Introduction

0.1 Climate Change, Hypoxia, and Upwelling

Due to human greenhouse gas emissions, the Earth's oceans are warming and losing oxygen (Pörtner et al. 2022; Takamitsu Ito et al. 2017). Over 90% of the excess heat trapped by greenhouse gases has been absorbed by the ocean, causing a significant increase in temperature in most regions in the ocean (N. Bindoff et al. 2013). According to the United Nations Intergovernmental Panel on Climate Change, the global sea surface temperature has risen 0.65 ^{o}C above pre-industrial levels as of 2022 (Pörtner et al. 2022). This warming has reduced the solubility of dissolved oxygen in water, which accounts for 15-50% of the climate change driven oxygen decrease in the global ocean (Helm, N. L. Bindoff, and Church 2011; Takamitsu Ito et al. 2017; Schmidtko, Stramma, and Visbeck 2017). The loss of 0.5-3.3% of the open ocean's oxygen down to 1000 m depth is very likely, and it is very likely that oxygen minimum zones (water masses with very low oxygen) are expanding (N. Bindoff et al. 2013). This decline has been particularly pronounced in the North Pacific, where this study is located (N. Bindoff et al. 2013; Takamitsu Ito et al. 2017).

A significant decrease in coastal oxygen is caused by decreased sea-air and surface ocean-deep ocean oxygen flux due to increasing stratification (Mancini 2024; Pörtner et al. 2022). Ocean stratification refers to the way that layers of ocean water with different densities form, restricting heat, carbon, and oxygen exchange between layers of different depths. This density difference is primarily caused by differences in temperature and salinity between water masses. As the ocean's surface water warms, it becomes less dense, increasing the the density difference between surface and deep waters and increasing stratification. This increased stratification decreases the flow of nutrients and oxygen to the deeper layers of the ocean. Stratification has increased by 5.3% globally between 1960 and 2018 (G. Li et al. 2020).

The surface ocean has a relatively high dissolved oxygen concentration, because the atmosphere and the surface ocean rapidly exchange oxygen in order to equilibrate the partial pressure of oxygen in both systems (Taka Ito and Deutsch 2010). According to a famous 1948 study, 40% of air-sea exchange at a study site in the Gulf of Maine was due to organisms producing and consuming oxygen, while the remaining 60% of the exchange was due to the solubility of oxygen in water (Redfield 1948). Because the upper ocean receives sunlight, phytoplankton live there and photosynthesize, producing oxygen that primarily remains in the ocean (C. Li et al. 2020). However, the rate at which oxygen leaves the ocean is increasing as the ocean's temperature increases, leading to lower gas solubility (C. Li et al. 2020).

The most oxygen-poor layer of the ocean is the Oxygen Minimum Zone (OMZ), between a few hundred and 1000 meters below the surface. Above the OMZ, oxygen concentration in seawater decreases with distance from the surface, as sunlight and photosynthesis decrease. Below the OMZ, the concentration of dissolved oxygen increases slightly because oxygen is more soluble in colder, higher-pressure water, and because of ocean circulation from polar regions. Surface seawater near the poles is extremely oxygen-rich due to its low temperature, and that oxygen-rich water sinks due to its high density and moves slowly over the seafloor over the course of centuries, where its oxygen is consumed via respiration and cannot be replenished by photosynthesis or atmospheric exchange (Karstensen, Stramma, and Visbeck 2008; Deutsch, Penn, and Lucey 2024; Taka Ito and Deutsch 2010). The OMZ in the middle of the Pacific Ocean water column ranges from less than 0.02 mg/L to 0.72 mg/L, compared to the 1.0-1.9 mg/L of shallower waters between 50 and 200m deep along the Oregon and Washington coasts in the summertime (Karstensen, Stramma, and Visbeck 2008; Deutsch, Penn, and Lucey 2024; Barth et al. 2024; S. D. Pierce et al. 2012; Wyrtki 1962) (see Figure 1).

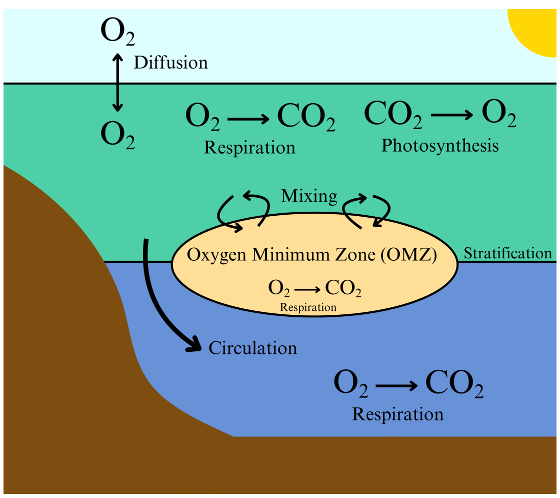


Figure 1: Oxygen sources and sinks in the ocean, adapted from (Deutsch, Penn, and Lucey 2024). The Oxygen Minimum Zone (OMZ) is relatively still, so circulation from surface waters does not bring it as much oxygen as the deep ocean. Photosynthesis and atmospheric diffusion increase the amount of oxygen in surface waters, and respiration decreases the amount of oxygen in the midwater and deep water.

As a result of these interactions, hypoxic events in which the oxygen concentration in water becomes low enough to harm or kill marine organisms are increasing in frequency, duration, and severity throughout the world's oceans. Worldwide, another major cause of hypoxia is eutrophication, which is when nutrient runoff results in a sudden population boom in phytoplankton, which then die and decompose, leading to a rapid decrease in dissolved oxygen levels. However, eutrophication is not a major cause of hypoxia off the northwest coast of Washington state, where this study is located. The vast majority of marine organisms require oxygen to survive, and these hypoxic events can cause severe fish die-offs or force migrations which change the distribution of species (Pihl, Baden, and Diaz 1991; D. Miller, Poucher, and Coiro 2002). Because different species have different tolerances to hypoxia and different levels of mobility, not all species distributions will be affected in the same way by changing hypoxic conditions. This could have large effects on marine ecosystems through direct effects (hypoxia tolerant species becoming more prevalent) or indirect effects (e.g. offsets in predator and prey populations) Thus, understanding the effects of this global decrease in ocean oxygen levels is crucial to predicting the overall effects of climate change on the oceans and to developing climate-aware management strategies for marine resources.

Studies of the effects of hypoxia on marine organisms use a variety of lab and fieldwork methods. Laboratory experiments on hypoxia's effects typically involve subjecting organisms to different levels of dissolved oxygen over a certain period of time, then measuring factors such as survival, growth rate, metabolism, and reproductive rate in order to determine whether the hypoxia has a negative effect (Steckbauer, Klein, and Duarte 2020). Field studies typically entail measuring dissolved oxygen levels and organismal prevalence and abundance, using methods such as satellite tagging and trawl surveys that vary by scale and organism type (for example, (Keister, Winans, and Herrmann 2020)). Field experiments have more ecological relevance, but present difficulties in untangling the effects of other environmental factors (Borges et al. 2022; Boyd et al. 2018). Therefore, linking experiments and field observations enables us to better demonstrate the connection between the lethal and sublethal effects of hypoxia shown in lab settings and real-world effects on organisms and changes in species distributions.

0.2 OCMNMS

This study will focus on hypoxia in Olympic Coast National Marine Sanctuary (OCNMS), off the coast of Washington state, U.S.A.. The Olympic Coast is the California Current Eastern boundary current upwelling zone (see Figure 2), meaning that when summer winds push the surface water offshore, nutrient-rich and oxygen-poor water flows upwards from the subsurface Pacific Ocean towards the coast (Office of National Marine Sanctuaries 2022; Hickey and Banas 2003). After this oxygen-poor water is advected onto the shallower waters of the continental shelf, respiration of decaying organic matter further reduces the water's oxygen content, leading to hypoxia (S. D. Pierce et al. 2012).

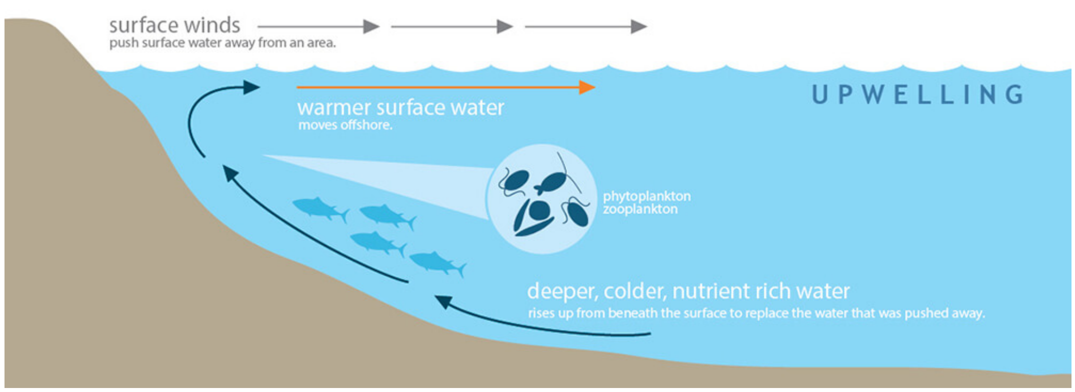


Figure 2: In the summer, winds move warmer surface water offshore, resulting in cold, nutrient-rich water flowing upwards into OCNMS. Image: NOAA, from (*Oceanography — Olympic Coast National Marine Sanctuary* 2025).

Hypoxic events in OCNMS often occur in the summer, are driven by upwelling, not nutrient runoff, and are increasing in severity (Barth et al. 2024). Hypoxic events have been observed annually in the US Pacific Northwest since 2002, and their increasing size and severity has been linked to climate change (Bograd et al. 2023; Parks 2009). These events have led to fish and crabs dying in large numbers, as well as moving to shallower waters temporarily (Grantham et al. 2004). According to Barth et al. 2024, the hypoxic fraction of near-bottom water inshore of the 200-m isobath increased from 2% to 24% between their two comparison periods, 1950-1980 and 2009-2018. During the first year of this study, 2021, this hypoxic fraction increased to 56% due to extreme upwelling conditions (Barth et al. 2024).

Nutrients from upwelling sustain a rich ecosystem with high primary productivity, but as oceans warm and the deep water gets progressively more hypoxic, the seasonal hypoxic events in OCNMS are increasing in their severity and duration, which will eventually harm organisms more than the nutrients help them. Additionally, as climate change warms the land and ocean at different rates, the summer winds that cause upwelling are getting stronger, resulting in upwelling events that push more deep water up towards the coast (Barth et al. 2024).

OCNMS is located off the Olympic Peninsula of Washington state, and extends 25 to 45 miles into the ocean. It regularly experiences rough seas, wave heights up to 49 feet, and intense winter storms. The sanctuary contains a wide variety of habitats, including rocky shores, kelp forests, sandy seafloor, and deep ocean canyons. The sanctuary is home to many marine mammals, fish, invertebrates, and seaweeds, and is a major feeding area for migrating seabirds. The sanctuary covers habitat for rockfish, salmon, halibut, Dungeness crab and other ecologically, commercially, and culturally important species of the Olympic Coast (Office of National Marine Sanctuaries 2022). Within the sanctuary, many activities that could damage the ocean environment are prohibited, such as moving or injuring historical resources, certain waste discharge from boats, military bombing activities, seabed drilling, and oil, gas, and mineral development. Activities that could harm wildlife, such as taking or disturbing marine mammals and birds, are also prohibited (*15 CFR Part 922 – National*

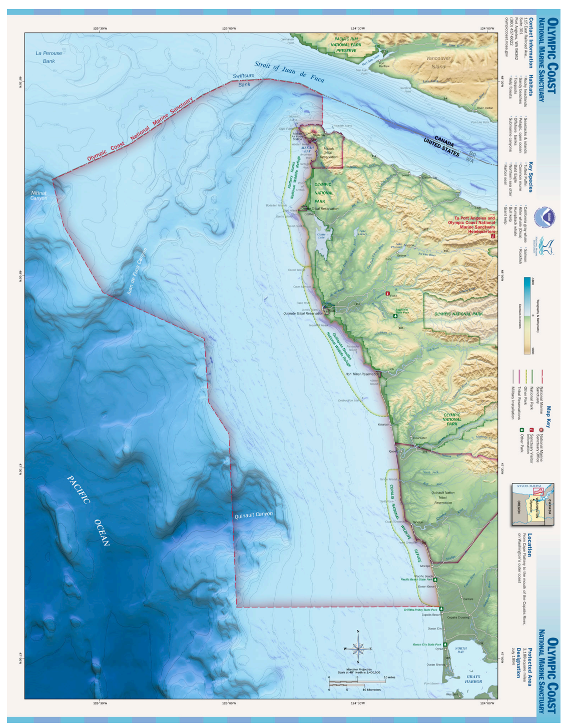


Figure 3: The coast of Washington State with the border of Olympic National Marine Sanctuary (OCNMS) in red. Image from *Olympic Coast National Marine Sanctuary* (2025). URL: `https://olympiccoast.noaa.gov/` (visited on 04/17/2025).

Marine Sanctuary Program Regulations
1995). The Olympic Coast National Marine Sanctuary Advisory Council provides advice on the sanctuary's management. The council is comprised of representatives of governments including Native American tribes, federal, state, and local. Local industries and interests are also represented, including the maritime industry, fishing, education, tourism, conservation organizations, and the community. The sanctuary lies within the Usual and Accustomed treaty fishing, hunting, and gathering areas of the Hoh Tribe, Makah Tribe, Quileute Tribe, and the Quinault Indian Nation, known collectively as the Coastal Treaty Tribes. Fisheries and other marine resources off the Olympic Coast are co-managed by the state of Washington, the United States (specifically the National Oceanographic and Atmospheric Administration's Office of National Marine Sanctuaries), and the Coastal Treaty Tribes, who formed the Olympic Coast Intergovernmental Policy Council (IPC) in 2007 to provide a forum for resource managers (*Intergovernmental Policy Council — Olympic Coast National Marine Sanctuary* 2025).

0.3 Copepods

Copepods are crustacean zooplankton of the class Copepoda, formerly combined with the class Thecostraca under the class Hexanauplia (Oakley et al. 2013; Lozano-Fernandez et al. 2019). Copepods are abundant in nearly every body of water on Earth, including oceans, freshwater, groundwater, and New York City's tap water (Vakati et al. 2023; Berger 2004). Copepods are an important component of marine and freshwater food chains, as they eat phytoplankton and are eaten by a wide variety of fish. This study will focus on free-living marine copepods found in OCNMS. In the northeast Pacific Ocean, copepods are important food sources for many pelagic fishes, including juvenile salmon (Brodeur and Pearcy 1990). The copepod species most commonly found in OCNMS are classified as Northern (cold-water) and Southern (warm-water) based on their seasonal pattern of occurrence (See Table 1,(NOAA Fisheries 2024; William T. Peterson and Keister 2003; Wiluam T Peterson and C. B. Miller 1977)). Northern copepods, especially Calanus marshalls and Pseudocalanus mimus, are known to be more lipid-rich. These fattier copepods serve as important food sources for salmon populations (NOAA Fisheries 2024). In the North Pacific, where this study is located, *Calanus pacificus* is the dominant *Calanus* copepod (Star and Mullin 1981), and in Arctic ecosystems *Calanus* copepods play an important role in food webs by consuming diatoms and being a large component of fish and bird diets (Falk-Petersen et al. 2007). In studies of the Oregon coast, *Pseudocalanus*, *Acartia*, *Oithona* and *Calanus* were the dominant genera in the summer, while *Paracalanus* and *Ctenocalanus* dominated in the winter (Wiluam T Peterson and C. B. Miller 1977; William T. Peterson and Keister 2003). Copepod species richness in the coastal Northeast Pacific Ocean is generally lower in the summer. Northern copepods are more common in the summer, and southern copepods are more common in the winter (NOAA Fisheries 2024). Copepod biomass off the Oregon coast is highest in the summer (William T. Peterson and Keister 2003). Copepods are eaten by herring and juvenile salmon, especially chum and sockeye salmon, off the west coast of North America. (Brodeur and Pearcy 1990; Friedenberg, Bollens, and Rollwagen-Bollens 2012). Because of their importance to the food chain, it is important to understand how copepod populations are affected by hypoxic events, and how they might be affected by worsening hypoxia in the future as climate change intensifies.

Previous work on copepod hypoxia tolerance has found that dissolved oxygen levels below 0.9-1.5 mg/L can kill many copepods within days, and levels below 0.66-0.9 mg/L kill nearly all copepods (He et al. 2021; Nancy H Marcus et al. 2004; Stalder and N. H. Marcus 1997; Grodzins, Ruz, and Keister 2016). However, hypoxia exposure was not found to impact the common model species *Acartia tonsa*'s eggs (Invidia, Sei, and Gorbi 2004). Oxygen levels above 1.7 mg/L are generally safe for copepods (Grodzins, Ruz, and Keister 2016). In situ zooplankton abundance is generally lower at sites with dissolved oxygen levels of 2 mg/L or lower, and copepod abundance is extremely low below 1 mg/L (Keister, Winans, and Herrmann 2020; Roman, Gauzens, et al. 1993). Additionally, copepods behaviorally avoid dissolved oxygen levels between 1.0 and 2.0 mg/L in lab and field observations (Keister, Winans, and Herrmann 2020; Roman, Gauzens, et al. 1993; He et al. 2021; Elliott, Pierson, and Roman 2012; Keister and Tuttle 2013), although Marcus & Stalder 1997 did not find this pattern (Stalder and N. H. Marcus 1997). These patterns differ between species and populations, which this study hopes to address (Grodzins, Ruz, and Keister 2016).

Even at nonlethal levels from 2.0-3.0 mg/L of dissolved oxygen, moderate hypoxia lowers copepod egg production, egg survival, and prey consumption. Egg production was delayed and reduced in studies that exposed copepods to 2.0 mg/L, 1.0 mg/L and 0.7 mL/L (Nancy H Marcus et al. 2004; Richmond et al. 2006; Roman, Gauzens, et al. 1993). This may be because energy reserves must be spent coping with the physiological effects of low oxygen instead of on egg production or digestion, and egg production is very energy-intensive (Nancy H Marcus et al. 2004; Elliott, Pierson, and Roman 2013; Lutz, N. H. Marcus, and Chanton 1992; Stalder and N. H. Marcus 1997; Roff 1992). At lower dissolved oxygen levels, studies have found that the copepods *A. tonsa* and *Temora turbinata* decrease their rate of feeding (He et al. 2021; Elliott, Pierson, and Roman 2013).

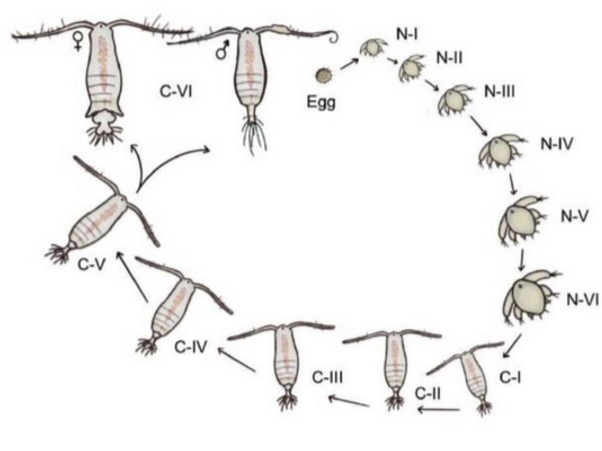


Figure 4: Simplified *Paracartia grani* life cycle, not to scale. N: nauplius, C: copepodite. Copyright C. Traboni. Claudia Traboni (May 11, 2022). "Trophic Interactions between Mixoplankton and Copepods". DOI: 10.13140/RG.2.2.24272.99849

The physiological problems that copepods face in hypoxic conditions are exacerbated by warmer temperatures, as crustaceans in general consume oxygen more rapidly at higher temperatures (Vaquer-Sunyer and Duarte 2011). Higher temperatures on their own are also a source of stress for copepods, although copepod thermal tolerances are understudied (M. Sasaki and Dam 2021). Copepods are capable of rapid thermal acclimation, but they can still be harmed or killed by high enough temperatures, and copepods acclimated to lower temperatures have lower thermal tolerances (M. Sasaki and Dam 2021; Hahn and Brennan 2024). Additionally, copepod body size decreases with increasing temperature (Hahn and Brennan 2024). In many copepod species acclimated to 15-20 $^{\circ}$C, about 50% of the population will die at 25 $^{\circ}$C, but the present study is at a site where water temperatures typically range from 8-12 $^{\circ}$C (M. C. Sasaki and Dam 2019; Sunar and Kır 2021; Jiang 2009). One study on Baltic Sea *Acartia hudsonica* copepods found that copepods collected in the winter from 7 $^{\circ}$C water had a critical thermal maximum of 25-27 $^{\circ}$C, and the same copepods acclimated to 11 $^{\circ}$C had a critical thermal maximum around 28 $^{\circ}$C, so it is (Hahn and Brennan 2024). The critical thermal maximum is defined at the temperature at which an individual is no longer capable of moving away from harmful conditions, eventually resulting in death. Therefore, it is likely that these copepods experience sublethal impacts at temperatures lower than 25 $^{\circ}$C, and that temperatures above that threshold are harmful to them.

Table 1: Classification of copepods as cold-water ("Northern," primarily occurring in the summer upwelling season) and warm-water ("Southern," primarily occurring in the winter) (NOAA Fisheries 2024; William T. Peterson and Keister 2003; Wiluam T Peterson and C. B. Miller 1977)

Species	Group
Acartia clausii	Cold-water
Acartia longiremis	Cold-water
Calanus marshallae	Cold-water
Centropages abdominales	Cold-water
Microcalanus pusillus	Cold-water
Pseudocalanus mimus	Cold-water
Pseudocalanus acuspes	Cold-water
Pseudocalanus spp.	Cold-water
Oithona similis	Year-round
Acartia tonsa	Warm-water
Calanus pacificus	Warm-water
Calocalanus spp.	Warm-water
Calocalanus styliremis	Warm-water
Clausocalanus spp.	Warm-water
Clausocalanus parapergens	Warm-water
Corycaeus anglicus	Warm-water
Ctenocalanus vanus	Warm-water
Mesocalanus tenuicornis	Warm-water
Metridia pacifica	Warm-water
Paracalanus spp.	Warm-water
Paracalanus parvus	Warm-water

0.4 Environmental DNA

Environmental DNA (eDNA) is the genetic material present in the environment, including extracellular DNA, fragments of cells and tissues, and entire small organisms (Taberlet et al. 2018). In marine environments, eDNA can be filtered out of the water and sequenced to detect many species present the place and time where the water sample was collected. eDNA is a powerful tool for detecting multiple species at once with relatively little sampling effort. Although eDNA is a sensitive tool, it is important to note it cannot detect demographic information such as size, sex, and age. The general process of eDNA sampling begins by physically filtering seawater, then extracting and amplifying the resulting DNA and chromosome fragments using polymerase chain reaction (PCR) (Power et al. 2023). eDNA metabarcoding is conducted using a set of primers designed to target a conserved region of DNA that flanks a hyper variable region of DNA across a broad set of species, thus enabling taxonomic classification of the resulting barcodes. The sequence fragments are compared to a reference database of genes sequenced from known species, allowing the species present in the water column at the time of sampling to be taxonomically identified (Miya 2022).

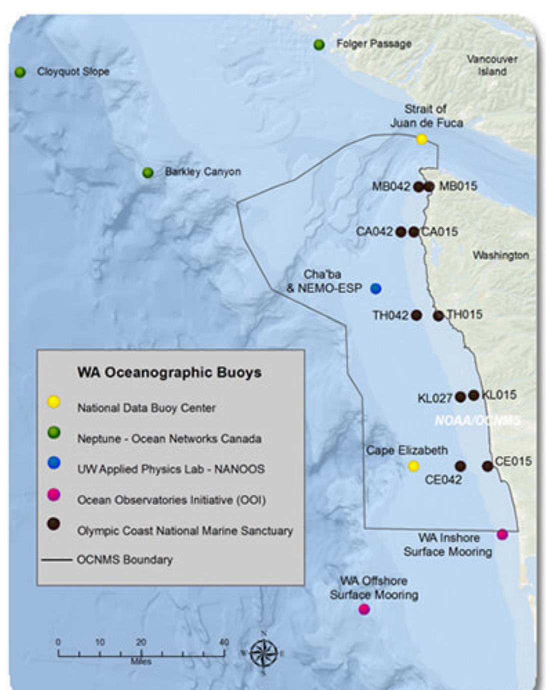
eDNA can detect species with relatively high spatial resolution, from as little as 60 meters in a kelp forest (Port et al. 2016) to 800 meters in deep-water habitats in Maizuru Bay, Japan (Yamamoto et al. 2017), because eDNA levels drop below the detection limit soon after being released via diffusion and decay. However, our understanding of how far eDNA can migrate in the environment is still relatively limited, and eDNA dispersal and decay can be affected by a number of environmental factors (Cristescu and Hebert 2018). Because of the high sensitivity of eDNA methods, contamination must be diligently avoided. Because metabarcoding requires reference sequences, any species whose barcoding region in question has not been sequenced cannot be detected by a study (Miya 2022). This is not an issue with heavily-studied species like salmon, but can present an issue in other areas. eDNA metabarcoding results provide read abundances, or numbers of DNA fragments, but these are affected by PCR replication efficiency, so they are difficult to compare between different species and methods (Miya 2022). While it is difficult to get quantitative results from eDNA, it is possible to calculate a metric of relative abundance within species known as eDNA index. Kelly et al. 2019 used simulations and found that this index accurately captures trends in the biomass of detected organisms. eDNA index is calculated in two steps. First, the read counts of each species within a sample are converted to a proportion of reads within the sample. Then, within each species, those proportions are normalized to range from zero to 1. After this, each species has one sample where it has an eDNA index of one, and that is the sample where that species was most abundant. This method cannot be used to compare different species, only to determine trends within each species, and it will be used in this study (Kelly, Shelton, and Gallego 2019).

Since 2021, the National Oceanic and Atmospheric Administration's Pacific Marine Environmental Laboratory (NOAA PMEL) Ocean Molecular Ecology (OME) lab has been collecting eDNA samples in OCNMS using a modified McLane phytoplankton and particulate sampler (PPS). The PPS was deployed for four, month-long intervals between May 2021 and August 2023, at the Teawhit Head 42 meter depth mooring (see Figure 5). This sampler collected, filtered, and preserved 1 L water samples every 36 hours. Samples were taken using 0.4 micron filters taken off the sampler, preserved in 95% ethanol, frozen, and taken to the lab. Additionally, some samples were taken using Niskin bottles during PPS deployment and recovery. These samples were filtered using Sterivex filters and a peristaltic pump. In total, 84 samples were collected in 2021, 122 in 2022, and 60 in 2023. 24 of these samples had three PCR technical replicates each. eDNA was extracted from the samples according to the DNeasy Blood and Tissue Kit protocols with adaptations from Spens et al. 2017 (Spens et al. 2017). The full protocol is available on the Ocean Molecular Ecology Zenodo (J. (Weinrich and Gold 2025). This study focuses on copepods, and only uses data from the CO1 (mitochondrially encoded cytochrome c oxidase I, a common barcoding gene for studies on eukaryotes) primer. Amplified regions were sequenced using the Leray F / mICOlinF forward primer and Folmer R / HCO2198 reverse primer, according to OME protocols (Gold, H. Weinrich, and Brown 2024; Spens et al. 2017). The CO1 sequences were then matched to available reference genomes, resulting in species detection data that includes the number of reads (DNA strands) of each species detected.

My summer 2024 research included combining the results of these eDNA detections with oceanographic measurements collected every ten minutes by the Teahwhit Head oceanographic mooring in 42 meter deep water (TH042), where the PPS sampler was deployed. I first matched the species detections to their associated metadata, including the sample date and time, using their unique sample IDs. I then matched each of those detections to the oceanographic mooring measurements that were closest to their sample date and time.

0.5 Study Plan

After calculating binomial regressions comparing oxygen saturation to species presence and absence, I found that a northern copepod, Pseudocalanus mimus, became less common during hypoxic events, and a southern copepod, Paracalanus sp. C AC-2013, became more common during hypoxic events. Because copepods are so important to the OCNMS food web, and because of the potentially interesting pattern of northern and southern copepods having different levels of hypoxia tolerance, further research into how these species respond to changes in dissolved oxygen and



temperature is warranted (NOAA Fisheries 2024). The combined eDNA and oceanographic data contains detections of 20 species of copepods, with 12 of those detected more than once in 2021-2022 and 13 detected more than once in 2021-2023.

In this study, I will assess the effects of hypoxia on copepod abundance and community composition. I will compare copepod prevalence and eDNA index, a measure of relative abundance using eDNA detections, to dissolved oxygen data in order to assess whether hypoxia decreases copepod abundance in OCNMS. I will also generate preferred ranges of environmental conditions for each copepod species, and compare northern and southern copepod abundance across normoxic and hypoxic conditions. I predict that copepod abundance will decrease with hypoxia, and that the northern copepods will be detected in colder conditions than the southern copepods. Based on preliminary data analysis from the previous research, I expect southern copepods to persist at lower oxygen levels than northern copepods (Crotty 2024). Understanding these patterns will inform predictions of climate change effects on OCNMS food chains and culturally and economically important species such as salmon.