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Brief Report

Point-of-use carbon-block drinking water filters change gut microbiome of larval zebrafish

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Summary

Activated carbon block (ACB) point-of-use (PoU) drinking water filters can change the bacterial composition in drinking water. Consuming ACB PoU filtered water may also influence gut microbiomes. This study uses the zebrafish model to evaluate how the ACB PoU filter affects the gut microbiomes and phenotypic responses in larvae and adulthood. An ACB PoU filter manifold system was constructed to feed larval and adult zebrafish tap and filtered water at the early and late stages of the filter operation period. Adult zebrafish gut microbiomes were not affected by exposure to water types and filter stages. Unlike the adult, gut microbiomes of the larvae exposed to filtered water at the late stage of filter operation were dominated by more filter-relevant bacterial taxa, including *Comamonadaceae* and *Brevundimonas*, than the early stage-filtered-water- and tap water-exposed larvae. We also found some fish that were either exposed to filtered water at early and late stages or tap water supplied to the filter toward the end of the experiment showed hyperactive locomotion behaviour, and had significantly lower relative abundances of a *Pseudomonas* spp. (OTU3) than the normally behaved fish. Our findings indicate that ACB PoU filtered water can alter gut microbiomes and affect the behaviour patterns in larval zebrafish.

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Introduction

Activated carbon block (ACB) point-of-use (PoU) filters are certified to remove chemicals from drinking water, including heavy metals, chlorine, disinfection byproducts and other contaminants of concern (NSF International/ANSI, 2015a; NSF International/ANSI, 2015b; NSF International, 2016). The ACB is a solid block of compressed activated carbon with low porosity and extensive surface area that removes unwanted chemicals through physical adsorption and mechanical filtration (Wu *et al*., 2017). However, these filters are known to support bacterial growth (Tobin *et al*., 1981; Reasoner *et al*., 1987) and change the drinking water microbiome significantly over time (Chaidez and Gerba, 2004; Wu *et al*., 2017). These microbial changes may influence gut microbiomes. Certain drinking water bacteria, such as the genus *Ralstonia*, *Bacillus* and *Escherichia*, can be selected for and form gut microbiota in germ-free mice (Lee *et al*., 2010). Consuming drinking water from different origins has been shown to affect the diversity of bacterial communities in mice intestines (Dias *et al*., 2018). Changes to gut microbiomes affect the metabolic, immunological, physiological and neurological development of hosts (Cresci and Bawden, 2015; Ihekweazu and Versalovic, 2018). Bacterial diversity and richness in gut can affect animal behaviours and locomotor activity through metabolic alterations that are implicated in inducing neurochemical changes in the central nervous system (Desbonnet *et al*., 2015; Borrelli *et al*., 2016; Schretter *et al*., 2018). Since ACB PoU filters can substantially change the microbial composition of drinking water, and drinking water microbial composition can affect mammalian host function, it is important to understand the potential health impacts of ACB PoU filtered water on the gut microbiome.

Zebrafish (*Danio rerio*) is a well-established model to study impacts of environmental exposure on gut microbiomes. The zebrafish gut has similar development, structure and function with the mammalian digestive system (Stroband and Debets, 1978; Rombout *et al*., 1984). Several bacterial divisions in humans, mice and other mammals intestines, such as *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Proteobacteria*, also reside in the zebrafish gut (Rawls *et al*., 2004; Eckburg *et al*., 2005; Bates *et al*., 2006). The host gene expression and gut microbial regulation in zebrafish are highly conserved in the mammalian gut (Rawls *et al*., 2004). Conventionally raised zebrafish are reared in conditioned water treated by reverse osmosis (RO). Under controlled environmental and dietary conditions, zebrafish gut microbiota evolves over development. During embryogenesis and larval stage, bacterial memberships and abundances are highly variable (Stephens *et al*., 2016). Zebrafish embryos are initially sterile and axenic. By 4 days post-fertilization, larvae hatch from their chorion and encounter environmental microbes for the first time (Bates *et al*., 2006). Initially, *Gammaproteobacteria* dominate the intestine community (Stephens *et al*., 2016). Entering adulthood, host selection mechanisms and exogenous factors become the main driver shaping intestine bacterial composition, where *Fusobacteria* join *Gammaproteobacteria* as the core class among the gut colonizers (Stephens *et al*., 2016). Other than lifecycle stages, recent studies have identified various external factors, such as diet (Koo *et al*., 2017), antibiotic exposure (Gaulke *et al*., 2016; Almeida *et al*., 2019) and chemical contaminants (Oliveira *et al*., 2016; Dahan *et al*., 2018; Xia *et al*., 2018; Xia *et al*., 2020) that can reshape gut microbiota in zebrafish.

In this study, we use the zebrafish model to evaluate how ACB PoU filters impact gut microbiomes, embryonic development and locomotive behaviour patterns. We constructed a sink manifold that had duplicate ACB PoU filters attached to a split faucet and operated to simulate diurnal usage patterns (Fig. S1). To compare the effects of water microbiome changes over the filter life, we exposed larval and adult zebrafish with tap or filtered water at either early or late stages during filter operation. Our findings fill a gap in understanding the interaction of water and gut microbiomes at different host ages. The phenotypic responses of zebrafish due to the exposure suggest potential health impacts of ACB PoU filters on humans.

Results and discussion

At both early and late stages, larval and adult zebrafish developed bacterial members that were previously identified as part of the core gut microbiota of conventionally reared zebrafish (Table S1). *Alpha*-, *Beta*- and *Gammaproteobacteria* constituted at least 70% in all larval gut communities. Among *Gammaproteobacteria*, *Pseudomonas* (OTU3), *Aeromonas* (OTU7), and *Vibrio* (OTU13) were the three most frequently detected bacterial taxa with relative abundances varying across exposure conditions. In adult guts, *Fusobacteria* made up of at least 65% of the community with the majority being classified in the genus *Cetobacterium*. The rest of the adult gut community was made up of *Aeromonas* (OTU7) and an unclassified Firmicutes (OTU8). Only a few zebrafish intestinal OTUs had greater than 5% relative abundances in water environments, such as *Blastomonas* (OTU22) in tap water, and *Comamonadaceae* (OTU4) as well as *Burkholderiales* (OTU6) in filtered water (Table S2). The remaining zebrafish intestinal OTUs were less than 10% of tap or filtered water bacterial populations (Table S2), and represent taxa that were selected for and colonized the zebrafish intestine environment.

The chemical and microbial properties of tap and filtered water were consistent with previous studies (Wu *et al*., 2017). Over the whole experiment, water quality remained stable in tap water and filtered water. The filters removed residual chlorine and reduced conductivity from tap water (Table S3). Both tap and filtered water were supplemented with salts, sodium bicarbonate and tap water conditioner (removes chlorine and detoxifies heavy metals) to ensure all water types were consistent and comparable. The bacterial community structure in filtered water changed significantly over the filter operating period (Fig. S2, ANOSIM, $R = 0.54$), whereas the microbial composition of tap water was consistent over time (Fig. S2, ANOSIM, $R = 0.33$). To characterize how the bacterial quality of PoU filter water affected zebrafish gut communities, OTUs were categorized as 'filter-relevant', 'tap-relevant', 'gut only' and 'irregular' according to their presence, abundance and timing in tap and filtered water throughout the operation (Table 1 and Table S2). Bacterial taxa that were more likely to be detected in filtered water are defined as 'filter-relevant' OTUs. This group met one of the following criteria: (i) higher relative abundances in filtered water than in tap water for at least three operational days, (ii) only detected in filtered water, or (iii) detected in filtered water at least one of the last two sampling days toward the end of filter operation. Similar logic rules apply to the 'tap-relevant' OTUs. 'Gut only' OTUs were only present in the gut microbiomes and OTUs that did not show consistent patterns throughout the operation are classified as 'irregular'.

Larva exposed to filtered water showed significantly different total richness and predominant OTUs between early and late stages, especially for filter and tap relevant OTUs (Table 1; Fig. 1). We used Chao 1 index to estimate the richness of the population and each OTU

Table 1. Estimated richness and relative abundances of OTUs in the gut of zebrafish larvae exposed to tap or filtered water.

	Tap water		Filtered water	
Estimated nonparametric diversity	Day 1–5	Day 45–49	Day 1–5	Day 45–49
Total	34 ± 9	53 ± 10	18 ± 9	44 ± 18**
Filter relevant OTUs	6 ± 3 (4% ± 8%)	7 ± 3 (4% ± 4%)	1 ± 1** (0.6% ± 0.5%)	9 ± 5 (48% ± 33%)**
Tap relevant OTUs	19 ± 11 (94% ± 8%)	24 ± 7 (60 ± 32%)	10 ± 3 (79% ± 22%)	17 ± 6 (37% ± 38%)
Gut only OTUs	7 ± 3 (1% ± 1%)	9 ± 8 (11% ± 15%)	1 ± 1 (9% ± 11%)	11 ± 10 (9% ± 11%)
Irregular OTUs	2 ± 1 (1% ± 2%)	9 ± 5** (25% ± 20%)	2 ± 1 (13% ± 12%)	5 ± 2 (6% ± 11%)

Values shown in parenthesis are the total relative abundances of the classified OTUs in the community.
Significant levels of the differences between operation periods within each water type are shown as $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$.

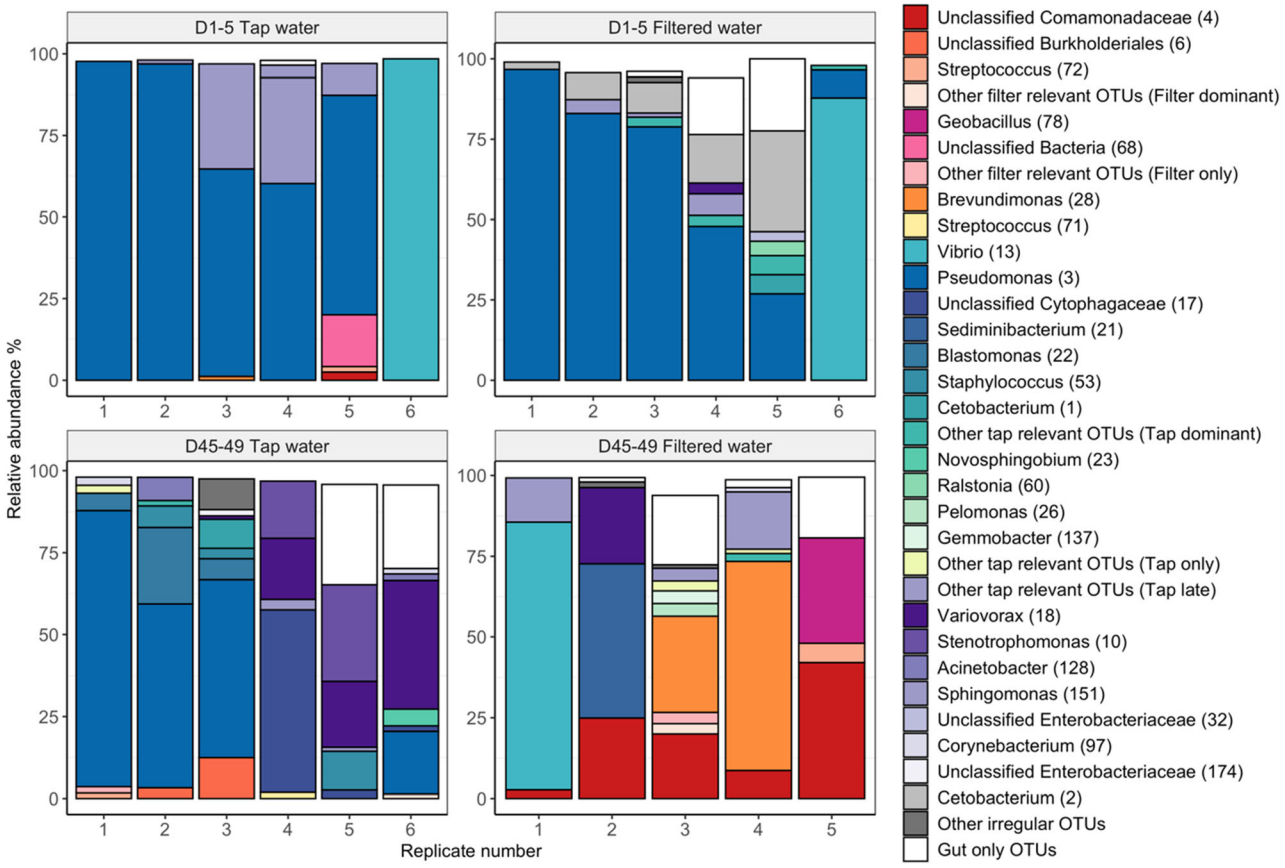

Fig. 1. Relative abundance of OTUs (>1%) in individual larval gut across exposure water types and filter stages. Numbers in the parenthesis represent the OTUs ID. Taxa in red and orange colours are 'filter relevant' OTUs. Taxa in blue, green and yellow colours are 'tap relevant' OTUs. Taxa in purple and grey colours are 'irregular' OTUs. Taxa in white are 'gut only' OTUs. Each exposure condition had six replicates except for D45-49 Filtered water, in which one replicate was discarded as outlier using CLOUD non-parametric method (Montassier *et al*., 2018).

category (Gotelli and Colwell, 2011). Early-filtered-water-exposed larval guts had the fewest bacterial taxa among exposure conditions. In late-filtered-water-exposed larval guts, eight times more richness of filter relevant OTUs developed and contributed to half of the community. Early-filtered-water larval gut microbiomes were dominated by a tap relevant OTU (*Pseudomonas*, OTU3) that comprised 9%–97% of the population, and an irregular OTU (*Cetobacterium*, OTU2) that comprised 2%–31% of the population. In late-filtered-water larval guts, these two bacterial taxa were replaced by filter relevant OTUs, *Comamonadaceae* (OTU4, classified as *Betaproteobacteria*) and *Brevundimonas* (OTU28, classified as *Alphaproteobacteria*), which made up 3%–42% and 0.02%–6% of the population respectively. Individual larval gut microbial communities within each condition had some interindividual variation, as commonly found in zebrafish larvae (Stephens *et al*., 2016) and other young vertebrates (Koenig *et al*., 2011), but late-filtered-water larvae

possessed relatively higher variation in the overall richness than others (Table 1). Significant variation in other filter relevant OTUs was observed, such as *Streptococcus* (OTU72) and *Geobacillus* (OTU78), and comprised up to 6% and 33% of the population respectively. Another predominant tap relevant OTU, *Vibrio* (OTU13), also varied with the exposure condition. The presence of *Vibrio* (OTU13) may have been excluded by *Pseudomonas* (OTU3) in the intestine of tap- and filter-water- larvae at early stages, since *Pseudomonas* spp. inhibits the growth of *Vibrio anguillarum* in fish guts (Spanggaard *et al*., 2001). Similar intermicrobial competition may occur between *Vibrio* (OTU13) and other bacterial taxa in late-filter water-exposed larval guts.

For tap-exposed larvae, numbers and total relative abundances of filter-relevant, tap-relevant and gut only OTUs were similar between early and late stages (Table 1; Fig. 1), except the total richness was about two times higher in late-tap-exposed larval gut. The increase in richness came from more irregular OTUs, including *Stenotrophomonas* (OTU10) and *Variovorax* (OTU18). A tap-relevant OTU, *Blastomonas* (OTU22), which was frequently detected in tap water during the operation period, also had significantly higher relative abundances in late-tap-exposed larval guts.

Overall, filtered-water-exposed larval guts had significantly different bacterial community structures between early and late filter stages (Fig. 2, ANOSIM, $R = 0.86$), whereas tap-exposed larval guts of both stages showed relatively similar composition (ANOSIM, $R = 0.18$). Within each stage, the gut communities from filtered-water-exposed larvae were significantly different from that of tap-exposed larvae (ANOSIM, $R = 0.70$ and 0.58 for early and late stages respectively). Since the bacterial compositions in the filtered water became more distinct over time than in the tap water, the differences in early- and late-filtered-water-exposed larval gut microbiomes are likely associated with bacterial dynamics in the water after PoU filtration. There is a possibility that this change could be effected by exposure to low levels of various regulated and unregulated chemical contaminants present in tap water (Detroit Water and Sewerage Department, 2019), although both tap and filtered water were supplemented with salts, sodium bicarbonate and tap water conditioner to ensure all water types were consistent and comparable.

The association between water and fish gut microbiomes has been found in other fish species (Giatsis *et al*., 2015; Kashinskaya *et al*., 2018; Nikouli *et al*., 2019). Fish embryos can ingest bacteria from the rearing water and establish initial microflora during larval development (Hansen and Olafsen, 1999). At the early larval development stage, the intestinal tract of larvae can be colonized by bacterial taxa randomly sourced from water and their loss and replacement in the gut occur stochastically (Burns *et al*., 2016). Larvae exposed to higher bacterial concentrations in water can develop a gut community with a higher abundance of bacteria (Tan *et al*., 2019). In our previous studies, we found that up to 60% of tap water bacteria can be removed in filtered water at the beginning of filter operation (Wu *et al*., 2021). Early-filtered-water-exposed larvae may have been exposed to fewer bacterial colonizers and thus developed the least diverse gut bacterial community. Over time, PoU filters generate filtered water with higher bacterial concentrations and different bacterial composition than tap water. Larvae exposed to late filtered water are more likely to encounter filter relevant OTUs, which then assemble into communities with considerable inter-individual variations. They are distinct from the gut microbiomes of larvae exposed to other conditions.

Compared to larval gut microbiomes, adult gut microbiomes were homogenous across exposure conditions and not affected by either water type or filter stage (ANOSIM, $R = 0.13$, Fig. 2). Our findings were similar to a study that showed adult zebrafish intestinal communities were more similar to each other than to the microbiomes of source water, tank surface, or food (Stephens *et al*., 2016). Adult zebrafish microbiota may become an anoxic environment that selects for certain obligate anaerobes, which are resilient to changes in host provenance and domestication status (Roeselers *et al*., 2011). This resilience in adult intestinal community therefore is less likely disturbed by changes in the bacterial composition and properties of PoU filtered water compared to larval fish.

Due to the changes of larval gut microbiomes over filter stages, we evaluated phenotypic responses including development and locomotion behaviour in zebrafish larvae after exposure to tap or filtered water (Fig. 3). Skeletal deformities, heart oedema, yolk sac oedema, inflated swim bladder, mortality and unhatched embryos were tracked and quantified throughout the 5-day exposure. Larvae exposed to filtered water of both stages had low developmental abnormality rates. However, those exposed to tap water at the end of the operation period were more likely to experience a delay in hatching and higher rates of uninflated swim bladder than conventionally reared larvae (Fig. 3A). The locomotor activity was assessed by tracking larva movements during light and dark alteration cycles. We found elevated average levels of locomotor activity in both early and late filtered water-exposed as well as late tap water-exposed larvae (Fig. 3B). Since relative abundances of detected OTUs varied across individual larvae within each exposure condition (Fig. 2), we found *Pseudomonas* (OTU3) was significantly lower in conditions when hyperactive larvae occurred ($12 \pm 22\%$) than those that behaved normally ($67 \pm 28\%$). According to its partial 16S rRNA gene

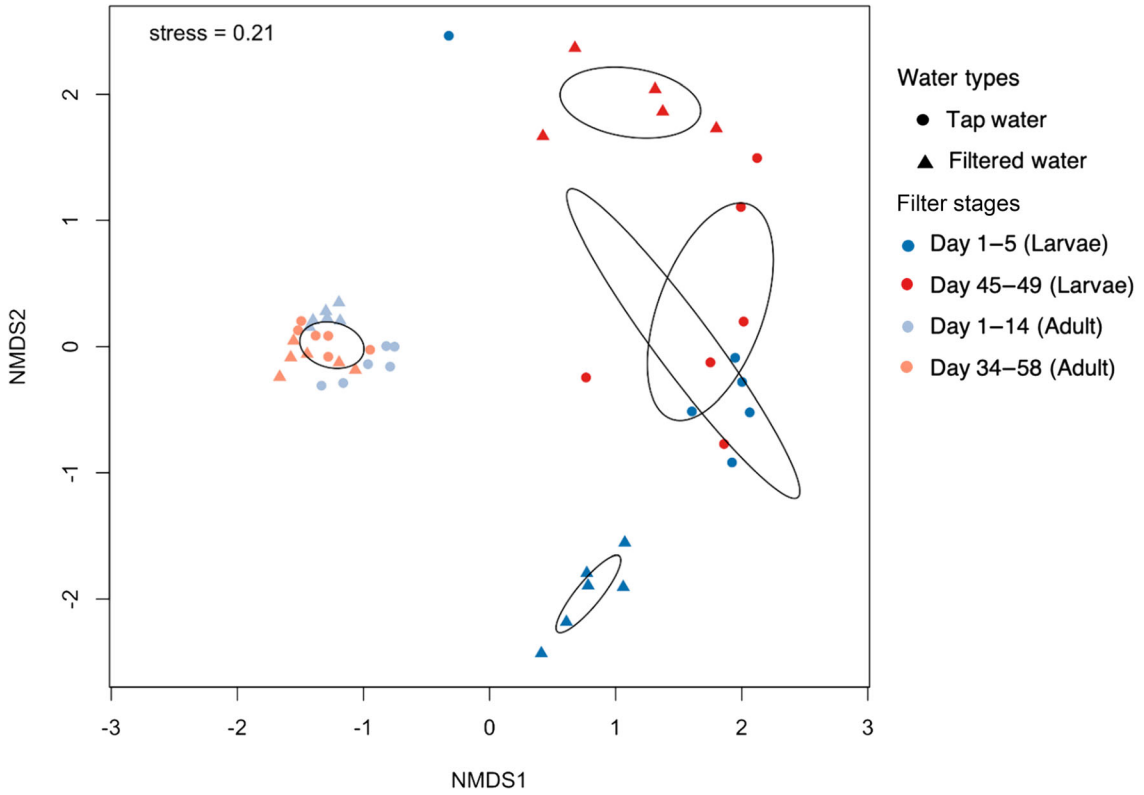

Fig. 2. Non-metric multidimensional scaling (NMDS) ordination plot of the gut microbiomes from larval (dark) and adult (light) zebrafish exposed to tap water (circle) and filtered water (triangle) at different filter stages. Filter stages are indicated by colour: early (Day 1–5 for larvae and Day 1–14 for adult) as blue and late (Day 45–49 for larvae and Day 34–48 for adult) as red. Ellipses show the 95% confidence interval around samples from each cluster. The analysis was based on Bray–Curtis dissimilarity coefficients calculated from the relative abundances of OTUs.

sequence, *Pseudomonas* (OTU3) includes taxa that degrade recalcitrant organics (Schaeffer *et al*., 1979; Kurzawova *et al*., 2012; Mangwani *et al*., 2016), which had been detected in zebrafish intestines (Rawls *et al*., 2004; Sundarraman *et al*., 2020) and include *Pseudomonas alcaliphila* and *P. mendocina*. Bacterial colonization of specific bacterial species and exposure concentrations are associated with neurobehavioral development of zebrafish larvae (Phelps *et al*., 2017; Tan *et al*., 2019). Colonizers such as *E. coli* (Tan *et al*., 2019), *Bacillus subtilis* (Tan *et al*., 2019), *Aeromonas veronii* (Phelps *et al*., 2017) and *Vibrio cholerae* (Phelps *et al*., 2017) can reverse the hyperactivity in germ-free zebrafish at larval development stage. *Pseudomonas* spp. were found to have a high affinity of gamma-aminobutyric acid (Guthrie *et al*., 2000), one of the neurotransmitters responsible for microbiota–gut–brain communication (Carabotti *et al*., 2015). The succession among *Pseudomonas* (OTU3) and filter relevant OTUs may influence the gut–brain axis and the neurobehaviour of zebrafish larvae.

Our findings indicate that the exposure to the water microbiome altered by ACB PoU filters during embryonic development changes the species richness and predominant OTUs in larvae gut. The association between water and fish gut microbiomes has been found in other fish species (Giatsis *et al*., 2015; Kashinskaya *et al*., 2018; Nikouli *et al*., 2019). Fish embryos can ingest bacteria from the rearing water and establish initial microflora during larval development (Hansen and Olafsen, 1999). The number and types of shared bacterial taxa between water and fish gut differ according to host genotypes, age and diet condition (Sullam *et al*., 2012; Legrand *et al*., 2020). After the inclusion of artificial formulated commercial diet, the intestinal bacterial community shifts to a more stable composition, which is less influenced by the rearing water and other environmental factors (Nikouli *et al*., 2019). Indeed, we found that the adult gut microbiomes were consistent regardless of their exposure to tap or filtered water at early or late filter stages. The exposed larval fish may establish new microbial flora after being introduced with commercial food; therefore, filter relevant OTUs may not reside in the gut as much as just after exposure. Since the succession of filter relevant OTUs in the gut affect larval behaviour patterns, future studies should focus on the effects of early exposure to ACB PoU filtered water. More work is needed to develop a comprehensive understanding of

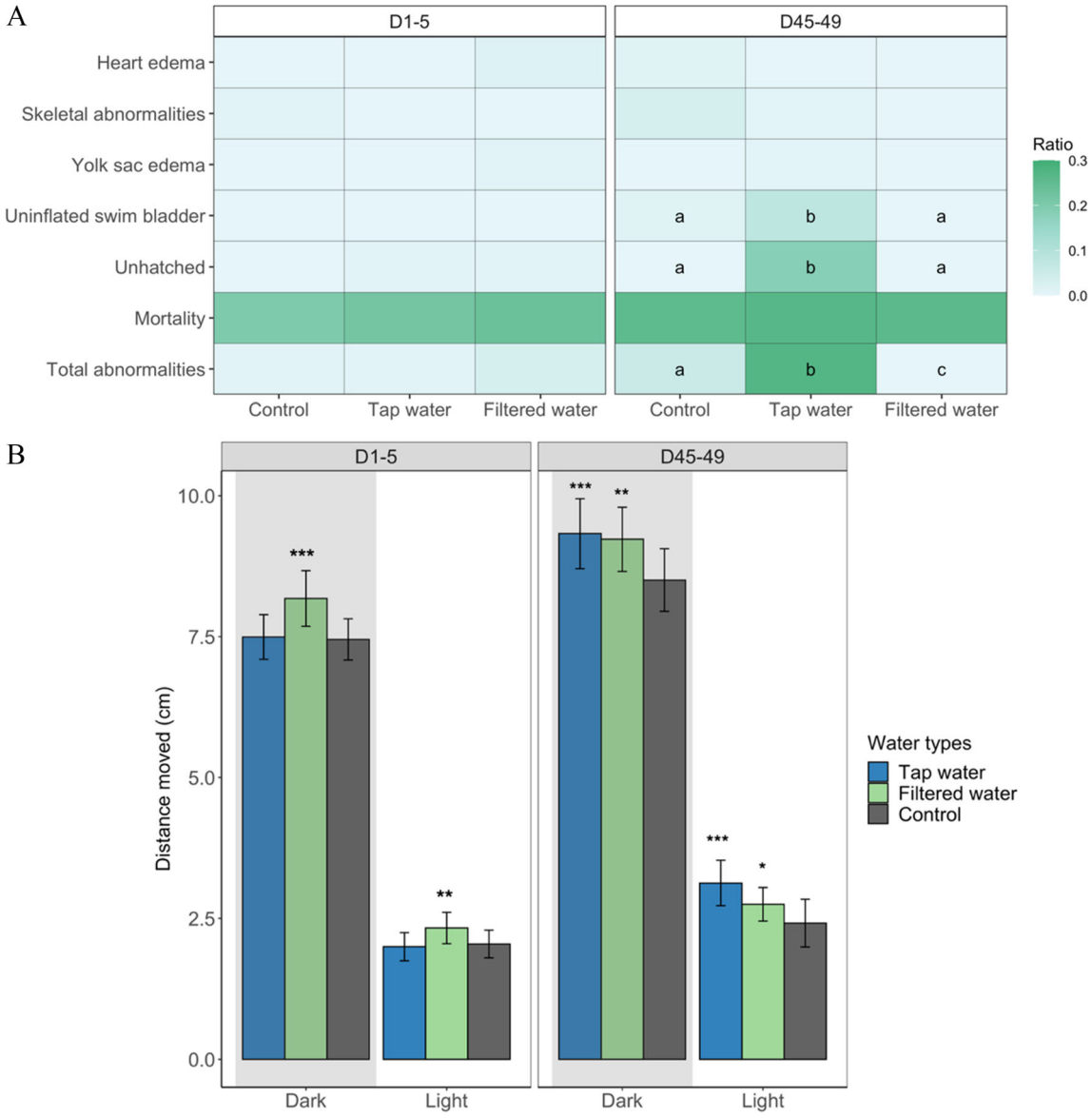

Fig. 3. Developmental behaviours of zebrafish larvae.A. Abnormality rates of larval zebrafish for each water type. Letters indicate significant differences in the abnormality rates across the water types in *post hoc* pairwise comparison ($p < 0.05$).B. Total distance moved at dark and light for zebrafish larvae exposed to tap water (blue), filtered water (green) and RO water (grey) after 5-day exposure at operational day 1–5 (D1-5) or day 45–49 (D45-49). Significant levels are shown as $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$.

interactions among the ACB PoU filtered water and gut microbiomes.

Experimental procedures

Fish husbandry. Domesticated AB wild-type zebrafish used in this study were housed in a recirculating system (Aquaneering, CA, USA) on a 14 h:10 h light/dark cycle with RO water buffered with sodium bicarbonate and Instant Ocean salts (Spectrum Brands, WI, USA). Water temperature was maintained between 27°C and 30°C. During the larval development period, embryos and larval fish were fed a combination of larval food and *Artemia nauplii*. The fish that were 8 weeks post-fertilization (wpf) were fed with a combination of Zeigler Zebrafish Adult Diet, O.S.I. Spirulina Flakes and Golden Pearl. Zebrafish use protocols were approved by the Institutional Animal Care and Use Committee at Wayne State University, according to the National Institutes of Health Guide to the Care and Use of Laboratory Animals.

Filter manifold system. The manifold system was connected to a faucet fed with municipal tap water (Detroit, Michigan) by a copper pipe that was split into two lines, each of which was connected to a commercial ACB PoU filter (Fig. S1). Each filter consisted of an annular block of activated carbon surrounded by a fabric prefilter. The system was fitted with a

solenoid controlled by a digital switchbox (ChronTrol Corporation, USA) programmed to simulate diurnal water use. Equal volumes of water were processed through the filters for 20 s intervals 10 times throughout the day and leaving a nightly stagnation period of 8 h, which is typical in premise plumbing (Lautenschlager *et al*., 2010). The total volume of water through each filter was simulated as daily potable water consumption of one adult person. It was assumed that the user consumed a volume of water between 1.9 L (based on the anecdotal '8 cups a day') (Valtin, 2002) and 3.7 L (based on the Mayo Clinic recommendation for an average adult male) (Mayoclinic, 2020). Flow rate and water quality, including chlorine residual, temperature, specific conductance, pH and oxygen-reduction potential of the tap and filtered water were monitored daily. Both tap and filtered water were supplemented with instant ocean salts, sodium bicarbonate and tap water conditioner (API; Mars Fishcare North America) to ensure all water types met the water quality thresholds that are known to be habitable for zebrafish. Water samples were collected and transferred to the aquarium lab for fish exposure testing. The manifold system was operated for 50 days and reached the manufacturer's rated design process volume (100 gal) on day 40. Each filter processed an average of 125 gal of water at the end of operation. The larval and adult fish were exposed for 5 and 14 days respectfully, to tap or filtered water at the early (Day 1–5 for larvae and day 1–14 for adult) and the late (Day 34–48 for adult and day 45–49 for larvae) stages of the operational period.

Larval exposures. Embryos were obtained by spawning AB zebrafish in 1 L crossing tanks and collecting the eggs 2 h post-fertilization (hpf) and subsequently sterilized with 58 ppm bleach (Clorox, Oakland, CA, USA). The washed embryos were then exposed in 10 ml of water samples in sterile 6-well plates at 28°C with a 14:10 light:dark cycle. Each water type had a total of 60 embryos that were exposed in three separate plates. Throughout the 5-day exposure period as embryos developed into zebrafish larvae, about 99% of water volume was replaced every day with a new aliquot of conditioned tap or filtered water without supplement food. On the last day of exposure, 25 larval fish were isolated for abnormality or behavioural analysis, and the remaining fish were sacrificed for DNA isolation.

Adult fish exposures. Six-month-old adult zebrafish were housed in 1.8 L aerated tanks at a density of five fish per 1.5 L for the duration of the exposure test. All adult fish were fed with the same food and light–dark cycle as in the recirculating system. Every tank received a 50% water change daily using sterile pipettes and autoclaved glassware. Water qualities were tested daily by sterile test strips (Tetra, Blacksburg, VA, USA; API, Chalfont, PA, USA) to ensure the conditions were at pH 7.0, 50–150 ppm hardness, 120–130 ppm alkalinity, 0–3 ppm ammonium, <0.05 ppm nitrite and <40 ppm nitrate. Exposures lasted 14 days and fish were sacrificed on the last day.

Abnormality test. Abnormalities were quantified and tracked daily throughout over the duration of all 5-day larval exposures before the daily water change. The abnormalities assessed were skeletal deformities, heart oedema, yolk sac oedema, inflated swim bladder, mortality and unhatched embryos. After all embryos were screened, the dead larvae were removed. The abnormality rates were compared over time using the chi-square test and pairwise comparison with Bonferroni corrections.

Behaviour test. Behavioural analysis was completed via a DanioVision Behavioural Chamber (Noldus Information Technology, Wageningen, Netherlands). Each larval fish was placed into an individual well of a 24-well plate with 2 ml saline prepared with RO water and 600 mg L^{-1} Instant Ocean Salt. After acclimating for 1 h at 27°C, the plate was placed into the DanioVision Observation Chamber set at 28.5 $\pm$ 0.5°C. The behavioural assay consisted of 3-min light and dark alternating periods with a total of four light–dark cycles for 24 min. The behavioural data were then analysed using ANOVA and Tukey's HSD tests. Significance was considered at p values smaller than 0.05. The quality control and statistics were conducted using R (http://www.r-project.org).

Gut microbiomes characterization. Both adult and larval zebrafish were euthanized by concentrated Tricaine (0.6 g/250 ml) for 10 min before sacrifice. The adult fish intestine from directly posterior to the oesophagus to the vent was removed intact with sterile technique. Five larval fish were pooled as one replicate for DNA isolation. A total of 25 larvae and 30 adult intestines per exposure condition were extracted. The obtained larval or intestine samples were immediately placed in RNA later and stored at 4°C until DNA isolation was performed using the Qiagen PowerFecal Kit (Qiagen, Hilden, Germany). DNA samples were characterized via targeted gene amplification of the 16S rRNA V4 region (515F/806R).(Caporaso *et al*., 2012; Kozich *et al*., 2013) Pooled and purified libraries were sequenced on an Illumina MiSeq sequencer for 250-bp paired-end reads (University of Michigan Medical School, Ann Arbor, MI, USA). Amplicon sequences for each sample are available on the Sequence Read Archive of the National Center for Biotechnology Information. All sequencing data processing and quality control were conducted using Mothur (v.1.41.3) following the MiSeq standard operating protocol (Schloss *et al*., 2009). Quality-filtered reads were aligned against SILVA v132 and clustered using the average neighbour approach to form operational taxonomic units (OTUs) with a sequence similarity cut-off of 97%. Any singleton across all samples was discarded from the analysis. One gut microbiome outlier was detected using a non-parametric detection test CLOUD (Montassier *et al*., 2018) and removed. Total richness of each sample was assessed by Chao1 Index (Chao, 1984). Dissimilarities between gut microbiomes OTUs diversity and abundances were compared by Bray–Curtis ordination (Beals, 1984). Significant association across exposure conditions were compared using ANOSIM. Total richness and relative abundances of OTUs across exposure conditions were compared using ANOVA and Tukey's HSD tests if normally distributed, or Kruskal–Wallis and Dunn's non-parametric comparison if not normally distributed.

References

Almeida, A.R., Tacão, M., Machado, A.L., Golovko, O., Zlabek, V., Domingues, I., and Henriques, I. (2019) Long-term effects of oxytetracycline exposure in zebrafish: a multi-level perspective. *Chemosphere* **222**: 333–344.

Bates, J.M., Mittge, E., Kuhlman, J., Baden, K.N., Cheesman, S.E., and Guillemin, K. (2006) Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Dev Biol* **297**: 374–386.

Beals, E.W. (1984) Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. *Adv Ecol Res* **14**: 1–55.

Borrelli, L., Aceto, S., Agnisola, C., De Paolo, S., Dipineto, L., Stilling, R.M., *et al*. (2016) Probiotic modulation of the microbiota-gut-brain axis and behaviour in zebrafish. *Sci Rep* **6**: 1–9.

Burns, A.R., Stephens, W.Z., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K., and Bohannan, B.J.M. (2016) Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME J* **10**: 655–664.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., *et al*. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6**: 1621–1624.

Carabotti, M., Scirocco, A., Maselli, M.A., and Severi, C. (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* **28**: 203–209.

Chaidez, C., and Gerba, C.P. (2004) Comparison of the microbiologic quality of point-of-use (POU)-treated water and tap water. *Int J Environ Health Res* **14**: 253–260.

Chao, A. (1984) Nonparametric estimation of the number of classes in a population. *Scand J Stat* **11**: 265–270.

Cresci, G.A., and Bawden, E. (2015) Gut microbiome: what we do and don't know. *Nutr Clin Pract* **30**: 734–746.

Dahan, D., Jude, B.A., Lamendella, R., Keesing, F., and Perron, G.G. (2018) Exposure to arsenic alters the microbiome of larval zebrafish. *Front Microbiol* **9**: 1323.

Desbonnet, L., Clarke, G., Traplin, A., O'Sullivan, O., Crispie, F., Moloney, R.D., *et al*. (2015) Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. *Brain Behav Immun* **48**: 165–173.

Detroit Water and Sewerage Department. (2019) Detroit water quality report.

Dias, M.F., Reis, M.P., Acurcio, L.B., Carmo, A.O., Diamantino, C.F., Motta, A.M., *et al*. (2018) Changes in mouse gut bacterial community in response to different types of drinking water. *Water Res* **132**: 79–89.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., *et al*. (2005) Diversity of the human intestinal microbial flora. *Science* **308**: 1635–1638.

Gaulke, C.A., Barton, C.L., Proffitt, S., Tanguay, R.L., and Sharpton, T.J. (2016) Triclosan exposure is associated with rapid restructuring of the microbiome in adult zebrafish. *PLoS One* **11**: 1–20.

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., and Verdegem, M. (2015) The impact of rearing environment on the development of gut microbiota in tilapia larvae. *Sci Rep* **5**: 1–15.

Gotelli, N.J., and Colwell, R.K. (2011) Estimating species richness. In *Biological Diversity: Frontiers in Measurement and Assessment*. United Kingdom: Oxford University Press, pp. 39–54.

Guthrie, G.D., Nicholson-Guthrie, C.S., and Leary, H.L., Jr. (2000) A bacterial high-affinity GABA binding protein: isolation and characterization. *Biochem Biophys Res Commun* **268**: 65–68.

Hansen, G.H., and Olafsen, J.A. (1999) Bacterial interactions in early life stages of marine cold water fish. *Microb Ecol* **38**: 1–26.

Ihekweazu, F.D., and Versalovic, J. (2018) Development of the pediatric gut microbiome: impact on health and disease. *Am J Med Sci* **356**: 413–423.

Kashinskaya, E.N., Simonov, E.P., Kabilov, M.R., Izvekova, G.I., Andree, K.B., and Solovyev, M.M. (2018) Diet and other environmental factors shape the bacterial communities of fish gut in an eutrophic lake. *J Appl Microbiol* **125**: 1626–1641.

Koenig, J.E., Spor, A., Scalfone, N., Fricker, A.D., Stombaugh, J., Knight, R., *et al*. (2011) Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A* **108**: 4578–4585.

Koo, H., Hakim, J.A., Powell, M.L., Kumar, R., Eipers, P.G., Morrow, C.D., *et al*. (2017) Metagenomics approach to the study of the gut microbiome structure and function in zebrafish Danio rerio fed with gluten formulated diet. *J Microbiol Methods* **135**: 69–76.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**: 5112–5120.

Kurzawova, V., Stursa, P., Uhlik, O., Norkova, K., Strohalm, M., Lipov, J., *et al*. (2012) Plant-microorganism interactions in bioremediation of polychlorinated biphenyl-contaminated soil. *N Biotechnol* **30**: 15–22.

Lautenschlager, K., Boon, N., Wang, Y., Egli, T., and Hammes, F. (2010) Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. *Water Res* **44**: 4868–4877.

Lee, J., Lee, C.S., Hugunin, K.M., Maute, C.J., and Dysko, R. C. (2010) Bacteria from drinking water supply and their fate in gastrointestinal tracts of germ-free mice: a phylogenetic comparison study. *Water Res* **44**: 5050–5058.

Legrand, T.P.R.A., Wynne, J.W., Weyrich, L.S., and Oxley, A. P.A. (2020) A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome. *Rev Aquac* **12**: 1101–1134.

Mangwani, N., Shukla, S.K., Kumari, S., Das, S., and Rao, T. S. (2016) Effect of biofilm parameters and extracellular polymeric substance composition on polycyclic aromatic hydrocarbon degradation. *RSC Adv* **6**: 57540–57551.

Mayoclinic (2020). Water: How much should you drink every day? Nutrition and healthy eating. URL https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/water/art-20044256.

Montassier, E., Al-Ghalith, G.A., Hillmann, B., Viskocil, K., Kabage, A.J., McKinlay, C.E., *et al*. (2018) CLOUD: a non-parametric detection test for microbiome outliers. *Microbiome* **6**: 1–10.

Nikouli, E., Meziti, A., Antonopoulou, E., Mente, E., and Kormas, K.A. (2019) Host-associated bacterial succession during the early embryonic stages and first feeding in farmed Gilthead Sea Bream (Sparus aurata). *Genes (Basel)* **10**: 1–15.

NSF International. (2016) NSF protocol P473: drinking water treatment units—PFOA and PFOS, Ann Arbor.

NSF International/ANSI. (2015a) NSF standard 42: drinking water treatment units—aesthetic effects, Ann Arbor.

NSF International/ANSI. (2015b) NSF standard 53 drinking water treatment units—health effects, Ann Arbor.

Oliveira, J.M.M., Almeida, A.R., Pimentel, T., Andrade, T.S., Henriques, J.F., Soares, A.M.V.M., *et al*. (2016) Effect of chemical stress and ultraviolet radiation in the bacterial communities of zebrafish embryos. *Environ Pollut* **208**: 626–636.

Phelps, D., Brinkman, N.E., Keely, S.P., Anneken, E.M., Catron, T.R., Betancourt, D., *et al*. (2017) Microbial colonization is required for normal neurobehavioral development in zebrafish. *Sci Rep* **7**: 1–13.

Rawls, J.F., Samuel, B.S., and Gordon, J.I. (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci U S A* **101**: 4596–4601.

Reasoner, D.J., Blannon, J.C., and Geldreich, E.E. (1987) Microbiological characteristics of third-faucet point-of-use devices. *J Am Water Work Assoc* **79**: 60–66.

Roeselers, G., Mittge, E.K., Stephens, W.Z., Parichy, D.M., Cavanaugh, C.M., Guillemin, K., and Rawls, J.F. (2011) Evidence for a core gut microbiota in the zebrafish. *ISME J* **5**: 1595–1608.

Rombout, J., Stroband, H.W.J., and Taverne-Thiele, J.J. (1984) Proliferation and differentiation of intestinal epithelial cells during development of Barbus conchonius (Teleostei, Cyprinidae). *Cell Tissue Res* **236**: 207–216.

Schaeffer, T.L., Cantwell, S.G., Brown, J.L., Watt, D.S., and Fall, R.R. (1979) Microbial growth on hydrocarbons: terminal branching inhibits biodegradation. *Appl Environ Microbiol* **38**: 742–746.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., *et al*. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Schretter, C.E., Vielmetter, J., Bartos, I., Marka, Z., Marka, S., Argade, S., and Mazmanian, S.K. (2018) A gut microbial factor modulates locomotor behaviour in Drosophila. *Nature* **563**: 402–406.

Spanggaard, B., Huber, I., Nielsen, J., Sick, E.B., Pipper, C. B., Martinussen, T., *et al*. (2001) The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. *Environ Microbiol* **3**: 755–765.

Stephens, W.Z., Burns, A.R., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K., and Bohannan, B.J.M. (2016) The composition of the zebrafish intestinal microbial community varies across development. *ISME J* **10**: 644–654.

Stroband, H.W.J., and Debets, F.M.H. (1978) The ultrastructure and renewal of the intestinal epithelium of the juvenile grasscarp, Ctenopharyngodon idella (Val.). *Cell Tissue Res* **187**: 181–200.

Sullam, K.E., Essinger, S.D., Lozupone, C.A., O'Connor, M. P., Rosen, G.L., Knight, R., *et al*. (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Mol Ecol* **21**: 3363–3378.

Sundarraman, D., Hay, E.A., Martins, D.M., Shields, D.S., Pettinari, N.L., and Parthasarathy, R. (2020) Higher-order interactions dampen pairwise competition in the zebrafish gut microbiome. *MBio* **11**: e01667–e01620.

Tan, F., Limbu, S.M., Qian, Y., Qiao, F., Du, Z.-Y., and Zhang, M. (2019) The responses of germ-free zebrafish (Danio rerio) to varying bacterial concentrations, colonization time points, and exposure duration. *Front Microbiol* **10**: 1–13.

Tobin, R.S., Smith, D.K., and Lindsay, J.A. (1981) Effects of activated carbon and bacteriostatic filters on microbiological quality of drinking water. *Appl Environ Microbiol* **41**: 646–651.

Valtin, H. (2002) "Drink at least eight glasses of water a day." really? Is there scientific evidence for "8×8"? *American Journal of Physiology-Regulatory, Integrative and Comparative Phys Ther* **283**: R993–R1004.

Wu, C.-C., Ghosh, S., Martin, K.J., Pinto, A.J., Denef, V.J., Olson, T.M., and Love, N.G. (2017) The microbial colonization of activated carbon block point-of-use (PoU) filters with and without chlorinated phenol disinfection by-products. *Environmental Science: Water Research and Technology*. **3**: 830–843.

Wu, C.-C., Love, N.G., and Olson, T.M. (2021) Bacterial transmission and colonization in activated carbon block (ACB) point-of-use (PoU) filters. *Environmental Science: Water Research and Technology*. **7**: 1114–1124.

Xia, J., Lu, L., Jin, C., Wang, S., Zhou, J., Ni, Y., Fu, Z., and Jin, Y. (2018) Effects of short term lead exposure on gut microbiota and hepatic metabolism in adult zebrafish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **209**: 1–8.

Xia, Y., Zhu, J., Xu, Y., Zhang, H., Zou, F., and Meng, X. (2020) Effects of ecologically relevant concentrations of cadmium on locomotor activity and microbiota in zebrafish. *Chemosphere* **257**: 127220.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Exposure scheme of larvae and adult zebrafish from ACB PoU filter manifold system

Fig. S2. Non-metric multidimensional scaling (NMDS) ordination plot of tap (circle) and filtered water (triangle) bacterial communities across operation day 1, 17, 30, 37, and 49. The analysis was based on Bray–Curtis dissimilarity coefficients calculated from the relative abundances of OTUs.

Table S1. Average relative abundances (%) of bacterial taxa found in greater than 80% of larval or adult intestines within each exposure condition

Table S2. Average relative abundances (%) of bacterial taxa in tap or filtered water bacterial community over filter operation

Table S3. Water quality changes in tap and filtered water during early and late stages of operation period