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Impacts of *Deepwater Horizon* Crude Oil on Mahi-Mahi (*Coryphaena hippurus*) Heart Cell Function

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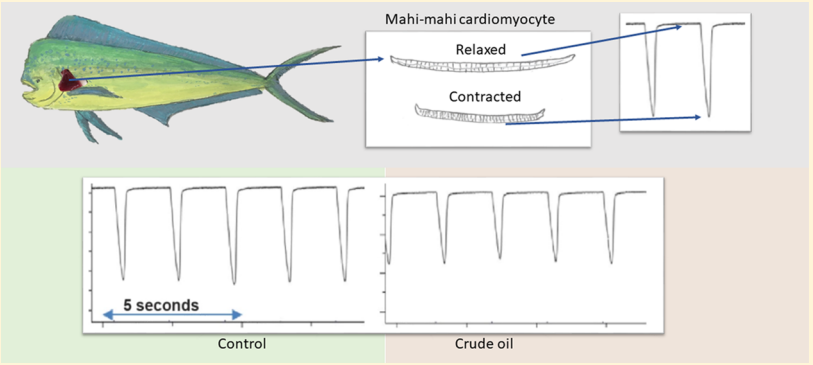
Supporting Information



ABSTRACT: *Deepwater Horizon* crude oil is comprised of polycyclic aromatic hydrocarbons that cause a number of cardiotoxic effects in marine fishes across all levels of biological organization and at different life stages. Although cardiotoxic impacts have been widely reported, the mechanisms underlying these impairments in adult fish remain understudied. In this study, we examined the impacts of crude oil on cardiomyocyte contractility and electrophysiological parameters in freshly isolated ventricular cardiomyocytes from adult mahi-mahi (*Coryphaena hippurus*). Cardiomyocytes directly exposed to oil exhibited reduced contractility over a range of environmentally relevant concentrations (2.8−12.9 $\mu g\ l^{-1} \sum$PAH). This reduction in contractility was most pronounced at higher stimulation frequencies, corresponding to the upper limits of previously measured in situ mahi heart rates. To better understand the mechanisms underlying impaired contractile function, electrophysiological studies were performed, which revealed oil exposure prolonged cardiomyocyte action potentials and disrupted potassium cycling (9.9−30.4 $\mu g\ l^{-1} \sum$PAH). This study is the first to measure cellular contractility in oil-exposed cardiomyocytes from a pelagic fish. Results from this study contribute to previously observed impairments to heart function and whole-animal exercise performance in mahi, underscoring the advantages of using an integrative approach in examining mechanisms of oil-induced cardiotoxicity in marine fish.

INTRODUCTION

The *Deepwater Horizon* oil spill (2010) was the largest offshore marine oil spill in U.S. history, releasing more than 3 million barrels of crude oil into the Gulf of Mexico over 87 days.[1−3] The location and timing of the *Deepwater Horizon* spill overlapped with the habitat utilization patterns of many important pelagic fish species in the Gulf of Mexico, including Atlantic bluefin tuna, yellowfin tuna, and mahi-mahi.[4−7] This is important because the polycyclic aromatic hydrocarbons (PAHs) present in crude oil have been demonstrated to impair cardiac function in marine fish, both across species and across life stages.[8−15]

Over the past decade, the mahi-mahi (*Coryphaena hippurus*: referred to herein as "mahi") has served as a valuable model for pelagic fish, facilitating studies on the impacts of oil exposure across levels of biological organization. Mahi exposed to oil

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have compromised swim performance, including reductions to maximal sustained swimming speed[16,17] and reduced maximal metabolic rate.[17] Further examination of heart function revealed that short-term oil exposure (24 h) was found to reduce stroke work, stroke volume, and cardiac output in an in situ preparation.[12]

Although cardiotoxic effects of oil have been widely reported, few studies have examined the cardiac cellular mechanisms underlying observed whole animal and organ-level impacts.[18,19] Cardiomyocyte function is dependent on excitation−contraction coupling which consists of three key events: (1) the generation of an action potential, (2) followed by a mobilization of calcium from extracellular and intracellular sources, (3) which initiates cross-bridge formation and contraction at the myofilaments.[20,21] Previous studies examining the impacts of oil on cardiomyocytes from Pacific bluefin tuna, yellowfin tuna, and Pacific mackerel, have exclusively focused on the first two components of excitation−contraction coupling.[18,19] These studies have demonstrated that PAH exposure causes prolonged action potentials, diminished calcium transients, disruptions to sarcoplasmic reticular calcium cycling, and a reduction of the potassium current (I_{Kr}) necessary for repolarization.[18,19] While these findings inferred reduced cardiomyocyte contractility, direct measurements of contractility following oil exposure have not previously been examined in any fish.

Accordingly, the first objective of this study was to examine mahi cardiomyocyte contractility over a range of environmentally relevant oil exposures, with the expectation that oil would cause a dose-dependent decrease in isolated cardiomyocyte contractile function. The second objective was to examine the impacts of oil on contractility over a range of stimulation frequencies representative of heart rates measured in whole-organ in situ studies on mahi,[12] with the expectation that oil would exacerbate impaired contractile function at higher stimulation frequencies. The third objective was to quantify electrophysiological properties of mahi cardiomyocytes over a range of oil concentrations to gain mechanistic insight into the oil-induced disruptions to excitation−contraction coupling.

MATERIALS AND METHODS

Experimental Animals. Adult mahi (F1 generation) (n = 10−15) ranging from 0.3 to 0.91 kg (Supporting Information, SI, Table S1) were raised from wild-caught broodstock and maintained in two 3000 L fiberglass tanks at the University of Miami Experimental Hatchery (UMEH).[22] Fish were maintained on flow-through seawater (25−29 °C) and fed daily with a mix of squid, mackerel, and silversides. All animal care and experimental procedures were approved by the University of Miami's Institutional Animal Care and Use Committee (IACUC protocol 15−019).

Ventricular Cardiomyocyte Isolation. Myocyte isolations were performed as in previous studies.[23,24] Mahi were stunned by a blow to the head and euthanized by pithing, and the heart (n = 10−15; SI Table S1) was extracted and retrograde perfused with isolation solution (SI Table S2) for 10 min, followed by perfusion with proteolytic enzymes (Trypsin Type 1X 0.5 mg mL^{-1}, Collagenase Type 1A 0.37−0.43 mg mL^{-1}; Sigma-Aldrich) and fatty-acid free bovine serum albumin (0.75 mg mL^{-1}; Sigma-Aldrich) for 10−25 min. The ventricle was then transferred into fresh isolation solution and triturated to free individual cardiomyocytes. Cardiomyocytes were stored in a fresh isolation solution up to 8 h following isolation.

Oiled Saline Preparation and PAH Analysis. Surface oil collected from the *Deepwater Horizon* oil spill (Sample ID: OFS-20100719-Juniper-001:00979, 00919) was transferred to the University of Miami under chain of custody and used to prepare all high energy water accommodated fractions (HEWAF). To prepare HEWAF saline needed for dilutions, 1 g of oil was added to 1 L of control extracellular saline (referred to herein as "saline," SI Table S2) at room temperature, blended in a Waring CB15 industrial blender (Torrington, CT) for 30 s at low speed, and transferred to 1 L separatory funnel for 1 h. The bottom 90% of this mixture was used as HEWAF saline. Unfiltered dilutions of HEWAF salines were used for Objective 3. For Objectives 1 and 2, each HEWAF saline was filtered through two stacked 0.3 μm 90 mm glass fiber filters with a vacuum pump under low suction[25] prior to preparing dilutions. Filtration was necessary to prevent oil microdroplets from interfering with the IonOptix imaging system.

Samples for ∑PAH analysis were taken immediately after HEWAF was made and sent to ALS Environmental (Kelso, WA) for analysis by gas chromatography/mass spectrometry with selective ion monitoring (GC/MS-SIM; based on EPA method 8270D). Reported ∑PAH values represent the sum of 50 selected PAH analytes dominated by 3-ring PAHs (SI Figures S1−S4). In a subset of samples (n = 4), unfiltered and filtered PAH analyses were performed from the same HEWAF saline to compare ∑PAH concentration and PAH composition (SI Figures S5−S7; "HEWAF salines")

Cardiomyocyte Oil Exposure. PAH concentrations corresponding to HEWAF dilutions are summarized in Table 1. Filtered HEWAF saline was diluted to nominal concentrations of 10, 20, or 40% in control saline for Objective 1, and based on these findings, a 10% filtered HEWAF was chosen for Objective 2. Since HEWAF saline was unfiltered for Objective 3, dilutions of 1, 2, and 5% were used. Validation of nominal dilutions (Table 1) were obtained using an established fluorescence method[26] as described in Esbaugh et al.[25] (SI, Fluorescence measurements). Samples for fluorescence in

Table 1. HEWAF Dilutions and Measured Concentrations for Each Experimental Objective

	HEWAF dilution (%)	Measured concentration (μg l^{-1} ∑ 50 PAH) Means ± s.e.m.
Objective 1: Dose response	10	2.8 ± 0.2
	20	6.1 ± 0.2
	40	12.9 ± 0.4
Objective 2: Force frequency	10	3.6 ± 0.1
Objective 3: Electrophysiology		
Action potentials	1	4.0 ± 1.6
	2	10.6 ± 3.2
	5	30.4 ± 11.9
I_{Kr}	1	9.3 ± 1.8
	2	9.9 ± 0.4
	5	17.5 ± 3.4
I_{K1}	1	3.0 ± 0.1
	2	5.0 ± 0.2
	5	7.4 ± 1.2

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Objectives 1 and 2 were collected directly from the microscope stage to calculate dilutions for each cell. In a small subset of volume-limited samples, nominal dilutions were used. In Objective 3, samples were collected directly from HEWAF saline nominal dilutions before and after they had passed through the perfusion chamber and measured using fluorescence. Procedures for cleaning and testing the experimental stage after oil exposure can be found in the SI section "Microscope stage cleaning."

Experimental Protocols: Objectives 1 and 2. Sarcomere length and other kinetic properties of mahi cardiomyocyte shortening were recorded in real-time using an IonOptix Myocyte Contractility system comprised of a perfusion chamber (FHDRCC1, IonOptix) mounted to an inverted microscope (Motic AE31) attached to a specialized fast digitizing dimensioning camera (MyoCam-S, IonOptix). The perfusion chamber was instrumented with electrodes connected to a MyoPacer stimulator (IonOptix) for field stimulation to induce myocyte shortening. The average sarcomere length was determined using SarcLens within IonWizard 6.0 software, which uses the A- and I- bands as a markers of sarcomere spacing within a user-defined region of interest and applies a fast Fourier transform to determine the mean spacing frequency. IonOptix software was used to analyze sarcomere shortening (our index of cell contractility) in addition to the other kinetic properties of mahi cardiomyocyte shortening including departure velocity, return velocity, time to peak contraction (50 and 70%), time to recovery (50% and 70%), time at maximal rate of departure, and time at maximal rate of return.

To obtain an individual cell recording, a small set of cardiomyocytes were gently transferred to the perfusion chamber and allowed to settle on the bottom for 5−10 min prior to perfusion with control saline (SI Table S2). Then, a randomly selected healthy cell was chosen for an individual cell recording. To assess changes in sarcomere shortening at different oil concentrations (Objective 1), cardiomyocytes were stimulated at 0.5 Hz and perfused with control saline followed by either an identical control saline or dilutions of HEWAF saline (10, 20, or 40%; Table 1). During Objective 2, all cardiomyocytes were stimulated at 0.5 Hz and perfused with control saline and followed by a switch to either an identical control saline or a 10% filtered HEWAF saline dilution at the same frequency (0.5 Hz; Table 1). After 1−2 min following the saline switch, cardiomyocytes were stimulated at progressively increasing frequencies (1.5, 2.0, 2.5, 3.0 Hz, 12 times/frequency) that represented a range of heart rates reported in anesthetized mahi in an in situ heart preparation (∼100−180 bpm).[12] The average of the middle 6 contractions from each frequency were used for analysis.[27] Cardiomyocytes were then returned to 0.5 Hz to assess whether or not the cell experienced reduced performance over the course of the experiment due to repeated stimulation.

Experimental Protocols: Objective 3. A sample of cardiomyocytes were placed in a recording chamber (RC-24, Warner Instruments) and superfused with control solution at room temperature (SI Table S2). Ventricular action potentials (APs), I_{Kr} (delayed rectifier current) and I_{K1} (inward rectifier current responsible for setting the membrane potential), were measured under control conditions followed by increasing dilutions of unfiltered HEWAF saline: 1, 2, and 5% (Table 1). Cardiomyocytes were then returned to control conditions to assess recovery from oil exposure.

Experiments were performed using Molecular Devices Axopatch 200A and 200B amplifiers connected to computers using 1140A A-to-D converters. Pipettes had a resistance of 4.3 ± 0.2 MΩ when filled with pipette solution. Pipette solutions for K^+ channel and action potential measurements are reported in SI Table S2. Junction potentials were zeroed prior to seal formation. Pipette capacitance was compensated after formation of a GΩ seal. Mean series resistance was 5.2 ± 0.8 MΩ. Membrane capacitance was measured using the calibrated capacity compensation circuit of the Axopatch amplifier. Signals were low-pass filtered using the 4-pole low pass Bessel filter at a frequency of 2 kHz and were then analyzed off-line using pClamp 10.0 software (Axon Instruments). To estimate cardiomyocyte surface area and normalize current density, total membrane capacitance (Cm) was recorded in each cardiomyocyte tested.

Action Potential and K^+ Channel Recordings. Action potentials from isolated cardiomyocytes were recorded in current clamp mode of the Warner patch clamp amplifier. Action potentials were elicited by 2 ms depolarizing voltage pulses (0.1−0.7 mV). K^+ channel recordings were obtained by adaptation of protocols previously designed by Vornanen and colleagues.[28,29] I_{Kr} and I_{K1} were isolated via pharmacological inhibition by blocking Na^+, Ca^{2+}, and ATP-sensitive K^+ channels with tetrodotoxin (0.5 μM), nifedipine (10 μM), and glibenclamide (10 μM), respectively.[29]

In preliminary experiments, the voltage eliciting maximum I_{Kr} tail currents were assessed by a depolarizing 4 s prepulse from 80 mV to +60 mV followed by a 3 s test pulse between −100 mV and +40 mV. Similar to results obtained from a previous study,[29] the maximum I_{Kr} tail current occurred at −20 mV. Therefore, when measuring the current−voltage relationship for I_{Kr}, −20 mV was applied as the test pulse voltage.

Current−voltage relationship for I_{Kr} was measured as the tail current at −20 mV following a series of prepulses between −80 and +80 mV in the absence and presence of the specific I_{Kr} inhibitor, E-4031 (2 μM). I_{Kr} was measured as an E-4031-sensitive current. Following blockade of I_{Kr}, I_{K1} was measured by repolarizing voltage ramps (1 s) from 30 mV to −120 mV with 5 s intervals, in the presence and absence of $BaCl_2$ (0.5 mM). I_{K1} was then determined as the barium (Ba^{2+}) sensitive difference in current.

Statistical Analyses. In Objective 1, the percent change in sarcomere shortening between saline 1 (control) and saline 2 (either control, 10%, 20% or 40% HEWAF saline) was assessed using a one-way ANOVA. All other parameters were statistically assessed using paired-t tests or signed-rank tests with Bonferonni corrections comparing raw values from the first and second saline (SI Table S5). Absolute values used to calculate percent changes are reported in SI Table S3 and Table S4 for Objective 1 and Objective 2, respectively. In Objective 2, the percent of sarcomere shortening during control saline 1 was set at 100% at 0.5 Hz, and all shortening values at subsequent frequencies were expressed as a percent decline from this original value. Transformed (squared) percent values were assessed using a two-way repeated measures ANOVA, using frequency and treatment as factors. All other biophysical parameters were also assessed using a two-way repeated measures ANOVA, using frequency and treatment as factors (SI Table S6). Holm-Sidak posthoc tests were performed following two-way repeated measures ANOVAs to assess treatment differences at specific frequencies. For Objective 3, differences in AP characteristics and I_{Kr}

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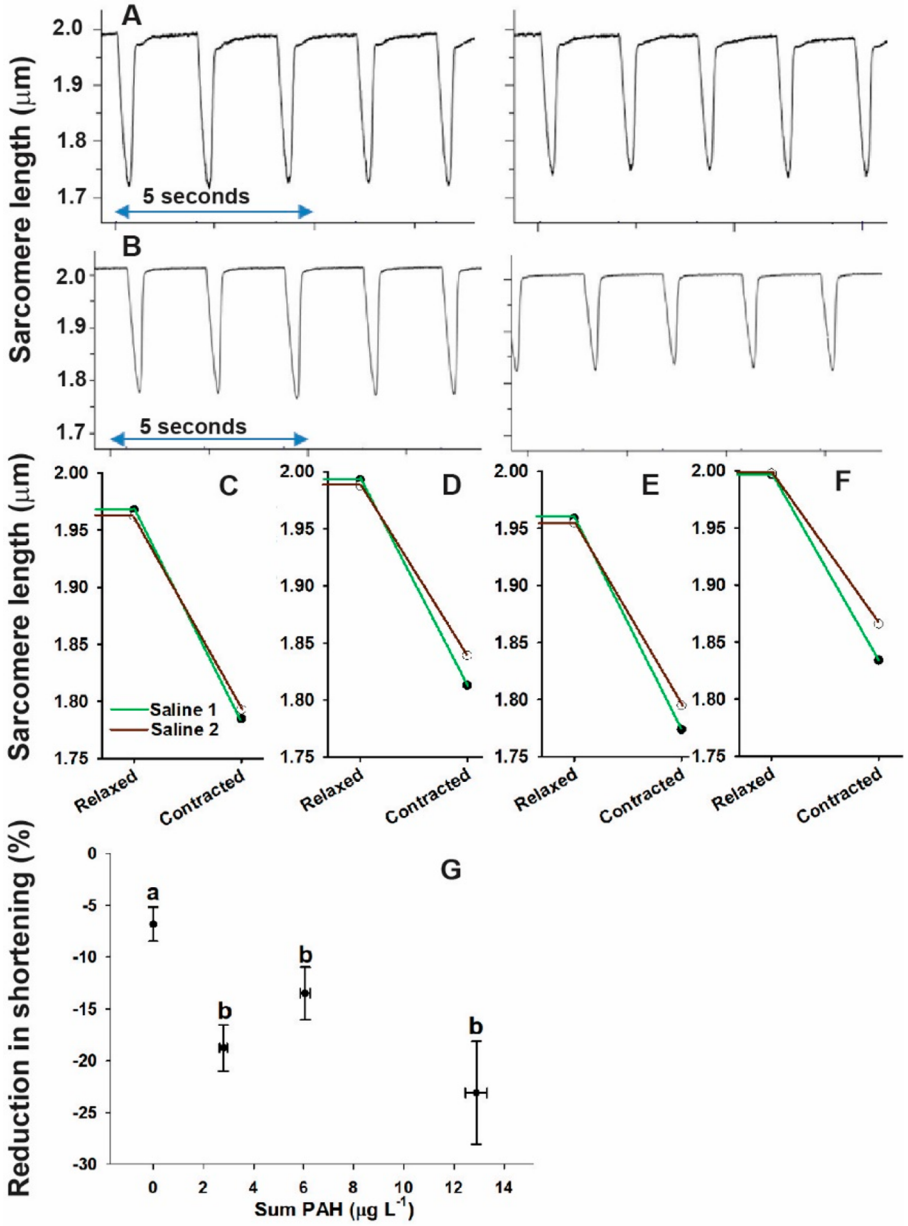

Figure 1. Representative traces of isolated mahi ventricular cardiomyocytes stimulated at 0.5 Hz showing the effect of a switch from a control saline to an identical control saline (A) or from a control saline to a HEWAF saline (B) containing polycyclic aromatic hydrocarbons (PAHs). Absolute relaxed and shortening lengths (C−F) and the percent reduction in shortening (G) are shown switching from a control saline to either (1) a second identical control saline (C) or (2) a saline containing one of three HEWAF dilutions (D: 2.8 ± 0.2, E: 6.1 ± 0.2, and F: 12.9 ± 0.4 $\mu g\ l^{-1}$ ∑PAHs, n = 17, 22, 22, and, 6, respectively). In C−F, green lines and closed symbols represent the first saline and brown lines and open circles represent the switch to the second saline. Different letters denote statistical significance $P < 0.05$ from control values (a). Values represent mean ± SEM. Absolute shortening lengths are summarized in SI Table S3.

and I_{K1} densities as a result of oil exposure were assessed with a one-way ANOVA followed by a Student-Neuman-Keuls posthoc test to identify all pairwise differences. All data are reported as means ± s.e.m. and were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

We sought to investigate the impacts of crude oil on ventricular cardiomyocyte function in an ecologically important pelagic fish species in the Gulf of Mexico, the mahi. To our knowledge, this is the first study to directly measure the impacts of oil on cardiomyocyte contractility in a marine fish. Our results show significant cardiomyocyte impairments at PAH concentrations that were observed in the water following the *Deepwater Horizon* oil spill[30,31] that include reduced cardiomyocyte contractility. Our data was also in agreement with other studies that found prolonged action potential duration and disruptions to potassium cycling.[18,19]

Impairments to Cardiomyocyte Contractile Function: Objectives 1 and 2. Cardiomyocytes exposed to oil showed a significant reduction in shortening across a range of concentrations (2.8, 6.1, and 12.9 $\mu g\ l^{-1}$ ∑PAH) when compared to control cardiomyocytes (Figure 1A−G). This decline in contractility due to oil likely accounts for the previously observed reductions in cardiac output[12] and swim

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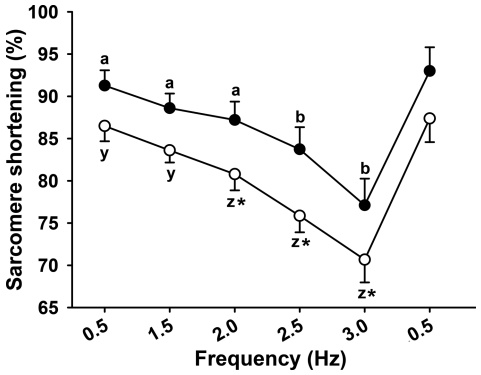

Figure 2. Reduction in shortening expressed as a percentage of control values at 0.5 Hz (100%). All cardiomyocytes were exposed to a control saline followed by a switch to either (1) a second identical control saline (filled circles) or (2) a saline containing HEWAF (open circles; 3.6 ± 0.2 $\mu g\ l^{-1}$ ∑PAH). Following the saline switch, cardiomyocytes were stimulated at progressively increased stimulation frequencies representing mahi heart rates reported in Nelson et al. (2016)[12] and then returned to resting frequency (0.5 Hz). Different letters represent a significant effect of frequency within a treatment, while an asterisk (*) represents significant differences between control and oil treatment at a given frequency (two-way repeated measures ANOVA, oil $P < 0.019$, frequency $P < 0.001$, interaction $P < 0.124$, $N = 12-13$). Values represent mean ± SEM. Sarcomere lengths used to calculate percent values are summarized in SI Table S4, and representative traces are presented in SI Figure S8.

performance[16,17] noted in oil-exposed mahi. Interestingly, the threshold necessary to induce impacts in isolated cardiomyocytes is lower than that needed to compromise whole animal performance. The present study notes reduced cardiomyocyte shortening at 2.8 $\mu g\ l^{-1}$ ∑PAH, while impairments to Ucrit (maximal sustained swimming speed) and maximal metabolic rate were noted at 8.4 but not 2.3 $\mu g\ l^{-1}$ ∑PAH in adults following 24 h of oil exposure.[17] This discrepancy could be due to a number of factors. First, this discrepancy could reflect that the intact animal has compensatory mechanisms that attenuate the deleterious effects of oil that are not evident at the cellular level. Such compensatory mechanisms have been recently noted in the pelagic cobia (*Rachycentron canadum*), where the administration of a high concentration of isoproterenol (10×10^{-5}), the isopropyl analog of adrenaline, was found to restore cardiac function in oil-exposed animals.[13] In many fish species, adrenaline has been previously demonstrated to increase force development,[32] increase action potential duration,[33] and increase intracellular calcium cycling in fish hearts.[33−35] Although acute experiments suggests adrenergic compensation is possible, it is important to note that at least in one species (red drum), impairments to swim performance in adults persisted 6 weeks after oil exposure, making it unclear whether adrenergic compensation is sustainable.[36] Second, the exposure of cardiomyocytes to saline containing crude oil may not reflect what is bioavailable in vivo in the blood. Understanding the concentration and composition of bioavailable PAHs in vivo would aid in anchoring cellular function studies to findings at higher levels of biological organization. Nonetheless, there is strong molecular, morphological, and physiological evidence that PAH exposure leads to circulation of PAH's in the blood. From a molecular perspective, increased

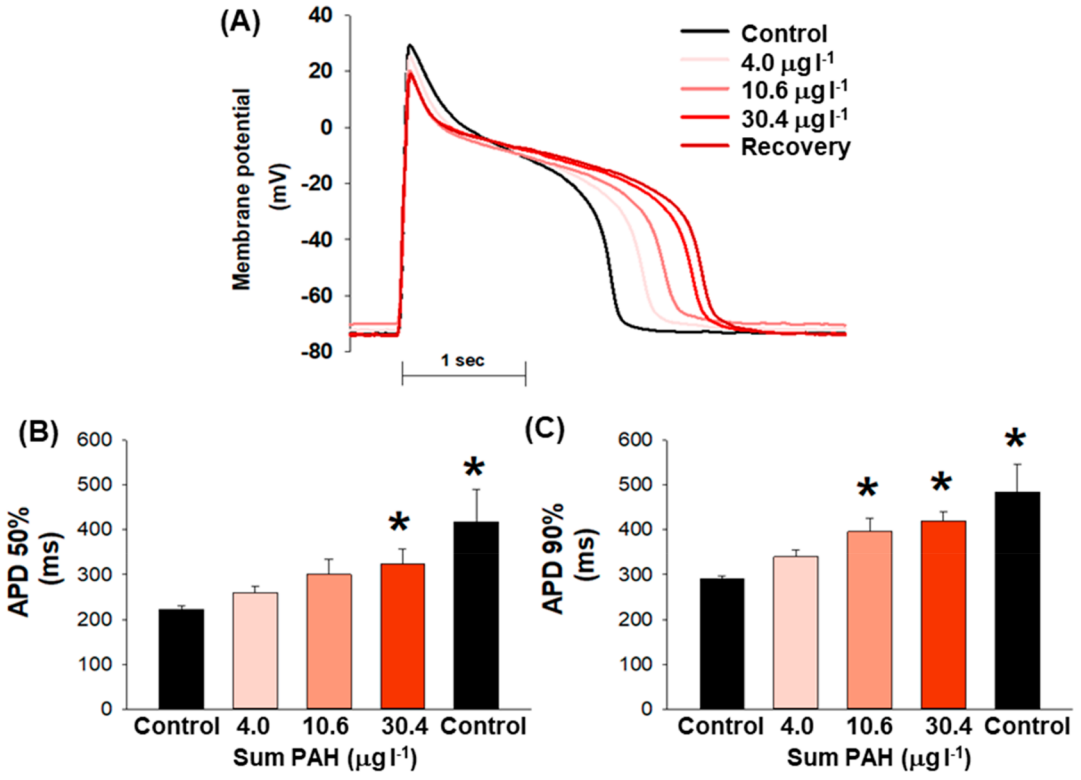

Figure 3. Dose response effects of acute HEWAF exposure on cardiomyocyte action potential (AP) characteristics. (A) Representative AP's recorded with current-clamp in a cardiomyocyte under control conditions (black) and with an increasing concentration of HEWAF (pink/red traces) followed by return to control conditions to assess recovery time to 50% (B) and 90% (C) AP duration (APD). An asterisk (*) indicates significant difference from initial control value ($P < 0.05$, one-way ANOVA, $n = 5-6$). Values represent mean ± SEM.

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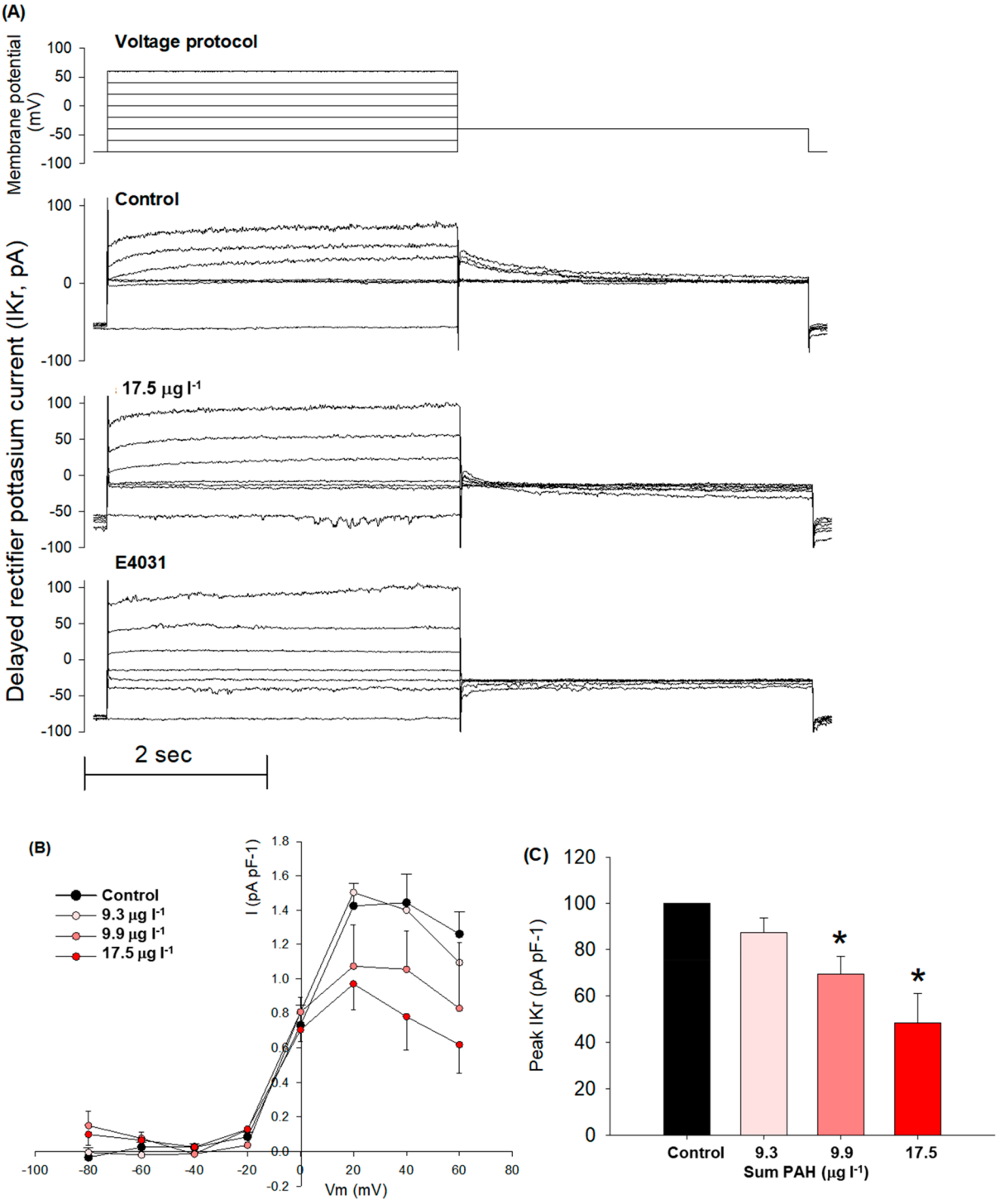

Figure 4. Effect of acute HEWAF exposure on current−voltage relationship on the delayed inward rectifier (I_{Kr}). (A) Voltage protocol to elicit I_{Kr}. (B) Representative current trace and voltage protocol from a cardiomyocyte showing current−voltage relationship of I_{Kr}. I_{Kr} was measured as the tail current at −20 mV following a series of prepulses between −80 and +80 mV in the absence and presence of the specific I_{Kr} inhibitor, E-4031, 2 μM. I_{Kr} was then calculated as the E-4031-sensitive current. (C) Mean ± SEM of I_{Kr} at different doses of HEWAF (n = 7−12 cardiomyocytes). An asterisk (*) indicates significant difference from initial control value ($P < 0.05$, one-way ANOVA).

CYP1A protein expression in the head kidney following PAH exposure suggested systemic circulation of PAHs.[37] Additionally, Alderman and colleagues (2017)[38] showed PAH's in the blood are the likely trigger for changes in the protein expression in the kidney and liver. From a morphological perspective, the heart of these high performing pelagic fish receives blood directly from the gill via the coronary artery with limited capacity for biotransformation. Finally, physiological evidence that supports the presence of circulating PAH's includes a reduction in cardiovascular function following whole animal exposure to PAH-contaminated seawater. This has been quantified in both in vivo and in situ studies.[12,13] In these studies, cardiovascular performance was measured in clean conditions (PAH-free) following

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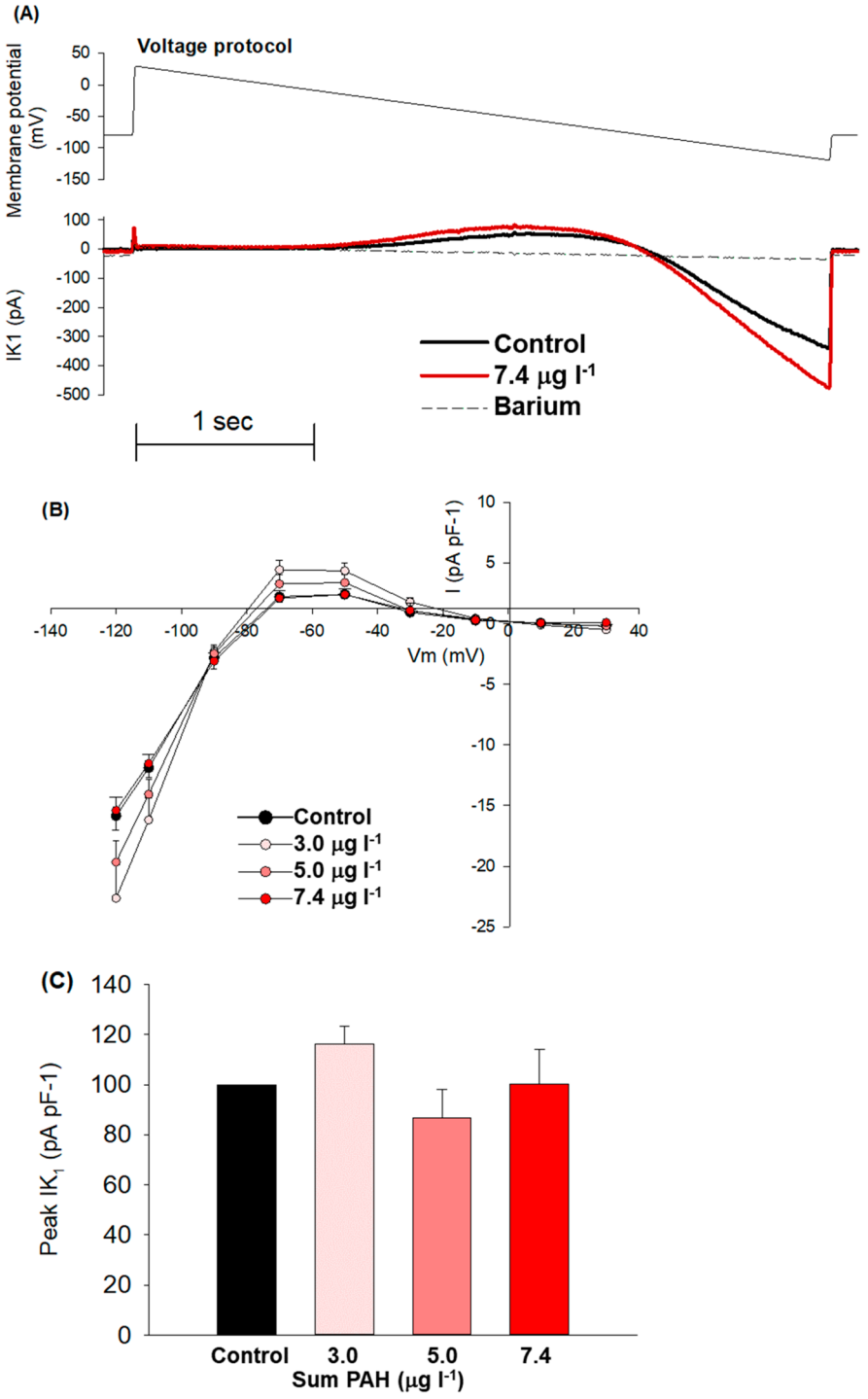

Figure 5. Effect of acute HEWAF exposure on current−voltage relationship of the background inward rectifier current (I_{K1}) from cardiomyocytes (n = 6−13 cardiomyocytes). (A) Voltage protocol for eliciting I_{K1}. (B) Original trace of I_{K1} in the absence and presence of 5% HEWAF (7.4 ± 1.2 μg l^{-1} ∑PAH) and the I_{K1} inhibitor, barium (Ba^{2+}). I_{K1} was then calculated as the Ba^{2+}-sensitive current. (C) Mean ± SEM results.

exposure, and the results clearly indicate that reduced performance resulted from direct deleterious effects of PAH's on organ function.

In agreement with a reduction in sarcomere shortening, both the sarcomere departure velocity and the return velocity were impacted at the low and intermediate levels of oil exposure (2.8, 6.1 μg l^{-1} ∑PAH; SI Table S5). Effects at the highest level of oil escaped statistical significance likely due to a high level of variance and a lower number of cardiomyocytes for this treatment (12.9 μg l^{-1} ∑PAH; SI Table S5). Control cardiomyocytes showed a small but significant decline in the return velocity, which likely reflects some degree of a reduction in sarcomere shortening in control-exposed cardiomyocytes noted in Figure 1C,G. This control shortening can be explained by a small amount of routine cell degradation and/or reflect changes due to switching saline wells. No other aspects of contractile function were significantly impacted (SI Table S5).

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As cardiomyocytes were subjected to progressively increased stimulation frequencies (1.5, 2.0, 2.5, and 3.0 Hz), both control and oil-exposed cardiomyocytes showed a decline in sarcomere shortening (Figure 2, SI Figure S8). This negative force frequency relationship is similar to that previously observed in fish cardiomyocytes.[39,40] Sarcomere shortening was significantly decreased in cardiomyocytes exposed to oil compared to controls indicating a treatment effect, and there was no statistically significant interaction between frequency and oil. Posthoc multiple comparison testing revealed that the effects of oil were most pronounced at the higher stimulation frequencies, 2.0, 2.5, and 3.0 Hz. This finding is particularly biologically relevant, because it suggests that the negative effects of oil are most pronounced at stimulation frequencies corresponding to higher heart rates. Higher heart rates would likely occur in response to stress or during periods of maximal activity. Thus, our findings provide a mechanistic link between reductions in contractility occurring at the cellular level to oil-induced impairments of mahi during maximal activity.[16,17] All other aspects of biophysical cardiomyocyte contractile function showed a significant effect of frequency but did not show an effect of treatment (SI Table S6), although an interactive frequency × treatment effect was noted in the time for cells to reach 50% and 75% of peak contraction ($P < 0.009$, 0.006, respectively).

Electrophysiological Studies: Objective 3. Ventricular action potential duration (APD) was significantly affected by oil in a concentration-dependent manner. APD at 50% and 90% repolarization was significantly longer at concentrations above 30.4 and 10.6 $\mu g\ l^{-1}$ ∑PAH, respectively (Figure 3A–C). The effects of oil on APD were not reversible. Prolonged action potential duration appears to be a common response to oil and has been noted in Pacific bluefin tuna (4 $\mu g\ l^{-1}$ ∑PAH; Slick A) and yellowfin tuna (22 $\mu g\ l^{-1}$ ∑PAH; Slick B).[19] Similar to what has been observed in other studies,[19] all other calculated action potential parameters, including resting membrane potential and peak amplitude, were unaffected by acute oil exposure (SI Figure S9).

To determine the mechanism of APD prolongation, we next examined I_{Kr}, the rapid component of the delayed rectifier K^+ current, which is considered the main repolarizing current in fish cardiomyocytes and has been shown to be inhibited by PAH exposure in at least two previous species (6 $\mu g\ l^{-1}$ ∑PAH).[19] As expected, oil-exposed cardiomyocytes exhibited significantly inhibited peak I_{Kr} density at concentrations above 9.9 $\mu g\ l^{-1}$ ∑PAH (Figure 4B,C). In the presence of E-4031 (I_{Kr} inhibitor), a repolarizing voltage ramp (1 s) from 30 to −120 mV led to an inward current at voltages from negative to −80 mV and a physiologically relevant outward current between −80 and 0 mV (Figure 5). Both currents were inhibited with $BaCl_2$, confirming their identities as the background inward rectifiers. I_{K1} outward density in the physiological range (−80 mV) was not affected by acute exposure to oil at any tested concentrations (3.0–7.4 $\mu g\ l^{-1}$ ∑PAH; Figure 5). Although these PAH concentrations were generally lower than in other measurements in the present study, no effect of oil on I_{K1} was consistent with the lack of a change in resting membrane potential following oil exposure (SI Figure S9).

Together, oil-induced reductions in cardiomyocyte contractility and I_{Kr} suggest that mechanisms underlying oil impairment in mahi are similar to what has been observed in a limited number of marine teleosts studied to date.[18,19] In these studies, oil or individual PAHs (phenanthrene) appear to disrupt excitation–contraction coupling and prolong action potentials by reducing the rapid delayed rectifier potassium current (I_{Kr}). In addition to causing cellular disruption in cardiomyocytes from adult marine teleosts,[18] phenanthrene causes cardiac dysfunction in developing teleost embryos.[41] Interestingly, compositional PAH profiles reported herein show high levels of PAHs in the phenanthrene family (SI Figure S1, S3). The reduction in cardiomyocyte shortening in the face of increased action potential duration suggests a decrease in calcium current (I_{Ca}) and/or a disruption in release and/or uptake of Ca^{2+} from the sarcoplasmic reticulum (SR). We did not directly measure these Ca^{2+} flux pathways, but such changes have been previously observed in mackerel and two tuna species.[18,19] One caveat is that in muscle strip preparations under certain conditions (0.5 Hz, SR inhibition with thapsigargin and ryanodine at 26 °C), mahi exhibited little reliance on the sarcoplasmic reticulum.[42] This suggests that the oil-induced reduction to contractile function is more likely due to either decreased calcium current (I_{Ca}) or impacts to myofilament sensitivity, providing an interesting avenue for future research. Notably, embryonic oil exposure led to significant downregulation in a number of genes related to calcium cycling in mahi at 48–96 h post hatch including ryanodine receptor 2, troponin T type 2, and L-type voltage-dependent calcium genes,[43] again suggesting that further research into calcium cycling in adult cardiomyocytes would be useful in further discerning mechanisms underlying contractile impairment.

Overall, findings from this study clarify the cardiac mechanisms underlying impairment of organ function[12] and reduced swim performance in mahi[17] following oil exposure. This study is the first to report reductions in cardiomyocyte contractility following oil exposure in a marine fish, providing a link between oil-induced impairments of electrophysiological parameters with a functional consequence. More broadly, our findings are similar to reported impacts in other species suggesting common mechanisms underlying oil cardiotoxicity across species.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b03798.

Morphometrics on mahi used in experiments, composition of saline solutions, sarcomere length during contraction and relaxation at various PAH concentrations and increasing stimulation frequencies, various biophysical aspects of cardiomyocyte contractile function at various PAH concentrations and increasing stimulation frequencies, PAH compositional profiles for all objectives, percent of PAHs grouped by ring structure for all objectives, unfiltered and filtered comparison of extracellular saline characteristics, representative traces of cardiomyocytes under increasing stimulation frequencies, other characteristics of action potential data including resting membrane potential, details of fluorescence measurements, results of comparison of filtered and unfiltered HEWAF PAH composition, and microscope cleaning procedures (PDF)

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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-s7d0-s364.[44]

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