International Association for Food Protection WEBINAR

Don’t be Shellfish!
Use Next Generation Sequencing to Improve Seafood Safety and Quality

Moderator: *Marlee Mims & Rachel Rodriguez, FDA*

Sponsored by the IAFP FOUNDATION

4 MILLION$ for 40 YEARS an IAFP FOUNDATION milestone event

Please consider making a contribution

This webinar is being recorded and will be available to IAFP members within one week.

International Association for Food Protection

Webinar Housekeeping

- It is important to note that all opinions and statements are those of the individual making the presentation and not necessarily the opinion or view of IAFP.
- All attendees are muted. Questions should be submitted to the presenters during the presentation via the Questions section at the right of the screen. Questions will be answered at the end of the presentations.
- This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

Don’t be Shellfish! Use Next Generation Sequencing to Improve Seafood Safety and Quality

Organized by: IAFP’s Seafood Safety and Quality PDG

Moderated by: Marlee Mims and Rachel Rodriguez

Introduction

Seafood safety and quality research primarily focuses on two areas: the presence of pathogens and spoilage. Spoilage is traditionally evaluated using microbiological, chemical, and/or sensory analyses of products. Pathogens, including bacteria, viruses, and parasites, are commonly detected with molecular methods, including real-time PCR and Sanger sequencing. However, as next-generation sequencing (NGS) approaches such as metagenomics and whole genome sequencing (WGS) are more readily available, their use can provide a broader perspective on seafood safety and quality compared with classic molecular techniques. While WGS and metagenomic tools are frequently applied to other food commodities, the application of these powerful tools towards seafood safety, quality, and outbreak investigations is not as widespread. Key questions remain about NGS and seafood research. Focusing on seafood safety and quality, this webinar will delve into metagenomics and WGS as tools for a better understanding of pathogens and spoilage organisms, and their associated public health risks.

Learning Objectives

- Explore recent advancements of NGS tools in seafood safety and quality research and application of these tools to seafood spoilage with an emphasis of comparison to traditional methods.
- Apply metagenomics to bacterial and viral analyses of seafood safety.
- Discuss lessons learned in overcoming the challenges of seafood matrices to generate impactful NGS data for practical applications.

Foteini ‘Fay’ F. Parlapani, Ph.D.

Dr. Parlapani is an Assistant Professor in Molecular Microbiology, Safety and Quality of Seafood at the University of Thessaly since January 2021. Her interests focus on the monitoring of seafood spoilers and foodborne pathogens including Antibiotic Resistant Bacteria, following the Farm to Fork strategy for transition to a sustainable, fair, healthy, and environmentally friendly food system, via pathways of new –omics approaches including technologies such as NGS and modern analytical techniques. Last three years, she has introduced new strategies to end all forms of hunger and malnutrition in Sub-Saharan Africa and other poor regions in the world, as well as to ensure access to safe, nutritious and sufficient food for all; and she has suggested a new approach to track microbial and chemical hazards of seafood in both artificial (aquaculture) and natural ecosystems at State and Country levels.

International Association for Food Protection Annual Meeting 2023

Webinar:

Don't be Shellfish! Use Next Generation Sequencing to Improve Seafood Safety and Quality

Update on the use of NGS for spoilage and safety in seafood: How far have we come? How far do we have to go?

Dr Foteini F. Parlapani

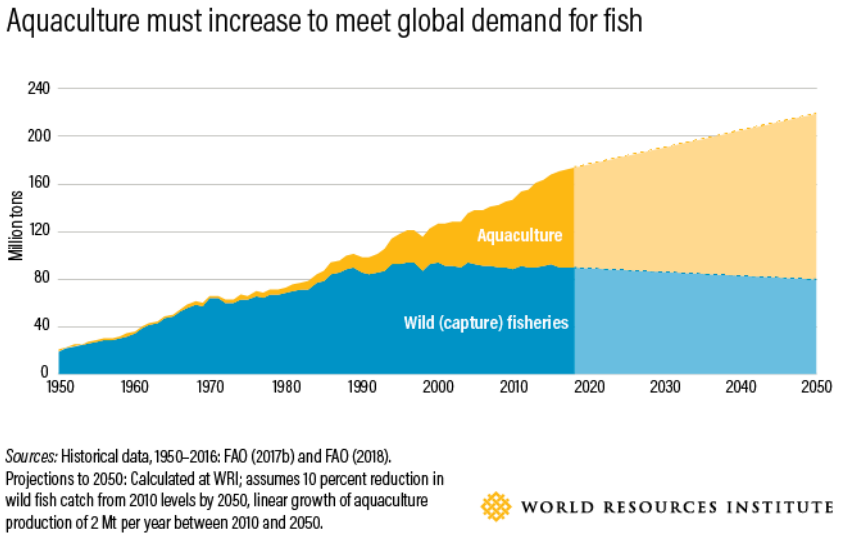
Assistant Professor, University of Thessaly, Volos, Greece

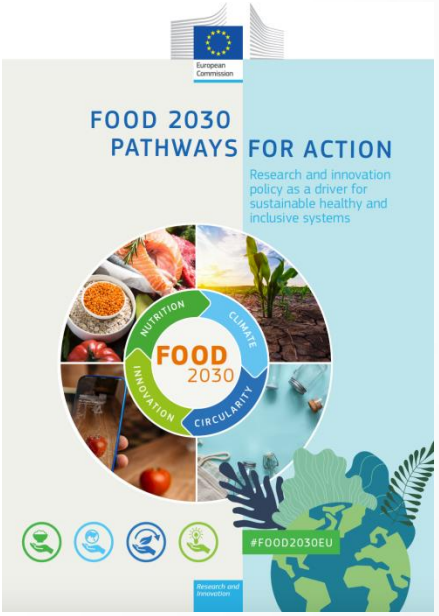
June, 2023

'...how to secure food for more than 9 billion people by 2050?'

To ensure Food Security and Nutrition for all:

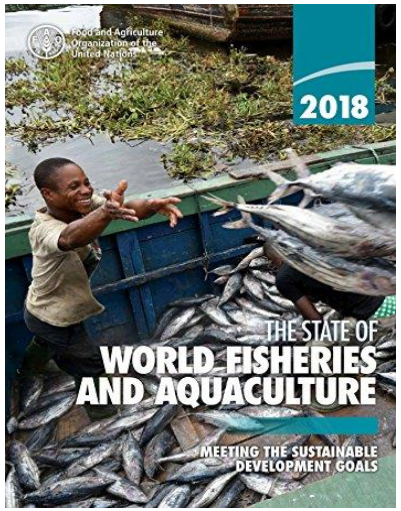
*we must **adopt** new **strategies** to **build** a **sustainable-fair**, **healthy** and **environmentally-friendly- food future for all***

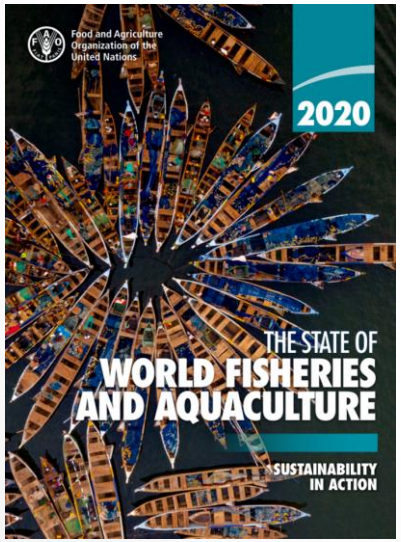


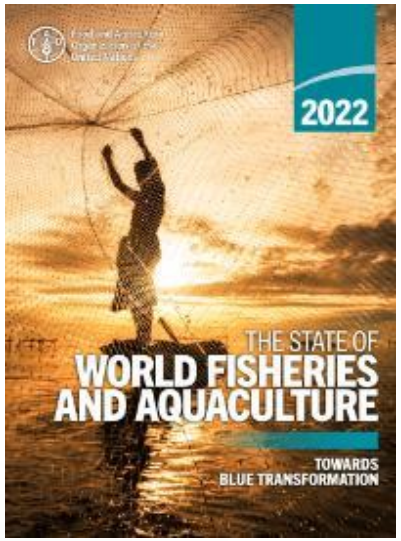


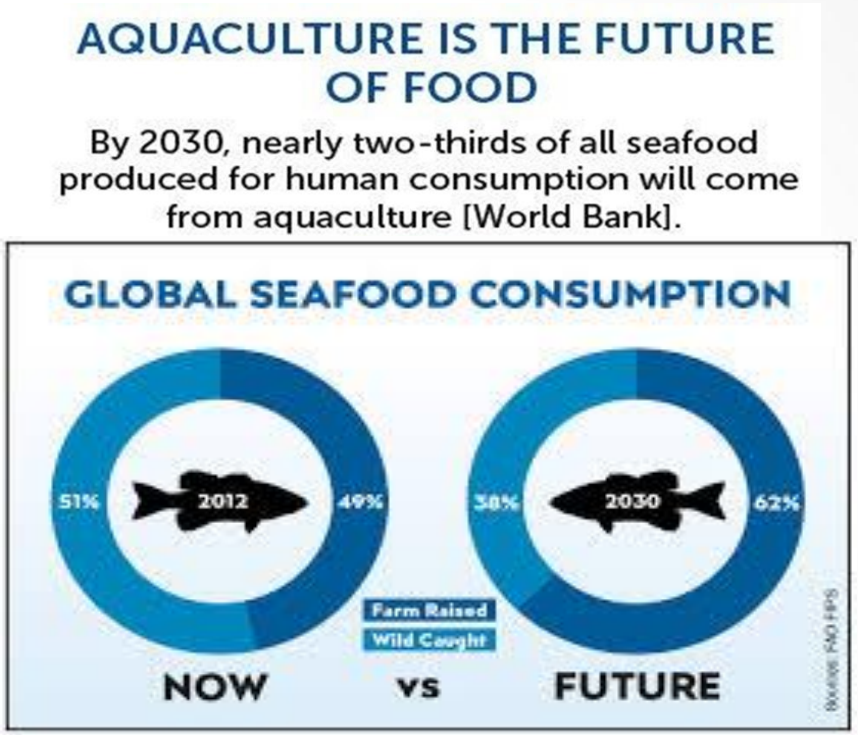
Research &
*Innovation as a **driver***

Fisheries and aquaculture



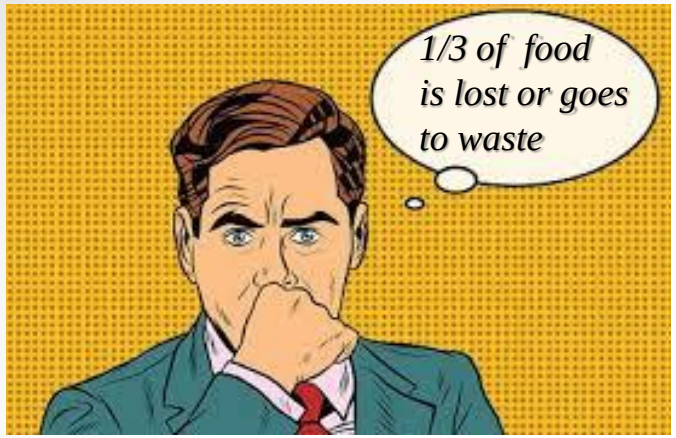






…both have tremendous potential to contribute significantly to Food Security and adequate Nutrition for a global population of 9.7 billion by 2050

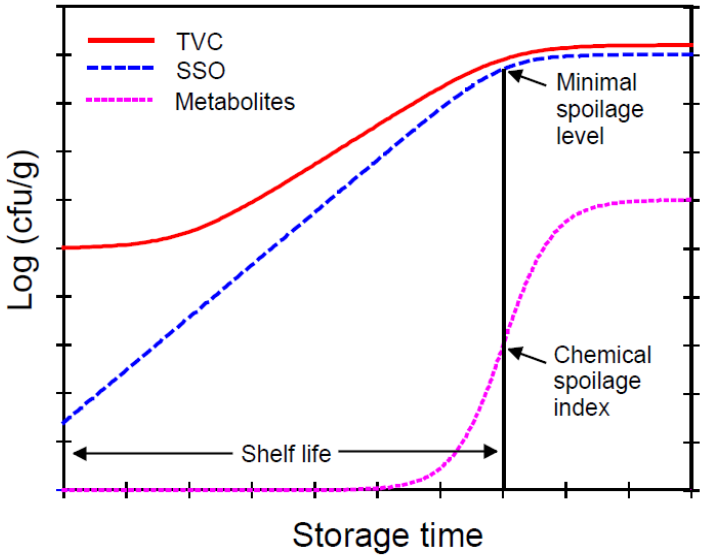
BUT
first, ensure Sustainability!!!

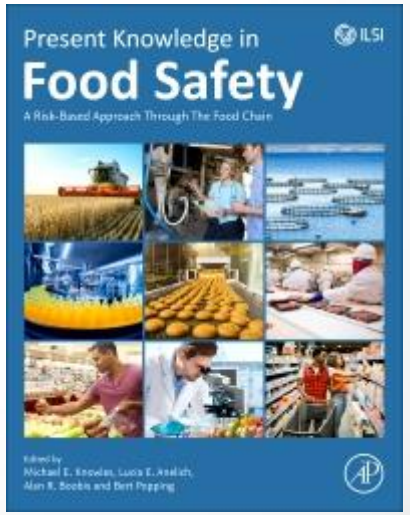


Major Reasons

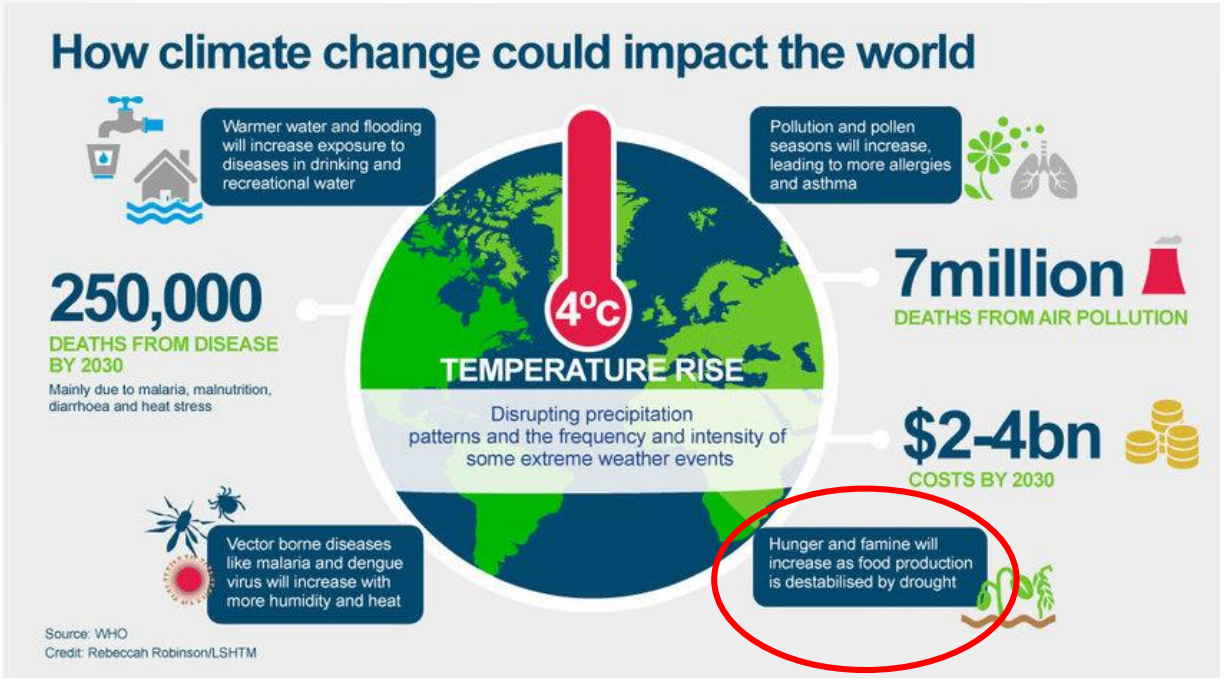
Microbial Spoilage & Microbial Safety

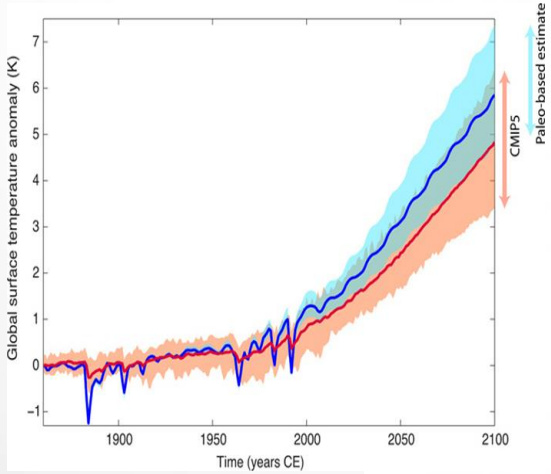
35%
FISH & SEAFOOD FOOD LOSSES

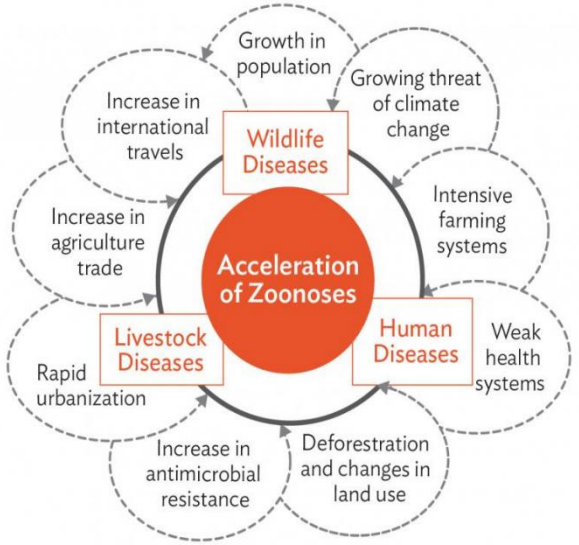


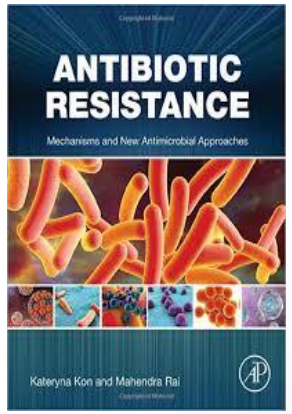


21st Century challenges accelerate our problems










CONSEQUENCES


FOOD
LOSS


Seafood Poisoning


BANKRUPTCY
BANKRUPTCY
BANKRUPTCY

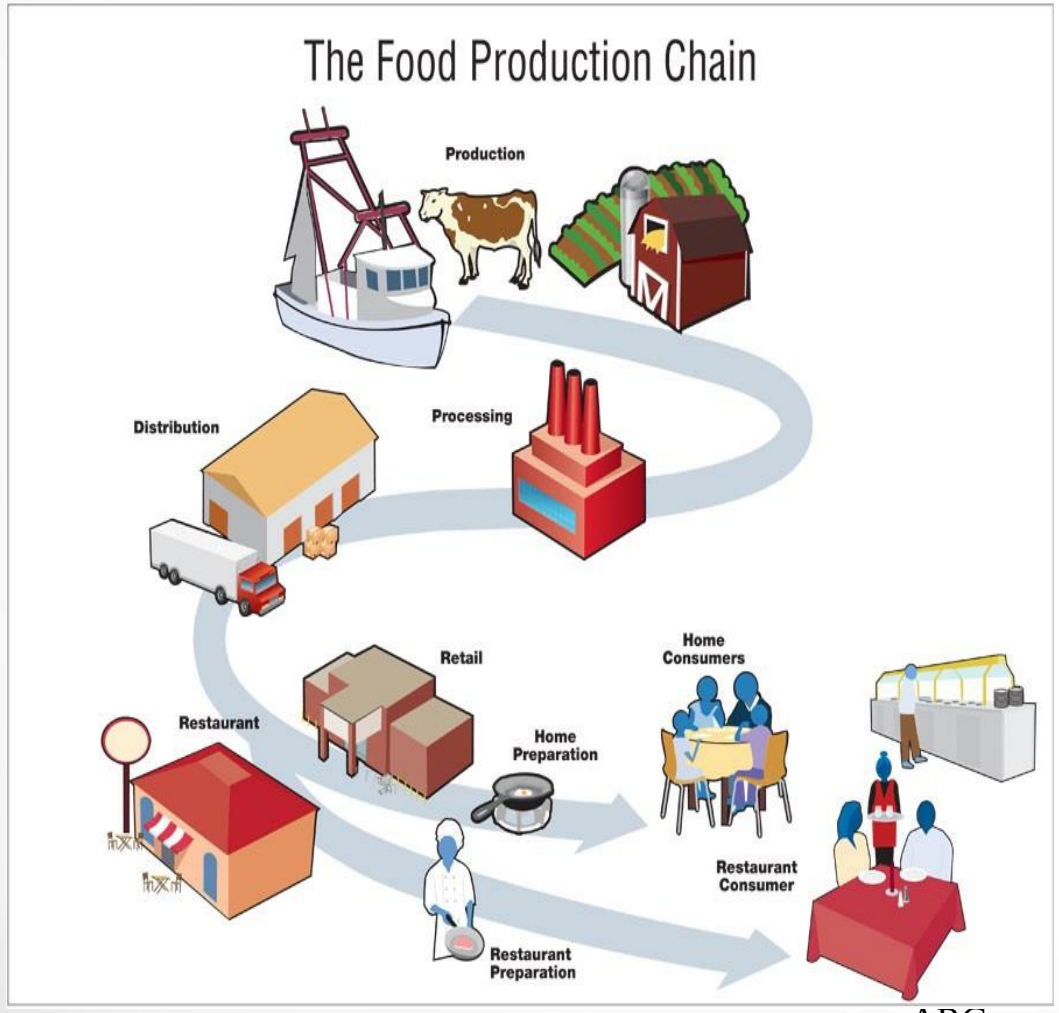

CLOSED


FIRED

Solutions?

Next Generation Sequencing along the whole food production chain

(from primary production to consumption)



NGS

provides theory-changing breakthroughs on

Microbiota
Microbial function
ARGs and MGEs

Gives answers on:

- *Who are there? (microbiota)*
- *What are they doing? (functionality)*

ARGs: antibiotic resistance genes, MGEs: mobile genetic elements

Why NGS vs others?

How far have we come?

Traditional Microbiology

- **We find less than 1% of the total microbiota present in an environmental sample (or higher in food samples)**

- laborious and time-consuming
- many fish spoilage microorganisms cannot grow on some general growth media
- might not allow stressed or sublethally injured cells to recover and grow on selective media

Molecular Microbiology (other than NGS)

- **We find fish microbiota able to grow on culture media (culture-dependent) or the dominants present in a sample (culture-independent)**

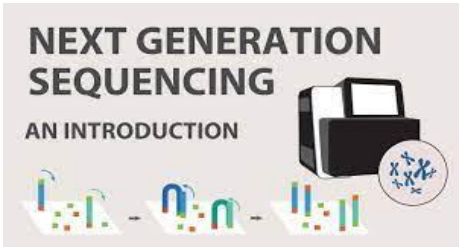
They allow a deeper and clearer picture about microorganisms existing or dominating in seafoods, compared to the classical ones, **but none of them have allowed a full description of the microbiota present in a sample** (e.g. finfish, shellfish).

Identify known spoilers and pathogens

NGS based Microbiology

- **We find all microorganisms or genes present in a sample - *a full description of the microbiome directly from the sample***

- Rapidly sequence whole genomes
- Deeply sequence target regions
- Utilize RNA sequencing (RNA-Seq) to discover novel RNA variants
- Utilize RNA sequencing (RNA-Seq) to quantify mRNAs for gene expression analysis
- Identify unknown/novel pathogens and spoilers



Identify unknown spoilers and pathogens

NGS: The Revolution in Microbiology

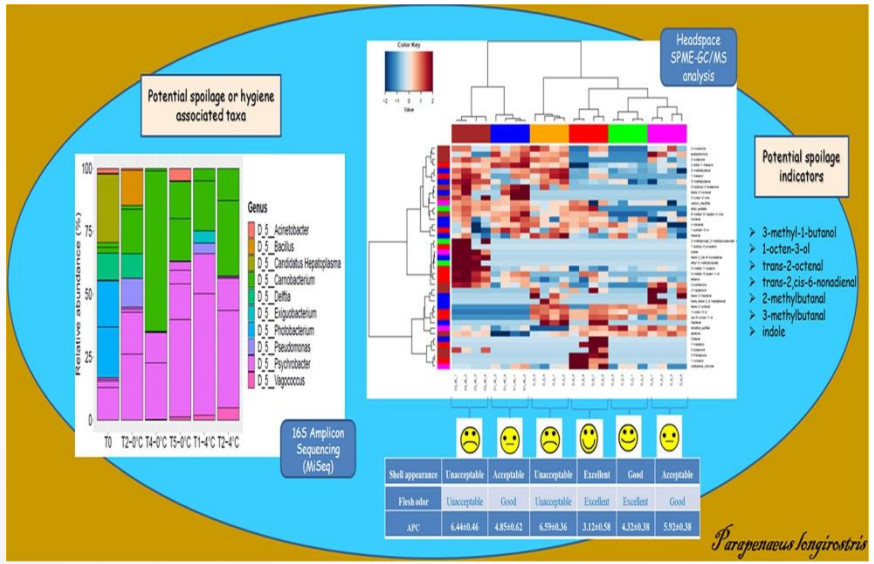
How far have we come?

Who are there?

Microbial structure/microbiota (Amplicon sequencing or Metabarcoding – Metataxonomics)

- Amplification and sequencing of marker/phylogenetic genes (e.g., 16S, 18S, ITS) of microbial communities

- *Identification of Operational Taxonomic Units (OTUs) or Amplicon sequence variants (ASVs)*



Parlapani et al. 2020 *Food Research International* 132, 109057

What are they doing?

Microbial function, ARGs and MGEs abundance & diversity (Metagenomics)

- Random shotgun sequencing of the whole genomic content of microbial communities

- *Prediction of function (DNA-seq)*
- *Function (RNA-seq)* encoded by microbial communities

**Whole Genome Sequencing (WGS)*

- determine the whole genome of **an organism**

✓ Characterize foodborne pathogens
✓ Track disease outbreaks

Link genotype with phenotype

We better understand '*what they are doing*' when metabarcoding & shotgun sequencing combined with:

- Meta-transcriptomics (gene expression)
- Proteomics (protein identification/profile)
- Metabolomics (metabolic identification/profile)

Tackle spoilage problems of fisheries, aquaculture & fish processing industry

How far do we have to go?

*Reveal **all** microbial potential **spoilers** and **sources** of contamination **using NGS***

↓

Study their function & link with quality parameters in seafood

↓

Develop manuals of potential spoilers and their control to minimize contamination of fish with fish spoilers along the whole production chain

↓

Develop simple, rapid and reliable methods to find rapidly spoilers and sources of contamination to control them

↓

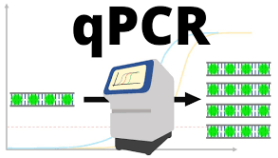
Educate stakeholders

↓

Maximize fish shelf-life
Minimize fish losses

Reveal spoilers & their sources

DO Research - NGS



Find & quantify Bacterial genera or Specific Spoilage Genes! (recommended by Prof. Boziaris)

Develop manuals for stakeholders

Educate stakeholders on new findings

Tackle safety problems of fisheries, aquaculture & fish processing industry

How far do we have to go?

Reveal hazards & their sources

DO Research - NGS

Update guidance & Standards based on NGS findings

Educate all on new findings

Stop fish losses

*Reveal **all** microbial potential **hazards** and sources of contamination using NGS*

↓

*Measure & evaluate risks **from farm to fork** (Risk Assessment)*

↓

***Update the guidance of hazards and their control based on NGS findings** to ensure safety of fish*
Develop manuals for sustainable seafood systems

↓

Develop simple, rapid and reliable methods to find rapidly hazards and sources of contamination to control them

↓

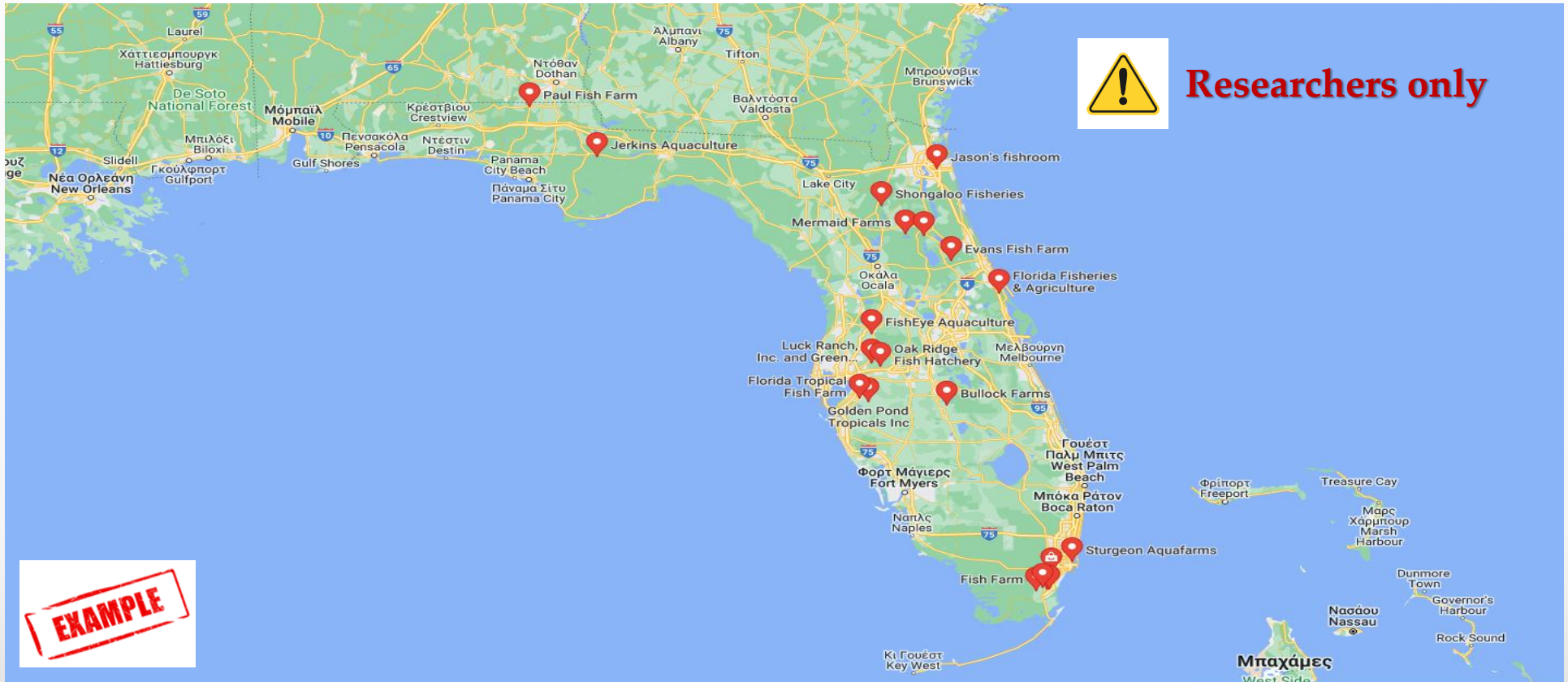
Educate stakeholders

↓

Produce safe fish
Minimize fish losses

*To **develop manuals for sustainable seafood systems** and to **update guidance & Standards** based on new/NGS findings... do research at large scale*

- ***Collect samples** (e.g., water, fish, fish feed, equipment, nets, vessels, vehicles, workers) from **various** fish (finfish, shellfish, etc.) farms*
- ***Analyze** the samples using **NGS** (metataxonomics, metagenomics) and **link** with **phenotype***
- ***Update guidance** and **Standards** & develop manuals **based on NGS findings***
- ***Educate** policymakers/regulators, officers and all relevant stakeholders on **(i)** new or updated guidance, Standards and manuals, and **(ii)** rapid methods for spoilers & hazards determination (detection & quantification)*



Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Food Science

Microbial diversity of seafood

Foteini F Parlapani

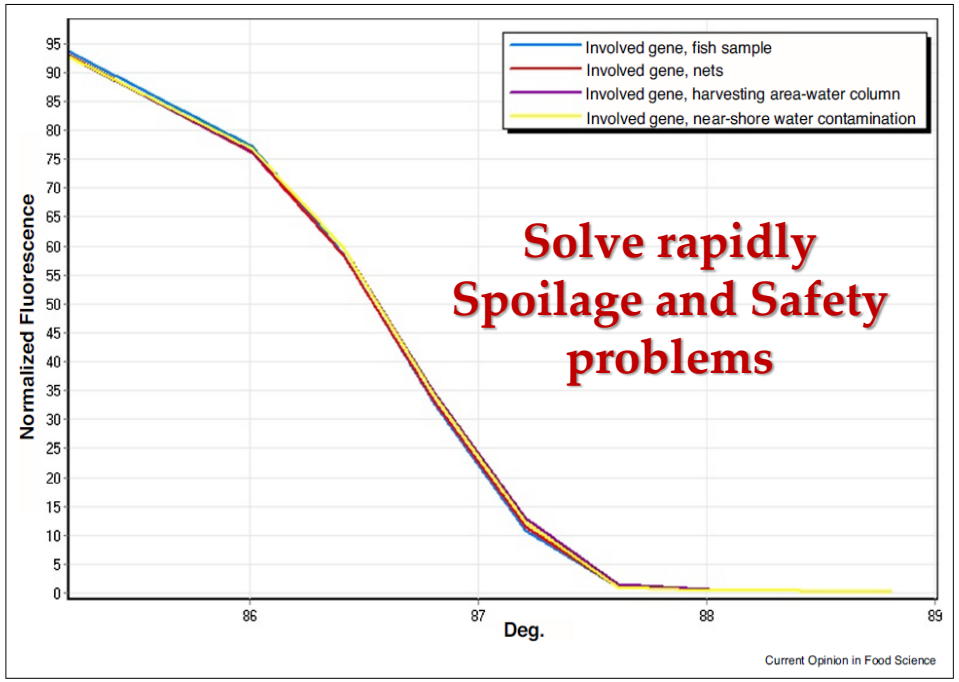
New challenges on seafood microbial quality and safety

Climate change and Antimicrobial Resistance are serious challenges of 21st century. The warming water can increase abundance of autochthonous *Vibrio*, including food-borne strains, and various enteric pathogens that end up into aquatic environments from inland so cause more seafood-borne illnesses in consumers due to the consumption of contaminated seafood [47]. Shellfish are expected to be the most significant means of transporting seafood-borne pathogens in humans since they are mainly

New strategies to ensure microbial safety and quality of seafood from farm to fork

To tackle new challenges, scientists attempt to establish intelligent strategies to follow. The development of reliable methodologies to detect, quantify and monitor spoilers, pathogens and antibiotic resistance genes, the updating of legislation and policy based on new insights on microbial diversity of seafood, the collaborative partnerships with stakeholders (e.g. research and education) and

Figure 1



Normalized HRM curves of a virulence or antimicrobial resistance gene in fish and three environmental samples (total samples number: 36, negative results: 32 samples). In diagram, the positive samples are only indicated.

For stakeholders use
Developed by researchers!!

Use rapid protocols to detect and quantify pathogens, antibiotic resistant bacteria and spoilers during fish production, processing & distribution

Reveal the sources of microbial contamination

Follow the guidelines of the new manuals or updated guidance/Standards to control spoilers and pathogens

Extension activities based on new or updated knowledge – *derived by NGS*

''School of Sustainable Fisheries, Aquaculture and Fish processing''

How much further do we have to go?

Updating the guidance & Standards using Next Generation Sequencing

Performing Extension activities based on new findings

Improve food systems & Create Sustainable Environments

Tackle hunger and malnutrition & Promote Health

Promote Economy & Create New Sources of Growth

Sustainability


Thank you

Marlee Hayes-Mims, M.S.

Marlee is a biological laboratory technician at the US FDA researching bacterial and chemical indicators of decomposition in seafood. Before joining the FDA in 2018, Marlee received her master's degree at the University of Alabama at Birmingham. At the Gulf Coast Seafood Lab in Dauphin Island, she studies molecular tools and methods to identify bio markers of decomposition in seafoods and will be working towards a PhD, starting this fall with the University of South Alabama and the Dauphin Island Sea Lab.

FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

Culture-dependent versus Culture-independent Sequencing Methods for Bacterial Community Analysis of Decomposing Shrimp

Marlee Hayes-Mims, Kristin Bjornsdottir-Butler, Ronald A. Benner, Jr.
U.S. FDA Gulf Coast Seafood Laboratory
Dauphin Island, AL

Identification of Microbial Biomarkers During Seafood Decomposition

- Bacterial communities during decomposition are influenced by storage conditions, including temperature and time of storage and pre-harvest and harvest conditions
- Understanding the bacterial community composition from pre-harvest through storage is critical for informing seafood decomposition guidance and mitigating potential human health risks associated with consumption of seafood products

Goal: Biomarker(s) of decomposition

Biomarkers - Shrimp Decomposition

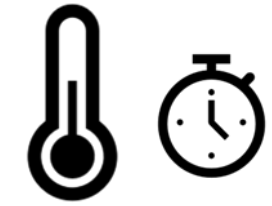
- Goal: Biomarker(s) of decomposition
- Objective: compare results from 16S Sanger sequencing (culture-dependent) and targeted metagenomic 16S sequencing (culture-independent)

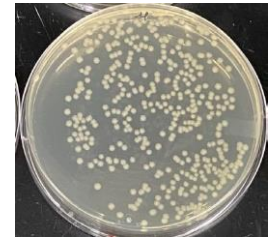
Methods

- Live shrimp were acquired from a local fisherman in the Gulf of Mexico/Mobile Bay and expired in an ice-water bath immediately
- Shrimp were then beheaded, rinsed, and weighed into plastic sample bags
- Incubated at 0, 12, 24, and 36^{o}C for 20 days, 72 hours, 24 hours, and 12 hours, respectively
  - Sampling every 4 days, 12 hours, 8 hours, and 4 hours at 0, 12, 24, and 36^{o}C, respectively

Methods
Culture-dependent

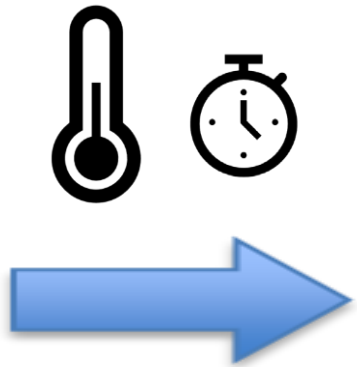
- Isolates cultured on TSA
- 48 single colonies picked per countable plate per time point
- Isolates purified and DNA extracted
- 16S Sanger Sequencing via 3500 XL Genetic Analyzer



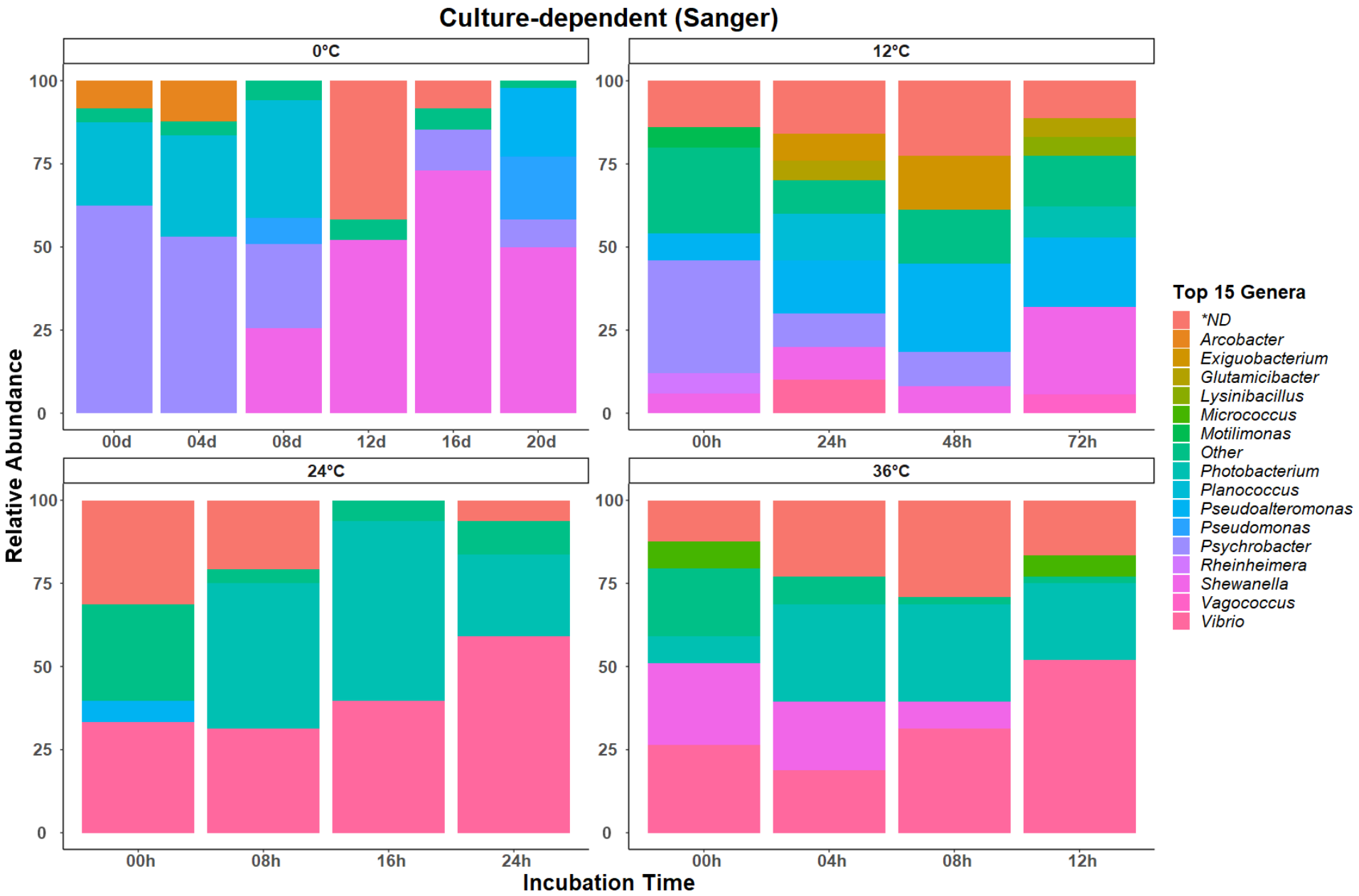


Methods
Culture-independent

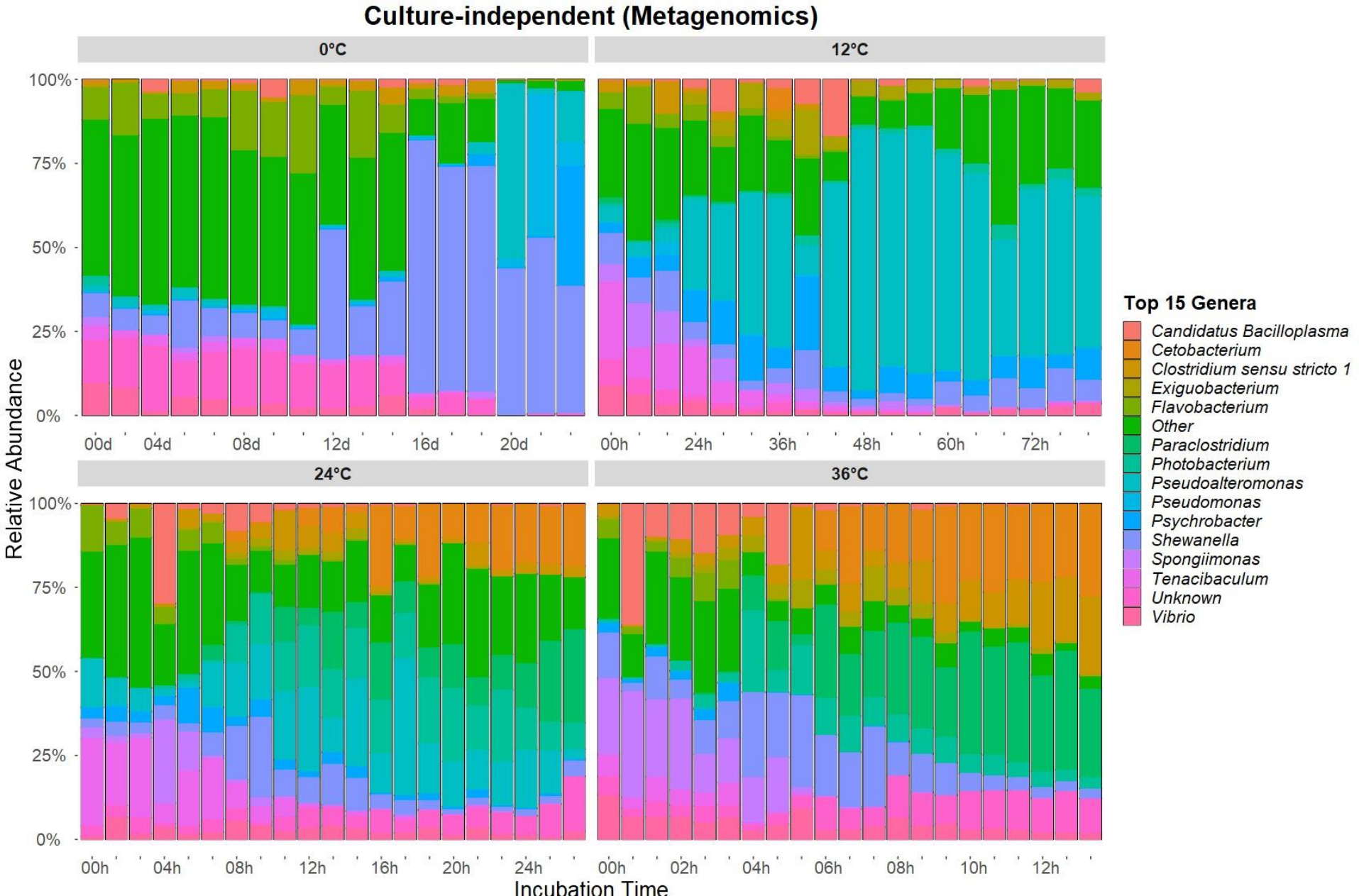
- Low spin of fish homogenate
- DNA extraction using ZymoBIOMICS Magbead DNA Kit
- 16S gene is barcoded and amplified
- Targeted 16S Metagenomic Sequencing via Illumina MiSeq platform (V3 chemistry)



Results


Culture-dependent (Sanger)
0°C
12°C
24°C
36°C
Relative Abundance
100
75
50
25
0
00d
04d
08d
12d
16d
20d
00h
24h
48h
72h
08h
16h
04h
12h
Incubation Time
Top 15 Genera
*ND
Arcobacter
Exiguobacterium
Glutamicibacter
Lysinibacillus
Micrococcus
Motilimonas
Other
Photobacterium
Planococcus
Pseudoalteromonas
Pseudomonas
Psychrobacter
Rheinheimera
Shewanella
Vagococcus
Vibrio

Results


Culture-independent (Metagenomics)
0°C
12°C
24°C
36°C
Relative Abundance
100%
75%
50%
25%
0%
00d
04d
08d
12d
16d
20d
00h
24h
36h
48h
60h
72h
00h
04h
08h
12h
16h
20h
24h
00h
02h
04h
06h
08h
10h
12h
Incubation Time
Top 15 Genera
Candidatus Bacilloplasma
Cetobacterium
Clostridium sensu stricto 1
Exiguobacterium
Flavobacterium
Other
Paraclostridium
Photobacterium
Pseudoalteromonas
Pseudomonas
Psychrobacter
Shewanella
Spongiimonas
Tenacibaculum
Unknown
Vibrio

Take-Aways

- Potential candidates for biomarkers were identified at all temperatures studied but varied across the temperatures

- Varying results depending on the sequencing method
  - Greater number of genera identified with culture-independent method
  - Culture-dependent may have limitations but may be better for species level identification

Future Directions

Continue research to understand bacterial communities and changes in decomposition dynamics for shrimp

Evaluating pre-harvest and harvest factors that influence bacterial community composition during decomposition

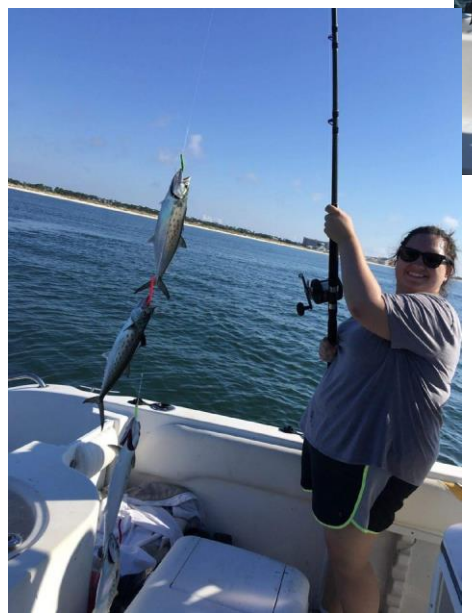
Continue work with other species (Snapper and Spanish Mackerel)

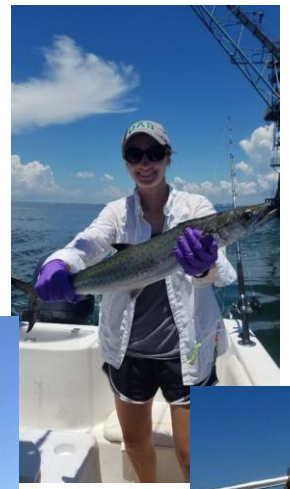
Poster(P3-267)- IAFP 2023 in Toronto Canada

Acknowledgements

FDA Gulf Coast Seafood Laboratory

- Dr. Ann Abraham
- Dr. Carolyn Simmons
- Madison McGough
- Sarah May
- Dr. Keri Lydon
- George Doup
- Ashley Frith






FDA
U.S. FOOD & DRUG
ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

Sabrina Mace, Ph.D.

Dr. Macé is a research scientist who has been working on seafood bacterial communities at the French Research Institute for Exploitation of the Sea (IFREMER) since January 2020. She studies seafood microbiota and its functionality in order to decipher bacterial spoilage of those products. With her colleagues, she also works on a biocontrol approach to propose new tools to improve seafood safety and quality.

IDENTIFICATION OF SPOILAGE ORGANISMS VIA 16S SEQUENCING: WHAT'S THE RELATION TO SENSORY ANALYSIS?

SABRINA MACÉ

IAFP WEBINAR

JUNE 27, 2023

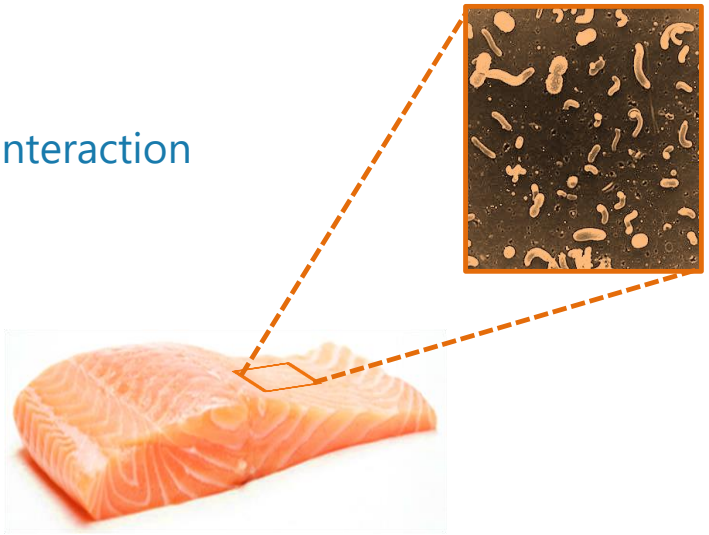
SEAFOOD PRODUCT SPOILAGE MICROBIOTA

Highly perissable – important economical lost - bacterial spoilage

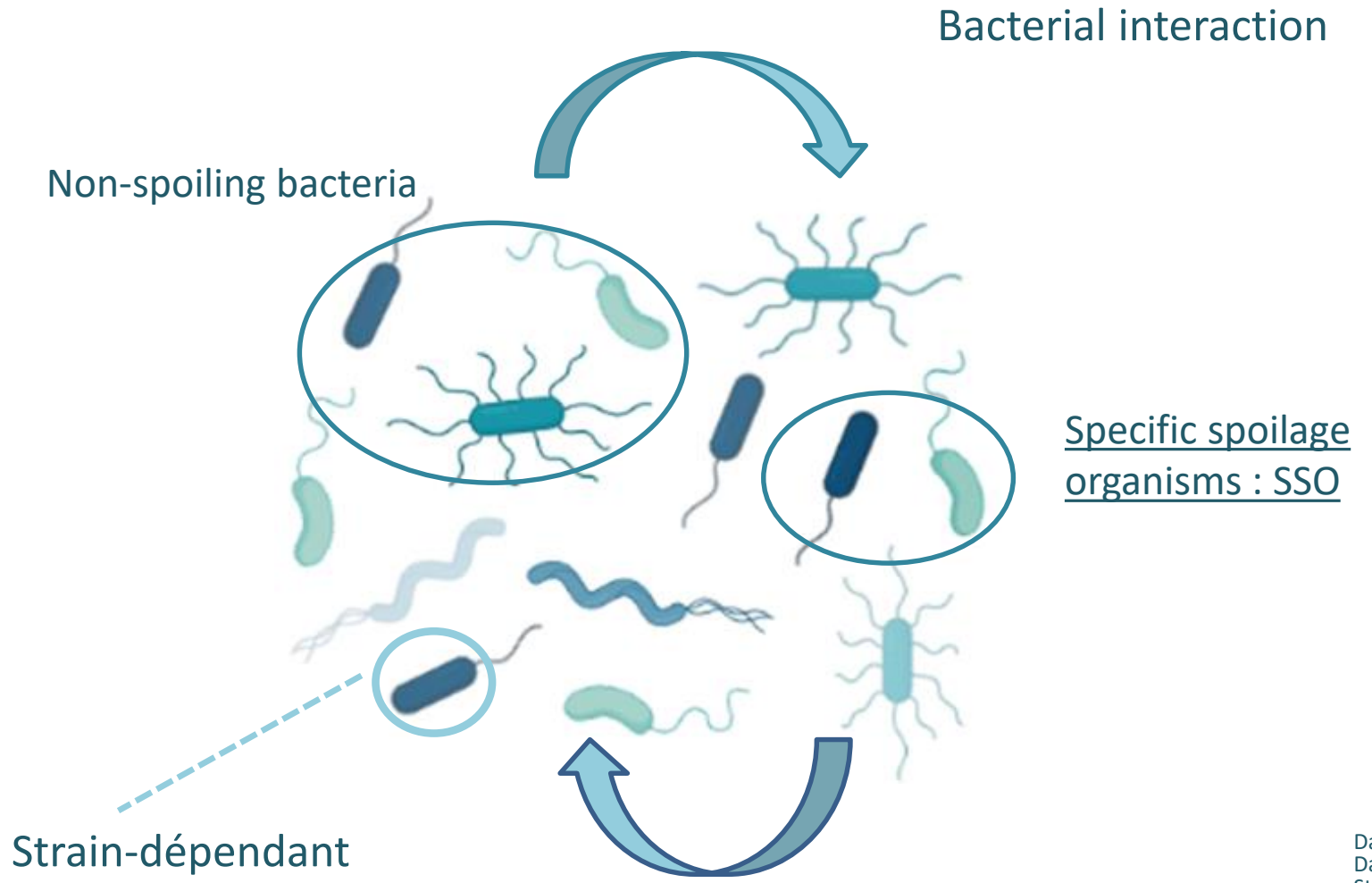
- Bacterial communities evolve during storage
- Microbiota specific of a given product

Abiotic : process, packaging, storage

Biotic : initial community structure, bacterial interaction



BACTERIAL COMMUNITY AND SPOILAGE



Dalgaard, 1995
Dalgaard, 2000
Storh *et al*. 2001
Gram *et al.*, 2002
Gram & Dalgaard, 2002
Laursen *et al,*. 2006

SSO IN SOME SEAFOOD AND THEIR AROMA CARACTERISTICS

Carnobacterium

Butter
Feet/cheese
Sour

Photobacterium phosphoreum

Sour
Amine

Brochothrix thermosphacta

Sour
Feet/Cheese

Carnobacterium

Shewanella baltica

Butter
Foot/cheese
Sour

Cabbage
Sour
Sulfur

Shewanella

Sulfur

Lactobacillus

Sour

Photobacterium phosphoreum

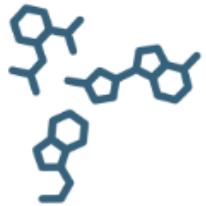
Sour
Amine

Leroi, 1998
Stohr, 2001,
Brillet, 2005
Macé, 2013
Fall,2012
Jaffres, 2011
Macé, 2014

from CULTURE-DEPENDENT to CULTURE-INDEPENDANT method

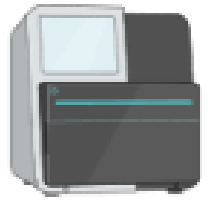
For more than two decades, studying bacterial ecosystems with combining approach :

- Classic microbiology
- Biochemistry
- Sensory analysis


In recent years, with the use of **16S rDNA gene metabarcoding**

Shrimp, tuna, salmon, sea bass, cuttle fish and blue crab




POWERFUL TOOL to DESCRIBE

- The global structure of bacterial communities
- Their dynamics during storage

What is the link with sensory analysis ?

EXPERIMENT EXAMPLE

3 Seafood product plant production (L1, L2, L3)

STORAGE: 7 days at 4°C, then at 8°C

D8 **D15** **D22** **D29**

4 time-points x 3 factories = **12 samples**

Sensory Analysis

Métabarcoding 16S

SENSORY ANALYSIS

- Expert jury= 11 panelists
- 12 samples (4 time-points *3 plants)

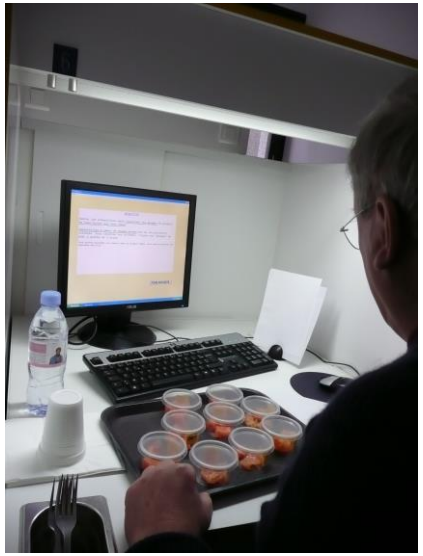
- Mean score of 11 panelists

- 10 Variables : Odor, Flavor, Texture

O-Global spoilage
O-Acid
O-Amine
O-Sulfur
O-Sour
O-Smoke

- **F-Smoke**
- **F-Acid**
- **F-Amine**

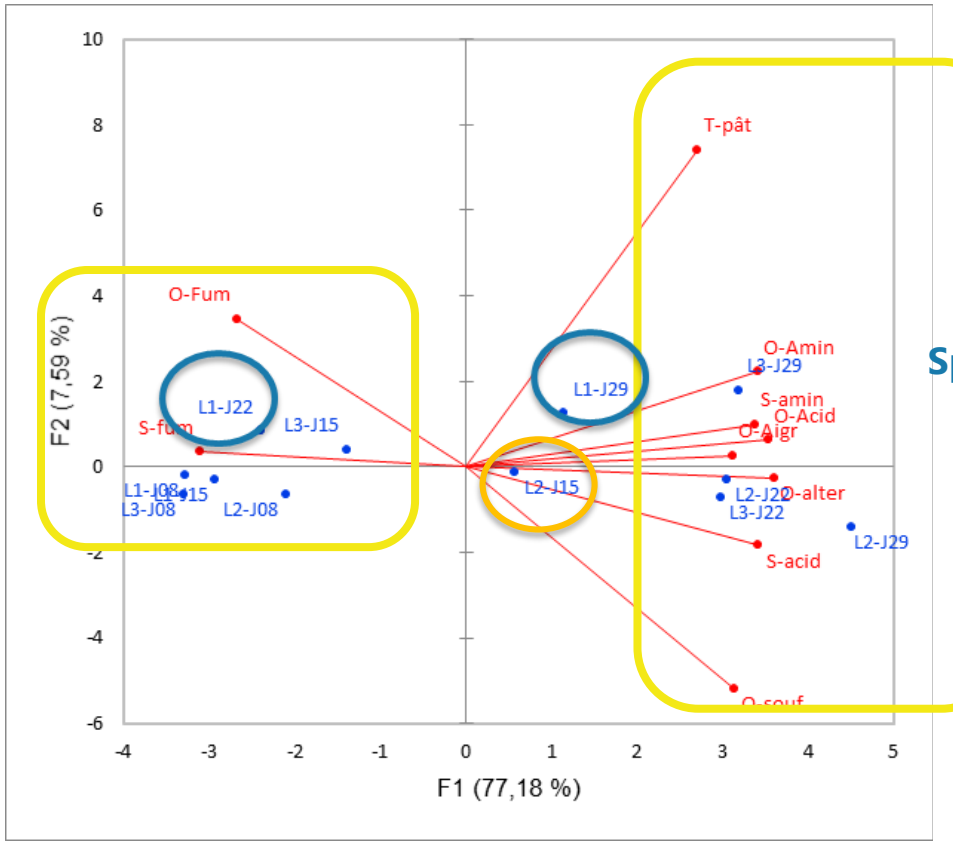
- **T-Pasty**
- **T-Firm**



SPOILAGE ASSESSEMENT

Principal Componant Analysis

Non-Spoiled (D08/D15)



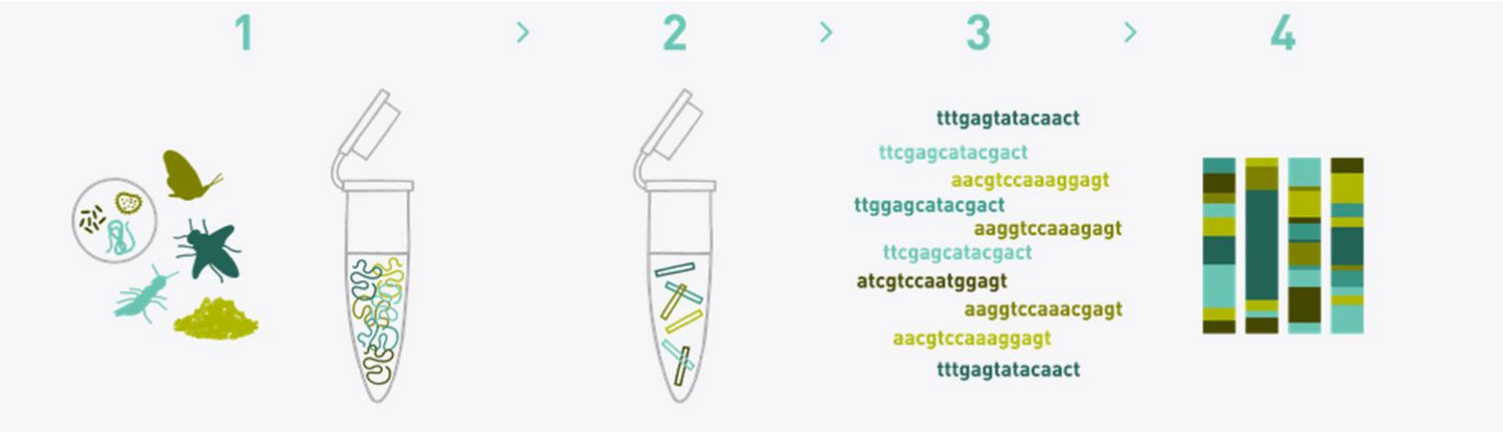
Spoiled (D22, D29)

L1 : Factory 1
L2 : Factory 2
L3 : Factory 3

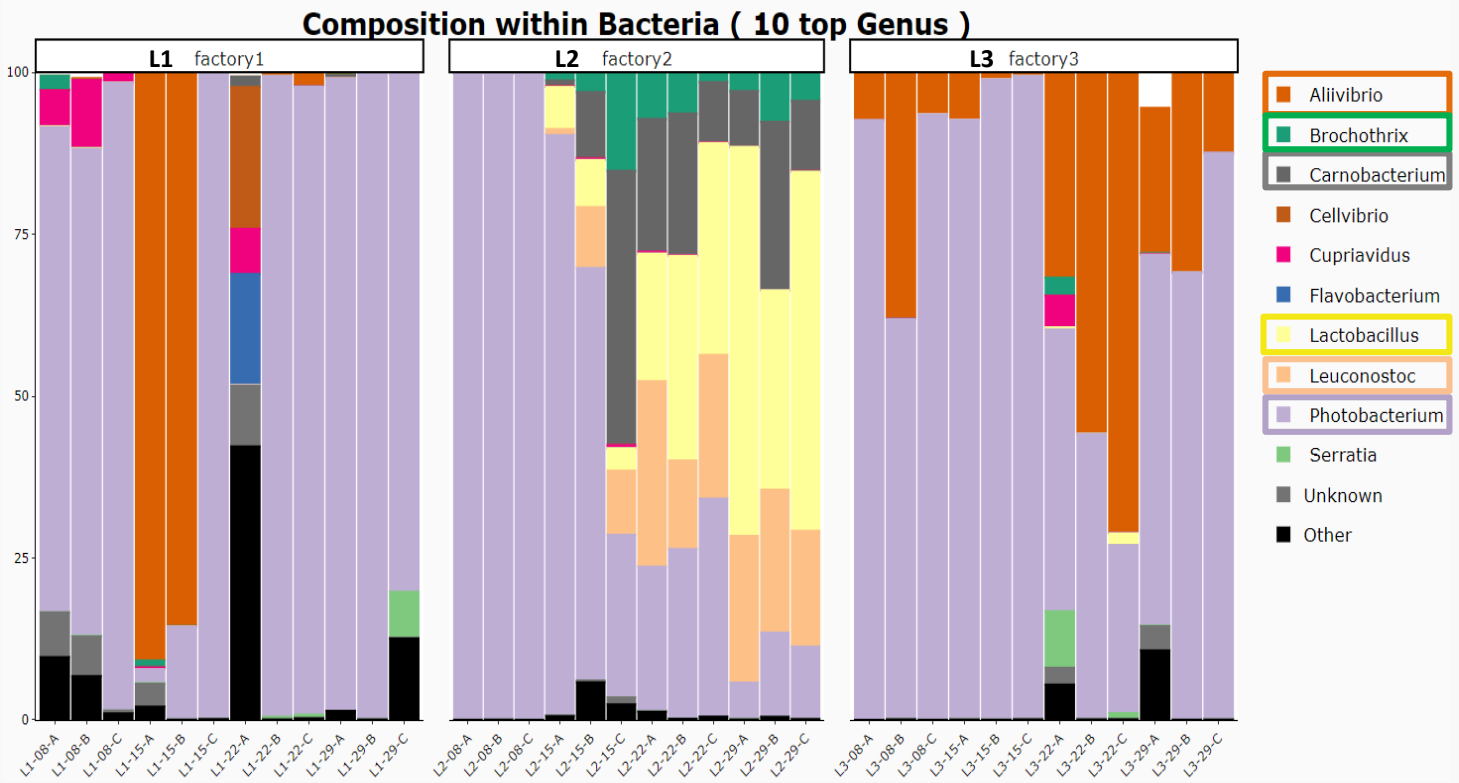
Spoilage :
L1 < L3 < L2

METABARCODING ANALYSIS

12 DNA samples isolated in the laboratoire (in triplicates)
Illumina by Microsynth platform sequencing- V3-V4 16S rDNA gene
Bioinformatic analysis with Samba (SebiMER-Ifremer bioinformatic service)



Bacterial community structure evolution during storage



L2 ≠ different profile : LAB and change during storage : Spoilage *Lactobacillus* ?

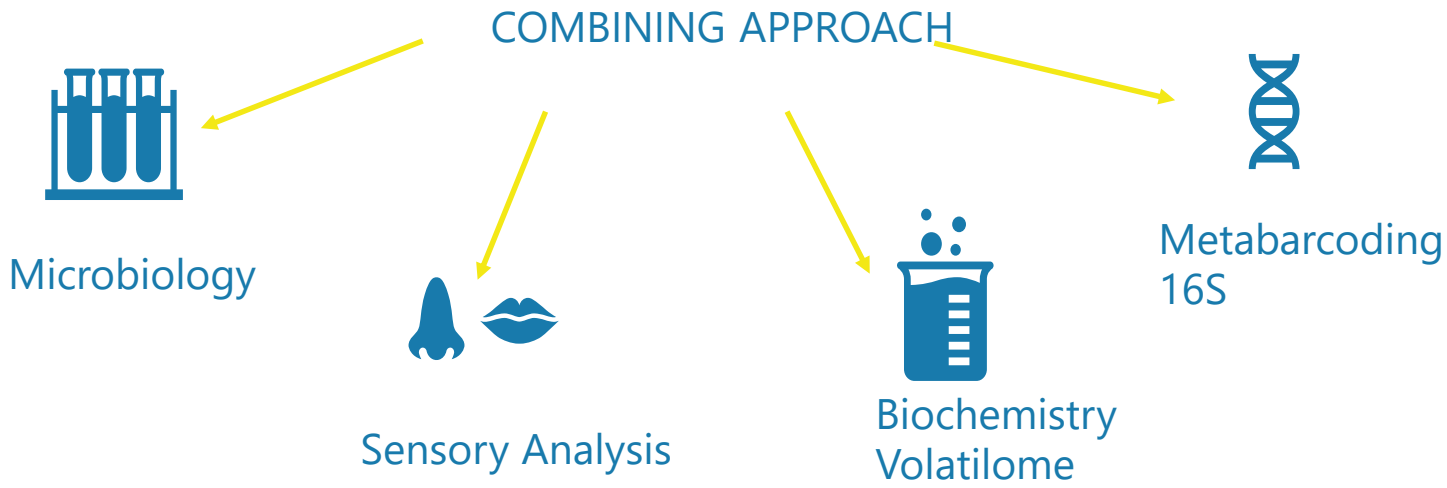
L1 & L3 close profiles : *Photobacterium, Allivibrio*

BUT L1 less spoiled … MISSING INFORMATION !

CONCLUSION and PERSPECTIVES

- QUANTIFICATION is required as it is link with off odors/metabolites production
- CORRECTION of NB of COPIES 16S gene
  i.e. Photobacterium (more than 10 copies de 16S rDNA gene) can hide some informations
- VIABILITY (amplification of dead bacteria)

FOR NOW



Relationship between these data ⇨ Multibloc statistical analysis (ComDIM)

Thanks to laboratory Microbial Ecosystems and Marine Molecules for Biotechnology (EM^{3}B) team

Thank you !

Christopher J. Grim, Ph.D.

Dr. Grim is a molecular biologist with over 20 years of experience in microbial ecology, bacterial genomics, metagenomics, infectious disease, public health, and food safety. Dr. Grim received his Ph.D. in Environmental Molecular Biology/Biotechnology from the University of Maryland, College Park. Dr. Grim joined FDA in 2009 and serves as a Research Microbiologist in the Center for Food Safety and Applied Nutrition. Dr. Grim's work is focused on integrating genomics and metagenomics into the areas of pathogen detection and subtyping, virulence assessment, improved method development, and source tracking of the foodborne pathogens, antimicrobial resistance and emerging hazards.

Metagenomics for *Vibrio* Illness Investigation? Lessons from Implicated Seafood

IAFP Webinar: “Don’t be Shellfish! Use Next Generation Sequencing to Improve Seafood Safety and Quality”

Christopher J. Grim, Ph.D.

Research Microbiologist

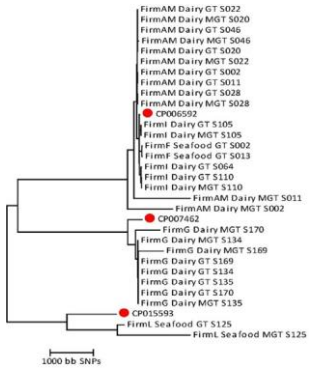
Center for Food Safety and Applied Nutrition

Office of Regulatory Science

Tel +240-402-3582

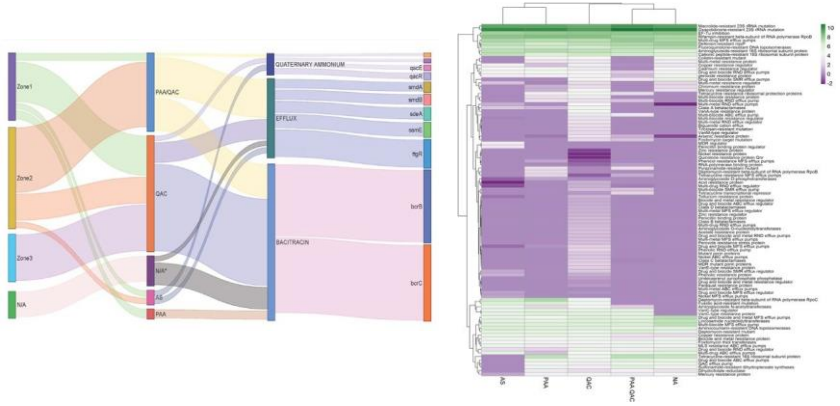
Christopher.Grim@fda.hhs.gov

Advancing Pathogen Detection and Subtyping Using Metagenomic Approaches



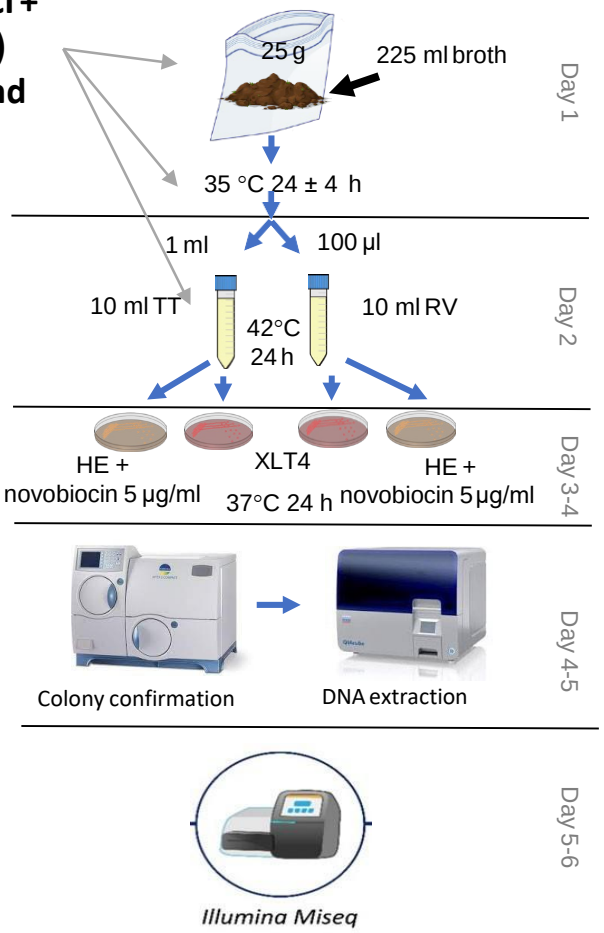
Culture independent- and quasi-metagenomics (CI + minimal enrichment) pathogen detection and subtyping

Functional Gene Content - Resistome

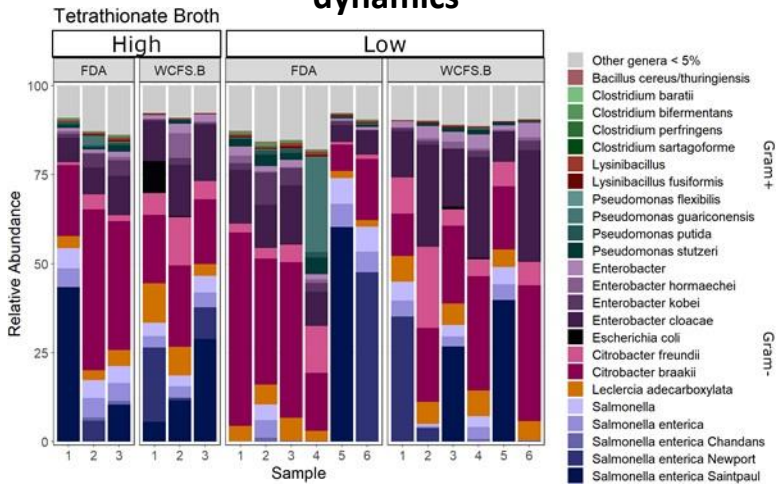


Long-read sequencing platform and chemistry improvements

FDA BAM protocol

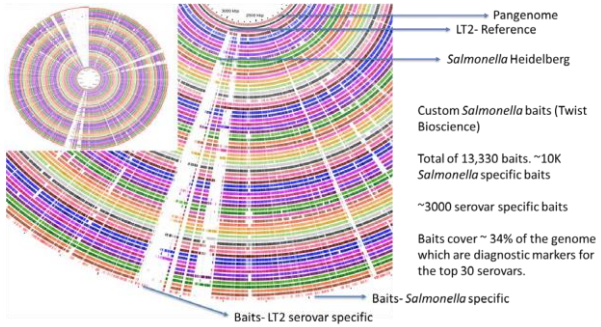


Evaluation and improvement of culture enrichment dynamics



Targeted, precision metagenomic approaches (hybridization capture)

***Salmonella* Baits Panel v3.0**



Two Case Studies: Crab and Scallop

Case Study 1: Implicated cooked **whole blue crabs**

- CIDT stool positive for "*Vibrio*"; single case
- Cooked, chilled crabs collected and analyzed by FDA BAM methods (Chapter 9); received chilled (6°C)
- Real-time PCR (pre- and post-enrichment) positive for *Vp* and *Vv*
- Pre-enrichment DNA positive for pathogenic *Vp* (*tdh*+ and *trh*+)
- Culture negative for *Vibrio* spp.

Two Case Studies: Crab and Scallop

Case Study 2: Implicated frozen **scallops**

- Consumer complaint, associated with consumption of frozen scallops
- Partial bag of frozen scallops collected and analyzed by FDA BAM methods (Chapter 9); shipped on dry ice
- Real-time PCR positive for *Vp* and *Vv* (pre-enrichment only), single *V. alginolyticus* (WGS identification) culture isolate

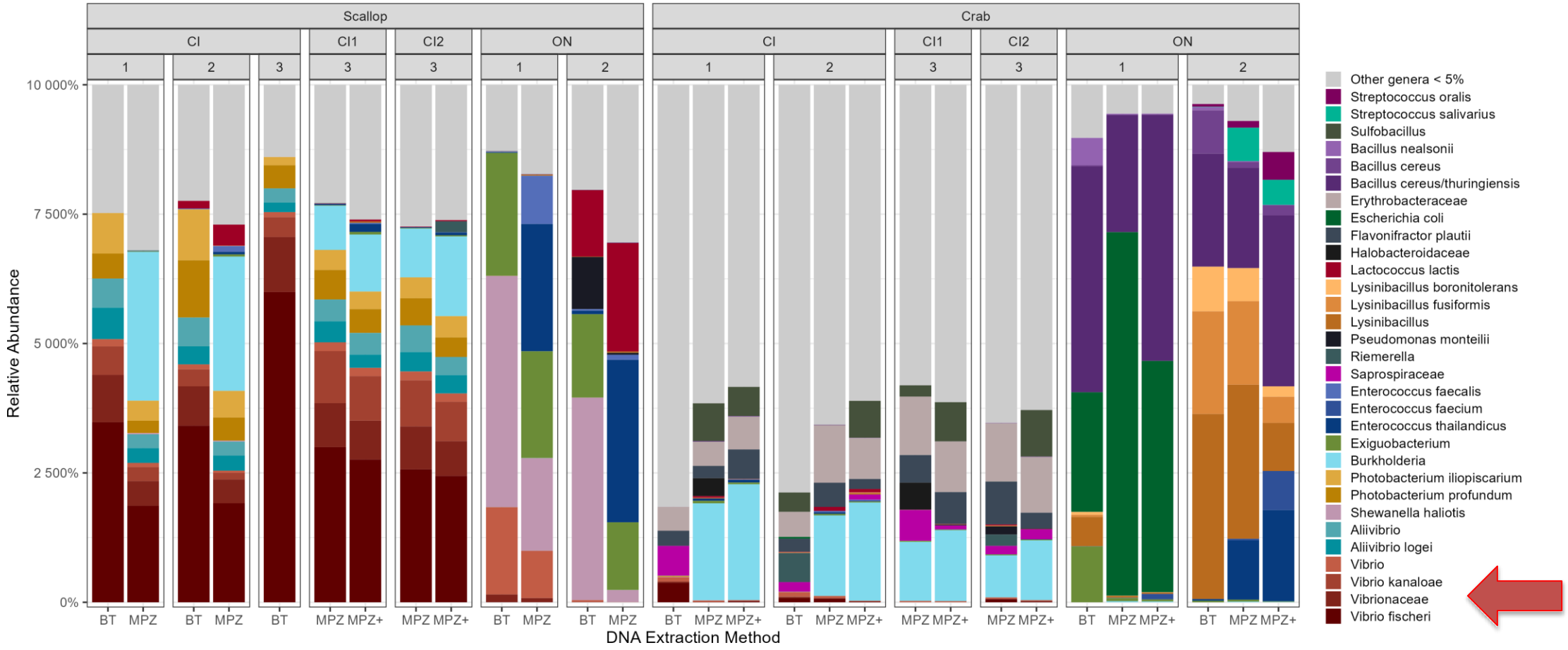
Samples Received and Processed

- CI (or -3): 1g samples 1:1 in PBS **no enrichment**; homogenized by blending; technical replicates
- CI-1/-2 : biological replicates; 1ml of CI samples 1:10 in APW or TSB broth; **pre-enrichment**; some technical replicates
- [CI-1/2]-**ON**: biological replicates; 1ml samples 1:10 in broth; post-enrichment; some technical replicates
- Extractions performed with Maxwell Promega + Metapolyzyme lysis
- Originating lab also sent extractions – Qiagen Blood and Tissue
- Long-read (ONT) sequencing performed on select samples; mostly scallop

CI = culture independent

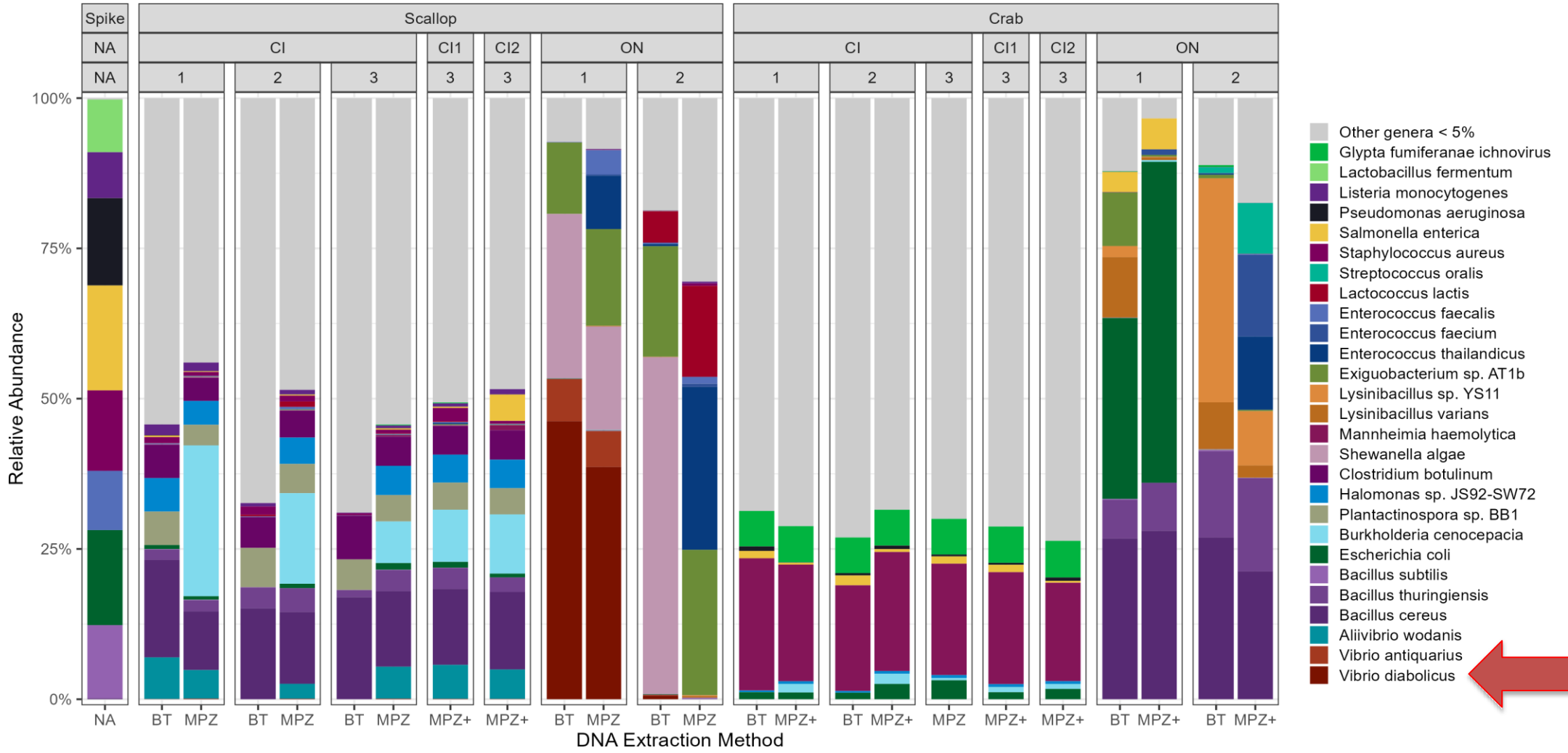
Bactikmer Results

Reduced foodborne pathogen database *k*-mer classification tool



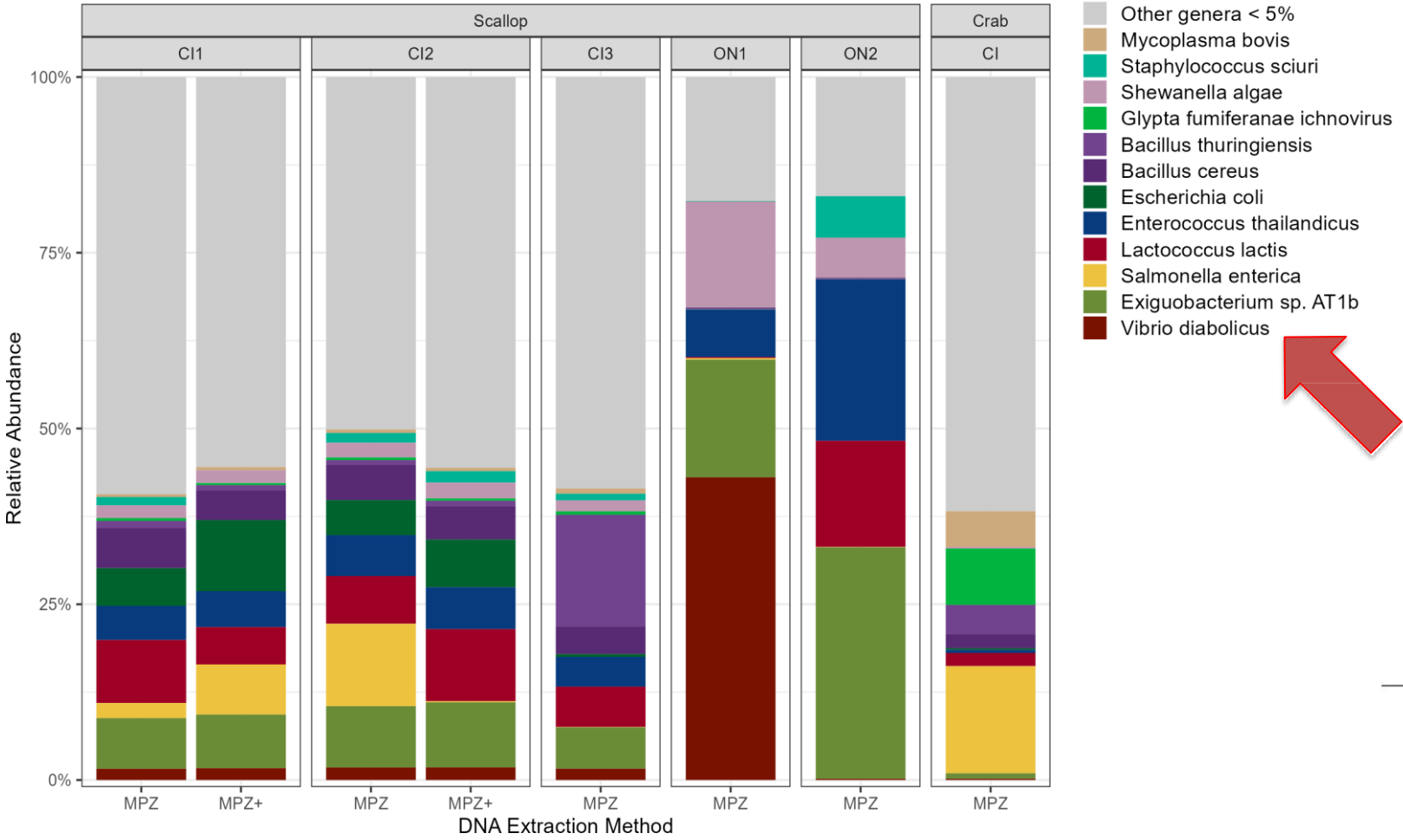
Kraken Results

Metagenomic read classification using exact-match *k*-mer querying

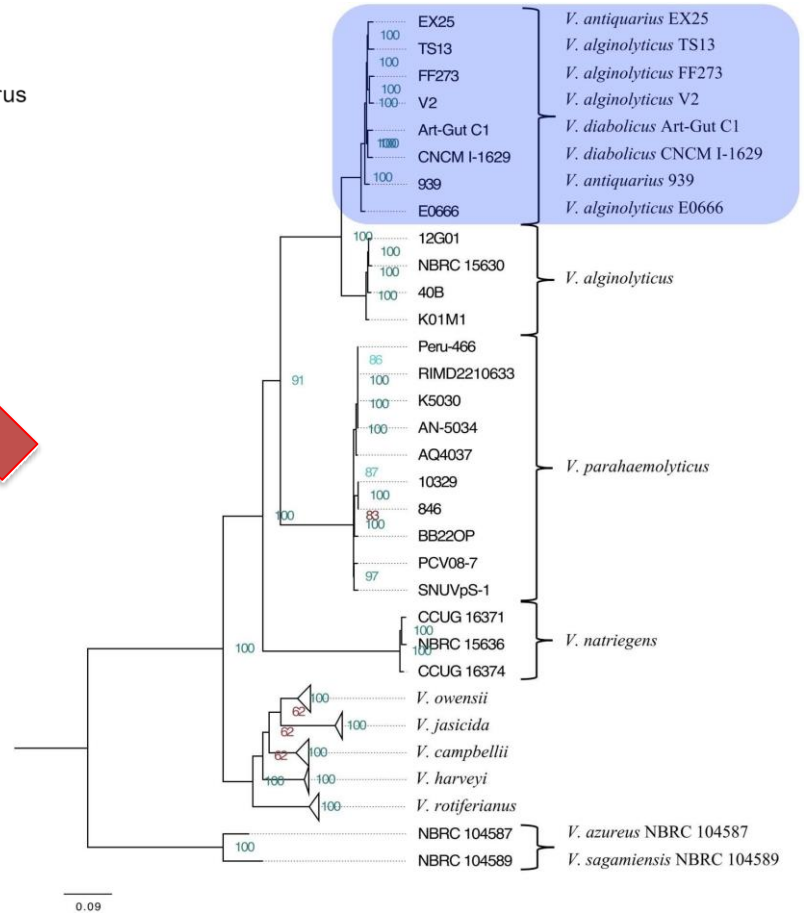


Long-read Metagenomic Sequencing

Oxford Nanopore MinIon sequencing and analysis with Kraken



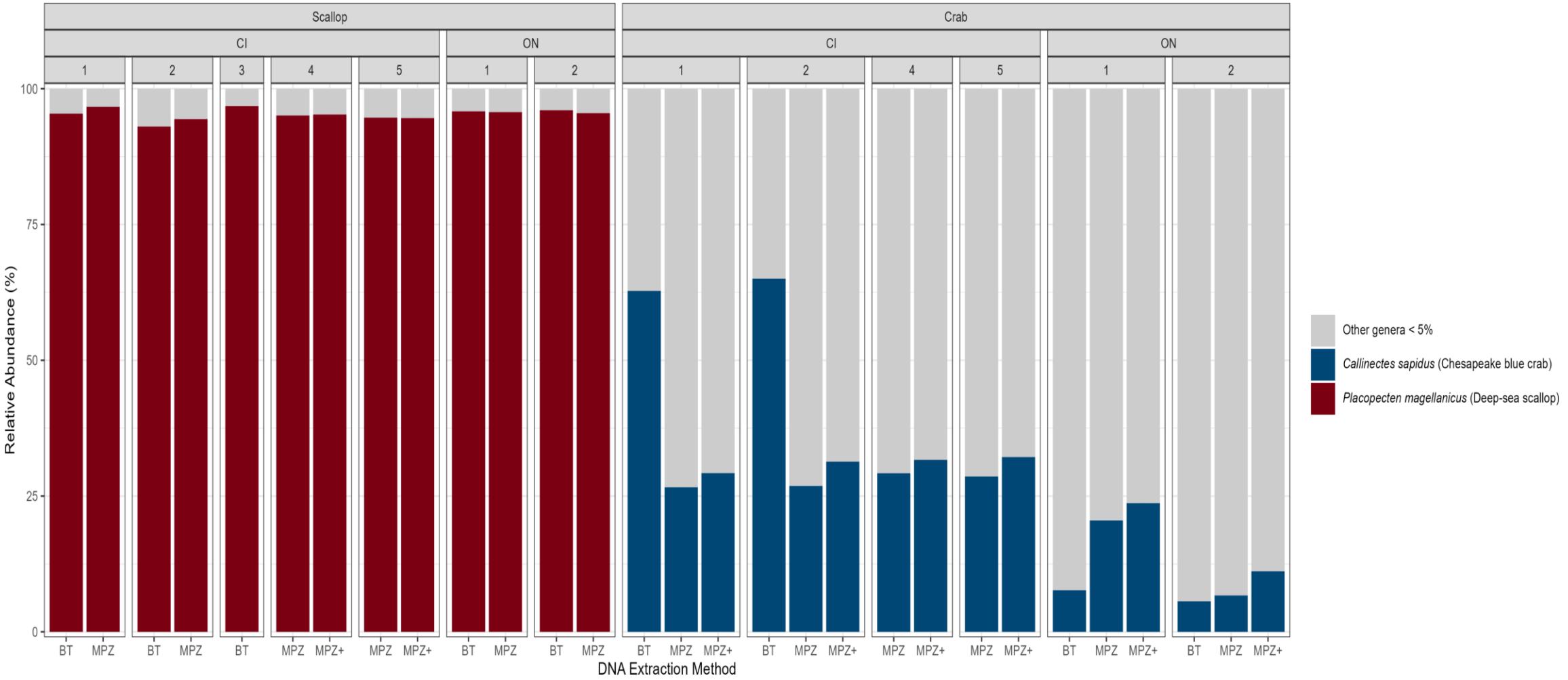
All (most) signs point to *V. diabolicus*



Turner et al. 2018; 8 | https://doi.org/10.3389/fmicb.2018.01893

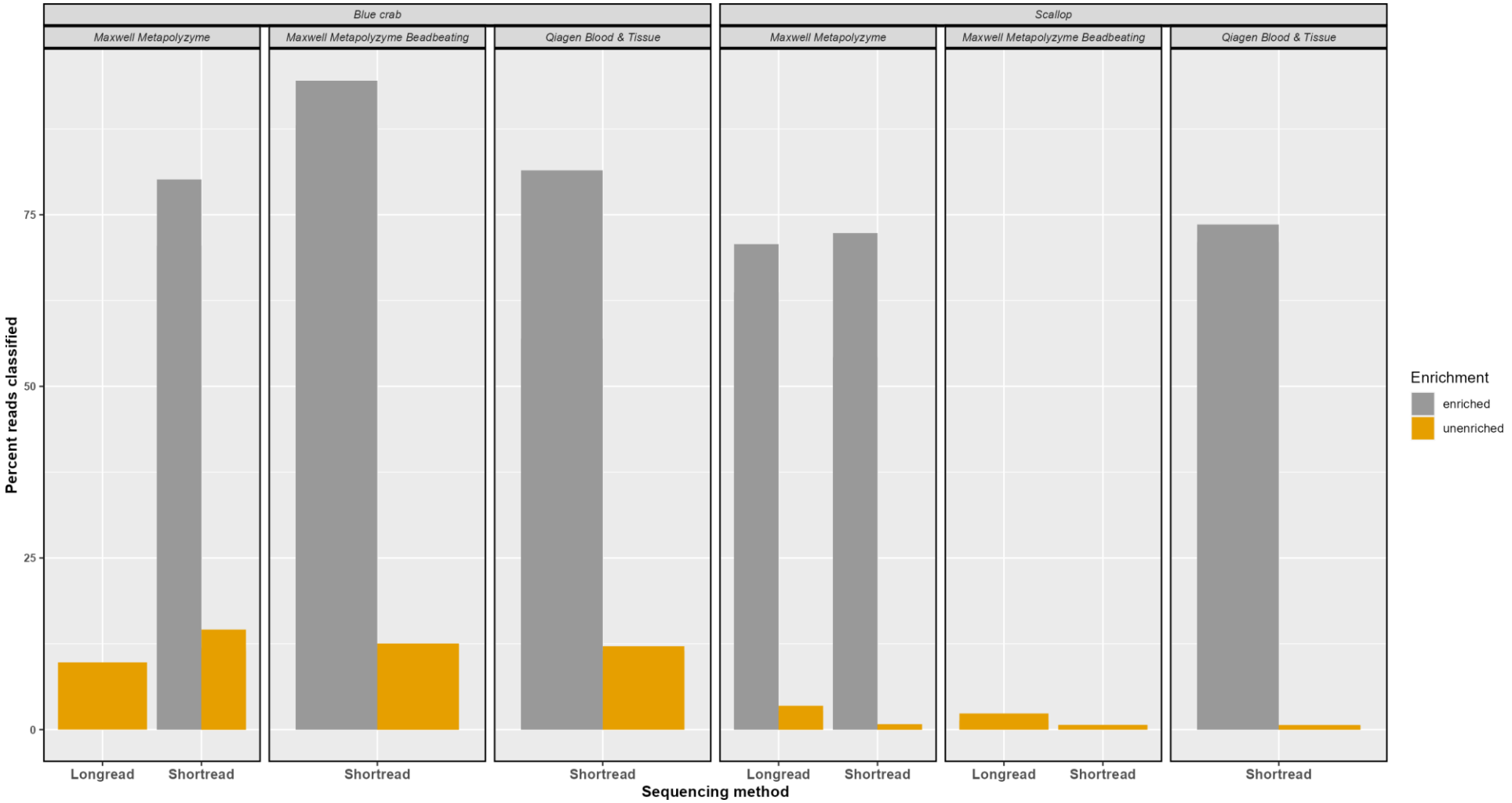
Mitokmer Results

Mitochondrial reads queried to determine/corroborate host species



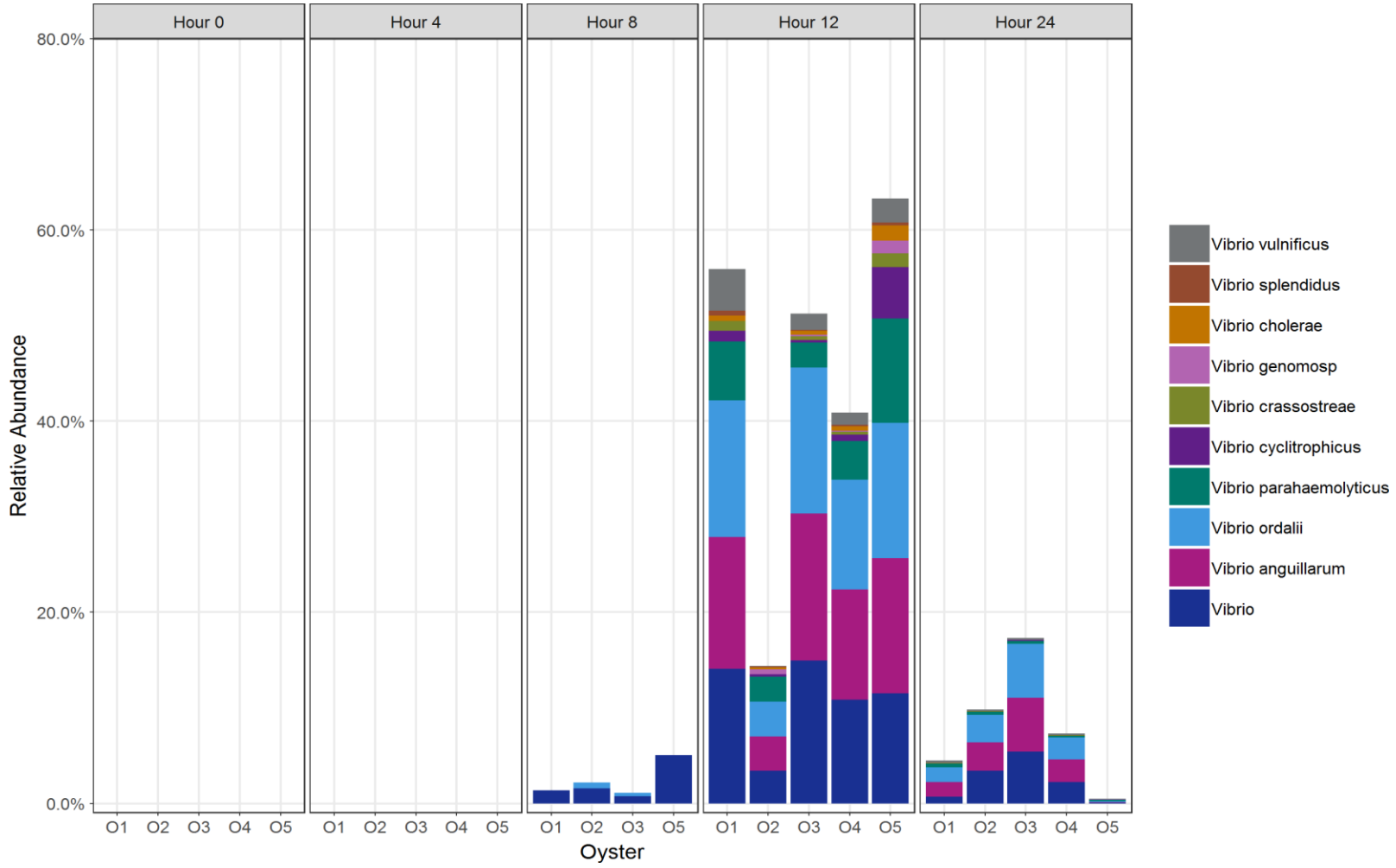
Extraction Method Comparison

Host Depletion Needed for Seafood CI samples

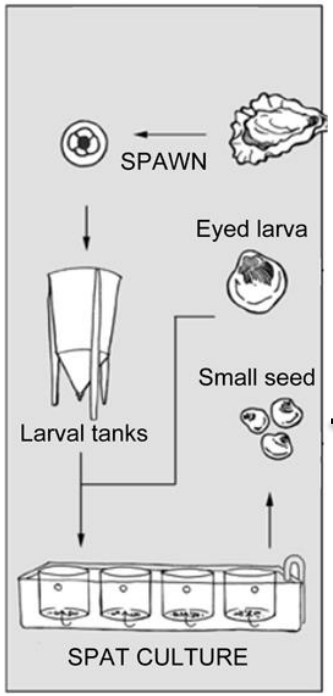


Vibrio Enrichment Dynamics - Oysters

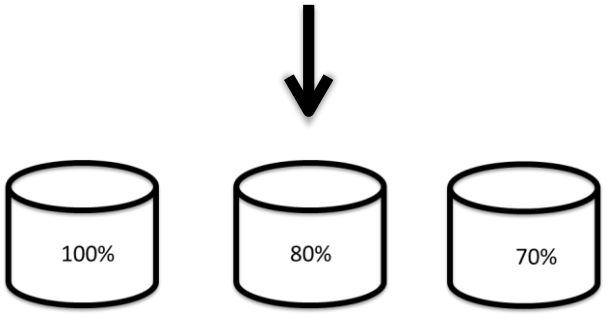
Vibrio Relative Abundances Peak around 12 hours



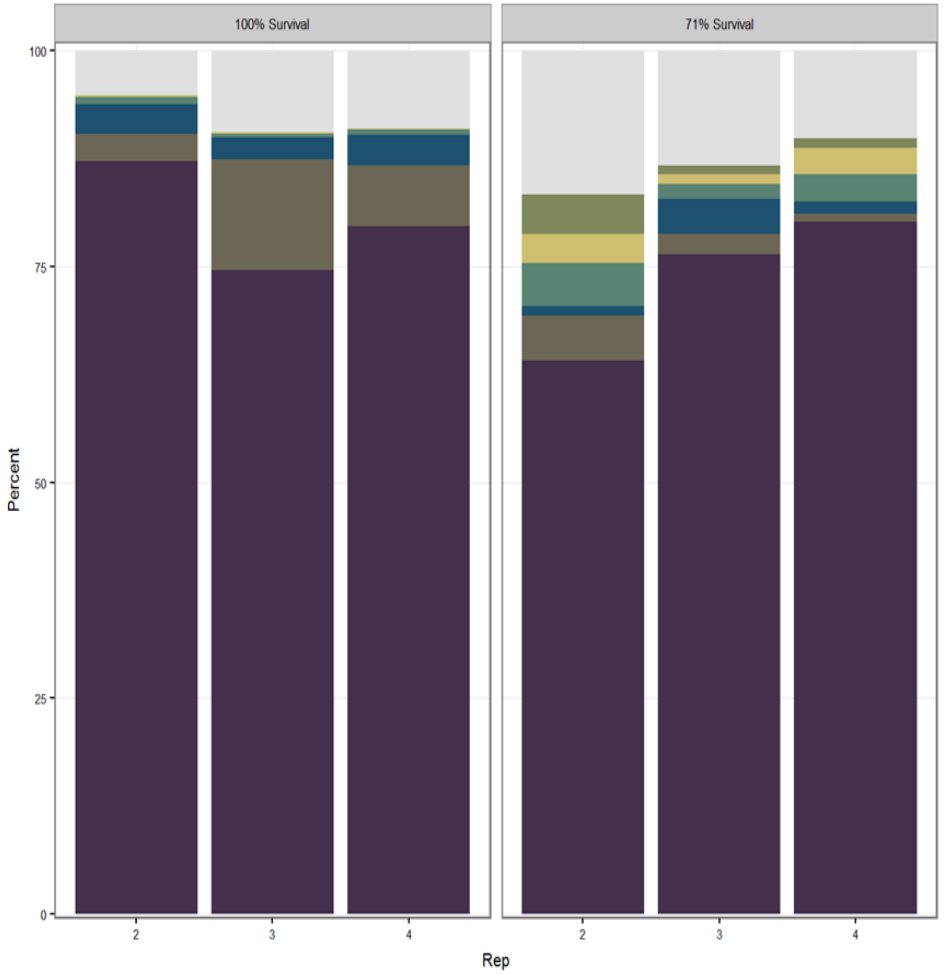
Metagenomic Sequencing for Spat Mortality Discovery

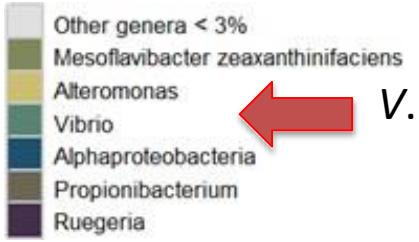


→ Nurseries



Survival Rates in Tanks





V. anguillarum

Ruegeria spp. produce tropodithietic acid; suppresses pathogens

Vibrio and *Listonella* phages in high abundance – *L. anguillarum*

Parasites:

Bonamia exitiosa (Bonamiasis) – 20 reference genes identified

H. nelsoni (MSX) – 8 hits

P. marinus (Dermo) – 13 hits

Acknowledgments

- **CFSAN/ORS/DM Metagenomics Team**:
  - Padmini Ramachandran
  - Amanda Windsor
  - Kathryn Judy
- **Elizabeth Reed** (CFSAN/ORS/DM) – data curation and visualizations
- **Jessica L. Jones** (CFSAN/OFS, GCSL) – provided crab and scallop samples
- **Angelo (Andy) DePaola** (Angelo DePaola Consulting) – oyster larval spat study
- **Leena Malayil** (UMCP, Public Health) – oyster-*Vibrio* enrichment study
- **Andrea Ottesen** (CVM) – metagenomics SME and collaborator

- ORS/DM/MMSB Management and Senior Scientists


FDA
U.S. FOOD & DRUG
ADMINISTRATION

Soizick Le Guyader, Ph.D.

In collaboration with her colleagues from the Microbiology laboratory at Ifremer, Soizick F. Le Guyader has been working in the field of coastal area contamination by human sewage for many years. Beside method development for viral detection and quantitation, the main research objectives are to understand how shellfish get contaminated, including sewage depuration efficiency, field study analysis, and outbreak investigations. Dr Le Guyader's research projects mainly focus on norovirus, which is the main pathogen implicated in shellfish related outbreaks worldwide. After the demonstration that oysters are able to actively select some norovirus strains, *via* specific ligands detected in digestive tissues, her work now focus on metagenomic approach to describe viral diversity.

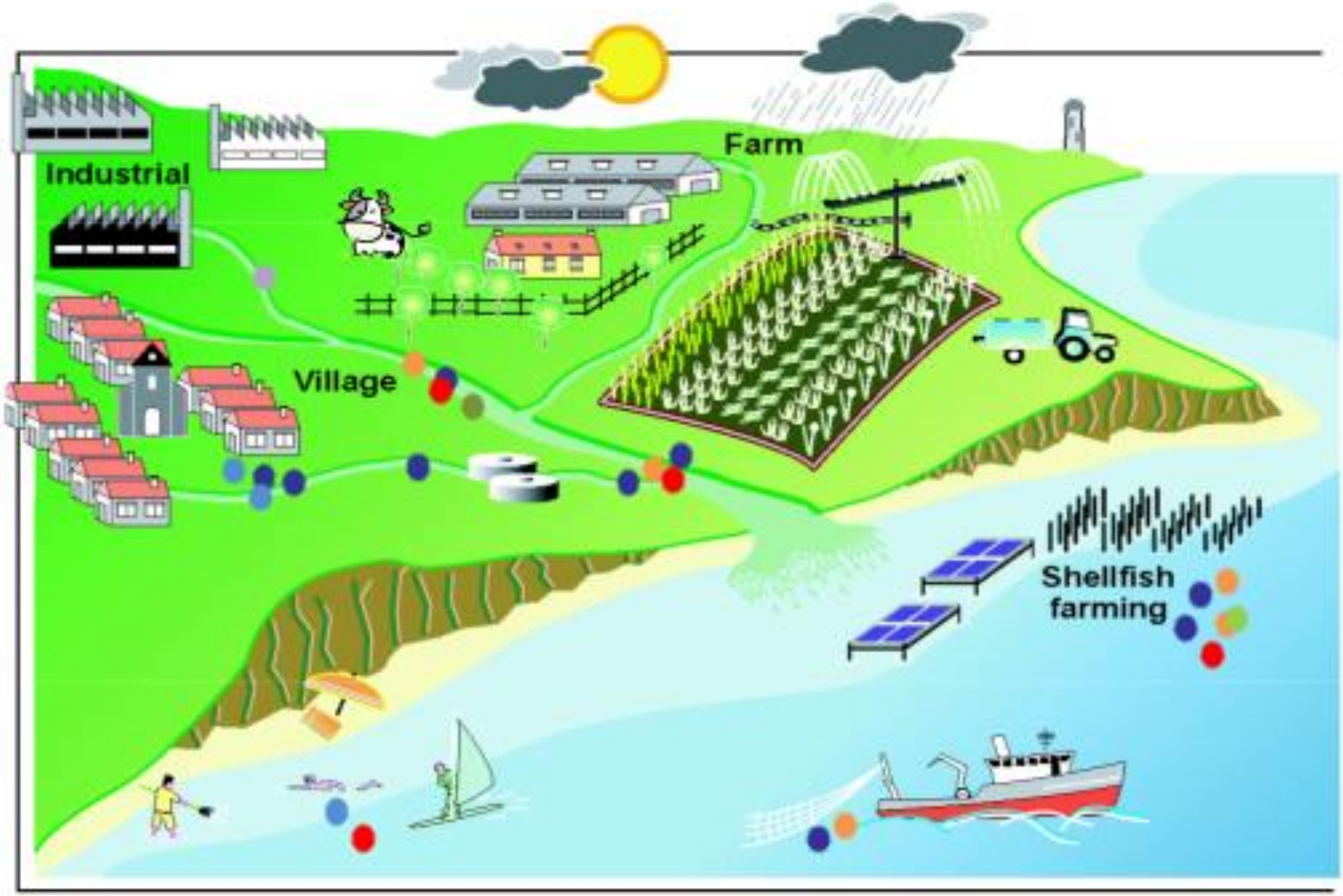
Dr Le Guyader is the head of the Microbiology laboratory at Ifremer and the head of the French Reference Laboratory for Shellfish Microbiology.

Molecular Epidemiology: Diversity of enteric viruses from contaminated shellfish using NGS

Soizick F. Le Guyader

Shellfish, unique food

- production,
- Filtering organisms
- Raw consumption


Industrial
Farm
Village
Shellfish
farming

Shellfish borne outbreak following a flooding event…

- ✓ 38 clusters following oyster consumption (205 cases identified …)
- ✓ 66 oyster samples collected

Diversity of virus detected in samples

Cluster date	Stool sample	Shellfish sample	Virus				Genotype(s)[a]	
			AiV	AV	EV	RV	NoV GI	NoV GII
8 February	73		−	−	+	−	+ GI.2	−
	74		+	−	+	−	+ GI.1	+ GII.2
		109	+	+	−	−	+ GI.4	−
		1739	+	+	−	+	+ GI	−
15–17 February	E1196		−	−	+	−	−	+ GII.7, GIIb
	E1197		+	+	+	−	−	−
	E1201		−	−	−	−	−	−
	E1202		+	−	−	−	−	−
		93	−	+	−	−	−	+ GII.4
		107	+	+	+	−	+ GI.1	+ GII
		110	−	+	−	+	+ GI.4	+ GII
18 February	E1203		+	+	+	+	+ GI.1	+ GII.17
	E1204		−	−	−	−	−	+ GII.4
	E1205		−	−	−	−	+ GI.1	+ GII.4
	E1206		−	−	+	−	+ GI.1	−
	E1207		+	−	−	−	+ GI.2	+ GII.7
	E1208		+	+	−	+	−	+ GIIb,
		140	+	+	−	+	+ GI	+ GII.4
		115	+	+	−	−	−	−
		130	−	+	−	−	+ GI.2	
		131	+	−	−	+	+ GI	+ GII.4

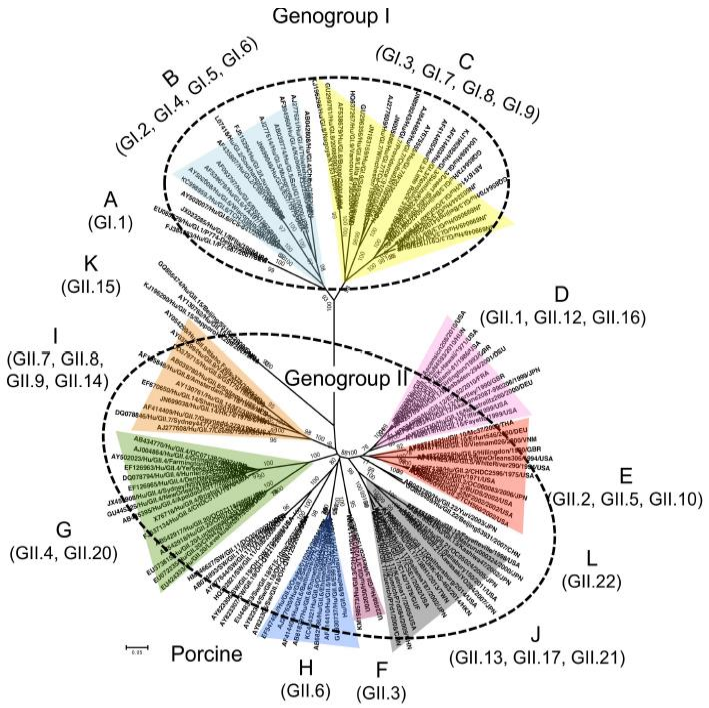
[a] Genotypes are listed (+) if genotypes were determined; − indicates genotype was undetermined.

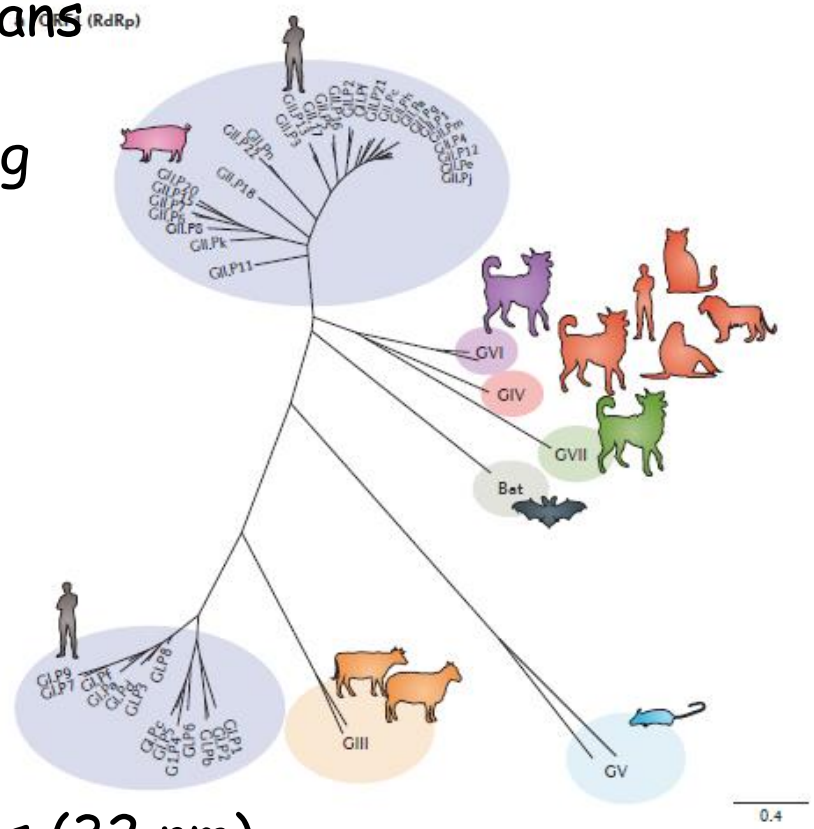
- Up to 7 different virus sequences detected in one stool sample
- Same sequences detected in several oyster samples

Le Guyader et al. AEM, 2008

Norovirus : a major public health issue

- ✓Main agent of acute gastroenteritis in Humans
- ✓Winter desease
- ✓High excretion in stool: 10^9-10^{11} particules/g
- ✓High infectivity (GI & GII)
- ✓Very stable and resistant to inactivation.





- ➢ Small viruses (32 nm),
- ➢ Genome ssRNA, 7.5 kb
- ➢ High genetic diversiy
- ➢ Numerous recombinant

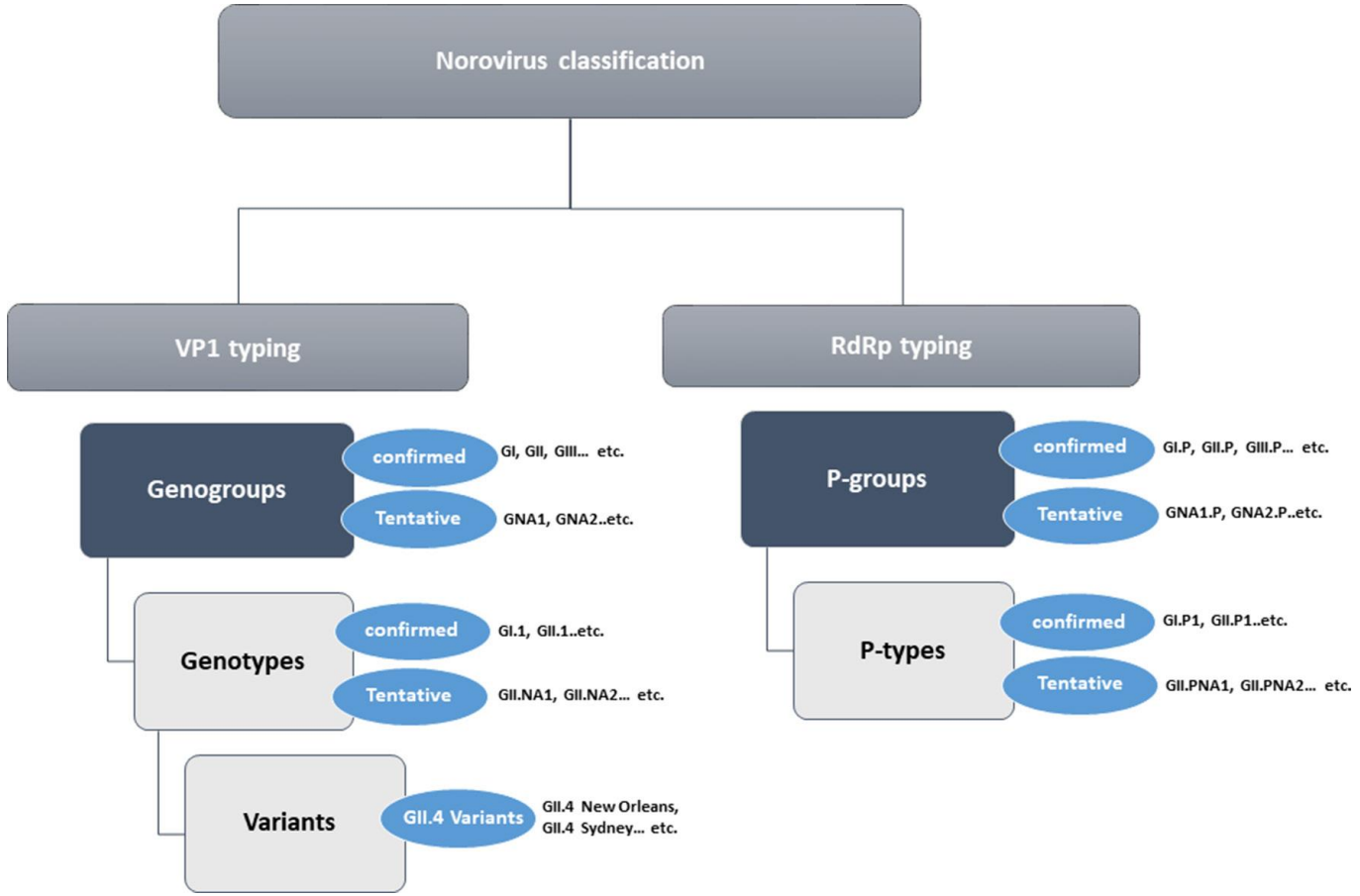


Fig. 1. Classification of noroviruses into genogroups, genotypes, variants, P-groups and P-types. Tentative genogroups, genotypes, P-groups and P-types are currently represented only by a single sequence or multiple non-identical sequences from a single geographic location and are therefore named as non-assigned (NA).

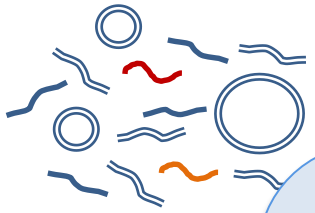
Chhabra et al. 2019, J. Gen. Virol

How can we describe all viruses that may contaminate shellfish??

Amplicon based metagenomic

CONTAMINATED FOOD

NA extraction



library preparation

raw reads

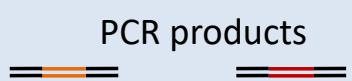
bioinformatic analysis

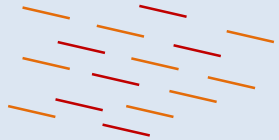
Frogs

« METABARCODING »

primers (degenerate) targetting typing regions

PCR products





mapping / assembly / clustering

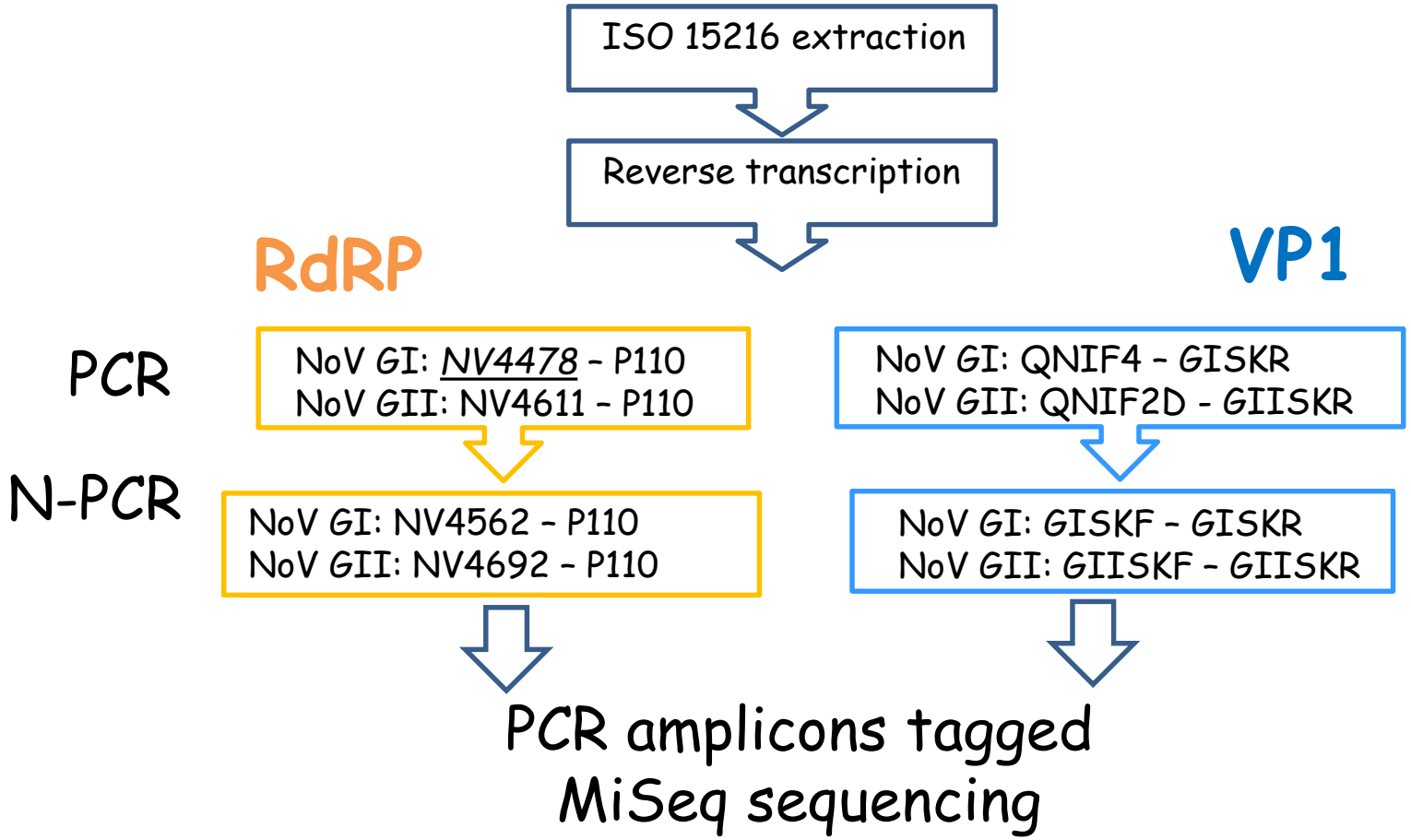
sequence of typing region

genotype identification and diversity

genotype A **genotype B**

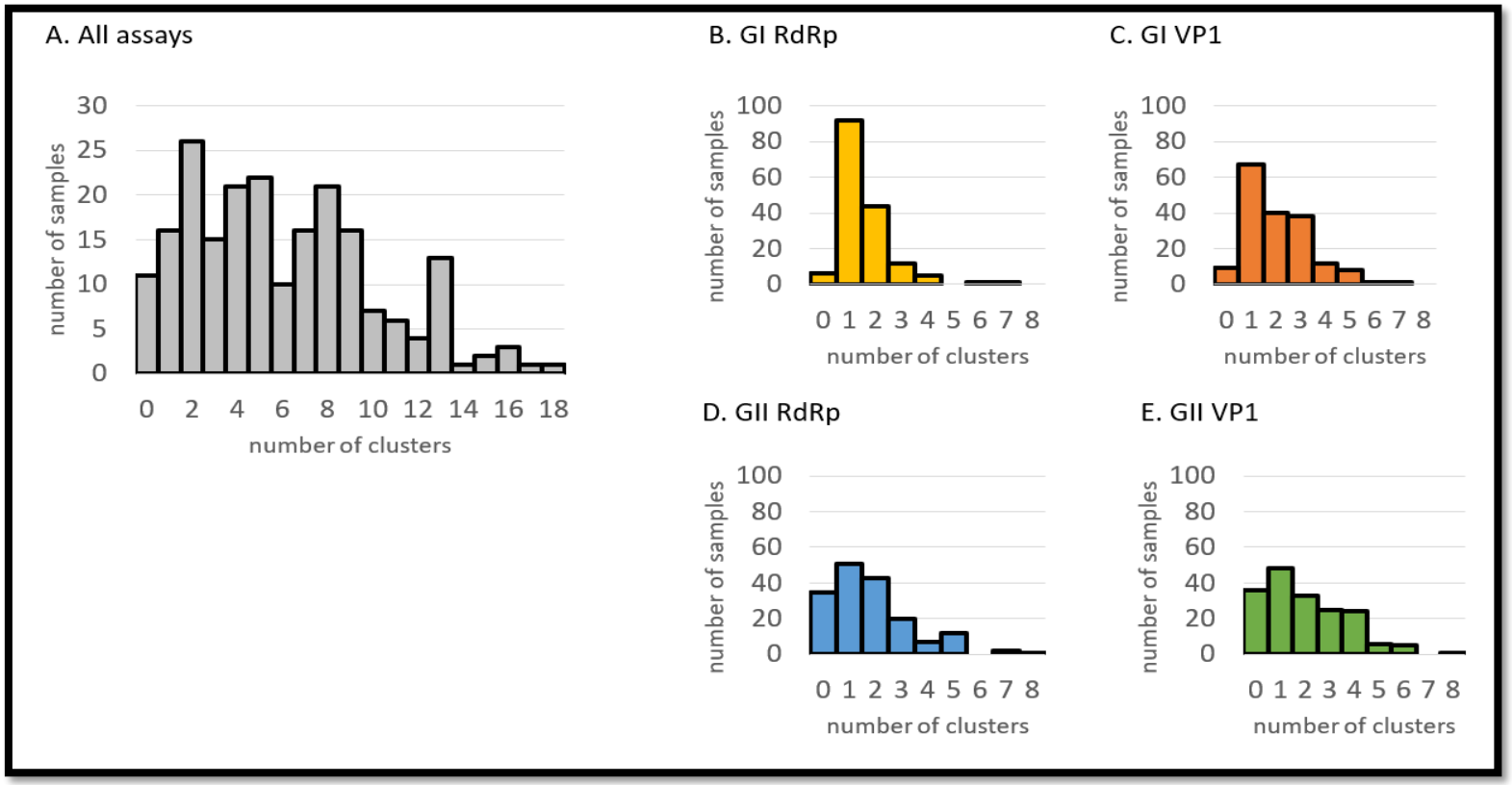
Desdouits et al. Onehealth Outlook 2020, 2:14

Amplicon-based metagenomic to describe norovirus diversity



Norovirus diversity in European oysters

- 200 samples (collected over a 2 year period)
- 242 unique NoV sequence clusters identified
- 95% of samples contained at least one cluster



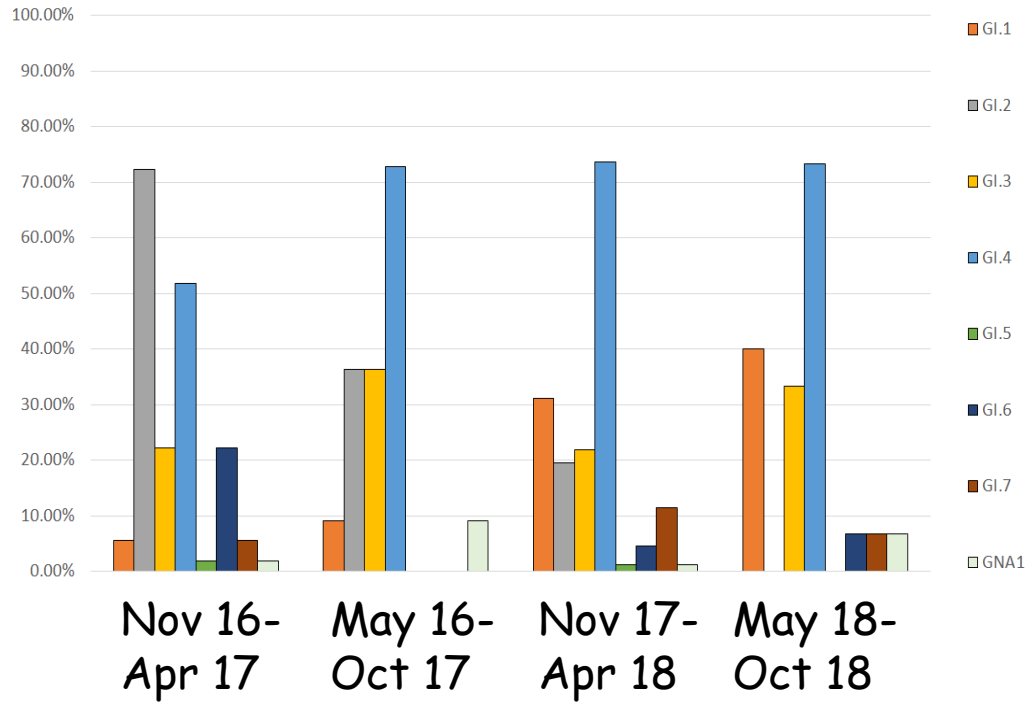
Up to 18 different clusters identified in a single sample

Ollivier et al. EFSA J. 2022

Distribution of genotypes over the study period

NoV GI

Data obtained for the VP1 typing

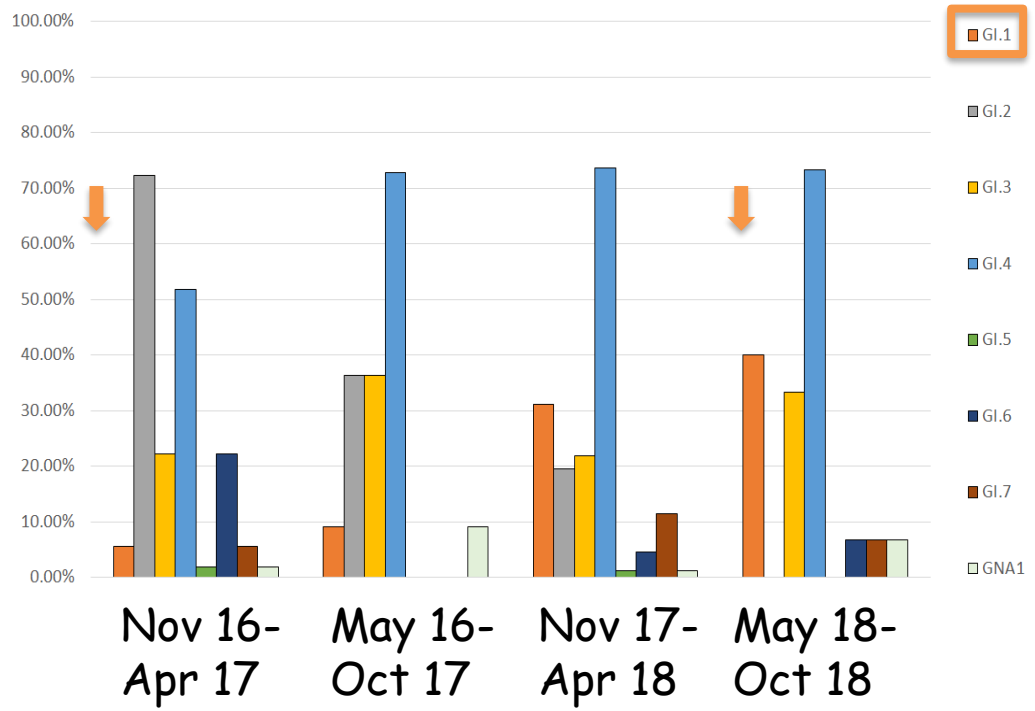


Ollivier et al. EFSA J. 2022

Distribution of genotypes over the study period

NoV GI

Data obtained for the VP1 typing

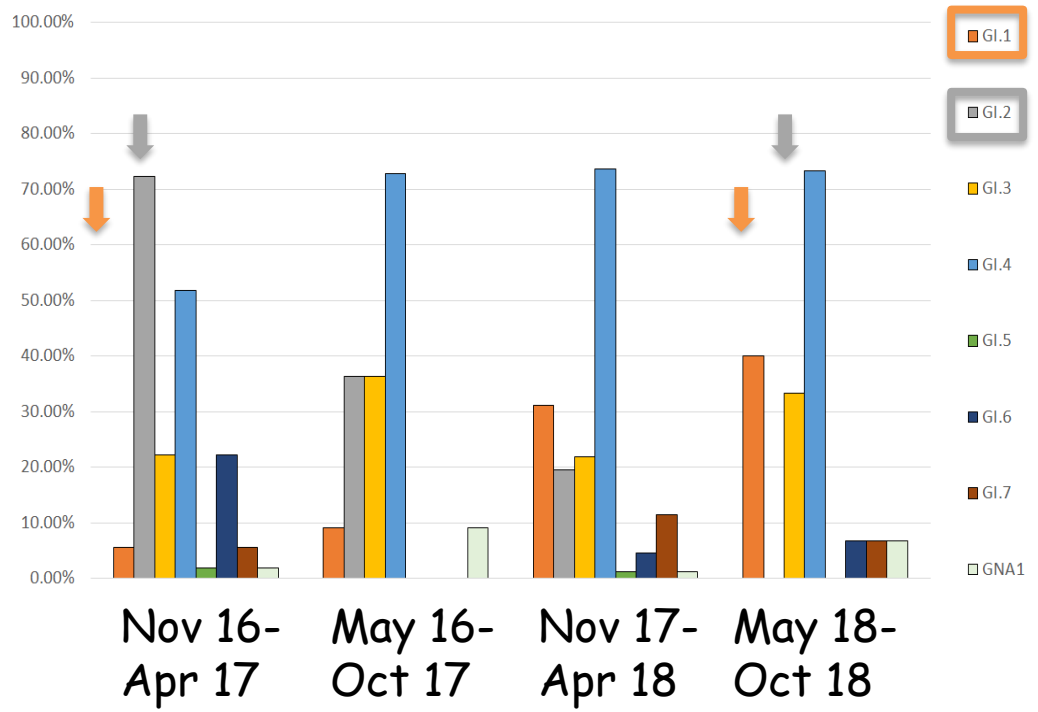


Ollivier et al. EFSA J. 2022

Distribution of genotypes over the study period

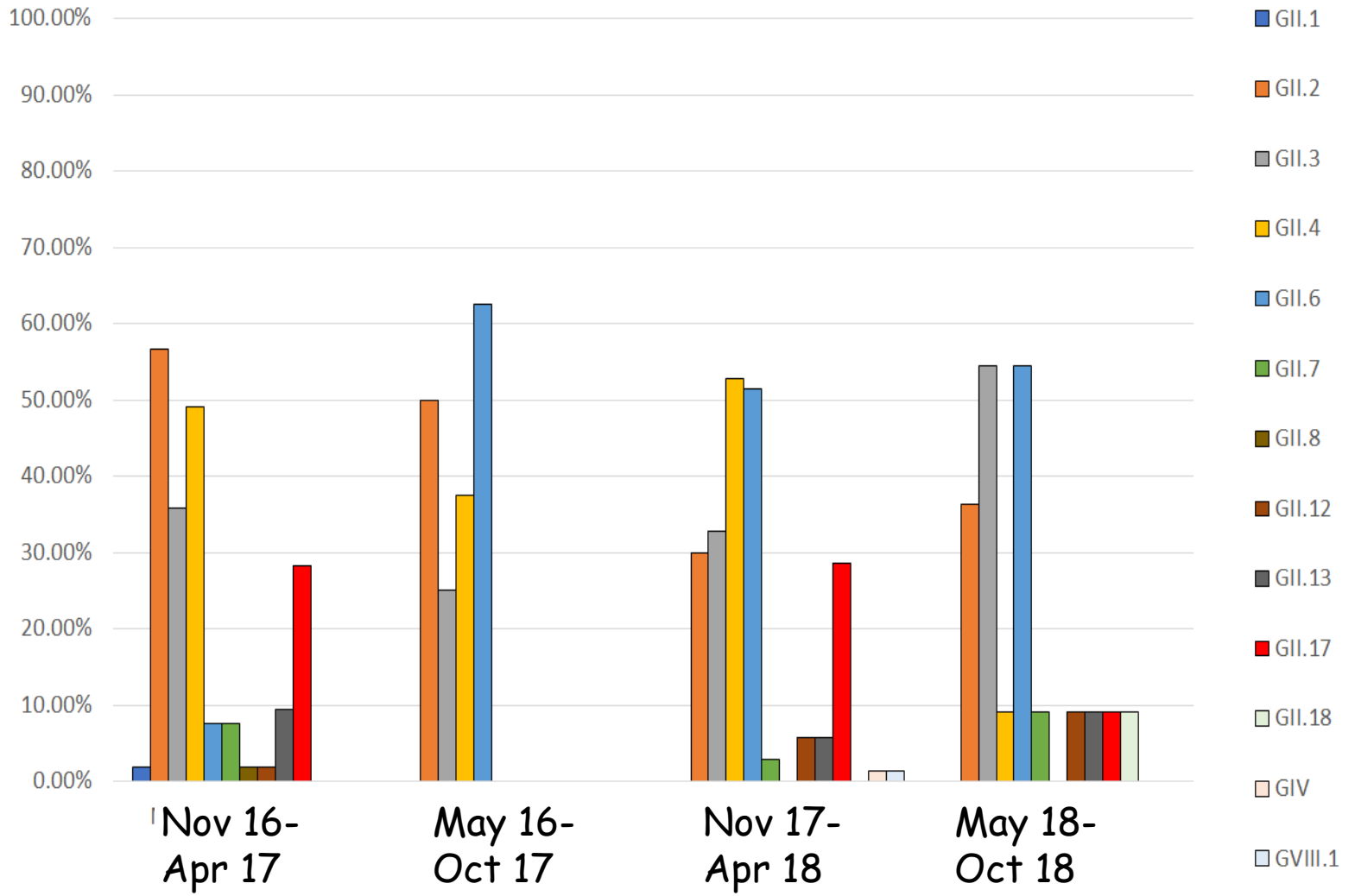
NoV GI

Data obtained for the VP1 typing



Ollivier et al. EFSA J. 2022

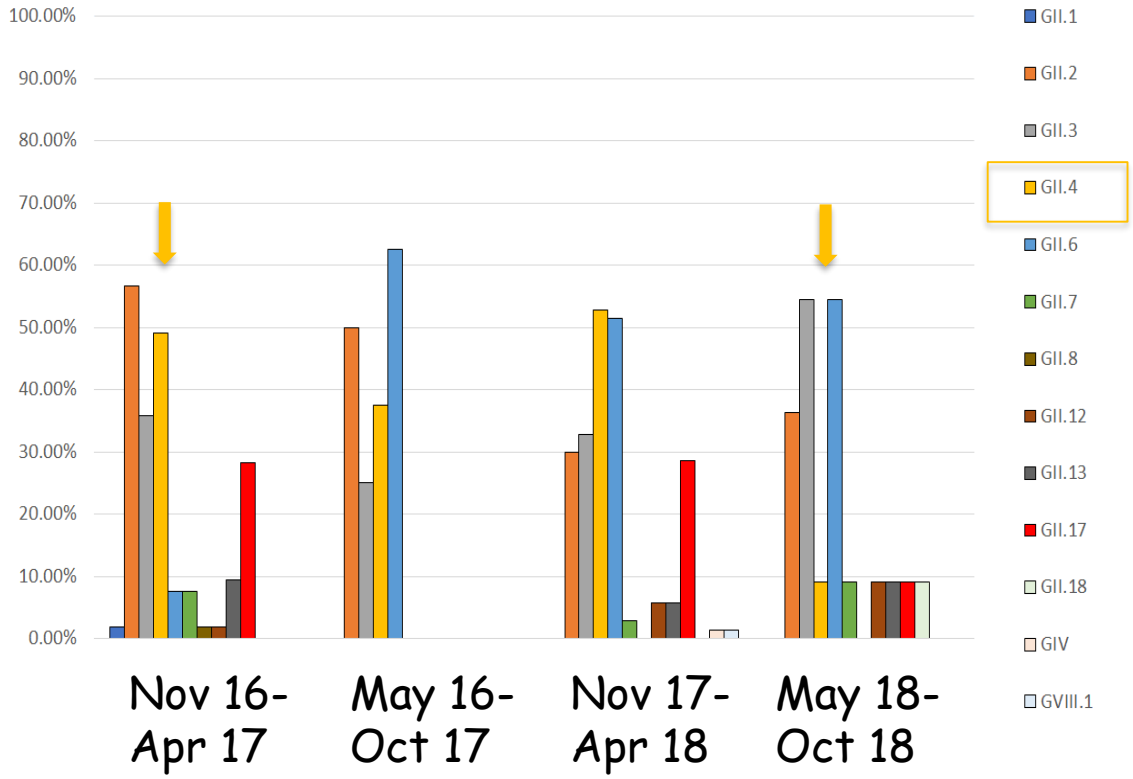
Distribution of genotypes over the study period



NoV GII

Ollivier et al. EFSA J. 2022

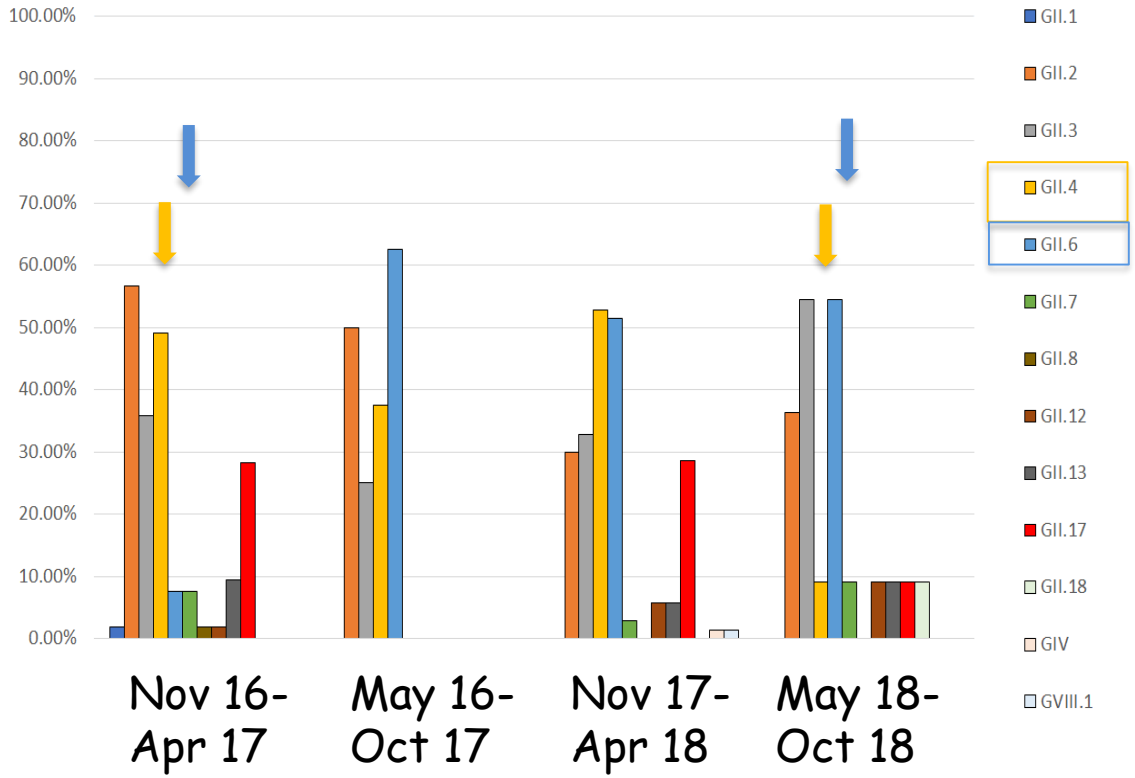
Distribution of genotypes over the study period



NoV GII

Ollivier et al. EFSA J. 2022

Distribution of genotypes over the study period



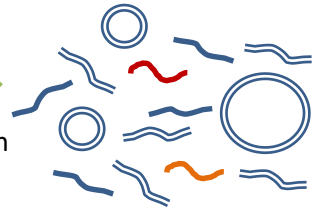
NoV GII

Ollivier et al. EFSA J. 2022

Metagenomic applied to oysters…

CONTAMINATED FOOD

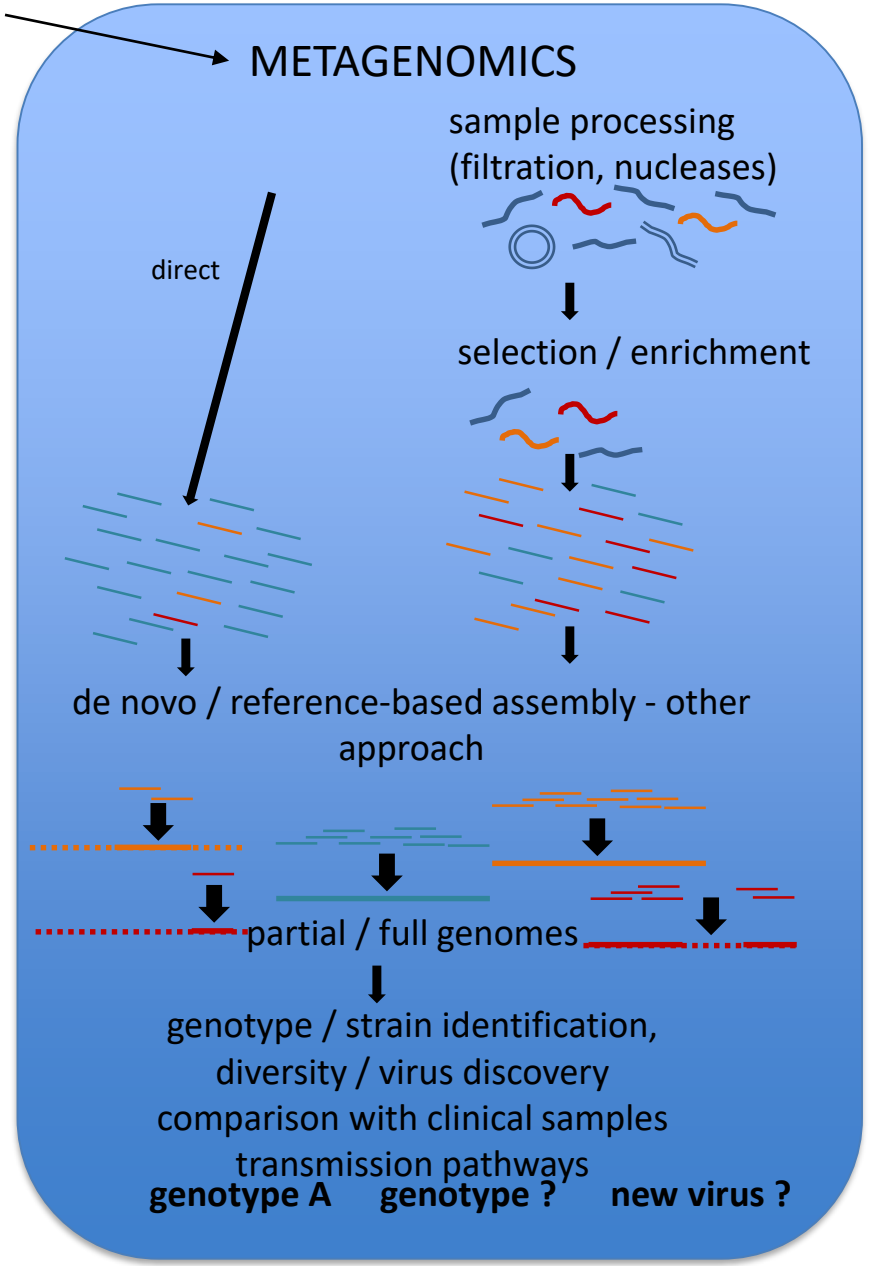
nucleic acids extraction



library preparation

raw reads

bioinformatic analysis



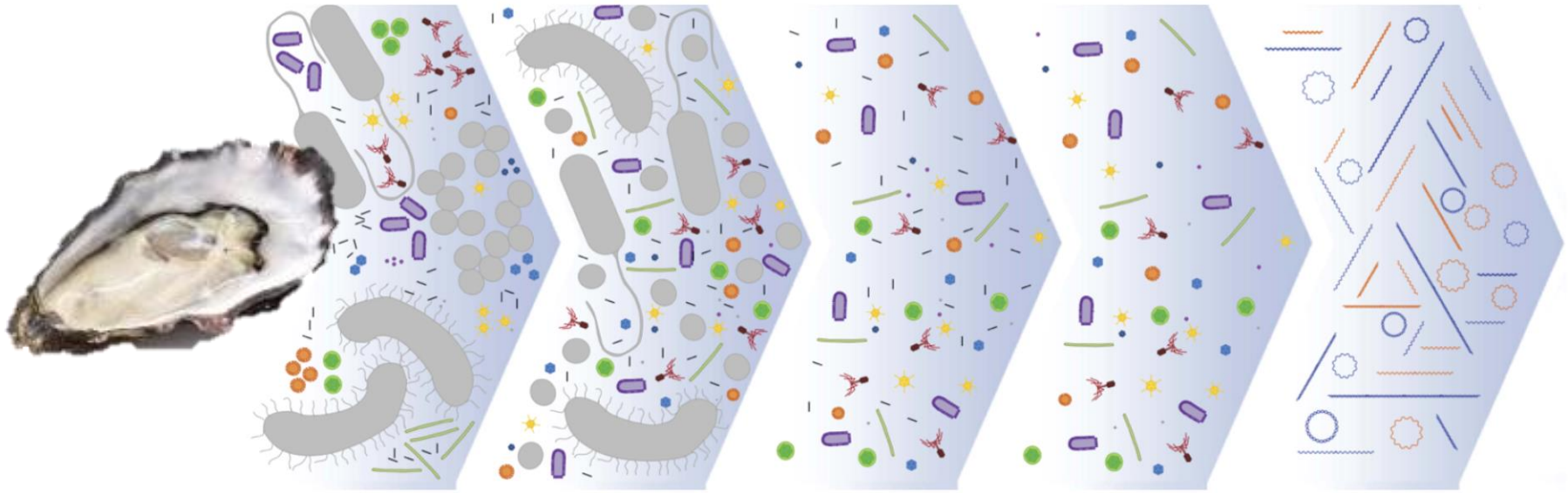
Desdouits et al. Onehealth Outlook 2020, 2.

Sample preparation

PK elution/PEG precipitation

Filtration

Nuclease treatment



Conceição-Neto *et al.*, 2015

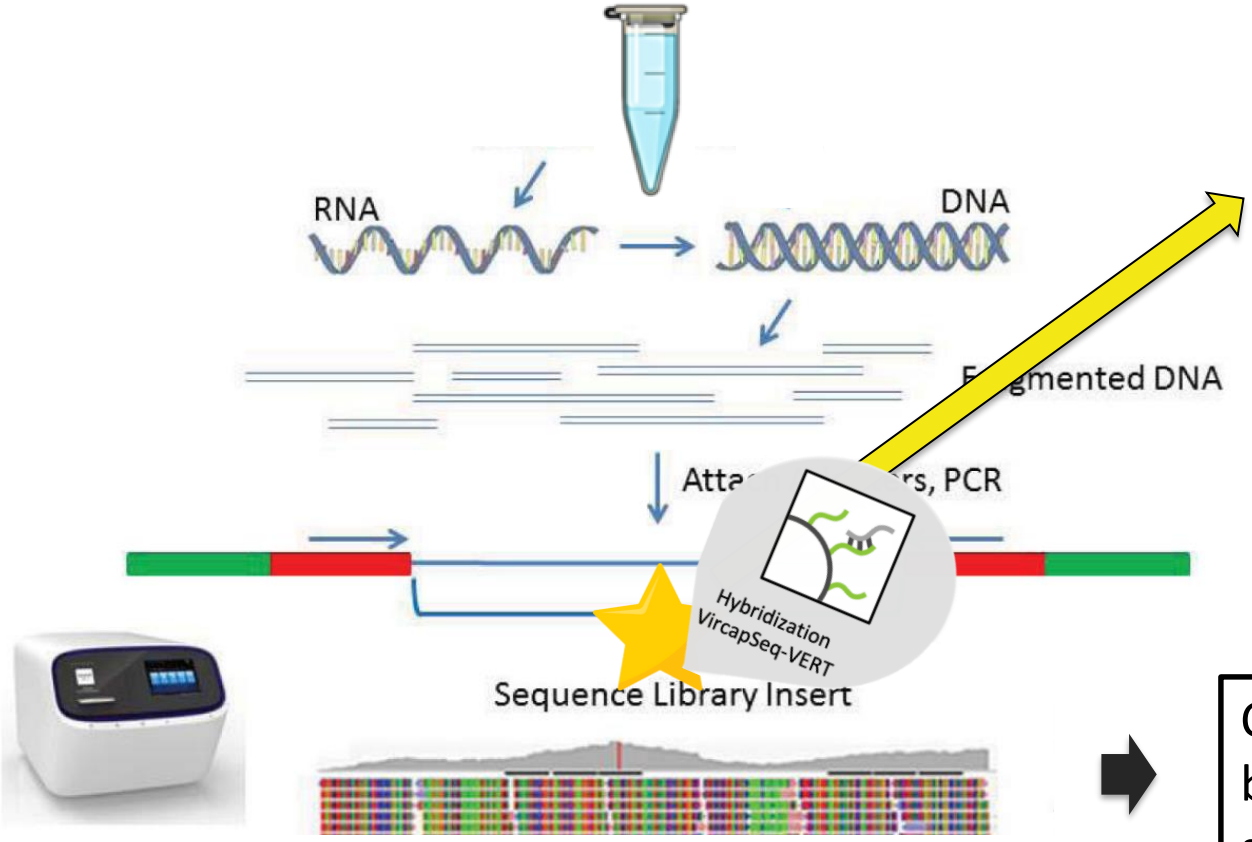
FROM
Complex « dirty » sample

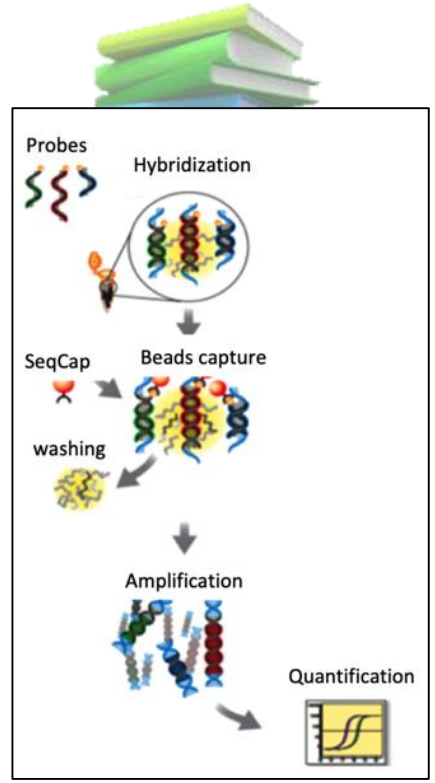
TO
« Clean » extract

Strubbia et al., Front. Microbiol; 2019, 10.

Library preparation

VirCap-Seq = ~2 million probes that cover the genomes of members of the 207 viral taxa known to infect vertebrates, including humans.



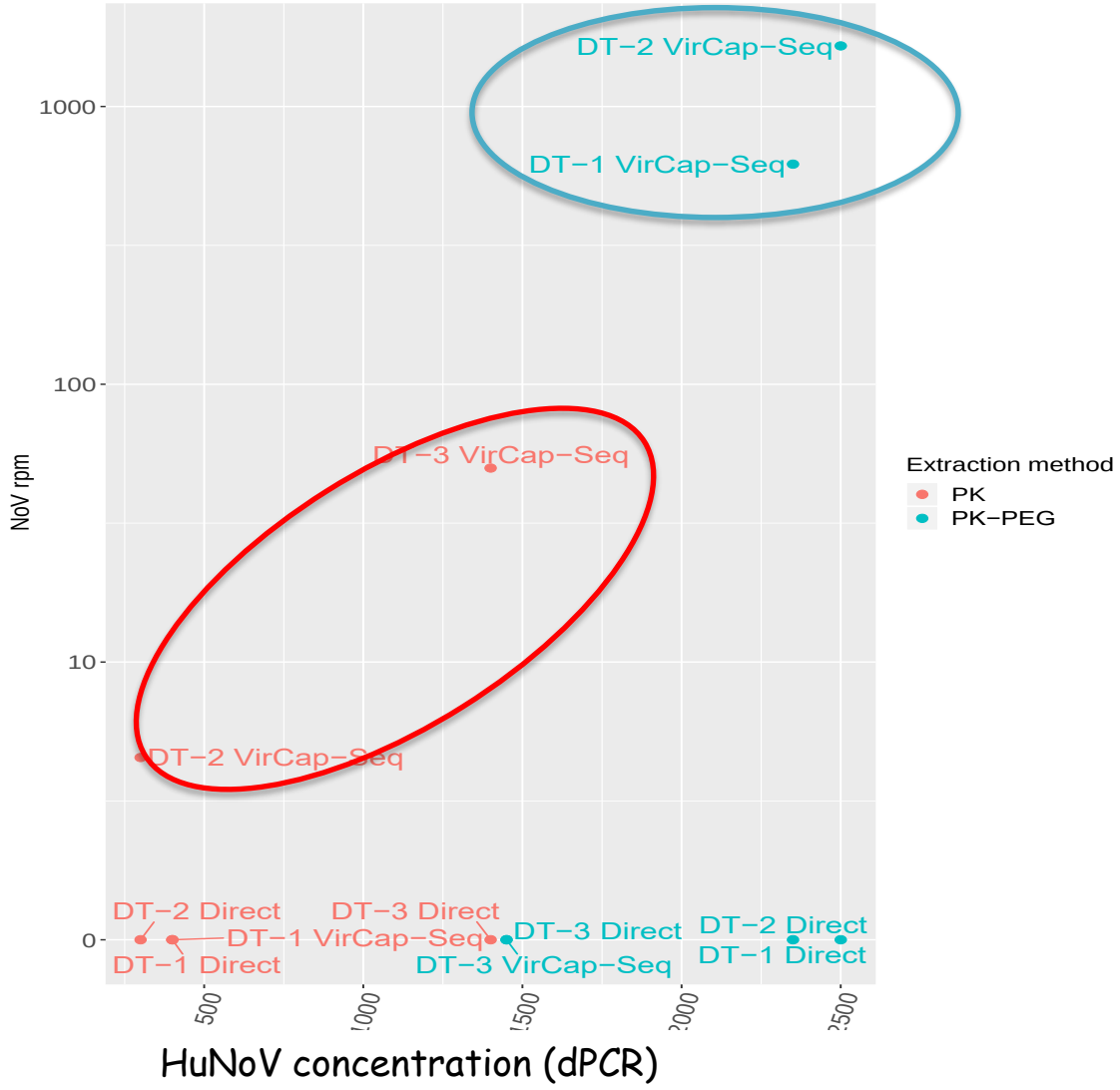


(Briese *et al.*, 2015)

Capture-based sequencing

Strubbia et al., Front. Microbiol; 2019, 10.

Interest of the enrichment during the library preparation

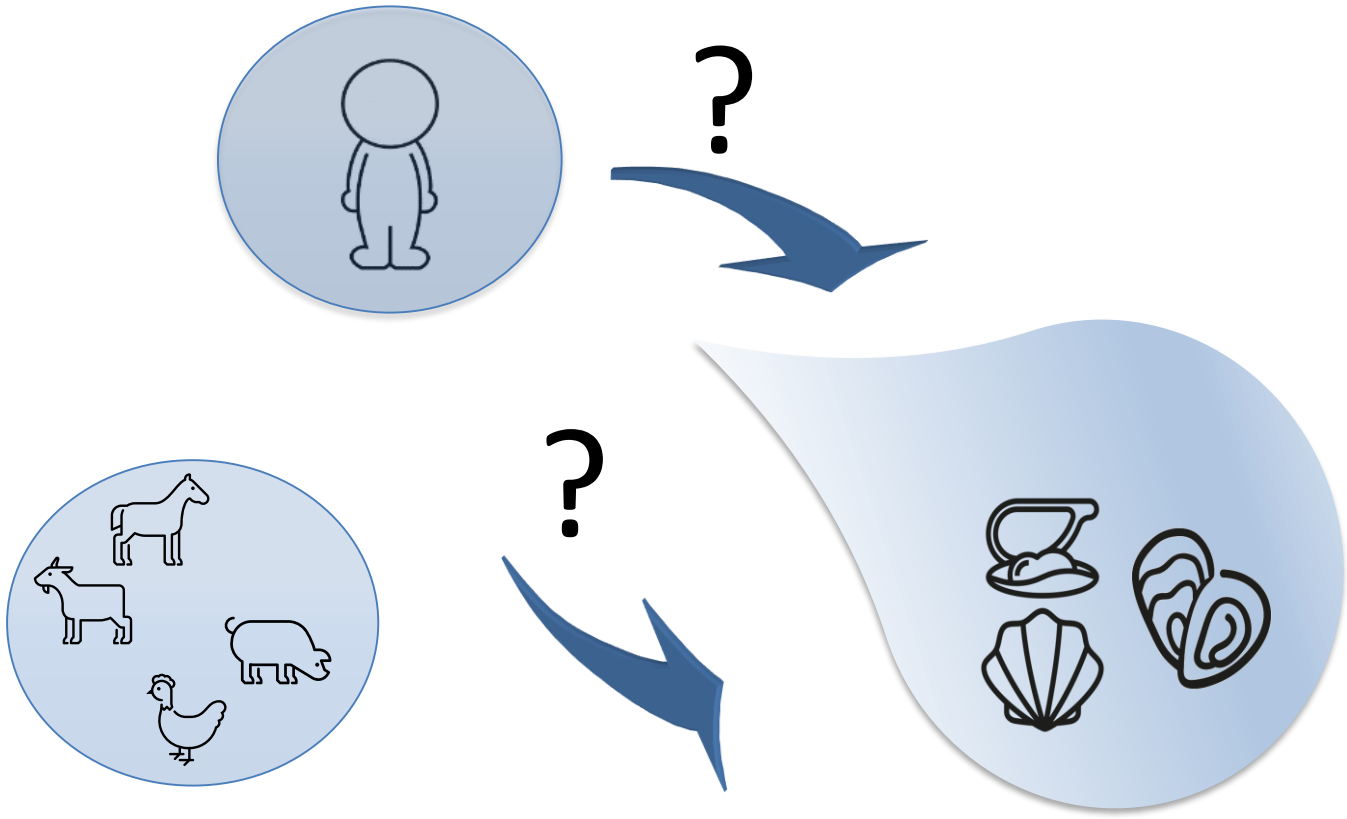


Strubbia et al., Front. Microbiol; 2019, 10.

Sample	Node	Reads	Length	ORF 1	ORF 2	Ref. sequence	Identity
C.gigas-1	34	560	2243	GII.P7	GII.6	MH218642.1	97.46
	38	241	2192	GI.P1	GI.4	LN854563.1	87.79
	156	1,082	1629	GI.P1	GI.1	MH638229.1	97.85
	192	52	1055	GII	-	MK073886.1	98.62
	209	94	1016	-	GI.1	MH638229.1	98.13
C.gigas-2	13	1951	**4205**	**GII.P7**	**GII.6**	MH218642.1	98.05
	16	2,341	**4023**	**GI.P4**	**GI.4**	LN854563.1	96.69
	17	1,385	**3960**	**GI.P1**	**GI.1**	MH638228.1	98.84
	20	2,296	**3677**	**GI.P2**	**GI.2**	KF306212.1	99.12
	26	619	3303	-	GII.6	MH218642.1	98.00
	216	176	1371	-	GII.3	MG601451.1	94.39
	77	464	1985	GII	-	MG601447.1	98.39
	346	174	1101	GII.P12	GII.3	MH260511.1	98.37
	85	338	1893	GI	-	MH218649.1	97.68
	86	445	1890	-	GI.2	KF306212.1	99.15
	98	261	1817	GII	-	MG601447.1	98.24
	326	167	1038	-	GI.4	GQ413970.1	97.15
	377	111	1072	GII	-	KU870455.1	94.96
	434	138	1019	GII	-	LN854563.1	95.49
O.edulis	2	2,858	**7445**	**GII.P7**	**GII.6**	MH218642.1	98.06
	4	2,261	**7288**	**GI.P2**	**GI.2**	LN854563.1	99.69
	5	1,722	**6422**	**GII.P12**	**GII.3**	MG601447.1	97.88
	25	1,594	**3852**	**GI.P4**	**GI.4**	LN854563.1	96.72
	30	387	3257	GII.P7	-	KU870455.1	95.42
	51	641	2479	-	GII.6	KU870455.1	95.60
	59	1,487	2274	GI.P1	GI.1	MH638228.1	98.55
	95	729	1741	-	GI.1	MH638229.1	97.97
	105	120	1618	GI	-	LN854563.1	96.89

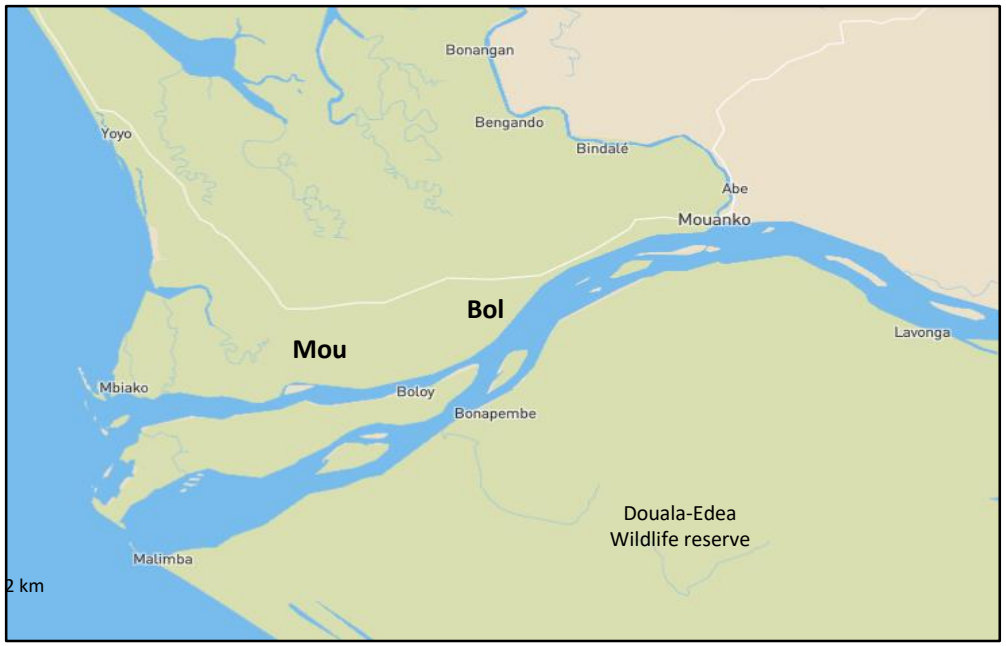
Strubbia et al., Int. J. Food Microbiol., 2020, 323.

Human and animal wastes can reach coastal areas where shellfish are growing

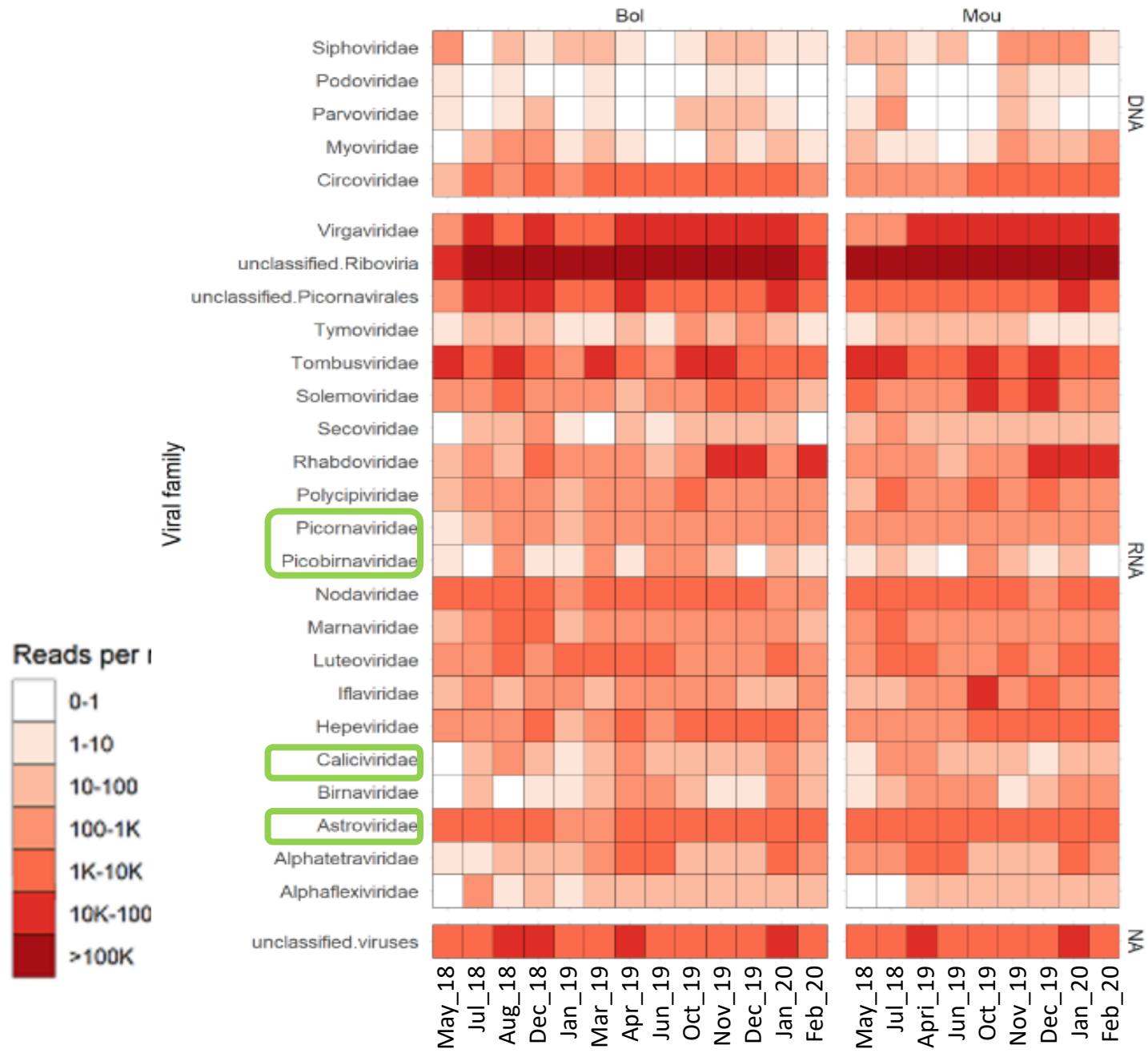


Analysis of clams collected in Sanaga river (Cameroon)

- Sites exposed to human and animal contamination
- 22 samples
- Analysis following the optimized protocol
- Triplicates of library for each sample
- Illumina NovaSeq sequencing

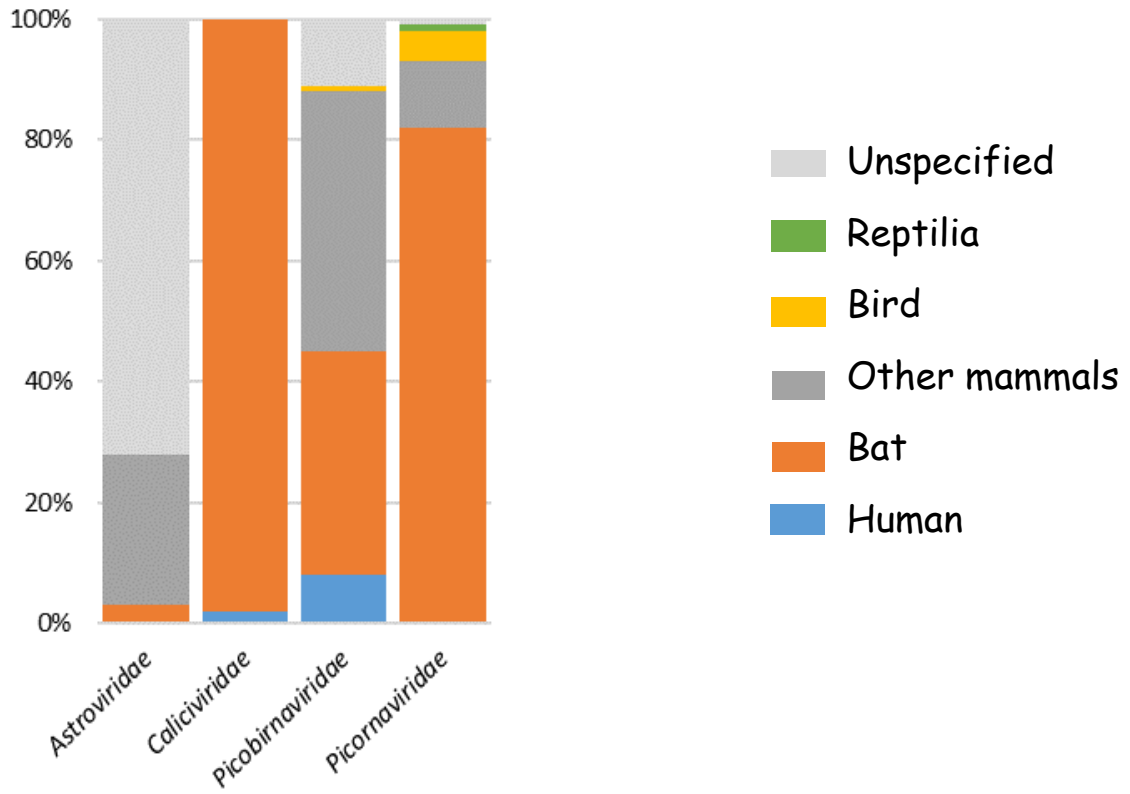


Bonny, et al. Front. Microbiol. 2021: 12.



Bonny, et al. Front. Microbiol. 2021: 12.

A variety of sequences identified



Bonny, et al. Front. Microbiol. 2021: 12.

Human and animal NoV sequences

Date	Length (nt)	Mapped reads	Nucleotide match	Identity (%)	Names
Sept. 18	367	176	366#	96.2	Norovirus GII.6[P6]
Sept. 18	353	101	353#	95.7	
Dec. 19	387	10	387#	97.1	Norovirus GII.2[Pe]
Jan. 20	1,833	1,003	699	87.1	Bat calicivirus A10
Apr. 19	1,328	846	1,281	71.0	
Sept. 18	1,026	670	1,026	51.3	
Apr. 19	1,653	769	1,389	70.8	
Apr. 19	1,086	155	294	70.4	

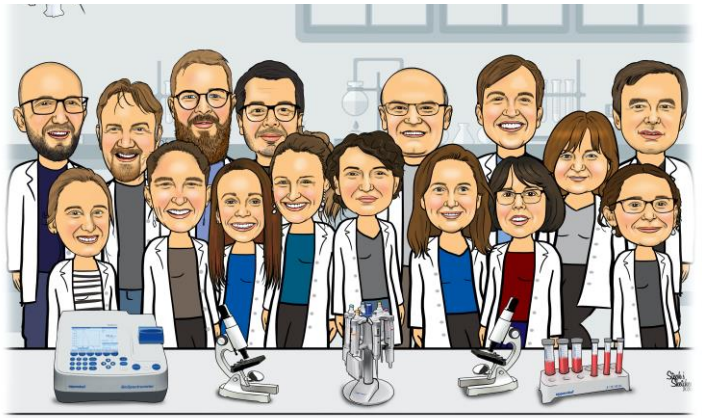
Bonny, et al. Front. Microbiol. 2021: 12.

Discussion…

- Finding short RNA genomes in food samples contaminated at low level is rather challenging,
- Depending on questions targeted metagenomic or agnostic metagenomic may help to describe viral diversity
- Method developments are still needed to select viral particles from a very diverse environment
- An enrichment step during the library preparation is important to obtain more reads (= longer contigs?)
- Quality criteria need to be included…

Sebimer

LSEM, PhD (P. Bonny, and post-docs.



Erasmus Medical Center
-M. Koopmans
- M. De Graaf,
- B Oude Munnich

U. Yaounde
- J-J Essia-Ngang
- P. Bonny

Funding : Ifremer, DGAL,
EU-H2020 Compare, VEO

Thank you!

Any questions?

- Marlee.Mims@fda.hhs.gov
- Rachel.Rodriguez@fda.hhs.gov

Final Days!

IAFP Offers Open Access to Webinars Until June 30!

Food standards save lives

World Food Safety Day was June 7, 2023.

In recognition of this day to increase awareness about food safety, IAFP is providing **open access from June 1–30, 2023,** to all recorded webinars in the IAFP archives for non-Members.

IAFP non-Members can browse the webinar archives on our **website** where **more than 100 webinars** dating back to 2009 are located (log-in not required).

One of the many benefits of IAFP Membership is access to the Association's **free webinars**, which are sponsored by the IAFP Foundation.

Not a Member? Consider joining today. Go here to learn more.

IAFP FOUNDATION

Upcoming Webinars

International Association for Food Protection WEBINAR

September 22, 2023	Modeling Salmonella Growth and Inactivation for Small and Very Small Processors with Limited Data
October 24, 2023	Managing Meat Shelf Life and Spoilage to Ensure Food Security

https://www.foodprotection.org/events-meetings/webinars/

IAFP FOUNDATION

Be sure to follow us on social media

International Association for Food Protection WEBINAR

InternationalAssociationforFoodProtection

@IAFPFOOD

IAFPFood

international-association-for-food-protection

International Association for Food Protection WEBINAR

This webinar is being recorded and will be available for access by **IAFP members** at www.foodprotection.org within one week.

Not a Member? We encourage you to join today.
For more information go to: www.FoodProtection.org/membership/

All **IAFP webinars** are supported by the IAFP Foundation with no charge to participants.

Please consider making a donation to the **IAFP Foundation** so we can continue to provide quality information to food safety professionals.

IAFP FOUNDATION