Hazard/Risk Assessment

A Comparison of Short-Term and Continuous Exposures in Toxicity Tests of Produced Waters, Condensate, and Crude Oil to Marine Invertebrates and Fish

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Abstract: Petroleum hydrocarbons can be discharged into the marine environment during offshore oil and gas production or as a result of oil spills, with potential impacts on marine organisms. Ecotoxicological assay durations (typically 24–96 h) used to characterize risks to exposed organisms may not always reflect realistic environmental exposure durations in a high-energy offshore environment where hydrocarbons are mixed and diluted rapidly in the water column. To investigate this, we adapted 3 sensitive toxicity tests to incorporate a short-term pulse exposure to 3 petroleum-based products: a produced water, the water-accommodated fraction (WAF) of a condensate, and a crude oil WAF. We measured 48-h mobility of the copepod *Acartia sinjiensis*, 72-h larval development of the sea urchin *Heliocidaris tuberculata*, and 48-h embryo survival and deformities of yellowtail kingfish *Seriola lalandi*, after exposure to a dilution series of each of the 3 products for 2, 4 to 12, and 24 h and for the standard duration of each toxicity test (continuous exposure). Effects on copepod survival and sea urchin larval development were significantly reduced in short-term exposures to produced water and WAFs compared to continuous exposures. Fish embryos, however, showed an increased frequency of deformities at elevated concentrations regardless of exposure duration, although there was a trend toward increased severity of deformities with continuous exposure. The results demonstrate how exposure duration alters toxic response and how incorporating relevant exposure duration to contaminants into toxicity testing may aid interpretation of more realistic effects (and hence an additional line of evidence in risk assessment) in the receiving environment. *Environ Toxicol Chem* 2021;40:2587–2600. © 2021 CSIRO. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Petroleum hydrocarbons; Bioassay; Early life stage; Risk assessment; Oil and Gas; Water-accommodated fraction

INTRODUCTION

Anthropogenic sources of petroleum hydrocarbons to the offshore marine environment include discharges of treated wastewater during oil and gas extraction and production and accidental releases (Neff et al. 2000; Saco-Alvarez et al. 2008; Bejarano et al. 2014; Negri et al. 2016). In an offshore oceanic environment where deep water is turbulently mixed by waves, currents, and wind, these hydrocarbons can be mixed from a surface slick into the water column, initially as entrained droplets and subsequently as dissolved constituents (Redman and Parkerton 2015). Once mixed in the water column, elevated oil concentrations may persist for only a few hours (Lee et al. 2013). Modeling and measurements taken following the *Deepwater Horizon* blowout in the Gulf of Mexico suggested residence times of petroleum products in surface waters in the order of hours, not days, with maximum persistence up to 24 h (Bejarano et al. 2013).

Produced waters are routinely released into the environment from oil and gas operations via permitted discharges (Neff et al. 2011). Produced water contains variable and sometimes elevated concentrations of low–molecular weight polycyclic aromatic hydrocarbons (PAHs); benzene, toluene, ethylene, and xylene (BTEX); metals and radionuclides from reservoir formations; as well as chemicals added in production. It is monitored for its chemical composition, and when oil content exceeds locally permitted limits, produced water is typically treated to remove the oil before being discharged into the marine environment (Neff et al. 2011). Often, produced water is discharged in varying quantities and over varying durations, and in some cases continuously, and is expected to rapidly dilute in the oceanic receiving environment (Brooks et al. 2011; Neff et al. 2011). The exact composition and volume of produced water are specific to the reservoir formation from which the resource is being extracted, and therefore will vary with production practices and over time (Brooks et al. 2011). Periodic chemical analysis and toxicity testing of the produced water discharge is often conducted to determine environmental risk, as set out by local regulatory agencies. Crude oil and condensate may enter the marine environment through accidental releases. The composition of crude oils varies depending on location, refining, and weathering conditions and can include a range of constituents from lighter hydrocarbons to higher–molecular weight compounds (Redman and Parkerton 2015). "Condensate" is defined as the hydrocarbon fraction of a gas or light crude well that is liquid at room temperature and 1 atmosphere pressure (Negri et al. 2016). The water-accommodated fractions (WAFs) of a condensate and crude oil were used to simulate the hydrocarbon content in the water column following an accidental spill, as is common practice in toxicity tests (Singer et al. 2000).

The risk that environmental contaminants, including petroleum hydrocarbons, pose to the environment is frequently evaluated using laboratory-based toxicity tests (see Bejarano et al. 2016). Marine ecotoxicity studies primarily use planktonic species, such as copepods, or planktonic juvenile life stages of taxa such as mussels, sea urchins, coral larvae, and fish (Neff et al. 2000; Saco-Alvarez et al. 2008; Negri et al. 2016). Plankton are in direct contact with contaminants in the water column, and juvenile life stages are often considered to be the most sensitive life stage. Tests use continuous exposure (typically 24–96 h) to a series of contaminant concentrations and identify the concentration at which a deleterious effect, such as mortality or developmental abnormality, is manifested (Doyle et al. 2003; Gissi et al. 2013; Angel et al. 2018). Such toxicity tests are based on standard, well-established protocols (e.g., Organisation for Economic Co-operation and Development, US Environmental Protection Agency [USEPA]), and the test duration is often chosen for logistical reasons (i.e., a typical work week allows enough time for toxicity to be observed, minimizes handling of the animals, and captures important stages of development). Standard toxicity tests with planktonic marine species may not be truly representative of the risks associated with petroleum products in an offshore environment, where exposure is likely to occur for <2 up to 24 h because of the mixing rates in high-energy environments (International Tanker Owners Pollution Federation 2011; Bejarano et al. 2013; Hodson et al. 2019). As suggested for other short-term discharge scenarios such as stormwater discharge and transient discharges (see Gordon et al. 2012), standardized toxicity tests (48–96 h) may overestimate the risk of petroleum compounds to planktonic organisms because of their comparatively long exposure times. This includes scenarios such as discharge, dispersion of oil during a spill, and the use of chemical oil dispersants as a spill response technology. Robertson et al. (2020) modeled produced water plume behavior discharged from the Chevron Wheatstone platform (northwest Australia) and found that, with local currents (0.23 m/s), it took just over 1 h for the plume to move 850 m from the platform and experience rapid dilutions, although the amount of dilution was not reported.

Previous studies have found conflicting results regarding the influence of exposure time on the toxicity of hydrocarbon compounds, with toxicity generally dependent on the individual hydrocarbon compound tested. Modeled predictions based on narcosis and baseline toxicity indicate minimal impacts from short exposures because very high concentrations would be required for organisms to acquire critical body burdens in 2 h (French-McCay 2002). A study using weathered crude oil measured a linear decrease in toxicity to fish embryos with decreasing exposure times (Greer et al. 2012). However, recent studies using dispersants and varied exposure durations have found that toxicity thresholds did not decrease in a time-proportional manner (Negri et al. 2018), and depending on the toxicokinetics of uptake and metabolism, critical body burdens may be reached in a short time (~6 h). Consequently, the extent to which standardized toxicity tests may overestimate the toxicity, and hence potential environmental impact, cannot be accurately estimated without further investigation.

The objective of the present study was to determine if toxicity following exposure to petroleum products differed in standardized testing protocols compared to short-term exposures. The aim was to adapt standardized toxicity tests using model organisms to represent a short-term pulse exposure followed by a recovery phase. We hypothesized that short-term exposures would significantly alter the magnitude of response of the test organisms. We exposed the tropical copepod *Acartia sinjiensis*, temperate sea urchin embryos of *Heliocidaris tuberculata*, and subtropical yellowtail kingfish embryos of *Seriola lalandi* to a produced water, a condensate WAF, and a crude oil WAF for different exposure durations (2, 4–12, 24 h) and continuous exposure (i.e., the toxicity test duration, according to standard protocols). These organisms were chosen based on their known sensitivity to petroleum-based hydrocarbon compounds (see Gissi et al. [2013] for copepods, Rial et al. [2013] for sea urchins, and Sweet et al. [2018] for yellowtail kingfish embryos) and because developing embryos are sensitive to petroleum products and robust to handling (Rial et al. 2013; Sweet et al. 2018). The different species chosen were also expected to have toxicological impacts via different modes of action: narcosis/baseline toxicity in all species (Di Toro et al. 2000; French-McCay 2002; Redman et al. 2012) as well as cardiotoxicity/interference with potassium pumps in fish embryos (Brette et al. 2014).

The additional modes of action in sea urchin embryos are unknown but may be related to inhibition of skeletogenesis (Sekiguchi et al. 2018).

METHODS

Preparation and chemical analysis of WAFs and produced water

Condensate from the Wheatstone facility and crude oil from Barrow Island (both northwest of Western Australia) were supplied by Chevron. For each petroleum product, WAFs (low energy) were made using standard Chemical Response to Oil Spills: Ecological Research Forum protocols (Singer et al. 2000) and prepared immediately prior to each toxicity test. Briefly, an aliquot (~5 g) of each petroleum product was collected in a glass syringe, weighed, and added to 1 L of natural filtered seawater (containing a glass pipette for siphoning) in an amber glass bottle. This was sealed with a Teflon-coated lid (with a hole to accommodate the pipette; the area around the hole was sealed with Teflon tape). The amber glass bottle was placed on a stir plate in a fume hood with a Teflon-coated stirrer bar, and the mixture was stirred for 18 to 22 h with minimal vortexing. After the allocated time, the stir plate was switched off to allow any entrained droplets to float back to the surface for at least 1 h; then, approximately 800 mL of the aqueous phase was siphoned from the bottom of the amber glass bottle, taking care to leave the oil layer intact.

The produced water was obtained from Chevron's Wheatstone Platform (collected on 6 December 2018). Aliquots of produced water had been stored according to Binet et al. (2011) for approximately 4 wk in amber glass vials with Teflon septa (no headspace), unopened at 4 °C in the dark until use in our experiments (in early January 2019) to prevent changes in produced water composition and toxicity during storage. Binet et al. (2011) demonstrated that when produced water was

stored under these conditions for 4 d, there was no change in toxicity to Microtox®. Although not published, data from our laboratory also indicate no change in toxicity if produced water is stored as described for several weeks. Each aliquot of produced water was used only once, with any surplus being discarded.

Toxicity test solutions of produced water and WAFs were prepared by dilution with filtered seawater in glass amber bottles. Seawater was collected from Oak Park, Cronulla, New South Wales, Australia, and filtered to 0.45 µm (Sartorius capsule filter with 0.65-µm prefilter) on the day of collection. It was stored at 1 to 4 °C in polyethylene carboys prior to use.

The undiluted produced water and neat condensate and crude oil, as well as an aliquot of each dilution for each test, were analyzed for BTEX, total recoverable hydrocarbons (TRHs), and total PAHs (TPAH$_{50}$) using USEPA standard methods at Chemcentre (Perth, WA). BTEX was analyzed using USEPA standard method ORG015W, TRH was analyzed using USEPA standard method ORG007W, and TPAH$_{50}$ was analyzed using USEPA ORG020WL, modified per Forth et al. (2017). The neat condensate and crude oil were also analyzed for BTEX, TRH, and TPAH$_{50}$. The total BTEX, TRH, TPAH, and total petroleum hydrocarbons (TPH; BTEX and TPAH) were calculated as the sum of detectable, measured analytes only, that is, analytes that were above the limit of reporting (LOR). Values of TPAH and TPH were used in graphical depictions of data and statistical analysis (see section, *Statistical analysis*).

Toxicity tests

Parallel toxicity tests, each with its own dilution series of petroleum product and control, were run for each exposure duration. A schematic of the exposure scenario used in all tests is shown in Figure 1. Taxa-specific test protocols are outlined in this section and in Supplemental Data, Table S1. The handling control was conducted in seawater and accounted for any

2 h exposure to FSW (control)	FSW (handling control)
2 h exposure to sample	FSW
4-6 h exposure to FSW (control)	FSW (handling control)
4-6 h exposure to sample	FSW
12-24 h exposure to FSW (control)	FSW (handling control)
12-24 h exposure to sample	FSW
Continuous exposure to FSW (control)	
Continuous exposure to sample	
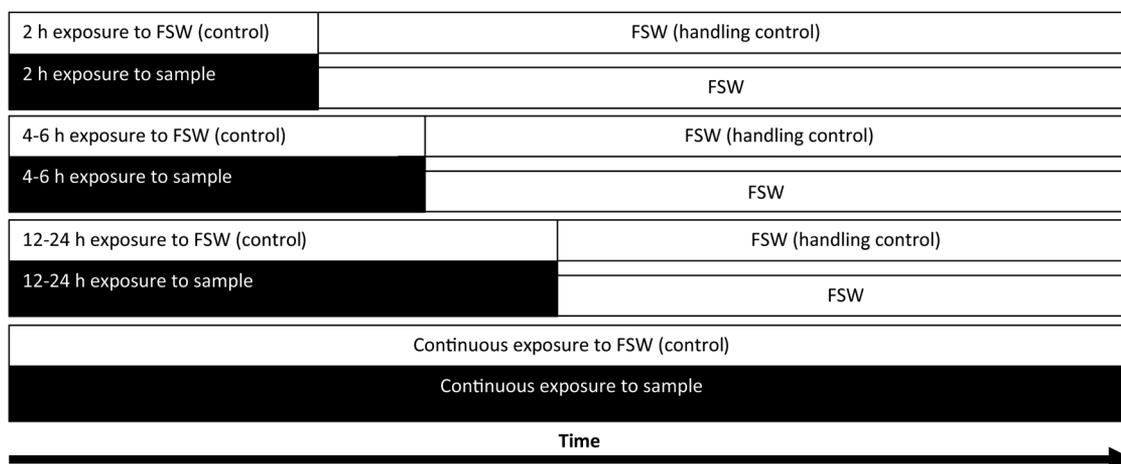
Time

FIGURE 1: A schematic diagram showing how exposure time was varied throughout the toxicity tests described for the copepod *Acartia sinjiensis*, the sea urchin *Heliocidaris tuberculata*, and the fish *Seriola lalandi* to produced water and condensate and crude oil water-accommodated fractions. Organisms were exposed to either control or treatment conditions for 2, 4 to 6, or 12 to 24 h and then transferred to filtered seawater. For comparison, a parallel treatment was run concurrently where the organisms were not transferred to new media during the assay. FSW = filtered seawater.

effects on the test organisms due to physically transferring them between the pulse and recovery solutions.

Copepod. This test protocol followed the method described in Gissi et al. (2013) and utilized the same culture of test organisms. This test assesses mobility (defined as forward swimming motion) in adult tropical copepods over a 48-h exposure in an incubator at $30 \pm 1\,°C$. Briefly, 5 adult copepods were added to one of 4 replicate vials per treatment (giving a total of 20 copepods per treatment) containing 20 mL of test solution and algal food (Gissi et al. 2013). At the end of the short-term exposure period (2 and 5 h for produced water and crude oil WAF, 2 and 4 h for condensate WAF), copepods were filtered onto a 30-µm cell strainer (Pluristrainer; Pluriselect), gently washed with filtered seawater (see section, *Preparation and chemical analysis of WAFs and produced water*), and then transferred into new test vials with filtered seawater and algal food. Mobile copepods were counted before and following transfer as well as at 24 and 48 h, following the standard protocol. Water quality (temperature, pH, salinity, conductivity, dissolved oxygen) was measured on day 0 in the filtered seawater prior to transferring organisms and in all test solutions at 48 h (test completion). Test parameters and conditions as well as a summary of physicochemical data are presented in Supplemental Data, Table S1.

Sea urchin. The sea urchin test was modified from Doyle et al. (2003). This test assesses larval development of temperate *H. tuberculata* following 72-h exposure in a temperature-controlled laboratory at $20 \pm 1\,°C$. Adult *H. tuberculata* were collected from South Maroubra, Sydney, Australia (permit P19/0014-1.0, Department of Primary Industries) and returned to the collection site on the same day. Spawning was induced by injecting potassium chloride (0.5 M KCl) through the peristomal membrane on the oral surface of adults. Gamete viability from one male and one female was assessed under the microscope at ×40 magnification (Olympus BX53; Olympus). Sperm quality was confirmed by the presence of motile sperm and egg quality, identified by the ability of healthy sperm to fertilize 90% of the eggs within 10 min of combining gametes. Sperm and egg densities were determined using a hemocytometer and a Sedgwick-Rafter cell, respectively and a final sperm to egg ratio of 100:1 was used to fertilize the egg solution (2000 eggs/mL) and ensure that >90% of eggs were fertilized. Approximately 500 fertilized eggs were added to each 20 mL of test solution in each of 4 replicate glass vials (20 mL) with Teflon-lined screw lids. For the short-term exposure, following 2 h, embryos were collected on 30-µm cell strainers, gently washed with filtered seawater, and then transferred into new 20-mL glass test vials with filtered seawater for the remaining 70 h of the test duration. The continuous exposure had no water renewals and was terminated at 72 h. Water quality (temperature, pH, salinity, conductivity, dissolved oxygen) was measured on day 0 in the filtered seawater prior to transferring organisms and in all test solutions at 72 h. A summary of the physicochemical data is presented in Supplemental Data, Table S1. After 72 h, all vials were fixed with buffered formalin

to reach a final concentration of 2.5% v/v formalin, and the proportion of normal larvae (first 100 observed under a microscope) was determined and expressed as a percentage of the control. Normal larvae had reached the pluteus stage of development, exhibited good symmetry, and had well-developed skeletal rods and a differentiated gut (Supplemental Data, Figure S1; Doyle et al. 2003). Test parameters and conditions are described in Supplemental Data, Table S1.

Fish. Experiments with fish embryos were conducted under CSIRO Animal Ethics Committee permit 2018-24 at the TAFE Maritime Aquaculture Facility (Perth, WA) using embryos of the yellowtail kingfish (also known as the amberjack), *S. lalandi*. Embryos were collected as soon after fertilization as feasible (typically < 6 h) and separated for viability gravimetrically. Viability of the brood was confirmed microscopically, and toxicity tests started when embryos were at the gastrula stage (Moran et al. 2007). Seawater used in test setup and control/diluent water was collected from the Indian Ocean Marine Research Centre in Perth, passed through sand filters, and stored at 4 °C in the dark. Twenty embryos were placed into glass beakers with treatment solutions (200 mL) for varying durations (2, 6, 12, 24, or 48 h) and then transferred to solutions with seawater by pipetting. There were 5 replicates per treatment. One set of exposures (2, 6, and 24 h for the condensate WAF and produced water and 2, 12 and 24 h for the crude oil WAF) were conducted alongside a continuous exposure (48 h) in a temperature-controlled room at 20 to 22 °C. Water quality (temperature, pH, dissolved oxygen) was measured daily. A summary of the physicochemical data is presented in Supplemental Data, Table S1. Immediately after hatching, approximately 48 h after the exposures commenced, survival was counted (mortality was determined by discoloration and inhibited development of embryos), and 5 surviving embryos were visualized stereoscopically, with deformities recorded. Selected embryos were photographed to further document malformations (as shown in Supplemental Data, Figure S2), per Sweet et al. (2018). At the completion of counting, all surviving fish were euthanized using clove oil. Test parameters and conditions are described in Supplemental Data, Table S1.

Statistical analysis

Statistical analysis to determine toxicity estimates was carried out using the free software R (Ver 3.6.0; R Development Core Team 2019) and R Studio (Ver 1.2.1335) with the drc package (Ritz et al. 2015). For simplicity, concentration–response curves were plotted for each treatment using measured TPAH concentrations. Toxicity estimates (effect concentrations to cause 10 and 50% effect relative to the control [EC10 and EC50, respectively]) were calculated for nominal (percentage) concentrations of produced water and using TPAH and TPH (micrograms per liter) concentrations for produced water, the condensate WAF, and the crude oil WAF using the Weibull 1.3 model. Hypothesis testing was performed using Sigmaplot, Ver 14.0. Following a check for normality and homogeneity of variance, analysis of variance

(ANOVA) with Tukey's post hoc test was used to determine significant differences ($p < 0.05$) at each of the test concentrations following the short-term and continuous exposures. When the assumptions of normality or equal variance was not met (i.e., fish data), a nonparametric Kruskal-Wallis ANOVA on ranks, followed by Tukey's multiple pairwise comparison was performed. Statistical outputs are presented in Supplemental Data, Table S2.

RESULTS

Chemical analysis of produced water and WAFs

Chemical analyses of the undiluted produced water and neat condensate and crude oil are shown in Supplemental Data, Table S3. The composition of the condensate and crude oil is shown in Supplemental Data, Figure S3. The BTEX component of the 2 products was largely similar (Supplemental Data, Figure S3A). The TRHs had a greater proportion of high–molecular weight components in the crude oil than in the condensate (Supplemental Data, Figure S3B). In accordance with this, more substituted and high–molecular weight PAHs were present in the crude oil than in the condensate (Supplemental Data, Figure S3C). These differences in high–molecular weight compounds account for the differences in solubility of the 2 products in seawater (WAFs and diluted toxicity test solutions), with the solubility of PAHs generally decreasing with increasing molecular weight. The results from the chemical analysis of the diluted produced water and the condensate and crude oil WAF are presented in Supplemental Data, Table S4. The produced water was derived from the same production facility as the condensate. The ratios of individual PAHs within all 3 petroleum products were very similar, with alkylated naphthalenes being the predominant compounds measured in all solutions (Supplemental Data, Figure S3C and Tables S3 and S4). A summary of the chemical analysis for total BTEX, TRH, TPAH, and TPH (TPAH + BTEX) concentrations used in the toxicity test solutions is presented in Table 1. For some sample dilutions, concentrations of BTEX, TRH, and TPAH differed between the tests for each species. This could be a result of experiments being conducted in different laboratories over a couple of months; it is expected that the composition of WAFs will vary slightly each time it is made. In addition, we cannot exclude the possibility of oil droplets.

Feasibility of short-term exposure durations

All toxicity tests with copepods and sea urchins met the acceptability criteria for control mobility in the copepods (>90%) and normal development (>70%) in the sea urchins (Supplemental Data, Table S1). Similar acceptability criteria for the species of fish used in the present study have not yet been established. For all tests and exposure durations, physicochemical parameters remained within set ranges (Supplemental Data, Tables S1 and S6).

For the control treatments (filtered seawater), there was no significant difference between the handling control (short-term exposure) and the continuous controls (standard toxicity test duration) for any of the species and test endpoints measured (Table 2). This demonstrated the feasibility of the short-term exposure durations and that the methods of transferring organisms (and the additional handling) did not influence the sensitivity of the test organism endpoints measured during the toxicity tests.

Differences in toxicity between short-term and continuous exposures

For simplicity, the responses of copepod, sea urchin, and fish following exposures to produced water, condensate WAF, and crude oil WAF were plotted against the TPAH concentrations (Table 1) measured in each test treatment so that the influence of exposure duration on toxic response could be readily visualized. Toxicity estimates were calculated based on TPAH and TPH. Concentration–response models are provided in Supplemental Data, Figures S4 through S6. Because TPAHs and TPHs in solution may not be the only cause of toxicity observed in each sample, other constituents of the oil/condensate/produced water may be contributing as well, but these were not investigated further for BTEX and TRHs because the measured concentrations of these compounds were below the LOR in many of the lower treatments tested.

Copepod. No toxic effects were observed on copepods following exposure to the crude oil WAF (Figure 2B) at any exposure concentration or duration (Kruskal-Wallis one-way ANOVA on ranks, $p > 0.05$). This is most likely due to the low solubility of the crude oil. However, exposure duration influenced the toxic response observed in copepods for both the condensate WAF and the produced water.

Following exposure to the condensate WAF, toxicity was significantly reduced (Kruskal-Wallis one-way ANOVA on ranks, $p < 0.05$) in 2- and 4-h exposures at TPAH concentrations ranging from 5.4 to 96 µg/L, compared to the continuous 48-h exposure to the same TPAH concentrations (Figure 2A; Supplemental Data, Table S5). Mobility of copepods (as percentage of control) was >70% at all concentrations of condensate WAF tested (Figure 2A), and there were no significant decreases in mobility between 2- and 4-h exposures (Kruskal-Wallis one-way ANOVA on ranks, $p > 0.38$). Continuous exposure to the same condensate WAF solutions caused a significant decrease in mobility (Kruskal-Wallis one-way ANOVA on ranks, $p < 0.001$); at TPAH concentrations ≥73 µg/L, all copepods were immobile (i.e., 0% mobility).

Following 2- and 5-h exposures to all concentrations of produced water, toxicity was significantly reduced at exposure concentrations ≥16 µg/L TPAH (Kruskal-Wallis one-way ANOVA on ranks, $p < 0.026$) compared to the continuous exposure (Figure 2C; Supplemental Data, Table S5). Mobility of copepods exposed to produced water was ≥60% (compared to controls) at all concentrations in the short-term exposures and was not statistically significantly different between 2- and 5-h exposures (Kruskal-Wallis one-way ANOVA on ranks, $p > 0.15$; Figure 2C). Continuous exposure to produced water caused a reduction in

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TABLE 1: Concentrations of benzene, toluene, ethylene, and xylene (BTEX), total recoverable hydrocarbons (TRH), total petroleum hydrocarbons (TPAHs), and total petroleum hydrocarbons (BTEX, TPAH) measured in the test concentrations for each species (copepod, sea urchin, and fish)[a]

Taxa	Product	Nominal exposure concentration (%)	BTEX (µg/L)	TRH (µg/L)	TPAH (µg/L)	TPH (µg/L)
Copepod, *Acartia sinjiensis*	Produced water	0.1	<LOR	<LOR	0.04	0.04
		0.5	<LOR	25	0.15	0.15
		1	<LOR	140	7.2	7.2
		3	20	952	16	36
		6	64	2553	41	105
		10	95	3920	59	154
	Condensate WAF	0.1	<LOR	<LOR	0.09	0.09
		0.5	<LOR	27	0.25	0.25
		1	108	1080	5.4	113
		5	448	4500	73	523
		10	604	4500	75	700
		25	941	4910	96	1014
	Crude oil WAF	1	<LOR	<LOR	0.07	0.07
		5	<LOR	<LOR	0.19	0.19
		10	<LOR	28	0.33	0.33
		20	53	170	16	69
Sea urchin, *Heliocidaris tuberculata*	Produced water	0.1	<LOR	<LOR	0.08	0.08
		1	<LOR	98	2.7	2.7
		3	13	690	15	28
		6	36	1701	42	78
	Condensate WAF	0.5	<LOR	<LOR	0.03	0.03
		1	<LOR	<LOR	0.20	0.20
		5	144	227	1.9	146
		10	277	443	3.5	280
		20	645	1040	13	658
	Crude oil WAF	0.1	<LOR	<LOR	0.04	0.04
		1	<LOR	<LOR	0.10	0.10
		10	<LOR	46	0.59	0.59
		20	38	261	1.3	39
Fish, *Seriola lalandi*	Seawater		<LOR	<LOR	<LOR	<LOR
	Produced water	0.1	<LOR	<LOR	0.003	0.003
		0.5	<LOR	130	0.39	0.39
		1	<LOR	610	1.1	1.1
		5	38	756	3.7	42
	Condensate WAF	0.5	<LOR	43	0.02	1.7
		1	15	128	0.37	18
		5	102	296	1.7	102
		10	397	599	3.1	397
	Crude oil WAF	0.5	<LOR	<LOR	0.04	0.04
		1	2	<LOR	0.04	2.0
		5	116	437	0.13	116
		10	287	1526	0.41	287

[a]Samples for analysis were collected from bulk treatment solution at test initiation ($n = 1$ per test concentration), $t = 0$; TPAH and TPH concentrations presented in Table 1 were used in all subsequent statistical analyses. The limits of reporting were 1 µg/L for each of the BTEX compounds, 25 µg/L for TRHs C6 through C10 and C10 through C16, and 100 µg/L for TRHs C16 through C34 and C34 through C40. For polycyclic aromatic hydrocarbons, the levels of reporting were 0.001 µg/L for the parent compounds 1-methylnaphthalene and 2-methylnaphthalene, 0.005 µg/L for the C2 and C3 alkylated compounds, and 0.01 for the C4 alkylated compounds.
BTEX = benzene, toluene, ethylene, and xylene; TRH = total recoverable hydrocarbons; TPAH = total polycyclic aromatic hydrocarbons; TPH = total petroleum hydrocarbons; LOR = limit of reporting; WAF = water-accommodated fraction.

mobility relative to controls (Kruskal-Wallis one-way ANOVA on ranks, $p < 0.001$) at concentrations ≥41 µg/L (Tukey's multiple pairwise comparison test, $p < 0.037$).

Because toxic responses were only significantly different from the control following continuous exposure to condensate WAF and produced water, toxicity estimates (EC10/50) were only calculated to compare the potency for continuous exposure to these products. Based on TPAH concentrations, the condensate WAF was more toxic than the produced water, with respective EC50 values of 7.9 (3.9–12) and 16 (12–20) µg TPAH/L (Table 3). Toxicity estimates based on TPH could not be reliably calculated for the condensate WAF; however, the EC50 value for the produced water was 31 (19–44) µg TPH/L.

Sea urchin larvae. Exposure duration influenced the toxic response observed in sea urchin larvae. All 3 petroleum products caused toxicity, with increasing concentration in the continuous exposure (Figure 3). Toxicity was substantially reduced in all 3 samples following the shortened exposure duration at the highest 2 to 3 concentrations tested relative to the continuous exposure (Figure 3).

Following a 2-h exposure, normal larval development was ≥69% (as percentage of control) in all concentrations of condensate WAF tested, although the mean ($n = 4$) measured percentage of sea urchins with normal development to the pluteus phase (80%) at a TPAH concentration of 0.27 µg/L was significantly less than control (ANOVA followed by Holm-Sidak pairwise

TABLE 2: Range of control mobility, normal larval development, and survival for the copepod, sea urchin, and fish tests, respectively, in the handling experiments (over short-term, 2–24 h, exposure) and continuous (standard test duration) exposures

Taxon (endpoint)	n	Exposure duration (pulse)				
		2 h	4–6 h	12 h	24 h	Continuous
Copepod (mobility)	8[a]	95–100%	95–100%	NA	NA	95–100% (48 h)
Sea urchin (normal larval development)	8[a]	90–94%	NA	NA	NA	92–96% (72 h)
Fish embryo (survival)	5	64–95%	50–100%	50–100%	78–100%	60–100% (48 h)

[a]Data compiled from 2 separate experiments, with 4 replicate controls within each experiment.
NA = not applicable, not tested at these exposure durations.

comparison, $p = 0.012$). Toxicity following 2-h exposures was significantly reduced relative to continuous exposures following 2-h exposures at TPAH concentrations ranging from 1.9 to 13 µg/L (Welch's t test, $p < 0.001$; Figure 3A). The proportion of normally

developed sea urchin larvae following continuous exposure to condensate WAF for 72 h was significantly different from the control (Kruskal-Wallis one-way ANOVA on ranks, $p > 0.001$), with TPAH concentrations between 1.9 and 13 µg/L causing 60 to 96%
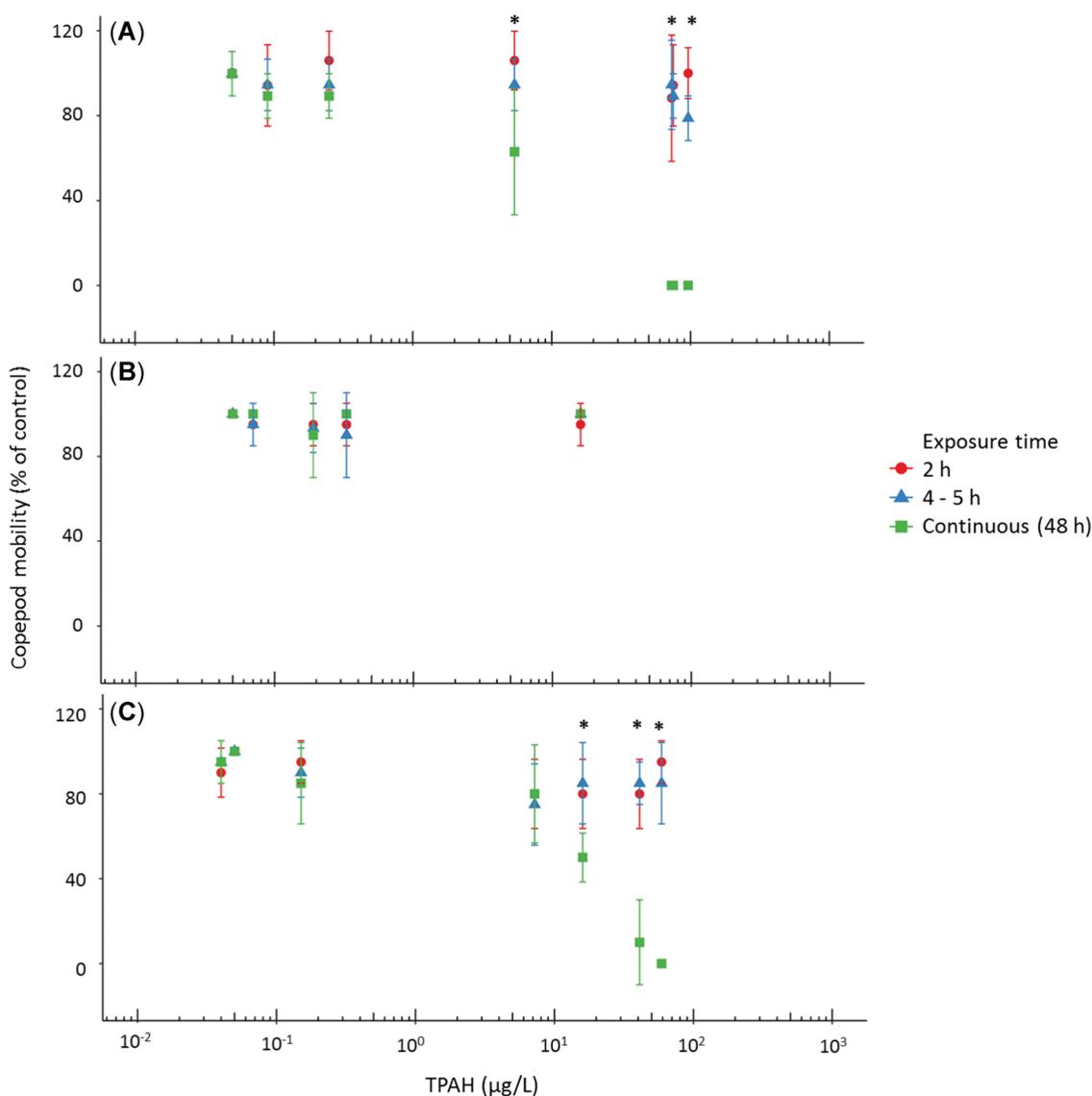
FIGURE 2: Effect of test duration on mobility of the marine adult copepod (*Acartia sinjiensis*) when exposed to condensate water-accommodated fraction (WAF; **A**), crude oil WAF (**B**), and produced water (**C**) as measured by total polycyclic aromatic hydrocarbons (TPAH) for 2, 4 to 5, or 48 h (continuous). Means and 1 standard deviation ($n = 4$) are plotted; for graphical representation on the log scale, controls were set to 0.05 µg TPAH/L. Exposure times for produced water and crude oil WAFs were 5 h, and condensate WAF exposure was 4 h; but they are grouped together for ease of comparison in Figure 2 as 4 to 5 h. * Indicates statistically significant difference in effect at TPAH concentration between the short-term (2- or 4- to 5-h) and the continuous exposures.

TABLE 3: Toxicity of produced water, condensate water-accommodated fraction (WAF), and crude oil WAF to the copepod, sea urchin, and fish[a]

Species	Endpoint	Product		Toxicity estimate (±95% CI)	
				EC10	EC50
Copepod, *Acartia sinjiensis*	48-h mobility	PW	Nominal (%)	0.56 (0.15–0.97)	2.6 (1.9–3.3)
			TPAH (µg/L)	4.0 (1.2–6.7)	16 (12–20)
			TPH (µg/L)	4.2 (0–9.2)	31 (19–44)
		Condensate WAF	TPAH (µg/L)	0.61 (0–1.4)	7.9 (3.9–12)
		Crude oil WAF	TPAH (µg/L)	NC	>16[b]
Sea urchin, *Heliocidaris tuberculata*	72-h larval development	PW	Nominal (%)	0.69 (0.40–0.98)	2.3 (2.0–2.6)
			TPAH (µg/L)	1.5 (0.53–2.6)	9.9 (7.7–12)
			TPH (µg/L)	1.5 (0.18–2.9)	16 (11–20)
		Condensate WAF	TPAH (µg/L)	0.05 (0.01–0.10)	1.4 (1.0–1.8)
			TPH (µg/L)	NC	143 (79–208)
		Crude oil WAF	TPAH (µg/L)	0.02 (0.01–0.04)	0.49 (0.40–0.58)
			TPH (µg/L)	0.04 (0–0.09)	0.41 (0.20–0.63)
Fish, *Seriola lalandi*[b]	24-h pericardial edema	Condensate WAF	TPAH (µg/L)	0.01 (0–0.03)	1.2 (0.63–1.8)

[a]Toxicity estimates were calculated based on continuous exposure to nominal concentrations (percentage, for produced water only), and measured concentrations of total polycyclic aromatic hydrocarbons and total petroleum hydrocarbons. Estimates were calculated using the Weibull model 1.3 in the drc package in R Studio; these models are presented in Supplemental Data, Figures S4 through S6. For each species, data are from one individual experiment, with 4 replicates per treatment for the copepod and urchin and 5 replicates per treatment for the fish. Values in parentheses are 95% confidence limits. If results have not been presented, reliable estimates could not be calculated.
[b]Fish embryo data were highly variable for all endpoints, and reliable estimates could only be calculated for the condensate WAF.
[c]Highest concentration tested.
EC10/EC50 = 10/50% effect concentrations; PW = produced water; CI = confidence interval; TPAH = total polycyclic aromatic hydrocarbons; TPH = total petroleum hydrocarbons (benzene, toluene, ethylene, and xylene; TPAH); NC = not calculated.

reduction in normal larval development (Supplemental Data, Table S5).

More than 80% of larvae developed normally in all concentrations of crude oil WAF tested following 2-h exposure, and they were not significantly impaired relative to the control development (Kruskal-Wallis one-way ANOVA on ranks, followed by Tukey's method for multiple pairwise comparison, $p = 0.06$; Figure 3B; Supplemental Data, Table S5). However, toxicity increased relative to the control with increasing crude oil concentration in the continuous exposures (Kruskal-Wallis one-way ANOVA on ranks, followed by Tukey's method for multiple pairwise comparison, $p < 0.006$; Figure 3B). Toxicity was significantly reduced following 2-h compared to continuous exposure at TPAH concentrations ranging from 0.59 to 1.3 µg/L (t test, $p < 0.0001$; Figure 3B).

At all concentrations of produced water tested, normal larval development was ≥82% following 2-h exposure; however, there was a slight but significant difference in the proportion of normal development at TPAH concentrations >2.7 µg/L (ANOVA, followed by Holm-Sidak pairwise comparison, $p < 0.017$; Supplemental Data, Table S5). Toxicity was significantly reduced in short-term compared to continuous exposures at TPAH concentrations ranging from 15 to 42 µg/L (Welch's t test, $p < 0.001$; Figure 3C). The proportion of normally developed sea urchin larvae was much lower for those continuously exposed to high concentrations of produced water, as shown in Figure 3C. Development was significantly impaired in the continuous exposure at test concentrations of 15 and 42 µg TPAH/L (Kruskal-Wallis one-way ANOVA on ranks, followed by Tukey's method for multiple pairwise comparison, $p < 0.04$), with a mean proportion of normal development of 4% at the highest concentration (42 µg TPAH/L; Figure 3C).

The EC10 and EC50 values were calculated for continuous exposure to TPAH and TPH concentrations. The EC50 values were 9.9 (7.7–12), 1.4 (1.0–1.8), and 0.49 (0.40–0.58) µg TPAH/L for the produced water, condensate WAF, and crude oil WAF, respectively (Table 3). Reliable estimates could not be calculated for the condensate WAF, based on TPH concentration; however, the EC50 values for the produced water (16 [11–20] µg TPH/L) and crude oil WAF (0.42 [0.20–0.63] µg TPH/L), based on TPH were similar to values calculated using TPAH.

Fish. Posthatch mortality was highly variable in fish embryos at all exposure durations (Supplemental Data, Table S5). Exposure to different petroleum products resulted in different frequencies of deformities, as shown in Figure 4, and further explained in this section.

Exposure to the condensate WAF did not have much influence on the incidence of spinal curvature (ANOVA, $p > 0.05$; Figure 4D). However, incidence of pericardial edema increased at all exposure durations (2-way ANOVA, $p < 0.01$), with 3.1 µg TPAH/L causing cardiotoxicity after 2- and 24-h exposures (Kruskal-Wallis one-way ANOVA on ranks, followed by Tukey's method for multiple pairwise comparison, $p = 0.33$ and 0.019, respectively) and concentrations >1.7 µg TPAH/L causing toxicity following continuous exposures (Kruskal-Wallis one-way ANOVA on ranks, followed by Tukey's method for multiple pairwise comparison, $p < 0.005$; Figure 4A).

Exposure to the crude oil WAF also increased the incidence of pericardial edema (Figure 4B) but not spinal curvature (Figure 4E). At exposure durations of 24 h or longer, incidence of pericardial edema increased slightly (but significantly; ANOVA, followed by Holm-Sidak pairwise
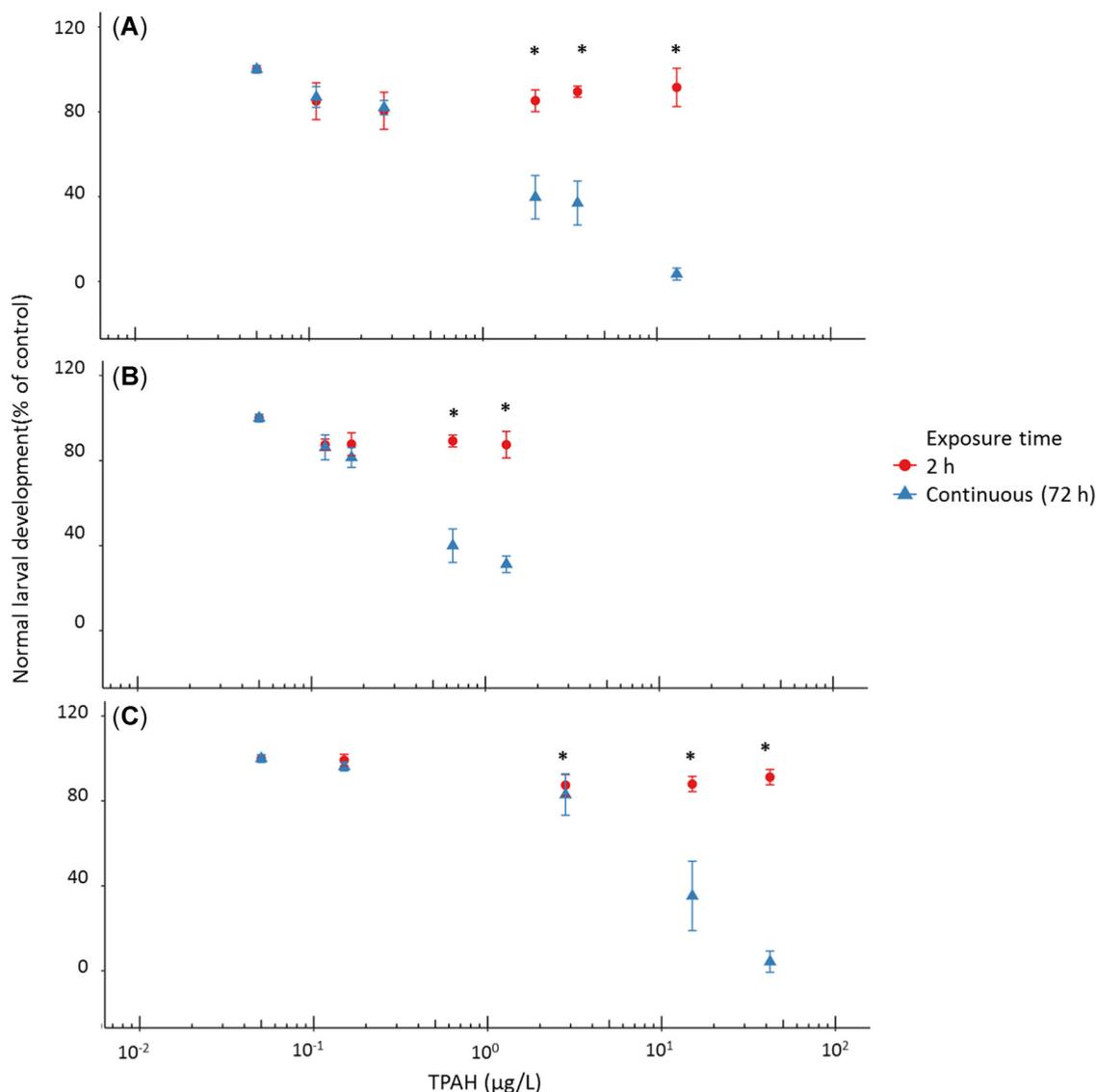
FIGURE 3: Effect of test duration on normal larval development of the sea urchin (*Heliocidaris tuberculata*) when exposed to condensate water-accommodated fraction (WAF; **A**), crude oil WAF (**B**), and produced water (**C**) as measured by total polycyclic aromatic hydrocarbons (TPAH) for 2 or 72 h (continuous). Means and 1 standard deviation (*n* = 4) are plotted; for graphical representation on the log scale, controls were set to 0.05 µg TPAH/L. *Indicates statistically significant difference in effect at TPAH concentration between the short-term (2-h) and the continuous exposures.

comparison, *p* < 0.028) in fish embryos exposed to 0.37 µg TPAH/L from crude oil WAF.

Exposure to produced water caused a trend, though insignificant, toward increased incidence of edema (Figure 4C). Exposure to produced water also caused increased incidence of spinal curvature (Figure 4F), following 2-h exposure to 3.1 µg TPAH/L (ANOVA, followed by Holm-Sidak pairwise comparison, *p* < 0.010); however, this response was not concentration-dependent; that is, the incidence of spinal curvature did not increase with increasing TPAH concentration. At the 6- and 24-h exposures, all exposure concentrations (>0.02 µg TPAH/L) caused toxicity (ANOVA, followed by Holm-Sidak pairwise comparison, *p* < 0.006; Figure 4C). For the continuous exposure, spinal curvature in only the 1.7 µg TPAH/L concentration was significantly different compared to control, though these results may be confounded by the variable

survival at this exposure duration (Kruskal-Wallis one-way ANOVA on ranks, followed by Tukey's method for multiple pairwise comparison, *p* < 0.01).

Toxicity estimates could only be calculated for the pericardial edema endpoint and only for continuous exposure to the condensate WAF. The EC50 value was 1.2 (0.63–1.8) µg TPAH/L (Table 3).

DISCUSSION

In the present study, we have demonstrated the feasibility of performing toxicity tests with shortened exposure durations, although these tests are more logistically challenging because of the handling and transferring of organisms within the standard test duration. We also note that only one or 2 experiments were conducted for each organism (and only one per sample,
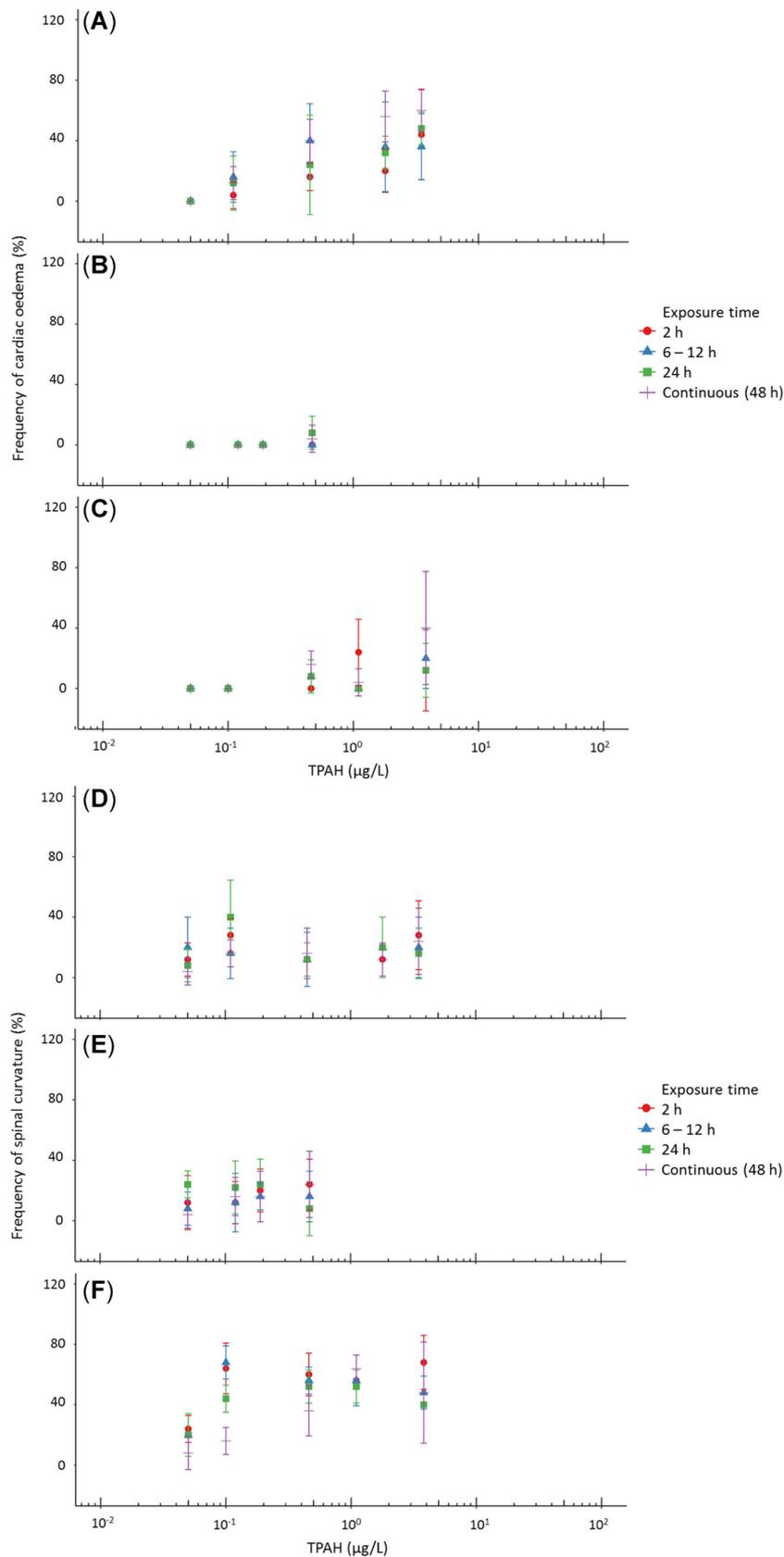
FIGURE 4: Effect of exposure duration on frequency of pericardial edema (**A–C**) and spinal curvature (**D–F**) of embryonic fish (*Seriola lalandi*) when exposed to condensate water-accommodated fraction (WAF; **A,D**), crude oil WAF (**B,E**), and produced water (**C,F**) as measured by total polycyclic aromatic hydrocarbons (TPAHs) for 2, 6 to 12, 24, or 48 h (continuous). Means and 1 standard deviation (*n* = 5) are plotted; for graphical representation on the log scale, controls were set to 0.05 μg TPAH/L. Exposure times for produced water and condensate WAFs were 6 h, and crude oil WAF exposure was 12 h; but they are grouped together for ease of comparison in Figure 4 as 6 to 12 h.

produced water, condensate WAF, and crude oil WAF), and we suggest further refinement and test development before these procedures involving short-term exposures can be adapted as standard procedures and used in routine risk assessments.

Changing the exposure duration did not impact control response of copepod mobility, sea urchin larval development, and fish embryo hatching survival and development (Table 2). Our results also clearly show that toxicity of 3 petroleum-based hydrocarbon products is often dependent on exposure duration for the organisms tested and that at exposure times that simulate environmentally realistic single exposure times for high-energy offshore environments (e.g., 2 up to 24 h) we did not observe statistically decreased mobility of copepods or impaired development in sea urchins relative to controls. The lack of a significant toxic response, even at high concentrations, following short exposures to contaminants followed by a recovery period is likely attributable to the slow uptake of PAHs and other analytes from solution (French-McCay 2002). Also, only adult copepods and sea urchin fertilized eggs (first 2 h of development) were exposed to the petroleum products, and these may not represent the most sensitive life stages for these organisms. However, for fish embryos, we observed significant deformities when exposed to produced water and condensate WAF even following short-term exposures.

Our study is not the first to explore the influence of exposure duration on toxic responses, though to our knowledge, it is the first to investigate the difference in short-term (2–24 h) and continuous (48–72 h) exposures to petroleum products, with the aim to better simulate the offshore environment (Hodson et al. 2019). Previous studies have found that rapid movement and dilution of petroleum products are likely to occur in the offshore environment (Robertson et al. 2020), and hence exposure times are likely to be in the range of <2 up to 24 h (International Tanker Owners Pollution Federation 2011; Bejarano et al. 2013). However, this will vary depending on the product (condensate, crude or produced water), the rate of release and the receiving environment (International Tanker Owners Pollution Federation 2011). Previous studies have examined either pulse (Angel et al. 2018) or episodic (reviewed in Gordon et al. [2012]) exposures as modifications to standardized toxicity tests. These studies have aimed to simulate stormwater discharge or episodic discharges (Gordon et al. 2012; Angel et al. 2018). These studies have generally, but not always, found that shortened exposure duration reduced the toxic impact (reviewed in Gordon et al. [2012]). Some of this work has found that variable exposure durations to metals, which are bioaccumulated quickly (within 1 h in some instances [Quigg et al. 2006]), are best simulated using time-averaged concentrations (Angel et al. 2018). Because of the slow kinetics of PAH uptake, with some high–molecular weight compounds taking >96 h to come to equilibrium in organism tissues (French-McCay 2002) as well as the metabolism of PAHs by some organisms (Whyte et al. 2000), a time-averaged concentration may not be appropriate for use with petroleum substances and would need to be carefully

validated, including by measuring critical body burden, which is beyond the scope of the present study. In addition, it should be noted that multiple pulse exposures of varying concentrations could occur as planktonic organisms swim (vertically or horizontally) and a plume moves with wave and ocean current. Pulse exposures could occur to organisms at different stages of development, and this could alter the toxic response (Vikebø et al. 2013).

The toxic response to the 3 petroleum products was different for each organism tested, most likely driven by the differences in the composition of the PAHs and hence in their solubilities (Di Toro et al. 2000; McGrath et al. 2005, 2018; Redman et al. 2012), although it is not certain that PAHs are the only contributors to oil toxicity (Meador and Nahrgang 2019) and nonaromatics such as ammonia, phenols, and metals could also contribute to the observed toxicity. The condensate was more soluble than the crude oil, as shown by the different TRH and TPAH compositions at comparable loadings and dilutions (Supplemental Data, Tables S3 and S4). The high concentrations of TRH in some samples could be indicative of oil droplets. The higher proportion of high–molecular weight compounds in the crude oil explains the relative insolubility of the product, and the differences in solubility may explain the differences in toxic response. Typically, a mixture of formation water and residual phase–separated hydrocarbons (oil/condensate), produced water may also contain production chemicals, if used, and is primarily a water-soluble emulsion (Neff et al. 2011). However, all compounds are extremely complex mixtures of numerous compounds, many of which have uncharacterized toxicity (Meador and Nahrgang 2019; Neff et al. 2011). Undoubtedly many of these compounds may be contributing to the observed toxicity, especially to the toxicity observed following exposures to produced water.

The differences in toxic response between species are likely driven by different modes of toxic action and the life stage of the organisms used. The toxicity we observed in copepods is likely due to narcotic (or nonspecific or baseline) toxicity (Veith and Broderius 1990). Narcosis occurs as hydrophobic compounds, such as PAH, are accumulated in the lipid cell membrane or in the membrane of tissues such as the gill that are in contact with water (Escher et al. 2008). Narcotic toxicity is commonly used in models predicting oil toxicity (Di Toro et al. 2000; French-McCay 2002; McGrath and Di Toro 2009; Redman and Parkerton 2015; Redman et al. 2017; McGrath et al. 2018). Although this mode of action is likely to affect all organisms, the 2 different embryos (sea urchin and fish) were likely also sensitive to developmental impairment.

Cardiac toxicity in fish embryos exposed to crude oil has been widely studied in recent years (Greer et al. 2012; Incardona et al. 2014; Esbaugh et al. 2016). Some recent literature has argued that this toxic impact occurs independently of narcosis and instead results from interference with potassium channels in the heart (Brette et al. 2014). This form of toxicity occurs primarily after exposure to 3- and 4-ringed PAHs (Incardona et al. 2005) and, as a consequence, is not well predicted by target lipid–type models. Other studies have

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hypothesized that the impacts observed could occur from narcotic or baseline toxicity and that many components of oil in addition to PAHs may be contributing (Meador and Nahrgang 2019). The EC50 we report for pericardial edema in fish exposed to condensate (1.2 µg/L TPAH) is within the range of EC50s reported elsewhere following exposure to crude oil WAFs (typically 1–10 µg/L TPAH; Incardona et al. 2014).

Our fish embryo data were confounded by high degrees of variability, particularly in survival after hatching, which is very low in yellowtail kingfish in natural conditions (Symonds et al. 2014). Many experiments in the literature do not report this endpoint (see Incardona et al. 2014; Esbaugh et al. 2016). Nevertheless, we were able to measure increased incidence of spinal curvature in fish embryos exposed to produced water and increased incidence of pericardial edema in fish embryos exposed to condensate WAF, even following the 2-h exposure period at the highest exposure concentrations. It has been argued that short exposures would not be sufficient to cause PAH-based toxicity because the uptake kinetics are slow and that the compounds would diffuse out of tissues once the exposure concentrations decreased (Di Toro et al. 2000; French-McCay 2002). However, the assumption of reversibility may not hold for exposures that result in disruption of a key event in development, such as the formation of the vertebrate heart. Pericardial edema did not occur as readily in fish embryos exposed to produced water, even though the PAH composition and concentrations were comparable (Supplemental Data, Table S4). The only components of oil that were specifically quantified were the PAHs; however, it is possible that other, unquantified compounds were also contributing to the observed toxicity (Meador and Nahrgang 2019). In addition, in testing WAFs we only consider the water-soluble component of oils, and the surface film of oil (rather than the WAF) may persist for longer.

The hypothesized mode of toxic action for crude oil in sea urchin embryos is inhibition of normal skeletogenesis and spicule formation (Sekiguchi et al. 2018); however, other modes of action, including narcosis, are likely to contribute (Meador and Nahrgang 2019). Unlike fish and copepods, crude oil was toxic to sea urchin larvae under continuous exposure. Despite the low solubility of the Barrow Island crude oil, impacts on embryonic development were measured at a lower TPAH threshold than was observed for the condensate WAF. Previous studies have attributed some of the toxicity that was observed in developing sea urchin embryos to the unresolved complex mixture (Neff et al. 2000; Saco-Alvarez et al. 2008). It is possible that some of these compounds are contributing to the developmental toxicity we observed as well, as has been hypothesized for other fish embryos (Meador and Nahrgang 2019).

The present study is one of the few in the peer-reviewed literature to compare the toxicity of crude oil and condensate, even though gas condensate is an increasingly common petroleum product. To date, many of the studies described in the literature for condensate and crude oil toxicity used coral as a test organism (Negri et al. 2016; Nordborg et al. 2018). Negri et al. (2016) reported an EC50 value of 339 µg/L TPH (TPAH + BTEX) for coral larvae exposed to a condensate WAF. Nordborg et al. (2018) also exposed coral larvae to a heavy fuel oil and estimated an EC50 value of 96 µg/L based on TPH. Because of limited data points on the slopes of the curves, we were unable to calculate reliable toxicity estimates using TPH concentration for all WAFs and species. For the sea urchin exposed to condensate WAF, we calculated an EC50 value of 143 µg/L TPH; this is within the range reported by Negri et al. (2016). Negri et al. (2016) reviewed the toxicity of various hydrocarbon products to a range of corals and sponges and reported effect concentrations ranging from 2 to 3800 µg/L. The most sensitive species/endpoint was fertilization of the coral *Acropora tenuis* exposed to a fuel oil, with a reported lowest observable effect concentration (LOEC) of 2 µg/L (Total hydrocarbon concentration) (Harrison 1999, reviewed in Negri et al. 2016). Neff et al. (2000) investigated the toxicity of 3 different types of crude oils to a range of marine species including crustaceans (mysids and shrimps, acute survival), echinoderms (sand dollars and sea urchins, larval development) and fish (acute survival of juveniles). Survival of shrimps and mysids was inhibited by 50% (LC50) between 2 to 210 µg/L (PAHs). In our study, mobility of the copepod was inhibited by 50% at a TPAH concentration of 7.9 µg/L in the condensate WAF; within the range reported by Neff et al. (2000) for other crustaceans. However for the crude oil WAF exposure, the EC50 for copepod mobility was above the highest concentration tested (16 µg/L TPAH). Neff et al. (2000) reported that larval development (after 60 h exposure to crude oil WAFs) of a sea urchin and sand dollar were inhibited (EC50) at concentrations between 50 to 200 µg/L (PAH) and fish showed similar sensitivity to crustaceans with LC50 values ranging from 2 to 160 µg/L (PAH). Out of the 3 species tested in our study, the sea urchin was the most sensitive to PW, condensate WAF and crude oil WAF, and the most sensitive species to crude oil WAF so far reported in the literature. It is important to note that different oils (and the source and solubility of those oils) were tested in the present study and that of Nordborg et al. (2018), Neff et al. (2000) and as reported in Negri et al. (2016). Differences in the test organisms, analytical procedures, and protocols likely contribute to the differences in toxicity reported between studies. However, future research should further investigate the sensitivity of the sea urchin *H. tuberculata* to crude oil WAFs. In addition, more studies are needed to properly characterize the environmental risk from an accidental release of condensate.

The findings of the present study highlight the need for site-specific environmental realism in toxicity studies. There have been numerous studies examining the toxicity of oil, but many of them use standardized exposure durations (e.g., continuous exposure for a period of >24 h) for both oil exposure (Hansen et al. 2012; Incardona et al. 2014; Johansen and Esbaugh 2017) and exposure to produced water (Meier et al. 2010). Although these studies provide invaluable information about the potential mode of toxic action for organisms exposed to oil (Incardona et al. 2005, 2014; Rial et al. 2013) and can be used to benchmark improvements in operational controls and compare between facilities internationally, it may not be appropriate to use them in

evaluating environmental risk for waterborne exposures in oceanic environments because exposures would be much shorter (Lee et al. 2013; Bejarano et al. 2014). Indeed, exposure duration may help to explain the discrepancy between toxicity measurements predicted based on laboratory studies and impacts measured in the field at either discharge locations or sites of uncontrolled release of oil (Fodrie et al. 2014). Once fully developed, validated, and accepted by the relevant regulators, short-duration toxicity tests may be more appropriate to use in risk analysis for activities in the offshore environment, or indeed other intermittent discharge scenarios, such as those being proposed for use in stormwater risk assessment. A pathway to incorporation into risk assessment would be an additional line of evidence to the standard duration bioassays.

CONCLUSIONS

In the present study, we compared short-term and continuous exposures to 3 petroleum products (produced water, condensate WAF, and crude oil WAF) to determine if exposure duration influenced ecotoxicological response of a copepod, sea urchin, and fish. We demonstrated that short-term exposures and transferring organisms during tests are feasible. However, further experimental development is required before the tests could be considered standard method. Toxicity of the petroleum products was only observed to copepods and sea urchins following the continuous test exposure duration (after 48 and 72 h, respectively). We observed a trend of increasing incidence of spinal curvature in fish embryos exposed to produced water or crude oil WAF and an increase in pericardial edema in embryos exposed to condensate WAF irrespective of exposure duration. These results highlight the need for increased environmental realism in toxicity test design and for more research in this area to further understand the impacts of short-term exposure on other species and life stages. Future studies could increase test concentrations where practical to enable possible estimation of ECx values at short-term exposure durations, and time-dependent toxicity data, presented in our study and in previous studies, could be used to develop toxicokinetic models which could predict how the toxic response of a species to a petroleum product changes with exposure duration.

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