Similar bacterial communities on healthy and injured skin of black tip reef sharks

Claudia Pogoreutz1, Mauvis A. Gore2,3, Gabriela Perna1, Catriona Millar2,3, Robert Nestler4, Rupert F. Ormond2,3,5*, Christopher R. Clarke6 and Christian R. Voolstra1,7*

Abstract

Background: Sharks are in severe global decline due to human exploitation. The additional concern of emerging diseases for this ancient group of fish, however, remains poorly understood. While wild-caught and captive sharks may be susceptible to bacterial and transmissible diseases, recent reports suggest that shark skin may harbor properties that prevent infection, such as a specialized ultrastructure or innate immune properties, possibly related to associated microbial assemblages. To assess whether bacterial community composition differs between visibly healthy and insulted (injured) shark skin, we compared bacterial assemblages of skin covering the gills and the back from 44 wild-caught black-tip reef sharks (Carcharhinus melanopterus) from the Amirante Islands (Seychelles) via 16S rRNA gene amplicon sequencing.

Results: Shark skin-associated bacterial communities were diverse (5971 bacterial taxa from 375 families) and dominated by three families of the phylum Proteobacteria typical of marine organisms and environments (Rhodobacteraceae, Alteromonadaceae, Halomonadaceae). Significant differences in bacterial community composition of skin were observed for sharks collected from different sites, but not between healthy or injured skin samples or skin type (gills vs. back). The core microbiome (defined as bacterial taxa present in ≥50% of all samples) consisted of 12 bacterial taxa, which are commonly observed in marine organisms, some of which may be associated with animal host health.

Conclusion: The conserved bacterial community composition of healthy and injured shark skin samples suggests absence of severe bacterial infections or substantial pathogen propagation upon skin insult. While a mild bacterial infection may have gone undetected, the overall conserved bacterial community implies that bacterial function(s) may be maintained in injured skin. At present, the contribution of bacteria, besides intrinsic animal host factors, to counter skin infection and support rapid wound healing in sharks are unknown. This represents clear knowledge gaps that should be addressed in future work, e.g. by screening for antimicrobial properties of skin-associated bacterial isolates.

Keywords: Skin microbiota, Pseudoalteromonas, Psychrobacter, Lesion, Injury, Wound healing, Immunity, Elasmobranch, Carcharhinus
organisms over recent decades [11]. While only a few documented infections of sharks in the wild are available [12, 13], sharks can often be observed bearing open wounds without any obvious sign of infection [14, 15]. In contrast, increased frequency and severity of bacterial and/or eukaryotic infection has been described for sharks in captivity, in particular when kept at high densities [16–23].

Like all other animals, sharks should be considered metaorganisms, i.e. animals hosts associated with a diverse microbial community collectively termed the microbiome [24, 25]. This microbiome typically consists of prokaryotes (Bacteria, Archaea), eukaryotes (fungi, protists, algae), and viruses [26, 27]. Skin in particular constitutes a large habitat for animal-associated bacteria, creating an abundance of niches for unique microbial communities [28]. Environmental stress can lead to a disturbance of associated microbiota, the structural and functional disruption of the entire community and, ultimately, disease [29, 30]. Consequently, skin diseases [31–37] as well as mechanic insult, disruption, or irritation of skin [38] may cause distinct changes in the associated bacterial microbiome.

The black-tip reef shark (Carcharhinus melanopterus), a medium-sized and relatively common Indo-Pacific predator [39], can often be observed in the wild bearing severe skin insults, such as deep open wounds (Fig. 1b; [15]). At the same time, this species seemingly exhibits a highly developed capacity for rapid wound healing after skin injury [15]. In this context, it is important to understand the contribution of the resident bacterial community on the skin to such properties. In the present study, we therefore investigated bacterial community structure of skin samples from a population of black-tip reef sharks (Carcharhinus melanopterus) in the Amirante Islands (Seychelles). Of the sharks sampled, a proportion were noticeably affected by skin insults (lesions) and surface irregularities, especially around and behind the gills. This allowed us to profile bacterial communities associated with visibly healthy skin and compare them to the skin from conspecifics bearing such injuries (Fig. 1c-d), so as to determine whether bacterial community shifts align with healthy and insulted skin samples of black-tip reef sharks.

**Results**

**Bacterial community composition of black-tip reef shark skin**

To assess bacterial community composition of healthy and compromised skin areas of the gills and the back,
we conducted amplicon sequencing of the V5 and V6 region of the bacterial 16S rRNA gene from wild-caught sharks from the Amirante Islands in the Seychelles (Fig. 1a). In total, 88 skin samples from 44 black-tip reef sharks (one mucus swab sample each from the skin covering over and around the gills and one from the back of each individual shark) were collected from five sites in the study area. Overall, 28 of the sampled sharks were visibly healthy and 16 exhibited marked insult(s) on the skin around the gill area (Table 1, Fig. 1c-d). A total of 18,022,131 16S rRNA gene amplicon sequences were determined, distributed over the 88 samples. After quality checks and removal of unwanted sequences, 2,034,047 sequences with an average length of 293 bp remained, and were clustered at 97% similarity into 5971 distinct bacterial Operational Taxonomic Units (OTUs; 'taxa') from 375 bacterial families (Additional file 4: Table S1, Additional file 5: Table S2). Plateauing rarefaction curves suggest sufficient sampling effort, higher variation in numbers of OTUs in gill samples than in back samples, and higher numbers of OTUs per sample for the sampling site North Side; for details, see Additional file 2: Figure S1).

The majority of bacterial sequences on the phylum level were assigned to Proteobacteria (63.4%), Bacteroidetes (24.0%), Actinobacteria (6.1%), Firmicutes (5.3%), and others (1.2%). On the class level, most sequences assigned to Gammaproteobacteria (34.8% of total sequences and 54.9% of Proteobacteria), Alphaproteobacteria (24.6% of total sequences and 38.8% of Proteobacteria), Acidimicrobia (3.6% of the total), and Bacilli (3.3% of the total); remaining bacterial sequences were assigned to low abundance classes, cumulatively making up 33.7% of the total. Overall, the three most abundant bacterial families observed (ranked by realtive abundance) included the Rhodobacteraceae (Gammaproteobacteria: Rhodobacterales; on average contributing 16.0 and 13.2% of the total bacterial community on the skin around the gills and back skin, respectively), Alteromonadaceae (Gammaproteobacteria: Alteromonadales; 10.7 and 12.1% of the total around gills and backs, respectively), and Halomonadaceae (Gammaproteobacteria: Oceanospirillales; 4.8 and 5.4% of the total around gills and backs, respectively). Other bacterial families individually contributed around 5% or less to the total (Additional file 4: Table S1).

Overall, the bacterial community composition was uneven (Simpson’s Evenness of the bacterial communities mean ± SE = 0.07 ± 0.003) (Table 2). No difference was observed in the most abundant bacterial OTUs between skin samples from visibly healthy and lesioned gill areas or control samples from the back (PERMANOVA; F = 83.592, R² = 0.0963, p = 0.5657, Table 3 a; Fig. 2; for bar plots showing bacterial community composition of individual samples, see Additional file 3: Figure S2). The core microbiome at a cut-off of 80% (i.e., present in 80% of samples) consisted of the two most abundant OTUs, i.e. OTU00001 (Rhodobacteraceae sp.) and OTU00002 (Alteromonas sp.). At a less stringent cut-off of 50% (i.e., present in 50% of samples), the core microbiome consisted of 11 OTUs, more specifically OTUs 00001–00006 (Rhodobacteraceae sp., Alteromonas sp., Pelagibacteraceae sp., Flavobacteriales sp., Vibionales sp., OCS155 sp.), OTUs 00010–00011 (Oceanospirillales sp., Psychrobacter pacificensis), 14 (Flavobacteriales sp.), OTUs 16 (Pseudoalteromononas porphyrae) and 19 (Halomonadaceae sp.) (OTUs 00001–00006, 00010, 00011, 00014, 00016, and 00019).

**Table 1 Overview of shark samples collected**

| Sample Location | Healthy Skin-Insulted | No. of gill and back samples |
|-----------------|----------------------|-------------------------------|
| Number of sharks | 27                   | 17                            |
| Number of sharks per site | 5/7                  | 12/13                         |
| West Ressource (St. Joseph) | 8                   | 3 | Each 11 |
| East Ressource (St. Joseph) | 5                   | 3 | Each 7 |
| Fouquet (St. Joseph) | 5                   | 2 | Each 7 |
| Benjamin (St. Joseph) | 0                   | 3 | Each 3 |
| North Side (D’Arros) | 9                   | 2 | Each 11 |

Shark skin microbiomes differ between collection sites, but not between location on skin or condition.

To assess whether community composition of skin-associated bacterial communities differed between health states of shark skin (visibly healthy and insulted) of black-tip reef sharks, and across the five sites in the Amirante Islands, Seychelles, we conducted a Permutational Analysis of Variance (PERMANOVA) on microbiome assemblages using the adonis function in the R package vegan [81]. Significant differences for shark skin bacterial communities were apparent for collection site, both for samples from gills (adonis PERMANOVA, Pseudo-F = 5.5281, R² = 0.3561, p < 0.0001, Table 3 b) and the back (adonis PERMANOVA; Pseudo-F = 4.9994, R² = 0.34319, p < 0.0001, Table 3 c). There were however no significant differences between the two health states of skin samples taken from gills (PERMANOVA, Pseudo-F = 1.1763; R² = 0.01942, p = 0.2666, Table 3 b), nor between those and samples from the back areas (PERMANOVA, Pseudo-F = 1.0906, p = 0.3035, Table 3 c). No significant interactions between any of the factors ‘health’, ‘site’, or ‘sex’ were observed for skin covering the gills (Table 3 b) or skin on the back (Table 3 c). However, pairwise ANOSIM comparisons for gill and back samples from different sites subsequently
Table 2 Statistics of 16S rRNA gene amplicon sequencing, and richness and diversity indices of bacterial communities associated with visibly healthy and infected skin around the gills and visibly healthy skin on the back of black-tip reef sharks (*Carcharhinus melanopterus*) collected in the Amirante Islands (Seychelles). BD = samples from skin on the back; GD = samples from skin around the gills; F = female; M = male; H = visibly healthy sharks; D = sharks with infected skin around the gills

| Sample     | Chao1 Index | Inverse Simpson Index | Simpson's Evenness | Number of Seqs |
|------------|-------------|-----------------------|--------------------|----------------|
| a) Skin around the gills |
| CM01_GD_F_H | 829.11      | 68.73                 | 0.1                | 22,837         |
| CM02_GD_F_H | 1225.4      | 113.21                | 0.1                | 22,612         |
| CM03_GD_F_I | 856.24      | 38.5                  | 0.05               | 23,567         |
| CM04_GD_F_H | 585.64      | 43.1                  | 0.09               | 23,337         |
| CM05_GD_F_I | 670.49      | 102.7                 | 0.18               | 21,951         |
| CM06_GD_F_I | 579.88      | 53.57                 | 0.11               | 23,006         |
| CM07_GD_F_I | 1428.6      | 106.33                | 0.09               | 23,823         |
| CM08_GD_M_H | 1037.1      | 98.46                 | 0.11               | 23,578         |
| CM09_GD_F_H | 1501.9      | 92.43                 | 0.07               | 23,743         |
| CM10_GD_F_H | 3181.1      | 123.1                 | 0.05               | 24,111         |
| CM11_GD_F_H | 1088.9      | 8.05                  | 0.01               | 23,495         |
| CM12_GD_F_I | 1612.1      | 133.24                | 0.1                | 23,782         |
| CM13_GD_F_H | 1853.1      | 130.33                | 0.09               | 23,751         |
| CM14_GD_F_H | 1476.1      | 14.87                 | 0.01               | 23,674         |
| CM15_GD_F_H | 1837.9      | 109.52                | 0.07               | 23,954         |
| CM16_GD_M_H | 3442.4      | 100.96                | 0.04               | 24,120         |
| CM17_GD_F_H | 2021.6      | 183.98                | 0.1                | 23,814         |
| CM18_GD_M_H | 546.05      | 51.66                 | 0.11               | 22,227         |
| CM19_GD_M_H | 790.74      | 53.95                 | 0.08               | 23,413         |
| CM20_GD_M_I | 508.86      | 30.46                 | 0.07               | 22,719         |
| CM21_GD_F_I | 854.37      | 25.41                 | 0.04               | 24,060         |
| CM22_GD_F_H | 623.35      | 28.13                 | 0.05               | 23,716         |
| CM23_GD_M_H | 567         | 34.65                 | 0.07               | 22,839         |
| CM24_GD_F_H | 554.29      | 33.12                 | 0.07               | 21,255         |
| CM25_GD_M_H | 486.78      | 39.75                 | 0.09               | 23,369         |
| CM26_GD_M_H | 615         | 31.26                 | 0.07               | 23,417         |
| CM27_GD_M_I | 792         | 34.57                 | 0.05               | 23,625         |
| CM31_GD_F_I | 509.08      | 26.13                 | 0.06               | 23,473         |
| CM32_GD_M_H | 437         | 14.2                  | 0.04               | 22,970         |
| CM33_GD_F_H | 567.85      | 34.52                 | 0.07               | 23,452         |
| CM34_GD_F_I | 904.58      | 31.56                 | 0.04               | 23,506         |
| CM35_GD_M_H | 559.91      | 29.73                 | 0.07               | 22,633         |
| CM36_GD_F_H | 574.42      | 18.28                 | 0.04               | 21,942         |
| CM37_GD_M_H | 755.85      | 38.93                 | 0.06               | 21,908         |
| CM38_GD_F_H | 671.21      | 13.45                 | 0.02               | 23,638         |
| CM39_GD_M_I | 641.87      | 27.18                 | 0.05               | 23,472         |
| CM40_GD_M_I | 620.64      | 26.61                 | 0.05               | 23,623         |
| CM41_GD_M_I | 609.35      | 24.07                 | 0.05               | 23,727         |
| CM42_GD_F_I | 236         | 29.44                 | 0.15               | 21,194         |
| CM43_GD_F_I | 786.4       | 39.58                 | 0.06               | 23,589         |
| CM44_GDD_F_I | 1090.6    | 63.38                 | 0.08               | 23,187         |
Table 2  Statistics of 16S rRNA gene amplicon sequencing, and richness and diversity indices of bacterial communities associated with visibly healthy and infected skin around the gills and visibly healthy skin on the back of black-tip reef sharks (Carcharhinus melanopterus) collected in the Amirante Islands (Seychelles). BD = samples from skin on the back; GD = samples from skin around the gills; F = female; M = male; H = visibly healthy sharks; D = sharks with infected skin around the gills (Continued)

| Sample       | Chao1 Index | Inverse Simpson Index | Simpson’s Evenness | Number of Seqs |
|--------------|-------------|-----------------------|-------------------|----------------|
| CM45_GD_F_H  | 921.89      | 28.71                 | 0.04              | 22,768         |
| CM47_GD_F_I  | 1266.1      | 66.5                  | 0.06              | 22,222         |
| CM48_GD_F_I  | 791.26      | 39.77                 | 0.06              | 23,720         |
| CM01_BD_F_H  | 424.05      | 22.19                 | 0.07              | 22,718         |
| CM02_BD_F_H  | 544.43      | 29.67                 | 0.07              | 23,087         |
| CM03_BD_F_I  | 399.3       | 61.82                 | 0.18              | 22,542         |
| CM04_BD_F_H  | 459.24      | 30.95                 | 0.07              | 23,172         |
| CM05_BD_F_I  | 502.25      | 51.18                 | 0.11              | 21,367         |
| CM06_BD_F_I  | 691.78      | 74.37                 | 0.12              | 20,901         |
| CM07_BD_F_I  | 1615.9      | 143.2                 | 0.1               | 23,496         |
| CM08_BD_M_H  | 1054.4      | 126                   | 0.13              | 23,287         |
| CM09_BD_F_H  | 501.1       | 51.88                 | 0.12              | 21,079         |
| CM10_BD_F_H  | 1013.0      | 94.43                 | 0.11              | 23,249         |
| CM11_BD_F_H  | 963.75      | 84.59                 | 0.11              | 23,299         |
| CM12_BD_F_I  | 908.87      | 137.11                | 0.18              | 23,033         |
| CM13_BD_F_H  | 2338.8      | 150.65                | 0.08              | 23,996         |
| CM14_BD_F_H  | 2374.2      | 226.81                | 0.12              | 23,992         |
| CM15_BD_F_H  | 2541.1      | 103.63                | 0.06              | 24,112         |
| CM16_BD_M_H  | 3500.8      | 81.1                  | 0.03              | 24,155         |
| CM17_BD_F_H  | 2987.9      | 144.14                | 0.07              | 23,952         |
| CM18_BD_M_H  | 454.36      | 38.21                 | 0.09              | 22,717         |
| CM19_BD_M_H  | 564.5       | 37.43                 | 0.08              | 23,157         |
| CM20_BD_M_I  | 507.23      | 48.92                 | 0.11              | 21,976         |
| CM21_BD_F_I  | 457.16      | 31.31                 | 0.08              | 23,450         |
| CM22_BD_F_H  | 480.28      | 34.59                 | 0.08              | 22,383         |
| CM23_BD_M_H  | 549.26      | 28.39                 | 0.06              | 23,067         |
| CM24_BD_F_H  | 650.16      | 33.82                 | 0.06              | 22,486         |
| CM25_BD_M_H  | 449.22      | 36.44                 | 0.09              | 22,757         |
| CM26_BD_M_H  | 533.81      | 35.57                 | 0.08              | 23,177         |
| CM27_BD_M_I  | 591.23      | 29.23                 | 0.06              | 23,476         |
| CM31_BD_F_I  | 531.63      | 29.43                 | 0.06              | 22,829         |
| CM32_BD_M_H  | 442.83      | 24.99                 | 0.06              | 22,781         |
| CM33_BD_F_H  | 519.56      | 29.86                 | 0.06              | 23,381         |
| CM34_BD_F_I  | 522         | 29.84                 | 0.06              | 22,841         |
| CM35_BD_M_H  | 598.38      | 30.95                 | 0.06              | 22,562         |
| CM36_BD_F_H  | 578.88      | 29.27                 | 0.06              | 23,251         |
| CM37_BD_M_H  | 408.6       | 13.95                 | 0.04              | 24,023         |
| CM38_BD_F_H  | 518         | 27.97                 | 0.06              | 23,295         |
| CM39_BD_M_I  | 506.66      | 26.52                 | 0.06              | 23,406         |
| CM40_BD_M_I  | 499.15      | 22.4                  | 0.05              | 23,491         |
| CM41_BD_M_I  | 614.56      | 23.54                 | 0.04              | 23,770         |
demonstrated significant differences in skin bacterial communities for the majority of sites (Table 3d). No significant differences were observed between male and female sharks (Table 3b, c). Principal Coordinate plots support the statistical analyses, showing the samples clustering by site, but not by health state (Fig. 3a,b).

In order to identify bacterial OTUs with differential abundance between study sites and in relation to skin location, a two-way ANOVA was conducted (Additional file 5: Table S2). It identified a total of 840 OTUs differentially abundant between collection sites, including 18 out of the 20 most abundant OTUs (Additional file 5: Table S2). Among these, several core microbiome taxa (OTU00001, OTU00002, OTU00004, OTU00010, OTU00011, OTU00014, OUT00016, OTU00019) exhibited higher relative abundances on sharks caught at sites located off of St. Joseph Atoll (i.e., East Ressource, Fouquet, and Benjamin), in contrast to the sites closer to the island d’Arros (i.e., North Side, West Ressource). Only one bacterial taxon (OTU00005; *Vibrio*ales sp.) was more abundant on the skin of sharks collected at West Ressource and North Side compared to the other three sites off St. Joseph. One OTU (OTU00006; OCS155 sp.) was more abundant at the four sites belonging to St. Joseph (i.e., West Ressource, East Ressource, Benjamin, Fouquet) compared to the North Side. The above pattern of relative abundances among sites was apparent for both sampled skin locations, i.e. skin covering the gills and the back of the sharks (for details, see Table 4a,b).

Notably, putative core microbiome members together constituted a larger relative proportion of total bacterial sequences associated with black-tip reef shark skin off the outer St. Joseph Islands, i.e. East Ressource, Fouquet, and Benjamin, compared to West Ressource and North Side (Table 4a, b). No OTU was significantly differentially abundant between the two locations of shark skin.

**Discussion**

The present study investigated the bacterial skin microbiome of wild-caught black-tip reef sharks, *C. melanopterus*, from the Amirante Islands in the Seychelles, comparing visibly healthy individuals with individuals exhibiting tissue insult on the skin around the gills. High throughput 16S rRNA gene amplicon sequencing on the Illumina HiSeq platform revealed that the bacterial communities in those specimens with visibly healthy skin and those with insulted skin on the gills were statistically indistinguishable, i.e. bacterial community composition remained highly conserved upon tissue insult. Similarly, no differences were observed between samples from skin around the gills and from skin on the posterior back of the same sharks. Significant differences were only observed with respect to the sampling sites where the sharks were caught. The observed patterns align with our current understanding of black-tip reef shark ecology and the unique cutaneous structure of shark skin, suspected to hinder bacterial infection. Potential links between bacterial taxa and immune properties of shark skin should be addressed in future work, as discussed below.

**Bacterial community composition of black-tip reef shark skin**

The bacterial community of black-tip reef shark skin investigated in the present study was comprised of a combination of several bacterial genera previously identified to be characteristic of shark skin [27], as well as bacterial taxa common in a range of marine organisms and environments [40–43]. Bacteria previously reported characteristic of the thresher shark (*Alopias vulpinus*) skin microbiome, but absent in corresponding seawater samples were *Erythrobacter, Idiomarina, Marinobacter, and Pseudoalteromonas* [27]. Shotgun sequencing suggested these bacteria harbor potentially important functions, including the synthesis of photosynthate (*Erythrobacter*), heavy metal detoxification (*Idiomarina*), and lipopolysaccharide degradation (*Marinobacter*), the latter of which may mediate and reduce host inflammatory responses [27, 44]. Several *Pseudoalteromonas* species produce compounds with bioactivity against prokaryotes and eukaryotes, affecting biofilm formation and biofouling [45, 46]. While these bacteria are metabolically

**Table 2** Statistics of 16S rRNA gene amplicon sequencing, and richness and diversity indices of bacterial communities associated with visibly healthy and infected skin around the gills and visibly healthy skin on the back of black-tip reef sharks (*Carcharhinus melanopterus*) collected in the Amirante Islands (Seychelles). BD = samples from skin on the back; GD = samples from skin around the gills; F = female; M = male; H = visibly healthy sharks; D = sharks with infected skin around the gills (Continued)
Table 3 Results of global and pairwise test statistics comparing differences in composition of bacterial communities associated with visibly healthy and insulted skin around the gills and visibly healthy skin on the back of black-tip reef sharks (*Carcharhinus melanopterus*) collected in the Amirante Islands (Seychelles). **a)** PERMANOVA results under unrestricted permutation to assess statistical differences of location (gills vs. back) of skin bacterial communities. **b)** Global PERMANOVA results with permutation of residuals under a reduced model to assess statistical differences of sampling site (**site**), health status (**health**), and sex of shark (**sex**) on bacterial community composition on skin around the gills. **c)** Global PERMANOVA results with permutation of residuals under a reduced model to assess statistical differences of sampling site (**site**), health status (**health**), and sex of shark (**sex**) on bacterial community composition on skin on the back. **d)** Summary of ANOSIM pairwise tests for ‘site’. Global $R = 0.551$, significance level $p < 0.0001$

| PERMANOVA table of results |
|-----------------------------|
| **a)** Pairwise PERMANOVA (gills vs. back) |

Terms added sequentially (first to last)

| Df | Su  | SS      | MS      | F.Model | R2    | Pr(>F) |
|----|-----|---------|---------|---------|-------|--------|
| Skin | 1   | 0.2019  | 0.20193 | 0.8359  | 0.0963 | 0.5657 |
| Residuals | 86   | 20.7746 | 0.24157 | 0 | 99,037 |
| Total | 87   | 20.9765 | 1       | 0       |        |        |

**b)** global PERMANOVA for gill samples

Terms added sequentially (first to last)

| Df | SS      | MS      | F.Model | R2    | Pr(>F) |
|----|---------|---------|---------|-------|--------|
| Site | 4     | 3.787   | 0.94675 | 5.5281 | 0.3651 | 0.0001 |
| Health | 1   | 0.2015  | 0.20145 | 1.1763 | 0.01942 | 0.2666 |
| Sex | 1     | 0.1692  | 0.16915 | 0.9877 | 0.01631 | 0.4169 |
| Site:Health | 3   | 0.4111  | 0.13705 | 0.8002 | 0.03964 | 0.7672 |
| Site:Sex | 4   | 0.7226  | 0.18064 | 1.0548 | 0.06966 | 0.3818 |
| Health:Sex | 1   | 0.1146  | 0.11461 | 0.6692 | 0.01105 | 0.7496 |
| Residuals | 29  | 4.9666  | 0.17126 | 0 | 0.47882 |
| Total | 43   | 10.3725 | 1       | 0       |        |        |

**c)** global PERMANOVA for back samples

Terms added sequentially (first to last)

| Df | SS      | MS      | F.Model | R2    | Pr(>F) |
|----|---------|---------|---------|-------|--------|
| Site | 4     | 3.5699  | 0.89246 | 4.9904 | 0.34319 | 0.0001 |
| Health | 1   | 0.195   | 0.19504 | 1.0906 | 0.01875 | 0.3035 |
| Sex | 1     | 0.2623  | 0.26227 | 1.4665 | 0.02521 | 0.1232 |
| Site:Health | 3   | 0.4142  | 0.13808 | 0.7721 | 0.03982 | 0.8355 |
| Site:Sex | 4   | 0.6674  | 0.16684 | 0.9329 | 0.06416 | 0.5962 |
| Health:Sex | 1   | 0.1071  | 0.10709 | 0.5988 | 0.01029 | 0.853  |
| Residuals | 29  | 5.1862  | 0.17884 | 0 | 0.49858 |
| Total | 43   | 10.4021 | 1       | 0       |        |        |

**d)** ANOSIM for gill/back samples

Pairwise Tests

| Groups | R Stats | Sig. Level | Act. Perm. |
|--------|---------|------------|------------|
| StJos_WRes, D’Arros_North | 0.8415/0.6811 | 0.001/0.001 | 9999 |
| StJos_WRes, StJos_ERes | 0.3809/0.4359 | 0.002/0.001 | 9999 |
| StJos_WRes, StJos_Fouq | 0.3692/0.2233 | 0.002/0.024 | 9999 |
| StJos_WRes, StJos_Ben | 0.4525/0.1103 | 0.001/0.241 | 9999 |
| D’Arros_North, StJos_ERes | 0.8476/0.8237 | 0.001/0.001 | 9999 |
| D’Arros_North, StJos_Fouq | 0.8923/0.7936 | 0.001/0.001 | 9999 |
| D’Arros_North, StJos_Ben | 0.9164/0.7095 | 0.003/0.007 | 9999 |
**Table 3** Results of global and pairwise test statistics comparing differences in composition of bacterial communities associated with visibly healthy and insulted skin around the gills and visibly healthy skin on the back of black-tip reef sharks (*Carcharinus melanopterus*) collected in the Amirante Islands (Seychelles). a) PERMANOVA results under unrestricted permutation to assess statistical differences of location (gills vs. back) of skin bacterial communities. b) Global PERMANOVA results with permutation of residuals under a reduced model to assess statistical differences of sampling site (‘site’), health status (‘health’), and sex of shark (‘sex’) on bacterial community composition on skin around the gills. c) Global PERMANOVA results with permutation of residuals under a reduced model to assess statistical differences of sampling site (‘site’), health status (‘health’), and sex of shark (‘sex’) on bacterial community composition on skin on the back. d) Summary of ANOSIM pairwise tests for ‘site’. Global $R = 0.551$, significance level $p < 0.0001$ (Continued)

| Site | Global $R$ | Significance level $p$ |
|------|------------|------------------------|
| StJos_North, StJos_Fouq | 0.01162/0.01265 | 0.364/0.371 | 9999 |
| StJos_ERes, StJos_Ben | 0.1076/0.3354 | 0.270/0.086 | 9999 |
| StJos_Fouq, StJos_Ben | 0.0119/0.1746 | 0.436/0.184 | 9999 |

Diverse and may exhibit different metabolic traits even at the strain level, they may have a potentially critical role in structuring the shark skin microbiome and aid in the prevention of bacterial infection of (injured) skin. Notably these four bacterial genera occur on both thresher shark and black-tip reef shark skin – two species of shark exhibiting very different ecological niches and lifestyles [47] – suggesting a potentially conserved role in shark skin health.

We identified eleven core microbiome members of black-tip reef shark skin. Two of these could be annotated to the species level: OTU11 *Psychrobacter pacificensis* and OTU16 *Psychroderma porphyrae*. *Psychrobacters* were previously identified as core microbiome members of humpback whale skin and have been linked with whale health and immunity [48, 49]. Notably, *Psychrobacters* occur in the skin mucus of bony fish [50] and pure isolates have shown inhibition to aquatic fungal pathogens [51]. The presence of *Psychrobacters* on the skin of whale [48], shark [27] and in the present study, and bony fish suggest *Psychrobacters* may be ubiquitous and functionally important skin microbiota of aquatic vertebrates. While it should be noted that the identification of the core microbiome is always only an approximation, biased by sample design and sample size, the present study features a reasonable number of samples covering a fairly comprehensive study area. This is further supported by the identification of *Pseudoalteromonas* and *Psychrobacter* as core microbiome members of black-tip reef shark skin, given the contemporary literature (see above). In this regard, future work should include the isolation of bacteria to assess their potential contribution to shark skin health. In particular, targeting the production and activity of antibiotics, antimicrobial peptides, and other bioactive compounds may provide clues as to the importance of bacteria.

In the present study, the bacterial communities of shark skin were conserved with regard to skin health state and sampled skin location, but exhibited differences between sampling locations within the Amirante Islands. While the sites are only a few kilometers away from each other, relative abundances of core microbiome members (Table 4) likely reflect oceanographic connectivity and movement of sharks between the three St. Joseph islands, i.e. East Ressource, Fouquet, and Benjamin, as opposed to the other two sites, North Side (off d’Arros) and West Ressource (belonging to the St. Joseph reef group, but situated closer to d’Arros). Thereby, the shark skin microbiome may be reflecting seawater properties, connectivity, and potentially anthropogenic impact of the respective sampling locations within the study area, while transmission of surface microbes between individual sharks using a reef area may also be a factor, since this species often feed in close proximity to one another. This observed location-specific pattern is in line with our understanding of the movement ecology of the black-tip reef shark, since the species exhibits the smallest known home range within the genus *Carcharhinus*, in some cases being known to not (or rarely) cross between adjacent habitats separated by channels of as little as 1.7 km [52–54]. Indeed, an acoustic tagging study undertaken in parallel at the same locations as the present study has shown that in contrast to other species, black-tip reef sharks rarely cross the deeper water between D’Arros and St. Joseph island [55], likely due to the risk of predation by larger shark species [56, 57]. The distances between the islands off St. Joseph reef (East Ressource, Fouquet, Benjamin) however are well within the home ranges reported for black-tip reef sharks, and cross-reef migration in this area has been observed [55]. The same may apply to the sites North Side and West Ressource. Hence, between-island movement of sharks likely explains observed patterns in skin-associated bacterial communities in the present study.

**Potential causes of skin insults in black-tip reef sharks**

The bacterial community composition conserved in both visibly healthy and insulted skin covering the gill area strongly suggests that despite sometimes extensive visible skin injury, there is no indication of severe bacterial infection as characterized by the propagation of opportunistic or pathogenic bacteria. Indeed, not every wound
progresses to being infected, and, even when inflammation is present, bacterial infection may not occur [61]. While the skin insult might have been caused by infection with fungi [17] or monogenean worms [16, 23], skin-associated bacteria likely would have exhibited a ‘secondary’ change in community composition in response to primary eukaryotic infection. Therefore, eukaryotic infection as the cause of skin insults may be unlikely. Rather, skin insults observed in the black-tip reef shark samples may have been a consequence of mechanic disruption of the skin. Due to the limitations of vessel-based field work, we could not directly observe the cause of skin insults, or track the development of skin insults over time, but as the behavior of black-tip

---

Fig. 2 Family-level stacked bar plots showing bacterial community composition of healthy and insulted skin samples of black-tip reef sharks (*Carcharhinus melanopterus*) collected at different sites in the Amirante Islands, Seychelles. **a** Samples from the skin around the gill area. **b** Samples from the skin on the back of the shark. There are no statistically significant differences at OTU level for health state (‘healthy’, ‘insulted’; PERMANOVA, Pseudo-F = 1.1031; p = 0.2646), and location on skin (‘gill’, ‘back’; PERMANOVA, Pseudo-F = 1.316; p = 0.2839). Community composition was significantly different at OTU level between study sites (PERMANOVA, Pseudo-F = 4.1429, p < 0.0001)
reef sharks is reasonably well understood, it is conceivable to interpret the insults as the result of inter- and intraspecific antagonistic interactions. In some cases, this could have occurred during the mating act, in which male sharks commonly injure females during courtship and intromission by biting on to one of their pectoral fins and gill area, or when entangled both partners may come into physical contact with nearby rocks or coral [54]. However, similar skin insults were observed in both female and male sharks, the two sexes exhibiting similar patterns of damage, being concentrated on the anterior flank, immediately around the gills. While this might be suggestive of damage inflicted by a gill parasite, none were evident on quick inspection in the field. Other causes of mechanical disruption of the skin in black-tip reef sharks are also possible, such as boat strike, or intraspecific aggressive behavior or predation attempts by larger sharks [15, 56], although most injuries did not suggest these causes in the present study.

Conserved bacterial communities on healthy and insulted skin: structural properties of shark skin and immune responses

Skin acts as a physical barrier to the surrounding environment, protecting against invasion by foreign substances and pathogens [26, 30]. Skin microbiomes are shaped in part by properties, such as topographical location, endogenous host factors, and exogenous environmental factors [27, 28, 58]. Skin insults, including injury, lesions, inflammation, infection, or disease, are commonly associated with microbiome shifts [31–33, 35]. Whether or not progression from bacterial colonization to infection occurs depends first and foremost on the host’s immune response [61]. In the present study, bacterial community composition and structure was highly conserved between healthy and insulted shark skin samples based on 16S rRNA gene amplicon sequencing. From the bacterial community profiles, any progression from bacterial colonization to severe infection (characterized by the propagation of potential pathogens) was notably absent, even though a mild bacterial infection may have gone undetected.

It is important to acknowledge that bacterial community profiles based on 16S rRNA gene amplicon sequencing alone cannot address mechanisms underlying the conserved bacterial community composition in visibly healthy and insulted shark skin. Nonetheless, the present study provides insight into the ecology of shark skin microbiomes and highlights that mechanistic studies will be required for a better understanding of bacterial infection and immunity in sharks. Specifically, future studies should target whether shark skin and its associated bacteria are able to maintain skin functioning under environmental stress or severe tissue insult, as previously suggested [27, 38], and whether this is linked to endogenous host factors.

Endogenous host factors encompass physical properties of the skin, such as its microtexture [59, 60] and cutaneous immune response repertoires, which may modulate skin-associated bacterial communities [28]. In sharks, skin microtexture potentially constitutes an important host factor that contributes to the structuring of bacterial communities. As described previously, shark skin exhibits a unique cutaneous structure, morphologically setting it
apart from the skin of bony fish. Specifically, shark skin is characterized by dermal denticles, which protrude through both the epidermis and mucus layer. This results in a textured surface with pronounced microscopic ridging, which appears to greatly reduce microbial settlement [59, 60] and which has likewise been found to reduce microbial settlement on a similarly textured experimental substrate [68]. Another potential factor mediating skin bacterial communities in black-tip reef sharks may be the production of antimicrobial compounds resident in the skin or skin mucus layer. While the presence of such compounds has been previously reported from other sharks (e.g., squalamines, a group of water-soluble antibiotics associated with shark organs and tissues) and from bony fish [62–64], their role in countering bacterial infection in hospite still needs to be assessed. Hence, the potential role of resident bacterial members in structuring the shark skin microbiome [30, 45] and supporting wound healing by mediating the inflammatory response [27, 44, 65, 66] should be a focus of future research efforts. Finally, as in all cartilaginous fish, the shark immune system encompasses adaptive components (e.g., an immunoglobulin system) and appears to be capable of immunological recall [67]. If and how the adaptive immune system plays into the significant capacity for wound healing in the black-tip reef shark [57] remains yet to be determined. Nevertheless, our finding of conserved bacterial community structures between healthy and injured black tip reef shark skin highlights the putative immense capacity to thwart bacterial infection and support rapid wound healing.

Table 4 Relative abundances (%) of putative core microbiome members of bacterial communities associated with visibly healthy and infected skin around the gills and visibly healthy skin on the back of black-tip reef sharks (Carcharhinus melanopterus) collected in the Amirante Islands (Seychelles), presented for a) gills and b) backs of sharks. Bacterial relative abundances are averaged within sites (data presented as means ± SD). Taxonomy: Numbers in brackets constitute bootstrap values; only bootstrap values < 100 are shown.

| Site       | West Ressource | North Side | East Ressouce | Fouquet | Benjamin | Taxonomy                          |
|------------|----------------|------------|---------------|---------|----------|-----------------------------------|
| a) Gills   |                |            |               |         |          |                                   |
| OTU00001   | 3.61 ± 2.95    | 2.86 ± 1.23| 10.49 ± 3.76  | 8.61 ± 3.83| 9.48 ± 5.54| f__Rhodobacteraceae_unclass.(86) |
| OTU00002   | 5.11 ± 4.62    | 1.76 ± 1.59| 4.24 ± 3.75   | 8.28 ± 7.16| 9.04 ± 2.72| g__Alteromonas_unclass.           |
| OTU00003   | 5.65 ± 1.87    | 1.21 ± 0.86| 3.11 ± 1.50   | 2.53 ± 1.55| 1.40 ± 1.20| f__Pelagibacteraceae_unclass.(99) |
| OTU00004   | 1.45 ± 1.54    | 0.09 ± 0.11| 3.19 ± 1.28   | 4.38 ± 2.57| 3.28 ± 0.63| o__Flavobacteriales_unclass.      |
| OTU00005   | 2.02 ± 1.13    | 3.62 ± 1.74| 1.22 ± 0.94   | 1.17 ± 0.50| 1.3 ± 0.46 | o__Vibrionales_unclass.(85)      |
| OTU00006   | 2.07 ± 1.05    | 0.24 ± 0.14| 2.08 ± 1.04   | 2.06 ± 1.07| 1.62 ± 1.17| f__OCS155_unclass.                |
| OTU00010   | 0.63 ± 0.70    | 0.07 ± 0.09| 2.42 ± 1.11   | 2.02 ± 1.02| 3.67 ± 1.96| o__Oceanospirillales_unclass.     |
| OTU00011   | 0.8 ± 0.67     | 0.28 ± 0.35| 1.69 ± 1.19   | 1.78 ± 0.74| 0.98 ± 0.57| s__Psychrobacter pacificensis     |
| OTU00014   | 0.98 ± 1.17    | 0.28 ± 0.33| 2.13 ± 1.13   | 1.81 ± 0.80| 0.96 ± 0.84| f__Flavobacteriaceae_unclass.     |
| OTU00016   | 0.06 ± 0.72    | 0.78 ± 0.55| 1.72 ± 0.96   | 1.42 ± 0.36| 0.67 ± 0.074| s__Pseudalteromonas porphyrae   |
| OTU00019   | 1.04 ± 0.98    | 0.03 ± 0.03| 1.57 ± 0.84   | 1.76 ± 0.89| 1.09 ± 0.82| f__Halomonadaceae_unclass.        |
| Others     | 75.95 ± 4.83   | 88.78 ± 2.36| 66.14 ± 8.33  | 64.17 ± 4.59| 66.47 ± 8.04|                                 |
| b) Back    |                |            |               |         |          |                                   |
| OTU00001   | 2.92 ± 2.39    | 2.55 ± 1.29| 10.96 ± 4.01  | 8.33 ± 3.77| 9.15 ± 4.74| f__Rhodobacteraceae_unclass.(86) |
| OTU00002   | 7.57 ± 7.78    | 2.88 ± 2.61| 4.49 ± 2.29   | 8.14 ± 3.86| 10.57 ± 6.65| g__Alteromonas_unclass.           |
| OTU00003   | 7.58 ± 5.39    | 1.36 ± 1.78| 3.40 ± 1.37   | 7.77 ± 1.30| 1.68 ± 1.26| f__Pelagibacteraceae_unclass.(99) |
| OTU00004   | 0.90 ± 0.61    | 0.08 ± 0.15| 3.36 ± 1.37   | 8.31 ± 1.56| 2.19 ± 0.82| o__Flavobacteriales_unclass.      |
| OTU00005   | 1.49 ± 1.28    | 2.36 ± 0.90| 1.81 ± 1.32   | 9.05 ± 0.42| 1.76 ± 1.30| o__Vibrionales_unclass.(85)      |
| OTU00006   | 2.41 ± 1.65    | 0.26 ± 0.28| 2.62 ± 0.91   | 10.15 ± 1.06| 1.96 ± 0.53| f__OCS155_unclass.                |
| OTU00010   | 0.68 ± 0.91    | 0.04 ± 0.06| 2.71 ± 1.10   | 11.44 ± 0.85| 4.74 ± 3.34| o__Oceanospirillales_unclass.     |
| OTU00011   | 1.00 ± 1.05    | 0.39 ± 0.48| 3.42 ± 3.06   | 13.18 ± 0.83| 1.64 ± 0.37| s__Psychrobacter pacificensis     |
| OTU00014   | 0.88 ± 0.81    | 0.28 ± 0.34| 2.15 ± 0.82   | 15.99 ± 0.89| 1.28 ± 0.80| f__Flavobacteriaceae_unclass.     |
| OTU00016   | 0.70 ± 0.63    | 1.34 ± 1.02| 2.08 ± 0.95   | 20.33 ± 0.45| 0.78 ± 0.30| s__Pseudalteromonas porphyrae   |
| OTU00019   | 1.08 ± 1.21    | 0.03 ± 0.05| 1.89 ± 0.79   | 3.00 ± 1.03| 1.50 ± 0.88| f__Halomonadaceae_unclass.        |
| Others     | 72.78 ± 7.39   | 88.43 ± 3.13| 61.10 ± 7.76  | 58.56 ± 19.04| 62.75 ± 4.52|                                 |
Conclusions
The present study employed high throughput 16S rRNA gene amplicon sequencing to characterize skin-associated bacterial communities of black-tip reef sharks from the Amirante Islands in the Seychelles. Comparison of visibly healthy and insulted skin samples from the gill areas, as well as healthy skin samples from the back of the sharks, showed no differences in bacterial community composition, suggesting conservation of microbiome structure even under injury. At present the relative contribution of animal host factors, such as the ultrastructure of the shark skin to limit bacterial settlement or factors attributable to the resident bacterial community, such as the production of antimicrobial compounds, is unknown. Both factors may help select and preserve the native bacterial community even upon tissue insult and may likewise counter infection. In contrast to the similarities between healthy and injured skin samples, differences related to collection sites suggest that bacterial community structure may respond to exogenous environmental factors. For a better understanding of the roles and properties of resident bacteria of shark skin, future studies should aim for a comprehensive approach combining bacterial community profiling with host immune assays and screening for bioactive compounds from bacterial isolates. Such a combined approach may help elucidate the mechanisms underlying the considerable capacity for wound healing and microbiome resilience prevalent in sharks.

Methods
Sampling sites, shark sampling, and swab collection
Black-tip reef sharks were wild-caught and sampled in the Amirante Islands, Seychelles, from 27 March – 19 April 2017 (Fig. 1a; Additional file 6: Table S3). Sampling locations included St. Joseph Atoll (Four Sites: Western Ressource, Eastern Ressource, Fouquet, and Benjamin) and D’Arros Island (North Site; Fig. 1a). Overall, the sites are located a few hundred meters (within the St. Joseph Island group) to a few kilometers away from each other (between North Site off D’Arros and the St. Joseph island group). Notably, Ressource is located about halfway between D’Arros (in the West) and St. Joseph (in the East), however its western reefs are facing D’Arros, and its eastern reefs are facing the St. Joseph island group. Likely, W. and E. Ressource are therefore more strongly oceanographically connected to D’Arros and St. Joseph, respectively.

A total of 44 black-tip reef sharks were caught alive by circle hook and line; the sharks remained partially submerged at the side of the boat during sampling and were then released unharmed. Skin sections from which mucus swab samples were taken were briefly exposed to air during the sampling. For each shark, the left side of the body was sampled. Specifically, one sample was taken from the skin covering and around the gill area, and a second sample from the skin on the back just below the first dorsal fin, by swabbing the surface with individual forceps-held sterile cotton swabs (Nuova Aptaca, Italy) so as to collect a sample of the mucus. Overall, 44 mucus swabs were collected from each of (a) the skin covering and around the gills (‘gills’) and (b) the dorsal part of the flank (‘back’), resulting in 88 swab samples in total. Swabs were selected as a means of non-invasive sampling [69]. Swab samples were immediately transferred into RNAlater and stored at 5 °C and subsequently –20 °C until further processing. Sampling the same shark twice was avoided by taking pictures of each side of the first dorsal fin to document individual markings on each shark, an approach which is commonly used for identification of individuals. In addition, all sharks sampled were marked by removing the extreme tip of the anal fin.

For each sampled shark, health condition (‘healthy’ and ‘insulted’) of the skin covering gills was recorded. ‘Healthy’ shark samples did not exhibit any visible signs of tissue insult on the skin surrounding the gill area. ‘Insulted’ shark samples exhibited marked tissue insult (Fig. 1c). None of the sharks exhibited any visible skin insults on the ‘back’ area, i.e., in the dorsal part of the flank. Sampling of insulted skin area entailed sampling directly across the insulted area on the skin covering the gills in order to determine whether bacterial community composition was different in insulted skin areas compared to visibly healthy skin. Due to practical considerations, time constraints, and the fact that observation of shark matings are very rare, we were not able to observe when individual skin insults were inflicted, nor to track the development of insults over time. Hence, the age of skin insults at the time of sampling is unknown.

DNA extraction, PCR conditions, sequencing library preparation
Prior to DNA extraction, swabs were thawed at room temperature, removed from RNAlater solution, each placed in a sterile 1.5 ml Eppendorf tube, and air-dried for 10 min. DNA extraction was conducted using a modified ‘Wayne’s’ protocol [70]. 375 μl of freshly prepared extraction buffer (100 mM Tris, 100 mM EDTA, 100 mM NaCl, 1% SDS) was added to each tube. Samples were vortexed and incubated at 65 °C for 2 h. 1 μl of RNase A was added 15 min before the end of the incubation. After the incubation samples were vortexed again, the swab removed, and the sample put on ice. 94 μl of 5 M KOAc was added to each tube, vortexed, and incubated on ice for 10 min. Samples were then centrifuged for 10 min (14,000 rpm, RT). The supernatant was transferred to a new tube and 300 μl of 100%
isopropanol added, mixed gently, and incubated for 5 min at RT. Samples were then spun at maximum speed at RT for 20 min. The supernatant was discarded by pipetting. 150 µl of 70% ethanol were added to each tube, mixed gently, and then tubes were centrifuged at maximum speed for 10 min. The resulting DNA pellet was air-dried for 15 min and subsequently resuspended overnight at 4 °C in 20 µl of 0.1 M Tris. Isolated DNA was quantified on the NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, USA). In addition to DNA extractions from samples, mock DNA extractions (no sample, reagents only) were conducted.

For all samples, PCR amplifications were performed in triplicates using Qiagen Multiplex PCR Kit (Qiagen, Germany) with primers containing Illumina adapters (underlined below). For the 16S rRNA gene sequencing, we amplified the hypervariable regions V5 and V6 of the bacterial 16S rRNA gene. Primers 16SMiSeqF-Andersson 5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCCTGGTA-3′ and 16SMiSeqR-Andersson 5′-GCTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATGCAGGCGCACACGCTCACTATAGATAAGAGACAGAGGATTAGATACCCTGGTA-3′ were used, which have previously been shown to amplify well with marine templates [41, 71]. Individual PCRs were run using 5 µl Qiagen Mix, 0.2 µl of each 10 µM primer mix, 1 µl of DNA template, and RNase-free water to adjust to a final reaction volume of 10 µl. In addition to samples, PCRs were run for templates from the mock DNA extraction, along with mock PCRs (no template input). Thermal cycling conditions for 16S rRNA gene PCRs were: 95 °C for 15 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 90 s, 72 °C for 30 s, and a final extension cycle of 72 °C at 10 min. Five µl of each PCR product were run on an 1% agarose gel for 30 s, and a final extension cycle of 72 °C for 10 min. The resulting DNA pellet was maximum speed for 10 min. PCR products were subjected to an indexing PCR (8 cycles) followed by 27 cycles of 95 °C for 15 min, 55 °C for 90 s, 72 °C for 30 s, and a final extension cycle of 72 °C at 10 min. Five µl of each PCR product were run on an 1% agarose gel to visualize successful amplification. Sample triplicates were subsequently pooled and then purified with Illustra Exo-ProStar 1-Step (GE Healthcare Life Sciences, UK). Purified PCR products were subjected to an indexing PCR (8 cycles) to add Nextera XT indexing and sequencing adapters (Illumina, USA) according to the manufacturer’s protocol. Indexed products were again purified and normalized with the SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, USA), followed by quantification on the BioAnalyzer (Agilent Technologies, USA) and Qubit (Quant-IT dsDNA High Sensitivity Assay Kit; Invitrogen, USA), and pooled in equimolar ratios. The library was sequenced at 15 pM with 2% phiX on the Illumina HiSeq 2500, 2 × 250 bp end, Rapid run, 500 cycles, according to the manufacturer’s specifications at the Bioscience Core Lab (BCL) at the King Abdullah University of Science and Technology (KAUST), Saudi Arabia. Libraries sequenced included samples along with PCR products from mock DNA extractions and mock PCRs as a negative control to account for environmental and laboratory contamination.

### Sequencing data analysis

To assess bacterial community composition of shark skin of different health states and from different locations on shark skin, we sequenced 88 16S rRNA gene amplicon libraries (44 gill + 44 back samples, distributed over 28 visibly healthy + 16 injured specimens (Additional file 4: Table S1). Bacterial 16S rRNA gene amplicon sequences were processed using mothur version 1.39.0 using the mothur MiSeq SOP (accession date: May 2018; [72] (Additional file 1: Methods S1). In brief, sequences were assembled into contigs and quality trimmed. Identical sequences (duplicates) were merged. Singletons and rare sequences (n < 10 over all samples) were removed. This resulted in 18,022,131 sequences distributed over 88 shark samples [44 gill and 44 back skin samples; distributed over 28 visibly healthy and 16 infected individuals]. After trimming, 14,320,306 sequences with average length of 292 bp remained. Remaining sequences were aligned against the SILVA database (release 119; [73]) and pre-clustered (2 bp difference; [74]). Chimeric sequences were removed using the VSEARCH command [75]. Unwanted sequences assigned to chloroplasts, mitochondria, archaea, and eukaryotes were removed, clustered into Operational Taxonomic Units (OTUs, 97% similarity cutoff), and annotated against the Greengenes database (release gg_13_8_99, [76]). Notably, the here-used primer pair 784F-1016R is not well suited for the amplification of archaeal 16S rRNA gene sequences, as assessed using the TestPrime tool in SILVA (https://www.arb-silva.de/search/testprime/): coverage and specificity of this primer pair against the SILVA database was 0 for archaea. For this reason, any sequences assigned as archaea were removed during the remove.lineages step in mothur (for details, please refer to Additional file 1: Methods S1). After removal of these unwanted sequences 10,674,925 sequences were retained. Subsequently, sequences were subsampled to 24,190 sequences per sample, and low abundance taxa (< 10 sequences across all samples) were removed. Environmental and laboratory contaminants were removed based on sequencing results of mock extractions and mock PCRs (Staphylococcus OTU 00008, Propionibacterium OTU00024, Caulobacter OTU00099, Pelomonas OTU00148, Sphingomonas OTU00196, Brevibacterium OTU00238, Sediminibacterium OTU00290, Corynebacterium OTU00333, Aquabacterium OTU00511, Microbyspora OTU00598, Bosea OTU00601, Delftia OTU00745, Rubricoccus OTU00949, Polyangiaceae sp. OTU01000 and OTU02727, Sapropiraceae sp. OTU01314, Myroides OTU02959, and Frankiaceae OTU04398, some of which are common lab or kit contaminants [77], along with Endozoicomonas OTUs 00022, 00065, 00121, 00301, a marine bacterium maintained in permanent culture in the processing lab). After removal of sequences related to contaminants,
a total of 2,034,047 sequences (on average 23,114 sequences per sample) were retained for subsequent analyses. Alpha diversity metrics were calculated with the `summary.single` command as implemented in `mothur` [78]. The bacterial ‘core’ microbiome was extracted with the `get.coremicrobiome` command as implemented in `mothur` at an 80 and 50% cut-off (i.e., present in at least 80 and 50% of all samples, respectively) [78]. All raw sequence data are accessible under NCBI’s BioProject PRJNA498626.

Statistical analysis
Sequence counts of the OTU abundance table were converted into relative abundance data, normalized, and square root transformed. Bray-Curtis similarity was applied on the square root transformed data [79]. Subsequently, permutational multivariate analysis of variance (PERMANOVA [80]) was conducted. To assess differences in bacterial community composition between sharks with visibly healthy and insulted skin covering the gill area, PERMANOVAs were run separately on samples from gills and back using `adonis` [80]. To assess differences in bacterial community composition for sampling sites in the Amirante Islands, ‘site’ was assigned a fixed factor and shark ‘sex’ was assigned a random factor nested in ‘site’. Subsequently, 9999 permutations of residuals under a reduced model were conducted based on Bray–Curtis distances between root transformed samples. In addition, pairwise Analysis of Similarity (ANOSIM) comparisons with 9999 permutations were run for factor sampling site (‘site’) to assess which sites were significantly different from each other. Beta diversity differences for bacterial community composition were visualized in a principal coordinate analysis based on a Bray–Curtis dissimilarity matrix. A two-way ANOVA run in R [81] revealed the main contributing bacterial families responsible for differences regarding shark health state and sampling site.

Additional files

Additional file 1: Table S1 OTU abundance table showing the distribution of bacterial 16S rRNA gene amplicon sequences for each OTU over samples. Bacterial communities were associated with visibly healthy and infected skin around the gills and visibly healthy skin on the back of black-tip reef sharks (Carcharhinus melanopterus) collected in the Amirante Islands (Seychelles). (TXT 3913 kb)

Additional file 2: Table S2 Detailed results of two-way ANOVA with subsequent FDR correction to test for differentially abundant bacterial OTUs of black-tip reef sharks (Carcharhinus melanopterus) between shark collection sites, location of shark skin tissue, and the interaction of both. (TXT 620 kb)

Additional file 3: Table S3 Details on collection date, location, health state, and sex of individual blacktip reef sharks collected in 2017 around the Amirante Islands; Seychelles. Shark identifiers printed in bold indicate individuals with observed skin insult. (DOCX 19 kb)

Abbreviations
ANOSIM: Analysis of Similarity; ANOVA: Analysis of Variance; bp: Base pair; DNA: Deoxyribonucleic acid; FDR: False discovery rate; MS: Mean of squares; NCBI: National Center for Biotechnology Information; OTU: Operational taxonomic unit; PCoA: Principal Coordinate Analysis; PCR: Polymerase Chain Reaction; PERMANOVA: Permutational Analysis of Variance; rpm: Rotations per minute; RT: Room temperature; SE: Standard error; SOP: Standard operation procedure; SS: Sum of squares

Acknowledgements
Thank you to N. Rädecker for assistance with statistics in R. Thank you to the KAUST Biosciences Core Lab for sequencing assistance. We thank an anonymous reviewer whose comments have helped improving the manuscript.

Authors’ contributions
RO, CRC, CRV designed and conceived the study. MG, CM, and RN collected the skin swabs. GP processed the samples and prepared the 16S rRNA sequencing libraries. CP, CRV analyzed and interpreted the data and wrote the manuscript with contributions from MG, RO, MG, RO, CRC, CRV provided tools and reagents. All authors provided input to the manuscript and approved the final version for submission.

Funding
Baseline funding by the King Abdullah University of Science and Technology to CRV; Specific project funding for fieldwork from the Marine Research Facility, Jeddah.

Availability of data and materials
Sequence data determined in this study are available under NCBI BioProject ID PRJNA498626 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA498626). Abundant shark skin bacterial microbiome OTU reference sequences are available under GenBank Accession numbers MK577282 - MK577302 (https://www.ncbi.nlm.nih.gov/nucleotide?term=MK577282:MK577302[accn]).

Ethics approval
The study was undertaken within the scope of a letter of approval from the Seychelles Ministry of the Environment. All sharks sampled were released unharmed.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Red Sea Research Center, Biological and Environmental Science and Engineering Division (BSE), King Abdullah University of Science and Technology (KAUST), Thuwal 23955, Saudi Arabia. 2Marine Conservation International, South Queensferry, Edinburgh, Scotland, UK, 3Centre for Marine Biodiversity & Biotechnology, Heriot-Watt University, Riccarton, Edinburgh, Scotland, UK. 4Veterinär-Physiologisch-Chemisches Institut, University of Leipzig, 04107 Leipzig, Germany. 5Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.
References

1. Baum JK, Myers RA. Shifting baselines and the decline of pelagic sharks in the Mediterranean sea. Conserv Biol. 2003;17:46–50.
2. Baum JK, Myers RA, Kehler DG, Worm B, Harley SJ, Doherty PA. Collapse and conservation of shark populations in the Northern Atlantic. Science. 2003;293:89–93.
3. Robbins WD, Hisano M, Connolly SR, Choat JH. Ongoing collapse of coral-reef shark populations. Curr Biol. 2006;16:2314–9.
4. Poynton SL, Campbell TW, Palm HW. Loss of large predatory sharks from the Mediterranean sea. Conserv Biol. 2008;22:952–64.
5. Graham NAJ, Spalding MD, Sheppard CRF. Reef shark declines in remote atolls highlight the need for multi-faceted conservation action. Aquat Conserv Mar Freshw Ecosyst. 2010;15:458–63.
6. Duply NA, Baum JK, Clarke S, Compagno LIV, Cortes E, Al E. You can swim but you can’t hide: the global status and conservation of oceanic pelagic sharks and rays. Conserv Mar Freshw Ecosyst. 2008;8:1459–502.
7. IUCN. UCN red list of threatened species. version 4, update 2016–2017. p. 7.
8. Ormond R, Gore M, Bladon A, Dubock O, Kohler J, Millar C. Protecting the world’s sharks: the first decade of the Global Shark Conservation Initiative. Marine Research Facility, North Carolina State University, Jeddah 21589, Saudi Arabia. 6Marine Research Facility, North Carolina State University, Jeddah 21589, Saudi Arabia. 6
9. Nigmatulina T, Chen TH, Narala S, Chun KA, Two AM, Yun T, et al. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci Transl Med. 2017;9:360ra1–12.
10. Cárdenas A. Rodríguez-R LM, Pizarro V, Cadavid LF, Arroyo-Cero F, Cárdenas A. et al. Shifts in bacterial communities of two Caribbean reef-building coral species affected by white plague disease. ISME J. 2012;6:502–12.
11. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beattson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res. 2012;22:580–9.
12. Roder C, Aifl C, Daniels C, Weil E, Voolstra CR. Bacterial profiling of white plaque disease across corals and oceans indicates a conserved and distinct disease microbiome. Mol Ecol. 2014;23:965–74.
13. 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:fx080.
14. Shedd EB Jr, Albert TF, Woolley RE, Brown J. Microflora associated with the skin of the bowhead whale (Balaena mysticetus). J Wildl Dis. 1990;26:635–9.
15. Becker MH, Harris RN. Cutaneous bacteria of the redback salamander prevent morbidity associated with a lethal disease. PLoS One. 2010;5:1–6.
16. Zaneveld JD, McIndoe R, Vega Thurber R. Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nat Microbiol. 2017;2:17121.
17. Reid G, Yone JA, Van der Me HC, Gooob G, Knight R, Busscher HJ. Microbiota restoration: natural and supplemented recovery of human microbial communities. Nat Rev Microbiol. 2011;9:277–83.
18. Vignaud T, Mourié J, Maynard J, Leblanc B, Spautz J, Claux E, et al. Blacktip reef sharks, Carcharhinus melanopterus, have high genetic structure and varying demographic histories in their Indo-Pacific range. Mol Ecol. 2014;23:5193–207.
19. Pfreundt U, Spungin D, Bonnet S, Berman-frank I, Hess WR. Global analysis of gene expression dynamics within the marine microbiome during the VAHINE mesocosm experiment in the Southwest Pacific. Bioseosciences. 2016;13:341–55.
20. Poogrez C, Rädecker N, Cárdenas A, Gárdes 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.
21. Raina JB, Tapolías D, Willis BL, Bourne DG. Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. Appl Environ Microbiol. 2009;75:3492–501.
22. Romanenko LA, Schumann P,Mohd B, Hijazi A. Shifting baselines and the decline of pelagic sharks in the Mediterranean sea. Conserv Biol. 2003;17:46–50.
23. Mahnich DF, Colwell RR, Stemmler J, Hada M, Meilawal D, Hettick MV, et al. Vibrio species as agents of elasmobranch disease. Helgolander Meeresuntersuchungen. 1984;31:135–9.
24. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2011;9:244–53.
25. Egan S, Gardiner M. Microbial dysbiosis: rethinking disease in marine ecosystems. Front Microbiol. 2016;7:991.

Page 15 of 16

Microbiota restoration: natural and supplemented recovery of human microbial communities. Nat Rev Microbiol. 2011;9:277–83.
20. Vignaud T, Mourié J, Maynard J, Leblanc B, Spautz J, Claux E, et al. Blacktip reef sharks, Carcharhinus melanopterus, have high genetic structure and varying demographic histories in their Indo-Pacific range. Mol Ecol. 2014;23:5193–207.
21. Pfreundt U, Spungin D, Bonnet S, Berman-frank I, Hess WR. Global analysis of gene expression dynamics within the marine microbiome during the VAHINE mesocosm experiment in the Southwest Pacific. Bioseosciences. 2016;13:341–55.
20. Poogrez C, Rädecker N, Cárdenas A, Gárdes 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.
21. Raina JB, Tapolías D, Willis BL, Bourne DG. Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. Appl Environ Microbiol. 2009;75:3492–501.
22. Romanenko LA, Schumann P, Riddle M, Mihallov W, Stackebrandt E. Halomonas halocynthiae sp. nov., isolated from the marine ascidian Halocynthia roretzi. Int J Syst Evol Microbiol. 2002;52:1767–72.
23. Gerdes J, Barlow J, Hales A, Ricketts TN, et al. Early emergence of bacterial communities from Caribbean coral reefs and rays (Carcharhinus limbatus). J Fish Dis. 2000;23:167–70.
24. Breen DJ, Colwell RR, Stemmler J, Hada M, Maneval D, Hettick MV, et al. Vibrio species as agents of elasmobranch disease. Helgolander Meeresuntersuchungen. 1984;31:135–9.
25. Mahnich DF, Colwell RR, Stemmler J, Hada M, Maneval D, Hettick MV, et al. Vibrio species as agents of elasmobranch disease. Helgolander Meeresuntersuchungen. 1984;31:135–9.
