Marine Biology (2020) 167:85
https://doi.org/10.1007/s00227-020-03698-7

ORIGINAL PAPER

Bacterial communities on the gills of bonefish (*Albula vulpes*) in the Florida Keys and The Bahamas show spatial structure and differential abundance of disease-associated bacteria

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Received: 21 September 2019 / Accepted: 28 April 2020 / Published online: 30 May 2020

Abstract

The Caribbean bonefish species *Albula vulpes* is an economically important nearshore marine sport fish that has notably declined in the Florida Keys over the past 20–30 years. The reasons for this decline are unclear, although habitat loss, water quality reductions, climate change, and other environmental drivers likely play a role. Infectious disease can also cause precipitous species-specific declines in wildlife populations, but virtually nothing is known about infection in bonefish. We analyzed communities of bacteria on the gills of bonefish from the Florida Keys, where declines are pronounced, and the islands of Eleuthera and Inagua in The Bahamas, where no such declines have been recorded. Bacterial community composition varied significantly among island location (Keys, Eleuthera, Inagua) and among sites within island locations (e.g., tidal creeks, coves, inlets). Seventeen times more bacterial taxa were over-represented in the Florida Keys than in The Bahamas, and several bacterial genera over-represented in the Florida Keys have been linked to environmental contamination and disease (e.g., *Corynebacterium*; *Acholeplasma*; *Staphylococcus*; and *Streptococcus*). These results show that gill bacterial community signatures may prove useful for investigating bonefish spatial ecology and that communities of microbes on bonefish gills contain differentially abundant and potentially pathogenic bacteria that covary with the overall "health" of the population.

Introduction

Bonefish (*Albula* spp.) are a circum-tropically distributed assemblage of genetically related species with a morphology and physiology adapted for benthivory and high-speed burst swimming (Colborn et al. 1997; Murchie et al. 2011). Bonefish are highly prized as sport fish and represent a rare example of a recreational fishery that is predominantly catch-and-release, although they are harvested for consumption in some areas (Danylchuk et al. 2007b; Adams and Cooke 2015; Filous et al. 2019). Much of the economic value of bonefish is tied to angling and eco-tourism, which can provide sustained revenue for communities close to the nearshore, shallow marine habitats ("flats") that bonefish inhabit (Adams et al. 2014). Bonefish also play important ecological roles in coastal marine food webs (Haak et al. 2019; Murchie et al. 2019). Sustainable management of bonefish populations is therefore a priority in both the tropics and sub-tropics (Adams and Cooke 2015).

Unfortunately, bonefish populations in certain locations are declining precipitously (Adams et al. 2014). In the Florida Keys, for example, anglers and guides have reported substantial reductions in catch rates and fish sizes over the last several decades (Brownscombe et al. 2018; Kroloft et al. 2019). Given the economic importance of flats fishing to Florida's economy (estimated at over $465 million in 2012,

Reviewed by Z. Pratte and M. Reverter.

Communicated by K. Clements.

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00227-020-03698-7) contains supplementary material, which is available to authorized users.

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much of this distributed locally; Fedler 2013), determining the causes of bonefish declines is paramount. Proposed factors include habitat loss, water quality declines, weather and hydrological events, reductions in forage, chemical/toxic inputs, overexploitation, and interactions among these factors (Brownscombe et al. 2018; Kroloft et al. 2019). Direct evidence for specific factors is scant, however, and other "flats" species that are also targeted by recreational anglers (e.g., permit, *Trachinotus falcatus*, and Atlantic tarpon, *Megalops atlanticus*) have shown no evidence of similar declines.

Infectious disease can reduce or cause the extinction of wildlife populations, either alone or in synergy with other factors (McCallum 2012; Cunningham et al. 2017). Such diseases include those caused by viruses (e.g., Ebola virus disease in gorillas; Bermejo et al. 2006), bacteria (e.g., hemorrhagic septicemia in antelopes; Robinson et al. 2019) fungi (e.g., white-nose syndrome in bats; Frick et al. 2010), eukaryotic parasites (e.g., trichomonosis in finches; Lawson et al. 2012), and even transmissible cancers (e.g., facial tumor disease in Tasmanian devils; McCallum 2008). Infectious diseases are emerging at accelerated rates globally, driven by forces such as pollution, habitat degradation, species invasions, and climate change (Cunningham et al. 2017; Young et al. 2017; Ogden et al. 2019), including in fish (e.g., Chen et al. 2018; Combe and Gozlan 2018; Reid et al. 2019). Southern Florida is noteworthy as an epicenter of invasive species, including their pathogens, due to high rates of introduction of exotic species and ecological conditions favorable for their persistence (Simberloff et al. 1997; Farrell et al. 2019). Furthermore, certain infectious agents have narrow host ranges or are pathogenic to only one or a few species within ecological assemblages (McCallum 2012). Infectious disease has not, to our knowledge, previously been considered among the panoply of factors contributing to bonefish declines.

The purpose of this study was to provide a provisional assessment of the hypothesis that microbes are contributing to the decline of bonefish in the Florida Keys (Goldberg 2019). We examined differences in bacterial communities on the gills of bonefish from several sites in the Florida Keys, where marked declines have occurred, and in The Bahamas, which are latitudinally and ecologically similar but where bonefish declines have not been observed. We then identified bacterial taxa that differed most significantly in abundance between the Florida Keys and The Bahamas, and we examined whether those taxa might be associated with disease. We chose to analyze gill bacterial communities because gills are immunologically active organs that are entry points for bacterial pathogens of teleost fishes (Secombes and Wang 2012). Previous studies have shown the gill microbial communities of fishes to be diverse and distinct from those of other body compartments (Merrifield and Rodiles 2015; Pratte et al. 2018), even serving as reservoirs of bacterial diversity for complex ecosystems such as coral reefs (Reverter et al. 2017). Fish gill microbiomes also reflect disease states, such as developmental abnormalities (Hess et al. 2015) and chronic gastroenteritis (Legrand et al. 2017). In bonefish, gills develop during the post-larval life stage, such that bonefish must acquire their gill microbial communities as they metamorphose between 41 and 71 days after fertilization (Mojica et al. 1995; Miller and Tsukamoto 2004; Diaz-Viloria et al. 2017). Gill microbiomes should therefore offer insights that are specific for the bonefish juvenile and adult life stages.

Methods

Sampling bonefish

Bonefish were sampled by angling (using spinning and fly-fishing tackle) from nearshore environments in the Florida Keys and the islands of Eleuthera and Inagua in The Bahamas (Fig. 1; Table S1). Within each of these three locations, fish were sampled from multiple sites that included tidal creeks, islets, coves, saltwater ponds, and similar features. To avoid physiological exhaustion and to maximize post-release survival, fish were angled for minimal duration (Danylchuk et al. 2007a). Once a fish was captured, it was briefly removed from the water and a sterile polyester swab with a Dacron tip (Fisher Scientific, Waltham, MA) was inserted briefly between the gill filaments and gently rotated approximately five times. Swabs were then immediately placed tip-down into a 1.2-ml cryovial (Fisher Scientific, Waltham, MA) containing 0.25 ml RNAlater nucleic acid stabilization solution (Qiagen,

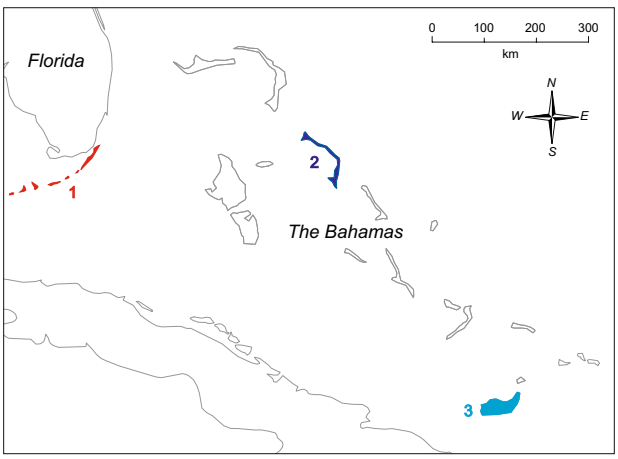

Fig. 1 Map of sampling locations. The Florida Keys are highlighted in red, the Bahamian island of Eleuthera in dark blue, and the Bahamian island of Inagua in light blue

Hilden, Germany) and swab shafts were cut flush with the tube opening using sterile scissors, after which tubes were capped and labeled. An approximately 1 × 1 cm fin clip was then removed from the fish for genetic analysis using sterile scissors and placed immediately into a separate 1.2-ml cryovial containing 0.5 ml RNAlater. Tubes containing swab tips and fin clips were stored at − 20 °C within 6 h of sampling, shipped at ambient temperature to the laboratory, and stored at − 80 °C thereafter.

Molecular analyses

In addition to *A. vulpes*, two other species of bonefish, *A. goreensis* and *A. sp. cf. vulpes*, occur in the areas sampled (Wallace and Tringali 2016). To identify the bonefish species used for analysis, we conducted microsatellite-based genetic species identification analyses of fin clips as previously described (Wallace and Tringali 2016).

To assess microbial communities, we first extracted nucleic acids from gill swabs using the Qiagen AllPrep PowerViral DNA/RNA Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions and eluting in a 50 µL volume. We then conducted polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA V4 gene region using protocols developed by the Earth Microbiome Project (Gilbert et al. 2014). Each PCR contained 2 µL DNA template, 9.5 µL of Qiagen Nuclease free water, 12.5 µL of Qiagen HotStarTaq master mix 0.5 µL of 10 mM forward primer 515f (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA-3′), and 0.5 µL of 10 mM reverse primer 806Rb (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACNVGGGTWTCTAAT-3′) (Caporaso et al. 2011; Walters et al. 2016) in a total volume of 25 µL. Cycling conditions consisted of an initial denaturation step of 15 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 60 °C and 1 min at 72 °C, and final extension of 10 min at 72 °C. We then used the Nextera XT Index v2 Kit (Illumina, San Diego, CA) to dual-index amplicons and electrophoresed them on 2% agarose gels with ethidium bromide. We cut amplicons from gels under ultraviolet light and extracted them using the Zymoclean gel DNA recovery kit (Zymo Research, Irvine, CA), and prepared them for 600 cycles of paired-end sequencing (MiSeq Reagent Kit v3) on an Illumina MiSeq instrument. To limit the potential for contamination, we included negative controls at all stages of our extraction and amplification procedures and used the resulting data to subtract potential contaminants in silico. Negative controls consisted of DNA extractions performed with no input swab and PCR amplifications with 2 µL of sterile molecular grade water substituted for DNA template.

Sequence processing

We used the R (R Core Team 2019) package DADA2 (Callahan et al. 2016) to quality-screen and trim sequence reads. We trimmed reads at the first appearance of a base with a quality score of two or lower. We then truncated forward reads at 240 bases in length and reverse reads at 160 bases in length to account for differences in the rate of per base quality degradation between forward and reverse reads. We also removed reads with non-assigned bases (N) and reads mapping to the PhiX sequencing standard. We then applied DADA2 to detect sequence variants (SVs) and merged paired reads into single consensus reads, after which we removed chimeric sequences and sequences from negative controls. We assigned SVs to taxonomic groupings at the genus level using the SILVA ribosomal RNA database (Quast et al. 2013). Following quality filtering and merging of overlapping reads, our final read data set consisted of 272,675 consensus reads originating from 6029 SVs.

We conducted subsequent analyses using the R package phyloseq (McMurdie and Holmes 2013). We first identified bacterial genera present in negative controls (*Acinetobacter*, *Arcobacter*, *Brevundimonas*, *Kingella*, *Pelagibacterium*, *Polaromonas*, *Rhodococcus*, *Thermus*, *and Veillonella*, together accounting for 11,770 reads) and removed them from the dataset. We also removed reads assigned as eukaryotic in origin at this stage. To avoid biases associated with extremely rare SVs, we applied an abundance-based filter to our read dataset. Sequence variants accounting for less than 100 total reads across all samples were removed from our dataset. Removal of potential contaminants and ultra-rare SVs yielded a dataset of 81,782 reads from 393 SVs.

To ensure a minimum number of reads per sample adequate for characterizing gill bacterial communities in the final dataset, we omitted any sampled fish for which fewer than 1000 reads were generated (n=7). To account for biases introduced by differences in individual library sizes we randomly subsampled (rarefied) all libraries to the size of the smallest set of reads in a sample (1200 reads) using the rarefy function in phyloseq. The rarefying procedure resulted in the removal of four additional SVs from the dataset. This resulted in a final dataset of 23 fish that accounted for 27,600 reads from 389 sequence variants.

Analyses of microbial communities

To quantify microbial community alpha diversity, we used phyloseq to calculate the Shannon index of SVs in the rarefied sequence set for each sampled fish. We subsequently converted Shannon indices to an effective number of species score (ENS) via exponentiation, because this is considered a more interpretable representation of alpha diversity (Jost 2006). We also considered the number of detected SVs per

sampled fish as a measure of the alpha diversity of each gill microbiome in terms of species richness alone (irrespective of species evenness). To investigate variation in both measures of alpha diversity among island locations, we conducted statistical hypotheses tests in R. We tested the normality of response variable distribution and the homogeneity of group variances for both diversity measures using Shapiro–Wilk and Bartlett's tests. Both ENS and number of detected SVs data were approximately normally distributed, however ENS data violated the parametric assumption of homogeneous group variances. We therefore analyzed differences between island groups in ENS and number of detected SVs using a Kruskal–Wallis test and an analysis of variance test, respectively. In both instances we fitted the alpha diversity metric for each sample as the response variable and sampling location (island) as the sole explanatory variable. We then used post hoc Wilcoxon (Benjamini–Hochberg correction for multiple testing) and Tukey's honest statistical differences (HSD) tests to assess pairwise differences in alpha diversity between locations for ENS and detected number of SVs, respectively.

To visualize differences among sampled fish based on the most abundant bacterial genera (across all detected sequence variants) and the most abundant bacterial sequence variants, we constructed a stacked bar graph of the rank abundance of the 20 most common bacterial genera per sampled fish (Fig. 4) and a heatmap of the relative abundance of the 50 most common bacterial SVs using the heatmap.2 function within the R package gplots (Fig. 3). We produced an associated dendrogram of the between-sample Bray–Curtis dissimilarity scores based on the relative abundance profiles of the 50 displayed sequence variants, as computed by the vegdist function within the R package vegan and the hclust algorithm within the base R package (Fig. 3)

To quantify beta diversity, we used the R package vegan (Oksanen et al. 2018) to produce a non-metric multidimensional scaling (NMDS) ordination using Bray–Curtis dissimilarity of between-sample differences in bacterial communities. We performed ordinations across two dimensions (k=2), yielding a stress of fit value of 0.12. We then tested for differences in community structure between sample groups using permutational analysis of variance tests (PERMANOVA) implemented using the adonis function in vegan. We modeled different geographic groupings separately and constructed individual PERMANOVAs using 1000 permutations for the Florida Keys versus The Bahamas (Eleuthera and Inagua combined) and to compare the Keys versus the two Bahamian islands separately. We constructed an additional PERMANOVA to compare gill bacterial communities between individual sampling sites across all three islands.

To detect differentially abundant SVs between the Florida Keys and The Bahamas, which might drive divergence between the gill bacterial communities from each island group we used the linear discriminant analysis effect size method (LEfSe; Segata et al. 2011), which is a two-stage process that first determines which SVs are differentially enriched between comparison groups and then determines which of those differentially enriched SVs are consistently represented among individuals within a group. To examine differentially abundant SVs between islands within The Bahamas, we also used LEfSE to compare Eleuthera and Inagua directly.

Results

Sampling and fish genetics

We captured, sampled and released bonefish from various sites in the Florida Keys, Eleuthera, and Inagua (Fig. 1; Table S1) and chose a subsample of 23 fish for microbiome analysis, to roughly equalize geographic representation and to exclude samples with inadequate sequence data (see above). This final sample set included six fish from the Florida Keys, 12 fish from Eleuthera, and 5 fish from Inagua (Table S1). All 23 fish used in microbiome analyses were genetically confirmed to be *A. vulpes*.

Analyses of microbial communities

Shapiro–Wilk tests showed that neither ENS nor the number of detected SVs violated assumptions of normality (ENS; W= 0.93, p= 0.095, detected number of SVs; W= 0.94, p= 0.203). Bartlett's tests demonstrated that variation in ENS between islands did violate the assumption of normality (k^2= 6.32, p= 0.043), whereas variation in number of detected SVs between islands did not (k^2= 4.86, p= 0.088).

Alpha diversity varied significantly among locations based on both ENS (Kruskal–Wallis X^2=8.55, p=0.014; Fig. 2) and number of detected sequence variants (F=6.726, p=0.005; Fig. 2). Post hoc analysis using a Wilcoxon test and a Tukey's HSD test, respectively, revealed this variation to have resulted from reduced alpha diversity in Inagua compared to both Eleuthera (ENS; p=0.045, number of detected SVs; p=0.03) and the Florida Keys (ENS; p= 0.018, detected number of SVs; p = 0.004; Fig. 2). Visual appraisal of abundance patterns of the top 50 most abundant SVs (Fig. 3) and the 20 most abundant bacterial genera (Fig. 4) suggested no clear location-level patterns in bacterial assemblages based on the most abundant genera and sequence variants. However, when the whole bacterial microbiome was considered Bray–Curtis ordination revealed strong separation of Florida Keys gill bacterial communities from those in The Bahamas (Fig. 4), which PERMANOVA showed to be highly statistically significant (F=2.14, p=0.001, R^2=0.17). PERMANOVA also showed that differences among all three

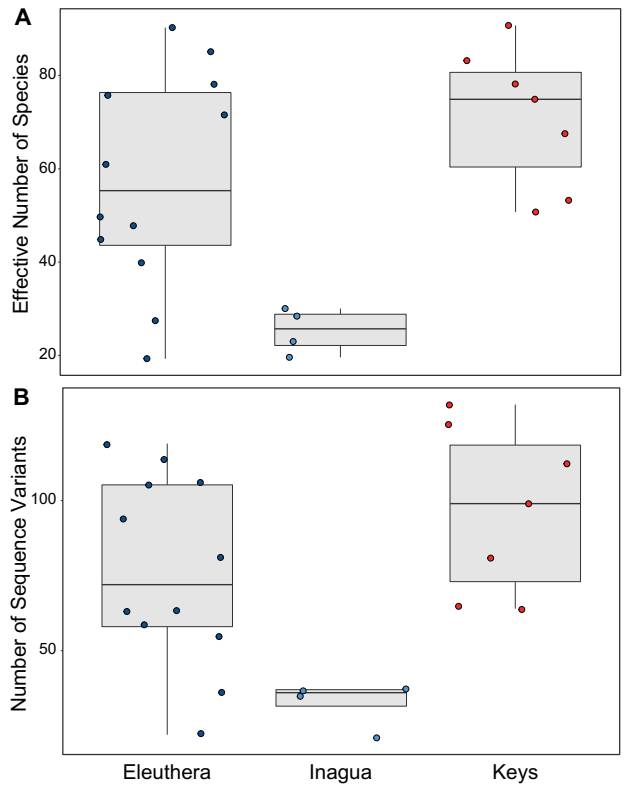

Fig. 2 Alpha diversity of each location. **a** Each point represents the effective number of species value (ENS, Y-axis) of an individual fish. The underlying boxplot shows the distribution of ENS values within a location. **b** Each point represents the number of detected sequence variants (Y-axis) of an individual fish. The underlying boxplot shows the distribution of number of detected SVs within a location. In both plots, dark blue points represent fish sampled on the Bahamian island of Eleuthera, light blue points represent fish sampled on the Bahamian island of Inagua, and red points represent fish sampled on the Florida Keys

island locations (Florida Keys vs Eleuthera vs Inagua) were highly statistically significant (F=2.66, p=0.001, R^2=0.11). The site at which a fish was sampled within each island was also an important predictor of bacterial community structure, accounting for the most variance in the model of any geographical scale analyzed (PERMANOVA F=1.49, p=0.005, R^2=0.51) (Fig. 5).

LEfSe analysis revealed a marked difference between the Florida Keys and The Bahamas, with 70 SVs over-represented in the Florida Keys, but only four SVs over-represented in The Bahamas (Fig. 6). Despite evidence of significant divergence in gill microbial communities between the Bahamian islands, only two SVs were differentially abundant between Eleuthera and Inagua. Both of these SVs (one belonging to the genus *Alteromonas* and the other unidentified at the genus level, but belonging to the family *Burkholderiaceae*) were enriched in the gill bacterial communities of fish sampled from Inagua.

Discussion

Our analyses show that bacterial communities on the gills of bonefish vary among geographic locations and, on a finer scale, among local sites within those locations. Gill microbiomes of Florida Keys bonefish clustered distinctly from gill microbiomes of Bahamian bonefish. Gill microbiomes from the two Bahamian islands (Eleuthera and Inagua) overlapped to a greater extent, but were still divergent. The Florida Keys and The Bahamas are separated by the Gulf Stream current, which exerts a major biogeographic influence on the distribution of nearshore fishes (Robertson and Cramer 2014). Indeed, estimates of connectivity of bonefish populations by larval transport have shown no detectable connection between the Florida Keys and The Bahamas (Zeng et al. 2019). Strong separation of bonefish gill microbiomes to the east and west of the Gulf Stream may reflect barriers to population connectivity, ecological differentiation, exposure to different sources of environmental microbes, or all three. We suspect that similar patterns will be evident for other species occurring on either side of this oceanic feature.

LeFSe analysis identified 17 times more over-represented SVs in the Florida Keys than in The Bahamas (70 SVs over-represented in the Florida Keys versus 4 SVs over-represented in The Bahamas). The majority of these SVs belong to genera *Litoricola* and *Catenococcus*, but several SVs that were more abundant in the Florida Keys are associated with disease, including *Corynebacterium* (Baya et al. 1992) and *Acholeplasma* (Francis-Floyd et al. 1997). We also detected SVs belonging to the genera *Staphylococcus* and *Streptococcus*, which are markers of environmental contamination (Lleò et al. 2005), cause disease in fish (Musharrafieh et al. 2014; Mishra et al. 2018), and are the most common causes of marine-associated skin and soft tissue infections in humans (Diaz 2014; Vasagar et al. 2018). Although the abundance of these genera did not differ significantly among locations, data from other systems suggest that such bacteria are useful indicators of exposure to sewage and wastewater, for example from sugar production (Popović et al. 2019), which has increased in the Caribbean in recent decades (Cramer et al. 2020). We caution that our microbial data are resolved mainly to the genus level, such that more specific inferences would require bacterial isolation (where possible), microbiological assessment of isolates, and more specific genetic markers.

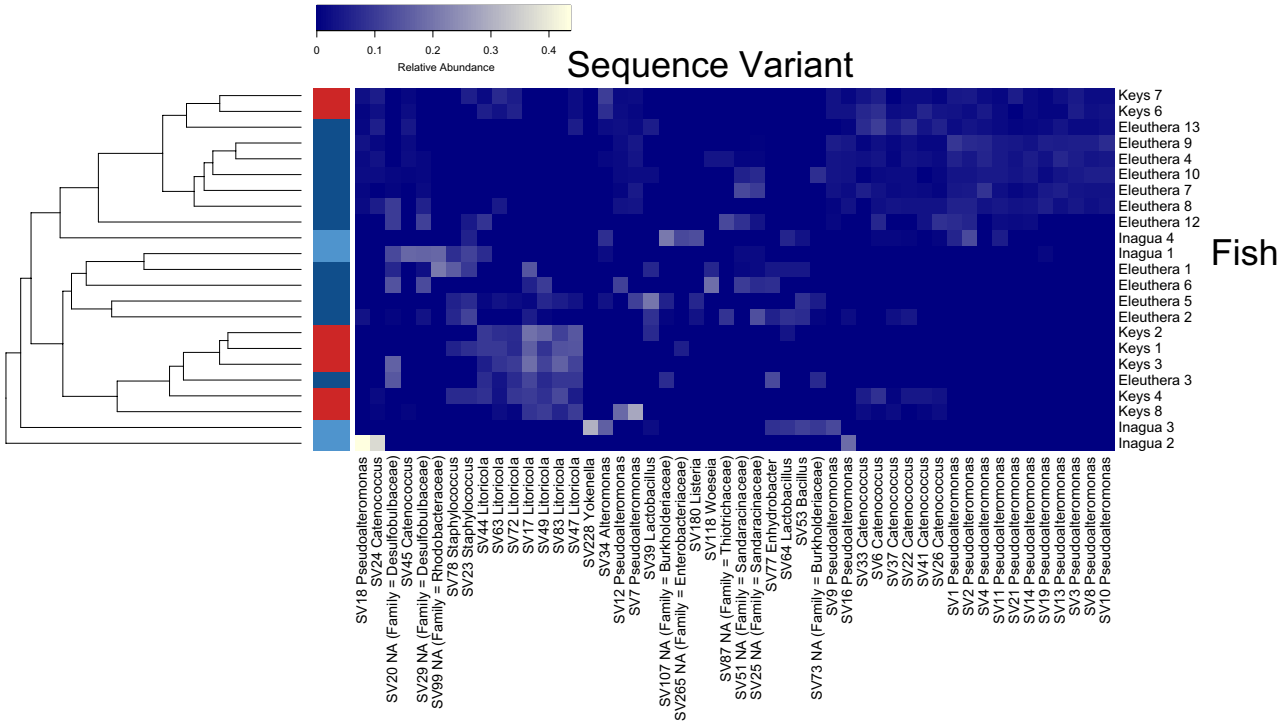

Fig. 3 Heatmap of the relative abundance of the 50 most common bacterial sequence variants per each sampled fish in the final dataset. The dendrogram represents the relationships between each fish based on the abundance profiles of the 50 displayed sequence variants, based on Bray–Curtis dissimilarity. End of row color blocks denote the location at which each fish was sampled. Dark blue blocks represent fish sampled on the Bahamian island of Eleuthera, light blue blocks represent fish sampled on the Bahamian island of Inagua, and red blocks represent fish sampled on the Florida Keys

Bonefish can migrate long distances, as shown using mark–recapture methods (Boucek et al. 2019; Perez et al. 2019). In this light, we note that gill microbiome data provide different information than genetics, which has been used to infer reproductive connectivity among bonefish populations (Wallace and Tringali 2016). Bonefish larvae (leptocephali) do not have gills; rather, gills develop as bonefish leptocephali exit their extended planktonic phase and metamorphose (Miller and Tsukamoto 2004; Diaz-Viloria et al. 2017). As a result, bonefish must acquire their gill microbiomes in the environments where they spend their post-metamorphic life stages (Zeng et al. 2019). Although fish gill microbiome composition depends on many factors, some of which are host-specific (Merrifield and Rodiles 2015; Pratte et al. 2018), local acquisition of bacteria also plays a role (Reverter et al. 2017). Pairing bonefish samples with water samples and physiochemical data from the same environments would clearly be useful for determining which bonefish gill microbes are acquired locally and possibly associated with disease. Furthermore, our observation that bacterial community composition varied significantly among sites within island locations suggests that microbes could potentially serve as markers for bonefish movement (i.e., a form of "microbial telemetry"), which could be a useful alternative to more invasive methods (e.g., surgical implantation of electronic devices or analysis of otolith microchemistry; Murchie et al. 2013; Murchie et al. 2015; Santos et al. 2019).

The patterns documented herein may have differed had we analyzed other body compartments, such as the gastrointestinal tract (Pratte et al. 2018). We specifically chose gills because of the unusual bonefish life cycle and the delayed development of gills until the post-metamorphic life stage (Miller and Tsukamoto 2004; Diaz-Viloria et al. 2017), and because the gill is a highly susceptible anatomic site for the adherence of pathogenic bacteria in teleost fishes (Secombes and Wang 2012). Moreover, fish gills are neither a sterile nor an enclosed anatomic site, but rather are exposed to the water (Reverter et al. 2017). Indeed, our data show a mix of both environmental and animal-associated microbial SVs on bonefish gills. As mentioned above, paired data on microbial communities of seawater would help shed light on this issue.

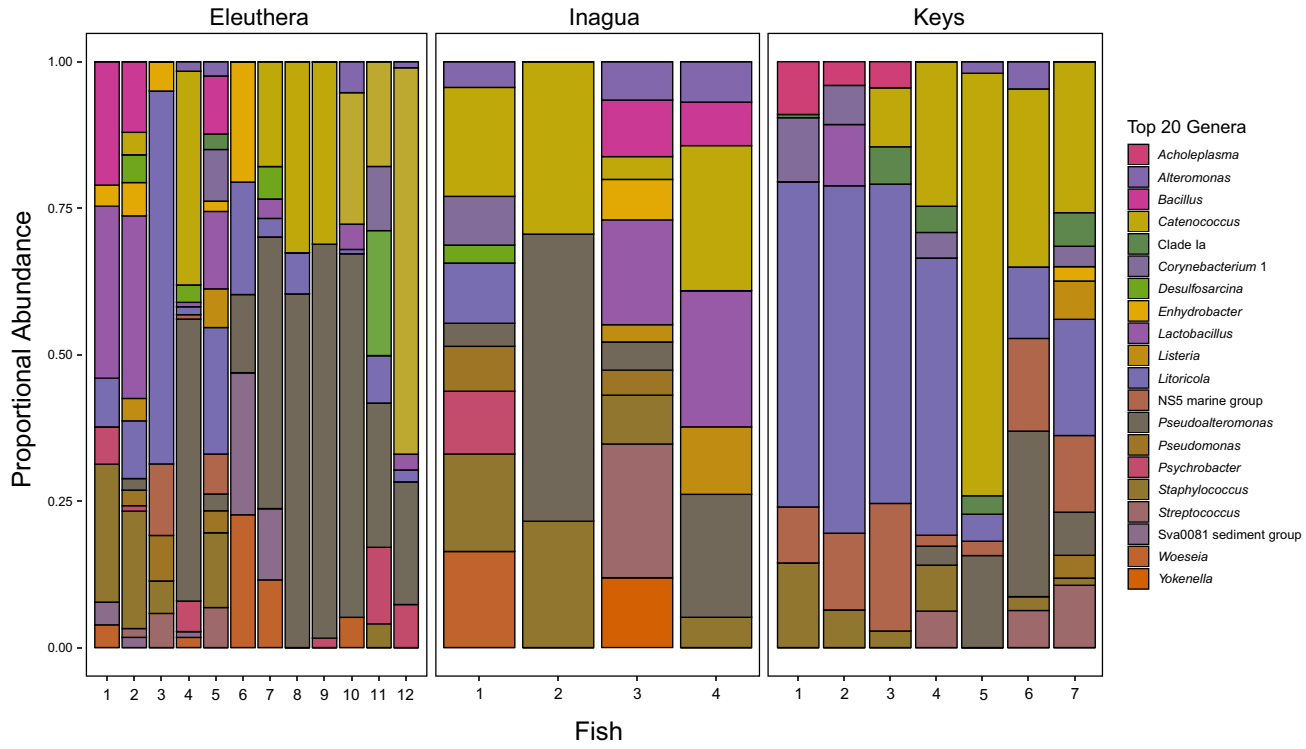

Fig. 4 Rank abundance of each of the 20 most common bacterial genera in each sampled fish. Individual fish are grouped by the location of capture to allow for visual assessment of the relationship between abundance of bacterial genera and geography. Reads mapping to sequence variants belonging to each genus were compiled and presented as a proportion of the total number of reads mapping to all members of the top 20 genera per sample

Overall, our findings provide proof of concept that assemblages of bacterial SVs differ among bonefish populations, especially between the declining population of the Florida Keys and the stable populations on the Bahamian islands of Eleuthera and Inagua. Additionally, we have identified bacterial SVs which are overly abundant in the declining population and that have previously been associated with disease. Further studies using expanded sample sets should provide a more complete picture of variation in the geographic occurrence and abundance of disease-associated bacteria on the gills of bonefish. Ultimately, experimental studies using bonefish or a suitable surrogate species will likely be necessary to assess the impact of any particular microbial taxon on bonefish health. Future studies may also clarify the usefulness of bonefish bacterial communities for inferring migration and movement patterns. The advantages of microbial community analysis over other methods for such purposes include non-invasiveness, non-lethality, specificity for the post-metamorphic life stages, and, perhaps, higher discriminatory power due to the inherent taxonomic richness of microbial communities. We advocate for additional exploration of this and related methods as tools for population health assessment and spatial ecology in bonefish and other species with similar life histories.

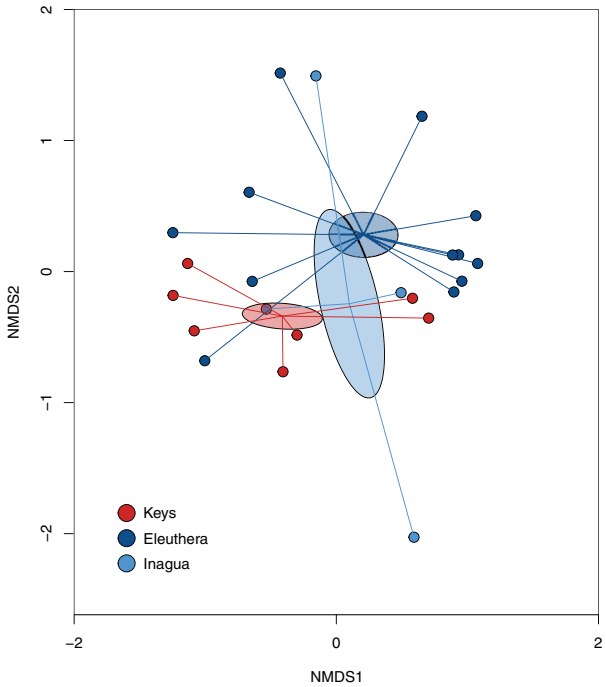

Fig. 5 Bray–Curtis dissimilarity non-metric multidimensional-scaling plot. Each point represents the bacterial community of a sampled fish. Points are connected to the group centroid (averaged community composition) of a given island location by lines. Shaded ellipses represent 95% confidence bounds around each group centroid

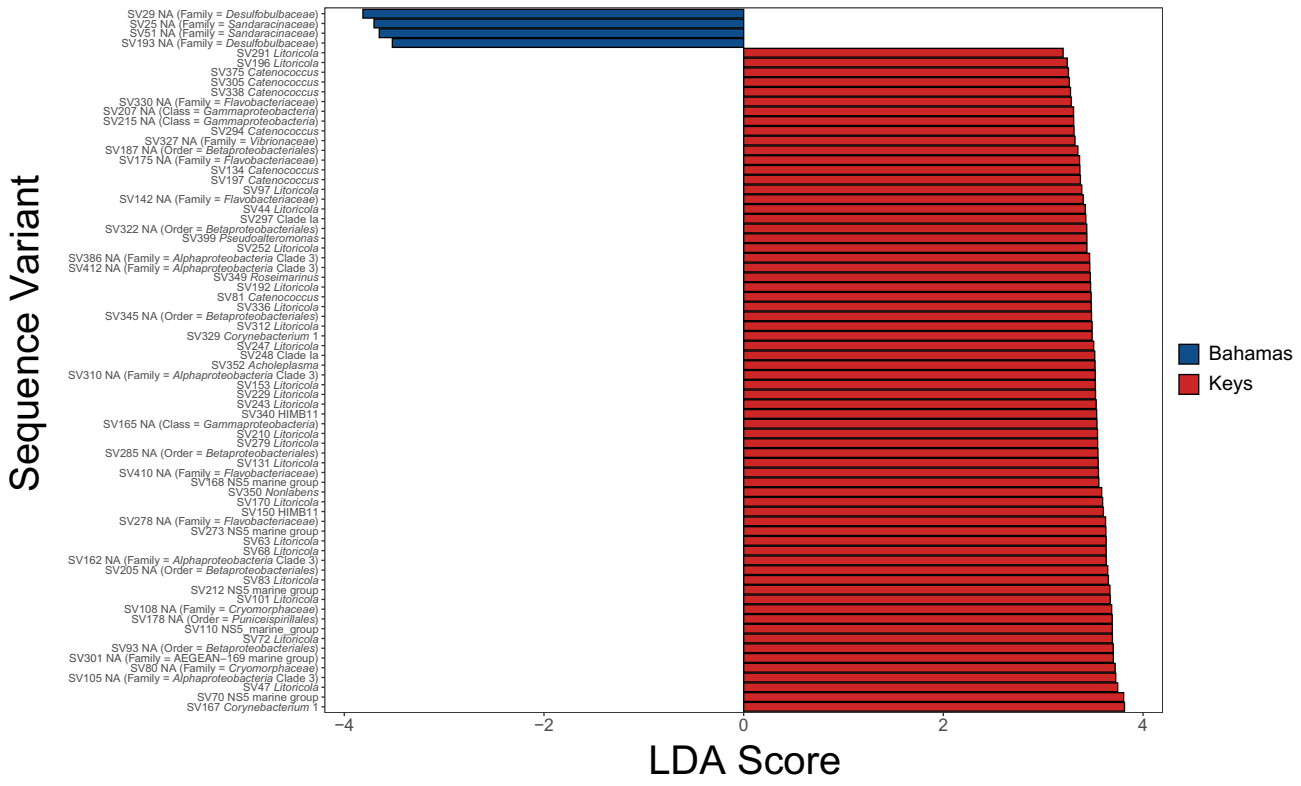

Fig. 6 Output of linear discriminant analysis effect size (LEfSe) analysis for detection of bacterial biomarkers that distinguish the Florida Keys from both Bahamian islands. Sequence variants with blue bars and negative LDA scores are considered biomarkers of bonefish sampled in The Bahamas, while sequence variants with red bars and positive LDA scores are considered biomarkers of bonefish sampled in the Florida Keys

Acknowledgements We gratefully acknowledge the Bonefish and Tarpon Trust for assisting with sampling and logistics in the Florida Keys, as well as the Fisheries Conservation Foundation, the faculty and staff of the Cape Eleuthera Institute, and D. Rankin and Z. Wasserman for assisting with sampling and logistics in The Bahamas.

Author contributions CDD, AJD, SJC, DPP, AJA and TLG contributed to the study conception and design. Sample and data collection were performed by AJD, ADS, BDB, JWB, LPG, and TLG. Analyses and writing were conducted by CDD, LJC, EMW, and TLG. All authors read and approved the final manuscript.

Funding This project was funded by the Bonefish and Tarpon Trust, the University of Wisconsin-Madison John D. MacArthur Fellowship Program, the Fisheries Conservation Foundation, and the individual scientists and their home institutions.

Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Compliance with ethical standards

Conflict of interest Author Christopher Dunn declares that he has no conflict of interest. Author Lewis Campbell declares that he has no conflict of interest. Author Elizabeth Wallace declares that she has no conflict of interest. Author Andy Danylchuk declares that he has no conflict of interest. Author Steven Cooke declares that he has no conflict of interest. Author Aaron Shultz declares that he has no conflict of interest. Author Brooke Black declares that she has no conflict of interest. Author Jacob Brownscombe declares that he has no conflict of interest. Author Lucas Griffin declares that he has no conflict of interest. Author David Philipp declares that he has no conflict of interest. Author Aaron Adams declares that he has no conflict of interest. Author Tony Goldberg declares that he has no conflict of interest.

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

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