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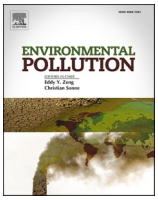

# Sensitivity of the Indo-Pacific coral *Acropora millepora* to aromatic hydrocarbons☆

Diane L. Brinkman [a,*,1], Florita Flores [a,1], Heidi M. Luter [a], F. Mikaela Nordborg [a,b,c], Maxime Brooks [a,b,c], Thomas F. Parkerton [d], Andrew P. Negri [a,b]

[a] *Australian Institute of Marine Science, Townsville, Queensland, 4810, Australia*
[b] *AIMS@JCU, Division of Research & Innovation, James Cook University and Australian Institute of Marine Science, Townsville, Queensland, 4810, Australia*
[c] *James Cook University, College of Science & Engineering, Townsville, Queensland 4810, Australia*
[d] *EnviSci Consulting, LLC, Austin, TX, USA*

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ABSTRACT

The risks posed by petroleum spills to coral reefs are poorly understood and quantifying acute toxicity thresholds for aromatic hydrocarbons to reef-building corals is required to assess their sensitivity relative to other taxa. In this study, we exposed *Acropora millepora* to toluene, naphthalene and 1-methylnaphthalene (1-MN) in a flow-through system and assessed survivorship and sublethal responses including growth, colour and the photosynthetic performance of symbionts. Median 50% lethal concentrations (LC50s) decreased over the 7-d exposure period, reaching asymptotic values of 22,921, 5,268, 1167 μg $L^{-1}$ for toluene, naphthalene and 1-MN, respectively. Corresponding toxicokinetic parameters ($\varepsilon_{LC50}$) defining the time progression of toxicity were 0.830, 0.692, and 0.256 $d^{-1}$, respectively. Latent effects after an additional 7-d recovery in uncontaminated seawater were not observed. Effect concentrations (EC50s) for 50% growth inhibition were 1.9- to 3.6-fold lower than the LC50s for each aromatic hydrocarbon. There were no observed effects of aromatic hydrocarbon exposure on colour score (a proxy for bleaching) or photosynthetic efficiency. Acute and chronic critical target lipid body burdens (CTLBBs) of 70.3 ± 16.3 and 13.6 ± 18.4 μmol $g^{-1}$ octanol (± standard error) were calculated for survival and growth inhibition based on 7-d LC50 and EC10 values, respectively. These species-specific constants indicate adult *A. millepora* is more sensitive than other corals reported so far but is of average sensitivity in comparison with other aquatic taxa in the target lipid model database. These results advance our understanding of acute hazards of petroleum contaminants to key habitat-building tropical coral reef species.

## 1. Introduction

Uncontrolled petroleum releases from platforms in the tropics (Storrie, 2011) and sub-tropics (Lubchenco et al., 2012), along with multiple recent shipping accidents in tropical waters (Daley, 2019; Gurumoorthi et al., 2021; Zacharias et al., 2021; Zúñiga and Faiola, 2020), have emphasised the need to better understand the risks posed by petroleum hydrocarbons to tropical marine organisms (reviewed in Turner and Renegar (2017) and Nordborg et al. (2020)). These risks are often represented as measured or modelled exceedances of ecological thresholds for harm to relevant species or ecosystems (French-McCay et al., 2018; NOPSEMA, 2019). Corals are the primary habitat-building species of tropical reefs, and an understanding of their sensitivity to petroleum spills is critical to assess the risks posed by spills to reefs of high conservation value.

Monocyclic aromatic hydrocarbons (MAHs) and the less volatile but more toxic polycyclic aromatic hydrocarbons (PAHs) are key hydrocarbon components in dissolved oil exposures that pose a hazard to aquatic receptors, including corals (French-McCay, 2002; Redman, 2015). Seawater concentrations of aromatic hydrocarbons can be highly dynamic but have been reported to reach 385 μg PAH $L^{-1}$ (Baum et al., 2016; Diercks et al., 2010) and 10,600 μg TRH $L^{-1}$ (Boehm and Fiest, 1982) in tropical waters polluted by ports, petroleum spills and blow-outs. Field studies following spills have documented a variety of impacts

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* Corresponding author. PMB No. 3, Townsville MC, Townsville, QLD, 4810, Australia.
*E-mail address:* d.brinkman@aims.gov.au (D.L. Brinkman).

[1] These authors contributed equally to this work.

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on coral populations and recruitment that can last decades (Guzman et al., 2020; Jackson et al., 1989). However, due to uncertain exposures and effects of additional stressors, field studies are not well suited to defining quantitative concentration-response relationships (Chapman, 2002). To address this issue several laboratory studies have quantified the effects of water accommodated fractions (WAFs) of a variety of oils and fuels to adult corals (reviewed in Turner and Renegar (2017); Nordborg et al. (2020)). These studies have reported an array of biological effects including mortality, tissue damage and effects on symbiosis, including reduced performance and/or loss of symbiotic algae. More recently, the effects of petroleum products have been reported on the early life stages of coral such as fertilisation, embryo development, larval settlement and survival, and on the survival and growth of coral recruits (Nordborg et al., 2022; Nordborg et al., 2021).

Direct comparisons of species and life-stage sensitivities to oil WAFs are not strictly valid when expressed in μg $L^{-1}$ as each oil is comprised of different proportions of MAHs, PAHs, soluble aliphatics and other unresolved compounds, all with different potencies (Di Toro et al., 2000; Redman and Parkerton, 2015). However, the acute toxicity of hydrocarbons to aquatic species is strongly correlated with the lipid solubility of individual compounds, with narcosis the presumed primary mode of acute action (Di Toro et al., 2000; French, 2000). In the target lipid model (TLM), each species (and life stage) has a specific critical target lipid body burden (CTLBB) that can be calculated from the linear relationship between the lethal or effect concentrations (LCx or ECx) and octanol-water partition constants ($K_{OW}$) for individual aromatics on a log scale (Eq. (1)) (Di Toro et al., 2000).

$$\log(\mathrm{LC}/\mathrm{EC}x_i) = m\log(K_{ow_i}) + \log(\mathrm{CTLBB}) + \Delta c_i \quad (1)$$

where for compound *i*, LC/ECx is the lethal or effect concentration affecting x percent of the test species population, *m* is the universal slope, $K_{OW}$ is the octanol-water partition coefficient, CTLBB is the critical target lipid body burden, and Δc is the chemical class correction.

CTLBBs are typically derived from the relationship between effect concentrations and $K_{OW}$ for at least three hydrocarbons with different $K_{OW}$ values (McGrath and Di Toro, 2009). However, *preliminary* CTLBBs can be calculated from a toxicity test with one hydrocarbon (Renegar and Turner, 2021), while *putative* CTLBBs can be calculated from observed oil effects data by applying an additive toxic unit model to complex oil WAF exposures of known composition (Negri et al., 2021; Nordborg et al., 2021). Furthermore, CTLBBs can be used to predict the toxicity of any neat oil or petroleum WAF of known composition in terms of toxic units (TU) or μg $L^{-1}$. The TLM databases published by McGrath et al. (2018) includes acute CTLBBs for 79 aquatic species (estimated from acute LC/EC50 data) and chronic CTLBBs for 36 species (calculated from longer duration survival, growth or reproduction LC/EC10 or NOEC/NEC data). These databases provide the opportunity to compare biological responses to hydrocarbon exposures between species based on CTLBB values.

Cumulative distributions of CTLBBs allow the calculation of hazard concentrations, including HC5 and HC50, that represent ecological threshold values above which 5% and 50% of the community of species with CTLBBs are predicted to be affected, respectively (McGrath et al., 2018). However, the suitability of HCx values modelled from CTLBBs in the TLM database for application in oil spill risk assessments in the tropics is uncertain (Negri et al., 2021) since only 10% of the 79 CTLBBs are representative of tropical marine species (McGrath et al., 2018). Recent studies have used measured 48 h LC50s to derive acute CTLBBs for five Atlantic corals, ranging from 181 to 572 μmol $g^{-1}$ octanol (Renegar and Turner, 2021; Turner et al., 2021), indicating these adult corals are less sensitive than the geometric mean of species in the TLM database (CTLBB of 71.1 μmol $g^{-1}$ octanol (McGrath et al., 2018)). Another recent study has derived acute putative CTLBBs ranging from 5.1 to 97 μmol $g^{-1}$ octanol for seven tropical marine species relevant to the Indo-Pacific which were exposed to a partially weathered condensate (Negri et al., 2021). The lowest CTLBBs were for early life stages of invertebrates, including inhibition of larval metamorphosis of the coral *Acropora millepora* (5.1 μmol $g^{-1}$ octanol) (Negri et al., 2021). A similar putative CTLBB of 4.1 μmol $g^{-1}$ octanol was obtained for metamorphosis inhibition of *A. millepora* larvae exposed to heavy fuel oil (HFO) (Nordborg et al., 2021). Low putative CTLBBs for other early life history stages of *A. millepora* have also been reported (Nordborg et al., 2022; Nordborg et al., 2021).

The objective of the current study was to derive acute and chronic CTLBBs for propagated adult colonies of the model Indo-Pacific coral *A. millepora* following 7-d exposures to three individual aromatic hydrocarbons. Establishing these CTLBBs will test hypotheses that the sensitivity of corals to dissolved hydrocarbons is life stage-dependent and that adult *A. millepora* is more sensitive than some Atlantic coral species. The *A. millepora* CTLBBs will also be valuable to assess the sensitivity of Indo-Pacific corals in comparison to species in the TLM databases, helping to validate whether ecological thresholds (i.e. HC5) for oil derived from the TLM are suitable to assess the likely consequences of oil spills to adult corals.

## 2. Methods

### 2.1. Experimental approach

A preliminary 4-d static renewal exposure of *A. millepora* to 1-methylnaphthalene (1-MN) was conducted to guide concentration selection for the flow-through experiment (Supplementary Materials, Section S1, Section S2). In the main experiment, experimental exposures to 8–10 concentrations of three individual aromatic hydrocarbons were conducted on small *Acropora millepora* (Ehrenberg, 1834) colonies (~11 mm fragments) in replicated flow-through exposures. Partial colony mortality was measured daily for 7 days and following an additional 7-d recovery in uncontaminated seawater to assess potential latent effects. Sublethal effects on colony growth, colour score, photosynthetic efficiency of symbionts (effective quantum yield) and damage to the photosystem II of symbionts (maximum quantum yield) were assessed after 7-d exposures as further detailed below.

### 2.2. Sample collection

Adult colonies (25–30 cm diameter) of *A. millepora* were collected in July 2020 and February 2021 from 5 to 8 m depth at Davies Reef, Great Barrier Reef (GBR; 18°49′52.7″S 147°38′07.8″E) under Great Barrier Reef Marine Park Authority (GBRMPA) permit G12/35236.1 and transported to the National Sea Simulator at the Australian Institute of Marine Science (AIMS, Townsville, Australia). Colonies were maintained in partially (50%) shaded outdoor flow-through tanks (1120 L) at 24 ± 1 °C (July 2020 colonies) and 28 ± 1 °C (February 2021 colonies) to match water temperatures at the collection sites.

Coral branches were cut longitudinally into small fragments (10–15 mm long × 6–8 mm wide) and affixed horizontally (Leal et al., 2016) with cyanoacrylate gel onto 20 mm square coloured glass tiles (Fig. S1). Coloured glass tiles were used to differentiate the coral colonies. Corals were allowed to heal in flow-through aquaria and acclimated over two weeks to the target experimental temperature and light conditions of 27 °C and 100 μmol photons $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR; blue and white dominated LED lights). During the acclimation period corals were fed daily *Artemia* (0.5 nauplii $mL^{-1}$) and a mix of microalgae (2000 cells $mL^{-1}$; *Tisochrysis lutea, Chaetoceros muelleri, Dunaliella* spp., *Nannochloropsis oceania, Proteomonas sulcata* cultured using AlgaBoost™ F/2 media (AusAqua, Australia). Fragments from five of the colonies that actively grew and suffered no mortality during the acclimation period were used for the experiments. Filamentous algae that had grown on the glass tiles over the acclimation period were manually removed immediately prior to toxicity test initiation.

### 2.3. Preparation of treatment solutions

Analytical grade toluene, naphthalene and 1-MN (Section S1, Table S1) were purchased from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany). For the flow-through exposure experiments, naphthalene and 1-MN were dissolved in dimethylsulfoxide (DMSO) to final concentrations of 200 and 167 mg $L^{-1}$ PAH, respectively. The concentrated PAH stocks were serially diluted in DMSO to produce a total of 7–9 stock solutions for each compound (Section S3, Table S3, Table S4). Treatment solutions for each compound were prepared at room temperature (22 ± 1 °C) and in darkness in solvent-rinsed glass aspirator bottles (5 or 10 L) pre-filled with 0.5 μm filtered natural seawater (FSW; 36 PSU, pH 8.1) to 80% capacity. Aliquots of the naphthalene and 1-MN stocks were added to FSW at a ratio of 1:10,000 (v/v; 0.01% DMSO final concentration). Variable volumes of neat toluene (40–4000 μL) were added directly to the FSW to achieve nominal treatment concentrations without the use of a carrier solvent (Table S5). The aspirator bottles were sealed with polytetrafluoroethylene (PTFE)-lined caps and the solutions were gently stirred on magnetic stirrer plates (40 mm stir bars; <5% vortex) for 18 h. Solutions were allowed to settle for 30 min and dispensed via glass/PTFE taps near the base of each aspirator bottle. All solutions were equilibrated to test temperature (27 °C) before application in toxicity tests. Fresh solutions were prepared every 48 h to replace treatment stocks in the exposure system.

### 2.4. Experimental exposures

Treatment solutions were delivered using a flow-through dosing system (Fig. S3) that pushed solutions through a titanium tube to the experimental glass chambers under positive air pressure (Negri et al., 2021). Flow rates were controlled by a programmable logic controller (Siemens PC7, Supervisory Control and Data Acquisition System) for a turnover rate of approximately one chamber per day. Treatment stock bottles were replaced every 48 h with freshly prepared solution. Based on results of the preliminary experiments, the exposure concentration ranges were chosen to ensure LC/EC50 values could be estimated for each aromatic hydrocarbon (required for calculation of CTLBB, see below). Seven-day exposures of *A. millepora* to 7-d time weighted average (TWA) concentrations of toluene (10 treatments up to 77,869 μg $L^{-1}$), naphthalene (10 treatments up to 8619 μg $L^{-1}$) and 1-MN (8 treatments up to 4427 μg $L^{-1}$) were conducted on three separate occasions (Section S3). Corals (n = 5 replicate coral fragments each from a different colony per chamber) were placed in custom glass chambers (450 mL; Negri et al. (2021)) with borosilicate lids and PTFE seals. Independent replicate chambers (n = 3–4) per treatment, seawater controls and solvent carrier controls (0.01% DMSO in FSW) were randomised throughout the experimental setup. Duplicate chambers containing treatment solutions with nominal concentrations of 44,000 μg $L^{-1}$ toluene, 10,000 μg $L^{-1}$ naphthalene or 6000 μg $L^{-1}$ 1-MN were randomly placed amongst the experimental chambers for chemical sampling during the tests. A separate experiment was performed to assess potential effects of the solvent carrier and consisted of eight seawater controls, ten DMSO controls (0.01% v/v) and four replicates of a mid-concentration of 1-MN (6000 μg $L^{-1}$ nominal), which was used as a positive control (refer to Section S3, Table S7). In all experiments, corals were returned to uncontaminated seawater for an additional 7-d recovery period to investigate the potential for latent effects, as recently recommended by Hook (2020).

Exposure to ultraviolet radiation (UVR) was included as a co-factor in the 1-MN experiment, since UVR can increase the toxicity of some aromatic hydrocarbons to corals (Negri et al., 2016; Nordborg et al., 2021; Nordborg et al., 2018). In this experiment, there were four replicate chambers per treatment including two replicate chambers exposed to visible light only (400–700 nm) and the other two replicate chambers exposed to visible light + UVR (300–400 nm). Corals were illuminated over 12:12 h light:dark cycles (blue and white dominated LED lights) with ramping up of light for the first 3 h to 100 μmol $m^{-2}$ $s^{-1}$ PAR then ramping down to darkness over the last 3 h (equivalent to 3.25 mol $m^{-2}$ $d^{-1}$ daily light integral, DLI). UVR channels were activated during the 1-MN experiments to provide UV-A (1.91 ± 0.35 mWatt $cm^{-2}$) and UV-B (0.038 ± 0.007 mWatt $cm^{-2}$) to half the chambers (Fig. S4, Table S9). Experimental chambers were partially submerged in water baths at 27 ± 1 °C to maintain temperature. UVR was not used as a co-stressor in the toluene and naphthalene exposures (n = 3 replicate chambers per concentration) since there was no significant effect of UVR exposure on coral survival exposed to 1-MN, and toluene and naphthalene are not considered phototoxic chemicals (Arfsten et al., 1996; NIST, 2023).

### 2.5. Sample collection and chemical analysis

Samples of each toluene and naphthalene treatment solution (n = 1) and duplicate samples of a mid-range 1-MN solution (6000 μg $L^{-1}$ nominal concentration) were collected for chemical analysis at test initiation. Control samples of FSW and 0.01% (v/v) DMSO in FSW were also collected. Sampling from designated chambers occurred at 4 h and 24 h after experiment initiation and thereafter at 24-h intervals until the end of the 7-d exposures. To monitor the consistency of 48-hourly treatment solution preparation, samples were collected from freshly prepared representative solutions (44,000 μg $L^{-1}$ toluene, 10,000 μg $L^{-1}$ naphthalene and 6000 μg $L^{-1}$ 1-MN nominal concentrations). All samples were collected in 40 mL solvent-rinsed amber vials with PTFE-lined septa. For toluene, the vials were pre-spiked with preservative (sulphuric acid; final pH < 2) and filled completely with sample (no headspace). Samples were stored at 4 °C until analysis at AIMS or ALS Environmental (Brisbane, Australia) as described in Section S1. The measured initial concentration of the mid-range 1-MN solution was used to estimate the initial concentrations in the other 1-MN treatment solutions (Table S6), assuming a similar solubility profile to that obtained in a separate 1-MN solubility study. TWA exposure concentrations in the chambers after 1, 2, 3, 4 and 7 days of exposure were calculated (refer to Section S3) and applied in statistical analyses to estimate toxicity thresholds at each of these timepoints.

### 2.6. Physico-chemical parameters

Irradiance (photosynthetically available spectrum (400–700 nm)) was measured with a LI-190 R quantum sensor with a LI-250 A light meter (LI-COR, Nebraska, USA) and UVR (UVA, UVB) was measured with a Model PMA2100 radiometer (Solar Light Company, Inc., Pennsylvania, USA). Salinity and pH were measured via a LAQUAact PC110 m (Horiba, Kyoto, Japan) and dissolved oxygen concentration was determined with a HQ30 d m equipped with IntelliCAL LDO101 oxygen sensor (Hach, Co., CO, USA). Temperature was logged in 15-min intervals via a HOBO pendant logger (Model UA-002-64; Onset Computer Corp, Massachusetts, USA). Water quality measurements were measured in the stock solution bottles at initiation of experiment, and in each experimental chamber at the completion of the 7-d exposure.

### 2.7. Coral survival and growth

Corals were photographed using a Nikon D810 digital camera at initiation of experiment, after 7-d exposures and after a further 7-d recovery. Lighting for photography was provided by a Nikon Speedlight SB-910 flash for 1-MN exposures and Ikelite DS161 strobes for the naphthalene and toluene exposures. An Olympus TG-6 camera and two waterproof LED lights (Lume Cube 2.0, Lume Cube, CA, USA), were used atop experimental chambers to photograph corals within the chambers from exposure days 1 through 6. Mortality and growth rates were assessed via the original images using the image processing package Fiji (Schindelin et al., 2012). Partial mortality was calculated by measuring the 2-dimensional area of the dead tissue relative to the total surface

area, while growth rate (in $mm^2\ day^{-1}$) was determined by the change in surface area after the 7-d exposures relative to the initial surface area (Fig. S1). Colour score and chlorophyll fluorescence measurements are described in the Section S3.

### 2.8. Statistical analysis

All statistical analyses and graphical results were performed in the software R (R Core Team, 2021). Concentrations that reduced coral tissue survivorship by 10% and 50% (LC10 and LC50, respectively) were estimated using the R package *bayesnec* V2.0.1 (Fisher et al., 2021) and its dependencies, including the package *brms* (Bürkner, 2017; Bürkner, 2018). *Bayesnec* uses a model averaging approach based on pseudoBMA weighted averaged predictions using a Bayesian bootstrap, with weights derived using the package *loo* (Vehtari et al., 2018; Vehtari et al., 2017) across a potential candidate model set composed of several commonly used concentration-response relationship models, including logistic, Weibull and hormesis models. Model fits were assessed visually through chain mixing, R hat values and an assessment of the number of divergent transitions. Models were excluded if they showed poor model fit. Modelled estimates for toxicity (LC/EC10 and LC/EC50 values) were calculated using weighted model averaged estimates across all successfully fitted models. See Section S3 for further details.

CTLBBs for coral survival and growth were compared to the CTLBBs in the current acute and chronic CTLBB databases (McGrath et al., 2018) and acute values for coral mortality (Renegar and Turner, 2021). Comparisons were performed by interpolating a percentile sensitivity ranking for each CTLBB from a species sensitivity distribution model (SSD) fitted to the CTLBBs included in the acute and chronic TLM databases (McGrath et al., 2018) on the log-normal distribution using the *ssdtools* package (Thorley and Schwarz, 2022) in R (R Core Team, 2021) as per Negri et al. (2021).

### 2.9. Toxicokinetics, chemical activity and CTLBB estimation

The minimum (incipient) lethal threshold ($LCx_\infty$) and first-order rate (ε), a lumped parameter that incorporates passive elimination, biotransformation, and damage repair processes (Jager et al., 2011), were simultaneously estimated for each test compound by fitting a one compartment toxicokinetic model (Eq. (2)) described by French-McCay et al. (2021) to LCx data for days 1, 2, 3, 4 and 7 using the R package *nlstools* (Baty et al., 2015). Plots were visualised using *ggplot2* (Wickham, 2016).

$$LCx_\infty = LCx_t\ (1 - e^{-\varepsilon t}) \quad 2$$

where $LCx_t$ is the lethal concentration affecting x percent of the test species population at time t, ε is the first-order rate of the test substance that characterises the time-dependent progression of observed toxicity, and t is the duration of exposure in days.

CTLBBs for coral mortality ($CTLBB_{LCx}$) and growth ($CTLBB_{ECx}$) were estimated by fitting a linear regression described by Eq. (1) to the experimental lethal or sublethal 7-d thresholds for the three aromatic hydrocarbons. Eq. (1) was parameterised as per McGrath et al. (2018) with a universal slope of −0.94 and Δc values of −0.025 (toluene) and −0.364 (naphthalene and 1-MN). Log ($K_{OW}$) values (Table S1) were sourced from EPI Suite v4.11 (USEPA, 2012). The model fit for each endpoint was optimised by minimising the sum of the residuals using the Goal Seek algorithm in Microsoft Excel, with the y-intercept representing the log CTLBB, expressed in mM. The standard error (SE) of each CTLBB on the arithmetic scale was calculated using Equation (3) (Di Toro et al., 2000).

$$SE_{CTLBB} = e^{\mu} \bullet \sqrt{e^{2\sigma^2} - e^{\sigma^2}} \quad 3$$

where $\mu = \ln(10) \times \log$ CTLBB and $\sigma = \ln(10) \times$ residual standard error [n–1 degrees of freedom].

The chemical activity of each aromatic hydrocarbon was calculated by dividing the 7-d LC50 with the subcooled liquid solubility ($S_L$) of the test compound. As toluene and 1-MN are liquids at experimental temperature (27 °C), $S_L$s were considered equal to the solubilities in seawater (Table S1). For naphthalene, $S_L$ was calculated from Eq. (4) (Prausnitz et al., 1999) using its solid solubility ($S_S$) in seawater (Table S1) and empirical thermodynamic data (McCullough et al., 1957).

$$S_L = S_S \bullet \exp\left(\frac{\Delta H_f}{RT_{tp}}\left(\frac{T_{tp}}{T} - 1\right) - \frac{\Delta C_p}{R} \bullet \left(\frac{T_{tp}}{T} - 1\right) + \frac{\Delta C_p}{R} \bullet \ln\left(\frac{T_{tp}}{T}\right)\right) \quad 4$$

where, $\Delta H_f$ is the enthalpy of fusion, R is the universal gas constant, $T_{tp}$ is the triple-point temperature, T is the experimental temperature, and $\Delta C_p$ is the difference in the heat capacities of the liquid and solid.

## 3. Results

### 3.1. Physicochemical conditions and treatment chemistry

Parameters, including temperature, dissolved oxygen, pH and salinity met acceptable criteria for all experiments (Table S9). In the preliminary 1-MN static-renewal test, the 4-d TWA concentration (410 μg $L^{-1}$) was 40% of the average initial measured concentration (Table S3). In the 7-d flow-through exposure experiments, initial measured concentrations of toluene and naphthalene correlated well with nominal concentrations and were 88% and 95% of nominal concentration, respectively, across the full concentration range (Fig. S5 and Fig. S6). The measured 1-MN treatment concentration (3823 μg $L^{-1}$) was initially 64% of nominal concentration (Table S6). Within the first 4–24 h of the experiments, measured concentrations in designated chambers (Table S6) decreased 78% (toluene), 43% (naphthalene) and 44% (1-MN), with losses likely to have occurred through volatility and adsorption to the interior surfaces of chambers. Thereafter, concentrations in these chambers stabilised and the average daily concentrations for toluene, naphthalene and 1-MN were 8317 ± 599, 4358 ± 653 and 1951 ± 220 μg $L^{-1}$, respectively, with each varying by 7.2, 15.0 and 11.3%, respectively (Table S6). The 7-d TWA concentrations were 10.1%, 6.6% and 0.8% higher, respectively (Table S8).

### 3.2. Lethal response

Survival of *A. millepora* colonies in the 4-d static renewal range-finding experiment followed a typical concentration response curve with exposure to 1-MN (Fig. S2), resulting in LC10 and LC50 values of 1481 (977–1630) and 1654 (1486–1860) μg $L^{-1}$, respectively. This guided selection of the concentration range for the 7-d flow-through experiments. There was no significant effect of UVR exposure on 1-MN concentrations on coral survival over the 7-d exposures ($p = 0.845$, Table S10), therefore replicates from the 1-MN experiment were pooled per concentration.

All control corals survived in the 7-d flow-through experiments for toluene, naphthalene, 1-MN and for the solvent carrier control experiment. Survival of *A. millepora* decreased with increasing 1-MN concentrations and increasing exposure duration (Fig. 1). Coral mortality was characterised by exposed coral skeleton, and over longer durations by algal overgrowth (Fig. S8). Similar concentration-response curves were obtained for toluene (Fig. S9) and naphthalene (Fig. S10). The LC50 value for 1-MN after 4-d exposure in the pilot static renewal experiment (Fig. S2) was within 10% of the 4-d LC50 derived from the flow-through experiment (Table S11), confirming the consistency of responses to the TWA concentrations between experiments. By day 7, the LC50s for toluene, naphthalene and 1-MN had reached minima of 22,921 (20,454–25,173), 5268 (4910–5583) and 1167 (1105–1227) μg $L^{-1}$, respectively (Table S11). After 7-d recovery in uncontaminated

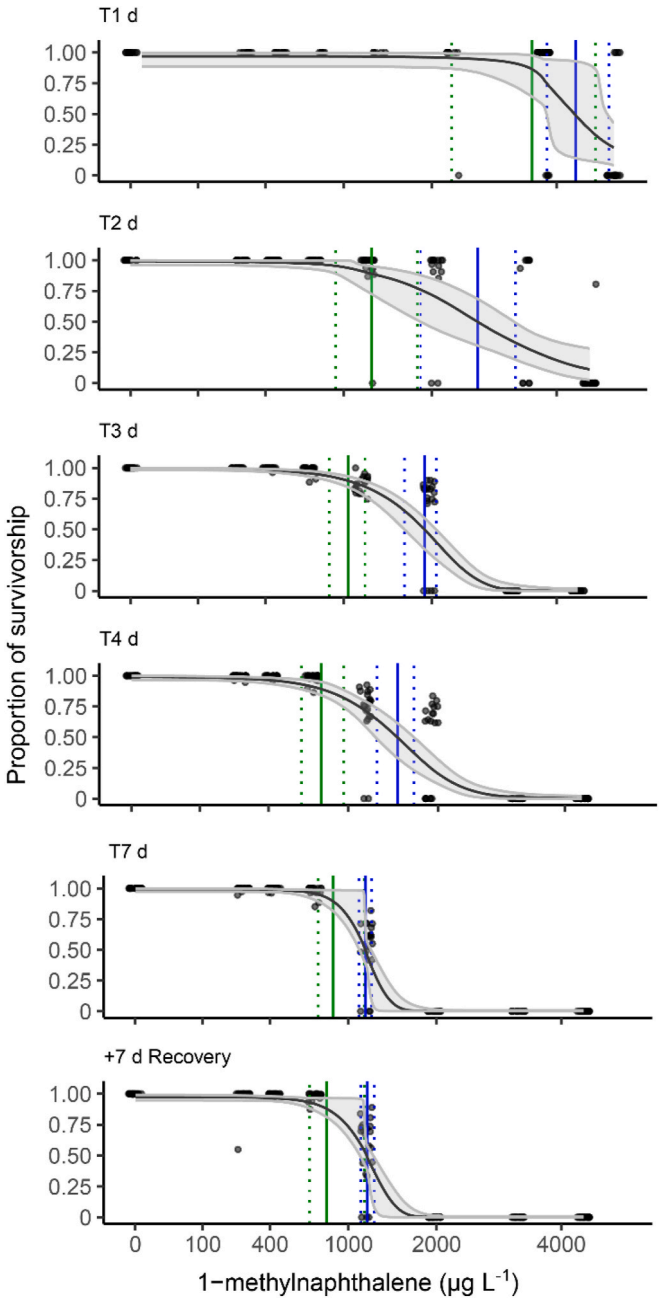


**Fig. 1.** Concentration-response curves for adult *Acropora millepora* exposures to increasing concentrations of 1-methylnaphthalene (1-MN) in flow-through chambers for 7 days, followed by 7 days of recovery in uncontaminated seawater. The proportion of survivorship was assessed at 5 timepoints during the exposure period, and after 7 days of post-exposure recovery. The solid black lines are the Bayesian non-linear Bernoulli (for t = T1 d only) and beta-binomial model fits on the proportional decline in coral tissue survivorship relative to initial tissue surface area of the coral fragment. Shaded grey areas are the 95% credible intervals (CIs) of the models. LC10s and LC50s (10% and 50% lethal concentrations) are depicted by green and blue lines, respectively, with corresponding 95% CIs indicated by dotted green and blue lines. n = 20 corals per treatment in 4 replicate test chambers. Data points are horizontally jittered to visualise all replicates. Refer to Fig. S9 and Fig. S10 for toluene and naphthalene exposure duration-dependent concentration-response curves, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

seawater, the LC50s of toluene and 1-MN had not changed significantly, while the LC50 for naphthalene increased 14% (p = 0.008) (Table S11, Fig. S11), indicating some recovery had occurred after 7 days in uncontaminated seawater. These results indicate no evidence of latent toxicity. The acute/chronic ratios (LC50/LC10) after 7-d exposures were low for all three aromatic hydrocarbons: 1.47 (toluene), 1.43 (naphthalene) and 1.35 (1-MN; Table S11) and highlights the steep response that is characteristically observed in hydrocarbon toxicity studies across diverse aquatic taxa (Redman et al., 2022).

### 3.3. Growth

The growth rates of *A. millepora* colonies were relatively slow, so to maximise precision only 7-d data were reported. Mean (± SD) coral growth rate in control treatments over 7 days was 2.16 ± 1.94, 5.51 ± 3.17 and 3.03 ± 1.84 mm$^2$ d$^{-1}$ for toluene, naphthalene and 1-MN, respectively (Fig. 2). Growth responses were far less consistent than survival between replicates, resulting in wide 95% credible intervals for EC10 and EC50 estimates. Acute to chronic ratios derived from the ratio

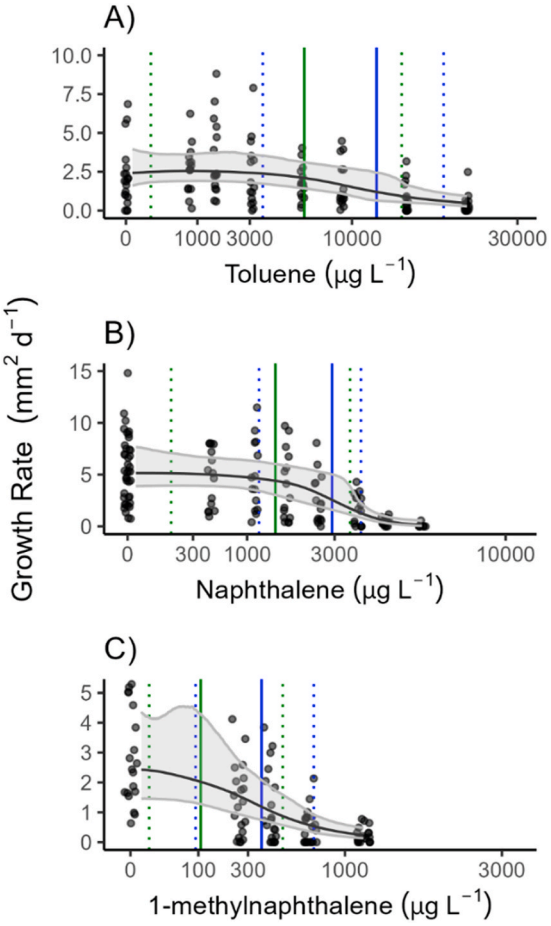


**Fig. 2.** Concentration-response curves for *Acropora millepora* growth rates following exposure to A) toluene, B) naphthalene and C) 1-methylnaphthalene (1-MN) in flow-through chambers for 7 days. Solid black lines are the Bayesian non-linear gamma model fits on the 7-d increase in surface area relative to the initial surface area of the coral fragment. Shaded grey areas are the 95% credible intervals (CIs) of the models. EC10s and EC50s (10% and 50% effect concentrations) are depicted by green and blue lines, respectively, with corresponding 95% CIs indicated by dotted green and blue lines. n = 15–20 corals per toluene treatment; n = 7–20 corals per naphthalene treatment; n = 20 corals per 1-MN treatment. Data points are horizontally jittered such that all replicates can be observed. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of 7-d LC50 to EC10 values were: 3.7 (toluene), 3.4 (naphthalene) and 10.9 (1-MN; Table S11). These estimates bracket the median ACR of 5.2 and fall within the 5th to 95th percentile range (2.3–11.9) included in the TLM database for other aquatic test species (McGrath et al., 2018).

### 3.4. Colour score and chlorophyll fluorescence

The mean colour scores for control corals at start of experiment were 6.4, 6.4 and 5.5 and after 7-d exposures were 6.2, 6.0 and 3.9 for the toluene, naphthalene and 1-MN experiments, respectively. There was no evidence of a treatment effect on coral colour for any of the three aromatic hydrocarbons (Table S10). Though there was a significant interaction between treatment and time (Table S10), the difference in colour between exposure initiation and termination was more likely due to the corals acclimating to the experimental conditions. In addition, the difference in colour score did not exceed 2 scale points of the 6-point Coral Health Chart reference card (scale = 1–6 points with 1 being the lightest colour code and 6 the darkest), which infers that no significant change in symbiont density occurred (Siebeck et al., 2006).

Effective quantum yields ($\Delta F/F_m'$) of control corals at the start of the experiment were 0.67, 0.65, 0.60 for toluene, naphthalene and 1-MN, respectively, while maximum quantum yields ($F_v/F_m$) were 0.70, 0.71, 0.64. In general, there was no treatment effect on $\Delta F/F_m'$ or $F_v/F_m$ after 7-d exposures (Table S10). Although there was a slight influence of treatment for $\Delta F/F_m'$ in corals exposed to naphthalene and toluene, deviations from the control values were less than 6% (Table S11).

### 3.5. Toxicokinetics and chemical activity

Lethal concentrations (LC10s and LC50s) for each aromatic hydrocarbon (Table S11) decreased most steeply within the first 48 h of exposures, then approached asymptote by day 7 (Fig. 3). $LCx_\infty$ and $\varepsilon$ values were estimated for each aromatic hydrocarbon by fitting the one-compartment toxicokinetic model described by French-McCay et al. (2021) to the LC50 (Fig. 3) and LC10 (Fig. S13) data at 5 timepoints during the experiment (Table S11). The 7-d lethal thresholds (LC10s and LC50s) for the three aromatic hydrocarbons were generally lower than or similar (overlapping 95% credible intervals) to the modelled incipient thresholds (Table 1), indicating that all toxicant concentrations had reached equilibrium in the corals between 4 and 7 days of exposure. Consequently, the experimental 7-d thresholds were used for the subsequent estimation of CTLBBs. The $LC10_{\blacklozenge}$ for 1-MN was an order of magnitude lower than the experimental LC10, but this was likely due to less robust LC10 thresholds and poor toxicokinetic model fit (Fig. S13). To explore the relationship between acute first-order rates ($\varepsilon_{LC50}$) and hydrophobicity of the test compounds, $\varepsilon_{LC50}$ values (Table 1) were plotted as a function of $K_{OW}$ on a log-log scale (Fig. S14). While $\varepsilon_{LC50}$ is slightly higher for toluene than naphthalene, the 95% confidence intervals overlap. In contrast, the $\varepsilon_{LC50}$ for 1-MN appears significantly lower, indicating a slower progression of toxicity at higher log $K_{ow}$. Using the mean estimates from all three test compounds in a log-log regression analysis with $K_{ow}$, the slope and intercept were estimated to be −0.425 and 1.059, respectively ($R^2$ = 0.83; Fig. S14). However, $\varepsilon_{LC50}$ estimates were better correlated to the square root of the $K_{OW}$ ($R^2$ = 0.98; Fig. 4) as proposed by Kooijman et al. (2004) for a well-mixed one-compartment toxicokinetic model based on mass transfer considerations. The calculated subcooled liquid solubility of naphthalene at experimental temperature was 72.4 mg $L^{-1}$, and aqueous exposures expressed as chemical activities causing 50% mortality (LA50) occurred in a narrow range from 0.046 to 0.073 (Table 1).

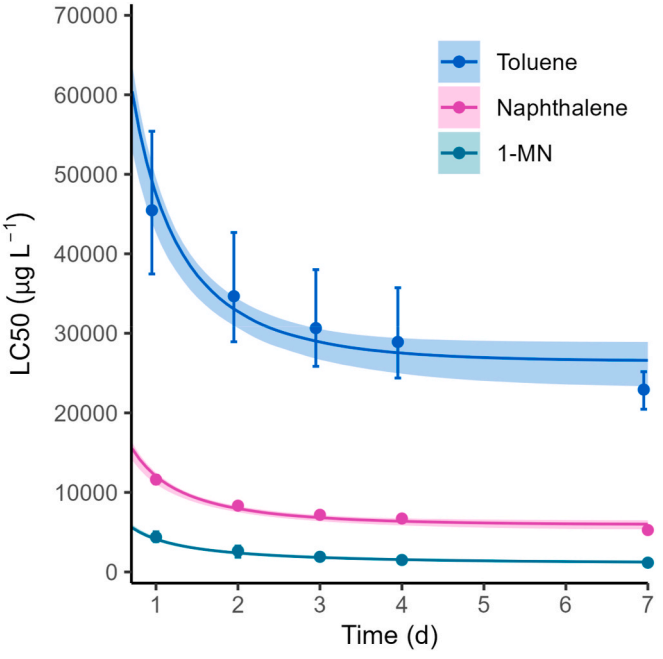


**Fig. 3.** Time-dependent, median acute 50% lethal concentrations (LC50) for *Acropora millepora* following exposures to toluene, naphthalene and 1-methylnaphthalene (1-MN) in flow-through chambers for 7 days. Solid circles indicate experimental LC50s after 1, 2, 3, 4 and 7 days of exposure. Vertical bars indicate the 95% credible intervals of the LC50s. Solid lines indicate the asymptotic regression model fits. Shaded areas represent the 95% bootstrap confidence intervals of the model.

### 3.6. Derivation of CTLBBs

Acute CTLBBs were derived from linear relationships of the experimental survivorship and growth thresholds (7-d LC50 and EC50) and the $K_{OW}$ of the single aromatic hydrocarbons on a log-log scale (Fig. 5, Table 2). Chronic CTLBBs were likewise derived from 7-d LC10 and EC10 values (Fig. S15, Table 2). The acute $CTLBB_{LC50}$ of 70.3 µmol $g^{-1}$ octanol, indicates *A. millepora* was of average sensitivity (49%) relative to 79 species in the TLM database (McGrath et al., 2018), while the $CTLBB_{EC50}$ of 32.1 µmol $g^{-1}$ octanol places growth inhibition of *A. millepora* among the 17% most sensitive endpoints for aquatic taxa (Table 2, Fig. 6). Similar comparisons were made for chronic $CTLBB_{LC10}$ and $CTLBB_{EC10}$ values, which indicated *A. millepora* exhibited moderate (growth) or less (survival) sensitivity compared to the 36 species in the chronic TLM database (Table 2, Fig. 6).

## 4. Discussion

### 4.1. Coral sensitivity to aromatic hydrocarbon exposure

Measured concentrations of toluene, naphthalene and 1-MN decreased substantially within the first 4–24 h after test initiation, then stabilised for the remainder of the 7-d exposures, varying by ≤ 15%. Future studies could incorporate passive dosing to generate more stable concentrations of individual aromatic hydrocarbons (Bera et al., 2018; Turner et al., 2021) and passive sampling to verify the TWA concentrations over the entire exposure (Letinski et al., 2022; Redman et al., 2018). Despite the initial drop in exposure concentrations, the current study was one of few truly flow-through systems used in coral-oil ecotoxicology; an advantage over alternative recirculating systems that may allow the build-up of mucous, metabolites and nutrients as well as deoxygenation from stressed corals.

Exposure to toluene, naphthalene and 1-MN over 7 days caused lethal and sublethal effects (growth) on *A. millepora,* but no effects were observed on microalgal symbionts. Increased tissue death with increasing aromatic concentrations is consistent with previous toxicity studies exposing adult coral colonies to individual aromatic hydrocarbons (e.g., Renegar et al. (2017); Renegar and Turner (2021); Turner et al. (2021); Bytingsvik et al. (2020)) or dissolved oil (May et al., 2020;

**Table 1**
Acute 50% lethal (LC50) and 50% effect (EC50) concentrations for adult *Acropora millepora* following 7-d flow-through exposures to toluene, naphthalene and 1-methylnaphthalene (1-MN), with 95% credible intervals (CIs) in brackets. Incipient LC50 ($LC50_{\infty}$) and first-order rate ($\varepsilon_{LC50}$) estimates, derived from toxicokinetic modelling, are reported with 95% CIs in brackets. The lethal toxicity of the aromatic hydrocarbons after 7-d exposures (7-d LA50), is expressed in terms of chemical activity causing 50% mortality, with 95% CIs in brackets. Acute LC10 (10% lethal concentrations) and LC50 values for other timepoints can be found in Table S11.

| Compound | 7-d LC50 (μg $L^{-1}$) | $LC50_{\infty}$ (μg $L^{-1}$) | 7-d EC50 (μg $L^{-1}$) | $\varepsilon_{LC50}$ ($d^{-1}$) | 7-d LA50 |
|---|---|---|---|---|---|
| Toluene | 22,921 (20,454–25,173) | 26,510 (23,196–28,906) | 12,294 (3658–19,747) | 0.830 (0.648–1.089) | 0.058 (0.052–0.064) |
| Naphthalene | 5268 (4910–5583) | 5991 (5244–6479) | 2926 (1210–3812) | 0.692 (0.558–0.865) | 0.073 (0.068–0.077) |
| 1-MN | 1167 (1105–1227) | 1000 (853–1184) | 374 (92–731) | 0.256 (0.206–0.317) | 0.049 (0.046–0.051) |

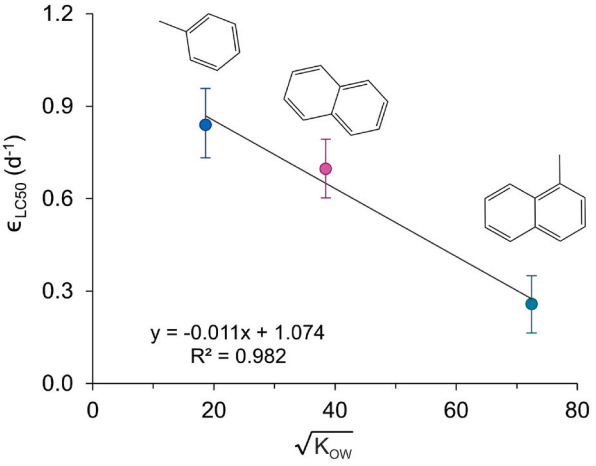


**Fig. 4.** Linear relationship between the first-order rate characterising time-dependence of 50% lethal concentrations (LC50s) for adult *Acropora millepora* ($\varepsilon_{LC50}$) and the square root of the octanol-water partition coefficients ($K_{ow}$) of toluene (blue), naphthalene (magenta) and 1-methylnaphthalene (teal). The solid line indicates the linear regression. Vertical bars indicate the 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

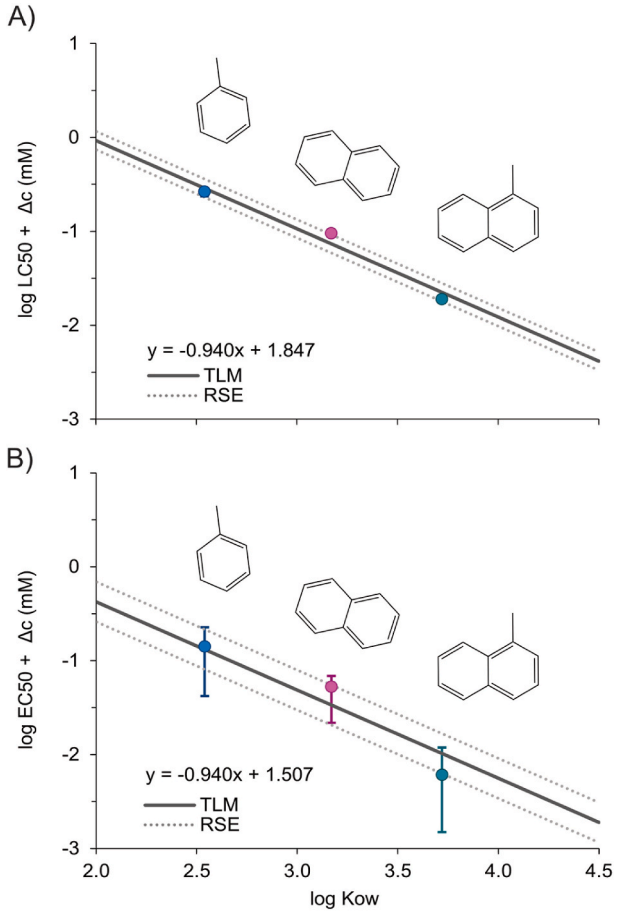


**Fig. 5.** Acute 7-d critical target lipid body burdens (CTLBBs) for adult *Acropora millepora* A) survivorship and B) growth based on median 50% lethal and effect concentrations, LC50 and EC50, respectively. Vertical bars indicate the 95% credible intervals of the thresholds and are smaller than the symbols in A). Dotted lines indicate the residual standard error of the regression fit. The chemical structures of each compound are above the corresponding datapoint.

Mercurio et al., 2004; Rinkevich and Loya, 1979; Villanueva et al., 2011). It is likely that the observed tissue death was due to non-specific narcosis, since the chemical activity of toluene, naphthalene and 1-MN to *A. millepora* were within the range expected for baseline toxicity (0.01–0.1; Mackay et al. (2011) and Schmidt and Mayer (2015)). Comparison of 2-d acute species-specific sensitivity constants for mortality ($CTLBB_{LC50}$) indicates that adult *A. millepora* may be more sensitive to petroleum hydrocarbons than other shallow water corals for which $CTLBB_{LC50}$ values have been derived (Table S14). The CTLBBs for mortality and coral growth of *A. millepora* advance our understanding of the relative sensitivity of tropical reef-building species for application in oil spill risk assessments in these habitats.

### 4.2. Time-dependence of toxicity

Mortality occurred within 24 h at higher concentrations of all aromatic hydrocarbons tested, indicating the potential for adult *A. millepora* mortality during short exposures to oil spills. Thereafter, LC50s continued to decline 2–3.5-fold, reaching minimum values between 4 and 7 days. The LC50s for aromatic hydrocarbons with $K_{OW} < 4$ typically reach minimum (incipient) values in toxicity tests with aquatic species within 4 days (French-McCay, 2002); however, for *A. millepora* LC50s continued to decrease after this period. The first-order rates ($\varepsilon_{L/EC50}$) derived from previous warm water coral toxicity studies are compared to the results of our study in Table S15. The $\varepsilon_{LC50}$ values obtained in the present work, ranging from 0.83 (toluene) to 0.26 (1-MN) $d^{-1}$, fall at the low end of previously reported values, indicating a generally slower progression of toxicity with *A. millepora* than other coral species investigated to date. Our experimental estimates are also consistently lower than predicted using the equation applied by French-McCay (2002) by a

**Table 2**
Acute and chronic 7-d critical target lipid body burdens (CTLBBs) ± standard error (SE) for *Acropora millepora* and their sensitivity percentile (± SE) relative to the 79 species in the target lipid model (TLM) acute toxicity database or the 36 species in the TLM chronic toxicity database (McGrath et al., 2018).

| | Acute | | Chronic | |
|---|---|---|---|---|
| | CTLBB (μmol $g^{-1}$ octanol) | Relative to database (%) | CTLBB (μmol $g^{-1}$ octanol) | Relative to database (%) |
| Lethal | 70.3 ± 16.3 | 49 (37–59) | 49.6 ± 10.9 | 88 (83–91) |
| Growth | 32.1 ± 18.8 | 17 (2.3–35) | 13.6 ± 18.4 | 54 (0–79) |

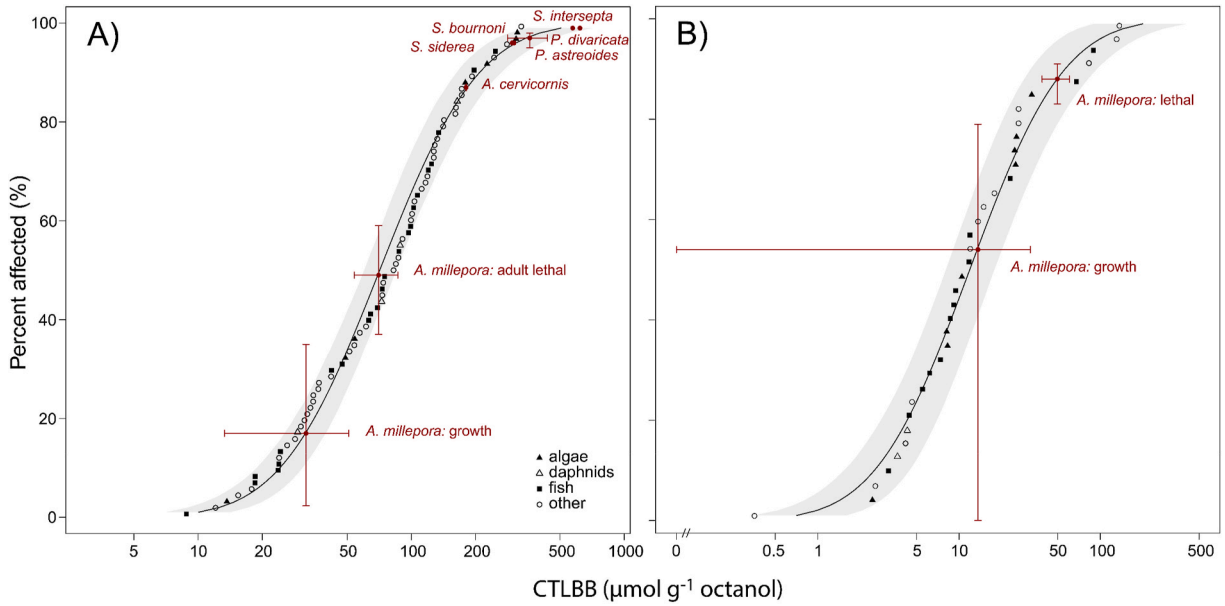


**Fig. 6.** Species sensitivity distributions of critical target lipid body burdens (CTLBBs) for A) 79 species in the acute target lipid model (TLM) toxicity database and B) 36 species in the chronic TLM database (McGrath et al., 2018). Ecologically relevant CTLBBs for corals (Table S14) are overlayed in red on the distributions, with red vertical and horizontal bars indicating standard error. Shaded grey area represents the 95% credible intervals of the models. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

factor of 3–4 across test compounds (Table S14). The propagated coral colonies used here were relatively small (~11 mm length), and durations for body burdens to reach steady state may be longer for corals with morphologies that have lower surface area to volume ratios (potentially lower $\varepsilon_{LC50}$ values). However, Turner et al. (2021) reported $\varepsilon_{LC50}$ values for the related coral *Acropora cervicornis* (2–3 cm branch tips) of 6.48 and 0.58 $d^{-1}$ for toluene and 1-MN respectively, indicating more rapid toxicokinetics compared to *A. millepora* (especially for toluene). In comparison, $\varepsilon_{EC50}$ values obtained using health scores rather than lethality yielded estimates for *A. cervicornis* of 1.42 and 0.88 $d^{-1}$ for toluene and 1-MN respectively (Turner et al., 2021). While there appears to be considerable variability in estimates between similar species and acute endpoints used in analysis, rates for toluene appear higher than 1-MN suggesting some dependence of this parameter on substance hydrophobicity. The $\varepsilon_{EC50}$ values derived in this work are comparable to estimates reported in a meta-analysis of 42 invertebrate species in which the 10th to 90th percentile ranged from 0.28 to 2.8 $d^{-1}$ (Redman et al., 2022). However, unlike the findings reported in this study (Fig. 4), no significant log-log dependence between $\varepsilon_{L/EC50}$ and test substance $K_{ow}$ was found across the diverse invertebrates evaluated. Given the limited number of hydrocarbons in this study, further toxicity data on additional hydrocarbons are needed to confirm the significance and nature of a $K_{ow}$ dependence on the rate parameter governing time-dependent toxicity in *A. millepora* and other coral species.

There were no negative latent effects of the 7-d exposures after an additional 7-d recovery in uncontaminated seawater, and small but significant recovery was observed for corals exposed to naphthalene. Recovery of corals, indicated by an increase in LC50, is possible since survival was normalised to colony area and polyps which had died could be partially overgrown by expanding or newly budding polyps. A continued decline in coral health during the recovery period might have indicated specific or irreversible toxicity mechanisms; however, the observed absence of latent effects could be interpreted as being consistent with baseline or narcotic toxicity, where cellular damage is considered reversible (Lee et al., 2002). Latent effects have previously been reported for the survival of *A. millepora* embryos and 1-week old recruits following exposure to HFO WAF (Nordborg et al., 2022; Nordborg et al., 2021). However, the lack of observed latent effects in this work is consistent with the findings of a previous adult coral toxicity study using 1-MN (Renegar et al., 2017), suggesting the occurrence of latent effects may be dependent on the coral life stage investigated or the presence of heavier aromatics in treatment solutions.

### 4.3. Lethal CTLBBs

Regression analysis of experimental log LCx against log $K_{OW}$ values produced slopes of −0.96 (LC50) and −0.93 (LC10), which were in good agreement with the universal slope (−0.94) for non-polar narcotic compounds and species in the TLM database (McGrath et al., 2018). This analysis further suggests that the observed lethal responses in *A. millepora* to toluene, naphthalene and 1-MN were due to non-specific membrane disruption (narcosis). The 7-d LC50s were generally lower than the incipient LC50s predicted from the asymptotic curve and therefore used to calculate acute and chronic CTLBBs under steady state conditions. The acute $CTLBB_{LC50}$ for *A. millepora* of 70.3 µmol $g^{-1}$ octanol is almost identical to the geometric mean of the 79 species in the TLM database (71 µmol $g^{-1}$ octanol; McGrath et al. (2018)), indicating this coral is of average sensitivity compared with other aquatic taxa. *A. millepora* is substantially more sensitive than five Atlantic species of adult corals which had $CTLBB_{LC50}$s ranging from 181 to 572 µmol $g^{-1}$ octanol (Renegar and Turner, 2021; Turner et al., 2021). *Putative* lethal CTLBBs for coral recruits have been estimated from the total dissolved aromatic LC50s of HFO (Nordborg et al., 2022). That study indicated that 1-week and 2-month-old recruits exposed to HFO for 7 and 14 days respectively, had very low putative CTLBBs (2.2 and 8.1 µmol $g^{-1}$ octanol, respectively). These very low putative CTLBBs place mm-sized coral recruits among the most sensitive aquatic species in the TLM database (McGrath et al., 2018). However, care should be taken in comparing putative CTLBBs, especially those derived from complex oils such as HFO, where unmeasured components such as tetralins, decalins, acenaphthenes, fluorenes, biphenyls, benzothiophenes, naphthobenzothiophenes, etc., may also contribute to observed effects. Further studies exposing coral recruits to individual aromatic hydrocarbons are required to validate these low values.

### 4.4. Sublethal CTLBBs

Along with lethal effects, inhibition of growth and disruption of reproductive processes are considered ecologically relevant responses to contaminants in aquatic organisms (van Straalen, 2003; Warne et al., 2018). The $CTLBB_{EC50}$ and $CTLBB_{EC10}$ for growth inhibition of *A. millepora* of 32.1 ± 18.8 and 13.6 ± 18.4 μmol $g^{-1}$ octanol was 2- and 4-fold lower than the lethal $CTLBB_{LC50}$ and $CTLBB_{LC10}$, respectively, indicating that effects on growth are a more sensitive, ecologically relevant indicator of stress in this species. However, the growth of these small coral colonies was relatively inconsistent, leading to larger uncertainties in the CTLBB estimates. Nevertheless, horizontal attachment of the laterally sliced branches exposed a large surface area to consistent light intensities and enabled highly precise surface area measurements of basal growth in two dimensions (Fig. S1). This method to measure growth is well suited to small, relatively flat microcolonies. However, for larger colonies, in particular those expected to exhibit vertical growth, new 3-D scanning techniques are likely to offer a greater precision (Koch et al., 2021) and should be considered in future ecotoxicological studies.

The effects of oil have now been reported for all life history stages of *A. millepora*, with putative CTLBBs estimated from HFO concentrations for ecologically relevant sub-lethal impacts on fertilisation, embryogenesis, larval settlement and recruit growth (Nordborg et al., 2022; Nordborg et al., 2021; Nordborg et al., 2018). Of these endpoints, inhibition of larval metamorphosis is among the most sensitive and consistent with acute putative CTLBB estimates of 5.1 and 4.4 μmol $g^{-1}$ octanol calculated from 48 h exposures to condensate (Negri et al., 2021) and HFO (Nordborg et al., 2022; Nordborg et al., 2021), respectively. The order of magnitude difference in sensitivity between acute responses (morality and growth) in adult colonies and larval metamorphosis inhibition in the same species of coral is consistent with other studies that have long demonstrated the greater sensitivity of early life stages (Connor, 1972; Rice et al., 1977), and should be an important consideration when selecting species thresholds for risk assessments.

Other sublethal acute CTLBBs have previously been presented for adult corals. For example, CTLBBs ranging from 170 to 363 μmol $g^{-1}$ octanol corresponding to *coral condition* (a semi-quantitative metric summing sub-lethal responses: polyp extension/retraction, tissue swelling, tissue attenuation and mucus production) have been reported for five Atlantic species (*A. cervicornis, P. astreoides, S. siderea, S. intersepta,* and *S. bournoni*) (Turner et al., 2021). A lower CTLBB of 51.6 μmol $g^{-1}$ octanol has been reported for the inhibition of polyp activity in the cold-water coral *Lophelia pertusa* (Bytingsvik et al., 2020). Collectively, these endpoints appear less sensitive than the sublethal effects (growth) examined in this study for *A. millepora* (Table S14). Further work is required to understand the relationship of coral condition and polyp activity with ecologically important whole-organism effects that are linked to population growth and reproduction.

### 4.5. Sensitivity of symbionts

No effects of 7-d exposure to aromatic hydrocarbons were observed on symbiont photochemical efficiency ($\Delta F/F_m'$ or $F_v/F_m$) or bleaching (colour score) at concentrations of toluene, naphthalene and 1-MN approaching LC50s. The lack of effect on $F_v/F_m$ indicates no evidence of damage to Photosystem II and suggests that the primary effects of these aromatic hydrocarbons over the 7-d exposures was on the animal host tissue. A recent study determined an estimated putative CTLBB of 45 μmol $g^{-1}$ in cultures of the coral symbiont *Cladocopium goreaui* exposed to condensate (light crude) (Negri et al., 2021). This indicates cultured Symbiodiniaceae should be almost twice as sensitive as adult *A. millepora* to non-polar narcotic compounds. However, Symbiodiniaceae live within the host cells (Wakefield and Kempf, 2001) and the contaminants would have to cross multiple cellular barriers, potentially limiting their exposure. Nevertheless, translocation of nutrients between the host and symbiont is well documented (Tremblay et al., 2012), and polar contaminants (e.g. herbicides) can rapidly access coral symbionts within host tissues (Jones et al., 2003). The lack of effect of aromatic compounds on coral symbionts *in hospite* and symbiosis (bleaching) is consistent with *A. millepora* recruits exposed to HFO (Nordborg et al., 2022), but studies with *A. millepora* and other species have reported effects on $F_v/F_m$, $\Delta F/F_m'$ and/or bleaching (Ashok et al., 2020; May et al., 2020; Renegar and Turner, 2021; Renegar et al., 2017; Turner et al., 2021). Differences may be due to species-specific symbiont sensitivities or to divergence in methodology. Higher light intensities and exposure to UV radiation may cause additional oxidative damage to symbionts in the presence of aromatic hydrocarbons, resulting in greater effects on $F_v/F_m$ and $\Delta F/F_m'$. Furthermore, it can be difficult to distinguish precise live-dead tissue boundaries, and reduced $F_v/F_m$, $\Delta F/F_m'$ or colour might result from the inclusion of coral tissue that had already died from exposure (Bessell-Browne et al., 2017). Nevertheless, functional symbiosis is critical for the health of scleractinian corals (Bessell-Browne et al., 2017; Muscatine, 1990) and assessing symbiont densities and health in response to xenobiotics, including aromatic hydrocarbons, remains a potentially valuable metric to understand coral health in ecotoxicological studies.

## 5. Conclusion

The exposure of small *A. millepora* colonies to toluene, naphthalene and 1-MN revealed slower toxicokinetics than previous coral toxicity studies, with steady state lethal effects occurring within 4–7 days. The chemical activities of these aromatic hydrocarbons, and the strong relationship between $K_{OW}$ and toxicity, implies a narcotic mode of action. Thus, the resulting CTLBBs derived for *A. millepora* in this work are suitable for inclusion in toxicity modelling of petroleum hydrocarbons for risk assessment purposes. Of the 79 species in the acute CTLBB database (McGrath et al., 2018), less than 10% are tropical marine taxa and none are reef-building corals. The acute $CTLBB_{LC50}$ calculated for adult *A. millepora* places this species among the more sensitive coral species to nonpolar narcotic compounds tested so far, but of average sensitivity in comparison to other aquatic taxa. Adult *A. millepora* were confirmed as being over an order of magnitude less sensitive to aromatic hydrocarbons than some early life stages of the same species, yet more sensitive than Atlantic species tested so far. The present study contributes to an improved understanding of the hazards of petroleum contaminants to key reef-building coral reef species, and indicates that HC5 values modelled from the TLM database (McGrath et al., 2018) would be protective of adult colonies of the hard coral *Acropora millepora*.

## Credit author statement

**Diane L. Brinkman** and **Florita Flores**: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing–original draft, Writing–review & editing, Visualization. **Heidi M. Luter, F. Mikaela Nordborg** and **Maxime Brooks:** Methodology, Investigation, Data curation, Formal analysis, Writing–review & editing. **Thomas F. Parkerton:** Methodology, Writing–review & editing. **Andrew P. Negri:** Conceptualization, Methodology, Writing–original draft, Writing–review & editing.

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## 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 acquired in the current study is available on the associated page of the Australian Institute of Marine Science data repository (AIMS, 2022).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121963.

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## Glossary

*1-MN:* 1-methylnaphthalene
*CTLBB:* critical target lipid body burden
*DMSO:* dimethylsulfoxide;
*ε:* first order rate
*ΔF/Fm′:* effective quantum yield
*Fv/Fm:* maximum quantum yield
*FSW:* filtered seawater
*GBR:* Great Barrier Reef
*HCx:* hazard concentration protective of x percent of species in a community
*HFO:* heavy fuel oil
*LC/ECx:* lethal or effect concentration, where x percent of organisms are affected
$K_{OW}$*:* octanol-water partition coefficient
*LA50:* chemical activity causing 50% mortality
*MAH:* monocyclic aromatic hydrocarbon
*NEC:* no effect concentration
*NOEC:* no observed effect concentration
*PAH:* polycyclic aromatic hydrocarbon
*PAR:* photosynthetically available radiation
*PTFE:* polytetrafluoroethylene
*TLM:* target lipid model
*TWA:* time-weighted average
*UV:* ultraviolet;
*UVR:* ultraviolet radiation
*WAF:* water accommodated fraction