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BIODIVERSITY
Published: 27 January 2023
doi: 10.3389/frym.2023.818373

THE UNSEEN MICROBES IN THE GUT, LIVER AND SKIN OF TUNAS

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YOUNG REVIEWERS:

MOAB CHARTER SCHOOL

AGES: 9–12

OLIVE

AGE: 12

The microbiome is the name for the collection of microbes that live on or in another organism. The human microbiome has received a lot of attention, but in fact every living thing hosts a community of microbes that can be helpful—even vital—or sometimes harmful. In this study, we investigated the microbiome of tuna to identify the specific bacteria that populate it and whether the microbiome varies depending on the type of tuna, the ocean it lives in, or the organ. We found that the gut microbiome differs according to species due to feeding habits, while the skin microbiome differs according to the environment and is linked to the bacteria in the surrounding seawater. Bacteria that can cause food poisoning in humans were concentrated in the gut and liver of tuna, highlighting the importance of removing the organs before consuming this fish, which is one of the most widely eaten in the world.

THE SECRET WORLD OF THE MICROBIOME

Although **bacteria** have a bad reputation as organisms that make us sick, we now know that bacteria are essential for human health and the health of other animals, both on and in the sea. Invisible to the naked eye, bacteria are found in the gut, mouth, nose, lungs, kidneys, liver, and even on the surface of the skin! While it is true that some bacteria can cause disease, many are extremely useful, if not essential, to the organisms they live in or on—with roles in digestion, the immune system, behavior, and more [1]. All these bacteria, together with other **microbes** such as viruses, **archaea** (primitive single-celled organisms) and fungi, make up what is called the **microbiome**, which means "small living community."

The microbiome has received a lot of attention in recent years. Scientists have begun to understand that an organism is not just an individual, but a complex community of organisms. Studying the microbiome of animals is interesting for several reasons. First, it teaches us about the microbes themselves—they are tiny but infinitely interesting—and about their interactions with other organisms. Second, studying the microbiome allows us to identify microbes that are dangerous to the humans who eat the animals that contain those microbes. This is why researchers track the presence of microbes in the animals—including fish—that end up on our plates.

There are still a lot of mysteries. For example, do all living organisms have their own specific microbiomes? Does an organism have the same bacteria everywhere in its body? Is the microbiome different between males and females? And does the microbiome vary depending on where an animal lives? These are some of the questions we wanted to answer with our research on one of the ocean's top predators—one that humans consume in large quantities: tuna!

In many coastal countries around the world, tuna is a major source of both income and food. Sometimes tuna can give humans food poisoning, caused by the presence of bacteria that produce harmful compounds such as **histamine**, which cause unpleasant reactions in humans [2]. Understanding where these bacteria live inside the tuna and how they cause histamine to build up in the fish's flesh are the first step toward preventing people from getting sick.

To unravel the mysteries of the tuna microbiome, we focused on two species that are among the most widely consumed: yellowfin and skipjack tuna. While an organism's microbiome contains multiple types of microbes, our study focused on just one type: the bacteria.

BACTERIA

Microscopic organisms present nearly everywhere on Earth. Some are dangerous and can cause infection, but others are essential for life—for example, bacteria in the gut help to digest food.

MICROBES

An organism so small that it cannot be seen with the naked eye. Microbes, which include bacteria, viruses, archaea, and fungi, are the most abundant organisms on earth and are found everywhere—in the air, in the water, and associated with all living organisms.

ARCHAEA

Microscopic, single-celled organisms, usually found in extreme environments. Archaea were initially considered to be bacteria, for they share many characteristics, but they are now considered as another domain of life.

MICROBIOME

A community of microbes present in a living organism. Our research focused on bacteria, so we use "microbiome" to refer only to the bacteria in an organ.

HISTAMINE

A chemical produced by certain bacteria in tuna and other fish. Ingestion of histamine can cause an allergic reaction in humans, with symptoms including diarrhea, vomiting, redness, itching, and fever.

TIME TO GO FISHING!

First, we needed fish. So, we went out with local professional fishers and caught 48 skipjack and yellowfin tuna in the waters off the Ivory Coast (Atlantic Ocean) and off Reunion Island (Indian Ocean) (Figure 1). Our aim was to examine the bacteria living on the tunas' skin and in two important organs: the gut and the liver. These organs are both involved in digestion, but the liver also performs other functions such as removing toxins from the blood and producing many chemicals essential for health. Once the fish were caught, stunned (to reduce stress) and killed, we swabbed their skin to collect mucus—fish skin is covered with a layer of slime that protects the fish from the external environment. The fish were then kept on ice until we could get back to the laboratory.

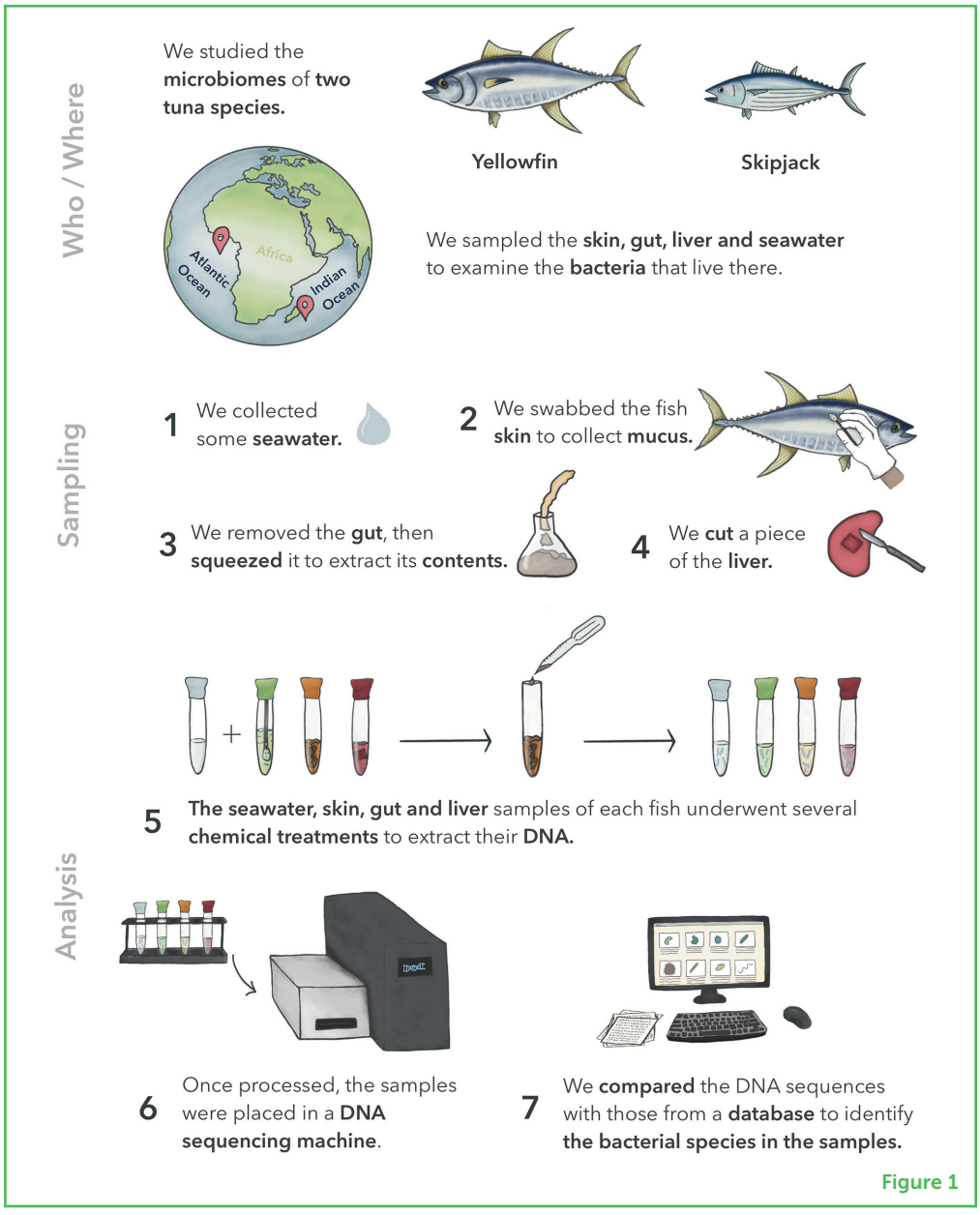


Figure 1

The different methodological steps of our study, from the sampling of tuna in the two oceans, to the identification by DNA sequencing of the bacteria living on their skin, gut, and liver.

In the lab, we dissected the tuna to remove the digestive tract, collect the gut contents, and to slice off a small piece of the liver so we could look for the presence of microbes. The fishers then took the meat, which was either sold to a local market or used to feed their families. Tuna is a precious resource, and it was not wasted! When we were at sea, we also sampled the seawater so we could compare the bacteria in the water to those in our tuna samples, to see if bacteria were transmitted to the fish through the water they lived in.

Since microbes are so small, advanced technologies must be used to identify their presence. To check which types of bacteria were present in the skin, gut, liver, and seawater samples, we looked at bacterial **DNA**, which contains all of the bacterias' genetic information. First, we treated the tissue samples with chemicals to remove the DNA from the bacterial cells. Then we put the DNA in a machine called a sequencer, which can read the genetic information like a barcode. We then compared our DNA samples with a database of bacterial DNA, which allowed us to identify which types of bacteria were present in our samples (Figure 1).

DNA

Found in every living organism on Earth, DNA is a molecule that carries the genetic instructions for that organism. The sequence of molecules in DNA is unique to each species.

A BOATLOAD OF BACTERIA

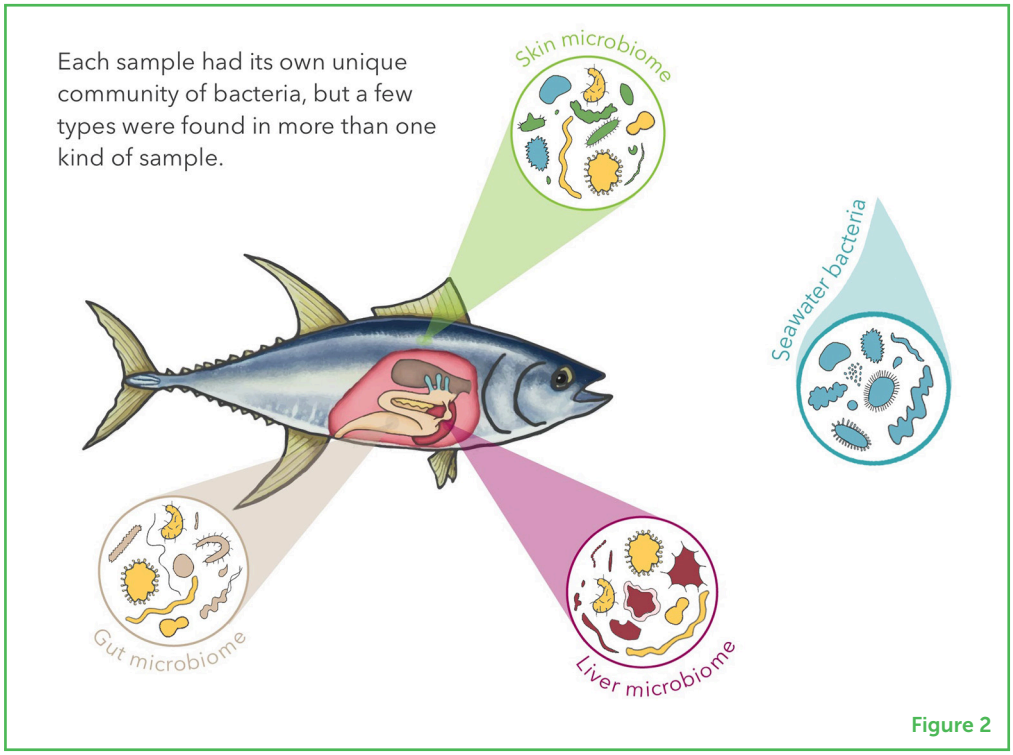
We found that tuna skin contained more than 2,000 different bacterial species—maybe not what you wanted to hear if you love tuna! The tuna gut had about 800 species. We were not surprised by this finding, as this diversity has been previously shown in humans and other animals. Remember, many bacteria are perfectly harmless. However, we did not know what to expect in the liver. We were surprised to find there were about 800 bacterial species in the liver as well! These results are very interesting because they show that, like the skin and the gut, the liver is an important reservoir of bacteria within the body. This had never been observed in tuna.

We also found that each organ hosted bacteria that were not present in the other organs (Figure 2). It seems likely that these different populations of bacteria are involved in the specific functions of each organ—helping the gut to digest food, the liver to eliminate waste products from the body, and the skin to serve as a protective shield.

We also found that a few species of bacteria were present in all three organs, suggesting that certain microbes can move from one organ to another. This is not surprising for the gut and the liver because they are connected by the circulatory system. A few types of bacteria found in the seawater samples were also found on fish skin, telling us that certain bacteria from the water can live on the surfaces of marine animals.

Figure 2

Representation of the diversity of the bacteria living in the gut, liver and skin of tunas, some are common to all three, others are specific to each organ.



Comparison is extremely important in science. This is why we tested two species of tuna (including both males and females), from two oceans, plus seawater samples from both oceans. The data allowed us to see if two tuna species, skipjack and yellowfin tuna, share the same microbiome, if the microbiome changes depending on where the fish live, and whether gender is linked to differences in the microbiome.

WHAT REALLY MATTERS TO MICROBES?

Comparing our data revealed several interesting findings (Figure 3). First, we found that there was no difference between the microbiomes of male and female tuna of either species, in either ocean. In fact, there are only a few differences between female and male tuna: they look the same, eat the same food, live in the same place, and basically have the same way of life. So microbes do not really care about gender!

We did observe differences in the microbiome when we looked closely at each organ: for example, the gut microbiomes of skipjack and yellowfin tuna were very different from each other. This makes sense because these two species have quite different lifestyles. Yellowfin can grow up to 2 meters (6.5 ft) long while skipjack are half that size, so yellowfin can swim deeper in the ocean to find more types of prey. Skipjack tuna stay closer to the ocean surface, where the food options are less diverse. Gut microbiomes did not differ between yellowfin (or skipjack) tuna living in the Atlantic and those living in the Indian Ocean,

Figure 3

Comparison of the bacterial communities in the tuna gut, skin and liver microbiome depending on sex, species and sampling site. A green tick indicates a significant difference, while a red cross indicates no difference.

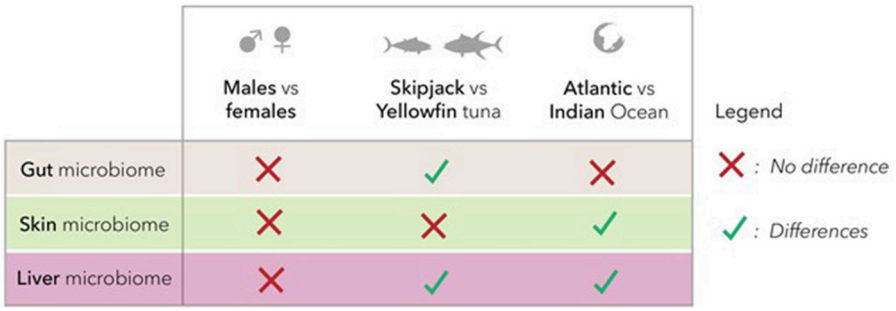


Figure 3

so we can conclude that the gut microbiome depends more on what fish eat than where fish live.

When we looked at the skin, the reverse was true: skipjack and yellowfin had the same skin microbiomes, but there were huge differences between fish caught in the two oceans. The skin of fish is in direct contact with seawater, and it acts as an "exchange zone" between the animal and its environment. This told us that the bacteria living on the skin of tuna are more dependent on the external conditions than they are on the organism that hosts them. This was confirmed by the fact that the skin microbiome samples shared numerous bacteria species with the seawater samples. For the skin microbiome, only location matters!

In the liver, the situation was more complex, as no clear trend was observed. It seems that both the ocean and the species influence the bacteria living in the liver, but more research will be necessary to solve this mystery.

PINPOINTING HARMFUL BACTERIA

So, what did our research tell us about bacteria that can cause food poisoning in humans? As we mentioned, some bacteria can produce a compound called histamine. Histamine production is limited when food is stored at low temperatures, but if fish is not fresh or has not been kept cold enough, eating it can cause histamine poisoning. This can result in rashes, vomiting, and diarrhea, and, in the most extreme cases, it can even require hospitalization. Histamine is not destroyed by cooking—it is almost indestructible. So, did we find harmful, histamine-producing bacteria in the tuna we studied? Indeed, we did, and they were particularly abundant and diverse in the guts and livers of tuna. This is why, apart from keeping fish cold, it is very important to remove the internal organs of fish before cooking and eating their meat!

CONCLUSION

Our study confirmed the results of studies in other animals—that microbes are found in every living thing, including those in the sea, and that microbes can have a big impact—sometimes positive and sometimes negative—on the animals they interact with. New techniques such as DNA sequencing have allowed scientists to identify the microbes present in the microbiome and to better understand their important roles.

Over thousands of years, people have been trying to understand the world by discovering previously unknown environments, species, and phenomena. Just as our ancestors before us analyzed and investigated their worlds, today's researchers have a vast new world of microbiomes to explore—an area of study that has just begun. The microbiome still has many secrets to reveal.

ORIGINAL SOURCE ARTICLE

Gadoin, E., Desnues, C., Roque d'Orbcastel, E., Bouvier, T., Auguet, T., Dagorn, L., et al. 2022. Fishing for the microbiome of tropical tuna. *Microbial Ecol.* doi: 10.1007/s00248-022-02096-4

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SUBMITTED: 19 November 2021; **ACCEPTED:** 10 January 2023;
PUBLISHED ONLINE: 27 January 2023.

EDITOR: Emily King, Xiamen University, China

SCIENCE MENTORS: Joan West and Marcus Yee

CITATION: Gadoin E, Desnues C, Roque d'Orbcastel E, Bouvier T, Auguet J-C, Boudin E and Bettarel Y (2023) The Unseen Microbes in the Gut, Liver and Skin of Tunas. Front. Young Minds 11:818373. doi: 10.3389/frym.2023.818373

CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

MOAB CHARTER SCHOOL, AGES: 9–12

We are a 5th and 6th grade class at the Moab Charter School located in...you guessed it...Moab, Utah! We have a wide range of interests: dogs, pickles, grammar, art, ghosts, football, beyblades, video games, hiking and watermelon. We feel lucky to live in such a beautiful place!

OLIVE, AGE: 12

My name is Olive and I am a sixth grader. I love reading, science, biology, medicine, and taking care of animals, and I hope to study veterinary medicine when I get into university. Our family has one dog, one blue tongue skink, and one busy fish tank. I do Aikido and Outschool online classes. My hobbies are: crocheting, reading books, cooking, reading things on the computer, watching cartoons, and walking our dog.

AUTHORS

ELSA GADOIN

Elsa Gadoin has just completed her Ph.D. at the MARBEC lab in Montpellier, France. She is interested in the ecology of marine microbes. She would like to specialize in the study of marine microbiomes to better understand their diversity and functions, as well as the role of the microbial communities living together with marine animals.

CHRISTELLE DESNUES

Christelle Desnues (CNRS Research Director) was passionate about viruses, their ecology, and human health. She studied the diversity, dynamics, and evolution of viruses at human-animal-ecosystem interfaces. With her dual research experience in clinical and environmental sciences, she actively made the bridge between physicians and ecologists, fully in lines with the "One Health, One Medicine" approach, to better understand the interactions between environmental, animal, and human health. We all miss her so much.

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Emmanuelle Roque d'Orbcastel is a marine biologist working at the Ifremer Institute, associated with the MARBEC laboratory (Montpellier, France). She works on aquaculture ecosystems, and studies how the environment influences the health of cultivated organisms. She is interested in working with fish farmers, proposing ways to reduce the environmental impact of fish farming.

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Thierry Bouvier is a marine microbial ecologist at the MARBEC lab located in Montpellier, France. He started to study the bacteria and viruses living in the sediment and water column of polar, temperate and tropical oceans.

JEAN-CHRISTOPHE AUGUET
Jean-Christophe Auguet (CNRS Researcher at MARBEC Lab) is an enthusiastic microbial ecologist. His current research focuses on the diversity of microorganisms associated with marine organisms, including pathogenic bacteria, which he studies using molecular tools and next-generation DNA sequencing.

ELYSE BOUDIN
Elyse Boudin graduated with a degree in Ecology Engineering, from the University of Montpellier, in 2019. Now, she is involved in several environmental and educational projects in the South of France. These projects combine research in marine and terrestrial ecology, photography, and the creation of environmental education tools.

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