

FLORIDA ATLANTIC UNIVERSITY: The Impact of Crude Oil and the Dispersant Corexit on Three Key Gulf of Mexico Invertebrate Species

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SCIENCE ACTIVITIES

1) General Summary

In response to the call for proposals for the FIO Rapid Response Research Grants we chose to focus on determining the impact on multiple larval life stages of GOM relevant invertebrates concentrating on those stages in which major metamorphic changes occur. Larvae were chosen as they are 1) typically assumed to be more sensitive to the effects of contaminants than juveniles or adults, therefore responses would represent a worst case scenario for these species, and 2) the Deepwater Horizon event coincided with the timing of spawning events for many marine organisms, including invertebrates. Species chosen were not only relevant to the GOM but were species that we had worked with previously and included a crustacean (*Litopenaeus duorarum*), a bivalve (*Crassostrea virginica*), and a gastropod (*Strombus gigas*) as well as phytoplankton (*Isochrysis galbana*, *Chaetoceros gracilis*), and zooplankton (*Brachionus plicatilis*) food sources.

Objectives

1) Establishment of LC₅₀ and EC₅₀ values for early larval stages of the queen conch, eastern oyster, and white shrimp exposed to oil and Corexit, 2) Establishment of LC₅₀ and IC₅₀ levels for planktonic food sources exposed to oil and Corexit, 3) To document the effect of sub-lethal exposures to dispersed oil on survival, growth and development of early life stages of queen conch, oysters, and shrimp and 4) To document the long term effect of sub-lethal exposure of larvae to dispersed oil on growth and development into juvenile and adult stages.

Progress to Date/Problems Encountered

Static acute toxicity (24-96 hour) and short-term (24 hour) sub-lethal studies were conducted. Survival and behavioral responses were assessed during the acute toxicity studies. Sub-lethal concentration exposures were based on LC₁₀ values obtained from 24 hour acute toxicity studies. Survival, development, and growth were followed until benthic metamorphosis. To determine the long term effect of short term sub-lethal exposure larvae were reared for an additional six months and histological evaluations were conducted monthly for six months.

All objectives have been completed with the exception of # 4. The only invertebrate that we were able to successfully culture through metamorphosis in sufficient numbers to conduct monthly histological evaluations was *Litopenaeus duorarum*. The shrimp were cultured for an additional six months post-benthic metamorphosis and representative samples of control and exposed shrimp have been fixed and embedded. Slide preparation is currently underway therefore no results are included in this report.

Lessons learned

Management problems encountered included funding delays (August start, funds delayed until November) that in turn resulted in delaying larval experiments until spring, as the fall spawning season had been missed. Logistical problems encountered included 1) problems in obtaining crude oil from BP (September request, obtained in March) resulting in the use of oil from other

sources (tar mats) in early experiments, 2) that larval experiments were dependent on spawning events (spring/fall for oysters and shrimp and summer for conch), and 3) that sublethal experiments were dependent on short term acute toxicity results and were delayed accordingly.

2) Results and scientific highlights

Objective 1: Acute toxicity larval experiments

Oysters

Dispersants had the greatest impact on D stage (24 hr LC₅₀, 9 mg/L) and eyed larvae (24 hr LC₅₀, 22 mg/L) survival while WAFs had the least. For eyed larvae no LC₅₀ values were obtained while a time dependent effect was seen with D stage larvae (24 hr LC₅₀, 1093 mg/L; 96 hr LC₅₀, 262 mg/L). CEWAFs exhibited greater toxicity than WAFs (nominal and PAH concentrations), but were less toxic than WAFs if TPH values were considered. Survival of D stage larvae exposed to CEWAFs was initially greater than eyed larvae but no different at 48 hours (44 mg/L). D stage larvae exhibited decreased swimming activity at lower concentrations than elicited mortality following exposure to WAFs and CEWAFs, but not dispersant.

Gametogenesis was impacted at lower concentrations, even though exposure times were reduced. Although exposure of gametes to WAFs did not affect fertilization success, exposure of fertilized eggs (100 mg/L) resulted in decreased numbers of trochophores. Likewise exposure of trochophores to WAFs (100 mg/L) resulted in fewer D stage larvae and greater abnormalities. Exposure to CEWAFs (12.5 mg/L) resulted in reduced fertilization, and decreased numbers of trochophores (25 mg/L) and D stage larvae (12.5 mg/L) but increased abnormalities. Dispersants likewise negatively impacted fertilization (1.25 mg/L), and development to trochophore (2.5 mg/L) and D stage larvae (1.25 mg/L).

Conch

Mortality impacts were life stage dependent with six lobed veligers (three weeks post hatch) being more sensitive than earlier (two lobed, four lobed) stages. All life stages were least sensitive to WAFs (24 hr LC₅₀ >800 mg/L; 96 hr LC₅₀ 425-661 mg/L). The dispersant and CEWAFs were equally toxic to four and six lobed veligers while two lobed veligers were more sensitive to the dispersant. CEWAFs were more toxic than WAFs (nominal and PAH concentrations). If TPH concentrations were compared WAF and CEWAF toxicity was life stage dependent, with CEWAFs having greater toxicity for four and six lobed veligers and WAFs having greater (48-72 hr) or equal (96 hr) toxicity for two lobed veligers.

Shrimp

Zoea were more sensitive to contaminants than other life stages. Zoea exposed to ≥ 2.5 mg/L Corexit 9500A and to ≥ 6.25 mg/L CEWAF were dead by 48 hours. For all stages exposure to the dispersant had the greatest impact on survival while WAFs had the least. Nauplii and mysis stages showed similar and intermediate responses to all contaminants, while postlarvae were the least effected. CEWAFs were more toxic than WAFs when nominal values were compared but were of equal toxicity using PAH concentrations. WAFs were more toxic than CEWAFs if TPH values were compared.

Significantly altered behavioral responses (activity, molting) of mysis shrimp were seen at lower concentrations than elicited mortality in WAFs (72-96 hr) and CEWAFs (24-96 hr). In contrast behavioral responses were not altered at lower concentrations in postlarval (PL8) shrimp, although water quality issues may have compromised results.

Objective 2: Planktonic Food Sources (Acute Toxicity)

Phytoplankton

Cell division (growth) of *Isochrysis galbana* or *Chaetoceros sp.* was not affected by exposure to either tar mat or crude oil (1200 mg/L), nor was the motility of *I. galbana* decreased. In contrast, dispersed oil solutions (tar mat, crude) inhibited cell division (48 hr IC₅₀ 174 mg/L *I. galbana*; 95 mg/L *Chaetoceros sp.*) and decreased motility in *I. galbana*. The dispersant alone likewise decreased cell growth (48 hr IC₅₀ 44 mg/L *I. galbana* and 2.5 mg/L *Chaetoceros sp.*) and motility. By 96 hours significant decreases in motility were seen at >0.5 mg/L. The diatom, *Chaetoceros sp.* was more susceptible to both the dispersed tar mat oil and the dispersant than *I. galbana* with total mortality occurring in all treatments by 72 hours. Although nominal CEWAF IC₅₀ concentrations were similar for both oils, tar mat CEWAF's were twice as toxic as crude CEWAFs if PAH values were compared. The toxicity of TPHs was similar.

Zooplankton

Initial rotifer, *Brachionus plicatilis*, mortality was greatest in the dispersant (24 hr LC₅₀ 1.9 mg/L), but significant mortality occurred by 48 hours in dispersed tar mat (<150 mg/L). Exposure to tar mat WAFs (48 hr LC₅₀ 588 mg/L) and CEWAFs (48 hr LC₅₀ <150 mg/L) was more toxic to rotifers than exposure to crude oil WAFs and CEWAFs (48 hr LC₅₀ >800 mg/L). LC₅₀ values for crude oil treatments could not be calculated after 24 hr due to unacceptable mortality (>20%) in the controls.

Objective 3: Short Term Sub-lethal CEWAF Exposure Effects

Oysters (D stage larvae)

A delayed mortality effect was seen one week post exposure for D stage larvae (24 hr LC₁₀, 16 mg/L. No differences in development from D-hinge through the late umbo or growth (length or width) were seen between exposed and control groups.

Conch (Two lobed veligers)

A two-three days lag in lobular development was seen (24 hr, LC_{10, 59} mg/L). This was most apparent during development from two to four lobed stage. At experimental termination (pre-settlement, four weeks) all surviving conch had six fully developed lobes. Growth (length) was not affected. Survival was not assessed due to complications from a ciliate infestation.

Shrimp (Nauplii)

Initial survival was lower in exposed nauplii (24 hour LC₁₀, 23 mg/L); however final survival was not evaluated due to issues encountered during culture. A slight two day lag was seen midway through larval stage development (zoea III to mysis I) in exposed groups. This initial delay in development continued through mysis III. However both groups reached postlarval stage by day eleven. No significant difference in growth (length) was noted.

Objective 4: Long Term Sub-lethal CEWAF Exposure Effects

Not accomplished for oysters and conch. Significant mortalities (unknown cause) occurred in both control and exposed oysters prior to and during metamorphosis. Significant mortalities (ciliate infestation) occurred in both control and exposed conch prior to metamorphosis. Accomplished for shrimp, but data has not yet been evaluated

Overall Conclusions

Mortality of larval invertebrates was life stage dependent; however, the earliest life stages were not necessarily the most sensitive. Clearly extrapolating that the impact of contaminant effects obtained with one larval life stage is applicable to all larval life stages should be approached with caution. Mortality was also species dependent. A comparison of two microalgal species clearly showed that *Chaetoceros* was more sensitive than *I. galbana*. Again, caution is warranted when using one species as a proxy for related species.

The dispersant was clearly more toxic than either the oil or the dispersed oil for all organisms tested. In general, the effect of the dispersant on survival was immediate, with the majority of mortalities occurring in the first 24 hours. Overall, CEWAFs had greater toxicity than WAFs whether nominal LC₅₀ values or polycyclic aromatic hydrocarbon (PAH) concentrations were compared although the degree of toxicity was lower when using PAH concentrations. The most likely explanation for this is that the addition of the dispersant increased the bioavailability, as CEWAFs had triple the PAHs of WAFs. This difference was most apparent in the concentrations of PAHs that contained two to four carbon rings, which are considered to be the most toxic PAHs. On the other hand, if total petroleum hydrocarbons (TPHs) were compared WAFs were usually more toxic than CEWAFs; even though the dispersant increased the availability of TPHs twenty-five fold. Exceptions were seen with conch larvae.

Tar mat CEWAFs were more toxic than crude CEWAFs. As the PAH concentration was less in the tar mats, and the majority were the less toxic three and four carbon rings we concluded that the method of preparation was responsible for the variation in toxicity. Low heat was applied to both the WAF and CEWAF tar mat stock solutions to allow the oil to go into solution, (Sara Edge, HBOI, *personal communication*) which may have released toxic contaminants that were not measured. Tar mat WAFs and CEWAFs had a greater concentration of TPHs than crude oil.

Short term sublethal CEWAF exposures had few lasting negative effects on survivors. Although the earliest stage post-hatch larval stage was used for all three experiments this was not necessarily the most sensitive stage for that species. Growth was not affected for any of the three invertebrate species examined. Delayed mortality occurred with exposed oysters and could not be evaluated for conch or shrimp. Slight developmental delays in development were seen in conch and shrimp, but exposed survivors eventually caught up with control groups. Whether such delays would impact survival in the field is speculative.

3) Cruises & field expeditions

None

4) Peer-reviewed publications, if planned

a. Manuscripts in preparation:

- Potential Impacts of the Deepwater Horizon on Gulf of Mexico Oyster Populations (Mar poll bull, submission date 2/10/2013)
- Potential Food Web Impacts Following the Deep Water Horizon Oil Spill: Toxicity Effects of Oil and Corexit on Phytoplankton and Zooplankton (Mar environ res, submission date 2/25/2013)
- Acute Toxicity and Sublethal Exposure of Deepwater Horizon Oil and Dispersed Oil to Queen Conch, *Strombus gigas*, Larvae (Environ tox, submission date 3/15/2013)
- Survival, Growth and Behavioral Responses of *Litopenaeus duorarum* Following Exposure to Acute and Sublethal Concentrations of MC252 Crude and Dispersed Oil and the Corexit Dispersant (Arch environ tox, submission date 3/30/2013)

5) Presentations and posters, if planned (Please provide copies of each)

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Toxic Effects of Crude Oil and the Corexit 9500 Dispersant on Conch (<i>Strombus Gigas</i>), Oyster (<i>Crassostrea Virginica</i>) and Shrimp (<i>Penaeus Duorarum</i>) Larvae	Laramore	Laramore, Krebs, Garr	104 th National Shellfisheries Association Meeting, 25-29 March 2012, Seattle, WA.	Y	March 2013, JSR
Acute and sublethal impacts of MC252 oil and dispersant on early life stages of <i>Crassostrea virginica</i>	Laramore	Laramore, Garr and Krebs	GOM Oil spill and Ecosystem Science Conference, 21-23 January 2013, New Orleans, LA.	N	Online, GOMARI website
Potential food web impacts following the deep water horizon oil spill: toxicity effects on phytoplankton and zooplankton	Garr	Garr, Laramore and Krebs	GOM Oil spill and Ecosystem Science Conference, 21-23 January 2013, New Orleans, LA.	N	Online, GOMARI website
Acute and sublethal impacts of MC252 oil and dispersant on early life stages of <i>Crassostrea virginica</i>	Laramore	Laramore, Garr and Krebs	Aquaculture 2013, 21-25 February 2013, Nashville, TN.	N	Online WAS website and CD
Potential food web impacts following the deep water horizon oil spill: toxicity effects on phytoplankton and zooplankton	Garr	Garr, Laramore and Krebs	Aquaculture 2013, 21-25 February 2013, Nashville, TN	N	Online WAS website and CD

6) Other products or deliverables

None

7) Data

Please provide a spreadsheet indicating the metadata and ancillary information on the location and status of the archived samples. Also, indicate if there are any issues with respect to data archiving schedule and plan. If you have a lot of metadata, representative samples will suffice. This will all be incorporated into the GoMRI database at some point in the future.

PARTICIPANTS AND COLLABORATORS

8) Project participants

First Name	Last Name	Role in Project	Institution	Email
Susan	Laramore	PI	FAU	Slaramo1@hboi.fau.edu
Amber	Garr	Co-PI	FAU	Agarr1@hboi.fau.edu
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Helen	Lopez	Technician	FAU	Hlopez3@hboi.fau.edu

MENTORING AND TRAINING

9) Student and post-doctoral participants

First Name	Last Name	Post-doc / PhD / MS / BS	Thesis or research topic	Institution	Supervisor	Expected Completion year
Richard	Jones	BS	Plankton exp.	FAU	Laramore	2013
Emily	Reynolds	BS	None, conch exp.	Emory Univ	Garr	2012
Heather	Kalish	BS	None, oyster exp.	FAU	Laramore	2013

10) Student and post-doctoral publications, if planned

None

11) Student and post-doctoral presentations and posters (Please provide copies of each)

None

12) Images

Please attach high-resolution images and provide details including a description of the image, location, credit, date, etc. Of note: Image may be used in FIO or GoMRI promotions, so please make sure you have rights to use the image.

13) Continuing Research

None

TOXIC EFFECTS OF CRUDE OIL AND THE COREXIT 9500 DISPERSANT ON CONCH (*STROMBUS GIGAS*), OYSTER (*CRASSOSTREA VIRGINICA*) AND SHRIMP (*PENAEUS DUORARUM*) LARVAE*

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Static acute toxicity tests were conducted on ecological and economically important invertebrate larvae with artificially weathered crude oil, dispersant and dispersed oil (1:10 ratio) to determine LC₅₀ values following short term exposure. Larvae exposed included conch veligers (2, 4 and 6 lobed stages), oyster veligers (D and “eyed” stages), shrimp larvae (nauplii, zoea 1, mysis 1 and PL6 stages). Dispersed oil was more toxic than crude oil for larvae at all stages of development and as toxic as the dispersant alone at 48 hours for most larvae. The dispersant was equally toxic to all invertebrate larvae at all life stages. LC₅₀ levels for the dispersant ranged from a high of 36 ppm (2 lobed conch veligers) at 24 hours to <1.25 ppm at 72 hours (shrimp zoea 1). Survival decreased with an increase in exposure time and younger stages were generally more sensitive than older stages. Shrimp were more sensitive to oil and dispersed oil than conch or oysters. Zoea 1 shrimp were the most sensitive to the contaminants (81 ppm oil, 15 ppm mix, 4 ppm dispersant; 24 hours). Eyed oyster larvae were the most resistant (>1200 ppm oil, 97 ppm mix, 23 ppm dispersant; 24 hours).

* Presented at the 104th National Shellfisheries Association Meeting, 25-29 March 2012, Seattle, WA.

ACUTE AND SUBLETHAL IMPACTS OF MC252 OIL AND DISPERSANT ON EARLY LIFE STAGES OF *Crassostrea virginica* *

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The impact of Deepwater Horizon on larval eastern oysters, *Crassostrea virginica* was assessed by static acute toxicity and short-term sublethal exposures to water accommodated fractions (WAF) of oil, dispersed oil (CEWAF) and dispersant (Corexit 9500A). Endpoints evaluated via static acute toxicity tests included LC₅₀'s, fertilization, trochophore and D stage development, and swimming ability. The impact of short-term (24 hour) sublethal exposure of D stage larvae to CEWAF (16 mg/L) on survival, growth, umbo development and settlement was also evaluated.

Fertilization was impacted by 2.5 mg/L dispersant, 100 mg/L CEWAF, but not by WAF (100-1200 mg/L). Trochophore development was affected by 2.5 mg/L dispersant, 25 mg/L CEWAF and 100 mg/L WAF. D stage development was impacted by 1.25 mg/L dispersant, 12.5 mg/L CEWAFs and 200 mg/L WAF. Abnormal shell development of D stage larvae was noted at higher concentrations than seen for D stage development. Swimming activity decreased at concentrations below LC₅₀ levels.

Survival and settlement of D stage oysters exposed to sublethal concentrations of CEWAF were negatively impacted compared to controls, but there was no significant impact on size or rate of umbo development.

*Presented at the GOM Oil spill and Ecosystem Science Conference, 21-23 January 2013, New Orleans, LA.

Potential Food Web Impacts Following the Deep Water Horizon Oil Spill: Toxicity Effects on Phytoplankton and Zooplankton*

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To better understand the potential impacts of the DWH oil spill on lower trophic level food sources, a series of experiments were conducted with rotifers and two microalgae species. The acute toxicity of oil, dispersant, and dispersed oil on *Brachionus plicatilis* survival (LC_{50}), and growth inhibition (IC_{50}) of *Isochrysis galbana* (clone T-iso) and *Chaetoceros sp.* were determined. There was no impact on cell division (growth) for either phytoplankton exposed to oil and mean motility of *Isochrysis sp.* never dropped below 79%. However, the addition of dispersant inhibited cell division and motility within 24 hrs. IC_{50} levels ranged from 44 to 638 mg/L for dispersed oil solutions. Initial rotifer mortality was greatest in those exposed to the dispersant, but within 48 hrs the impact of exposure to dispersed tar mat oil was equally detrimental. LC_{50} values ranged from 7 to 707 mg/L for oil exposure and 1 to 140 mg/L for dispersed oil over the 96 hr period. Results from these studies indicate that phytoplankton motility and rotifer survival can be negatively impacted by oil spills.

*Presented at the GOM Oil spill and Ecosystem Science Conference, 21-23 January 2013, New Orleans, LA.

ACUTE AND SUBLETHAL IMPACTS OF MC252 OIL AND DISPERSANT ON EARLY LIFE STAGES OF *Crassostrea virginica* *

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The impact of the Deepwater Horizon spill on embryogenesis and development of the eastern oyster, *Crassostrea virginica* was assessed using a combination of static acute toxicity tests and short-term sublethal exposures to water accommodated fractions (WAF) of oil, dispersed oil (CEWAF) and dispersant. In addition to LC₅₀ determination, endpoints evaluated using static acute toxicity tests were fertilization success, trochophore and D stage development, abnormal development and decreased swimming ability. The impact of short-term (24 hour) sublethal exposure of D stage larvae to CEWAF (16 mg/L) on survival, growth, umbo development and settlement rate was also evaluated.

Fertilization success was impacted after four hours of exposure to 2.5 mg/L dispersant, 100 mg/L CEWAF, but not for WAF (100-1200 mg/L) (Figure 1). Development from fertilized egg to trochophore was affected after twelve hours of exposure to 2.5 mg/L dispersant, 25 mg/L CEWAF and 100 mg/L WAF. Development from trochophore to D stage was impacted after 24 hours of exposure to 1.25 mg/L dispersant, 12.5 mg/L CEWAFs and 200 mg/L WAF. Abnormal shell development of D stage larvae was noted at higher concentrations than seen for D stage development. Swimming activity decreased at concentrations below LC₅₀ levels.

Long term survival and settlement rates of D stage oysters exposed to sublethal concentrations of CEWAF was negatively impacted compared to controls, but there was no significant difference in size or rate of umbo development.

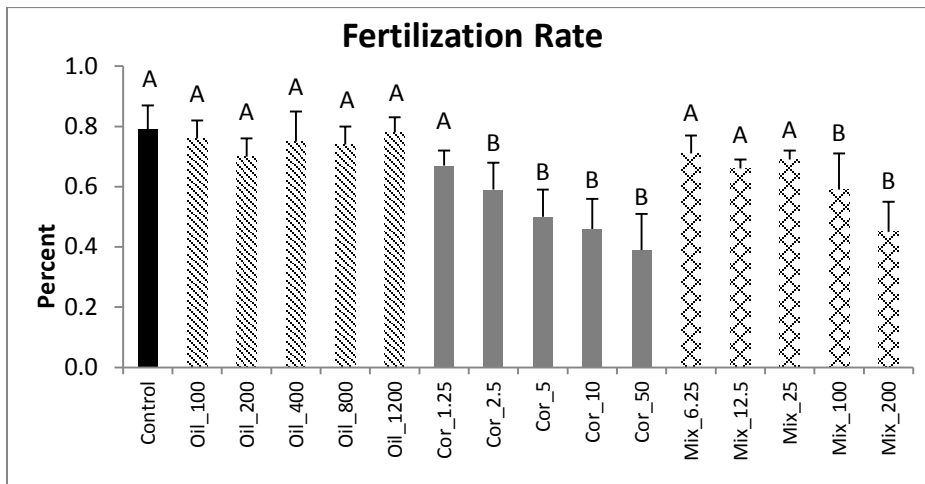


Figure 1. Percent of fertilized oyster eggs following exposure of gametes to oil, dispersant and dispersant mixtures. Letters indicate significant differences in relation to controls (p<0.001).

* To be presented at Aquaculture 2013, 21-25 February 2013, Nashville, TN.

POTENTIAL FOOD WEB IMPACTS FOLLOWING THE DEEP WATER HORIZON OIL SPILL: TOXICITY EFFECTS ON PHYTOPLANKTON AND ZOOPLANKTON

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On April 20, 2010, the Deepwater Horizon (DWH) oil rig exploded, releasing 200 million gallons of oil into the Gulf of Mexico over a three month period. Up to 100,000 km² of the waterways were impacted by the spill, and over 1,000 total linear miles of coastlines were oiled in Louisiana, Alabama, Mississippi, and Florida. Acute and chronic exposure to oil will often negatively impact coastal and oceanic marine life and may have severe implications for food web dynamics.

To better understand the potential impacts of the DWH oil spill on lower trophic level food sources, a series of experiments were conducted with rotifers and two microalgae species. The acute toxicity (24, 48, 72, and 96 hr) of oil (tar mat and crude), dispersant, and dispersed oil on *Brachionus plicatilis* survival (LC₅₀), and growth inhibition (IC₅₀) of the marine dinoflagellate *Isochrysis galbana* (clone T-iso) and the diatom *Chaetoceros sp.* were determined. All three species are a food source for fish and shellfish larvae and are commonly found throughout Florida and Caribbean waters.

There was no impact on cell division (growth) for *Isochrysis sp.* exposed to tar mat oil and crude oil, and therefore IC₅₀ levels could not be calculated. Likewise, exposure to both the tar mat and crude oil alone did not impact the diatom *Chaetoceros sp.* throughout most of the exposure periods. However, there was a drastic impact on cell division for diatoms exposed to the tar mat oil after 96 hrs. Motility of *Isochrysis sp.* exposed to the crude oil was also affected, however it never dropped below 79% for the duration of the experiment. The addition of dispersant to both oil solutions inhibited cell division and motility of the phytoplankton. IC₅₀ levels for both species ranged from 44 to 81 mg/L for dispersed tar mat solutions and were slightly higher at 114 to 638 mg/L for dispersed crude oil. There was also a marked decrease in *Isochrysis sp.* motility for all concentrations above 150 mg/L after 48 hrs. When the phytoplankton was exposed to the dispersant alone, motility decreased after 24 hrs at concentrations of 50 mg/L.

Initial rotifer mortality was greatest in those exposed to the dispersant alone, but within 48 hrs the impact of exposure to dispersed tar mat oil was equally detrimental. LC₅₀ values ranged from 7 to 707 mg/L for oil exposure and 1 to 140 mg/L for dispersed oil over the 96 hr period. Results from these studies indicate that phytoplankton growth and motility along with rotifer survival can be negatively impacted following an oil spill.

* To be presented at Aquaculture 2013, 21-25 February 2013, Nashville, TN.