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IMPACTS OF *DEEPWATER HORIZON* CRUDE OIL EXPOSURE ON ADULT MAHI-MAHI (*CORYPHAENA HIPPURUS*) SWIM PERFORMANCE

JOHN D. STIEGLITZ,* EDWARD M. MAGER, RONALD H. HOENIG, DANIEL D. BENETTI, and MARTIN GROSELL
Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida, USA

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Abstract: The temporal and geographic attributes of the *Deepwater Horizon* incident in 2010 likely exposed pelagic game fish species, such as mahi-mahi, to crude oil. Although much of the research assessing the effects of the spill has focused on early life stages of fish, studies examining whole-animal physiological responses of adult marine fish species are lacking. Using swim chamber respirometry, the present study demonstrates that acute exposure to a sublethal concentration of the water accommodated fraction of *Deepwater Horizon* crude oil results in significant swim performance impacts on young adult mahi-mahi, representing the first report of acute sublethal toxicity on adult pelagic fish in the Gulf of Mexico following the spill. At an exposure concentration of $8.4 \pm 0.6\ \mu g\ L^{-1}$ sum of 50 selected polycyclic aromatic hydrocarbons (PAHs; mean of geometric means $\pm$ standard error of the mean), significant decreases in the critical and optimal swimming speeds of 14% and 10%, respectively ($p < 0.05$), were observed. In addition, a 20% reduction in the maximum metabolic rate and a 29% reduction in aerobic scope resulted from exposure to this level of ΣPAHs. Using environmentally relevant crude oil exposure concentrations and a commercially and ecologically valuable Gulf of Mexico fish species, the present results provide insight into the effects of the *Deepwater Horizon* oil spill on adult pelagic fish. *Environ Toxicol Chem* 2016;9999:1–10. © 2016 SETAC

Keywords: *Deepwater Horizon* Oil spill Environmental toxicology Swim performance Fish indices

INTRODUCTION

The Gulf of Mexico supports one of the most prolific marine fisheries in the United States. A number of high-value and ecologically important marine fish species, such as mahi-mahi (*Coryphaena hippurus*) or dolphin fish, utilize the variety of Gulf of Mexico habitats for many or all life stages [1–4]. Mahi-mahi are well known worldwide to commercial and recreational fishermen as well as seafood consumers. The *Deepwater Horizon* oil spill in the summer of 2010 released over 3 million barrels of crude oil into the Gulf of Mexico over the course of 87 d [5] and likely impacted native Gulf of Mexico pelagic fish such as mahi-mahi [6,7]. The impacts caused by exposure of fish early life stages to *Deepwater Horizon* crude oil include disruptions to cardiac form and function, as well as a host of other ontogenetic developmental defects [6,7] that are consistent with those well documented for a number of other teleosts and oil types [8–13]. Such negative impacts are of great concern for apex pelagic predatory species, such as mahi-mahi, that possess high metabolic demands to support robust feeding and migratory abilities needed for survival, growth, and reproduction in the oceanic pelagic environment [14–17]. Because of the positive scaling of aerobic scope with size [18] as well as the increased metabolic capabilities and reduced cumulative body burdens of pollutant exposure in larger fish, it is generally thought that adult individuals may be able to withstand higher levels of environmental stressors than those at early life stages. To test the hypothesis that whole-animal physiological impacts of sublethal *Deepwater Horizon* crude oil exposure extend to later–life stage pelagic fish, swim performance and swimming efficiency studies were conducted on adult mahi-mahi following transient exposure to *Deepwater Horizon* crude oil. Swim performance studies of fish have been used to reveal the sublethal impacts of a number of environmental stressors [19–21] including marine pollution events such as crude oil spills [6,22,23] that lead to exposure to toxic polycyclic aromatic hydrocarbons (PAHs). When combined with respirometric analysis, swim performance studies allow for determination of aerobic scope, which is defined as the energy available for sustained activity. Through analysis of aerobic scope, loading and limiting stressors on animals can be revealed which may otherwise not be apparent in traditional impact quantification efforts. In addition, given the suggestion that reductions in swim performance following transient crude oil exposure to juvenile mahi-mahi (mean mass 0.40–0.81 g) [6] may result at least partially from a reduced swimming efficiency [6,24], video analysis of swimming kinematics was incorporated to look at a potential contribution of this effect. Given the long migrations and vast areas covered by these animals in search of prey and spawning sites, sublethal impacts to swim performance may have significant impacts on stocks of these high-value species.

MATERIALS AND METHODS

Experimental animals

Mahi-mahi embryos were obtained as described [6] from wild adult broodstock spawning volitionally in 80-m^3 seawater tanks at the University of Miami Experimental Hatchery and raised to young adult stages. All mahi-mahi used in the present study were maintained in flow-through tanks and fed daily a diet of freshly thawed natural prey (squid, sardines, and silversides), along with regular additions of vitamin and mineral supplements. Fish used in the swim study were randomly selected from the holding tank and briefly examined to make sure there were no visible injuries or damage to the caudal fin. Following use in

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* Address correspondence to jstieglitz@rsmas.miami.edu

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the swim study, necropsies were performed on each fish to confirm the lack of any external or internal abnormalities.

Preparation of water accommodated fractions

The crude oil used in the present study (referred to as "slick A"; sample ID CTC02404-02) was collected at the site of the *Deepwater Horizon* oil spill on 29 July 2010 from a barge (CTC02404) receiving oil from a number of skimmer vessels and subsequently transferred under custody to the University of Miami. High-energy water accommodated fraction (HEWAF) preparations were made within 24 h of the start of each exposure period and prepared as described [6] using a loading rate of 1 g of oil/L of 1 μm filtered, ultraviolet (UV)-sterilized seawater. The resulting slick A HEWAF preparations used in the present study had proportional PAH characterizations nearly identical to those reported in the supporting information section of Mager et al. [6] (Supplemental Data, Figure S3).

Mahi-mahi 24-h exposures

The static exposure methods used in the present study are based on those described by Mager et al. [6]. Exposures were conducted in 2500-L cylindrical fiberglass tanks at the University of Miami Experimental Hatchery with a total exposure volume of approximately 360 L to 900 L, depending on exposure concentration. The test medium was natural, filtered, and UV-sterilized seawater. On the day of each exposure, the treatment tank was filled with seawater, after which a circulation pump (Danner Supreme Mag Drive Pump, model MD12) attached to the center drain outflow pipe was turned on to generate a directional current of approximately 1 body length s^{-1} within the treatment tank to facilitate ram ventilation by the fish. A short center standpipe was used in each tank to reduce the chance of fish becoming trapped in the bottom drain, and a light amount of aeration was provided at the base of the standpipe to maintain dissolved oxygen (DO) levels at or near saturation. Individual fish were exposed to control seawater or nominal HEWAF dilutions of 0.4% and approximately 1.7% for 24 h. Exposures were administered by adding HEWAF to the treatment tank following initiation of the directional current. After a short period of mixing (~5 min), fish were randomly selected from the holding tank using a long-handled net and immediately placed into the treatment tank. Mortalities during the 24-h exposure were minimal and only occurred at the highest concentration (3 individuals at the 1.7% dilution). The total number for each of the 3 treatment groups is listed in Table 1. Because of the rapid growth of mahi-mahi, fish remained at the desired test size (~250 g) for only a matter of a week or 2. Consequently, a total of 5 different cohorts of mahi-mahi were used for testing, with at least 1 individual from each cohort tested for control performance to minimize the potential for confounding factors because of batch variability. Fish were fed on the morning of each exposure in their holding tank, approximately 2.5 h to 3 h prior to exposure, and were not fed during the 24-h exposure period.

Water quality and ΣPAH analysis

Water quality parameters measured at the beginning and end of each exposure period for each replicate were as follows: ΣPAH, temperature, DO, pH, total ammonia, and salinity. The initial (0 h) water samples for ΣPAH analysis were obtained following the HEWAF addition and short mixing period in the treatment tank, and the final samples were obtained just prior to transfer of the fish to the swim chamber respirometer. Samples for ΣPAH analysis were collected in 250-mL amber glass bottles that were shipped on ice overnight to ALS Environmental for analysis by gas chromatography/mass spectrometry with selective ion monitoring (GC/MS-SIM, according to US Environmental Protection Agency method 8270D). The reported ΣPAH(50) values represent the sum of 50 selected PAH analytes (Supplemental Data, Table S2). Given the number of individual exposures used in the present study, whereby each fish was exposed individually prior to each swim performance test, the results of the GC-MS/SIM water chemistry analysis for each treatment group were analyzed statistically to determine if there were any outliers in the water chemistry data. Individuals with water chemistry results deemed to be significant outliers ($p < 0.05$, Grubb's test) were removed from further analysis, and no data for these individuals are included in any of the following analyses. Salinity was measured using a refractometer, and pH was measured using a PHM201 meter (Radiometer) fitted with a glass electrode. Water temperature and DO were measured using a ProODO handheld optical DO probe and meter (YSI), and total ammonia was measured using a colorimetric assay [25].

Swimming performance

A 90-L Brett-type swim tunnel respirometer and AutoR-esp™ 2.1.0 software (Loligo Systems ApS) were used to assess swim performance. Using this system, a critical swim velocity (U_{crit}) test was performed [26], and oxygen consumption (MO_2) was measured with a Pt100 fiber-optic probe connected to a Fibox 3 minisensor oxygen meter (PreSens Precision Sensing) using intermittent respirometry (20-min measurement periods). Details on swim tunnel calibration methods used in the present study can be found in the Supplemental Data.

Following the 24-h exposure period, fish were gently transferred and acclimated to the swim tunnel by introducing a slow water flow to encourage swimming while they were manually prevented from contacting the sides. Once the fish

Table 1. Treatment group exposure concentrations, water temperatures, and biometric data for mahi-mahi used in the present study[a]

Treatment	ΣPAH(50) (μg L^{-1})	Water temperature (°C)	*n*	Mass (g)	Fork length (cm)	Age (d posthatch)
Control	0.09 ± 0.01 (0.05 ± 0.01)	27.9 ± 0.5	16 (16^a;14^b;6^c)	278 ± 23	29.1 ± 0.8	129 ± 10
0.4% HEWAF	2.30 ± 0.10 (7.33 ± 0.35)	28.9 ± 0.3	7 (7^a;6^b;0^c)	196 ± 9	26.5 ± 0.5	119 ± 10
1.7% HEWAF	8.40 ± 0.59 (24.19 ± 1.35)	26.6 ± 0.4	18 (15^a;17^b;16^c)	298 ± 15	30.7 ± 0.6	121 ± 5

[a]Values are expressed as mean ± standard error of the mean. Only individuals with exponential (standard metabolic rate, maximum metabolic rate) or polynomial (cost of transport) regression r^2 values ≥0.7 were used for the respective analyses. In column *n*, numbers in parentheses indicate the number of fish from each treatment group used for [a]metabolic rate (standard metabolic rate, maximum metabolic rate), [b]cost of transport, and [c]kinematics (tail beat frequency, stride length) analyses. The ΣPAH(50) values represent the mean of the geometric means of initial and final exposure concentrations, with values in parentheses indicating only the initial exposure concentrations.

HEWAF = high-energy water accommodated fraction; ΣPAH(50) = sum of 50 selected polycyclic aromatic hydrocarbons.

were swimming steadily in the chamber at a slow speed without contacting the sides, the lid of the swim tunnel working section was bolted closed and MO_2 measurements were initiated during the acclimation period. When necessary during the early portion (i.e., initial few measurement periods or "loops") of the acclimation period, supplemental O_2 was used to rapidly reestablish approximately 95% to 100% O_2 saturation in the water following MO_2 measurement intervals to minimize the time that acclimating fish spent in <95% O_2 saturated water. The brief use of O_2 aided in the recovery of the fish following handling, after which ambient aeration in the buffer tank was used for the remainder of the acclimation period and during the U_{crit} swim performance test. The duration of the acclimation period was determined by conducting a preliminary experiment in which young adult mahi-mahi were fed, isolated in the control treatment tank for 24 h, and transferred to the swim tunnel using the methods described previously. The MO_2 of the fish was monitored for 18 h to 24 h as the fish swam at a low acclimation speed (~0.6 body lengths s^{-1}). Results indicate that MO_2 stabilizes after approximately 3 h, and thereafter MO_2 readings are interpreted as routine metabolic rate (Supplemental Data, Figure S1), defined as the average energy utilization at a minimal swim speed following acclimation. Therefore, all fish used in swimming performance tests were acclimated for a minimum of 2.5 h to 3 h, with the ramped speed portions of the U_{crit} test not commencing until acclimation MO_2 readings were within approximately 10% of each other over 2 consecutive 20-min measurement periods. A custom-built enclosure for the entire swim tunnel respirometer allowed for stable experimental conditions within the chamber at all times, while eliminating disturbance from monitoring activities. During the U_{crit} test, fish were monitored in real time using 2 different camera angles, as well as by peering into an opening at the top rear portion of the enclosure that was not visible to the fish.

Following the acclimation period, water flow velocity was increased approximately 0.5 body lengths s^{-1} (12–13 cm s^{-1}) every 20 min until the fish fatigued. Fatigue was confirmed using video analysis and defined as the point at which the fish began to continuously rest on the rear screen of the swim tunnel, pushed off the rear screen in a burst-and-glide form of locomotion, or became pinned sideways against the rear screen and unable to recover to normal swimming behavior. Following completion of the swim test, the fish were euthanized by tricaine methanesulfonate (MS-222) overdose, after which the mass (grams) and fork length in centimeters were obtained. Using the equation described by Brett [26], U_{crit}, expressed in body lengths s^{-1}, was calculated:

$$U_{crit} = [U_f + (T/t)dU]/\text{cm for k length}$$

where U_f (centimeters per second) is the highest swim velocity maintained for a full interval, T (seconds) is the time spent at the final velocity, t is the time interval (seconds), and dU is the increment in swim speed (centimeters per second). Not all fish completed the swim performance test successfully as a result either of handling-induced mortality occurring during the exposure period (11% of fish exposed to 1.7% HEWAF and 0 mortalities in either of the other 2 treatments) or of failure to acclimate to the swimming chamber, which occurred for fish in both the control and the 1.7% HEWAF treatment groups (6% and 11% of fish, respectively). Within the 1.7% HEWAF treatment group, an additional 11% of exposed fish acclimated to the swim chamber but were unable to complete the U_{crit} test given that they only completed a partial swim interval above acclimation speed. Such fish are not included in any of the following analyses.

Metabolic rates and aerobic scope

The combination of MO_2 (milligrams of O_2 per kilogram per hour) data at each swim speed and determination of U_{crit} allowed for calculation of aerobic scope. Aerobic scope is defined as the difference between the maximum metabolic rate and the standard metabolic rate (see Mager et al. [6] for definition of these parameters). The MO_2 data were log-transformed and plotted versus swim speed (body lengths per second) to obtain these values [6]. Given that empirical data of this type have been modeled in fish swim performance studies using both least squares linear regression and exponential regression [27], we performed both modeling analyses to determine which best fit the data (highest r^2 values). The exponential regression best modeled the data for 74% of the individuals compared to the linear regression (26%), though differences in r^2 were minor between the 2 methods. To maintain uniformity in analytical methods between individuals, the exponential regression was used and the standard metabolic rate (y intercept) and maximum metabolic rate (extrapolated MO_2 value at U_{crit}) were derived from the resulting equation. As outlined by Mager et al. [6], only individuals with regression $r^2 \geq 0.7$ were used for the aerobic scope analysis. Because metabolic rate is known to scale with mass and there was variation in the body sizes of fish used in the present study, the standard metabolic rate and maximum metabolic rate data for each individual were normalized for the effect of mass by scaling all such values to a standard mass of 250 g before calculating aerobic scope. To eliminate the influence of treatment effects on metabolic allometric scaling relationships, only data from the control treatment group were used to generate the metabolic scaling coefficients (Supplemental Data, Figure S2). Standard metabolic rate, maximum metabolic rate, and aerobic scope were estimated for each individual fish; and mean values of each parameter for each treatment group are presented in *Results*.

Cost of transport

The energetic expense of movement over a distance (milligrams of O_2 per kilogram per meter), termed the cost of transport, was quantified for each individual by dividing the MO_2 by swimming velocity at each velocity increment. The resulting parabola-shaped plot was fit to a second order ($k = 2$) polynomial regression model and used to determine the cost of transport at U_{crit}. In addition, the model was used to calculate the optimal swimming speed (U_{opt}), which is the speed at which swimming required the minimum cost of transport and was determined by fitting the first derivative of the polynomial model equation to 0 [28]. Only individuals with regression $r^2 \geq 0.7$ were used for the cost of transport analysis (Table 1). As described in the previous section, *Metabolic rates and aerobic scope*, minimum cost of transport and cost of transport at U_{crit} data were scaled to a standard fish mass of 250 g using only data from the control group to generate the metabolic scaling coefficients (Supplemental Data, Figure S2).

Swimming kinematics

A subsample of fish from the 1.7% HEWAF and control treatments (see Table 1 for n) was analyzed for tail beat frequency and stride length using 30 frames/s video recordings (GoPro Hero 2) of individual fish at different swim speed

intervals. Tail beat was defined as 1 complete oscillation of the tail. Frequency was determined by completing 3 separate analyses of 5 beat intervals, whereby the time (tenths of a second) to complete 5 tail beats was quantified for each interval. The number of tail beats (5) was then divided by the average of the 3 times to obtain tail beat frequency in beats per second, expressed as hertz. The distance traveled per tail beat, also known as stride length, was calculated as the swim speed (centimeters per second) divided by the tail beat frequency and is expressed as fractions of the body length of each individual fish. Tail beat frequency and stride length values for each treatment group are presented as mean ± standard error of the mean (SEM).

Statistical analysis

All data are presented as mean ± SEM. Statistical differences were analyzed using either one-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA), with differences between treatment groups determined with appropriate post hoc tests noted specifically in each section. Outliers within individual treatment groups were detected using the Grubbs outlier test ($\alpha = 0.05$). Statistical analysis was performed using XLSTAT (Ver 2014.3.02; Addinsoft). Values were considered significantly different at $p < 0.05$.

RESULTS

Experimental animals, water quality, and PAH exposures

The GC-MS/SIM analyses revealed concentrations of ΣPAHs in the 0.4% and 1.7% slick A HEWAF treatments of $2.3 \pm 0.1\ \mu g\ L^{-1}$ and $8.4 \pm 0.6\ \mu g\ L^{-1}$ ΣPAH(50), respectively (represented as means ± SEMs of the geometric means of the initial and final ΣPAH(50) concentrations from each individual exposure; Table 1). The primary groups used in analyses are the control and $8.4\ \mu g\ L^{-1}$ ΣPAH(50) treatment groups because of temperature and size variation in the $2.3\ \mu g\ L^{-1}$ ΣPAH(50) treatment group. There were no significant differences in size (mass and length) and water temperature conditions between the control and 1.7% HEWAF treatment groups. At the $2.3\ \mu g\ L^{-1}$ ΣPAH(50) exposure, the fish were smaller than those in the other treatment groups (ANOVA $F_{(2,38)} = 5.241$, $p = 0.01$; Tukey's honestly significant difference post hoc test $p < 0.05$), and water temperatures during the swim performance analyses of these fish were warmer (ANOVA $F_{(2,38)} = 9.682$, $p < 0.001$; Tukey's honestly significant difference post hoc test $p < 0.01$) compared to the other treatment groups (Table 1).

Swimming performance

Swimming performance, measured as U_{crit}, of the $2.3\ \mu g\ L^{-1}$ ΣPAH(50) treatment group (3.94 ± 0.35 body lengths s^{-1}) was similar to that of the control treatment group (4.08 ± 0.12 body lengths s^{-1}). However, the U_{crit} of the $8.4\ \mu g\ L^{-1}$ ΣPAH(50) treatment group was reduced by 14% (3.51 ± 0.14 body lengths s^{-1}) compared to controls (ANOVA $F_{(2,38)} = 3.706$, $p = 0.034$; Tukey's honestly significant difference post hoc test $p < 0.031$; Figure 1). Similarly, there was no apparent effect of the $2.3\ \mu g\ L^{-1}$ ΣPAH(50) treatment on U_{opt} (2.80 ± 0.18 body lengths s^{-1}) compared to the control group (2.80 ± 0.09 body lengths s^{-1}), yet at the higher concentration ($8.4\ \mu g\ L^{-1}$ ΣPAH(50)), the U_{opt} was decreased by 10% to 2.52 ± 0.07 body lengths s^{-1} compared to controls (ANOVA $F_{(2,34)} = 3.263$, $p = 0.05$; Dunnett's post hoc test $p = 0.035$; Figure 1 and Figure 3).

Metabolic rates and aerobic scope

There was no difference in standard metabolic rate among treatment groups (ANOVA $F_{(2,35)} = 1.607, p = 0.215$; Figure 2). However, there was a decrease in maximum metabolic rate for the $8.4\ \mu g\ L^{-1}$ ΣPAH(50) treatment group ($1327 \pm 75\ mg\ O_2\ kg^{-1}\ h^{-1}$) compared to controls ($1652 \pm 78\ mg\ O_2\ kg^{-1}\ h^{-1}$; ANOVA $F_{(2,35)} = 4.129$, $p = 0.025$; Tukey's honestly significant difference post hoc test $p = 0.028$; Figure 2). This reduction in maximum metabolic rate contributed to a reduced aerobic scope ($849 \pm 85\ mg\ O_2\ kg^{-1}\ h^{-1}$) compared to controls ($1194 \pm 77\ mg\ O_2\ kg^{-1}\ h^{-1}$; ANOVA $F_{(2,35)} = 3.814$, $p = 0.032$; Tukey's honestly significant difference post hoc test $p = 0.027$; Figure 2).

Cost of transport

No impact of transient crude oil exposure on cost of transport at U_{crit} was observed. The minimum cost of transport was highest in the $2.3\ \mu g\ L^{-1}$ ΣPAH(50) treatment ($0.38 \pm 0.04\ mg\ O_2\ kg^{-1}\ m^{-1}$; ANOVA $F_{(2,34)} = 5.062$, $p = 0.012$; Tukey's honestly significant difference post hoc test $p < 0.05$), whereas the mean minimum cost of transport values in the control and the $8.4\ \mu g\ L^{-1}$ ΣPAH(50) treatment groups were identical ($0.29 \pm 0.02\ mg\ O_2\ kg^{-1}\ m^{-1}$ and $0.29 \pm 0.01\ mg\ O_2\ kg^{-1}\ m^{-1}$, respectively; Figure 3).

Swimming kinematics

Measurements of tail beat frequency and stride length in a subsample of control and $8.4\ \mu g\ L^{-1}$ ΣPAH(50) treatment group fish revealed no effects ($p > 0.05$, ANCOVA; Figure 4). Both groups exhibited linear increases in tail beat frequency with increasing swimming velocity, whereas stride length followed a parabolic pattern with the highest values, expressed as body length per tail beat, in the midrange of swimming velocities.

DISCUSSION

Given the spatiotemporal aspects of the *Deepwater Horizon* oil spill and the documented contamination of the Gulf of Mexico pelagic environment [3,5,29–33], top trophic level pelagic species such as mahi-mahi encountered transient crude oil exposures well above those tested in the present study. Field samples of *Deepwater Horizon* crude oil ΣPAH concentrations in the pelagic environment have been reported as high as $85\ \mu g\ L^{-1}$ ΣPAH [34,35], which is 10 times greater than the high experimental dose used in the present study. Furthermore, the *Deepwater Horizon* slick A HEWAFs used in the present study are virtually identical in composition to those of previous studies and samples obtained from the Gulf of Mexico during the spill [6,7]. Therefore, the present study's findings that swimming performance (U_{crit}), optimal swim speed (U_{opt}), maximum metabolic rate, and aerobic scope were significantly decreased in the $8.4 \pm 0.6\ \mu g\ L^{-1}$ ΣPAH(50) treatment are relevant to the PAH exposure conditions experienced in the Gulf of Mexico during the *Deepwater Horizon* spill. These findings add to previous work suggesting that juvenile and early life stages of pelagic fish were negatively impacted by the oil spill [6,36,37]. It should be noted that when comparing the total ammonia levels observed during oil exposure in the present study (Supplemental Data, Table S1) on the basis of NH_3, the toxic form of ammonia/ammonium was approximately 1% of the mean acute ammonia toxicity values for marine fish [38] and thus, with little uncertainty, did not contribute to reduced swimming performance.

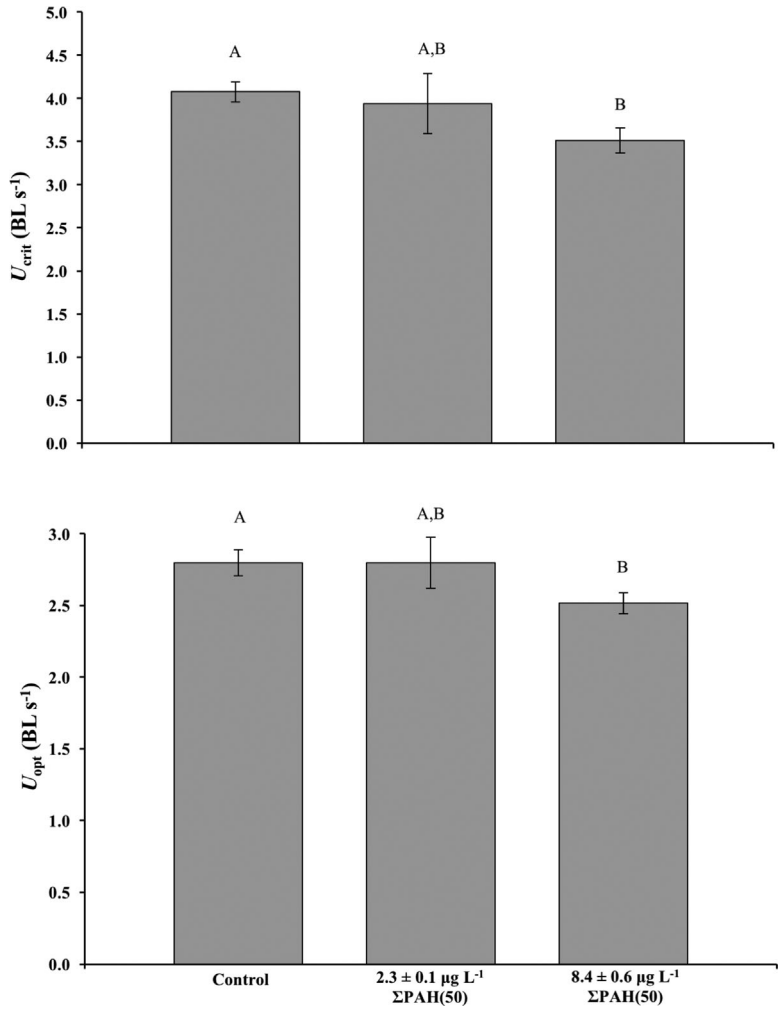

Figure 1. Exposure to *Deepwater Horizon* crude oil reduced swimming performance (critical swimming speed and optimal swimming speed). Different letters indicate significant differences between treatment groups ($p < 0.05$) for each respective swim performance parameter. BL = body lengths; ΣPAH(50) = sum of 50 selected polycyclic aromatic hydrocarbons; U_{crit} = critical swimming speed; U_{opt} = optimal swimming speed.

There is extensive literature documenting the importance of focusing on PAHs when investigating crude oil toxicity in fish. This class of hydrocarbons is extremely toxic to aquatic organisms, especially fish early life stages, even at relatively low levels of exposure [11,12,39–42]. In particular, 3-ring PAHs are known to cause a suite of cardiotoxic effects in fish embryos [40,43]. The slick A HEWAFs used in the present study (Supplemental Data, Figure S3) and other recent studies have an increased proportion of 3-ring PAHs, relative to less weathered or source *Deepwater Horizon* crude oil [6,7,44]. Although volatile organic compounds such as benzene, toluene, ethylbenzene, and xylenes and the lower–molecular weight PAHs have historically been associated with hydrocarbon narcosis [45,46], these compounds are gradually lost during the weathering process of crude oil and greatly reduced in the slick A HEWAFs used in the present study and other recent studies [6,7,36,44]. Therefore, the swim performance and metabolic effects seen in the present study are likely not reflective of a narcosis response but instead attributable to the toxicity of nonvolatile and higher–molecular weight compounds, possibly the 3-ring PAHs, found in the *Deepwater Horizon* slick A HEWAFs, as has been documented for earlier life stages of native Gulf of Mexico pelagic fish [6,7,36].

To date, many of the efforts aimed at investigating the effects of the *Deepwater Horizon* spill on high-value pelagic species have focused on the early life stages [6–8], whereas impacts to later life stages have been more challenging to quantify accurately. Interestingly, when compared with juvenile mahi-mahi (0.4–0.8 g, 28–37 d posthatch) exposed and tested under similar conditions as those employed in the present study, the larger young adult mahi-mahi appear more sensitive because a significant decrease in U_{crit} occurs at 8.4 µg L^{-1} ΣPAH(50) versus 30 µg L^{-1} ΣPAH(50) for juveniles [6]. However, the magnitude of the decrease in U_{crit} is approximately 14% at the 8.4 µg L^{-1} ΣPAH(50) exposure concentration used in the present study compared to a 22% decrease in U_{crit} observed at the 30 µg L^{-1} ΣPAH(50) exposure concentration [6]. The lack of significant impacts to U_{crit} on juvenile mahi-mahi exposed to similar concentrations [6] may simply reflect the fact that aerobic scope positively scales with fish mass, thereby allowing aerobic scope impacts to be more easily detected in the larger young adult mahi-mahi of the present study. Furthermore, the absence of significant impacts in cost of transport at U_{crit} of the oil-exposed young adult mahi-mahi in the present study echo the lack of significant impacts seen in this parameter following acute 24-h *Deepwater Horizon* crude oil exposure at the juvenile stage [6]. Given the significant differences in both U_{opt} and U_{crit}

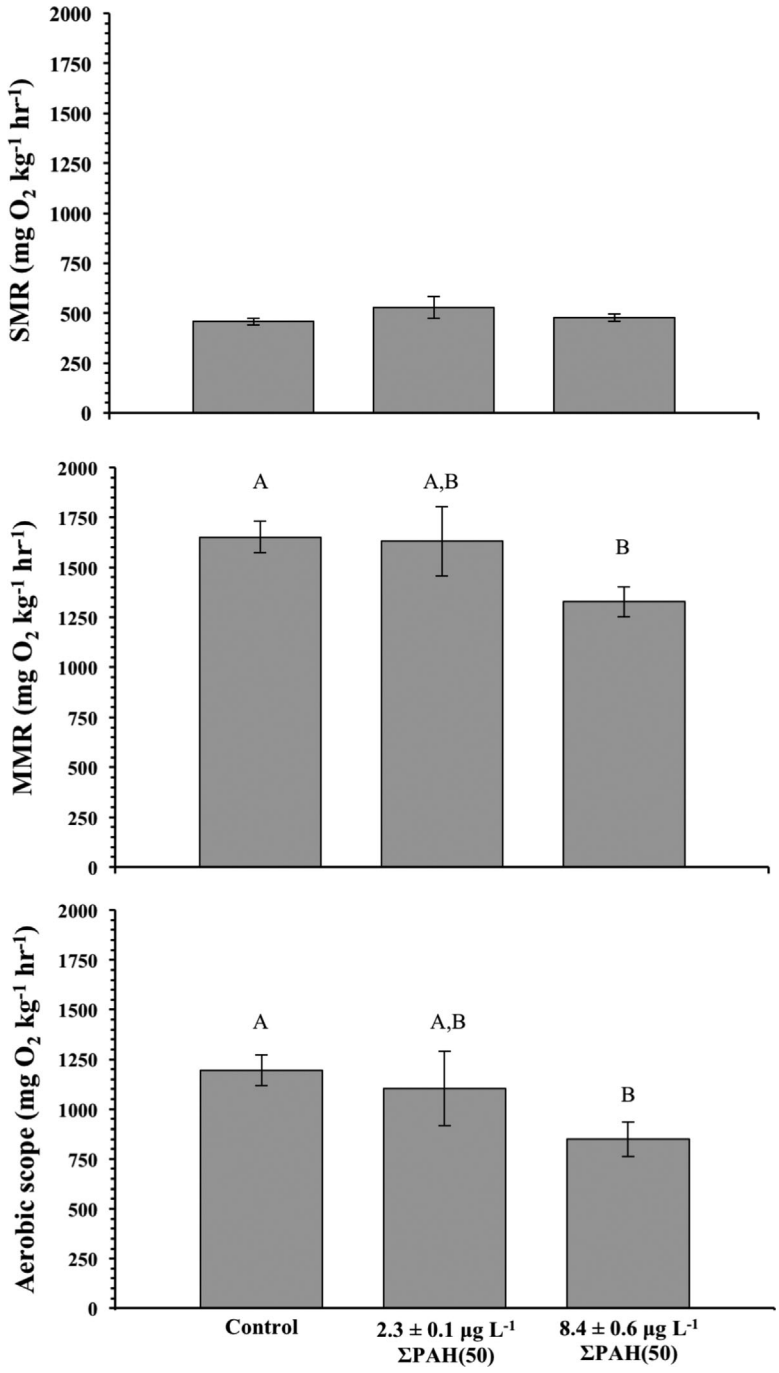

Figure 2. *Deepwater Horizon* crude oil exposure reduced maximum metabolic rate but not standard metabolic rate of young adult mahi-mahi. Top, standard metabolic rate; middle, maximum metabolic rate; bottom, aerobic scope. Data normalized for mass, as described in *Materials and Methods* and in Supplemental Data, Figure S2. Different letters indicate significant differences between treatment groups ($p < 0.05$). ΣPAH(50) = sum of 50 selected polycyclic aromatic hydrocarbons; MMR = maximum metabolic rate; SMR = standard metabolic rate.

between the control and 8.4 $\mu g\,L^{-1}$ ΣPAH(50) treatment groups, the present study reveals that oil-exposed young adult mahi-mahi are significantly slower. In addition, given the increased rate of pre-test handling-induced mortality at the 8.4 $\mu g\,L^{-1}$ ΣPAH(50) exposure level, it is hypothesized that this concentration may represent a threshold above which few fish are able to survive long enough to fully complete swim performance testing. Assuming that the most impacted individuals suffered mortality, the present study's findings, which stem from survivors, and conclusions for the 8.4 $\mu g\,L^{-1}$ ΣPAH(50) treatment group are likely conservative.

The observed 10% decrease in U_{opt} is similar in magnitude to the 14% decrease in U_{crit}, indicating a significant impact of transient crude oil exposure on not only the high end of the young adult mahi-mahi swimming ability but also on the optimal, or cruising, speed of the fish (Figure 3). Impacts to U_{crit} can affect the fish's ability to feed and flee effectively because young adult mahi-mahi rely on speed and endurance to capture prey in a pelagic environment as well as to avoid becoming prey to larger predatory pelagic species such as billfish, sharks, tuna, and larger mahi-mahi [47]. Of similar importance to life in the pelagic environment is the ability to swim at a high cruising

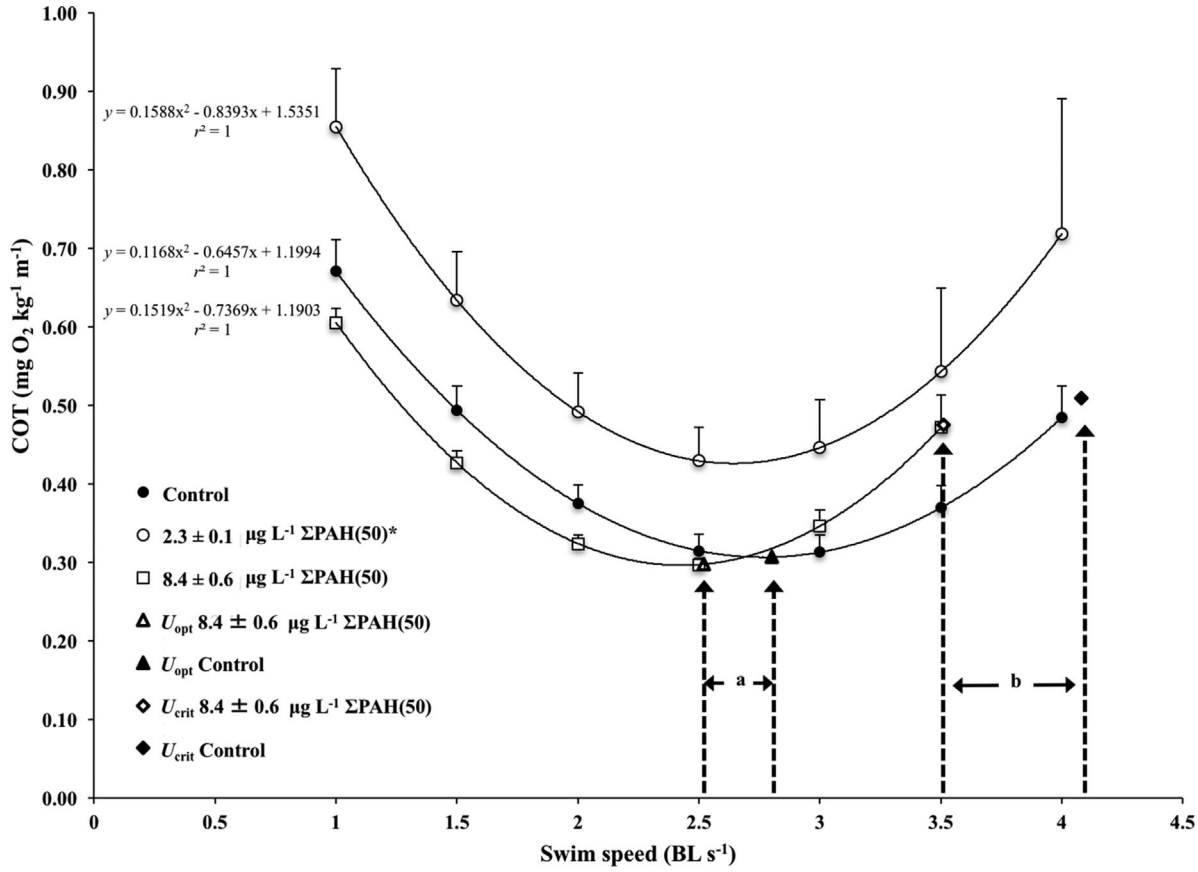

Figure 3. Cost of transport of young adult mahi-mahi at different swimming speeds. Each data point represents the cost of transport (mean ± standard error of the mean) at intervals of 0.5 body lengths s^{-1} beginning at 1 body length s^{-1}. Both the 10% decrease in optimal swimming speed (U_{opt}; *a*) and the 14% decrease in critical swimming speed (U_{crit}; *b*) in the 8.4 $\mu g L^{-1}$ ΣPAH(50) treatment group (open squares) compared to control fish (filled circles; see Figure 1) occur at nearly the same cost of transport for each respective endpoint (U_{opt} and U_{crit}). *Elevated cost of transport of the 2.3 $\mu g L^{-1}$ ΣPAH(50) treatment group (open circles) is likely the result of significant differences ($p < 0.05$) in mean size and swimming temperature of this group (see *Experimental animals, water quality, and PAH exposures*). BL = body lengths; COT = cost of transport; ΣPAH(50) = sum of 50 selected polycyclic aromatic hydrocarbons; U_{crit} = critical swimming speed; U_{opt} = optimal swimming speed.

speed (U_{opt}) to facilitate ram ventilation and allow for the great distances traveled by pelagic predators in search of prey and suitable reproductive environments [2,48,49]. Reduced swimming efficiency resulting from *Deepwater Horizon* oil exposure was recently documented for juvenile mahi-mahi [6] where cost of transport at U_{crit} was significantly elevated following embryonic oil exposure, yet a similar difference in cost of transport at U_{crit} was not documented in the present study (Figure 3).

Research suggests that migratory animals predominantly utilize the most efficient swimming speed (i.e., U_{opt}) to travel long distances [28,50–52], and the cruising speed of unexposed young adult mahi-mahi in the present study (2.9 km h^{-1}) was similar, when size and scaling relationships were accounted for, to data reported for other highly migratory species [27,51,53,54]. This notable high-speed cruising ability, combined with elevated metabolic rates, specialized physiology and biochemistry, and substantial aerobic capacities, allows for these apex pelagic predators to survive in the energy depauperate oceanic pelagic environment [15,55–57]. Therefore, the significant reduction in U_{opt} of mahi-mahi in the present study indicates that crude oil–exposed fish may be unable to keep up with other, nonexposed fish in a school, potentially leaving them open to higher rates of predation and reduced foraging and spawning opportunities. Although the scope for recovery from *Deepwater Horizon* crude oil exposure is unknown, such effects are likely to impact survival at least in the period between the onset of effect and potential recovery.

As previously mentioned, determination of aerobic scope allows for insight into whether a stressor or pollutant—in this case, *Deepwater Horizon* crude oil—is causing a loading stress (increased standard metabolic rate) or limiting stress (reduced maximum metabolic rate) [6]. The observed reduction of aerobic scope is attributable to a significant decrease in maximum metabolic rate following transient crude oil exposure at exposure levels in the low parts per billion range ($8.4 \pm 0.6\ \mu g\ L^{-1}$ ΣPAH(50)), whereas there was no significant difference in standard metabolic rate among treatment groups, indicating that *Deepwater Horizon* crude oil acts as a limiting stressor (Figure 2). The limiting stress indicates that crude oil exposure impairs oxygen uptake and/or oxygen transport capabilities, reducing oxygen delivery and thus limiting the overall metabolic capability of the fish. Reduced oxygen delivery in *Deepwater Horizon* oil-exposed fish is consistent with recent reports of impaired isolated myocyte function in tuna following in vitro exposure to PAHs [44]. Both the isolated myocyte study and the present study show effect levels in the low parts per billion ΣPAH range of slick A crude oil exposure [44]. Future studies may include measurement of plasma ΣPAH concentrations to quantify the comparison between intact animals and studies of isolated myocytes. Although there are no long-term studies on the effects of

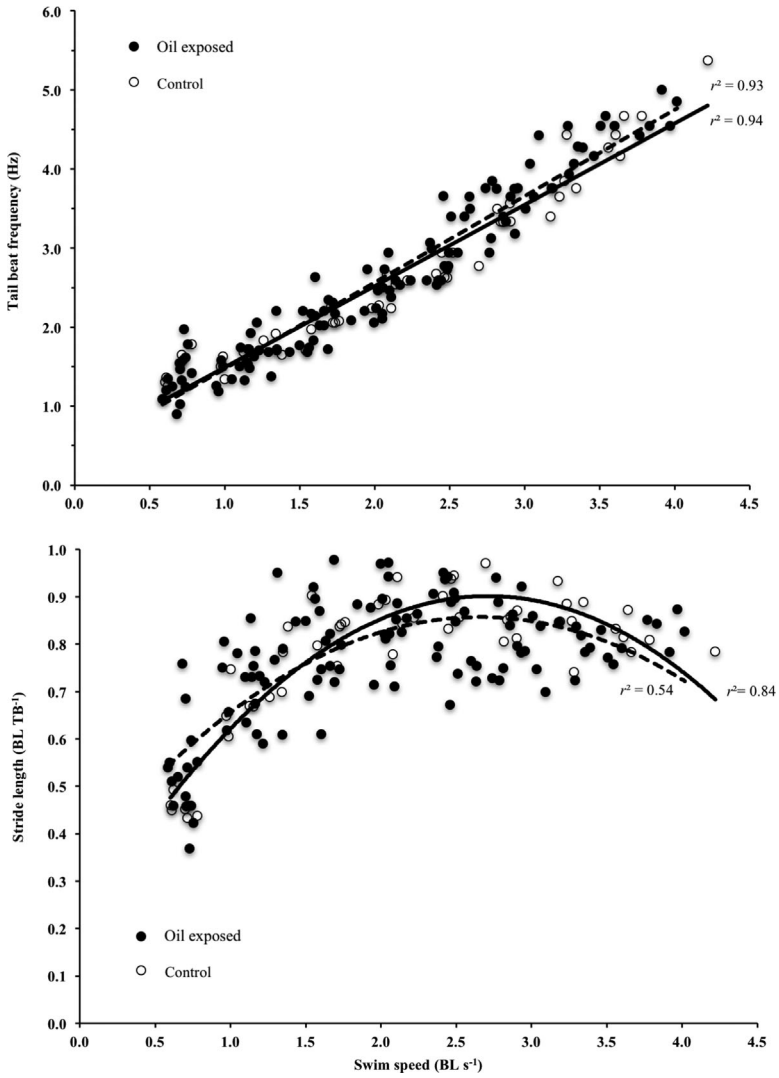

Figure 4. Tail beat frequency and stride length of young adult mahi-mahi at different swimming speeds following acute 8.4 $\mu g\,L^{-1}$ ΣPAH(50) exposure (dashed line) and no oil (control) treatment (solid line). ΣPAH(50) = sum of 50 selected polycyclic aromatic hydrocarbons; BL = body lengths; TB = tail beat.

reduced metabolic capacity in predatory pelagic fish species, such impacts are likely to reduce the overall fitness of the exposed animals.

To provide insight on whether decreases in swimming speed might be the result of excitation–contraction uncoupling of skeletal muscle contractions that may occur in a similar manner as described for cardiac myocytes [44], video analysis was incorporated into the analysis of a subset of individuals in the present study. The lack of a significant relationship between oil exposure and tail beat frequency or stride length at a variety of swimming speeds suggests that the reduced maximum metabolic rate and reduced aerobic scope are the primary drivers of the oil-induced reductions in swim performance seen at this advanced life stage. Mechanisms behind these drivers may include crude oil–induced damage to gill oxygen uptake and/or cardiac output. Gill damage is a documented effect of crude oil exposure, commonly resulting in reduced oxygen uptake from damage such as filament thickening, hyperplasia, and hemorrhaging [39,58–61]. However, post hoc analysis of fixed gill samples imbedded, sectioned, and examined microscopically for differences in interlamellar distances and epithelial damage from a subset of individuals from both the control and 8.4 $\mu g\,L^{-1}$ ΣPAH(50) treatment groups did not reveal any definitive signs of gill damage (data not shown). Such findings lend support to the notion that reduced cardiac output may be the primary mechanism responsible for the swim performance impacts noted in the present study, although other possible explanations may exist. As previously mentioned, *Deepwater Horizon* crude oil has been shown to disrupt excitation–contraction coupling in cardiomyocytes [44]. Such cardiotoxic effects are believed to cause arrhythmias that likely reduce cardiac output, a notion supported by the reduced maximum metabolic rate limiting aerobic scope in the present study. These effects may be more pronounced in "high-performance" pelagic teleosts, such mahi-mahi and tuna, because of the larger gill surface areas and the thinner gill water–blood barrier in these species compared with other active fishes and finally the oxygen-dependent energetic requirements necessary for maintaining such specialized features [62].

Given the physiological and anatomical adaptations of apex pelagic predators, such as mahi-mahi, tuna, and billfish, which require the rapid cycling of metabolic substrates in the body to support their life processes, these species may be more sensitive to sublethal crude oil exposure than other teleosts with more limited metabolic demands. This is supported by results of the present study revealing significant impacts to swim

performance from a relatively short exposure to *Deepwater Horizon* crude oil at ΣPAH concentrations that are low compared with values reported from the field [33–35]. The documented effects provide insight into the effects of sublethal *Deepwater Horizon* crude oil on whole-animal physiology of these high-performance pelagic teleosts at a life stage hypothesized to be rather impervious to such damages when compared with earlier life stages of these species. Clearly, determination of the sublethal effects of events such as the *Deepwater Horizon* oil spill on all life stages of potentially impacted species is beneficial to understanding and quantifying injury to natural resources.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3436.

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Data Availability—The data presented in the present study are a subset of a larger toxicological database that is being generated as part of the *Deepwater Horizon* Natural Resource Damage Assessment; therefore, these data will be subject to additional analysis and interpretation, which may include interpretation in the context of additional data not presented in the present study. For further information, please contact the corresponding author (jstieglitz@rsmas.miami.edu).

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