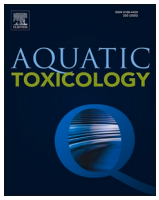
Aquatic Toxicology 242 (2022) 106045

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox



Exposure of zebrafish larvae to water accommodated fractions of weathered crude oil alters steroid hormone concentrations with minimal effect on cholesterol

Edwin R. Price [a,*], Fabrizio Bonatesta [a], Victoria McGruer [b], Daniel Schlenk [b], Aaron P. Roberts [a], Edward M. Mager [a]

[a] Department of Biological Sciences and the Advanced Environmental Research Institute, University of North Texas, Denton, TX 76203, United States
[b] Department of Environmental Sciences, University of California, Riverside, CA, 92521, United States

ARTICLE INFO

Keywords:
Early life stages
Deepwater Horizon
Estradiol
Androstenedione
Polycyclic aromatic hydrocarbons, cortisol

ABSTRACT

Crude oil has multiple toxic effects in fish, particularly during their early life stages. Recent transcriptomics studies have highlighted a potential effect on cholesterol homeostasis and biosynthesis, but have not investigated effects on steroid hormones, which are biosynthetically downstream metabolites of cholesterol. We exposed zebrafish (*Danio rerio*) embryos and larvae to 3 concentrations of a high energy water accommodated fraction (HEWAF) of crude oil and measured effects on cholesterol and steroid hormones at 48 and 96 h post fertilization (hpf). HEWAF exposure caused a small decrease in cholesterol at 96 hpf but not 48 hpf. HEWAF-exposed larvae had higher levels of androstenedione, testosterone, estradiol, cortisol, corticosterone, and progesterone at 96 hpf compared to controls, while effects at 48 hpf were more modest or not present. 2-Methoxyestradiol was lower following HEWAF exposure at both time points. Dihydrotestosterone was elevated in one HEWAF concentration at 48 hpf only. Our results suggest that hormone imbalance may be an important toxic effect of oil HEWAF exposure despite no major effect on their biosynthetic precursor cholesterol.

1. Introduction

Crude oil exposure can cause acute toxicity in fish, with early life stages being particularly sensitive (Pasparakis et al., 2019). The mechanism of this toxicity is often ascribed to pericardial and yolk sac edema and reduced cardiac function (Edmunds et al., 2015; Incardona et al., 2013; Khursigara et al., 2017; Li et al., 2019), although other mechanisms may be important, including effects on sensory organs, swim bladder development and inflation, metabolic rate, among others (Pasparakis et al., 2019; Price and Mager, 2020). Recent transcriptomics approaches have suggested additional putative mechanisms based on studies of haddock, mahi-mahi, and red drum (Sørhus et al., 2017; Xu et al., 2017a, 2017b, 2016). These include effects of crude oil on craniofacial development, ionoregulation, water balance, steroid biosynthesis, ribosome biogenesis, and cholesterol synthesis and homeostasis (Sørhus et al., 2017; Xu et al., 2016).

Upregulation of cholesterol synthesis was a common finding from several of these transcriptomics studies (Sørhus et al., 2017; Xu et al., 2017a, 2016). In mahi-mahi, steroid biosynthesis (particularly the cholesterol biosynthetic pathway) was one of the top pathways altered, with all steroid biosynthesis genes upregulated in 96 h post fertilization (hpf) larvae exposed to water accommodated fractions of oil (Xu et al., 2017b, 2016). Similarly, in Atlantic haddock, genes related to cholesterol biosynthesis showed upregulated transcription during embryonic development following exposure to weathered oil (Sørhus et al., 2017). Nonetheless, a study of zebrafish found significant downregulation of cholesterol synthesis – and lower cholesterol esters – after exposure to diesel, which shares many of the same toxic components (polycyclic aromatic hydrocarbons; PAHs) with crude oil (Mu et al., 2018).

Cholesterol homeostasis is essential for early fish embryo development, and disruption of cholesterol by crude oil could therefore underlie components of the well-described developmental toxicity phenotype caused by oil. Cholesterol reduction in developing zebrafish embryos results in a cardiotoxic phenotype similar to that observed with crude oil exposure, including pericardial edema and reduced heart rate (Barros et al., 2018; Maerz et al., 2019). Recently, pretreatment with cholesterol was shown to partially mitigate the effects of the cardiotoxic PAH phenanthrene on heart rate in zebrafish (McGruer et al., 2021b).

* Corresponding author.
E-mail address: Edwin.Price@unt.edu (E.R. Price).

https://doi.org/10.1016/j.aquatox.2021.106045
Received 21 July 2021; Received in revised form 5 October 2021; Accepted 22 November 2021
Available online 27 November 2021

Although the mechanism of cholesterol-mediated cardiotoxicity is not fully understood, reduced cholesterol attenuates hedgehog signaling, which can ultimately lead to heart defects. Cholesterol is also a key component of membranes, and altered cholesterol can influence ion-channel function either by changing membrane properties or through sterol-protein specific interactions (reviewed by Levitan et al., 2010). Crude oil and the single PAH phenanthrene alone have been shown to disrupt ion channel function in isolated fish cardiomyocytes (Brette et al., 2014, 2017), and therefore disruption of membrane cholesterol levels has been proposed as a potential mechanism through which ion channels in the cardiomyocytes could be affected (McGruer et al., 2019).

Cholesterol also serves as the precursor molecule for steroid hormones. The rate-limiting step for hormone production is the transport of cholesterol to the inner mitochondrial matrix where it is converted to pregnenolone (Rone et al., 2009). Pregnenolone then enters the endoplasmic reticulum for further enzymatic reactions to produce the various steroid hormones. Steroid hormones are important for organization and development of sexual organs and behavior, immune function, growth, and response to stress (Wilson et al., 2016). Moreover, previous studies on adult fish of a range of species have shown altered plasma steroid hormone concentrations following exposure to oil or PAHs (Arukwe et al., 2008; Collier et al., 2014; Monteiro et al., 2000a; Reddam et al., 2017; Roy et al., 2003; Seruto et al., 2005). Changes in cholesterol and steroid hormone concentrations could therefore represent significant mechanisms of both lethal and sublethal effects of oil exposure.

Despite this importance, the effects of oil on cholesterol homeostasis and biosynthesis, and particularly the effects on steroid hormones, have been poorly studied in fish at early life stages. Here, we measure the effects on cholesterol and 8 steroid hormones following early life exposure to a high energy water accommodated fraction (HEWAF) (Forth et al., 2017) of naturally weathered crude oil.

2. Methods

2.1. Animals

Adult zebrafish (*Danio rerio*) were obtained from a local pet shop and maintained in a colony at the University of North Texas under standard conditions and were fed TetraMin tropical flakes. Males and females were isolated, and later paired to induce spawning. All experimental procedures were approved by the Institutional Animal Care and Use Committee (protocol #19–016) at the University of North Texas.

2.2. Exposure and toxicity

HEWAF preparations are designed to reflect exposure scenarios involving vigorous mixing of oil and water and physical dispersion of oil (Forth et al., 2017). We prepared a HEWAF of naturally weathered slick oil collected from the sea surface during the *Deepwater Horizon* spill (sample ID: OFS-20,100,719-Juniper-001 A0087Q), as previously described (Sweet et al., 2017), using 1 g oil in 1000 mL embryo water (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$). This 100% HEWAF was further diluted with embryo water to create 40%, 20%, and 10% HEWAF treatment solutions, and embryo water was used for control. Chemical analysis (see below) showed these HEWAF treatments to be equivalent to 516, 258, and 129 μg/L ΣPAH_{50}, respectively, at the beginning of each exposure day. Reported concentrations of typical ΣPAH concentrations during the DWH oil spill ranged as high as 240 μg/L (DWH NRDA Trustees, 2016). While some of the exposure concentrations used herein are near or above this concentration, zebrafish are known to be more tolerant to crude oil exposures compared to species native to the Gulf of Mexico. Thus, these higher concentrations are needed to relate effects observed in zebrafish to those that likely occurred in more sensitive fish that were actually exposed to the spill (Pasparakis et al., 2019).

After spawning, eggs were collected and inspected for quality, and then placed into 200 mL glass crystalizing dishes, with 40 eggs per dish and methylene blue (0.1%) added. There were 5 replicate dishes for each exposure concentration and collection timepoint (48 or 96 hpf; for a total of 40 statistically independent dishes). Exposure began at 5 hpf and continued until collection. HEWAF and control solutions were renewed daily, at which time we counted and removed dead embryos/larvae. Embryos and larvae were deemed dead if overtly opaque or unresponsive to gentle prodding, respectively. The light cycle was 14L:10D. Water parameters were measured daily and were within the following ranges throughout development: 23.5–26.1 °C (mean ± s.e.m: 24.7 ± 0.05), pH 5.88–7.38 (6.60±0.03), and 6.13–6.95 (6.56±0.01) mg/L dissolved oxygen.

2.3. Water chemistry analysis

Given that PAHs are believed to be the main drivers of crude oil toxicity, water samples were collected for measurement of 50 select PAHs (ΣPAH_{50}) commonly reported for crude oil studies since the DWH oil spill (Forth et al., 2017). Water from the control was sampled at the beginning of the exposure. Water from the 40% HEWAF solution was sampled daily when it was prepared ('initial') as well as just prior to renewal ('final'; pooled from all 40% treatments). These samples (250 mL) were collected in amber bottles with no headspace and stored at 4 °C until they were shipped to ALS Environmental (Kelso, WA, USA) for PAH analysis by GC/MS-SIM (EPA method 8270D).

2.4. Cholesterol analysis and sterol panel

Larvae were collected at 48 and 96 hpf, evenly divided into two cryovials for each replicate (about 10 larvae were pooled from each replicate dish and placed in each of 2 vials), and frozen at −80 °C. Later, one vial was thawed on ice and the larvae were homogenized in 200 μL Dulbecco's PBS using a bead beater homogenizer and then refrozen. We measured total cholesterol (free cholesterol + cholesterol esters) with a fluorometric assay using a kit (Item 10007640; Cayman Chemical; Ann Arbor MI) after diluting homogenates 10-fold with assay buffer. All samples were in range of the standard curve. Protein was measured in the same homogenates diluted 10-fold using a spectrophotometer (Pierce Coomassie (Bradford) Protein Assay Kit, Cat #23200, Thermo Fisher Scientific, Waltham MA). Cholesterol is presented as μmol cholesterol per μg protein.

The other vial (also containing about 10 pooled larvae) from each replicate was sent to the VCU Massey Cancer Center lipidomics shared resource (Richmond, VA) for analysis of a standard panel of steroid hormones. Steroid hormones are presented as ng steroid per nmol lipid phosphate.

2.5. Statistics

Each vial of pooled larvae (which was derived from an independent crystalizing dish) was treated as the statistically independent sampling unit. Steroid hormones and cholesterol data were analyzed using ANOVA and Tukey's HSD for post-hoc comparisons. The decline of ΣPAH_{50} over each day was tested using a paired *t*-test. These analyses were conducted using R statistical software (R Core Team, 2019). Additionally, we conducted a metabolite set enrichment analysis to detect altered patterns of functionally related metabolites using MetaboAnalyst 5.0 (Xia and Wishart, 2016). Data are presented as means ± s.e.m.

3. Results

Total PAH analysis revealed only a small concentration (0.030 μg/L) of biphenyl in the control, and no other PAHs. In the 40% HEWAF, ΣPAH_{50} averaged 516 ± 47.5 μg/L, with 3-ringed PAHs representing

about two thirds of the total (Fig. 1). Sum PAH_{50} declined over 24 h (Paired *t*-test; t_2=5.768, $p = 0.028$), but was still high at the time of renewal, averaging 388 ± 26.9 μg/L.

Survival at 96 hpf was 91% for both the control and 10% HEWAF treatments, was non-significantly lower in the 20% HEWAF treatment at 78% survival (Tukey HSD, $p = 0.079$ versus control), and was significantly reduced in the 40% HEWAF treatment to 56% survival ($p < 0.001$ versus control; data not shown).

Cholesterol concentration (range: 51.1–133.1 μmol per μg protein) was unaffected by treatment at 48 hpf (ANOVA, $F_{3,16}$=1.109, $p = 0.374$) (Fig. 2). At 96 hpf, ANOVA detected a difference among treatments ($F_{3,16}$=3.366, $p = 0.045$), tending downward with increasing HEWAF concentration, but Tukey's post-hoc test revealed no significant differences among groups ($p > 0.077$ for all comparisons).

Exposure to the HEWAF caused hormone-specific changes at both 48 and 96 hpf (Fig. 3). Androstenedione did not vary amongst treatments at 48 hpf, but was elevated in the 20% ($p < 0.001$) and 40% ($p < 0.001$) HEWAF groups at 96 hpf. Testosterone was only elevated over control in the 40% HEWAF group at 48 hpf ($p = 0.345$), while it was elevated in only the 20% group at 96 hpf ($p = 0.002$). 2-Methoxyestradiol decreased substantially in all treatment groups at both timepoints ($p<0.029$ for all comparisons). Dihydrotestosterone showed a mixed response, with only the 10% HEWAF group elevated over control ($p < 0.020$) and only at the 48 hpf timepoint. Estradiol was elevated in the 40% HEWAF group at 96 hpf only ($p = 0.035$). Cortisol was elevated over control in the 20% ($p = 0.007$) and 40% ($p = 0.014$) groups at 96 hpf only. Similarly, corticosterone was elevated over control in the 20% ($p <0.001$) and 40% ($p <0.001$) HEWAF at 96 hpf only. Finally, progesterone was increased in the 20% and 40% HEWAF groups at both timepoints ($p <0.026$ for all comparisons)(Fig. 3).

We confirmed the effect of HEWAF exposure on metabolic pathways using metabolite set enrichment analysis for control versus 40% HEWAF at 96 hpf. This concentration of HEWAF induced altered pathways for steroidogenesis (Holm $p<0.001$), androgen and estrogen metabolism (Holm $p = 0.004$), and androstenedione metabolism (Holm $p = 0.015$) (Supplementary Fig. S1).

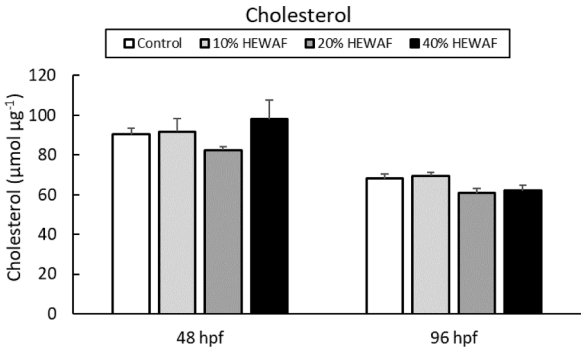


Fig. 2. Cholesterol (μmol cholesterol per μg protein) in 48 and 96 hpf zebrafish larvae after exposure to 0, 10, 20, or 40% HEWAF of *Deepwater Horizon* oil. Data are means + s.e.m.; $n = 5$ for each bar. There were no significant pairwise differences among treatments at either age (Tukey HSD, $p > 0.05$), although ANOVA detected a significant difference among treatments at 96 hpf.

4. Discussion

Our results demonstrate that crude oil can stimulate steroidogenesis in zebrafish larvae. This effect was broad and included increased estradiol, androstenedione, and cortisol.

Interestingly, our results differ from the effect that PAHs have been shown to have on adult fish, particularly females (Collier et al., 2014). PAH exposure in adult females is associated with smaller ovarian growth and gonadosomatic index, lower vitellogenin production, and reduced fecundity (Collier et al., 2014; Nicolas, 1999). Plasma estradiol, and its secretion by the ovary, are also reduced by PAH exposures in several species (Monteiro et al., 2000a, 2000b; Pollino et al., 2009; Sol et al., 2000; Tintos et al., 2006). Even exposures in larvae, followed by a grow-out period to adulthood in clean water, generally result in lower plasma estradiol concentrations in adults (Chen et al., 2021; Gao et al., 2018). In adult males, PAH exposure has been associated with declines in androgen production and testicular development, although this has not always been a consistent effect (Collier et al., 2014). And in adult male hornyhead turbot (*Pleuronichthys verticalis*) exposed to oil-contaminated sediment, plasma concentrations of estradiol were also

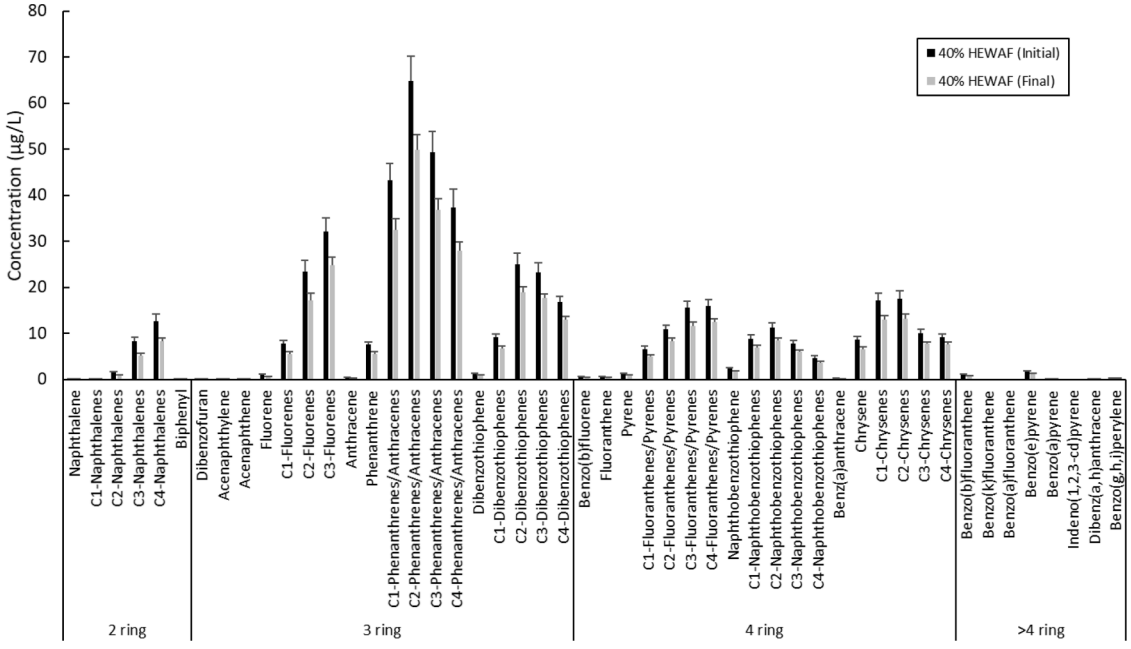


Fig. 1. Concentration of 50 polycyclic aromatic hydrocarbons (PAHs) in the 40% HEWAF treatment at initial exposure and just prior to renewal, 24 h later ("Final"). Concentrations are μg/L and are means + s.e.m.

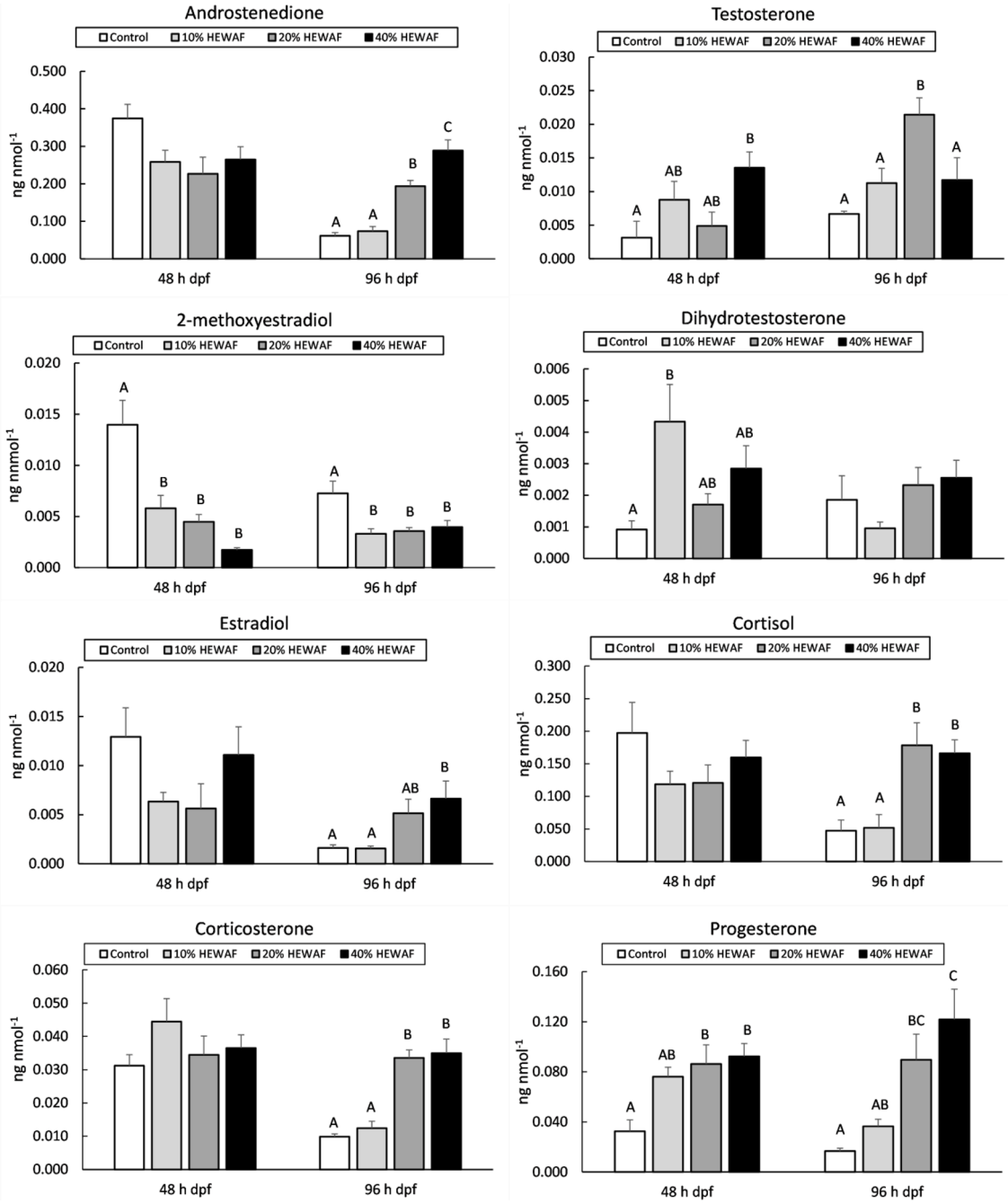


Fig. 3. Steroid hormone concentrations (ng steroid per nmol lipid phosphate) in 48 and 96 hpf zebrafish following exposure to 0, 10, 20 and 40% HEWAF from *Deepwater Horizon* oil. Data are means + s.e.m.; $n = 5$ for each bar. Within an age, bars sharing letters or without letters do not significantly differ from each other (Tukey HSD $p > 0.05$).

reduced (Roy et al., 2003). In adult toadfish (*Opsanus beta*), cortisol production was not affected acutely by exposure to oil HEWAF (Cartolano et al., 2021).

In developing fish larvae, few studies have measured steroid hormones directly; rather, many studies have instead measured an estrogenic effect of PAHs. For example, Philibert et al. (2019) demonstrated that larval sheepshead minnows exposed to *Deepwater Horizon* HEWAF had higher vitellogenin mRNA expression (vitellogenin is a downstream target of estrogen and a commonly used marker of estrogen disruption), although vitellogenin expression was not upregulated in zebrafish in the same study. Nonetheless, He et al. (2018) showed that exposure to flowback and produced water from hydraulic fracturing increased vitellogenin mRNA expression in zebrafish larvae. Additionally, early life exposure to PAHs from contaminated sediments caused a female-biased sex ratio in medaka (Mu et al., 2017). These results have led investigators to conclude that PAHs are mild estrogen disruptors (Chen et al., 2021; Gao et al., 2018; Mu et al., 2017). Our data suggest that PAHs may act by stimulating endogenous estradiol production, which could account for the downstream effects on vitellogenin expression and sex ratio observed in these other studies. One previous study found that estradiol was instead decreased by exposure to the PAH benzo(a)pyrene in zebrafish (Alharthy et al., 2017). However, those measurements were made at 48 hpf, a time point at which we saw no significant effect of HEWAF exposure on estradiol (and in fact observed a non-significant decrease at low HEWAF dilutions). Our most consistent and statistically significant effects for all hormones occurred at 96 hpf.

While there were notable increases after oil exposure in progesterone, which sits near the beginning of the biosynthetic pathway for multiple steroids, not all steroids showed elevated concentrations. The combination of high androstenedione and estradiol but no consistent increases in testosterone nor dihydrotestosterone at 96 hpf suggests high aromatase activity. Similarly, the decreased concentration of 2-methoxyestradiol suggests either diminished cytochrome P450 activity or diminished catechol-O-methyltransferase activity, although we cannot verify this as we have not measured these activities in the present study.

Although we observed elevated concentrations of most steroids at 96 hpf after exposure to high HEWAF dilutions, we did not observe elevated concentrations of these steroids' biosynthetic precursor, cholesterol. Previous studies on early life stage fish have shown mixed effects of oil or PAH exposure on cholesterol metabolism. Based on transcription studies, cholesterol biosynthesis is upregulated after exposure in several species (Loughery et al., 2018; McGruer et al., 2019; Sørhus et al., 2017; Xu et al., 2017a, 2017b). However, total cholesterol itself was decreased in larval mahi-mahi at high HEWAF concentration (McGruer et al., 2019); the authors concluded that HEWAF depressed cholesterol concentration, which then stimulated upregulation of the cholesterol biosynthetic pathway. Sørhus et al. (2017) similarly concluded that yolk absorption (and associated cholesterol absorption) was diminished in early life stage Atlantic haddock after oil exposure, which then led to upregulation of gene expression for biosynthesis of cholesterol and steroids. In contrast, a study on zebrafish reported lower expression of the cholesterol biosynthesis pathway following diesel exposure at early life stages (Mu et al., 2018), along with depressed cholesterol esters. Free cholesterol was not affected in zebrafish (24 – 72 hpf) following exposure to phenanthrene (McGruer et al., 2021b). Free cholesterol levels are tightly regulated and can be converted into cholesterol esters, a storage form of cholesterol, or metabolized into steroid hormones. Nonetheless, when red drum (*Sciaenops ocellatus*) embryos were exposed to oil HEWAF, increases in free cholesterol were observed, but without a change in total cholesterol (McGruer et al., 2021a). Moreover, Li et al. (2021) showed decreased expression of steroidogenic enzymes after exposure to low energy WAF of oil in zebrafish. It is unclear why these studies observed differing results; perhaps it can be attributed to differences among species, exposures, cholesterol form, or particular PAH mixtures. Nonetheless, by measuring the steroids themselves – as opposed to changes in transcription – our study demonstrates that oil WAF has measurable effects on steroid concentrations in early life stage fish.

Because hormones are major regulators of development, the alterations in their concentrations we observed have the potential to have important long term and perhaps sub-lethal effects. This includes, but is not limited to, failed or delayed swim bladder inflation (Alharthy et al., 2017), altered sex ratios, altered behavior and responses to stressors, changes in growth (Wilson et al., 2016), and diminished reproductive output. Future studies should confirm these results in other ecologically relevant species and determine how they interact with other mechanisms of toxicity.

CRediT authorship contribution statement

Edwin R. Price: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Fabrizio Bonatesta:** Conceptualization, Investigation, Writing – review & editing. **Victoria McGruer:** Writing – review & editing. **Daniel Schlenk:** Conceptualization, Writing – review & editing, Funding acquisition. **Aaron P. Roberts:** Conceptualization, Writing – review & editing, Funding acquisition. **Edward M. Mager:** Conceptualization, Writing – review & editing, Investigation, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Corey Green, Rachel Leads, Cameron Emadi, and Mehrnaz Moghimi for their generous assistance in the lab. Barney Venables and Ed Dzialowski graciously shared their equipment and lab space. This research was supported by a grant from the Gulf of Mexico Research Initiative (15–067). Additionally, services in support of this project were generated by the VCU Massey Cancer Center Lipidomics Shared Resource, supported, in part, with funding from NIH—NCI Cancer Center Support Grant P30 CA016059 and NIH shared resource grant S10RR031535.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2021.106045.

References

Alharthy, K.M., Albaqami, F.F., Thornton, C., Corrales, J., Willett, K.L., 2017. Mechanistic evaluation of benzo[a]pyrene's developmental toxicities mediated by reduced Cyp19a1b activity. Toxicol. Sci. 155, 135–147.

Arukwe, A., Nordtug, T., Kortner, T.M., Mortensen, A.S., Brakstad, O.G., 2008. Modulation of steroidogenesis and xenobiotic biotransformation responses in zebrafish (*Danio rerio*) exposed to water-soluble fraction of crude oil. Environ. Res. 107, 362–370.

Barros, S., Montes, R., Quintana, J.B., Rodil, R., André, A., Capitão, A., Soares, J., Santos, M.M., Neuparth, T., 2018. Chronic environmentally relevant levels of simvastatin disrupt embryonic development, biochemical and moecular responses in zebrafish (*Danio rerio*). Aquat. Toxicol. 210, 47–57.

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

Brette, F., Shiels, H.A., Galli, G.L.J., Cros, C., Incardona, J.P., Scholz, N.L., Block, B.A., 2017. A novel cardiotoxic mechanism for a pervasive global pollutant. Sci. Rep. 7, e41476.

Cartolano, M.C., Alloy, M.M., Milton, E., Plotnikova, A., Mager, E.M., McDonald, M.D., 2021. Exposure and recovery from environmentally relevant levels of waterborne polycyclic aromatic hydrocarbons from *Deepwater Horizon* oil: effects on the gulf toadfish stress axis. Environ. Toxicol. Chem. 40, 1062–1074.

Chen, Y., Zhang, Y., Yu, Z., Guan, Y., Chen, R., Wang, C., 2021. Early-life phenanthrene exposure inhibits reproductive ability in adult zebrafish and the mechanism of action. Chemosphere 272, 129635.

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 A. P. Farrell, K.B., Brauner, C.J. (Eds.), Organic Chemical Toxicology of Fishes. Academic Press, Amsterdam, pp. 195–255.

DWH NRDA Trustees. (2016). Chapter 4: injury to Natural Resources. In Deepwater Horizon Oil Spill: final programmatic damage assessment and restoration plan and final programmatic environmental impact statement. Available at: https://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan/.

Edmunds, R.C., Gill, J.A., Baldwin, D.H., Linbo, T.L., French, B.L., Brown, T.L., Esbaugh, A.J., Mager, E.M., Stieglitz, J., Hoenig, R., et al., 2015. Corresponding morphological and molecular indicators of crude oil toxicity to the developing hearts of mahi mahi. Sci. Rep. 5. Article 17326.

Forth, H.P., Mitchelmore, C.L., Morris, J.M., 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.

Gao, D., Lin, J., Ou, K., Chen, Y., Li, H., Dai, Q., Yu, Z., Zuo, Z., Wang, C., 2018. Embryonic exposure to benzo(a)pyrene inhibits reproductive capability in adult female zebrafish and correlation with DNA methylation. Environ. Pollut. 240, 403–411.

He, Y., Sun, C., Zhang, Y., Folkerts, E.J., Martin, J.W., Goss, G.G., 2018. Developmental toxicity of the organic fraction from hydraulic fracturing flowback and produced waters to early life stages of zebrafish (*Danio rerio*). Environ. Sci. Technol. 52, 3820–3830.

Incardona, J.P., Swarts, T.L., Edmunds, R.C., Linbo, T.L., Aquilina-Beck, A., Sloan, C.A., Gardner, L.D., Block, B.A., Scholz, N.L., 2013. Exxon Valdez to Deepwater Horizon: comparable toxicity of both crude oils to fish early life stages. Aquat. Toxicol. 142-143, 303–316.

Khursigara, A.J., Perrichon, P., Martinez Bautista, N., Burggren, W.W., Esbaugh, A.J., 2017. Cardiac function and survival are affected by crude oil in larval red drum, *Sciaenops ocellatus*. Sci. Total Environ. 579, 797–804.

Levitan, I., Fang, Y., Rosenhouse-Dantsker, A., Romanenko, V., 2010. Cholesterol and ion channels. In: Harris, J. (Ed.), Cholesterol Binding and Cholesterol Transport Proteins. Springer, Dordrecht.

Li, X., Xiong, D., Ding, G., Fan, Y., Ma, X., Wang, C., Xiong, Y., Jiang, X., 2019. Exposure to water-accomodated fractions of two different crude oils alters morphology, cardiac function and swim bladder development in early-life stages of zebrafish. Chemosphere 235, 423–433.

Li, X., Xiong, D., Ju, Z., Xiong, Y., Ding, G., Liao, G., 2021. Phenotypic and transcriptomic consequences in zebrafish early-life stages following exposure to crude oil and chemical dispersant at sublethal concentrations. Sci. Total Environ. 763, 143053.

Loughery, J.R., Kidd, K.A., Mercer, A., Martyniuk, C.J., 2018. Part A: temporal and dose-dependent transcriptional responses in the liver of fathead minnows following short term exposure to the polycyclic aromatic hydrocarbon phenanthrene. Aquat. Toxicol. 199, 90–102.

Maerz, L.D., Burkhalter, M.D., Schilpp, C., Wittekindt, O.H., Frick, M., Philipp, M., 2019. Pharmacological cholesterol depletion disturbs ciliogenesis and ciliary function in developing zebrafish. Commun. Biol. 2. Article 31.

McGruer, V., Khursigara, A.J., Magnuson, J.T., Esbaugh, A.J., Greer, J.B., Schlenk, D, 2021a. *Deepwater Horizon* crude oil increases free cholesterol in larval red drum (*Sciaenops ocellatus*) with implications for developmental toxicity. Aquat. Toxicol.

McGruer, V., Pasparakis, C., Grosell, M., Stieglitz, J.D., Benetti, D.D., Greer, J.B., Schlenk, D, 2019. Deepwater horizon crude oil exposure alters cholesterol biosynthesis with implications for developmental cardiotoxicity in larval mahi-mahi (*Coryphaena hippurus*). Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 220, 31–35.

McGruer, V., Tanabe, P., Vliet, S.M.F., Dasgupta, S., Qian, L., Volz, D.C., Schlenk, D, 2021b. Effects of phenanthrene exposure on cholesterol homeostasis and cardiotoxicity in zebrafish embryos. Environ. Toxicol. Chem. 40, 1586–1595.

Monteiro, P.R.R., Reis-Henriques, M.A., Coimbra, J., 2000a. Plasma steroid levels in female flounder (*Platichthys flesus*) after chronic dietary exposure to single polycyclic aromatic hydrocarbons. Mar. Environ. Res. 49, 453–467.

Monteiro, P.R.R., Reis-Henriques, M.A., Coimbra, J., 2000b. Polycyclic aromatic hydrocarbons inhibit in vitro ovarian steroidogenesis in the flounder (*Platichthys flesus* L.). Aquat. Toxicol. 48, 549–559.

Mu, J., Chernick, M., Dong, W., Di Giulio, R.T., Hinton, D.E., 2017. Early life co-exposures to a real-world PAH mixture and hypoxia result in later life and next generation consequences in medaka (*Oryzias latipes*). Aquat. Toxicol. 190, 162–173.

Mu, X., Liu, J., Yang, K., Huang, Y., Li, X., Yang, W., Qi, S., Tu, W., Shen, G., Li, Y., 2018. 0# Diesel water-accomodated fraction induced lipid homeostasis alteration in zebrafish embryos. Environ. Pollut. 242, 952–961.

Nicolas, J.M., 1999. Vitellogenesis in fish and the effects of polycyclic aromatic hydrocarbon contaminants. Aquat. Toxicol. 45, 77–90.

Pasparakis, C., Esbaugh, A.J., Burggren, W., Grosell, M., 2019. Physiological impacts of Deepwater Horizon oil on fish. Comparat. Biochem. Physiol. Part C Toxicol. Pharmacol. 224, e108558.

Philibert, D.A., Lyons, D., Philibert, C., Tierney, K.B., 2019. Field-collected crude oil, weathered oil and dispersants differentially affect the early life stages of freshwater and saltwater fishes. Sci. Total Environ. 647, 1148–1157.

Pollino, C.A., Georgiades, E., Holdway, D.A., 2009. Physiological changes in reproductively active rainbowfish (*Melanotaenia fluviatilis*) following exposure to naphthalene. Ecotoxicol. Environ. Saf. 72, 1265–1270.

Price, E.R., Mager, E.M., 2020. The effects of exposure to crude oil or PAHs on fish swim bladder development and function. Comp. Biochem. and Physiology, Part C: Toxicology & Pharmacology 238, 108853.

R Core Team, 2019. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reddam, A., Mager, E.M., Grosell, M., McDonald, M.D., 2017. The impact of acute PAH exposure on the toadfish glucocorticoid stress response. Aquatic. Toxicol. 192, 89–96.

Rone, M.B., Fan, J., Papadopoulos, V., 2009. Cholesterol transport in steroid biosynthesis: role of protein-protein interactions and implications in disease states. Biochim. Biophys. Acta 1791, 646–658.

Roy, L.A., Steinert, S., Bay, S.M., Greenstein, D., Sapozhnikova, Y., Bawardi, O., Leifer, I., Schlenk, D, 2003. Biochemical effects of petroleum exposure in hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of sediments collected from a natural petroleum seep in CA, USA. Aquatic. Toxicol. 65, 159–169.

Seruto, C., Sapozhnikova, Y., Schlenk, D, 2005. Evaluation of the relationships between biochemical endpoints of PAH exposure and physiological endpoints of reproduction in male California Halibut (*Paralichthys californicus*) exposed to sediments from a natural oil seep. Mar. Environ. Res. 60, 454–465.

Sol, S.Y., Johnson, L.L., Horness, B.H., Collier, T.K., 2000. Relationship between oil exposure and reproductive paramaters in fish collected following the *Exxon Valdez* oil spill. Mar. Pollut. Bull. 40, 1139–1147.

Sørhus, E., Incardona, J.P., Furmanek, T., Goetz, G.W., Scholz, N.L., Meier, S., Edvardsen, R.B., Jentoft, S., 2017. Novel adverse outcome pathways revealed by chemical genetics in a developing marine fish. Elife 6, e20707.

Sweet, L.E., Magnuson, J., Garner, T.R., Alloy, M.M., Stieglitz, J.D., Benetti, D., Grosell, M., Roberts, A.P., 2017. Exposure to ultraviolet radiation late in development increases the toxicity of oil to mahi-mahi (*Coryphaena hippurus*) embryos. Environ. Toxicol. Chem. 36, 1592–1598.

Tintos, A., Gesto, M., Alvarez, R., Míguez, J.M., Soengas, J.L., 2006. Interactive effects of naphthalene treatment and the onset of vitellogenesis on energy metabolism in liver and gonad, and plasma steroid hormones of rainbox trout *Oncorhynchus mykiss*. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 144, 155–165.

Wilson, K.S., Tucker, C.S., Al-Dujaili, E.A.S., Holmes, M.C., Hadoke, P.W.F., Kenyon, C.J., Denvir, M.A., 2016. Early-life glucocorticoids programme behaviour and metabolism in adulthood in zebrafish. J. Endocrinol. 230, 125–142.

Xia, J., Wishart, D.S., 2016. Using MetaboAnalyst 3.0 for comprehensive metabolomics data analysis. Curr. Protoc. Bioinform. 55, 14.10.1-14.10.91.

Xu, E.G., Khursigara, A.J., Magnuson, J., Hazard, E.S., Hardiman, G., Esbaugh, A.J., Roberts, A.P., Schlenk, D, 2017a. Larval red drum (*Sciaenops ocellatus*) sublethal exposure to weathered Deepwater Horizon crude oil: developmental and transcriptomic consequences. Environ. Sci. Technol. 51, 10162–10172.

Xu, E.G., Mager, E.M., Grosell, M., Hazard, E.S., Hardiman, G., Schlenk, D, 2017b. Novel transcriptome assembly and comparative toxicity pathway analysis in mahi-mahi (*Coryphaena hippurus*) embryos and larvae exposed to deepwater horizon oil. Sci. Rep. 7, e44546.

Xu, E.G., Mager, E.M., Grosell, M., Pasparakis, C., Schlenker, L.S., Stieglitz, J.D., Benetti, D., Hazard, E.S., Courtney, S.M., Diamante, G., et al., 2016. Time- and oil-dependent transcriptomic and physiological responses to *Deepwater Horizon* oil in mahi-mahi (*Coryphaena hippurus*) embryos and larvae. Environ. Sci. Technol. 50, 7842–7851.