

RESEARCH ARTICLE

Elevated temperatures increase microbiome similarity between host and environment in a freshwater cnidarian

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Abstract

Bacterial communities associated with animals show complex spatial and temporal variation. The main driving forces behind this variation are still to be deciphered. Differences in microbiome composition could be caused by stochastic changes, such as random gain and loss of microbiome components, as well as deterministic factors, such as variation in temperature (or other abiotic factors), diet, or the availability of microbes with the potential to colonize the hosts in the surrounding environment. Here we investigated seasonal variation in the microbiome of *Hydra* polyps and the bacterioplankton surrounding them to test the hypothesis that the contribution of environmental microorganisms to host-associated microbial communities varies seasonally. Sampling was performed for two consecutive years in three distinct temperate water bodies in Eastern Hungary: a shallow lake, a deep lake, and a river. We found that the microbiomes of polyps differed from their surrounding environment and varied seasonally. The similarity of polyp and water microbiomes changed seasonally in a population-specific way: microbial communities associated with polyps became markedly more similar to that of their surrounding environment during the summer in the shallow lake habitat, but not in the other populations. Our results suggest that environmental and host-associated microbiomes change independently during most of the year, but high temperature increases the impact of environmental microbiome on host-associated microbial communities.

Animals coexist with complex microbial communities that have an increasingly recognized effect on their survival and reproduction, driving variation in Darwinian fitness and ultimately affecting evolutionary trajectories within species (Kolodny et al. 2020, and references therein). Despite consistent and ubiquitous effects on host physiology, animal microbiomes vary substantially under different climatic conditions (Kueneman et al. 2019; Woodhams et al. 2020; Härer and Rennison 2023). For instance, in a global analysis of amphibians, higher microbial species richness was found in environments with colder winters and more variable annual temperatures (Kueneman et al. 2019). Comparing seasons, species richness of frog and axolotl microbiomes did not differ too much, but compositionally they were different (Douglas

et al. 2021; Martínez-Ugalde et al. 2022). Among aquatic animals, latitudinal patterns in microbiome diversity and composition were detected in sponges (Griffiths et al. 2019), sea anemones (Morelan et al. 2019; Baldassarre et al. 2023), and damselflies (Theys et al. 2023). In birds, little geographical variation in gut microbiome composition was found in a pan-European study of great tits (*Parus major*) (Liukkonen et al. 2024), and in another study comparing the gut microbiome of multiple European and African birds (Schmiedová et al. 2023), yet both studies found seasonal differences in gut bacterial species richness and/or composition. Similar seasonal differences in microbiome composition were detected in a range of organisms from cnidarians to mammals (Carey et al. 2013; Li et al. 2022; Baldassarre et al. 2023). These observations suggest that microbiome compositions, rather than being solely determined by the neutral processes of bacterial dispersal between hosts (Sieber et al. 2019), are also shaped by environmental conditions.

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Several mechanisms have been suggested to explain how environmental factors, such as those observed during seasonal changes, alter microbiome composition. Temperature variation is the most prominent explanation. Temperature is a key regulator of physiological performance, affecting both host performance and microbial growth (Sepulveda and Moeller 2020). Variation in temperature is consistently associated with differences in microbiome composition, sometimes convergently in distantly related host taxa (Sepulveda and Moeller 2020). Animals exposed to distinct thermal conditions (e.g., winter vs. summer) might differ in microbiota composition because these thermal conditions are conducive to the growth of different microbial species (especially likely in ectothermic species that do not maintain a constant body temperature; Ferguson et al. 2018; Moeller et al. 2020) or exhibit seasonal changes in physiological state (e.g., due to hibernation or hypothermia; Carey et al. 2013; Lee et al. 2024).

Changes in diet across the seasons could also contribute to microbiome differences by altering the biochemical properties of food and thereby providing contrasting selection pressures on gut microbial communities (Wu et al. 2017; Baniel et al. 2021). For instance, in wild geladas, seasonal fluctuations in rainfall result in changes in the availability of cellulose vs. starch-based food, resulting in shifts in the diet between the rainy and dry seasons, with concomitant changes in gut microbiome composition. Similar diet-related shifts in gut microbiome communities have been inferred in other mammal and bird species (Góngora et al. 2021; Fan et al. 2022).

Finally, the environmental reservoir of microbes might change as a function of seasonal environmental differences, providing an altered source of microbial diversity that becomes encompassed into host-associated microbial communities. In this context, the altered host microbiome could reflect neutral processes of microbial dispersal, as described by neutral ecological models (Sloan et al. 2006; Venkataraman et al. 2015; Huang et al. 2021), whereby bacteria from environmental reservoirs stochastically colonize host-associated microbial communities without strong selection. An increasingly large number of studies suggest that these environmental microbes can contribute to the assembly of host-associated microbial communities (Adair and Douglas 2017; Mushegian et al. 2019; Bathia et al. 2024), especially in the case of skin/epithelial microbiomes that are directly exposed to the external environment and microbes residing within. One prominent example of environmentally-acquired microbial symbiont is the squid-*Vibrio* relationship, where bacteria are obligately acquired during early development from the environment (Nyholm et al. 2000). Bacteria colonizing hosts from environmental sources were detected in a range of different aquatic animal species (Mushegian et al. 2019; Chen et al. 2022; Bathia et al. 2024). However, whether their contribution is constant or varying in response to environmental conditions and whether these environmental bacteria can

explain patterns of seasonal variation in the composition of host-associated microbial communities is still unclear.

We investigated these questions employing the freshwater cnidarian *Hydra oligactis* (Pallas 1766) as a model host species. *Hydra oligactis* is a small, sedentary invertebrate (height of body column 10–30 mm excluding tentacles; Schuchert 2010) that is generally found attached to aquatic macrophytes, preying on zooplankton. It reproduces asexually through budding for much of the year and has a limited thermal tolerance, preferring colder water and experiencing stress at temperatures above 23–25°C (Tökölyi 2023). Due to its thermal preferences, the population size of *H. oligactis* in the littoral zone tends to be low during summer, while it increases substantially during winter (Bryden 1952).

Hydra form complex associations with microorganisms that mostly reside on the external epithelial surface of the animal, where they are directly exposed to the environment (Fraune et al. 2015). The composition and stability of these communities are of key importance for maintaining proper physiological function of the host as evidenced from the fact that experimental disruption of the microbiome alters development, movement, and reproduction (Rahat and Dimentman 1982; Murillo-Rincon et al. 2017; Taubenheim et al. 2020) and increases susceptibility of *Hydra* to pathogens (Fraune et al. 2015). To understand how microbiome composition changes seasonally, we sampled *Hydra* polyps and lake water from three distinct populations in Eastern Hungary four times a year over a 2-year period and compared between-season changes, as well as the similarity of polyp microbiome to water microbiome, which can act as a microbial reservoir for host colonization (Bathia et al. 2024). By comparing the *Hydra* microbiome with that of the surrounding lake water across seasons, we aim to uncover the extent to which environmental microbes drive seasonal shifts in host-associated microbial communities.

Materials and methods

Sample collection

Hydra polyps were collected on 9 distinct sampling events spanning a 2-year period starting from June 2021 to June 2023. Sampling campaigns were organized in both years once during the summer, autumn, winter, and spring. Three populations were followed: site M52 is a small shallow lake near Apagy (47.98556N, 21.94776E), with a surface area of 7 ha and an average depth of 1.7 m (henceforth called “Shallow lake”). Site M25 is a deeper lake near Debrecen (47.54955N, 21.45163E) with a surface area of 60 ha and an average depth of 4.5 m (henceforth called “Deep lake”). Site M26 is a canalized river near Hortobágy (47.58846N, 21.15045E) with an average depth of ~2 m (henceforth called “River”). All three water bodies can be classified as eutrophic to hypereutrophic. They are situated within agricultural landscapes, where runoff from surrounding fields may carry excess

nutrients and pesticides into the water. Water quality measurements were conducted at these sites in a previous study (Bathia et al. 2024) at three time points—July 2021, November 2021, and April 2022. The mean chlorophyll-*a* concentrations, used as a proxy for primary production, were $50.8 \mu\text{g L}^{-1}$ in the deep lake, $93.4 \mu\text{g L}^{-1}$ in the shallow lake, and $18.1 \mu\text{g L}^{-1}$ in the river, all indicative of eutrophic conditions (Bathia et al. 2024).

In all three populations, *Hydra* were collected from the littoral zone, from an approximate depth of 1–1.5 m. We collected animals from three distinct locations, at least 10 m from each other, within each population. From each distinct location, we collected three polyps, aiming at $n = 9$ polyps from a population at each specific sampling event. We placed pieces of aquatic vegetation into autoclaved plastic boxes to detect the presence of polyps and used sterile Pasteur pipettes to remove them from the vegetation. Subsequently, they were collected in sterile containers and brought to the laboratory in a cooled box within a few hours after collection. In the laboratory, polyps were washed in sterile-filtered water to remove debris attached to them and frozen overnight at -20°C before DNA extraction was carried out the next day.

At each location where *Hydra* were sampled, we also collected water samples in sterile bottles. In the laboratory, these water samples were filtered with $0.2 \mu\text{m}$ PES membranes (FilterBio®, Nantong, P. R. C.) on the day of collection. Between 100 and 200 mL water was filtered, due to the very large amount of suspended material in the water. Membranes were frozen overnight at -20°C and DNA was extracted the next day. Water samples were not collected on the first sampling (in June 2021) due to technical limitations.

Water temperature was measured on each sampling occasion approximately 30 cm below the surface with an ExTech TM20 temperature logger (Supporting Information Fig. S1).

DNA extraction and sequencing

DNA was extracted using a modified phenol–chloroform extraction protocol (Miklós et al. 2021). Subsequently, extracted DNA was purified with Vazyme VAHTS DNA Clean Beads (Nanjing, P. R. C.), following manufacturer's instructions. *Hydra* species identity was verified by running PCR reactions with three species-specific primer pairs (Bathia et al. 2024) on the extracted DNA. Samples were shipped to Novogene (Cambridge, UK) for 16S amplicon sequencing of the V4 region of the bacterial 16S gene.

We had a total of $n = 211$ polyp DNA samples on which 16S amplicon sequencing could be performed. The average number of samples per population per sampling event was 7.8 (range 2–9). A lower sample size was obtained in some cases due to our failure to find sufficient numbers of individuals in the field. Out of the $n = 81$ polyps we aimed to collect throughout the sampling period per population (9 sampling events, with 9 polyps per event), we were able to collect $n = 75$ polyps in the shallow lake, $n = 61$ from the deep lake

and $n = 74$ from the river. All polyps yielded high quality DNA on which sequencing could be performed.

Polyp DNA samples were matched with $n = 72$ water DNA samples (3 water samples per sampling event per population, with 8 sampling events in total). However, some of these samples were not of sufficient quality for DNA sequencing and could not be purified, making the final sample size lower. There were $n = 24$ successful water samples collected from the shallow lake, $n = 22$ from the deep lake, and $n = 15$ from the river, where none of the samples collected in September 2021, December 2021, and February 2023 were of sufficient quality.

Data analyses

Raw sequences underwent quality filtering (Phred scores > 20) and chimera removal. An average of 60,533 reads/sample remained after these filtering steps (minimum = 3059, maximum = 163,274, SD = 26,681). Operational Taxonomic Units (OTUs) were identified by the sequencing facility using Uparse v7.0.1001 (Edgar 2013) and the Silva 138.1 database (Quast et al. 2013), with a $\geq 97\%$ similarity threshold. The resulting OTU table contained 17,806 taxa. This OTU table was filtered as follows: chloroplast and mitochondria sequences were removed, OTUs were clustered at the genus level, then samples with ≤ 5000 reads were removed. Due to this last step, $n = 12$ samples with low read counts (4.2% of the total) were excluded, all of which were polyp samples. We filtered out bacterial genera with a prevalence $< 5\%$ across samples and abundance $< 0.05\%$ fraction of total abundance. Filtering and subsequent analyses were done in R v4.4.1 (R Core Team 2024), using the packages *phyloseq* v1.48.0, *metagMisc* v0.5.0, *microViz* v0.12.4 and *fantaxtic* v0.2.0 (McMurdie and Holmes 2013; Barnett et al. 2021; Teunisse 2022; Mikryukov 2023). The OTU table was also utilized for longitudinal analysis in QIIME2 (Bolyen et al. 2019). Samples were rarefied to a read depth of 5019 (the read count of the sample with the fewest reads after filtering) before performing subsequent analyses.

We calculated the Chao1 index to characterize the α diversity of samples using the *alpha* function in the *microbiome* R package v.1.26.0 (Lahti and Shetty 2019). Factors determining α diversity of *Hydra* samples were analyzed employing Linear Mixed-effects Models (LMMs). Temperature or season were included as predictors, and sampling site was included as a random factor to control for the fact that *Hydra* polyps collected from the same location are likely to be more similar to each other than expected by chance. We considered both temperature and season as predictors in our models, since these provide complementary information on environmental conditions: season captures the accumulated, longer-term environmental context, whereas temperature provides a more immediate, albeit temporary, snapshot. However, we did not include both temperature and season in the same models as the two variables were very strongly related to each other.

Instead, models were run either with temperature or season as predictors. LMMs were implemented in the *glmmTMB* v1.1.10 R package (Brooks et al. 2017). Water samples were analyzed in the same way, with the difference that random factors were not included in the models (because we had a single sample from each location). To estimate the strength of relationship between sampling season or temperature on one hand and microbiome α diversity on the other, we calculated marginal R^2 -values, as implemented in the *r.squaredGLMM* function in the *MuMIn* v1.48.4 R package (Bartoń 2024).

Core microbiome analysis was performed with the *plot_core* function in *microbiome* R package v.1.26.0 (Lahti and Shetty 2019) to identify bacterial genera that consistently occur in *Hydra* polyp samples in the three habitat types. Core taxa were defined as those present above a given detection threshold (relative abundance) in a specified percentage of samples (prevalence). We explored a range of detection thresholds (0.2–20% relative abundance) and prevalence levels (10–100% of samples). Core microbiome analysis was performed for water samples as well, but in this case, the detection threshold was increased to >0.6% due to the very large number of microbial genera with the lower threshold. We used higher detection thresholds (1% and 5% for polyp and water samples, respectively) for plotting temporal change in the top core microbiome components.

For β diversity, we calculated Bray–Curtis distance and used it for Principal Coordinates Analysis (PCoA), as implemented in the *phyloseq* package. Statistical difference of microbiome composition between season and sample types (polyp vs. water) was analyzed with PERMANOVA, employing the *adonis2* function in the *vegan* package v2.6-8 (Oksanen et al. 2022). For this analysis, sampling done in different years but in the same season was categorized in the same group. We analyzed the effect of temperature (a continuous variable) on β diversity distance with Distance-based Regression Analysis, as implemented in the *capscale* function from the *vegan* v2.6-8 R package (Oksanen et al. 2022). We extracted R^2 values to characterize the strength of the relationship between season or temperature on one hand and β diversity on the other.

For longitudinal analysis to determine the change in β diversity over time, we utilized the “qiime2-longitudinal” function and determined the changes for the Bray–Curtis dissimilarity index over sampling time for the first principal coordinates.

We used Mantel tests implemented in the *vegan* package v2.6-8 (Oksanen et al. 2022) to test the hypothesis that differences in polyp samples were correlated with differences in water samples by running Mantel tests with $n = 999$ permutations between the Bray–Curtis distance matrix of water samples on one hand and the Bray–Curtis distance matrix of polyp samples on the other hand. Because each water sample was associated with multiple polyp samples, we averaged the distance of polyp samples associated with a given water sample.

To estimate similarity of polyp and water microbiome composition, we calculated β diversity distance (Bray–Curtis index) of the microbial communities of polyps from their respective water samples. We used LMMs to analyze how sampling season or temperature affects the differentiation of polyp-associated microbiome communities from bacterioplankton composition. Sampling site was included as a random factor in the models.

Finally, to gain further insight into the role of temperature in the assembly of *Hydra* microbiome, we performed Sloan Neutral Model analysis (Sloan et al. 2006; Sieber et al. 2019) on samples collected at low or high temperatures. The Sloan Neutral Model can be considered a null model for microbiome diversity across hosts, assuming random population dynamics and dispersal as main drivers of community assembly. It investigates the relationship between the abundance and prevalence of bacterial taxa in a set of samples and predicts that, if the distribution of bacteria in a set of samples follows a neutral process, then the relationship between abundance and prevalence should follow a logistic curve. Taxa with high abundances are predicted to occur frequently across habitats or samples within a metacommunity due to their greater likelihood of dispersing and colonizing multiple patches, whereas low-abundance taxa are less likely to do so, and deviations from this pattern suggest that certain taxa possess traits under selection rather than following neutral expectations. These taxa are expected to have large residuals, being either over-represented (more prevalent than expected based on abundance) or under-represented (less prevalent than expected based on abundance). The Sloan Neutral Model was run in R by fitting a General Linear Model via non-linear least squares minimization between log10-transformed abundance values and prevalence, employing the *nls* R function. We used two kinds of abundance data as predictors: bacterial abundance on polyp samples (reflecting inter-host dispersal) and bacterial abundance in water samples (reflecting colonization from environmental sources). To see how temperature affects the fit of microbial distributions to these neutral models, the analysis was repeated for two distinct datasets: samples collected at low temperature (< 5°C, the lower quartile of temperature values recorded in this study) and high temperature (> 25°C, the upper quartile of temperature values recorded). To estimate the fit of bacterial prevalence and abundance data to a neutral model of distribution, we calculated determination coefficients based on the formula presented in Sieber et al. (2019). The model was run on samples from all three populations combined due to the relatively low number of samples in each population.

Results

The core microbiome of *Hydra oligactis*

Water and polyp microbiomes were significantly differentiated from each other in all three habitat types in terms of both α and β diversity (Supporting Information Table S1).

Core microbiome analysis, considering both abundance and prevalence, identified the following taxa that were consistently found in *Hydra* polyp samples in all three habitats: *Acidovorax*, *Polynucleobacter*, *Rhodoferrax*, Unknown Comamonadaceae, Unknown Oxalobacteraceae, Unknown Pirellulaceae, Unknown Rhodobacteraceae (Supporting Information Figs. S2a, S3).

In water samples, the core microbiome analysis identified an unknown Sporichthyaceae genus, an unknown Comamonadaceae genus, hgcI_clade, Cyanobium_PCC-6307, LD29, *Polynucleobacter*, CL500-29_marine_group, *Flavobacterium*, *Rhodoferrax*, and an unknown Pirellulaceae. From these, Cyanobium_PCC-6307, *Flavobacterium*, LD29, and unknown Pirellulaceae were only identified in some of the populations (Supporting Information Figs. S2b, S4).

Rhodoferrax, *Acidovorax*, *Polynucleobacter*, Unknown Oxalobacteraceae, and Unknown Comamonadaceae were taxa identified to be part of the core microbiome of both *Hydra* polyps and water samples in all three populations. In addition, *Flavobacterium* was part of the core microbiome of both polyp and water samples in all three populations except the shallow lake bacterioplankton (Supporting Information Figs. S3, S4).

The abundance of top bacteria in the water samples was relatively similar within and between sampling events (although their abundance fluctuated from one sampling to the next), while polyp samples showed substantial, apparently stochastic variation between sampling events (Fig. 1b; Supporting Information Fig. S2).

Seasonality has a stronger effect on water than polyp microbiome α diversity

We found a significant seasonal difference in α diversity of *Hydra* microbiomes in the shallow lake, but not in the deep lake or the river (Table 1). By contrast, bacterioplankton α diversity showed significant seasonal differences in all three habitat types, generally with substantially larger marginal R^2 values than in the case of polyp microbiomes (Table 1). In water samples, α diversity was lower in the winter than in other seasons (Fig. 1a). The relationship between microbiome α diversity and water temperature showed similar patterns, though temperature seemed to explain a lower proportion of variation in α diversity than seasonality (e.g., 0.405 vs. 0.066 in the shallow lake), likely because of apparent non-linear effects of temperature on α diversity of *Hydra* microbiomes (increased α diversity observed both in summer and winter compared to other seasons; Fig. 1a).

Hydra and water microbiome compositions change seasonally

Season and temperature both significantly predicted variation in β diversity of *Hydra* and water samples in all three populations (Table 1; Fig. 2a). Bacterioplankton composition changed more strongly with season and temperature than *Hydra*-associated bacterial communities, as judged by the substantially larger R^2 values (Table 1).

This variation in β diversity was also visible on the longitudinal analysis performed along the sampling time points (Fig. 2b). While the shallow lake water showed seasonal variation, for the other 2 habitats, there was little or no seasonal variation visible for water samples from other two sites along the primary axis (Axis-1) for Bray–Curtis dissimilarity. For these two habitats, the polyp samples had an inconsistent pattern, but overall, they showed seasonal variation.

Environmental and *Hydra*-associated microbiome communities are more similar during the summer

Overall, α diversity of *Hydra* microbiomes correlated with α diversity of bacterioplankton in the deep lake ($R^2 = 0.117$, $p = 0.012$); but not in the other two populations.

In terms of β diversity, differences in microbiome composition between polyp and water samples across different sampling sites were significantly or marginally significantly correlated with each other (Mantel-test, Bray–Curtis index, Shallow lake: $r = 0.555$, $p = 0.001$; Deep lake: $r = 0.151$, $p = 0.080$; River: $r = 0.444$, $p = 0.002$), implying that more differentiated bacterioplankton communities were associated with more differentiated *Hydra* microbiomes.

To see whether this correlation between the microbiome composition of polyp and water samples is constant or changing with time, we compared Bray–Curtis dissimilarity of polyp samples from their respective water samples with respect to season or as a function of temperature. Sampling season and temperature were significantly associated with the distance of polyp samples from their matching water samples in the shallow lake environment, but not the deep lake or river habitats (Table 2). Polyp microbiomes in the shallow lake environment were more similar to surrounding bacterioplankton communities during the summer (Fig. 2c).

Neutrality at low and high temperature

The fit of *Hydra*-associated bacterial prevalence and abundance data to a logistic curve, as predicted by the neutral model of dispersal between hosts, showed an overall R^2 value of 0.63 (all polyp samples included). Conversely, the neutral model of dispersal from water to polyp samples had an R^2 value of 0.29, indicating that environmental bacterial sources were less important than transmission between hosts. In both inter-host dispersal (*Hydra* microbiome abundances as predictor) and environmental colonization (water microbiome abundances as predictor), R^2 was higher at low temperature ($\leq 5^\circ\text{C}$, $R^2 = 0.67$ and 0.31 , respectively) than at high temperature ($\geq 25^\circ\text{C}$, $R^2 = 0.59$ and 0.24 , respectively), indicating a reduced conformity to a neutral model at high temperature (Fig. 3).

Discussion

In this study, we investigated seasonal dynamics of microbial communities associated with *Hydra* polyps in three populations and compared microbiome composition of polyps

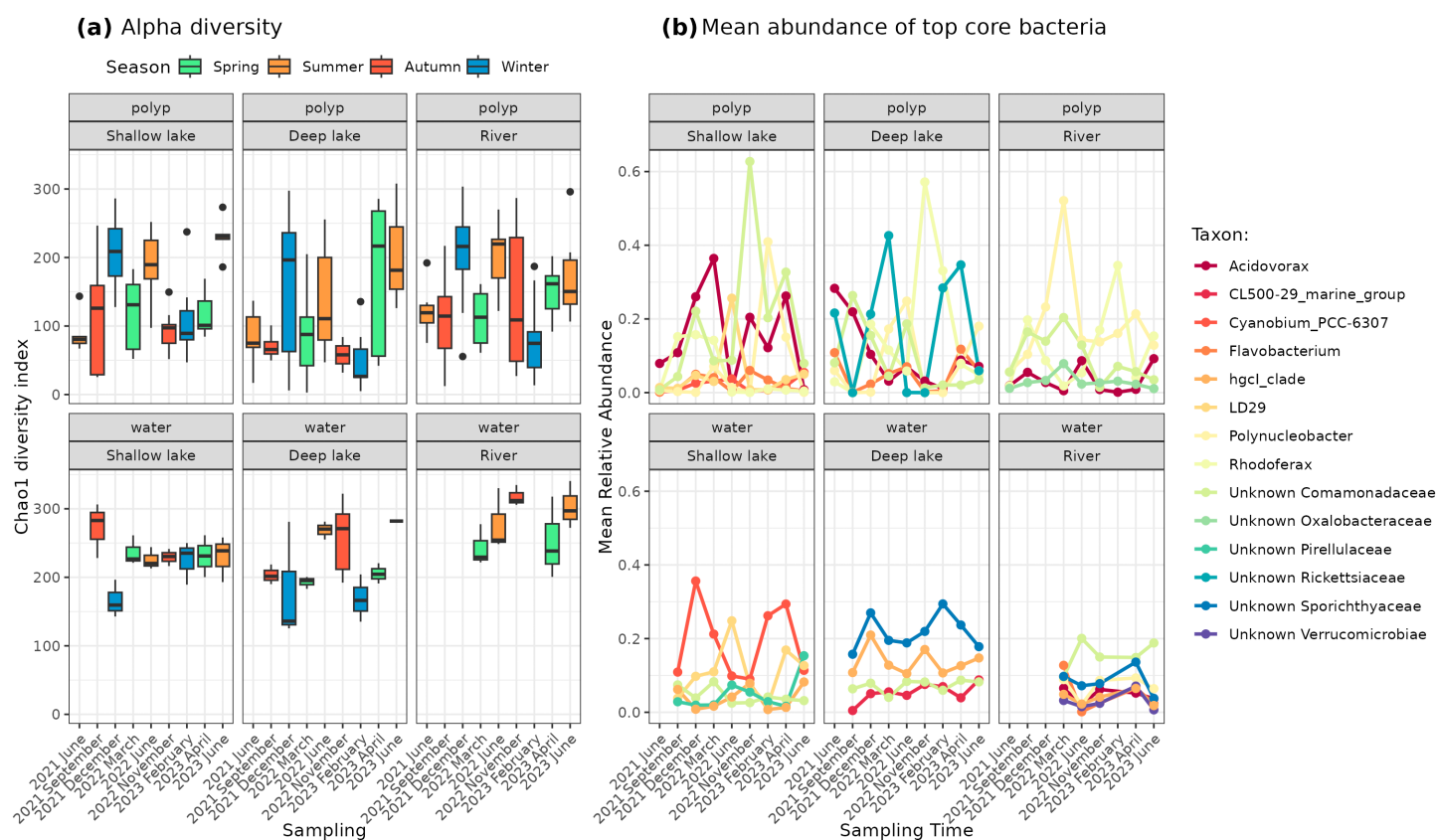


Fig. 1. Seasonal patterns of microbiome diversity in the freshwater cnidarian *Hydra oligactis* and bacterioplankton in three distinct Hungarian populations. Panel (a) shows α diversity, as measured by the Chao1 index in polyp and water samples across nine sampling events spanning a 2-year period. Panel (b) shows changes in mean abundance of core microbiome members in *Hydra*-associated bacterial communities and bacterioplankton across sampling events. The core microbiome components shown were identified with a prevalence threshold of 25% and abundance threshold of 1% in *Hydra* samples and 5% in water samples (a higher threshold was used in water samples because of the very large number of core taxa with a threshold of 1%; that is, in this case only the top core taxa are shown).

Table 1. Marginal R^2 -values and p -values (in parentheses) showing the effects of sampling season or temperature on variation in α diversity (Chao1 index) and β diversity (Bray–Curtis index) of bacterial communities associated with hydra polyps or water samples. In case of α diversity, results are from single-predictor Gaussian LMMs (hydra microbiome, with collection site as a random factor) or LMs (water microbiome). In case of β diversity, results are from PERMANOVA analysis (for season) or from Distance-based Regression analysis (for temperature).

		α diversity		β diversity	
		Season	Temperature	Season	Temperature
Shallow lake	Hydra	0.405 (0.001)	0.066 (0.152)	0.206 (0.001)	0.127 (0.001)
	Water	0.542 (< 0.001)	0.173 (0.036)	0.648 (0.001)	0.368 (0.001)
Deep lake	Hydra	0.064 (0.341)	0.024 (0.267)	0.151 (0.001)	0.068 (0.002)
	Water	0.402 (0.025)	0.443 (0.001)	0.386 (0.001)	0.224 (0.001)
River	Hydra	0.106 (0.239)	0.144 (0.047)	0.107 (0.001)	0.039 (0.005)
	Water	0.388 (0.031)	0.001 (0.889)	0.657 (0.001)	0.492 (0.001)

Statistically significant relationships are highlighted in bold.

to bacterial diversity of the surrounding environment. We found that the *Hydra* microbiome changed seasonally, albeit to a different degree in the three populations. The relative abundance of core microbiome components appeared to

change stochastically through time. During the summer, the *Hydra* microbiome of the shallow lake, but not the deep lake or the river, changed more substantially and became similar to the microbial communities of the surrounding environment.

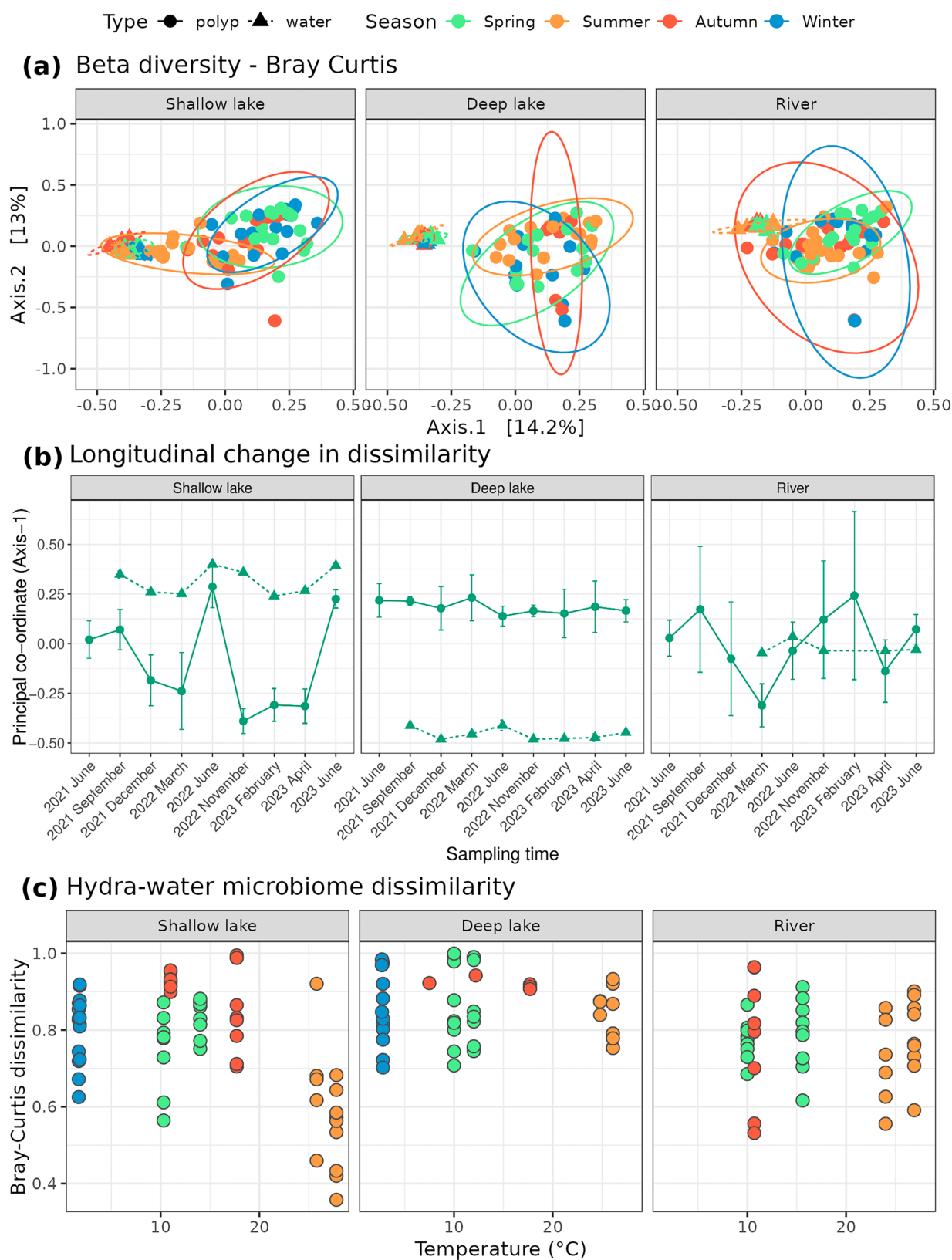
**Fig. 2.** Legend on next page.

Table 2. Effect of season or temperature on Bray–Curtis dissimilarity of the microbiomes of polyps and their matching water samples (Linear Mixed-effects Models).

	Season		Temperature	
	Marginal R^2	p -value	Marginal R^2	p -value
Shallow lake	0.501	< 0.001	0.238	0.005
Deep lake	0.090	0.349	0.041	0.234
River	0.020	0.792	0.006	0.705

Statistically significant relationships are highlighted in bold.

The *Hydra* microbiome is seasonally variable

We found significant differences in the *Hydra* microbiome both in overall diversity and in composition. In all three populations, the highest α diversity was found in the summers of 2022 and 2023. Interestingly, we also found high diversity during December 2021 (though this was not repeated in the winter of 2022/23). These results suggest the presence of fluctuations in microbiome diversity, potentially due to climatic factors (e.g., temperature), although other factors, such as seasonal changes in the surrounding nutrient input could have been involved as well. Similar seasonal differences in microbiome α diversity have been detected in both ectothermic and endothermic animals (Sharp et al. 2017; Xiao et al. 2019; Hernández et al. 2024), though not all long-term studies detected seasonality in diversity (e.g., Epstein et al. 2019; Lamb and Watts 2023).

While diversity varied between sampling events, the patterns detected by us were not discernible enough to clearly identify their cause. Temperature differences could provide a straightforward explanation (Sepulveda and Moeller 2020) and our analysis provided some support for the effect of temperature on microbiome diversity, especially on β diversity of the water microbiome. This analysis showed that as temperature difference increased, both polyp and water microbiome composition changed more strongly. By contrast, α diversity showed only weak correlation with temperature, which depended on the sampling site. Therefore, even if temperature was the reason for differences in bacterial diversity, the relationship between temperature and α diversity is likely non-linear, as both summer and winter samples had high diversity. Such non-linear responses might exist, as a recent meta-analysis found that both increases and decreases in temperature can be associated with altered microbiome diversity and composition (Li et al. 2023). More studies are needed under experimentally controlled temperature to explain seasonality in the microbiome diversity of *Hydra*.

The relative abundance of *Hydra* core microbiome components changes through time

Among the most abundant genera found in the polyp samples were *Polynucleobacter*, *Acidovorax*, *Rhodferax*, and *Flavobacterium*, taxa that are commonly found in association with *Hydra* under both field and laboratory conditions (Fraune and Bosch 2007; Taubenheim et al. 2022; Lachnit et al. 2025). Interestingly, these taxa did not show a constant abundance through time, but fluctuated considerably between sampling events. For instance, in the shallow lake, polyp microbiomes were dominated by *Rhodferax*, *Polynucleobacter*, or an unknown Comamonadaceae, and all three could be dominant taxa in subsequent sampling events. Such stochastic changes could happen in the microbial community itself, for example, due to the random increase or decrease, or dispersal of specific taxa. However, they could also happen due to the random increase or decrease of the polyp lineages harboring these communities. *Hydra* polyps reproduce asexually throughout much of the year via budding, and if asexual offspring inherit the microbiome of their parents, then the expansion or contraction of clonal lineages can lead to the concomitant change of the microbial populations, an assumption that needs to be tested in the future. Nonetheless, our observations align with other seasonal studies that inferred a mix of stochastic and deterministic processes in animal microbiome assembly and maintenance (Xu et al. 2022, 2024). The functional consequences of variation in microbiome composition on the *Hydra* host are still to be deciphered, but in other cnidarians, like sea anemones, bacterial plasticity in response to temperature changes contributes to stress resistance of the organism (Baldassarre et al. 2022).

The contribution of aquatic bacteria increases during the summer

Comparing the similarity of *Hydra* polyps to their respective water samples, we found that their differences were not constant in time. On some sampling occasions, polyps were much more similar to their surrounding aquatic environments than on others. This effect was most discernible in the shallow lake habitat, where polyps collected during the summer showed high similarity to water samples. Investigation of the taxonomy plots showed that these summer samples had different microbiome compositions than samples collected immediately before or after the summer, with a higher diversity of bacterial taxa, a lower abundance of core *Hydra* microbiome components, and the presence of non-core taxa. One of the prominent non-core taxa was an LD29 bacterium. LD29

Fig. 2. Similarity of the microbiome composition of *Hydra* and water samples. Panel (a) shows the first two axes of a Principal Coordinates Analysis (PCoA) based on the Bray–Curtis dissimilarity, with 95% confidence ellipses. Panel (b) shows beta-volatility plots based on Bray–Curtis dissimilarity matrix. The plots represent the mean position of the polyp or water samples along the first principal coordinate axis for all the sampling time points for three sites. (c) Seasonal change in the pairwise dissimilarity of the microbiome of polyps and their respective water samples, as measured by the Bray–Curtis index.

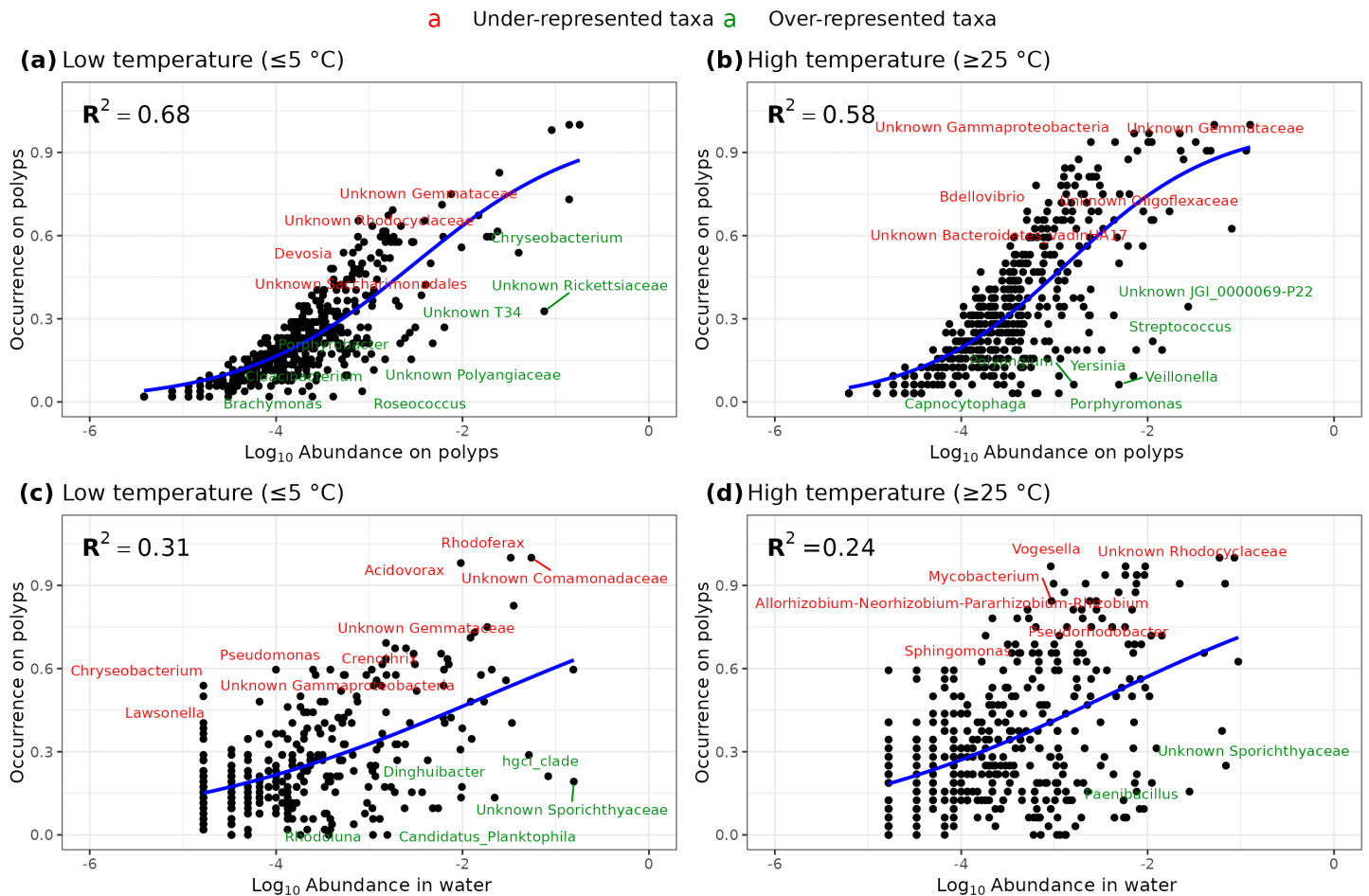


Fig. 3. Sloan neutral models showing the fit of bacterial prevalence to a neutral model of distribution where prevalence (occurrence on polyps) is determined by abundance in *Hydra* samples (**a**, **b**) or in bacterioplankton (**c**, **d**). The model was calculated for low temperature ($\leq 5^{\circ}\text{C}$) and high temperature ($\geq 25^{\circ}\text{C}$). Bacterial genera with residuals outside the 95% confidence intervals are highlighted with a color; taxa with positive residuals are considered over-represented (more prevalent than expected based on abundance), while those with negative residuals are considered under-represented (less prevalent than expected based on abundance).

in this lake was among the top bacterioplankton taxa, and their increased appearance on the polyps suggests that aquatic bacteria colonized the *Hydra*.

Several factors might explain the accumulation of aquatic bacteria on *Hydra* polyps. First, the availability of bacterial taxa having the ability to colonize *Hydra* polyps might increase seasonally. Indeed, the abundance of LD29, which increased on polyps during summer 2022 in the shallow lake, also seemed to have a higher abundance in the water samples at the same time. LD29 is a clade of bacteria belonging to Verrucomicrobia that was initially discovered in a shallow, eutrophic lake in the Netherlands (Zwart et al. 1998). While not much is known about their seasonal dynamics, a genome assembly from metagenome sequences of *Spartobacteria baltica* belonging to the LD29 lineage suggests they might be involved in the degradation of polysaccharides from Cyanobacteria (Herlemann et al. 2013). Hence, the summer proliferation of phytoplankton (Padisák 1992) and the release of organic matter

originating from their death might result in the build-up of heterotrophic bacteria that could also survive and proliferate on the surface of *Hydra* polyps.

While shifts in the composition of environmental microbiota—serving as a source pool for colonizing taxa—could contribute to the observed patterns, it is unlikely that these reflect a purely neutral accumulation of aquatic bacteria on *Hydra* hosts. If environmental changes alone were responsible, we would expect the fit to a neutral model to remain consistent across temperatures. However, this was not the case. Instead, we found that environmental bacterial abundances became less predictive of *Hydra*-associated bacterial prevalences at higher temperatures. This decoupling indicates that changes in environmental bacterial abundance are not directly mirrored in host-associated communities, suggesting that additional, deterministic processes influence host–environment microbiome similarity. Such processes may include temperature-dependent shifts in *Hydra*'s core microbiome components allowing opportunistic or stress-tolerant taxa to

dominate, alterations in host immune function and hence filtering strength of the host favoring specific taxa, temperature-driven changes in microbe–microbe interactions, or differences in the colonization success of bacterioplankton taxa under varying thermal conditions. Further experimental work needs to be done to discern these mechanistic hypotheses.

While the above pattern was clearly visible in the shallow lake, the other populations did not show the same pattern. In particular, we did not detect a substantially increased similarity of polyp and water samples during the summer in these two populations. This lack of difference could be explained by technical issues: especially in the river population, water samples from three sampling events could not be sequenced, preventing us from clearly testing the seasonal difference. However, it could also be an ecological difference: both the deep lake and river populations are likely to represent more diverse ecosystems that are less exposed to effects of summer heat than the shallow lake habitat, potentially resulting in more buffered and stable water temperatures. Although polyps were collected from the same shoreline zone, assumed to experience uniform warming, we cannot rule out the possibility that some originated from deeper water. However, since our sampling was not replicated at the habitat level, we cannot clearly tell whether the differences we observed are due to habitat differences or some other factor.

Overall, our results suggest that the contribution of environmental bacteria to the *Hydra* microbiome varies throughout the year. During the summer, *Hydra* polyps in some populations experience an increase in the diversity of their bacterial communities due to apparent colonization by aquatic bacteria. A key question in this area is the role of temperature in mediating the colonization of hosts by environmental bacteria. If high water temperature is causing increased similarity of animal-associated and environmental microbiomes, then global warming due to recent climate change might result in the accumulation of aquatic bacteria in the *Hydra* microbiome. The functional effects of these aquatic bacteria on the animal host are currently unclear. Nonetheless, our results suggest an underappreciated, intricate relationship between animals, their microbial communities and microorganisms of the surrounding environment. Further experimental work is required to understand variation among habitats and the causative role of different abiotic conditions on this tripartite interaction.

Author Contributions

Jácint Tökölyi: Conceptualization, methodology, formal analysis, visualization, writing – original draft preparation, writing – review and editing. **Máté Miklós:** Conceptualization, methodology, writing – review and editing. **Jay Bathia:** Methodology, formal analysis, visualization, writing – review and editing. **Sebastian Fraune:** Methodology, formal analysis, visualization, writing – review and editing.

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Conflicts of Interest

None declared.

Data Availability Statement

Raw sequence reads are available on the NCBI Short Read Archive, BioProject PRJNA1119407 (<https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1119407>). R code to analyze data is available on GitHub (<https://github.com/jtokolyi/HydraMicrobiomeSeasonal>).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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