Environmental Pollution 268 (2021) 115715

Contents lists available at ScienceDirect

Environmental Pollution

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

Microbiome analysis and predicted relative metabolomic turnover suggest bacterial heme and selenium metabolism are altered in the gastrointestinal system of zebrafish (*Danio rerio*) exposed to the organochlorine dieldrin☆

Qing Hua [a, b, 1], Ondrej Adamovsky [a, c, 1], Hana Vespalcova [c], Jonna Boyda [a], Jordan T. Schmidt [a], Marianne Kozuch [a], Serena L.M. Craft [d], Pamela E. Ginn [d], Stanislav Smatana [c, g], Eva Budinska [c], Maria Persico [c], Joseph H. Bisesi Jr. [e], Christopher J. Martyniuk [a, f, *]

[a] Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, 32611, USA
[b] Inner Mongolia Key Laboratory of Environmental Pollution Control & Waste Resource Reuse, School of Ecology and Environment, Inner Mongolia University, Hohhot, 010021, China
[c] Masaryk University, Research Centre for Toxic Compounds in the Environment (RECETOX), Brno, Czech Republic
[d] University of Florida, Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, Gainesville, USA
[e] Department of Environmental & Global Health and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, 32611, USA
[f] University of Florida Genetics Institute and Interdisciplinary Program in Biomedical Sciences Neuroscience, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611, USA
[g] Faculty of Information Technology, IT4Innovations Centre of Excellence, Brno University of Technology, Brno, Czech Republic

ARTICLE INFO

Article history:
Received 20 May 2020
Received in revised form
29 August 2020
Accepted 20 September 2020
Available online 5 October 2020

Keywords:
Microbiome
Pesticide
Aquatic
Ecotoxicology
Pathology

ABSTRACT

Dietary exposure to chemicals alters the diversity of microbiome communities and can lead to pathophysiological changes in the gastrointestinal system. The organochlorine pesticide dieldrin is a persistent environmental contaminant that bioaccumulates in fatty tissue of aquatic organisms. The objectives of this study were to determine whether environmentally-relevant doses of dieldrin altered gastrointestinal morphology and the microbiome of zebrafish. Adult zebrafish at ~4 months of age were fed a measured amount of feed containing either a solvent control or one of two doses of dieldrin (measured at 16, and 163.5 ng/g dry weight) for 4 months. Dieldrin body burden levels in zebrafish after four-month exposure were 0 (control), 11.47 ± 1.13 ng/g (low dose) and 18.32 ± 1.32 ng/g (high dose) wet weight [mean ± std]. Extensive histopathology at the whole organism level revealed that dieldrin exposure did not induce notable tissue pathology, including the gastrointestinal tract. A repeated measure mixed model analysis revealed that, while fish gained weight over time, there were no dieldrin-specific effects on body weight. Fecal content was collected from the gastrointestinal tract of males and 16S rRNA gene sequencing conducted. Dieldrin at a measured feed dose of 16 ng/g reduced the abundance of Firmicutes, a phylum involved in energy resorption. At the level of class, there was a decrease in abundance of Clostridia and Betaproteobacteria, and an increase in Verrucomicrobiae species. We used a computational approach called predicted relative metabolomic turnover (PRMT) to predict how a shift in microbial community composition affects exchange of metabolites. Dieldrin was predicted to affect metabolic turnover of uroporphyrinogen I and coproporphyrinogen I [enzyme]–cysteine, hydrogen selenide, selenite, and methyl-selenic acid in the fish gastrointestinal system. These pathways are related to bacterial heme biosynthesis and selenium metabolism. Our study demonstrates that dietary exposures to dieldrin can alter microbiota composition over 4 months, however the long-term consequences of such impacts are not well understood.

☆ This paper has been recommended for acceptance by Dr. Sarah Harmon.

* Corresponding author. Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, 32611, USA.
E-mail address: cmartyn@ufl.edu (C.J. Martyniuk).

[1] authors contributed equally to the manuscript.

https://doi.org/10.1016/j.envpol.2020.115715
0269-7491/

1. Introduction

Organochlorine pesticides (OCPs) were among some of the earliest commercial pesticides developed for pest control in the 1940s–50s. Following multiple reports of adverse effects in wildlife and humans, these pesticides were gradually replaced over time with safer chemicals. Despite restrictions on the use of OCPs in several developed countries (e.g. USA), there remains wildlife and human health risks associated with exposure, as developing nations continue to use these relatively cheaper pesticides. Other reasons for continued concern include the high potential for bioaccumulation in lipid rich tissues and resistance to microbial and UV degradation compared to other pesticides (i.e. long half-life) (Chopra et al., 2011; Jayaraj et al., 2016). In fact, the Substance Priority List put forth by the Agency for Toxic Substances and Disease Registry (ATSDR) in 2019 (https://www.atsdr.cdc.gov) includes many OCPs within the top 50 chemicals that are human health priority areas.

One OCP on the ATSDR list is dieldrin, which has been ranked at position #18 for the past several years. Dieldrin bioaccumulates significantly in large predatory animals within the food chain and can negatively impact the endocrinology and physiology of fish species. Organochlorine pesticides have multiple effects on endocrine systems and can act as thyroid disruptors, anti-androgens and estrogens, in addition to modulating the immune system and metabolism (Cowie et al., 2017; Martyniuk et al., 2020). In the central nervous system of largemouth bass (*Micropterus salmoides*), dieldrin affects the expression of receptors of different neurotransmitters and can alter neurotransmitter concentrations in specific brain regions (Martyniuk et al., 2013, 2010a). Dieldrin can also modify the relative abundance of proteins associated with neurodegeneration and neurotoxicity (Martyniuk et al., 2010b) and can change transcript and protein levels of signaling molecules related to mTOR (mammalian target of rapamycin) in the heart of zebrafish (*Danio rerio*) (Slade et al., 2017). However, no study to date has determined whether or not dieldrin impacts the gastrointestinal system of fish at a molecular/microbial level, despite the fact that oral exposure through food and particulate matter is the most relevant exposure scenario. This is important because the epithelium of the gastrointestinal system acts as a physical barrier to chemicals, and loss of intestinal structure and integrity can have detrimental effects on the individual.

The microbiome has now been established as a major health factor, and disruption of the microbiome in terms of diversity, richness, and function can lead to significant negative health outcomes. Research now demonstrates that the microbiota of aquatic organisms can be adversely affected by aquatic pollutants (Adamovsky et al., 2020, 2018; Evariste et al., 2019). Studies also demonstrate that aquatic contaminants such as antibiotics (Claus et al., 2016) and anti-bacterial agents such as triclosan (Gaulke et al., 2016a, 2016b) can shift the composition of the microbiota in the gastrointestinal tract. Investigations into the biological impacts of the Deepwater Horizon oil spill also showed that weathered Macondo MC252 oil containing polycyclic aromatic hydrocarbons mixed with sediment altered the microbiome of benthic dwelling juvenile flounder (Brown-Peterson et al., 2015). Thus, dietary exposure to chemicals in the environment can modify the microbiome of fish, which may have consequences for intestinal integrity and function. However, data are lacking on the toxicity of OCPs in the gut of fish. This is a significant knowledge gap as bioaccumulation studies in fish demonstrate that organochlorine pesticides such as p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) and dieldrin accumulate to relatively high levels in the gastrointestinal (Dang et al., 2016), surpassing other tissue concentrations due to direct contact with the chemicals.

In this study, we aimed to determine the effects of environmentally-relevant dietary exposures of dieldrin on the gastrointestinal microbiome and structure/anatomy of zebrafish. Studies in mammals report that OCPs can alter the microbiome of the gut (Liu et al., 2017), enhancing *Lactobacillus* with bile salt hydrolase (BSH) activity but such data are lacking in fish. Fish are exposed to aquatic contaminants and ingest chemicals through the intake of food; this can be problematic for highly lipophilic molecules such as OCPs. We hypothesized that dieldrin would also shift the microbiome towards higher abundance of *Lactobacillus*. Along with microbiome analysis, we conducted a rigorous histopathological assessment of the gastrointestinal tract and other tissues to determine if there were tissue pathologies associated with dieldrin exposure.

2. Methods

2.1. Feed preparation

Twelve milligrams of dieldrin (MERCK, cat.no. 33491, CAS 60-57-1, PESTANAL®, analytical standard) were dissolved into 4 mL of menhaden oil. The solution was mixed for 1 h (stock solution) on an orbital shaker in a sterile glass flask. A serial dilution was performed by initially adding 0.5 mL of the stock solution into 4.5 mL of fresh oil into sterile glass flask to generate two treatments that differed by a factor of 10-fold. Feed pellets (Zeigler Adult Zebrafish Diet, Zeigler Bros, PA, USA) were prepared by mixing ~400 g of feed in a KitchenAid® Stand Mixers with 1 mL of oil with dieldrin over a 3-h period for a total of 3 mL oil. Control feed was prepared in the same way but with 3 mL of oil only. The target nominal feed concentrations were 22.5 ng/g dry weight (d.w.) and 225 ng/g (d.w). A menhaden oil control group was also prepared using the same amount of feed and the same volume of oil. The concentration of dieldrin in feed was analytically verified (see section below describes the analytical methods).

The rationale for the time point and the doses examined is derived from research conducted at Lake Apopka in Florida, USA. This environment contains heavy contamination of OCPs. Largemouth bass placed into experimental ponds in the North Shore of Lake Apopka accumulated dieldrin rapidly after four months to measured levels of 500 ng/g wet weight (Martyniuk et al., 2016). A follow study conducted in largemouth bass collected by electroshocking from Lake Apopka revealed levels of aldrin and its metabolite dieldrin of ~3–13 ng/g wet weight (w/w) in different tissues (Dang et al., 2016). In laboratory-based studies, feeding zebrafish the same doses resulted in the incorporation of dieldrin into tissues within a month, reaching levels between 11 and 58 ng/g w/w (Cowie et al., 2017). These doses were also selected because they are environmentally relevant (ng levels are reported in sediment and fish tissues) (Sapozhnikova et al., 2004). Thus, based on these studies, we elected to feed zebrafish as per Cowie et al. (2017) over four months to achieve environmentally realistic body burden levels. As detailed below, we were successful in targeting a relevant range of what is expected in fish in a natural environment.

Twelve milligrams of dieldrin (MERCK, cat.no. 33491, CAS 60-57-1, PESTANAL®, analytical standard) were dissolved into 4 mL of menhaden oil. The solution was mixed for 1 h (stock solution) on an orbital shaker in a sterile glass flask. A serial dilution was performed by initially adding 0.5 mL of the stock solution into 4.5 mL of fresh oil into sterile glass flask to generate two treatments that differed by a factor of 10-fold. Feed pellets (Zeigler Adult Zebrafish Diet, Zeigler Bros, PA, USA) were prepared by mixing ~400 g of feed in a KitchenAid® Stand Mixers with 1 mL of oil with dieldrin over a 3-h period for a total of 3 mL oil. Control feed was prepared in the same way but with 3 mL of oil only. The target nominal feed

concentrations were 22.5 ng/g dry weight (d.w.) and 225 ng/g (d.w). A menhaden oil control group was also prepared using the same amount of feed and the same volume of oil. The concentration of dieldrin in feed was analytically verified (see section below describes the analytical methods).

2.2. Animals and experimental design

An animal use protocol for the experiment was carried out ethically in accordance with approval from the University of Florida Institutional Animal Care and Use Committee (Study #201509038). Adult wildtype zebrafish (AB x Tü strain, *Danio rerio*, ~4 months of age) were obtained from a breeding culture of fish housed by Animal Care Services (Cancer and Genetics Research Complex, University of Florida, Gainesville, Fl, USA). Zebrafish were maintained in a Pentair Aquatic Eco-systems Z-hab mini stand-alone recirculating system (Pentair, Minneapolis, MN). Water parameters (mean ± standard deviation) measured daily during the experiment included temperature = 26.9 ± 0.50 °C, dissolved oxygen = 7.78 ± 0.68 mg/L, and pH = 7.9 ± 0.29. Other parameters were measured every two or three weeks and included alkalinity = 37.33 ± 5.50 ppm and ammonia = 0.011 ± 0.006 ppm. Fish were subjected to 14-h light and 10-h dark daily cycles.

For the dietary exposure, zebrafish were divided into 9 tanks (10 L water) per experimental group (n = 3 for control, n = 3 for low dose, n = 3 for high dose) in a flow through system. The organization of the tanks was such that the contaminated feed could not enter the control tanks. Twelve fish were added to each tank, with the goal of maintaining an equal ratio of female to male sex ratio. Zebrafish in designated tanks were fed a measured 0.065 g of the control dose or one of the two contaminated feeds twice a day over the course of the experiment. This represented a daily intake of 0.13 g feed (~2–4% of individual body weight per tank based at different times as the fish grew). Feeding took place at ~10 a.m. and ~4 p.m. The mean weight of the fish per tank (tank biomass) was recorded on a monthly basis over the 4-month dietary exposure.

After 4 months, zebrafish were euthanized in a sodium bicarbonate buffered solution of MS-222 (250 mg/L; Sigma-Aldrich, St. Louis, MO, USA) and their spinal cords were immediately severed. Under a microscope, the gastrointestinal system of zebrafish was extracted with sterile forceps and micro-scissors and the feces was extracted by gently scraping the intestinal lining with forceps to move food through the gastrointestinal system and into a tube for DNA processing. The remaining fish carcass was frozen in liquid nitrogen and stored at −80 °C for dieldrin measurements in the whole body. For histopathology and microbiome analysis, only males were analyzed, as we did not obtain sufficient fecal material or DNA from female fish to continue, and we decided to focus efforts on the male fish. One to two males per replicate tank were taken for histopathology. Microbiome studies in fish show that separating the sexes is important and sexes should be analyzed separately (DeBofsky et al., 2020).

2.3. Analytical chemistry

Fish body burden measurements followed the methods outlined by Hong et al. (2004). Briefly, frozen fish were homogenized in liquid nitrogen with a pestle. Homogenized samples (whole fish, ~0.4–0.5 g wet weight) were spiked with 20 ng internal standard ($^{13}C_{12}$-dieldrin, Cambridge Isotope Laboratory). The samples were then twice extracted using acetone:hexane (5:2, vol/vol), followed by sonication and centrifugation. Pooled supernatants were transferred to glass tubes, dried under nitrogen, and reconstituted into acetonitrile. The extracts were placed to −20 °C for 1 h to precipitate lipids. Soluble extracts were transferred to new glass tubes and the remaining material was re-extracted with additional 2 mL of acetonitrile followed by lipid precipitation in −20 °C for 1 h. Lipid extraction was done according to (Ahn et al., 2007). The combined acetonitrile extracts were concentrated and purified on Florisil SPE column (PreSep, 6 cc, 1 g). Final extracts were solvent exchanged into 1 mL hexane prior to analysis. Fish control, matrix spike (control fish spiked with 10 ng and 100 ng dieldrin), and matrix spike duplicates were also included. For details of the whole extraction methodology, see Supplemental Materials.

Samples were analyzed with a gas chromatograph coupled with a tandem mass spectrometer (GC-MS/MS; Agilent 7890B GC and 7000C QQQ MS; Santa Clara, CA). The analytical column used for chromatographic separation was a Zebron ZB-5MS, 30 m × 0.25 mm x 0.25 μm film thickness (Torrance, CA). The carrier gas was 99.999% pure helium at a constant flow of 1.5 mL/min. One μL sample was injected into a 280 °C inlet in splitless mode. The GC oven was initially 70° and held for 2 min, after which the first temperature gradient was 40°/min to 170° (no hold time), the second temperature gradient was 5°/min to 200° (no hold time), and final temperature gradient was 20°/min to 280°, (1 min hold).

The GC transfer line temperature was 280°, MS source temperature at 280°, and Quad temperatures at 150°. The MS operated in EI mode; MS/MS collision gas was 99.999% pure nitrogen with a flow of 1.5 mL/min, and MS/MS quench gas was 99.999% pure helium at 2.25 mL/min. Post-run helium backflush was 50 mL/min for 1 min. For details of the monitored (MRN) ions used for detection and quantitation, see Supplemental Materials. Dieldrin concentration in the samples was determined by comparison to authentic dieldrin calibrators ranging between 0.1 and 100 ng/mL. The instrument limit of quantitation was 0.2 ng/mL.

2.4. Histopathology

Five control male zebrafish and 7 treated fish (n = 4 low, n = 3 high dose) were selected for histopathological evaluation. The fish, selected from each of the experimental replicates, were labeled to blind the evaluator to the group of origin. Post euthanasia, zebrafish whole bodies were preserved in Davidson's solution for 1 week and then transferred to a 70% ethanol solution. Whole fish were sent to the histology laboratory at Histology Tech Services (Gainesville, Florida) for sectioning and staining. Each fish was paraffin embedded and sectioned 5 μm in both a mid-high plane and a sagittal plane, mounted onto slides, and deparaffinized. All mid to high cuts were stained with a standard hematoxylin and eosin stain (H & E). Shallow sagittal sections of each fish were stained with a modified McManus Method for Glycogen (Periodic acid-Schiff histochemical stain (PSA)). Slides were placed into 1% periodic acid for 5 min, washed in running tap water for 1 min, and placed in Schiff's Solution (Poly Scientific R&D) for 15 min. Slides were again washed in running tap water for 10 min and placed in Hematoxylin (Statlab) for 30 s. Rinsing occurred in running water for 1 min, followed by 5 dips in Clarifier reagent (High Def by Statlab). Slides were then again rinsed in tap water for 1 min and dipped 5 times in Bluing reagent (Statlab). Lastly, slides were rinsed in tap water for 1 min, followed by 10 dips in 95% alcohol, 30 s in 3 changes of 100% alcohol, and 30 s in 3 changes of Xylene prior to cover-slipping. The sections of each fish were examined.

Zebrafish histopathology was evaluated by two board certified anatomic pathologists (Drs. Craft and Ginn) in the College of Veterinary Medicine, University of Florida for abnormalities. An inventory of tissues was compiled and each investigated when possible for abnormalities. The tissue inventory included the following: telencephalon containing the olfactory bulb, dorsal and ventral cerebrum, diencephalon including the thalamcephalon,

pineal body, epithalamus, dorsal thalamus, ventral thalamus, posterior tuberculum, and the hypothalamus. Mesencephalon including the tectum opticum (5 layers deep to cellular gray matter), periventricular gray zone of the optic tectum, as well as the bulging tract (torus longitudinalis) which forms the roof of tectal ventricle. Other tissues examined included the myelencephalon, spinal cord, pituitary, thyroid, ultimobranchial gland, corpuscles of stannious, urophysis, interrenal cells, chromaffin cells, skin/lateral line, gastrointestinal tract, anterior segment, mouth, buccal cavity, oropharynx (callous pad and pharyngeal teeth), posterior segment, esophagus, intestinal bulb mid-intestine, posterior intestine, heart (atrium), head kidney, trunk kidney, opisthonephric duct, spleen, thymus, liver, gall bladder, pancreas, gills, pseudobranch, testes, ovaries, eye, ear, olfactory sac, air bladder, and skeletal muscle.

2.5. Microbiome analysis and data processing

Fecal samples (N = 7 to 9 male fish/experimental group; 2 or 3 males collected from each of the three replicate tanks) were extracted using the Zymo Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (D6010) and extracted DNA quantified using the Qubit (ThermoFisher). Adjusted samples to 5 ng/μL were then used to amplify the microbial 16S (V3 and V4) region using AccuPrime Taq DNA Polymerase (ThermoFisher Scientific Cat. No: 12346-086) according to the manufacturer's procedure. The amplicon size (~500−600 base pairs) was confirmed by agarose gel electrophoresis. The amplicon was purified by magnetic beads AMPure XP Beads (A63880) and indexed using Nextera XT DNA Library Preparation Kit (FC-131-2004) with High Fidelity AccuPrime Taq DNA Polymerase (12346-086) and Nextera XT Index primers (N716−N729, S520−S522). The indexed samples were purified by magnetic beads, quantified (Qubit Fluorometer) and diluted using 10 mM Tris (pH 8.5) to a final concentration 4 nM. Samples were combined and pooled library quantified by NEBNext NGS Library Quant Kit (Illumina, New England). The pooled library was sequenced on an Illumina MiSeq (2 × 300 bp/600 cycles) at University of Florida according to established protocols. For more details deals with preparation of the library for the analysis, used chemicals and regents and sequencing run i.d., see the Supplemental Materials.

It was necessary to preprocess the raw sequencing reads, before any further downstream analysis could be performed. As a first step of this process, low-quality ends of reads were trimmed using the tool Trimmomatic (Bolger et al., 2014) with a phred quality score threshold of 20. Reads left too short by the trimming process (<200 bp for forward reads and <190 for reverse reads) were discarded from subsequent analysis. Next, the trimmed sequence pairs were joined using the program fastq-join from the ea-utils toolkit (Aronesty, 2011) and analyzed for chimeras using UCHIME (v 6.1). Joined sequence were subjected to closed OTU picking using the Uclust algorithm (Edgar, 2010) with 97% sequence similarity threshold and Silva (v123) (Pruesse et al., 2007) as the 16s rRNA reference database. As a final step, representative sequences of OTUs were assigned taxonomy by Uclust-consensus taxonomy assignment method (threshold 0.51, n-hits 3). The taxonomy assignment, as well as OTU picking, were performed using the Quantitative Insights into Molecular Ecology (1.9.1.) (Caporaso et al., 2010) framework and used Silva (v123) as the 16s rRNA reference database.

2.6. Generation of functional and metabolome estimates of the microbiota

Starting from the OTU table obtained in the pre-processing step, we obtained a predicted "functional profile" of the microbial community under study using the workflow known as PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (Langille et al., 2013), conveniently retrained for the Silva database: in fact the original PiCRUST tool (v 1.1.3) has been developed using as a reference, the Greengene database. The pipeline aimed at functional profiling and the community was then expanded by a normalization step based on the algorithm MUSICC (Manor and Borenstein, 2015) to re-scale the KOs abundances to average gene copy number, with the final aim to correct the predicted KOs counts derived from the original OTU table, taking into account some known biases in the downstream analysis of OTUs table. We then use this normalized functional profile to move a step forward and to predict putative shifts in metabolic potential of the microbial community under study, by using a re-adapted and re-implemented version of the original PRMT/MIMOSA methods (Predicted Relative Metabolomic Turnover, (Larsen et al., 2011); MIMOSA (Noecker et al., 2016). Both these approaches produce microbial community-wide metabolic potential scores (CMP or PRMT scores) for each metabolite annotated in the reference knowledge base KEGG. The output of the re-implemented workflow is a matrix whose values are community-wide estimated metabolic potential scores for each metabolite per metagenomics sample. Those metabolite scores can be regarded as the relative capacity of the metagenome content of each sample to create or deplete each metabolite.

2.7. Statistical analysis

A One-Way ANOVA followed by a Holm-Sidak's multiple comparisons test was conducted to evaluate differences in body burden levels. A repeated measures ANOVA (mixed model) and Dunnett's multiple comparison test was conducted to determine if body mass varied by time, treatment, or subject (tank). The mean biomass of each tank replicate was recorded every month and the data represent the mean of three replicates (i.e. tanks) at each time point. A simple linear regression was conducted to evaluate the slopes in body weight over time in each of the three treatment groups. Statistical analysis and graphing were conducted with GraphPad v8.4 (La Jolla, CA, USA).

For microbiome analysis, QIIME and PICRUST data were transformed using centered log-ratio transformation (Jones and Aitchison, 1987) and an ANOVA test with Tukey's HSD (Honestly Significant Difference) post-hoc tests were used for comparison of the differences between the groups, for each taxa or KO term separately. Details deals with the data processing, data selection for QIIME and PICRUST analysis and applied thresholds, see the supplemental materials.

All resulting p-values were adjusted for multiple hypothesis testing using the Benjamini-Hochberg procedure with significance threshold of 0.05 for QIIME taxonomy estimates and metabolome estimates and with significance threshold of 0.1 for the PICRUST metabolic pathway estimates. The statistical analyses were performed in R version 3.4.4 (R Development Core Team, 2018).

3. Results

3.1. Dieldrin accumulation in zebrafish and growth rate

Adult male zebrafish at 4 months of age were fed a measured amount of feed containing either a solvent control or one of two doses of dieldrin. The feed containing the solvent control was below detection limit, while dieldrin at the lower dose measured 16.0 ng/g [nominal target = 22.5 ng/g (recovery of 71.3%)] while the higher feed diet measured 163.5 ng/g [nominal target = 225 ng/g (recovery of 72.7%)]. Body burden levels in zebrafish at the end of

the four-month experiment were 0 ng/g (no samples reached above detection limit), 11.47 ± 1.13 ng/g and 18.32 ± 1.32 ng/g wet weight [mean (±s.d.)] (Fig. 1). Each group was different than the next in terms of dieldrin burden ($F_{(2, 6)} = 254.6$, $p < 0.0001$).

While zebrafish gained weight over time, exposure to dietary dieldrin did not result in any difference in body mass among groups. Tanks (subjects) differed in body mass but this was randomized across the treatments and reflected differences in the starting biomass at the beginning of the experiment: Time x Treatment ($F_{(2, 9)} = 0.43$, $p = 0.66$), Time ($F_{(2, 9)} = 93.86$, $p=<0.0001$), Treatment ($F_{(2, 9)} = 1.31$, $p = 0.30$) (Fig. 2). A regression analysis also revealed that dieldrin in the diet did not result in any difference among slopes between groups ($F_{(2, 9)} = 0.43$, $p = 0.67$) (Fig. 2), indicating that growth trajectories were not different among treatments.

3.2. Histopathology

Histopathology was assessed by two board certified veterinarians. There were no discernible changes in any tissue examined, including the gastrointestinal tissue (Fig. 3A, control and Fig. 3B, dieldrin treated). The only tissue where some abnormalities were detected were in areas of the brain (Fig. 3C–E), mostly metencephalon (cerebellar region), but this was present in all groups and was not different between the control nor dieldrin-treated fish (Hematoxylin and Eosin). These abnormalities were approximately 15 × 20-μm PAS negative amorphous, acellular globules (Arrow in Fig. 3E). Globules were present throughout all sections of the brain, with the majority of the globules found in the metencephalon of the brain. The total number of globules varied between fish, ranging from 5 to 40. The significance of this finding is unknown and pathologists were unable to determine the reason for such structures in the brain.

3.3. Microbial communities

All metagenomics data has been submitted to ENA (European Nucleotide Archive (Accession no. PRJEB39690)). Intriguingly, the lower dose of dieldrin (i.e. 16 ng/g feed d.w.) had a more pronounced effect on the microbiome than the higher dose. At the phylum level (Fig. 4), dieldrin decreased the abundance of *Firmicutes* (log2 fold change (FC) of mean centered log-ratio transformed

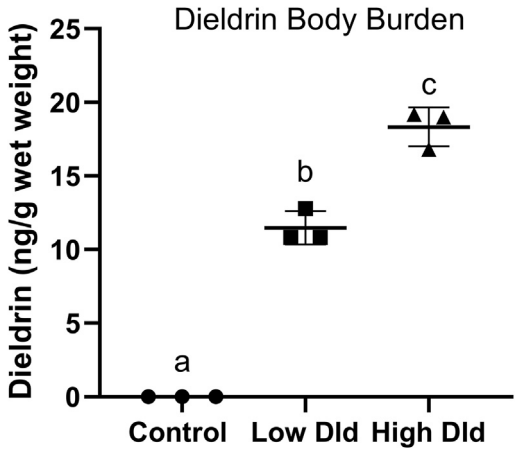

Fig. 1. Body burden levels of dieldrin (Dld) in zebrafish fed dieldrin-enriched feed at a low (16 ng/g d.w.) and high (160 ng/g d.w.) dose at the end of a four-month experiment. Each bar represents mean ± standard deviation (ng/g/wet weight). Data analyzed using a One-Way ANOVA followed by a Holm-Sidak's multiple comparisons test ($p < 0.05$, $n = 3$/group).

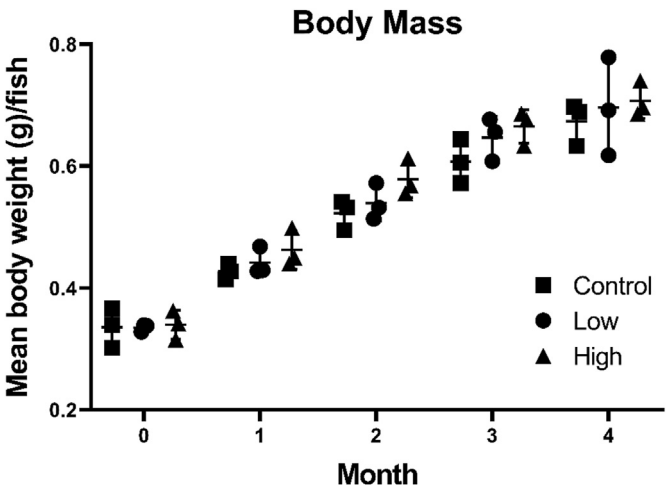

Fig. 2. The mean body weight of zebrafish exposed to low (16 ng/g d.w.) and high (160 ng/g d.w.) dieldrin dose during 4 months exposure. Values represents mean ± standard deviation. Data analyzed using a repeated measures ANOVA (mixed model) followed by a Dunnett's multiple comparison test ($p < 0.05$, $n = 3$/group).

abundance of control and dieldrin group) (log2 FC = −1.96, FDR-corrected p-value = 0.022), decreased the classes *Clostridia* (log2 FC = −1.89, FDR-corrected p-value = 0.037), *Betaproteobacteria* (log2 FC = −1.13, FDR-corrected p-value = 0.01), increased the class *Verrucomicrobiae* (log2 FC = 2.44, FDR-corrected p-value = 0.043) (Fig. 4) and decreased order *Clostridiales* (log2 FC = −2.04, FDR-corrected p-value = 0.038) (Fig. 5). Only uncultured family NKB5_1 differed between low and high dieldrin dose (log2 FC = 3.21, FDR-corrected p-value = 0.017) (Fig. S1).

The low dose of dieldrin affected the microbial abundance of genus *Defluviimonas* (*log2* FC = 2.28, FDR-corrected p-value = 0.039) and *Sphingomonas* (*log2* FC = −2.01, FDR-corrected p-value = 0.047) and uncultured genus NKB5_1 significantly differs between Low and High dieldrin dose (*log2* FC = 3.12, FDR-corrected p-value = 0.018) (Fig.S2). At the OTU level, the low dieldrin dose differed from the high dieldrin dose in OTU KJ475025.1.1452 (*Pseudomonas*) (*log2* FC = −2.38, FDR-corrected p-value = 0.015) (Fig.S3). The higher dose of dieldrin did not affect the microbiome in any of the phylogenetic levels. Dieldrin did not cause any changes in microbial diversity according to alpha diversity indexes (number of observed species, Shannon and Simpson indexes (Fig. S4) and beta diversity (PCA plot, Fig. S5).

The computational approach aimed at functional profiling the community, suggested a statistically significant reduction in functional capabilities of the community in fish treated with the 160 ng dieldrin/g feed d.w. treatment (Fig. 6). Dieldrin exposure was predicted to decrease the community's gene content of enzymes 3−methyl−2−oxobutanoate hydroxymethyltransferase (*log2* FC −1.17) and aminomethyltransferase (*log2* FC −1.17), whereas fish exposed to the lower dieldrin dose of 16 ng dieldrin/g feed d.w. were predicted to exhibit lower gene content of enzymes for pyrimidine/purine−5−nucleotide nucleosidase (*log2* FC = −0.15), F−type H+−transporting ATPase subunit b (*log2* FC = −0.16), branched−chain amino acid aminotransferase (*log2* FC = −0.16), phosphoribosylformylglycinamidine synthase (*log2* FC = −0.17) and transketolase (*log2* FC = −0.22) (Fig. 6).

These enzymatic shifts were used to computationally predict the statistically significant shift in the microbial community metabolome (Fig. 7). The lowest dose of dieldrin decreased relative metabolic turnover (PRMT) of uroporphyrinogen I (*log2* FC = −0.41), dephospho−CoA (*log2* FC = −2.68),

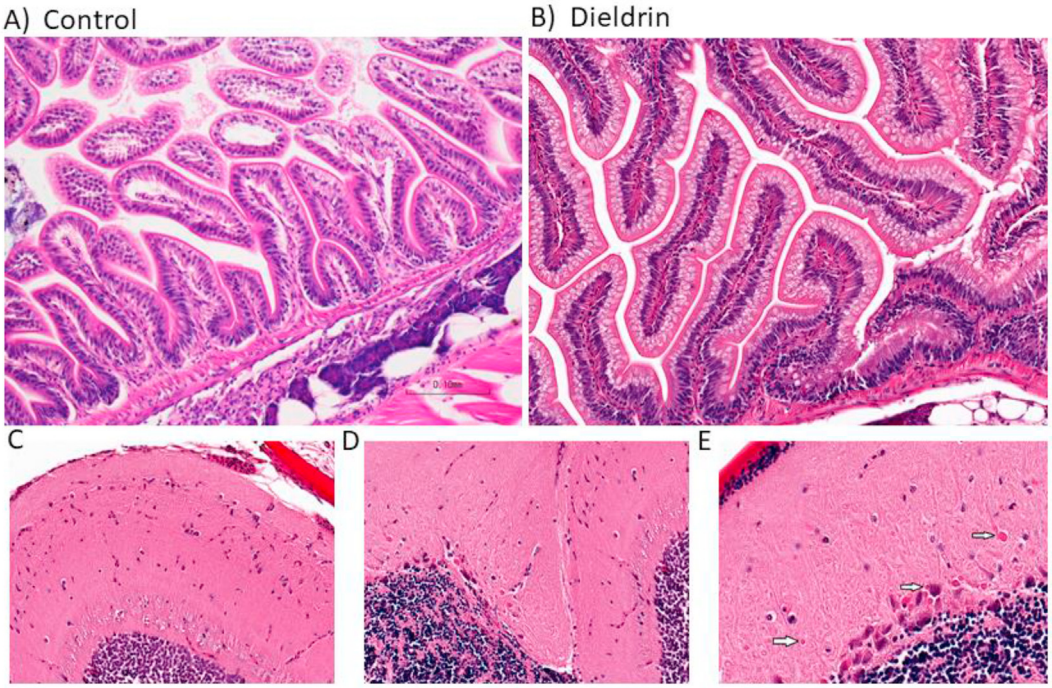

Fig. 3. Dieldrin did not cause any discernible changes in the gastrointestinal tissue (A-control fish, B-dieldrin treated fish). Several non-significant dieldrin induced abnormalities were observed in areas of the brain (C–E). Arrows point on PAS negative amorphous, acellular globules (Arrow in Fig. 3E).

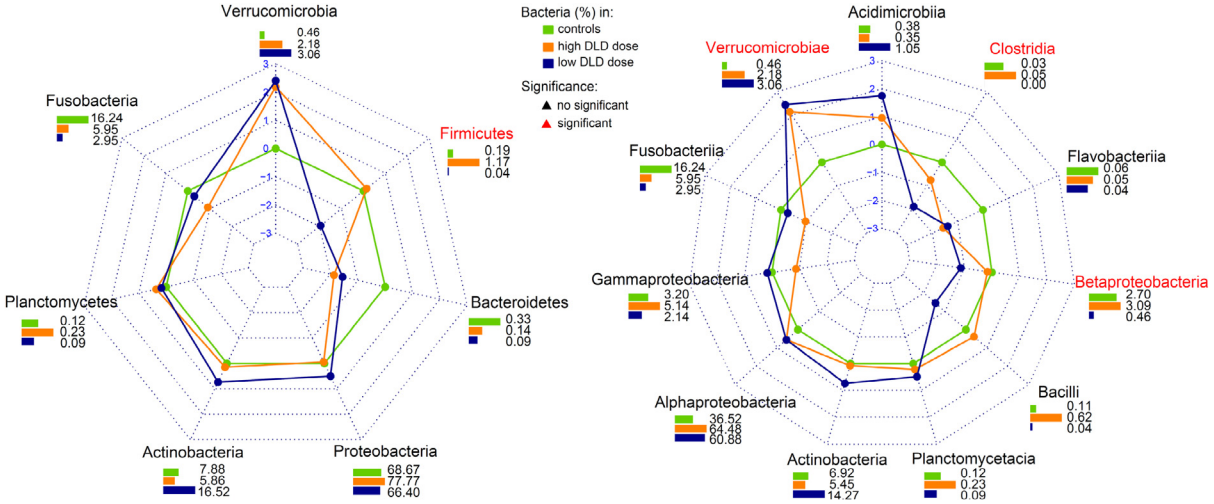

Fig. 4. Comparison of the effect of dieldrin on microbial phyla (left) and classes (right) between control and exposed groups (high dose 160 ng/g, low dose 16 ng/g). Radar charts represent the shift in the specific microbial phyla or classes expressed as the difference between median abundance of central log ratio(clr)-normalized read counts of control and exposed groups. The bars under each microbial class represent medians of the precentral abundance (%) of each phyla/class within the control or exposed group. Solely low dieldrin dose statistically significantly affected the microbial abundance (the affected phyla and classes with low dieldrin dose are marked red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

undecaprenyl–diphospho–N–acetylmuramoyl (*log2* FC = −1.80), [enzyme]–S–sulfanylcysteine (*log2* FC = −1.86), 2′-deoxyguanosine 5′-monophosphate (dGMP) (*log2* FC = −1.78), hydrogen selenide (*log2* FC = −1.86), and increased the relative metabolic turnover of selenite (*log2* FC = 1.74), xanthosine 5′-triphosphate (XTP) (*log2* FC = 1.78), methylselenic acid (*log2* FC = 1.86), coproporphyrinogen I (*log2* FC = 2.41) and [enzyme]–cysteine (*log2* FC = 1.95). The predicted shift of coproporphyrinogen I, uroporphyrinogen I and dephospho–CoA remained significant following an adjusted p-value FDR (false discovery rate < 0.05). The higher dose of dieldrin decreased the relative metabolic turnover of metabolites L–Arginyl–tRNA(Arg) (*log2* FC = −2.14) and D–Fructose 6–phosphate (*log2* FC = −2.15) (p value < 0.05) in zebrafish.

4. Discussion

The gut microbiome of the gastrointestinal system is well established to be a major factor involved in the etiology and pathogenesis of wildlife and human disease. Environmental chemicals can either negatively impact the microbiota due to

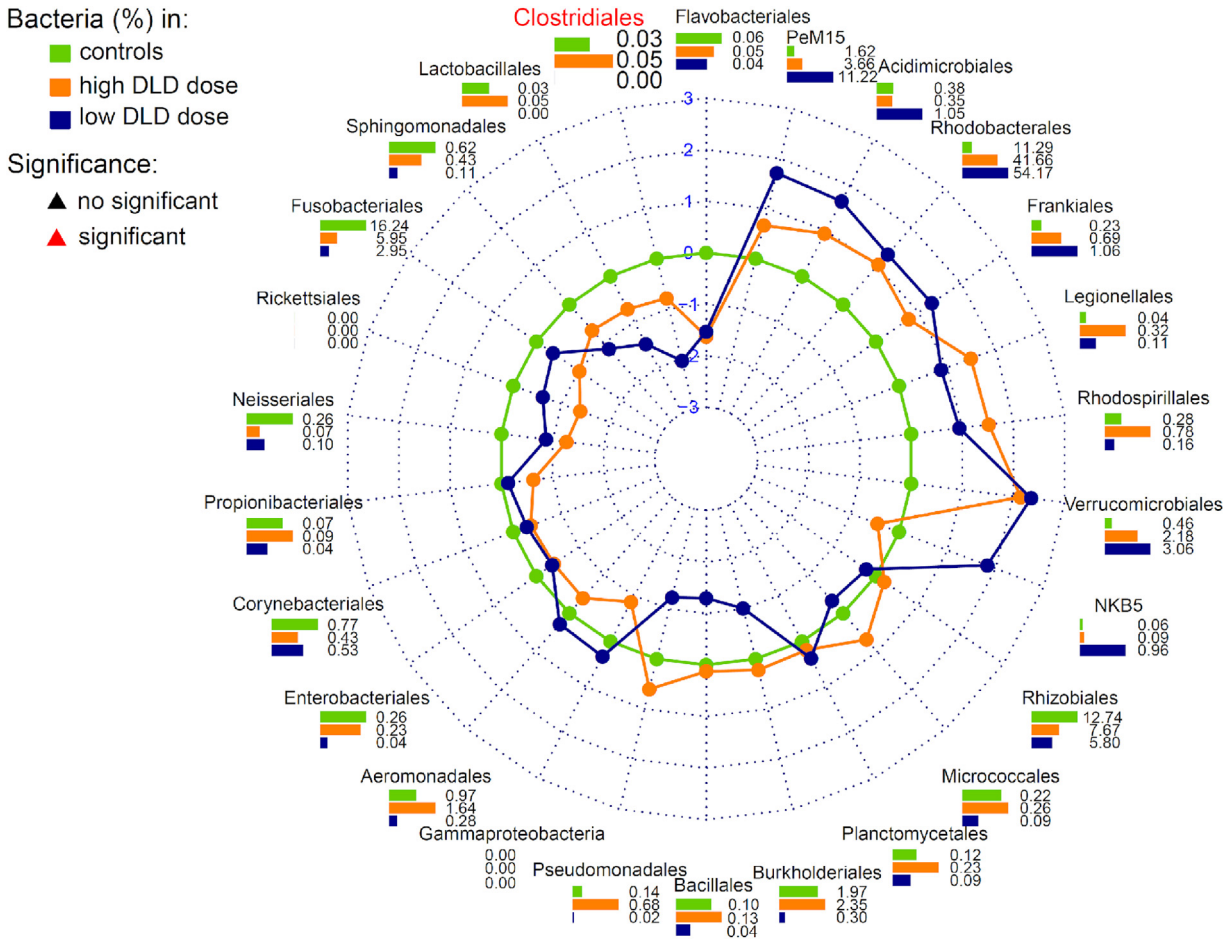

Fig. 5. Comparison of the effect of dieldrin on microbial orders between control and exposed groups (high dose 160 ng/g, low dose 16 ng/g). Radar charts represent the shift in the specific microbial orders expressed as the difference between median abundance of clr-normalized read counts of control and exposed group. The bars under each microbial order represent medians of the precentral abundance (%) of each order within the control or exposed group. Solely low dieldrin dose statistically significantly affected the microbial abundance (the affected order is marked red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

toxicity to the community, or these chemicals can be further metabolized into bioactive moieties that affect host gut interactions (Adamovsky et al., 2018). Moreover, evidence suggests that gut microbial composition and richness can affect a plethora of hormone pathways in animals and vice versa (Williams et al., 2020), which is relevant for dieldrin as organochlorine pesticides are known to be potent endocrine disruptors in fish (Martyniuk et al., 2020). Here we investigated the effects of the organochlorine pesticide dieldrin on the gastrointestinal system of zebrafish, to more completely discern the toxicity of this chemical at low, environmentally-relevant levels of dietary exposure.

While dieldrin was incorporated into zebrafish over the four-month period, there was no discernible pathology in any of the tissues examined (e.g. gastrointestinal system and all major organs). The levels of dieldrin that were incorporated into the zebrafish were 11.47 ± 1.13 ng/g and 18.32 ± 1.32 ng/g wet weight. This is similar to another feeding study in zebrafish, in which dieldrin was mixed into the feed at measured doses of 0.03, 0.15, 1.8 μg dieldrin/g d.w. feed (Cowie et al., 2017). Our targets were comparable for the two lower doses in (Cowie et al., 2017). In that study, after 21 days, body burden levels of dieldrin were calculated at 11 ± 0.005 and 58 ± 0.016 ng/g dry weight for feed doses within range or our feed. Adult largemouth bass, a much larger fish compared zebrafish, fed ~3.0 μg dieldrin/g feed for 2 months contained 360 ng dieldrin/g w.w. in the muscle (Martyniuk et al., 2010b). Thus, fish will rapidly uptake OCPs such as dieldrin and reach a steady state body burden level that can remain relatively high (Dang et al., 2016). We also did not observe any significant effect of dieldrin at these doses on growth nor histopathology. The lack of an effect on growth is consistent with another study investigating the effects of 10, 50, and 200 ng/L β-endosulfan on sexually mature 120-d-old male and female zebrafish over 21 days (Han et al., 2011), which revealed that waterborne exposure to β-endosulfan did not affect the growth of animals. However, unlike the feeding study here, Han and colleagues reported effects in the liver in terms of pathology, and there were vacuolization and some evidence of cell damage. This may be due to differences in route of exposure, OCP used, or the internal dose.

While dieldrin did not result in any specific pathology in the gastrointestinal tract, it did modulate the gastrointestinal microbiome at the lowest dose. The *Clostridia* is primarily responsible for the observed decrease in *Firmicutes* as *Clostridia* represent 95% of the *Firmicutes* phyla (Rinninella et al., 2019). The most abundant bacterial phyla *Fusobacteria* and *Alphaproteobacteria* were not sensitive to dieldrin. However, *Firmicutes* are not the dominant phyla in fish, but are one of the major bacterial phyla in human and mouse gut microbiota (Nguyen et al., 2015). These microbes are known for their major role in short chain fatty acids (SCFA) production that are beneficial metabolites for immune host homeostasis (Tremaroli and Bäckhed, 2012) and their reduced proportion

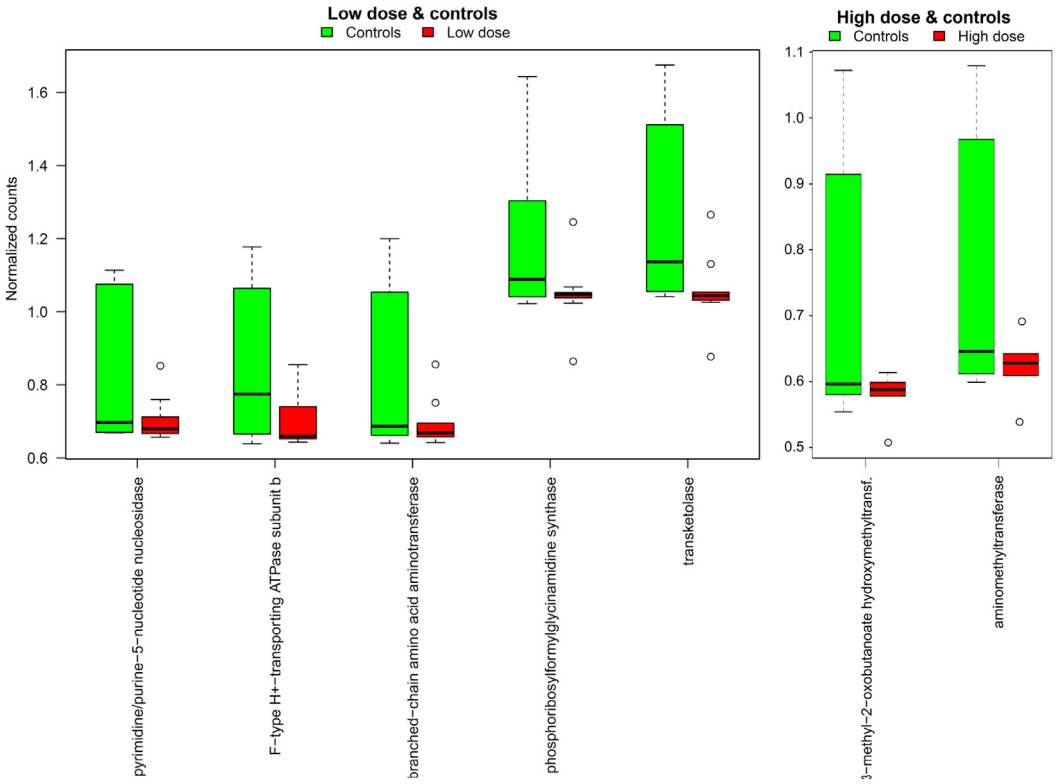

Fig. 6. The shift of the microbial community's functional capability based on predictive functional profiling (PICRUSt) as a result of dieldrin induced effect on host intestinal microbiome.

may have a significant impact on the gastrointestinal system of zebrafish. We show for the first time that *Firmicutes* in zebrafish are sensitive to organochlorine pesticides exposure. Data also suggest that *Firmicutes* are affected by exposure to other studied chemical stressors such as antibiotics (Rinninella et al., 2019) and environmental chemicals (Evariste et al., 2019) including OCPs (Kan et al., 2015). However low and high dieldrin dose share similar trends in lower taxonomical levels (family-genus, Fig. S1-S3). Diversity indexes and PCA analysis indicate that dieldrin did not cause a significant shift in overall microbial diversity (Fig.S4-S5). Taken together, while some bacterial communities were altered in abundance, the impact of environmentally-relevant dietary exposure may be subtle on the microbiome. A possible reason for these subtle impacts may be that the pesticide has reached steady state levels after 4 months of dietary exposure; as such, the microbiome may have adjusted or compensated to the steady intake and metabolism of dieldrin. We point out that the value of the study is that these doses, and body burden levels achieved in the experiment, are reflective of an ecological scenario in polluted environments such as Lake Apopka, Florida.

While the microbial shift had comparable trends between high and low doses (Figs. 4 and 5), the microbial functional content (Fig. 6) and related predicted metabolic profile (Fig. 7) differ. We implemented our concept of metabolic potential modeling for the microbial community under study, being aware of its difference from the actual realization of the model (the *gut metabolome*), since the latter is the result of a complex interplay between molecular machines of the cell hosts and of the cells constituting the gut microbiome, mainly at the transcription and expression levels of both kind of cells. The derived PRMT scores represent metabolites consumed and produced by the gut microbiome in the different experimental conditions characterizing our experimental design.

The predicted shift in the enzymatic profile indicates modulation of several microbial biosynthetic pathways. Based on the KEGG database of enzymes, the shift may target CoA biosynthesis, pyrimidine, cysteine and methionine metabolism, and metabolic pathways related to the biosynthesis of secondary metabolites. These changes may have an effect on metabolic profiles and on related metabolites. Similarly, with the taxonomical data, we revealed a more profound effect in fish from the lower dieldrin dose group compared to the higher dose. The majority of the identified affected metabolites by dieldrin belong to either to bacterial Heme biosynthesis pathway or selenium metabolism.

Employed predictive models indicate, that the lower dieldrin treatment significantly decreased Uroporphyrinogen I (Ugen I) levels and increased Coproporphyrinogen I (Cgen I) levels. Ugen I and Cgen I are closely related metabolites, precursors of heme which is an essential part of hemoglobin (Hb). In contrast to vertebrates, several types of bacteria can synthesize heme via Ugen I that is transformed to the precursor of Protoheme, Cgen I, via HemE enzyme (Dailey et al., 2015). Cgen I and its precursor Ugen I are the isomers of Ugen III and Cgen III, the metabolic intermediates in the vertebrate's biosynthesis of heme. Cgen I and Ugen I are not normally produced by the vertebrates/human body; their production and accumulation cause a type of porphyria (Di Pierro et al., 2016). Interestingly, chlorine-based chemicals like dieldrin, which include polychlorinated biphenyl mixture and the pesticide hexachlorobenzene, are used as porphyria inducing agents in vertebrates studies (Chaufan et al., 2005; Franklin et al., 1997). Our results indicate that dieldrin may affect microbial heme biosynthesis and the level of porphyrins in the host, and therefore target similar pathways that were identified in vertebrate tissues exposed

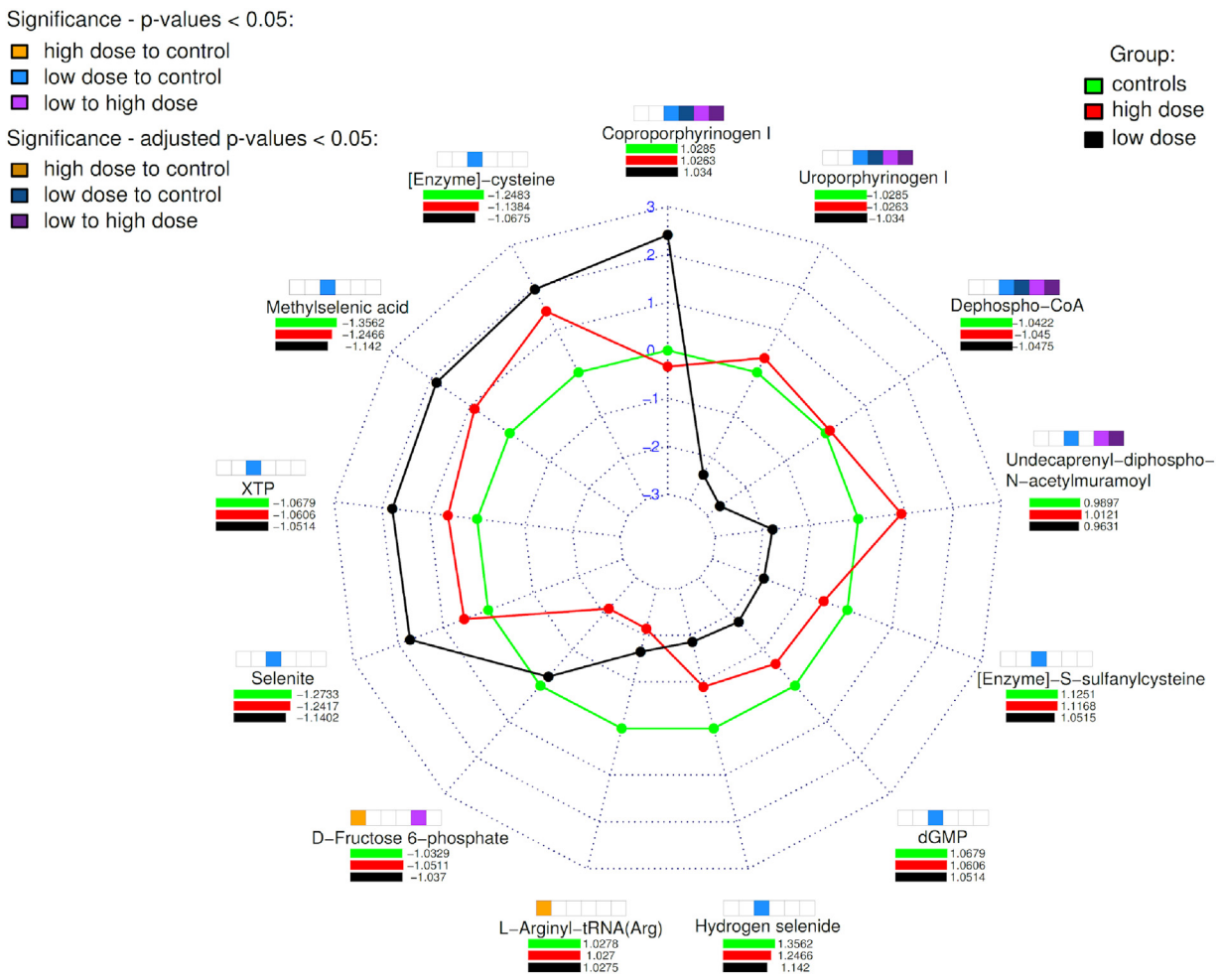

Fig. 7. Significant changes in predicted relative metabolomic turnover (PRMT) of microbial metabolites in control and dieldrin exposed groups (low dose 16 ng/g d.w., higher dose 160 ng/g d.w.). Radar charts represent the shift in the microbial metabolites expressed as the difference between cumulative distribution functions of PRMT scores between control and exposed groups. The bars under each metabolite represent medians of estimated PRMT scores of each metabolite within the control or exposed group.

to organochlorine compounds. Dieldrin may have a propensity to modulate heme biosynthesis in the host and its microbiome. The potency of OCPs to modulate the host level of heme would be the next step in strengthening this hypothesis.

Further, we identified selenium metabolism as a process significantly affected by dieldrin. Our analysis predicts decreased selenite (Se) and its reduced form hydrogen selenide (HSe), as well as an increase in methylselenic acid (MSeA). Selenium is an important element in selenocysteine-containing selenoproteins, selenium-containing tRNAs, as well as selenoenzymes (Stolz and Oremland, 1999). A decrease of selenium is associated with the decreased activity of selenium-containing enzymes such as glutathione peroxidase and its protective role against oxidative stress (Stolz and Oremland, 1999). Further, a recent study suggests that selenoproteins play essential roles in protecting specific cells (e.g. neurons) from oxidative damage (Cardoso et al., 2015).

Our computational approach also indicates other notable modulated metabolites involved in microbial energy metabolism and biosynthesis of bacterial cell wall peptidoglycan. Specifically, two metabolites are predicted to decrease, and both are involved in the acetyl coenzyme A (acetyl CoA) pathway. Dephospho-Coenzyme A (Dephospho-CoA) is an intermediate of CoA and D−Fructose 6−phosphate is a precursor of pyruvate (Wolfe, 2015). Pyruvate and CoA creates acetyl CoA, a key metabolite in energy-rich molecules generating citric cycle. Additionally, we determined that the lower dose of dieldrin may affect Undecaprenyl−diphospho−N−acetylmuramoyl, a subunit of glycans that is a part of heteropolymer peptidoglycan (Bouhss et al., 2008). The identified changes in enzymes and metabolites involved in bacterial energy metabolism may perturb the overall performance of the host-microbiome with major consequences for their intrinsic activity. Bacterial energy metabolism is a target of a wider spectrum of chemicals e.g. antibiotics that inhibit processes that deals with cellular energy output with the important downstream consequences on bacterial metabolism and bacterial growth (Lobritz et al., 2015). These hypotheses are intriguing and must be further examined in terms of animal physiology.

5. Conclusion

We show that dietary exposure to dieldrin results in bioaccumulation in zebrafish in a dose-dependent manner but did not affect the body mass or growth trajectory of the fish. Despite a lack of significant histologic changes in fish tissue, the microbiome was altered by dieldrin. The potency of OCPs to change the composition and function of host-microbiota has also been reported by others in mice (Liu et al., 2017). In agreement with our study, Liu et al. (2017) showed that OCPs change the composition and abundance of

intestinal microbiota, showing that OCPs significantly enhance the abundance of *Lactobacillus* spp. and modulate microbial function in mice (e.g. bile salt hydrolase (BSH) activity, which may affect energy homeostasis and metabolic disorder).

However, while changes were subtle in this study, composition and functional changes were observed with an environmentally relevant exposure regime. The analysis determined that the metabolic state of host-microbiome may be impacted, specifically heme, selenium, and energy metabolism. These pathways, e.g. selenium and heme metabolism, are crucial for many health aspects and their dysregulation is a marker of diseases. The long-term effect of dieldrin induced changes in microbiome on the health of wildlife should be investigated further, for example achieving a better understanding of host-microbe relationships through metatranscriptomics of the active microbiome or targeted metabolome analysis.

Author statement

Conception and design of study: Joseph H. Bisesi Jr., Christopher J. Martyniuk. Acquisition of data: Qing Hua, Ondrej Adamovsky, Jonna Boyda, Jordan T. Schmidt, Marianne Kozuch, Serena L. M. Craft, Pamela E. Ginn, Analysis and/or interpretation of data: Hana Vespalcova, Stanislav Smatana, Eva Budinska, Maria Persico. Drafting / revising the manuscript: Ondrej Adamovsky, Maria Persico, Eva Budinska, Joseph H. Bisesi Jr., Christopher J. Martyniuk

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.

Acknowledgements and Funding

The authors have no conflict of interest to declare. We thank Edward Flynn and the Animal Care Services at the University of Florida for zebrafish husbandry and technical support. This research is funded by start-up funding from the University of Florida and the College of Veterinary Medicine (CJM). The bioinformatics and statistical analyses were supported by the RECETOX research infrastructure (the Czech Ministry of Education, Youth and Sports: LM2018121), by CETOCOEN EXCELLENCE Teaming 2 project supported by Horizon2020 (857560), by the Czech ministry of Education, Youth and Sports (02.1.01/0.0/0.0/18_046/0015975) and by grant FIT-S-20-6309 (Design, Optimization and Evaluation of Application-Specific Computer Systems).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115715.

References

Adamovsky, O., Buerger, A.N., Wormington, A.M., Ector, N., Griffitt, R.J., Bisesi, J.H., Martyniuk, C.J., 2018. The gut microbiome and aquatic toxicology: an emerging concept for environmental health. Environ. Toxicol. Chem. 37, 2758–2775. https://doi.org/10.1002/etc.4249.

Adamovsky, O., Buerger, A.N., Vespalcova, H., Sohag, S.R., Hanlon, A.T., Ginn, P.E., Craft, S.L., Smatana, S., Budinska, E., Persico, M., Bisesi, J.H., Martyniuk, C.J., 2020. Evaluation of microbiome-host relationships in the zebrafish gastrointestinal system reveals adaptive immunity is a target of bis(2-ethylhexyl) phthalate (DEHP) exposure. Environ. Sci. Technol. 54, 5719–5728. https://doi.org/10.1021/acs.est.0c00628.

Ahn, Y.G., Shin, J.H., Kim, H.Y., Khim, J., Lee, M.K., Hong, J., 2007. Application of solid-phase extraction coupled with freezing-lipid filtration clean-up for the determination of endocrine-disrupting phenols in fish. Anal. Chim. Acta 603, 67–75. https://doi.org/10.1016/j.aca.2007.09.045.

Aronesty, E., 2011. ea-utils: Command-line tools for processing biological sequencing data. Expr. Anal. Durham. http://code.google.com/p/ea-utils.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bouhss, A., Trunkfield, A.E., Bugg, T.D.H., Mengin-Lecreulx, D., 2008. The biosynthesis of peptidoglycan lipid-linked intermediates. FEMS Microbiol. Rev. 32, 208–233. https://doi.org/10.1111/j.1574-6976.2007.00089.x.

Brown-Peterson, N.J., Krasnec, M., Takeshita, R., Ryan, C.N., Griffitt, K.J., Lay, C., Mayer, G.D., Bayha, K.M., Hawkins, W.E., Lipton, I., Morris, J., Griffitt, R.J., 2015. A multiple endpoint analysis of the effects of chronic exposure to sediment contaminated with Deepwater Horizon oil on juvenile Southern flounder and their associated microbiomes. Aquat. Toxicol. 165, 197–209. https://doi.org/10.1016/j.aquatox.2015.06.001.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pēa, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. https://doi.org/10.1038/nmeth.f.303.

Cardoso, B.R., Roberts, B.R., Bush, A.I., Hare, D.J., 2015. Selenium, selenoproteins and neurodegenerative diseases. Metall 7, 1213–1228. https://doi.org/10.1039/C5MT00075K.

Chaufan, G., Corvi, M.M., San Martín De Viale, L.C., Cárdenas, M.L., Ríos De Molina, M.D.C., 2005. Abnormal kinetic behavior of uroporphyrinogen decarboxylase obtained from rats with hexachlorobenzene-induced porphyria. J. Biochem. Mol. Toxicol. 19, 19–24. https://doi.org/10.1002/jbt.20055.

Chopra, A.K., Sharma, M.K., Chamoli, S., 2011. Bioaccumulation of organochlorine pesticides in aquatic system—an overview. Environ. Monit. Assess. 173, 905–916. https://doi.org/10.1007/s10661-010-1433-4.

Claus, S.P., Guillou, H., Ellero-Simatos, S., 2016. The gut microbiota: a major player in the toxicity of environmental pollutants? NPJ Biofilms Microbiomes 2, 16003. https://doi.org/10.1038/npjbiofilms.2016.3.

Cowie, A.M., Sarty, K.I., Mercer, A., Koh, J., Kidd, K.A., Martyniuk, C.J., 2017. Molecular networks related to the immune system and mitochondria are targets for the pesticide dieldrin in the zebrafish (Danio rerio) central nervous system. J. Proteomics 157, 71–82. https://doi.org/10.1016/j.jprot.2017.02.003.

Dailey, H.A., Gerdes, S., Dailey, T.A., Burch, J.S., Phillips, J.D., 2015. Noncanonical coproporphyrin-dependent bacterial heme biosynthesis pathway that does not use protoporphyrin. Proc. Natl. Acad. Sci. U.S.A. 112, 2210–2215. https://doi.org/10.1073/pnas.1416285112.

Dang, V.D., Kroll, K.J., Supowit, S.D., Halden, R.U., Denslow, N.D., 2016. Tissue distribution of organochlorine pesticides in largemouth bass (Micropterus salmoides) from laboratory exposure and a contaminated lake. Environ. Pollut. 216, 877–883. https://doi.org/10.1016/j.envpol.2016.06.061.

DeBofsky, A., Xie, Y., Grimard, C., Alcaraz, A.J., Brinkmann, M., Hecker, M., Giesy, J.P., 2020. Differential responses of gut microbiota of male and female fathead minnow (Pimephales promelas) to a short-term environmentally-relevant, aqueous exposure to benzo [a] pyrene. Chemosphere 126461.

Di Pierro, E., Brancaleoni, V., Granata, F., 2016. Advances in understanding the pathogenesis of congenital erythropoietic porphyria. Br. J. Haematol. 173, 365–379. https://doi.org/10.1111/bjh.13978.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Evariste, L., Barret, M., Mottier, A., Mouchet, F., Gauthier, L., Pinelli, E., 2019. Gut Microbiota of Aquatic Organisms: a key endpoint for ecotoxicological studies. Environ. Pollut. 248, 989–999. https://doi.org/10.1016/j.envpol.2019.02.101.

Franklin, M.R., Phillips, J.D., Kushner, J.P., 1997. Cytochrome P450 induction, uroporphyrinogen decarboxylase depression, porphyrin accumulation and excretion, and gender influence in a 3-week rat model of porphyria cutanea tarda. Toxicol. Appl. Pharmacol. 147, 289–299. https://doi.org/10.1006/taap.1997.8282.

Gaulke, C.A., Barton, C.L., Proffitt, S., Tanguay, R.L., Sharpton, T.J., 2016a. Triclosan exposure is associated with rapid restructuring of the microbiome in Adult zebrafish. PloS One 11, e0154632. https://doi.org/10.1371/journal.pone.0154632.

Gaulke, C.A., Barton, C.L., Proffitt, S., Tanguay, R.L., Sharpton, T.J., 2016b. Triclosan exposure is associated with rapid restructuring of the microbiome in Adult zebrafish. PloS One 11, e0154632. https://doi.org/10.1371/journal.pone.0154632.

Han, Z., Jiao, S., Kong, D., Shan, Z., Zhang, X., 2011. Effects of β-endosulfan on the growth and reproduction of zebrafish (Danio rerio). Environ. Toxicol. Chem. 30, 2525–2531. https://doi.org/10.1002/etc.646.

Hong, J., Kim, H.Y., Kim, D.G., Seo, J., Kim, K.J., 2004. Rapid determination of chlorinated pesticides in fish by freezing-lipid filtration, solid-phase extraction and gas chromatography-mass spectrometry. J. Chromatogr. A. https://doi.org/10.1016/j.chroma.2004.03.003.

Jayaraj, R., Megha, P., Sreedev, P., 2016. Review Article. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdiscipl. Toxicol. 9, 90–100. https://doi.org/10.1515/intox-2016-0012.

Jones, M.C., Aitchison, J., 1987. The statistical analysis of compositional data. J. R. Stat. Soc. Ser. A 150, 396. https://doi.org/10.2307/2982045.

Kan, H., Zhao, F., Zhang, X.-X., Ren, H., Gao, S., 2015. Correlations of gut microbial community shift with hepatic damage and growth inhibition of Carassius auratus induced by pentachlorophenol exposure. Environ. Sci. Technol. 49, 11894–11902. https://doi.org/10.1021/acs.est.5b02990.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31, 814–821. https://doi.org/10.1038/nbt.2676.

Larsen, P.E., Collart, F.R., Field, D., Meyer, F., Keegan, K.P., Henry, C.S., McGrath, J., Quinn, J., Gilbert, J.A., 2011. Predicted Relative Metabolomic Turnover (PRMT): determining metabolic turnover from a coastal marine metagenomic dataset. Microb. Inf. Exp. 1, 4. https://doi.org/10.1186/2042-5783-1-4.

Liu, Q., Shao, W., Zhang, C., Xu, C., Wang, Q., Liu, H., Sun, H., Jiang, Z., Gu, A., 2017. Organochloride pesticides modulated gut microbiota and influenced bile acid metabolism in mice. Environ. Pollut. https://doi.org/10.1016/j.envpol.2017.03.068.

Lobritz, M.A., Belenky, P., Porter, C.B.M., Gutierrez, A., Yang, J.H., Schwarz, E.G., Dwyer, D.J., Khalil, A.S., Collins, J.J., 2015. Antibiotic efficacy is linked to bacterial cellular respiration. Proc. Natl. Acad. Sci. Unit. States Am. 112, 8173–8180. https://doi.org/10.1073/pnas.1509743112.

Manor, O., Borenstein, E., 2015. MUSiCC: a marker genes based framework for metagenomic normalization and accurate profiling of gene abundances in the microbiome. Genome Biol. 16, 1–20. https://doi.org/10.1186/s13059-015-0610-8.

Martyniuk, C.J., Feswick, A., Spade, D.J., Kroll, K.J., Barber, D.S., Denslow, N.D., 2010a. Effects of acute dieldrin exposure on neurotransmitters and global gene transcription in largemouth bass (Micropterus salmoides) hypothalamus. Neurotoxicology 31, 356–366. https://doi.org/10.1016/j.neuro.2010.04.008.

Martyniuk, C.J., Kroll, K.J., Doperalski, N.J., Barber, D.S., Denslow, N.D., 2010b. Genomic and proteomic responses to environmentally relevant exposures to dieldrin: indicators of neurodegeneration? Toxicol. Sci. 117, 190–199. https://doi.org/10.1093/toxsci/kfq192.

Martyniuk, C.J., Doperalski, N.J., Kroll, K.J., Barber, D.S., Denslow, N.D., 2013. Sexually dimorphic transcriptomic responses in the teleostean hypothalamus: a case study with the organochlorine pesticide dieldrin. Neurotoxicology. https://doi.org/10.1016/j.neuro.2012.09.012.

Martyniuk, C.J., Doperalski, N.J., Prucha, M.S., Zhang, J.L., Kroll, K.J., Conrow, R., Barber, D.S., Denslow, N.D., 2016. High contaminant loads in Lake Apopka's riparian wetland disrupt gene networks involved in reproduction and immune function in largemouth bass. Comp. Biochem. Physiol. Genom. Proteonomics 19, 140–150.

Martyniuk, C.J., Mehinto, A.C., Denslow, N.D., 2020. Organochlorine pesticides: agrochemicals with potent endocrine-disrupting properties in fish. Mol. Cell. Endocrinol. 507, 110764. https://doi.org/10.1016/j.mce.2020.110764.

Nguyen, T.L.A., Vieira-Silva, S., Liston, A., Raes, J., 2015. How informative is the mouse for human gut microbiota research? Dis. Model. Mech. 8, 1–16. https://doi.org/10.1242/dmm.017400.

Noecker, C., Eng, A., Srinivasan, S., Theriot, C.M., Young, V.B., Jansson, J.K., Fredricks, D.N., Borenstein, E., 2016. Metabolic model-based integration of microbiome taxonomic and metabolomic profiles elucidates mechanistic links between ecological and metabolic variation. mSystems 1, 1–17. https://doi.org/10.1128/mSystems.00013-15.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glockner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. https://doi.org/10.1093/nar/gkm864.

R Development Core Team, R., 2018. R: A Language and Environment for Statistical Computing, R Core Team. https://doi.org/10.1007/978-3-540-74686-7.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G., Gasbarrini, A., Mele, M., 2019. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 7, 14. https://doi.org/10.3390/microorganisms7010014.

Sapozhnikova, Y., Bawardi, O., Schlenk, D., 2004. Pesticides and PCBs in sediments and fish from the salton sea, California, USA. Chemosphere 55, 797–809. https://doi.org/10.1016/j.chemosphere.2003.12.009.

Slade, L., Cowie, A., Martyniuk, C.J., Kienesberger, P.C., Pulinilkunnil, T., 2017. Dieldrin augments mTOR signaling and regulates genes associated with cardiovascular Disease in the Adult zebrafish heart (Danio rerio). J. Pharmacol. Exp. Therapeut. 361, 375–385. https://doi.org/10.1124/jpet.116.239806.

Stolz, J.F., Oremland, R.S., 1999. Bacterial respiration of arsenic and selenium. FEMS Microbiol. Rev. 23, 615–627. https://doi.org/10.1016/S0168-6445(99)00024-8.

Tremaroli, V., Bäckhed, F., 2012. Functional interactions between the gut microbiota and host metabolism. Nature 489, 242–249. https://doi.org/10.1038/nature11552.

Williams, C.L., Garcia-Reyero, N., Martyniuk, C.J., Tubbs, C.W., Bisesi, J.H., 2020. Regulation of endocrine systems by the microbiome: perspectives from comparative animal models. Gen. Comp. Endocrinol. 292, 113437. https://doi.org/10.1016/j.ygcen.2020.113437.

Wolfe, A.J., 2015. Glycolysis for microbiome generation. Microbiol. Spectr. 3 https://doi.org/10.1128/microbiolspec.MBP-0014-2014.