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Cardio-respiratory function during exercise in the cobia, *Rachycentron canadum*: The impact of crude oil exposure

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ABSTRACT

Aerobic exercise capacity is dependent on the cardiorespiratory system's ability to supply oxygen at a rate that meets energetic demands. In teleost fish crude oil exposure, with the associated polycyclic aromatic hydrocarbons (PAH's), reduces exercise performance and this has been hypothesized to be due to compromised cardiovascular function. In this study, we test this hypothesis by simultaneously measuring cardiovascular performance, oxygen consumption, and swim performance in a pelagic teleost, the cobia (*Rachycentron canadum*). Metabolic rate increased over 300% in both groups during the swim trial but as the fish approached the critical swim speed (U_{crit}) MO_2 was 12% lower in the oil exposed fish. Further, stroke volume was initially 35% lower while heart rate was 15% higher in the oil exposed compared to control fish. Our findings suggested, while aspects of cardiovascular and metabolic function are altered by oil exposure, additional studies are needed to further understand the homeostatic mechanisms that may sustain cardiovascular function at higher exercise intensities in cobia.

1. Introduction

All teleost species increase cardiac output and associated oxygen delivery to support increases in swim performance (Farrell, 1991). Increased convective transport by the cardiovascular system is essential to ensure oxygen transport from the respiratory media to the aerobically active tissues (Hillman et al., 2013; Hedrick et al., 2015; Hillman and Hedrick, 2015). As cardiac output is the product of stroke volume and heart rate, adjustments in either or both parameters can modulate convective transport to meet the increasing oxygen demands that accompany aerobic exercise. However, the role of heart rate or stroke volume in driving adjustments in cardiac output may differ between fish species.

Previous studies of several fish species suggest there are differences in the primary parameter used to modulate cardiac output. In brown trout (*Salmo trutta*), cardiac output increases with swimming speed due to increases in both heart rate and stroke volume with a notable approximate doubling of heart rate (Altimiras et al., 2002). In the largemouth bass (*Micropterus salmoides*) increases in cardiac output during exercise are attributed to increases in heart rate while stroke volume remains constant. (Cooke et al., 2007). Sockeye salmon (*Oncorhynchus nerka*) exhibit marked increases in stroke volume as swim speed increases over a wide range of temperatures (Eliason et al., 2013). Atlantic salmon (*Salmo salar*) exhibit strong correlations between swim speed and heart rate, suggesting heart rate is the primary parameter used to adjust cardiac output in this species (Lucas, 1994). Hence, the dependence on changing heart rate and/or stroke volume to adjust cardiac output to meet increased convective oxygen transport demands appears to be dictated by species, as well as the conditions, exercise or temperature, that increased metabolic rate. In addition to these naturally occurring environmental factors, anthropogenic challenges, such as crude oil exposure, could also impact cardiovascular function in vivo.

The impact of crude oil exposure, and associated polycyclic aromatic hydrocarbons (PAHs), on organismal physiology has been investigated at multiple levels of biological organization in marine ecosystems (Eisler, 1987; McDowell Capuzzo et al., 1988; Edmunds et al., 2015; Esbaugh et al., 2016; Incardona et al., 2014; Stieglitz et al., 2016; Xu et al., 2016; Mager et al., 2014). Specifically, the detrimental impacts on fish cardiovascular systems have been reported from studies ranging from gene expression to overall organismal function (Edmunds et al., 2015; Incardona et al., 2014; Esbaugh et al., 2016; Stieglitz et al., 2016; Xu et al., 2016; Brette et al., 2014). At the level of the whole organism, our prior studies of the mahi-mahi (*Coryphaena hippurus*; "mahi" in the following) have documented that PAH exposure

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compromises swim performance and maximal metabolic rate (Mager et al., 2014; Stieglitz et al., 2016). In our recent study of anesthetized mahi we found that crude oil exposure decreased stroke volume and cardiac contractility (Nelson et al., 2016). These studies provided data suggesting that the deleterious effects of crude oil on swim performance and metabolic rate may be due to compromised cardiac function. However, in vivo investigations of swimming fish are needed to determine if impairments in cardiac function account for the overall reduction in maximal metabolic rate, aerobic scope and swim performance previously documented (Mager et al., 2014; Stieglitz et al., 2016).

Given the variability in the literature regarding the parameters fish use to increase cardiac output and the clear consequences of crude oil exposure on fish aerobic performance, this study was undertaken to first; investigate how cobia (*Rachycentron canadum*) adjusts heart rate and stroke volume with exercise, and, second; study impacts of oil exposure on cardiac performance during stepwise increases in exercise intensity. We hypothesized that exposure to crude oil would decrease critical swim speed accompanied by reduced cardiac performance.

2. Methods

2.1. Animals

Juvenile cobia *Rachycentron canadum*, (control 382.94 ± 33.13 g n = 8, experimental 354.89 ± 26.15 g n = 9) were maintained in 3000 l fiberglass tanks supplied with flow-through seawater (24–26 °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 study. All study procedures were approved by the University of Miami Institutional Animal Care and Use Committee (IACUC protocols #12-064 and #15-019).

2.2. Surgery protocol

Two days prior to study, individual fish were removed from the holding tanks, anesthetized in seawater with MS222 (tricaine methanesulfonate) 80 mg·l^{-1} (Sigma-Aldrich, St. Louis, MO) buffered to neutral pH with sodium bicarbonate ($NaHCO_3$, Sigma-Aldrich, St. Louis, MO). Anesthetized fish were transferred to a custom surgical table and placed on their right side. 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 an anesthetic reservoir containing 80 mg l^{-1} MS222 buffered to neutral pH with $NaHCO_3$. The left operculum and gills were retracted to expose the thin epithelium lining on the left side of the isthmus. Approximately 1 cm up from the ventral surface a 1 cm cut was made and the underlying tissue was separated under a dissection microscope (Leica M60 Leica Microsystems, Waukegan, IL, USA) exposing the ventral aorta. After the ventral aorta was isolated, a silicone cuff flow probe, 1.6 or 2.0 mm, (Iowa Doppler Products Model ES, Iowa City, IA, USA) was placed around the ventral aorta and the leads were anchored with silk suture to the isthmus, the lateral body wall and the dorsal surface. Once instrumentation was completed, the fish was placed in a recovery tank with 600 l of fresh circulating, aerated seawater maintained at 26 °C by a 1000-watt submersible heater (Innovative Heat Concepts QDPTY1-1, Homestead, FL, USA). The recovery tank was covered to reduce stress and prevent disturbances during the 24 h recovery period. All surgical preparations were completed within 1 h.

2.3. Experimental conditions

Following the 24 h surgical recovery period, cobia were exposed to control seawater or seawater containing a 20% dilution of a high energy water accommodated fraction (HEWAF) of oil for 24 h in static 300 l tanks (2 fish per tank). This exposure period has previously been shown to impact swim performance of fish species (Mager et al., 2014; Stieglitz et al., 2016). Preliminary studies using 10% HEWAF of oil, geometric mean of polycyclic aromatic hydrocarbons = 5.24 ± 4.5 μg/L, had no impact on the measured parameters. Seawater conditions were maintained as outlined above. The crude oil used in this study was a highly weathered slick oil (sample ID CTC02404-02) 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 the University of Miami. The stock HEWAF solution was prepared at a loading rate of 2 g of oil per 1 l of UV sterilized, 1 μm filtered seawater by blending for 30 s in a Waring CB15 blender (Torrington, CT, USA). The mixture was poured into a glass separation 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 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. Total ammonia analysis was conducted using colorimetric assay (Ivančič and Degobbis, 1984). In addition, initial and final water samples were collected for total PAH analysis from oil exposures. 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 chromatography and selective ionic monitoring mass spectrometry within six days of collection. Reported geometric mean (initial and final values) of polycyclic aromatic hydrocarbons (ΣPAH) values represent the sum of 50 PAH analytes, selected by the EPA based on individual toxicity and concentration.

2.4. Swimming study

On the day of study, animals were individually moved from the control or exposure tanks to 90-L Brett-type swim chamber respirometers (Loligo Systems, Viborg, Denmark). The flow probe leads were then connected to a directional pulsed doppler blood flow meter (Model 545C-4, Bioengineering, University of Iowa, Iowa City, IA, USA). Fish were allowed to recover from handling and acclimated for 2.5–4 h at a swim speed of approximately 0.5 body lengths·s^{-1} (BL·s^{-1}). Oxygen consumption (MO_2) was monitored using a Pt100 fiber-optic probe connected to a Fibox 3 minisensor oxygen meter (PreSens Precision Sensing GmbH) that was operated with AutoResp™ 2.1.0 software (Loligo Systems. Viborg, Denmark). Intermittent flow respirometry, with 20-minute loop durations, was utilized to monitor MO_2 (mg·O_2·kg^{-1}·h^{-1}) and real-time video monitoring of each swim chamber allowed for continuous tracking of each individual fish during the testing period.

Acclimation (2.5–4 h) was defined as at least two consecutive MO_2 readings were within ~10% of each other. In addition to the stable MO_2 readings, heart rate was stable for at least 1 h prior to the onset of the swim trial, data not shown. Following acclimation, the critical swimming speed test (U_{crit}) was initiated, which consisted of 0.5 BL·s^{-1} swim speed increases every 20 min until failure. Failure was defined as the point at which fish were no longer able to maintain a steady swimming position in front of the back grate of the chamber working section, or when they became pressed against the back grate and were unable to regain steady swimming behavior. U_{crit}, expressed in BL·s^{-1}, was calculated as previously described (Brett, 1964): $U_{crit} = [U_f + (T/t)dU]$·cm·FL^{-1}, where U_f (cm·s^{-1}) is the highest swim velocity maintained for a full interval, T (s) is the time spent at the final velocity, t is the time interval (s), FL is the fish length from anterior of the animal to the midpoint of the tail fork and dU is the increment in swim speed (cm·s^{-1}). MO_2 and U_{crit} data were used to calculate metabolic rates (standard metabolic rate: 'SMR'; maximum metabolic rate: 'MMR'), and aerobic scope standard methodology (Mager et al., 2014; Stieglitz et al.,

2016). Briefly MO_2 data were log transformed and plotted vs swim speed. An exponential regression was fitted to the data with the SMR (y axis intercept) and MMR (extrapolated MO_2 at U_{crit}) derived from the resulting equation. Cost of transport (COT), the energetic consumption of movement over a distance, was calculated by dividing MO_2 by swim velocity, resulting in a parabola-shaped plot (Palstra et al., 2008). COT at U_{crit} (COT_{Ucrit}) was determined by fitting a second order (k = 2) polynomial regression model. Optimal swim speed (U_{opt}), the swim speed that requires the minimum cost of transport (COT_{min}), was determined by fitting the first derivative of the polynomial model equation to 0. Individuals with a regression $r^2 > 0.55$ were used for cost of transport analysis. This metabolic data was allometrically scaled to a standard fish mass of 400 g in order to eliminate the influence of varying fish size on the metabolic endpoints. Scaling coefficients were determined from control fish only in order to not be confounded by oil treatment effects on these endpoints. Cardiovascular data was sampled during the last 5 min of the 20-minute swim interval as conducted in prior studies (Farrell, 2007; Kiceniuk and Jones, 1977). Water was maintained at 26 °C throughout the swim study.

2.5. Data acquisition, calibration and calculations

At completion of the study all animals were euthanized by an overdose of buffered MS222 (190 mg·l^{-1}, 190 mg·l^{-1} $NaHCO_3$) and blow to the head. Blood flow probes were then calibrated in place using an infusion pump (PHD 2000, Harvard Apparatus Company Inc., Millis, MA, USA). Briefly, approximately 10 ml of blood was withdrawn by ventricular puncture into a heparinized syringe. The ventral aorta was cut from the ventricle and cannulated with PE 90 tubing. A second cut was made downstream of the blood flow probe placement. Blood was then infused through the ventral aorta at 7 different flow rates with the infusion pump and flow signals recorded. Mean voltage signals were plotted versus flow rate and a regression line was best fitted to the function of flow (ml·min^{-1}) versus flowmeter output (V). Absolute cardiac output (Q) was calculated using the calibration and corrected for 1 kg mass. Heart rate (f_H) was calculated based on the blood flow pulse interval and stroke volume (V_s) was calculated as $Q \cdot f_H^{-1}$. All instrument signals during the study were recorded with a PowerLab16/35 data acquisition system (ADInstruments, Colorado, USA) with data collected on a computer (Mac Mini, Apple Inc., Cupertino, CA, USA) running LabChart data acquisition software (Chart 8.1.2, ADInstruments, Colorado, USA) and data recorded at 100 Hz.

2.6. Statistical analysis

U_{crit} data was analyzed with t-tests. To account for differences in U_{crit} both within and between the experimental groups, swim velocity for each animal was converted to a percentage of calculated U_{crit} as previously conducted with similar data (Swanson et al., 1998). These values were then grouped into percentage bins of 15–25, 45–60, 70–80, 85–100 representing the range of values at each step increase in swim velocity. f_H, V_s, Q, and MO_2 values were analyzed with a one-way repeated measures ANOVA with the bin percentage of U_{crit} used as the repeated treatment and oil exposure as the independent variable. Correlations between MO_2, and f_H, V_s, Q, were analyzed with linear regression with MO_2 as the dependent variable. SMR, MMR, aerobic scope, COT_{min}, $COT_{Ucrit,}$ and U_{opt} were compared with t-tests. Water parameters were analyzed with a one way repeated measure ANOVA with initial and final samples used as the repeated treatment and oil exposure as the independent variable. All data are presented as mean ± standard error. ANOVA tests were followed by a LSD post hoc test to compare individual means ($p < 0.05$) (Statistica v13.0; StatSoft, Tulsa, OK, USA).

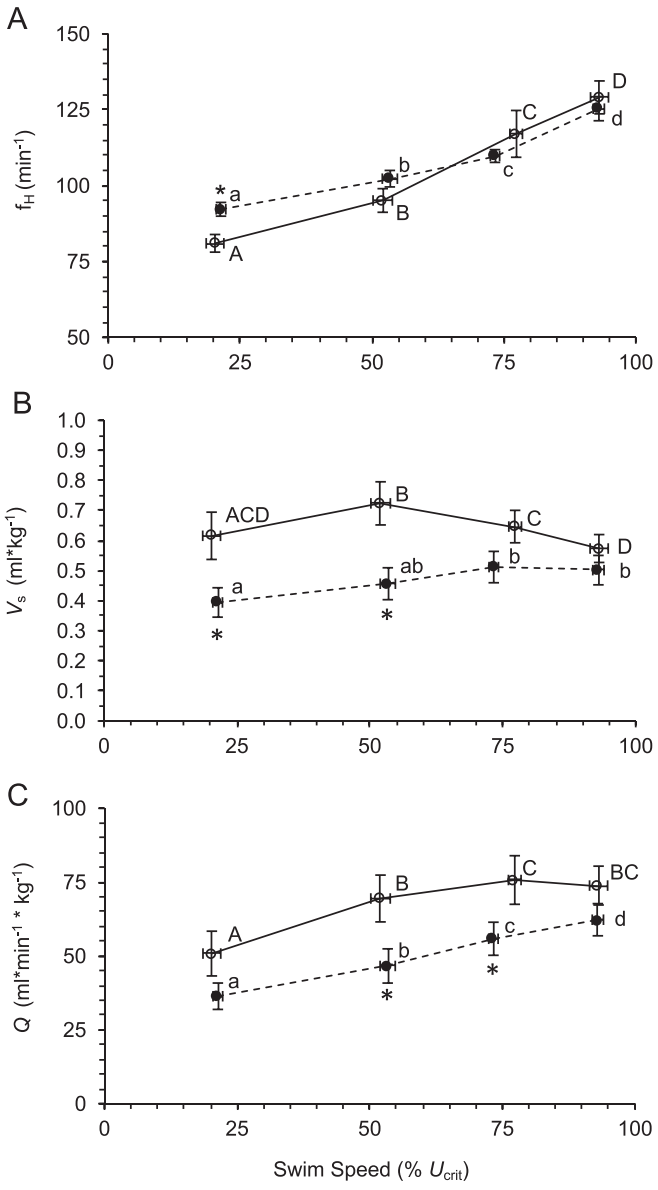

Fig. 1. Heart rate (A), stroke volume (B) and cardiac output (C) of control (open circles) and 24 h oil exposed (10.52 ± 6.22 µg·l^{-1} ΣPAH; closed circles) cobia at percentage of critical swimming speed (U_{crit}). An * indicates significant difference between control and oil treated fish ($p < 0.05$). Differences in letters indicate significant difference across percentages of U_{crit} within experimental groups, upper case controls and lower case oil exposed fish ($p < 0.05$). Values represent mean ± SEM. In all cases sample size for the control fish is 8 and for the oil exposed fish is 9.

3. Results

3.1. Routine cardiorespiratory performance

Oil exposed fish had an 18% higher heart rate (f_H) compared to the control fish (92 ± 2 and 80 ± 3 beats·min^{-1}, respectively) ($p = 0.046$; Fig. 1A). In contrast, stroke volume (V_s) was 36% lower (0.39 ± 0.05 ml·kg^{-1}, 0.61 ± 0.07 ml·kg^{-1} respectively) in oil-exposed fish relative to controls ($p = 0.02$; Fig. 1B). The combined effect was that cardiac output (Q) was similar, control 49.5 ± 6.9 ml·min^{-1}·kg^{-1} and oil exposed 36.4 ± 4.5 ml·min^{-1}·kg^{-1}, between the two experimental groups ($p = 0.12$; Fig. 1C). Likewise, calculated standard metabolic rates (SMR), were comparable between control and oil exposed fish, 152.3 ± 9.1 vs 166.7 ± 9.6 mg·O_2·kg^{-1}·h^{-1} respectively (Table 1).

Table 1
Standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope, cost of transport at U_{opt} (COT_{min}), cost of transport at U_{crit} (COT_{Ucrit}) and optimal swim speed (U_{opt}) of control and 24 h High Energy Water Accommodated Fraction (HEWAF) exposed juvenile cobia. Values represent mean ± SE.

Measure	Control	PAH exposed
SMR ($mg{\cdot}O_2{\cdot}kg^{-1}{\cdot}h^{-1}$)	152.3 ± 9.1 (8)	166.7 ± 9.6(9)
MMR ($mg{\cdot}O_2{\cdot}kg^{-1}{\cdot}h^{-1}$)	1017.0 ± 99.6(8)	845.9 ± 61.7(9)
Aerobic scope ($mg{\cdot}O_2{\cdot}kg^{-1}{\cdot}h^{-1}$)	864.8 ± 101.2(8)	679.2 ± 59.7(9)
COT_{min} ($mg{\cdot}O_2{\cdot}kg^{-1}{\cdot}m^{-1}$)	0.18 ± 0.01(6)	0.22 ± 0.01(8)*
COT_{Ucrit} ($mg{\cdot}O_2{\cdot}kg^{-1}{\cdot}m^{-1}$)	0.25 ± 0.01(6)	0.27 ± 0.02(8)
U_{opt} ($BL{\cdot}s^{-1}$)	2.01 ± 0.11(6)	1.89 ± 0.10(8)

* Indicates significant difference between control and oil treated fish ($p < 0.05$). Sample size for each parameter is noted in parentheses.

3.2. Cardiorespiratory performance during swimming

During the swim trial, control fish increased f_H by 61% reaching a maximum of 129 ± 5 min^{-1} at the highest U_{crit} percentage (Fig. 1A). Oil exposed fish also responded to increasing swim speeds by increasing f_H to 125 ± 5 min^{-1} (Fig. 1A). However, unlike the control animals, f_H increased 36% in the oil exposed fish (Fig. 1A). In contrast to f_H, maximal V_s in the control fish occurred at the midpoint of the swim trial (+19%) and then fell to values similar to those measured at the onset of the swim study (Fig. 1B). Oil exposed animals initially had lower V_s than the control animals but it increased during the swim trial, and remained elevated reaching a maximum value 27% above the values measured at the onset of the swim study (Fig. 1B).

Cardiac output (Q), the product of f_H and V_s, increased by 53% in the control group during the course of the swim trial while the oil exposed group increased Q by 71% (Fig. 1C). The groups differed in speed of maximal Q response, with the control animals reaching a maximum at 70–80% of U_{crit} while the oil exposed animals increased throughout the swim trials (Fig. 1C). Further, Q of the oil exposed fish was lower at the intermediate swim percentages than the controls (Fig. 1C).

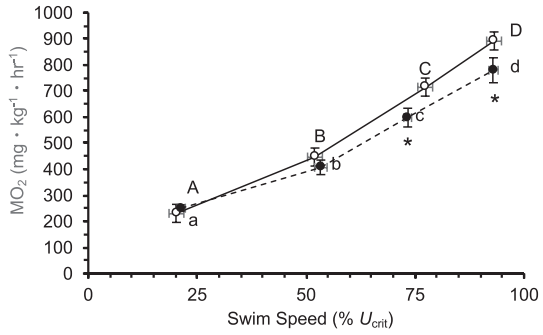

Fig. 3. Oxygen consumption (MO_2) at increasing percentage of critical swimming speed of control (open circles) and 24 h 10.52 ± 6.22 $\mu g{\cdot}l^{-1}$ ΣPAH exposed (closed circles) juvenile cobia. Differences in letters indicate significant difference across percentages of U_{crit} within experimental groups, upper case controls and lower case oil exposed fish ($p < 0.05$). Values represent mean ± SEM. In all cases sample size for the control fish is 8 and for the oil exposed fish is 9.

The blood flow profile, cyclic maximum and minimum flow, changed differently in the control and oil exposed fish as swim speed increased (Fig. 2A & B). Average peak systolic blood flow increased with swim speeds in both groups (Fig. 2C). Control animals peaked at the 45–60% interval of U_{crit} and then fell as the swim trial progressed (Fig. 2C) while the oil exposed fish increased until 70–80% interval and remained elevated (Fig. 2C). There was no difference in peak systolic flow between the treatment groups. Average diastolic flow increased in the control group until the animals reached the 70–80% of U_{crit} (Fig. 2C). In the oil exposed fish average diastolic flow increased throughout the trial and flow values were lower than the control fish (Fig. 2C).

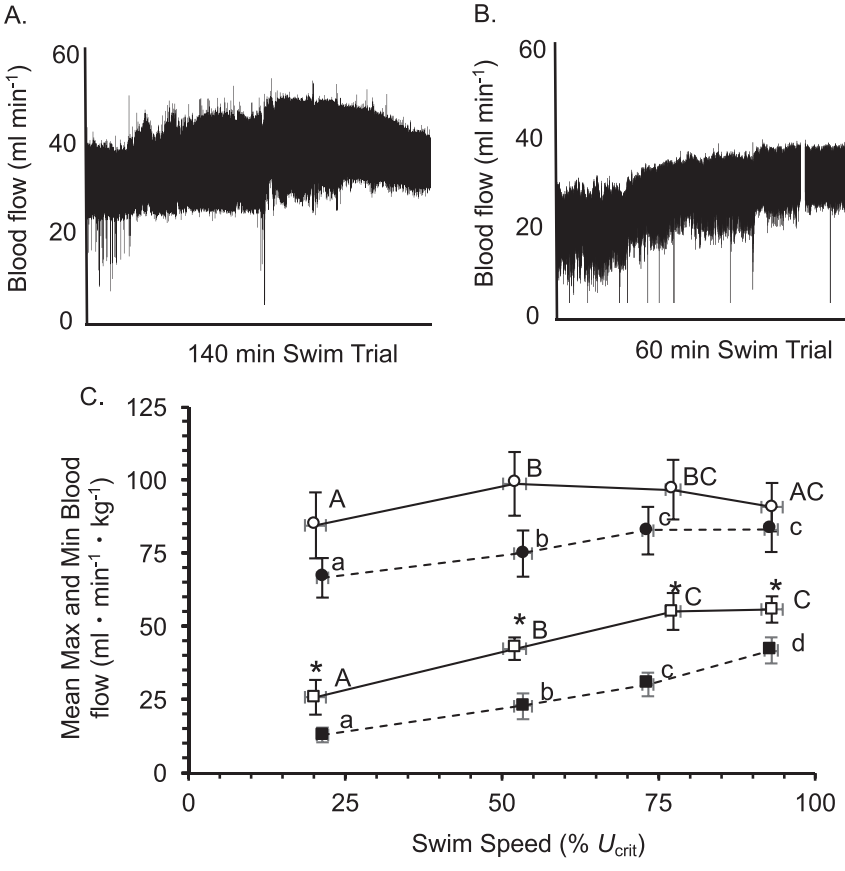

Fig. 2. Representative traces of blood flow from a control (A), and an oil exposed fish (B) during the course of a swim trial. Mean systolic blood flow (C) of control (open circles) and oil exposed fish (closed circles) at percentage of critical swimming speed. Mean diastolic blood flow (C) of control (open squares) and oil exposed fish (closed squares) at percentage of critical swimming speed. An * indicates significant difference between control and oil treated fish ($p < 0.05$). Differences in letters indicate significant difference across percentages of U_{crit} within experimental groups, upper case controls and lower case oil exposed fish ($p < 0.05$). Values represent mean ± SEM. In all cases sample size for the control fish is 8 and for the oil exposed fish is 9.

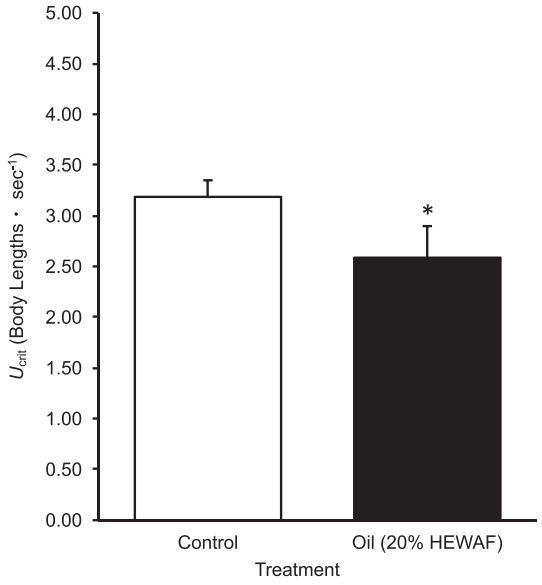

Fig. 4. Critical swimming speed of control (open bar) and 24 h 10.52 ± 6.22 μg·l^{-1} ΣPAH exposed (closed bar) juvenile cobia. An * indicates significant difference between control and oil treated fish ($p < 0.05$). Values represent mean ± SEM. In all cases sample size for the control fish is 8 and for the oil exposed fish is 9.

As swim speed increased, oxygen consumption (MO_2) increased by 390% and 310% in the control and oil exposed fish respectively (Fig. 3). Fish swimming at speeds above 50% of U_{crit}, MO_2 was significantly lower in the oil exposed group ($p = 0.032$ Fig. 3). Although standard and maximal metabolic rates did not differ significantly between treatment groups, COT_{min} was greater in the oil exposed fish (Table 1). Finally control cobia reached a U_{crit} value significantly higher than the oil exposed fish (Fig. 4).

3.3. Parameter correlations

There was a positive correlation between MO_2 and f_H in the control ($MO_2 = -544.16 + 10.37 \cdot f_H$, $r^2 = 0.77$) and oil exposed fish ($MO_2 = -833.67 + 12.39 \cdot f_H$, $r^2 = 0.69$) (Fig. 5A). There was no correlation between MO_2 and V_s (Fig. 5B), however, there was a weak correlation between MO_2 and Q (Fig. 5C) in the control ($MO_2 = 171.75 + 5.1059 \cdot Q$, $r^2 = 0.19$) and oil exposed fish ($MO_2 = 133.29 + 7.24 \cdot Q$, $r^2 = 0.31$) (Fig. 5C).

3.4. Water chemistry

Initial mean PAH concentration of the 20% HEWAF was 38.29 ± 4.84 μg·l^{-1} and was dominated by 3-ringed compounds at 68.2%, followed by 4-ringed compounds at 26.4%, 2-ringed compounds at 5%, and ≥5-ringed compounds at < 1%. After the 24 h period, PAHs were depleted by 90%, decreasing mean PAH concentration to 3.70 ± 1.24 μg·l^{-1}. PAH composition decreased by 61.2%, 8.4% and 83.3%, in 2-ringed, 3-ringed and ≥5-ringed compounds respectively, meanwhile, 4-ringed compounds increased by 30.6%. Geometric mean of polycyclic aromatic hydrocarbons (ΣPAH) was 10.52 ± 6.22 μg·l^{-1} ΣPAH over the 24 h exposure. Control water ΣPAH totaled 0.09 ± 0.02 μg·l^{-1} (Fig. 6A & B). All other water chemistry parameters are reported in Table 2.

4. Discussion

Adjustments in convective oxygen transport during exercise can be achieved through changes in cardiovascular function, blood oxygen saturation and increased arterial venous O_2 content difference (Farrell, 2007). In fish species, as in other vertebrates, cardiovascular function adjustments are mediated by changes in both heart rate and stroke volume, however differences in the primary modulator of cardiac output have been reported (Farrell, 1991). While the role of heart rate and stroke volume in altering cardiovascular function have been studied in several fish species, the degree to which past findings are representative of unstudied taxa and the impact of environmental toxins, such as crude oil, on these parameters remain poorly understood. Our aims in this study were to investigate the cardiovascular response to exercise in the cobia and determine how oil impacts cardiovascular function. Collectively the data suggest that cobia primarily adjust heart rate during exercise (Fig. 1A & B). Oil exposed fish appeared to increase their dependence on stroke volume and decrease their dependence on heart rate to elevate cardiac output during exercise (Fig. 1A–C). The reduction in stroke volume observed in oil exposed fish appeared to be buffered by an increase in heart rate. Further, while cardiac output of

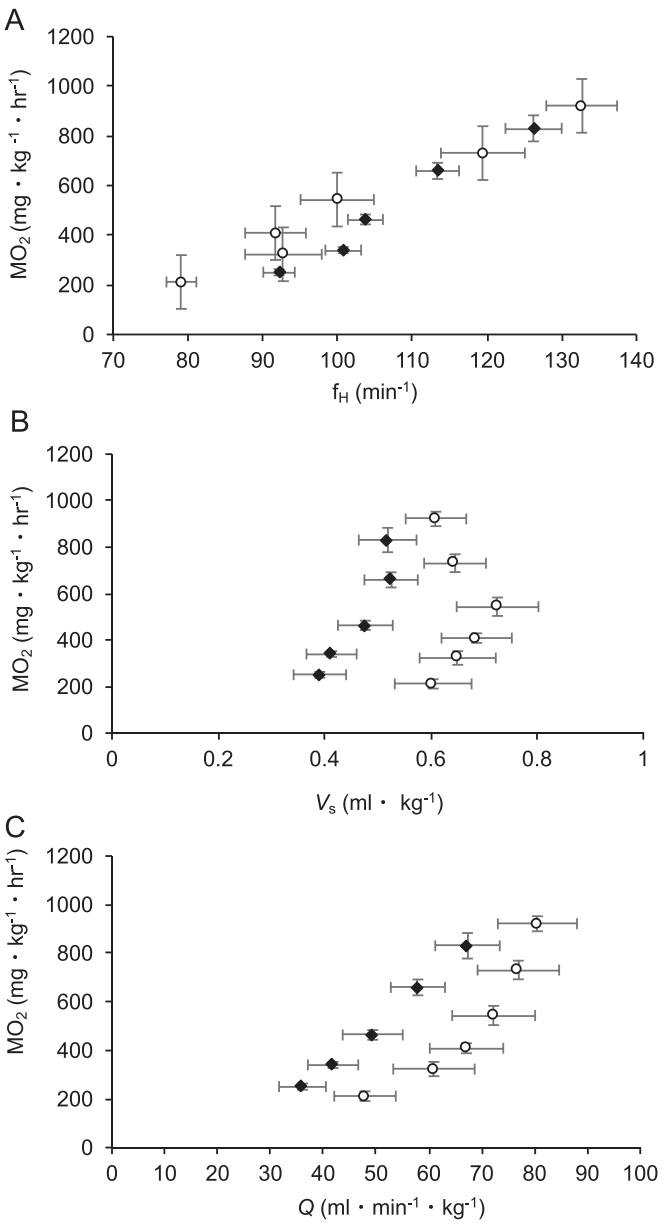

Fig. 5. Relationship of oxygen consumption (MO_2) and heart rate (A), stroke volume (B) and cardiac output (C) of control (open circles) and 24 h 10.52 ± 6.22 μg·l^{-1} ΣPAH exposed (closed diamonds) juvenile cobia. Values represent mean ± SEM. In all cases sample size for the control fish is 8 and for the oil exposed fish is 9.

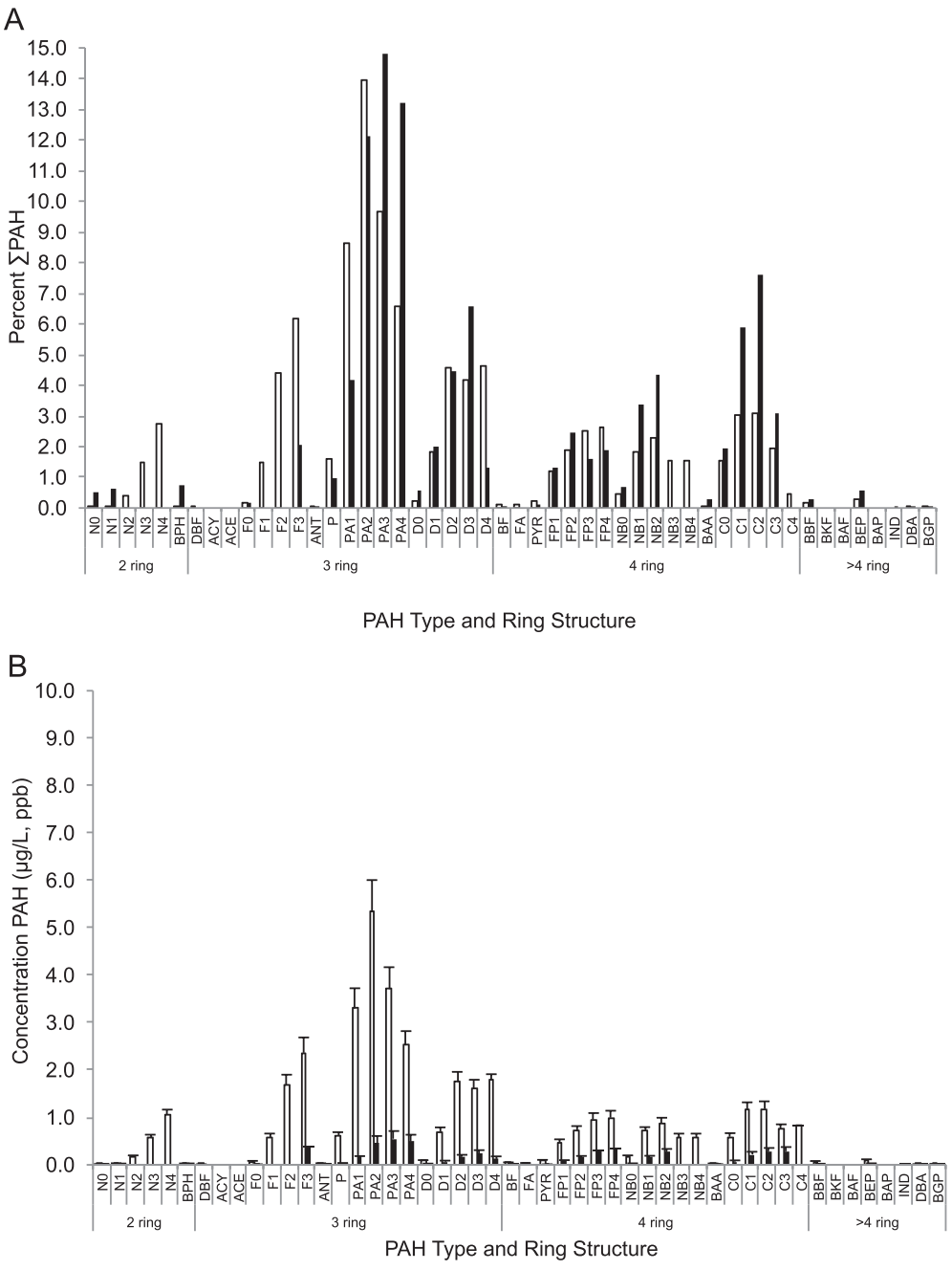

Fig. 6. Individual PAH percentages (A) and, concentrations (B) of initial (open bars) and final (close bars) samples of 20% HEWAF at 10.52 ± 6.22 µg·l^{-1} ΣPAH (N = 9). Values represent mean ± SEM. Abbreviations: naphthalenes (N), biphenyl (BPH), dibenzofuran (DBE), acenaphthylene (ACY), acenaphthene (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.

Table 2
Water parameters for control (N = 9) and 24 h 10.52 ± 6.22 µg·l^{-1} ΣPAH exposed (N = 9) juvenile cobia. Values represent mean ± SEM.

Measure	Control	PAH exposed
pH initial (S.U.)	7.93 ± 0.04	8.11 ± 0.06
pH final (S.U.)	7.49 ± 0.12	7.75 ± 0.18
DO initial (mg·l^{-1})	6.72 ± 0.21	6.82 ± 0.34
DO final (mg·l^{-1})	5.80 ± 0.16	5.39 ± 0.27
Salinity initial (ppt)	34.38 ± 0.32	34.50 ± 0.22
Salinity final (ppt)	34.14 ± 0.40	34.60 ± 0.24
Ammonia initial (µM)	5.19 ± 1.03	0.00 ± 1.23*
Ammonia final (µM)	38.57 ± 9.70	43.30 ± 5.96

* Indicates significant difference between control and oil treated fish ($p < 0.05$).

the oil exposed fish was significantly lower in the middle of the swim trial, it was generally lower throughout the study (Fig. 1C). These findings suggest the depression in stroke volume may impair the heart's capacity to adjust cardiac output to sustain swim speeds in oil exposed fish relative to control fish.

4.1. Cardiac performance

Prior to this investigation, cardiac function of the cobia was largely unknown. The initial cardiac output of cobia, 49 ± 7 ml·min^{-1}·kg^{-1}, was similar to a closely related species mahi, 55 ± 6 ml·min^{-1}·kg^{-1}, studied under anesthesia at similar temperatures (Nelson et al., 2016). Compared to prior studies of surgically recovered fish species

investigated at a similar temperature, the control routine f_H, V_s and Q values (Fig. 1A–C) were within the range reported for skipjack tuna, *Katsuwonus pelamis*, (Ishimatsu et al., 1990; Lee et al., 2003). Interestingly, while heart rate of cobia was lower and stroke volume almost two-fold higher than reported for yellowtail kingfish *Seriola lalandi*, cardiac output was similar between these two species (Clark and Seymour, 2006).

During swim trials, control cobia increased cardiac output 53%, with an increase in heart rate of 61% acting as the greatest contributor to that change, whereas stroke volume increased to a minor degree (Fig. 1A–C). This dependence on heart rate is further illustrated in the strong correlation with MO_2 (Fig. 5A). A similar contribution of heart rate to increase cardiac output has been reported for multiple fish species from different taxonomic lineages, different water types, and different temperatures (Axelsson et al., 1992; Clark and Seymour, 2006; Farrell, 1991; Lucas, 1994; Altimiras et al., 2002; Axelsson et al., 2002; Cooke et al., 2007). In cobia, stroke volume increased marginally at the onset of the swim trials but then dropped to pre-trial values at the highest swim speeds (Fig. 1B). This exercise stroke volume response was accompanied by a reduction or plateau of systolic flow and rise in diastolic flow (Fig. 2C). This change in blood flow profile suggests that the exercise tachycardia of cobia may physically constrain stroke volume due to reduced heart filling times or venous return limits at high heart rates as previously suggested for other species (Korsmeyer et al., 1997; Jones et al., 1993). While additional studies of diverse taxa are needed to elucidate factors that determine species dependence on heart rate or stroke volume to adjust cardiac output during activity, our data indicates cobia may share similarities to other apex pelagic species (Farrell, 1991).

4.2. Metabolic parameters

Cobia maintained standard metabolic rate that was 50–70% lower than a closely related species, the mahi (Stieglitz et al., 2016; Gray et al., 2009; Benetti et al., 1995). Maximal metabolic rate of the cobia (Table 1) was also lower than that reported for the mahi (~1000 vs ~1650 $mg{\cdot}O_2{\cdot}kg^{-1}{\cdot}h^{-1}$), but cobia were able to increase metabolic function 6.3 times (Fig. 3) compared to 3.6 times in the mahi (Stieglitz et al., 2016). The difference in capacity to increase metabolic function may reflect an increased resting metabolic cost in mahi ascribed to elevated protein synthesis (Brill, 1996).

4.3. Effect of oil exposure

Crude oil exposure has been suggested to potentially diminish cardiac output by changes in heart rate and/or stroke volume in fish (Brette et al., 2014). In the current study, crude oil exposure significantly reduced stroke volume initially, however, it recovered as the animals neared their U_{crit} (Fig. 1B). This initial reduction in blood flow was accompanied by higher heart rates in the oil exposed compared to the control fish (Fig. 1A). Decreased cardiac contractility may play a role in the oil induced reduction in stroke volume of cobia as previously suggested for the mahi (Nelson et al., 2016). Interestingly, while oil exposed cobia had reduced stroke volume initially it increased as swim speed approached U_{crit} (Fig. 1B). While additional studies are needed to understand how aspects of cardiovascular regulation may be effected, changes in cholinergic and adrenergic stimulation could account for the cardiovascular findings following oil exposure.

Critical swim speed (U_{crit}) was 22% lower in the oil exposed cobia compared to the control fish (Fig. 4). Similarly, adult mahi exposed to similar concentrations of oil also showed a reduction in U_{crit} (14%) and reduced maximal oxygen uptake. The findings in mahi were proposed to be due to oil induced impairments to cardiac output that negatively affected convective oxygen transport (Stieglitz et al., 2016). However, an earlier study of juvenile mahi reported a decreased U_{crit} in oil exposed fish without affecting maximal oxygen uptake (Mager et al., 2014). Similarly, red drum have been reported to display a reduced U_{crit} despite unaltered maximal oxygen uptake at lower PAH concentrations, albeit, both were reduced at higher PAH concentrations (Johansen and Esbaugh, 2017). We found that calculated MMR of control and oil exposed cobia was not different ($p = 0.15$). However, oil exposed fish did have lower MO_2 values at equivalent percentages of U_{crit} relative to the control animals at the end of the swim trial (Fig. 3). Further, calculated minimum cost of transport was higher in the oil exposed fish suggesting that oil exposure impacted metabolic function at calculated optimal swim speed (Table 1). The apparent disconnect between limits to aerobic activity and MMR in the cobia may be attributed to multiple effects of oil exposure and suggests that multiple factors contribute to reduced U_{crit}. In regards to contribution of the cardiovascular system, additional studies during activity are needed to assess blood oxygen saturation and tissue oxygen extraction to definitively outline the role of convective oxygen in maintaining equivalent MMR in the oil exposed fish.

5. Conclusions

Cobia have a large scope for adjusting heart rate and MO_2 as they increased by 61% and 390%, respectively, from routine swimming to U_{crit}. U_{crit} was significantly reduced by oil exposure, which was accompanied by a general reduction in cardiac outputs that was significant at intermediate swim speeds. Collectively, the decreased stroke volume in oil exposed fish was offset by increases in heart rate suggesting homeostatic mechanisms were able to rescue function. Our findings emphasize the need for future studies to investigate the adjustments in cardiovascular regulation, as well as blood properties, that may preserve function following oil exposure in pelagic species.

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