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Exposure to Crude Oil from the *Deepwater Horizon* Oil Spill Impairs Oil Avoidance Behavior without Affecting Olfactory Physiology in Juvenile Mahi-Mahi (*Coryphaena hippurus*)

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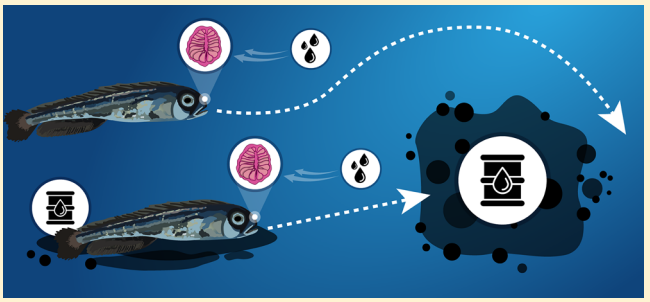
Supporting Information

ABSTRACT: The understanding of the detection threshold and behavioral response of fishes in response to crude oil is critical to predicting the effects of oil spills on wild fish populations. The *Deepwater Horizon* oil spill released approximately 4.9 million barrels of crude oil into the northern Gulf of Mexico in 2010, overlapping spatially and temporally with the habitat of many pelagic fish species. Yet, it is unknown whether highly migratory species, such as mahi-mahi (*Coryphaena hippurus*), might detect and avoid oil contaminated waters. We tested the ability of control and oil-exposed juvenile mahi-mahi (15−45 mm) to avoid two dilutions of crude oil in a two-channel flume. Control fish avoided the higher concentration (27.1 μg/L Σ_{50}PAH), while oil-exposed (24 h, 18.0 μg/L Σ_{50}PAH) conspecifics did not. Electro-olfactogram (EOG) data demonstrated that both control and oil-exposed (24 h, 14.5 μg/L Σ_{50}PAH) juvenile mahi-mahi (27−85 mm) could detect crude oil as an olfactory cue and that oil-exposure did not affect the EOG amplitude or duration in response to oil or other cues. These results show that a brief oil exposure impairs the ability of mahi-mahi to avoid oil and suggests that this alteration likely results from injury to higher order central nervous system processing rather than impaired olfactory physiology.



INTRODUCTION

The assessment of an organism's capacity to detect toxins and the evaluation of their behavioral responses to such compounds is essential for estimating the exposure of organisms in the wild and predicting the likely biological impacts of large-scale pollution events. The 2010 *Deepwater Horizon* oil spill released 4.9 million barrels of crude oil into the northern Gulf of Mexico in the largest marine oil spill in the United States history.[1,2] Crude oil was released for 87 days and overlapped spatially and temporally with the habitats of many highly migratory pelagic fish such as mahi-mahi (*Coryphaena hippurus*).[3−5] Mahi-mahi exposed to sublethal concentrations of crude oil in the laboratory have been shown to have reduced cardiac output,[6] impaired swim performance,[7,8] and reduced visual acuity;[9] however, it is not known whether mahi-mahi are capable of detecting crude oil as a chemosensory cue and whether they avoid oiled water and thus modulate their exposure.

The behavioral responses of marine organisms to petroleum-based hydrocarbons include both attraction and avoidance with variation based on concentration and chemical components. Whole kerosene has been shown to be an attractant and feeding stimulus for the American lobster (*Homarus americanus*), and kerosene-soaked bricks have even been used in some regions as bait in the commercial lobster fishery.[10] In contrast, several species of marine flatfish, a sciaenid species, and several estuarine minnow species have been shown to avoid sediments that are heavily contaminated with polycyclic aromatic hydrocarbons (PAHs),[11−13] the primary toxic components of crude oil. There is also evidence that the prevalence of one or more compounds in some forms

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of crude oil may drive behavioral patterns. Crude oil is a complex mixture containing tens of thousands of individual chemical compounds. Fresh source oil becomes weathered as it loses low molecular weight hydrocarbons through evaporation, therefore producing weathered oil with proportionately higher molecular weight and low solubility.[14] In a recent study, gulf killifish (*Fundulus grandis*), sailfin mollies (*Poecilia latipinna*), and sheepshead minnow (*Cyprinodon variegatus*) showed increasing avoidance of sediments mixed with source crude oil as the concentration of PAHs increased, but avoidance was not observed at any tested concentration when weathered crude oil was used.[12] Several studies have also investigated the behavioral responses to the water accommodated fraction (WAF) of crude oil and found avoidance in pink salmon fry (*Oncorhynchus gorbuscha*) at a threshold of 1.6 mg/L,[15] Caspian roach (*Rutilus caspicus*) at a 2 mg/L threshold,[16] and at 8.54 μg/L in the European seabass (*Dicentrarchus labrax*).[17] Further, it has been demonstrated in Caspian roach that when the nares are surgically obstructed, fish no longer avoid crude oil suggesting that avoidance is based on detection of crude oil as a chemosensory cue at the olfactory epithelium.[16]

Olfactory cues provide information to marine teleosts about predators,[18] prey,[19] migratory pathways,[20,21] and conspecifics[22] that are critical to survival. Although pelagic fish are better known for their visual acuity,[23,24] they have morphological adaptations that suggest olfactory senses may be important,[25] and yet almost nothing is known about the olfactory physiology of pelagic fishes. Mahi-mahi have olfactory epithelia with 60 lamellae in each rosette, each with 32000 olfactory sensory neurons per square mm, a similar density to bigeye tuna (*Thunnus obesus*) and striped marlin (*Kajikia audax*).[25] The pelagic environment is inherently patchy with prey distributed over great distances;[26] and in this heterogeneous environment, chemosensory cues are essential for informing the movements of pelagic fishes.[19] Even when striking prey at close range, where vision is heavily relied upon, olfactory cues influence predation strategies of pelagic fishes, affecting swimming patterns and strike behavior.[19,27] Ram ventilators, such as mahi-mahi, are constantly receiving olfactory cues from the environment as they swim, and during slower swimming, they have the capability to pump water into the olfactory chamber by modifying the position of the jaw.[28]

In order to rapidly detect chemical cues in the aquatic environment, the olfactory sensory neurons of marine organisms are directly exposed to the water and therefore, also directly exposed to any water-borne contaminants such as crude oil.[29] In multiday exposures, crude oil has been shown to cause necroses, hyperplasia, and lesions in the olfactory epithelium of marine teleosts.[30,31] Using the electro-olfactogram (EOG) technique, oil sands process affected water (OSPW), a mixture containing PAHs, has been shown to reduce olfactory function of rainbow trout (*Oncorhynchus mykiss*).[32] Additionally, Atlantic stingrays (*Hypanus sabinus*) exposed to a 10% WAF of crude oil for 48 h demonstrated a decreased amplitude and initial slope and an increased duration of the EOG response to amino acid cues prepared with a concentration of crude oil that matched exposure conditions.[33] Bicolor damselfish (*Stegastes partitus*) exposed to crude oil for 24 h (11.2 μg/L $\sum_{50}$ PAH) have also been shown to have reduced detections of a conspecific alarm cue at the olfactory epithelium.[34]

Injury resulting from exposure to a toxicant can impair detection and/or behavioral responses to olfactory cues, and an understanding of where those changes may occur is fundamental to our knowledge of the effects of oil-exposure on marine life. When odorant molecules enter the nasal cavity, they bind to G-protein coupled receptor proteins on the olfactory sensory neurons and stimulate the formation of secondary messenger molecules that open gated ion channels allowing Ca^{2+} and Na^{+} to enter the cell. Increased levels of Ca^{2+} in the neuron trigger the opening of a Ca^{2+} activated Cl^{-} channel, and the resulting efflux of Cl^{-} depolarizes the cell. This change in membrane potential, known as the generator potential, may lead to an action potential in the axons.[35] If action potentials occur, signals are sent to the olfactory bulb for signal processing before transmission to secondary neurons and higher brain centers, ultimately leading to behavioral responses.[36] Therefore, any observed behavioral modifications in response to olfactory cues following oil exposure may be the result of either impacts to the olfactory sensory neurons at the olfactory epithelium or central nervous system processing.

The successful detection of chemosensory cues and avoidance of harmful compounds is essential for individual fitness following a pollution event. Mahi-mahi habitat overlapped with surface oil after the 2010 *Deepwater Horizon* oil spill making it likely that mahi-mahi of various life stages were exposed to oil,[3−5] and yet, our understanding of whether they detect and avoid crude oil is nonexistent. For the first time, we evaluate whether control and oil-exposed mahi-mahi avoid two concentrations of oiled seawater using a two-channel flume behavioral test. Additionally, we examine whether control and oil-exposed mahi-mahi detect the WAF of crude oil, a food cue, an amino acid cue, and a bile salt cue at the olfactory epithelium using the EOG technique. Our goals were to understand if previous oil-exposure modified the behavior of mahi-mahi in response to crude oil and/or affected their olfactory physiology. An understanding of detection mechanisms and the behavioral response of marine fishes to crude oil is critical for modeling efforts to understand how fish move in space and time in response to oil spills and to predict the likely impacts of oil exposure on wild fish populations.

METHODS

Test Animals. Mahi-mahi embryos were spawned volitionally from wild captured broodstock maintained at the University of Miami Experimental Hatchery and raised to an early juvenile stage.[37] Fish used in two-channel flume experiments were from two separate spawns and were used from October 23 to November 2 in 2015. All fish used in flume experiments were 18−30 days post hatch and ranged in size from 15 to 45 mm fork length. Fish used in EOG experiments were from three separate spawns and were used from November 25−29 in 2018 and from May 5−29 in 2019. Fish used in EOG experiments were 33−50 days post hatch and ranged in size from 27 to 85 mm fork length. Seawater used in all experiments was UV-sterilized and collected from the experimental hatchery to match the temperature, pH, and salinity profiles of the water in which mahi-mahi were raised. All experiments on mahi-mahi were performed under the University of Miami IACUC protocol # 15−019.

Oil Preparation and Exposure. The high-energy water accommodated fraction (HEWAF) was prepared the day that exposures were initiated from slick oil collected from the sea surface during the *Deepwater Horizon* spill (sample ID: OFS-

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Table 1. Mean Water Chemistry Parameters ± Standard Deviation for 24 h Control and Oil Exposure Incubations of Juvenile Mahi-Mahi Used in Two-Channel Choice Flume and EOG Experiments

Treatment	Temperature (°C)	pH_{NBS}	Dissolved Oxygen(mg/L)	Salinity (ppt)	Ammonia(μMol/L)	Experiment Type
Control	24.7 ± 0.3	8.05 ± 0.02	6.12 ± 0.24	33 ± 0	NA	Choice Flume
Oil-exposed	25.0 ± 0.6	8.06 ± 0.02	6.23 ± 0.08	33 ± 0	NA	Choice Flume
Control	25.8 ± 0.7	8.07 ± 0.07	6.95 ± 0.17	36 ± 0	8.42 ± 5.12	EOG
Oil-exposed	25.7 ± 0.2	8.04 ± 0.05	6.56 ± 0.30	36 ± 0	12.68 ± 4.52	EOG

20100719-Juniper-001 A00884). To make the HEWAF, 1 g of oil per liter of seawater was blended at low speed for 30 s in a Waring CB 15 blender (Torrington, CT). The seawater and oil blend was immediately transferred to a glass separatory funnel where the mixture settled for 1 h before 90% of the volume was drained and used as the 100% HEWAF. The stock HEWAF was diluted with seawater to a 6% static exposure treatment, and mahi-mahi were immediately introduced to either a control or 6% HEWAF 24 h exposure. A 6% HEWAF exposure was chosen because it is within the range of concentrations measured in the Gulf of Mexico after the spill,[38] and it is typically sublethal but contains a sufficient concentration of PAHs to observe detrimental effects on morphology and/or physiology in mahi-mahi.[7]

The HEWAF of crude oil was additionally used as a cue in both two-channel flume choice and EOG experiments. In flume experiments, a 2% HEWAF was used to represent a low concentration cue and a 6% HEWAF was used to represent a high concentration cue. For EOG experiments, a full strength HEWAF was made as described previously with the exception that 5 g of oil was blended with 1 L of seawater and this 100% HEWAF was used full strength as a cue. This increased loading rate was used in order to account for both the dilution that occurs as cues are introduced into the experimental recording tank in an underwater EOG, and the signal loss that is inherent in seawater EOGs.[39]

For both two-channel flume choice and EOG tests, fish were housed identically prior to experimentation. Throughout 24 h control and oil exposures, mahi-mahi were fasted and maintained in a temperature-controlled room with a photoperiod of 16:8 h of light dark cycle. Initial and final water quality metrics were recorded for temperature and dissolved oxygen using a ProODO optical probe (YSI, Inc.,Yellow Springs, OH); pH was measured with a PHM201 meter (Radiometer, Copenhagen, Denmark), and salinity was assessed with a refractometer. Final water samples were collected from all tanks to evaluate ammonia concentrations in the static exposure and ensure that ammonia levels did not exceed toxic thresholds. A micromodified colorimetric assay with indophenol blue was used for all ammonia measurements.[40] For all experiments, mahi-mahi were exposed to the HEWAF of crude oil for 24 h, used in experiments immediately following exposure, and euthanized by pithing immediately following testing.

Water samples were collected directly into 250 mL amber bottles from each oil-exposure tank at the beginning and end of 24 h exposures for both behavior and EOG experiments to quantify Σ_{50}PAHs. For two-channel flume experiments, one water sample was additionally collected for each concentration used in flume experiments from each batch of HEWAF used and a matching sample was collected of control seawater. These samples were collected from the flume test arena where fish were tested so that the PAHs fish encountered in the flume were measured precisely. A sample was collected of the 100% HEWAF (5g/L) used for the EOG oil cue. Water samples were chilled and shipped on ice overnight to ALS environmental (Kelso, WA) for gas chromatography/mass spectrometry—selective ion monitoring (GC/MS−SIM; based on EPA method 8270D). In our EOG experiments, we assumed that some quantity of PAHs were absorbed by our EOG cue delivery tubing and therefore we collected samples of the EOG oil cue from the terminal end of the tubing throughout a typical EOG experiment. The concentrations of these samples were calculated using an established fluorescence method[41] and were related to the concentration of the 100% HEWAF (5g/L) as described in Esbaugh et. al.[42] Exposure concentrations are reported as the geometric mean of 50 individual PAHs. A summary of all mahi-mahi sizes, measured water quality parameters, and a representative PAH profile are available in Supporting Information (Figure S1; Tables S1 and S2).

Two-Channel Flume Experiments. Flume experiments were designed to test whether juvenile mahi-mahi would avoid the HEWAF of crude oil at either a low or a high PAH concentration and whether a previous oil-exposure would modify that behavior. The two-channel choice flume (13 × 4 cm) was gravity fed from two separate tanks and was divided down the midline for half the length with fish able to move freely between both streams of water.[43] Laminar flow and separation of the two water streams through the test arena was previously verified in this exact flume[44] and was confirmed before and throughout behavior testing. Water flow was maintained throughout experiments at a constant flow rate of 200 mL per minute using a Dwyer Mini-Master Flowmeter (MMA-38, Michigan City, Indiana) and was temperature controlled to match exposure conditions (Table 1, Table S2).

Four tests were conducted in the flume and the Σ_{50}PAH concentrations for each 24 h exposure and HEWAF cue used in each test are presented in Table 2. A negative control group (n = 20) was tested with control mahi-mahi and untreated seawater on either side of the flume to evaluate whether any side bias existed. A low (2% HEWAF, n = 52) and a high (6% HEWAF, n = 42) concentration HEWAF test were additionally run with fish held in control conditions for 24 h. In these tests, control seawater was used on one side of the flume and the HEWAF cue on the other side. These fish encountered the HEWAF of crude oil in the flume but were held in control conditions before testing and are therefore referred to as control mahi-mahi. An additional high concentration HEWAF cue (6% HEWAF) test was completed for fish exposed to oil for 24 h (6% HEWAF, n = 26), and these mahi-mahi are referred to as oil-exposed. It was not possible to switch the side of the flume that held the oil cue within a test due to the fact that substantial cleaning was required to eliminate PAHs from the flume. Therefore, fish were tested with the oil cue only on one side of the flume; the flume was thoroughly cleaned at the completion of each day's tests, and the side of the flume that held the oil cue was alternated daily. On days where both the

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Table 2. $\sum_{50}$ PAH Data for Two-Channel Flume Choice and EOG Experiments[a]

Experiment Type	HEWAF Application (Cue or 24 h Exposure?)	Target Dilution (% HEWAF)	Geometric Mean $\sum_{50}$PAH (μg/L) ± SEM
Choice Flume	Exposure	0 (Control)	0.03 ± 0.001
Choice Flume	Exposure	6	18.0 ± 3.2
Choice Flume	Cue for control and oil-exposed fish	0 (Control)	0.03 ± 0.005
Choice Flume	Cue for control fish	2	7.5 ± 0.4
Choice Flume	Cue for control fish	6	27.1 ± 1.0
Choice Flume	Cue for oil-exposed fish	6	31.1 ± 0.5
EOG	Exposure	6	14.5 ± 8.3
EOG	Cue for both control and oil-exposed fish	100	818.3 ± 37.4

[a]Samples for 24 h exposures were collected at the beginning and end of 24 h exposures. Samples for the HEWAF cues used in two-channel flume choice experiments were collected at the base of the flume test arena for each new batch of HEWAF. EOG oil cue samples were collected from 100% HEWAF and from the terminal end of the cue-delivery tubing.

low concentration oil cue and the high concentration oil cue were tested, the low concentration oil tests were always completed first.

All of the fish tested with the oil cues were centered in the downstream end of the flume and given a two-minute habituation period. Following habituation, the position of the fish in the flume was noted every five seconds for two minutes. The negative control fish that were tested with seawater on both sides of the flume were given this same protocol twice, such that at the end of the two-minute test the water streams were switched and the fish were recentered in the flume, allowed a second two-minute habituation period, and following the habituation, their position in the flume was again recorded every five seconds for a two-minute test period.

Electro-Olfactogram Experiments. EOG experiments were designed to evaluate whether observed behavioral responses of oil-exposed mahi-mahi were explained by reduced sensitivity of the olfactory epithelium to crude oil and whether oil-exposure affected the detection of other olfactory cues. Underwater EOG recordings for control (n = 12) and oil-exposed (n = 12, Table 2) mahi-mahi commenced immediately following a control or oil exposure and were done blind such that the researcher performing EOG recordings did not know whether a fish was a control or oil-exposed individual. Fish were anesthetized with 0.2 g/L MS-222 (tricaine methanesulfonate, Western Chemical, Inc., Ferndale, WA) buffered with $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO) and moved to a submerged recording chamber where they were ventilated with aerated culture water containing 0.1 g/L MS-222.

The EOG technique measures the potential change in the water immediately above the surface of the olfactory epithelium that is induced by odors. To access the olfactory epithelium, the septum was surgically removed, and a recording electrode was placed adjacent to the longest lamella on the ventral side of the epithelium. A reference electrode was placed into the skin just posterior to the eye. To make electrodes, 1.5 mm glass capillary tubes (Warner Instruments, Hamden, CT) were heat-pulled to a thin tip, filled with 3 M potassium chloride (Sigma-Aldrich), and fitted to electrode holders with nonpolarizable Ag-AgCl electrodes (Warner Instruments).

The 100% HEWAF (5 g/L loading rate, Table 2) of crude oil was used as a cue to determine whether oil-exposed and control mahi-mahi could detect crude oil at the olfactory epithelium. Additionally, 10^{-2} M L-alanine (Ala 10^{-2}; Sigma-Aldrich), 10^{-3} M L-alanine (Ala 10^{-3}; Sigma-Aldrich), a food pellet extract (PT, Otohime C2 Pellet, Reed Mariculture, Inc., Campbell, CA), a 10-fold dilution of the food pellet extract (10% PT), and 10^{-3} M taurocholic acid (TCA; Sigma-Aldrich) were used as olfactory cues to determine the effect of oil-exposure on a variety of cue types. The food pellet extract was made by soaking 5.4 g of pellets in 800 mL of seawater for 12 h and filtering it through 100 μm mesh. This batch of pellet cue was frozen in 10 mL aliquots at −20 °C, and each aliquot was further diluted with an additional 40 mL of seawater upon thawing.

Olfactory cues were selected to represent a variety of types and concentrations. L-alanine binds to microvillous olfactory receptor neurons and represents a prey cue, while taurocholic acid binds to ciliated olfactory receptor neurons and represents an alarm cue.[45] Seawater was mixed with 0.1 g/L MS-222 to match the water chemistry of the submerged recording chamber and was used to perfuse the olfactory epithelium between cue delivery, as a negative control cue, and to make all cues. Cues were delivered to the olfactory epithelium for three seconds via an eight-channel perfusion valve control system (Warner Instruments) that allowed smooth transition from perfusion, to cue delivery, and back to perfusion without interrupting flow speed or volume. All cues were administered in three blocks with each cue spaced by 90 s and delivered once per block in a randomized order. One blank, or negative control cue, was administered with each block of cues.

The EOG amplitude (mV), duration (s), integral (mV·s), mean (mV), average slope (mVs^{-1}), and maximum slope (mVs^{-1}) were measured by differentially amplifying 1000x (DP-311, Warner Instruments), filtering (0.1 Hz-0.1 kHz, 50/60 Hz; DP-311, Warner Instruments), digitizing and filtering (60 Hz, Power Lab, AD Instruments, Dunedin, NZ), and recording (Chart Software v. 8.1.3, AD Instruments, Colorado Springs, CO) the output from the recording and reference electrodes. For each EOG response, the negative control response was subtracted.

Statistical Analysis. All statistical analyses were conducted in R.[46] A significant difference was determined a priori to be α = 0.05. Akaike's Information Criterion (AIC) was used to discriminate among competing formulations when multiple models were assessed.[47,48]

For two-channel flume choice test data, the proportion of time spent in the cue, or the proportion of time on the left side of the flume for negative control fish, was arcsine transformed. It was our intent to test oil-exposed mahi-mahi at the same high concentration of PAHs in the flume at which the control fish had been tested; however, the concentration in the flume for the 6% HEWAF cue was measured to be slightly, but significantly, higher for the oil exposed fish (Table 2). Therefore, we used a one-way analysis of variance (ANOVA) to test whether each of the test groups (negative control mahi-mahi, control fish with 2% HEWAF, control fish with 6% HEWAF, and oil-exposed fish with 6% HEWAF) predicted the transformed time in the cue, and a Bonferroni correction was used for pairwise comparisons. A Student's t test was used to

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compare the time that the negative control group spent in the cue to the expected no preference statistic of 50% time on a side.

The amplitude and duration of the EOG response were selected as the variables of interest as they were not correlated with one another, and a principle components analysis showed they were expressing different types of information (Figure S2). The "lme4" package was used to create linear models assessing the effect of the cue type, 24 h exposure, and their interaction on the EOG amplitude and duration with individual fish added as a random effect.[49] The "multcomp" package was used *posthoc* to examine differences in the response to cues between the oil-exposed and control treatment groups.[50]

RESULTS AND DISCUSSION

This study is the first to examine the ability of control and oil-exposed mahi-mahi to detect and avoid crude oil, and our results demonstrate that time spent on one side of the flume was significantly affected by treatment group (Figure 1, Table S3). Control fish showed increasing avoidance of the oil cue as the concentration of crude oil increased in the flume spending an average of 43.0 ± 4.5% of their time in the 2% HEWAF cue (7.5 μg/L Σ_{50}PAH) and 34.4 ± 4.5% of their time in the 6% HEWAF cue (27.1 μg/L Σ_{50}PAH, Figure 1). However, only control mahi-mahi tested with the 6% HEWAF cue behaved significantly differently than the negative control fish, demonstrating that control mahi-mahi in our study only avoided the higher concentration cue (Figure 1). These data demonstrate a much lower avoidance threshold than has been shown for pink salmon (1.6 mg/L)[15] or Caspian roach (2 mg/L)[16] and avoidance on the same order of magnitude as European seabass (8.54 μg/L).[17] In addition, the present study demonstrates avoidance of the HEWAF of weathered crude oil, which was not seen in gulf killifish, sailfin mollies, or sheepshead minnows when weathered oil was mixed into sediment.[12] These findings may suggest that weathering state, exposure mechanism, and interspecific differences can affect detection processes, although it cannot be ruled out that differences in the composition of each test oil or variations in oil preparations contributed to differences among studies. Additionally, large differences in overall sensitivity to oil exposure between coastal species and pelagic fishes, such as mahi-mahi, may also contribute to this discrepancy in detection thresholds.[51]

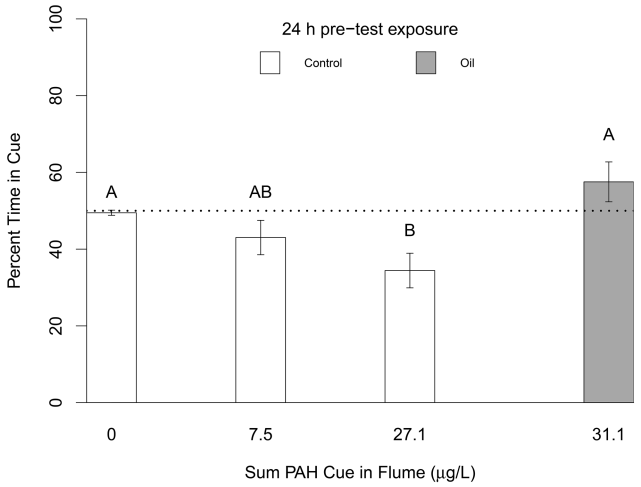

Figure 1. Control juvenile mahi-mahi were tested in a two-channel flume with control seawater on both sides of the flume (n = 20), with control seawater against a 2% high energy water accommodated fraction (HEWAF) of crude oil (7.5 μg/L Σ_{50}PAH, n = 52) and with control seawater against a 6% HEWAF of crude oil (27.1 μg/L Σ_{50}PAH, n = 42). Oil-exposed juvenile mahi-mahi (24 h, 18.0 μg/L Σ_{50}PAH, n = 26) were tested with control seawater against a 6% HEWAF of crude oil (31.1 μg/L Σ_{50}PAH). The dotted line shows the expected no preference statistic of 50% time on each side. Letters denote significant differences between groups using a one-way ANOVA with pairwise comparisons with a Bonferroni correction. Values are mean ± standard error.

Two-channel flume data show that control mahi-mahi avoid concentrations of PAHs that have been shown to cause reductions in maximum aerobic swimming speed in mahi-mahi of the same age class.[7] However, although our study demonstrates avoidance of the 6% HEWAF (27.1 μg/L Σ_{50}PAH) cue, control mahi-mahi in our study still spent 34% of their time in the 6% HEWAF cue indicating that their ability to detect and avoid crude oil may reduce, but will not prevent, harmful exposures entirely. Moreover, mahi-mahi exposed to 0.67 μg/L Σ_{50}PAH from 6 to 36 h post fertilization have been shown to have a significantly reduced optomotor response compared to control fish when they were assessed at seven to 10 days post hatch.[9] Additionally, juvenile red drum (*Sciaenops ocellatus*) exposed to only 5.7 μg/L Σ_{50}PAH for 24 h were more likely to be subordinate in paired behavior experiments.[52] These recent studies illustrate that deleterious behavioral and physiological effects can take place at concentrations below the avoidance threshold for mahi-mahi.

In contrast to the control mahi-mahi, following a 24 h oil exposure (18.0 μg/L Σ_{50}PAH) mahi-mahi spent 57.5 ± 5.2% of their time in the 6% HEWAF cue (31.1 μg/L Σ_{50}PAH), significantly more time than control fish spent in the 6% HEWAF cue (Figure 1). Oil-exposed mahi-mahi did not spend significantly more time in the 6% HEWAF cue than the negative controls spent on a side, demonstrating that they showed neither attraction nor avoidance of the cue. Negative control fish spent 49.5% ± 0.7% of their time on the left side of the flume, which was not statistically different than the expected no preference statistic of 50%, confirming that side bias was not a factor in these experiments.

Several studies have examined the crude oil avoidance threshold of freshwater and marine fish,[11,12,15−17] but we are not aware of any prior study that has examined the effect of oil exposure on oil-avoidance. Care is needed in extrapolating lab-based studies to wild fish populations; however, electronic tag data from adult blue marlin (*Makaira nigricans*) suggests that despite an overlap of suitable habitat with the area affected by the *Deepwater Horizon* oil spill, blue marlin spent less time within the oiled region in the northern Gulf of Mexico in the summer of 2010 than they had in previous years, indicating they may have avoided heavily oiled areas.[4] In contrast, adult bluefin tuna (*Thunnus thynnus*) tagged with electronic tags spent several weeks around the Macondo well during the spill and were predicted to have spawned in the region, suggesting that not all large pelagic fishes avoided oiled waters.[53] While electronic tagging data can provide powerful insight into behaviors of fish in the wild it is important to remember that these observations come from very few individuals and that large errors are inherent in estimating locations from tag data.[54] Empirical observations of oil avoidance are therefore a

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much more powerful tool to understand the effect of oil spills. However, the observations of blue marlin and bluefin tuna during the *Deepwater Horizon* spill are not inconsistent with our findings, which show that mahi-mahi can detect oil and may adjust behavior to reduce exposure without completely avoiding the oil. While species and life history specific differences likely exist, it remains to be seen how migration patterns, foraging behavior, or oceanographic conditions would influence oil avoidance in the wild.

The EOG results demonstrate that control and oil-exposed (24 h, 14.5 μg/L Σ_{50}PAH) juvenile mahi-mahi detect crude oil at the olfactory epithelium (Figure 2). Interestingly, we

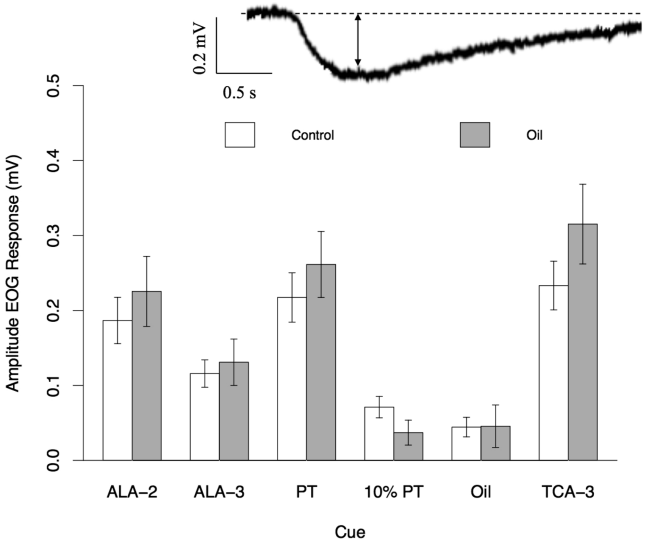

Figure 2. Mean (±standard error) electro-olfactogram (EOG) response amplitude (mV) with the negative control cue subtracted for control and oil-exposed mahi-mahi. A representative EOG response is shown to indicate the measured parameter. ALA-2 = L-alanine 10^{-2} M, ALA-3 = L-alanine 10^{-3} M, PT = food pellet cue, 10% PT = 10% dilution food pellet cue, Oil = high energy water accommodated fraction of crude oil, TCA-3 = taurocholic acid 10^{-3} M. No significant differences were found between the EOG amplitudes of oil-exposed and control individuals ($p > 0.05$). Across treatment groups, all olfactory cue amplitudes were significantly different from one another ($p < 0.05$) with the exception of the food pellet cue and L-alanine 10^{-2} M ($p > 0.05$), taurocholic acid 10^{-3} M and L-alanine 10^{-2} M ($p > 0.05$), 10% food pellet and L-alanine 10^{-3} M ($p > 0.05$), taurocholic acid 10^{-3} M and food pellet ($p > 0.05$), and 10% food pellet and oil ($p > 0.05$).

detected no differences in either the amplitude or the duration of the EOG response to any of the cues used between control and oil-exposed fish (Figures 2 and 3, Tables S4–S7). These results suggest that a 24 h oil exposure at 14.5 μg/L Σ_{50}PAH does not damage the ability of the olfactory sensory neurons to detect olfactory cues, at the cue concentrations we applied, when fish are assessed in control water. These results stand in contrast to several recent studies that have found disruptions to olfactory physiology after exposure to crude oil or to OSPW, a byproduct from the surface mining of oil sands.[32,33] However, several of these studies delivered olfactory cues mixed into the same concentration of toxicant that was used in exposures, rather than testing toxicant-exposed fish in clean water as was done in the present study. Delivering olfactory cues mixed into toxicants is done to mimic exposure conditions but has the confounding effect of the possible

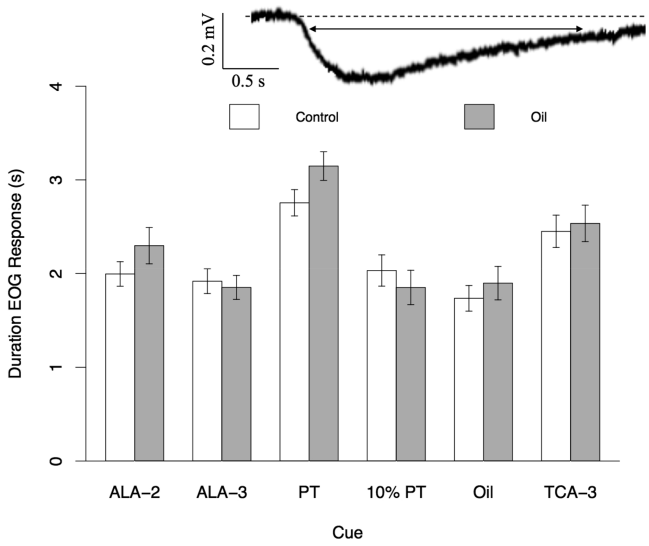

Figure 3. Mean (±standard error) electro-olfactogram (EOG) response duration (s) for control and oil-exposed mahi-mahi. A representative EOG response is shown to indicate the measured parameter. ALA-2 = L-alanine 10^{-2} M, ALA-3 = L-alanine 10^{-3} M, PT = food pellet cue, 10% PT = 10% dilution food pellet cue, Oil = high energy water accommodated fraction of crude oil, TCA-3 = taurocholic acid 10^{-3} M. No significant differences were found between the EOG durations of oil-exposed and control individuals ($p > 0.05$). Across treatment groups, the duration of the food pellet cue was significantly different than the duration of all other cues ($p < 0.05$) and the duration of taurocholic acid 10^{-3} M was significantly different than the duration of L-alanine 10^{-3}, 10% pellet cue, and oil ($p > 0.05$).

interaction of the toxicant and the cue either in the exposure medium or when interacting with olfactory sensory neurons at the olfactory epithelium.[33] Additionally, while profound disturbances to the olfactory physiology of oil-exposed Atlantic stingrays have been found, the crude oil exposure concentrations were substantially greater than those used in the present study. Moreover, OSPW, while it contains alkylated PAHs, is fundamentally different than the crude oil spilled in the *Deepwater Horizon* disaster and therefore may have different effects on the olfactory sensory neurons. In another recent study that identified reduced detections of conspecific alarm cue in oil-exposed bicolor damselfish using the EOG technique, effects were specific to detections of the alarm cue, illustrating that impacts of oil exposure may be specific to particular cues and/or particular olfactory sensory neurons.[55] More work is required to determine which components of petrogenic contamination are responsible for altered responses to olfactory cues.

Although our EOG oil cue was measured to be substantially more concentrated than the oil cues we used in our behavioral experiments (Table 2), our EOG results nonetheless suggest that control fish in our flume choice experiments were detecting one or more components of crude oil as an olfactory cue and choosing to avoid the more concentrated HEWAF cue in the flume (Tables S8 and S9). In our EOG experiment, cues were delivered just above the olfactory epithelium into the seawater filled experimental chamber and were therefore diluted below our measured concentration before reaching the olfactory epithelium. Additionally, due to the high conductance of seawater, signal loss is inherent in seawater EOGs, and that signal loss dictates that cues be more

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concentrated to make successful recordings.[39] Further, even in freshwater, it has been previously shown that behavioral responses take place at a concentration 10-fold lower than can be measured using the EOG technique.[45,56]

Our EOG data demonstrate that after a 24 h oil exposure, fish detected crude oil at the olfactory epithelium but did not avoid the HEWAF cue in our behavior assay (Figures 1 and 2). These data suggest that if mahi-mahi are unable to avoid an initial oil exposure they will no longer seek to do so, despite being able to detect the crude oil cue, and this pattern has the potential to increase the PAH exposure that mahi-mahi experience. These results indicate that the altered behavior we observed in flume choice tests between oil-exposed and control fish is likely due to an impact on some aspect of central nervous system processing. It is additionally possible that gustation played some role in the avoidance of the crude oil cue, and impairment of gustatory receptors could have contributed to the altered behavioral results in our oil-exposed fish. However, because gustation was not found to contribute to oil avoidance in Caspian roach,[16] we find it more likely that alterations to central nervous system processing explain our behavioral results.

Despite the fact that we observed no significant differences in the EOG amplitude or duration between oil-exposed and control mahi-mahi, we did observe interesting differences in the variation in the EOG amplitude between treatment groups. The coefficient of variation (CV) for EOG amplitude responses was consistently greater in the oil-exposed group within a particular olfactory cue, but for the most weakly detected cues (10% pellet cue and the oil cue), the CV was more than twice as large in the oil-exposed group as in the control group (Table S10). These data suggest that oil-exposed mahi-mahi have a more variable response at the olfactory epithelium than control fish, and this greater inconsistency in detecting the more dilute chemosensory cues may contribute to the altered behavior that we observed in our flume choice experiments. Similarly, in our flume choice experiments, we saw at least a 9-fold increase in the CV in all the test groups that were tested with the WAF of crude oil in the flume compared to the negative control group. However, the oil exposed group tested in the flume had a lower CV than either of the control groups tested with crude oil (Table S10). These findings suggest a complex relationship between input from olfactory sensory neurons and behavioral outcomes that merits further investigation.

Taken together the results of our flume and EOG trials suggest an effect on nervous system processing of signals elicited by olfactory cues following a 24 h oil exposure that did not affect olfactory physiology. A recent study examining the effects of CO_2 exposure on behavior, olfactory function, and central nervous system processing in ocean phase coho salmon (*Oncorhynchus kisutch*) similarly saw behavioral impacts with no significant disruptions at the olfactory epithelium.[57] Williams and colleagues found that salmon exposed to CO_2 did not avoid a skin extract as controls did, but they did not detect any significant alterations to the olfactory physiology or gene expression within the olfactory epithelium. However, they did find significant differences in signaling and gene expression in the olfactory bulb illustrating that the behavioral alterations they observed were due to central nervous system processing injuries rather than diminished olfactory function. Although CO_2 and crude oil are fundamentally different, the results of our study suggest a similar injury to juvenile mahi-mahi following a brief environmentally realistic oil-exposure. Additionally, this research highlights the importance of using multiple assessment tools to evaluate the effect of a toxicant in order to better understand the mechanisms involved in behavioral alterations.

For the first time, we demonstrate that mahi-mahi avoid oiled water in favor of unoiled seawater and that avoidance seems to increase proportionately to the concentration of crude oil in the water. However, a 24 h oil exposure is sufficient to disrupt that behavior, and mahi-mahi will no longer avoid oiled water following a brief oil exposure. We further demonstrate that both oil-exposed and control mahi-mahi can detect crude oil as an olfactory cue and that oil-exposed mahi-mahi do not show any differences in their physiology at the olfactory epithelium as compared to controls, suggesting that the observed behavioral alterations are occurring in the olfactory bulb or higher brain centers rather than at the olfactory epithelium. These data suggest that mahi-mahi approaching heavily oiled areas may be able to avoid those areas, but the fish that are unable to escape oil-exposure will make no attempt to choose clean water and will likely experience continued exposure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b05240.

> Profile and concentrations of the 50 measured polycyclic aromatic hydrocarbons (PAHs) from a representative 24 h oil-exposure, a principle components analysis for mahi-mahi electro-olfactogram (EOG) data, morphometric data on mahi-mahi used in two-channel flume choice and EOG experiments, water chemistry data for water used in two-channel flume experiments, model fit for linear model fitted to flume data, model fit and comparison statistics for models fitted to EOG amplitude and duration data, parameters of best models fitted to EOG amplitude and duration data, parameters for linear mixed effect models for EOG amplitude and duration, data comparing responses from oil-exposed and control mahi-mahi, linear mixed effect model comparisons for EOG amplitude and duration data across treatments, and the coefficient of variation of flume choice and EOG amplitude data PDF)

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Notes

The authors declare no competing financial interest.

Additional data for this article are publicly available through the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC) available at https://data.gulfresearchinititative.org DOI: 10.7266/n7-jhhx-4k44[58] and DOI: 10.7266/N7KH0KQ9.[59]

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