**Determining the acute toxicity of current and alternative oil spill chemical dispersants to early life-stage blue crabs (*Callinectes sapidus*).**

**Hannah Pie and Carys Mitchelmore**
UMCES, Chesapeake Biological Laboratory

## 1. Introduction

The rising demand for petroleum-based products over the last few decades has resulted in an increase in oil pollution within marine environments from multiple sources, including oil spills/leaks with transportation and extraction processes (e.g. Deepwater Horizon Incident). Exposure to oil and its constituents can be highly detrimental to marine organisms and can result in acute toxicity (death) as well as sublethal and delayed effects that can impact an organism's growth, reproduction and potentially have population-level consequences [1, 2]. Multiple means of remediation are used to reduce the overall impacts from oil spills and leaks on marine ecosystems. While the primary means of clean-up remains mechanical containment and recovery of the oil slick, chemical dispersants are used as a secondary mechanism to reduce the environmental and economic impact of an oil spill. Chemical dispersant use in oil spill response strategies has always been controversial, particularly given the data gaps and uncertainties regarding their chronic, sublethal, and delayed impacts [3, 4]. The decision to use dispersants on both surface and underwater source oil is based upon minimizing the overall environmental harm of oil, coupled with human health and economic considerations. For example many of these environmental tradeoff decisions are aimed at reducing the amount of oil reaching sensitive and highly productive shoreline habitats whilst potentially increasing oil impacts for pelagic (water column) and benthic (bottom dwelling) organisms. Dispersants are generally pre-approved for use in waters beyond 3 miles of the shoreline and water depths greater than 10m [2]. Dispersants increase the generation of small droplets of oil, which enter into the water column by wave energy, dissipating and diluting the oil rapidly [4]. These oil droplets remain suspended in the water column and are bioavailable to water column organisms especially within the top 10m before they dilute in three dimensions over time post-spill.

Corexit 9500 and the older formulation, Corexit 9527, have been the primary dispersants utilized in the U.S. and their toxicity has been extensively studied worldwide in multiple species and life-stages [4]. However, very little is known about the impacts of oil, chemically dispersed oil, or chemical dispersants on blue crabs, *Callinectes sapidus*, especially to the sensitive larval stages. Blue crabs are a keystone species for the Atlantic coast and Gulf of Mexico both ecologically and economically. Juvenile and adult blue crabs are benthic dwelling organisms that live in estuaries like the Chesapeake, Chincoteague and Delaware Bays, which are not pre-approved areas for dispersant use and would therefore be less likely to be exposed to dispersants and chemically dispersed oil. Blue crab zoea (larval stage), however, migrate from the spawning grounds at the mouths of estuaries to the open waters of the continental shelves where they live a planktonic existence on the surface, particularly the upper 3m including the neuston [5,6,7]. Therefore, the blue crab zoea have a potential to be impacted by the use of chemical dispersants in an oil spill event, especially those like the Deep Water Horizon (DWH) incident that co-occur with crab spawning. Furthermore, in most species the most sensitive stages of organisms to pollution impacts are the younger life stages, such as larvae [8].

Given the extensive use of dispersants Corexits 9500 and 9527 during the DWH incident, there has been an increased emphasis on the potential use of less toxic, more effective dispersant alternatives [9]. While Corexits 9500 and 9527 are the most commonly used dispersants, numerous alternative dispersants listed under the EPA's National Contingency Plan (NCP) Product Schedule are also available [10]. However, very few toxicity studies have been conducted on these alternatives beyond the data directly supplied to the EPA from the manufacturers [10] and the EPA's recent toxicity tests by Hemmer et al. [11]. Considering the potential impacts of Corexit products and more importantly, oil dispersed by Corexit products on many invertebrate species as reviewed in the NRC 2005 report [4], it is important to fully investigate the toxicity of alternative dispersants from the NCP Product Schedule to know if any of these dispersants are less harmful to blue crabs, and therefore, could be more suitable alternatives to use in remediation of oil spills. Therefore, this project sought to examine the acute toxicity of multiple chemical dispersants used for oil spill remediation on early life-stage blue crabs, *Callinectes sapidus*. The overall goal of this study was to provide data on the sensitivity of early life-stage blue crabs to the currently used chemical dispersant, Corexit 9500A, in comparison to four alternative chemical dispersants, including one microbially-based dispersant, to determine if a less toxic option might be available for use in oil spill remediation in areas containing larval blue crabs. Examining acute toxicity of dispersants is a crucial first step in understanding the impacts of dispersants, oil, and dispersed oil on larval blue crabs.

## 2. Methods

### 2.1 *Study Design*

In the study, the acute toxicity of five chemical dispersants was examined using blue crab, *Callinectes sapidus,* larvae. The larvae used for the experiment were stage II zoea. Stage II zoea were utilized as they are the earliest life-stage of larvae found predominantly in the open ocean environment along the continental shelf [7]. Stage I zoea are found in substantial numbers at the mouths of estuaries like the Chesapeake Bay where spawning occurs and are then transported out to open waters of the continental shelf [7]. The blue crab larvae used for testing were provided by the Institute of Marine Environmental Technology-Aquatic Research Center (IMET-ARC)'s blue crab hatchery. The larvae were hatched and raised in-house until the experiments.

Three of the dispersants were selected based upon their current availabilities and their NCP Product Schedule results [12] and the results of the study by Hemmer et al. [11] that both examined their acute toxicities (48 h $LC_{50}$ values) to juvenile mysid shrimp. The chosen dispersants include Corexit 9500A, Sea Brat #4 (Alabaster Corp.), and Dispersit SPC 1000 (US Polychemical Corp.). Two additional dispersants were provided by Alabaster Corp. for analysis including a microbially-based dispersant/surface washing agent, Petro-Clean, and a new formula dispersant, Orca. Orca is not on the NCP Product Schedule nor was it tested in the study by Hemmer et al [10]. Petro-Clean is a pH-neutral blend of nonionic surfactants and emulsifiers used for petroleum bioremediation, but is classified by the NCP Product Schedule as a surface-washing agent [9]. Petro-Clean contains naturally-occurring, non-pathogenic microorganisms used to aid in the degradation process and is the least toxic surface-washing agent listed on the NCP Product Schedule [12].

The experiment was a 48-hr static exposure testing a range of dispersant concentrations that were prepared with filtered artificial seawater (ASW) as well as a control of ASW only. The ASW used in the experiments was prepared by the ARC facility staff and adjusted to 30ppt with charcoal filtered municipal water then filtered with a 0.2µm filter. Exposures were carried out in 20mL glass scintillation vials with each vial containing 10mL of test exposure solution and 5 total larvae. There were 8 replicate scintillation vials (so total N=40 zoea) for each dispersant test solutions and 9 replicate scintillation vials (N=45 zoea) for ASW only control. Scintillation vials were cleaned and baked at 500°C for 4 hours prior to use.

The vials were not sealed for experiments, but lightly covered with saran wrap to allow light penetration and air transfer. No water change was needed as water quality parameters, including dissolved oxygen (D.O.), pH, salinity, ammonia and temperature, did not change appreciably over the 48 hour period in preliminary trials. Organisms were fed daily with 10µL of concentrated rotifers. Scintillation vials were maintained at 21-23°C under a photoperiod of 14:10 hr light:dark. Water quality parameters were monitored at time 0- and 48-hr.

Dispersant solutions were made by dilution from a stock of the highest concentration tested using filtered ASW at 30ppt. The highest dispersant stock concentration was prepared using a glass syringe to add the dispersant to a glass aspirator bottle with 1 L ASW stirring (in the dark) with a standard 25% vortex for 18 hours. The exact loading rate of each dispersant was determined by pre-weighing an empty glass syringe, weighing the syringe with dispersant added, re-weighing the syringe after adding the dispersant to the filtered seawater, and then calculating the mass difference. The experiments were conducted using a range of concentrations based on previous work done by the Mitchelmore lab with blue crab larvae exposed to Corexit 9500 and the $LC_{50}$ concentrations of the alternative dispersants on mysid, *Americamysis bahia* [11]. Preliminary range finding tests in 2012 showed that the $LC_{50}$ concentrations for Corexit 9500 and Sea Brat #4 were between 100 and 200 parts per million (ppm) (unpublished results), so the definite experimental ranges were expanded to include multiple test concentrations around those two values. The concentrations used for the experiment are detailed in the table below (Table 1).

**Table 1. Nominal concentrations of chemical dispersants used in experiments**

| Dispersant Type | Concentrations (ppm) |
|---|---|
| Corexit 9500A | 10, 25, 50, 75, 100, 125, 150 |
| Seat Brat #4 | 10, 50, 75, 100, 125, 150, 175, 200, 225 |
| Orca | 10, 50, 75, 100, 150, 175, 200 |
| Petro-Clean | 10, 25, 50, 75, 100 |
| Dispersit SPC 1000 | 0.5, 1, 10, 25, 50, 75, 100, 125, 150 |

2.2 *Mortality*

Mortality of zoea in each treatment was recorded at 24- and 48-hr by examining the larvae under a dissecting microscope at 2x without removal from the scintillation vials. The time spent under the light of the microscope was minimized to reduce stress on the larvae. A blue crab zoea was designated dead if there was no longer movement in maxillipeds or a visible heart-beat. All deceased larvae were removed from the vials as soon as possible to minimize impacts on water quality.

2.3 *Statistics*

        The lethal dispersant concentration at which 50% of individuals exposed are dead ($LC_{50}$) compared to the blue crabs in the controls after 48-hr exposure were determined for all five dispersants. No mortalities occurred in the control (ASW only) zoea, so the mortalities of crabs in exposure treatments did not need to be normalized to the control mortalities. The $LC_{50}$ values and their 95% confidence intervals were calculated using a probit linked binomial generalized linear model in R using the MASS package. Plots of proportion of dead larvae over log dispersant concentrations were produced and lines of the regression model and its 95% confidence values were fitted to the data. McFadden's Pseudo R² was calculated for each model.

3. **Results:**

        Control performance (ASW only) met all criteria for acceptable toxicity test exposure (×90% survival) with 100% survival of larvae for the 48-hr exposure. All water quality parameters were within acceptable ranges. The $LC_{50}$ values ranged from 10.1 ppm for Dispersit SPC 1000 to 76.5 ppm for Orca (Table 2). Concentration-response curves with regression models and McFadden's Pseudo-R² values are presented in Figure 1. Using the U.S. EPA toxicity classification system [12], all dispersants would be classified as slightly toxic, except Dispersit SPC 1000, which bordered between slightly and moderately toxic to *C. sapidus* larvae. By comparing $LC_{50}$ values and 95% confidence intervals, the rank order toxicity (most to least toxic) of the dispersants to *C. sapidus* larvae was Dispersit SPC 1000 > Sea Brat #4 > Petro-Clean > Corexit 9500A > Orca.

**Table 2. Median lethal concentration ($LC_{50}$, ppm) values and 95% confidence intervals in brackets for *Callinectes sapidus* 48-hr static acute toxicity tests with five dispersants**

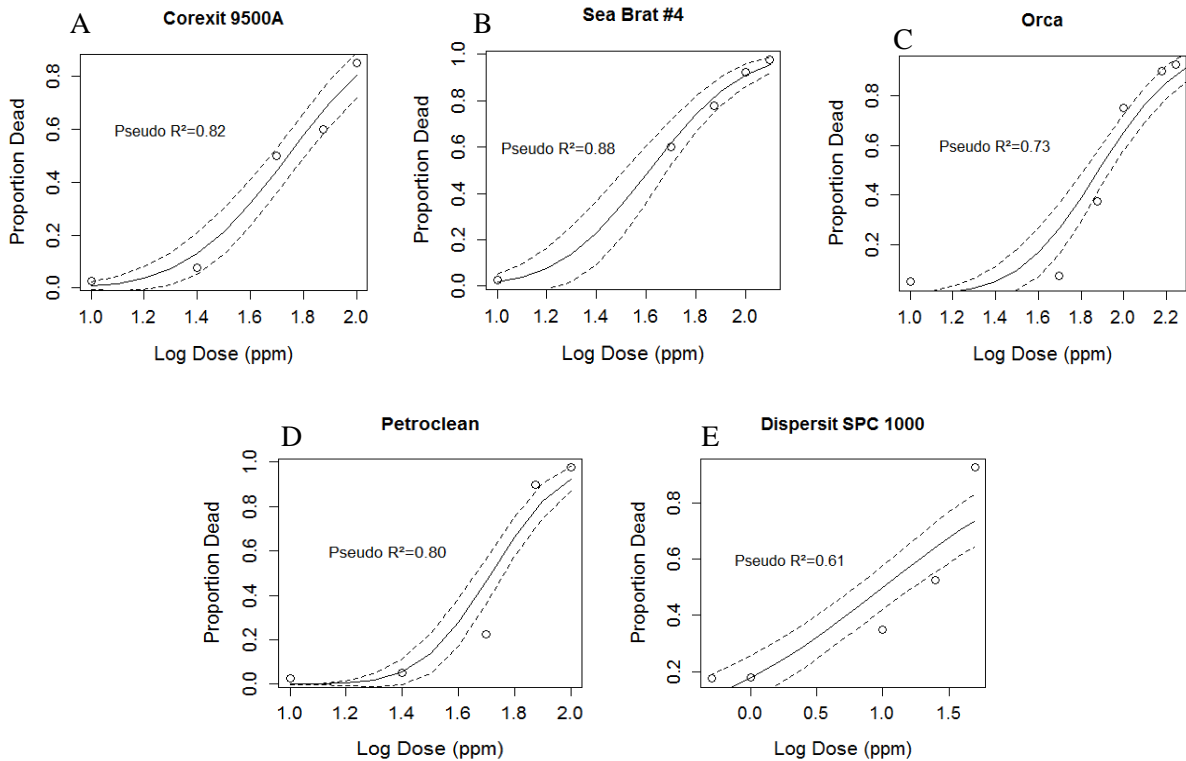| Dispersant | $LC_{50}$ ppm [95% Confidence Interval] |
|---|---|
| Corexit 9500A | 55.0 [47.5-63.8] |
| Dispersit SPC 1000 | 10.1 [6.2-16.4] |
| Orca | 76.5 [66.4-88.1] |
| Petro-Clean | 52.0 [46.5-58.2] |
| Sea Brat #4 | 41.0 [33.6-50.1] |

Fig. 1 Concentration-response curves for blue crab, *Callinectes sapidus*, stage II zoea exposed for 48-hr to Corexit 9500A (A), Dispersit SPC 1000 (B), Orca (C), Petro-Clean (D), and Sea Brat #4 (E). The probit linked binomial generalized linear model (solid line) and the 95% confidence intervals (dashed lines) for each model are shown for each dispersant. McFadden's Pseudo $R^2$ value is included for each model.

## 4. Discussion

Blue crabs, *Callinectes sapidus,* are an important species to the ecology and economy of the Atlantic coast and Gulf of Mexico. In spite of this, very little is known about the impacts of oil, chemically dispersed oil, or chemical dispersants on any stage of the blue crab life cycle. The distribution of *C. sapidus* zoea on the surface of open, coastal waters [7] would put them at risk of exposure to high concentrations of dispersants and chemically dispersed oil in an oil spill event. *C. sapidus* recruitment and subsequent fishery abundance depends on the survival of larvae in the coastal, open ocean. Therefore, it is imperative to understand the biological impacts of such chemicals on early stage zoea.

Similar to Hemmer et al. [11], the toxicity of Corexit 9500A was essentially equivalent to most of the dispersants tested with the exception of Dispersit SPC 1000, to which the larvae showed increased sensitivity. The new formula dispersant, Orca, from Alabaster Corp. was the least toxic of the dispersants tested ($LC_{50}$=76.5 ppm). With the exception of Corexit 9500A, very few studies have examined the toxicity of the other four dispersants on aquatic organisms. Currently, Gulf mysid shrimp, *Americamysis (Mysidopsis) bahia,* is the most closely related species to compare the toxicity of dispersants with blue crab larvae. Hemmer at al. [11] examined the acute toxicity (48-hr static exposures) of eight commercial oil dispersants, including Corexit

9500A, Sea Brat #4, and Dispersit SPC 1000, on mysid shrimp (*Americamysis bahia*). The 48-hr $LC_{50}$ values with 95% confidence intervals for *A. bahia* exposed to Corexit 9500A, Dispersit SPC 1000, and Sea Brat #4 were 42 ppm (38-47 ppm), 12 ppm (10-14 ppm), and 65 ppm (57-74 ppm ), respectively [11]. These results were similar to those found on the NCP Product Schedule results except for Sea Brat #4, which showed high toxicities that were closer to Dispersit SPC 1000 [12]. The NCP Product Schedule resultsø 48-hr $LC_{50}$ values for Corexit 9500A, Dispersit SPC 1000, and Sea Brat #4 were 32.2 ppm (26.5-39.2 ppm; 95% CI), 16.6 ppm(14.1-19.6 ppm; 95% CI), and 14.0 ppm($\pm$10.4 ppm; SE), respectively [12]. Additional studies by Clark et al. [14] resulted in a 96-hr $LC_{50}$ value of 35.9 ppm (32.2-41.3 ppm, 95% CI) for *Mysidopsis bahia* continuously exposed to Corexit 9500A. The stage II blue crab zoea in this study show similar sensitivity to Dispersit SPC 1000 and slightly less sensitivity to Corexit 9500A than Gulf mysid shrimp. The sensitivity of larval blue crab to Sea Brat #4 was between the two study results above for Gulf mysid shrimp.

Overall, the dispersants examined in this study are only slightly toxic to the earliest life stage of blue crabs that might likely be exposed to dispersants in the environment, with the exception of Dispersit SPC 1000 that bordered between slightly and moderately toxic. However, the $LC_{50}$ values for all the dispersants are well above the estimated and measured concentrations of Corexit 9500 applied in the DWH [14, 15] and all but Dispersit SPC 1000 are above the maximum measured field concentration of dispersants, 13 ppm, described in George-Ares and Clark [16]. Dispersant concentrations in DWH applications were estimated to be 30 parts per billion (ppb) to a depth of 10m [14]. The highest surface concentration of dioctyl sodium sulfosuccinate (DOSS), a major surfactant component of Corexit 9500, measured in ocean waters during the DWH incident was 229 ppb at one site with the rest of the sites examined having concentrations ranging from below the detection limit of <0.25 ppb to 24.5 ppb[15]. Additionally, previous studies have indicated that Corexit 9500 is expected to decrease in concentration by dilution below detection limits within hours of application [3, 16]. Therefore, stage II blue crab zoea are unlikely to be exposed to concentrations of dispersants that will result in extensive acute toxicity.

Although the dispersants themselves appear to not cause substantial acute toxicity, this study did not address chronic or sublethal impacts from dispersant exposure, particularly impacts on growth, molting and behavior, which could also result in the removal of blue crab larvae from the population. Additionally, it is generally not the dispersants alone, but the oil/dispersed oil that is the driving factors in acute toxicity when chemical dispersion is used to clean-up an oil spill [2]. For *A. bahia* exposed to the Chemically-Enhanced Water Accommodated Fraction (CE-WAF) of dispersant-Louisiana Sweet Crude (LSC) oil mixtures, the 48-hr $LC_{50}$ values ranged from 1.4-5.4 mg total petroleum hydrocarbons (TPH)/L for Corexit 9500A-, Dispersit SPC 1000-, and Sea Brat #4-LSC mixtures, resulting in these dispersant-LSC mixtures being classified as moderately toxic [11, 13]. The WAF of LSC oil alone resulted in a 48hr $LC_{50}$ value of 2.7 mg TPH/L (2.5-3.0mg TPH/L, 95% CI) for *A. bahia,* which would also be considered moderately toxic [11, 13]. This present study only addresses the acute toxicity of chemical dispersants, but a study including examining the impacts of oil and chemically dispersed oil as well would need to be conducted to have a more complete understanding of the impacts of using chemical dispersion in oil spill remediation on blue crab larvae.

To account for this, further work is ongoing by researchers at CBL and IMET to examine the sublethal impacts of oil, chemically dispersed oil, and Corexit 9500A on blue crab zoea. These studies will give a more comprehensive examination of sublethal impacts including molecular (endocrine disruption, DNA damage, and oxidative stress) and behavioral (disruption of phototaxis, geotaxis, or vertical migration) endpoints. Additionally, full chemical analysis will be performed examining PAH composition and particle counts of all exposure solutions and stocks.

**5. References**

1. Hylland K., K.E. Tollefsen, A. Ruus, G. Jonsson, C.R. Sundt, S. Sanni, S. et al. (2008). Water column monitoring near oil installations in the North Sea 2001ó2004. *Marine Pollution Bulletin,* 56: 414ó 429.
2. National Research Council  Committee on Oil in the Sea: Inputs, Fates, and Effects. (2003). Oil in the sea III: inputs, fates, and effects. The National Academics Press, Washington DC.
3. National Research Council (1989). Using Oil Dispersants on the Sea. National Academy Press, Washington, D.C.
4. National Research Council (2005). Oil Spill Dispersants: Efficacy and Effects. National Academy Press, Washington, D.C.
5. Epifanio C.E. (1988). Dispersal strategies of two species of swimming crab on the continental shelf adjacent to Delaware Bay. *Marine Ecology Progress Series,* 49:243-248
6. Epifanio C.E., Mass A.K. and R.W. Garvine (1989). Transport of blue crab larvae by surface currens off Delaware Bay, USA. *Marine Ecology Progress Series,* 54:35-41
7. Epifanio C.E. (1995) Transport of Blue Crab (*Callinectes sapidus*) Larvae in the Waters Off Mid-Atlantic States. *Bulletin of Marine Science*, 57:713-725.
8. Rand G.M. and S.R. Petrocelli (1985). Introduction. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic Toxicology, Hemisphere, New York
9. USEPA (2010, May 20). Dispersant Monitoring and Assessment Directive ó Addendum. Retrieved from http://www.epa.gov/bpspill/dispersants/directive-addendum2.pdf
10. USEPA (2010, October). National Contingency Plan Product Schedule. Retrieved from http://www.epa.gov/emergencies/docs/oil/ncp/schedule.pdf
11. Hemmer M.J., Barron M.G. and R.M. Green (2011). Comparative Toxicity of Louisiana Sweet Crude Oil (LSC) and Chemically Dispersed LSC to Two Gulf of Mexico Aquatic Test Species. *Environmental Toxicology and Chemistry*, 30: 2244-2252.
12. USEPA (2013, November 21). National Contingency Plan Product Schedule Toxicity and Effectiveness Summaries. Retrieved from http://www.epa.gov/ceppo/web/content/ncp/tox_tables.htm
13. USEPA (2012, May 9).  Ecotoxicity Categories for Terrestrial and Aquatic Organisms. Retrieve from http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm
14. Nalco (2010). Seven Facts about Corexit 9500 Oil Dispersant. Retrieved from http://www.nalcoesllc.com/nes/1601.htm
15. Gray J.L., Kanagy, L.K., Furlong E.T., Kanagy C.J., McCoy J.W. et al. (2014). Presence of the Corexit Compoent Dioctyl Sodium Sulfosuccate in Gulf of Mexico Waters After the 2010 Deepwater Horizon Oil Spill. *Chemosphere,* 95: 124-130

16. George-Ares A. and J.R. Clark (2000). Aquatic Toxicity of Two Corexit Dispersants. *Chemosphere,* 40:897-906

Reasoning for Changes to Model Species Used for Ruth Mathes Scholarship Project

While applying for the Ruth Mathes Scholarship, I anticipated that a majority of my Ph.D. thesis would involve the use of oysters as a model species for examining oil pollution and the use of chemical dispersants in oil spill clean-up. My advisor, Dr. Carys Mitchelmore, and I had submitted a few grants to examine the impacts of oil, chemically dispersed oil, and chemical dispersant alone (Corexit 9500) on the early life stages of the Eastern oyster, *Crassostrea virginica*. I was interested in adding onto this project by examining the impacts of alternative chemical dispersants and how they compared to the commonly used Corexit 9500. Therefore, the project I submitted to the Ruth Mathes scholarship committee was on examining the impacts of alternative dispersants on larval oysters, *Crassostrea virginica.*

The main experiment examining the impacts of oil on oysters was not subsequently funded. However, the Mitchelmore lab did receive funding to conduct such an experiment with blue crab, *Calinectes sapidus,* larvae in a collaborative project between the Chesapeake Biological Laboratory and the Institute of Marine Environmental Technology (IMET) in Baltimore, MD. Additionally, in another one of my thesis research projects I used juvenile *C. sapidus* as a model species for examining the impacts of oil contaminated sediments. Therefore, I decided to use the scholarship funding from the Ruth Mathes Scholarship to examine the impacts of alternative dispersants on larval blue crabs, *Callinectes sapidus*, to better fit this project into the body of research for the thesis.

Larval blue crabs were as easy to obtain as the oysters would have been. IMET has a blue crab hatchery within its Aquatic Research Center (ARC). The staff in ARC have extensive experience raising blue crab larvae and allowed me access to facilities there to conduct my experiments in house with access to all of their seawater and food supplies for maintaining the larvae. Preliminary studies by the Mitchelmore lab in 2011 showed that the sensitivity of blue crab larvae to Corexit 9500A decreased as the larvae aged with the earliest stage zoea (stages I-III) displaying the most sensitivity (Unpublished results). Stage II zoea are the earliest life-stage of larvae found predominantly in the open ocean environment, where they could be exposed to dispersants and dispersed oil during an oil spill clean-up (Epifanio 1995). Therefore, given their sensitivity and potential risk of exposure, stage II zoea were chosen for this experiment to examine the impacts of chemical dispersants on early-life stage blue crabs.

Preliminary experiments exposing blue crabs to two dispersants, Corexit 9500A and Sea Brat #4, to determine the concentration ranges to use for exposure were conducted in late summer of 2012. These tests were conducted at the tail end of the natural spawning period of blue crabs (usually May-August), which meant only a limited number of larvae were available for testing. Therefore, the definitive experiment examining the full five dispersants was not conducted until the next spawning period in early summer of 2013 when there were enough healthy larvae to use. The plan was to repeat the definitive experiment with the five dispersants using a second batch of larvae to address inter-batch variation. However, the ARC hatchery had difficulties this summer/fall in getting mature female crabs to sponge or to hatch viable, healthy larvae. Many of the mature females that were brought in did not sponge. The few that did either produced a low larvae count or had low survival rates from stage I to stage II zoea. Therefore, a repeat experiment could not be conducted this spawning season before the funding period for the scholarship ended. The final report shows the results from the full definitive experiment.

Epifanio C.E. (1995) Transport of Blue Crab (*Callinectes sapidus*) Larvae in the Waters Off Mid-Atlantic States. *Bulletin of Marine Science*, 57:713-725.