

Individual to population level effects of South Louisiana crude oil water accommodated hydrocarbon fraction (WAF) on a marine meiobenthic copepod

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Abstract

Acute toxicities of crude oil and crude oil water accommodated hydrocarbon fraction (WAF) are relatively well documented, but data on the biological effects of chronic exposures to WAF on species and populations are scarce. South Louisiana Sweet crude oil was used to assess the effects of crude oil WAF on the copepod *Amphiascus tenuiremis*' survival, development and reproduction. Effects were evaluated using a 96-well microplate full life-cycle toxicity test, a test that allows tracking of individuals from the nauplius stage to sexual maturation and reproduction. Briefly, 24-h hatched nauplii were followed to adulthood ($n_i \geq 120$ nauplii/treatment) in individual glass-coated microplate wells containing 200 μ L of seawater solution. Treatments consisted of 10%, 30%, 50% and 100% Louisiana WAF, with seawater used as control. Nauplii were monitored through development to adulthood, and sexually mature virgin copepods were mated pairwise in wells containing original rearing treatments. Nauplius-to-copepodite survival was reduced by 57% in exposures to 100% WAF, relative to controls ($88 \pm 3\%$), and copepodite-to-adult survival was reduced by 18% in the 50% WAF, relative to controls ($98 \pm 3\%$). Analysis of development curves showed that nauplii in the 10% WAF developed significantly faster into copepodites, while nauplii in the 50% WAF developed significantly slower than controls. Although the naupliar developmental rate in the 100% WAF was not significantly different from the control, these nauplii showed an average 1.4 day delay in development into copepodites. Similarly, copepodite development into mature females and males was significantly enhanced by 1.2 to 1.8 days and delayed by 1.9 to 2.2 days ($p < 0.05$) in the 10% and 50% WAFs, respectively, compared to controls. Although the copepodite developmental rate in the 100% WAF was not significantly different from the control, these copepodites still showed an average 1.5 and 2.1 day delay in development into females and males, respectively. Analysis of reproductive endpoints showed that fertility was the only endpoint negatively affected by WAFs; reproductive failure increased by 30% and 41% in exposures to 30% and 100% WAF, respectively, compared to controls ($3.33 \pm 4.71\%$). Leslie matrix population projections based on empirical microplate data indicated lower production rates through three generations of exposure to WAFs. Furthermore, a comparison between NIST and Louisiana crude oil WAFs using the same life-cycle approach indicated a greater chronic toxicity for the Louisiana WAF and an overall developmental delay in exposures to high WAFs (50% and 100% WAFs) from both crude oil types.

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1. Introduction

Coastal ecosystems are subject to potentially long lasting ecological and environmental effects following oil spills. From a toxicological perspective, the degree of spill damage to marine and coastal fauna and flora depends on the amount, composition and persistence of water-soluble components in the seawater phase (NRC, 1985). The acute toxicity of crude oil and various crude oil water accommodated hydrocarbon fractions (WAFs) has been evaluated on aquatic organisms (Bonsdorff et al., 1990; Eadsforth, 1997). Among aquatic fauna, crustaceans have shown high susceptibility to crude oil and crude oil WAF exposures (Anderson et al., 1974; Bonsdorff et al., 1990; Stark et al., 2003; Coull and Chandler, 1992). While studies with fish (Eadsforth, 1997) have shown hydrocarbon WAF median lethal concentrations (96-h LC₅₀) greater than 100 mg/L, studies with crustaceans, principally shrimp (i.e., *Mysidopsis almyra*; *Paleomonetes pugio* and *Penaeus aztecus*), have shown WAF LC₅₀'s ranging from 0.9 to 3 mg/L (Anderson et al., 1974; Tatem et al., 1978). Chronic exposures under laboratory and field conditions (Bonsdorff et al., 1990; Elmgren et al., 1983; Linden, 1976; Stark et al., 2003) have also corroborated an elevated crustacean sensitivity to crude oil WAF exposure. Stark et al. (2003) assessed the effect of field marine sediments contaminated with hydrocarbons on the recruitment and development of soft-sediment assemblages. In that study hydrocarbon contaminated sediments significantly reduced crustacean abundance (i.e., gammarid, ostracods, tanaids and copepods) compared to reference sediment. In a similar study, Bonsdorff et al. (1990) investigated the effects of North Sea (Ekofisk) crude oil WAF on recruitment of zoobenthos to shallow soft bottoms. This exposure showed that hydrocarbon WAF had a negative effect on population density of the amphipod (i.e., *Corophium bonelli*), while recruitment of polychaetes was not affected. The authors suggested that WAF may have negatively affected amphipod embryos leading to reduced juvenile survival. Also, Linden (1976) indicated that long term exposure to low oil concentrations not only resulted on decreased brood size of exposed amphipod *Gammarus oceanicus* females, but also decreased female–male mating encounters during reproduction. Also, the abundance of crustaceans such as amphipods (*Pontoporeia*) and meiobenthic ostracod and harpacticoid copepods were substantially depressed within 2 weeks after a major oil spill (Elmgren et al., 1983). Among meiobenthic fauna,

harpacticoid copepods are particularly sensitive to hydrocarbon and crude oil contamination (Coull and Chandler, 1992; Carman et al., 1997) partially due to their relatively short life cycles (15 to 25 days, species dependent). For example, WAF at a low concentration (200 µg/L) caused 50% mortality of *Halectinosoma curticorne* after 6 days of continuous exposure (Gyllenberg, 1986), while reducing by 43% the brood size of *Nitocira affinis* (Ustach, 1979). The high susceptibility, short generation time and ecological relevance of harpacticoid copepods makes them ideal models for assessing contaminant WAF effects at the individual and population levels.

In the current study we utilized the rapidly maturing harpacticoid copepod *Amphiascus tenuiremis* as a model for assessing the life-history effects from exposure to petroleum crude oil WAFs. This model copepod allows the evaluation of WAF impacts on several life-cycle endpoints (i.e., development and reproduction), as well as the assessment of potential population level effects under controlled laboratory conditions. This chronic WAF toxicity test provides a rapid and sensitive measurement of crude oil WAF effects on an ecologically important crustacean group (Hicks and Coull, 1983).

2. Materials and methods

2.1. Test organism

A. tenuiremis is a sediment-dwelling harpacticoid copepod that inhabits muddy estuarine sediments from the Baltic and Black Seas to the southern Gulf of Mexico (Lang, 1948). The life cycle of *A. tenuiremis* consist of six nauplius, five copepodite stages and sexually dimorphic adults. The short generation time (21 days at 20 °C) and ease of culture in sediment and water-only exposure conditions (Chandler and Green, 1996; ASTM, 2004) make this species suitable for evaluating sublethal reproductive and developmental effects resulting from exposure to sediment-associated or water-borne contaminants (Bejarano et al., 2005, 2004; Chandler et al., 2004; Chandler and Green, 1996).

2.2. Crude oil WAF extraction

Crude oil water accommodated hydrocarbon fractions (hereafter WAF) were prepared using South Louisiana sweet petroleum crude oil. WAFs were made following NRC (1985) recommendations (Fig. 1). Briefly, 60 mL of 0.22-µm filtered seawater (30‰

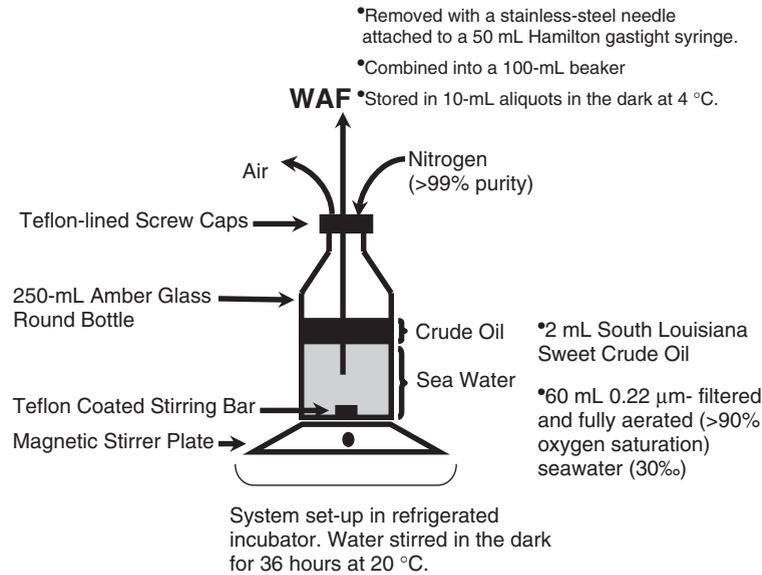


Fig. 1. Schematic representation of the methodology used to extract crude oil water accommodated fractions (WAF) from South Louisiana Sweet Crude Oil.

salinity; >99% dissolved oxygen), and a Teflon-coated stirring bar (1 cm), were placed into an amber round glass flask (250 mL), following the layering of Louisiana crude oil (2 mL) on the top of the seawater surface by means of a glass tight syringe. The flask was sealed tight, headspace air purged through the Teflon septa with a stainless-steel needle attached to a gas tight syringe, and the 190-mL headspace filled with nitrogen (>99% purity) to prevent oil degradation/oxidation. The flask was placed in a refrigerated incubator (20 ± 1.5 °C) on a magnetic stirrer plate, with stirring speed adjusted to avoid a large vortex and formation of oil droplets. WAF from the flask was collected after 36 h, transferred into a clean beaker and aliquoted out into 10-mL glass vials. Single aliquots containing extracted WAF were covered with aluminum foil and stored at 4 °C. WAF stocks for chronic copepod exposures were made fresh every 2 weeks. All glassware used for WAF extraction and storage was cleaned thoroughly with methylene chloride (>99.5 DCM, HPLC grade; Fisher, Pittsburgh, PA, USA).

WAF treatment solutions were made by combining 10-mL freshly extracted WAF aliquots with appropriate amounts of fully aerated (30‰) seawater into clean 100-mL beakers. WAF treatments included 10%, 30%, 50%, and 100% WAF treatments, with seawater used as a control (0% WAF). Microplate wells were loaded with 200 µL of WAFs or control solution and microplates placed in an incubator under cool-fluorescent light and 12:12 light/dark cycles.

2.3. Crude oil SWF chronic exposures

A full life-cycle bioassay, following ASTM E-2317-04 protocol, was conducted to assess the effects of South Louisiana sweet crude oil WAF on *A. tenuiremis* survival, development and reproduction (Fig. 2). Gravid *A. tenuiremis* were collected from clean laboratory cultures and transferred to a 12-well tissue culture plate containing seawater and 75-µm mesh cup inserts. The inserts retain the females while allowing hatching nauplius to fall to the well bottom over a 24-h period.

A minimum of 35 nauplii were placed individually into 250-µL well microplates (i.e., glass coated 96-well microplates; Sun-SRI, Duluth, GA, USA), and microplates assigned in triplicate to each treatment. Excess transfer water was removed from each well following the addition of 200 µL control or WAF treatment solutions. To ensure proper water quality (>90% DO) and consistent WAF or control exposures, every third day throughout the exposure period, exposure solutions were removed from all treatments and replaced with 200 µL of fresh WAF or control solutions (>90% water replacement). Water quality (salinity, temperature, dissolved oxygen and pH) was recorded from fresh solutions prior to each water change. Individuals were fed every 6 days with 3 µL of a fresh 1:1 of *Isochrysis galbana*/*Dunaliella tertiolecta* algae (10^7 cells/mL). Covered microplates were held in an incubator (Revco, Asheville, NC, USA) at 25 ± 1 °C and 12:12 light/dark conditions.

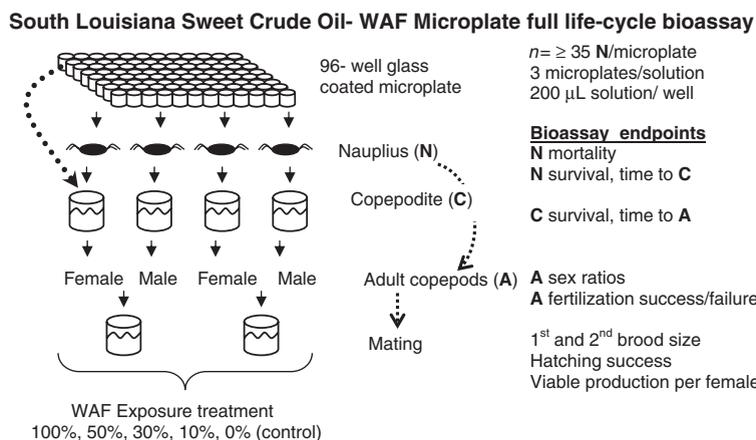


Fig. 2. Experimental set-up of the full life-cycle bioassay exposing the copepod *A. tenuiremis* to South Louisiana Sweet Crude Oil water accommodated fraction (WAF) dilutions and seawater control (0% WAF). N=nauplius, C=copepodite, A=adult copepod.

Individual nauplii were monitored daily through the 1st copepodite stage and to sexual maturity via inverted stereomicroscopes. Upon reaching sexual differentiation virgin copepods were removed from wells and mated pairwise in new wells containing original WAF exposures. Individual mating pairs were also monitored daily during the mating period, which lasted up to 9 days post-mating to accommodate potential delays in reproduction. Full life-cycle test endpoints included naupliar mortality through time, naupliar survival and development time to 1st copepodite stage, copepodite survival and development time to successful sexual differentiation, and adult sex ratios. Reproductive endpoints included fertilization success/failure, hatching success, and total viable offspring production over two consecutive broods. Fertilization failure was defined as any mating pairs unable to extrude viable embryos over a 9-day mating period.

2.4. Stage-structured population growth model

Crude oil WAF population-level effects over multiple generations were estimated using empirical microplate data fitted to a matriarchal stage-structured Leslie matrix model (RAMAS[®] EcoLab 2.0, Applied Biomathematics, Setauket, NY, USA) Akçakaya et al., 1999; Caswell, 2001). A 5-stage (i.e., embryo-to-nauplius, nauplius-to-copepodite, copepodite-to-virgin female and virgin female-to-gravid female) matrix model was used to project naupliar production through 3 generations. Naupliar production projections by treatment were based on (1) stage-specific survival rates, (2) the proportion of virgin females, (3) the proportion of females becoming fertile; and (3) female fecundity (i.e., viable offspring/female) through two broods.

Model constraints included demographic stochasticity and an arbitrarily carrying capacity set to 20,000 individuals. Empirical data from each microplate per treatment were used to simulate naupliar production through 3 generations (i.e., 10 separate runs of the model per microplate per treatment) allowing subsequent statistical comparisons of model-derived population-growth predictions across WAF treatments and controls (Bejarano et al., 2005; Chandler et al., 2004).

2.5. Water chemistry analysis

Three freshly made 100% WAF stocks (100 mL/each) were analyzed for polycyclic aromatic hydrocarbons (PAHs) following EPA SW-846 Method 3510C. Briefly, 100 mL WAF stocks and seawater control samples were each transferred to 250 mL separation funnels followed by additions of 50 μ L of surrogate standard solutions (2-fluorobiphenyl (96%) and *p*-terphenyl-d14 (98%); 200 μ g/mL; Aldrich[®], St. Louis, MO, USA). PAHs from all solutions were extracted three times with methylene chloride (6 mL) by shaking the funnel vigorously for 2 min each time, and collecting the organic phase into a 40-mL borosilicate glass vial. The three extracts were combined and filtered through a 60° Pyrex glass funnel equipped with filter paper loaded with 2 g anhydrous sodium sulfate. Each filtered extract was collected into 40-mL vials, blown down to 1 mL with a gentle stream of nitrogen, and the remaining sample transferred to a 2-mL volumetric flask. Following the addition of 50 μ L internal standard (i.e., phenanthrene-d10) and careful mixing of the volumetric flask contents, samples were transferred to 2-mL Target I-D[™] vials and blown down with nitrogen to exactly 1 mL.

Sixteen polycyclic aromatic hydrocarbon (PAH) analytes (2–6 ring structures) were quantified based on six-point calibration curves of a standard mixture (Supelco, St. Louis, MO, USA) and 2 surrogate standards. A continuous calibration check of standards and an instrument blank sample were analyzed before and after each sample analysis. The extracts containing PAHs were analyzed using a Varian 3800 gas chromatograph (GC) coupled to a Varian Saturn 2000 MS/MS ion trap mass selective detector system (ITMS). Injection port and transfer line temperatures were set at 280 °C. The GC column oven was programmed to 50 °C (2 min hold) and ramped to 290 °C at 12 °C/min (10 min hold). The ion trap and the manifold temperature were set at 220 °C and 80 °C, respectively. A fused silica capillary column (30 m (L) × 0.25 mm (i.d.) × 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) was used to separate target PAH analytes.

2.6. Statistical analysis

Naupliar-to-copepodite and copepodite-to-adult development curves of WAFs and controls were compared using Generalized Linear Interactive Modeling, GLiM (Piegorisch and Bailer, 1997) via PROC GENMOD (SAS® Institute, Inc., Cary, NC, USA). GLiM uses generalizations of normality-based linear models (i.e., ANOVA and linear regression) to account for non-normal responses (Piegorisch and Bailer, 1997). All life-cycle bioassay endpoints were tested for normality and homogeneity of variance using the Shapiro–Wilk “goodness of fit test” and Levene test, respectively. Data failing normality were transformed accordingly. Differences in stage-specific survival, percent mating success, hatching success and total viable offspring production between WAF-exposed individuals and controls were assessed by a one-way analysis of variance (PROC GLM, SAS), and comparisons were made using Student’s paired *t*-test with the Bonferroni adjustment for multiple comparisons.

Additionally Probit Analysis (PROC PROBIT, SAS) on naupliar mortality curves over time were employed to estimate median time to lethality (LT₅₀) values (Piegorisch and Bailer, 1997). The log likelihood ratio Chi-square test statistic was used to evaluate the goodness of fit between naupliar mortality curves and the predicted Probit model.

Population-level effects of South Louisiana WAF were analyzed using population projections from individual microplates per WAF treatment and control, and with variance estimates computed at the level of

individual microplates. Data were logarithmically transformed (i.e., $\text{Log}_{10}(x+1)$), and differences in projections across South Louisiana WAFs and controls were determined by a one-way analysis of variance (PROC GLM, SAS). All tests for significance were performed using an alpha level of 0.05.

3. Results

The full life-cycle exposure (i.e., 24-h hatched nauplii to extrusion of their second broods) of the copepod *A. tenuiremis* to South Louisiana sweet petroleum crude oil water accommodated hydrocarbon fractions (WAF) lasted 32 days. Naupliar survival to the 1st copepodite stage across most WAF treatments was >75% and not significantly different from controls ($88 \pm 3\%$; $p > 0.05$; Fig. 3); however, nauplius survival in the 100% WAF was reduced by 57% relative to controls ($p < 0.001$). The only treatment showing acute, time dependent naupliar toxicity was the 100% WAF where the estimated median time to lethality (LT₅₀; Fig. 3A) was 4 days. Copepodite survival to adults, on the other hand, was >90% in most WAF treatments and only significantly reduced in the 50% WAF ($88 \pm 3\%$; $p = 0.04$; Fig. 3B) compared to control survival ($98 \pm 3\%$).

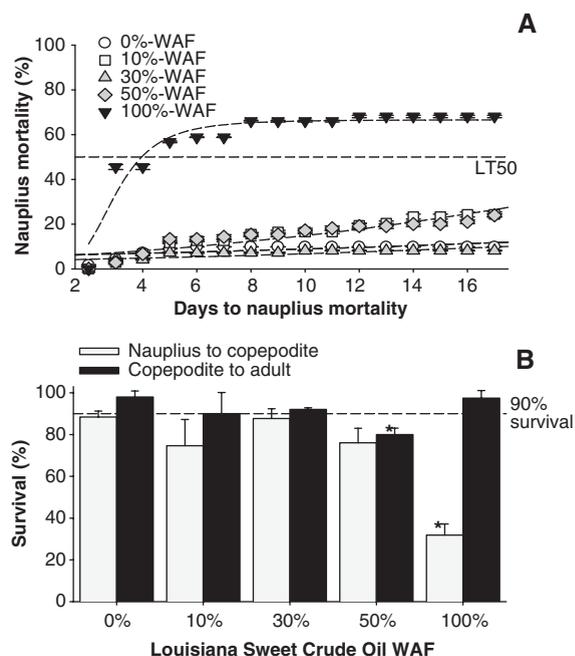


Fig. 3. Nauplius mortality through time (A) and nauplius-to-copepodite ($n = 99$ –111 nauplius/treatment) and copepodite-to-adult ($n = 31$ –98 copepodites/treatment) survival (B) in individuals exposed to South Louisiana Sweet Crude Oil water accommodated fraction (WAF). WAF included 10%, 30%, 50% and 100% WAF and seawater control (0%). *Represents significant difference ($\alpha = 0.05$) vs. control.

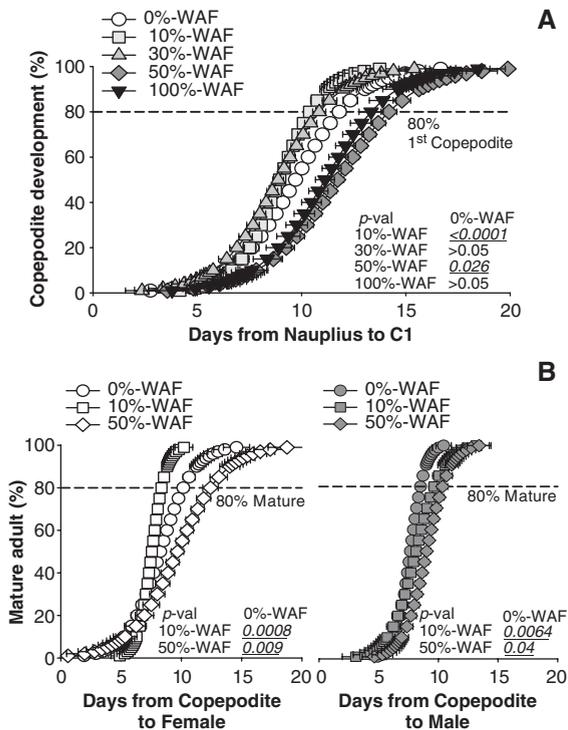


Fig. 4. Estimated nauplius-to-1st copepodite ($n=31$ –98 nauplius/treatment) (A); and copepodite to female (F) or male (M) (B) development curves of *A. tenuiremis* ($n=30$ –96 copepodites/treatment) chronically exposed to South Louisiana Sweet Crude Oil water accommodated fraction (WAF) serial dilutions. Developments into F and M in 30% and 100% WAF are not shown. p -values represent regression differences compared to controls.

Generalized Linear Interactive Modeling (GLiM) of naupliar-to-1st copepodite stage development curves estimates 10 to 14 days for 80% development across WAF dilutions. Statistical comparisons of naupliar development curves (i.e., slopes and intercepts; Fig. 4A) in the 10% and 50% WAFs were significantly different from control copepods. The majority of nauplii exposed to 10% WAF developed into copepodites

on average 46% faster, while in exposures to 50% WAF they developed on average 15% slower than controls. Consistently, the time to 80% 1st copepodites was 11.8 ± 0.3 days for control copepods, and 10.4 ± 0.3 and 14.2 ± 0.4 days for copepods exposed to 10% and 50% WAFs, respectively. Although the developmental rate of the fewer surviving nauplii in the 100% WAF was not significantly different from the control, these nauplii showed a mean 1.4 day delay in development into copepodites (80% 1st copepodites 13.3 ± 0.4 days) relative to controls.

Similar patterns were also observed in the copepodite-to-adult development window (Fig. 4B). Time to 80% copepodite development into female and male in controls were 10.2 ± 0.5 and 8.5 ± 0.3 days, respectively. Relative to controls, copepodites exposed to 10% and 50% WAF developed on average 1.8 days earlier and 2.2 days later into females, and on average 1.2 and 1.9 days later into males, respectively. Similarly, although the developmental rate into adults in the 100% WAF was not significantly different from controls, these surviving copepodites showed a mean 1.5 and 2.1 day delay in development into females and males (data not shown), respectively, compared to controls.

Female-to-male ratios were variable across microplates and not significantly different among any WAF treatment and control ($p > 0.05$). The number of mating pairs per treatment was variable across treatments, with the lowest number of pairs (7 pairs) in the 100%, resulting primarily from low nauplius-to-copepodite survival. Compared to controls, percentage of females able to produce at least two viable broods (i.e., fertilization success) was significantly decreased by 30% and 41% in exposures to 30% and 100% WAF, respectively (Table 1). Despite this decrease in fertilization success, embryo hatching and total viable production of those mating pairs successfully producing two broods was not statistically different among any WAF and control ($p > 0.05$).

Table 1

Reproductive endpoints of *A. tenuiremis* chronically exposed to South Louisiana Sweet Crude Oil water accommodated fraction (WAF) serial dilutions

WAF treatment	Fertilization success (%)	Hatching success (%)	Total viable production	Time from N-to-2nd brood extrusion (days)
0% ($n=33$)	96.67 ± 4.71	99.47 ± 3.03	18.18 ± 3.55	26.06 ± 2.32
10% ($n=29$)	76.30 ± 12.08	99.79 ± 0.95	16.09 ± 4.51	$24.3 \pm 1.98^*$ (0.005)
30% ($n=22$)	$67.20 \pm 8.43^*$ (0.03)	$92.11 \pm 12.39^*$ (0.02)	15.47 ± 5.19	25.72 ± 2.26
50% ($n=19$)	88.57 ± 8.41	96.08 ± 6.32	15.41 ± 2.75	$30.03 \pm 1.71^*$ (<0.0001)
100% ($n=7$)	$55.56 \pm 7.86^*$ (0.015)	93.06 ± 7.22	15 ± 1.22	$30.45 \pm 2.44^*$ (0.001)

* p -values (enclosed in parentheses) represent statistical difference vs. control. N=nauplius, F=female. 'n' represents the number of mating pairs per treatment.

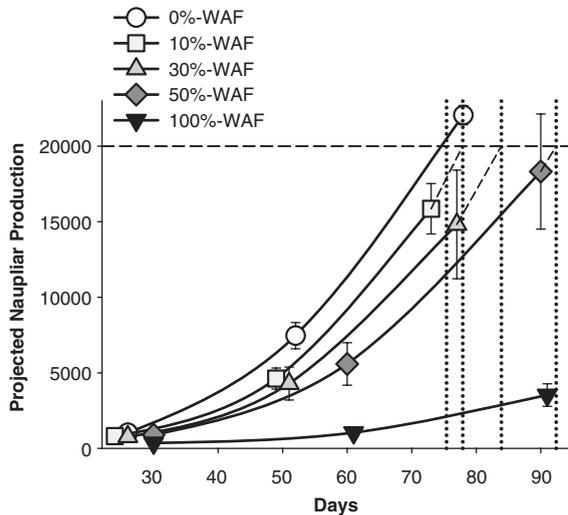


Fig. 5. Leslie model projected naupliar production through three generations in *A. tenuiremis* exposed to South Louisiana Sweet Crude Oil water accommodated fraction (WAF).

Considering the developmental times for each of the crucial life and reproductive stages (i.e., naupliar-to-1st copepodite stage, copepodite-to female, mating-to-2nd brood hatch), all WAF treatments except the 30% WAF showed a significantly different full life-cycle development time (i.e., nauplius to 2nd brood extrusion) ($p < 0.05$) compared to controls (Table 1). Individuals exposed to 10% WAF showed on average a full life-cycle development time 1.8 days shorter than controls, while individuals exposed to 50% and 100% WAF showed on average a full life-cycle development time 4 and 4.4 days longer than controls, respectively.

Table 2

Survival, developmental and reproductive effects of NIST and South Louisiana Sweet Crude Oil water accommodated fractions (WAF) on *A. tenuiremis*

Bioassay endpoints	Effect relative to control	NIST WAF (Bejarano et al., in review)	Louisiana WAF
N mortality	Increased	100%	100%
C mortality	Increased	None	50%
N to C development	Delayed	50%, 100%	50%, 100%
	Enhanced	10%	10%
C to F development	Delayed	100%	50%, 100%
	Enhanced	None	10%
C to M development	Delayed	30%, 100%	50%, 100%
	Enhanced	None	10%
Fertilization failure	Increased	None	30%, 100%
Hatching success	Reduced	100%	30%
Total life-cycle time	Shorter	10%	10%
	Longer	30%, 100%	50%, 100%
Estimated population size	Increased	10%, 30%, 50%	None
	Reduced	100%	10%, 30%, 50%, 100%

Treatments include 10%, 30%, 50% and 100% WAF and a seawater control (0% WAF). Only endpoints where differences were found vs. controls are included in the table. N=nauplius, C=copepodite, F=adult female and M=adult male.

3.1. Stage-structured population growth modeling

A staged-based Leslie matrix model (Akçakaya et al., 1999; Caswell, 2001) was used to predict potential population level responses of *A. tenuiremis* following exposures to South Louisiana crude oil WAFs. All model population projections were calculated using empirical data from each microplate per treatment, and then presented using estimated generation times (i.e., nauplius-to-2nd brood extrusion; Table 1) as comparative timeframes for projected generations F_1 , F_2 and F_3 . Since sex ratios were not influenced by WAF treatments, and were not significantly different across WAFs and control microplates, all population projections were performed assuming a 50:50 male/female ratio. Naupliar projections through three generations were significantly lower in WAF treatments than in the control ($p < 0.0001$; Fig. 5). Compared to controls, naupliar projections in the 10%, 30%, 50% and 100% WAFs across all generations were $29 \pm 7\%$, $34 \pm 7\%$, $18 \pm 5\%$ and $79 \pm 9\%$ lower, respectively. In addition, while the estimated time to reach an arbitrary carrying capacity of 20,000 individuals was 75.4 days for the control population, these estimated times were increased by 2.5, 8.5, 17 and 394 days in 10%, 30%, 50% and 100% WAF populations, respectively.

3.2. Comparisons between NIST and Louisiana WAF crude oil chronic effects

In a similar study (Bejarano et al., in press) we evaluated effects of a benchmark reference crude oil

(National Institute of Standards and Technology, NIST) WAF on *A. tenuiremis*. A summary of all the survival, developmental and reproductive endpoints where favorable or adverse effects on *A. tenuiremis* were observed following exposure to NIST and South Louisiana Sweet Crude Oil WAF is presented in Table 2. The most consistent shared effects from exposures to NIST and Louisiana WAF were delayed development in the highest WAFs and enhanced nauplius-to-copepodite development rates in the lowest WAFs. However, these and other effects were more pronounced in exposures to Louisiana WAF (Table 2). We found favorable or adverse effects in most full life-cycle endpoints evaluated in copepod exposures to Louisiana WAF.

3.3. Water chemistry analysis

NIST and South Louisiana 100% WAFs were analyzed for a total of 16 PAH analytes (2–6 rings). Percent recovery of the surrogate standards, 2-fluorobiphenyl and *p*-terphenyl-d14, was $90 \pm 3\%$ and $143 \pm 4\%$, respectively, in NIST 100% WAFs samples ($n=3$), and $79 \pm 2\%$ and $155 \pm 6\%$, respectively, in South Louisiana samples ($n=3$). Of all the 16 quantified PAHs, 6 were detected in NIST WAF, while 9 in South Louisiana WAF. Naphthalene (2 rings), phenanthrene, fluoranthene and acenaphthene comprised over 95% of the total PAHs in NIST and South Louisiana 100% WAF, with high predominance (50%) of naphthalenes. The PAHs benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, benzo [g, h, i] perylene, dibenzo [a, h] anthracene, fluorene and ideno [1,2,3-c, d] pyrene

were below instrument detection limits for individual PAHs or were not detected in NIST or Louisiana WAF. Seawater controls had non-detectable levels of PAHs. Although total PAH concentration in Louisiana WAF was six times greater ($878 \pm 28 \mu\text{g/L}$) than that in NIST WAF ($134 \pm 4 \mu\text{g/L}$), the composition and relative proportions of analytes were similar between these two WAFs (Fig. 6).

4. Discussion

Crude oil comprises a myriad of chemicals from structurally simple methane molecules to extremely large hydrocarbon molecules (>16 carbon atoms) (NRC, 1985). Chemical analysis of Prudhoe Bay, South Louisiana and Kuwait crude oils (see NRC, 1985) indicated that nearly a quarter of the total chemicals present in these crude oils were aromatic hydrocarbons (25%, 16.5% and 21.9%, respectively). Within the aromatic fraction, naphthalenes comprise 9.9%, 1.3% and 0.7%, respectively. Moreover, the aromatic hydrocarbon fraction of 10% hydrocarbon water accommodated fraction (WAF) extracted from Prudhoe Bay, South Louisiana and Kuwait crude oils was dominated by benzene and toluene (>25% and >35%, respectively), while that of 10% WAF extracted from No. 2 fuel oil and Bunker C refined oils was dominated by naphthalenes (>40%; see NRC, 1985). The same study indicated that naphthalenes comprised only 2.5% of the total aromatics present in 10% South Louisiana WAF. In the current study, although total PAH concentration in 100% South Louisiana WAF was six times greater ($878 \pm 28 \mu\text{g/L}$) than that in 100% NIST WAF

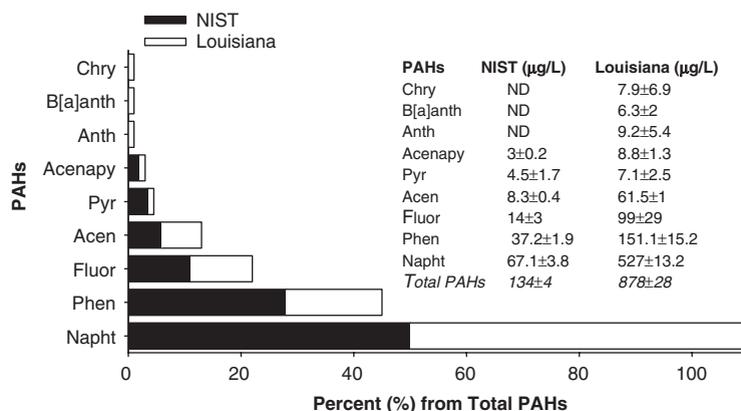


Fig. 6. PAH analysis of freshly extracted 100% water accommodated fraction (WAF) from NIST and South Louisiana Sweet Crude Oil, using EPA SW-846 Method 3510C ($n=3$ replicates per WAF). PAH analytes include: acenaphthene (Acena), acenaphthylene (Acenapy), anthracene (Anth), benzo [a] anthracene (B[a]anth), chrysene (Chry), fluoranthene (Fluor), naphthalene (Napht), phenanthrene (Phen), pyrene (Pyr). PAH analyzed but non-detected or below instrument detection limits included: benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, benzo [g, h, i] perylene, dibenzo [a, h] anthracene, fluorene and ideno [1,2,3-c, d] pyrene.

($134 \pm 4 \mu\text{g/L}$), the composition and proportion of analytes was similar between these two WAFs. In both of these fractions naphthalene was the most abundant aromatic hydrocarbon (~50% and ~60%, respectively). The proportion of naphthalene in 100% South Louisiana WAF found in this study was considerably higher than that reported elsewhere (NRC, 1985) possibly due to differences in crude oil lots, freshness, WAF extraction method and/or chemical analysis.

Earlier studies with crude oil have shown that the toxicity of soluble petroleum hydrocarbons to several *Palaemonetes pugio* life stages, particularly to larvae, was related to concentration of naphthalenes (Tatem et al., 1978). Similarly, WAFs rich in naphthalenes (i.e., refined oils) were more acutely toxic to several marine and estuarine species (*Cyprinodon variegatus*, *Menidia beryllina*, *Fundulus similis*, *P. aztecus* post-larvae, *P. pugio* and *M. almyra*) than crude oil WAFs containing higher total hydrocarbons (Anderson et al., 1974). Consistently, in the present study with naphthalene rich Louisiana crude oil WAFs, naupliar mortality in the 100% WAF and copepodite mortality in the 50% WAF were significantly greater than in controls. Interestingly, surviving copepodites exposed to 100% WAF exhibited survival rates into adults similar to controls suggesting that these individuals may be selectively more resistant to WAF acute toxicity.

This and a similar study (Bejarano et al., in press) showed consistent crude oil WAF effects on copepod development. In both studies, and particularly in exposures to Louisiana WAF, we found enhanced development (nauplius-to-copepodite and copepodite-to-adult) in the low 10% WAF, and delayed development mainly in the 50% and 100% WAFs. These effects may be the result of growth hormesis. Hormesis is a dose–response phenomenon characterized by low dose stimulation and high dose inhibition, resulting from disruption of homeostasis (Calabrese and Baldwin, 1997; Stebbing, 1982). Biological systems exposed to low levels of potentially toxic agents may exhibit an over-compensatory response resulting in apparent low-dose stimulations (i.e., growth and reproduction); while at higher doses, these systems exhibit a reduced capacity for a compensatory response (see Calabrese and Baldwin, 1997). As suggested elsewhere (Bejarano et al., in press) this over-compensatory response (i.e., enhanced growth rates) in exposures to 10% WAF may be the result of induction of cytochrome P450-dependent xenobiotic monooxygenase isozymes, which in turn may have accelerated the molt cycle (i.e., development rate) by

increasing titers of the molting regulating hormone 20-hydroxyecdysone (Mothershead and Hale, 1992; Oberdorster et al., 1999; Snyder, 1998). However, this over-compensatory response did not confer a concurrent reproductive advantage or disadvantage to fast-growing individuals.

Consistently, these enhancements/delays in development resulted in a shorter life-cycle development time in exposures to 10% WAF, while longer in exposures to 50% and 100% WAFs. Significant time delays in naupliar development and copepodite molting to reproductive maturity (i.e., 4 to 5 days total in exposures to higher WAFs) would be critical for a species with a fairly short life-span (i.e., ~38 days on average in seawater; 49 days in sediments (Green et al., 1996)). Under microplate conditions and in seawater-only exposures, a 14-day mating period of *A. tenuiremis* virgin individuals will result in the extrusion of nearly 6 clutches with 6 to 9 embryos each (Bejarano and Chandler, 2003). A 4-day delay in development would translate into 12 to 18 fewer embryos per female over a 14-day mating period in exposures to 50% and 100% WAFs. Therefore, developmental delays to maturity solely could result in significant negative effects on population growth; effects that could be further exacerbated by increased fertilization failure (i.e., 30% and 41% failure in exposures to 30% and 100% WAF, respectively). In fact, projected population level suppressions via Leslie stage matrix modeling in exposures to all Louisiana WAFs were attributed primarily to differences in early-life stage survival and developmental delays (50% and 100% WAFs), and secondarily to increased fertilization failures.

Meiobenthic fauna are highly productive and abundant in estuarine sediments where they exhibit fast turnover rates and densities of $>10^6$ individuals/m², resulting in elevated secondary production (see Giere, 1993; Platt, 1981). They also play an important role in the diets of juvenile fish such as salmon, flaxfish and spot. For instance, the diet of spot (*Leiostomus xanthurus*) is largely comprised (>50%) of harpacticoid copepods (Nelson and Coull, 1989). Estimates of meiofaunal production from various intertidal aquatic environments in the Atlantic showed productions ranging from 5.1 g C/m² year to 29.4 g C/m² year (Escaravage et al., 1989). The same study showed a meiofaunal production of 19.5 g C/m² year in estuarine mud flats. Harpacticoid copepods are estimated to contribute nearly 50% of the total meiofauna production (Danovaro et al., 2002). Within this context, the ecological consequences of the projected population level responses presented here could have serious conse-

quences for the secondary production of marine habitats holding a predominance of harpacticoid copepods such as *A. tenuiremis*.

As a final comment, the comparison between NIST and Louisiana WAFs using the 96-well microplate copepod life-cycle bioassay showed a greater toxicity of Louisiana WAF and an overall developmental delay in exposures to high WAFs from both sources. The consistency in these results for two compositionally similar but distinctively different crude oils shows the utility and reliability of this logistically simple bioassay in assessing crude oil WAF acute and chronic toxicities to a marine harpacticoid copepod.

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References

- Akçakaya, H.R., Burgman, M.A., Ginzburg, L.R., 1999. Applied Population Ecology: Principles and Computer Exercises using RAMAS EcoLab 2.0, 2nd edition. Applied Biomathematics, Setauket, NY.
- American Society for Testing Materials (ASTM), 2004. Standard guide for conducting renewal microplate-based life-cycle toxicity tests with a marine meiobenthic copepod. ASTM Standard No. E2317-04. ASTM, Philadelphia, pp. 1–16.
- Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., Hightower, G.M., 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar. Biol.* 27 (1), 75–88.
- Bejarano, A.C., Chandler, G.T., 2003. Reproductive and developmental effects of atrazine on the estuarine meiobenthic copepod *Amphiascus tenuiremis*. *Environ. Toxicol. Chem.* 22 (12), 3009–3016.
- Bejarano, A.C., Maruya, K.A., Chandler, G.T., 2004. Toxicity assessment of sediments associated with various land-uses in coastal South Carolina, USA, using a meiobenthic copepod bioassay. *Mar. Pollut. Bull.* 49 (1–2), 23–32.
- Bejarano, A.C., Chandler, G.T., Decho, A.W., 2005. Influence of natural Dissolved Organic Matter (DOM) on acute and chronic toxicity of the pesticides chlorothalonil, chlorpyrifos and fipronil to the meiobenthic estuarine copepod *Amphiascus tenuiremis*. *J. Exp. Mar. Biol. Ecol.* 321 (1), 43–57.
- Bejarano, A.C., Chandler, G.T., He, L., Cary, T.L., Ferry, J.L., in press. Risk assessment of the NIST petroleum crude oil standard water accommodated fractions (WAFs) on a meiobenthic copepod: further application of a copepod-based full life-cycle bioassay. *Environ. Toxicol. Chem.*
- Bonsdorff, E., Bakke, T., Pedersen, A., 1990. Colonization of amphipods and polychaetes to sediments experimentally exposed to oil hydrocarbons. *Mar. Pollut. Bull.* 21 (7), 355–358.
- Calabrese, E.J., Baldwin, L.A., 1997. The dose determines the stimulation (and poison): development of a chemical hormesis database. *Int. J. Toxicol.* 16 (6), 545–559.
- Carman, K.R., Fleeger, J.W., Pomarico, S.M., 1997. Response of a benthic food web to hydrocarbon contamination. *Limnol. Oceanogr.* 42, 561–571.
- Caswell, H., 2001. Matrix Population Models—Construction, Analysis, and Interpretation, 2nd edition. Sinauer Associates, Sunderland, MA.
- Chandler, G.T., Green, G.T., 1996. A 14-day harpacticoid copepod reproduction bioassay for laboratory and field contaminated muddy sediments. In: Ostrander, G.K. (Ed.), *Techniques in Aquatic Toxicology*, pp. 23–39.
- Chandler, G.T., Cary, T.L., Bejarano, A.C., Pender, J., Ferry, J.L., 2004. Population consequences of fipronil and degradates to copepods at environmental concentrations: an integration of life-cycle testing with Leslie-matrix population modeling. *Environ. Sci. Technol.* 38 (23), 6407–6414.
- Coull, B.C., Chandler, G.T., 1992. Pollution and meiofauna: field, laboratory and mesocosm studies. *Oceanogr. Mar. Biol.: Annu. Rev.* 30, 191–271.
- Danovaro, R., Gambi, C., Mirto, S., 2002. Meiofaunal production and energy transfer efficiency in a seagrass *Posidonia oceanica* bed in the western Mediterranean. *Mar. Ecol. Prog. Ser.* 234, 95–104.
- Eadsforth, C.V., 1997. Heavy fuel oil: acute toxicity toxicity of water accommodated fractions to *Oncorhynchus mykiss*, *Daphnia magna* and *Raphidocelis subcapitata*. Study conducted by Sittingbourne Research Centre. Report No. OP.97.47002. Thornton: Shell Research Ltd.
- Elmgren, R., Hansson, S., Larsson, U., och Boehm, P.D., 1983. The 'tseisis' oil spill: acute and long-term impact on the benthos. *Mar. Biol.* 73 (1), 51–65.
- Escaravage, V., Garcia, M.E., Castel, J., 1989. The distribution of meiofauna and its contribution to detritic pathway in tidal flats (Arcachon Bay, France). In: Ros, J.D. (Ed.), *Topics in Marine Biology*, Scientifica Marina, vol. 53, pp. 551–559.
- Giere, O., 1993. Meiobenthology. The Microscopic Fauna in Aquatic Sediments. Springer Verlag, Heilderberg, Germany.
- Green, A.S., Chandler, G.T., Piegorsch, W.W., 1996. Life-stage-specific toxicity of sediment-associated chlorpyrifos to a marine infaunal copepod. *Environ. Toxicol. Chem.* 15, 1182–1188.
- Gyllenberg, G., 1986. The influence of oil pollution on three copepods at Helsinki, Finland. *Ann. Zool. Fenn.* 23, 395–399.
- Hicks, G.R.F., Coull, B.C., 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. Mar. Biol.: Annu. Rev.* 21, 67–175.
- Lang, K., 1948. Monographie der Harpacticiden. Nordiska-Bokhändeln, Stockholm, Sweden.
- Linden, O., 1976. Effects of oil on the reproduction of the amphipod *Gammarus oceanicus*. *AMBIO* 5 (1), 36–37.
- Mothershead II, R.F., Hale, R.C., 1992. Influence of ecdysis on the accumulation of polycyclic aromatic hydrocarbons in field exposed blue crabs. *Mar. Environ. Res.* 33, 145–156.
- National Research Council (NRC), 1985. Oil in the Sea: Inputs, Fates, and Effects. National Academy Press, Washington, DC.
- Nelson, A.L., Coull, B.C., 1989. Selection of meiobenthic prey by juvenile spot (Pisces): an experimental study. *Mar. Ecol. Prog. Ser.* 53, 51–57.

- Oberdorster, E., Cottam, D.M., Wilmot, F.A., Milner, M.J., McLachlan, J.A., 1999. Interaction of PAHs and PCBs with ecdysone-dependent gene expression and cell proliferation. *Toxicol. Appl. Pharmacol.* 160 (1), 101–108.
- Piegorsch, W.W., Bailer, A.J., 1997. *Statistics for Environmental Biology and Toxicology*. Chapman & Hall, London, UK.
- Platt, H.M., 1981. Meiofauna dynamics and the origin of the metazoan. In: Forey, P.L. (Ed.), *The Evolving Biosphere: Chance, Change and Challenges*. Cambridge University Press, London, pp. 207–216.
- Snyder, M.J., 1998. Identification of a new cytochrome P450 family, CYP45, from the lobster, *Homarus americanus*, and expression following hormone and xenobiotic exposures. *Arch. Biochem. Biophys.* 358 (2), 271–276.
- Stark, J.S., Snape, I., Riddle, M.J., 2003. The effects of petroleum hydrocarbon and heavy metal contamination of marine sediments on recruitment of Antarctic soft-sediment assemblages: a field experimental investigation. *J. Exp. Mar. Biol. Ecol.* 283 (1–2), 21–50.
- Stebbing, A.R.D., 1982. Hormesis: the stimulation of growth by low levels of inhibitors. *Sci. Total Environ.* 22, 213–234.
- Tatem, H.E., Cox, B.A., Anderson, J.W., 1978. The toxicity of oils and petroleum hydrocarbons to estuarine crustaceans. *Estuar. Coast. Mar. Sci.* 6 (4), 365–373.
- Ustach, J.F., 1979. Effects of sublethal oil concentrations on the copepod *Nitocra affinis*. *Estuaries* 2, 273–276.