Title: Combined Effects of Hypoxia or Elevated Temperature and *Deepwater Horizon* Crude Oil Exposure on Juvenile Mahi-Mahi Swimming Performance

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Abstract

This study examined potential interactive effects of co-exposure to *Deepwater Horizon* (*DWH*) crude oil (~30 µg L^{-1} ΣPAHs) for 24 h and either hypoxia (2.5 mg O_2 L^{-1}; 40% O_2 saturation) or elevated temperature (30 °C) on the swimming performance of juvenile mahi-mahi (*Coryphaena hippurus*). Additionally, effects of shorter duration exposures to equal or higher doses of oil alone either prior to swimming or during the actual swim trial itself were examined. Only exposure to hypoxia alone or combined with crude oil elicited significant decreases in critical swimming speed (U_{crit}) and to a similar extent (~20%). In contrast, results indicate that elevated temperature might ameliorate some effects of oil exposure on swimming performance and that effects of shorter duration exposures are either reduced or delayed.

Keywords: Gulf of Mexico; PAHs; pelagic fish; *Coryphaena hippurus*; U_{crit}; aerobic scope

1.1 Introduction

The 2010 *Deepwater Horizon* (*DWH*) incident overlapped in time and space with the spawning of commercially important predatory fish such as mahi (*Coryphaena hippurus*; hereafter referred to as mahi) and others (Brown-Peterson et al., 2001; McEachran et al., 1980; Palko et al., 1982; Rooker et al., 2013; Teo and Block, 2010). Given the protracted nature of the spill (87 d), it is likely that exposure to crude oil constituents, including toxic polycyclic aromatic hydrocarbons (PAHs), occurred over a range of life stages for these fishes (DWH NRDA Trustees, 2016). While exposure at any stage might lead to outright mortality for some, there is also risk that survivors could sustain acute and potentially persistent or latent effects that alter physiological, and potentially ecological, performance. For example, previous work has shown that 48 h exposures to crude oil during the embryonic/larval stages caused latent effects that manifested in reduced swimming performance as juveniles and adults (Hicken, et al., 2011; Mager et al., 2014). Additionally, 24 h oil exposures to young adult red drum (*Sciaenops ocellatus*) elicited reduced swimming performance that persisted for 6 weeks (Johansen and Esbaugh, 2017). Hence, there is mounting evidence that even transient exposure to crude oil at various life stages can elicit long-lasting sublethal physiological impacts that may translate to higher-order ecological effects as a result of impaired swimming performance (e.g., prey capture, predator evasion, migration).

Fish residing in the northern Gulf of Mexico (GoM) face a number of natural environmental stressors, such as hypoxia and elevated temperature, each of which had potential to interact with the crude oil exposure stress imposed by the *DWH* event. The occurrence of hypoxia is common and widespread in the northern GoM and is largely coupled to eutrophication from nutrient discharge from the Mississippi and Atchafalaya Rivers (Diaz and Rosenberg, 2008;

Rabalais et al., 2007). Adult and juvenile fish can detect and actively avoid hypoxic regions, although the nature (i.e., graded vs. threshold) of the hypoxic avoidance response appears to vary with species (Wannamaker and Rice, 2000). Previous work indicates that such avoidance leads to habitat compression and aggregation of fish at the hypoxic zone edges, potentially increasing predator-prey interactions (Zhang et al., 2009). Fish living at such edges may experience moderate hypoxia or make occasional forays into more severe hypoxic waters to chase prey or avoid predation. Considering that predator-prey interactions and other ecological activities (e.g., migration, settlement) critical to the life history of fishes are largely a function of swimming performance, standard measures of swimming performance such as critical swimming speed (U_{crit}) are therefore useful for assessing the potential ecological impacts of hypoxia exposure and how crude oil exposure may further influence such effects (Plaut, 2001).

Elevated temperature represents an additional natural stressor in the northern GoM, with annual sea surface temperatures reaching upwards of 30 – 32 °C during summer months (NOAA, 2017). Temperature plays an important role in the metabolic performance of ectothermic fishes and hence is expected to impact the uptake, metabolism and depuration of crude oil constituents as well as U_{crit} through alterations in aerobic scope. However, it remains to be determined how the aerobic performance of a native pelagic juvenile fish with high energetic demand, such as mahi, is influenced by the upper range of GoM water temperatures and how crude oil exposure might interact with elevated temperature to affect performance.

Another important consideration is that previous studies of GoM fish swimming performance during the juvenile and young adult stages revealed effects following exposure periods of 24 h (Johansen and Esbaugh, 2017; Mager et al., 2014; Stieglitz et al., 2016). However, it remains unknown whether such effects can be elicited by shorter exposures to

environmentally relevant PAH concentrations. Moreover, previous studies have utilized exposures prior to the actual swim trial itself. While such studies are relevant to understanding the impacts of pre-exposure to oil on subsequent swimming performance, it is possible that direct physical interaction with crude oil while swimming (e.g., through a large plume of oil) may also reduce swimming performance. If so, this would likely indicate a reduced ability to escape from oil-contaminated water in the event of entrainment; yet, this is an area of study that remains virtually unexplored.

The primary aim of the present study was to assess the impacts of hypoxia and elevated temperature individually and combined with acute *DWH* crude oil exposure on the swimming performance of juvenile mahi. Additional aims were to: (1) determine whether reduced swimming performance could be elicited by a shorter duration (12 h), but higher concentration exposure to crude oil than used previously and (2) investigate the potential impact of swimming directly within oil-contaminated water on swimming performance.

2.1 Materials and Methods

2.1.1 Experimental animals.

All experimental fish were F1 generation mahi from volitional (non-induced) spawns of wild-caught broodstock that were raised to the juvenile stage at the University of Miami Experimental Hatchery (UMEH) as previously described (Stieglitz et al., 2017). The handling and use of animals complied with the guidelines of the Institutional Animal Care and Use Committee of the University of Miami. Biometric data for all experimental fish are provided in Table 1.

Table 1. Biometric data (mean ± SEM) for mahi-mahi used in swimming performance tests.

Treatment	*n*	Mass (mg)	BL (cm)	Age (dph)
Hypoxia				
Control	23	501 ± 42	4.2 ± 0.1	28 ± 1
Hypoxia	21	485 ± 26	4.2 ± 0.1	27 ± 1
Hypoxia + 2% HEWAF	22	448 ± 39	4.2 ± 0.1	27 ± 1
High Temp.				
Control	18	433 ± 31	4.1 ± 0.1	33 ± 1
High Temp.	17	328 ± 24*	3.7 ± 0.1*	30 ± 1
High Temp. + 2% HEWAF	28	287 ± 19*	3.6 ± 0.1*	31 ± 1
Swim Tunnel (ST) & 12 h Exposures				
Control	18	254 ± 18	3.4 ± 0.1	29 ± 1
20% HEWAF (ST)	6	352 ± 28	3.9 ± 0.1*	32 ± 1
12 h 4% HEWAF	14	340 ± 38	3.9 ± 0.1*	34 ± 1*

Abbreviations: body length (BL); days post-hatch (dph). *Significantly different from treatment matched control by one-way ANOVA and Holm-Sidak or Dunn's multiple comparison procedure.

2.1.2 Experimental design.

Four experiments were performed (see Fig. 1 for an overview of the experimental design for each). The first two experiments assessed the impacts of hypoxia or high temperature either alone or in combination with crude oil exposure on the swimming performance of juvenile mahi. Both experiments utilized a 24 h exposure period to either control seawater or seawater spiked with a 2% high energy water accommodated fraction (HEWAF) of oil immediately prior to the swim trials (no oil exposure during the swim trials). For the hypoxia experiments, fish were maintained in normoxic conditions (6.2 mg O_2 L^{-1}) during the 24 h pre-exposure period. Fish were exposed to hypoxia (2.5 mg O_2 L^{-1}) only during the swim trials, whereas the swim trials for controls were performed in normoxia. For the high temperature experiments, fish were maintained at 30 ºC (27 °C for controls) during the 24 h exposure period as well as during the

swim trials. A third experiment was performed to evaluate whether the previously observed impairment to swimming performance following 24 h exposure to 2% HEWAF (Mager et al., 2014) could be similarly induced by a shorter (12 h) exposure to a higher (4%) HEWAF dilution. Fish were held in treatment vessels for 24 h, but only exposed to HEWAF during the final 12 h. The fourth and final experiment was designed to assess potential impacts on swimming performance arising from direct exposure to diluted HEWAF during the habituation and active phases of a swim trial. For these last experiments, fish were first exposed for 24 h to control seawater at 27 °C prior to introduction into the swim tunnel to maintain consistency with the other experiments. HEWAF exposures occurred only within the swim tunnel apparatus. A pilot experiment was first performed using 6 different HEWAF dilutions in the swim tunnels: 2, 4, 6, 10, 15 and 20%. Two fish were exposed per day (alongside two controls) using these increasing dilutions of HEWAF with the intention of rapidly identifying a dilution that elicited an obvious impairment to swimming performance. However, no clear impairment was observed up to and including 20% HEWAF and therefore efforts were devoted to obtaining a greater sample size (n=6) to anchor the results for the highest dilution (data not shown for other dilutions).

Because of the rapid growth rates of mahi, multiple cohorts of fish were required to provide sufficient sample sizes. Two and four cohorts were used for the hypoxia and temperature experiments, respectively; no cohorts were shared between these experiments. All fish used for the 12 h 4% HEWAF and swim tunnel exposure experiments originated from a single, shared cohort. Controls were included from each cohort.

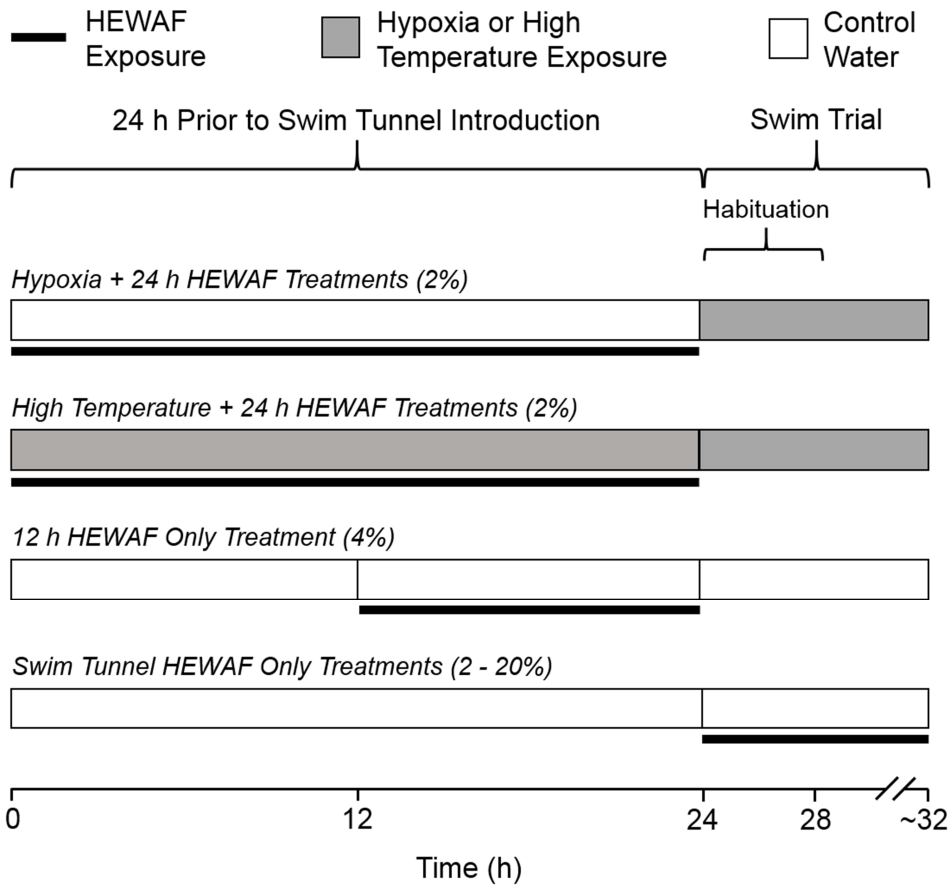

Figure 1. Schematic representation of the different exposure regimes described herein. To simplify, only exposures combined with HEWAF are illustrated for the hypoxia and high temperature treatments. Hypoxia alone and high temperature alone treatments were also performed in an identical fashion as illustrated except without HEWAF exposure during the initial 24 h. Controls without HEWAF, hypoxia and high temperature were also performed but are not illustrated. Note that final hypoxia and temperature levels were gradually achieved over time, beginning at the habituation stage of the swim trial or the initiation of the 24 h exposure period prior to swim tunnel introduction, respectively. Please refer to Materials and Methods for further details.

2.1.3 Preparation of water accommodated fractions of oil.

The oil used in this study (referred to herein as slick A) 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. For all exposures, a high-energy water accommodated fraction (HEWAF) of oil was prepared on the day of use as previously described (Mager et al., 2014). All of the water used in the experiments described herein was 1 µm-filtered, UV-sterilized seawater.

2.1.4 Exposure regimes.

All pre-swimming exposures were performed using 12 L volumes within 20 L glass jars held in a temperature controlled environmental chamber. Temperature and photoperiod within the chamber were 27 °C and 16:8 h of light:dark, respectively. For the hypoxia and temperature experiments, juvenile mahi were exposed for 24 h to either control seawater or seawater spiked with freshly prepared 2% HEWAF. For the high temperature exposures, fish were added to exposure media at 27 °C and then the temperature was slowly raised (~1 °C per hour) to 30 °C using an aquarium heater. For the 12 h 4% HEWAF exposures, fish were held within exposure vessels for 24 h to remain consistent with prior exposures; however, fish were only exposed to HEWAF during the final 12 h. Light aeration was provided to each exposure using an air stone. Although only a maximum of four fish were swum per replicate, six fish were typically exposed to safeguard against losses due to mortality. When selecting fish for the swim trials, only healthy appearing individuals were selected (i.e., actively swimming with normal ventilation) and were collected at random. Although rare, any fish exhibiting overt signs of stress were avoided (e.g., remaining stationary on the bottom or aberrant ventilation pattern). Fish were fed in the morning

at the UMEH before transfer to an exposure chamber, but not fed during the 24 h exposure period. For the HEWAF exposures in the swim tunnels, fish were initially held for 24 h in control seawater as described above. Swim tunnel exposures were prepared by thoroughly mixing the appropriate volume of HEWAF with seawater in the swim tunnel reservoir. Two of the four swim tunnels were dedicated for HEWAF exposures and two for controls to prevent potential cross-contamination of residual HEWAF constituents. For all HEWAF exposures (swim tunnels and glass jars), HEWAF dilutions were prepared and mixed well with a Teflon stir bar just prior to introducing the fish. Number of replicates and sample sizes for all exposures and swim trials are provided in Table S1.

2.1.5 Swimming Performance and Metabolic Rate Measurements.

Four miniature Blazka-style swim tunnel respirometers (0.17 L) from Loligo Systems (Denmark) were used in parallel to assess critical swimming speed (U_{crit}) and obtain metabolic rate measurements for determination of standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS = MMR – SMR) via automated intermittent flow respirometry (Blazka et al., 1960; Steffenson, 1989). Flow velocity was calibrated for each swim tunnel by tracking fluorescent microspheres using the DPTV Flow Tracking System and velocimetry software (version 1; Loligo Systems, Denmark). Oxygen consumption (or ambient O_2 concentrations for the hypoxia trials; see below), was measured within each swim chamber 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% oxygen saturation, established by vigorous aeration with an air stone, and 0% saturation, achieved using a solution of 10 g L^{-1} Na_2SO_3 (Sigma-Aldrich, St. Louis, MO). All data were collected using AutoResp2 version 2.2.2 (Loligo Systems, Denmark). Temperature (27 or 30 ºC) was

maintained in all swim chambers using an aquarium heater placed in the reservoir bath surrounding the chamber and was measured through the Fibox meter using a separate probe. Fish were transferred directly from treatment vessels to the swim tunnel respirometers immediately following the 24 h exposures described above (i.e., no recovery period was permitted prior to transfer).

For the hypoxia experiments, hypoxic conditions (2.5 mg O_2 L^{-1}; 40% O_2 saturation) were achieved in the swim tunnel by gradually decreasing the O_2 content from 100% to ~40% O_2 saturation (~10% decrease every 10 min) during the final hour of the habituation phase (described below). This saturation level was selected to be just above a level inducing overt hypoxia stress as indicated by loss of the righting reflex and heavy ventilation. From a preliminary experiment, these signs became evident when air saturation levels reached ~35 – 37% (data not shown). Ambient O_2 levels were regulated by N_2 gas using a solenoid controlled by the OxyCTRL system (Loligo Systems, Denmark). Because measurements of O_2 consumption require a period during which the respirometer is closed and ambient O_2 levels are drawn down due to consumption, metabolic rate measurements were not collected for the hypoxia experiments so that stable hypoxia levels would be maintained throughout the ramped velocity stages of each trial.

U_{crit} and metabolic rate measurements were collected as previously described using 20 min intervals (Brett, 1964; Mager et al., 2014). Each interval was comprised of a flush, wait and measure period of 600, 10 and 590 s, respectively. SMR (y intercept) and MMR (extrapolated at U_{crit}) were derived from least-squares linear regressions of the logarithm of oxygen consumption (mg O_2 g^{-1} h^{-1}) versus swimming speed (BL s^{-1}). To remain consistent with previous studies (Mager et al., 2014; Stieglitz et al., 2016), only individuals yielding a regression with an $r^2 \geq 0.7$

were used (see Table S1 for *n*). All metabolic rate data were normalized for the effect of mass before calculating aerobic scope by scaling to a standard mass of 1 g using scaling coefficients for juvenile mahi as previously described (Mager et al., 2014).

2.1.6 Water quality and PAH analysis.

Initial PAH samples were collected immediately after preparing HEWAF dilutions and thorough mixing with a Teflon stir bar and just prior to addition of fish. All samples (initial and final) were collected several inches below the water surface in 250 mL amber bottles as grab samples 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 USEPA method 8270D). Reported ΣPAH values represent the sum of 50 select PAH analytes (Table S2). Initial and final samples were collected for all HEWAF exposures; typically, only initials were collected for controls. For all exposures, temperature, pH, dissolved oxygen (DO) and salinity were measured daily and total ammonia was measured at the conclusion of each test. 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 (Ivančič and Degobbis, 1984). A summary of measured ΣPAH concentrations and water quality parameters is provided in Table S3.

2.1.7 Statistical analyses.

Results from the 20% HEWAF swim tunnel exposures and 12 h 4% HEWAF exposures were each statistically compared to controls using a Student's t-test. All other swim trial statistical comparisons were made using one way analysis of variance (ANOVA) followed by

Holm-Sidek multiple comparison procedure. All ANOVAs passed tests for normality and equal variance. Differences were tested for statistical significance using SigmaStat version 3.5 (Systat Software, Inc., San Jose, CA) and were deemed significant at $p < 0.05$.

3.1 Results & Discussion

3.1.1 PAH concentrations and composition.

The compositional PAH profiles for oil exposures from all four experiments were consistent with those previously reported for slick A HEWAFs (Esbaugh et al., 2016; Forth et al., 2017; Mager et al., 2014; Stieglitz et al., 2016) as well as from samples collected from the active spill zone (Incardona et al., 2014; Table S2). All ΣPAH concentrations are summarized in Table S3, including both initial concentrations and the geometric means of initial and final concentrations. Reported values in the text and figures represent the geometric means of initial and final concentrations.

3.1.2 Hypoxia and crude oil exposure effects on swimming performance.

The primary objective of the present study was to assess the impacts of acute exposure to hypoxia and high temperature individually and combined with acute crude oil exposure on the swimming performance of juvenile mahi. Hypoxia is typically defined as DO concentrations of ≤ 2 mg O_2 L^{-1} (Diaz and Rosenberg, 2008). However, this value serves as a somewhat arbitrary threshold as clear signs of hypoxic distress are evident in some fish species at higher concentrations, likely reflecting differences in metabolic demands associated with different lifestyles and inherent activity levels (Diaz, Robert J. and Breitburg, Denise L., 2009; Gray et al., 2002). Interestingly, acute exposure to a hypoxic level for mahi (2.5 mg L^{-1} O_2; 40% O_2 saturation) alone or combined with 2% HEWAF (29 µg L^{-1} ΣPAHs) exposure reduced U_{crit} by a similar extent of ~20% ($F = 3.792$; $p = 0.028$; Fig. 2). Although in the current study aerobic

scope was not measured for the hypoxia experiments, previous studies suggest that reductions in U_{crit} with crude oil exposure might not be directly linked to aerobic scope. For example, a reduced U_{crit} was previously observed with 2% HEWAF that did not coincide with a reduced aerobic scope, thus not supporting an oxygen delivery limitation as the primary mechanism of impairment (Mager et al., 2014). Other work has similarly revealed a reduction in U_{crit} without a corresponding decrease in aerobic scope following transient crude oil exposure to red drum (Johansen and Esbaugh, 2017). Additionally, exposure to a complex PAH mixture from sediment extract reduced U_{crit} of Atlantic killifish (*Fundulus heteroclitus*; Brown et al., 2017). While aerobic scope was not explicitly measured, the reported metabolic rate measurements did not clearly support a parallel reduction in aerobic scope. Such results indicate that this phenomenon is, therefore, not unique to mahi. Factors other than cardiovascular function must act to limit swimming performance in these cases and might include, for example, neurological effects or impaired recruitment of white muscle fibers when approaching U_{crit}. Nevertheless, it should be noted that oil exposure does reduce aerobic scope by suppressing maximum metabolic rate resulting in reduced swimming performance in adult mahi (Stieglitz et al., 2016) and at higher concentrations in red drum (Johansen and Esbaugh, 2017). The mechanism associated with hypoxia exposure is likely reduced oxygen delivery to the working muscles stemming from insufficient oxygen loading at the gills. Determining the oxygen equilibrium curve for juvenile mahi hemoglobin and monitoring changes in hematocrit would help clarify the role of gill oxygen loading in this regard or indicate whether other physiological responses (e.g., reduced cardiac output, peripheral vasoconstriction) are potentially contributing to a deficit in oxygen supply to the swimming muscles in response to hypoxia.

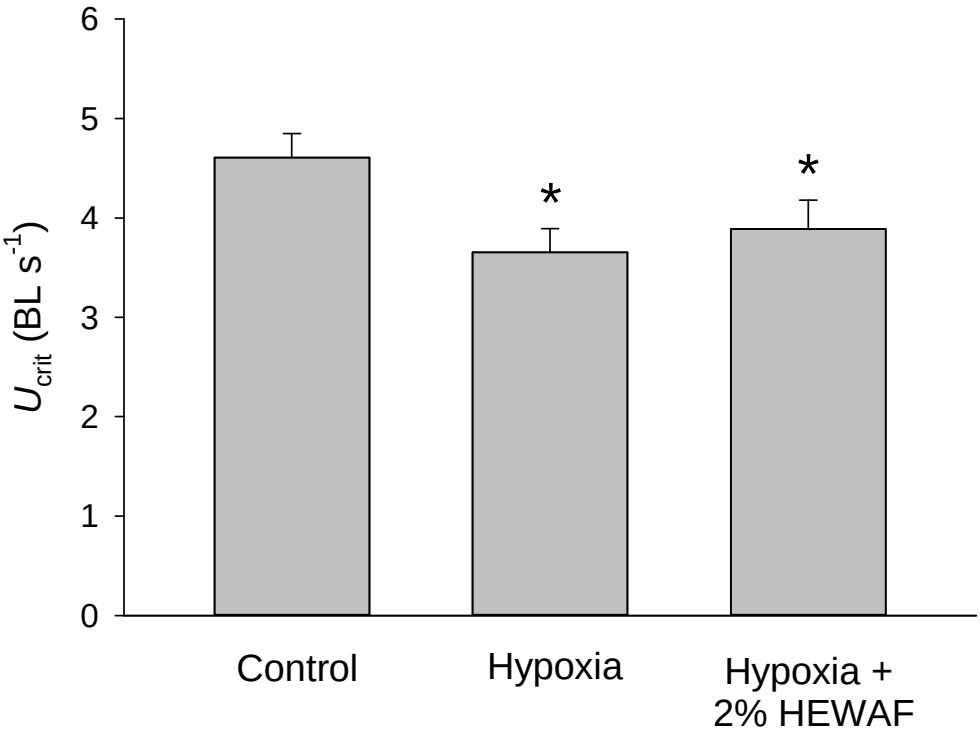

Figure 2. Mean ± SEM critical swimming speeds (U_{crit}) of juvenile mahi exposed to control conditions, hypoxia (2.5 mg L^{-1} O_2) or hypoxia + 2% slick A HEWAF (29 µg L^{-1} ΣPAHs) for 24 h prior to swim trial. Sample sizes are 23, 21 and 22, respectively. *Significantly different from controls by one-way ANOVA (p = 0.028) and Holm-Sidek multiple comparison procedure.

3.1.3 Temperature and crude oil exposure effects on swimming performance.

In contrast to hypoxia, acute exposure to elevated temperature or a combined exposure of elevated temperature and 2% HEWAF (30 µg L^{-1} ΣPAHs) did not elicit a change in U_{crit} (F = 0.466; p = 0.63; Fig. 3), SMR, MMR or AS (Fig. S1). The relationship of aerobic scope and temperature has been characterized by generalized curves, the shapes of which vary for reasons outside the scope of this paper (Clark et al., 2013; Pörtner and Farrell, 2008). Nevertheless, the relationship is generally represented by an initial trend of increasing aerobic scope with

increasing temperature until an optimal temperature for peak aerobic performance is reached. Beyond this optimal temperature, aerobic scope decreases, either gradually or precipitously as the critical lethal limit is reached (i.e., CT_{max}). Thus, a possible explanation for the observed lack of effect of temperature in the present study is that the temperatures of 27 °C and 30 °C occupy similar crossing points on the upward and downward slopes of the curve, respectively. Additional experiments employing more temperatures across this range would be needed to confirm or refute this notion. The lack of a temperature effect might also reflect, to some extent, a selection event considering that increased mortality was observed at 30 °C (Table S1) and that presumably only the most fit individuals survived. Furthermore, such mortality likely indicates that this temperature is approaching the lethal limit for juvenile mahi, a finding that would appear consistent with 30 °C representing a position on the downward slope of the temperature-aerobic scope relationship curve. This would seem surprising considering that 30 °C falls within the normal temperature range of surface waters in the northern GoM; however, it is unknown whether juvenile mahi normally reside in the upper surface waters at this temperature or if they seek cooler temperatures at lower depths. It should be noted that fish from both high temperature treatments (+/- HEWAF) were on average slightly smaller (~11%) than the controls (Table 1). When expressed in terms of body length, smaller fish typically have higher U_{crit} values than larger fish of the same species (Beamish et al., 1978). Thus, the smaller average size of fish used in the high temperature treatments might have confounded the ability to detect a mild decrease in swimming performance at elevated temperature.

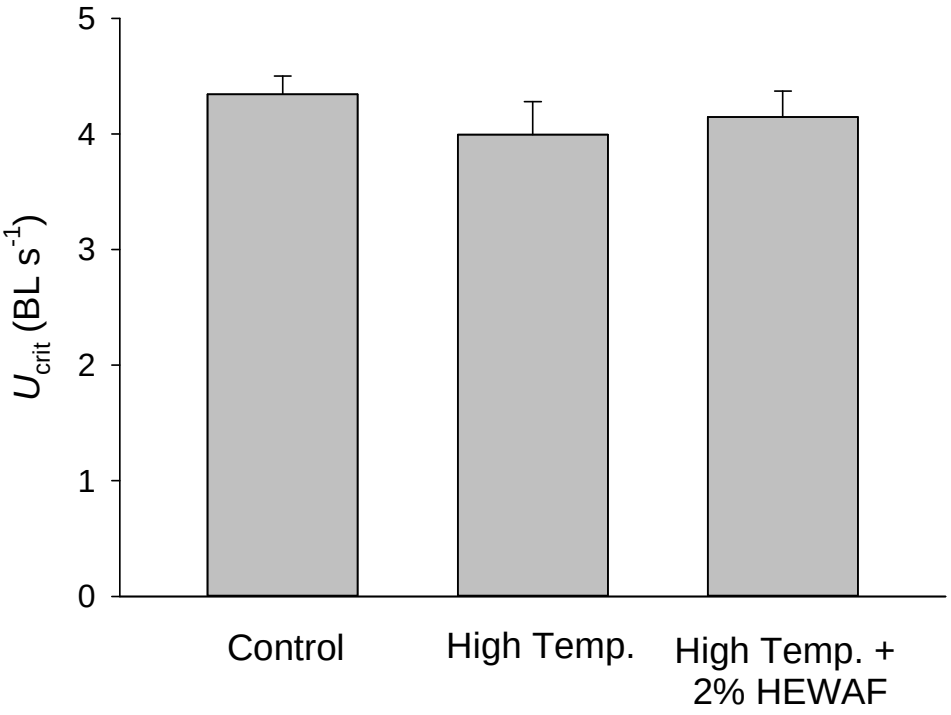

Figure 3. Mean ± SEM critical swimming speeds (U_{crit}) of juvenile mahi exposed to control conditions (27 ºC), high temperature (30 ºC) or high temperature + 2% slick A HEWAF (30 µg L^{-1} ΣPAHs) for 24 h prior to swim trial. Sample sizes are 18, 17 and 28, respectively.

With respect to the combined exposures of HEWAF and elevated temperature, the absence of an effect was surprising considering that the same dilution of HEWAF with the same PAH concentration (~30 µg L^{-1} ΣPAHs) reduced U_{crit} at 27 °C previously (Mager et al., 2014). A change in PAH chemistry at elevated temperature seems an unlikely explanation considering that PAH composition and concentration were remarkably similar among the 30 °C exposure and the 27 °C exposures of the present (Table S2) and previous studies. Instead, these differences may reflect batch effects associated with using different cohorts of fish or temperature dependent toxicokinetics. Results from isolated PAH studies suggest that, despite a more rapid uptake rate

in warmer temperature, PAHs are also more quickly metabolized and eliminated in warmer temperature (Collier et al., 1978; Jimenez et al., 1987; Varanasi et al., 1981). It is also interesting to note that rainbow trout and European sea bass showed signs of increased thermal tolerance following crude oil exposure using multiple temperature tolerance indices (Anttila et al., 2017). Thus, there appears to be growing evidence that elevated temperature may be protective to some extent against crude oil exposures to fish during the juvenile stage, although the mechanism of protection is unclear at this time. A final point of consideration is that only the effects of acute hypoxia or elevated temperature exposure were analyzed by the current study. It remains unknown how acclimation to such conditions over longer periods might influence the current findings.

3.1.4 Effect of shorter (12 h) crude oil exposure on swimming performance.

Another objective of the present study was to discern whether reduced swimming performance could be elicited by a shorter duration (12 h) exposure to a higher, 4% HEWAF dilution (55 µg L^{-1} ΣPAHs). This concentration is still environmentally relevant considering that reported PAH concentrations in the upper subsurface waters during the spill ranged as high as 59 – 240 µg L^{-1} ΣPAHs (Diercks et al., 2010; DWH NRDA Trustees, 2016; Wade et al., 2011). However, U_{crit} (Fig. 4) and O_2 consumption (Fig. S2) results revealed no effects arising from this exposure, indicating that a longer duration (up to 24 h) is potentially required to impair swimming performance of juvenile mahi for PAH exposures in the range of ~30 – 55 µg L^{-1} ΣPAHs. It is unknown at this time why a shorter 12 h exposure is insufficient to elicit the effects observed following 24 h of exposure, but a greater time needed to bioaccumulate crude oil toxicants, or simply batch effects, could represent potential explanations. Alternatively, such toxicants might have bioaccumulated to sufficient levels to induce effects; however, the effects

might have been indirect and required additional time to manifest. Another potential explanation is that the effects are due at least in part to toxic metabolites produced from phase 1 enzyme induction and additional time is needed for these metabolites to reach effective concentrations. Additional experiments examining bioaccumulation rates, timing and production of phase 1 metabolites and varying exposure durations in relation to swim trial initiation will be needed to clarify the effects of timing and duration of crude oil exposures on swimming performance.

3.1.5 Effect of direct crude oil exposure while swimming on swimming performance.

A final objective was to investigate the potential impact on swimming performance associated with swimming directly within oil-contaminated water. Direct interaction with crude oil constituents (dissolved or particulate) while swimming might elicit effects that disrupt maximum swimming performance through a potentially different mechanism(s) than that following an exposure prior to swimming. Moreover, during the post-exposure swim trial the fish is effectively afforded a 4 h depuration period during the habituation phase that may help to clear any direct effects of swimming within oil contaminated water. Examples of direct effects of swimming through oil might include stress or behavioral effects due to potential sensory impairment (e.g., vision, neuromast function) or stimulation (e.g., olfaction) that alter swimming performance. Alternatively, oil droplets could potentially interact with the gill to affect ventilation efficiency. Evidence from the present study, however, did not indicate a detrimental effect on U_{crit} (Fig. 4) or O_2 consumption (Fig. S2) when swimming directly within a HEWAF dilution of 20%. Considering that the PAH concentration (320 µg L^{-1} ΣPAHs) exceeded the upper concentrations measured in the upper surface waters of the GoM during the spill, it seems unlikely that the swimming performance of juvenile mahi was impaired by a brief incursion into oil-contaminated water during the *DWH* event. However, as mentioned above, the possibility

exists that an impairment to U_{crit} might not manifest until sometime after the exposure, although this awaits further examination. Another possibility is that the propeller used to generate flow within the swim tunnel reduced mean oil droplet size, potentially lessening the impact of oil droplets on fish ventilation efficiency. As a final note, fish from both the 20% HEWAF and 4% 12 h HEWAF exposures were significantly larger than the controls (~13%; Table 1). However, as mentioned previously, smaller fish typically have higher U_{crit} values than larger fish of the same species when expressed in terms of body length (Beamish et al., 1978). Thus, this potential confounding effect of size would bias toward an expected decrease in U_{crit} for these treatments, which was not observed.

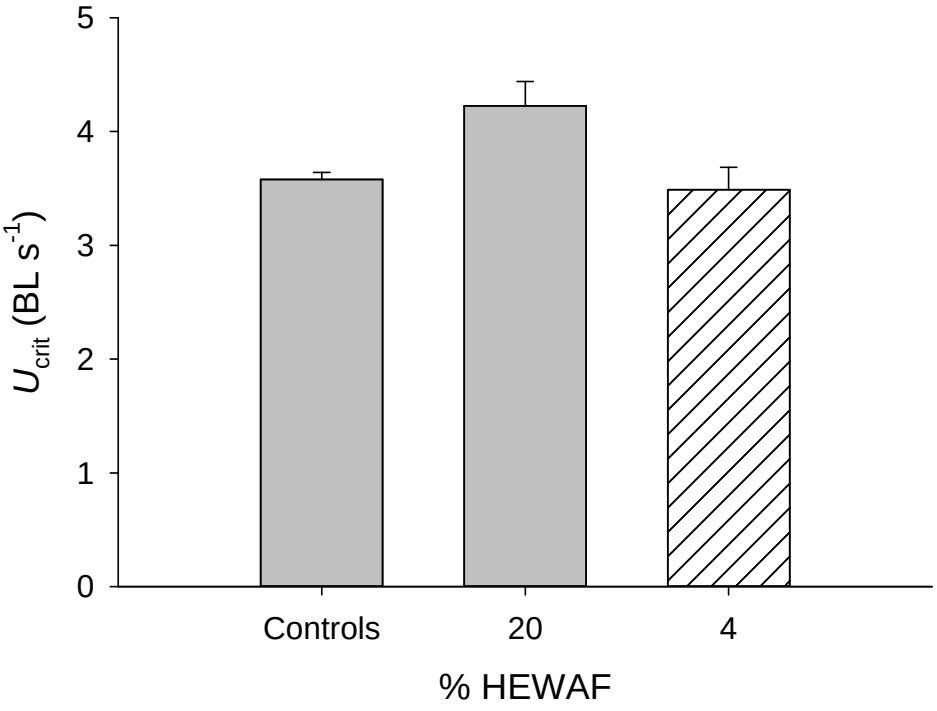

Figure 4. Mean ± SEM critical swimming speeds (U_{crit}) of juvenile mahi exposed to control conditions or 20% slick A HEWAF (320 µg L^{-1} ΣPAHs) during a swim trial or to 4% HEWAF

379 (55 µg L^{-1} ΣPAHs) for 12 h prior to swim trial (hatched bar). Sample sizes are 18, 6 and 14,
380 respectively.

381 **3.1.6 Caveats**

382 While in many respects mahi represents an exceptional model for studying the impacts of
383 the *DWH* oil spill on native pelagic fish from the GoM, there also exist a number of inherent
384 caveats that arise from using this species. For one, rearing mahi to the juvenile stage and beyond
385 is quite challenging and there is typically a paucity of fish that successfully reach the juvenile
386 stage within a cohort. Additionally, mahi have exceptionally high growth rates, limiting the time
387 during which experiments can be performed for a given cohort to approximately 2 weeks.
388 Consequently, experiments must frequently be performed using multiple cohorts from different
389 spawns. Given the limited number of fish available and fast growth rate of mahi, it is often
390 difficult or impossible to include fish from a given cohort within all desired treatments, or to do
391 so without introducing the confounding effect of size associated with rapid growth rates. Every
392 attempt was made to minimize these effects by including fish from each cohort for all treatments
393 within a given experiment and spacing treatments more-or-less evenly over time. Nevertheless,
394 cohort effects cannot be ruled out for explaining differences observed across experiments.
395 Moreover, there is a natural limit to the number of treatments that can be included per
396 experiment given the above logistical challenges. Thus, a more thorough experimental design
397 inclusive of additional treatments or more concentrations for evaluating dose-response effects
398 was precluded. Such experiments, while important, are likely more amenable using standard test
399 species (e.g., sheepshead minnow, zebrafish). Despite these challenges and limitations, the
400 information reported herein on mahi swimming performance (from a total of 177 swim trials)

represents an important first step on which to anchor future research on the interactive effects of natural stressors with crude oil exposure to native high-performing GoM fishes.

3.1.7 Conclusions

In conclusion, exposure to hypoxia alone or combined with crude oil elicits significant impairment to the swimming performance of juvenile mahi. By contrast, elevated temperature appears to ameliorate at least some effects of oil exposure on swimming performance. This study also shed additional light on the timing and duration of exposure required to elicit acute effects of oil on the swimming performance of juvenile mahi. Evidence suggests either that exposures must exceed 12 h or that there is a delay between the exposure period and the manifestation of a physiological impact. Finally, there was no indication from the present study that briefly swimming directly within crude oil contaminated water impairs swimming performance of juvenile mahi within the range of PAH concentrations that were likely to be encountered during the spill. While the present findings help illuminate the roles of combined natural stressors likely to have coincided with the *DWH* spill, clearly more research is needed to gain a more comprehensive understanding of these interactions as well as the timing and duration of exposures that elicit effects. Nevertheless, these findings advance our knowledge about the impacts of crude oil exposure on pelagic fish native to the GoM and should help inform about potential impacts from future oil spills on such commercially and ecologically important species.

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- Hypoxia alone or combined with 2% HEWAF elicited similar decreases in U_{crit}
- Elevated temperature alone or combined with 2% HEWAF did not affect U_{crit}
- Shorter 12 h exposure to 4% HEWAF did not affect U_{crit}
- 20% HEWAF exposure during swim trials did not affect U_{crit}