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Acute Embryonic or Juvenile Exposure to Deepwater Horizon Crude Oil Impairs the Swimming Performance of Mahi-Mahi (Coryphaena hippurus)

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Title: Acute Embryonic or Juvenile Exposure to *Deepwater Horizon* Crude Oil Impairs the Swimming Performance of Mahi-Mahi (*Coryphaena hippurus*)

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Abstract

The *Deepwater Horizon* incident likely resulted in exposure of commercially and ecologically important fish species to crude oil during the sensitive early life stages. We show that brief exposure of a water-accommodated fraction of oil from the spill to mahi-mahi as juveniles, or as embryos/larvae that were then raised for ~25-d to juveniles, reduces their swimming performance. These physiological deficits, likely attributable to polycyclic aromatic hydrocarbons (PAHs), occurred at environmentally realistic exposure concentrations. Specifically, a 48-h exposure of 1.2 ± 0.6 μg L^{-1} ΣPAHs (geometric mean ± SEM) to embryos/larvae that were then raised to juvenile stage, or a 24-h exposure of 30 ± 7 μg L^{-1} ΣPAHs (geometric mean ± SEM) directly to juveniles resulted in 37% and 22% decreases in critical swimming velocities (U_{crit}), respectively. Oil-exposed larvae from the 48-h exposure showed a 4.5-fold increase in the incidence of pericardial and yolk sac edema relative to controls. However, this larval cardiotoxicity did not manifest in a reduced aerobic scope in the surviving juveniles. Instead, respirometric analyses point to a reduction in swimming efficiency as a potential alternative or contributing mechanism for the observed decreases in U_{crit}.

Introduction

The largest marine oil spill in U.S. history spanned 87 days during the spring and summer of 2010 during which the blown-out *Deepwater Horizon* (*DWH*) Macondo wellhead released approximately 4 million barrels (6 x 10^8 L) of crude oil into the northern Gulf of Mexico (GoM)[1-3]. The *DWH* incident oiled large portions of the pelagic zone, including the upper surface waters, and overlapped both spatially and temporally with the spawning of a number of commercially and ecologically important fish species, including yellowfin and bluefin tuna[4, 5], mahi-mahi[6, 7] and other large pelagics[8-10]. An unusual aspect of the spill was that it originated at

depth and under high energy which facilitated the dissolution of toxic polycyclic aromatic hydrocarbons (PAHs) by increasing mixing and contact time with the water during ascent to the surface[11, 12]. Thus, PAH exposures likely occurred for adult fish breeding in the area and during the far more sensitive early life stages (ELS; i.e., embryos and larvae) of the developing offspring. Besides immediate impacts on survival, such PAH exposures, even if brief, may have imparted subtle developmental and physiological impairments with ensuing ramifications of ecological importance[13]. Here we provide novel information on sublethal exposures of the PAHs found in this oil spill to a resident GoM fish (mahi-mahi).

Numerous studies have investigated the developmental toxicity of crude oil to fish embryos and larvae, and also identified the constituent PAHs responsible for ELS impacts[14-27]. These functional studies have identified cardiac development as a key process impaired by PAHs, particularly 3-ring (tricylic) compounds, causing anatomical malformations and functional defects that likely diminish cardiac output in association with bradycardia and arrhythmia. Oil-exposed embryos that develop cardiac failure and jaw deformities are unlikely to survive past the yolk sac stage to become free swimming and feeding larvae[14]. These findings are perhaps more worrisome than the longstanding paradigm wherein lower molecular weight and more volatile 2-ring PAHs drive crude oil toxicity through acute and nonspecific narcosis[13]. The nervous system has also been highlighted as a target of oil toxicity in fish by recent work showing that acute oil exposure disrupts normal sensory and motor axon pathfinding as well as locomotor behavior during early development[28-30]. While severe oil effects culminate in cardiac failure, as evident from the accumulation of fluid around the heart or yolk sac (i.e., pericardial or yolk sac edema)[17, 19], more subtle changes in cardiac morphogenesis can cause permanent shape

changes that persist to adulthood[16]. However, the physiological consequences of cardiac and/or neurological defects in fish that survive transient PAH exposures remain poorly understood.

Given that ecologically important behaviors such as foraging, predator avoidance and migration are limited by the maximum swimming velocity that a fish can sustain for a prolonged period (U_{crit}), tests of swimming performance provide a relevant measure of physiological fitness for fish exposed to relatively low levels of PAHs derived from crude oil[31, 32]. A recent study showed that embryonic exposure of zebrafish to North Slope crude oil (ANSCO) conferred reduced U_{crit} in adulthood along with changes in ventricular shape, suggesting impaired cardiac output may have limited swimming performance in these fish[16]. Still, similar studies are lacking for pelagic species with high aerobic demand, including the large open ocean fish that spawned in the surface spill zone during the *DWH* incident. In addition to the unknown latent effects following embryonic exposure, it remains to be seen whether transient oil exposure to GoM pelagic species during later stages of development (e.g., juvenile) might also cause immediate impairment to swimming performance.

The recent addition of a successful mahi-mahi husbandry program at the University of Miami Experimental Hatchery (UMEH) presents a rare opportunity to assess the short- and long-term impacts that the *DWH* incident may have imparted to this commercially and recreationally important GoM species during any life stage and under controlled laboratory conditions. In the present study we tested the hypotheses that transient exposure to *DWH* oil prepared as a high-energy water-accommodated fraction (HEWAF) would: (1) cause direct impairment to the swimming performance of juvenile mahi-mahi and (2) manifest in latent impairment as juveniles when exposed ~25 days earlier during the embryonic/larval stage. The oil used for exposures was collected on July 29, 2010 from a barge hold receiving slick oil from various skimmer

vessels (hereafter referred to as slick A). Additionally, we sought to further investigate the role of reduced cardiac output as a potential underlying cause limiting swimming performance by assessing aerobic scope from respirometry measurements collected during the swim trials.

Materials and Methods

Experimental animals. Mahi-mahi broodstock (*Coryphaena hippurus*) were captured off the coast of Miami, FL using hook and line angling techniques. The fish were subsequently transferred to the University of Miami Experimental Hatchery (UMEH) where they were acclimated in 80 m^3 fiberglass maturation tanks (typically 5-7 per tank) equipped with recirculating aquaculture systems for water quality and temperature control. All embryos used in the experiments described herein were collected within ~2-10 h following a volitional (non-induced) spawn using standard UMEH methods [33]. A prophylactic formalin (37% formaldehyde solution) treatment was administered to the embryos (100 ppm for 1 h), followed by a 0.5 h rinse with a minimum of 300% water volume in the treatment vessel using filtered, UV-sterilized seawater. A small sample of eggs was collected from each spawn to microscopically assess fertilization rate and embryo quality. Spawns with low fertilization rate (<85%) or frequent morphological abnormalities (>5%) were not used.

Preparation of water-accommodated fractions. The oil (referred to herein as slick A) used to prepare all HEWAFs was collected during the *DWH* spill on July 29, 2010 from the hold of barge number CTC02404, which was receiving slick oil from various skimmer vessels (sample ID CTC02404-02), and was subsequently transferred under chain of custody to the University of Miami. Each HEWAF was prepared on the day of use at a loading rate of 1 g of oil per liter of 1 μm filtered, UV-sterilized seawater and mixed in a Waring CB15 blender (Torrington, CT) at low speed for 30 s. The mixture was immediately transferred to a glass

separatory funnel, allowed to settle for 1-h, and the lower ~90% carefully drained and retained for subsequent use as 100% WAF (unfiltered) that was diluted for test exposures.

Embryo-larval exposures (48-h). Embryos from the same cohort were exposed to control seawater or a 0.2% HEWAF dilution (1.2 μg L^{-1} ΣPAH) for 48-h and then raised for ~25-d to the early juvenile stage in clean seawater. The 0.2% HEWAF dilution was selected to target a concentration that minimized acute mortality yet was high enough to induce cardiotoxicity based on results from a previous slick A HEWAF bioassay. Exposures were established in a 2500 L cylindrical fiberglass tank (1 per treatment) coated with standard marine gelcoat paint on the interior and fitted with a PVC stand pipe that was filled with filtered (5-10 μm), UV-sterilized seawater. For the oil exposure, HEWAF was added when the tank was approximately ⅓ filled by even distribution along a concentric path midway between the standpipe and the tank wall to ensure adequate mixing. Once the tanks were filled (~2200 L final volumes) flow was stopped and ~30,000-80,000 viable mahi-mahi embryos were stocked into each at <12-h post-fertilization (hpf). A low level of aeration was provided to each tank using an air stone to distribute the embryos throughout the tank during static exposure. Following the 48-h exposure period, inflow of clean water to both tanks resumed and each remained under flow-through conditions with gentle aeration for the remainder of the experiment. Larvae were reared using UMEH intensive marine fish larviculture protocols and collected for swim trials at 25-26 days post-hatch (dph). Specifically, larvae were pulse-fed enriched rotifers (*Brachionus plicatilis*) and *Artemia* nauplii during the first 2-3 weeks post-hatch, followed by weaning onto an inert diet (Otohime, Marubeni Nisshin Feed Co., Ltd.). Waste was removed daily from each tank by siphoning.

Juvenile exposures (24-h). Juveniles raised entirely in clean seawater (mean age of 31 d) were exposed to control seawater or 0.4, 1.2 or 2% HEWAF dilutions (4.2, 17 or 30 μg L^{-1} ΣPAH, respectively) for 24-h. Exposures were performed in a temperature controlled environmental chamber by placing six fish into 10-12 L of control seawater or seawater spiked with freshly-prepared HEWAF in a 20 L glass jar with light aeration provided by an air stone. Temperature and photoperiod within the chamber were set at 27ºC and 16:8 h of light:dark, respectively. Fish were transferred directly from treatment tanks to the swimming respirometers (i.e., no recovery period was permitted prior to transfer). All swim trials were performed with clean seawater. Although only four fish were swum per replicate, six fish were exposed to safeguard against losses to mortality. With the exception of 2 fish that died within one of the 30 μg L^{-1} ΣPAH replicates, no other mortalities were observed for the 24-h exposures. In all other replicates, the remaining two fish not used in the swim trials were euthanized. In sum, the number of replicates (and total *n*) for the control, 4.2, 17 and 30 μg L^{-1} ΣPAH 24-h exposures were 5 (20), 2 (8), 3 (12) and 3 (12), respectively. Due to the rapid growth rates of juvenile mahi-mahi[34], control exposures were interspersed among oil exposures to minimize potential confounding factors due to size on swimming performance and aerobic scope. Fish were obtained from three cohorts, with at least one control replicate included from each. Fish were fed in the morning before transferring to an exposure chamber, but not fed during the 24-h exposure period.

Water quality and PAH analysis. In addition to samples collected for ΣPAH analysis, the following water quality parameters were monitored: temperature, pH, dissolved oxygen (DO), salinity and total ammonia. Temperature and DO were measured using a ProODO handheld optical DO probe and meter (YSI, Inc., Yellow Springs, OH) and pH was measured

using a PHM201 meter (Radiometer, Copenhagen, Denmark) fitted with a combination glass electrode. The pH and DO probes were calibrated daily. Salinity was measured using a refractometer and total ammonia determined using a colorimetric assay[35]. All samples for PAH analysis were collected directly from exposure tanks as grab samples in 250 mL amber bottles and shipped overnight on ice to ALS Environmental (Kelso, WA) for analysis by gas chromatography/mass spectrometry – selective ion monitoring (GC/MS-SIM; based on EPA method 8270D). Reported ΣPAH values represent the sum of 50 select PAH analytes (Table S1; Fig. S1).

For the 48-h embryo-larval exposures, ΣPAH samples and measurements for all other water quality parameters except ammonia (final only) were collected daily during the exposure period (i.e., 0, 24 and 48-h) and at 24 and 48-h of the ensuing washout period when flow was returned to the tanks. For the 24-h juvenile exposures, initial and final measurements were taken for all parameters except ammonia (final only) and initial and final samples were collected for ΣPAH analysis. A summary of all measured water quality parameters and ΣPAH concentrations is provided in Table S2.

Assessment of cardiac abnormalities. Immediately following the 48-h exposure period, a subsample of larvae was collected from each of the grow-out tanks for assessment of pericardial edema and heart rate. Larvae (n = 20 per tank) were mounted 2-3 at a time over 2% methylcellulose in seawater and imaged using a Fire-i400 digital camera (Unibrain, San Ramon, CA) mounted on a Nikon SMZ800 stereomicroscope. Images were collected on a MacBook laptop using iMovie software and calibrated using a stage micrometer. Larvae were scored blind for presence or absence of pericardial edema by morphological assessment of the yolk mass which became distorted with pericardial edema. Presence of edema was noted if fluid

accumulation was sufficient to distort the normal smooth bullet shape of the anterior yolk mass. Edema typically occurred in the form of a concave or pointed wedge shape of the anterior yolk mass and/or contained a number of smaller indentations indicated by dark, angular lines. Pericardial area was measured using ImageJ version 1.46r (rsbweb.nih.gov/ij/) from a perimeter drawn with the freehand tool enclosing this area (Fig. S2). Lines were drawn across the boundary of the yolk sac only if distortion of the yolk mass was clearly evident by a sharp dark line. Heart rate was measured by counting the total number of heart beats for each larva in a ≥10-s digital video clip played back at half speed.

Swimming performance and aerobic scope. Four miniature (0.17 L) Blazka-style swim respirometers (Loligo Systems, Denmark) were used to asses U_{crit} and aerobic scope via automated intermittent flow respirometry[36, 37]. Oxygen consumption within each swim chamber was measured using a Pt100 fiber-optic probe connected to a Fibox 3 minisensor oxygen meter (PreSens Precision Sensing GmbH, Germany). The oxygen sensor was calibrated daily using 100% air saturation, established by vigorous aeration with an air stone, and 0% O_2 saturation, achieved using a solution of 10 g L^{-1} Na_2SO_3 (Sigma-Aldrich, St. Louis, MO). Temperature was maintained at 27 ± 1°C using an aquarium heater submerged in the reservoir water surrounding each swim chamber and was measured through the oxygen meter using a separate probe. All data were collected using AutoResp2 version 2.1.2 (Loligo Systems, Denmark).

A preliminary study was performed with four juvenile fish to determine a minimum habituation period sufficient to permit recovery from handling stress and obtain a stable routine metabolic rate (RMR) prior to initiating a swim trial. The fish were each held in a swim chamber for 20 h at a minimal flow speed to maintain water mixing within the chamber without

forcing the fish to swim. From these experiments a stabilized RMR was established for each fish by 3-4 h. Thus, all subsequent swim trials followed a 4-h habituation period.

To measure U_{crit}, fish were forced to swim for 20 min intervals until exhausted using fixed velocity increments that, depending on size, ranged between 1-2.5 cm s^{-1} (~0.5 body lengths (BL) s^{-1}) per interval. Unlike many other teleosts, mahi-mahi rarely exhibit fatigue by becoming pinned parallel to the rear screen. Rather, juveniles tend to maintain a position perpendicular to the rear screen while resting on a bent caudal fin, sometimes with intermittent swim bursts off the screen. Therefore, fatigue was designated as when the fish began to consistently rest on its caudal fin for several seconds at a time. U_{crit} (in BL s^{-1}) was calculated using the following equation originally described by Brett[38]: $U_{crit} = [U_f + (T/t)dU]/cm$, where U_f (cm s^{-1}) is the highest swim velocity maintained for a full interval, T (s) is the time spent at the final velocity, t is the time interval (s) and dU is the increment in swim speed (cm s^{-1}). Fish mass and BL were determined post-swimming to minimize stress pre-testing and to convert U_{crit} to BL s^{-1}. Food was withheld for all fish for at least 24-h prior to introduction into a swim chamber to eliminate digestion (i.e., specific dynamic action) as a confounding factor.

Although juveniles from the embryonic grow-out exposures were collected at a younger age and thus were significantly smaller (Table 1; $P < 0.05$), there was no significant difference in U_{crit} among any of the control cohorts. Furthermore, given that the controls were represented by a total of six treatment replicates from four cohorts, there was no indication of tank or batch effects on swimming performance. Therefore, fish from all of the control treatments were pooled for statistical comparisons of U_{crit}. This represents a conservative approach since smaller fish typically have higher U_{crit} values than larger fish when expressed in terms of body length[39]

and the oil-exposed juveniles from the grow-out exposure were of smaller size than the combined controls (Table 1; $P < 0.05$).

A standardized approach was used to estimate aerobic scope for each fish. First, the logarithm of oxygen consumption (mg O_2 g^{-1} h^{-1}) versus swimming speed (BL s^{-1}) was plotted for each fish and a least squares linear regression performed (see Fig. S3 for example). Standard metabolic rate (SMR; y-intercept) and maximum metabolic rate (MMR; extrapolated at U_{crit}) were then derived from the resulting equations. Only individuals yielding a regression with an $r^2 \geq 0.7$ were used for these analyses (see Table 1 for n). Given the variation in observed body masses (Table 1), and that metabolic rate is known to scale with mass, we normalized for the effect of mass in calculating SMR and MMR by scaling to a standard mass of 1 g before calculating aerobic scope (MMR – SMR; Fig. S4).

Cost of transport at U_{crit} (COT_{Ucrit}) was calculated by dividing the MMR (mg O_2 g^{-1} s^{-1}) for each fish by the corresponding U_{crit} (BL s^{-1})[40].

Statistical analyses. Data are presented as means ± 1 standard error of the mean (SEM). Differences were tested for statistical significance using SigmaStat version 3.5 (Systat Software, Inc., San Jose, CA). Unless otherwise noted, see figure legends and table footnotes for the exact statistical method used for each test. In all cases, differences were deemed significant at $P < 0.05$.

Results

PAH concentrations and composition of HEWAF preparations. Because ΣPAH concentrations decreased over time, both the initial and geometric mean concentrations are reported (Table S2). Previous work has demonstrated consistency between PAH profiles obtained from our HEWAF preparations and samples collected from the active spill zone[27]. The

percent composition of PAHs was similar among all oil exposures, with the bicyclic naphthalenes and tricyclic fluorenes, dibenzothiophenes and phenanthrenes/anthracenes predominating (Fig. S1). Nominal doses of HEWAF produced nearly proportional changes in ΣPAHs, although this relationship appeared to weaken at lower doses (Fig. S1). Initial C1-phenanthrene concentrations (a PAH class well represented in *DWH* crude oil; Fig. S1) ranged from 4.6 – 0.33 μg L^{-1} in the 2% and 0.2% HEWAF dilutions, respectively (Table S1). Notably, the latter concentration falls below a previously reported level of ≥0.36 μg L^{-1} representing 4.6% (25 of 548) of samples collected within a ~96,000 km^2 region centered over the wellhead[27].

Evidence of 48-h larval cardiotoxicity. Larvae that were exposed to oil for 48-h during early development (1.2 μg L^{-1} ΣPAHs) were microscopically examined for defects in heart form (increased pericardial area due to edema) and function (heart rate) immediately following exposure using blind scoring of keyed images and videos. The incidence of pericardial edema increased 4.5-fold in oil-exposed larvae relative to controls (Fig. 1A). Pericardial area also increased nearly 2-fold in larvae that were previously exposed to HEWAF (Fig. 1B; $P = 0.003$). Analysis of heart rates revealed greater variability in larvae exposed to HEWAF (Fig. 1C), although the mean heart rate was not significantly different from control (Student's *t*-test; $P = 0.128$). Gross malformations that typify the more severe oil injury syndrome in fish ELS, including spinal, craniofacial and fin deformities[19], were not observed.

Swimming performance. Results from the swim trials revealed a significant 37% ($P < 0.05$) decrease in mean U_{crit} for juveniles that were transiently exposed to 1.2 μg L^{-1} ΣPAH as embryos and larvae. By comparison, among mahi-mahi exposed as juveniles, only the highest concentration (30 μg L^{-1} ΣPAH) elicited a significant decrease of 22% ($P < 0.05$) (Fig. 2A).

Metabolic rates, aerobic scope and cost of transport. Respirometry was conducted during each swim trial to determine the potential impacts of slick A HEWAF exposure on aerobic scope (i.e., maximum metabolic rate (MMR) - standard metabolic rate (SMR)). To account for variation in metabolic rates due to variation in size, calculations of SMR and MMR were first normalized for mass using empirically determined scaling coefficients and constants (see Materials and Methods). Non-normalized data are provided in the Supporting Information (Fig. S5).

No significant effect on SMR, MMR or aerobic scope was evident for the fish exposed to 1.2 μg L^{-1} ΣPAH as embryos/larvae and then tested as juveniles ~25 days later (Fig. 2). Accordingly, cost of transport at U_{crit} (COT_{Ucrit}), which represents the mass-specific amount of aerobic energy expended to traverse a given distance[40], increased significantly by 40% (P = 0.0085) to a level nearly matching the 37% decrease in U_{crit} (Fig. 3). Similarly, no significant effect on SMR, MMR or aerobic scope was evident for the fish exposed to HEWAF as juveniles (Fig. 2). Again, however, there was an upward trend in COT_{Ucrit} (21%, P = 0.147) at the 30 μg L^{-1} ΣPAH concentration that closely mirrored the 22% decrease in U_{crit} (Fig. 3).

Discussion

Results from the present study demonstrate that exposure to crude oil collected from surface slicks during the *DWH* incident impairs the swimming performance of juvenile mahi-mahi, an ecologically and commercially important pelagic predator in the northern GoM. Perhaps most significant was the finding that such impairment during the juvenile stage manifested as a latent effect ~25 days following a 48-h exposure during the embryonic/larval stage to a ΣPAH concentration of 1.2 μg L^{-1}. By contrast, a nearly 30-times higher, yet still environmentally relevant, concentration of 30 μg L^{-1} ΣPAH was required to elicit a similar

decrease in U_{crit} for fish exposed for 24-h as juveniles. While these results are consistent with an expected decrease in sensitivity with increasing developmental stage, they also support the suggestion that PAH exposure during the embryonic/larval stage caused latent developmental effects. The effective embryonic/larval exposure was 1/30th that for the juveniles and the larval body burden would be expected to decrease over time through growth dilution and hepatic metabolism, potentially eliminating an effect of a residual body burden of PAHs that persisted to the juvenile stage. The observed larval effects of pericardial and yolk sac edema, which were similar to those previously described for a number of different teleosts exposed to various sources of crude oil[14, 18, 20, 23, 27, 41-43], lend further credence to a likely early developmental origin for the latent effects on swimming performance.

The available data to date indicate that our exposure concentrations, which ranged from 1.2 – 30 μg L^{-1} ΣPAHs, likely represent environmentally realistic exposure scenarios. For example, Bejarano et al. reported a range of <0.01 – 77 μg L^{-1} ΣPAHs from samples collected at 1 m and 10 m depths[44] and other studies have reported similar values ranging as high as 59 μg L^{-1} and 85 μg L^{-1} ΣPAHs from samples collected both at depth and at the surface within the active spill zone[45, 46]. However, it should be noted that comparisons among ΣPAH measurements are somewhat complicated by analytical and sample variations (e.g., the number and selection of analytes included in ΣPAH calculations and collection depths and locations). Additional perspective can be gained by comparing measurements of a single analyte, C1-phenanthrene, which is well-represented within *DWH* crude oil. It was previously found that 4.6% of 548 samples collected from a region centered above the wellhead met or exceeded a concentration of 0.36 μg L^{-1} for C1-phenanthrene, a value near the initial concentration of 0.33 μg L^{-1} measured for the 0.2% HEWAF embryonic/larval exposure[27]. The fertilized eggs of mahi-mahi float near

the surface of the open waters of the northern GoM where they are spawned and putatively remain to develop throughout their ELS history[7]. Thus, given that the spill likely overlapped temporally and spatially with mahi-mahi spawning events[6, 7, 10], it seems highly probable that detrimental PAH exposures occurred for these fish during the sensitive ELSs.

Considering the above, the current findings likely indicate an additional level of reduced survival to maturity in the wild than might be anticipated from acute mortality alone due to the spill. For example, the latent effects exhibited by juveniles exposed as embryos/larvae support a potential mechanism of impaired swimming performance for the delayed mortality and, by extension, population-scale impacts shown previously by mark-recapture studies of pink salmon exposed to ANSCO (~5-19 μg L^{-1} initial ΣPAH concentrations)[47, 48]. The more direct effects on fish exposed transiently as juveniles may contribute yet a further level of mortality due to impaired swimming performance; however, the long-term impact on survival in this scenario is less clear as the likely duration of such impairment remains unknown at this time. Nevertheless, it is important to note that the effective concentrations reported herein may underestimate toxicity in the natural environment where other stressors with the potential to increase toxicant sensitivity (e.g., hypoxia, temperature and UV exposure) are commonly present. Furthermore, such effects on mahi-mahi likely also span to other important pelagic GoM species, such as billfish and tunas, which also resided in the spill path during the ELSs[4, 5, 9, 10].

There are several physiological/behavioral mechanisms that could potentially contribute to a reduced U_{crit}. Firstly, U_{crit} may decrease as result of diminished aerobic scope deriving from a limitation in oxygen uptake or delivery that decreases MMR (e.g., reduced cardiac output, anemia or increased lamellar diffusion distance) and/or an effect that adds to the basal metabolic load and thus increases SMR (e.g., specific dynamic action, metabolism of xenobiotics or

318 increased ionoregulatory costs)[49]. Secondly, glycolytically fuelled white muscles may be
319 recruited to sustain high swimming speeds approaching and including U_{crit}. Thus, any
320 impairment to this recruitment may also limit U_{crit}[50]. Finally, there is evidence that behavior
321 plays a role in setting swimming speed when fish cannot generate or sense a ground speed, a
322 particular concern when using swim tunnels where forward movement is greatly limited[50].
323 Although each of these potential mechanisms could conceivably play a role in reducing the U_{crit}
324 of oil-exposed fish in our experiments, we chose to focus our investigation on the first
325 mechanism of diminished aerobic scope in light of the well-known cardiotoxic effects of PAHs.
326 A recent study revealed that zebrafish that survived to adulthood following a 48-h
327 embryonic exposure to ANSCO (24-36 μg L^{-1} ΣPAHs) developed defects in ventricular shape
328 likely to impair cardiac output[16]. Consistent with this, swim trials with the affected adult
329 zebrafish revealed an 18% decrease in U_{crit}, thereby supporting a link between impaired cardiac
330 output, reduced aerobic scope, and impaired swimming performance[16]. However, respirometry
331 was not performed in the zebrafish trials, and we did not examine the hearts of juvenile mahi-
332 mahi for permanent changes in heart shape in response to embryonic PAH exposure.
333 Nevertheless, larval evidence for developmental cardiotoxicity (pericardial and yolk sac edema)
334 following the 48-h exposure period would suggest that subsequent reductions in juvenile
335 swimming performance might be a consequence of a reduced aerobic scope, with a diminished
336 cardiac output limiting MMR. Yet our respirometry results did not clearly reveal a reduced
337 MMR as the underlying mechanism for the impaired swimming ability of juvenile mahi-mahi.
338 Although direct measurements of cardiac performance were not made, the lack of an effect on
339 aerobic scope strongly indicates that cardiac output was maintained, thereby suggesting a

mechanism(s) for the reduced U_{crits} other than, or in addition to, one that limits aerobic scope for juvenile mahi-mahi exposed to *DWH* crude oil.

The apparent discrepancy between potential aerobic scope effects observed in zebrafish but not juvenile mahi-mahi may relate to species-specific differences in life-histories, most notably the aggressive predatory nature of the latter. Mahi-mahi exhibit a high degree of cannibalism when reared at high density under standard culture conditions. This was frequently observed during our rearing procedures and undoubtedly introduced a baseline level of selection that would have only been exacerbated with the addition of oil. Thus, the absence of a corresponding reduced aerobic scope by juvenile fish from the same cohort exhibiting larval cardiotoxicity may indicate that the most severely impaired fish with cardiac defects were selected out during the rearing process. Similar fish in the wild, however, would not have been subjected to the same high selective pressures due to confinement, but rather pressures from other species and environmental stressors as discussed above. In the end, impaired juvenile swimming performance by feral fish exposed to crude oil may result both from an as of yet undetermined physiological deficit, as indicated by the present study, and a reduced aerobic scope owing to developmental cardiac defects as indicated by other studies. Nevertheless, considering that in all likelihood the most highly fit fish were used for the swim trials, in addition to the fact that the exposures were performed in the absence of environmental stressors, lends further support that our results are likely conservative.

The observed decreases in U_{crit} without corresponding decreases in aerobic scope as described herein suggest that the former are due to reductions in swimming efficiency (i.e., greater energy is required to swim a given distance or maintain a given velocity). This is well illustrated by the proportional inverse relationships for U_{crit} and COT_{Ucrit} exhibited by both the

juveniles swam ~25 days following exposure to HEWAF as embryos/larvae and the juveniles swam 24-h after exposure to the highest concentration of HEWAF. Thus, it is plausible that the latent and acute effects are related to direct toxic effects in the red and/or white swimming muscles, or the neural control of swimming muscle contractions, that cause dysfunction in the proper coordination of muscles required for efficient swimming. For example, this might occur due to a PAH-induced disruption in the excitation-contraction coupling mechanism of skeletal muscles in manner consistent with that recently described for bluefin and yellowfin tuna[26] cardiomyocytes. Alternatively, PAH-induced ROS production could result in the uncoupling of mitochondrial oxidative mechanisms that diminish the amount of ATP generated per unit oxygen[51, 52]. Considering that both sustained aerobic activity and anaerobic burst swimming are utilized in achieving U_{crit}, a failure in gait transition to anaerobic burst swimming by white muscles might represent another possible explanation as greater demand would be placed on aerobic metabolism to sustain U_{crit}[53]. Finally, a recent report described a number of neurological and muscular defects that coincided with impaired locomotor responses in larval zebrafish exposed to WAF treatments derived from *DWH* riser oil[28]. Moreover, a separate study revealed flow sensing by superficial neuromasts of the lateral line is required for efficient swimming by the yellowtail kingfish[54], thus highlighting the importance of a functional sensory system in coordinating swimming motions. Taken together, these studies may implicate a role for neurological dysfunction in limiting the swimming performance of pelagic fish species exposed to *DWH* crude oil. Clearly, more research is needed to test these hypotheses and elucidate the underlying mechanism(s) of impaired swimming performance by juvenile mahi-mahi following transient exposure to *DHW* crude oil.

Irrespective of mechanism, our findings of reduced U_{crit} for mahi-mahi exposed to *DWH* crude oil nevertheless reveal significant physiological impairment at PAH concentrations well below some measurements taken during the spill (i.e., as low as 1.2 μg L^{-1} ΣPAHs). These results indicate that the impacts on survival of mahi-mahi to maturity are likely to be greater than estimates that consider acute mortality alone. Still, there remains much to be learned regarding the effects of *DWH* oil exposure on the swimming performance of resident GoM pelagic fishes, including for example the ability to recover from transient exposures and the significance of natural environmental stressors in contributing additional detriment. Such information would provide further insight relevant to assessing the damage that the *DWH* spill imparted to these important natural resources.

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Supporting Information Available. Further information is available that provides PAH compositions and measurements (Table S1, S2; Figure S1), water quality measurements (Table S2), an example of larval pericardial edema (Figure S2), an example linear regression of metabolic rate vs. swimming speed (Figure S3), methods for determining mass scaling constants

and coefficients (Figure S4) and non-mass scaled metabolic rate and COT data (Figure S5). This information is available free of charge via the Internet at http://pubs.acs.org/.

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Figure Legends

Figure 1. Evidence of cardiac toxicity in larval mahi-mahi exposed to 1.2 μg L^{-1} ΣPAHs (geometric mean) for 48-h initiated <12 hpf (n = 20 each, except n = 19 for the oil-exposed group in (B)) as assessed by (A) percent incidence of pericardial edema, (B) pericardial area and (C) heart rate. Data in (B) are presented as means ± SEM. Data in (C) are presented as box plots indicating the 25th and 75th percentiles; whiskers indicate the 90th and 10th percentiles; filled circles indicate outliers; solid and dashed lines indicate the median and mean, respectively. *Significantly different from controls by Mann-Whitney rank sum test.

Figure 2. Comparisons of (A) critical aerobic swimming speed (U_{crit}), (B) standard metabolic rate (SMR), (C) maximum metabolic rate (MMR) and (D) aerobic scope from swimming respirometry experiments of juvenile mahi-mahi exposed to 4.2, 17 or 30 μg L^{-1} ΣPAHs (n = 7, 9 and 8, respectively) for 24-h prior to swimming (shaded bars) or to 1.2 μg L^{-1} ΣPAHs (n = 5) for 48-h initiated <12 hpf (hatched bar) and then raised for ~25-d to the juvenile stage. The control group represents fish from all 4 control cohorts combined (n = 21). Data in (B-C) were normalized for body mass by scaling to the respective SMR and MMR values predicted for a 1 g

fish (aerobic scope = normalized MMR- normalized SMR). Data are presented as means ± SEM. *Significantly different from controls by one-way ANOVA and Holm-Sidak multiple comparison procedure.

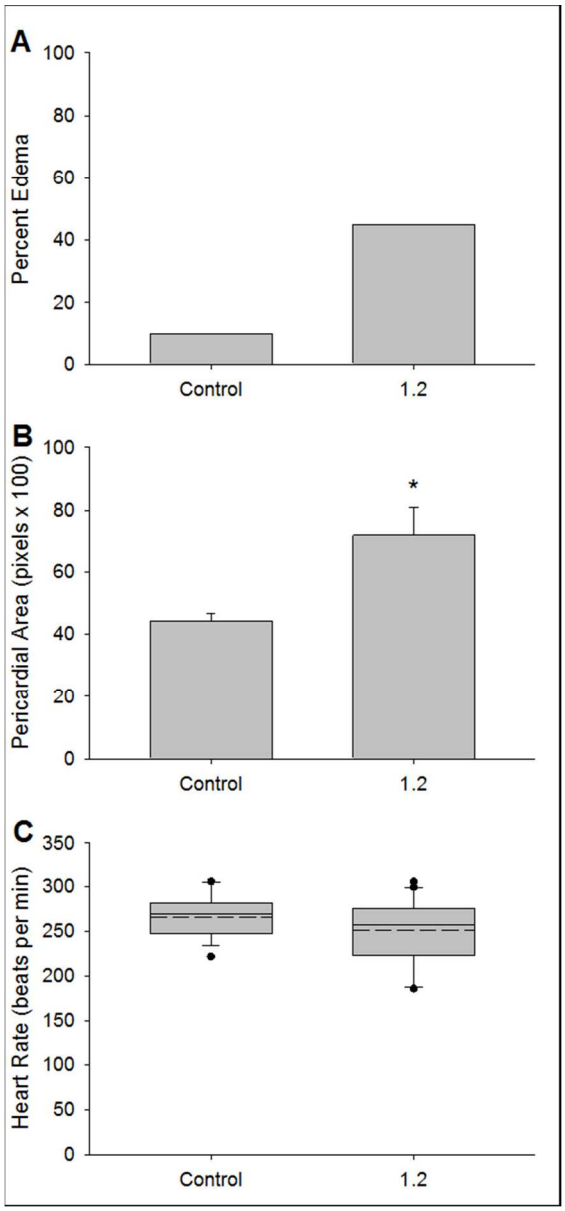
Figure 3. Cost of transport at U_{crit} for juvenile mahi-mahi exposed to 4.2, 17 or 30 µg L^{-1} ΣPAHs (n = 7, 9 and 8, respectively) for 24-h prior to swimming (shaded bars) or to 1.2 µg L^{-1} ΣPAHs (n = 5) for 48-h initiated <12 hpf (hatched bar) and then raised for ~25-d to the juvenile stage. The control group represents fish from all 4 control cohorts combined (n = 21). Data are presented as means ± SEM. *Significantly different from controls by one-tailed Student's t-test and Bonferroni multiple comparison procedure.

Table 1. Biometric data for mahi-mahi used in swimming performance tests.

Treatment	*n*	Mass (mg)	BL (cm)	Age (dph)
24-h Juvenile				
Control	20 (17)	645 ± 97	4.4 ± 0.2	32 ± 2
0.4% HEWAF	8 (7)	404 ± 33	4.0 ± 0.1	28 ± 0
1.2% HEWAF	12 (9)	497 ± 29	4.3 ± 0.1	30 ± 0
2% HEWAF	12 (8)	812 ± 180	4.8 ± 0.3	35 ± 2
48-hpf				
Control	5 (4)	283 ± 29	3.4 ± 0.1	26 ± 0
0.2% HEWAF	5 (5)	204 ± 35	3.1 ± 0.1	25 ± 0

Numbers in parentheses are *n* used in respirometry calculations ($r^2 \geq 0.7$ for linear regression of log O_2 consumption vs. swimming speed). Values represent mean ± SEM.

Abbreviations: hpf; hours post-fertilization



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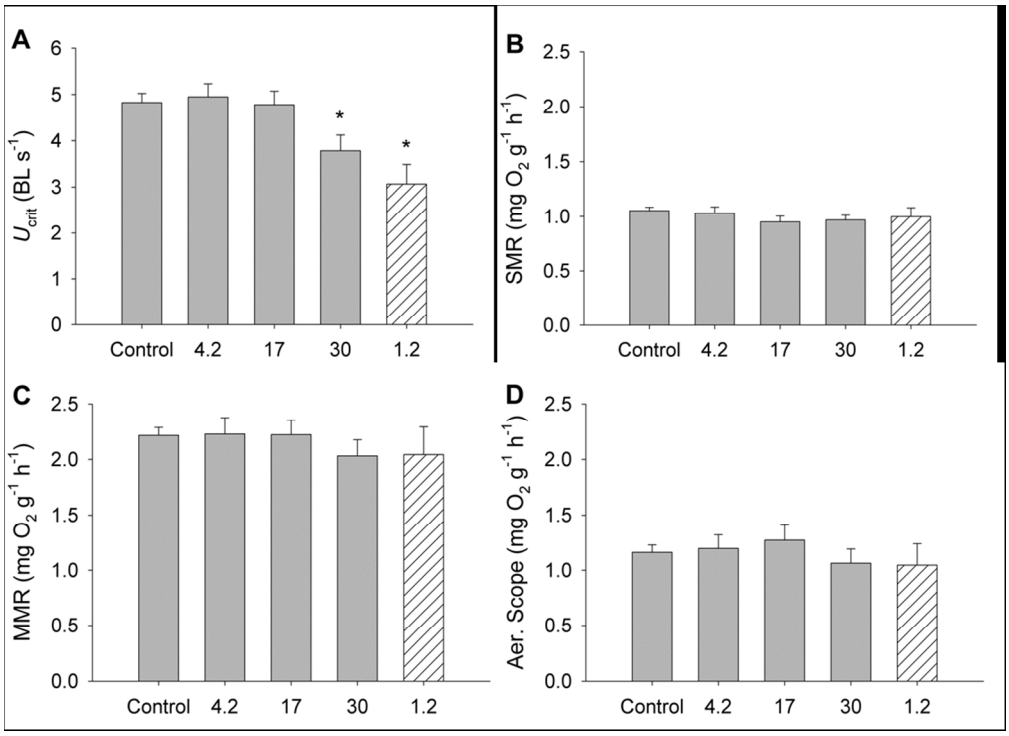

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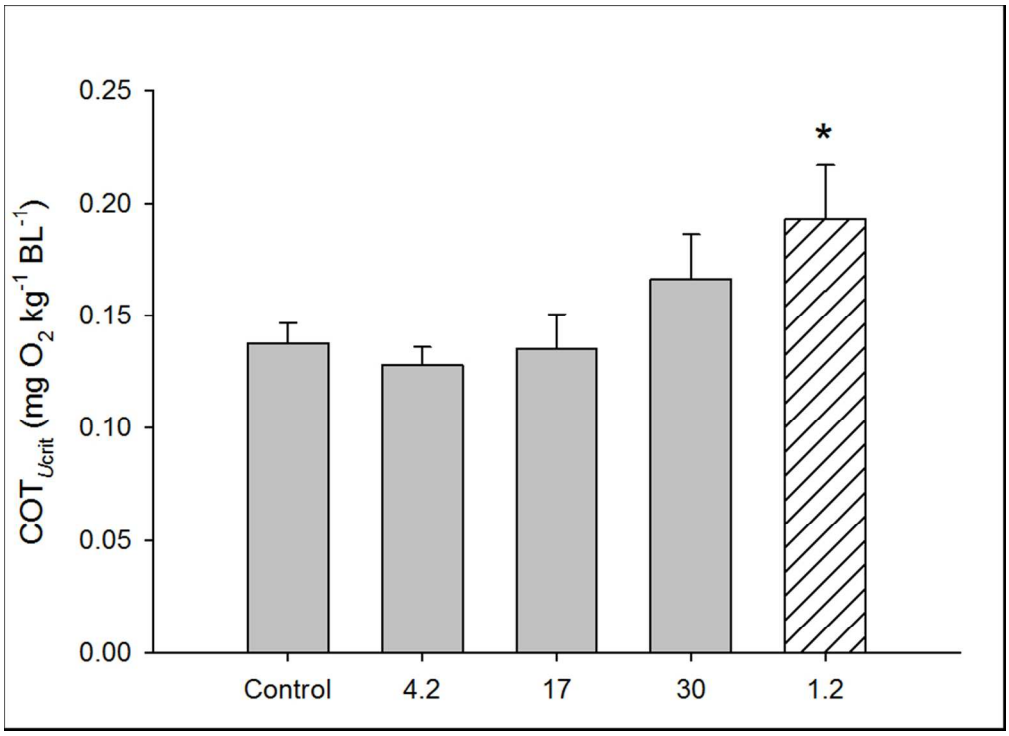

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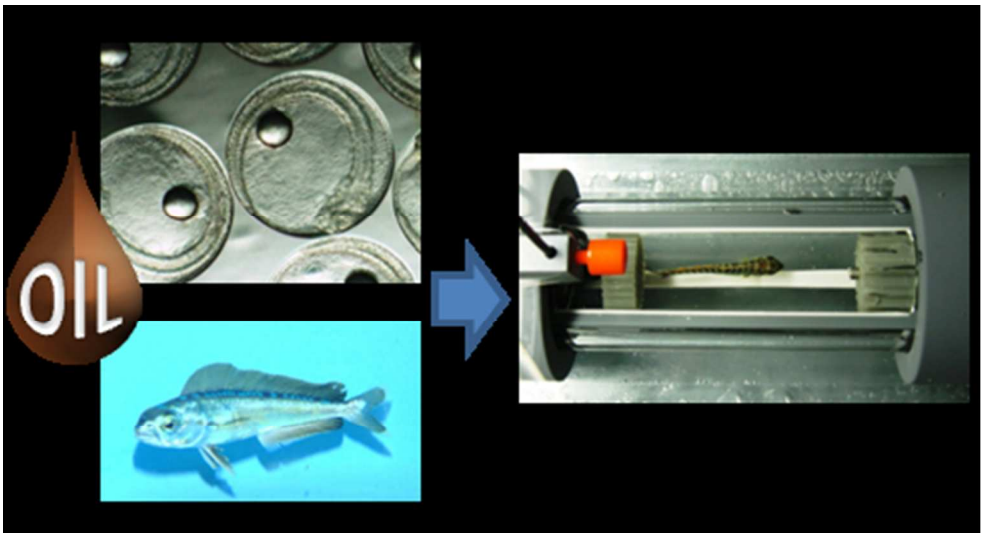

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