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Differences in the microbiota of native and non-indigenous gelatinous zooplankton organisms in a low saline environment

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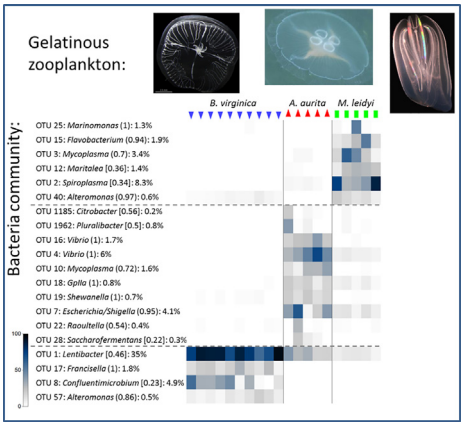
HIGHLIGHTS

- Non-indigenous species (NIS) are increasingly recognized as a matter of concern.
- The microbiome of native and NIS gelatinous zooplankton organisms are compared.
- Next generation sequencing confirms sign. Species specific microbiome differences.
- Indicator OTUs include bacteria which contain known pathogenic strains.
- Microbiome monitoring of NIS should be considered for aquaculture risk assessments.

GRAPHICAL ABSTRACT



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ABSTRACT

The translocation of non-indigenous species (NIS) around the world, especially in marine systems, is increasingly being recognized as a matter of concern. Species translocations have been shown to lead to wide ranging changes in food web structure and functioning. In addition to the direct effects of NIS, they could facilitate the accumulation or translocation of bacteria as part of their microbiomes. The Baltic Sea harbours many non-indigenous species, with most recent detection of the jellyfish *Blackfordia virginica* and the comb jelly *Mnemiopsis leidyi* in the low saline southwestern Baltic Sea. In this study, we used a multidisciplinary approach and investigated three gelatinous zooplankton species that co-occur in the same environment and feed on similar zooplankton food sources but show different histories of origin. The aim was to conduct a comparative microbiome analysis of indigenous and non-indigenous gelatinous zooplankton species in the low-saline southwestern Baltic Sea. Next-generation 16S rRNA marker gene sequencing of the V1/V2 region was employed to study the bacterial microbiome compositions. All tested species showed significant differences in their microbiome compositions (one way ANOSIM, $R = 1, P < 0.008$) with dissimilarities ranging from 85 to 92%. The indigenous jellyfish *Aurelia aurita* showed the highest bacterial operational taxonomic unit (OTU) richness. The overall differentiation

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between microbiomes was driven by eight indicator OTUs, which included *Mycoplasma* and *Vibrio* species. These bacteria can be problematic, as they include known pathogenic strains that are relevant to human health and aquaculture activities. Our results suggest that the impact assessment of NIS should consider potential pathogenic bacteria, enriched in the environment due to invasion, as potential risks to aquaculture activities.
Data availability:
The sequencing data were submitted to the European Nucleotide Archive and can be assessed under Accession No. PRJEB30924 for *Aurelia aurita* and *Mnemiopsis leidyi* (Rausch et al., 2019) and under Accession No. PRJEB34823 for *Blackfordia virginica*.

1. Introduction

The human-aided translocation of organisms outside their natural dispersal ranges is steadily increasing without showing signs of reaching a plateau (Seebens et al., 2017). The distribution of these non-indigenous species (NIS) can directly impact ecosystem structures and services (Walsh et al., 2016) and poses indirect effects by functioning as vectors for pathogens, parasites (Hohenadler et al., 2018) and novel, allochthonous bacteria. Bacteria are an important component of all multicellular organisms and form so-called metaorganisms along with their hosts (Bosch and McFall-Ngai, 2011; Esser et al., 2019). It has been discussed that associated bacteria can be introduced to new environments along with non-indigenous species as part of the microbiome (Basso et al., 2019). Additionally, it has been shown that the fish pathogen *Tenacibaculum maritimum,* a Gram-negative bacterium of the family Flavobacteriaceae, is a core microbiome member of the jellyfish species *Pelagia noctiluca* and *Phialella quadrata* (Ferguson et al., 2010; Delannoy et al., 2011). Similarly, other jellyfish microbiome members, such as certain *Flavobacterium* species and *Vibrio* species, have been shown to cause harm to farmed fish (Ferguson et al., 2010; Delannoy et al., 2011).

Jellyfish and comb jellies include many successful non-indigenous species (Bayha and Graham, 2014; Jaspers et al., 2018a) and have been hypothesized to increase in number due to global-change induced stressors (Richardson et al., 2009). However, the increase in jellyfish populations is currently under debate (Condon et al., 2012). In the low-saline environment of the southwestern Baltic Sea, two non-indigenous macro-zooplankton species, namely, the comb jelly *Mnemiopsis leidyi* (Costello et al., 2012; Jaspers et al., 2018a) and the jellyfish *Blackfordia virginica* (Jaspers et al., 2018b), as well as the native jellyfish *Aurelia aurita* (Möller, 1980a) are known.

Mnemiopsis leidyi is characterized by a long history of invasion in western Eurasia (as reviewed in Jaspers et al., 2018a) and has caused great and widespread impacts on the ecosystems it has invaded (e.g. Kideys, 2002; Tiselius and Møller, 2017). This species can reach very high abundances in invaded habitats, which is in part attributed to its high reproduction rate of >11,000 eggs individual^{-1} d^{-1} and the observed selection for earlier reproduction with higher size-specific reproduction rates in invaded habitats (Jaspers et al., 2015; Jaspers et al., 2018c). The newly discovered non-indigenous jellyfish species *B. virginica* is a prominent non-native species in many low saline environments around the world (Bardi and Marques, 2009). This species is characterized by a preference for brackish water bodies (Marques et al., 2017) and therefore the entire Baltic Sea is at risk for potential colonization. In addition to the two non-indigenous gelatinous macro-zooplankton species, the native jellyfish species *Aurelia aurita* is also present in the southwestern Baltic Sea. This native species is a common and voracious predator in the Baltic Sea (Möller, 1980b) but is invasive in many other areas of the world (Bayha and Graham, 2014).

Jellyfish and comb jellies can occur in great numbers and form blooms or aggregations during certain periods. High-abundance patches of jellyfish are often associated with spawning events and subsequent death after which the carcasses rapidly sink to the seafloor. These deposition events can lead to massive carbon export in certain areas from the surface to the seafloor (Steinberg and Landry, 2017) and dramatically impact microbial community compositions. It has been documented that rapid decomposition of jellyfish carcasses alters bacterial communities (West et al., 2009; Tinta et al., 2010) and leads to increases in *Vibrio* and *Pseudoalteromonas* species in the surrounding water (Tinta et al., 2012). Additionally, jellyfish and comb jellies are known to exude large amounts of dissolved organic carbon, which are rapidly consumed by the microbial community (Condon et al., 2011). In this manner, jellyfish and comb jellies fuel and restructure microbial communities (Riemann et al., 2006) in the pelagic zone in both native and invasive habitats (Condon et al., 2011; Dinasquet et al., 2012). For example, the arrival of non-indigenous gelatinous zooplankton species was shown to impact the bacterioplankton community composition in a coastal Italian lagoon in July 2013 (Manzari et al., 2015). Hence, jellyfish and comb jelly species have the potential to alter bacterial community compositions and additionally, to introduce allochthonous bacterial strains (Basso et al., 2019), such as fish pathogens. To date, this property of non-indigenous species as potential sources of microbial pathogen translocation and accumulation has largely been neglected in marine ecology. In this study, we compare the microbiota community compositions of native and non-indigenous jellyfish and comb jelly species in the low saline environment of the southwestern Baltic Sea.

The first objective of the study was to characterize the microbiome of three gelatinous macro-zooplankton species that share a similar environment and food source. The second objective was to investigate if potential fish pathogens are present in the microbiomes of the respective native and non-indigenous bloom-forming gelatinous zooplankton species of the low saline southwestern Baltic Sea. The accumulation of certain bacteria could be of high relevance to aquaculture activities, and microbiome monitoring could be used to guide risk assessments to sustain fish health during global change.

2. Materials and methods

2.1. Sampling and tissue preparation

Three gelatinous macro-zooplankton species, namely, the jellyfish *Aurelia aurita* (native scyphozoan, n = 5), *Blackfordia virginica* (non-indigenous hydrozoan, n = 10) and the comb jelly *Mnemiopsis leidyi* (non-indigenous ctenophore, n = 5) were sampled in low saline environments of the southwestern Baltic Sea to characterize their microbiomes (see Table 1 for metadata).

Aurelia aurita (Linnaeus, 1758) medusae (average diameter 23 cm, n = 5) were individually collected with a 1-cm mesh dip net attached to a long stick in the Eckernförder Bight, southwestern Baltic Sea (54.463 N, 9.843 E) in June 2016 (Rausch et al., 2019). Individuals of the non-indigenous jellyfish species *Blackfordia virginica* (Mayer, 1910) were caught (average size ca. 1 cm, n = 10) with a ladle in the Kiel Canal (54.345 N, 9.983 E) on 28.6.2017. Additionally, five

Table 1
Metadata of native (*Aurelia aurita*, n = 5), invasive (*Blackfordia virginica*, n = 10) jelly fish and invasive comb jelly (*Mnemiopsis leidyi*, n = 5) species used for microbiome analyses.

Species	Sampling location	Date	Temp. (°C)	Salinity (ppt)
Aurelia aurita	54.463N, 9.843E	28.6.2016	17.1	16.8
Blackfordia virginica	54.345N, 9.983E	28.6.2017	18.4	10
Mnemiopsis leidyi	54.330N, 10.15E	6.9.2016	20.0	15.9

individuals of the non-indigenous and invasive comb jelly *Mnemiopsis leidyi* (Agassiz, 1865) (average oral-aboral length ca. 4 cm) were sampled with a ladle in Kiel Bight, Baltic Sea (54.330 N, 10.150 E) on 6.9.2016 (Rausch et al., 2019). All the organisms were caught at their peak abundance, which occurs during summer in the SW Baltic Sea. The sampling regions are in close proximity to each other and are characterized by similar physical and biological conditions throughout summer. As indicated by a previous bacterioplankton investigation conducted along the entire salinity gradient of the Baltic Sea, the southwestern Baltic shows very high similarity in the bacterial community composition, which forms one cluster (Herlemann et al., 2011). Although seasonality might impact bacterial community compositions, this investigation explicitly focused on gelatinous zooplankton as potential vectors for allochthonous bacteria. After capture, all the animals were transported individually in containers with >15 L ambient seawater to the laboratory at the Institute for General Microbiology, University of Kiel, Germany. In the laboratory, all the animals were confirmed to be healthy and actively swimming and were individually washed three times with sterile artificial seawater of the appropriate salinity before DNA isolation. The washing procedure consisted of transferring the animals with a soup spoon into sterile glass beakers filled with sterile filtered sea water, and this procedure was repeated three times. New water was used for each wash to remove transient bacteria and bacteria from the surrounding seawater. All the animals had empty guts to avoid bacterial contamination from food before the tissues were dissociated overnight at 4 °C with 1 mg/mL collagenase (Sigma-Aldrich, St. Louis, USA). Collagenase was used to dissociate the tissues because physical homogenization has been shown to be insufficient for gelatinous tissue samples. The homogenates were filtered through sterile 10-μm nylon gauze followed by the addition of 0.1% IGEPAL CA-630 (Sigma-Aldrich). The samples were centrifuged for 25 min at 300 xg at 4 °C. The supernatants, including the prokaryotic fraction, were centrifuged for 5 min at 7500 xg.

2.2. DNA isolation and 16S rRNA gene amplicon sequencing

DNA was extracted using the Wizard genomic purification kit (Promega, Madison, WI, USA). The pellets from eukaryotic/prokaryotic cell separation were homogenized in 480 μL 50 mM EDTA and incubated at 37 °C for 30 min after the addition of 10 mg/mL lysozyme (Carl Roth, Karlsruhe, Germany) and 60 units Proteinase K (Life Technologies, Darmstadt, Germany). The remaining preparation steps were performed according to the manufacturer's protocol.

The 16S rRNA gene was amplified using uniquely barcoded primers, which flank the V1 - V2 hypervariable regions (27F-338R) and are fused to MiSeq adapters and heterogeneity spacers (Fadrosh et al., 2014). PCRs were performed in either a 20 L reaction volume (*B. virginica*: including 0.4 μL of each forward and reverse primer (5 μM), 0.4 μL dNTP mixture (10 mM), 0.2 μL Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, USA) (2 U/μL), 4 μL HF buffer (Thermo Fisher Scientific) and 2 μL 10 ng/μL DNA template) or a 25 μL reaction volume for *A. aurita* and *M. leidyi* (see Rausch et al., 2019). PCRs were conducted with the following cycling conditions: *B. virginica*, 1 min 95 °C, 30× [15 s 95 °C, 30 s 52 °C, 30 s 72 °C], 5 min 72 °C and for *A. aurita* and *M. leidyi*, 0.1 min 98 °C, 30× [9 s 98 °C, 60 s 55 °C, 90 s 72 °C], 10 min 72 °C. The PCR products were examined on agarose gel.

The concentrations of the amplicons were estimated using a Gel Doc System with 2 μL of GeneRulerTM Low Range DNA Ladder or 3 μL of O'GeneRulerTM 100 bp Plus DNA Ladder (Thermo Fisher Scientific) as the internal standard for band intensity measurements. Size-checked amplicons were excised from the gel and cleaned using a MinElute Gel Extraction kit (Qiagen, Hilden, Germany). The samples of individual gels were pooled into approximately equimolar sub-pools as indicated by band intensity and were measured with the Qubit dsDNA br Assay Kit (Life Technologies GmbH, Darmstadt, Germany).

The sub-pools were mixed in an equimolar fashion and stored at −20 °C until sequencing. Amplicon sequencing was performed on the Illumina MiSeq platform with v3 chemistry (2 × 300 bp) at the Institute of Clinical Molecular Biology's Sequencing Center (Kiel University, Germany) and Max-Planck Institute for Evolutionary Biology (Germany). The sequence data for *A. aurita* and *M. leidyi* have been re-analysed from Rausch et al. (2019).

A comparison of sequencing runs using slightly modified protocols, as outlined above, showed negligible sequencing run effects of 5.8%, while the biological effect size was 1 order of magnitude greater (58.5%). In detail, we compared the sequencing results using water from one distinct salinity experiment, which was replicated with 7 groups. Each of the 7 groups was sampled at the same time with 2 to 3 replicates per group. Statistical analyses confirmed a significant group effect; therefore, all the biological groups (biological replicates) were significantly different (PERMANOVA: F-pseudo$_{6,36}$ = 6.5985, P = 0.001, 997 permutations) without significant interaction between "group x sequencing run" (F-pseudo$_{6,36}$ = 0.66, P = 0.99, 999 permutations). The additional post hoc analyses with pairwise testing showed that within each of the 7 groups, no effects from the sequencing runs were detected (Supplementary Table S1, P > 0.09).

2.3. Bio-informatics and statistical analyses

All the raw reads were analysed together with the same pipeline. Demultiplexing was performed using the Bcl2fastq module in CASAVA 1.8.2, and no mismatches in the forward and reverse index reads were allowed. Sickle (Joshi and Fass, 2011) was used with default settings in paired-end mode for the trimming of the forward and reverse amplicon reads. The downstream data processing (e.g., read merging, quality control, OTU clustering and chimaera filtering) was performed using VSEARCH (v.2.5) (Rognes et al., 2016). Assignments of taxonomic annotations from the phylum to genus levels (where possible) to the clean OTU sequences were performed using the SINTAX (Edgar, 2016) algorithm implemented in USEARCH software (v.9) (Edgar, 2010) and the ribosomal database project (RDP) (Cole et al., 2014) training set 14 as a reference.

All the raw reads were rarefied to 10 K reads for further statistical analyses. Taxonomic assemblages were inferred from non-chimeric operational taxonomic units using mothur at a 97% pairwise similarity cut-off. The OTU abundances were summarized at the genus level, and the bar plots were grouped according to species or according to the lowest possible taxonomic entity (genus level 6). Taxa with relative abundances of <0.7% across all the samples were subsumed under "other bacteria". The average effective OTU richness was subsequently calculated as the number of different OTUs per individual and presented as the average ± standard deviation (SD). Significant differences in OTU richness between groups were tested using one-way ANOVA and a Student-Newman-Keuls post hoc comparison. To account for the large number of zeros in the dataset and very high abundance reads for few operational taxonomic units (OTUs), raw read abundances were square-root transformed before calculation of the Bray-Curtis similarity matrix (Clarke and Green, 1988), and subsequent statistical analyses using the software package PRIMER-e V7. One-way SIMPER analyses were performed using a 70% cut-off. One-way ANOSIM was performed using species as group levels and a pairwise Spearman rank test with a total number of permutations of 999. Multivariate analyses using clustering and ordination techniques to indicate relationships between sample groups were employed. We used principal coordinate analyses (PCoA) with vector overlays using multiple correlation types and a vector correlation >0.2 along with a PERMANOVA to test for significant bacteria OTU differences between species using 998 permutations. Additionally, a heat map was generated using the twenty most abundant OTUs, which were representative of >75% of all raw sequences reads. Beta diversity analysis demonstrated that the bacterial communities of the examined gelatinous macro-zooplankton species were

Table 2
Average microbiome % dissimilarity between the native and invasive jellyfish/comb jelly species in the SW Baltic Sea. One-way SIMPER analysis (70% cut-off) showed high microbiome dissimilarity (> 85%) with a very high separation of all the groups tested (one-way ANOSIM: $R = 1$, $P < 0.008$).

Group comparisons	Average dissimilarity (%)
B. virginica vs *M. leidyi*	91.70
B. virginica vs *A. aurita*	91.16
M. leidyi vs *A. aurita*	85.16

different from the community present in the water column and the control samples (Supplementary Figure - Fig. S1).

3. Results

The characterization of the microbiomes of both the invasive and native comb jelly and jellyfish species in the low saline southwestern Baltic Sea indicates that all the species exhibit distinct bacterial communities. Based on the analyses of 1800 operational taxonomic units (OTUs), all three gelatinous macro-zooplankton species were characterized by a significant separation between their respective microbiomes (one-way ANOSIM $R = 1$, $P < 0.008$) with an average dissimilarity between species ranging from 85 to 92% (Table 2). Analyses of the general bacterial community composition by use of the species identities of the most abundant OTUs showed that the key taxa that characterized the three zooplankton species differed considerably. Identity clustering was based on a 97% cut-off with posterior probabilities calculated with a specific taxon level cut-off of 80% for each OTU and classification to the lowest possible taxon level (genus) if possible; otherwise, higher taxonomic grouping is presented. Hence, Fig. 1 depicts single high-abundance OTUs that have been identified to the lowest possible taxonomic level. The results indicate that *Blackfordia virginica* was characterized by a high percentage of unclassified Rhodobacteraceae and Proteobacteria while the native jellyfish *Aurelia aurita* was characterized by a high fraction of *Vibrio*, *Mycoplasma* and unclassified Enterobacteriaceae (Fig. 1). For the invasive comb jelly *Mnemiopsis leidyi*, *Spiroplasma*, *Mycoplasma* and unclassified Alphaproteobacteria dominated the community composition (Fig. 1). For all three gelatinous zooplankton species, unclassified Rhodobacteraceae were among the most important taxa; however, their abundance only dominated the microbiome community of the invasive jellyfish *B. virginica* (Fig. 1).

The in-depth investigation of microbiome similarity within each species showed that 28% of the similarity of the invasive jellyfish *B. virginica* was driven by two unclassified OTUs from the family Rhodobacteraceae and the class Alphaproteobacteria (Table 3). For the native jellyfish *A. aurita*, > 9% of the similarity was due to one OTU from the genus *Vibrio*, and > 11% of the microbiome similarity of the invasive comb jelly *M. leidyi* was due to an unclassified OTU from the phylum Tenericutes (Table 3). In general, few abundant OTUs per species contributed to the similarities. Among all three gelatinous macro-zooplankton species, a total of 15 OTUs contributed three or more percent to the average similarity per species (see Table 3 for details and OTU identities). Alpha diversity was measured as the difference between the effective OTU richness per species (Fig. 2). The microbiome diversities showed significant differences between all species (one-way ANOVA $F_{2,19} = 4.52$, $P = 0.027$). We found the highest diversity in the bacterial community composition of the native jellyfish *A. aurita* (Fig. 2). Regardless of the dominance of unclassified Rhodobacteraceae in the invasive jellyfish *B. virginica*, their overall effective OTU richness

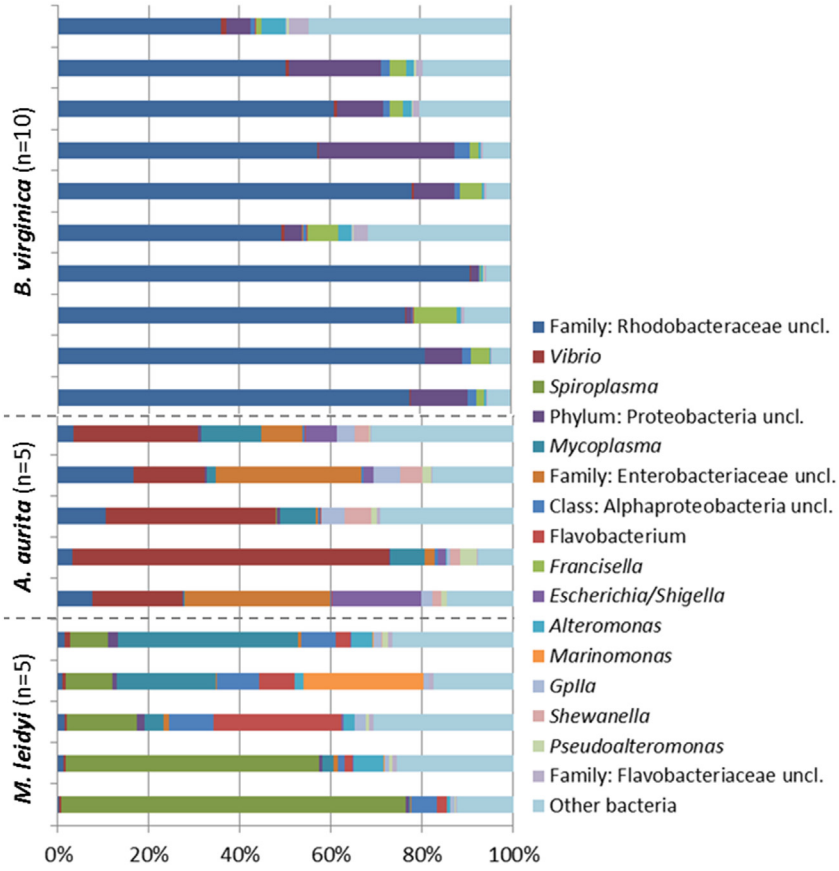

Fig. 1. Bar plot of the most important bacterial OTUs contributing to the microbiome of native and invasive jellyfish/comb jelly species in low saline areas of the Baltic Sea. Identity clustering is based on a 97% cut-off with posterior probabilities calculated with a specific taxon level cut-off of 80% for each OTU and classification to the lowest possible taxon level (genus), if possible; otherwise, a higher taxonomic grouping is presented. The lower-abundance bacterial OTUs are grouped under "other bacteria".

Table 3
Key bacterial OTUs responsible for >3% of the average similarity of the microbiomes of the jellyfish a) *Blackfordia virginica* (n = 10, av. similarity = 54.7%), b) *Aurelia aurita* (n = 5, av. similarity = 45.3%) and c) the comb jelly *Mnemiopsis leidyi* (n = 5, av. similarity = 42.7%), including the respective percent similarity contribution (% contr.) and taxonomic identity of the respective bacterial OTU, which is shown in descending order from the highest taxon level 2 (phylum) to the lowest taxon level 6 (genus) along with bootstrap values (in brackets).

Group	OTU	% contr.	OTU identity
a) *Blackfordia virginica*	1*	22.9	Proteobacteria (1), Alphaproteobacteria (1), Rhodobacterales (1), Rhodobacteraceae (1), *Lentibacter* (0.46)
	8*	4.7	Proteobacteria (0.99), Alphaproteobacteria (0.93), Rhodobacterales (0.43), Rhodobacteraceae (0.43), *Confluentimicrobium* (0.23)
	17	3.9	Proteobacteria (1), Gammaproteobacteria (1), Thiotrichales (1), Francisellaceae (1), *Francisella* (1)
b)	2*	11.7	Tenericutes (0.58), Mollicutes (0.58), Entomoplasmatales (0.5), Spiroplasmataceae (0.34), *Spiroplasma* (0.34)
Mnemiopsis leidyi	12	5.5	Proteobacteria (0.99), Alphaproteobacteria (0.68), Rhizobiales (0.4), Hyphomicrobiaceae (0.36), *Maritalea* (0.36)
	3*	4.6	Tenericutes (0.71), Mollicutes (0.71), Mycoplasmatales (0.7), Mycoplasmataceae (0.7), *Mycoplasma* (0.7)
	15*	4.14	Bacteroidetes (1), Flavobacteriia (1), Flavobacteriales (1), Flavobacteriaceae (1), *Flavobacterium* (0.94)
	40	3.07	Proteobacteria (1), Gammaproteobacteria (1), Alteromonadales (1), Alteromonadaceae (1), *Alteromonas* (0.97)
c) *Aurelia aurita*	4*	9.15	Proteobacteria (1), Gammaproteobacteria (1), Vibrionales (1), Vibrionaceae (1), *Vibrio* (1)
	1	5.3	Proteobacteria (1), Alphaproteobacteria (1), Rhodobacterales (1), Rhodobacteraceae (1), *Lentibacter* (0.46)
	16	5.3	Proteobacteria(1), Gammaproteobacteria(1), Vibrionales(1), Vibrionaceae(1), *Vibrio*(1): OTU16
	7*	4.9	Proteobacteria (1), Gammaproteobacteria (1), Enterobacteriales (1), Enterobacteriaceae (1), *Escherichia/Shigella* (0.95)
	10*	3.6	Tenericutes (0.72), Mollicutes (0.72), Mycoplasmatales (0.72), Mycoplasmataceae (0.72), *Mycoplasma* (0.72)
	19	3.5	Proteobacteria (1), Gammaproteobacteria (1), Alteromonadales (1), Shewanellaceae (1), *Shewanella* (1)
	18	3.1	Cyanobacteria/Chloroplast (1), Cyanobacteria (1), Family II (1), Family II (1), *GpIIa* (1)

* Indicator species from vector overlay of principal coordinate analyses for axis 1 and 2 (see Fig. 3) are highlighted.

was intermediate compared to *A. aurita* and the invasive comb jelly *M. leidyi*, which was characterized by the lowest diversity of the bacterial community found across all three investigated species (Fig. 2).

Additionally, we found very high separation of the microbiomes for the three gelatinous macro-zooplankton species (e.g., one-way ANOSIM $R = 1$, $P < 0.008$, Table 2), which was corroborated by the results from multivariate statistical analysis (Fig. 3). Based on principal coordinate analysis using 1800 bacteria OTUs per individual, we confirmed distinct species clusters that were significantly different from each other (PERMANOVA: F-pseudo$_{2,17} = 14.0$, $P = 0.001$). The first two coordinates of the principal coordinate analysis explained 62.5% of the total variation (Fig. 3). Vector overlay with multiple correlation type analyses and vector selection based on a correlation >20% resulted in eight identified indicator OTUs that drove this differentiation. Among those indicator OTUs for the native jellyfish *A. aurita* and the invasive comb jelly *M. leidyi* are *Vibrio* species, Flavobacteriia as well as *Mycoplasma* species. The indicator OTUs for the invasive jellyfish *B. virginica* showed a very low identity bootstrap value, which indicated that these two bacteria OTUs are thus far unknown members from the family Rhodobacteraceae (OTU1) and the class Alphaproteobacteria (OTU8). Interestingly, the latter OTU was not present in either

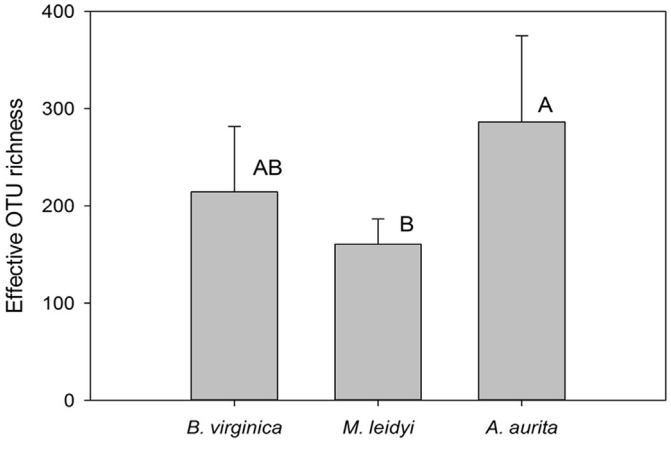

Fig. 2. Comparison of the average effective OTU richness (± SD) of the microbiomes of three different jellyfish/comb jelly species of native and invasive origins in the low saline SW Baltic Sea showed significant differences between groups (one-way ANOVA $F_{2,19} = 4.52$, $P = 0.027$). *A. aurita* harboured the most diverse microbiome with the highest average OTU richness, which was significantly different from the lowest richness found in *M. leidyi* (Student-Newman-Keuls post hoc comparison, $P = 0.021$, A vs B), while the newly invasive jellyfish *B. virginica* showed intermediate richness without significant differences relative to the other species (Student-Newman-Keuls post hoc comparison, $P > 0.07$, AB vs A and B).

M. leidyi or *A. aurita* (Fig. 4, Supplementary Table S2 and S3). When examining the raw sequence read abundances, > 75% of all the OTU reads were attributed to 20 different OTUs, for which 6 were characteristic for *M. leidyi*, 10 for *A. aurita* and 4 for *B. virginica* (Fig. 4). Hence, even though we observed high diversity in the bacterial community with 1800 bacteria OTUs, only relatively few were responsible for driving the major differences between the investigated native and invasive jellyfish and comb jelly species.

4. Discussion

Bacteria are important components of all multicellular organisms (Bosch and McFall-Ngai, 2011; Esser et al., 2019), as has recently been highlighted by a paradigm shift in ecology that reflects the overwhelming importance of microbes for the functioning and fitness of animal hosts (McFall-Ngai et al., 2013; Rees et al., 2018). For marine animals, it has been demonstrated that bacteria can modulate life history decisions, such as metamorphosis, and induce settlement to the benthos (as reviewed in Wahl et al., 2012). Additionally, for marine chanoflagellates, it has been shown that bacterial exudates can trigger sexual reproduction (Woznica et al., 2017) as well as multicellularity development (Alegado et al., 2012) and cnidarian-associated microbes potentially engage in functional cross-talk (Stabili et al., 2018). Regardless of the importance of microbes for the fitness and survival of multicellular hosts, the functioning of non-indigenous species as potential vectors or for the accumulation of potential pathogenic bacteria in marine habitats has received little attention. This phenomenon can be especially widespread in gelatinous zooplankton species, as they are known to form blooms with very high abundance and include some highly potent invasive species. In the present study, we showed that our three investigated native and non-indigenous gelatinous zooplankton species all harbour specific and distinct bacterial communities during summer in the low saline environments of the southwestern Baltic Sea. Even though these species co-occur, share the same environment, and consume similar zooplankton food sources, we identified significant differences in their microbiomes. However, further time points should be sampled to confirm this pattern throughout different seasons. The identified indicator bacteria OTUs, which are responsible for the microbiome differentiations of the gelatinous macro-zooplankton species, include *Flavobacterium, Vibrio* and *Mycoplasma* species. Bacteria from these genera are known to contain species that are pathogenic to fish and shellfish and can cause potential conflict with aquaculture activities (Austin et al., 1997; Loch and Faisal, 2015; Dubert et al., 2017). In total, we identified one *Flavo*-bacterium (OTU15), which is characteristic of the invasive comb jelly *M. leidyi*, and two different *Vibrio*-bacteria (OTU16 and 4), which are characteristic of the native jellyfish *A. aurita*.

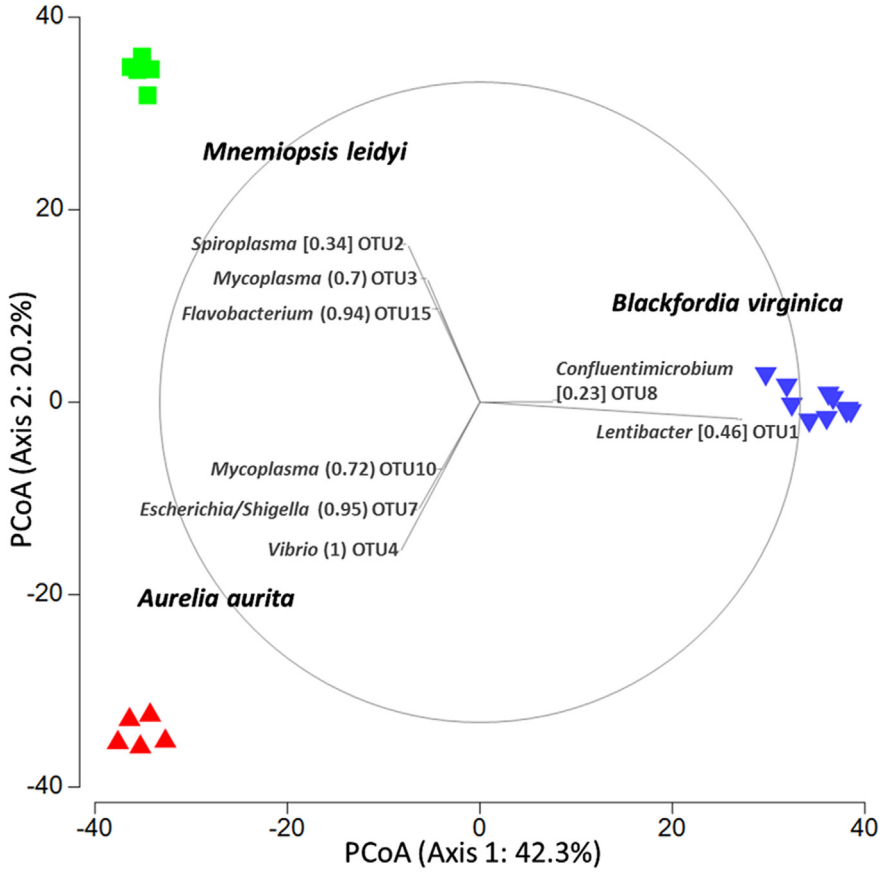

Fig. 3. Principal coordinate analyses (PCoA) of bacterial diversity of the most important bacterial OTUs that contributed to the distinct microbiome of native (*Aurelia aurita*, red triangle) and invasive (*Blackfordia virginica*, blue triangle) jellyfish as well as one invasive comb jelly (*Mnemiopsis leidyi*, green square) in low saline environments of the SW Baltic Sea. The first two axes explained 62.5% of the total variation with significant differences between the microbiomes of all three groups tested (PERMANOVA: F-pseudo$_{2,17}$ = 14.0, P = 0.001, 998 permutations with a pairwise post hoc test of all groups $P < 0.005$). Overlay vectors using multiple correlation types with a vector correlation >0.2 indicated eight bacteria OTUs driving the differentiation between jellyfish/comb jelly species (specified by bacteria OTU number and genus). Note: respective bootstrap values for OTUs are given in brackets, and the squared brackets indicate values < 0.7 – see Table 3 for full identities. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

One of the *Vibrio* species was traced to species level and was classified as *Vibrio splendidus* or *V. crassostreae* (both 100% query coverage and identity match), which are suspected shellfish and fish pathogens (Austin et al., 1997). None of the other OTUs led to a direct pathogen hit. In other European water systems, native jellyfish have been hypothesized to potentially harm aquaculture activities, as their microbiomes harbour potential fish pathogens (Basso et al., 2019), which have been shown to lead to secondary infections in farmed salmon (Ferguson et al., 2010). In this context, the bacterium *Tenacibaculum maritimum*, which is associated with the hydrozoan jellyfish *Phialella quadrata* (Ferguson et al., 2010) and the jellyfish *Pelagia noctiluca* (Delannoy et al., 2011), has received considerable attention. Although we did not precisely classify *T. maritimum* (belonging to Bacteroidetes – Flavobacteria – Flavobacteriales – Flavobacteriaceae -Tenacibaculum) as a member of the microbiome of our investigated native and invasive jellyfish and comb jelly species, it is conceivable that this known fish pathogen hides behind unclassified OTUs belonging to the phylum Bacteroidetes, as it has been documented as a core member of the microbiome of other jellyfish species (Ferguson et al., 2010; Delannoy et al., 2011). As jellyfish and comb jellies form blooms or aggregations during certain periods, these high density accumulations could act as modulators for the microbial community. For example, it has been shown that the decaying jellyfish species *Aurelia aurita*, *Pelagia noctiluca*, and *Rhizostoma pulmo* impact microbial communities. The rapid degradation of jellyfish carcasses has been documented to lead to an increase in jellyfish-associated bacteria and a dramatic change in the free-living bacterial community composition with e.g., an increase in *Vibrio* and *Pseudoalteromonas* species in the surrounding waters (Tinta et al., 2012). Similarly, it has been shown that the non-indigenous comb jelly *M. leidyi* has an impact on free-living microbial community compositions in invaded areas. This includes a positive growth response and selection for certain bacteria, such as Flavobacteriaceae,in the vicinity of the comb jelly (Dinasquet et al., 2012). In detail, when comparing waters that had been in contact with *M. leidyi* to control waters, an increase in Flavobacteriaceae and *Alteromonas* species was observed (Dinasquet et al., 2012). Due to the high densities of *M. leidyi* in invaded areas, e.g., Limfjorden, Denmark (Riisgård et al., 2007) and the Dutch Wadden Sea (van Walraven et al., 2013; Jaspers et al., 2018a), it can be hypothesized that this non-indigenous species impacts local bacterioplankton community compositions. Similarly, it has been shown that jellyfish blooms can lead to significant changes in bacterial community compositions due to the release of jellyfish-derived dissolved organic carbon, which favours rapid growth of certain bacterial strains, such as Gamma-proteobacteria (Condon et al., 2011).

We identified *Mycoplasma*-bacteria as a key component and indicator taxa for the microbiome of the jellyfish *A. aurita*. Similar results have previously been presented by Weiland-Bräuer et al. (2015), who suggested that *Mycoplasma*-bacteria are important endosymbionts of *A. aurita*. Interestingly, we additionally confirmed that *Mycoplasma*-bacteria are important members of the microbiome of the invasive comb jelly *M. leidyi* and have similarly been documented as members of the microbiome of *M. leidyi* in native habitats (Daniels and Breitbart, 2012). In our study, *Mycoplasma* bacteria were among the three indicator bacteria OTUs that characterized the differentiation

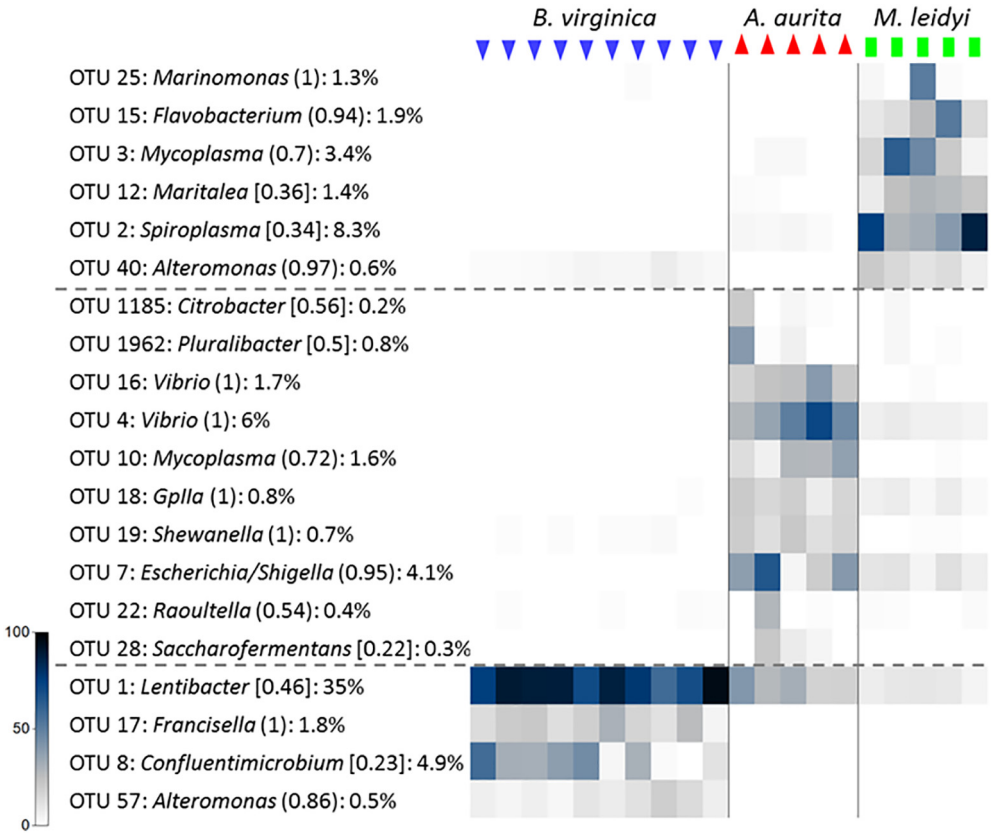

Fig. 4. Heat map of the twenty most abundant representative OTUs (> 75% of all sequences) of the core microbiomes of the invasive and native jellyfish species *Blackfordia virginica* (blue triangle, n = 10) and *Aurelia aurita* (red triangle, n = 5) and the invasive comb jelly *Mnemiopsis leidyi* (green square, n = 5). OTUs are specified at the genus level, and the respective bootstrap values are given in brackets [squared brackets indicate values <0.7], along with the % contribution of each OTU to the total sequence read numbers. The heat map indicates standardized (square root transformed) read numbers per bacteria OTU ranging from zero (white) to 100 (dark blue). See Table 3 and Supplementary Table S1 for detailed OTU identities. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

between our analysed gelatinous zooplankton species. To date, the function of *Mycoplasma* bacteria on gelatinous zooplankton remains unknown.

In conclusion, although all three investigated species of gelatinous zooplankton shared the same water and food sources, the differences in their respective microbiome compositions were remarkable and warrant future studies to address the potential function of particular bacterial groups. For future investigations, dedicated experiments are needed to understand the microbiota-host interactions, the importance of *Mycoplasma* bacteria, and the mechanisms of transfer of the pathogenic *Flavobacterium* and *Vibrio* species to e.g., caged fish or shellfish aquaculture facilities. Although microbiome changes throughout the seasons should be considered in future investigations, our results suggest that the potential spread or accumulation of pathogenic bacteria from the environment via the jellyfish could lead to potential risk for aquaculture activities, especially during jellyfish blooms.

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CRediT authorship contribution statement

Cornelia Jaspers: Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing - original draft, Writing - review & editing. **Nancy Weiland-Bräuer:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Malte C. Rühlemann:** Data curation, Formal analysis, Writing - review & editing. **John F. Baines:** Funding acquisition, Writing - review & editing. **Ruth A. Schmitz:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing. **Thorsten B.H. Reusch:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139471.

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