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Bacterial communities associated with scyphomedusae at Helgoland Roads

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Abstract

Different modes of asexual and sexual reproduction are typical for the life history of scyphozoans, and numerous studies have focused on general life history distribution, reproductive strategies, strobilation-inducing factors, growth rates, and predatory effects of medusae. However, bacteria associated with different life stages of Scyphozoa have received less attention. In this study, bacterial communities associated with different body compartments and different life stages of two common scyphomedusae (*Cyanea lamarckii* and *Chrysaora hysoscella*) were analyzed via automated ribosomal intergenic spacer analysis (ARISA). We found that the bacterial community associated with these two species showed species-specific structuring. In addition, we observed significant differences between the bacterial communities associated with the umbrella and other body compartments (gonads and tentacles) of the two scyphomedusan species. Bacterial community structure varied from the early planula to the polyp and adult medusa stages. We also found that the free-living and particle-associated bacterial communities associated with different food sources had no impact on the bacterial community associated with fed polyps.

Introduction

Jellyfish represent a conspicuous element of the zooplankton as a major component of the pelagic system (Brodeur 2002). Scyphomedusae utilize a wide variety of zooplankton prey, including fish larvae and eggs, copepods, small ctenophores,

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and can have a strong impact on zooplankton standing stocks in all parts of the world (Brodeur 1998; Barz and Hirche 2007; Decker et al. 2007). In recent decades, the composition of phyto- and zooplankton communities changed considerably (Beaugrand et al. 2002; Beaugrand 2004) accompanied by increasing frequency and intensity of scyphomedusa outbreaks around the world (Brodeur et al. 1999; Brodeur 2002; Beaugrand 2004; Hay 2006; Barz and Hirche 2007). In the North Sea, the abundance of scyphomedusae has shown interannual fluctuations and large variability between regions (Hay et al. 1990).

Marine microorganisms, especially marine symbiotic bacteria, are thought to be the true producers of some active metabolites found in marine invertebrates (Peraud 2006). The epimicrobial community has been found to be important in the larval settlement processes of many marine invertebrates (Wieczorek and Todd 1998), including sponges (Woollacott and Hadfield 1996), cnidarians (Bosch 2013), ascidians (Wahl et al. 1994; Schuett et al. 2005), and bryozoans (Pukall et al. 2001; Kittelmann and Harder 2005), where bacteria and/or their products induce the settlement and metamorphosis of these organisms (Müller and Leitz 2002). Interestingly, jellyfish have

diverse symbiotic relationships with various creatures, ranging from macro-organisms such as shrimp (Bruce 1995) and crustaceans (Sal Moyano et al. 2012; Alvarez-Tello et al. 2013) to microorganisms such as dinoflagellates (Lampert et al. 2012; Mellas et al. 2014). These jellyfish-associated microorganisms may be an untapped source of new marine natural products. Meanwhile, bottom-up effects of bacteria associated with the presence and activity of different life stages of scyphozoans have received less attention. Endobiotic bacteria were detected in the tentacles of jellyfish species *Cyanea capillata* and *Cyanea lamarckii* (Schuett and Doepke, 2010). Fraune and Cooper (2010) described bacterial colonization during the early embryonic development of *Hydra* (Cnidaria, Hydrozoa). Cleary et al. (2016) investigated bacterial community composition associated with the scyphozoan *Mastigias* cf. *papuaetpisoni* and box jellyfish *Tripedalia cf. cystophora*. Viver et al. (2017) reported the gastric microbiome of the jellyfish *Cotylorhiza tuberculata* using catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH). Cortés-Lara et al. (2015) presented data on the microbiota associated with the gastric cavity of jellyfish *C. tuberculata*, which showed low diversity and included the representatives of the genera *Spiroplasma*, *Thalassospira*, *Tenacibaculum*, and *Vibrio*. Weiland-Bräuer et al. (2015) analyzed the microbiota community structure associated with *Aurelia aurita* regarding to different life stages, compartments, and populations. Daley et al. (2016) focused on the bacterial communities associated with the invasive hydrozoan *Nemopsis bachei* and the moon jellyfish *A. aurita* (Cnidaria). However, little is known about the early steps of bacterial colonization in planula larvae or in scyphozoan polyps, the establishment and duration of the associated microbiota, especially after strobilation. This information would represent a first step in understanding bacterial involvement in controlling reproductive mechanisms related to jellyfish blooms. The knowledge on the interrelations between bacteria communities and different life stages in Scyphozoa could help to provide profound insights into understanding microbe-dependent life histories and their evolutionary consequences.

The present study focused on *Cyanea lamarckii* and *Chrysaora hysoscella* (Russell, 1970), which are common scyphomedusan species at Helgoland Roads in the German Bight. The medusae usually occur during the summer season (Möller 1980; Hay et al. 1990; Barz and Hirche 2007). The bacterial communities present in planulae, polyps, and medusae were determined using automated ribosomal intergenic spacer analysis (ARISA). We aimed to answer the following questions: (1) Do different body compartments of these scyphomedusan species contain different bacterial communities? (2) Is there a transition of the bacterial community from larvae to medusae during the different life stages? (3) Do different food types influence the bacterial community associated with polyps? (4) Do different scyphomedusan species harbor different bacterial communities?

Materials and methods

Sample collection and preparation

Intact individual medusae of the scyphozoan species *Cyanea lamarckii* and *Chrysaora hysoscella* were collected around the Helgoland Road station in the German Bight (54°11.3′N, 7°54.0′E) from May to July 2011 twice a week using a 500-µm mesh trawl towed by the research vessel "Aade" or by using a bucket with a long handle. The samples were transferred to the laboratory within 1 h after collection. The specimens were identified to the species level based on morphological features according to Russell (1970) and Holst (2012). Five intact individuals of each species were collected on each day of sampling which applied to analyze the bacterial community of the different body compartments. Medusae were dissected under the stereomicroscope using sterile forceps and scissors. Umbrella, gonad, tentacle, and mouth arm were separated, and then rinsed five times with sterilized seawater (0.2 µm filtration and autoclaving) in order to eliminate transient and loosely attached microorganisms from the surface of the scyphomedusae. All samples were frozen at −20 °C and lyophilized prior to molecular manipulation. In addition, at least one mature medusa carrying planula larvae was chosen at each sampling day and transferred into a 30-L aquarium (sterilized seawater) for planula collection. Released planulae were collected from the bottom of the aquarium using a pipette, and then rinsed with sterile seawater. Parallel samples of single planulae were separated using a sterile capillary pipette for subsequent molecular analysis, and the remaining rinsed planulae were retained for subsequent larval settlement experiments.

Planula settlement

Larval settlement was performed as described in the protocol of Holst and Jarms (2010) with slight modifications. Three replicates were set up for each medusa specimen. Twenty milliliters of concentrated planula suspension was pipetted into 100 mL sterile plastic jars containing 15–20 mL sterile seawater at situ temperature (15 ± 2 °C). The lid of one polystyrene petri dish (47 mm diameter) was placed on the water surface in each jar to provide a settlement substrate (Brewer 1976, 1984; Holst and Jarms 2007). The water from each jar was carefully replaced with fresh sterile seawater every 48 h after planulae had settled on the undersides of the floating lids.

Feeding of polyps

Settled polyps of each species were fed twice a week using two different food sources, with one group receiving brine shrimp (*Artemia salina*) nauplii (hereinafter referred to as *Artemia*) reared in sterile seawater from eggs in the laboratory,

and one group receiving natural zooplankton collected at Helgoland Roads using a 75-μm mesh size plankton net on the day of feeding. Feeding with both food types was conducted at the same time and in the same manner for both jellyfish species. Until polyps developed four tentacles (usually 5–6 days after settlement), the food organisms were mashed prior to feeding. At the eight-tentacle stage, polyps were fed with intact living food organisms. After each feeding, jars were cleaned and the water was replaced with sterile seawater to maintain a debris-free environment. During the strobilation stage, only half of the water was changed, and uneaten food was removed from the jars with a pipette to avoid disturbing the strobilation process.

Only well-developed polyps with extended tentacles were used in the subsequent experiments. Two days after final feeding, polyps were carefully removed from the underside of the settlement lids using a needle under the stereomicroscope. Polyps were rinsed and frozen in the same manner as described above for larvae. In order to estimate the impact of each food source on the bacterial community associated with polyps during the feeding process, the free-living and food particle-attached bacteria were collected from each food source. Accordingly, 500 mL of the different food source (*Artemia* hatching water and plankton seawater water) was subjected to sequential filtration. Food particle-associated bacteria were collected on 3-μm-pore size filters (TCTP, 47 mm, Millipore, Germany). To collect free-living bacteria, the resulting filtrate was subsequently filtered on a 0.2-μm-pore size filter (GTTP, 47 mm, Millipore, Germany). Filters with bacterial biomass were stored at − 20 °C until further processing.

DNA extraction

Total genomic DNA of the bacterial community associated with different body compartments was extracted from freeze-dried tissue using cetyl-trimethyl-ammoniumbromide (CTAB) according to the modified protocol of Gawel and Jarret (1991).

For the analysis of the bacterial community composition associated with polyps, white-colored polyps and polyps with empty gut were collected 2 days after feeding. DNA extraction of the bacterial community associated with larvae and polyps was performed as previously described (Sapp et al. 2007) with minor modifications. Briefly, the tube containing larva or polyp was centrifuged for 1 min at 2504×g to get rid of the supernatant water, then extracted bacterial biomass was resuspended in STE buffer (stock concentrations 6.7% saccharose, 50 mM Tris, 1 mM EDTA, pH 8). Lysozyme (10 mg/mL) and proteinase K (10 mg/mL) were added and samples were incubated for 30 min at 37 °C. Cell lyses was performed by adding Tris-EDTA (50 mM Tris, 250 mM EDTA, pH 8) and SDS-Tris-EDTA (20% sodium dodecyl sulfate, 50 mM Tris, 20 mM EDTA, pH 8) for 60 min at 50 °C with slow

agitation. DNA extraction was performed using 1/10 volume NaCl (5 M) and 1 volume phenol–chloroform–isoamylalcohol (25:24:1). After precipitation of the DNA with isopropanol, DNA was washed with 75% ethanol and finally dried on sterile bench.

DNA of particle-attached and free-living bacteria of the two food sources extracted from the 3 and 0.2 μm filters was done as described previously (Sapp et al. 2007). Briefly, frozen filters were cut with sterilized scissors into small strips and cells were lysed using lysozyme/SDS. DNA was obtained by phenol–chloroform extraction and subsequent isopropanol precipitation. All DNA extracts were dissolved in 30–50 μL sterile water and were used as template DNA in subsequent PCR. DNA concentration in each sample was quantified using the Invitrogen (Carlsbad, CA, USA) Quant-iTPicoGreen ® dsDNA Reagent as per manufacturers' instructions.

Automated ribosomal intergenic spacer analysis

The ARISA fingerprinting method, a PCR method based on the length polymorphism of the internal transcribed spacer (ITS) (Fisher and Triplett 1999; Ranjard et al. 2000) was applied to characterize the bacterial communities associated with the different life stages and different body compartments of the two scyphozoan species. Briefly, the ITS region was amplified using the forward primers L-D-Bact-132-a-A-18 and the fluorescently labeled reverse primer S-D-Bact-1522-b-S-20 (Ranjard et al. 2000). The PCR reaction and cycling conditions were performed as described previously (Hao et al. 2015). PCRs were performed in volumes of 25 μL containing 5 ng template DNA. PCR products were diluted (1:5) with autoclaved ultrapure water, then mixed with an equal volume of formamide containing loading buffer. Finally, 0.25 μL of the product mixture was separated in 5.5 % polyacrylamide gels at 1500 V for 14 h on a LI-COR4300 DNA Analyzer. A 50–1500-bp size standard was run as a size reference on each gel (all materials: LI-COR Bioscience, USA).

Statistical analysis

Analysis of ARISA data

ARISA gel images were analyzed by using BioNumerics 6.6 software (Applied Maths, Sint-Martens-Latem, Belgium). Bands with intensities lower than 2% of the maximum value of the respective lane and bands smaller than 262 bp were neglected. Normalization of band patterns was conducted automatically referencing by the size standard and the presence or absence of each band was determined based on the normalized minimum threshold density (5%). Depending on the length of the detected fragment, bins of 3 bp were used for fragments up to 700 bp in length, bins of 5 bp for fragments

between 700 and 1000 bp, and bins of 10 bp for fragments larger than 1000 bp. Binning to band classes was performed according to Kovacs (2010). Each band class is referred to as an ARISA operational taxonomic unit (OTU). Peak intensities of ARISA OTUs were translated to binary data reflecting the presence or absence of the respective OTU. The alpha diversity (OTU richness) (operational taxonomic units) of each sample which obtained from ARISA fingerprints was calculated by summing the total number of remaining bands. ARISA-OTUs were analyzed based on a constructed binary table. Differences between the groups were tested by one-way analysis of variance (ANOVA) with the software package Statistica (Version 6.0).

Multivariate analyses

Multivariate statistical analyses were conducted using the software package PRIMER v.6 and the add-on PERMANOVA+ (both PRIMER-E Ltd, Plymouth, UK) (Clarke and Warwick 2001). To test for statistically significant variance among the bacterial communities associated of two scyphozoan species regarding different life stage and different body compartments respectively, permutational multivariate analysis of variance (PERMANOVA) with fixed factor was accomplished at a significance level of $p < 0.05$. Principal co-ordinate analysis (PCO) was performed to visualize patterns of the bacterial community influenced by different life stage and body compartment. A distance-based test for homogeneity of multivariate dispersions (PERMDISP) was used to assess the differences in bacterial communities (BCs) between the groups on the PCO plot. PERMDISP relies on permutation methods to compute p values for distances of each sample to the group centroids. For both permutation tests, we performed a resemblance measure and used 999 permutations on the basis of Jaccard index.

Results

BCs associated with body compartments of *Cyanea lamarckii* and *Chrysaora hysoscella*

The BCs associated with four different body compartments, including umbrella, gonad, mouth arm, and tentacle, were analyzed separately to obtain a general overview on the bacterial community associated with the investigated scyphozoan species. In total, 44 specimens of *Cyanea lamarckii* and 17 specimens of *Chrysaora hysoscella* were applied in the bacterial community analysis.

The PCO plots based on the ARISA fingerprints depict the bacterial communities associated with different body compartments (umbrella, gonad, mouth arm, and tentacle) of these two analyzed scyphomedusaen species (Fig. 1). The PERMANOVA

main test indicated significant difference among the bacterial communities associated with the body compartments in *C. lamarckii* ($p = 0.001$, Table 1), where pair-wise comparison indicated that the bacterial community associated with the umbrella was significantly different from the communities associated with gonad, mouth arm, and tentacle (Supplementary Table 1, $p = 0.001$). In *C. hysoscella*, the bacterial community showed no differences regarding different body compartments ($p = 0.119$, Table 2) according to PERMANOVA main test. The bacterial community associated with the umbrella only differed from the community of gonad and tentacle (Supplementary Table 2, $p = 0.017$ and 0.015, respectively) based on pair-wise comparison. Within each group, the PERMDISP overall test for homogeneity revealed a difference in dispersion among the four body compartments in both *C. lamarckii* ($F = 6.35$, $p = 0.002$) and *C. hysoscella* ($F = 5.18$, $p = 0.016$). Notably, the umbrella group was significantly different from the gonad and tentacle groups in terms of their variability in bacterial community composition (Supplementary Table 3 and Table 4).

Bacterial OTU richness (alpha diversity) of different body compartments varies from each other in both scyphomedusaen species. The highest OTU richness was observed in the community of umbrella, with similar numbers of 23 for *C. lamarckii* and 25 for *C. hysoscella* (see Supplementary Fig. 1), followed by the community of mouth arm (S = 17 and 18, respectively) and tentacle (S = 15 for both species). The lowest richness was observed in the community of gonad with both species showing a richness of S = 14.

BCs associated with different life stages of *Cyanea lamarckii* and *Chrysaora hysoscella*

The bacterial community associated with different life stages was analyzed in the present study based on the metagenetic nature of scyphozoans. For purposes of comparison, the four compartments were considered together as representing adult medusae, and the bacterial community associated with different body compartments were therefore considered in combination for each specimen. Larvae were collected from adult medusae and polyps were reared from the larvae with two different kinds of food in laboratory conditions.

The ARISA fingerprint pattern indicated that bacterial communities associated with different stages of *C. lamarckii* were clearly separate from one another (Fig. 2a), where the PERMANOVA main test and pair-wise comparisons showed that BCs of different life stages differed significantly ($p = 0.001$, Table 3 and Supplementary Table 5). The overall test comparing all four groups was also statistically different in PERMDISP analysis ($F = 72.8$, $p = 0.001$). Individual pair-wise tests of PERMDISP showed significant difference in dispersion between each pair, except for the comparison between larvae and polyps fed with plankton (Supplementary Table 7).

Fig. 1 Principal coordinate (PCO) analysis presenting the bacterial communities associated with different body fractions of scyphomedusae **a** *Cyanea lamarckii* and **b** *Chrysaora hysoscella* based on Jaccard co-efficient from ARISA profiles
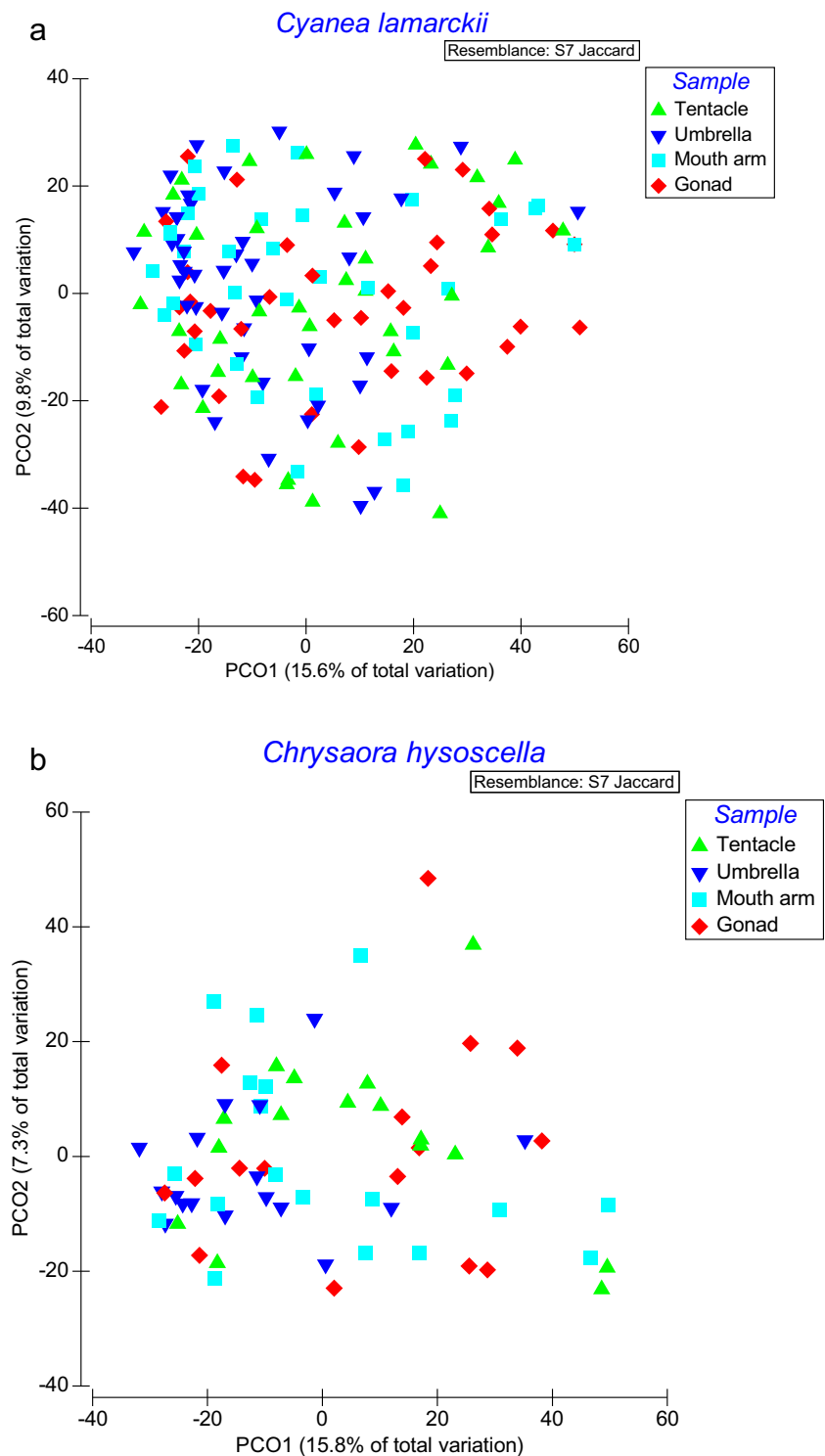
In *C. hysoscella*, the PCO plot displays a clear separation between each life stage (Fig. 2b). Each of the bacterial community corresponding to larvae, medusae, and polyps form a very tight cluster. The analyses of PERMANOVA main test and pair-wise comparisons revealed significant differences between each of the stages ($p = 0.001$) (Table 4 and Supplementary Table 6).

Consistent with PERMDISP analysis, the overall test shows that the dispersions were significantly different within four tested groups ($F = 41.06$, $p = 0.001$). Pairwise comparisons of PERMDISP indicate that these differences among the four stages were highly significant ($p = 0.001$) except for the comparison between larvae and polyps fed with plankton ($p = 0.646$; Supplementary Table 8).

Table 1 PERMANOVA main tests of bacterial community structure of different body fractions of *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	*pseudo F*	*p* (*perm*)	Perms	Sq. root
Sam	3	19,091	2.1109	*0.001*	998	9.2795
Res	152	4.5822E5				54.906
Total	155	4.7731E5				

Significant results (*p* (*perm*) < 0.05) are highlighted in italics. Displayed are tests for the factor "sample" and the partitioning of multivariate variation. *p* values were obtained using type III sums of squares

d.f. degrees of freedom, *SS* sums of squares, *Sq. root* square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

The alpha diversity of each stage of both scyphomedusan species is shown as box plot of mean values with 25–75% variation of ARISA OTU numbers (see Supplementary Fig. 2). Generally, the lowest richness in *C. lamarckii* was observed in the polyp stage (S = 20). By contrast, for *C. hysoscella*, the lowest bacterial richness was observed in the adult medusa (S = 19). The significantly highest ARISA OTU number was detected in the medusa stage of *C. lamarckii* (S = 69). However, the highest ARISA OTU number was found in the larva stage of *C. hysoscella* (S = 37). In both scyphomedusaen species, the bacterial communities of life stages were significantly different and both species were distinct from each other. Richness increased in *C. lamarckii* from larva to medusa, but decreased in *C. hysoscella*.

Impact of food source on the BCs of polyps

For both scyphozoan species, there was strong separation in the bacterial assemblage between the polyps and their food sources (Fig. 3a, b). The bacterial communities associated with polyps of *C. lamarckii* fed with two kinds of different food are clearly distinguishable from each other (Fig. 3a). However, for *C. hysoscella*, the bacterial communities of polyps fed with different food sources were difficult to distinguish (Fig. 3b). Moreover, the PERMANOVA main tests of the BCs of polyps and food sources showed significant differences in both scyphozoan species (*p* = 0.001, Tables 5 and 6). Consistent with the PERMANOVA pairwise comparison, there is fairly strong evidence to suggest that all of the groups differ from each other (*p* = 0.001 for most comparisons,

Supplementary Tables 9 and 10) in both species. For the pairwise comparison using PERMDISP, both scyphozoan species showed similar variation among each group (Supplementary Tables 11 and 12). Overall, the BCs of polyps fed either with plankton or *Artemia* nauplii were significantly different from the BCs of the food sources themselves, including both the particle-attached and the free-living communities either on the location aspect (PERMANOVA) or dispersion aspect (PERMDISP).

The alpha diversity of bacterial community associated with polyps, the particle-attached and free-living communities of two kinds of food sources is depicted as box plot of median values with respective 25–75% variation of ARISA OTU numbers (see Supplementary Fig. 3). Generally, among the food sources, the highest ARISA OTU number was detected in the free-living community of plankton water (S = 50), followed by the particle-attached community of plankton (S = 31). The particle-attached community of *Artemia* (S = 26) had a higher diversity than the comparative free-living community (S = 20). Among the polyps, the highest OTU number was observed in plankton-fed *C. lamarckii* polyps (S = 36), followed by plankton-fed *C. hysoscella* polyps (S = 30). Polyps fed with *Artemia* displayed a lower diversity, at 20 for *C. lamarckii* and 23 for *C. hysoscella*.

Comparison of BCs associated with *Cyanea lamarckii* and *Chrysaora hysoscella*

For the BCs of planula larvae, a clear separation between *C. lamarckii* and *C. hysoscella* could be observed in the PCO plot (Fig. 4a). In accordance with PERMANOVA

Table 2 PERMANOVA main tests of bacterial community structure of different body fractions of *C. hysoscella* based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	*pseudo F*	*p* (*perm*)	Perms	Sq. root
Sample	3	11,280	1.2061	0.119	993	6.1956
Res	63	1.964E5				55.835
Total	66	2.0768E5				

Significant results (*p* (*perm*) < 0.05) are highlighted in italics. Displayed are tests for the factor "sample" and the partitioning of multivariate variation. *p* values were obtained using type III sums of squares

d.f. degrees of freedom, *SS* sums of squares, *Sq. root* square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

Fig. 2 Principal coordinate (PCO) analysis presenting the bacterial communities associated with different life stages of Scyphozoan **a** *Cyanea lamarckii* and **b** *Chrysaora hysoscella* based on Jaccard coefficient from ARISA profiles
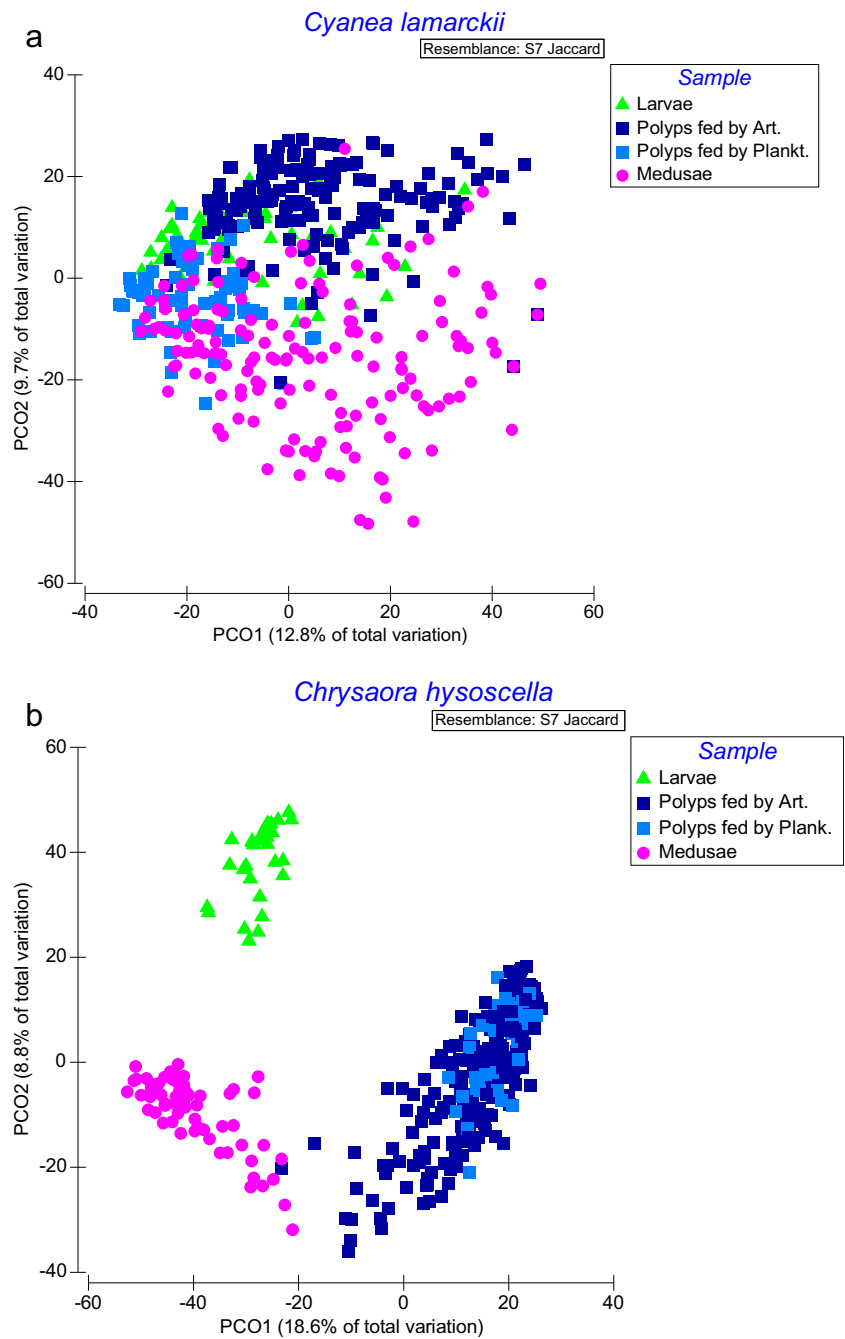

Table 3 PERMANOVA main tests of bacterial community structure of different life stages of *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Sample	3	1.8504E5	26.29	*0.001*	998	23.937
Res	439	1.03E6				48.438
Total	442	1.215E6				

Significant results (*p* (*perm*) < 0.05) are highlighted in italics. Displayed are tests for the factor "sample" and the partitioning of multivariate variation. *p* values were obtained using type III sums of squares

d.f. degrees of freedom, *SS* sums of squares, *Sq. root* square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

Table 4 PERMANOVA main tests of bacterial community structure of different life stages of *C. hysoscella* based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Sample	3	2.7552E5	40.386	*0.001*	999	38.36
Res	339	7.709E5				47.687
Total	342	1.0464E6				

Significant results (*p (perm)* < 0.05) are highlighted in italics. Displayed are tests for the factor "sample" and the partitioning of multivariate variation. *p* values were obtained using type III sums of squares

d.f. degrees of freedom, *SS* sums of squares, *Sq. root* square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

Fig. 3 Principal coordinate (PCO) analysis presenting the bacterial communities associated with polyps of two scyphozoan species **a** *Cyanea lamarckii* and **b** *Chrysaora hysoscella* (including fed with *Artemia* and plankton) and food resource (including attached community of food itself: *Artemia* and plankton, and free-living community of food: *Artemia* water and plankton water) based on Jaccard coefficient from ARISA profiles
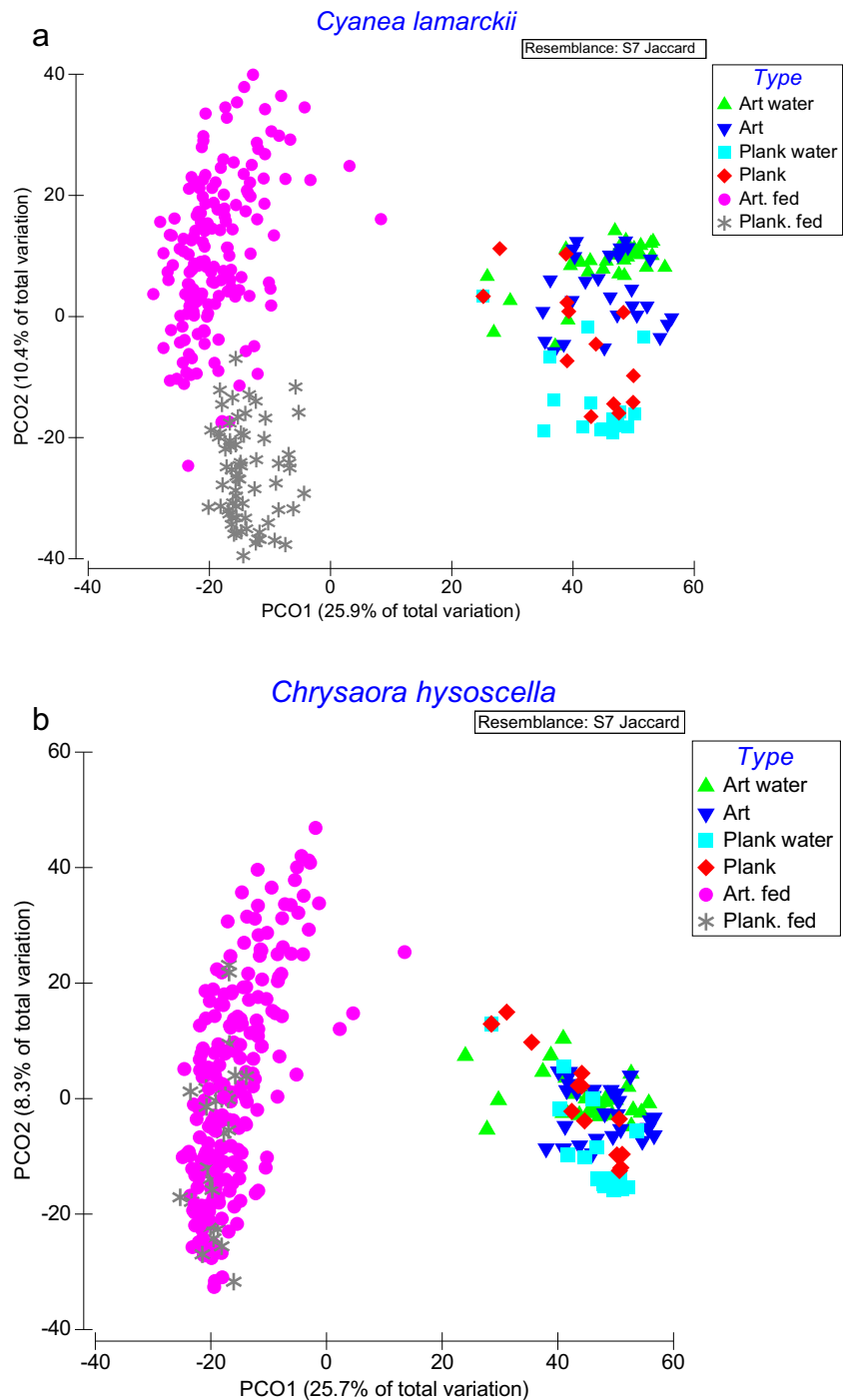
Table 5 PERMANOVA main tests of bacterial community structure of polyps of *C. lamarckii* and food source based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	*pseudo F*	*p* (perm)	Perms	Sq. root
Food type	5	3.5405E5	35.232	*0.001*	999	40.065
Res	311	6.2506E6				44.831
Total	316	9.7911E5				

Significant results (*p* (perm) < 0.05) are highlighted in italics. Displayed are tests for the factor "food type" and the partitioning of multivariate variation. *p* values were obtained using type III sums of squares

d.f.:degrees of freedom, *SS* sums of squares, *Sq. root* square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

analysis, the species factor significantly influenced the bacterial community structure associated with planulae (*p* = 0.001, Table 7). BCs of polyps compared between two scyphozoan species are specified by different food source in Fig. 4b. Both experimental factors (species and food source) and their interactions significantly influenced the bacterial community structure (PERMANOVA Table 8), with species explaining the greatest amount of variation (sq. root, Table 8). For the community of adult medusae, distinct differences were evident in the PCO plot (Fig. 4c), where both bacterial communities of the adults belonging to the two scyphozoan species showed clear separation. The PERMANOVA analysis revealed that the communities in *C. lamarckii* and *C. hysoscella* were significantly different from one another (*p* = 0.001, Table 9). The results of the PERMDISP analysis indicated that the dispersion varies significantly in the community of polyps (*F* = 14.48, *p* = 0.001, see Supplementary Table 13). Overall, the BCs of all three life stages (planulae, polyps, and medusae) differed significantly between *C. lamarckii* and *C. hysoscella*, indicating a species-specific bacterial association in these scyphozoans.

Discussion

Bacterial communities associated with different body parts

We found that the bacterial community associated with the umbrella of both scyphomedusa species differs significantly from those of tentacle and gonad, with consistent diversity in both species. A similar result has been investigated in the scyphozoan *Aurelia aurita*, in which Weiland-Bräuer et al. (2015) reported a difference in bacterial community among the outer, mucus-covered surface of the exumbrella and gastral cavity. Kramar et al. (2018 preprint version) focused on the bacterial community composition of different body parts of medusa including exumbrella surface, oral arms ("outer" body parts), and the gastric cavity ("inner" body part). They found that the bacterial communities differed significantly between different *Aurelia* sp. medusa body parts, especially within the gastral cavity.

In the present study, we analyzed the complete mesoglea of the umbrella, including the surface (exumbrella and subumbrella ectoderm) and inner surface (gastric cavity entoderm). The mesoglea is an extracellular matrix (ECM) situated between the epidermal and gastrodermal layers (Shaposhnikovaa et al. 2005), containing collagen-like proteins which are associated with mucopolysaccharides (Hoeger 1983; Hsieh and Rudloe 1994). Sorokin (1973) showed that in corals (Cnidaria, Anthozoa), the surface mucopolysaccharide layers (SML) serve as a food source for the associated bacterial community, and Ritchie and Smith (2004) subsequently found a correlation between bacterial community structure and the coral SML, with changes in the coral surface mucopolysaccharide layer composition leading to changes in the bacterial population structure. In the present study we found that bacterial communities associated with complete mesoglea of the umbrella presented a distinct structure with the highest alpha diversity. Additionally, Megill (2002) reported that the medusozoan mesoglea is a fiber-reinforced mucopolysaccharide gel, where mesoglea in the umbrella seems to be the main

Table 6 PERMANOVA main tests of bacterial community structure of polyps of *C. hysoscella* and food resource based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	*pseudo F*	*p* (perm)	Perms	Sq. root
Food type	5	3.1079E5	28.798	*0.001*	998	40.541
Res	332	7.1661E5				46.459
Total	337	1.0274E6				

Significant results (*p* (perm) < 0.05) are highlighted in italics. Displayed are tests for the factor "food type" and the partitioning of multivariate variation. *p* values were obtained using type III sums of squares

d.f. degrees of freedom, *SS* sums of squares, *Sq. root* square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

Fig. 4 Principal coordinate (PCO) analysis presenting the bacterial communities associated with different life stages between two scyphozoan species (*Cyanea lamarckii* and *Chrysaora hysoscella*) based on Jaccard coefficient from ARISA profiles **a** Larvae **b** Polyps **c** Medusae
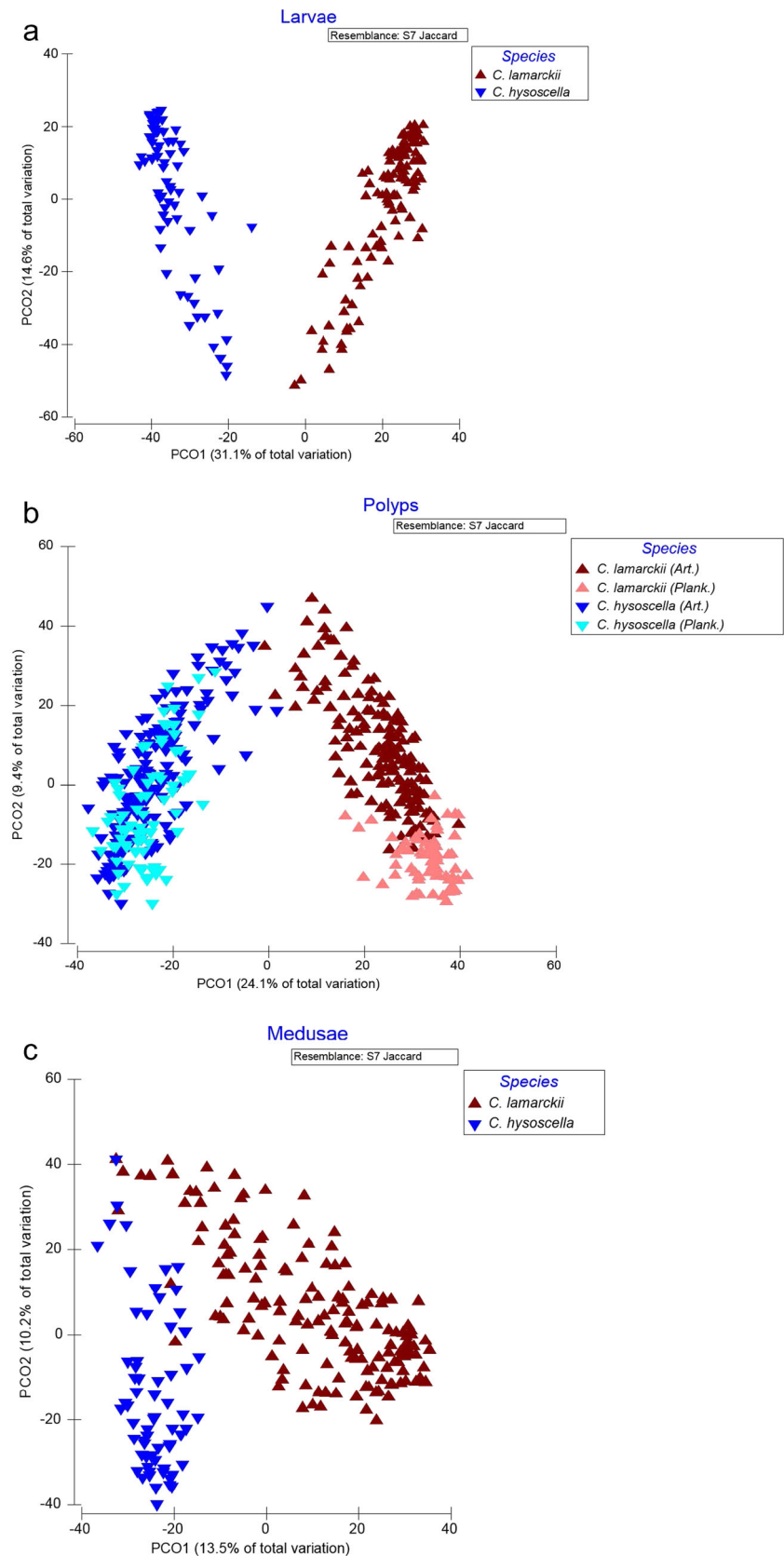
metabolically active tissues (Thuesen et al. 2005). The surface of bacterial colonization is determined by the availability of nutrients, host immune responses, and competition between bacteria from the surrounding environment for attachment space (Bosch 2013). These factors are also reasonable explanations for our findings, wherein the bacterial community associated with the umbrella not only presented higher diversity, but also significantly differed from the communities in the gonad, mouth arm, and tentacle. The pronounced difference in bacterial community structure among the umbrella, mouth arm, gonad, and tentacle surfaces could be the consequence of different surfaces or epithelial structures and their function. Cortés-Lara et al. (2015) and Viver et al. (2017) focused on the microbiota associated with gastric cavity of jellyfish *C. tuberculata*. Both reported that *C. tuberculata* harbored low-diversity microbial communities with a similar dominant group, possibly related to food digestion and protection from pathogens (Cortés-Lara et al. 2015; Viver et al. 2017).

A direct interaction between the cellular tissue composition has already been reported for the genus of *Hydra* and the microbiota by removing the interstitial stem cells (Fraune et al. 2009). In jellyfish, different types of cells are described in different body compartments (Lesh-Laurie and Suchy 1991). The umbrella, tentacle, and oral arm surfaces are in constant contact with bacteria in the surrounding ambient seawater, and their secreted mucus is potentially a high quality energy source and settling niche. Hosts recruit bacteria, which are beneficial for their development or contribute to their well-being (Shnit-Orland and Kushmaro 2009). Selection of certain bacteria in different medusa body parts could be done strategically to harbor bacteria with specific functions needed in different body compartments. The sequencing and functional analysis of microbial community diversity associated with jellyfish should be investigated further in order to examine the holobiome of the scyphozoan species.

Bacterial communities associated with different life stages

Our investigation of the bacterial communities associated with different life stages of two scyphozoan species *Cyanea* *lamarckii* and *Chrysaora hysoscella* mainly focused on three representative life stages: planula larvae, polyps, and adult medusae. Overall, the bacterial communities associated with these life stages significantly differed among each other in both scyphozoan species accompanying with species-specific. The bacterial communities in *C. lamarckii* underwent a clear transition from larvae to polyps. We assume that in *C. lamarckii*, each life stage serves as a passive substrate colonized by a different and increasingly diverse bacterial community, with the highest alpha diversity observed in medusa adults. In *C. hysoscella*, the bacterial community from each stage showed a strong selective colonization process, with a highly assembled and separated community structure. The diversity of the bacterial communities of the three development stages decreased from larvae to medusa adult, indicating a selective process of bacterial colonization.

There are few studies on the development of bacteria associated with different life stages in the Scyphozoa. Weiland-Bräuer et al. (2015) reported that significant restructuring of the *A. aurita*-associated microbiota occurred during strobilation from the benthic polyp to planktonic life stages, and suggested that associated bacteria may play important functional roles during the life cycle. Fraune and Cooper (2010) investigated the bacterial colonization during early embryogenesis in *Hydra* and found that bacterial communities associated with early embryos were significantly different from those in the subsequent polyp development stages. *Hydra* appears to select and shape the bacterial community in both adult polyps and embryos, wherein embryos are protected by a maternally produced antimicrobial peptide (AMP) of the periculin peptide family, which controls the establishment of the microbiota during embryogenesis (Fraune and Bosch 2007; Fraune and Cooper 2010).

The development of the animal host shapes a new microbiota due to changes in the surface architecture or the production of host compounds, such as in the differential expression patterns of antimicrobial peptides during different life stages. In adult *Hydra* polyps, additional AMPs including Hydramacin and Arminin contributed to the selection of bacterial colonization (Augustin et al. 2009; Jung et al. 2009), and the novel antimicrobial peptide Aurelin found in *A. aurita* may

Table 7 PERMANOVA main tests of bacterial community structure of larvae based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Species	1	1.4141E5	77.928	*0.001*	999	39.404
Res	188	3.4114E5				42.598
Total	189	4.8254E5				

Significant results (*p (perm)* < 0.05) are highlighted in italics. Displayed are tests for the factor "species" and the partitioning of multivariate variation. *p* values were obtained using type III sums of squares

d.f. degrees of freedom, *SS* sums of squares, *Sq. root* square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

Table 8 PERMANOVA main tests of bacterial community structure of polyps based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	pseudo F	p (perm)	Perms	Sq. root
Species	1	1.8153E5	86.654	0.001	998	37.912
Food	1	37,302	17.807	0.001	999	16.793
Species × Food	1	32,021	15.286	0.001	998	21.896
Res	473	9.9085E5				45.769
Total	476	1.3853E6				

Significant results (p (perm) < 0.05) are highlighted in italics. Displayed are tests for the factor "species" and the partitioning of multivariate variation. p values were obtained using type III sums of squares

d.f. degrees of freedom, SS sums of squares, Sq. root square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

contribute a similar function (Ovchinnikova et al. 2006). It was reported that half core bacteria of *Chrysaora plocamia* were also present in life stages of the jellyfish *A. aurita*. This might suggest that bacterial community represent an intrinsic characteristic of scyphozoan jellyfish, contributing to their evolutionary success (Lee et al. 2018). AMPs are known to be strong tools of the innate immune system that are often secreted in response to external stimulation (Bosch 2013). Thus, the colonized bacterial community adapted to different AMP repertoires of different species might result in specific associations between hosts and bacteria.

It is also widely reported that bacteria are important in the larval settlement processes in most marine invertebrates (Wieczorek and Todd 1998), such as sponges (Woollacott and Hadfield 1996), cnidarians (Bosch 2013), ascidians (Wahl et al. 1994; Schuett et al. 2005), and bryozoans (Pukall et al. 2001; Kittelmann and Harder 2005). Scyphozoan polyps require specific cues to induce settlement and metamorphosis, with those emanating from various substrates mostly attributable to bacteria. In marine environments, almost all substrates are covered by biofilms where certain bacteria are suggested to deliver metamorphosis-inducing stimuli (Clare et al. 1998; Müller and Leitz 2002). In accordance with these findings, planulae of the scyphozoan *Cassiopea andromeda* did not settle in the absence of microbes, but inoculation with *Vibrio* sp. induced settlement and metamorphosis (Hofmann et al. 1996). As such, it may be worth investigating various other bacterial taxa for their function during the course of metamorphosis in different life stages. Interestingly, there may be scope for examining the relationship between medusae and their microbiota from a disease-vectoring perspective. Cortés-Lara et al. (2015) proposed that many of the jellyfish-associated *Vibrio* sp. bacteria may be potential pathogens of other marine species, with the medusozoan host possibly serving to disperse them.

Ambient bacterial community and the impact of food source on the bacterial community of polyp

As described above, many cnidarian species require the involvement of specific bacterial biofilms to be present prior to their larval settlement. Since our experiment involved planula larvae cultured in sterile seawater, there were only two means of accessing the required bacteria: either they were carried endogenously or they were obtained during feeding. Our results indicate that the BCs of the provided food sources were clearly separate from the communities associated with polyps. Additional, we proved that food source had no impact on the selection of bacterial communities associated with polyps in both scyphozoan species, with those fed with plankton showing greater bacterial richness than those fed with *Artemia* (Fig. 4b and Supplementary Fig. 2). There is always a discussion concerning the origin of the associated BC in cnidarians and other jellyfish species. With regard to the surrounding seawater, a number of studies examining both cnidarians and ctenophores showed that the composition of the jellyfish microbiota is generally highly distinct from the composition of communities present in ambient seawater (Daniels and Breitbart 2012;

Table 9 PERMANOVA main tests of bacterial community structure of medusae based on Jaccard dissimilarities of ARISA profiles

Source of variation	d.f.	SS	pseudo F	p (perm)	Perms	Sq. root
Species	1	77,150	24.877	0.001	999	28.052
Res	223	6.9159E5				55.689
Total	224	7.6874E5				

Significant results (p (perm) < 0.05) are highlighted in italics. Displayed are tests for the factor "species" and the partitioning of multivariate variation. p values were obtained using type III sums of squares

d.f. degrees of freedom, SS sums of squares, Sq. root square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities

Hao et al. 2015; Dinasquet et al. 2012; Daley et al. 2016; Cleary et al. 2016; Cortés-Lara et al. 2015; Viver et al. 2017; Weiland-Bräuer et al. 2015). Although we assume that this cannot be generalized, in our study, the BC associated with the zooplankton fraction also indicated a significant different structure (free-living and particle-attached) compared to BCs associated with two different scyphomedusa species (data not shown). In addition, detailed analyses of recurring patterns of seawater bacteria from the same study site at the same time (Lucas et al. 2015 and Chafee et al. 2018) also indicate clear differences to the BCs of scyphomedusae. Taken together, we conclude that the structure of the associated bacterial community of the investigated scyphozoan species is generally distinct from the structure of communities present in ambient water.

The polyps may select for specific bacteria by expressing different metabolic activity according to food source, thereby providing different nutrients for the bacteria (Bosch 2013). This might also indicate that the BCs of scyphozoan polyps interact with the inherent immune systems of the polyps, which play a pivotal role for selecting and shaping the associated bacterial community (Fraune and Bosch 2007). Therefore, since the BCs of polyps are significantly distinct between the food sources, we believe that although the bacterial community of the food source has no direct influence on the BCs of polyps, there may be other factors shaping the bacterial communities which cannot be determined based on the present data.

In conclusion, we might assume that so far unknown factors are involved in shaping the bacterial communities which cannot be determined based on this preliminary screening study. Studies on additional life stages such as strobilae and ephyrae will help to clarify whether changes in the jellyfish-associated bacterial communities may primarily depend on host-specific selective controls or on environmental microbial diversity. We assume that our results cannot be extrapolated to other jellyfish species; hence, it is very important to verify our results with regard to other jellyfish species. As Lee et al. (2018) proposed, the bacterial community presents an intrinsic characteristic of scyphozoan jellyfish. There is a need for further studies addressing also an in-depth DNA sequencing analyses of the accompanying BC in order to better understand species-specificity of bacterial communities as well as function and composition of bacterial communities associated with jellyfish.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable.

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