Effect of Louisiana Sweet Crude Oil on a Pacific Coral, *Pocillopora damicornis*

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Highlights

- Tissue regeneration of a shallow-water, branching coral was significantly reduced after a 96 h exposure to a crude oil HEWAF with EC_{10} = 3 µg/L and EC_{50} = 10 µg/L tPAH50.
- Percent tissue regeneration was negatively correlated with exposure duration during short-term exposures followed by a week-long recovery in clean artificial seawater.
- Chlorophyll *a* fluorescence of the algal symbiont decreased following oil exposure: dark-adapted EC_{10} = 330 and EC_{50} = 913 µg/L tPAH50; light-adapted EC_{10} = 176 and EC_{50} = 428 µg/L tPAH50.
- Polyp behavior (retraction) was the driver for reduced gross health assessments in oil-exposed coral.

Abstract

Recent oil spill responses such as the Deepwater Horizon event have underscored the need for crude oil ecotoxicological threshold data for shallow water corals to assist in natural resource damage assessments. We determined the toxicity of a mechanically agitated oil-seawater mixture (high-energy water-accommodated fraction, HEWAF) of a sweet crude oil on a branched stony coral, *Pocillopora damicornis*. We report the results of two experiments: a 96 h static renewal exposure experiment and a "pulse-chase" experiment of three short-term exposure durations followed by a recovery period in artificial seawater. Five endpoints were used to determine ecotoxicological values: 1) algal symbiont chlorophyll fluorescence, 2) a tissue regeneration assay and a visual health metric with three endpoints: 3) tissue integrity, 4) tissue color, and 5) polyp behavior. The sum of 50 entrained polycyclic aromatic hydrocarbons was used as a proxy for oil exposure. For the 96 h exposure dose response experiment, dark-adapted maximum quantum yield (Fv/Fm) of the dinoflagellate symbionts was least affected by crude oil (EC_{50} = 913 µg/L tPAH50); light-adapted effective quantum yield (EQY) was more sensitive (EC_{50} = 428 µg/L tPAH50). In the health assessment, polyp behavior (EC_{50} = 27 µg/L tPAH50) was more sensitive than tissue integrity (EC_{50} = 806 µg/L tPAH50) or tissue color (EC_{50} = 926 µg/L tPAH50). Tissue regeneration proved to be a particularly sensitive measurement for toxicity effects (EC_{50} = 10 µg/L tPAH50). Short duration (6-24 h) exposures using 62.5 mg/L nominal crude oil load resulted in negative impacts to *P. damicornis* and its symbionts. Recovery of chlorophyll *a* fluorescence levels for 6-24 h oil exposures was observed in a few hours (Fv/Fm) to several days (EQY) following recovery in fresh seawater. The coral health assessments for tissue integrity and tissue color were not affected following short-term oil exposure durations, but the 96 h treatment duration resulted in significant decreases for both. A reduction in polyp behavior (extension) was observed for all treatment durations, with recovery observed for the short-term (6-24 h) exposures within 1-2 days following placement in fresh seawater. Wounded and intact fragments exposed to oil treatments were particularly sensitive, with significant delays observed in tissue regeneration. Estimating ecotoxicological values for *P. damicornis* exposed to crude oil HEWAFs provides a basis for natural resource damage assessments for oil spills in reef ecosystems. These data, when combined with ecotoxicological values for other coral reef species, will contribute to the development of species sensitivity models.

Keywords: coral; crude oil; high-energy water-accommodated fraction (HEWAF); *Pocillopora damicornis*; pulse amplitude modulated fluorometry; tissue regeneration assay

Abbreviations: artificial seawater, ASW; confidence interval, C.I.; Deepwater Horizon, DWH; effective quantum yield, EQY; high-energy water-accommodated fraction,

HEWAF; maximum quantum yield, Fv/Fm; parts per thousand, ppt; pulse amplitude modulated, PAM; photosystem II, PSII; photosynthetically active radiation, PAR; polycyclic aromatic hydrocarbons, PAH; toluidine blue O, TBO; sum of 50 polycyclic aromatic hydrocarbons, tPAH50

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1. Introduction

Scleractinian coral species are exposed to oil from both chronic input into shallow marine waters and from acute exposures (e.g., spills) (National Research Council, 2003). Annually, approximately 390 million gallons of petroleum products enter the world's oceans, with 54 % coming from human-derived activities such as extraction, transportation and consumption of oil products (National Research Council, 2003). Recent studies indicate that chronic hydrocarbon exposure (e.g., natural seeps, land-based runoff) may impact both the coral animal and its dinoflagellate symbionts (Downs

et al., 2012; Jafarabadi et al., 2018). Highly polluted areas such as the Persian Gulf (Sinaei and Mashinchian, 2014) and Jakarta Bay, Indonesia (Baum, et al., 2016) have chronic PAH levels between 10-385 µg/L (Σ16 and Σ15 PAHs, respectively). Most reported levels of chronic oil input to coral reef waters have been in the ng/L (parts per trillion) range, however (Cheng et al., 2010; Jafarabadi et al., 2018; Zhou et al., 2000). By contrast, the sudden exposure of corals to large oil spill events such as DWH can have catastrophic short-term effects, as the capacity to acclimatize quickly to the petroleum hydrocarbon load is limited, and cellular metabolic processes can be overwhelmed (Downs et al., 2006).

Evaluations of coral physiological condition in response to xenobiotics have included monitoring the loss of the dinoflagellate symbionts (zooxanthellae) or 'bleaching' (Brown, 2000; Douglas, 2003; Jones, 1997), physiological changes such as polyp retraction (Reimer, 1975; Renegar et al., 2016; Wyers et al., 1986) and estimation of percent tissue loss during disease events or exposure to stressors (Reimer, 1975; Renegar et al., 2016; Thompson et al., 1980). It is estimated that stony corals acquire as much as 90 % of the fixed carbon required for growth from zooxanthellae (Muscatine and Porter, 1977); therefore, it is important to evaluate possible oil effects on the coral symbionts. Measuring algal photosynthetic quantum yield under dark- and light-adapted conditions using a pulse amplitude modulating (PAM) fluorometer provides a method to evaluate chlorophyll *a* fluorescence, providing insight on carbon fixation and photosystem II (PSII) damage (Ralph et al., 2005). Coral condition also has been evaluated by monitoring tissue repair of experimentally induced wounds, as slower regeneration rates are associated with increased exposure to stressors (Fisher et al.,

2007; Kramarsky-Winter and Loya, 2000; Meesters and Bak, 1993; Moses and Hallock, 2016; Rodriguez-Villalobos et al., 2016; Traylor-Knowles, 2016). Since coral reefs are often in close proximity to sources of oil such as marinas, drilling rigs, tanker routes and shoreline oil refineries, it is important to understand how petroleum spill exposures impact these critical ecosystems. Oil spills are dependent upon ocean currents and tides, forces that may move the slick away within a few hours, or hold the oil in place for days to weeks. While decades of research have documented negative impacts of crude oil exposure on stony coral species, there has been little evidence linking crude oil effects using hydrocarbon chemistry measurements (Negri, et al., 2016; Turner and Renegar, 2017).

Pocillopora damicornis (Linnaeus 1758), an Indo-Pacific branching stony coral species, was used to determine oil exposure effects (Hoeksema et al., 2014). It is found in areas with oil-related activities (e.g., shipping lanes, oil rigs) and its relatively fast growth rate makes it particularly suitable for use in tissue regeneration assessments (Rodriguez-Villalobos et al., 2016). Previous studies of petroleum hydrocarbon effects on *P. damicornis* indicate that this species is relatively sensitive to exposure compared to other corals (e.g., *Porites*, *Montipora*, *Fungia*), with documented effects on both the coral animal and the algal symbiont (Johannes et al., 1972; Peachey and Crosby, 1995; Reimer, 1975; Rougee et al., 2006; Te, 1992; Villanueva et al., 2011).

Recently the negative effects of measured petroleum hydrocarbons on coral larvae have been demonstrated (Hartmann et al., 2015; Negri et al., 2016; Overmans et al., 2018). Our study focuses on short-term effects of oil spills in coral reef environments on

adult *P. damicornis* fragments. We have coupled analytical chemistry measurements of tPAH50 as a proxy for oil exposure with behavioral and physiological endpoints for coral and associated dinoflagellate symbionts to determine ecotoxicological concentration values of acute oil exposures using a 96 h static renewal dose-response experiment. The effect of shorter-term (6-24 h) oil exposure scenarios was tested by varying exposure duration of coral to a single high-energy water-accommodated fraction (HEWAF) oil load (62.5 mg/L) over three separate short exposure durations (6, 12, and 24 h) and including an additional 96 h exposure for comparison. In addition, recovery responses in fresh artificial seawater were evaluated by following exposed fragment responses for up to 184 h post treatment. We hypothesize that acute crude oil exposures will result in 1) health effects (polyp retraction, bleaching, tissue loss), 2) a reduction in symbiont chlorophyll *a* fluorescence, and 3) reduced wound healing ability of *P. damicornis*. We expect that recovery of the measured health parameters may be delayed once the stressor is removed.

2. Materials and Methods

2.1 Chemicals

Solvents (pesticide-free acetone, isopropanol, and pesticide-free hexane), Liquinox detergent, hydrochloric acid, sodium sulfate, toluidine blue O and dimethyl sulfoxide were purchased from Thermo Fisher Scientific (Waltham, MA). Dichloromethane and GF/F paper were acquired from VWR International (Radnor, PA). Louisiana sweet crude oil (Mississippi Canyon Block 252) was a gift from the NOAA Office of Response and Restoration and was stored at 4 °C until use. Internal polycyclic aromatic

hydrocarbon (PAH) standards used in the quantitative analysis of PAHs were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA).

2.2 Equipment Preparation

Precleaned glassware was used for all coral exposures and HEWAF preparation and storage. Straight-sided, wide mouth glass jars (500 mL) were used for coral acclimation and dosing (VWR, Radnor, PA, part #89093-982, cleaning protocol A, level PC). Amber glass bottles for the analysis of semivolatiles and pesticides (group 2) were used for HEWAF storage and all dilutions (I-Chem Certified, Chase Scientific Glass, Rockwood, TN). All vessels were rinsed with Tropic Marin artificial seawater (ASW, Tropic Marin Sea Salts, Wartenburg, Germany) prior to use. Glassware, Teflon and metal utensils not previously exposed to crude oil were cleaned thoroughly according to Chapman et al. (1995). Oiled glassware and Teflon-ware reused in generating HEWAFs (graduated cylinders, funnels, etc.) were cleaned according to the method of Forth (2017).

2.3 Coral Culture

Pocillopora damicornis, colonies were purchased from Pacific East Aquaculture (Mardela Springs, MD) and held under the South Carolina Department of Natural Resources non-indigenous species permit #NI17-0401 at the NOAA Coral Culture Facility in Charleston, SC. Eight weeks prior to experiment initiation, colonies were fragmented to generate approximately 120 small (~2.0 cm height) nubbins. Coral nubbins for the tissue regeneration experiment were 3.0 cm, so that when cut, they would meet the target (2.0 cm) height. Each nubbin was attached with cyanoacrylate gel (e.g., superglue gel) to a custom-made Teflon mounting peg (Supplement A). Mounted

187 nubbins were cultured in a glass and Teflon aquarium system (26.0 +/- 0.5 °C)
188 containing ASW (36 ppt) under a 10 h:14 h light:dark cycle. Lighting was provided by
189 two 4 X 39 W T-5 HO light fixtures, each with two AquaSun (UV Lighting Co., Avon, OH)
190 and two Blue Plus (ATI, Denver, CO) 54 W bulbs. The photosynthetically active
191 radiation (PAR) at coral depth ranged from 68–77 μmol m^{-2} s^{-1}. Spectral analysis (JAZ
192 spectrometer, Ocean Optics, Largo, FL) of the lighting system showed three major
193 peaks at 430-440, 540-550 and 610-620 nm. Corals were fed a mixture of Bio-Pure®
194 frozen brine shrimp nauplii, rotifers, and cyclopods (Hakari, Hayward, CA), Grow Fry
195 Starter (New Life Spectrum® , Homestead, FL) and Reef-Roids (Polyp Lab, Lenexa,
196 KS) three times per week until oil exposure initiation. Accumulated algal biofilms were
197 manually debrided from the Teflon pegs 2-3 days before starting the experimental
198 acclimation.

199 2.4 Coral Acclimation

200 *Pocillopora damicornis* fragments were acclimated in an environmentally controlled
201 room (26 °C) for 72 h prior to starting the experiment. Coral nubbins on Teflon pegs
202 were placed in custom-made Teflon jar stands (Supplement A) and Teflon stands with
203 coral were transferred to clean 500 mL jars. Each was filled with 400 mL ASW (36.0 +/-
204 0.5 ppt, 26 °C) during the acclimation period to accommodate the 3.0 cm tissue
205 regeneration nubbins. A Teflon air line (attached to house air line and vinyl tubing via a
206 quick-release valve) was placed in each jar and the bubble rate was adjusted (~1-2/s).
207 Lighting and seawater temperature were as described for coral culture conditions.
208 Water changes (100 %) were performed every 12 h. Salinity and temperature on fresh
209 and spent treatment solutions were monitored at each water change.

2.5 Crude oil high-energy water-accommodated fraction

The HEWAF was generated using Louisiana sweet crude oil (Macondo source oil collected during the Mississippi Canyon 252 oil spill response) using a Waring™ CB commercial blender according to the methods of Forth (2017). This method generates an oil suspension containing both dissolved hydrocarbon fractions and small oil droplets (Redman, 2015). Briefly, 3.75 L of ASW (36 ppt, 26.0 °C) and 3.75 g of crude oil (1 g/L) were added to the blender under reduced (red) lighting and agitated on low speed for 30 s. The oil-seawater suspension immediately was transferred to a 2.0 L separatory funnel and allowed to separate for 1 h (26 °C). The bottom layer of the unfiltered HEWAF from each vessel (1.5 L total volume) was collected into a clean, rinsed (ASW) 4 L amber glass bottle and used immediately to make the required dilutions (two-fold series, 3.9-250.0 mg/L, nominal oil concentrations). The sum of 50 individual polycyclic aromatic hydrocarbons and alkylated homolog groups (tPAH50) was used as a surrogate for crude oil exposure. The tPAH50 results are presented graphically as the geometric mean of fresh and 12 h spent treatment concentrations (see section 2.11 Analytical Chemistry). We also present effect concentrations calculated from chemistry analysis of the fresh (time 0) treatments.

2.6 HEWAF 96 h Exposure

Corals were exposed to HEWAF dilutions (3.9-250 mg/L oil load) in a 96-h static renewal experiment. Treatments were changed every 12 h to keep salinity in check. This 12 h cycle also mimics tidal ebb and flow in the marine environment, which could act to move spilled oil back and forth over a reef. Following the acclimation period and at the onset of the dark period, Teflon stands with coral were transferred with clean

Teflon-coated metal tongs to dosing jars containing 350 mL of freshly prepared treatment solution (n = 4). Jars remained open for the duration of the experiment. To reduce placement effects, jars were organized randomly under light fixtures in the temperature-controlled dosing room. One jar with no coral at the highest nominal HEWAF concentration (250 mg/L) was included in the dosing experiment to monitor water quality. Temperature and salinity were measured every 12 h on fresh and spent treatment solutions. Daily pH was measured on spent treatment solutions using a probe connected to a Thermo Orion 5-Star multimeter. Samples (400 µL) for ammonia quantification (sodium salicylate method) were removed from each vessel once daily (12 h spent treatment) and stored at -20 °C until further analysis (Bower and Holm-Hansen, 1980). Water changes (100 %) occurred every 12 h, with newly made HEWAF in clean dosing vessels. Water samples were collected for chemistry analysis at experiment initiation (T0, fresh ASW and fresh HEWAF dilutions). Spent treatment solutions from pooled replicates were also analyzed for tPAH50 at 12 h and 96 h.

2.7 Pulse-Chase Experiment

A pulse-chase experiment was designed to determine effects of acute short-term exposures of coral to oil and to evaluate coral recovery following varying exposure durations. A single HEWAF oil load (62.5 mg/L nominal oil concentration) was selected for this experiment based on effects observed during the 96 h dose-response experiment and reported levels of PAHs documented following oil spills (DIVER, 2017). The five treatment regimens (n = 4) included: an ASW control, 6 h, 12 h, 24 h and 96 h HEWAF exposures (pulse) followed by a recovery period (chase) of 7-11 days in ASW. Coral nubbins were acclimated as above (single replicate for each endpoint per dosing

jar), then exposed to the single oil load across treatment times. Coral fragments were transferred to fresh treatment solutions in clean jars every 12 h. Fragments in the 6 h and 12 h treatments received a single pulse of oil treatment, while the 24 h and 96 h treatments were subjected to repeated pulses (2 and 8, respectively). Samples for chemical analysis were collected: fresh control ASW (T0 and 84 h, n = 2) fresh HEWAF (T0, 12 h, 24 h and 84 h, n = 8), spent ASW (6 h, 12 h, 24 h and 96 h, n = 4) and spent HEWAF (6 h, 12 h, 24 h and 96 h, n = 4). Each treatment jar contained two intact 2.0 cm fragments (for health score and photosynthetic quantum yield endpoints) and two 3.0 cm nubbins for tissue regeneration. One tissue regeneration fragment per treatment replicate was used to determine oil effects on wounded coral fragments (cut prior to oil exposure). A second tissue regeneration fragment was exposed to oil intact, cut at the beginning of the recovery period and evaluated when fragments reached the approximate percent tissue regeneration of control fragments. A comparison between control and exposed fragments was used to estimate approximate recovery times following oil exposure. Temperature and salinity were measured on fresh and spent treatment solutions every 12 h, and pH and ammonia were measured every 24 h (during light cycle).

2.8 PAM Fluorometry

One coral nubbin per treatment replicate was used to determine dark-adapted maximum quantum yield (Fv/Fm) and light-adapted effective quantum yield (EQY) of dinoflagellate symbionts using an Imaging PAM M-series chlorophyll fluorometer (MAXI version, Walz GmbH, Effeltrich, Germany). Each coral fragment was analyzed 2 h before the end of the dark cycle (Fv/Fm) and 2 h before lights went off (EQY).

279 Experimental time points for the 96 h dose-response experiment were -14 h, 34 h and
280 82 h (dark adaption) and -4, 44 and 92 h (light adaption). Experimental time points for
281 the pulse-chase experiment were -14, 10, 34, 58, 82, 106, 130, 154 and 250 h (dark
282 adaption) and -4, 20, 44, 68, 92, 116, 140, 164 and 260 h (light adaption).

283 Replicate fragments were evaluated as previously reported (Ralph et al., 2005)
284 (Supplement A). Data were collected on three areas of interest placed on a flat surface
285 in the center of a given fragment and averages for each replicate fragment were
286 calculated.

287 2.9 Health Assessment

288 One 2.0 cm coral fragment in each dosing jar was visually scored daily (middle of light
289 period) for changes in any of the three parameters associated with health condition
290 (Table 1). The scoring criteria were refined to distinguish gradations of change in tissue
291 integrity, tissue color and polyp behavior (modified from De Leo et al., 2016). Care was
292 taken to avoid disturbing the coral during the assessment (e.g., bumping jars), so that
293 maximum polyp extension could be determined.

294 2.10 Tissue Regeneration Assay

295 Immediately prior to experiment initiation, 3.0 cm fragments were cropped using clean,
296 stainless steel bone cutters and leaving a flat apical surface. Corals exposed to oil
297 intact during the pulse-chase experiment were cropped immediately following oil
298 exposure (along with control fragments). Each cut fragment (~2.0 cm) was placed into
299 a dosing jar filled with ASW and a bright field image was taken of the cut surface next to
300 a Teflon centimeter rule using a MVX10 research macro zoom microscope with a 0.63x

objective (Olympus, Melville, NY) and equipped with a DP71 digital camera (Olympus, Center Valley, PA). Since new tissue growth is translucent (lacks symbionts), coral tissue was stained prior to imaging at experiment termination. Coral fragments were removed from the treatment solution, rinsed in ASW and placed in a jar filled with ASW approximately 1 cm below the cut surface of the coral fragment. The translucent new tissue growth was dyed with a vital stain prior to imaging. Toluidine blue O (TBO, 1 % in DMSO) was diluted to 0.1 % in ASW. Approximately 30 µL of the dilute TBO was placed onto the cut surface of the coral and incubated at room temperature for 3 min. Stain was reapplied as needed (i.e., if it drained away from the coral surface). Fragments were rinsed in ASW and imaged as previously described.

Image analysis was performed using Adobe Photoshop CC 2017. Pixel units were calibrated to the centimeter ruler in each image. Total area of bare skeleton was recorded for each fragment at time 0 and experiment termination. Percent tissue regeneration was determined from the difference in skeletal area between the two values.

2.11 Analytical Chemistry

The sum of 50 polycyclic aromatic hydrocarbon compounds were determined for fresh and spent treatment solutions (as detailed above for each experiment) and used as a surrogate for crude oil exposure (Supplement B). Water samples (130-1000 mL) were collected in solvent rinsed, pre-acidified (0.2-1.5 mL 18 % HCl), amber bottles and stored at 4°C until analysis. Samples were spiked with internal standards (18 deuterated polycyclic aromatic hydrocarbons) and extracted using a liquid/liquid

extraction adapted from methods detailed in Reddy and Quinn (1999). Samples were passed through silica solid phase extraction (SPE) cartridges and eluted from the cartridge with hexane and dichloromethane. A recovery standard, d_{14}-p-terphenyl, was added prior to GC/MS analysis to measure internal standard recovery. Samples were analyzed on an Agilent 6890/5973N GC/MS with split/splitless injector and a DB17ms 60m x 0.25 mm x 0.25 µm analytical column. All analytes had a coefficient of determination (r^2) greater than or equal to 0.995. Data analyses were performed using MSD Chemstation software. A procedural blank and a PAH reagent spike sample were included in all sample extraction batches (n = 8).

2.12 Statistical Analyses

Fresh (time 0) and the geometric mean of fresh and 12 h spent tPAH50 measurements were used for statistical analyses of each endpoint. All analyses were performed using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). The response variables for the dose-response experiment (health metric scores, percent tissue regeneration, and PAM fluorometry measurements) met the assumptions for parametric statistics. The model residuals followed the normal distribution and the residual variances were homogeneous. A single factor ANOVA (PROC GLM) was performed on each variable using the ASW treatment as the experimental control. A Dunnett's test for multiple comparisons versus control was performed post-hoc to determine significant differences between treatment groups and control. Alpha was set to 0.05 for all statistical tests. To determine the effective concentration to cause a 50% effect (EC_{50}) in the aforementioned response variables, a three-parameter normal probability (fitted probit)

345 equation was constructed (PROC NLIN METHOD=GAUSS) using the PROBNORM
346 function.

347 For the pulse-chase experiment, tissue regeneration and PAM fluorometry
348 measurements were analyzed using a two-factor ANOVA with a test for interaction. The
349 two factors were pulse duration (6 h, 12 h, 24 h, and 96 h) and treatment (control or
350 dosed). The model residuals followed the normal distribution and the residual variances
351 were homogeneous. Finding no significant interaction, a test for simple effects was
352 performed at each level of pulse duration to examine if control treatments were
353 significantly different from dosed treatments. Alpha was set to 0.05 for all statistical
354 tests. The health score data from the pulse-chase experiment were analyzed using the
355 non-parametric Kruskall-Wallis test followed by non-parametric multiple comparison
356 tests for treatments versus a control (Zar, 1999).

357 **3. Results**

358 3.1 Water quality

359 Temperature and salinity levels remained within normal growth parameters for the
360 course of each experiment (26.0 +/- 0.5 °C, 36 +/- 1 ppt). Total ammonia nitrogen (12 h
361 spent treatment sample) ranged from 0.00-0.05 for the initial dose response experiment
362 and 0.00-0.16 mg/L for the pulse-chase experiment. The pH values for the 96 h
363 exposure were between 8.12 and 8.42 and for the pulse-chase experiment ranged from
364 8.17-8.59.

365 3.2 Analytical chemistry

366 Crude oil high-energy WAF mixtures consisted of both dissolved PAHs and droplet oil.
367 Chemical analysis of tPAH50 concentrations in HEWAFs followed expected trends,
368 based on degree of stock dilution. Extraction of Louisiana sweet crude oil HEWAF
369 medium at time zero (T0, 250 mg/L nominal oil load) resulted in 3971 µg/L tPAH50 with
370 roughly 50% reduction in each subsequently lower treatment dilution (Supplement B.1).
371 After 12 h in the dosing jar, HEWAF tPAH50 concentrations were reduced by at least 88
372 % in all treatments. For example, the 7.8 mg/L oil loading treatment tPAH50 was
373 measured in the fresh treatment solution as 62 µg/L and decreased to 8 µg/L tPAH50
374 after 12 h under experimental conditions (Supplement B.2).

375 Chemical analysis of the pulse-chase experimental treatment also exhibited ~86 %
376 reduction of tPAH50 concentration over 12 h. Initial (fresh, load 62.5 mg/L) average
377 tPAH50 concentrations in eight HEWAF preparations was 503 µg/L (Supplement B.3;
378 percent relative standard deviation ~8 %). The 6 h spent treatment solution retained
379 131 µg/L tPAH50. Repeated spent treatment analysis after 12 h resulted in average
380 tPAH50 concentrations ranging from 64 to 79 µg/L (Supplement B.3). Spent media
381 tPAH50 concentration was reduced by approximately 74 % after 6 h vs 86 % after 12 h.
382 Individual PAH50 concentrations from the pulse-chase experiment are detailed in
383 Supplement B.4.

384 <u>3.3 Effect of 96 h HEWAF concentrations on coral and symbionts</u>

385 *3.3.1 HEWAF impacts to symbiont chlorophyll a fluorescence*

386 Following a static 12 h renewal exposure of *P. damicornis* fragments to dilutions of
387 HEWAF (12-945 µg/L tPAH50, geometric mean of fresh and 12 h spent treatment), oil-

388 exposed symbiont Fv/Fm and EQY were decreased significantly compared to controls
389 ($p<0.05$, Figure 1). A reduction in dark-adapted chlorophyll *a* fluorescence was
390 observed with an 82 h exposure (Panel A). Decreased fluorescence was noted for EQY
391 vs Fv/Fm (Panel B). Table 2 shows the effect concentrations for the tPAH50
392 exposures. At 82 h the oil exposure resulted in an Fv/Fm EC_{50} value of 913 µg/L
393 tPAH50 (95% C.I. = 862-968), reported as the geometric mean of T0 and 12 h spent
394 treatment. The EC_{50} value for EQY at 92 h was 428 µg/L tPAH50 (95% C.I. = 375-488).
395 The EC_{50} values calculated using the time zero (fresh) treatment chemistry data were
396 3804 and 1640 µg/L tPAH50 for Fv/Fm and EQY, respectively (Table 2). Response
397 curves for Fv/Fm and EQY are shown in Supplement C.1.

398 *3.3.2 General coral health effects of HEWAF concentration exposure*
399 *Pocillopora damicornis* fragments exposed to increasing concentrations of HEWAF
400 resulted in significantly decreased ($p<0.001$) health rubric scores at 89 h (Figure 2,
401 Supplement D). Tissue loss was first observed for three fragments at 65 h (245 and
402 456 µg/L tPAH50, geometric mean). Tissue loss continued for the three highest
403 treatments until experiment termination. Tissue loss was most severe for the 945 µg/L
404 tPAH50 treatment, with three of four fragments losing 50-75 % tissue by 89 h (Figure 2,
405 Panel A). Coral fragments started bleaching by 31 h for the 22-945 µg/L tPAH50
406 treatments, but at 89 h was significant only for the 945 µg/L tPAH50 treatment (Figure 2,
407 Panel B). A decline in health was driven by polyp behavior (reduced 3-4 points within
408 the first 17 h of exposure), with treatments of 127 µg/L tPAH50 and higher significantly
409 different from the control at 89 h (Figure 2, Panel C). Some polyp recovery was noted
410 in the 12 µg/L tPAH50 treatment at 41 h post treatment initiation. The EC_{50} values for

411 tissue integrity were 806 µg/L tPAH50 (geometric mean) and 3296 µg/L tPAH50 (fresh
412 treatment), while tissue color resulted in EC_{50} values of 926 (geometric mean) and 3280
413 (fresh treatment) µg/L tPAH50, respectively (Table 2). Polyp behavior was the most
414 sensitive endpoint in the visual health assessment, with EC_{50} values ranging from 27
415 (geometric mean) to 84 (fresh) µg/L tPAH50 (Table 2). Dose-response curves with 95
416 % confidence intervals for each health metric are presented in Supplement C.2.

417 *3.3.3 Inhibition of coral tissue regeneration by crude oil HEWAF concentrations*
418 The tissue regeneration assay was most sensitive to crude oil HEWAF exposure.
419 Tissue regeneration was inhibited in the 96 h dose response experiment at all tested
420 concentrations (Figure 3, Panel A, $p<0.005$). Figure 4 shows representative coral
421 fragments at time zero (after initial cut) and at 96 h (with TBO tissue staining).
422 Treatments of 12-127 µg/L tPAH50 (geometric mean of fresh and 12 h spent
423 treatments) had little to no tissue regeneration compared to the control. Increasing
424 tissue loss was observed for fragments subjected to 245-945 µg/L tPAH50. The tissue
425 regeneration EC_{10} values ranged from 3 (geometric mean) to 7 (fresh) µg/L tPAH50 and
426 EC_{50} values were 10 (geometric mean) and 29 (fresh) µg/L tPAH50 (Table 2). The
427 response curve for tissue regeneration is shown in Supplement C.3.

428 <u>3.4 Pulse-Chase Experiment: Effects of oil exposure duration</u>
429 *3.4.1 Symbiont chlorophyll* a *fluorescence effects*
430 Shorter durations of a single HEWAF dose (266 µg/L HEWAF tPAH50 concentration, 6
431 h geometric mean; 189 µg/L HEWAF tPAH50 concentration, 12 h geometric mean)
432 significantly affected ($p<0.05$) symbiont chlorophyll *a* fluorescence (Fv/Fm and EQY,

433 Figure 5). Maximum quantum yield (Fv/Fm) impacts were variable at the 10 h time
434 point with significant reduction for the 12 h and 96 h exposure durations (Figure 5,
435 Panel A). The 12 h exposure fragments fully recovered within 24 h. The Fv/Fm of the
436 96 h HEWAF exposure required 34 h to recover to the level of the controls following
437 placement in fresh ASW. The EQY for 6, 12, 24 and 96 h HEWAF exposures was
438 significantly decreased at the 20 h time point (Figure 5, Panel B). Complete EQY
439 recovery for the 6, 12 and 24 h HEWAF exposures occurred by 92 h (68-86 h) after
440 placement in fresh ASW. Recovery time for *P. damicornis* symbionts with significant
441 impacts to EQY after a 96 h HEWAF exposure was between (68-164 h post-treatment).

442 *3.4.2 Coral health score effects*

443 Decreased health effects were observed for *P. damicornis* fragments exposed to crude
444 oil HEWAF. Tissue integrity and tissue color were not affected by the short-term (6-24
445 h) oil exposures; however, immediate polyp retraction was observed in response to all
446 pulse (exposure) durations compared to the no pulse control (Figure 6). There was little
447 (<5 %) to no tissue loss for coral fragments in the 6, 12 or 24 h oil treatments and these
448 treatment durations did not result in significant effects when compared to the no
449 treatment control (Figure 6, Panels A, D). Coral fragments in the 96 h oil treatment
450 exhibited a loss of tissue integrity after 89 h of exposure and this tissue loss increased
451 with time. Coral tissue for all oil treatment durations partially bleached over time; the 96
452 h treatment duration resulted in significant differences ($p<0.05$) from the control
453 between 65 and 185 h (Figure 6, Panels B, E). *Pocillopora damicornis* fragments in the
454 control also showed a very slight loss of color over time, especially after 185 h. Low
455 initial polyp behavior scores at – 7 h (before treatment started) were due to disturbing

fragments during photography (Figure 6, Panel C). Control fragments returned to full extension at 17 h. Polyps exhibited normal behavior (fully extended) for the 6, 12 and 24 h HEWAF following placement in fresh ASW; the normal response was delayed for 24-53 h (Figure 6, Panel C and Supplement E). Fragments exposed to oil for 96 h did not fully extend polyps in the recovery period (up to 233 h). This resulted in a significant difference ($p<0.05$) for the 96 h oil exposure when compared to the no pulse control after 65 h HEWAF treatment (Figure 6, Panel F).

3.4.3 Impacts to tissue regeneration

Pocillopora damicornis fragments cut prior to exposures and subjected to short-term HEWAF exposures (189 µg/L, 12 h geometric mean; 266 µg/L, 6 h geometric mean tPAH50) showed significantly ($p<0.0001$) decreased tissue regeneration after 72 h, with tissue loss noted for the longest exposure (Figure 7, Panel A). Following placement in fresh seawater, delayed tissue regeneration times were observed for all HEWAF treatments compared to the controls at 72 h (approximately 50 % tissue regeneration). The 6 h oil treatment resulted in a 48 h lag in recovery, the 12 h treatment had a 72 h lag and the 24 h treatment resulted in 84 h delayed recovery. The cut coral fragments in the 96 h exposure did not recover to the level of the 72 h controls by the end of the experiment (168 h post treatment). Significant impacts ($p<0.0001$) to oil-exposed intact coral fragments were also noted (Figure 7, Panel B). Intact fragments cut following oil exposure and placed in fresh seawater resulted in significantly slowed tissue regeneration 72-84 h post treatment compared to controls cut at the same time.

4. Discussion

Oil exploration, production and transport often intersect spatially with shallow coral reef ecosystems throughout the tropics and sub-tropics, presenting numerous threats of exposure through spills, groundings or wellhead blowouts, as in DWH. This is the first study to demonstrate ecotoxicological effects of acute crude oil HEWAF exposures on the adult life stage of the ecologically important shallow water coral species, *P. damicornis,* using the surrogate, tPAH50. Total PAH50 has been used as a proxy for oil toxicity to other marine organisms (Esbaugh et al., 2016; Incardona et al., 2014; Stieglitz et al., 2016), providing a platform to compare toxicity between species and contaminant type that are suitable for oil spill-related ecological risk assessments and natural resource damage assessments. We emphasize that our experiments were performed under laboratory light conditions (no ultraviolet light, UV). As it has been demonstrated that UV light enhances toxicity of hydrocarbon compounds for marine organisms (Alloy et al., 2016; Finch et al., 2017; Sweet et al., 2017), the coral toxicity data we report are likely conservative values.

4.1 Ecotoxicological Potential of Louisiana sweet crude oil to coral

4.1.1 Oil effects on symbiont photosynthesis

We observed that symbiont Fv/Fm was impacted by crude oil exposure (EC_{10} = 330 µg/L tPAH50, geometric mean) and this was further reduced to 176 µg/L tPAH50 following *P. damicornis* exposure to light. The reported effect concentration values for *P. damicornis* symbionts were 1-2 orders of magnitude higher than for tissue regeneration, indicating that PAHs are less toxic to the algal symbiont than to the coral animal. Chlorophyll *a* fluorescence measurements are valuable indicators of the state of PSII in plants. Exposure to light naturally damages PSII reactions centers

(photoinhibition) and dark adaptation provides time for photosynthetic reaction centers in the chloroplast to repair damage normally resulting from exposure to sunlight. Photoinhibition is exacerbated when light is combined with various other environmental stressors, yielding an increase in damaged PSII reaction centers that cannot be repaired during the dark cycle (Nishiyama et al., 2001; Ralph, 2000; Takahashi and Murata, 2005). Since symbiotic zooxanthellae have been reported to contribute as much as 90 % of the fixed carbon required for growth of scleractinian corals (Muscatine and Porter, 1977), impacts to PSII also have severe impacts on coral nutrition, and in turn on growth and other physiological processes (e.g., reproduction, defense, wound repair, etc.).

Oil exposure has been shown to impact photosynthetic output in several ways. Physical barriers created by the oil can block sunlight required for photosynthesis or decrease gas exchange (Baker, 1970). Reductions in chlorophyll content have been observed in response to oil exposure of corals (Baruah et al., 2014; Jafarabadi et al., 2018; Olaranont et al., 2018). In addition, Jafarabadi et al. (2018) have shown that PAHs can penetrate both coral tissues and zooxanthellae membranes. Since chlorophyll-peridinin complexes of many autotrophic dinoflagellates incorporate both hydrophilic and lipophilic proteins, cellular penetration of PAH compounds could act to rupture the photosynthetic assembly, with pigments subsequently degraded.

Similar to our study, decreases in Fv/Fm from oil exposure have been reported for *Acropora formosa* (Mercurio et al., 2004) and *Porites divaricata* (Guzman Martinez et al., 2007) symbionts with effective concentrations of hydrocarbon in the ppb (µg/L)

range. However *Pocillopora verrucosa* exposure to a diesel fuel WAF (415 µg/L total PAH, geometric mean, *our calculation*) resulted in no significant effect on symbiont Fv/Fm in a static 84-h test (Kegler et al., 2015). Additionally, Renegar et al. (2016) found no effect on Fv/Fm following a 48-h exposure of *P. divaricata* to a single PAH compound, 1-methylnaphthelene in a continuous-flow system (640-25,832 µg/L). While experimental design (static, continuous flow, or static renewal test), exposure duration, lighting and the use of different PAH mixtures may have played a role in the contrasting results, it is possible that variable rates of PSII electron transport among zooxanthellae species are also a factor (Cantin, et al. 2009). Additionally, variable optical properties of corals (fluorescent proteins, chromoproteins) can confound PAM-based fluorescence measurements (Mayfield et al., 2014; Wangpraseurt et al., 2019). We observed significant tissue color loss in the visual health score only at the highest HEWAF treatment (945 µg/L tPAH50), but significant decreases in Fv/Fm at much lower concentrations (to 48 µg/L tPAH50, Figure 1). While it is possible that symbionts could be dead or dying within the coral tissues, it also is possible that reduction in Fv/Fm for oil-exposed corals may be due to PAH effects on the photosynthetic machinery. Further research to understand mechanisms of coral symbiont photosynthetic responses to crude oil exposure is needed.

4.1.2 Oil effects on polyp behavior and tissue integrity

Pocillopora damicornis exhibited severe polyp retraction in response to crude oil HEWAF exposure (EC_{10} = 3 µg/L tPAH50, geometric mean). Corals can respond in a limited number of ways to xenobiotic exposure. Mucocytes located in the epidermal cell layer produce a lipopolysaccharide mucus layer, which acts as a buffer to the immediate

546 environment. Mucus production can be stimulated, and it can bind and remove
547 potentially harmful chemicals such as oil (Bak and Elgershuizen, 1976). Corals also
548 retract polyps in response to irritants such as crude oil (Knap, 1987; Lewis, 1971;
549 Reimer, 1975). This action closes off the gastrodermal cavity, possibly delaying or
550 limiting effects of toxic compounds on the gastrodermal cells not protected by a mucus
551 layer. Since coral species such as *P. damicornis* extend polyp tentacles as a normal
552 heterotrophic feeding behavior, sustained polyp retraction can reduce feeding activity,
553 and potentially nutrient intake. When the concentration of a contaminant reaches a
554 threshold that overcomes these natural defense mechanisms, biochemical and cellular
555 processes break down, which can result in tissue degradation.

556 We also observed that *P. damicornis* lost tissue in response to crude oil HEWAF
557 exposure (EC_{10} = 202 µg/L tPAH50). Adult coral mortality is usually associated with
558 complete tissue loss from the skeleton. While corals can recover from partial tissue
559 losses (Kramarsky-Winter and Loya, 2000), even small tissue lesions can have a
560 negative impact on colony health by increasing chances of disease by opportunistic
561 microorganisms (Page and Willis, 2008). In the wild, tissue integrity changes likely
562 affect feeding activity, increase susceptibility to disease or encourage algal overgrowth,
563 and/or decrease photoprotective mechanisms due to chromoprotein or fluorescent
564 protein loss (Lamb et al., 2014; Lirman, 2001; Salih et al., 2000).

565 Results from this study and others indicate that corals elicit an innate protective
566 response to oil exposure (Bak and Elgershuizen, 1976; De Leo et al., 2016;
567 Elgershuizen and De Kruijf, 1976; Frometa et al., 2017; Lewis, 1971; Reimer, 1975;

568 Renegar et al., 2016; White et al., 2012; Wyers et al., 1986). We observed significant
569 negative gross health effects for *P. damicornis* in an acute 96 h static renewal test.
570 Renegar et al. (2016) recently reported a health score EC_{50} value of 7442 µg/L tPAH50
571 for *P. divaricata* exposure using a single PAH analyte, 1-methylnaphthalene. We can
572 make no direct comparison with our study due to differences in health metric reporting,
573 PAH composition and exposure duration, however. *Porites divaricata and P.*
574 *damicornis* are both 'weedy' coral species, often found in disturbed environments or
575 when reef species complexity is low (Darling et al., 2012; Newman et al., 2015; Smith et
576 al., 2013). Increased relative sensitivity of *P. damicornis* compared to other corals has
577 been demonstrated for both natural and anthropogenic stressors, however (Reimer,
578 1975, Te et al., 1998; Stimson et al., 2002; Downs et al., 2016). Our method of static
579 renewal (vs continuous flow for the *P. divaricata* experiment) also may have contributed
580 to increased toxicity of crude oil to *P. damicornis*. As the 12 h static renewal mimics
581 tidal ebb and flow in shallow marine waters, it suggests that the action of tidal forces to
582 move oil slicks back and forth over a coral reef may increase detrimental effects of oil
583 exposure to stony corals.

584 *4.1.3 Oil effect on tissue regeneration*

585 Tissue regeneration in *P. damicornis* was significantly affected in a 96 h HEWAF
586 exposure at all tested doses (as low as 12 µg/L tPAH50), demonstrating that it is a
587 particularly sensitive quantitative assay for coral toxicology evaluations. Corals are
588 susceptible to wounds from a variety of natural (e.g., fish bites, storms, disease) and
589 anthropogenic (e.g., boat groundings, diver interactions, pollution) causes and
590 regeneration of tissue over bare skeleton requires significant energy input. Wound

591 healing has been used previously as a field assessment tool to gauge coral condition
592 (Dustan et al., 2008; Fisher et al., 2007; Moses and Hallock, 2016). Coral colonies that
593 cannot undergo lesion repair are susceptible to infection, infiltration by boring organisms
594 or algal overgrowth, often leading to poor reproductive output, slowed growth and death
595 (Meesters and Bak, 1993; Rinkevich and Loya, 1979). Kramarsky-Winter and Loya
596 (2000) first demonstrated how tissue repair is dependent upon intrinsic (size,
597 reproductive state) and extrinsic (season, water temperature) factors by comparing field
598 and laboratory wound healing rates. We have shown that this assay is amenable to
599 laboratory toxicology studies with fast-growing, branching corals, which are easily
600 fragmented. Slower-growing corals, such as *Orbicella, Dendrogyra,* or *Diploria* spp.,
601 may not be easily wounded, nor exhibit enough tissue regrowth to be utilized in shorter
602 timeframe toxicological studies using this assay, however.

603 While the precise mechanism of oil toxicity is not well understood, we know that corals
604 can bioaccumulate hydrocarbons (Burns and Knap, 1989; Jafarabadi et al., 2018; Ko et
605 al., 2014; Peters et al., 1981; Sabourin et al., 2013) and uptake of these hydrophobic
606 compounds can result in disruption of cellular biochemical and physiological processes.
607 Exposure to oil is linked with a decrease in lipid biosynthesis (Cook and Knap, 1983)
608 and disruption of cellular protein production (Rougee et al., 2006), thus inhibiting cell
609 membrane biosynthesis. Peters et al. (1981) observed that crude oil exposure impaired
610 development of reproductive tissues and resulted in atrophied muscle bundles, further
611 evidence that hydrocarbon exposure negatively impacts coral tissue growth. Combined
612 with a possible reduction in fixed carbon availability or energy reserves from
613 photosynthetic processes, we would expect oil-exposed corals to have greatly

614 decreased capacity for tissue regeneration compared to unexposed corals.
615 Furthermore, our study did not incorporate natural sunlight (with UV), thus we again
616 emphasize that our reported toxicity threshold for this assay may represent a
617 conservative value.

618 **4.2 Time-dependent toxicity of Louisiana sweet crude oil to coral**

619 To examine the effect of exposure duration, coral fragments were challenged with a
620 single concentration HEWAF load over three short time periods (6-24 h), representing
621 possible scenarios during an oil spill. We also included one longer exposure of 96 h for
622 comparison to the 96 h dose-response experiment. Corals in the 6 h treatment duration
623 were exposed in total darkness, representing the most conservative estimate of oil
624 effects. We selected a median oil load (62.5 mg/L) from the 96 h dose-response
625 experiment, resulting in environmentally relevant tPAH50 concentrations. PAH loss is
626 relatively rapid due to volatilization or degradation, thus at 6 h, the tPAH50 geometric
627 mean of fresh and spent treatment solutions was higher than at 12 h. For example, our
628 calculated tPAH50 dose for the 6 h crude oil exposure was 266 µg/L, while the 12 h
629 duration resulted in an exposure of 189 µg/L tPAH50. Treatments longer than 12 h
630 were dosed repeatedly (static renewal) at 189 µg/L tPAH50.

631 We found that 12 h and 96 h durations of HEWAF exposure significantly affected
632 photosynthetic quantum yield in *P. damicornis* symbionts. We did not observe any
633 significant effect to chlorophyll fluorescence with either the 6 h or 24 h oil exposure.
634 Variable numbers of symbionts in each coral fragment or varying amounts of chlorophyll
635 *a* in each algal cell may explain these results, especially if 189 µg/L tPAH50 is at the

lower limit for chlorophyll fluorescence effects. Additionally, the timing of the 6 h oil exposure may have resulted in symbiont recovery prior to the PAM fluorometry evaluation, particularly since this treatment was not exposed to light and oil, concurrently.

Recovery of chlorophyll *a* fluorescence was observed within 24 h after placement in fresh ASW for the fragments exposed to oil for 12 h and coral fragments in the 96 h exposure recovered after 34 h in ASW, demonstrating resilience of *P. damicornis* algal symbionts to shorter-term oil exposures. Similar quick recoveries of photosynthetic effects have been observed with placement in fresh seawater following short-term (8-48 h) hydrocarbon exposures (Cook and Knap, 1983; Jones et al., 2003). Cook and Knap (1983) postulated that had damage occurred to cellular membranes, it would take much longer to recover and proposed that the most likely explanation of the decrease in photosynthetic output was due to interference with enzymatic processes. They did not measure chlorophyll or chlorophyll fluorescence, however, and admitted that the loss and resynthesis of chlorophyll *a* could be another likely explanation. Jones et al. (2003) observed reduced chlorophyll fluorescence (Fv/Fm) from freshly-isolated symbionts (1 x 10^7 cells/mL) exposed to metal- and hydrocarbon-contaminated water and gradual recovery was observed for the algal cells following placement in fresh seawater. The results from our experiments and these works support a theory of oil destruction of photosynthetic pigments. Further studies should incorporate chlorophyll measurements and enumeration of algal symbionts to provide a more definitive answer to this question.

Pocillopora damicornis tissue integrity and tissue color were not significantly affected by 6-24 h oil treatments. Timing of the health data collection (2:00 pm daily, mid-light

659 cycle) did not coincide with HEWAF exposures for the 6, 12 and 24 h treatments, which
660 may have resulted in underreported effects. Additionally, reduction in tissue color
661 scores for all *P. damicornis* fragments over time may have resulted in the observed
662 'recovery' of the 96 h treatment duration after 209 h (Figure 6, Panels B, E).

663 Polyp behavior was significantly affected by oil exposure for all treatment durations and
664 was the principal driver of decreased health scores. This was not unexpected, since
665 polyp retraction has been reported following coral exposure to various types of
666 hydrocarbons (DeLeo, et al., 2016; Reimer, 1975; Renegar et al., 2016; Ruis-Ramos et
667 al., 2017). Recovery for the 6-24 h treatments was within 53 h (Figure 6, Panel C).
668 Coral fragments subjected to the 96 h HEWAF exposure exhibited severe polyp
669 retraction at 89 h, mirroring effects noted in the 96 h dose-response experiment, and
670 polyp retraction continued throughout the recovery period. As mentioned above,
671 bioaccumulation of hydrocarbons has been reported for oil-exposed corals (Burns and
672 Knap, 1989; Jafarabadi et al., 2018; Ko et al., 2014, Peters et al., 1981; Sabourin et al.,
673 2013) and hydrocarbons can remain in coral tissues weeks after placement in clean
674 seawater (Peters et al., 1981). The continued polyp retraction for the longest oil
675 exposure duration may result from PAH accumulation in the lipophilic coral polyp
676 tissues. Polyp behavior provided a key visual clue to decreasing coral health and this
677 health metric took days to recover to the level of the controls once the stressor was
678 removed.

679 The ability to regenerate tissue over wounded areas was inhibited following 6 h of crude
680 oil exposure (266 µg/L tPAH50) to pre-wounded *P. damicornis* fragments,

demonstrating that significant health impacts do occur with short-term oil exposures. The lower tPAH50 dose (189 µg/L tPAH50) represented in the 12-24 h crude oil exposures also impeded coral tissue regeneration. Recoveries for all short-term exposures were delayed for 48-84 h after transferring fragments to fresh ASW. Complete recovery did not occur following 7 days in fresh ASW for the fragments treated with crude oil HEWAF for 96 h. This result is not unprecedented: Johannes et al. (1972) observed complete tissue breakdown for several coral species exposed directly to crude oil for 1.5 h, with no signs of regeneration on the oil-affected parts up to 4 weeks later. It is not clear if a longer recovery time would result in total recovery for these fragments, or if eventual death would occur. While there has been a recent report of polyp rejuvenescence in presumably totally 'dead' corals following a bleaching event (Kersting and Linares, 2019), significant coral tissue loss from oil exposure may be more difficult to repair, since bioaccumulated oil may continue to alter normal cellular processes long after the environmental exposure has ended.

Significantly decreased tissue regeneration was observed for intact fragments exposed to moderate tPAH50 levels and wounded in the recovery phase, indicating that oil is not simply affecting abraded tissue. This is further evidence that crude oil has effects at the cellular or subcellular level as detailed in section 4.1.3. The implications of oil damage to corals are lowered resistance to disease or predation and possible colony death, with increased negative impacts predicted for corals with lesions prior to exposure.

4.3 Conclusion

Following the DWH oil spill, measured tPAH50 concentrations in the 0-10 m depth (shallow water coral habitat) ranged from undetectable to over 100 µg/L, with the highest concentration observed in surface waters (0-1 m), 10-20 miles northwest of the wellhead (Boehm et al., 2016). Crude oil was released from the site for nearly three months, significantly damaging natural resources across many species extending hundreds of kilometers from the release site. Total PAH50 concentrations in our study were between 12 and 945 µg/L, with coral and zooxanthellae effects well within reported environmental levels for this event. The toxic responses of *P. damicornis* to the 96 h oil exposure are in the same range (low ppb) found for other marine organisms such as fish, shrimp and echinoderms (Esbaugh et al., 2016; Hemmer et al., 2011; Incardona et al., 2014; Neff et al., 2000; Stieglitz et al., 2016). We emphasize that care should be taken when comparing studies, however, since the varying analyte composition in different oil types may result in altered toxicity profiles. This is important especially when using the data in risk assessments. We note that our study results likely underestimate oil effects due to the omission of UV and the nature of the HEWAF (containing small droplets, which may not be bioavailable). Our work shows that short exposure times with moderate tPAH50 levels can negatively affect *P. damicornis* and its symbionts, providing evidence that both acute and longer-term (chronic) PAH exposures in the ppb range can impair coral health. Finally, we demonstrated that coral tissue regeneration is a very sensitive indicator of petroleum hydrocarbon exposure. With appropriate controls, wound healing could be implemented as part of a field assessment to determine impacts of oil exposure to corals.

We have limited our initial investigation to a single species and life stage and have used standardized laboratory settings (e.g., artificial light without UV, artificial seawater, etc). Photo-enhanced oil toxicity has been reported for several species of marine organisms (Alloy et al., 2016; Alloy et al., 2015; Boese et al., 1998; Newsted and Giesy, 1987; Oris and Giesy Jr, 1987; Sweet et al., 2017) and coral larvae (Negri et al., 2016; Nordborg et al., 2018), however. We would expect that under field conditions (e.g., UV light) that oil toxicity thresholds for *P. damicornis* would be even lower than those reported in this work. Further studies should include additional species representing other distinctive morphologies, environmentally relevant levels of UV, as well as gametes or larvae, as early life stages often exhibit higher sensitivity to xenobiotic stressors.

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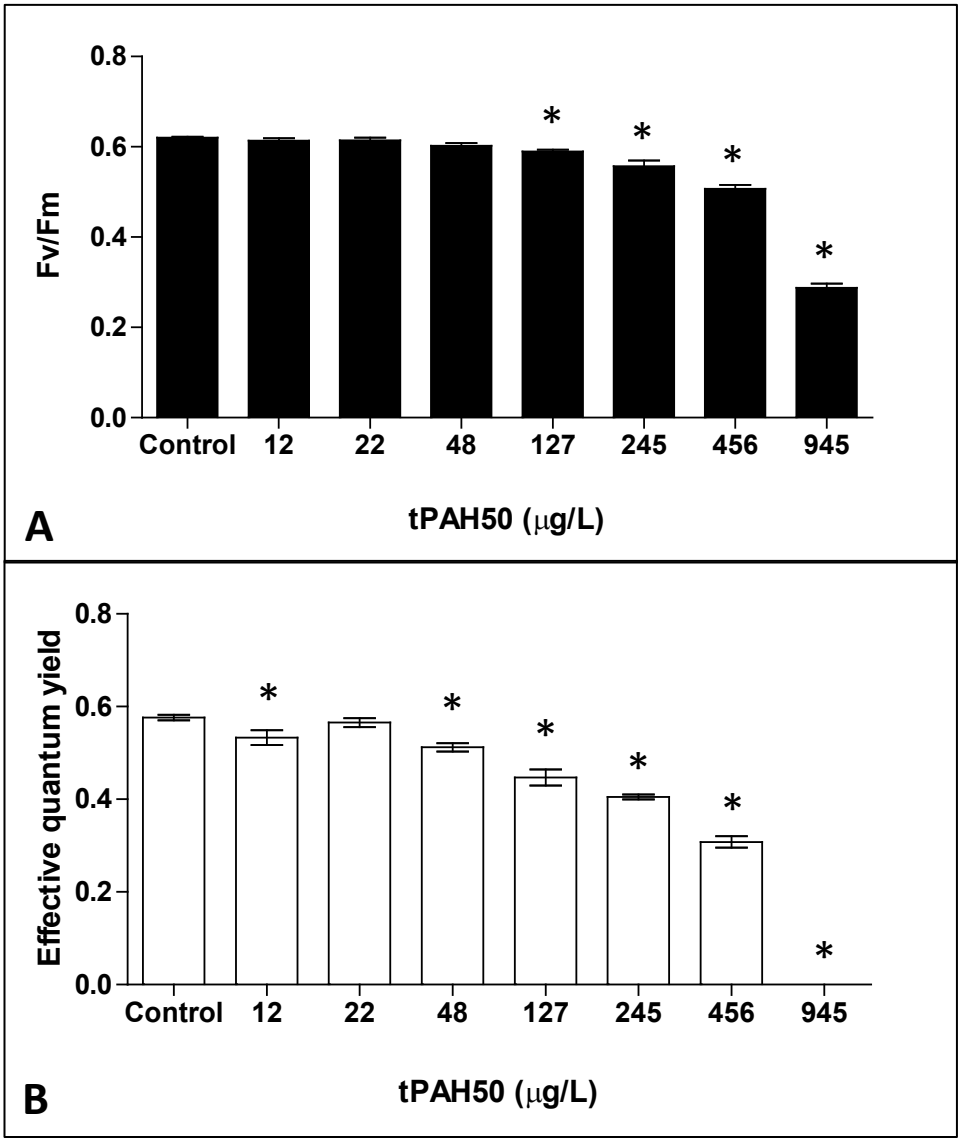

Figure 1. *Pocillipora damicornis* symbiont chlorophyll *a* fluorescence measurements following crude oil exposure. Panel A: dark-adapted maximum quantum yield after 82 h; Panel B: light-adapted effective quantum yield after 92 h. Total PAH50 (tPAH50) is reported as the sum of 50 polycyclic aromatic hydrocarbons. Maximum quantum yield (Fv/Fm, panel A) was reduced significantly (indicated by *) for treatments of 127 µg/L tPAH50 and higher after 82 h of exposure ($p<0.05$). Effective quantum yield (EQY, panel B) was significantly lower ($p<0.05$) following exposure with 12 µg/L tPAH50 for 92 h. Effective quantum yield was zero for the 945 µg/L tPAH50 treatment. Total PAH50 values are reported as the geometric mean of fresh and spent treatment solution (refreshed every 12 h).

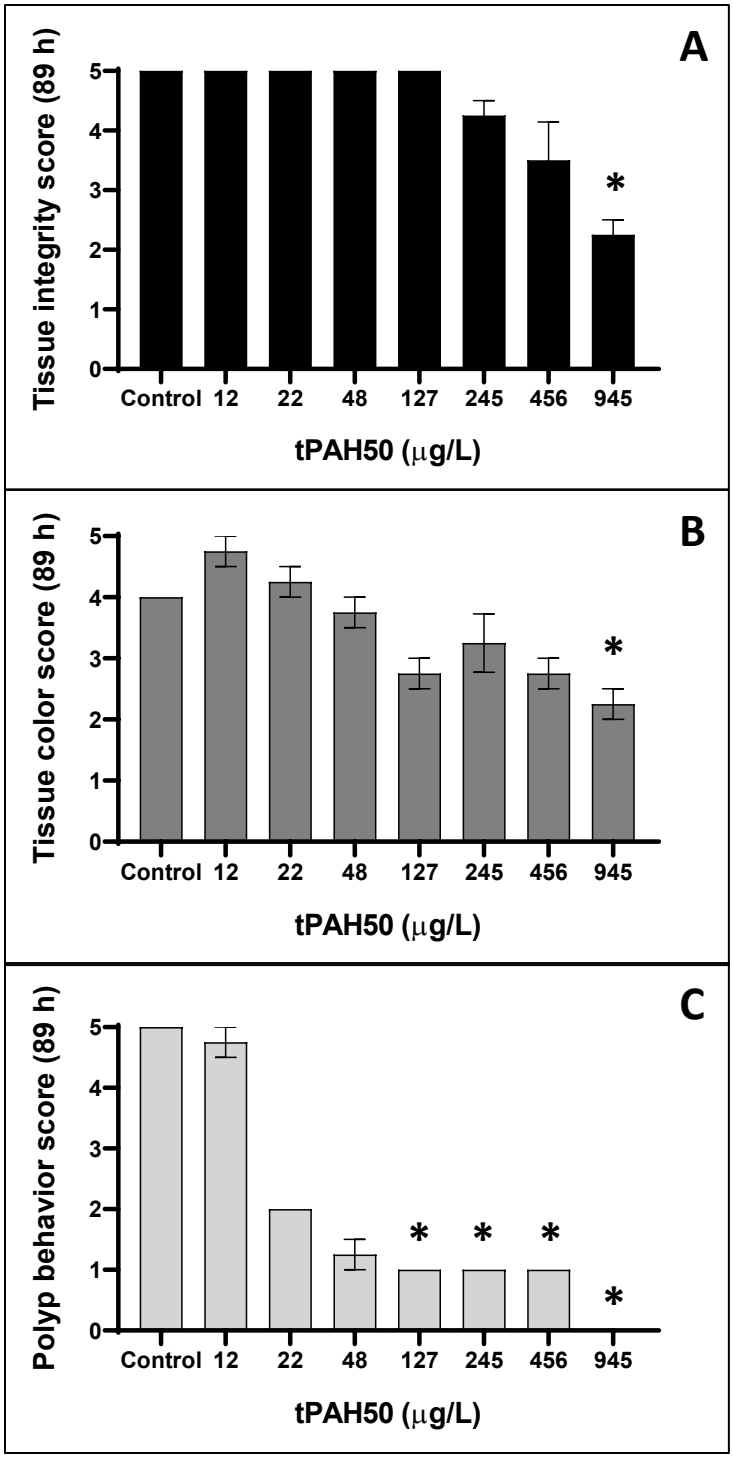

Figure 2. *Pocillopora damicornis* health status effects after 96 h HEWAF exposure. Oil treatments are reported as the sum of 50 polycyclic aromatic hydrocarbons (tPAH50). Panel A: tissue integrity metric, Panel B: tissue color metric and Panel C: polyp behavior metric. Treatments marked with an asterisk (*) were significantly different from the

artificial seawater control at $p<0.001$. Concentrations of tPAH50 are reported as the geometric mean of fresh and spent HEWAF treatment, which was refreshed every 12 h.

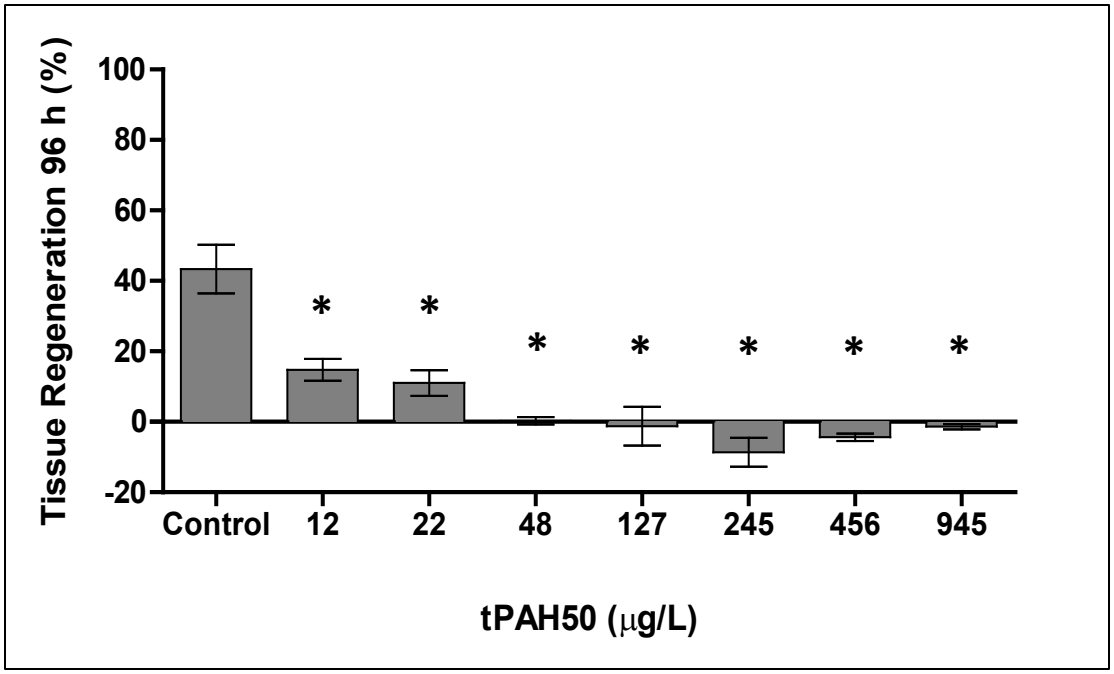

Figure 3. Percent tissue regeneration for *Pocillopora damicornis* following 96 h HEWAF exposure. Oil dose is reported as the sum of 50 polycyclic aromatic hydrocarbons (tPAH50). Treatments designated with an asterisk (*) were significantly different from the control ($p<0.005$). Total PAH50 values are reported the geometric mean of fresh and spent treatment solution (refreshed every 12 h).

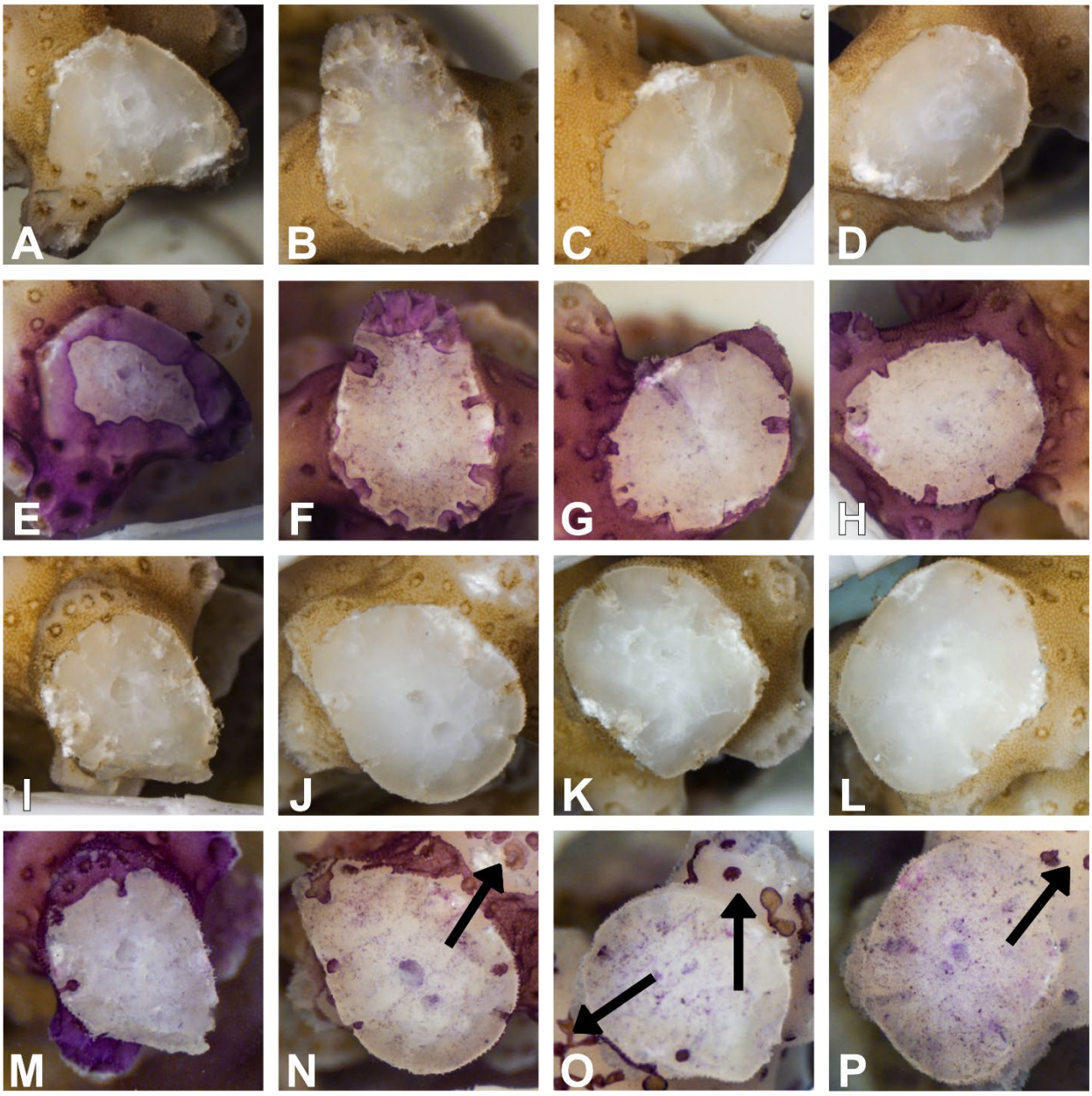

Figure 4. *Pocillopora damicornis* tissue regeneration images. Images are shown at time 0 following initial cut (Panels A-D and I-L) and after 96 h treatment, stained with toluidine blue O (Panels E-H and M-P). Panels A and E = ASW control, panels B and F = 12 µg/L tPAH50, panels C and G = 22 µg/L tPAH50, panels D and H = 48 µg/L tPAH50, panels I and M = 127 µg/L tPAH50, panels J and N = 245 µg/L tPAH50, panels K and O = 456 µg/L tPAH50 and panels L and P = 945 µg/L tPAH50. Areas of tissue loss for treatments at and above 245 µg/L tPAH50 are indicated by arrows in panels N, O and P. Total PAH50 values are reported as the geometric mean of fresh and spent treatment solution (refreshed every 12 h).

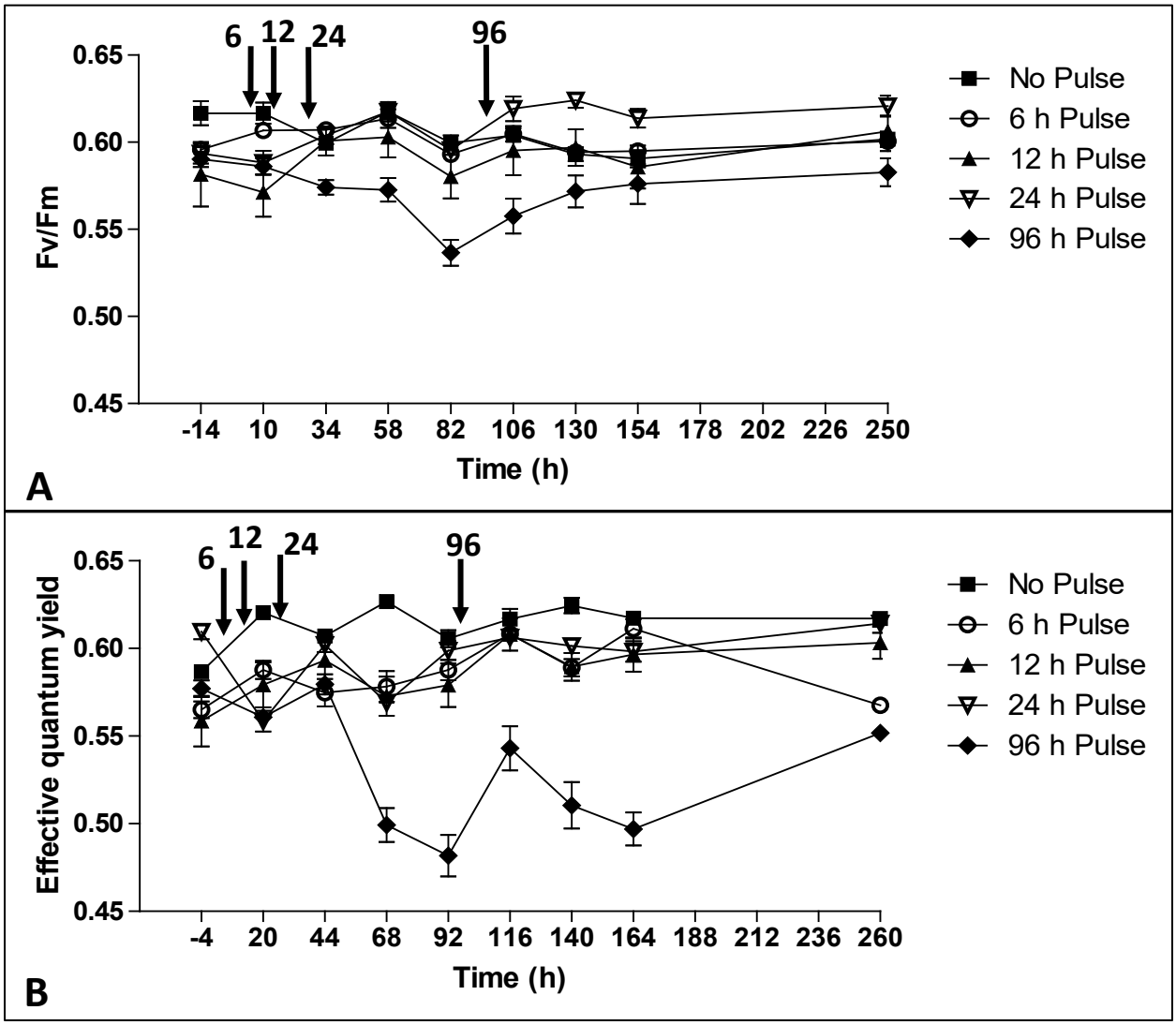

Figure 5. Chlorophyll *a* fluorescence measurements of *Pocillopora damicornis* algal symbionts following short-term crude oil exposure. The HEWAF exposure durations of 6, 12, 24 and 96 h (indicated by arrows) contained 266 µg/L tPAH50 (6 h duration) or 189 µg/L tPAH50 (12, 24 or 96 h duration) (geometric mean of fresh and spent HEWAF treatment). Maximum quantum yield (Fv/Fm, evaluated two hours before the light cycle began) was reduced significantly ($p<0.05$) for the 12 h and 96 h pulse durations at the 10-h time point (Panel A). No significance was observed for Fv/Fm in the 6 h and 24 h pulse durations. Fragments exposed to a 12 h pulse recovered Fv/Fm within 24 h after treatment ended and fragments exposed for 96 h recovered to the level of the controls within 34 h post treatment (130 h time point). Effective quantum yield (EQY, measured two hours before light cycle ended) for all oil pulse durations was impacted significantly compared to controls at the 20 h time point (Panel B, $p<0.05$). The EQY for 6, 12 and 24 h pulse exposures recovered to the level of the controls by 92 h. The 96 h pulse

duration was significantly different from the control up to 68 h post treatment and recovered by the end of the experiment (260 h).

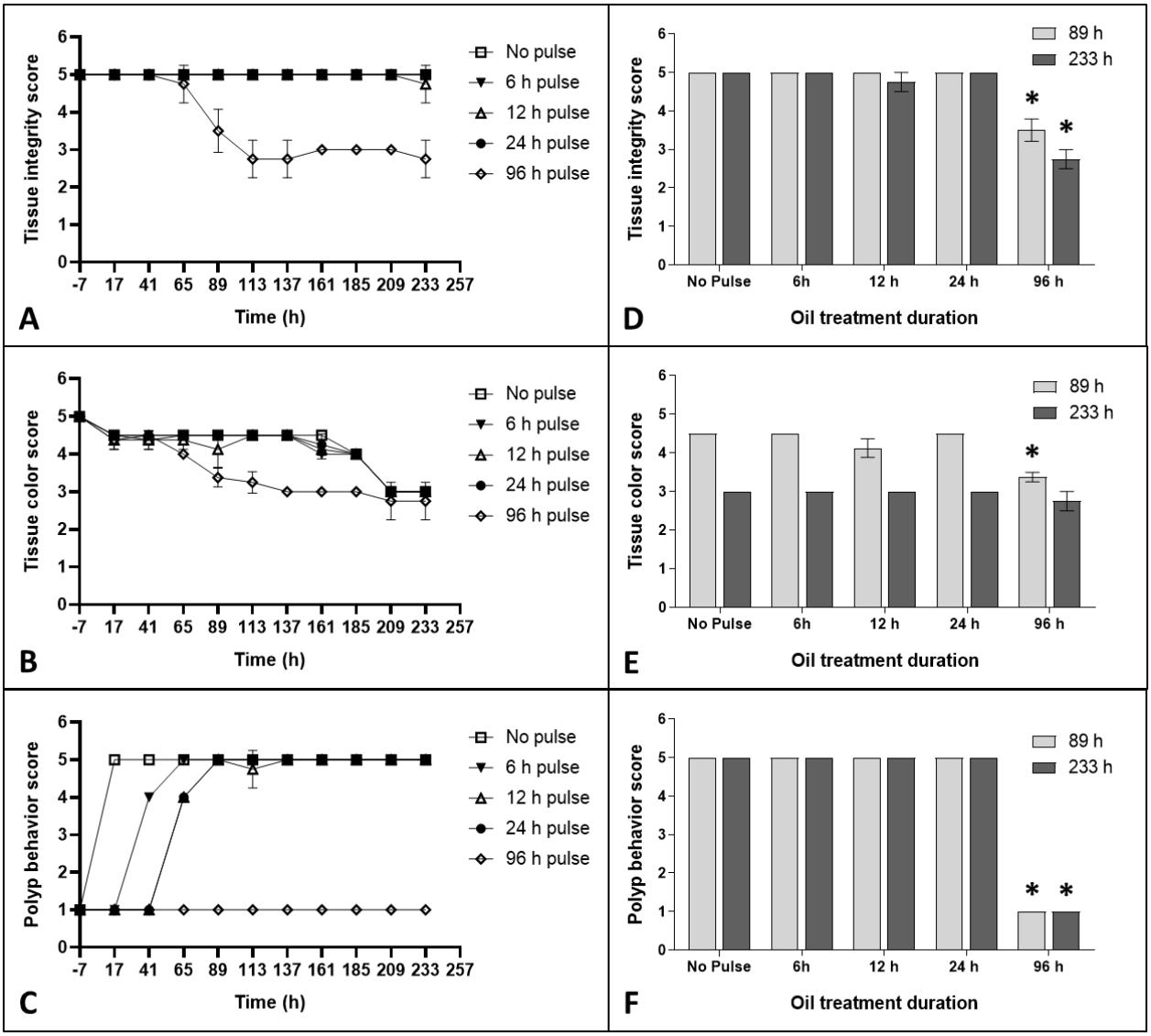

Figure 6. *Pocillopora damicornis* health status scores following short-term HEWAF pulse durations (189-266 µg/L tPAH50, refreshed every 12 h). Coral fragments were evaluated at 2 pm daily. Initial evaluation at -7 h included polyp retraction for all treatments following image analysis. Tissue integrity (Panels A and D) and tissue color (Panels B and E) were not affected by short-term (6-24 h) oil exposures. Coral fragments exposed to HEWAF for 96 h lost tissue and did not recover to the level of the controls after one week in fresh artificial seawater (Panel D, * = significant at $p<0.05$). Tissue color for the 96 h treatment duration was significantly reduced (indicated by *, $p<0.05$) from 65 to 185 h (Panel E). All treatments (including controls) had reduced tissue color scores after 185 h. Polyp behavior was significantly affected by all treatment durations (Panels C and F). Recovery for the 6 h (266 µg/L tPAH50), 12 h and 24 h (189 µg/L tPAH50) exposures occurred within 53 h after treatment ceased (Panel C). The 96 h pulse (189 µg/L tPAH50) exposure had significantly reduced

(indicated by *, $p<0.05$) polyp behavior at 89 h and at 137 h post treatment (233 h) time point (Panel F). Total PAH50 is the geometric mean of fresh and spent (6 or 12 h) HEWAF solutions.

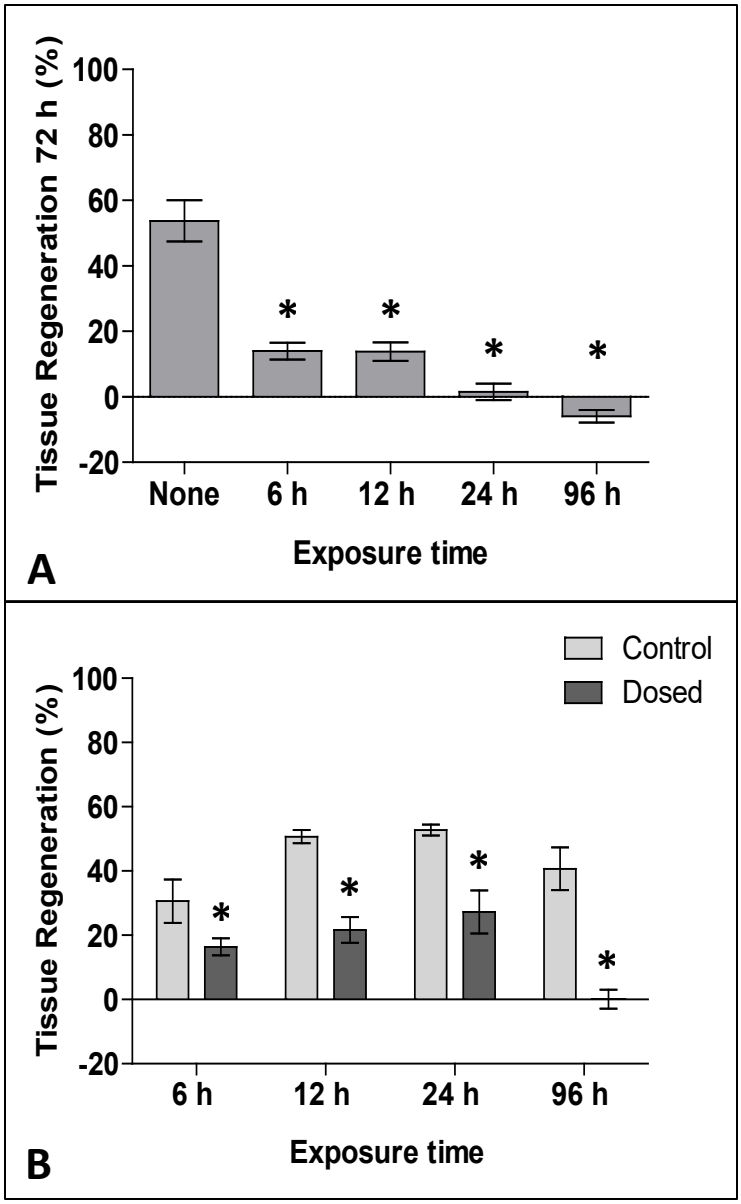

Figure 7. Percent tissue regeneration for *Pocillopora damicornis* fragments after short-term HEWAF exposures (189-266 µg/L tPAH50, refreshed every 12 h). Panel A: tissue regeneration for cut fragments exposed to HEWAF. At 72 h, all treatments had significantly reduced (*) tissue regeneration when compared to the artificial seawater control ($p<0.0001$). Fragments exposed for 96 h did not recover to the level of the controls by experiment termination (168 h post treatment). Panel B: Results of intact fragments exposed to HEWAF (cut and placed in fresh artificial seawater). Dosed and control treatments for each exposure time were imaged when controls for each treatment duration reached 30-50 % tissue regeneration (72-84 h post exposure). All dosed fragments exhibited significantly reduced ($p<0.05$) tissue regeneration when compared to the controls (indicated by *)

Table 1. Coral health score rubric. Maximum cumulative score for a healthy coral is 15: five points for color, five points for tissue integrity and five points for polyp extension.

Score	Polyp Behavior	Color	Tissue loss (%)
5	All fully extended	Normal, 100% color	0
4	Fully extended, few withdrawn	25% color loss	1-25
3	Polyps extended 50%	Pale, 50% color loss	26-50
2	Polyps extended 10%	75% color loss	51-75
1	No polyps extended	Bleached, with pale areas	76-99
0	Polyp bailout	Totally bleached	100

Table 2. Reported effect concentrations for the 96 h HEWAF dose-response exposure using fresh (time 0) treatments or the geometric mean (Geomean) of fresh and 12 h spent treatment solutions (units = µg/L tPAH50). C. I. = 95 % confidence interval, Fv/Fm = maximum quantum yield, EQY = effective quantum yield, Tissue reg. = tissue regeneration, Tissue integ. = tissue integrity, Polyp retract. = polyp retraction.

Endpoint	Fresh EC_{10} (C. I.)	Fresh EC_{50} (C. I.)	Geomean EC_{10} (C. I.)	Geomean EC_{50} (C. I.)
Fv/Fm (82 h)	**1193** (1051-1355)	**3804** (3575-4049)	**330** (292-372)	**913** (862-968)
EQY (92 h)	**632** (472-847)	**1640** (1429-1882)	**176** (13-234)	**428** (375-488)
Tissue reg. (96 h)	**7** (1-52)	**29** (15-57)	**3** (0.4-16)	**10** (6-19)
Tissue integ. (89 h)	**711** (431-1174)	**3296** (2595-4187)	**202** (128-319)	**806** (649-1000)
Tissue color (89 h)	**111** (15-853)	**3820** (1741-8385)	**36** (5-234)	**926** (450-1907)
Polyp retract. (89 h)	**8** (2-23)	**84** (50-139)	**3** (1-9)	**27** (17-43)

Supplement A. Detailed design for the construction of custom Teflon coral mounting pegs and coral fragment support stands.

A Teflon® coral mounting peg was fabricated from a 9.5 mm Teflon rod with a 4.6 mm section of Teflon tubing (ID=9.5 mm, OD=12.7 mm, wall=1.6 mm) capping one end (Figure A.1.1, panel A).

Round Teflon coral jar stands (Figure A.1, panels B, C) were constructed from a 50.8 mm dia PTFE disk (cut from a 1.6 mm thick sheet, Industrial Plastic Supply (Anaheim, CA) with alternating 9.5 mm (4) and 4.8 mm (4) holes around the edge equidistant from one another. A center 7.6 mm hole was used to insert a 6.4 mm diameter Teflon rod (38.1 mm long), used as a grip. Teflon tubing sections (OD=9.5 mm, ID=6.4 mm, wall=1.6 mm, length=4.6 mm) on each end of the center rod were added to hold the grip in place. Four 6.4 mm dia x 19.1 mm long rods were grooved with a lathe, approximately 3.2 mm from one end, then snapped into the 4.8 mm holes as support legs. A notch was cut into the support disk next to one 9.5 mm hole as an orientation reference.

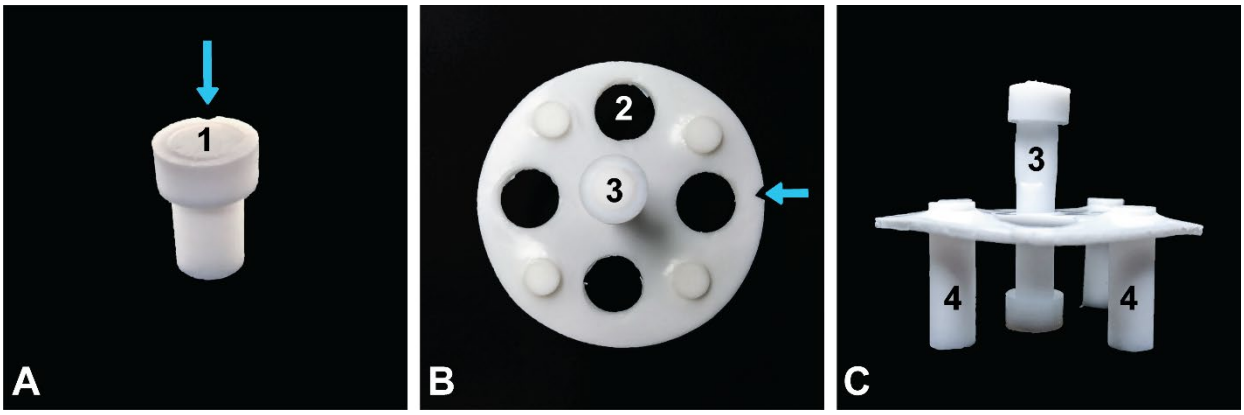

Figure A.1. Teflon coral mounting peg (panel A) and Teflon coral fragment stand (panel B, top view; panel C, side view) custom-constructed for coral toxicology experiments. 1=coral mounting surface, 2=hole for coral peg, 3=grip, 4=support legs, blue arrow=orientation notch.

A rectangular Teflon stand was constructed for PAM fluorometry (Figure A.2). The stand measured 120 x 28 x 5.5 mm and four 9.5 mm dia holes were cut in the top to fit four Teflon pegs with coral. On the side of the stand and in the center of each hole, an orientation notch was cut for aligning with the notch on the Teflon coral peg during imaging (blue arrows). Two 30 x 20 mm pieces with notches were cut from the same Teflon sheet and inserted over the stand to keep the stand from tilting when filled with coral.

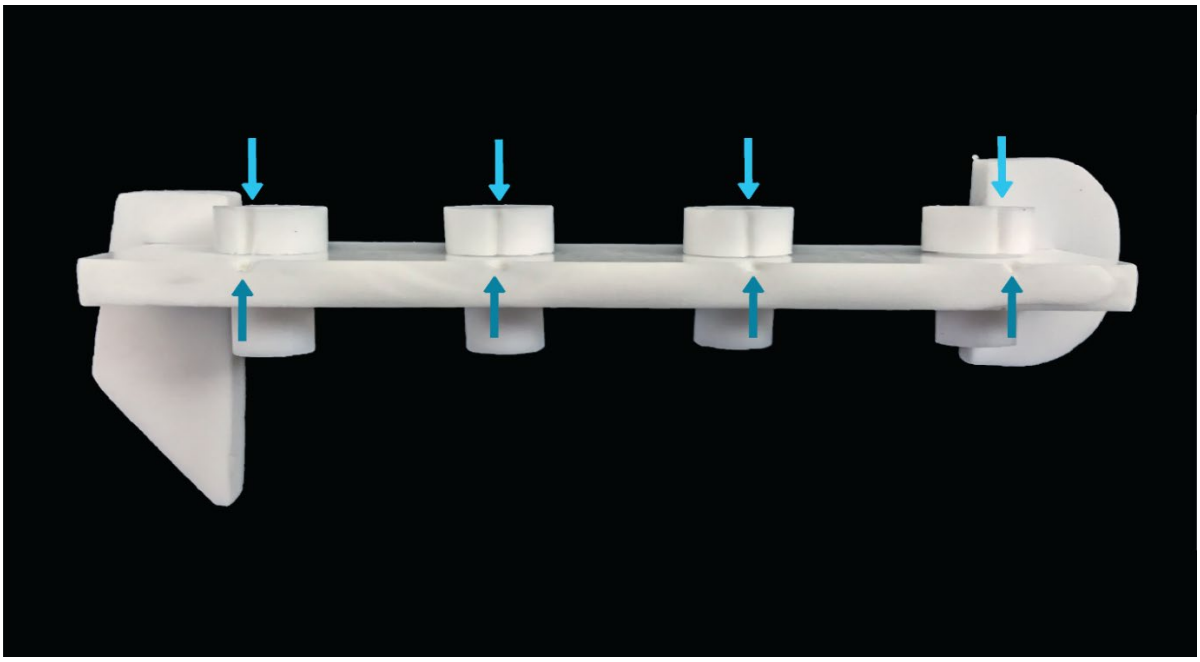

Figure A.2. Teflon PAM fluorometry stand. Teflon pegs (with coral) were placed in the stand so that orientation notches on both peg and stand were aligned. The stand was placed on its side in a crystallizing dish, so that side-view measurements of the coral could be collected. Data was collected from the same surface of each fragment during repeated measurements.

PAM fluorometry detailed methods:

Instrument settings were optimized for *P. damicornis*: measuring light intensity = 1, frequency = 1, gain = 1, damping = 2, saturation pulse intensity = 10 and absorptivity, red gain = 10, red intensity = 2 and NIR intensity = 9. The PAM imaging system was white-balanced and a standard fluorescence color card was used to calibrate the instrument before each use. Teflon-mounted coral nubbins from each treatment (n = 4) were set in a rectangular Teflon fluorometry stand (Supplement A.2), aligned using notches, and placed horizontally in a glass crystallizing dish (150 x 75 mm) filled with 500 mL ASW (-14 h and -4 h), or the respective treatment solution (all other time points).

Supplement B. Analytical chemistry data.

Table B.1. Fresh (Time 0) tPAH50 results from dose-response experiment. Total PAH50 concentrations in µg/L. ('-' represents individual PAHs below the limit of detection)

HEWAF nominal Oil Concentrations (Time 0h)								
PAH Analyte	***250 mg/L***	***125 mg/L***	***62.5 mg/L***	***31.3 mg/L***	***15.6 mg/L***	***7.8 mg/L***	***3.9 mg/L***	***0 mg/L***
Naphthalene	76.1	39.1	22.2	13.7	5.81	2.26	1.23	-
C1-Naphthalenes	468	156	89.6	50.8	19.4	7.41	3.98	-
C2-Naphthalenes	918	350	194	100	37.4	12.44	7.46	-
C3-Naphthalenes	1064	468	221	97.5	32.6	12.71	7.45	-
C4-Naphthalenes	88.1	38.5	20.8	10.6	3.62	1.22	0.61	-
Biphenyl	24.9	9.81	5.93	3.29	1.28	0.43	0.27	-
Acenaphthene	-	-	-	-	-	-	-	-
Acenaphthylene	11.9	4.32	2.34	1.29	0.41	0.16	0.07	-
Fluorene	24.5	9.27	5.02	2.63	0.99	0.35	0.19	-
C1-Fluorenes	151	73.9	45.2	12.4	8.05	2.76	1.38	-
C2-Fluorenes	105	52.2	25.5	13.3	4.61	1.24	0.73	-
C3-Fluorenes	83.4	37.6	19.5	10.0	3.99	1.01	0.54	-
Dibenzofuran	4.79	1.88	1.04	0.56	0.21	0.06	0.05	-
Dibenzothiophene	5.87	2.42	1.45	0.71	0.26	0.10	0.05	-
C1-Dibenzothiophenes	37.2	16.9	10.6	5.33	1.90	0.72	0.37	-
C2-Dibenzothiophenes	31.1	16.2	9.54	4.82	1.82	0.67	0.34	-
C3-Dibenzothiophenes	19.8	10.2	5.77	3.19	1.27	0.42	0.21	-
C4-Dibenzothiophenes	8.66	5.04	3.03	1.65	0.58	0.22	0.11	-
Phenanthrene	50.3	19.6	11.2	5.63	2.06	0.76	0.40	-
Anthracene	2.28	1.17	0.57	0.27	0.09	0.06	0.02	-
C1-Phenanthrenes/Anthracenes	187	95.9	56.1	27.8	10.3	3.75	1.84	-
C2-Phenanthrenes/Anthracenes	366	201	82.4	34.4	11.6	7.20	3.61	-
C3-Phenanthrenes/Anthracenes	76.8	37.4	22.7	11.7	4.50	1.74	0.87	-
C4-Phenanthrenes/Anthracenes	41.2	19.6	13.3	6.50	2.64	1.11	0.49	-
Fluoranthene	1.93	0.80	0.47	0.24	0.09	0.03	0.02	-
Pyrene	1.92	0.80	0.47	0.23	0.09	0.03	0.02	-
C1-Fluoranthenes/Pyrenes	6.82	2.83	2.12	0.91	0.35	0.13	0.06	-
C2-Fluoranthenes/Pyrenes	24.8	10.5	8.54	4.15	1.63	0.65	0.31	-
C3-Fluoranthenes/Pyrenes	19.5	10.0	7.58	3.66	1.37	0.67	0.29	-
C4-Fluoranthenes/Pyrenes	9.80	4.84	3.90	1.97	0.79	0.42	0.17	-
Benzo(a)anthracene	1.55	0.63	0.38	0.18	0.07	0.02	0.01	-
Chrysene+Triphenylene	8.88	3.49	2.06	1.00	0.37	0.13	0.07	-
C1-Chrysenes/Benzanthracenes	8.90	3.65	2.34	1.16	0.42	0.11	0.08	-
C2-Chrysenes/Benzanthracenes	7.46	2.94	2.03	1.02	0.37	0.11	0.08	-
C3-Chrysenes/Benzanthracenes	3.83	1.57	1.17	0.58	0.22	0.07	0.06	-
C4-Chrysenes/Benzanthracenes	1.16	0.45	0.34	0.18	0.06	0.02	0.02	-
Benzo[b]naphtho[2,1-d]thiophene	3.03	1.19	0.66	0.32	0.12	0.03	0.02	-
C1-Naphthobenzothiophenes	8.79	3.57	2.24	1.10	0.39	0.13	0.08	-
C2-Naphthobenzothiophenes	8.81	3.64	2.49	1.24	0.44	0.12	0.09	-
C3-Naphthobenzothiophenes	2.94	1.19	0.84	0.45	0.16	0.05	0.04	-
C4-Naphthobenzothiophenes	1.37	0.61	0.39	0.21	0.07	0.02	0.02	-
Benzo(b)fluoranthene	0.87	0.34	0.19	0.10	0.04	0.01	0.01	-
Benzo(k)fluoranthene	-	-	-	-	-	-	-	-
Benzo[j]fluoranthene	-	-	-	-	-	-	-	-
Benzo[a]fluoranthene	0.21	0.10	0.06	0.02	0.01	-	-	-
Benzo(e)pyrene	1.98	0.78	0.44	0.21	0.08	0.03	0.01	-
Benzo(a)pyrene	0.25	0.14	0.08	0.04	0.01	-	-	-
Dibenz(a,h)anthracene	-	0.05	0.03	-	-	-	-	-
Indeno(1,2,3-cd)pyrene	-	-	-	-	-	-	-	-
Benzo(g,h,i)perylene	0.31	0.12	0.07	0.03	0.01	-	-	-
TPAH50	**3971**	**1721**	**907**	**438**	**163**	**61.6**	**33.7**	**0.00**

Table B.2. Spent (12 h) HEWAF tPAH50 concentrations (µg/L) from dose-response experiment. ('-' represents individual PAHs that were below the limit of detection)

HEWAF nominal Oil Concentrations (Time 0h)								
PAH Analyte	***250 mg/L***	***125 mg/L***	***62.5 mg/L***	***31.3 mg/L***	***15.6 mg/L***	***7.8 mg/L***	***3.9 mg/L***	***0 mg/L***
Naphthalene	13.2	6.5	3.5	1.7	0.63	0.32	-	-
C1-Naphthalenes	34	19	10.4	5.4	2.0	1.12	0.46	-
C2-Naphthalenes	48	27	15	8	3.2	1.75	0.76	-
C3-Naphthalenes	37	19	11	5.6	2.0	1.20	0.65	-
C4-Naphthalenes	3.2	1.5	1.6	0.4	0.14	0.07	0.04	-
Biphenyl	2.4	1.40	0.79	0.43	0.17	0.08	0.04	-
Acenaphthene	-	-	-	-	-	-	-	-
Acenaphthylene	0.3	0.18	0.10	0.05	0.03	0.01	-	-
Fluorene	1.8	1.07	0.62	0.37	0.13	0.08	0.04	0.01
C1-Fluorenes	10	5.3	2.7	1.6	0.61	0.30	0.17	0.01
C2-Fluorenes	5	2.6	1.4	0.7	0.28	0.15	0.09	-
C3-Fluorenes	4.7	2.0	1.0	0.5	0.18	0.08	0.05	-
Dibenzofuran	0.39	0.23	0.13	0.07	0.03	0.02	0.01	-
Dibenzothiophene	0.51	0.31	0.19	0.11	0.04	0.03	0.01	-
C1-Dibenzothiophenes	2.6	1.4	0.8	0.45	0.17	0.09	0.05	-
C2-Dibenzothiophenes	2.3	1.2	0.59	0.37	0.11	0.06	0.04	0.01
C3-Dibenzothiophenes	1.5	0.8	0.34	0.25	0.07	0.03	0.02	-
C4-Dibenzothiophenes	0.77	0.39	0.15	0.11	0.03	0.02	0.01	-
Phenanthrene	3.7	2.2	1.3	0.87	0.33	0.19	0.10	0.01
Anthracene	0.14	0.10	-	-	-	-	-	-
C1-Phenanthrenes/Anthracenes	13	7.4	4.1	2.5	0.9	0.49	0.26	0.04
C2-Phenanthrenes/Anthracenes	23	12	6.6	4.6	1.4	0.70	0.36	0.05
C3-Phenanthrenes/Anthracenes	5.2	3.0	1.5	1.1	0.36	0.23	0.07	-
C4-Phenanthrenes/Anthracenes	3.1	1.6	0.8	0.64	0.24	0.13	0.10	-
Fluoranthene	0.10	0.05	0.03	0.02	0.01	-	-	-
Pyrene	0.11	0.06	0.04	0.03	0.01	0.01	-	-
C1-Fluoranthenes/Pyrenes	0.44	0.24	0.11	0.08	0.03	0.02	0.01	-
C2-Fluoranthenes/Pyrenes	1.8	0.9	0.43	0.26	0.12	0.07	0.04	-
C3-Fluoranthenes/Pyrenes	1.8	0.8	0.35	0.30	0.13	0.07	0.05	-
C4-Fluoranthenes/Pyrenes	0.96	0.51	0.27	0.17	0.08	0.04	0.02	-
Benzo(a)anthracene	0.07	0.04	0.02	0.01	-	-	-	-
Chrysene+Triphenylene	0.41	0.22	0.12	0.09	0.03	0.02	0.01	-
C1-Chrysenes/Benzanthracenes	0.51	0.27	0.14	0.10	0.03	0.02	0.01	-
C2-Chrysenes/Benzanthracenes	0.58	0.34	0.24	0.15	0.06	0.05	0.02	-
C3-Chrysenes/Benzanthracenes	0.33	0.17	0.09	0.07	0.02	0.01	0.01	-
C4-Chrysenes/Benzanthracenes	0.10	0.07	0.04	0.02	0.01	0.01	-	-
Benzo[b]naphtho[2,1-d]thiophene	0.13	0.07	0.03	0.03	0.01	-	-	-
C1-Naphthobenzothiophenes	0.47	0.24	0.14	0.10	0.03	0.02	0.01	-
C2-Naphthobenzothiophenes	0.60	0.32	0.20	0.12	0.04	0.03	0.02	-
C3-Naphthobenzothiophenes	0.25	0.13	0.07	0.05	0.02	0.01	0.01	-
C4-Naphthobenzothiophenes	0.12	0.07	0.03	0.02	0.01	-	-	-
Benzo(b)fluoranthene	0.04	0.02	0.01	0.01	-	-	-	-
Benzo(k)fluoranthene	-	-	-	-	-	-	-	-
Benzo[j]fluoranthene	-	-	-	-	-	-	-	-
Benzo[a]fluoranthene	0.01	-	-	-	-	-	-	-
Benzo(e)pyrene	0.09	0.05	0.02	0.02	0.01	-	-	-
Benzo(a)pyrene	0.02	0.01	-	-	-	-	-	-
Dibenz(a,h)anthracene	-	-	-	-	-	-	-	-
Indeno(1,2,3-cd)pyrene	-	-	-	-	-	-	-	-
Benzo(g,h,i)perylene	0.02	0.01	-	-	-	-	-	-
TPAH50	**225**	**121**	**66**	**37**	**14**	**7.5**	**3.5**	**0.13**

Table B.3. Total PAH50 from selected time points in the pulse-chase experiment (62.5 mg/L nominal oil HEWAF).

Total PAH50 (μg/L)

Test Duration	Time 0 (Fresh)	Time 6 h	Time 12h
0 h	504		64.5
0 h	555		
0 h	569		
6 h		131	
12 h	506		
12 h	461		
24 h	465		71.2
24 h	485		
84 h	479		
96 h			78.6
Average	503	131	71.5
Standard Deviation	40.0		7.06

Table B.4. Average PAH concentrations from the 62.5 mg/L oil HEWAF measured in the pulse-chase experiment (concentrations in µg/L).

	62.5 mg/L HEWAF Chemistry					Control Water Chemistry				
	Fresh HEWAF (t=0hr)		Spent HEWAF (t=6hr)	Spent HEWAF (t=12hr)		Control water (t=0hr)		Spent Control (t=6hr)	Spent Control (t=12hr)	
PAH Analyte	*Average conc. µg/L (n=8)*	*Standard Deviation*	*conc. µg/L (n=1)*	*Average conc. µg/L (n=3)*	*Standard Deviation*	*Average conc. µg/L (n=2)*	*Standard Deviation*	*conc. µg/L (n=1)*	*Average conc. µg/L (n=3)*	*Standard Deviation*
Naphthalene	21.2	0.71	5.36	2.97	0.61	0.04	0.01	0.00	0.00	0.00
C1-Naphthalenes	58.7	2.60	15.4	8.94	1.31	0.06	0.02	0.04	0.00	0.00
C2-Naphthalenes	102	8.06	21.4	15.0	2.30	0.02	0.03	0.05	0.01	0.02
C3-Naphthalenes	117	13.3	22.8	13.6	1.82	0.00	0.00	0.02	0.01	0.01
C4-Naphthalenes	18.1	4.74	3.91	1.62	0.39	0.00	0.00	0.00	0.00	0.00
Biphenyl	3.92	0.30	1.00	0.66	0.12	0.00	0.00	0.00	0.00	0.00
Acenaphthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acenaphthylene	0.90	0.12	0.13	0.10	0.02	0.00	0.00	0.00	0.00	0.00
Fluorene	3.10	0.31	0.96	0.63	0.08	0.00	0.00	0.00	0.00	0.00
C1-Fluorenes	20.4	2.27	5.00	2.89	0.28	0.00	0.00	0.01	0.00	0.00
C2-Fluorenes	13.1	1.30	3.51	1.68	0.23	0.00	0.00	0.00	0.00	0.00
C3-Fluorenes	9.63	1.20	3.06	1.36	0.23	0.00	0.00	0.00	0.00	0.00
Dibenzofuran	0.68	0.09	0.18	0.12	0.02	0.00	0.00	0.00	0.00	0.00
Dibenzothiophene	0.94	0.07	0.29	0.20	0.03	0.00	0.00	0.00	0.00	0.00
C1-Dibenzothiophenes	6.26	0.37	1.73	0.98	0.16	0.00	0.00	0.00	0.00	0.00
C2-Dibenzothiophenes	4.95	0.34	1.50	0.71	0.10	0.00	0.00	0.00	0.00	0.00
C3-Dibenzothiophenes	3.02	0.20	0.99	0.46	0.05	0.00	0.00	0.00	0.00	0.00
C4-Dibenzothiophenes	1.55	0.14	0.26	0.24	0.01	0.00	0.00	0.00	0.00	0.00
Phenanthrene	6.97	0.43	2.20	1.55	0.21	0.00	0.00	0.01	0.01	0.00
Anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-Phenanthrenes/Anthracenes	29.5	1.87	8.56	4.93	0.67	0.00	0.00	0.02	0.01	0.00
C2-Phenanthrenes/Anthracenes	40.3	4.07	17.9	6.78	1.13	0.01	0.00	0.02	0.02	0.01
C3-Phenanthrenes/Anthracenes	13.7	1.26	4.60	1.93	0.10	0.00	0.00	0.00	0.00	0.00
C4-Phenanthrenes/Anthracenes	5.74	0.99	2.56	0.88	0.14	0.00	0.00	0.00	0.00	0.00
Fluoranthene	0.23	0.02	0.07	0.04	0.01	0.00	0.00	0.00	0.00	0.00
Pyrene	0.29	0.02	0.09	0.05	0.01	0.00	0.00	0.00	0.00	0.00
C1-Fluoranthenes/Pyrenes	0.91	0.10	0.30	0.15	0.01	0.00	0.00	0.00	0.00	0.00
C2-Fluoranthenes/Pyrenes	3.65	0.29	1.09	0.54	0.03	0.00	0.00	0.00	0.00	0.00
C3-Fluoranthenes/Pyrenes	3.77	0.27	1.28	0.58	0.04	0.00	0.00	0.00	0.00	0.00
C4-Fluoranthenes/Pyrenes	2.19	0.17	0.83	0.32	0.04	0.00	0.00	0.00	0.00	0.00
Benzo(a)anthracene	0.20	0.01	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Chrysene+Triphenylene	1.19	0.07	0.37	0.19	0.03	0.00	0.00	0.00	0.00	0.00
C1-Chrysenes/Benzanthracenes	1.39	0.08	0.46	0.22	0.01	0.00	0.00	0.00	0.00	0.00
C2-Chrysenes/Benzanthracenes	1.37	0.11	0.50	0.22	0.04	0.00	0.00	0.00	0.00	0.00
C3-Chrysenes/Benzanthracenes	0.85	0.05	0.36	0.13	0.02	0.00	0.00	0.00	0.00	0.00
C4-Chrysenes/Benzanthracenes	0.30	0.03	0.15	0.05	0.01	0.00	0.00	0.00	0.00	0.00
Benzo[b]naphtho[2,1-d]thiophene	0.37	0.03	0.11	0.06	0.01	0.00	0.00	0.00	0.00	0.00
C1-Naphthobenzothiophenes	1.36	0.07	0.46	0.22	0.01	0.00	0.00	0.00	0.00	0.00
C2-Naphthobenzothiophenes	1.61	0.07	0.59	0.28	0.01	0.00	0.00	0.00	0.00	0.00
C3-Naphthobenzothiophenes	0.47	0.05	0.21	0.08	0.01	0.00	0.00	0.00	0.00	0.00
C4-Naphthobenzothiophenes	0.30	0.04	0.12	0.05	0.01	0.00	0.00	0.00	0.00	0.00
Benzo(b)fluoranthene	0.11	0.01	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Benzo(k)fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo[j]fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo[a]fluoranthene	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo(e)pyrene	0.24	0.02	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Benzo(a)pyrene	0.04	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Dibenz(a,h)anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Indeno(1,2,3-cd)pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo(g,h,i)perylene	0.03	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
TPAH50	**503**		**131**	**71.5**		**0.14**		**0.16**	**0.06**	

Supplement C. Response curves.

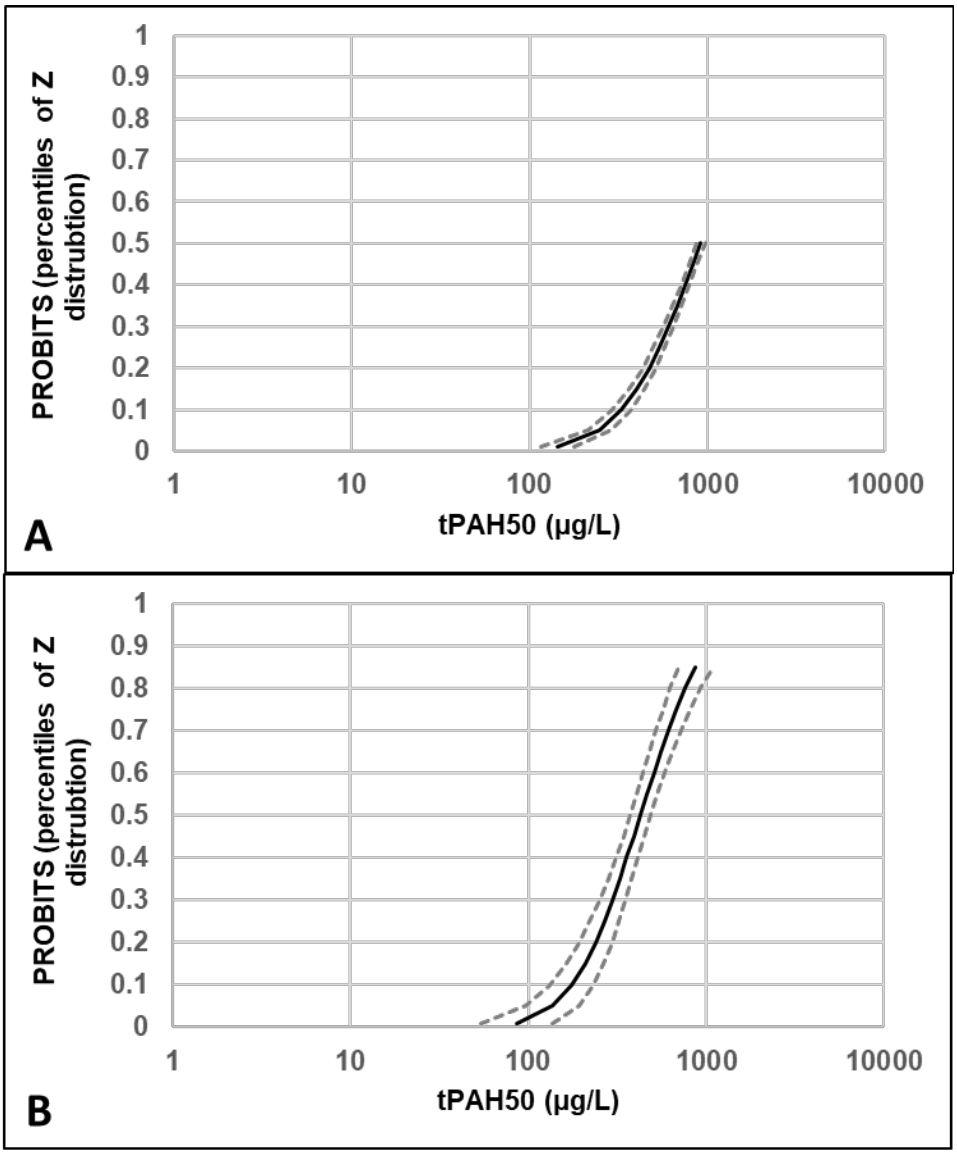

Figure C.1. Results of the probit analyses for chlorophyll *a* fluorescence. Panel A: dark-adapted maximum quantum yield (Fv/Fm) at 82 h; Panel B: light-adapted effective quantum yield at 92 h. Response curves (black lines) are shown with 95 % confidence intervals (gray, dashed lines).

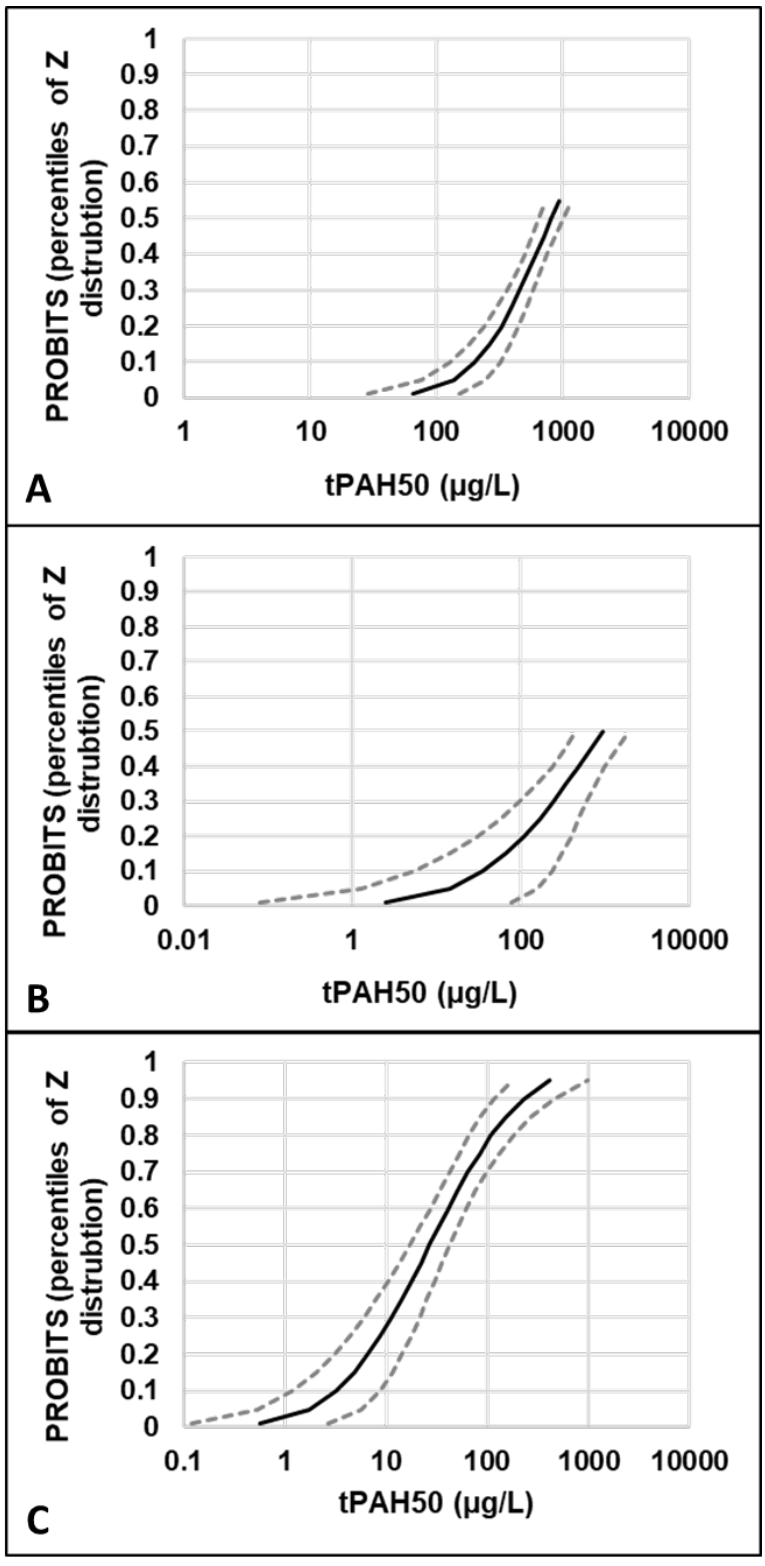

Figure C.2. Results of the probit analyses for the health assessments. Panel A: tissue integrity metric; Panel B: tissue

color metric; Panel C: polyp behavior metric. Response curves (black lines) are shown with 95 % confidence intervals (gray, dashed lines).

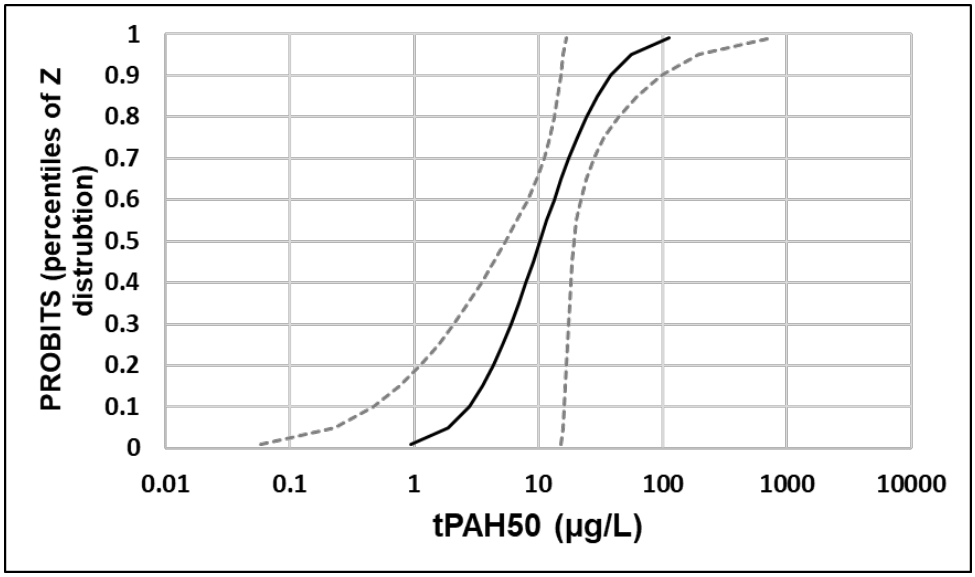

Figure C.3. Results of the probit analysis for tissue regeneration. Response curves (black lines) are shown with 95 % confidence intervals (gray, dashed lines).

Supplement D. HEWAF 96 h exposure health score data. Scores ranged from zero (poor condition) to five (excellent condition) in three categories: tissue integrity, tissue color and polyp extension.

tPAH50 Treatment	Replicate	Condition	- 7 h	17 h	41 h	65 h	89 h
ASW	1	Tissue	5	5	5	5	5
		Color	5	5	5	4	4
		Polyp	5	5	5	5	5
	2	Tissue	5	5	5	5	5
		Color	5	5	5	4	4
		Polyp	5	5	5	5	5
	3	Tissue	5	5	5	5	5
		Color	5	5	4	4	4
		Polyp	5	5	4	5	5
	4	Tissue	5	5	5	5	5
		Color	5	5	5	4	4
		Polyp	5	5	5	5	5
12 µg/L	1	Tissue	5	5	5	5	5
		Color	5	5	5	5	5
		Polyp	5	1	3	4	4
	2	Tissue	5	5	5	5	5
		Color	5	5	5	5	5
		Polyp	5	1	3	5	5
	3	Tissue	5	5	5	5	5
		Color	5	5	5	5	4
		Polyp	5	2	4	5	5
	4	Tissue	5	5	5	5	5
		Color	5	5	5	5	5
		Polyp	5	1	4	4	5
22 µg/L	1	Tissue	5	5	5	5	5
		Color	5	5	4	4	4
		Polyp	5	2	1	1	2
	2	Tissue	5	5	5	5	5
		Color	5	5	5	5	5
		Polyp	5	2	2	2	2
	3	Tissue	5	5	5	5	5
		Color	5	5	4	4	4
		Polyp	5	1	1	1	2
	4	Tissue	5	5	5	5	5
		Color	5	5	4	4	4
		Polyp	5	3	1	2	2
48 µg/L	1	Tissue	5	5	5	5	5
		Color	5	5	4	4	4
		Polyp	5	2	2	1	1
	2	Tissue	5	5	5	5	5
		Color	5	5	4	4	4
		Polyp	5	1	1	1	2
	3	Tissue	5	5	5	5	5
		Color	5	5	5	5	4
		Polyp	5	1	1	1	1
	4	Tissue	5	5	5	5	5
		Color	5	5	4	4	3
		Polyp	5	2	1	1	1

tPAH50 Treatment	Replicate	Condition	- 7 h	17 h	41 h	65 h	89 h
127 µg/L	1	Tissue	5	5	5	5	5
		Color	5	5	4	4	3
		Polyp	5	1	1	1	1
	2	Tissue	5	5	5	5	5
		Color	5	5	5	5	3
		Polyp	5	1	1	1	1
	3	Tissue	5	5	5	5	5
		Color	5	5	4	4	3
		Polyp	5	2	1	1	1
	4	Tissue	5	5	5	5	5
		Color	5	5	4	3	2
		Polyp	5	2	1	2	1
245 µg/L	1	Tissue	5	5	5	4	4
		Color	5	5	4	4	4
		Polyp	5	1	1	1	1
	2	Tissue	5	5	5	5	4
		Color	5	5	4	4	4
		Polyp	5	2	1	1	1
	3	Tissue	5	5	5	5	5
		Color	5	5	4	4	3
		Polyp	5	1	1	1	1
	4	Tissue	5	5	5	5	4
		Color	5	5	4	3	2
		Polyp	5	2	1	1	1
456 µg/L	1	Tissue	5	5	5	5	4
		Color	5	5	4	4	3
		Polyp	5	1	1	1	1
	2	Tissue	5	5	5	5	3
		Color	5	5	4	4	3
		Polyp	5	1	1	1	1
	3	Tissue	5	5	5	5	5
		Color	5	5	4	4	3
		Polyp	5	1	1	1	1
	4	Tissue	5	5	5	4	2
		Color	5	5	4	3	2
		Polyp	5	2	1	1	1
945 µg/L	1	Tissue	5	5	5	5	2
		Color	5	5	4	4	2
		Polyp	5	1	1	1	0
	2	Tissue	5	5	5	5	2
		Color	5	5	4	3	3
		Polyp	5	1	1	1	0
	3	Tissue	5	5	5	5	3
		Color	5	5	4	3	2
		Polyp	5	2	1	1	0
	4	Tissue	5	5	5	5	2
		Color	5	5	4	4	2
		Polyp	5	1	1	1	0

Supplement E. Pulse-chase experiment health score raw data. *A score of 3.5 or 4.5 for color indicates that the polyp tentacles are dark, but the surrounding tissue is increasingly pale.

			17h	41h	65h	89h	113 h	137 h	161 h	185 h	209 h	233h
Treatment	**Rep**	**Condition**	**21-Mar**	**22-Mar**	**23-Mar**	**24-Mar**	**25-Mar**	**26-Mar**	**27-Mar**	**28-Mar**	**29-Mar**	**30-Mar**
No Pulse control	**A**	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5*	4	4.5	4.5	4.5	4.5	4.5	4	3	3
		Polyp ext (0-5	5	5	5	5	5	5	5	5	5	5
	B	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4	3	3
		Polyp ext (0-5	5	5	5	5	5	5	5	5	5	5
	C	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4	3	3
		Polyp ext (0-5	5	5	5	5	5	5	5	5	5	5
	D	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4	3	3
		Polyp ext (0-5	5	5	5	5	5	5	5	5	5	5
6h pulse	**A**	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	4	5	5	5	5	5	5	5	5
	B	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	4	5	5	5	5	5	5	5	5
	C	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	4	5	5	5	5	5	5	5	5
	D	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	4	5	5	5	5	5	5	5	5
12h pulse	**A**	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4	4	4	4	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	1	4	5	5	5	5	5	5	5
	B	Tissue (0-5)	5	5	5	5	5	5	5	5	5	4
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4	3	3
		Polyp ext (0-5	1	1	4	5	5	5	5	5	5	5
	C	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	1	4	5	4	5	5	5	5	5
	D	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	3.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	1	4	5	5	5	5	5	5	5
24h pulse	**A**	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4	3	3
		Polyp ext (0-5	1	1	4	5	5	5	5	5	5	5
	B	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	1	4	5	5	5	5	5	5	5
	C	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4	4	3	3
		Polyp ext (0-5	1	1	4	5	5	5	5	5	5	5
	D	Tissue (0-5)	5	5	5	5	5	5	5	5	5	5
		Color (0-5)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4	3	3
		Polyp ext (0-5	1	1	4	5	5	5	5	5	5	5
96h pulse	**A**	Tissue (0-5)	5	5	5	4	3	3	3	3	3	2
		Color (0-5)	4	4.5	4	3.5	3	3	3	3	2	2
		Polyp ext (0-5	1	1	1	1	1	1	1	1	1	1
	B	Tissue (0-5)	5	5	5	3	3	3	3	3	3	3
		Color (0-5)	4.5	4.5	4	3.5	3.5	3	3	3	3	3
		Polyp ext (0-5	1	1	1	1	1	1	1	1	1	1
	C	Tissue (0-5)	5	5	4	3	2	2	3	3	3	3
		Color (0-5)	4.5	4.5	4	3.5	3.5	3	3	3	3	3
		Polyp ext (0-5	1	1	1	1	1	1	1	1	1	1
	D	Tissue (0-5)	5	5	5	4	3	3	3	3	3	3
		Color (0-5)	4.5	4.5	4	3	3	3	3	3	3	3
		Polyp ext (0-5	1	1	1	1	1	1	1	1	1	1