Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2019, 53, 10993−11001

pubs.acs.org/est

# Damsels in Distress: Oil Exposure Modifies Behavior and Olfaction in Bicolor Damselfish (*Stegastes partitus*)

Lela S. Schlenker,[*,†] Megan J. Welch,[‡] Tricia L. Meredith,[§] Edward M. Mager,[†,∥] Ebrahim Lari,[⊥,$] Elizabeth A. Babcock,[†] Greg G. Pyle,[⊥] Philip L. Munday,[‡] and Martin Grosell[†]

[†]Department of Marine Biology and Ecology, University of Miami, Rosenstiel School of Marine and Atmospheric Sciences, 4600 Rickenbacker Causeway Miami, Florida 33149, United States

[‡]ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD, Australia 4811

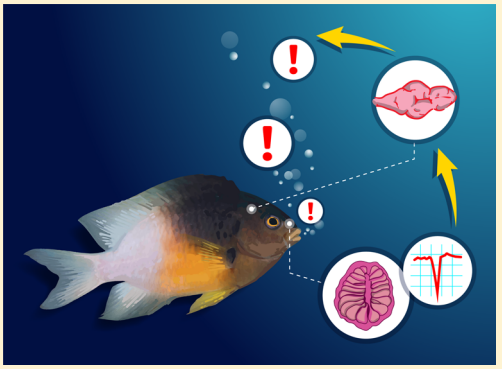
[§]Florida Atlantic University, 777 Glades Road, Boca Raton, Florida 33431, United States

[∥]Department of Biological Sciences and Advanced Environmental Research Institute, University of North Texas, 1511 W. Sycamore Street, Denton, Texas 76203, United States

[⊥]Department of Biological Sciences, University of Lethbridge, Lethbridge, AB T1K 3M4, Canada

Supporting Information

**ABSTRACT:** In fishes, olfactory cues evoke behavioral responses that are crucial to survival; however, the receptors, olfactory sensory neurons, are directly exposed to the environment and are susceptible to damage from aquatic contaminants. In 2010, 4.9 million barrels of crude oil were released into the northern Gulf of Mexico from the *Deepwater Horizon* disaster, exposing marine organisms to this environmental contaminant. We examined the ability of bicolor damselfish (*Stegastes partitus*), exposed to the water accommodated fraction (WAF) of crude oil, to respond to chemical alarm cue (CAC) using a two-channel flume. Control bicolor damselfish avoided CAC in the flume choice test, whereas WAF-exposed conspecifics did not. This lack of avoidance persisted following 8 days of control water conditions. We then examined the physiological response to CAC, brine shrimp rinse, bile salt, and amino acid cues using the electro-olfactogram (EOG) technique and found that WAF-exposed bicolor damselfish were less likely to detect CAC as an olfactory cue but showed no difference in EOG amplitude or duration compared to controls. These data indicate that a sublethal WAF exposure directly modifies detection and avoidance of CAC beyond the exposure period and may suggest reduced predator avoidance behavior in oil-exposed fish in the wild.



## INTRODUCTION

The olfactory sensory neurons (OSNs) of marine organisms are directly exposed to the environment, which allows for rapid detection of olfactory cues essential for navigation,[1,2] settlement,[3] social interactions,[4] spawning behavior,[5] assessing habitat quality,[6] and evading predation.[7] Olfactory cues provide information critical to survival and can be detected over great distances, at low concentrations, and in conditions that can make other senses ineffective.[8] However, while this direct connection between the OSNs and the environment is essential, it also exposes OSNs to aquatic contaminants such as crude oil.[9]

In 2010, the *Deepwater Horizon* (*DWH*) blowout released approximately 4.9 million barrels of crude oil into the northern Gulf of Mexico over an 87 day period, resulting in the largest documented oil spill in United States history.[10,11] While some of the oil evaporated at the sea surface, formed surface slicks, or was mechanically removed or burned, as much as 31% of the total oil spilled is estimated to have ended up in shallow and deep-water sediments.[12,13] Demersal reef fishes in the northern Gulf of Mexico, such as the bicolor damselfish (*Stegastes partitus*), were heavily impacted by the spill and experienced severe population declines,[14−16] likely due in part to their close association with the benthos, affinity to a specific territory, and consumption of benthic algae and prey.[17,18] In addition to the outright mortality that occurred following the oil spill,[15,16] transcriptomic and morphological data from marine teleosts exposed to sublethal concentrations of crude oil have suggested sensory and nervous system impairment.[19−21] However, despite the expansive range of research completed following the spill and the link between physiology, behavior, and ecological processes, very little is known about the effects of crude oil exposure on olfactory physiology and associated impacts on behavior.

Received: July 1, 2019
Revised: August 22, 2019
Accepted: August 26, 2019
Published: August 26, 2019






DOI: 10.1021/acs.est.9b03915
Environ. Sci. Technol. 2019, 53, 10993−11001

Table 1. Mean Water Chemistry Parameters ± Standard Deviation for 24 h Control and WAF-Exposure Treatments of Bicolor Damselfish Used in Two-Channel Flume and Electro-Olfactogram Experiments

| | Temperature (°C) | $pH_{NBS}$ | Dissolved Oxygen (mg/L) | Salinity (ppt) | Ammonia (μMol/L) |
|---|---|---|---|---|---|
| Control | 25.8 ± 0.6 | 8.10 ± 0.05 | 6.76 ± 0.21 | 32.6 ± 1.2 | 20.82 ± 9.68 |
| WAF-Exposure | 25.8 ± 0.6 | 8.10 ± 0.06 | 6.66 ± 0.28 | 32.9 ± 1.4 | 19.14 ± 6.29 |

To assess the lasting implications of the 2010 spill on marine fishes, it is essential to understand the impacts of crude oil on key sensory systems, like the olfactory system, and behavior of marine teleosts. In vertebrates, the process of olfactory detection begins when olfactory molecules in the water bind to G-protein-coupled receptor proteins on olfactory sensory neurons (OSNs) covering the olfactory epithelium (OE).[22] Binding releases second messengers that open gated ion channels which indirectly leads to cell depolarization and the increased likelihood of action potentials.[22,23] If they occur, resulting action potentials are transmitted to the olfactory bulb (OB) where signal processing takes place before they are relayed to secondary neurons and higher brain centers.[22] Therefore, behavioral disruptions that may occur as a result of an exposure to a toxicant[24−26] could result from either impairment peripherally at the OE or an effect on higher order central nervous system (CNS) processing. The link between olfaction and behavior has been demonstrated for fish exposed to contaminants,[9,27] and both metrics are critical predictors of fitness in marine fishes.[28,29]

Exposure to crude oil or individual polycyclic aromatic hydrocarbons (PAHs), the primary toxic constituents of crude oil, are known to be detrimental to the health of OSNs and the OE.[30−33] Pink salmon (*Oncorhynchus gorbuscha*) exposed to sublethal concentrations of benzene showed an 83% reduction in OE cilia and increased mucus production compared to control fish.[30] Killifish (*Fundulus heteroclitus*) exposed to naphthalene[31] developed necrotic areas throughout the OE, a condition also observed in conjunction with OE hyperplasia in inland silversides (*Menidia beryllina*) and hogchoker (*Trinectes maculatus*) exposed to crude oil.[33] Similarly, Atlantic silversides (*Menidia menidia*) exposed to crude oil exhibited lesions and hyperplasia of the OE.[32]

Chemical alarm cue (CAC) released from injured conspecifics has long been shown to elicit behavioral responses in teleost fishes[34,35] and has been established as an olfactory cue in marine and freshwater teleosts.[36−38] Several species of damselfish are known to display an innate avoidance of CAC,[29,35] a behavior that is hypothesized to have an evolutionary role as a protective mechanism against predation.[29] Fish rely on the detection of CAC as an olfactory cue to avoid predation;[39] therefore, impairment of OSNs that would interfere with CAC detection has the potential to have direct effects on the survival of wild fish.[40,41]

Recent studies have separately demonstrated behavioral and olfactory impacts to marine fishes briefly exposed to the water accommodated fraction (WAF) of crude oil from the *DWH* spill. WAF-exposed coral reef fish display reduced settling to an experimental reef, less shoaling, increased boldness, and a 2.7-fold increase in mortality as a result of predation.[24] Similarly, WAF-exposed juvenile red drum (*Sciaenops ocellatus*) were found to be more likely to be subordinate than controls in dyad experiments.[25] Use of the electro-olfactogram (EOG) technique to measure the odor-evoked extracellular field potential, an indirect measurement of the OE generator potential, has shown that WAF-exposed Atlantic stingrays (*Hypanus sabinus*) demonstrate an average 46% reduction in response magnitude when stimulatory amino acids are introduced.[28] Additionally, WAF-exposed Atlantic stingrays had slower EOG response onset and longer EOG response duration compared to unexposed animals.[28]

To more completely understand the physiological and ecological implications of the 2010 *DWH* oil spill, it is important to examine behavior and olfactory responses in WAF-exposed individuals of the same species. The present study is the first to examine both the olfactory physiology and behavior of a marine teleost exposed to the WAF of crude oil. The primary goals of this study were, first, to examine the behavioral responses of control and WAF-exposed bicolor damselfish to CAC using a two-channel flume behavioral test and second, to evaluate whether control and WAF-exposed bicolor damselfish were able to detect CAC, a food cue, a bile salt cue, and amino acid cues at the olfactory epithelium using the EOG technique. By studying both behavior and olfaction within a species, we can begin to probe into the mechanisms through which oil-exposure affects marine teleosts.

## MATERIALS AND METHODS

**Test Animals.** Bicolor damselfish were hand netted from Emerald reef (25°40′27.0 N, 80°05′55.2 W) east of Key Biscayne, Florida (SAL-18−303-SR) by divers using SCUBA. Collections occurred on September 29, 2015, for flume choice experiments and on August 13, 2018, for EOG experiments. Fish were transported to the University of Miami Rosenstiel School of Marine and Atmospheric Science and maintained in flow-through seawater aquaria for 1−8 weeks before use in experiments. Temperature and salinity were measured daily with a ProODO optical probe (YSI, Inc., Yellow Springs, OH) and a refractometer, respectively, in order to monitor water chemistry. Damselfish were fed daily ad libitum until transfer into 24 h treatment or control exposures where they were fasted. Photoperiod was maintained with 16 h of light and 8 h of darkness in all holding conditions. The seawater used in all experiments matched the temperature, pH, and salinity profiles in which fish were housed.

For both two-channel flume and EOG experiments, bicolor damselfish were transferred to static 24 h exposures of either control seawater or the WAF of crude oil, 1 day prior to experimentation. During static 24 h treatments, initial and final water chemistry measurements were recorded for the following: temperature and dissolved oxygen using a ProODO optical probe (YSI, Inc., Yellow Springs, OH), pH measured with a PHM201 m (Radiometer, Copenhagen, Denmark), and salinity determined with a refractometer (Table 1). To evaluate ammonia concentrations and to ensure they did not accumulate above toxic thresholds in static exposures, the final ammonia concentration of each tank was assessed using Micromodified Indophenol Blue with a colorimetric assay (Table 1).[42] All experiments on bicolor damselfish were performed under the University of Miami IACUC protocol # 15−019.



DOI: 10.1021/acs.est.9b03915
Environ. Sci. Technol. 2019, 53, 10993−11001

**Oil Preparation.** Slick oil collected from surface skimming operations during the *DWH* spill (sample ID: OFS-20100719-Juniper-001 A00884) was used to prepare a high-energy water accommodated fraction (HEWAF) of crude oil by mixing 1 g of oil per liter of seawater at low speed for 30 s in a Waring CB 15 blender (Torrington, CT). The blended oil and seawater mixture was immediately transferred to a glass separatory funnel, allowed to settle for 1 h, and the lower 90% was drained and retained as the 100% WAF. This WAF was then diluted with control seawater to make a 6% static exposure treatment, and damselfish were immediately introduced into either control or WAF-exposure treatment tanks. A 6% WAF exposure was chosen because it is within the range of concentrations measured in the Gulf of Mexico following the *DWH* disaster[43] and because it is typically sublethal but sufficient to cause detrimental effects on morphology and/or physiology in other marine teleosts.[24]

Water samples were collected in 250 mL amber bottles directly from each tank at the beginning and end of 24 h exposures to quantify ∑PAHs. Samples were shipped overnight on ice to ALS Environmental (Kelso, WA) for gas chromatography/mass spectrometry−selective ion monitoring (GC/MS−SIM; based on EPA method 8270D). Reported ∑PAH values are the sum of 50 individual PAHs averaged using the geometric mean of initial and 24 h samples. Both experiments exposed fish to a 6% WAF exposure, but due to the inherent variability of preparing crude oil, the 24 h exposures used in behavior experiments ($\sum_{50}$ PAH 31.7 ± 4.31 μg/L) were more concentrated than those used in EOG experiments ($\sum_{50}$ PAH 11.22 ± 5.55 μg/L). Control exposures had a negligible quantity of PAHs present ($\sum_{50}$ PAH 0.02917 μg/L). A summary of all bicolor damselfish sizes, ∑PAH initial and final concentrations, and a representative ∑PAH profile are available in Supporting Information (Figure S1; Tables S1−S4).

**Behavioral Experiments.** Behavioral experiments were designed to test the hypothesis that exposure to the WAF of crude oil would modify behavioral responses to CAC. Immediately following static exposures, control ($n$ = 15) and WAF-exposed ($n$ = 12) bicolor damselfish were tested in a two-channel flume (13 cm × 4 cm) divided down half of the length so that the two water streams, CAC and untreated seawater, remained separate.[44] An additional test with control bicolor damselfish ($n$ = 8) used untreated seawater on both sides of the flume to test for any possible side bias in the test. A previous study demonstrated near laminar flow and separation of flows between the two sides in this exact flume.[35] Water flow to both sides of the flume was gravity fed at a constant flow rate of 200 mL per minute. Water flow was maintained and monitored throughout experiments using a Dwyer Mini-Master Flowmeter (MMA-38, Michigan City, Indiana).

Following established protocol,[35,45] conspecific CAC was produced by euthanizing a control bicolor damselfish with a quick blow to the head, making six superficial cuts on the lateral sides of the fish, and rinsing each side of the fish with 15 mL of seawater. This rinsewater was added to 5 L of seawater, mixed, and added to one side of the gravity flow system immediately prior to the start of each flume test. The side of the flume to initially hold CAC was alternated between fish. One donor damselfish was used to make CAC for each experimental damselfish trial.

For each trial, a fish was placed directly in the center of the downstream side of the flume and given 2 min to habituate. After the habituation period, the position of the fish in the flume (CAC side or untreated seawater side) was noted every five seconds for 2 min. The side on which the CAC and the untreated seawater were located was switched during a 1 min rest period to eliminate the confounding effect of a potential side-bias. The fish was recentered at the end of the rest period, and the entire habituation and trial process were repeated with the cue on the opposite side of the flume. The flume choice test observer was not blinded to the treatment group.

Following the completion of two-channel flume experiments, all damselfish were returned to clean seawater and were fed daily ad libitum. WAF-exposed damselfish were retested in the same manner as described above 3 and 8 days following exposure and were again fasted for the 24 h prior to experimentation. The identity of individual WAF-exposed fish was tracked, but in tests performed 3 and 8 days postexposure, the observer was blinded to individual fish identification. Experiments were halted once the WAF-exposed fish had been tested three times.

**Electro-Olfactogram Experiments.** EOG experiments were designed to test the hypothesis that observed reduced behavioral responses of WAF-exposed bicolor damselfish were associated with diminished sensitivity of OSNs to CAC and other olfactory cues. All EOG recordings for control ($n$ = 14) and WAF-exposed ($n$ = 11) bicolor damselfish were performed blind such that the researcher did not know whether the fish was a control or WAF-exposed individual. Underwater EOG experiments commenced immediately following 24 h exposures. Bicolor damselfish 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 transferred to a submerged experimental chamber where they were ventilated with aerated control seawater containing 0.1 g/L MS-222. To access the OE, the septum that separates the anterior and posterior nares was surgically removed and a recording electrode was placed on the longest lamellae of the anterior dorsal section of the OE and a reference electrode was placed directly posterior to the eye. Recording and reference electrodes were built identically with nonpolarizable Ag-AgCl electrodes (Warner Instruments, Hamden, CT) fitted with a 1.5 mm glass capillary tube (Warner Instruments), pulled to a fine tip, and filled with 3 M potassium chloride (Sigma-Aldrich).

In addition to CAC, the following olfactory cues were used to assess the effect of WAF exposure on detection of a variety of cue types: a brine shrimp rinse (brine shrimp; San Francisco Bay Brand, Newark, CA), a 10-fold dilution of the brine shrimp rinse (10% brine shrimp), $10^{-1}$ M L-alanine (Ala $10^{-1}$; Sigma-Aldrich), $10^{-2}$ M L-alanine (Ala $10^{-2}$), and $10^{-3}$ M taurocholic acid (TCA; Sigma-Aldrich). Brine shrimp rinse was made by soaking 0.5 g of frozen brine shrimp in 200 mL of control seawater for 30 min and filtering it through a 100 μm mesh. CAC for EOG experiments was made as described for flume choice experiments with the modification that the rinsewater was added to 20 mL of control seawater. This concentration of CAC was selected for EOG work following initial trials that showed a consistent, measurable EOG response to this dilution of CAC. CAC was made immediately following a successful recording for each individual, and one control donor fish was used for each experimental fish. Cues were selected to represent both natural cues, e.g., CAC and a concentrated and dilute brine shrimp rinse, as well as standard olfactory cues of varying concentrations, e.g., Ala $10^{-1}$, Ala $10^{-2}$, and TCA. L-


DOI: 10.1021/acs.est.9b03915
Environ. Sci. Technol. 2019, 53, 10993−11001

alanine cues were used to examine the effect of WAF exposure on microvillous OSNs and represent food cues while TCA was used to evaluate the effect of WAF exposure on ciliated OSNs and represents an alarm cue.[27]

All cues were delivered for 3 s using an eight-channel perfusion valve control system (Warner Instruments) that allowed seamless transitions from OE perfusion, to cue delivery, and back to OE perfusion without interruption in flow or change in flow volume. Seawater was used to perfuse the OE for 90 s between cues and was also used as a negative control cue. Perfusate, as well as all cues, contained 0.1 g/L MS-222 to match the submerged experimental chamber and ventilation water. Cues were administered in a randomized order with each cue delivered three times per experiment with a paired negative control cue following each cue. At the conclusion of the experiment, fish were euthanized by pithing.

During EOG experiments, the output from the recording and reference electrodes were differentially amplified 1000× (DP-311, Warner Instruments), filtered (0.1 Hz-0.1 kHz, 50/60 Hz; DP-311, Warner Instruments), digitized and filtered (60 Hz, Power Lab, AD Instruments, Dunedin, NZ), and recorded (Chart Software v. 8.1.3, AD Instruments, Colorado Springs, CO). For each EOG response, amplitude (mV), duration (s), integral (mV·s), mean (mV), average slope ($mVs^{-1}$), and maximum slope ($mVs^{-1}$) were measured, and the corresponding negative control response was subtracted. When the negative control response was greater than the corresponding cue response, the cue was considered to be a nondetection.

**Statistical Analysis.** All data were analyzed in R,[46] and all linear models were performed with the "lme4" package.[47] Akaike's Information Criterion (AIC)[48,49] was used to discriminate among competing formulations when multiple models were assessed.

In behavior trials, percent time spent in the CAC was arcsine transformed for all analyses. Treatment (WAF or control) was used in a linear model to predict the time that bicolor damselfish spent in the CAC with individual fish identity added as a random effect and a Bonferroni correction for pairwise comparisons (Table S5). A Student *t* test was used to compare the time that negative control fish (seawater on both sides of the flume) spent on one side of the flume to 50% time on a side to evaluate whether any side bias occurred.

For EOG data, the amplitude and duration of the response were chosen as the variables of interest out of the subset measured, as they were not highly correlated with one another and a principle components analysis demonstrated they were conveying different types of information (Figure S2). For both EOG amplitude and duration, linear models were used to assess the effect of the olfactory cue, treatment (WAF or control), and their interaction on the EOG amplitude and duration with individual fish as a random effect. The "multcomp" package in R[50] was used posthoc to examine differences in responses to particular cues between control and WAF-exposed bicolor damselfish.

For EOG cues that resulted in both detections and nondetections, individual fish were defined as "detectors" of a particular cue if they successfully detected the cue at least 33% of the time, while individual fish were conversely defined as "non-detectors" if they did not meet that criteria. A linear model using individual fish as a random effect was performed to determine whether a fish was a detector for a particular cue was predicted by WAF exposure. To estimate confidence intervals for these models, the Monte Carlo method was used to examine the fixed[51] effects for a typical fish.

## RESULTS AND DISCUSSION

CAC is a biologically relevant and evolutionarily significant cue for fish species. The present study utilized a paired approach to examine both the behavior and detection capacity of control and WAF-exposed bicolor damselfish when they were presented with CAC. Our results demonstrate that WAF-exposed bicolor damselfish do not avoid CAC in a two-channel flume as control fish do and that this exposure reduced the probability of CAC detections at the OE. Neither the detection probability of the other cues used in this study nor the amplitude or duration of the EOG responses to any cue were affected by WAF exposure. Our results suggest that modifications to behavior that occur following an environmentally relevant 24 h exposure to WAF may be driven in part by reductions in neuronal signaling that could be specific to particular cues.

When faced with a predator, prey may respond to a variety of cues including auditory distress calls,[52] visual detection of a predator or fleeing prey,[52] and detection of CAC.[39] Chemical cues often provide an early warning over visual or auditory cues and have been shown to increase survival time in experimental interactions between predator and prey fish.[53] Avoidance of CAC has also been shown across a range of animal taxa from flatworms[54] to marine teleost embryos[55] to mammals,[56] demonstrating the evolutionary significance of this cue. Control fish in our experiment that were tested with CAC spent 17% of their time on the side of the flume with CAC demonstrating that under control conditions, bicolor damselfish will avoid CAC (Figure 1). In contrast, a 24 h WAF exposure ($\sum_{50}$ PAH 31.7 ± 4.31 μg/L) significantly increased the amount of time that bicolor damselfish spent in the CAC, a normally deterrent olfactory cue, compared to control individuals ($p < 0.0001$, Figure 1, Table S5). Negative control fish that were tested with seawater on both sides of the flume

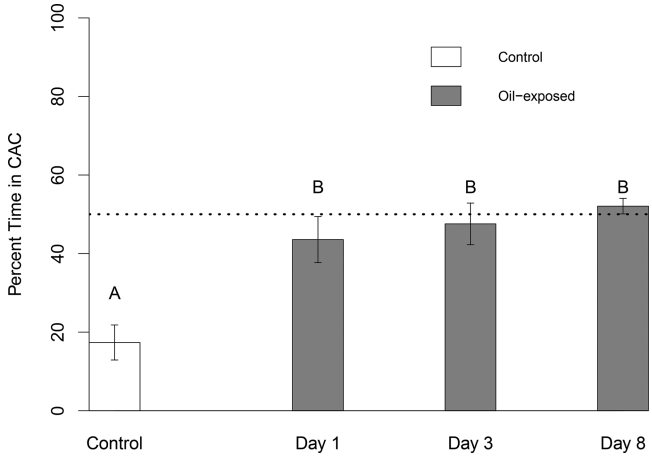



**Figure 1.** Control ($n$ = 15) and oil-exposed ($n$ = 12) bicolor damselfish were tested in a two-channel flume with seawater against chemical alarm cue. Oil-exposed fish were tested immediately following a 24 h oil exposure ($\sum_{50}$ PAH 31.7 ± 4.31 μg/L) and after 3 days and 8 days recovery in clean seawater. The dashed line shows the expected no preference statistic of 50% time on each side. Letters denote significant differences in time spent in the CAC ($p < 0.0001$). Means are plotted with standard error.



DOI: 10.1021/acs.est.9b03915
Environ. Sci. Technol. 2019, 53, 10993−11001

spent equivalent time on both sides of the flume demonstrating side preference was not a factor in our experiments ($p$ = 0.104). Disruption of CAC avoidance has been shown in fish exposed to other types of toxins and is implicated in their reduced survival in the wild.[9,35,38,41] The ability of small reef fishes to recognize predators and hide is critical for their survival in the wild, and WAF-exposure has previously been shown to modify several predator avoidance mechanisms in coral reef fishes.[24]

Impaired CAC avoidance was evident when WAF-exposed bicolor damselfish were tested immediately following exposure and this lack of avoidance behavior persisted 8 days after the initial exposure. WAF-exposed fish tested immediately following exposure spent 44% of their time in the CAC, while fish that were retested in the flume following 3 and 8 days of recovery spent 48% and 52% of their time, respectively, in the CAC. The behavior of the WAF-exposed fish was significantly different from the behavior of control fish at all three time points ($p$ < 0.001 for all comparisons), but the behavior of the WAF-exposed fish was not significantly different between day 1, day 3, or day 8 ($p$ = 1.0 for all comparisons). These data suggest that this behavioral disruption is persistent and is not a transient effect of crude oil compounds temporarily binding to OSNs. The impaired CAC avoidance that was observed in WAF-exposed individuals suggests either the loss of the ability to detect CAC at the OE and/or the loss of downstream ability to process and interpret the neuronal signals at the OB.

We observed persistent behavioral effects in WAF-exposed bicolor damselfish; however, in a lab setting these effects were sublethal. WAF-exposed fish used in this experiment resumed eating immediately during depuration. Moreover, following the experiment, WAF-exposed fish were kept in the lab for over one year with no mortality occurring in the group. Sustained effects on swim speed[57,58] and visual acuity[20] have previously been measured in marine teleosts as many as 6 weeks following an acute WAF exposure, further demonstrating that sublethal effects resulting from crude oil can be long lasting. However, mesocosm experiments on WAF-exposed coral reef fish showed a 2.7-fold increase in mortality due to predation over control fish,[24] suggesting that in the wild, impaired CAC avoidance would not be a sublethal effect.

The EOG technique demonstrated substantial individual variation and, consequently, we found no significant differences in either the amplitude or the duration of the EOG response for any of the cues tested between WAF-exposed ($\sum_{50}$ PAH 11.22 ± 5.55 μg/L) and control bicolor damselfish (Tables S6–S9). Though not statistically significant, we observed smaller or more negative (e.g., saltwater negative control cue larger than response) EOG amplitudes in WAF-exposed individuals than in control fish for the more dilute olfactory cues (CAC, brine shrimp, 10% brine shrimp, and TCA; Figure 2). Similarly, we saw shorter duration responses to all cues except for Ala $10^{-2}$ in WAF-exposed individuals, though there were no significant differences between the treatment groups. Cave and Kajiura[28] recently published the only other study to use an electrophysiological assay to examine the effect of WAF exposure on olfaction in a marine fish. The authors found that after a 48 h exposure to a 10% WAF, the amplitude and the initial slope of the EOG response of Atlantic stingrays to various amino acid cues was significantly decreased and the duration of the EOG response was significantly increased.[28]

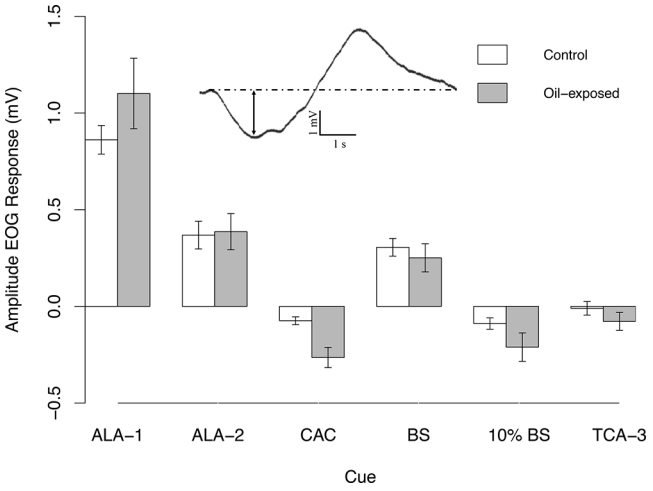



**Figure 2.** Bicolor damselfish EOG response amplitude (mV) with negative control cue subtracted for 24 h control ($n$ = 14) and oil-exposure ($n$ = 11, $\sum_{50}$ PAH 11.22 ± 5.55 μg/L) treatments. A representative EOG response is shown to indicate the measured parameter. ALA-1 = L-alanine $10^{-1}$ M, ALA-2 = L-alanine $10^{-2}$ M, CAC = chemical alarm cue, BS = brine shrimp rinse, 10% BS = 10% dilution brine shrimp rinse, TCA-3 = taurocholic acid $10^{-3}$ M. Means are plotted with standard error. No significant differences were found between oil-exposed and control individuals ($p$ > 0.05). Across treatment groups, all cues were significantly different from one another ($p$ < 0.05) with the exception of brine shrimp and L-alanine $10^{-2}$ M ($p$ > 0.05), 10% dilution brine shrimp rinse and CAC ($p$ > 0.05), taurocholic acid $10^{-3}$ M and CAC ($p$ > 0.05), and 10% dilution brine shrimp rinse and taurocholic acid $10^{-3}$ M ($p$ > 0.05).

In contrast to our study design, Cave and Kajiura prepared amino acid cues with the identical crude oil concentration as exposure conditions.[28] Similarly, Lari and Pyle[27] reported that in control rainbow trout (*Oncorhynchus mykiss*) L-alanine and taurocholic acid cues had EOG amplitude responses that were reduced 60 and 29%, respectively, when they were administered together with oil sands process-affected water (OSPW), a mixture containing petroleum hydrocarbons. These previous studies[27,28] administered olfactory cues together with toxins; however, the aim of our work was to examine whether effects at the OE were measurable when assessed in clean seawater. Without testing the interaction of the WAF of crude oil with olfactory cues to assess effects on EOG amplitude and duration, it is difficult to compare the results of the present study to those of Cave and Kajiura.[28] Additionally, it is possible that the longer exposure period used in Cave and Kajiura,[28] the higher WAF concentration,[28] or interspecific differences contributed to divergent findings. It is also important to note that, despite our intentions to make the exposures similar, WAF exposures for fish used in our EOG experiments had a lower geometric mean of PAHs than those used in behavioral experiments (Tables S3 and S5), and this may have also contributed to the lack of significant differences in EOG amplitude and duration in our study. Rainbow trout exposed to OSPW at two concentrations and across three exposure periods showed increasing olfactory inhibition as both exposure time and concentration increased,[59] suggesting the possibility that either a longer exposure or an higher concentration of PAHs may be needed to see significant differences in EOG amplitude and duration.

In the present study, fish showed a concentration-dependent EOG amplitude and duration to alanine and brine shrimp cues,


DOI: 10.1021/acs.est.9b03915
Environ. Sci. Technol. 2019, 53, 10993−11001

the only cues delivered in multiple concentrations, across treatment groups demonstrating the efficacy of the EOG recordings (Figures 2 and 3). Similarly, the response amplitude

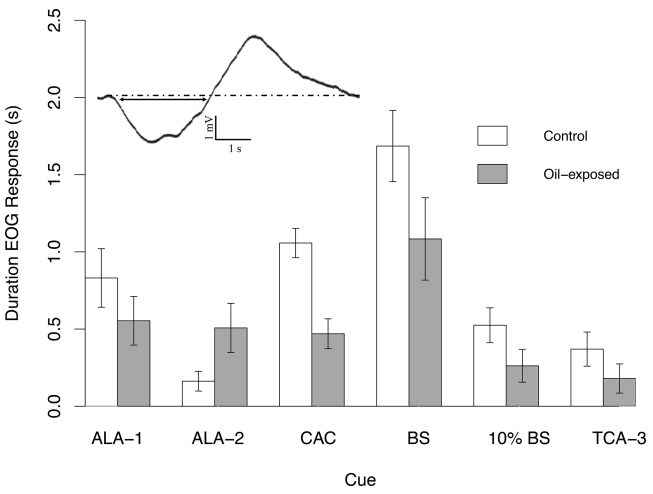



**Figure 3.** Bicolor damselfish EOG response duration (s) with negative control cue subtracted for 24 h control ($n$ = 14) and oil-exposure ($n$ = 11, $\sum_{50}$ PAH 11.22 ± 5.55 $\mu$g/L) treatments. A representative EOG response is shown to indicate the measured parameter. ALA-1 = L-alanine $10^{-1}$ M, ALA-2 = L-alanine $10^{-2}$ M, CAC = chemical alarm cue, BS = brine shrimp rinse, 10% BS = 10% dilution brine shrimp rinse, TCA-3 = taurocholic acid $10^{-3}$ M. Means are plotted with standard error. No significant differences were found between oil-exposed and control individuals ($p$ > 0.05). Across treatment groups, the duration of the response to the brine shrimp rinse cue was significantly different than the response duration to L-alanine $10^{-1}$ M ($p$ < 0.05), L-alanine $10^{-2}$ M ($p$ < 0.05), CAC ($p$ < 0.05), 10% dilution brine shrimp rinse ($p$ < 0.05), and taurocholic acid $10^{-3}$ M ($p$ < 0.05).

of all cues was significantly different from one another (Table S10) with the exception of brine shrimp and Ala $10^{-2}$, 10% brine shrimp and CAC, TCA and CAC, and 10% brine shrimp and TCA. Response duration to the brine shrimp rinse cue was significantly different from Ala $10^{-1}$, Ala $10^{-2}$, CAC, 10% brine shrimp, and TCA (Table S11).

For both control and WAF-exposed bicolor damselfish, the average EOG amplitude response was smaller than the paired negative control cue, thus generating a mean negative ΔmV, for CAC, 10% brine shrimp, and TCA, indicating that these cues were at the edge of detection by the technique or the organism (Figure 2). Indeed, the coefficient of variation was amplified for these less concentrated cues and fish ranged from robust- to nonresponders. Fish that were nonresponders to CAC demonstrated a greater coefficient of variation than those that responded to CAC, suggesting that increased variation occurred near the detection threshold.

The classification of individual fish as detectors or nondetectors of the CAC, 10% brine shrimp, and TCA cues allowed an analysis of the relationship between individual detector status and WAF exposure. Linear mixed effect models examining the effect of WAF exposure on detection probability for CAC suggest that WAF-exposed individuals are less likely to detect CAC at the olfactory epithelium than control fish; however, these results have borderline statistical significance ($p$ = 0.055, Table S12). The confidence intervals for a typical fish in this model demonstrate that WAF-exposed fish were less likely than control fish to detect CAC at the OE but that large amounts of individual variation were present in both groups (Figure 4). The detection probability of the 10% brine shrimp

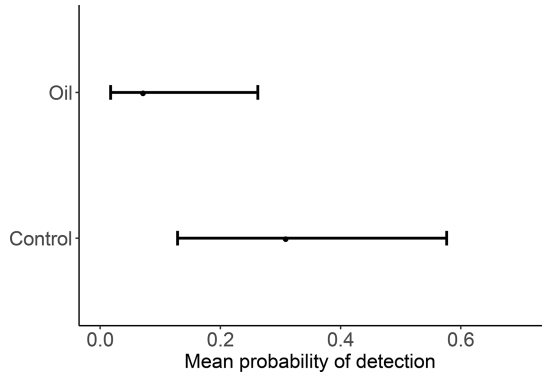



**Figure 4.** Confidence intervals for a typical fish from a linear model examining the effect of 24 h control ($n$ = 14) and oil-exposure ($n$ = 11, $\sum_{50}$ PAH 11.22 ± 5.55 $\mu$g/L) treatments on detection of chemical alarm cue (CAC) at the olfactory epithelium of bicolor damselfish. Individual fish were defined as CAC detectors if the EOG technique showed that they successfully detected CAC at least 33% of the time. A linear model suggested control fish were more likely to detect CAC, though individual variation was large for both groups ($p$ = 0.055).

and TCA cues were not significantly affected by WAF exposure (Table S12). The specificity of different odorants to primarily bind to a particular class of OSN is well documented,[60] and similarly, it is understood that a particular toxicant can impact binding of one OSN class and not another.[61] In fathead minnows (*Pimephales promelas*) the response to CAC has been shown to be dependent on ciliated OSNs, and it was observed that copper exposure impairs detection of CAC and taurocholic acid, but not L-alanine.[61] Our results suggest a similar specificity of crude oil to impair detection of CAC while not reducing the detection likelihood of the other cues in our study.

Our strong behavioral responses to CAC are not at odds with our EOG data showing variable responses to CAC. Signal loss in saltwater EOGs due to high seawater conductance[62] may have contributed to the small and variable responses to CAC and other less concentrated cues. To attempt to mitigate this, the concentration of CAC was increased in EOG experiments relative to our behavior trials; however, the bicolor damselfish OE was still relatively insensitive to CAC, TCA, and the 10% brine shrimp cue. It is also established that the EOG technique does not capture the full detection capacity for chemical cues, as it represents a summated response measured some small distance above the OE after part of the signal is presumably shunted away from the electrode. It has been demonstrated that rainbow trout show behavioral differences at a 10-fold lower concentration than that detected using the EOG technique.[27,63] Further, using an electroencephalogram (EEG) technique to measure detection at the OB, rainbow trout have been shown to detect olfactory cues at a 10- to 100-fold lower concentration than the concentration at which behavioral changes are observed.[64] Regardless of its limitations, the utility of the EOG technique is that it is uniquely capable of measuring physiological detection at the OE and therefore providing insight into the target of a particular toxin.


DOI: 10.1021/acs.est.9b03915
Environ. Sci. Technol. 2019, 53, 10993−11001

Previous studies have identified both excessive mucus production and cilia damage at the OE[30] as well as hyperplasia and necrosis of the OE[33] after exposure to the WAF of crude oil or the constituents of crude oil. Our hypotheses regarding the mechanisms for the reduced detection of CAC in WAF-exposed bicolor damselfish are (1) crude oil compounds bind irreversibly to the OE, thereby reducing binding sites, (2) a secondary response to WAF-exposure generates excessive mucus that coats the OE, similarly reducing binding sites, or, (3) WAF-exposure causes apoptosis of OSNs specific to the signaling for CAC. Our behavioral data demonstrates impairment out to at least 8 days of depuration suggesting that cell death of OSNs, and potentially cell death of the stem cells responsible for generating new OSNs, may be a more likely hypothesis than either a crude oil or mucus coating on the OE, which would likely abate in that period and may be less likely to target specific OSNs. However, causes of the reduced CAC detection at the OE are by no means mutually exclusive. Additionally, while our EOG results show impaired detection of CAC at the OE following WAF exposure, our findings do not rule out additional impairment in the CNS contributing to the substantial behavioral alterations we observed in WAF-exposed bicolor damselfish.

Regardless of the precise mechanism, WAF exposure reduces detections of CAC at the OE and eliminates CAC avoidance behavior out to at least 8 days postexposure. When assessed in control seawater, we did not find significant differences in the amplitude or duration of the EOG response between control and WAF-exposed fish or a significant reduction in detection probability for the other cues that had both detections and nondetections, suggesting that WAF exposure may target OSNs specific to CAC. A loss of the ability to detect and avoid CAC, a critical predator-avoidance strategy, likely has implications for oil-exposed populations in the wild. A failure to recognize fitness enhancing olfactory stimuli may, in part, explain observations in the Gulf of Mexico of large declines in damselfish populations as late as 2014[14−16] and points to the importance of evaluating sublethal effects of oil exposure, which may secondarily increase mortality.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b03915.

Representative profile and concentrations of the 50 measured polycyclic aromatic hydrocarbons (PAHs) in a 24 h WAF exposure, a principle components analysis for electro-olfactogram data, morphometric data on bicolor damselfish used in two-channel flume choice and electro-olfactogram experiments, $\sum_{50}$ PAH data for both experiments, model fit for linear model fitted to flume choice data, model fit and comparison statistics for linear mixed effects models fitted to electro-olfactogram amplitude and duration data, parameters of the best linear mixed effects models fitted to electro-olfactogram amplitude and duration data, multiple comparison model parameters from electro-olfactogram amplitude and duration data between treatments, multiple comparison model parameters from electro-olfactogram amplitude and duration data across treatments, and model fit for linear models fitted to electro-olfactogram detection data (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

*E-mail: lschlenker@rsmas.miami.edu. Phone: (305) 421-4366.

### ORCID

Lela S. Schlenker: 0000-0002-8261-0923

### Present Address

[$]Department of Cell and Systems Biology, University of Toronto, Toronto, ON M5S 3G5 Canada.


### Notes

The authors declare no competing financial interest.

Additional data for this article may be accessed through the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC) available at https://data.gulfresearchinititative.org, DOI: 10.7266/n7−48jn-7a47[65] and DOI: 10.7266/N7KH0KQ9.[66]

## ■ ACKNOWLEDGMENTS

This research was supported by a grant from the Gulf of Mexico Research Initiative (Grant No. SA-1520 to the RECOVER consortium (Relationship of effects of cardiac outcomes in fish for validation of ecological risk). We thank Dr. Michael Schmale for his expertise and assistance in collecting and housing bicolor damselfish. We thank Dr. Rachael Heuer for blinding L.S.S. with science. We thank Emma Esch for processing ammonia samples and assisting with water chemistry. We are grateful to three anonymous reviewers for their assistance in improving this manuscript. M.G. is a Maytag Professor of Ichthyology.

## ■ REFERENCES


(1) Paris, C. B.; Atema, J.; Irisson, J. O.; Kingsford, M.; Gerlach, G.; Guigand, C. M. Reef Odor: A Wake Up Call for Navigation in Reef Fish Larvae. *PLoS One* **2013**, *8* (8), 1−8.

(2) Scholz, N. L.; Truelove, N. K.; French, B. L.; Berejikian, B. A.; Quinn, T. P.; Casillas, E.; Collier, T. K. Diazinon disrupts antipredator and homing behaviors in chinook salmon (Oncorhynchus tshawytscha). *Can. J. Fish. Aquat. Sci.* **2000**, *57* (9), 1911−1918.

(3) Foretich, M. A.; Paris, C. B.; Grosell, M.; Stieglitz, J. D.; Benetti, D. D. Dimethyl Sulfide is a Chemical Attractant for Reef Fish Larvae. *Sci. Rep.* **2017**, *7*, 1−10.

(4) Meredith, T. L.; Caprio, J.; Kajiura, S. M. Sensitivity and specificity of the olfactory epithelia of two elasmobranch species to bile salts. *J. Exp. Biol.* **2012**, *215* (15), 2660−2667.

(5) Frade, P.; Hubbard, P. C.; Barata, E. N.; Canario, A. V. M. Olfactory sensitivity of the Mozambique tilapia to conspecific odours. *J. Fish Biol.* **2002**, *61* (5), 1239−1254.

(6) Smith, R. J. F. Alarm signals in fishes. *Reviews in Fish Biology and Fisheries* **1992**, *2* (1), 33−63.

(7) Ferrari, M. C.; Wisenden, B. D.; Chivers, D. P. Chemical ecology of predator−prey interactions in aquatic ecosystems: a review and prospectus. *Can. J. Zool.* **2010**, *88* (7), 698−724.

(8) Tierney, K. B.; Baldwin, D. H.; Hara, T. J.; Ross, P. S.; Scholz, N. L.; Kennedy, C. J. Olfactory toxicity in fishes. *Aquat. Toxicol.* **2010**, *96* (1), 2−26.

(9) Mirza, R. S.; Green, W. W.; Connor, S.; Weeks, A. C. W.; Wood, C. M.; Pyle, G. G. Do you smell what I smell? Olfactory impairment in wild yellow perch from metal-contaminated waters. *Ecotoxicol. Environ. Saf.* **2009**, *72* (3), 677−683.

(10) McNutt, M. K.; Chu, S.; Lubchenco, J.; Hunter, T.; Dreyfus, G.; Murawski, S. A.; Kennedy, D. M. Applications of science and engineering to quantify and control the Deepwater Horizon oil spill. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (50), 20222−20228.

(11) Camilli, R.; Di Iorio, D.; Bowen, A.; Reddy, C. M.; Techet, A. H.; Yoerger, D. R.; Whitcomb, L. L.; Seewald, J. S.; Sylva, S. P.;




DOI: 10.1021/acs.est.9b03915
Environ. Sci. Technol. 2019, 53, 10993−11001

Fenwick, J. Acoustic measurement of the Deepwater Horizon Macondo well flow rate. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (50), 20235−20239.
(12) Romero, I. C.; Schwing, P. T.; Brooks, G. R.; Larson, R. A.; Hastings, D. W.; Ellis, G.; Goddard, E. A.; Hollander, D. J. Hydrocarbons in Deep-Sea Sediments following the 2010 Deepwater Horizon Blowout in the Northeast Gulf of Mexico. *PLoS One* **2015**, *10* (5), 1−23.
(13) Valentine, D. L.; Fisher, G. B.; Bagby, S. C.; Nelson, R. K.; Reddy, C. M.; Sylva, S. P.; Woo, M. A. Fallout plume of submerged oil from Deepwater Horizon. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (45), 15906−15911.
(14) Murawski, S. A.; Fleeger, J. W.; Patterson, W. F.; Hu, C. M.; Daly, K.; Romero, I.; Toro-Farmer, G. A. How Did the Deepwater Horizon Oil Spill Affect Coastal and Continental Shelf Ecosystems of the Gulf of Mexico? *Oceanography* **2016**, *29* (3), 160−173.
(15) Trustees, D. H. N. R. D. A., Deepwater Horizon oil spill: Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. In 2016.
(16) Dahl, K. A.; Patterson, W. F.; Snyder, R. A. Experimental assessment of lionfish removals to mitigate reef fish community shifts on northern Gulf of Mexico artificial reefs. *Mar. Ecol.: Prog. Ser.* **2016**, *558*, 207−221.
(17) Rooker, J.; Dokken, Q.; Pattengill, C.; Holt, G. Fish assemblages on artificial and natural reefs in the Flower Garden Banks National Marine Sanctuary, USA. *Coral Reefs* **1997**, *16* (2), 83−92.
(18) Snyder, S. M.; Pulster, E. L.; Wetzel, D. L.; Murawski, S. A. PAH Exposure in Gulf of Mexico Demersal Fishes, Post-Deepwater Horizon. *Environ. Sci. Technol.* **2015**, *49* (14), 8786−8795.
(19) Xu, E. G.; Mager, E. M.; Grosell, M.; Pasparakis, C.; Schlenker, L. S.; Stieglitz, J. D.; Benetti, D.; Hazard, E. S.; Courtney, S. M.; Diamante, G.; Freitas, J.; Hardiman, G.; Schlenk, D. Time- and Oil-Dependent Transcriptomic and Physiological Responses to Deepwater Horizon Oil in Mahi-Mahi (Coryphaena hippurus) Embryos and Larvae. *Environ. Sci. Technol.* **2016**, *50* (14), 7842−51.
(20) Xu, E. G.; Magnuson, J. T.; Diamante, G.; Mager, E.; Pasparakis, C.; Grosell, M.; Roberts, A. P.; Schlenk, D. Changes in microRNA−mRNA Signatures Agree with Morphological, Physiological, and Behavioral Changes in Larval Mahi-Mahi Treated with Deepwater Horizon Oil. *Environ. Sci. Technol.* **2018**, *52* (22), 13501−13510.
(21) Xu, E. G. B.; Mager, E. M.; Grosell, M.; Hazard, E. S.; Hardiman, G.; Schlenk, D. Novel transcriptome assembly and comparative toxicity pathway analysis in mahi-mahi (Coryphaena hippurus) embryos and larvae exposed to Deepwater Horizon oil. *Sci. Rep.* **2017**, *7*, 1−13.
(22) Hamdani, E. H.; Doving, K. B. The functional organization of the fish olfactory system. *Prog. Neurobiol.* **2007**, *82* (2), 80−86.
(23) Restrepo, D. The ins and outs of intracellular chloride in olfactory receptor neurons. *Neuron* **2005**, *45* (4), 481−482.
(24) Johansen, J. L.; Allan, B. J. M.; Rummer, J. L.; Esbaugh, A. J. Oil exposure disrupts early life-history stages of coral reef fishes via behavioural impairments. *Nature Ecology & Evolution* **2017**, *1* (8), 1146−1152.
(25) Khursigara, A. J.; Johansen, J. L.; Esbaugh, A. J. Social competition in red drum (Sciaenops ocellatus) is influenced by crude oil exposure. *Aquat. Toxicol.* **2018**, *203*, 194−201.
(26) Lari, E.; Abtahi, B.; Hashtroudi, M. S.; Mohaddes, E.; Doving, K. B. The effect of sublethal concentrations of the water-soluble fraction of crude oil on the chemosensory function of Caspian roach, Rutilus caspicus (YAKOVLEV, 1870). *Environ. Toxicol. Chem.* **2015**, *34* (8), 1826−1832.
(27) Lari, E.; Pyle, G. G. Rainbow trout (Oncorhynchus mykiss) detection, avoidance, and chemosensory effects of oil sands process-affected water. *Environ. Pollut.* **2017**, *225*, 40−46.
(28) Cave, E. J.; Kajiura, S. M. Effect of Deepwater Horizon Crude Oil Water Accommodated Fraction on Olfactory Function in the Atlantic Stingray, Hypanus sabinus. *Sci. Rep.* **2018**, *8* (1), 15786.
(29) Holmes, T. H.; McCormick, M. I. Smell, learn and live: the role of chemical alarm cues in predator learning during early life history in a marine fish. *Behav. Processes* **2010**, *83* (3), 299−305.
(30) Babcock, M., Morphology of olfactory epithelium of pink salmon, Oncorhynchus gorbuscha, and changes following exposure to benzene: a scanning electron microscopy study. 1985.
(31) DiMichele, L.; Taylor, M. H. Histopathological and physiological responses of Fundulus heteroclitus to naphthalene exposure. *J. Fish. Res. Board Can.* **1978**, *35* (8), 1060−1066.
(32) Gardner, G., Chemically induced lesions in estuarine or marine teleosts. 1975.
(33) Solangi, M. A.; Overstreet, R. M. Histopathological changes in two estuarine fishes, Menidia beryllina (Cope) and Trinectes maculatus (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. *J. Fish Dis.* **1982**, *5* (1), 13−35.
(34) Chivers, D. P.; Brown, G. E.; Smith, R. J. F. Familiarity and shoal cohesion in fathead minnows (Pimephales promelas): implications for antipredator behaviour. *Can. J. Zool.* **1995**, *73* (5), 955−960.
(35) Welch, M. J.; Watson, S. A.; Welsh, J. Q.; McCormick, M. I.; Munday, P. L. Effects of elevated CO2 on fish behaviour undiminished by transgenerational acclimation. *Nat. Clim. Change* **2014**, *4* (12), 1086−1089.
(36) Hamdani, E. H.; Doving, K. B. Sensitivity and selectivity of neurons in the medial region of the olfactory bulb to skin extract from conspecifics in crucian carp, Carassius carassius. *Chemical Senses* **2003**, *28* (3), 181−189.
(37) Porteus, C. S.; Hubbard, P. C.; Webster, T. M. U.; van Aerle, R.; Canário, A. V.; Santos, E. M.; Wilson, R. W. Near-future CO 2 levels impair the olfactory system of a marine fish. *Nat. Clim. Change* **2018**, *8* (8), 737−743.
(38) Williams, C. R.; Dittman, A. H.; McElhany, P.; Busch, D. S.; Maher, M. T.; Bammler, T. K.; MacDonald, J. W.; Gallagher, E. P. Elevated CO2 impairs olfactory-mediated neural and behavioral responses and gene expression in ocean-phase coho salmon (Oncorhynchus kisutch). *Global Change Biology* **2019**, *25*, 963−977.
(39) Chivers, D. P.; Smith, R. J. F. Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Ecoscience* **1998**, *5* (3), 338−352.
(40) Sandahl, J. F.; Baldwin, D. H.; Jenkins, J. J.; Scholz, N. L. A sensory system at the interface between urban stormwater runoff and salmon survival. *Environ. Sci. Technol.* **2007**, *41* (8), 2998−3004.
(41) Scott, G. R.; Sloman, K. A. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquat. Toxicol.* **2004**, *68* (4), 369−392.
(42) Ivancic, I.; Degobbis, D. An Optimal Manual Procedure for Ammonia Analysis in Natural-Waters by the Indophenol Blue Method. *Water Res.* **1984**, *18* (9), 1143−1147.
(43) Boehm, P. D.; Murray, K. J.; Cook, L. L. Distribution and attenuation of polycyclic aromatic hydrocarbons in Gulf of Mexico seawater from the Deepwater Horizon oil accident. *Environ. Sci. Technol.* **2016**, *50* (2), 584−592.
(44) Gerlach, G.; Atema, J.; Kingsford, M. J.; Black, K. P.; Miller-Sims, V. Smelling home can prevent dispersal of reef fish larvae. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (3), 858−863.
(45) Heuer, R.; Welch, M.; Rummer, J.; Munday, P.; Grosell, M. Altered brain ion gradients following compensation for elevated CO 2 are linked to behavioural alterations in a coral reef fish. *Sci. Rep.* **2016**, *6*, 1−10.
(46) R Core Team *R: A language and environment for statistical computing 3.5.3*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
(47) Bates, D.; Maechler, M.; Bolker, B.; Walker, S.; Christensen, R. H. B.; Singmann, H.; Dai, B.; Grothendieck, G.; Green, P., Package 'lme4'. *Convergence* 2015, *12*(1).
(48) Akaike, H. In Information theory and an extention of the maximum likelihood principle *2nd International Symposium on*


DOI: 10.1021/acs.est.9b03915
*Environ. Sci. Technol.* 2019, 53, 10993−11001

*Information Theory*, 1973, 1973; Akademiai Kiado: 1973; pp 267−281.
(49) Burnham, K. P.; Anderson, D. R. *Model selection and multimodel inference: A practical information-theoretic approach*. Springer-Verlag: New York, 2002.
(50) Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. *Biom. J.* **2008**, *50* (3), 346−363.
(51) Tewfik, A.; Babcock, E. A.; Gibson, J.; Perez, V. R. B.; Strindberg, S. Benefits of a replenishment zone revealed through trends in focal species at Glover's Atoll, Belize. *Mar. Ecol.: Prog. Ser.* **2017**, *580*, 37−56.
(52) Blumstein, D. T.; Armitage, K. B. Alarm calling in yellow-bellied marmots: I. The meaning of situationally variable alarm calls. *Anim. Behav.* **1997**, *53* (1), 143−171.
(53) Mirza, R. S.; Chivers, D. P. Response of juvenile rainbow trout to varying concentrations of chemical alarm cue: response thresholds and survival during encounters with predators. *Can. J. Zool.* **2003**, *81* (1), 88−95.
(54) Wisenden, B. D.; Millard, M. C. Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk and to associate risk with novel cues. *Anim. Behav.* **2001**, *62* (4), 761−766.
(55) Atherton, J. A.; McCormick, M. I. Active in the sac: damselfish embryos use innate recognition of odours to learn predation risk before hatching. *Anim. Behav.* **2015**, *103*, 1−6.
(56) Herzog, M.; Hopf, S. Behavioral responses to species-specific warning calls in infant squirrel monkeys reared in social isolation. *Am. J. Primatol.* **1984**, *7* (2), 99−106.
(57) Johansen, J. L.; Esbaugh, A. J. Sustained impairment of respiratory function and swim performance following acute oil exposure in a coastal marine fish. *Aquat. Toxicol.* **2017**, *187*, 82−89.
(58) Mager, E. M.; Esbaugh, A. J.; Stieglitz, J. D.; Hoenig, R.; Bodinier, C.; Incardona, J. P.; Scholz, N. L.; Benetti, D. D.; Grosell, M. Acute embryonic or juvenile exposure to Deepwater Horizon crude oil impairs the swimming performance of mahi-mahi (Coryphaena hippurus). *Environ. Sci. Technol.* **2014**, *48* (12), 7053−61.
(59) Lari, E.; Steinkey, D.; Razmara, P.; Mohaddes, E.; Pyle, G. G. Oil sands process-affected water impairs the olfactory system of rainbow trout (Oncorhynchus mykiss). *Ecotoxicol. Environ. Saf.* **2019**, *170*, 62−67.
(60) Hansen, A.; Rolen, S. H.; Anderson, K.; Morita, Y.; Caprio, J.; Finger, T. E. Correlation between olfactory receptor cell type and function in the channel catfish. *J. Neurosci.* **2003**, *23* (28), 9328−9339.
(61) Dew, W. A.; Azizishirazi, A.; Pyle, G. G. Contaminant-specific targeting of olfactory sensory neuron classes: Connecting neuron class impairment with behavioural deficits. *Chemosphere* **2014**, *112*, 519−525.
(62) Silver, W. L.; Caprio, J.; Blackwell, J. F.; Tucker, D. Underwater Electro-Olfactogram - Tool for Study of Sense of Smell of Marine Fishes. *Experientia* **1976**, *32* (9), 1216−1217.
(63) Valentinčič, T.; Caprio, J. Visual and chemical release of feeding behavior in adult rainbow trout. *Chem. Senses* **1997**, *22* (4), 375−382.
(64) Hara, T. J. Olfactory responses to amino acids in rainbow trout, Salmo gairdneri. *Comparative Biochemistry and Physiology Part A: Physiology* **1973**, *44* (2), 407−416.
(65) Schlenker, L., Electro-olfactogram bicolor damselfish (Stegastes partitus). *Relationship of Effects of Cardiac Outcomes in Fish for Validation of Ecological Risk II (RECOVER II)* 2019 .
(66) Schlenker, L., Grosell, M., Flume choice experiments with bicolor damselfish and mahi-mahi. *Relationship of Effects of Cardiac Outcomes in Fish for Validation of Ecological Risk (RECOVER)* 2018 .


DOI: 10.1021/acs.est.9b03915
*Environ. Sci. Technol.* 2019, 53, 10993−11001