Quantifying Active Carbon Flux from Euphausiid Respiration in the Clarion-Clipperton Zone

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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For Eric, Stacie, and Christian Westerhausen for always supporting me no matter what.

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ABSTRACT

The process of moving carbon from the atmosphere into the ocean is called carbon flux. Within the oceans, carbon flux has two main components: active flux and passive flux. Passive flux considers only the sinking of non-living matter such as detritus and waste. Active flux is the movement of carbon to depth through respiration of animals during vertical migration. The Clarion Clipperton mining zone falls directly below an oxygen minimum zone (OMZ). During euphausiid diel vertical migration, their metabolism burns the carbon from phytoplankton consumed at the surface, then they respire carbon dioxide. This process contributes to active carbon flux. Euphausiid carbon flux has been measured only a few times in the central Pacific and no research has been done within an OMZ. Euphausiid respiration has an almost negligible effect on total carbon flux with 0 - 0.0748 (mg C/day/m^2) which is less than 1% of passive flux in the testing area, however, there is still respiratory flux occurring. The majority of carbon flux in this area comes from passive flux and respiratory flux from other migrating organisms. This is a valuable study to estimate active carbon flux of euphausiids within an OMZ, and this study provides important baseline observations before deep sea mining begins. More research is needed on respiratory flux of other organisms to create a full representation of active carbon flux within this OMZ.

Keywords: Biological carbon pump, Active carbon flux, Euphausiid, Oxygen minimum zone, Diel migration.

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1.0 INTRODUCTION

1.1 Carbon Flux

The Ocean is a massive carbon sink from the Earth's atmosphere. Phytoplankton fix carbon during photosynthesis, converting it to particulate organic carbon (POC). The process of moving carbon to deeper depths in the water column is often referred to as the biological carbon pump (Steinberg and Landry, 2017). This process is part of what allows the oceans to continue to absorb carbon dioxide from the atmosphere. There are two types of carbon flux, passive flux and active flux. Passive flux is a physical mechanism to export carbon to the deep ocean through the sinking of decaying matter, fecal pellets, remnants of messy grazing and other detritus. If this matter is not eaten by organisms, it will sink to the bottom of the ocean. Passive carbon flux is measured using sediment traps that capture the falling detritus. Active carbon flux is a process that involves mobile organisms such as zooplankton and micronekton vertically migrating, feeding in shallower waters during the night and respiring at depth during the day. As phytoplankton are eaten by zooplankton and other micronekton including euphausiid, the organic carbon is transferred to the predator and respired during vertical migration. The zooplankton and other predators convert it to dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) by respiration of carbon dioxide at depth. In this way, they transport carbon from surface waters to greater depths.

Early studies of vertical carbon flux focused on measurements of passive flux, but more recent ones suggest that active flux could be very important. For instance, in (Ducklow et al., 2001), they discovered active carbon flux made up around 2-35% of

carbon flux, while passive flux was up to 70%. More recent studies of zooplankton and micronekton have shown that respiratory flux can account for upwards of 53% of total flux, equaling or even exceeding the passive flux at times (Ariza et al., 2015, Saba et al., 2021, Takahashi et al., 2009, Hidaka et al., 2001). However, there are limited studies, so more estimates in different parts of the world are needed.

1.2 Oxygen Minimum Zones (OMZs)

OMZs are regions of the ocean where persistent low oxygen levels exist in the mid water column. Oxygen concentrations within an OMZ are below 0.5 ml l^{-1} or below 7.5% saturation (Levin, 2003). These areas are unlike fjords or other, mostly coastal areas that experience seasonal low oxygen: OMZs have consistently low oxygen concentrations over geological time and can span through different depth ranges. OMZs are caused by biochemical oxygen consumption, circulation affecting the position and distribution of microbes and other biological organisms throughout the water column, and the age of the water mass (Levin, 2003). In an OMZ, oxygen levels deplete rapidly with depth in midwaters and rise again, often below a depth of about 1000 m. The largest OMZ region occurs in the eastern tropical Pacific spanning thousands of miles (Busecke et al., 2022). These ecosystems are large, open ocean habitats for fisheries that supply the global market, as well as migratory species such as whales, tuna, marlin, and swordfish. These ecosystems also provide stable environments for phytoplankton that fix carbon from the atmosphere, and zooplankton and other micronekton than transport that carbon to deeper depths. All kinds of organisms exist and migrate in and out of OMZs throughout the global oceans.

There are currently no measurements of active vertical carbon flux in the Clarion Clipperton Zone OMZ. However, Steinberg and Landry (2017) proposed that hypoxic areas in an OMZ can suppress the metabolism of vertically migrating animals that would reduce their respiration rates and thus active respiratory carbon flux compared to higher oxygen areas around the world. There are organisms, such as copepods, euphausiids, doliolids, appendicularians, heteropods, midwater fishes, and myctophids that have high tolerance for hypoxia (Saba et al., 2021; Wishner et al., 2020). Organisms typically prefer to inhabit well oxygenated areas to breathe and diurnally migrate. A high oxygen and high productivity environment allows for high metabolic and respiration rates while migrating to and from the surface. Within an OMZ, however, the oxygen levels are extremely low and cause metabolic suppression in euphausiids (Seibel et al., 2016). Euphausiid travel down into the OMZ during the day to avoid predation; here, they have adapted to slow their metabolism to reduce their oxygen demand in relation to the surrounding hypoxic environment. Then at night they speed up their metabolism as they migrate back up to the surface to feed. As a result of their slowed metabolism at depth, the active carbon flux is expected to be very low (Steinberg and Landry, 2017). Some species of euphausiid found in this area are localized and some cases endemic to the eastern tropical Pacific where the largest OMZs are found, and others are more broadly distributed around the Pacific. These euphausiid have adapted to the OMZ by slowing their metabolism. Active flux relies on respiration at depth, so if species' metabolisms are slowed within an OMZ core, there will be lower active flux (Steinberg and Landry, 2017). Although there is metabolic suppression occurring within these areas, active acoustic measurements of migrating animals suggest that a large proportion of them do

vertically migrate in the eastern tropical Pacific OMZ (Pereleman et al., 2021). Clearly, more study is required to measure active respiratory flux of animals within OMZ regions.

1.3 Importance of Euphausiids

Krill (aka euphausiids) are found in all oceans and they are an abundant and ecologically important group (Ikeda, 2013). Furthermore, they are some of the most abundant small micronekton that can survive in extremely low oxygen environments (Maas et al., 2014). They are primary consumers and also provide food to higher trophic levels. Since they diurnally migrate throughout the water column, they transport carbon from the surface ocean to deeper depths. By consuming carbon at the surface then metabolizing and converting it to DIC at depth, they likely contribute to active carbon flux in an OMZ. Euphausiid abundance could heavily influence the amount of active flux occurring within an OMZ, among other factors including temperature, biomass, metabolic suppression, and productivity levels. Therefore, studies are needed to understand their role in the biological carbon pump and other biochemical processes, particularly in OMZ regions.

1.4 Deep Sea Mining

In the Clarion Clipperton Zone (CCZ) region, there is interest in mining polymetallic nodules from the deep-sea floor (Cooley et al., 2020). Deep sea mining is a threat to the biological carbon pump due to the massive sediment plumes that may be released into the mid water column. The sediment plume will be made up of fine sediment that is sucked up by a hydraulic pump along with the polymetallic nodules.

After the nodules are recovered onboard a ship, the excess sediment will be pumped back out to the mid water column. This sediment plume will likely have major negative ecological consequences (Drazen et al., 2020). It will introduce heavy metals to the water column including copper and cobalt, cause respiratory distress by clogging respiratory surfaces and gills, and dilute food material with inorganic introduced sediment. The sediment plume could also disrupt diurnal migration patterns. The sediment plume will likely suffocate animals residing in this area, especially if the plume lingers and travels with the currents (Drazen et al., 2020). Active flux relies on vertically migrating animals that respire. Since the plume could be released into their midwater resting depth habitat, this would disrupt the natural process of active carbon flux in the upper water column. However, there are no measurements of active carbon flux in nodule mining areas, which are needed as a baseline before mining begins. This region also coincides with the largest OMZ in the world.

The goal of this study is to quantify the amount of active carbon flux that euphausiid contribute to total carbon flux at a site in the eastern CCZ before mining begins. This information will greatly help us understand the effect euphausiids have on the biological carbon pump in this OMZ before it is altered by mining. By understanding as much about this environment as possible, this research can provide important baselines for monitoring and evaluation of the importance of ecosystem services provided by organisms and communities within mining areas.

2.0 METHODS

2.1 Study Site and Field Collections

Samples were collected from the Clarion Clipperton Zone in the eastern Tropical Pacific Ocean, specifically at a mining license area called NORI-D (Fig. 1)

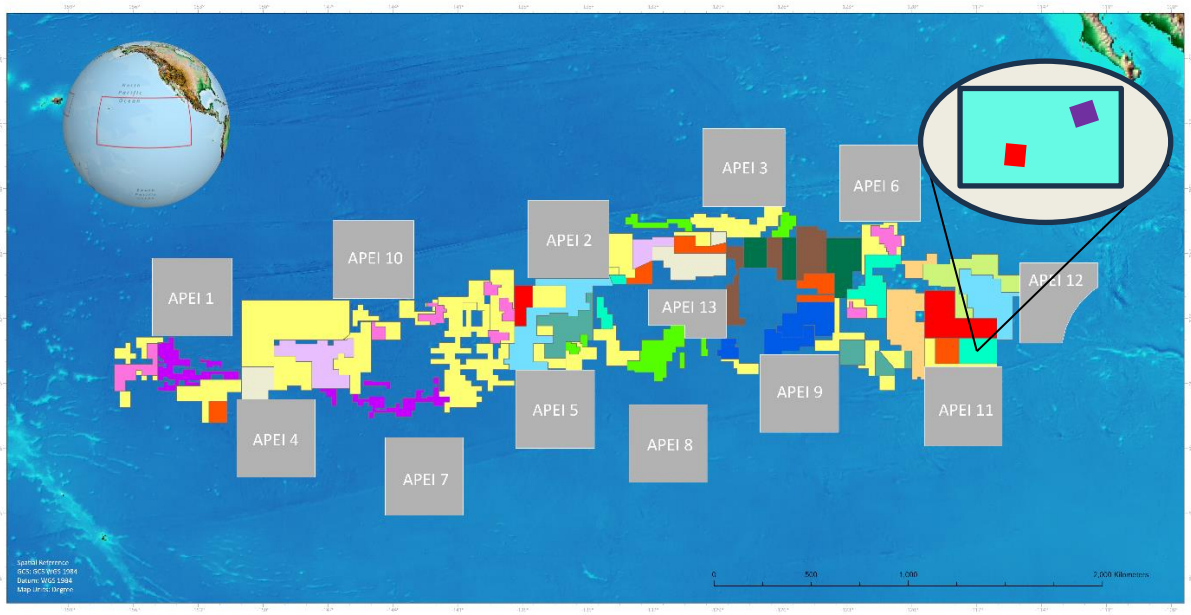


Figure 1: The NORI-D mining site where sampling took place is shown in relation to the rest of the CCZ license areas. In the expanded NORI-D area, the Preservation Reference Zones (PRZ) and the Collector Test Area (CTA) are shown in purple and red boxes, respectively. The other CCZ license areas are indicated by the other colored areas around the map, and the Areas of Particular Environmental Interest (APEIs) are marine protected areas where licenses are not allowed.

There were two cruises, one in the spring of 2021(March and April) and one in fall 2021 (September and October). During both cruises, two locations were sampled: the preservation reference zone (PRZ), and the mining collector test area (CTA). The PRZ is

meant to be a control site compared to the CTA where test mining activities were conducted in November of 2022. Unfortunately, no pelagic biological sampling was conducted during the tests of the mining collector vehicle.

Euphausiids were collected using the 10-meter MOCNESS (Multiple Opening Closing Net Environmental Sampling System). This system is used for sampling micronekton and other organisms. With multiple 3 mm mesh size nets, there were 5 depth-discrete samples for each tow. The opening of the net mouth was roughly 10 meters^2 (Wiebe et al., 1985). The MOCNESS also recorded temperature, conductivity, depth, and oxygen throughout all tows. The upper 1500m of the water column was sampled, however, data in this study is only from nets 4 and 5. Net 5 depths ranged from 0-70/90 and net 4 from 70/90-450 meters. Net 5 represented the well oxygenated upper part of the epipelagic zone and net 4 sampled the upper mesopelagic zone into the upper and core OMZ.

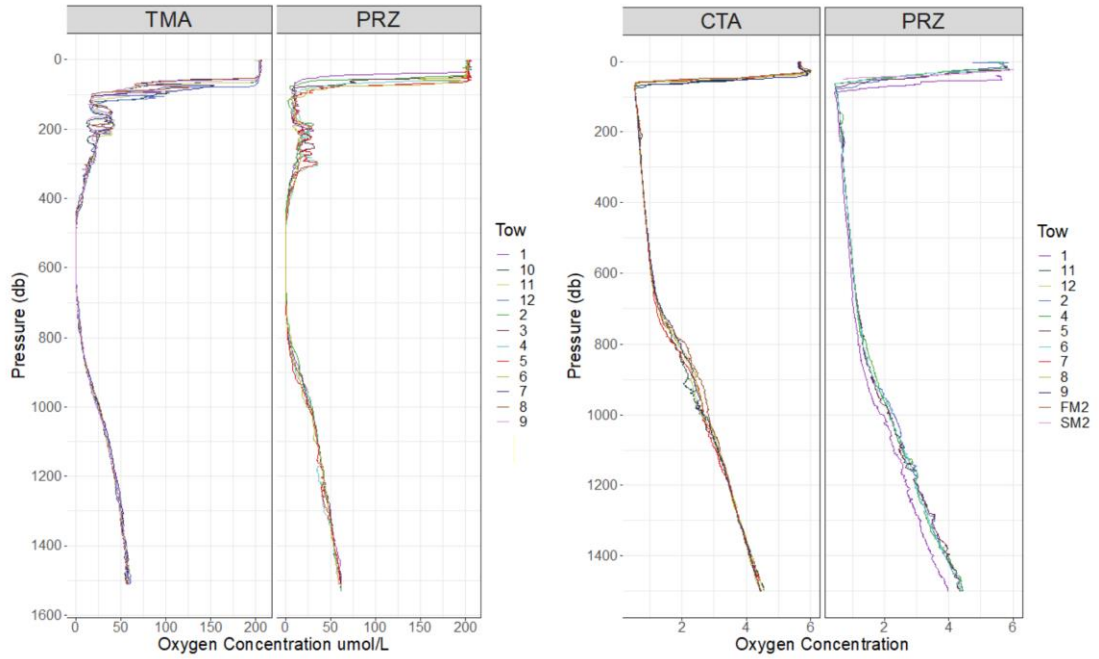


Figure 2: Oxygen profiles from spring (left) and fall (right) cruises in both CTA and PRZ sites. Oxygen concentrations were recorded by the SBE O_2 sensor on the $1m^2$ MOCNESS measured in µmol/l in spring and ml/l in the fall. All data was collected from the surface to 1500m (Goetze et al., 2023).

These regions of the water column are within the path that diurnal migrators travel through to get to the surface at night. Nets were towed at about 1.5 knots during the day and night to obtain diurnal changes in micronekton communities, including euphausiid. The tow time at each depth varied, however, the goal was to tow for an acceptable amount of time, while keeping the correct ship speed and winch speed to create a 45-degree net angle. The volume sampled for each net was calculated using a calibrated flow meter and continuous measurements of the effective mouth opening.

2.2 Sample Processing

Organisms were sorted to the family level or the lowest taxonomic category possible at sea. However, euphausiid, small shrimp, and gelatinous animals were unable to be processed on the ship. These organisms were preserved together in 10% borax buffered formalin in seawater and returned to the lab for further sorting. A few samples were fixed in 95% nondenatured ethanol.

In the laboratory, samples were separated by cruise and further processed. Specimens were retained on a strainer to remove formalin and delicately moved into a petri dish for sorting and identification. The identification process involved utilizing a microscope to distinguish various morphotypes, including Round-eyed euphausiid, Lobed-eyed euphausiid, diverse shrimp species, unknown crustaceans, squid, juvenile shrimp, the amphipod *Phronima* spp., Crab Zoea, stomatopod larvae, euphausiids with

parasites, the giant ostracod *Gigantocyprus* sp., lobster larvae, juvenile fishes, octopus, leptocephalus eel larvae, and the shrimp *Lucifer* spp. The euphausiids were not individually identified to species because there were over 10,000 individuals. Instead, euphausiids were sorted into two categories based on their morphology: Round-eyed euphausiid and Lobed-eyed euphausiid. The Round-eyed species most likely to be located in this area within this OMZ are *Euphausia diomedeae, Euphausia distinguenda, Euphausia eximia, Euphausia recurve, Euphausia tenera, Euphausia hemigibba, Thysanopoda aequalis, Thysanopoda Astylata, Thysanopoda orientalis,* and *Thysanopoda pectinate.* The Lobed-eyed euphausiid most likely found in this area and found in the samples are *Nematoscelis gracilis, Nematobrachion flexipes, Stylocheiron abbreviatum, Stylocheiron carinatum, Stylocheiron longicorne, Stylocheiron maximum, Stylocheiron suhmi,*and *Nematobrachion flexipe* (Brinton, 2021).

Each taxon was placed onto a separate, clean petri dish and enumerated. Each taxon was gently patted dry with clean paper towels before being weighed on a scale. The specimens were subsequently transferred to new jars containing fresh formalin, with each jar labeled comprehensively with cruise and sample ID, collection date, and species name. This process was repeated for each scoop of specimens from the original jar, ensuring that specimens of the same species were stored together for future reference. Comments pertinent to the specimens were incorporated into a spreadsheet, and the cumulative counts and weights were calculated once all specimens within the original sample jar were processed. This comprehensive methodology ensured the preservation, systematic organization, and accurate documentation of specimens.

The same process was applied to ethanol-preserved samples with minor differences. The specimen sorting did not take place under the fume hood and was done on the laboratory countertop. The processed samples were stored in fresh ethanol, and jars marked with a DNA barcode indicator so they could be located later for genetic analysis.

2.3 Calculations

Estimation of active carbon transport from euphausiid respiration below the euphotic zone was obtained by combining empirically derived migration data from the net sampling and published values for euphausiid respiration that use biomass inputs. Using the euphausiid counts and mass from each net, combined with the volumes of seawater filtered by the net, euphausiid abundance and biomasses were calculated and presented as mean and standard deviation of number per 10,000m^{-3} and grams per 10,000m^{-3}. To determine the total abundance and biomass of euphausiid migrating from the surface to below 70- 90 m, these values were integrated over this depth horizon to estimate number or mass of euphausiid per 10,000m^2. Night minus daytime values were used to estimate the total abundance and mass of migrating euphausiid.

Respiration of euphausiid at depth during the day was estimated using the global comprehensive meta-analysis and empirical equation from (Ikeda, 2013) . This equation is as follows:

$$\ln Y = 0.392 + (0.753)\ln X_1 + (0.046)X_2 + (-0.107)\ln X_3$$

In this equation, Y is equal to respiration (μlO_2 $ind.^{-1}$ h^{-1}), X_1 is body mass (mg dry weight $ind.^{-1}$), X_2 is temperature (1000/K), X_3 is depth (m), and the constants are from the

multiple regression. This equation was selected because it considered all variables including depth and dry mass. This made it possible for the equation to be used in different parts of the world and was not species specific. Respiration of the average sized Round- Eyed euphausiid and Lobed-eyed euphausiid were determined for their daytime depth and temperature.

The average individual size of surface migrating euphausiid was determined by estimating the average total abundance in surface waters 0-70/90 meters (net 5) at night divided by the average total biomass, for both seasons and sites. The body mass calculated was wet weight so to create a conversion to dry mass, required for the empirical equation, 50 randomly selected euphausiid from 0-70/90 meter samples were weighed both wet and after drying at 60°C for 24 hours. The average ratio of dry weight to wet weight was then used as a conversion factor to transform wet weights to dry weights.

The temperature value used comes from the middle depth of their daytime depth range. Based on early investigation of the trawl data the euphausiid resided at ~80 m to 450 m during the day. The middle depth, or the depth closest to this value, was located in the data and the MOCNESS recorded temperature at this depth was recorded for each net sample. The middle depth value from 70/90-450 meters (net 4) was also used in the respiration equation for the depth (X_3).

Total individual respiration (µl CO_2/individual/hr) was calculated by taking the individual respiration (µl O_2/individual/hr) and multiplying it by a respiratory quotient for crustaceans of 0.97 (Pakhomov et al., 1999). This number was then multiplied by the abundance of migrating euphausiid (net 5, night – day abundance). Near the equator it

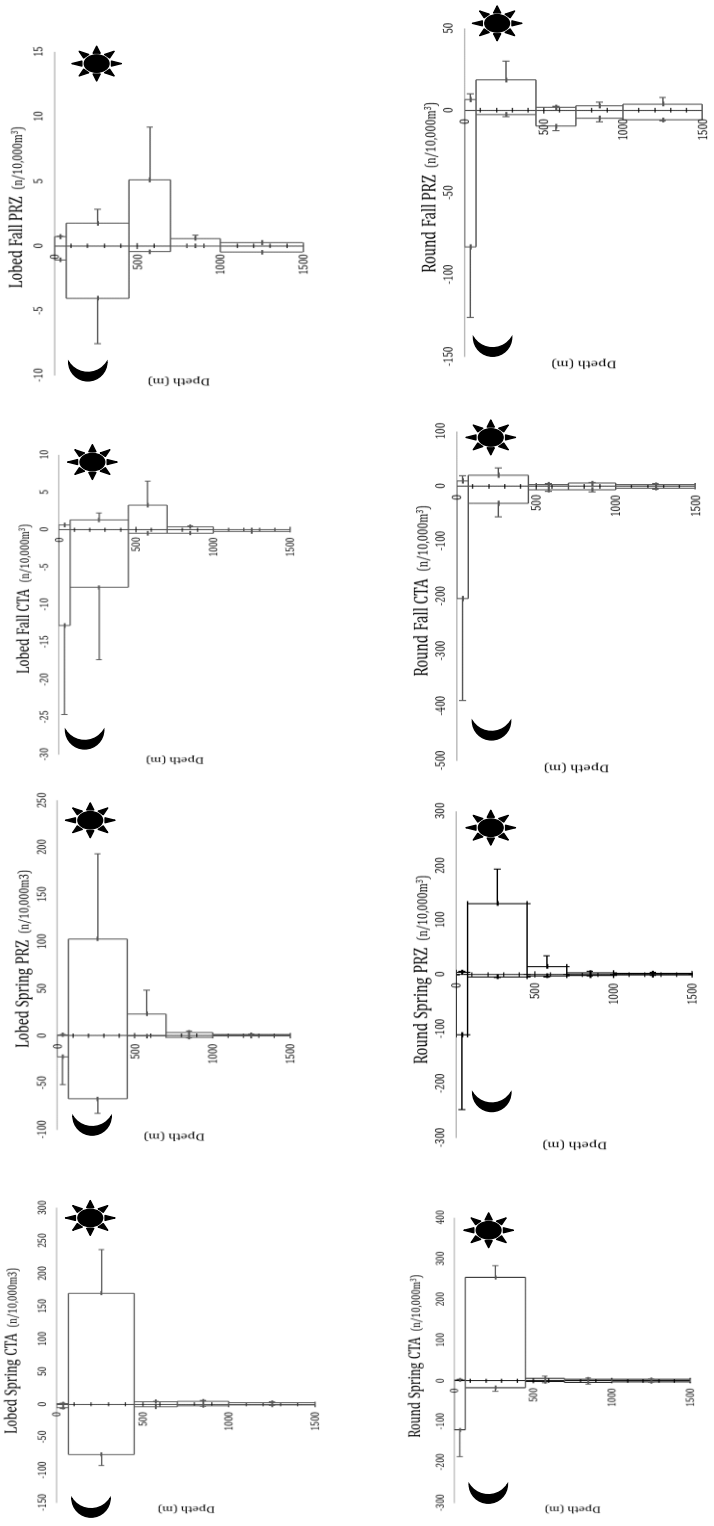
was assumed that the euphausiid occupy their nighttime depths for about 12 hours per day, so individual respiration was then multiplied by 12 hours to obtain the total respiration at depth (µl CO_2 /day/m^2). Microliters of CO_2 were converted to mg of CO_2 using the gas law (22.4 mols of CO_2 l^{-1}) and the molecular weight of CO_2 (44 g/mol). The resulting respiration in mg CO_2 respired at depth/day/ m^2 was then multiplied by the molar mass of carbon:12(g/mol) and divided by the molar mass of CO_2: 44 (g/mol). This gives the final total respiratory flux in mg of carbon per day per m^2 (total respiratory flux mg C /day/ m^2). Carbon flux was calculated for each morphotype of euphausiid, each season, and location.

3.0 RESULTS

The data obtained consisted of two seasons, spring and fall, two sites, the PRZ and the CTA, and day/night samples. Throughout the whole sampling process, there were 24 tows, 12 in the Spring and 12 in the Fall. Within each season and location there were ~2-4 day tows and ~2-4 night tows. Throughout all nets, there were 13,594 euphausiid processed for this study. This study only considered the migrating abundance and biomass of Lobed-eyed and Round-eyed euphausiid of the surface waters (0-70-90m, net 5) for respiratory flux calculations.

3.1 Abundance and Biomass of Euphausiid Diurnal Migration

a.)



b.)

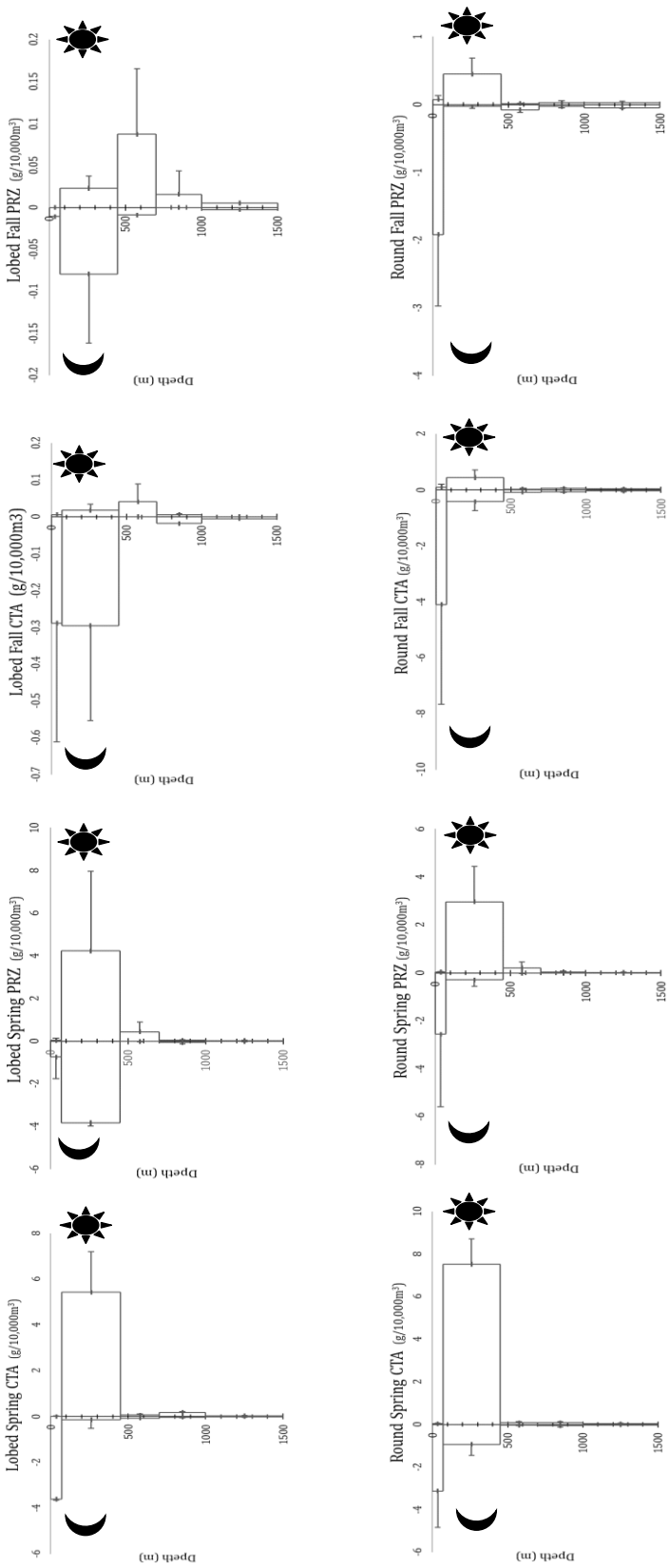

Lobed Fall PRZ (g/10,000m³)
Round Fall PRZ (g/10,000m³)
Lobed Fall CTA (g/10,000m3)
Round Fall CTA (g/10,000m³)
Lobed Spring PRZ (g/10,000m³)
Round Spring PRZ (g/10,000m³)
Lobed Spring CTA (g/10,000m³)
Round Spring CTA (g/10,000m³)
Depth (m)

Figure 3: Kite diagrams showing the diurnal vertical abundance (a) and biomass (b) migration of Round-eyed and Lobed-eyed euphausiid in the fall PRZ and CTA, and the spring PRZ and CTA. The y-axis represents the depth intervals of each net in meters. In each plot, the left side represents abundance/biomass at night with day shown on the right side. Note that negative values are used only to represent the difference between day and night values.

Diel vertical migration was common for Round-eyed and Lobed-eyed euphausiid (Fig. 3). The Round-eyed euphausiid seem to have a more pronounced migration from ~80-500m to the surface, evident in both seasons. In contrast, the Lobed-eyed euphausiid seem to stay at deeper depths even during the night and exhibited vertical migration mostly during the Fall. Lobed-eyed euphausiid diel vertical migration also occurred to greater depths and they frequently stay below the surface euphotic zone. There are varying smaller amounts of both types of euphausiid below 450m, however, migration to the surface does not appear to be strong. The most migration to the surface for Round-eyed euphausiid occurred in the Fall CTA with 204/10,000m^3 compared to Lobed-eyed that had the most abundance in the spring PRZ with 22/10,000m^3. The lowest amount of migration to the surface for Round-eyed euphausiid occurred in the Fall PRZ with 83/10,000m^3 in the Fall PRZ and Lobed-eyed euphausiid with ~1/10,000m^3 in both the Fall PRZ and Spring CTA. The Spring CTA and PRZ have similar migration patterns for both species.

The biomass of migrating euphausiid largely followed the patterns in abundance. The Round-eyed euphausiid have a much more prominent, and consistent biomass migration pattern to the surface than the Lobed-eyed euphausiid. There still seems to be biomass migration of Lobed-eyed euphausiid, however, it is just at deeper depths. The

body weight in table 1 follows the biomass of the first 70/90m of the water column with Lobed-eyed having the least mg dry mass of 1.997 ± 0 in the fall PRZ and the most in the spring CTA of 6.218 ±2.84. Round-eye body mass was less variable with the most mg dry mass in the spring CTA of 5.137 ± 0.13, and the least in the fall CTA of 3.732 ± 1.15. The Round-eyed euphausiid showed a clear biomass difference in net distribution during the day and night in the spring and fall. During the day most of the biomass resides in 70/90-450 meters, however, during the night the biomass travels up to the surface (0-70/90 m). There are smaller amounts of euphausiid biomass at deeper depths, but most of the euphausiid biomass is within 0-450 meters consistently. A more defined pattern occurs in the spring with a sharper distribution of biomass for Round-eyed euphausiid. There is also more biomass in the spring than in the fall for Round-eyed euphausiid.

The fall biomass distribution of Lobed-eyed euphausiid differed greatly between night and day. In the Fall, Lobed-eyed euphausiid had significantly less biomass in the fall CTA (<0.08 g/10,000m^3) and PRZ (<0.3 g/10,000m^3) than in the spring CTA (<5 g/10,000m^3) and PRZ (<4 g/10,000m^3) compared to Round-eye overall. Round-eye biomass ranged from ~7 to ~0.2 g/10,000m^3. Most of the biomass during the day resides between 70 and 1000m. Biomass follows the same pattern of diel migration as abundance does. During the spring there is diurnal migration to the surface at night, however, the fall is a bit different. The biomass existed at the surface down to 500m at night and 500-750m during the day in the fall CTA. Then in the PRZ during the fall, there was almost no biomass at the surface at night, almost all of it was between 70-500m. During the day, Lobed-eyed biomass existed between 70-1000m. There is more than twice the biomass between 70-450 meters in the spring than in the fall for both species. There is diurnal

migration of biomass for both species, however the amount of biomass is significantly higher in the spring than in the fall.

3.2 Euphausiid Respiration

Respiration of both types of euphausiid was estimated from their mean body mass, temperature at depth during the day, and median daytime depth. Round-eyed body mass stayed relatively consistent throughout all the seasons and sites with a slight spike in the spring CTA. Lobed-eyed body mass varied greatly by season and site with the greatest body mass in the spring CTA and lowest in the fall PRZ. Since respiration was multiplied by the abundance, the respiratory flux was directly altered by the abundance. The higher the abundance was, the more respiratory flux there was. Since the depth profiles were selected for specific nets throughout the study, there was little temperature variation between site and season. Individual respiration stayed relatively consistent in Round-eyed euphausiid with the lowest value in the fall CTA and highest range in the fall PRZ, whereas Lobed-eyed euphausiid were more variable. The highest individual respiration rate for Lobed-eyed euphausiid was in the spring PRZ and lowest in the fall PRZ. Migrating abundance levels varied greatly between site and season for both species. Round-eyed euphausiid had the highest abundance in the fall CTA leading to the highest respiration rate. Lobed-eyed euphausiid had substantially lower abundances compared to Round-eyed euphausiid with the highest abundance in the spring PRZ and lowest in the fall PRZ. Round-eyed euphausiid had similar respiratory flux rates for the spring PRZ and CTA. However, the fall CTA had the most respiratory flux due to the greater abundance than any other season or site (13313.68 ± 13496.03 #/10,000m^2), and the fall

PRZ had the least respiratory flux. This can all be seen in table 1. Lobed-eyed euphausiid respiratory flux varied greatly with almost no flux in the fall PRZ and the most flux in spring PRZ. The Lobed-eyed euphausiid respiratory flux rates were a magnitude lower than Round-eyed euphausiid respiration.

The biggest factors affecting respiratory flux were individual body mass, abundance, temperature, and depth. Body mass, temperature from depth, and abundance directly affect respiratory flux rates. The more biomass and abundance there is, the more respiratory flux there will be. Higher temperatures also support higher metabolic rates, meaning more flux as well. Depth inversely affects respiratory flux rates, as depth increases, temperature decreases, and respiration decreases. For estimates of individual body mass, a conversion from wet mass to dry mass was used based on specimens from this study. 80 ± 2.4% of an individual's body weight was made from water (n=50).

Table 1: Euphausiid respiration, abundance and total respiratory flux. Values are means +/- standard deviations. Mean and ranges are given for individual respiration and total respiratory flux values based on standard deviations of input variables temperature, body mass and depth.

morphotype	season	site	day/ night	Body mass (mg dry mass)	Temperature at median depth (°C)	median Depth (m)	individual respiration range (µl CO_2/ind/h)	Abundance of migrating euphausiid (#/10,000m^2)	Total respiratory flux range (mg C/day/m^2)
Round-eyed	fall	PRZ	night	4.539 ± 0.18	11.279 ± 0.18	259.5	4.156 (4.0 - 4.31)	5029.03 ± 2918.67	0.0132 (0.0054 - 0.022)
	fall	CTA	night	3.732 ± 1.15	10.931 ± 0.11	260.0	3.529 (2.66 - 4.34)	13313.68 ± 13496.03	0.0302 (0 - 0.0748)
	spring	PRZ	night	4.73 ± 0.98	11.332 ± 0.4	240.7	4.333 (3.63 - 5.02)	7433.33 ± 9639.01	0.207 (0 - 0.0551)
	spring	CTA	night	5.137 ± 0.13	11.215 ± 0.05	265.6	4.538 (4.45 - 4.62)	9942.90 ± 5847.1	0.029 (0.0117 - 0.0469)
Lobed-eyed	fall	PRZ	night	1.997 ± 0	11.279 ± 0.18	259.5	2.24 (2.22 - 2.26)	24.49 ± 2.17	0 (3.19E-5 - 3.87E-5)
	fall	CTA	night	3.573 ± 1.54	10.931 ± 0.11	260.0	3.416 (2.23 - 4.49)	823.59 ± 845.5	0.0018 (0 - 0.0048)
	spring	PRZ	night	4.913 ± 2.36	11.332 ± 0.4	240.7	4.458 (2.71 - 6.03)	1487.82 ± 2073.33	0.0043 (0 – 0.0138)
	spring	CTA	night	6.218 ±2.84	11.215 ± 0.05	265.6	5.239 (3.31 - 6.96)	314.06 ± 158.54	0.0011 (0.0003 – 0.0021)

3.3 Active Carbon Flux from Euphausiid Respiration:

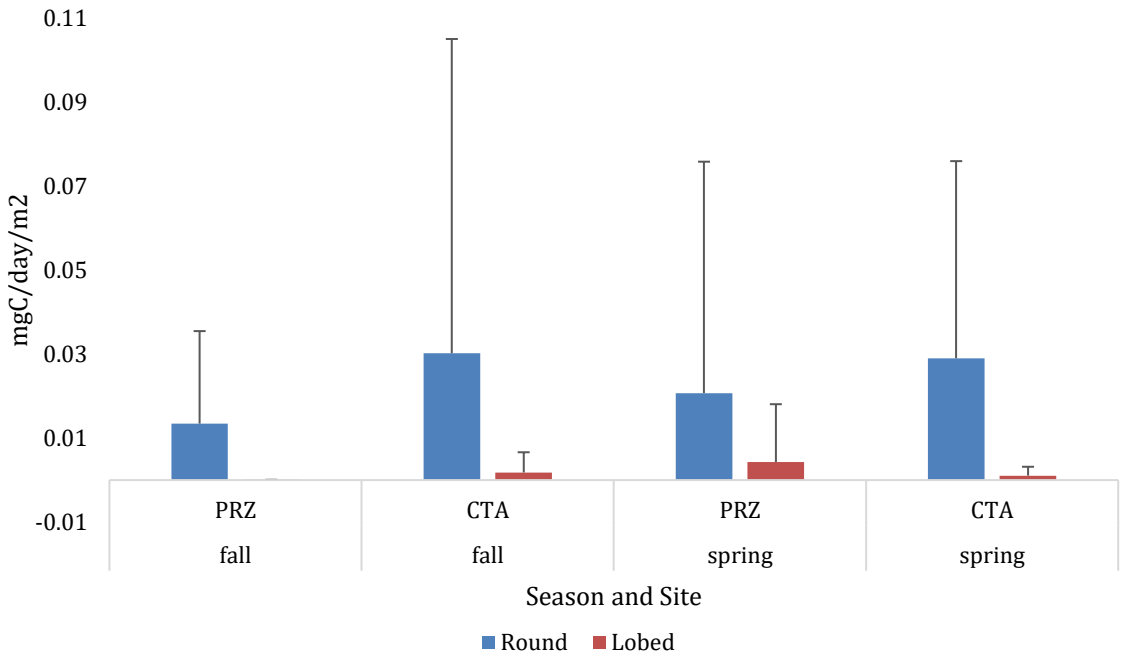


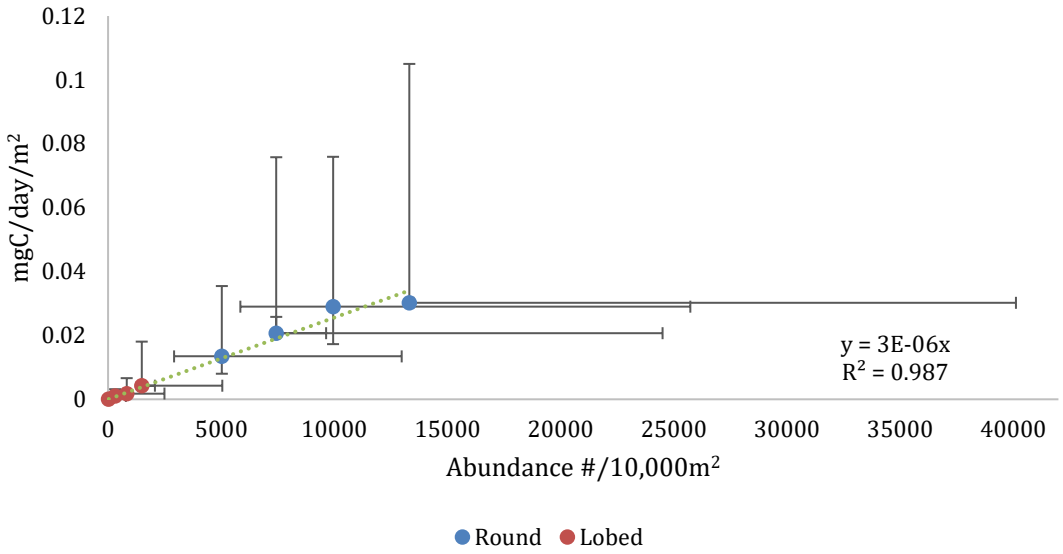
Figure 4. Respiratory carbon flux (mg of C/day/m^2) in the Fall and Spring, PRZ and CTA locations in the first 90m of the water column. Blue represents the mean amount of carbon Round-eyed euphausiid transport through respiration per day and red represents Lobed-eyed euphausiid. The black bars represent the upper range of respiratory flux driven by respiration and abundance ranges.

The respiratory flux varied by taxa most strongly and then by site and season (Fig. 4). There is much less Lobed-eyed euphausiid carbon flux from respiration as they have a much smaller migrating abundance and biomass (Table 1). For Round-eyed euphausiid, the most carbon flux came from the fall CTA samples and was higher in the CTA than the PRZ in both seasons, being lowest in the fall in the PRZ. There were large amounts of variability throughout all seasons and sites. The most variability occurred in the fall CTA,

however, that season and site also had the most respiratory flux. Total respiratory flux across all euphausiid ranged from about 0 to 0.1005 (mg C/day/m^2).

3.4 Relationship Between Abundance/Biomass and Respiratory Flux

a.)



b.)

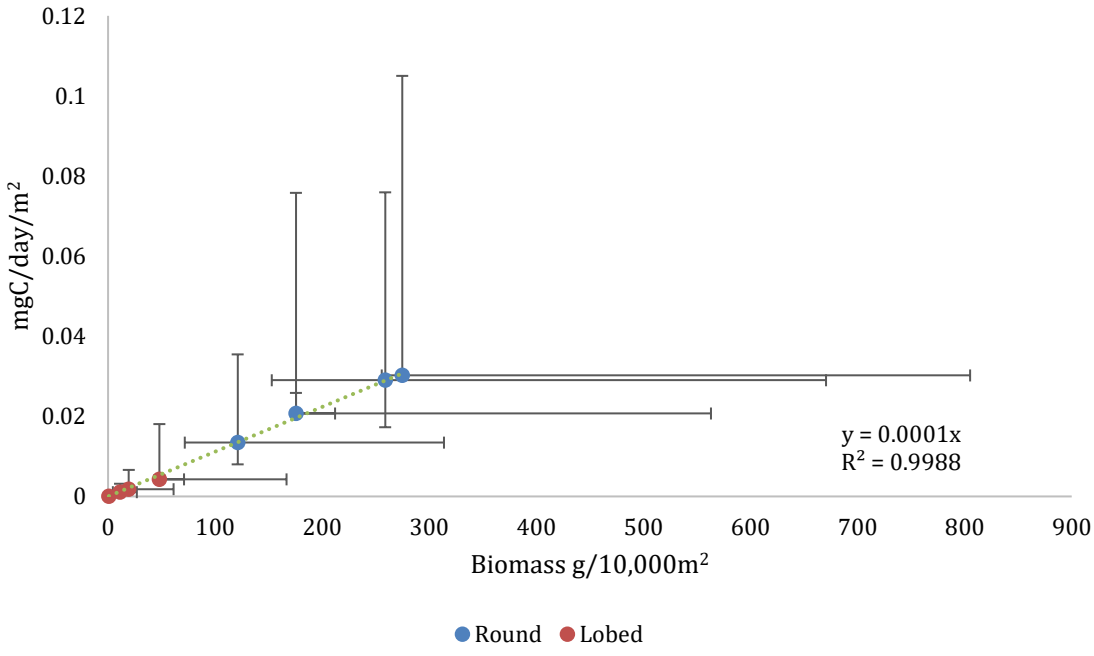


Figure 5: (a) The relationship between respiratory flux (mg of C per day per m^2) and migrating euphausiid abundance (#/10,000m^2). (b) The liner relationship between the biomass dry weight (g) of migrating euphausiid per 10,000 m^2 and the respiration in mg of C per day per m^2. The blue dots are Round-eyed euphausiid, and the orange dots are Lobed-eyed euphausiid.

There is a clear positive relationship between abundance and respiratory flux as expected. When there is a higher abundance of any breathing organism, the respiratory flux will be higher. For every migrating euphausiid there is about 3E-06 mg of C per day per m^2 being transported from the surface to below the photic zone. There were significantly more migrating Round-eyed euphausiid than Lobed-eyed euphausiid, largely because the Lobed-eyed euphausiid did not migrate into the surface euphotic zone. The dominating contributor to active carbon flux within euphausiid is biomass. The trend follows a direct positive linier relationship as well that shows for every gram of biomass there is approximately 0.0001 mg of C per day per m^2. This value is two magnitudes higher than the respiratory flux amount for every migrating euphausiid. The Round-eyed euphausiid make up the majority of the biomass with up to 275 g/10,000m^2 (dry weight). Lobed make up a smaller biomass up to 48 g/10,000m^2 (dry weight), however, this could be due to the is due to the amount of Lobed-eyed recorded, not necessarily their actual size. This pattern is also seen throughout nature, the more biomass a group has, the more total respiratory flux there will be.

4.0 DISCUSSION

4.1 Respiratory Flux Variation in NORI-D

The respiratory flux of euphausiid in NORI-D varied to some extent largely as a result of changes in abundances of migrating euphausiid. The Fall PRZ has substantially less respiration occurring than the other site and seasons due to its lower abundance and biomass (Fig. 3). Abundance and biomass are controlled by the amount of food available for euphausiids, so if the food supply is low then the abundance and biomass will be too. The net primary productivity (NPP) was recorded through satellite observations and model-based estimates during this study as well. During the fall, the PRZ was located in a very low NPP site while the CTA was in a slightly higher NPP site. Then in the spring, there was a strong seasonality difference and shows a large amount of NPP for both the PRZ and the CTA. Although there is large variation of NPP in the satellite data, there is not much variation in respiratory flux.

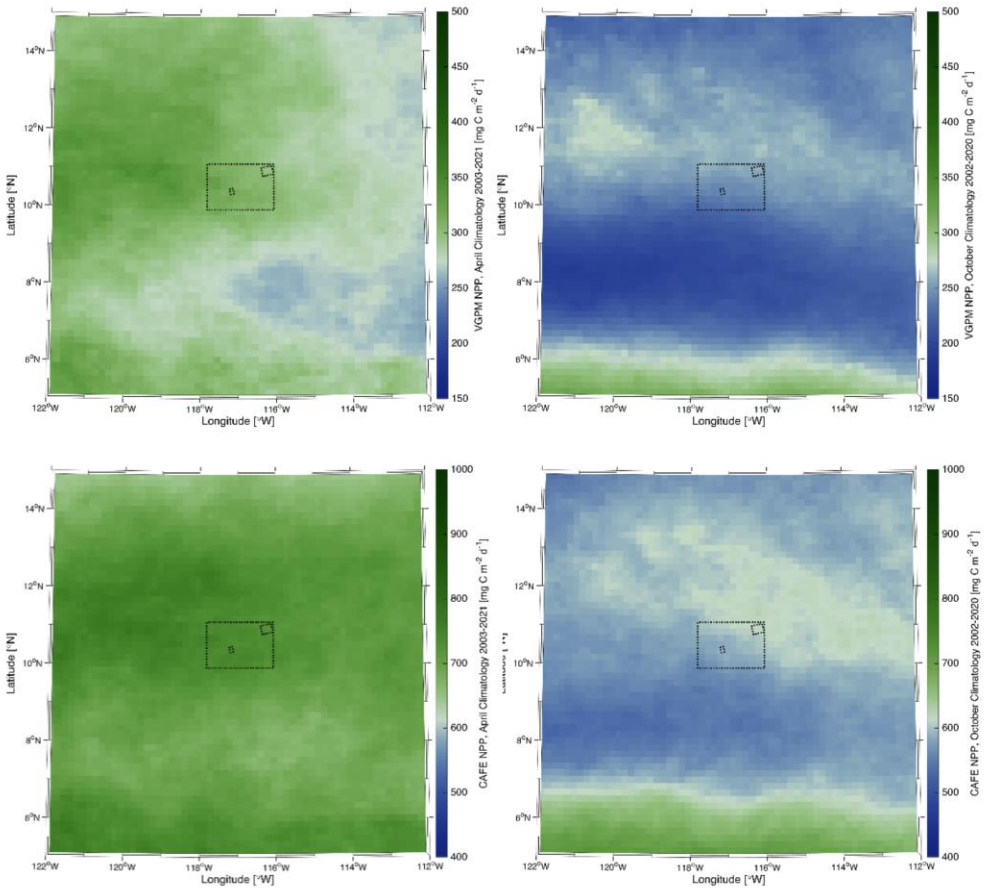


Figure 6: NORI-D climatology maps for spring (left) and fall (right) from the Final Report Characterization and Monitoring of the Water Column Ecosystem in the Eastern CCZ (NORI-D). Estimations were made from the VGPM (top) and CAFÉ (bottom) models of net primary productivity. Both approaches predicted strong seasonality in the study region as well as having magnitude variations (White et al., 2023).

On a larger scale, nutrient levels play a significant role in controlling the phytoplankton populations, therefore controlling micronekton populations. However, in this study in the fall, PRZ had the only major difference in low abundance, biomass, and carbon flux values. The fall CTA had respiration, abundance, and biomass values as high

as the spring. This could be due to an influx of nutrients available in the CTA location compared to the PRZ in both seasons.

4.2 Respiratory flux in NORI-D and Comparison to Other Respiratory Flux Measurements

The respiratory flux values presented here are similar to, but on the lower end of, the range presented in the few other studies around the world. For instance, Ariza et al., (2015) findings had a migratory flux of 0.06-0.19 (mg C/day/m^2) by euphausiid, whereas in this study our estimates were 0 to 0.1005 (mg C/day/m^2) (Fig. 4). The values conducted in this study were about 10-50% smaller than Ariza's findings. Differences in this study are most likely due to large differences in abundances and biomass of migrating euphausiid. The euphausiid migrating abundance and biomass in this study were much smaller than that reported in Ariza et al., (2015). Differences in biomass may be due to productivity differences. Along the Canary Islands, the island mass effect could play a role in the greater abundance. Typically, the Island mass effect leads to an increased abundance of organisms around islands from nutrient addition to the water from runoff and other physical processes. The CCZ is at the southern edge of the oligotrophic subtropical gyre meaning there could be less productivity compared to areas with the island mass effect. Another important difference between the present study and others is the net capture efficiencies. Nets frequently do not capture all micronektonic organisms because they are mobile and can avoid the net to some extent (Davison et al., 2013). The capture efficiency used in Ariza, 2015 is 80% for euphausiid derived from (Davison et al., 2013). Using this same capture efficiency rate, the respiratory fluxes

increase the present estimates to between 0-0.036 (mg C/day/m^2). Capture efficiency was not used in this study because further research is needed on capture efficiency within this specific geographic area.

4.3 Sources of Error, Respiration and Abundance

Respiratory flux is the product of individual respiration rates and the abundance of the individuals. Variability of respiratory flux comes from variability within abundance and individual respiration. Since abundance has a nearly linear relationship to respiratory flux (R^2>0.9) (Figure 5a) this indicates abundance has a strong influence on respiratory flux. Individual respiration variability comes from variability within oxygen availability, body mass, temperature, and depth. Within these calculations, temperature and depth have little variability in the first 90m of the water column and body mass have high variability. Some of the most important factors to consider while calculating respiratory flux are body mass, abundance, and biomass. Biomass has been investigated to be an obvious driver of active carbon flux through diurnal vertical migration (Takahashi et al., 2009, Steinberg and Landry, 2017). Variation within abundance and biomass alter the total respiratory flux greatly. There are also environmental error components that affect the driving factors of active carbon flux from respiration that need to be taken into account such as nutrient availability, net avoidance, oxygen content, water temperature, currents, metabolic suppression, etc.

4.4 OMZs and Flux

The carbon flux values in this study compared to the other studies presented support (Steinberg and Landry, 2017) predictions that active transport of respiratory CO_2 by euphausiid diel vertical migration may be reduced in an OMZ. Within these findings, respiration and carbon transport is reduced by up to an entire magnetude. This is not only due to oxygen content, but also lower migrating biomass to the surface.

Metabolic suppression is also a major factor in active flux measurements. Euphausiid feed at night near the surface, then migrate down during dawn and daytime so they won't be preyed upon. During migration to depth, they travel through the OMZ where respiration occurs. Due to the low oxygen concentrations, animals living in OMZs during the day often undergo metabolic suppression that could reduce the amount of active carbon flux from their respiration (Kim et al., 2010).

A metabolic suppression rate has been calculated before in Seibel et al., (2016). They found that the oxygen consumption rates of euphausiids decreased by 40-80% within OMZs. In the present study, however, metabolic suppression was not considered. If this suppression had been included, a large reduction in the calculated respiratory flux would be expected due to the decreased oxygen consumption rates. When considering the calculated carbon flux and the suppressed metabolic rate, there is a better understanding of the role micronekton and other vertical migrators play in the biological carbon pump within OMZs.

Carbon flux is made up of active flux and passive flux. A drifting sediment trap array conducted by others (Popp et al., 2023) within this study recorded the passive flux occurring at a depth of about 70m on the same sampling cruises. Sediment trap flux was

very high in the spring with ~ 320 mg C per m^2 per day falling to ~10 mg C per m^2 per day in the fall. This extreme difference was most likely due to the spring season being more productive than the fall. However, active flux continues deeper in the water column than passive flux does, and the sediment trap measurements were taken in shallow depths. Active flux is more significant deeper in the water column than passive flux (Stukel et al., 2023). Active flux by euphausiids is therefore around 0.13% to 0.35% of passive flux in the Fall and in the Spring when active flux was exceptionally high. By these numbers, active flux from euphausiid respiration is almost negligible to total carbon flux within this OMZ. This is not to say that there is no active flux occurring though.

The active carbon pump is still occurring in this OMZ mining region, but the contribution from euphausiid is very small compared to the passive pump. The numbers estimated could be higher due to poor net capture efficiency and underestimation of euphausiid abundance, but this is offset by metabolic suppression of individual respiration in this OMZ region. More importantly, euphausiids are only one of many vertically migrating organisms that contribute to active carbon flux within this area. There are currently more studies being done to quantify the amount of carbon flux from different organisms within this OMZ such as Cyclothone, Melamphaes, Scopelogadus, and other fish along with crustaceans and cephalopods (Assad et al., 2023). Larger, and more abundant animals likely play a large role in total active carbon flux. Zooplankton along with other micronekton and small fish respiration accounts for a major carbon flux around the globe (Saba et al., 2021, Hidaka et al., 2001, Steinberg and Landry, 2017, Hays, 2003).

4.5 Deep Sea Mining

These results enable an evaluation of the importance of potential deep-sea mining disruptions to the active biological carbon pump in the CCZ mining region. It has been suggested that if sediment plumes are released in the mid water column, they could introduce toxic metals like copper and cobalt, clog gills and respiratory mechanisms causing suffocation, cause buoyancy issues with gelatinous migrating animals, block visible light which might disrupt diurnal vertical migrating patterns, result in sediment ingestion, and noise disruption (Drazen et al., 2020). If large amounts of mortality occur within vertically migrating creatures, this may very well disrupt and slow the biological carbon pump. At least for euphausiid, their contribution to carbon flux is very small, so disruption of their behavior is not likely to affect carbon flux overall. However, future studies must focus on measuring the active flux of all organisms in this region to fully appreciate the potential impact of deep-sea mining on this part of the carbon cycle.

5.0 CONCLUSION

This study is the first to estimate active carbon flux of any animals group in the CCZ. Round-eyed and Lobed-eyed euphausiid were found to have a low active carbon flux, compared to the few other studies done, due to their low migrating abundance and biomass. This study represents the OMZs throughout the CCZ (4,500,000 km^2) and provides an important part of a baseline study conducted before mining begins. It has also helped us understand active carbon flux from euphausiid respiration within an OMZ and is rather low as previously hypothesized (Steinberg and Landry, 2017). The active biological carbon pump is a crucial part of earth's system to transport and store carbon. Though euphausiid respiratory flux is low in the NORI-D region, further research is needed to better understand the contribution of all micronekton and zooplankton to carbon transport. It is essential to protect these delicate areas and provide more baseline studies before mining begins and any major ecological changes occur.

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