Comparative toxicity of hydrocarbons for evaluation of *Lysmata boggessi* as an experimental proxy for deep-water column micronekton

D. Abigail Renegar [a],[*], Nicholas R. Turner [a], Gopal Bera [b], Eileen G. Whitemiller [a],
Bernhard M. Riegl [a], José L. Sericano [b], Anthony Knap [b]

[a] *Nova Southeastern University, Halmos College of Natural Sciences and Oceanography, Dania, FL, USA*
[b] *Texas A&M University, College Station, TX 77845, USA*

A B S T R A C T

The potential impacts of sub-surface hydrocarbon plumes to deep-water column micronekton are an important consideration in a more complete understanding of ecosystem effects resulting from deep-sea oil spills. However, evaluating toxicity in these organisms presents multiple challenges, and the use of a shallow-water proxy species allows comparison and validation of experimental results. This study thus examined the suitability of the peppermint shrimp, *Lysmata boggessi*, as an experimental proxy for ecologically important deep-sea zooplankton/micronekton in hydrocarbon toxicity assays. This crustacean species occurs in shallow coastal marine environments throughout the western Atlantic, Caribbean and Gulf of Mexico, is similar in size to the mesopelagic organisms previously tested and is readily available via commercial aquaculture. The effects of 1-methylnaphthalene and fresh Macondo oil (MC252) on *L. boggessi* were assessed in 48-h constant-exposure toxicity tests, and acute thresholds were compared to previously determined LC50s for oceanic mid water Euphausiidae, *Janicella spinacauda, Systellaspis debilis, Sergestes sp., Sergia sp.* and the mysid shrimp *Americamysis bahia*. Acute thresholds and the calculated critical target lipid body burden (CTLBB) for the shallow-water *L. boggessi* were comparable to the deep-water species tested, suggesting that *L. boggessi* may be a suitable proxy for some mesopelagic micronekton species in acute hydrocarbon exposures.

1. Introduction

Deep-water column micronekton (e.g. shrimp and plankton) are a key component of the Gulf of Mexico ecosystem, representing a significant trophic link between deep- and shallow-water ecosystems and food webs [1]. As the vertical range of their nightly migration from the mesopelagic to the epipelagic can overlap subsurface hydrocarbon plumes, such as those which resulted from subsea dispersant use during the Deepwater Horizon spill [2,3], it is important to consider these organisms in the context of potential ecosystem impacts from deep-sea oil spills.

Despite their significance, studies on these species are rare due to the difficulties associated with collecting live animals at sea and their maintenance in the laboratory. In order to address this data gap, the Deep-sea Risk Assessment and Species Sensitivity to WAF, CEWAF and Dispersant project (D-TOX), was designed to advance the understanding of hydrocarbon toxicity in several ecologically important deep-sea

micronekton species. Results to date have indicated some species-specific variability in sensitivity to 1-methylnaphthalene (1-MN) [4], phenanthrene and crude oil exposures in these marine crustaceans [5]). However, there are multiple challenges to conducting experiments with these organisms. The cost of ship time can be prohibitive, and collection requires specialized equipment to maintain the animals at the necessary temperature (7–10 °C) and dark conditions. Additionally, collection of adequate numbers of animals in good condition is not guaranteed, which can reduce the applicability of generated results and overestimate toxicity. The use of a proxy organism allows comparison and validation of experimental results when similar protocols and exposures are used.

Although uncertainties exist regarding the behavior and solubility of oils and dispersed oils in the deep sea resulting from temperature and pressure, and the potential impact that this may have on subsequent toxicity, some studies have demonstrated the potential for shallow-water species to be used as ecotoxicological proxies for deep water species [6,7]. The sole study examining toxicity of a single hydrocarbon

to deep-sea micronekton found similar acute toxicity thresholds for 1-MN to the commonly used test species *Americamysis bahia* [4] at ambient temperature and salinity conditions for each species, however a fully marine shallow-water crustacean species with a similar body size was needed for comparison.

This study examines the suitability of the peppermint shrimp, *Lysmata boggessi*, both as an experimental proxy for deep-sea micronekton and as a substitute for estuarine organisms (such as *A. bahia*) in hydrocarbon toxicity studies for the marine environment. This crustacean species occurs in shallow coastal marine environments throughout the western Atlantic, Caribbean and Gulf of Mexico, is similar in size to the mesopelagic organisms previously tested and is readily available via commercial aquaculture. Acute and sub-acute effects of 1-MN and fresh Macondo oil (MC252) on *L. boggessi* were assessed in 48-h constant-exposure toxicity tests, and acute thresholds were compared to results of similar experiments previously conducted with *A. bahia* and deep-sea micronekton species *Janicella spinacauda*, Euphauisiidae, *Systellaspis debilis*, *Sergestes sp.*, and *Sergia sp.* [4,5].

2. Materials and methods

2.1. Experimental organisms

Aquacultured individuals of *L. boggessi* were obtained from ORA®, in Fort Pierce, Florida. All shrimp were of similar size (2 cm in length) and age. Specimens were acclimated to laboratory conditions in a 750 L indoor seawater system; the system was maintained at 26 °C and 35 PSU, and shrimp were held for 24–72 h before use in experimental exposures.

2.2. 1-methylnaphthalene exposure

Exposure to 1-MN was conducted in the same continuous-flow, recirculating exposure system used for similar experiments with deep-sea crustacean species as described in Knap et al. [4]. This system utilizes a passive-dosing methodology which is designed to determine toxicity of individual oil constituent hydrocarbons and has been used successfully with a variety of other species [8,9]. For each dosing system, water was continuously recirculated from a 2-L dosing vessel to a corresponding exposure chamber (750-mL Pyrex bottle) via Viton tubing with a Cole-Parmer multichannel peristaltic pump (flow rate 7.5 mL/min). Five concentrations of 1-MN (nominally 150 µg/L, 300 µg/L, 600 µg/L, 1200 µg/L and 2400 µg/L) and a seawater control (with O-rings) were tested, with 3 independent replicate dosing systems per treatment and 2 shrimp per replicate. Treatments were randomly assigned to individual dosing systems.

Before the start of the exposure period, polydimethylsiloxane (PDMS) O-rings (O-Rings West) were cleaned by rinsing in ethyl acetate (Fisher Scientific) (24 h), methanol (Fisher Scientific, HPLC grade) (3x in 24 h), and deionized water (3x in 24 h), then dried at 100°C for 1 h. Stock solutions of 1-MN (Acros Organics, 97%) in methanol were prepared using the equation:

$$C_{MeOH} = \left[K_{MeOH-PDMS} + \left[\frac{V_{PDMS}}{V_{MeOH}} \right] \right] \times \left[K_{PDMS-Water} + \left[\frac{V_{Water}}{V_{PDMS}} \right] \right] \times C_{Target}$$

where C_{MeOH} is the concentration of 1-MN added to methanol (mg/L); $K_{MeOH-PDMS}$ is the partition coefficient of 1-MN between methanol and PDMS (log $K_{MeOH-PDMS}$= 0.43); V_{PDMS} is the volume of PDMS O-rings in the mixing vessel (mL); C_{target} is the target concentration in seawater (mg/L); V_{MeOH} is the volume of the methanol dosing solution (mL); $K_{PDMS-Water}$ is the partition coefficient of 1-MN between PDMS and water (log $K_{PDMS-Water}$= 2.98) and V_{water} is the volume of water in the recirculating flow-through system (mL) [10].

Stock solutions were made by adding the amount of 1-MN calculated for each experimental concentration in 500 mL of methanol and mixing for 1 h. Clean PDMS O-rings were then added to the methanol stock

solutions and equilibrated for 72 h on an orbital shaker for partitioning of 1-MN into the O-rings (mean mass 1.06 g, 140 for each concentration/treatment, 35 per replicate). Calculated depletion of 1-MN in both reservoirs was 4.83% in the methanol loading solution, and 4.44% in the PDMS O-rings.

Dosing systems were filled with seawater sourced from the laboratory system and filtered to 1 µm (Polymicro); a total of 2750 mL was used in each system, resulting in < 10% headspace to limit volatile loss. Dosing vessels were aerated prior to addition of O-rings to ensure that the seawater was 100% saturated with oxygen; no aeration took place during the exposure. After loading, O-rings were rinsed 3x in seawater and added to the appropriate dosing vessels. The peristaltic pumps were started and the systems were allowed 20 h for equilibration; dosing vessels were vigorously stirred on magnetic stir plates throughout the equilibration and exposure periods.

After equilibration, 2 randomly assigned shrimp were added to each dosing system chamber, and the 48-h exposure was initiated. All systems and equipment were monitored for continuous operation within designated limits throughout the duration of exposure. Shrimp mortality was recorded every hour for the first 8 h and every 12 h thereafter for the remainder of the 48-h exposure.

2.3. Macondo 252 oil exposure

Exposure to fresh Macondo (MC252) oil (obtained from AECOM, product code 0000003291, and stored in a sealed amber glass jar at 4 °C until use) was conducted in the same continuous-flow, recirculating exposure system used for a similar experiment with deep-sea species [5], and for the 1-MN exposures. Passive dosing of oil via silicone tubing produces a water accommodated fraction (WAF) with a concentration and composition of dissolved oil in the exposure media similar to that produced by other methods of WAF preparation, whilst limiting the presence of droplets [11]. Five oil loadings (nominally 15 mg/L, 60 mg/L, 130mg/L, 240 mg/L and 960 mg/L) and a seawater control were tested, with 4 independent replicate dosing systems per concentration and 2 shrimp per replicate. Treatments were randomly assigned to individual dosing systems.

Dosing systems were filled with seawater sourced from the laboratory system and filtered to 1 µm (Polymicro); a total of 2750 mL was used, resulting in < 10% headspace. Dosing vessels were aerated prior to addition of oil-loaded tubing to ensure that the seawater was 100% saturated with oxygen; no aeration took place during the exposure. The oil WAF was prepared following the method of Redman et al. [12] and Bera et al. [11]. For each treatment replicate, a predetermined amount of oil was injected into medical grade silicone tubing (A-M Systems Inc., WA, dimensions of 0.058 ×0.077 ×0.0095-inch) using a gas tight Hamilton syringe, and both ends were knotted tightly. The loaded silicone tubing was coiled and suspended, fully submerged, in the 2 L dosing vessel. The peristaltic pumps were started, and the systems were given 20 h for equilibration. Dosing vessels were vigorously stirred throughout the equilibration and exposure periods.

After equilibration, 2 randomly selected shrimp were added to each dosing system chamber, and the 48-h exposure was initiated. All systems and equipment were monitored for continuous operation within designated limits throughout the duration of exposure. Shrimp mortality was recorded every hour for the first 8 h and every 12 h thereafter for the remainder of the 48-h exposure.

2.4. Hydrocarbon chemistry

Water samples for 1-MN analysis were collected from the outflow of each chamber, in certified volatile organic analyte vials (Thermo Scientific) (with no headspace) at the beginning (0 h, immediately prior to addition of shrimp), middle (24 h) and end (48 h) of the exposure to verify concentration stability during the exposure. Samples were preserved at 4 °C and the concentration of 1-MN was quantified in a Horiba

Aqualog-UV-800 Spectrofluorometer after extraction with dichloromethane (DCM) (Sigma Aldrich). A calibration curve with at least five points was run daily (analytical standard, Supelco); the coefficient of determination (r^2) was required to be greater than 0.99 before the samples were run. Blanks were run vs air and tested to determine that no emission was observed at the wavelengths (excitation and emission) used for 1-MN. Fluorescence emissions of the water samples were measured at the optimal wavelengths (Ex=284 nm, Em=335.19 nm) and the concentrations of 1-MN were calculated using the calibration curve.

Water samples from MC252 oil exposure experiments were collected for analysis of estimated oil equivalents (EOE), volatile organic carbons (VOCs), total petroleum hydrocarbons (TPAH), and alkanes. The EOE was monitored at 0 h, 24 h and 48 h, VOCs, PAHs and alkanes were only measured at 48 h. Because passive dosing systems were used for these experiments, concentrations of petroleum hydrocarbons from oil were expected to be constant during the exposure period. The EOE measurements allowed confirmation of the stability of hydrocarbon concentrations over time.

Measurement of EOE followed the methods described in detail by Wade et al. [13] and Bera et al. [14]. Briefly, the maximum intensity at optimal wavelengths (Ex=260, Em=372.05 nm) for MC252 oil were determined. A six-point calibration curve was generated using a range of oil concentrations (0.1 mg/L – 10 mg/L). Different amounts (0.1 mg/L – 10 mg/L) of MC252 oil were dissolved in DCM to make the calibration standards. The water samples were extracted with DCM and their fluorescence emissions were measured at optimal wavelengths (Ex=260, Em=372.05 nm). EOE concentrations were calculated using the calibration curve.

Water samples for VOCs were collected in 40 mL certified volatile organic analyte vials (Thermo Scientific) with no headspace, and acidified with 70 μL of 6 M hydrochloric acid (HCl). Samples were analyzed by EPA Method 8260 for VOCs by GC/MS (Shimadzu QP2010SE with EST Purge & Trap).

For PAH and alkane measurements, the methods of Wade et al. [15] and Bera et al. [11] were followed. Water samples (~1 L) were collected at 48 h and 100 mL DCM was added to each sample for preservation. Samples were spiked with aromatic and aliphatic surrogates (d8-naphthalene, d10-acenaphthene, d10-phenanthrene, d12-chrysene, and d12-perylene for PAHs and d26-nC$_{12}$, d42-nC$_{20}$, d50-nC$_{24}$, and d62-nC$_{30}$ for aliphatic) before extraction with DCM (total 200 mL) in a separatory funnel. The extracts were evaporated in a water bath at 55 °C to 1 mL. Aliphatic and aromatic fractions were separated using a silica gel column. For the aliphatic fraction, 50 mL pentane was passed through the column, and a mixture of pentane and DCM (50%/50%) was used to collect the aromatic fraction. These extracts were evaporated to a final volume of 1 mL and GC internal standards (e.g., d10-Fluorene and d12-Benzo(a)pyrene for PAHs and d54-nC$_{26}$ for aliphatic hydrocarbons) were added. Aliphatic hydrocarbons and PAHs were quantitatively analyzed by GC with mass spectrometric detection (Agilent 6890 N GC/5975 C inert MSD); the details of temperature program, column used, and quantification method are described in Bera et al. [11].

2.5. Statistical analysis

Threshold concentrations were determined for both toxicity tests (1-MN and MC252 oil) with the *drc* package in RStudio statistical software (R V3.4.3) [16]. The log-logistic 2-parameter dose response model for binary data was used to estimate the acute lethality threshold (LC50). This model is a self-starting function that initially estimates the model parameters using the maximum likelihood principle. Estimates of all threshold levels were made with the effect dose (ED) function, which utilizes the delta method to estimate 95% confidence intervals.

The dependent variable used in each model is lethality, but the independent variable was adjusted to accurately reflect the composition of the exposure media. Single compound effects were modelled using the

geometric means of the 0, 24 and 48-h 1-MN individual chamber concentrations to determine the LC50 values at each time point. Multiple independent variables (each hydrocarbon characterization method) were utilized to assess the effects of oil in order to improve comparisons with other studies. Estimated oil equivalents and total National Status & Trend (NS&T) PAHs were each used to estimate lethality thresholds, and the initial oil loadings were used to estimate the lethal loading (LL50). To further increase comparative ability to other studies, the target lipid model (TLM) was used to calculate a critical target lipid body burden (CTLBB) (μmol chemical/g lipid) following determination of the LC50 for 1-MN using the equation: [17].

$$\log LC_{50} = \log CTLBB - 0.940 \times \log K_{OW} + \Delta c$$

The TLM relates the experimentally determined toxicity of a substance, in terms of aqueous concentration, to a species-specific CTLBB and the target-lipid water partition coefficient, which is estimated from the chemical's octanol-water partition coefficient (K_{OW}), the universal narcosis slope (− 0.940), and the chemical class correction factor (Δc) [17]. Typically, the CTLBB is estimated by fitting calculated thresholds from multiple single hydrocarbon exposures (>2) to the TLM, and reducing residuals between the values and the TLM with the universal narcosis slope. However, in situations where only one threshold is available, the CTLBB can be calculated by rearranging the TLM with the calculated acute toxicity threshold and log K_{OW} for that specific chemical as inputs. In this study, the threshold and log K_{OW} for 1-MN was input to the TLM to calculate the CTLBB.

The calculated acute toxicity thresholds were then used to generate species sensitivity distributions with the USEPA SSD Generator V1, available at https://www.epa.gov/caddis-vol4/caddis-volume-4-data-analysis-download-software.

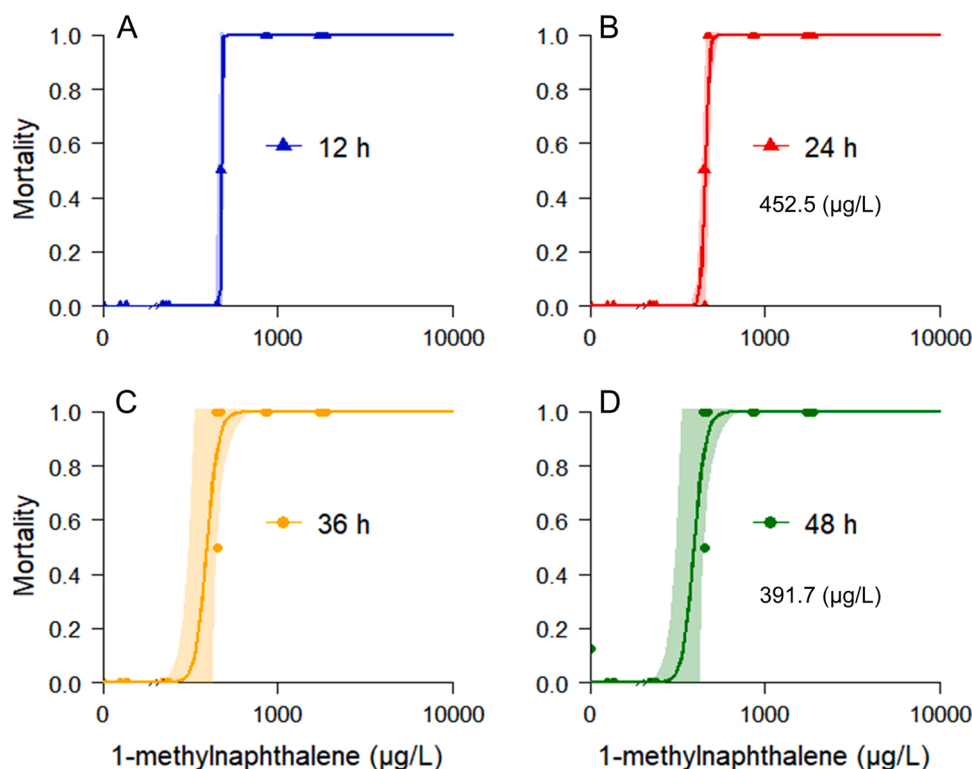## 3. Results

3.1. 1-methylnaphthalene

Measured concentrations of 1-MN over the exposure period for each treatment are shown in Table 1 (and Supplemental Data, Table S1). The aqueous concentrations were in general agreement with predicted values, with a mean variability in concentration of 6.4%, and a maximum mean loss of 11.7% over 48 h for all exposure levels. Similar to previous experiments utilizing passive dosing, the present study demonstrates the value of this methodology in achieving and maintaining relatively stable hydrocarbon concentrations during dosing experiments, with limited variation in concentration over the test period.

Acute toxicity thresholds for *L. boggessi* exposed to 1-MN were determined at 12 h, 24 h, 36 h and 48 h (Fig. 1, Table S5). Control performance was acceptable, with no mortality observed from 0 to 36 h and 12.5% mortality at 48 h (with 1 of 6 control shrimp lost due to apparent predation). Mortality was initially observed in the two highest concentrations tested after 2 h of exposure. The *drc* model created for each time point was used to determine the LC50; calculated LC50s (Table S5) for *L. boggessi*, *A. bahia* and deep-sea micronekton. The

Table 1

Measured concentrations of 1-methylnaphthalene for each treatment during the exposure period.

Treatment	Measured concentrations of 1-methylnaphthalene[a]		
	0 h	24 h	48 h
Seawater Control	ND	ND	ND
150 μg/L	138.6 ± 4.5	137.6 ± 3.1	122.5 ± 8.0
300 μg/L	218.1 ± 17.8	230.7 ± 14.3	230.6 ± 11.0
600 μg/L	446.1 ± 19.8	465.5 ± 11.6	448.5 ± 19.5
1200 μg/L	845.7 ± 13.7	876.3 ± 20.8	861.9 ± 7.6
2400 μg/L	1737.2 ± 97.3	1850.0 ± 53.1	1832.4 ± 77.2

[a] mean ± SD (μg/L). h= hour. ND=Below detection limit

Fig. 1. Dose response curves produced from mortality data at A) 12 h, B) 24 h, C) 36 h, D) 48 h exposure to 1-methylnaphthalene. Solid lines = model estimate, Dashed lines= 95% CI, Symbols= average mortality in each chamber.

similarity of model outputs between 24 and 36 h suggest the hydrocarbon partitioned into tissue lipid and reached the acute toxicity threshold concentration after 24 h. For this reason, the 24-h LC50 was used in the TLM to calculate a CTLBB of 24.8 μmol/g octanol for *L. boggessi*.

3.2. Macondo 252 oil

Mean measured concentrations of EOE over the exposure period for each treatment are shown in Table 2 (and Supplemental Data, Table S2). The mean variability in EOE concentrations over time was 8.8%, with a maximum mean loss of 0.32% over 48 h for all of the exposure levels. Aqueous concentrations in most chambers increased slightly over time, as hydrocarbons continuously partitioned through the silicone tubing regardless of loss to the system. The passive dosing of oil therefore produced consistent aqueous concentrations of TPAH and VOCs between replicates (Tables S3 and S4, respectively) (Table 3).

Acute toxicity thresholds for *L. boggessi* exposed to passively-dosed MC252 oil were determined at 12 h, 24 h, 36 h and 48 h using EOE concentrations (μg/L), total NS&T PAHs (μg/L), and oil loading (mg/L) (Fig. 2, Table S6). Control performance was acceptable, with no mortality observed from 0 to 48 h.

Table 2
Measured estimated oil equivalents (EOE) for each oil loading over time.

Oil loading	Mean measured EOE concentrations[a]		
	0 h	24 h	48 h
Seawater Control	61.5 ± 6.0	72.1 ± 3.0	80.5 ± 4.4
15 mg/L	79.5 ± 4.5	98.8 ± 8.0	97.8 ± 11.0
60 mg/L	99.0 ± 5.2	126.2 ± 2.7	135.5 ± 2.5
130 mg/L	192.3 ± 18.6	234.4 ± 10.1	240.7 ± 17.3
240 mg/L	250.1 ± 67.5	250.2 ± 63.7	290.2 ± 26.5
960 mg/L	259.1 ± 12.2	319.4 ± 21.6	346.2 ± 32.4

[a] mean ± SD (μg/L). h= hour.

Table 3
Measured TPAH concentrations at 48 h for each treatment.

Oil loading	Mean measured TPAH concentrations[a]		
	w/perylene	w/out perylene	Total NS&T
Seawater Control	0.053 ± 0.005	0.0051 ± 0.041	0.045 ± 0.006
15 mg/L	20.89 ± 2.63	20.87 ± 2.63	14.46 ± 1.83
60 mg/L	75.73 ± 13.74	75.73 ± 13.74	57.45 ± 11.27
130 mg/L	142.92 ± 6.14	142.92 ± 6.14	114.70 ± 3.89
240 mg/L	230.98 ± 11.53	230.98 ± 11.53	192.88 ± 6.93
960 mg/L	367.84 ± 7.42	367.84 ± 7.42	324.82 ± 8.07

[a] mean ± SD (μg/L). h= hour.

For *L. boggessi*, the 36- and 48-h LC50s were the same, as no additional mortality occurred after 36 h. The similarity in models between 24, 36, and 48 h suggest the CTLBB was achieved within 24 h of exposure, consistent with observations from the 1-MN exposure. The 24-h LC50s ranged from 108.8 μg/L (91.8–123.4 μg/L) TPAH (NS&T) (Fig. 2E) to 195.2 μg/L (160.3–230.2 μg/L) EOE (Fig. 2B).

3.3. Relative species sensitivity

Acute toxicity thresholds for 1-MN exposures (Table S6) were used to generate a species sensitivity distribution (Fig. 3A) comparing *L. boggessi* (this study) to *A. bahia, J. spinacauda*, Euphauisiidae, *Sergia* sp., *Sergestes* sp. and *S. debilis* (data from [4]). *Lysmata boggessi* falls near the 50th percentile of these species.

Acute toxicity thresholds for passively-dosed MC252 oil exposures were used to generate a species sensitivity distribution (Fig. 3B) comparing *L. boggessi* (this study) to *A. bahia, J. spinacauda*, and Euphauisiidae [5]. *Lysmata boggessi* falls at the 63rd percentile of these species. The 24- and 48-h acute thresholds for 1-MN, and the 24-h acute thresholds for crude oil for *L. boggessi* were very similar to *A. bahia*. However, the 48-h LC50 for *L. boggessi* was 55.3% greater than the 48 h LC50 for *A. bahia*, possibly due to the loss of *A. bahia* from the
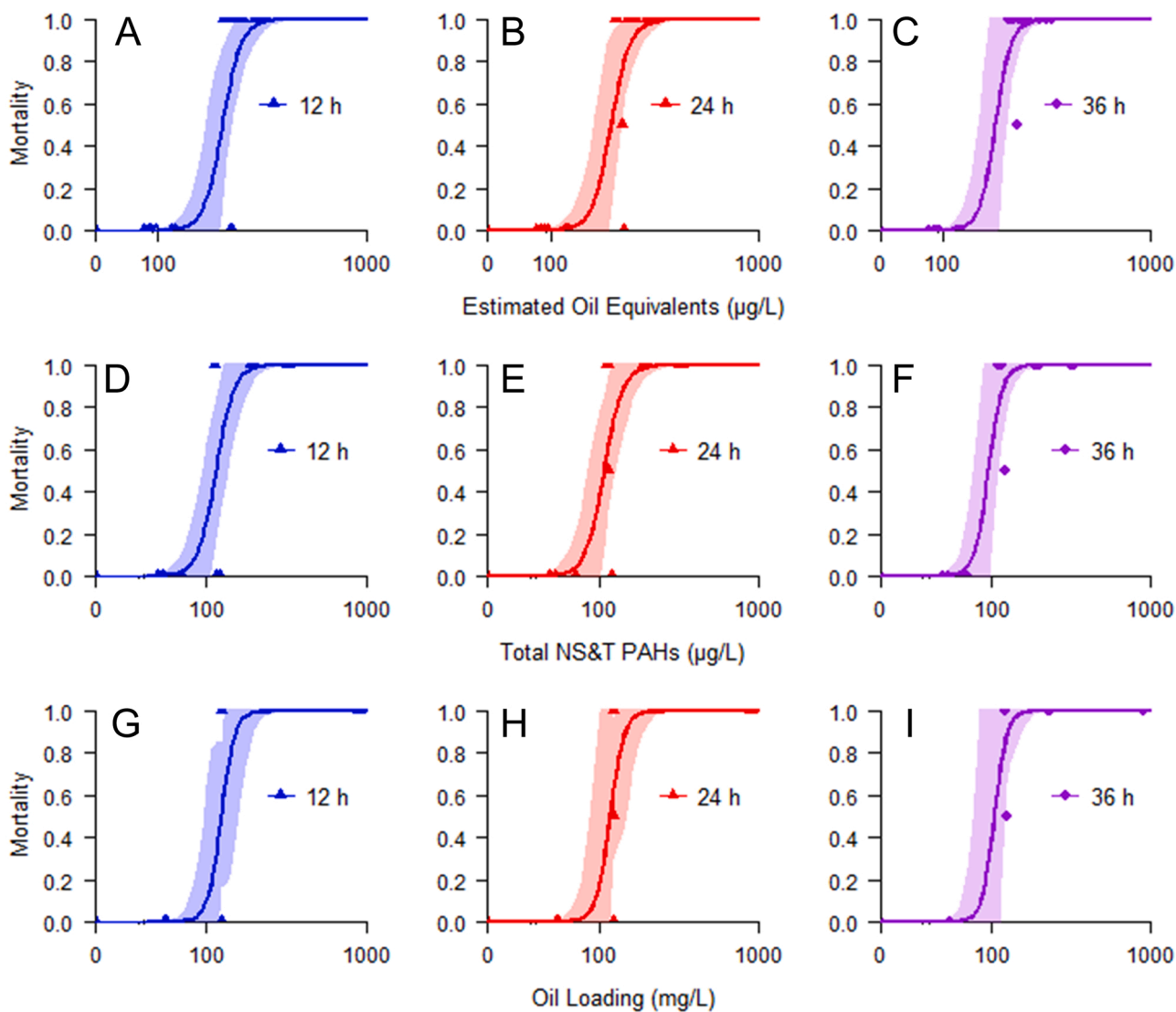
Fig. 2. Dose response curves produced from mortality data at 12 h, 24 h, and 36 h exposure to passively-dosed MC252 oil. Threshold concentrations were calculated using estimated oil equivalents (A-C), Total NS&T PAHs (D-F), and oil loading (G-I). Solid lines = model estimate, Dashed lines= 95% CI, Symbols= average mortality in each chamber.
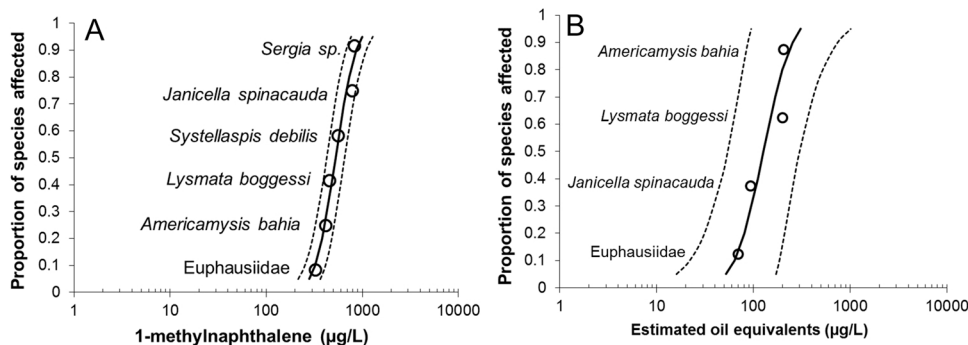

Fig. 3. A) Species sensitivity distribution (SSD) of 24 h acute toxicity for 1-methylnaphthalene. Symbols represent the 24-h LC50 for each species, and solid and dashed lines represent the mean and 95% CI of the SSD [data from Knap et al. [4] and this study]. B) Species sensitivity distribution (SSD) of 24 h acute toxicity for MC252 oil. Symbols represent the 24-h LC50 for each species, and solid and dashed lines represent the mean and 95% CI of the SSD [data from Turner et al. [5] and this study].

recirculating experimental system, or due to observed cannibalism.

The acute CTLBB for *L. boggessi* and 5 deep-sea species (Table S6) was compared to calculated values for other species for which this data is available (Fig. 4). The CTLBBs for the mesopelagic crustaceans (red squares) and *L. boggessi* (blue circle) are within the same range, and are in the lower range of acute CTLBBs and thus relatively more sensitive [4, 18,17].

4. Discussion

The objective of the present study was assessment of a shallow-water marine crustacean species, *L. boggessi*, as an experimental proxy for Gulf
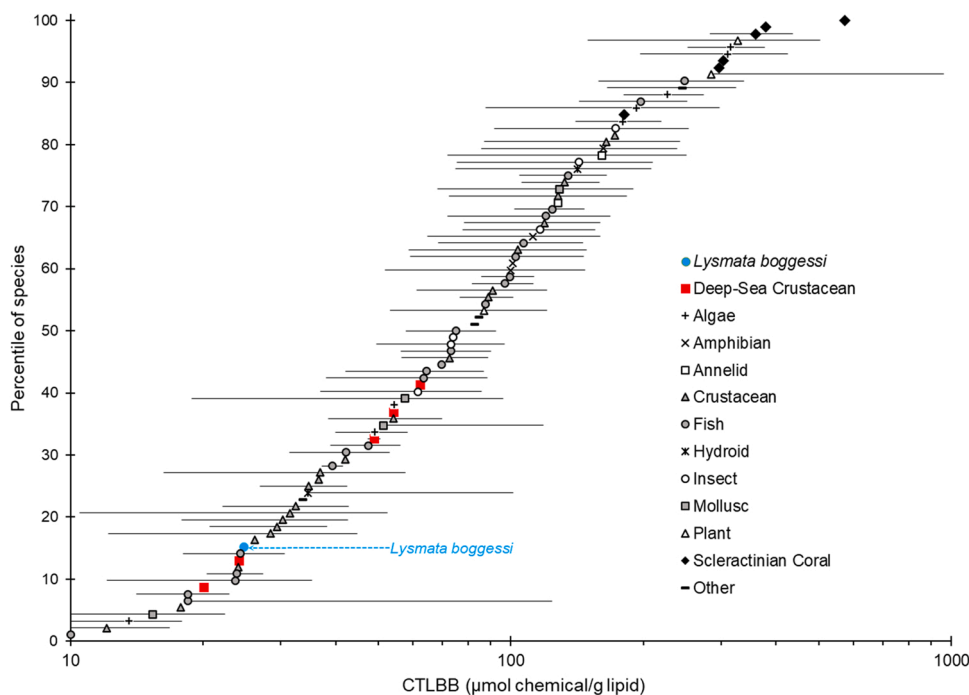
Fig. 4. Comparison of critical target lipid body burdens (CTLBB) for species with available data. Data for *Lysmata boggessi* (solid blue circle) is from this study; data for deep-sea crustaceans (solid red squares) from [4,5]; data for scleractinian corals from [19,20]; all other data from [21]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of Mexico mesopelagic crustacean species in hydrocarbon toxicity studies. The measured acute thresholds and CTLBB for *L. boggessi* were in the same range as those of the deep-sea species (Figs. 3 and 4), and more conservative than other commonly used test species *A. bahia* and *Palaemonetes pugio,* suggesting that *L. boggessi* could potentially be a suitable proxy for some mesopelagic micronekton species in acute hydrocarbon exposures.

When comparing relative sensitivity among species, comparisons should be made at a steady state exposure duration and should be based on the concentration at which the toxicity endpoint reaches an asymptote and does not change with a longer exposure [22]. For chemicals such as 1-MN, the mode of action underlying baseline toxicity is narcosis, or the non-specific partitioning of chemicals in biological membranes and membrane-protein interfaces; bioaccumulation is dependent on the hydrophobicity of the chemical [23,24]. The function of the lipid membranes is altered due to an increase in fluidity of the membranes which accompanies solubilization of the narcotic chemical [25].

The lipid content of the organism has been observed to have a significant positive linear relationship to the acute toxicity threshold [26, 27]. The lipid content of proxy species should therefore be comparable to the target species. Donnelly et al. [28] assessed the composition of 25 mesopelagic crustacean species collected from the Gulf of Mexico, including shallow, deep and non migrating Oplophorids and Sergestids. Variability in composition was related to depth of occurrence and migration pattern, with lower lipid and higher protein in migratory species, and compositional consistency among cogeners with similar migration patterns [28]. Mean total lipid for the shallow-migrating Sergestids ranged from 6.6 to 7.0 (% AFDW), and from 9.2 to 10.4 (% AFDW) for the Oplophordids. Mean total lipids in four Euphausiid species from the eastern Gulf of Mexico ranged from 4.4 to 5.4 (% AFDW) [29]. No published data on lipid composition of *A. bahia* or *L. boggessi* was found, however the lipid content of 3.8 (% dw) in wild *Lysmata seticaudata* [30] is less than that reported for mesopelagic crustaceans, and the 3.99 (% ww) for *Neomysis integer* (another estuarine mysid) [31] is higher than the 0.88–2.3 (% ww) reported for mesopelagics [28,29]. Thus, the lipid composition of *L boggessi* may be more similar than that of

A. bahia to shallow migrating mesopelagic crustaceans.

For the exposure to 1-MN, the 24-h LC50s for *L. boggessi* and the deep-sea crustaceans were within a factor of 2, and the 24-h LC50 for *L. boggessi* fell at the 50th percentile of the acute SSD which included 4 deep-sea species (Fig. 3). The acute CTLBB for *L. boggessi* (24.8 μmol/g octanol) based on the 1-MN exposure was in the same range as those for deep-sea crustaceans (9.4–48.7 μmol/g octanol) for the same chemical [4]. Only two of the mesopelagic species are included in the acute SSD for MC252 oil exposure (Fig. 3B), and a different pattern of sensitivity is observed in which *L. boggessi* is relatively less sensitive. Relatively small differences in mortality around the LC50 may be due to statistical distribution of tolerance in the population, or related to variance in individual lipid content or energy stores [23]. The observed increase in the comparative sensitivity of the deep-sea species in the oil exposures may also be due to the low numbers of replicate animals and/or collection and transport stress in the animals collected for the MC252 oil exposures. In contrast, a larger number of replicate deep-sea animals in good condition were collected for the 1-MN exposures, with some surviving for more than two weeks in the laboratory holding system. It is therefore assumed that the results of the 1-MN experiment are more representative of accurate acute thresholds.

In comparison to *A. bahia*, *L. boggessi* may be more suitable as a proxy for several reasons. While acute toxicity thresholds are comparable between all species tested, the ability to conduct toxicity assays at the same native salinity as the mesopelagic crustaceans may be significant in terms of greater compositional similarity in exposure media. Not all of the chemical components of crude oil act as baseline narcotics; non-narcotic toxicity of other constituents and/or the altered toxicity of metabolites or oxygenated derivatives may be significantly influenced by differential solubility at marine vs estuarine salinities. This can prevent determination of accurate toxicity estimates required for robust comparisons of relative risk [32]. This, coupled with the factors described above, supports the use of this species instead of *A. bahia* for both hydrocarbon toxicity assays related to the marine environment and for the migratory mesopelagic crustaceans tested in Knap et al. [4].

While collection or transport stress may be a complicating factor in

interpretation of results for deep-sea species, it is possible to assess that to some degree by comparison with a proxy or reference species. Overall, *L. boggessi* has demonstrated potential as an experimental proxy for some mesopelagic crustacean species. Subsequent experimentation with additional single hydrocarbons and dispersed oil will provide further information on the use and applicability of hydrocarbon toxicity data determined for *L. boggessi* as a possible conservative alternative for mesopelagic crustacean data in hydrocarbon risk assessments.

CRediT authorship contribution statement

DA Renegar: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing – original draft preparation. **NR Turner**: Methodology, Software, Investigation, Formal analysis, Data curation, Validation, Visualization, Writing – original draft preparation. **G Bera**: Methodology, Validation, Formal analysis, Investigation, Visualization, Writing – original draft preparation. **EG Whitemiller**: Investigation, Resources. **BM Riegl**: Writing – reviewing and editing. **JL Sericano**: Writing – reviewing and editing. **A Knap**: Conceptualization, Writing – reviewing and editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (DOI: 10.7266/N77M06F3; DOI:10.7266/N7WS8RN2, DOI:10.7266/N7S46QBC.

Acknowledgements

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.03.023.

References

[1] T.L. Hopkins, M.E. Flock, J.V. Gartner Jr, J.J. Torres, Structure and trophic ecology of a low latitude midwater decapod and mysid assemblage, Mar. Ecol. Prog. Ser. (1994) 143–156.

[2] A.R. Diercks, R.C. Highsmith, V.L. Asper, D. Joung, Z. Zhou, L. Guo, A.M. Shiller, S. B. Joye, A.P. Teske, N. Guinasso, Characterization of subsurface polycyclic aromatic hydrocarbons at the Deepwater Horizon site, Geophys. Res. Lett. (2010) 37.

[3] S.B. Joye, I.R. MacDonald, I. Leifer, V. Asper, Magnitude and oxidation potential of hydrocarbon gases released from the BP oil well blowout, Nature Geoscience 4 (2011) 160–164.

[4] A. Knap, N.R. Turner, G. Bera, D.A. Renegar, T. Frank, J. Sericano, B.M. Riegl, Short-term toxicity of 1-methylnaphthalene to *Americamysis bahia* and 5 deep-sea crustaceans, Environ. Toxicol. Chem. 36 (2017) 3415–3423.

[5] N.R. Turner, G. Bera, D.A. Renegar, T.M. Frank, B.M. Riegl, J.L. Sericano, S. Sweet, A.H. Knap, Measured and predicted acute toxicity of phenanthrene and MC252 crude oil to vertically migrating deep-sea crustaceans, Environ. Sci. Pollution Res. 27 (2020) 45270–45281.

[6] A. Brown, S. Thatje, C. Hauton, The effects of temperature and hydrostatic pressure on metal toxicity: insights into toxicity in the deep sea, Environ. Sci. Technol. 51 (2017) 10222–10231.

[7] A. Brown, R. Wright, L. Mevenkamp, C. Hauton, A comparative experimental approach to ecotoxicology in shallow-water and deep-sea holothurians suggests similar behavioural responses, Aquat. Toxicol. 191 (2017) 10–16.

[8] J.D. Butler, T.F. Parkerton, D.J. Letinski, G.E. Bragin, M.A. Lampi, K.R. Cooper, A novel passive dosing system for determining the toxicity of phenanthrene to early life stages of zebrafish, Sci. Total Environ. 463 (2013) 952–958.

[9] D.A. Renegar, N.R. Turner, B.M. Riegl, R.E. Dodge, A.H. Knap, P.A. Schuler, Acute and subacute toxicity of the polycyclic aromatic hydrocarbon 1-methylnaphthalene to the shallow-water coral *Porites divaricata*: Application of a novel exposure protocol, Environ. Toxicol. Chem. 36 (2017) 212–219.

[10] J.D. Butler, The application of a passive dosing system for determining zebrafish early life stage toxicity of hydrocarbons for use in calibrating a predictive model to acute and chronic endpoints, Graduate Program of Environmental Sciences. Rutgers University, New Brunswick, NJ, USA, 2013, p. 157.

[11] G. Bera, T. Parkerton, A. Redman, N.R. Turner, D.A. Renegar, J.L. Sericano, A. H. Knap, Passive dosing yields dissolved aqueous exposures of crude oil comparable to the CROSERF (Chemical Response to Oil Spill: Ecological Effects Research Forum) water accommodated fraction method, Environ. Toxicol. Chem. 37 (2018) 2810–2819.

[12] A.D. Redman, J.D. Butler, D.J. Letinski, T.F. Parkerton, Investigating the role of dissolved and droplet oil in aquatic toxicity using dispersed and passive dosing systems, Environ. Toxicol. Chem. 36 (2017) 1020–1028.

[13] T.L. Wade, S.T. Sweet, J.L. Sericano, N.L. Guinasso, A.-R. Diercks, R.C. Highsmith, V.L. Asper, D. Joung, A.M. Shiller, S.E. Lohrenz, Analyses of water samples from the Deepwater Horizon oil spill: documentation of the subsurface plume, Monitoring and Modeling the deepwater horizon oil spill: a record-breaking enterprise 195 (2011) 77–82.

[14] G. Bera, G. Gold-Bouchot, U. Passow, T.L. Wade, D. Shi, M. Morales-McDevitt, N. Ramirez-Miss, A.H. Knap, A. Quigg, Inter-laboratory calibration of estimated oil equivalent (EOE) concentrations of a water accommodated fraction (WAF) of oil and a chemically enhanced WAF (CEWAF), Heliyon 5 (2019) e01174.

[15] T.L. Wade, M. Morales-McDevitt, G. Bera, D. Shi, S. Sweet, B. Wang, G. Gold-Bouchot, A. Quigg, A.H. Knap, A method for the production of large volumes of WAF and CEWAF for dosing mesocosms to understand marine oil snow formation, Heliyon 3 (2017), e00419.

[16] C. Ritz, F. Baty, J.C. Streibig, D. Gerhard, Dose-response analysis using R, PloS one (2015) 10.

[17] J.A. McGrath, D.M. Di Toro, Validation of the target lipid model for toxicity assessment of residual petroleum constituents: Monocyclic and polycyclic aromatic hydrocarbons, Environ. Toxicol. Chem. 28 (2009) 1130–1148.

[18] M.M. McConville, J.P. Roberts, M. Boulais, B. Woodall, J.D. Butler, A.D. Redman, T.F. Parkerton, W.R. Arnold, J. Guyomarch, S. LeFloch, J. Bytingsvik, L. Camus, A. Volety, S.M. Brander, The sensitivity of a deep-sea fish species (Anoplopoma fimbria) to oil-associated aromatic compounds, dispersant, and Alaskan North Slope crude oil, Environ. Toxicol. Chem. 37 (2018) 2210–2221.

[19] D.A. Renegar, N.R. Turner, Species sensitivity assessment of five Atlantic scleractinian coral species to 1-methylnaphthalene, Sci. Rep. 11 (2021) 529.

[20] N.R. Turner, T.F. Parkerton, D.A. Renegar, Toxicity of two representative petroleum hydrocarbons, toluene and phenanthrene, to five Atlantic coral species, Mar. Pollut. Bull. 169 (2021), 112560.

[21] J.A. McGrath, C.J. Fanelli, D.M. Di Toro, T.F. Parkerton, A.D. Redman, M. L. Paumen, M. Comber, C.V. Eadsforth, K. den Haan, Re-evaluation of target lipid model–derived HC5 predictions for hydrocarbons, Environ. Toxicol. Chem. 37 (2018) 1579–1593.

[22] L.J. Schuler, P.F. Landrum, M.J. Lydy, Time-dependent toxicity of fluoranthene to freshwater invertebrates and the role of biotransformation on lethal body residues, Environ. Sci. Technol. 38 (2004) 6247–6255.

[23] R. Ashauer, I. O'Connor, A. Hintermeister, B.I. Escher, Death dilemma and organism recovery in ecotoxicology, Environ. Sci. Technol. 49 (2015) 10136–10146.

[24] B.I. Escher, R. Ashauer, S. Dyer, J.L. Hermens, J.H. Lee, H.A. Leslie, P. Mayer, J. P. Meador, M.S. Warne, Crucial role of mechanisms and modes of toxic action for understanding tissue residue toxicity and internal effect concentrations of organic chemicals, Integrated Environ. Assess. Manag. 7 (2011) 28–49.

[25] A.Pv Wezel, A. Opperhuizen, Narcosis due to environmental pollutants in aquaticorganisms: residue-based toxicity, mechanisms, and membrane burdens, Crit. Rev. Toxicol. 25 (1995) 255–279.

[26] H.J. Geyer, I. Scheunert, R. Bruggemann, M. Matthies, C.E. Steinberg, V. Zitko, A. Kettrup, W. Garrison, The relevance of aquatic organisms′ lipid content to the toxicity of lipophilic chemicals: toxicity of lindane to different fish species, Ecotoxicol. Environ. Saf. 28 (1994) 53–70.

[27] H.J. Geyer, C.E. Steinberg, I. Scheunert, R. Brüggemann, W. Schütz, A. Kettrup, K. Rozman, A review of the relationship between acute toxicity (LC50) of γ-hexachlorocyclohexane (γ-HCH, Lindane) and total lipid content of different fish species, Toxicology 83 (1993) 169–179.

[28] J. Donnelly, D. Stickney, J. Torres, Proximate and elemental composition and energy content of mesopelagic crustaceans from the Eastern Gulf of Mexico, Mar. Biol. 115 (1993) 469–480.

[29] M.J. Morris, T.L. Hopkins, Biochemical composition of crustacean zooplankton from the eastern Gulf of Mexico, J. Exp. Mar. Biol. Ecol. 69 (1983) 1–19.

[30] R. Calado, R. Rosa, S. Morais, M.L. Nunes, L. Narciso, Growth, survival, lipid and fatty acid profile of juvenile monaco shrimp Lysmata seticaudata fed on different diets, Aquacult. Res. 36 (2005) 493–504.

[31] T. Verslycke, C.R. Janssen, Effects of a changing abiotic environment on the energy metabolism in the estuarine mysid shrimp Neomysis integer (Crustacea: Mysidacea), J. Exp. Mar. Biol. Ecol. 279 (2002) 61–72.

[32] L. McCarty, J. Arnot, D. Mackay, Evaluation of critical body residue data for acute narcosis in aquatic organisms, Environ. Toxicol. Chem. 32 (2013) 2301–2314.