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Jellyfish blooms through the microbial lens: temporal changes, cross-species and Jellyfish-water comparisons

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Abstract

Jellyfish blooms have significant ecological and economic impacts, yet the microbial communities associated with these blooms remain poorly understood, despite their potential influence on host fitness and microbial communities in the surrounding water. In this study, we explored temporal and tissue-specific variations in the microbiota of *Rhopilema nomadica*, the dominant jellyfish species in the Eastern Mediterranean Sea, across winter and summer blooms. During late summer blooms, microbial richness declined, coinciding with an increase in *Endozoicomonas* and unclassified *Rickettsiales*, while *Tenacibaculum* predominantly characterized winter blooms. Tissue-specific analyses revealed bacterial groups that were more consistently associated with different jellyfish tissues (e.g., *Bacteroides* in the bell and *Simkaniaceae* in the gonads), suggesting different microbial niches within the host. Furthermore, some key bacteria associated with *R. nomadica*, including *Endozoicomonas*, unclassified *Rickettsiales*, and *Bacteroides* were detected in the surrounding bloom water but absent from remote seawater, suggesting potential localized transmission dynamics between jellyfish and their immediate marine environment. Finally, a comparative analysis with nine additional jellyfish species identified recurring microbial taxa, including *Endozoicomonas*, *Mycoplasma*, and *Spiroplasma*, though no universal core microbiota was observed. This study represents the first exploration of microbial dynamics within *R. nomadica* blooms and the most comprehensive analysis of jellyfish-associated microbiomes across bloom stages and tissues to date. Our findings reveal complex relationships between jellyfish species, bloom progression, their microbial communities, and the surrounding seawater.

Keywords *Rhopilema nomadica*, 16S analysis, Microbiome, Cnidaria, Bloom, Jellyfish

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Background

Jellyfish blooms, particularly those caused by species within the Phylum Cnidaria, specifically the Class Scyphozoa, are a widespread and dynamic global phenomenon with broad ecological and economic implications. While other jellyfish classes can also form blooms [1], this study primarily focuses on Scyphozoa blooms. The effects of such blooms include damage to tourism, clogging of water intake of power stations and desalination plants, the disruption of local fisheries and, potentially, the alteration of marine food webs [2, 3, 4, 5]. These usually ephemeral jellyfish blooms, characterized by significant, often seasonal increases in jellyfish populations, are caused by a complex interplay of environmental factors and human activities [3, 6, 7, 8]. Typically, these blooms are more common during mid-spring until mid-autumn [1, 7] and decline rapidly, leading at times to "jelly-falls", where large amounts of biomass are exported to the seafloor [9, 10, 11]. Despite their frequent occurrence and substantial ecological and societal impact, the reasons why blooms appear and decline rapidly remain elusive.

One potential factor in jellyfish blooms is the interplay between jellyfish and their associated bacteria, viewing the bloom-forming jellyfish as a holobiont—a symbiotic unit of the jellyfish and its bacteria, or microbiome. This symbiosis can be crucial for the emergence of blooms, as evidenced by recent studies showing that bacteria are involved in various stages of early jellyfish development, such as larva settlement and the initiation of the strobilation process [12, 13, 14]. As the jellyfish mature, these bacteria are hypothesized to play essential roles in nutrient acquisition, digestion, and immune response support [15, 16]. However, the disappearance of jellyfish blooms could be triggered by massive mortality events, potentially due to physiological changes associated with natural aging processes [1] or the introduction of harmful pathogens. Such events likely cause significant shifts in the microbial community associated with the jellyfish. These bacteria may be specifically adapted to different parts of the jellyfish or various stages of its life cycle [16], each serving specialized roles. Alternatively, some may be opportunistic, thriving in the jellyfish environment without a definitive symbiotic relationship. These complex interactions highlight the need to closely examine the jellyfish microbiome throughout different stages of blooms to discern the microbial compositions that might influence the onset and decline of these events.

In the Eastern Mediterranean Sea, *R. nomadica* stands out as the predominant jellyfish species, known for forming significant blooms. First documented in the Mediterranean in 1977, this species showed a notable population increase during the 1980s. It was initially observed off the coast of Israel, then spread along the Eastern Mediterranean coastline, reaching as far west as Sardinia and Sicily [8, 17, 18, 19, 20, 21]. The blooms appear predominantly during the summer months, characterized by a defined progression from peak to decline stages. Secondary blooms are observed in winter but tend to be less predictable and lack clearly delineated stages [8, 17]. Here, we asked whether the *R. nomadica* microbiome differs between the winter and summer blooms, and whether it changes across different summer bloom stages — specifically, between the early peak bloom in June and the late bloom stages in July, after which large jellyfish aggregations are rarely observed until the next winter bloom.

To answer this question, we considered factors influencing microbiome structure, including jellyfish size, sex, tissue type, and overall health. We investigated whether different jellyfish species, despite their unique characteristics, host common bacteria with identical 16S rRNA sequences, potentially revealing core microbiome members. Additionally, we explored whether jellyfish-associated bacteria are present in surrounding bloom water and in a time-series dataset from a jellyfish-free reference site (SoMMoS cruise series [22], providing clues about bacterial origins and transmission. Specifically, we examined whether the jellyfish microbiome composition is shaped by horizontal acquisition from the environment, by potential host filtering effects, or by a combination of both processes.

Methods

Sample collection and DNA extraction

A total of 32 *R. nomadica* jellyfish were collected during different bloom events in and around Haifa Bay, Israel, with 9–13 individuals sampled from each bloom stage: winter bloom, summer peak, and late-stage bloom (Fig. 1). Winter blooms were sampled in two consecutive years (February 2020 and 2021), while the summer bloom was sampled twice during the peak (mid-June 2020) and once toward the end of the bloom (mid-July 2020). We note that jellyfish are present almost year-round along the Israeli coast but are rare and hard to sample outside of the major bloom events. We also note that the jellyfish distribution along the coast is patchy, and the samples collected at different time points likely do not represent the same physically aggregated population.

Each jellyfish was carefully collected into an 80 L container filled with ambient seawater [23]. The jellyfish were dissected on shore immediately following each research cruise (typically at most 2 h after collection). To mitigate the risk of cross-contamination, a separate sterile toolkit was utilized for each jellyfish, with rigorous cleaning procedures implemented between the handling of different tissues. These included cleaning the tools with 1% sodium hypochlorite, DNA AWAY™ (Thermo Fisher Scientific), 70% ethanol, and ultrapure water after each use.

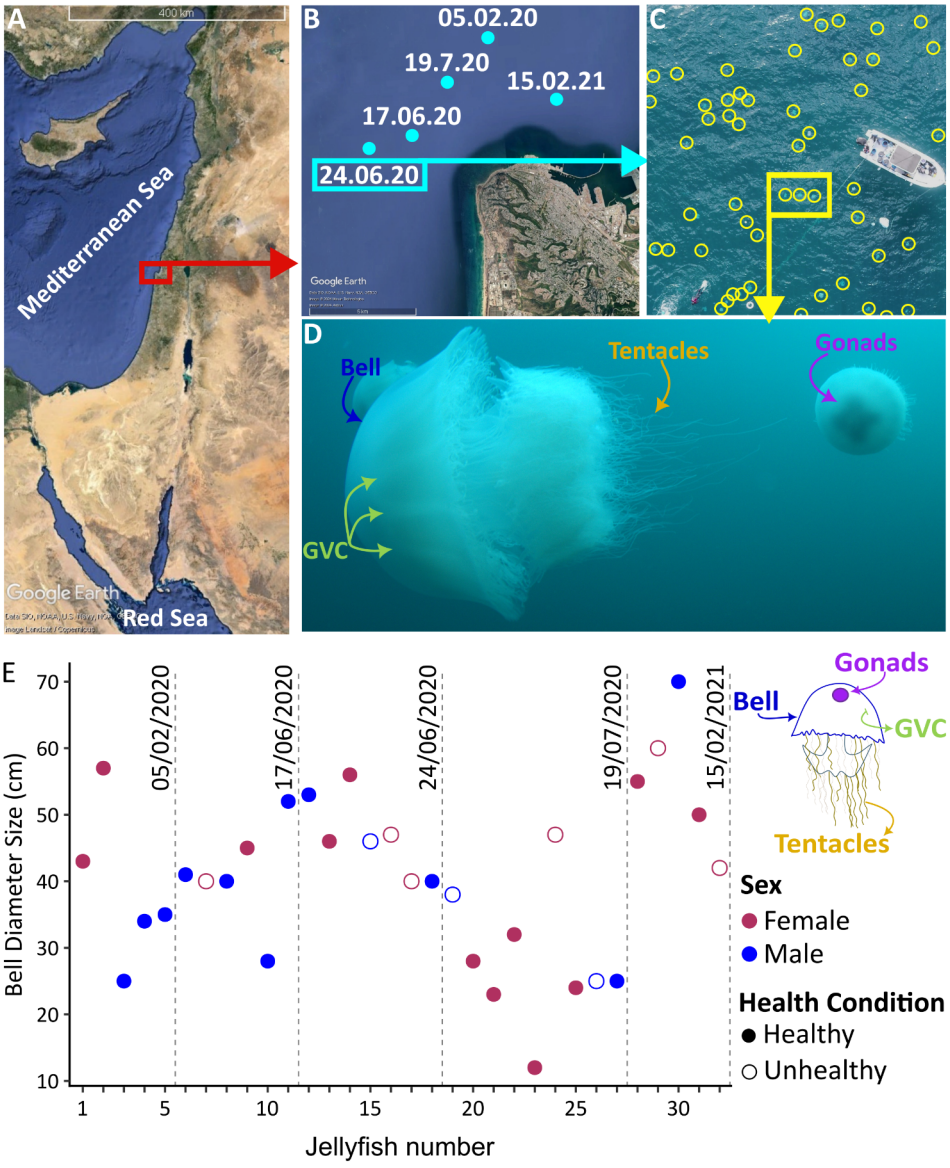

Fig. 1 Jellyfish Sample Collection Overview (**A**, **B**) Maps of the sampling locations and the dates of sampling. (**C**) A photograph taken from a drone deployed during the jellyfish sampling on 24.6.2020 [25], demonstrating the density of the jellyfish bloom. Individual jellyfish are marked by yellow circles. (**D**) Underwater bloom perspective of a jellyfish bloom. Circles indicate the different tissues sampled from the jellyfish. Photo by Hagai Nativ. (**E**) Collection data graph displays size, sex, health condition, and collection date for the 32 jellyfish. When jellyfish were identified as "unhealthy", e.g. exhibited visible lesions on the bell, samples were collected from both visibly unhealthy bell tissue and healthy tissue from the same individual

For each jellyfish, we collected three technical replicates of four distinct tissues; bell, gonads, tentacles, and gastrovascular canals (GVC), into sterile 0.5 ml Eppendorf tubes and immediately froze them on dry ice. For microbiome analyses, one replicate per tissue was selected for each individual. Additionally, jellyfish exhibiting visible signs of damage (e.g., lesions, holes, cracked bells, or abnormal morphology) were sampled. For these individuals, tissues were collected from both damaged areas and visually healthy regions to compare microbial communities between them. The diameter of each jellyfish was recorded, unique characteristics were noted, and gonad tissue was collected for sex determination in the lab. Additionally, at each location, three replicates of five liters of seawater were filtered through a Sterivex filter cartridge (0.22 µm), 1 ml of preservation/lysis solution was added (40mM EDTA, 50mM Tris pH 8.3, 0.75 M Sucrose), and the filters were frozen on dry ice. Upon arrival at the lab, the samples were transferred to a ⁻80 °C freezer for long-term storage until further analysis.

Samples from the bell, gonads, tentacles and GVC were thawed, homogenized using a bead beater (TissueLyser II, Qiagen), treated with 100 mg/ml lysozyme at 37°C (Merck) for one hour, and then incubated for

an additional hour at 55°C after adding 20 mg/ml proteinase K (Promega). DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research), according to the manufacturer's protocol. The Sterivex filter (Millipore) was processed as described previously [24], with the filter being placed in the ZR Bashing-Bead™ lysis tubes from the ZymoBIOMICS DNA Miniprep Kit. The subsequent DNA extraction was carried out as described for the tissue samples. For standardization purposes, a commercially available mock microbial community standard (ZymoBIOMICS™, Zymo Research) was utilized. The mock community DNA was extracted using 75 µl per preparation, following the manufacturer's recommendations and employing the same protocol as for the other samples. The mock community served as a quality control to evaluate sequencing accuracy and detect potential contamination. Negative controls for PCR and DNA extraction were also analysed, confirming the absence of significant contamination.

16S rRNA gene amplification and sequencing

The V3V4 hypervariable region of the bacterial 16S rRNA gene was amplified using a two-stage polymerase chain reaction (PCR) protocol, using the 341 F and 806R primers [26] and a protocol described previously [23]. The final PCR libraries were pooled and sequenced using a 15% phiX spike-in on an Illumina MiSeq sequencer with V3 chemistry for 2 × 300 base paired-end reads. This sequencing was performed at the Genomics and Microbiome Core Facility (GMCF; Rush University, IL, USA). The raw sequences were deposited in NCBI PRJNA1107792.

Raw data processing

Raw paired-end 16S rRNA gene sequences were processed using the DADA2 [27] pipeline (v1.20) in R (v4.1.0) with customized parameters. Quality control was first assessed using FastQC (v0.11.9), followed by trimming of forward and reverse reads to 260 bp and 220 bp, respectively. Reads were filtered based on quality scores, maximum expected errors (maxEE), and the removal of ambiguous bases. Error rates were learned separately for forward and reverse reads, which were then denoised and dereplicated. Paired-end reads were merged with a minimum overlap of 12 bp, and chimeric sequences were removed. An amplicon sequence variant (ASV) table was constructed, retaining sequences between 400 and 430 bp and excluding singletons. Taxonomy was assigned using the SILVA v138.1 database [28] and the naïve Bayesian classifier. ASVs unclassified at the phylum level or not assigned to bacteria were excluded from downstream analyses.

Data and statistical analysis

Data management and organization were conducted using the tidyverse v2.0.0 package [29], and results were plotted using ggplot2 v3.4.2 [30]. α and β-diversity were calculated using vegan v2.6.4 [31], mia v1.6.0 [32], and phyloseq v1.40.2 [33]. We used the Bray-Curtis index to generate a dissimilarity matrix by rarefying the samples to 14,500 reads across 1,000 iterations using the avgdist function from the vegan package. The rarefaction depth was chosen based on rarefaction curve analysis (Fig. S1). The dissimilarity matrix was then applied to Non-metric Multidimensional Scaling (NMDS) and Variation Partitioning Analysis (VPA). VPA was used to assess the contribution of bloom and jellyfish-related factors to variation between the samples. For α-diversity indices, we applied the estimateRichness function from the mia package on the rarefied dataset. To test for differences in microbial community composition among groups identified in the NMDS, we performed two-way PERMANOVA. To compare mean Bray-Curtis dissimilarities and α-diversity indices across multiple factors, we used ARTool (Aligned Rank Transform), a non-parametric method that enables ANOVA-like tests on aligned and ranked data [34, 35]. To identify significant taxonomic variations in relative abundance across different tissues and bloom stages, we analysed ASVs using two approaches. For the bar plot visualization, we included ASVs with a mean relative abundance exceeding 1% across months and tissue types. For the second analysis, which aimed to detect significant variations, we focused on taxa with a median relative abundance above 1% in the monthly analysis and 3% in the tissue-based analysis. The elevated threshold for tissues was set to minimize the influence of bell-only taxa, as this tissue type was significantly richer than the others. Taxa were further analysed only if they had a p-value below 0.05 in both the Kruskal-Wallis test and the subsequent Wilcoxon test. The host phylogenetic tree was constructed using 18S rRNA gene sequences from each jellyfish species. The tree was built in MEGA11 using the Maximum Likelihood method with the General Time Reversible (GTR) model plus Invariant Sites (GTR + I) for nucleotide substitution. Bootstrap support was estimated with 1000 replications to assess node reliability.

R. nomadica vs. baseline water comparison

We compared our *R. nomadica* jellyfish microbiome data with microbial community data from the Southeastern Mediterranean Monthly Cruise Series (SoMMoS) [22]. The SoMMoS dataset includes seawater samples collected between February 2018 and January 2019 from two offshore sites: one located at the edge of the continental shelf (125 m, 10 km from the coast) and another in open water (1500 m, 50 km from the coast). These sites

are approximately 22 and 55 Km from the site where the jellyfish were sampled, and are in deeper water. Therefore, they should not be interpreted as representatives of the coastal water where the jellyfish were collected, but as waters potentially connected to those where the jellyfish are found through the regional currents and mesoscale structures [36, 37]. Additionally, in contrast to the water collected from inside the jellyfish blooms, in the SoMMoS cruises, two size fractions were collected and analyzed separately (> 11 µm and 5–0.22 µm), precluding a direct comparison [38]. Therefore, we focus here only on ASVs whose sequences are identical to those from jellyfish, to examine potential models of microbial community transmission [40].

Jellyfish species comparison

In addition to our primary *R. nomadica* dataset, we analyzed microbiome data from nine other jellyfish species: *Rhopilema esculentum*, *Nemopilema nomurai*, *Rhizostoma pulmo*, *Cassiopea xamachana*, *Mastigias papua*, *Aurelia coerulea*, *Cyanea nozakii* (all belong to Scyphozoa) *and Tripedalia cystophora* (Cubozoa) [39, 40, 41, 42]. Raw sequencing reads for these datasets were downloaded from NCBI. For the jellyfish microbiomes, the 16S rRNA datasets were originally sequenced using different primer sets targeting various regions of the 16S rRNA gene (V3V4, V4V5, or V5V6). Previous work on *R. nomadica* has shown that different primer sets yield similar genus-level community compositions, supporting this approach for cross-study comparisons [23]. To minimize study-related biases and enable meaningful comparisons, each dataset was re-processed using the same general pipeline, with adjustments for the specific primer set used. Following processing, sequences containing the V4 region were subjected to BLAST (Nucleotide-Nucleotide BLAST 2.12.0+ [BLASTn] algorithm [43]) and identical sequences were collapsed to a single ASV. We then trimmed all the sequences to retain only the overlapping V4 region. Datasets without V4 coverage were excluded from ASV-level analyses but were included in genus-level comparisons [23]. Mean relative abundances for each ASV or genus was calculated to get a representative dataset for each jellyfish species. For additional jellyfish species, *Cotylorhiza tuberculata*, raw sequence data were only available from metagenomic samples, which did not allow for re-analysis using the standard 16S rRNA gene pipeline. Instead, we compared the top 25 ASVs from *R. nomadica* directly against the *C. tuberculata* metagenomic dataset using BLAST, to assess the presence of shared bacterial taxa.

To define the 'core' microbiome across jellyfish species, we applied two thresholds. The first, a strict definition, identified taxa with a mean relative abundance of ≥ 0.1% in at least 80% of the samples from each jellyfish species. The second, a more lenient threshold, accounted for the transient nature of the microbiome and the high variability between species, where the prevalence was reduced to at least 50% of the samples. The top taxa composition was visualized with a heatmap, and core taxa were identified using the microbiome package in R [44].

Results

The *R. nomadica* microbiome differs between tissues and over time

First, we aimed to examine whether *R. nomadica* microbiome differs between jellyfish tissues and changes across different stages of the bloom (Fig. 2). We observed significant differences in the microbiome composition among tissues, underscoring the presence of distinct microbial communities across them (PERMANOVA: $F_{(3,116)} = 56.36$, $p < 0.001$). Pairwise comparisons revealed that the bell and gonads hosted significantly distinct communities ($p < 0.01$), while the gastrovascular canals (GVC) and tentacles exhibited substantial overlap ($p > 0.5$, Fig. 2A). However, clear temporal differences in microbial community dispersion were also observed (PERMDISP2: $F_{(2,117)} = 11.83$, $p < 0.001$, Fig. 2B). Indeed, Variation Partitioning Analysis (VPA) analysis showed that tissue type and month of bloom are the most influential factors on the variation between samples, explaining 19% and 11% of the variation, respectively (Fig. 2C). When repeated for individual tissues separately, the VPA revealed even clearer temporal changes in microbiome structure, accounting for 17–24% of the variations ($p < 0.003$, Fig. S2). Here, again, the bell and gonad communities varied significantly between months (PERMANOVA: bell $F_{(2,25)} = 6.03$, $p = 0.001$; gonads $F_{(2,28)} = 5.47$, $p = 0.001$), while the GVC and tentacles differed only between July (towards the end of the bloom) and the other months (PERMANOVA: GVC $F_{(2,27)} = 6.52$, $p = 0.001$; tentacles $F_{(2,28)} = 7.21$, $p = 0.001$, Fig. 2D). Jellyfish size and sex were also tested but did not significantly affect the microbial population structure (Fig. 2C).

Notably, the samples from July formed tighter clusters in the NMDS ordination than those from other months, particularly in the tentacles and the GVC, suggesting more uniform communities across individual jellyfish towards the end of the bloom (Fig. 2B, D, Fig. S3). Taken together, these results reveal distinct patterns within *R. nomadica* across different tissues and bloom stages, with the most pronounced shifts occurring in July.

Key bacteria (ASVs) shaping variability across tissues and bloom stages

Next, we aimed to identify the bacterial taxa responsible for the observed dissimilarities between tissues and months. A relatively small group of ASVs dominated the entire jellyfish microbial community, with 68

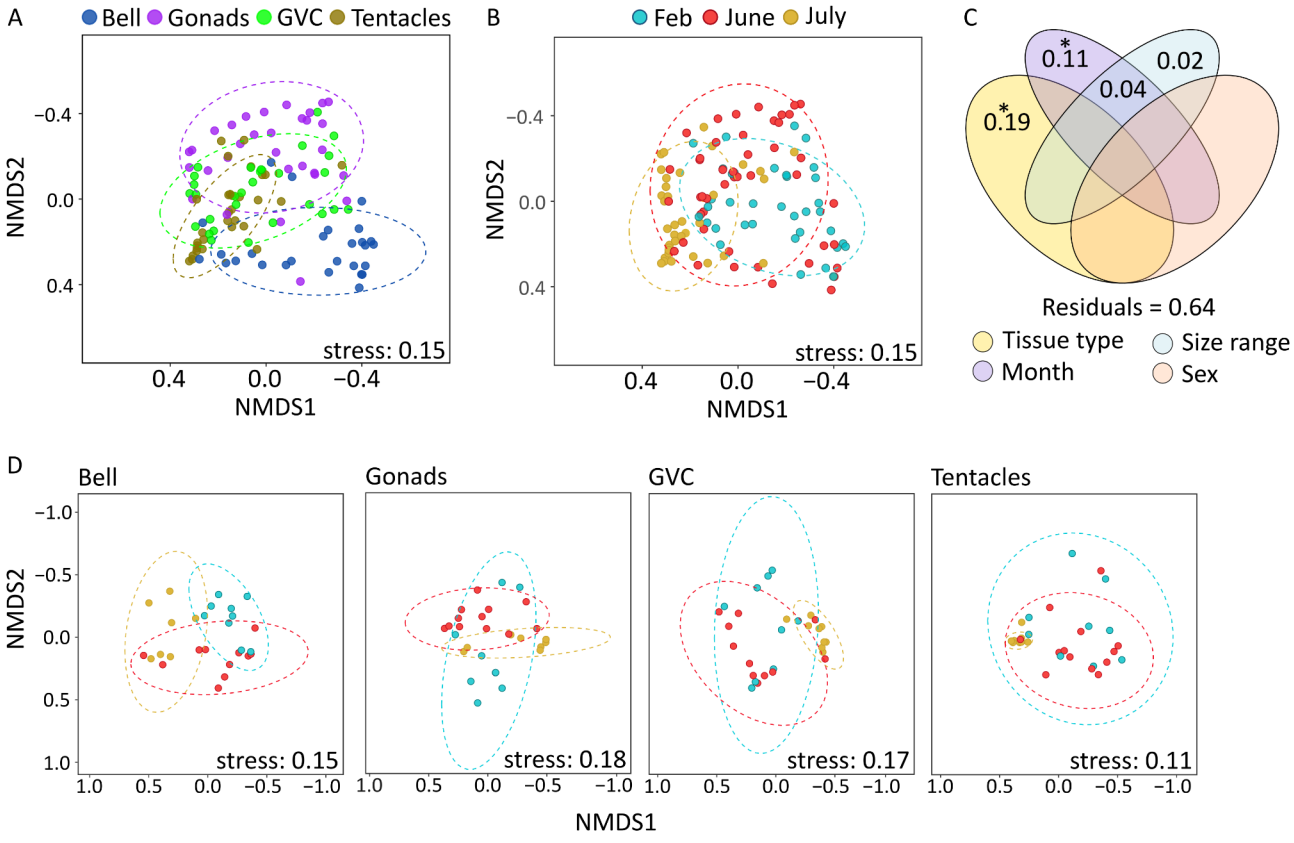

Fig. 2 Variation in microbiome composition. (**A**, **B**) Nonmetric multidimensional scaling (NMDS) analysis for all samples, colored by tissue type (**A**) or month of sampling (**B**). Additional panels representing the third dimension can be found in Fig. S2. (**C**) VPA analysis performed on all data. Asterisks represent statistical significance $p < 0.001$ as estimated using CCA analysis. Colors represent different factors that were tested. The total percentage of variance explained by the factors is calculated as 1 minus the residuals. (**D**) NMDS analysis for each tissue separately. Tissue type is specified on top of each panel and the colors represent different months as specified in panel B.

ASVs representing taxa with a relative abundance above 1%. Notably, the bell tissue contributes to most of these ASVs making it the most diverse tissue (Fig. 3A, Fig. S4). The differences between tissues and month of sampling were primarily associated with six types of bacteria, which, while present to some extent in all tissues, show clear temporal patterns (Fig. 3B). The most relatively abundant ASV, an *Endozoicomonas* (ASV1), was associated primarily with the tentacles, and to a lesser extent with the bell and GVC. ASV1 was also highly seasonal, peaking in all tissues during July, when it comprised, on average, more than 89% of the tentacle community. The second most abundant ASV was an unclassified *Rickettsiales* (ASV2), with high relative abundance mostly in the gonads and GVC during July (Fig. 3A, Fig. S4). Other noteworthy associations were between an ASV belonging to the *Entomoplasmatales* (ASV3) with the tentacles and GVC, mostly during February and June; a member of the *Simkaniaceae* (ASV4) with the gonads during June, and a member of the *Bacteroides* (ASV6) with the bell primarily in February. The gonads were the only tissue that had a high relative abundance of *Mycoplasma* (ASV11/21) (Fig. 3A-C). Finally, a *Tenacibaculum* (ASV5) was mostly associated with the winter bloom, without any clear tissue patterns (Fig. 3C).

Microbial alterations during jellyfish bloom decline

To investigate the link between microbial alterations and the decline of the bloom, we concentrated on two potential scenarios: the emergence of pathogenic bacteria or a significant shift in microbial community structure leading to an imbalanced microbiome. Both scenarios could potentially disrupt the physiology of the jellyfish, thereby affecting the bloom.

First, we sought to examine how the health of jellyfish influences the diversity and composition of their microbiome. We compared both healthy and unhealthy individuals, as well as specific damaged areas versus healthy areas within the same individual. Our analysis revealed no notable distinction between groups. Monthly clustering patterns remained consistent, with samples from both healthy and unhealthy individuals interspersed (Fig. S6), and no specific taxa were found to be exclusive to either group. These observations remained consistent

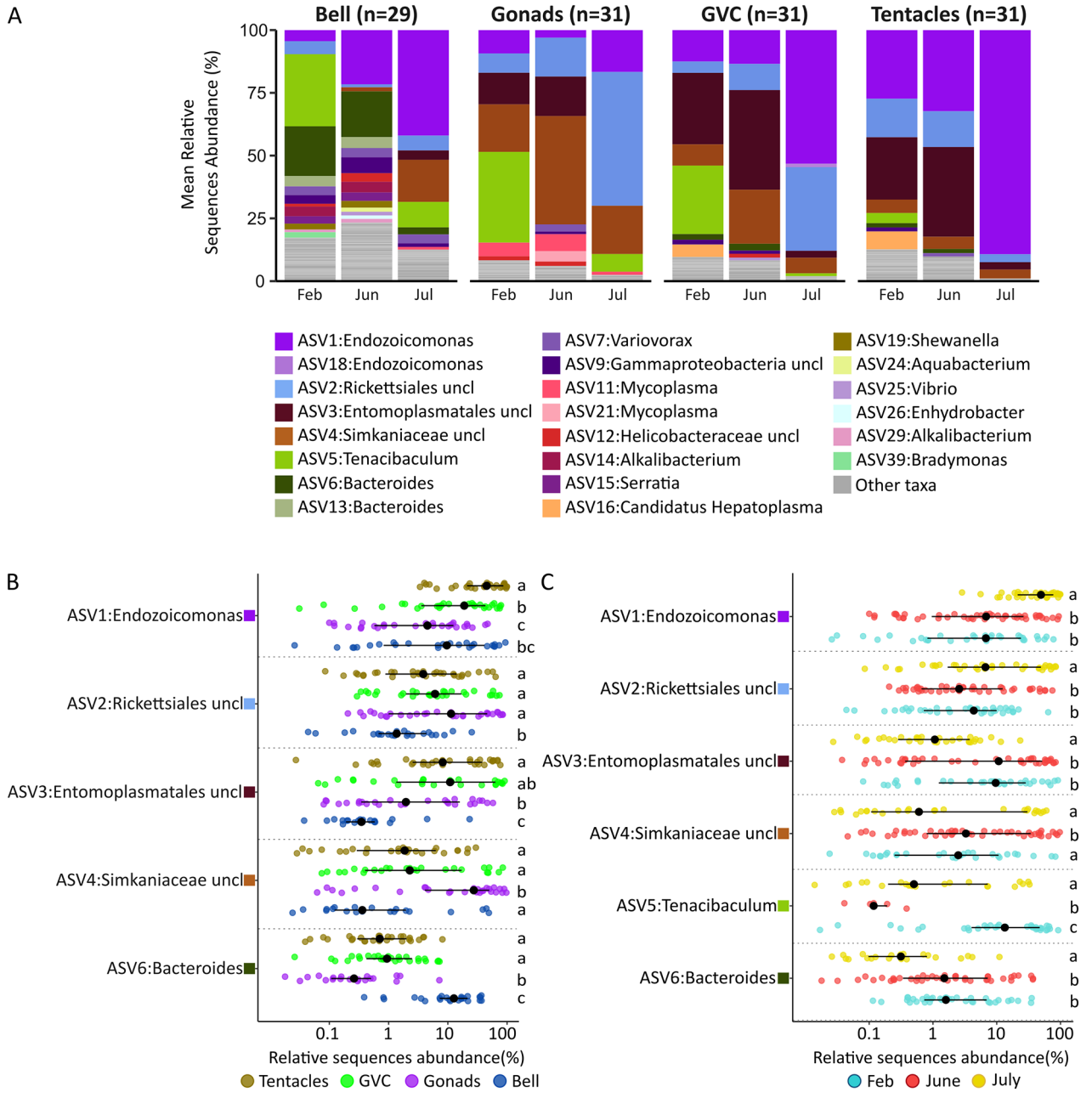

Fig. 3 Jellyfish bacteria composition analysis. (**A**) Mean relative abundance analysis for tissues across bloom months. Type of tissue is specified above each graph. ASVs belonging to the same genus are color coded with similar colors and grouped together, otherwise taxa are ordered by mean relative abundance. Each ASV is classified to the highest level of phylogenetic identification achieved. The data for all individual samples are shown in Fig. S5. (**C**) Taxa with significant differences between tissues (**B**) or months (**C**). Each dot represents a single sample; black dots indicate the mean ± standard deviation. Colors are the same as those used in Fig. 2. Letters represent significant groups found in with $p < 0.05$ as determined by pairwise comparisons -Wilcoxon tests following a Kruskal-Wallis test.

even when focusing specifically on damaged vs. undamaged areas within the same individual jellyfish (Fig. S6).

Next, we examined the α-diversity across various stages of the bloom considering that shifts in microbial diversity could critically impact the functionality and resilience of the host. The Shannon diversity during July (late-stage bloom) was notably lower than in June (early-stage bloom) or February (winter bloom based on the ART ANOVA test (p-value < 0.001, Fig. 4A). We also examined the Bray-Curtis distances to explore differences between bacterial communities of each month. Our results indicated that the communities in July were significantly more homogeneous compared to those from other months (ART ANOVA test, p-value < 0.001, Fig. 4B).

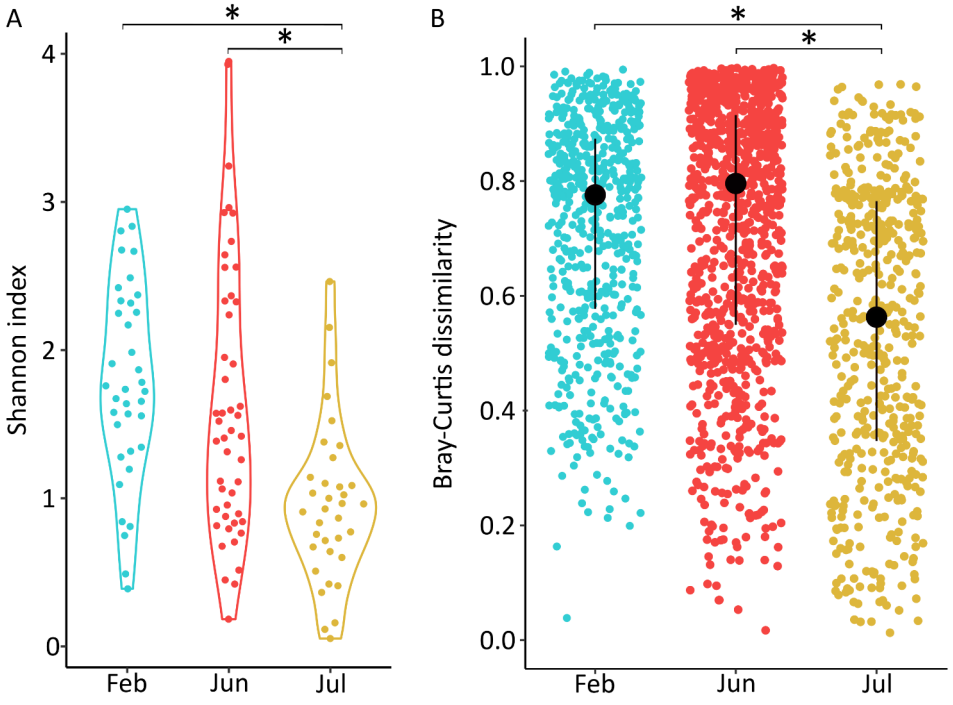

Fig. 4 Variation in bacterial community diversity over different bloom events. (**A**) Shannon diversity of individual samples. (**B**) Bray Curtis dissimilarity between samples from the same month. Asterisks represent statistically significant differences (ART ANOVA test, p-value < 0.001)

Upon further examination, we found that in July, the bell and tentacles exhibited lower diversity compared to February and June, while more uniform communities were observed in the gonads, tentacles, and GVC (Fig. S3). Overall, no specific taxa or patterns could differentiate healthy and unhealthy jellyfish, yet a significant decline in microbiome diversity was observed during the late bloom stage.

Microbial connectivity of *R. nomadica* with its environment and other jellyfish species

In this section, we address two key questions. First, we ask whether a 'core' microbiome exists across jellyfish species. Second, we ask whether ASVs found in *R. nomadica* were also present in the surrounding water or in a 'baseline' time-series, where jellyfish were not observed during sampling. These questions provide a foundation for exploring how jellyfish acquire their microbiome - specifically, whether they are colonized by bacteria commonly found in the surrounding water.

There was no clear correlation between the phylogeny of the jellyfish species tested and the structure of their microbiomes (Procrustean superimposition test, $r = 0.5157$, $p = 0.166$, based on 999 permutations), although we note that the data had multiple limitations that could affect this analysis (supplementary text 1). Additionally, when the most dominant bacterial community members across all jellyfish were examined, no single ASV (Fig. 5A, Table S1) or even genus (Fig. 5B, Table S2) was found, thus no clear 'core' could be identified. However, when we consider a less strict core definition some genus, such as *Endozoicomonas*, *Spiroplasma*, *Mycoplasma* and *Rasltonia*, were identified with prevalence of over half of the jellyfish (5–6/9), where they often exhibit high relative abundance (Fig. 5B, Table S6). In contrast, while *Vibrio* was the most prevalent genus, appearing in 8/9 jellyfish, it is found in very low relative abundance in all except *A. coerulea* (Fig. 5B).

Within the predominant genera, specific ASVs of *Endozoicomonas* (ASV1) and *Ralstonia* (ASV23) were identified across diverse geographical locations, highlighting a potential widespread distribution. *R. nomadica* from the Eastern Mediterranean shared an identical *Endozoicomonas* ASV with *C. xamachana* from Nichupté Lagoon in Mexico (maintained in the lab), and with *M. papua* and *T. cystophora* from Indonesian marine lakes (Fig. S7). This indicates a shared symbiont among geographically distant species. In contrast, ASVs from other genus showed associations with specific geographical locations. For example, the ASVs identified in the four jellyfish species (*R. esculentum*, *N. nomurai*, *C. nozakii*, and *A. coerulea*) from the Yellow Sea appeared to correlate with their respective habitats, suggesting a degree of localization (Fig. S7).

The microbial diversity in the seawater collected from within the jellyfish bloom was higher than that of the jellyfish tissues and was clearly different from it in an NMDS analysis (Fig.S3). Of the 25 most abundant ASVs

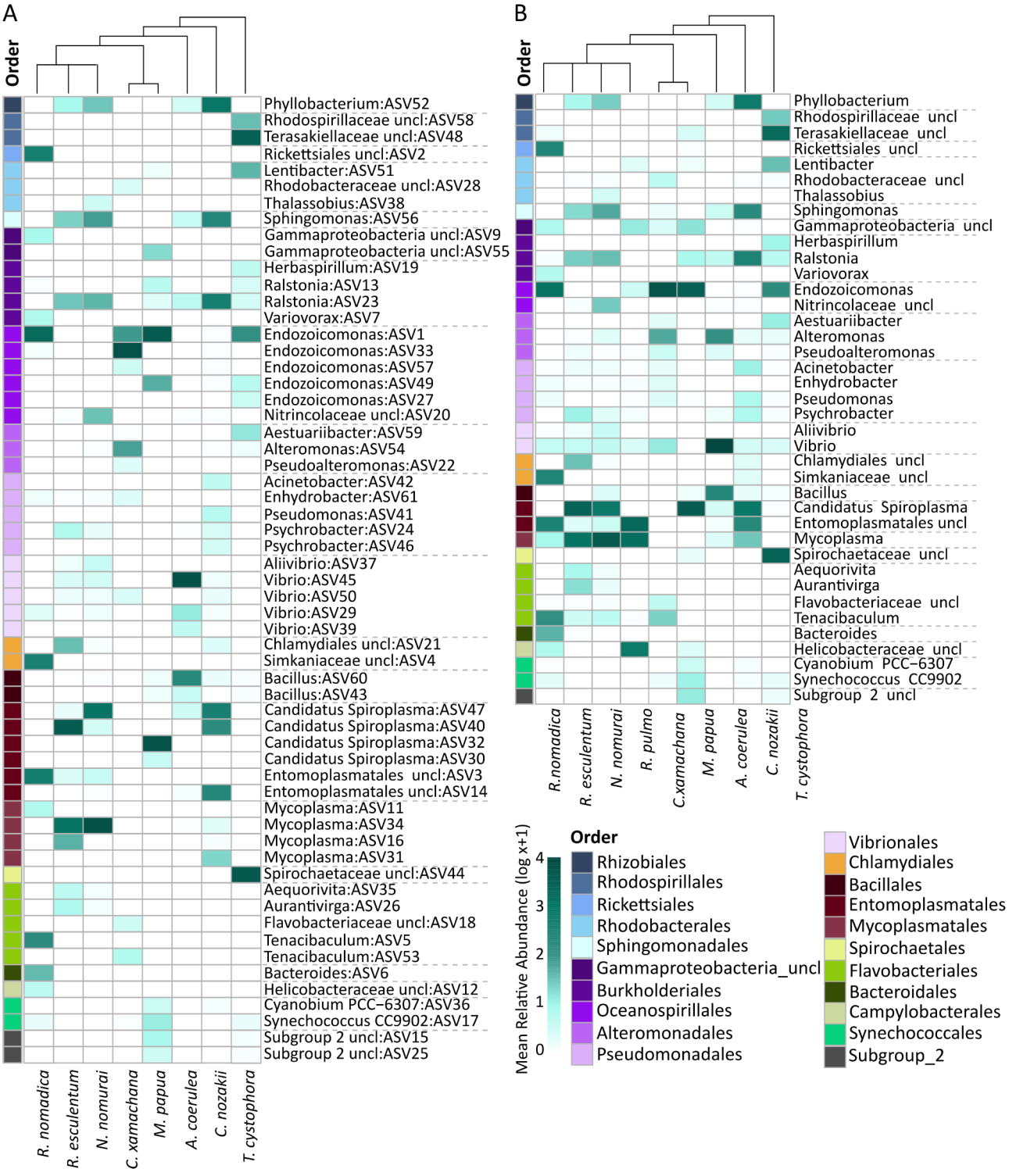

Fig. 5 Comparative analysis of microbial populations in different jellyfish species. (**A**) ASV-level relative abundance. The heatmap displays the top 10 ASVs for each jellyfish species after a 100% sequence similarity BLAST match, yielding 59 unique ASVs defined based on a 250 bp segment of the conserved V4 region. When more than one ASV has identical V4 regions (but, for example, differ in other sequenced regions) they are combined to include a single representative ASV from each species. (**B**) Genus-level relative abundance heatmap. Displays the aggregation of all ASVs at the genus level identified in panel A and incorporating additional *Rhisoztoma pulmo* sequences from the V5V6 16S region (which do not overlap with the V4 region and thus could not be included in the ASV comparison in panel A). Mean relative abundances per jellyfish species are represented through a color gradient, normalized using log (x + 1) to accommodate both low and high values. A phylogenetic tree above the heatmaps orders jellyfish species by their evolutionary relationships, aiding in the detection of microbial composition patterns

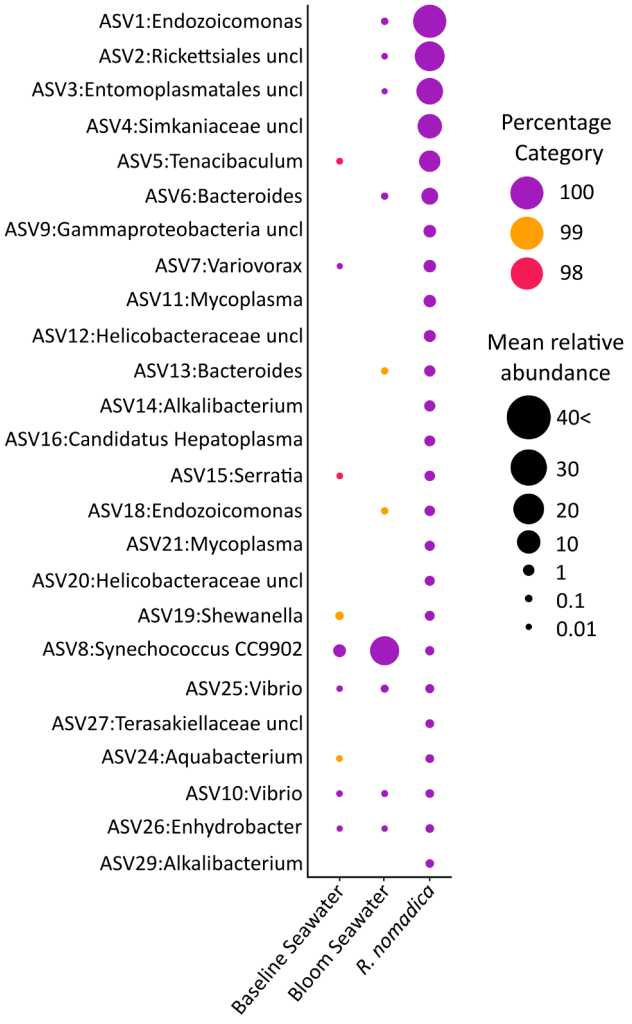

Fig. 6 Comparative analysis of top R. nomadica ASVs against seawater from different locations. The comparison was done on ASV level. The size of each circle indicates the mean relative abundance of the corresponding ASV, while the color denotes the percentage identity among the ASVs examined. The blue background represents the two seawater types: water from the bloom and baseline water collected from a remote location

in the *R. nomadica* microbiome, 8 were shared with the water surrounding the bloom, and 5 were shared with the water collected from the "baseline" locations (Fig. 6). Most of these shared ASVs were rare (<0.1% average relative abundance) in both jellyfish tissues and seawater, with the exception of *Synechococcus* CC9902 (ASV8). This ASV formed ~20% of the reads within the sampled jellyfish blooms but is commonly found at high relative abundances in samples from the Eastern Mediterranean and likely represents a signature of the coastal water where the jellyfish bloom was found [36]. It is noteworthy that the three ASVs with the highest mean relative abundance in the jellyfish tissues were identified in the water within the bloom but not in the baseline sites (*Endozoicomonas* ASV1, *Rickettsiales* (ASV2) and unclassified *Entomoplasmatales* (ASV3). The unclassified *Entomoplasmatales* (ASV3), which closely matches (99.5%) a complete 16S sequence from *C. tuberculata* [15], has been further identified as *Spiroplasma* and will henceforth be referred to as *Spiroplasma*.

Discussion

In this study, we examine the microbiome dynamics of *R. nomadica* across various tissues and bloom stages. We observed a decrease in microbial diversity as the bloom progressed, with taxa like *Endozoicomonas* and *Rickettsiales* becoming dominant in specific tissues. Extending our analysis to other jellyfish species revealed that although certain clades, and even specific ASVs, such as *Endozoicomonas*, are common, they are not universal, indicating a lack of a "core jellyfish microbiome". Interestingly, certain ASVs, including the three most abundant, were present only in the bloom water and were absent in Baseline water where jellyfish were not present.

Temporal microbial shifts in *R. nomadica* are characterized by reduced communities diversity

Throughout the months of February, June, and July, we observed significant shifts in the microbial communities of *R. nomadic*a, especially noticeable between the early bloom in June and the late bloom in July. The late bloom stages featured less diverse, more homogenous bacterial communities across all tissues. These shifts were predominantly driven by two taxa, *Endozoicomonas* (ASV1) and Unclassified *Rickettsiales* (ASV2), which were present in over 90% of our samples. Their high prevalence and relative abundance suggest they may play a significant role in the jellyfish microbiome—similar to the roles proposed for *Rickettsiales*, and especially *Endozoicomonas*, in other cnidarians [45, 46, 47]. However, this dominance may lead to an imbalanced microbial community, which, in other marine hosts such as corals, sponges, seaweeds, fish and a few marine mammals, has been linked to tissue necrosis, bleaching, reduced nutrient uptake, and increased susceptibility to pathogens [48, 49, 50, 51, 52]. Additionally, these changes could correlate with physiological deterioration in the jellyfish, such as degradation of feeding structures, potentially affecting food intake and further altering the microbiome [53]. These observations indicate that changes in microbial dominance and diversity might not necessarily reflect the health status of jellyfish but may be part of normal developmental processes the jellyfish go through as the blooms evolve. This raises questions about the dynamic roles these bacteria play in jellyfish, particularly how their interactions with the host might change during different bloom stages.

Endozoicomonas are known for their interactions with multiple marine hosts, particularly cnidarians, and have been extensively studied in corals. They often appear in high abundance, forming aggregates, and are considered

important to coral health and resilience [54, 55, 56, 57, 58, 59]. However, their relationship may not be solely mutualistic. Genomic traits such as eukaryote-like proteins, secretion systems, and antioxidant properties suggest the potential for pathogenic or context-dependent lifestyles [60, 61]. Recent research linking coral microbiomes to disease susceptibility suggests that *Endozoicomonas* may mediate trade-offs between host growth and immune defense. Under stable conditions, they could enhance host metabolism, but during environmental stress or microbiome shifts, they may increase susceptibility to pathogens or other stressors [62]. These findings support the idea that *Endozoicomonas* span a range of roles along the symbiosis spectrum. Moreover, specific *Endozoicomonas* clades are associated with different cnidarian families, and their responses to stress—such as heat—vary. For example, some studies report decreased abundance during coral bleaching, while others observe stable or increased presence [47, 57, 63, 64, 65].

The role of Unclassified *Rickettsiales* (ASV2) in jellyfish remains speculative, given their classification only to the order level in *R. nomadica*. Generally, *Rickettsiales* are considered obligate intracellular bacteria, often host-specific and potentially pathogenic [66, 67], with occurrences reported across various marine hosts, including bivalves and corals [68, 69]. For example, in the coral *Acropora cervicornis*, a low abundance of *Rickettsiales* spp. (*Candidatus Aquarickettsia*) is generally harmless. However, changes in environmental conditions can cause the Rickettsiales spp. population to increase, leading to nutrient and energy depletion in the host. This, in turn, has been linked to increased disease risk and slower growth in the coral [69]. Interestingly, the *Rickettsiales* sequence in *R. nomadica* did not match any other jellyfish, suggesting a species-specific relationship.

During the late bloom stage, we observed significant shifts in the *R. nomadica* microbiome, marked by the dominance of *Endozoicomonas* and *Rickettsiales* and reduced microbial diversity. This change suggests a transition from a balanced host-microbe interaction during the peak bloom—when jellyfish are in their prime condition—to a more imbalanced community, which could reflect physiological deterioration as the jellyfish age or weaken. It is also possible that these shifts in the jellyfish microbiome are linked to changes occurring in the surrounding bloom water, as has been shown in coral reefs [70]. While more research is needed to identify the exact drivers of these shifts, we propose that microbial imbalances may play role, or at least mark, the decline of the bloom and the eventual disappearance of the jellyfish.

Tenacibaculum spp. dominate *R. nomadica* winter blooms

Unlike the well-documented summer blooms, the dynamics, and connections of winter blooms to their summer counterparts remain less understood. Our analysis over consecutive winters (2020 and 2021) revealed a consistent winter bacterial community, which is different from the summer one. Population genetic analysis show that the winter and summer blooms appear to be part of the same genetically diverse population indicated by high gene flow and lack of genetic differentiation between seasons and months [71]. This suggests that the microbial differences between seasons are driven by environmental conditions or jellyfish physiology rather than distinct cryptic species of *R. nomadica* [72, 73].

The winter community has a high relative abundance of *Tenacibaculum* spp (ASV5), which is mostly absent in early summer and sporadically observed in late summer. *Tenacibaculum* species, part of the *Flavobacteriaceae* family, show a range of interactions with marine hosts, from symbiosis to pathogenicity. Although their exact roles in jellyfish are not fully understood, they are observed across various jellyfish species, with different ASVs present in each. In *C. tuberculata*, *Tenacibaculum*-like bacteria within the gastric filaments may enhance carbon and energy transfer, suggesting a symbiotic relationship. Their genome encodes pathways for glycolysis, the Krebs cycle, and synthesis of essential amino acids and vitamins such as thiamine (B1), biotin (B7), and riboflavin (B2), providing significant metabolic benefits to the host [15]. Additionally, species like *Pelagia noctiluca* and *Phialella quadrata* are potential reservoirs for *Tenacibaculum maritimum*, a known pathogen of farmed fish [74, 75]. However, in *R. nomadica*, the presence of *Tenacibaculum* appears non-obligatory and possibly influenced by environmental factors.

Key microbial taxa in *R. nomadica*– putative roles and cross-species presence

Our analysis reveals that the *R. nomadica* microbiome is dominated by a few bacterial taxa with high relative abundance and prevalence, consistent with other jellyfish species [15, 39, 42, 76]. We investigated whether key taxa are shared across jellyfish with different phylogenetic and geographic backgrounds but found no consistent 'core' microbiota at the ASV or genus level. Additionally, phylogenetic classification did not show any distinct patterns indicative of phylosymbiosis, aligning with studies suggesting that shared bacteria among cnidarians and marine invertebrates often reflect environmental rather than phylogenetic similarities [47, 59, 77]. In this regard, we observed that certain ASVs were exclusive to four jellyfish species from the Northern Yellow Sea [39]. However, these four jellyfish were from a single study, and thus further work is needed to determine if the sampling location influences the microbiome composition of different jellyfish species.

Despite the absence of a clear shared core microbiome our analysis identified several bacterial taxa common to various jellyfish species and locations. Notably, an *Endozoicomonas* ASV (ASV1) and a *Ralstonia* ASV (ASV23) were widespread across diverse environments ASV1 was particularly abundant in *R. nomadica* from the Eastern Mediterranean and had high relative abundance in other species like *M. papua* and *T. cystophora* in Indonesian Marine lakes, and *C. xamachana* from Mexico's Nichupté Lagoon, which was cultured in a lab. Previous studies have suggested that *Endozoicomonas* ASVs can be divided into generalist and host-specific clades, with some groups, such as those associated with Atlantic Rhizostomeae, exhibiting strong host specificity [47, 78]. Our *Endozoicomonas* ASV1 matches ASV59, 97, 1987, 2517, and 96,488, which McCauley et al. classified as specific to this group. This supports the association of the clade with jellyfish, but also suggests its adaptability beyond previously observed geographic constraints.

Focusing only on ASV-level bacteria might miss broader patterns evident at higher taxonomic levels, leading us to also examine genus common among jellyfish. Notably, *Mycoplasma*, *Spiroplasma*, and *Vibrio* emerged as highly prevalent taxa. This prevalence warrants further exploration into their roles in jellyfish, which might explain their widespread presence across different geographical and phylogenetic backgrounds.

While the specific functions of *Spiroplasma* in jellyfish remain unclear, they are recognized for their intracellular lifestyle and defensive roles in host interactions [78, 79]. Observations in *C. tuberculata* jellyfish suggest that these *Spiroplasma* possess small genomes and limited metabolic capabilities, consistent with a commensal or mutualistic association rather than a pathogenic one [15]. *Mycoplasma*, typically viewed as intracellular parasites, have recently been identified as part of the core microbiome in healthy cnidarian tissues. However, their role may shift from beneficial to harmful due to changes in the community composition or environmental factors [47, 80, 81]. Lastly, *Vibrio* present an interesting case as while very prevalent among the jellyfish species, they appear in low abundance and are consistently found in all our seawater samples. This finding underscores their generalist nature and consistent with the prevalence of the *Vibrionaceae* family in Anthozoa, Cubozoa, and Scyphozoa as well as many other marine organisms [82].

Origin and transmission of Jellyfish-associated bacteria

Previous studies indicated that cnidarian and other invertebrate microbiomes are distinct from the surrounding seawater [16, 47, 77]. However, ASVs within cnidarian microbiomes often show region-specific patterns [47, 83], hinting at potential organism-to-organism transfer. How, then, are cnidarian-associated bacteria acquired? Are they present in seawater at low abundances, possibly associated with marine particles, thus potentially "inoculating" cnidarians? Or, are they exclusively around cnidarians, transferred vertically or through contact with other cnidarians or their mucus? Our comprehensive sampling of jellyfish and surrounding waters, including a control site without jellyfish, allowed us to explore these modes of transmission. To contextualize the patterns of microbiome acquisition, we categorized the main *R. nomadica* ASVs into three groups based on the previously presented results: 1. shared between jellyfish, bloom seawater, and 'control' seawater; 2. shared between jellyfish and bloom seawater; and 3. found only in the jellyfish.

The first group includes marine bacteria like two *Vibrio* ASVs (ASV10 and ASV25), commonly found in the jellyfish microbiome and are likely opportunistic, adapting to various niches such as marine particles and different jellyfish species, including those in this study. *Vibrio* are known to inhabit diverse marine niches, including various cnidarian hosts [16, 47, 82, 84]. Notably, *Vibrio* increase in abundance during the decay of *A. aurita* jellyfish blooms, underscoring their role as opportunistic decomposers [7, 85]. Additionally, *Vibrio* produce transporters that aid in nutrient uptake from decomposing jellyfish, leveraging substances released by others and creating niche partitioning within the bacterial community [85].

The second group includes bacteria found on jellyfish and in bloom water, but absent in jellyfish-free water, featuring four primary ASVs from *R. nomadica—Endozoicomonas* (ASV1), unclassified *Rickettsiales* (ASV2), *Spiroplasma* (ASV3), and *Bacteroides* (ASV6). *Endozoicomonas* may have a free-living stage as suggested by their large genome, metabolic diversity, and ability to utilize diverse carbon sources [55, 56, 63]. However, our findings, reinforced by coral studies, indicate that their presence in seawater primarily depends on proximity to hosts. For example, *Endozoicomonas* abundance decrease with increasing distance from coral reefs [86]. Additionally, two generations of adult *Pocillopora acuta* displayed consistent *Endozoicomonas* ASVs, which were absent in their larvae [54]. This suggests horizontal acquisition at the adult stage, emphasizing a primarily host-associated lifestyle rather than a truly free-living existence.

In contrast to *Endozoicomonas*, neither *Rickettsiales*—primarily intracellular pathogens or symbionts—nor *Spiroplasma* and *Bacteroides*, both considered anaerobic, are expected to have a free-living stage [15, 87, 88]. *Spiroplasma*, identified as anaerobic fermenters in *C. tuberculata* (showing 99.53% identity with ASV3 from *R. nomadica* microbiome), were found primarily in the tentacles and gastric vascular cavity (GVC), and also in the mucus of *R. pulmo* [15, 89]. Similarly, *Bacteroides*,

mostly studied in mammalian gastrointestinal tracts and considered obligate anaerobes has limited known environmental survival abilities [90]. Here, they were mostly found in the bell of *R. nomadica*, and we speculate that they may be associated with the bell mucus layer, akin to their role as mucosal bacteria in animal guts [91]. However, the identification of putative anaerobic organisms in the jellyfish microbiome raises the question of whether and where these animals have anaerobic environments. While decomposition of jellyfish blooms can cause localized hypoxic or anoxic conditions, particularly in areas like the seafloor where organic material accumulates [1, 92], this has not been observed during the active bloom stages when jellyfish are healthy. Their presence in the water surrounding the jellyfish bloom could be explained by the shedding of tissue or mucus, as suggested in corals [86].

The final group of ASVs, found exclusively on the jellyfish, includes unclassified *Simkaniaceae* (ASV4) and *Mycoplasma* (ASV11). These were primarily identified in the gonads of *R. nomadica*, raising the possibility for vertical transmission. Similarly, in *P. acuta*, a member of the *Simkania* (a *Simkaniaceae* genus) appeared not only in adults but consistently across larval samples, indicating vertical transmission in asexually reproducing larvae [54]. Likewise, in *A. aurita*, the presence of *Mycoplasma* in adults and various developmental stages also supports the likelihood of vertical transmission among these cnidarian species [13, 84].

In addition to inoculation by waterborne bacteria and vertical transmission, food-borne bacteria (e.g. associated with copepods or other zooplankton [53], could also be a source for jellyfish-associated communities. Intriguingly, *Simkania*-like cells, corresponding to our ASV4 unclassified *Simkaniaceae*, were found inside eukaryotic ciliates in the gastric cavity of *C. tuberculata* [15], suggesting potential tripartite symbioses [93]. Investigating environmental conditions within jellyfish niches, like oxygen levels in mucus, and conducting broader sampling of surrounding waters including prey- and other eukaryote-associated bacteria, could clarify these transmission hypotheses. Additionally, studying other jellyfish life cycle stages, such as polyps and ephyrae [12, 13, 94], might provide deeper insights into the mechanisms governing jellyfish-associated communities and how these microbiomes influence jellyfish fitness.

Conclusion

This study represents the first comprehensive exploration of microbial dynamics in *R. nomadica* blooms, offering insights into jellyfish-associated bacterial communities across different bloom stages and tissues. We documented significant temporal changes during late summer blooms, including a decline in microbial richness and shifts in dominant taxa, underscoring how microbial communities change during bloom progression. Tissue-specific analyses revealed distinct microbial niches within jellyfish tissues, highlighting the complexity of host-microbe interactions in bloom ecosystems. By comparing *R. nomadica* microbiomes to those of nine other jellyfish species, we identified a limited number of recurring microbial taxa shared across species, while demonstrating the absence of a universal core microbiome. Additionally, the detection of some bacterial taxa in bloom-associated water, but not in remote seawater, suggests localized transmission dynamics between jellyfish and their immediate marine environment. This study establishes a robust foundation for understanding the microbial ecology of jellyfish blooms, shedding light on the intricate relationships between bloom progression, microbial communities, and surrounding waters. These findings provide a basis for future research to explore microbial contributions to bloom stability and decline, as well as their broader roles in marine ecosystem processes.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

N.B. Conceptualization, Data Curation, Formal Analysis, Investigation, Visualization, Writing– Original Draft Preparation, Writing– Review & Editing. T.L. and D.S. Supervision, Conceptualization, Funding Acquisition, Resources, Writing– Review & Editing. V.B. and D.A. Project Administration, Resources.

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Data availability

Raw 16S sequencing data from this study are available in the NCBI Sequence Read Archive under Accession Number PRJNA1107792. The analysis code is accessible on GitHub at https://github.com/NogaBarak/R.nomadica_microbiome. Samples metadata and ASVs table can be found in Tables S3-S6.

Declarations

Conflict of interest

The authors declare no conflict of interest.

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References

1. Pitt KA, Budarf AC, Browne JG, Condon RH. Bloom and bust: why do blooms of jellyfish collapse? Jellyfish blooms. Springer Netherlands; 2014. pp. 79–103.
2. Angel DL, Edelist D, Freeman S. Local perspectives on regional challenges: jellyfish proliferation and fish stock management along the Israeli mediterranean Coast. Reg Environ Change. 2016;16:315–23.
3. Dong Z, Liu D, Keesing JK. Jellyfish blooms in China: dominant species, causes and consequences. Mar Pollut Bull. 2010;60:954–63.
4. Lucas CH, Gelcich S, Uye S-I. Living with jellyfish: management and adaptation strategies. In: Pitt KA, Lucas CH, editors Jellyfish blooms. Springer. p. 129–50.
5. Bosch-Belmar M, Milisenda G, Basso L, Doyle TK, Leone A, Piraino S. Jellyfish impacts on marine aquaculture and fisheries. Reviews Fisheries Sci Aquaculture. 2020;29:242–59.
6. Purcell JE, Uye S, Lo W. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Mar Ecol Prog Ser. 2007;350:153–74.
7. Kramar MK, Tinta T, Lučić D, Malej A, Turk V. Bacteria associated with Moon jellyfish during bloom and post-bloom periods in the Gulf of Trieste (northern Adriatic). PLoS ONE. 2019;14.
8. Edelist D, Guy-Haim T, Kuplik Z, Zuckerman N, Nemoy P, Angel DL. Phenological shift in swarming patterns of *Rhopilema nomadica* in the Eastern mediterranean sea. J Plankton Res. 2020;42:211–9.
9. Lebrato M, Pahlow M, Frost JR, Küter M, de Jesus Mendes P, Molinero JC, et al. Sinking of gelatinous zooplankton biomass increases deep carbon transfer efficiency globally. Global Biogeochem Cycles. 2019;33:1764–83.
10. Sweetman AK, Chapman A. First observations of jelly-falls at the seafloor in a deep-sea Fjord. Deep Sea Res 1 Oceanogr Res Pap. 2011;58:1206–11.
11. Guy-Haim T, Rubin-Blum M, Rahav E, Belkin N, Silverman J, Sisma-Ventura G. The effects of decomposing invasive jellyfish on biogeochemical fluxes and microbial dynamics in an ultra-oligotrophic sea. Biogeosciences. 2020;17:5489–511.
12. Jensen N, Weiland-Bräuer N, Joel S, Chibani CM, Schmitz RA. The life cycle of *Aurelia aurita* depends on the presence of a Microbiome in polyps prior to onset of strobilation. Microbiol Spectr. 2023;11.
13. Weiland-Bräuer N, Pinnow N, Langfeldt D, Roik A, Güllert S, Chibani CM, et al. The native Microbiome is crucial for offspring generation and fitness of *Aurelia aurita*. mBio. 2020;11:1–20.
14. Peng S, Ye L, Li Y, Wang F, Sun T, Wang L et al. Microbiota regulates life-cycle transition and nematocyte dynamics in jellyfish. iScience. 2023;26.
15. Viver T, Orellana LH, Hatt JK, Urdiain M, Díaz S, Richter M, et al. The low diverse gastric Microbiome of the jellyfish Cotylorhiza tuberculata is dominated by four novel taxa. Environ Microbiol. 2017;19:3039–58.
16. Tinta T, Kogovšek T, Klun K, Malej A, Herndl GJ, Turk V. Jellyfish-associated Microbiome in the marine environment: exploring its biotechnological potential. Mar Drugs. 2019;17.
17. Lotan A, Fine M, Ben-Hillel R. Synchronization of the life cycle and dispersal pattern of the tropical invader scyphomedusan *Rhopilema nomadica* is temperature dependent. 1994.
18. Galil BS, Spanier E, Ferguson Galil WW, Ferguson WW. The Scyphomedusae of the Mediterranean coast of Israel, including two Lessepsian migrants new to the Mediterranean.Zoologische mededelingen. 1990;64:95-105
19. Brotz L, Cheung WWL, Kleisner K, Pakhomov E, Pauly D. Increasing jellyfish populations: trends in large marine ecosystems. Hydrobiologia. 2012;690:3–20.
20. Yahia MND, Yahia OKD, Gueroun SKM, Aissi M, Deidun A, Fuentes V, et al. The invasive tropical scyphozoan *Rhopilema nomadica* Galil, 1990 reaches the Tunisian Coast of the mediterranean sea. Bioinvasions Rec. 2013;2:319–23.
21. Madkour FF, Safwat W, Hanafy MH. Record of aggregation of alien tropical schyphozoan *Rhopilema nomadica* Galil, 1990 in the mediterranean Coast of Egypt. Int Mar Sci J. 2019;1:1–7.
22. Reich T, Ben-Ezra T, Belkin N, Tsemel A, Aharonovich D, Roth-Rosenberg D et al. A year in the life of the Eastern mediterranean: monthly dynamics of phytoplankton and bacterioplankton in an ultra-oligotrophic sea. Deep Sea Res 1 Oceanogr Res Pap. 2022;182.
23. Barak N, Fadeev E, Brekhman V, Aharonovich D, Lotan T, Sher D. Selecting 16S rRNA primers for Microbiome analysis in a Host–Microbe system: the case of the jellyfish *Rhopilema nomadica*. Microorganisms. 2023;11.
24. Cruaud P, Vigneron A, Fradette MS, Charette SJ, Rodriguez MJ, Dorea CC, et al. Open the sterivextm casing: an easy and effective way to improve DNA extraction yields. Limnol Oceanogr Methods. 2017;15:1015–20.
25. Malul D, Berman H, Solodoch A, Tal O, Barak N, Mizrahi G et al. Directional swimming patterns in jellyfish aggregations. Curr. Biol. 2024;34:4033-4038
26. Apprill A, Mcnally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015;75:129–37.
27. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from illumina amplicon data. Nat Methods. 2016;13:581–3.
28. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, et al. The SILVA and all-species living tree project (LTP) taxonomic frameworks. Nucleic Acids Res. 2014;42:643–8.
29. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the tidyverse. J Open Source Softw. 2019;4:1686.
30. Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer; 2016.
31. Oksanen J, Simpson GL, Blanchet FG. Accessed April 17,. Vegan: Community Ecology Package. R package version 2.5–7. 2022.
32. Felix GM, Ernst SA, Shetty. Tuomas Borman, Leo Lahti. mia: Microbiome analysis. 2022.
33. McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of Microbiome census data. PLoS ONE. 2013;8.
34. Elkin LA, Kay M, Higgins JJ, Wobbrock JO. An Aligned Rank Transform Procedure for Multifactor Contrast Tests. The 34th Annual ACM Symposium on User Interface Software and Technology. Association for Computing Machinery, 2021.
35. Jacob O, Wobbrock L, Findlater D, Gergle JJ. Higgins. The Aligned Rank Transform for Nonparametric Factorial Analyses Using Only ANOVA Procedures. 2011.
36. Haber M, Roth Rosenberg D, Lalzar M, Burgsdorf I, Saurav K, Lionheart R et al. Spatiotemporal variation of microbial communities in the Ultra-Oligotrophic Eastern mediterranean sea. Front Microbiol. 2022;13.
37. Efrati S, Lehahn Y, Rahav E, Kress N, Herut B, Gertman I, et al. Intrusion of coastal waters into the pelagic Eastern mediterranean: in situ and satellite-based characterization. Biogeosciences. 2013;10:3349–57.
38. Roth Rosenberg D, Haber M, Goldford J, Lalzar M, Aharonovich D, Al-Ashhab A, et al. Particle-associated and free-living bacterial communities in an oligotrophic sea are affected by different environmental factors. Environ Microbiol. 2021;23:4295–308.
39. Peng S, Hao W, Li Y, Wang L, Sun T, Zhao J et al. Bacterial communities associated with four blooming scyphozoan jellyfish: potential Species-Specific consequences for marine organisms and humans health. Front Microbiol. 2021;12.
40. Carabantes N, Cerqueda-García D, García-Maldonado JQ, Thomé PE. Changes in the bacterial community associated with experimental symbiont loss in the mucus layer of *Cassiopea xamachana* jellyfish. Front Mar Sci. 2022;9.
41. Cleary DFR, Becking LE, Polónia ARM, Freitas RM, Gomes NCM. Jellyfish-associated bacterial communities and bacterioplankton in Indonesian marine lakes. FEMS Microbiol Ecol. 2016;92.
42. Basso L, Rizzo L, Marzano M, Intranuovo M, Fosso B, Pesole G, et al. Jellyfish summer outbreaks as bacterial vectors and potential hazards for marine animals and humans health? The case of *Rhizostoma pulmo* (Scyphozoa, Cnidaria). Sci Total Environ. 2019;692:305–18.
43. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. NCBI BLAST: a better web interface. Nucleic Acids Res. 2008;36:5–9.
44. Lahti L. Sudarshan Shetty. Tools for microbiome analysis in R.752 Microbiome package version 1.23.1. 2017.
45. da Silva DMG, Pedrosa FR, Ângela Taipa M, Costa R, Keller-Costa T. Widespread occurrence of Chitinase-encoding genes suggests the endozoicomonadaceae family as a key player in Chitin processing in the marine benthos. ISME Commun. 2023;3.
46. Pogoreutz C, Oakley CA, Rädecker N, Cárdenas A, Perna G, Xiang N, et al. Coral holobiont cues prime Endozoicomonas for a symbiotic lifestyle. ISME J. 2022;16:1883–95.
47. McCauley M, Goulet TL, Jackson CR, Loesgen S. Systematic review of cnidarian microbiomes reveals insights into the structure, specificity, and fidelity of marine associations. Nat Commun. 2023;14.
48. Egan S, Gardiner M. Microbial dysbiosis: Rethinking disease in marine ecosystems. Front Microbiol. 2016;7:991.
49. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, et al. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVa and IV clusters. PLoS ONE. 2015;10:1–16.

50. Apprill A. Marine animal microbiomes: toward Understanding host-microbiome interactions in a changing ocean. Front Mar Sci. 2017;4:222.
51. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current Understanding of the human Microbiome. Nat Med. 2018;24:392–400.
52. Krabberød AK, Deutschmann IM, Bjorbækmo MFM, Balagué V, Giner CR, Ferrera I et al. Long-term patterns of an interconnected core marine microbiota. Environ Microbiomes. 2022;17.
53. Kuplik Z, Angel DL. Diet composition and some observations on the feeding ecology of the rhizostome *Rhopilema nomadica* in Israeli coastal waters. J Mar Biol Association United Kingd. 2020;100:681–9.
54. Maire J, Tandon K, Collingro A, Van De Meene A, Damjanovic K, Gotze CR et al. Colocalization and potential interactions of Endozoicomonas and chlamydiae in microbial aggregates of the coral *Pocillopora acuta*.Sci Adv. 2023.
55. Neave MJ, Michell CT, Apprill A, Voolstra CR. *Endozoicomonas* genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. Sci Rep. 2017;7:1–12.
56. Neave MJ, Apprill A, Ferrier-Pagès C, Voolstra CR. Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. Appl Microbiol Biotechnol. 2016;100:8315–24.
57. Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Wild C, Voolstra CR. Dominance of *Endozoicomonas* bacteria throughout coral bleaching and mortality suggests structural inflexibility of the *Pocillopora verrucosa* Microbiome. Ecol Evol. 2018;8:2240–52.
58. Hamner W, Dawson M. A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages. Hydrobiologia. 2009;616:161–91.
59. Hochart C, Paoli L, Ruscheweyh HJ, Salazar G, Boissin E, Romac S et al. Ecology of *Endozoicomonadaceae* in three coral genera across the Pacific ocean. Nat Commun. 2023;14.
60. Pogoreutz C, Ziegler M. Frenemies on the reef? Resolving the coral–*Endozoicomonas* association. Trends Microbiol. 2024.
61. Ide K, Nishikawa Y, Maruyama T, Tsukada Y, Kogawa M, Takeda H et al. Targeted single-cell genomics reveals novel host adaptation strategies of the symbiotic bacteria *Endozoicomonas* in *Acropora tenuis* coral. Microbiome. 2022;10.
62. Epstein HE, Brown T, Akinrinade AO, McMinds R, Pollock FJ, Sonett D, et al. Evidence for microbially-mediated tradeoffs between growth and defense throughout coral evolution. Anim Microbiome. 2025;7:1.
63. Tandon K, Lu CY, Chiang PW, Wada N, Yang SH, Chan YF, et al. Comparative genomics: dominant coral-bacterium *Endozoicomonas* acroporae metabolizes Dimethylsulfoniopropionate (DMSP). ISME J. 2020;14:1290–303.
64. Gignoux-Wolfsohn SA, Aronson FM, Vollmer SV. Complex interactions between potentially pathogenic, opportunistic, and resident bacteria emerge during infection on a reef-building coral. FEMS Microbiol Ecol. 2017;93.
65. McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL. Responses of coral-associated bacterial communities to local and global stressors. Front Mar Sci. 2017;4:AUG.
66. Khamesipour F, Dida GO, Anyona DN, Razavi M, Rakhshandehroo E. Tick-borne zoonoses in the order *Rickettsiales* and Legionellales in Iran: A systematic review. PLoS Negl Trop Dis. 2018;12.
67. Thomas S, *Rickettsiales*. Biology, molecular biology, epidemiology, and vaccine development. Springer International Publishing; 2016.
68. Cruz-Flores R, Cáceres-Martínez J. *Rickettsiales*-like organisms in bivalves and marine gastropods: a review. Reviews Aquaculture. 2020;12:2010–26.
69. Klinges JG, Rosales SM, McMinds R, Shaver EC, Shantz AA, Peters EC, et al. Phylogenetic, genomic, and biogeographic characterization of a novel and ubiquitous marine invertebrate-associated *Rickettsiales* parasite, candidatus Aquarickettsia Rohweri, gen. Nov., Sp. nov. ISME J. 2019;13:2938–53.
70. Glasl B, Herndl GJ, Frade PR. The Microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. ISME J. 2016;10:2280–92.
71. Douek J, Giallongo G, Harbuzov Z, Galil BS, Rinkevich B. Long-Term population genetic features of the *Rhopilema nomadica* jellyfish from the Israeli mediterranean Coasts. J Mar Sci Eng. 2024;12.
72. Derycke S, De Meester N, Rigaux A, Creer S, Bik H, Thomas WK, et al. Coexisting cryptic species of the litoditis marina complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability. Mol Ecol. 2016;25:2093–110.
73. Thomas T, Moitinho-Silva L, Lurgi M, Björk JR, Easson C, Astudillo-García C et al. Diversity, structure and convergent evolution of the global sponge Microbiome. Nat Commun. 2016;7.
74. Delannoy CMJ, Houghton JDR, Fleming NEC, Ferguson HW. Mauve stingers (*Pelagia noctiluca*) as carriers of the bacterial fish pathogen *Tenacibaculum maritimum*. Aquaculture. 2011;311:255–7.
75. Ferguson HW, Delannoy CMJ, Hay S, Nicolson J, Sutherland D, Crumlish M. Jellyfish as vectors of bacterial disease for farmed salmon (*Salmo salar*).
76. Cortés-Lara S, Urdiain M, Mora-Ruiz M, Prieto L, Rosselló-Móra R. Prokaryotic microbiota in the digestive cavity of the jellyfish *Cotylorhiza tuberculata*. Syst Appl Microbiol. 2015;38:494–500.
77. Boscaro V, Holt CC, Van Steenkiste NWL, Herranz M, Irwin NAT, Àlvarez-Campos P, et al. Microbiomes of microscopic marine invertebrates do not reveal signatures of phylosymbiosis. Nat Microbiol. 2022;7:810–9.
78. Ballinger MJ, Perlman SJ. The defensive *Spiroplasma*. Curr Opin Insect Sci. 2019;32:36–41.
79. Masson F, Rommelaere S, Marra A, Schüpfer F, Lemaitre B. Dual proteomics of Drosophila *melanogaster hemolymph* infected with the heritable endosymbiont *Spiroplasma poulsonii*. PLoS ONE. 2021;16 4 April.
80. Van De Water JAJM, Allemand D, Ferrier-Pagès C. Host-microbe interactions in octocoral holobionts - recent advances and perspectives. Microbiome. 2018;6.
81. Holm JB, Heidelberg KB. Microbiomes of *Muricea californica* and *M. fruticosa*: Comparative analyses of two co-occurring Eastern Pacific Octocorals. Front Microbiol. 2016;7:917..
82. Takemura AF, Chien DM, Polz MF. Associations and dynamics of vibrionaceae in the environment, from the genus to the population level. Front Microbiol. 2014;5: 38.
83. Pollock FJ, McMinds R, Smith S, Bourne DG, Willis BL, Medina M et al. Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. Nat Commun. 2018;9.
84. Weiland-Bräuer N, Neulinger SC, Pinnow N, Künzel S, Baines JF, Schmitz RA. Composition of bacterial communities associated with *Aurelia aurita* changes with compartment, life stage, and population. Appl Environ Microbiol. 2015;81:6038–52.
85. Tinta T, Zhao Z, Bayer B, Herndl GJ. Jellyfish detritus supports niche partitioning and metabolic interactions among pelagic marine bacteria. Microbiome. 2023;11.
86. Weber L, Gonzalez-Díaz P, Armenteros M, Apprill A. The coral ecosphere: A unique coral reef habitat that fosters coral–microbial interactions. Limnol Oceanogr. 2019;64:2373–88.
87. Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G. Environmental and gut *Bacteroidetes*: the food connection. Front Microbiol. 2011;2:93.
88. Salje J. Cells within cells: *Rickettsiales* and the obligate intracellular bacterial lifestyle. Nat Rev Microbiol. 2021;19:375–90.
89. Stabili L, Rizzo L, Fanizzi FP, Angilè F, Coco L, Del, Girelli CR et al. The jellyfish *Rhizostoma pulmo* (Cnidaria): biochemical composition of ovaries and antibacterial lysozyme-like activity of the oocyte lysate. Mar Drugs. 2019;17.
90. Ballesté E, Blanch AR. Persistence of Bacteroides species populations in a river as measured by molecular and culture techniques. Appl Environ Microbiol. 2010;76:7608–16.
91. Nishiyama K, Yokoi T, Sugiyama M, Osawa R, Mukai T, Okada N. Roles of the cell surface architecture of Bacteroides and Bifidobacterium in the gut colonization. Front Microbiol. 2021;12.
92. Qu CF, Song JM, Li N, Li XG, Yuan HM, Duan LQ, et al. Jellyfish (*Cyanea nozakii*) decomposition and its potential influence on marine environments studied via simulation experiments. Mar Pollut Bull. 2015;97:199–208.
93. Brinker P, Fontaine MC, Beukeboom LW, Falcao Salles J, Host. Symbionts, and the microbiome: the missing tripartite interaction. Trends Microbiol. 2019;27:480–8.
94. Lee MD, Kling JD, Araya R, Ceh J. Jellyfish life stages shape associated microbial communities, while a core microbiome is maintained across all. Front Microbiol. 2018;9 JUL.

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