Journal of
Marine Science and Engineering

MDPI

Article

Effects of Exposure of Pink Shrimp, *Farfantepenaeus duorarum*, Larvae to Macondo Canyon 252 Crude Oil and the Corexit Dispersant

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Academic Editor: Merv Fingas
Received: 1 January 2016; Accepted: 2 February 2016; Published: 8 March 2016

Abstract: The release of oil into the Gulf of Mexico (GOM) during the Deepwater Horizon event coincided with the white and pink shrimp spawning season. To determine the potential impact on shrimp larvae a series of static acute (24–96 h) toxicity studies with water accommodated fractions (WAFs) of Macondo Canyon (MC) 252 crude oil, the Corexit 9500A dispersant, and chemically enhanced WAFS (CEWAFs) were conducted with nauplii, zoea, mysid, and postlarval *Farfantepenaeus duorarum*. Median lethal concentrations (LC_{50}) were calculated and behavior responses (swimming, molting, light sensitivity) evaluated. Impacts were life stage dependent with zoea being the most sensitive. Behavioral responses for all stages, except postlarvae, occurred at below LC_{50} values. Dispersants had the greatest negative impact while WAFs had the least. No short-term effects (survival, growth) were noted for nauplii exposed to sub-lethal CEWAFs 39 days post-exposure. This study points to the importance of evaluating multiple life stages to assess population effects following contaminant exposure and further, that the use of dispersants as a method of oil removal increases oil toxicity.

Keywords: *Farfantepenaeus duorarum*; shrimp; DWH; MC252 crude oil; Corexit 9500A dispersant

1. Introduction

The Gulf of Mexico (GOM) has some of the most productive coastal bodies of water in the world, making it a major source for the U.S. seafood industry and the most economically important of all domestic commercial seafood harvesting sectors [1]. One of the most important GOM fisheries is the shrimp industry, extending from Brownsville, Texas to Key West, Florida. In 2010 the GOM provided 68% of U.S.-harvested shrimp with a total dockside value of $281 million [2]. The fishery consists of three major species: brown shrimp (*Farfantepenaeus aztecus*), pink shrimp (*Farfantepenaeus duorarum*), and white shrimp (*Litopenaeus setiferus)* [3]. Both the pink and white shrimp began migrating offshore to spawn in the spring, with continued spawning migration throughout the summer (pink shrimp) and fall (white shrimp), while the spawning season for brown shrimp is less defined in terms of season [4–6]. Fertilized eggs pass through nauplii, zoea, and mysis stages in offshore waters before migrating back to coastal estuaries as postlarvae within three to four weeks, throughout the spring and summer, dependent on species. On April 20, 2010 the Deepwater Horizon (DWH) oil platform exploded resulting in 200 million gallons of oil being released into the GOM until the well was capped on July 15 [7,8]. It is estimated that 100,000 km^2 of the GOM was affected by the spill, which coincided

with the spring spawning season of a number of key GOM species, including shrimp [3–6,8]. In an effort to contain the spill and prevent the oil from reaching the shoreline, booms, skimmers, burning, direct recovery, and dispersants were used [7]. It has been calculated that 1.9 million gallons of dispersant (Corexit 9527 and Corexit 9500A) were used [9]. Dispersants do not remove oil from water but act to break the oil into smaller droplets that are more readily dispersed into the water column [10]. While dispersant use decreases the amount of surface oil lessening the amount of oil that reaches shorelines, the small dispersed droplets that remain in the water column are now made available to pelagic organisms that inhabit the water column [7].

Several studies have shown negative impacts of oil or dispersed oil exposure on various invertebrates, including mollusks [11–15], echinoderms [16–18], and crustaceans [11,13,19–22]. Other studies have focused on determining the effect of dispersants on marine organisms [10,23–26]. Most studies concentrate on one stage of development. Early life stages are typically more sensitive to pollutants than juveniles or adults and may be impacted at concentrations that, at least on the surface, do not cause acute mortality in juveniles or adults. Yet, in the long term survival may be impacted by behavioral modifications such as reduced activity that may affect predator avoidance and food intake [27–31]. The aim of our study was to determine what concentration of MC252 oil, Corexit dispersant and chemically dispersed MC252 would adversely affect the survival, development, and behavioral responses of the four major larval stages of shrimp (nauplii, zoea, mysis, and postlarvae). Behavioral responses included swimming activity, light response, feeding, and molting.

2. Experimental Section

2.1. Animals

Various life stages: nauplii (stage N_1, N_5), proto-zoea (stage Z_1, Z_3), mysis (stage M_1, M_2), and six-day-old post-larvae (Pl_6) of shrimp (*Farfantepenaeus duorarum*) were obtained from two commercial shrimp facilities in Florida (Scientific Associates, Indiantown and Pine Island Aquafarms, St. James City, FL, USA).

2.2. Solution Preparation

Oil and dispersant solutions for all experiments were prepared with MC252 oil (British Petroleum Company, BP PLC, London, UK) or Corexit 9500A dispersant (Nalco/Exxon Energy Chemicals, Sugarland, TX, USA). Solutions were prepared following CROSERF procedures [32,33]. Prior to solution preparation, crude oil was physically weathered in the lab for 24 h by placement of oil in a beaker on a stir plate and mixing with a magnetic stir bar in the dark in a chemical fume hood. Stock solutions of water accommodated fractions (WAFs) of crude oil (2 g L^{-1}), dispersant (2 g L^{-1}) and chemically enhanced WAFs (CEWAFs) (1:10 ratio) were prepared in 2 L flasks of filtered, UV treated seawater (28 ppt), covered, and mixed at moderate intensity (25% vortex) for 24 h. Stock solutions were allowed to settle for 3 h prior to preparation of working solutions.

2.3. PAH Analysis

Samples of oil and dispersed oil stocks (2 g L^{-1}) used in the acute toxicity experiments were preserved in glass jars with dichloromethane (1:10 v/v) and extracted using modified EPA method 3510C (Mote Marine Laboratory, Sarasota, FL). Polycyclic aromatic hydrocarbons (PAHs, parent compounds, and homologues) were analyzed using GC/MS (Agilent 7890A/5975C), modified EPA method 8260. Total petroleum hydrocarbons (TPH) *n*-C9 to *n*-C42 were analyzed using a GC with a flame ionization detector (FID, Agilent 7890A, Agilent Technologies Inc., Santa Clara, CA, USA).

2.4. Acute Toxicity Bioassays

2.4.1. Survival (Determination of LC_{50} Values)

Acute static toxicity tests (May 2011) were conducted with N_2, Z_1, and M_1 using nominal WAF concentrations of 0, 6.25, 12.5, 25, 50, and 100 mg L^{-1}, and for Pl_6 shrimp using nominal WAF concentrations of 0, 50, 100, 200, and 400 mg L^{-1} . CEWAF concentrations of 0, 6.25, 12.5, 25, 50, and 100 mg L^{-1}, and dispersant concentrations of 0, 1.25, 2.5, 5, 10, and 50 mg L^{-1} were used for all four life stages, with five replicates per treatment for each of the three solutions. Nauplii (N = 15) were placed in finger bowls containing 50 mL of the appropriate solution. All other life stages (N = 15 Z_1; N = 12 M_1, Pl_6) were placed in 1000 mL beakers containing 600 mL of the appropriate solution. All containers were placed in incubators (28 °C, 12:12 h light:dark cycle). Shrimp were fed once per day. Nauplii and zoea were fed a mixture of *Chaetocerous gracilis* and *Isochrysis galbana*, mysis were fed rotifers, and postlarvae were fed a pelleted diet (Shrimp PL 40-9, Zeigler Bro. Inc., Gardners, PA, USA). Survival was assessed at 24, 48, 72, and 96 h. Lethal concentrations (LC_{50}) were determined using the trimmed Spearman-Karber method (ToxCalcv5.0).

2.4.2. Behavioral Responses

Several experiments were conducted to evaluate behavioral responses. Activity level (swimming behavior) and molting frequency were evaluated for M_1 and Pl_6 stages for both WAF (0, 100, 200, 400, 800, and 1200 mg L^{-1}) and CEWAF (0, 6.25, 12.5, 25, 50, and 100 mg L^{-1}) exposures, with five replicates per treatment group, and 12 shrimp per replicate. Activity level was scored on a scale of 1–4: 1 = actively moving, 2 = moderately active, 3 = lethargic/moving appendages only, 4 = dead. Molting frequency was calculated as the percent of shrimp that molted compared to the total number of shrimp.

Subsequent behavioral response experiments were conducted for CEWAF exposures only for N_5, Z_1, Z_3, and M_2 stages. Concentrations used varied based on life stage evaluated, with five replicates per treatment group, 15 shrimp per replicate. CEWAF concentrations of 0, 6.25, 12.5, 25, 50, and 100 mg L^{-1} were used for nauplii and mysis stages, while concentrations were adjusted to 0, 3.125, 6.25, 12.5, and 25 mg L^{-1} for the more sensitive proto-zoeal stages as determined by the LC_{50} experiments. Behavioral parameters assessed included activity and molting as defined above, and feeding and photo-taxic response. Feeding was scored on a 1–4 scale: 1 = actively feeding (food in gut, fecal strands), 2 = 50% or less feeding, 3 = 25% or less feeding, 4 = 0% feeding. Photo-taxic response was evaluated by placing a light source to one side of the container and noting the proportion of shrimp that were attracted to the light (N_5, Z_1) or avoided the light (Z_3, M_2). Photo-taxic response was scored on a 1–3 scale: for N_5, Z_1—1 = actively moving towards light, 2 = sluggish response, 3 = no response; for Z_3, M_2—1 = actively moving away from light, 2 = slow avoidance response, 3 = no response. The proportion of shrimp that underwent metamorphosis to the next stage was also noted in these experiments.

2.5. Sub-Lethal Toxicity Bioassays

Approximately 10,000 *L. duorarum* nauplii were evenly divided between one of six 13 L buckets containing either filtered, UV treated HBOI salt well water (N = 3) or 23 mg L^{-1} CEWAF (N = 3). During the 24 h exposure, shrimp were fed *Isochrysis galbana* during experimental exposure at a rate of 15,000 (or $\times 10^3$) cells/mL. Surviving shrimp from both control buckets and treatment buckets were sieved, combined and then redistributed into one of four 400-L larval rearing tanks (two control, two treatment) containing filtered, UV treated HBOI salt well water. On day 1 (24 h exposure) and on alternate days, seven to eight shrimp were randomly removed from each of the four tanks (15 control, 15 exposed) for 39 days, collected and placed in vials containing 10% NBT formalin. After a 24 h fixation period, shrimp were placed in 70% ethanol, examined microscopically, and photographed (Infinity 2 digital camera, Luminera Co., Sachse, TX, USA). Developmental stage was recorded and length measurements averaged for each data point using Infinity Analyze (Luminera Co.).

3. Results

3.1. PAH Analysis

The total PAH level in the CEWAF stock solution (1429 μg L^{-1}) was three times greater than that of the WAF stock solution (452 μg L^{-1}) while the TPH level (62,613 μg L^{-1}) in the CEWAF solution was 25 times greater than that of the WAF stock solution (2467 μg L^{-1}) (Table 1). The predominant compound was napthalene, which made up 83.5% of the compounds in the WAF and 65% of the compounds in the CEWAF stock solution. Compounds containing three and four carbon rings (e.g., anthracene, fluorene, pyrene, chrysene, and phenanthrene) were approximately two times greater in the CEWAF compared to the WAF solution.

Table 1. Individual PAH and total TPH and PAH concentrations (μg L^{-1}) of 2 g L^{-1} stock solutions of water accommodated fractions (WAF) and chemically enhanced water accommodated fractions (CEWAF) used to prepare working solutions used in the acute toxicity experiments.

Target Compounds	C rings	2 ppt CEWAF	2ppt WAF
		μg L^{-1}	μg L^{-1}
Napthalene (C0-C4)	2	925.96	377.66
Acenaphthylene	2	6.40	0.05
Acenaphthene	2	0.61	0.67
Fluorene (C0-C4)	3	102.92	14.65
Anthracene (C0-C4)	3	235.38	30.14
Phenanthrene	3	34.79	8.36
Fluoranthene	3	1.53	0.18
Chrysene (C0-C4)	4	32.6	4.02
Pyrene (C0-C4)	4	61.5	6.71
Benzo[A]anthracence	4	0.21	0.14
Napthobenzothiophene (C0-C4)	4	1.32	0.16
Dibenzothiophene (C0-C4)	5	5.47	5.6
Benzo[B]fluorene	5	0.72	0.09
Benzo[B]fluoranthene	5	0.00	0.07
Benzo[K]fluoranthene	5	0.43	0.00
Benzo[E]pyrene	5	0.61	0.11
Benzo[A]pyrene	5	0.00	0.02
Perylene	5	0.77	0.13
Dibenzo[A,H]anthracene	5	0.00	0.01
Indeno[1,2,3-Cd]pyrene	6	0.01	0.00
Benzo[G,H,I]perylene	6	0.00	0.02
Total PAH in μg L^{-1}		1428.64	451.92
Total Petroleum Hydrocarbon C9-C42 in μg L^{-1}		62,613.50	2466.57

3.2. Acute Toxicity Bioassays

3.2.1. Survival (Nominal LC_{50} Values)

Dispersants had the greatest impact on survival of all larval stages while WAFs had the least, with the proto-zoeal (Z_1) stage exhibiting the greatest sensitivity and the postlarval (Pl_6) stage the least sensitivity to all three contaminants (Table 2). The dispersant had the greatest impact on Z_1 shrimp (3.1 mg L^{-1}, LC_{50}, 24 h; 2.5 mg L^{-1} LC_{50}, 48 h; 100% mortality, 72 h), with all other stages having similar LC_{50} values at 24 h (21–33 mg L^{-1}) (Table 2). LC_{50} values continued to decrease for nauplii (N_2) and mysis (M_1) over time, but not for Pl_6 (22–28 mg L^{-1}). CEWAFs, likewise, had the greatest impact on Z_1 shrimp (15.4 mg L^{-1}, LC_{50}, 24 h; 100% mortality, 48 h), with all other stages having similar LC_{50} values at 24 h (81.5–100 mg L^{-1}) (Table 2). LC_{50} values continued to decrease for all stages over time, but less for Pl_6 (44 mg L^{-1}, LC_{50}, 96 h) than for M_1 (8.5 mg L^{-1}, LC_{50}, 96 h). WAFs had the least

impact on all life stages, with Z_1 being the most sensitive (67.4 mg L^{-1}, LC_{50}, 24 h; 25.5 mg L^{-1} LC_{50}, 48 h; 100% mortality, 72 h) and Pl_6 the least, with no LC_{50} value determined for concentrations tested (>400 mg L^{-1}, 96 h) (Table 2).

Table 2. Lethal concentration (LC_{50}) values for shrimp exposed to oil (WAF), dispersant (Corexit 9500A) and oil/dispersant mixture (CEWAF) as determined by a trimmed Spearman-Karber method (ToxCalc). Reported values include nominal LC_{50} (95% CL) (mg L^{-1}) and corresponding PAH and TPH levels (µg L^{-1}). Non-determined values are indicated by ND; Non-calculated values are indicated by NC.

Time	WAF LC_{50}	PAH	TPH	CEWAF LC_{50}	PAH	TPH	Corexit LC_{50}
24 h							
Nauplii	>100	NC	NC	81.5 (75.3, 88.1)	58	2,551	33.3 (31.8, 34.9)
Zoea 1	67.4 (39, 100)	15	83	15.4 (11.6, 20.4)	11	470	3.1 (0.7, 13.7)
Mysis 1	>100	NC	NC	84.6 (74.9, 95.7)	60	2,649	20.9 (19.2, 22.7)
PL 6	>400	NC	NC	99.7 (77.3, 100)	71	3,121	28.4 (24.2, 33.3)
48 h							
Nauplii	>100	NC	NC	41.5 (36.3, 47.5)	30	1,299	18.6 (16.8, 20.5)
Zoea 1	25.5 (22.5, 28.9)	6	31	ND	NC	NC	<2.5
Mysis 1	>100	NC	NC	47.4 (41.3, 54.3)	34	1,484	18.3 (16.1, 20.8)
PL 6	>400	NC	NC	70.4 (56.4, 87.9)	50	2,204	26.5 (22.4, 31.3)
72 h							
Nauplii	ND	NC	NC	ND	NC	NC	ND
Zoea 1	21.2 (17.7, 25.5)	5	26	ND	NC	NC	ND
Mysis 1	>100	NC	NC	31.9 (28.6, 35.7)	23	999	8.3 (6.8, 10.1)
PL 6	>400	NC	NC	49.2 (40, 60.5)	35	1,002	22.4 (20.8, 23.9)
96 h							
Nauplii	ND	NC	NC	ND	NC	NC	ND
Zoea 1	23.3 (20.9, 26)	5	29	ND	NC	NC	ND
Mysis 1	29.7	7	37	8.5 (7.1, 10.1)	6	266	2.6 (2.2, 3.0)
PL 6	>400	NC	NC	44 (36.5, 53.2)	31	1,377	22.5 (21.4, 23.8)

3.2.2. Survival (Determined PAH and TPH LC_{50} Values)

PAH and TPH values for nominal CEWAFs and WAFs could only be compared at 24 h for Z_1 and 96 h for M_1 (Table 2). Toxicity of CEWAFs and WAFs were similarly toxic when PAH concentrations were compared, however WAFs were more toxic than CEWAFs when TPH concentrations were compared.

3.2.3. Behavioral Response—WAF & CEWAF (M_1,Pl_6)

Activity (swimming ability) was significantly decreased for M_1 exposed to CEWAF and WAF (Figures 1 and 2). CEWAFs decreased M_1 activity at 50 mg L^{-1} (36 µg L^{-1} PAH) at 24 h ($F_{5,24}$ = 103.6, $p < 0.0001$), 12.5 mg L^{-1} (18 µg L^{-1} PAH) at 48 and 72 h ($F_{5,24}$ = 25.93, $p < 0.0001$; $F_{5,24}$ = 26.53, $p < 0.0001$) and 6.25 mg L^{-1} (4 µg L^{-1} PAH) at 96 h (($F_{5,24}$ = 119.73, $p < 0.0001$ (Figure 1). WAFs decreased M_1 activity at 800 mg L^{-1} (181 µg L^{-1} PAH) at 24 h ($F_{5,24}$ = 28.11, $p < 0.0001$), 400 mg L^{-1} (90 µg L^{-1} PAH) at 48 h ($F_{5,24}$ = 9.62, $p < 0.0001$) and 100 mg L^{-1} (23 µg L^{-1} PAH) at 72 and 96 h ($F_{5,24}$ = 12.38, $p < 0.0001$; $F_{5,24}$ = 25.05, $p < 0.0001$) (Figure 2).

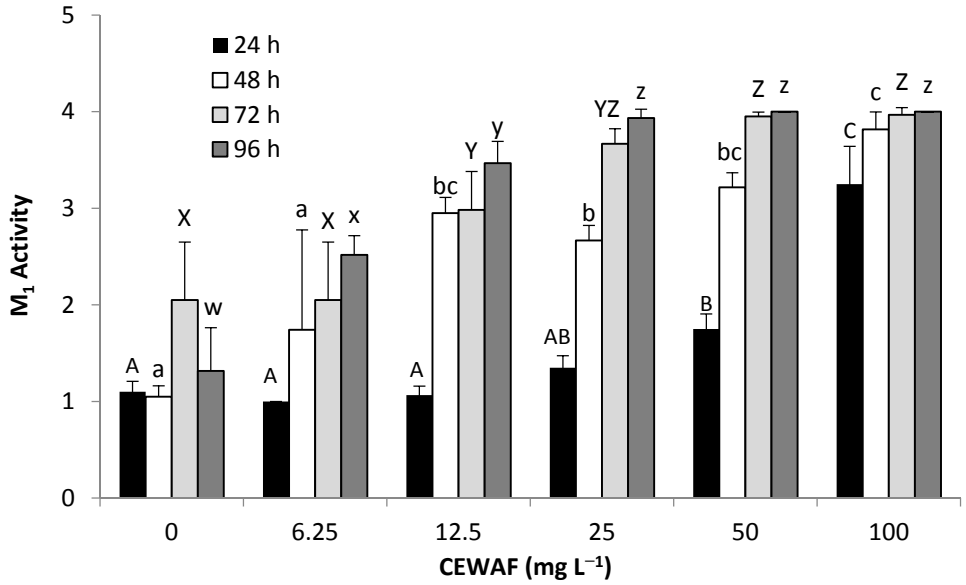

Figure 1. Average activity level (±S.D.) of *F. duorarum* mysis 1 (M_1) shrimp larvae exposed to chemically enhanced water accommodated fractions of MC252 crude oil (CEWAF). Treatment groups consisted of five replicates with 12 shrimp each: 1 = active, 2 = moderately active, 3 = lethargic, and 4 = dead. Numerical representations indicate statistical comparisons of exposure periods. Statistical differences were seen at all exposure times ($p < 0.0001$).

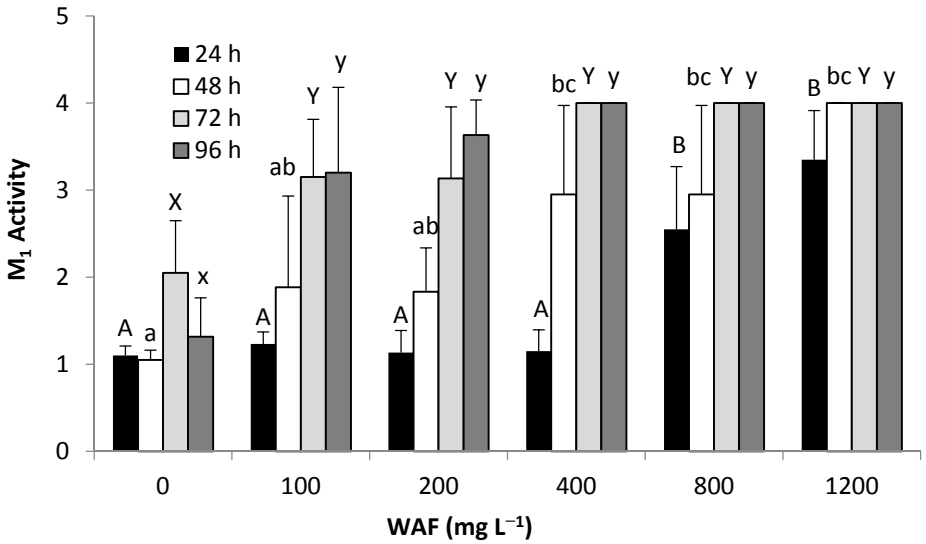

Figure 2. Average activity level (±S.D.) of *F. duorarum* mysis 1 (M_1) shrimp larvae exposed to water accommodated fractions of MC252 crude oil (WAF). Treatment groups consisted of five replicates with 12 shrimp each: 1 = active, 2 = moderately active, 3 = lethargic, and 4 = dead. Numerical representations indicate statistical comparisons of exposure periods. Statistical differences were seen at all exposure times ($p < 0.0001$).

Activity of Pl_6 was not affected by exposure to CEWAFs or WAFs at concentrations tested (Figures 3 and 4). There were significant differences in activity in Pl_6 exposed to CEWAFs at 48 h ($F_{5,24} = 4.56$, $p = 0.0046$) and 96 h ($F_{5,24} = 15.73$, $p = 0.0001$), however this was not dose dependent (Figure 3). There were significant differences in activity in Pl_6 exposed to WAFs at 48 h ($F_{5,24} = 3.81$, $p = 0.0111$), however activity was not dose dependent (Figure 4).

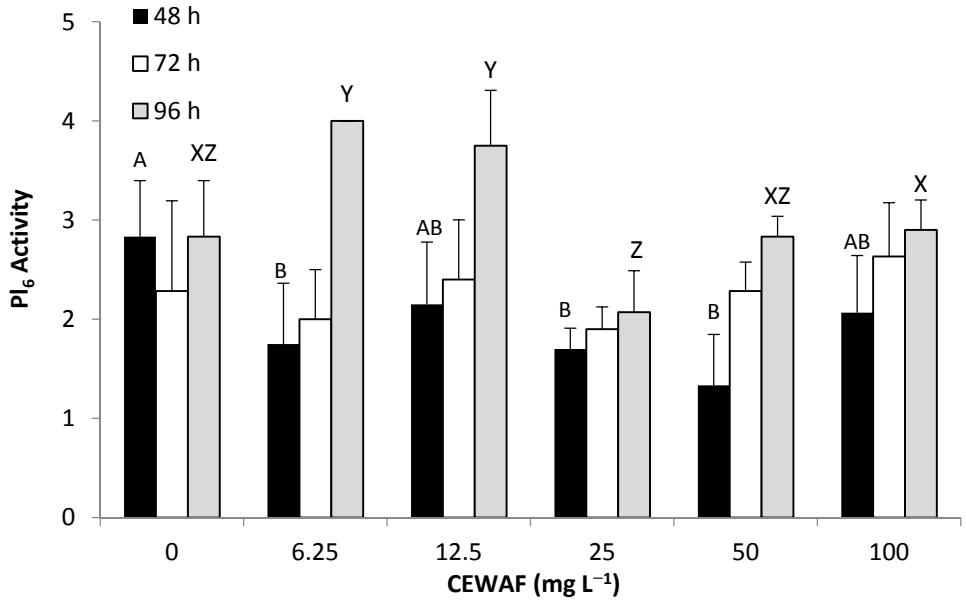

Figure 3. Average activity level (±S.D.) of *F. duorarum* postlarval (Pl_6) shrimp exposed to chemically enhanced water accommodated fractions of MC252 crude oil (CEWAF). Treatment groups consisted of five replicates with 12 shrimp each: 1 = active, 2 = moderately active, 3 = lethargic, and 4 = dead. Numerical representations indicate statistical comparisons of exposure periods ($p \leqslant 0.0046$, 48 h; $p = 0.3642$, 72 h; $p \leqslant 0001$, 96 h).

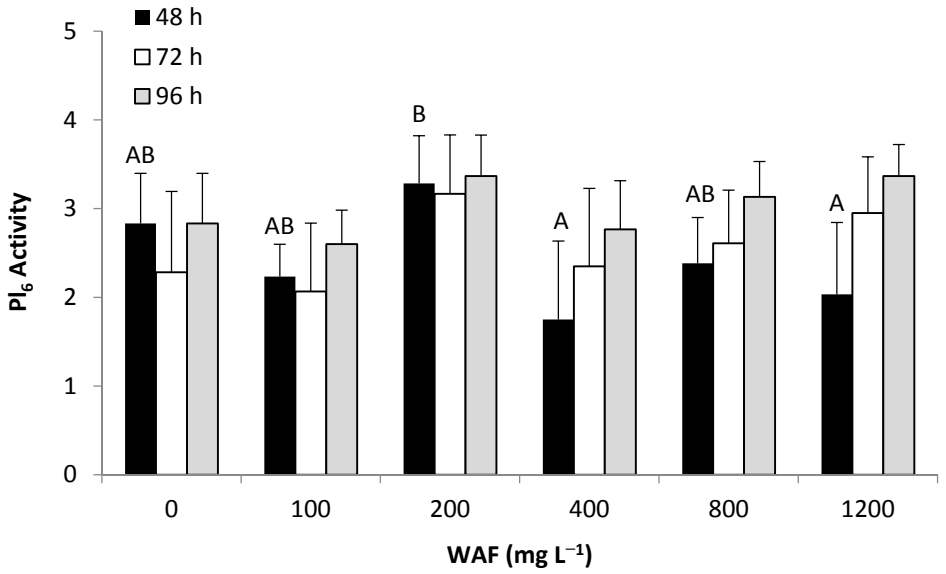

Figure 4. Average activity level (±S.D.) of *F. duorarum* postlarval (Pl_6) shrimp exposed to MC252 water accommodated fractions of crude oil (WAF). Treatment groups consisted of five replicates with 12 shrimp each: 1 = active, 2 = moderately active, 3 = lethargic, and 4 = dead. Significant differences were seen at 48 h ($p = 0.0111$).

CEWAFs $\geqslant$ 12.5 mg L^{-1} caused a significant increase in molting of M_1 (Figure 5). Significant differences were seen at both 24 ($F_{5,17} = 7.71$, $p = 0.0006$) and 48 h ($F_{5,17} = 63.79$, $p < 0.0001$).

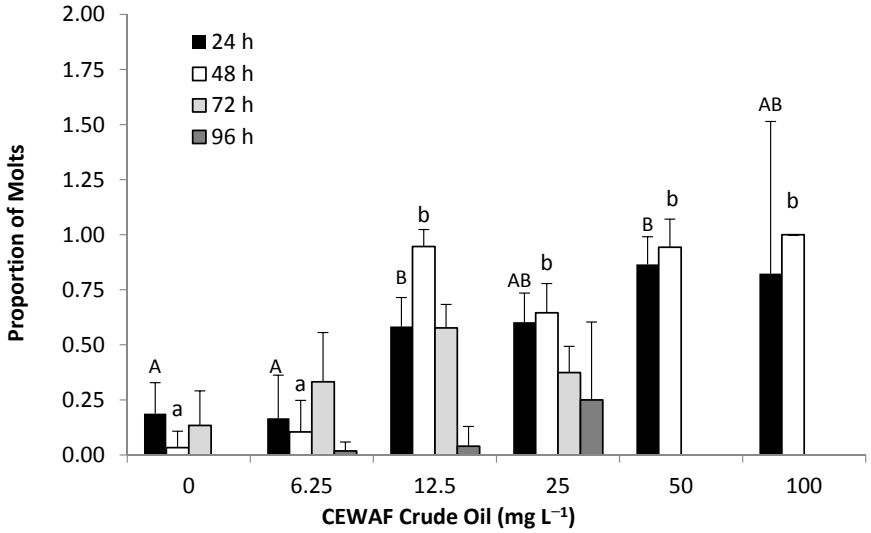

Figure 5. Proportion of *F. duorarum* M_1 shrimp larvae (±S.D.) that molted following exposure to CEWAFs. Treatment groups consisted of five replicates with 12 shrimp each. Numerical representations indicate statistical comparisons of exposure periods. Significant differences were seen at 24 and 48 h ($p \leqslant 0.0006$).

3.2.4. Behavioral Response—CEWAF (N_5, Z_1, Z_3, M_2)

Exposure of nauplii (N_5) to CEWAFs impacted swimming ability, feeding activity and phototaxic response (Table 3). Activity (swimming ability) of N_5 shrimp significantly decreased ($F_{4,25}$ = 98.8, $p < 0.0001$) after 24 h exposure to all concentrations of CEWAF tested (12.5–100 mg L^{-1}) in a dose dependent matter. At 48 h, activity of N_5 shrimp was significantly different ($F_{4,25}$ = 98.8, p=.0262) only at 100 mg L^{-1}. Feeding activity was likewise reduced ($F_{4,10}$ = 35.75, $p < 0.0001$) at 24 h at all CEWAF concentrations. Phototaxtic response was also reduced at both 24 ($F_{4,25}$ = 81.45, $p < 0.0001$) and 48 h ($F_{4,15}$ = 7.67, p = 0014), in a dose dependent manner, with exposed shrimp, being slower to respond at both 24 and 48 h. There was no difference in metamorphosis from nauplii to zoea stages by 48 h, except for at 100 mg L^{-1}.

Table 3. Behavioral response of various larval stages of *F. duorarum* exposed to nominal CEWAF concentrations. Activity level is ranked on a 1–4 scale (1 = active, 4 = no response); feeding is ranked on a 1–4 scale (1 = 100% feeding, 4 = 0% feeding); phototaxic response is ranked on a 1–3 scale, which indicates attraction to light (1 = 100% attracted, 3 = 0% response) for nauplii (N_5) and zoea (Z_1), but light avoidance (1 = 100% avoidance, 3 = 0% response) for zoea (Z_3) and mysis (M_2). For each concentration a total of five replicates consisting of 15 shrimp each were averaged. Letters indicate significant differences between behavioral responses for concentrations at each time point.

Larval Stage	Time (h)	Concentration (mg L^{-1})	Activity Level (1–4) ± S.D.	Feeding (1–4) ± S.D.	% molts	Phototaxic (1–3) ± S.D.	Metamorphosis
N5	24	0	1.03±0.05 ^{a}	1.0 ± 0.0 ^{a}	-	1.02 ± 0.05 ^{a}	N5-Z1
		12.5	2.13 ± 0.05 ^{b}	3.33 ± 0.5 ^{b}	-	2.13 ± 0.05 ^{b}	N5-Z1
		25	2.35 ± 0.35 ^{bc}	3.67 ± 0.5 ^{b}	-	2.27 ± 0.41 ^{b}	N5-Z1
		50	2.28 ± 0.14 ^{bc}	4.0 ± 0.0 ^{b}	-	2.28 ± 0.14 ^{b}	N5-Z1
		100	2.98 ± 0.04 ^{c}	4.0 ± 0.0 ^{b}	-	3.0 ± 0.0 ^{c}	N5-Z1
	48	0	1.33 ± 0.52 ^{a}	-	-	1.5 ± 0.58 ^{a}	Z1-Z2
		12.5	2.17 ± 0.98 ^{a}	-	-	2.25 ± 0.29 ^{b}	Z1-Z2
		25	2.23 ± 0.74 ^{a}	-	-	2.34 ± 0.45 ^{bc}	Z1-Z2
		50	3.19 ± 0.95 ^{ab}	-	-	2.53 ± 0.67 ^{bc}	Z1-Z2
		100	3.83 ± 0.41 ^{b}	-	-	3.0 ± 0.0 ^{c}	Z1

Table 3. *Cont.*

Larval Stage	Time (h)	Concentration (mg L^{-1})	Activity Level (1–4) ± S.D.	Feeding (1–4) ± S.D.	% molts	Phototaxic (1–3) ± S.D.	Metamorphosis
Z1	24	0	1.67 ± 0.82 ^{a}	-	4%	1.67 ± 0.82 ^{a}	Z1-Z2 (4:1)
		3.125	2.29 ± 0.56 ^{ab}	-	12%	2.33 ± 0.58 ^{ab}	Z1
		6.25	2.54 ± 0.56 ^{b}	-	21%	2.63 ± 0.38 ^{b}	Z1
		12.5	2.92 ± 0.13 ^{bc}	-	21%	2.92 ± 0.13 ^{bc}	Z1
		25	3.0 ± 0.0 ^{c}	-	21%	3.0 ± 0.0 ^{c}	Z1
	48	0	2.0 ± 1.55	-	-	1.67 ± 1.03 ^{a}	Z1-Z2
		3.125	2.17 ± 1.47	-	-	1.83 ± 0.98 ^{a}	Z1-Z2
		6.25	3.0 ± 1.55	-	-	2.5 ± 0.77 ^{ab}	Z1-Z2
		12.5	3.17 ± 1.33	-	-	2.5 ± 0.84 ^{ab}	Z1-Z2
		25	4.0 ± 0.0	-	-	3.0 ± 0.0 ^{b}	-
	72	0	2.5 ± 1.64 ^{a}	-	-	2.0 ± 1.1	Z2
		3.125	2.75 ± 1.47 ^{a}	-	-	2.33 ± 1.03	Z1-Z2
		6.25	3.75 ± 0.61 ^{ab}	-	-	2.75 ± 0.61	Z1-Z2
		12.5	4.0 ± 0.0 ^{b}	-	-	3.0 ± 0.0	-
		25	4.0 ± 0.0 ^{b}	-	-	3.0 ± 0.0	-
Z3	24	0	1.0 ± 0.0 ^{a}	1.0 ± 0.0	0.80%	1.0 ± 0.0 ^{a}	-
		3.125	1.0 ± 0.0 ^{a}	1.0 ± 0.0	1.60%	1.0 ± 0.0 ^{a}	-
		6.25	1.0 ± 0.0 ^{a}	1.0 ± 0.0	0%	1.0 ± 0.0 ^{a}	-
		12.5	2.0 ± 0.0 ^{b}	1.0 ± 0.0	2.80%	3.0 ± 0.0 ^{b}	-
		25	2.0 ± 0.0 ^{b}	1.0 ± 0.0	1.60%	3.0 ± 0.0 ^{b}	-
	48	0	1.02 ± 0.04	1.0 ± 0.0	9.1%	-	Z3-M1 (3:2)
		3.125	1.0 ± 0.0	1.0 ± 0.0	15%	-	Z3-M1 (1:4)
		6.25	1.0 ± 0.0	1.0 ± 0.0	19.0%	-	Z3-M1 (1:4)
		12.5	1.0 ± 0.0	1.0 ± 0.0	13.3%	-	M1
		25	1.02 ± 0.04	1.0 ± 0.0	14.1%	-	Z3-M1 (2:3)
	72	0	1.13 ± 0.31	-	0%	1.29 ± 0.71 ^{ab}	-
		3.125	1.7 ± 1.1	-	0%	1.7 ± 1.1 ^{ab}	-
		6.25	1.03 ± 0.05	-	0%	1.03 ± 0.05 ^{a}	-
		12.5	1.12 ± 0.12	-	0%	2.39 ± 0.47 ^{b}	-
		25	1.07 ± 0.05	-	1.60%	2.03 ± 0.03 ^{b}	-
M2	24	0	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	4%	1.0 ± 0.0 ^{a}	M2
		12.5	1.2 ± 0.45 ^{a}	1.0 ± 0.0 ^{a}	1%	1.0 ± 0.0 ^{a}	M2
		25	1.84 ± 0.19 ^{b}	2.2 ± 0.45 ^{b}	28%	3.0 ± 0.0 ^{b}	M2
		50	1.9 ± 0.12 ^{b}	3.6 ± 0.55 ^{c}	37%	3.0 ± 0.0 ^{b}	M2
		100	2.0 ± 0.0 ^{b}	4.0 ± 0.0 ^{c}	51%	3.0 ± 0.0 ^{b}	M2
	48	0	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	2%	1.0 ± 0.0 ^{a}	-
		12.5	1.2 ± 0.45 ^{a}	1.0 ± 0.0 ^{a}	4%	2.0 ± 0.0 ^{b}	-
		25	1.35 ± 0.41 ^{a}	2.0 ± 0.0 ^{b}	28%	2.0 ± 0.0 ^{b}	-
		50	1.98 ± 0.08 ^{b}	2.6 ± 0.55 ^{bc}	2%	3.0 ± 0.0 ^{c}	-
		100	2.28 ± 0.04 ^{b}	3.4 ± 0.55 ^{c}	0%	3.0 ± 0.0 ^{c}	-
	72	0	1.8 ± 1.1 ^{a}	1.0 ± 0.0 ^{a}	0%	1.0 ± 0.0 ^{a}	M3
		12.5	2.29 ± 0.40 ^{a}	1.0 ± 0.0 ^{a}	0%	2.2 ± 0.45 ^{b}	-
		25	2.6 ± 0.55 ^{ab}	3.2 ± 1.1 ^{b}	3%	2.8 ± 0.45 ^{bc}	-
		50	3.0 ± 0.0 ^{b}	4.0 ± 0.0 ^{b}	0%	3.0 ± 0.0 ^{c}	-
		100	3.0 ± 0.0 ^{b}	4.0 ± 0.0 ^{b}	0%	3.0 ± 0.0 ^{c}	-

The behavior responses of proto-zoeal larvae (Z_1, Z_3) were impacted at lower concentrations of CEWAFs than were nauplii and mysis larvae (see below), and Z_1 larvae tended to exhibit these responses at levels lower than did Z_3 larvae (Table 3). Activity of Z_1 ($F_{4,25}$ = 6.68, p = 0.0008) and Z_3 ($F_{4,25}$ = 707, p < 0.0001) shrimp was significantly decreased at 6.125 and 12.5 mg L^{-1}, respectively, at 24 h. No significant differences were seen for either zoeal stage at 48 h, or for Z_3 shrimp at 72 h, however, Z_1 shrimp exposed to 12.5 and 25 mg L^{-1} for 72 h ($F_{4,25}$ = 2.96, p = 0.039) were dead or moribund. Feeding activity was not monitored for Z_1 shrimp, however no difference was seen with Z_3 shrimp (Table 3). Molting frequency increased following exposure to 3.125 mg L^{-1} CEWAFs at 24 h for Z_1 and 48 h for Z_3 shrimp (Table 3). Phototaxic response was reduced for Z_1 shrimp at both 24 ($F_{4,25}$ = 7.78, p = 0.003) and 48 h ($F_{4,25}$ = 2.77, p = 0.05) at 6.125 and 12.5 mg L^{-1} respectively, and for Z_3 shrimp at 24 ($F_{4,25}$ = 3751, p < 0.0001) and 72 h ($F_{4,25}$ = 5.12, p = 0.0036) at 12.5 mg L^{-1}. All Z_1 control shrimp developed to Z_2 stage by 72 h, while some of the shrimp in all exposed groups were still in stage Z_1. An interesting pattern was seen in Z_3 exposed shrimp. A greater percentage of shrimp

exposed to the highest CEWAF concentrations (12.5 and 25 mg L^{-1}) developed to M_1 stage than did control Z_3 shrimp, or shrimp exposed to lower CEWAF concentrations (Table 3).

Exposure of M_2 larvae to CEWAFs affected swimming ability, feeding response, phototaxic response and molting (Table 3). Swimming ability was affected at 25 mg L^{-1} at 24 h ($F_{4,20}$ = 21, p = 0.0007) and at 50 mg L^{-1} at 48 ($F_{4,20}$ = 13.8, $p < 0.0001$) and 72 h ($F_{4,20}$ = 3.0, p = 0.043). Feeding behavior was affected at 25 mg L^{-1} at all exposure times ($F_{4,20}$ = 99, $p < 0.0001$, 24 h; $F_{4,20}$ = 45, $p < 0.0001$, 48 h; $F_{4,20}$ = 48.9, $p < 0.0001$, 72 h) and shrimp exposed to higher concentrations had ceased feeding at 72 h. Molting frequency increased following exposure at 24 h for concentrations 25–100 mg L^{-1} (Table 3). Light avoidance was affected at 25 mg L^{-1} at 24 h ($F_{4,20}$ = 956, $p < 0.0001$) and at 12.5 mg L^{-1} at 48 ($F_{4,20}$ = 1483, $p < 0.0001$) and 72 h ($F_{4,20}$ = 44.2, $p < 0.0001$). No apparent lag in development to M_3 was noted between control and exposed groups.

3.3. Long Term Sublethal Effects

3.3.1. Survival

No difference in survival was seen between control and treatment groups. Survival was approximately 25% for both groups at day 39 post exposure.

3.3.2. Growth

No significant difference was seen in growth. Growth, as defined by total body length, was not significantly different between control and exposed groups from N_5 to Pl_{28} ($F_{1,12}$ = 0.42, p = 0.5302) (Figure 6).

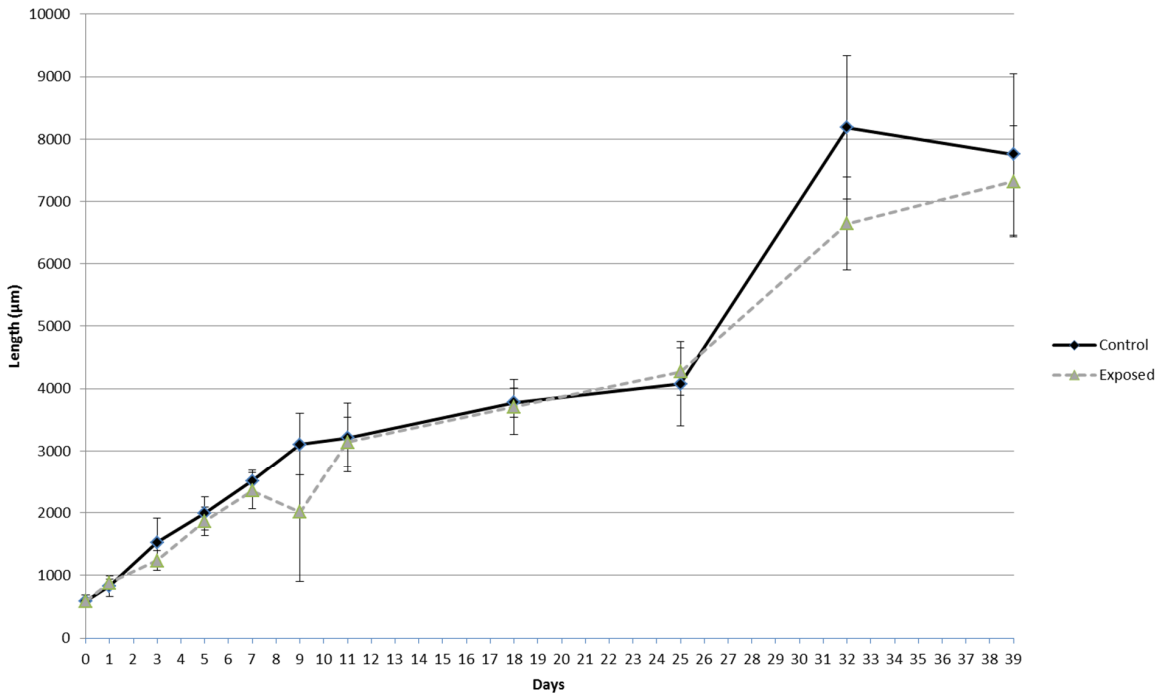

Figure 6. Average growth (±S.D.) of *F. duorarum* shrimp nauplii (N = 15) exposed to sub-lethal concentrations (23 mg L^{-1}) of CEWAFs for 24 h ($F_{1,12}$ = 0.42, p = 0.5302).

3.3.3. Developmental Stages

A slight developmental delay was seen between the control and exposed treatments on day five. Development from Z_3 to M_1 proceeded at a slower pace in the exposed groups resulting in a delayed

development from M_2 to M_3 from day seven to nine. By day 11, development was similar and both groups reached Pl_1 (Figure 7).

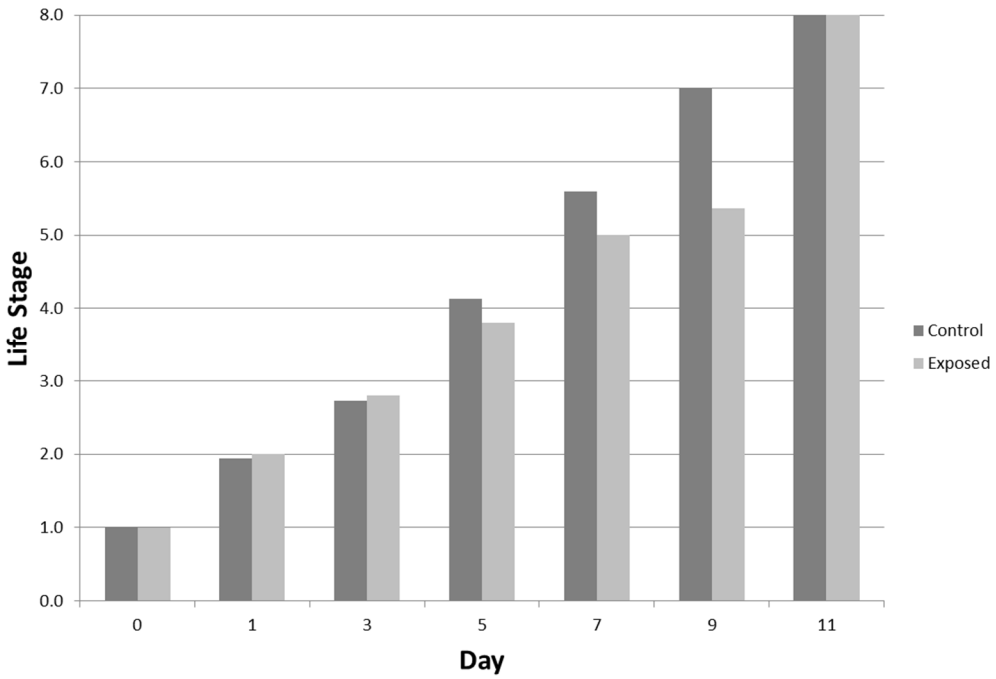

Figure 7. Development of *F. duorarum* nauplii (N = 15) exposed to sublethal concentrations of dispersed oil (23 mg L^{-1}) for 24 h. Life stage, (y axis), were assigned a numerical function for graphical representation. 1 = nauplii V, 2 = zoea 1, 3 = zoea 2, 4 = zoea 3, 5 = mysis 1, 6 = mysis 2, 7 = mysis 3, 8 = postlarvae.

4. Discussion

Exposure of various stages (nauplii, zoea, mysis, and postlarvae) of *F. duorarum* shrimp larvae to MC252 surrogate oil from the Deepwater Horizon well, and the primary dispersant, Corexit 9500A, used during the spill, adversely affected survival and behavior. Zoea (Z_1) were more sensitive to contaminant effects than other life stages. Dispersant exposure had a more pronounced affect, than did water accommodated fractions of crude oil (WAFs) or chemically enhanced WAFs (CEWAFs), and affected all larval stages equally and negatively. CEWAFs generally had a more negative impact than WAFs. Effects were dose and exposure dependent, with short-term sublethal effects resulting in slight developmental delays, with no longer term consequences to growth or survival seen in the laboratory.

The water column is the most likely route of contaminant uptake following an oil spill and therefore the toxicity of oil within the water column are commonly measured by analyzing the amount of oil contaminants within the WAFs and CEWAFs. Oil used was artificially weathered, to decrease the amount of volatile organic compounds (benzene, toluene, ethylbenzene, xylene) that tend to evaporate readily, in order to more closely mimic the type of oil that most organisms in the water column would likely encounter.

Concentrations of WAFs and CEWAFs used in this study represent moderate to maximum environmentally relevant levels based on reported literature. Oil levels ranging from 20 to 600 mg L^{-1} and dispersed oil levels ranging from 25 to 75 mg L^{-1} have been reported in the water column 24 h after a spill and may be a magnitude greater immediately following a spill [34]. Dispersants are used in relatively few spill events due in part to unfavorable conditions that are necessary for dispersants to work effectively [35]. Due to the magnitude of the Deepwater Horizon event large amounts of dispersant were used. Although the majority of water column organisms were likely exposed to either oil or dispersant and oil mixtures during the event it is possible that some organisms were

inadvertently exposed to dispersants alone. Levels of dispersant used in these experiments were considered relevant based on reported literature. Dispersant concentrations of 0.1–15 mg L^{-1} have been reported in the field [10,31,36], although initial dispersant concentrations are generally below 10 mg L^{-1} (maximum range 5–15 mg L^{-1}), dropping to less than 1 mg L^{-1} in a few hours [24,36].

4.1. Acute Toxicity Effects

4.1.1. Survival

Survival is often used as the endpoint to determine oil toxicity effects. Previous studies have reported lethal concentration (LC_{50}) values for crustaceans, including shrimp, following acute exposure to oil, however, researchers typically focus on only one life stage and one contaminant (oil, dispersed oil, or dispersant). Early life stages tend to be more susceptible to toxic compounds than adults, which may negatively affect populations in affected areas, especially in the short-term. This study is unique in studying the effects of various shrimp larval stages simultaneously, to oil, dispersed oil, and a dispersant, and reporting behavioral responses along with LC_{50} values. We found that larval shrimp mortality varied dependent on developmental stage, and was not age dependent as zoea were more sensitive than nauplii. This is likely the result of differing feeding modalities at these two larval stages. Nauplii have undeveloped mouth parts and rely on their yolk sac for nutrition, while zoea are indiscriminate feeders and consume anything large enough to enter their mouth, and mysis seek out and capture their food [37]. Feed sources provided during this study varied: nauplii and zoea were provided with algae, mysis with rotifers, and postlarvae with commercial pellets. Exposure of WAFs or CEWAFs through either the water column or exposed feed resulted in similar alterations of metabolic enzymes in fish [38]. The addition of small quantities of feed may have resulted in larvae being exposed to oil contaminants through both the water column via the gills and the digestive tract via ingestion. We believed that administration of some feed was necessary to eliminate the likelihood of starvation as the cause of death as larvae, unlike postlarvae and juvenile shrimp, need to eat continuously. Preliminary experiments conducted with untreated and unfed larvae resulted in notable lethargy at 24 h and 50%–90% mortality of nauplii and zoea, respectively, at 48 h.

We noted that dispersant exposure negatively impacted all four larval stages at similar concentrations, although zoea were the most adversely affected, with all Z_1 shrimp dead by 48 h. Our reported values for 96 h exposures for *F. duorarum* M_1 and Pl_6 for Corexit 9500A, were similar to those previously reported for Corexit 9527 for *L. setiferus* postlarvae (96 h LC_{50}, 12–31 mg L^{-1}) [11,13]. Similar LC_{50} values (3.5–83 mg L^{-1}) have been reported following 48 to 96 h of exposure of other postlaval and juvenile crustaceans to Corexit 9500 [10,26,32,39,40].

Exposure to nominal concentrations of CEWAFs resulted in increased mortality compared to WAFs in our study. The majority of researchers have concluded that chemically dispersed oil is more toxic than physically dispersed oil [35]. However, reporting methods (nominal, PAH, TPH), may impact researchers conclusions [41]. In our study, when PAH, rather than nominal values were compared, the toxicity of WAFs and CEWAFs were equivalent, although WAFs appeared to be more toxic when TPHs were compared, similar to that seen for *L. setiferus* juveniles [13]. The increase in toxicity of CEWAFs is attributed to increased availability of PAHs in the water column through the creation of a large number of small oil droplets [35]. The PAH levels in the prepared CEWAF stocks in our study were three times greater and the TPH levels 25 times greater than in the WAF stocks. Oil droplets were observed in fecal strands and the digestive tract and fecal strands of some zoea and mysis larvae exposed to CEWAF concentrations $\geqslant$ 25 mg L^{-1} indicating ingestion of oiled particulates in larvae that were still feeding. At 50–100 mg L^{-1} oil was noted on appendages and molts of some shrimp larvae, implicating narcosis (PAH toxicity) and perhaps restricted motility as likely causes of mortality.

Although previous researchers have compared survival of crustaceans exposed to WAFs and CEWAFs by reporting LC_{50} values, there is little consistency in the reporting method (nominal, TPH,

PAH) which makes comparison difficult. In this study, we attempted to make cross-comparison easier by listing LC_{50} values for all three parameters. The majority of early crustacean studies were conducted with larval mysid shrimp, *Americanus* (*Mysiopsis*) *bahia*, where TPH values were reported, and 96 h LC_{50} values ranged from 0.15–83 mg L^{-1} WAFs and 0.5–120 mg L^{-1} CEWAFs [26,42,43]. That these results differ somewhat may be explained by variation in exposure methods used (constant, spiked, static renewal), however, each study reported a similar toxicity for WAFs and CEWAFs based on comparison of TPHs. Similar results have been reported for *Americamysis* (*Holmesimysis*) *costata* (1–35 mg L^{-1} WAF, 8–33 mg L^{-1} CEWAF, 96 h) and *L. setiferus* juveniles (6.5 mg L^{-1} WAFs, 5–7.5 mg L^{-1} CEWAFs, 96 h) [13,25,44]. In contrast, we report a 96 h TPH toxicity with larval *F. duorarum* (0.029–0.037 mg L^{-1} WAFs, 0.27–1.38 mg L^{-1} CEWAFs), indicating that WAFs were more toxic. This is in contrast to 96 h LC_{50} PAH values, in which little difference in toxicity was seen for WAFs and CEWAFs. In the wake of the Deepwater Horizon event, reported concentrations of TPAHs in May 2010 varied greatly dependent on site and sampling depth and ranged from 0 to 146 mg L^{-1} at the wellhead, 0 to 0.9 mg L^{-1} in field collected WAFs, 0 to 18 mg L^{-1} in field collected CEWAFs, and 0 to 0.17 µg L^{-1} in shoreline samples [45–47]. Following capping of the well in July concentrations in collected sample were significantly lower at all sites and depths.

4.1.2. Behavior

Factors in addition to mortality need to be considered when assessing contaminant effects, as behavioral responses, such as swimming ability, and response to stimuli affect the ability to locate prey or escape predation. Some researchers have reported behavioral inhibitory (IC_{50}) effects following exposure to lower levels of oil contaminants than at which mortality (LC_{50}) occurs. Changes in behavior due to sublethal exposures are considered to be the most sensitive indicators of environmental disturbance, and yet are among the least studied effects with regards to toxicity [48]. A variety of behavioral responses of marine organisms to pollutants, including oil, such as motivation (e.g., feeding response), sensory responses (e.g., phototaxis), and motor activity (e.g., swimming performance) are given in a summary of early work [49]. Examples of depressed feeding responses associated with PAHs have been shown in a variety of invertebrates including rotifers, crabs, and shrimp [50–52]. Examples of differential phototaxic responses associated with invertebrates have been reported with crabs and barnacles [27,53]. Invertebrates, such as crabs, shrimp, and barnacles, have also been shown to exhibit erratic swimming behavior in response to oil contaminants [27,53,54] and it has been postulated that differential sensory and motor responses that resulted in differential depth distribution might affect larval distribution and recruitment via directional current activity [53].

Some researchers have reported behavioral effects, such as reduction of swimming ability, at lower than LC_{50} concentrations [15,28]. Others have reported similar LC_{50} and EC_{50} values following exposure to oil contaminants, including swimming ability, settlement behavior and burying behavior [27,31,39]. Decreased swimming behavior is likely a result of narcosis typically seen in acute toxicity of high short-term exposures to naphthalene [55]. Narcotic chemicals affect the lipid bilayer in membranes reducing activity and the ability to react to stimuli, which may ultimately lead to mortality [56]. However narcosis does not account for other reported toxic effects such as deformities, edema, and cardiovascular effects [57] Regardless of whether this is the result of narcosis, or some other phenomenon the end result is that decreased swimming ability results in decreased ability to find food or escape predation, either of which will likely reduce survival.

In this study, swimming ability was significantly decreased for M_1 larvae exposed to both CEWAF and WAF at all exposure times. Exposure to CEWAFs caused an initial decrease in activity at concentrations that were two times less than LC_{50} values at 24 h and three to four less than LC_{50} values at 48 and 72 h. Similar results were seen with N_5, Z_1, Z_3, and M_2 larvae exposed to CEWAFs. However, results were stage dependent, in that Z_1 larvae were the most sensitive, whereas, older shrimp (Pl_6) did not exhibit reduced swimming ability at sublethal concentrations of either CEWAFs or WAFs. Although significant differences in activity in Pl_6 shrimp exposed to CEWAFs at 48 and

96 h and to WAFs at 48 h occurred, they were not dose dependent and thought to be due to water quality issues. Phototaxic response, whether attraction to light (N_5, Z_1) or avoidance (Z_3, M_2) followed a similar pattern to that seen with swimming ability, and this manifested itself in reduced feeding response for N_5, Z_1 stages.

4.1.3. Molting

Molting frequency increased in response to CEWAFs at 24 or 48 h post-exposure. Response was stage dependent with Z_1 and Z_3 larvae responding at lower concentrations than M_1 or M_2 larvae. It is postulated that this behavior is a stress response to compounds present in CEWAFs or an attempt by the shrimp to rid itself of oil adhering to the carapace or appendanges. Molting was not associated with metamorphosis to the next stage, as, except for the Z_3 stage, metamorphosis occurred at the same rate as the controls or was somewhat delayed, and control shrimp molted less frequently. An unwanted side effect of this response might be increased susceptibility to oil contaminants. Crustaceans are more susceptible to environmental stressors, including oil pollutants during molting, and may experience increased mortality [58,59]. In the present study, molting frequency was not followed after 72–96 h post-exposure. PAHs has been shown to increase the length of the intermolt period, resulting in decreased molting in a variety of invertebrates [58,60,61]. The use of additional measurements, such as biochemical endpoints, provide researchers with another set of tools for evaluating oil toxicity, allowing additional means of assessing the potential consequences of oil exposure for marine organisms, such as shrimp.

4.2. Sublethal Effects

Survival following sublethal exposures of invertebrates varies based on species and life stage [21,54,62,63]. In the present study, *F. duorarum* nauplii exposed to sub-lethal amounts (23 mg L^{-1}, LC_{10}) of CEWAFs for 24 h showed no difference in survival compared to controls over 39 days and was approximately 25% for both groups. Shrimp were cultured in larval tanks specific to the penaeid shrimp industry and industry operational procedures followed. Due to the sensitivity of handling the zoeal stage, tanks are not drained, water is instead added to partially filled tanks to alleviate water quality issues. Unfortunately, *Artemia* proliferated in the tanks, competing with shrimp for resources, and causing low survival in tanks regardless of exposure. Delayed morality has been reported by other researchers in shrimp and crab larvae and embryos exposed to low levels of WSF for short periods with zoea being more sensitive than later developmental stages [21,54,62]. Other research has shown no survival effects in crab zoea following either short term exposure or continuous exposure to low concentrations of oil [63].

Developmental delays have been reported for invertebrate larvae exposed to PAHs [21,63–65]. Developmental delays in invertebrates are typically accompanied by an increased period of intermolt following exposure to oil [58,64]. We saw a slight developmental lag in the exposed group between Z_3 and M_1 resulting in delayed development to subsequent mysis stages, but both groups developed in postlarvae at roughly the same time. Other researchers have reported similar findings. Zoeal stages of the mud crab *Rhithropanopeus harrisii* increased following prolonged exposure to chronic, low levels of WSF, however short term exposure had no impact [63,64]. Minor differences (one day lags) in development times were also reported in *Pandalus borealis* larvae in early stages of development but effects decreased at later stages [21]. The more time spent in pelagic larval stages, as would occur as a result of delayed development, may result in increased likelihood of predation, impact dispersion, increase time to maturity, and therefore negatively affect population growth rate [66].

In our study, despite slight developmental lags, no lasting growth effects were seen in the exposed group at day 39 (Pl_{28}). This is consistent with results reported in the literature for *Cancer irroratus* larvae exposed to WAFs, *R. harrisii* larvae exposed to low concentrations of naphthalene, and grass shrimp *Palaemonetes pugio* exposed to sublethal WAFs [59,67,68]. Other research has indicated decreased growth following exposure to oil. Decreased growth was reported in DWH exposed juvenile brown

shrimp *Farfantepenaeus aztecus* but not in juvenile white shrimp *L. setiferus* exposed to the same waters [22].

5. Conclusions

This study shows that the concentrations of oil released and dispersant used during the DWH event could have negatively affected penaeid shrimp in the GOM, whether through altered behavioral responses, delayed development, or mortality. Even though the spill occurred during the spring spawning season and likely affected shrimp larvae at select locations, GOM shrimp populations as a whole do not appear to have been affected long term, perhaps in part due to fishery closures that were put in place following the spill [69].

Acknowledgments: This research was made possible by a grant from BP/The Gulf of Mexico Research Initiative through the Florida Institute of Oceanography #4710-1101-00B. We thank Sloane Wendell, BP GCRO Reference Material Account Manager for providing us with surrogate MC252 oil #SO-20110212-HMPA9-008; David Giddings, Strategic Technology Manager of Nalco Co., Sugarland Texas, for providing us the Corexit 9500A dispersant; and to FAU student Kaitlin Gallagher (currently UConn). This is HBOI-FAU publication # 1989.

Author Contributions: Susan Laramore and Amber Garr conceived and designed the experiments; Susan Laramore and William Krebs performed the experiments; Amber Garr analyzed the data; Susan Laramore and William Krebs wrote the paper; Amber Garr edited the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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