FishMed-PhD
28 Febbraio 2023

Microbial food webs, Safety of fish and fisheries products, and teleost microbiome

Gian Marco Luna

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Istituto per le Risorse Biologiche e le Biotecnologie Marine
IRBIM CNR

Outline for today

- The microbial (unseen) majority on Earth
- Use of DNA-based techniques to study marine microbes
- The teleost microbiome
- Safety of fish and fisheries products

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 6578–6583, June 1998

Perspective

Prokaryotes: The unseen majority

*William B. Whitman**†, *David C. Coleman*‡, *and William J. Wiebe*§

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ABSTRACT **The number of prokaryotes and the total amount of their cellular carbon on earth are estimated to be 4–6 × 10^{30} cells and 350–550 Pg of C (1 Pg = 10^{15} g), respectively. Thus, the total amount of prokaryotic carbon is 60–100% of the estimated total carbon in plants, and inclusion of prokaryotic carbon in global models will almost double estimates of the amount of carbon stored in living organisms. In addition, the earth's prokaryotes contain 85–130 Pg of N and 9–14 Pg of P, or about 10-fold more of these nutrients than do plants, and represent the largest pool of these nutrients in living organisms. Most of the earth's prokaryotes occur in the open ocean, in soil, and in oceanic and terrestrial subsurfaces, where the numbers of cells are 1.2 × 10^{29}, 2.6 × 10^{29}, 3.5 × 10^{30}, and 0.25–2.5 × 10^{30}, respectively. The numbers of heterotrophic prokaryotes in the upper 200 m of the open ocean, the ocean below 200 m, and soil are consistent with average turnover times of 6–25 days, 0.8 yr, and 2.5 yr, respectively. Although subject to a great deal of uncertainty, the estimate for the average turnover time of prokaryotes in the subsurface is on the order of 1–2 × 10^{3} yr. The cellular production rate for all prokaryotes on earth is estimated at 1.7 × 10^{30} cells/yr and is highest in the open ocean. The large population size and rapid growth of prokaryotes provides an enormous capacity for genetic diversity.**

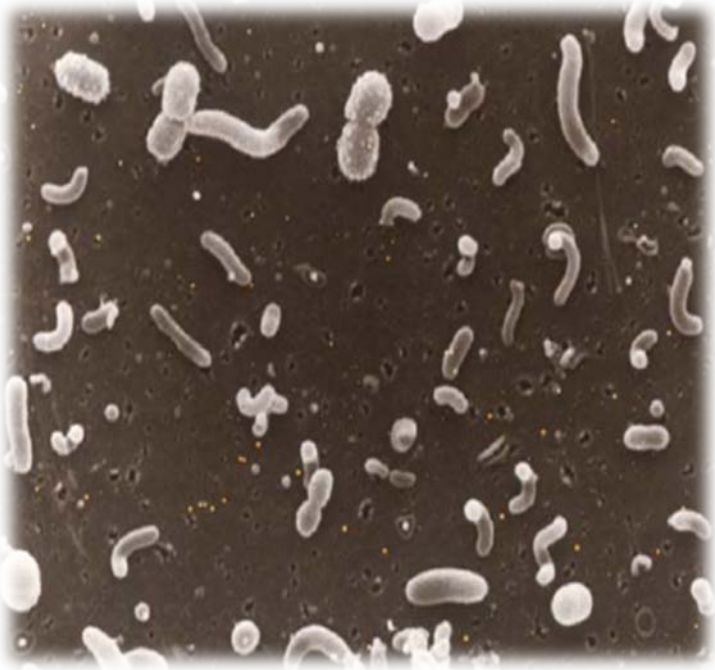
portion of these cells are the autotrophic marine cyanobacteria and *Prochlorococcus* spp., which have an average cellular density of 4 × 10^{4} cells/ml (6). The deep (>200 m) oceanic water contains 5 × 10^{4} cells/ml on average. From global estimates of volume, the upper 200 m of the ocean contains a total of 3.6 × 10^{28} cells, of which 2.9 × 10^{27} cells are autotrophs, whereas ocean water below 200 m contains 6.5 × 10^{28} cells (Table 1).

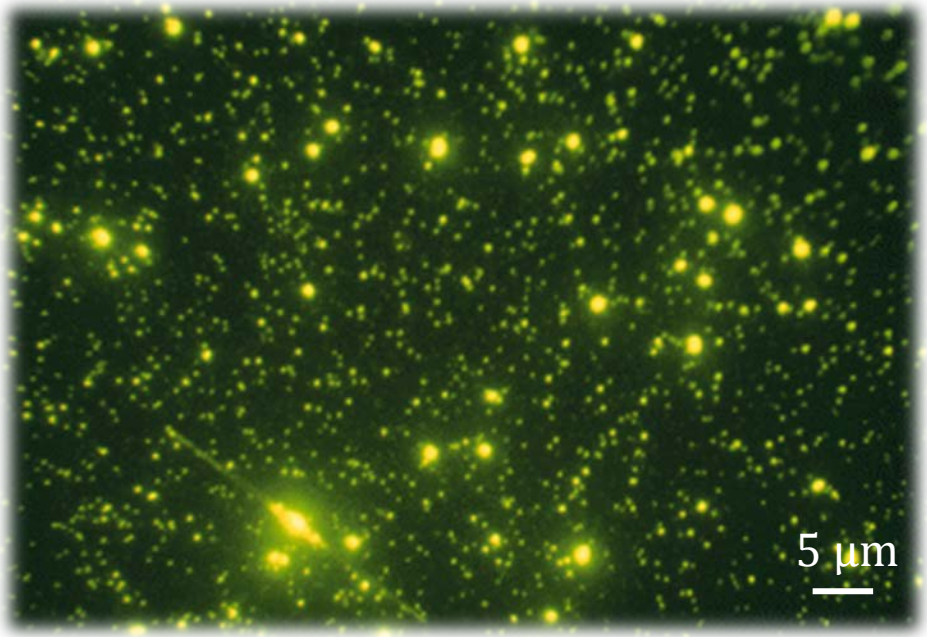
The upper 10 cm of sediment in the open ocean is included in the oceanic habitat because, as a result of animal mixing and precipitation, it is essentially contiguous with the overlying water column. Most of the marine sediment is found in the continental rise and abyssal plain, so the numbers of prokaryotes were calculated from an arithmetic average of the cellular densities in the studies cited by Deming and Baross (ref. 9; Table 1). The Nova Scotian continental rise was excluded from this calculation because of its unusual hydrology (10).

There are fewer estimates of the number of prokaryotes in freshwaters and saline lakes (5). Given an average density of 10^{6} cells/ml, the total number of cells in freshwaters and saline lakes is 2.3 × 10^{26}. This value is three orders of magnitude below the numbers of prokaryotes in seawater.

In the polar regions, a relatively dense community of algae and prokaryotes forms at the water–ice interface in annual sea

The «microbial» ocean



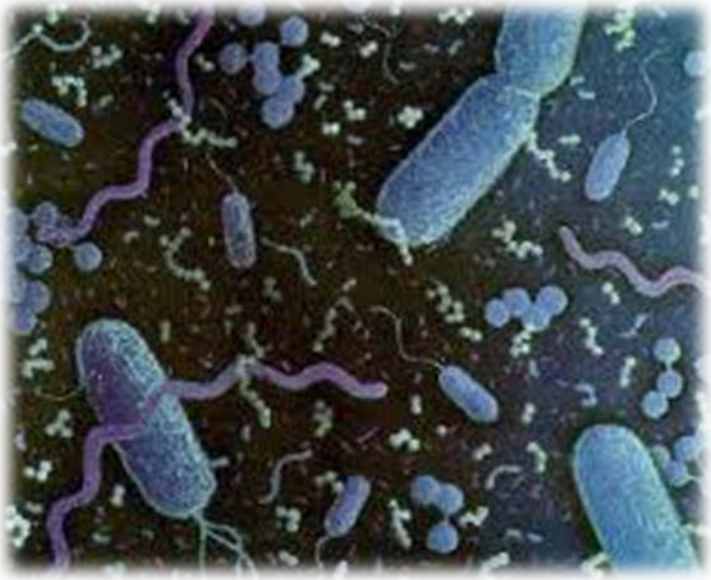


One drop of ocean water hosts more than 1,000,000 microbial cells and viruses

More than 1,000,000,000 in one gram of sediment

The microbial ocean is grand

2×10^{29} bacteria in the Ocean

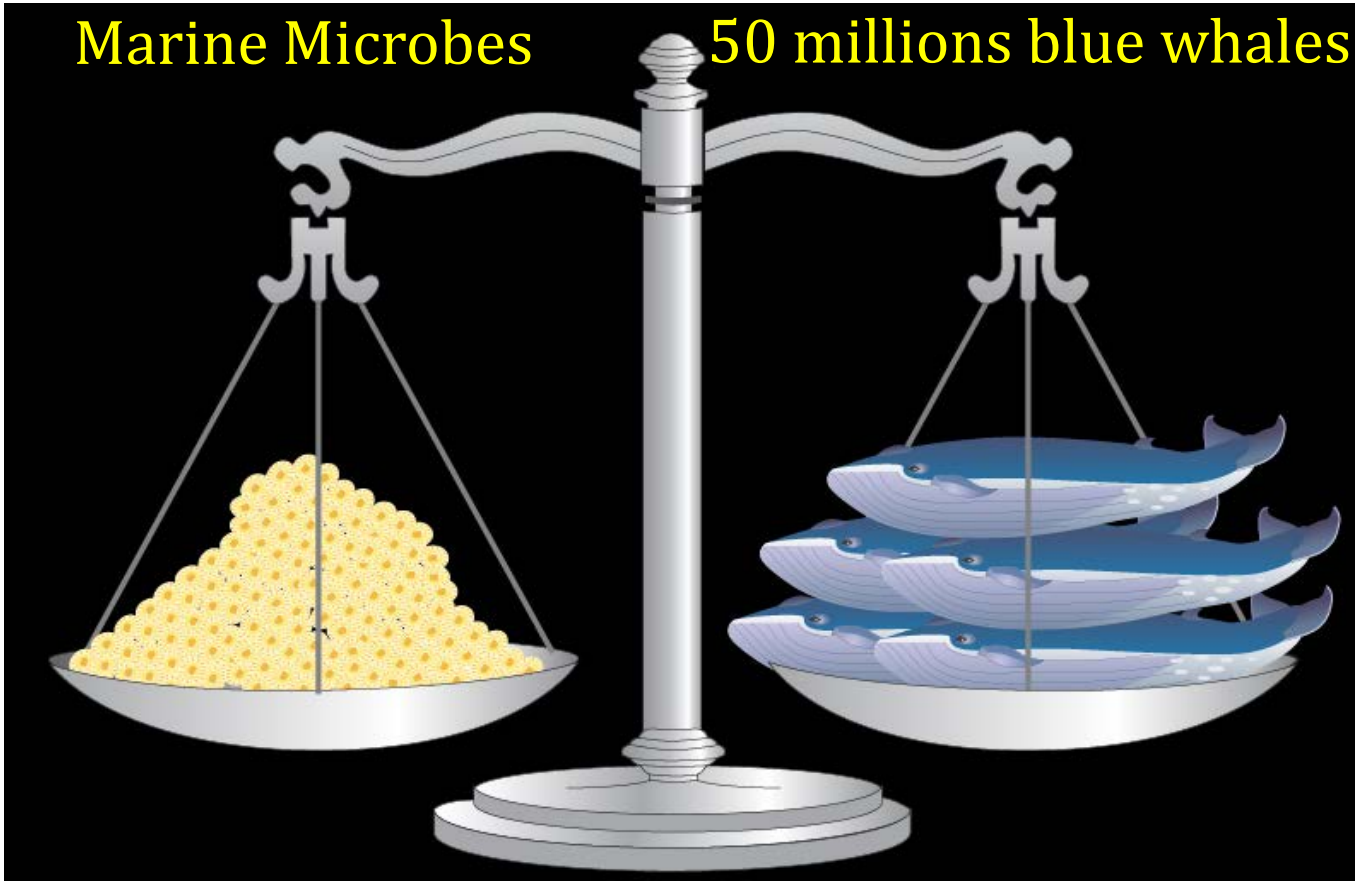


3×10^{23} stars in the Universe

Aligning oceanic viruses (average diameter 50 nm), the viral string-of-pearls

… would be 400,000 light years long
(the diameter of our galaxy [Milky Way] is only 25,000 light years)

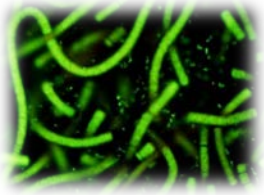
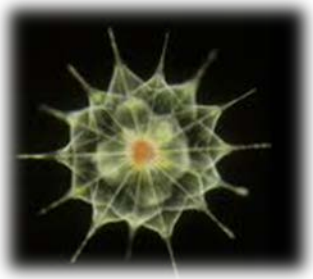
Invisible, yet heavy …

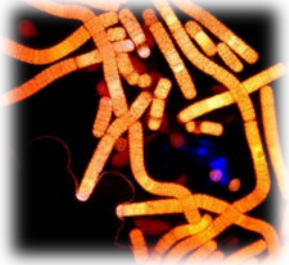
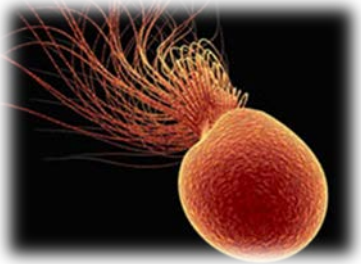
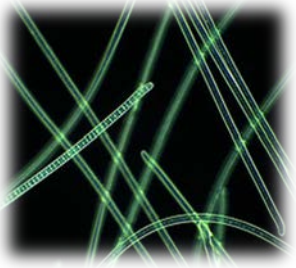
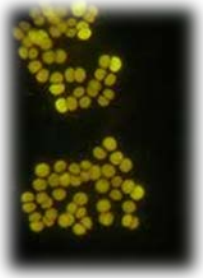


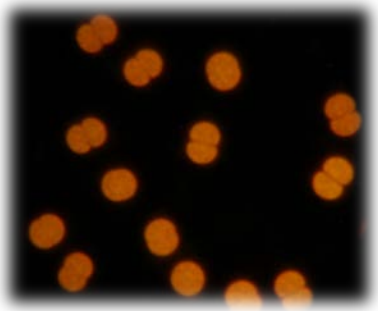
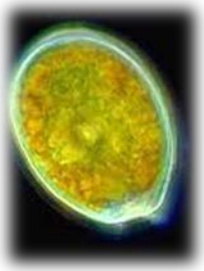
The cumulative biomass of marine microbes = 2.2 Pg of C ($1\ Pg = 10^{15}$)

Whiman et al 1998 PNAS

Microbial diversity is immense
More than one million species on Earth?


commentary

More than meets the eye

Earth's real biodiversity is invisible, whether we like it or not.

Abundant yet important: the GOOD side of marine microbes

Microbes play an essential role for the ocean functioning

- Life originated in the oceans 3.5 billion years ago, and microbes were the only form of life for two thirds of the planet's existence

-The development and maintenance of all forms of life depend on the past/present activities of marine microbes

- Yet the vast majority of humans - including many marine scientists - live their lives completely unaware of the diversity and importance of marine microbes

Mediate the energy and matter fluxes along the trophic web

(channel up to 90% of OM in the ocean)

https://ocean.si.edu/ocean-life/microbes/microbial-loop

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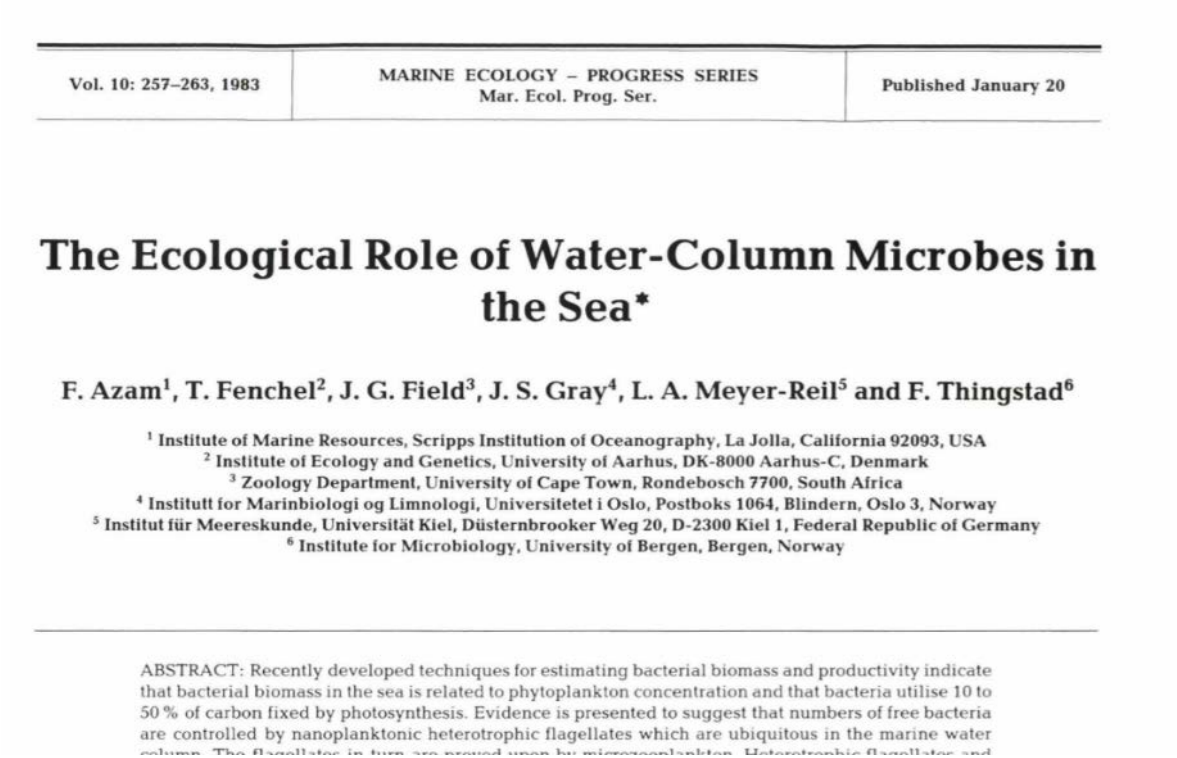

Vol. 10: 257–263, 1983 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published January 20

The Ecological Role of Water-Column Microbes in the Sea*

F. Azam[1], T. Fenchel[2], J. G. Field[3], J. S. Gray[4], L. A. Meyer-Reil[5] and F. Thingstad[6]

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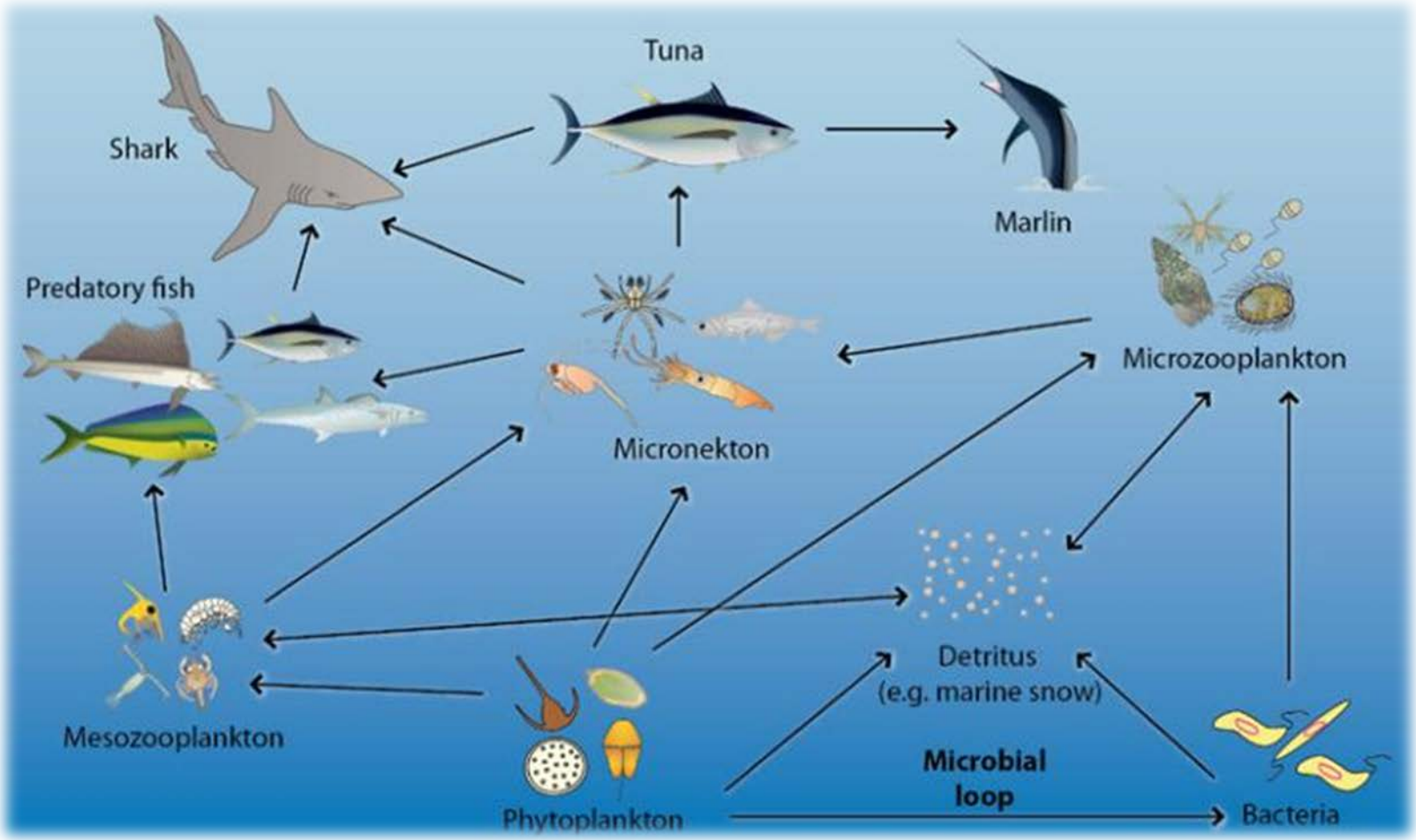
[2] Institute of Ecology and Genetics, University of Aarhus, DK-8000 Aarhus-C, Denmark
[3] Zoology Department, University of Cape Town, Rondebosch 7700, South Africa
[4] Institutt for Marinbiologi og Limnologi, Universitetet i Oslo, Postboks 1064, Blindern, Oslo 3, Norway
[5] Institut für Meereskunde, Universität Kiel, Düsternbrooker Weg 20, D-2300 Kiel 1, Federal Republic of Germany
[6] Institute for Microbiology, University of Bergen, Bergen, Norway

ABSTRACT: Recently developed techniques for estimating bacterial biomass and productivity indicate that bacterial biomass in the sea is related to phytoplankton concentration and that bacteria utilise 10 to 50 % of carbon fixed by photosynthesis. Evidence is presented to suggest that numbers of free bacteria are controlled by nanoplanktonic heterotrophic flagellates which are ubiquitous in the marine water

https://www.int-res.com/articles/meps/10/m010p257.pdf

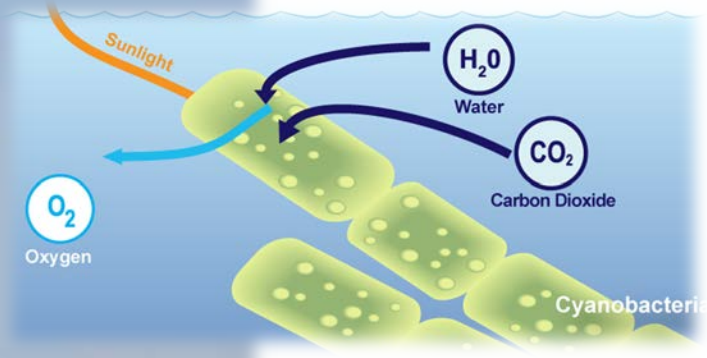
Bacteria and other micro-organisms have long been known to play a part in marine ecosystems (Sorokin, 1981), but it has been difficult to study them quantitatively

Mediate the energy and matter fluxes along the trophic web
(channel up to 90% of OM in the ocean)

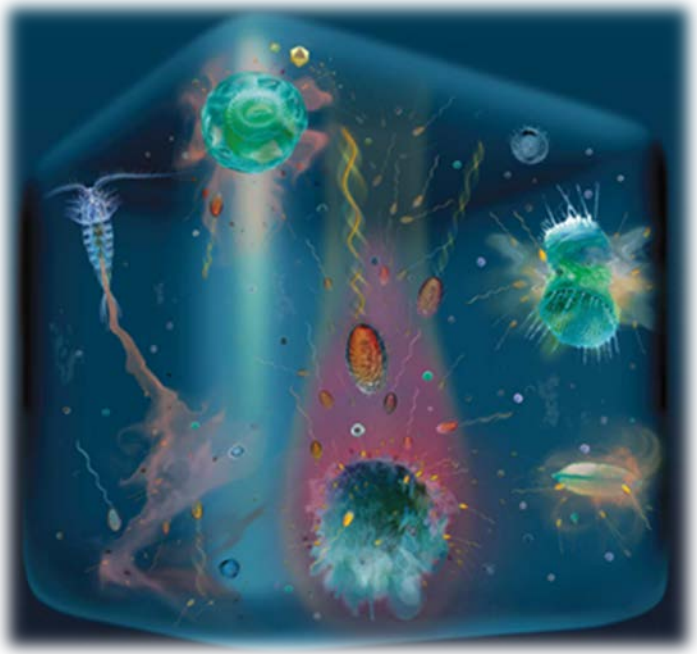


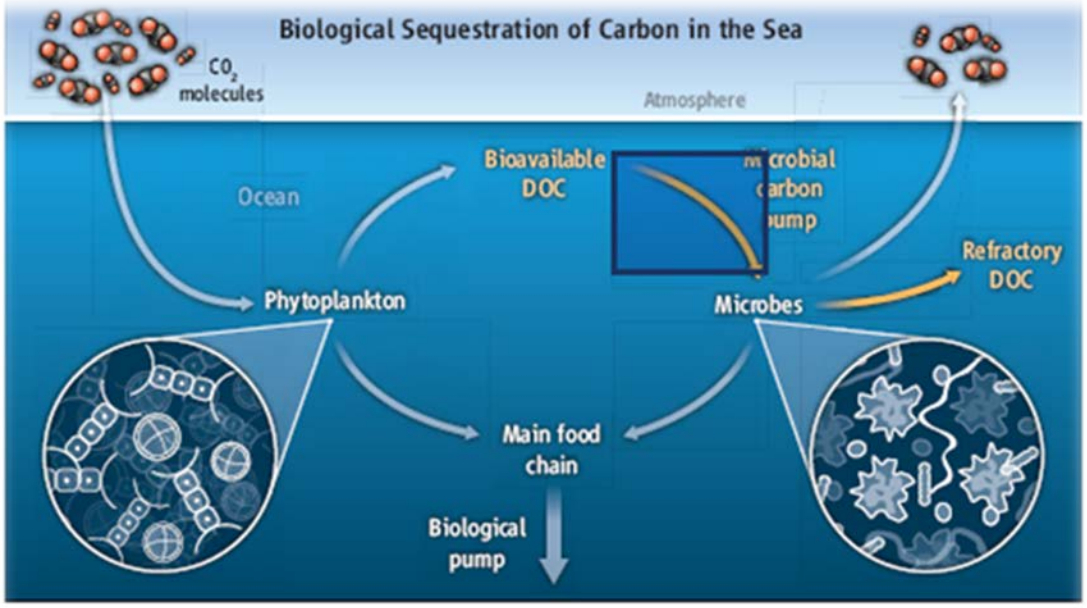
The “microbial loop” concept

Produce half of the oxygen that we breathe



Drive the global biogeochemical cycles

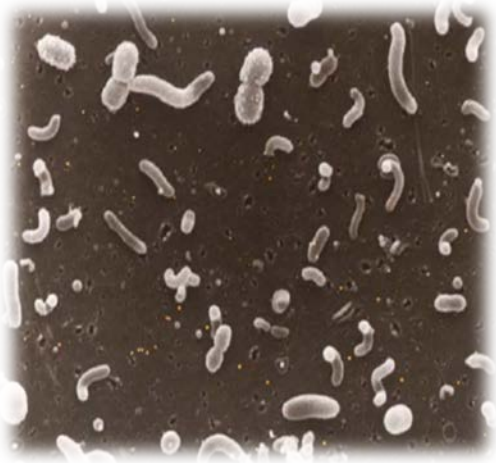




Double-barrel pump. Each year, the biological pump deposits some 300 million tons of carbon in the deep ocean sink. Even more massive amounts are suspended in the water column as dissolved organic carbon, much of which is converted into refractory forms by the microbial carbon pump.

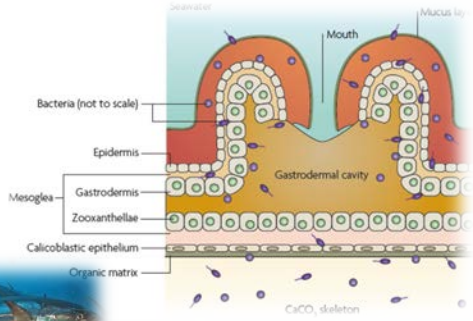
Result of nearly infinite interactions occurring at the mm, µm, nm and molecular scale

Marine microbial habitats

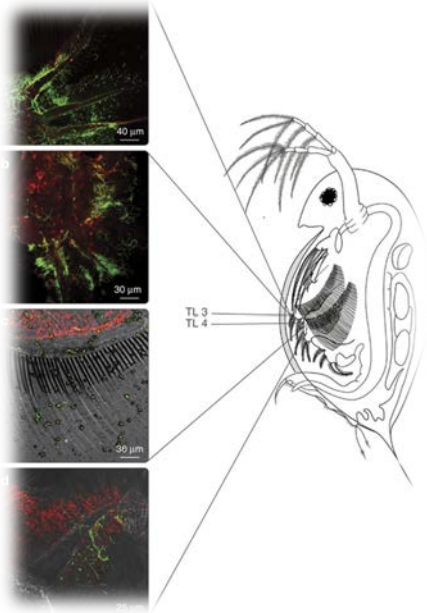


Water column (free-living vs. attached)

Sediment (down to the deep biosphere)



The surface of marine organisms (epibionts & co)



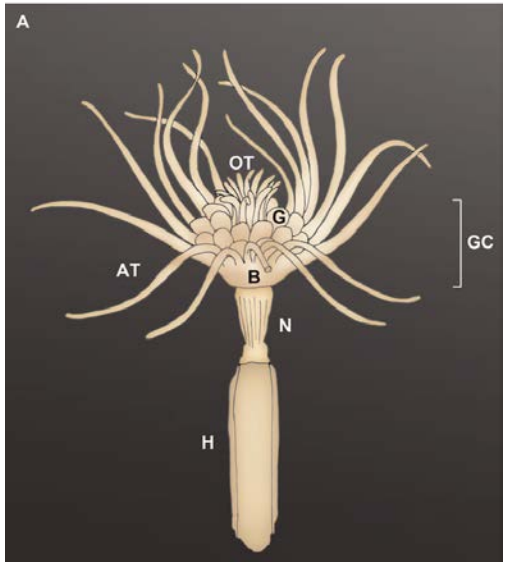
All marine organisms have an associated microbiome (essential for their life!)

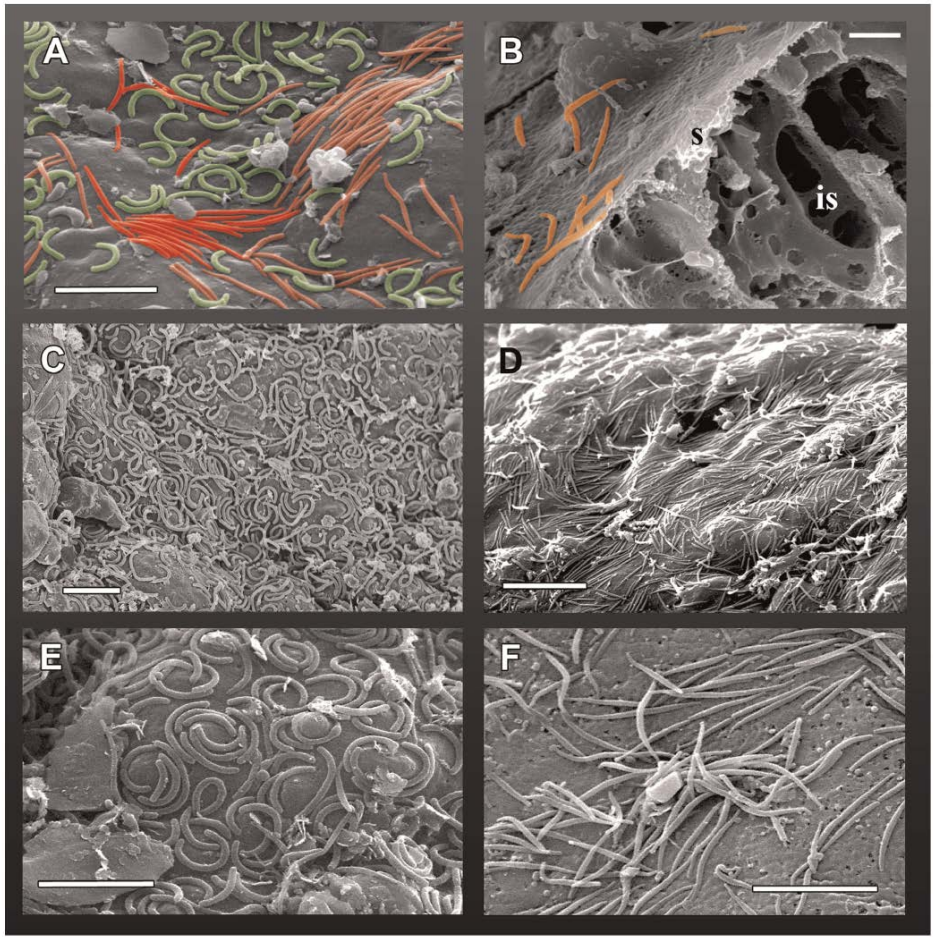
Microbial epibionts

OPEN ACCESS Freely available online PLoS one

Biodiversity of Prokaryotic Communities Associated with the Ectoderm of *Ectopleura crocea* (Cnidaria, Hydrozoa)

Cristina Gioia Di Camillo[1]*, Gian Marco Luna[2], Marzia Bo[3], Giuseppe Giordano[1], Cinzia Corinaldesi[1], Giorgio Bavestrello[3]





Two microbial morphotypes:
Type I horseshoe-shaped
Type II fusiform, worm-like
Prokaryotes present all around the epidermis. The two morphos often simultaneously present on the hydroid's surface (Type II most common)

Microbial epibionts

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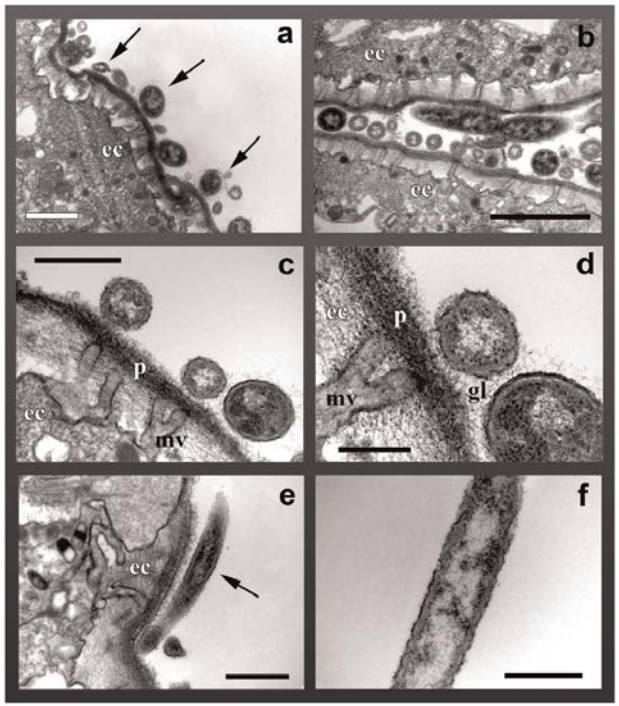
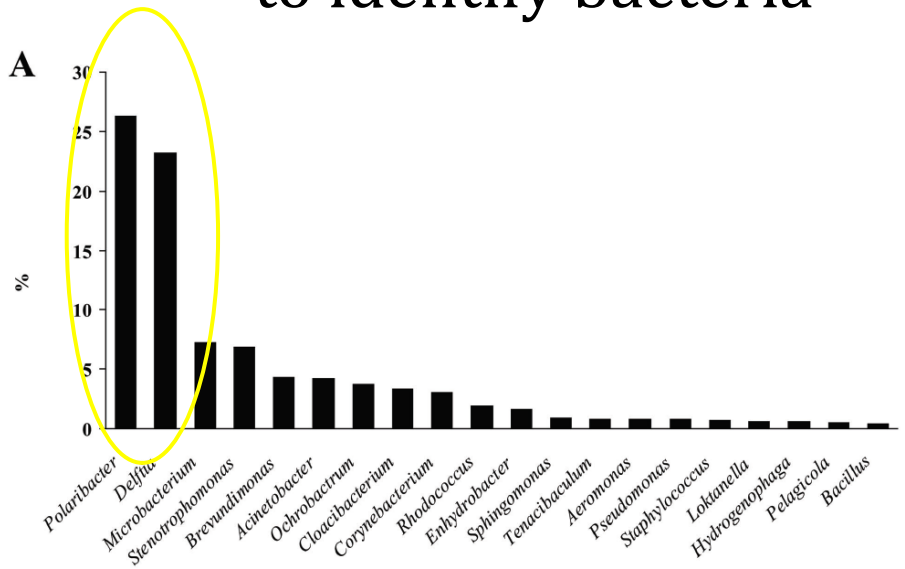


Figure 4. TEM pictures of *Type II* bacteria associated with *Ectopleura crocea*. A) Numerous bacteria in transve on the hydroid ectoderm (ec). B) Bacteria (longitudinal and tranversal sections) present in a groove of the hydroid ect hydroid periderm (p) are often found in correspondence to the microvilles (mv) of the ectodermal cells. D) Close-up v surrounded the microorganisms. e–f. Longitudinal section of a bacterium. Scale bars: a, c, e 1 µm; b 2 µm; d, f 0.5

doi:10.1371/journal.pone.0039926.g004

16S rDNA NGS sequencing to identify bacteria



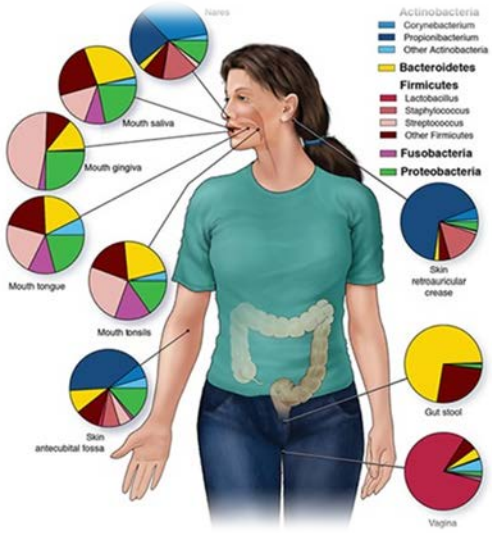
Type I *Delftia*

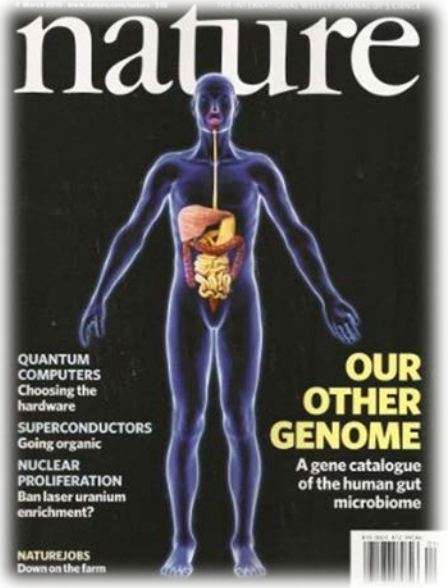
Type II *Polaribacter*

Which role they play?

The microbiome revolution

Siamo più «microbi» che «uomini»?





Siamo un “super-organismo” composto da cellule umane e da microbi.

Sorprendentemente, le cellule batteriche sono 10-100 volte le cellule «propriamente» umane

Ognuno di noi trasporta un chilo e trecento grammi di microbi (più pesanti del cervello)

The microbiome revolution

Scienza

I batteri siamo noi

Michael Specter, The New Yorker, Stati Uniti
Foto di Martin Oeggerli

Possono ucciderci, ma sono anche indispensabili per tenerci in vita. Gli scienziati studiano questi organismi che vivono dentro di noi per capire come usarli nella cura delle malattie. Dopo la mappatura del genoma umano, è cominciata l'era del microbioma

Probabilmente l'*Helicobacter pylori* è l'agente patogeno più efficiente della storia dell'umanità. Non è letale come i batteri che provocano la tubercolosi, il colera e la peste, ma infetta un numero di persone maggiore di tutti gli altri messi insieme. Emigrato dall'Africa come i nostri antenati, è legato alla storia della specie umana da almeno duecentomila anni. Anche se è ospitato dalla metà degli stomaci del pianeta, il suo ruolo non era mai stato chiaro. Poi nel 1982 due scienziati, Barry Marshall e J. Robin Warren, scoprirono che l'*H. pylori* è la causa principale della gastrite e delle ulcere peptiche: da allora, il batterio è stato anche associato a un aumento del rischio di cancro allo stomaco. Fino a quella scoperta si pensava che la causa principale delle ulcere peptiche non fosse un'infezione, ma lo stress. Nel 2005 Marshall e Robin hanno ricevuto il premio Nobel per le loro ricerche.

L'*H. pylori* ha la forma di un cavatappi ed è lungo tre micron (un granello di sabbia misura circa trecento micron). È anche uno dei rari microbi che vivono tranquillamente nelle zone più acide dello stomaco. I medici si sono resi conto che si poteva curare l'ulcera eliminando il batterio con gli antibiotici: la cura era così efficace che ogni tanto qualcuno proponeva di provare a debellare totalmente l'*Helicobacter*. Su una cosa erano tutti d'accordo: "L'unico *Helicobacter pylori* buono è quello morto", scriveva un eminente gastroenterologo nel 1997. Ma debellarlo si è rivelato più complicato e costoso del previsto e i tentativi hanno perso slancio. Eppure quasi nessuno metteva in discussione la validità dell'obiettivo: "L'*Helicobacter* causava il cancro e l'ulcera", mi ha detto Martin J. Blaser, direttore del dipartimento di medicina e docente di microbiologia alla scuola di medicina della New York university. "Bisognava eliminarlo il più rapidamente possibile. Che io sappia, nessuno all'epoca si sarebbe mai fermato a riflettere sulle possibili conseguenze".

E nessuno era ansioso di eliminare quell'organismo più di Blaser, che ha dedicato gran parte della sua vita professionale allo studio dell'*H. pylori*. Il suo laboratorio alla New York university ha sviluppato i primi esami per identificare il microbo, molti dei quali sono usati ancora oggi. Ma Blaser – una mente curiosa che, oltre a fare il ricercatore è stato tra i fondatori della Bellevue Literary Review – si chiedeva come era possibile che un organismo vecchio quanto la specie umana era potuto sopravvivere se faceva solo danni. "Non è così che funziona l'evoluzione", sostiene Blaser. "L'*H. pylori* è una componente ancestrale dell'umanità". Alla fine degli anni novanta Blaser ha cominciato a studiare più da vicino il comportamento molecolare del batterio e nel 1998 ha pubblicato uno studio sul British Medical Journal in cui sosteneva, contro l'opinione corrente, che forse non era così pericoloso. L'anno dopo ha creato la Foundation for batteriology, con l'obiettivo

Batteri intestinali

I nostri microbi influiscono in maniera significativa sulle nostre principali funzioni vitali (meccanismi immunitari, digestione), ma anche sulla nostra personalità!

The microbiome revolution

https://www.google.it/search?q=ted+microbiome&oq=ted+microbiome&aqs=chrome..69i57j69i60

App | Venezia Gestione Pr... | Biblioproxy - Risorse | Come iscriversi ai co... | Marea Venezia | ADRION Programm... | The Em

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ted microbiome

Tutti | Notizie | Immagini | Video | Shopping | Altro | Strumenti di ricerca

Circa 104.000 risultati (0,41 secondi)

Rob Knight: How our microbes make us who we are | TED ...

https://www.**ted**.com/.../rob_knight_how_our_micro...

How the **microbiome** shapes our world. Rob Knight talks to biologist Jonathan Eisen and biodiversity scientist ...

Jonathan Eisen: Meet your microbes | TED Talk | TED.com

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How the **microbiome** shapes our world. Jonathan Eisen talks to biologist Rob Knight and biodiversity scientist ...

Rob Knight | Speaker | TED.com

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Using scatological research methods that might repel the squeamish, microbial researcher Rob Knight uncovers the secret ecosystem (or "**microbiome**") of ...

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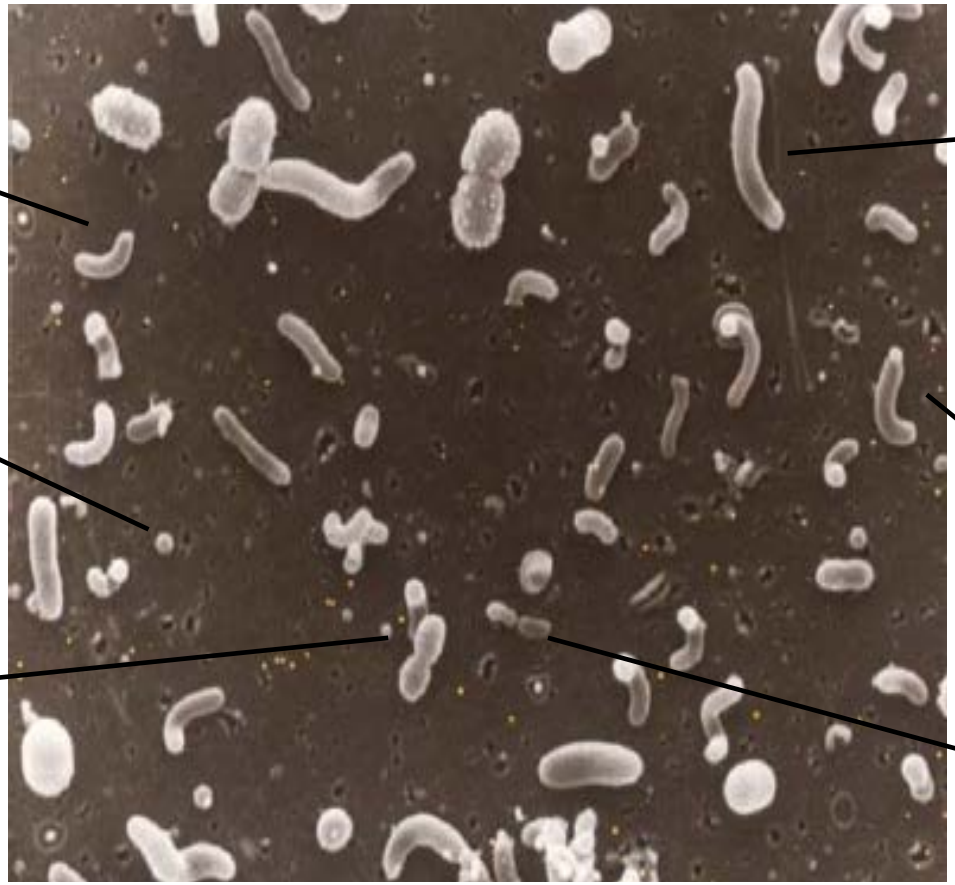
how the microbiome affects brain and behavior - YouTube

https://www.youtube.com/watch?v=FWT_BLVOASI

How to study the diversity of microbes?

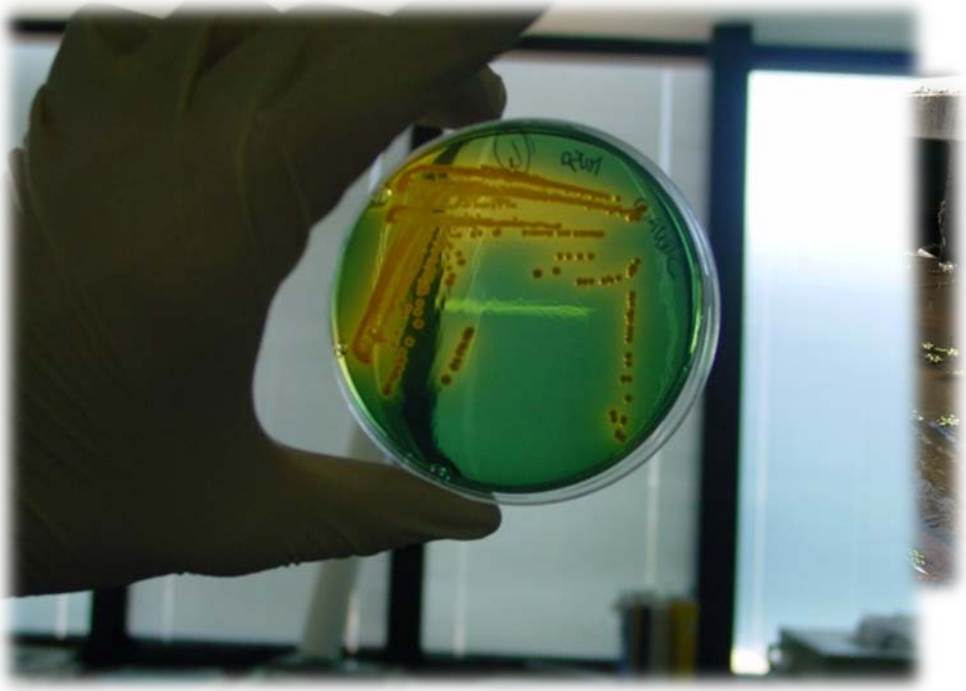
How to study the diversity of microbes?

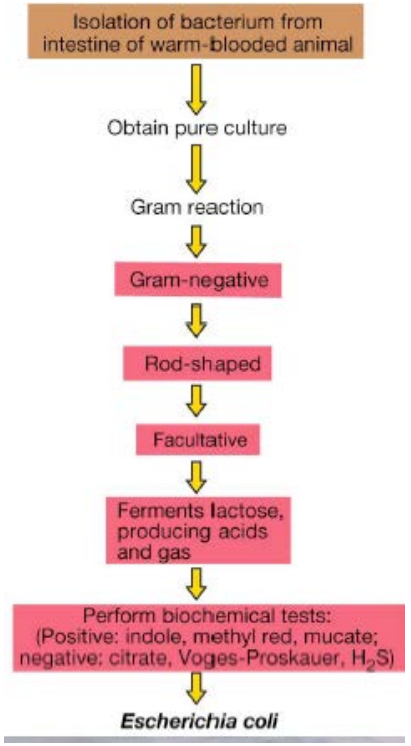
Microbes under the microscope all look more or less the same



Contrariously to higher organisms, morphology is not or poorly informative…..

Traditionally, cultivation in the lab was a prerequisite to identify microbes





Isolate the microbe(s) on agar plates, then identify through phenotipic/metabolic features

Problem: won't work for environmental microbes.
Only <0.01% of bacteria from one sample will grow

Recognizing microbes from their DNA sequence is easier

In the last decades, alternative ways to identify microbes from their DNA sequences have been developed

No need to cultivate, DNA extracted directly from the environmental sample

Based on DNA or RNA extraction, sequencing of specific («marker») or all genes, bioinformatics analyses

The 16S rRNA gene hystorically the first (and best?) marker gene for phylogenetic studies

Carl Woese pioneered the use of 16S rRNA to identify microbes

Winner of the Crafoord Prize in 2003

Ribosomal RNA: a key to phylogeny

GARY J. OLSEN[1] AND CARL R. WOESE
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ABSTRACT **As molecular phylogeny increasingly shapes our understanding of organismal relationships, no molecule has been applied to more questions than have ribosomal RNAs. We review this role of the rRNAs and some of the insights that have been gained from them. We also offer some of the practical considerations in extracting the phylogenetic information from the sequences. Finally, we stress the importance of comparing results from multiple molecules, both as a method for testing the overall reliability of the organismal phylogeny and as a method for more broadly exploring the history of the genome.—Olsen, G. J., Woese, C. R. Ribosomal RNA: a key to phylogeny. *FASEB J.* 7: 113–123; 1993.**

Key Words: molecular phylogeny · maximum parsimony · maximum likelihood · least squares distance

WHEN DEALING WITH MOLECULAR SEQUENCES an evolutionist feels a sense of liberation; he is no longer confined to the world of "higher forms." From the vantage point provided by molecular data, he now gazes over the Cambrian "wall" that had obstructed his temporal perspective. He can now scan the full panorama of Earth's four-billion-year evolutionary history. Color has been added to his monochromic, morpho-

However, molecular sequences are a vast and rich mine of evolutionary information, a fact first brought home by Zuckerkandl and Pauling (4) in their article "Molecules as documents of evolutionary history." With this publication the handwriting was on the wall: the basis for phylogenetic inference would now shift from the level of gross cellular and organismal properties to that of molecular characteristics. Here, then, was the solution to microbiology's chronic problem. On the molecular level the history of the prokaryote is just as evident, just as richly informative, as is that of the higher forms. Yet for reasons we will not discuss here, microbiologists did not harken to the molecular message, and so another decade or more would pass before prokaryotes came in for the molecular phylogenetic characterization they so badly needed (5). The results of these molecular studies are now widely known: phylogenies are being inferred today for all microorganisms, and the old determinative classification is rapidly being supplanted by a natural, phylogenetic one.

MOLECULAR PHYLOGENY

A molecular phylogeny is the phylogeny of a portion of a ge-

A revolution is occurring in biology: perhaps it is better characterized as a revolution within a revolution. I am, of course, referring to the impact that the increasingly rapid capacity to sequence nucleic acids is having on a science that has already been radically transformed by molecular approaches and concepts (Woese, 1987).

First studies to test the usefulness of the approaches in the early '90s

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16S Ribosomal DNA Amplification for Phylogenetic Study

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A set of oligonucleotide primers capable of initiating enzymatic amplification (polymerase chain reaction) on a phylogenetically and taxonomically wide range of bacteria is described along with methods for their use and examples. One pair of primers is capable of amplifying nearly full-length 16S ribosomal DNA (rDNA) from many bacterial genera; the additional primers are useful for various exceptional sequences. Methods for purification of amplified material, direct sequencing, cloning, sequencing, and transcription are outlined. An obligate intracellular parasite of bovine erythrocytes, *Anaplasma marginale*, is used as an example; its 16S rDNA was amplified, cloned, sequenced, and phylogenetically placed. Anaplasmas are related to the genera *Rickettsia* and *Ehrlichia*. In addition, 16S rDNAs from several species were readily amplified from material found in lyophilized ampoules from the American Type Culture Collection. By use of this method, the phylogenetic study of extremely fastidious or highly pathogenic bacterial species can be carried out without the need to culture them. In theory, any gene segment for which polymerase chain reaction primer design is possible can be derived from a readily obtainable lyophilized bacterial culture.

The comparison of rRNA sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria, archaebacteria, and eucaryotic organisms. These sequences have been derived previously by methods including oligonucleotide cataloging (6), sequencing of clones, direct sequencing of RNA by using reverse transcriptase (11), and sequencing of material amplified by polymerase chain reaction (PCR) (3, 5, 15). The present study expands on the use of DNA amplification technology for the study of rRNA sequences within the eubacteria. Several primers are

5% of this resuspended DNA was put into the PCR amplification.

PCR amplification and purification of product. Approximately 1 to 3 μg of genomic DNA was amplified in a 100-μl reaction by using the Geneamp kit (U.S. Biochemicals, Cleveland, Ohio; presently, these kits are only available from Perkin-Elmer Cetus, Norwalk, Conn.). When the lyophilized ampoule DNA was amplified, 1 μl was routinely used. Conditions consisted of 25 to 35 cycles of 95°C (2 min),

First studies to test the usefulness of the approaches in the early '90s

The Analysis of Natural Microbial Populations by Ribosomal RNA Sequences

NORMAN R. PACE, DAVID A. STAHL, DAVID J. LANE, and GARY J. OLSEN

1. Introduction

Recombinant DNA methodology and rapid nucleotide sequence determinations have changed the face of cell biology in the past few years. This technology offers powerful new tools to the microbial ecologist as well. In this chapter we describe technical strategies we are developing which use these methods to analyze phylogenetic and quantitative aspects of mixed, naturally occurring microbial populations.

The procedures we are developing use ribosomal RNA (rRNA) sequences to define and enumerate the components of mixed, natural populations. In one approach, suitable for mixed populations of limited complexity (less than about ten different organisms), we isolate 5S rRNA, sorting out the various species-specific molecules by high-resolution gel electrophoresis. Individual 5S rRNA types then are sequenced and, with reference to existing files of 5S rRNA sequences, the phylogenetic affinities of organisms contributing the analyzed 5S rRNAs are defined.

In a second approach toward analyzing mixed populations, an approach that seems to have no upper limit as to the complexity of the

Pace and colleagues used direct analysis of 5S and 16S rRNA gene sequences in the environment to describe the diversity of microorganisms in environmental samples without culturing

First studies to test the usefulness of the approaches in the early ‘90s

letters to nature

Nature **345**, 60 - 63 (03 May 1990); doi:10.1038/345060a0

Genetic diversity in Sargasso Sea bacterioplankton

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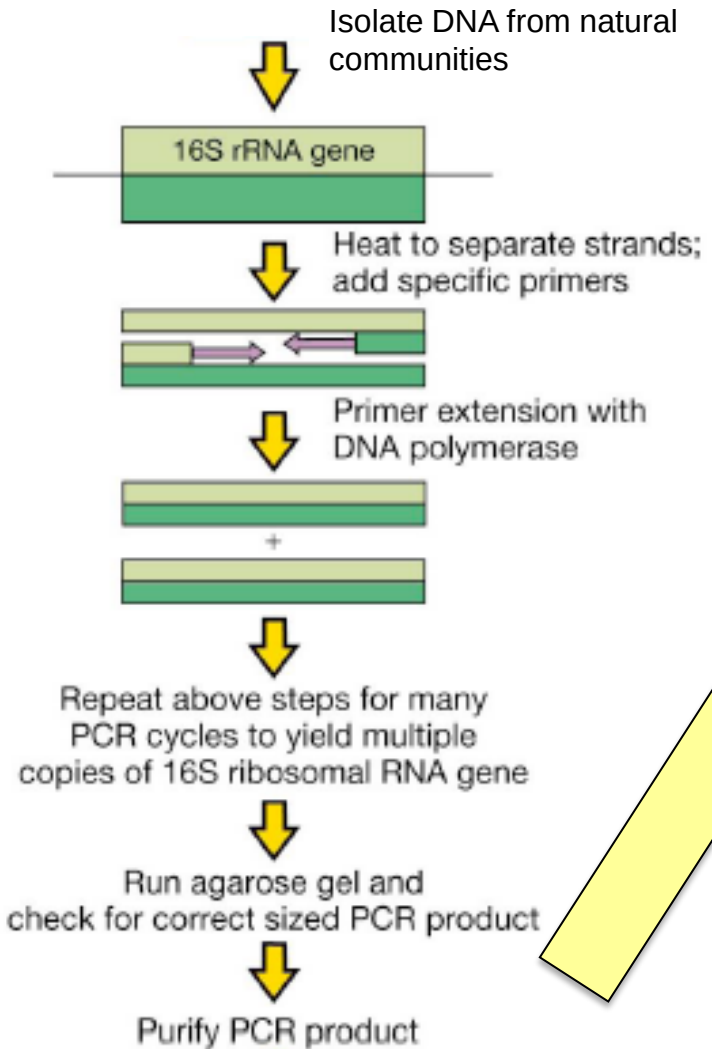
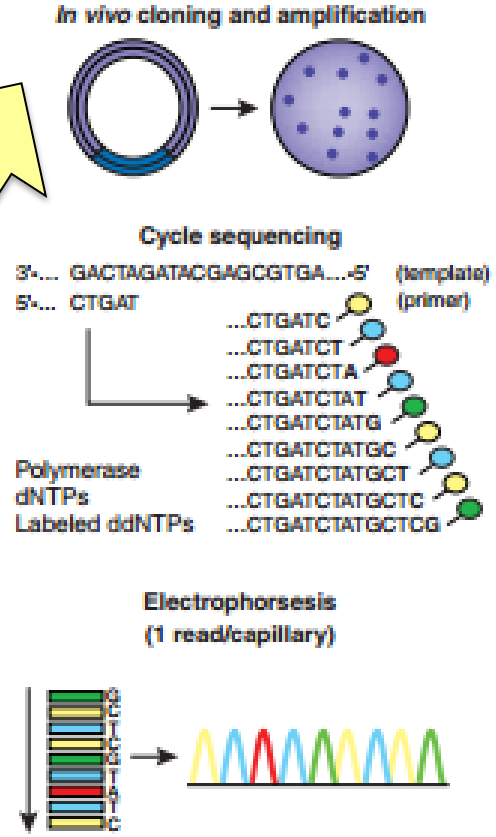
BACTERIOPLANKTON are recognized as important agents of biogeochemical change in marine ecosystems, yet relatively little is known about the species that make up these communities. Uncertainties about the genetic structure and diversity of natural bacterioplankton populations stem from the traditional difficulties associated with microbial cultivation techniques. Discrepancies between direct counts and plate counts are typically several orders of magnitude, raising doubts as to whether cultivated marine bacteria are actually representative of dominant planktonic species[1–3]. We have phylogenetically analysed clone libraries of eubacterial 16S ribosomal RNA genes amplified from natural populations of Sargasso Sea picoplankton by the polymerase chain reaction[4]. The analysis indicates the presence of a novel microbial group, the SAR 11 cluster, which appears to be a significant component of this oligotrophic bacterioplankton community. A second cluster of lineages related to the oxygenic phototrophs—cyanobacteria, prochlorophytes and chloroplasts—was also observed. However, none of the genes matched the small subunit rRNA sequences of cultivated marine cyanobacteria from similar habitats. The diversity of 16S rRNA genes observed within the clusters suggests that these bacterioplankton may be consortia of independent lineages sharing surprisingly distant common ancestors.

One of the first microbial molecular surveys in the ocean. They phylogenetically analysed clone libraries of bacterial 16S rRNA genes amplified from natural populations by the PCR. They reported novel microbial groups, none of the genes matched the ss rRNA sequences of cultivated marine cyanobacteria from similar habitats

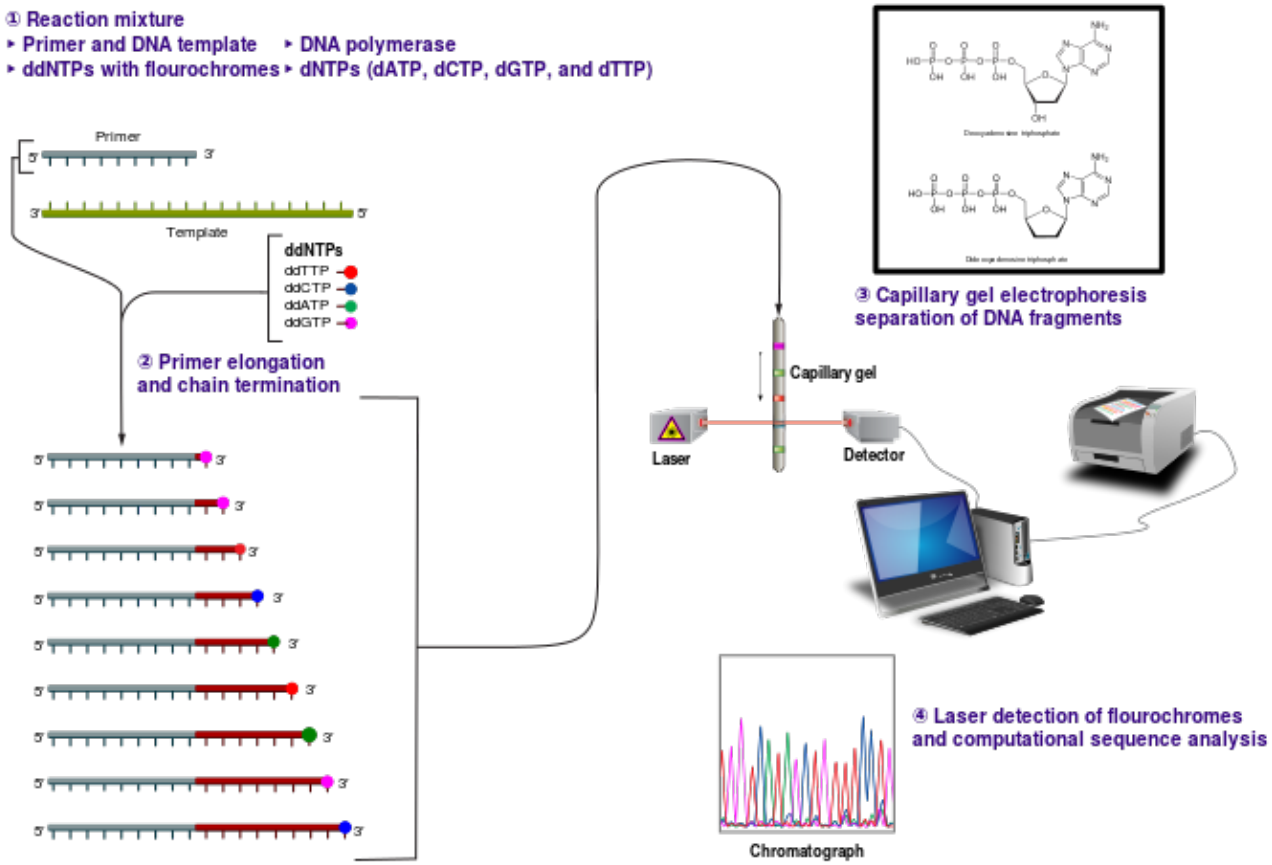
Rationale of the first approach





16S fragments cloned into a vector that was transformed (usually into *E. coli*) and subsequently sequenced by the Sanger method

Sanger sequencing



The chain-termination method requires a ssDNA template, a DNA primer, a DNA polymerase, normal dNTPs, and modified di-deoxyNTPs, terminating DNA strand elongation (lack 3'-OH group required for the formation of phosphodiester bond between nucleotides), causing polymerase to cease extension when a modified ddNTP is incorporated.

The ddNTPs may be radioactively or fluorescently labeled

The advent of Next (or Second) Generation Sequencing .... everything changes

nature biotechnology

REVIEW

Next-generation DNA sequencing

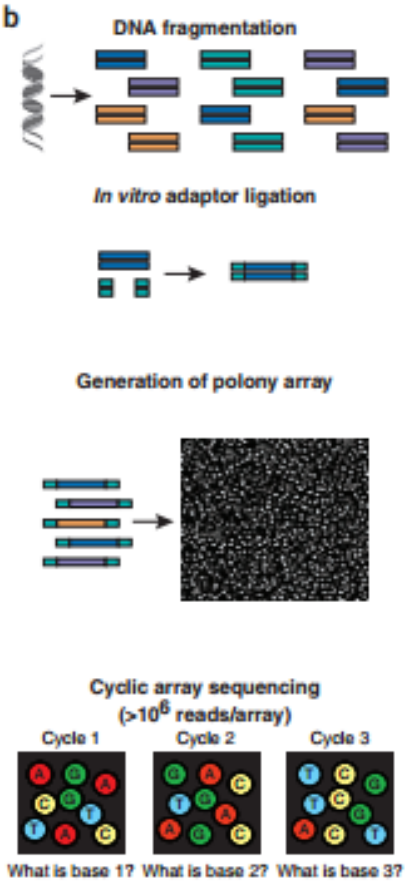
Jay Shendure[1] & Hanlee Ji[2]

DNA sequence represents a single format onto which a broad range of biological phenomena can be projected for high-throughput data collection. Over the past three years, massively parallel DNA sequencing platforms have become widely available, reducing the cost of DNA sequencing by over two orders of magnitude, and democratizing the field by putting the sequencing capacity of a major genome center in the hands of individual investigators. These new technologies are rapidly evolving, and near-term challenges include the development of robust protocols for generating sequencing libraries, building effective new approaches to data-analysis, and often a rethinking of experimental design. Next-generation DNA sequencing has the potential to dramatically accelerate biological and biomedical research, by enabling the comprehensive analysis of genomes, transcriptomes and interactomes to become inexpensive, routine and widespread, rather than requiring significant production-scale efforts.

The field of DNA sequencing technology development has a rich and diverse history[1,2]. However, the overwhelming majority of DNA sequence production to date has relied on some version of the Sanger biochemistry[3]. Over the past five years, the incentive for developing entirely new strategies for DNA sequencing has emerged on at least four levels, undeniably reinvigorating this field (for a review,

Sanger sequencing

Since the early 1990s, DNA sequence production has almost exclusively been carried out with capillary-based, semi-automated implementations of the Sanger biochemistry[3,5,6] (**Fig. 1a**). In high-throughput production pipelines, DNA to be sequenced is prepared by one of two approaches: first, for shotgun *de novo* sequencing, randomly frag-



is used to generate a sequencing trace. (b) In shotgun sequencing with cyclic-array methods, common adaptors are ligated to fragmented genomic DNA, which is then subjected to one of several protocols that results in an array of millions of spatially immobilized PCR colonies or 'polonies'[15]. Each polony consists of many copies of a single shotgun library fragment. As all polonies are tethered to a planar array, a single microliter-scale reagent volume (e.g., for primer hybridization and then for enzymatic extension reactions) can be applied to manipulate all array features in parallel. Similarly, imaging-based detection of fluorescent labels incorporated with each extension can be used to acquire sequencing data on all features in parallel. Successive iterations of enzymatic interrogation and imaging are used to build up a contiguous sequencing read for each array feature.

Different technologies available for NGS

Pyrosequencing (or 454)

Sequencing by Synthesis (e.g. Illumina)

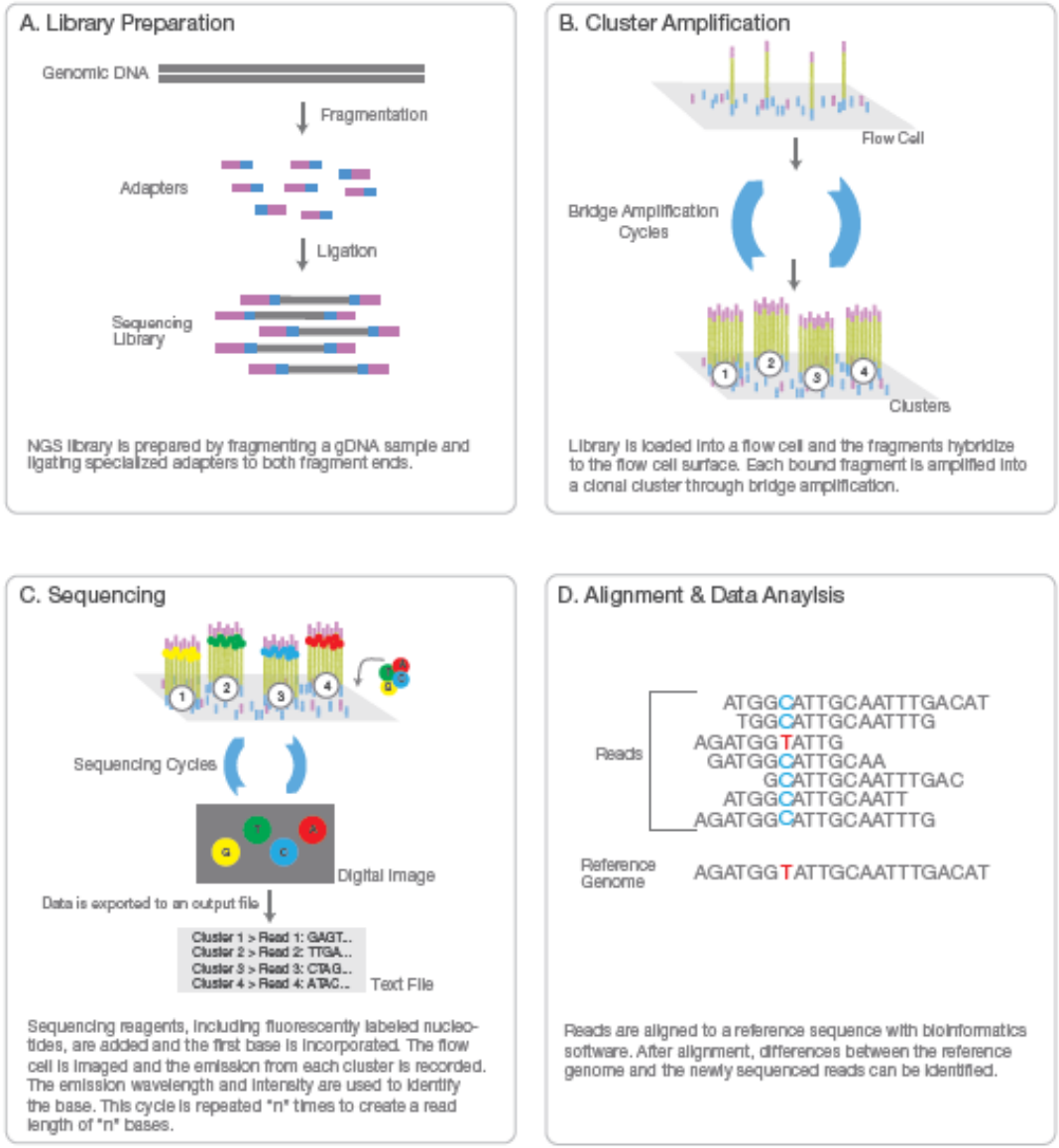
Ion Seminconductor Sequencing (e.g. Ion Torrent)

Sequencing by Ligation (e.g. ABI)

Illumina NGS chemistry overview

Today, the most widely adopted chemistry in microbial ecology studies

As opposed to Sanger that sequences one single DNA fragment, it extends the process across millions of fragments



Illumina paired-end

Involves sequencing of both ends of the DNA fragments to be sequenced, and aligning the *for* and *rev* reads as read pairs

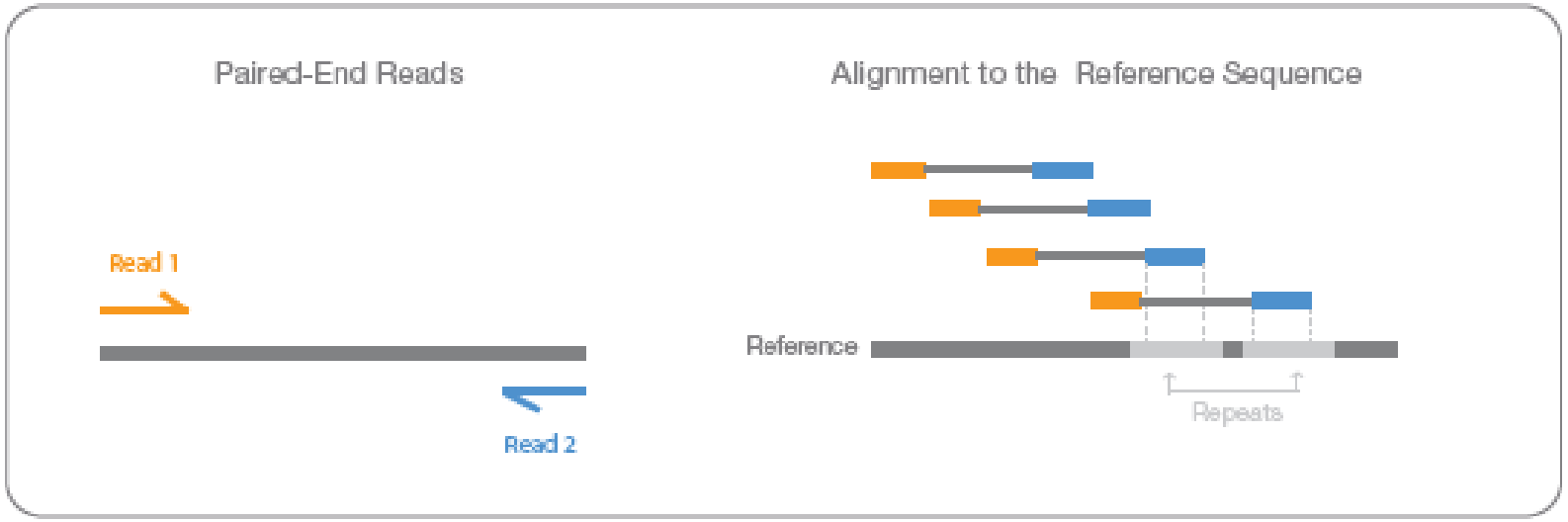


Figure 4: Paired-End Sequencing and Alignment—Paired-end sequencing enables both ends of the DNA fragment to be sequenced. Because the distance between each paired read is known, alignment algorithms can use this information to map the reads over repetitive regions more precisely. This results in much better alignment of the reads, especially across difficult-to-sequence, repetitive regions of the genome.

Longer sequences can be produced (today up to 600 bp)

Longer size allows better sequence comparisons

Multiplexing

In addition to the rise of data output per run, the sample throughput per run has also increased over time

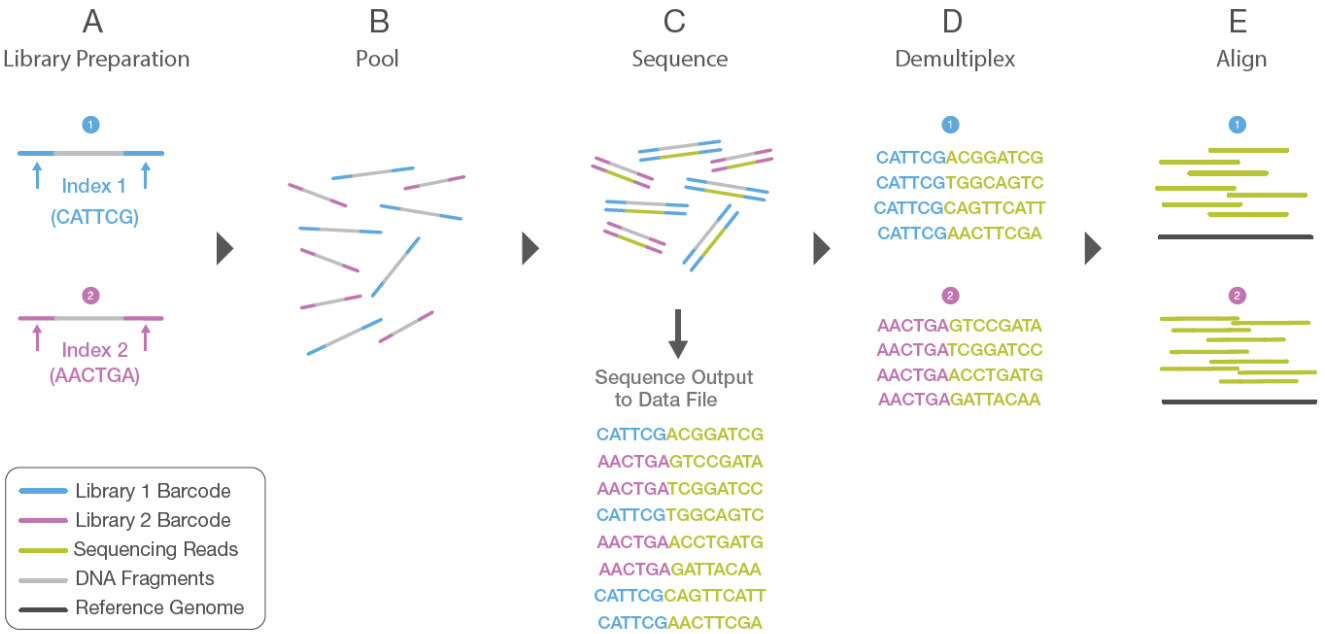


Figure 5: Library Multiplexing Overview.

a. Two distinct libraries are attached to unique index sequences. Index sequences are attached during library preparation.

b. Libraries are pooled together and loaded into the same flow cell lane.

c. Libraries are sequenced together during a single instrument run. All sequences are exported to a single output file.

d. A demultiplexing algorithm sorts the reads into different files according to their indexes.

e. Each set of reads is aligned to the appropriate reference sequence.

Multiplexing allows large numbers of libraries to be pooled and sequenced simultaneously during a single sequencing run

I. Welcome to Next-Generation Sequencing

a. The Evolution of Genomic Science

DNA sequencing has come a long way since the days of two-dimensional chromatography in the 1970s. With the advent of the Sanger chain termination method[1] in 1977, scientists gained the ability to sequence DNA in a reliable, reproducible manner. A decade later, Applied Biosystems introduced the first automated, capillary electrophoresis (CE) based sequencing instruments—the AB370 in 1987 and the AB3730xl in 1998—instruments that became the primary workhorses for the NIH-led and Celera-led Human Genome Projects.[2] While these "first-generation" instruments were considered high throughput for their time, the Genome Analyzer emerged in 2005 and took sequencing runs from 84 kilobase (kb) per run to 1 gigabase (Gb) per run.[3] The short read, massively parallel sequencing technique was a fundamentally different approach that revolutionized sequencing capabilities and launched the "next-generation" in genomic science. From that point forward, the data output of next-generation sequencing (NGS) has outpaced Moore's law—more than doubling each year (Figure 1).

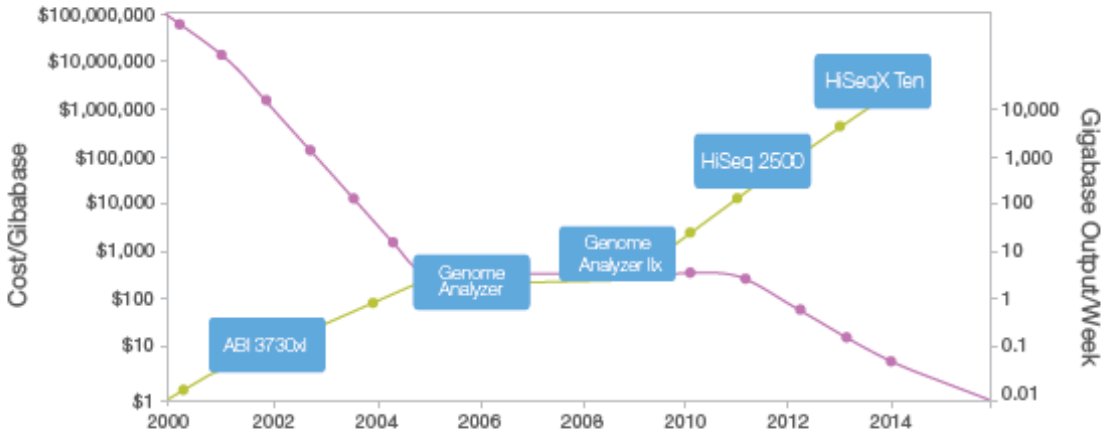


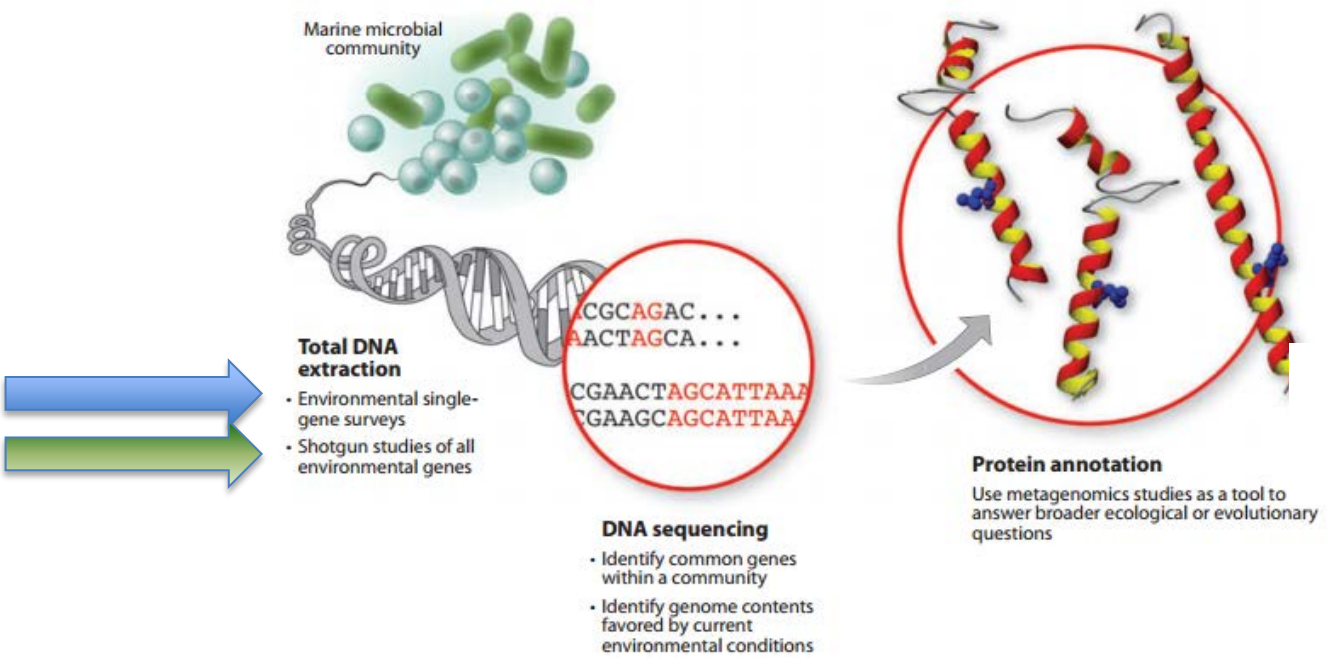
Figure 1: Sequencing Cost and Data Output Since 2000—The dramatic rise of data output and concurrent falling cost of sequencing since 2000. The Y-axes on both sides of the graph are logarithmic.

2000 Primo genoma umano (*Human Genome Project*), 3 miliardi di $, 13 anni di lavoro

2014 Costo per sequenziare il genoma di ognuno di noi è 1,000 $, 16 genomi in 3 giorni

NGS has lead to the rapid development of METAGENOMICS

(Also called "environmental" or "community" genomics) Genomic analysis of microbes by direct extraction and sequencing of DNA from a natural assemblage



Microbial Metagenomics: Beyond the Genome

Jack A. Gilbert[1,2,3] and Christopher L. Dupont[4]

Different from genomics (analysis of genomic DNA from an individual organism or cell). Meta in Greek is "beyond," the term means "beyond the single genome study." Coined in 1998 (a study of soil microbes using random cloning of eDNA; Handelsman *et al.*). Subsequent definitions have varied to include any study whereby a whole community is analyzed, e.g., **studies of 16S rDNA diversity** from an environment to analysis of **all genes from environmental samples** without cultivation

Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample

J. Gregory Caporaso[a], Christian L. Lauber[b], William A. Walters[c], Donna Berg-Lyons[b], Catherine A. Lozupone[a], Peter J. Turnbaugh[d], Noah Fierer[b,e], and Rob Knight[a,f,1]

[a]Department of Chemistry and Biochemistry, [b]Cooperative Institute for Research in Environmental Sciences, [c]Department of Molecular, Cellular, and Developmental Biology, and [e]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309; [d]Harvard FAS Center for Systems Biology, Cambridge, MA 02138; and [f]Howard Hughes Medical Institute, Boulder, CO 80309

Edited by Jeffrey I. Gordon, Washington University School of Medicine, St. Louis, MO, and approved April 30, 2010 (received for review February 27, 2010)

The ongoing revolution in high-throughput sequencing continues to democratize the ability of small groups of investigators to map the microbial component of the biosphere. In particular, the coevolution of new sequencing platforms and new software tools allows data acquisition and analysis on an unprecedented scale. Here we report the next stage in this coevolutionary arms race, using the Illumina GAIIx platform to sequence a diverse array of 25 environmental samples and three known "mock communities" at a depth averaging 3.1 million reads per sample. We demonstrate excellent consistency in taxonomic recovery and recapture diversity patterns that were previously reported on the basis of meta-analysis of many studies from the literature (notably, the saline/nonsaline split in environmental samples and the split between host-associated and free-living communities). We also demonstrate that 2,000 Illumina single-end reads are sufficient to recapture the same relationships among samples that we observe with the full dataset. The results thus open up the possibility of conducting large-scale studies analyzing thousands of samples simultaneously to survey microbial communities at an unprecedented spatial and temporal resolution.

massive datasets to produce new biological insight, but in turn the availability of these software tools prompts new experiments that could not previously have been considered, which lead to the production of the next generation of datasets, starting the process again. However, we would argue that the situation is not precisely that of a "Red Queen" coevolutionary process (in which one must run faster and faster to remain in the same place), because each advance really does provide a new level of insight into a range of biological phenomena. The increase in number of sequences per run from parallel pyrosequencing technologies such as the Roche 454 GS FLX (5×10^5) to Illumina GAIIx (1×10^8) is on the order of 1,000-fold and greater than the increase in the number of sequences per run from Sanger (1×10^3 through 1×10^4) to 454. The transition from Sanger sequencing to 454 sequencing has opened new horizons in microbial community analysis by making it possible to collect hundreds of thousands of sequences spanning hundreds of samples. A transition to the Illumina platform will similarly allow for deeper sequencing than has previously been feasible, with the possibility of detecting even phylotypes that are very rare (11). By using a variant of the barcoding

The issue: how to analyze and interpret the vast amounts of sequence/tree data!

first 9 of 130,000 sequences

~0.1% of the tree file

naive visualization of tree...

The issue: how to analyze and interpret the vast amounts of sequence/tree data!

The same approach can be used for microbial eukaryotes (18S rRNA as target)

Patterns of Rare and Abundant Marine Microbial Eukaryotes

Ramiro Logares,[1,*] Stéphane Audic,[2,3] David Bass,[4] Lucie Bittner,[2,3,5] Christophe Boutte,[2,3] Richard Christen,[6,7] Jean-Michel Claverie,[8] Johan Decelle,[2,3] John R. Dolan,[9] Micah Dunthorn,[5] Bente Edvardsen,[10] Angélique Gobet,[2,3] Wiebe H.C.F. Kooistra,[11] Frédéric Mahé,[2,3,5] Fabrice Not,[2,3] Hiroyuki Ogata,[8,12] Jan Pawlowski,[13] Massimo C. Pernice,[1] Sarah Romac,[2,3] Kamran Shalchian-Tabrizi,[10] Nathalie Simon,[2,3] Thorsten Stoeck,[5] Sébastien Santini,[8] Raffaele Siano,[14] Patrick Wincker,[15] Adriana Zingone,[11] Thomas A. Richards,[16] Colomban de Vargas,[2,3] and Ramon Massana[1]

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[12]Education Academy of Computational Life Sciences,

investigate abundant and rare s
microbial eukaryotes, a crucial
remains among the least-explore
of the biosphere. We surveyed
separate coastal locations in Euro
ering the picoplankton, nanoplan
mesoplankton organismal size frac
Results: Deep Illumina sequencing
that the abundant regional commu
by organismal size fraction, where
munity was mainly structured by g
some abundant and rare taxa prese
pointing to spatiotemporal structu
yote biosphere. Abundant and r
sented regular proportions across samples, indicating similar species-abundance distributions despite taxonomic compositional variation. Several taxa were abundant in one location and rare in other locations, suggesting large oscillations in abundance. The substantial amount of metabolically active lineages found in the rare biosphere suggests that this subcommunity constitutes a diversity reservoir that can respond rapidly to environmental change.
Conclusions: We propose that marine planktonic microeukaryote assemblages incorporate dynamic and metabolically active abundant and rare subcommunities, with contrasting structuring patterns but fairly regular proportions, across space and time.

Introduction

Microbes are the dominant form of life in the oceans, playing fundamental roles in ecosystem functioning and biogeochemical processes on local and global scales [1–4]. However, limited knowledge of their diversity and community structure

Research article

Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing

Ramon Massana ✉, Angélique Gobet, Stéphane Audic, David Bass, Lucie Bittner, Christophe Boutte, Aurélie Chambouvet, Richard Christen, Jean-Michel Claverie, Johan Decelle, John R. Dolan, Micah Dunthorn, Bente Edvardsen, Irene Forn, Dominik Forster, Laure Guillou, Olivier Jaillon, Wiebe H. C. F. Kooistra, Ramiro Logares, Frédéric Mahé, Fabrice Not, Hiroyuki Ogata, Jan Pawlowski, Massimo C. Pernice, Ian Probert, Sarah Romac, Thomas Richards, Sébastien Santini, Kamran Shalchian-Tabrizi, Raffaele Siano, Nathalie Simon, Thorsten Stoeck, Daniel Vaulot, Adriana Zingone, Colomban de Vargas

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Volume 17, Issue 10
October 2015
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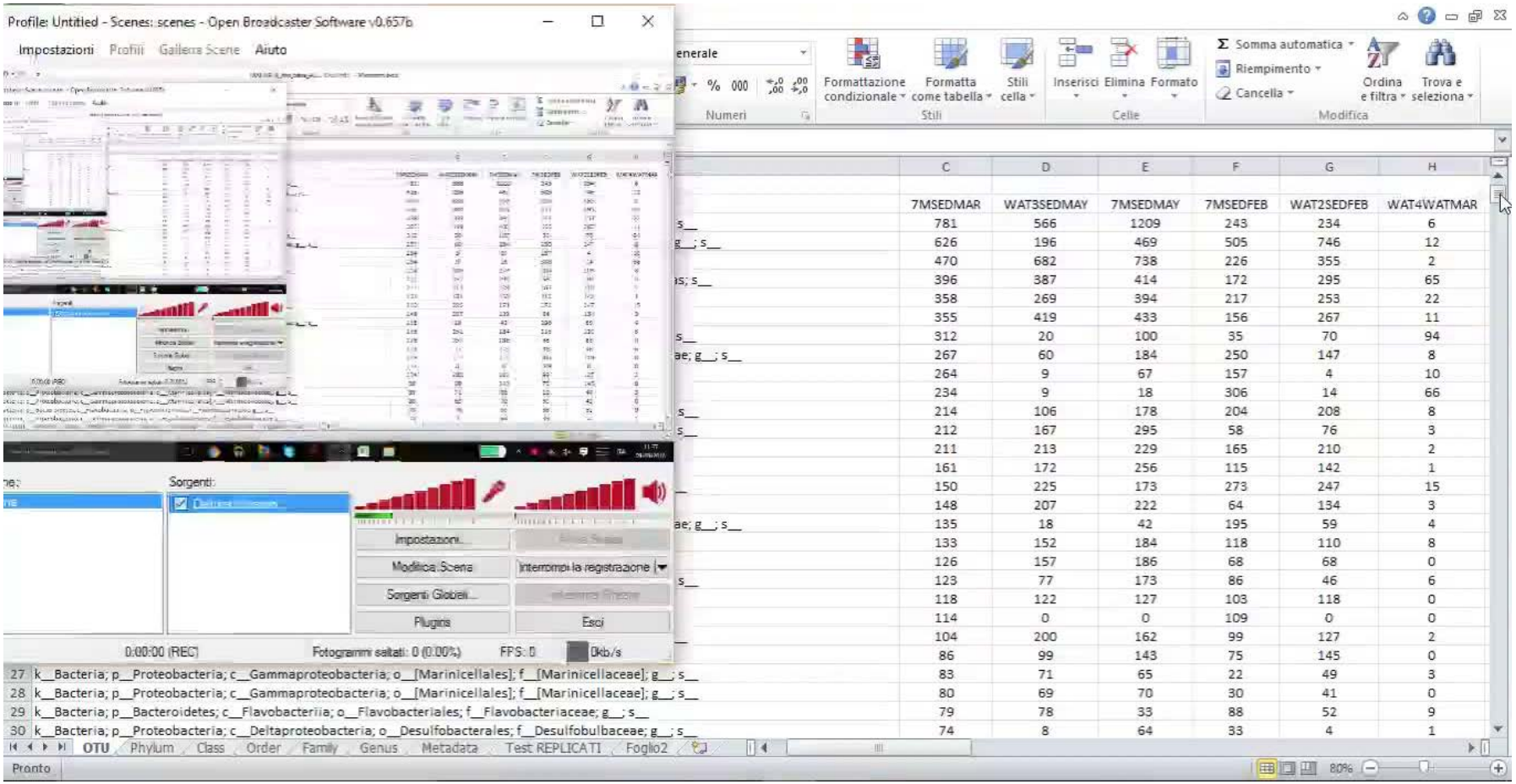
View issue TOC
Special Issue:
Marine Microbes

How sequences from one sample look like…

How sequence data look like

ATCG…

Sequences have to be analyzed and classified into microbial taxonomic units (OTUs)



Ecologists need good bioinformatic tools (and even more good bioinformaticians…)

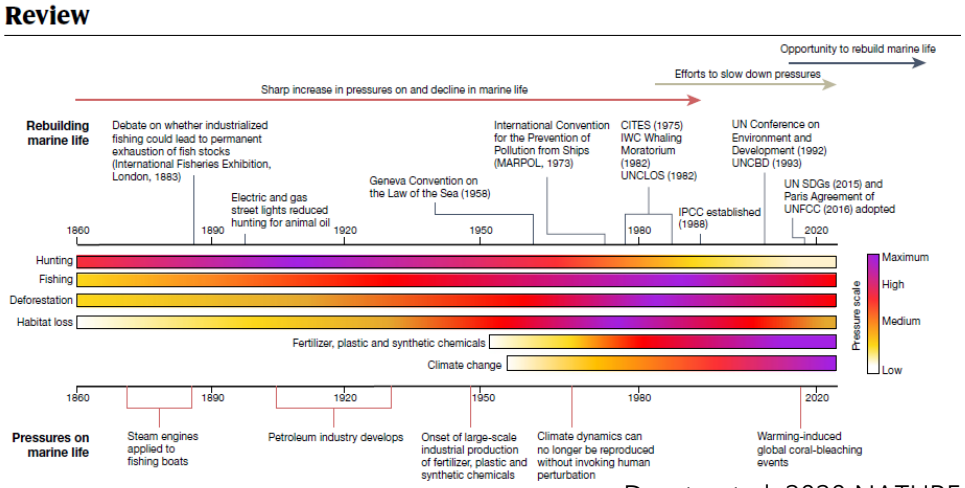
Contesto internazionale

Temi e sfide (mediterranee e planetarie)

Declino degli ecosistemi e delle (bio)risorse marine a causa del crescente impatto antropico. Pressione globale sul biota marino

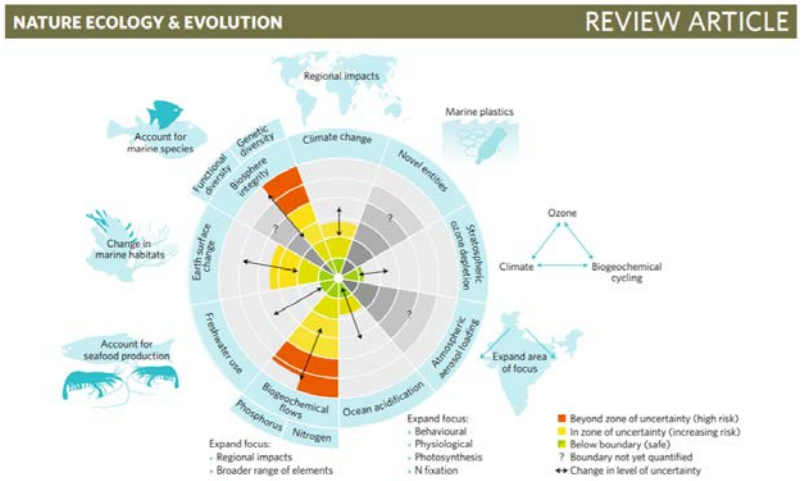
Stretta interconnessione tra salute dell'oceano e salute dell'uomo

The ability of the ocean to support human wellbeing is at a crossroads



Duarte et al. 2020 NATURE

Planetary boundaries for a blue planet



Nash et al. 2017 NATURE ECOL&EVOL

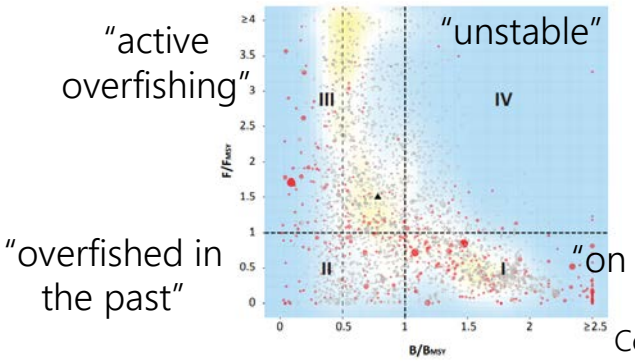
Necessità di definire anche i confini planetari blu

Necessity to rebuild the marine life-support systems that deliver the benefits that society receives from a healthy ocean

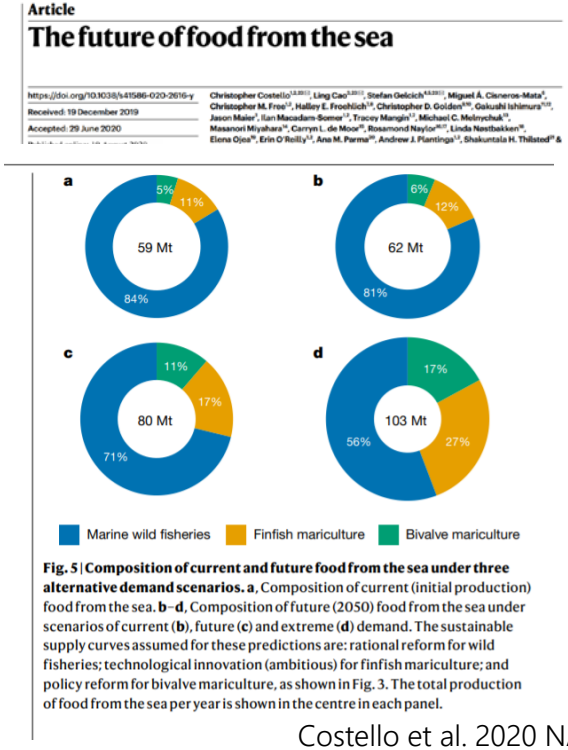
CNR IRBIM
ISTITUTO PER LE RISORSE BIOLOGICHE E LE BIOTECNOLOGIE MARINE

Contesto internazionale
Temi e sfide (mediterranee e planetarie)

PESCA: rapida crescita nelle ultime decadi, attualmente in profonda crisi (sovra-sfruttamento stock, degradazione degli habitat), richiede gestione più razionale e sostenibile



Costello et al. 2016 PNAS



Article

The future of food from the sea

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Fig. 5 | Composition of current and future food from the sea under three alternative demand scenarios. a, Composition of current (initial production) food from the sea. **b**–**d**, Composition of future (2050) food from the sea under scenarios of current (**b**), future (**c**) and extreme (**d**) demand. The sustainable supply curves assumed for these predictions are: rational reform for wild fisheries; technological innovation (ambitious) for finfish mariculture; and policy reform for bivalve mariculture, as shown in Fig. 3. The total production of food from the sea per year is shown in the centre in each panel.

Costello et al. 2020 NATURE

Cibo dal mare aumenterà di 21–44 milioni tonnellate nel 2050 (aumento di 36–74% rispetto ad oggi)

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Contesto internazionale
Temi e sfide (mediterranee e planetarie)

ACQUACOLTURA: importante alternativa alla pesca, rapida crescita, ha davanti a sé numerose sfide di tipo economico, ambientale e sociale (in ottica di economia circolare, sicurezza/qualità prodotti, rispetto dell'ambiente)

nature food

PERSPECTIVE

https://doi.org/10.1038/s43016-020-0127-5

Check for updates

Sustainable aquaculture through the One Health lens

G. D. Stentiford[1,2 ✉], I. J. Bateman[3], S. J. Hinchliffe[2,4], D. Bass[1,2], R. Hartnell[5], E. M. Santos[2,6], M. J. Devlin[7], S. W. Feist[1], N. G. H. Taylor[1,2], D. W. Verner-Jeffreys[1,2], R. van Aerle[1,2], E. J. Peeler[1,2], W. A. Higman[1], L. Smith[1], R. Baines[1], D. C. Behringer[8,9], I. Katsiadaki[1,2], H. E. Froehlich[10,11] and C. R. Tyler[2,6]

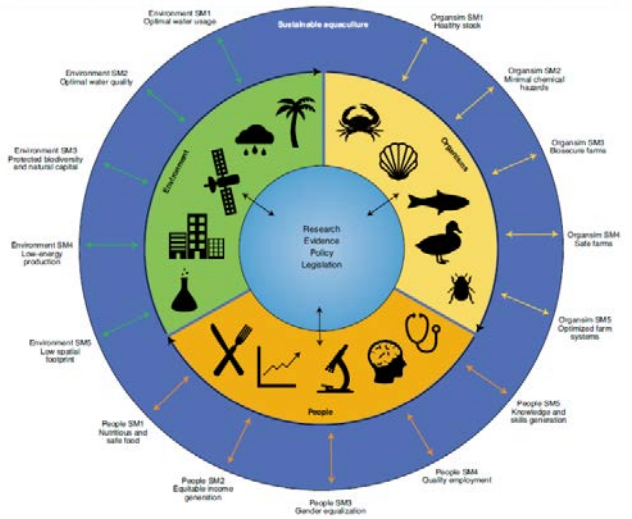

Fig. 2 | One Health success metrics for sustainable aquaculture. A One Health approach (Fig. 1) to the design and assessment of ESP in aquaculture and related sub-sectors requires success metrics (SMs) spanning environment, organism and human health. Descriptors for SMs (Table 1) are applied to hypothetical sub-sectors of the aquaculture industry in Fig. 3.

Predicted to supply the majority of aquatic dietary protein by 2050

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10 BILLION PEOPLE

ON PLANET EARTH BY 2100

17%

THE GLOBAL SUPPLY OF «FOOD FROM THE SEA» PROVIDED BY THE OCEAN TODAY (REACHING >50% IN COUNTRIES IN ASIA AND AFRICA)

214 MILLION TONNES

TOTAL FISHERIES & AQUACULTURE PRODUCTION IN 2020

(90.3 MILLION TONNES FROM GLOBAL CAPTURE FISHERIES)

Data from:

Free et al., 2022 Nature

Costello et al., 2020 Nature

FAO The State of World Fisheries & Aquaculture 2022

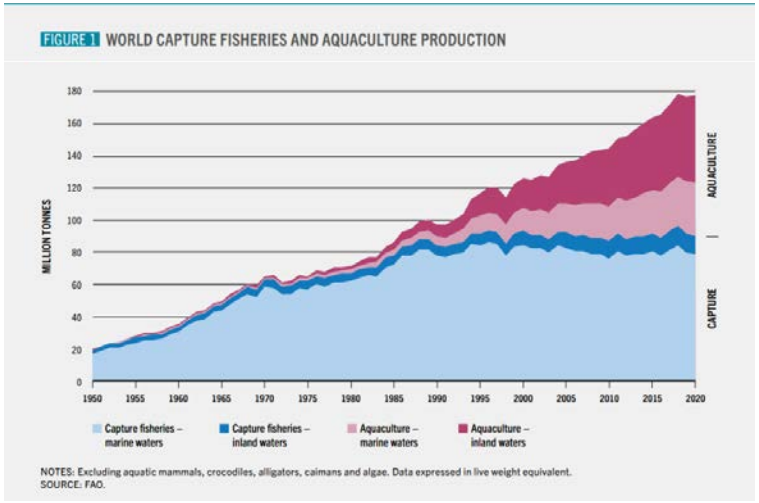
20.5 KG PER CAPITA

CONSUMPTION OF AQUATIC FOOD IN 2019 (PROJECTED TO A 15% INCREASE IN 2030)

90% GLOBAL FISH STOCKS

FULLY OR OVEREXPLOITED. MARINE FISHERY RESOURCES HAVE CONTINUED TO DECLINE (REBUILDING OVERFISHED STOCKS COULD INCREASE PRODUCTION BY 16.5 MTONNES)

AQUATIC FOODS INCREASINGLY RECOGNIZED FOR THEIR ROLE IN FOOD SECURITY AND NUTRITION, NOT ONLY AS SOURCE OF PROTEIN, BUT ALSO AS UNIQUE & EXTREMELY DIVERSE PROVIDER OF ESSENTIAL OMEGA-3 FATTY ACIDS AND BIOAVAILABLE MICRONUTRIENTS



AQUACULTURE PREDICTED TO **DOUBLE** ITS PRODUCTION BY **2050**

ALL REGIONS, EXCEPT AFRICA, EXPERIENCED CONTINUED AQUACULTURE GROWTH IN 2020, DRIVEN BY EXPANSION IN CHILE, CHINA AND NORWAY (THE TOP PRODUCERS IN THEIR RESPECTIVE REGIONS)

Low Carbon Economy

Mitigate Effects of Climate Change

Reduce Pollution

Circular Economy

Preserve Ecosystem

AQUAFARM CNR IRBIM Circles

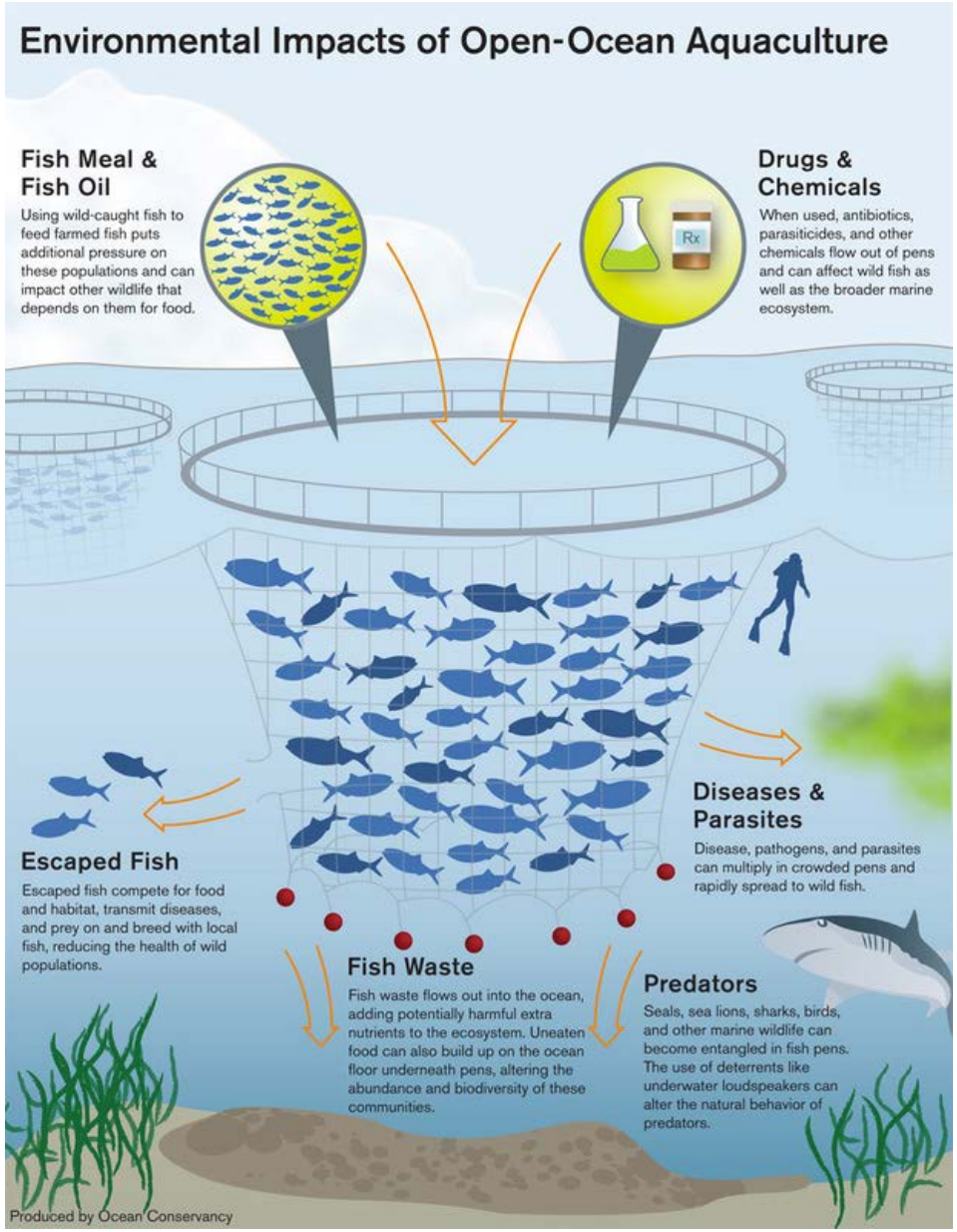
Aquaculture and sustainability

- Fish and shellfish production is limited by several emerging diseases caused by viruses, bacteria, fungi, oomycetes, amoebas and other ectoparasites.

- Bacterial fish diseases are typically addressed by antibiotics (Romero *et al.*2012; Cabello *et al.*2013), viral diseases by vaccination (Evensen and Leong 2013) and parasitic diseases by chemical treatment (Burridge *et al.*2010).

- Impact on the environment and the quality/safety of aquacultured fish food due to:
  - risk of antibiotic resistance development
  - transfer of antibiotic resistance genes to other animal pathogens
  - concerns for environmental impact and consumer safety
  - organic matter enrichment in aquacultured sediments

Need for additional/alternative strategies for sustainable disease



A promising alternative: the exploitation of fish microbiome

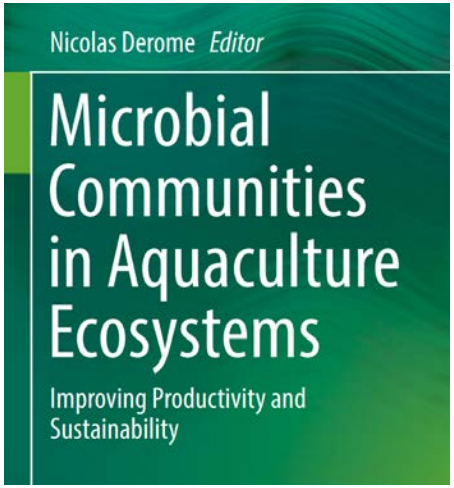
The microbiome is defined as described by Joshua Lederberg: '***the totality of microbes, their genomes and their interactions in a particular environment***'. It is a concept born for studies on humans and it is now applied to a plethora of terrestrial and marine organisms, including fish

The impact of microbial consortia on early **development and health of their eukaryotic hosts** is gaining increased attention, with new '**omics**'-based technologies allowing for in-depth characterizations of microbial communities and functions in diverse ecosystems.

The human gut microbiomes can significantly drive or suppress disease development, whereas environmental changes or infections can substantially influence the human gut microbiome by causing blooms of microbes that otherwise are present at low abundance.

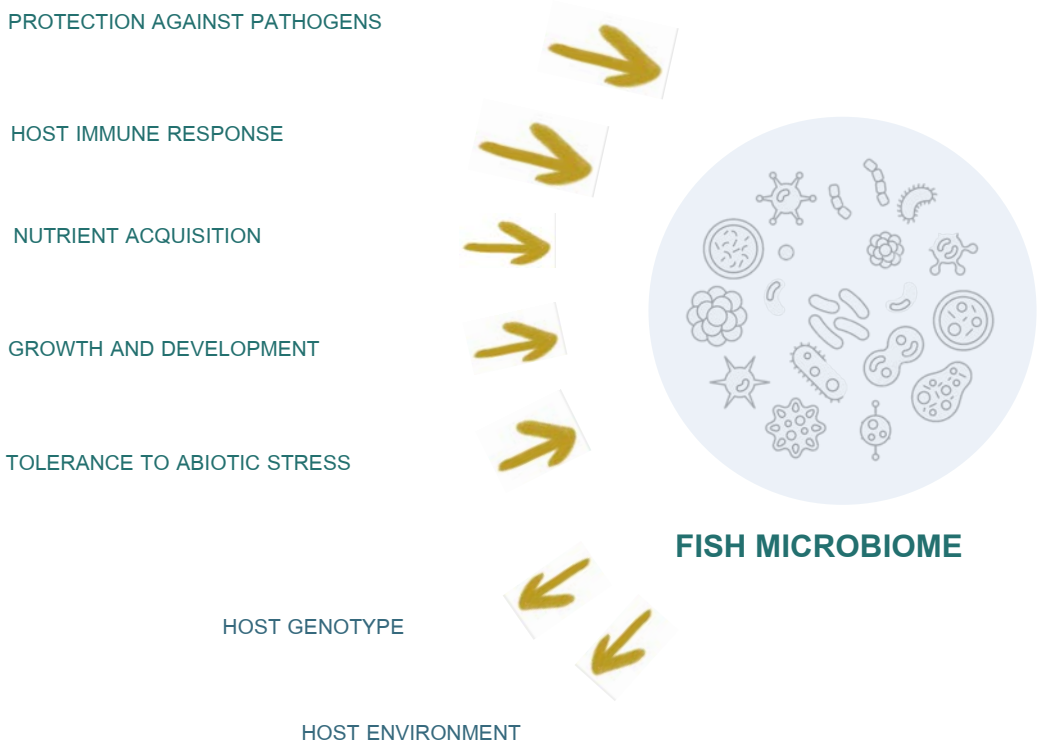
<u>What about fish?</u>

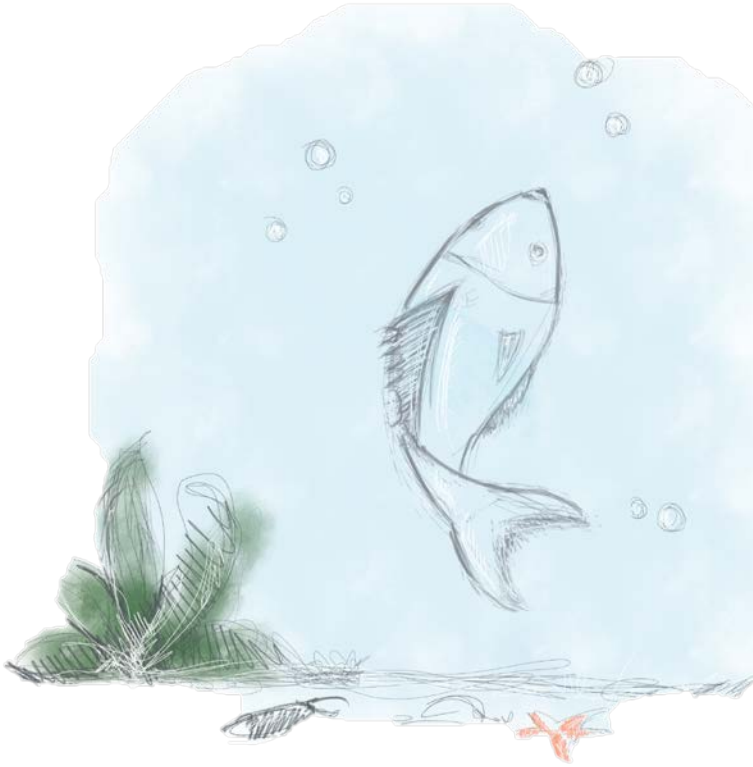
To become truly sustainable, the aquaculture industry has no choice but to adopt alternative strategies to control disease occurrence and promote optimal host–microbiota functional interactions (Derome, 2019).



Microbiota research is paving the way to a highly integrated approach to understand complex relationships between farmed fish and their associated and environmental microbial communities at the frontier between health and disease

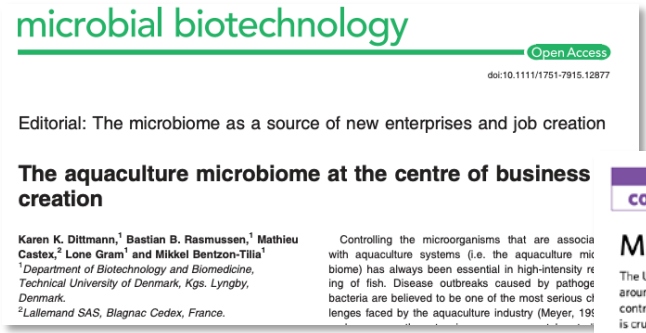
Role of fish microbiome





Like for other vertebrates, the fish microbiome is critical to the health of its host and has complex and dynamic interactions with the surrounding environment

Fish microbiome: a promising strategy to make aquaculture more sustainable?


microbial biotechnology

Open Access

doi:10.1111/1751-7915.12877

Editorial: The microbiome as a source of new enterprises and job creation

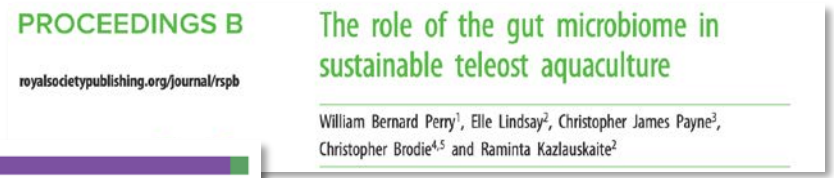
The aquaculture microbiome at the centre of business creation

Karen K. Dittmann,[1] Bastian B. Rasmussen,[1] Mathieu Castex,[2] Lone Gram[1] and Mikkel Bentzon-Tilia[1]

[1]*Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark.*

[2]*Lallemand SAS, Blagnac Cedex, France.*

Controlling the microorganisms that are associa with aquaculture systems (i.e. the aquaculture mic biome) has always been essential in high-intensity re ing of fish. Disease outbreaks caused by pathoge bacteria are believed to be one of the most serious cl lenges faced by the aquaculture industry (Meyer, 19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The role of the gut microbiome in sustainable teleost aquaculture

William Bernard Perry[1], Elle Lindsay[2], Christopher James Payne[3], Christopher Brodie[4,5] and Raminta Kazlauskaite[2]


comment

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Microbiome innovations for a sustainable future

The United Nations Sustainable Development Goals (SDGs) are being integrated into bioeconomy strategies around the world, including the European Green Deal. We highlight how microbiome-bi contribute to policies that interface with the SDGs and argue that international coopera is crucial for success.

Kathleen D'Hondt, Tanja Kostic, Richard McDowell, Francois Eudes, Brajesh K. S Markakis, Bettina Schelkle, Emmanuelle Maguin and Angela Sessitsch

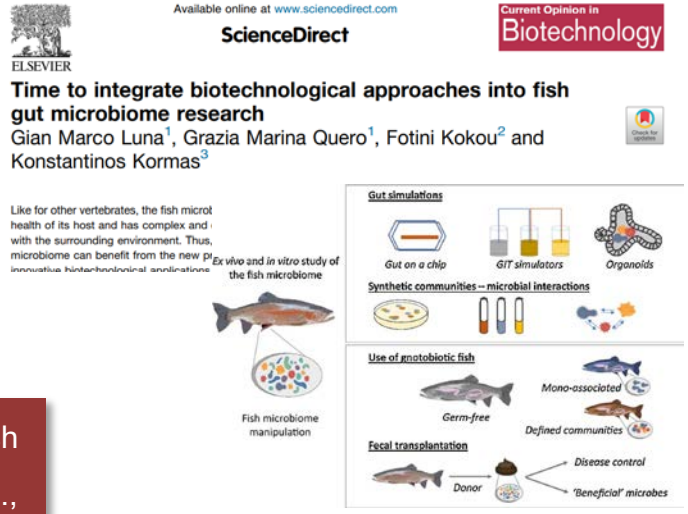

OXFORD FEMS JOURNALS

FEMS Microbiology Ecology, 94, 2018, fix161

MINIREVIEW

Exploring fish microbial communities to mitigate emerging diseases in aquaculture

Irene de Bruijn[1,†], Yiying Liu[1,*,†], Geert F. Wiegertjes[2] and Jos M Raaijmakers[1,3]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Current Opinion in Biotechnology

Time to integrate biotechnological approaches into fish gut microbiome research

Gian Marco Luna[1], Grazia Marina Quero[1], Fotini Kokou[2] and Konstantinos Kormas[3]

Like for other vertebrates, the fish microt health of its host and has complex and with the surrounding environment. Thus, microbiome can benefit from the new pl innovative biotechnological applications

Ex vivo and *in vitro* study of the fish microbiome

Fish microbiome manipulation

Gut simulations

Gut on a chip

GIT simulators

Organoids

Synthetic communities – microbial interactions

Use of gnotobiotic fish

Germ-free

Mono-associated

Defined communities

Fecal transplantation

Donor

Disease control

'Beneficial' microbes

''Integration of microbiomes in smart farming practices (including animal/fish production and urban farming) for reduced GHG emissions'' (D'Hondt et al., 2021)

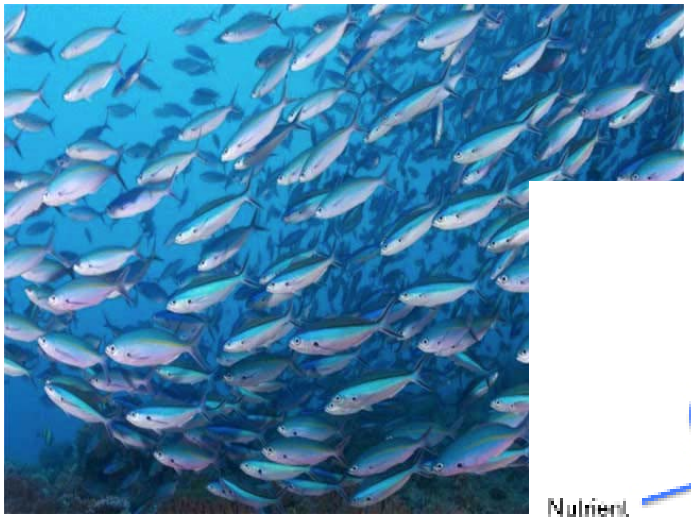
AQUAFARM

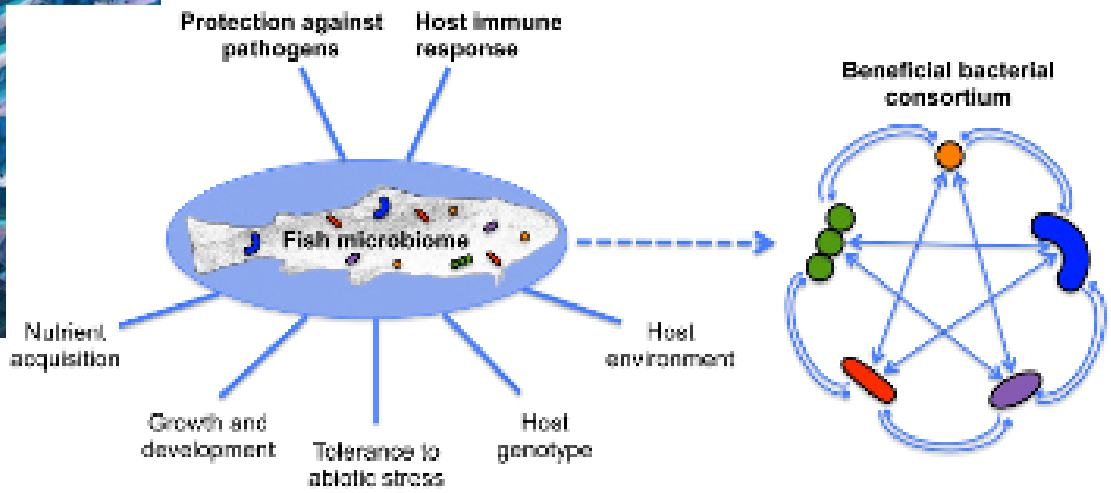
CNR IRBIM

Circles

The hypothesis behind the study of fish microbiome

Microbial communities of fish may harbor **substantial potential to modulate health and disease**. Due to the complex structure of microbial communities, disentangling interactions and identifying keystone species for specific functions is enormously challenging, especially when environmental influences on population dynamics and activities are taken into account.





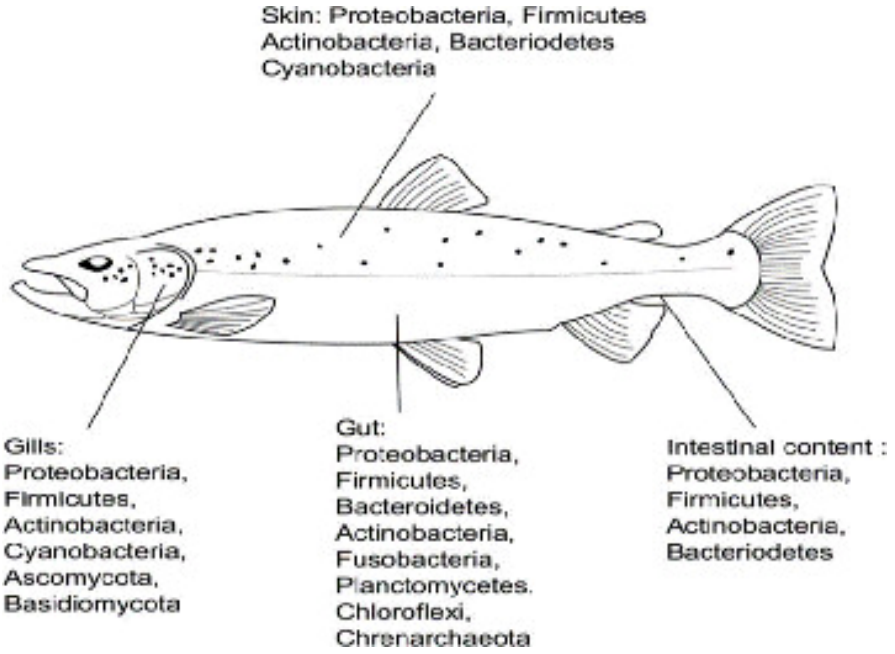
Fish microbiome differs according to the different fish tissues

- Mucosal tissues (skin, olfactory system, gills and also the gut) are in direct contact with the environment and thus are **the first contact points of the microbes with their host**.

- The mucus provides a **carbon source for commensal microbes** that can subsequently form a protective shield against invading pathogens

- The mucus of the fish skin and gills generally contains more aerobic than anaerobic microbes

- **fish** skin typically harbors 10^2–10^4 bacteria per cm^2
- **gills** 10^3–10^6 bacteria per gram of tissue
- **gut** up to 10^8 aerobic heterotrophic bacteria and up to 10^5 anaerobic bacteria per gram of gut tissue



Fish microbiome differs according to the different fish tissues
Skin and gills

- The mucosal surfaces and associated microbiota of fish are an important **primary barrier** and provide the first line of defense against potential pathogens.
- An understanding of the skin and gill microbial assemblages and the factors which drive their composition may provide **useful insights into the broad dynamics of fish host–microbial relationships**, and may reveal underlying changes in health status.
- This is particularly pertinent **to cultivated systems** whereby various stressors may led to conditions (like enteritis) which impinge on productivity.

FIGURE 1 | Study site and sampling approach. Farmed Yellowtail Kingfish (YTK, *Seriola lalandi*) were obtained from **(A)** sea cages from a commercial enterprise from temperate waters in southern Australia, where swab samples were taken from **(B)** gills and **(C)** skin within the regions denoted by dashed lines from **(D)** healthy individuals, and those with **(E)** early and late stages of enteritis.

Fish microbiome differs according to the different fish tissues
Skin and gills

- First studies indicate that the composition of the microbial communities of the gills and skin is different

- The protected niches of the gill lamellae contain more microbes that putitatively favor gas exchange

- High influence of surrounding environment in defining skin and gill microbiome

- *Examples*:

- the gill microbiota of rainbow trout (*Oncorhynchus mykiss*) contains mostly Proteobacteria and Bacteroidetes (*Flectobacillus* and *Flavobacterium*), while the skin contains more Actinobacteria and Firmicutes

- the gills of common carp (*Cyprinus carpio*) and zebrafish (*Danio rerio*) contained ammonia oxidizing and denitrifying bacteria, such as *Nitrosomas*-like bacteria that are thought to play an important role in detoxifying the excreted ammonia

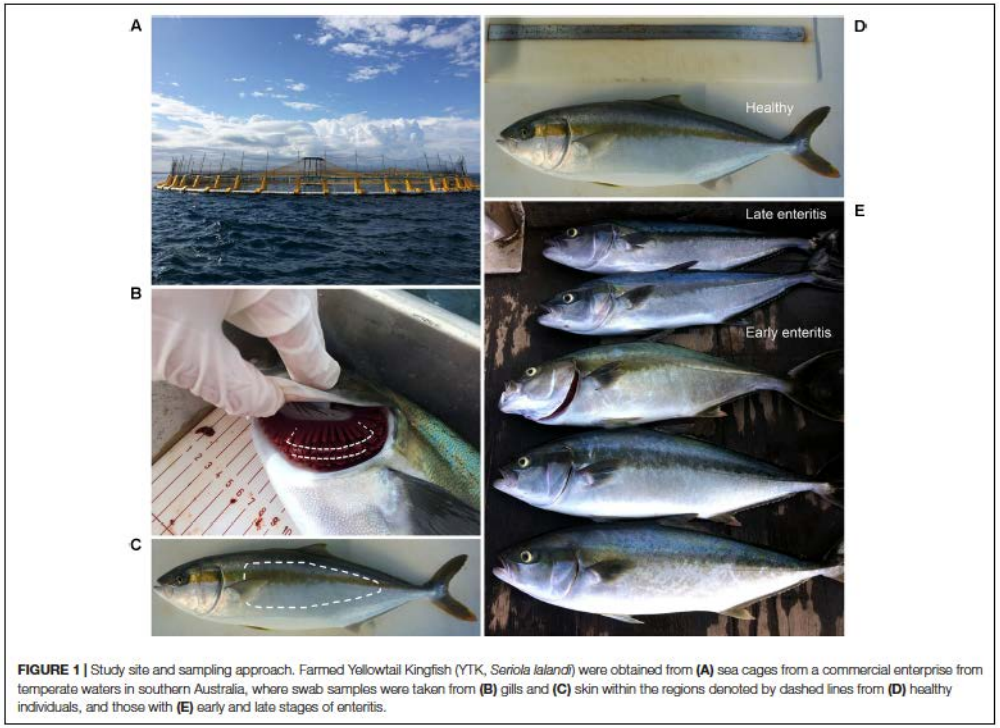
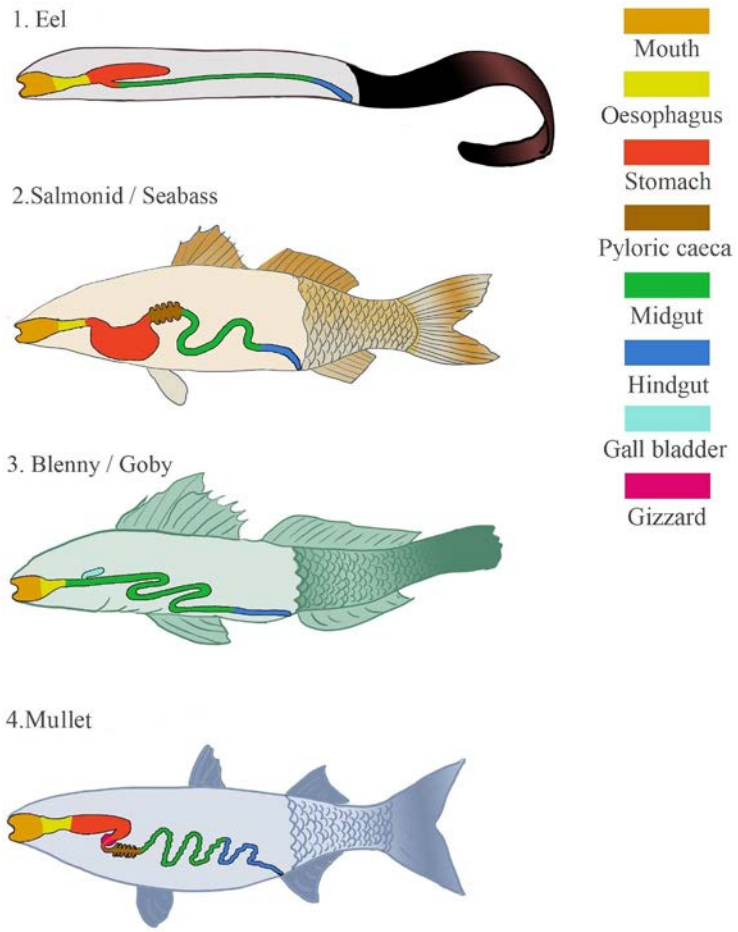


FIGURE 1 | Study site and sampling approach. Farmed Yellowtail Kingfish (YTK, *Seriola lalandi*) were obtained from **(A)** sea cages from a commercial enterprise from temperate waters in southern Australia, where swab samples were taken from **(B)** gills and **(C)** skin within the regions denoted by dashed lines from **(D)** healthy individuals, and those with **(E)** early and late stages of enteritis.

Fish microbiome differs according to the different fish tissues
Gut



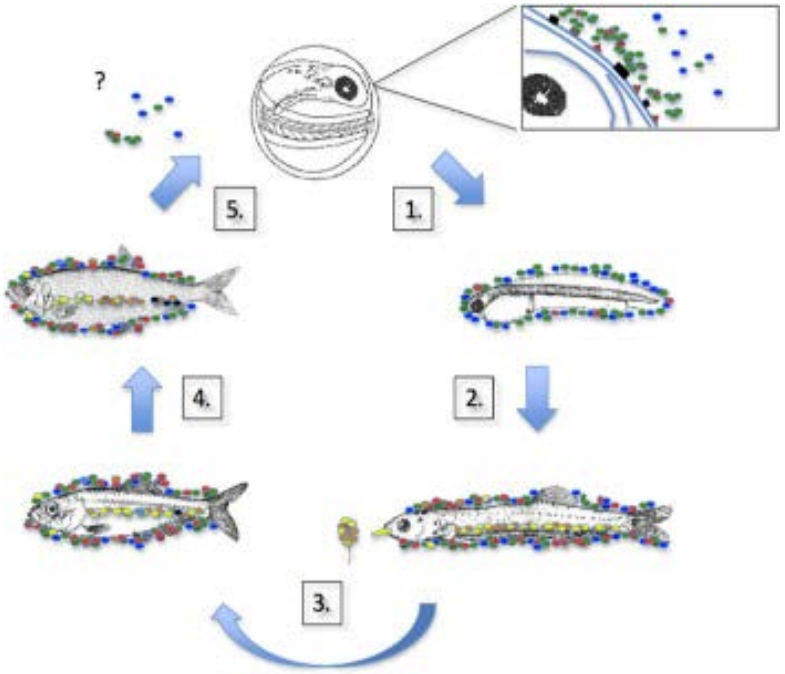
- Influence of numerous factors influencing gut microbiome: diet, age, genetics, environment
- For most fish species, the most abundant phyla found in fish guts are typically Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes.
- Some study suggest that the microbial community gut is much less diverse than that of the skin or gills

Microbial community changes during fish development

- Most fish species lay eggs that are fertilized externally in the aquatic ecosystem.
- Microbes associated with fish eggs can range from 10^3 to 10^6 CFU g^{-1}, with *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Flavobacterium*, *Moraxella*, *Pseudomonas* and *Streptomyces* as the major bacterial genera , although, to date many unknown microbial species reside on the fish eggs
- It is likely that part of the fish egg microbiota is obtained via vertical transmission from the mother. Also the internal tissue of eggs of freshwater and marine fish may contain bacteria
- Already before the yolksac is consumed, fish larvae start to ‘drink’ water and the gut becomes colonized by microorganisms from the water. At later developmental stages, larvae are exposed to other microbiota, for example by ingestion of egg debris or by microorganisms present in live and artificial feed

Thus, these initial studies suggest that profound changes can already occur in the microbiome during early development of the fish.

Need for further studies!



Teleost microbiome during development, Source: (Derome et al., 2014)

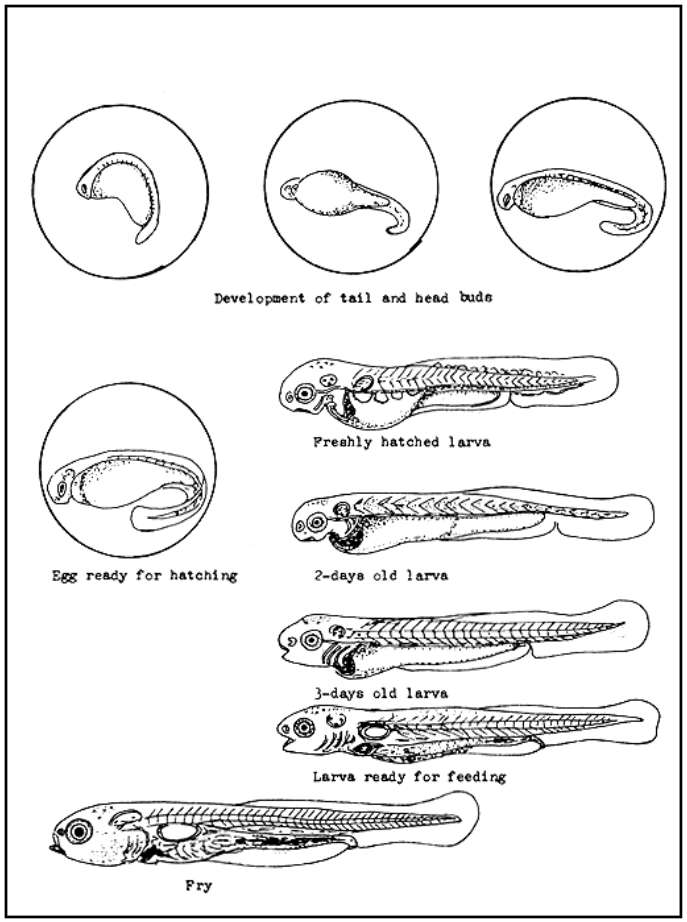
Microbial community changes during fish development

- First studies reveal that microbial diversity in decreased from larval to adult stages and showed no similarity to the ambient water microbiota

- Because larvae have incomplete digestive systems and have more diverse diets, including various planktonic organismsit has been suggested that they strongly rely on microbes for the digestion of feed

- The diet is an important factor in colonization of the guts, as was shown for the guts of rainbow trout larvae where an increase in bacterial abundance and diversity was observed upon first feeding and where Firmicutes were most abundant in plant fed fish and Proteobacteria were most abundant in marine fed fish).

- In gilthead seabream (Sparus aurata), Firmicutes and Bacteroidetes were more abundant in the late, Rotifer and Artemis fed larval stage compared to the early, non-feeding larvae



Impact of host genotype on fish microbiota

- Host genetics is known to be important in shaping the microbial community of fish

- The importance of the host genetics in shaping the microbiome was demonstrated by identifying a **core microbiota** in the guts of laboratory-reared and wild fish

- The core microbiota often comprises a small number of operational taxonomic units (OTUs), but which are highly abundant. Example: the core microbiota of three species of laboratory-reared or wild carp (*Hypophthalmichthys nobilis*, *Hypophthalmichthys molitrix*, *Cyprinus carpio*) comprised only five OTUs classified into the orders Aeromonadales, Xanthomonadales and Fusobacteriales but made up 35–40% of the total fecal microbiome (Eichmiller *et al.*2016).

- However, the underlying mechanisms of how host genetics influences microbial community structure or whether genetic information is transferred from microbiome to host are not yet understood.

Effect of environmental conditions on the fish microbiota

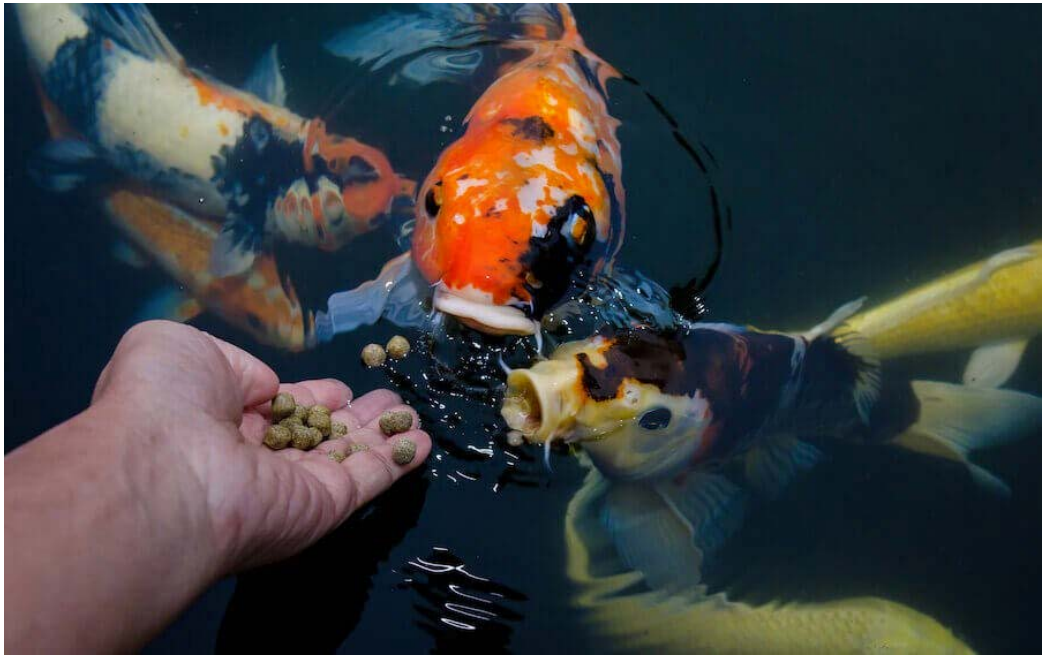
- The composition of the gut microbiota is also determined by the microbiota present in the ambient water and sediment

- Due to environmental fluctuations, the ambient water conditions like temperature and nutrient levels change and affect microbiome composition. The microbial community composition and densities of seawaters are influenced by the seasons, and this may have an effect also on fish microbiome

- A second environmental factor determining the fish microbiome composition is water chemistry (pH, salinity)

Effect of feeding strategy on the fish microbiota

- Diet can have a major impact on the fish gut microbiota
- The gut microbiota of different wild fish species caught from the same river showed different community between omnivorous, herbivorous, carnivorous and filter-feeding fish
- Recent studies support the findings that also the type of feed, including lipid and fatty acid content is an important factor in shaping the gut microbial community.



Effect of feeding strategy on the fish microbiota

Examples showing differences in gut microbiome of fish with different diets

TABLE 2 | Dominant bacterial species isolated from the intestinal tracts of marine fish species at different trophic levels.

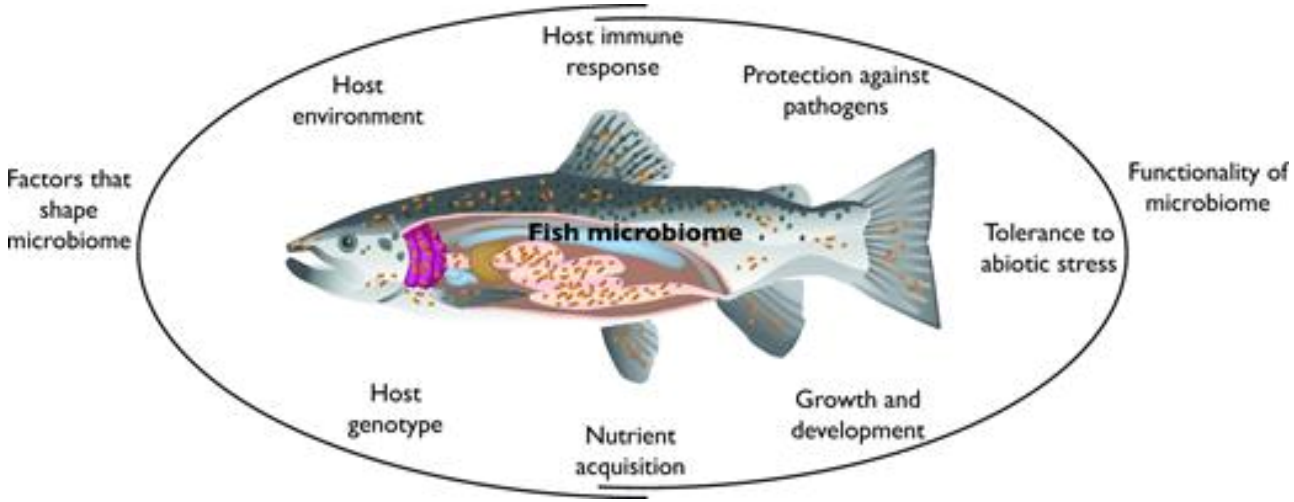
Trophic level	Fish species	Dominant bacteria genera	Reference
Herbivores			
	Butterfish, *Odax pullus*	*Clostridium*	Clements et al., 2007
	Marblefish, *Aplodactylus arctidens*	*Clostridium, Eubacterium desmolans, Papillibacter cinnaminovorans*	Clements et al., 2007
	Parrotfish, *Chlorurus sordidus*	*Vibrio, Photobacterium*	Smriga et al., 2010
	Silver drummer, *Kyphosus sydneyanus*	*Clostridium*	Moran et al., 2005
	Surgeonfish, *Acanthurus nigricans*	Bacteroidetes, non-vibrio Proteobacteria, Firmicutes	Smriga et al., 2010
	Surgeonfish, *Acanthurus sp.*	*Epulopiscium*	Miyake et al., 2015
	Zebraperch, *Hermosilla azurea*	Enterovibrio, Bacteroides, Faecalibacterium, Desulfovibrio	Fidopiastis et al., 2006
Omnivores			
	Pinfish, *Lagodon rhomboides*	*Clostridium, Mycoplasma*	Ransom, 2008
		Photobacterium, Propionibacterium, Staphylococcus, Pseudomonas, Corynebacterium	Givens et al., 2015
	Long-jawed mudsucker, *Gillichthys mirabilis*	*Mycoplasma*	Bano et al., 2007
Carnivores			
	Atlantic cod, *Gadus morhua*	*Clostridium perfringens*	Aschfalk and Müller, 2002
		Vibrio	Star et al., 2013
	Atlantic halibut, *Hippoglossus hippoglossus*	Vibrionaceae (larvae, juveniles), *Photobacterium phosphoreum* (adults)	Verner-Jeffreys et al., 2003
	Atlantic salmon, *Salmo salar*	*Acinetobacter junii, Mycoplasma*	Holben et al., 2002
		Lactobacillus, P. phosphoreum, Lactococcus, Bacillus	Hovda et al., 2007
	Blackfin icefish, *Chaenocephalus aceratus*	*Photobacterium*	Ward et al., 2009
	Black rockcod, *Notothenia coriiceps*	*Photobacterium, Vibrio*	Ward et al., 2009
	Bluefish, Pomatomus saltatrix	Vibrio, Pseudomonas, Enterobacteraceae	Newman et al., 1972
	Gilthead sea bream, Sparus aurata	Pseudomonas	Floris et al., 2013
	Grass puffer, Fugu niphobles	Vibrio, Pseudomonas, Flavobacterium	Sugita et al., 1989
	Grouper, Epinephelus coioides	Bacillus, Vibrio, Delftia, Psychroacter, Acinetobacter, Pseudomonas	Sun et al., 2009
	Red drum, Sciaenops ocellatus	Mycoplasmataceae	Ransom, 2008
		Photobacterium, Cetobacterium, Clostridiaceae, Vibrio	Givens et al., 2015
	Sea trout, Salmo trutta trutta	Aeromonas sobria, Pseudomonas	Skrodenytė-ArbačIauskiene et al., 2008
	Siberian sturgeon, Acipenser baerii	Cetobacterium somerae	Geraylou et al., 2013
	Snapper, Lutjanusn bohar	Vibrio, Photobacterium	Smriga et al., 2010
	Southern flounder, Paralichthys lethostigma	Clostridium	Ramirez and Dixon, 2003
		Clostridium	Ransom, 2008
		Photobacterium, Clostridiaceae, Clostridium	Givens et al., 2015
	Speckled trout, Cynoscion nebulosus	Escherichia coli	Ransom, 2008

Effects of the microbiota on fish health

- Similar to mammals and plants, pathogens can become more prevalent and cause **infection and disease**, a process referred to as dysbiosis, when the fish commensal microbial community balance is disturbed

- The imbalances in the protective commensal microbial community can be induced by **changes in the environment, including water conditions, temperature, seasonal changes**, climate change, **antibiotic usage** or changes in rearing conditions

- **Commensal microbes** play important functions that contribute to host health and protection against pathogens. These functions include not only **direct protective effects** against pathogens via antibiosis, competition for resources or niche exclusion, but also **indirect effects** by stimulating the host immune response and nutrient uptake thereby increasing fish health

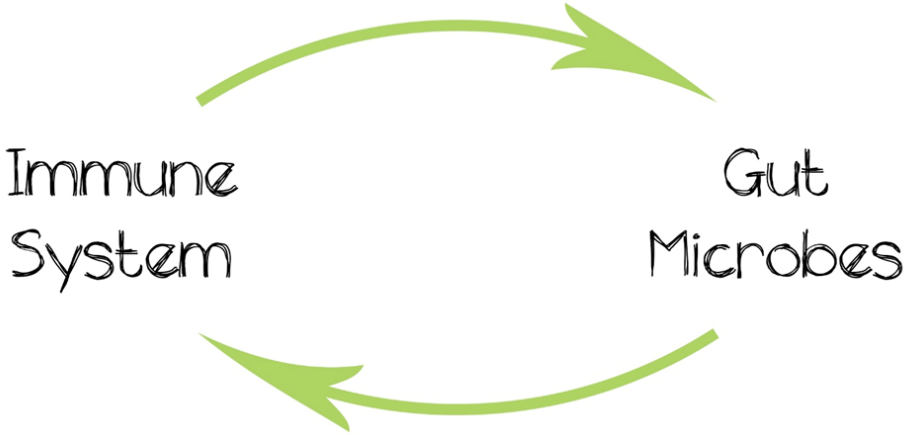


Interesting indirect effects of microbiota on fish health: modulation of nutrient uptake

- Commensal gut microbes also aid the fish in nutrient acquisition since they can produce exogenous enzymes to facilitate the digestion of food and degradation of large and complex molecules, such as chitin, protein and starch

- The gut microbes can also produce vitamins and eicosapentaenoic acid (EPA, an omega-3 fatty acid that is essential for metabolism) to enhance the health of the host

- The type of diet influences the microbial population and could potentially promote the microbial subpopulation providing protection against pathogens.



Wild fish microbiome

- Studies on wild (thus, likely healthy) fish in comparison with aquacultured fish species are lacking

- Some of the few studies performed so far highlighted that there are **significant differences between fish belonging to the same species but of wild and aquaculture origin**

- This comparison is key to shed light on the **beneficial microorganisms**

- First results show that the most abundant microbial functional categories (as highlighted by metagenomic analyses) were those corresponding to the metabolism of cofactors and vitamins, amino acid metabolism and carbohydrate metabolism

Fish microbiome: a growing research field

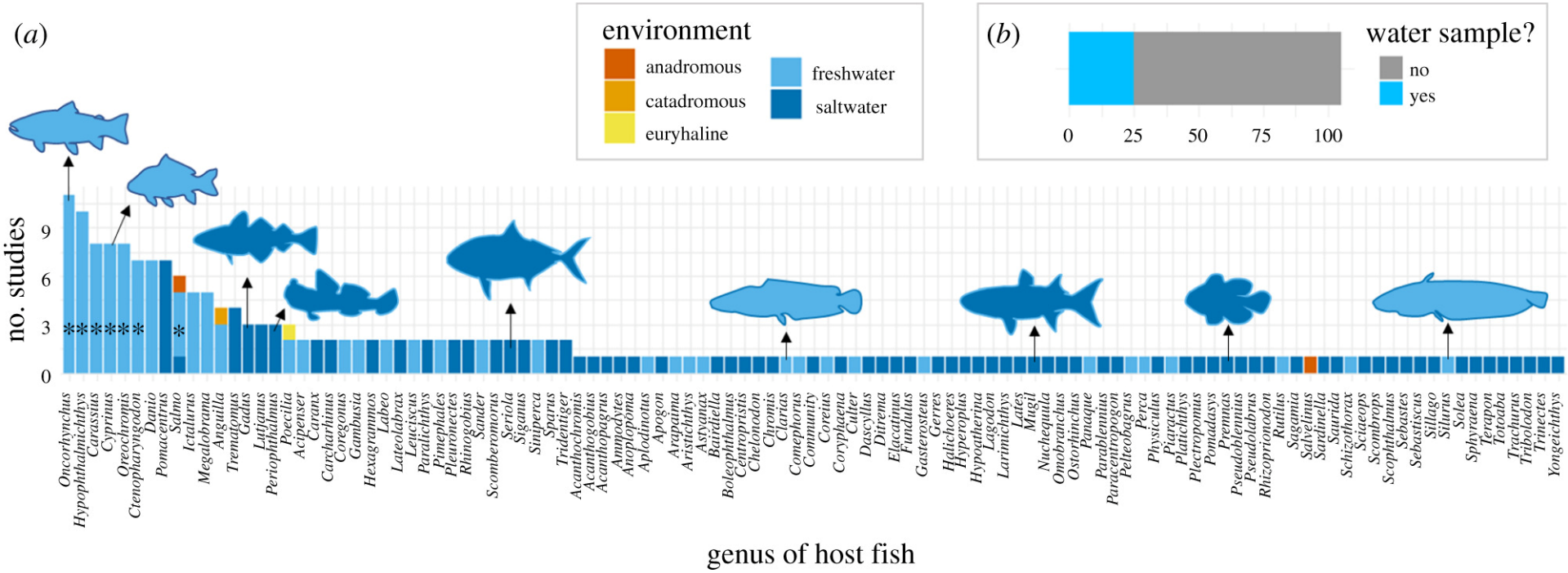


Figure 1. (*a*) Number of studies on the gut microbiome using NGS broken down by the genus of fish that the study was conducted on, as well as the environment those fish same from. Asterisk represents salmonid, carp and talapia. (*b*) The number of studies that assessed the water microbial communities. Gut microbiome studies were compiled using Web of Science [4] and only include studies that implemented NGS. It is acknowledged that total microbiome research extends further than this. Further information on search terms and filtering can be found in the electronic supplementary material. (Online version in colour.)

Fish microbiome: a growing research field

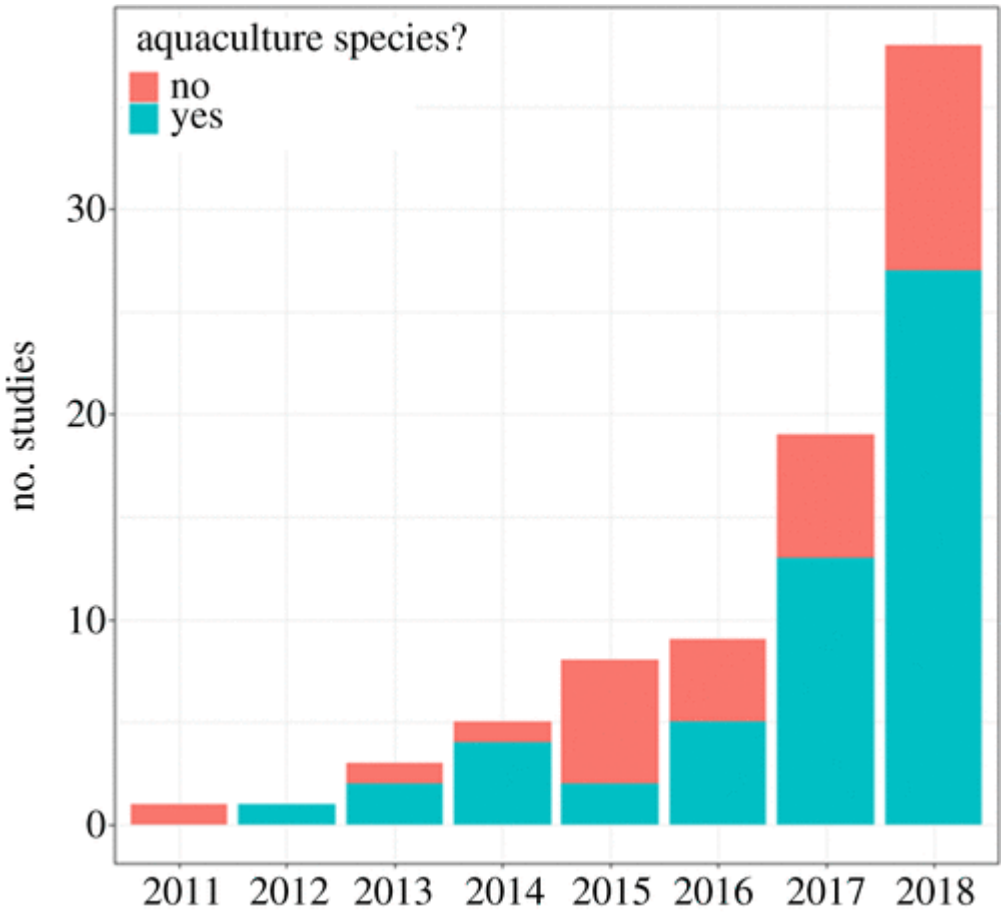


Figure 2. Growth in the studies using NGS on fish gut microbiomes, including food aquaculture species (aquaculture status taken from FishBase [12]). Further information on search terms and filtering can be found in the electronic supplementary material. (Online version in colour.)

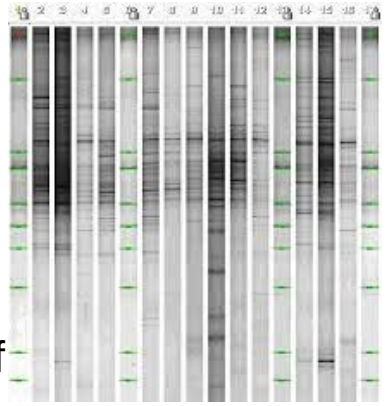
The role of fish microbiome in sustainable teleost production

- Rapid growth of the aquaculture industry has led to mounting pressure to make it more sustainable, and one way is the study and modulation of microbiome
- The teleost gut microbiome has a clear role in the future of aquaculture, and although research has come a long way in recent decades, there are still many areas of microbiome research that require further development
- Progression in teleost gut microbiome research will depend on: combining function, composition and spatial distribution of microbes; understanding the role of host genetic diversity; incorporating environmental variation.
- Understanding and manipulating microbial–host–environmental interactions and associated functional capacity in these areas could contribute substantially towards achieving a more sustainable aquaculture industry (for example, dramatic reduction of antibiotics)

Tools to study the diversity of fish microbiome

- Microbes associated with aquatic animals and their environment have been triditionally isolated on agar media

- Only a proportion of the viable microbes in various aquatic environments are culturable



- Culture-independent methods have been developed: qPCR, DGGE, TGGE, T-RFLP, clone library sequencing

- Today the more advanced ‘omics’ techniques such as 16S/18S rRNA gene and internal transcribed spacer (ITS) sequencing, metatranscriptomics and metagenomics are now rapidly advancing providing a more in-depth insight in the composition and functions of microbiomes

International Scientific Efforts to the study of fish microbiome: the example of the CIRCLES project


msbiotech
ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA
UNIVERSITÀ DEGLI STUDI DI GENOVA
DSM
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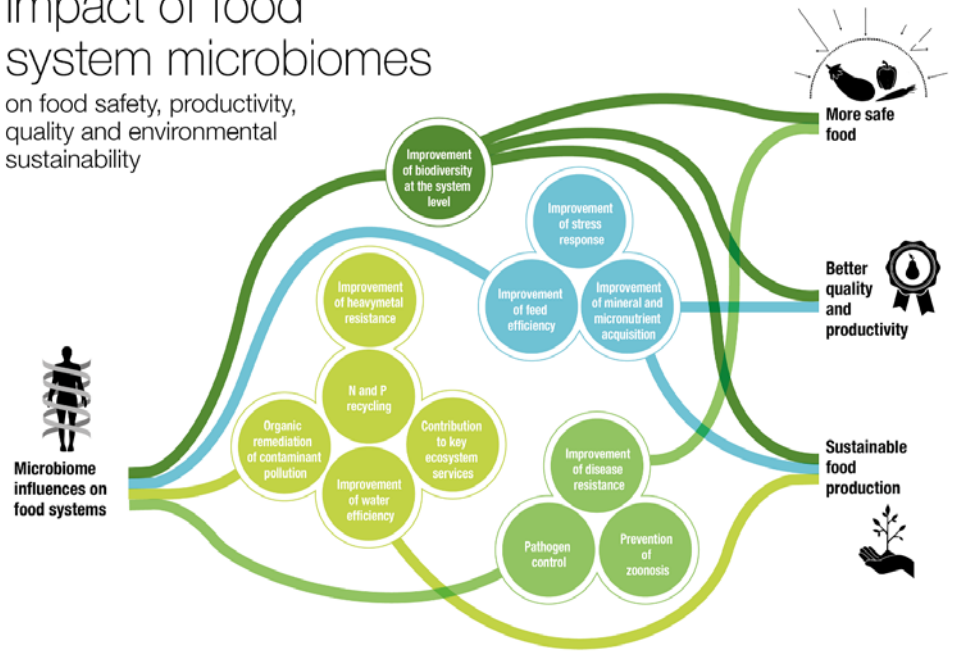
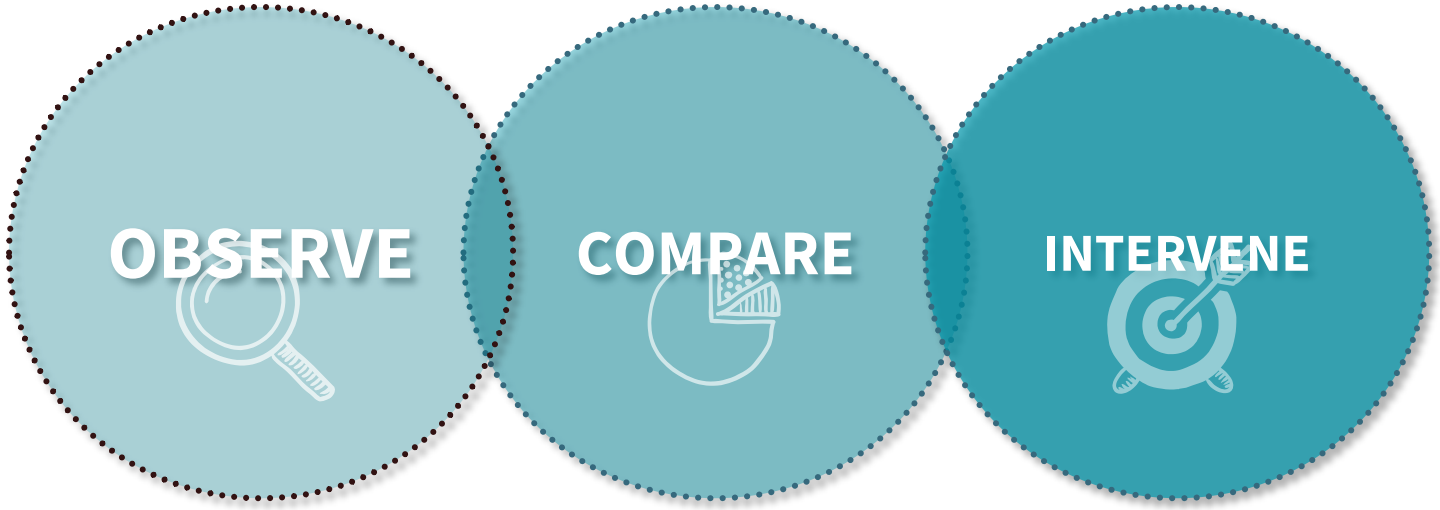
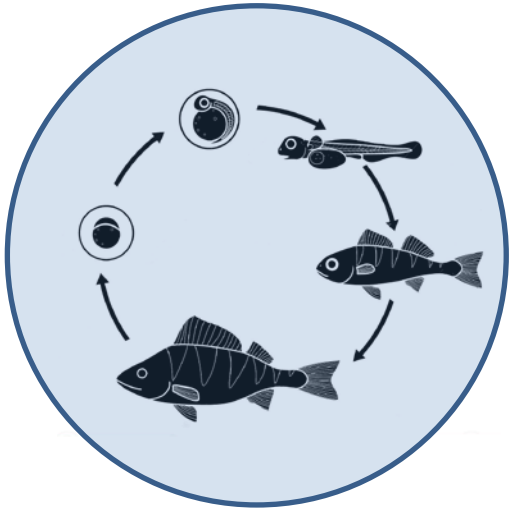
Centro Ricerche per la Chimica Fine
OROGEL
GATE 2 GROWTH
HAGUE
FCiências.ID
University of Dundee
EmmeFOOD
GruppoVeronesi
INRA SCIENCE & IMPACT
wellmicro
Luke NATURAL RESOURCES INSTITUTE FINLAND
Marine Institute
eurovix BIOTECNOLOGIE PER LA VITA
UNIVERSITY of STIRLING
Veterinærinstituttet
SOIL FOOD
PREVIWO
eufic
Bolton Alimentari
nordlaks
CSIC
CRCF

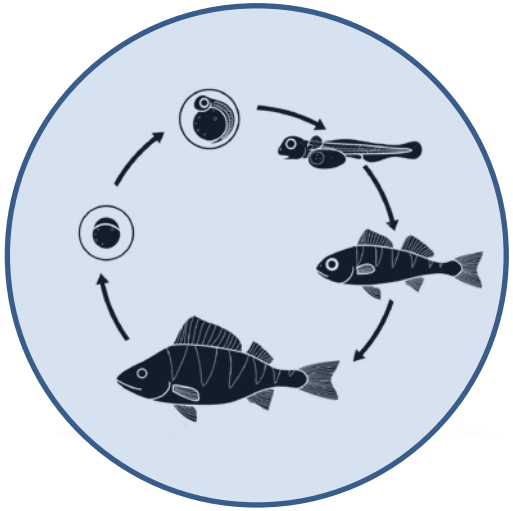

Impact of food system microbiomes
on food safety, productivity, quality and environmental sustainability
Microbiome influences on food systems
Improvement of biodiversity at the system level
Improvement of stress response
Improvement of heavymetal resistance
Improvement of feed efficiency
Improvement of mineral and micronutrient acquisition
N and P recycling
Organic remediation of contaminant pollution
Contribution to key ecosystem services
Improvement of water efficiency
Improvement of disease resistance
Pathogen control
Prevention of zoonosis
More safe food
Better quality and productivity
Sustainable food production


OBSERVE
COMPARE
INTERVENE

No action can be taken if we miss a complete knowledge of fish microbiome in current conditions, i.e., if we don't have BASELINE data

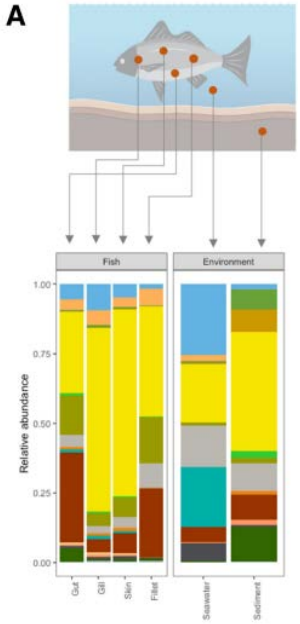


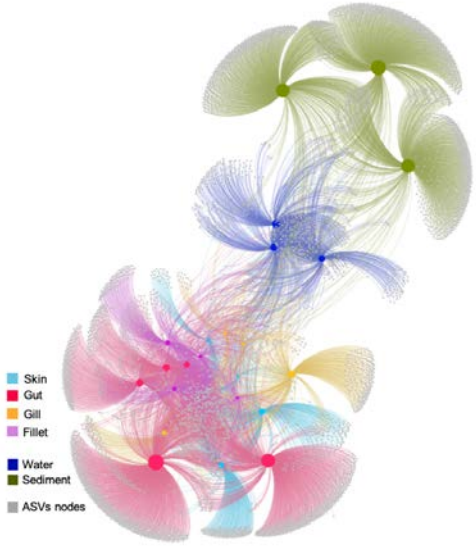
To determine the **microbiome dynamics and circulations** in farmed seabream froduction, from farm to fork (egg to fish product, feeds, production environment, workers) in order **to validate specific actions on microbiome** to improve quality, performance, safety and sustainability in acquaculture



Analyses still in progress

Pictures from IRBIM CNR





Microbial Ecology
https://doi.org/10.1007/s00248-022-02120-7

HOST MICROBE INTERACTIONS

Host-associated and Environmental Microbiomes in an Open-Sea Mediterranean Gilthead Sea Bream Fish Farm

Grazia Marina Quero[1] · Roberta Piredda[2] · Marco Basili[1,3] · Giulia Maricchiolo[4] · Simone Mirto[5] · Elena Manini[1] · Anne Mette Seyfarth[6] · Marco Candela[3] · Gian Marco Luna[1]

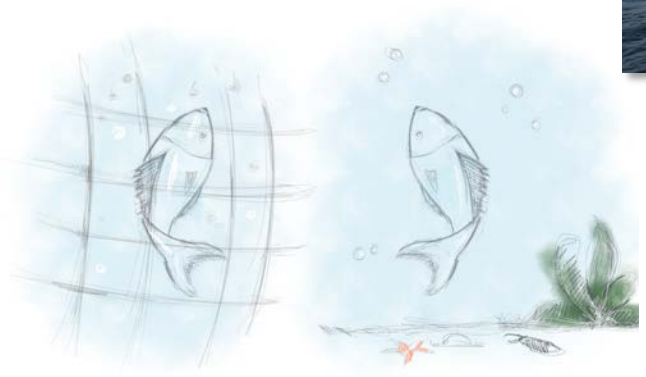
A first study produced with CIRCLES data focused on the relationships occurring between **adult farmed seabream microbiome and the surrounding environment**

Main outcomes

- Seabream had **distinct microbiome** associated to the host's tissues and compared to the marine environment
- Seabream microbiomes reflected only partially those in their surrounding environment suggesting that the **host is the primary driver shaping seabream microbiome**
- Overall, we observed a **greater influence of seawater than sediment** in shaping fish microbiome, especially when considering **gills and seawater**

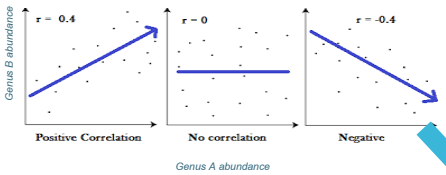
COMPARE

Need to understand the differences between wild and farmed fish microbiome



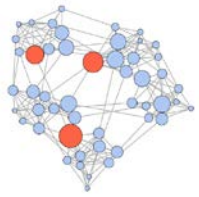
COMPARE

Modelling (UNIBO-WP8) observational data to design the Intervention Phase

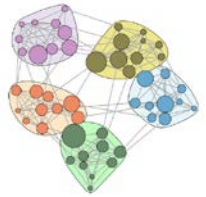


Significant & sparse correlations

Differences in composition

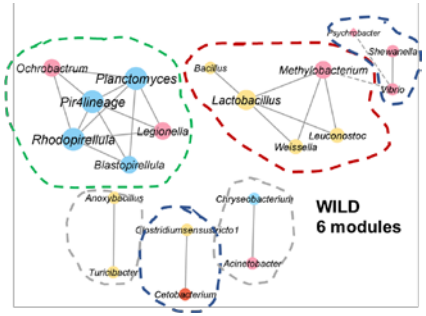


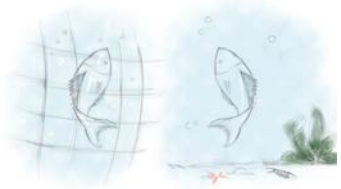
Hub genera



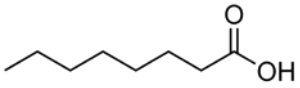
Genus modules

Network analysis



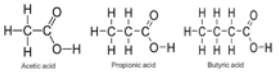


Identification of bacterial genera to focus on for POSITIVE (growth) and NEGATIVE (inhibition) actions to design microbiome-modulating diets (WP9)



Caprylic acid

Short Chain Fatty Acids



Tannins

Pre/probiotics

Modulate fish microbiome

INTERVENE

Testing experimental feedings to modulate microbiome towards a ''good'' microbiome based on the observation and modelling

Design **alternative diets** with nutraceuticals substances able to **modulate seabream microbiome**.

Evaluate efficacy of the alternative diets in modulate seabream microbiome and affect their **quality, welfare, safety and growth performances**

INTERVENE

IRBIM CNR Aquaculture Facility in Messina

Stabilimento utilizzatore di pesci ai sensi del D. lgs 26/2014
Autorizzazione Ministeriale n° 105/2014-A

INDOOR SECTION

12 twin tanks

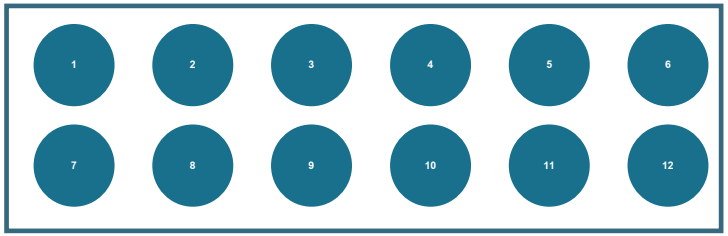
(volume 1.4 m^3)

Open system

Natural photoperiod

Twenty-four daily water changes

Incoming sea water filtered (sand filter) and sterilized (UV lamp)



Modulate fish microbiome

INTERVENE

180 DAYS

FEEDING TRIAL - Experimental design

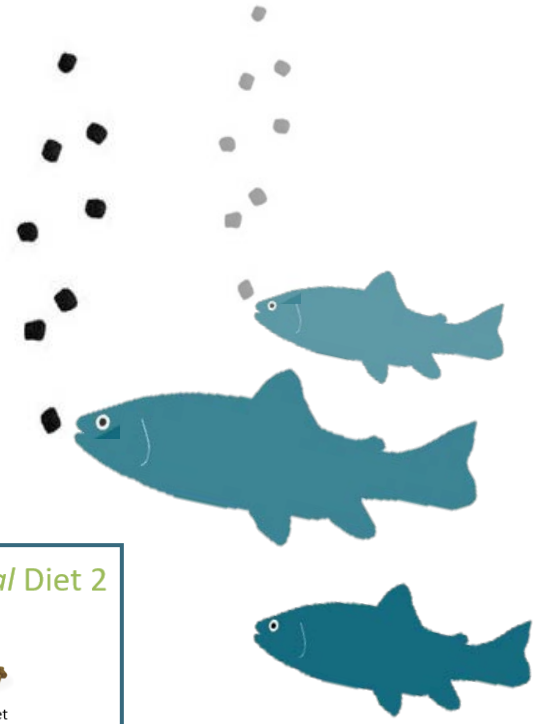
Number of fish: 476 gilthead sea bream
(Autorizzazione n° 740/2020-PR)

Fish weight: 120-150 gr

Beginning: 7 November 2021

Diets: control diet *vs* two experimental diets

Feeding: daily ration

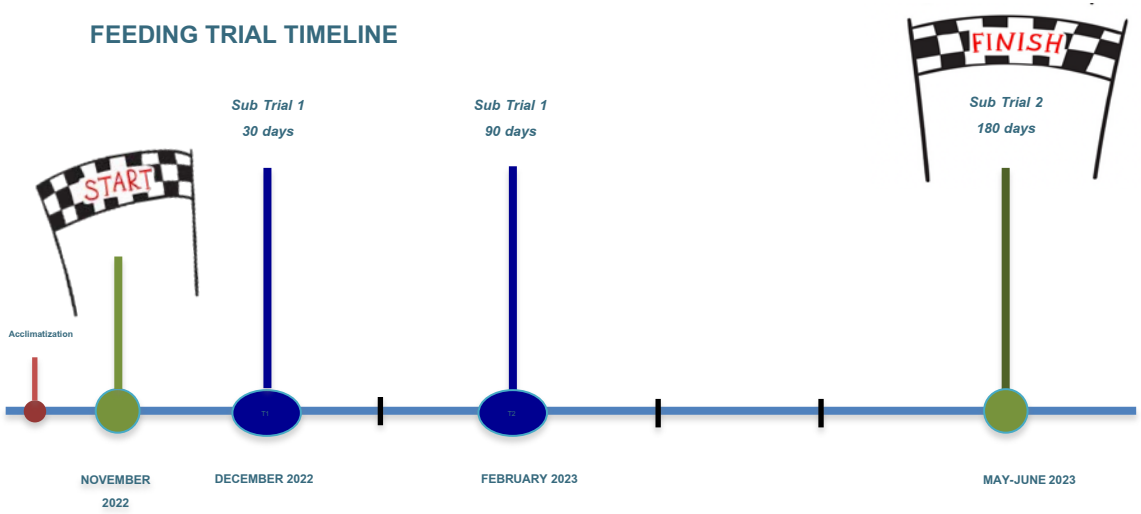


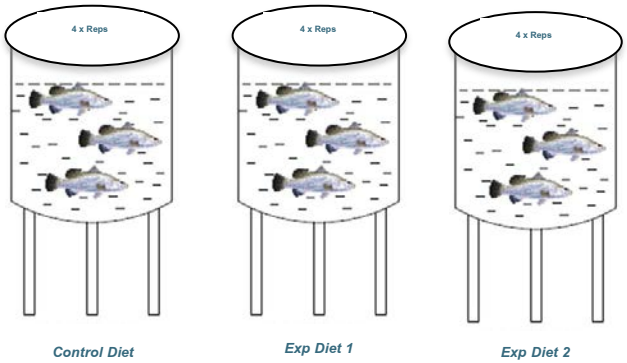
Control Diet	*Experimental* Diet 1 (D1)	*Experimental* Diet 2 (D2)
		
Commercial Diet for Gilthead Seabream	Control Diet enriched with **Fatty Acid (SCFA)** and **Tannins**	Control Diet enriched with **prebiotics** and **Tannins**

VERONESI CRCF SILVATEAM

INTERVENE

FEEDING TRIAL TIMELINE





Modulate fish microbiome

INTERVENE

FEEDING TRIAL

Control Diet

4 x Reps

Exp Diet 1

4 x Reps

Exp Diet 2

4 x Reps

Sub Trial 1
90 days

Sub Trial 2
180 days (350 g)

FEBRUARY 2023

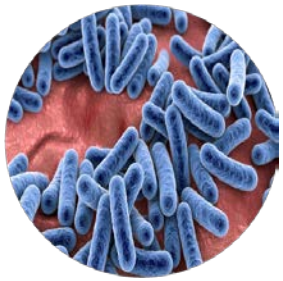
MAY-JUNE 2023

- Growth performances
- Microbiome
- Intestinal health
- Welfare
- Immunity

QUALITY

Modulate fish microbiome

INTERVENE



Ancona

Microbiome in gut, gills, skin and fillets

Fillets Quality

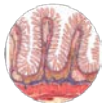
Nutrizional composition
Reological analysis

Mazara del Vallo

Messina

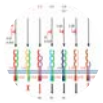
ciimar

Growth performance



Gut Health

Welfare – Stress indicators



Immunity

The team work

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Maria Bonsignore
Antonio Bellante
Marianna Del Core
Vincenzo Tancredi
Massimo De Luca

UNIBO

Marco Candela
Pier Luigi Martelli
Giulia Babbi

Some useful references:

- de Bruijn, et al. 2018.Exploring fish microbial communities to mitigate emerging diseases in aquaculture. FEMS Microbiology Ecology https://doi.org/10.1093/femsec/fix161

- Egerton et al. 2018. The Gut Microbiota of Marine Fish. Frontiers in Microbiology. doi: 10.3389/fmicb.2018.00873

- Legrand et al., 2018. The Inner Workings of the Outer Surface: Skin and Gill Microbiota as Indicators of Changing Gut Health in Yellowtail Kingfish. Frontiers in Microbiology doi:10.3389/fmicb.2017.02664

- Ramirex and Romero. 2017. The Microbiome of Seriola lalandi of Wild and Aquaculture Origin Reveals Differences in Composition and Potential Function. Front. Microbiol https://doi.org/10.3389/fmicb.2017.01844

- Perry et al. 2020. The role of the gut microbiome in sustainable teleost aquaculture. Proc. Royal Soc. B https://doi.org/10.1098/rspb.2020.0184

Safety of fish and fisheries products

- Food safety has become a worldwide concern that affects international trade and relations due to its impact on human health and economics, especially in recent years when the number and complexity of food safety issues has increased substantially

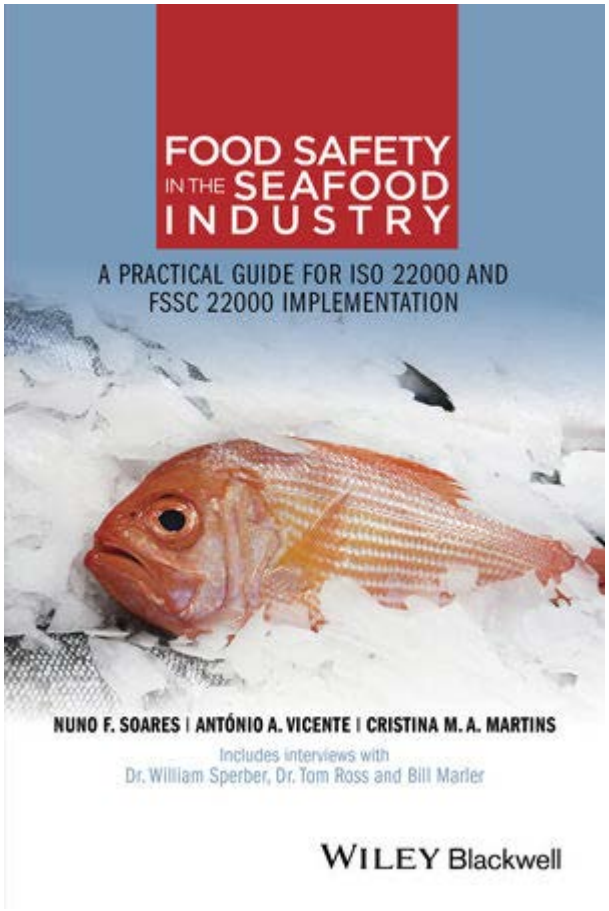
- This is evidenced by the large number of new, emerging, reemerging, or evolving pathogenic microorganisms (e.g., Escherichia coli O157:H7 and other Shiga toxin– producing E. coli serotypes, Salmonella serotypes Enteritidis and Typhimurium DT 104, Campylobacter jejuni/coli, Yersinia enterocolitica, Listeria monocytogenes, and Enterobacter sakazakii, parasitic agents such a Cryptosporidium and Cyclospora, Noroviruses) which have become food safety concerns after the 1970s, 1980s, and even 1990

- Modern food safety issues and concerns appear to multiply and become more significant when considered in association with societal changes and our transformation as consumers

Safety of fish and fisheries products

TABLE 2 Specific Spoilage Organisms of Cod

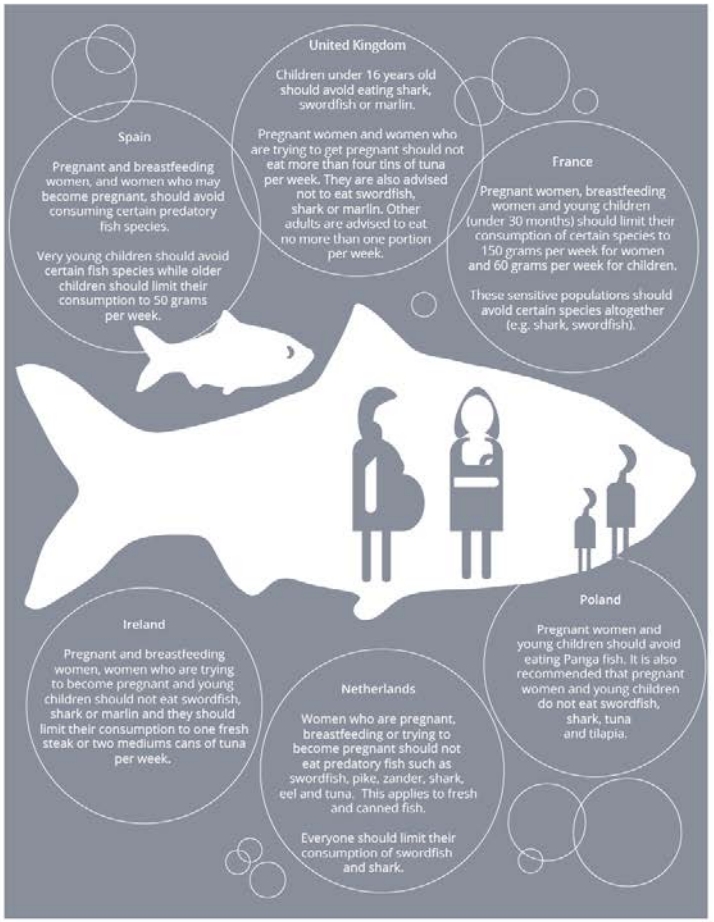
Storage Temperature (°C)	Specific Spoilage Organisms	Packing Method[a]
0	Gram-negative psychrotrophs, nonfermentative rods, *Pseudomonas* spp., *Shewanella putrefaciens*, *Moraxella* spp., *Acinetobacter* (*Pseudomonas*) spp.	Aerobic
	Gram-negative rods; psychrotrophs and psychrophiles (*S. putrefaciens*, *Photobacterium phosphoreum*)	Vacuum
	Gram-negative fermentative rods with psychrophilic character (*Photobacterium phosphoreum*), *Pseudomonas* spp., *S. putrefaciens*, gram-positive rods (lactic acid bacteria)	MAP
5	Psychotrophic gram-negative rods, Vibrionaceae (*Aeromonas* spp., *S. putrefaciens*)	Aerobic
	Psychotrophic gram-negative rods; Vibrionaceae (*Aeromonas* spp., *S. putrefaciens*)	Vacuum
	Gram-negative psychotrophic rods (*Aeromonas* spp.)	MAP
20–30	Gram-negative mesophilic fermentative rods, Vibrionaceae, Enterobacteraceae	Aerobic



Safety of fish and fisheries products

Examples of food safety advice on fish consumption from EU Member States

The European Food Safety Authority (EFSA) recommends that countries provide specific and relevant national advice to their citizens on safe consumption of fish (EFSA, 2015), as the nature of fish consumption — including the type and quantity — varies significantly across EU Member States.



Sources: Spain — AECOSAN, 2011; United Kingdom — NHS, 2015; France — Anses, 2016; Ireland — FSAI, 2017; Netherlands — NVWA, 2016, Voedingscentrum, 2018; Poland — Serwis Zdrowie, 2018.