Ecological filtering drives rapid spatiotemporal dynamics in fish skin microbiomes

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Abstract

Skin microbiomes provide vital functions, yet knowledge about their species assemblages is limited - especially for non-model organisms. In this study, we conducted in situ manipulations and repeated sampling on wild-caught individuals of Rutilus rutilus. Treatments included translocation between fresh and brackish water habitats to investigate the role of environment; community rebooting by disinfection to infer host-microbe interactions; and housing in pairs to study the role of inter-host dispersal for the structure of microbiomes colonizing animals. Results revealed that fish skin microbiomes were biodiversity hotspots with highly dynamic composition that were distinct from bacterioplankton communities. External environmental conditions and individual-specific factors jointly determined the colonization-extinction dynamics, whereas inter-host dispersal had negligible effects. The dynamics of the microbiome composition was seemingly non-affected by reboot treatment, pointing to high resilience to disturbance in these microbial communities. Together, the manipulations demonstrate that host individual characteristics and environment interactively shapes the skin microbiome of fish. The results emphasize the role of inter-individual variability for the unexplained variation found in many host-microbiome systems, although the mechanistic underpinnings remain to be identified.

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Abstract

Skin microbiomes provide vital functions, yet knowledge about the drivers and processes structuring their species assemblages is limited - especially for non-model organisms. In this study, fish skin microbiomes were assessed by high throughput sequencing of amplicon sequence variants from metabarcoding of V3-V4 regions in the 16S rRNA gene on fish hosts subjected to the following experimental manipulations: *i*) translocation between fresh and brackish water habitats to investigate the role of environment; *ii*) treatment with an antibacterial disinfectant to reboot the microbiome and investigate community assembly and priority effects; and *iii*) maintained alone or in pairs to study the role of social environment and inter-host dispersal of microbes. The results revealed that fish skin microbiomes harbor a highly dynamic microbial composition that was distinct from bacterioplankton communities in the ambient water. Microbiome composition first diverged as an effect of translocation to either the brackish or freshwater habitat. When the freshwater individuals were translocated back to brackish water, their microbiome composition converged towards the fish microbiomes in the brackish habitat. In summary, external environmental conditions and individual-specific factors jointly determined the community composition dynamics, whereas inter-host dispersal had negligible effects. The dynamics of the microbiome composition was seemingly non-affected by reboot treatment, pointing towards high resilience to disturbance. The results emphasized the role of inter-individual variability for the unexplained variation found in many host-microbiome systems, although the mechanistic underpinnings remain to be identified.

Keywords: aquatic, ecology, environmental translocation, skin microbiota, teleost, 16S rRNA amplicons

Introduction

Understanding the ecological and evolutionary processes that shape and contribute to the variation and changes within (intra-individual) and the differences among (inter-individual) host-associated microbial communities (i.e., microbiomes) is critically important as microbiomes affect the host phenotype and health by modulating immune responses (Popkes & Valenzano, 2020; Schommer & Gallo, 2013), metabolism (Blanton et al., 2016), stress tolerance (Compant, Samad, Faist, & Sessitsch, 2019), social behaviors (Soares, Cable, Lima-Maximino, Maximino, & Xavier, 2019), and mate choice (Sharon et al., 2010). Insights into the processes shaping microbiomes can be gained by utilizing the same theoretical framework applied in community ecology (Vellend, 2010), which was originally developed for multicellular organisms in macroscale ecosystems (Keddy, 1992), but is now increasingly also applied for microbiomes (Berggren et al., 2022; Bonilla-Rosso, Eguiarte, Romero, Travisano, & Souza, 2012; Coyte, Rao, Rakoff-Nahoum, & Foster, 2021; Miller & Bohannan, 2019). Conceptually, the four main processes involved in shaping species composition and diversity of communities are selection, drift, speciation, and dispersal, although there are many ways in which they interact (Kohl, 2020; Vellend, 2010). To simplify, as posited by the community assembly theory, colonization and accumulation of species are either the result of stochastic events and interchangeability of species, or driven by deterministic processes that favor certain functional traits and niches (Diamond, 1978; Keddy, 1992; Kraft, Cornwell, Webb, & Ackerly, 2007; Rosenzweig, 1995). However, empirical evidence rather indicate patterns in between those two extremes which can be deduced to ecological filtering caused by the interplay among abiotic environmental factors and biotic inter-specific interactions (Cadotte & Tucker, 2017; Coyte et al., 2021; Kraft et al., 2007; Rosenzweig, 1995; Stegen, Lin, Konopka, & Fredrickson, 2012).

Ecological filtering of fish skin microbiomes may be imposed by host characteristics, interactions among host conspecifics, and host-microbe interactions, as well as by interactions between the host and its environment, including movements between habitats (Berggren et al., 2023; Coyte et al., 2021; Kohl, 2020). Previous studies suggest that host species and

populations, mediated by ecology and genetic make-up, are important determinants of fish skin microbiomes (Chiarello et al., 2018; Franzenburg et al., 2013; Larsen, Tao, Bullard, & Arias, 2013; Larsen, Bullard, Womble, & Arias, 2015; Smith, Danilowicz, & Meijer, 2007). There are also studies reporting that skin microbiomes vary among different fish individuals within populations (Berggren et al., 2023; Berggren et al., 2022). However, little is known about temporal dynamics of skin microbiome composition within individual animal hosts other than humans in which intra-individual temporal variability was low whereas the inter-individual variability was high (Apprill, 2017; Costello et al., 2009; Oh et al., 2016; Risely, 2020; Ross, Hoffmann, & Neufeld, 2019).

If fish host species exert strong filtering, this should lead to a core microbiome among individuals of the same species. While several studies have emphasized the presence of species-specific core microbiomes (Shade & Handelsman, 2012; Sullam et al., 2012), they are often limited to a small fraction of the overall community, and most taxa are not shared among all individual hosts (Berggren et al., 2022; Burns et al., 2016; Chiarello, Villeger, Bouvier, Bettarel, & Bouvier, 2015). Such a pattern is consistent with the notion that individual host characteristics may be an important part of the filtering process. However, few studies have evaluated this by following the same individuals over time, or between habitats, such that the relative impact of environment and host characteristics could be disentangled (see Berggren et al. (2023); Uren Webster et al. (2020)). Individual-specific filtering may result from different inherent individual properties, such as physiological changes associated with spawning, osmotic changes, or shifts in water quality (Hess, Wenger, Ainsworth, & Rummer, 2015; Kueltz, 2015; Wotton, 2004) that modify the amount and biochemical composition of the mucus (Ángeles Esteban, 2012), or reflect genetic characteristics such as immune defense or sex (Boutin, Sauvage, Bernatchez, Audet, & Derome, 2014; Tort, Balasch, & Mackenzie, 2003). Differences among host individuals in behaviors, movements, and habitat use may also contribute to specific microbiomes via exposure to different environments and potential colonizers from surrounding microbial communities (Berggren et al., 2023; Sadeghi, Chaganti, Johnson, & Heath, 2023). Moreover, contrasting abiotic conditions (e.g., salinity, pH, and/or temperature) in different habitats are also likely to

induce behavioral and physiological responses of the fish hosts and thereby influence the structure and dynamics of the associated microbiomes (Ángeles Esteban, 2012; Boutin et al., 2014; Tort et al., 2003).

The assembly of skin microbiome communities may also be affected by priority effects mediated by inter-specific interactions among microbiome members, whereby the initial establishing species that make up the pioneer community preempt or modify niches for later arrivers, thereby potentially affecting the successional trajectory and composition of the climax community (Debray et al., 2022). Theory and empirical evidence largely concur that the ability of populations to establish in island habitats, which fish hosts constitute to microbes, is influenced both by the characteristics of species in the pool of potential colonizers and by the number, density, and identity of species already present in the community (Cadotte & Tucker, 2017; Fukami, 2015; MacArthur & Wilson, 1967; Simberloff & Wilson, 1970). As such, comparisons between rebooted and non-treated host individuals can inform whether the early established microbiome offers resistance to further colonization by restricting the available niches (Fukami, 2015). Furthermore, the community assembly process is influenced in part by the pool of potential colonizers. Adjacent populations and communities are in many systems more similar to one another than to more distant ones, owing to the homogenizing effect of dispersal (Miller, Svanback, & Bohannan, 2018; Wright, 1943). In this context, effects of inter-host dispersal on the assembly of host-associated microbiomes have been inferred previously (Burns et al., 2017; Song et al., 2013), but experimental evaluations remain rare.

One way to evaluate the joint effects of host-microbe interactions and environmental factors on community assembly and dynamics (Miller et al., 2018) is to conduct experimental studies and repeated sampling of individual hosts under natural conditions. Here, we have studied a Baltic Sea (southeast Sweden) roach (*Rutilus rutilus*) population that migrates from foraging grounds in a coastal brackish environment to spawn in freshwater (**Fig. 1a** and Supplementary information **S1**). In this study, we aimed to investigate the assembly and temporal

dynamics of the skin microbiome composition of fish *in situ*. To this end, we performed a field experiment during three consecutive weeks that involved three types of manipulations. To disentangle the relative roles of the external environment and individual fish host characteristics, we used a split-environment design by translocating fish between the fresh and the brackish water habitat, with putatively different bacterioplankton communities, and monitored their skin microbiome composition over three weeks. Furthermore, to investigate the processes involved in the assembly of the microbiome and whether it is affected mostly by individual host intrinsic factors, priority effects, colonization by microbes originating from the ambient water, or by the microbiome of conspecific hosts we first "rebooted" the microbiome of half of the individuals by treating them with a disinfectant agent (benzalkonium chloride) and then manipulated their social setting by housing them in cages either alone or together in pairs. The combination of manipulations also enabled evaluation of interactive effects of different drivers.

Materials and Methods

Evaluating the rebooting effect of benzalkonium chloride (BKC) on colony forming bacteria in fish skin microbiomes. To evaluate the effect of bathing fish in BKC on viable bacteria, a laboratory study was performed on roach using a culture dependent technique (Marine Zobell agar; 5 g/L peptone, 1 g/L yeast extract, filtered seawater, and 1.5% agar). Although this method does not reflect the whole community due to the small proportion of bacteria able to grow on agar (Amann, Ludwig, & Schleifer, 1995; Hugenholtz, Goebel, & Pace, 1998; Torsvik, Goksoyr, & Daae, 1990), it was reliable in determining viable bacteria compared to 16S rRNA gene amplicon sequencing that will include both dead and live cells. BKC is a disinfecting agent that efficiently lyses bacterial cells without harming the host and is therefore extensively used within aquaculture for treating fish with bacterial infections on skin and gills (Anderson & Conroy, 1969). The fish is bathed in a solution with 1-2 mg of BKC per liter H_2O for up to 60 min (Anderson & Conroy, 1969; Bullock & Conroy, 1971).

All individuals (n = 10) were sampled with sterile cotton swabs before being placed into a BKC-bath (concentration 1.5 mg/L) and then sampled again after 10, 20, and 30 min. A new spot was sampled at each time-point to avoid being affected by the previous sampling (**Fig. S1**). The person handling the fish strictly avoided touching the hind dorsal areas. The surface of the fish was washed with sterile MilliQ water prior to each sampling to minimize the possibility that loosely attached microbes belonging to the water column would be sampled in the initial sampling. This also minimized the risk for the dis-infecting agent present in the water to affect the colonies on the agar plates during subsequent sampling. This further ensured that a reduction in colony forming units (CFU) was a result of reduced viable bacteria on fish rather than a reduced microflora in the water column. After 24 h of incubation in 20°C, pictures were taken of each agar plate. CFUs were counted twice and blind with respect to the first count on computer screen using GIMP2 (v2.8). The mean number of CFUs at the initial sampling was 163 (median = 122, range = 28-330). Results showed that a 10 min bath in 1% solution of BKC reduced the number of CFUs with an average of 96% (median = 97%, range = 85-99%; **Fig. S2**). The effect of BKC on the number of viable bacteria was significant when testing for differences in the number of CFUs among time points (generalized linear mixed model: $\chi^2_{3, 34} = 124.8$, $p < 0.001$), time point was included as a fixed effect and individual as a random effect to account for the dependency of repeatedly sampling the same individuals. Fixed effect was evaluated with type II Wald chi-square test using the *glmer* function in the lme4 R package (Bates, Maechler, Bolker, & Walker, 2015). Moreover, considering the well-being of the fish included in the study and hence subjected to BKC, no negative effects were noticed either directly or two weeks post treatment. Based on the results from the laboratory study, it was decided that individuals assigned for microbiome reboot in the field experiment were to be subjected to BKC bath for approximately 10 min at the initiation of the experiment.

Capture of fish and collection of microbiome samples. Fish (*n* = 80) were captured with fyke nets on two consecutive days (12-13 April 2016, day 1: 36 individuals, and day 2: 44 individuals) close to the outfall of freshwater stream Oknebäcken into the Baltic Sea (57°016,569'N; 16°451,018'E) (**Fig. 1a).** Captured individuals were distributed among 30 L containers that were kept shaded and well-oxygenated through oxygen pumps. For each experimental unit, we selected four individuals of similar size (mean = 22.82 cm ± SD = 1.91 cm, measured after the last sampling occasion to minimize handling time), and sex as determined by the presence/absence of "breeding tubercles" that male roach develop during the spawning period (Kortet, Taskinen, Vainikka, & Ylonen, 2004; Kortet, Vainikka, Rantala, Jokinen, & Taskinen, 2003). Both males and females were used in the experiment but in different proportions (72 females and 8 males) due to a skewed sex ratio of individuals of the suitable size class. All applicable national guidelines for the care and use of animals were followed. Ethical approval for the study was granted by the Ethical Committee on Animal Research in Linköping, Sweden (Dnr. 33-14 and 10-14).

After capture, microbiome samples were taken before exposure to any manipulation. Each fish was rinsed with MilliQ-water and subsequently sampled on the right dorsal area (2×2 cm) with a sterile cotton swab (Nordic Biolabs, CP167KS01, Sweden). All samples were collected in Eppendorf tubes with 750 µL TE-buffer (Tris-EDTA, 10:1) and stored on ice until arrival at the laboratory where they were placed in a -80°C freezer. To enable subsequent identification of individuals the tail fin was marked with a scissor. Following initial sampling and marking, all individuals were transferred to plastic bags in separate, non-transparent boxes to receive either BKC treatment (2 l BKC) or control treatment (2 L of either brackish or freshwater from incubation sites). After approximately 10 min, the fish were transferred to a new plastic bag filled with 2 L of well-oxygenated water for transportation to either of the two field incubation sites (see **Fig. 1a**).

Split-environment experimental design. To evaluate effects of the external environment and host characteristics, fish individuals were divided into two main groups and translocated to either fresh- or brackish water. Individuals were further distributed in replicate units, with each unit consisting of four individuals (of same gender) distributed among three cages (cage size: L520×Ø250 mm, mesh of nylon with grid size of ~10 mm). Within each replicate unit, two fish were treated (T) with BKC and the other two were left untreated as controls (C) ($N_T = 40$, $N_C = 40$) to investigate the role of community assembly and priority effects. To manipulate social environment and inter-host dispersal, two individuals from each treatment (T and C) were housed together in one cage, whereas the other two were housed in separate cages (**Fig. 1b**). In each habitat, ten replicate units were distributed among five blocks located approximately 3 m apart and secured in the bottom with wooden poles (Ø12 cm, L300 cm). The experiment lasted for three weeks (April 12th-May 3rd, 2016) and fish were sampled with a sterile swab near the dorsal fin repeatedly on four different occasions. To avoid samples of the fish microbiome potentially being affected by previous sampling, a different part of the dorsal area near the dorsal fin was chosen for each sampling occasion (**Fig. S1**). Due to the loss of 13 individuals during the experimental period, sampling resulted in 175 microbiome samples from 44 individuals (we strove to process samples from complete replicate units that lasted throughout the experiment). Of the eleven replicate units used in the end, six were initially placed in the freshwater environment and five in the brackish environment. The experimental design and sampling scheme are illustrated in **Fig. 1b**. According to animal ethics prescriptions, all experimental animals were euthanized by decapitation after being anesthetized by a blow to the head after the last sampling occasion.

To enable comparisons of fish microbial communities with the bacterial communities in the surrounding water, water samples (1 L) were taken at the locations of each experimental unit at the onset of the experiment when replicate units were distributed in the two environments (day 1: $n = 5$, day 2: $n = 6$), on each microbiome sampling occasion (three occasions, 1ten0 locations: $n = 30$), and at the time of translocation of units between environments (one water sample from each of the new locations, $n = 5$) resulting in total of 46 water samples.

DNA extractions, library preparation and sequence data processing. DNA from microbiome samples was extracted using QIAmp DNA Mini Kit (QIAGEN, Germany), following the protocol by the manufacturer (QIAmp ® DNA Mini and Blood Mini Handbook, Protocol: DNA Purification from Tissues (QIAamp DNA mini-Kit, 2016), starting from step 3 and eluting in 100 µL elution buffer (TE-buffer, (Tris-EDTA, 10:1). Water samples were vacuum-filtered through a 0.22 µm pore size 47 mm Supor® membrane filter (Pall Corporation) that was stored 1.8 mL TE-buffer in -80°C freezer. DNA was extracted with the DNeasy PowerWater kit (Qiagen, Germany) following the protocol provided by the manufacturer (2017). To minimize cross-contamination of samples, the tweezer used to take out swabs was first rinsed in 70% ethanol and flame sterilized between each sample (for both mucus and water). Obtained concentrations from all DNA extractions were measured using NanoDrop 2000. Extracted DNA was stored at -20°C until 16S rRNA gene amplification and library preparation.

Sequencing libraries were prepared with the primer pair 341F and 805R that target the V3-V4 hypervariable regions of the bacterial 16S rRNA gene complex (Herlemann et al., 2011). Amplification followed the Illumina PCR-protocol by Hugerth et al. (2014); Lindh et al. (2015) and subsequently adding five cycles to a total of 25 cycles in PCR1. The process of adding Illumina adapters and index sequences was conducted according to Lindh et al. (2015) and concentrations of PCR2 products were measured with a Qubit ® 2.0 Fluorometer. The resulting purified (individually barcoded) amplicons were pooled with normalized concentrations of each sample. Four negative controls for the extraction kits were also included in the sequencing libraries. The library pools were purified with E.Z.N.A. ® Gel purification kit (Omega Bio-Tek, Inc.) following the protocol from the manufacturer and sequenced on the Illumina MiSeq platform (Illumina, USA) with 2×300 bp paired-end settings at Science for Life Laboratory (SciLifeLab, Stockholm, Sweden). All samples were processed blindly during DNA extractions and library preparations.

Microbiome samples (*n* = 175) and water samples (*n* = 46) were sequenced on three runs yielding 23.5, 24, and 17.6 million raw reads, respectively. Samples were randomly distributed among sequencing runs. The Ampliseq workflow (v2.4.0), available at https://nf-co.re/ampliseq/2.4.0 (Bolyen et al., 2019; Straub et al., 2020), was employed for the conversion of raw reads into Amplicon Sequence Variants (ASVs) count- and taxonomy tables. In summary, this pipeline identifies complete amplicon reads through primer sequences and eliminates the primer-derived sequences using Cutadapt (v3.4) (Martin, 2011). The remaining sequences were subsequently standardized to uniform length, subjected to denoising, and converted to ASV tables using DADA2 with pipeline default parameters (v1.22.0) (Callahan et al., 2016). The Silva taxonomy (v138.1) (Quast et al., 2013) was used to determine taxonomy with DADA2's assignTaxonomy function. This reduced the total number of reads from 65 067 574 to 44 228 626. Sequences present in the negative controls were excluded, as were sequences not assigned to any domain and sequences that were taxonomically assigned to chloroplasts or mitochondria.

Statistical analyses. All statistical analyses were performed in R studio (v2022.07.2) with R (v11.28.45) if not stated otherwise (R Core Team, 2013; RStudio Team, 2019). Technical details on statistical approach and generation of included plots can be found in Supplementary information **S2**. For beta diversity analyses, we performed a centered log ratio (CLR) transformation of the raw count of each ASV to account for the compositional nature of data sets obtained from high throughput sequencing (Aitchison, Barcelo-Vidal, Martin-Fernandez, & Pawlowsky-Glahn, 2000; Gloor, Macklaim, Pawlowsky-Glahn, & Egozcue, 2017; Pawlowsky-Glahn, Egozcue, & Lovell, 2015). This approach focuses on the variance-based components of the data (Chao, Chazdon, Colwell, & Shen, 2006).

Testing the impact of environmental translocation, individual characteristics of fish hosts, and reboot treatment on microbiome composition and dynamics. To investigate whether fish

microbiome (*n* =175) composition shifted and whether such shifts depended on main group (initial water habitat that fish were allocated to), we performed a constrained redundancy analyses (hereafter RDA) (Dixon, 2003; Oksanen, 2012) using the vegan package (v2.6-4) to evaluate whether there was an interaction between the initial location (brackish or freshwater group) and sampling occasion (week0-week3). To evaluate whether microbiome composition differed depending on treatment (BKC or control), fish id (*n* = 44), and sampling occasion (week0-week3), we conducted a second RDA with permutations restricted to initial location (due to significant interaction between initial location and sampling occasion in the first analysis),

Pairwise comparisons of fish microbiome composition between each sampling occasion. Next, we analyzed the temporal dynamics in more detail by performing pairwise comparisons between sequential sampling occasions for each of the four groups (rebooted brackish, control brackish, rebooted freshwater, and control freshwater) by performing a PERMANOVA in PRIMER-E v7 (Anderson, Gorley, & Clarke, 2008) on Euclidean distance matrix (based on CLR values). In this analysis, sampling occasion (week0-week3) was included as a fixed factor, and fish id (*n* = 44) was included as a random factor to account for repeated samples from the same individual. The null hypothesis of homogeneity of multivariate dispersions among groups was tested by permutational analysis of multivariate dispersions (PERMDISP) (Anderson, 2006) based on mean distance to group centroids to clarify the dispersion effect (**Table 1**).

Comparing resemblance in microbiome composition depending on social setting. To evaluate whether individuals that shared a cage had a higher resemblance in their microbiome composition compared to the single individuals we first compared the dispersion from the group centroid between single and paired individuals one and two weeks after experiment started, because that represented a similar treatment for all individuals: two weeks in the same environment. For this, the group means/centroids were based on each replicate unit consisting of four individuals that were housed singles or in pairs (**Fig. 2b**). Data was analyzed using a linear mixed model in the lme4 package (v1.1-31) with individual as random factor, to control for

dependent observations. Next, we investigated whether the rebooted microbiomes (n = 22) converged to the microbiome composition of untreated control hosts in the same cage after the first week (week 1). For this analysis, we used a paired t.test to compare the Euclidean distance of rebooted hosts to their cage mate with their average distance to the microbiomes of untreated control hosts in the same environment.

Comparisons of microbial communities associated with water and fish skin. To compare the community composition of samples collected from water and fish, respectively, we performed an RDA with sample type as a constraining factor. To investigate whether the dynamics of community composition water microbial communities depended on habitat (brackish and freshwater) we performed an RDA with interaction between location and sampling occasion (week0-week3). Next, we investigated whether water microbial communities shifted over time, and whether they differed in community composition according to spatial separation. Thus, the variables included in this analysis thus were sampling occasion (week0-week3) and position in water (pole nr) and permutations nested within sampling location (brackish/freshwater) due to a significant interaction between sampling location and occasion.

Results

Impacts of environmental translocation, individual characteristics of fish hosts, and reboot treatment on microbiome composition and dynamics

The temporal dynamics of community composition depended on whether the fish were translocated to brackish or freshwater habitats, as indicated by the significant interaction effect between habitat and sampling week (n = 175, RDA, effect of interaction: $F_{3, 167}$ = 1.38, P = 0.001, **Fig. 2**). There was no difference in the overall community composition between the rebooted and the control group (RDA, effect of reboot: $F_{1, 127}$ = 1.07, P = 0.19), but differences in microbiome composition between fish individuals were repeatable across both time and space (RDA, effect of fish individual: $F_{43, 127}$ = 1.04, P = 0.007), pointing to a role of host characteristics. We therefore

evaluated whether the intrinsic factors of sex or size (length) of the host was associated with its microbiome composition at the initial sampling (before the fish were subjected to any manipulations), but the results showed no statistically significant association with either trait (n = 44, RDA, effect of sex: $F_{1, 43}$ = 1.03, P = 0.44; effect of length: $F_{1, 43}$ = 1.13, P = 0.23).

Next, we analyzed the temporal dynamics in more detail by performing pairwise comparisons between sequential sampling occasions for each of the four groups (rebooted brackish, control brackish, rebooted freshwater, and control freshwater). All fish were spatially translocated between brackish- and freshwater water habitats between week 0 and week 1 and this manipulation resulted in a shift in microbiome composition in all groups (P < 0.001; **Table 1, Fig. 2**). No translocations were performed between weeks 1 and 2 and the community composition did not change during this period in any of the freshwater groups (P > 0.05; **Table 1, Fig. 2**) but did alter in both brackish groups (P < 0.05; **Table 1)**. Finally, between week 2 and 3, fish in the brackish habitat were not subjected to any manipulation whereas fish housed in the freshwater habitat were translocated back to the brackish habitat. This resulted in a concomitant and statistically significant shift in microbiome composition among translocated fish hosts (i.e., both freshwater groups, P < 0.01; **Table 1**), but no significant shift occurred in the resident brackish groups (**Table 1, Fig. 2**).

Comparing resemblance in microbiome composition depending on social setting

Host individuals that shared a cage (n = 22) did not show a higher resemblance in microbiome composition compared with individuals that were housed alone, but the resemblance varied significantly according to sampling occasion (Linear mixed model [with individual as random factor to control for dependent observations], effect of social setting: χ^2 = 0.05, df = 1, P = 0.82; effect of sampling occasion: χ^2 = 6.67, df = 1, P = 0.01; **Fig. 3**). The interaction effect between social setting and sampling occasion was non-significant (χ^2 = 0.05, df = 1, P = 82). In addition, a paired t-test was used to evaluate whether rebooted hosts that shared a cage (n = 11) had higher

resemblance to their control cage mate (i.e., if the Euclidean distance was less) than to other control hosts in the same environment. The result indicated that the microbiome composition of rebooted hosts did not converge to the microbiome composition of their control cage mate (paired *t*.test: $t(10) = 0.58$, $p = 0.57$, **Fig. S3**).

Comparisons of microbial communities associated with water and fish skin

The microbial communities associated with water and fish were significantly different ($n = 221$; RDA: $F_{1, 219} = 16.56$, $P < 0.001$; **Fig. 4**). The composition of the bacterioplankton community shifted significantly over time and exhibited significant small-scale spatial heterogeneity in both brackish and freshwater (RDA, effect of sampling occasion: $F_{3, 23} = 1.70$, $P = 0.019$; effect of location: $F_{14, 23} = 1.86$, $P = 0.001$). However, no such pattern according to location in the water was found among skin microbiome samples ($F_{4, 83} = 1.05$, $P = 0.251$). Only 2.5 % of the identified microbes present both in water and fish skin (469 of a total 19104 ASVs, **Fig. S4**). The phylogenetic diversity represented in fish skin microbiome samples far exceeded that in water samples, both in general (66 versus 31 phyla) and in terms of enriched taxa (6 versus 3 phyla; **Fig S5**).

Discussion

We report on findings from repeated and longitudinal sampling of translocated fish host individuals that provide important insights on how the skin microbiomes of an anadromous fish species are individual-specific, how they differ from the microbial communities in the surrounding water, and how they rapidly respond to environmental conditions. This is an important contribution to the knowledge of the ecological and evolutionary processes of fish skin microbiomes, especially since skin microbiomes rarely have been characterized in fish species

associated with freshwater systems (Chiarello et al., 2019; Llewellyn, Boutin, Hoseinifar, & Derome, 2014).

Translocation between environments induced shifts in microbiome composition. Results from longitudinal sampling revealed a species turnover within a week, demonstrating that the skin-microbiomes of fish were highly dynamic over time. To our knowledge, such rapid shifts of fish skin microbiomes within individuals have never been reported. Uren Webster et al. (2020) stated that the microbiomes of Atlantic salmon showed dynamics over a six-week period associated with shifts in environment and diet, but they also report signs of individual-specific effects on microbiome composition when comparing pre- and post-translocation microbiomes. The intra-individual repeatability found in the present study reflected that the differences in microbiome composition among individuals persisted over time. The repeatability of microbiomes within individual hosts pointed to ecological filtering consistent with a growing body of research (Berggren et al., 2023; Figueiredo & Kramer, 2020; Nicholson et al., 2012; Rawls, Mahowald, Ley, & Gordon, 2006). This result could be due to individual characteristics of the hosts, to microbe interactions during colonization and succession of the microbiome, or to a combination of the two. In the present study, we did not detect any association with sex or body size and microbiome composition. This result was partly coherent with results from a recent study of carp (*Cyprinus carpio*) that did not find any association between microbiome and sex, but showed that variation in microbiome composition among fish hosts was significantly associated with body site (i.e., dorsal or ventral), sun-basking behavior, vertical habitat switches, and bodily growth (Berggren et al., 2023).

Another explanation for the high variation in microbiome composition among host individuals is functional similarity (Risely, 2020), meaning that ecological functions can be maintained even though taxonomic composition differs, as shown by (Louca et al., 2017). Diamond (1978) hypothesized that competition is the main force structuring species assemblages

as total niche overlap means that the species cannot coexist. In agreement with this last notion, interspecific competition can either lead to competitive exclusions or result in evolutionary modifications of resource utilization with increased specialization and reduced niche overlap as a result. It has been suggested that the short generation time in bacteria enables rapid adaptation and evolution of interchangeability, especially in open and changing environments such as fish skin (Philippot et al., 2010). To investigate whether such processes are at play, future studies could use transcriptomics in combination with amplicon sequencing.

The reboot treatment had negligible effects on microbiome composition and dynamics. Evaluations of disrupting treatments (e.g., antibiotics or other disinfectant) of skin microbiomes are rather rare compared to studies investigating their effect on the gut microbiome (Merrifield & Rodiles, 2015; Ross et al., 2019; Sadeghi, Chaganti, & Heath, 2023). Previous studies on gut microbiomes in humans show that there are both transient and long-lasting effects of disrupting treatments on the community composition (Langdon, Crook, & Dantas, 2016; Willing, Russell, & Finlay, 2011). However, no long-lasting effects of reboot on the fish skin microbiome composition were detected in the current study, and neither did the community composition dynamics differ between rebooted versus control individuals. This partly contradicted earlier studies on fish microbiomes although these have not been conducted at the level of individuals (Carlson, Leonard, Hyde, Petrosino, & Primm, 2017; Langdon et al., 2016; Rosado et al., 2019; Willing et al., 2011). Based on our results, we thus contend that repeated sampling of individuals is necessary to fully evaluate how disruptions affect microbiome composition. Moreover, that the reboot treatment had no detectable effects on the microbiome may either reflect that microbial interactions were of limited importance for community assembly, or that the succession of the microbial community was very rapid relative to the sampling interval used (one week) (Carlson et al., 2017). Still, the results implied that the fish skin microbiomes recovered from the reboot treatment without any detectable long-lasting effects, indicating that the resilience of fish skin

microbiome was high. That hosts subjected to similar environmental regimes developed similar microbiomes and differences among individual hosts were repeatable over time, regardless of reboot treatment, points to that external environmental conditions and host-specific filtering jointly contributed to the structuring of these communities, resulting in a highly dynamic microbiome composition.

On the role of social environment and inter-host dispersal for microbiome composition. Under the assumption that co-housing increases connectivity and inter-host dispersal, as inferred from island biogeography theory (MacArthur & Wilson, 1967), the results did not support our prediction that the microbiomes of co-housed hosts would converge. As demonstrated by the distribution of the Euclidean distances (**Fig. S3**), the negative outcome reflected that microbiome similarity was truly independent of whether hosts were co-housed and was not an example of a difference that falls below the threshold of statistical significance due to insufficient sample size or low power. Furthermore, the finding that individuals that shared a cage did not exhibit higher similarity in microbiome composition compared to single individuals, but that microbiome variation decreased significantly for all individuals between sampling occasions, was noteworthy. This indicated that the ability of fish hosts to move around freely might be an important part of the explanation of varying microbiome composition among individuals (Berggren et al., 2023; Larsen et al., 2015). Experimental investigations of inter-host dispersal are rare (but see (Schmidt, Smith, Melvin, & Amaral-Zettler, 2015) and therefore, the nature and context specificity of such phenomena are not well understood. Our findings did not support convergence of skin-associated microbiomes between co-housed individuals which was in congruence with a previous study by (Schmidt et al., 2015) that found that microbiomes of fish sharing a tank were no more similar to each other than to those in different tanks, so long as both tanks shared the same salinity. However, studies of fish gut microbiomes did find such patterns (Burns et al., 2017). Such opposing findings might be attributed to the type of microbiome studied (skin versus gut) or the

life stage of the fish host (Burns et al., 2017; Sylvain et al., 2020; Yan et al., 2016). Skin microbiomes are highly variable compared to gut microbiomes that more often show strong filtering (Sylvain et al., 2020), and perhaps early life stages of fish hosts are more open for colonization (Burns et al., 2016). Future studies of both gut and skin-associated microbiomes should aim at discriminating between alternative explanations and identify if specific factors affect the contribution of dispersal (Chen, Fischbach, & Belkaid, 2018; Voelkl et al., 2020).

Low similarity between fish skin microbiome and bacterioplankton in the water support ecological filtering as driver of microbiome assembly. Comparisons between fish microbiomes and water bacterioplankton communities showed that fish skin housed microbial communities that were remarkably different, with regards both to composition (only a very small fraction of identified microbes was shared) and phylogenetic diversity, from those in the water pointing to strong ecological filtering. Furthermore, the bacterioplankton communities in both the brackish and the freshwater habitats showed signs of small-scale spatial heterogeneity that was not paralleled by the variation in the microbiomes among the fish hosts that were experimentally housed at the corresponding locations within each habitat, adding to previous conception that fish skin harbor unique microbiota compared to the water column (Chiarello et al., 2020; Ross et al., 2019). The higher phylogenetic diversity in the microbiome samples, compared with the bacterioplankton diversity in the water samples, might suggest that the fish skin environment was more complex, and that the assembly and dynamics of the microbiome was strongly influenced by species interactions (Kohl, 2020). Despite our current understanding, the dispersal over the host-water interface and the biotic interactions that influence colonization-extinctions in fish skin mucus remain largely unexplored. Experimental approaches, which allow for the manipulation of the microbiome and ambient environment, and the tracking of effects within and among hosts over time, are vital for advancing our comprehension of these processes. The integration of host physiology, particularly the properties of skin mucosa, and its interaction with the environment,

could be a significant progression (Berggren et al., 2023; Wang et al., 2023). This could help elucidate how host characteristics contribute to the differences in microbiome composition observed between water and fish, among hosts, and within hosts over time.

Concluding remarks. Besides demonstrating rapid dynamics, a strong signature of ecological filtering driven by external factors, and high resilience, the results showed that the heterogeneity of microbiomes among hosts was repeatable over time. The realization that the dynamics of skin microbiomes were both host individual-specific and affected by external conditions has important implications and can ultimately contribute to increased reproducibility of research findings (Voelkl et al., 2020) because it emphasizes the importance of taking individual-specific effects into account in future studies. If high variation among individuals is not accounted for by combining studies with experimental manipulations, high n-values, and replicates, this might lead to misinterpretation of observed patterns. The finding that fish skin microbiomes shared little microbial diversity with the surrounding environment calls for consideration when discussing conservation of biodiversity in aquatic habitats, given that the loss of an animal species will result in the concomitant loss of its associated unique microbial diversity.

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Data Accessibility statement

All raw sequence data and metadata from this study are available in NCBI Short Read Archive under BioProject ID PRJNA714685 and PRJNA673155.

Author Contributions: AF and HB conceived the study; AF, HB, PT, JP, MD, and PL contributed to the study design; HB, ON, PT, and AF performed the field work; HB and ON performed the laboratory study; HB and YY performed the laboratory work; HB and DL performed downstream processing of samples; HB and YY performed statistical analyses under supervision of AF and DL; and HB and AF drafted the manuscript. All authors contributed to interpreting the results, read and approved the final manuscript version.

Figures and Tables

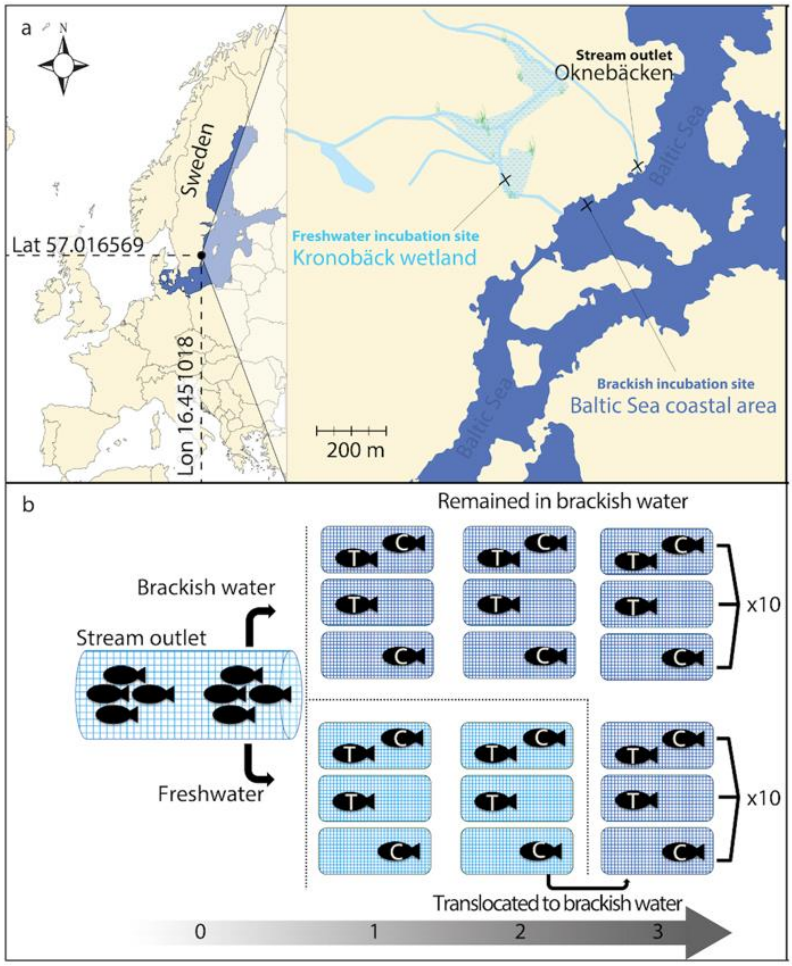


Figure 1. Study area and experimental design. a) Map illustrating the study site in Sweden. The roach population migrates from Baltic Sea coastal brackish environment to spawn in a freshwater stream Oknebäcken and its inundated floodplains. Fish were captured with a fyke-net placed in the stream mouth during spawning migration from brackish to freshwater. The incubations sites in fresh- and brackish water are marked with light blue and blue, respectively. **b**) The experimental setup comprised 80 individuals divided in two main groups that were translocated either to brackish (n = 40) or freshwater (n = 40). The individuals were further distributed among replicate units. Each unit consisted of four individuals, two were treated (T) with the BKC disinfecting agent, whilst the other two were left untreated as controls (C). The four

individuals were then distributed among three cages, two individuals were housed together (one BKC treated and one control) and the other two were housed alone in separate cages (one BKC treated and one control). In each habitat, ten replicate units were distributed among five wooden poles located 3 m apart. Sampling occurred at week 0, 1, 2, and 3. After week two, freshwater replicates were translocated to brackish water for one week before the last sampling occasion.

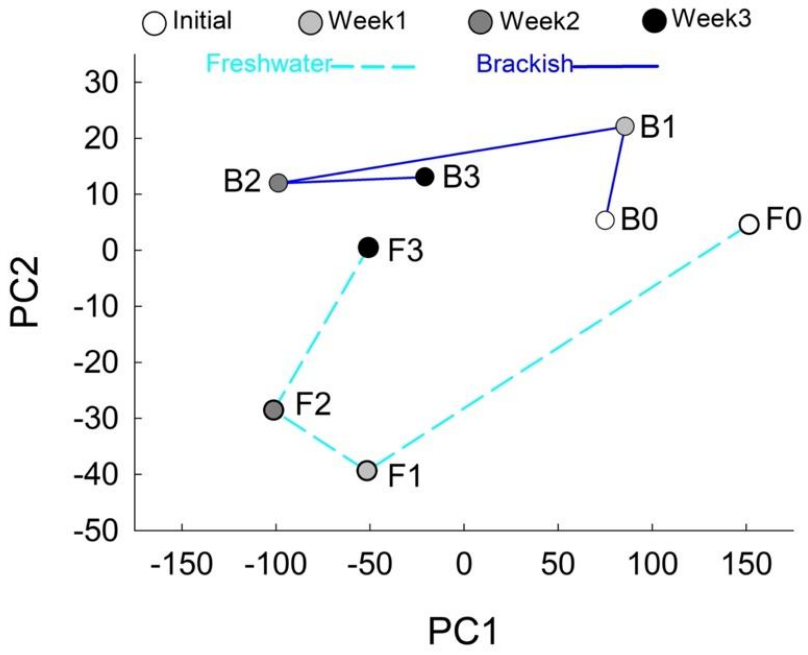
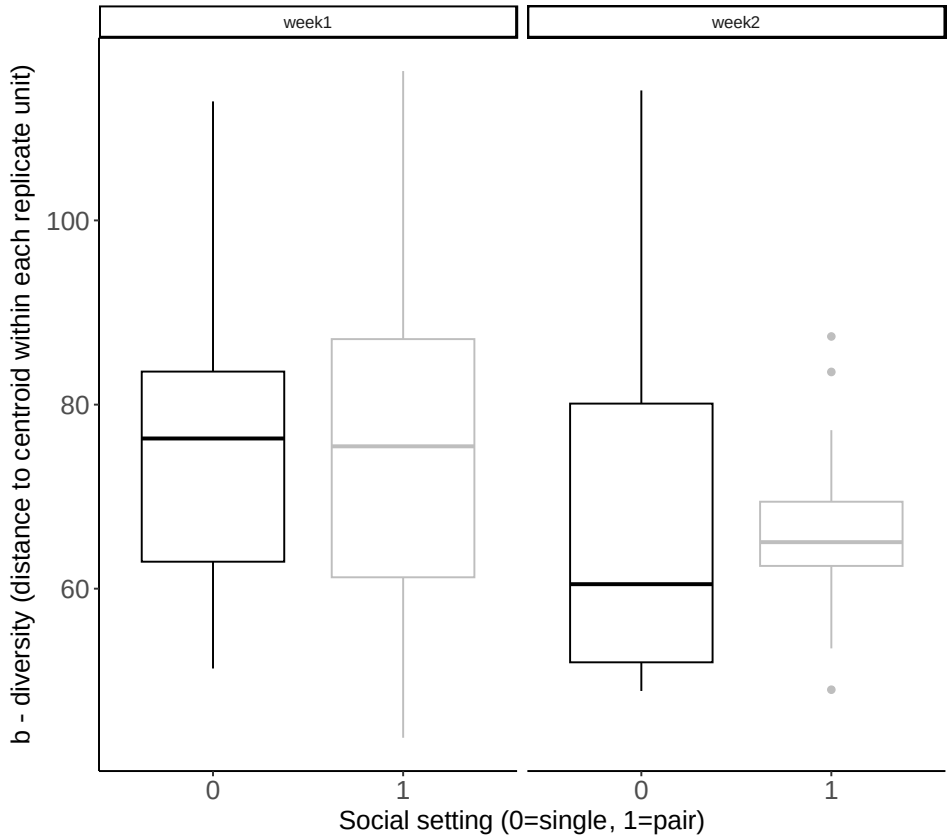


Figure 2. Variation and change in fish skin microbiome according to habitat (brackish versus freshwater) and time (sampling occasion). Figure shows mean PC1 and PC2 scores for brackish (solid line) and freshwater (dashed line) habitat and sampling occasion based on Euclidean distance matrix.



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808 **Figure 3. Comparisons of microbiome resemblance among individuals subjected to**
809 **different social settings.** The comparison includes the first and second week of the experiment
810 when all replicates were kept in constant conditions (i.e., fresh- or brackish water environment).
811 Black boxes represent single individuals, whereas grey boxes represent individuals that shared
812 cage with a conspecific.

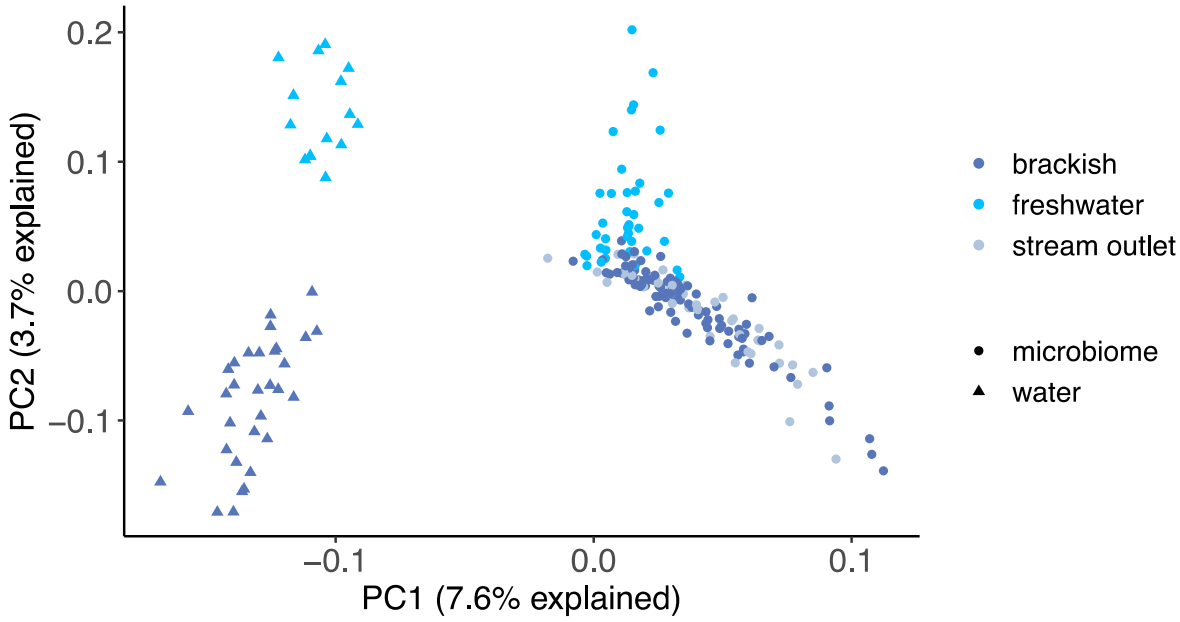

Figure 4. Comparison of community composition among water and microbiome samples. Plot is based on principal component analysis on all water samples (*n* = 46) and fish skin microbiome samples (*n* = 175).

Table 1. Shifts in fish microbiome community composition vary according to environment. Pairwise comparisons of community structure between sampling occasions were performed with PERMANOVA on four separate groups of fish hosts subjected to different combination of experimental treatment: environment (brackish or freshwater) and disinfectant treatment (reboot or control). Pairwise comparisons of dispersion of microbiomes among different hosts (within treatment groups) between sampling occasions were tested using PERMDISP. Significant results are indicated in bold.

	PERMANOVA; PERMDISP		
	week 0-week 1	*week 1- week 2*	*week 2- week 3*
Brackish rebooted	***t* = 1.10, *P* = 0.002**;	***t* = 1.067, *P* = 0.0266**;	*t* = 1.01, *P* = 0.28;
n = 12	*t* = 0.35, *P* = 0.7	*t* = 1.58, *P* = 0.18	*t* = 1.19, *P* = 0.30
Brackish control	***t* = 1.16, *P* = 0.0005**;	***t* = 1.11, *P* = 0.014**;	*t* = 1.01, *P* = 0.37;
n = 12	*t* = 0.46, *P* = 0.68	***t* = 3.98, *P* = 0.0014**	*t* = 0.98, *P* = 0.41
Freshwater rebooted	***t* = 1.29, *P* = 0.0001**;	*t* = 1.07, *P* = 0.0957;	***t* = 1.10, *P* = 0.0015**;
n = 10	*t* = 2.20, *P* = 0.065	*t* = 2.21, *P* = 0.067	*t* = 1.02, *P* = 0.45
Freshwater control	***t* = 1.16, *P* = 0.0001;**	*t* = 0.99, *P* = 0.62;	***t* = 1.12, *P* = 0.0026**;
n = 10	***t* = 3.20, *P* = 0.0078**	*t* = 0.87, *P* = 0.48	*t* = 0.17, *P* = 0.89