

Utilizing Environmental DNA to Investigate the Effects of Hypoxia on Copepod
Abundance

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List of Abbreviations

OCNMS	Olympic Coast National Marine Sanctuary
eDNA	Environmental DNA
DO	Dissolved oxygen
ENSO	El Niño Southern Oscillation
NWFSC	Northwest Fisheries Science Center
OMZ	Oxygen Minimum Zone
IPC	Intergovernmental Policy Council
NOAA	National Oceanic and Atmospheric Administration
PMEL	Pacific Marine Environmental Laboratory
OME	Ocean Molecular Ecology
PPS	Phytoplankton and Particle Sampler
TH042	Teawhit Head, 42 meter deep mooring
ESV	Exact Sequence Variant
NCBI BLAST	National Center for Biotechnology Information Basic Local Alignment Search Tool
UW	University of Washington
PDO	Pacific Decadal Oscillation

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Abstract

Hypoxic events are increasing in severity and duration in Olympic Coast National Marine Sanctuary (OCNMS) off the coast of Washington State. Hypoxia, generally defined as a dissolved oxygen concentration below 2 mg/L, can have lethal and sublethal effects on marine life with potentially severe consequences for marine ecosystems. Understanding the effects of hypoxic events is critical as OCNMS habitats support vital recreation, fisheries, and four sovereign tribal communities: the Hoh Tribe, Makah Tribe, Quileute Tribe, and Quinault Indian Nation. I focus on understanding the impacts of hypoxia on copepods, small crustacean zooplankton, that are both an important foundational food source for fish such as juvenile salmon and sentinels for changing ocean conditions. Here, I pair mooring-based autonomous environmental DNA (eDNA) sampling and dissolved oxygen (DO) measurements in OCNMS to track changes in copepod prevalence and relative abundance across DO levels. Copepods were classified as northern (cold-water) and southern (warm-water) species following the established NOAA NWFSC local juvenile salmon biological indicators analysis, and classified by size (NOAA Fisheries 2024). Northern copepods are generally more lipid-rich, and represent an important food source for juvenile salmon and other fish. I found that most copepod species, regardless of group, were more abundant at higher dissolved oxygen levels, but southern copepods were detected proportionally more in hypoxic conditions than northern copepods. Smaller copepods were detected proportionally more at lower dissolved oxygen levels, indicating a higher hypoxia tolerance that is supported by metabolic theory and previous studies. Additionally, northern copepods were generally found in colder conditions than southern copepods, which is consistent with the premise of the grouping scheme. These results suggest northern copepods may be more sensitive to hypoxia than southern copepods, and that larger copepods may be more sensitive to hypoxia than smaller copepods. As hypoxia continues to increase, these results predict that the copepod community in OCNMS will become dominated by smaller copepods and Southern copepods, both of which are less optimal food sources for fish that consume zooplankton. Therefore, the increasing frequency of hypoxic events may result in a decline in essential food resources that support the growth and survival of many key pelagic fishes.

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 °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; Li et al. 2020). 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). The vast majority of marine organisms require oxygen to survive, and severe hypoxic events have caused mass mortality events of marine species, so understanding the potential effects of decreasing oxygen levels in the world's ocean is crucial in order to predict the future of marine ecosystems and fisheries (D. Miller, Poucher, and Coiro 2002; Deutsch, Penn, and Lucey 2024).

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 (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).

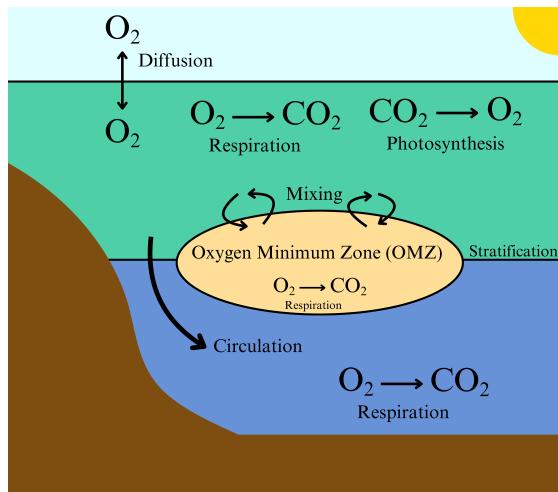


Figure 1: Oxygen sources and sinks in the ocean, adapted from (Deutsch, Penn, and Lucey 2024). The Oxygen Minimum Zone (OMZ) is relatively still, so circulation from surface waters does not bring it as much oxygen as the deep ocean. Photosynthesis and atmospheric diffusion increase the amount of oxygen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

effects of climate change on the oceans and to developing climate-aware management strategies for marine resources.

As a result of ocean warming and deoxygenation, 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. 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 (e.g. mass die-offs of hypoxia sensitive species and hypoxia tolerant species becoming more prevalent) or indirect effects (e.g. offsets in predator and prey populations). Hypoxic conditions can force migrations between depths or geographic regions, which change the distribution of species (Deutsch, Penn, and Lucey 2024; Pihl, Baden, and Diaz 1991). Thus, understanding the effects of this global decrease in ocean oxygen levels is crucial to predicting the overall

0.2 Study Location: OCNMS

0.2.1 Upwelling in OCNMS

This study will focus on hypoxia in Olympic Coast National Marine Sanctuary (OC-NMS), off the coast of Washington state, U.S.A.. The Olympic Coast is the California

Current Eastern boundary current upwelling zone, meaning that when summer winds push the surface water offshore, nutrient-rich and oxygen-poor water flows upwards from the 150-200m deep waters of the Pacific Ocean towards the coast (see Figure 2), (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 in the bottom of the water column (S. D. Pierce et al. 2012; Landry and Hickey 1989). Another major cause of hypoxia worldwide is eutrophication, which occurs 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 (Deutsch, Penn, and Lucey 2024). However, eutrophication is not a major driver of hypoxia in OCNMS (Office of National Marine Sanctuaries 2022).

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). Eastern boundary current upwelling zones are being affected by climate change in a variety of complex ways, so studying the effects of extreme oceanographic conditions in an upwelling zone in detail will inform our future understanding of how these extremely productive ecosystems will respond to climate change (Bograd et al. 2023).

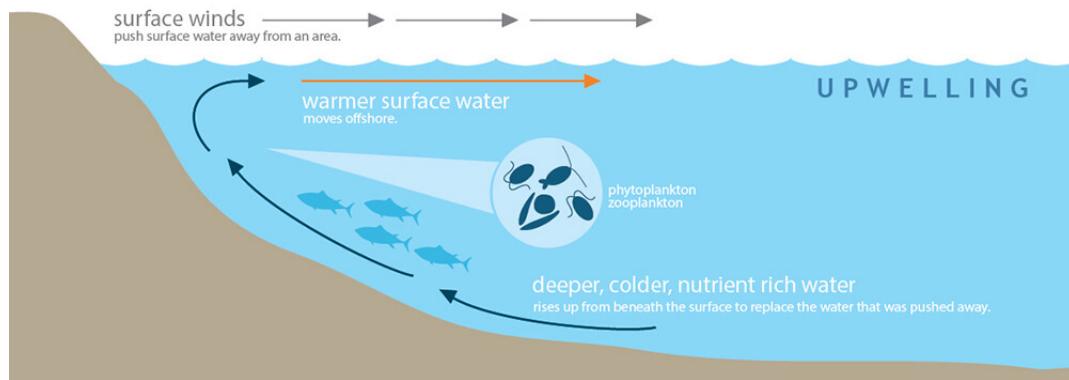


Figure 2: In the summer, winds move warmer surface water offshore, resulting in cold, nutrient-rich water flowing upwards into OCNMS. Image: NOAA, from (*Oceanography — Olympic Coast National Marine Sanctuary* 2025).

Hypoxic events 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).

0.2.2 OCNMS Ecosystems and Policies

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 protects 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 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 tribal, federal, state, and local government representatives, as well as local industries and interests including maritime industry, fishing, education, tourism, and conservation. 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

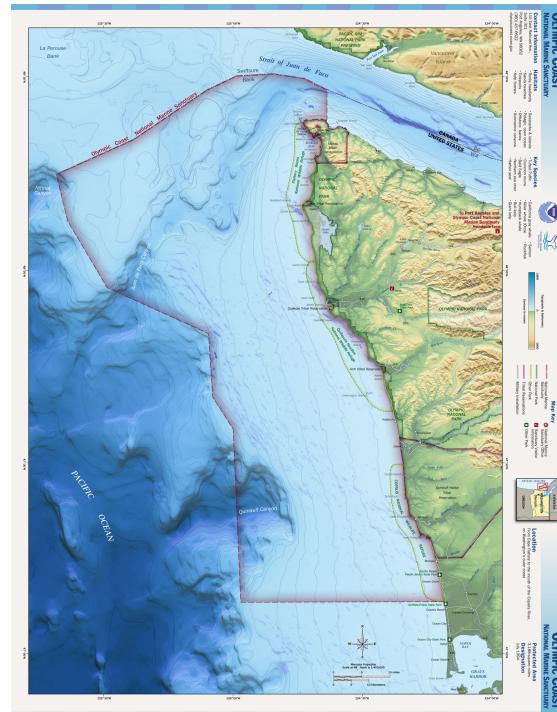


Figure 3: The coast of Washington State with the border of Olympic National Marine Sanctuary (OCNMS) in red. Image from *Olympic Coast National Marine Sanctuary* (2025). URL: <https://olympiccoast.noaa.gov/> (visited on 04/17/2025).

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). They 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). 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, especially as prey for culturally and economically important fish such as salmon, 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.

0.3.1 Seasonal Classifications

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; William T Peterson and C. B. Miller 1977)). Northern copepods are more common in the summer, and southern copepods are more common in the winter (NOAA Fisheries 2024). In studies of the Oregon coast, which is part of the same California Current upwelling system as OCNMS, *Pseudocalanus*, *Acartia*, *Oithona* and *Calanus* were the dominant genera in the summer, while *Paracalanus* and *Ctenocalanus* dominated in the winter (William T Peterson and C. B. Miller 1977; William T. Peterson and Keister 2003). Northern copepods, especially Calanus marshallae and Pseudocalanus mimus, are known to be more lipid-rich. These fattier copepods serve as important food sources for salmon populations (NOAA Fisheries 2024). Copepod species richness in the coastal Northeast Pacific Ocean is generally lower in the summer, and copepod biomass off the Oregon coast is highest in the summer (William T. Peterson and Keister 2003).

0.3.2 Effects of Hypoxia on Copepods

Previous lab 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;

Species	Group
<i>Acartia clausii</i>	Cold-water
<i>Acartia longiremis</i>	Cold-water
<i>Calanus marshallae</i>	Cold-water
<i>Centropages abdominalis</i>	Cold-water
<i>Microcalanus pusillus</i>	Cold-water
<i>Pseudocalanus mimus</i>	Cold-water
<i>Pseudocalanus acuspes</i>	Cold-water
<i>Pseudocalanus spp.</i>	Cold-water
<i>Oithona similis</i>	Year-round
<i>Acartia tonsa</i>	Warm-water
<i>Calanus pacificus</i>	Warm-water
<i>Calocalanus spp.</i>	Warm-water
<i>Clausocalanus spp.</i>	Warm-water
<i>Clausocalanus parapergens</i>	Warm-water
<i>Ctenocalanus vanus</i>	Warm-water
<i>Metridia pacifica</i>	Warm-water
<i>Paracalanus spp.</i>	Warm-water
<i>Paracalanus parvus</i>	Warm-water

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; William T Peterson and C. B. Miller 1977)

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 lab experiments by He et al., copepods have been observed to behaviorally avoid dissolved oxygen levels between 1.0 and 2.0 mg/L, although other experiments by Marcus & Stalder did not find this pattern (He et al. 2021; Stalder and N. H. Marcus 1997). In Puget Sound, an estuary very close to OCNMS, lower *in situ* copepod abundance was observed at sites with dissolved oxygen concentrations of 2 mg/L or lower, and copepods were observed to behaviorally avoid dissolved oxygen levels below 2 mg/L by migrating vertically (Keister, Winans, and Herrmann 2020; Keister and Tuttle 2013).

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 lab 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).

Higher temperatures 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 °C, about 50% of the population will die at 25 °C, but the present study is at a site where water temperatures typically range from 8-12 °C (M. C. Sasaki and Dam 2019; Sunar and Kir 2021; Jiang 2009). One study on Baltic Sea *Acartia hudsonica* copepods found that copepods collected

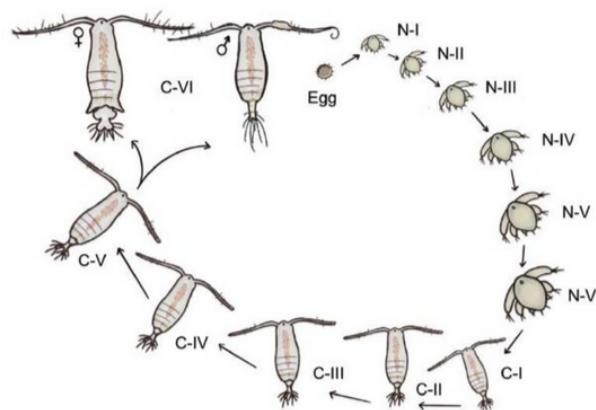


Figure 4: Simplified *Paracartia grani* life cycle, not to scale. N: nauplius, C: copepodite. Copyright C. Traboni. Claudia Traboni (May 11, 2022). “Trophic Interactions between Mixoplankton and Copepods”. DOI: [10.13140/RG.2.2.24272.99849](https://doi.org/10.13140/RG.2.2.24272.99849)

in the winter from 7 °C water had a critical thermal maximum of 25-27 °C, and the same copepods acclimated to 11 °C had a critical thermal maximum around 28 °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 °C, and that temperatures above that threshold are harmful to them.

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). A combination of heat and hypoxia results in a lower hypoxia tolerance in most marine taxa, because organisms' metabolisms accelerate at higher temperatures, increasing their demand for oxygen (Deutsch, Penn, and Lucey 2024). Therefore, the combination of dissolved oxygen and temperature is a major limiting factor to the geographical range of many marine species (Deutsch, Ferrel, et al. 2015; Deutsch, Penn, and Seibel 2020). As global oceans warm and lose oxygen, it is important to understand the interaction between these two environmental stressors on a variety of marine organisms in order to understand possible future changes to marine ecosystems.

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 different species present at 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 studies may take multiple approaches to sequencing, depending on their goals. Targeted assays are species-specific and measure absolute DNA concentrations, metabarcoding assays target multiple species, and metagemonics looks at functional genes present in the environment. The goal of this study was to understand the overall community composition, and so eDNA samples were processed using metabarcoding methods in order to detect a wide variety of species.

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 that accounts for the effects of amplification bias across taxa, known as eDNA index. Kelly et al. 2019 found that this index accurately captures trends in the biomass of detected organisms using simulations. eDNA index is calculated in two steps. First, the read counts of each species within a sample are used to calculate the proportional relative abundance of that species within the sample. Then, within each species, those relative abundances are all divided by the highest relative abundance for the species, so that the resulting eDNA index ranges from 0 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 (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, see Figure 5). 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 6). This sampler collected, filtered, and preserved

1 L water samples every 36 hours using 0.4 micron filters. Filters were then 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, and replicates on the same date were combined for this study. 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 (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, 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

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.

Because copepods are so important to the OCNMS food web, further research into how these species respond to changes in dissolved oxygen and temperature is warranted (NOAA Fisheries 2024). In this study, I will assess the effects of hypoxia on copepod abundance and community composition. I will compare copepod prevalence



Figure 5: Modified McLane phytoplankton and particulate sampler (PPS) used for automated eDNA sampling in OCNMS. This sampler was placed at TH042 (See Figure 6.)

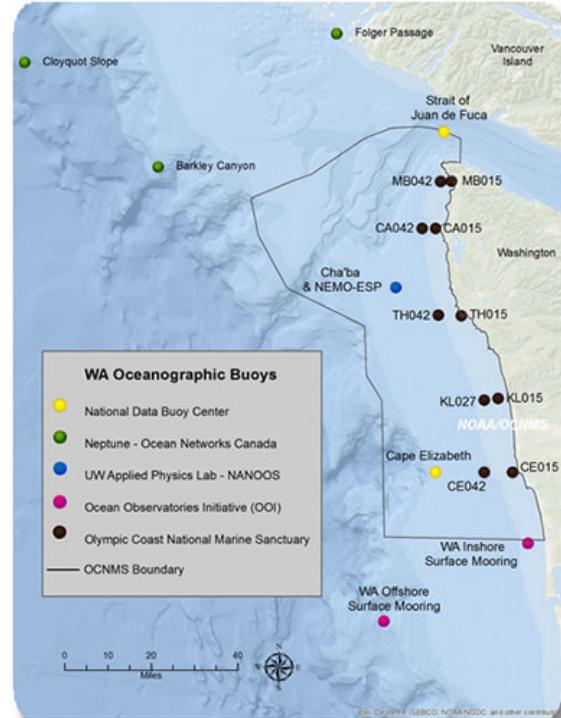


Figure 6: Map of the Washington state coastline and Olympic Coast National Marine Sanctuary, featuring locations of Washington state oceanographic buoys. This study was conducted at the buoy labeled TH042 *Oceanographic Moorings — Olympic Coast National Marine Sanctuary* (2025). URL: <https://olympiccoast.noaa.gov/science/oceanographic-moorings/> (visited on 02/14/2025).

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 a foundational species of OCNMS food chains, which will inform predictions of the effects of climate change on entire OCNMS ecosystems.

Chapter 1

Methods

1.1 eDNA Index Preparation

Data analysis for this study was conducted using R version 4.3.0 “Already Tomorrow.” I downloaded eDNA sample data and environmental data from the TH042 mooring from previous OME research. Some *P. mimus* samples were misidentified as *P. acuspes*, so I re-identified the copepod sequence samples using NCBI BLAST and corrected the species identifications using those results. eDNA index was calculated in order to obtain a measure of the relative abundance of each species over time. Before calculating the eDNA index, I averaged the number of reads across technical replicates so that each observation represented one sampling date. Technical replicates included multiple samples taken at the same time, as well as multiple polymerase chain reaction (PCR) runs performed on the same sample. Additionally, each species has multiple characteristic variants of each gene that was used in the metabarcoding process, and each of those variants is called an ESV (Exact Sequence Variant). Because each individual observation in the data was one ESV, there were many observations of each species per sample. I combined these by adding up the total number of reads across all ESVs for each species in each sample.

I calculated eDNA index by grouping by sample, then calculating the proportion of reads of each species in each sample. I then normalized each species’ proportions on a scale of 0 to 1, such that the sample with the most reads of that species had a value of 1, and any sample where that species was not detected had a value of 0. It is important to note that eDNA index cannot be used to make comparisons between species, but can be used to make comparisons between different time points in the same species. After calculating eDNA index, the data was filtered to only include copepods by selecting any observations in the genera *Calocalanus*, *Clausocalanus*, or *Paracalanus*, as well as any observations in the class Hexanauplia, which contains Thecostraca and Copepoda (Oakley et al. 2013). While the class Hexanauplia is no longer recognized (*WoRMS - World Register of Marine Species - Copepoda* 2024; Lozano-Fernandez et al. 2019), and Thecostraca and Copepoda are now recognized as classes, it was used when the eDNA for this study was processed, so I used it for data processing as well. This study will not discuss species in Thecostraca.

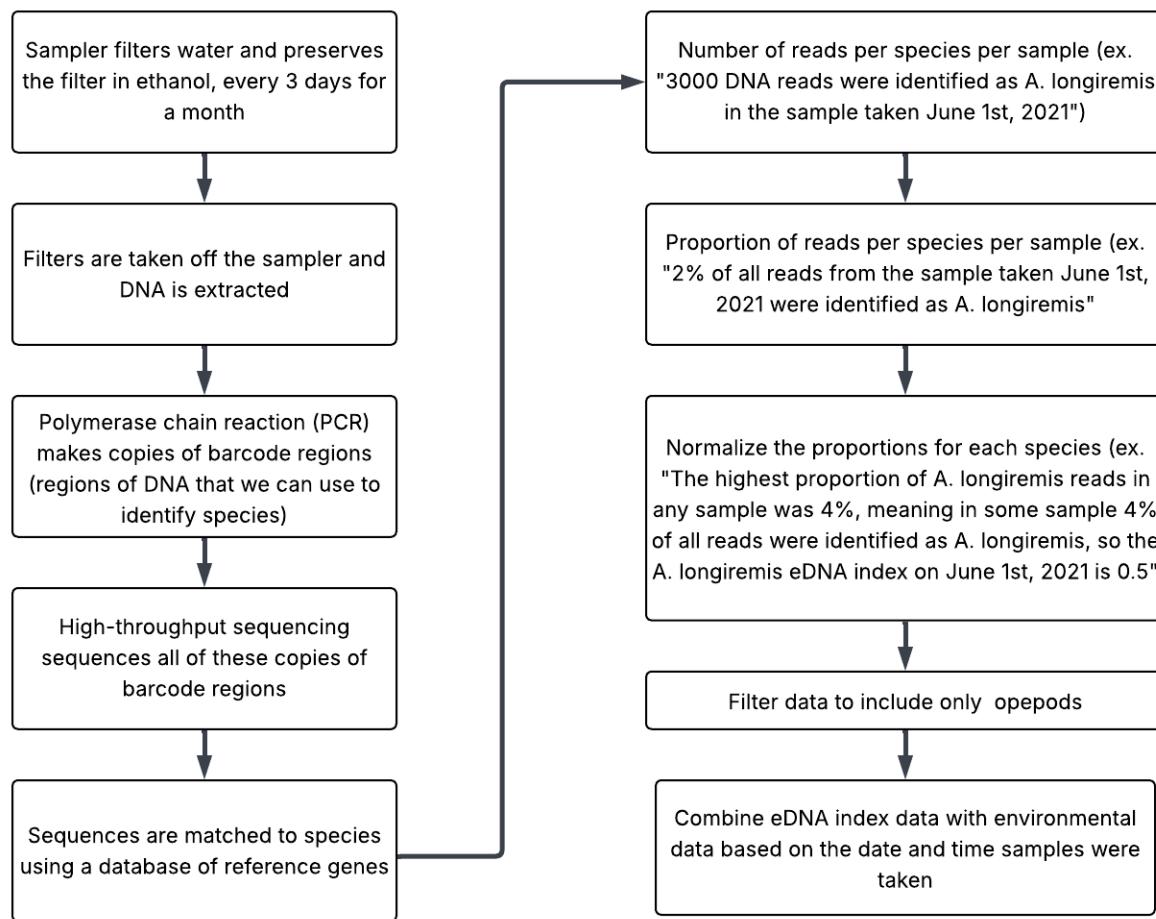


Figure 1.1: Flowchart detailing the steps to calculating eDNA index as laid out in sections 0.4 and 1.1.

I combined the eDNA index data with its corresponding oceanographic data, using code from the previous study to attach each sample ID to its corresponding date and time, then joining it with the closest mooring observation. Additionally, I downloaded environmental data from the LiveOcean model at TH042 during the study period in order to fill in a data gap in 2023 during which the TH042 mooring was not functional, but the automated eDNA sampler was deployed (Siedlecki et al. 2015; Fatland, MacCready, and Oscar 2016; *LiveOcean Homepage* 2025). I matched the LiveOcean model's hourly dissolved oxygen and temperature data to the eDNA sample data by date and time, and also compared the results of the LiveOcean model's 2021-22 data to the TH042 mooring's 2021-22 data. The model data matched the mooring data closely enough to be useable (See Figure 1.2). When mooring and model data from the same hour was compared via linear model, the dissolved oxygen data had an R^2 value of 0.39 and a root mean square error (RMSE) of 0.98, and the temperature data had an R^2 of 0.82 and a RMSE of 0.41. The 2021-22 mooring-only

data is still used for some of the statistical analyses in this thesis, as the mooring data captures short and extreme hypoxic events more accurately (See Figure 1.2, orange spikes downwards are hypoxic events captured by the mooring but not the model). When modeling data is used in this thesis, it is used for all years 2021-23, in an effort to ensure that the different years are comparable and utilize all available eDNA sample dates.

1.2 Data Visualization

In order to determine the relationships between copepod abundance and environmental factors, I compared eDNA index to temperature and dissolved oxygen. I conducted preliminary analysis using time series plots, where dissolved oxygen was plotted as a time series and each sample was plotted as a dot on top of the associated dissolved oxygen measurement (See Figure 1.3). This allowed for visual identification of hypoxic events in July 2021, October 2021, September 2022, and October 2023, as well as a heatwave in September 2021. Analysis of abundance relative to both dissolved oxygen and temperature was first conducted using scatterplots of dissolved oxygen and temperature at each sampling time, with color corresponding to eDNA index. To compensate for the different sampling frequencies in different environmental conditions, I then made density plots of the detection frequency within bins of environmental conditions, each spanning 0.5 mg/L of dissolved oxygen and 0.5 degrees Celsius. Each square of the plot represents a varying number of eDNA samples, and the darker squares have a higher proportion of species detections relative to

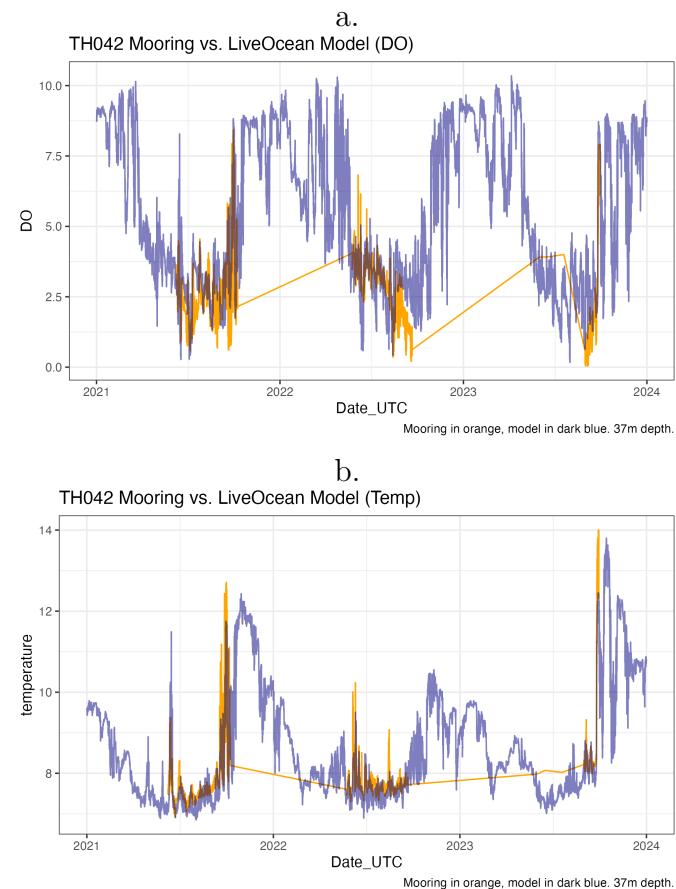


Figure 1.2: Time series of (a) dissolved oxygen (mg/L) and (b) temperature (Celsius) over the study period. TH042 mooring data is displayed in orange, while LiveOcean model data is displayed in blue.

non-detections.

In order to compare copepod hypoxia tolerances by size, I divided the species in the dataset into size bins based on the size ranges listed in the Marine Planktonic Copepods database (*WoRMS - World Register of Marine Species - Copepoda* 2024). Copepods with a minimum size smaller than 1.5 mm were classified as small, 1.5-2.5 mm copepods were classified as medium, 2.5-4 mm copepods were classified as large, and copepods exceeding 4 mm were classified as huge.

1.3 Statistical Analysis

I conducted statistical analyses of the relationship between species abundance and dissolved oxygen by using binomial regression models and zero-inflated beta regression models. I generated binomial regression models relating dissolved oxygen to species presence for each species of copepod in the dataset, excluding one data point that occurred during a short heatwave in September 2021. Because only one sample was taken during the heatwave and no other samples were from temperatures that high, it was difficult to draw conclusions about the effects of the heatwave on species presence, and the datapoint was excluded from the binomial regressions. Using the LiveOcean model data, two of the data points fell during the heatwave, because the model estimated that the heatwave started days earlier than the mooring indicated, and so those two sampling dates were excluded from statistical analyses. I then used the p-values for these binomial regressions to analyze individual species responses to hypoxia.

I attempted to generate zero-inflated beta regression models using the zoib R package, but due to difficulties in visualizing the model output I switched to the brms package, a more general modeling package that uses Stan (Bürkner et al. 2024). I chose a zero-inflated beta regression because eDNA index ranges from zero to one, necessitating a beta regression, and the data is zero-inflated because each species was absent from many samples. I used the output of these models to generate and plot mean predicted data. I then compared these models to the binomial regression models, which related the presence and absence of eDNA to dissolved oxygen. Based on the Akaike information criterion (AIC), the binomial regression models performed better (lower AIC) for *A. longiremis*, *C. pacificus*, *O. similis*, *Paracalanus sp.* C AC-2013, *P. mimus*, and *P. newmani*. Based on AIC again, the zero-inflated beta regression models performed better for *C. parapergens*, *Lucicutia flavigornis*, *Metridia lucens*, *M. pacifica*, and *P. acuspes*. The zero-inflated beta regression for *C. pacificus* performed better with the mooring data, but the binomial regression model for *C. pacificus* performed better with the modeled data. Overall, in species with 5 or fewer detections, the binomial regression models performed better, but in species with more than 5 detections, the zero-inflated beta regression models performed better. I considered there to be a significant response for a species to hypoxia using binomial regression based on alpha = 0.05, and I considered zero-inflated beta regressions to be significant if their 95% confidence interval showed an upward or downward trend and did not include zero for every DO value (e.g. the 95% confidence interval left

the x-axis at any point, see Appendix B for visualized model confidence intervals). I did not perform significance tests directly comparing size categories or northern and southern copepods in aggregate, because the number of species with more than 5 detections was too low.

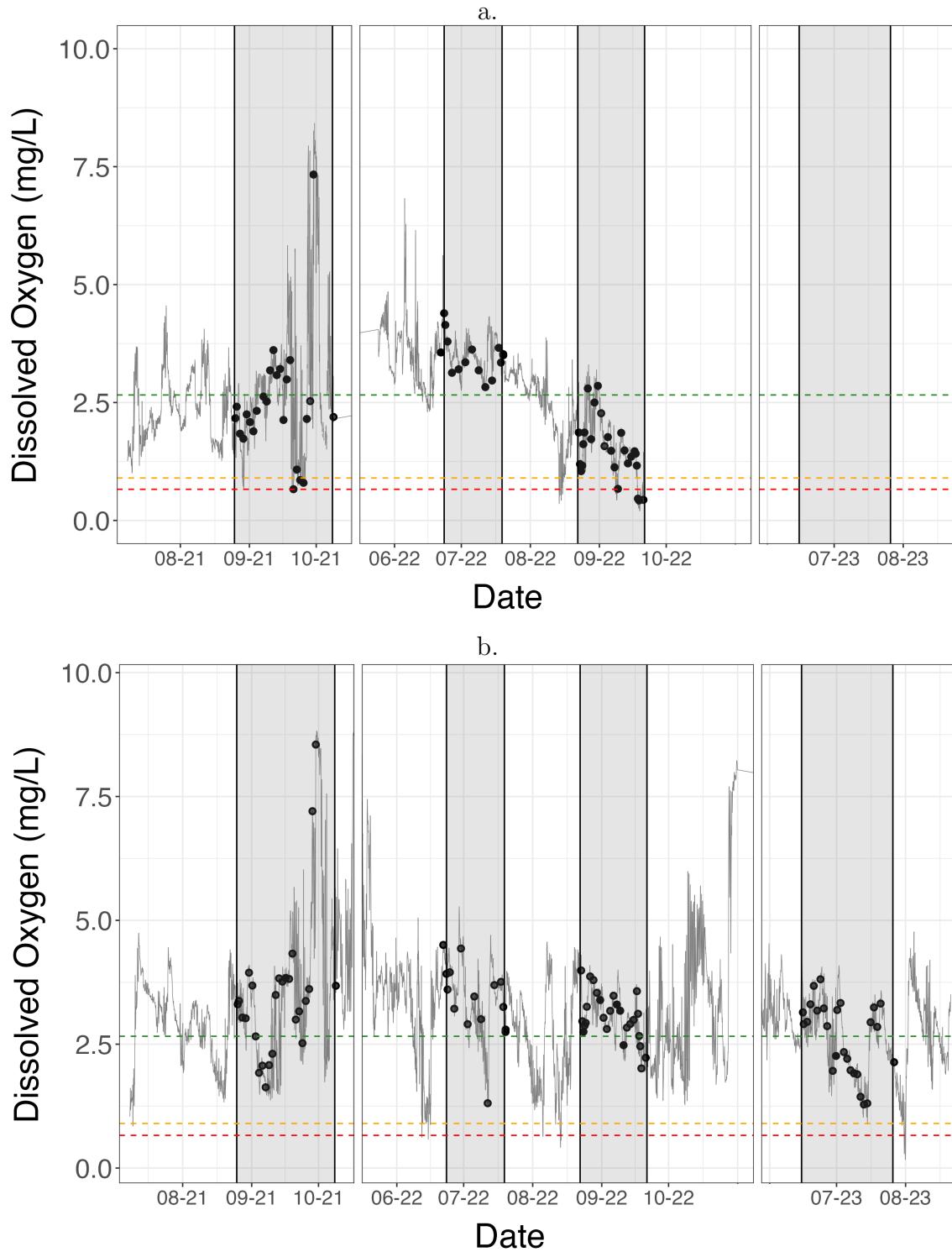


Figure 1.3: Time series of dissolved oxygen (mg/L) over the study period. Plot (a) displays TH042 mooring data, plot (b) displays LiveOcean model data. Black dots represent eDNA sampling dates. Gray boxes represent automated eDNA sampling periods. Red line represents 0.6 mg/L DO, a level known to kill most copepods in lab experiments, orange line represents 0.9 mg/L DO, a level known to kill approximately 50% of copepods in lab experiments, and green line represents 2.66 mg/L DO, a level above which copepods have not shown negative effects in lab studies.

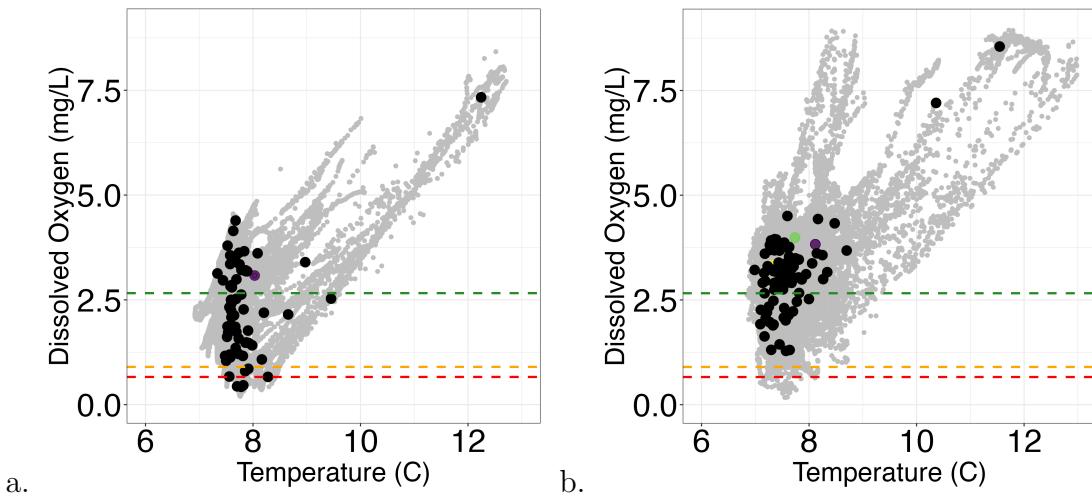


Figure 1.4: Distribution of samples versus the distribution of temperature and dissolved oxygen during the entire sampling period, of May-October 2021-23. LiveOcean model output from winter months is not shown, since the eDNA sampler and TH042 mooring were not deployed during winter months. Gray dots indicate any environmental data sampling time, from the TH042 mooring (a) or from the LiveOcean model (b). Black dots indicate environmental conditions at which eDNA samples were taken. Note the high outliers, which are during a short heatwave in September 2021 and will not be shown in future scatterplots or considered in statistical analyses due to their small sample size.

Chapter 2

Results

After bioinformatics and quality filtering, the total number of reads from the CO1 primer was 2,454,571, with 611,924 from copepods. From the CO1 primer reads, 569 unique species were identified, of which 16 were copepods with useable DNA reads in the dataset. The highest number of reads in one sample was 62,940, and the highest number of copepod reads in a sample was 19,335. The copepod eDNA detections dataset contained 26 samples from 2021, 35 samples from 2022, and 26 samples from 2023. In total, there were 556,748 DNA reads from copepods detected more than once during the study period, 364,980 of which were from 2021 and 2022.

Of the copepod species detected in the data, 5 species had more than 10 detections: *A. longiremis*, *P. mimus*, *Paracalanus* sp. C AC-2013, *P. newmani*, and *O. similis*. *C. abdominales* had 7 detections overall, with one in 2023. *C. pacificus* had 5 detections, none of which were in 2023, and *P. acuspes* had 5 detections, 2 of which were in 2023. All other copepod species had fewer than 5 detections overall.

2.1 Dissolved Oxygen and Species Abundance

Most of the northern copepods studied had higher eDNA index at higher dissolved oxygen levels, with a positive correlation between eDNA index and DO according to zero-inflated beta regressions (See Appendix B). All northern copepods except *P. newmani* were more abundant at higher dissolved oxygen levels according to zero-inflated beta regressions based on the TH042 mooring data from 2021-22 (See Table 2.1). Of these northern copepods, *A. longiremis* and *P. mimus* had significant positive correlations between eDNA and DO.

2.2 Dissolved Oxygen and Species Prevalence

The data suggests that northern copepods have a comparatively lower tolerance to hypoxia. Southern copepods and *O. similis*, which is present year-round, were detected proportionally more at dissolved oxygen levels below 0.9 mg/L (see Figure 2.2a). However, the northern copepod *P. newmani* had fewer detections at high dissolved oxygen levels according to a binomial regression comparing LiveOcean modeled

Species	Group	Detections		Regression Results	
Species	Group	2021-22	2021-23	2021-22	2021-23
<i>Acartia longiremis</i>	Cold-water	61	86	Positive*	Neutral
<i>Pseudocalanus mimus</i>	Cold-water	28	46	Positive*	Negative*
<i>Pseudocalanus newmani</i>	Cold-water	20	25	Neutral	Neutral*
<i>Centropages abdominalis</i>	Cold-water	6	7	Positive	Neutral
<i>Pseudocalanus acuspes</i>	Cold-water	3	5	Positive	Neutral
<i>Oithona similis</i>	Year-round	11	11	Negative*	Neutral
<i>Paracalanus</i> sp. C. AC-2013	Warm-water	33	33	Neutral	Positive*
<i>Calanus pacificus</i>	Warm-water	5	5	Negative	Negative

Table 2.1: Trends of zero-inflated beta regressions relating dissolved oxygen concentration to eDNA index for different species of copepods. Regressions were computed using the TH042 mooring data from 2021-22, and again using LiveOcean model data from 2021-23, so both are shown. I considered trends to be significant (marked with “*” in this table) if their 95% confidence interval showed an upward or downward trend and did not contain zero at some DO value. See Appendix B for plotted regression results. Cold-water copepods (blue) are northern copepods, warm-water copepods (orange) are southern.

DO to species presence, but the effect was not significant (-0.30, df = 2, 89, 2, p = 0.36). *P. newmani* did have significantly fewer detections at high dissolved oxygen levels when the binomial regression was calculated using the TH042 mooring data for all three years (-0.64, df = 2, 63, 2, p = 0.03), see Figure 2.1). *P. newmani* also had a relatively consistent eDNA index across DO levels according to a zero-inflated beta regression, so while its prevalence was higher in hypoxic conditions, its abundance was not (See Appendix B).

Small copepod species showed a higher hypoxia tolerance than large copepod species in this study. Large and huge copepods (*C. pacificus* and *M. pacifica*) were rarely detected below 0.9 mg/L DO, although *C. pacificus*, the only huge (larger than 4 mm) copepod in the dataset, was detected once below 0.6 mg/L DO with a high eDNA index (see Appendix C). Medium copepods were rarely detected below 0.9 mg/L DO compared to small copepods (See Figure 2.2b). Small copepods were frequently detected below 0.6 mg/L DO, especially *A. longiremis* and *Paracalanus* sp. (see Figure 2.2b). *O. similis*, a small copepod, was present below 0.6 mg/L DO, but generally had a higher eDNA index at higher values of DO (see Appendix C).

2.3 Temperature

During this study, northern copepods were generally detected at lower temperatures than southern copepods. Most of the northern copepods in this study were not detected at temperatures above 8 °C, except for *P. mimus*, which was detected above 8 °C but was not detected at the highest temperature in the dataset, 12.2 °C. Southern

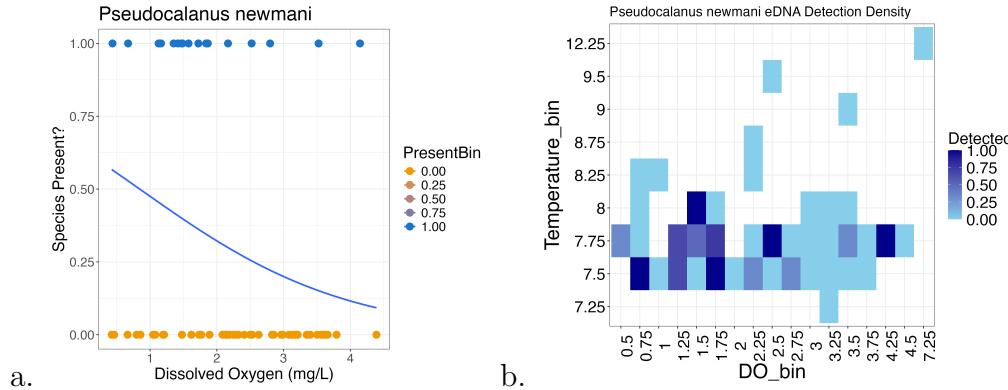


Figure 2.1: Dissolved oxygen data from the TH042 mooring in 2021-22 compared to *P. newmani* detections. (a) *P. newmani* binomial regression comparing species presence to dissolved oxygen. *P. newmani* was detected significantly less frequently at higher dissolved oxygen levels. (b) Density plot of *P. newmani* detections. Each pixel represents a range of dissolved oxygen and temperature values, and the shade of each pixel represents the proportion of eDNA samples in that environmental range in which *P. newmani* was primarily detected below 2.5 mg/L DO.

Species	Group	Binomial Regression		Beta Regression
Species	Group	Coefficient	P-value	(LiveOcean Data)
<i>Acartia longiremis</i>	Cold-water	-0.03	0.9	Neutral
<i>Pseudocalanus mimus</i>	Cold-water	0.07	0.5	Negative*
<i>Pseudocalanus newmani</i>	Cold-water	-0.3	0.3	Neutral*
<i>Centropages abdominalis</i>	Cold-water	-0.005	0.9	Neutral
<i>Pseudocalanus acuspes</i>	Cold-water	1.48	0.2	Neutral
<i>Oithona similis</i>	Year-round	0.68	0.4	Neutral
<i>Paracalanus</i> sp. C. AC-2013	Warm-water	0.6	0.03	Positive*
<i>Calanus pacificus</i>	Warm-water	-0.15	0.044	Negative

Table 2.2: Trends of binomial regressions relating dissolved oxygen concentration to species detections and zero-inflated beta regressions relating dissolved oxygen concentration to eDNA index for different species of copepods. Regressions were computed using LiveOcean model data from 2021-23. I considered beta regression trends to be significant (marked with “*” in this table) if their 95% confidence interval showed an upward or downward trend and did not contain zero at some DO value. See Appendix B for plotted regression results. Cold-water copepods (blue) are northern copepods, warm-water copepods (orange) are southern.

copepods were primarily detected above 7.5 °C, and the southern copepod *Paracalanus* sp. C AC-2013 had higher eDNA index values measured above 8 °C, despite being detected many times below 8 °C (see Figure 2.3).

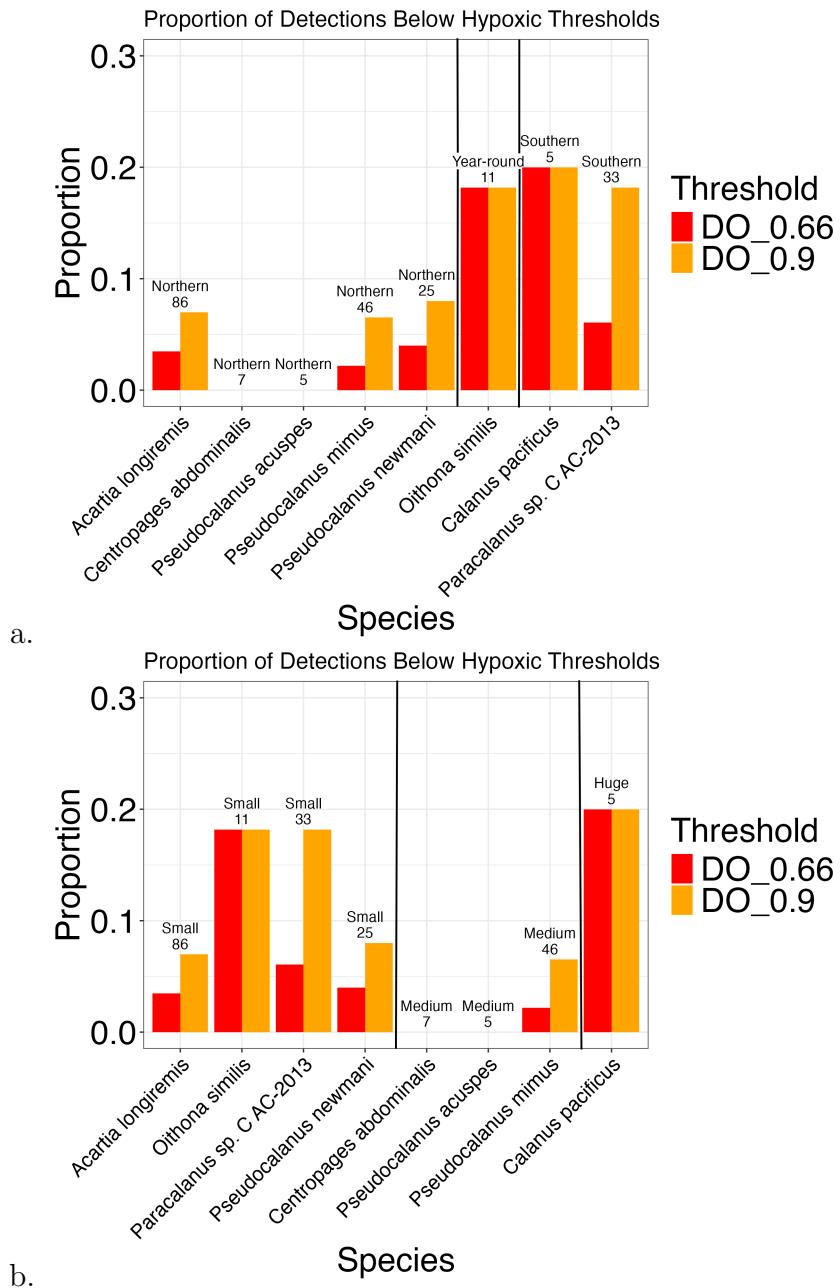


Figure 2.2: A comparison of different copepod groups and their rates of occurrence below certain hypoxic thresholds. Red bars indicate the proportion of detections of that species that were in conditions below 0.66 mg/L of oxygen, and orange bars indicate the proportion of detections of that species that were in conditions below 0.9 mg/L of oxygen. Numbers above bars are the total number of detections of that species. In plot (a) copepods are labeled by seasonal groupings. In plot (b), copepods are labeled by size category. Species with a maximum size of less than 1.5 mm are Small, maximum size 1.5-2.5 mm is Medium, maximum size 2.5-4 mm is Large, and maximum size >4 mm is Huge (*WoRMS - World Register of Marine Species - Copepoda 2024*)

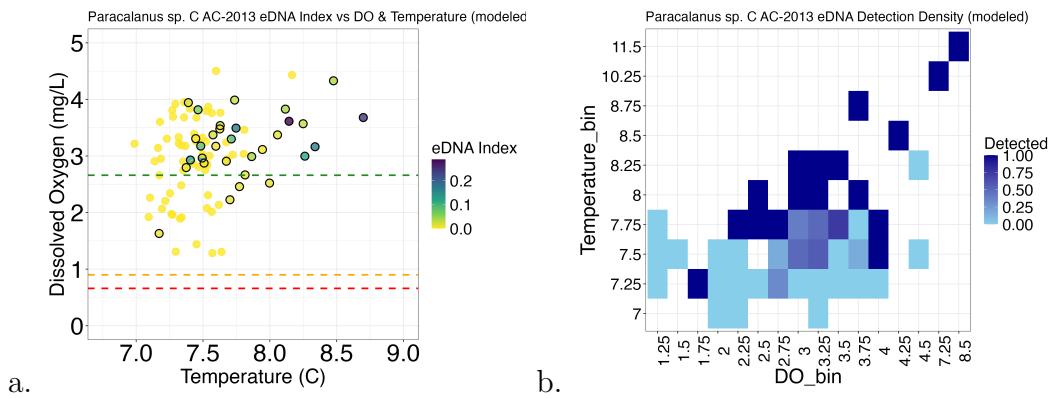


Figure 2.3: *Paracalanus* sp. C AC-2013 abundance and prevalence plots using LiveOcean model data from 2021-23.(a) Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *Paracalanus* sp. C AC-2013 scatterplot detections circled in black. Color represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *Paracalanus* sp. C AC-2013 was not detected. (b) Density plot of *Paracalanus* sp. C AC-2013 detections. Each pixel represents a range of dissolved oxygen and temperature values, and the shade of each pixel represents the proportion of eDNA samples in that environmental range in which *Paracalanus* sp. C AC-2013 was detected.

Chapter 3

Discussion

Most of the copepods in this dataset with sufficiently large sample sizes were less abundant at lower dissolved oxygen levels, indicating that the levels of hypoxia in OCNMS during this study period were low enough to negatively impact copepod populations, regardless of whether the copepods were northern or southern. My results demonstrate that there was substantial inter-specific variability in whether copepod abundance changed with dissolved oxygen. Northern copepods were generally more abundant at higher dissolved oxygen levels. Only one southern copepod, *Paracalanus* sp. C AC-2013, was detected more than 5 times in the dataset, and so it is difficult to draw overall conclusions about the hypoxia sensitivity of southern copepods.

The only copepod with more than 5 detections to have abundance negatively correlated with dissolved oxygen was *O. similis*, which is generally considered neither northern nor southern and is abundant year-round off the coasts of Oregon and Washington. *O. similis* was detected 11 times in the dataset, so future study is warranted before drawing conclusions, but it is possible that *O. similis* has a higher tolerance to hypoxia than other copepods, and its abundance increases due to decreased competition during hypoxic events. The genus *Oithona* has been used as an indicator of low oxygen conditions (Richard and Jamet 2001) and is known to have high hypoxia tolerance (Roman, Gauzens, et al. 1993), so this result is unsurprising.

The size patterns found in this study are generally consistent with the existing literature on copepod hypoxia tolerance. Previous studies have found that smaller copepods are more hypoxia tolerant, which is likely due to their larger surface area to volume ratio because copepods take up oxygen all over their bodies (Pörtner 2010; Roman, Brandt, et al. 2019). This study found that most smaller copepods were detected below 0.6 mg/L of oxygen, while several medium and large copepods were not. This supports the theory that smaller copepods are more hypoxia-tolerant and indicates that eDNA index can be a useful tool for investigating copepod environmental tolerance. However, this result should be interpreted with caution, as smaller copepods were detected many more times in this dataset than medium or large copepods. Copepod size depends on sex and age, which eDNA cannot discern, but we can still consider smaller and larger species in aggregate based on their known size ranges.

Observed patterns of detection and abundance at different temperatures aligned

with the expected difference between northern and southern copepod ranges. Northern copepods were generally not detected above 8 °C, and southern copepods were primarily detected above 7.5 °C. Northern copepods are known to inhabit cold water, and southern copepods are known to inhabit warm water, so this aligns with existing knowledge of these two groups of copepods.

Global climate change models predict that upwelling in the northern region of the California Current system, where this study was located, will increase in strength and event duration in the future (Bograd et al. 2023). While there is significant uncertainty in these models, upwelling has been increasing in severity in OCNMS, and it is important to consider the potential impacts if this trend continues. Increased upwelling would lead to colder temperatures and lower dissolved oxygen in OCNMS (Bograd et al. 2023). Based on the results of this study, decreasing dissolved oxygen levels in OCNMS could lead to a decrease in overall copepod abundance, and a copepod community dominated by smaller species, southern copepods, and *Oithona similis*. Lower temperatures due to increased upwelling could buffer this effect, as southern copepods would likely become less abundant and copepod hypoxia tolerance declines in warmer conditions (Vaquer-Sunyer and Duarte 2011). These trends would result in less copepod biomass available for juvenile salmon and other fish in OCNMS, which could lead to a decrease in abundance of culturally and economically important fish species. Future studies should investigate the effects of hypoxia on other fish prey species, to determine if juvenile salmon and other fish could be expected to switch to more hypoxia-tolerant prey in a future with increased upwelling.

It is worth noting that the entire period of this study, 2021-2023, took place during negative Pacific Decadal Oscillation (PDO) and negative Oceanic Niño Index (ONI) years, meaning that this study took place in relatively cold conditions. The Pacific Decadal Oscillation (PDO) is a climatological index used to describe broad shifts in the Pacific Ocean and the atmosphere above it. Cooler periods are described as negative PDO periods, and warm periods are described as positive PDO years. ONI is an index used to describe the El Niño Southern Oscillation (ENSO), a climate pattern in the tropical Pacific that changes every two to seven years. ONI is the three-month running mean of sea surface temperature anomalies in a specific region of the Pacific Ocean (Climate Prediction Center Internet Team 2025). During El Niño events, characterized by a positive ONI, the central and eastern tropical Pacific Ocean warm and the west coast of North America warms (L'Heureux 2014). During positive PDO or positive ONI (El Niño events) periods, copepod species richness on the west coast of North America is high (NOAA Fisheries 2024). Because of this, we expect lower species richness during the period of this study than during positive ENSO years, so some species may be missing from this study. In the future, the automated eDNA sampler in OCNMS will hopefully collect data during a positive ENSO year and acquire more data, improving the generalizability of any studies conducted using this data.

While eDNA index is not a perfect metric of abundance, it is still useful for broad studies such as this one when it is the only data available. 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).

Copepods in the genera *Paracalanus* and *Acartia* in Puget Sound, an estuary close to this study's site, are known to behaviorally avoid hypoxia by remaining above hypoxic bottom water (Keister and Tuttle 2013). Therefore, the lower abundance in these genera found in this study could be the result of behavioral avoidance, and copepod populations may be moving to different depths. This study was limited to one point in space, but future studies using multiple automated eDNA samplers at different depths could determine whether declining copepod abundance in hypoxic conditions is due to population declines or population movements.

Copepod populations do not exist in isolation, and future studies should also consider the taxonomic effects of hypoxia. For example, if fish that eat copepods are more sensitive to hypoxia than the copepods themselves, decreased predation due to fish kills may lead to increased copepod populations at certain dissolved oxygen levels. If copepods are more sensitive to hypoxia than the fish that eat them, decreased prey availability may impact fish at levels of hypoxia that would otherwise be tolerable. Crustaceans and fish are both known to be highly sensitive to hypoxia compared to other taxonomic groups, so future studies into copepod hypoxia tolerance and copepod predator hypoxia tolerance will be important and should take individual species tolerances into account.

Appendix A

Software and Data Availability

A.1 Data Availability Statement

All code used in this thesis can be found at <https://github.com/DolphinCoder/Reed-Thesis-Copepod-Populations>.

The results of the overall automated eDNA sampler study in OCNMS are actively being organized into a peer-reviewed manuscript, and all data will be made available upon publication. Reach out to Zachary Gold (zachary.gold@noaa.gov) for more information or to request raw data.

A.2 R Packages Used

Pedro J. Aphalo, Kamil Slowikowski, and Samer Mouksassi (Nov. 14, 2024). *Ggpmisc: Miscellaneous Extensions to 'Ggplot2'*. Version 0.6.1. URL: <https://cran.r-project.org/web/packages/ggpmisc/index.html> (visited on 04/26/2025)

Hadley Wickham and RStudio (Feb. 22, 2023). *Tidyverse: Easily Install and Load the 'Tidyverse'*. Version 2.0.0. URL: <https://cran.r-project.org/web/packages/tidyverse/index.html> (visited on 04/26/2025)

Kirill Müller and Jennifer Bryan (Dec. 13, 2020). *Here: A Simpler Way to Find Your Files*. Version 1.0.1. URL: <https://cran.r-project.org/web/packages/here/index.html> (visited on 04/29/2025)

Hadley Wickham, Thomas Lin Pedersen, et al. (Apr. 24, 2025). *Scales: Scale Functions for Visualization*. Version 1.4.0. URL: <https://cran.r-project.org/web/packages/scales/index.html> (visited on 04/26/2025)

Paul-Christian Bürkner et al. (Sept. 23, 2024). *Brms: Bayesian Regression Models Using 'Stan'*. Version 2.22.0. URL: <https://cran.r-project.org/web/packages/brms/index.html> (visited on 04/26/2025)

Hadley Wickham and Posit Software (Mar. 22, 2023). *Modelr: Modelling Functions That Work with the Pipe*. Version 0.1.11. URL: <https://cran.r-project.org/web/packages/modelr/index.html> (visited on 04/26/2025)

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Appendix B

Zero-Inflated Beta Regression Plots

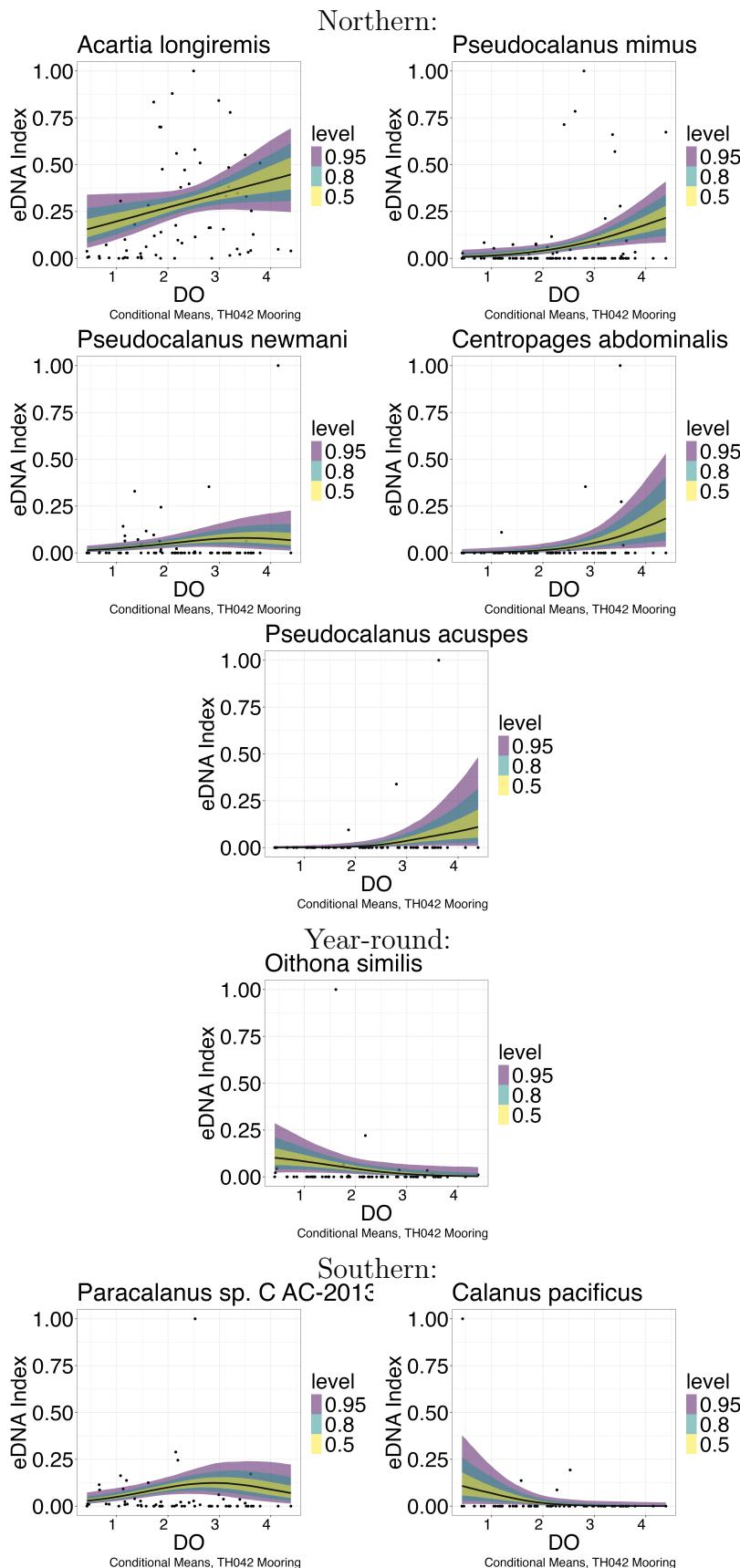


Figure B.1: Zero-inflated beta regression model means, with bands showing 0.5, 0.8, and 0.95 confidence levels, using data from 2021-22 from the TH042 mooring.

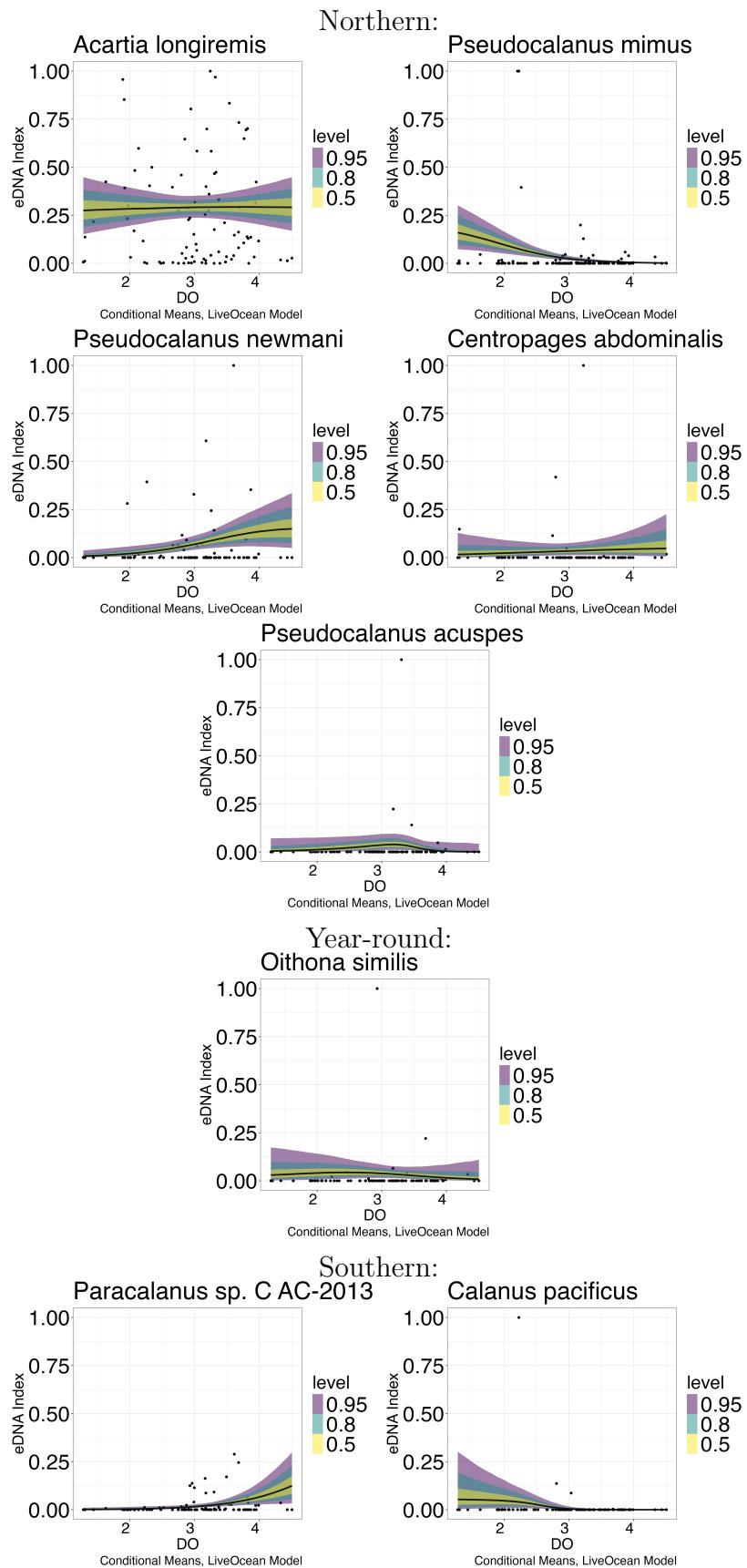


Figure B.2: Zero-inflated beta regression model means, with bands showing 0.5, 0.8, and 0.95 confidence levels, using data from 2021-23 from the LiveOcean model.

Appendix C

Additional Plots of Dissolved Oxygen vs. Temperature vs. eDNA Index

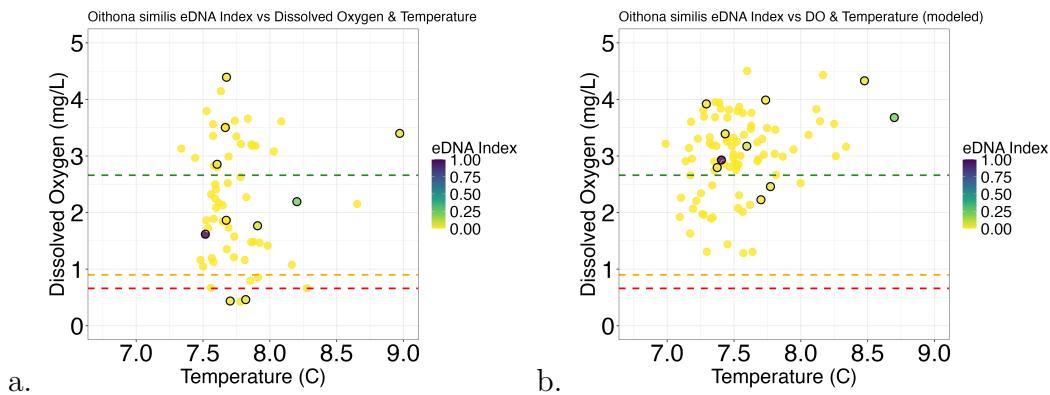


Figure C.1: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *O. similis* detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *O. similis* was not detected. Plot (a) uses TH042 mooring data from 2021-22, (b) uses LiveOcean model data from 2021-23.

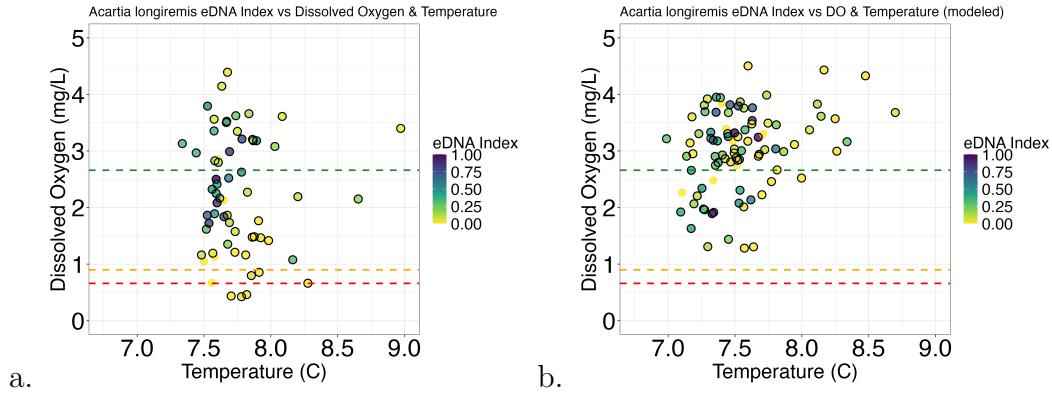


Figure C.2: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *A. longiremis* detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *A. longiremis* was not detected. Plot (a) uses TH042 mooring data from 2021-22, (b) uses LiveOcean model data from 2021-23.

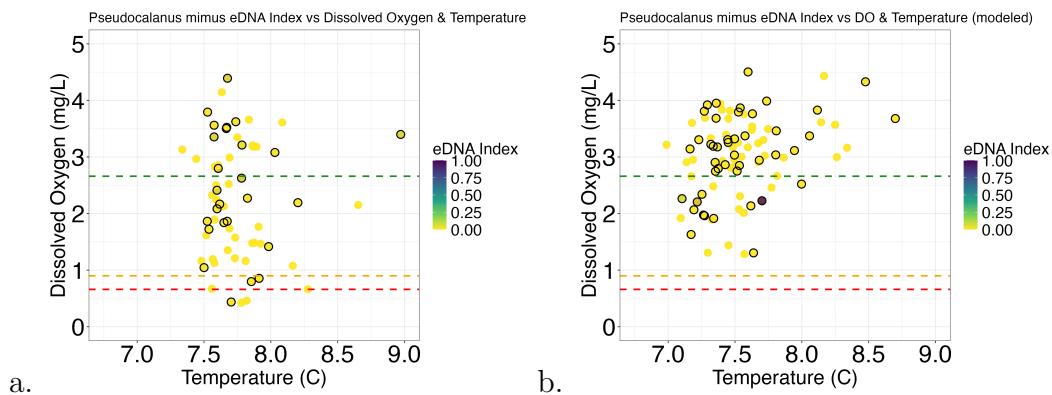


Figure C.3: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *P. mimus* detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *P. mimus* was not detected. Plot (a) uses TH042 mooring data from 2021-22, (b) uses LiveOcean model data from 2021-23.

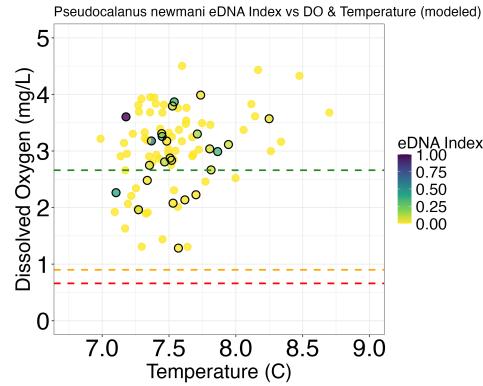


Figure C.4: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *P. newmani* detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *P. newmani* was not detected. This plot uses LiveOcean model data from 2021-23.

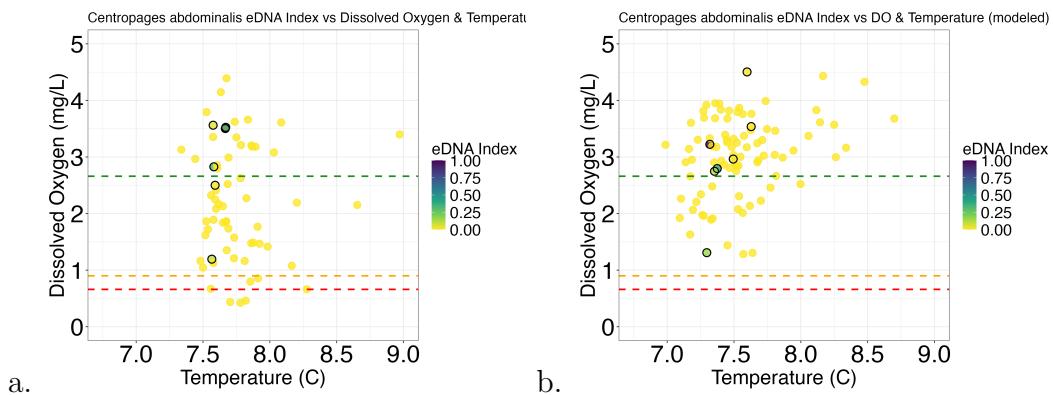


Figure C.5: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *C. abdominalis* detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *C. abdominalis* was not detected. Plot (a) uses TH042 mooring data from 2021-22, (b) uses LiveOcean model data from 2021-23.

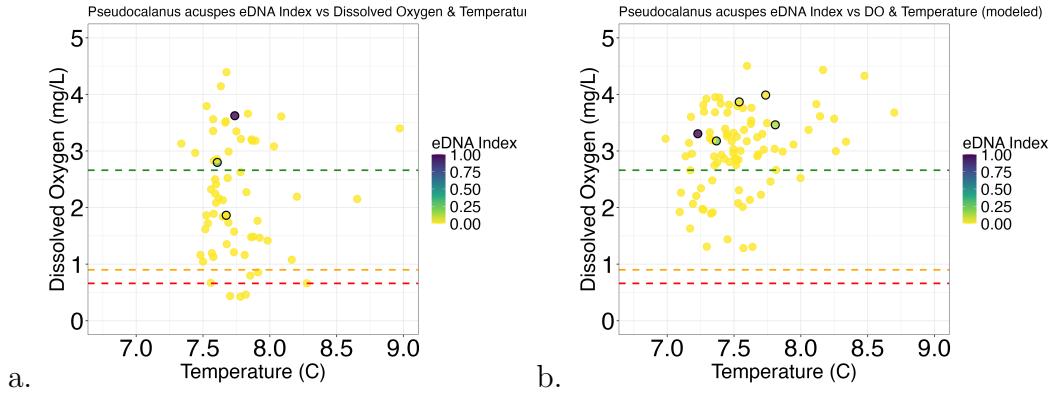


Figure C.6: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *P. acuspes* detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *P. acuspes* was not detected. Plot (a) uses TH042 mooring data from 2021-22, (b) uses LiveOcean model data from 2021-23.

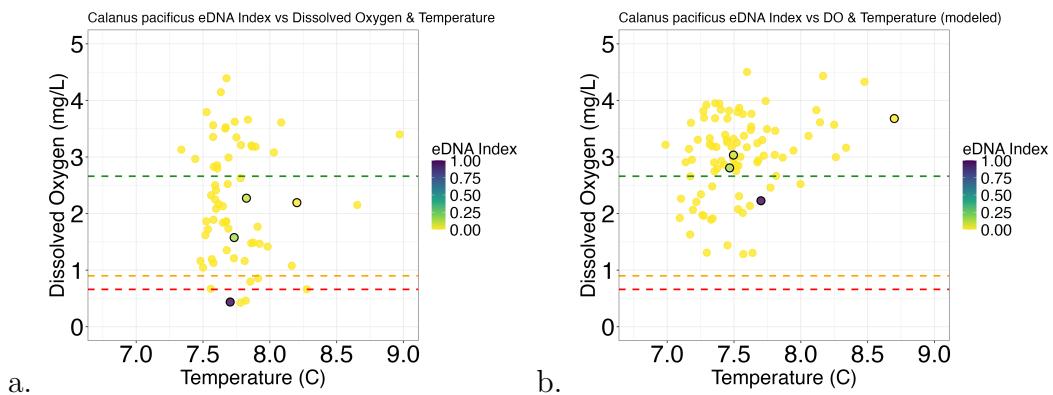


Figure C.7: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *C. pacificus* detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *C. pacificus* was not detected. Plot (a) uses TH042 mooring data from 2021-22, (b) uses LiveOcean model data from 2021-23.

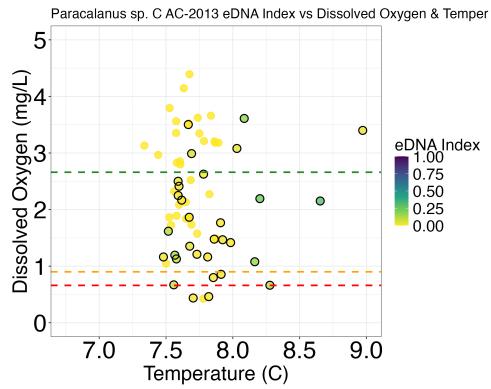


Figure C.8: Scatterplot of dissolved oxygen (mg/L) and temperature (Celsius) for each environmental DNA sampling time, with *Paracalanus* sp. C AC-2013 scatterplot detections circled in black. Color of dots represents eDNA index, a normalized measure of abundance that can compare abundance within a species. Yellow, uncircled dots represent sampling dates when *Paracalanus* sp. C AC-2013 scatterplot was not detected. This plot uses TH042 mooring data from 2021-22.

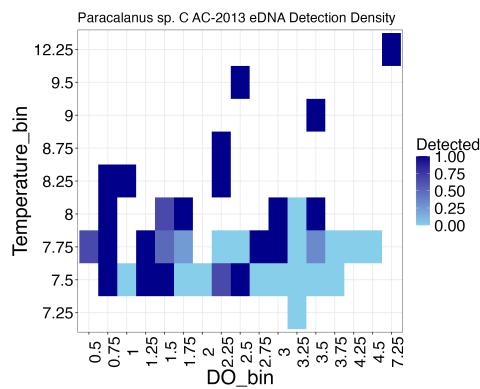


Figure C.9: Density plot of *Paracalanus* sp. C AC-2013 detections. Each pixel represents a range of dissolved oxygen and temperature values, and the shade of each pixel represents the proportion of eDNA samples in that environmental range in which *Paracalanus* sp. C AC-2013 was detected. This plot uses TH042 mooring data from 2021-22.

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