



Dietary Exposure to Low Levels of Crude Oil Affects Physiological and Morphological Phenotype in Adults and Their Eggs and Hatchlings of the King Quail (*Coturnix chinensis*)

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Despite the current knowledge of the devastating effects of external exposure to crude oil on animal mortality, the study of developmental, transgenerational effects of such exposure has received little attention. We used the king quail as an animal model to determine if chronic dietary exposure to crude oil in a parental population would affect morpho-physiological phenotypic variables in their immediate offspring generation. Adult quail were separated into three groups: (1) Control, and two experimental groups dietarily exposed for at least 3 weeks to (2) Low (800 PAH ng/g food), or (3) High (2,400 PAH ng/g food) levels of crude oil. To determine the parental influence on their offspring, we measured metabolic and respiratory physiology in exposed parents and in their non-exposed eggs and hatchlings. Body mass and numerous metabolic (e.g., O₂ consumption, CO₂ production) and respiratory (e.g., ventilation frequency and volume) variables did not vary between control and oil exposed parental groups. In contrast, blood PO₂, PCO₂, and SO₂ varied among parental groups. Notably, water loss through the eggshell was increased in eggs from High oil level exposed parents. Respiratory variables of hatchlings did not vary between populations, but hatchlings obtained from High oil-exposed parents exhibited lower capacities to maintain body temperature while exposed to a cooling protocol in comparison to hatchlings from Low- and Control-derived parents. The present study demonstrates that parental exposure to crude oil via diet impacts some aspects of physiological performance of the subsequent first (F₁) generation.

Keywords: Transgenerational Inheritance, parental effects, crude oil, oxygen consumption, development, epigenetics, physiology, bird

Abbreviations: dph, days post-hatching; *f*, breathing frequency; F_EO₂, excurrent O₂ fraction of dry gas; F_IO₂, incurrent O₂ fraction of dry gas; F_ECO₂, excurrent CO₂ fraction of dry gas; F_ICO₂, incurrent CO₂ fraction of dry gas; \dot{V}_I , incurrent flow rate; T_a, air temperature; T_b, body temperature; \dot{V}_{O_2} , oxygen consumption; \dot{V}_{CO_2} , carbon dioxide production; \dot{V}_E , minute ventilation; \dot{V}_E/\dot{V}_{O_2} , air convection requirement; V_T, tidal volume; RER, respiratory exchange ratio; W_{loss}, Water loss across the eggshell.

INTRODUCTION

The impacts of crude oil exposure on wild animal populations have been a subject of study since the 1970s when major oil spills first appeared. Oil spills such as The Deepwater Horizon (DWH) extended from the pelagic zone in the ocean to estuaries along the ocean's coast potentially impacting animal life negatively (Michel et al., 2013; Pasparakis et al., 2019). The weathering process separates crude oil into different phases with different physicochemical characteristics, leading to obvious harmful effects on aquatic species (Dubansky et al., 2013; Tierney et al., 2013; Mager et al., 2014). However, crude oil also contacts shorelines, where sediments remain oil-laden (Pennings et al., 2014; Dubansky et al., 2018). Consequently, oil represents a constant threat for marine and especially marsh species of birds that could be exposed dermally, orally and/or via oil vapor inhalation (Horak et al., 2017; Perez et al., 2017; Harr et al., 2017a,b; Dubansky et al., 2018).

Crude oil exposure in avian species has acute lethal effects on heavily exposed individuals (Perez et al., 2017). Short of proving lethal, however, external (dermal) oil-contamination and respiratory exposures in birds induces increased lung-epithelial CYP1A expression, skin irritation, conjunctivitis and corneal ulcers, and also reduces the feathers' capacity to repel water, which leads to increased heat loss, and reductions in insulating capacities, buoyancy, and flight performance (Leighton, 1993; Jenssen, 1994; O'Hara and Morandin, 2010; Munilla et al., 2011; Fiorello et al., 2016; Maggini et al., 2017b; Dubansky et al., 2018). Beyond these effects of dermal and respiratory oil exposures, after an oil spill many birds continue being orally exposed for months and even for decades to varying concentrations of petroleum leading also for sublethal effects (Alexander et al., 2017; Esler et al., 2018). Oral exposure to oil occurs via ingestion of contaminated prey, grit and water during foraging, and also occurs during attempts for cleaning (preening) of oil-contaminated feathers (Hartung and Hunt, 1966; Pritsos et al., 2017b). Dietary oil exposure has been reported to induce effects on birds at different levels of biological organization and different developmental stages. For example, loss of body mass has been reported in herring gulls, laughing gull, and kestrels (Pattee and Franson, 1982; Leighton, 1986; Puddephatt, 2014; Horak et al., 2017). Morphological changes and inflammation of the kidney have been reported in mallard ducklings after dietary oil-exposure (Szaro et al., 1980). Hematological responses to dietary oil exposure led to hemolytic anemia, as indicated by increased numbers of Heinz bodies and degenerated erythrocytic organelles in exposed organisms (Pattee and Franson, 1982; Horak et al., 2017; Fallon et al., 2018). Additionally, dietarily oil exposed laughing gulls and cormorants exhibited increased oxidative stress in the liver (Horak et al., 2017; Pritsos et al., 2017a). With respect to physiological effects, potential impairment of blood oxygen carrying capacity was reported in sandpipers, cormorants, homing pigeons, and laughing gulls (Dean et al., 2017; Bursian et al., 2017a). Overall, the results from these studies suggest that dietary/oral exposure to crude oil has significant metabolic implications for bird populations. To our knowledge, this are the first measurements of metabolic rate in oil-exposed birds,

which opens a new avenue for exploring the physiology of avian toxicant exposure.

Remarkably, studies aiming to understand the physiological effects of dietary exposure to crude oil in oil-exposed birds – and especially the transgenerational effects on the physiology of their offspring – are scarce. Exposure to crude oil, or to its individual components, induces parent-to-offspring transfer of effects in fish, even when the *first* (F_1) generation itself has not experienced exposure (Corrales et al., 2014; Bautista et al., 2020). Consequently, it is possible that petroleum exposure on bird parental populations could potentially lead to developmental effects in their subsequent generations. Performing this type of transgenerational study on wild birds is rarely practical because of the large amounts of animal rearing space and the time required for birds to develop. Not surprisingly, researchers have turned to the use of animal models or domesticated species (Guerrero-Bosagna et al., 2018; Burggren, 2020).

In the present study, we have used the king quail, *Coturnix chinensis*, as a proxy species to evaluate the effects of dietary exposure to crude oil on the parental P_0 generation and subsequent effects on their eggs and hatchlings comprising the F_1 generation. First, we determined whether the P_0 generation *per se* was affected by assessing multiple physiological and morphological traits. Then, we assessed physiological and morphological traits of F_1 eggs and hatchling king quail. We hypothesized that chronic exposure to low levels of crude oil in adult king quail will alter the morphology and physiology of both the P_0 adults and their F_1 offspring.

MATERIALS AND METHODS

Ethics Statement

All experiments in this study were approved and performed in strict accordance with the Institutional Animal Care and Use Committee (IACUC-Protocol #18022) at the University of North Texas.

Animal Husbandry

Fifteen pairs of adult king quail *Coturnix chinensis* were obtained from a local supplier (Elm Ridge Enterprises, Temple, United States) and kept at the avian facilities at the University of North Texas. All birds were maintained at $\sim 24^\circ\text{C}$ and $\sim 60\%$ humidity in a 14:10 h light:dark cycle, and provided with food and water *ad libitum*. *C. chinensis* exhibit monogamous reproductive behavior. Thus, one male and one female (five pairs per experimental group) were kept together in a ventilated plastic cage that was 60 cm long \times 48 cm wide \times 35 cm deep. The bottom of the containers were covered with wood shavings to prevent injuries in the feet of the birds and to absorb moisture (MacDonald, 2010; Puddephatt, 2014). Cleaning of the containers was performed on a weekly basis, but all containers were checked, stocked, and cleaned (if necessary) every morning and afternoon.

Eggs collected from the adults' cages were placed inside a cycle-rotating incubator (GQE, 3258N Digital Command Center, Georgia, United States) and maintained at 37.5°C and 60% RH.

King quail eggs take approximately 16 days to hatch (MacDonald, 2010). On day 13 the eggs were removed from the rotation motion of the incubator and placed, until hatch, into individual cells of a custom-built hatcher at 37.5°C and 60% relative humidity. Hatchlings were individually labeled by attaching a piece of tape (<1 cm²) to the feathers on the back and transferred into an environmental chamber with the bottom coated with wood flakes to prevent feet injury and absorb moisture. The hatchlings were maintained in similar conditions as in the hatcher under 14:10 h dark/light cycle and provided with water and food *ad libitum*, until seven to 8 days post hatching (dph), at which time respirometry measurements were made (see below). Eggs laid before at least 21 days of parental exposure were not incubated for respirometry nor hematological measurements.

Diet Preparation

Dietary exposure to crude oil in adult quail was used as the stressor in this study. To prepare oiled diets, solutions of High Energy Water Accommodated Fractions of crude oil (HEWAF) – essentially oil blended into water – were prepared following standard protocols (Mager et al., 2014; Forth et al., 2017; Reddam et al., 2017; Bautista et al., 2019). Source oil “B” (SOB) sampled from the Gulf of Mexico MC252 well on May 22–23, 2010 was used for this experiment. British Petroleum acknowledges the use of a defoamer (Nalco EC9323A), oxygen scavenger (Nalco VX9831), and methanol during the collection of this type of crude oil. Although their presence in our SOB cannot be dismissed, the direct sampling of oil from the riser insertion tube reduces the possibility of incorporation of these compounds into the oil (de Soysa et al., 2012). HEWAF preparation was performed by adding 1,000 mg of crude oil into 1 L of deionized water and blending for 30 s in a commercial blender (Waring TM CB15, Torrington, United States). After blending, the mixture was placed into a separation funnel for 1 h, after which 100 mL of the solution was taken out through a bottom port of the funnel and discarded. 600 mL of the remaining mixture (considered as 100% HEWAF) and two diluted solutions, 1 and 10% HEWAF, in deionized water were used for diet preparation.

Three dietary treatments were used for adult quail (P₀) exposures: (1) Control group, (2) “Low” exposure (1% HEWAF), and (3) “High” exposure (10% HEWAF). To make these dietary treatments, 100 g of quail food was prepared from equal parts of 24% protein chick starter (Dumor poultry #5078197, St. Louis, United States), Reptile diet (Mazuri 006267, Gentofte, Denmark), and wild bird mixed seeds (Blain’s Farms and Fleet, Janesville, United States). This mixture was then evenly distributed across the bottom of aluminum trays (100 cm long × 30 cm wide). The food mixture was sprayed with 60 mL of water (Control), or with 60 mL of one of the two HEWAF solution concentrations described above. The treated food was allowed to dry for ~12 h. The dried food was then collected from the trays and stored at room temperature in glass bottles (Bautista et al., 2019).

Representative samples of each treatment diet were analyzed by ALS Environmental (ALS Environmental, Kelso, United States) to obtain total polycyclic aromatic hydrocarbons (PAH) concentrations (Σ_{totPAH}). PAHs are petroleum components affecting morphology, cardiac system physiology,

and swimming performance of vertebrates (Harr et al., 2017b,c; Incardona and Scholz, 2018; Perrichon et al., 2018; King et al., 2020). Thus, determination of [PAH] in dietarily experimental treatments is widely accepted as a valid proxy indication of toxicity level of each treatment in experimental protocols involving animal exposures (Mager et al., 2014; Incardona and Scholz, 2018; Bautista et al., 2020). In addition to dietary samples, eggs obtained from each parental group were also sent for PAH determination analysis to ALS Environmental. Total sum of PAH concentrations in the dietary treatments was 0, 800 and 2,400 ng/(g of food) for the Control, Low and the High exposed groups diets, respectively, (**Supplementary Figure 1**). These doses are below concentrations used in studies with wild marine species (for a review, see King et al., 2020). In contrast, none of the egg samples from the parental groups exhibited any detectable content of PAHs after analysis (**Supplementary Table 1**).

Exposure Protocol

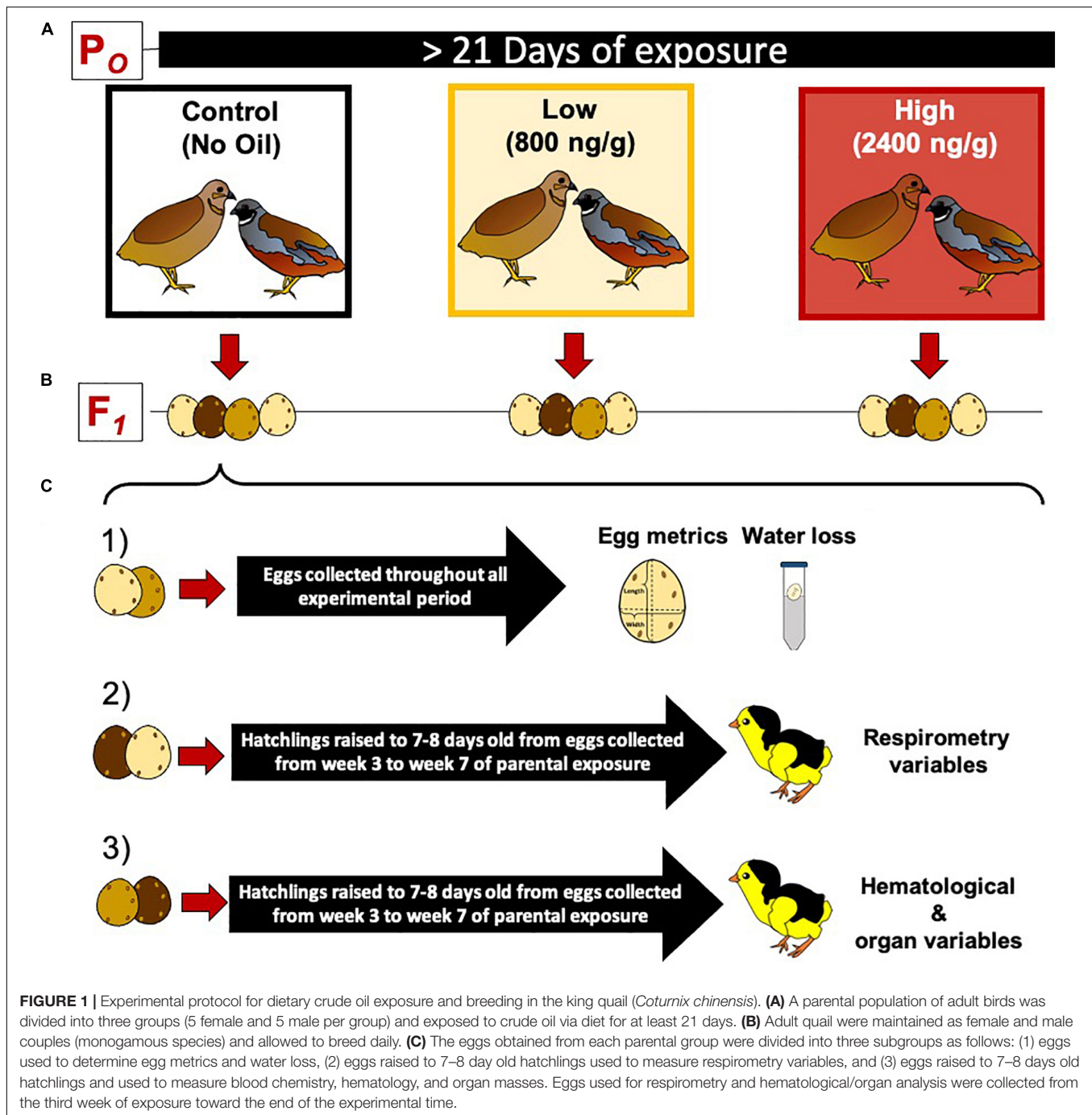
Adult quail were allowed to acclimate for 14 days prior to dietary exposure. Before the beginning of exposure, all birds were screened to ensure healthy condition. Dietary exposures began at day 15 of the experiment. Food and water were provided *ad libitum*. Eggs variables were determined throughout all the experimental weeks, however, analysis of respirometry variables in the first generation was performed in hatchlings obtained from eggs collected from adult quail dietarily exposed to crude oil for at least 21 consecutive days (3 weeks; **Figure 1**). Feeding with control or crude oil-treated food continued until the seventh week of the experiment when the desired number of F₁ offspring was obtained. None of the dietary treatments induced any mortality in the experimental P₀ groups.

Assessments of Parental Population (P₀)

Body mass (g) was measured by placing each bird on a top pan balance (Mettler Toledo, XA105 DualRange, Columbus, United States). Body mass was recorded at the end of the acclimation period, on weeks 1, 2, 3 5, and 7 and every time that oxygen consumption measurements were made.

Respirometry

Measurements of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) of all adult quail were recorded at the end of the acclimation period, before any dietary exposure, and on weeks 1, 2, 3 5, and 7 after the start of the exposure. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured by flow-through respirometry as previously reported (Amaral-Silva et al., 2020; Sirsat and Dzialowski, 2020). In brief, individual adult quail were placed inside 500 mL opaque respirometers that were maintained in an incubator to control temperature at 24 ± 0.5°C. All individuals were allowed to acclimate to the respirometer for at least 30 min before measurements, during which time a flow of humidified air was maintained through the respirometer. Up to three respirometers were operated simultaneously and individuals from different experimental groups were randomized to minimize effects due to any variation between respirometers. A gas mixture of 20.8–21.2% O₂, balanced with N₂ generated



with a gas mixer (Model 0154 Brooks Instruments, Hatfield, PA, United States) was delivered as the inflow gas to each respirometer and maintained at a flow rate of $\sim 370 \text{ mL} \cdot \text{min}^{-1}$. The flow of the gas mixture was controlled with a flow controller Model 5850E (Brook Instruments Hatfield, United States) and measured with a mass-flow meter FlowBar1 (Sable Systems, Las Vegas, United States). The gas from the outlet of the respirometers was passed through Drierite (Sigma Aldrich, St. Louis, United States) to absorb any humidity before gas analysis and then pulled through the gas analyzers using a flow controller

R1 (AEI Technologies, Bastrop, TX, United States). O_2 and CO_2 concentrations in outflow respirometer gas were measured using a FC-1B O_2 and a CA-10 CO_2 analyzer, respectively, (Sable Systems Las Vegas, United States). Sequential individual sampling of the respirometers was performed for five min using a custom built Arduino based four-channel solenoid multiplexer controlled by LabChart V.7 (ADIInstruments, Colorado Springs, United States). Respirometry data were calculated and recorded using LabChart V.7 and Powerlab 16SP (ADIInstruments, Dunedin, New Zealand). Two or three measurements per

individual quail were averaged to generate $\dot{V}O_2$ and $\dot{V}CO_2$ values used for analysis.

Cloacal temperature (T_b) of each quail was immediately determined after respirometry measurements using a silicon-coated thermocouple inserted approximately 2 cm into the bird's cloaca. The birds were checked for any signs of injury (not observed in any bird) and returned to their respective housing containers.

$\dot{V}O_2$ and $\dot{V}CO_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$ at standard temperature and pressure in dry conditions, STDP) were calculated with equations Eq. (1) and Eq. (2), respectively, (Lighton, 2018):

$$\dot{V}O_2 = \dot{V}_I * \left(\frac{(F_I - O_2 - F_E O_2) - F_E O_2 (F_E CO_2 - F_I CO_2)}{(1 - F_E O_2)} \right)$$

$$\dot{V}CO_2 = \dot{V}_I * \left[\left(\frac{F_E CO_2 * (1 - F_I O_2 - F_I CO_2)}{(1 - F_E O_2 - F_E CO_2)} \right) - F_I CO_2 \right]$$

where \dot{V}_I = incurrent flow rate ($mL \cdot min^{-1}$), $F_I O_2$ = incurrent O_2 fraction of dry gas, $F_I CO_2$ = incurrent CO_2 fraction of dry gas, $F_E O_2$ = excurrent O_2 fraction of dry gas, and $F_E CO_2$ = excurrent CO_2 fraction of dry gas. After calculating $\dot{V}O_2$ and $\dot{V}CO_2$, the respiratory exchange ratio (RER) was calculated as $\dot{V}CO_2/\dot{V}O_2$.

Pulmonary ventilation (\dot{V}_E) was estimated by plethysmography, using a FD141 pressure transducer-based spirometer (ADI Instruments Dunedin, New Zealand) connected to each respirometer. Breathing frequency (f , breaths $\cdot min^{-1}$) was determined with the cycle measurements function of LabChart 7 based on the changes in the pressure waves of the recordings. Tidal volume (V_T) was calculated as previously reported (Drorbaug and Fenn, 1955; Amaral-Silva et al., 2020; Sirsat and Dzialowski, 2020). Volume calibration of the system was performed by comparison with changes in pressure produced by injection of 250 μ l volumes of air into the chambers using a Hamilton glass syringe (Hamilton, Reno, United States; Szdzuy and Mortola, 2007). Relative humidity in the chambers was determined with a humidity sensor (HIH 4021, Honeywell, Minneapolis, United States). Minute ventilation (\dot{V}_E) was then calculated from tidal volume and breathing frequency as $\dot{V}_E = V_T * f$. Air convection requirement was calculated as $\dot{V}_E/\dot{V}O_2$.

Blood and Organ Sampling

At the end of the crude oil exposure period, adult quail were initially anesthetized by placing them into a sealed container with a cotton ball soaked with isoflurane. Anesthetized birds were placed in a dissection tray, and the head of the bird was inserted into a custom -built mask connected to a vaporizer to maintain constant flow of isoflurane (1.5% in air) vapors to maintain light anesthesia. An incision was made at the top of the breast area to obtain an unobstructed view of the heart under the sternum. Blood sampling and analysis was performed as described elsewhere (Tazawa et al., 2002). Briefly, blood samples of $400 \pm 50 \mu$ l were obtained directly from the right brachiocephalic artery using a one ml syringe previously heparinized. Sampled blood was placed in sterile 1.5 mL centrifuge tube. Blood collection was performed within a 1-min sampling period. Immediately after collection, blood samples were processed and

analyzed, in the following sequence: (a) 120 μ l of blood were analyzed for pH, partial pressure of oxygen (PO_2 , mmHg), partial pressure of carbon dioxide (PCO_2 , mmHg), blood oxygen saturation (SO_2 , %), and bicarbonate concentration ($[HCO_3^-]$, mmol/L) using a Radiometer blood gas analyzer ABL5 (Diamond Diagnosis, Holliston, United States); (b) 10 μ l were used to estimate mean corpuscular hemoglobin concentration ($[MCHb]$, g%) using an hematology analyzer (Beckman Coulter *diff*, Indianapolis, United States); (c) 10 μ l were used to estimate osmolality with a vapor pressure osmometer (Vapro 5520, EliTech, Puteaux, France); (d) 10 μ l were used to estimate lactate concentration (mmol/l) with a lactate meter (Nova biomedical – 39654, Waltham, United States); (e) and 140 μ l of blood were used to measure hematocrit (Hct, %) in duplicate using 70 μ l capillary tubes (Thermo Fisher Scientific, 22-362-566 Waltham, United States) and spun for 5 min in a micro hematocrit centrifuge (LW Scientific M24, Lawrenceville, United States). With the exception of hematocrit, all measurements were obtained within 2 min after blood was sampled.

Immediately after blood extraction, the deeply anesthetized birds were euthanized by decapitation. The major organs (brain, eyes, lungs, heart, liver, spleen, kidneys, stomach and intestines, and gonads) were excised and weighed to the closest mg using a micro balance (XA105 DualRange, Mettler Toledo, Columbus, United States).

Assessments of Offspring Population (F_1) Egg Variables

All adult housing containers were daily checked for newly laid eggs 2 h after the beginning of the light cycle during the morning and 2 h prior to the dark cycle during the evening. Collected eggs were labeled and immediately weighed on a digital top-loading balance (Symmetry EC-series, Cole Parmer, Vernon Hills, IL, United States) to the closest mg. Egg length and width were obtained to the nearest mm using a digital caliper. Egg mass, length, and width were determined throughout the experiments. Offspring eggs obtained from each parental group (control, Low HEWAF and High HEWAF diets) were divided into one of three different experimental protocols (**Figures 1B,C**).

Egg Water Loss

The first egg group (**Figure 1C.1**) was used to measure water loss (W_{loss} , mL/day) through the eggshell, determined by measuring the egg mass changes, representing water loss, over time. Mass of the intact egg was determined immediately after collection, and then the egg was immediately placed into a sealed 50 mL Falcon tube filled with the desiccant Drierite (Sigma Aldrich St. Louis, United States) up to the 25 mL mark and the egg was left undisturbed for 24 h. After this period, water loss throughout the eggshell was calculated as the difference in egg mass before and after the 24 h desiccation period (Paganelli, 1980; Ackerman and Rahn, 1981; Spotila et al., 1981).

F1 Hatchling Respirometry

The second group of eggs was used in metabolic measurements. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured in 7–8 dph old F1 hatchlings to determine if parental dietary crude oil exposure had an effect

on offspring metabolic traits. $\dot{V}O_2$ and $\dot{V}CO_2$ were determined in the F_1 under a cooling protocol to assess if physiological variables associated with thermogenic capacities were affected by the parental exposure. F_1 quail offspring from the three parental populations were deprived from food 1 h prior to start of the $\dot{V}O_2$ measurement. Each hatchling was individually placed into a ~ 250 ml metal respirometer with an inlet and outlet port affixed in the lid of the chamber. Respirometer temperature was controlled by placing the respirometer into a $37^\circ C$ incubator and the birds were allowed to acclimate within the respirometer for a 45 min period. Body temperature (T_b) was recorded during measurements using a silicon coated thermocouple adapted with a plastic base that facilitated attachment to the feathers, passing through the lid of the chamber, inserted into the hatchling's cloaca (~ 1 cm), and glued to the surrounding feathers. $\dot{V}O_2$ and $\dot{V}CO_2$ were estimated using the same equipment arrangement as for the parental population described above. After the acclimation period, chamber temperature was gradually decreased at a rate of $3^\circ C$ every 30 min (Swanson and Liknes, 2006), until reaching $19^\circ C$. Two – three measurements per individual were obtained at each temperature step and averaged for analysis. Additionally, pulmonary ventilation (\dot{V}_E), f , V_T and air convection requirement for the F_1 hatchling groups were calculated as described above for the parental populations. After $\dot{V}O_2$ and $\dot{V}CO_2$ measurements were finished, the hatchlings were deeply anesthetized with isoflurane by placing them into a sealed container, and then decapitated.

F₁ Hatchling Blood and Organ Sampling

The third group of eggs was incubated and maintained until 7–8 dph as described above (Figure 1C). At this stage, the hatchlings were anesthetized by placing them into a sealed container with a cotton ball soaked with isoflurane. Anesthetized hatchlings were placed in a dissection tray, and the head of the bird was inserted into a custom -built mask connected to a vaporizer to maintain constant flow of isoflurane (1.5% in air) vapors to maintain anesthesia. Similar to the parental population, an incision was made at the top of the breast area to obtain an unobstructed view of the heart under the sternum and blood was withdrawn from the brachiocephalic artery using a previously heparinized syringe. Hematological variables were measured as described above for the parental population. Because of the small size of the animals (~ 8.1 g) the maximum blood sample size was 300 μ l. After blood sampling, the offspring quail were immediately decapitated while still under anesthesia and all major organs were excised, weighed and fixed. An attempt was made to determine the sex of each hatchling during dissection of the organs, however, the stage gonadal development in 7–8 dph king quails do not allowed for determination of the sex with 100% certainty, therefore, hatchlings sex was not considered in statistical analysis.

Statistical Analysis

Parental Population

Statistical analysis and graphing were performed on R software version (4.0.3) and Rstudio (1.4.1106), and Prism V.8 (GraphPad), respectively. Normality of the residuals was interpreted from graphical analysis and from statistical output

of Shapiro–Wilk test. Homogeneity of variance in residuals was analyzed with Levene's homogeneity test. Adult body mass and T_b were compared among groups using a general linear model (GLM) from the lme4 R package, in which experimental group (Control, Low, or High exposure level), sex, and experimental period (time in weeks) were used as fixed effects. Similarly, $\dot{V}O_2$, $\dot{V}CO_2$, RER, \dot{V}_E , V_T , f , and $\dot{V}_E/\dot{V}O_2$ were compared among groups using a similar GLM model, however, body mass and T_b of the quail were considered as covariates.

Hematological variables of the parental population were compared between groups with GLM model using experimental group and the sex as fixed effects, and considering body mass and body temperature as covariates. Similarly, organ masses were analyzed with a similar mode as for blood variables, but T_b was not considered as covariate. Organ mass data were expressed as percentage of body mass.

Offspring Population (F₁)

Egg mass, egg length, and egg width, were compared among F_1 offspring groups with GLM model considering experimental group and experimental time as fixed effects. Egg W_{loss} was compared between groups considering experimental group and experimental time as fixed effects and initial egg mass as covariate.

Body mass of hatchling quails was compared between experimental groups considering week of collection as random effect. T_b , $\dot{V}O_2$, $\dot{V}CO_2$, RER, \dot{V}_E , V_T , f , and $\dot{V}_E/\dot{V}O_2$ were analyzed using repeated measurements mixed effects model considering experimental group and cooling temperature as fixed effects, body mass as covariate, and week of collection as random effect.

Hematological variables in offspring population were compared between groups considering body mass and body temperature as covariates and week of collection as random effect. Finally, organ masses in the offspring generation were compared among groups considering body mass as covariate and week of collection as random effect.

The *a posteriori* Tukey's honest significant difference (HSD) test was used to determine mean differences. In addition, when one specific factor induced significant effects on the analyzed variable, all pairwise multiple comparisons were performed with Holm-Sidak procedure to explore differences between the different levels within the factor. Statistical significance was considered with a p value ≤ 0.05 and α value of 0.05. Data are expressed as mean \pm s.e.m, unless otherwise stated.

RESULTS

Parental Population

None of the dietary treatments induced any mortality in the experimental groups. Moreover, we did not observe changes in appetite or notice any difference in the behavior of the birds. However, the experimental group fed with the High dietary treatment diet exhibited moderate diarrhea.

Adult Body Mass

Crude oil exposure in either amount had no significant effect ($P > 0.05$) on adult body mass for either males (range 44.5 to 45.8 g) or females (range 58.7 to 63.8 g) across all experimental weeks. However, body mass was significantly lower in males compared to females at all measurement points ($P < 0.001$).

Adult Body Temperature (T_b)

Similarly to body mass, T_b of the adult populations, which averaged $40.7 \pm 0.18^\circ\text{C}$, was also not significantly different between sexes in any groups ($P = 0.193$).

$\dot{V}O_2$ and $\dot{V}CO_2$ in the P_0 Adults

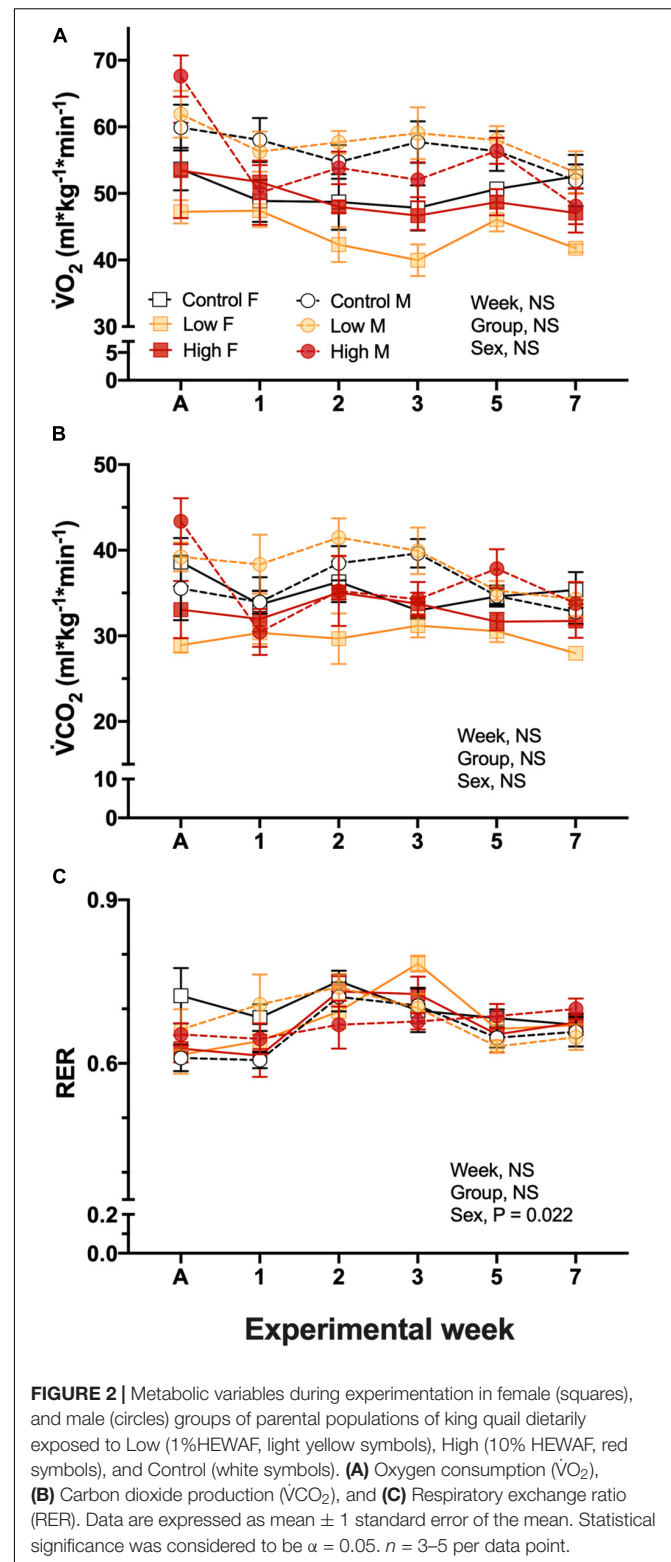
Resting $\dot{V}O_2$ in the parental populations was $\sim 50\text{--}60$ mL $O_2 \cdot \text{kg} \cdot \text{min}^{-1}$, and was not significantly affected by experimental dietary treatment, quail sex, nor experimental week, and there was not significant interactions among the factors (Figure 2A). $\dot{V}O_2$ was not related to T_b , however, it was significantly related to body mass of the quail ($P = 0.0005$). Similar to $\dot{V}O_2$, none of the considered factors affected $\dot{V}CO_2$ values and were maintained in a range between 28.9 ± 0.9 and 41.5 ± 2.2 mL/kg/min, similarly to $\dot{V}O_2$ this variable was also significantly related to body mass ($P = 0.0015$, Figure 2B).

Respiratory exchange ratio, RER calculated by the ratio of $\dot{V}CO_2/\dot{V}O_2$, ranged between 0.61 ± 0.04 and 0.75 ± 0.02 and no interactions were found among the factors. Additionally, neither exposure treatment nor experimental period had an effect on this variable, however, the sex of the quail had an individual effect ($P = 0.022$). RER varied with experimental week, but no clear pattern of change emerged (Figure 2C).

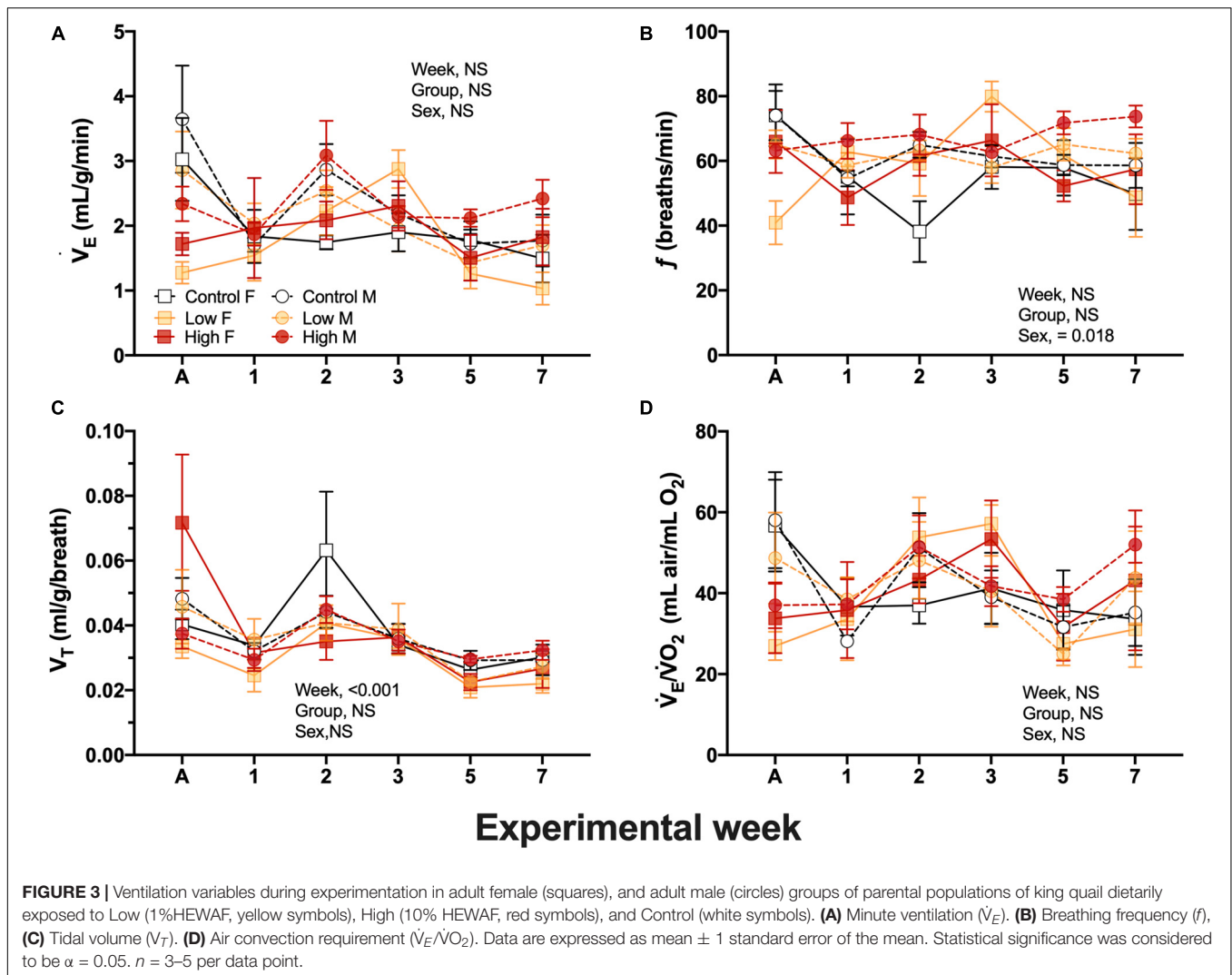
\dot{V}_E and the air convection requirement did not differ among experimental groups, the sex of the quail, nor experimental time and ranged between 2.01 ± 0.52 and 1.45 ± 0.23 mL/g/min in female, and between 2.94 ± 0.37 and 1.96 ± 0.22 mL/g/min in males with no clear trend in the results (Figure 3A). Breathing frequency, f , did not differ between experimental groups and ranged from 63.4 ± 2.2 to 73.7 ± 3.4 breaths/min in males and 40.9 ± 6.7 to 79.8 ± 4.7 breaths/min in females during experimentation (Figure 3B). f did not significantly differ between sex ($P > 0.05$). Similarly, tidal volume did not significantly vary between the sex of the quail ($P > 0.05$) and dietary treatment ($P > 0.05$), but V_T varied significantly with experimental week ($P < 0.018$) without clear trend (Figure 3C). V_T values ranged from 0.048 ± 0.011 to 0.026 ± 0.002 mL/g/breath in female and in 0.043 ± 0.003 to 0.029 ± 0.001 mL/g/breath in male quail (Figure 3C). Similarly to V_T , the air convection requirement did not varied based on dietary treatment, sex, week, or neither of the covariates. $\dot{V}_E/\dot{V}O_2$ averaged 40.4 ± 1.7 mL air/g/mL O_2 across experimental time (Figure 3D).

Adult Blood Chemistry and Hematology

Results of the different blood variables measured are summarized in Table 1. Dietary exposure to crude oil induced significant changes in three of the measured blood parameters. The Control group, both female and male birds, exhibited the lowest PCO_2 mmHg values in their blood (24.5 ± 0.3 and 27 ± 1.4



for male and female, respectively) when compared to the Low (27.4 ± 2.3 , 27.6 ± 1.6) and High oil (27.4 ± 2.3 , 31.2 ± 1.5) exposed groups ($P < 0.008$). Arterial blood PO_2 was also affected by the dietary treatment, with the Low and High oil-exposed



groups exhibiting the higher and lower values, respectively, (88.4 ± 1 and 85.8 ± 1 , $P < 0.02$). Oxygen saturation values from oil-exposed groups were significantly lower ($\sim 96\%$, $P = 0.0003$) in comparison to the Control group ($\sim 97.5\%$). With exception of Hb (10.7 ± 0.2 g%) and Hct (31.6%) values, where female quail from the High oil-exposed group exhibited significantly lower values in comparison to males, the rest of the measured blood chemistry variables ($[\text{HCO}_3^-]$, OSM, Lactate, and pH) were not significantly different (Table 1).

Organ Mass in the P_0

Organ masses, expressed as percentage of body mass, are presented in Table 2. Heart mass was smaller in female and male quail in both oil-exposed groups compared to the Control group (Table 2). Kidney mass was higher in the Low-oil exposed group in comparison to the Control and the High-oil exposed group and differed between sex in all experimental groups (Table 2). Liver, digestive tract and ceca masses were not affected by oil exposure, but differences between sexes were found within all experimental groups in which female quail exhibited higher masses of these

organs in comparison to males (Table 2). These three variables were related with body mass. Brain mass as a percentage of body mass was also not affected by exposure to crude oil, but in female quail the values were smaller in comparison to the male groups in all three experimental groups. Lungs and eye mass did not differ neither between groups nor on the basis of sex within treatments (Table 2).

Offspring Population (F_1)

Egg Variables

Egg mass obtained from the Low and High concentration groups exhibited higher values (5.2 ± 0.4 , 5.1 ± 0.8 g, respectively) in comparison with the eggs from the Control parental group (4.8 ± 0.7 g, $P = 0.047$). These egg mass differences were present from the beginning of the experimental week and were constant throughout all the experiments. Thus, they cannot be attributable to the dietary parental oil exposure (Figure 4A). Egg length (2.5 ± 0.012 cm) and egg width (1.9 ± 0.011 cm) were not significantly different among experimental groups (Figures 4B,C). Egg water loss did not differ between groups

TABLE 1 | Blood chemistry and hematology in the parental adult populations (P₀), and in the first generation offspring populations (F₁) of king quail.

	Group	Parental adult population (P ₀)						Offspring population (F ₁)		
		Control		Low oil diet		High oil diet		Control	Low oil diet	High oil diet
		Female	Male	Female	Male	Female	Male			
		<i>n</i>	4	4	3–5	5	5	4–5	16–17	20–22
Arterial blood chemistry	pH	7.42 ± 0.02	7.46 ± 0.04	7.45 ± 0.02	7.43 ± 0.01	7.41 ± 0.02	7.45 ± 0.01	7.43 ± 0.04	7.43 ± 0.02	7.48 ± 0.02
	PCO ₂ (mmHg)	24.5 ± 0.3^a	27 ± 1.4^a	27.4 ± 2.3^b	27.6 ± 1.6^b	31.2 ± 1.5^c	32.4 ± 0.9^c	39.5 ± 3.7	36.2 ± 3.3	31.5 ± 0.8
	PO ₂ (mmHg)	88.3 ± 0.5^{ab}	87 ± 1.1^{ab}	88.4 ± 1.0^a	88.8 ± 0.7^a	85.8 ± 1.0^b	85.7 ± 0.9^b	99.5 ± 4.8	99.3 ± 5.1	98.9 ± 3.2
	SO ₂ (%)	98 ± 0.4^a	97 ± 0.4^a	96.6 ± 0.7^b	95.8 ± 0.9^b	94.6 ± 0.2^b	95.2 ± 0.5^b	95.5 ± 1.8	94.0 ± 2.6	97.2 ± 0.5
	HCO ₃ ⁻ (mmol/L)	23.2 ± 0.5	22.2 ± 1.8	23.8 ± 0.6	22.4 ± 1.0	21.6 ± 0.7	22.8 ± 1.0	22.4 ± 1.1	21.7 ± 0.5	24.5 ± 0.7
Hematology	OSM (mmol/Kg ⁻¹)	305.5 ± 1.4	306 ± 1.4	307 ± 3.2	303.8 ± 0.9	305 ± 1.5	302.4 ± 2.9	297 ± 2.8	298.8 ± 3.2	291.3 ± 2.1
	Lac (mmol/L)	3.3 ± 0.6	3.3 ± 0.6	3.7 ± 1.3	3.94 ± 0.6	4.5 ± 0.6	3.3 ± 0.6	4.1 ± 0.6	4.7 ± 0.5	5.5 ± 0.9
	Hct (%)	31.8 ± 3.1	33.9 ± 2.9	36.7 ± 1.9	37.7 ± 1.7	*31.6 ± 1.0	40.9 ± 2.4	29.2 ± 2	32.9 ± 0.7	32.3 ± 0.8
	Hb (g%)	11.8 ± 0.6	13.2 ± 0.6	12.2 ± 0.4	11.6 ± 0.5 ^a	*10.7 ± 0.2	13.5 ± 0.6	10.3 ± 0.3	10.5 ± 0.2	10.4 ± 0.3

Different superscript letters indicate significant differences among experimental groups. “*” in Hct and Hb in the High oil diet group of the parental population indicates significant differences between sex. Significantly different values are also indicated in bold.

TABLE 2 | Organ masses (% of body mass) in the parental adult population (P₀), and in the offspring population (F₁).

Organ/n	Adult parental generation (P ₀)						Offspring population (F ₁)		
	Control		Low oil diet		High oil diet		Control	Low oil diet	High oil diet
	Female	Male	Female	Male	Female	Male			
	4	4	5	5	5	5	8	11	10
Heart	0.74 ± 0.05^a	0.9 ± 0.05^a	0.65 ± 0.06^b	0.7 ± 0.04^b	0.68 ± 0.04^b	0.7 ± 0.02^b	1.2 ± 0.04	1.2 ± 0.04	1.1 ± 0.03
Liver	*3.05 ± 0.42	1.84 ± 0.1	*2.9 ± 0.18	1.92 ± 0.07	*3.02 ± 0.16	1.86 ± 0.11	4.3 ± 0.4	4.4 ± 0.2	4.7 ± 0.3
Lungs	0.87 ± 0.11	0.8 ± 0.07	0.74 ± 0.06	0.88 ± 0.06	0.68 ± 0.03	0.74 ± 0.07	0.7 ± 0.04	0.8 ± 0.04	0.8 ± 0.02
Gut	*2.38 ± 0.16	1.77 ± 0.09	*2.48 ± 0.28	2.18 ± 0.35	*2.7 ± 0.15	1.65 ± 0.18	5.7 ± 0.9	6.3 ± 0.4	6.3 ± 0.5
Eyes	0.53 ± 0.05	0.97 ± 0.12	0.65 ± 0.05	0.8 ± 0.02	0.57 ± 0.02	0.81 ± 0.04	2.5 ± 0.2	2.3 ± 0.12	2.2 ± 0.1
Brain	*0.84 ± 0.11	1.11 ± 0.04	*0.77 ± 0.03	1.03 ± 0.03	*0.73 ± 0.02	1.01 ± 0.03	3.3 ± 0.2	3.2 ± 0.2	2.9 ± 0.1
Ceca	*0.43 ± 0.02	0.4 ± 0.03	*0.82 ± 0.1	0.64 ± 0.15	*0.48 ± 0.06	0.37 ± 0.03	1.0 ± 0.04	1.0 ± 0.08	1.1 ± 0.03
Kidneys	*0.9 ± 0.08^a	0.67 ± 0.04^a	*1.03 ± 0.08^b	0.71 ± 0.03^b	*0.99 ± 0.03^a	0.62 ± 0.02^a	1.3 ± 0.2	1.1 ± 0.08	1.3 ± 0.2

“*” indicates differences between sex within the adult experimental groups. Significantly different values are indicated in bold font and with different superscript letters.

during the first 4 weeks of exposure (Figure 4D). However, at week five the eggs from the Control parental group exhibited lower water loss volumes (0.04 ± 0.004 mL/day) in comparison with eggs obtained from Low and High-exposed groups (0.05 ± 0.003 , and 0.051 ± 0.003 mL/day). This difference was also observed at the last week of experimental time (Figure 4D).

Body Mass

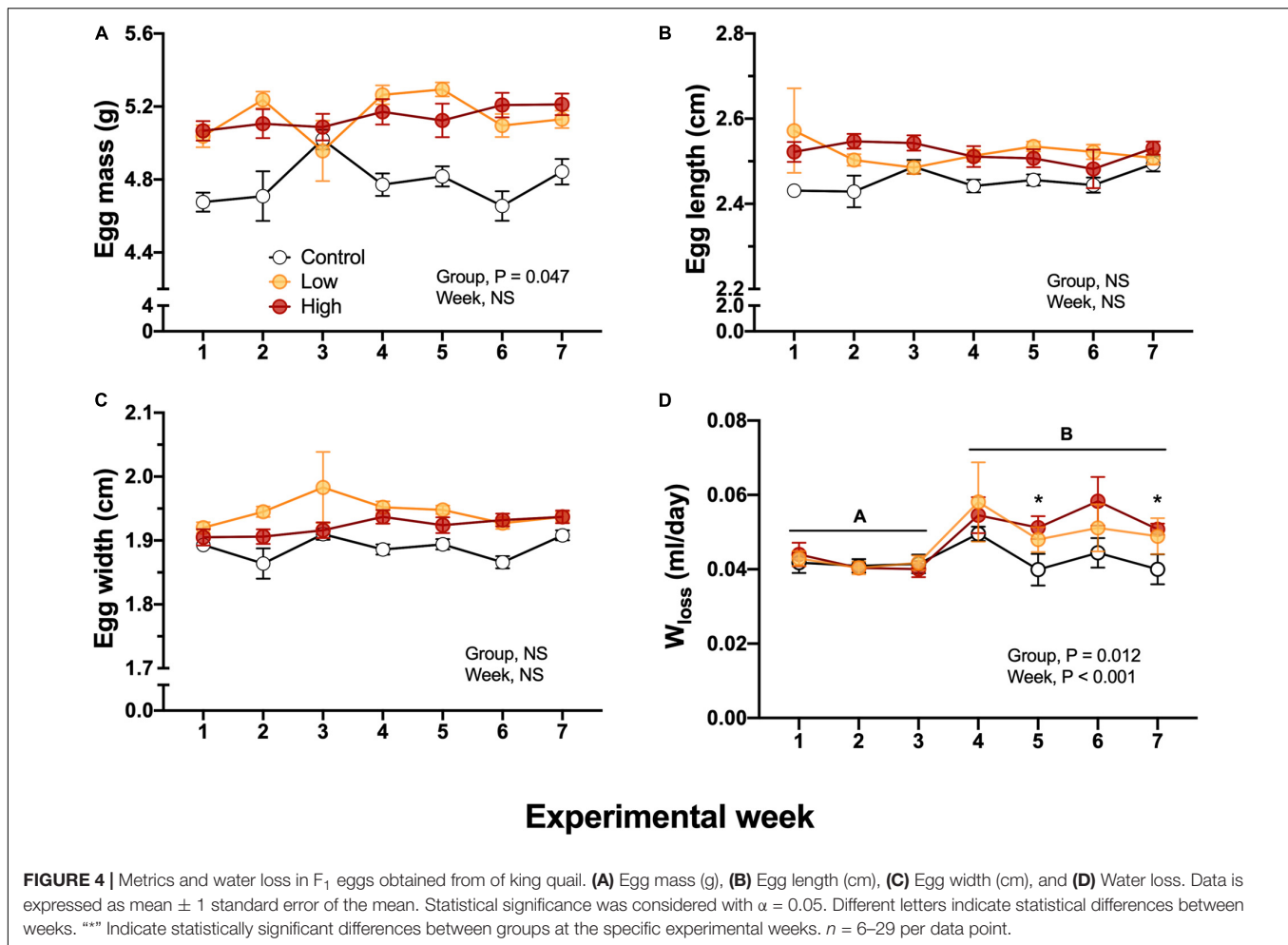
Body mass of 7–8 days old hatchlings did not differ among experimental groups ($P = 0.287$), including when week of collection was used as random effect ($P = 1.0$). Body mass averaged 7.9 ± 0.27 , 8.2 ± 0.24 , and 8.6 ± 0.25 g for Control, Low and High groups.

Body Temperature and Metabolism

Body temperature of hatchlings was not related with body mass and the High-oil-exposed P₀ exhibited lower values in

comparison with the Low or the Control groups (Figure 5). This difference became evident when ambient temperature was reduced to 25°C and was maintained until the end of the experimental test. Similarly to body mass, there were no significant differences ($P = 1.0$) when week of collection was used as a random effect.

Parental experimental dietary exposure to Low or High levels of oil did not affect $\dot{V}O_2$ of the F₁ offspring during the cooling challenge (Figure 6A). However, the decrease in temperature induced significant increases in oxygen consumption at 25 and 22°C in the F₁ quail in general (Figure 6A). Although $\dot{V}CO_2$ followed a similar trend as $\dot{V}O_2$ (Figure 6B), the decrease in temperature did not significantly affect this variable ($P > 0.05$). Both of these variables were significantly associated with body mass of the hatchlings. Additionally, RER was not affected by parental exposure, but did change overall as a function of temperature, generally decreasing as the temperature of



the challenge was decreased (Figure 6C). There were also no significant differences ($P > 0.05$) when week of collection was used as a random effect.

Ventilatory Variables and Body Temperature

Breathing frequency, f , was unaffected by diet or by ambient temperature (Figure 7A). While not affected by parental diet, V_T increased during the cooling challenge, reaching a value more than 600% higher than at 37°C (Figure 7B). Unsurprisingly, then \dot{V}_E also increased from 3.48 ± 0.43 to 23.92 ± 1.49 mL/g/min, a nearly 700% increase. This sharp rise in \dot{V}_E occurred equally in all groups until 22°C, but at 19°C \dot{V}_E was lowest in the Control group and highest in the High exposure group (Figure 7C). Finally, air convection requirement followed a similar trend to \dot{V}_E , significantly and sharply increasing at 19°C (Figure 7D). V_T , \dot{V}_E and $\dot{V}_E/\dot{V}O_2$ were significantly associated with body mass ($P < 0.05$). No significant differences ($P > 0.05$) emerged when the week of collection used as random effect.

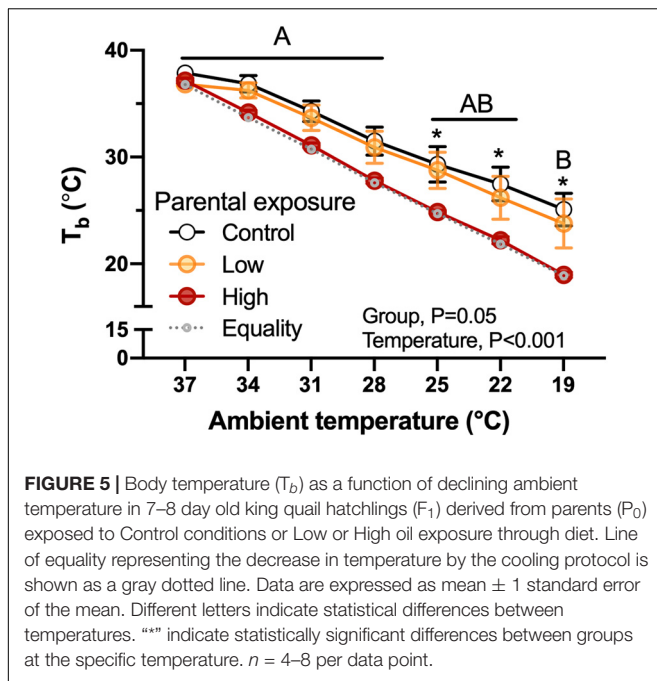
Blood Chemistry and Hematological Variables and Organ Masses

None of the blood chemistry or hematological variables measured in the offspring population varied significantly between

experimental groups (Table 1). With exception of PO_2 and PCO_2 values that were associated with Tb of the hatchling, none of the variables were associated with the covariates for any of the hematological variables (the random effect of week of collection was not statistically significant, $P > 0.05$), with the exception of Osm ($P < 0.002$). Noteworthy is the fact that the values obtained in this study for the king quail fall within the ranges previously reported for hatchlings of the bobwhite quail (*Colinus virginianus*), the common quail (*Coturnix coturnix*), and the chicken (*Gallus gallus*; Burgren and Elmonoufy, 2017; Flores-Santin et al., 2018; Amaral-Silva et al., 2020). Similarly, none of the organ masses significantly differed between hatchlings from the Control, Low or High oil-exposed parental groups (Table 2) and, for none of the organ masses when the random effect of week of collection was included, with the exception of gut mass ($P < 0.002$).

DISCUSSION

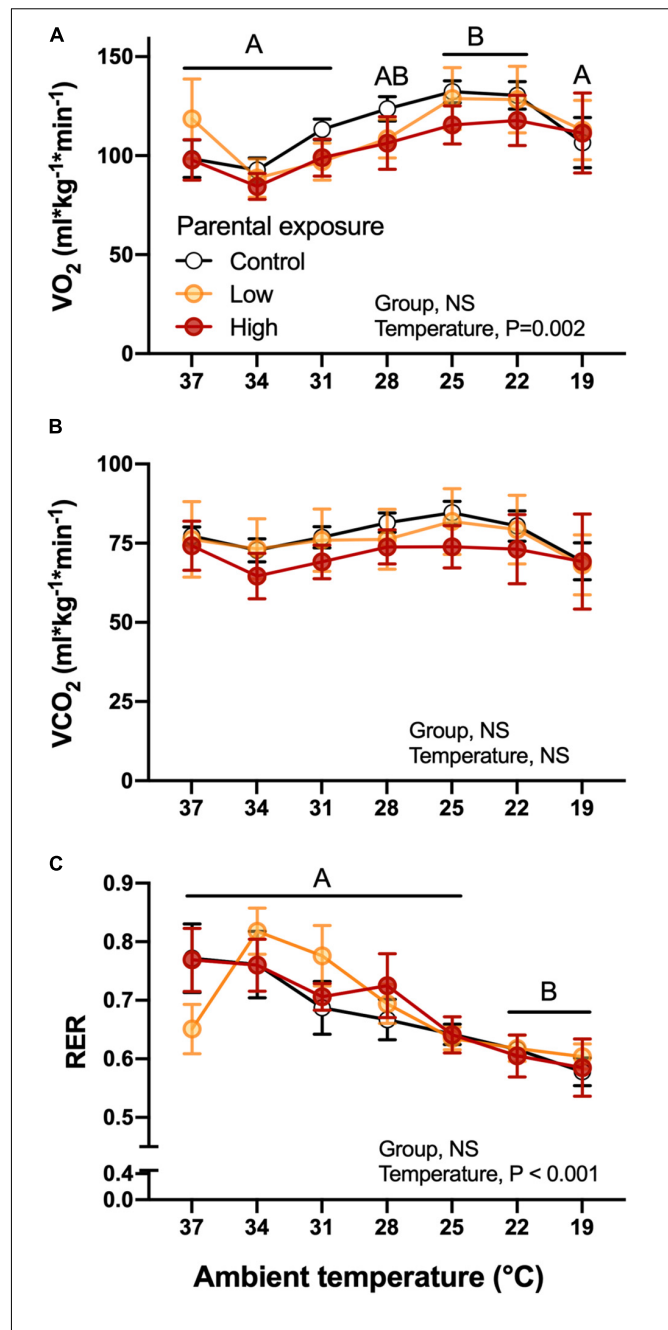
Dietary oil exposure induces severe effects on directly exposed adult bird populations. Compounding the problem, oil spills may also coincide with the breeding season of numerous bird species,



as was the case for the DWH oil spill (Bernardo, 1996; Vidal et al., 2011; Horak et al., 2017; Perez et al., 2017; Harr et al., 2017a,b). Exposed adult populations have exhibited reductions of clutch size and egg abandonment (Azkona et al., 2006). Thinning of the eggshell was also reported in mallard ducks (Stubblefield et al., 1995). Direct oil exposure on bird eggs has been reported to lead to increased mortality in chickens and gulls (Leighton, 1993). Reduced hatching success has also been reported in mallard ducks (Albers, 1977; Szaro et al., 1980). Along with these effects, crude oil exposure leads to heart, liver, and spleen enlargement in bird embryos (Couillard and Leighton, 1989, 1991). However, beyond the effects that direct exposure to crude oil induce in adult and young bird populations, oil exposure in adults during breeding season could potentially lead to transgenerational effects in their offspring generations. In the present study, we explored whether P_0 exposure led to phenotype change in their immediate F_1 generation that was itself was not exposed to crude oil, by measuring morphological and respiratory-related variables in eggs and hatchlings obtained from the oil-exposed parental populations. To the authors' knowledge, this is the first study investigating whether crude oil exposure in a P_0 generation could influence offspring phenotype in birds.

Direct Effects of Crude Oil Exposure in the P_0 Parental Generation

Exposure to crude oil in avian species induces life-threatening effects. Dermal exposure to crude oil leads to impairment of thermoregulatory capacities, decrease in buoyancy and changes in navigational abilities in cormorants, gulls, and western sandpipers (Maggini et al., 2017a; Harr et al., 2017c). In addition, dietary exposure to crude oil induced loss of body mass, loss of appetite, loss of weight, and hepatic and renal diseases



in sanderlings and semipalmated plovers, cormorants, and American kestrels (Pattee and Franson, 1982; Burger, 1997; Harr et al., 2017a,b). In contrast, in the present study neither of the dietary treatments induced mortality in the experimental groups.

The actual concentration of oil load may have contributed to these differences. Noteworthy is that at the concentration levels of crude oil used in this study, which are lower than concentrations used in other studies (for a review see King et al., 2020), we did not observe changes in appetite or loss of body mass in the exposed groups. However, we did observe diarrheic feces in the High-oil-exposed group beginning on the third week of exposure, suggesting a potential disruption of the hepatic function and the digestive tract integrity (Leighton, 1986). Our observations align with previously reported results where watery diarrhea was observed in cormorants and gulls after dietary exposure to crude oil exposure (Leighton, 1986; Cunningham et al., 2017; Harr et al., 2017a).

Studies investigating the effects of crude oil exposure on the oxygen consumption and carbon dioxide production in adult birds are scarce and have been overshadowed by those focusing on the effects of thermoregulatory variables. In the present study $\dot{V}O_2$ and $\dot{V}CO_2$ were not affected by any of the dietary crude oil treatments in the adult populations. These results align with the results previously reported in nestling adult Storm-petrels (*Oceanodroma leucorhoa*; Butler et al., 1986). Respiratory distress due to crude oil exposure, exhibited as changes in ventilation rates, has been reported previously in birds (Mazet et al., 2002). In contrast, we found no differences induced by the dietary oil treatments in any measured ventilatory variables (Figure 2). This result suggests that the levels of dietary crude oil exposures used did not compromise resting metabolic rates at the organismal level. However, our measurements were performed in standard controlled conditions (i.e., room temperature, food, and water *ad libitum*) where the adults birds were not exposed to additional stressors beyond oil exposure. Consequently, possible alterations in metabolic rates induced by dietary oil exposure could occur when the animals face constantly changing environments such as natural daily changes of environmental temperature.

We additionally analyzed the effects of dietary crude oil exposure on blood parameters (Table 1). From the 9 measured blood chemistry and hematology variables, we found differences between experimental groups just in three of them: arterial PO_2 , PCO_2 , and SO_2 . Arterial PO_2 and SO_2 were higher in the Control group in comparison with the Low and High oil-exposed groups and PCO_2 was higher in oil-exposed groups compared with the Control group. However, interpretation of these results is complicated by the fact that blood pH, HCO_3^- , and lactate did not change (Table 1). In addition, it is widely known that anesthesia can induce respiratory depression and bradycardia in birds, however, at least to the authors knowledge, virtually nothing is known about if crude oil exposure may increase susceptibility to anesthesia, and if those effects could have been reflected in the results. Furthermore, studies implementing surgical techniques are warranted to explore these possibilities.

Additionally, we found no differences in [Hb] and Hct values between experimental groups. These results contrast with oil-induced changes in blood parameters, accompanied with hemolytic anemia, caused by ROS oxidative damage and the subsequent lysis of RBC in chickens, American oystercatchers, black skimmers, brown pelicans, cormorants, and great egrets

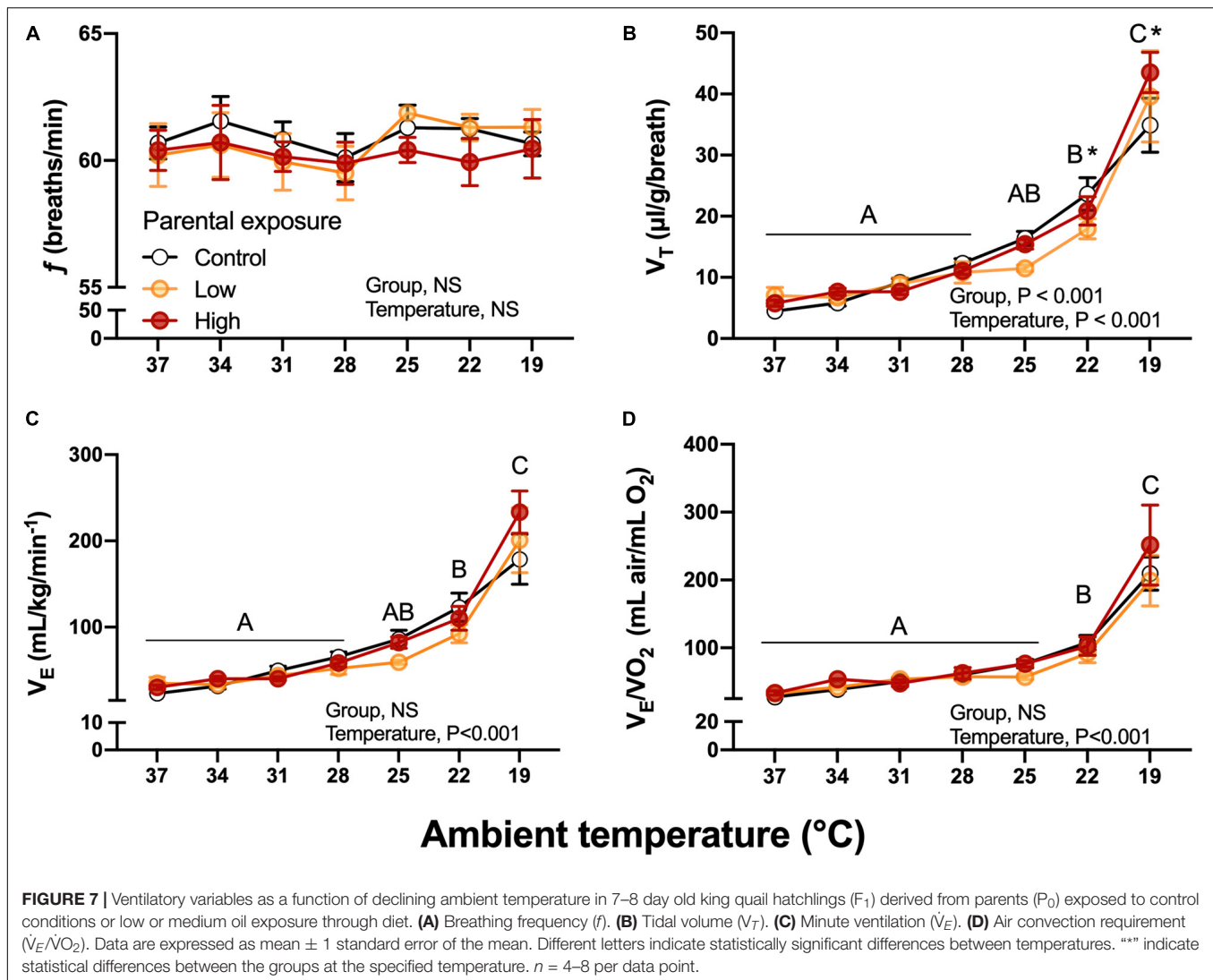
(Couillard and Leighton, 1991; Harr et al., 2017a; Fallon et al., 2018). Overall, these results suggest a possible impairment of blood oxygen transport by crude oil exposure. However, to test this hypothesis, future studies focused on determining the molecular implications of oil exposure in blood and hemoglobin, including specific mechanisms of O_2 -binding affinity and genetic control on these variables are warranted.

Heart mass of the exposed adults was smaller than in the Control group, while kidney mass was larger in the exposed groups (Table 2). The heart and the kidney were the only organs in which we observed differences between experimental groups. Crude oil exposure in laughing gulls (*Leucophaeus atricilla*) and in double crested cormorants (*Phalacrocorax auritus*) led to increased loss of structural integrity and cardiac damage (Horak et al., 2017; Harr et al., 2017b). Therefore, it is possible that the loss of heart mass observed in this study in both of the oil exposed groups could be the result of similar effects. Additionally to effects in heart mass, the decreased kidney mass in oil exposed quail observed in this study suggest that kidney tissue was damaged as reported in oil exposed Western (*Calidris mauri*), and double crested cormorants, which exhibited cell hypertrophy, including adrenal gland hypertrophy and inflammatory lesions in renal tubules/ductules (Bursian et al., 2017b; Harr et al., 2017b). Future histological studies are warranted to further explore these possibilities.

Surprisingly, in the present study we found no differences in liver mass between experimental groups. The liver is one of the most important organs for metabolic regulation and detoxification during toxicant exposure and increases in liver mass in response to crude oil ingestion have been reported previously (Dean et al., 2017; Horak et al., 2017; Harr et al., 2017b). Histopathological studies would reveal if dietary crude oil exposure at the levels used in this study induced effects at a deeper level (e.g., necrosis, neoplasia). Most of the organ masses, including that of the liver, differed between sex within the groups, a reflection of sexual dimorphism evident in body mass differences between sexes (Table 2). Overall, the results from the parental population suggest that 3 weeks of dietary exposure to specific crude oil doses is sufficient to generate significant differences in adults, helping to define further investigations. Additionally, we found inter-sex differences in the measured respirometry variables, highlighting the importance of considering sex as a factor when designing experimental protocols.

Transgenerational Developmental Effects of Crude Oil in the F₁ Generation

An organism's developmental trajectory depends upon both its own genetic instructions as well as phenotypes deriving from non-genetic inheritance related to parental environmental experiences (Burggren and Dubansky, 2018). Crude oil exposure and its components has been shown to induce transgenerational effects in fish (Corrales et al., 2014; Bautista and Burggren, 2019). However, the study of transgenerational effects of crude oil exposure in avian species has received little attention (Paiva et al., 2018; King et al., 2020). To address



this, we demonstrated some altered phenotypes related to thermoregulation, respiratory physiology, and gas exchange in non-exposed eggs and hatchlings quail obtained from the parental adult exposed groups.

Measurements of egg mass, length, and width revealed no differences between experimental groups induced by the parental dietary exposure (Figure 3). However, at the physiological level egg water loss was increased in eggs obtained from the Low and the High-level of oil-exposed parental groups when compared to controls (Figure 3D). Maintenance of humidity and temperature during egg incubation is of critical importance for eggshell quality and for the calcification process during the egg's passage through the female bird's oviduct, thus affecting survival during early development in the embryos (Yang et al., 2010; Branum et al., 2016). Our results showing modified water vapor flux across the eggshell in the High oil level exposed group suggests a functional disturbance of the female oviduct due to stress that potentially arose from dietary oil exposure. These data are aligned with reported reduction in egg production, yolk deposition,

eggshell thinning, and fertilization reported after oil exposure in several bird species (Lewis and Malecki, 1984; Leighton, 1993; Stubblefield et al., 1995; Azkona et al., 2006; Alonso-Alvarez et al., 2007). The present study did not analyze changes in eggshell thickness, nor pore size in the eggshells. However, these factors are likely to be a cause of the increased W_{loss} in eggs from High-oil-exposed adults. Furthermore, embryonic survival could be compromised in the face of fluctuating environmental conditions that challenge the maintenance of adequate humidity and embryonic oxygen consumption in the wild (King et al., 2020). Therefore, further investigations to elucidate if dietary exposure to crude oil at the levels used in this study will induce similar effects in fecundity and eggshell characteristics are warrant.

The hatchlings of all experimental quail groups exhibited a phenotype typical of precocial avian hatchlings characterized by insufficient thermal insulation to efficiently maintain metabolically generated heat, and that is reflected in the decrease in body temperature as ambient temperature decreased (Figure 5). However, the hatchlings of the High

oil-exposed parental population exhibited statistically lower body temperatures compared to the Control and the Low groups at almost all ambient temperatures. This result suggests that insulation and heat retention capacities in the F₁ offspring were potentially impaired as a result of parental oil exposure. Therefore, it is possible that natural populations in the wild may experience increased risk of mortality. This is a significant finding, because oil exposure through fouling can reduce the ability of adult birds to maintain body temperature. Future experiments will be necessary to determine if the impaired thermoregulatory abilities evident in hatchlings are retained into adulthood. Impaired thermoregulatory capacities may be result from the disruption of hormonal signaling (thyroid hormones) due to oil exposure. These effects have been reported previously in mammals (Fowles et al., 2016).

$\dot{V}O_2$, $\dot{V}CO_2$, and RER did not differ significantly at any ambient temperature or between any experimental group (Figure 7). These results suggest that the parental oil-exposure did not affect the offspring's capacity to maintain whole organismal metabolic variables that reflect the integrated function of the respiratory and cardiovascular system. Therefore, we investigated whether these results were due to the adjustment of subordinate components.

At the most challenging temperatures of the cooling experimental protocol (19–22°C), f was unaffected, but V_T and thus \dot{V}_E were significantly elevated in all populations, with hatchlings from High oil-exposed parents showing the highest increase (Figure 7). Regarding the efficiency of gas exchange, air convection requirement increased at the two lower temperatures, but was unaffected by parental history. This effect could be the result of different scenarios, for example reduced ability of the lungs to acquire oxygen at the lowest ambient temperatures. However, this possibility is contradicted the decrease in body temperature would result in the left shift of the oxygen equilibrium curve, increasing hemoglobin-oxygen binding affinity and aiding pulmonary oxygen uptake. Clearly, further studies are required to determine the mechanism underlying the decrease in gas exchange effectiveness at the lower temperature, and in particular why this was greatest in hatchlings from oil-exposed parents.

To our knowledge, this is the first study investigating how dietary crude oil exposure in the P_O influences offspring's physiological and morphological phenotypes in developing birds that, themselves, had not been directly exposed to oil. Overall, our findings of relatively few physiological effects (except involving thermoregulation) are surprising, given that embryonic developmental stages represent periods of high sensitivity to effects elicited by the parental dietary oil exposure, a characteristic also seen in other organisms (Auge et al., 2017; Burggren and Dubansky, 2018; Bautista et al., 2020). Notably, we incubated the eggs in strictly controlled conditions, and so it is possible that some effects in early hatchling development were attenuated or unnoticed. Thus, future studies that consider "wild" incubation patterns are needed to explore and reveal the possible impairment of thermogenic capacities during early embryonic development.

Finally, it is widely accepted for birds that the development of the subsequent generation is affected by the yolk components deposited by the mother (Ho and Burggren, 2010; Guerrero-Bosagna et al., 2018; Jenni-Eiermann et al., 2020). That there were no PAHs detected in the offspring eggs from the experimental parental groups in the present study suggests that there were no direct effects of PAHs in the offspring. However, it is possible that components other than the analyzed PAHs may be inherited from the mother through the yolk (Hagmayer et al., 2018). Molecular studies focused on identifying the presence and the inheritance of epigenetic markers are needed to determine the mechanism underlying the inheritance of altered phenotypes in the F₁ generation.

CONCLUSION AND FUTURE DIRECTIONS

Despite the devastating effects of external exposure to petroleum in bird populations, only a few studies have attempted to understand the effects of different routes of exposure and their consequences. Although dietary exposure to crude oil may not lead to increased mortality in the exposed birds, it may affect their physiological functions, as well as the normal development of their offspring. The present study has demonstrated that, indeed, parental exposure to crude oil via diet in the king quail may impact embryonic development of their offspring, even when they themselves have not been directly exposed to oil. To better understand the transgenerational impacts of environmental stressors such as crude oil, future studies should focus on exposure effects of the offspring throughout their life span, and also track potential phenotypic effects across multiple generations. In addition, as responses at the whole organismal level may not be complementary with molecular responses, a multi-level approach aimed to decipher the molecular basis of these effects will provide more complete answers.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://data.gulfresearchinitiative.org/data/R6.x804.000:0025>.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

NB: conceptualization, data acquisition, processing, analysis, and interpretation, performance of the experiments, drafting of the manuscript, and editing. LA-S: conceptualization, data acquisition, performance of the experiments, data acquisition and

interpretation, and manuscript editing. ED: equipment, data interpretation, and manuscript editing. WB: conceptualization, data interpretation, manuscript editing, and Funding. All authors contributed to the article and approved the submitted version.

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REFERENCES

- Ackerman, R. A., and Rahn, H. (1981). In vivo O₂ and water vapor permeability of the hen's eggshell during early development. *Respir. Physiol.* 45, 1–8. doi: 10.1016/0034-5687(81)90044-x
- Albers, P. H. (1977). "Effects of external applications of fuel oil on hatchability of mallard eggs," in *Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms*, D. A. Wolfe, and J. W. Anderson (Amsterdam: Elsevier) 158–163. doi: 10.1016/b978-0-08-021613-3.50020-6
- Alexander, C. R., Hooper, M. J., Cabela, D., Smelker, K. D., Calvin, C. S., Dean, K. M., et al. (2017). Reprint of: CYP1A protein expression and catalytic activity in double-crested cormorants experimentally exposed to deepwater horizon mississippi canyon 252 oil. *Ecotoxicol. Environ. Safety* 146, 68–75. doi: 10.1016/j.ecoenv.2017.05.015
- Alonso-Alvarez, C., Pérez, C., and Velando, A. (2007). Effects of acute exposure to heavy fuel oil from the prestige spill on a seabird. *Aquat. Toxicol.* 84, 103–110. doi: 10.1016/j.aquatox.2007.06.004
- Amaral-Silva, L., Tazawa, H., Bicego, K. C., and Burggren, W. W. (2020). Metabolic and hematological responses to endotoxin-induced inflammation in chicks experiencing embryonic 2,3,7,8-tetrachlorodibenzodioxin exposure. *Environ. Toxicol. Chem.* 39, 2208–2220. doi: 10.1002/etc.4832
- Auge, G. A., Leverett, L. D., and Edwards, B. R. (2017). Tansley insight adjusting phenotypes via within- and across- generational plasticity. *New Phytol.* 216, 343–349. doi: 10.1111/nph.14495
- Azkona, A., Zuberogoitia, I., Martínez, J., Etxezarreta, J., Iraeta, A., Castillo, I., et al. (2006). Short-term effects of the prestige oil spill on a colony of European storm-petrels *Hydrobatas pelagicus*. *Dong wu xue bao.[Acta Zoologica Sinica]* 52, 1042–1048.
- Bautista, N. M., and Burggren, W. W. (2019). Parental stressor exposure simultaneously conveys both adaptive and maladaptive larval phenotypes through epigenetic inheritance in the zebrafish (*Danio rerio*). *J. Exp. Biol.* 222:jeb208918. doi: 10.1242/jeb.208918
- Bautista, N. M., Crespel, A., Crossley, J., Padilla, P., and Burggren, W. (2020). Parental transgenerational epigenetic inheritance related to dietary crude oil exposure in *Danio rerio*. *J. Exp. Biol.* 223:jeb222224. doi: 10.1242/jeb.222224
- Bautista, N. M., Pothini, T., Meng, K., and Burggren, W. W. (2019). Behavioral consequences of dietary exposure to crude oil extracts in the Siamese fighting fish (*Betta splendens*). *Aquatic Toxicol.* 207, 34–42. doi: 10.1016/j.aquatox.2018.11.025
- Bernardo, J. (1996). Maternal effects in animal ecology. *Integr. Comp. Biol.* 36, 83–105. doi: 10.1093/icb/36.2.83
- Branum, S. R., Tazawa, H., and Burggren, W. W. (2016). Phenotypic developmental plasticity induced by preincubation egg storage in chicken embryos (*Gallus gallus domesticus*). *Physiol. Rep.* 4:e12712. doi: 10.14814/phy2.12712
- Burger, J. (1997). Effects of oiling on feeding behavior of sanderlings and semipalmated plovers in New Jersey. *Condor* 99, 290–298. doi: 10.2307/1369935
- Burggren, W., and Dubansky, B. (2018). *Development and Environment*. Berlin: Springer. 467.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2021.661943/full#supplementary-material>

- Burggren, W. W. (2020). Putting the august krogh principle to work in developmental physiology. *Comparat. Biochem. Physiol. A Mol. Integr. Physiol.* 252:110825. doi: 10.1016/j.cbpa.2020.110825
- Burggren, W. W., and Elmonoufy, N. A. (2017). Critical developmental windows for morphology and hematology revealed by intermittent and continuous hypoxic incubation in embryos of quail (*Coturnix coturnix*). *PLoS One* 12:e0183649. doi: 10.1371/journal.pone.0183649
- Bursian, S. J., Alexander, C. R., Cabela, D., Cunningham, F. L., Dean, K. M., Dorr, B. S., et al. (2017a). Reprint of: overview of avian toxicity studies for the deepwater horizon natural resource damage assessment. *Ecotoxicol. Environ. Safety* 146, 4–10. doi: 10.1016/j.ecoenv.2017.05.014
- Bursian, S. J., Dean, K. M., Harr, K. E., Kennedy, L., Link, J. E., Maggini, I., et al. (2017b). Effect of oral exposure to artificially weathered Deepwater Horizon crude oil on blood chemistries, hepatic antioxidant enzyme activities, organ weights and histopathology in western sandpipers (*Calidris mauri*). *Ecotoxicol. Environ. Safety* 146, 91–97. doi: 10.1016/j.ecoenv.2017.03.045
- Butler, R. G., Peakall, D. B., Leighton, F. A., Borthwick, J., and Harmon, R. S. (1986). Effects of crude oil exposure on standard metabolic rate of leach's storm-petrel. *Condor* 88:248. doi: 10.2307/1368924
- Corrales, J., Thornton, C., White, M., and Willett, K. L. (2014). Multigenerational effects of benzo [a] pyrene exposure on survival and developmental deformities in zebrafish larvae. *Aquat. Toxicol.* 148, 16–26. doi: 10.1016/j.aquatox.2013.12.028
- Couillard, C. M., and Leighton, F. A. (1989). Comparative pathology of prudhoe bay crude oil and inert shell sealants in chicken embryos. *Fundament. Appl. Toxicol.* 13, 165–173. doi: 10.1093/toxsci/13.1.165
- Couillard, C. M., and Leighton, F. A. (1991). Bioassays for the toxicity of petroleum oils in chicken embryos. *Environ. Toxicol. Chem.* 10, 533–538. doi: 10.1002/etc.5620100414
- Cunningham, F., Dean, K., Hanson-Dorr, K., Harr, K., Healy, K., Horak, K., et al. (2017). Development of methods for avian oil toxicity studies using the double crested cormorant (*Phalacrocorax auritus*). *Ecotoxicol. Environ. Saf.* 141, 199–208. doi: 10.1016/j.ecoenv.2017.03.025
- de Soysa, T. Y., Ulrich, A., Friedrich, T., Pite, D., Compton, S. L., Ok, D., et al. (2012). Macondo crude oil from the Deepwater Horizon oil spill disrupts specific developmental processes during zebrafish embryogenesis. *BMC Biol.* 10:40. doi: 10.1186/1741-7007-10-40
- Dean, K. M., Cabela, D., Carney, M. W., Cunningham, F. L., Ellis, C., Gerson, A. R., et al. (2017). Testing of an oral dosing technique for double-crested cormorants, *Phalacrocorax auritus*, laughing gulls, *Leucophaeus atricilla*, homing pigeons, *Columba livia*, and western sandpipers, *Calidris mauri*, with artificially weathered MC252 oil. *Ecotoxicol. Environ. Saf.* 146, 11–18. doi: 10.1016/j.ecoenv.2017.07.003
- Drorbaug, J. E., and Fenn, W. O. (1955). A barometric method for measuring ventilation in newborn infants. *Pediatrics* 16:81.
- Dubansky, B., Verbeck, G., Mach, P., and Burggren, W. (2018). Methodology for exposing avian embryos to quantified levels of airborne aromatic compounds associated with crude oil spills. *Environ. Toxicol. Pharmacol.* 58, 163–169. doi: 10.1016/j.etap.2018.01.005

- Dubansky, B., Whitehead, A., Miller, J. T., Rice, C. D., and Galvez, F. (2013). Multitissue molecular, genomic, and developmental effects of the Deepwater Horizon oil spill on resident Gulf killifish (*Fundulus grandis*). *Environ. Sci. Technol.* 47, 5074–5082. doi: 10.1021/es400458p
- Esler, D., Ballachey, B. E., Matkin, C., Cushing, D., Kaler, R., Bodkin, J., et al. (2018). Timelines and mechanisms of wildlife population recovery following the Exxon Valdez oil spill. *Deep Sea Res. II Top. Stud. Oceanogr.* 147, 36–42. doi: 10.1016/j.dsr2.2017.04.007
- Fallon, J. A., Smith, E. P., Schoch, N., Paruk, J. D., Adams, E. A., Evers, D. C., et al. (2018). Hematological indices of injury to lightly oiled birds from the Deepwater Horizon oil spill. *Environ. Toxicol. Chem.* 37, 451–461. doi: 10.1002/etc.3983
- Fiorello, C. V., Freeman, K., Elias, B. A., Whitmer, E., and Ziccardi, M. H. (2016). Ophthalmic effects of petroleum dispersant exposure on common murrelets (*Uria aalge*): an experimental study. *Mar. Pollut. Bull.* 113, 387–391. doi: 10.1016/j.marpolbul.2016.10.027
- Flores-Santin, J., Rojas Antich, M., Tazawa, H., and Burggren, W. W. (2018). Hematology from embryo to adult in the bobwhite quail (*Colinus virginianus*): Differential effects in the adult of clutch, sex and hypoxic incubation. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 218, 24–34. doi: 10.1016/j.cbpa.2018.01.005
- Forth, H. P., Mitchelmore, C. L., Morris, J. M., and Lipton, J. (2017). 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, 1450–1459. doi: 10.1002/etc.3672
- Fowles, J. R., Banton, M. I., Boogaard, P. J., Ketelslegers, H. B., and Rohde, A. M. (2016). Assessment of petroleum streams for thyroid toxicity. *Toxicol. Lett.* 254, 52–62. doi: 10.1016/j.toxlet.2016.05.001
- Guerrero-Bosagna, C., Morisson, M., Liaubet, L., Rodenburg, T. B., de Haas, E. N., Košťál, I., et al. (2018). Transgenerational epigenetic inheritance in birds. *Environ. Epigenet.* 4:dvy008.
- Hagmayer, A., Furness, A. I., Reznick, D. N., and Pollux, B. J. A. (2018). Maternal size and body condition predict the amount of post-fertilization maternal provisioning in matrotrophic fish. *Ecol. Evol.* 8, 12386–12396.
- Harr, K. E., Cunningham, F. L., Pritsos, C. A., Pritsos, K. L., Muthumalage, T., Dorr, B. S., et al. (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.* 146, 29–39. doi: 10.1016/j.ecoenv.2017.07.030
- Harr, K. E., Reavill, D. R., Bursian, S. J., Cacula, D., Cunningham, F. L., Dean, K. M., et al. (2017b). Organ weights and histopathology of double-crested cormorants (*Phalacrocorax auritus*) dosed orally or dermally with artificially weathered Mississippi Canyon 252 crude oil. *Ecotoxicol. Environ. Saf.* 146, 52–61. doi: 10.1016/j.ecoenv.2017.07.011
- Harr, K. E., Rishniw, M., Rupp, T. L., Cacula, D., Dean, K. M., Dorr, B. S., et al. (2017c). Dermal exposure to weathered MC252 crude oil results in echocardiographically identifiable systolic myocardial dysfunction in double-crested cormorants (*Phalacrocorax auritus*). *Ecotoxicol. Environ. Saf.* 146, 76–82. doi: 10.1016/j.ecoenv.2017.04.010
- Hartung, R., and Hunt, G. S. (1966). Toxicity of some oils to waterfowl. *J. Wildl. Manag.* 30, 564. doi: 10.2307/3798748
- Ho, D. H., and Burggren, W. W. (2010). Epigenetics and transgenerational transfer: a physiological perspective. *J. Exp. Biol.* 213, 3–16. doi: 10.1242/jeb.019752
- Horak, K. E., Bursian, S. J., Ellis, C. K., Dean, K. M., Link, J. E., Hanson-Dorr, K. C., et al. (2017). Toxic effects of orally ingested oil from the Deepwater Horizon spill on laughing gulls. *Ecotoxicol. Environ. Saf.* 146, 83–90. doi: 10.1016/j.ecoenv.2017.07.018
- Incardona, J. P., and Scholz, N. L. (2018). “The 2010 deepwater horizon oil spill and its environmental developmental impacts,” in *Development and Environment*, 1 Edn, eds W. W. Burggren and B. Dubansky (Berlin: Springer), 235–283. doi: 10.1007/978-3-319-75935-7_10
- Jenni-Eiermann, S., Jenni, L., Olano Marin, J., and Homberger, B. (2020). Seasonal changes in yolk hormone concentrations carry-over to offspring traits. *Gen. Comp. Endocrinol.* 287:113346. doi: 10.1016/j.ygcen.2019.113346
- Jenssen, B. M. (1994). Review article: effects of oil pollution, chemically treated oil, and cleaning on thermal balance of birds. *Environ. Pollut.* 86, 207–215. doi: 10.1016/0269-7491(94)90192-9
- King, M. D., Elliott, J. E., and Williams, T. D. (2020). Effects of petroleum exposure on birds: a review. *Sci. Total Environ.* 755:142834. doi: 10.1016/j.scitotenv.2020.142834
- Leighton, F. A. (1986). Clinical, gross, and histological findings in herring gulls and Atlantic puffins that ingested Prudhoe Bay crude oil. *Vet. Pathol.* 23, 254–263. doi: 10.1177/030098588602300305
- Leighton, F. A. (1993). The toxicity of petroleum oils to birds. *Environ. Rev.* 1, 92–103. doi: 10.1139/a93-008
- Lewis, S. J., and Malecki, R. A. (1984). Effects of egg oiling on larid productivity and population dynamics. *Auk* 101, 584–592. doi: 10.1093/auk/101.3.584
- Lighton, J. R. (2018). *Measuring Metabolic Rates: A Manual for Scientists*. Oxford: Oxford University Press.
- MacDonald, J. (2010). *A Closer Look At "Button quail*. Grass Valley, CA: Bracken Ridge Ranch.
- Mager, E. M., Esbaugh, A. J., Stieglitz, J. D., Hoenig, R., Bodinier, C., Incardona, J. P., et al. (2014). Acute embryonic or juvenile exposure to deepwater horizon crude oil impairs the swimming performance of mahi-mahi (*Coryphaena hippurus*). *Environ. Sci. Technol.* 48, 7053–7061. doi: 10.1021/es501628k
- Maggini, I., Kennedy, L. V., Bursian, S. J., Dean, K. M., Gerson, A. R., Harr, K. E., et al. (2017a). Toxicological and thermoregulatory effects of feather contamination with artificially weathered MC 252 oil in western sandpipers (*Calidris mauri*). *Ecotoxicol. Environ. Saf.* 146, 118–128. doi: 10.1016/j.ecoenv.2017.04.025
- Maggini, I., Kennedy, L. V., Macmillan, A., Elliott, K. H., Dean, K., and Guglielmo, C. G. (2017b). Light oiling of feathers increases flight energy expenditure in a migratory shorebird. *J. Exp. Biol.* 220, 2372. doi: 10.1242/jeb.158220
- Mazet, J. A. K., Newman, S. H., Gilardi, K. V. K., Tseng, F. S., Holcomb, J. B., Jessup, D. A., et al. (2002). Advances in oiled bird emergency medicine and management. *J. Avian Med. Surg.* 16, 146–150. doi: 10.1647/1082-6742(2002)016[0146:aiobem]2.0.co;2
- Michel, J., Owens, E. H., Zengel, S., Graham, A., Nixon, Z., Allard, T., et al. (2013). Extent and degree of shoreline oiling: deepwater horizon oil spill, Gulf of Mexico, USA. *PLoS One* 8:e65087. doi: 10.1371/journal.pone.0065087
- Munilla, I., Arcos, J. M., Oro, D., Álvarez, D., Leyenda, P. M., and Velando, A. (2011). Mass mortality of seabirds in the aftermath of the Prestige oil spill. *Ecosphere* 2:art83. doi: 10.1890/es11-0002.1
- O’Hara, P. D., and Morandin, L. A. (2010). Effects of sheens associated with offshore oil and gas development on the feather microstructure of pelagic seabirds. *Mar. Pollut. Bull.* 60, 672–678. doi: 10.1016/j.marpolbul.2009.12.008
- Paganelli, C. V. (1980). The physics of gas exchange across the avian eggshell. *Am. Zool.* 20, 329–338. doi: 10.1093/icb/20.2.329
- Paiva, J. T., De Resende, M. D. V., Resende, R. T., Oliveira, H. R., Silva, H. T., Caetano, G. C., et al. (2018). A note on transgenerational epigenetics affecting egg quality traits in meat-type quail. *Br. Poultry Sci.* 59, 624–628. doi: 10.1080/00071668.2018.1514582
- Pasparakis, C., Esbaugh, A. J., Burggren, W., and Grosell, M. (2019). Impacts of deepwater horizon oil on fish. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 224:108558. doi: 10.1016/j.cbpc.2019.06.002
- Pattee, O. H., and Franson, J. C. (1982). Short-term effects of oil ingestion on american kestrels (*Falco sparverius*). *J. Wildl. Dis.* 18, 235–241.
- Pennings, S. C., McCall, B. D., and Hooper-Bui, L. (2014). Effects of oil spills on terrestrial arthropods in coastal wetlands. *BioScience* 64, 789–795. doi: 10.1093/biosci/biu118
- Perez, C. R., Moyer, J. K., Cacula, D., Dean, K. M., and Pritsos, C. A. (2017). Low level exposure to crude oil impacts avian flight performance: the deepwater horizon oil spill effect on migratory birds. *Ecotoxicol. Environ. Saf.* 146, 98–103.
- Perrichon, P., Mager, E. M., Pasparakis, C., Stieglitz, J. D., Benetti, D. D., Grosell, M., et al. (2018). Combined effects of elevated temperature and Deepwater Horizon oil exposure on the cardiac performance of larval mahi-mahi, *Coryphaena hippurus*. *PLoS One* 13:e0203949. doi: 10.1371/journal.pone.0203949
- Pritsos, K. L., Perez, C. R., Muthumalage, T., Dean, K. M., Cacula, D., Hanson-Dorr, K., et al. (2017a). Dietary intake of Deepwater Horizon oil-injected live food fish by double-crested cormorants resulted in oxidative stress. *Ecotoxicol. Environ. Saf.* 146, 62–67.

- Pritsos, K. L., Perez, C. R., Muthumalage, T., Dean, K. M., Cacula, D., Hanson-Dorr, K., et al. (2017b). Dietary intake of Deepwater Horizon oil-injected live food fish by double-crested cormorants resulted in oxidative stress. *Ecotoxicol. Environ. Saf.* 146, 62–67.
- Puddephatt, K. J. (2014). *Urban Quail-Keeping*, 1st Edn. Scotts Valley, CA: CreateSpace Independent Publishing Platform.
- Reddam, A., Mager, E. M., Grosell, M., and McDonald, M. D. (2017). The impact of acute PAH exposure on the toadfish glucocorticoid stress response. *Aquat. Toxicol.* 192, 89–96.
- Sirsat, T. S., and Dzialowski, E. M. (2020). Manipulating plasma thyroid hormone levels at hatching alters development of endothermy and ventilation in Pekin duck (*Anas platyrhynchos domestica*). *J. Exp. Biol.* 223:jeb237701.
- Spotila, J. R., Weinheimer, C. J., and Paganelli, C. V. (1981). Shell resistance and evaporative water loss from bird eggs: effects of wind speed and egg size. *Physiol. Zool.* 54, 195–202.
- Stubblefield, W. A., Hancock, G. A., Prince, H. H., and Ringer, R. K. (1995). Effects of naturally weathered Exxon valdez crude oil on mallard reproduction. *Environ. Toxicol. Chem.* 14, 1951–1960.
- Swanson, D. L., and Liknes, E. T. (2006). A comparative analysis of thermogenic capacity and cold tolerance in small birds. *J. Exp. Biol.* 209:466.
- Szaro, R. C., Coon, N. C., and Stout, W. (1980). Weathered petroleum: effects on mallard egg hatchability. *J. Wildl. Manag.* 44, 709–713.
- Szdzuy, K., and Mortola, J. P. (2007). Monitoring breathing in avian embryos and hatchlings by the barometric technique. *Respir. Physiol. Neurobiol.* 159, 241–244.
- Tazawa, H., Akiyama, R., and Moriya, K. (2002). Development of cardiac rhythms in birds. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 132, 675–689.
- Tierney, K. B., Kennedy, C. J., Gobas, F., Gledhill, M., and Sekela, M. (2013). *Organic Contaminants and Fish, Fish Physiology*. Elsevier, 1–52.
- Vidal, M., Domínguez, J., and Luís, A. (2011). Spatial and temporal patterns of polycyclic aromatic hydrocarbons (PAHs) in eggs of a coastal bird from northwestern Iberia after a major oil spill. *Sci. Total Environ.* 409, 2668–2673.
- Yang, D.-j., Sasaki, O., Minezawa, M., Nirasawa, K., and Takahashi, H. (2010). Mapping of quantitative trait loci affecting eggshell quality in an F2 population derived from strong and weak eggshell lines of the white leghorn chicken breed. *Agric. Sci. China* 9, 593–597.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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