Large-Scale, Multi-Year Microbial Community Survey of a Freshwater Trout Aquaculture Facility

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Abstract

Aquaculture is an important tool for solving growing worldwide food demand, but infectious diseases of the farmed animals represent a serious roadblock to continued industry growth. Therefore, it is essential to understand the microbial communities that reside within the built environments of aquaculture facilities to identify reservoirs of bacterial pathogens and potential correlations between commensal species and specific disease agents. Here, we present the results from three years of sampling a commercial rainbow trout aquaculture facility. The sampling was focused on the early-life stage hatchery building and included sampling of the facility source water and outdoor production raceways. We observed that the microbial communities residing on the abiotic surfaces within the hatchery were distinct from those residing on the surfaces of the facility water source as well as the production raceways, despite similar communities in the water column at each location. Within the hatchery building, most of the microbial classes and families within surface biofilms were also present within the water column, suggesting that these biofilms are seeded by a unique subgroup of microbial taxa from the water. Lastly, we detected a common fish pathogen, *Flavobacterium columnare*, within the hatchery, including at the source water inlet. Importantly, the relative abundance of this pathogen was

correlated with clinical disease. Our results characterized the microbial communities in an aquaculture facility, established that the hatchery environment contains a unique community composition, and demonstrated that a specific fish pathogen resides within abiotic surface biofilms and is seeded from the natural source water.

Importance

The complex microbial consortium residing in the built environment of aquaculture facilities is poorly understood. In this study, we provide a multi-year profile of the surface- and water-associated microbial communities of this biome. The results demonstrated that distinct community structures exist in the water and on surfaces. Furthermore, it was shown that a common and economically impactful bacterial pathogen, *F. columnare*, is continually introduced via the source water, is widespread within surface biofilms in the hatchery environment, and is likely amplified within these raceways but does not always cause disease despite being present. These results advance our understanding of pathogen localization at fish farms, show the interplay between host and environmental microbiomes, and reveal the importance of microbial community sequencing in aquaculture for identifying potential beneficial and harmful microbes. This study adds to the aquaculture microecology dataset and enhances our ability to understand this environment from a “One Health” perspective.

Introduction

Aquaculture has grown extensively over the past three decades, but disease caused by microbes poses one of the greatest threats to the continued growth of this industry (1). While the microbiomes of many farmed fish have been studied (2, 3), the microbial communities existing within the built environments of these animals, including raceway surfaces, have garnered less attention. These biomes are important as they offer potential insight into the distributions and abundances of specific pathogens. For example, in farms where untreated surface water is used, microbes from local upstream ecosystems flow into

raceways and form biofilms on their surfaces (4). A mature biofilm is typified by a sturdy and stable architecture, a protective matrix, and often, a highly dense and heterogenous assembly of microbial species. These features allow biofilms to serve as microbial banks, providing stable sites for genetic exchange that are relatively undisturbed by environmental hazards, including antibiotic and chemical treatments (5). Previous studies have demonstrated that most biofilm-associated microbes in aquaculture facilities are not harmful, and many may in fact be beneficial to the farmed fish. However, a subset of species are opportunistically pathogenic and can cause rapid and deadly disease outbreaks depending on host health, age, and environmental factors (6, 7).

Numerous methods are used to farm rainbow trout (*Oncorhynchus mykiss*) in the United States, with a common approach being the flow-through system in which natural spring or river water is diverted through the facility and eventually flows out (8). This type of system can provide relatively stable temperatures and consistent water quality based on the farm's location, source water type, flow, and distance the water travels above ground level (8). These farms are typically configured such that eggs and young fish are housed within a hatchery building, or early life-stage rearing facility, where they mature for several months (9). After reaching a specified size, fish are moved into outdoor raceways for grow-out before harvesting. Although disease can occur at any step in this process, generally the younger individuals are at greatest risk relative to the older individuals. Due to an immature immune system and difficulties with vaccination, rapid and significant mortality events occur often in the hatchery setting (10).

Two bacterial species, *Flavobacterium columnare* and *Flavobacterium psychrophilum*, have been shown to impact the early life stages of farmed rainbow trout. *F. columnare* causes columnaris disease (CD) (11) and has recently been divided into four species based on genomic differences, with each species being associated with varying virulence levels in laboratory studies (12). *F. psychrophilum* causes bacterial cold-water disease (BCWD), which is often observed in immature cold water fish species (13). There is

only one licensed vaccine for CD in catfish (14), and no licensed vaccines are available for BCWD within the United States (although a live-attenuated vaccine is in development (15–17). The lack of potent, universal, and well-tolerated vaccines for fish, especially for the youngest fish (fry), means that early and accurate detection of pathogens is essential for reducing the impacts of an outbreak. Current mitigation and prevention methods heavily rely on animal health surveillance targeting fish that exhibit symptoms of CD or BCWD (i.e., lethargy, abnormal swimming, lack of appetite, and skin lesions) for culturing and diagnostic PCR. If a pathogen is identified, intervention efforts commence. However, while important, this strategy focuses on identifying pathogenic *Flavobacterium* spp. during the period of clinical disease, when bacterial load is high and peak shedding occurs, overlooking initial infection events, the incubation period, the window of subclinical disease, asymptomatic shedding, and importantly, the point source.

Outbreaks of *F. psychrophilum* and *F. columnare* frequently re-occur in the hatchery environment between different production lots despite disinfection procedures. Such observations suggest that disinfection protocols are not entirely effective at removing these pathogens or that they are regularly re-introduced into this environment. Both of these flavobacterial pathogens have been shown to survive for long periods of time in freshwater at cool temperatures in the absence of their fish host (18, 19). They have also been shown to readily form biofilms, which may increase their ability to persist and survive treatment with some of the common disinfectants used in aquaculture (20, 21). Understanding the reservoirs and sources of these pathogens will be an important step in developing management practices for their control.

High throughput sequencing of the 16S rRNA gene in samples collected at various sites within raceways and the inflowing water provides crucial insights into the sites of pathogen localization within farms along with potential correlations with collected metadata. Coupled with standard health surveillance and culturing strategies, microbiome research at fish farms allows us to characterize the transient versus resident flora of the farm, identify possible interactions between microbiota, and direct the

development of new methods of pathogen control by identifying beneficial microbes for probiotic studies. Microbiome research at fish farms can thus inform and enhance outbreak control measures.

In this work, we present a study of the microbiome of a rainbow trout facility spanning three consecutive years of sampling. We performed sequencing of a hypervariable region in the 16S rRNA gene to characterize the native microbial communities flowing into and out of the hatchery raceways as well as those growing within biofilms formed on structures in the raceways. We sequenced water and surface samples collected from three sites: Box Canyon Spring (BCS), the hatchery facility, and the outdoor raceways, with a primary focus on the hatchery facility. We also aimed to leverage our 16S rRNA survey data to simultaneously evaluate the presence of two pathogenic flavobacterial species and better understand pathogen refuge and transmission within the hatchery.

Materials and Methods

Sample Sites and Site Metadata

Samples were collected from and around a rainbow trout aquaculture facility in Idaho, USA. Sites included BCS (where the source water is obtained), the hatchery building, and the outdoor raceways. Collection was performed in October 2017, 2018, and 2019 and August 2019. The water temperature at each of the times of sampling was consistently 14-15°C. The upstream source water runs approximately 1 mile from its spring source, receives ample natural sunlight, and possesses a population of wild rainbow trout and other fish species (Supplementary Figure 1). The water from this area is diverted through an enclosed pipe that flows ~0.5 miles directly into the hatchery building and outdoor raceways. The outdoor raceways remain uncovered and receive abundant sunlight. The hatchery building is entirely covered (minimal natural light) and contains 20 individual epoxy-painted concrete raceways into which water is piped before flowing out of the building and into the river. Hatchery raceway surfaces are regularly scrubbed with a coarse brush and visible debris are removed. Between

production lots, hatchery raceways are drained, pressure washed, soaked in a bleach solution (200 parts per thousand), and then allowed to dry. The available water quality parameters of the influent are as follows; 2017: 0.018 mg/L total phosphorus (TP) and 2.116 mg/L nitrate/nitrite. The available water quality parameters of the effluent are as follows; 2017: 0.092 mg/L TP, 2.083 mg/L nitrate/nitrite, 7.2 mg/L dissolved oxygen (DO), < 31 mg/L chloride, and 1.0 mg/L ammonia (pH 8); 2018:– 0.156 mg/L TP, 7.4 mg/L DO, < 31 mg/L chloride, and 0.5 mg/L ammonia (pH 8.1); 2019: 0.074 mg/L TP, 7.8 mg/L DO, < 31 mg/L chloride, and 0.5 mg/L ammonia (pH 7.3).

Surface Samples

Surface samples were collected using sterile Whatman OmniSwabs (Maidstone, United Kingdom; cat. # WHAWB100035). In the indoor hatchery and outdoor raceways, an approximately 1-m long strip of the target surface was swabbed until the swab was visibly discolored. For the hatchery, polycarbonate baffles suspended in the center of the raceway were swabbed on both the front (flow facing) and back sides at the air/water interface and at 1 swab length below the surface (15 cm) (Supplementary Figure 2). The metal tailscreens were only swabbed on the front side at the air/water interface. The epoxy-coated concrete walls of the raceways were typically swabbed at four depths, including the air/water interface, 1 swab length below the surface (15 cm), 2 swab lengths below the surface (30 cm), and at the bottom portion of the wall adjacent to the floor (variable depth as water levels change during production cycle). The walls along the length of the raceway were typically swabbed towards the front third, directly preceding the location of the first baffle. In 2019, a second location of the wall was also sometimes swabbed toward the back third of the raceway, directly behind the final baffle. For the outdoor raceways, swab samples were only collected from the air/water interfaces of walls. For BCS, wall swabs refer to any surfaces that were swabbed (typically rocks) below the water line at various depths. All swabs were immediately placed on ice and then frozen at -20°C for subsequent shipment and storage.

147 *Water Samples*

148 Six liters of water were collected for each water sample using sterile Whirlpak bags (Madison, WI, USA;
149 cat. # B01447WA). In the hatchery, inflowing and outflowing water samples were typically collected at
150 the inflow and outflow pipes of individual raceways. A few samples were collected from the "global"
151 inflow, which refers to water collected from the primary water pipe before being diverted to individual
152 raceways. Global outflow samples were collected from the main water pipe leading from the hatchery to
153 the river. All water samples were filtered in 1-L aliquots using a previously described system and
154 protocol (22). A 2 µm pore size prefilter (Sigma Aldrich, St. Louis, MO, USA; cat. # AP2502500) and a 0.2
155 µm pore size Sterivex filter unit (Fisher Scientific, Waltham, MA, USA; cat. # SVGPL10RC) were collected.
156 Attempts to filter the water with a Sterivex filter unit alone (to attain a single representative water
157 sample) proved unfeasible, as clogging was a common occurrence. Prefilters and Sterivex units were
158 immediately frozen at -20°C for shipment and storage.

159 *Fish Samples*

160 Fish from the outdoor raceways were captured via a net. Gill swabs were collected by direct swabbing of
161 the outward facing gill tissue. Intestinal samples were collected following dissection of euthanized adult
162 fish. Sampling of farmed and wild fish were approved under IACUC Animal Protocol A16-040.

163 *Diagnosis of CD and BCWD*

164 CD and BCWD were diagnosed by assessing the presence or absence of rod-shaped, oxidase-positive
165 bacteria on gills and within the spleen and kidney tissues of multiple sampled fish.

166 *DNA Extraction*

167 DNA was isolated from the swab and tissue samples using a Qiagen PowerSoil DNA Isolation kit (Qiagen,
168 Hilden, Germany; cat. # 12888-100) following the manufacturer's protocol (23). For the prefilter

samples, DNA was isolated using a Qiagen PowerWater DNA Isolation kit (cat. # 14900-50-NF). DNA was extracted and isolated from Sterivex filters using the Qiagen Sterivex PowerWater DNA Isolation kit (cat. # 14600-50-NF) following the manufacturer's protocol. DNA was eluted in 50 µL of buffer EB (Qiagen) and quantified with a Qubit HS dsDNA Assay kit (Life Technologies, Carlsbad, CA, USA). DNA extracts were stored at -20°C until ready for further processing.

PCR Amplification

For each sample, the V4 hypervariable region of the 16S rRNA gene was amplified using previously validated primers that contained dual-end adapters for indexing as previously described (22, 23). Briefly, PCR reactions were set-up and performed in triplicate using the 515F forward and 806R reverse rRNA gene V4 primers (24) with Illumina MiSeq adaptors. A Bio-Rad C1000 Touch Thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used for PCR amplification with the following parameters: 94°C for 3 min followed by 30 cycles of 94°C for 45 sec, 50°C for 60 sec, and 72°C for 90 sec, with a final incubation at 72°C for 10 min.

Library Preparation and Sequencing

PCR amplicons were prepared for sequencing as previously described (23). Briefly, reactions were pooled, and product presence and size were verified on a QIAxcel (Qiagen). PCR products were then cleaned using a GeneRead Size Selection kit (Qiagen; cat. # 180514) and diluted to 4 nM for sequencing on an Illumina MiSeq (Illumina, San Diego, CA, USA). A 500 cycle MiSeq Reagent kit v2 (Illumina; cat. # MS-102-2003) was used for paired-end sequencing of all libraries.

Bioinformatic Processing

16S rRNA V4 gene sequence data were demultiplexed through BaseSpace (basespace.illumina.com), after which paired-end data were downloaded and imported into QIIME 2 (version 2021.4) (25). Then,

191 the reads underwent filtering, denoising, and dereplication using the DADA2 denoise-paired plugin (26).
192 Sequences were taxonomically classified and compiled using the feature-classifier (27) and taxa
193 (https://github.com/qiime2/q2-taxa) plugins. Taxonomy was assigned using a pre-trained Naïve Bayes
194 classifier based on the Silva 132 99% OTU database (https://www.arb-silva.de/documentation/release-
195 138/) (28), with the reference trimmed down to the region bound by the 515F/806R primer pair. The
196 default QIIME 2 processing parameters were used throughout the workflow except where otherwise
197 noted.

198 ASV counts, taxonomy information, and a phylogeny were imported into RStudio (version 1.1.447) (29)
199 from QIIME 2 using the qiime2R package (30). Phyloseq (version 1.36.0) (31), ggplot2 (version 3.3.5)
200 (32), and microViz (version 0.7.9) (33) were used to calculate alpha and beta diversity as well as to
201 generate barplot, boxplot, and ordination visualizations. Statistical testing was performed primarily
202 using the vegan (version 2.5-7) (34) package.

203 *Controls and QC*

204 DNA extraction controls were processed for every extraction kit lot and used to assess potential kit
205 contaminants and contamination occurring during DNA extraction. PCR negative controls were run with
206 every PCR plate using molecular grade water in place of DNA to assess PCR reagent contaminants and
207 contamination occurring during PCR. Mock community DNA controls (ZymoBIOMICS, Irvine, CA, USA;
208 cat. # D6306) were also amplified during each PCR and used to assess PCR amplification biases.

209 QC steps performed during library preparation included the previously mentioned use of a QIAxcel to
210 verify product presence and size. For the 2019 samples, 16S-targeted qPCR (35) was performed to verify
211 that the total bacterial load was sufficient to overcome potential well-to-well contamination that may
212 occur in plate-based PCR setups. For this specific group of samples, any sample with more than 10,000
213 copies of the 16S rRNA gene (as determined by qPCR) was retained (total samples retained, 431/446,

97%). A number of samples did not return reads following sequencing, possibly due to failed PCR reactions (357/431, 83%). Following sequencing and initial processing, only samples with at least 10,000 reads were retained for downstream analyses to guard against the effects of potential contamination on low biomass samples and increase the robustness of downstream analyses (163/357, 46%). Sequence quality filtering and chimeric sequence removal were also performed within QIIME 2 using the default parameters. Five separate MiSeq sequencing runs yielded 17,226,236 paired reads, and after the aforementioned QC steps were 6,131,752 with an average of 46,103 reads and a median of 39,325 reads per sample. The minimum and maximum read counts were 10,720 and 172,746, respectively. A total of 25,790 ASVs were recovered across all samples.

Statistical Analysis

Alpha and beta diversity index calculations were performed on data rarefied to 10,000 reads. PERMANOVA testing was used to determine statistical significance of group differences for beta diversity indices. Shapiro-Wilk normality testing was used to justify specific statistical testing choices. Normally and non-normally distributed data were analyzed with t-tests and Wilcoxon signed-rank tests, respectively. Holm corrections were used to correct for testing with multiple comparisons, where appropriate. ANCOM-BC default parameters were used, and as recommended, the structural zero flag (neg_lb) was set to “True” only for comparisons in which both groups had 30 or more samples per group. Tested classes had to be present in 10% of samples to be included. For all tests, a corrected p-value of 0.05 was required to achieve significance.

Results

Sample Summary

A total of 448 samples were collected at an aquaculture facility between 2017-2019, 163 of which were analyzed using an amplicon sequencing approach. As such, this study represents one of the most

extensive microbial surveys conducted on an aquaculture facility and provides important insight into microbial community composition across various sites at the farm, including but not limited to those colonizing the surfaces of raceway walls and other raceway structures, those residing within the water column, as well as those of fish skin and gills. The large and diverse dataset provides an opportunity to compare and correlate microbial communities according to specific farm site (hatchery, outdoor raceway, source water location, inflow, outflow, etc.), sample type (water, surface, etc.), and clinically diseased versus healthy fish populations.

Microbial Communities of Box Canyon Spring, the Hatchery, and the Outdoor Raceways

We first compared the three sampling sites to determine whether the surface or water communities at each location were distinct from one another and assess if the type of sample or year of sampling were correlated with microbial community structure. The microbial communities of BCS (n = 15), the indoor hatchery facility (n = 134), and the outdoor raceways (n = 14) were compared, with water and surface biofilm samples sampled at all three sites. Additionally, intestine and gill swab samples were included from fish contained in the outdoor raceways to assess the degree of relatedness between host-associated and host-independent samples. Weighted Unifrac distances were calculated for all samples, and PCoA plots were prepared (Figure 1). Microbial community composition was largely dependent on sample type and sampling site. Sterivex and prefilter samples clustered separately from the surface (baffle, wall, tailscreen) samples (Figure 1A) indicating a different overall community composition in biofilms. Fish-associated samples (gills and intestines) clustered closer to the water samples (prefilters and Sterivex). Given the observed differences in microbial community composition between the surfaces (walls, baffles, and tailscreens) and the water (prefilter and Sterivex), these sample types were plotted and analyzed independently. Figure 1B only contains the surface samples (excludes water and fish samples) and shows distinct surface community clustering based on sampling site with BCS and the indoor hatchery resolving into separate clusters, whereas most of the outdoor raceways fell in between

these clusters. Figure 1C shows only water samples (prefilters and Sterivex) and lacks obvious clustering by sampling site, whereas a clear separation was noted between the prefilter and Sterivex sample types. This result indicates that the compositions of the communities residing in the water are fairly consistent from the source water to the hatchery and outdoor raceways. Bray-Curtis, Jaccard, and Unweighted Unifrac metrics were also calculated and are displayed in Supplementary Figures 3-5. These results showed overall agreement with those of the weighted Unifrac analysis, with the most obvious divergence being that the hatchery surface samples appeared more variable (larger confidence interval). However, the observed separation between surfaces at different sites and the lack of separation noted for water samples was still evident.

Next, we tested for the significance of these observed community differences that were attributable to sample site, sample type, and year of sampling (as well as their interactions) using PERMANOVA analysis of surface and water samples (Table 1). For surface biofilm samples, significant differences were observed when testing the effects of sample type, sample site, and year of sampling as well as the interaction of sample type and year and sampling site and year. The largest proportion of variance explained was due to sample site ($R^2 = 0.2048$), with all other variables and interactions having R^2 values <0.10. Significant differences were noted for year but also for the interaction between sampling year and sample site/sample type, indicating that differences due to year of sampling may be due to differences in the types or locations samples were collected from in a given year as opposed to differences solely based on time. Beta dispersion testing was also performed to evaluate differences in dispersion between groups and aid in the interpretation of the PERMANOVA results. Significant differences in dispersion were noted between the walls and baffles ($p = 0.0002$) and the years 2017 and 2018 ($p = 0.003$) as well as 2017 and 2019 ($p = 0.011$). No significant differences were observed between the dispersion levels of sampling sites, further supporting the conclusion that the surface biofilm communities at the three sampled locations are distinct.

For water samples, sample type (prefilter vs. Sterivex) and sampling year were both significant factors, with sample type explaining a large amount of variance and sampling year explaining a smaller portion (Table 1). No interactions between variables were found to be significant. The beta dispersion results showed significant differences in dispersion between BCS and both the outdoor raceways ($p = 0.0014$) and hatchery ($p = 0.0132$). Additionally, dispersion differences were noted between the years 2017 and 2019 ($p = 0.0355$).

Similar results to the ones found from weighted Unifrac were obtained from testing on Bray-Curtis, Jaccard, and unweighted Unifrac distances as well (Supplementary Tables 2- 4). The primary differences between the non-phylogenetic and phylogenetic metrics were smaller R^2 values contributed by sampling site for surface samples and smaller R^2 values contributed by sample type for water samples. These results indicate that sampling site has a significant and strong impact on the microbial community structure of the tested surfaces. This contrasts with the water samples where sampling site was not a significant factor influencing the microbial communities. Sample type was the primary significant variable explaining close to 50% of the variance in water samples (particle-associated prefilter vs. planktonic Sterivex). The beta dispersion analysis showed non-significant effects for these aforementioned variables, indicating that uneven variance between groups was not the driver of these significant differences. Significant results were obtained for the sampling year variable for both water and surfaces samples. However, uneven sampling on a yearly basis (significantly more samples in 2019 than 2017 and 2018 as well as only sampling certain sites on a given year) may have been a driving factor for this detected difference and this could be reflected in the significant differences in the beta dispersion results.

Pairwise differential abundance testing using the ANCOM method showed that 36 bacterial classes were present at significantly higher relative abundances within the surface biofilms at the individual sampling sites (Supplementary Table 5). Between the hatchery and outdoor raceways, a significantly higher

relative abundance of Cyanobacteria (W = 22.49) was noted in the outdoor raceways. This difference in Cyanobacteria abundance was also observed when testing BCS and the hatchery (W = 14.58) with significantly higher levels found at BCS. This result is likely due to the hatchery receiving minimal natural sunlight, whereas BCS and the outdoor raceways receive ample amounts to support Cyanobacteria and algae. Additionally, significantly higher relative abundances of Deinococci were found at BCS (W = 3.51) and the outdoor raceways (W = 16.61) compared to the hatchery, possibly owing to the survivability of this bacterial class in high UV environments (36). Alphaproteobacteria were detected at significantly higher relative abundances in the hatchery compared to BCS (W = 9.61) and the outdoor raceways (W = 5.06), possibly indicating that this group fills the niche of photosynthetic microbes in low light environments and/or that high densities of fish support and seed Alphaproteobacteria.

Microbial Communities of the Indoor Hatchery Structure, Biofilms and Water

Three primary structural components of each hatchery raceway [the epoxy-coated concrete walls of the raceways (n = 57), the plastic baffles (n = 43), and the metal tailscreens (n = 7)] were extensively sampled, and the associated microbial communities were analyzed. The inflowing and outflowing water was also filtered, and the microbial communities were profiled in two fractions [a 2 µm or larger prefilter fraction (n = 8) and a 0.2-2 µm Sterivex fraction (n = 19)]. Since the prefilters comprised larger pore diameters than the Sterivex filters, we expected the microbial community sequenced from there to be more enriched in aggregate-lifestyle or particle-attached microbial cells, while the Sterivex data would show the remaining size-filtered fraction, largely represented by planktonic cells.

The total ASV count amongst these samples following filtering was 19,061. Twenty-five classes belonging to 18 phyla comprised the bulk of the microbial communities along with a group of reads unable to be classified to the phylum level (Bacteria_Kingdom) (Figure 2, Supplementary Figure 6). The most dominant classes (totaling >90% of the community composition of each sample type) were also

compared using boxplots (Supplementary Figure 7). The surface samples were predominantly composed of the classes Bacteroidia, Gammaproteobacteria, Alphaproteobacteria, and Verrucomicrobiae (Figure 2). These classes were also major components of the microbial community within the water samples, although at generally lower relative abundances, and additional classes were present that are typically found in water (Figure 2, Supplementary Figure 7).

Phylogenetic (weighted and unweighted Unifrac) and non-phylogenetic (Bray-Curtis and Jaccard) beta diversity metrics were used to compare sample types within the hatchery (Figure 3). Sterivex and prefilter samples clustered independently from one another and all other sample types while maintaining a consistent microbial community profile. Surface biofilm samples typically showed greater variation in composition as demonstrated by larger confidence ellipses. Global PERMANOVA testing showed that sample type was a significant variable (Supplementary Table 6). Pairwise PERMANOVA results with multiple testing correction was performed for Bray-Curtis and Jaccard distances and showed significant differences between all sample types (all comparisons, $p = 0.01$).These results indicate that the communities that reside on the surfaces in the hatchery are compositionally distinct from the seeding water communities, and they are also significantly different from one another.

Alpha diversity was calculated for all sample types (Figure 4). The water samples had significantly higher alpha diversity index values compared to the surface samples with the Sterivex samples having the highest. Amongst the surface samples, the walls had the highest Shannon index values followed by the baffles and then the tailscreens. Wilcoxon signed-rank tests were performed in a pairwise fashion for all groups and multiple comparisons correction was applied. For Shannon diversity, all comparisons were shown to have statistical significance ($p < 0.05$) (Supplementary Table 7). For Faith phylogenetic diversity, all comparisons yielded significant differences ($p < 0.05$), excluding the comparison between baffles and tailscreens ($p = 0.125$) and Sterivex and prefilters ($p = 0.125$) (Supplementary Table 8). These results indicate that the microbial diversity of the water communities was typically greater than that of

356 those residing on the surfaces and that surface type has a significant effect on the community richness
357 and evenness, as the walls had consistently and significantly higher alpha diversity values than baffles or
358 tailscreens.

359 A presence/absence comparison of the class composition by sample type (only including the inflowing
360 prefilter and Sterivex water samples) showed that most of the microbial classes (13 out of 24) were
361 shared between every sample type (Figure 5A). However, there were 7 classes detected in all samples
362 excluding the tailscreens, 2 classes detected in only the water and on the walls, 1 class detected in only
363 the water and on baffles, and 1 class only detected in water samples. At the family level, 88 of the 211
364 represented families were present in all sample types (Figure 5B). An additional 28 families were only
365 detected in the water samples, with 8 of those 28 being found exclusively in Sterivex samples. There
366 were also 8 families not observed in Sterivex samples but present in prefilters and surface biofilm
367 samples. There were 57 families detected in all sample types except tailscreen swabs, potentially due to
368 the lower number of tailscreen samples analyzed compared to baffle and wall samples or actual
369 differences in composition. These results reinforce the above alpha diversity results showing that the
370 water communities are far richer than the surface communities. In addition, these findings revealed that
371 28 microbial families seemingly fail to colonize the surface biofilm communities and that specific
372 surfaces such as the walls contain microbial families (4 present only in water and walls) that baffles and
373 tailscreens do not.

374 ANCOM-BC testing was used to compare the microbial communities of the three surface types at the
375 class level (Supplementary Figure 8). This comparison showed that 8 bacterial classes were at a
376 significantly higher relative abundance on walls than baffles, 9 were significantly higher on baffles than
377 walls, 12 were significantly higher on walls than tailscreens, 1 was significantly higher on tailscreens than
378 walls, and 11 were significantly higher on baffles than tailscreens. Classes consistently detected at
379 significantly higher levels on walls compared with baffles and tailscreens included OM190,

Desulfuromonadia, Anaerolineae, and NB1-j. Classes consistently observed at significantly higher levels on baffles compared with walls and tailscreens included Verrucomicrobiae, Armatimonadia, Fimbriimonadia, and Blastocatellia. No classes were consistently detected at significantly higher levels on tailscreens compared with both walls and baffles (Bacteroidia was found at significantly higher levels on tailscreens than walls but not baffles). These results indicate that not only are there differences in the presence/absence of specific microbial groups but also that many shared groups are present at significantly different relative abundances depending on the surface type profiled.

Pathogenic Flavobacterium Detection in the Hatchery

F. columnare and *F. psychrophilum* species-level detection was performed on the same 16S rRNA dataset described above using a previously validated method (23). A total of 8,854 *F. columnare* reads were detected across 64 samples, and 9 *F. psychrophilum* reads were detected from a single sample, with the relative abundances of *F. columnare* ranging from 0.01 to 6.24%. *F. columnare* was detected for every sampling year and was detected in all sample types. In total, 8/20 Sterivex, 6/8 prefilters, 20/43 baffle swabs, 29/69 wall swabs, and 2/7 tailscreen swabs tested positive for the presence of *F. columnare* (Table 2, Supplementary Table 9).

Based on recent taxonomic restructuring, *F. columnare* has been divided into four species: *F. columnare, Flavobacterium davisii*, *Flavobacterium oreochromis*, and *Flavobacterium covae* (12). A 16S rRNA V4 gene sequence comparison was performed between the four type strain sequences and the 7 ASVs within this dataset that were assigned to *F. columnare* based on the Silva database (Supplementary Figure 9). The primary ASV in this dataset, comprising 74% of the recovered *F. columnare* reads, was an exact match to the type strain sequence for *F. columnare* as defined within the new taxonomy. An additional two ASVs comprising 13% of the *F. columnare* reads had a 1 bp difference from the type strain of *F. columnare.* Based on this alignment, confidence in discerning between *F. columnare, F.*

403 *davisii*, and *F. covae* solely using the V4 region of the 16S rRNA gene is tenuous at best, as each species
404 differs by at most 2 bp.

405 The co-occurrence of *F. columnare* and the clinical manifestation of columnaris within a given raceway
406 was also examined. Of the 12 raceways where *F. columnare* was detected, 3 had active columnaris
407 infections, 1 had previously experienced a large columnaris outbreak but had no known active cases, 2
408 had no active cases during sampling and no history of columnaris but experienced an outbreak following
409 sampling, and 6 had no diagnosed columnaris infections during the production cycle. Typically, raceways
410 where no columnaris was detected over the course of the production cycle had lower levels of *F.*
411 *columnare* compared to raceways where columnaris was identified (Table 2). The relative levels of *F.*
412 *columnare* in samples in which *F. columnare* reads were recovered (one or more reads) are compared in
413 Figure 6. The levels of *F. columnare* in surface biofilm samples in the 6 healthy raceways was shown to
414 be significantly lower than those detected in the 6 diseased raceways (Wilcoxon, $p = 0.0028$) (Figure 6A).
415 In contrast, the levels of *F. columnare* in water samples in the 6 healthy raceways were not significantly
416 different than those detected in the 6 diseased raceways (Wilcoxon, $p = 0.095$) (Figure 6B). This result
417 may be in part due to the difference in sample size for these comparisons, as only 9 water samples were
418 included versus 48 total surface samples (5 water samples were global inflow/outflow samples and were
419 not included as disease status could not be attributed).

420 Inflowing and outflowing water samples both contained *F. columnare*, and in multiple cases, *F.*
421 *columnare* was detected in both the inflow and outflow of the same individual raceway. However, a key
422 difference in these specific cases and with inflow/outflow samples in general was consistently higher
423 levels of *F. columnare* in the outflowing water. In the positive inflow samples, the median percent
424 abundance of *F. columnare* was 0.016 and 0.038% for the Sterivex ($n = 3$) and prefilter samples ($n = 3$),
425 respectively. In the positive outflow samples, the median percent abundance of *F. columnare* was 0.114
426 and 1.294% for the Sterivex ($n = 5$) and prefilter samples ($n = 3$), respectively. As these are relative

abundances, it is possible *F. columnare* is not truly increasing but that other microbial taxa are decreasing in abundance, causing the percent abundance of *F. columnare* to increase. However, qPCR quantitation of the total microbial load within a subset of 2019 samples showed no significant differences in the total bacterial abundance between inflowing and outflowing prefilter samples (t-test, $p = 0.454$), indicating that *F. columnare* is likely being amplified in the raceway. Additionally, significantly higher relative abundances of *F. columnare* were detected in the outflowing water samples ($n = 8$) than in the inflowing water samples ($n = 6$) (Wilcoxon, $p = 0.008$; Figure 6C). Furthermore, despite a limited number of samples collected at BCS, *F. columnare* was detected on the piping that flows water to the farm (7 reads) as well as on a submerged rock surface (44 reads). The detection of this pathogen in both the upstream location and the inflowing water reveals BCS as a likely source for recurrent introduction of *F. columnare* into the hatchery and outdoor raceways.

Discussion

This study focused on the characterization and comparison of the microbial communities associated with the source water, outdoor raceways, and hatchery at a freshwater trout aquaculture facility over three years. The microbial communities of incoming water at inflow, outflow, and within raceways, as well as of surfaces of baffles, tail screens, and raceway walls were characterized. As young fish are particularly vulnerable to *F. columnare* and *F. psychrophilum*, the hatchery building was the most extensively sampled site, and five different sampling location types were characterized, compared, and screened for the presence of these specific flavobacterial pathogens.

The three environments in water-flow succession were compared to assess site-based variation of microbial communities within water and on surfaces. The water source location (BCS), hatchery, and outdoor raceways of the farm had consistent and mostly indistinguishable microbial communities within the water. Sampling site was not shown to be a significant variable contributing to differences in

450 microbial community structure in either the prefilter or Sterivex water fractions. This result indicates
451 that the overall composition of the water community from the natural source spring through to the
452 outflow from the farm is stable and not significantly different despite variations in light availability,
453 animal abundance, and water quality parameters between the three sampled sites. Additionally,
454 sampling year was noted as a significant variable influencing microbial community structure for water
455 samples while only contributing a small amount of variance. Considering the uneven site sampling of
456 water between years (e.g., the outdoor raceways were only sampled in 2017 while the hatchery was
457 sampled 2017-2019), we consider the microbial communities in the water to be consistent on a yearly
458 basis, providing the opportunity for greater reproducibility and stronger study conclusions. As the vast
459 majority of samples were collected during October of each year (a small subset was from August 2019),
460 seasonal variance in community composition may exist that was not explored in the present study.

461 Compared to the water samples, the surface biofilm communities were significantly different between
462 the three sites. Hatchery surfaces contain a distinct community that differs from the communities
463 residing within BCS and the outdoor raceways. A determining factor in this difference may well be that
464 the hatchery raceways are a poor environment for bacterial (Cyanobacteria) and eukaryotic phototrophs
465 (algae), whereas BCS and the outdoor raceways receive plentiful sunlight. ANCOM results indicated that
466 Cyanobacteria were present at significantly higher levels on BCS surfaces and the outdoor raceway
467 surfaces compared to hatchery surfaces.

468 Focusing on the hatchery environment specifically, water and surface communities were shown to be
469 highly distinct. Alpha diversity calculations showed that the water communities were significantly more
470 diverse than surface biofilm communities. Additionally, beta diversity results exhibited significantly
471 different clustering of prefilter and Sterivex samples from wall, baffle, and tailscreen biofilm samples.
472 Presence/absence comparison plotting showed every microbial class detected within the hatchery
473 environment was present within the inflowing water community, and all but two microbial families were

474 detected in the inflowing water. This result indicates that while most biofilm constituents are likely
475 seeded directly from the water community, only a distinct subset of this water community can establish
476 itself within the surface biofilm community. It is also worth noting that the potential microbial
477 contributions from the feed as well as the fish themselves was not accounted for in the present study.
478 The two microbial families not detected in the water (Streptococcaceae and Nakamurellaceae) but
479 detected on the surfaces may have originated from one of these two sources. Interestingly,
480 Streptococcaceae were found at moderate levels in fish-associated samples from the outdoor raceways
481 and were observed in outflowing water samples (data not shown), possibly implicating the farmed
482 species as the source of these bacteria. Alternatively, it is possible these microbial classes are present in
483 the water at low enough levels where detection through sequencing is not possible and amplification
484 within the surface biofilms then allows for detection.

485 Specific surface types within the hatchery were also unique from one another. Walls were shown to
486 have significantly higher alpha diversity than baffles and tailscreens. All three surface types were also
487 shown to cluster in a significantly different manner regardless of the beta diversity metric used. The vast
488 majority of the microbial classes and families detected were present in all three surface type
489 communities but at different relative abundances. This result indicates that despite having identical
490 sources (the water, fish, and feed), specific microbial taxa favor unique surfaces based on potential
491 factors such as material type, surface roughness, nutrient concentrations, hydrodynamics, or other
492 factors(37). Hydrodynamics, surface materials, and exposure time may well explain why the wall, a
493 rough concrete surface located on the periphery of the raceway, has a greater community richness
494 compared to corrugated polycarbonate baffles and stainless steel tailscreens (smoother surfaces located
495 perpendicular to the flow of water(38)). In addition, the baffles are placed into the raceways several
496 weeks after the raceway is seeded with fish, and the water level increases as the fish mature. Microbes
497 with less adept adhesion mechanisms may be able to withstand this reduced flow force along the walls

compared to the medial flow force experienced on baffles and tailscreens. There was also an interesting subset of wall swabs that generally clustered distinctly from other wall swabs and surfaces (Figure 3). After reviewing available farm metadata, we are currently investigating the potential for fish age or water contact time as contributors to microbial community composition that may explain this unique clustering pattern.

The lone hatchery sample in which *F. psychrophilum* was detected was a baffle swab from a raceway towards the end of a columnaris outbreak. The detection of *F. psychrophilum* in only this sample may indicate that a coinfection was occurring within this raceway, but diagnosis was not undertaken as an infection cause was already established. A previous study at this farm site (but not at the hatchery facility) identified *F. psychrophilum* on sampled fish at a high frequency using qPCR (39). The lack of *F. psychrophilum*-positive samples in our dataset may be at least partly due to the reduced sensitivity of MiSeq sequencing for pathogen detection compared to traditional qPCR methods (23). *F. psychrophilum* may in fact be present but is simply at too low of a level to be reliably detected using this method.

The sampling of inflowing water shows a clear source of *F. columnare* that can act to reintroduce this and other pathogens from the untreated inflowing water into cleaned and disinfected raceways. *F. columnare* was detected over multiple years in both prefilter and Sterivex samples for inflowing source water. *F. columnare* was also detected within two surface biofilms in the upstream source environment, establishing its presence there. The populations of resident trout and other fish species in BCS together with the ability of *F. columnare* to survive independent of a host provides a reasonable scenario for reintroduction into the hatchery via the inflow. This reinforces the idea that this and other pathogens are likely endemic and the continued use of any approved and available vaccines, even when disease is not present, should be continued to avoid future outbreaks. We also observed that outflowing water samples from specific raceways where *F. columnare* was detected in surface biofilms showed higher relative abundances of *F. columnare* than the inflow. This result indicates the possible amplification of

this pathogen in surface biofilms within the raceway followed by shedding into the water where it is then detected in the outflow. Higher levels of *F. columnare* in biofilms and high levels in outflowing water was observed in raceways with no clinical signs of columnaris This finding supports the idea that this shedding likely occurs from either infected, subclinical fish (more fish-associated samples would be needed to investigate this) or from the surface biofilms themselves.

F. columnare was detected in all 5 sample types, including, most interestingly, within surface biofilms in both infected and healthy raceways. Intriguingly, *F. columnare* was detected in two swabbed locations of a raceway that had been emptied and mostly dried (data not shown). This result further solidifies the idea that *F. columnare* is ubiquitous in the environment and is adequately equipped with molecular mechanisms to reside within a complex biofilm community. This finding also highlights that the mere presence of these opportunistic pathogens does not necessarily have a direct correlation with a disease phenotype in the farmed species. Fish immune factors and general stress within the farm environment likely predispose fish to infection by opportunistic pathogens residing in the surrounding communities. However, this phenomenon does not completely invalidate the idea that preventing potentially pathogenic microbes from residing within the hatchery environment would be beneficial, as bacterial load likely plays a crucial role in disease occurrence as well. Increased exposure events due to higher levels of *F. columnare* entering the water from surface biofilm sources may also increase the likelihood for infections to occur. Our results showed that infected raceways had significantly higher levels of *F. columnare* in surface biofilms than healthy ones, but more work will be needed to establish whether this is a precursor to infection or the consequence of one.

In the present study, the communities residing on surfaces within a rainbow trout aquaculture facility were shown to be distinct from the water community that initially seeds these communities and are distinct from one another. The presence of the causative agent of a recurrent disease within the facility was also shown to be widespread, with inflowing water and surface biofilms acting as source and sink

546 for *F. columnare*. These results provide valuable insight into a mostly uncharacterized setting and
547 provides a backdrop for future studies concerning microbial ecology of the built environment, biofilm
548 maturation, antibiotic resistance, probiotic strain sourcing, and disease prevention.

549 **Data Availability**

550 Raw read data are available in the NCBI SRA database under project ID PRJNA821905.

551 **Code Availability**

552 Code is available at https://github.com/joerggraflab/Code-for-Testerman-Beka-2022.

553 **Acknowledgements**

554 We thank the University of Connecticut Microbial Analysis, Resources, and Services (MARS) facility for
555 performing the sequencing work. We thank Chris Jackson and Dr. Jesse Trushenski for providing farm
556 metadata. We thank Dr. Jeremiah Marden for providing feedback on the manuscript.

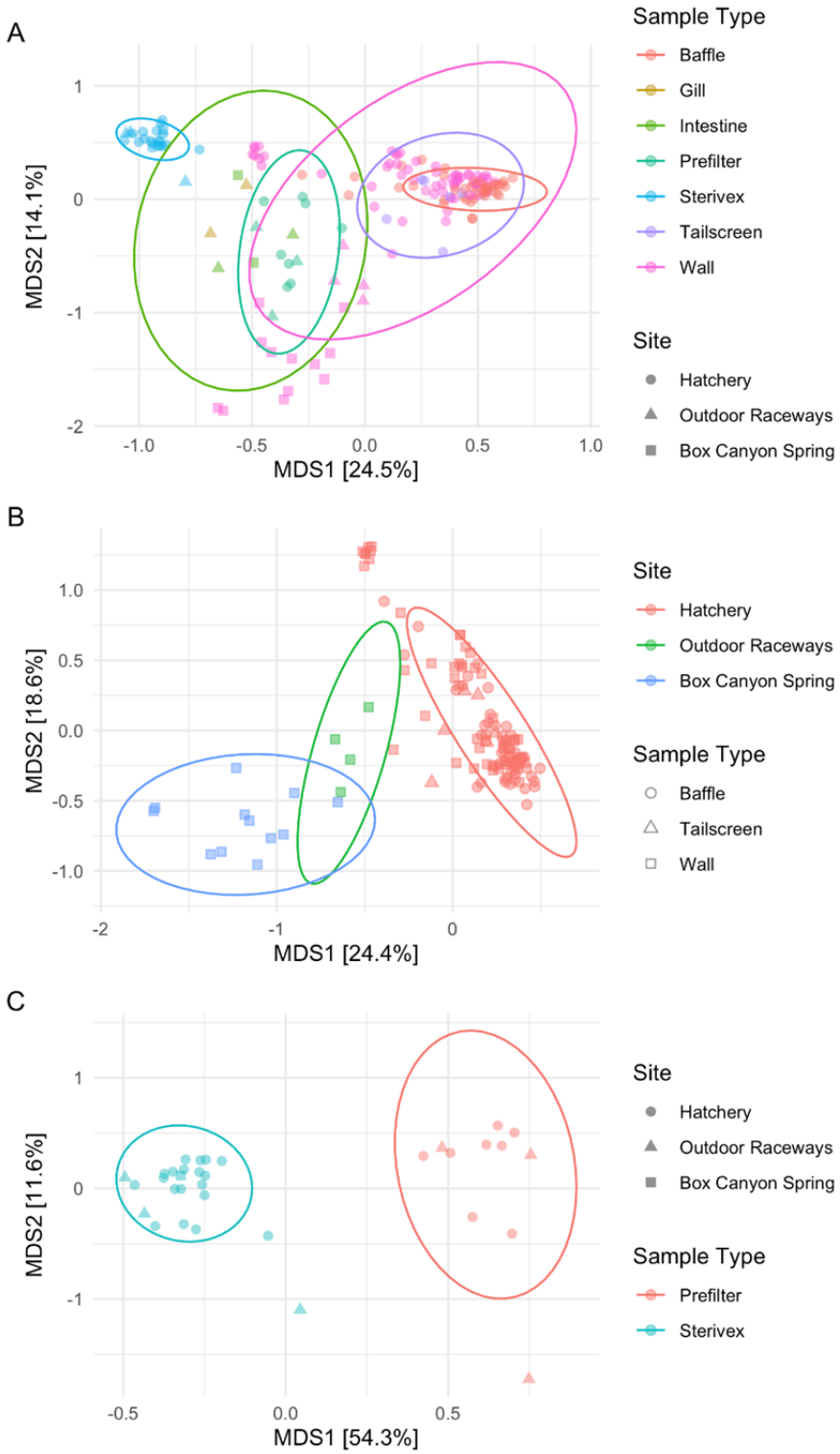

Figure 1: Weighted Unifrac Ordinations of the Three Sampling Sites. (A) All sample types, (B) surface samples only, and (C) water samples only from three sampling sites with 95% confidence ellipses. Three samples or more were required for an ellipse to be drawn for a particular category. Samples are colored by most significant contributing variables.

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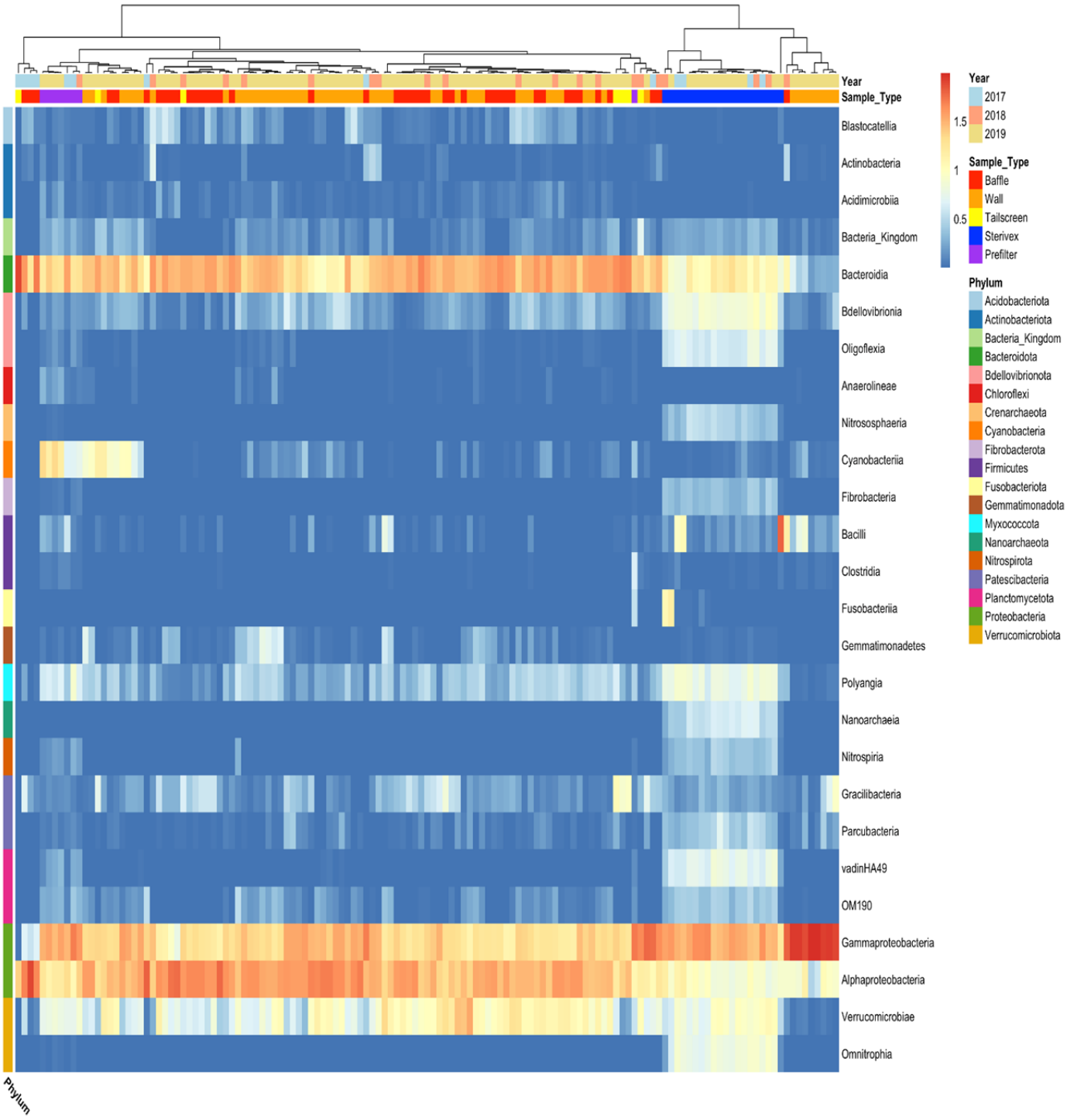

563

564 **Figure 2: Log-Scaled Heatmap of Bacterial and Archaeal Abundances at the Class Level in the Hatchery.**
565 Per sample composition is presented vertically, and samples have been clustered using Pearson
566 correlation values. Phyla are annotated on the left, and classes are grouped based on phylum.

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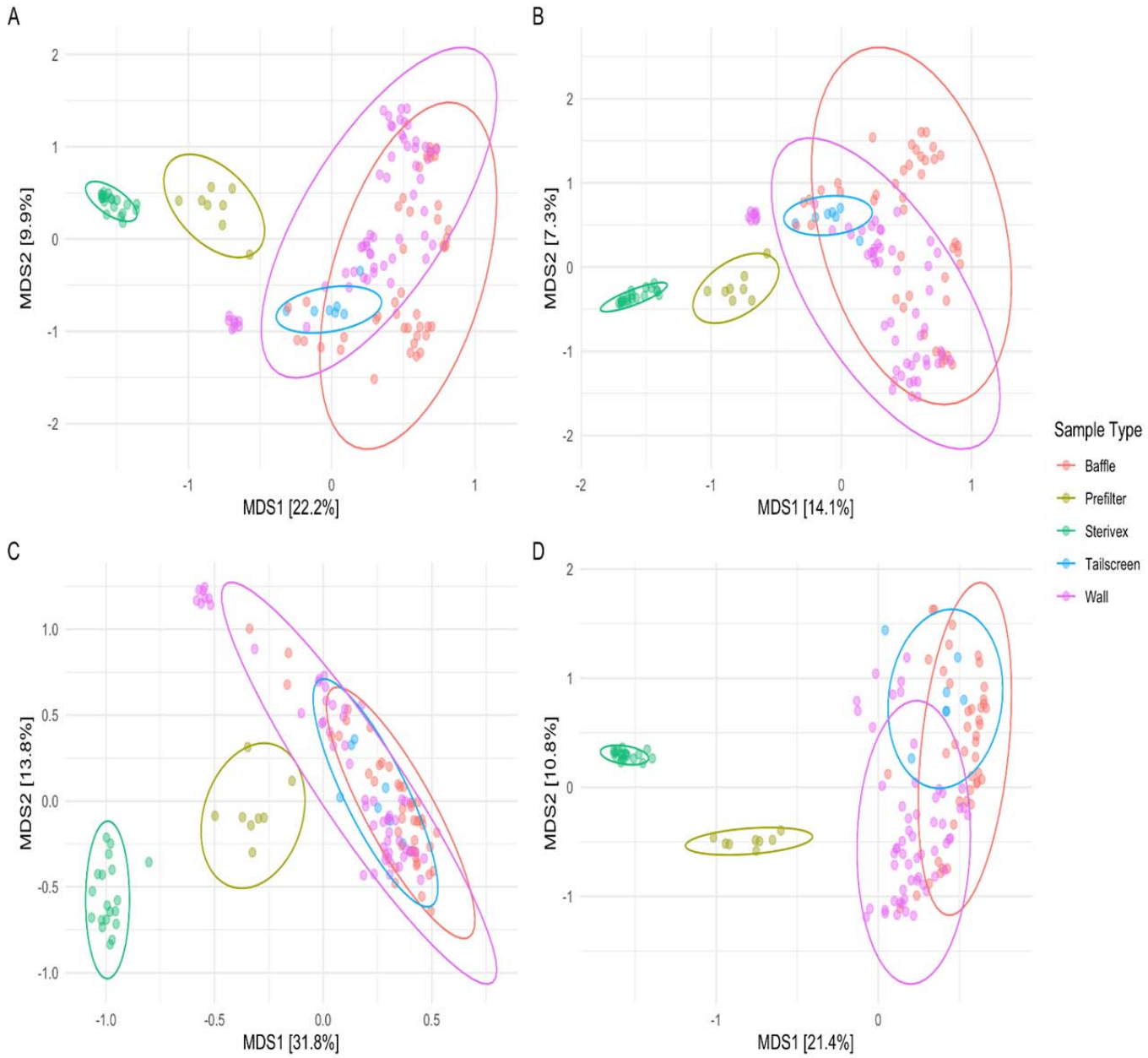

Figure 3: Beta Diversity by Sample Type in the Hatchery. (A) Bray-Curtis, (B) Jaccard, (C) Weighted Unifrac, and (D) Unweighted Unifrac distance metrics colored by sample type with 95% confidence ellipses.

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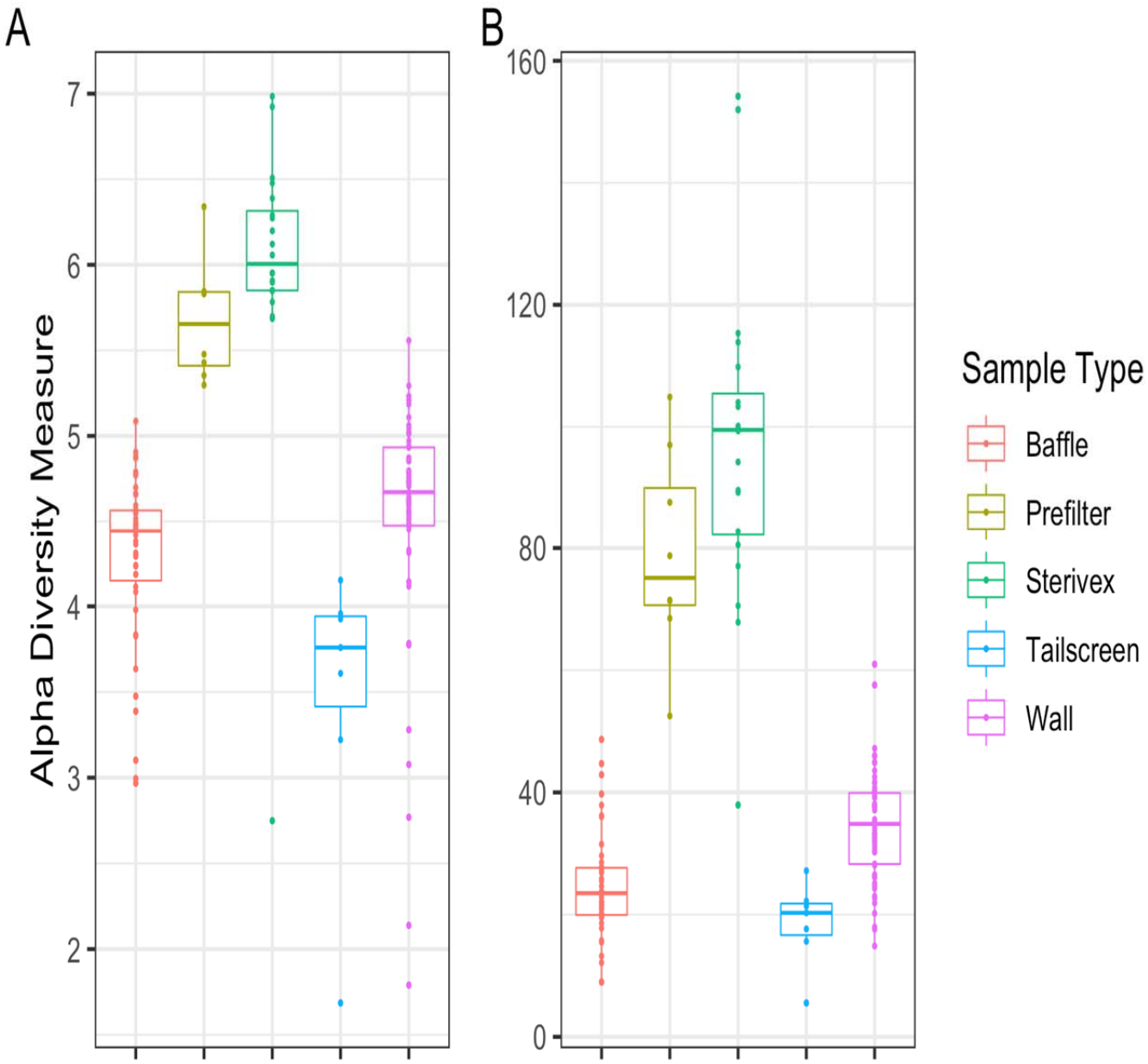

578

579 **Figure 4: Alpha Diversity by Sample Type in the Hatchery.** (A) Shannon diversity index and (B) Faith
580 phylogenetic diversity values were calculated for all samples and arranged by sample type. Samples
581 were rarefied to 10,000 reads before calculating alpha diversity index values.

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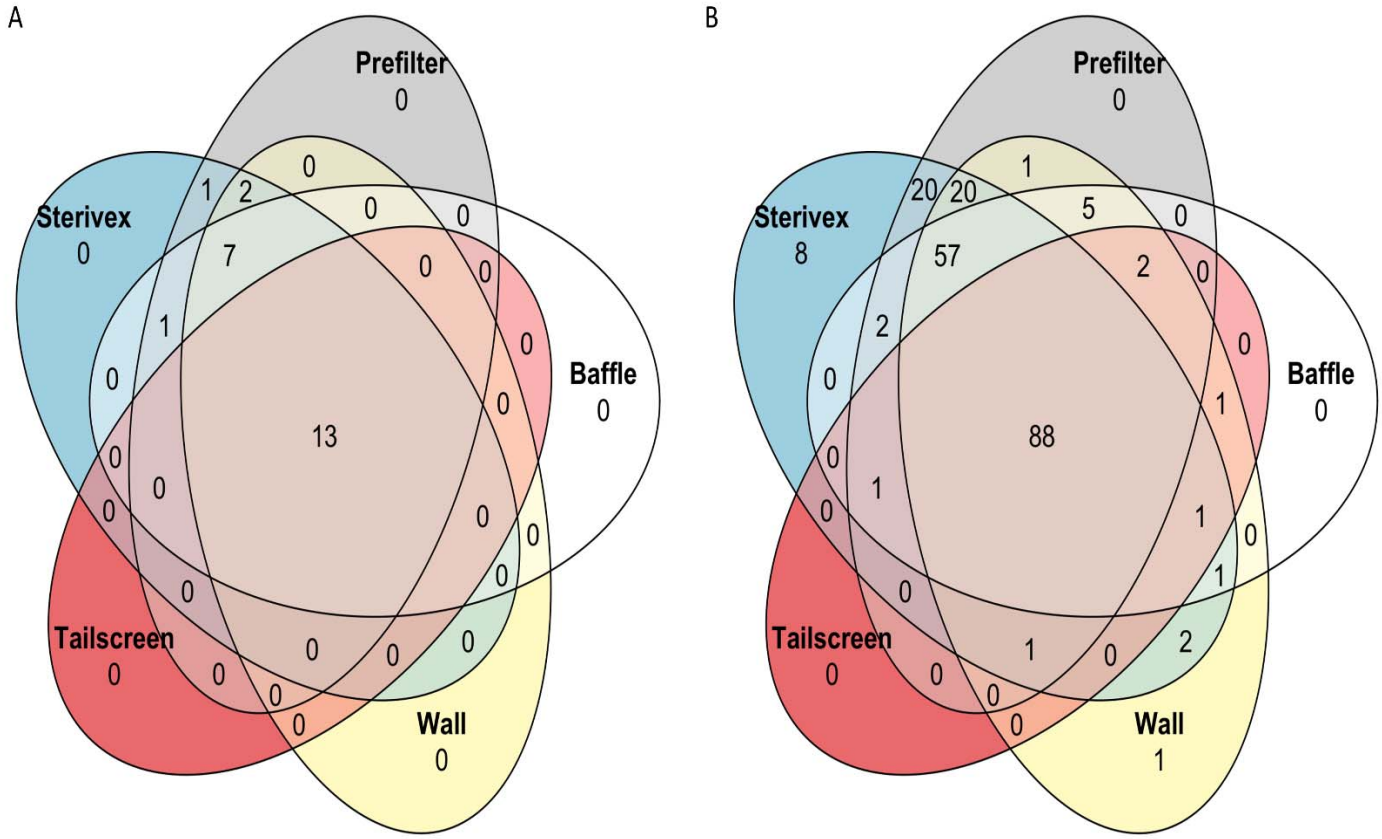


Figure 5: Venn Diagrams of Shared Microbial Classes and Families by Sample Type in the Hatchery. Presence/absence summary of (A) shared microbial classes and (B) shared microbial families stratified by sample type. Microbial classes had to contribute at least 1,000 reads to one or more samples to be included in this comparison. Microbial families had to contribute at least 100 reads to one or more samples to be included in this comparison. Only inflowing water samples were included.

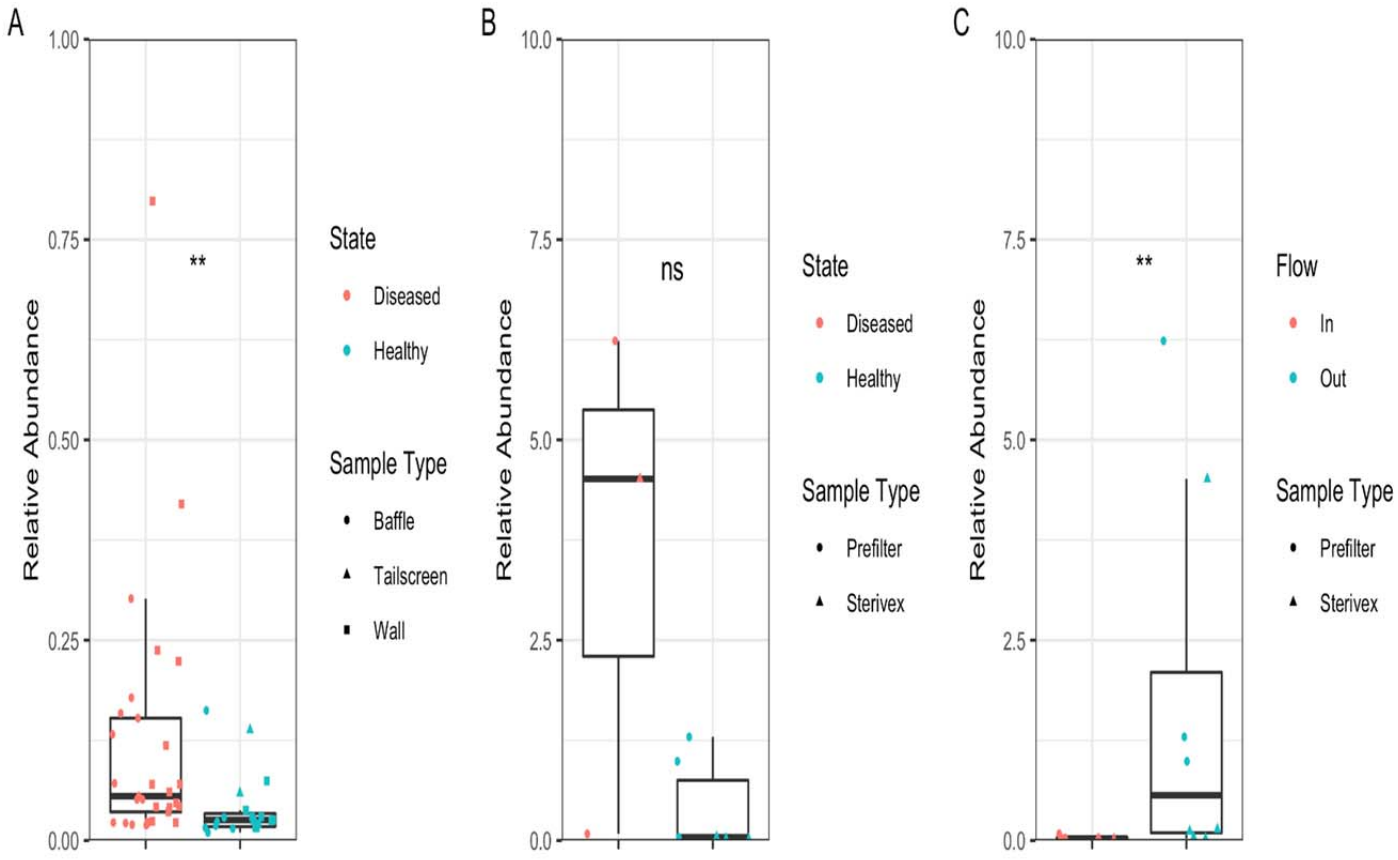


Figure 6: Relative Abundances of *F. columnare* in the Hatchery. Boxplots display the distribution of relative abundances from (A) surface samples grouped by raceway disease status and (B) water samples grouped by raceway disease status and (C) flow direction. Only samples with one or more *F. columnare* reads were included. Global inflow and outflow water samples were not included in disease state figures as water from healthy and diseased raceways would be collected in these samples. Wilcoxon signed-rank tests were performed to test for differences. ns = not significant.

Table 1: PERMANOVA Testing of Weighted Unifrac Distances for Surface and Water Samples at the Three Sites.

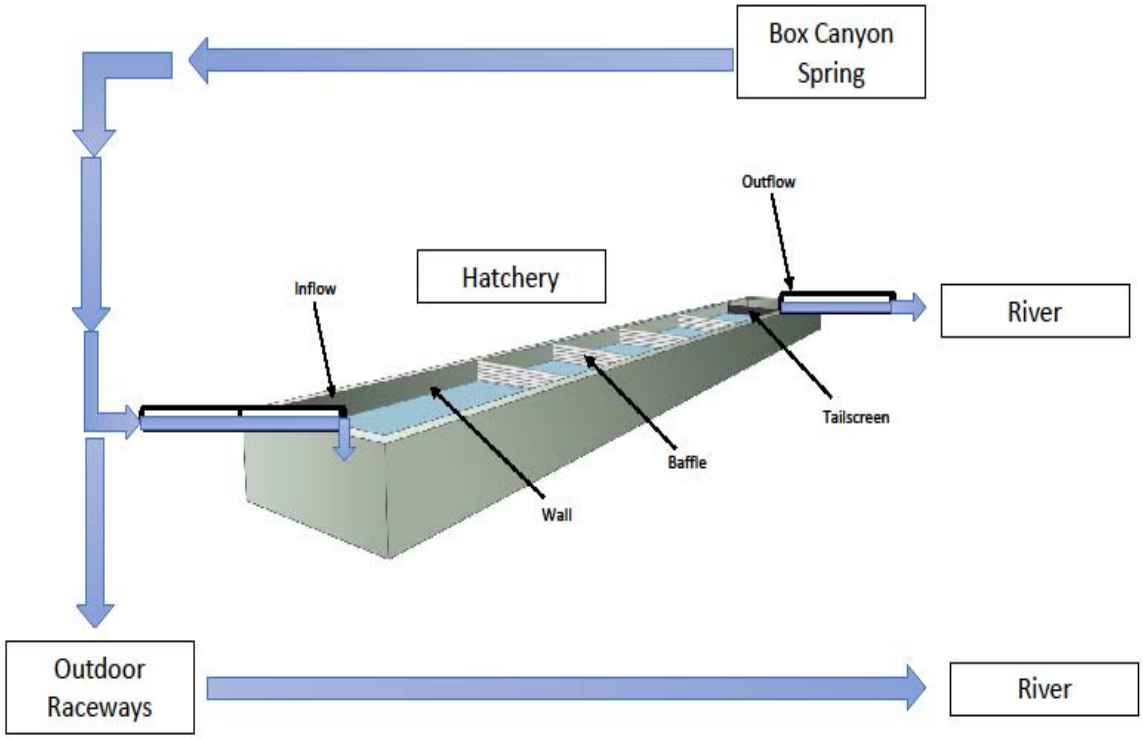
	Surfaces		Water	
	p	**R^2**	**p**	**R^2**
Sample Type	0.0001	0.0817	0.0001	0.4918
Sampling Year	0.0001	0.0320	0.0489	0.0374
Sampling Site	0.0001	0.2048	0.3606	0.0298
Sample Type: Year	0.0003	0.0390	0.0626	0.0325
Sampling Site: Year[1]	0.0002	0.0251		
Sample Type: Site[1]			0.2200	0.0178

[1]Due to sample sparsity for specific sites, testing could not be performed on these interactions

Table 2: Percent Abundance and Frequency of Detection of *F. columnare*.

Year	Raceway ID	Wall Fc%	Baffle Fc%	Tailscreen Fc%	Inflow Sterivex Fc%	Inflow Prefilter Fc%	Outflow Sterivex Fc%	Outflow Prefilter Fc%	Days from First Feed at Sampling	Daily % Mortality at Sampling	Mortality % at Sampling	Cumulative % Mortality	Columnaris Diagnosed
2017	6	N/A	0 (0/2)	0.06 (1/1)	N/A	N/A	N/A	1.29 (1/1)	71	0.017	1.93	1.97	-
	8	N/A	0 (0/1)	N/A	N/A	N/A	N/A	N/A	50	0.045	1.68	1.94	-
	14	N/A	0 (0/1)	N/A	N/A	N/A	N/A	N/A	64	0.119	2.96	3.57	(-)
	19	N/A	0.16 (1/1)	N/A	N/A	N/A	N/A	N/A	57	0.007	0.99	1.05	-
	20	N/A	0 (0/1)	N/A	N/A	N/A	N/A	N/A	57	0.009	1.20	1.27	
	Global	N/A	N/A	N/A	0.02 (2/2)	0.03 (1/1)	0.13 (2/2)	N/A	N/A	N/A	N/A	N/A	N/A
2018	9	0.04 (2/3)	0.02 (3/4)	0 (0/1)	0 (0/1)	0 (0/1)	0.02 (1/1)	0.99 (1/1)	42	0.002	8.30	9.25	-
	11	0 (0/3)	0.01 (1/3)	0.14 (1/1)	N/A	N/A	N/A	N/A	71	0.004	3.61	3.65	-
	16	0 (0/1)	N/A	N/A	N/A	N/A	N/A	N/A	28	0.002	0.14	0.93	-
	Global	N/A	N/A	N/A	0 (0/1)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2019	1	0.05 (7/7)	0.02 (3/7)	N/A	0 (0/1)	0 (0/1)	0 (0/1)	N/A	62	0.017	4.10	4.25	(+)
	2	0.31 (6/6)	0.15 (7/7)	N/A	0 (0/1)	0.08 (1/1)	4.51 (1/1)	6.24 (1/1)	50	2.562	24.22	28.84	+
	4	0.02 (3/5)	0.02 (3/6)	0 (0/1)	0 (0/1)	N/A	0 (0/1)	N/A	64	0.006	14.26	14.42	(+)
	8	0 (0/3)	0 (0/3)	0 (0/3)	N/A	N/A	N/A	N/A	73	0.012	1.34	1.45	-
	9	0.01 (1/3)	N/A	N/A	0 (0/1)	0.04 (1/1)	0 (0/1)	N/A	20	0.004	0.45	3.13	-
	10	0 (0/4)	N/A	N/A	0.02 (1/1)	N/A	0.04 (1/1)	N/A	7	0.000	0.00	9.59	(-)
	11	0.01 (1/4)	N/A	N/A	N/A	N/A	N/A	N/A	6	0.000	0.00	8.47	-
	12	0.03 (4/4)	0.02 (2/7)	N/A	N/A	N/A	N/A	N/A	79	0.004	45.40	45.45	[-]
	16	0 (0/4)	N/A	N/A	0 (0/1)	N/A	0 (0/1)	N/A	20	0.086	0.90	8.82	(-)
	17	0 (0/4)	N/A	N/A	N/A	N/A	N/A	N/A	20	0.063	0.92	6.63	(-)
	18	0.01 (2/6)	N/A	N/A	N/A	N/A	N/A	N/A	20	0.072	0.83	12.81	(-)

(+) = active columnaris cases but post peak, (-) = no active cases but infection occurred sometime following sampling during production cycle, [-] = no active cases but previous large outbreak had occurred, N/A = not applicable due to samples not being collected or being excluded during processing.



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656 **Supplementary Figure 1: Diagram of Water Flow Progression and Hatchery Raceway Structure.** Blue
657 arrows indicate the flow of water starting from the upstream, Box Canyon Spring and proceeding
658 through the hatchery and outdoor raceways before flowing out into the river. This is a simplified layout
659 and is only meant to show the basic progression of water as it pertains to this study.

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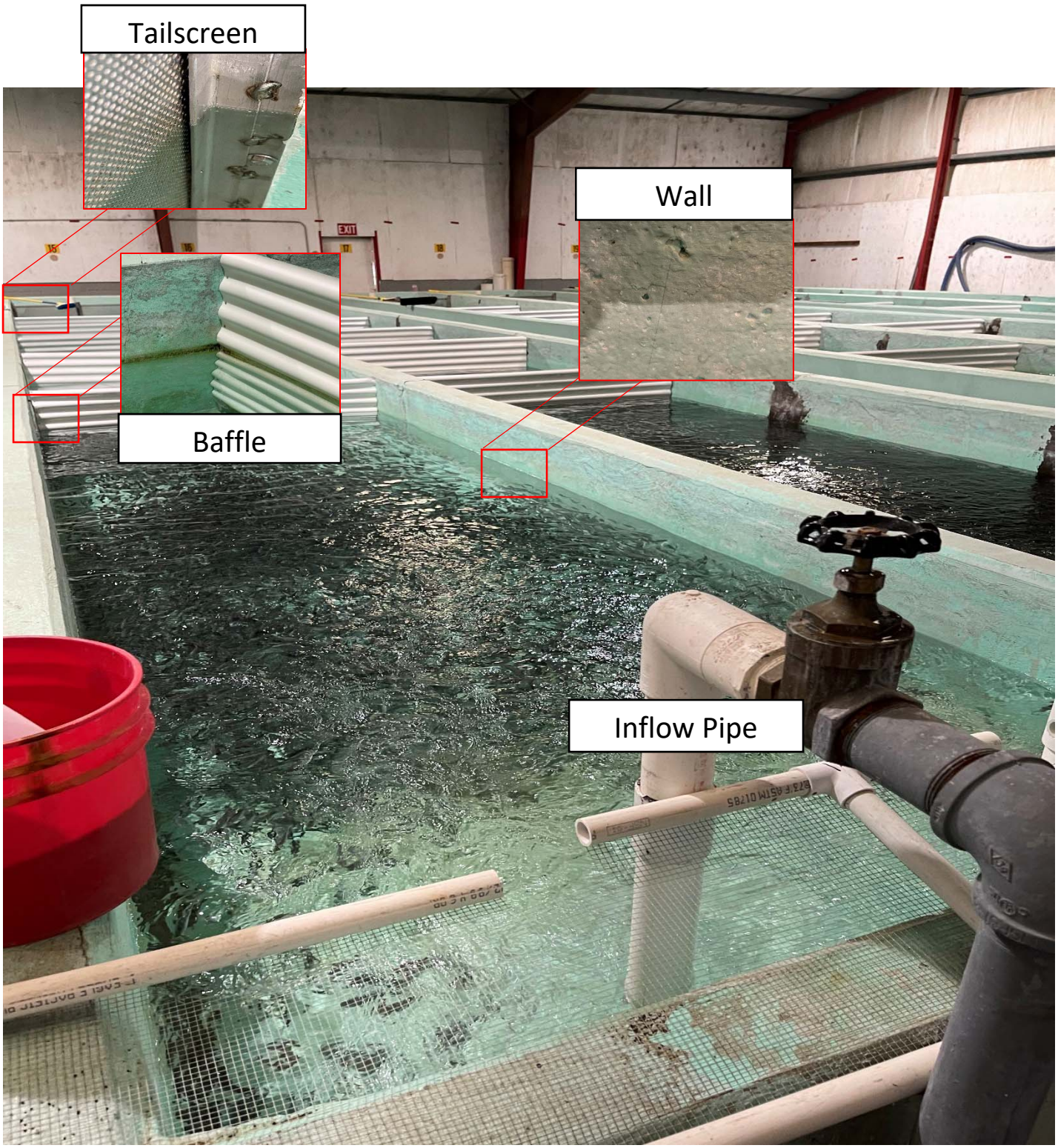
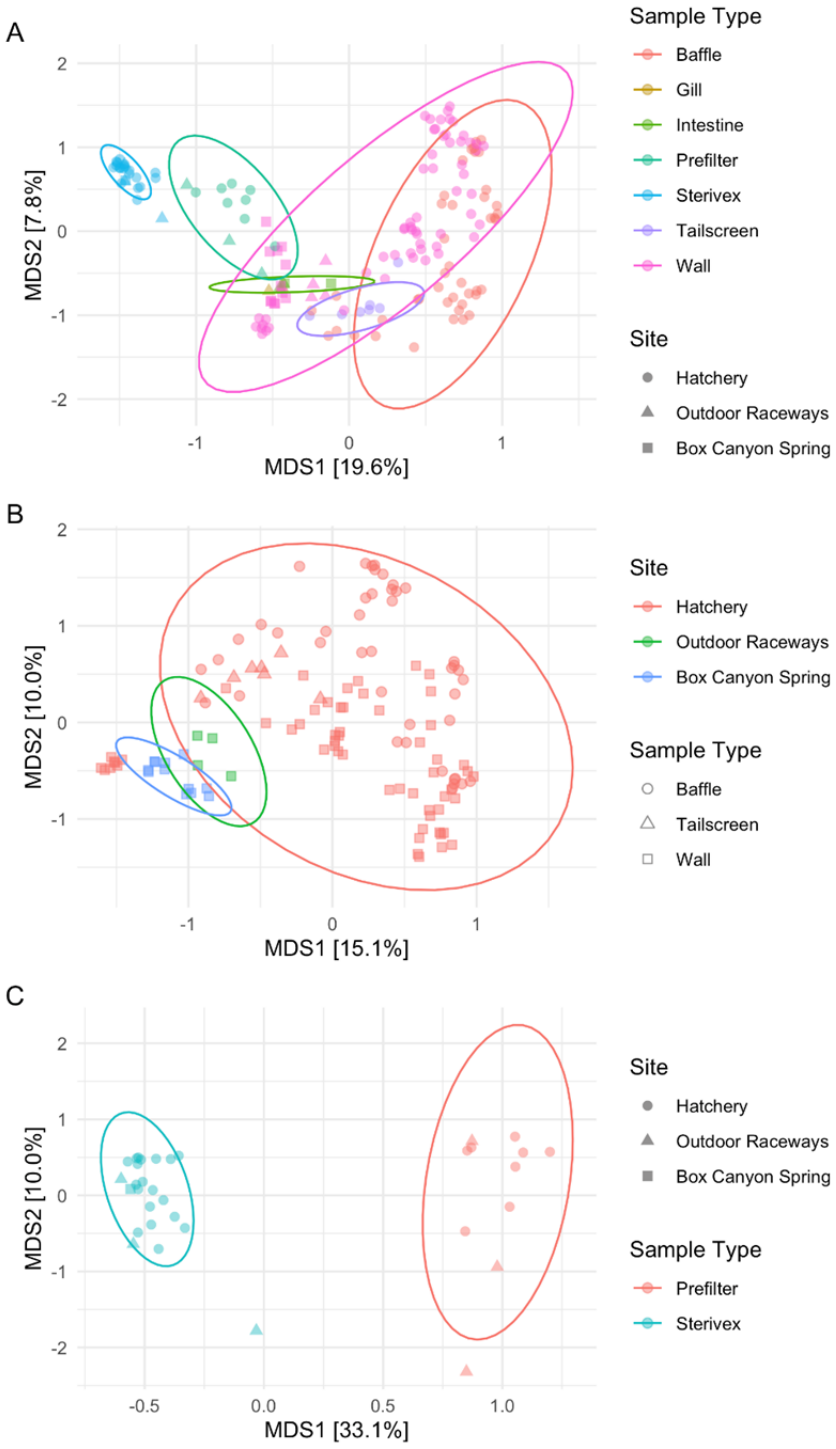
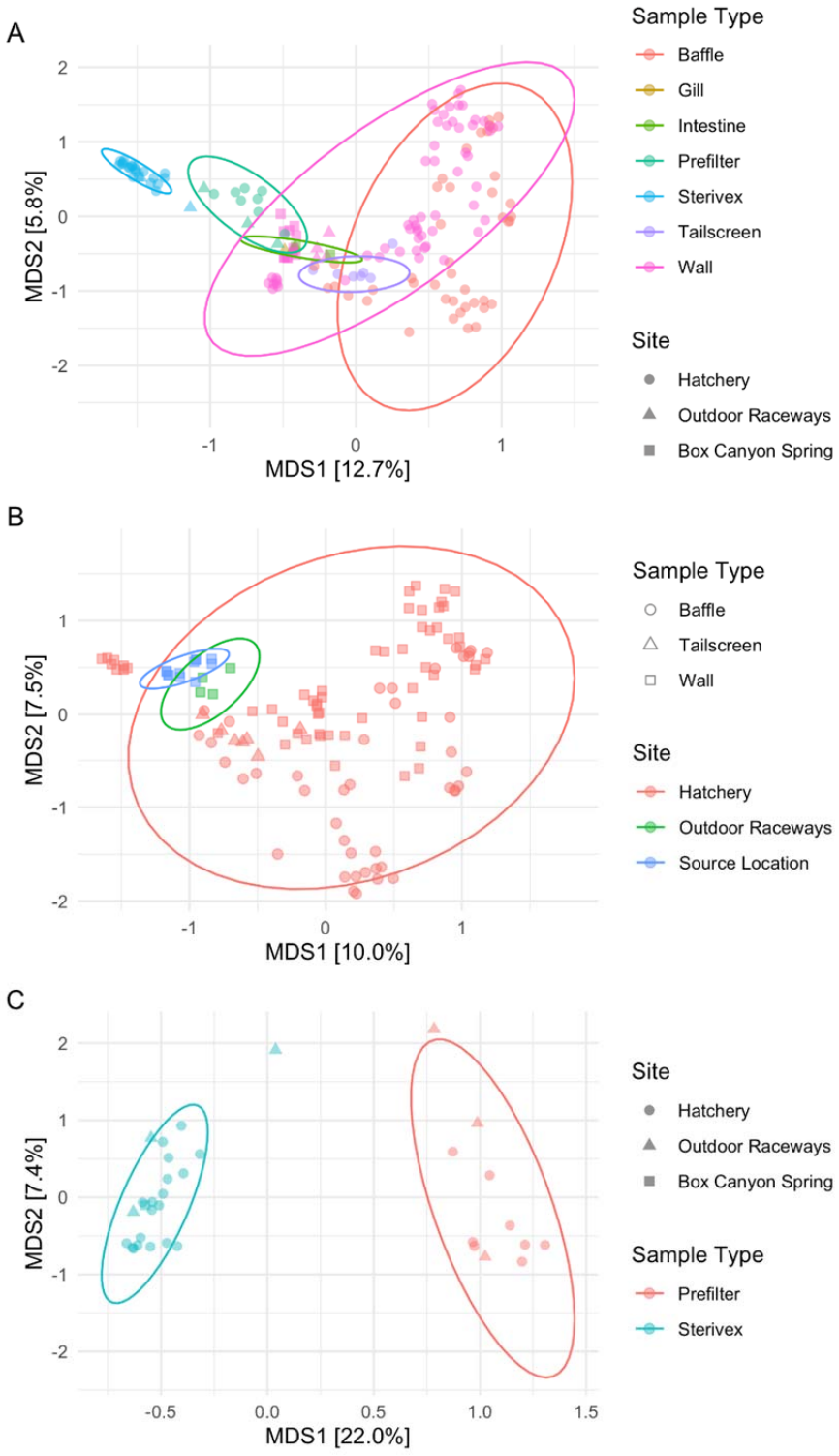


Supplementary Figure 2: Raceway Layout and Structures. The raceway layout is displayed with the inflowing water pipe and three sampled surface types (wall, baffle, and tailscreen) highlighted. Water flows away from the inflow pipe towards the end of the raceway, where it flows out through a pipe (not shown).

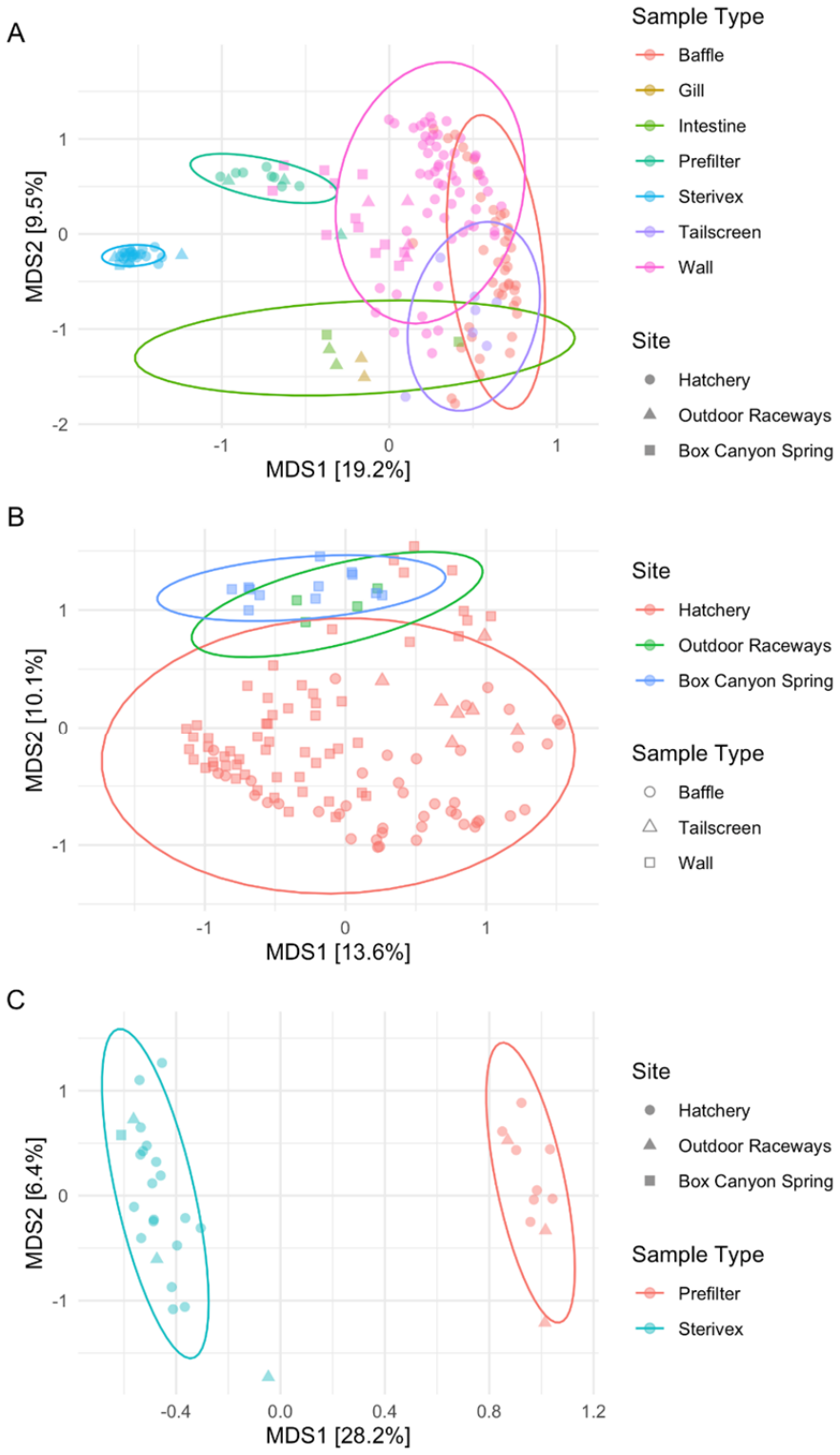


669

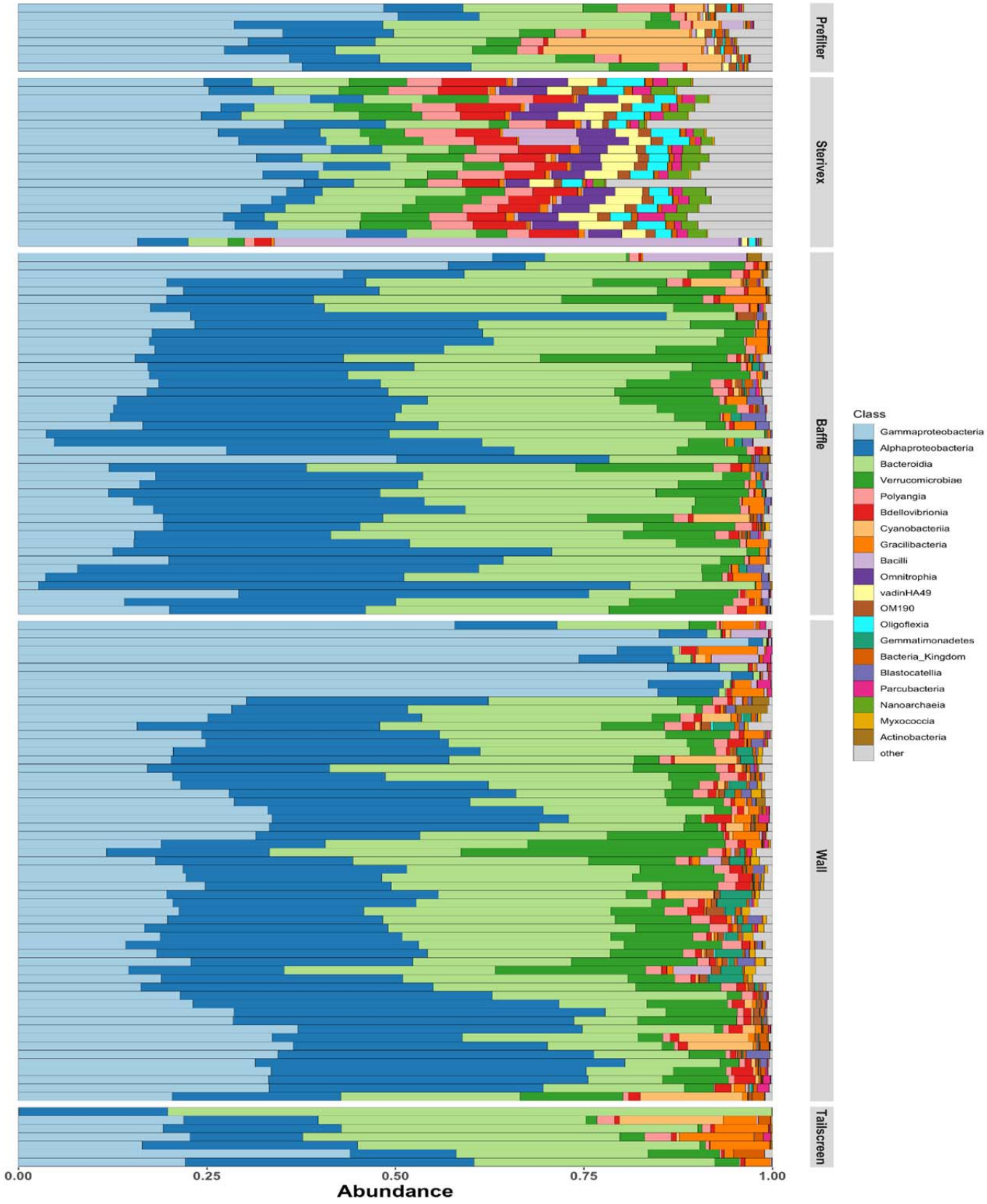
670 **Supplementary Figure 3: Bray-Curtis Ordinations of the Three Sampling Sites.** (A) All sample types, (B)
671 surface samples only, and (C) water samples only from three sampling sites with 95% confidence
672 ellipses. Three samples or more were required for an ellipse to be drawn for a particular category.
673 Samples are colored by most significant contributing variables.



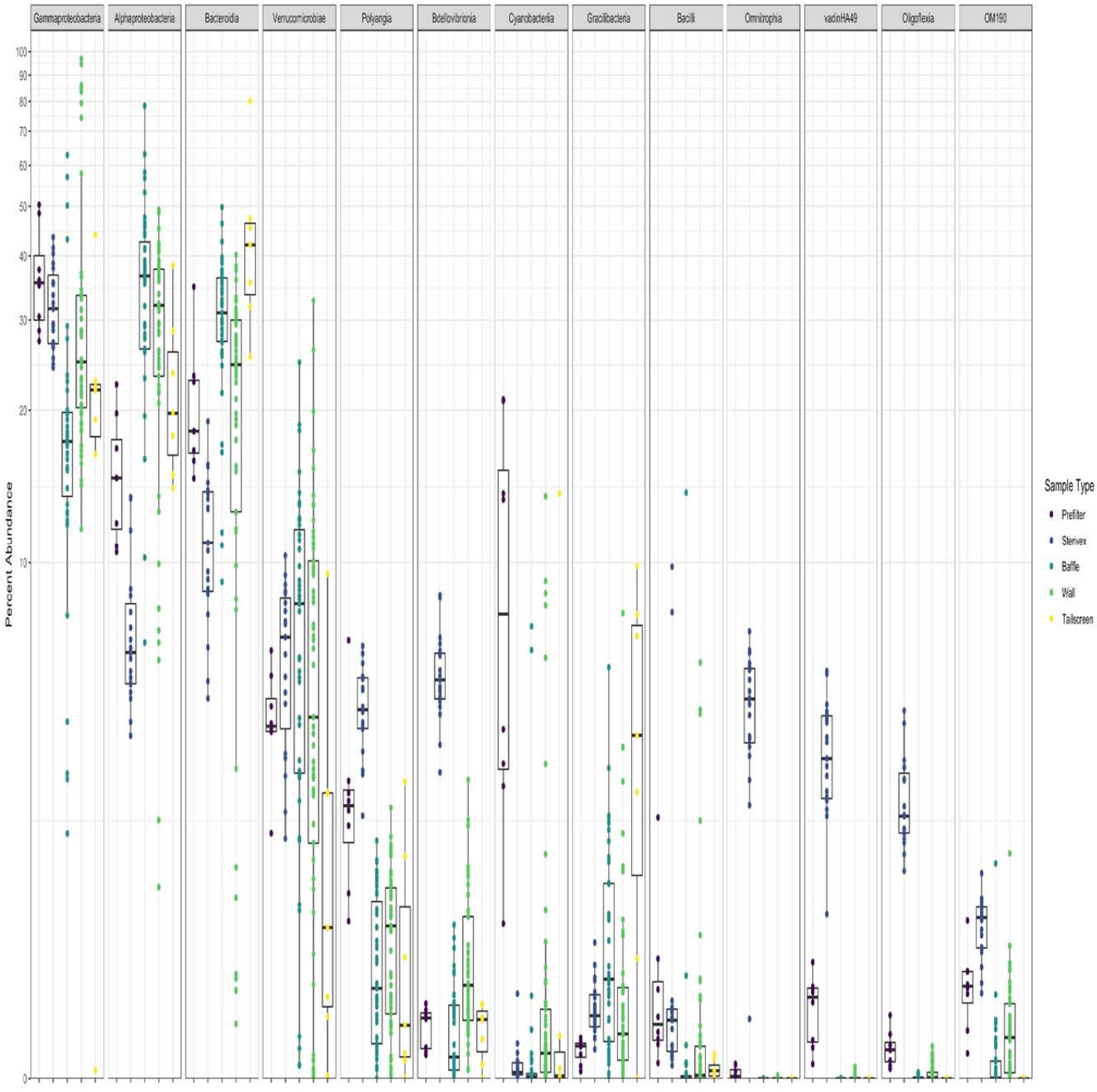
Supplementary Figure 4: Jaccard Ordinations of the Three Sampling Sites. (A) All sample types, (B) surface samples only, and (C) water samples only from three sampling sites with 95% confidence ellipses. Three samples or more were required for an ellipse to be drawn for a particular category. Samples are colored by most significant contributing variables.



Supplementary Figure 5: Unweighted Unifrac Ordinations of the Three Sampling Sites. (A) All sample types, (B) surface samples only, and (C) water samples only from three sampling sites with 95% confidence ellipses. Three samples or more were required for an ellipse to be drawn for a particular category. Samples are colored by most significant contributing variables.



Supplementary Figure 6: Class Level Relative Abundance Bar Plots Grouped by Sample Type.



686

687 **Supplementary Figure 7: Boxplots of Dominant Bacterial Classes by Sample Type.** Percent abundances
688 are shown on the y-axis on a log scale.

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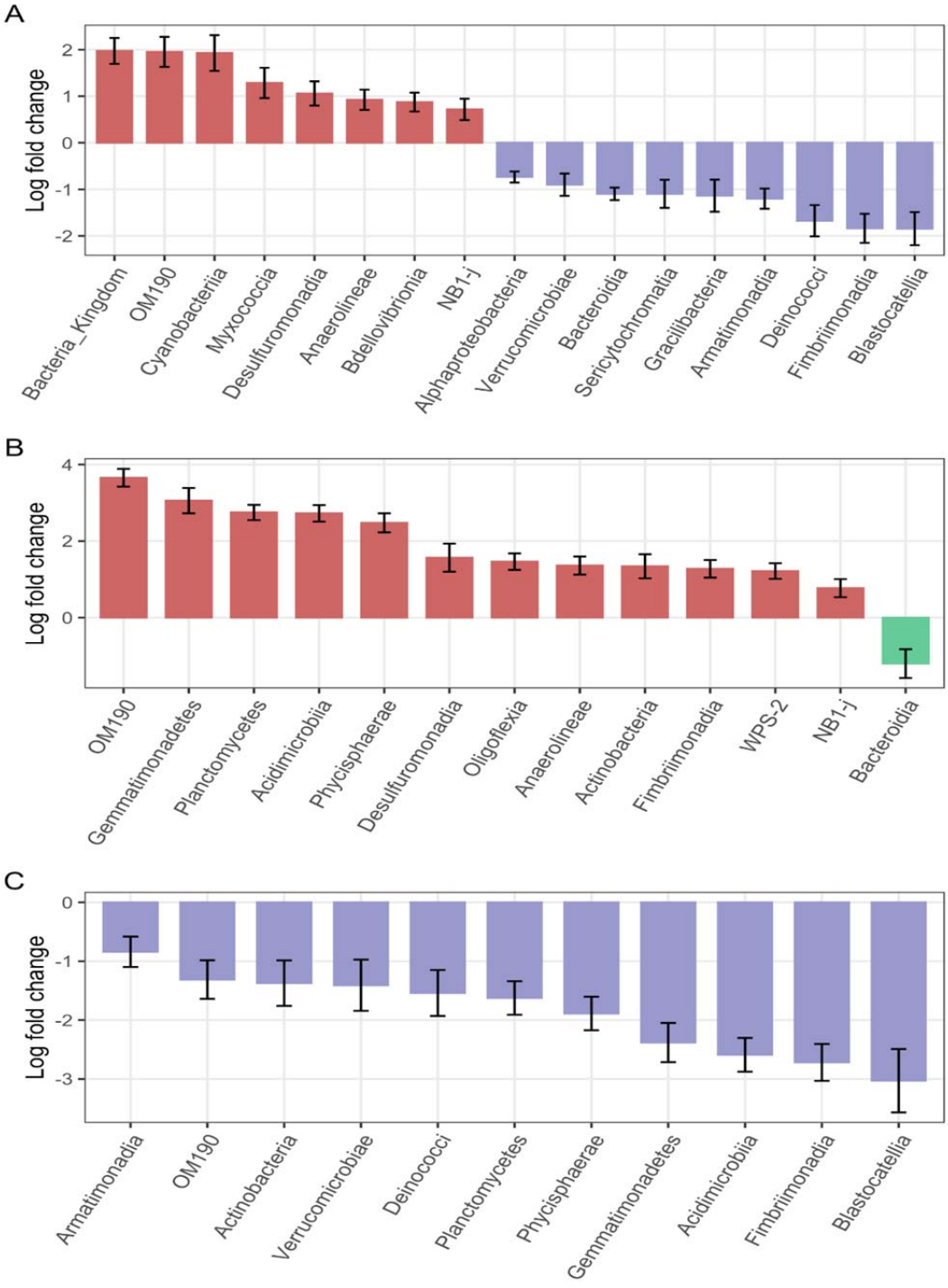
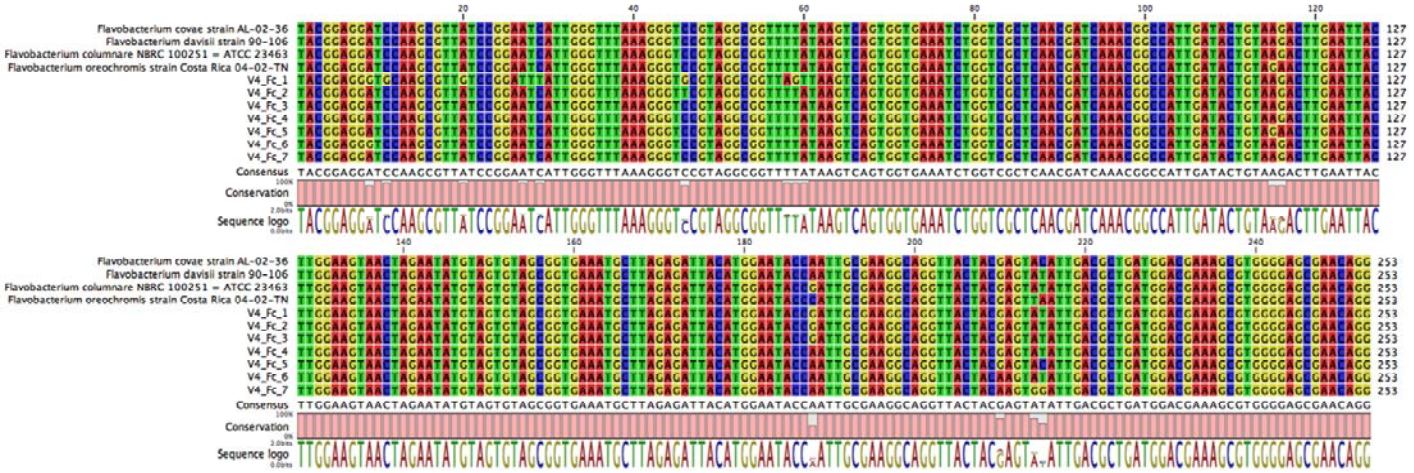


691

692 **Supplementary Figure 8: Waterfall Plots for Hatchery Surfaces.** (A) Wall and baffle swabs, (B) wall and
693 tailscreen swabs, and (C) tailscreen and baffle swabs were compared in pairwise fashion using ANCOM-
694 BC at the class level. Log2-fold differences are plotted from classes found to be significantly different.
695 Red bars – elevated in wall swabs, blue bars – elevated in baffle swabs, green bars – elevated in
696 tailscreen swabs. Bacteria_Kingdom indicates reads that were not assigned past the kingdom level.

A



B

		1	2	3	4	5	6	7	8	9	10	11
Flavobacterium covae strain AL-02-36	1		0	0	0	0	0	0	0	0	0	0
Flavobacterium davisii strain 90-106	2	1		0	0	0	0	0	0	0	0	0
Flavobacterium columnare NBRC 100251 = ATCC 23463	3	2	1		0	0	0	0	0	0	0	0
Flavobacterium oreochromis strain Costa Rica 04-02-TN	4	5	5	5		0	0	0	0	0	0	0
V4_Fc_1	5	2	1	0	5		0	0	0	0	0	0
V4_Fc_2	6	3	3	4	6	4		0	0	0	0	0
V4_Fc_3	7	1	0	1	5	1	3		0	0	0	0
V4_Fc_4	8	3	2	1	6	1	5	2		0	0	0
V4_Fc_5	9	3	2	3	7	3	3	2	4		0	0
V4_Fc_6	10	2	3	4	3	4	5	3	5	5		0
V4_Fc_7	11	11	10	9	14	9	13	10	9	10	13	

Supplementary Figure 9: *F. columnare* 16S rRNA V4 Region Gene Sequence Comparison. 16S rRNA gene sequences from the four type strains previously comprising the single species *F. columnare* were compared with the 7 ASVs detected within the hatchery dataset. An alignment (A) and distance matrix (B) were generated for this comparison. ASVs from the hatchery dataset are in decreasing order by total read count from 1 to 7.

708 **Supplementary Table 1: Summary of Samples**

Sample Number	BioSample Accession	Site	Raceway ID	Year	Sample Type	Final Read Count
1	SAMN27156050	Hatchery	6	2017	Baffle	97335
2	SAMN27156051	Hatchery	6	2017	Baffle	24975
3	SAMN27156090	Hatchery	6	2017	Prefilter	36639
4	SAMN27156117	Hatchery	6	2017	Tailscreen	52382
5	SAMN27156052	Hatchery	8	2017	Baffle	45411
6	SAMN27156047	Hatchery	14	2017	Baffle	19054
7	SAMN27156048	Hatchery	19	2017	Baffle	65294
8	SAMN27156049	Hatchery	20	2017	Baffle	36761
9	SAMN27156091	Hatchery	Global Inflow	2017	Prefilter	80237
10	SAMN27156098	Hatchery	Global Inflow	2017	Sterivex	61951
11	SAMN27156101	Hatchery	Global Inflow	2017	Sterivex	104949
12	SAMN27156099	Hatchery	Global Outflow	2017	Sterivex	16695
13	SAMN27156100	Hatchery	Global Outflow	2017	Sterivex	17882
14	SAMN27156183	Outdoor Raceways	11	2017	Intestine	52719
15	SAMN27156184	Outdoor Raceways	11	2017	Intestine	10967
16	SAMN27156186	Outdoor Raceways	11	2017	Prefilter	37641
17	SAMN27156191	Outdoor Raceways	11	2017	Wall	35347
18	SAMN27156192	Outdoor Raceways	11	2017	Wall	42876
19	SAMN27156182	Outdoor Raceways	15	2017	Gill	14805
20	SAMN27156181	Outdoor Raceways	15	2017	Gill	17319
21	SAMN27156187	Outdoor Raceways	15	2017	Prefilter	39936
22	SAMN27156193	Outdoor Raceways	15	2017	Wall	18868
23	SAMN27156194	Outdoor Raceways	15	2017	Wall	42581

24	SAMN27156185	Outdoor Raceways	Global Inflow	2017	Prefilter	41543
25	SAMN27156188	Outdoor Raceways	Global Inflow	2017	Sterivex	51162
26	SAMN27156189	Outdoor Raceways	Global Outflow	2017	Sterivex	17298
27	SAMN27156190	Outdoor Raceways	Global Outflow	2017	Sterivex	19492
28	SAMN27156198	BCS	N/A	2017	Wall	49281
29	SAMN27156199	BCS	N/A	2017	Wall	46696
30	SAMN27156056	Hatchery	9	2018	Baffle	100379
31	SAMN27156057	Hatchery	9	2018	Baffle	53660
32	SAMN27156058	Hatchery	9	2018	Baffle	121706
33	SAMN27156059	Hatchery	9	2018	Baffle	35652
34	SAMN27156092	Hatchery	9	2018	Prefilter	10896
35	SAMN27156093	Hatchery	9	2018	Prefilter	64545
36	SAMN27156103	Hatchery	9	2018	Sterivex	114452
37	SAMN27156104	Hatchery	9	2018	Sterivex	55537
38	SAMN27156119	Hatchery	9	2018	Tailscreen	16367
39	SAMN27156128	Hatchery	9	2018	Wall	46967
40	SAMN27156129	Hatchery	9	2018	Wall	47685
41	SAMN27156130	Hatchery	9	2018	Wall	29580
42	SAMN27156053	Hatchery	11	2018	Baffle	89277
43	SAMN27156054	Hatchery	11	2018	Baffle	111266
44	SAMN27156055	Hatchery	11	2018	Baffle	38021
45	SAMN27156118	Hatchery	11	2018	Tailscreen	12311
46	SAMN27156124	Hatchery	11	2018	Wall	123608
47	SAMN27156125	Hatchery	11	2018	Wall	22624
48	SAMN27156126	Hatchery	11	2018	Wall	17685
49	SAMN27156127	Hatchery	16	2018	Wall	88742
50	SAMN27156102	Hatchery	Global Inflow	2018	Sterivex	55104
51	SAMN27156195	BCS	N/A	2018	Intestine	22981
52	SAMN27156196	BCS	N/A	2018	Intestine	13416
53	SAMN27156197	BCS	N/A	2018	Sterivex	86709
54	SAMN27156200	BCS	N/A	2018	Wall	56506
55	SAMN27156070	Hatchery	1	2019	Baffle	49124
56	SAMN27156071	Hatchery	1	2019	Baffle	20848
57	SAMN27156072	Hatchery	1	2019	Baffle	32048
58	SAMN27156073	Hatchery	1	2019	Baffle	47895

59	SAMN27156074	Hatchery	1	2019	Baffle	45974
60	SAMN27156075	Hatchery	1	2019	Baffle	21094
61	SAMN27156076	Hatchery	1	2019	Baffle	42022
62	SAMN27156094	Hatchery	1	2019	Prefilter	24436
63	SAMN27156105	Hatchery	1	2019	Sterivex	30241
64	SAMN27156106	Hatchery	1	2019	Sterivex	27031
65	SAMN27156160	Hatchery	1	2019	Wall	66874
66	SAMN27156161	Hatchery	1	2019	Wall	27823
67	SAMN27156162	Hatchery	1	2019	Wall	47713
68	SAMN27156163	Hatchery	1	2019	Wall	31361
69	SAMN27156164	Hatchery	1	2019	Wall	40100
70	SAMN27156165	Hatchery	1	2019	Wall	24135
71	SAMN27156166	Hatchery	1	2019	Wall	69859
72	SAMN27156083	Hatchery	2	2019	Baffle	83995
73	SAMN27156084	Hatchery	2	2019	Baffle	34789
74	SAMN27156085	Hatchery	2	2019	Baffle	72400
75	SAMN27156086	Hatchery	2	2019	Baffle	72046
76	SAMN27156087	Hatchery	2	2019	Baffle	23188
77	SAMN27156088	Hatchery	2	2019	Baffle	17014
78	SAMN27156089	Hatchery	2	2019	Baffle	68713
79	SAMN27156095	Hatchery	2	2019	Prefilter	24046
80	SAMN27156096	Hatchery	2	2019	Prefilter	19179
81	SAMN27156113	Hatchery	2	2019	Sterivex	15861
82	SAMN27156114	Hatchery	2	2019	Sterivex	22509
83	SAMN27156175	Hatchery	2	2019	Wall	46866
84	SAMN27156176	Hatchery	2	2019	Wall	80912
85	SAMN27156177	Hatchery	2	2019	Wall	38752
86	SAMN27156178	Hatchery	2	2019	Wall	42984
87	SAMN27156179	Hatchery	2	2019	Wall	24812
88	SAMN27156180	Hatchery	2	2019	Wall	34524
89	SAMN27156077	Hatchery	4	2019	Baffle	52165
90	SAMN27156078	Hatchery	4	2019	Baffle	38246
91	SAMN27156079	Hatchery	4	2019	Baffle	65713
92	SAMN27156080	Hatchery	4	2019	Baffle	41429
93	SAMN27156081	Hatchery	4	2019	Baffle	79989
94	SAMN27156082	Hatchery	4	2019	Baffle	41698
95	SAMN27156107	Hatchery	4	2019	Sterivex	48223
96	SAMN27156108	Hatchery	4	2019	Sterivex	11237
97	SAMN27156123	Hatchery	4	2019	Tailscreen	33234

98	SAMN27156167	Hatchery	4	2019	Wall	31368
99	SAMN27156168	Hatchery	4	2019	Wall	28743
100	SAMN27156169	Hatchery	4	2019	Wall	44824
101	SAMN27156170	Hatchery	4	2019	Wall	40921
102	SAMN27156171	Hatchery	4	2019	Wall	58156
103	SAMN27156060	Hatchery	8	2019	Baffle	27750
104	SAMN27156061	Hatchery	8	2019	Baffle	10978
105	SAMN27156062	Hatchery	8	2019	Baffle	10720
106	SAMN27156120	Hatchery	8	2019	Tailscreen	52232
107	SAMN27156121	Hatchery	8	2019	Tailscreen	58755
108	SAMN27156122	Hatchery	8	2019	Tailscreen	26943
109	SAMN27156131	Hatchery	8	2019	Wall	44277
110	SAMN27156132	Hatchery	8	2019	Wall	31499
111	SAMN27156133	Hatchery	8	2019	Wall	42472
112	SAMN27156097	Hatchery	9	2019	Prefilter	39325
113	SAMN27156115	Hatchery	9	2019	Sterivex	19687
114	SAMN27156116	Hatchery	9	2019	Sterivex	32062
115	SAMN27156172	Hatchery	9	2019	Wall	54288
116	SAMN27156173	Hatchery	9	2019	Wall	30401
117	SAMN27156174	Hatchery	9	2019	Wall	15257
118	SAMN27156109	Hatchery	10	2019	Sterivex	28559
119	SAMN27156110	Hatchery	10	2019	Sterivex	25746
120	SAMN27156134	Hatchery	10	2019	Wall	30488
121	SAMN27156135	Hatchery	10	2019	Wall	55582
122	SAMN27156136	Hatchery	10	2019	Wall	65335
123	SAMN27156137	Hatchery	10	2019	Wall	83071
124	SAMN27156138	Hatchery	11	2019	Wall	18941
125	SAMN27156139	Hatchery	11	2019	Wall	41404
126	SAMN27156140	Hatchery	11	2019	Wall	40264
127	SAMN27156141	Hatchery	11	2019	Wall	37745
128	SAMN27156063	Hatchery	12	2019	Baffle	38024
129	SAMN27156064	Hatchery	12	2019	Baffle	41947
130	SAMN27156065	Hatchery	12	2019	Baffle	53911
131	SAMN27156066	Hatchery	12	2019	Baffle	47991
132	SAMN27156067	Hatchery	12	2019	Baffle	55628
133	SAMN27156068	Hatchery	12	2019	Baffle	118206
134	SAMN27156069	Hatchery	12	2019	Baffle	80312
135	SAMN27156142	Hatchery	12	2019	Wall	50708
136	SAMN27156143	Hatchery	12	2019	Wall	50835

137	SAMN27156144	Hatchery	12	2019	Wall	172746
138	SAMN27156145	Hatchery	12	2019	Wall	164648
139	SAMN27156111	Hatchery	16	2019	Sterivex	26260
140	SAMN27156112	Hatchery	16	2019	Sterivex	26428
141	SAMN27156146	Hatchery	16	2019	Wall	30427
142	SAMN27156147	Hatchery	16	2019	Wall	24192
143	SAMN27156148	Hatchery	16	2019	Wall	34118
144	SAMN27156149	Hatchery	16	2019	Wall	21829
145	SAMN27156150	Hatchery	17	2019	Wall	33660
146	SAMN27156151	Hatchery	17	2019	Wall	45161
147	SAMN27156152	Hatchery	17	2019	Wall	16197
148	SAMN27156153	Hatchery	17	2019	Wall	23562
149	SAMN27156154	Hatchery	18	2019	Wall	22460
150	SAMN27156155	Hatchery	18	2019	Wall	50796
151	SAMN27156156	Hatchery	18	2019	Wall	16011
152	SAMN27156157	Hatchery	18	2019	Wall	52423
153	SAMN27156158	Hatchery	18	2019	Wall	31999
154	SAMN27156159	Hatchery	18	2019	Wall	30377
155	SAMN27156201	BCS	N/A	2019	Wall	46605
156	SAMN27156202	BCS	N/A	2019	Wall	12823
157	SAMN27156203	BCS	N/A	2019	Wall	76585
158	SAMN27156204	BCS	N/A	2019	Wall	42970
159	SAMN27156205	BCS	N/A	2019	Wall	87642
160	SAMN27156206	BCS	N/A	2019	Wall	95144
161	SAMN27156207	BCS	N/A	2019	Wall	42303
162	SAMN27156208	BCS	N/A	2019	Wall	78732
163	SAMN27156209	BCS	N/A	2019	Wall	42722

718 **Supplementary Table 2: PERMANOVA Testing of Bray-Curtis Distances for Surface and Water Samples**
719 **at the Three Sites.**

	Surfaces		Water	
	p	R^2	p	R^2
Sample Type	0.0001	0.0909	0.0001	0.3117
Sampling Year	0.0001	0.0328	0.0186	0.0476
Sampling Site	0.0001	0.0882	0.1408	0.0517
Sample Type: Year	0.0001	0.0351	0.1421	0.0268
Sampling Site: Year[1]	0.0001	0.0217		
Sample Type: Site[1]			0.3472	0.0193

720 [1]Due to sample sparsity for specific sites, testing could not be performed on these interactions

721

722 **Supplementary Table 3: PERMANOVA Testing of Jaccard Distances for Surface and Water Samples at**
723 **the Three Sites.**

	Surfaces		Water	
	p	R^2	p	R^2
Sample Type	0.0001	0.0696	0.0001	0.2062
Sampling Year	0.0001	0.0257	0.0194	0.0452
Sampling Site	0.0001	0.0588	0.2006	0.0534
Sample Type: Year	0.0001	0.03	0.0892	0.0323
Sampling Site: Year[1]	0.0001	0.0173		
Sample Type: Site[1]			0.3441	0.0235

724 [1]Due to sample sparsity for specific sites, testing could not be performed on these interactions

725

726 **Supplementary Table 4: PERMANOVA Testing of Unweighted Unifrac Distances for Surface and Water**
727 **Samples at Three Sites.**

	Surfaces		Water	
	p	R^2	p	R^2
Sample Type	0.0001	0.0894	0.0001	0.2598
Sampling Year	0.0001	0.0232	0.2348	0.0254
Sampling Site	0.0001	0.0723	0.3346	0.0463
Sample Type: Year	0.0002	0.0282	0.3479	0.0221
Sampling Site: Year[1]	0.0006	0.0163		
Sample Type: Site[1]			0.3877	0.0214

728 [1]Due to sample sparsity for specific sites, testing could not be performed on these interactions

729

730

731 **Supplementary Table 5: Significant Class-Level ANCOM Results Between Sampling Sites.**

Hatchery vs. Outdoor Raceways		
Class	***W***	***q-value***
Alphaproteobacteria	5.06	1.67E-05
OM190	3.81	5.29E-03
Myxococcia	3.79	5.58E-03
Vampirivibrionia	-5.76	3.36E-07
Armatimonadia	-5.83	2.27E-07
Bacteria_Kingdom	-5.83	2.27E-07
Sericytochromatia	-7.70	6.06E-13
Chlamydiae	-10.86	8.04E-26
Deinococci	-16.61	2.72E-60
Cyanobacteriia	-22.49	2.32E-110
BCS vs. Hatchery		
Class	***W***	***q-value***
Cyanobacteriia	14.58	1.80E-46
Kapabacteria	5.71	4.76E-07
Oligoflexia	4.53	2.17E-04
Deinococci	3.51	1.40E-02
Intramacronucleata	-3.16	4.64E-02
Gemmatimonadetes	-3.32	2.73E-02
Phycisphaerae	-3.77	5.18E-03
Gracilibacteria	-4.01	2.00E-03
Campylobacteria	-4.21	8.56E-04
OM190	-4.25	7.49E-04
Omnitrophia	-4.59	1.60E-04
Firmicutes_Phylum	-4.75	7.69E-05
Bacteroidia	-4.91	3.60E-05
Fusobacteriia	-4.96	2.89E-05
Blastocatellia	-5.29	4.98E-06
Bdellovibrionia	-6.09	4.78E-08
Polyangia	-6.85	3.22E-10
WPS-2	-7.38	7.20E-12
Myxococcia	-8.13	2.03E-14
Gammaproteobacteria	-8.39	2.33E-15
Alphaproteobacteria	-9.61	3.63E-20
Outdoor Raceways vs. BCS		
Class	***W***	***q-value***
Oligoflexia	5.40	3.28E-06
Vicinamibacteria	4.95	3.55E-05
Cyanobacteriia	4.18	1.38E-03
vadinHA49	3.86	5.26E-03
Actinobacteria	3.28	4.71E-02

732 Positive W values indicate higher levels of the corresponding class in the first mentioned site group and
733 negative W values indicate higher levels in the second mentioned site group within the comparison.

734

Supplementary Table 6: PERMANOVA Testing Results of Sample Type Variable within Hatchery Samples for All Methods.

	p	R^2
Weighted Unifrac	0.0001	0.398
Unweighted Unifrac	0.0001	0.317
Bray-Curtis	0.0001	0.31
Jaccard	0.0001	0.214

Supplementary Table 7: Pairwise Wilcoxon Signed-Rank Test Statistics for Shannon Index Diversity by Sample Type.

group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Baffle	Prefilter	43	8	0	3.14E-09	2.51E-08	****
Baffle	Sterivex	43	19	0	4.67E-16	4.67E-15	****
Baffle	Tailscreen	43	7	260	0.001	0.002	**
Baffle	Wall	43	57	714	0.000374	0.002	**
Prefilter	Sterivex	8	19	25	0.005	0.005	**
Prefilter	Tailscreen	8	7	56	0.000311	0.002	**
Prefilter	Wall	8	57	452	8.09E-06	4.85E-05	****
Sterivex	Tailscreen	19	7	133	3.04E-06	2.13E-05	****
Sterivex	Wall	19	57	1083	8.60E-11	7.74E-10	****
Tailscreen	Wall	7	57	39	0.000578	0.002	**

Supplementary Table 8: Pairwise Wilcoxon Signed-Rank Test Statistics for Faith's Phylogenetic Diversity by Sample Type.

group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Baffle	Prefilter	43	8	1	6.28E-09	5.02E-08	****
Baffle	Sterivex	43	19	0	4.67E-16	4.67E-15	****
Baffle	Tailscreen	43	7	219	0.056	0.059	ns
Baffle	Wall	43	57	534	1.50E-06	1.05E-05	****
Prefilter	Sterivex	8	19	35	0.029	0.059	ns
Prefilter	Tailscreen	8	7	56	0.000311	0.000933	***
Prefilter	Wall	8	57	454	6.71E-06	3.36E-05	****
Sterivex	Tailscreen	19	7	133	3.04E-06	1.82E-05	****
Sterivex	Wall	19	57	1083	8.60E-11	7.74E-10	****
Tailscreen	Wall	7	57	27	0.000216	0.000864	***

745 **Supplementary Table 9: *F. columnare*-positive Samples.**

Sample Number	BioSample Accession	Sampling Location	Sample Type	Sampling Year	*F. columnare* Reads	*F. columnare* Relative Abundance	Days from First Feed at Sampling	Daily % Mortality at Sampling	Mortality % at Sampling	Cumulative % Mortality	Columnaris Diagnosed
3	SAMN27156090	Raceway #6 Outflow	Prefilter	2017	474	1.29	71	0.017	1.93	1.97	-
4	SAMN27156117	Raceway #6	Tailscreen	2017	31	0.06	71	0.017	1.93	1.97	-
7	SAMN27156048	Raceway #19	Baffle	2017	106	0.16	57	0.007	0.99	1.05	-
9	SAMN27156091	Global Hatchery Inflow	Prefilter	2017	25	0.03	N/A	N/A	N/A	N/A	N/A
10	SAMN27156098	Global Hatchery Inflow	Sterivex	2017	10	0.02	N/A	N/A	N/A	N/A	N/A
11	SAMN27156101	Global Hatchery Inflow	Sterivex	2017	15	0.01	N/A	N/A	N/A	N/A	N/A
12	SAMN27156099	Global Hatchery Outflow	Sterivex	2017	19	0.11	N/A	N/A	N/A	N/A	N/A
13	SAMN27156100	Global Hatchery Outflow	Sterivex	2017	25	0.14	N/A	N/A	N/A	N/A	N/A
30	SAMN27156056	Raceway #9	Baffle	2018	16	0.02	42	0.002	8.30	9.25	-
31	SAMN27156057	Raceway #9	Baffle	2018	8	0.01	42	0.002	8.30	9.25	-
32	SAMN27156058	Raceway #9	Baffle	2018	35	0.03	42	0.002	8.30	9.25	-
35	SAMN27156093	Raceway #9 Outflow	Prefilter	2018	638	0.99	42	0.002	8.30	9.25	-
37	SAMN27156104	Raceway #9 Outflow	Sterivex	2018	13	0.02	42	0.002	8.30	9.25	-
40	SAMN27156129	Raceway #9	Wall	2018	18	0.04	42	0.002	8.30	9.25	-
41	SAMN27156130	Raceway #9	Wall	2018	22	0.07	42	0.002	8.30	9.25	-
44	SAMN27156055	Raceway #11	Baffle	2018	7	0.02	71	0.004	3.61	3.65	-
45	SAMN27156118	Raceway #11	Tailscreen	2018	17	0.14	71	0.004	3.61	3.65	-
55	SAMN27156070	Raceway #1	Baffle	2019	11	0.02	62	0.017	4.10	4.25	(+)
59	SAMN27156074	Raceway #1	Baffle	2019	10	0.02	62	0.017	4.10	4.25	(+)
61	SAMN27156076	Raceway #1	Baffle	2019	30	0.07	62	0.017	4.10	4.25	(+)
65	SAMN27156160	Raceway #1	Wall	2019	16	0.02	62	0.017	4.10	4.25	(+)
66	SAMN27156161	Raceway #1	Wall	2019	13	0.05	62	0.017	4.10	4.25	(+)
67	SAMN27156162	Raceway #1	Wall	2019	20	0.04	62	0.017	4.10	4.25	(+)
68	SAMN27156163	Raceway #1	Wall	2019	19	0.06	62	0.017	4.10	4.25	(+)
69	SAMN27156164	Raceway #1	Wall	2019	9	0.02	62	0.017	4.10	4.25	(+)
70	SAMN27156165	Raceway #1	Wall	2019	17	0.07	62	0.017	4.10	4.25	(+)
71	SAMN27156166	Raceway #1	Wall	2019	49	0.07	62	0.017	4.10	4.25	(+)
72	SAMN27156083	Raceway #2	Baffle	2019	43	0.05	50	2.562	24.22	28.84	+
73	SAMN27156084	Raceway #2	Baffle	2019	62	0.18	50	2.562	24.22	28.84	+

74	SAMN27156085	Raceway #2	Baffle	2019	40	0.06	50	2.562	24.22	28.84	+
75	SAMN27156086	Raceway #2	Baffle	2019	110	0.15	50	2.562	24.22	28.84	+
76	SAMN27156087	Raceway #2	Baffle	2019	70	0.30	50	2.562	24.22	28.84	+
77	SAMN27156088	Raceway #2	Baffle	2019	27	0.16	50	2.562	24.22	28.84	+
78	SAMN27156089	Raceway #2	Baffle	2019	91	0.13	50	2.562	24.22	28.84	+
79	SAMN27156095	Raceway #2 Inflow	Prefilter	2019	20	0.08	50	2.562	24.22	28.84	+
80	SAMN27156096	Raceway #2 Outflow	Prefilter	2019	1196	6.24	50	2.562	24.22	28.84	+
82	SAMN27156114	Raceway #2 Outflow	Sterivex	2019	1016	4.51	50	2.562	24.22	28.84	+
83	SAMN27156175	Raceway #2	Wall	2019	20	0.04	50	2.562	24.22	28.84	+
84	SAMN27156176	Raceway #2	Wall	2019	181	0.22	50	2.562	24.22	28.84	+
85	SAMN27156177	Raceway #2	Wall	2019	92	0.24	50	2.562	24.22	28.84	+
86	SAMN27156178	Raceway #2	Wall	2019	51	0.12	50	2.562	24.22	28.84	+
87	SAMN27156179	Raceway #2	Wall	2019	198	0.80	50	2.562	24.22	28.84	+
88	SAMN27156180	Raceway #2	Wall	2019	145	0.42	50	2.562	24.22	28.84	+
89	SAMN27156077	Raceway #4	Baffle	2019	10	0.02	64	0.006	14.26	14.42	(+)
91	SAMN27156079	Raceway #4	Baffle	2019	13	0.02	64	0.006	14.26	14.42	(+)
93	SAMN27156081	Raceway #4	Baffle	2019	41	0.05	64	0.006	14.26	14.42	(+)
98	SAMN27156167	Raceway #4	Wall	2019	13	0.04	64	0.006	14.26	14.42	(+)
100	SAMN27156169	Raceway #4	Wall	2019	16	0.04	64	0.006	14.26	14.42	(+)
102	SAMN27156171	Raceway #4	Wall	2019	13	0.02	64	0.006	14.26	14.42	(+)
112	SAMN27156097	Raceway #9 Inflow	Prefilter	2019	15	0.04	20	0.004	0.45	3.13	-
115	SAMN27156172	Raceway #9	Wall	2019	14	0.03	20	0.004	0.45	3.13	-
118	SAMN27156109	Raceway #10 Inflow	Sterivex	2019	5	0.02	7	0	0.00	9.59	(-)
119	SAMN27156110	Raceway #10 Outflow	Sterivex	2019	11	0.04	7	0	0.00	9.59	(-)
126	SAMN27156140	Raceway #11	Wall	2019	10	0.02	6	0	0.00	8.47	-
132	SAMN27156067	Raceway #12	Baffle	2019	13	0.02	79	0.004	45.40	45.45	[-]
134	SAMN27156069	Raceway #12	Baffle	2019	8	0.01	79	0.004	45.40	45.45	[-]
135	SAMN27156142	Raceway #12	Wall	2019	15	0.03	79	0.004	45.40	45.45	[-]
136	SAMN27156143	Raceway #12	Wall	2019	13	0.03	79	0.004	45.40	45.45	[-]
137	SAMN27156144	Raceway #12	Wall	2019	28	0.02	79	0.004	45.40	45.45	[-]
138	SAMN27156145	Raceway #12	Wall	2019	26	0.02	79	0.004	45.40	45.45	[-]
152	SAMN27156157	Raceway #18	Wall	2019	11	0.02	20	0.072	0.83	12.81	(-)
154	SAMN27156159	Raceway #18	Wall	2019	9	0.03	20	0.072	0.83	12.81	(-)

(+) = active columnaris cases but post peak, (-) = no active cases but infection occurred sometime following sampling during production cycle, [-] = no active cases but previous large outbreak had occurred, N/A = not applicable.

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