Fishing for the Bacteriome of Tropical Tuna

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FISHING FOR THE BACTERIOME OF TROPICAL TUNA

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ABSTRACT

Background: Although tunas represent a significant part of the global fish economy and a major nutritional resource worldwide, their consumption poses a risk of food poisoning through the development of particular bacterial pathogens. However, their microbiome still remains poorly documented. Here, we conducted a multi-compartmental analysis of the taxonomic composition of the bacterial communities inhabiting the gut, skin and liver of two most consumed tropical tuna species (skipjack and yellowfin), from individuals caught in the Atlantic and Indian oceans.

Results: Our results revealed that the composition of the microbiome was independent of fish sex, regardless of the species and ocean considered. Instead, the main determinants were (i) tuna species for the gut and (ii) sampling site for the skin mucus layer, and (iii) a combination of both parameters for the liver. Interestingly, only 4.5% of all ASVs were shared by the three compartments, raising numerous questions about the circulation of microorganisms within the tuna body. Our results also revealed the presence of a unique and diversified bacterial assemblage within the liver, comprising a substantial proportion of histamine-producing bacteria, well known for their potential pathogenicity and their contribution to fish poisoning cases.

Conclusions: These results indicate that the tuna liver is an unexplored microbial niche whose role in the health of both the host and consumers remains to be elucidated.

BACKGROUND

Like their terrestrial counterparts, marine organisms live in close association with microbial communities composed of a diverse assemblage of viruses, bacteria, archaea, fungi and protists. Mammals, corals and, to a lesser extent, fish have been primarily targeted by marine microbiologists in microbiome studies, and we now have a body of evidence that these diverse and abundant microbes play a vital role in the health and fitness of their hosts, participating in functions as important as digestion, defence, and nutrition, among others [1–3]. Most such studies show that the composition of these microbial communities remains highly variable and multi-factorial and is subject, in a still unclear way, to the influence of different parameters associated with the host, including species [4], age [5], sex [6], and diet [7], as well as external environmental conditions such as salinity [8], seasonality [9], geographical location [10], temperature [11], and chlorophyll *a* concentration [12]. However, these commensal microbes are not evenly distributed throughout the body of their marine hosts, where similar to those in humans, they form complex bacterial consortia mainly in the digestive tract [13], skin [14], and respiratory system [15]. To date, most studies investigating marine microbiomes have examined a single biological compartment at a time, often the digestive tract or the skin mucus, but the microbiome of other essential organs such as the liver has never been investigated, despite the central role of this organ in metabolic and immune functions within the organism [16]. Moreover, recent findings of bacterial genes in the human liver suggest that this organ could be a neglected bacterial habitat in vertebrates [17,18]. Additionally, we still lack information about the potential microbial links or connections between the different organs of a given marine animal. Recent studies on the human microbiome demonstrated the existence of communication axes

between biological compartments, such as the gut-brain, gut-liver and gut-skin axes [19–21]. While many questions remain unanswered about the mechanisms of these interactions, it is clear that microbial communities, because of their composition and the metabolites that they can generate, are at the center of a complex communication system between different organs, which may influence not only the health of the host but also its behaviour [22,23].

In this integrative study, we conduct a simultaneous multi-compartmental analysis of the microbial communities in the gut, liver and skin mucus layer of an emblematic fish: tuna. Tuna is a pelagic teleost fish distributed in tropical waters that plays a key role in the ecosystem as a top predator [24]. It is one of the most widely consumed fish in the world and a crucial source of animal protein in many countries, therefore having major social, nutritional and economic value [25]. The annual catch of tuna reached 7.7 million tons in 2017, with skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) representing more than 70% of the captures [26]. However, the consumption of tuna also poses a health risk, with the occasional development of histamine-producing bacteria (HPB) responsible for frequent fish poisoning cases [27,28]. Finally, despite the considerable nutritional value of this resource as well as the health hazard associated with its consumption, knowledge of the microbiome of tuna remains rudimentary [29].

In this study, our main objectives were to (i) describe the composition of the skin, gut and liver microfloras in two major tropical tuna species, (ii) identify shared and endemic bacterial taxa in these three organs, (iii) elucidate the influences of phylogeny, sex and environmental conditions on the composition of their respective microbiomes and (iv) examine the diversity and location of HPB.

MATERIAL AND METHODS

Sampling procedure.

Tuna. Tunas of the species *Thunnus albacares* (yellowfin, YLF) and *Katsuwonus pelamis* (skipjack, SKJ) were captured around FADs located in the Atlantic (Ivory Coast, Gulf of Guinea, N04°55'00", W03°42'19.97) and Indian (Réunion Island, S20°57'816", E55°04'457") oceans in July (10-11th) and September (26-29th) 2018, respectively. Sampling and euthanasia of animals were performed by professional fishers working for the IRD's Exploited Tropical Pelagic Ecosystems Observatory (certified **ISO 9001:2015)**. In the Gulf of Guinea, 6 skipjack tunas (3 females, 3 males) (min-max: 56-66 cm) and 15 yellowfin tunas (8 females, 7 males) (min-max: 46-66 cm) were collected. On Réunion Island, 27 tunas were captured: 18 skipjack tunas (14 females, 4 males) (min-max: 41-60 cm) and 9 yellowfin tunas (6 females, 3 males) (min-max: 61-69 cm). To avoid contamination during sampling, fish were caught using hook lines and euthanized by professional fishers immediately after capture by cervical dislocation (following European directive 2010/63/UE). Fishes were handled by the mouth using a clamp, and all the participants wore gloves.

Sampling of the skin mucus, gut and liver.

Skin mucus layer. After euthanasia, individuals were laid down, and the skin superficial mucus layer was immediately sampled by swabbing the entire untouched side of the body (from the back of the operculum to the caudal peduncle, i.e., head not included) using buccal swabs (SK-2S swabs, Isohelix, Harrietsham, UK) [14].

Gastrointestinal content. Following skin sampling, fish were individually placed in plastic bags and immediately stored on ice before dissection (within 5 h after sampling) [30]. Briefly, the gastrointestinal tract was extracted from each individual

and cut from below the stomach to the rectum using sterile tools. Each gut was squeezed to expel the contents (minimum volume of 5 mL) on a sterile surface, and the contents were homogenized before sampling.

Liver. For each tuna, a longitudinal piece of approximately 1 x 0.2 x 0.2 cm was trimmed from the right lobe (the largest) of the liver by using sterile cutter and forceps. Liver samples were then rinsed with distilled water filtered on 0.2 µm to avoid any contamination from other internal organs or fluids.

Ambient water. In addition to tuna samples, triplicate samples of surface seawater were collected at both sampling sites (within the FAD area at 1 m below the surface) by using a Niskin bottle. Triplicates of 500 mL of seawater were filtered through 0.2-µm-porosity polycarbonate membranes (ø47 mm, Whatman® Nucleopore, Maidstone, UK).

Storage. All mucus, gut, liver and seawater samples were placed in 5 mL sterile cryovials, frozen in liquid nitrogen onboard, and stored at -80°C in the laboratory until bacterial nucleic acid extraction.

DNA extraction, amplification and sequencing.

Bacterial DNA was extracted from 250 ± 0.5 mg of gut (n= 48) and liver samples (n= 48) and from the entire swabs and filters for skin mucus (n= 48) and seawater (n=6). All extractions were performed with the PowerSoil DNA Isolation Kit (Qiagen®, Hilden, Germany) following the manufacturer's instructions. DNA quality and quantity were assessed by spectrophotometry (NanoDrop®, Wilmington, DE, USA). The V3-V4 region of the 16S rDNA gene was amplified using universal bacterial primers modified for Illumina sequencing: 343F (5'- ACGGRAGGCAGCAG) [31] and 784R (5'- TACCAGGGTATCTAATCCT) [32]. The reaction mixture consisted of 12.5 µL of

2X Phusion Mix (New England Biolabs®, Ipswich, MA, USA), 1 µL of each primer at 10 µM (Eurofin®, Luxembourg), 10 ng of DNA template and enough molecular-grade H_2O (Qiagen®) to reach a final volume of 25 µL. All samples were amplified in triplicate to avoid PCR bias in the taxonomic diversity of the community [33]. Triplicate PCR products were pooled and purified with a NucleoSpin Kit (Macherey-Nagel®, Düren, Germany) following the manufacturer's instructions. Successfully amplified samples (n=103) were sequenced on the Illumina platform (GenoToul®, Toulouse, France) using 2×250 bp MiSeq chemistry.

Bacterial sequence processing and analysis.

A total of 8 295 541 reads were obtained. Raw reads were processed with RStudio (R version 3.5.3) using the DADA2 package (v1.10.1) [34] following the authors' tutorial (https://benjjneb.github.io/dada2/tutorial.html). Briefly, the quality of forward and reverse reads was analysed before removing adaptors and primers, based on their length. Using the DADA2 tutorial with default parameters, reads were then filtered, trimmed and merged into 5 269 075 amplicons sequence variants (ASVs), which have a higher resolution than operational taxonomic units (OTUs) [34]. Chimaeras were removed, and sequences were aligned to the SILVA 123 database [35] to access their taxonomy. Analyses were performed on a random subsample of 6 847 sequences per sample, corresponding to the sample with the smaller number of sequences, after trimming and quality processing. Using the *phyloseq* package [36], final taxonomic and ASV tables were linked to sample metadata (tuna species, sex, biological compartment and ocean). The relative abundances of ASVs in each sample were assessed by *phyloseq,* and ASVs assigned to non-prokaryotes, archaea, chloroplasts and mitochondria were removed. Using the *phyloseq* package

[36], taxonomic richness was calculated for each sample and tested for differences between biological compartments (skin mucus, gut and liver), tuna species (yellowfin and skipjack), oceans (Atlantic and Indian oceans) and sexes (female and male) using the non-parametric Kruskal-Wallis ANOVA test. Statistical significance was assumed when $p < 0.05$. Within *phyloseq*, the composition and diversity of bacterial communities were represented at the class level, based on the relative abundances of ASVs in each sample. Within each sample, the detection of histamine-producing bacteria (HPB) was based on the presence/absence of bacterial species reported to produce histamine in the literature. Dissimilarities between bacterial communities were assessed using Bray-Curtis distances, which were calculated with the *vegan* package [37] and represented in a principal coordinate analysis (PCoA) plot built with the *ape* package [38]. The effect of biological compartment, tuna species and sampling site on the composition of bacterial communities was determined by one-factor PERMANOVA with 999 permutations of the Bray-Curtis matrix using the "adonis" function of the *vegan* package [39]. To compare the compositions of the bacterial communities between the three organs (i.e., skin, gut and liver), a Venn diagram was constructed using the *VennDiagram* package [40]. From the Venn calculations, the list of specific ASVs within each biological organ was sorted in RStudio. The occurrence of each ASV, i.e., the frequency of its observation in the samples of a dataset, was calculated. For each biological compartment, the five most frequent ASVs were identified to the lowest taxonomic level available.

RESULTS

Alpha diversity.

The taxonomic richness of bacterial communities, defined as the number of amplicon sequence variants (ASVs), showed important differences and similarities between sexes, tuna species (skipjack and yellowfin), biological compartments (skin mucus, gut and liver) and sampling sites (Atlantic and Indian oceans).

Variability between sexes. Regardless of the tuna species, ocean and biological compartment considered, the taxonomic richness of the bacterial communities did not show significant differences between male and female individuals (Fig. 1; Tab. 1).

Variability between tuna species. In the gut and liver samples, bacterial richness was significantly higher in yellowfin than in skipjack tuna in both oceans (Fig. 1). Statistical analysis confirmed that bacterial *alpha* diversity differed significantly between the two tuna species, while no effect of sampling site was observed (Tab. 1).

Variability between oceans. In the skin mucus samples, the opposite pattern was observed for *alpha* taxonomic richness, which was significantly lower in tuna captured in the Indian Ocean (Fig. 1) but not significantly different between skipjack and yellowfin (Tab. 1).

Variability between compartments. The skin mucus layer hosted a significantly higher bacterial richness than the gut and liver of both tuna species, regardless of the sampling site (Fig. 1). However, ASV richness did not differ significantly between the gut and liver samples (Tab. 1).

***Beta* diversity.**

As observed for *alpha* diversity, the composition of the bacterial communities (*beta* diversity) did not show significant differences between sexes, regardless of the tuna species, sampling site and biological compartment (PERMANOVA, $p > 0.05$).

Skin microbiome. Skin samples showed significant similarities between tuna species but large dissimilarities between the two sampling sites (Fig. 2A,D). In both the Indian and Atlantic oceans, the skin bacterial communities greatly differed from those examined in the surrounding seawater (Fig. 2D, Supplementary material Fig. 1). In Atlantic yellowfin and skipjack tunas, the skin bacteriome was dominated by *Gammaproteobacteria,* representing up to 83% of the sequences (Fig. 3A). Several other bacterial classes, such as *Actinobacteria, Alphaproteobacteria, Bacilli, Bacteroidia* and *Mollicutes,* were also present, together representing less than 50% of the sequences in most samples. In Indian Ocean yellowfin and skipjack, the same bacterial classes were observed in much greater proportions, representing more than 50% of the sequences in some samples (Fig. 3B).

Gut microbiome. By contrast with the skin microflora, the gut microflora included a bacterial assemblage that was clearly distinct between the two tuna species, while sampling site had no significant effect (Fig. 2B,E). In skipjack tunas, the gut bacteriome was dominated by *Mollicutes* (Fig. 3C,D), whereas that of yellowfins tunas was more diversified, with higher proportions of *Gammaproteobacteria* and, to a lesser extent, *Alphaproteobacteria* and *Actinobacteria* (Fig. 3C,D). Although *Gammaproteobacteria* were generally more abundant in the gut of tuna collected in the Indian Ocean, no significant differences were observed between the two oceans (Fig. 2E).

Liver microbiome. Liver samples exhibited an intermediate outcome since hepatic bacterial communities were significantly affected by both tuna species and sampling site (Fig. 2C,F). *Gammaproteobacteria* were highly abundant in most of the samples, and *Mollicutes* were generally more represented in skipjack than in yellowfin tuna (Fig. 3E,F). By contrast, the proportions of *Actinobacteria*, *Alphaproteobacteria* and *Bacilli* were, on average, lower in skipjack than in yellowfin. Tuna from the Indian Ocean hosted a liver microflora that was globally less diversified than that of their Atlantic counterparts (Fig. 3F). However, no clear pattern was observed, and the composition of hepatic bacterial communities in the liver seemed to be slightly more influenced by the sampling site.

Shared taxa and specific ASVs among the three organs.

The Venn diagram revealed that a relatively small proportion of all ASVs (4.5%) were common to the skin, gut and liver (Fig. 4). Among these 138 common ASVs, the five most common (observed in 60% to 90% of the samples) corresponded to three species of the genus *Photobacterium* (i.e., *P. leiognathi, P. damselae* and *P. angustum*), which are histamine-producing bacteria (HPB); *Mycoplasma* sp.; and *Cutibacterium* sp. In addition, each compartment hosted a specific and diversified assemblage of taxa. The skin microflora, with 1661 specific ASVs, accounted for half of the total microbiome diversity (i.e., 53.7%). The five most common taxa were *Flavobacterium frigidarium, Psychrobacter* sp*., Rothia muciloginosa, Streptococcus* sp*.* and *Alkanindiges* sp. Comparatively, the gut and liver hosted 560 and 440 specific ASVs, respectively. These relatively similar numbers were unexpected and show that the liver harbours a unique bacterial assemblage that is almost as large as that found in the digestive tract of tunas. In this organ, the five most common taxa

were *Photobacterium* sp., *Vibrio* sp*., Mycoplasma* sp*., Sulfitobacter pontiacus* and *Corynebacterium-1 aurimucosum.*

Diversity and location of HPB.

In the variety of samples analysed, the community of HPB comprised 7 known taxa, namely, *Aliivibrio fischeri, Klebsiella oxytoca, Photobacterium angustum, Photobacterium damselae, Photobacterium leiognathi, Photobacterium phosphoreum* and *Vibrio harveyi* (Fig. 5). In general, HPB were largely dominated by species of the genus *Photobacterium,* but their respective proportions greatly varied between the biological compartments. The liver showed the greatest occurrence of HPB in both tuna species and ocean, with a total relative abundance reaching up to 68%. *Photobacterium damselae* was rather abundant in the liver of Atlantic Ocean tuna, whereas *P. angustum* was more prevalent in the Indian Ocean, mainly in yellowfin. Conversely, the gut generally hosted the lowest abundance of HPB, especially in tuna from the Atlantic, which exhibited nearly undetectable levels of HPB (Fig. 5A). In the skin mucus, the diversity of HPB varied between the two oceans, as *Photobacterium angustum* and *Photobacterium leiognathi* were found in large proportions in Atlantic Ocean tuna while *Photobacterium angustum* was rather dominant in fishes from the Indian Ocean (Fig. 5A,B).

DISCUSSION

The tuna microbiome is not sex-specific. An important result of this study was that, invariably, the bacteriome of tuna did not show significant differences between sexes, regardless of the tuna species, sampling site and biological compartment (Tab. 1). Skipjack and yellowfin tunas typically do not show sexual dimorphism: males and females share the same ecological niche as well as anatomical and

behavioural similarities, with only the gonads able to differentiate them [41–43]. The same results were reported in both sticklebacks and salmon, for which the gut and skin microflorae did not vary between male and female individuals [44,45]. Conversely, Bolnick et al. (2014) reported sex-related variability in the gut microbiome of the threespine stickleback and Eurasian perch, which was explained by a differential diet between males and females [6]. During reproduction, the levels of sex hormones usually increase, and the production of gametes can lead to higher energy expenditure, especially in females [42,46,47]. During this period, females are likely to modify their diet [48], which could alter the composition of their gut microflora. In our study, although all the yellowfin were smaller than 70 cm and therefore sexually immature [48,49], the skipjack in their size class are considered mature and with the ability to reproduce throughout the year [46]. Therefore, the strong microbiological homogeneity between sexes for this species strongly suggests that the composition of the tuna microbiome is likely not subject to the influence of sex hormones.

The gut microbiome of tropical tuna is species-specific. Our results showed that the composition of the gut microflora differed between the two tuna species but not between the sampling sites (i.e., for a given species) (Fig. 2). Skipjack and small yellowfin tunas (size classes sampled in our study) are very similar anatomically, physiologically and behaviourally [50]. They also share the same habitat in the water column [51] and usually feed on the same prey (i.e., mostly fish, crustaceans and cephalopods) [52,53]. In addition, individuals in this study were caught around fish aggregating devices (FADs), under which both tuna species tend to gather, feed on the same bait used by the fishers, and therefore consume similar diets. Thus, considering the strong similarities between these two species, especially regarding

their diets, one could expect similar gut microbiota compositions. In our study, the enteric flora of yellowfin tuna was dominated by *Proteobacteria*, which is often the case with carnivorous fishes [1]. By contrast, the gut of skipjack tuna hosted a majority of *Mollicutes* of the genus *Mycoplasma* sp. (Fig. 3), which also form a major component of the gut microbiome of salmons, mackerels and gobies [4,11,12]. Such species-specific composition of the gut bacteriome is also well known in vertebrates, including birds, primates, reptiles, fishes and mammals, and is thought to be driven by host genotype, physiology and diet [2]. Here, for the reasons cited above, the diet and physiology hypotheses were discarded. Our results are in agreement with the phylosymbiosis hypothesis, which assumes that the host phylogeny reflects the composition of its microbiome [54]. Although genetically closely related, yellowfin (of the genus *Thunnus*) and skipjack (of the genus *Katsuwonus*) have followed two distinct evolutionary trajectories over time (5 millions years ago) [50]. Therefore, the composition of a tuna's enteric flora could be tightly linked to its evolutionary history [55,56], but further analysis including more tuna species is needed. The lack of a difference between the two oceans (i.e., for the same species) also revealed the weak influence of physico-chemical conditions in the water column. Given the negligible inter-oceanic genetic differences typically reported for both skipjack and yellowfin tunas [57], our results support the hypothesis that host phylogeny might be a major driver of the composition of the gut microbiome in tropical tuna.

The skin microbiome is influenced by external conditions. The composition of the skin microbiota showed completely different patterns and greatly varied between the two oceans but not between the tuna species (Fig. 2). *Proteobacteria, Actinobacteria* and *Bacteroidetes* were the main phyla in both species, but their relative abundances were highly variable between the Indian and Atlantic Ocean

sampling sites (Fig. 3). These phyla typically dominate within the skin microbiome of fish species [14,29,58–60]. Geographic and seasonal variations in the composition of the skin microflora have been recently reported in marine mammals, corals and fishes [10,12,61], suggesting that environmental conditions (biotic and abiotic) are strong determinants of the skin microbiome. Most of the commensal bacteria inhabiting the fish mucus layer are thought to play an essential role in protecting the host from colonization by surrounding pathogens [3]. Such bacteria could be capable of adapting to changing conditions in the ocean's water column to maintain this role. The strong microbial similarities found between skipjack and yellowfin tunas in both oceans in this study are interesting and tend to minimize the role of parameters related to the host (i.e., genetic, physiology, immune system, and diet) in shaping the surface microbiome, unlike what was observed in the digestive tract. By contrast, several other studies suggested that host species, as well as physiology or diet, could be a major driver of skin microbiome composition in marine organisms [14,62]. However, those studies compared species belonging to different families and orders, with contrasting physiologies and feeding habits (omnivorous *vs* herbivorous), which is not the case between skipjack and yellowfin tunas.

The liver microbiome: an unexpected niche of high bacterial diversity. The most striking result in this study was the discovery of a highly diversified and unique bacterial assemblage in the tuna liver (Fig. 4). Since the liver is a highly vascularized organ, the presence of such bacteria could be the result of exchanges with the gut via blood circulation, as recently hypothesized in humans and mice [63]. However, the observation of a significant proportion of ASVs in the liver that were not found in any other compartments (Fig. 4) demonstrated that this organ should be considered a major microbial niche, as important as the gut microflora, from the strict point of

view of diversity. This vital organ in vertebrates has attracted increasing attention since the recent finding of bacterial DNA and active bacterial genes in human hepatic tissues [17,64]. Such bacteria are thought to synthesize important metabolic compounds or enzymes useful for various biological processes occurring in this organ, including detoxification, digestion and immune responses [65,66]. However, the role of hepatic bacteria in tuna still remains to be explored, as this is to date the first report of liver-associated bacterial communities in fish.

Interestingly, HPB were present in relatively large quantities in the liver of most individuals of the two tuna species compared with the two other organs (Fig. 5). HPB are well-known human pathogens in fish of the *Scombridae* family and have long been studied in tuna since they represent the most frequent cause of fish poisoning cases [28]. Previous studies reported the occurrence of HPB in the digestive tract, skin, gills and anal vents of tuna [67,68], but to the best of our knowledge, this is the first report of HPB in the tuna liver. Interestingly, HPB belonging to the *Photobacterium* genus (*P. angustum*, *P. damselae*, *P. leiognathi* and *P. phosphoreum*) represented up to 50% of the liver-associated bacterial communities in several of our samples (Fig. 5), and the first three were among the top five taxa present in the “common microbiome” comprising ASVs shared by the three organs (Fig. 4). Altogether, these results raise the hypothesis of active circulation of HPB between the different organs of tuna, which might be mediated by the bloodstream. Our results thus provide new perspectives by describing the liver as another major reservoir of HPB, where these bacteria may not only transit temporarily but also proliferate. Our results also show the need to include this organ in animal microbiome investigations in order to respond to health issues that might be posed by the consumption of animals by humans.

The core and meta-microbiomes in tuna. In our study, although endemic microbiotas were detected in the skin, gut and liver of tuna, our results also highlighted the existence of a common microbiome shared by the three compartments. These shared taxa (mostly represented by the genera *Photobacterium*, *Mycoplasma* and *Cutibacterium*) represented only less than 5% of all ASVs (Fig. 4); however, their ubiquity raises various questions about the circulation, establishment and connectivity of bacterial communities within the fish body. It is now recognized that enteric or epibiotic bacterial communities can interact with other organs, such as the liver, the brain and the lungs, via complex pathways involving blood circulation, immune system components, hormones and various metabolites [22,64,69]. Mono- and bidirectional communication pathways, such as the gut-skin axis or the gut-liver axis, have been described in humans and are thought to be strongly involved in the development of diseases [23,70,71]. For example, the gut-liver axis is now the subject of much speculation in relation to human health [18]. Recently, modification of the gut microbiota was shown to alter the tightness of the epithelial barrier, allowing the transfer of microbes and various other metabolites into the blood and triggering the inflammation of liver tissue [64,65]. Similarly, changes in the intestinal microflora could have a direct effect on the production of neurotransmitters, hormones and other bioactive molecules capable of acting on cutaneous receptors, thus altering the skin structure and its functions [19,72].

CONCLUSION

Finally, the results of our study suggest that the tuna microbiome is composed of distinct microbial niches, comprising both specific and ubiquitous bacterial communities, probably relevant for their respective functioning. The results of this

study led to the first characterization of the meta-microbiome of the two most consumed tuna species worldwide and highlight the importance of the liver as an unexplored microbial niche in fish.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

B.Y. conceived and obtained the funding of this study. Sampling expeditions were performed by B.Y., G.E., B.T., and R.- O.E. G.E. performed all laboratory procedures and data analysis. G.E. and B.Y. wrote the first draft which was revised and discussed with D.C., A. J.-C., R.-O. E., B.T., M. J.-L, A.A. and D.L.

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FIGURE AND TABLE LEGENDS

Figure 1. Alpha taxonomic richness of bacterial communities in the three biological compartments of yellowfin and skipjack tunas. Boxplots represent the distribution of alpha taxonomic richness within each biological compartment. Each circle corresponds to a fish, and the sex (female/male) of the fish is represented by the colour (white/black). Different letters indicate significant differences (KW, $p < 0.05$) between groups within each square.

Figure 2. Compositional dissimilarity between the bacterial communities in the skin (A,D), gut (B,E) and liver (C,F) of tropical tuna, presented along the two first axes from principal coordinates analyses based on Bray-Curtis dissimilarity. Each dot represents an individual tuna or seawater samples, whose species and sampling site are represented by different shapes and colours. In the top panels, samples are gathered according to tuna species, while they are connected according to their ocean of origin in the lower panels. The results of PERMANOVAs (999 permutations) performed on Bray-Curtis dissimilarity matrices to test the variation in bacterial community composition with respect to species and sampling site are indicated in each panel. Values marked with an asterisk indicate a significant effect of the tested factor ($p< 0.05$).

Figure 3. Relative abundances of the main bacterial classes in the skin (A,B), gut (C,D), and liver (E,F) of yellowfin and skipjack tunas at the two sampling sites. Each bar corresponds to an individual fish. Bacterial classes showing a relative abundance lower than 1% were pooled and designated "Other".

Figure 4. Venn diagram representing the number of shared and specific ASVs in tuna skin, gut and liver. For each category, the five most abundant ASVs are indicated at the lowest taxonomic level available (genus or species).

Figure 5. Relative abundance of the main histamine-producing bacteria found in the skin, gut and liver of yellowfin and skipjack tunas from the Atlantic (A) and Indian (B) oceans. Each bar corresponds to an individual fish.

Table 1. Results of Kruskal-Wallis tests between bacterial alpha taxonomic richness and tuna sex, tuna species and sampling site. Bold values indicate a significant effect of the tested factor ($p < 0.05$).

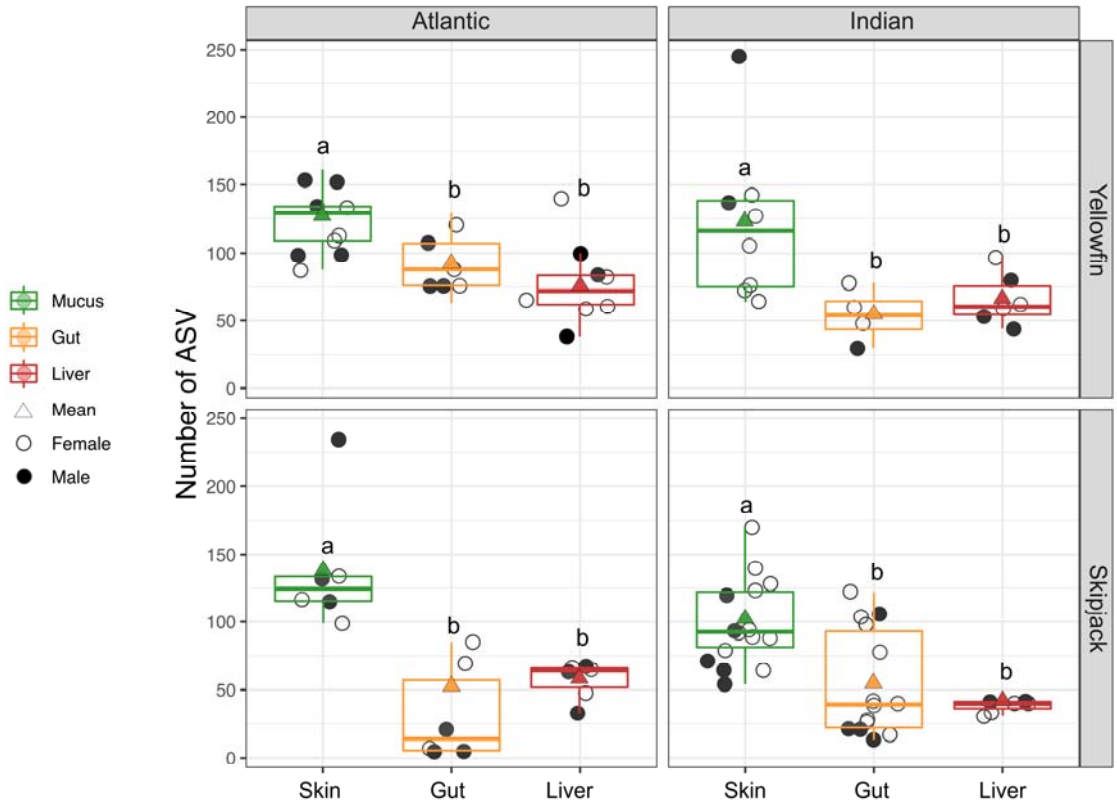
Atlantic
Indian
Yellowfin
Skipjack
Number of ASV
250
200
150
100
50
0
a
b
b
a
b
b
a
b
b
a
b
b
Skin
Gut
Liver
Skin
Gut
Liver
Mucus
Gut
Liver
Mean
Female
Male

Figure 1.

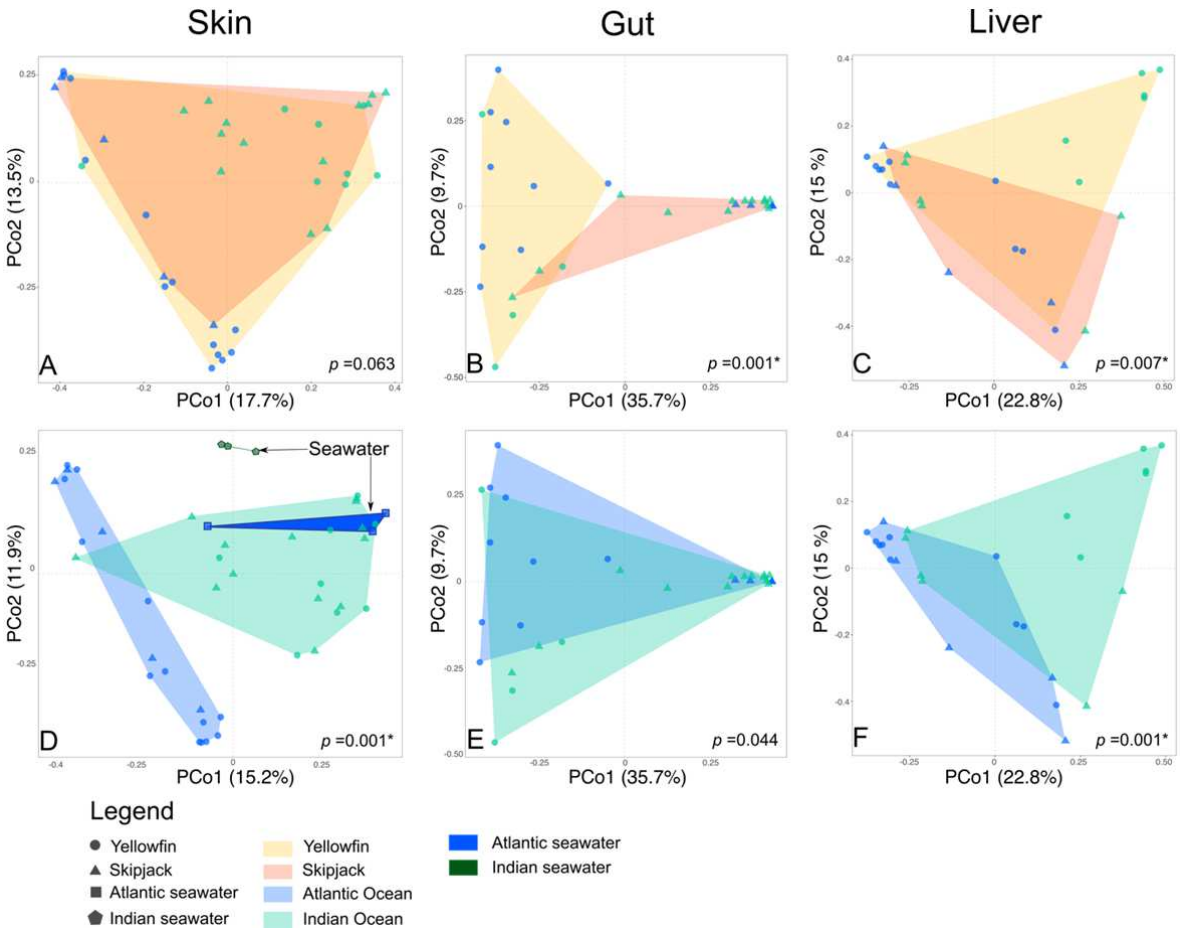
Skin
Gut
Liver
PCo2 (13.5%)
PCo1 (17.7%)
A
p =0.063
PCo2 (9.7%)
PCo1 (35.7%)
B
p =0.001*
PCo2 (15 %)
PCo1 (22.8%)
C
p =0.007*
Seawater
PCo2 (11.9%)
PCo1 (15.2%)
D
p =0.001*
PCo2 (9.7%)
PCo1 (35.7%)
E
p =0.044
PCo2 (15 %)
PCo1 (22.8%)
F
p =0.001*
Legend
Yellowfin
Skipjack
Atlantic seawater
Indian seawater
Yellowfin
Skipjack
Atlantic Ocean
Indian Ocean
Atlantic seawater
Indian seawater

Figure 2

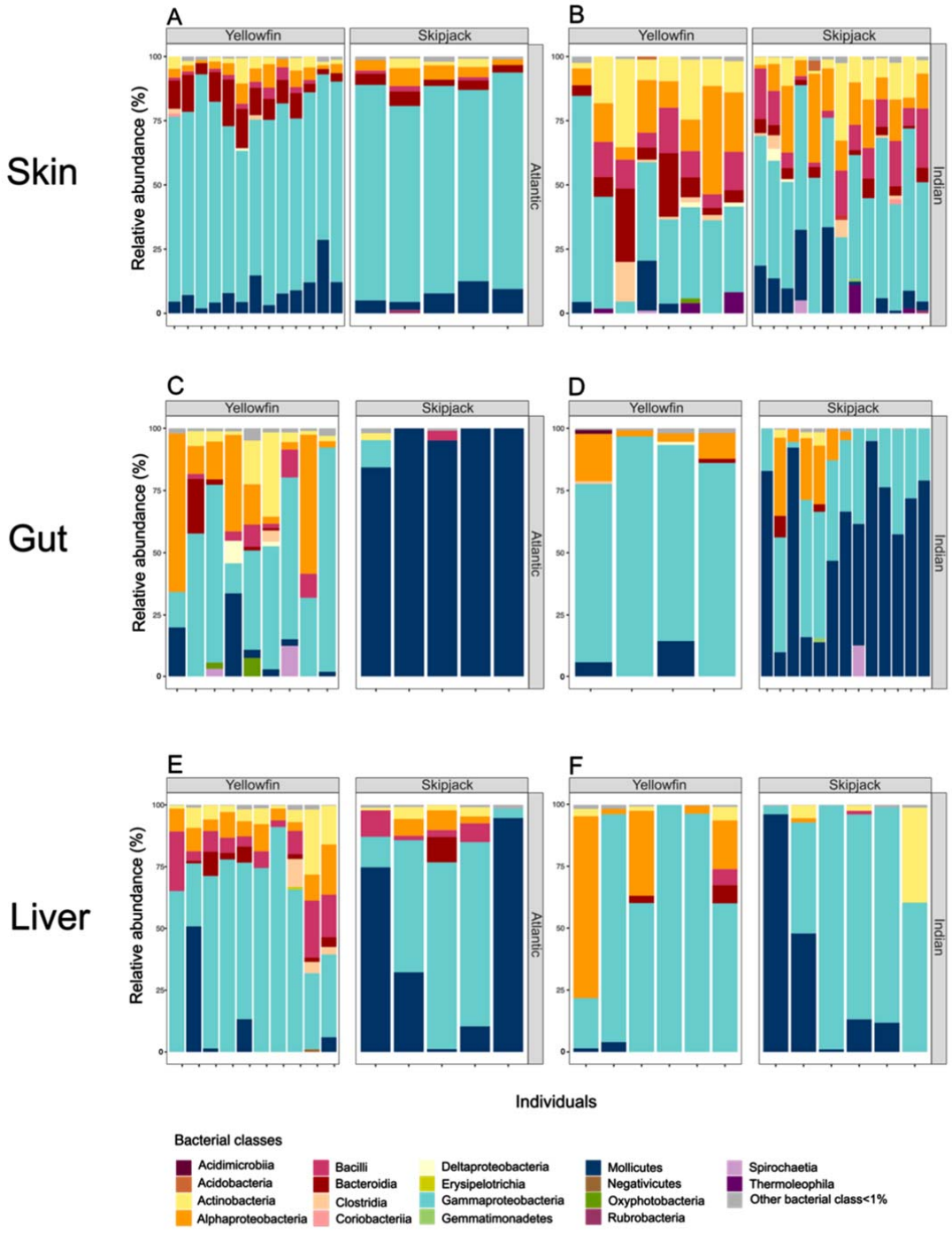
Skin
Gut
Liver
A
B
C
D
E
F
Yellowfin
Skipjack
Atlantic
Indian
Relative abundance (%)
Individuals
Bacterial classes
Acidimicrobiia
Acidobacteria
Actinobacteria
Alphaproteobacteria
Bacilli
Bacteroidia
Clostridia
Coriobacteriia
Deltaproteobacteria
Erysipelotrichia
Gammaproteobacteria
Gemmatimonadetes
Mollicutes
Negativicutes
Oxyphotobacteria
Rubrobacteria
Spirochaetia
Thermoleophila
Other bacterial class<1%

Figure 3

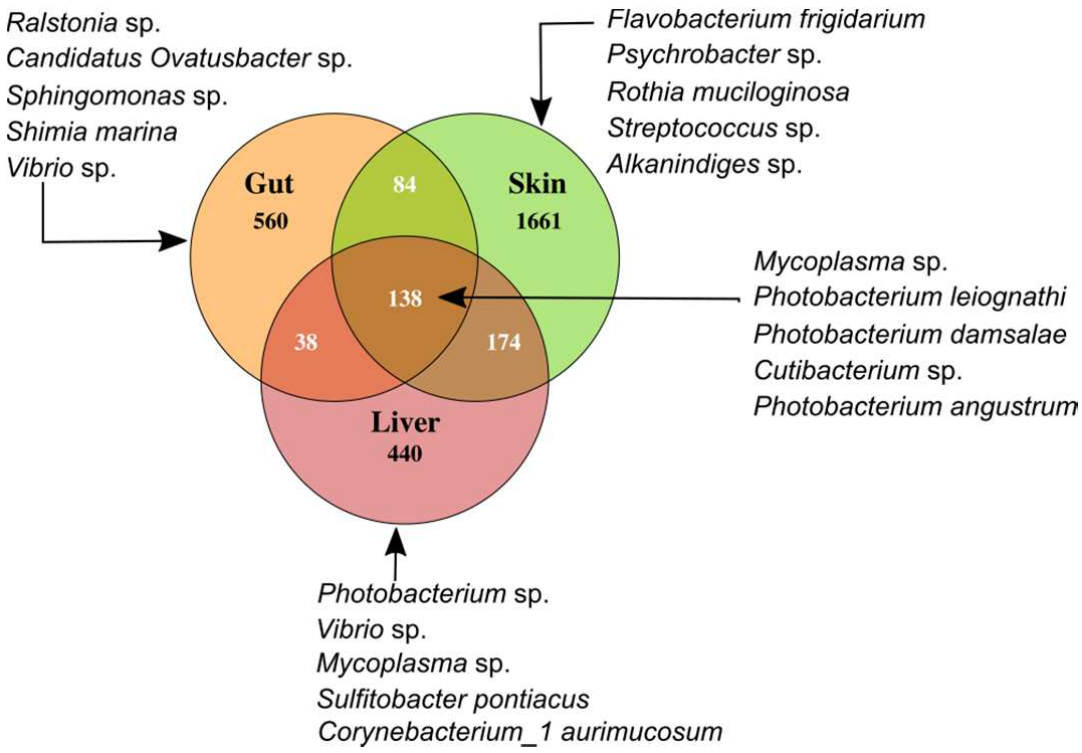
Ralstonia sp.
Candidatus Ovatusbacter sp.
Sphingomonas sp.
Shimia marina
Vibrio sp.
Flavobacterium frigidarium
Psychrobacter sp.
Rothia muciloginosa
Streptococcus sp.
Alkanindiges sp.
Gut
560
84
Skin
1661
138
38
174
Liver
440
Mycoplasma sp.
Photobacterium leiognathi
Photobacterium damsalae
Cutibacterium sp.
Photobacterium angustrum
Photobacterium sp.
Vibrio sp.
Mycoplasma sp.
Sulfitobacter pontiacus
Corynebacterium_1 aurimucosum

Figure 4

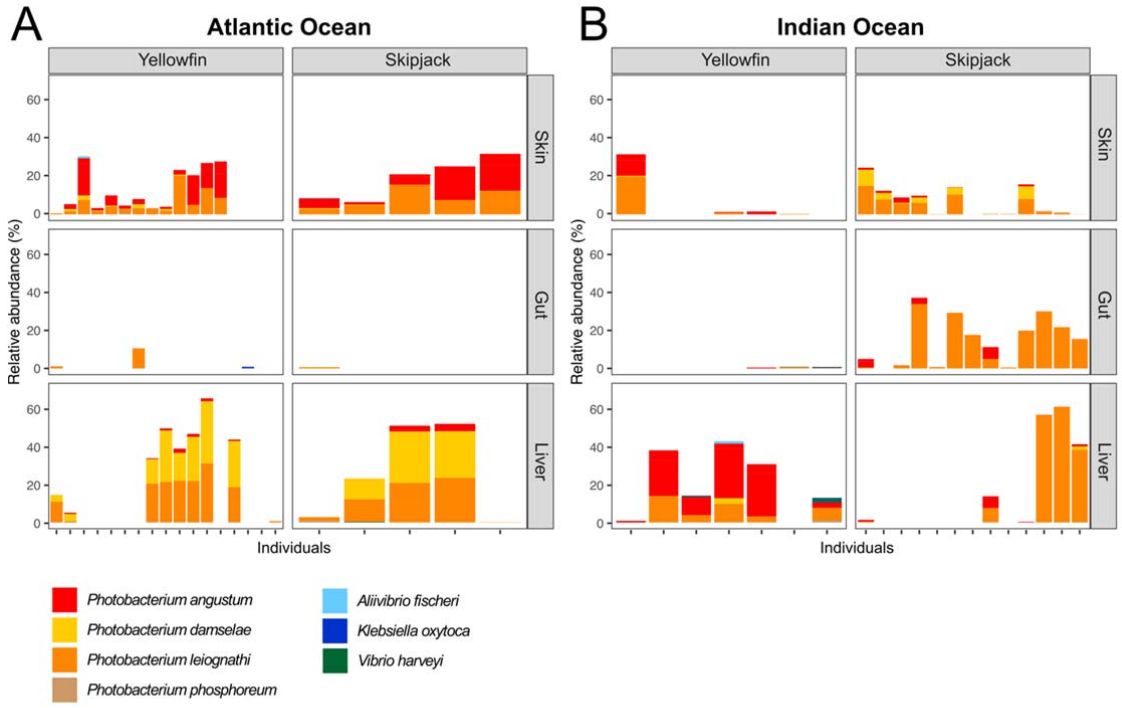
A
Atlantic Ocean
B
Indian Ocean
Yellowfin
Skipjack
Skin
Gut
Liver
Relative abundance (%)
Individuals
Photobacterium angustum
Photobacterium damselae
Photobacterium leiognathi
Photobacterium phosphoreum
Aliivibrio fischeri
Klebsiella oxytoca
Vibrio harveyi

Figure 5

Table 1. Results of Kruskal-Wallis tests between bacterial alpha taxonomic richness and tuna sex, tuna species and sampling site. Bold values indicate a significant effect of the tested factor ($p < 0.05$).

	Number of ASV		
	Sex	Ocean	Species
Skin	$p = 0.063$	***p* = 0.024**	$p = 0.183$
Gut	$p = 0.086$	$p = 0.426$	***p* = 0.015**
Liver	$p = 0.419$	***p* = 0.043**	***p* = 0.009**

Supplementary Figure 1. Relative abundances of the main bacterial classes in surface seawater samples of the Atlantic (A) and Indian (B) sampling sites. Each bar corresponds to a replicate sample. Bacterial classes showing a relative abundance lower than 1% were pooled and designated as “Other”.