

Fishing for Tropical Tuna's Virome

Subjects: Virology

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The tuna virome was mainly dominated by eukaryotic viruses in the digestive organs (gut and liver), while bacteriophages were predominant in the mucus. We observed the presence of specific viral families in each organ, some previously identified as fish or human pathogens (e.g., Iridoviridae, Parvoviridae, Alloherpesviridae, Papillomaviridae). Interestingly, we also detected a 'core virome' that was shared by all the organs and was mainly composed of Caudovirales, Microviridae and Circoviridae. These results show that tuna host a mosaic of viral niches, whose establishment, role and circulation remain to be elucidated.

Keywords: tuna ; virome diversity ; microbiome

1. Introduction

Marine animals live in close and complex interactions with an abundant and diverse assemblage of viruses, typically made up of bacteriophages and eukaryotic viruses. Both are suspected to play an essential role in the health of the host, either by contributing to its immune system ^{[1][2]} or, conversely, by transmitting infections in tissues ^[3]. In recent years, only a handful of studies have explored the virome of marine invertebrates such as corals ^{[4][5][6]} or sponges ^[7], while those studying marine vertebrates are even scarcer ^{[8][9]}. This is particularly true for marine fish, the viromes of which the ecological determinants, variability between species, organs, sex, areas of distribution, as well as risks and benefits to the host, are virtually unknown.

Tuna are an interesting model for investigating the fish virome since they are (i) a keystone species and top predators in pelagic ecosystems, (ii) one of the most consumed fish species worldwide ^[10], and (iii) microbiologically sensitive, as the consumption of tuna presents a risk of food poisoning linked to the presence of pathogenic—histamine-producing—bacteria ^[11].

In this study, we aimed to describe the taxonomic diversity of viral communities inhabiting the skin mucus, gut and liver of two major tropical tuna species (skipjack and yellowfin) in individuals of similar sizes sampled in the Atlantic and Indian Oceans. We sought to identify shared and specific viral taxa in these three key organs and to characterize the influences of species, sex and sampling site on the composition of their virome.

2. Fishing for the Virome of Tropical Tuna

2.1. Sampling Procedure

Tuna. All skipjack (*Katsuwonus pelamis*, SKJ) and yellowfin (*Thunnus albacares*, YLF) tuna used in this study were sampled around fish aggregating devices (FAD), in the Atlantic (Ivory Coast, Gulf of Guinea, N04°55'00", W03°42'19.97) and Indian Oceans (Reunion Island, S20°57'816", E55°04'457"), during campaigns in July (10–11) and September (26–29) 2018, respectively. In total, 21 tuna were sampled in the Gulf of Guinea (6 skipjack and 15 yellowfin) and 27 off Reunion Island (18 skipjack and 9 yellowfin). In both locations the sampling and euthanasia of the fish were performed by professional fishermen working for the French National Research Institute for Sustainable Development (IRD) Exploited Tropical Pelagic Ecosystems Observatory (certified ISO 9001:2015). A hook and line were used, and the tuna always handled by the mouth using a clamp; euthanasia was performed by cervical dislocation (following European directive 2010/63/UE) immediately after capture. All participants wore gloves to avoid contamination during sampling.

2.2. Sampling of the Skin Mucus, Gut and Liver

Skin mucus layer. The superficial mucus layer was sampled immediately after the death by swabbing one side of the body surface, which remained untouched. Avoiding the head, which was manipulated during euthanasia, buccal swabs (Isohelix®, Harrietsham, UK SK-2S swabs) were rubbed from the back of the operculum to the caudal peduncle.

Gastrointestinal content. After the sampling of the skin mucus, fish were individually placed in plastic bags and immediately stored on ice before dissection (within 5 h after sampling). Back in the laboratory, each individual was dissected using sterile tools: the gastrointestinal tract was extracted and cut from below the stomach to the rectum. Placed on a sterile surface, the content of each gut was expelled by squeezing, and the collected contents (minimum volume of 5 mL) were homogenized before sampling.

Liver. Using a sterile cutter and forceps, a longitudinal piece of about $1 \times 0.2 \times 0.2$ cm was cut from the right lobe (the largest) of the liver in each individual tuna. To avoid contamination from any other internal organs or fluids, the collected liver samples were rinsed with distilled water (through 0.2- μ m filters).

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

2.3. Viral Nucleic Acid Extraction and Sequencing

Samples preparation. Three individuals (triplicate) of each sex (male and female) of each species (skipjack and tuna) in each ocean (Atlantic and Indian) were selected (a total of $n = 3 \text{ indiv} \times 2 \text{ sex} \times 2 \text{ species} \times 2 \text{ oceans} = 24 \text{ indiv}$). In each of the 24 tuna, skin mucus, gut and liver viral communities were sampled as described above (for a total of $n = 3 \text{ organs} \times 24 \text{ indiv} = 72 \text{ biological samples}$).

To separate virus from bacteria and organic matter, all biological samples ($n = 72$) were suspended in 0.02 μm filtered PBS. For gut ($n = 24$) and liver ($n = 24$), 1 g sub-samples were placed in 2 mL Eppendorf and completed with 0.02 μm filtered PBS. For skin mucus samples ($n = 24$), individual swabs were placed in 1.5 mL Eppendorf with 500 μL of 0.02 μm filtered PBS. Gut and skin mucus samples were vortexed at maximum speed, respectively, 10 and 5 min, while liver samples were crushed with a FatsPrep for 45 s at 6 ms/ms. All samples were then centrifugated 10 min at $1500\times g$ at 4°C and supernatants were collected. After a second centrifugation at $14,000\times g$ at 4°C for 15 min, supernatants were collected and put in clean Eppendorf (protocol adapted from Monteil-Bouchard et al., 2018) [12]. All resuspended samples were then digested 1 h at 37°C with a cocktail of enzymes composed of 100 μL of 10X TurboDNase buffer ($^{\circ}$ Invitrogen, Carlsbad, CA, USA), 20 μL of TurboDNase (2 U/ μL , $^{\circ}$ Invitrogen), 5 μL of RNase A (3.6 U/ μL , $^{\circ}$ Macherey Nagel, Düren, Germany), 5 μL of Exonuclease I (20 U/ μL , $^{\circ}$ ThermoFisher Scientific, Waltham, MA, USA) and 5 μL of Benzonase (25 U/ μL , $^{\circ}$ Merck Millipore, Molsheim, France) for 1 mL of sample [12].

Nucleic acid extraction. Viral nucleic acids were extracted from each sample ($n = 72$) using the Roche High Pure Viral Nucleic acid extraction kit ($^{\circ}$ Roche Diagnostics, Meylan, France) following the manufacturer's protocols. Following the extraction, 1 μL of RNase OUT (40 U/ μL , $^{\circ}$ Life Technologies, Saint Aubin, France) was added in all samples, then nucleic acid concentration was assessed using Qubit dsDNA high sensitivity ($^{\circ}$ Life Technologies), following the manufacturer's instructions.

After quantification, triplicate individuals were pooled prior to the sequencing. Subsamples of 100 ng of extracted nucleic acid were taken from each of the three individuals replicates and pooled. Thus, for each organ a single replicate per sex (male and female), per species (skipjack and yellowfin) and per sampling site (Atlantic or Indian ocean) was obtained, for a total of 24 samples to be sequenced ($n = 3 \text{ organs} \times 2 \text{ species} \times 2 \text{ sex} \times 2 \text{ sampling site} = 24 \text{ samples}$).

DNA amplification and purification. DNA amplification was then performed in duplicate, using Genomiphi V3 kit ($^{\circ}$ GE Healthcare, Vélizy-Villacoublay, France) following the manufacturer's protocols. Duplicate amplifications of each sample were pooled, diluted with 60 μL of biomolecular water and purified using NucleoFast plates ($^{\circ}$ Macherey Nagel). Plates were centrifugated 30 min at $4000\times g$, 100 μL of molecular water were added in each well before a second 30 min centrifugation at $4000\times g$. After the addition of 30 μL of molecular water in each well, plates were agitated during 10 min before the collection of the eluted sample placed in clean Eppendorf. Samples were sequenced using the Illumina MiSeq Technology with Nextera XT library kit in $2 \times 250 \text{ bp}$ format.

2.4. Viral Sequences Treatment and Analysis

A total of 70,552,236 raw paired reads were obtained and trimmed, filtered, mapped and assembled into contigs/scaffolds using CLCgenomics7. Trimming parameters allowed the selection of sequences with a minimum length of 50 nucleotides and a quality above 0.05, resulting in 70,124,886 trimmed paired-reads. After assembly, the 828,771 contigs, with an average length of 596 bp (range 17 bp–117,982 bp), were aligned (BLASTx) against the *nr* database using DIAMOND [13]. The taxonomical annotations were then determined with MEGAN (LCA top percent = 0.1) [14]. Contigs assigned as bacteria or eukaryotes as well as non-assigned contigs were discarded and only the contigs assigned as viruses were analyzed (107,657 contigs). Viral sequences assigned as unknown viruses or unknown environmental marine viruses

(45.64% of the assigned viral sequences) were removed from the analysis of viral communities' diversity. Composition of the viral communities was represented at the family level using graphic tools of the *ggplot* package in RStudio (R version 3.5.3).

2.5. Statistical Analysis

The effect of tuna species, sex, sampling site and organs on the composition of viral communities was determined by a one-factor PERMANOVA with 999 permutations on Bray–Curtis matrix, using the “adonis” function of the *vegan* package [15]. For all tests statistical significance was assumed when p value < 0.05.

3. Conclusions

These results allow a pioneering characterization of the tuna meta-virome and provided new knowledge on the microbiome of marine organisms. Demonstrating the remarkable diversity of viruses associated with tuna, these findings raise questions about the role and implication of these viruses in the health of the host. Public health questions are also emerging as to whether these viruses can affect the health of fish consumers.

References

1. Barr, J.J. A bacteriophages journey through the human body. *Immunol. Rev.* 2017, 279, 106–122.
2. Knowles, B.; Silveira, C.; Bailey, B.A.; Barott, K.; Cantu, V.A.; Cobián-Güemes, A.G.; Coutinho, F.; Dinsdale, E.; Felts, B.; Furby, K.A.; et al. Lytic to temperate switching of viral communities. *Nature* 2016, 531, 466–470.
3. Laffy, P.W.; Botté, E.S.; Wood-Charlson, E.M.; Weynberg, K.D.; Rattei, T.; Webster, N.S. Thermal stress modifies the marine sponge virome. *Environ. Microbiol. Rep.* 2019, 9.
4. Cárdenas, A.; Ye, J.; Ziegler, M.; Payet, J.P.; McMinds, R.; Thurber, R.V.; Voolstra, C.R. Coral-Associated Viral Assemblages from the Central Red Sea Align with Host Species and Contribute to Holobiont Genetic Diversity. *Front. Microbiol.* 2020, 11, 572534.
5. Thurber, R.V.; Payet, J.P.; Thurber, A.R.; Correa, A.M.S. Virus–host interactions and their roles in coral reef health and disease. *Nat. Rev. Microbiol.* 2017, 15, 205–216.
6. Van Oppen, M.J.H.; Leong, J.-A.; Gates, R.D. Coral-virus interactions: A double-edged sword? *Symbiosis* 2009, 47, 1–8.
7. Pascelli, C.; Laffy, P.W.; Botté, E.; Kupresanin, M.; Rattei, T.; Lurgi, M.; Ravasi, T.; Webster, N.S. Viral ecogenomics across the Porifera. *Microbiome* 2020, 8, 1–22.
8. Filipa-Silva, A.; Parreira, R.; Martínez-Puchol, S.; Bofill-Mas, S.; Barreto Crespo, M.T.; Nunes, M. The Unexplored Virome of Two Atlantic Coast Fish: Contribution of Next-Generation Sequencing to Fish Virology. *Foods* 2020, 9, 1634.
9. Geoghegan, J.L.; Di Giallonardo, F.; Wille, M.; Ortiz-Baez, A.S.; Costa, V.A.; Ghaly, T.; Mifsud, J.C.; Turnbull, O.M.; Bellwood, D.R.; Williamson, J.E.; et al. Virome composition in marine fish revealed by meta-transcriptomics. *Virus Evol.* 2021, 7, veab005.
10. FAO. The State of World Fisheries and Aquaculture 2018—Meeting the Sustainable Development Goals; FAO: Rome, Italy, 2018.
11. Hungerford, J.M. Scombroid poisoning: A review. *Toxicon* 2010, 56, 231–243.
12. Monteil-Bouchard, S.; Temmam, S.; Desnues, C. Protocol for Generating Infectious RNA Viromes from Complex Biological Samples. In *The Human Virome*; Moya, A., Pérez Brocal, V., Eds.; Springer: New York, NY, USA, 2018; Volume 1838, pp. 25–36.
13. Buchfink, B.; Xie, C.; Huson, D.H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* 2015, 12, 59–60.
14. Huson, D.H.; Beier, S.; Flade, I.; Górski, A.; El-Hadidi, M.; Mitra, S.; Ruscheweyh, H.J.; Tappu, R. MEGAN Community Edition-Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. *PLoS Comput. Biol.* 2016, 12, e1004957.
15. Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* 2003, 14, 927–930.

