



HAL
open science

Tracking spoilage bacteria in the tuna microbiome

Elsa Gadoin, Christelle Desnues, Thierry Bouvier, Emmanuelle Roque d'Orbcastel, Jean-Christophe Auguet, Sandrine Crochemore, Antoinette Adingra, Yvan Bettarel

► **To cite this version:**

Elsa Gadoin, Christelle Desnues, Thierry Bouvier, Emmanuelle Roque d'Orbcastel, Jean-Christophe Auguet, et al.. Tracking spoilage bacteria in the tuna microbiome. *FEMS Microbiology Ecology*, 2022, 98 (10), pp.fiac110. 10.1093/femsec/fiac110 . hal-03860695

HAL Id: hal-03860695

<https://hal.umontpellier.fr/hal-03860695>

Submitted on 5 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL
open science

Tracking spoilage bacteria in the tuna necrobiome

Elsa Gadoin, Christelle Desnues, Thierry Bouvier, Emmanuelle Roque d'Orbcastel, Jean- Christophe Auguet, Sandrine Crochemore, Adingra Antoinette, Yvan Bettarel

► **To cite this version:**

Elsa Gadoin, Christelle Desnues, Thierry Bouvier, Emmanuelle Roque d'Orbcastel, Jean- Christophe Auguet, et al.. Tracking spoilage bacteria in the tuna necrobiome. FEMS Microbiology Ecology, 2022. hal-03869406

HAL Id: hal-03869406

<https://hal.archives-ouvertes.fr/hal-03869406>

Submitted on 24 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Tracking spoilage bacteria in the tuna necrobiome

Elsa Gadoin¹, Christelle Desnues², Thierry Bouvier¹, Emmanuelle Roque d'Orbcastel¹, Jean-Christophe Auguet¹, Sandrine Crochemore¹, Adingra Antoinette³, Yvan Bettarel^{1*}

¹MARBEC, Université de Montpellier, CNRS, Ifremer, IRD, Montpellier, France

²Institut Méditerranéen d'Océanologie (MIO), Aix-Marseille Université, Université de Toulon, CNRS, IRD, Marseille, France

³Centre de Recherche Océanologique, Abidjan, Côte d'Ivoire

Abstract

Like other seafood products, tuna is highly perishable and sensitive to microbial spoilage. Its consumption, whether fresh or canned, can lead to severe food poisoning due to the presence of histamine-producing bacteria and other specific spoilage organisms (SSOs) found in the tuna microbiome. Such bacteria generally develop in dead fish after their capture if conservation conditions are deficient. However, many grey areas persist regarding their ecology, their conditions of emergence and proliferation, and their distribution within different organs. In this study, we used 16S rDNA barcoding to investigate post-mortem changes in the tuna necrobiome until the advanced stages of decomposition (i.e. 120 h). The analyses were performed on fresh and brine-frozen yellowfin tuna (*Thunnus albacares*) captured in the tropical Atlantic Ocean. The results revealed that despite standard refrigeration storage conditions (i.e. 4°C), a diverse and complex spoilage bacteriome continued to develop in the gut and liver. In general, the relative abundance of SSOs increased rapidly in both organs, representing 82% of the bacterial communities in fresh yellowfin tuna, and less than 30% in brine-frozen ones. Interestingly, *Photobacterium* was identified as a major bacterial genus, and its temporal dynamics were positively correlated with histamine concentrations, which ultimately, in fresh tunas, exceeded the recommended sanitary level of 50 ppm established by the United States Food and Drug Administration. Finally, the study of the tuna necrobiome shows that the sanitary risks associated with the consumption of this widely eaten fish is strongly influenced by the post-capture storage conditions.

Keywords: necrobiome, tuna, histamine, *Photobacterium*, microbiome, spoilage microorganisms

*corresponding author

1 Introduction

2 Like other living organisms, fish live in close association with a diverse assemblage of
3 microorganisms, including bacteria, viruses, archaea and microeukaryotes, which constitute
4 their microbiome. Increasing attention has been paid to the fish microbiome in recent years,
5 and we now know that it ensures a number of essential functions for the health and fitness
6 of the host (Egerton et al. 2018; Sehnal et al. 2021). It has also been shown to be highly
7 heterogeneous in the body, with specific microbial signatures in different fish organs,
8 including the gut, gills, skin, liver, etc. (Aprill 2017; Egerton et al. 2018; Ross et al. 2019;
9 Gadoin et al. 2021). Numerous studies have reported that the composition of the fish
10 microbiome depends on various factors such as species (Chiarello et al. 2015, 2018; Givens
11 et al. 2015; Larsen et al. 2013), stage of development (Hansen & Olafsen 1999), sex
12 (Dhanasiri et al. 2011), diet (Cordero et al. 2015; Parata et al. 2019), geographical location
13 (Chiarello et al. 2019; Xavier et al. 2020) or captive state (Dhanasiri et al. 2011; Parata et al.
14 2019). However, little is known about the evolution of this microbiome in the different
15 organs after the death of the fish, which nevertheless partly conditions its sanitary quality
16 for consumption. After a fish dies, numerous physical and chemical alterations take place in
17 the body (i.e. decrease in pH, cellular lysis), inducing taxonomic and functional shifts in the
18 bacterial community initially present in the organism (Boziaris & Parlapani 2017; Duarte et
19 al. 2020; Gram & Huss 1996). The microbial assemblage that grows in a dead fish and leads
20 to its decomposition is known as the necrobiome, from the Greek word *nekrós* for 'death'.

21 In the last three decades, numerous studies have analysed the diversity and activity of
22 spoilage microorganisms in many seafood products, mainly using a culture-based approach
23 (reviewed in Boziaris & Parlapani 2017; Gram & Huss 1996; Gram & Dalgaard 2002). These
24 microorganisms, referred to as specific spoilage organisms (SSOs), typically belong to the
25 bacterial genera *Aeromonas*, *Vibrio*, *Photobacterium*, *Shewanella* or *Enterobacteriaceae*, to
26 cite a few, and they are commonly found in the flesh of fish and seafood products (Boziaris
27 & Parlapani 2017; Gram & Dalgaard 2002). In general, most SSOs are known to produce
28 specific metabolites (trimethylamine oxide, ammonia, biogenic amines, organic acids,
29 acetate and sulphur) leading to the organoleptic rejection of the seafood product during
30 quality control checks (Boziaris & Parlapani 2017; Gram & Dalgaard 2002). Among SSOs,
31 several species such as *Shewanella* spp., *Vibrio* spp., *Salmonella* and *Listeria monocytogenes*

1 are also human pathogens (Parlapani 2021). The levels of these SSOs in the host organism
2 are mainly dependent on storage conditions and the previous fish evisceration (Huss 1995).
3 Chilling, freezing and vacuum storage can reduce the production of degrading metabolites
4 by SSOs and thus increase the shelf-life of seafood products (Dawson, Al-Jeddawi &
5 Remington 2018; Ghaly 2010; Sivertsvik, Jeksrud & Rosnes 2002). The diversity of SSOs also
6 varies according to the fish species (Parlapani et al. 2013; Parlapani et al. 2018; Reynisson et
7 al. 2010), the geographical location (Parlapani et al., 2018), and the composition of the initial
8 microbiome (Boziaris & Parlapani 2017; Gram & Dalgaard 2002).

9 The majority of studies on the spoilage microbiome in fish have been conducted on flesh
10 (Antunes-Rohling et al. 2019; Chytiri et al. 2004; Eliasson et al. 2019; Taliadourou et al. 2003;
11 Wang et al. 2017; Zotta et al. 2019), while the viscera such as the gut and liver have received
12 less attention. Yet the latter are recognized as important microbial reservoirs: the digestive
13 tract of fish is known to host specific bacterial taxa that play key roles in the digestion,
14 immunity and fitness of the host (Egerton et al. 2018; Ghanbari, Kneifel & Domig 2015).
15 More recently, diverse microbial communities have also been discovered in the liver of
16 several fish species, including tuna, mullet, sardinella and Randall's threadfin bream (Gadoin
17 et al. in rev.; Meron et al. 2020), showing the importance of including this organ in
18 microbiome studies on marine organisms.

19 Of the main fish species consumed worldwide, tuna show one of the highest risks of food
20 poisoning (Hungerford 2010; Tortorella et al. 2014). From a microbiological perspective,
21 tuna, like other members of the *Scombridae* family, is an interesting study model, as the
22 consumption of this species can lead to histamine poisoning (Hungerford 2010, 2021).
23 Histamine is produced by specific SSOs (Gram & Dalgaard 2002; Jørgensen et al. 2000) called
24 histamine-producing bacteria (HPB), from a precursor amino acid (histidine) present in high
25 concentrations in *Scombridae*, that HPB catalyse with the enzyme histidine decarboxylase
26 (HDC) (Prester 2011). It has been clearly established that storage temperature is a major
27 factor influencing the production of histamine by HPB (Economou et al. 2007; Guizani et al.
28 2005; Hungerford 2021; Mahusain et al. 2017; Silva et al. 1998). Yet, while these histamine-
29 producing bacteria have been identified and the production mechanisms of this biogenic
30 amine are relatively well known, the ecology and development of HPB within the post-
31 mortem microbiome of tuna remain poorly understood.

1 In this study, we chose to conduct our investigations on a particular species: the yellowfin
2 tuna (*Thunnus albacares*), which is found in tropical waters worldwide and is the second
3 most consumed tuna species in the world (FAO 2020). Our objective was to understand how
4 the necrobiome of this key species evolves after fish capture/death by examining two major
5 bacterial reservoirs: the gut and the liver. We used a metabarcoding approach depict the
6 dynamics of the whole bacterial community as well as the emergence of more specific SSOs
7 and HPBs. The results are discussed in the light of fish conditioning process by comparing the
8 development of the necrobiome in fresh and brine-frozen tuna fished by artisanal and
9 industrial techniques, respectively.

10

11 **Material and methods**

12 **Sampling**

13 The yellowfin tuna (*Thunnus albacares*) were captured using two different fishing techniques
14 and post-capture storage conditions: (1) artisanal fishing with immediate storage on ice of
15 fresh individuals, and (2) industrial fishing followed by immediate brine-freezing treatment.
16 For freshly caught yellowfin, 12 individuals were captured around fish-aggregating devices
17 (FADs) located in the Gulf of Guinea (Ivory Coast, N04°55'00", W03°42'19.97") on 20–21
18 November 2019. The capture and euthanasia of the fish were performed by professional
19 fishermen. The tuna were individually placed in plastic bags and kept on ice until they
20 reached the laboratory, less than 5 h after death. The mean fork length of the individuals
21 was 49.5 cm (min 45.7 cm – max 52.3 cm) and the average weight was 2.1 kg (min 1.7 kg –
22 max 2.6 kg).

23 For the brine-frozen yellowfin tuna, 12 individuals were collected at the Abidjan tuna port
24 (Ivory Coast) by the Exploited Tropical Pelagic Ecosystem Observatory (IRD, Ob7, certified
25 ISO 9001:2015) within the framework of multiannual European fishery data collection (DCF,
26 financed by the European Maritime and Fisheries Fund, Article 77). All individuals were
27 caught by purse seine vessels between May and December 2019 in the Eastern Atlantic
28 Ocean (Gulf of Guinea and off the coast of Senegal) and immediately chilled brine to lower
29 their temperature to around -15°C. The fish remained frozen in the tanks until their landing
30 in the Port of Abidjan and were then thawed at 4°C in the laboratory, 24 hours before the

1 beginning of the experiment (Fig. 1). The mean fork length of these individuals was 63.4 cm
2 (min 58.0 cm – max 70.0 cm) and the average weight was 4.4 kg (min 3.1 kg – max 5.9 kg).

3 **Experimental design**

4 For each fresh and brine frozen lots, three yellowfin individuals were dissected and sampled
5 at the beginning of the experiment (T_0) to analyse their liver and gut microbiota, as well as
6 the histamine concentration (see sampling procedure below) (Fig. 1). For brine-frozen tuna,
7 T_0 corresponded to 24 h after thawing at 4°C, which is considered as the standard
8 temperature for home-storage. For fresh tuna, T_0 corresponded to the time of death of the
9 fish since they were dissected directly onboard. The 12 remaining fish in each batch were
10 kept at 4°C in temperature-controlled refrigerators. Every 48 h until the end of the 120-h
11 experiment (i.e. T_{120}), three individuals from each batch were randomly selected to sample
12 their hepatic and intestinal microbiota (Fig. 1).

13

14 **Sampling the gut and liver microbiota**

15 *Gut*

16 The tuna were dissected, extracting the gastrointestinal tract after cutting from below the
17 stomach to the rectum using sterile tools. Each gut was opened, squeezed, and its inner
18 surface entirely rubbed to expel the contents (minimum volume of 5 mL) on a sterile
19 surface. The contents were homogenized before sampling (Gadoin et al. 2021).

20 *Liver*

21 A 2 x 0.2 x 2 cm (L x W x H) piece was trimmed from the right lobe of each tuna liver using
22 sterile tools. Liver samples were rinsed with distilled water filtered through a 0.2 µm filter to
23 avoid any contamination from other internal organs or fluids.

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

26

27 **Bacterial DNA extraction, amplification and sequencing**

28 The bacterial DNA was extracted from 250 ± 0.5 mg of the gut (n= 30) and liver (n= 30)
29 samples. All extractions were performed with the PowerSoil DNA Isolation Kit (Qiagen®,
30 Hilden, Germany) following the manufacturer's instructions. DNA quality and quantity were
31 assessed by spectrophotometry (NanoDrop®, Wilmington, DE, USA). The V3-V4 region of the

1 16S rDNA gene was amplified using universal bacterial primers modified for Illumina
2 sequencing: 343F (5'- ACGGRAGGCAGCAG) (Economou et al. 2007; Guizani et al. 2005;
3 Hungerford 2021; Mahusain et al. 2017) and 784R (5'- TACCAGGGTATCTAATCCT) (Andersson
4 et al. 2008). The reaction mixture consisted of 12.5 µL of 2X Phusion Mix (New England
5 Biolabs®, Ipswich, MA, USA), 1 µL of each primer at 10 µM (Eurofin®, Luxembourg), 10 ng of
6 DNA template and enough molecular-grade H₂O (Qiagen®) to reach a final volume of 25 µL.
7 All samples were amplified in triplicate to avoid PCR bias in the taxonomic diversity of the
8 community (Perreault et al. 2007). Successfully amplified samples (n= 30) were sequenced
9 on the Illumina platform using 2x250 bp MiSeq chemistry.

10

11 **Bacterial sequence processing and analysis**

12 A total of 16,277,785 reads were obtained. Raw reads were processed with RStudio (R
13 version 3.5.3) using the DADA2 package (v1.10.1) (Callahan et al. 2016) following the
14 authors' tutorial (<https://benjjneb.github.io/dada2/tutorial.html>). The quality of forward
15 and reverse reads was analysed before removing adaptors and primers based on their
16 length. Using the DADA2 tutorial with default parameters, reads were then filtered, trimmed
17 and merged into 8312 amplicon sequence variants (ASVs). Chimaeras were removed, and
18 sequences were aligned to the SILVA 123 database (Quast et al. 2012) to access their
19 taxonomy. Analyses were performed on a random subsample of 2337 sequences per
20 sample, corresponding to the sample with the smallest number of sequences after trimming
21 and quality processing. Using the *phyloseq* package (McMurdie & Holmes 2013), final
22 taxonomic and ASV tables were linked to sample metadata (including biological
23 compartment, sampling time and conservation conditions). The relative abundance of ASVs
24 in each sample were assessed by *phyloseq*, and ASVs assigned to non-prokaryotes, archaea,
25 chloroplasts and mitochondria were removed. Using the *phyloseq* and *ggplot2* packages, the
26 composition and diversity of bacterial communities were then represented at the class level,
27 based on the relative abundance of ASVs in each sample. Referring to the literature, a list of
28 putative histamine-producing bacteria (HPB) genera was established, and their
29 presence/absence in our samples was assessed by comparing the list of HPB to our
30 taxonomy table.

31

32

1 **Histamine concentration**

2 In all the gut and liver samples, histamine concentration was assessed by enzyme-linked
3 immunosorbent assays (ELISA) using the Veratox[®] kit for tuna histamine (Neogen[®], Lansing,
4 MI, USA) following the manufacturer's instructions. Samples were suspended in distilled
5 water, filtered and diluted 10X prior to the ELISA tests. Assays were performed under sterile
6 conditions and the optical density was measured at 650 nm using a TECAN Infinite M200 Pro
7 (Tecan[®], Männedorf, Switzerland). The optical densities of the six standards available in the
8 kit allowed us to trace the standard curve against which the optical density of a sample was
9 plotted to calculate its histamine concentration in parts per million (ppm).

10

11 **Statistical analysis**

12 All statistical analyses were performed with RStudio. The effect of time and post-catch
13 storage conditions on the composition of hepatic and intestinal bacterial communities was
14 determined by single-factor and multiple-factor PERMANOVA with 999 permutations on the
15 Bray–Curtis dissimilarity matrix, using the “adonis” function of the *vegan* package (Dixon
16 2003). Correlations between histamine concentration and the relative abundance of
17 potential HPB were evaluated using a Spearman correlation test performed in RStudio.

18

19 **Results**

20 ***Short-term dynamics of the tuna necrobiome***

21 The results revealed that the composition of the tuna necrobiome changed significantly over
22 time in both the gut and liver (Tab. 1).

23 *The gut microbiome*

24 In fresh tuna, the initial enteric microbiome was comprised of numerous taxa that included
25 the genera *Cutibacterium*, *Enhydrobacter*, *BD1-7 clade* and *Neorickettsia*, as well as several
26 potential SSO genera such as *Photobacterium*, *Shewanella*, *Pseudomonas*, *Novosphingobium*
27 and *Vibrio*. Over the 120-h period, the abundance of *Photobacterium* then rapidly increased
28 to reach almost 90% of the total abundance of bacteria, while most of the other genera
29 decreased (Fig. 2).

30 In brine-frozen yellowfin, significant changes in the composition of the gut necrobiome were
31 also observed during the experiment (Fig. 2). In addition, in these fish, the presence of

1 several potential SSOs that were not found in fresh tuna were identified (*Lactococcus*,
2 *Lactobacillus*, *Psychrobacter*, *Psychrilyobacter* and *Proteus*). The occurrence of certain SSOs
3 such as *Psychrobacter*, *Lactococcus* and *Shewanella* increased throughout the experiment.
4 At T₉₆, *Photobacterium*, *Lactobacillus*, *BD1-7 clade* and *Mycoplasma* were the most
5 abundant bacterial genera, but taxa with a relative abundance of less than 2% represented
6 more than 25% of the community. Their proportion increased at T₁₂₀, when the potential
7 SSO genera *Shewanella*, *Psychrobacter*, *Proteus*, *Pseudomonas*, *Photobacterium*,
8 *Lactobacillus* and *Psychrilyobacter* were detected and together represented 22.6% of the
9 bacterial community.

10

11 *The liver microbiome*

12 The composition of the bacterial community in the liver was highly diverse and was
13 significantly different from that of the intestine (Fig. 2, Permanova, $p = 0.003$). At T₀, the
14 microbiome in fresh tuna was mostly comprised of *Enhydrobacter*, *Micrococcus*,
15 *Neorickettsia* and *Massilia*. In contrast with gut samples, the liver of fresh yellowfin initially
16 hosted few SSOs, but these proliferated rapidly over time. The only SSO genus observed in
17 liver samples at the beginning of the experiment was *Pseudomonas*, but at T₄₈ the relative
18 abundance of other SSO genera such as *Photobacterium*, *Shewanella*, *Psychrobacter* and
19 *Vibrio* began to increase. By T₉₆, SSO genera were dominant within the liver necrobiome,
20 representing 76% of the bacterial community. They remained the major component of the
21 liver microbiota until the end of the experiment, when other genera such as *Salegentibacter*,
22 *Sporosarcina*, *Enhydrobacter* and *Cutibacterium* were also detected.

23 The storage conditions greatly impacted the composition of the necrobiome in this organ.
24 The liver-associated bacteria in brine-frozen tuna evolved in a different way than in fresh
25 tuna (Tab. 1, Fig. 2). For example, the genus *Photobacterium*, which was highly dominant in
26 the liver microbiome of fresh fish, was much less abundant in brine-frozen fish. Generally,
27 although the relative abundance of SSO genera increased over time, their occurrence
28 remained lower in brine-frozen than in fresh samples. At the beginning of the experiment,
29 hepatic bacterial communities were composed of *Enhydrobacter*, *Cutibacterium*,
30 *Brachybacterium*, *Macrococcus*, *Halomonas*, *Acinetobacter* and *Methylobacterium*, as well as
31 two main SSO genera (*Photobacterium* and *Pseudomonas*), and potential pathogens such as

1 *Staphylococcus* and *Corynebacterium*. At the end of the experiment (T₁₂₀), the liver
2 microbiome hosted several other potential SSO genera including *Proteus*, *Psychrobacter*,
3 *Photobacterium*, *Shewanella* and *Psychrilyobacter*, which together represented 29% of the
4 bacterial community.

5

6 ***Diversity of histamine-producing bacteria and histamine concentrations***

7 In general, the relative abundance of HPB was much higher in fresh than in brine-frozen
8 tuna. *Photobacterium* ASVs were generally dominant in gut samples, while other HPB genera
9 (*Pseudomonas* and *Acinetobacter*) were also present in the liver in equivalent proportions
10 (Fig. 3). Interestingly, the genus *Proteus* was only detected at the late stage of fish
11 decomposition (T₁₂₀) and exclusively in brine-frozen samples.

12 In fresh yellowfin, the temporal dynamics of *Photobacterium* ASVs were significantly
13 correlated with histamine concentration in both gut and liver samples (Pearson, $p < 0.05$).
14 Other potential HPB genera such as *Pseudomonas*, *Vibrio*, *Acinetobacter* and *Enterobacter*
15 were also detected, but at low levels (Fig. 3).

16 Fresh and brine-frozen tuna exhibited contrasting patterns of histamine concentration. In
17 fresh fish, histamine concentration increased abruptly after T₄₈ to reach a maximum at T₉₆ in
18 the gut (mean = 676 ppm) and at T₁₂₀ in the liver (mean = 59 ppm), thus exceeding the 50
19 ppm sanitary threshold established by the United States Food and Drug Administration (FDA
20 2021)(Fig. 3). Conversely, in brine-frozen fish, histamine concentrations remained below that
21 threshold throughout the experiment.

22

23 **Discussion**

24 Modifications in animal's microbiome composition are normal phenomena following their
25 death, resulting from physical and chemical changes, as well as the loss of immune response
26 (Benbow, Receveur & Lamberti 2020). In fish, however, the evolution of the post-mortem
27 bacteriome over time has been poorly studied. In this study, we explored the tuna
28 necrobiome by examining the dynamics of the main SSOs together with the production of
29 histamine in two major bacterial reservoirs: the gut and the liver. We compared the
30 incidence of post-capture storage conditions (fresh and brine-frozen individuals) on the
31 development of these spoilage bacteria.

1 **Occurrence and diversity of SSOs**

2 As expected, the proportion of potential specific spoilage organisms (SSOs) increased
3 significantly throughout the experiment in the gut and the liver (Fig. 2). This trend was
4 particularly marked in fresh tunas. In both organs, we observed an increase in abundance of
5 *Psychrobacter*, *Pseudomonas*, *Proteus*, *Aeromonas*, *Lactobacillus*, *Shewanella* and
6 *Photobacterium*, which have all been previously detected in the flesh of various fish species
7 such as haddock, Atlantic salmon, gilthead sea bream, European sea bass and yellowfin tuna
8 (Dalgaard et al. 2006; Fogarty et al. 2019; Jääskeläinen et al. 2019; Parlapani et al. 2018;
9 Reynisson et al. 2010; Syropoulou et al. 2020) (Fig. 2). The development of SSOs in fish and
10 seafood products is well documented, and their proliferation typically depends on
11 conservation conditions (Boziaris & Parlapani 2017). Several studies have demonstrated that
12 SSO diversity in the flesh of different fish species varies between chilling, vacuum packaging
13 or thawing temperature (Antunes-Rohling et al. 2019; Odeyemi et al. 2018; Reynisson et al.
14 2010; Syropoulou et al. 2021). Bacteria from the *Shewanella*, *Photobacterium* and
15 *Pseudomonas* genera are known for their ability to produce high quantities of H₂S,
16 trimethylamine and volatile nitrogenous compounds respectively (Boziaris & Parlapani 2017;
17 Carrascosa et al. 2014; Chinivasagam et al. 1998), while species such as *S. putrefaciens*,
18 *Proteus mirabilis* and *P. damsela* are bacteria potentially pathogenic to humans (Gennari,
19 Tomaselli & Cotrona 1999; Ozogul et al. 2020; Speranza et al. 2013).

20 It should be noted that the spoilage activity of SSOs is a relatively complex mechanism that
21 has multiple determinants. The production of spoiling metabolites is clearly species-
22 dependent and varies according to the storage conditions, such as temperature (Antunes-
23 Rohling et al. 2019; Parlapani & Boziaris 2016), atmosphere conditions (Emborg, Laursen &
24 Dalgaard 2005; Silbande et al. 2016; Sivertsvik et al. 2002), as well as microbial interactions
25 between communities (Joffraud et al. 2006; Zotta et al. 2019). Various analyses such as the
26 detection of spoilage genes, the growth of SSOs on selective media and the quantification of
27 spoilage metabolites are usually required to assess the spoilage potential of SSOs (Fu et al.
28 2018; Syropoulou et al. 2020; Tang et al. 2019). Although these analyses were not performed
29 in this study, the taxonomic identification of SSO genera in the two digestive organs raises
30 questions about their dispersion from the viscera to the flesh after fish death (Shen & Wang
31 2020).

1

2 ***The effect of storage conditions on the tuna necrobiome***

3 One of the main findings of this study was that the relative abundance and dynamics of SSOs
4 greatly varied according to the initial storage conditions (Fig. 2). At the end of the
5 experiment, they represented on average (for the two organs) 82% of the bacterial
6 community in fresh tuna, in contrast to less than 30% in brine-frozen samples (Fig. 2). The
7 influence of storage conditions on the composition of the fish microbiome has long been
8 investigated (Ghaly 2010; Zhuang et al. 2021). For example, a delayed development of SSOs
9 was reported in frozen fillets of Atlantic cod, mackerel and salmon compared to fresh
10 samples (Fagan, Ronan Gormley & Mhuircheartaigh 2003; Sørensen et al. 2020). While low-
11 temperature chilling is known to decrease the growth of microorganisms, freezing between -
12 18 and -30°C kills between 10% and 60% of viable bacteria (Berkel, Boogaard & Heijnen
13 2004; Rahman 1999). In addition, the presence of sodium chloride is also known to
14 inactivate autolytic enzymes in fish, as well as to negatively impact the growth of several
15 spoilage bacteria (Ghaly 2010; Henney et al. 2010; Mejlholm, Devitt & Dalgaard 2012; Turan
16 & Erkoyuncu 2012). This may partially explain why brine-frozen yellowfin exhibited a limited
17 abundance of SSOs in the gut and liver microbiota compared to fresh tuna.

18

19 ***SSOs and histamine production in tuna***

20 Among the potential SSO genera detected in the tuna necrobiome, histamine-producing
21 bacteria (HPB) are of particular interest, as they have been implicated in cases of food
22 poisoning worldwide (Hungerford 2010, 2021). We identified several HPB genera in the gut
23 and liver samples, including *Acinetobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Pseudomonas*
24 and *Vibrio*, but *Photobacterium* was the most abundant, especially in fresh fish, where it
25 rapidly dominated the bacterial community in both organs (Fig. 3). The genus
26 *Photobacterium* is ubiquitous in marine environments and is composed of several species
27 (Thyssen & Ollevier 2005). It has been described as commensal in various fish species
28 (Egerton et al. 2018; Estruch et al. 2015; Givens et al. 2015), but some *Photobacterium*
29 species such as *P. damsela* and *P. piscicida* are known as fish and human pathogens (Rivas,
30 Lemos & Osorio 2013; Romalde 2002). *Photobacterium* has also been identified as an SSO in
31 Atlantic cod (Kuuliala et al. 2018), haddock (Reynisson et al. 2010) and Atlantic salmon
32 (Jääskeläinen et al. 2019). Indeed, several *Photobacterium* species are able to synthesize

1 histamine, including *P. angustum*, *P. aquimaris*, *P. kishitanii*, *P. damselae* and *P.*
2 *phosphoreum*, which are designated as high histamine producers (> 200ppm) (Bjornsdottir-
3 Butler et al. 2018). While histamine-production capacity has been demonstrated to vary
4 across different *Photobacterium* species, this capacity is also influenced by temperature
5 (Bjornsdottir-Butler et al. 2018; Morii & Kasama 2004; Takahashi et al. 2015). Insufficiently
6 cold temperatures are known to favour the production of histamine and maintaining the
7 cold chain is essential to prevent its formation (Hungerford 2010, 2021). Some
8 psychrotrophic HPB, such as *P. phosphoreum* and *Morganella psychrotolerans*, are able to
9 synthesize histamine at temperatures between 0° and 5°C (Bjornsdottir-Butler et al. 2018;
10 Emborg et al. 2005; Kanki et al. 2004; Wang et al. 2020). Although our data did not allow us
11 to identify the potential HPB down to the species level, we can consider that the
12 *Photobacterium* taxa observed in both the gut and liver of fresh yellowfin tuna were HPB, as
13 their temporal dynamics were positively correlated with the increase in histamine
14 concentration in the different incubations (Fig. 3). As early as 96h after their capture,
15 histamine concentration in the gut and liver of fresh yellowfin exceeded the United States
16 Food and Drug Administration (FDA 2021) recommendations of 50 ppm.

17 The vast majority of studies investigating Scombroid (histamine) poisoning have been
18 conducted on tuna flesh or in processed products such as filets or canned tuna (Emborg et
19 al. 2005; Guizani et al. 2005; Kim et al. 2002; Kung et al. 2009; Silva et al. 2011). Our study
20 extends this by revealing the presence of histamine and HPB in both gut and liver samples.
21 These organs have been previously identified as important reservoirs of HPB in tuna
22 (Bjornsdottir-Butler et al. 2015; Taylor & Speckhard 1983; Gadoin et al. 2021), but few
23 studies have considered the liver and gut in their investigations on histamine formation in
24 scombroid fish. Glória et al. (1999) observed that the intestinal wall of yellowfin tuna
25 contained a substantial concentration of histamine. Similarly, Fernández-Salguero & Mackie
26 (1979) reported significant histamine concentration in the liver of mackerel, in an even
27 greater proportion than in muscles.

28 Another key finding was that histamine was not detected in the gut or liver samples of brine-
29 frozen tuna, despite the presence of potential HPB genera (Fig. 3). This suggests that the
30 brine-freezing treatment may alter the capacity of HPB to produce histamine in these two
31 organs. Freezing has been previously observed to limit the production of this biogenic amine

1 in tuna fillets (Tahmouzi et al. 2013). In addition, brine immersion is known to inhibit the
2 activity of the histidine decarboxylase enzyme in HPB, and therefore to limit the synthesis of
3 histamine from its precursor histidine (Hwang et al. 2020; Morii & Kasama 2004; Tabanelli et
4 al. 2012). Overall, in line with previous studies, our results confirm the usefulness of applying
5 a brine-freezing treatment to tuna to prevent the formation of histamine, and thus reduce
6 the health risk associated with their consumption (Hungerford 2021).

7

8 **Conclusion**

9 Our results highlight the sanitary risks associated with the development of SSOs and of
10 histamine concentrations in tuna's digestive organs, reminding the importance of removing
11 their viscera prior to consumption. Such sanitary risks were much more elevated with fresh
12 than brine-frozen fishes, confirming the incidence of the storage conditions on the evolution
13 of the tuna necrobiome. Finally, this study confirms the need to take into account the gut
14 and liver in further investigations on the ecology of HPB in scombroid fish.

15

16 **Acknowledgements**

17 We thank the Montpellier University of Excellence (I-site MUSE; Project The MOME) and the
18 Insitute of Research for Development (JEAI; Project MOSANE) for their financial support. he
19 data used here was collected within the framework of the Data Collection Framework
20 program co-financed by the IRD and Measure 77 of the European Maritime and Fisheries
21 Fund (EMFF). We are grateful to Justin Aurélie Guillou for their assistance during sampling.
22 We would like to thank the members of the IRD's Exploited Tropical Pelagic Ecosystems
23 Observatory in Abidjan (ie, Aurélie, Guillou, Pascal Bach) for providing the brine frozen
24 tunas. We also grateful to the CRO staff for providing access to their laboratory and
25 facilities.

26

27

28

29

30

31

1 REFERENCES

- 2 Andersson, A. F., Lindberg, M., Jakobsson, H., Bäckhed, F., Nyrén, P., & Engstrand, L. (2008).
3 Comparative analysis of korean human gut microbiota by barcoded pyrosequencing. *PLoS ONE*,
4 3(7), e2836.
- 5 Antunes-Rohling, A., Calero, S., Halaihel, N., Marquina, P., Raso, J., Calanche, J., Beltrán, J. A., Álvarez,
6 I., & Cebrián, G. (2019). Characterization of the Spoilage Microbiota of Hake Fillets Packaged
7 Under a Modified Atmosphere (MAP) Rich in CO₂ (50% CO₂/50% N₂) and Stored at Different
8 Temperatures. *Foods*, 8(10), 489.
- 9 Apprill, A. (2017). Marine Animal Microbiomes: Toward Understanding Host–Microbiome
10 Interactions in a Changing Ocean. *Frontiers in Marine Science*, 4, 222.
11 <https://doi.org/10.3389/fmars.2017.00222>
- 12 Benbow, M. E., Receveur, J. P., & Lamberti, G. A. (2020). Death and Decomposition in Aquatic
13 Ecosystems. *Frontiers in Ecology and Evolution*, 8, 17.
- 14 Berkel, B. M., Boogaard, B. V., & Heijnen, C. (2004). *Preservation of Fish and Meat* (p. 78-80).
- 15 Bjornsdottir-Butler, K., Abraham, A., Harper, A., Dunlap, P. V., & Benner, R. A. (2018). Biogenic Amine
16 Production by and Phylogenetic Analysis of 23 Photobacterium Species. *Journal of Food*
17 *Protection*, 81(8), 1264-1274.
- 18 Boziaris, I. S., & Parlapani, F. F. (2017). Specific Spoilage Organisms (SSOs) in Fish. In *The*
19 *Microbiological Quality of Food* (p. 61-98). Elsevier.
- 20 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016).
21 DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods*, 13(7),
22 581.
- 23 Carrascosa, C., Millán, R., Saavedra, P., Jaber, J. R., Montenegro, T., Raposo, A., Pérez, E., & Sanjuán,
24 E. (2014). Predictive models for bacterial growth in sea bass (*Dicentrarchus labrax*) stored in ice.
25 *International Journal of Food Science & Technology*, 49(2), 354-363.
- 26 Chiarello, M., Auguet, J.-C., Bettarel, Y., Bouvier, C., Claverie, T., Graham, N. A. J., Rieuvilleneuve, F.,
27 Sucre, E., Bouvier, T., & Villéger, S. (2018). Skin microbiome of coral reef fish is highly variable
28 and driven by host phylogeny and diet. *Microbiome*, 6(1), 147.
- 29 Chiarello, M., Paz-Vinas, I., Veyssiére, C., Santoul, F., Loot, G., Ferriol, J., & Boulêtreau, S. (2019).
30 Environmental conditions and neutral processes shape the skin microbiome of European catfish
31 (*Silurus glanis*) populations of Southwestern France. *Environmental Microbiology Reports*,
32 11(4), 605-614.
- 33 Chiarello, M., Villéger, S., Bouvier, C., Bettarel, Y., & Bouvier, T. (2015). High diversity of skin-
34 associated bacterial communities of marine fishes is promoted by their high variability among
35 body parts, individuals and species. *FEMS Microbiology Ecology*, 91(7), fiv061.
- 36 Chinivasagam, H. N., Bremner, H. A., Wood, A. F., & Nottingham, S. M. (1998). Volatile components
37 associated with bacterial spoilage of tropical prawns. *International Journal of Food Microbiology*,
38 42), 45-55.
- 39 Chytiri, S., Chouliara, I., Savvaadis, I. N., & Kontominas, M. G. (2004). Microbiological, chemical and
40 sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology*,
41 21(2), 157-165.
- 42 Cordero, H., Guardiola, F. A., Tapia-Paniagua, S. T., Cuesta, A., Meseguer, J., Balebona, M. C.,
43 Moriñigo, M. Á., & Esteban, M. Á. (2015). Modulation of immunity and gut microbiota after
44 dietary administration of alginate encapsulated *Shewanella putrefaciens* Pdp11 to gilthead
45 seabream (*Sparus aurata* L.). *Fish & shellfish immunology*, 45(2), 608-618.
- 46 Dalgaard, P., Madsen, H. L., Samieian, N., & Emborg, J. (2006). Biogenic amine formation and
47 microbial spoilage in chilled garfish (*Belone belone belone*)—Effect of modified atmosphere
48 packaging and previous frozen storage. *Journal of Applied Microbiology*, 101(1), 80-95.
- 49 Dawson, P., Al-Jeddawi, W., & Remington, N. (2018). Effect of Freezing on the Shelf Life of Salmon.
50 *International Journal of Food Science*, 2018, 1-12.
- 51 Dhanasiri, A. K. S., Brunvold, L., Brinchmann, M. F., Korsnes, K., Bergh, Ø., & Kiron, V. (2011). Changes

- 1 in the Intestinal Microbiota of Wild Atlantic cod *Gadus morhua* L. Upon Captive Rearing.
2 *Microbial Ecology*, 61(1), 20-30.
- 3 Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation*
4 *Science*, 14(6), 927-930.
- 5 Duarte, A. M., Silva, F., Pinto, F. R., Barroso, S., & Gil, M. M. (2020). Quality Assessment of Chilled and
6 Frozen Fish—Mini Review. *Foods*, 9(12), 1739.
- 7 Economou, V., Brett, M. M., Papadopoulou, C., Frillingos, S., & Nichols, T. (2007). Changes in
8 histamine and microbiological analyses in fresh and frozen tuna muscle during temperature
9 abuse. *Food Additives and Contaminants*, 24(8), 820-832.
- 10 Egerton, S., Culloty, S., Whooley, J., Stanton, C., & Ross, R. P. (2018). The Gut Microbiota of Marine
11 Fish. *Frontiers in Microbiology*, 9, 873.
- 12 Eliasson, S., Arason, S., Margeirsson, B., Bergsson, A. B., & Palsson, O. P. (2019). The effects of
13 superchilling on shelf-life and quality indicators of whole Atlantic cod and fillets. *LWT*, 100, 426-
14 434.
- 15 Emborg, J., Laursen, B. G., & Dalgaard, P. (2005). Significant histamine formation in tuna (*Thunnus*
16 *albacares*) at 2 °C—effect of vacuum- and modified atmosphere-packaging on psychrotolerant
17 bacteria. *International Journal of Food Microbiology*, 101(3), 263-279.
- 18 Estruch, G., Collado, M. C., Peñaranda, D. S., Tomás Vidal, A., Jover Cerdá, M., Pérez Martínez, G., &
19 Martínez-Llorens, S. (2015). Impact of Fishmeal Replacement in Diets for Gilthead Sea Bream
20 (*Sparus aurata*) on the Gastrointestinal Microbiota Determined by Pyrosequencing the 16S rRNA
21 Gene. *PLOS ONE*, 10(8), e0136389.
- 22 Fagan, J. D., Ronan Gormley, T., & Mhuirheartaigh, M. U. (2003). Effect of freeze-chilling, in
23 comparison with fresh, chilling and freezing, on some quality parameters of raw whiting,
24 mackerel and salmon portions. *LWT - Food Science and Technology*, 36(7), 647-655.
- 25 FAO, *The State of World Fisheries and Aquaculture 2020*. (2020).
- 26 FDA. (2021). *Appendix 5 : FDA and EPA safety levels in regulations and guidance*.
- 27 Fernández-Salguero, J., & Mackie, I. M. (1979). Histidine metabolism in mackerel (*Scomber*
28 *scombrus*). Studies on histidine decarboxylase activity and histamine formation during storage of
29 flesh and liver under sterile and non-sterile conditions. *Journal of Food Science & Technology*,
30 14(2), 131-139.
- 31 Fogarty, C., Whyte, P., Brunton, N., Lyng, J., Smyth, C., Fagan, J., & Bolton, D. (2019). Spoilage
32 indicator bacteria in farmed Atlantic salmon (*Salmo salar*) stored on ice for 10 days. *Food*
33 *Microbiology*, 77, 38-42.
- 34 Fu, L., Wang, C., Liu, N., Ma, A., & Wang, Y. (2018). Quorum sensing system-regulated genes affect
35 the spoilage potential of *Shewanella baltica*. *Food Research International*, 107, 1-9.
- 36 Gadoin, E., Durand, L., Guillou, A., Crochemore, S., Bouvier, T., Roque, E. R., Dagorn, L., Auguet, J.-C.,
37 Adingra, A., Desnues, C., & Bettarel, Y. (2021). Does the Composition of the Gut Bacteriome
38 Change during the Growth of Tuna? *Microorganisms*, 9(6), 1157.
- 39 Gadoin, E., Desnues, C., d'Orbcastel, E. R., Bouvier, T., Auguet, J.-C., Dagorn, L., Moroh, J.-L., Adingra,
40 A., & Bettarel, Y. (2021). *Fishing for the Bacteriome of Tropical Tuna* [Preprint]. In Review.
41 <https://doi.org/10.21203/rs.3.rs-586887/v1>
- 42 Gennari, M., Tomaselli, S., & Cotrona, V. (1999). The microflora of fresh and spoiled sardines (*Sardina*
43 *pilchardus*) caught in Adriatic (Mediterranean) Sea and stored in ice. *Food microbiology*, 16(1),
44 15-28.
- 45 Ghaly. (2010). Fish Spoilage Mechanisms and Preservation Techniques : Review. *American Journal of*
46 *Applied Sciences*, 7(7), 859-877.
- 47 Ghanbari, M., Kneifel, W., & Domig, K. J. (2015). A new view of the fish gut microbiome : Advances
48 from next-generation sequencing. *Aquaculture*, 448, 464-475.
- 49 Givens, C., Ransom, B., Bano, N., & Hollibaugh, J. (2015). Comparison of the gut microbiomes of 12
50 bony fish and 3 shark species. *Marine Ecology Progress Series*, 518, 209-223.
- 51 Glória, M. B. A., Daeschel, M. A., Craven, C., & Hilderbrand, K. S. (1999). Histamine and Other
52 Biogenic Amines in Albacore Tuna. *Journal of Aquatic Food Product Technology*, 8(4), 55-69.

- 1 Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria – problems and solutions. *Current Opinion in*
2 *Biotechnology*, 13(3), 262-266.
- 3 Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International*
4 *Journal of Food Microbiology*, 33(1), 121-137.
- 5 Guizani, N., Al-Busaidy, M. A., Al-Belushi, I. M., Mothershaw, A., & Rahman, M. S. (2005). The effect
6 of storage temperature on histamine production and the freshness of yellowfin tuna (*Thunnus*
7 *albacares*). *Food Research International*, 38(2), 215-222.
- 8 Hansen, G. H., & Olafsen, J. A. (1999). Bacterial Interactions in Early Life Stages of Marine Cold Water
9 Fish. *Microbial Ecology*, 38(1), 1-26.
- 10 Henney, J. E., Taylor, C. L., Boon, C. S., & Intake, I. of M. (US) C. on S. to R. S. (2010). Preservation and
11 physical property roles of sodium in foods. In *Strategies to reduce sodium intake in the United*
12 *States*. National Academies Press (US).
- 13 Hungerford, J. M. (2010). Scombroid poisoning : A review. *Toxicon*, 56(2), 231-243.
- 14 Hungerford, J. M. (2021). Histamine and Scombrottoxins. *Toxicon*, S0041010121002245.
- 15 Huss, H. H. (1995). *Quality and quality changes in fresh fish* (Vol. 348). FAO Rome.
- 16 Hwang, C.-C., Lee, Y.-C., Huang, C.-Y., Kung, H.-F., Cheng, H.-H., & Tsai, Y.-H. (2020). Effect of Brine
17 Concentrations on the Bacteriological and Chemical Quality and Histamine Content of Brined
18 and Dried Milkfish. *Foods*, 9(11), 1597.
- 19 Jääskeläinen, E., Jakobsen, L. M. A., Hultman, J., Eggers, N., Bertram, H. C., & Björkroth, J. (2019).
20 Metabolomics and bacterial diversity of packaged yellowfin tuna (*Thunnus albacares*) and
21 salmon (*Salmo salar*) show fish species-specific spoilage development during chilled storage.
22 *International Journal of Food Microbiology*, 293, 44-52.
- 23 Joffraud, J.-J., Cardinal, M., Cornet, J., Chasles, J.-S., Léon, S., Gigout, F., & Leroi, F. (2006). Effect of
24 bacterial interactions on the spoilage of cold-smoked salmon. *International Journal of Food*
25 *Microbiology*, 112(1), 51-61.
- 26 Jørgensen, L. V., Huss, H. H., & Dalgaard, P. (2000). The effect of biogenic amine production by single
27 bacterial cultures and metabiosis on cold-smoked salmon. *Journal of Applied Microbiology*,
28 89(6), 920-934.
- 29 Kanki, M., Yoda, T., Ishibashi, M., & Tsukamoto, T. (2004). *Photobacterium phosphoreum* caused a
30 histamine fish poisoning incident. *International Journal of Food Microbiology*, 92(1), 79-87.
- 31 Kim, S. H., Price, R. J., Morrissey, M. T., Field, K. G., Wei, C. I., & An, H. (2002). Histamine Production
32 by *Morganella morganii* in Mackerel, Albacore, Mahi-mahi, and Salmon at Various Storage
33 Temperatures. *Journal of Food Science*, 67(4), 1522-1528.
- 34 Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013).
35 Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation
36 sequencing-based diversity studies. *Nucleic acids research*, 41(1).
- 37 Kung, H.-F., Wang, T.-Y., Huang, Y.-R., Lin, C.-S., Wu, W.-S., Lin, C.-M., & Tsai, Y.-H. (2009). Isolation
38 and identification of histamine-forming bacteria in tuna sandwiches. *Food Control*, 20(11), 1013-
39 1017.
- 40 Kuuliala, L., Al Hage, Y., Ioannidis, A.-G., Sader, M., Kerckhof, F.-M., Vanderroost, M., Boon, N., De
41 Baets, B., De Meulenaer, B., Ragaert, P., & Devlieghere, F. (2018). Microbiological, chemical and
42 sensory spoilage analysis of raw Atlantic cod (*Gadus morhua*) stored under modified
43 atmospheres. *Food Microbiology*, 70, 232-244.
- 44 Larsen, A., Tao, Z., Bullard, S. A., & Arias, C. R. (2013). Diversity of the skin microbiota of fishes :
45 Evidence for host species specificity. *FEMS Microbiology Ecology*, 85(3), 483-494.
- 46 Mahusain, N. A. S., Bayoi, F., Karim, N. U., Zainol, M. K., & Danish-Daniel, M. (2017). Changes of
47 histamine levels and bacterial growth in longtail tuna, *Thunnus tongsol* stored at different
48 temperature. *Journal of Sustainability Science and Management*, 3, 38-46.
- 49 McMurdie, P. J., & Holmes, S. (2013). phyloseq : An R Package for Reproducible Interactive Analysis
50 and Graphics of Microbiome Census Data. *PLoS ONE*, 8(4).
- 51 Mejlholm, O., Devitt, T. D., & Dalgaard, P. (2012). Effect of brine marination on survival and growth of
52 spoilage and pathogenic bacteria during processing and subsequent storage of ready-to-eat

- 1 shrimp (*Pandalus borealis*). *International Journal of Food Microbiology*, 157(1), 16-27.
- 2 Morii, H., & Kasama, K. (2004). Activity of Two Histidine Decarboxylases from *Photobacterium*
3 *phosphoreum* at Different Temperatures, pHs, and NaCl Concentrations. *Journal of Food*
4 *Protection*, 67(8), 1736-1742.
- 5 Odeyemi, O. A., Burke, C. M., Bolch, C. C. J., & Stanley, R. (2018). Seafood spoilage microbiota and
6 associated volatile organic compounds at different storage temperatures and packaging
7 conditions. *International Journal of Food Microbiology*, 280, 87-99.
- 8 Ozogul, Y., Boğa, E. K., Akyol, I., Durmus, M., Ucar, Y., Regenstein, J. M., & Köşker, A. R. (2020).
9 Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-
10 borne pathogens. *Food Bioscience*, 36, 100635.
- 11 Parata, L., Nielsen, S., Xing, X., Thomas, T., Egan, S., & Vergés, A. (2019). Age, gut location and diet
12 impact the gut microbiome of a tropical herbivorous surgeonfish. *FEMS Microbiology Ecology*,
13 fiz179.
- 14 Parlapani, F. F. (2021). Microbial diversity of seafood. *Current Opinion in Food Science*, 37, 45-51.
- 15 Parlapani, F. F., & Boziaris, I. S. (2016). Monitoring of spoilage and determination of microbial
16 communities based on 16S rRNA gene sequence analysis of whole sea bream stored at various
17 temperatures. *LWT - Food Science and Technology*, 66, 553-559.
- 18 Parlapani, F. F., Meziti, A., Kormas, K. Ar., & Boziaris, I. S. (2013). Indigenous and spoilage microbiota
19 of farmed sea bream stored in ice identified by phenotypic and 16S rRNA gene analysis. *Food*
20 *Microbiology*, 33(1), 85-89.
- 21 Parlapani, F. F., Michailidou, S., Anagnostopoulos, D. A., Sakellariou, A. K., Pasentsis, K.,
22 Psomopoulos, F., Argiriou, A., Haroutounian, S. A., & Boziaris, I. S. (2018). Microbial spoilage
23 investigation of thawed common cuttlefish (*Sepia officinalis*) stored at 2 °C using next generation
24 sequencing and volatilome analysis. *Food Microbiology*, 76, 518-525.
- 25 Parlapani, F. F., Michailidou, S., Pasentsis, K., Argiriou, A., Krey, G., & Boziaris, I. S. (2018). A meta-
26 barcoding approach to assess and compare the storage temperature-dependent bacterial
27 diversity of gilt-head sea bream (*Sparus aurata*) originating from fish farms from two
28 geographically distinct areas of Greece. *International Journal of Food Microbiology*, 278, 36-43.
- 29 Perreault, N. N., Andersen, D. T., Pollard, W. H., Greer, C. W., & Whyte, L. G. (2007). Characterization
30 of the prokaryotic diversity in cold saline perennial springs of the Canadian high arctic. *Applied*
31 *and Environmental Microbiology*, 73(5), 1532-1543.
- 32 Prester, L. (2011). Biogenic amines in fish, fish products and shellfish : A review. *Food Additives &*
33 *Contaminants: Part A*, 28(11), 1547-1560.
- 34 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O.
35 (2012). The SILVA ribosomal RNA gene database project : Improved data processing and web-
36 based tools. *Nucleic acids research*, 41, 590-596.
- 37 Rahman, S. F. (1999). Food Preservation by Freezing. In *Handbook of Food Preservation* (Marcel
38 Dekker).
- 39 Reynisson, E., Lauzon, H. L., Thorvaldsson, L., Margeirsson, B., Rúnarsson, Á. R., Þór Marteinsson, V.,
40 & Martinsdóttir, E. (2010). Effects of different cooling techniques on bacterial succession and
41 other spoilage indicators during storage of whole, gutted haddock (*Melanogrammus aeglefinus*).
42 *European Food Research and Technology*, 231(2), 237-246.
- 43 Rivas, A. J., Lemos, M. L., & Osorio, C. R. (2013). *Photobacterium damsela* subsp. *Damsela*, a
44 bacterium pathogenic for marine animals and humans. *Frontiers in Microbiology*, 4.
- 45 Romalde, J. L. (2002). *Photobacterium damsela* subsp. *piscicida* : An integrated view of a bacterial
46 fish pathogen. *International Microbiology*, 5(1), 3-9.
- 47 Ross, A. A., Rodrigues Hoffmann, A., & Neufeld, J. D. (2019). The skin microbiome of vertebrates.
48 *Microbiome*, 7(1), 79.
- 49 Sehna, L., Brammer-Robbins, E., Wormington, A. M., Blaha, L., Bisesi, J., Larkin, I., Martyniuk, C. J.,
50 Simonin, M., & Adamovsky, O. (2021). Microbiome composition and function in aquatic
51 vertebrates : Small organisms making big impacts on aquatic animal health. *Frontiers in*
52 *microbiology*, 12, 358.

- 1 Sheng, L., & Wang, L. (2020). The microbial safety of fish and fish products : Recent advances in
2 understanding its significance, contamination sources, and control strategies. *Comprehensive*
3 *Reviews in Food Science and Food Safety*, 20(1), 738-786.
- 4 Silbande, A., Adenet, S., Smith-Ravin, J., Joffraud, J.-J., Rochefort, K., & Leroi, F. (2016). Quality
5 assessment of ice-stored tropical yellowfin tuna (*Thunnus albacares*) and influence of vacuum
6 and modified atmosphere packaging. *Food Microbiology*, 60, 62-72.
- 7 Silva, C. C. G., Ponte, D. J. B., & Dapkevicius, M. L. N. E. (1998). Storage Temperature Effect on
8 Histamine Formation in Big Eye Tuna and Skipjack. *Journal of Food Science*, 63(4), 644-647.
- 9 Silva, T. M., Sabaini, P. S., Evangelista, W. P., & Gloria, M. B. A. (2011). Occurrence of histamine in
10 Brazilian fresh and canned tuna. *Food Control*, 22(2), 323-327.
- 11 Sivertsvik, M., Jeksrud, W. K., & Rosnes, J. T. (2002). A review of modified atmosphere packaging of
12 fish and fishery products—Significance of microbial growth, activities and safety. *International*
13 *Journal of Food Science and Technology*, 37(2), 107-127.
- 14 Sørensen, J. S., Ørnfeld-Jensen, O., Bøknæs, N., Mejlholm, O., Jessen, F., & Dalgaard, P. (2020).
15 Thawed and chilled Atlantic cod (*Gadus morhua* L.) from Greenland—Options for improved
16 distribution. *LWT*, 131, 109473.
- 17 Speranza, B., Bevilacqua, A., Conte, A., Del Nobile, M. A., Sinigaglia, M., & Corbo, M. R. (2013). Use of
18 Desirability Approach to Predict the Inhibition of *Pseudomonas fluorescens*, *Shewanella*
19 *putrefaciens* and *Photobacterium phosphoreum* in Fish Fillets Through Natural Antimicrobials
20 and Modified Atmosphere Packaging. *Food and Bioprocess Technology*, 6(9), 2319-2330.
- 21 Syropoulou, F., Parlapani, F. F., Bosmali, I., Madesis, P., & Boziaris, I. S. (2020). HRM and 16S rRNA
22 gene sequencing reveal the cultivable microbiota of the European sea bass during ice storage.
23 *International Journal of Food Microbiology*, 327, 108658.
- 24 Syropoulou, F., Parlapani, F. F., Kakasis, S., Nychas, G.-J. E., & Boziaris, I. S. (2021). Primary Processing
25 and Storage Affect the Dominant Microbiota of Fresh and Chill-Stored Sea Bass Products. *Foods*,
26 10(3), 671.
- 27 Tabanelli, G., Torriani, S., Rossi, F., Rizzotti, L., & Gardini, F. (2012). Effect of Chemico-Physical
28 Parameters on the Histidine Decarboxylase (HdcA) Enzymatic Activity in *Streptococcus*
29 *thermophilus* PRI60. *Journal of Food Science*, 77(4), M231-M237.
- 30 Tahmouzi, S., Ghasemlou, M., Aliabadi, F. S., Shahraz, F., Hosseini, H., & Khaksar, R. (2013). Histamine
31 formation and bacteriological quality in Skipjack tuna (*Katsuwonus pelamis*): effect of defrosting
32 temperature. *Journal of Food Processing and Preservation*, 37(4), 306-313.
- 33 Takahashi, H., Ogai, M., Miya, S., Kuda, T., & Kimura, B. (2015). Effects of environmental factors on
34 histamine production in the psychrophilic histamine-producing bacterium *Photobacterium*
35 *iliopiscarium*. *Food Control*, 52, 39-42.
- 36 Taliadourou, D., Papadopoulos, V., Domvridou, E., Savvaidis, I. N., & Kontominas, M. G. (2003).
37 Microbiological, chemical and sensory changes of whole and filleted Mediterranean
38 aquacultured sea bass (*Dicentrarchus labrax*) stored in ice. *Journal of the Science of Food and*
39 *Agriculture*, 83(13), 1373-1379.
- 40 Tang, R., Zhu, J., Feng, L., Li, J., & Liu, X. (2019). Characterization of LuxI/LuxR and their regulation
41 involved in biofilm formation and stress resistance in fish spoilers *Pseudomonas fluorescens*.
42 *International Journal of Food Microbiology*, 297, 60-71.
- 43 Thyssen, A., & Ollevier, F. (2005). Genus II. *Photobacterium*. In *Bergey's manual of systematic*
44 *bacteriology* (2^e éd., Vol. 1, p. 546-552). Springer.
- 45 Tortorella, V., Masciari, P., Pezzi, M., Mola, A., Tiburzi, S. P., Zinzi, M. C., Scozzafava, A., & Verre, M.
46 (2014). Histamine Poisoning from Ingestion of Fish or Scombroid Syndrome. *Case Reports in*
47 *Emergency Medicine*, 2014, 1-4.
- 48 Turan, H., & Erkoyuncu, İ. (2012). Salting technology in fish processing. *Progress in food preservation*,
49 297-13.
- 50 Wang, D., Yamaki, S., Kawai, Y., & Yamazaki, K. (2020). Histamine Production Behaviors of a
51 Psychrotolerant Histamine-Producer, *Morganella psychrotolerans*, in Various Environmental
52 Conditions. *Current Microbiology*, 77(3), 460-467.

- 1 Wang, H., Liu, X., Zhang, Y., Lu, H., Xu, Q., Shi, C., & Luo, Y. (2017). Spoilage potential of three
2 different bacteria isolated from spoiled grass carp (*Ctenopharyngodon idellus*) fillets during
3 storage at 4 °C. *LWT - Food Science and Technology*, 81, 10-17.
- 4 Xavier, R., Pereira, A., Pagan, A., Hendrick, G. C., Nicholson, M. D., Rosado, D., Soares, M. C., Perez-
5 Losada, M., & Sikkel, P. C. (2020). The effects of environment and ontogeny on the skin
6 microbiome of two *Stegastes* damselfishes (*Pomacentridae*) from the eastern Caribbean Sea.
7 *Marine Biology*, 167(7), 1-12.
- 8 Zhuang, S., Hong, H., Zhang, L., & Luo, Y. (2021). Spoilage-related microbiota in fish and crustaceans
9 during storage : Research progress and future trends. *Comprehensive Reviews in Food Science
10 and Food Safety*, 20(1), 252-288.
- 11 Zotta, T., Parente, E., Ianniello, R. G., De Filippis, F., & Ricciardi, A. (2019). Dynamics of bacterial
12 communities and interaction networks in thawed fish fillets during chilled storage in air.
13 *International Journal of Food Microbiology*, 293, 102-113.
- 14
15
16
17
18

19 FIGURE LEGENDS

20

21 **Figure 1.** Experimental design to study the post-mortem microbiome of yellowfin tuna
22 stored at 4°C. Gut and liver samples were collected in triplicate at the beginning of the
23 experiment (T_0) and after 48, 96 and 120 hours, on fresh (A) and brine-frozen (B) individuals.

24

25 **Figure 2.** Temporal variation in the relative abundance of the main bacterial genera in the
26 gut and liver samples of fresh and brine-frozen yellowfin tuna. The size of the dot is
27 proportional to the relative abundance of each bacterial genus from T_{0h} to T_{120h} . Genera
28 identified as potential specific spoilage organisms (SSOs) are coloured in red. Arrows
29 represent the overall development of each bacterial genus during the experiment. Bacterial
30 genera with a relative abundance inferior to 2% were grouped and designated as < 2%
31 abund.

32

33 **Figure 3.** Dynamics of histamine concentration (ppm) (right abscissa) and relative abundance
34 (left abscissa) of the main putative histamine-producing bacteria (HPB) found in the gut (A,B)
35 and liver (C,D) of fresh (B,D) and brine-frozen (A,C) yellowfin tuna. The red horizontal bar
36 represents the sanitary threshold of 50 ppm established by the United States Food and Drug
37 Administration (FDA 2021).

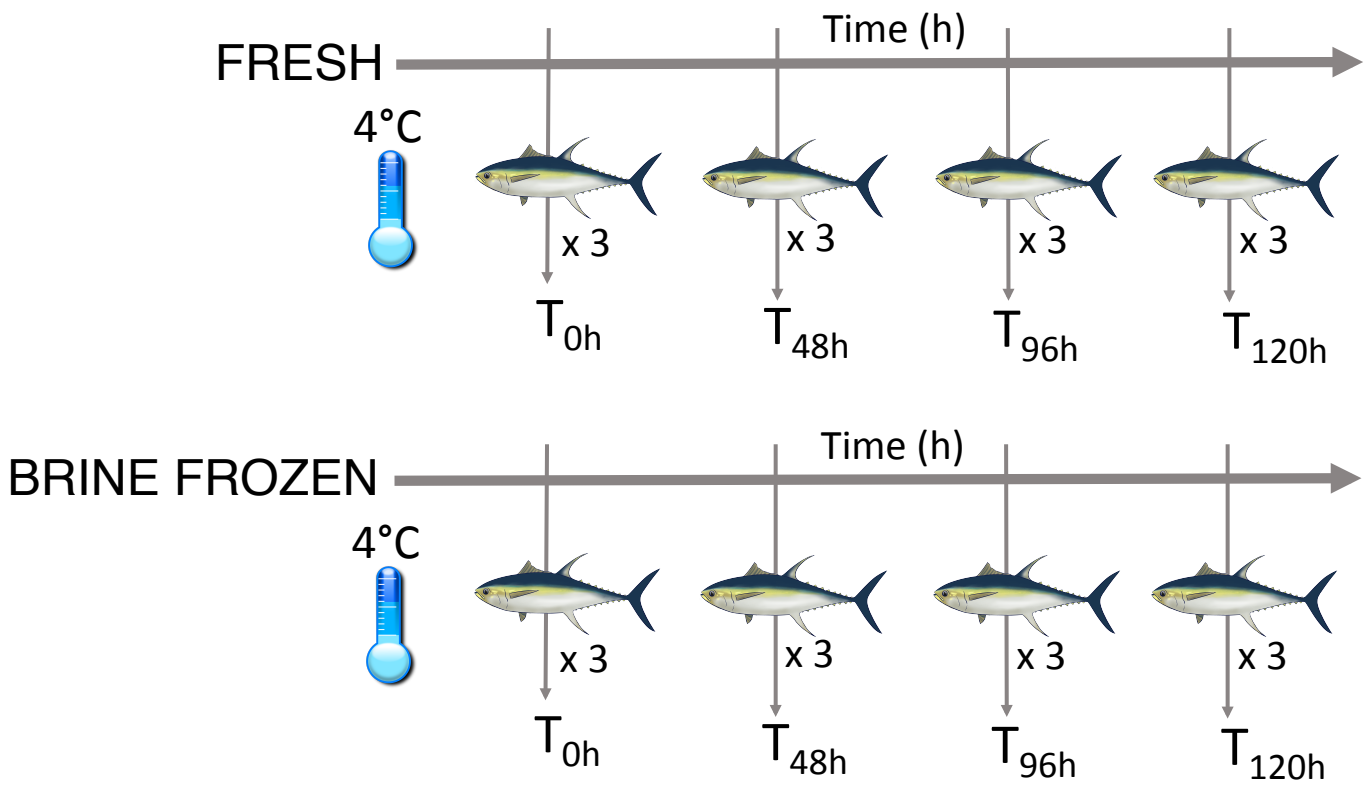
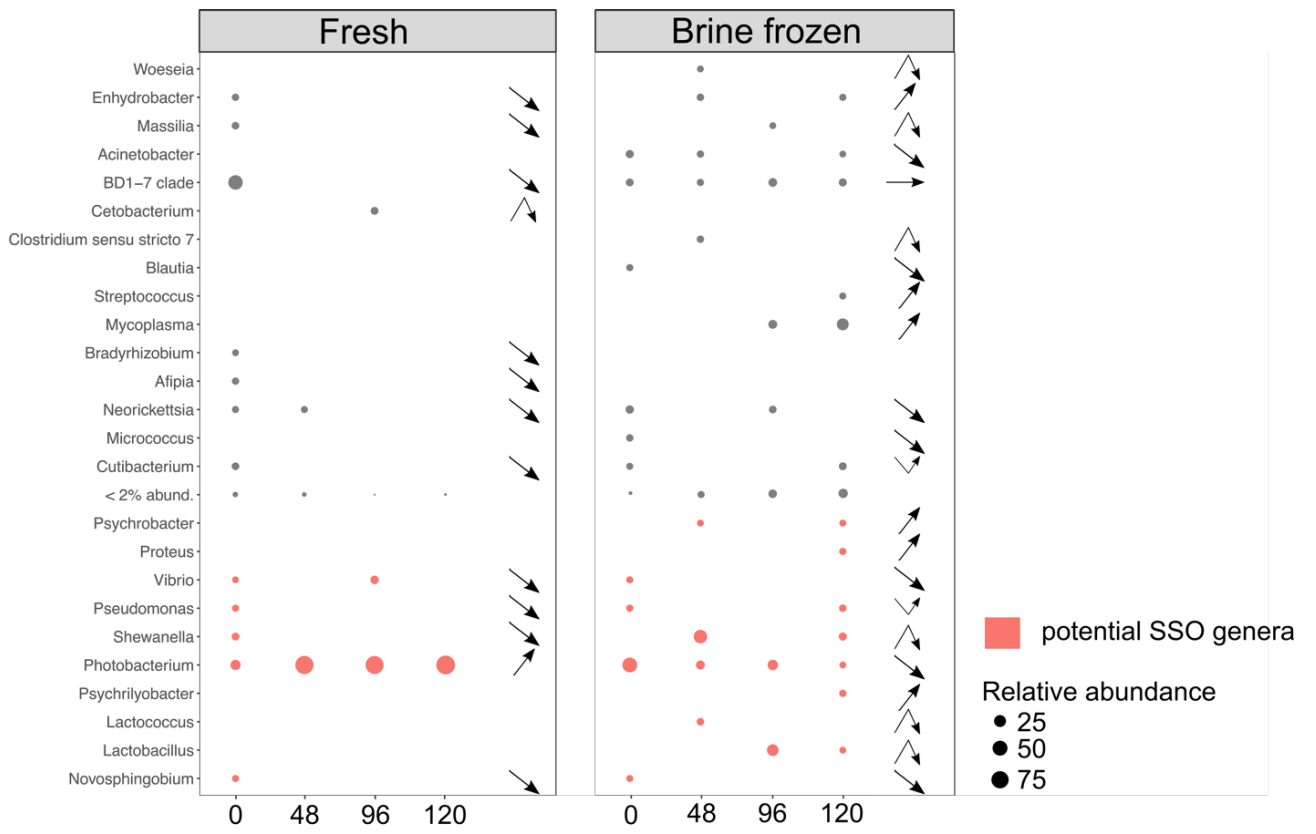


Figure 1

Gut



Liver

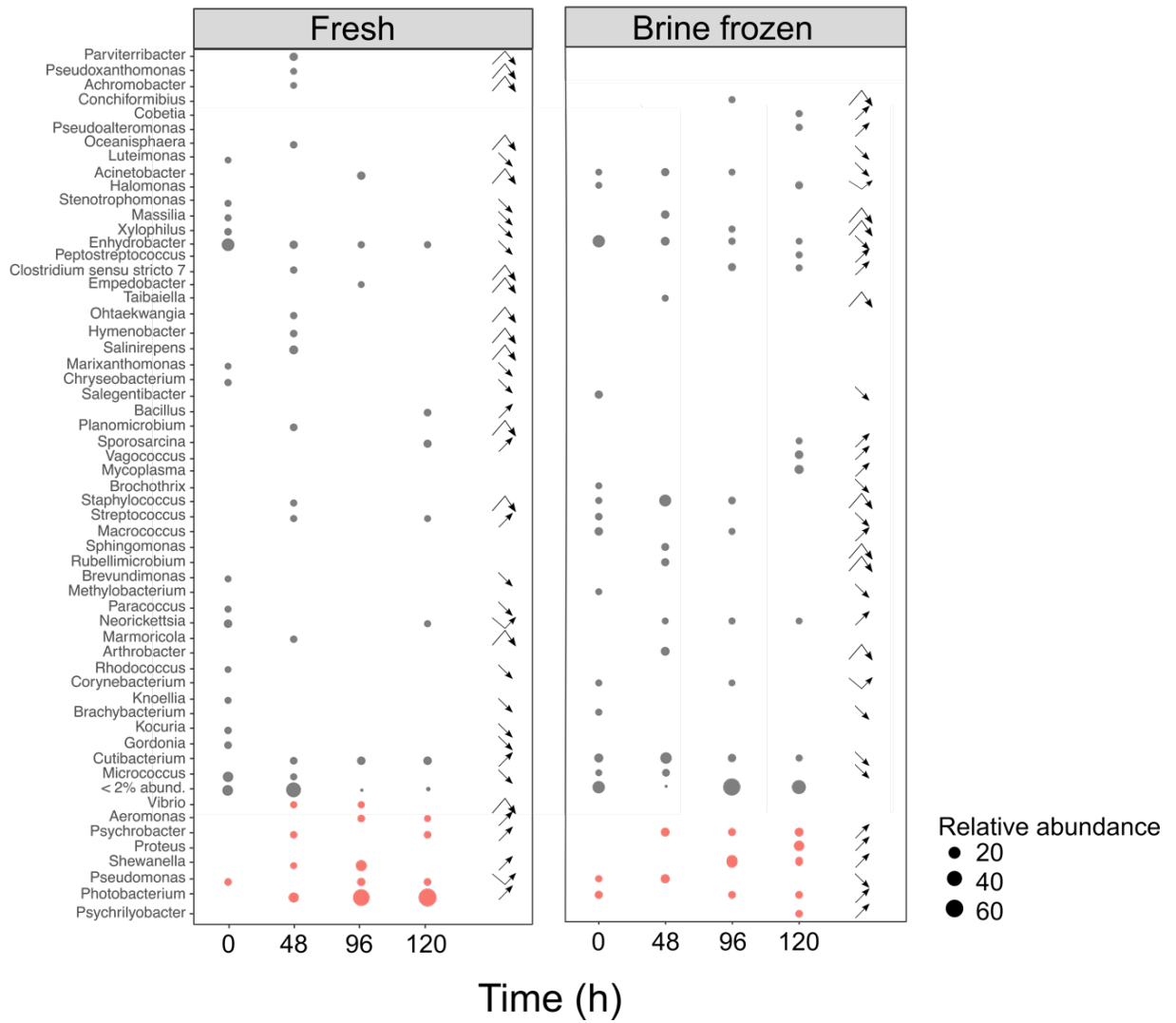
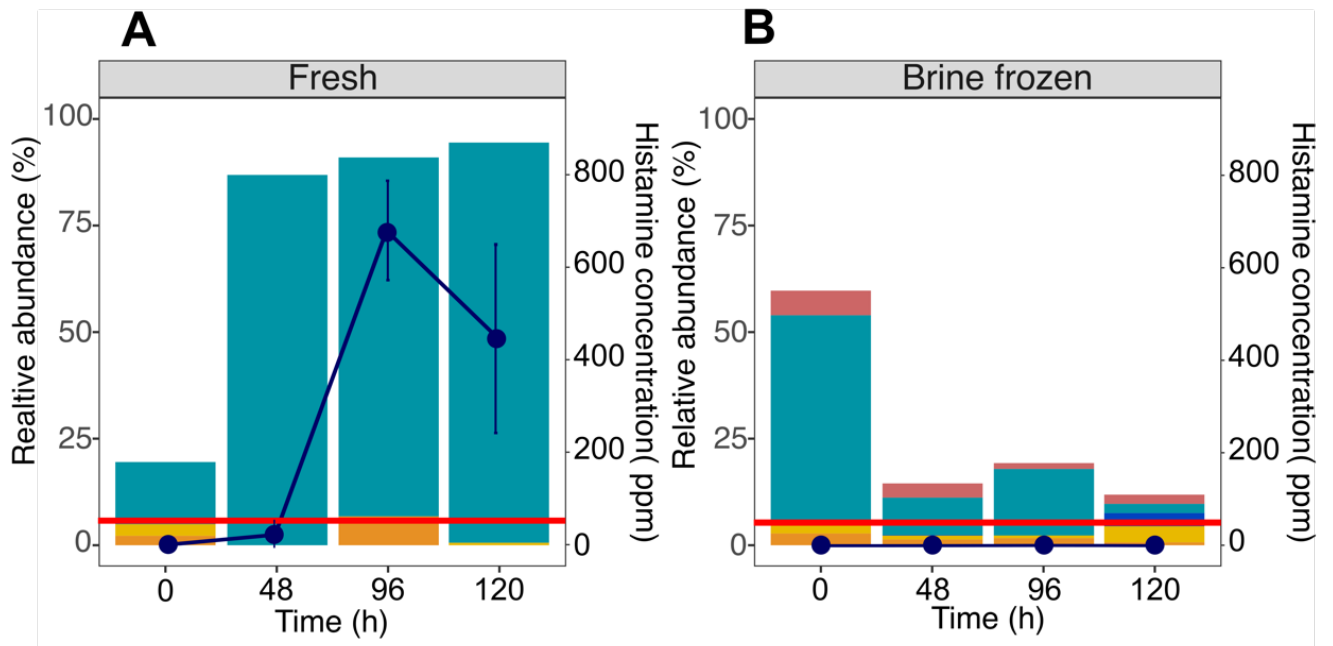
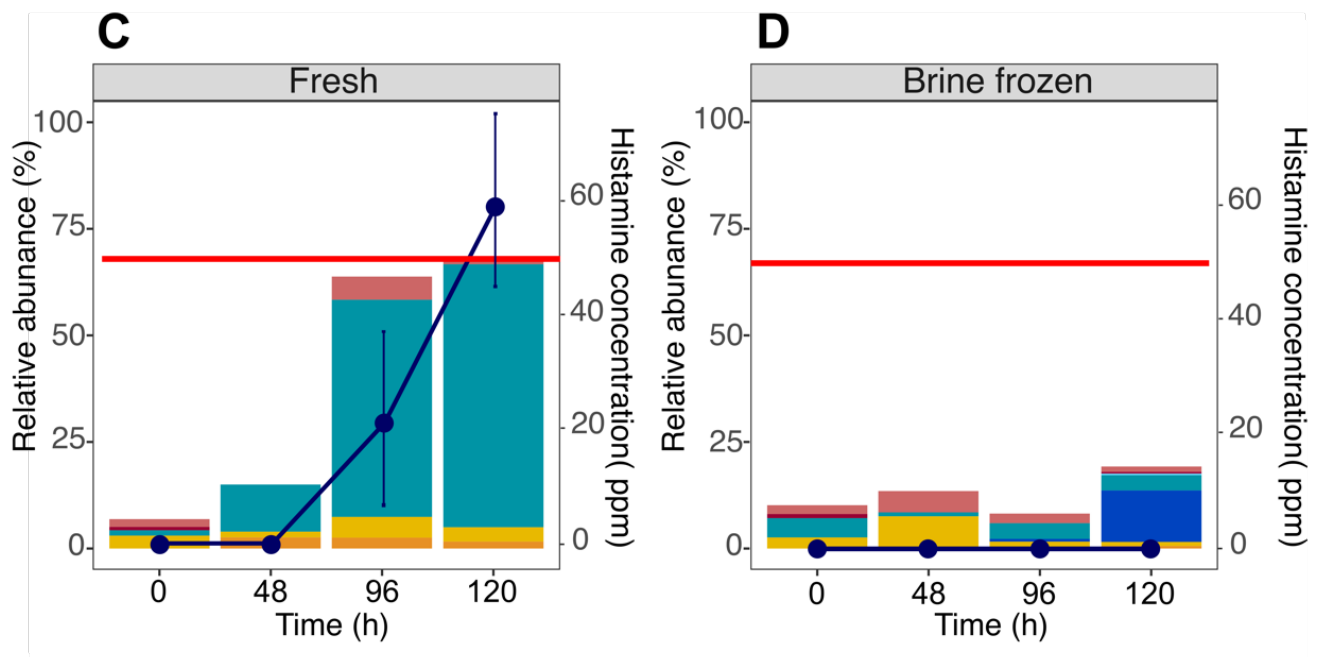


Figure 2

GUT



LIVER



Putative histamine producing bacteria genera

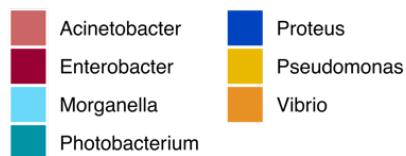


Figure 3

Table 1: Results of permutational ANOVAS (PERMANOVA, 999 permutations) performed on Bray-Curtis dissimilarities matrices to test the variation of bacterial community composition with time and post-capture conservation conditions in gut and liver samples. Bold values indicate a significant effect of the tested factor ($p < 0.05$).

Community dissimilarity						
	Time			Conservation		
	p value	r^2	df	p value	r^2	df
Gut	0.001	0.21	2	0.255	0.05	1
Liver	0.023	0.13	2	0.003	0.09	1