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## Fishing for the Microbiome of Tropical Tuna

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### Abstract :

Although tunas represent a significant part of the global fish economy and a major nutritional resource worldwide, their microbiome still remains poorly documented. Here, we conducted an 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. We hypothesized that each organ harbors a specific microbial assemblage whose composition might vary according to different biotic (sex, species) and/or abiotic (environmental) factors. Our results revealed that the composition of the tuna microbiome was totally independent of fish sex, regardless of the species and ocean considered. Instead, the main determinants of observed diversity 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, 4.5% of all amplicon sequence variants (ASV) were shared by the three organs, highlighting the presence of a core-microbiota whose most abundant representatives belonged to the genera *Mycoplasma*, *Cutibacterium*, and *Photobacterium*. Our study also revealed the presence of a unique and diversified bacterial assemblage within the tuna liver, comprising a substantial proportion of potential histamine-producing bacteria, well known for their pathogenicity and their contribution to fish poisoning cases. These results indicate that this organ is an unexplored microbial niche whose role in the health of both the host and consumers remains to be elucidated.

**Keywords** : Microbiome, Bacteria, Histamine, Tuna, Liver

## INTRODUCTION

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, defense, and nutrition, among others [1–3]. Most of these 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 microbial associates 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 bacterial communities associated with other essential potential “microbial organs” such as the liver have been poorly investigated, despite its essential role in metabolic and immune functions within the host 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 organs, 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].

Here, we conduct a simultaneous multi-compartmental analysis of the microbial communities from the gut, liver and skin mucus layer of two emblematic tropical tuna species, skipjack (*Katsuwonus pelamis*) and yellowfin tunas (*Thunnus albacares*). Tuna is a pelagic teleost fish distributed in tropical and temperate 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 and yellowfin 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 fish poisoning cases [27,28], typical with most the fish species belonging to the *Scombridae* family, which contain high levels of histidine, the histamine precursor. Finally, despite the considerable nutritional value of this resource as well as the health hazard associated with its consumption (nausea, diarrhea, hypotension, vomiting, faintness, etc.), knowledge of the tuna microbiome remains rudimentary [29].

In this study, our main objectives were to (i) describe the composition of the skin, gut and liver microbiota 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 sampling site on the composition of their respective microbiota and (iv) examine the diversity and location of potential HPB.

## **MATERIAL AND METHODS**

### **Sampling procedure.**

*Tunas.* Tunas of the species *Thunnus albacares* (yellowfin, YFT) and *Katsuwonus pelamis* (skipjack, SKJ) were captured around Fish Aggregating Devices (FAD) 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 (10-11<sup>th</sup>) and September (26-29<sup>th</sup>) 2018, respectively (Fig. 1). Sampling and euthanasia of animals were performed by professional fishermen working for the Exploited Tropical Pelagic Ecosystems Observatory (certified ISO

9001/2015). In the Gulf of Guinea, 6 skipjack tuna (3 females, 3 males) (fork length min-max: 56-66 cm) and 15 yellowfin tuna (8 females, 7 males) (fork length min-max: 46-66 cm) were collected. On Réunion Island, 27 tunas were captured: 18 skipjack tuna (14 females, 4 males) (fork length min-max: 41-60 cm) and 9 yellowfin tuna (6 females, 3 males) (fork length min-max: 61-69 cm) (Supplementary Tab. 1). The fish sex was determined by visual examination of the gonads. With regards to the size and species of the different individuals, all the skipjack are considered as sexually mature adults, while all the yellowfin are sexually immature sub-adults [30,31]. 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.

*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].

*Gastro-intestinal content.* Following skin sampling, fish were individually placed in plastic bags and immediately stored on ice before dissection (within 5 h after sampling) [32]. Briefly, the gastrointestinal tract was extracted from each individual and cut from below the stomach to the rectum using sterile tools. Each gastrointestinal tract was opened, squeezed and its inner surface was entirely rubbed with sterile dissection tools to expel the contents (minimum volume of 5 mL) on a sterile surface, and the gut contents (eg, the digesta microbiota) were homogenized before sampling. The generic term "GUT" was used here to refer to the intestinal contents and not the entire gut, which includes the tissue of the digestive tract.

*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- $\mu$ m-porosity polycarbonate filters membranes ( $\phi$ 47 mm, Whatman<sup>®</sup> 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  $250 \pm 0.5$  mg of gut (n= 48) and liver samples (n= 48) and from the entire swabs and filter for skin mucus (n= 48) and seawater (n=6). All extractions were performed with the PowerSoil DNA Isolation Kit (Qiagen<sup>®</sup>, Hilden, Germany) following the manufacturer's instructions. DNA quality and quantity were assessed by spectrophotometry (NanoDrop<sup>®</sup>, Wilmington, DE, USA). To identify potential contaminants within the reagents, negative controls were obtained by performing blank extraction (n=2). Similarly, blank DNA extractions were also performed on two virgin membranes (used to filter seawater samples) to serve as negative controls. The V3-V4 region of the 16S rDNA gene was amplified using universal bacterial primers modified for Illumina sequencing: 343F (5'-ACGGRAGGCAGCAG) [33] and 784R (5'- TACCAGGGTATCTAATCCT) [34]. The reaction mixture consisted of 12.5  $\mu$ L of 2X Phusion Mix (New England Biolabs<sup>®</sup>, Ipswich, MA, USA), 1  $\mu$ L of each primer at 10  $\mu$ M (Eurofin<sup>®</sup>, Luxembourg), 10 ng of DNA template and enough molecular-grade H<sub>2</sub>O (Qiagen<sup>®</sup>) to reach a final volume of 25  $\mu$ L. All samples were amplified in triplicate to avoid PCR bias in the taxonomic diversity of the community [35]. Triplicate PCR products were pooled and purified with a NucleoSpin Kit (Macherey-Nagel<sup>®</sup>, Düren, Germany) following the manufacturer's instructions. Negative controls for contamination of the PCR reactions were performed in duplicate. Successfully amplified samples (n=103) as well as negative controls (n=6) were sequenced on the Illumina platform (GenoToul<sup>®</sup>, Toulouse, France) using 2x250 bp MiSeq platform.

## **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) [36]. The quality of forward and reverse reads was plotted and analysed, then the sequencing adapters were removed from the reads. To do so, the length of each adapter being known, the corresponding number of bases was removed from the respective ends of the reads. Using the DADA2 tutorial with default parameters, reads were then filtered, trimmed and merged into 4 934 amplicons sequence variants (ASVs), which have a higher resolution than operational taxonomic units (OTUs) [36]. Chimeras were removed, and sequences were aligned to the SILVA 123 database [37] to assess 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 (maximum = 71 339 sequences, mean = 47 217 sequences). Using the *phyloseq* package [38], final taxonomic and ASV tables were linked to sample metadata (tuna species, sex, organ 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. To explore the alpha diversity, the Shannon index was calculated for each sample with the *phyloseq* package [38], and was then tested for differences between organs (skin mucus, gut content and liver), tuna species (yellowfin and skipjack), oceans (Atlantic and Indian oceans) and sexes (female and male) using a one-way ANOVA test (the data were normally distributed and the homoscedasticity was respected). 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. 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 [39]. 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.

Dissimilarities between bacterial communities were assessed using Bray-Curtis distances, which were calculated with the *vegan* package [40] and represented in a principal coordinate analysis (PCoA) plot built with the *ape* package [41]. The effect of organs, tuna species, sex and sampling site on the composition of bacterial communities, as well as the interactions between these factors were determined by using a one-factor and a multifactorial PERMANOVA with 999 permutations of the Bray-Curtis matrix using the “adonis” function of the *vegan* package [42]. Statistical significance was assumed when  $p < 0.05$ .

A list of potential histamine-producing bacteria (HPB) genera was also established, based on the literature (Supplementary Materials, Tab. 2), and their presence in our samples was assessed by comparing this list to our taxonomic table.

## RESULTS

On average the relative abundance of bacterial ASVs observed in the 3 types of negative sequencing controls remained below 5% of the total abundance of ASVs, whatever the organ considered. The low level of contamination was confirmed by performing a one-factor PERMANOVA (with 999 permutations of the Bray-Curtis matrix [40]), which measured the dissimilarity between the results obtained in the negative sequencing controls and those in skin mucus samples ( $p$  value = 0.006), gut ( $p$  value = 0.01), and liver ( $p$  value = 0.02).

### ***Alpha diversity.***

The Shannon alpha diversity of bacterial communities, considering the number of ASVs and their respective relative abundance, 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) (Tab.1).

*Variability between sexes.* Regardless of the tuna species, ocean and biological compartment considered, the Shannon alpha diversity of the bacterial communities did not show significant differences between male and female individuals (Fig. 2).

*Variability between tuna species.* In the gut and liver samples, Shannon index was significantly higher in yellowfin than in skipjack tuna (Fig. 2). Statistical analysis confirmed that bacterial *alpha* diversity differed significantly between the two tuna species.

*Variability between oceans.* In the liver samples, the Shannon index was significantly lower in tuna captured in the Indian Ocean (Fig. 2).

*Variability between compartments.* The skin mucus layer showed a significantly higher Shannon index than the gut and liver of both tuna species, regardless of the sampling site (Fig. 2). However, it did not differ significantly between the gut and liver samples (Fig. 2).

### **Shared taxa and specific ASVs among the three organs.**

The Venn diagram revealed that among all the ASVs identified within tuna microbiota, a relatively small proportion (4.5% = 138 ASVs) were common to the skin, gut and liver (Fig. 3). Among these common taxa, the five most represented ASVs (observed in 60% to 90% of the samples) were identified as *Mycoplasma* sp., *Cutibacterium* sp. and three species of the genus *Photobacterium* (i.e., *P. leiognathi*, *P. damsela* and *P. angustum*), which are potential histamine-producing bacteria (HPB) (Supplementary Tab. 2)[43]. In addition, each compartment hosted a specific and diversified assemblage of taxa. The skin microbiota, with 1661 specific ASVs, accounted for half of the total microbiota diversity (i.e., 53.7%). The five most common taxa were *Flavobacterium frigidarium*, *Psychrobacter* sp., *Rothia muciloginosa*, *Streptococcus* sp. and *Alkanindiges* sp. The gut and liver hosted less specific ASV, 560 and 440, respectively. These relatively similar numbers were unexpected and show that the liver harbors a unique bacterial assemblage that is almost as large as that found in the digestive tract of tunas. The five most representative taxa found in the liver were *Photobacterium* sp., *Vibrio* sp., *Mycoplasma* sp., *Sulfitobacter pontiacus* and *Corynebacterium-1 aurimucosum*. Several of the genera cited above, including

*Corynebacterium*, *Flavobacterium*, *Psychrobacter* and *Streptococcus* which are known to be common contaminants in bacteria metabarcoding studies [44], although they were not detected in our negative sequencing controls.

### **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,  $p > 0.05$ , Tab. 2).

*Skin microbiota.* Skin samples showed significant similarities between tuna species but large dissimilarities between the two sampling sites (Fig. 4A,D, Tab. 2). In both the Indian and Atlantic oceans, the skin bacterial communities greatly differed from those examined in the surrounding seawater (Fig. 5A,B, Supplementary Fig. 1). For Atlantic yellowfin and skipjack tunas, the skin bacteriome was dominated by *Gammaproteobacteria*, representing up to 83% of the sequences (Fig. 5A,B). Several other bacterial classes, such as *Actinobacteria*, *Alphaproteobacteria*, *Bacilli*, *Bacteroidia* and *Mollicutes* were also present. The relative abundance of the latter classes was higher for skipjack and yellowfin tunas samples from the Indian Ocean (Fig. 5A, B).

*Gut microbiota.* By contrast with the skin microbiota, the gut microbiota included a bacterial assemblage that was clearly distinct between the two tuna species, while sampling site had no significant effect (Fig. 4B,E, Tab. 2). In skipjack tuna, the gut microbiota was dominated by *Mollicutes* (Fig. 5C), whereas that of yellowfin tuna was more diversified, with higher proportions of *Gammaproteobacteria* and, to a lesser extent, *Alphaproteobacteria* and *Actinobacteria* (Fig. 5D). 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. 4E).

*Liver microbiota.* Liver samples exhibited an intermediate outcome since hepatic bacterial communities were significantly affected by both tuna species (PERMANOVA,  $p$  value = 0.001) and sampling site (PERMANOVA,  $p$  value = 0.001) (Fig. 4C,F, Tab. 2).

*Gammaproteobacteria* were highly abundant in most of the samples, and *Mollicutes* were generally more represented in skipjack than in yellowfin tunas (Fig. 5E,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 microbiota that was globally less diversified than that of their Atlantic counterparts (Fig. 5E,F). However, no clear pattern was observed, and the composition of the bacterial communities in the liver seemed to be more influenced by the sampling site than by the tuna species (Tab. 2).

### **Diversity and location of potential HPB.**

In the variety of samples analysed, 7 taxa were identified as potential HPB (based on the literature, see Supplementary Table 2), namely, *Aliivibrio fischeri*, *Klebsiella oxytoca*, *Photobacterium angustum*, *Photobacterium damselae*, *Photobacterium leiognathi*, *Photobacterium phosphoreum* and *Vibrio harveyi* (Fig. 6). In general, potential HPB were largely dominated by species of the genus *Photobacterium*, but their respective proportions greatly varied between the different organs. The liver showed the greatest occurrence of potential HPB in both tuna species and oceans (Kruskal Wallis,  $p < 0.05$ ), 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 putative HPB, especially in tuna from the Atlantic, which exhibited nearly undetectable levels of potential HPB (Fig. 6A). 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. 6A,B).

## **DISCUSSION**

***The tuna microbiome is not sex-specific.*** An important result of this study was that, invariably, the tuna microbiome did not show significant differences between sexes,

regardless of the tuna species, sampling site and organ. For both skipjack and juvenile yellowfin (< 70 cm) tunas, males and females share the same ecological niche as well as anatomical similarities, and only the gonads able to differentiate them [45–48]. The same results were reported in both sticklebacks and salmon, for which the gut and skin microbiota did not vary between male and female individuals [49,50]. Conversely, Bolnick et al. (2014) reported sex-related variability in the gut microbiota 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 [31,46,51]. During this period, females are likely to modify their diet [30], which could alter the composition of their gut microbiota. In our study, although all the yellowfin were smaller than 70 cm and therefore sexually immature [30,52], the skipjack in their size class are considered mature and with the ability to reproduce throughout the year [51]. 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 skin microbiota 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. *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. These phyla typically dominate within the skin microbiota of fish species [14,29,53–55]. Geographic and seasonal variations in the composition of the skin microflora have been recently reported in marine mammals, corals and fishes [10,12,56], suggesting that environmental conditions (biotic and abiotic) are strong determinants of the skin microbiota. Nevertheless, it has been long reported that the composition of surface microbiota of marine organisms strongly differ from that of the surrounding planktonic bacterial communities [57–60], and which was also the case in our study (Fig. 4). Such differences might be explained by the specificity of the fish skin which,

due to the presence of mucus, organic residues and immune components, represents a very selective medium for microorganisms [57,61,62]. 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 microbiota, 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 microbiota composition in marine organisms [14,63]. 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 gut microbiota of tropical tuna is species-specific.*** Our results showed that the composition of the gut microbiota differed between the two tuna species but not between the sampling sites (i.e., for a given species). Skipjack and juvenile yellowfin tunas (size classes sampled in our study) are relatively close physiologically and behaviourally [64]. They also share the same habitat in the water column [65] and usually feed on the same prey (i.e., mostly fish, crustaceans and cephalopods) [66,67]. In addition, individuals in this study were caught around fish aggregating devices (FADs), under which both tuna species tend to gather 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 piscivorous fishes [1]. By contrast, the gut of skipjack tuna hosted a majority of *Mollicutes* of the genus *Mycoplasma* sp., which also form a major component of the gut microbiota of salmon, 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 might be discarded. Our results are in agreement with the phylosymbiosis hypothesis, which assumes that the host phylogeny reflects the composition of its microbiota [68]. In a previous study investigating the composition of gut microbiota on three tuna species (skipjack, yellowfin and bigeye), we also observed a species-specific composition of the microbiota [69]. 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) [64,70]. Therefore, the composition of a tuna's enteric flora could be tightly linked to its evolutionary history [71,72], 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 of the water column and of the surrounding planktonic communities. Given the negligible inter-oceanic genetic differences typically reported for both skipjack and yellowfin tunas [73], our results support the hypothesis that host phylogeny might be a major driver of the composition of the gut microbiota in tropical tuna.

***The liver microbiota: an unexpected reservoir 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. 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 [74]. However, the observation of a significant proportion of ASVs in the liver that were not found in any other organ demonstrated that this one 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 [17,75]. 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 [76–78]. 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. One should stress out that the sequencing approach used in this study can be sensitive to potential contamination and crosstalk [44]. However, a low level of contamination was observed in our samples, as the ASVs identified in the negative controls represented less than 5% of their total abundance. Thus, by demonstrating the existence of a unique bacterial signature in the liver, our study suggests that this organ should be considered with more attention in further studies on fish microbiome.

Interestingly, potential HPB were observed in significantly larger quantities in the liver of most individuals of the two tuna species compared with the two other organs (Fig. 6). 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, Supplementary Table 1]. Previous studies reported the occurrence of HPB in the digestive tract, skin, gills and anal vents of tuna [43,79], but to the best of our knowledge, this is the first report of potential HPB genera in the tuna liver. Interestingly, potential HPB belonging to the *Photobacterium* genus (*P. angustum*, *P. damsela*, *P. leiognathi* and *P. phosphoreum*) represented up to 50% of the liver-associated bacterial communities in several of our samples (Fig. 6), and the first three were among the top five taxa present in the “common microbiome” comprising ASVs shared by the three organs (Fig. 3). Altogether, these results raise the hypothesis of active circulation of potential HPB between the different organs of tuna, which might be mediated by the bloodstream. Although our data do not allow stating about the production of histamine production by the identified HPB taxa, they do allow us mapping their distribution within the tuna microbiome. Finally, our results thus provide new perspectives by describing the liver as another major reservoir of potential 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 the health issues that might be posed by the consumption of animals by humans.

***The core and meta-microbiome 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 microbiota 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; however, their ubiquity raises various questions about the circulation, establishment and connectivity of bacterial communities within the fish body. Indeed, the detection of taxa shared by the three organs might suggest the existence of an active circulation of bacteria including HPB amongst organs. However, such mechanisms still remain to be confirmed. 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 [22,75,80]. 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 [23,81,82]. 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 [75,76]. Similarly, changes in the intestinal microbiota 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 [19,83].

## **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.

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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.

*Ethics approval.* This is an observational study. The IRD Ethics Committee has confirmed that no ethical approval is required

*Consent to participate.* Informed consent was obtained from all individual participants included in the study

*Consent to publish.* Not applicable.

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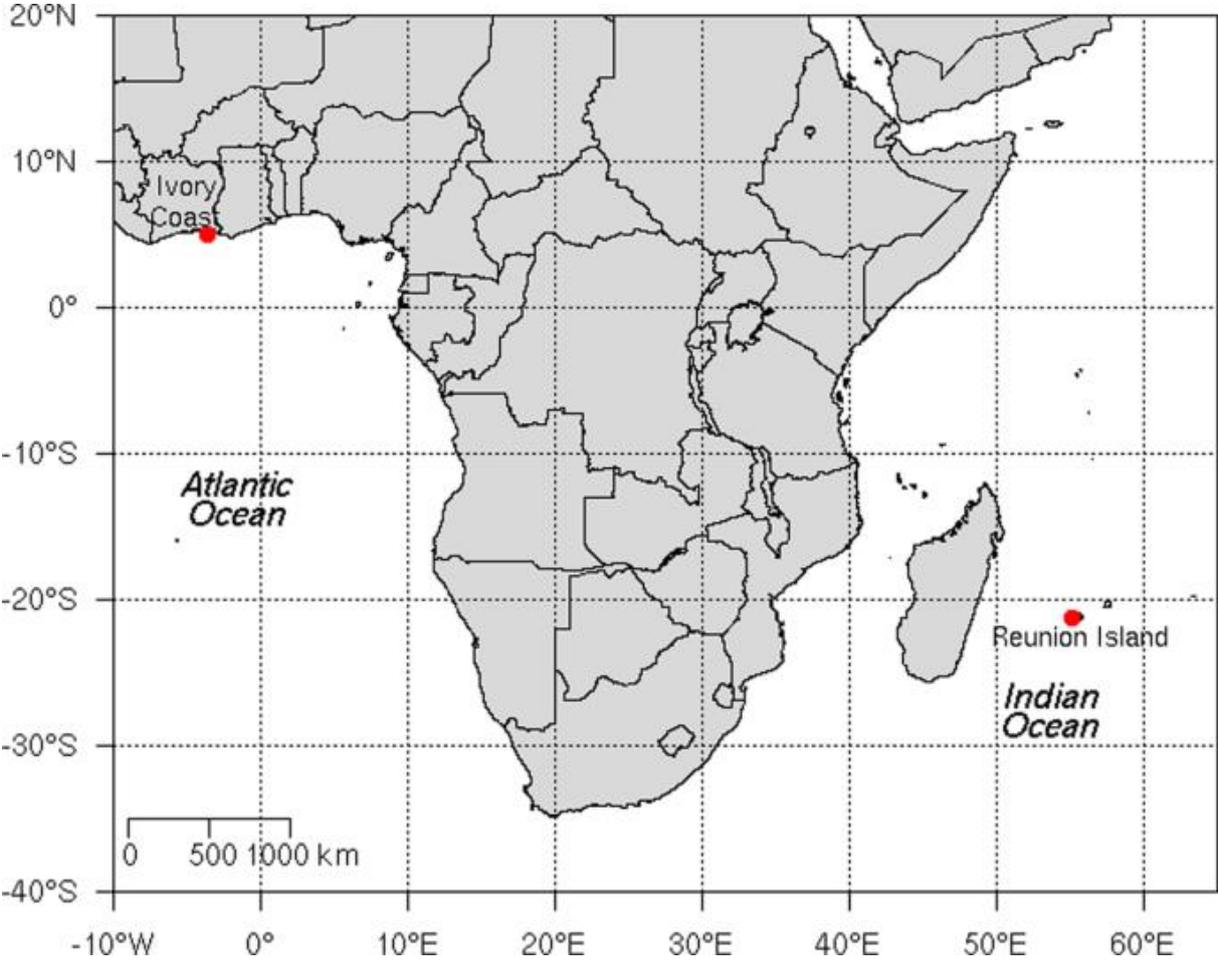
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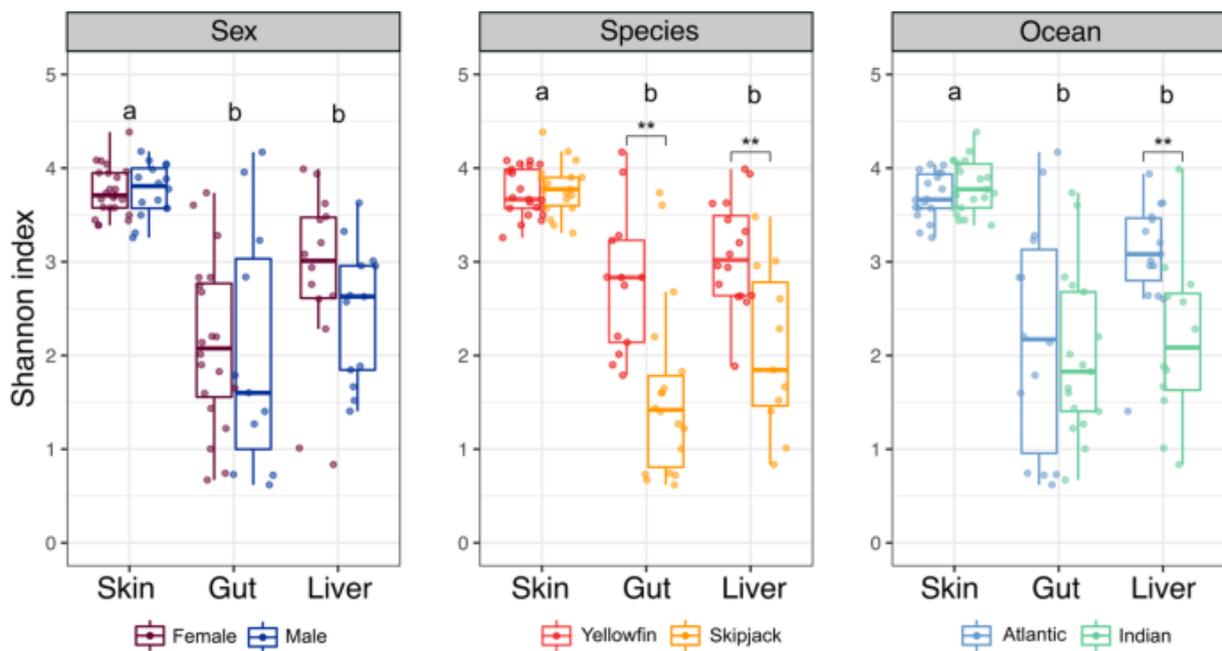
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**FIGURES**

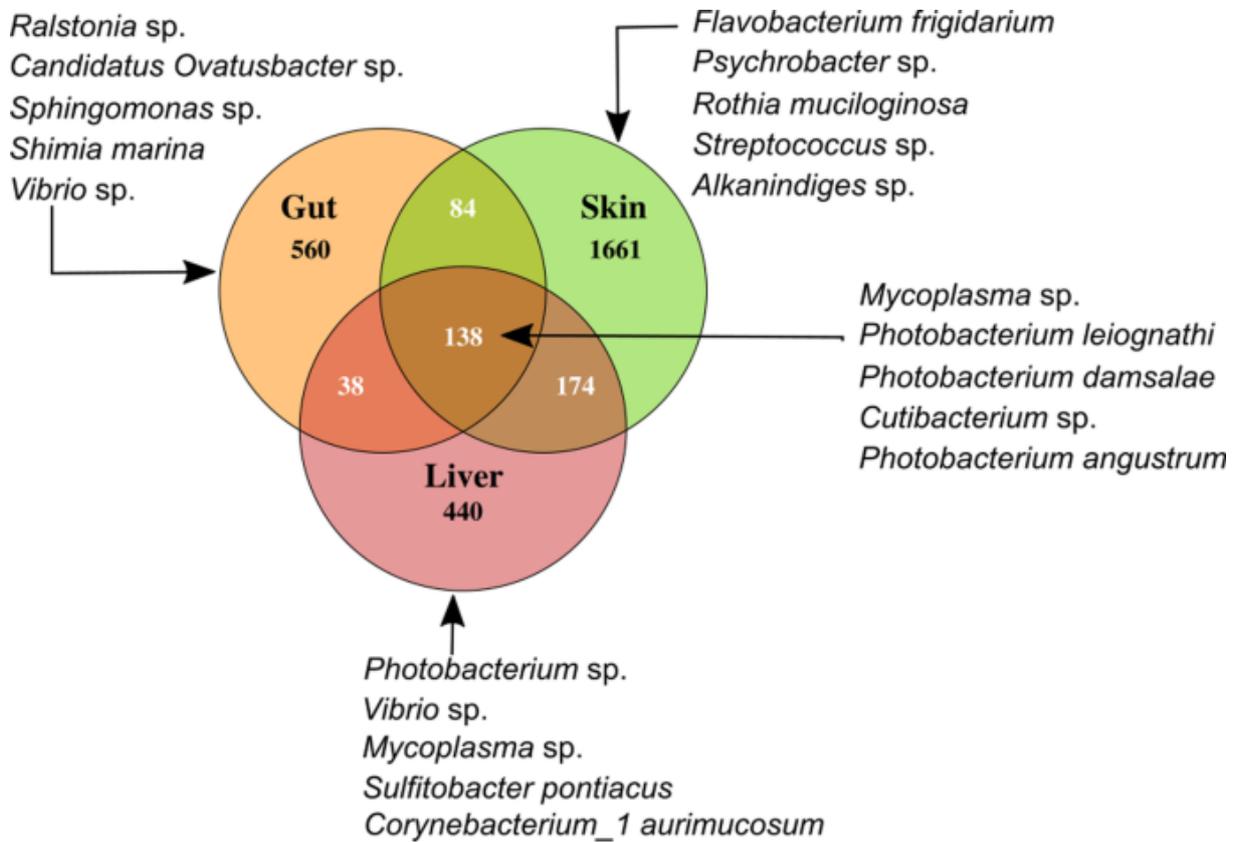
**Figure 1.** Location of the two sampling sites (red dots) in Ivory Coast (Atlantic Ocean) and Reunion Island (Indian Ocean) where skipjack and yellowfin tunas were captured.



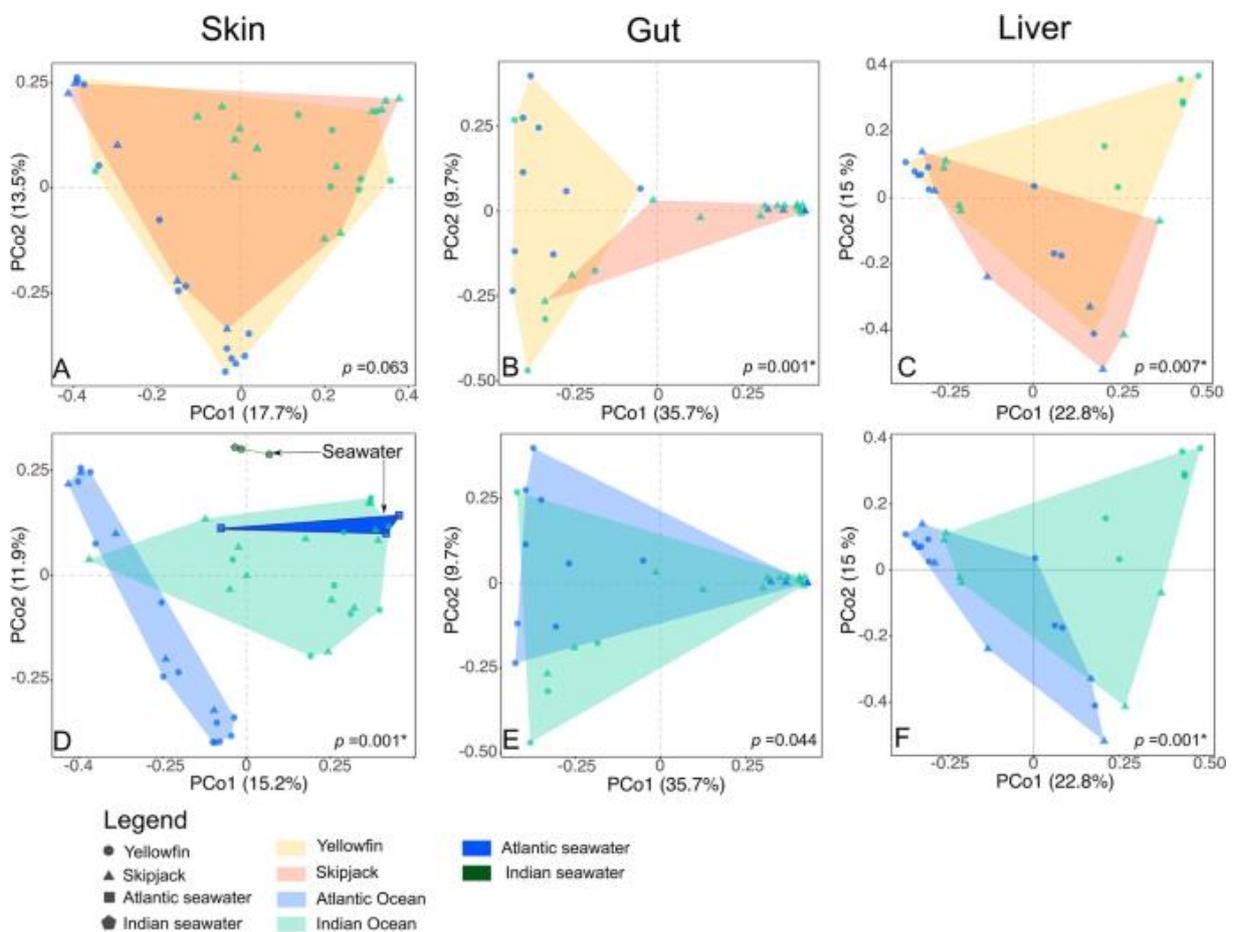
**Figure 2.** Distribution of the Shannon index for the three organs (eg, skin, gut and liver) according to the sex (female/male), species (yellowfin/skipjack) and sampling site (Atlantic/Indian ocean) of the individuals. At the organ levels, significant differences between sex, species or sampling site are indicated by brackets with asterisk (One-way ANOVA,  $p < 0.05$ ). Different letters indicate significant differences (One-way ANOVA,  $p < 0.05$ ) between each organs.



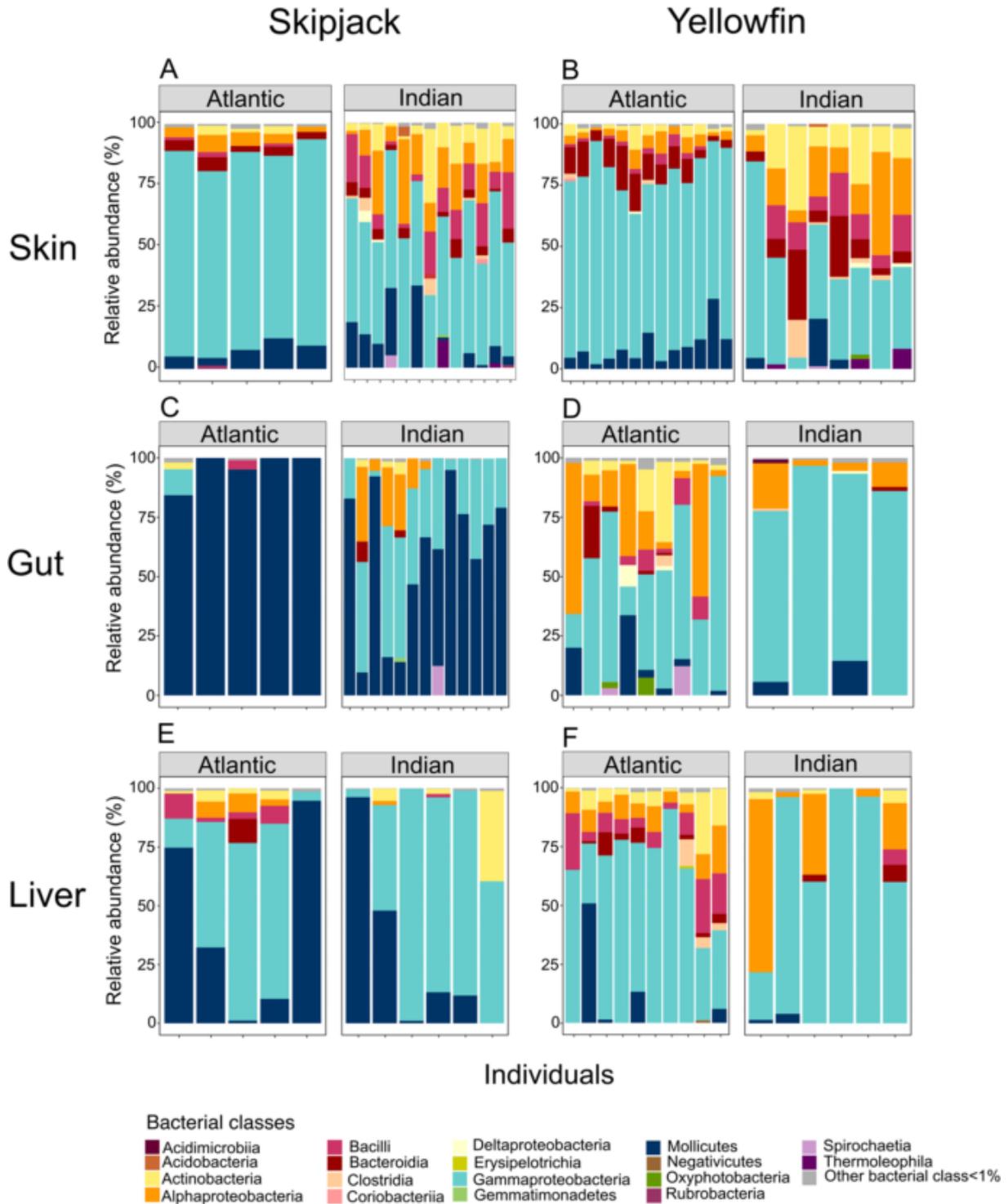
**Figure 3.** Venn diagram representing the number of shared and specific ASVs in tuna skin, gut and liver of yellowfin and skipjack tunas from the two sampling sites. For each category, the five most abundant ASVs are indicated at the lowest taxonomic level available (genus or species).



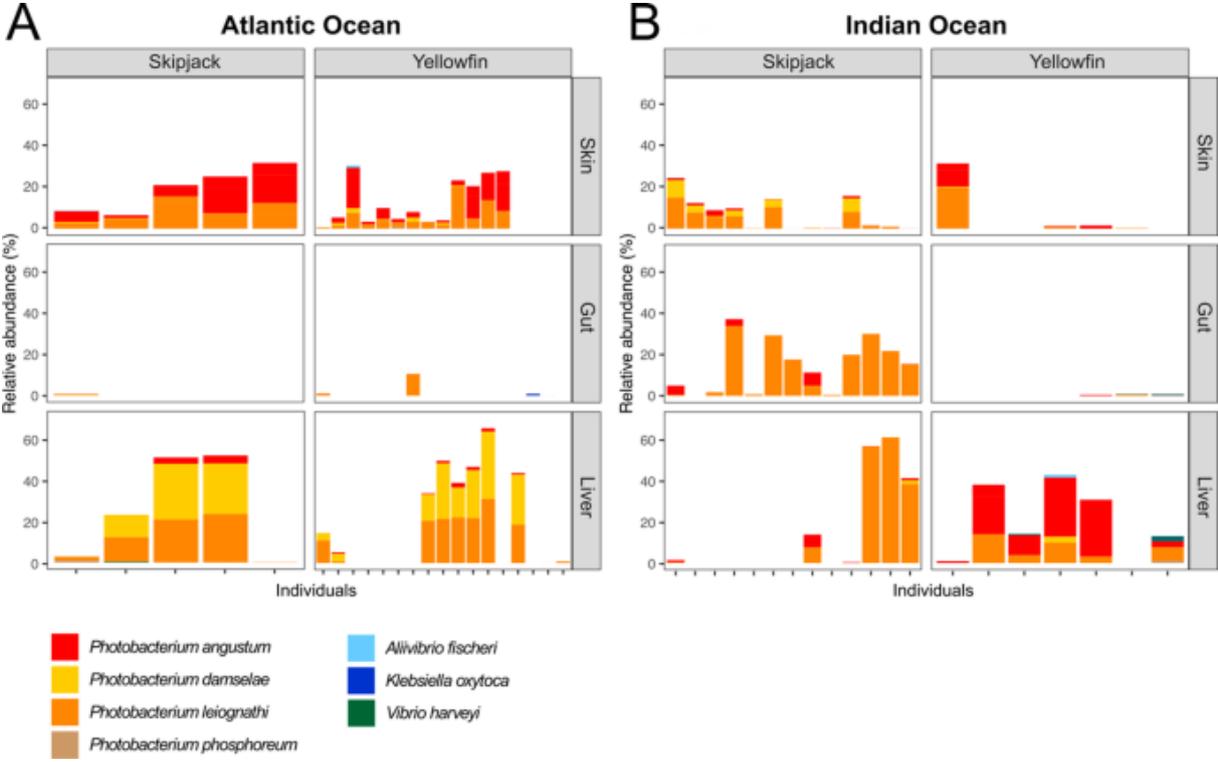
**Figure 4.** Compositional dissimilarity between the bacterial communities in the skin (A,D), gut (B,E) and liver (C,F) of tropical tuna, presented along the two first axes from principal coordinates analyses based on Bray-Curtis dissimilarity. Each dot represents an individual tuna or seawater samples, whose species and sampling site are represented by different shapes and colors. 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 5.** Relative abundances of the main bacterial classes in the gut (A,B), liver (C,D), and skin (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 6.** 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 one-way ANOVA tests between bacterial Shannon alpha diversity index and tuna sex, species and sampling site. Bold values indicate a significant effect of the tested factor ( $p < 0.05$ ).

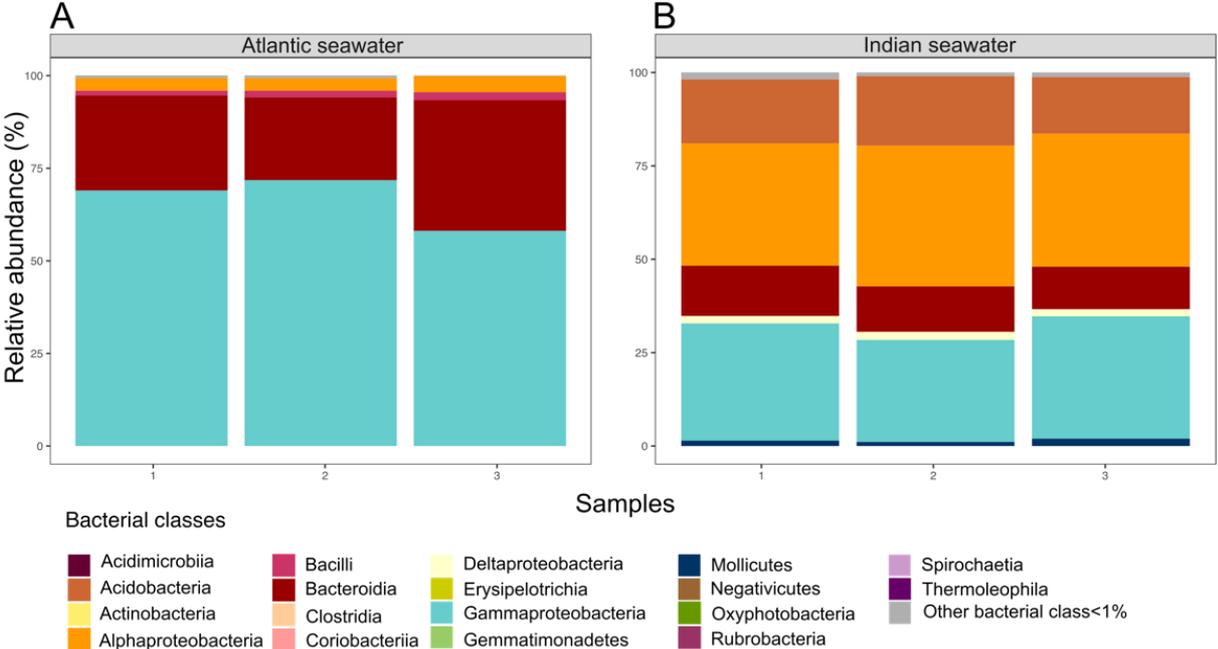
	Shannon alpha diversity		
	Sex	Species	Ocean
Skin	$p = 0.875$	$p = 0.579$	$p = 0.232$
Gut	$p = 0.828$	<b><math>p = 0.0008</math></b>	$p = 0.573$
Liver	$p = 0.258$	<b><math>p = 0.001</math></b>	<b><math>p = 0.004</math></b>

**Table 2:** Results of permutational ANOVAS (PERMANOVA, 999 permutations) performed on Bray-Curtis dissimilarity matrices to test the variation in the bacterial community composition in skin, gut and liver samples, with respect to the tuna sex, species and sampling site and the interactions between them. Bold values indicate a significant effect of the tested factor ( $p < 0.05$ ).

	Community dissimilarity																				
	Sex			Species			Ocean			Sex * Species			Sex * Ocean			Species * Ocean			Sex * Species * Ocean		
	<i>P</i>	<i>r</i> <sup>2</sup>	df	<i>P</i>	<i>r</i> <sup>2</sup>	df	<i>P</i>	<i>r</i> <sup>2</sup>	df	<i>P</i>	<i>r</i> <sup>2</sup>	df	<i>P</i>	<i>r</i> <sup>2</sup>	df	<i>P</i>	<i>r</i> <sup>2</sup>	df	<i>P</i>	<i>r</i> <sup>2</sup>	df
<b>Skin</b>	0.640	0.02	1	0.074	0.03	1	<b>0.001</b>	0.11	1	0.34	0.02	1	0.48	0.02	1	0.17	0.02	1	0.63	0.04	1
<b>Gut</b>	0.113	0.06	1	<b>0.001</b>	0.21	1	0.088	0.03	1	0.07	0.04	1	0.31	0.02	1	0.20	0.03	1	0.57	0.01	1
<b>Liver</b>	0.381	0.05	1	<b>0.001</b>	0.08	1	<b>0.001</b>	0.13	1	0.05	0.05	1	0.64	0.02	1	<b>0.007</b>	0.08	1	0.61	0.02	1

**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 “Other”.

Seawater



**Supplementary Table 1:** Number of male and female individuals sampled and successfully sequenced for each biological compartment, tuna species and sampling sites.

	Atlantic				Indian			
	Skipjack		Yellowfin		Skipjack		Yellowfin	
	Samples	Successfully sequenced	Samples	Successfully sequenced	Samples	Successfully sequenced	Samples	Successfully sequenced
Skin	♀ 3	♀ 2	♀ 8	♀ 5	♀ 14	♀ 10	♀ 6	♀ 6
	♂ 3	♂ 3	♂ 7	♂ 8	♂ 4	♂ 3	♂ 3	♂ 2
Gut	♀ 3	♀ 2	♀ 8	♀ 5	♀ 14	♀ 10	♀ 6	♀ 3
	♂ 3	♂ 3	♂ 7	♂ 4	♂ 4	♂ 3	♂ 3	♂ 1
Liver	♀ 3	♀ 2	♀ 8	♀ 6	♀ 14	♀ 3	♀ 6	♀ 3
	♂ 3	♂ 3	♂ 7	♂ 4	♂ 4	♂ 3	♂ 3	♂ 3

**Supplementary Table 2.** List of the major histamine producing bacteria (HPB) species previously identified in various fish species and seafood products.

Bacterial species	Fish species and seafood products	References
<i>Acinetobacter baumannii</i>	Swordfish ( <i>Xiphias gladius</i> )	[84]
<i>Aeromonas hydrophila</i>	Jack mackerel ( <i>Trachurus symmetricus</i> )	[85]
<i>Bacillus subtilis</i>	Mahi-mahi ( <i>Coryphaena hippurus</i> )	[86]
<i>Citrobacter freundii</i>	Tuna fillets	[87]
<i>Citrobacter koseri</i>	Yellowfin fillet ( <i>Thunnus albacares</i> )	[88]
<i>Clostridium perfringens</i>	Longtail tuna ( <i>Thunnus tonggoh</i> )	[89]
	Skipjack tuna ( <i>Katsuwonus pelamis</i> )	[90,91]
<i>Enterobacter aerogenes</i>	Mahi-mahi ( <i>Coryphaena hippurus</i> ), Striped marlin ( <i>Tetrapturus audax</i> ), Sailfish ( <i>Istiophorus platypterus</i> ), Milkfish ( <i>Chanos chanos</i> ), Yellowfin tuna ( <i>Thunnus albacares</i> ), Indian anchovy ( <i>Stolephorus indicus</i> )	[86][92] [93][94] [95]
<i>Enterobacter cloacae</i>	Mahi-mahi ( <i>Coryphaena hippurus</i> ) Yellowfin tuna ( <i>Thunnus albacares</i> )	[96]
<i>Hafnia alvei</i>	Tuna fillets, Skipjack tuna ( <i>Katsuwonus pelamis</i> )	[87] [97]
<i>Klebsiella oxytoca</i>	Tuna fillets, Yellowfin tuna ( <i>Thunnus albacares</i> ) Swordfish ( <i>Xiphias gladius</i> )	[87] [94][84]
<i>Klebsiella pneumoniae</i>	Tuna fillets	[87]
	Skipjack tuna ( <i>Katsuwonus pelamis</i> )	[98,99]
	Salted semi-preserved anchovies ( <i>Engraulis encrasicolus</i> var. <i>mediterraneus</i> )	[100] [101][94]
<i>Morganella morganii</i>	Yellowfin tuna ( <i>Thunnus albacares</i> )	[102]
	Bigeye Tuna ( <i>Thunnus obesus</i> )	[95]
	Indian anchovy ( <i>Stolephorus indicus</i> )	[103]
	Mahi-mahi ( <i>Coryphaena hippurus</i> )	[104]
	Sardine ( <i>Sardina pilchardus</i> )	
<i>Morganella psychrotolerans</i>	Yellowfin tuna ( <i>Thunnus albacares</i> )	[101]
	Cold-smoked tuna	[105]
	Garfish ( <i>Belone belone</i> )	[106]
<i>Photobacterium angustum</i>	Yellowfin tuna ( <i>Thunnus albacares</i> ), Skipjack tuna ( <i>Katsuwonus pelamis</i> ), Albacore tuna ( <i>Thunnus alalunga</i> )	[107]
<i>Photobacterium aquimaris</i>	Horse mackerel ( <i>Trachurus trachurus</i> ) Herring ( <i>Clupea harengus</i> )	[107][108]
<i>Photobacterium damsela</i>	Jack mackerel ( <i>Trachurus symmetricus</i> ) Mullet ( <i>Mugil cephalus</i> )	[109] [107]
<i>Photobacterium kishitanii</i>	Yellowfin tuna ( <i>Thunnus albacares</i> ) Skipjack tuna ( <i>Katsuwonus pelamis</i> ) Albacore tuna ( <i>Thunnus alalunga</i> )	[107]
<i>Photobacterium leognathi</i>	Oil sardine and mackerel	[110]
	Garfish fillets ( <i>Belone belone belone</i> )	[106]
<i>Photobacterium phosphoreum</i>	Yellowfin tuna ( <i>Thunnus albacares</i> ) Mackerel ( <i>Scomber</i> or <i>Trachurus</i> spp.) Bigeye Tuna ( <i>Thunnus obesus</i> ) Salmon ( <i>Salmo salar</i> )	[101] [111] [102] [112]
<i>Proteus mirabilis</i>	Sardine ( <i>Sardina pilchardus</i> )	[104]
	Mahi-mahi ( <i>Coryphaena hippurus</i> )	[103]
<i>Proteus penneri</i>	Swordfish ( <i>Xiphias gladius</i> )	[84]
	Jack mackerel ( <i>Trachurus symmetricus</i> )	[85]
<i>Proteus vulgaris</i>	Indian anchovy ( <i>Stolephorus indicus</i> ) Yellowfin tuna ( <i>Thunnus albacares</i> )	[95] [94]
<i>Pseudomonas fluorescens</i>	Salted semi-preserved anchovies ( <i>Engraulis encrasicolus</i> var. <i>mediterraneus</i> ), Tuna fillets	[100] [113]
<i>Rahnella agnatilis</i>	Swordfish ( <i>Xiphias gladius</i> )	[84]
<i>Raoultella ornithinolytica</i>	Tuna, Bonito, Sardine	[114]
<i>Raoultella planticola</i>	Tuna, Bonito, Sardine	[114]
<i>Serratia fonticola</i>	Yellowfin tuna ( <i>Thunnus albacares</i> ), Tuna fillets	[94] [87]
<i>Serratia marcescens</i>	Tuna fillets	[87]
<i>Staphylococcus kloosii</i>	Mahi-mahi ( <i>Coryphaena hippurus</i> ) and Yellowfin tuna ( <i>Thunnus albacares</i> )	[96]
<i>Staphylococcus xylosus</i>	Salted semi-preserved anchovies ( <i>Engraulis encrasicolus</i> var. <i>mediterraneus</i> )	[100]
<i>Vibrio harveyi</i>	Oil sardine and mackerel	[110]

