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Combined effects of Corexit EC 9500A with secondary abiotic and biotic factors in the rotifer *Brachionus plicatilis*

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ABSTRACT

We examined lethality and behavioral effects of Corexit EC 9500A (C-9500A) exposure on the model marine zooplankton *Brachionus plicatilis* singularly and in combination with abiotic and biotic factors. C-9500A exposure at standard husbandry conditions (17.5 ppt, 24 °C, 200 rotifer*mL^{-1} density) identified the 24 h median lethal concentration, by Probit analysis, to be 107 ppm for cultured *B. plicatilis*. Rotifers surviving exposure to higher concentrations (100 and 150 ppm) exhibited a decreased swimming velocity and a reduced net to gross movement ratio. Significant interaction between C-9500A exposure and temperature or salinity was observed. Upper thermal range was reduced and maximal salinity stress was seen as ca. 25 ppt. Increased or decreased nutritional availability over the exposure period did not significantly alter mortality of *B. plicatilis* populations at the concentrations tested. Results from this study may be useful for predicting possible outcomes on marine zooplankton following dispersant application under diverse natural conditions.

1. Introduction

Corexit EC9500A (C-9500A) is a chemical dispersant of the Corexit product line produced by Nalco Environmental Solutions LLC of Sugarland Texas. Like many dispersants, C-9500A is a mixture of chemicals designed to allow increased degradation of large hydrocarbon masses such as oil rafts (Nalco, 1995). During the Deepwater Horizon oil spill event of April 2010, an estimated 7 million liters of C-9500A and Corexit 9527 (C-9527), also a Nalco product, were applied by surface spraying and subsea pumps (C-9500A only) into Gulf of Mexico waters. This was part of an attempt to reduce the ecological impact of an estimated 700 million liters of crude oil released from the Mississippi Canyon Block 252 well (On Scene Coordinator Report DWH, 2011). For dispersant application operations the net benefit of application must outweigh any detrimental ecological impacts and considerations are best applied on a case by case basis (Prince, 2015). Toxicity studies of C-9500A and C-9527 on various marine species indicate survivorship is affected by dispersant exposure even in the absence of crude oil/dispersant interactions (reviewed by George-Ares and Clark, 2000). In areas of overspray and spray drift due to wind and sea current, dispersant independent of crude oil may harm marine life. There is inadequate dispersant toxicity data to clearly predict outcomes of application of dispersants on various ecosystems (Singer et al., 1998).

The complex interactions of multiple stressors make development of predictive models challenging, highlighting the need for future toxicity studies to consider possible positive or negative co-tolerance outcomes. Comparing several toxicity studies of two Corexit dispersants (C-9500A and the previously used C-9527) suggests physical factors that alter the effectiveness of C-9527 to disperse crude oil may also alter singular toxicity of the dispersant material. In the grass shrimp, *Palaemonetes pugio*, there were differences between the 96 h LC50s (concentration causing lethality to 50% of the organisms) of C-9527 exposures at 17 and 27 °C, with the latter showing higher toxicity and being outside the optimal rearing temperature (National Research Council, 1989 and Anderson, 1954). For the medaka, *Oryzia latipes*, a difference can be seen between 24 h C-9527 exposure LC50s when reared in freshwater or seawater (Lessard, 1995). This indicates that C-9527 exposure may disrupt normal functions of homeostasis pathways leading to higher mortality in suboptimal or changing environments.

Brachionus plicatilis is a representative of primary consumer species that are required for the flow of carbon and energy to higher trophic level species in aquatic environments (Wong et al., 2003). Decreased survivorship of wild populations of *B. plicatilis* or other zooplankton could have a direct effect on the survival of larger marine and estuarine species that are largely unaffected by direct C-9500A exposure (Calbet, 2008). *B. plicatilis* has been used to evaluate direct exposure effects, trophic transfer of PAHs, and combinatorial effects following application of oil dispersants (Rico-Martínez et al., 2013; Wolfe et al., 2000). In evaluating co-tolerances, *B. plicatilis* has the benefit of being a model organism with tolerance over a wide range of abiotic and biotic conditions (Epp and Winston, 1977; Yoshinaga et al., 2000, and Smith et al.,

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2012). This tolerance to various environments allows the use of this species as a model to evaluate C-9500A toxicity when zooplankton populations are exposed to changes in environmental parameters including temperature, salinity, population density, and nutrient availability. This study compares effects of Corexit exposure in combination with changes in environmental parameters on *B. plicatilis* populations. We hypothesize that laboratory maintained populations of *B. plicatilis* will be susceptible to increasing concentrations of C-9500A. We further hypothesize that exposure to naturally occurring abiotic and biotic factors will interact to increase mortality in C-9500A exposed populations.

2. Materials and methods

2.1. Maintenance of stock populations

A stock population of the rotifer species *Brachionus plicatilis* was established from populations obtained originally from Reed Mariculture Inc. (Campbell, CA). Rotifer stock was maintained under constant aeration and a 12 h light, 12 h dark photoperiod at a 10 L volume, 17.5 ppt salinity, and 25 °C. Rotifer stock was provided an AM (09:00) and PM (17:00) feeding of a concentrated mixed alga paste (14.8% dry weight, ca. $5.5 * 10^{10}$ cells* mL^{-1}) (Reed Mariculture Inc. Campbell, CA) at a concentration of 0.3 $mL*L^{-1}$ and 0.6 mL*L respectively. To maintain water quality baseline (<0.1 mg/L Cl, <3 mg/L P, and <1 mg/L NO_2^-) and exponential growth in the population, prior to the AM feeding 20% of the volume with its associated rotifers was removed and replaced with 2 L of 17.5 ppt artificial sea water (ASW) (Instant Ocean, United Pet Group, Blacksburg, VA).

2.2. Determination of LC 50

A 1 L volume of *B. plicatilis* was removed from stock population prior to the AM feeding. Average population density from that 1 L collected was calculated from samples collected between 10:00 and 11:00 h ($n = 5$; 1 mL samples were counted and averaged (average and SEM, 326.57 ± 20.15 ind.$*mL^{-1}$)). Initial density counts represent population numbers when samples were collected, usually 4 to 5 h before they were exposed to Corexit. The separated 1 L of rotifers was provided 0.3 mL/L concentrated *Nannochloropsis* and maintained at 24 °C until time of exposure. Exposures were always initiated at 15:30 in the afternoon. ASW (17.5 ppt) was added to increase the volume to reach a final population density of ca. 200 ind.$*mL^{-1}$. This population represents various life stages including juveniles and adults. *B. plicatilis* were exposed in 20 mL glass vials at concentrations of C-9500A ranging from 0 to 300 ppm (following initial range finding bioassays) ($n = 5$ vials/treatment concentration) produced by serial dilution (concentrations are calculated) and all vials capped with plastic paraffin film. *B. plicatilis* populations were exposed for a 24 h period at 12 h light, 12 h dark photoperiod in a 24 °C incubator. Over the 24 h exposure period vials received an AM and PM feeding of concentrated mixed alga paste (14.8% dry weight, ca. $5.5 * 10^{10}$ cells* mL^{-1}) (Reed Mariculture Inc. Campbell, CA) at a concentration of 0.3 $mL*L^{-1}$ and 0.5 $mL*L^{-1}$, respectively, and an O_2 gas application (bubbled 5 s directly into each vial using a glass pipet). To ensure oxygen under these conditions was adequate a set of unexposed rotifers were prepared and monitored using an YSI 5300 Clark type oxygen probe over a 24 h time period. Oxygen saturation varied from 18.40% (±0.48%) (1.42 mg/L) at the initiation of exposure to 6.02% (±0.55%) (0.47 mg/L) at the longest time period between vials being oxygenated. Following the exposure period vials were shaken and rinsed with ASW and contents emptied onto a glass Petri dish. The contents were again mixed with a glass pipet to homogenize the contents and 1 mL was removed to assess % alive and % dead. The % alive and % dead was determined for each replicate by observation of the first 100 rotifers to be located in the field of view under light microscope (Nikon SMZ1000, Nikon Inc. Melville, NY). Alive was determined by motility through the water or movement of the mastax or foot, any of which are common indicators of viability (ASTM, 1998). The same experiment was replicated in its entirety in 3 separate trials ($n = 5$ vials/treatment concentration) and outcomes among trials were averaged for further analysis.

2.3. Behavioral response

In this experiment we used the same exposure methodologies as were used to determine medial lethal concentration with some modifications. *B. plicatilis* populations were exposed in 22 °C, incubator to 0, 50, 100, or 150 ppm concentrations of C-9500A for 6, 12, or 24 h ($n = 5$ vials/treatment concentration at each exposure period). Following exposure periods the vials were removed from the incubator and moved to 23–24 °C, ambient temperature and lighting. Five 1 mL aliquots from each treatment vial were placed into 3 mL of clean ASW of matching temperature and salinity on glass petri dishes (radius of 23 mm). A field of view observing 1–12 rotifers was recorded for 5–7 s at 45 frames per second (fsp) at a resolution of 600 × 800 pixels by light microscope using a Moticam 2000 microscope camera and Motic Images Plus 2.0 software (Motic North America, British Columbia Canada). Video was analyzed in CellTrak 1.5 motion analysis software (Motion Analysis Co. Santa Rosa, CA) for average swimming velocity ($\mu m * s^{-1}$) and average net to gross movement (distance from starting location divided by total distance traveled, where a value of 1 would represent perfectly straight line of travel) over the time period.

2.4. C-9500A lethality at variable temperature

In this experiment we used the same exposure and mortality methodologies used to determine medial lethal concentration with some modifications. *B. plicatilis* populations were exposed to 0, 50, 100, or 150 ppm concentrations of C-9500A ($n = 2$ vials/treatment concentration). Exposures were completed in either low (11–26 °C) or high (24–40 °C) temperature range maintained by an aluminum block (61 × 23 × 8 cm) that is heated from one side and cooled from the other to create a linear temperature gradient extending an ca. 15 °C range. The same experiment was replicated in its entirety in 2 trials and outcomes among trials were averaged for further analysis.

2.5. C-9500A lethality at variable salinity

In this experiment we used the same exposure and mortality observation methodologies used to determine median lethal concentration with some modifications. Prior to the experiment the *B. plicatilis* stock was segregated into 4 separate stocks and acclimated over a 21 day period to salinities of 5, 17.5, 25, or 32 ppt by adding ASW of appropriate salinity (separate stocks maintain equal population growth during acclimation). Once acclimated and raised to a 10 L volume they were maintained as described previously. *B. plicatilis* populations at each of these 4 salinities were exposed in a 22 °C incubator (Percival) to 0, 50, or 100 ppm concentrations of C-9500A ($n = 3$ vials/treatment concentration). The same experiment was replicated in its entirety in 2 trials and outcomes among trials were averaged for further analysis.

2.6. C-9500A lethality at variable population density

In this experiment we used the same exposure and mortality observation methodologies used to determine medial lethal concentration with some modifications. ASW (17.5 ppt) was added to increase water volume to reach approximate population densities of 50, 200, and 350 ind.$*mL^{-1}$ at the initial time of exposure. *B. plicatilis* populations at each of these 3 population densities were exposed in a 22 °C incubator to 0, 50, or 100 ppm concentrations of C-9500A ($n = 5$ vials/treatment concentration). The same experiment was replicated in its entirety in 2 trials and outcomes were averaged for analysis.