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EX5069-000001-TRB

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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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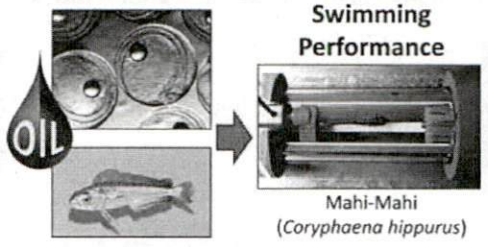
Supporting Information

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 days 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 $\mu g\ L^{-1}$ ΣPAHs (geometric mean ± SEM) to embryos/larvae that were then raised to juvenile stage or a 24 h exposure of 30 ± 7 $\mu g\ L^{-1}$ 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 ($6 \times 10^8$ 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.[14−28] 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 contravened a longstanding paradigm wherein lower molecular weight and more volatile 2-ring PAHs drive crude oil toxicity through acute and nonspecific narcosis.[13] Recent studies have also shown that the nervous system may be a target of oil toxicity in fish, as

Received: April 2, 2014
Revised: May 21, 2014
Accepted: May 23, 2014
Published: May 23, 2014

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EX5069-000002-TRB

heavy fuel oil exposure in marine fish embryos led to abnormal projections of cranial and peripheral nerves.[29,30] Although 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 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 Alaska 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 (noninduced) 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 $\mu$m 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 $\mu g\ L^{-1}$ ΣPAH) for 48 h and then raised for ~25 days 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 $\mu$m), UV-sterilized seawater. For the oil exposure, HEWAF was added when the tank was approximately one-third 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 postfertilization (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 posthatch (dph). Specifically, larvae were pulse-fed enriched rotifers (*Brachionus plicatilis*) and *Artemia* nauplii during the first 2–3 weeks posthatch, 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 $\mu g\ L^{-1}$ 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 27 °C 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 two fish that died within one of the 30 $\mu g\ L^{-1}$ ΣPAH replicates, no other mortalities were


dx.doi.org/10.1021/es501628k | Environ. Sci. Technol. 2014, 48, 7053–7061
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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 $\mu$g $L^{-1}$ 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 hand held 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 (Supporting Information Table S1; Figure S1).

For the 48 h embryo–larval exposures, ΣPAH samples and measurements for all 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 Supporting Information 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 (Supporting Information Figure 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 Fibox 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 fatigued using fixed velocity increments that, depending on size, ranged between 1 and 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 postswimming to minimize stress pretesting 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


dx.doi.org/10.1021/es501628k | Environ. Sci. Technol. 2014, 48, 7053–7061
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**Table 1. Biometric Data for Mahi-Mahi Used in Swimming Performance Tests[a]**

| 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 |

[a]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 postfertilization; dpf, days postfertilization.

each fish and a least-squares linear regression performed (see Supporting Information Figure 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; Supporting Information Figure 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 (Supporting Information 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 (Supporting Information Figure S1). Nominal doses of HEWAF produced nearly proportional changes in ΣPAHs, although this relationship appeared to weaken at lower doses (Supporting Information Figure S1). Initial C1-phenanthrene concentrations (a PAH class well represented in *DWH* crude oil; Supporting Information Figure S1) ranged from 4.6–0.33 μg $L^{-1}$ in the 2% and 0.2% HEWAF dilutions, respectively (Supporting Information 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 (Figure 1A). Pericardial area also increased nearly 2-fold in larvae that were previously exposed to HEWAF (Figure 1B; $P = 0.003$). Analysis of heart rates revealed greater variability in larvae exposed to HEWAF (Figure 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.

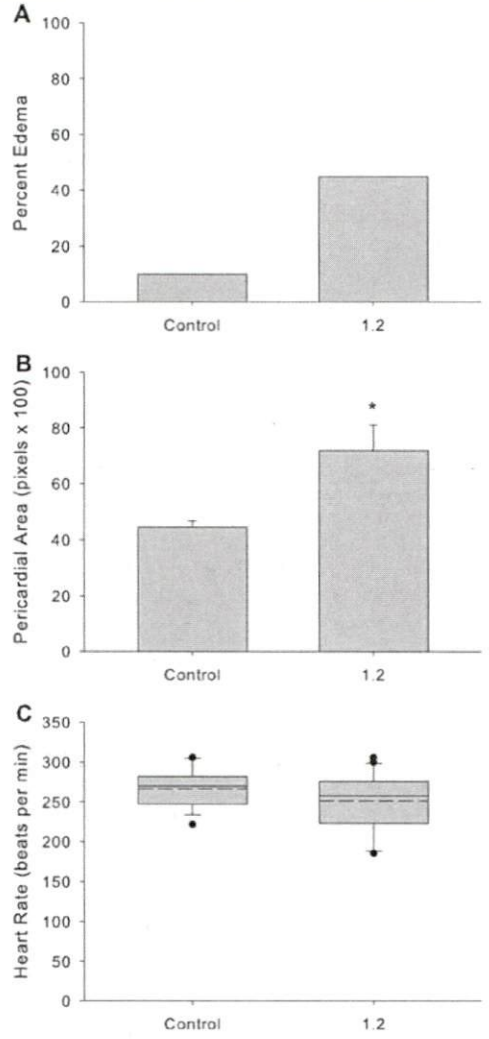


**Figure 1.** Evidence of cardiac toxicity in larval mahi-mahi exposed to 1.2 μg $L^{-1}$ ΣPAHs (geometric mean) for 48 h initiated <12 hpf ($n$ = 20 each, except $n$ = 19 for the oil-exposed group in (B)) as assessed by (A) percent incidence of pericardial edema, (B) pericardial area and (C) heart rate. 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.


dx.doi.org/10.1021/es501628k | Environ. Sci. Technol. 2014, 48, 7053–7061
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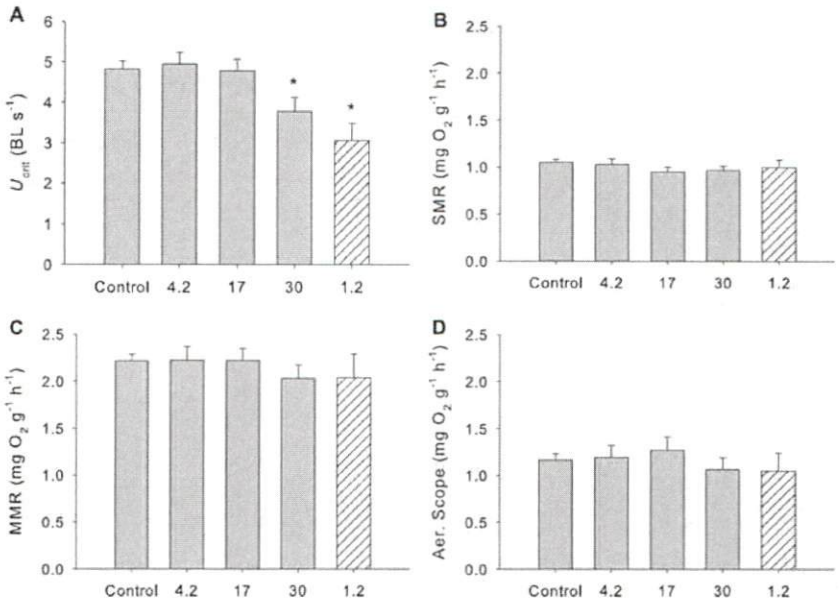


**Figure 2.** Comparisons of (A) critical swimming speed ($U_{crit}$), (B) standard metabolic rate (SMR), (C) maximum metabolic rate (MMR), and (D) aerobic scope from swimming respirometry experiments of juvenile mahi-mahi exposed to 4.2, 17, or 30 $\mu$g L$^{-1}$ ΣPAHs ($n$ = 7, 9, and 8, respectively) for 24 h prior to swimming (shaded bars) or to 1.2 $\mu$g L$^{-1}$ ΣPAHs ($n$ = 5) for 48 h initiated <12 hpf (hatched bar) and then raised for ~25 days to the juvenile stage. The control group represents fish from all four 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.

**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 $\mu$g L$^{-1}$ ΣPAH as embryos and larvae. By comparison, among mahi-mahi exposed as juveniles, only the highest concentration (30 $\mu$g L$^{-1}$ ΣPAH) elicited a significant decrease of 22% ($P < 0.05$) (Figure 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 (Figure S5).

No significant effect on SMR, MMR, or aerobic scope was evident for the fish exposed to 1.2 $\mu$g L$^{-1}$ ΣPAH as embryos/larvae and then tested as juveniles ~25 days later (Figure 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 $U_{crit}$ (Figure 3). Similarly, no significant effect on SMR, MMR or aerobic scope was evident for the fish exposed to HEWAF as juveniles (Figure 2). Again, however, there was an upward trend in $COT_{Ucrit}$ (21%, $P$ = 0.147) at the 30 $\mu$g L$^{-1}$ ΣPAH concentration that closely mirrored the 22% decrease in $U_{crit}$ (Figure 3).

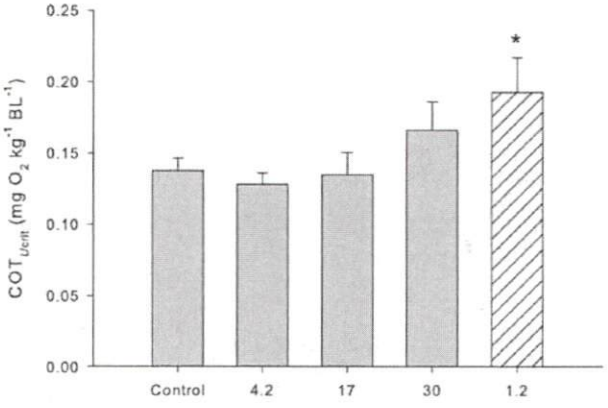


**Figure 3.** Cost of transport at $U_{crit}$ for juvenile mahi-mahi exposed to 4.2, 17, or 30 $\mu$g L$^{-1}$ ΣPAHs ($n$ = 7, 9, and 8, respectively) for 24 h prior to swimming (shaded bars) or to 1.2 $\mu$g L$^{-1}$ ΣPAHs ($n$ = 5) for 48 h initiated <12 hpf (hatched bar) and then raised for ~25 days to the juvenile stage. The control group represents fish from all four 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.

## ■ 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 $\mu$g L$^{-1}$. By contrast, a nearly 30-times higher, yet still environmentally relevant, concentration of 30 $\mu$g L$^{-1}$ ΣPAH was required to elicit a similar decrease in $U_{crit}$ for fish exposed for 24 h as juveniles. Although these results are consistent with an expected decrease in sensitivity with increasing developmental stage, they also support the suggestion that PAH exposure


dx.doi.org/10.1021/es501628k | Environ. Sci. Technol. 2014, 48, 7053−7061
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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 $\mu g\ L^{-1}$ ΣPAHs, likely represent environmentally realistic exposure scenarios. For example, Bejarano et al. reported a range of <0.01−77 $\mu g\ L^{-1}$ ΣPAHs from samples collected at 1 and 10 m depths[44] and other studies have reported similar values ranging as high as 59 $\mu g\ L^{-1}$ and 85 $\mu g\ L^{-1}$ 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 $\mu g\ L^{-1}$ for C1-phenanthrene, a value near the initial concentration of 0.33 $\mu g\ L^{-1}$ 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 $\mu g\ L^{-1}$ 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 $U_{crit}$. First, $U_{crit}$ 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) or an effect that adds to the basal metabolic load and thus increases SMR (e.g., specific dynamic action, metabolism of xenobiotics, or increased ionoregulatory costs).[49] Second, glycolytically fuelled white muscles may be recruited to sustain high swimming speeds approaching and including $U_{crit}$. Thus, any impairment to this recruitment may also limit $U_{crit}$.[50] Finally, there is evidence that behavior plays a role in setting swimming speed when fish cannot generate or sense a ground speed, a particular concern when using swim tunnels where forward movement is greatly limited.[50] Although each of these potential mechanisms could conceivably play a role in reducing the $U_{crit}$ of oil-exposed fish in our experiments, we chose to focus our investigation on the first mechanism of diminished aerobic scope in light of the well-known cardiotoxic effects of PAHs.

A recent study revealed that zebrafish that survived to adulthood following a 48 h embryonic exposure to ANSCO (24−36 $\mu g\ L^{-1}$ ΣPAHs) developed defects in ventricular shape likely to impair cardiac output.[16] Consistent with this, swim trials with the affected adult zebrafish revealed an 18% decrease in $U_{crit}$, thereby supporting a link between impaired cardiac output, reduced aerobic scope, and impaired swimming performance.[16] However, respirometry was not performed in the zebrafish trials, and we did not examine the hearts of juvenile mahi-mahi for permanent changes in heart shape in response to embryonic PAH exposure. Nevertheless, larval evidence for developmental cardiotoxicity (pericardial and yolk sac edema) following the 48 h exposure period would suggest that subsequent reductions in juvenile swimming performance might be a consequence of a reduced aerobic scope, with a diminished cardiac output limiting MMR. Yet, our respirometry results did not clearly reveal a reduced MMR as the underlying mechanism for the impaired swimming ability of juvenile mahi-mahi. Although direct measurements of cardiac performance were not made, the lack of an effect on 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


dx.doi.org/10.1021/es501628k | Environ. Sci. Technol. 2014, 48, 7053−7061
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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 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, an intact lateral line is necessary for efficient swimming,[54] and an oil-induced abnormality in lateral line development could underlie the observed changes in cost of transport. Taken together, these considerations 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 $\mu g\ L^{-1}$ 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.

## ■ ASSOCIATED CONTENT

### Supporting Information

Further information is available that provides PAH compositions and measurements (Tables S1 and 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 nonmass scaled metabolic rate and COT data (Figure S5). This material is available free of charge via the Internet at http://pubs.acs.org/.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors would like to thank Dr. Anthony Farrell for his insightful review and suggestions for the manuscript, as well as Stratus Consulting for their valuable assistance and feedback, Kathleen Munley and Maria Rodgers for their technical support, and the students and staff at the UMEH for their assistance in the culture of mahi-mahi used in this study. This work was supported by funds provided as part of the Natural Resource Damage Assessment (NRDA) for the *DWH* oil spill. Data presented here are a subset of a larger toxicological database that is being generated as part of the *DWH* NRDA, therefore, these data will be subject to additional analysis and interpretation, which may include interpretation in the context of additional data not presented here.

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