



Project Knowledge Product: Mitigating the Damage to Arctic Copepods from Surface Oil Spills: When to Apply Dispersants

A Project of the Arctic Domain Awareness Center (ADAC)

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1. Summary

The use of dispersants to mitigate damage from oil spills in Arctic environments requires an understanding of the positive and negative consequences for offshore and coastal ecosystems. In this study we investigated the impact of treating a surface oil spill with dispersants. Translocating oil from the surface of the ocean into deep water could negatively impact aquatic organisms that reside below the surface. To investigate the effect of oil and dispersants on the base of the Arctic food web, we used an ecologically important marine animal, called copepods (*Calanus sp.*), that play an essential role in bridging primary production (algae) with higher trophic levels such as fish and marine mammals. The important characteristics of these small (2-5 mm) aquatic crustaceans comes from their ability to store lipids (up to 80% of their biomass) and to actively swim 100s of meters vertically in the water column. Previous work investigating the impact of oil and dispersants on these animals have ignored the behavioral capabilities.

To address these knowledge gaps, we constructed a 2m tall “copepod” tower equipped with 5 distinct sampling ports and 4 video cameras such that we could simultaneously monitor the dynamics of the hydrocarbon and the copepod’s behavioral response. These two variables are very important in the environment, but are often not considered laboratory experiments. We used this experimental system to directly test the effects of non-dispersed and dispersed oil on copepods and by regionally cooling the lower portion of the tower, we quantified the effects in an unstratified as well as in a stratified water column. *Calanus finmarchicus* was used as a model *Calanus* copepod.

The following conclusions were drawn from the results of these experiments:

- Copepods react rapidly (within a few hours) to sublethal oil concentrations. The quickly moved deeper to avoid the dissolving hydrocarbons.
- The addition of dispersants transported dispersed oil down the water column, thereby increasing the hydrocarbon concentration and the exposure of copepods to oil.
- Copepods exposed to oil high concentrations of oil (sublethal) become temporarily incapacitated, causing them to sink into deeper water.
- Some of the incapacitated copepods recover when they sink into clean water.
- The interaction of oil droplets and copepods led to adhesion or ingestion of oil droplets.

The results imply that the application of dispersants have the following seasonal consequences for Arctic calanus copepods:

- There is a high risk that dispersed oil would negatively affect copepods in their early development stages (early spring).

- During late spring, there is a moderate risk that the application of dispersants will negatively affect copepods. Many copepods could avoid the oil by rapidly swimming deeper. The copepods that are exposed to high concentrations of oil may sink with the potential to recover in deeper, clean water.
- During summer, the application of dispersants represents a relatively low risk to the copepod population, since water column stratification can act as a barrier between dispersed oil and copepods.
- During the Fall copepods descend to great depths (300-500m) and would be unaffected by the oil spill itself or the added dispersant.

2. Overview: Motivation and Goals of this Project

While dispersants are a highly efficient and proven tool for oil spill response in sub-Arctic and Arctic environments (Lewis and Prince 2018), there is concern about negative impacts on organisms at the base of the marine food web. This study focused on lipid rich copepods that serve as consumers of primary production (phytoplankton) and in turn serve as food for larval fish, seabirds and charismatic megafauna including whales (Gardiner et al. 2013; Cohen et al. 2014; Almeda et al. 2014a, c, 2016; Nordtug et al. 2015a; Hansen et al. 2015, 2016). Previous (laboratory) experiments measured the toxicity of oil on copepods by placing them in a homogenous solution of oil and dispersants. However, such an experimental design does not allow for the vertical movement of copepods and may over (or under) estimate the potential toxicity for the copepods. We propose to investigate the impacts of dispersed oil on vertically migrating copepods. The main hypothesis of this project is that, in the ocean, calanoid copepods can spatially separate themselves from surface oil spills and may not be present at all during portions of their developmental cycle (Figure 2.1). As a result, copepods may be exposed to much lower concentrations of oil than found at the surface and for much less time than has been assumed based on previous laboratory studies.

This report describes the investigation of this migration behavior in a novel mesocosm system, which allowed for the determination of effects of dispersants and oil in a more environmentally-relevant condition than the traditionally-used systems using water-accommodated fraction (WAF) or chemically enhanced WAF (CEWAF; i.e., oil with dispersants). Experiments were performed using 2 meter vertical mesocosms in which copepods can move vertically in the water column. Furthermore, this system allowed for investigating the effects of water column stratification on the distribution of oil and copepods. As a model copepod species, *Calanus finmarchicus* was used to test our hypothesis. The presented results fill critical knowledge gaps about the toxicity of oil and dispersants on *Calanus spp.* copepods, which are essential organisms for the Arctic food web as well as for commercial fishery.

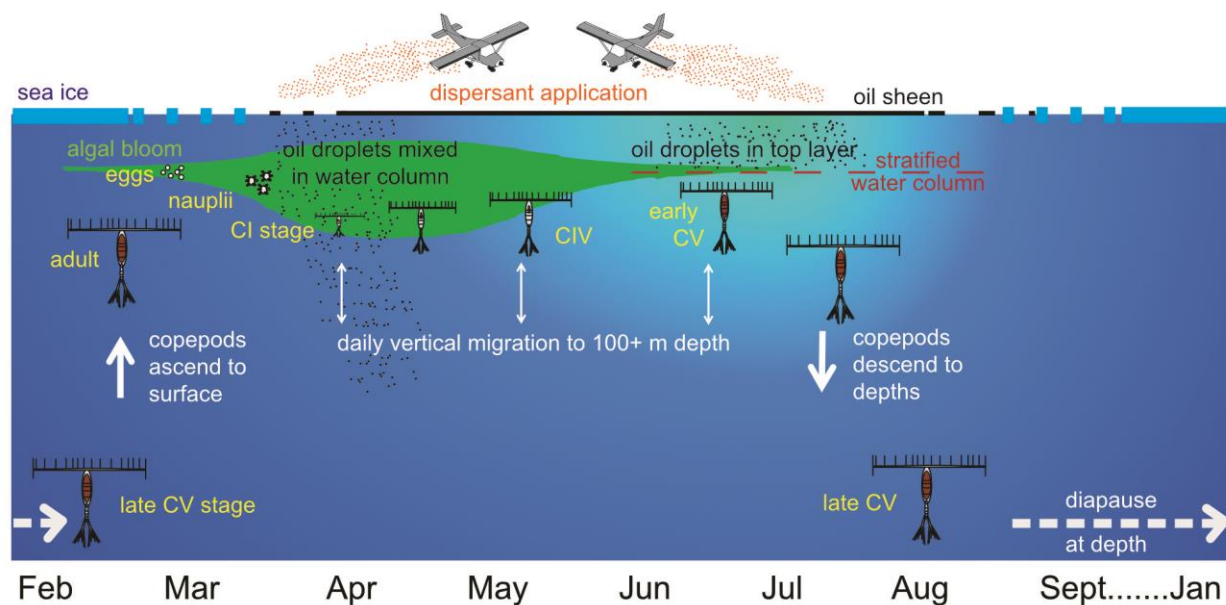


Figure 2.1: Life cycle of the sub-Arctic copepods *C. finmarchicus*. During the Spring and early Summer, copepods ascend at night to feed on the algal bloom, and then migrate down to 100m+ during the day. During the late summer until early spring, they reside at great depths. Other *Calanus* species have a similar cycle. The extent to which copepods are affected by dispersant application depends on when in the season the dispersants are applied, and whether copepods can actively avoid hydrocarbons.

3. Background and State of Knowledge

3.1. Risks of and Response to Arctic Oil Spills

There is a Growing Risk of Oil Spills in the Arctic Region. The rising demand for petroleum products has led to an increase in Arctic oil drilling as well as in shipping traffic within the Arctic waterways. Over the past 50 years, oil spills have become a major environmental concern (Kennish 1996; National Research Council and Committee on Oil in the Sea: Inputs, Fates, and Effects 2003; Dalsøren et al. 2007). With the decreased ice coverage in the Arctic and sub-Arctic, there has been a substantial increase in trans-Arctic shipping and renewed interest in Arctic oil and gas exploration. This poses a growing risk for oil pollution in the Bering and Beaufort Sea (National Research Council of the National Academies 2014) and raises concern about its impact on Arctic food webs. The opening of passage through the Arctic Ocean has increased interest in the Northern Sea Route and the Northwest Passage. Transits through the Bering Strait are already growing from 220 transits in 2008 to roughly 500 per year in recent years. This increased shipping brings the inherent risk of oil spills. This risk comes not just from oil tankers, but from the heavy fuel oil that all ships carry. In addition to increased shipping, Arctic oil exploration and drilling is expected to increase. Current estimates suggest that 30 billion barrels of undiscovered oil lie in the U.S. Arctic (Gautier et al. 2009). While oil exploration in the Chukchi and the Beaufort Seas came to a temporary stop in 2016, the U.S. Congress recently opened Area 1002 in the Arctic National Wildlife Refuge (ANWR) for oil exploration. Extracted oil from these sites would be transported through the trans-Alaskan pipeline to Port Valdez in the Gulf of Alaska, where it is loaded onto tanker ships and shipped for refining.

Dispersants as a Response Options to Arctic Oil Spills. Dispersants are an established and effective tool in oil spill response (Lessard and DeMarco 2000; Chapman et al. 2007; Li et al. 2009a, b). A recent comprehensive literature review argued that dispersants are especially useful in the Arctic since they can be deployed by airplanes in remote locations (Lewis and Prince 2018). By breaking up oil into droplets (< 100 µm), dispersants fulfill two primary functions: they increase the surface area of the oil which enhances the biodegradation rate, and they displace oil from the water surface and dilute it into the water column. New types of dispersants (e.g. Corexit) are considered less toxic than crude oil alone (National Research Council 2005; Judson et al. 2010; Nwaizuzu et al. 2015), and have been suggested to have minimal effects on marine life (Lessard and DeMarco 2000; Lewis and Prince 2018). However, recent studies of Corexit and chemically dispersed crude oil provide evidence of its toxicity for *Calanus spp.* copepods and other planktonic species (Gardiner et al. 2013; Cohen et al. 2014; Almeda et al. 2014a, c, 2016; Nordtug et al. 2015a; Hansen et al. 2015, 2016).

3.2. Biology of *Calanus* spp.

***Calanus* spp. Are Essential Arctic Species:** Copepods (0.5-5 mm) are the most numerically abundant multicellular zooplankton in the water column. They are a primary consumer of microalgae, and are a major food source for young fish and krill, which also serve as prey for higher trophic levels. Lipid-rich copepods of the *Calanus* species are a critical foundation species that provides an energy-rich food source for higher trophic level organisms in boreal and high latitude regions (Melle et al. 2014; Runge et al. 2016). These copepods serve as a primary source of energy to consumers such as herring (*Clupea harengus*), mackerel (*Scomber scombrus*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes* spp.). These species are in turn prey for top predators including groundfish, tuna (*Thunnus thynnus*), marine mammals, and many seabirds (e.g., Saetre and Skjoldal 2004; Baumgartner and Fratantoni 2008; Johnson et al. 2011; Trenkel et al. 2014). Copepods often dominate gut contents of wild-caught larval fishes (Gregory Lough and Mountain 1996; Østergaard et al. 2005; Carassou et al. 2009; Llopiz 2013). Since *Calanus* spp. play such a crucial role in sub-Arctic and Arctic ecosystems, oil-related impacts would likely have important consequences for coastal and shelf ecosystems of the North Pacific, the North Atlantic and the Arctic Ocean (Figure 3.2.1).

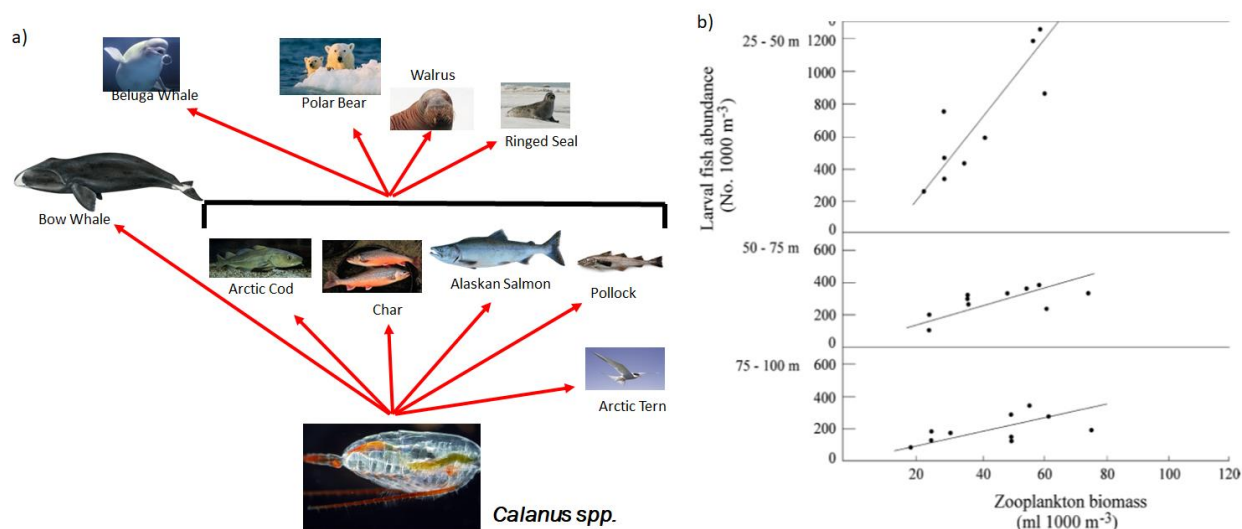


Figure 3.2.1. Diagram depicting a) the role of lipid-rich *Calanus* spp. in marine food-webs. b) Relationship between zooplankton biomass and fish recruitment in marine fisheries (adapted from Sanvicente-Anorve 2006)

Though specifics and phenology vary by species, copepods in the genus *Calanus* share a general life history pattern (Figure 3.2.2). Adult females release eggs in the spring, preceding the annual vernal phytoplankton bloom. Eggs hatch in the surface waters and development is largely dependent on water temperature. The organisms undergo development through six naupliar stages at shallow depths and four to six copepodite in the waters between 0 and 100 meters (Mauchline 1998; Sanvicente-Añorve et al. 2006). During these later stages, copepods demonstrate a diel vertical migration behavior, in which they migrate to 100 m during daylight hours to avoid predation and move to shallower depths at night to feed on phytoplankton (Mauchline, 1998). Later stages of copepodites will accumulate lipid sacs. Depending on species, a proportion of the population will complete an entire life cycle within the surface waters, and may have multiple generations within a season (Mauchline, 1998). For *Calanus* species it is also common for late season CV stages to sink to deep water and enter a diapause nonfeeding phase for the winter months, accompanied with a depressed metabolic rate. These lipid stores are used for winter survival and production of eggs at the start of the next season (Mauchline, 1998). For *Calanus* with longer generation times, this cycle can be extended multiple years.

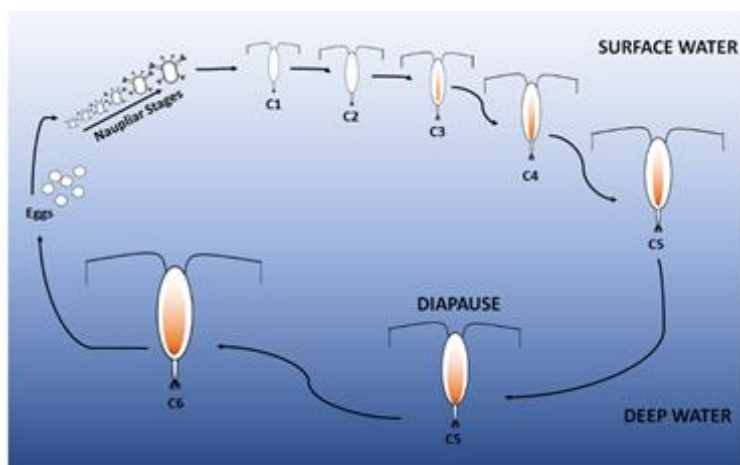


Figure 3.2.2. General life history of *Calanus* spp, from egg, through six naupliar stages, and 6 copepodite stages. Orange hue inside the body of the organism represents growing lipid reserves.

A handful of *Calanus* species are the major players in Arctic and sub-Arctic ecosystems (Berge et al. 2012): *C. finmarchicus* and *C. marshallae* are sibling species; *C. finmarchicus* is restricted to the North Atlantic Ocean and *C. marshallae* to the North Pacific Ocean and the Bering Sea. These latter two species are closely related and were identified as the same species until 1974 (Frost 1974). Another species, *C. glacialis*, is slightly larger and resides primarily in the Arctic but is found in sub-arctic regions in early Spring. Finally, *C. hyperboreus* occupies the high Arctic and is the largest of all the *Calanus* species with a life cycle (egg to adult) that spans several years (Hirche 1997).

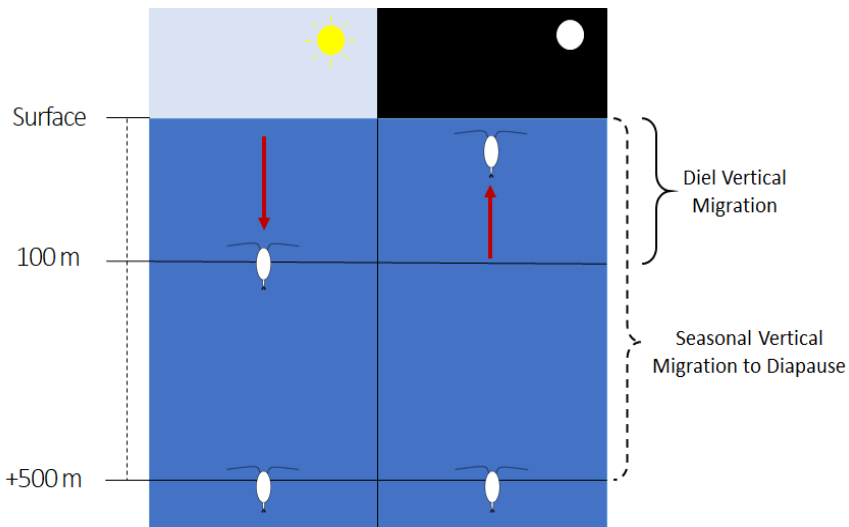


Figure 3.2.3. Vertical movement of *Calanus* spp. within the water column during day/night and over a seasonal cycle. During productive seasons (spring-fall) copepods migrate daily to occupy the upper water column at night to feed and then descend down to 100+ meters at night to avoid visual predation. During winter months, a large proportion of the *Calanus* population will sink to over 300 meters, become stationary and enter a diapause state until the next season.

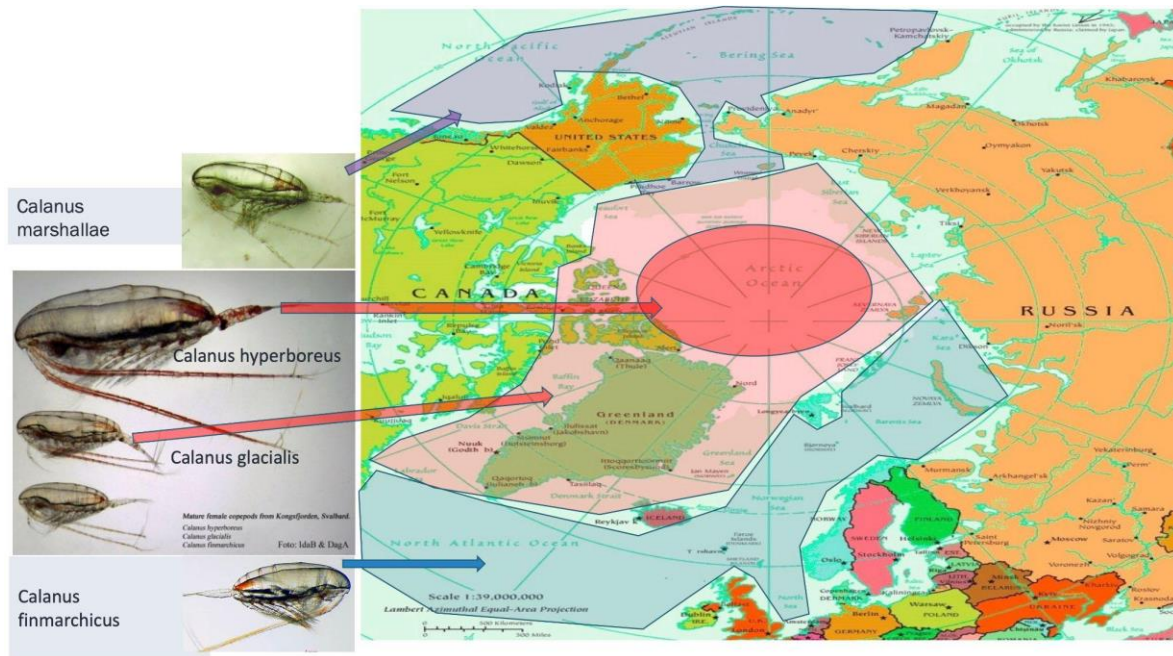


Figure 3.2.4. General ranges for arctic and subarctic *Calanus* species.

For this project, we investigated *C. finmarchicus* as a model *Calanus* species to test the hypothesis that the copepod's ability to move vertically in the water column will minimize their interaction with surface oil spills (Figure 3.2.5). *C. finmarchicus* is a good model species because (i) it is closely related to the other Arctic *Calanus* species (Berge et al. 2012), and (ii) because the toxicity of oil and dispersants to this species has been reported in a number of studies (Hansen et al. 2008a, 2011a, 2012a, 2013a; Jensen et al. 2008a, 2016a; Hjorth and Nielsen 2011a; Olsen et al. 2013a; Tarrant et al. 2014; Nordtug et al. 2015a; Jager et al. 2017a), which will facilitate the interpretation and comparison of our results to these published WAF- or CEWAF-based studies.



Figure 3.2.5. Image of *Calanus finmarchicus* with scale bar for relative size.

3.3. Effects of Oil on Copepods

Among the biological components of marine ecosystems, planktonic organisms are particularly vulnerable to crude oil pollution (Walsh 1978; Graham et al. 2010). More than 90 percent of the biological processes in the ocean are due to planktonic organisms (Hays et al. 2005). Therefore, any attempt to assess the ecological impact of oil spills in marine environments requires a good understanding of the effects of crude oil pollution on planktonic communities, as well as a clear understanding of the organisms natural vertical distribution pattern and their behavioral response to chemical pollutants.

The impact of oil hydrocarbons on copepods has been studied in literature (Table 3.3.1). Most crude oil toxicological studies on plankton have been conducted using water-accommodated fractions (WAF) of crude oil individual dissolved petroleum hydrocarbons (Barata et al. 2005; Calbet et al. 2007; Saiz et al. 2009; Echeveste et al. 2010; Jiang et al. 2010, 2012). However, petroleum is present in the water column in both dissolved and particulate forms (i.e. crude oil droplets) after a crude oil spill in the natural environment. Plumes of small crude oil droplets generated by wind and waves or by treatment with chemical dispersants (Lichtenthaler and Daling 1985; Delvigne and Sweeney 1988; Mukherjee and Wrenn 2009) are frequently observed after crude oil spills. These dispersed oil droplets can be transported deeper in the water column and serve as a reservoir of dissolved oil hydrocarbons that can affect copepods. Furthermore, these droplets can be in the same size spectra of the food for many zooplankton species, and there is evidence that they ingest crude oil droplets suspended in the water column (Conover 1971; Mackie et al. 1978; Lee et al. 2012).

Table 3.3.1. Overview of select literature investigating toxic effect of petroleum and PAHs on *Calanus* copepods.

#	Reference	Copepod sp.	Exposure	Oil / Exposure	Endpoint	Comment
1	Duesterhoj et al. 2002	<i>C. finmarchicus</i> <i>C. glacialis</i> <i>C. hyperboreus</i> <i>C. marshallae</i> <i>A. tonsa</i> Other species	Soluble / WAF CEWAF mechan. disp. pure PAH	weathered ANS ANS Flu, Phe, Pyr Pyr Pyr Naph North Sea oil Pyr North Sea oil North Sea oil North Sea oil marine diesel Pyr fresh ANS Light LA sweet Phe, Pyr, BAP North Sea oil North Sea oil North Sea oil mx. PAH/phenol C2-Naph Pyr Pyr and C12 Pyr Pyr North Sea oil Pyr Field exposed North Sea, 2 other	LC50 Egg Fecal P Grazing Hatch TPH Other	Photoenhanced toxicity; generator column Also: <i>Pseudocalanus</i> spp. and other species Also: Photoenhanced toxicity Endpoint: grazing and reproduction Copepod: <i>Microsetella</i> spp Endpoint: gene expression Endpoint: gene expression Also: effect of warmer temp Comparison lipid-rich vs lipid-poor Comparing mechanic. and chem. dispersion Exposure and depuration period Other endpoint: gene expression Also measured bioaccumulation Soluble PAHs correlate with toxicity Oil ingestion. Also: <i>T. turbinata</i> and <i>P. crassirostris</i> Bioaccumulation w/ and w/o dispersed oil Accumulation of oil droplets; effect of dispersant Recovery after mech. disp. (but not chem. disp) oil exposure Role of lipids; Data from Hansen et al. 2012 & 2015 Exposure: artificial produced water Accumulation in structural vs. storage lipids vs. toxicity Adaptation in 2nd gen Short exposure of three stages. Depuration slow. Delayed effect during exposure of overwintering copepods Delayed effect during exposure of overwintering copepods Lipid metabolism impacts after diapause Effect of temperature Lipid- and metabolomics of field-collected <i>Calanus</i> sp. WAFs from refined oil (fuel oil, marine gas oil) more toxic.
2	Shiley et al. 2004	x	x	x	x	Also: Photoenhanced toxicity
3	Bellas et al. 2007	x	x	x	x	Also: Photoenhanced toxicity
4	Jensen et al. 2008	x	x	x	x	Endpoint: grazing and reproduction
5	Hjorth et al. 2008	x	x	x	x	Copepod: <i>Microsetella</i> spp
6	Hansen et al. 2008	x	x	x	x	Endpoint: gene expression
7	Hansen et al. 2009	x	x	x	x	Endpoint: gene expression
8	Hjorth et al. 2011	x	x	x	x	Also: effect of warmer temp
9	Hansen et al. 2011	x	x	x	x	Comparison lipid-rich vs lipid-poor
10	Hansen et al. 2012	x	x	x	x	Comparing mechanic. and chem. dispersion
11	Olsen et al. 2013	x	x	x	x	Exposure and depuration period
12	Hansen et al. 2013	x	x	x	x	Other endpoint: gene expression
13	Nøregaard et al. 2013	x	x	x	x	Also measured bioaccumulation
14	Gardner et al. 2013	x	x	x	x	Soluble PAHs correlate with toxicity
15	Almeida et al. 2014	x	x	x	x	Oil ingestion. Also: <i>T. turbinata</i> and <i>P. crassirostris</i>
16	Nøregaard et al. 2015	x	x	x	x	Bioaccumulation w/ and w/o dispersed oil
17	Nordug et al. 2015	x	x	x	x	Accumulation of oil droplets; effect of dispersant
18	Hansen et al. 2015	x	x	x	x	Recovery after mech. disp. (but not chem. disp) oil exposure
19	Hansen et al. 2016	x	x	x	x	Role of lipids; Data from Hansen et al. 2012 & 2015
20	Jensen et al. 2016	x	x	x	x	Exposure: artificial produced water
21	Jäger et al. 2017	x	x	x	x	Accumulation in structural vs. storage lipids vs. toxicity
22	Krause et al. 2017	x	x	x	x	Adaptation in 2nd gen
23	Ågersted et al. 2018	x	x	x	x	Short exposure of three stages. Depuration slow.
24	Toxvard et al. 2018	x	x	x	x	Delayed effect during exposure of overwintering copepods
25	Toxvard et al. 2019	x	x	x	x	Delayed effect during exposure of overwintering copepods
26	Skottene et al. 2019	x	x	x	x	Lipid metabolism impacts after diapause
27	Van Dinh et al. 2019	x	x	x	x	Effect of temperature
28	Hansen et al. 2020	x	x	x	x	Lipid- and metabolomics of field-collected <i>Calanus</i> sp.
29	Hafiez et al. 2021	x	x	x	x	WAFs from refined oil (fuel oil, marine gas oil) more toxic.

3.4. Knowledge Gaps: Movement of Organisms and Dynamics of Hydrocarbons.

The existing literature of copepod toxicity is very useful and provides knowledge of the toxic effects when copepods and dispersed oil are brought together. In these studies, copepods are exposed to WAFs or CEWAFs at constant concentrations for hours to days to assess toxicity. While these studies provide toxicity information at discrete concentrations, they likely overestimate the actual toxicity of the oil to copepods in the field for two reasons:

First, these studies do not allow copepods to avoid hydrocarbons by vertically migrating. It is currently not known whether such an avoidance behavior of copepods occurs. Second, existing studies do not account for the vertical hydrocarbon gradients of oil or dispersed oil. Especially during the critical copepod developmental time of lipid accumulation in early summer. Additionally, during summer months, the water column is often stratified, which may constrain the oil to the surface and separate it from the copepods (Figure 2.1). It is currently not known whether this stratification is an effective barrier between oil and copepods.

4. Copepod Tower: Description of the Novel Experimental System

4.1. Rationale and Features

To close existing knowledge gaps in our understanding of oil toxicity to the lower trophic level, we conducted experiments with oil (with and without dispersants) and copepods in an environmentally-relevant scenario that differed from traditional systems in two main aspects

Oil Concentration Gradients and Stratification. We created a system where the oil is introduced at the surface and allowed to create a concentration gradient with depth during the experiment. We investigated the penetration of specific hydrocarbons in the water column with and without dispersants and under stratified (Summer) and unstratified (Late Fall through Early Spring) conditions.

Vertical Migration of Copepods: We built a mesocosm system where copepods can migrate vertically, as they do in the ocean. Copepods have seasonal and daily distribution patterns (Figure 3.2.3.) and they can also change their vertical distribution in response to attractive (such as food and mates) (Lass and Spaak 2003) and aversive (predators) chemical signals (Poulet and Marsot 1978). In our proposed system, the position of the copepods was monitored to determine their vertical movement within the tank relative to the concentration gradient of the hydrocarbons.

Based on these parameters, we hypothesized that if copepods were able to detect and avoid PAH pollutants, they would change their vertical distribution to depths that minimize exposure. Furthermore, we hypothesized that a stratified water column would impede the movement of PAHs into the water column, and copepods would distribute themselves below a thermocline when present to avoid contact with pollutants (Figure 4.1.)

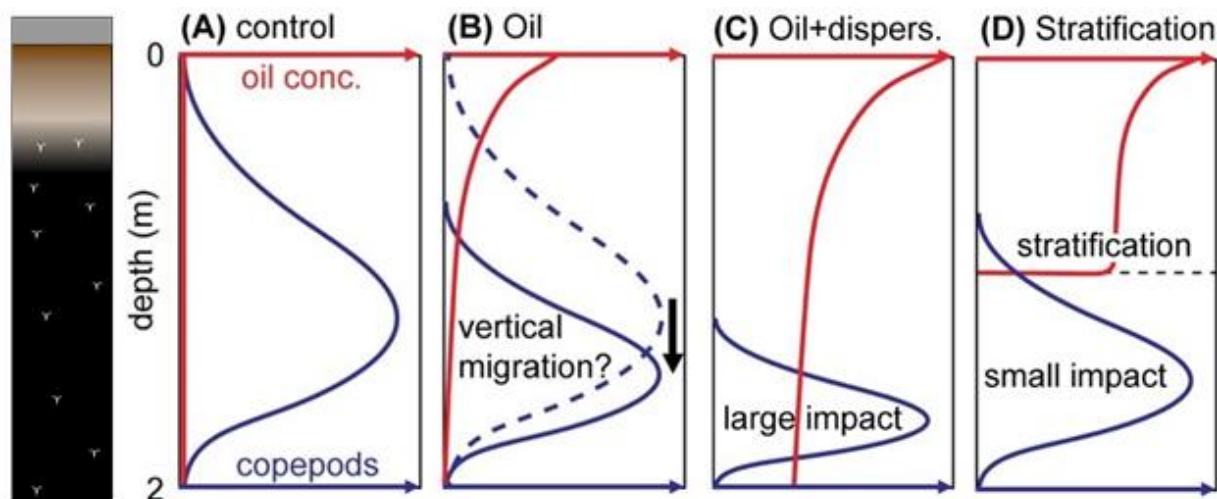


Figure 4.1. Visualization of hypothesis: PAH and *C. finmarchicus* vertical distribution. Depiction of 2 meter column with copepods and oil distribution with schematics showing different treatment scenarios. All diagrams show the hypothesized vertical distribution (depth) of oil and copepods in each treatment. **A) Control:** Under this scenario, we assume oil concentration will be 0 across all depths. Copepods will vertically distribute themselves naturally within the column, represented here with a bell curve. **B) Oil:** Under this scenario, oil is added to the top and diffuses into the water column creating a gradient of concentration. If copepods can detect and avoid oil pollutants, we hypothesize they will migrate to deeper water where concentration remains the lowest. **C) Oil+dispersant:** Under this scenario, the application of dispersant drives a higher concentration of oil into the column and down to the bottom of the chamber. In this case the copepods can migrate lower but cannot avoid the dispersant, so we hypothesize that this scenario would result in a higher proportion of copepods being driven out of the column relative to oil only. **D) Stratification:** Under this scenario, the presence of a thermocline prevents the dispersed oil from reaching the lower half of the column. If copepods can detect and avoid pollutants, we hypothesize that they will migrate below the thermocline, where they are protected from high levels of oil.

4.2. Copepod Mesocosm: Tank and Experimental Setup

A rectangular plexiglass column (23 cm x 23 cm x 167.5 cm) was filled with 89 L of sand-filtered seawater (Figure 4.2.1). All experiments were conducted in a temperature controlled dark room. Hobo loggers (HOBO MX2202; Onset MA USA) captured light and temperature data continuously at 1x/minute in the columns regularly spaced intervals: bottom (5 cm), middle (85 cm), and surface (166 cm). Room temperature was kept between 17-19 °C. An air bubbler was split to accommodate four air tubes blowing on the surface at 45° angles, in a clockwise orientation along each tank side to simulate surface wind energy (Figure 4.2.1).

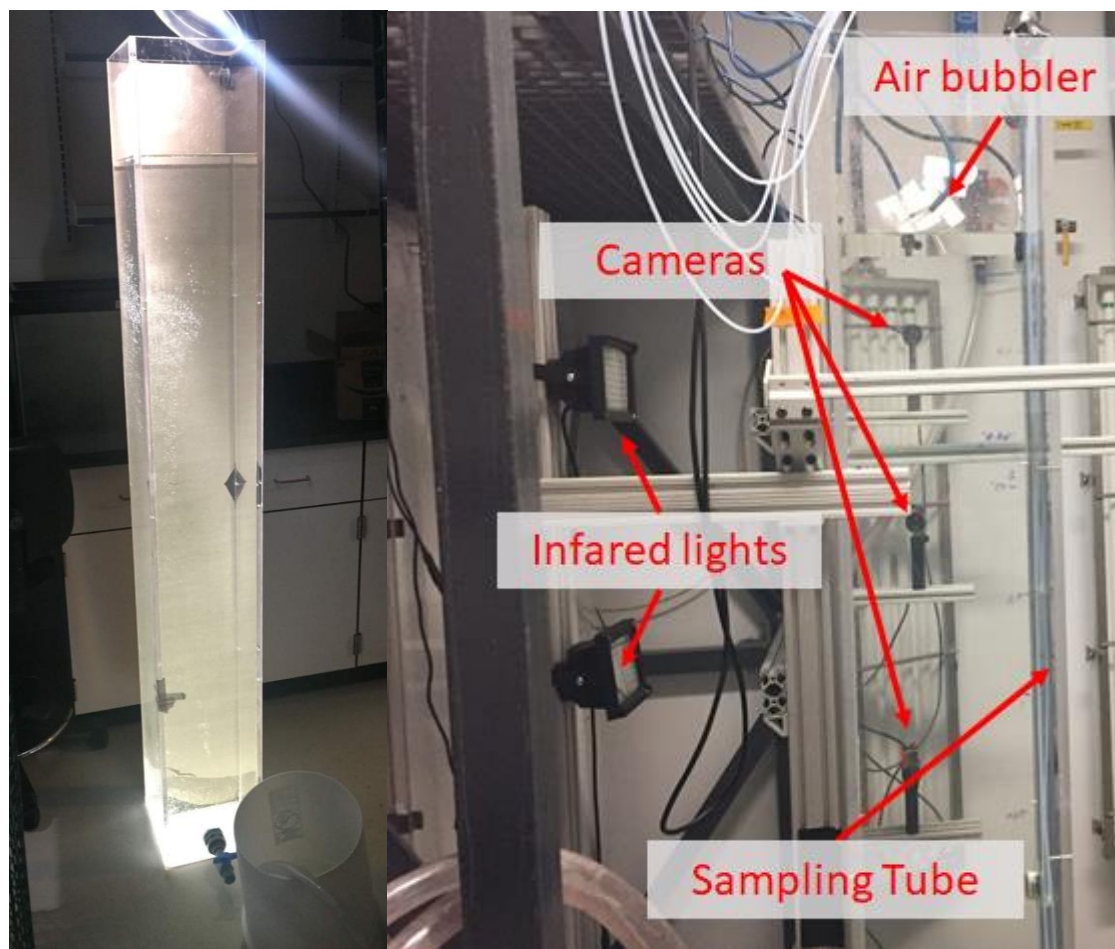


Figure 4.2.1. Experimental chamber set up. a). Image of the experimental chamber: 167.5cm deep plexiglass column filled with 89 L of seawater and illuminated from the top to represent the water column. b). View of the upper half of the system looking adjacent and through the experimental chamber. Highlighted are the air bubbler (air stream) tubes placed at 45° angles to the surface to simulate wave action movement, the sampling tube to extract water and oil from different depths, infrared lights placed orthogonally to cameras 1-3 situated ~1.5m behind the experimental chamber.

4.3. Unstratified Water Column

For a homogenous water column, temperature was kept in equilibrium to room temperature. Tanks were filled with sand-filtered estuarine water from the Bigelow dock (salinity ~31-32 ppt) and left for 12-15 hours to reach room temperature. Controls and experiments were started when the temperature throughout the experimental chamber reached a stable equilibrium within 1°C of room temperature (Figure 4.3.1.).

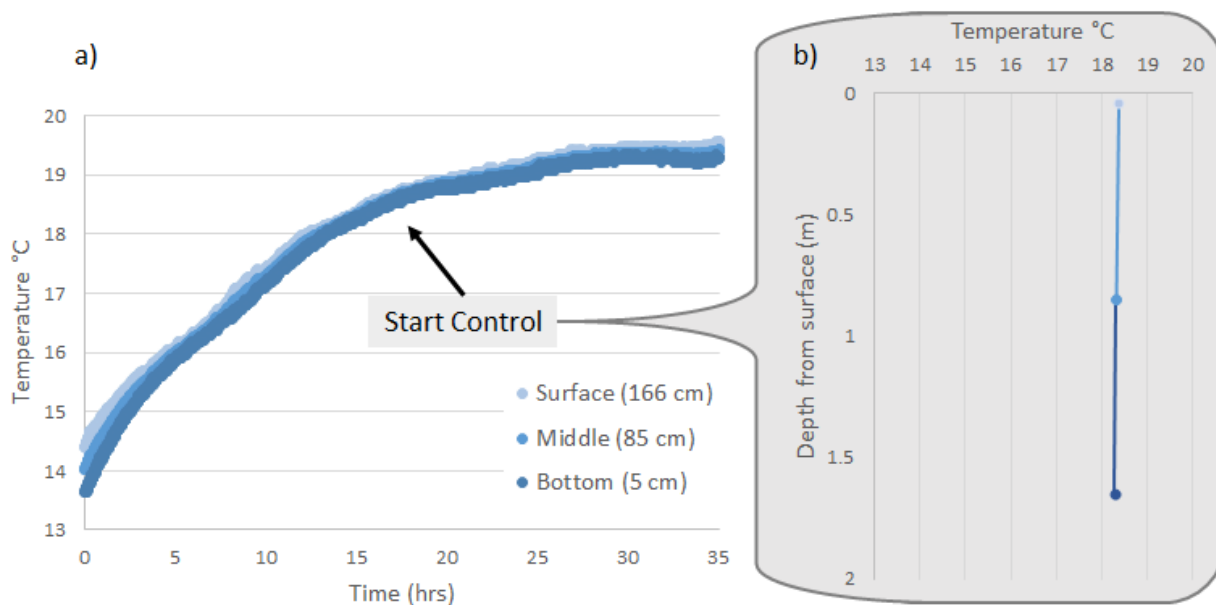


Figure 4.3.1. Temperature profile in the unstratified experimental chamber. a) Graph depicting the time required for the water column to reach stable temperature after being filled. Colors represent the location of the temperature sensors (HOBO logger) from the bottom. Controls were started when temperature leveled off within 1 °C of room temperature. b) depicts the temperature structure of the chamber at the start of the control. Temperature profile was the same from surface to bottom (unstratified).

4.4. Stratified Water Column

For the second phase of the experiment, a temperature-stratified column was simulated. A stainless steel chilling coil was situated between cameras #3 & #4, plumbed with Tygon tubing feeding out of the column into a water chiller system outside the tank (Figure 4.4.1). Deionized water was chilled to 8°C and circulated through the closed cooling system to chill the bottom layer of the water column. Two additional HOBO temperature loggers were added to the column for a 5-point temperature resolution for water-column structure: bottom (5 cm), mid-lower (40 cm), middle (85 cm), mid-upper (125 cm) and surface (166 cm) (Figure 4.4.2). The lower half of the experimental chamber was wrapped in insulated bubble wrap to reduce heat loss to air exchange.

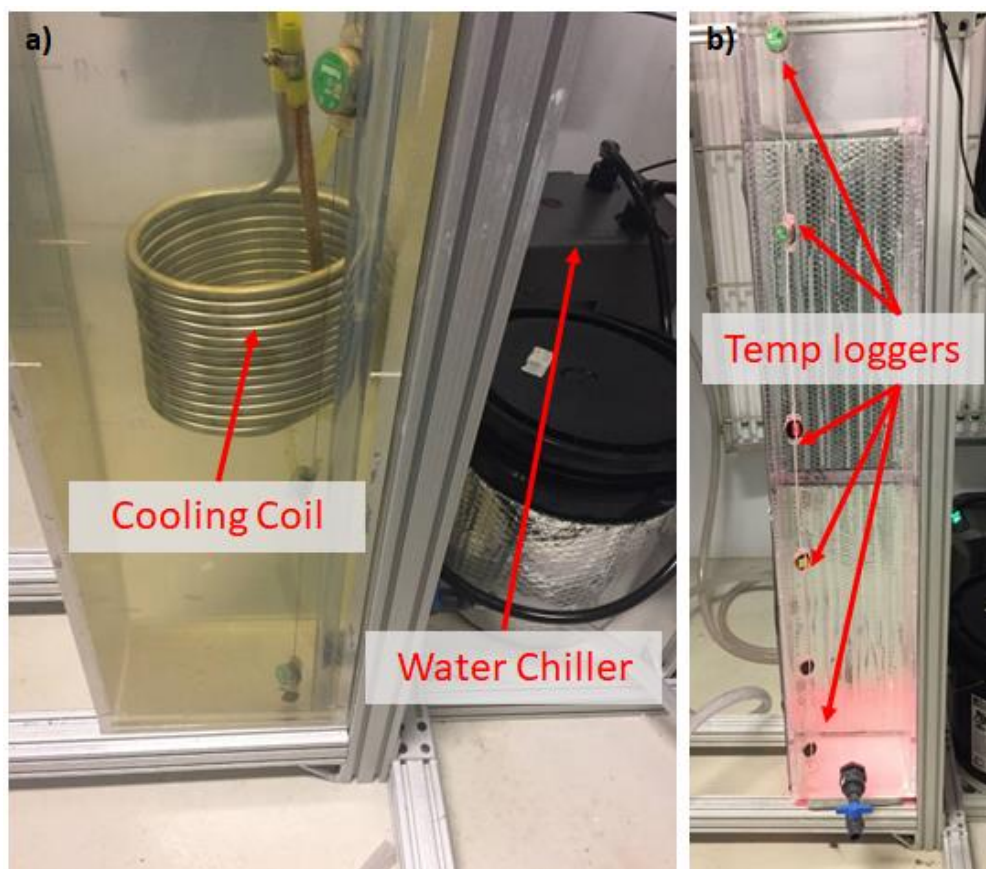


Figure 4.4.1 Stratified experimental chamber set-up. a) Image depicting cooling coil placed between cameras 3 & 4. Yellow tygon tubing was fed up through the chamber and connected to a water chiller. The closed system circulates DI water chilled to 8 °C. b) Image depicts temperature loggers stationed within the water column to generate a 5-temp resolution of water column temperature structure.

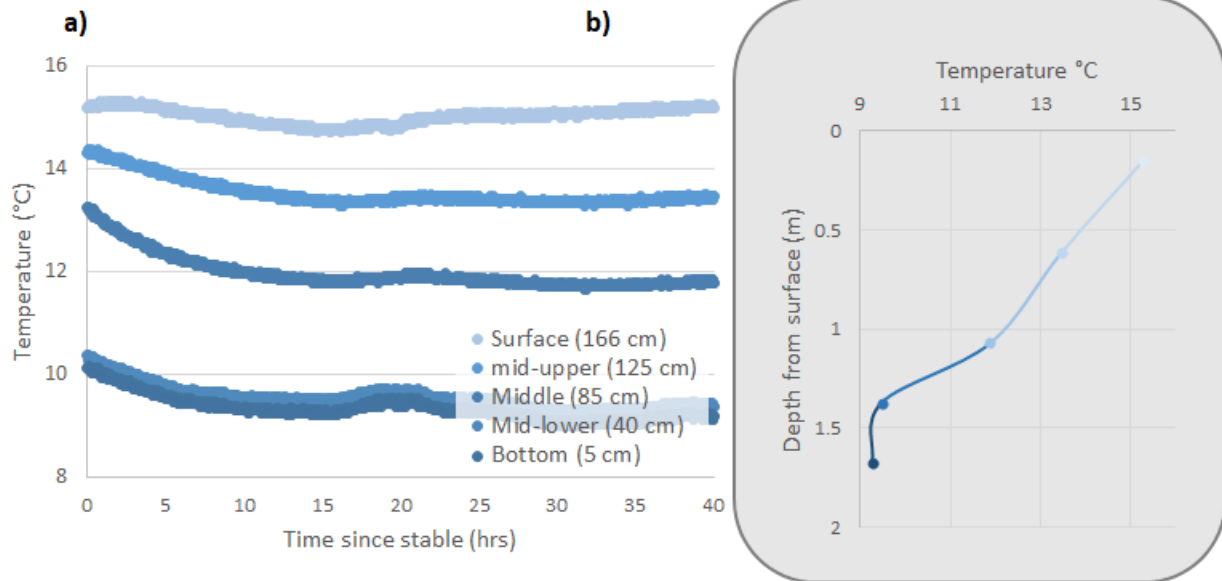


Figure 4.4.2. Temperature structure of stratified experimental chamber. a) Graph depicting experimental chamber temperature profile with established thermocline. After 20h the temperature became stable at all five depths. Colors represent the location of the HOBO logger from the bottom. Controls were started when a stratified water column was stable. b) The temperature structure of the chamber at the start of the control. The water column was stratified between the Middle (85 cm) and mid-lower (40 cm) temperature loggers with a steady temperature gradient from 40cm to the surface.

4.5. Video Imaging

Four cameras (FLIR, USA; FL3-U3-32S2C-CS) situated (from bottom), captured images of 2.5cm-30.5cm (Bottom), 60cm-87.5cm (Midlower), 106.5cm-134.5cm (Mid-upper), 141cm-165cm (top), capturing a total of 56.8 liters in frame. Infrared light (16 LED bulb panels) was placed orthogonally to each camera to illuminate the tank in a spectrum imperceivable to the copepods, but visible to the cameras.

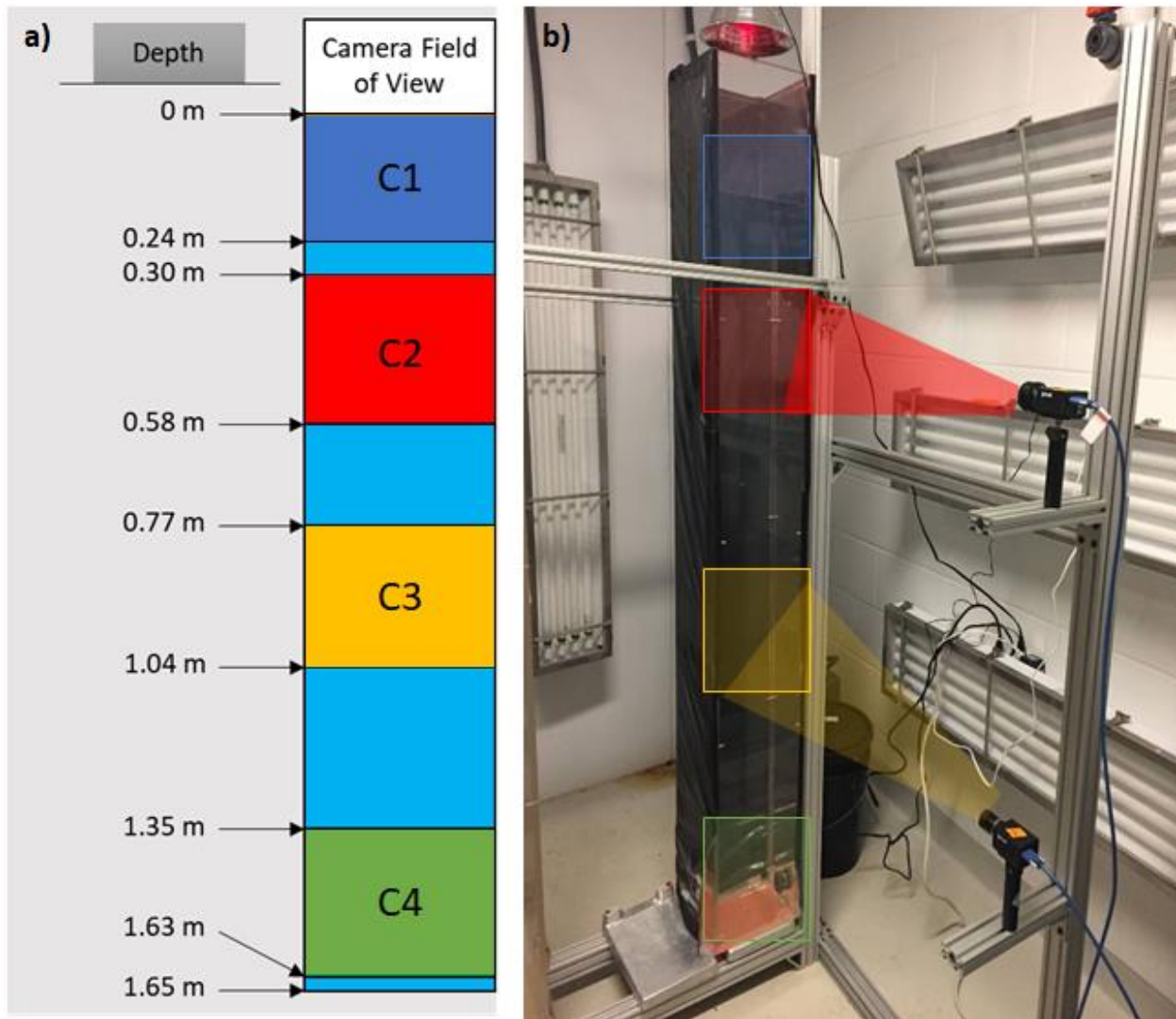


Figure 4.5.1. Camera placement and set-up. a) Diagram depicting relative positions of the cameras' (C) field of view vertically up the experimental chamber. b) Photograph demonstrating the cameras positioned and viewing into the chamber, with representations of view fields of the situated cameras. Colors correspond to camera number: C1=Blue, C2=Red, C3=Yellow, C4=Green.

4.6. Ports for Chemical Sampling.

A PVC tube with 5 ports drilled into it was installed into each tower. Within this tube were placed 5 teflon tubes (1/16" diameter), one reaching to each port for sampling at standard depths: 10 cm, 46 cm, 81 cm, 117 cm, and 152 cm from the water surface. Teflon tubes were fitted with Luer-lok tips outside of the tower in order to attach the 20mL gas-tight glass syringes used for water sampling (Figure 4.6.1). The longest teflon tube had an internal volume of 4.7 mL, therefore 5mL of water was taken as waste from each port and each time point, to avoid sampling stagnant water inside the tubing.

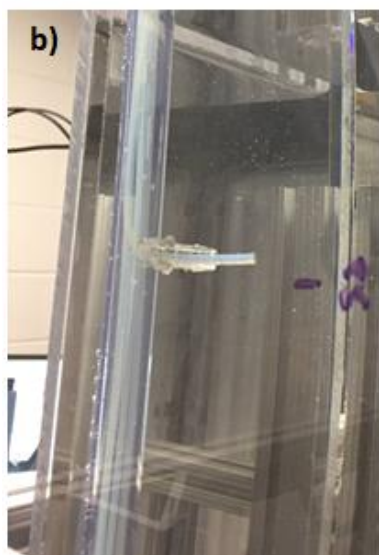
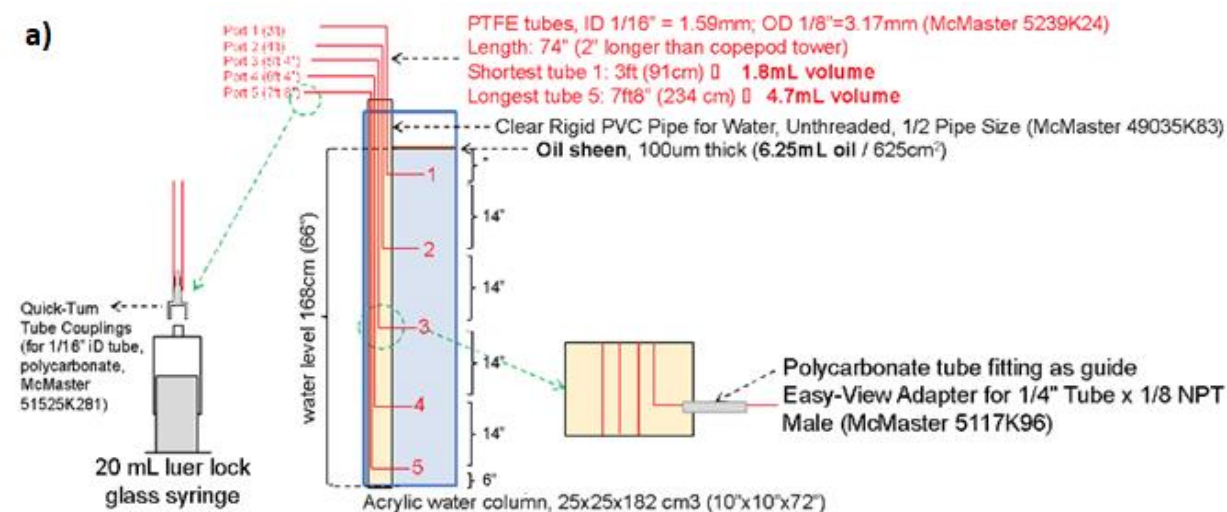


Figure 4.6.1 Chemical sampling system. a) Diagram shows the sampling port system throughout the experimental chamber. b) Image of individual sampling port situated near the surface of the experimental chamber. c) glass syringe used to extract water samples attached to the end of a teflon tube corresponding to the sampling port in image b.

4.7. Calanus Behavior Monitoring

4.7.1. Data Collection

Four cameras situated (from bottom), captured images of 2.5cm-30.5cm (Bottom), 60cm-87.5cm (Midlower), 106.5cm-134.5cm (Mid-upper), 141cm-165cm (top), capturing a total of 56.8 liters in frame. Images were captured at 10 Hz, and saved 1 Hz for analysis using Flycapture2 video software (Figure 4.7.1.1). Cameras were triggered to run simultaneously for automatic temporal match, frame by frame. Cameras fed back to monitors situated outside experimental dark-room (Figure 4.7.1.2). Brightness, contrast and shutter speed were manipulated to allow the camera to detect all *C. finmarchicus* within each frame to be counted in software. Video data was collected for 15-17 hours preceding treatment and 24 hours after the oil was added. Control conditions were determined to be the 2 hour period leading up to the addition of oil for a paired control/treatment design. Most distribution changes were noted within the first 5 hours post treatment, so this time frame was used to analyze behavioral response.

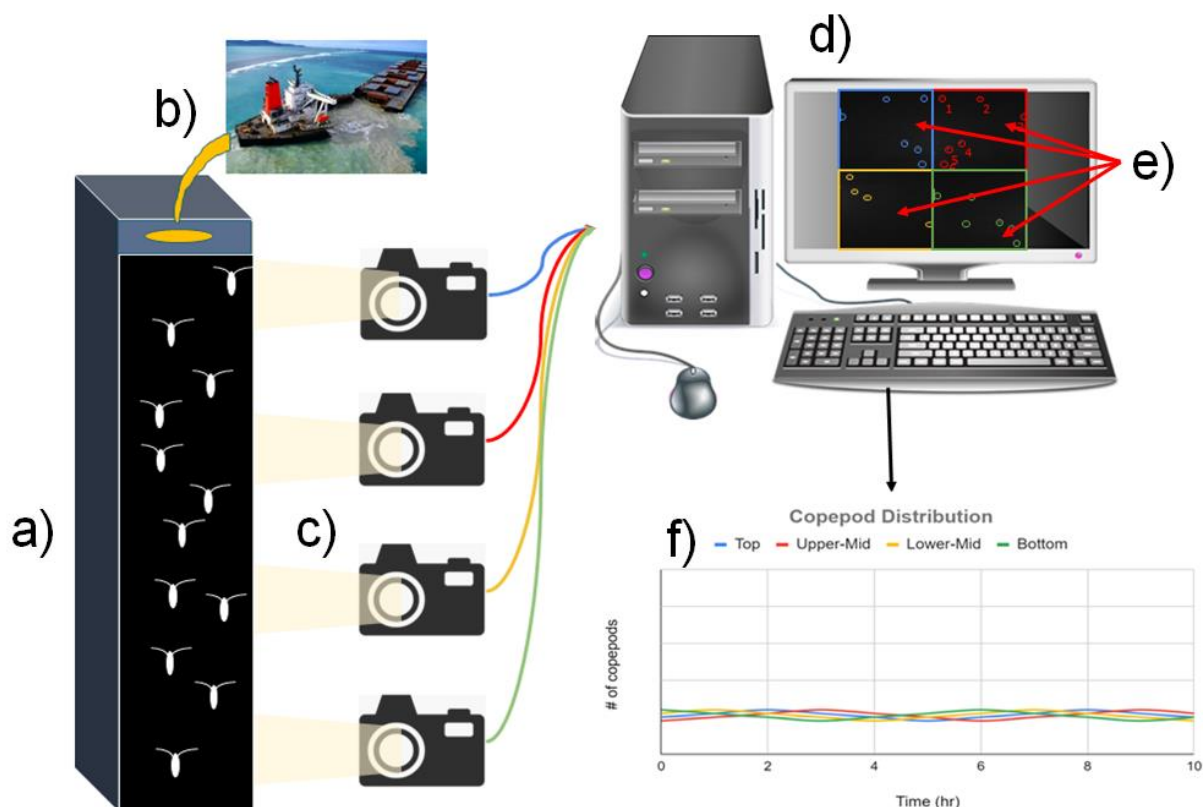


Figure 4.7.1.1. Video data collection set-up: a) Copepods were allowed to distribute themselves naturally throughout the experimental chamber for 15-17 hours. b) At the beginning of treatment, oil and dispersant were added at the surface of the chamber. c) Four video cameras placed vertically capturing distinct quadrants of the column and sending an image back to a d) computer monitor outside the temperature

controlled, dark room. e) Copepods were analyzed and counted as “particles” in view of each quadrant. f) Relative abundance in vertical space could then be shown over time.



Figure 4.7.1.2. Monitors to inspect camera settings and live video feeds. a) Image depicts monitors linked to cameras outside of the experimental room with b) view of the computer monitor capturing images from 4 cameras simultaneously from four quadrants along the experimental chamber.

4.7.2. Video Data Analysis

Video data was exported to ImageJ software (NIH; v1.52a) and converted to an 8-bit substacks collecting data from one frame every 2 minutes. For each stack of images, threshold was adjusted to create greater contrast between copepods and backgrounds. Background was deleted. The “analyze particles” tool was used to count discrete particles, size $>3 \text{ pixels}^2$ (seen as bubbles or *Calanus*) in each frame for the duration of the substack. The result was a sum of particles counted over each frame (Figure 4.7.2.1).

One issue we needed to resolve was the tendency for the particle counting software to confuse air bubbles with copepods of similar size, particularly in the upper column near the surface. To control for accumulating bubbles over the course of the experiment, bubbles within frame were manually counted at 4 hour intervals throughout the course of the entire video. These counts were fitted with a linear curve to calculate a “bubble correction” (Figure 4.7.2.2). This value was calculated with x equivalent to hour, and subtracted from the total particle count as a “true” value of copepods in each frame for that corresponding time point (Table 4.7.2.1). This correction was only needed in the oil only replicates, when the seawater incoming was colder than the room and released gas while reaching room temperature. By the later treatments, we had implemented a bubble sweep that cleared bubbles before treatment, bubble accumulation was less than 2 per 10 hr period, and we could forgo the correction. For treatments including dispersants, frames were deleted when reflection from the dispersant plume obstructed tank view and prevented accurate counts, resulting in

some gaps of time coverage for top and mid-upper video frames. This also reduced the bubble accumulation in the experimental chamber.

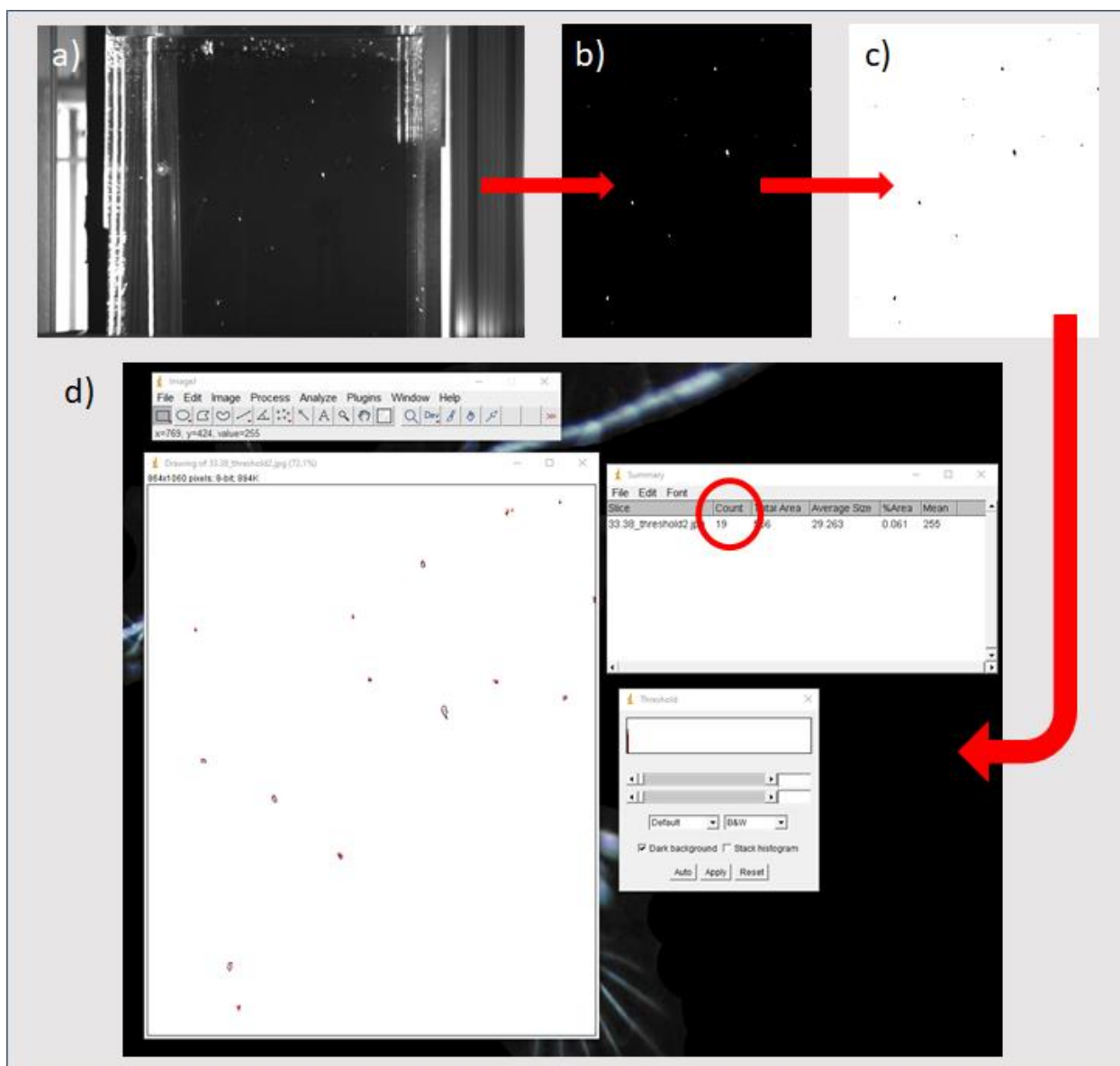


Figure 4.7.2.1. Diagram of video analysis methodology. a) Original video was converted to image stacks of 1 frame per two minutes of video. b) Each image was cropped, and manipulated to create better contrast between copepods and background. c) Threshold was adjusted to separate copepods from background and image was converted to white background. d) Particle analyzer counted discrete copepods, and generated a final count on each frame (circled) This was completed for the entirety of the stack.

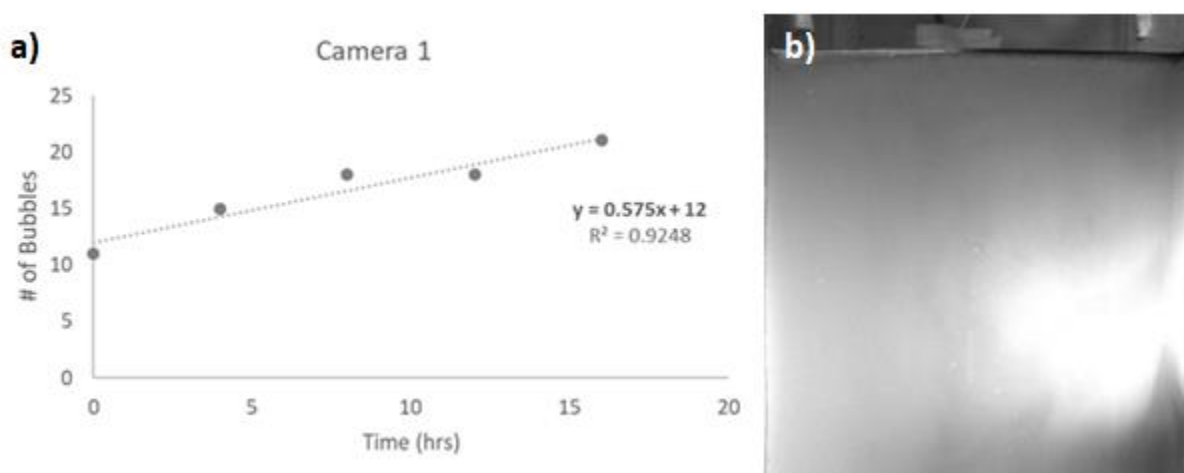


Figure 4.7.2.2. Example of “bubble correction” calculation for oil only treatments. a) For each video, we manually counted bubbles one frame per four hours experiment. We then fit a linear curve through the line to calculate a rate of change of bubble accumulation or loss. This value was subtracted from the overall count of copepods. b) Video frame from camera 1 after dispersant is released and view of column is obstructed.

Table 4.7.2.1. Equations used to calculate bubble corrections in count analysis.

Bubble Corrections in Oil Only Treatments			
Treatment	Camera	Equation	R2
OilR1	1	$y = -0.125x + 11.6$	0.2717
	2	$y = -1.875x + 23.167$	0.9985
	3	$y = 0.0268x + 7.8214$	0.0469
	4	$y = 0.875x + 8.5$	0.9423
OilR2	1	$y = 0.1696x + 24.107$	0.1556
	2	$y = -0.2589x + 23.536$	0.3967
	3	$y = -0.1607x + 13.929$	0.263
	4	$y = -0.4643x + 18.143$	0.7562
OilR3	1	$y = -0.4107x + 15.214$	0.8266
	2	$y = -0.1518x + 9.3929$	0.5827
	3	$y = 0.0268x + 2.25$	0.1875
	4	$y = 0.0625x + 2.3929$	0.3603
OilR4	1	$y = 0.2214x + 1.4$	0.704
	2	$y = -0.1071x + 3.7143$	0.1233
	3	$y = -0.0357x + 3.7143$	0.1667
	4	$y = 0.0179x + 2.9286$	0.0111

4.8. Oil Treatments

4.8.1. Oil and Weathering

Alaska North Slope (ANS) oil was artificially weathered by evaporation on a rotary evaporator. On two separate occasions, ANS oil in a round bottom flask was placed on a rotary evaporator for approximately 17 hours with the water bath set to 80°C until 22% of the mass had been evaporated. This process removed volatile compounds that would evaporate quickly during an oil spill (Ryerson et al. 2012), and are, therefore, of less relevance for the water column. See Figure 4.8.1.1 for a GC/MS chromatogram of the evaporated oil.

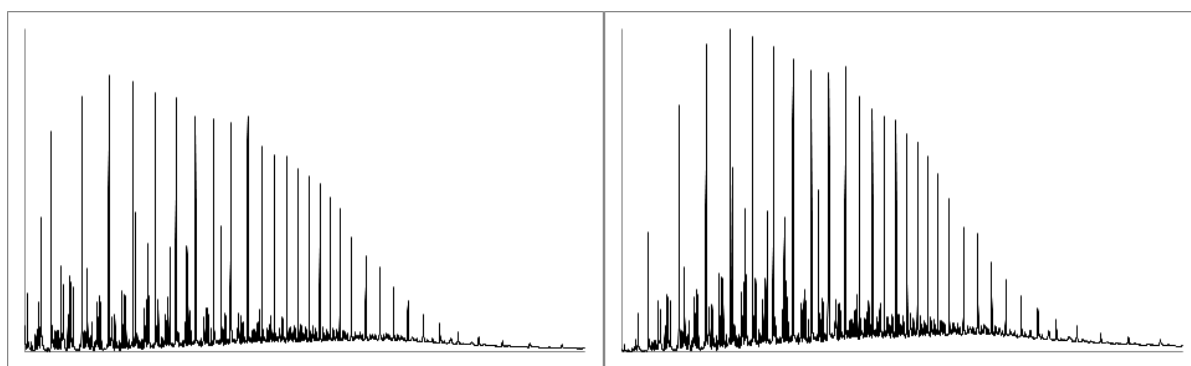


Figure 4.8.1.1. GCMS Chromatograms showing alkanes of unevaporated Alaska North Slope crude oil (left) and Alaska North Slope oil evaporated by 22% by mass (right). The evaporated oil has compounds < nC10, including benzene, toluene, ethylbenzene, and xylenes (BTEX) removed. The chromatogram is of the m/z 57 ion trace.

4.8.2. Dispersed Oil Formation and Mixing Energy.

To produce dispersed oil, evaporated ANS oil was premixed with dispersant (Corexit EC9500B; Corexit Environmental Solutions LLC, Sugar Land, TX) at a dispersant/oil ratio of 1:20 (v/v), and added to the experimental chamber in the same manner as the for the non-dispersed oil treatment. To allow dispersion of oil, mixing energy needs to be added to the water column. This was achieved by directing four air streams at a 45 ° angle at the water surface (one air jet at each side of the tank; see [Fig 4.2.1](#)). The air stream was delivered using an aquarium pump delivering air through silicone tubes capped by 100-μL pipette tips. The intensity of the air stream was adjusted so that it created an approx. 1-cm depression in the water surface (see Fig 4.8.2.1). This energy created a dispersion of the oil within minutes of oil addition. The same setup and mixing energy was used for dispersed as well as for non-dispersed treatments.

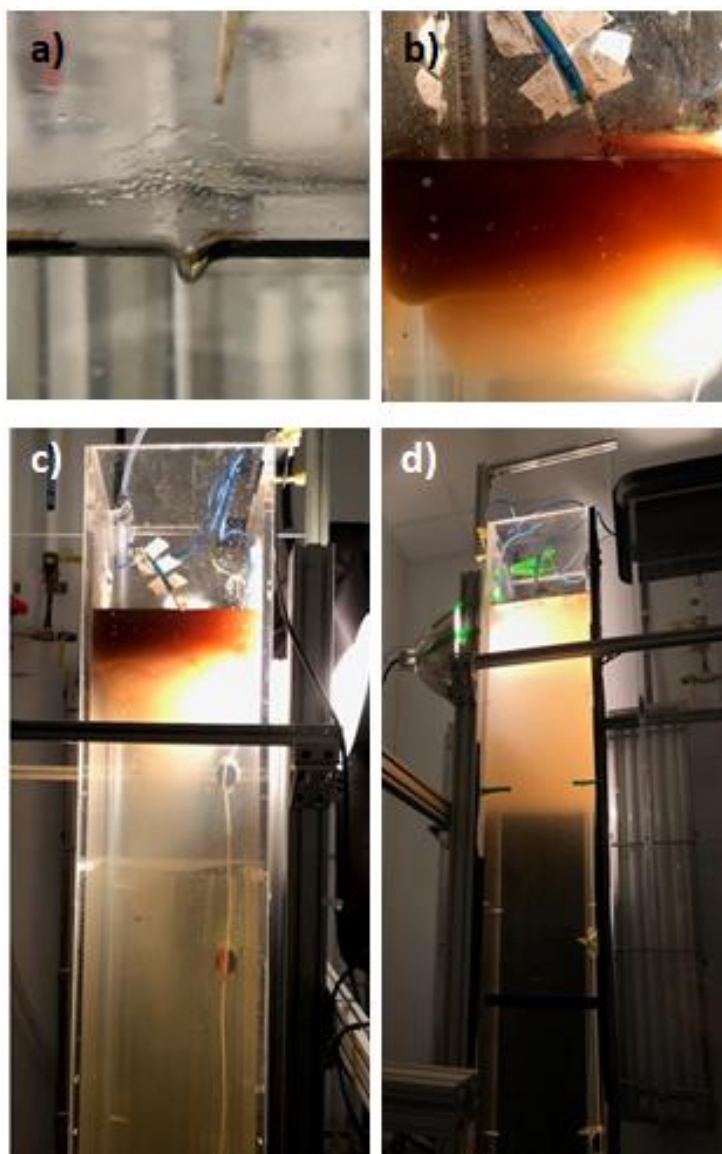


Figure 4.8.2.1 Surface energy and dispersant plume distribution. a-b) Close up photographs of the air stream (air jet) outlet creating surface energy in the oil-only (a) and oil+dispersant (b) treatments. c-d) Image of the oil plume within (c) minutes and (d) two hours of dispersant addition.

4.8.3. Initiation of Experiments,

Approximately 5 g of oil (6.25 mL) was added to the top of each tower at time 0 of the experiment, forming an oil film of a nominal thickness of 118 μm (Figure 4.8.3.1). To start the oil treatments, 6.5 mL of oil was measured into a graduated cylinder then poured into a pre-weighed 20 mL vial. If the experiment included dispersant, 310 μL of Corexit EC9500B was added to the vial before capping and was premixed with the oil by vortexing, leading to a dispersant/oil ratio of 1:20 (v/v). The contents of the vial was

poured slowly onto the top of the column, the vial was capped and the residual contents were weighed for accurate estimation of the mass of oil added.

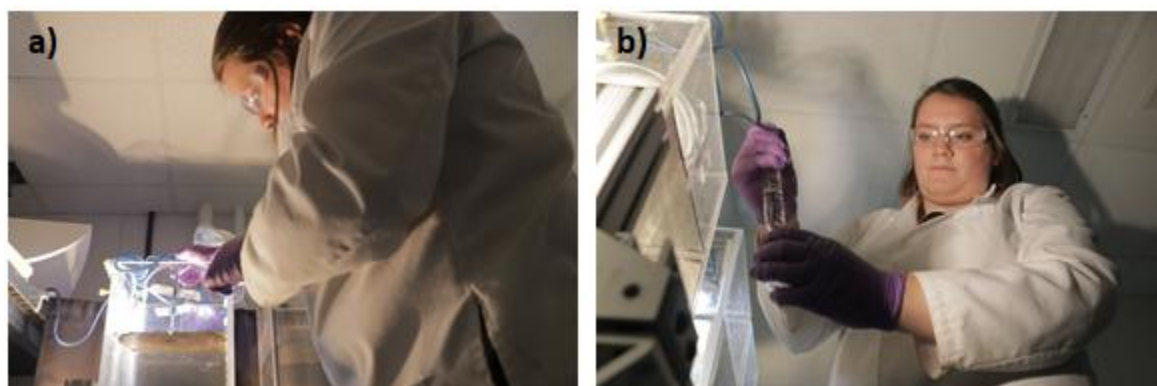


Figure 4.8.3.1. Oil addition and water sample extraction of oil treatment. a) 5 g of oil is added to the top of the water column at the beginning of the experimental treatment. b) Water samples are then collected using a 20 mL glass syringe from the five sampling port tubes at 0.5, 1, 2, 4, and 24 hour time points.

4.8.4. Sampling and Extraction for Oil Hydrocarbon Analysis

Water samples for chemical characterization were taken from each experiment directly before the addition of oil (Control), and then after the addition of oil or oil and dispersant at 0.5, 1, 2, 4, and 24 hours. The control was taken from only Port 1 (10cm below the surface), but at subsequent time points, samples were taken from all 5 ports along the length of the experimental chamber. The specific types of samples taken at each port during each experiment are listed in the Appendix. Samples were analysed by spectrofluorometer, gas chromatography-mass spectrometry (GC-MS), solid phase microextraction on two-dimensional gas chromatography-time of flight mass spectrometry (GCxGC/TOFMS-SPME), and fluorescence microscopy. Aqueous samples could be directly analysed by GCxGC-SPME and, if no dispersant was present, by fluorometry. However, samples measured by GC-MS, or dispersed samples measured by fluorometry, had to be solvent extracted prior to analysis. Generally, 30mL of aqueous sample was collected in a 40mL glass vial. 5mL of hexanes was added and the sample was shaken for 2 minutes, and then allowed to separate. The hexanes layer was removed and stored in a refrigerator until analysis (details of the corrections applied to analyses to account for sample concentration can be found in the Appendix).

5. General Materials and Methods

5.1. Copepod Collection and Husbandry

Calanus finmarchicus were collected at a location 5 nautical miles offshore of East Boothbay, Maine, USA (43°45'13"N, 69°30'20"W; Figure 5.1.1). A vertical zooplankton ring net (330µm mesh) was lowered to 100 meters, targeting a deepwater basin where *C. finmarchicus* are advected en masse. Large quantities of mixed zooplankton samples were held at surface temperature and transported back to the laboratory for sorting.

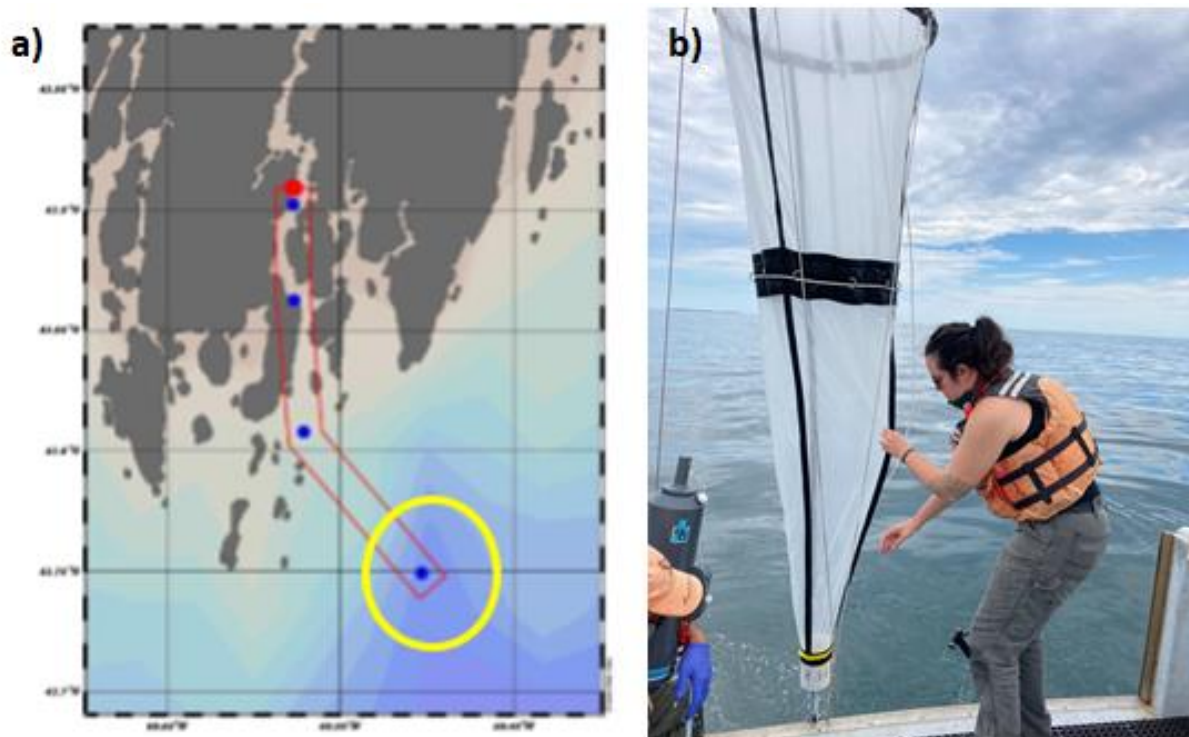


Figure 5.1.1. Location and method of *Calanus finmarchicus* collection. a) Chart of collection site off the Boothbay ME Peninsula (yellow circle). b) Image of 330µm vertical zooplankton net dropped to 100 m for copepod collection.

In the laboratory, newly collected zooplankton samples were transferred to a 16 °C incubator and allowed to acclimate for 15 hours. *Calanus finmarchicus* were then identified visually and sorted using 500µm mesh and hand-sorting and kept in dark, cool conditions until experiment (Figure 5.1.2). Animals were fed every other day a mixed diet of diatom (*Thalassiosira weissflogii*) and dinoflagellate (*Rhodomonas salina*) cultures and kept in culture for up to 1 month.

Before treatment, 500-700 animals were acclimated to the treatment temperature for 2 hours, and gently transferred into the experimental tank. After acclimating for 30

minutes, the video recording was started. Animals were held in treatment tanks for 13-17 hours overnight to allow them to distribute themselves before oil was added. Preliminary data showed that the distribution did not change after 4 hours and that the number of animals in each camera remained stable during the final 2 hours. As a result, the number of the copepods in each of the cameras was enumerated every 2 minutes during the final 2 hours and this average abundance was used as the pretreatment value (control; Figure 5.1.2.). After the experimental treatments were added to the chamber, the change in the number of animals was normalized to the control (on a proportional scale) to generate a paired control for each treatment of animal distribution before and after oil addition. This allowed us to compare replicates with different initial abundances.

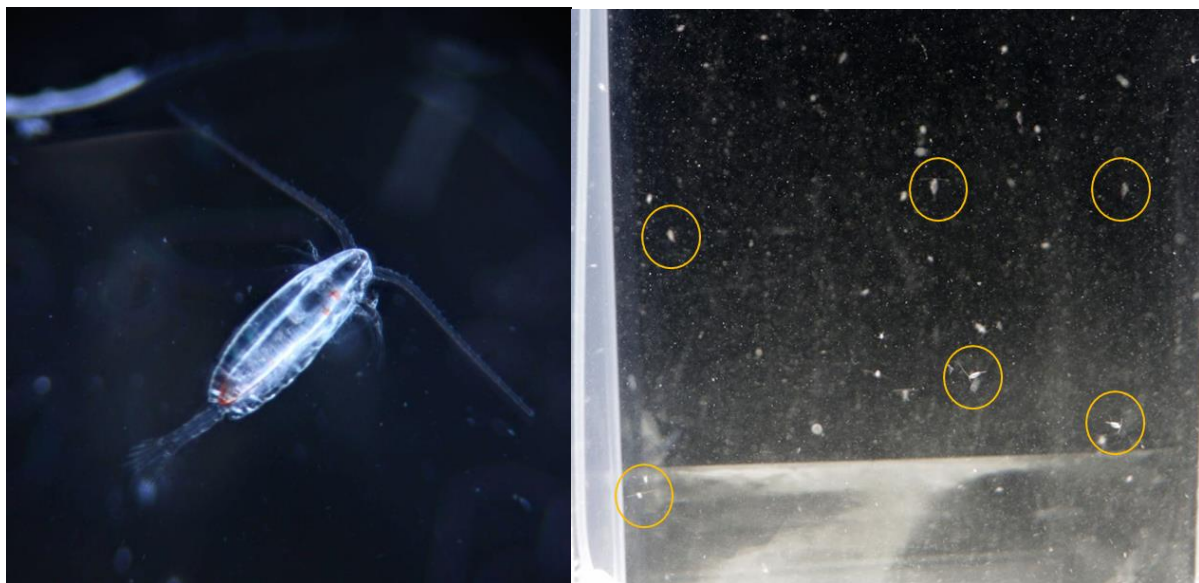


Figure 5.1.2. Identifying *Calanus* and enumerating individuals in the experimental chambers. a) Close up image of a *Calanus finmarchicus* individual taken shortly after collection. b) Image looking into an experimental chamber at *C. finmarchicus* after redistributing for 30 minutes. Individuals appear white against a black background. Several copepods are circled for reference, but more appear in frame.

5.2. Analysis Methods

5.2.1. GC/MS total PAH Analysis

Liquid-liquid extracted samples (primarily from treatments that included dispersant; see Section 4.8.4. for details of this procedure) were analyzed for a suite of polycyclic aromatic hydrocarbons (PAHs) and n-alkanes, nC10-nC40, on an Agilent GC-MS (7890B GC coupled to a 5977A MS). The GC was equipped with an 30-m Agilent DB-XLB column (0.25mm I.D., 0.25um film thickness). The GC oven was programmed at 40°C for 5 min and ramped to 340 °C at 10 °C min⁻¹ (held for 20 min). Ultrapure helium (99.999%) was used as a carrier gas with a constant flow of 1.2 mL min⁻¹. The inlet was held at 320 °C and a 1uL splitless sample injection was used.



Figure 5.2.1.1. Samples in the process of SPME analysis on the GCxGC/TOFMS.

5.2.2. SPME-GCxGC/TOF dissolved PAH Analysis

Samples taken for GCxGC SPME analysis were acidified with 50-100uL of Phosphoric acid, 85%, depending on the volume of the sample, and stored in a refrigerator at 10°C until analyzed. These samples were taken in duplicate, directly into SPME vials. A comprehensive two dimensional gas chromatography system coupled to a time-of-flight mass spectrometer (GCxGC-TOFMS; Leco Pegasus 4D; Leco Corp, St. Joseph, MI) was used to quantify the PAHs in each sample after equilibration with a polyacrylate fiber. An 85µm polyacrylate fiber was inserted into each sample and equilibrated with

the aqueous solution (Figure 5.2.1.1), incubated at 30°C for 100 minutes within an agitator. The fiber was washed in milli-Q water, and then desorbed in the inlet of the GC (Agilent 7890B) for 5 minutes at 280° C.

For GCxGC-TOFMS analysis, the first-dimension column was a 60-m Restek Rxi-1 column (0.25 mm ID, 0.25 µm film thickness), and the 2nd dimension column was a 1-m Restek Rxi-17sil column (0.15mm ID, 0.15 µm film thickness). The GC oven was programmed isothermal at 70 °C for 5 min and ramped to 340 °C at 4 °C min⁻¹ (held for 15 min). The second oven was programmed with a constant offset to the GC oven of + 5 °C, and the modulator with an offset to the GC oven of +15 °C. The modulation period was 5 seconds, and 100 spectra were acquired per second. Ultrapure He (99.999%) with a constant flow of 2 mL min⁻¹ was used.

5.2.3. Fluorescence Hydrocarbon Analysis

Samples from oil only experiments were analysed primarily by fluorescence in order to calculate the concentration of total dissolved oil compounds. Excitation scans were run between wavelengths of 250 and 390 nm to determine the best excitation wavelength for analysis (typically 280 nm). Once the excitation wavelength was determined, all samples, controls, blanks (i.e. artificial seawater and filtered seawater), and standards were analysed with emission scans between 290 and 450 nm. The area beneath the curve for each sample was approximated by taking the sum of the area of 1nm unit rectangles along the length of the emission spectra, and then subtracting the sum of the areas beneath the corresponding control or blank (Figure 5.2.3.1). Samples from “Oil Only” replicates were analyzed in their aqueous form, therefore, a dilution series was made using a WAF made from Alaska North Slope (ANS) evaporated oil. The concentration of these WAF dilutions was determined by GCxGC-SPME analyses and ranged from 0.1-200 µg/L. “Oil and Dispersant” replicates required liquid-liquid extraction prior to analysis by fluorometry. Therefore, samples were in a hexanes matrix, and the corresponding standard curve was made by dissolving evaporated ANS oil in hexanes (concentrations of standards ranging from 10µg/L to 10mg/L).

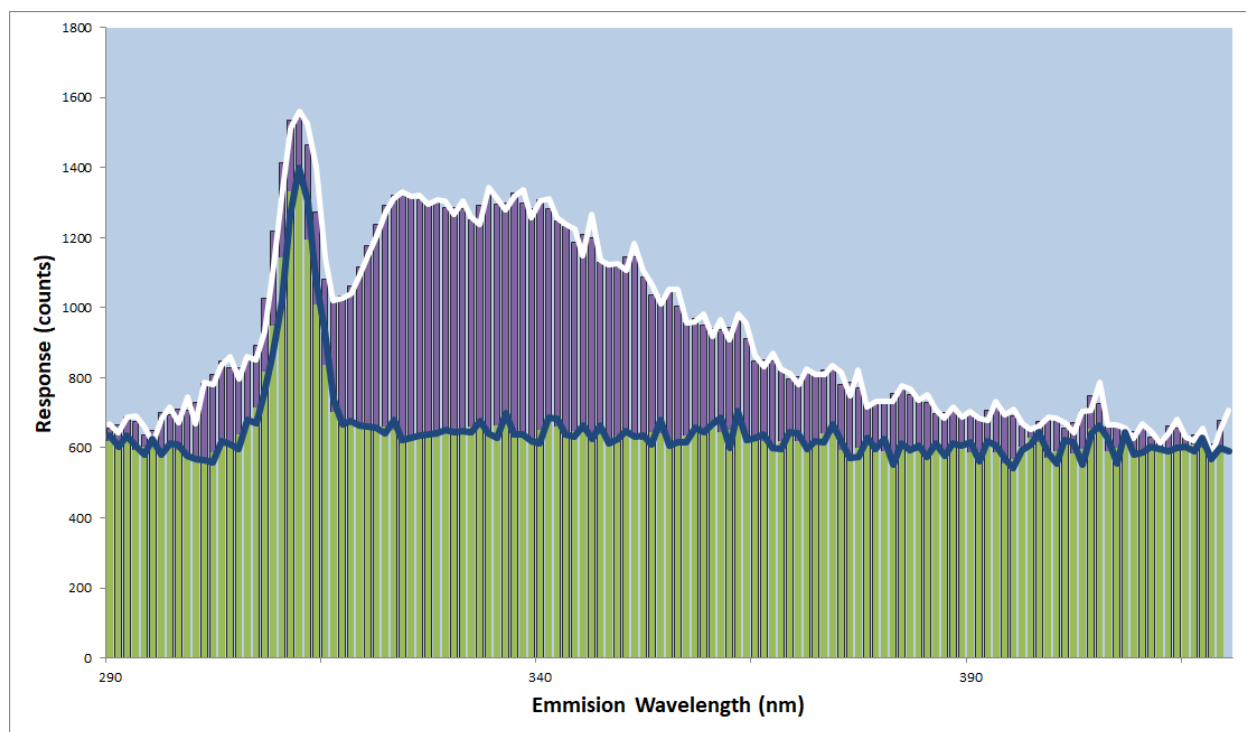


Figure 5.2.3.1. Fluorescence hydrocarbon analysis. Areas of samples were approximated by taking the sum of the areas of 1 nm unit rectangles of the analyte (purple bars), and subtracting the areas of the corresponding control or blank (green bars). The same process was conducted with a standard dilution series in order to correlate area to dissolved oil concentration.

5.3. Accompanying Small-Scale Experiments to Determine Copepod Inactivation and Recovery

5.3.1. *Preparation of Water-Accommodated Fractions of Oil (WAF)*

Experiment 1: WAF In order to test the effect of unevaporated versus evaporated Alaska North Slope oil dissolved in seawater, we created two water-accommodated fractions (WAF) of ANS. The same source of filtered seawater was used for these WAFs as was used in the tower experiments. Oil was placed in an aspirator bottle along with filtered seawater to achieve a concentration of 1 gram oil per liter of seawater. This was done for fresh, unevaporated ANS oil, as well as 22% by mass evaporated ANS oil. A solvent-cleaned stir bar was added to each bottle and they were set at a low speed on a magnetic stir plate to stir for 21 hours. At this point, the bottles were removed from the stir plate and the bottom $\frac{4}{5}$ of the solution was drained, leaving any surface oil slick intact. Both treatments were filtered through a 0.7 μ m pore size glass fiber filter to remove any remaining oil droplets. The WAFs were then diluted to 30% of their original strength with filtered seawater in order to mimic the concentrations that we had seen in initial tower experiments.

Experiment 2: Oil Slick: A small-scale experiment was conducted by adding a small slick of oil to the top of each replicate containing 10 copepods and 250mL filtered seawater. Half of the experimental replicates had evaporated ANS oil alone added to the top of the water, and the other half had evaporated ANS oil mixed with the dispersant, Corexit 9500 (20:1 v/v, oil to dispersant ratio). The replicates and controls were then monitored over the course of 4 hours, at which time individuals were moved into fresh seawater (see Section 5.3.3.)

Experiment 3: Filtered and Unfiltered CEWAFs: Another small-scale experiment was performed in order to test the effect of droplets produced by a chemical dispersant on the activity of copepods. A chemically enhanced WAF (CEWAF) was made by adding 1.5 grams of evaporated ANS oil to an aspirator bottle along with 1.5 liters of filtered seawater (from the same source of filtered seawater used for tower experiments), as well as 95 μ L of Corexit 9500 dispersant. A solvent-cleaned stir bar was added to the bottle and it was set at a low speed on a magnetic stir plate for 2 hours. The bottle was then removed from the stir plate and the bottom $\frac{4}{5}$ of the solution was drained. Half of the solution was filtered through a 0.7 μ m pore size glass fiber filter to remove any droplets. Both the filtered and unfiltered CEWAFs were subsequently diluted to 30% concentration with filtered seawater.



Figure 5.3.1.1. Prepared WAF and CEWAF water for small-scale experiments

5.3.2. Experimental Setup of Small-Scale Experiments to test Copepod Inactivation

In all three small-scale experiments, five replicates of control and treatment water were prepared as per section 5.3.1., and chilled to a temperature of 15 °C in 250 mL clear, plastic containers. Ten *Calanus* were carefully transferred into each container totaling 50 individuals per treatment. During treatment, containers were kept at low light and constant temperature. At the time points, 0.5 hr, 1 hr, 2 hr, and 4 hr, containers were visually analyzed to determine the status of all *Calanus*. Individuals were categorized into three groups: active, inactive and dead (Figure 5.3.2.1). Individuals were categorized as “active” if visually moving or suspended mid-water columns. “Inactive/deactivated” individuals were categorized as such if they were laying motionless on the bottom of the container, or floating at the top, and still showed signs of being alive. Dead individuals were determined by the opacity of the organism, as well as antennae placement, as dead individuals typically draw antennas to rest on sides. In the oil slick experiment, individuals trapped in oil were categorized as “inactive”.

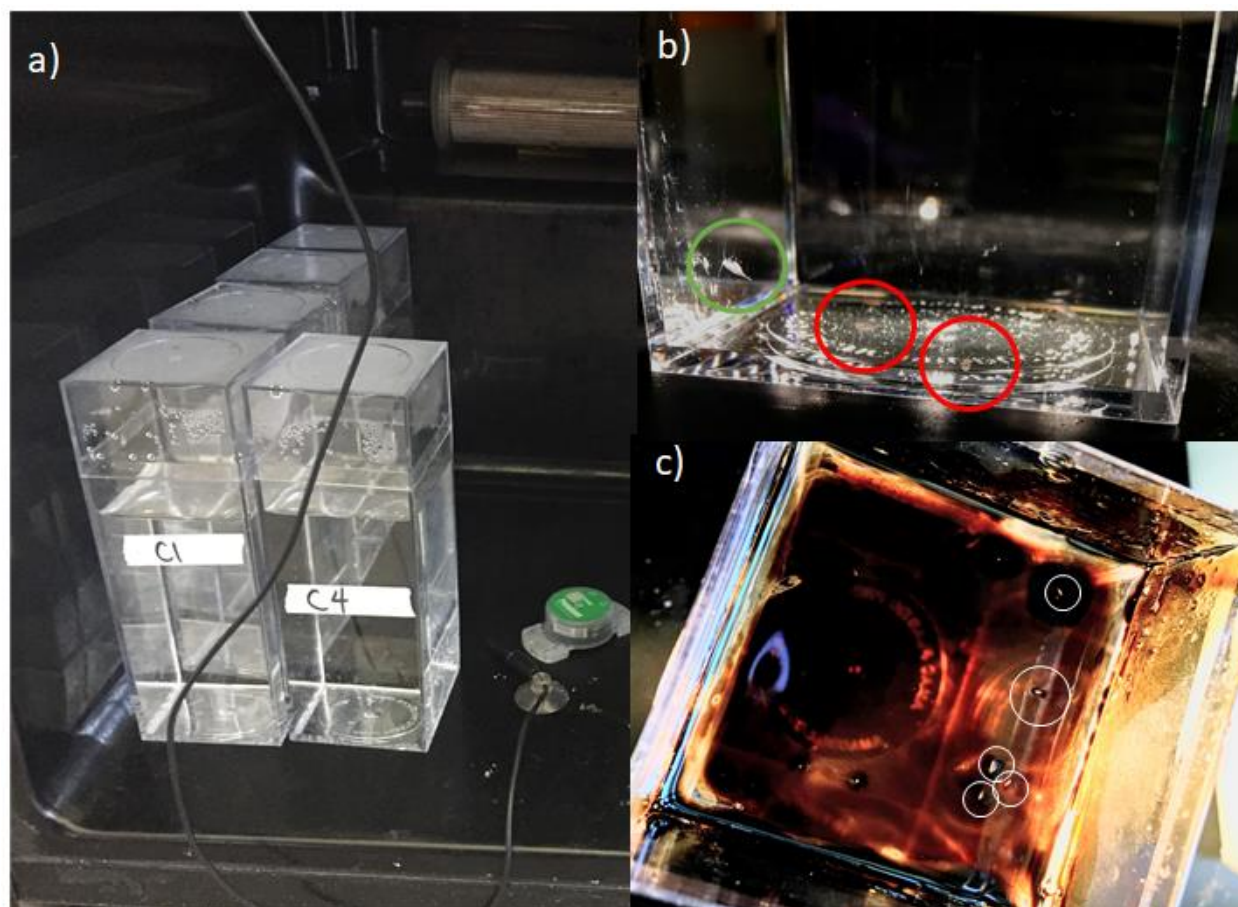


Figure 5.3.2.1. Categorization criteria for the accompanying small-scale experiments. a) Five replicates of 250mL chambers held in a temperature controlled incubator. b) Side view into chamber depicting a copepod categorized as active (green circle), and two copepods lying on the bottom of chamber, categorized as inactive (red circle). c) Top view into experimental replicate with oil. White circles identify individual *Calanus finmarchicus* trapped in the oil slick. These individuals were categorized as inactive.

5.3.3. Methods to Test Copepod Recovery

In the experiment testing effects of filtered and unfiltered CEWAF on copepod deactivation, we also tested the potential for recovery after exposure. After copepods were counted and categorized at the 4 hour mark, individuals were pipetted out and placed into fresh, pre-equilibrated seawater to test recovery rate. Individuals were visually inspected again using the same methodology at time periods 5 hr, 6 hr, and 24 hr after experimental start (1 hr, 2 hr, and 20 hr post water change). A two-way ANOVA was performed to compare the number of inactivated copepods between treatments and at each time point pre and post water change. Tukey-Kramer post hoc test was used to compare means within groups when significant factors were identified.

5.4. Data Evaluation

5.4.1. Copepod Behavioral Data Analysis & Statistics

For detailed description on the methodology of data collection and analysis of copepod behavior, see sections 4.2 (Copepod Mesocosm), 4.5 (Video Imaging) and 4.7 (Calanus behavior monitoring).

We calculated the average abundance of copepods in each region of the tank (C1-C4) for control periods by averaging the quantity of copepods in the view of the camera every 2 minutes for the 2 hours just prior to the addition of the oil treatment. This value, for each camera, was assumed to represent the pretreatment abundance of *Calanus*. All subsequent counts recorded after the start of the treatment were normalized to the control values to calculate the change in the proportion of individuals in frame, over time, relative to the initial control value. The normalized changes in the number of animals at all time points were then averaged between replicates. To determine the change in the abundance of copepods in the entire column over time, the process was repeated using the sum of counts of all cameras and normalizing them to the total of the controls. Tukey-Kramer post hoc tests were used to compare means between cameras.

To compare control distributions between treatments and cameras in an unstratified chamber, a two-way ANOVA was conducted on all individual replicates of unstratified control periods. In unstratified controls (oil and oil+dispersant) no difference was found between control distribution based on treatments. Therefore we pooled oil and oil+dispersant controls. A one-way ANOVA was then run to examine the effect of camera placement on average relative distribution to determine preferential vertical distribution of copepods in control conditions. Tukey-Kramer post hoc tests were used to compare means between cameras. This process was repeated to analyze change in abundance of copepods in the whole column.

For all treatment groups, simple linear regression analysis was used to investigate the relationship between time since start of experiment and proportion in frame relative to control when averaged across all replicates. All time points were included at a resolution of 30 counts per hour per replicate. Results were analyzed for each camera frame and all cameras summed, as described above. For the noteworthy replicate, described in section 6.2.4., data is not averaged with other replicate values and only represents a single experimental replicate.

To account for variability between replicates, a one-way ANOVA was also run for each treatment group at eight sampling time points. The mean of five discrete proportional counts (-4min, -2min, exact time, +2min, +4min) representing eight time points (start (0 hours), 10 min, 30 min, 1 hr, 2hr, 3 hr, 4 hr, and 5 hr) were extracted from each replicate. This allowed for preservation of variance between replicates. Within each treatment, a one-way ANOVA was run between the averages at the eight time points to determine where proportions in each camera differed over time. For replicates where video was missing due to obstruction from dispersant clouds, time points were excluded when there wasn't coverage from multiple replicates.

5.4.2. Hydrocarbon Data Evaluation

Oil hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs) and *n*-alkanes were analyzed both as total concentrations (sum of compounds dissolved in the water and present in oil droplets) and as dissolved concentrations (only the part that is dissolved in the aqueous phase).

Liquid/liquid extraction (using dichloromethane, DCM, as organic solvent for extraction), followed by GC/MS analysis was used to determine the total oil concentration (dissolved and particulate oil in the aqueous phase). GC/MS gave concentrations of individual PAHs and *n*-alkanes. Subsequently, these concentrations were summed as total PAHs (sum of all PAHs) and total alkanes (sum of all measured *n*-alkanes). Similarly, fluorescence analysis of the DCM extracts led to the total concentration of PAHs (since PAHs are the main fluorophores).

The SPME analysis was representative of the dissolved fraction of oil. This method is based on accumulation of soluble species on a hydrophobic fiber. See section xxx for details.

All results were converted to total PAHs per volume of seawater (μg total PAHs per L of seawater). We also measured *n*-alkanes in the samples. However, since alkanes are very insoluble compared to PAHs, we focused on the latter species. The *n*-alkane concentrations (along with that of the PAHs) are given in the Tables in the Appendix.

6. Results

6.1. Calanus Distribution in an unstratified water column without oil addition

Between all experiments within an unstratified water column, average water temperature during controls was $18.35\text{ }^{\circ}\text{C} \pm \text{SD } 7.6\text{ }^{\circ}\text{C}$ throughout the column. Average light intensity was $7.62\text{ lux} \pm \text{SD } 1.5\text{ lux}$. All treatment controls maintained temperatures between $17.41\text{ }^{\circ}\text{C}$ and $19.65\text{ }^{\circ}\text{C}$, with the exception of the “noteworthy replicate” as detailed below (section 6.2.4) which averaged at $16.4\text{ }^{\circ}\text{C}$ (Table 6.1.1).

Table 6.1.1. Average temperature and light levels (+/- SD) in each of the control periods within an unstratified experimental chamber.

Controls in unstratified column		
Replicate	Temperature ($^{\circ}\text{C}$)	Light Intensity, lux
O1	18.7 ± 0.07	9.21 ± 9.9
O2	18.5 ± 0.08	7.68 ± 3.22
O3*	16.4 ± 0.29	9.48 ± 6.48
O4	19.45 ± 0.14	7.80 ± 6.24
D1	19.47 ± 0.14	7.89 ± 6.30
D2	17.2 ± 0.45	5.62 ± 6.33
D3	17.2 ± 0.45	5.62 ± 6.33

Number of *Calanus finmarchicus* enumerated in a control period among all four cameras ranged from 32 to 179 individuals (Figure 6.1.1). When analyzed in a two-way ANOVA, there was no interactive effect between treatment and camera ($F_{(6,27)}=0.1.8526$, $p=0.1518$) (Table 6.1.2). However, camera location caused a statistically significant effect ($F_{(3,27)}=0.8.4923$, $p=0.0013$), indicating that there is a non-random distribution of copepods vertically in the experimental chamber. There was no significant difference between treatments, therefore we pooled all paired controls to determine a preferred distribution of copepods before the addition of any pollutants (See table 6.1.2).

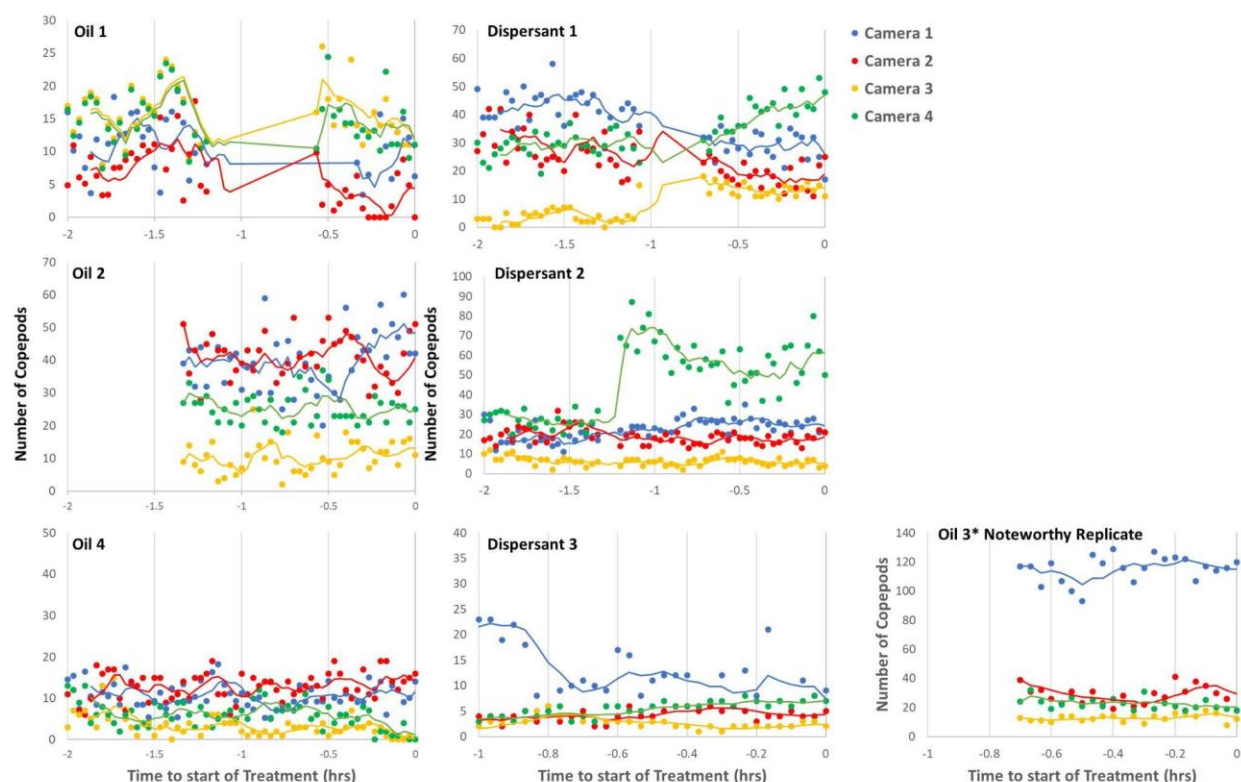


Figure 6.1.1. Raw total number of *Calanus finmarchicus* in each of the camera views depicted for each of the control period replicates in an unstratified water column. Counts are depicted every 2 minutes of footage. Line represents a 5-point moving average. *Oil 3 is included in this control data, but is marked as an outlier, and is removed from treatment analysis as a “noteworthy replicate.”

Table 6.1.2. Two-way ANOVA results testing the effect of subsequent treatment and camera placement on the average relative (vertical) distribution of *Calanus finmarchicus* in unstratified experimental chamber during control period.

2-way ANOVA, all unstratified controls					
Analysis of Variance					
Source of Variation	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	11	0.43877131	0.039888	2.9247	0.0253
Error	16	0.21821214	0.013638		
C. Total	27	0.65698345			
Effects Test					
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	2	2	3.33E-19	0	1
Camera	3	3	0.34747303	8.4926	0.0013
Treatment*Camera	6	6	0.15159886	1.8526	0.1518

Among all controls, an average of 40.0% (SD=+/-17.4%) of individuals occupied the top of the water column captured by Camera 1 (Figure 6.1.2). The upper-mid column (Camera 2) held an average of 23.5% (SD=+/- 9.9%) of the total *Calanus*, the lower-mid column (Camera 3) held 11.4% (SD=+/- 9.3%), and the bottom of the chamber (Camera

4) held 25.1% (SD= $\pm 11.5\%$). Though Cameras 2 and 4 did not differ significantly from cameras 1 and 3, Camera 1 had a significantly higher occupation of individuals than Camera 3 (ANOVA, $F_{(3,27)}=6.2123$, $p=0.0028$; Table 6.1.3). Furthermore, if grouped into halves of the water column, during control conditions 37% of individuals occupied the upper half of the water column, while 40% stayed in the bottom, indicating a preference for upper water column.

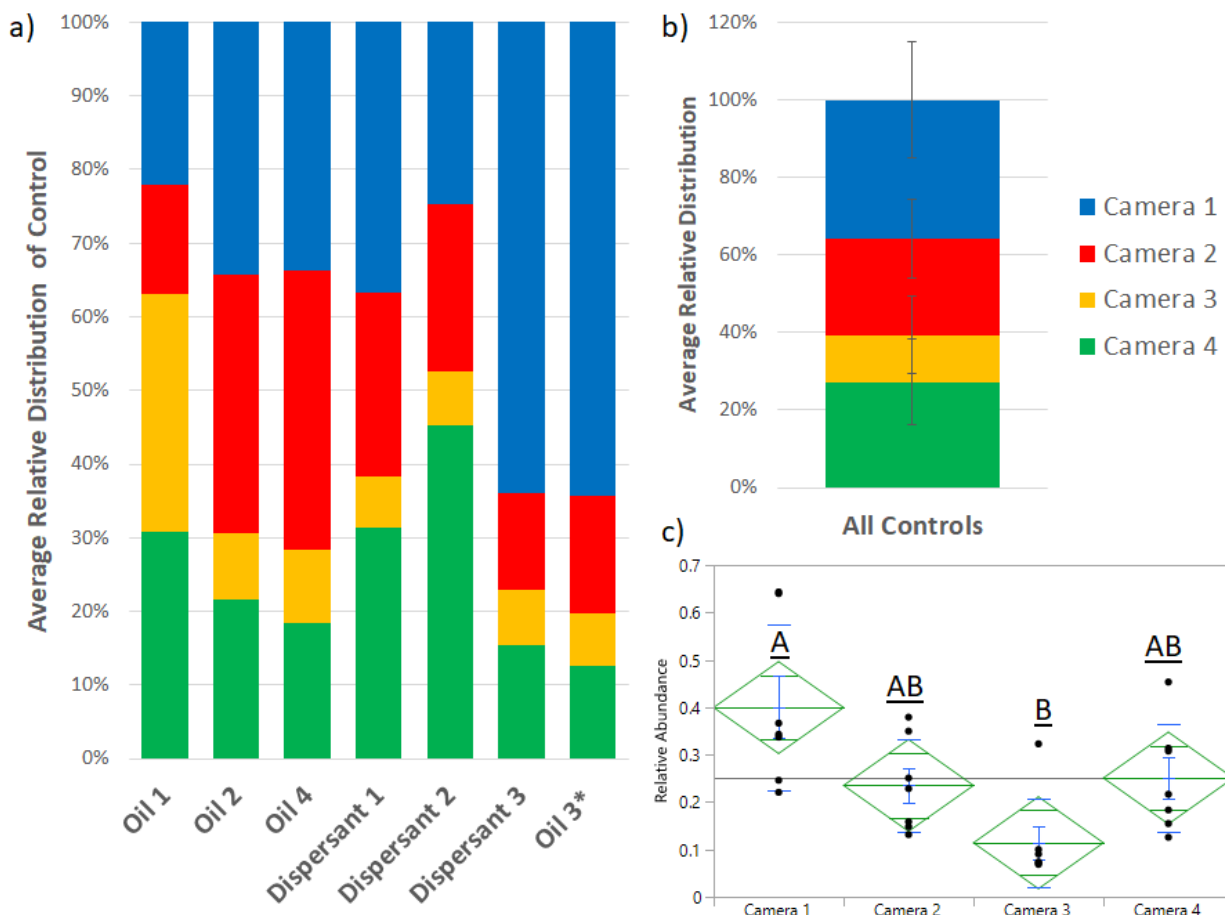


Figure 6.1.2. Distribution of *Calanus finmarchicus* in unstratified controls a) Average relative distribution of copepods in each replicate in the 2 hours prior to addition of oil or oil and dispersant. *Oil 3 is included in this control data, but is marked as an outlier, and is removed from later analysis as a “noteworthy replicate.” Camera 1 captures the top of the water column, while camera 4 captures the bottom. b) Average relative distribution across cameras views of all unstratified controls, with error bars representing standard deviation. c) Graph depicting relative abundance mean and standard deviations by camera. Variables not connected by same letter are significantly different, as determined by a post-hoc Tukey-Kramer test.

Table 6.1.3. One-way ANOVA results that tested the effect of camera placement on the average relative (vertical) distribution of *Calanus finmarchicus* in an unstratified experimental chamber during the control period. Camera 1 represents the top of a water column, and Camera 4 represents deeper in a water column. Post hoc Tukey-Kramer test also shown.

1-Way ANOVA, pooled controls by camera					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Camera	3	0.28717245	0.095724	6.2123	0.0028
Error	24	0.369811	0.015409		
C. Total	27	0.65698345			
Means for Oneway Anova					
Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Camera 1	7	0.399606	0.04692	0.30277	0.49644
Camera 2	7	0.235279	0.04692	0.13845	0.33211
Camera 3	7	0.11426	0.04692	0.01743	0.21109
Camera 4	7	0.250856	0.04692	0.15402	0.34769
Ordered Differences Report (Tukey-Kramer HSD)					
Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
C1 v. C3	0.2853456	0.0663514	0.102308	0.4683833	0.0013
C1 v. C2	0.164327	0.0663514	-0.018711	0.3473648	0.0895
C1 v. C4	0.14875	0.0663514	-0.034288	0.3317877	0.1407
C4 v. C3	0.1365956	0.0663514	-0.046442	0.3196333	0.1953
C2 v. C3	0.1210186	0.0663514	-0.062019	0.3040563	0.2871
C4 v. C2	0.015577	0.0663514	-0.167461	0.1986147	0.9953

6.2. Non-Dispersed Oil in an Unstratified Water Column

6.2.1. Water Conditions within the tank

During the oil-only treatment, in replicates 1, 2, and 4, water temperature averaged at $18.7\text{ }^{\circ}\text{C} \pm \text{SD } 7.9\text{ }^{\circ}\text{C}$ throughout the column (Table 6.2.1.1). Average light intensity was $8.7\text{ lux} \pm \text{SD } 2.8\text{ lux}$. Replicate 3 was removed from later pooled analysis as an outlier due to differences in oil and *Calanus* behavior. However, this replicate also held a lower temperature average ($\mu = 16.5\text{ }^{\circ}\text{C} \pm \text{SD } 0.43\text{ }^{\circ}\text{C}$).

Table 6.2.1.1. Average temperature and light levels (+/- SD) in each of the Oil treatment periods within an unstratified water column. *Oil 3 is marked as an outlier, and is removed from treatment analysis as a “noteworthy replicate.”

Oil Only		
Replicate	Temperature ($^{\circ}\text{C}$)	Light Intensity, lux
1	18.7 ± 0.16	7.0 ± 4.4
2	18.6 ± 0.10	11.9 ± 1.6
3*	16.5 ± 0.43	11.0 ± 7.8
4	18.7 ± 0.18	7.1 ± 5.7

6.2.2. Oil Distribution in the Copepod Tower

In the unstratified water column, an oil slick added without dispersants led to a characteristic hydrocarbon profile in the water column. Upon addition of an approx. $100\text{ }\mu\text{m}$ oil slick on top of the water column, PAH concentrations in the underlying water column slowly started to increase. In the uppermost sampling port (Port 1), PAH concentrations remained below $5\text{ }\mu\text{g L}^{-1}$ for at least 5 h in three out of four replicates. One replicate had a different PAH distribution than the other treatments, and will be discussed separately below (“noteworthy replicate”, see section 6.2.4.). Over time, samples throughout the water column (Ports 1 through 5) increased in PAHs, reaching values between 5 and $60\text{ }\mu\text{g L}^{-1}$ after 24 h.

In the three replicates shown in Figures 6.2.2.1-3, no large hydrocarbon gradient was observed with depth. The mixing energy introduced with the air stream blowing on top of the water column apparently led to vertical mixing that was faster than the mass transfer of hydrocarbons from the oil film into the water column.

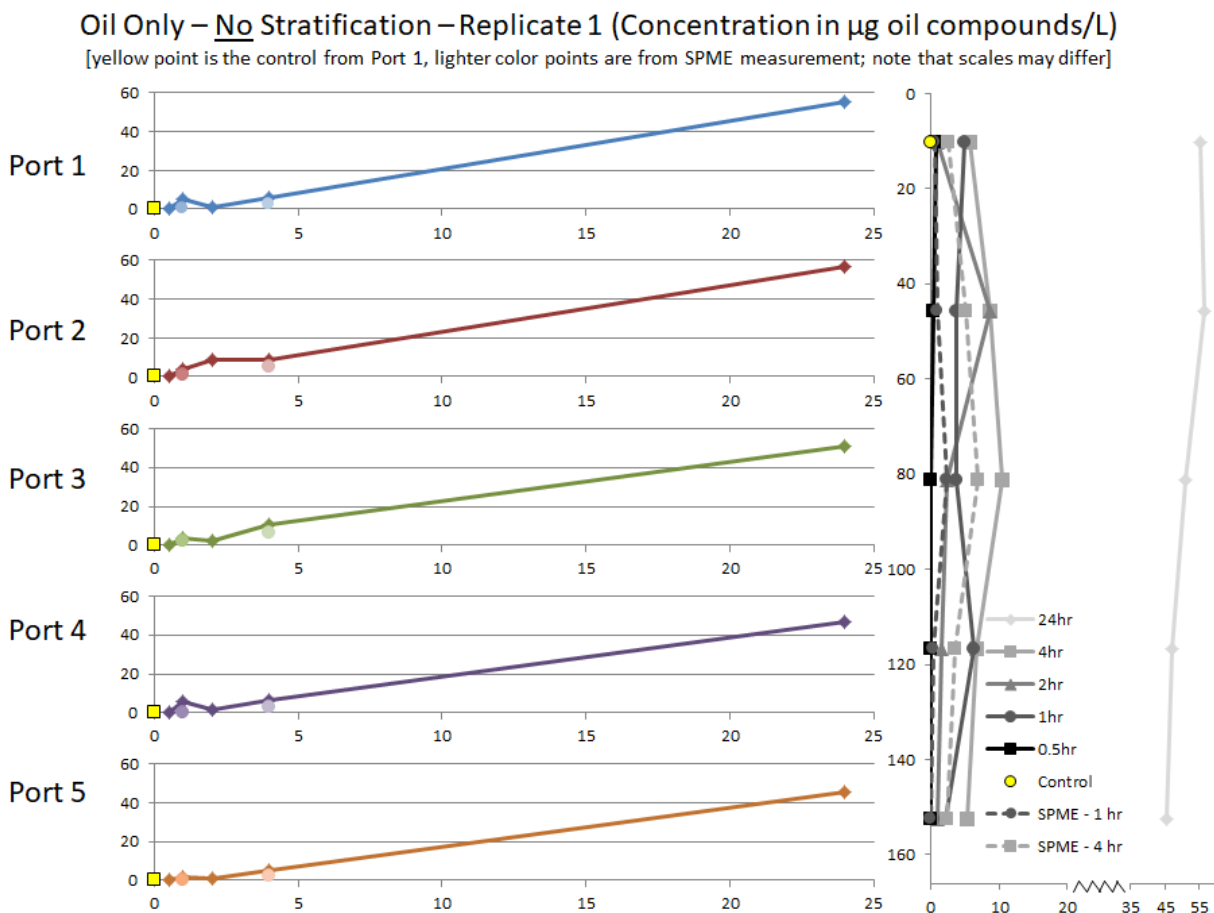


Figure 6.2.2.1. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil Only - Replicate 1 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from fluorometry analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

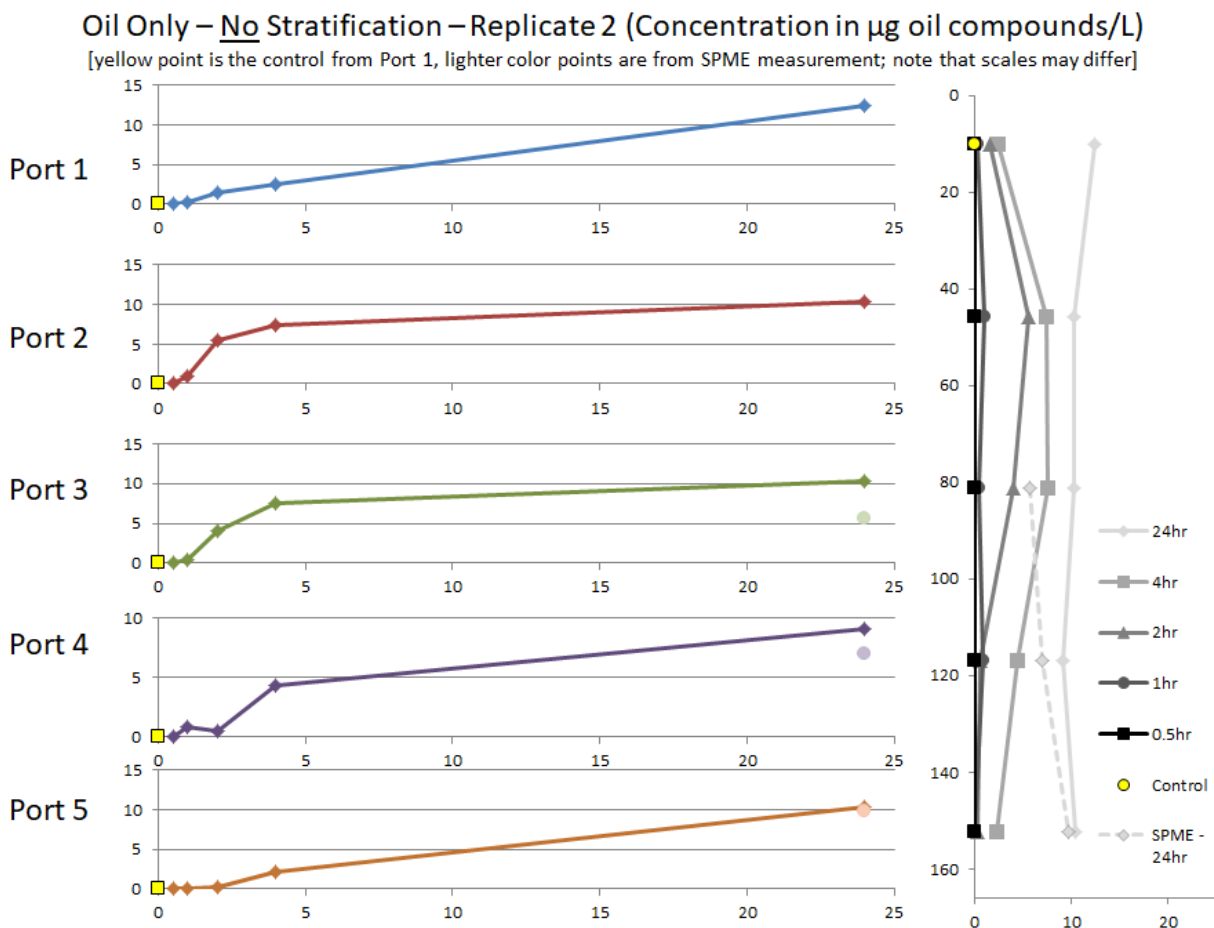


Figure 6.2.2.2. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil Only - Replicate 2 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from fluorometry analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

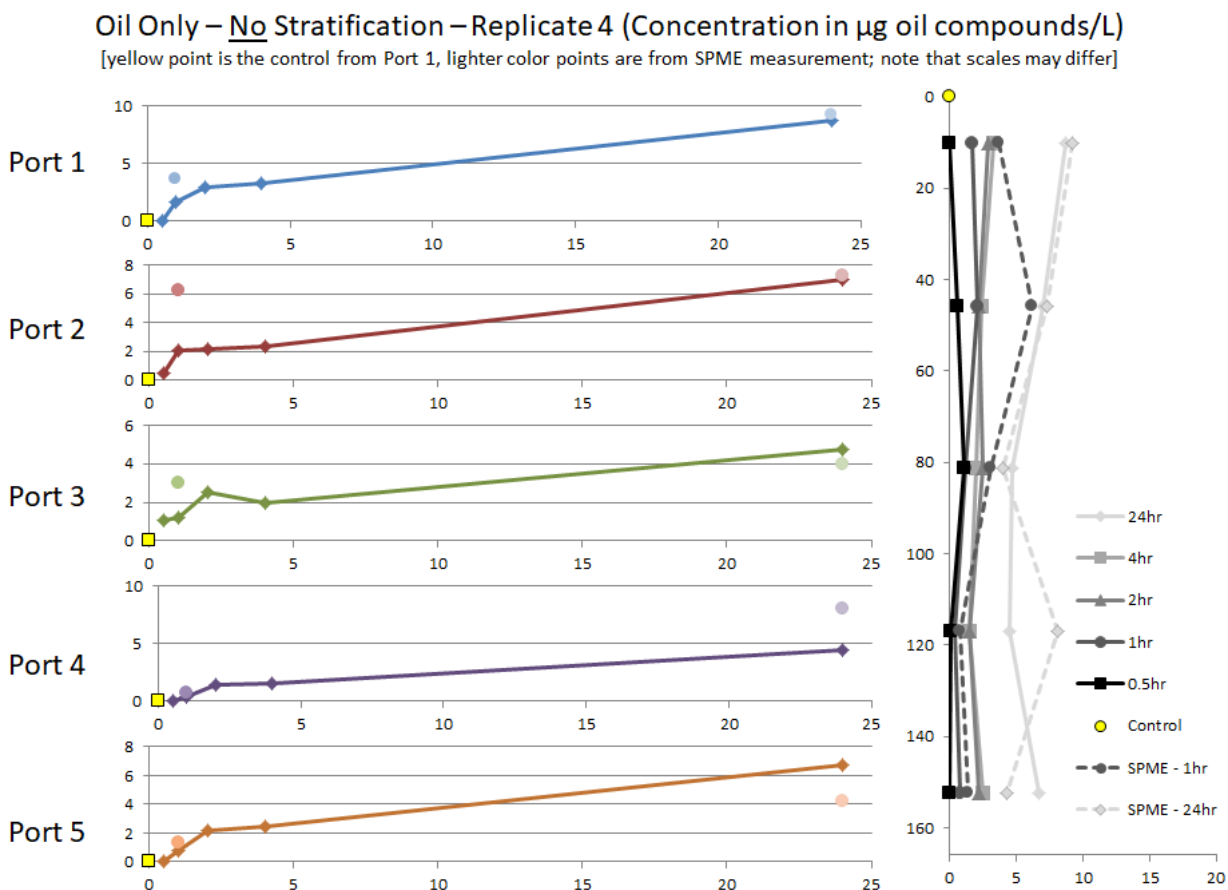


Figure 6.2.2.3. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil Only - Replicate 4 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from fluorometry analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

6.2.3. *Calanus finmarchicus* Distribution

Over the 5 hour observation period, behavior varied between the four replicates. Though the number of animals observable in all four frames at the first time point varied between 28 and 168 (Figure 6.2.3.1). The third replicate had a different behavior pattern than the others as well as a different oil behavior as described in the next section. For analysis, we removed this replicate from the pool of unstratified oil treatments as an outlier and analyzed it independently as a “noteworthy replicate”.

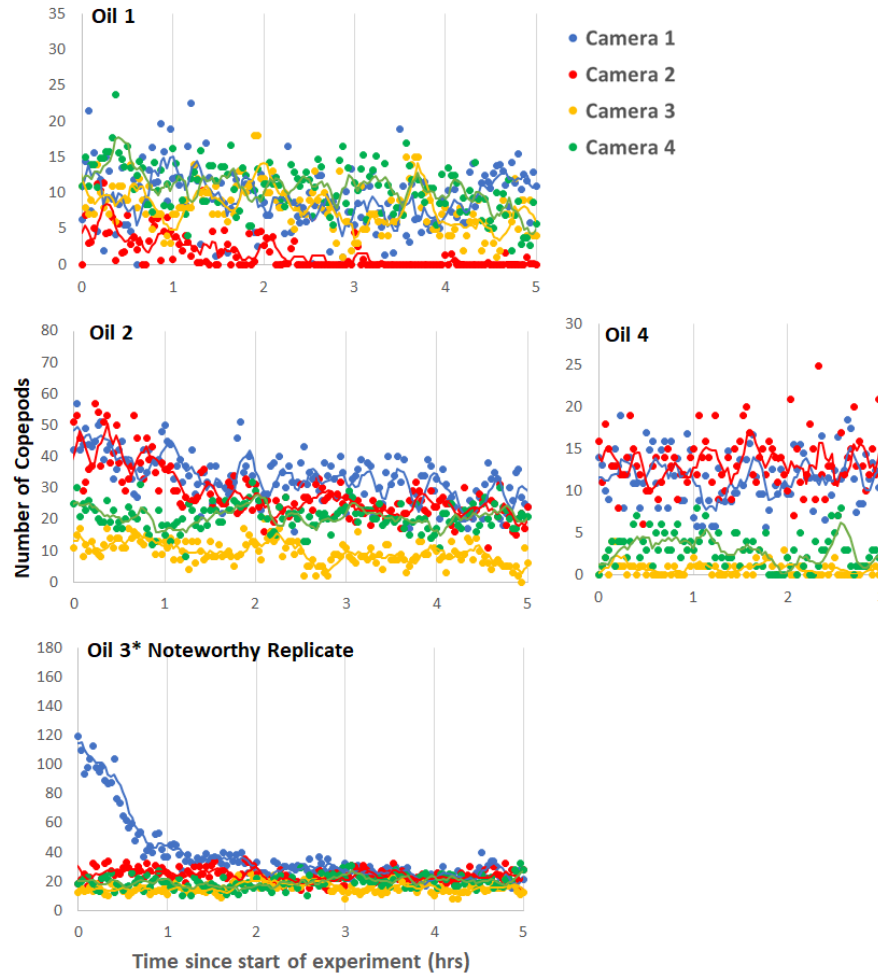


Figure 6.2.3.1. Total number of *Calanus finmarchicus* in each of the camera views depicted for each of the oil only replicates in an unstratified water column. Counts were taken every 2 minutes for the entirety of the video. Line represents a 5-point moving average.

During the five hour observation period after the addition of oil, linear regression was used to test if hours since addition of oil significantly predicted the proportion of *Calanus finmarchicus* observable across all four cameras, when averaged between three replicates (Figure 6.2.3.2). We found a significant negative decline since the start of the experiment of the proportion of copepods present in view ($R^2 = 0.18$, $F_{(1, 150)} = 32.3603$, $p < .0001$), indicating a drop of 27% from all frames. When separated by camera view, there was a significant negative relationship between time since addition of oil and relative proportion of *C. finmarchicus* in frame in Camera's 1-3 (C1: 28% drop; C2: 74% drop; C3: 13% drop), but Camera 4 saw no statistically significant decline in proportional abundance (Table 6.2.3.1).

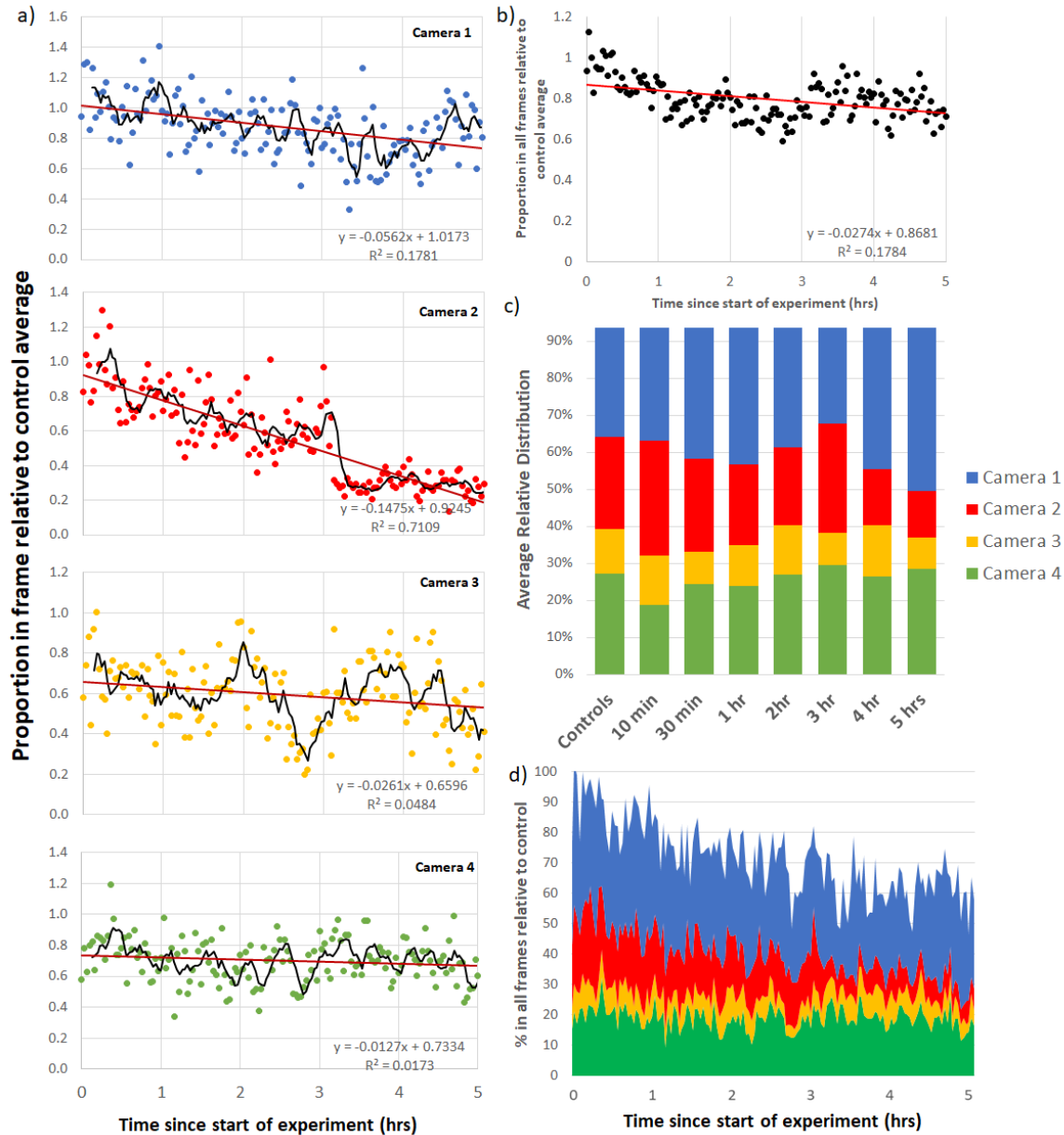


Figure 6.2.3.2 Distribution of *Calanus finmarchicus* over time and between camera view fields (vertical distribution in the experimental chambers) after oil is added to the top of the unstratified water column. For all figures, data is averaged between three replicates (O1, O2, O4). a) Proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period, separated by camera. Colored dots depict a count every 2 minutes of footage. Black line represents a 5-point moving average. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. b) Total proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period when all four camera field views are included. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. c) Average relative distribution of *Calanus finmarchicus* between the four camera views during the controls, at 10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr. d) Percent abundance of *Calanus finmarchicus* in view of each camera over the five hour observation period relative to the average of all camera viewfields combined.

Table 6.2.3.1. Summary of regression analysis used to evaluate the predictive power of time after oil-only addition on the averaged proportional abundance relative to control in each camera and summed cameras. Regression statistics and ANOVA tables for each grouping shown.

Oil Camera 1						
Regression Statistics		ANOVA				
RSquare	0.178129	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.172613	Model	1	1.0085685	1.00857	32.2936
Root Mean Square Error	0.176723	Error	149	4.6534456	0.03123	Prob > F
Mean of Response	0.876655	C. Total	150	5.6620141		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	1.0172759	0.028621	35.54	<.0001
		Time	-0.056248	0.009898	-5.68	<.0001
Oil Camera 2						
Regression Statistics		ANOVA				
RSquare	0.710902	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.708962	Model	1	6.9333479	6.93335	366.3963
Root Mean Square Error	0.137561	Error	149	2.8195392	0.01892	Prob > F
Mean of Response	0.555838	C. Total	150	9.7528871		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.9245329	0.022278	41.5	<.0001
		Time	-0.147478	0.007705	-19.14	<.0001
Oil Camera 3						
Regression Statistics		ANOVA				
RSquare	0.048409	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.042022	Model	1	0.2178551	0.217855	7.5798
Root Mean Square Error	0.169533	Error	149	4.2824772	0.028741	Prob > F
Mean of Response	0.594237	C. Total	150	4.5003323		0.0066
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.6595921	0.027456	24.02	<.0001
		Time	-0.026142	0.009495	-2.75	0.0066
Oil Camera 4						
Regression Statistics		ANOVA				
RSquare	0.017254	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.010659	Model	1	0.0512425	0.051242	2.616
Root Mean Square Error	0.139957	Error	149	2.9186263	0.019588	Prob > F
Mean of Response	0.701721	C. Total	150	2.9698687		0.1079
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.733417	0.022666	32.36	<.0001
		Time	-0.012679	0.007839	-1.62	0.1079
Oil ALL Cameras						
Regression Statistics		ANOVA				
RSquare	0.178431	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.172917	Model	1	0.239644	0.239644	32.3603
Root Mean Square Error	0.086055	Error	149	1.1034187	0.007405	Prob > F
Mean of Response	0.799554	C. Total	150	1.3430628		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.8680995	0.013937	62.29	<.0001
		Time	-0.027418	0.00482	-5.69	<.0001

To account for high variation between replicates, we also tested 8 time points (0 m, 10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr) as a one-way ANOVA between the three replicates. When exposed to oil only treatment, there was no statistically significant change in the number of animals within frame relative to the control in any of the cameras during the first 5 hours (Figure 6.2.3.3; Table 6.2.3.2). Though there is some indication that numbers were decreasing over that period. When averaged across replicates, the proportion of animals in camera 1 dropped 30% , camera 2 dropped 63%, camera 4 dropped 35% and camera 4 dropped 25% . There were very few animals in camera frames 2 and 3 during the course of the experiment, possibly impacting the variability of the results to make a change undetectable.

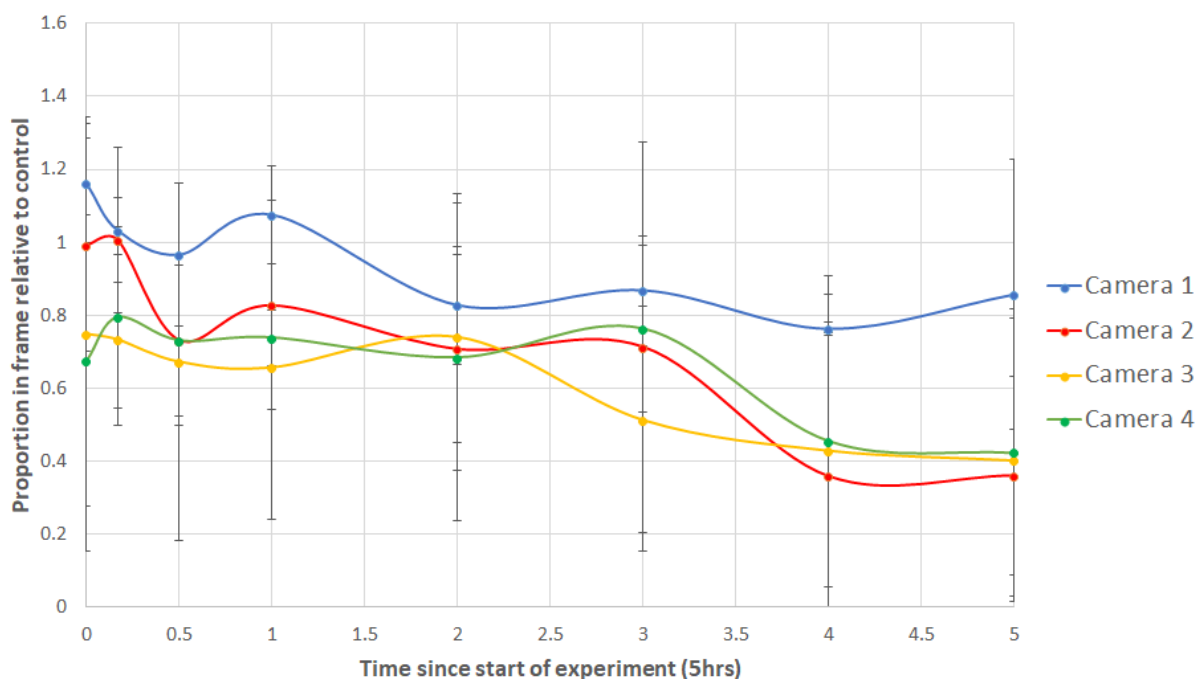


Figure 6.2.3.3. Average proportion in frame (relative to paired control) of replicate 1, 2, and 4 of oil-only treatments at eight discrete time points (10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr) after addition of oil to experimental chamber. Error bars represent standard deviation. No statistically significant difference was found in any camera (representative of depth in water column) over any time point.

Table 6.2.3.2 One-way ANOVA results by camera, testing the effect of eight discrete time points on proportion of animals in frame in oil-only, unstratified treatments.

Camera 1					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	0.35422879	0.050604	1.3965	0.2811
Error	14	0.50731667	0.036237		
C. Total	21	0.86154545			
Camera 2					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	1.0104167	0.144345	1.3848	0.2855
Error	14	1.4593333	0.104238		
C. Total	21	2.46975			
Camera 3					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	0.4239833	0.060569	0.3361	0.9256
Error	16	2.8834667	0.180217		
C. Total	23	3.30745			
Camera 4					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	0.4114625	0.05878	0.5594	0.7777
Error	16	1.6812	0.105075		
C. Total	23	2.0926625			

6.2.4. Noteworthy Replicate

The conditions in one of the replicates led to a strong hydrocarbon gradient through the column (Figure 6.2.4.1) and provides interesting observations of copepod response during a hydrocarbon gradient.

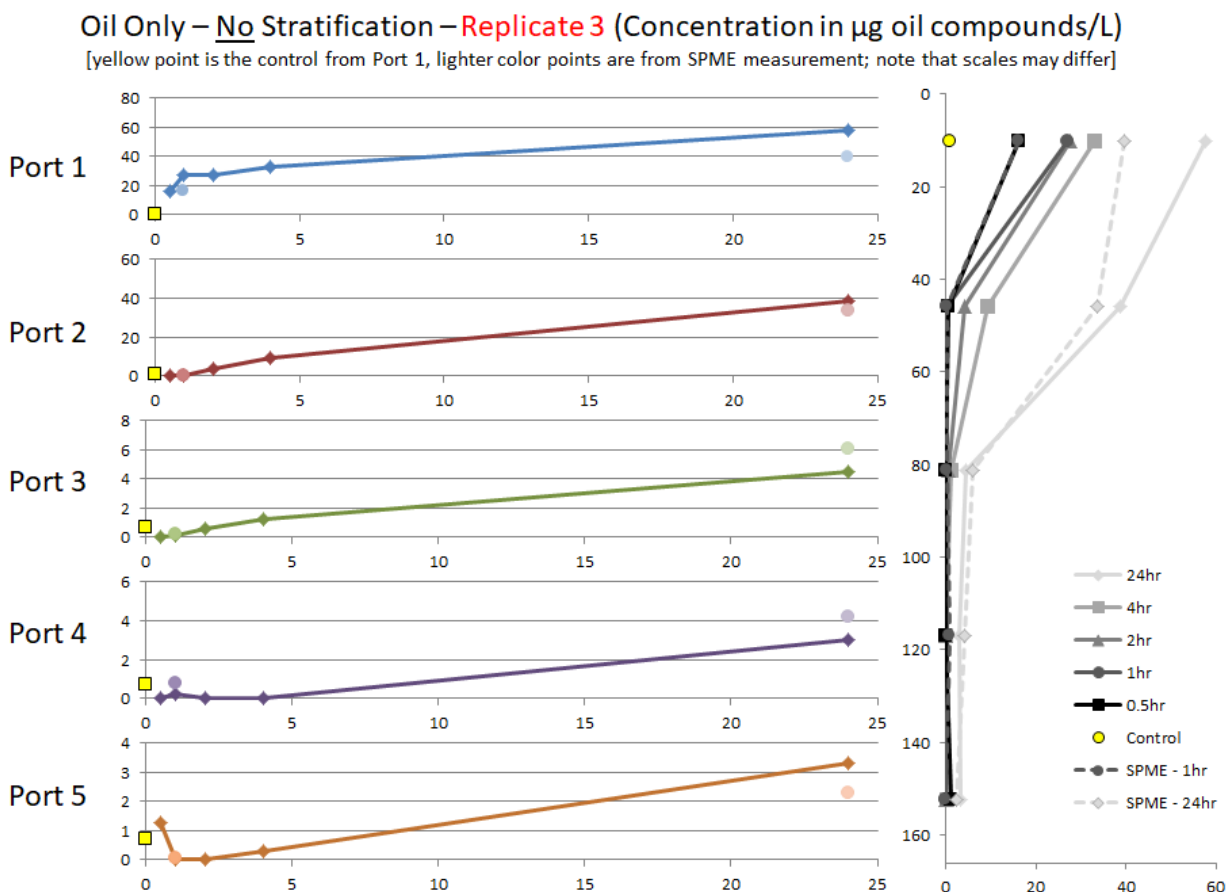


Figure 6.2.4.1. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil Only - Replicate 3 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from fluorometry analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses. In this replicate, a concentration gradient was established, and persisted throughout the experimental period.

Calanus behavior:

In addition to oil distribution and behavior, *Calanus finmarchicus* behavior also differed in the replicate 3 of the oil only treatment, leading us to separate this replicate from the other oil-only treatments as a “noteworthy replicate”, and exclude it from pooled analysis. During the control period, abundance was proportionally higher in camera 1, (near the surface of the column) compared to other cameras, but was not statistically different from the other controls. However, when oil was introduced to the tank, the number of individuals in the upper water column (camera 1) dropped precipitously within the first hour, unlike a gradual drop experienced in the other oil-only replicates (Figure 6.2.4.2).

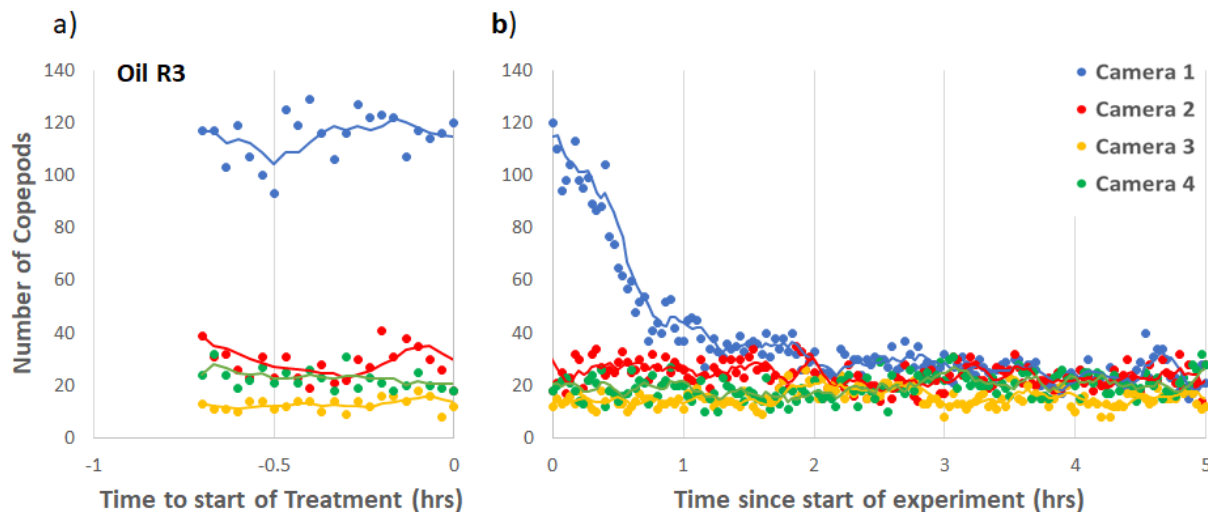


Figure 6.2.4.2. Total number of *Calanus finmarchicus* in each of the camera views depicted for the noteworthy, oil-only replicate (#3) in an unstratified water column. Graphs represent a) the control period and b) during the five hour observation period after oil was added. Counts are depicted every 2 minutes of footage. Line represents a 5-point moving average.

During the five-hour observation period after the addition of oil, linear regression was used to test if hours since addition of oil significantly predicted the proportion of *Calanus finmarchicus* observable across all four cameras. We found a significant negative decline, due to time after oil addition, in the proportion of copepods present in view ($R^2 = 0.47$, $F_{(1, 150)} = 134.64$, $p < .0001$), indicating a 61% drop across the whole chamber (Figure 6.2.4.3; Table 6.2.4.1).

When separated by camera view, there was a significant negative relationship between time since addition of oil and relative proportion of *C. finmarchicus* in frame in Camera's 1 and 2 (C1: 92% drop; C2: 22% drop), but abundance in Camera 3 could not be predicted by time since oil addition. Camera 4 had a significantly positive regression, and the abundance in that frame grew 20%, though the R^2 value was weak ($R^2 = 0.085$). However, the implications of that growth are that *Calanus finmarchicus* may have been accumulating at the bottom of the water column to avoid high oil concentrations at the surface (Figure 6.2.4.3; Table 6.2.4.1).

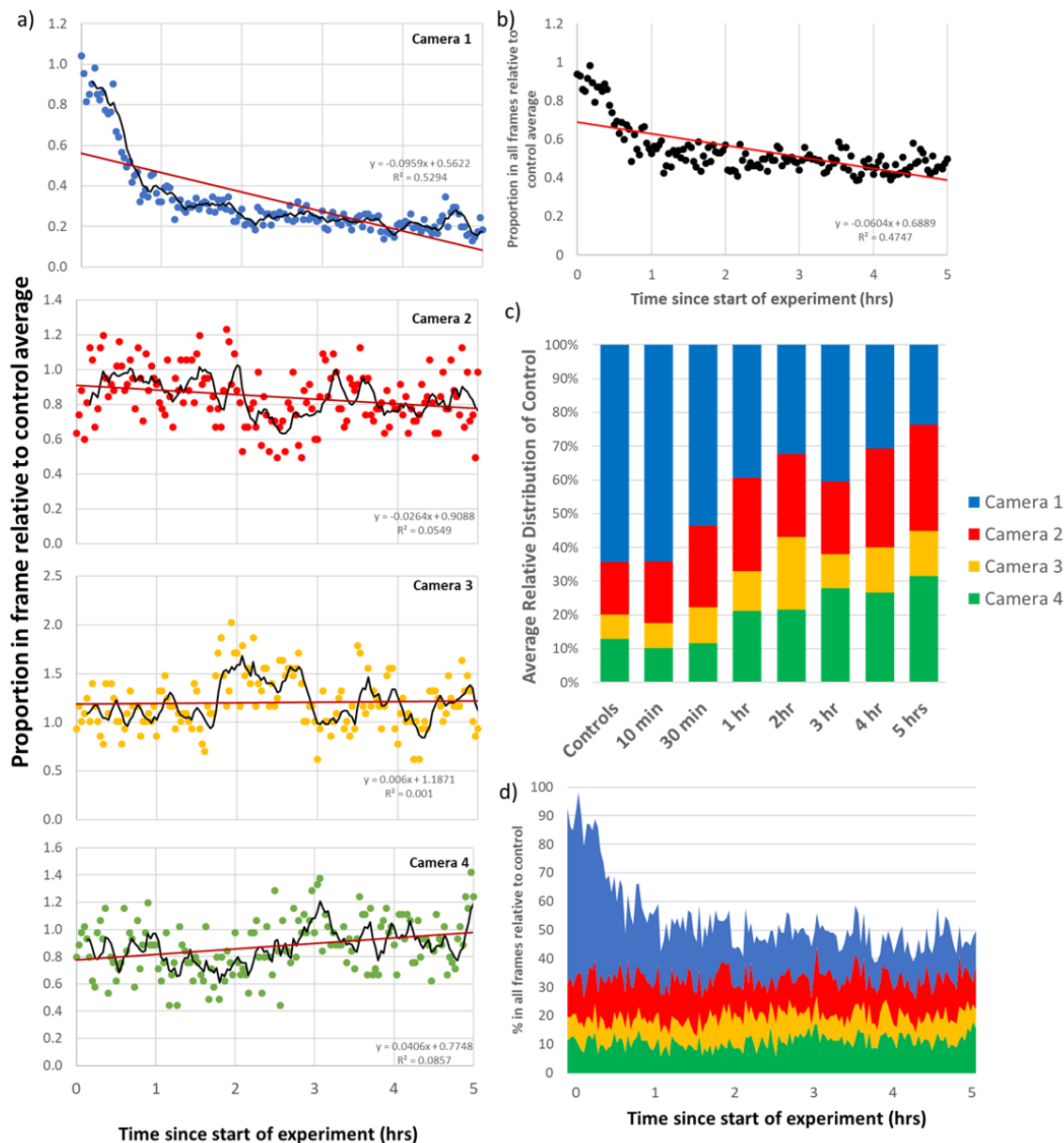


Figure 6.2.4.3. Distribution of *Calanus finmarchicus* over time and between camera view fields (vertical distribution in the experimental chambers) after oil is added to the top of the water column in “noteworthy replicate 3”. Data is from the “noteworthy replicate” only (O3). a) Proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period, separated by camera. Colored dots depict a count every 2 minutes of footage. Black line represents a 5-point moving average. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. b) Total proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period when all four camera field views are included. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. c) Average relative distribution of *Calanus finmarchicus* between the four camera views during the controls, at 10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr. d) Percent abundance of *Calanus finmarchicus* in view of each camera over the five hour observation period relative to the average of all camera viewfields combined.

Table 6.2.4.1 Summary of regression analysis used to evaluate the predictive power of time after oil-only addition on the averaged proportional abundance relative to control in each camera and summed cameras of the “noteworthy replicate”. Regression statistics and ANOVA tables for each grouping shown[1]

Oil R3* Camera 1						
Regression Statistics		ANOVA				
RSquare	0.529422	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.526264	Model	1	2.9307589	2.93076	167.6321
Root Mean Square Error	0.132224	Error	149	2.6050089	0.01748	Prob > F
Mean of Response	0.322484	C. Total	150	5.5357678		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.5621938	0.021414	26.25	<.0001
		Time	-0.095884	0.007406	-12.95	<.0001
Oil R3* Camera 2						
Regression Statistics		ANOVA				
RSquare	0.054851	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.048508	Model	1	0.2229504	0.22295	8.6472
Root Mean Square Error	0.160571	Error	149	3.8416752	0.025783	Prob > F
Mean of Response	0.842702	C. Total	150	4.0646256		0.0038
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.908817	0.026005	34.95	<.0001
		Time	-0.026446	0.008993	-2.94	0.0038
Oil R3* Camera 3						
Regression Statistics		ANOVA				
RSquare	0.001032	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	-0.00567	Model	1	0.011472	0.011472	0.1539
Root Mean Square Error	0.273008	Error	149	11.105436	0.074533	Prob > F
Mean of Response	1.202115	C. Total	150	11.116908		0.6954
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	1.1871178	0.044214	26.85	<.0001
		Time	0.0059991	0.015291	0.39	0.6954
Oil R3* Camera 4						
Regression Statistics		ANOVA				
RSquare	0.085711	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.079575	Model	1	0.5244101	0.52441	13.9682
Root Mean Square Error	0.19376	Error	149	5.5939234	0.037543	Prob > F
Mean of Response	0.876228	C. Total	150	6.1183336		0.0003
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.7748299	0.03138	24.69	<.0001
		Time	0.0405594	0.010852	3.74	0.0003
Oil R3* ALL Cameras						
Regression Statistics		ANOVA				
RSquare	0.474685	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.471159	Model	1	1.1616215	1.16162	134.6392
Root Mean Square Error	0.092885	Error	149	1.2855216	0.00863	Prob > F
Mean of Response	0.537952	C. Total	150	2.4471432		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.6888657	0.015043	45.79	<.0001
		Time	-0.060365	0.005202	-11.6	<.0001

6.3. Dispersed Oil in an Unstratified Water Column

6.3.1. Water Conditions within the tank

During the oil+dispersant treatment, water temperature averaged at $17.9\text{ }^{\circ}\text{C} \pm \text{SD } 0.18\text{ }^{\circ}\text{C}$ throughout the column (Table 6.3.1.1). Average light intensity was $4.5\text{ lux} \pm \text{SD } 2.2\text{ lux}$.

Table 6.3.1.1. Average temperature and light levels (+/- SD) in each of the Oil+Dispersant treatment periods within an unstratified water column.

Oil + Dispersant		
Replicate	Temperature ($^{\circ}\text{C}$)	Light Intensity, lux
1	18.7 ± 0.18	7.0 ± 5.72
2	17.5 ± 0.59	3.24 ± 2.15
3	17.5 ± 0.59	3.24 ± 2.15

6.3.2. Oil Dispersion

As described in the Method Section (Section [4.8.2](#)), the addition of dispersant led to a formation of dispersed oil in the water column. See [Figure 4.8.2.1](#) for an example.

6.3.3. Oil Distribution in the Copepod Tower

In the unstratified water column, an oil/dispersant mixture (20:1 v/v), led to a markedly different hydrocarbon distribution than the undispersed oil treatment discussed above. A large pulse of oil was detected in the top sampling port (Port 1) shortly after the initiation of the experiment, with PAH concentration of 3,000 to 13,000 $\mu\text{g L}^{-1}$ measured 0.5 to 1 h after the oil addition (Figures 6.3.3.1-5). This was at least a factor of 100 higher than measured in Port 1 in the undispersed treatments.

The measured PAHs were distributed between the dissolved phase (i.e., freely dissolved PAHs in water) and the oil phase in oil droplets (i.e., PAHs dissolved in oil droplets that were entrained in the water column). We used SPME measurements to estimate the amount of freely dissolved PAHs. After 1 h, approx. 100 to 300 $\mu\text{g L}^{-1}$ PAHs were dissolved in samples from Port 1. This was still approx. 10 times higher than in the non-dispersed treatments.

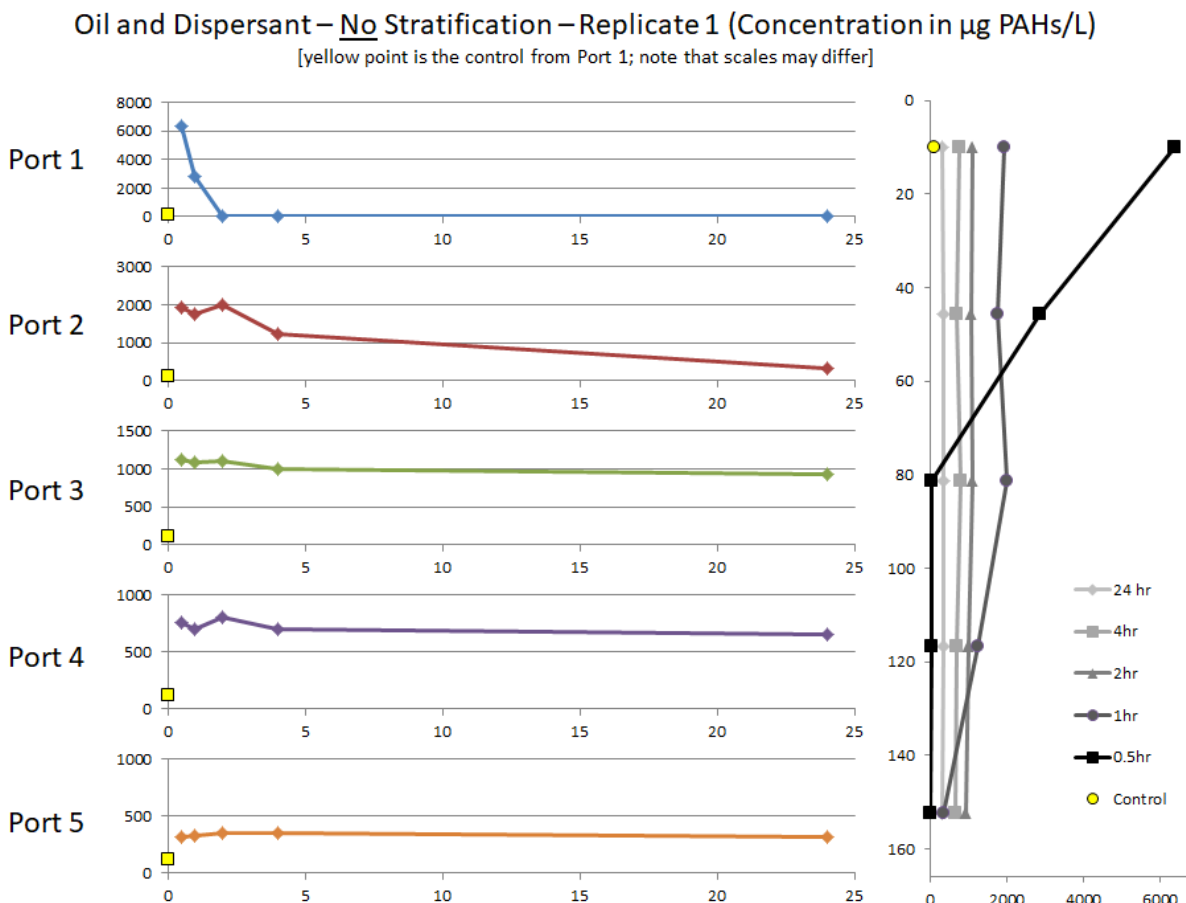


Figure 6.3.3.1. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Replicate 1 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm).

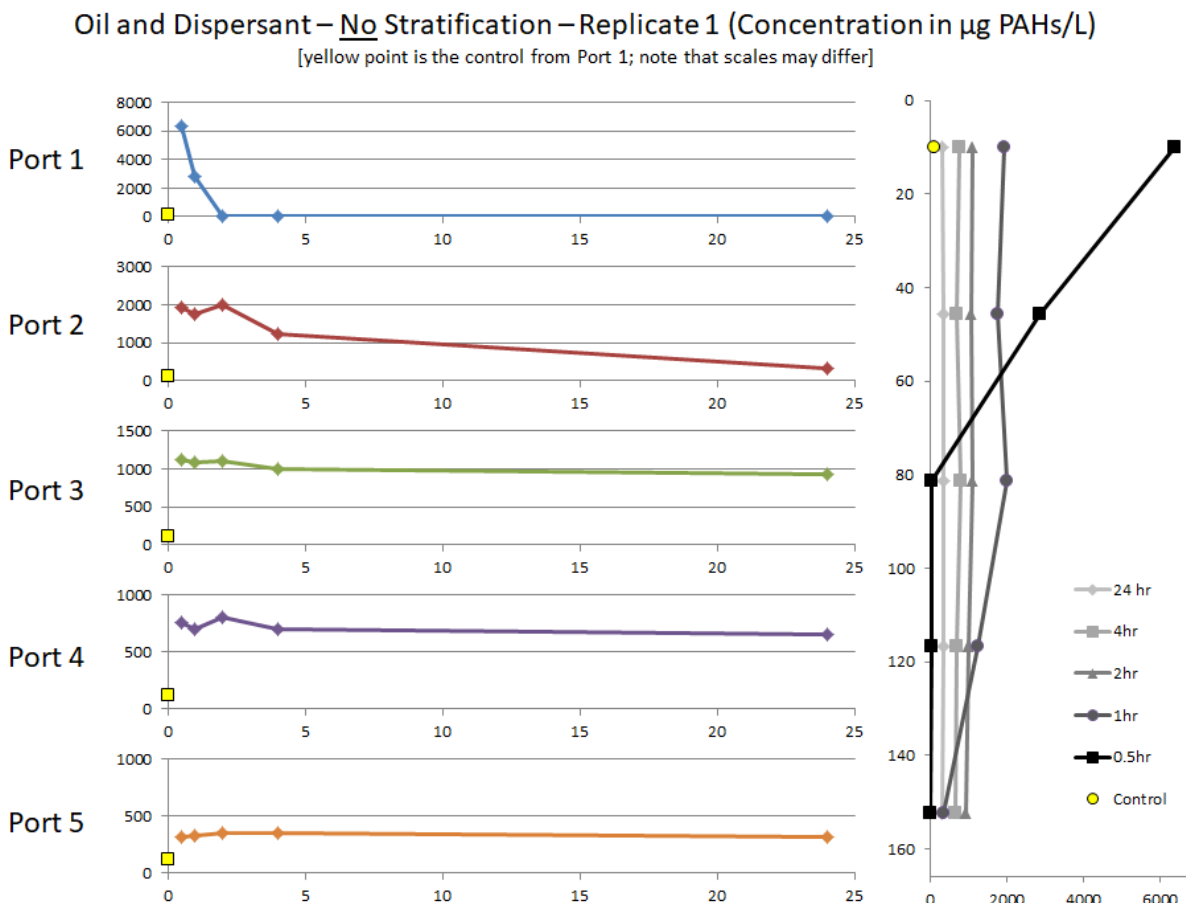


Figure 6.3.3.2. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Replicate 2 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

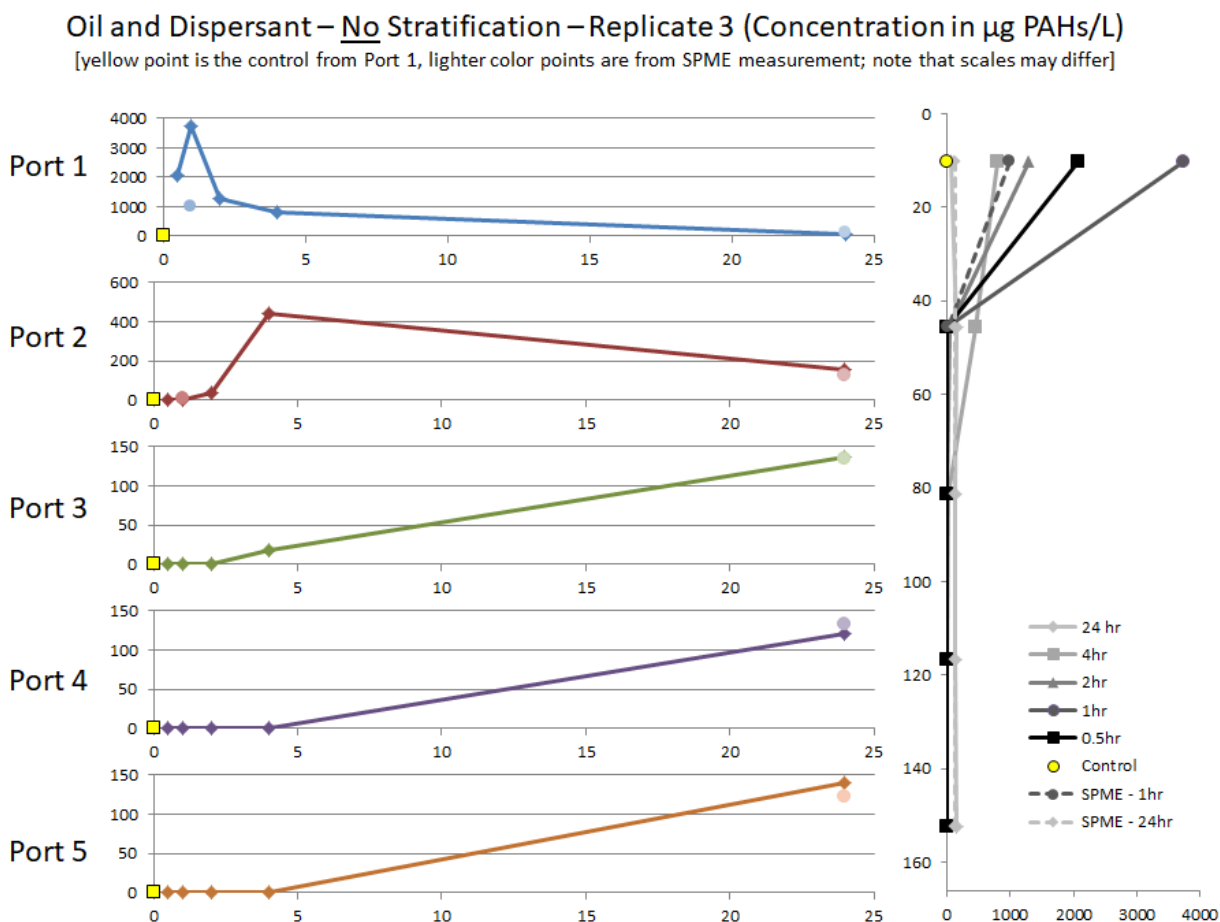


Figure 6.3.3.3. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Replicate 3 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

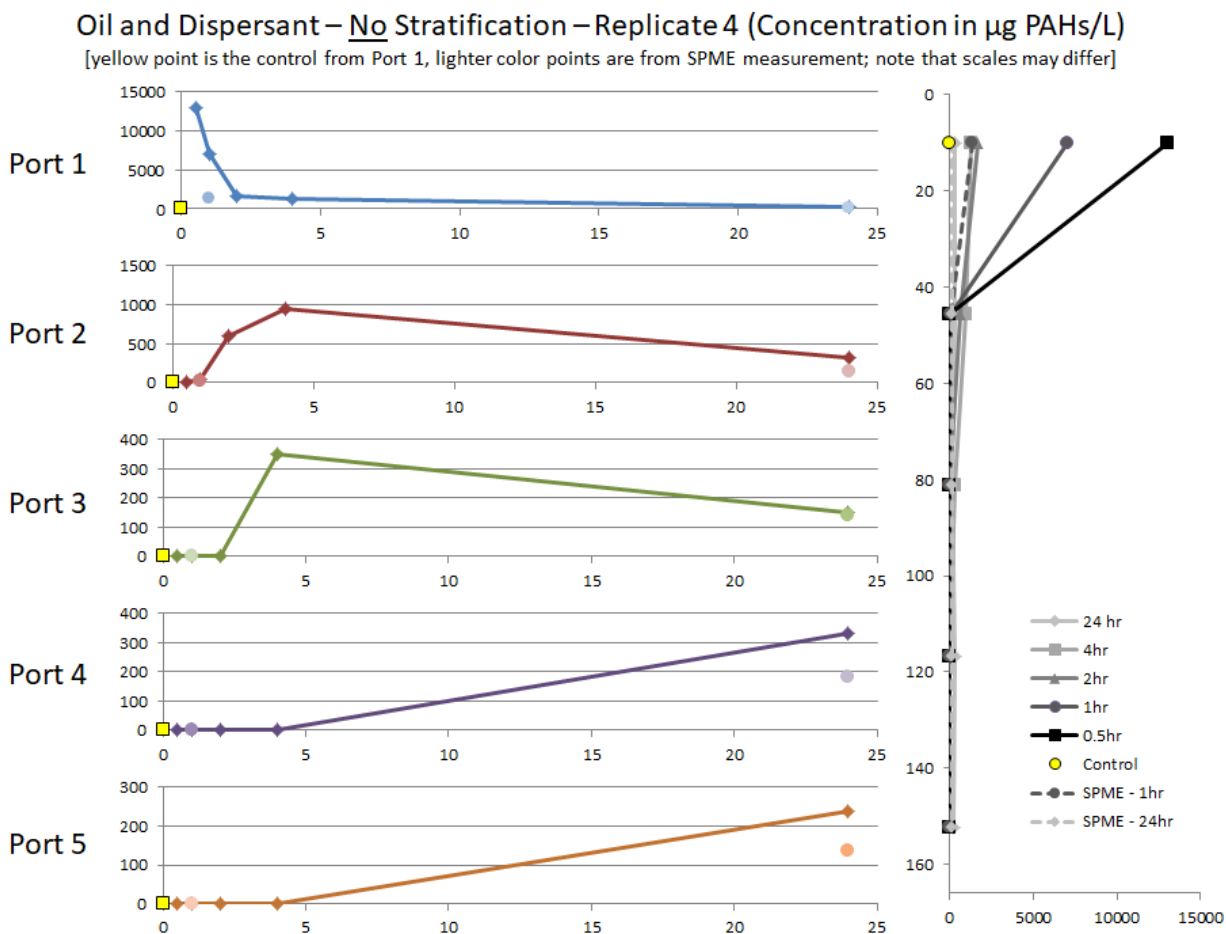


Figure 6.3.3.4. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Replicate 4 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

Oil and Dispersant – No Stratification – Replicate 5 (Concentration in $\mu\text{g PAHs/L}$)

[yellow point is the control from Port 1, lighter color points are from SPME measurement; note that scales may differ]

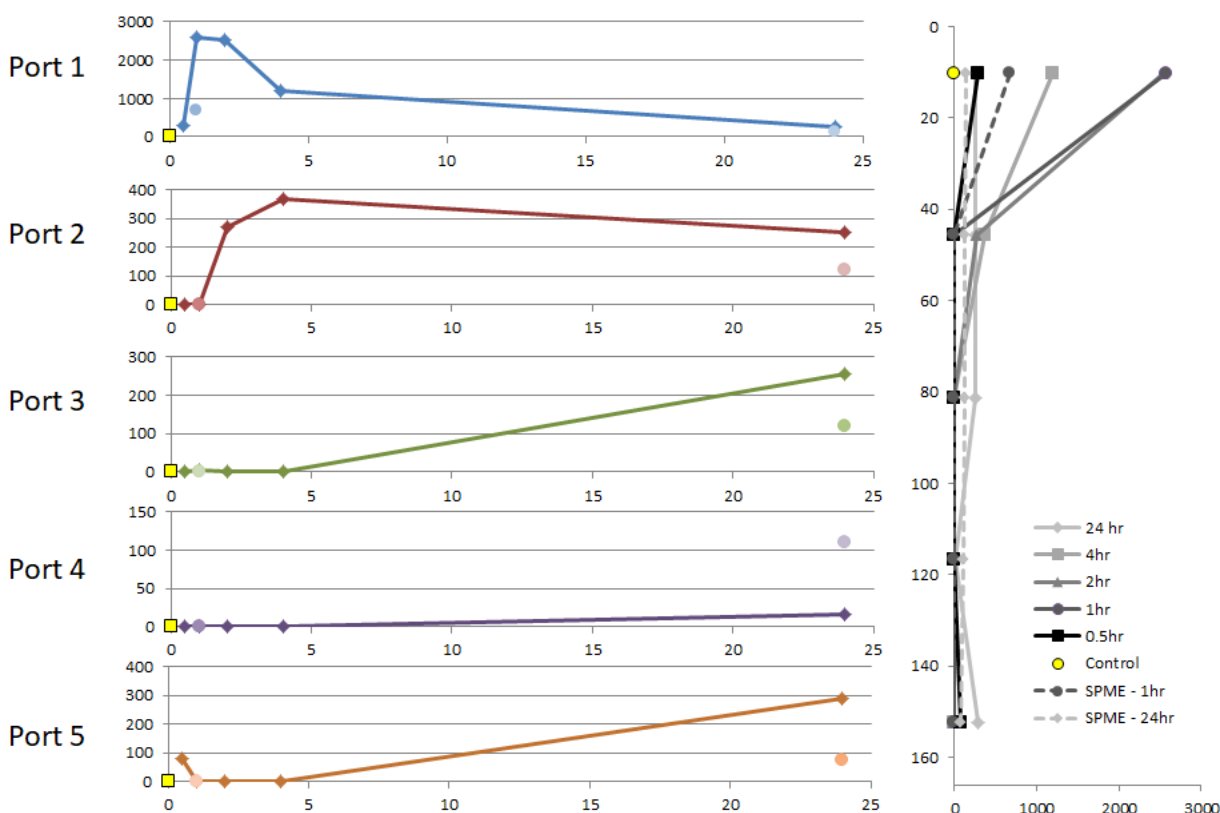


Figure 6.3.3.5. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Replicate 5 (with no temperature stratification). Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

6.3.4. *Calanus finmarchicus* Distribution

Within experiments that exposed copepods to dispersant in an unstratified column, reaction in the upper water column was notably faster than in treatments with oil only added. Over the 5-hour observation period, the number of animals observable in all four frames varied between 5 and 124 (Figure 6.3.4.1).

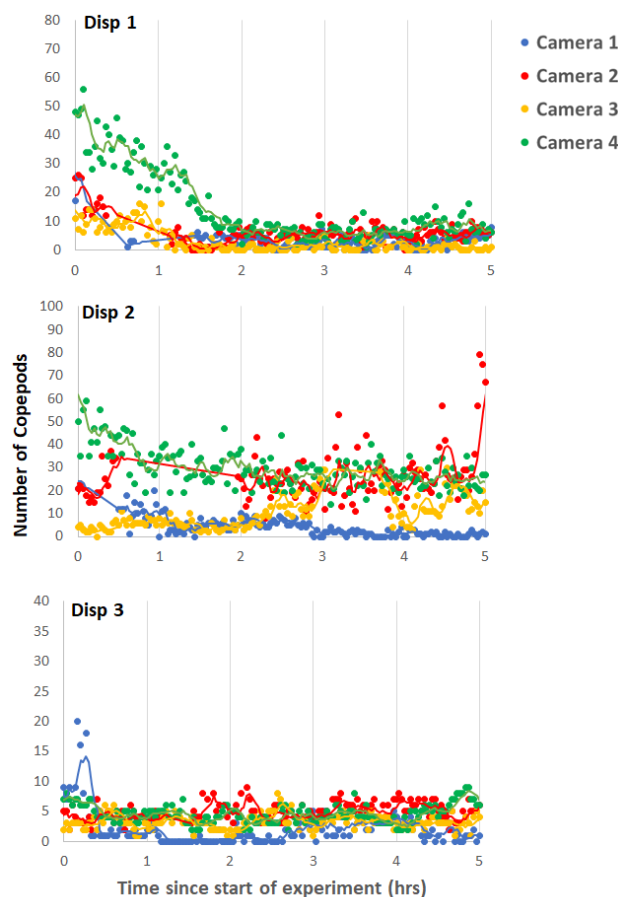


Figure 6.3.4.1. Total number of *Calanus finmarchicus* in each of the camera views depicted for each of the oil + dispersant replicates in an unstratified water column. Counts are taken every 2 minutes of footage. Line represents a 5-point moving average.

During the five-hour observation period after the addition of dispersant, linear regression was used to test if hours since addition of oil predicted the proportion of *Calanus finmarchicus* observable across all four cameras, when averaged between three replicates. We found a significant negative decline since the start of the experiment of the proportion of copepods present in view ($R^2 = 0.36$, $F_{(1, 150)} = 84.0553$, $p < .0001$), indicating a drop of 66% from all frames (Figure 6.3.4.2; Table 6.3.4.2). When separated by camera view, there was a significant negative relationship between time since addition of oil and relative proportion of *C. finmarchicus* at the top of the column,

in frame in Cameras 1 (C1: virtually 100% drop). Similarly, the average proportion of copepods in the bottom of the column, Camera 4, also declined (C4: 52% drop). Camera 2 saw no decline statistically attributable to time since dispersant, whereas camera 3 saw a mild increase (C3: 52%), though it should be noted that, though statistically significant, the power of time as a predictor of proportion in camera 3 was very weak ($R^2 = 0.083$, $F_{(1, 150)} = 13.5587$, $p = 0.0003$) possibly due to high variability and low numbers of animals that occupied the mid-lower water column (Figure 6.3.4.2; Table 6.3.4.2).

To account for high variation between replicates, we also tested 8 time points (0 m, 10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr) as a one-way ANOVA between the three replicates. When exposed to oil with a dispersant, there was a steep drop in number of animals in camera frame 1, resulting in a significant decline between hour 0 and all hours after and including hour 3 (Figure 6.2.3.3; Table 6.3.4.2) The number of animals on average dropped by 58% within the first two hours and ended at 9% of the control value at hour 3. Time points between 0 and 2 hours were often partially missing from treatments due to visual obscurity caused by the dispersant “plume” across the frame. Therefore, we can not confidently say that there was not an effect within those time points as well. Cameras 2 & 3, again, suffered low animal counts and did not see any differences in proportional changes across time points. Camera 4, however did see a drop of 90% between hours 0 and 4, resulting in a significant difference (Camera 4: 2-way ANOVA, $F_{(7,23)} = 3.547$, $p = 0.017$; Table 6.3.4.2). This change happened slower than in camera 1, with differences seen statistically between hour 0 and 4.

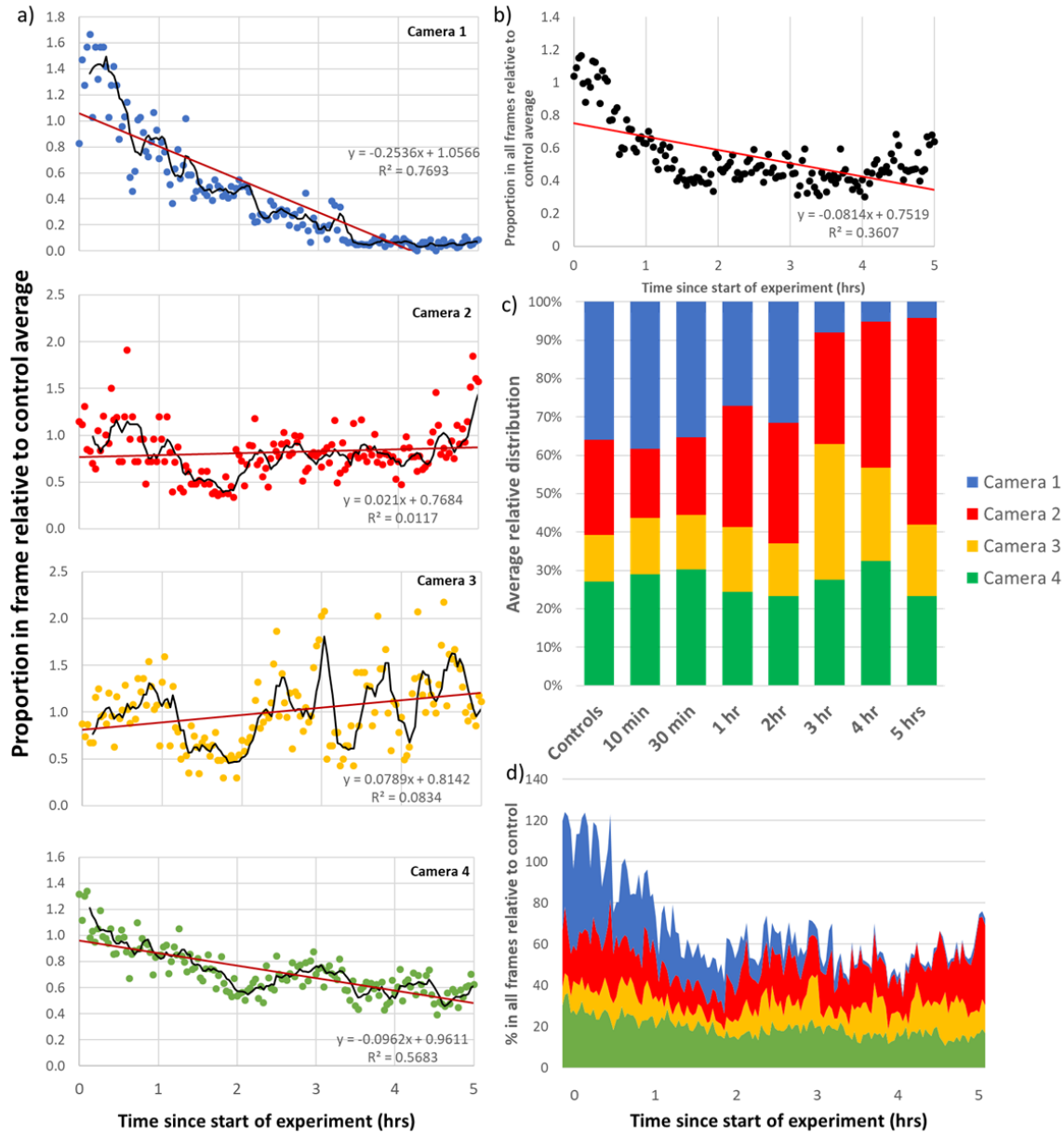


Figure 6.3.4.2. Distribution of *Calanus finmarchicus* over time and between camera view fields (vertical distribution in the experimental chambers) after oil+dispersant is added to the top of the unstratified water column. For all figures, data is averaged between three replicates (D1, D2, D3). a) Proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period, separated by camera. Colored dots depict a count every 2 minutes of footage. Black line represents a 5-point moving average. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. b) Total proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period when all four camera field views are included. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. c) Average relative distribution of *Calanus finmarchicus* between the four camera views during the controls, at 10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr. d) Percent abundance of *Calanus finmarchicus* in view of each camera over the five hour observation period relative to the average of all camera viewfields combined.

Table 6.3.4.1. Summary of regression analysis used to evaluate the predictive power of time after oil+dispersant addition on the averaged proportional abundance relative to control in each camera and summed cameras. Regression statistics and ANOVA tables for each grouping shown.

Dispersant Camera 1						
Regression Statistics		ANOVA				
RSquare	0.769334	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.767786	Model	1	20.505275	20.5053	496.9562
Root Mean Square Error	0.20313	Error	149	6.147999	0.0413	Prob > F
Mean of Response	0.422523	C. Total	150	26.653274		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	1.056581	0.032897	32.12	<.0001
		Time	-0.253623	0.011377	-22.29	<.0001
Dispersant Camera 2						
Regression Statistics		ANOVA				
RSquare	0.011741	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.005109	Model	1	0.14017	0.14017	1.7703
Root Mean Square Error	0.28139	Error	149	11.797864	0.07918	Prob > F
Mean of Response	0.820779	C. Total	150	11.938034		0.1854
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.7683557	0.045572	16.86	<.0001
		Time	0.0209693	0.01576	1.33	0.1854
Dispersant Camera 3						
Regression Statistics		ANOVA				
RSquare	0.083408	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.077256	Model	1	1.982825	1.98282	13.5587
Root Mean Square Error	0.382413	Error	149	21.789745	0.14624	Prob > F
Mean of Response	1.011408	C. Total	150	23.772569		0.0003
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.8142389	0.061933	13.15	<.0001
		Time	0.0788675	0.021418	3.68	0.0003
Dispersant Camera 4						
Regression Statistics		ANOVA				
RSquare	0.568347	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.56545	Model	1	2.9503864	2.95039	196.1848
Root Mean Square Error	0.122633	Error	149	2.2407828	0.01504	Prob > F
Mean of Response	0.720596	C. Total	150	5.1911693		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.9611076	0.019861	48.39	<.0001
		Time	-0.096205	0.006869	-14.01	<.0001
Dispersant ALL Cameras						
Regression Statistics		ANOVA				
RSquare	0.360667	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.356376	Model	1	2.1107614	2.11076	84.0553
Root Mean Square Error	0.158466	Error	149	3.7416236	0.02511	Prob > F
Mean of Response	0.548489	C. Total	150	5.8523849		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.7519197	0.025664	29.3	<.0001
		Time	-0.081372	0.008876	-9.17	<.0001

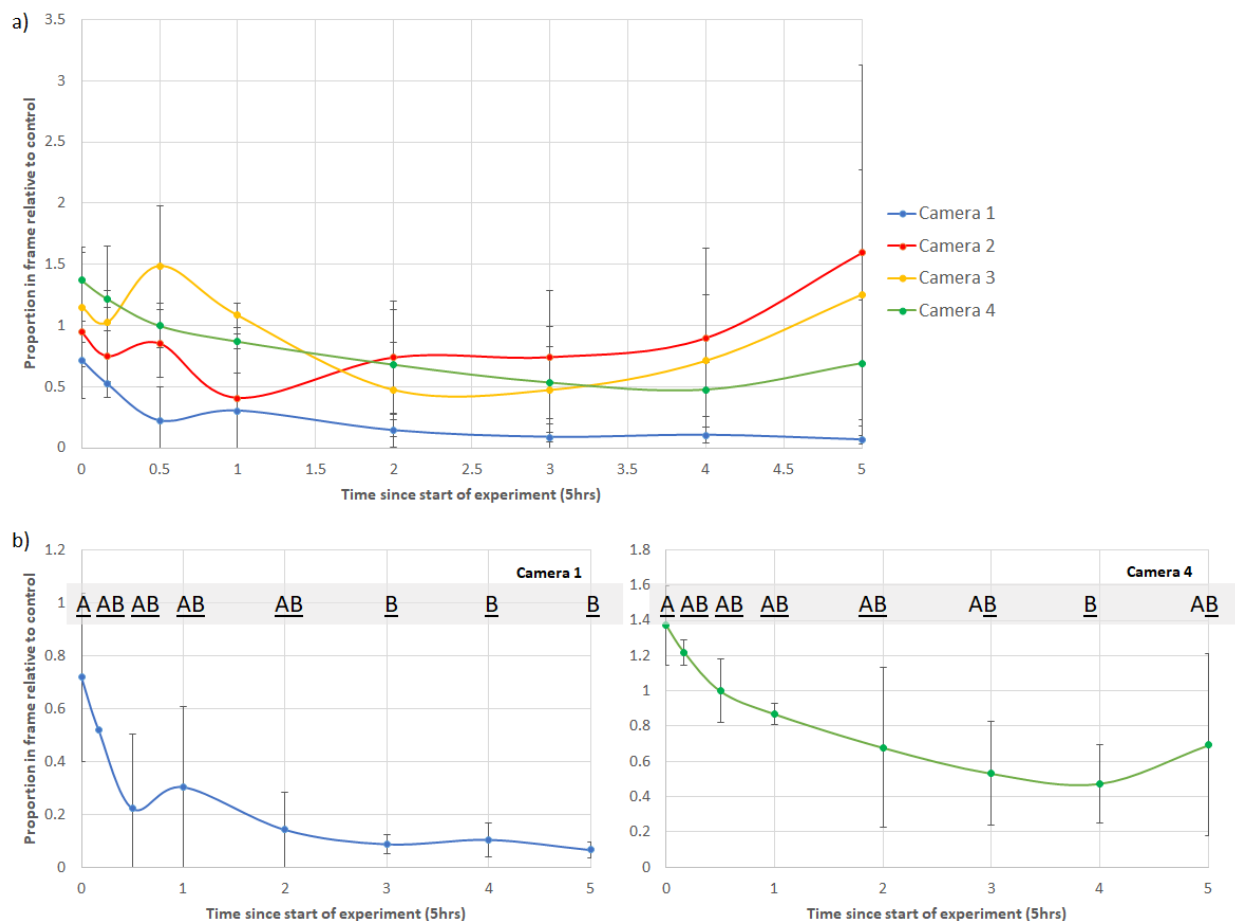


Figure 6.3.4.3. Average proportion in frame (relative to paired control) of all replicates of oil + dispersant treatments at eight discrete time points (10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr) after oil and dispersant is added to the top of the unstratified water column. a) All cameras after addition of dispersant to experimental chamber. Error bars represent standard deviation. b) Isolated line graphs of cameras (camera 1 and 4; top and bottom) where statistically significant differences were found between time points. Variables not connected by the same letter are significantly different, as determined by a post-hoc Tukey-Kramer test.

Table 6.3.4.2. One-way ANOVA results by camera, testing the effect of eight discrete time points on proportion of animals in frame in oil + dispersant, unstratified treatments.

Camera 1					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	1.0134738	0.144782	3.6989	0.0201
Error	13	0.50885	0.039142		
C. Total	20	1.5223238			
Camera 2					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	2.1715905	0.310227	0.5964	0.7492
Error	14	7.2828424	0.520203		
C. Total	21	9.4544329			
Camera 3					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	2.8433489	0.406193	1.2417	0.3376
Error	16	5.2341777	0.327136		
C. Total	23	8.0775266			
Camera 4					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	2.1587833	0.308398	3.516	0.0177
Error	16	1.4034	0.087712		
C. Total	23	3.5621833			

6.4. Stratified Water Column: No-Oil Controls

The copepod distribution and temperature profiles were recorded in treatments where no oil was added (Figures 6.4.1 & 6.4.2).

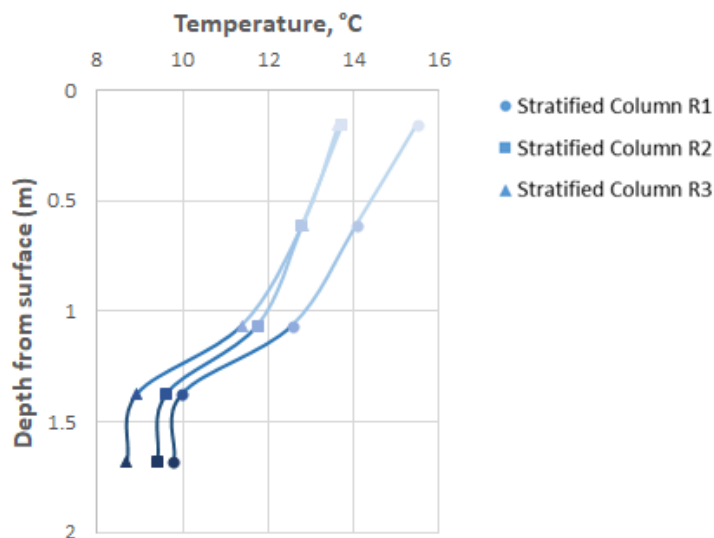


Figure 6.4.1. Temperature profiles of the three stratified column replicates (R1-3) during control period, showing temperature as a function of depth. Thermocline is apparent between 1 and 1.5 m from the surface.

During control periods, within the stratified columns, average water temperature across the entire column was $11.6^{\circ}\text{C} \pm \text{SD } 2.1^{\circ}\text{C}$. Average light intensity was $5.0 \text{ lux} \pm \text{SD } 4.4 \text{ lux}$. Across replicates, average temperature down the column (given in measurements from bottom) averaged at: surface (166cm): $14.2^{\circ}\text{C} \pm \text{SD } 1.0^{\circ}\text{C}$; Mid-upper (125 cm) $13.2^{\circ}\text{C} \pm \text{SD } 0.7^{\circ}\text{C}$; Mid (85 cm): $11.9^{\circ}\text{C} \pm \text{SD } 0.6^{\circ}\text{C}$; Mid-lower (40 cm): $9.5^{\circ}\text{C} \pm \text{SD } 0.5^{\circ}\text{C}$; Bottom (5 cm): $9.3^{\circ}\text{C} \pm \text{SD } 0.6^{\circ}\text{C}$ (Figure 6.4.1; Table 6.4.1)

Number of *Calanus finmarchicus* enumerated in a control period among all four cameras ranged from 40 to 110 individuals. In the stratified column, distribution differed based on vertical placement in the water column ($F_{(3,11)}=6.9537$, $p=0.0128$) indicating that there is a non-random distribution of copepods in the experimental chamber (Figure 6.4.3; Table 6.4.2). Tukey-Kramer post hoc analysis indicated that, similarly to the unstratified column, there were more *C. finmarchicus* distributed in the top of the water column (Camera 1) than cameras 2 and 3 (Table 6.4.2).

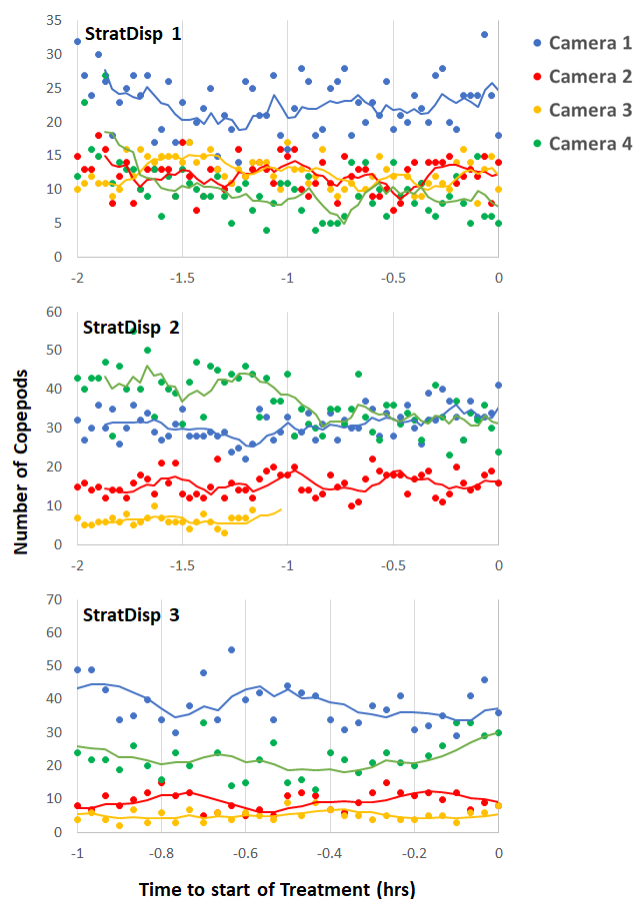


Figure 6.4.2. Raw total number of *Calanus finmarchicus* in each of the camera views depicted for each of the control period replicates in a stratified water column. Counts are depicted every 2 minutes of footage. Line represents a 5-point moving average.

Table 6.4.1. Average temperature and light levels (+/- SD) in each of the control periods within a stratified water column.

Controls					
Temperature, °C					
Replicate	Surface	Mid-upper	Middle	Mid-lower	Bottom
StratDisp 1	15.48 ± 0.03	14.06 ± 0.02	12.56 ± 0.03	9.98 ± 0.03	9.79 ± 0.02
StratDisp 2	13.73 ± 0.13	12.79 ± 0.08	11.74 ± 0.03	9.63 ± 0.07	9.42 ± 0.07
StratDisp 3	13.63 ± 0.03	12.82 ± 0.02	11.39 ± 0.02	8.93 ± 0.03	8.69 ± 0.03
Light Intensity, lux					
Replicate	Surface	Mid-upper	Middle	Mid-lower	Bottom
StratDisp 1	14.32 ± 0.29	5.9 ± 0.1	5.17 ± 0.09	1.73 ± 0.05	0.64 ± 0
StratDisp 2	1.98 ± 0.13	12.92 ± 0.34	6.1 ± 0.55	3 ± 0	10.01 ± 0.09
StratDisp 3	1.38 ± 0.25	1.37 ± 0.13	0.3 ± 0.1	7.1 ± 1.22	3.81 ± 0.28

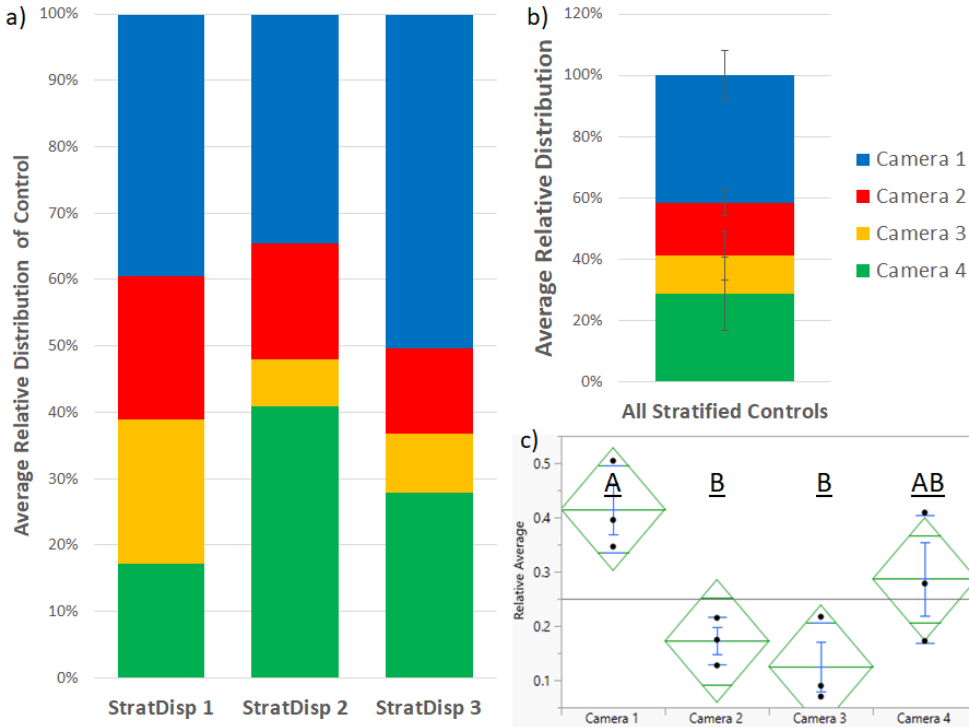


Figure 6.4.3. Control distributions of *Calanus finmarchicus* in a stratified water column. a) Average relative distribution of copepods in each replicate in the 2 hours prior to addition of oil and dispersant. Camera 1 captures the top of the water column, while camera 4 captures the bottom. b) Average relative distribution across cameras views of all stratified controls, with error bars representing standard deviation. c) Graph depicting relative abundance mean and standard deviations by camera. Variables not connected by the same letter are significantly different, as determined by a post-hoc Tukey-Kramer test.

Table 6.4.2. One-way ANOVA table testing difference of relative distribution of *Calanus finmarchicus* between the four camera placements in stratified experimental chamber. Camera 1 represents the top of a water column, and Camera 4 represents deeper in a water column. Post hoc Tukey-Kramer test shown.

1-way ANOVA, pooled controls by camera					
Analysis of Variance					
Source of Variation	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	3	0.15050221	0.050167	6.9537	0.0128
Error	8	0.05771554	0.007214		
C. Total	11	0.20821775			
Means for Oneway Anova					
Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Camera 1	3	0.415353	0.04904	0.30227	0.52844
Camera 2	3	0.172405	0.04904	0.05932	0.28549
Camera 3	3	0.125641	0.04904	0.01256	0.23873
Camera 4	3	0.286601	0.04904	0.17352	0.39968
Ordered Differences Report (Tukey-Kramer HSD)					
Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
C1 v. C3	0.2897117	0.0693515	0.067624	0.511799	0.0132
C1 v. C2	0.2429486	0.0693515	0.020861	0.465036	0.0328
C4 v. C3	0.1609595	0.0693515	-0.061128	0.383047	0.1721
C1 v. C4	0.1287523	0.0693515	-0.093335	0.35084	0.3168
C4 v. C2	0.1141963	0.0693515	-0.107891	0.336284	0.4076
C2 v. C3	0.0467631	0.0693515	-0.175324	0.26885	0.9039

6.5. Stratified Water Column: Undispersed Oil Treatment

6.5.1. Oil Distribution in the Copepod Tower

When oil without dispersant is added to a stratified water column, a similar initial oil distribution as in the non-stratified water column was observed for the upper portion of the water column (Figure 6.5.1.1): Oil hydrocarbons slowly dissolved from the oil slick, leading to relatively small (low $\mu\text{g L}^{-1}$ range) oil concentration in Port 1 after 1h. These oil concentrations subsequently increase over 24 h, to reach equilibrium between the aqueous and oil phase. A notable difference can be observed in the lower sampling ports: Whereas all ports experience increasing oil concentrations in the non-stratified water column (Figures 6.2.2.1-3), the lower two ports stay at relatively low oil concentrations ($< 2 \mu\text{g L}^{-1}$) (Figure 6.5.1.1).

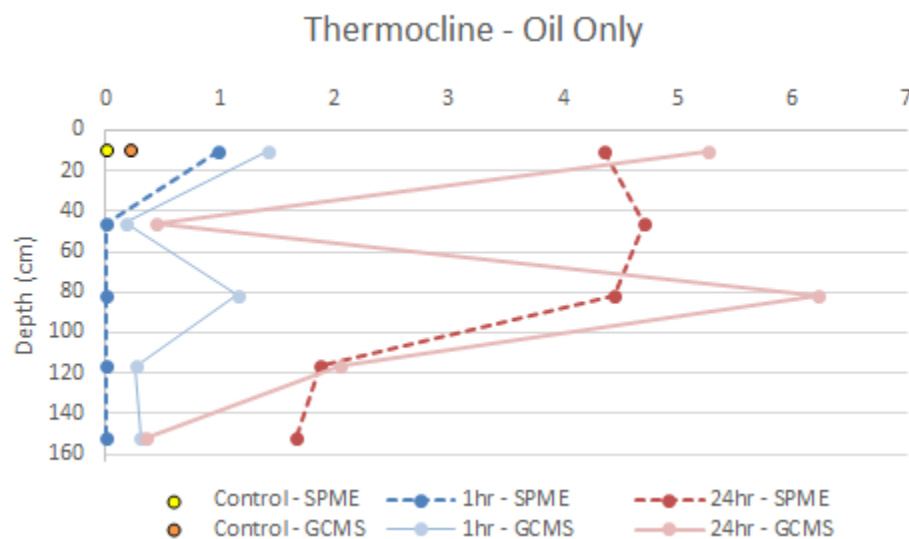


Figure 6.5.1.1. Oil concentrations ($\mu\text{g/L}$) from 3 time points (0=control, 1, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for an oil only replicate in a stratified system. Figure on shows concentrations (x-axis; $\mu\text{g/L}$) at two time points (blue = 1 hr, red = 24 hr) along the depth of the experimental tank (y-axis; cm). Solid lines depict concentrations obtained from GCMS analysis. Dashed lines depict concentrations obtained from GCxGC-SPME analyses. Controls (time = 0) for each analysis are depicted as yellow and orange circles, respectively.

6.6. Stratified Water Column: Dispersed Oil Treatments

6.6.1. Water Conditions within the tank

During treatment periods, within the stratified columns, average water temperature across all replicates in the entire column was $11.6^{\circ}\text{C} \pm \text{SD } 2.3^{\circ}\text{C}$ (Figure 6.6.1.1). Average light intensity was $5.2 \text{ lux} \pm \text{SD } 4.5 \text{ lux}$. Across replicates, average temperature down the column (given in measurements from bottom) averaged at: surface (166cm): $14.5^{\circ}\text{C} \pm \text{SD } 0.9^{\circ}\text{C}$; Mid-upper (125 cm) $13.3^{\circ}\text{C} \pm \text{SD } 0.6^{\circ}\text{C}$; Mid (85 cm): $11.8^{\circ}\text{C} \pm \text{SD } 0.6^{\circ}\text{C}$; Mid-lower (40 cm): $9.3^{\circ}\text{C} \pm \text{SD } 0.5^{\circ}\text{C}$; Bottom (5 cm): $9.1^{\circ}\text{C} \pm \text{SD } 0.5^{\circ}\text{C}$; (Table 6.6.1.1).

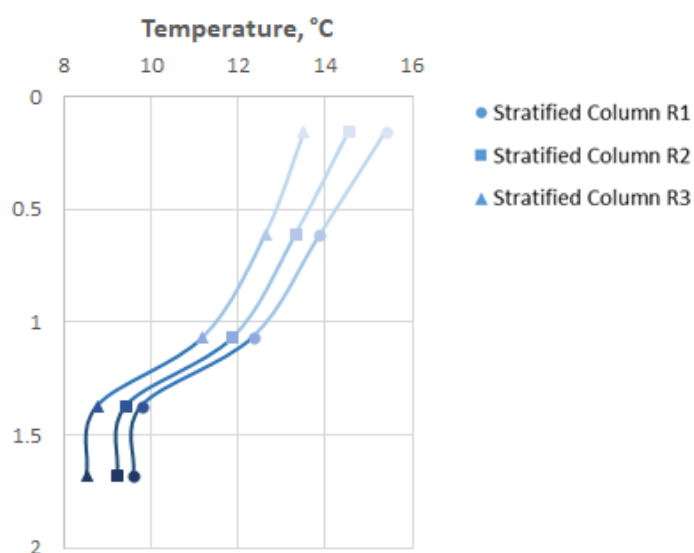


Figure 6.6.1.1. Temperature profiles of the three stratified column replicates (R1-3) during treatment period, showing temperature as a function of depth. Thermocline is apparent between 1 and 1.5 m from the surface.

Table 6.6.1.1. Average temperature and light levels (+/- SD) in each of the treatment periods within a stratified water column.

Experimental					
Temperature, °C					
Replicate	Surface	Mid-upper	Middle	Mid-lower	Bottom
StratDisp 1	15.39 ± 0.14	13.87 ± 0.15	12.35 ± 0.18	9.78 ± 0.23	9.59 ± 0.23
StratDisp 2	14.54 ± 0.19	13.33 ± 0.13	11.88 ± 0.06	9.42 ± 0.04	9.21 ± 0.04
StratDisp 3	13.51 ± 0.12	12.62 ± 0.14	11.18 ± 0.16	8.76 ± 0.13	8.51 ± 0.13
Light Intensity, lux					
Replicate	Surface	Mid-upper	Middle	Mid-lower	Bottom
StratDisp 1	14.35 ± 0.29	5.96 ± 0.08	5.34 ± 0.15	1.75 ± 0.07	0.81 ± 0.08
StratDisp 2	1.75 ± 0.44	13 ± 0	6.25 ± 0.43	3.2 ± 0.4	10 ± 0
StratDisp 3	1.27 ± 0.03	1.47 ± 0.06	0.42 ± 0.02	8.16 ± 0.14	3.72 ± 0.31

6.6.2. Oil Distribution

Adding oil and dispersant to the stratified water column led to characteristic hydrocarbon profiles (Figures 6.6.2.1-3). Similar to the dispersed oil in the unstratified water column the top sampling port showed a rapid initial increase of PAH concentration of up to $1,000 \mu\text{g L}^{-1}$ within the first hour after the addition of the oil/dispersant mixture, followed by slowly declining oil concentrations 3 to 5 hours after the addition (Figures 6.6.2.1-3). This peak of high PAHs traveled down the water column, and reached a maximum concentration in Port 3 of all treatments after 2 to 5 hours. This was a reproducible finding for all three replicates performed.

Similar to the unstratified water column experiments, dissolved PAHs, as well as PAH present in oil droplets, contributed to these oil concentrations. The purely dissolved PAH concentration can be estimated by using the SPME measurement, which showed a peak PAH concentration of approx. $1000 \mu\text{g L}^{-1}$ in Port 1 in the three replicates.

The decreasing PAH concentration in the upper ports can be explained by a decreasing oil droplet volume over time. Initially-formed large oil droplets likely drift to the surface over time, thereby reducing the volume of particulate oil in the water column. This reduction in oil volume is likely due to coalescing of oil droplets to form more buoyant droplets, in combination with the leaching of oil dispersants from the oil into the water column. This leaching reduced the concentration of dispersants in the oil film on top of the water column, thereby making dispersion with the relatively low energy input (air pump) less likely over time.

In contrast to the dispersed oil in the unstratified water column, the oil droplets do not seem to reach the lower ports in the stratified water column. Total PAH concentrations reached up to approx. $50 \mu\text{g L}^{-1}$ in the lowest port. The fact that total PAH concentration (PAHs in the aqueous and oil droplet phase) and the dissolved PAH concentration (PAHs in the aqueous phase only, measured by SPME) agreed in the lowest port is an indication that only dissolved hydrocarbons but no oil droplets reached these lowest ports.

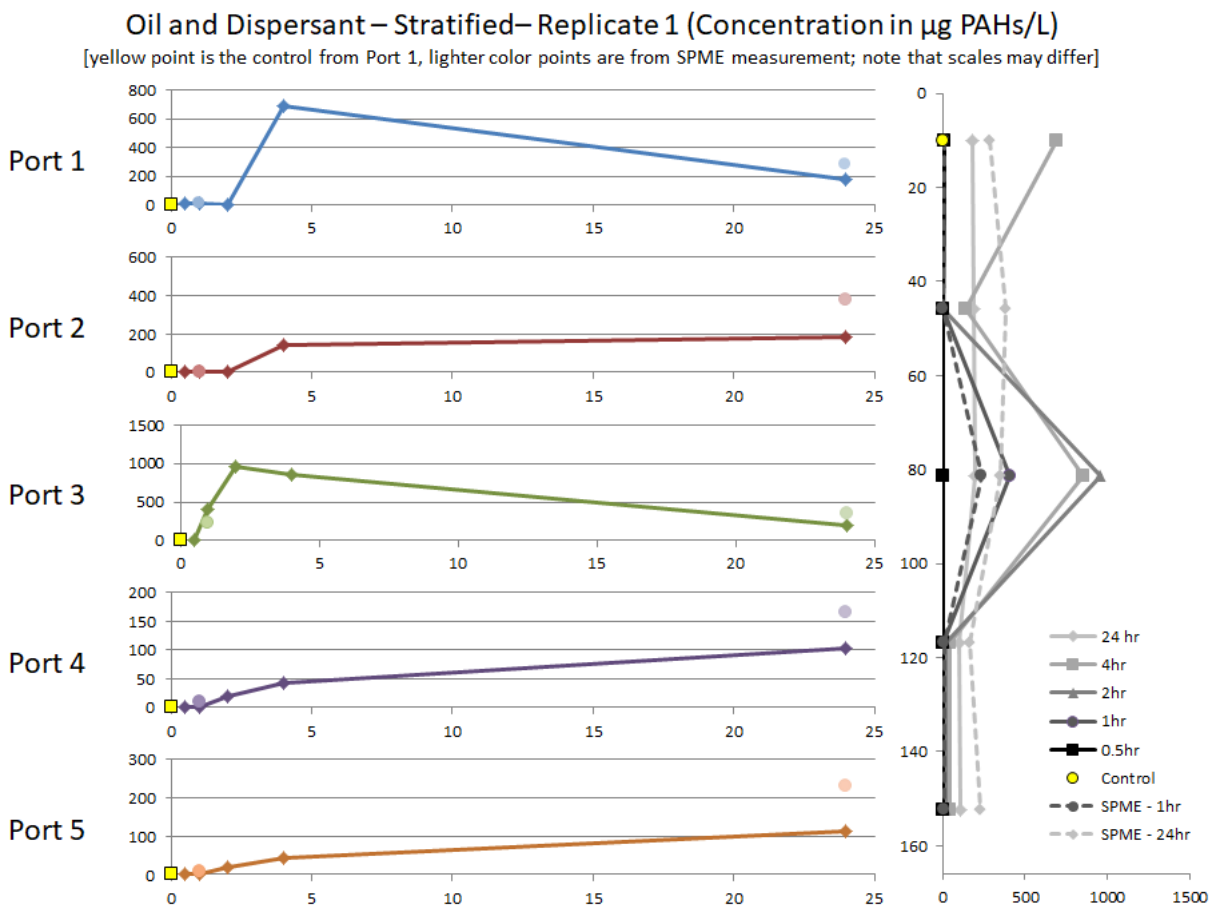


Figure 6.6.2.1. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Stratified - Replicate 1. Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

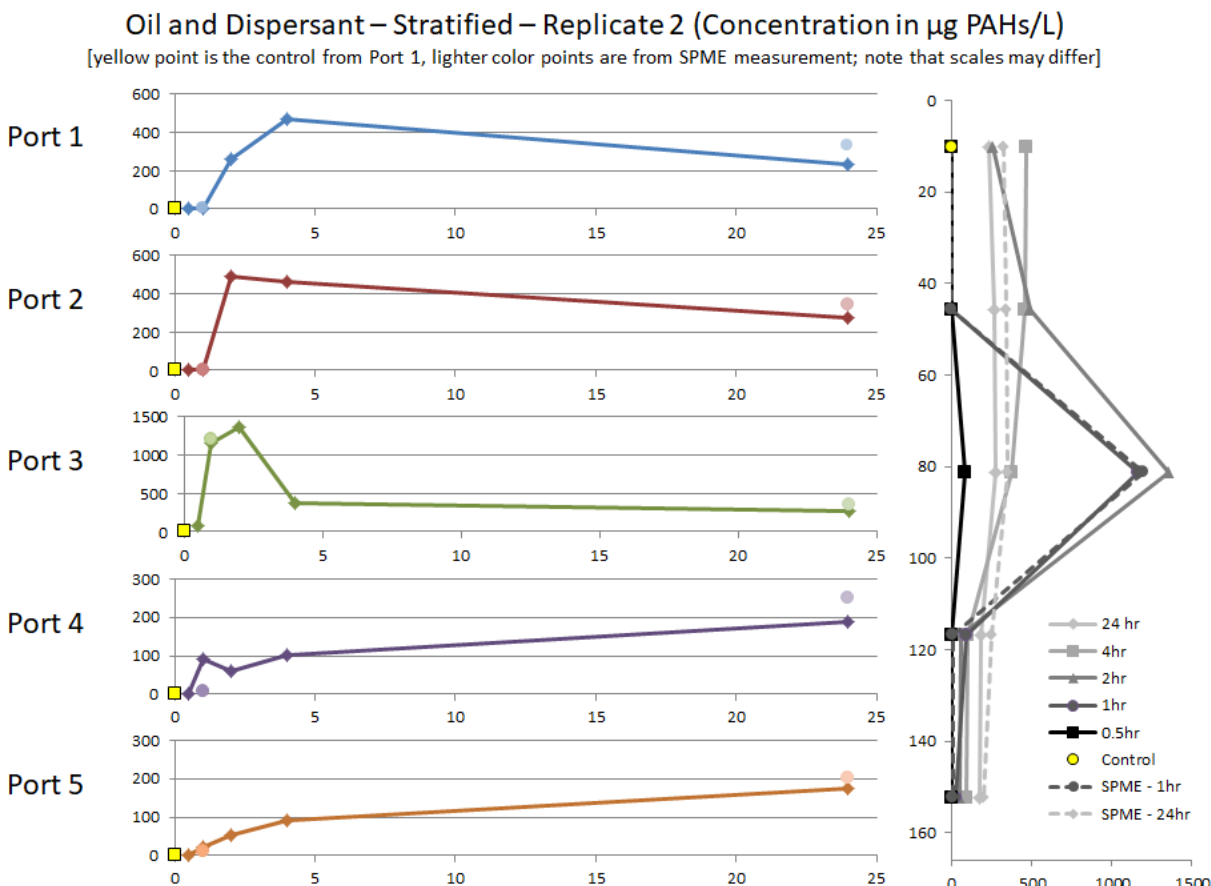


Figure 6.6.2.2. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Stratified - Replicate 2. Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

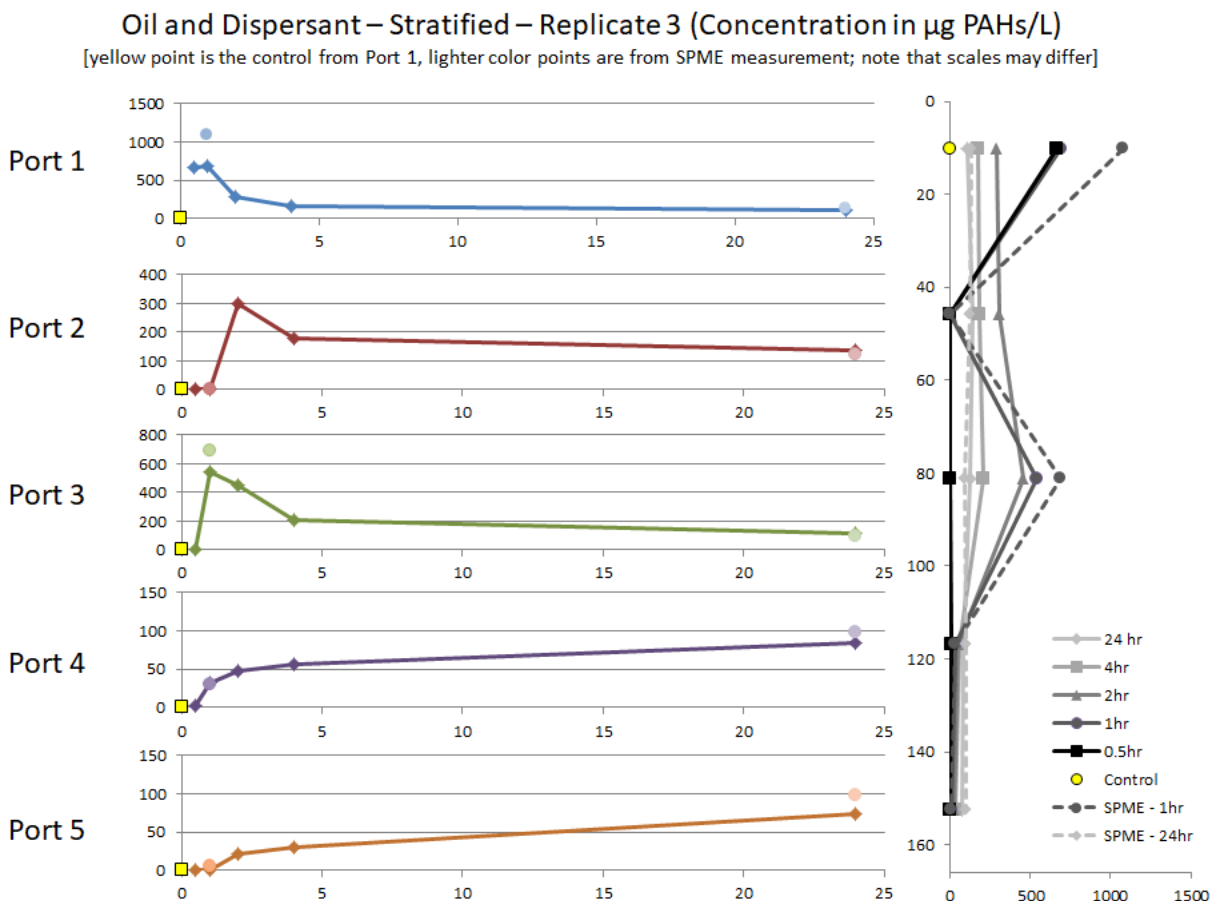


Figure 6.6.2.3. Oil concentrations ($\mu\text{g/L}$) from 6 time points (0, 0.5, 1, 2, 4, and 24 hours) and 5 depths (10, 46, 81, 117 and 152 cm below the water surface) for Oil and Dispersant - Stratified - Replicate 3. Figures on the left show concentration (y-axis; $\mu\text{g/L}$) from GC-MS analyses at individual depths across the experimental period (x-axis; hours). Lighter colored, round points are concentrations obtained from GCxGC-SPME analyses. The figure on the right shows concentrations (x-axis; $\mu\text{g/L}$) at all time points (differentiated by shade of gray) along the depth of the experimental tank (y-axis; cm). Dashed lines depict concentrations obtained from GCxGC-SPME analyses.

6.6.3. *Calanus finmarchicus* Distribution

Within experiments that exposed copepods to dispersant in an stratified column, over the 5-hour observation period, the number of animals observable in all four frames varied between 25 and 103 (Figure 6.6.3.1).

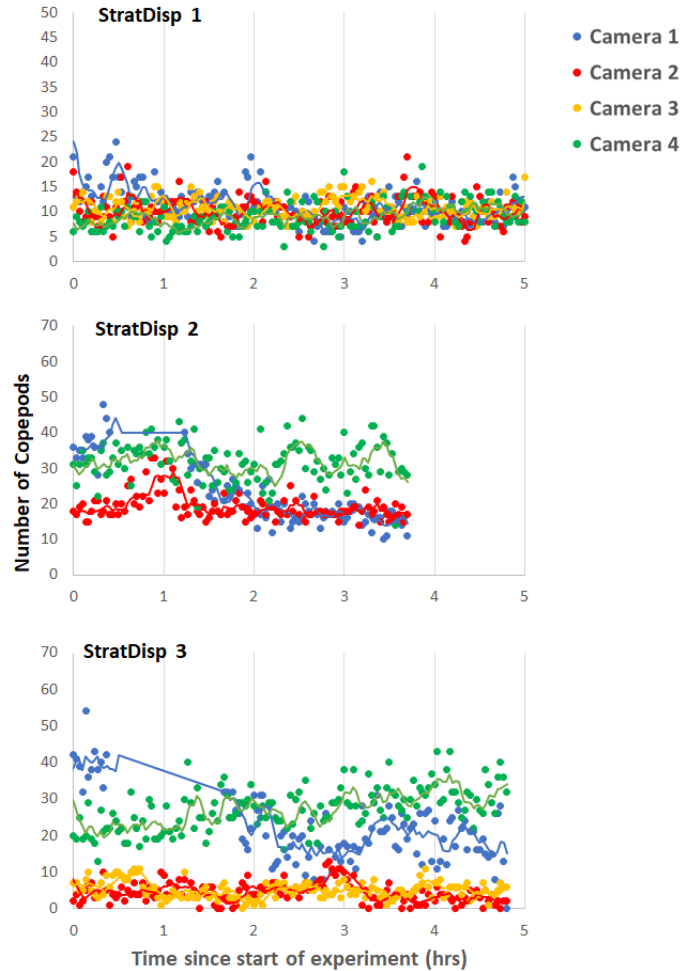


Figure 6.6.3.1. Total number of *Calanus finmarchicus* in each of the camera views depicted for each of the oil + dispersant replicates in a stratified water column. Counts are taken every 2 minutes of footage. Line represents a 5-point moving average.

During the five-hour observation period after the addition of oil + dispersant, linear regression was used to test if hours since addition of dispersant significantly predicted the proportion of *Calanus finmarchicus* observable across all four cameras, when averaged between three replicates in stratified columns (Figure 6.6.3.2). We found a significant negative decline since the start of the experiment of the proportion of copepods present in view ($R^2 = 0.13$, $F_{(1, 150)} = 22.15$, $p < .0001$), indicating a drop of 32% from all frames (Table 6.6.3.1). When separated by camera view, there was a significant

negative relationship between time since addition of dispersant and relative proportion of *C. finmarchicus* at the top of the column, in frame in Cameras 1 (C1: 67% drop relative to control). Camera 2 also experienced a mild decrease in relative abundance over the 5 hour period (C2: 26% drop relative to control) No relationship was detected between time since dispersant added and relative abundance in Camera 3. Camera 4 had a significantly positive regression, and the abundance in that frame grew 20%, implicating that *Calanus finmarchicus* may position themselves below a thermocline to avoid high oil concentrations brought deeper by dispersants (Figure 6.6.3.2; Table 6.6.3.1).

When a thermocline was present, and both oil and dispersant was applied, the proportional number of animals dropped 50% in camera 1 across 4 hours of treatment (Camera 1: 2-way ANOVA, $F_{(4,13)}=4.897$, $p=0.023$; Figure 6.6.3.3, Table 6.6.3.2). Again, time points between 0 and 2 hours were compromised due to obstruction of the dispersant plume. But a significant drop was observed by hour 3. Camera 2 saw no difference, perhaps owing to a small number of animals present in that frame, leading to great variability obstructing measurable change. Camera 3 suffered a malfunction in one trial, leading to an incomplete set to run an ANOVA set, but based on the other two trials, we observe little difference across the treatment time period. Finally, camera 4 indicated no change in the number of animals present in the frame (Camera 4: 2-way ANOVA, $F_{(7,22)}=0.351$, $p=0.917$), differing from the dispersant treatment (Figure 6.6.3.3, Table 6.6.3.2).

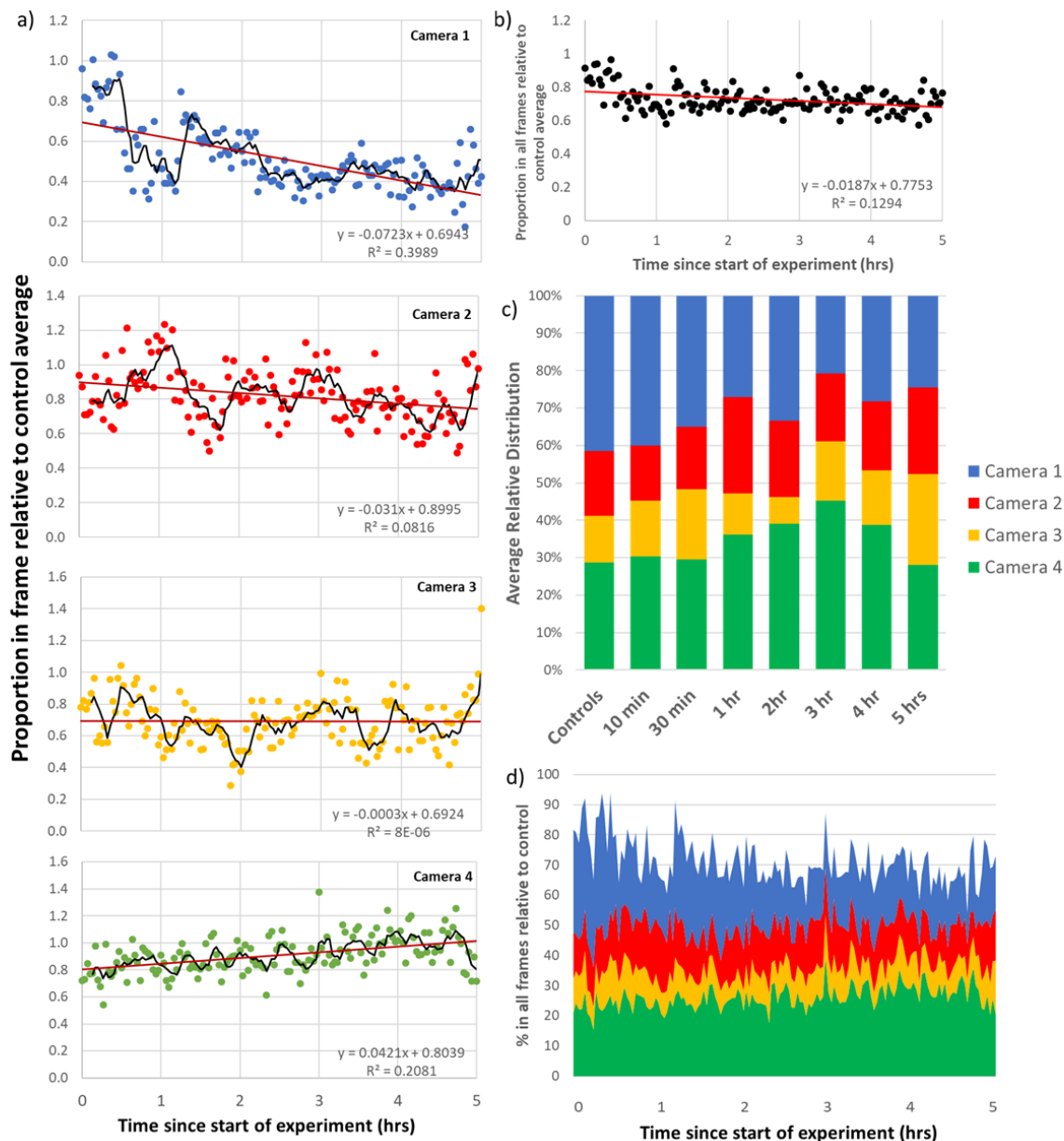


Figure 6.6.3.2. Distribution of *Calanus finmarchicus* over time and between camera view fields (vertical distribution in the experimental chambers) after oil and dispersant is added to the top of a stratified water column. For all figures, data is averaged between three replicates (SD1, SD2, SD3). a) Proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period, separated by camera. Colored dots depict a count every 2 minutes of footage. Black line represents a 5-point moving average. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. b) Total proportion of *C. finmarchicus* in frame relative to paired control period over the five hour observation period when all four camera field views are included. Red line represents a linear fit line used to run regression analysis with the accompanying equation and R^2 outputs listed adjacent. c) Average relative distribution of *Calanus finmarchicus* between the four camera views during the controls, at 10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr. c) Percent abundance of *Calanus finmarchicus* in view of each camera over the five hour observation period relative to the average of all camera viewfields combined.

Table 6.6.3.1. Summary of regression analysis used to evaluate the predictive power of time after oil and dispersant addition on the averaged proportional abundance relative to control in each camera and summed cameras in a stratified column. Regression statistics and ANOVA tables for each grouping shown.

Stratified + Dispersant Camera 1						
Regression Statistics		ANOVA				
RSquare	0.401368	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.397351	Model	1	1.6862192	1.68622	99.901
Root Mean Square Error	0.129919	Error	149	2.514957	0.01688	Prob > F
Mean of Response	0.515535	C. Total	150	4.2011762		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.69736	0.021041	33.14	<.0001
		Time	-0.07273	0.007277	-10	<.0001
Stratified + Dispersant Camera 2						
Regression Statistics		ANOVA				
RSquare	0.081624	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.07546	Model	1	0.3059006	0.305901	13.2429
Root Mean Square Error	0.151984	Error	149	3.4417771	0.023099	Prob > F
Mean of Response	0.822069	C. Total	150	3.7476777		0.0004
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.8995129	0.024614	36.54	<.0001
		Time	-0.030977	0.008512	-3.64	0.0004
Stratified + Dispersant Camera 3						
Regression Statistics		ANOVA				
RSquare	0.002513	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	-0.00418	Model	1	0.0115884	0.011588	0.3754
Root Mean Square Error	0.175693	Error	149	4.5993602	0.030868	Prob > F
Mean of Response	0.759242	C. Total	150	4.6109486		0.541
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.7743151	0.028454	27.21	<.0001
		Time	-0.006029	0.00984	-0.61	0.541
Stratified + Dispersant Camera 4						
Regression Statistics		ANOVA				
RSquare	0.208058	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.202743	Model	1	0.5644298	0.56443	39.145
Root Mean Square Error	0.120079	Error	149	2.1484211	0.014419	Prob > F
Mean of Response	0.909061	C. Total	150	2.7128509		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.8038646	0.019447	41.34	<.0001
		Time	0.0420786	0.006725	6.26	<.0001
Stratified + Dispersant ALL Cameras						
Regression Statistics		ANOVA				
RSquare	0.129434	Source	DF	Sum of Squares	Mean Square	F Ratio
RSquare Adj	0.123591	Model	1	0.1117881	0.111788	22.153
Root Mean Square Error	0.071036	Error	149	0.75188008	0.005046	Prob > F
Mean of Response	0.72845	C. Total	150	0.86366818		<.0001
Observations (or Sum Wgts)	151	Term	Estimate	Std Error	t Ratio	Prob> t
		Intercept	0.7752662	0.011505	67.39	<.0001
		Time	-0.018726	0.003979	-4.71	<.0001

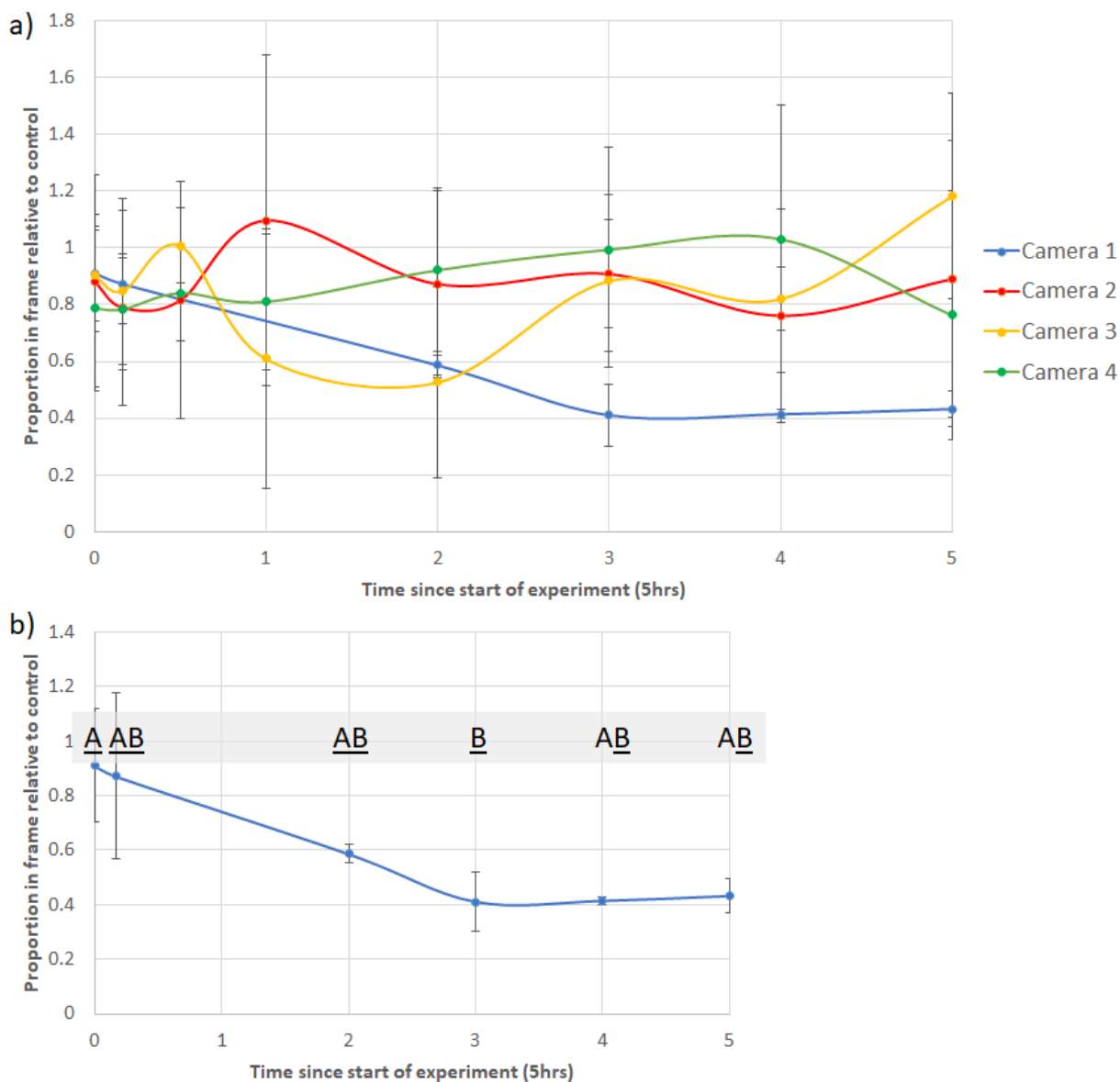


Figure 6.6.3.3. Average proportion in frame (relative to paired control) of all replicates of oil + dispersant treatments at eight discrete time points (10 m, 30 m, 1 hr, 2 hr, 3 hr, 4 hr, and 5 hr) in a stratified chamber. a) All cameras after addition of dispersant to stratified experimental chamber. Error bars represent standard deviation. b) Isolated line graphs of cameras (camera 1 and 4; top and bottom) where statistically significant differences were found between time points. Variables not connected by the same letter are significantly different, as determined by a post-hoc Tukey-Kramer test.

Table 6.6.3.2. ANOVA table comparing the means of proportion of animals relative to paired control between eight discrete time points by camera.

Camera 1					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	5	0.7696155	0.153923	5.1227	0.0137
Error	10	0.3004721	0.030047		
C. Total	15	1.0700876			
Camera 2					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	0.3759609	0.053709	0.3315	0.928
Error	16	2.59232	0.16202		
C. Total	23	2.9682809			
Camera 3					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	0.37695597	0.053851	0.7022	0.6728
Error	8	0.61349856	0.076687		
C. Total	15	0.99045454			
Camera 4					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	0.2316638	0.033095	0.3178	0.935
Error	16	1.6663345	0.104146		
C. Total	23	1.8979983			

6.7. Experiments to Determine Inactivation and Recovery of Copepods

Based on iterative feedback gained from the project advisory team during the project champions, we conducted a series of “small scale experiments” to determine the fate of the copepods exposed to the oil (see Methods Section for experimental details).

6.7.1. Acute Toxicity and Inactivation of Copepods

Experiment 1: Exposure to WAF: A 30% dilution of a WAF prepared with ANS was prepared (see Methods Section 5.3). The total PAH concentration in these dilutions was $180 \mu\text{g L}^{-1}$. When held in the WAF treatment for 24 hours, $74\% \pm \text{SD } 5\%$ of *Calanus* were inactivated compared to $28\% \pm \text{SD } 8\%$ of controls (Figure 6.7.1.1). The proportion of animals swimming actively was significantly different from the initial after 1 hour (compared to hour 4 in the control). Hour 4 and 24 did not differ from each other, but were significantly higher than any other time point in either treatment (Table 6.7.1.1). Over the 24 hour experiment, 8% ($n=4$) of individuals in the WAF treatment died, whereas 4% ($n=2$) died in the control treatment. This indicates that the effect of WAF exposure at $180 \mu\text{g L}^{-1}$ PAHs was only marginally lethal, and that the majority of the observed effects (inactivation) was of sub-lethal nature.

Experiment 2: Oil Slick: Over the 24 hours of the experiment, 2% ($n=1$) of individuals in the oil slick treatment died, and 4% ($n=2$) died in the control. When an oil slick (see Methods [Section 5.3](#)) was directly introduced to the chambers with fresh *Calanus*, after 24 hours, $38\% \pm \text{SD } 16\%$ of individuals were inactivated versus $22\% \pm 8\%$ in the control (Figure 6.7.1.1). The proportion of active animals was significantly dropped after 2 hours in the treatment versus 4 hours in the control. Hour 4 and 24 did not differ significantly between oil and control treatments ([Table 6.7.1.1](#)).

In the oil slick treatment, several individuals would become inactivated by interacting with and getting trapped in the slick (57% of deactivated individuals in the slick treatment were trapped in oil).

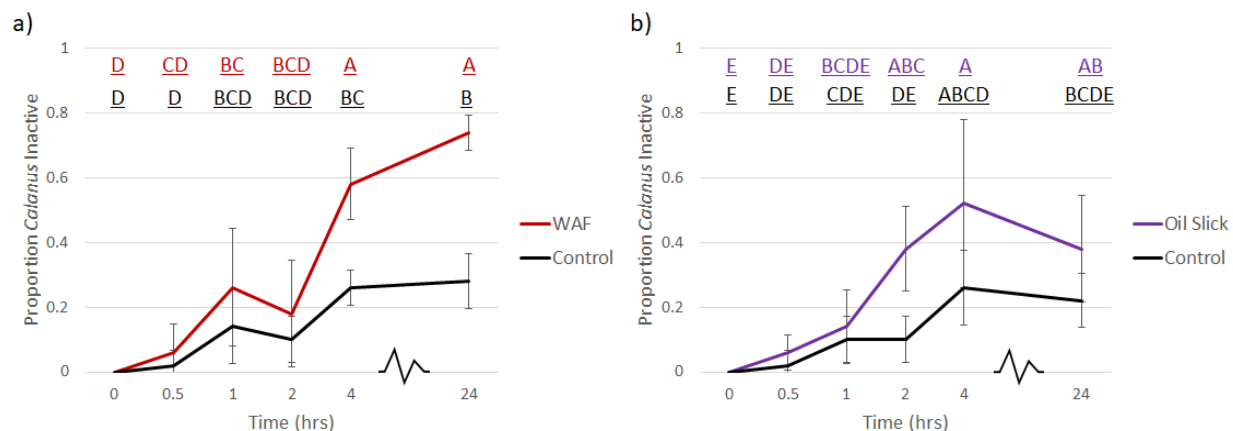


Figure 6.7.1.1. Proportion of *Calanus* inactivated over 24 hours in two small-scale experiments comparing a) filtered seawater control (black) and WAF treatment (red), and b) filtered seawater control (black) versus oil slick (purple). Error bars represent standard deviation. Variables not connected by the same letter are significantly different, as determined by a post-hoc Tukey-Kramer test.

Table 6.7.1.1. Two-way ANOVA results testing effect of time and treatment on proportion *Calanus* inactivated in experiments testing a) WAF vs control filtered seawater and b) Oil slick vs control filtered seawater.

a)

Two-way Analysis Prop Inactive by time and treatment					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	11	3.506	0.318727	30.5978	Prob > F
Error	48	0.5	0.010417		<.0001
C. Total	59	4.006			
Analysis of Variance					
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	4.4497E-33	0	1
Time	5	5	2.536	48.6912	<.0001
Treatment*Time	5	5	0.484	9.2928	<.0001

b)

Two-way Analysis Prop Inactive by time and treatment					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	11	1.7152847	0.155935	10.386	Prob > F
Error	48	0.7206722	0.015014		<.0001
C. Total	59	2.4359569			
Analysis of Variance					
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	7.2288E-33	0	1
Time	5	5	1.3103932	17.4556	<.0001
Treatment*Time	5	5	0.188649	2.513	0.0423

6.7.2. Recovery of Copepods in Clean Water

Experiment 3: Filtered and Unfiltered CEWAFs: The inactivation of copepods was tested in PAH-containing water-accommodated fractions of oil that contained oil droplets (unfiltered CEWAF) and in solutions with a matching dissolved PAH concentration that did not contain oil droplets (filtered CEWAF; Figure 6.7.2.1). In both cases, the dissolved PAH concentration was approximately 150 to 340 $\mu\text{g L}^{-1}$. Compared to the control, an increasing number of copepods became inactive within the first 3 h of the exposure. After 4 h of exposure, the recovery of copepods was tested by transferring all copepods into clean seawater. Over the next 20h, the number of inactive copepods steadily decreased in the both CEWAF treatments to a similar extent.

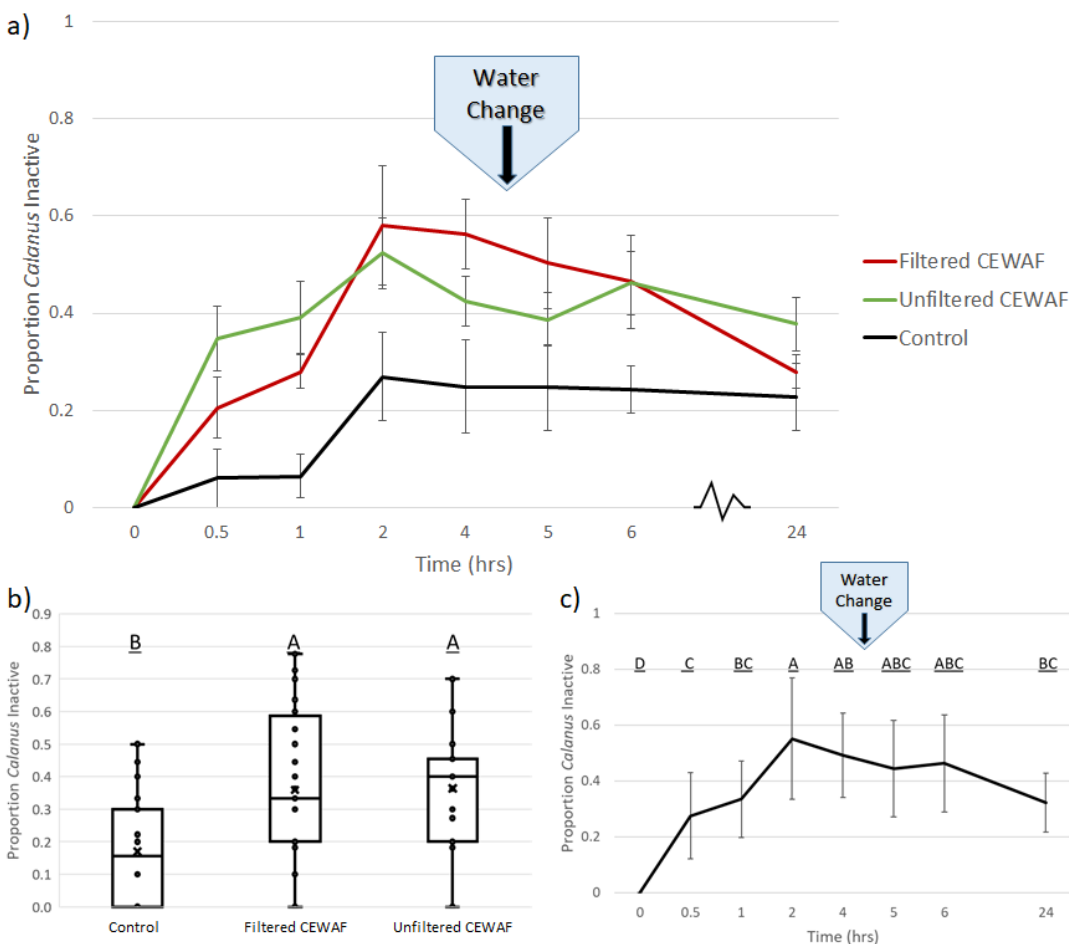


Figure 6.7.2.1. Proportion of *Calanus* inactivated in filtered and unfiltered CEWAF before and after water change. Error bars represent standard error. b) Proportion of *Calanus* deactivated by treatment. Variables not connected by the same letter are significantly different, as determined by a post-hoc Tukey-Kramer test. c) Proportion of *Calanus* in filtered and unfiltered CEWAF inactivated by time point. Variables not connected by the same letter are significantly different, as determined by a post-hoc Tukey-Kramer test.

A 2-way ANOVA was run with the variable factors as time point and treatment (Table 6.7.2.1). Both time and treatment found significant effects but there were no interactive effects between the two variables. The unfiltered and filtered CEWAF treatments resulted in a higher proportion of inactivated *Calanus* than the control, but did not differ significantly from each other. These two treatments were pooled and a one-way ANOVA was run to determine the effect of time point on proportion *Calanus* inactivated in two experimental treatments (Table 6.7.2.2). Over the first 2 hours, inactivation increased statistically at every measured time point until reaching a high of 57% inactivated at hour 2 (Figure 6.7.2.1c). After water change, the proportion inactivated declined slowly, resulting in a 23% decrease at 24 hours from the highest level at 2 hrs. We take this to indicate that there is some capacity for recovery in *Calanus finmarchicus* from dispersant exposure.

Table 6.7.2.1. Two-way ANOVA results testing effect of time and treatment on proportion *Calanus* inactivated in unfiltered CEWAF, filtered CEWAF, and control filter seawater.

2-way ANOVA					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	23	3.837916	0.166866	7.2985	<.0001
Error	95	2.172	0.022863		
C. Total	118	6.009916			
Effects Tests					
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	2	2	1.0941454	23.9281	<.0001
Time	7	7	2.3266667	14.5378	<.0001
Treatment*Time	14	14	0.4192811	1.3099	0.2162

Table 6.7.2.2. One-way ANOVA results testing effect of time point on proportion *Calanus* inactivated in the two CEWAF treatments pooled.

1-way ANOVA					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	7	2.1108023	0.301543	13.0583	<.0001
Error	71	1.6395374	0.023092		
C. Total	78	3.7503397			

6.7.3. Oil Accumulation of Oil on Copepods

Within the oil slick experiment, we observed some copepods interacting with the oil at the surface. Some would get trapped and immobilized by the oil, while others accumulated oil droplets on their carapace (Figure 6.7.3.1). We observed this in the large scale mesocosms as well. Although the oil entrapment wasn't quantified, this is an important observation since it shows that copepods do not consistently avoid a slick. The attachment or ingestion of oil provides a pathway for crude oil to become bioavailable to higher trophic levels. The importance of this pathway to higher trophic levels is currently unknown. Field work during an active oil spill is needed.

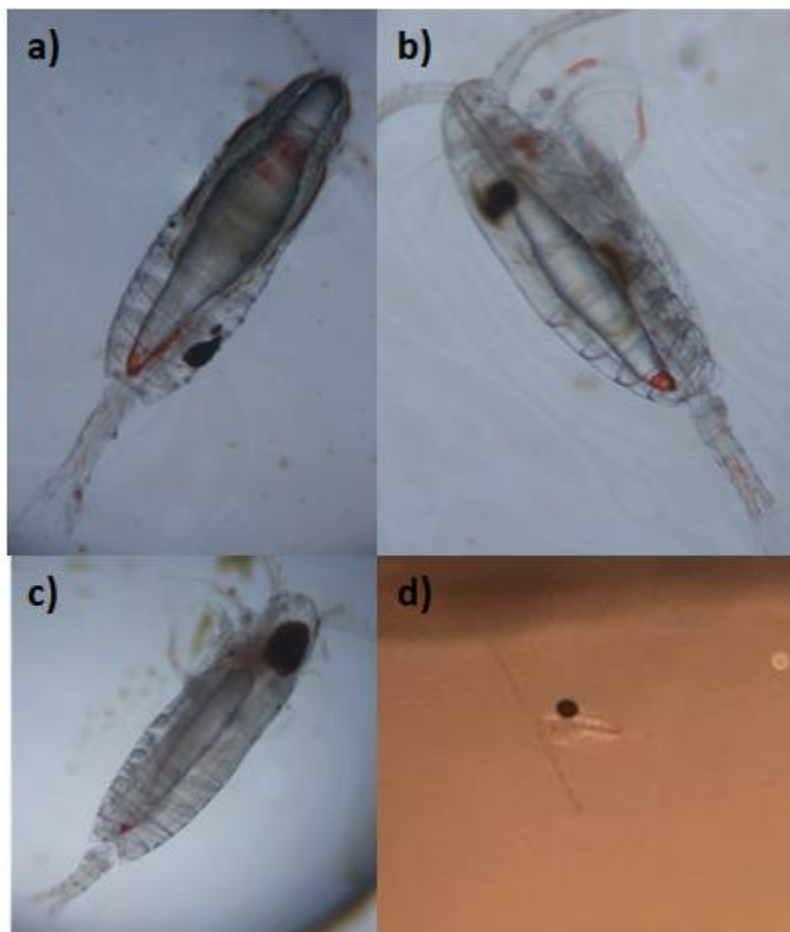


Figure 6.7.3.1. Oil droplets stuck on carapaces of *Calanus finmarchicus* in small scale oil slick experiment, and in large mesocosm experiment. (a) oil adheres to the outer carapace of the copepod. b) oil is entrained in the “heart” between the carapace and the body. c) ingested oil is the foregut of the copepod. d) Image of a copepod with an attached oil bubble.

7. Discussion

7.1. Copepod Tower as a Novel Exposure System

The developed and employed experimental system allowed for investigating the response of aquatic organisms to undispersed and dispersed oil in a new way. The experiments were designed to understand the behavioral response of a foundational food web organism (copepods) to a surface oil spill. The copepod tower, chemical sampling ports and video observation system allowed us to determine the behavior of copepods when they encounter an oil spill.

Novelty of the Copepod Tower: in comparison to commonly-used experimental systems that are based on constant concentration and homogeneous animal distribution, our copepod towers have three novel features: First, we were able to monitor changes in the PAH concentrations over time at a single location and with depth. Second, we were able to simultaneously characterize the movement of the copepods. Third, the two meter tanks provided sufficient vertical space to develop gradients in the water soluble fraction of the oil over the 24h experimental cycle. At 16 °C, the PAH concentrations in the water developed a measurable vertical gradient and provided ample time to resolve the dynamics of the oil as it partitioned into the water column. Similarly, the 2-meter tank allowed the movement of the copepod population to be followed as the oil penetrated deeper into the water column.

The narrow aspect ratio of the column (10 cm wide and 200 cm deep) allowed for the creation of a stable thermocline. The water at the base of the tower was cooled using a small stainless steel coil to generate a temperature gradient of 4 °C simulating a summertime scenario in the Arctic. This feature allowed testing of both the movement of copepods and the oil/dispersant through the density “barrier”. This flexibility of this experimental system lends itself to a wide range testing possibilities.

Monitoring Dynamics of Undispersed and Dispersed Oil: The five sampling ports allowed for a high spatial resolution. Given the total volume of the tanks were > 100L and we typically sampled approx. 50 mL per sampling point (0.05% of the total volume), sampling is expected to only minimally disturb any gradients in the column.

To control the mixing energy in the tower, the tunable air stream was used. Our mixing energies were internally set relatively low, which allowed to temporarily follow a hydrocarbon gradient, while providing sufficient energy to lead to approx. 50 to 90% initial dispersion of the oil slick (based on visual observations of the oil slick coverage).

We used the same energy input for the dispersed and non-dispersed oil. In the case of non-dispersed oil, we were able to monitor the slow mass transfer of PAHs from the oil phase into the aqueous phase. When dispersants were added, we were able to see the formation and downward movement of a front of oil droplets over approx. 4 h.

Characterization of Copepod Dynamics: The USB Machine vision camera (FLIR, USA; Flea) provided 3.2 MP (2048*1536) resolution. The cameras were mounted vertically to view approximately 28 cm of the water column. The end resolution was 14 $\mu\text{m}/\text{pixel}$ (~ 200 pixels/copepod). This resolution worked well to differentiate the copepods from other objects in the optical path. Although a lower resolution could be used if needed higher frame rates.

The 8 bulb array of IR LEDs (850 nm; Univivi Technology) provided ample lighting for each camera viewing the tank. The use of IR lights is a key element of the video setup. IR light is detected by the cameras, they generate uniform lighting within the filming towers and the wave length (~ 850 nm) is undetectable by the visual systems of the copepod.

7.2. Copepod Response to Undispersed oil

Oil Behavior: By applying non-dispersed oil into the unstratified water column, we were able to simulate low concentrations of PAHs (up to approx. $10 \mu\text{g L}^{-1}$) that slightly increased over 24 h. Depending on the exact condition in each replicate experiment, the 24-h concentrations varied somewhat (between 10 and $60 \mu\text{g L}^{-1}$), likely due to differences in advective mixing in the towers caused by small differences in direction and intensity of the air stream that was applied at the top of the towers. This increase in concentration was due to the slow mass transfer from the oil phase into the aqueous phase or an undispersed oil slick in low-energy conditions while reaching equilibrium concentrations. A comparison with theoretical equilibrium concentrations, as well as with measured concentration of small-scale WAFs prepared with evaporated suggests that equilibrium was not reached within 24 h (measured WAF concentrations were $390 \mu\text{g L}^{-1}$ at a loading of 1 g oil L^{-1} ; theoretical calculation for $5 \text{ g oil per } 100 \text{ L water}$ were $260 \mu\text{g L}^{-1}$).

The PAH concentrations at 5h exposure were generally below acute toxicity effects. Reported 96h lethal concentrations for *C. finmarchicus* (LC_{50} concentrations) are 10 to $16 \mu\text{g L}^{-1}$ total PAHs for dispersed crude oil (Hansen et al. 2012b), 480 to $1,000 \mu\text{g L}^{-1}$ PAHs for 96-h tests using marine diesel (Hansen et al. 2013b), and 370 to $1,000 \mu\text{g L}^{-1}$ total PAHs for dissolved PAHs (WAF treatments) for 12-day tests of *C. Glacialis* (Gardiner et al. 2013). We can, therefore, assume that all observed effects were sub-lethal responses of copepods.

Interestingly, we did not observe a pronounced vertical hydrocarbon gradient in three out of four experimental replicates. The hydrocarbon concentration almost simultaneously increased in all five sampling ports over the 24-h observation period. This behavior suggests that the advective mixing within the column was greater than the phase transfer of hydrocarbons between the oil phase and the aqueous phase. However, in one of the replicates (“noteworthy replicate” in Section 6.2.4) we did see a hydrocarbon gradient forming. It is possible that the air stream at the top of the column was more pronounced in the horizontal vs. the vertical direction compared to the other replicates, leading to a faster mass transfer while simultaneously causing a slower vertical mixing.

For the PAH profile, we observed that the two-ring PAHs naphthalene, methyl naphthalenes (i.e., C₁-naphthalenes), and C₂-naphthalenes contributed to approx. 95% of the total PAH concentration. The remaining 5% were mostly C₃- and C₄-naphthalene, followed by phenanthrenes, fluorenes, and dibenzothiophenes. This PAH profile remained rather constant for all the experiments with non-dispersed oil.

Copepod Response: Copepods respond to an untreated surface oil spill with a slow but consistent descent in the water column. The data suggests that as the oil components slowly solubilize, the copepods descended in the water column which decreased their surface population by 40% within the first 4 hours of the experiment. Since the PAH concentrations within the upper water column did not reach levels that cause mortality (~300 ug L⁻¹ - 96h), it is unlikely that the movement of the population into deep waters was a result of dead animals sinking to the bottom of the tank. Rather, these results suggest that copepods can actively avoid a surface oil spill.

An alternate hypothesis is that the exposed copepods were incapacitated and unable to maintain their vertical position in the water column. Since *Calanus spp.* are negatively buoyant, the exposed animals would have sunk from the surface to deeper less toxic water. Based on our finding on the recovery of copepods (section 7.5), when incapacitated copepods are transferred from a sublethal toxic solution to fresh water, they may be able to recover within 24 hrs.

Ecological Implications: If an oil spill occurred during the day, when copepods are in deep water, it is unlikely that adult vertically migrating copepods would be exposed to a surface oil spill. In the night, as the copepods moved toward the surface to feed, the findings of this study suggest that the copepods would detect the solubilized oil and would remain deeper in water and avoid the surface. As a result, the behavioral response allows the copepods to actively avoid the oil and minimize their encounter with

the elevated toxicity. If the spill occurred in the evening, it is likely that some of the surface dwelling copepods would be exposed. These animals may become temporarily incapacitated and sink out of the surface water into less contaminated deep water, where they may be able to recover.

7.3. Copepod Response to Dispersed Oil

Oil Behavior: The hydrocarbon profiles in the treatments with dispersed oil were characterized by high oil concentration due to the formation and downwards transport of oil droplets (Section 6.3.3). The sampling port closest to the surface (Port 1) showed total PAH concentrations $> 2,000 \mu\text{g L}^{-1}$ after 0.5 to 1 h. The presence of the insoluble *n*-alkanes, as well as the visual observation of oil droplets ([Figure 4.8.2.1](#)), shows that compounds dissolved in water as well as in oil droplets contribute to these concentrations. The PAH profile was similar to that of the pure oil, with C_0 to C_2 -naphthalenes contributing to 31% of the total PAH concentrations, C_3 to C_4 -naphthalenes to 20%, total three-ring PAHs to 43%, and total four-ring PAHs to 7%.

This PAH profile was unlike the profile in the non-dispersed oil treatments, where the C_0 to C_2 naphthalenes contributed to 95% of the total PAH concentration. This difference in PAH concentration is a consequence of the different water solubility of the various PAHs, with smaller and less-alkylated PAHs being more water-soluble than larger and higher alkylated compounds.

It is often thought that the dissolved hydrocarbons are the important fraction for a biological response, as they can readily be transported and taken up by organisms. We estimated this dissolved fraction of oil using an analytical technique based on the sorption of dissolved compounds (SPME coupled to GC; see Section [5.2](#)). The resulting dissolved concentrations of PAHs were in the 150 to $1,000 \mu\text{g L}^{-1}$ range 1 h after the addition of oil and dispersants in Port 1. These concentrations are at least a factor of 30 higher than that in the corresponding non-dispersed treatments at the same time. The effect of dispersed oil, therefore, does not only entrain oil droplets in the water column but also facilitates the phase transfer of compounds from the oil into the aqueous phase. This faster mass transfer is facilitated by a high surface area-to-volume ratio of the small oil droplets, as compared to an oil film that was present in the non-dispersed treatments.

Over time, the front of dispersed oil traveled down the water column. Port 2 saw a total PAH concentration $> 300 \mu\text{g L}^{-1}$ within 4 hours of oil addition. In three out of the five replicate tests, Port 3 saw the same increase in concentration, while the remaining two replicates Port 3 remained at concentrations of 0.5 to $18 \mu\text{g L}^{-1}$ in the first 4 hours and

reached maximum values of 130 to 280 $\mu\text{g L}^{-1}$ in the 24-h sampling point. In three of the replicates, PAHs in Ports 4 and 5 stayed at non-detect for 4 hours, while the front of oil droplets increased the PAH concentrations to $> 50 \mu\text{g L}^{-1}$ in the remaining two replicates. Overall, all treatments showed a front of dispersed oil traveling downwards the water column, reaching between Port 2 and Port 5 within 4 hours of oil addition.

After an initial pulse of hydrocarbons, a decrease in total oil concentrations over time was observed in most ports. Specifically, Ports 1 and 2 showed a rapid increase in total PAHs at the beginning of the experiments in all five replicates, followed by a steady decline in concentrations. This decrease in total oil concentration can be attributed to decline in oil droplet volume in the water column over time: larger droplets that were observed at the beginning of the experiments floated to the surface, leaving behind the less-buoyant droplets.

Copepod Response: Exposure to oil and dispersant caused a rapid (within seconds) increase in oil concentration at the surface volume (C1). The oil-dispersant mixture quickly sank deeper into the water column (C2/3). When mixed with the dispersant, the oil concentrations were nearly 100 times higher than without the dispersant and reached concentrations that were orders of magnitude higher than the lethal levels for copepods. Within minutes, the copepod population in the upper layer decreased by 67%. The copepod population remained constant in C2. Camera 3 in contrast showed a slow accumulation of copepods. It is unlikely that the copepods in the upper water column were able to avoid the rapid rise in oil concentrations. The sinking of the oil deep into the water column was much faster than the swimming speed of the individual copepods. What remains unclear is whether the copepods suffered mortality or were incapacitated by high oil concentrations. There remains a large knowledge gap in our understanding of the effects of short term exposure of copepods to oil. Toxicology experiments are typically concerned with gradations in concentration rather than exposure time. Data from our experiments (section 7.5) suggests that there is a clear gradient in toxicity with exposure time. Within the first 4 hour of exposure the toxicity to the copepod increases up to nearly 60% of the copepods affected.

Ecological Implications: Our experiments suggest that copepods that are in contact with dispersed oil (total PAH concentrations $> 200 \mu\text{g L}^{-1}$) sink to greater depth within hours. In an ecological context, 4 hours provides ample time for copepods to move deeper in the water. Based on our small-scale experiments (see discussion below, Section 7.5), it appears that the copepods can recover, to some extent, when transferred to clean seawater. Thus by sinking out of the water column, a proportion of the copepods may recover and previous predictions of high mortality may not be warranted.

7.4. Copepod Response in a Stratified Water Column

Oil Behavior: A water column stratification caused by temperature gradient restricted the transport of oil hydrocarbons (Sections 6.5.1 & 6.6.2). When using non-dispersed oil, the lower sampling ports (Ports 4 and 5) remained $< 2 \mu\text{g L}^{-1}$ PAHs during the 24-h observation, while the upper ports (Port 1 to 3) showed increased PAH concentration of up to $4 \mu\text{g L}^{-1}$ (6.5.1). Although the concentration difference was rather small, it suggests that the thermal gradient (present between Ports 3 and 4) limited the diffusion of PAHs to greater depths.

For dispersed oil, a similar limitation of oil movement was observed (Section 6.6.2). A hydrocarbon front traveled down the water column and reached peak total PAH concentrations in the range of 600 to $1,300 \mu\text{g L}^{-1}$ in Port 3 within 4 h of the oil/dispersant addition. However, this peak concentration did not migrate to Ports 4 and 5, and total PAH concentrations stayed in the range of 20 to $100 \mu\text{g L}^{-1}$ at the 4-hour sampling point in these ports. Thereafter, a gradual slow increase was observed in these lower ports, reaching the 100 to $200 \mu\text{g L}^{-1}$ range for the 24-h sampling point. While these Port 4 and 5 concentrations were still substantially higher than in any of the non-dispersed oil treatments, they were approx. 50% lower than that of the unstratified dispersed oil experiments. This signifies a restriction of oil droplet movement to water depth below the thermocline.

A noticeable feature of the dispersed oil profiles in the stratified water column was a peak concentration at the location of the thermocline (Port 3) around 0.5 to 1 h after the addition of the oil (Figures 6.6.2.1-3). During this time, lower total PAH concentration existed above and below Port 3. It appears that after the initial cloud of oil was formed on the surface and transported downward, a reduction in oil droplet formation occurred on the surface. Such a reduction in oil droplet formation is consistent with the decreasing total PAH concentration at Port 1 over time (see also Section 7.3). The environmental relevance of such a subsurface hydrocarbon maximum is not clear. While there existed a subsurface maximum during the subsurface *Deepwater Horizon* oil spill, such a subsurface maximum would generally not be expected during a surface oil spill.

Copepod Response: The stratified water column presented a clear barrier to vertical movement of oil in the water column. However, the copepods moved freely through the temperature gradient when the oil and dispersant was added to the top of the water column. The data showed a rapid decline in the number of copepods in the surface

layer (C1) analogous to the unstratified treatment. Copepod abundance decreased by 45% within the first 3 hours of the experiment. Two of the replicates showed a decrease in copepod numbers similar to the unstratified treatment. However, in contrast to the unstratified water column, the copepods did not descend all the way to the bottom of the tank. Interestingly, in the stratified water column the copepods accumulated in the lower water column resulting in an increase of 20% population abundance. These results suggest that the copepods actively avoided the oil/dispersant and descended in the water until they reached low oil concentrations.

Ecological Implications: Our results show that the thermocline limits the vertical transport of oil and provides a place of refuge below the thermocline for copepods to escape the high concentrations of oil. During stratified water column conditions, copepods are protected from dispersed oil. Depending on the time of year, a shallow thermocline will retain the oil in the upper few meters of the water column. If the thermocline is more shallow than the depth of the chlorophyll maximum, where copepods would feed during night time, the copepods may be unaffected. If the thermocline is as deep as the subsurface chlorophyll maximum, then the copepods may become food limited.

7.5. Inactivation and Recovery of Copepods upon Oil Exposure

Acute Toxicity of Copepods: Concentrations of 100 to 200 $\mu\text{g L}^{-1}$ dissolved PAHs do not lead to significant mortality in the copepods within 24 h (compared to the control). The presence of oil droplets did not exacerbate effects, pointing to the dissolved fraction of oil as responsible for causing effects.

Inactivation of Copepods: Copepods exposed to sub-lethal concentration of 100 to 200 $\mu\text{g L}^{-1}$ dissolved PAH became incapacitated. This response occurred within a short timescale (within hours). Since *C. finmarchicus* is negatively buoyant, when they stop swimming they sink. This sinking of non-swimming animals is common to other *Calanus* spp. copepods.

Recovery of Copepods in Clean Water: From small-scale experiments there is an indication that incapacitated copepods have the ability to recover when transferred to clean water. This behavior was observed in the small-scale experiment, but can also be inferred from the copepod tower experiments. These results suggest that copepods exposed to elevated oil concentrations in the surface may recover from the oil when they reach deeper/cleaner waters. Previous experiments that restrained the copepods

in the toxic layers may have overestimated the ecological impact of the oil on free swimming copepods in an oil gradient.

Accumulation or Uptake of Oil Droplets: We saw three general patterns in the accumulation of droplets in the copepods. Copepods accumulate oil on the surface of their carapace, between the carapace and the body and in their foregut, when the oil droplets are ingested ([Figure 6.7.3.1.](#)). This attachment of ingestion of oil droplets could have implications for bioaccumulation of oil hydrocarbons into the biomass of copepods. Since copepods are important for the Arctic food web ([Figure 3.2.1](#)), such an bioaccumulation would lead to transfer of hydrocarbons into higher trophic levels. However, it was beyond the scope of this study to investigate these effects.

8. Implications for Application of Dispersants in the Field

The data presented in this report fills a knowledge gap in our understanding of how copepods (small planktonic crustaceans) respond to surface oil spills under non-stratified and stratified water columns, and with and without dispersants. The interpretations of these experiments provide a framework for assessing the potential risks to copepods of oil spills and dispersants at different times of the year, and can guide decisions on application of oil spill response options to minimize damage to copepod populations.

8.1. General Recommendations for Dispersant Application

[Figure 8.1](#) summarizes the implications of the presented study for application of dispersants. Our study suggests there are three time windows to consider.

First, when *Calanus* spp. copepods are in their early development stage (egg, nauplii, and CI stage; early spring), there is a high risk that dispersed oil would negatively affect copepods (RED in the Figure). Young stages are confined to the upper water column. The toxicity of dispersed oil to young copepods may limit copepod recruitment. Furthermore, oil that is ingested or attached to copepods could be consumed by higher trophic levels. Dispersants should not be added.

Second, during late spring, there is a moderate risk that the application of dispersants will negatively affect copepods (YELLOW in the Figure). The water column is unstratified and dispersants would increase the interaction of oil with copepods. Also, oil that is ingested or attached to copepods can be consumed by higher trophic levels. However, copepods that come in contact with oil can sink to cleaner water and potentially recover. The use of dispersants should be used conservatively depending on the developmental stage of the copepods and the structure of the water column.

Third, during summer, the application of dispersants represents a relatively low risk to the copepod population (GREEN in the Figure). In June and July the water column is highly stratified limiting the depth that the oil/dispersant will reach. Furthermore, should copepods be exposed to oil, they would likely sink to lower depth, where they can recover in clean water conditions. However, a caveat needs to be added: Potential long-term effects of oiling, as described as a possibility in literature (Toxværd et al. 2018, 2019) were not considered in this present study.

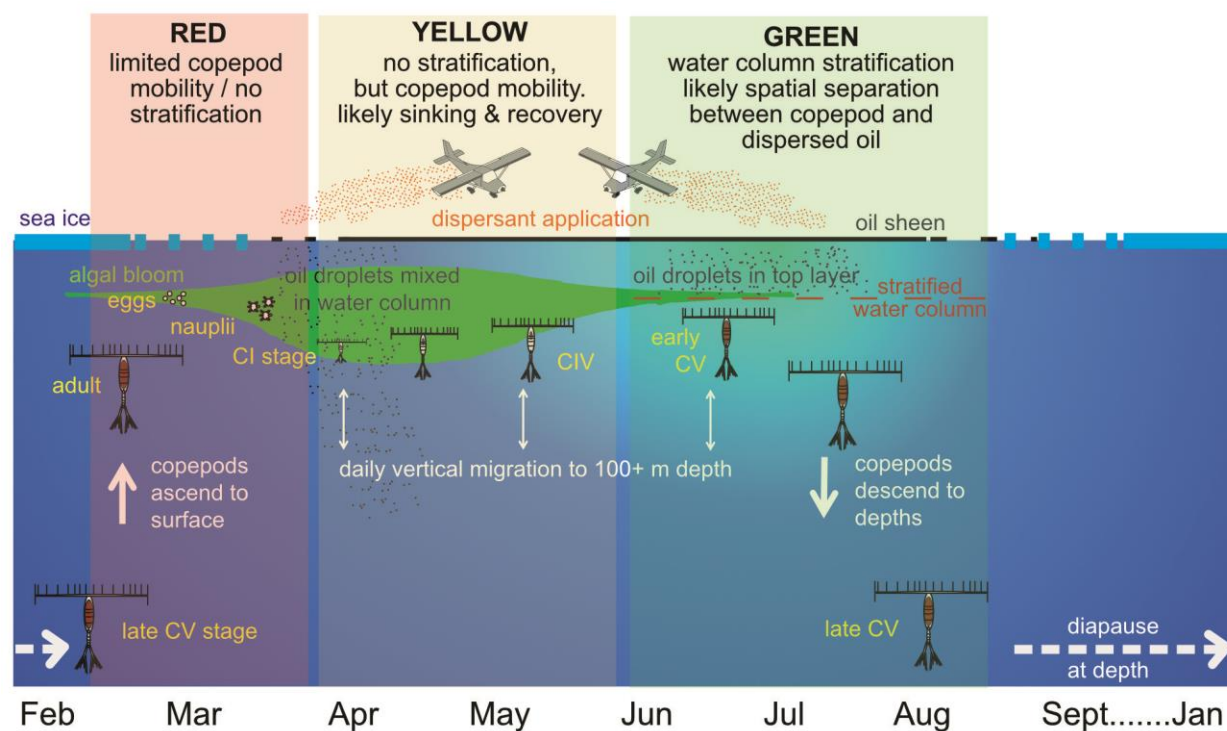


Figure 8.1. Risk of dispersant application to a model arctic copepod over its life cycle. Colors represent the relative risk to the copepod population of adding dispersants to an oil spill. RED represents high risk, YELLOW medium risk, GREEN low risk of direct copepod impacts.

8.2. Considerations for Dispersant Use in the U.S. Arctic Waters

[Figure 8.2.](#) shows a map of U.S. Arctic waters. Copepods of the genus *Calanus* are keystone organisms in the Bering Sea, the Arctic Ocean, and the Gulf of Alaska. Thereby, *Calanus marshallae*, the closely-related sister species of the studies *Calanus finmarchicus* (Frost 1974), is predominantly present in the Bering Sea and the Gulf of Alaska. Due to the similarity of these species we can expect that the effects we observed in our study are directly applicable to *C. marshallae*. Our recommendations would therefore apply to a large area of U.S. waters, including the areas of pre-approved dispersant applications (AK Department of Environmental Conservation 2019).

The larger *Calanus Glacialis* and *Calanus hyperboreus* are present in the Arctic Ocean. Due to their similar behavior (lipid-rich species, diapause to overwinter, similar feeding behavior) we expect that the generalized recommendation of restricted dispersant use in early development stage, and a likely reduced risk of dispersant use during stratified water column conditions, would still apply to these species. However, the timing of ice melt, copepod development, and phytoplankton bloom would be different from that in [Figure 8.1.](#)

Lastly, the species present in the shallow shelf include a mixed population of copepods with different ecological strategies. While *Calanus glacialis* are similar to *C. finmarchicus*, *Pseudocalanus spp.*, *Acartia tumida* and *Oithona similis* have different life cycles than the investigated *C. finmarchicus*. We therefore, cannot make recommendations based on our study. It is likely that early-stages of these species are also vulnerable to dispersed oil and the surface dwelling species may suffer a similar fate. However, due to the shallow nature of the shelf, the copepods can generally not escape to great depth.

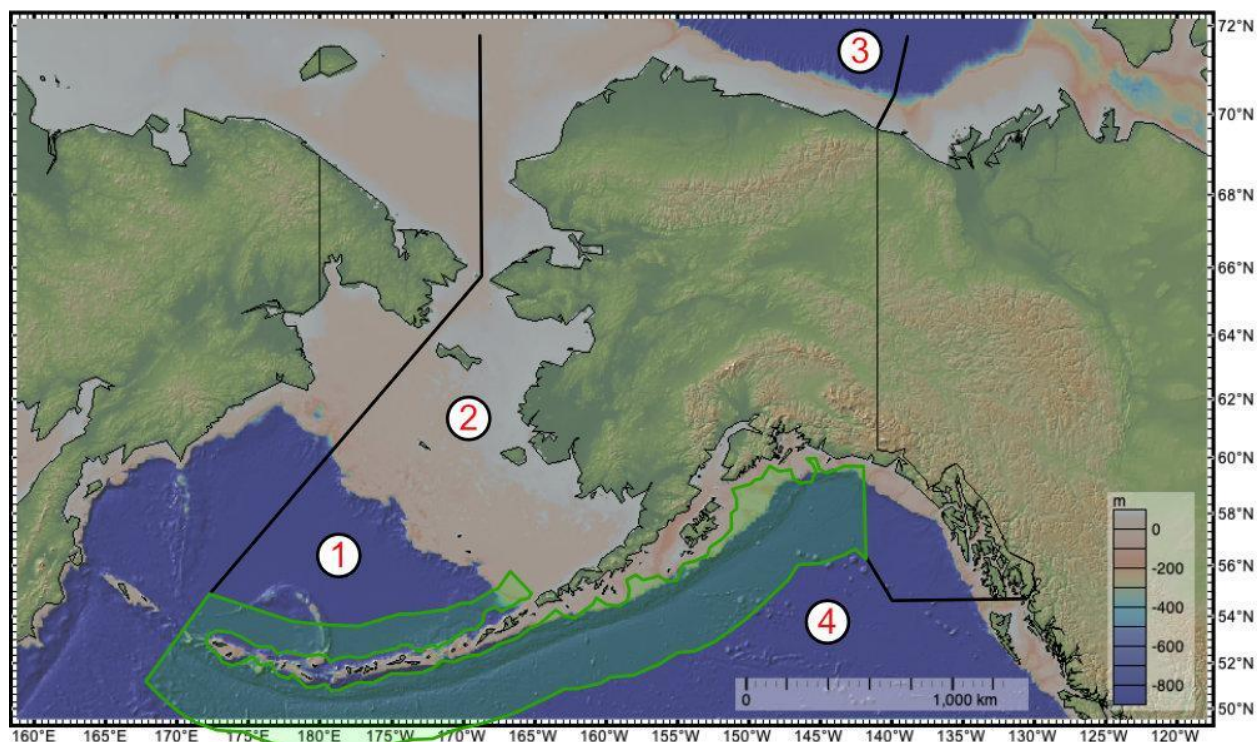


Figure 8.2.: Map showing copepod distribution and dispersant pre-authorization zones in U.S. Arctic Waters. The black lines show the borders of the Alaska planning area for oil spill response, and the green area depicts pre-approved dispersant application areas (AK Department of Environmental Conservation 2019). The dominant copepod species in different marine zones are indicated by numbers, as follows (Llinás et al. 2009): **1:** *Calanus marshallae* and *Calanus glacialis* in the Bering Sea; **2:** *Calanus glacialis* and *Pseudocalanus* spp. throughout the shelf; **3:** *Calanus hyperboreus*, *Calanus glacialis* and *Metridia longa* in the Arctic Ocean; **4:** *Calanus pacifica* (in the summer) and *Calanus marshallae* in the Gulf of Alaska.

8.3. Remaining Research Needs

Bioaccumulation: More work is needed to determine the bioaccumulation of oil in the copepods. Bioaccumulation includes the attachment of oil to the exterior of the animals. This may have little effect on the animal physiology but still provide a vector for oil to higher trophic levels. Oil can also be ingested. This would potentially affect the copepod's physiology. Finally, the oil may become incorporated into the lipid stores of the copepod. This oil would affect the physiology of the copepod, the development of the eggs of the copepod and could be biomagnified in higher trophic levels.

Interestingly, oil that is incorporated into the copepods lipid stores may be retained for months. For copepods that diapause (hibernate) through the winter at great depths, the stored oil in the fat of the animal could decouple the timing of the oil spill from when the toxicity appears in the food web. Determining the fate of ingested oil remains an important knowledge gap in our understanding of the ecological implications of an oil spill in the Arctic.

Lethal and Sublethal Threshold Concentrations: Determine the toxicity of oil to copepods over time and concentration. Current toxicity experiments calculate toxic concentrations (LC_{50} values) based on concentration over a constant exposure time. However, for animals such as copepods that are mobile and can escape the oil, an LC_{50} value may significantly overestimate the toxic levels. Additional experiments that reevaluate the toxicity of oil on time scales that are consistent with ecological exposure time would help to parameterize the effect of an oil spill on the population.

Other Oil Response Options: This study investigated the effects of crude oil and dispersants on the behavior of *C. finmarchicus*. In the open ocean, in-situ burning the surface oil slick provides an additional remediation tool to remove the oil. Burn oil produces a unique suite of residues or oxygenated transformation products with unknown effects on zooplankton populations.

Behavioral Impact of Other Planktonic Water Column Organisms: Understanding the behavior of other key species in the Arctic ecosystem to oil spills (and their remedies) is key to predicting the magnitude of impact of the spill. The application of the techniques used in this study to other economically and ecologically important species is warranted. In the marine environment nearly all vertebrate and invertebrate species begin their life in the plankton including larval fish, benthic crustaceans and a suite of benthic invertebrates which support an economically diverse fishing industry. As the Arctic ecosystem opens up to greater shipping and extraction, a well developed mitigation plan should include the response of several key organisms.

9. Broader Impacts: Outreach - Educational Outcomes

The knowledge products of this work were used in talks to other scientists and to the general public. Bigelow engages in outreach through its numerous public tours, our teacher workshop (<https://www.bigelow.org/education/professional-development.html>) our high school program (<https://www.bigelow.org/education/bloom.html>) and our undergraduate program (<https://www.bigelow.org/education/reu/>). Students in our undergraduate program developed focused projects on specific aspects of the hypotheses addressed in this larger study. The educational impacts of this work are shown below (section 9.1). The findings of this work have been presented in multiple round table discussions with project champions and partners of ADAC (8 meetings), Bigelow undergraduate symposiums (5 presentations), ADACs undergraduate symposium (2 presentations) and 3 national meetings (5 presentations). The inclusion of student projects in this work is an important component of the societal impact provided by this funding.

9.1. Student Research Projects

This project has supported 6 student projects during the 3 years. In 2020 two students (Sam McNeely and Cameron Carlson) participated in the ADAC program and completed 2 independent projects with research conducted at Bigelow. A third student participated through Bigelow's REU program. Due to COVID-19, the projects were conducted virtually.

The two ADAC fellows worked directly on the tower experiments being conducted at the lab. Sam McNeely (Junior; University of North Carolina) investigated the change in the distribution of copepods in the towers when Alaskan Slope Crude Oil was introduced to the top of the column. The raw video files were sent to Sam every 3 days for him to extract the copepod abundance in each camera over time. During the summer Sam learned image analyses, basic programming (Python, R) and large dataset management.

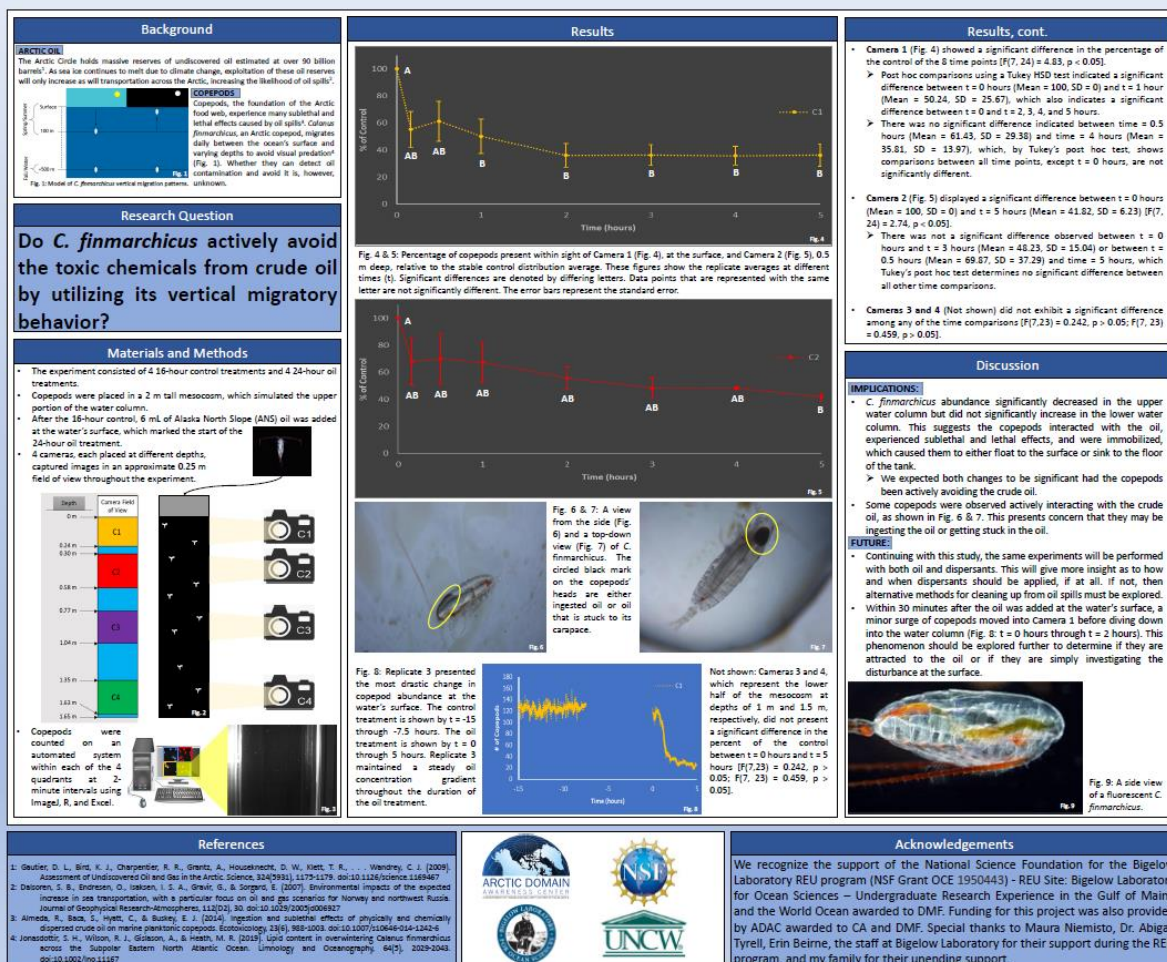
9.1.1. *Tiny Travelers: Behavioral Response of Copepod (Calanus finmarchicus) to Crude Oil Spills*

Authors: *Sam McNeely^{1,3}, *Cameron Carlson^{2,3}, Maura Niemisto³, Erin Beirne³, Abigail Tyrell³, Christoph Aepli³, David Fields³; (1)University of North Carolina Wilmington, Wilmington, NC, United States, (2) University of Alaska Anchorage, Anchorage, AK, **Affiliation:** United States, (3)Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, United States. *ADAC Fellows

The Arctic Circle holds massive reserves of undiscovered oil estimated at over 90 billion barrels, and, as sea ice continues to melt due to climate change, exploitation of these oil reserves will only increase as will transportation across the Arctic, increasing the likelihood of oil spills. Copepods, the foundation of the Arctic food web, experience many sublethal and lethal effects from crude oil. An Arctic copepod (*Calanus finmarchicus*), makes daily vertical migrations between the ocean's surface and a depth of 100 m. Our hypothesis is that *C. finmarchicus* can use its vertical migratory behavior to actively avoid the toxic chemicals that come from crude oil. *C. finmarchicus* are placed into a 2 m tall mesocosm, simulating the upper water column, with 4 cameras positioned at different depths. The cameras capture images throughout the 16-hour controls and the 24-hour oil treatments. The videos from the experiment are analyzed in ImageJ, R, and Excel. Our results show that copepod abundance in the upper mesocosm experienced significant decreases in proportion of the control ($p < 0.05$). The two cameras at depth displayed slight changes, however, they were insignificant ($p > 0.05$). This indicates that *C. finmarchicus* interacted with the oil, experienced sublethal and lethal effects, and were immobilized. This provides insight into the behavior of *C. finmarchicus* following the introduction of crude oil indicating the foundation of the Arctic food web are vulnerable to oil spills, highlighting the necessity of proper cleanup methods to minimize the environmental impact.

Tiny Travelers: Behavioral Response of Copepod (*Calanus finmarchicus*) to Crude Oil Spills

Sam McNeely^{1,3}, Cameron Carlson^{2,3}, Maura Niemisto³, Erin Beirne³, Abigail Tyrell³, Christoph Aeppli³, David Fields³
¹University of North Carolina Wilmington¹, ²University of Alaska Anchorage², ³Bigelow Laboratory for Ocean Sciences³



9.1.2. Simulating hydrocarbon gradients in the water column with mesocosms

Cameron Carlson (Senior; University of Alaska) used optical fluorescence to measure oil concentrations in the towers. Raw data was collected by our technician and sent to Cameron to analyse.

Authors: *Cameron Carlson^{1,2}, Dr. Christoph Aeppli², David Fields²

Affiliation: University of Alaska Anchorage¹, Bigelow Laboratory for Ocean Sciences²

*ADAC Fellows

Abstract: To conduct experiments on zooplankton response to oil spill treatments, we need a laboratory system which can simulate the gradient of hydrocarbon concentration down a water column. For this purpose, we constructed two-meter-tall tanks to hold marine water and copepods, to which treatments of undispersed and dispersed oil are added. These mesocosms simulated the expected small gradient of undispersed oil and more pronounced gradient of dispersed oil. Additionally, the system corroborated the increased concentration of crude oil hydrocarbons in seawater exposed to dispersed oil, as compared to undispersed oil.



Simulating hydrocarbon gradients in the water column with mesocosms

Cameron Carlson^{1,3}, Dr. Christoph Aeppli³, Dr. David Fields³, Sam McNeely^{2,3}
University of Alaska Anchorage¹, University of North Carolina Wilmington², Bigelow Laboratory for Ocean Sciences³



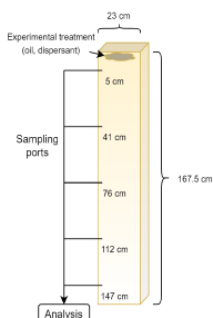
Abstract

To conduct experiments on zooplankton response to oil spill treatments, we need a laboratory system which can simulate the gradient of hydrocarbon concentration down a water column. For this purpose, we constructed two-meter-tall tanks to hold marine water and copepods, to which treatments of undispersed and dispersed oil are added. These mesocosms simulated the expected small gradient of undispersed oil and more pronounced gradient of dispersed oil. Additionally, the system corroborated the increased concentration of crude oil hydrocarbons in seawater exposed to dispersed oil, as compared to undispersed oil.

Introduction

In the event of an oil spill in a marine environment, a common technique for remediation is the application of chemical dispersants: surfactants which disperse the oil into droplets in the water column. This technique is effective at removing most of the spilled oil by increasing the rate of biodegradation, a consequence of the dispersion and increased surface area of the lipid-aqueous interface. At the same time, this remediation technique could potentially be posing a greater risk to marine life than untreated oil. Dispersed oil is more likely to impart toxic effects to plankton such as copepods. When dispersed, oil more easily releases toxic hydrocarbon compounds, such as polycyclic aromatic hydrocarbons (PAHs) into the water. Additionally, the reduced size of oil droplets increases the risk of oil being directly ingested by copepods (such as *Calanus finmarchicus*). This risk suggests that application of chemical dispersants may not be the most ecologically friendly technique. However, the ability of *Calanus spp.* to dive several hundred meters in the water column in a single day potentially mitigates this risk. If *Calanus spp.* can detect crude oil gradients in the water column and respond by diving, then it may be appropriate to apply dispersants to oil spills even during the active season of copepods. This interaction is difficult to simulate in normal toxicity experiments, which are performed in shallow systems. We are testing the potential for copepod oil detection and vertical movement by simulating oil gradients in the marine environment with mesocosms. These mesocosms are two meters tall aquaria, to which *C. finmarchicus* are added, and at the top of which oil and dispersants are applied.

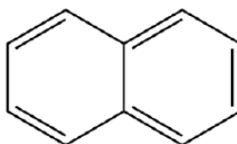
Methods



Experimental treatments: We added 300 *C. finmarchicus* to the mesocosm for each experiment. Four experiments received crude oil to the surface of the water, and one experiment received a mixture of crude oil and dispersant.



Data collection: Sample ports collected water at each depth at timepoints of 0, 0.5, 2, 4, and 24 hours. Additionally, cameras constantly captured images of copepods to observe vertical movement.

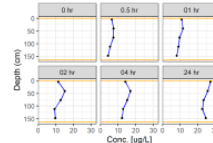


Analysis of PAHs: A fluorometer quantified the concentration of PAHs for each sample.

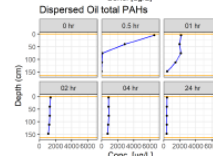
Results and Conclusion

As of July 24, 2020, we have performed four replicates of mesocosms tested with oil and no dispersant, as well as one experiment of a mesocosm tested with oil and dispersants. One of the replicates of oil only is an outlier, probably due to insubstantial air flow mixing. While this replicate will be revelatory when compared with copepod vertical distribution data, it is unimportant for evaluating the establishment of hydrocarbon gradients in this novel mesocosm system.

Undispersed Oil total PAHs



Oil only: undispersed oil established a small PAH gradient between 2 – 4 hours, after which the concentration became relatively constant down the water column.



Oil and dispersant: dispersed oil quickly established an extreme gradient, which leveled out after 1 hour.

The mesocosm apparatus was capable of simulating a hydrocarbon gradient, although, as expected, at a shorter timescale than would be expected in a true marine environment. The gradient, as well as the overall concentration of PAHs, was much more pronounced for the samples of dispersed crude oil, as compared to undispersed crude oil.

Acknowledgments

We recognize the support of the National Science Foundation for the Bigelow Laboratory REU program (NSF Grant OCE 1950443). REU Site: Bigelow Laboratory for Ocean Sciences - Undergraduate Research Experience in the Gulf of Maine and the World Ocean awarded to DMF. Funding for this project was also provided by ADAC awarded to CA and DMF. Special thanks to Erin Belme, Mauna Niemisto, and the staff at Bigelow Laboratory for their support during the REU program.

9.1.3. Predicting the Dissolved Organic Matter-Water Partitioning for Short-Chain Chlorinated Paraffins

Elizabeth Westbrook (Junior; University of Maryland) investigated the degree that chlorinated hydrocarbons are solubilized. Chlorinated hydrocarbons are highly toxic to living organisms.

Authors: Elizabeth Westbrook², Christoph Aeppli¹, Brian DiMento¹

Affiliation: 1: Bigelow Laboratory for Ocean Sciences; 2: University of Maryland, College Park

Abstract: Short-chain chlorinated paraffins (SCCPs) were declared a persistent organic pollutant by the United Nations Environment Programme in 2017 due to their potential carcinogenicity, ability to bioaccumulate, and persistence in the environment. The goal of this project was to better understand the fate of these molecules in the ocean by studying their dissolved organic matter (DOM) mediated photodegradation. Previous studies involving other hydrophobic organic compounds (HOCs) show that a molecule's affinity for the hydrophobic micro-environment created by DOM molecules is an important factor in its ability to degrade by this pathway. In order to predict the ability of a variety of SCCPs to degrade in the presence of DOM, a method for predicting the DOM-water partitioning coefficient (K_{dom}) of any SCCP molecule was determined. COSMOtherm calculated octanol-water partitioning coefficients (K_{ow}) were found to be the most accurate method of predicting K_{dom} when adjusted based on the linear relationship that exists between K_{ow} and K_{dom} for HOCs. This method was then used to predict K_{dom} for a variety of SCCPs and identify characteristics that correlated with higher affinity for DOM. The primary characteristics identified were longer carbon chains and generally increased chlorine content. Results also show that the distribution of chlorine substituents effects affinity for DOM. While K_{dom} values were highly variable for SCCPs, most of them are expected to be capable of at least some DOM mediated photodegradation based on previous studies of this pathway

Predicting the Dissolved Organic Matter-Water Partitioning for Short-Chain Chlorinated Paraffins



Bigelow Laboratory for Ocean Sciences

Elizabeth Westbrook², Christoph Aeppli, Ph. D.¹, Brian DiMento, Ph. D.¹

¹Bigelow Laboratory for Ocean Sciences

²University of Maryland, College Park



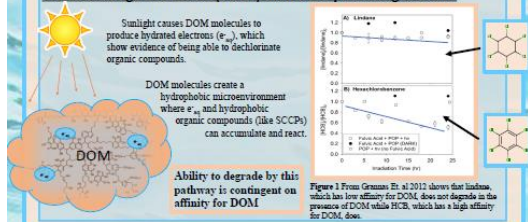
Background Information

Short-chain chlorinated paraffins (SCCPs)

- Structures:**
- 10-13 carbon chain
 - 30-70% chlorine by mass
 - Exemplifies over 4000 different molecules
- Uses:**
- Industrial metal working lubricants
 - Paint additives
 - Flame retardants
- History:**
- Used since around the 1930's
 - Declared a persistent organic pollutant by the United Nations Environment Programme in 2017



Dissolved organic matter (DOM) mediated photodegradation

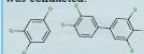


Research Goals

1. Determine the best method for predicting DOM partitioning coefficients for a variety of SCCPs
2. Use this method to identify characteristics that make certain SCCPs more capable than others of degradation in the presence of DOM

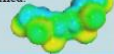
Methods

A literature search for K_{DOM} (dissolved organic matter / water partitioning coefficients) values of hydrophobic organic compounds like SCCPs was conducted.



K_{DOM} values for these molecules were calculated using COSMOtherm using different methods of representing DOM in the computer. The most accurate method was identified.

K_{DOM} of a variety of SCCPs were calculated using the method found to be the most reliable and characteristics that lead to higher K_{DOM} values were identified.



COSMOlogic software suite:

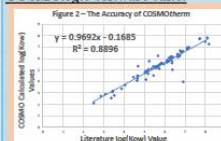


Figure 2 Shows the accuracy of COSMOtherm when calculating octanol/water partitioning coefficients for hydrophobic organic compounds by comparing calculated values to literature values.

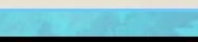
COSMOconf –

Optimized the molecular geometry of all molecules used in calculations done by COSMOtherm

COSMOtherm –

Calculated partitioning coefficients for HOCs using parameters generated by COSMOconf

Partitioning Coefficients:



Results- Best Method of Predicting K_{DOM}

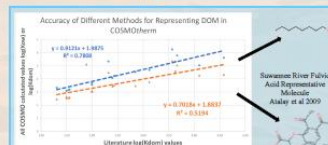


Figure 3 shows the accuracy of different methods for representing DOM in COSMOtherm when calculating partitioning coefficients of hydrophobic organic compounds by comparing calculated values to experimentally determined literature values.

Conclusions Based on Figure 3:

- K_{DOM} values are best predicted by linear relationship between K_{DOM} and K_{OW} values to extrapolate predicted K_{DOM} values.
- The equation to be used when adjusting K_{OW} values to be predicted Suwannee River Fulvic Acid K_{DOM} values is: $\log(K_{DOM}) = 0.99\log(K_{OW}) - 1.98$
- While this relationship is only applicable when the type of DOM being considered is Suwannee River Fulvic Acid, Burkhard et al. 2000 showed that this linear relationship exists when many types of DOM are being considered after minor adjustments to the above equation.

Results – Identifying SCCP Characteristics that Lead to Higher K_{DOM}

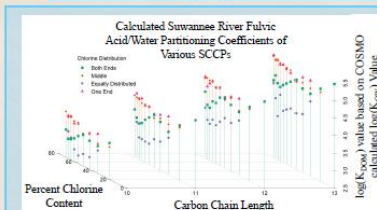


Figure 4 uses K_{OW} values for various SCCPs calculated by Dr. Juliana Grassano et al in 2013 to predict the partitioning of these molecules to Suwannee River Fulvic Acid. The scatter plot reveals the characteristics that lead to increased K_{OW} values (and therefore increased K_{DOM} values)

Conclusions Based on Figure 4:

- As carbon chain length increases, affinity for DOM increases
- For SCCPs with chlorines distributed on both ends of the molecule or equally distributed over the molecule, K_{DOM} changes in a very interesting pattern as chlorine content increases.
- SCCPs that have chlorines concentrated in the middle of the molecule or on one end of the molecule tend to have higher affinity for DOM, especially as chlorine content increases.

K_{DOM} values in figure 4 were calculated from K_{OW} values originally calculated in Grassano et al, 2013 using COSMOtherm.

Discussion

What these results mean for SCCP ability to degrade in the presence of DOM:

The $\log(K_{DOM})$ value of Hexachlorobenzene (HCB), when calculated by this method, is 3.2. Since HCB has been shown to be capable of degradation by this pathway due to its tendency to associate with DOM molecules, we expect that SCCP molecules with a calculated $\log(K_{DOM})$ over 3.2 will also associate with DOM and be able to degrade by this pathway.

Broader Impacts:

The Purpose of this project was to better understand the indirect photodegradation of SCCPs in the presence of DOM, specifically which SCCP congeners were more capable of degradation by this pathway. Understanding which congeners will persist longer in natural waters may lead to more informed policy regarding their use and production. Finally this project helps to further our understanding of what a typical DOM system looks like and how the properties of DOM and the molecules around it influence that system.

Further Investigation:

- A closer look at the folding patterns of these molecules is needed to better understand why COSMO calculated K_{OW} values change in this pattern as chlorine content increases
- Future projects should focus on marine DOM and the relationship between K_{OW} values and K_{DOM} values of marine DOM as to coincide with the larger photodegradation study
- Another avenue for potential research may be determining if higher affinity for DOM also indicates ability to bioaccumulate.

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Acknowledgements

We recognize the support of ADAC awarded to CA and DF, the National Science Foundation for the Bigelow Laboratory REU program (NSF Grant OCE 1950443) - REU Site: Bigelow Laboratory for Ocean Sciences - Undergraduate Research Experience in the Gulf of Maine and the World Ocean awarded to DMF and for this project (NSF ECS - 1808054). Special thanks to Dr. David Fields, Dr. Chris Aeppli, Dr. Brian DiMento, and the staff at Bigelow Laboratory for their support during the REU program.

In 2021 we accepted 3 students into our internship program to work on ADAC supported/related projects

9.1.4. Copepod Ingestion Rate At Sublethal Oil Concentrations

Authors: *Sam McNeely^{1,2}, Maura Niemisto², Christoph Aeppli,² & David Fields,²

Affiliation: 1 University of North Carolina Wilmington, 2 Bigelow Laboratory for Ocean Sciences. *ADAC Fellow

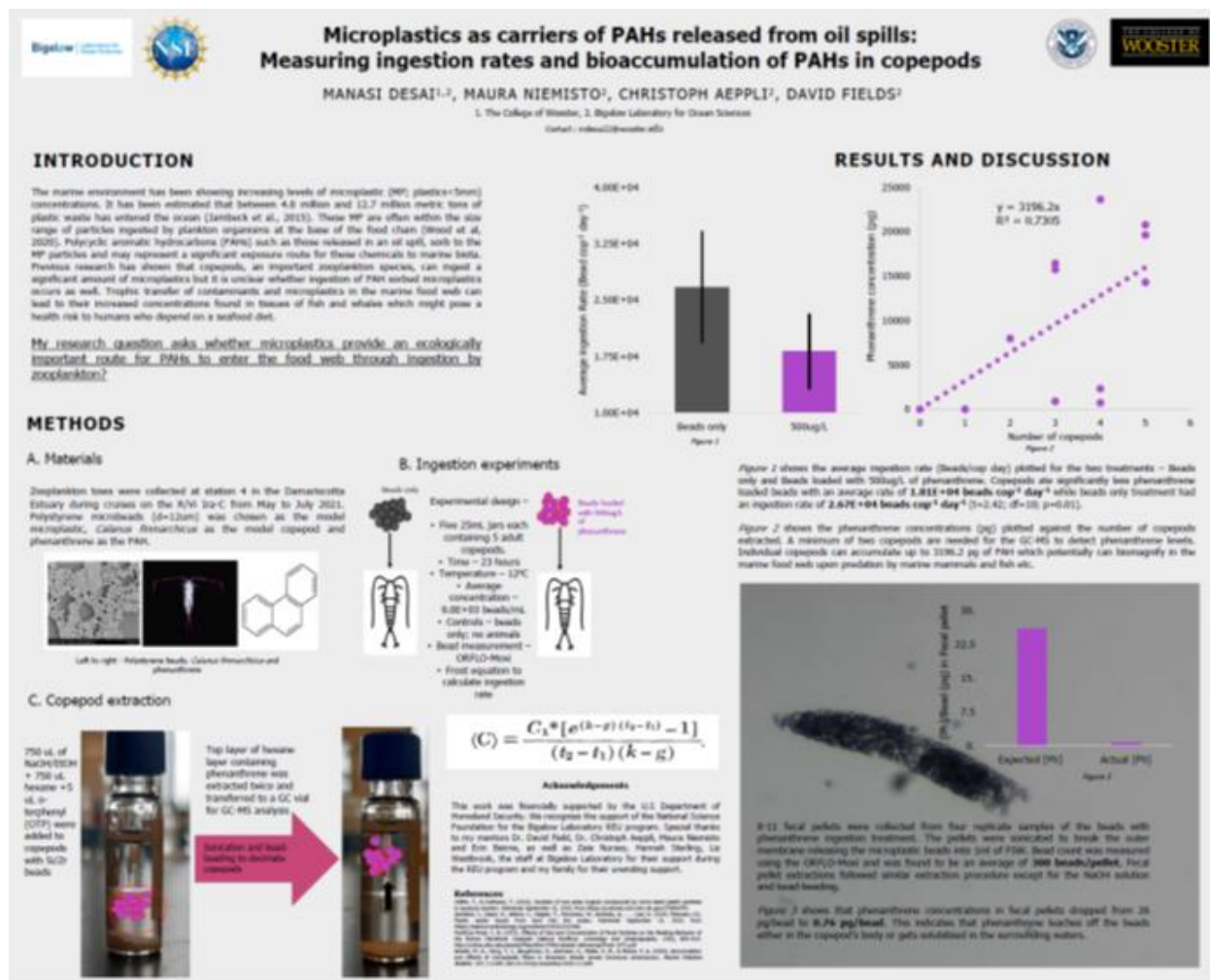
Abstract: Receding sea ice in the Arctic opens the gate for exploitation of Arctic oil reserves as well as transportation, thereby increasing the likelihood for an oceanic oil spill to occur. When exposed to sublethal crude oil and dispersant concentrations, copepods experience decreases in offspring production and fecal pellet production rates, suggesting a decrease in ingestion rates. This study examined the effects that sublethal crude oil and dispersant concentrations have on ingestion rates in a common North Atlantic copepod, *Calanus finmarchicus*, a valuable component in the Arctic food web. The survival rate of adult *C. finmarchicus* was examined on a concentration gradient for a water accommodated fraction (WAF), dispersants, and a chemically enhanced water accommodated fraction (CEWAF). The highest sublethal concentration became the concentration at which the copepods were exposed to when examining their ingestion rates of a centric diatom, *Thalassiosira weissflogii*. Even at a high WAF concentration of 10 $\mu\text{L oil L}^{-1}$ water and a dispersant concentration of 0.5 $\mu\text{L dispersant L}^{-1}$ water, *C. finmarchicus* did not show a significant difference in survivability. *C. finmarchicus* ingestion rates at the sublethal WAF, dispersant, and CEWAF concentrations did not differ significantly. This suggests that the dietary health of *C. finmarchicus* would not be affected by the soluble components of crude oil and dispersants. However, *T. weissflogii* may act as a vector for the pollutants to enter the digestive tract of *C. finmarchicus*.

9.1.5. *Microplastics as carriers of PAHs released from oil spills:
Measuring ingestion rates and bioaccumulation of PAHs in copepods*

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Abstract: Microplastics (MP; plastics < 5mm) are a growing problem in the marine environment due to a large influx of plastics entering the marine environment through rivers, wastewater and litter from land. (Ziccardi et al., 2016). MP are known to adsorb nonpolar compounds in the water including polycyclic aromatic hydrocarbons (PAHs) released from oil spills. In this study, we quantified the ingestion rates of a marine copepod (*Calanus finmarchicus*) on clean polystyrene MP beads (12 μm) and PAH loaded MP beads (26 pg PAH pellet⁻¹) to measure the role of MP as a vector for PAHs into marine food webs. Copepods ate significantly lower PAH loaded MP ($1.81\text{e}+04$ beads cop⁻¹ day⁻¹) than microplastics only ($2.67\text{E}+04$ beads cop⁻¹ day⁻¹) suggesting that copepods can reject particles based on chemical content. Accumulation of phenanthrene within the copepods (GC-MS analysis) showed individual copepods can accumulate up to 3196 pg of PAH which potentially can biomagnify in the marine food web upon predation. Fecal pellet analysis showed that PAH concentrations within the beads decreased from 26 pg/bead to 0.76 pg/bead which suggests the PAH desorb off and are retained in either the copepod's body or solubilizes in the surrounding waters.

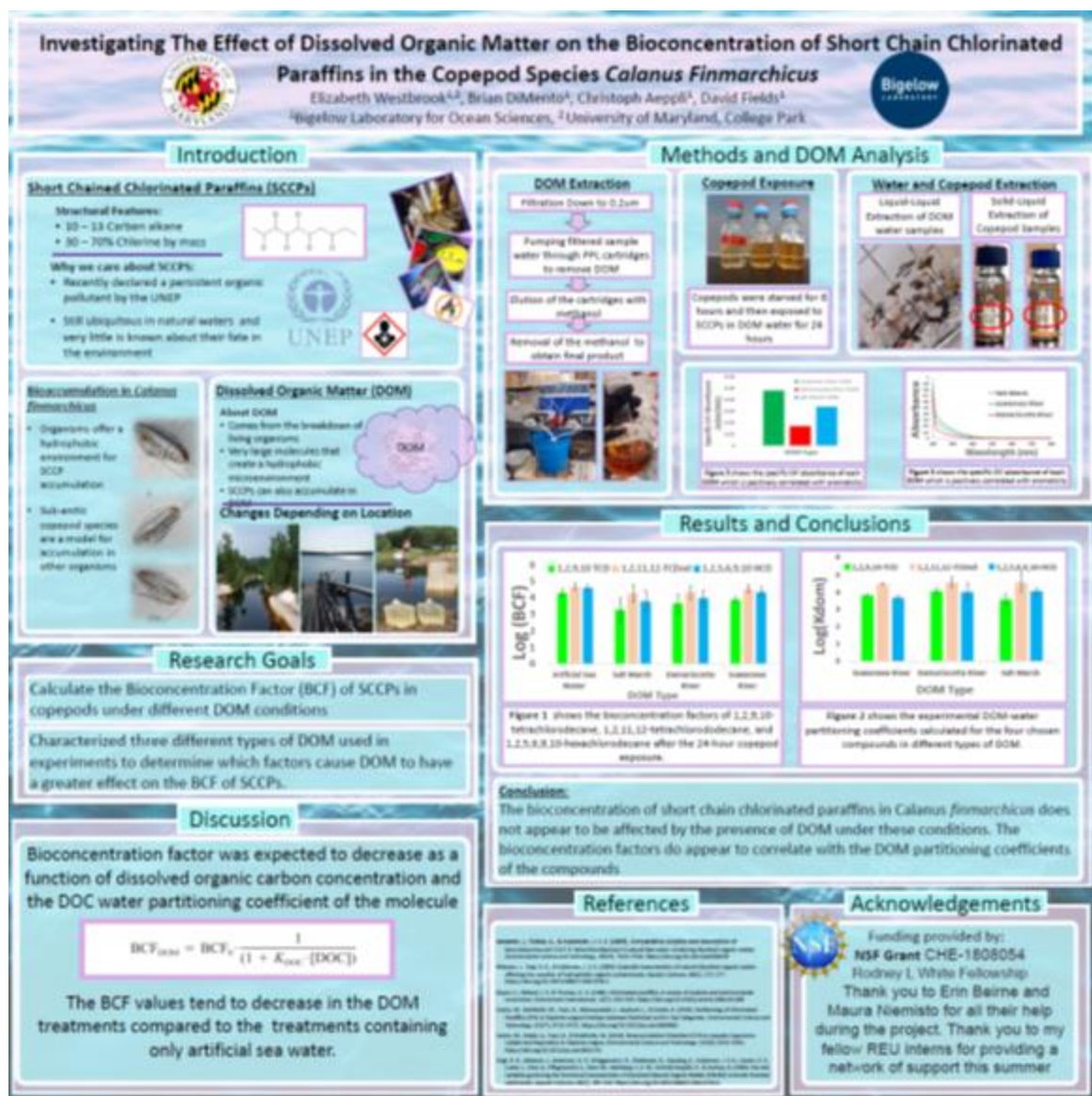


9.1.6. *Investigating the Effects of Dissolved Organic Matter on the Bioaccumulation of Short Chained Chlorinated Paraffins in the Copepod Species Calanus finmarchicus*

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Abstract: Short chain chlorinated paraffins (SCCPs) are a class of polychlorinated alkanes that have been used in industrial processes since around the 1930s, particularly as metal-working lubricants and plasticizers. In 2017, SCCPs were declared a persistent organic pollutant by the United Nations Environment Programme due to their toxicity, their potential for bioaccumulation, and their potential to be a carcinogen. Unfortunately, SCCPs remain ubiquitous in natural waters. To better understand their fate in the environment, we investigated how the partitioning of SCCPs in to three different types of dissolved organic matter (DOM) affected their bioaccumulation in a model organism, which was the subarctic copepod species *Calanus finmarchicus*. DOM creates a hydrophobic microenvironment wherein smaller hydrophobic molecules like SCCPs can sorb, potentially influencing their ability to accumulate in biota. The bioconcentration factor (BCF), a measure of the amount of SCCPs cumulated from the water by organisms, was expected to decrease as a function of the DOM concentration and the DOM water partitioning coefficient of the SCCPs. Results showed that the BCF values tend to decrease in the presence of DOM. High levels of DOM therefore appear to have an impact on the accumulation of SCCPs in aquatic organisms.



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