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Effects of crude oil on *in situ* cardiac function in young adult mahi–mahi (*Coryphaena hippurus*)

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ABSTRACT

Exposure to polycyclic aromatic hydrocarbons (PAH) negatively impacts exercise performance in fish species but the physiological modifications that result in this phenotype are poorly understood. Prior studies have shown that embryonic and juvenile mahi–mahi (*Coryphaeus hippurus*) exposed to PAH exhibit morphological abnormalities, altered cardiac development and reduced swimming performance. It has been suggested that cardiovascular function inhibited by PAH exposure accounts for the compromised exercise performance in fish species. In this study we used *in-situ* techniques to measure hemodynamic responses of young adult mahi–mahi exposed to PAH for 24 h. The data indicate that stroke volume was reduced 44% in mahi–mahi exposed to $9.6 \pm 2.7\ \mu g\ l^{-1}$ geometric mean PAH ($\sum$PAH) and resulted in a 39% reduction in cardiac output and a 52% reduction in stroke work. Maximal change in pressure over change in time was 28% lower in mahi–mahi exposed to this level of $\sum$PAH. Mean intraventricular pressures and heart rate were not significantly changed. This study suggests exposure to environmentally relevant PAH concentrations impairs aspects of cardiovascular function in mahi–mahi.

1. Introduction

The impact of anthropogenic factors on organismal physiology is widely documented and exposure to crude oil as the result of industrial disasters is a prime example. Acute and chronic exposures to polyaromatic hydrocarbons (PAH), toxic components of crude oil, have been investigated across multiple levels of biological organization in marine ecosystems (Eisler, 1987; McDowell Capuzzo et al., 1988). Fish species have been targeted for studying the effects of PAH due to the possible deleterious consequences on species abundances and the potential economic impacts on local and global recreational and commercial fisheries (Incardona et al., 2013, 2012a; Heintz et al., 1999; Hulson et al., 2008; Thorne and Thomas, 2008). Recent work has focused on the specific effects of PAH exposure on organ system development, and deleterious or abnormal organ phenotypes have been identified in several fish species (Incardona et al., 2013, 2012b; Hicken et al., 2011; Jung et al., 2013; Brette et al., 2014; Klinger et al., 2015; Heintz et al., 2008). Specifically, cardiovascular perturbations have been reported across multiple levels of organization, from the molecular level to overall organismal swim performance (Edmunds et al., 2015; Esbaugh et al., 2016; Incardona et al., 2014; Stieglitz et al., 2016; Xu et al., 2016).

Acute exposures to sublethal concentrations of crude oil containing PAH reduces swim performance in both juvenile and adult mahi–mahi (Mager et al., 2014; Stieglitz et al., 2016) and reduces maximal metabolic rate and aerobic scope in adult mahi–mahi (*Coryphaena hippurus*). Stieglitz et al. (2016) suggested these phenotypic responses could directly impact the ability of the animal to survive in its natural environment, limiting their capacity to migrate, capture prey and avoid predation. While there are clear negative effects on exercise performance associated with PAH exposure, questions remain regarding the mechanisms that underlie this detrimental phenotype.

The cardiovascular system plays a key role in the oxygen cascade from the respiratory media to the tissues (Hillman et al., 2013; Hedrick et al., 2015; Hillman and Hedrick, 2015) and meets the metabolic demands of active tissues by modulating cardiac output (Farrell, 1991). In all teleost species studied to date, increases in swim performance are accompanied by increases in cardiac output, but the parameters that contribute to this increase in heart rate or stroke volume differ between studies (Farrell, 1991). While

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the importance of increasing cardiac output to sustain increased swimming activity in fish is widely demonstrated, the impact of exposure to PAH on direct cardiac function at the organ system level has yet to be documented. Given its central role in meeting the oxygen demands of active tissue, the cardiovascular system is a strong candidate for study and may correlate with the known organismal response to PAH exposure.

Investigations of impacts of PAH exposure on fish cardiac function have primarily focused on anatomical changes observed during ontogeny (McIntyre et al., 2016; Esbaugh et al., 2016; Scholz and Incardona, 2015; Jung et al., 2015; Incardona et al., 2014, 2015) or on effects at the cellular level (Brette et al., 2014). At the level of the cardiomyocyte, PAHs disrupt potassium as well as calcium currents and calcium cycling in cells isolated from bluefin tuna (Thunnus *orientalis*) and yellowfin tuna (Thunnus *albacares*) (Brette et al., 2014). The consequences of these cardiomyocyte disruptions in membrane biophysical properties at the heart level could include reductions in cardiac contractile force due to critical role of ion channels in this process (Shiels et al., 2004, 2015; Galli et al., 2011). For the purpose of investigating these possible higher order effects, *in situ* measurements of cardiac function are useful by providing an index of cardiomyocyte function while maintaining the native structural and fluid environment composition. Further, intraventricular measurements of pressure combined with quantifications of total blood flow are essential to understanding the physiological changes that may correlate with the known compromise in swim performance in fish species exposed to PAH (Kennedy and Farrell, 2006; Mager et al., 2014; Stieglitz et al., 2016) and provide an important link between the cellular and organismal level of organization.

Mahi–mahi are active pelagic fish known to inhabit waters in the Gulf of Mexico (Gibbs and Collette, 1959; Palko et al., 1982) at the site of the *Deepwater Horizon* oil spill, the largest oil spill in U.S. history (Mager et al., 2014; Incardona et al., 2013). The present study aims to investigate the effects of 24 h acute exposure to High Energy Water Accommodated Fraction (HEWAF) crude oil on *in situ* cardiac function in adult mahi–mahi. Based on the previously reported reductions of swim performance in juvenile and adult mahi–mahi and disturbances in both calcium cycling and ion flux on non-pacemaker cells (Brette et al., 2014), we hypothesized that oil exposure would reduce cardiac contractility. To test this hypothesis, we utilized an anesthetized *in situ* preparation to investigate various aspects of cardiac function, including but not limited to cardiac output, contractility, cardiac cycle duration and intraventricular pressure indices. Our findings demonstrate that brief exposure to crude oil negatively impacts stroke volume and cardiac contractility in mahi–mahi.

2. Methods

Fish

Hatchery raised F1 mahi–mahi were maintained in two 3000 l fiberglass tanks equipped with flow-through seawater (25–28 °C) at the University of Miami Experimental Hatchery (UMEH). Fish were fed daily to satiation with a mixture of chopped squid, mackerel, and sardines. All fish were fasted for 24 h prior to the study. Young adult mahi–mahi, 90–120 days of age were used in this study (N = 7 for control 1.01 ± 0.11 kg, N = 8 for oil 1.00 ± 0.05 kg).

2.1. Experimental conditions

Prior to *in situ* measurements, mahi–mahi were maintained in either a control seawater tank (N = 7) or oiled seawater containing 10% HEWAF tank (N = 8) for 24 h in static 300 l tanks (2 fish per tank in both conditions) (Table 1). Temperature in experimental tanks was maintained at 26° C using a 1000-W submersible heating system (Innovative Heat Concepts QDPTY1-1, Homestead, FL, USA) placed in the center of the tank along with an air stone and an oxygen stone at a flow rate of 1 l min^{-1}. A small submersible aquarium pump (Rio© + 800, Technological Aquatic Associated Manufacturing, Camarillo, CA USA) ensured adequate water circulation. During experimentation, both tanks were covered with ventilated tops to reduce stress and prevent disturbances during exposures. The crude oil used in this study (sample ID CTC02404-02) was collected from a barge (#CTC2404) on July 29, 2010 at the site of the *Deepwater Horizon* oil spill and transferred under chain of custody to University of Miami. A 10% HEWAF solution was prepared by adding 2 g of oil from the surface per 1 l of UV sterilized, 1 μm filtered seawater and blending it for 30 s in a Waring CB15 blender (Torrington, CT, USA). The mixture was poured into a glass separatory funnel to settle for 1 h. The lower 90% of the mixture was used for the experiment, as previously described (Mager et al., 2014). This HEWAF mixture was added to the experimental tanks within 24 h of preparation.

Water quality measurements of temperature and dissolved oxygen (ProODO, YSI, Inc., Yellow Springs, OH), pH (PHC3005, Radiometer, France), salinity (Pentair Aquatic Ecosystems, Apopka, FL), and total ammonia were taken at the beginning and end of the 24 h experimental exposures. Water samples were collected by submerging a sample bottle 15 cm deep in the water column. Total ammonia analyses were conducted by colorimetric assay (Verdouw et al., 1978) (Table 3). In addition, initial and final water samples were collected for total ($\sum$) PAH analysis from oil exposures. $\sum$PAH samples were also taken from control tanks during initial water quality sampling. Samples were analyzed by ALS Environmental (ALS Environmental, Kelso, WA, USA) using gas

Table 1
Body Temperature, animal mass, and organ masses of control ($n = 7$) and 24 h High Energy Water Accommodated Fraction (HEWAF) exposed ($n = 8$) young adult mahi–mahi. Values represent mean ± SE.

Measure	Control	PAH exposed
Body temperature(°C)	25.95 ± 0.12	25.3 ± 0.31
Fish mass (kg)	1.00 ± 0.12	1.00 ± 0.06
Heart mass (g)	1.11± 0.11	1.16 ± 0.10
Ventricle mass (g)	0.92 ± 0.09	1.02 ± 0.07

Table 2
Intraventricular timed indices of control (n = 7) and exposed (24 h, 9.6 ± 2.7 μg l^{-1} $\sum$PAH) exposed (n = 8) young adult mahi–mahi. Values represent mean ± SEM.

Measure	Control	PAH exposed
Systolic Duration (s)	0.220 ± 0.006	0.197 ± 0.004*
Diastolic Duration (s)	0.192 ± 0.035	0.186 ± 0.017
Cycle Duration (s)	0.413 ± 0.040	0.383 ± 0.176

An asterisk indicates significant differences from control values.

Table 3
Water parameters of control (n = 7) and exposed (n = 8) adult mahi–mahi. Values represent mean ± SEM.

Measure	Control	PAH exposed
pH initial (S.U.)	8.06 ± 0.07	7.95 ± 0.10
pH final (S.U.)	7.51 ± 0.08	7.43 ± 0.04
DO initial (mg l^{-1})	11.20 ± 1.43	14.03 ± 1.47
DO final (mg l^{-1})	15.52 ± 2.35	15.42 ± 0.67
Salinity initial (ppt)	31.50 ± 0.76	32.57 ± 0.61
Salinity final (ppt)	31.00 ± 0.77	32.00 ± 0.58
Ammonia initial (μM)	10.31 ± 8.54	7.42 ± 3.62
Ammonia final (μM)	143.05 ± 47.96	115 ± 13.51

chromatography and selective ionic monitoring mass spectrometry within six days of collection.

2.1.1. Surgery protocol

All measurements were conducted on anesthetized fish using similar methodologies to those previously described (Chin Lai et al., 1987; Jones et al., 1993; Mendonça et al., 2007). Briefly, following the 24 h exposure to either control conditions or 10% HEWAF, each fish was transferred to a 19 l plastic tank containing seawater with 100 mg l^{-1} tricaine methane sulphonate (MS-222, Sigma-Aldrich, St. Louis, MO) buffered to neutral pH with 100 mg l^{-1} sodium bicarbonate ($NaHCO_3$, Sigma-Aldrich, St. Louis, MO) for anesthesia. Once the righting reflex was lost, the fish was transferred to a custom surgical table and placed ventral side up. A plastic tube was placed in the mouth of the fish to perfuse the gills at a rate of 6 l min^{-1} with oxygenated seawater pumped (Rio© + 800, Technological Aquatic Associated Manufacturing, Camarillo, CA USA) from a reservoir containing a surgical anesthetic (100 mg/l). To retain gill buoyancy and deter heat loss, the surgical table was submerged in a 40 l plastic box with water temperature maintained at 26° C by a VWR temperature controlled circulating bath (VWR 1160s, Radnor, PA, USA). To measure body temperature, a thermocouple (RET-1, Physitemp Instruments, Clifton, NJ, USA) was advanced into the animal's anus and connected to an electronic thermometer (BAT-12, Physitemp Instruments, Clifton, NJ, USA).

The heart and ventral aorta were then exposed with a single ventral incision at the midline from the pectoral fins anterior to the midpoint of the isthmus. Tissue was blunt dissected apart to expose the structures under a dissection microscope (Leica M60 Leica Microsystems, Waukegan, IL, USA). The pericardium was then cut to expose the ventricle for introduction of a pressure catheter. After the structures were isolated, a vascular blood flow probe (Transonic 2.5PS or 3PS, Transonic Systems, Ithaca, NY, USA), calibrated for the study temperature, was placed around the ventral aorta and then connected to a Transonic T402 blood flow meter (Transonic Systems, Ithaca, NY, USA). Next, a puncture was made at the apex of the ventricle using a 22-gauge needle. A Scisense ADVantage conductance catheter (Scisense FTH-3518B, Transonic Scisense, London, ON, Canada) connected to a Scisense ADVantage 5.0 control unit (Transonic Scisense, London, ON, Canada) was then inserted into the ventricle for intraventricular pressure measurements. Once instrumentation was completed, the preparation was allowed to stabilize for 10 min. All preparations were completed within 1.5 h. Throughout the study, care was taken to avoid direct contact of saltwater with the exposed heart and tissue. Tissue was superfused with 0.9% NaCl periodically to prevent desiccation. At the completion of the study all animals were euthanized by an overdose of buffered MS-222 (200 mg l^{-1}; 200 mg l^{-1} HCO_3) and blow to the head. Body mass and organ masses were recorded to the nearest 0.01 g. All study procedures were approved by University of Miami animal care and use committee (IACUC protocol # 12-064).

2.1.2. Data acquisition and calculations

Output from the flow meter, pressure catheter, and temperature meter were connected to a PowerLab 8/35 data acquisition system (ADInstruments, Colorado Springs, CO, USA) linked to a Macintosh computer (Mac Mini, Apple Inc, Cupertino, CA, USA) running LabChart data acquisition software (Chart v 8.1.2, ADInstruments, Colorado Springs, CO, USA). Data were recorded at 1000 Hz. Heart rate (f_H) was calculated based on the blood pressure pulse interval. Cardiac output (Q) was calculated as the mean blood flow through the ventral aorta per min and kg body mass. Stroke volume (V_s) was calculated as the quotient of Q and f_H. Pressure- and time-dependent parameters were collected using Scisense ADVantage conductance catheter. Stroke work (SW) was calculated as the product of V_s and mean intraventricular pressure (IVP). End systolic pressure (P_{es}) mean intraventricular pressure (IVP), end diastolic pressure (P_{ed}), peak developed pressure (P_{dev}) were all direct measures form the transducer. Calculated pressure and cardiac cycle parameters were maximal rate of pressure generation (dP/dt_{Max}), isovolumic relaxation constant (Tau), contractility index (dP/dt max divided by the pressure at the time of max dP/dt), systolic duration (elapsed time between the start of the cycle (P_{ed}) and the time of min dP/dt), diastolic duration (the elapsed time between the time of min dP/dt and the end of the cycle (P_{ed})) and cycle duration (time from P_{ed} the following P_{ed}). Cardiac power was calculated as the product Q and the difference between ventricular P_{es} and P_{ed} after converting pressure to mW.

2.1.3. Statistical analysis

Hemodynamic parameters were analyzed using a one-way ANOVA with treatment condition as the independent variable. Significant effects were followed by Fisher LSD *post-hoc* comparisons. Body temperature and animal mass were analyzed using a one-way ANOVA. Organ masses were analyzed with a one way ANCOVA with animal mass as the covariate. All data are presented as mean ± standard error. A significance level was assigned at 95% probability ($P < 0.05$) in all cases. All statistical analyses were carried out with Statistica v13 with all data sets tested to ensure ANOVA assumptions were met (StatSoft, Tulsa, OK, USA).

3. Results

3.1. Exposures

The geometric mean of polycyclic aromatic hydrocarbons ($\sum$PAH) during the 24-h exposure period was 9.6 ± 2.7 μg l^{-1} $\sum$PAH. Measurements of control water revealed 0.07 ± 0.02 μg·l^{-1} $\sum$PAH. Initial HEWAF samples of PAH consisted of 69% of 3-ringed compounds, 26% of 4-ringed compounds, 4.3% of 2-ring compounds and less than 1% of 5-ring or 6-ring compounds. There was a percentage drop in 3-ring, 5-ring, and 6-ring PAH by 35%, 20% and 89%, respectively, over the 24-h period. However, 4-ring compounds increased by 56% during the 24-h exposure period (Fig. 5A). PAH concentration decreased 81% over the 24-h exposure period from an initial PAH mean concentration of 24.9 ± 1.0 μg l^{-1} to a final PAH mean concentration of 4.7 ± 1.1 μg l^{-1} (Fig. 5B). Overall, the fish from this study were exposed to PAH levels that replicated prior study of the impact of HEWAF on swim performance and metabolic function (Stieglitz et al., 2016) (Table 3).

3.2. Hemodynamics

Mahi–mahi exposed to 9.6 ± 2.7 μg l^{-1} $\sum$PAH for 24 h exhibited a 44% reduction in stroke volume (V_s), (0.27 ± 0.04 ml $beat^{-1}$ kg^{-1}) compared to the control group (0.37 ± 0.03 ml $beat^{-1}$ kg^{-1}) (Fig. 1A). This reduction in V_s accounted for the 39% reduction in cardiac output (Q) in exposed mahi–mahi (33.6 ± 6.8 ml min^{-1} kg^{-1}) in comparison to the control group (55.4 ± 6.2 ml min^{-1} kg^{-1}) (Fig. 1B). Heart rate (f_H) was similar in the exposed mahi–mahi (158 ± 7 bpm) and the control group (152 ± 12 bpm) (Fig. 1C). Like the other factors of cardiac function, stroke work (SW), was 52% lower in the exposed mahi–mahi (0.71 ± 0.17 kPa ml^{-1}) compared to the control group (1.62 ± 0.37 kPa ml^{-1}) (Fig. 1D).

Mean intraventricular pressure (IVP), peak developed pressure (P_{dev}), end diastolic pressure (P_{ed}) and end systolic pressure (P_{es}) were similar between the experimental and control groups and (Fig. 2A–D). However, maximal change in pressure over change in time (dP/dt_{max}) was 28% lower in the exposed mahi–mahi (93.95 ± 13.61 kPa s^{-1}) compared to the control group (130.66 ± 10.47 kPa s^{-1}) (Fig. 3A). The isovolumic

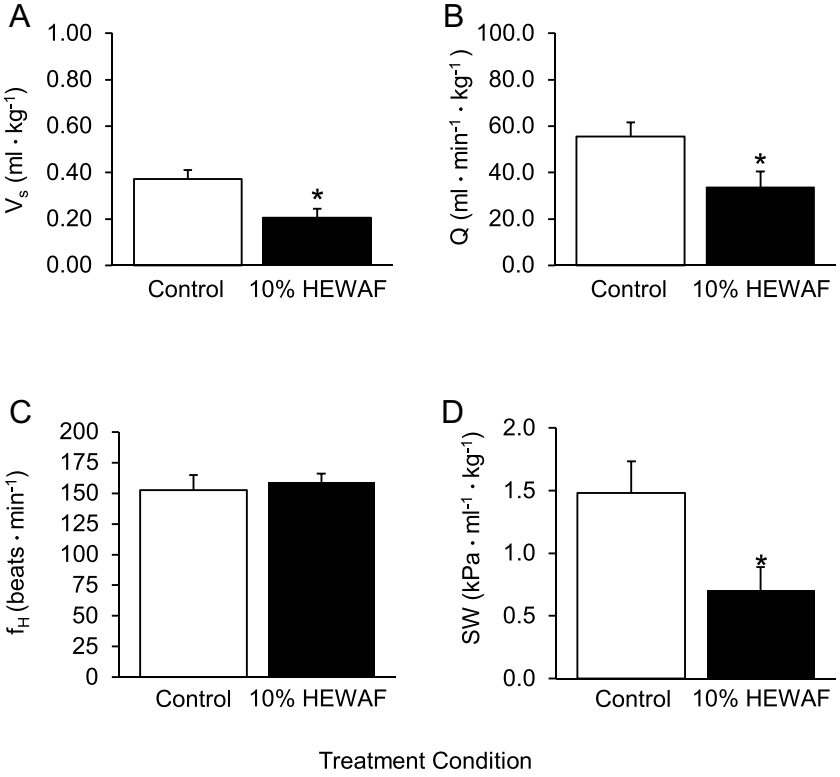

Fig. 1. Intraventricular parameters A. stroke volume (V_s), B cardiac output (Q), C. heart rate (f_H) and D. stroke work (SW) of control (open bars, n = 7) and 24 h PAH exposed (closed bars, n = 8) young adult mahi–mahi. An asterisk indicates significant difference between control and treated fish ($p < 0.05$). Values represent mean ± SEM.

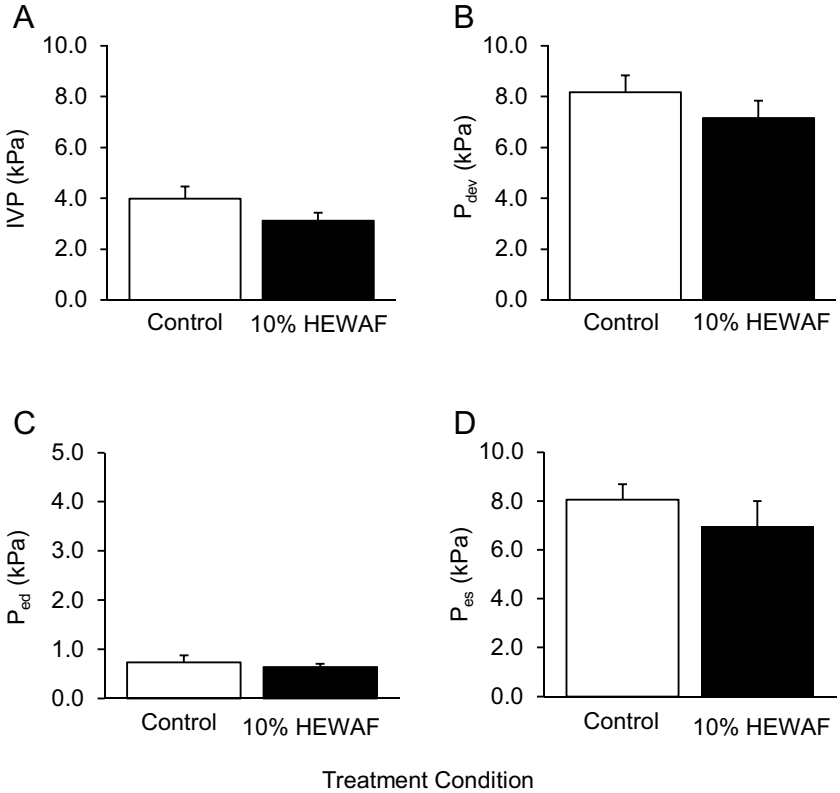

Fig. 2. Pressure parameters – A. intraventricular pressure (IVP), B. developing pressure (P_{dev}), C. end diastolic pressure (P_{ed}), and D. end systolic pressure (P_{es}), of control (open bars, n = 7) and 24 h PAH exposed (closed bars, n = 8) young adult mahi–mahi. Values represent mean ± SEM.

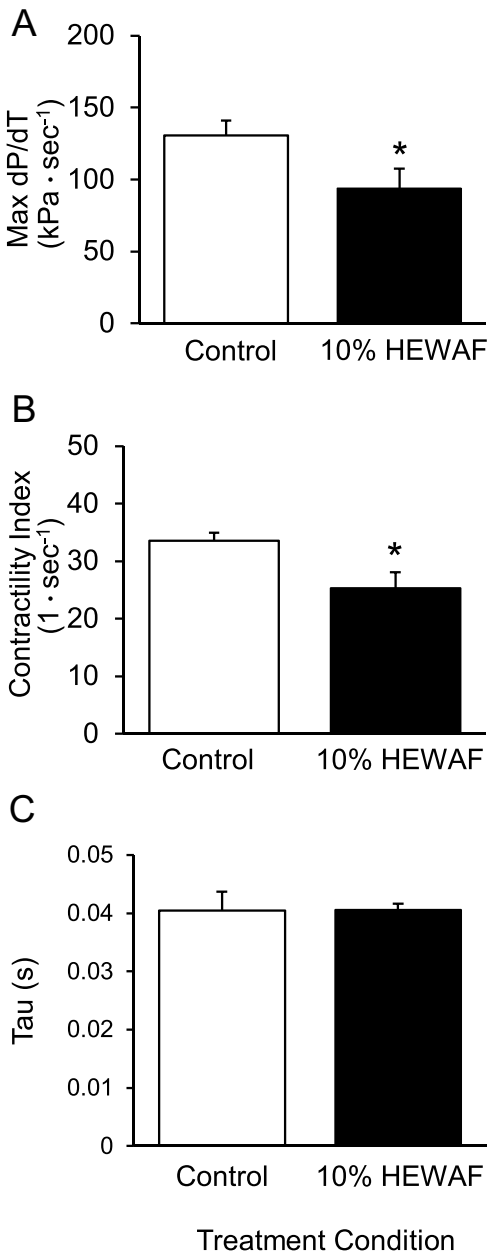

Fig. 3. Timed parameters – A. ventricle contractility (dP/dt), B. ventricle relaxation (Tau) and contractility index of control (open bars, n = 7) and 24 h PAH exposed (closed bars, n = 8) young adult mahi–mahi. An asterisk indicates significant difference between control and treated fish ($p < 0.05$). Values represent mean ± SEM.

relaxation constant (Tau) was similar between the groups (Fig. 3C). The contractility index decreased 24% in exposed mahi–mahi ($25.29 \pm 2.82\ s^{-1}$) compared to the controls ($33.50 \pm 1.40\ s^{-1}$) (Fig. 3B). Systolic duration was significantly shortened by 10% in exposed mahi–mahi (0.197 ± 0.004 s) compared to controls (0.220 ± 0.006 s) (Table 2). However, while both diastolic and cycle duration appeared short end, there was no significant difference (Table 2).

Mass specific cardiac power output showed a trend toward depression by 38% in exposed mahi–mahi ($4.07 \pm 0.95\ mW\ kg^{-1}$) compared to the control group ($6.58 \pm 1.32\ mW\ kg^{-1}$) ($P = 0.070$) (Fig. 4A). However, there was 41% decrease in ventricle mass specific cardiac power output in mahi–mahi exposed to PAH ($4.12 \pm 0.94\ mW\ g^{-1}$) compared to the control group ($7.07 \pm 1.40\ mW\ g^{-1}$) (Fig. 4B).

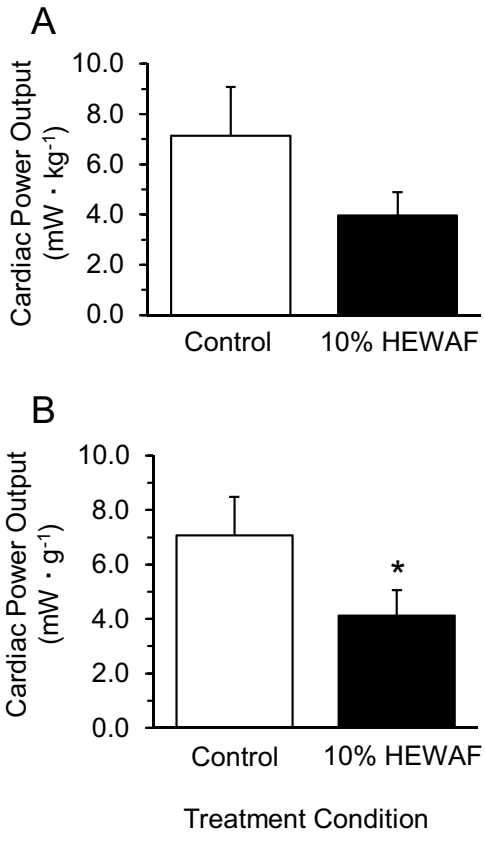

Fig. 4. Cardiac power output A. per unit body mass, and B. ventricle mass in fish from the control conditions (open column) and after 24 h exposure to 10% HEWAF (filled column). An asterisk indicates significant difference between control and treated fish ($p < 0.05$). Values represent mean ± SEM.

4. Discussion

Cardiovascular function is tightly correlated with exercise performance and the coupling of cardiac output to metabolic demand ensures that the convective transport of oxygen and nutrients meets tissue demand (Farrell and Steffensen, 1987; Farrell et al., 1990, 1991; Kolok et al., 1993; Keen and Farrell, 1994; Kolok and Farrell, 1994). Prior studies on mahi–mahi have reported that exposure to PAH impacts swim performance in both juvenile ($30\ \mu g\ l^{-1}$ ∑PAH) and adult mahi–mahi ($8.4\ \mu g\ l^{-1}$ ∑PAH) as well as maximal oxygen uptake as aerobic scope in adult mahi–mahi ($8.4\ \mu g\ l^{-1}$ ∑PAH) (Mager et al., 2014; Stieglitz et al., 2016). These findings are in agreement with reported actions of PAH on cardiomyocyte function (Brette et al., 2014) which could translate to depression of cardiac output via changes in heart rate and/or stroke volume. Our results show that indices of cardiac contractility and ejection are negatively impacted by 24 h of exposure to $9.6 \pm 2.7\ \mu g\ l^{-1}$ ∑PAH, values that fall within concentrations reported from the proximity of the Deepwater Horizon oil spill (Wade et al., 2013; Diercks et al., 2010). These findings support the hypothesis that crude oil exposure negatively impacts cardiac output due to reductions in stroke volume which may account for the previously documented compromised swim performance in mahi–mahi exposed to $8.4\ \mu g\ l^{-1}$ ∑PAH.

The ventricular contractile cycle consists of an isovolumic contractile phase, an ejection phase, an isovolumic relaxation phase, and a diastolic filling phase (Stekelenburg-de Vos et al., 2005). The exposed Mahi–mahi exhibited a marked reduction in stroke volume, cardiac output, and stroke work (Fig. 1A, B, D), all related to the ejection phase of the ventricle. Determination of the basis

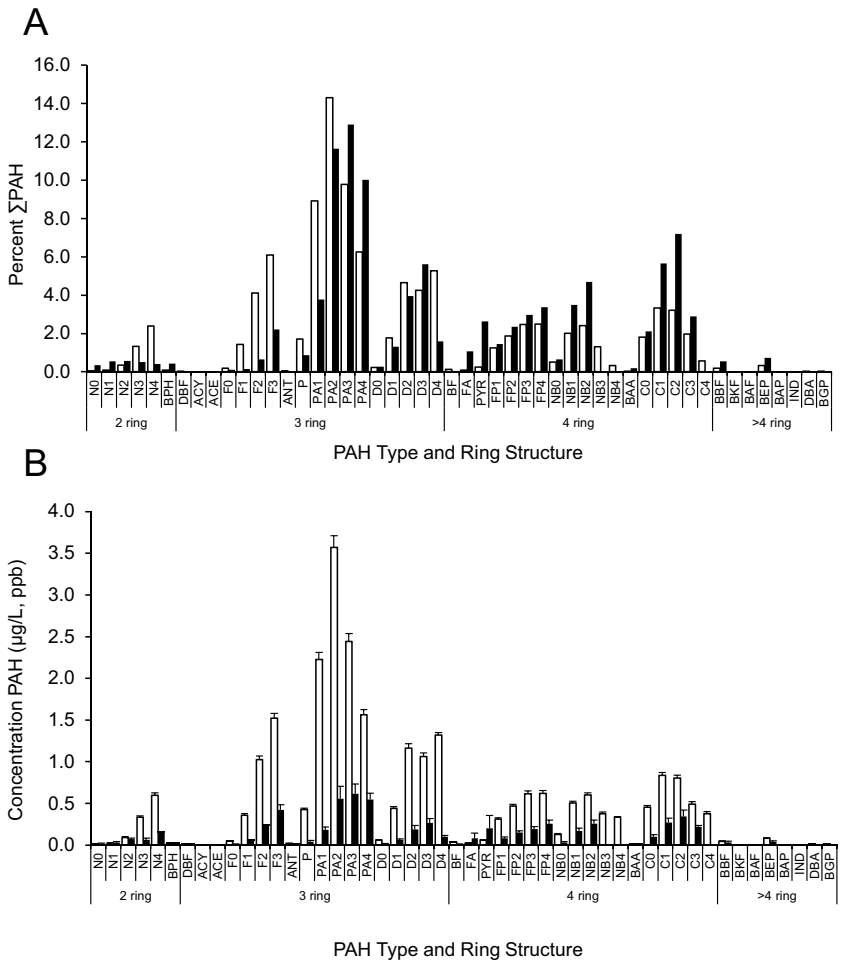

Fig. 5. A. Individual PAH percentages and, B. concentrations of initial (open bars) and final (close bars) samples of 10% HEWAF at 9.6 ± 2.7 µg l^{-1} ∑PAH (n = 8). Values represent mean ± SEM. Abbreviations: naphthalenes (N), biphenyl (BPH), dibenzofuran (DBE), acenaphthylene (ACY), acenaphthene (ACE), fluenes (F), anthracene (ANT), phenanthrene (P), phenanthrenes/anthracenes (PA), dibenzothiophenes (D), benzo(b)fluorine (BF), fluoranthenes (FA), pyrene (PYR), fluoranthenes/pyrenes (FP), naphthobenzothiophenes (NB), benz(a)anthracene (BAA), chrysenes (C), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(a)fluoranthene (BAF), benzo(e)pyrene (BEP), benzo (a)pyrene (BAP), indeno(1,2,3,cd)pyrene (IND), dibenz (a,h) anthracene (DBA), benzo(g,h,i)perylene (BGP). Numbers indicate additional carbons on alkylated homologues.

for changes in ejection phase of the ventricular contractile cycle lay outside the scope of this study, but may be attributed to changes in membrane biophysical properties impacting ion flux. At present, electrophysiological measurements on mahi–mahi cardiomyocytes exposed to PAH have yet to be performed.

All pressure parameters were similar between exposed and control mahi–mahi (Fig. 2A–D). While intraventricular pressures were similar between the experimental groups of mahi–mahi, maximal change in pressure over change in time (dP/dt_{max}), a key component of the isovolumic contractile phase of the ventricle contractile cycle, was lower in the treated group of mahi–mahi (Fig. 3A). Also, the contractility index, which is the quotient of dP/dt_{max} and the pressure at time of dP/dt_{max}, was reduced in the treated group of mahi–mahi accounting for the diminished stroke volume (Fig. 1A, B, D). The combined reduction in maximal change in pressure over change in time (dP/dt_{max}), and contractility index, and systolic duration, which contribute to the isovolumetric contraction phase and ejection phase, account for the diminished stroke volume, leading to reduced cardiac output in exposed mahi–mahi. Interestingly, while contractility was compromised by the exposure (Fig. 3A), the isovolumic relaxation constant (Tau) was unaffected by treatment (Fig. 3C). Therefore, at the organismal level, the active mechanisms of ventricular relaxation were unaffected by the treatment.

In addition to exploring the impacts of PAH, the present study adds to the body of knowledge of cardiac function in high performing apex pelagic finfish species. The temperature impact on heart rate necessitates comparisons of this parameter be limited to species that experience temperatures similar to those of mahi–mahi. The control group of mahi–mahi had heart rate values similar to those reported previously for chemically immobilized mahi–mahi, yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) (Benetti et al., 1995; Farrell et al., 1992). However, mahi–mahi appear to have heart rates 25–33% higher than perfused Pacific bluefin tuna (*Thunnus orientalis*) at similar temperatures (Jones et al., 1993; Blank et al., 2004; Chin Lai et al., 1987). Stroke volume in control mahi–mahi from this *in situ* anesthetized study was lower than those previously reported in anesthetized yellowfin tuna, but similar to values reported for anesthetized albacore tuna (*Thunnus alalunga*) (Jones et al., 1993; Chin Lai et al., 1987). Intraventricular pressure of the control mahi–mahi was below those previously reported for anesthetized yellowfin tuna, albacore tuna, actively swimming yellowfin tuna (Chin Lai et al., 1987; Jones et al., 1993) and the two perfused isolated hearts of yellowfin and skipjack tuna species (Farrell et al., 1992). In addition, ventricular pressure generation (Fig. 3A) of control mahi–mahi was 75% lower than previously reported for yellowfin tuna (Jones et al., 1993). The relative hypotension in the ventricle in control mahi–mahi compared to the tuna species is not surprising given a prior study reporting lower force generation in mahi–mahi ventricle muscle strips compared to tuna (Galli et al., 2009).

It is important to note that translation of our findings of the impact PAH exposure has on cardiac function in anesthetized fish to the possible effects on swimming performance in recovered animals would have been strengthened by an initial swim study trial. However, the high performance and fragile nature of mahi–mahi completely prevents such an approach as the majority of adult mahi–mahi subjected to ucrit tests die following the test as a result of the exhaustive exercise (Stieglitz et al., 2016). Further, a recent publication on adult mahi–mahi swim performance included several batches of fish spaced apart by months and there was no among-batch-variation in performance (Stieglitz et al., 2016). Therefore, we elected the experimental approached outlined in this study.

4.1. Effect of anesthesia

It should be recognized that the heart rate determined in this study may be elevated relative to that of non-anesthetized mahi–mahi. Prior studies assessing heart rate and electrocardiogram (ECG) characteristics have reported that MS222 increases baseline heart rate to value similar to that following parasympathetic inhibition (Cotter and Rodnick, 2006). While the effects of anesthesia should be considered when translating the current findings to free-swimming animals, the *in situ* preparation provides fundamental information on cardiac function in the absence of central nervous system control (Hill et al., 2002). Further studies are needed to determine the possible compensatory cardiovascular adjustments, via regulatory mechanisms, that occur in surgically recovered mahi–mahi exposed to similar PAH concentrations.

4.2. Conclusion and perspectives

Mahi–mahi are apex pelagic predators with high metabolic demand that supports their active foraging behaviors, predator avoidance and migratory patterns. Previous work has documented that adult mahi–mahi exposed to PAH have reduced critical swim speed, reduced optimal swim speed, reduced maximal metabolic rate, and reduced aerobic scope (Stieglitz et al., 2016). Our *in situ* findings clearly show an overall reduction in cardiovascular function including decreases in ventricular ejection, stroke volume, stroke work, cardiac output, cardiac power output, and contractility, following PAH exposure (Figs. 1, 3 and 4). If present in surgically recovered animals, impairments in ventricular function may account for the overall reduction in maximal metabolic rate and aerobic scope previously documented in mahi–mahi exposed to 8.4 $\mu g\,l^{-1}$ $\sum$PAH (Stieglitz et al., 2016). While further studies on surgically recovered mahi–mahi are critical to ascertaining the effects of PAH exposure, the data in the present study suggest that PAH exposure compromises aspects of cardiovascular function which may alter mahi–mahi swim performance and their capacity to survive post-exposure.

Disclosures

No conflicts of interests, financial or otherwise, are declared by the authors.

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