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Dermal exposure to weathered MC252 crude oil results in echocardiographically identifiable systolic myocardial dysfunction in double-crested cormorants (*Phalacrocorax auritus*)

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Dermal exposure to weathered MC252 crude oil results in echocardiographically identifiable systolic myocardial dysfunction in double-crested cormorants (*Phalacrocorax auritus*)

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ABSTRACT

During the Deepwater Horizon Natural Resource Damage Assessment, gross morphologic cardiac abnormalities, including softer, more distensible musculature, were noted upon gross necropsy in hearts from laughing gulls and double-crested cormorants exposed to weathered MC252 crude oil. A species specific, echocardiographic technique was developed for antemortem evaluation of function that was used to evaluate and better characterize cardiac dysfunction. Control (n = 12) and treated (n = 13) cormorant groups of similar sex-ratio and ages were dermally treated with approximately 13 ml of water or weathered MC252 crude oil, respectively, every 3 days for 6 dosages. This resulted in a low to moderate external exposure. Upon visualization and clinical assessment of the hearts of all test subjects, comprehensive diagnostic cardiographic measurements were taken twice, prior to oil application and after a 21 day dermal oil exposure. Oil-treated birds showed a decrease in cardiac systolic function, as characterized by an increased left ventricular internal dimension-systole and left ventricular stroke volume as well as concurrent decreased left ventricular ejection fraction and left ventricular fractional shortening when compared to both control birds' and the treated birds' time zero values. These changes are indicative of a possible dilative cardiomyopathy induced by oil exposure, although further elucidation of possible collagen damage is recommended. Arrhythmias including tachycardia in two treated birds and bradycardia in all treated birds were documented, indicating further clinically significant abnormalities induced by MC252 oil that warrant further investigation. A statistically significant increase in free calcium concentration, important to muscular and neurologic function in treated birds was also noted. This study documents that weathered MC252 oil caused clinically significant cardiac dysfunction that could result in mortality and decrease recruitment.

1. Introduction

The *Deepwater Horizon* (DWH) oil spill in 2010 released 3.2 million barrels of crude oil into the northern Gulf of Mexico, exposing numerous species of animals to the toxic components of oil. A comprehensive assessment of morbidity and mortality caused by DWH oil exposure was undertaken by the DWH Natural Resource Damage Assessment (NRDA) Trustees to characterize ecosystem damages that encompasses damage to animal health (Bursian et al., 2017; DWH Trustees - Deepwater Horizon Natural Resource Damage), 2015). While the cardiotoxic effects of oil are well documented in fish, especially during embryonic development, there is minimal information of the cardiotoxic effects of crude oil in other vertebrates (Collier et al., 2014; Incardona et al., 2014). In birds, anemia, disrupted feather function,

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hypothermia, respiratory distress, seizures, diarrhea, hepatic disease and renal disease have all been reported secondary to exposure to petroleum products (Mazet et al., 2002). However, to the best of the authors' knowledge, cardiac dysfunction has never been documented in birds post-petroleum exposure. Necropsies performed as part of a preliminary oral exposure study using weathered MC252 oil uncovered as yet undocumented gross morphological cardiac changes, consisting of softer, more distensible ventricular walls, in exposed double-crested cormorants (DCCOs) compared to control birds. This prompted further evaluation of live DCCOs for the presence of systolic myocardial dysfunction. Therefore, we sought to evaluate cardiac function echocardiographically in DCCOs to determine if cardiac dysfunction could be identified in live DCCOs subjected to dermal oil exposure.

2. Materials and methods

2.1. Toxicant

MC252 (DWH7937, batch# B030112) oil was collected during the 2010 Deepwater Horizon oil spill and artificially weathered by TDI-Brooks International, (College Station, TX) prior to use in the studies as previously described (Forth et al., 2015).

2.2. Animal husbandry

A total of 31 DCCO's were captured and retained in captivity under the authority of USFWS MBPO Federal Permit #MB019065-3, Mississippi and Alabama state (#8017) scientific collection permits, and Institutional Animal Care and Use Committee (IACUC) under NWRC protocol QA-2326. Cunningham et al. (2017) in this issue provides a detailed description of animal capture and handling in March 2014.

Birds were allowed to acclimate to captivity for a minimum of 21 days prior to initiation of the study. A total of 25 subadult DCCOs allocated to a control group (n = 12, 5 male, 7 female) and an exposed group (n = 13, 6 males, 7 females) were used in this trial. DCCOs were assigned to treatment groups based on the results of blood samples collected at the initiation of the three-week quarantine period. Complete blood count (CBC) values were used to ensure equal division of birds with potential health concerns between groups. DCCO's with monocyte counts greater than 2.0 x 10^9 cells/l were considered abnormal (severe monocytosis); and were divided between control (n = 4) and treatment (n = 3) groups. Additionally, a small oil spill took place one year prior to the study, not far from where 6 of the DCCOs were collected and were evenly distributed between groups. During the course of the trial, one bird from the control group and two birds from the treatment group died and were not replaced. Therefore, the final number of birds in the control and exposed group was 11 birds each to total 22 in the study. Oil on exposed birds (13 ml) and water on control birds (13 ml) was applied every three days through Day 15 of the trial (on Days 0, 3, 6, 9, 12, and 15). Detailed description of application is available in Cunningham et al. (2017).

Plasma ionized calcium was measured using heparinized whole blood by the potentiometric method using an iSTAT (Abaxis Diagnostics, Co, Union City, CA) at necropsy on days 23 and 24 of this study.

2.3. Echocardiography

During the quarantine period (Day = −3) and prior to oil application, a board-certified veterinary cardiologist (TLR) developed a method for species-specific DCCO echocardiography modified for DCCO anatomy. DCCO hearts were visualized in real time and chambers and wall thickness were measured using a standardized, quantitative technique to evaluate the four chambers of the heart during systole and diastole. Birds were hooded using a towel with a band around bill, avoiding the nares, but allowing sufficient space (~1 cm) for open-mouth breathing. Birds were restrained in sternal position, supported at mid thorax holding the wings with the head well restrained. The keel remained free and was never compressed and feet were retracted from the coelomic window. The head was angled up with the tail pointing towards the floor so that the bird lay at approximately a 75° angle. Ultrasound gel and alcohol were used to part the feathers at the coelomic window caudal to the keel just to the right of ventral midline. The probe was placed at the coelomic window and angled towards the head or to the shoulder/thoracic vertebra to obtain the optimal cardiac image. This positioning provided an apical 3-chamber or 4-chamber view (Fig. 1). An axillary view was also used and images could be obtained; but, during the quarantine echocardiography, our cardiologist's impressions were confirmed by repetitive quantitative measurements. The coelomic window provided access that produced more precise repetitive measurements. Therefore, the coelomic window was considered the preferable technique.

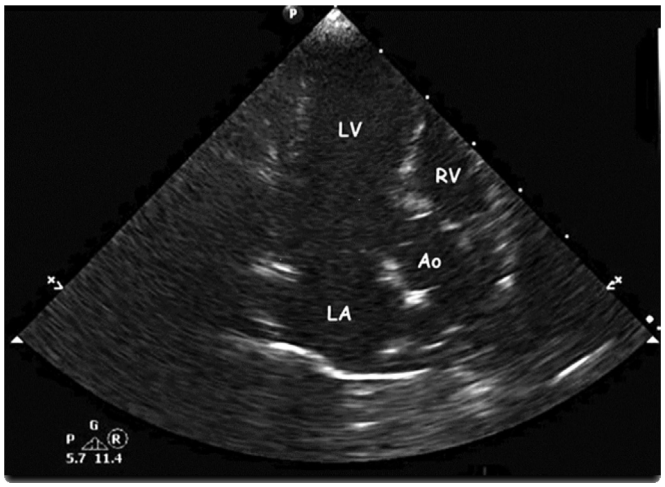

Fig. 1. Echocardiographic view of left atrium (LA), left ventricle (LV), right ventricle (RV), and aorta (Ao).

All imaging was performed using a portable ultrasound unit with a 12-4 phased array probe (Model CX50, Philips, Andover, MA).

The echocardiographic evaluations followed a consistent protocol including an apical three chamber view. First, the right atrium and tricuspid valve were examined using two-dimensional imaging. Color Doppler was then applied to interrogate the tricuspid valve for tricuspid regurgitation. Next, left ventricular outflow and transvalvular aortic velocities were obtained from the coelomic window with care to align with the left ventricular outflow tract so that optimal outflow velocities were obtained using spectral Doppler. Third, an apical three chamber image was obtained, optimized for the left ventricle and left atrium and allowing clear visualization of the ascending aorta and aortic valve. Color Doppler was applied to interrogate the mitral valve for mitral regurgitation. If regurgitation of any valve was detected with color Doppler, spectral Doppler was applied to confirm the observation.

Following the live data collection, the video loops of 2-dimensional images were reviewed utilizing analysis software (Philips Xcelera PACS). Dimensions and measurements obtained included:

I. heart rate (HR)
II. interventricular septal dimension diastole (IVSd)
III. left ventricular internal dimension diastole (LVIDd)
IV. left ventricular posterior wall dimension diastole (LVPWd)
V. interventricular septal dimension systole (IVSs)
VI. left ventricular internal dimension systole (LVIDs)
VII. left ventricular posterior wall dimension systole (LVPWs)
VIII. left atrial (LA) diameter
IX. left atrial volume
X. aortic root diameter

The following variables were calculated from the measurements as follows:

1) left ventricular fractional shortening

$$LVFS(\%) = \frac{(LVIDd - LVIDs)}{LVIDd} * 100$$

2) left ventricular ejection fraction (LVEF)

$$LVEF(\%) = \frac{(LVVol_d - LVVol_s)}{LVVol_d} * 100$$

where

$$oLVVol_d = \left(\frac{7}{2.4 + LVIDd}\right) * LVIDd^3$$

and

$$LVVol_s = \left(\frac{7}{2.4 + LVIDs}\right) * LVIDs^3$$

Standard gross necropsy was performed 24 days after baseline echocardiograph measurements and a complete set of tissues was collected for histopathologic analysis by a boarded anatomic pathologist (DRR).

2.3.1. Statistical analysis

Plots of echocardiographic variables were examined visually. Because of small sample sizes, data were analyzed non-parametrically. The post-exposure values of echocardiographic variables between exposed and unexposed DCCO were compared with Mann Whitney *U* Tests.

Because of the small sample size and no prior evidence of cardiac injury in other species, we made no adjustment of the alpha values for experiment-wise error. All comparisons were considered significantly different at $p < 0.05$.

Dot and box-and-whisker plots were used to display individual data for all endpoints.

Calculations were performed using TIBCO Spotfire S-PLUS 8.2 for Windows and MedCalc version 14.12.0 (Ostend, Belgium).

3. Results

Birds XU11 (control), CU09 (exposed) and CU18 (exposed) died prior to Day 21 of the study. A chronic, necrotizing granuloma was found at the heart base of the control bird at necropsy. Upon complete necropsy, exposed bird (CU09) died with probable septicemia (underlying etiologic agent not identified). Exposed bird (CU18), died with no significant lesions that could be assessed as a cause of death.

All pretreatment values measured in the control and exposed DCCOs had no significant difference based on ANOVA and were similar at baseline based on visual inspection of the dot plots. At necropsy, gonad identification revealed 5 males and 7 females in the control group and 6 males and 7 females in the treated group. No developed ovaries were found in any of the females at necropsy indicating a subadult classification.

After 21 day dermal exposure, DCCOs showed a decrease in cardiac systolic function, as characterized by an increased left ventricular internal dimension in systole (LVIDs, $P = 0.02$) and left ventricular systolic volume (LVSV, $P = 0.02$); consequently, left ventricular fractional shortening (LVFS, $P = 0.04$) and left ventricular ejection fraction (LVEF, $P = 0.04$) were decreased when compared to unexposed DCCO (Fig. 2a-d, Table 1). After exposure, DCCOs showed an increase in interventricular wall thickness in both diastole (IVSd, $P = 0.002$) and systole (IVSs, $P = 0.0006$), when compared to unexposed DCCOs. Conversely, left ventricular internal dimension in diastole (LVIDd, $P = 0.1$) and cardiac weight ($P = 0.4$) did not differ between control and exposed

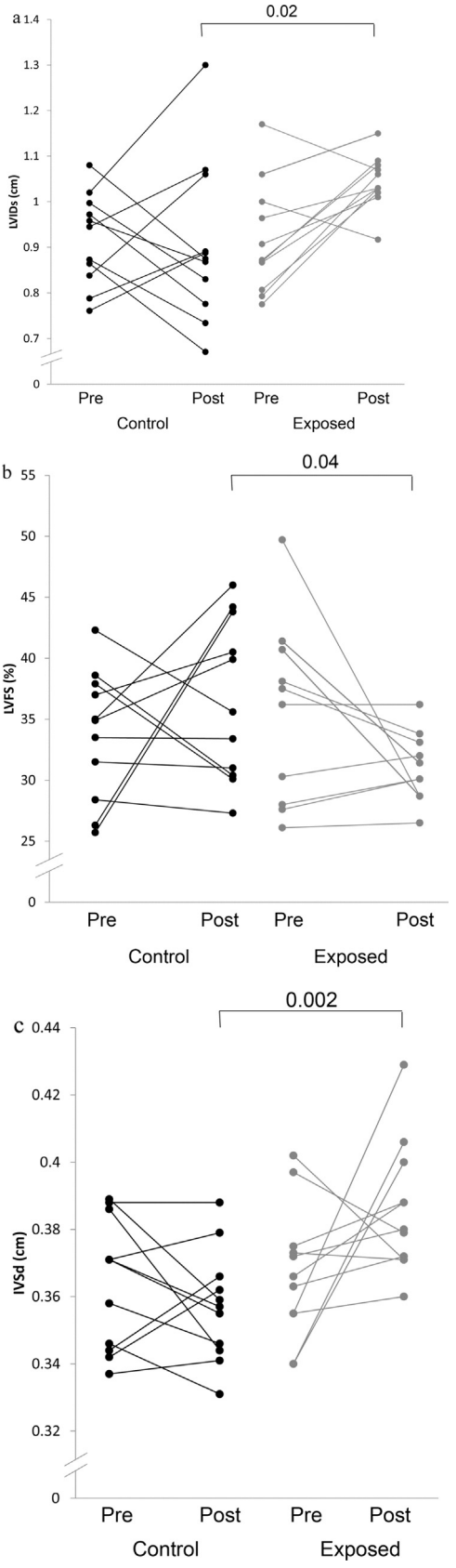

Fig. 2. a. Pre-exposure and post- 21 day exposure left ventricular internal dimension in systole (LVIDs) in control and exposed DCCO. b. Pre-exposure and post-exposure left ventricular fractional shortening (LVFS) in control and exposed DCCO. c. Pre-exposure and post-exposure interventricular septal thickness in diastole (IVSd) in control and exposed DCCO.

Table 1
Summary statistics for measures cardiac variables in DCCO.

Measurement	Treatment	n	median	min	max	p value
Aortic root dimension (AoR) (cm)	exposed	11	1.0	1.0	1.1	0.7
	control	11	1.0	0.9	1.0	
End diastolic volume (EDV 2D-Teich) (ml)	exposed	11	6.2	4.2	8.2	0.1
	control	11	4.9	3.3	9.6	
Heart rate mean (BPM)	exposed	11	140	80	263	0.8695
	control	11	135	108	173	
Interventricular septal dimension diastole (IVSd) (cm)	exposed	11	0.38	0.36	0.43	0.002
	control	11	0.36	0.36	0.38	
Interventricular septal dimension systole (IVSs) (cm)	exposed	11	0.49	0.47	0.55	0.0006
	control	11	0.46	0.42	0.5	
Left atrial area (cm^2)	exposed	11	3.1	2.4	3.5	0.2
	control	11	2.6	2.1	3.5	
Left atrial circumference (cm)	exposed	11	6.4	5.3	6.9	0.2
	control	11	5.9	5.6	6.8	
Left atrial dimension (LA Dimen) (cm)	exposed	11	1.4	1.3	1.6	0.02
	control	11	1.5	1.3	1.8	
Left ventricular ejection fraction (EF 2D-Teich) (%)	exposed	11	62	56	70	0.04
	control	11	69	57	81	
Left ventricular fractional shortening (FS 2D-Teich) (%)	exposed	11	30	27	36	0.04
	control	11	36	27	46	
Left ventricular internal dimension diastole (LVIDd) (cm)	exposed	11	1.51	1.31	1.69	0.1
	control	11	1.38	1.19	1.79	
Left ventricular internal dimension systole (LVIDs) (cm)	exposed	11	1.03	0.92	1.15	0.02
	control	11	0.88	0.67	1.30	
Left ventricular posterior wall dimension diastole (LVPWD) (cm)	exposed	11	0.45	0.43	0.55	0.045
	control	11	0.43	0.40	0.46	
Maximum aortic valve outflow velocity (AV Vmax) (cm/sec)	exposed	11	113	87	149	0.017
	control	11	137	101	191	
Systolic volume (ESV 2D-Teich) (ml)	exposed	11	2.26	1.63	2.97	0.02
	control	11	1.40	0.69	4.15	
Heart weight (g)	exposed	12	20	16	25	0.4
	control	11	21	15	32	

DCCOs.

Heart rates did not differ between exposed and unexposed DCCOs ($P = 0.7$). However, exposed DCCOs had clinically significant arrhythmia that included marked bradycardia, defined as < 70 BPM, (100%, 11/11 exposed DCCOs) and tachycardia, defined as > 200BPM (18%, 2/11 exposed DCCOs) (Fig. 3).

Control DCCOs had a sinus arrhythmia, which is typical in diving animals (Fig. 4a). No other arrhythmia was found in control DCCOs. A tachycardia was documented in two exposed DCCOs (Fig. 4b). Electrocardiography was unavailable, so the exact nature of the tachyarrhythmia could not be determined.

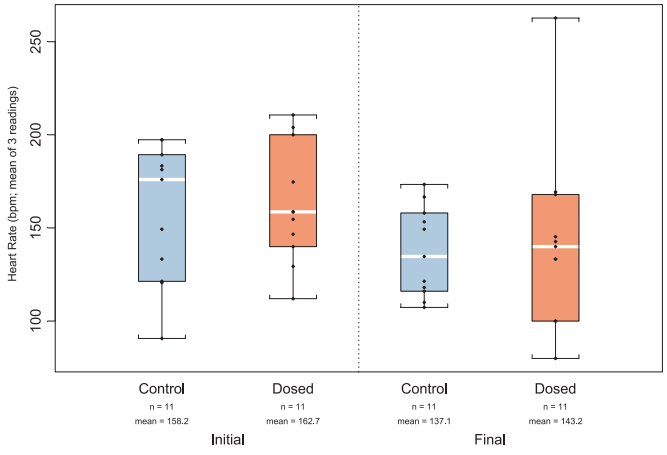

Fig. 3. Average heart rate of control and exposed DCCOs. No statistically significant difference was present as outliers in the treated group were on both ends of the dataset.

Pericardial effusion was documented echocardiographically and confirmed at gross necropsy in two exposed DCCOs (Addendum Video 3 – Echocardiograph video of a heart from a treated bird exhibiting pericardial effusion. Control DCCOs did not have evidence of any excess fluid around the heart at necropsy. When microscopically evaluated, the fluid contained within the pericardial sac differed slightly between the two exposed DCCOs in that one was a low protein transudate while the second, classified as an exudate, had a component of suppurative inflammation without evidence of an infectious agent.

Dyspnea (labored breathing) was also noted in exposed DCCOs but not controls. During the baseline evaluation, all DCCOs were able to breathe comfortably with a band placed around the beak to avoid the DCCOs biting the handlers. Comparison of the same population of DCCOs after oil exposure yielded notable differences between the control and exposed DCCOs. The oil-exposed DCCOs demonstrated severe dyspnea when handled. When the band was placed on the beak of several exposed DCCOs for short periods of time, the breathing was so labored that the safety of the DCCOs was threatened. We modified the beak restraint for the completion of the exams so that the DCCOs had their mouths open to breath at all times. All control DCCOs tolerated the beak band with mouths restrained with no breathing distress.

Gross necropsy revealed several hearts from dermally exposed DCCOs appeared to have softer cardiac musculature than the hearts from control DCCOs as in the oral exposure (Fig. 5a, b).

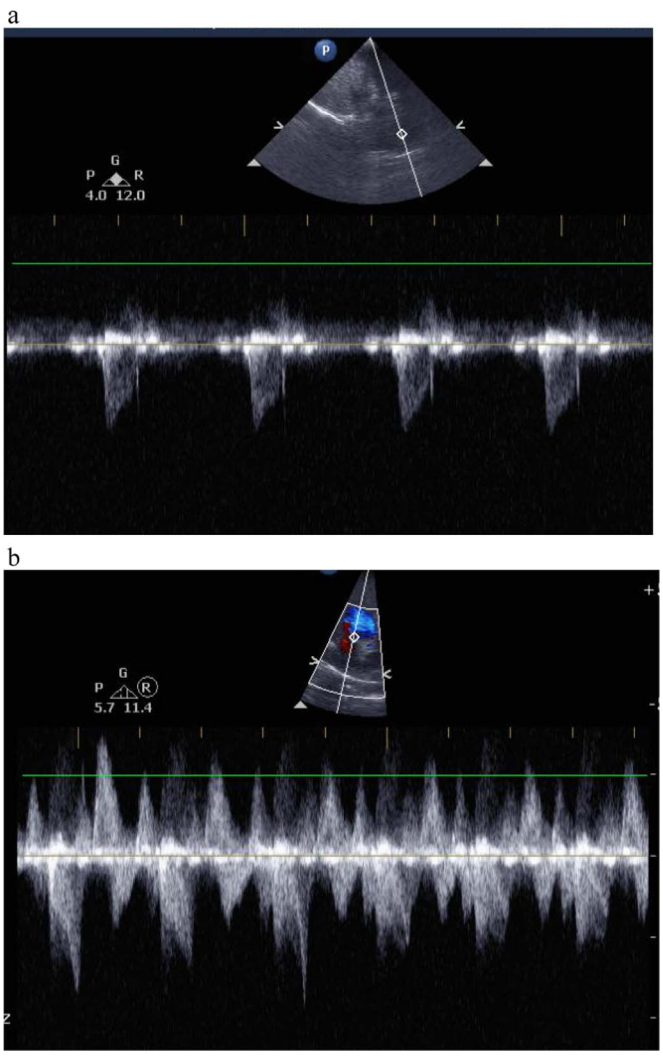

Fig. 4. a. Example of typical heart rate in a control DCCO. Addendum 1 – DCCO echocardiograph video illustrating a normal heart from a control DCCO. This figure represents 4 beats in 2 s or 120 beats per minute. b. Example of tachycardia found in two oil-exposed DCCOs. Addendum 2 – DCCO echocardiograph video illustrating tachycardia (> 200BPM). Note the disorganized electrical stimulation and contractions only found in treated birds.

Upon histopathologic examination, three hearts from exposed DCCOs had myocardial fibrosis, while no myocardial fibrosis was found in the control DCCOs. Other histologic lesions, including septicemia and inflammation, were considered to be representative of background disease in the population. Special stain for collagen have not been completed at the time of this report (Table 2).

4. Discussion

In this study, a reproducible echocardiographic technique to evaluate cardiac morphology and function was developed in DCCOs. The DCCOs dermally exposed to weathered MC252 oil had echocardiographic findings indicating decreased ventricular myocardial contractility, as evidenced by increased ventricular dimensions during systole; but no change in atrial dimensions was detected in this low sample number and low statistical power, short term study. This was consistent with necropsy findings of soft, flaccid, and enlarged hearts although the weight of the hearts of control and treated birds were not statistically different and myocardial fibrosis was observed in only some hearts from exposed DCCOs. Further study is needed to confirm possible dilative cardiomyopathy postulated by clinicians. Only exposed DCCOs exhibited arrhythmias other than a sinus arrhythmia, including marked bradycardia in all exposed DCCOs and marked tachycardia in two exposed DCCOs. Additionally, a statistically significant increase in ionized calcium in the treated birds was present.

In comparison to other avian species, the DCCOs have a larger keel and therefore a smaller coelomic viewing window where the ultrasound probe may be placed to view the cardiac silhouette. As the probe is more caudal, the angle to the head is more acute and more closely parallels the spine. Psittacines can be assessed in dorsal recumbency, but the preferred position to consistently view the cardiac silhouette in DCCOs was ventral recumbency. In psittacines, all four chambers of the heart can be seen in one window, but in DCCOs, the right atrium and ventricle were imaged with a slightly more cranial angle than the left atrium and left ventricle.

To the authors' knowledge, oil exposure has not been reported to cause cardiac dysfunction in birds, either in adults or developing life stages. The data in our study are strongly suggestive of cardiotoxicity and our findings mirror those observed in other species. Oil exposure has been shown to cause cardiac damage in a broad range of developing fish species including sole, herring, zebrafish, seabass and tuna (Brette et al., 2014; Claireaux and Davoodi, 2010; Marty et al., 2011; Jung et al., 2013; Tissier et al., 2015). The cardiac damage is theorized to occur by an impairment of the cardiac excitation-contraction coupling mechanism via blocking of the delayed rectified potassium current and a decrease in calcium current and calcium cycling (Brette et al., 2014). In this study, there was a statistically significant increase in plasma ionized calcium which warrants further investigation as a pathogenic mechanism of disease. Severe hypercalcemia may induce arrhythmia but this has not been shown to be causative in this study. All of the gonads in this study were assessed as immature at necropsy indicating that the bird's sex did not play a role in calcium values. In other studies, activation of the aryl hydrocarbon receptor is listed as the cause of ventricular remodeling and cardiac damage (Incardona et al., 2004). Similarly, developing birds exposed to dioxin-like compounds, which also activate the aryl hydrocarbon receptor, undergo changes in cardiac modeling (DeWitt et al., 2006; Kopf and Walker, 2009; Carro et al., 2013). Exposure to weathered MC252 oil and unweathered slick A oil collected during the Deepwater Horizon spill induced pericardial edema, atrial arrhythmia, dose dependent bradycardia, and an inversion of the systolic and diastolic phases in isolated tuna ventricular myocytes (Brette et al., 2014). It is interesting to note that very similar cardiac pathology including arrhythmia, bradycardia, pericardial edema/effusion, and decreased myocardial contractility are found in both cold and warm water species including tuna, amberjack, herring, salmon, and zebra fish when exposed to either source or weathered Louisiana sweet crude oil or source or weathered Exxon Valdez crude oil or Iranian heavy crude. While there is some variability in the prominence of effect between species and possibly between forms of oil, the effects themselves are consistent. It is also striking that not only does this crude oil cause cardiac abnormalities in delicate developing life stages but did produce clinically significant changes in adult birds after only 3 weeks of moderate exposure.

Similar findings have also been found in mammals exposed to crude oil. Flaccid hearts were observed upon gross examination and edema and coagulative necrosis of cardiac myofibers were found on histopathologic examination of sheep necropsied after crude oil exposure in Brazil (Batista et al., 2013). Mostrom and Campbell reported enlarged and "flabby" hearts upon gross necropsy of cattle exposed to sour multiphase crude petroleum mixed with river water and soil during a pipeline spill and then burned during cleanup in the Red Deer River, Alberta, Canada. It should be noted that this crude petroleum (sour gas and sour gas condensate) contains hydrogen sulfide and has a higher percentage of volatiles than the weathered MC252 oil in our study. Upon histopathologic evaluation, exposed cattle were found to have hemorrhage at valves and on epicardium and endocardium. Hemorrhagic lesions throughout the body were reported by the anatomic

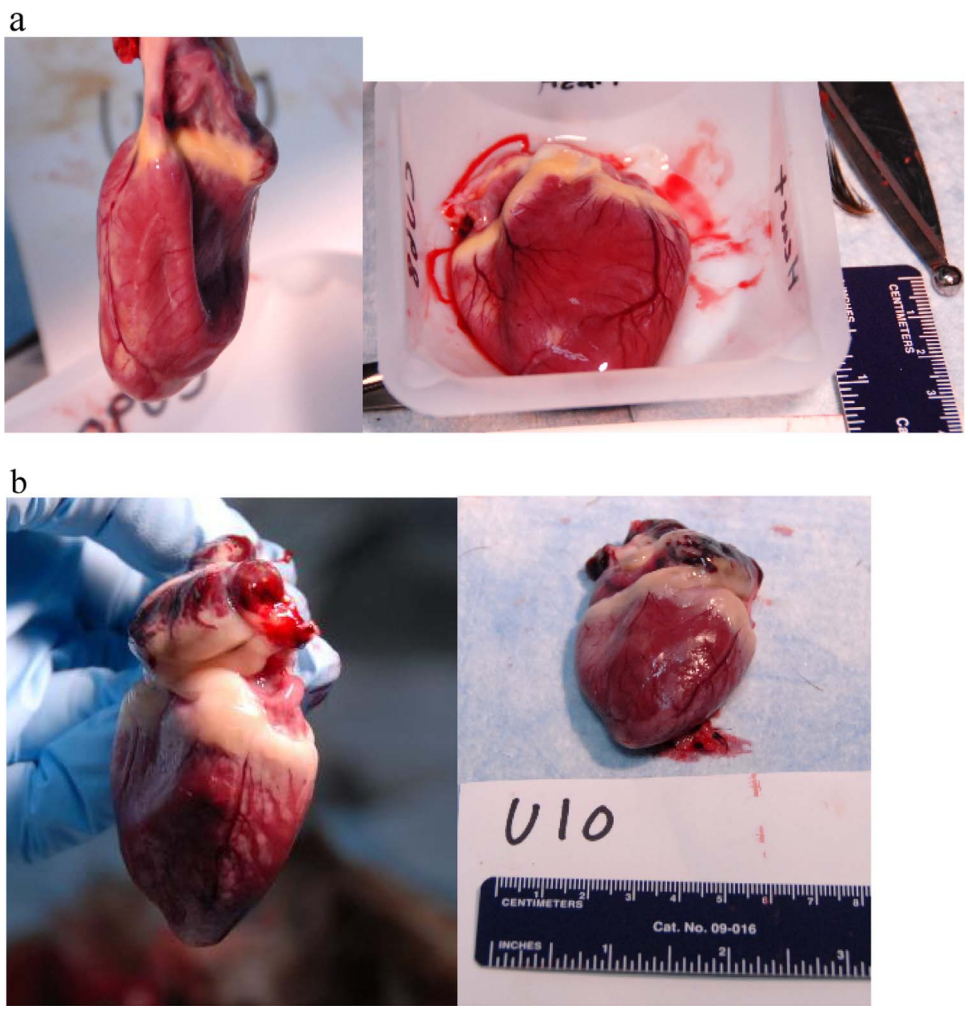

Fig. 5. a. Heart from dermally exposed DCCO. b. Heart from a normal control DCCO.

pathologist as being consistent with *Salmonella* infection, however, *Salmonella* was not cultured from any cattle (Mostrom and Campbell, 1994). The generalized hemorrhagic lesions found in many tissues including the heart in the exposed cattle indicated that an induced coagulopathy was possible, similar to that which was documented in the cormorants (Harr et al., 2017a). Depressed or rapid, weak pulses were noted clinically in cattle exposed to crude oil and upon complete necropsy fibrinous pericarditis was noted in occasional exposed animals only. No lesions were noted in the heart itself upon histopathology and heart rate/pulse were the only clinical measurement of the heart assessed in this study (Bystrom, 1989). This is consistent with the findings in the DCCOs in this study where average heart rates did not differ between exposed and control DCCOs, but clinically significant, and possibly life-threatening arrhythmias, including bradycardia (n = 11/11) and tachycardia (n = 2/11), were noted only in exposed DCCOs upon echocardiographic evaluation. Cardiac arrhythmias in humans have been observed in cases of oil exposure and are attributed to the volatile components as well as polycyclic aromatic hydrocarbons (PAHs) found in oil and oil byproducts. The tissue concentrations of these components in an ingested complex mixture required to produce arrhythmias is unknown (Bradley et al., 2013).

Many of the piscine studies suggested that humans might also suffer cardiac myocyte damage secondary to petroleum exposure however this has not been well-documented in studies of people exposed to crude oil products (D'andrea and Reddy, 2014; Goldstein et al., 2011; Solomon and Janssen, 2010). In humans, diesel exhaust particles, which also contain concentrated PAHs similar to weathered MC252 oil, increases the risk of significant arrhythmias and cardiac failure in urban residents (Brook et al., 2010). In Sprague Dawley rats, diesel exhaust particles resulted in eccentric left ventricular dilation with systolic dysfunction similar to that which we found in the DCCOs (Bradley et al., 2013). Bradley et al. (2013) postulated that induction of aryl hydrocarbon reductase induces left ventricular dilation due to loss of collagen. Further histopathologic evaluation of the cormorant heart

Table 2
Histopathologic findings in cormorants dermally exposed to weathered MC252 oil. Harr et al. (2017b), this edition.

Tissue	Treatment	Lesion description	Lesion distribution	Grade	Lesion grade	Animals affected
Heart						
	Control	Epicardial hemorrhage	focal	minimal	1	1/11
		Myocardial hemorrhage	multifocal	mild	2	1/11
		Myocarditis, lymphoplasmacytic	focal	minimal	2	1/11
	Externally oiled	Bacterial granulomas	multifocal	moderate	3	1/11
		Myocardial fibrosis	multifocal	mild to moderate	2.3	3/11
		Septic and suppurative thrombi	focal	mild	2	1/11

samples using special stains for collagen are warranted to assess this potential pathogenic mechanism of disease.

The pathogenesis of the cardiotoxicity may also be by direct oxidative damage to the cardiomyocytes causing decreased cellular function. Studies of specific components of crude oil have shown that significant concentrations can be found in cardiac tissue, although this is time-dependent. The chronic effects of oxidant damage caused by these components is not well studied, though mitochondrial damage in cardiac myocytes has been postulated. Aromatics and ^{14}C-naphthalene are detectable in cardiac tissue at 72 h (the last time point of the study) after a single administration of aromatic hydrocarbons in hens and pigs (Eisele et al., 1985). Aromatics have also been found in cardiac tissue from ducks dosed singly and multiple times with sweet Louisiana crude oil (Gay et al., 1980). It was beyond the scope of this study to assess PAH concentrations in all tissues.

Other pathologies documented at necropsy in other organ systems including the kidney, liver, and hematopoietic tissues likely compounded direct cellular damage as well as aryl hydrocarbon receptor binding to contribute to cardiac dysfunction. The treated DCCO in this study were found to be suffering from hemolytic anemia resulting from oxidative damage to the red blood cells (Harr et al., 2017a) and were likely to be under thermoregulatory stress due to feather disruption and feather plucking (Cunningham et al., 2017). Therefore, stresses on the heart prior to necropsy were multifactorial and could have synergistically resulted in decompensation.

In summary, external exposure of double-crested cormorants to artificially weathered MC252 crude oil collected during the Deepwater Horizon oil spill resulted in cardiac damage and dysfunction manifested as decreased ventricular contractility and arrhythmia, including both bradycardia and tachycardia. To our knowledge, this is the first report of oil-induced cardiac damage in oil-exposed birds. Dilative cardiomyopathy was postulated by clinicians and supported by echocardiography but further investigation is warranted to confirm this diagnosis. Further investigation to define the type of arrhythmia is warranted to understand the pathogenesis of oil-induced cardiac damage. The findings are similar to those reported in humans, rats, and other mammalian species typically attributed to the PAH component of oil. The changes in cardiac function reported here could impact acute mortality, long-term survivability, and recruitment of individuals and populations of birds.

Addendum

Three videos are listed in the text.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.04.010.

References

Batista, J.S., Câmara, A.C.L., Almeida, R.D., Olinda, R.G., Silva, T.M.F., 2013. Poisoning by crude oil in sheep and goats. Rev. Med. Vet. 11, 217–520.

Bradley, J.M., Cryar, K.A., Hajj, M.C., Hajj, E.C., Gardner, J.D., 2013. Exposure to diesel exhaust particulates induces cardiac dysfunction and remodeling. J. Appl. Physiol. 115, 1099–1106.

Brette, F., Machado, B., Cros, C., Incardona, J.P., Scholz, N.L., Block, B.A., 2014. Crude oil impairs excitation-contraction coupling in fish. Science 343 (February), 772–776.

Brook, R.D., Rajagopalan, S., Pope, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., Holguin, F., Hong, Y., Luepker, R.V., Mittleman, M.A., Peters, A., 2010. Particulate matter air pollution and cardiovascular disease an update to the scientific statement from the American Heart Association. Circulation 121 (21), 2331–2378(Retrieved from: ⟨http://circ.ahajournals.org/content/121/21/2331.long/feed⟩).

Bursian, S., Cacela, D., Cunningham, F.L., Dean, K.M., Dorr, B.S., Ellis, C., Guglielmo, C.G., Hanson-Dorr, K.C., Harr, K.E., Healy, K.A., Hooper, M.J., Horak, K.E., Isanhart, J., Kennedy, L., Link, J.E., Maggini, I., Moye, J., Perez, C.R., Pritsos, C.A., Shriner, S., Trust, K., Tuttle, P., 2017. Overview of avian toxicity studies for the Deepwater Horizon Natural Resource Damage Assessment. Ecotoxicol. Environ. Saf (in press) (Citation to be finalized during editorial review).

Bystrom, J.M., 1989. Study of the acute toxicity of ingested crude petroleum oil to cattle. Masters of Science Thesis accepted by University of Saskatchewan, Saskatoon, Canada.

Carro, T., Dean, K., Ottinger, M.A., 2013. Effects of an environmentally relevants polychlorinated biphenyl (PCB) mixture on embryonic survival and cardiac development in the domestic chicken. Environ. Toxicol. Chem. 32 (6), 1317–1324.

Claireaux, G., Davoodi, F., 2010. Effect of exposure to petroleum hydrocarbons upon cardio-respiratory function in the common sole (Solea solea). Aquat. Toxicol. 98 (2), 113–119(Retrieved from: ⟨http://www.sciencedirect.com/science/article/pii/S0166445X10000469⟩).

Collier, T.K., Anulacion, B.F., Arkoosh, M.R., Dietrich, J.P., Incardona, J.P., Johnson, L.L., Ylitalo, G.M., Myers, M.S., 2014. Effects on Fish of Polycyclic Aromatic Hydrocarbons (PAHS) and Naphthenic Acid Exposures. In: Tierney, C.J., Farrell, K.B., Brauner, A.P. (Eds.), Organic Chemical Toxicology of Fishes: Fish Physiology Vol. 33. Elsevier Inc, London, pp. 195–255. http://dx.doi.org/10.1016/B978-0-12-398254-4.00004-2.

Cunningham, F., Dean, K., Hanson-Dorr, K., Harr, K.E., Healy, K., Horak, K., Shriner, S., Link, J., Bursian, S., Dorr, B., 2017. Development of Methods for the study of Avian Oil Toxicity Studies using the Double Crested Cormorant (Phalacrocorax auritus). Ecotoxicol. Environ. Saf (in press) (Citation to be finalized during editorial review.).

D'andrea, M.A., Reddy, G.K., 2014. Hematological and hepatic alterations in nonsmoking residents exposed to benzene following a flaring incident at the British Petroleum plant in Texas City. Environ. Health 13, 115–132. http://dx.doi.org/10.1186/1476-069X-13-115.

DeWitt, J.C., Millsap, D.S., Yeager, R.L., Heise, S.S., Sparks, D.W., Henshel, D.S., 2006. External heart deformities in passerine birds exposed to environmental mixtures of polychlorinated biphenyls during development. Environ. Toxicol. Chem. 25 (2), 541–551.

DWH Trustees (Deepwater Horizon Natural Resource Damage), 2015. Deepwater Horizon oil spill draft programmatic damage assessment and restoration plan and draft programmatic environmental impact statement. ⟨Http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan⟩, pp. 1–685 (accessed 1 March 2017).

Eisele, G.R., Tsai, S.C., Payne, J., Kyle, B., Urso, I., 1985. The distribution of orally administered C-Aniline HCl in tissues of dairy cattle, swine and laying pullets. J. Anim. Sci. 61 (6), 1492–1497. http://dx.doi.org/10.2527/JAS1985.6161492X.

Forth, H.P., Mitchelmore, C.L., Morris, J.M., Lipton, J., 2015. Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon oil spill natural resource damage assessment. Environ. Toxicol. Chem. 36 (6), 1450–1459. http://dx.doi.org/10.1002/etc.3672. (Citation to be editorial review) (Epub 2016 Dec 9).

Gay, M.L., Belisle, A.A., Patton, J.F., 1980. Quantification of petroleum-type hydrocarbons in avian tissue. J. Chromatogr. A 187 (1), 153–160. http://dx.doi.org/10.1016/S0021-9673(00)87881-4.

Goldstein, B.D., Osofsky, H.J., Lichtveld, M.Y., 2011. The Gulf Oil Spill. N. Engl. J. Med. 364 (14), 1334–1348. http://dx.doi.org/10.1056/NEJMra1007197.

Harr, K.E., Cunningham, F.L., Pritsos, C., Pritsos, K., Muthumalage, T., Dorr, B., Horak, K., Hanson-Dorr, K., Dean, K., Cacela, D., Link, J.E., Healy, K., Tuttle, P., Bursian, S.J., 2017a. Weathered MC252 crude oil-induced anemia and abnormal erythroid morphology in double-crested cormorants (Phalacrocorax auritus) with light microscopic and ultrastructural description of Heinz bodies. Ecotoxicol. Environ. Saf (in Press) (Citation to be finalized during editorial review.).

Harr, K.E., Bursian, S.J., Cacela, D., Cunningham, F.L., Dean, K.M., Dorr, B.S., Hanson-Dorr, K.C., Healy, K.A., Horak, K.E., Link, J.E., Reavill, D.R., Shriner, S.A., Schmidt, R.E., 2017b. Comparison of organ weights and histopathology between double-crested cormorants (Phalacrocorax auritus) dosed orally or externally with artificially weathered Mississippi Canyon 252 crude oil. Ecotoxicol. Environ. Saf (in press) (Citation to be finalized during editorial review.).

Incardona, J.P., Collier, T.K., Scholz, N.L., 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 196 (2), 191–205. http://dx.doi.org/10.1016/j.taap.2003.11.026.

Incardona, J.P., Gardner, L.D., Linbo, T.L., Brown, T.L., Esbaugh, A.J., Stieglitz, J.D., French, B.L., Labenia, J.S., Laetz, C.A., Mager, E.M., 2014. Deepwater Horizon crude oil impacts the developing hearts of large predatory pelagic fish. Proc. Natl. Acad. Sci. 111 (15), E1510–E1518. http://dx.doi.org/10.1073/pnas.1320950111.

Kopf, P.G., Walker, M.K., 2009. Overview of developmental heart defects by dioxins, PCBs and pesticides. J. Environ. Health Sci. C: Environ. Carcinog. Ecoloxicol. Rev. 27 (4), 276–285.

Mazet, J.A.K., Newman, S.H., Kirsten, V.K., Tseng, F.S., Holcomb, J.B., Jessup, D.A., Ziccardi, M.H., 2002. Advances in oiled bird emergency medicine and management. J. Avian Med. Surg. 16 (2), 146–149.

Mostrom, M.S., Campbell, C., 1994. Livestock Field Investigations of Two Ranches Associated with a Pipeline Break. Alberta Research Council; Alberta Agriculture, Food and Rural Development, Edmonton.

Solomon, G.M., Janssen, S., 2010. Health effects of the Gulf Oil Spill. JAMA 304 (10), 1118–1119. http://dx.doi.org/10.1001/jama.2010.1254.

Tissier, F., Dussauze, M., Lefloch, N., Theron, M., Lemaire, P., Le Floch, S., Pichavant-Rafini, K., 2015. Effect of dispersed crude oil on cardiac function in seabass Dicentrarchus labrax. Chemosphere 134, 192–198. http://dx.doi.org/10.1016/j.chemosphere.2015.04.026.