>
<td>9.60E + 07</td>
</tr>
<tr>
<td>950 m, OMZ</td>
<td>3.4</td>
<td>0.2</td>
<td>0.0</td>
<td>7.03E + 07</td>
</tr>
<tr>
<td>2000 m, Bathypelagic</td>
<td>1.9</td>
<td>1.3</td>
<td>0.0</td>
<td>5.08E + 07</td>
</tr>
<tr>
<td>2560 m, BNL</td>
<td>1.7</td>
<td>1.8</td>
<td>0.0</td>
<td>5.85E + 07</td>
</tr>
</tbody>
</table>

Range: 1.7 – 11.1 0.2 – 6.5 0.0 – 1.3 5.08E+07 – 1.06E+09

3.2.2 Enzyme activities at the deep ocean site

At the deep ocean site, potential hydrolysis rates of alkaline phosphatase (AP) were the highest of all the enzymes assayed, with the exception of higher potential rates of LAP in the surface water sample (Figure 7). Overall, enzyme hydrolysis rates occurred in the following order of magnitude AP > LAP > NAG > BG > XYL. Activity by carbohydrate hydrolyzing enzymes (BG, NAG, XYL) were on the order of 0.1 nM/h whereas peptide (LAP) and phosphate (AP) hydrolysis
rates were on the order of 1 nM/h. Most enzymatic activity occurred in the top ocean layers and decreased with increased depth.

**Alkaline Phosphatase (AP)**

Hydrolysis rates by AP ranged from 2 – 6 nM/h, performing the maximum hydrolysis in the surface layer and minimum in the mesopelagic layer. According to the Tukey HSD connecting letters report, AP hydrolysis rates were statistically similar among the surface, deep chlorophyll maximum zone, oxygen minimum zone, and bottom nepheloid layer (M = 5.26 nM/h, SD = 0.52), and distinctly lower in the mesopelagic zone.

**Leucine Aminopeptidase (LAP)**

The activity of LAP had a maximum of 10.60 nM/h in the surface layer and a minimum of 0.72 nM/h in the OMZ. Hydrolysis rates by LAP were substantially different between the surface, deep chlorophyll maximum zone, and mesopelagic layers, decreasing by each layer, respectively. From the mesopelagic to the bottom nepheloid layer, activity remained at relatively the same low rate, supported by Tukey HSD tests (M = 0.78 nM/h SD = 0.04).
**N-Acetyl-β-D-Glucosaminidase (NAG)**

NAG hydrolysis rates ranged from 0.09 – 0.18 nM/h., with distinctly different levels of activity in the top ocean layers compared to the bottom ocean layers. The activity was highest in the surface layer, and although slightly lower, the activity in the deep chlorophyll maximum zone and mesopelagic were comparable to the surface layer (M = 0.17 nM/h SD = 0.008). Hydrolysis rates in the oxygen minimum zone, bathypelagic, and bottom nepheloid layer were distinctly lower.
than the top three layers, yet within these regions the activity of NAG was statistically similar (M = 0.09 nM/h SD = 0.003).

\( \beta \)-Glucosidase (BG)

The range of hydrolysis rates by BG was 0.24 – 0.01 nM/h. The maximum level of activity was in the surface layer, followed by the deep chlorophyll maximum zone, whereas activity remained low from the mesopelagic to the bottom nepheloid layer. According to Tukey HSD tests, the activity in the mesopelagic, oxygen minimum zone, bathypelagic, and bottom nepheloid layer are not significantly different (M = 0.04, SD = 0.023), however, a notable increase in activity was observed between the bathypelagic layer and the bottom nepheloid from 0.01 nM/h to 0.06 nM/h.

Xylosidase (XYL)

Xylosidase hydrolysis remained <0.1 nM/h overall, ranging from 0.0 – 0.1 nM/h. The maximum activity was observed in the mesopelagic layer and was statistically alike the level of activity in surface layer (M = 0.08 nM/h, SD = 0.024), however, between the two layers, no activity was detected at the deep chlorophyll maximum zone. Similarly, moderately high activity occurred in the oxygen minimum zone and the bottom nepheloid layer (M = 0.05 nM/h, SD = 0.00), but no activity was measured in the layer between (bathypelagic). The rates of hydrolysis by XYL increased in the bottom nepheloid layer compared to the bathypelagic from 0 to 0.05 nM/h.
High LAP/BG ratios were observed at all depth layers, indicating a preferential flux of proteins over polysaccharides throughout the water column (Table 10). The highest LAP/BG ratio quotient occurred in the bathypelagic layer, followed by the lowest LAP/BG ratio quotient at the bottom nepheloid layer, suggesting there was a shift of organic matter composition between these layers. High AP activity at depth decreased the LAP/AP ratio, indicating a P-limitation from the deep chlorophyll max to the bottom nepheloid layer.

Table 10: Selected water column enzymatic hydrolysis rates (BG, LAP, AP) and ratios. Mean enzymatic hydrolysis rates by BG, LAP, and AP at sampled water column depths. Enzyme ratios were rounded to the nearest whole value. (Ratio bold when <1).

<table>
<thead>
<tr>
<th>Depth</th>
<th>BG (nM h⁻¹)</th>
<th>LAP (nM h⁻¹)</th>
<th>AP (nM h⁻¹)</th>
<th>LAP/BG ratio</th>
<th>LAP/BG ratio quotient</th>
<th>LAP/AP ratio</th>
<th>LAP/AP ratio quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>0.2</td>
<td>10.7</td>
<td>5.9</td>
<td>107:2</td>
<td>43.8</td>
<td>11:6</td>
<td>1.8</td>
</tr>
<tr>
<td>DCM</td>
<td>0.1</td>
<td>3.6</td>
<td>5.5</td>
<td>36:1</td>
<td>24.9</td>
<td>2:3</td>
<td>0.6</td>
</tr>
<tr>
<td>Meso</td>
<td>0.1</td>
<td>0.8</td>
<td>2.3</td>
<td>16:1</td>
<td>14.9</td>
<td>1:2</td>
<td>0.3</td>
</tr>
<tr>
<td>OMZ</td>
<td>0.0</td>
<td>0.7</td>
<td>4.8</td>
<td>36:1</td>
<td>43.4</td>
<td>1:5</td>
<td>0.2</td>
</tr>
<tr>
<td>Bathy</td>
<td>0.0</td>
<td>0.8</td>
<td>3.3</td>
<td>80:1</td>
<td>60.3</td>
<td>1:3</td>
<td>0.2</td>
</tr>
<tr>
<td>BNL</td>
<td>0.1</td>
<td>0.8</td>
<td>4.9</td>
<td>40:3</td>
<td>14.1</td>
<td>1:5</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Figure 8: Contribution by β-Glucosidase (BG), N-Acetyl-β-D-Glucosaminidase (NAG), and Xylosidase (XYL) to total potential carbohydrate hydrolysis in Juan De Fuca water column. NAG contributed most to total hydrolysis overall. In the BNL, contribution to total hydrolysis is nearly equally distributed among all three enzymes. DCM, Deep Chlorophyll Maximum; Meso, Mesopelagic; OMZ, Oxygen minimum Zone; Bath, Bathypelagic; BNL, Bottom Nepheloid Layer.

Table 11: Hydrolysis by carbohydrate hydrolyzing enzymes (BG, NAG, XYL) and ratios. Mean enzymatic hydrolysis rates by BG, NAG, and XYL at sampled water column depths. Enzyme ratios were rounded to the nearest whole value. DCM, deep chlorophyll max; Meso, mesopelagic; OMZ, oxygen minimum zone; Bath, bathypelagic; BNL, bottom nepheloid layer.

<table>
<thead>
<tr>
<th>Depth</th>
<th>BG (nM h⁻¹)</th>
<th>NAG (nM h⁻¹)</th>
<th>XYL (nM h⁻¹)</th>
<th>BG/NAG/XYL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>4:3:1</td>
</tr>
<tr>
<td>DCM</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>14:17:0</td>
</tr>
<tr>
<td>Meso</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>5:16:10</td>
</tr>
<tr>
<td>OMZ</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>2:9:5</td>
</tr>
<tr>
<td>Bathy</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>1:9:0</td>
</tr>
<tr>
<td>BNL</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>6:9:5</td>
</tr>
</tbody>
</table>

3.2.3 Relative contributions of three carbohydrate hydrolases (BG, NAG, XYL) to total carbohydrate hydrolysis rates

In the surface layer, nearly 50% of potential carbohydrate hydrolysis was attributed to hydrolysis by BG, 40% of hydrolysis was performed by NAG, and the remaining 10% of activity
was the hydrolysis by XYL (Figure 8), represented by a BG/NAG/XYL ratio of 4:3:1 (Table 11). In the deep chlorophyll max, no xylosidase hydrolysis was measured, and NAG activity prevailed over BG activity with a higher BG/NAG/XYL ratio of 14:17:0. In the deep chlorophyll max, NAG accounted for about 60% of total hydrolysis with the remaining activity being that of glucosidase hydrolysis. In the mesopelagic and OMZ, xylosidase accounted for more of the total hydrolysis than it had in other ocean layers, but the majority of hydrolysis in these regions was by NAG. In the bathypelagic, NAG hydrolysis accounts for nearly all of the total hydrolysis, with only about 15% attributed to glucosidase activity, represented by a BG/NAG/XYL ratio of 1:9:0. In the bottom nepheloid layer, the BG/NAG/XYL ratio increases to 6:9:5, where NAG is still the most active however the activity by BG and XYL significantly increased compared to the previous layer. In the bottom nepheloid layer the contribution to total hydrolysis is almost evenly distributed amongst each carbohydrate hydrolyzing enzyme.

3.2.4 Correlations between enzyme activities and selected water column properties

Several significant correlations were found between environmental parameters (temperature, oxygen, and bacterial cell abundance) and enzymatic activities in the water column at the Juan De Fuca site (Table 12). The potential activity of BG was significantly and strongly correlated positively with all three parameters. The potential activity of BG was significantly and strongly correlated positively with all three parameters (i.e., highest rates in the depths with highest temperatures, oxygen concentrations, and cell abundances). LAP activity was also positively correlated with temperature and cell abundance but not with oxygen concentrations. Despite having the highest overall activity (Figure 7), AP did not have any significant correlations with any parameter (Table 12). XYL activity also had no significant correlation to any parameter. NAG activity was only correlated to temperature.
Table 12: Linear correlations between enzymatic activity and temperature, oxygen, and bacterial cell counts. Regression coefficients ($R^2$) are reported, as well as corresponding P-values, significant when $P<0.05.$

<table>
<thead>
<tr>
<th></th>
<th>Temperature ($^\circ$C)</th>
<th>Oxygen (mg/L)</th>
<th>Bacterial Cells (L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>P-Value</td>
<td>$R^2$</td>
</tr>
<tr>
<td>BG</td>
<td>0.89</td>
<td>0.01</td>
<td>0.84</td>
</tr>
<tr>
<td>NAG</td>
<td>0.76</td>
<td>0.02</td>
<td>0.57</td>
</tr>
<tr>
<td>XYL</td>
<td>0.01</td>
<td>0.90</td>
<td>0.06</td>
</tr>
<tr>
<td>LAP</td>
<td>0.79</td>
<td>0.02</td>
<td>0.65</td>
</tr>
<tr>
<td>AP</td>
<td>0.35</td>
<td>0.22</td>
<td>0.48</td>
</tr>
</tbody>
</table>

4. Discussion

Climate change may lead to warmer oceans that hold less oxygen and receive different inputs of freshwater and continental derived organic matter. This research set out to examine if there are correlations between marine microbial activities (as measured by extracellular enzyme hydrolysis rates) and environmental parameters such as temperature, salinity, phytoplankton biomass, oxygen, and bacterial cell abundance, in order to increase predictive power of how marine systems may respond to climate change perturbations. To address this, the potential rates of peptide, phosphate, and carbohydrate hydrolyzing enzymes were measured at three coastal sites in the Gulf of Maine over a period of two years, as well as the rates of activity with water depth at an open ocean site in the northeastern Pacific. It was hypothesized that enzymatic activities will follow seasonal changes in sea surface temperature in the Gulf of Maine, where higher temperatures enable greater rates of enzymatic hydrolysis. Additionally, variations in enzyme activity will correspond to variations in salinity, i.e. more active hydrolysis by microbial
communities in higher salinity water. Timepoints with high phytoplankton biomass will correspond to high enzymatic activity. A strong relationship between enzymatic activity and oxygen concentration will be prevalent in the water column at the deep ocean site where reduced oxygen conditions slow microbial activity. Out of the enzymes measured in this study, AP is the only enzyme that can be primarily produced by other marine organisms (microeukaryotes), not just heterotrophic bacteria. For this reason, it is hypothesized that all other measured enzymatic activities will correlate to bacterial cell abundance except AP, which will be decoupled from bacterial cell numbers. I observed that potential hydrolysis rates were overall poorly correlated with contemporaneous environmental parameters at the coastal sites (Table 4). The strongest correlations were between enzyme activity and bacterial cell abundance, but this correlation was not observed at all sites. Temperature and salinity only moderately correlated with enzyme activity, though not consistently across sites. Contemporaneous phytoplankton biomass did not correlate with enzyme activities, but this may be due to a lag in time of when enzymatic breakdown of phytoplankton-derived organic matter happens post phytoplankton blooms. In contrast, at the open ocean site, some enzyme activities had strong positive correlations with temperature, oxygen, and bacterial biomass, although the overall most active enzyme was not correlated with these parameters (Table 7). In aggregate, these results indicate that under current climate conditions, no one environmental parameter is significantly affecting enzymatic activity, however, a microbial response is expected under more extreme variations of temperature and greater loss of oxygen. These findings provide the basis for making predictions of ecosystem response to climate change perturbations.
4.1 Hypothesis 1: Relations to temperature

The rates of BG, LAP, and AP recorded at the coastal sites in this study did not strongly correlate with temperature. Although other coastal studies conclude that temperature is a driving factor (Caruso et al., 2013), the temperature sensitivity of enzymes can change seasonally, and by geographic region, thus, temperature alone may not drive enzymatic activity (Cunha & Almeida, 2010). The results of this study suggest that the enzymes in the Gulf of Maine are functioning in temperatures ranges well below the temperature ranges that facilitate maximum hydrolysis rates. Warm temperatures in the Gulf of Maine are not maintained for long periods, and do not approach optimal temperatures for maximum enzymatic activity. The optimal temperature for maximum hydrolysis by BG is in the range of 40-50°C (Fang et al., 2010; Hernández-Guzmán et al., 2016; King, 1986; Pyeon et al., 2019), LAP temperature optima ranges from 30-60°C (Zhang et al. 2018; Liew, Tay, and Puthucheary 2013; Matsushita-Morita et al. 2011; Lee et al. 1998), and the optima for AP is around 40°C (Chu et al., 2019; Mobley et al., 1984). Among the coastal areas, the highest recorded temperature was 23.5°C at JEL, a temperature well below that of maximum hydrolysis by BG, LAP, or AP. Yet a moderate correlation was found between the activity of LAP and temperature at JEL, indicating that temperature influenced the activity of LAP. Out of the three coastal sites, JEL experienced temperatures nearest to the optimal temperature for LAP, where the warmest temperatures facilitated greater hydrolysis by LAP. However, the temperatures at JEL were too far from the optima of BG and AP to detect any significant influence. At CML, sea surface temperatures ranged from 1.7 to 15.4°C. The enzymatic activities of BG, LAP, and AP remained relatively low throughout the sampling period until the final timepoint (July 11) when the maximum hydrolysis rates were recorded. On July 11, the maximum temperature was also recorded at 15.4°C, indicating that water temperatures had not begun to affect enzymatic activity.
until they became much warmer. Overall, the water temperatures recorded at the coastal sites remained in a high enough range to foster activity yet were not close enough to optimal temperatures to result in any strong effects on the hydrolysis rates of BG, LAP and AP.

In regions where temperature and enzymatic activities are closely correlated, such as in the Mediterranean Sea, higher temperature ranges are maintained throughout the year. One study, for example, considered several coastal sites in the Mediterranean Sea experiencing yearly ranges from 11 - 27°C and found that temperature was a main driver of enzymatic activity (Caruso et al., 2013). Though the maximum temperatures in the Gulf of Maine are not much lower than in the Mediterranean, the higher range of temperatures is maintained for longer periods of time at temperatures closer to the optimal temperatures for maximum enzymatic activity. Yearly average temperatures reported in the Mediterranean Sea study are in the range of 18 – 20°C, whereas in this study average temperatures at each coastal site were 7.6°C, 11°C, and 13.6°C. Overall, the Gulf of Maine does not sustain temperature ranges close enough to enzymatic optimal temperatures. By the time that Gulf of Maine waters reach maximum temperatures in summer, the seasons in this region are already transitioning into fall and winter, cooling the water to much lower temperatures than experienced in the Mediterranean Sea. Considering differences in temperature ranges, this may explain why a close correlation was found in the Mediterranean and not in the Gulf of Maine.

At the deep ocean site, water temperature decreased from the surface to the seafloor, as did the activities of BG, NAG, LAP, and AP. Although strong correlations were found between temperature and the activities of BG, NAG, and LAP, the variation in enzymatic activity cannot solely be explained by temperature. Deep sea microbial communities are reliant on sinking organic matter from the surface, thus making substrate availability a driving factor of enzymatic activity.
However, the concentration of substrates as well as the lability of organic matter also decreases with depth, reducing the quantity and quality of available substrates for enzymatic hydrolysis.

4.2 Hypothesis 2: Relations to salinity

The coastal study areas included one site with minimal salinity variation (BBH), one with a moderate salinity range (CML), and one estuarine site that experienced a wide range of salinities (JEL), (Tables 1, 2, and 3). Salinity at JEL ranged from 12.8 - 28.6 ppt due to the fluctuation between riverine sources and oceanic tides. At JEL, there were two moderately strong positive correlations between enzymatic activities of BG and LAP with salinity, demonstrating that at higher salinities BG and LAP were more active. At JEL, a higher measurement of salinity signifies that high tide brought oceanic waters into the estuary, along with the oceanic microbial communities and therefore, potential changes in enzymatic activities. The relationship to salinity suggests that microbial communities in the ocean waters were more active in hydrolysis by BG and LAP than the communities in the lower salinity water.

The fractionated enzyme assays were able to decipher that this correlation was driven by activity of the small size class (<1.2 µm). At JEL, strong correlations were found between salinity and the activity of BG and LAP by the small size class. When salinities were higher, so was the activity by the small size class, and in low salinity water, activity by the small size class was lower. This further indicates that the flux of ocean tides had shifted the microbial community within the estuary.

4.3 Hypothesis 3: Relations to chlorophyll-a

Correlations to chlorophyll-a indicate a relationship between phytoplankton biomass and enzymatic activity. At CML and BBH, anticorrelation relationships between chl-a and the activities of AP arose.
A negative correlation between chl-a and AP activity was found at CML. At this site, results indicate that during certain timepoints, phytoplankton were the main source of AP, rather than microbes. In early spring (March), when the maximum chl-a measurement was recorded at CML, the activity of AP was lowest. Combined, the high chl-a measurement and low AP activity suggests that a community of phytoplankton was active, and growth was not limited by available P. However, following this timepoint, and through the rest of spring, phytoplankton biomass diminished, and the activity of AP increased. During spring, the contribution to total AP hydrolysis by >1.2 µm fraction ranged from 25-60%, with the exception of one timepoint on which the contribution was 0%. The greater contribution to total AP hydrolysis rates by the >1.2 µm size class suggests that AP activity was attributed to phytoplankton, microalgae, and/or other larger organisms, rather than bacteria. High AP activity by the >1.2 µm fraction coupled with low chl-a activity is indicative that phytoplankton growth had been limited by P, thus, the communities were producing more AP.

This is further supported by historical data regarding phytoplankton community structure at CML. Historically, communities of small flagellates and dinoflagellates make up a large fraction of the phytoplankton biomass during April; although during one April a diatom bloom took the majority (Figure A - 3; (Moore, 2008)). Based on seasonal phytoplankton community patterns, it is expected that springtime communities at CML consist mostly of small flagellates and dinoflagellates. Dinoflagellates can be responsible for AP activity. A previous study in Monterey Bay California, found that despite dinoflagellates comprising only 14% of all cells counted, the dinoflagellates accounted for 78% of AP producing cells (Nicholson et al., 2006). At CML, the assumed springtime phytoplankton communities are made up of AP producing organisms, which supports that AP activity at CML was attributed to phytoplankton.
At BBH, however, the opposite scenario is driving a correlation between AP and chl-a. A positive relationship between chl-a and AP activity indicates that when chl-a concentrations are high, so is the activity of AP, yet the >1.2 µm size class did not contribute to total AP hydrolysis. At BBH, the maximum chl-a measurement took place in October, coinciding with the timepoint at which maximum AP hydrolysis rates were recorded. Additionally, on this date the <1.2 µm fraction made up 100% of AP hydrolytic activity, indicating that at BBH, phytoplankton were not responsible for the production of AP. Instead, heterotrophic bacteria were responsible for AP activity. At BBH, the seasonal LAP/AP ratios indicated that communities became limited by P during fall. When coupled with a high chl-a recording in October, this suggests that the bacterial communities suddenly faced a P limitation in the fall when a phytoplankton bloom reduced available P. The phytoplankton community composition at BBH consisted of a combination of ciliates, dinoflagellates and other phytoplankton during summer months of 2019, followed by a large diatom bloom in October (Figure A - 4). Studies have shown that diatoms infrequently produce AP, and generally synthesize AP to a lesser extent compared to other phytoplankton (Dyhrman & Ruttenberg, 2006; Nicholson et al., 2006). At BBH, evidence indicates that the large diatom bloom resulted in a depletion of nutrients, and because these organisms do not often produce AP, microbial communities synthesized more AP in response to a nutrient limitation.

*Relations to chl-a: considering a delayed microbial response*

Relationships between chl-a and enzymatic activity are not always directly correlated due to an uncoupling between the development of phytoplankton blooms and response time by microbial communities. A delay in microbial activity relative to phytoplankton biomass is often found, implying that in some cases, the post-bloom breakdown of phytoplankton cells produces more substrates for hydrolytic processing than during the bloom itself (Middelboe et al., 1995).
Middelboe observed that the activities of BG and LAP by free-living bacteria increased during the late stage of a phytoplankton bloom, suggesting that dissolved organic carbon and polymeric carbohydrates were released as the bloom collapsed (Middelboe et al., 1995). Results at JEL displayed similar instances of delayed responses by BG and LAP. Figure A - compares the concentration of chl-a at JEL to the hydrolysis rates by BG and LAP. At JEL, the activities of BG and LAP both reach maximum rates of hydrolysis on July-11, however, the chl-a concentration on the same timepoint is relatively low. Similarly, on the date for which the maximum chl-a concentration was recorded (Jun-11), the activities of BG and LAP were considerably lower than other timepoints, therefore resulting in no correlation between chl-a and enzymatic activities (BG: \( R^2 = -0.03, p = 0.712, N=7 \), LAP: \( R^2 = -0.01, p = 0.753, N=12 \)). Bacterial response delay times have been observed on timescales of 7-14 days in temperate regions, and up to 30 days in Antarctic waters (Billen, 1990; Middelboe et al., 1995). In this study, there was a difference of 30 days between the date of maximum chl-a and maximum hydrolysis rates. When a time lag of 30d is considered, there are strengthened relationships between chl-a and the activities of BG and LAP (BG: \( R^2 = 0.975, p = 0.100, N=7 \), LAP: \( R^2 = 0.9783, p = 0.94, N=3 \)). The statistical support of the P-value is diminished due to the limited availability of buoy data when including a 30d delay.

This is further supported by fractionated enzyme activity data. The fractionated enzymatic activity on July 11 demonstrates that 100% of the hydrolysis by BG and LAP was attributed to the <1.2 \( \mu \)m size class, indicating that activity was by free-living bacteria (Figure A - 5) after the phytoplankton bloom. No contribution by the larger size class (>1.2 \( \mu \)m) to total hydrolysis suggests that phytoplankton and algae were no longer a source of enzymatic activity. Instead, enzymatic activity was mainly attributed to free-living bacteria responding to newly provided substrates through the termination of a bloom.
4.4 Hypothesis 4: Relations to oxygen

Variations in oxygen concentration were tightly coupled with variations in enzymatic activity, driving strong correlations at the deep ocean site. In the surface layer where oxygen saturates the water, enzymatic activity was high, but at the OMZ, when oxygen concentration was lowest (0.2 mg/L), the functions of BG, NAG, and LAP significantly decreased. Deeper in the water column, oxygen saturation slightly increased, as did some enzymatic activities. While an abundance of marine microbes respire oxygen to carry out metabolic activities, there are also many species of anaerobic bacteria. Due to the methodological capabilities of this study, only aerobic microbial activities were assayed. Since anaerobic processes are less efficient at hydrolyzing structurally complex macromolecules, and because the abundance of labile organic material decreases with increased depth, it is assumed that microbial activity within the OMZ is lower compared to the aerobic processes in surface layers (Jiao et al., 2010; Kristensen et al., 1995). A similar study off the coast of northern Chile observed that while a portion of degradation occurs in the OMZ, most organic matter is degraded in the oxygen rich surface layer (Pantoja & Sepu, 2004). When there are regions of low oxygen supply in the water column, such as the OMZ, it becomes more likely that undegraded organic matter reaches the seafloor (Pantoja & Sepu, 2004). Results from the deep ocean site provide evidence that lower enzymatic activity occurred in the OMZ compared to layers with greater oxygen saturation.

Sinking material was minimally degraded as it passed through OMZ present at the deep ocean site. This sinking material delivers a higher quantity of less refractory compounds to the bottom nepheloid layer thereby stimulating nepheloid layer enzymatic activities. Figure 8 displays a change in relative activity within the carbohydrate hydrolyzing group (BG, NAG, and XYL) supporting that a fluctuation of organic matter flux occurred between the bathypelagic layer and
the bottom nepheloid layer. The relative BG/NAG/XYL ratios increased from 1:9:0 in the
bathypelagic layer to 6:9:5 in the bottom nepheloid year, demonstrating an increase in activity by
both BG and XYL. The relative shift in BG/NAG/XYL ratios at the bottom nepheloid layer
indicate a shift either in nutrient requirements, available organic substrate, microbial community
composition, and/or the enzymatic capabilities of the bacteria present.

Since results indicate that at the time of sampling this site, activity at the sea floor was
stimulated by sinking organic material, satellite chl-a data was used to investigate if a recent
phytoplankton bloom contributed to a heightened flux of organic matter to the seafloor.
Considering that organic matter sinking velocities occur within rates from 50 to 100 meters per
day, organic matter from the surface would reach the bottom nepheloid layer (2650 m) between
27 – 53 days (Alldredge & Gotschalk, 1988; Karakaş et al., 2009; McDonnell & Buesseler, 2010).
Satellite derived chl-a concentration indicates that the surface waters at the deep ocean site
experienced greater phytoplankton biomass from April 01 – April 09, with chl-a ranges within 1-2
mg/m³ compared to relatively lower chl-a concentrations on the day of sampling (~0.2 mg/m³)
(accessed via the web-based ERDDAP NOAA CoastWatch, Aqua MODIS, Figure 9). Sampling
at the deep ocean site occurred about 48 - 49 days after the high chl-a concentrations in early April,
falling within the timeframe for which it takes products of a phytoplankton bloom to reach the
seafloor. The time of sampling coincided with an accumulation of sinking particles depicted in
Figure 9. Sampling results demonstrate an increase in enzymatic response by the deep-sea
microbial communities to a flux of organic matter.
Figure 9: Timeline of deep ocean sampling in relation to sinking organic matter. A: Satellite derived chl-a concentration on April 01, 2019. (prior to sampling date) B: Satellite derived chl-a concentration on March 19, 2019. (date of sampling) C: Organic matter from surface is exported to the deep sea. Date of sampling at Juan de Fuca site coincided with a time in which fresh organic matter had reached the deep sea. Activity in the deep ocean is being fueled by sinking matter.
4.5 Hypothesis 5: Relations to bacterial cell abundance

The activities of BG and LAP were correlated to bacterial cell abundance at CML and JEL, and AP had a moderately strong relationship at CML. A positive correlation between bacterial cell abundance and enzymatic activity indicates that the selected enzyme was mainly produced by the bacterial community during the sampling period, as opposed to being produced by microalgae, or being associated with cell-free enzymatic activity. Bacterial cell abundance had the strongest effect on the activity of BG, aligning with the established notion that BG is primarily associated with bacteria (Chróst, 1989; Rath & Herndl, 1994). During this study, results indicate that LAP was also predominantly produced by the bacterial communities present. For the activity of AP at CML, 60% of the variance was coupled with variance in bacterial cell abundance, indicating that AP activity was associated with bacteria for most of the study period.

Cell abundance and enzymatic activity at the deep ocean site decreased with increasing depth, pointing out that where there were lower bacterial abundances, there were also less enzymes being produced. The activities of BG and LAP were strongly correlated to bacterial cell abundance, indicating that bacteria were the main source of these enzymes. Weaker relationships were found between cell abundance and the activities of NAG and AP, implying that a portion of the enzymatic activity was attributed to the presence of bacteria, however, additional cell-free or particle associated enzymatic activity contributed to the total activity of these enzymes. Previous studies have assumed that in the deep sea, the preferred, and most efficient method of substrate acquisition is via cell-associated enzymatic hydrolysis, however, mostly dissolved AP has been found in the deep sea (Zhao et al., 2020). In this study, the uncoupling between bacterial cell abundance and the enzymatic activity by AP support this finding by indicating that cell-free, dissolved, enzymatic activity contributed to total hydrolysis in the deep sea.
The level of AP activity remained relatively high throughout the entire water column. According to Tukey HSD comparison of means, the activity of AP in the bottom ocean layers were statistically like those in the upper layers. Although AP is typically induced in response to P limitation, high levels of AP activity and high inorganic P concentrations have often been observed in deep waters, resulting in an alkaline phosphatase paradox (Hoppe, 2003; Thomson et al., 2019; Zaccone & Caruso, 2019). With readily available P in the deep sea, AP activity would be expected to be low. Despite relatively few deep-sea studies investigating this, the AP paradox is assumed to be a ubiquitous pattern. A reasonable explanation for this paradox was provided in Thomson et al. 2019, with evidence of long-lived cell free AP activity. Extracellular AP can remain active for months, and can accumulate in the environment (Thomson et al., 2019). Biological activity in the upper layers of the ocean requires the production of AP, however, AP can be transported to the deep via sinking particles adding to the AP load of deep currents (Hoppe, 2003). In this study, the maintained high activity of AP throughout the water column and a decoupling from bacterial cell abundance implies that cell-free AP had descended and accumulated in the deep sea where it remained active.

High AP activity in the deep sea can also be attributed to the functional diversity of the enzyme. Baltar et al. 2016 found that AP in the deep ocean is also involved with C acquisition in C limited prokaryotes (Baltar et al., 2016). Considering that deep sea communities are reliant on carbon export from the upper layers, deep sea communities can be using AP for the acquisition of C rather than inorganic P, as the process of AP hydrolysis is capable of releasing both P and C from complex matter (Baltar et al., 2010).
4.6 Considering other drivers of enzymatic activity

Nitrate:

A weak and non-statistically significant correlation was reported between nitrate concentration and the activity of LAP. However, an inverse relationship is observed between nitrate concentration and the activity of LAP at JEL, as illustrated in Figure A - 1. When concentrations of nitrate were high, the activity by LAP, the enzyme associated with peptide and protein hydrolysis, was relatively low implying that sources of N were available to the microbial community therefore not requiring the production of LAP. At the last timepoint, a peak in LAP activity was recorded that coincides with a low nitrate concentration, indicating that the spike in LAP activity is a response to the depletion of available N.

Microbial community composition:

Although information on the microbial community structure was not included in this study, it is expected that shifts in community structure occurred throughout the sampling period, both spatially and temporally. For example, taxa associated with phytoplankton blooms thrive during spring, whereas taxa known to thrive in low-nutrient environments dominate during fall and winter (Teeling et al. 2012; Partensky and Garczarek 2010). In the Gulf of Maine, Pelagibacter and Flavobacteria have been more abundant during spring, whereas cyanobacteria such as Prochlorococcus and Synechococcus are the main taxa found more often in the fall (Zorz et al., 2019). For the cases when environmental factors do not strongly correlate to enzyme activity, it is important to consider that community structure could have played a role in variances. However, some studies suggest that shifts in community composition are decoupled from enzymatic activity rates, where instead, variability in substrate supply regulates enzymatic activity (Kamalanathan et al., 2020). Kamalanathan et al 2020, observed that activities of BG and LAP were decoupled from
shifts in microbial composition, suggesting that regardless of community composition, BG and LAP were expressed in response to substrate availability, thus, functional redundancy for enzyme production existed (Kamalanathan et al., 2020). In Shi et al. 2019, results suggested that different groups of bacterial communities hydrolyzed the same polysaccharides, highlighting a parallel in the enzymatic capabilities of microbial communities. The term functional redundancy also refers to the production of the same enzyme for acquisition of more than one nutrient. For example, LAP is important to both the carbon cycle and nitrogen cycle because the hydrolysis of protein containing particles releases both C and N (Zaccone & Caruso, 2019). Due to the functional redundancy of LAP, a microbial community can produce this enzyme for the acquisition of C and/or N depending on which substrates are available. Similarly, AP has been found to be expressed to acquire C when communities are limited by C (Baltar et al., 2016). Therefore, shifts in microbial community structure do not always strongly affect patterns of enzymatic activity.

5. Summary and conclusions

No one parameter was the sole driver of patterns in enzymatic activity based on the environmental conditions experienced throughout this study. The activities of BG, LAP, and AP were driven by a combination of environmental factors that varied spatially and temporally in the coastal Gulf of Maine. Diversity in the patterns of enzymatic activity between the coastal sites revealed site-specific variation, despite studying a relatively narrow region.

The relationships between enzymatic activity and temperature revealed that the current range of temperatures do not affect enzymatic activity, because they are well below the optimal temperature for maximum BG, LAP, and AP hydrolysis, unlike studies at warmer sites. Increased rates of activity by LAP were observed at the warmest coastal site (JEL), where temperatures were
nearest to that of the optimal temperature for LAP. As the Gulf of Maine continues to rise in temperature, it will approach optimal temperatures for maximum enzymatic activity.

Relationships between enzymatic activity and salinity indicated that in higher salinity water, greater rates of enzymatic activity were present. Land runoff, erosion, and additional fluvial input are expected to increase as a result of climate change, impacting coastal salinity and thus the activity of microbial communities.

Relationships with chl-a described the complex interactions with phytoplankton biomass that shape seasonal patterns of enzymatic activities. Environmental influences on phytoplankton biomass have a direct relationship to enzymatic activity as phytoplankton-derived organic matter provides substrates for hydrolytic breakdown by microbial communities.

Enzymatic activity was depressed under the low oxygen concentrations present in the oxygen minimum zone within the water column at the deep ocean site. Warming oceans reduce oxygen solubility and enhance water column stratification, a result of which is the expansion of oxygen minimum zones at depth. Further decreases of dissolved oxygen concentration could slow the rates of enzymatic activity, and therefore reduce organic matter cycling in the ocean.

Microbes are essential to a functioning oceanic ecosystem and understanding how microbes interact with their environment becomes increasingly important as climate change threatens to alter the physical, chemical, and biological environment of microbial communities. Results of this study provide insight on the current relationships between enzymatic activities and environmental parameters. This information contributes to the understanding necessary to make accurate predictions of ecosystem response to climate change.
References


Pantoja, S., & Sepu, J. (2004). Decomposition of sinking proteinaceous material during fall in the oxygen minimum zone off northern Chile. 16.


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https://doi.org/10.3389/fmicb.2019.00281
Table A-1: Volumetric and cell specific hydrolysis rates by BG, LAP, and AP at CML.

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Bacterial Cells (L⁻¹)</th>
<th>Volumetric Rate (nM/h)</th>
<th>Rate Per Cell (amol/cell∙h)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>BG</td>
<td>LAP</td>
</tr>
<tr>
<td>5-Mar-19</td>
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<td>0.16</td>
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</tr>
<tr>
<td>19-Mar-19</td>
<td>2.9 x 10⁸</td>
<td>6.74</td>
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<td>14.82</td>
<td>88.12</td>
</tr>
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<td>2-Apr-19</td>
<td>2.9 x 10⁸</td>
<td>5.85</td>
<td>178.11</td>
</tr>
<tr>
<td>9-Apr-19</td>
<td>2.6 x 10⁸</td>
<td>5.82</td>
<td>129.34</td>
</tr>
<tr>
<td>16-Apr-19</td>
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<td>21.78</td>
<td>451.39</td>
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</tr>
<tr>
<td>7-May-19</td>
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<td>6.23</td>
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<tr>
<td>6-Jun-19</td>
<td>2.7 x 10⁸</td>
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<td>11-Jul-19</td>
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<td>0.16 – 102.20</td>
<td>3.03 – 652.85</td>
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Table A - 2: Volumetric and cell specific hydrolysis rates by BG, LAP, and AP at JEL.

<table>
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<th>Sampling Date</th>
<th>Bacterial Cells (L⁻¹)</th>
<th>Volumetric Rate (nM/h)</th>
<th>Rate Per Cell (amol/cell-h)</th>
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<tbody>
<tr>
<td></td>
<td>BG</td>
<td>LAP</td>
<td>AP</td>
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<td>25-Mar-19</td>
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<td>15.56</td>
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<td>8.84</td>
<td>373.24</td>
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<td>2.5 x 10⁸</td>
<td>8.03</td>
<td>122.34</td>
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<td>15-Apr-19</td>
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<td>7.33</td>
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<td>4.28</td>
<td>246.38</td>
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<td>29-Apr-19</td>
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<td>2.72</td>
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</tr>
<tr>
<td>6-May-19</td>
<td>2.9 x 10⁸</td>
<td>6.99</td>
<td>132.00</td>
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<tr>
<td>13-May-19</td>
<td>2.9 x 10⁸</td>
<td>3.25</td>
<td>170.86</td>
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<tr>
<td>6-Jun-19</td>
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<td>11.30</td>
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<td>2.7 x 10⁸</td>
<td>3.14</td>
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<td>11-Jul-19</td>
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<td>61.86</td>
<td>378.36</td>
</tr>
<tr>
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<td>2.72–</td>
<td>44.9–</td>
</tr>
<tr>
<td></td>
<td>8.1 x 10⁸</td>
<td>61.86</td>
<td>378.36</td>
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Table A-3: Volumetric and cell specific hydrolysis rates by BG, LAP, and AP at BBH.

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<th>Bacterial Cells (L-1)</th>
<th>Volumetric Rate (nM/h)</th>
<th>Rate Per Cell (amol/cell∙h)</th>
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<td></td>
<td></td>
<td>BG</td>
<td>LAP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BG</td>
<td>LAP</td>
</tr>
<tr>
<td>2-Apr-19</td>
<td>1.6 x 10^9</td>
<td>1.26</td>
<td>1083.27</td>
</tr>
<tr>
<td>9-Jul-19</td>
<td>2.9 x 10^9</td>
<td>157.74</td>
<td>90.51</td>
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<tr>
<td>30-Jul-19</td>
<td>8.6 x 10^9</td>
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<td>259.71</td>
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<td>13-Aug-19</td>
<td>4.6 x 10^9</td>
<td>44.64</td>
<td>168.58</td>
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<td>9-Oct-19</td>
<td>1.9 x 10^9</td>
<td>17.07</td>
<td>146.61</td>
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<tr>
<td>Range:</td>
<td>1.6 x 10^9 – 8.6 x 10^9</td>
<td>1.26 – 157.74</td>
<td>90.51 – 1083.27</td>
</tr>
</tbody>
</table>
Figure A - 1: Fractionated LAP activity at JEL overlayed with daily average Nitrate data from the great bay buoy.
Figure A - 2: Environmental Parameters. A: SST; Sea Surface Temperature (°C), measured at time of sampling and/or obtained via buoy data. B: Salinity (ppt), measured at time of sampling and/or obtained via buoy data. C: Chlorophyll-a (µg/L) values are hourly averages from time and date of sampling, obtained from buoy measurements.
Figure A - 3: Historic phytoplankton abundances at CML from 2004-2007. Contribution to total plankton biomass by small flagellates (2 to 20 µm), dinoflagellates, cyanobacteria, and diatoms. Adapted from Timothy S. Moore, 2008.
Figure A - 4: Phytoplankton community composition at BBH from selected sampling dates. Large diatom bloom in October drives correlation between AP and Chl-a. A: Percent contribution to total phytoplankton biomass. B: Diatom biomass on selected sampling dates. Although diatoms make up ~90% of biomass in April, a larger biomass of diatoms dominate the community in October.
Figure A - 5: Delayed response to chl-a at JEL. A, B, C: Daily average chlorophyll-a concentrations at JEL overlayed with LAP (A) and BG (B) hydrolysis rates on each of the sampling dates. D, E: Hourly average (at time of sampling) chlorophyll-a concentrations at JEL on sampling dates only with size fractionated LAP (D) BG (E), and AP (F) hydrolysis rates. Peak chl-a measurement occurs on 11-Jun, 30 days prior to maximum hydrolysis rate by LAP and BG on 11-July. Chl-a concentration and LAP activity become strongly correlated when a delay of 30d is assumed. (With delay, LAP: $R^2=0.978$ $P=0.094$ $N=3$, BG: $R^2=0.975$ $P=0.099$, $N=3$). AP hydrolysis rates do not show the same pattern as BG and LAP. When a 30d delay is assumed, the strength of the relationship with chl-a does not increase.
Figure A - 6: Juan De Fuca Site CTD Water Column Profiles: Temperature (°C), Oxygen (mg/L), & Fluorescence (mg/m³). Temperature decreased with increased depth. At ~900m an oxygen minimum zone (OMZ) was identified. The fluorescence profile exposed a deep chlorophyll maximum at ~65m.