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Effects of *in situ* experimental selenium exposure on finescale dace (*Phoxinus neogaeus*) gut microbiome

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ABSTRACT

Selenium (Se) is an environmental contaminant of global concern that can cause adverse effects in fish at elevated levels. Fish gut microbiome play essential roles in gastrointestinal function and host health and can be perturbed by environmental contaminants, including metals and metalloids. Here, an *in-situ* Se exposure of female finescale dace (*Phoxinus neogaeus*) using mesocosms was conducted to determine the impacts of Se accumulation on the gut microbiome and morphometric endpoints. Prior to this study, the gut microbiome of finescale dace, a widespread Cyprinid throughout North America, had not been characterized. Exposure to Se caused a hormetic response of alpha diversity of the gut microbiome, with greater diversity at the lesser concentration of 1.6 μg Se/L, relative to that of fish exposed to the greater concentration of 5.6 μg Se/L. Select gut microbiome taxa of fish were differentially abundant between aqueous exposure concentrations and significantly correlated with liver-somatic index (LSI). The potential effects of gut microbiome dysbiosis on condition of wild fish might be a consideration when assessing adverse effects of Se in aquatic environments. More research regarding effects of Se on field-collected fish gut microbiome and the potential adverse effects or benefits on the host is warranted.

1. Introduction

Selenium (Se) is an essential micronutrient for most aquatic organisms, but elevated Se exposure can cause adverse effects in fish, with a narrow range between essential and toxic levels (Janz et al., 2010). Several anthropogenic activities including coal and metal mining, oil and gas extraction, and agricultural and irrigation practices can lead to elevated amounts of Se in aquatic environments (Yudovich and Ketris, 2006; Presser and Luoma, 2010; Young et al., 2010). Release of excess Se during these activities, typically in the form of the oxyanions selenate (SeVI) or selenite (SeIV), followed by efficient uptake, biotransformation (to organoselenium compounds) and trophic transfer from biofilm to invertebrates can lead to bioaccumulation of organoselenium compounds in fishes (Meseck and Cutter, 2006; Presser and Luoma, 2006, 2010). Greater exposures to Se can result in teratogenicity of fishes. Selenium is transferred maternally to eggs, and larval fish are exposed after hatch and during the absorption of egg yolk, leading to spinal and fin deformities or edema (Lemly, 1993; Hamilton, 2003; Janz et al., 2010). While the reproductive effects of Se on fish have been well documented, effects of excess Se on other aspects of fish health, such as gut microbiota, have rarely been examined in wild fish.

Effects resulting from Se exposure is a prototypical example of hormesis, wherein Se deficiency is lethal to most organisms, and small amounts of Se are necessary for physiological functioning, particularly in responses to oxidative stress; however, excess amounts of Se are deleterious to most organisms (Hodson and Hilton, 1983; Lobanov et al.,

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2009). Low-dose supplementation with Se can enhance growth and prevent oxidative stress in mice and carp (*Cyprinus carpio*) (Kasaikina et al., 2011; Saffari et al., 2017; Zhai et al., 2018; Liu et al., 2021), as well as increase alpha-diversity of gut microbiota and proportion of beneficial bacteria associated with production of short-chain fatty acids (SCFA) (Kasaikina et al., 2011; Zhai et al., 2018; Liu et al., 2021). Fish are exposed to Se mainly through their diet (Hamilton, 2004), making the gut an important site of interaction with Se. Other than nutrition, there are still knowledge gaps regarding the ecotoxicological effects of Se on gut microbiota of wild fish.

Gut microbiomes of wild fishes could become a key part of ecotoxicological assessments of aquatic environments. Fish have complex gut microbiomes that provide essential functions for their host, such as acquisition of nutrients, performance of gut mucosal barrier, and immune response (Sehnal et al., 2021). Regardless of host factors, gut microbiota can be influenced by environmental factors, including habitat, diet, and pollution (Romero et al., 2014; Egerton et al., 2018; Kim et al., 2021). Dysbiosis of gut microbiomes can arise from exposure to contaminants, which then results in impairment of normal functioning of the gut microflora, which can lead to adverse effects on condition of the host fish (Adamovsky et al., 2018; Sehnal et al., 2021). Studies on wild fishes are needed to incorporate the gut microbiome into ecotoxicological assessments. To our knowledge, this is the first study to describe the gut microbiome of the finescale dace while also being in a natural environment. The results of the study provide insight into the gut bacterial community of a Cyprinid that is widespread in waterbodies across Canada (Scott and Crossman, 1973).

The objective of this study was to evaluate the response of the gut microbiome of female finescale dace (*Phoxinus neogaeus*), to an ecologically realistic, whole food-web exposure of Se added to water as selenite. The present study was done as part of a larger project conducted at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA), Ontario, Canada to investigate bioaccumulation, trophic transfer, and toxic effects of Se in boreal lake food webs (Graves et al., 2021a, 2021b). Only female finescale dace were used in the present study because the most relevant exposure route of fishes to Se is via maternal transfer of Se from adult females to eggs (Janz et al., 2010). Selenite (Se(IV)) was used because it is one of the most common aqueous forms of Se present in boreal lakes (Ponton and Hare, 2013; Graves et al., 2021a). Finescale dace were exposed to incremental concentrations of waterborne Se(IV) in limnocorrals (*in situ* enclosures) located in Lake 239 at IISD-ELA for 63 days (Graves et al., 2021a). The specific objectives of this study were to: (1) profile the gut microbiome of female finescale dace; (2) assess alpha-diversity of the gut microbiome in response to aqueous Se exposure and dietary exposure from invertebrate prey (exposure modeled as average total Se) (TSe); (3) detail responses of the microbiome community and taxa after exposure to Se and co-variance with selected morphometric endpoints. Gut microbial communities of fish were characterized using 16S rDNA amplicon sequencing. It was hypothesized that exposure to Se would alter the gut microbiome of fish through shifts in alpha and beta-diversity and community composition.

2. Materials and methods

2.1. Experimental design

The present study was conducted at the IISD-ELA, an area of 58 boreal lakes located in northwestern Ontario, Canada set aside for whole-ecosystem experimentation (49°41′45.0″ N, 93°46′03.4" W) (Schindler et al., 1996; Kidd et al., 2007). The experimental design and treatment regime have been previously described in detail (Graves et al., 2021a). Briefly, nine limnocorrals (2 m wide x 1 m deep, ~3100 L) were set up in a sheltered bay within the small, oligotrophic Lake 239 in May of 2018. Six limnocorrals were randomly assigned nominal treatments of 0.5, 1, 2, 4, 7 or 10 μg Se/L as selenite (SeO_3^{2-}; Se(IV)), and three limnocorrals were left untreated as controls with background levels of TSe of 0.08, 0.08 or 0.09 μg Se/L. In June 2018, Se was added to limnocorrals as an aqueous solution of sodium selenite (Na_2SeO_3; CAS = 10102-18-8) (Sigma Aldrich, Oakville, ON, Canada). Throughout the 63-day experiment, dissolved water TSe was measured, and selenite was added as needed to maintain nominal concentrations. Measured mean aqueous concentrations for the respective treatments were 0.4, 0.8, 1.6, 3.4, 5.6, and 7.9 μg TSe/L. Throughout the 63-day experiment, water, sediment, benthic macroinvertebrates, zooplankton, and fish were collected to measure bioaccumulation of Se, as described previously (Graves et al., 2021a).

2.2. Fish exposure, sample collection, assessment of exposure to Se and fish condition

On day 21 of the study, five mature female finescale dace were added to each limnocorral so that fish were exposed to the "selenized" food web for 42 days. Fish were added on day 21 to allow lower trophic level organisms to reach pseudo-steady state concentrations of Se. On days 35 and 49 of the experimental period, one fish was re-captured and on day 63, three fish were re-captured from each enclosure using baited "gee" type minnow traps, with bait held in inaccessible containers so that it could not be consumed by fish, that were suspended in the water column. Fish were transported to the laboratory where they were weighed, fork length measured, and then euthanized via overdose (0.4 g/L) of pH (=7.0) buffered MS-222 followed by spinal severance. A portion of dorsal muscle tissue was taken for TSe analysis, liver was excised and weighed, and ovary tissue was excised, weighed, and frozen at −20 °C for TSe analysis. Length and weight measurements were used to calculate Fulton's condition factor (K), liver-somatic index (LSI) and gonadosomatic index (GSI; equations (1)–(3)).

$$K = 100 \times (\text{body mass (g)}/\text{fork length (mm)}^3) \quad (1).$$

$$LSI = 100 \times (\text{liver mass (g)}/\text{body mass(g)}) \quad (2).$$

$$GSI = 100 \times (\text{ovary mass (g)}/\text{body mass(g)}) \quad (3).$$

Gut tissue and contents were excised using decontaminated equipment (15% bleach solution and 70% ethanol) and methods outlined previously (DeBofsky et al., 2020a). Guts including tissue and contents were preserved in LifeGuard® Solution (Qiagen, Germany) and stored at −20 °C prior to extraction of DNA for 16S rRNA amplicon sequencing. All experiments that used live fish were approved by the Animal Research Ethics Board at the University of Saskatchewan (protocol # 20170046) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Concentrations of TSe in water, invertebrates and fish tissues were measured by use of inductively coupled plasma – mass spectrometry (ICP-MS). Detailed protocols and quality assurance/quality control measures are provided in Graves et al. (2021a). Concentrations of TSe dissolved in water were reported as μg/L, while those in tissue were reported as μg/g dry mass (dm). To determine the relationships between accumulation of Se and the and fish gut microbiome, mean concentrations of TSe for all analyzed invertebrate taxa were used as a proxy for dietary TSe.

2.3. Isolation of DNA, PCR amplification, and next-generation sequencing

Total DNA was extracted from collected gut tissues using the DNeasy Powersoil Kit (Qiagen) utilizing extraction blanks for quality control (QC). PCR amplification was performed on normalized DNA samples using unique dual-tagged primer set targeting the V3–V4 hypervariable region of the 16S rRNA gene, with the forward primer Bact-341: (5′-CCTACGGGNGGCWGCAG-3′) (Klindworth et al., 2013) and reverse primer Bact-806: (5′-GGACTACNVGGGTWTCTAAT-3′) (Fadrosh et al., 2014; Apprill et al., 2015). PCR products were checked with agarose gel electrophoresis and purified using the QIAquick PCR Purification kit (Qiagen). Construction of the sequencing library and next-generation

sequencing by use of Illumina MiSeq platform (Illumina, USA) and 600-cycle MiSeq Reagent Kit v3 was performed as described previously (DeBofsky et al., 2020a), with extraction and PCR blanks included. Sequencing data can be accessed at https://doi.org/10.20383/102.0529 with sequence read output included in the supporting information (SI-1, Table S1).

2.4. Bioinformatics

Raw reads were demultiplexed based on dual tags of both forward and reverse primers for each sample using fastq-multx (version 1.3.1). Paired-end sequences were then merged using VSEARCH (version 2.14.2), with forward and reverse primers removed and sequences filtered to remove lesser quality (expected error >1.0), chimeras, and shorter length (<400 bp) sequences thereafter (Rognes et al., 2016). Zero-radius operational taxonomic units, or exact sequence variants (ESVs), were generated using unoise3, with a minimum frequency of 5 (Edgar, 2016). Taxonomic annotation was conducted using classify-consensus-vsearch against the Silva database (version 132) (Quast et al., 2013; Bolyen et al., 2019). ESVs annotated as Bacteria were retained, with subsequent ESVs assigned to either Chloroplast or Mitochondria removed. ESVs with an overall frequency of less than 10 or found in only one sample were filtered out. Rarefaction at 14,226 was conducted to avoid bias introduced by uneven sequencing depth. Details on sequence read output can be found in the supporting information (SI-1).

2.5. Statistical analyses

All statistical and graphical analyses were performed using the R software environment (version 4.0.3: RStudio Team, 2021) using ggplot2 (version 3.3.3) for data visualization (Wickham, 2011). Alpha was set at 0.05 for all statistical tests. The relationships between continuous variables, including morphometric endpoints and concentrations of TSe in muscle or ovary, were assessed using simple linear regression (LR). Choa1 diversity index for fish microbiome was computed using package iNEXT (version 2.0.20) function ChaoRichness (Hsieh et al., 2016). Analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) was used to test for differences in varables among treatments, with assumptions of normal distribution and homogeneity of variance being met.

Due to abnormally high Chao1 diversity for one fish collected from a lesser exposure limnocorral (i.e., 0.80 μg/L), the sample was removed from subsequent analysis (Fig. S1) with anticipation of analytical artifact or abnormally perturbed fish. The greatest exposure group (7.9 μg/L) and two least concentration exposure groups (0.4 and 0.8 μg/L) were also entirely removed from subsequent analysis, as only one fish ($n = 1$) was successfully collected and sequenced to adequate depth for each treatment (rarefied sequence depth = 14,226). Final sample counts were $n = 17$, with treatment groups 1.6 μg/L ($n = 3$), 3.4 μg/L ($n = 3$), 5.6 μg/L ($n = 3$), and control ($n = 8$). Model selection for Choa1 diversity in relation to average invertebrate TSe was done using Akaike's Information Criterion (AIC) for selection of quadratic polynomial. ANOVA was used to test for the significance of the best-fit model terms.

Principal coordinate analysis (PCoA) of unweighted UniFrac distance was used to present beta-diversities in feature-level composition between treatments (McMurdie and Holmes, 2013). PERMANOVA was used to test for differences in beta-diversities for treatments (9999 permutations, unweighted UniFrac distance matrix) followed by pairwise PERMANOVA using false discovery rate (FDR) adjusted p-values (Martinez Arbizu, 2017). To determine differential proportion of abundant taxa at family-level or genus-level (>0.1%) between treatments including control, a linear discriminant analysis (LDA) effect size (LEfSe) was performed using the non-strict approach to differentiate taxa, using one-against-all method with threshold for LDA score and alpha values set to default of 2.0 and 0.05, respectively (Segata et al., 2011). Spearman correlations with adjusted FDR p-values were used to test relationships between proportion of abundant families and genera (mean relative abundance ≥1%), with TSe, K, and LSI. K and LSI were chosen using constrained principal coordinates analysis (CAP; function capscale) and were included in the best fit model using function ordistep and forward selection (SI-2).

3. Results

3.1. Overall fish condition and exposure

After 42 days of exposure to Se, fish condition factor ranged from 0.78 to 1.01, GSI ranged from 0.48 to 2.33% and LSI ranged from 0.65 to 2.02% among Se treatments (Fig. S2). None of these apical endpoints were significantly related to concentrations of Se in muscle (LR, $p = 0.507$ to 0.859, Fig. S2) or ovary (LR, $p = 0.685$ to 0.971, Fig. S2).

3.2. Gut microbiome of wild female finescale dace

The gut microbiome of female finescale dace was dominated by phyla *Proteobacteria* (average ± standard deviation; 54.5 ± 25.6%, Fig. 1A), *Firmicutes* (26.2 ± 22.3%), *Cyanobacteria* (7.17 ± 15.6%), *Planctomycetes* (4.69 ± 8.49%), and *Fusobacteria* (3.66 ± 14.1%). The most abundant families were *Aeromonadaceae* (31.1 ± 26.2%, Fig. 1B), *Bacillaceae* (14.6 ± 19.5%), *Enterobacteriaceae* (8.93 ± 11.4%), *Clostridiaceae* (8.30 ± 15.1%), and *Phormidiaceae* (4.33 ± 14.4%). *Fusobacteriaceae* had the greatest relative abundance in limnocorrals with the greatest concentrations of Se (5.6 μg/L; 19.6 ± 33.4%) compared to the rest of the treatment groups and the control (0.225 ± 0.109%). Genera with greater relative abundances among experimental groups were *Aeromonas* (31.1 ± 6.36%, Fig. 1C), *Exiguobacterium* (9.40 ± 4.20%), *Clostridium* (8.28 ± 3.65%), *Raoultella* (5.70 ± 2.62%), and *Bacillus* (5.15 ± 3.19%).

3.3. Effects of selenium on alpha-diversity of gut microbiome

Exposure to Se altered Chao1 indices among select treatments. The Chao1 diversity index differed significantly among experimental groups (ANOVA, $F_{3,13} = 4.46$, $p = 0.0230$, Fig. 2A). Microbiomes in guts of fish exposed to 1.6 μg Se/L exhibited greater Chao1 diversity index relative to 5.6 μg/L and Control (Tukey HSD, $p < 0.05$). A quadratic polynomial fitted response of Chao1 diversity index relative to mean concentration of TSe in invertebrates was observed for the gut bacterial community (Fig. 2B). The linear relationship (i.e., first term) was not significant ($F_{1,17} = 0.216$, $p = 0.649$), but the second, quadratic term was significant ($F_{1,16} = 11.7$, $p = 0.0041$) (Table S2).

3.4. Responses of the microbiota community and taxa to selenium

Exposure to Se significantly altered beta-diversities, as indicated by unweighted UniFrac distance of the gut microbiome (PERMANOVA, $F_{3,13} = 1.69$, $p = 0.0052$, Fig. 3A). The control and 1.6 μg/L groups differed in centroid position (Table S3, pairwise PERMANOVA $F_{1,9} = 2.11$, $p = 0.0217$), with the control and 5.4 μg/L treatment groups being marginally different although not significant (pairwise PERMANOVA, $F_{1,9} = 1.47$, $p = 0.0548$). Using LEfSe analysis, two families, *Chitinibacteraceae* and *Lactobacillaceae*, were identified as being associated with the control and 1.6 μg Se/L, respectively (Figs. S3 and S4). Four genera were found to be associated with various treatments, including *Iodobacter* for control, *Exiguobacterium* and *Lactobacillus* for 1.6 μg Se/L, and *Plesiomonas* for 3.4 μg Se/L (Figs. S3 and S5). Families *Chromobacteriaceae* (Fig. 3B, LR, R = 0.649, Adj-p = 0.0337) and *Peptostreptococcaceae* (Fig. 3B, R = −0.766, Adj-p = 4.70e-3) were positively and negatively correlated with LSI, respectively. Three genera were significantly correlated with LSI, with *Raoultella* and *Paludibacterium* being positively correlated (Fig. 3C, R ≥ 0.727, Adj-p ≤ 6.47e-3) and

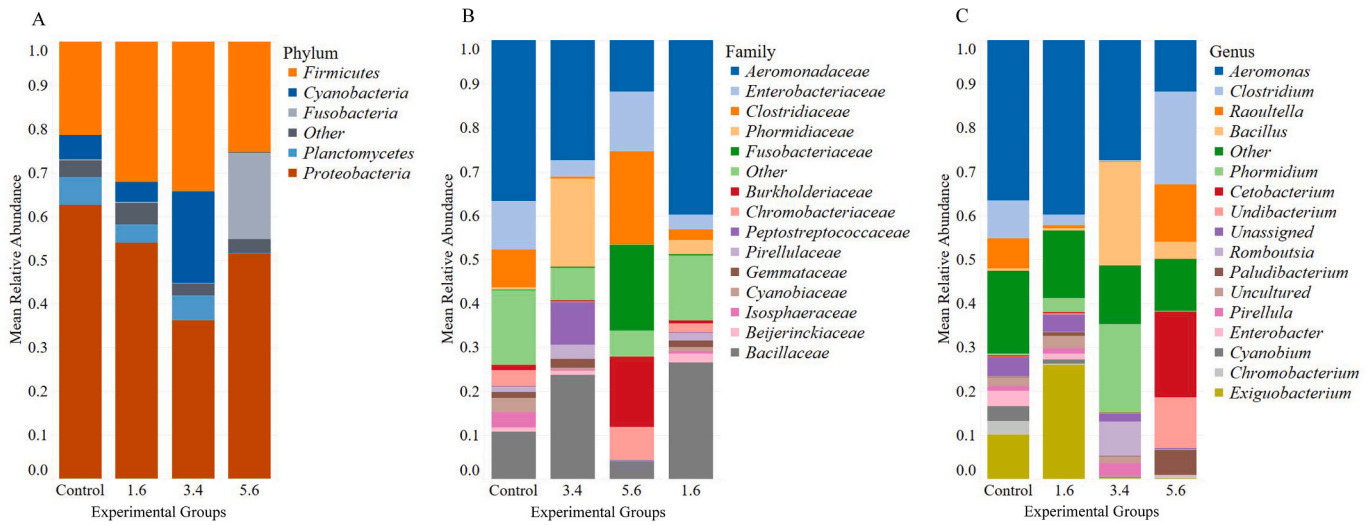

Fig. 1. Relative abundances of gut microbiota among experimental groups (i.e., Control (n = 8), 1.6 (n = 3), 3.4 (n = 3), or 5.6 (n = 3) μg Se/L). (A) Phylum-level relative abundance for individual samples. "Other" indicates taxa for each respective level with overall mean relative abundance less than 3%. (B) Family-level relative abundance data for respective treatments. (C) Genus-level relative abundance data for respective treatments. "Other" indicates taxa for each respective level with overall mean relative abundance less than 1%.

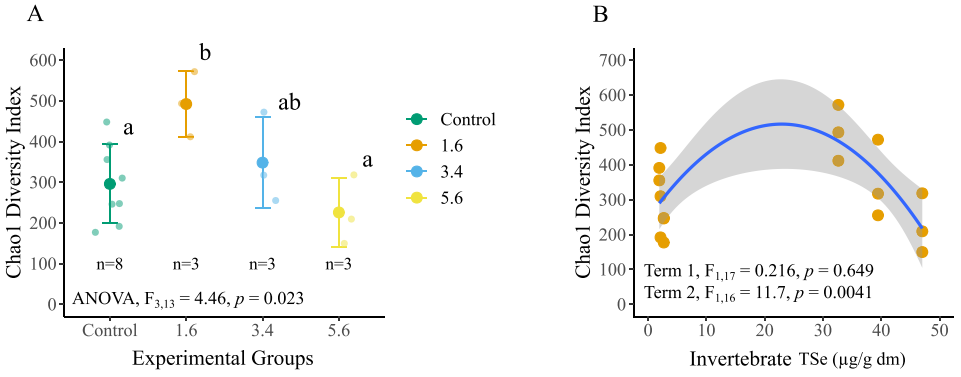

Fig. 2. (A) Chao1 diversity index among experimental groups (i.e., Control, 1.6, 3.4, and 5.6 μg/L). (B) Chao1 diversity index in response to mean concentration of TSe (μg/g dm) in invertebrates, with quadratic polynomial nonlinear model with 95% confidence intervals.

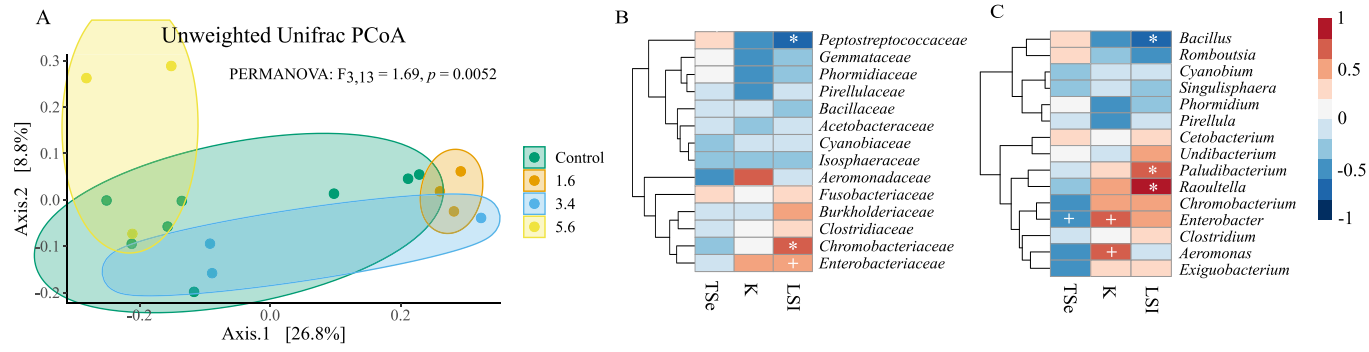

Fig. 3. (A) Principal coordinates analysis of the unweighted UniFrac distance matrix. Experimental groups consist of control (0.08, 0.08, and 0.09 μg Se/L), and treatments 1.6, 3.4, and 5.6 μg Se/L. Significantly correlated relative abundance of (B) family-level and (C) genus-level taxa ($P_{FDR} < 0.05$) with nominal treatment exposure concentration (TSe), Fulton's condition factor (K), and LSI. Scale indicates value for Spearman correlation coefficient. * indicates FDR-adjusted p-value < 0.05 and + indicates FDR-adjusted p-value < 0.1.

Bacillus negatively correlated (Fig. 3C, R = −0.714, Adj-p = 6.47e-3).

4. Discussion

Studies of toxic effects of Se have generally focused on apical outcomes in aquatic vertebrates, such as teratogenicity and reproductive

impairment, but recent advancements in next-generation sequencing techniques have allowed exploration of molecular ecotoxicological effects, including microbiota of the gut. Herein, the dominant phyla, families, and genera comprising the female finescale dace gut microbiome are described and changes in alpha- and beta-diversity of the female finescale dace microbiome following exposure to Se investigated. Fish morphometric endpoints varied slightly, with no relationship detected with ovary or muscle TSe concentration. Diversity of the fish gut microbiome exhibited a hormetic-like relationship with Se exposure. This suggested that small differences in Se exposure caused observable effects on the microbiome, some of which might be beneficial to the host fish.

The phyla *Proteobacteria* and *Firmicutes* are known to dominate guts of wild fishes and those maintained under laboratory conditions (Wu et al., 2012; Dulski et al., 2020; Kim et al., 2021). Overall, the gut microbiome of female finescale dace herein were dominated by the core phyla *Proteobacteria* and *Firmicutes*. *Proteobacteria* and *Firmicutes* also dominated microbiomes of guts of wild walleye (*Sander vitreus*), goldeye (*Hiodon alosoides*), northern pike (*Esox lucius*), and shorthead redhorse (*Moxostoma macrolepidotum*) from the North Saskatchewan River (DeBofsky et al., 2020b). Like the gut microbiome of previously studied fathead minnow, the family *Aeromonadaceae* exhibited a large relative abundance in finescale dace gut microbiome (Narrowe et al., 2015; DeBofsky et al., 2020a). The families *Bacillaceae* and *Enterobacteriaceae* also exhibited relatively large relative abundances in the microbiome of the gut of finescale dace. This observation was similar to the microbiome of the gut of wild and captive Atlantic salmon (*Salmo salar*) parr collected in Quebec, Canada (Lavoie et al., 2018). A relatively greater abundance of the genus *Aeromonas* was also observed, which was similar to that in the gut microbiome of tench (*Tinca tinca*), another cyprinid, collected during summer from Kortowskie Lake, Poland (Dulski et al., 2020). Similarities in dominant phyla and genera observed between finescale dace and other fishes studied to date suggest that these core gut microbes play a critical role in gut and host health.

Exposure to Se altered the gut microbiome of female finescale dace. Similar patterns of increased microbiome diversity at the lesser dietary Se supplementation, relative to greater dietary supplementation have been observed in mice (Zhai et al., 2018). Greater Shannon diversity, an alpha diversity metric, was observed in mice exposed to lesser amounts of Se (0.15 mg Se/kg) in the diet compared to that of those given a greater dietary supplementation (0.40 mg Se/kg), which led to decreased Shannon diversity (Zhai et al., 2018). Diversity of the gut microbial community is important for functional attributes in hosts, including regulation and development of the immune system (Kamada and Núñez, 2014) and nutrient processing and metabolic homeostasis (Oliphant and Allen-Vercoe, 2019). The community composition of the microbiome of bees was shifted when they were fed 50% sucrose spiked with 0.6 mg sodium selenate/L and 6.0 mg sodium selenite/L spiked pollen patties (Rothman et al., 2019b). Previous results overall indicate that small supplementation of the diet with Se might be beneficial to the gut microbiome, while exposure to greater concentrations of Se could potentially lead to dysbiosis of the gut microbiome.

Approximately 25% of bacteria, some of which colonize guts of animals, have genes that encode seleno-proteins (Ferreira et al., 2021). The essentiality of Se to some bacteria might influence the effect of Se on the gut microbiome; species with requirements for greater amounts of Se or greater tolerance to adverse effects of Se, might flourish under conditions of greater Se exposure. Other compounds, such as therapeutic drugs, have been found to bioaccumulate within gut bacteria (Klünemann et al., 2021). Selenium also has the potential to bioaccumulate in these gut bacteria. Therefore, the fish gut microbiome could likely play a role in the fate of ingested Se, and subsequent responses of the host to dietary Se. Selenium-supplementation has been associated with increases in health-relevant taxa in guts of the mouse, such as the families *Christensenellaceae* and *Ruminococcaceae*, and genus *Lactobacillus* (Callejón-Leblic et al., 2021). In bees, several ESVs assigned to genus *Lactobacillus* increased following selenate supplementation, while other ESVs in the same genus decreased (Rothman et al., 2019b). The gut microbiome could respond differently depending on the speciation of Se leading to distinct responses observed. Organic Se-supplemented diets via Se-enriched yeast (Sel-Plex®) had potentially greater benefits to functioning of the gut microbiome leading to higher concentration of total volatile fatty acids, propionate and butyrate, of beagle puppies, than did inorganic Se (sodium selenite, Na_2SeO_3) (Pereira et al., 2020). In common carp, dietary nano-Se (i.e., nano red elemental Se) at 0.7 mg/kg was more efficient for growth performance and antioxidant defense compared to organic (selenomethione) or inorganic (Na_2SeO_3) sources of Se (Saffari et al., 2017). Results of previous studies have also indicated potential competition between gut microbes and their host, such as Wistar rats, for assimilation of selenite (Takahashi et al., 2020), thus the microbes could serve as a buffer against excessive exposure to Se (Rothman et al., 2019a).

The relative abundance of *Fusobacteria* was greater in guts of fish exposed to 5.6 μg Se/L than in guts of the control fish, or those exposed to 1.6 or 3.4 μg Se/L. Greater abundance of *Fusobacteria* in response to dietary Se, or changes in overall fish diet caused by Se toxicity to lower trophic levels, could lead to this result (Graves et al., 2021a). Similarly, an increase in the relative abundance of *Fusobacteria* was observed in juvenile grass carp (*Ctenopharyngodon idella*) fed a diet supplemented with 0.6 mg/kg of nano-Se (Liu et al., 2021). Fusobacteria are considered beneficial bacteria that can activate a gut inflammatory response and could increase as a response to gut inflammation caused by Se toxicity (Kelly et al., 2018). Taxonomic composition can fluctuate in core microbiomes leading to potential changes in functional capacity and fundamental roles of the gut microbiota (Huang et al., 2020; Kim et al., 2021). Lesser diversity of the gut microbiota could lead to impairment of gut function including decreases in metabolism of SCFAs and increases in intestinal permeability (Kriss et al., 2018), whereas more diverse gut microbiomes can prevent unfavourable microbial colonization (Xiong et al., 2019; Perry et al., 2020). In the future, gathering more information on the specific functions of microbial taxa using metatranscriptomics, metaproteomics, or metabolomics in response to Se exposure could help to elucidate potential effects of Se on gut microbiome and resulting overall health of Se-exposed fishes.

The gut microbiome of fish can be influenced by a variety of factors, including the host species and their environment. Microbiomes of field-collected fish have been shown to be heavily influenced by species and their respective life history, including habitat and diet (DeBofsky et al., 2020b; Kim et al., 2021). In Se risk assessment, dietary concentrations of TSe are considered a reasonable indicator of fish Se exposure (Graves et al., 2021a). In the present study, dietary TSe was related to predicted diversity, albeit via a quadratic relationship, which suggested that concentrations of Se in the diet might be predictive of potential changes of the microbiota in guts of wild fishes. More studies are underway using gnotobiotic fish and advanced culturing methods (e.g., microfluidic intestine-on-a-chip) (Jalili-Firoozinezhad et al., 2019) to better understand host-microbiome interactions with environmental contaminants. Previous research revealed that the gut microbiome metabolizes many orally administered drugs (Zimmermann et al., 2019), and it may interact with Se in similar ways. Understanding the potential effects of dysbiosis on fish health is an emerging area of environmental research and is important to understand when assessing ecological risks of pollutants to fish (Adamovsky et al., 2018; Chen et al., 2018). In terms of Se toxicity, considering the potential effects of Se on gut microbiome as an additional factor impacting fish health may be an important consideration in Se risk assessment.

5. Conclusions

Effects of Se on reproduction and larval development of fishes have been well documented in the field, but the effect of elevated Se on wild fish gut microbiome was yet to be explored. Most previous research has

focused on evaluating the beneficial effects of low-dose Se on gut health, while the potential ecotoxicological effects of Se on the fish microbiome remain relatively unstudied. Here, we detailed the gut microbiome community in female finescale dace and reveal a hormesis pattern of microbiome diversity: lower concentrations of dietary Se increased diversity, while higher dietary Se exposure maintained baseline diversity. More research regarding the effects of Se on fish gut microbiome and the potential adverse effects or benefits on the host is warranted and focusing on field-collected fish is important to increase the environmental relevance of such studies.

Credit author statement

Phillip J. Ankley: Investigation, Methodology, Formal analysis, Data curation, Writing – original draft. **Stephanie Graves:** Conceptualization, Resources, Methodology, Data curation, Writing – review & editing. **Yuwei Xie:** Methodology, Resources, Writing – review & editing. **Abigail DeBofsky:** Resources. Writing – review & editing. **Alana Weber:** Resources, Writing – review & editing. **Markus Brinkmann:** Resources. Writing – Review & Editing. **Vince P. Palace:** Resources, Writing – review & editing. **Karsten Liber:** Project administration, Writing – review & editing. **Markus Hecker:** Funding acquisition, Project administration, Resources, Writing – review & editing. **David M. Janz:** Conceptualization, Project administration, Resources, Writing – review & editing. **John P. Giesy:** Funding acquisition, Resources, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.113151.

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Supporting Information for

Effects of *in situ* experimental selenium exposure on finescale dace (*Phoxinus neogaeus*) gut microbiome

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Number of figures: 5

Number of tables: 3

CONTENT

SI-1: Sequence output for 16S rRNA metabarcoding.

Sequence output for the 16S rRNA metabarcoding data consisted of a total of 1477837 sequence reads after demultiplexing, quality filtering, and merging of forward and reverse reads. Individual samples had sequence reads of 59113.5 ± 40266.6 (average ± standard deviation (SD)) (Table S1). After denoising, a total of 1435023 sequence reads remained, with individual samples have sequence read counts of 57400.9 ± 37459. A total of 1059125 sequence reads were assigned to target bacteria with individual samples having 42365 ± 33435.3. Final sequence reads consisted of 1041352 with removal of rare sequences (e.g., --p-min-frequency 10 and --p-min-samples 2), and individual samples had sequence read counts of 41654.1 ± 32950.4.

SI-2: Selection of environmental variables for Spearman correlation.

Constrained analysis of principal coordinates (CAP) was applied using Bray-Curtis distance matrix using feature-level data, with lingoes correction, being implemented with the function capscale. Function ordistep and forward selection was then used for decision of scaled environmental variables (i.e., Treatment + Condition + Ovary_Tse + Avg_Invert_Tse + LSI + GSI + fork_length) to be included in the final model of: capscale(bact.feat ~ Condition + liverd13c, distance = "bray", data = scale.env, add = TRUE).

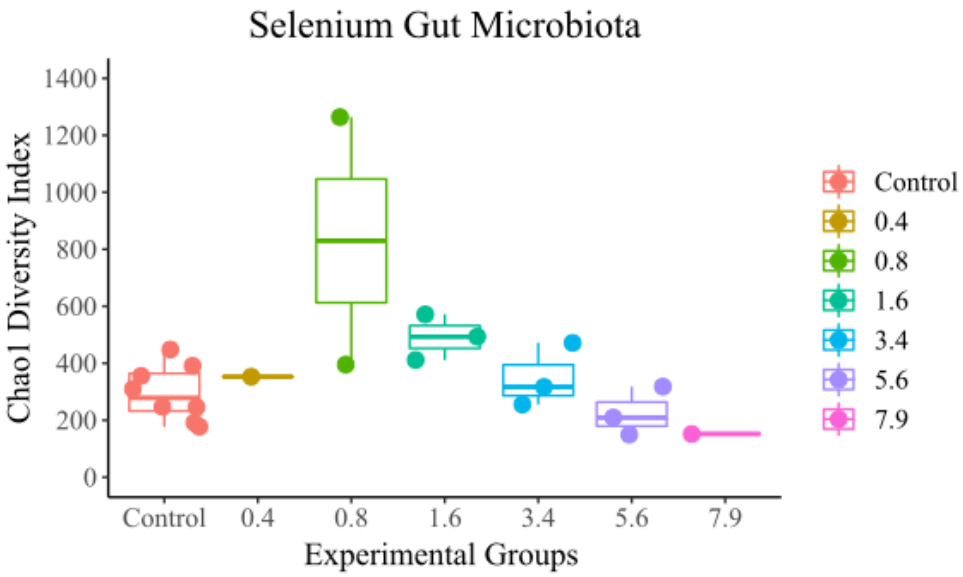


Figure S1: Choa1 diversity index for female finescale dace for all samples between experimental groups (Control and 0.4, 0.8, 1.6, 3.4, 5.6, and 7.9 µg/L TSe). From the boxplot, a large outlier can be visually detected. The outlier was a fish collected from the 0.8 µg/L treatment group – enclosure M5. The resulting Choa1 diversity index for the outlier was equal to 1263.8, 692.2 larger than the next highest resulting estimated richness. Experimental groups 0.4, 0.8, and 7.9 µg/L TSe were removed for downstream analysis.

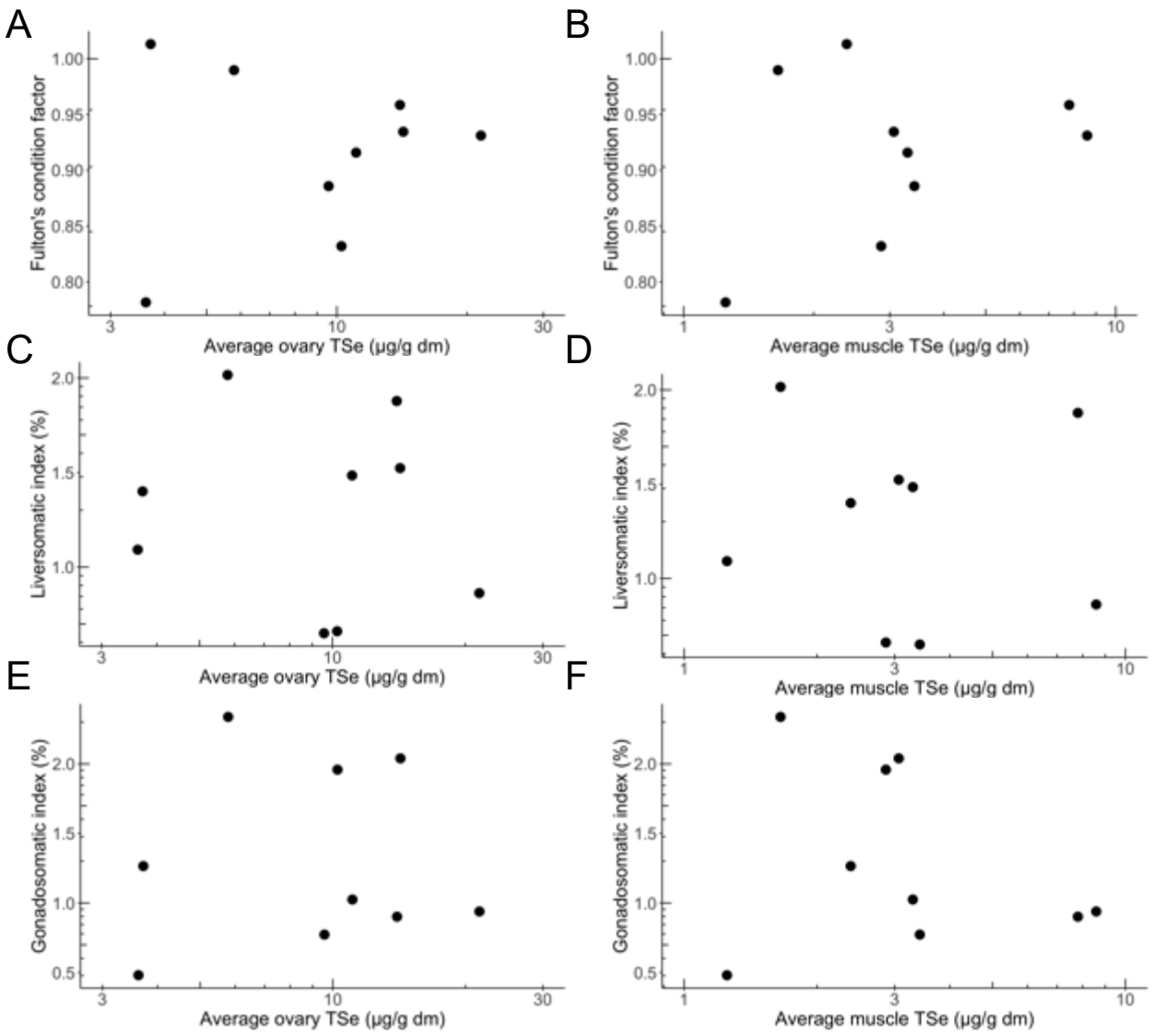


Figure S2: Relationships between female finescale dace (*Phoxinus neogaeous*) morphometric endpoints and ovary or muscle TSe concentrations after 42 d of exposure in limnocorrals ranging in aqueous Se concentration from 0.08 to 7.9 µg/L.

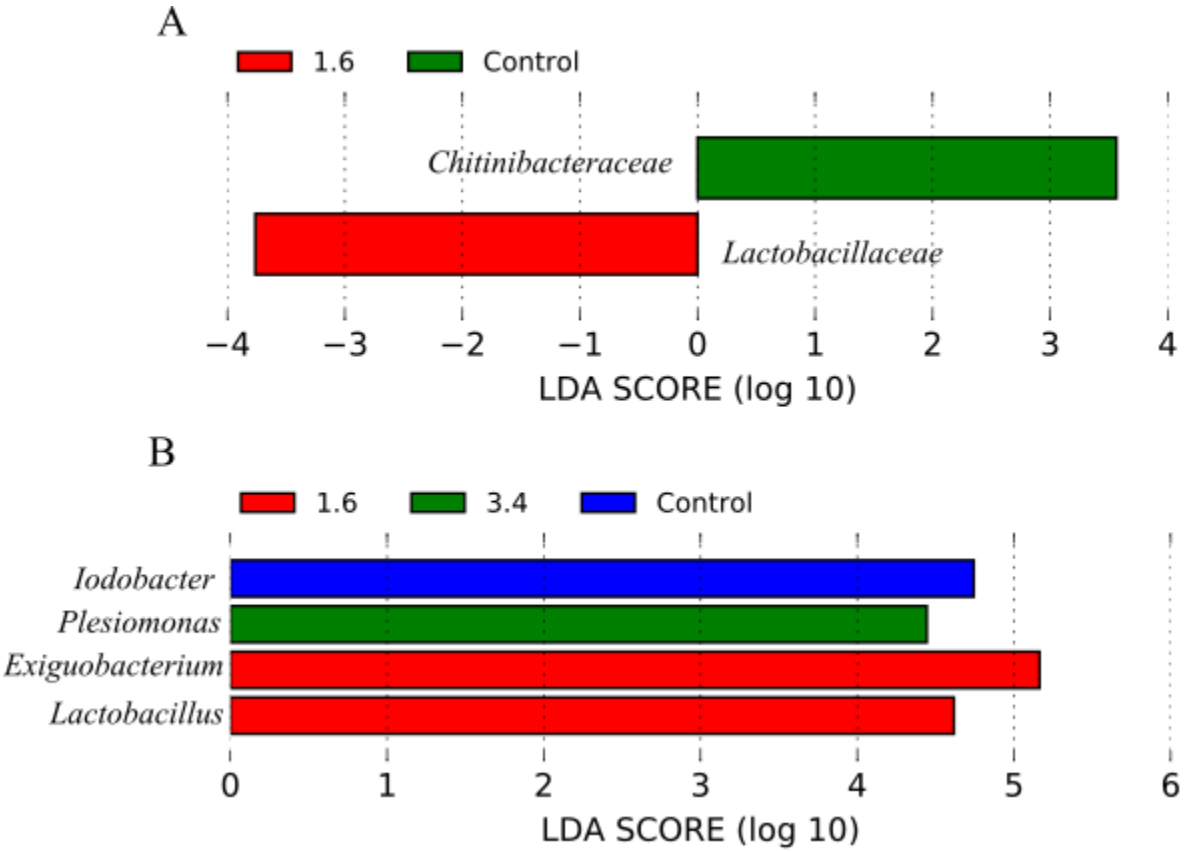


Figure S3: Associated taxa detected from LEfSe analysis for (A) family-level and (B) genus-level data. Control refers to untreated and 1,6 and 3.4 refers to µg/L TSe.

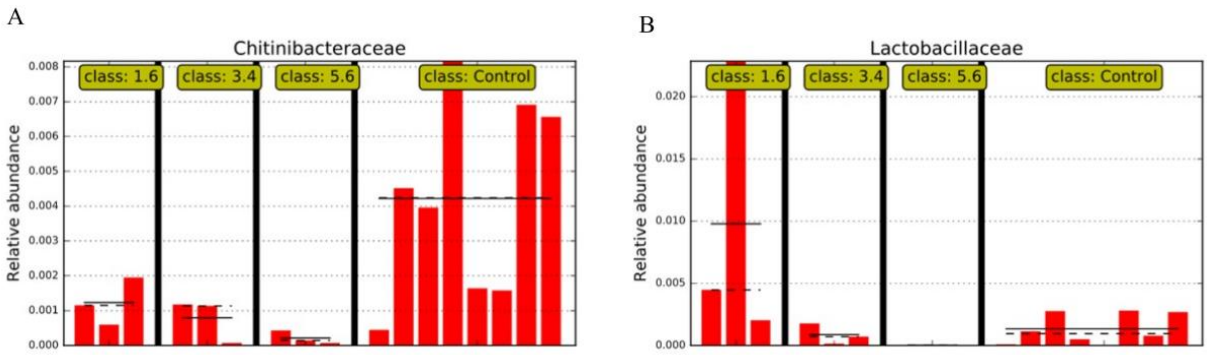
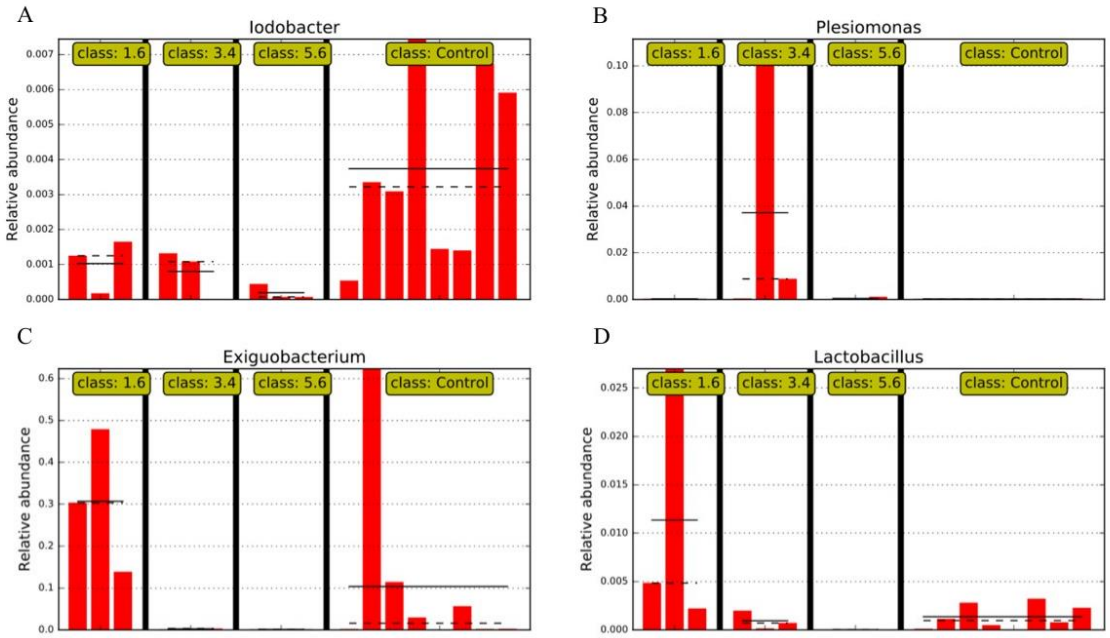


Figure S4: Relative abundance of associated taxa inferred from LEfSe analysis for family-level for different treatments. Treatment groups consist of Control (0.08 and 0.09 µg/L), 1.6, 3.4, and 5.6 µg/L TSe.



89

90 **Figure S5:** Relative abundance of associated taxa inferred from LEfSe analysis for genus-level

91 for different treatments. Treatment groups consist of Control (0.08 and 0.09 µg/L), 1.6, 3.4, and

92 5.6 µg/L.

93

Table S1: Sequence read counts of merged, denoised, assigned, and cleaned reads for each sample.

SampleID	Treatment	Merged_Reads	Denoised_Reads	Assigned_Reads	CleanedReads
PCRNC	NA	223	217	NA	NA
PCRNTC	NA	107	102	NA	NA
sg1	0.09	58545	58263	22434	21476
sg10	7.9	36760	36446	36233	36161
sg11	7.9	77812	77457	5267	5054
sg12	0.08	32711	32430	28729	28652
sg13	0.8	63979	63558	28474	27843
sg14	0.8	155271	131602	81860	71663
sg15	1.6	44494	44077	34722	34459
sg16	1.6	106445	105500	42399	41243
sg17	1.6	46581	45975	39214	38907
sg18	0.08	102109	99790	95896	95447
sg19	0.08	58655	57925	57406	57328
sg2	0.09	42695	42417	25182	24619
sg20	0.08	58120	57474	45040	44537
sg21	0.8	190	187	152	146
sg22	0.08	57346	56636	51044	50922
sg23	0.08	36646	36299	34215	34173
sg24	NA	104	101	NA	NA
sg25	5.6	113524	107983	106789	105939
sg26	5.6	22939	22523	22233	22123
sg3	0.09	4530	4510	484	405
sg32	NA	117	113	NA	NA
sg4	0.4	15252	15135	14272	14226
sg5	0.4	649	641	508	468
sg6	3.4	54001	53558	40576	39938
sg7	3.4	86422	85037	67954	67793
sg8	3.4	66262	65522	45252	45093
sg9	5.6	135899	134078	132790	132737

Table S2: Quadratic model summary output for microbiota Chao1 diversity index in response to invertebrate TSe content.

Quadratic Polynomial Model (Chao1 Diversity and Invert Tse)				
Term	Deviance	DF	F-value	*p*-value
(Invert Tse)	1992	1,15	0.216	0.649
(Invert Tse)^2	108340	1,14	11.7	0.00410

Table S3: Function pairwise.adonis2 (i.e., pairwise PERMANOVA) results to test for differences between treatments and respective centroid position (9999 permutations, Unweighted unifrac distance) using FDR to correct for multiple testing.

	DF	**R^2**	**F-value**	***p*-value**
Control vs. 1.6	1,9	0.190	2.11	0.0217
Control vs. 5.6	1,9	0.140	1.47	0.0548
Control vs 3.4	1,9	0.0903	0.894	0.568
1.6 vs. 5.6	1,4	0.456	3.36	0.1
1.6 vs. 3.4	1,4	0.276	1.53	0.2
5.6 vs. 3.4	1,4	0.292	1.65	0.1