The effects of deep sea mining on midwater organisms

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Abstract

The midwater ecosystem is the largest living space on earth and provides a variety of important ecosystem services. Deep sea mining (DSM), the exploitation and retrieval of valuable minerals found on the seafloor, will create sediment plumes that will impact the midwater ecosystem. There is currently an overwhelming lack of information regarding the exact effects that DSM will have on the midwater environment. This study intends to bridge that gap by first creating a methodology and proceeding to quantify the effects that sediment discharge, similar to the effluent that will be discharged in the midwaters by DSM operations, have on several taxa of midwater organisms collected via remotely operated vehicle (ROV). Replicated, controlled laboratory experiments are conducted on two species of midwater jellies, the shallower *Aurelia aurita* and the deep living narcomedusae*, Aegina sp.* as well as the fish *Melanostigma pammelas*. Sediment concentrations are maintained at three different dilutions intended to simulate the midwater discharge of mining effluent at increasing distances from the discharge pipe. A diverse range of stress responses are observed and quantified over the course of 48 hours for *Aurelia aurita* and *Melanostigma pammelas* and 96 hours for *Aegina sp.* The results of this experiment provides some of the first experimental data on the effects of deep-sea mining sediment plumes on midwater organisms and develops a base methodology which will be instrumental in future dose response experimentation. These experiments are crucial for creating scientifically valid policies and regulations regarding midwater discharge from deep sea mining activities.

Introduction

The mesopelagic, or midwater, defined as open ocean habitat between 200m and 4000m depths, is the largest living space on earth (Robison 2004, Sutton et al. 2017). It is an extremely stable environment as it is unaffected by wind-driven and surface currents. It was previously thought that the midwater was an expanse of empty ocean with little life but recent advancements have shown that the midwater has a thriving biotic community. This area accounts for the largest amount of biomass in the world (Robison 2004). It is home to a myriad of organisms, ranging from different species of jellies to fish with unique adaptations for survival in this dark, 3D environment.

The midwater is a critical ecosystem for a healthy planet, contributing in a variety of ways. Mesopelagic fish biomass is approximately one hundred times that of the annual fish catch (Irigoein 2014). This biomass supports a diversity of marine mammals and apex fish that dive in order to catch their prey. The carbon pump, the cycle in which the ocean sequesters carbon from the atmosphere, regulates the earth's temperature, ensuring that deep waters also act as the planet's largest carbon sink, absorbing 38,000 Pg of CO_2 a year (Long 2021).

Deep sea mining (DSM) is a relatively new exploit that focuses on the mining of valuable sediments found on the seabed. These sediments are typically metals that are considered valuable such as manganese, cobalt, or others that can be used in technology (Christensen 2019). The exact method of extraction varies on the mineral type that is being targeted (Drazen 2020). It typically involves the use of an extraction vehicle on the sea bed with a sediment transport tube that runs up to a surface support ship. After collection, the minerals are separated from the water and sediment and a slurry of water, sediment, and particulates is released into the midwater at an unspecified and unregulated depth (Drazen 2020).

Sediment plumes generated by DSM pose a significant threat to the midwater ecosystem. These animals live in an environment devoid of such sedimentation and it is unclear how or even if they will deal with it. Many gelatinous deep sea organisms utilize mucus in some capacity, including feeding; resuspended sediments can easily stick to the mucus net that many of these organisms create, either negatively impacting their ability to filter feed by clogging filtering apparatuses or diluting organic particles with inorganic sediment or decreasing their buoyancy (Drazen 2020). Jellies comprise a significant portion of the total biomass in this zone; they are an integral part of the food web as both predators and prey for larger organisms. Compromising the base of the food web could lead to drastic effects for the rest of the water column.

Understanding the full effects of deep sea mining on the midwater is a great challenge that will not be easily accomplished. However, in order to inform policies and regulations that are currently being drafted by the International Seabed Authority, attempts towards understanding the effects of this new endeavor on the midwater ecosystem must be made. The purpose of this study is to create a baseline understanding of some of the possible effects that sediment plumes created by deep sea mining will have on midwater animals through controlled laboratory experiments.

Methods

General Design

The experimental tanks were constructed by using acrylic cylinders secured to a 64L funnel, creating a total capacity of 8L (Fig. 1). Inside the funnel is a mesh intended to keep any organisms from being sucked downward while the motors are running. The funnel was then connected to an adapter that was attached to a three-way valve. This valve allows the water to

flow from the funnel into the Flodos NF10 KPDC 12V 3.7W motor and back into the tank. The pumps recirculated water and sediments through the tank, maintaining a consistent concentration throughout the trial.

All tanks were connected to a timer and power supply. The timer ran on a one minute cycle: 6 seconds on, 54 seconds off. This was intended to disturb the water enough for the sediment to remain suspended while attempting to mitigate any stress on the animal induced by the current.

Once the experimental tanks were attached to the motors, the flow rate was measured and recorded at 1.69mL/s. To test water flow in the tank, phosphorescent dye was injected into the three way valve and monitored to observe how the water mixed together. Trial runs were done by placing the output tube both at the surface of the tank and aiming the output from the bottom of the tank. The flow of water was determined to be best coming from under the mesh towards the top of the tank. A hard plastic tube was bent into a 'J' shape and fixed onto the side of each tank.

Sediments

The sediment used for this experiment was collected from the Monterey Bay seafloor at a depth of 538m. Sediments released from DSM operations are typically within the size range of 10-25µm (Munoz-Royo et al. 2021). For the purpose of this study, particles $\leq$ 63µm were used (Munoz-Royo et al. 2021).

The sediments collected were rinsed with reverse osmosis (RO) water through two different stacked sieves with mesh sizes of 300µm and 64µm. RO water was used to remove salt from the sediments so that when sediments were added to the experimental tanks, there would be no change in salinity in the tanks. All sediment that passed through the 64µm sieve was preserved for use in the experiments. The sieves were placed over a bucket to collect the water

and sediment. Sieving was done slowly over multiple rounds to gather as much sediment as possible.

After sieving, the sediment solution was allowed to settle out. The clear top water was siphoned out and the sediment was transferred to an Isotemp oven at 50℃ for the duration of three days to dry completely. At the end of the three days, the resulting clay block was ground into a powder using a mortar and pestle.

The concentration of sediment in each tank was a dilution factor of the initial 8kg/m^3 found at the CCFZ site (Munoz-Royo et al. 2021). Dilutions by a factor of 10, 100, and 1000 were chosen for the experiment setup, representing an increasing distance away from the effluent release site. (Table 1).

Observations during the Experimental Trials

Observational trials were conducted in an ambient temperature lab as well as a cold room (~6℃). Each trial had a control, x1000 dilution, x100 dilution, and x10 dilution tank. The organisms were allowed to acclimate for 2-4 hours in their tanks prior to the start of the experiment. After acclimation, a baseline observation was taken for each metric designated for each different species (Table 2). Observations were then recorded for two minutes every hour possible. Benchmark observations were made at hours 24 and 48.

Microscopy and Preservation

After the trials concluded, the organisms were humanely euthanized and preserved for further study. The *Aegina sp.* were placed into a small glass container filled with 70% ethanol to humanely dispatch them. They were then placed under the dissecting microscope to examine for damage. All organisms gathered on the Western Flyer study were preserved using a similar method. The *Aurelia aurita* were taken directly from their tanks using a coffin, an acrylic water

tight container, and observed under the microscope prior to being preserved in formaldehyde. After initial preservation, *A. aurita* were then transferred into a container of 70% ethanol. Due to its status as a vertebrate, *Melanostigma pammelas* were taken from the tanks and snipped at the base of the notochord. The head was preserved in formaldehyde and later transferred to 70% ethanol. The body was frozen in liquid nitrogen for future cortisol tissue analysis.

Western Flyer Study

The opportunity to conduct immediate research was presented in the form of a research cruise on the R/V Western Flyer. Midwater trawls were conducted and pairs of organisms in prime condition were collected from the net on two occasions: July 25th and July 27th. The organisms collected included *Lanceola*, *Eusergestes similis*, *Pasipheia*, *Pleurobrachia*, *Gennadas*, and *Aegina*. Once collected, each individual for each pair was placed into a 500mL open container and one was exposed to 0.8g/L (0.4g/500mL) of pre-weighed benthic sediments. Observations were taken before adding sediment (baseline), zero hour (the moment of adding sediment), 12 hours, 24 hours, 36 hours, and the final 48 hours into the trial. The metrics observed were similar to those measured in the laboratory observations (Table 2).

Results

Methodology

Overall, the design of this experiment worked. The acrylic cylinder and funnel provided ample volume to conduct the trials. The connection from the funnel to the motor posed somewhat of a problem as the amount of adapters created several areas that resulted in sediment accumulation and clogging. Throughout the trials, there were only two instances of clogging in the adapters and both were dealt with easily.

The use of an in-line pump has several benefits. The in-line system allows the sediment concentration to remain constant and resuspended during the duration of the trials. The largest drawback was from the constant clogging and overall weakening of the motors rather than the system setup. The 12V 3.7W motor had too narrow of an inner diameter which created a clogging issue. The motors had to be flushed out several times both during and after the experiments. The stream of water from the motors was weak and allowed for partial settling of the sediment towards the middle and bottom of the tank.

After several clogging events, it was noted that the drying process created fine clumps of clay that required more attention. Though the clay was grinded up after drying, the small inner diameter of the motors and the clumps that remained proved to be problematic. By dry-sieving the ground sediment a second time before use, internal clogging was reduced. The second round of sieving should be done immediately after the initial grounding. Coupled with a larger motor, clogging events could be reduced substantially.

Preliminary findings

Aurelia aurita is a proxy species for deep sea gelatinous medusa. Observations were made regarding their pulse rate, physical damage, and height in the water column (Table 3). High variability in pulse rate was observed in both the 0.0064g and 6.4g tanks (Fig. 2a). Tissue damage was noted to increase over time in all tanks (Fig. 2b). The highest tissue damage and most variability was seen in the 6.4g tank.

The *Melanostigma pammelas* did not curl into an o-shape as expected. Their height in the water column was variable over all concentrations and time stamps. Observation for ventilation rates was not started immediately so there are large gaps in the data. It was noted that the fish exposed to high concentrations had a more rhythmic and frequent ventilation rate (Table 4).

Turbidity in the 6.4g tank presented a problem when trying to take observations during the trials. Ventilation rates for the 6.4g tank were unable to be recorded due to the murkiness of the water.

Aegina sp. showed considerable damage. Similar metrics were used to observe *Aegina* and *A. aurita* (Table 5). In the experimental tanks, the tentacles of the *Aegina* detached from the medusa. The medusa itself was filled with sediment from the 6.4g tank and a large hole could be seen at the top center of the bell. Small spots of damage could be seen around the body of the aegina.

Data collected from the Western Flyer expedition was unable to be processed at the time of this paper. It is expected that all gelatinous organisms would see some level of physical damage. The arthropods collected would be more resilient to sediment exposure due to their hard exoskeleton.

Discussion

The new interest in deep sea mining is coupled with a lack of knowledge about the effects of DSM in the midwater. This lack of knowledge presents a great danger to the integrity of the midwater ecosystem. The International Seabed Authority is currently endeavoring to create a universal DSM mining code in order to regulate this new industry (Drazen et al., 2020).

There have been no prior studies conducted on DSM and the midwater, making this a novel study. While studies have been conducted on DSM and the benthic environment, the ISA has no data to inform guidelines on how to protect the midwater from sediment plumes or other effects of DSM. The need for this knowledge from the ISA is becoming an urgent matter and further research must be done to protect the midwater ecosystem.

The overall purpose of this study was to create a methodology for observing the impacts of DSM on midwater organisms. Future experiments must take into account clogging by sediment and use a stronger in-line pump system and/or one with a larger diameter. This would prevent clogging and potentially keep the sediment more evenly resuspended. Trials should start and stop on a more rigid schedule, allowing for easy comparison and consistent results. Any gelatinous organisms should be in a pseudo-kreisel for experimentation. This would eliminate damage to the medusa from sharp corners and the mesh at the mouth of the funnel. Many of the organisms that were used for this experiment were used for their convenience. By targeting specific organisms that have known stress responses, the metrics used to test the effects of DSM would be more revealing. Additionally, a larger sample size, both experimental and control, would allow for more reliable findings.

The basic methodology described above should serve as a basis for future research into this field. Deep-sea mining will most likely continue to grow as an industry and needs to be regulated properly. This first step allows for more advanced research to start with a simple understanding of what type of design is functional and what equipment is necessary.

Preliminary findings support the hypothesis that resuspended sediment in the water column will have a negative effect on midwater organisms, particularly gelatinous bodied organisms. More trials with deep sea gelatinous organisms would provide a better understanding about the extent of these effects.

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Tables and Figures

Table 1: Dilution factors chosen for the trials and concentrations for each

Dilution Factor	Concentration (g/L)	Total Concentration in Experimental Tanks (g)
x0 (control)	0	0
x1000	0.008	0.064
x100	0.08	0.64
x10	0.8	6.4

Table 2: The observations and units recorded for each species present in the trials

Species	Observations	Measurements
Aurelia aurita	Pulse Rate	pulse/minute
	Tissue Damage	0- no visible tissue damage, 1- small dots of damage, 2- consistent spots of damage, 3- tears or perforations
	Height in water column	cm
	Mucus Secretion	0- no mucus, 1- small trails of mucus, 2- large nets/trains of mucus, 3- mucus highly visible / catching sediment
	Other	
Melanostigma pammelas	O-Shape	yes / no
	Height in water column	cm
	Ventilation rate	(gill pulse/min)
Aegina sp.	Pulse rate	pulse/minute
	Height in water column	cm
	Tissue Damage	0- no visible tissue damage, 1- small dots of damage, 2- consistent spots of damage, 3- tears or perforations
	Bioluminescence	yes, no, sporadic, consistent
Other	Height in water column	cm
	Visible Damage	0- no visible tissue damage, 1- small dots of damage, 2- consistent spots of damage, 3- tears or perforations
	Other	Notes on behavior

Table 3: Observations taken on *Aurelia aurita* during the trial

Concentration (g)	Time	Pulse Rate (pulse/min)	Height in Water Column (cm)	Tissue Damage (0-3)	Mucus Secretion (0-3)
0 - control	Baseline	1	5-10	0	0
	0	1	0-5	0	0
	24	6	10-15	1	0
	48	4	15-20	1	0
0.064	Baseline	29	0-5	1	0
	0	18	0	1	0
	24	15	0-5	1	0
	48	26	15-20	1	0
0.64	Baseline	6	0-5	0	0
	0	0	>20	0	0
	24	5	>20	2	1
	48	6	>20	1	1
6.4	Baseline	8	0-5	0	0
	0	*Aurelia* is not visible			
	24	22	0	Unable to clearly see any damage	1
	48	0	0-5	3	Unable to clearly see mucus

Table 4: Observations made on *Melanostigma pammelas* during the trial

Concentration (g)	Time	O-Shape	Height in Water Column (cm)	Ventilation Rate
0 - control	Baseline	no	5-10	N/A
	0	no	15-20	N/A
	24	no	0-5	15
	48	no	0	5
0.64	Baseline	no	10-20	N/A
	0	no	15-20	N/A
	24	no	0	13
	48	no	0	15
6.4	Baseline	no	5-10	N/A
	0	no	0-5	N/A
	24	unable to see melanostigma due to cloudiness of the water		
	48			

Table 5: Observations made on *Aegina sp.* during the trials. There were two control tanks and one experimental tank

Concentration (g)	Time	Pulse Rate (pulse/min)	Height in Water Column (cm)	Tissue Damage (0-3)
Control 1	0	37	0-1	0
	1	34	1	0
	2	27	0	0
	67	*Aegina* not visible		
Control 2	0	26	2-3	0
	1	32	0	0
	2	39	1	0
	67	0	0	0
0.64 g	0	0	0	0
	1	6	0	0
	2	0	0	0
	67	0	0	2

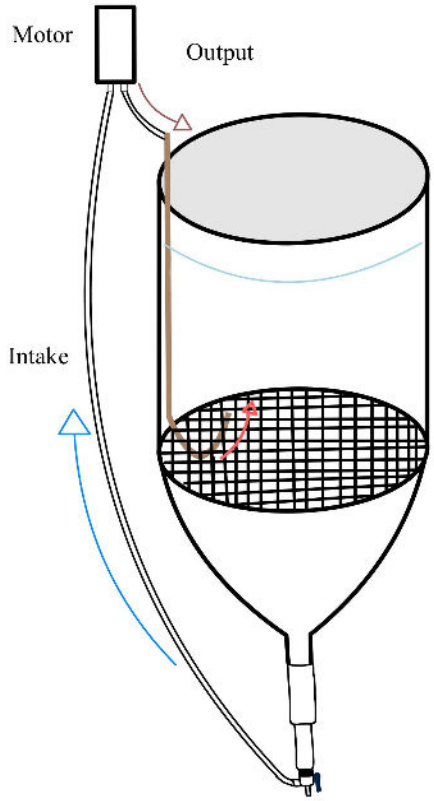
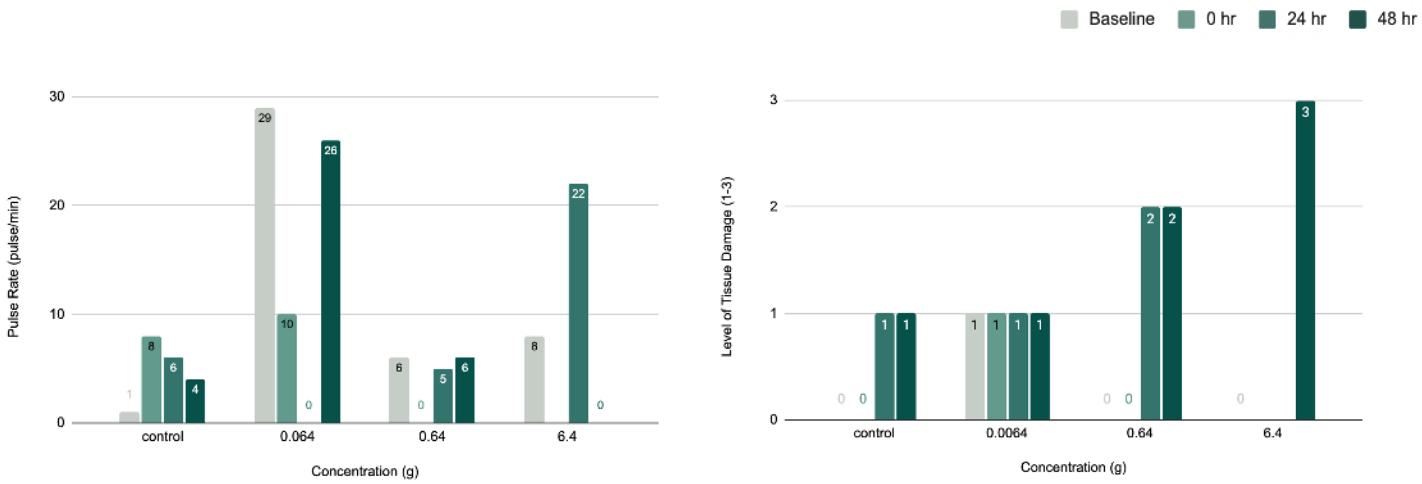


Figure 1: A simple schematic showing the general design of the tanks constructed for the trials



a) b)

Figure 2: *a)* Graph depicting the level of tissue damage of *Aurelia* in each concentration over time *b)* Pulse rate per minute of *Aurelia* in each concentration over the trial duration