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Coral recruits are highly sensitive to heavy fuel oil exposure both in the presence and absence of UV light☆

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ABSTRACT

Oil pollution remains a prominent local hazard to coral reefs, but the sensitivity of some coral life stages to oil exposure remains unstudied. Exposure to ultraviolet radiation (UVR), ubiquitous on coral reefs, may significantly increase oil toxicity towards these critical habitat-forming taxa. Here we present the first data on the sensitivity of two distinct post-settlement life stages of the model coral species *Acropora millepora* to a heavy fuel oil (HFO) water accommodated fraction (WAF) in the absence and presence of UVR. Assessment of lethal and sublethal endpoints indicates that both 1-week-old and 2-month-old recruits (1-wo and 2-mo) were negatively affected by chronic exposures to HFO (7 and 14 days, respectively). Relative growth (1-wo and 2-mo recruits) and survival (1-wo recruits) at end of exposure were the most sensitive endpoints in the absence of UVR, with no effect concentrations (NEC) of 34.3, 5.7 and 29.3 μg L^{-1} total aromatic hydrocarbons (TAH; ∑39 monocyclic- and polycyclic aromatic hydrocarbons), respectively. On average, UVR increased the negative effects by 10% for affected endpoints, and latent effects of exposure were evident for relative growth and symbiont uptake of recruits. Other sublethal endpoints, including maximum quantum yield and tissue colour score, were unaffected by chronic HFO exposure. A comparison of putative species-specific sensitivity constants for these ecologically relevant endpoints, indicates *A. millepora* recruits may be as sensitive as the most sensitive species currently included in oil toxicity databases. While the low intensity UVR only significantly increased the negative effects of the oil for one endpoint, the majority of endpoints showed trends towards increased toxicity in the presence of UVR. Therefore, the data presented here further support the standard incorporation of UVR in oil toxicity testing for tropical corals.

1. Introduction

Coral reefs are under increasing pressure from both global climate and local anthropogenic influences, putting at risk their inherent ecological value and the many ecosystem services they provide (Hoegh-Guldberg et al., 2017; Hughes et al., 2018; Woodhead et al., 2019). Large oil spill events are uncommon but the consequences for coral reef environments can be severe and persist for decades (Guzman et al., 2020; Jackson et al., 1989). Oil spill events therefore represent a significant hazard for many shallow tropical coral reefs, and by extension the human populations that rely on them. Recently there have been several oil spills on or near coral reefs across various geographical locations, including in the tropical West Atlantic Ocean, Caribbean Sea, Indian Ocean and Pacific Islands (Daley, 2019; Singh Khadka, 2020;

Abbreviations: 1-wo, one week old *A. millepora* recruits; *2-mo*, two months old *A. millepora* recruits; *CCA*, crustose coralline algae; *CTLBB*, critical target lipid body burden; *EC/LC$_x$*, effect- or lethal concentration; *FSW*, filtered seawater; *Fv/Fm*, maximum quantum yield; *GBR*, Great Barrier Reef (Australia); *GC-MS*, gas chromatography mass spectrometry; *GC-FID*, gas chromatography flame ionization detection; *HFO*, heavy fuel oil; *MAA*, mycosporin-like amino acids; *MAH*, monocyclic aromatic hydrocarbons; *MANEC*, model-averaged no effect concentration model; *NEC*, no effect concentration; *PAH*, polycyclic aromatic hydrocarbons; *PAM*, pulse amplitude-modulated chlorophyll fluorometry; *PTFE*, polytetrafluoroethylene; *TAH*, total aromatic hydrocarbons (the sum of all analysed mono- and polycyclic aromatic hydrocarbons); *TLM*, target lipid model; *TWA*, time-weighted average concentration; *UVR*, ultraviolet radiation; *WAF*, water accommodated fraction.

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Zacharias et al., 2021; Zúñiga and Faiola, 2020).

Despite the ongoing threat of oil pollution to coral reefs, the information available on the sensitivity of coral reef taxa is limited compared to more traditionally studied ecosystems (Hook (2020); Nordborg et al. (2020); Turner and Renegar (2017)). Methodological differences often prevent the direct comparison of species sensitivity or experimental results of oil exposure (Hodson et al., 2019; Redman and Parkerton, 2015), but recent research suggests that some early coral life stages may be among the most sensitive taxa assessed to date (Negri et al., 2021; Nordborg et al., 2021). For example, the ability of coral larvae to settle is more sensitive than endpoints reported for 79 species in the Target Lipid Model (TLM) database, and is > 10-fold more sensitive to oil exposure than survival in adult corals (McGrath et al., 2018; Negri et al., 2021; Nordborg et al., 2021). A single study indicated negative effects on early coral recruits in uncontaminated water, following exposure to dissolved oil during the motile larval stage (Hartmann et al., 2015). However, the direct effects of oil pollution on the potentially vulnerable 1–10 mm sized recruits have not been reported to date (Nordborg et al., 2020; Turner and Renegar, 2017). The survival rates of coral recruits are far lower than those for adult colonies, until they reach a site- and species-specific escape threshold size (Doropoulos et al., 2012). Therefore, additional stress from contaminants during this period can increase early mortality (Smith et al., 2003) and may reduce the capacity of reefs to replenish coral populations and recover from disturbances.

Tropical, shallow-water coral reef species are also exposed to environmental conditions that may increase oil toxicity, including high ultraviolet radiation (UVR) and heatwaves (Nordborg et al., 2020). Increased oil toxicity in the presence of UVR (phototoxicity) has been demonstrated for tropical corals in several studies (Guzmán Martínez et al., 2007; Negri et al., 2016; Nordborg et al., 2021; Nordborg et al., 2018; Overmans et al., 2018; Peachey and Crosby, 1995). Oil phototoxicity occurs through two main pathways, photooxidation and photosensitisation, with oxidative modification or damage to molecules and cell structures the primary modes of action for both pathways (Barron, 2017). Mounting evidence that UVR can have a large influence on oil toxicity to aquatic organisms has led to recommendations that UVR should be included in oil toxicity studies, as well as risk assessments, for clear water environments where co-exposure to oil pollutants and UVR is likely (Barron, 2017; French-McCay et al., 2018; Nordborg et al., 2021; Roberts et al., 2017). While phototoxic effects have been demonstrated for coral gametes, embryos, larvae and adults following exposure to oil pollutants and UVR, the influence of UVR on the sensitivity of coral recruits to oil exposure has not been assessed to date (Nordborg et al., 2021; Nordborg et al., 2020).

The present study aims to: (i) assess the sensitivity of 1-week old, single polyp- (1-wo) and 2-month old, symbiotic (2-mo) recruits of the model coral species *Acropora millepora* to the WAF of a heavy fuel oil (HFO); (ii) quantify any potential effects of UVR co-exposure on HFO toxicity towards *A. millepora* recruits; (iii) identify relevant endpoints for use in future oil toxicity research; and (iv) compare the sensitivity of coral recruits to previously assessed *A. millepora* life stages and other marine taxa.

2. Methods

2.1. Coral collection, spawning and recruit rearing

Gravid *Acropora millepora* colonies (n = 6) were collected from Backnumbers Reef (S18°30.788′ E147°9.094′), on the central Great Barrier Reef (GBR), Australia, on December 5, 2017 under Great Barrier Reef Marine Park Authority permit G12/35236.1. Colonies were transported to the National Sea Simulator at the Australian Institute of Marine Science (Townsville, Australia), where they were maintained in aquaria until spawning. Larval cultures were generated and maintained in slow flow-through 1 μm filtered seawater (FSW) at 27 °C as previously described (Nordborg et al., 2018). Briefly, parent colonies were monitored on nights when spawning was predicted and isolated once early signs of spawning were evident. Egg bundles were gently collected from each parent colony (n = 6; Table A1, Supplementary materials A) and separated through agitation. Rinsed gametes were combined in ~30 L of 1 μm FSW and allowed to fertilise for ~4 h. Following fertilisation, embryos were gently transferred to 400 L larval culture tanks. Cultures were manually cleaned at least twice daily for the duration and gentle aeration started ~20 h after fertilisation.

A. millepora larvae (~2500 per assay) from an individual mass culture were added to a 50 L aquarium containing clear glass tiles (16 × 16 or 20 × 20 mm) and 8 L of 1 μm FSW (27 ± 0.5 °C). Larvae were settled on the tiles in the absence of light using the crustose coralline algae (CCA) *Porolithon onkodes* as small live chips (~3 mm^{-2}; Heyward and Negri (1999); Whitman et al. (2020)) or finely ground and washed live tissue (Heyward and Negri, 1999; Meyer et al., 2009). The CCA was carefully spread across the tiles and after ~20 h the aquarium was slowly filled with FSW and flushed to remove larvae that had not settled. This process was repeated until sufficient larvae had settled on tiles. Newly settled recruits were maintained in flow-through aquaria until start of assays. For further details on culture preparation, larval settlement, recruit maintenance and inoculation with symbiotic algae refer to and Section A1, Supplementary materials A.

2.2. Preparation and chemical analysis of treatment solutions

Neat HFO chemistry (International Bunker Supplies Pty Ltd, Gladstone, Australia) was characterised as '2018 batch' by Nordborg et al. (2021). Low-energy WAFs were freshly prepared at the start of each assay as previously described (Negri et al., 2016; Nordborg et al., 2021; Nordborg et al., 2018). Briefly, WAF was prepared at room temperature (22 ± 1 °C) by addition of ~20 g L^{-1} HFO to the surface of 0.5 μm FSW in 10 L glass aspirator bottles (20% headspace), followed by gentle, low-energy stirring overnight (<5% vortex; ~18 h) while sealed and protected from light, in accordance with standardised procedures (Aurand and Coelho, 2005; Barron and Ka'aihue, 2003; Singer et al., 2000).

Fresh WAF was prepared every 48 h for each experimental assay and gently drained via the aspirator stopcock into solvent cleaned borosilicate bottles. Half of the WAF was immediately used to prepare 7 treatment solutions (0, 1.0, 2.6, 6.4, 16, 40 and 100% WAF) by dilution of the 100% WAF with 0.5 μm FSW. The remainder was stored in borosilicate bottles sealed with PTFE-lined caps without headspace at 4 °C overnight, then equilibrated to room temperature and used to prepare a second batch of treatment solutions as outlined above within 24 h of collection from the aspirator (Aurand and Coelho, 2005).

Samples for chemical analysis were collected for the highest treatment concentration (100% WAF) at the start and end of at least half of the individual 24-h (+UVR and -UVR) static exposures (Barron and Ka'aihue, 2003). Briefly, samples were collected in solvent cleaned amber vials (40 mL) and bottles (500 mL), acidified to pH 2 using 6 M hydrochloric acid and stored at 4 °C (no head space) until analysed. Samples were analysed for 39 individual mono- and polycyclic aromatic hydrocarbons (MAH and PAH, respectively) using GC-MS (USEPA method 8260 and 8270), and total recoverable hydrocarbons (TRH) using GC-FID (USEPA method 8270) at ChemCentre (Perth, Australia) and the Australian Institute of Marine Science (Townsville, Australia). The time-weighted average (TWA) concentrations of individual aromatics in the 100% WAF were calculated for each exposure duration as per Equation (1) and used to derive putative species-specific sensitivity constants. The TWA TAH concentration in the 100% WAF was calculated as the sum of the individual aromatic TWA concentrations. TWA TAH concentrations in more dilute treatments were calculated relative to the TWA TAH concentration in the 100% WAF and applied in subsequent statistical analyses.

$$TWA = \frac{\sum_{i=1}^{n} c_i t_i}{\sum_{i=1}^{n} t_i} \quad \text{(Eq.1)}$$

where, c_i is the average concentration during the *i*th time interval, t_i is the duration of the *i*th time interval and *n* is the total number of time intervals.

2.3. Experimental assays

Two individual experimental assays were performed using the newly settled 1-wo aposymbiotic and 2-mo symbiotic *A. millepora* recruits. The two separate age groups were selected to ensure that potential differences between young (aposymbiotic, single polyp) and more developed (symbiotic, multiple polyp) recruits could be detected and accounted for in future work. Similarly, the differing exposure durations were selected to ensure each exposure constituted a substantial time period relative to the total time spent at each life stage (Warne et al., 2018).

Recruits were exposed to 7 concentrations of HFO WAF for 7 days (1-wo) or 14 days (2-mo) in sealed, 330 mL glass exposure chambers with clear lids ($n_{chamber} = 5$ per treatment combination; $n_{chamber} = 7$ for FSW controls) containing 200 mL WAF (~40% headspace) and an average of 15 (1-wo) or 20 (2-mo) recruits. Exposure chambers were placed randomly in orbital shaker incubators (Thermoline Scientific, Australia) fitted with LEDs emitting in the visible spectrum (-UVR) or LEDs complemented with fluorescent tubes emitting in the UVR spectrum (+UVR) under a 12:12 h visible light and 6:18 h UVR light regime (for UVA, UVB and photosynthetically active radiation intensities for each assay see Table A2) while gently shaken (<70 rpm). Treatment solutions were renewed daily. Recruit survival was assessed during solution changes and tiles where all recruits had died were removed. At the end of exposure, recruits were transferred to flow-through FSW holding tanks and monitored for post-exposure effects for 6 weeks. Recruits were placed randomly in the holding tanks and fed newly hatched *Artemia* once daily. All recruits were photographed at the start and end of exposure, as well as weekly during post-exposure monitoring. Additionally, the photosynthetic efficiency (maximum quantum yield, Fv/Fm; Ferrier-Pagès et al. (2007)) of algal symbionts was assessed for 2-mo recruits at the start and end of exposure using an image pulse amplitude-modulated chlorophyll fluorometer (PAM; MAXI imaging PAM, Heinz Walz GmbH, Germany). For further details on the individual experimental assays refer to Section A2.

Water quality parameters (temperature, salinity, pH and dissolved oxygen) of new and discarded solutions (after individual 24 h exposures) were measured for at least one replicate per treatment combination, but measured for all replicates at start and end of the full exposure periods (7 or 14 d). Water quality parameter estimates are detailed in Table A3.

2.4. Image analysis

Coral survival, colour score and growth rate were assessed from captured images using FIJI ImageJ (version 2.0.0-rc-68; Schindelin et al. (2012)) by manually tracing the outline of live tissue for each recruit in scaled and/or colour calibrated images (refer to Section A2 for full details). Coral colour score was measured as the mean across the entire surface area of live tissue for each recruit. Growth rate was calculated as the percent increase in live tissue area for each recruit relative to start of exposure (Hughes and Connell, 1987). Analysed images are available through the associated data repository (AIMS, 2022).

2.5. Statistical analysis

Statistical analysis was performed using the *baysenec* package (version 2.0.1 for 2-mo and 2.0.2 for 1-wo; Fisher et al. (2021)) and it's dependencies (including the brms package; Bürkner (2017, 2018)) in R (version 4.1.1; R Core Team (2021)) through the RStudio interface (version 1.4.1717; RStudio Team (2020)) as previously described (Nordborg et al., 2021). Briefly, several commonly used concentration-response models were fitted using Markov-Chain Monte Carlo methods and candidate models were combined into a *model averaged no effect concentration model* (MANEC; Fisher et al. (2021); Fisher (2020)) for each dataset using the bnec-function and the cmdstanR package (Gabry and Cesnovar,) to interface with the Stan C++ compiler (Stan Development Team, 2019). The model assumptions for, and fit of, each candidate model was assessed using the inbuilt functions in *baysenec* and the no effect concentration (NEC), 10% and 50% effect or lethal concentrations (EC/LC_{10}, EC/LC_{50}) relative to control performance (USEPA, 2012 ; Warne et al., 2018) were derived from the respective MANEC. For further details on the statistical analysis and a full list of candidate models included in each MANEC refer to Section A3 and Table A4–Table A5. All decisions and evaluations performed during analysis have been recorded in the corresponding R script files (Nordborg, 2022).

2.6. Derivation of putative CTLBBs for sensitivity comparisons

The dissolved oil composition in a WAF is unique to the original oil composition and differs for the same oil type depending on the loading and environmental conditions. Therefore, direct comparisons of species sensitivities based on concentration-response relationships (i.e. EC/LC_{50}s in μg L^{-1}) are difficult to interpret, and are often inappropriate (Redman and Parkerton, 2015; Redman et al., 2012). To overcome this issue, objective comparisons of the relative sensitivity of the two assessed coral life stages with other taxa required the estimation of sensitivity constants (critical target lipid body burdens; CTLBBs) which are specific for each life stage and species, but independent of oil composition (Di Toro et al., 2000; Di Toro et al., 2007; Redman et al., 2012). Definitive CTLBBs are derived from the established linear relationship between the $\log_{10}EC_{50}$ and the $\log_{10}$octanol-water partitioning coefficient (K_{OW}) for several pure narcotic chemicals, including aromatic hydrocarbons, where the slope is considered universal for all species and the CTLBB is the y-intercept (Di Toro et al., 2000; Di Toro et al., 2007). However, in the absence of pure aromatic hydrocarbon toxicity data, a *putative* acute CTLBB can be approximated from the experimental EC_{50} and aromatic hydrocarbon composition of a complex oil WAF, as previously described (Negri et al., 2021; Nordborg et al., 2021). Putative CTLBBs are useful for comparing the sensitivities among species but are termed *putative* since they should be confirmed using EC/LC_{50} values for pure aromatic hydrocarbons. Putative chronic CTLBBs can be calculated in the same manner using the oil WAF EC_{10}. Low CTLBB values represent species and/or life stages that are more sensitive to hydrocarbon exposure. Here, putative acute CTLBBs were derived for each endpoint where an EC/LC_{50} could be determined in the absence of UVR and the TWA concentrations of individual aromatic hydrocarbons in the HFO WAF at various timepoints. Derived putative CTLBBs for coral recruits were compared to the CTLBBs in the current acute CTLBB database (McGrath et al., 2018) and recently published putative (Nordborg et al., 2021) and definitive (Renegar and Turner, 2021) CTLBBs for tropical corals. 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, 2018) in R (R Core Team, 2021) as per Negri et al. (2021). Resulting sensitivity rankings were then plotted against the definitive (McGrath et al., 2018; Renegar and Turner, 2021) and putative (present study; Nordborg et al., 2021) CTLBBs using the inbuilt functions of Microsoft Excel.

3. Results

3.1. Chemical composition of HFO WAFs

Freshly prepared 100% HFO WAFs contained 483 ± 146 μg TAH L^{-1} (n=5) and 424 ± 122 μg TAH L^{-1} (n = 7) for the 1-wo and 2-mo recruit experiments, respectively (Table B1–Table B2, Supplementary materials B). TAH constituted 32–42% of TRH in freshly prepared WAFs for both experiments (n = 8). MAH and PAH concentrations decreased 67–88% and 86–95% in the absence and presence of UVR, respectively, during each 24-h exposure period to successive batches of WAF across both assays. There was no significant difference in TAH losses between light regimes during the 1-wo recruit experiment ($t(4) = 1.84, p = 0.14$) or the 2-mo recruit experiment ($t(2) = -1.01, p = 0.42$). Individual hydrocarbon TWA concentrations were calculated after 7-d exposures for the 1-wo recruits and after 9 d and 14 d exposures for the 2-mo recruits (Table A6). TAH TWA-concentrations applied in statistical analyses are summarized in Table A7.

3.2. 1-Week-old (aposymbiotic) recruits

3.2.1. Survival

Survival of 1-wo recruits in control treatments over the 7-d exposure period was 50% and 41% in the absence and presence of UVR, respectively, with surviving recruits typically showing high tissue retention (Fig. 1a and Fig. 2a). Early recruit survival decreased significantly following 7-d exposure to low–mid concentrations, with an LC_{50} of 51.8 μg TAH L^{-1} in the absence of UVR (Fig. 1a and Table 1). Co-exposure to UVR did not change the LC_{10} and LC_{50} values but did depress survival further (~10%) at high concentrations (>100 μg TAH L^{-1}; Fig. 1a and Fig. A3b). A high degree of variability in survivorship was observed across all treatment combinations, which is typical for recently settled coral recruits (as reviewed in Randall et al. (2020) and Ritson-Williams et al. (2009)).

At the end of the 6-week recovery period, the average survival of control recruits was 63% (-UVR) and 48% (+UVR; Fig. 1b) for each light regime, respectively. Recruits exposed to concentrations <20 μg TAH L^{-1} which exhibited partial tissue loss at end of exposure (quantified as part of the survival metric; Figs. 2b and 1a) typically recovered during the post-exposure period, resulting in an increase in LC_{50}s and a higher overall survival at 6 weeks post-exposure (Fig. 1b). In contrast, for corals exposed to treatment concentrations above 40 μg TAH L^{-1}, tissue loss continued after transfer to uncontaminated FSW and no recruits remained alive at the highest treatment concentration (Fig. 1b and Fig. A4). While many recruits progressed from partial to complete mortality (Fig. 2b and c), the LC_{50} did not significantly decrease during the post-exposure period (Table 1 and Table A8). There was no significant difference in toxicity between light regimes during the recovery

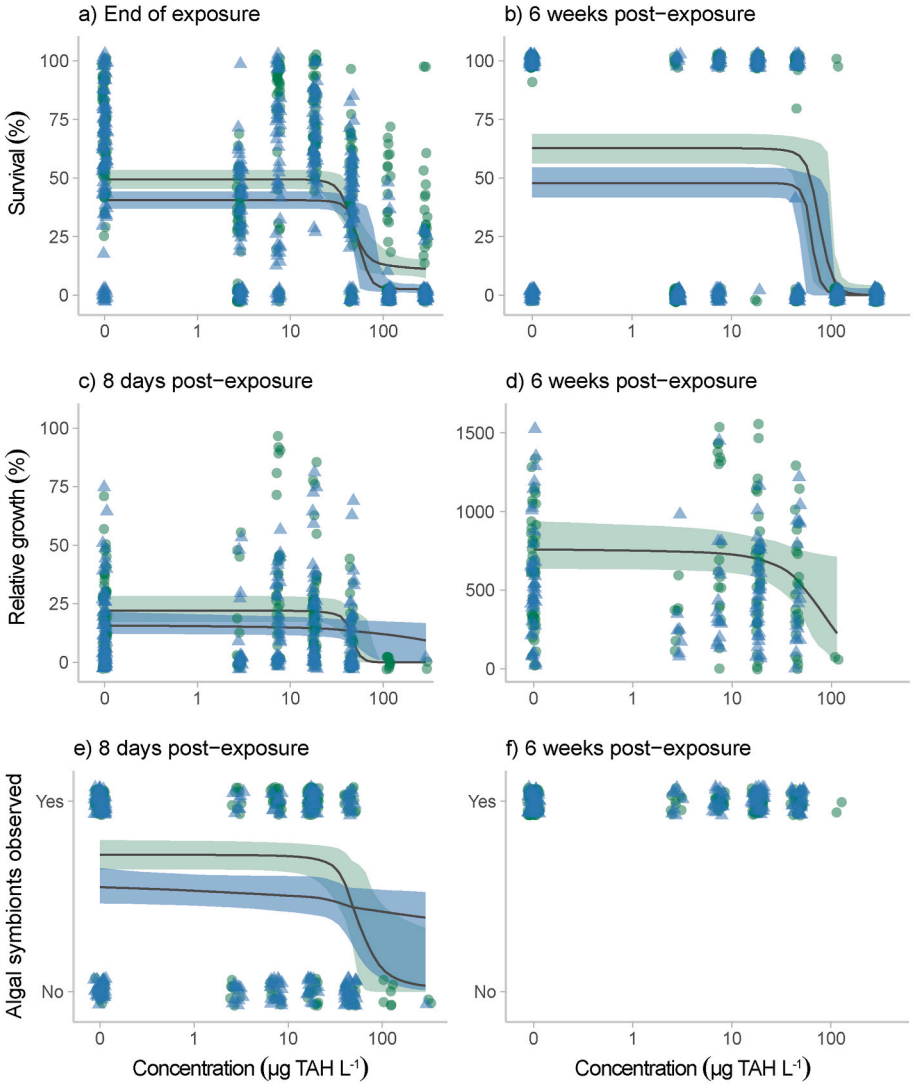

Fig. 1. Concentration-response relationship for 1-week-old *A. millepora* recruits exposed to the water accommodated fractions of heavy fuel oil for 7 days in the presence (+UVR; blue) or absence (-UVR; green) of ultraviolet radiation at the end of exposure (a), 8 days post-exposure (c) or 6 weeks post-exposure (b d and f). The model median (solid line), 95% credible intervals (shaded areas) and values for individual recruits within replicate chambers: $n_{chambers}$ = 5 or 7, $n_{recruits}$ = 10–20 per chamber (+UVR = triangle; -UVR = circle) shown for the full model averaged no effect concentration (MANEC) model for end of exposure survival (a), latent survival (b), latent relative growth (c and d) and whether algal symbionts were visually observed (e and f). Refer to Fig. A3–Fig. A6 and Table A8–Table A10 for survival, relative growth and observations of symbiotic algae results for all post-exposure periods. For interpretation of the references to colour, the reader is referred to the online version of this article.

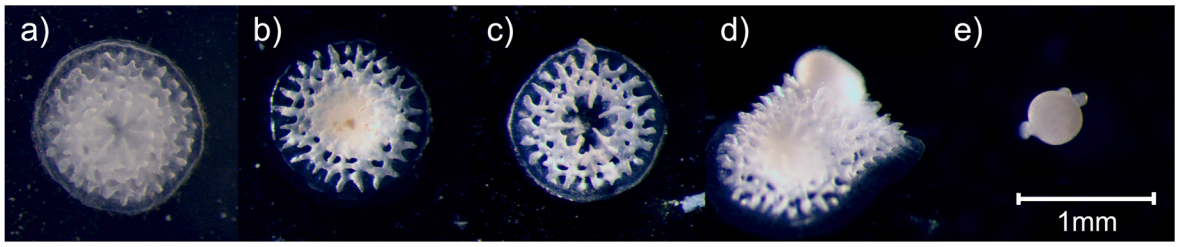

Fig. 2. Examples of 1-wo *A. millepora* recruits during and following 7 d exposure to the water accommodated fractions of heavy fuel oil in the presence or absence of ultraviolet radiation. Examples include a healthy recruit at end of exposure (a), a recruit showing partial mortality at end of exposure (b), an empty skeleton (c), a suspected polyp bail-out observed at end of exposure (d) and a bailed-out recruit/polyp removed from a treatment replicate following 4 d of exposure (e).

Table 1
Threshold concentrations (NEC, EC/LC_{10}, EC/LC_{50}) for 1-wo *A. millepora* recruits following 7 d exposure to heavy fuel oil WAF in the presence (+UVR) or absence (-UVR) of ultraviolet radiation. Toxicity increases in the presence of UVR also shown as -fold change compared to -UVR conditions. No significant difference in EC/LC_{50} detected between light regimes for any endpoint. For further details on NEC model subset and posterior comparisons of light regimes refer to Fig. A3–Fig. A6 and Table A8–Table A10.

Endpoint	Light regime	NEC (μg TAH L^{-1})	EC/LC_{10} (μg TAH L^{-1})	EC/LC_{50} (μg TAH L^{-1})	-fold change (EC/LC_{50})
7 d Survival	-UVR	29.3 (18.1–42.6)	33.7 (22.1–43.4)	52.6 (44.1–75.2)	*0.9*
	+UVR	37.7 (29.8–44.0)	41.0 (29.1–76.9)	56.3 (47.7–86.6)	
Post-exposure survival (8 d post-exposure)	-UVR	38.6 (29.2–67.4)	37.9 (26.9–59.7)	59.3 (47.8–84.1)	*0.9*
	+UVR	57.4 (43.8–96.4)	55.0 (37.5–94.6)	65.0 (49.2–99.2)	
Post-exposure growth (8 d post-exposure)	-UVR	34.3 (27.1–41.4)	36.5 (16.1–70.6)	45.2 (36.8–75.2)	*0.2*
	+UVR	33.7 (0.27–253.6)	42.3 (0.63–>286.2)	286.2 (42.0–>286.2)	
Symbiont uptake (8 d post-exposure)	-UVR	34.3 (12.6–75.1)	32.9 (8.56–64.7)	55.6 (41.7–187.9)	*0.2*
	+UVR	23.6 (0.19–189.3)	26.2 (0.47–>286.2)	286.2 (43.0–>286.2)	

period (Fig. A4 and Table A8). However, no recruits co-exposed to UVR and the second highest treatment concentration retained any live tissue from 2 weeks post-exposure (Fig. A4c, e and g).

3.3. Polyp bail-out

Polyp bail-out is a stress response where coral recruits reverse initial metamorphosis, or adult polyps detach from the colony, and leave the skeleton due to adverse environmental conditions (Negri et al., 2005; Sammarco, 1982; Schweinsberg et al., 2021). During exposure, polyp bail-out was observed on 7 different occasions in the low-to mid-range treatment concentrations (2.8–45.8 μg TAH L^{-1}; Fig. 2d, Table A11). The bail-out process was difficult to observe directly but was inferred from the presence of new, free-swimming pseudo-larvae or polyps, and corresponding empty skeletons at the time of daily solution changes (Fig. 2e and c). It is possible that a higher proportion of recruits may have undergone bail-out, but that the released polyps died and disintegrated between observations. None of the free-swimming larvae/polyps transferred from exposure chambers to clean FSW successfully re-attached.

3.3.1. Relative growth post-exposure

No growth of 1-wo recruits occurred during the 7-d exposure period, but control recruits grew on average 760% and 670% in the absence and presence of UVR, respectively, over the 6-week post-exposure period (Fig. 1d). For surviving recruits exposed to mid–high concentrations in the absence of UVR, growth was inhibited for the duration of the 6-week recovery period (EC_{50} = 75.8 μg TAH L^{-1}; Fig. 1d, and Table 1). A slight decrease in relative growth with increasing treatment concentration was also observed for surviving recruits co-exposed to UVR for the first 3 weeks post-exposure (Fig. A5 and Table A10). However, the statistical models fitted for recruits co-exposed to UVR were highly influenced by the lack of survivors at mid–high treatment concentrations (Fig. A5) and may therefore have obscured differences in growth between light treatments post-exposure.

3.3.2. Algal symbiont uptake post-exposure

At the end of the 7-d exposure, recruits were transferred to clean FSW and inoculated with cultured symbiotic algae (*C. goreaui*) prior to transfer to holding tanks for recovery and post-exposure observations (for details see Section A1, Supplementary materials A). At 8 d post-exposure (7 d post-inoculation), early symbiont uptake was typically observed in >50% of recruits exposed to low–mid concentrations. However, little uptake of symbionts (<30%) was observed for surviving recruits exposed to high concentrations (>100 μg TAH L^{-1}; Fig. 1e, Table 1). At 6 weeks post-exposure, symbionts were clearly visible in the tissues of all surviving recruits (Fig. 1f and Fig. A6).

3.4. 2-Month-old (symbiotic) recruits

3.4.1. Survival

The survival of 2-mo recruits at low WAF concentrations over the 14-d exposure was very high (97% and 98% in the absence and presence of UVR, respectively; Fig. 3a). Survival was negatively affected by exposure to HFO WAF at mid–high concentrations (>30 μg TAH L^{-1}), both in the presence and absence of UVR (Fig. 3a). However, no latent effects were apparent at 6 w post-exposure (Fig. 3b). This was also evidenced by the increase in LC_{50}s after the 6-week recovery period (Table 2 and Table A12), indicating that most of these older recruits exposed to <100

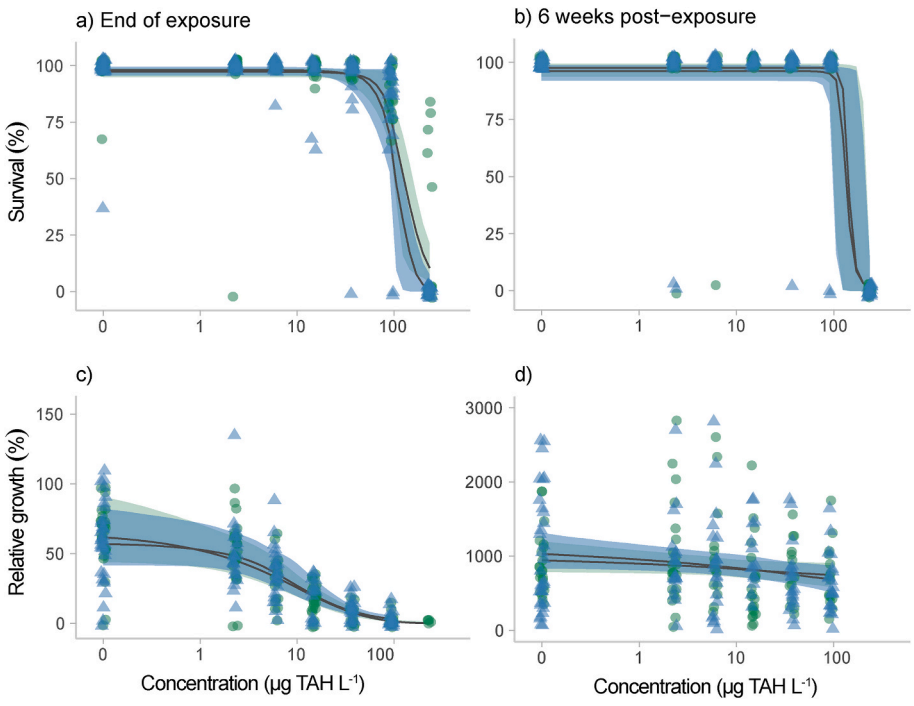

Fig. 3. Concentration-response relationships for 2-month-old *A. millepora* recruits exposed to the water accommodated fractions of heavy fuel oil for 14 days in the presence (+UVR; blue) or absence (-UVR; green) of ultraviolet radiation. The model median (solid line), 95% credible interval (shaded areas) and values for individual recruits within replicate chambers: $n_{chamber}$ = 5 or 7, $n_{recruits}$ = 15–24 per chamber (+UVR = triangle; -UVR = circle) shown for the full model averaged no effect concentration (MANEC) model for coral survival (a and b) and relative growth (c and d) at the end of exposure (a and c) and following the 6-w recovery period (b and d). Refer to Fig. A7–Fig. A8 and Table A12–Table A13 for survival and relative growth results for all post-exposure periods. For interpretation of the references to colour the reader is referred to the online version of this article.

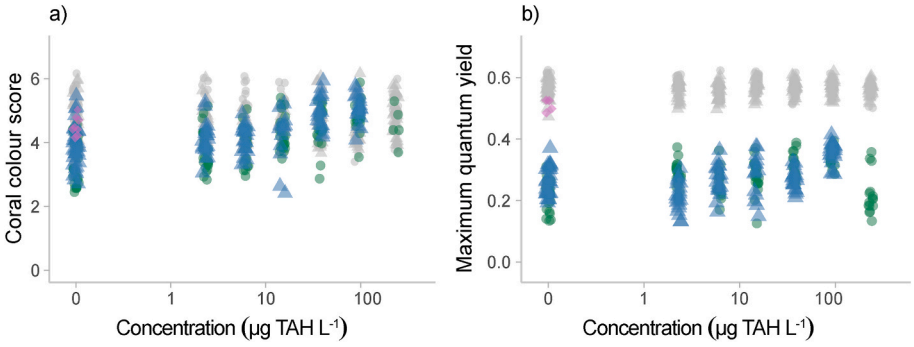

Fig. 4. Effects of the water accommodated fractions of heavy fuel oil on *A. millepora* algal symbionts following 14 d exposure under visible light in the presence (+UVR; blue) or absence (-UVR; green) of ultraviolet radiation. Individual replicate values (+UVR = triangle; -UVR = circle) shown for coral colour score (a) or maximum quantum yield (Fv/Fm) (b). Start of exposure values (grey) and values for holding tank controls at end of the exposure period (magenta) also shown for coral colour score and maximum quantum yield. For interpretation of the references to colour the reader is referred to the online version of this article.

Table 2
Threshold concentrations (NEC, EC/LC10, EC/LC50) for 2-mo *A. millepora* recruits following 14 d exposure heavy fuel oil WAF in the presence (+UVR) or absence (-UVR) of ultraviolet radiation. Toxicity increases in the presence of UVR also shown as -fold change compared to -UVR conditions. No significant difference in EC/LC_{50} detected between light regimes for any endpoint. For further details on NEC model subset and posterior comparisons of light regimes refer to Fig. A7–Fig. A9 and Table A12–Table A15.

Endpoint	Light regime	NEC (µg TAH L^{-1})	EC/LC_{10} (µg TAH L^{-1})	EC/LC_{50} (µg TAH L^{-1})	-fold change (EC/LC_{50})
14 d Survival	-UVR	85.4 (72.5–91.6)	76.4 (46.8–92.7)	128.5 (101.8–158.5)	*1.2*
	+UVR	88.3 (80.4–91.7)	63.6 (37.5–91.6)	104.6 (93.8–125.0)	
Post-exposure survival (6 w post-exposure)	-UVR	121.5 (93.7–200.3)	122.6 (0–195.4)	141.0 (101.8–206.3)	*1.1*
	+UVR	119.2 (90.2–199.0)	112.1 (74.3–193.0)	134.0 (100.6–203.8)	
14 d relative growth	-UVR	5.71 (2.27–11.7)	0.60 (0.26–5.62)	6.99 (3.09–15.1)	*0.8*
	+UVR	4.49 (1.60–11.9)	1.87 (0.29–9.59)	9.15 (3.41–19.8)	
Post-exposure relative growth (6 w post-exposure)	-UVR	3.80 (0.14–77.5)	5.57 (0.27–>93.5)	>93.5	*1.0*
	+UVR	5.27 (0.14–61.8)	1.92 (0.32–>93.0)	>93.0 (42.3–>93.0)	

μg TAH L^{-1} had recovered from partial tissue mortality (Fig. 3 and Fig. A7). There was no statistically significant difference in toxicity in the presence of UVR (Table 2 and Fig. A7). However, the +UVR LC_{50} was 1.2-fold lower than the -UVR LC_{50}, and complete mortality occurred for corals co-exposed to UVR in the highest treatment concentration (232.5 μg TAH L^{-1}; Fig. 3a and Table 2).

3.4.2. Relative growth

The relative growth of 2-mo recruits in control conditions increased by 60% and 56% over the 14-d experimental exposure period in the absence and presence of UVR, respectively (Fig. 3c). Growth was severely affected by 14-d exposure to low concentrations of HFO WAF with EC_{50}s of 7.0 and 9.2 μg TAH L^{-1} in the absence and presence of UVR, respectively (Fig. 3c and Table 2). Surviving corals exposed to the highest treatment concentrations showed limited or no growth over the 14-d exposure period, and growth was partially inhibited at concentrations as low as 0.6 μg TAH L^{-1} (Fig. 3c and Table 2). However, there was no statistically significant difference between the two light treatments (Fig. 3 and Table 2). After 6 w of recovery, control corals had grown by ~1020% (-UVR) and 940% (+UVR), and the growth of surviving recruits that had been severely affected by HFO WAF <100 μg TAH L^{-1} approached those of the controls (750% and 690% −/+UVR; Fig. 3d). The severity of impacts decreased gradually over the course of the recovery period with no significant differences detected between light regimes (Fig. A8 and Table A13). No negative effects of HFO exposure were observed for the onset of vertical growth in 2-mo recruits post-exposure (Fig. A2e; Fig. A9; Table A15 and Section A5).

3.4.3. Effects on symbiotic algae

No negative effects were observed for coral colour score or maximum quantum yield (Fv/Fm) with increasing WAF concentrations (14 d exposure) in the surviving 2-mo recruits (Fig. 4). While no concentration-response, or difference between light regimes, was observed for Fv/Fm, the values observed at end of exposure were lower than those recorded at start of exposure (Fig. 4). The mean coral colour score across the entire live tissue area of each recruit remained similar to the values recorded at the start of exposure, as well as the values for holding tank controls (Fig. 4). As no effects were observed for endpoints associated with the health of the symbiotic algae at the end of the 14-d exposure latent effects were not assessed.

3.5. Comparison of relative sensitivity across life stages and endpoints

The derived sensitivity constants (putative CTLBBs, Table 3) indicate that relative growth of 2-mo recruits after 14-d exposure to HFO WAF was more sensitive than survival of 1-wo and 2-mo recruits at the end of the exposure periods. However, the survival CTLBBs derived for 1-wo and 2-mo A. *millepora* recruits are more sensitive than most taxa included in the current acute TLM database and adults of previously assessed scleractinian corals (Table 3, Fig. 5a). Although not often reported, CTLBBs were also calculated for post-exposure or latent responses (Fig. 5b and Table A16). All putative acute CTLBBs derived for *A. millepora* recruits were among the lowest CTLBBs currently included in the acute TLM database (<10th percentile; Fig. 5a and Table A16; McGrath et al. (2018)). Seven of the putative acute CTLBBs derived also fell within the 10th percentile of the current chronic TLM database (McGrath et al., 2018).

4. Discussion

4.1. Effects on recruits

4.1.1. Survival

The survival of *A. millepora* recruits was severely affected by HFO exposure, regardless of recruit age or light treatment. While survival is the most frequently assessed endpoint in investigations of the effects of oil pollutants towards coral, the results can be highly variable and dependent on the methods and pollutants used (Haapkylä et al., 2007; Turner and Renegar, 2017). For adult tropical corals, toxicity has previously been reported for eight species exposed to dissolved aromatic hydrocarbons, either from oils (Mercurio et al., 2004; Rinkevich and Loya, 1979) or individual compounds (Guzmán Martínez et al., 2007; Renegar and Turner, 2021; Renegar et al., 2017; Turner et al., 2021). Examples of negative effects of oil exposure to adult corals include high mortality in *Stylophora pistillata* colonies repeatedly exposed to Iranian crude oil for an extended period (>2 months; Rinkevich and Loya (1979)) and necrosis in adult *Acropora microphthalma* after 48 h exposure to mineral lubricating oil WAF (Mercurio et al., 2004). Several previous studies have also reported no lethal impacts on adult corals following exposures involving both floating oil and WAFs (Dodge et al., 1984; Peters et al., 1981; Shafir et al., 2007), but the exposure periods used were often short and chemical characterisation of treatment solutions lacking.

The most objective way to compare the sensitivity between different life stages and species is to contrast their species-specific sensitivity constants (e.g. CTLBBs), as these are independent of oil composition (McGrath et al., 2018; Redman and Parkerton, 2015; Redman et al., 2012). Based on their putative CTLBBs, 1-wo recruits (7-d exposure) were 3.7-fold more sensitive than 2-mo recruits at the end exposure (14 d exposure; Table 3 and Table A16). The 1-wo recruit survival endpoint was also more sensitive than fertilisation, embryonic survival, larval metamorphosis and larval survival endpoints in *A. millepora* (Nordborg et al., 2021). The sensitivity of 2-mo recruit survival (after 9 or 14 d exposure) was similar to fertilisation success and embryonic survival, but was less sensitive than larval metamorphosis, the most sensitive life stage previously reported for this species (Nordborg et al., 2021).

Table 3
Species-specific toxicity constants: putative, acute critical target lipid body burdens (CTLBB; using EC/LC_{50}) and sensitivity rankings for key endpoints of the early life stages and recruits of *A. millepora*. CTLBBs only derived using data for corals exposed in the absence of ultraviolet radiation (UVR). Sensitivity ranking in relation to the acute CTLBB database (McGrath et al., 2018). All CTLBBs shown as μmol g^{-1} octanol. For complete list of putative CTLBBs derived refer to Table A16 (1-wo and 2-mo recruits) and Nordborg et al. (2021).

Life stage	Exposure length	Endpoint	Putative $CTLBB_{EC/LC50}$ (μmol g^{-1} octanol)	Sensitivity ranking (%)
Planula larvae[a]	2 d	Metamorphosis success	4.4 (3.8–5.1)	0.05 (0.03–0.09)
	2 d	Survival	21.6 (20.6–26.0)	7.8 (7.0–12.0)
1-wo recruits	7 d	Survival	2.2 (1.8–3.1)	0.002 (0.0006–0.01)
2-mo recruits	14 d	Survival	8.1 (6.4–10.0)	0.490 (0.21–0.98)
		Relative growth	0.4 (0.2–1.0)	$3.7{\cdot}10^{-8}$ ($1.5{\cdot}10^{-10}$–$2{\cdot}10^{-5}$)

[a] Nordborg et al. (2021).

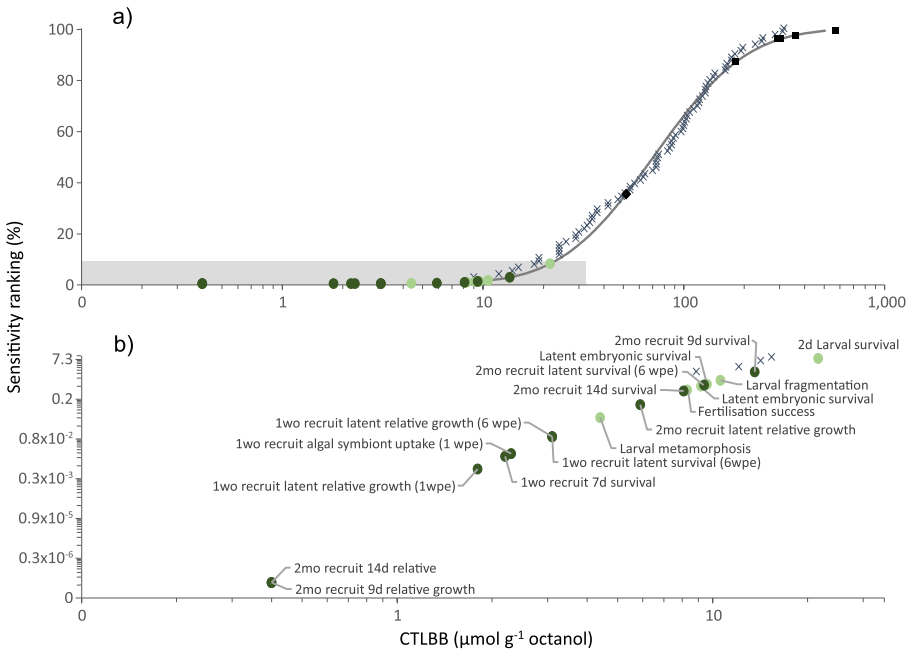

Fig. 5. Sensitivity ranking of A. millepora life stages compared to the acute species-specific sensitivity constant database and previously assessed scleractinian corals based on putative critical target lipid body burdens (CTLBBs; a) and relative sensitivity of individual life stages and endpoints assessed for *A. millepora* (b). Percentiles for early life stages (light green circles; (Nordborg et al., 2021)) and recruits (dark green circles; present study) of *A. millepora*, adult *Lophelia pertusa* (black diamond; Bytingsvik et al. (2020)) and 5 adult Atlantic coral species (black squares; Turner et al. (2021)) derived by fitting a SSD model (grey line) to CTLBBs from the acute TLM database (blue crosses; McGrath et al. (2018)). Grey shading (a) corresponds to range shown in Fig. 5b. Note scale differences between a and b. For interpretation of the references to colour the reader is referred to the online version of this article.

However, the exposure period was longer for the 1-wo recruits than earlier life stages (7 d compared to ≤48 h), and the greater sensitivity of recruits may be due to this longer exposure, if uptake and incorporation of the hydrocarbons into lipid membranes had not reached equilibrium after 48 h of exposure (French-McCay, 2002). Additionally, it is possible that components which were not analysed for in the WAFs (e.g. photomodification or phototoxidation products) could have contributed to the observed toxicity, leading to low putative CTLBB estimates. The LC_{50}s for 2-mo recruits decreased by 1.6- and 1.7-fold (-/+UVR, respectively) as the exposure period increased from 9 to 14 days, further highlighting the influence of exposure duration on oil toxicity. Assessments of the time-dependent toxicity of representative early and post-settlement coral life stages would further improve these comparisons of relative sensitivity.

Early life stages of aquatic organisms have long been assumed to be more sensitive to both the narcotic and phototoxic effects of oil exposure (Barron, 2017; Peters et al., 1997; Roberts et al., 2017). This assumption appears to be supported by the work on tropical reef-building corals to date (Negri et al., 2021; Nordborg et al., 2021; Nordborg et al., 2020; Turner and Renegar, 2017). A comparison of lethal CLTBBs indicates that *A. millepora* recruits are >20-fold more sensitive than five species of adult Atlantic corals exposed for 48 h (Table A16 and Turner et al. (2021)). In addition to exposure time-dependence the sensitivity to oil exposure may also be size-dependent, as the surface area-to-volume ratio of an organism decreases with increasing size. Therefore the time required to reach equilibrium for a given aqueous concentration increases with increasing size, as previously illustrated for other organic pollutants (Del Vento and Dachs, 2002). Size may also affect the available lipid stores, which could affect both the duration and dissolved hydrocarbon concentration required to reach the critical body burden. Size-dependent sensitivity has been previously reported for metals in aquatic invertebrates (Cadmus et al., 2020) and for coral larvae exposed to Louisiana crude oil (Goodbody-Gringley et al., 2013). This suggests that the size of propagated adult corals used in laboratory studies may partially explain the variability observed in response to oil exposure and that size-dependent sensitivity of corals to oil exposure warrants further investigation.

4.1.2. Post-exposure survival

The post-exposure increases in LC_{50}s indicate that at least some of the 1-wo and 2-mo recruits that exhibited partial tissue mortality and tissue retraction from the skeleton recovered following transfer to uncontaminated seawater. The potential for recovery following hydrocarbon exposure has varied across coral species and life stages in previous investigations. Latent mortality has been observed for up to 10 d post-exposure in recently settled recruits of the corals *Orbicella faveolata* and *Agaricia humilis* exposed to crude oil WAF during the larval stage (Hartmann et al., 2015). Latent mortality has also been shown for embryos of *A. millepora* exposed to HFO WAF (Nordborg et al., 2021), adult corals submerged in neat fuel oil or diesel (Reimer, 1975), adult *Porites divaricata* exposed to dissolved fluoranthene (Guzmán Martínez et al., 2007), and following an oil spill in Panama (Guzmán et al., 1994). However, no latent effects on survival were reported for *P. divaricata* exposed to dissolved 1-methylnaphthalene (Renegar et al., 2017), *Diploria strigosa* exposed to Arabian light crude WAFs and chemically enhanced WAFs (Knap, 1987), or *S. pistillata* and *Pocillopora damicornis* exposed to Eqyptian crude oil WAF (Shafir et al., 2007). This variability in responses suggests that latent mortality may be dependent on the oil type and exposure length, and that by excluding post-exposure assessments of coral health, the longer-term outcomes of exposure may be misinterpreted.

4.1.3. Polyp bail-out

Polyp bail-out was observed in 1.4% of 1-wo recruits during exposure to HFO WAF containing 2.8–45.8 µg TAH L^{-1}, but not for any of the 2-mo recruits. Polyp bail-out is a recognised stress response to detrimental environmental conditions and has been observed in recently settled recruits and established adult colonies across several anthozoan groupings (as reviewed by Schweinsberg et al. (2021)). This 'reverse metamorphosis', where stressed polyps revert to a planktonic state is proposed as a survival or risk-spreading strategy. However, the reattachment rate appears to generally be low (Schweinsberg et al., 2021). In the present study, none of the bailed-out polyps that were transferred to uncontaminated FSW reattached. There is evidence that coenosarc apoptosis and the separation of soft tissues from the skeleton may represent key steps in successful polyp bail-out for reef-building corals (Schweinsberg et al., 2021). This, combined with the observations of the present study, raises the question as to whether some of the previously reported polyp retraction, tissue swelling, lesions, tissue rupture and rapid tissue loss in adult corals exposed to oil pollutants (e.g. Jackson

et al. (1989); Knap (1987); Renegar and Turner (2021); Renegar et al. (2017); Silva et al. (2016); Wyers et al. (1986)) may have partially resulted from similar successful, or interrupted, polyp bail-out processes. Given the demonstrated bioaccumulation of aromatic hydrocarbons in coral tissues (Jafarabadi et al., 2018; Kennedy et al., 1992; Knap et al., 1982; Solbakken et al., 1984) and strong inhibition of larval metamorphosis success by oil pollutants (Epstein et al., 2000; Goodbody-Gringley et al., 2013; Hartmann et al., 2015; Negri et al., 2016; Negri et al., 2021; Negri and Heyward, 2000; Nordborg et al., 2021; Nordborg et al., 2018; Overmans et al., 2018; Te, 1991; Villanueva et al., 2008; Villanueva et al., 2011), the likelihood of successful reattachment of polyps may be lower than for other stressors. Nonetheless, the potential for polyp bail-out to increase survival of corals exposed to oil warrants further investigation.

4.2. Growth

Growth of reef-building corals exposed to oil pollutants has only been assessed in a handful of previous studies and is generally reported to be unaffected (e.g. Dodge et al. (1984) as reviewed by Turner and Renegar (2017)). However, in the present study, growth of 2-mo recruits decreased significantly with increasing treatment concentration during the 14-d exposure. It is plausible that less energy is allocated to growth during exposure to petroleum hydrocarbons to enable increased mucous production, upregulation of antioxidants and stress response enzymes, for example, to prevent or repair the damage caused by exposure (Downs et al., 2006; Overmans et al., 2018; Ramos and García, 2007; Renegar and Turner, 2021; Rougée et al., 2006; Xiang et al., 2019). Decreased growth rate may also result from increased energy demands for removal of aromatic hydrocarbons present in tissues, indicated by upregulation of proteins associated with xenobiotic processing, response and excretion, as observed in *P. damicornis* exposed to IFO 180 fuel oil WAF (Rougée et al., 2006), *Porites lobata* collected from a reef 3 months after a fuel oil spill (Downs et al., 2006) and *Montastrea faveolata* exposed to benzo[a]pyrene (Ramos and García, 2007).

4.2.1. Post-exposure growth

In contrast to the post-exposure recovery observed from partial mortality, inhibition of the relative growth of recruits continued for the duration of the recovery period following exposure to HFO WAF. The magnitude of the effects decreased over time but did not disappear before the final assessment at 6 w post-exposure. The onset of vertical growth in 2-mo recruits also showed a slight concentration-response, with vertical growth generally delayed in recruits exposed to mid–high treatment concentrations (Fig. A9 and Table A15), providing further evidence that negative effects from oil pollutants towards tropical corals can persist long after the end of exposure. This is also supported by the relatively short accumulation times, but often long depuration times, previously reported for e.g. PAHs in coral tissues (Knap et al., 1982; Solbakken et al., 1984). Delayed tissue regeneration in adult *P. damicornis* following exposure to Louisiana sweet crude oil was suggested to result from either effects on cellular protein production and decreased lipid biosynthesis, or limited fixed carbon availability due to decreases in photosynthetic efficiency of algal symbionts (May et al., 2020). In the present study, the latent effects on growth observed for the aposymbiotic 1-wo recruits and the 2-mo recruits, despite no observed negative effects on the photosynthetic efficiency of their symbionts, suggests that reduced growth is more likely due to biochemical and cellular effects on the host than reduced photosynthetic efficiency of symbiotic algae.

4.3. Impacts on symbiotic algae

The photosynthetic capacity, as quantified using F_v/F_m, and coral colour score of 2-mo A. *millepora* recruits were not affected by HFO exposure, suggesting that the coral host is more sensitive to oil exposure than the algal symbionts. This is consistent with a recent study on the effects of a weathered gas condensate towards *A. millepora* larvae and free-living *C. goraui*, where the putative CTLBB for *C. goraui* growth (45 μmol g^{-1} octanol) was almost 10-fold higher than that for *A. millepora* larval settlement (5.1 μmol g^{-1} octanol; Negri et al. (2021)). Several studies have reported that algal symbiont-associated endpoints such as F_v/F_m and tissue colour were among the least sensitive responses (May et al., 2020), or were completely unaffected (Kegler et al., 2015; Negri et al., 2021; Turner et al., 2021). Other studies on the effects of oil pollutants on adult corals have reported bleaching (expulsion of algal symbionts) or reduced photosynthetic efficiency following exposure to 1-methylnaphthalene, toluene and phenanthrene (Renegar and Turner, 2021; Turner et al., 2021), fluoranthene (+UVR only; Guzmán Martínez et al. (2007)), lubricant oil (Mercurio et al., 2004), produced formation water (Jones and Heyward, 2003) and No. 2 fuel oil (Peters et al., 1981). It is also possible that there are species-specific sensitivities to oil exposure among the diverse array of Symbiodiniaceae associated with corals and that culturing and/or protection by the host, or physiological differences between free-living and symbiotic alga, contribute to differences in sensitivity. The low F_v/F_m values observed across treatment combinations at the end of the 14-d exposure have previously been reported for cultured *C. goreaui*, with values between 0.3 and 0.5 observed for this genotype in culture and *in hospite* (Chakravarti et al. (2019), Carlos Alvarez Roa *pers. commun.*), but the effect of these low values on the experimental outcomes for the host is unknown. Nevertheless, the generally low response of coral symbionts to HFO exposure, in comparison to effects on the early life stages of coral, indicates that the assessment of symbiosis and symbiont health in response to oil exposure may not be a sensitive indicator of sub-lethal stress in tropical corals.

4.4. Potential for phototoxicity

Oil pollutant phototoxicity has previously been reported for several life stages and species of coral (Nordborg et al., 2020). In the present study, the only statistically significant increase in HFO WAF toxicity due to UVR co-exposure was for 2-mo recruit survival after 9 d of exposure. No significant difference in growth was detected between light treatments, indicating that HFO phototoxicity either does not affect growth, or the UVR dose applied was too low to detect any difference. HFO phototoxicity has previously been reported for early coral life stages using the same artificial light sources (Nordborg et al., 2021; Nordborg et al., 2018; Overmans et al., 2018) but the UVR intensities inside the exposure chambers used in the present study were lower due to higher UVR attenuation, primarily as a result of the thickness of the chamber lids. Corals also have highly effective mechanisms for protection against UVR exposure, for example through biochemical UVR filters such as mycosporin-like amino acids (MAAs; Dunlap and Shick (1998)), which can modify the spectrum reaching relevant tissue compartments and thereby potentially reduce the phototoxicity resulting from photosensitisation of oil components within tissues. Further research on the potential effects of MAAs and other UVR protection mechanisms on oil phototoxicity towards coral would help clarify the sensitivity of corals to phototoxicity relative to more traditionally studied taxa.

4.5. Ecological relevance

The low putative lethal CTLBBs derived for *A. millepora* recruits highlights the high potential of oil spills to cause ecological impacts in coral reef environments. Survival of young coral recruits is a significant ecological bottleneck (Randall et al., 2020) and further reductions due to oil exposure may lead to failed recruitment and replenishment of local coral populations, in addition to negative effects on established adult corals. The observed inhibition of growth and delayed uptake of algal symbionts is also likely to increase the time recruits spend below their critical size-escape thresholds, thereby increasing the risk of mortality through predation or competition (Doropoulos et al., 2012). While

latent mortality from the oil exposure was minimal for the *A. millepora* recruits, and recovery from partial mortality was evident for low exposure concentrations, the latent effects on growth and symbiont uptake could further exacerbate delays in reaching size-escape thresholds, even if direct exposure has ended. This supports the position that ecological outcomes may be underestimated by ignoring latent or long-term responses, as previously noted for other taxa and ecosystems (Hook, 2020).

The phototoxicity of HFO towards the recruits was lower than that previously reported for earlier life stages of *A. millepora* (Nordborg et al., 2021). However, the applied UVR exposures were also substantially lower than those reported for shallow reef environments (Nordborg et al., 2021; Nordborg et al., 2018), suggesting that the phototoxic effects of HFO may be more severe under field conditions. Future investigations on the effects of oil pollutants towards tropical, shallow reef species should therefore apply a higher intensity of UVR, within the ecologically relevant range. However, the results highlight the importance of oil phototoxicity in determining the outcomes of exposure, particularly for shorter exposure periods. This was exemplified by the statistically significant phototoxicity observed following 9 d exposure of 2-mo recruits, and further supports the need to consider light conditions in oil spill risk assessments for tropical reef environments.

In the context of broadcast-spawning corals, oil spills coinciding with spawning events may also cause cumulative impacts across both pre- and post-settlement life stages, depending on the temporal extent of the spill. Both larval metamorphosis success and recruit fitness have been identified as highly sensitive to oil exposure, particularly when compared to short-term exposures of adult corals (Fig. 5). Assessment of recruitment and recruit survival is therefore a highly appropriate method of impact assessment to complement adult coral cover assessments *in situ* during or following a spill event. The deployment of settlement devices to assess coral recruitment (Harriott and Fisk, 1987) may also offer opportunities to more broadly assess spill effects on coral reef community recruitment dynamics, including for sponges, calcareous algae, molluscs, bryozoans and other anthozoans.

5. Conclusions

This first assessment of the effects of oil exposure on coral recruits highlight their high sensitivity and the importance of selecting suitable, ecologically relevant endpoints for risk assessments. However, further investigations of the time-, size- and light dependence of coral sensitivity to oil exposure are required to further clarify the variability in responses observed across species, life stages and studies to date. Coral recruit survival and growth are key processes in coral reef regeneration following disturbance events, and their sensitivity to dissolved oil components indicate that oil spills during or after mass coral spawning events may significantly reduce recruitment success. The reduction of local pressures affecting coral recruitment and reef recovery are likely to become increasingly important to maintain reef function and ecosystem services under continued climate change, as well as other environmental and anthropogenic pressures. Therefore it is recommended that oil spill risk assessment and management consider the high sensitivity of early coral life stages, as well as information on the timing of coral spawning events and the protection of identified larval source-reefs, as previously applied for other anthropogenic disturbances such as dredging (Jones et al., 2016; Jones et al., 2015).

Author contributions

F. Mikaela Nordborg: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Visualization; Writing - original draft; Writing - review & editing. **Diane L. Brinkman**: Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing - review & editing. **Andrew P. Negri**: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

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Data statement

Data used in the current study is available as CSV and MS Excel files on the associated page of the Australian Institute of Marine Science Data Centre portal (AIMS, 2022. R rmd files containing statistical analysis and derivation of putative CTLBB values for the present study are available on the GitHub repository associated with the publication (Nordborg, 2022). All images used are available on request from the AIMS Data Centre.

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.

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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.2022.119799.

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