

Metabarcoding en comunidades de eucariontes

Día 1

24 al 28 de noviembre del 2025

Instituto de Ciencias del Mar y Limnología - UNAM

¡Bienvenidos!

Día 1: ¿Quiénes somos?

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Dr. Fausto Valenzuela Quiñonez
Investigador Titular B CIBNOR

Día 1: ¿Quiénes son ustedes?

- Nombre
- Universidad-Posgrado
- Intereses en investigación
- Experiencia en laboratorio, campo o análisis de datos
- Motivación por tomar el curso
- Expectativas del curso

Objetivos del curso

- Comprender los fundamentos del eDNA y metabarcoding aplicado a comunidades de eucariontes.
- Conocer y aplicar técnicas de muestreo, extracción y amplificación de ADN ambiental.
- Aprender el flujo bioinformático: desde datos crudos (FASTQ) hasta análisis ecológicos.
- Desarrollar habilidades prácticas en diseño experimental, control de calidad y visualización.
- Fomentar el pensamiento crítico sobre limitaciones, controles y decisiones metodológicas.

Dinámica y expectativas

- Principalmente marino

Temas principales por día:

- Lunes: Fundamentos de eDNA, diseño experimental, muestreo y laboratorio
- Martes: Bioinformática: conexión al servidor, Linux, importación y control de calidad
- Miércoles: Procesamiento de secuencias: denoising, quimeras, asignación taxonómica
- Jueves: Análisis ecológico: tablas, visualización, diversidad, comparación de barcodes
- Viernes: Controles negativos, OTUs, temas auxiliares y presentación de proyectos

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Introducción

La diversidad de vida en los océanos es crucial para la resiliencia y el mantenimiento de las funciones ecosistémicas y los servicios que estos brindan.

La biodiversidad marina se encuentra amenazada debido a las actividades humanas como el cambio climático, la sobrepesca, la introducción de especies invasoras y la contaminación.

Cambios en:

- Distribución de las especies
- Temporadas reproductivas
- Distribución y abundancia de stocks pesqueros
- Biodiversidad: riqueza, diversidad, estructura de las comunidades
- Blooms de algas nocivas y organismos patógenos
- Entre otras...

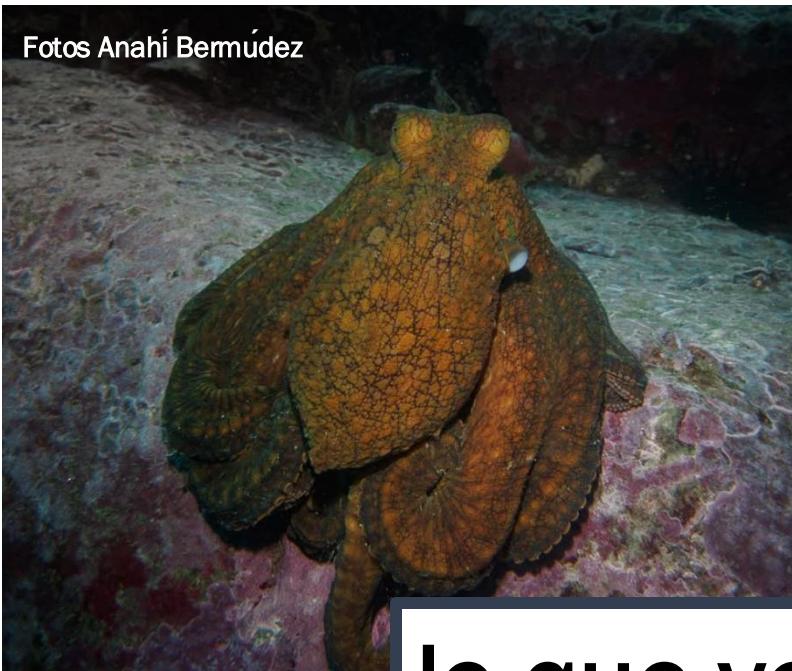
Métodos tradicionales

Se han obtenido avances significativos en el estudio de las comunidades marinas a partir de métodos tradicionales

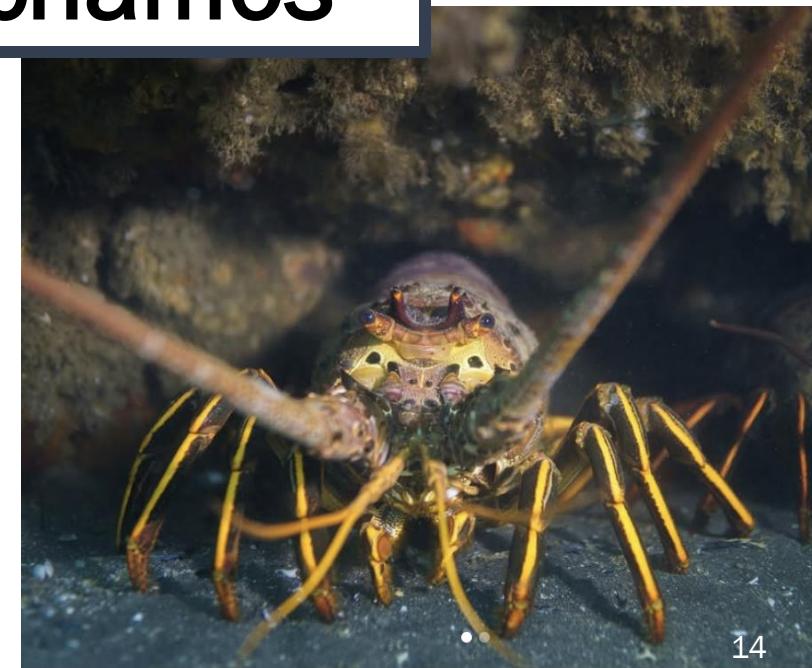
Características y limitaciones:

- Expertos en identificación morfológica
- Alta replicación para tener una representación completa de la comunidad estudiada
- Costos elevados (tiempo, \$)
- Invasivos



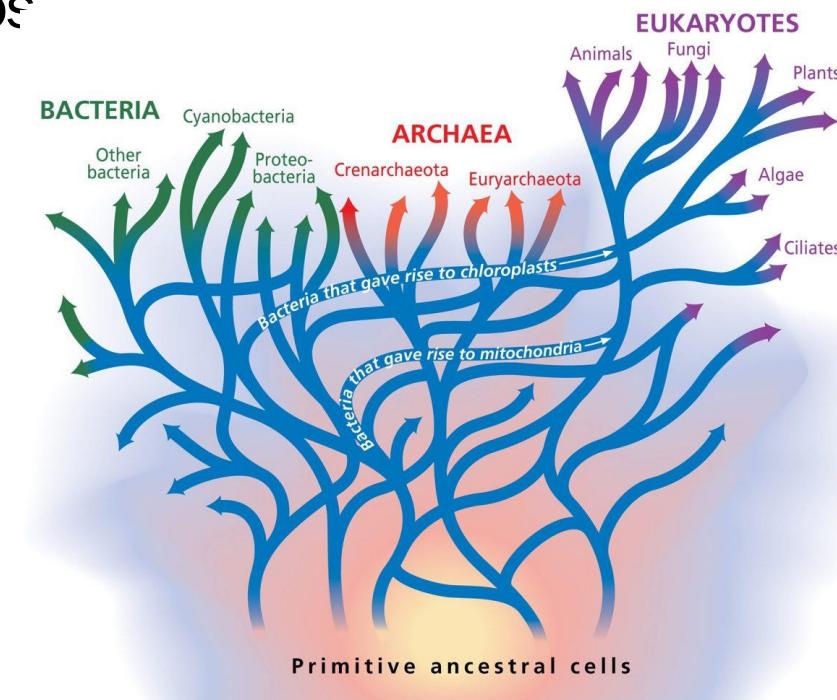


lo que vemos o lo que escuchamos

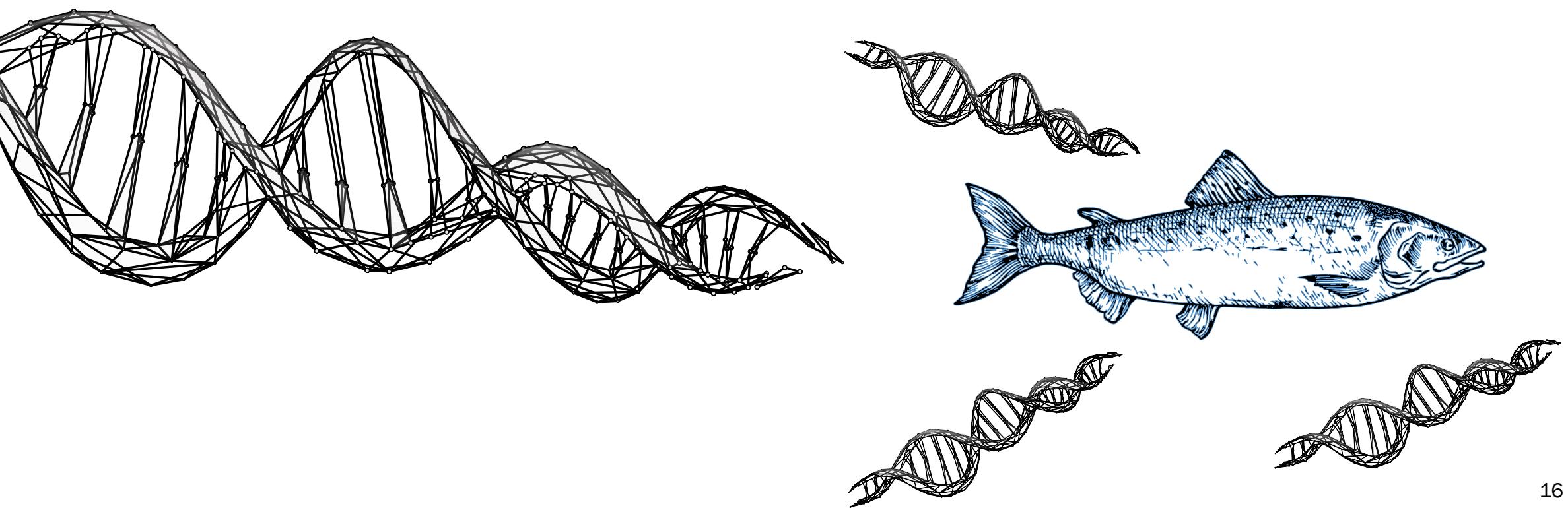


¿Existen métodos de detección de biodiversidad ideales?

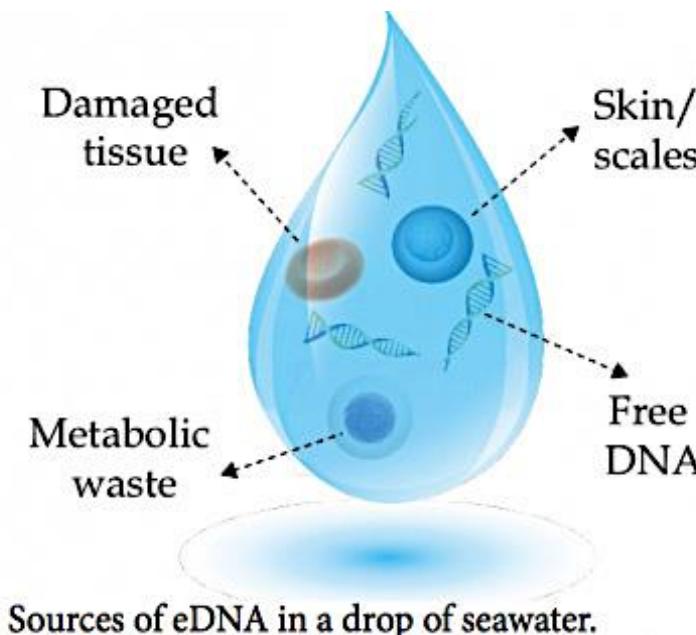
- Capturan comunidades enteras de manera no invasiva
- Información amplia de grupos taxonómicos
- Escalables
- Sensibles
- Resultados rápidos, a menor costo
- Sencillo de implementar



ADN ambiental environmental DNA (eDNA)

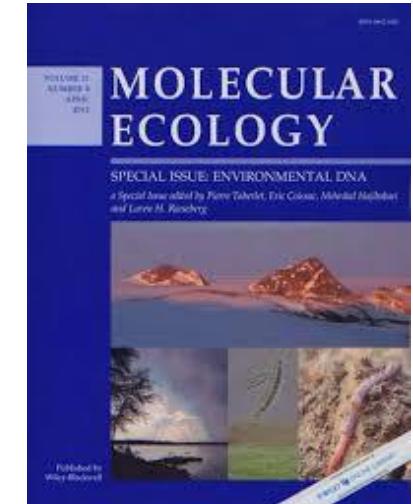
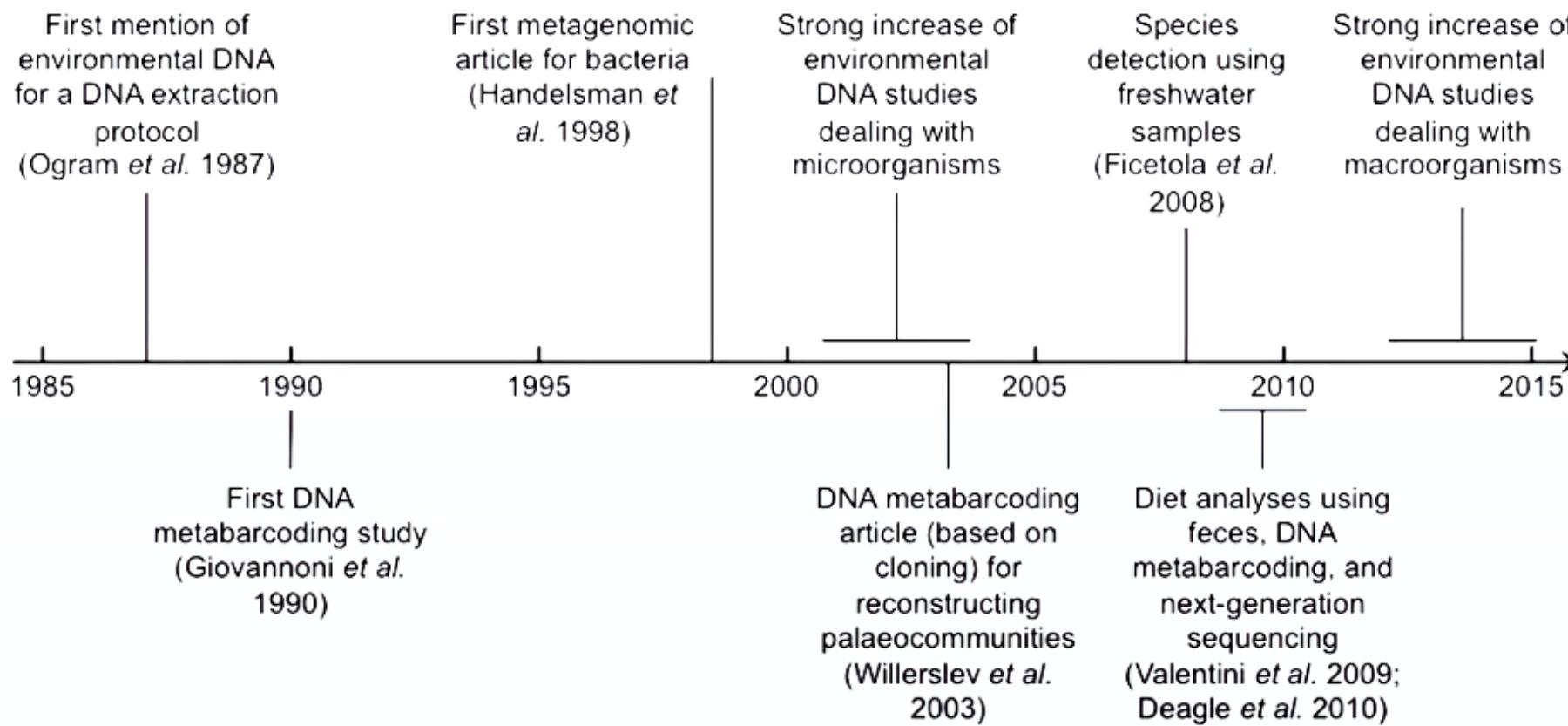


¿Qué es el ADN ambiental, o eDNA?



- ✓ Material genético que se obtiene de una muestra ambiental, sin organismos “evidentes”.
- ✓ Ventajas operativas sobre los métodos tradicionales
- ✓ No invasivo
- ✓ **Rentable y escalable:** la toma de agua y filtrado es relativamente barata y puede automatizarse o integrarse en ciencia ciudadana, multiplicando la cobertura espacial y temporal.
- ✓ **Alta sensibilidad:** detecta especies crípticas, raras o en etapas larvarias que pasan inadvertidas en censos visuales.

Historia





biology
letters

Population genetics

Biol. Lett. (2008) 4, 423–425

doi:10.1098/rsbl.2008.0118

Published online 9 April 2008

Species detection using environmental DNA from water samples

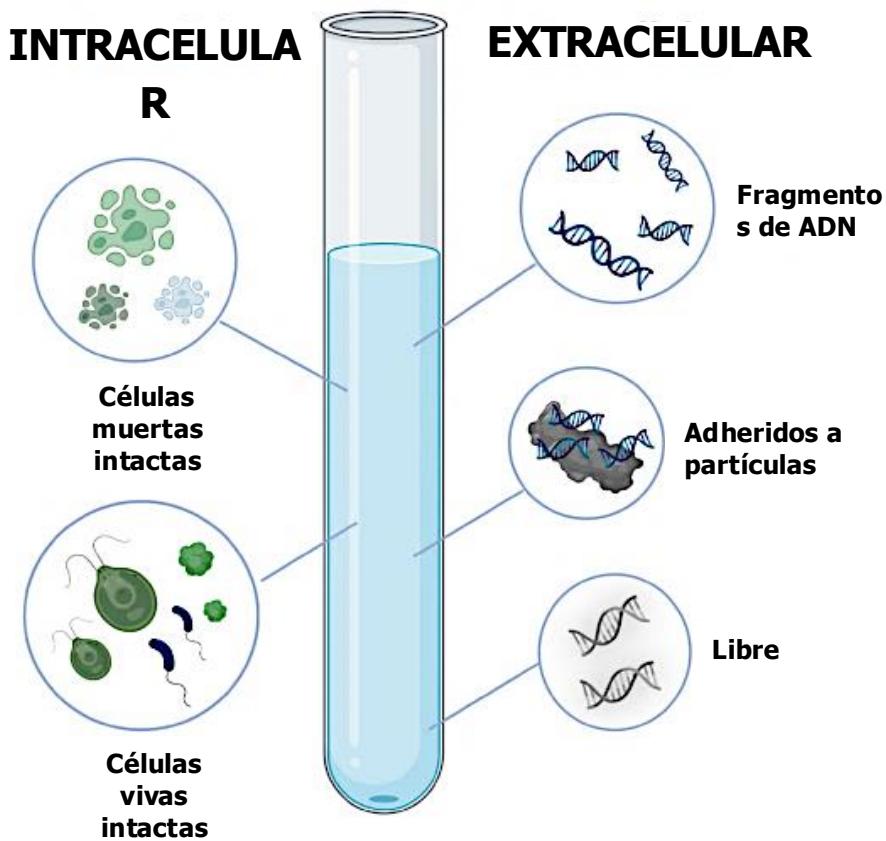
Gentile Francesco Ficetola^{1,2,*}, Claude Miaud²,
François Pompanon¹ and Pierre Taberlet¹

¹Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 09, France

²Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université de Savoie, 73376 Le Bourget du Lac Cedex, France

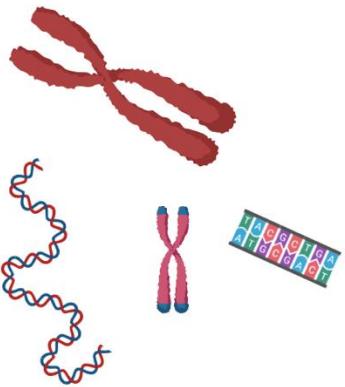
*Author and address for correspondence: Dipartimento di Scienze dell'Ambiente e del Territorio, Università Milano Bicocca, Piazza della Scienza 1, 20126 Milano, Italy (francesco.ficetola@unimi.it).

¿Qué es el ADN ambiental, o eDNA?



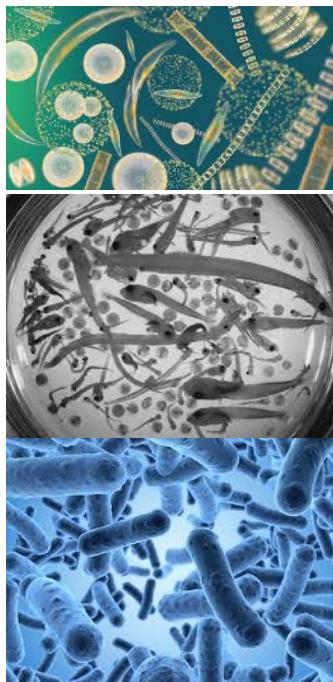
- ✓ Es una mezcla compleja de ADN de diferentes orígenes

Todas las muestras de eDNA son una combinación de dos fracciones:



DNA extraorganismo
“libre”, “disuelto”,
“extracelular”, “traza”

+



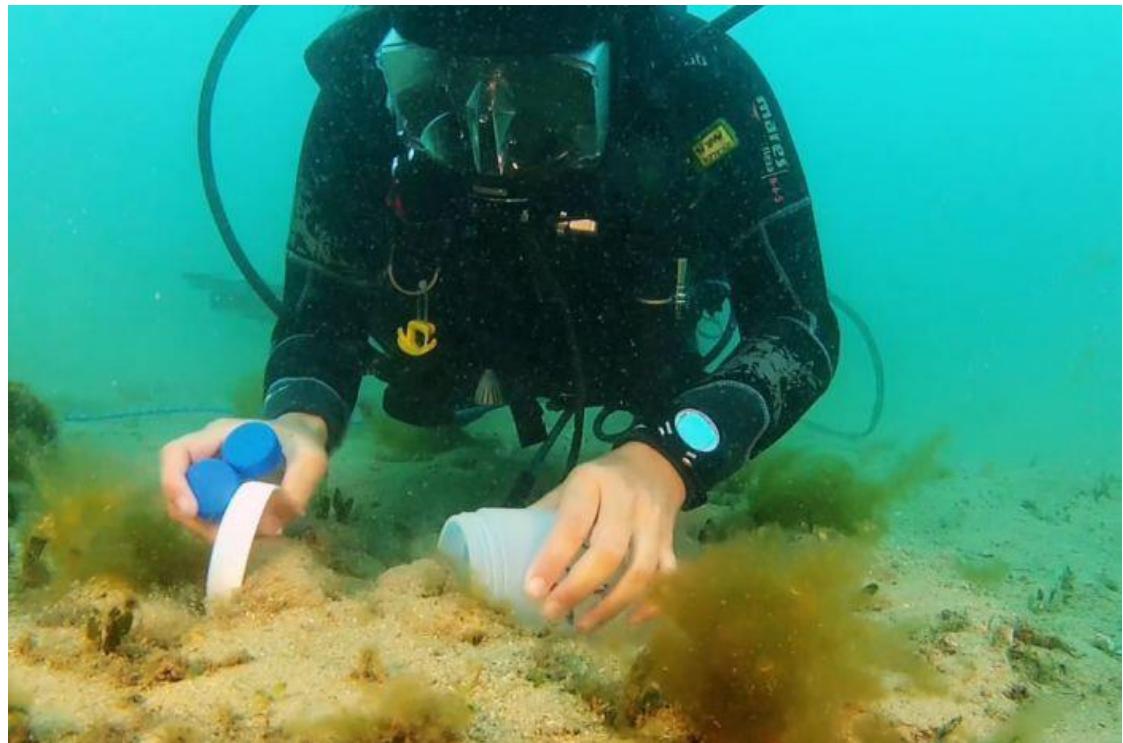
DNA de comunidades:
Algas, larvas,
microorganismos,
bacterias, etc.

=

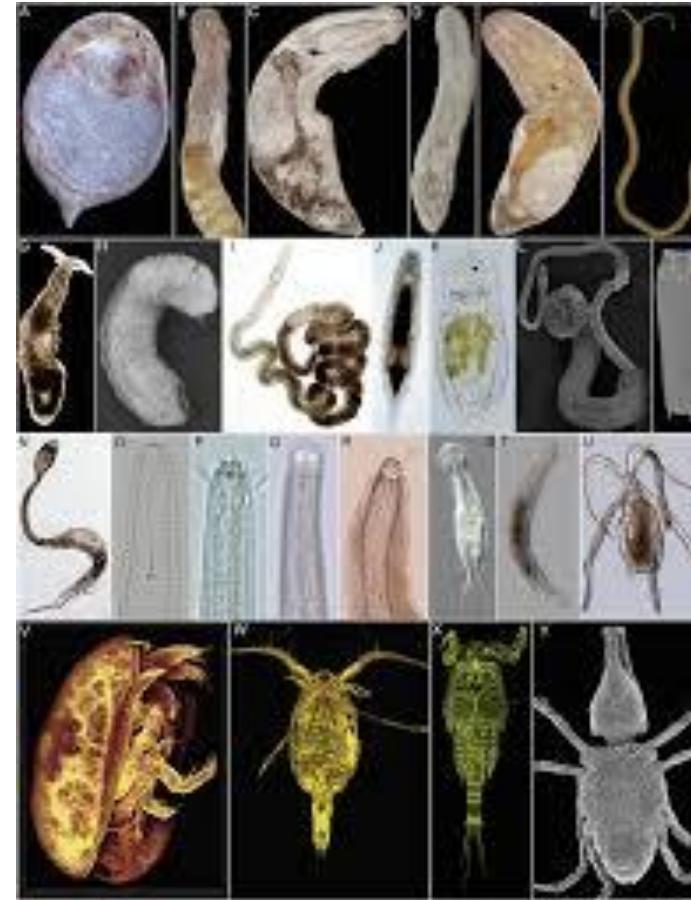


eDNA

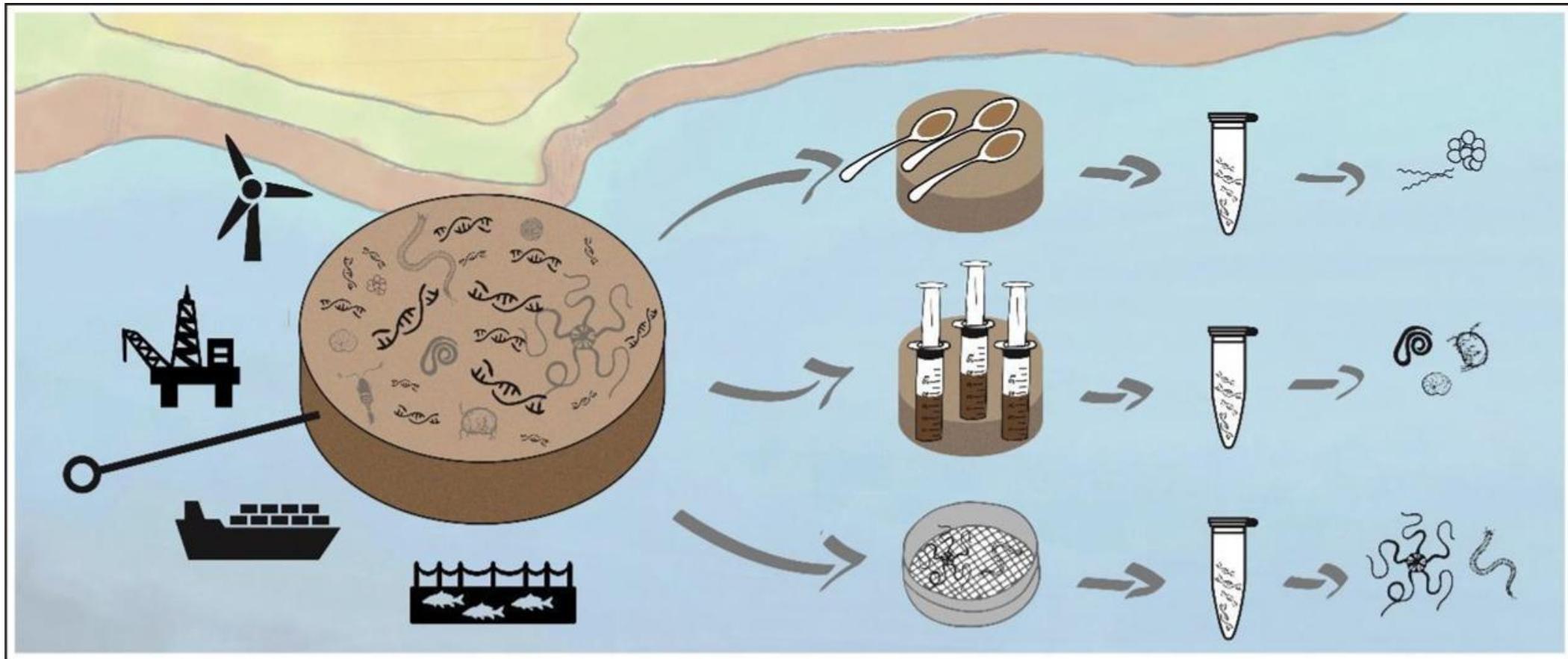
Esta distinción puede ser complicada:



DNA ambiental



DNA comunidades

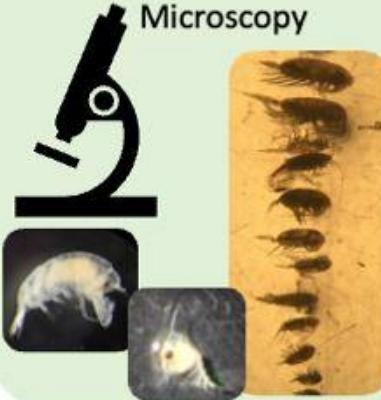


Pawlowski et al. 2022

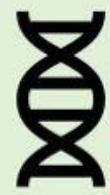
Zooplankton Net Tow



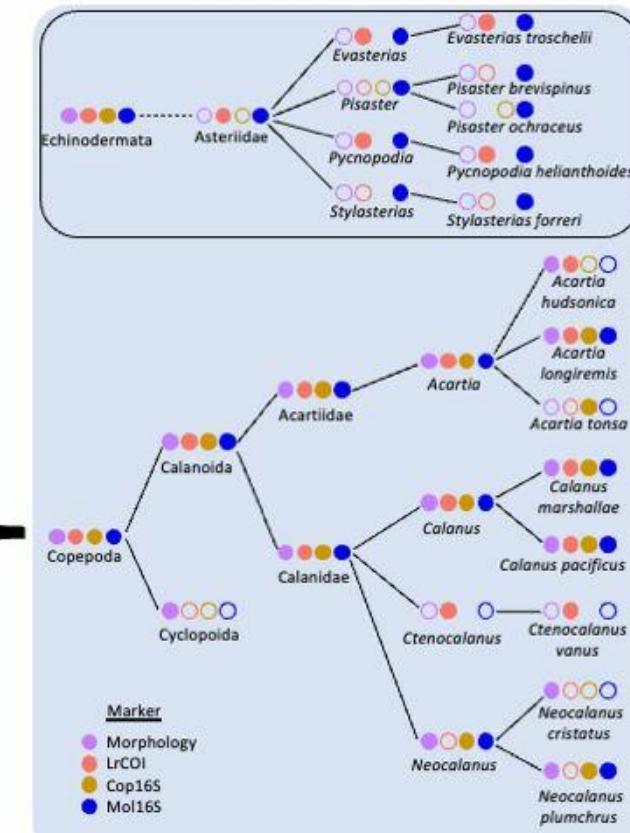
Morphological Identification Using Microscopy



DNA Metabarcoding: five general and targeted markers



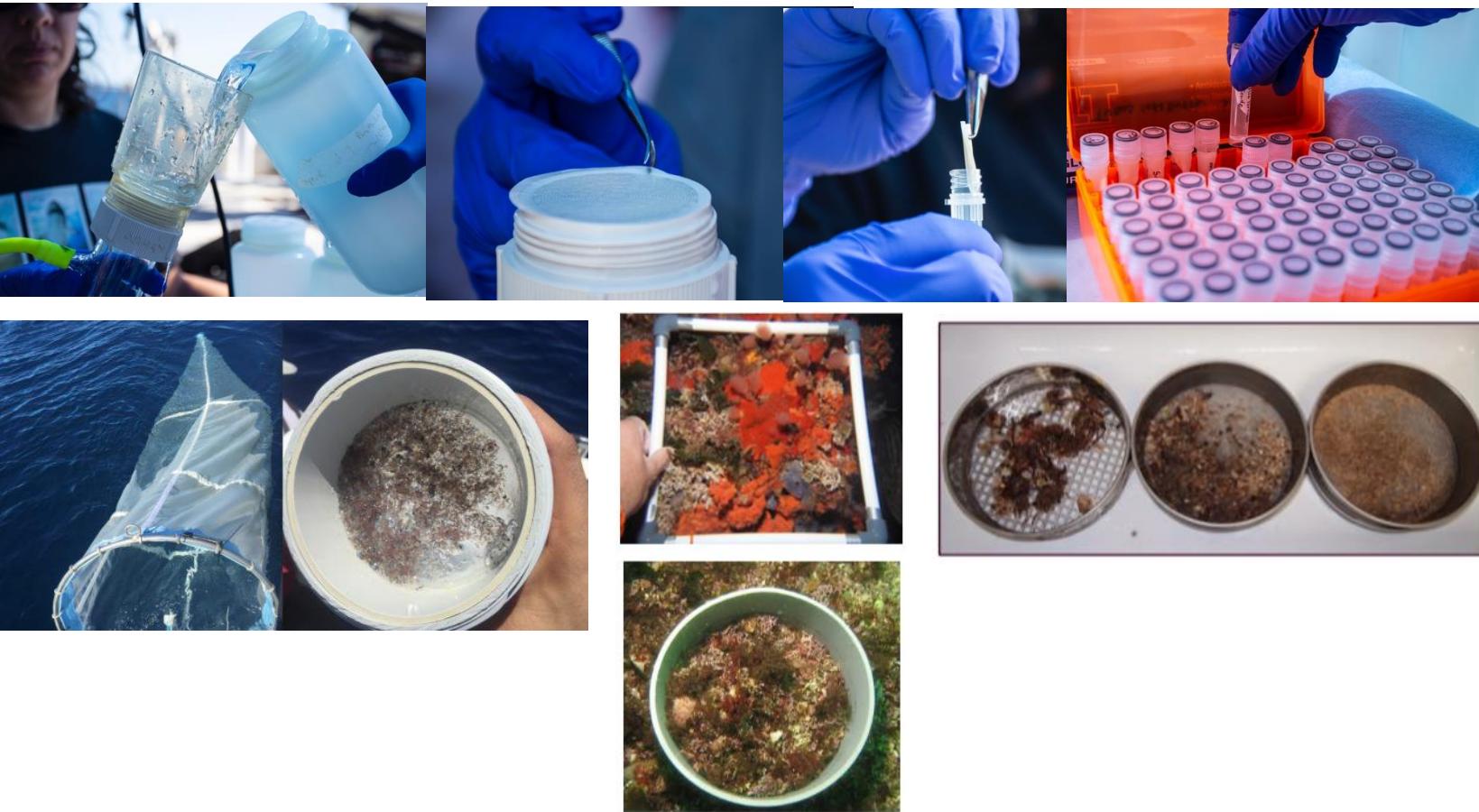
- LrCOI
- Cop16S
- Mol16S
- MiFish12S
- FishCytb



Conclusion: Coupling targeted and general metabarcoding markers can aid in resolving patterns and changes in zooplankton communities.



Muestreo

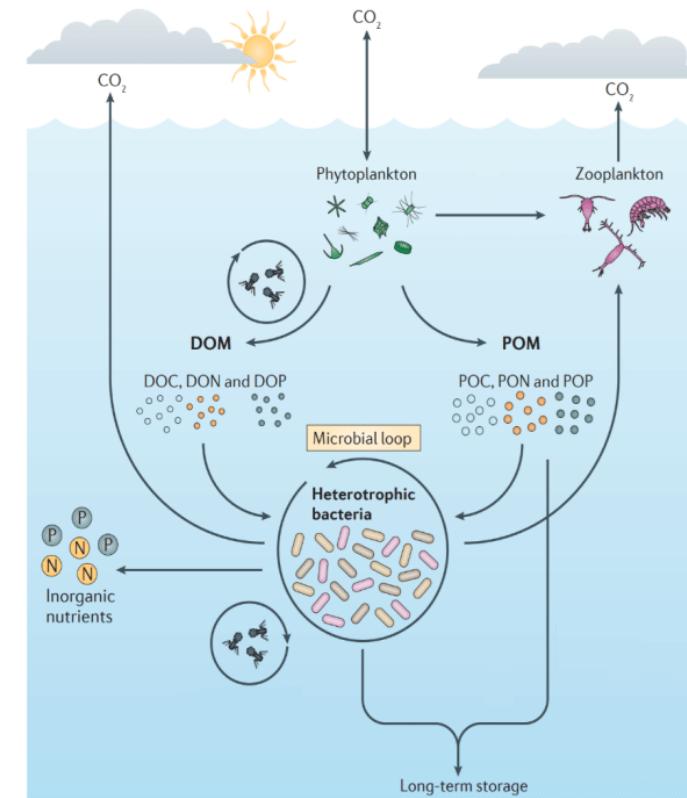


Dada la sensibilidad de la técnica es fundamental evitar contaminación

Esta distinción puede ser complicada:

DNA comunidades

DNA ambiental



Tipos de muestras

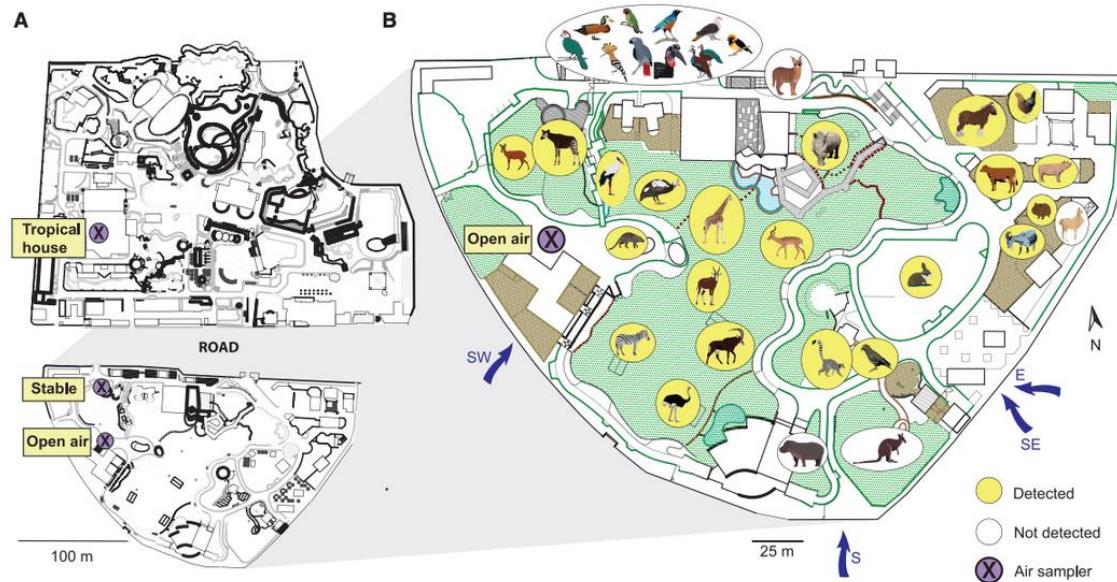
Agua



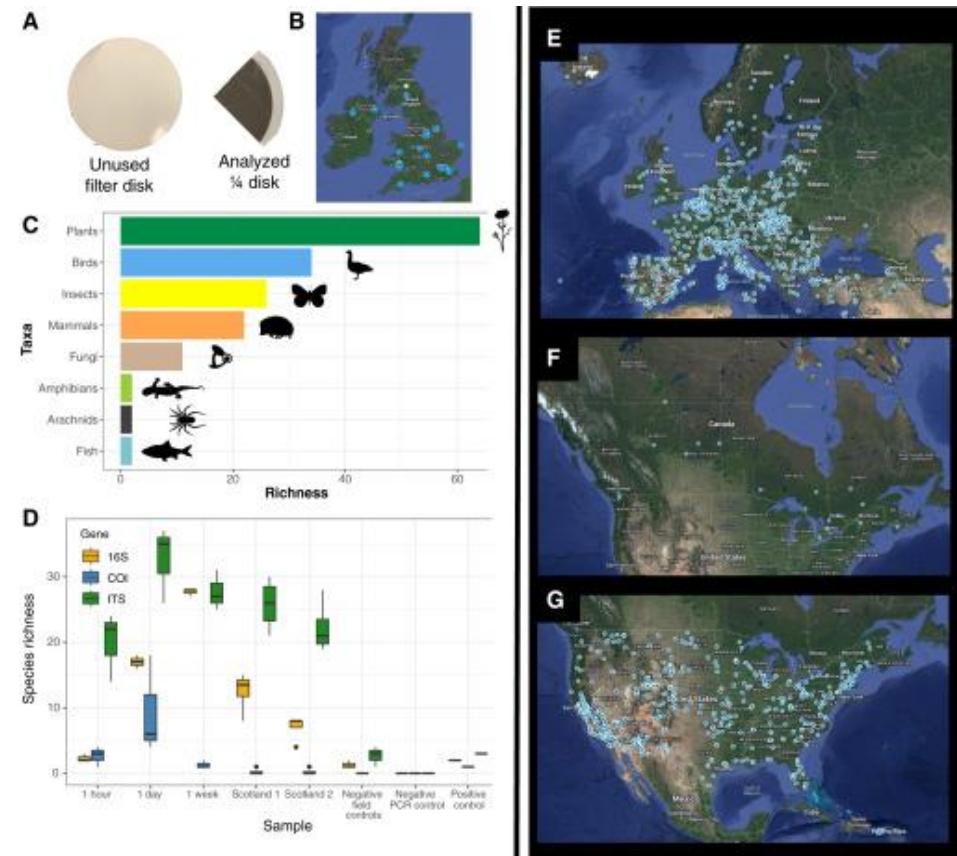


11/9/2025

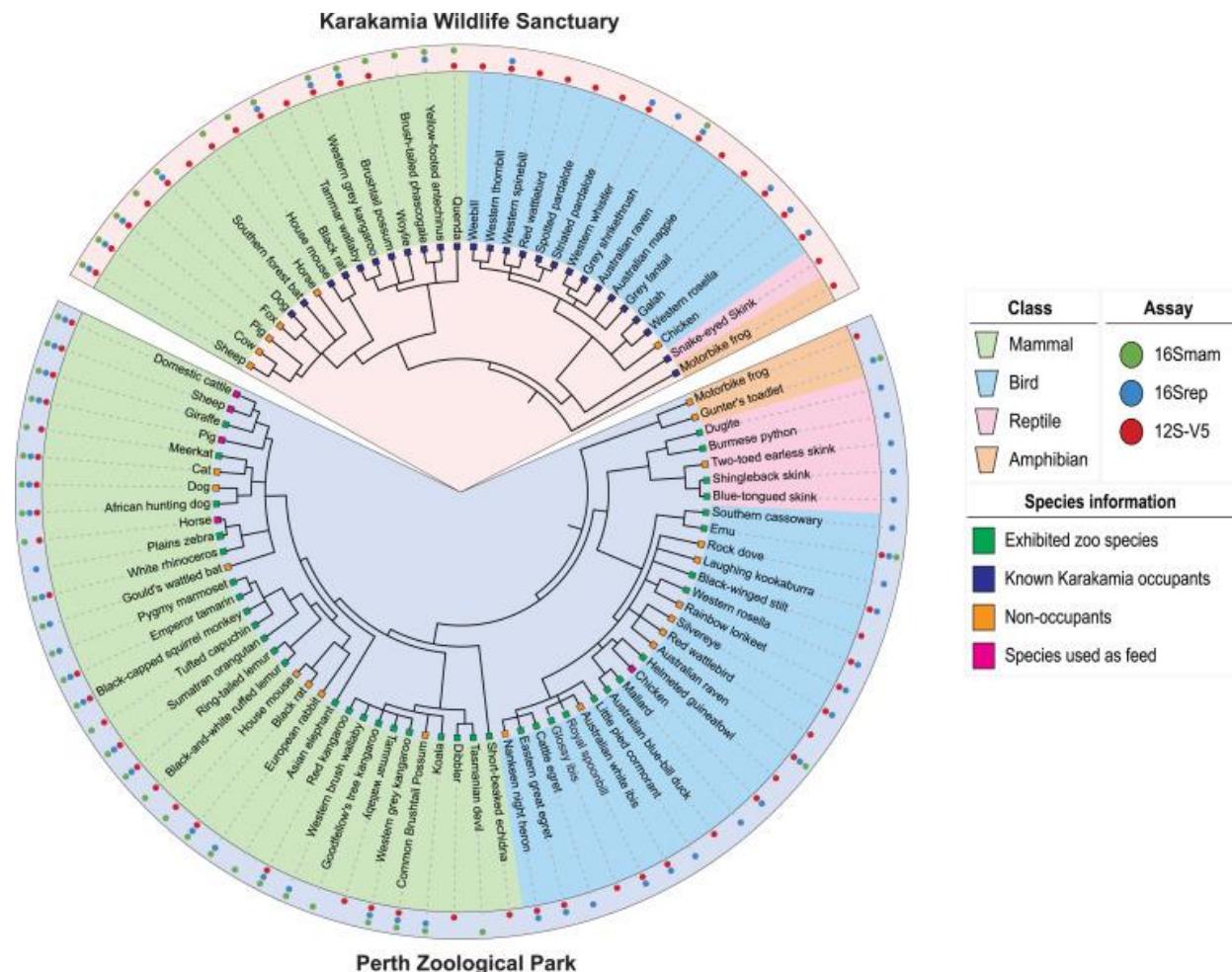
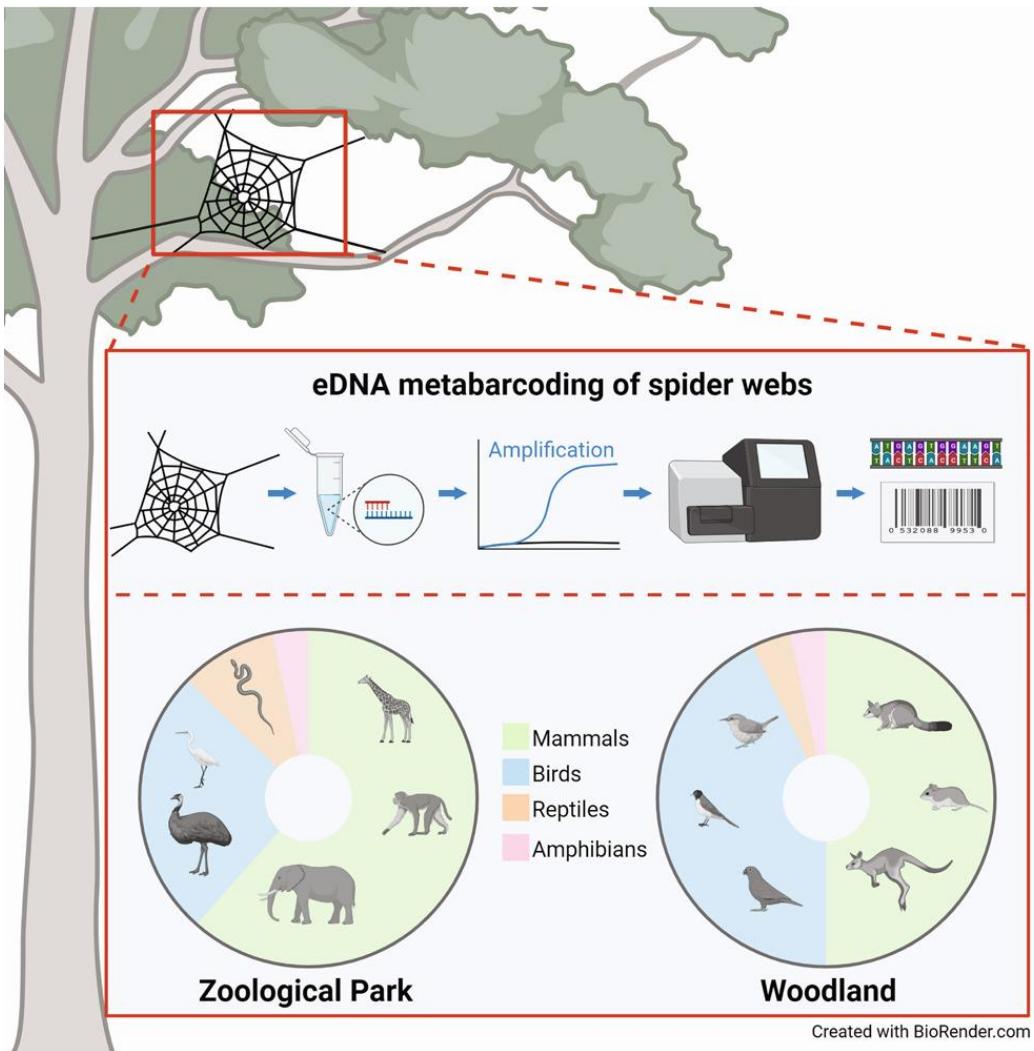
Aire (airborne eDNA)



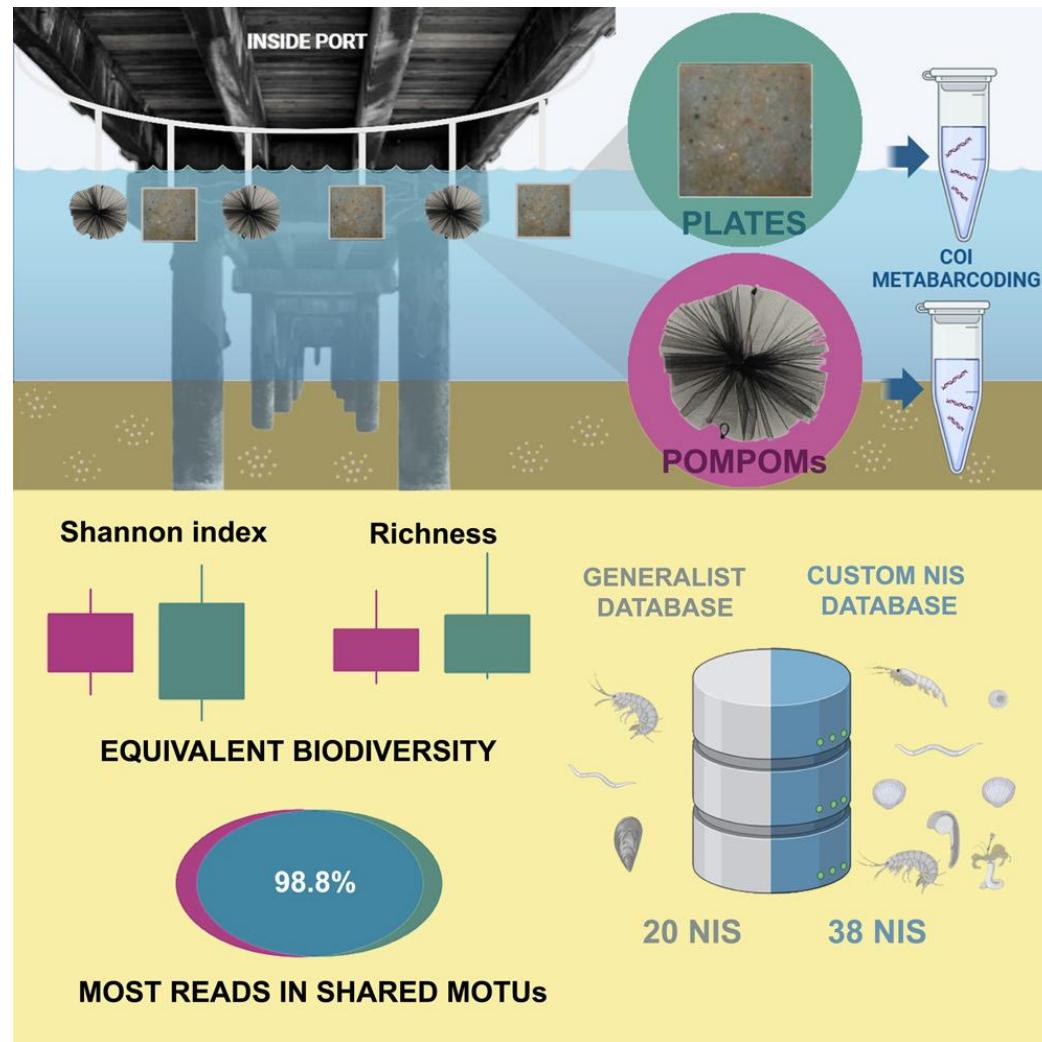
Lyndgaard et al. 2022 Current Biology



Littlefair et al. 2023 Current Biology

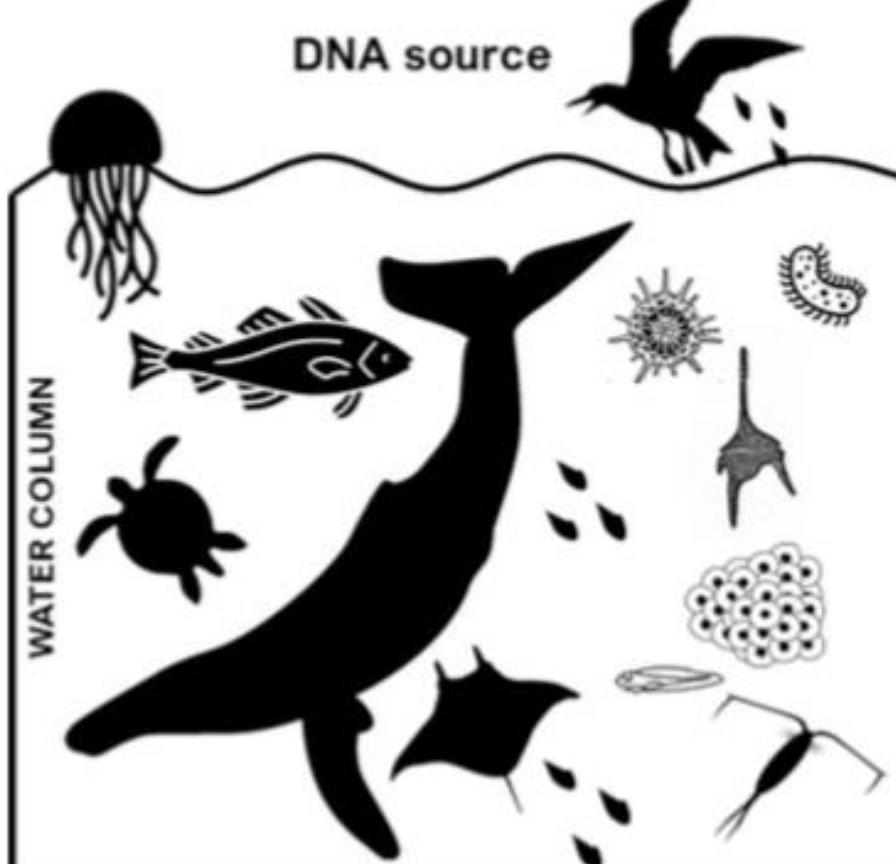


non-indigenous species



Sedimento

DNA source

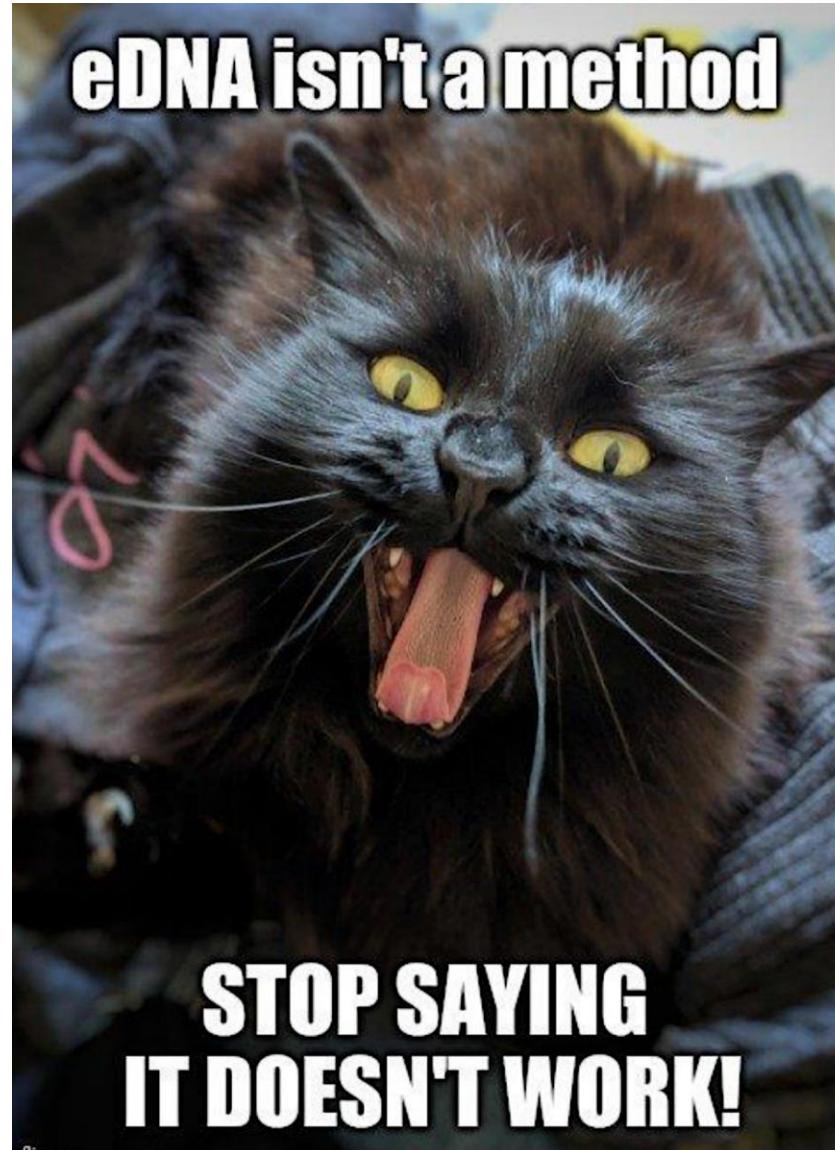


WATER COLUMN

Sampling method	Obtained sample	Target organisms	
		Community DNA	Environmental DNA
Water Bottle	Filter	Microeukaryotes Bacteria	Megafauna Macrofauna Benthic Invertebrates
Plankton Net	Bulk organisms	Meroplankton Zooplankton	
Bulk organisms		Benthic Invertebrates Macroalgae	
Van Veen Grab	Sediment	Microeukaryotes Bacteria	Megafauna Macrofauna Benthic Invertebrates

SEDIMENT

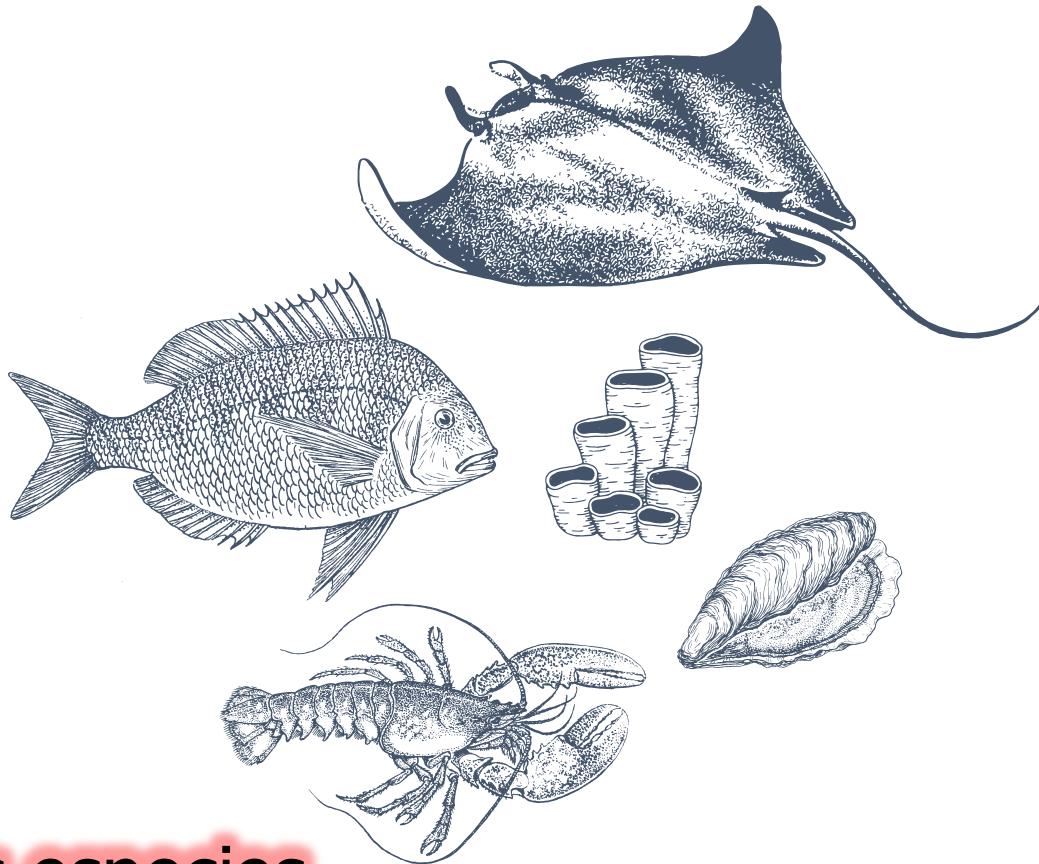
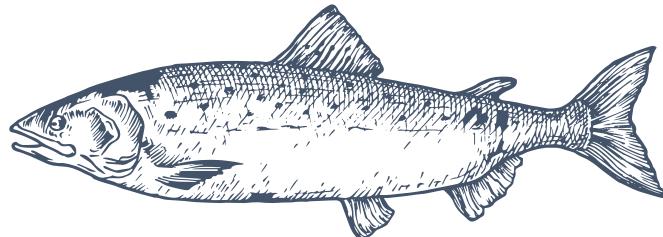




Detección de especies con eDNA: ¿qué nos interesa conocer?

una especie

método dirigido, o targeted en inglés



múltiples especies

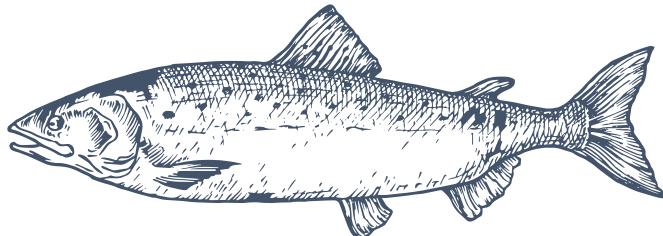
método no dirigido o non-targeted

Métodos dirigidos

Ensayo de detección especie-específico

una especie

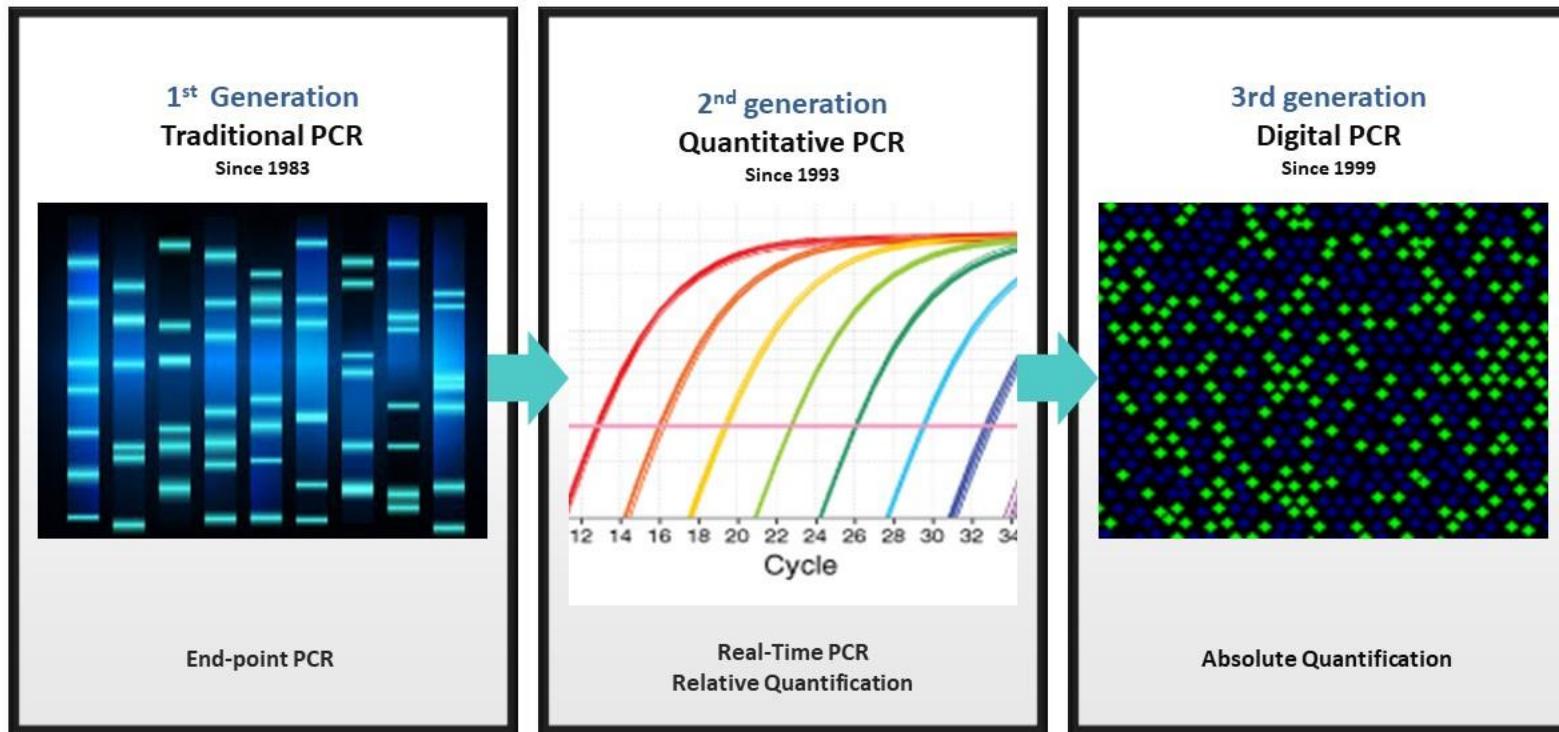
método dirigido, o targeted en inglés



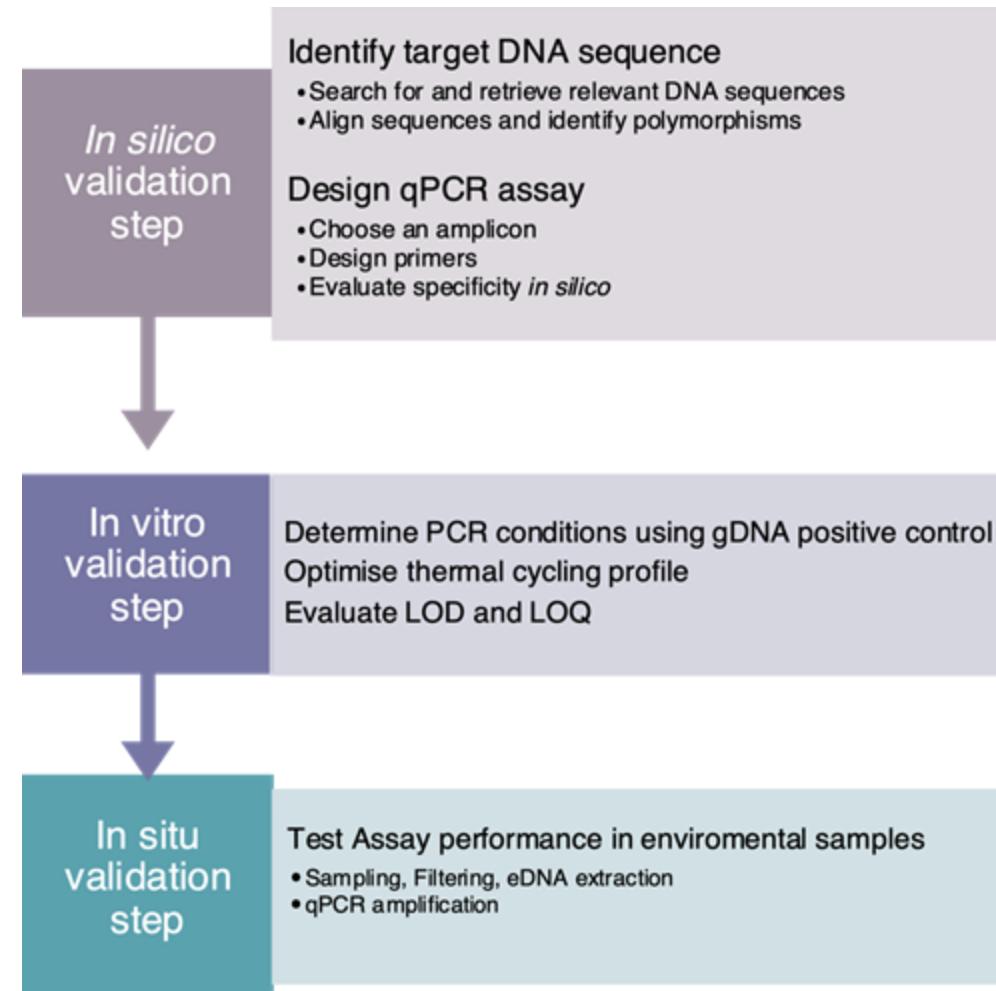
- ✓ Primers específicos
- ✓ Alta sensibilidad
- ✓ Estimación de abundancia
- ✓ Detección de especies:
 - invasoras
 - en peligro
 - de importancia pesquera

Métodos dirigidos

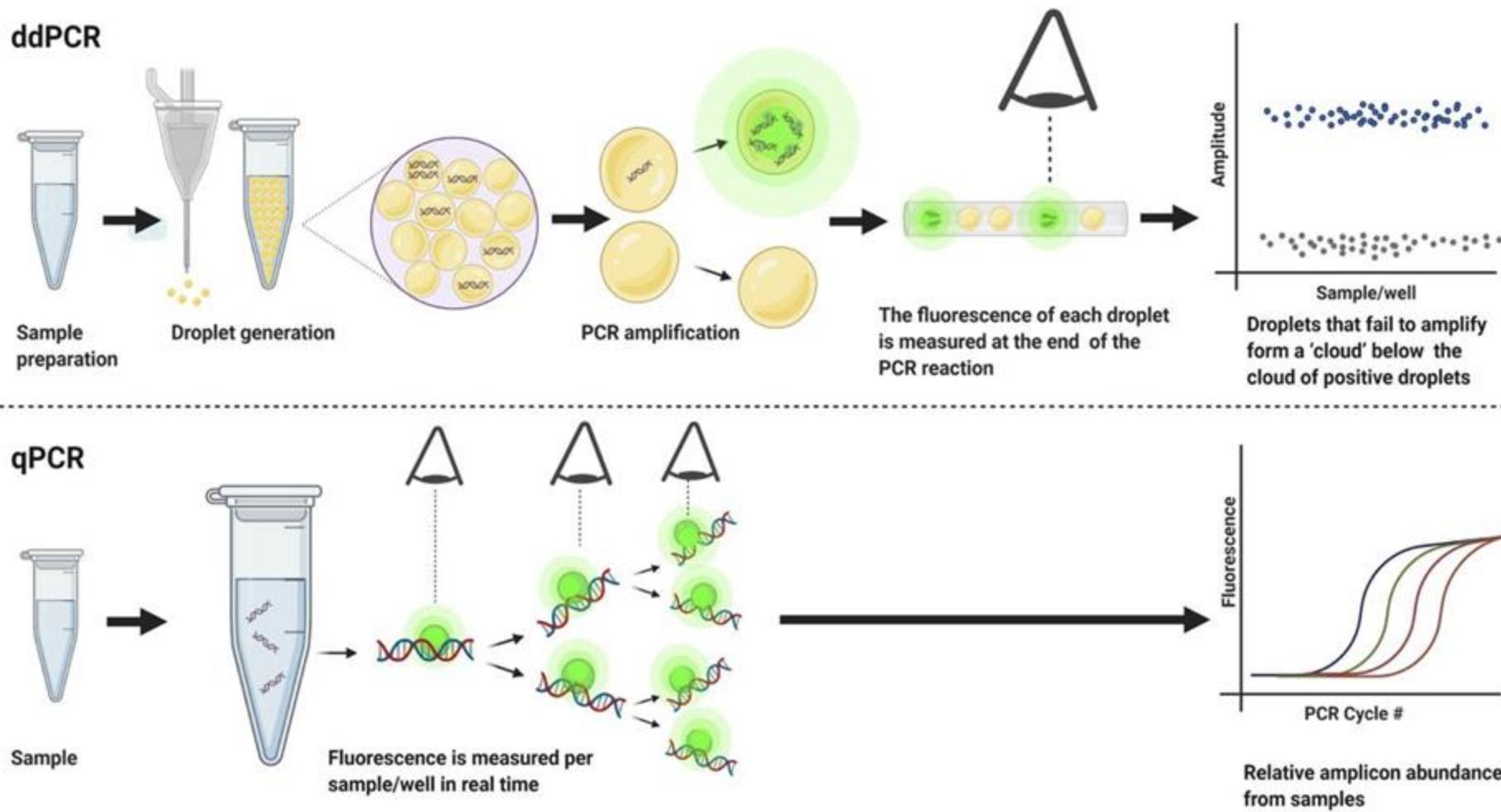
Ensayo de detección especie-específico



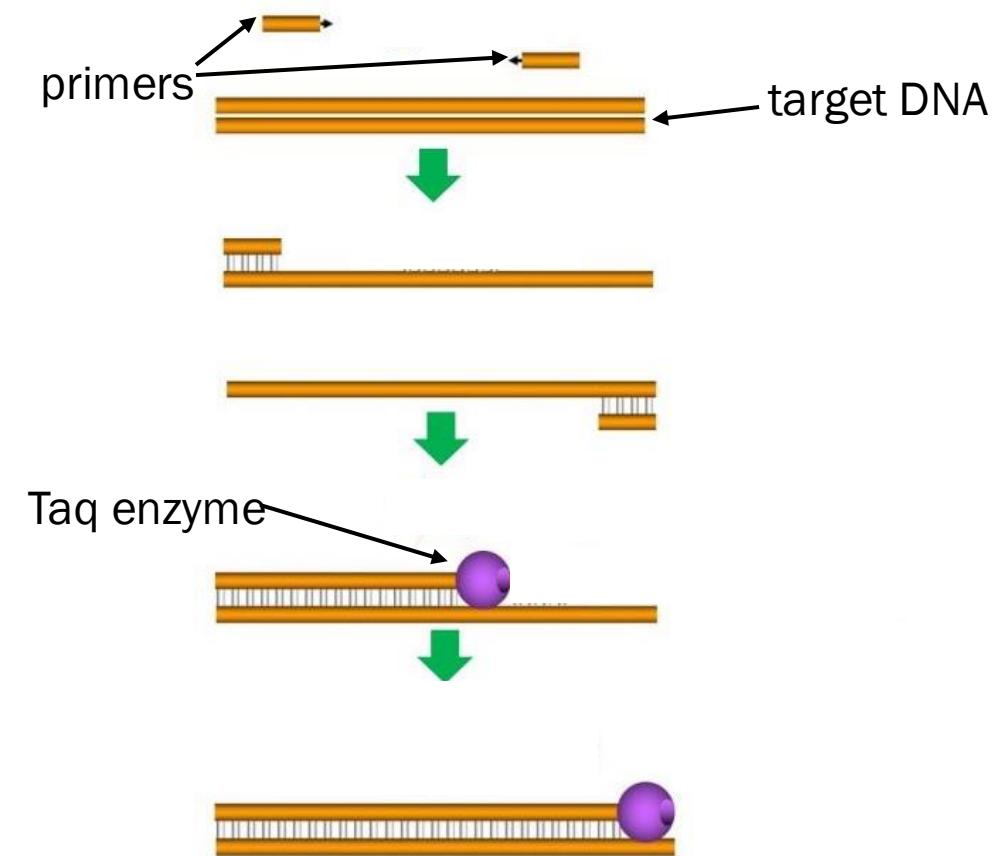
Detección especie-específica: Diseño y validación de un ensayo de detección



Detección especie-específica: dPCR o qPCR

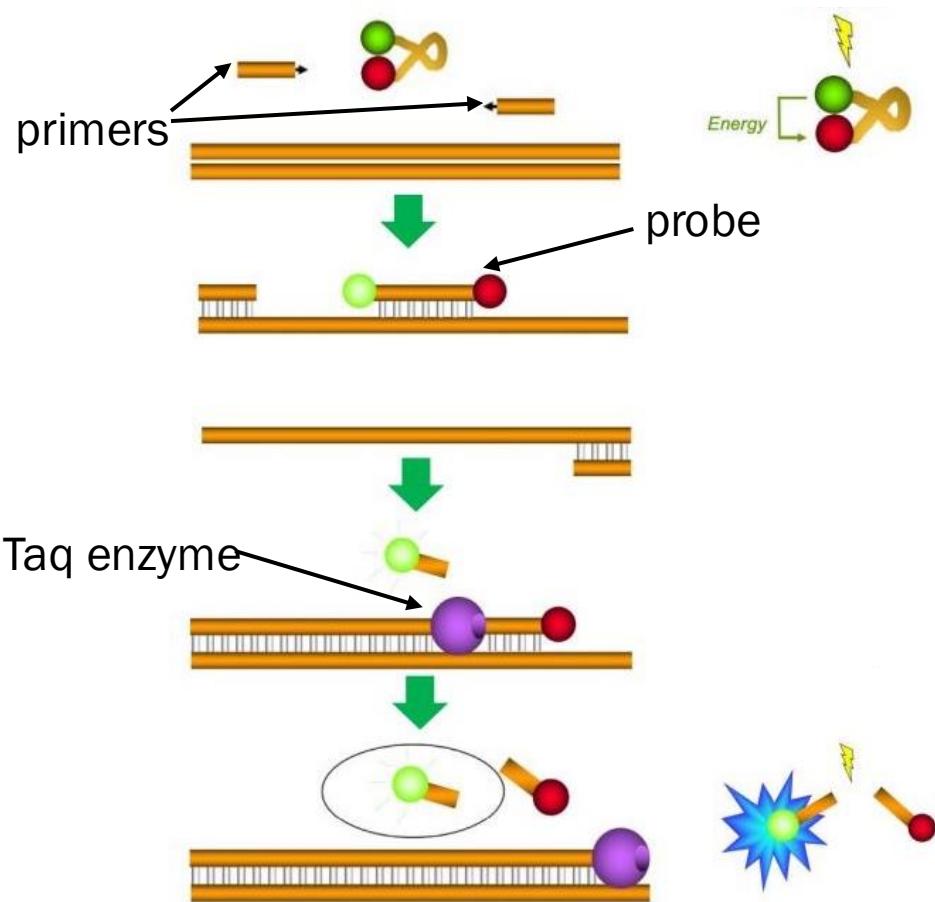


Crash course in qPCR



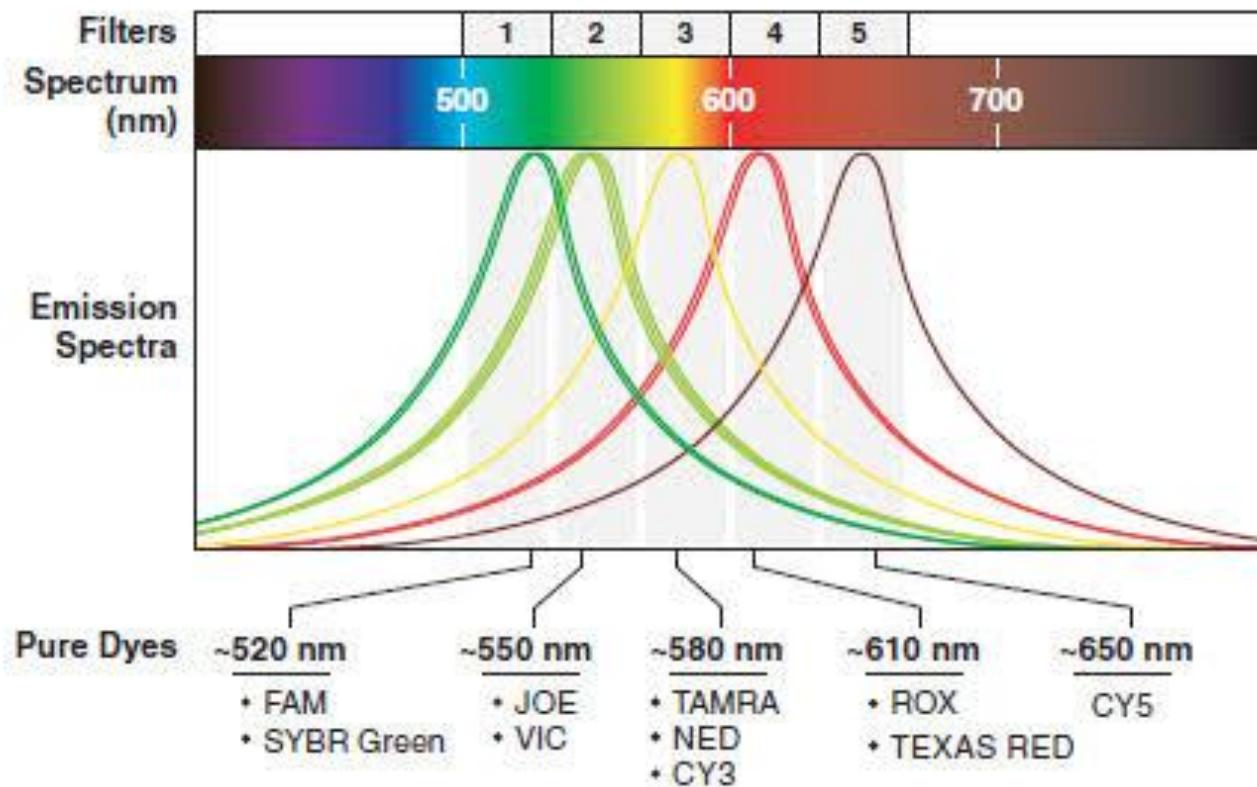
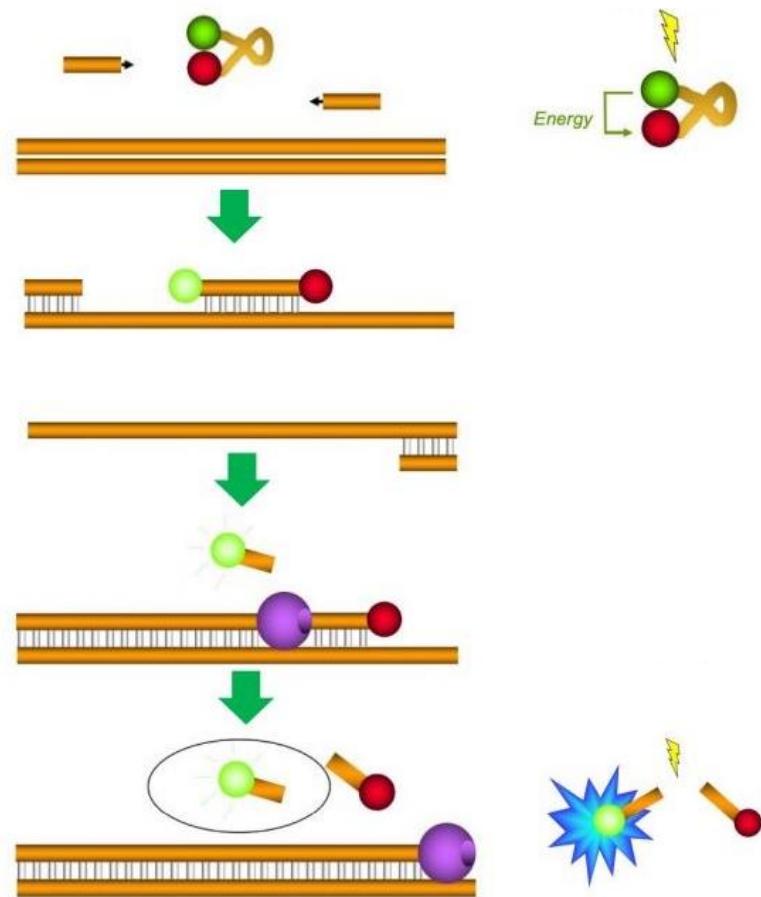
Crash course in qPCR

TaqMan®



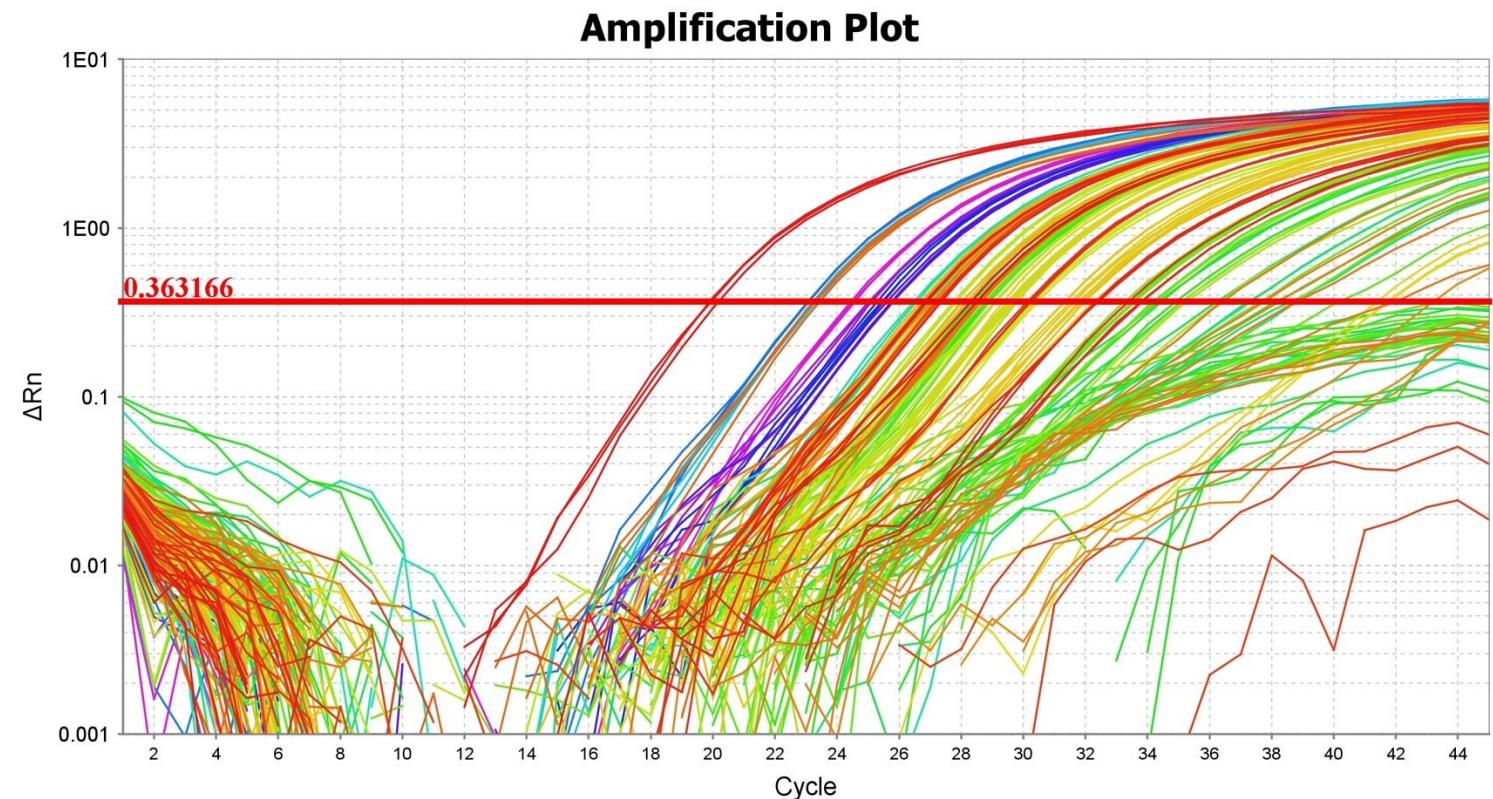
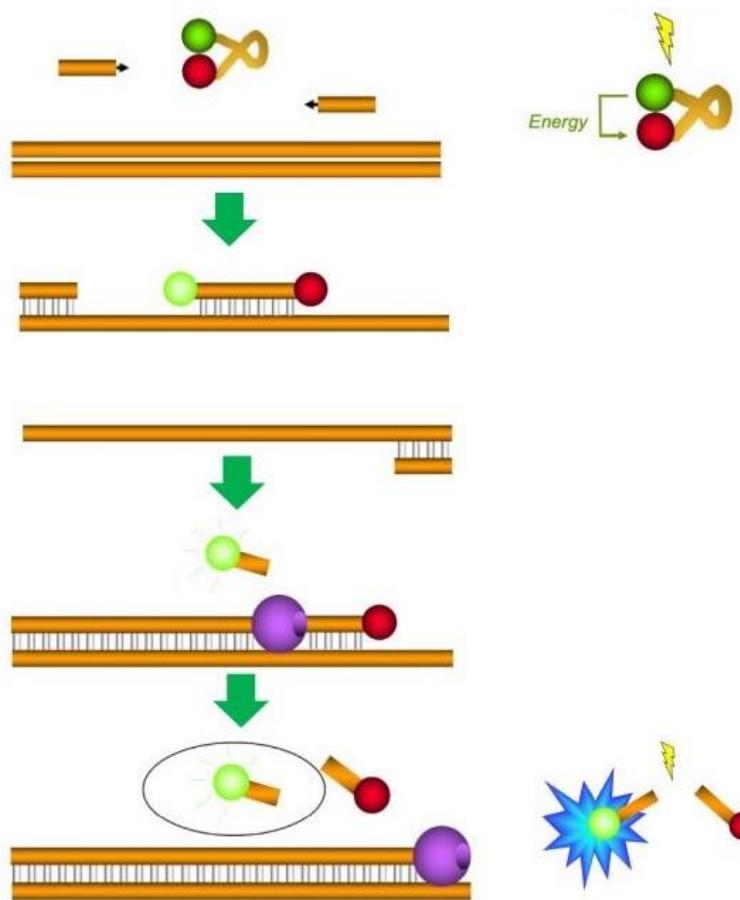
Crash course in qPCR

TaqMan®

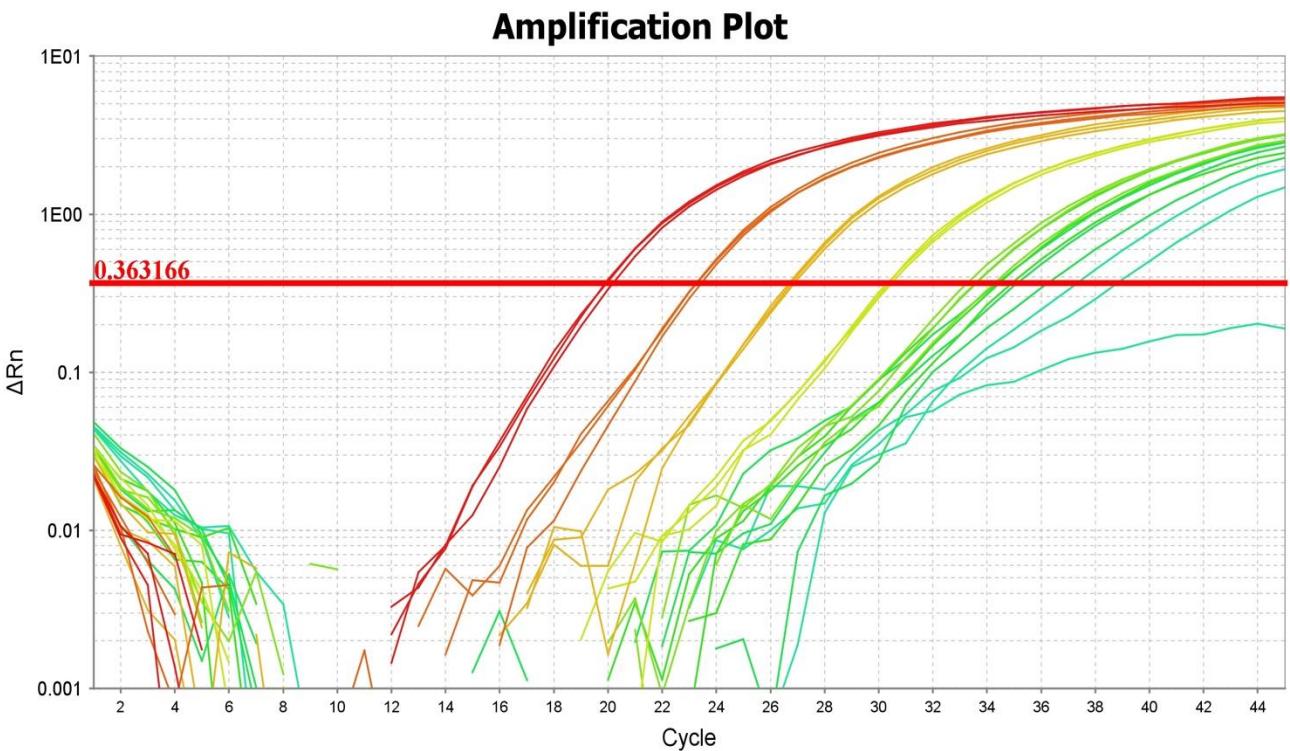


Crash course in qPCR

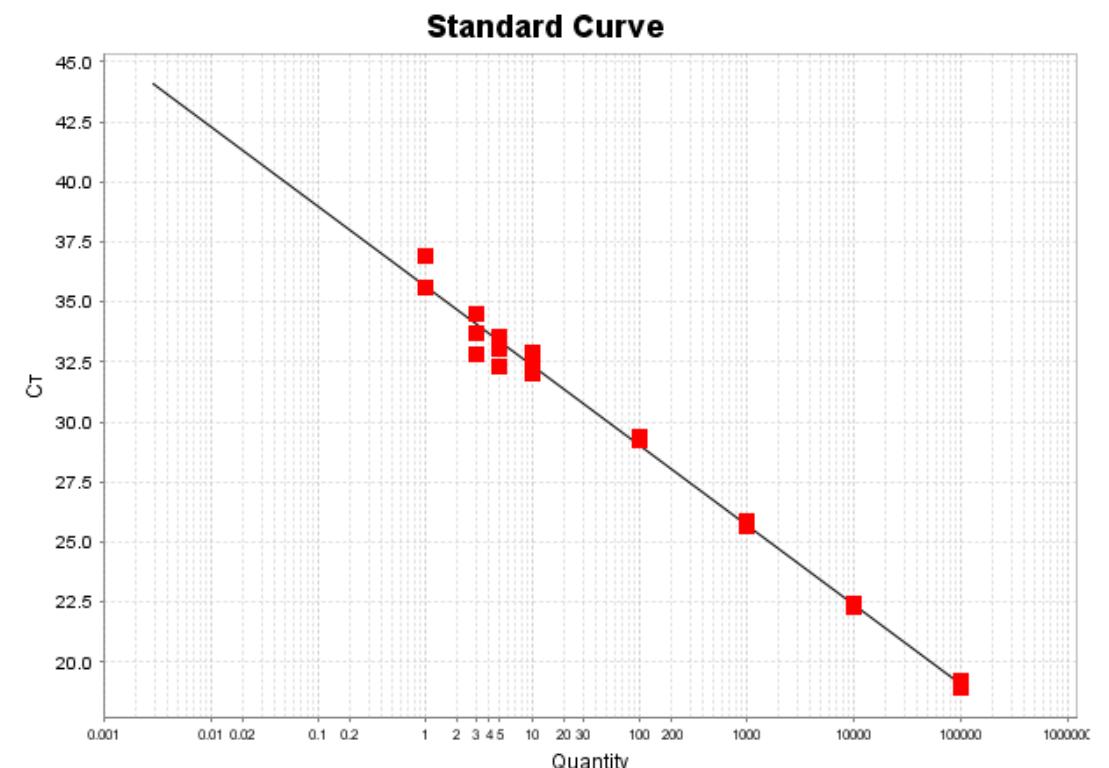
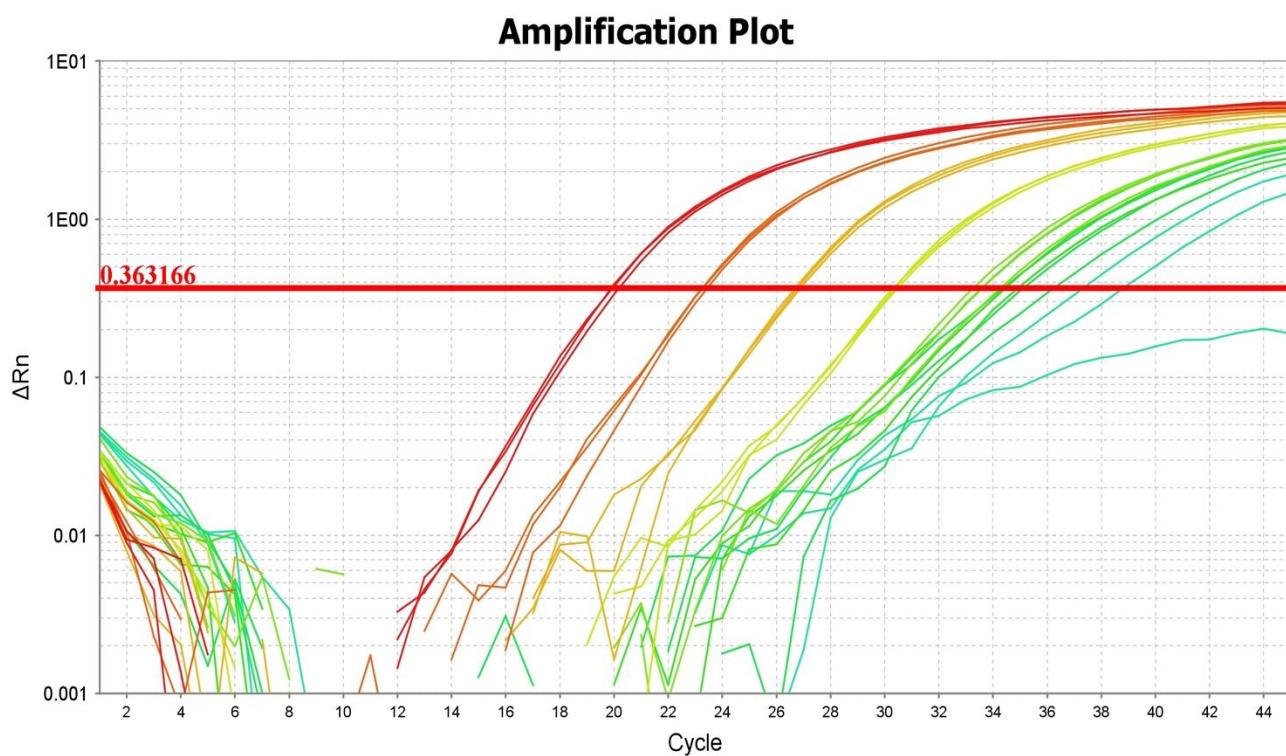
TaqMan®



Standard curve: Ct to [DNA]

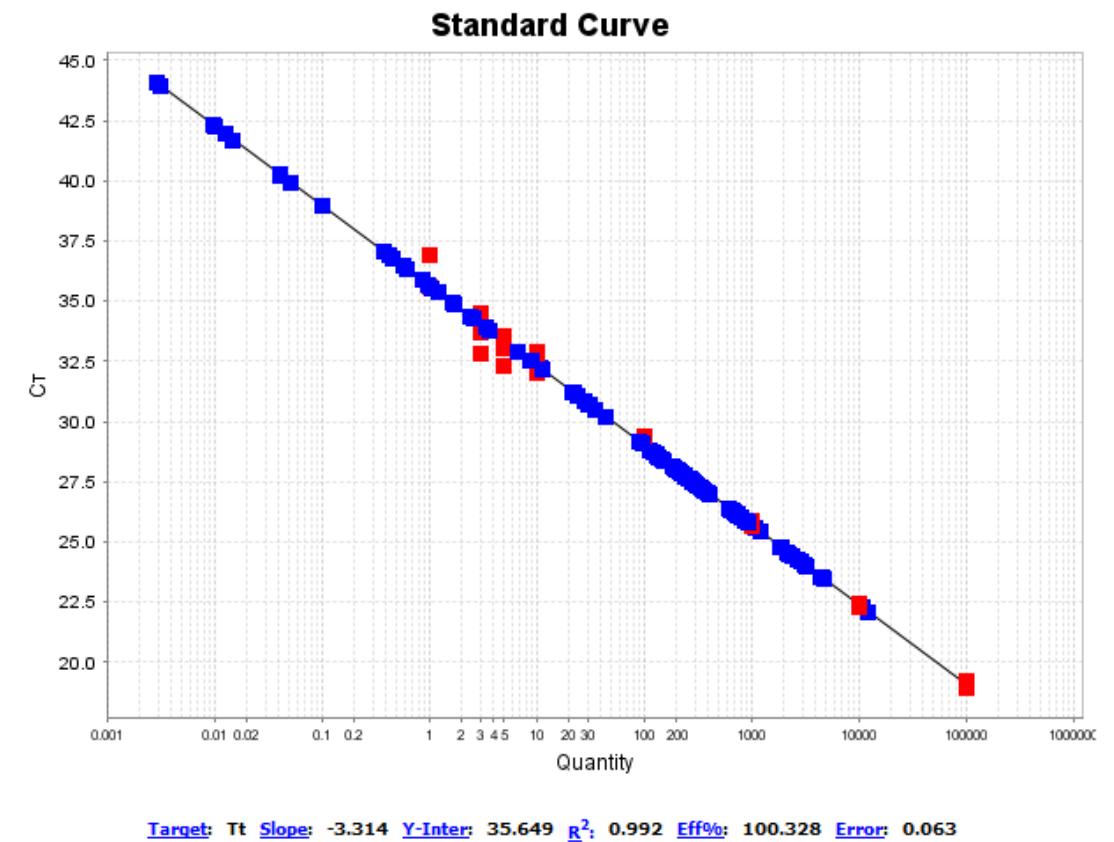
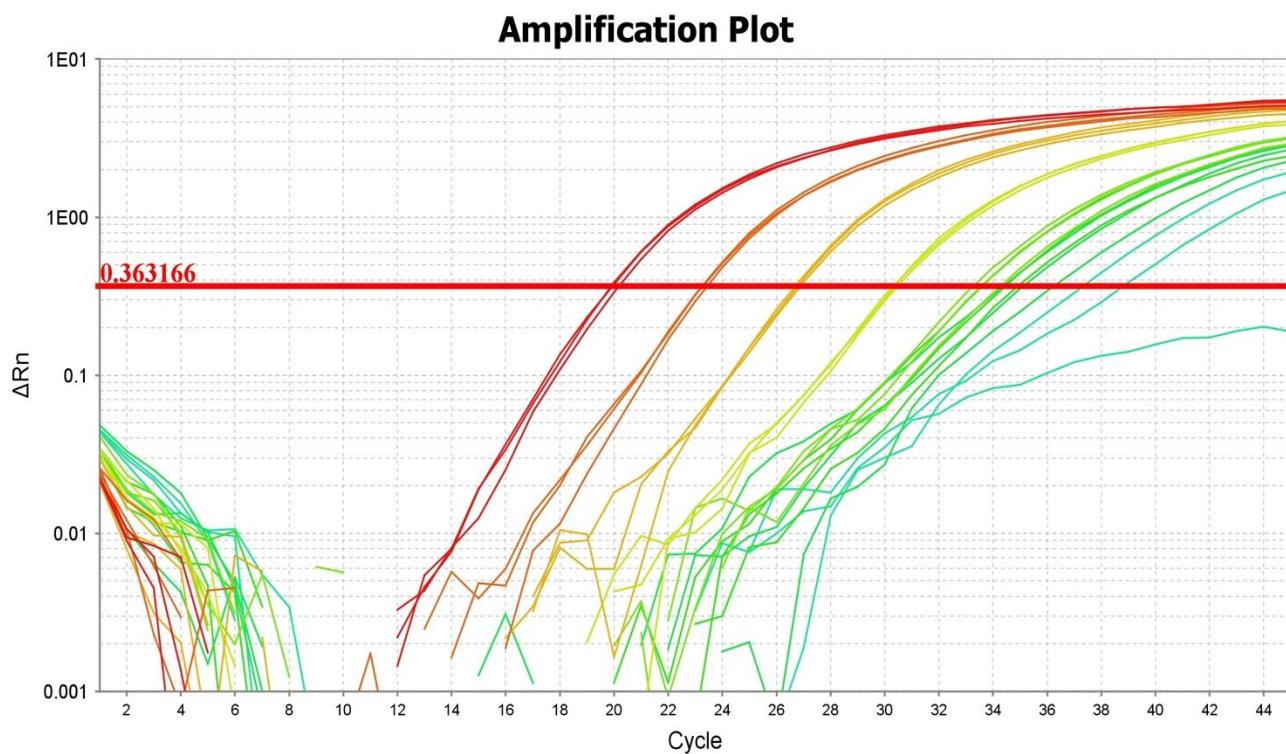


Standard curve: Ct to [DNA]

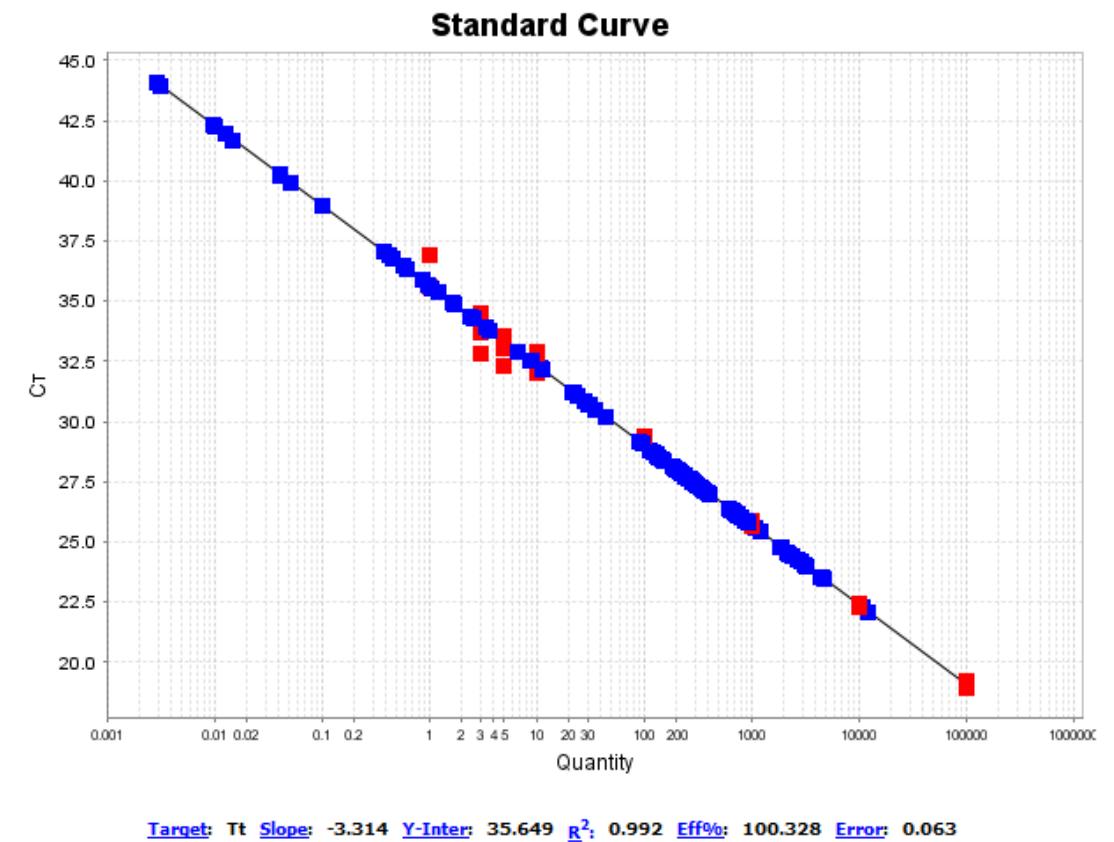
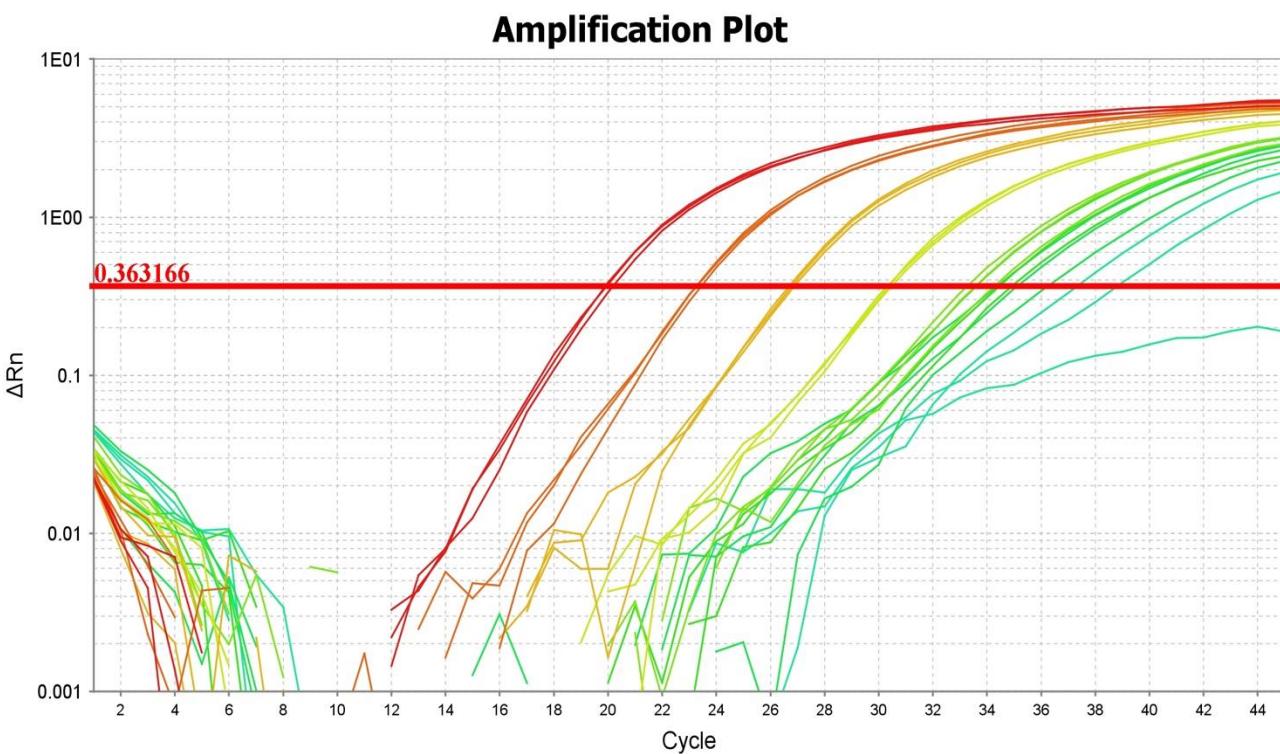


Target: Tt Slope: -3.314 Y-Inter: 35.649 R²: 0.992 Eff%: 100.328 Error: 0.063

Standard curve: Ct to [DNA]



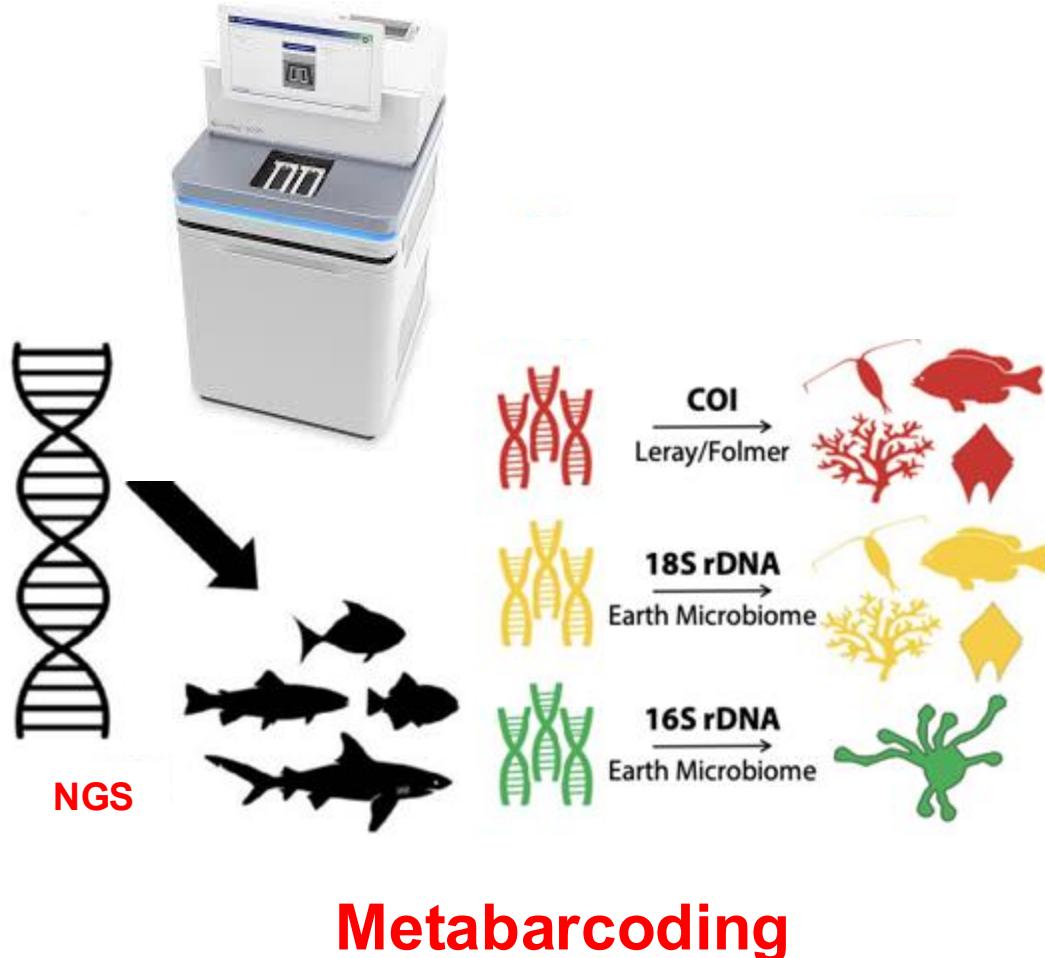
Standard curve: Ct to [DNA]



note: orders of magnitude

note: limit of detection (LOD)

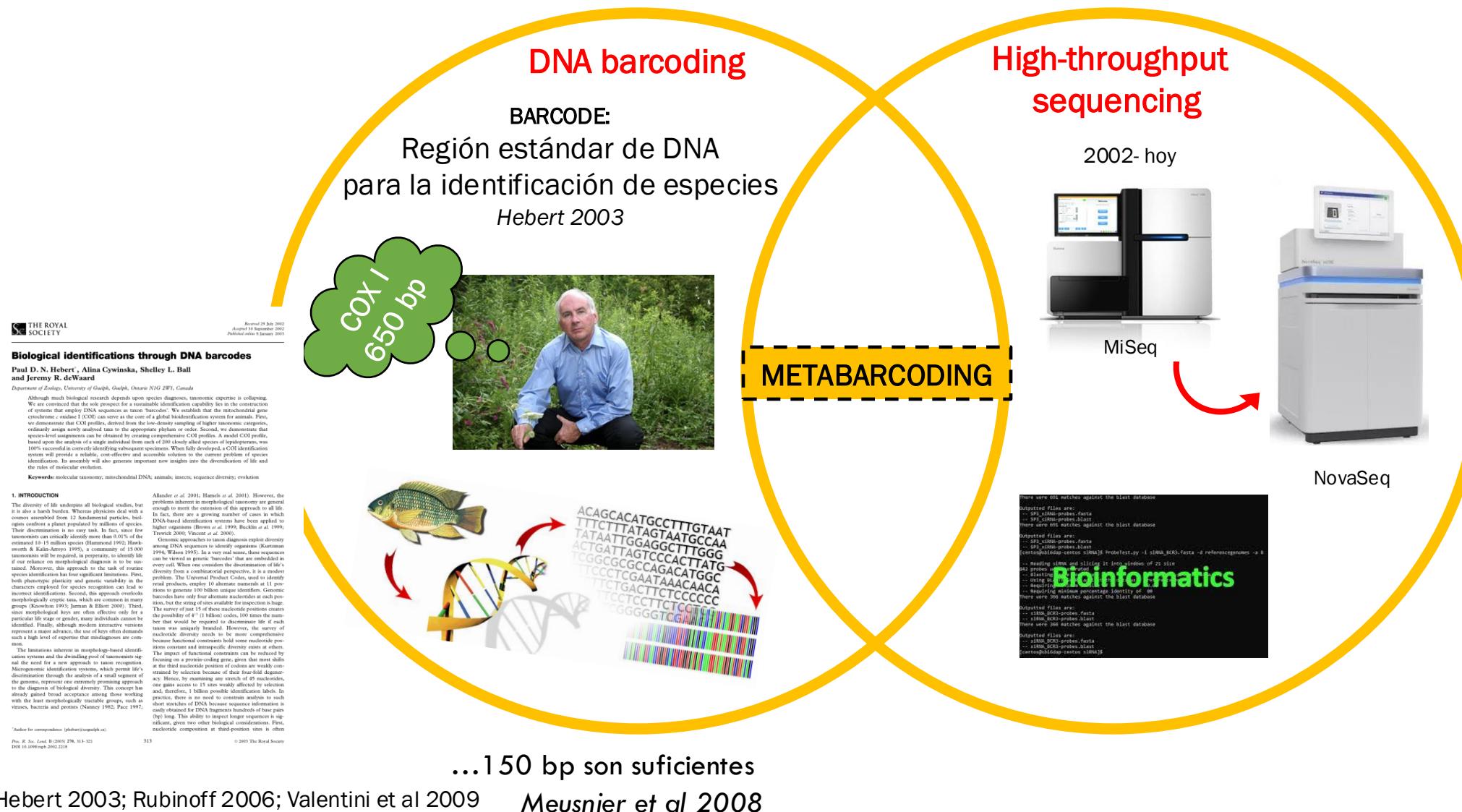
Métodos no dirigidos



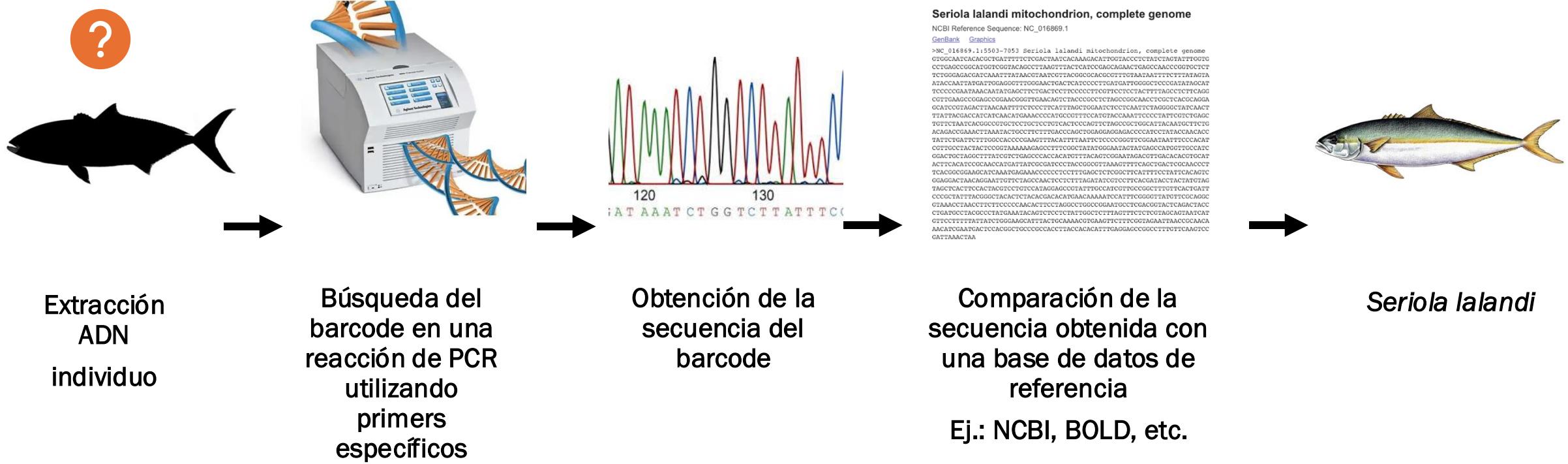
- ✓ Primers universals
- ✓ Alta sensibilidad
- ✓ Estimación de abundancia relativa
- ✓ Estimación de biodiversidad de comunidades

- ✓ Estudios de biodiversidad
- ✓ Análisis de dieta, contenido estomacal, heces

¿De dónde surge la técnica de metabarcoding de ADN?



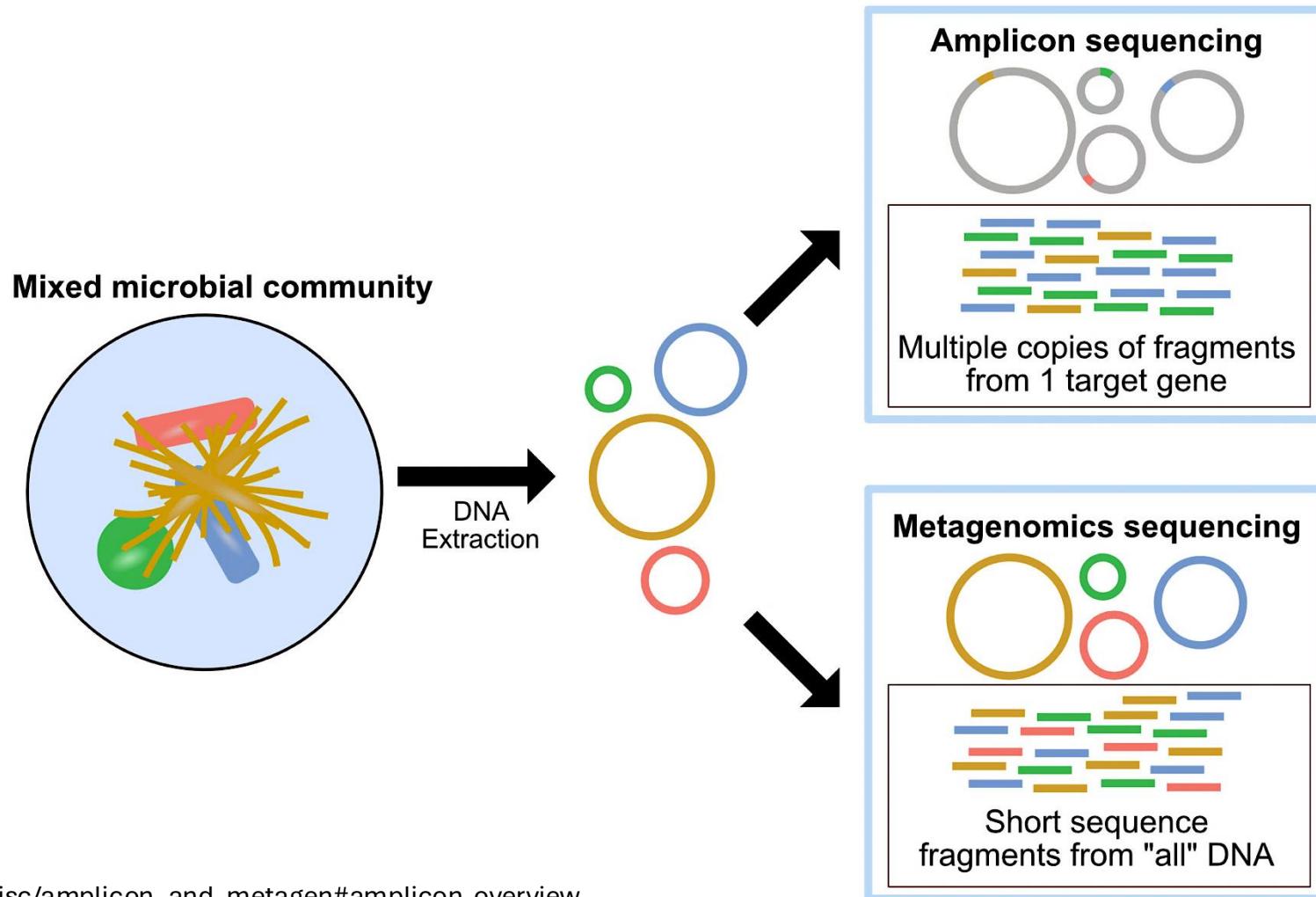
DNA barcoding



Detección de multiples especies: Metabarcoding



Metagenómica - Metabarcoding



A **metagenome** is a collection of genomes or genes from the members of a microbiota. A **microbiota** is an assemblage of microorganisms present in a defined environment. A **microbiome** refers to an entire habitat, including the microorganisms, their genomes, and the surrounding environmental conditions.

Marchesi and Ravel *Microbiome* (2015) 3:31
DOI 10.1186/s40168-015-0094-5



Open Access

EDITORIAL

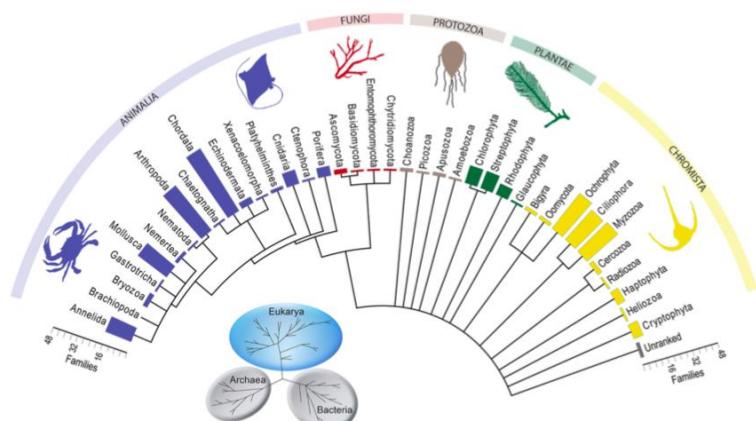
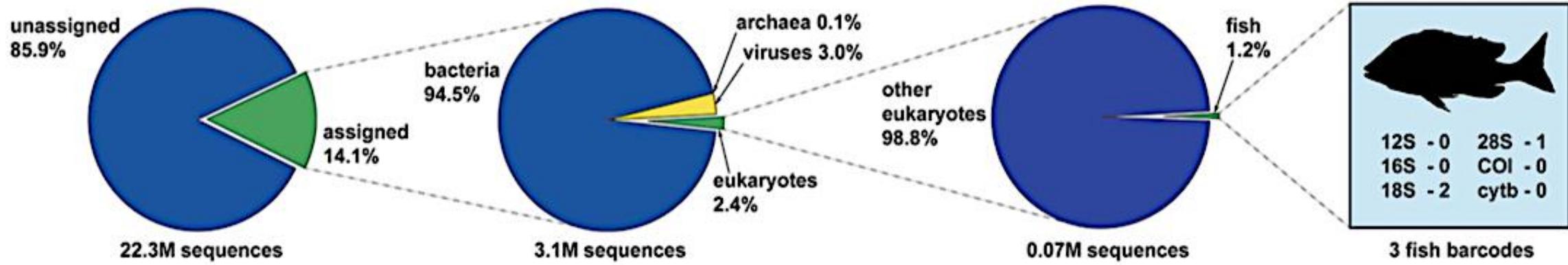
The vocabulary of microbiome research: a proposal



Julian R. Marchesi^{1,2} and Jacques Ravel^{3,4*}

Una aguja en un pajar

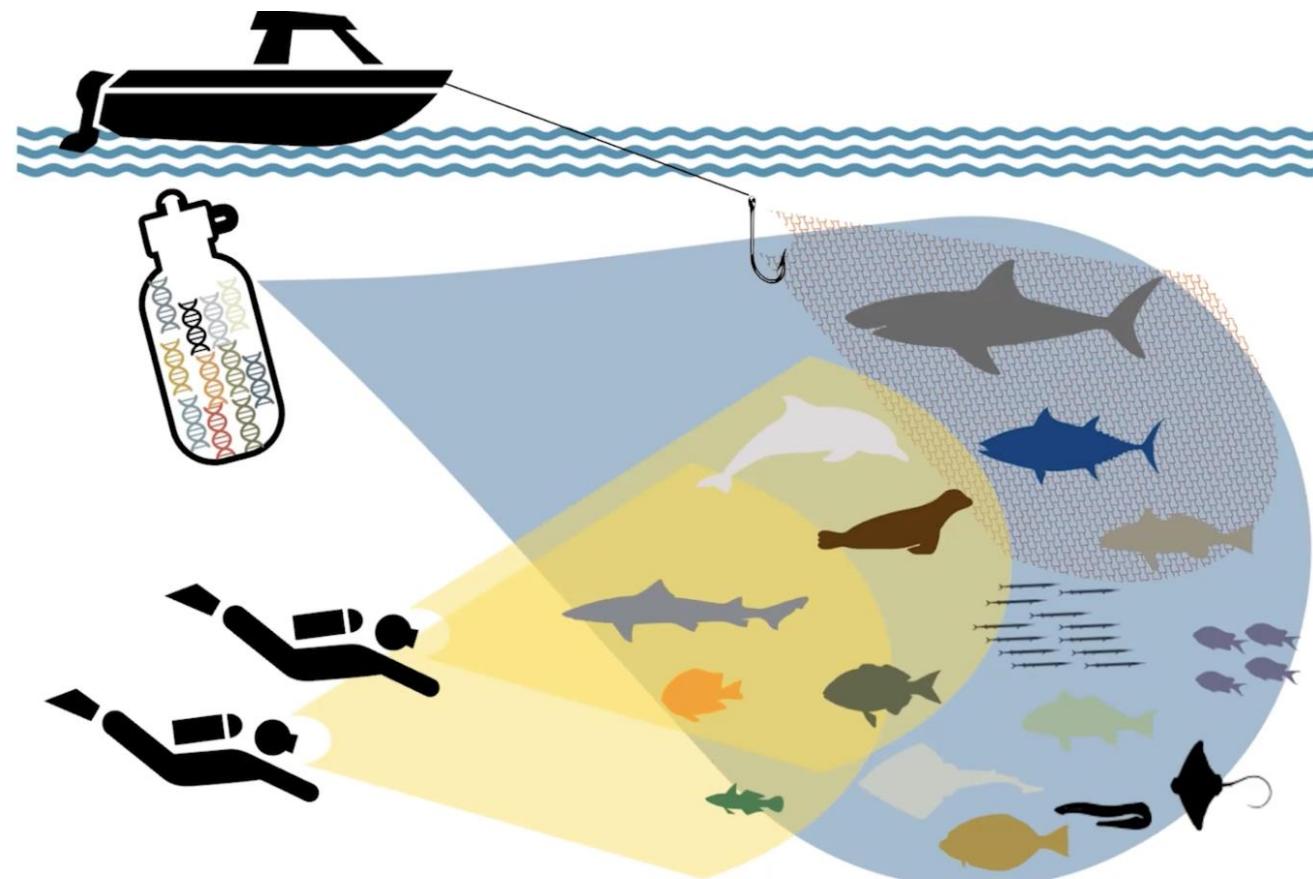
¿Por qué hacer una búsqueda dirigida en la muestra compleja?



- **Coral Bay, Australia**
 - 434 eukaryotic taxa: 38 phyla, 88 classes, 186 orders and 287 families

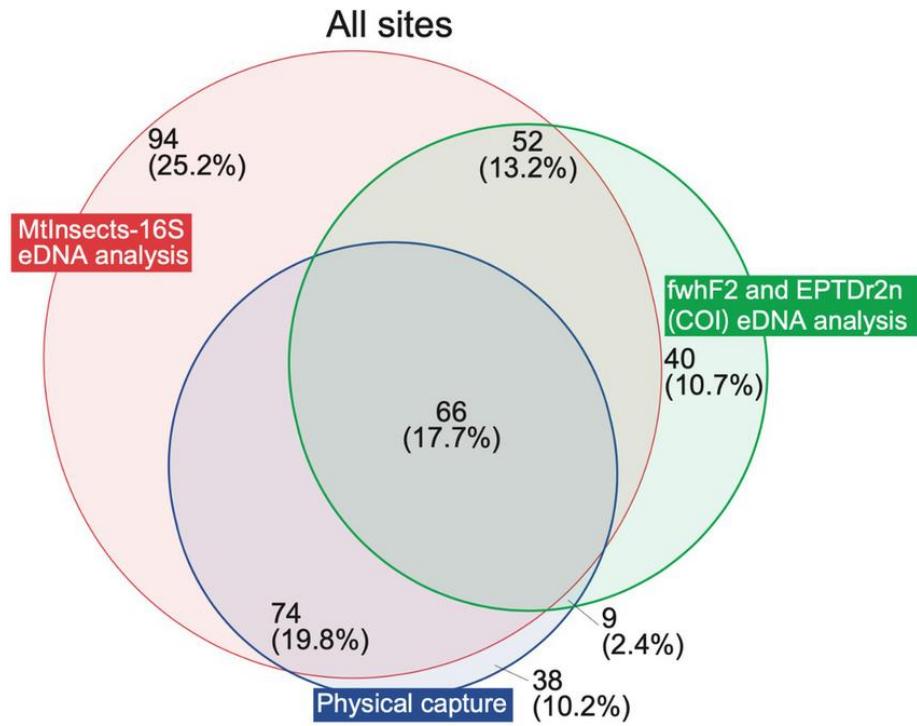
Stat et al 2017

eDNA como método de monitoreo

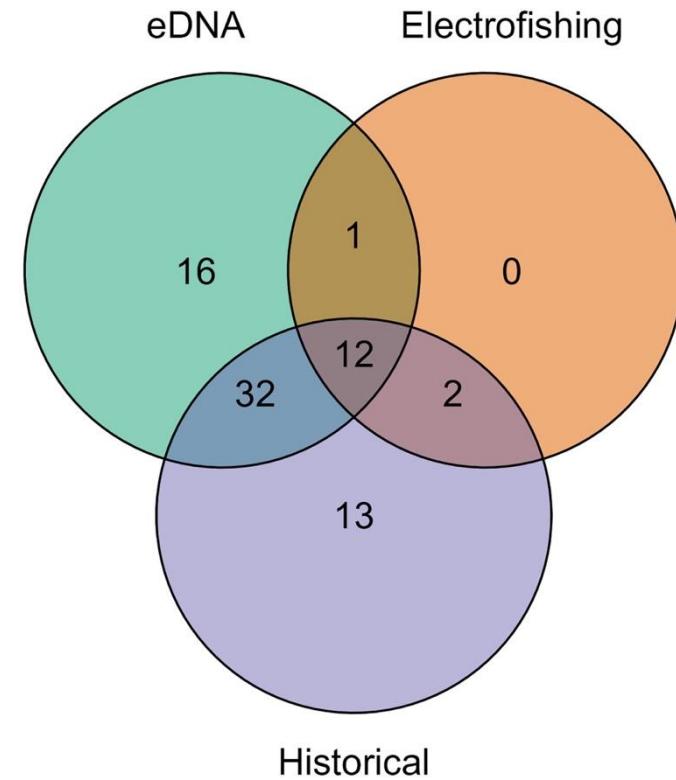


Figuia tomada de <https://oceandecadenortheastpacific.org/events/mobilizing-edna-for-management-in-the-northeast-pacific>

¿Qué método de biomonitordeo es mejor?



Lui et al. 2024



Morey et al. 2024

TABLE 1 Representative studies comparing richness estimates with traditional sampling or historical data for a geographic location to that of eDNA metabarcoding

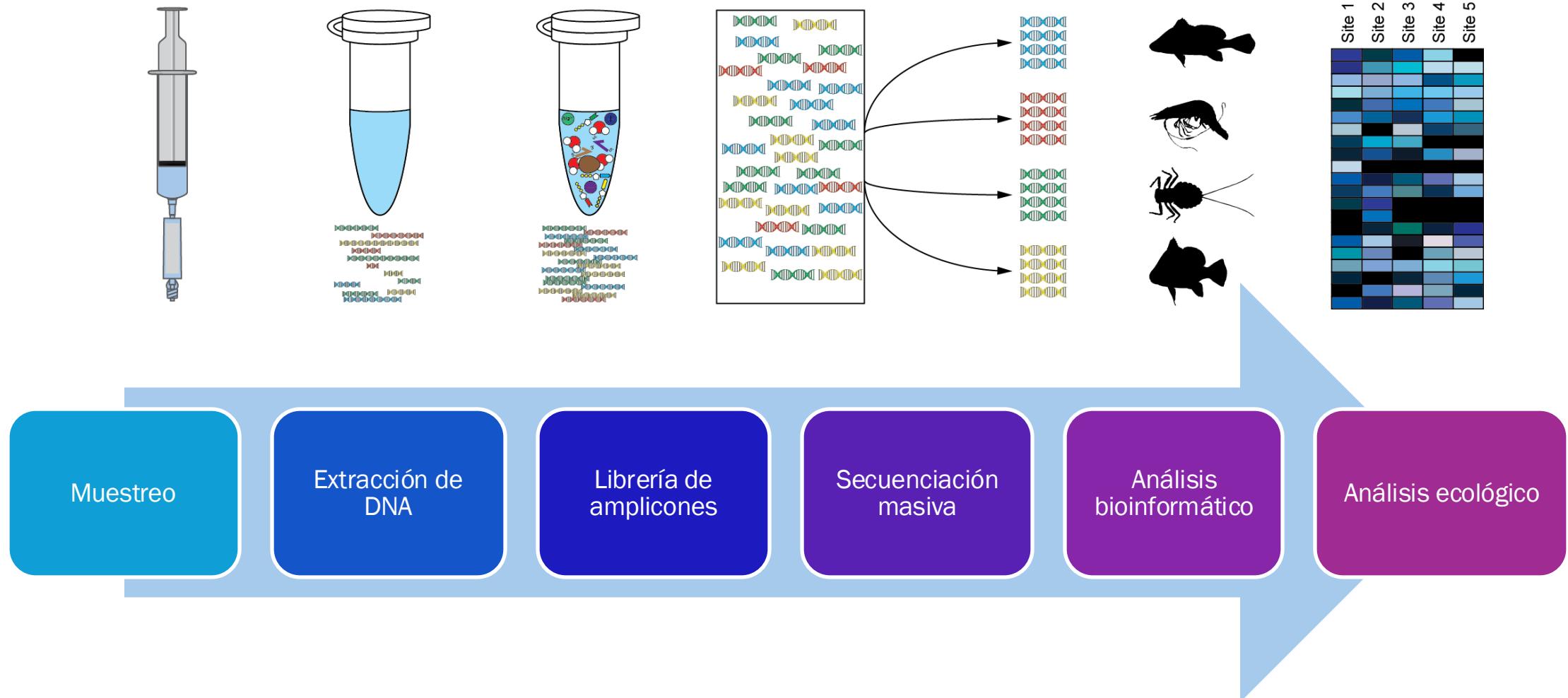
Habitat	Macro-organism taxonomic focus	eDNA sample type	Traditional sampling method	eDNA efficacy finding ^a	Authors, Year
Air	Plants	Air pollen trap	Morphological identification	Better taxonomic resolution	Kraaijeveld et al. (2015)
Freshwater	Fish	Flowing water	Depletion-based electrofishing	Higher diversity	Olds et al. (2016)
Freshwater	Invertebrates	Flowing water	Kicknet in stream and historical data	Higher diversity	Deiner et al. (2016)
Freshwater	Fish	Stagnant water	Gillnet, trapping, hydroacoustics, analysis of recreational anglers' catches	Complementary	Häneling et al. (2016)
Freshwater	Reptiles, amphibians	Stagnant water	Species distribution model based on historical data (i.e., distribution range and habitat type)	Increase species distribution knowledge	Lacoursière-Roussel, Dubois, Normandeau & Bernatchez, (2016)
Freshwater	Amphibians, fish	Stagnant water; flowing water	Amphibians: visual encounter survey, mesh hand-net; fish: electrofishing, and/or netting protocols (fyke, seine, gill)	Greater detection probability	Valentini et al. (2016)
Freshwater	Amphibians, fish, mammals, invertebrates	Stagnant water; flowing water	Active dip-netting, fresh tracks or scat, electrofishing with active dip-netting	Complementary	Thomsen, Kielgast, Iversen, Wiuf et al. (2012)
Freshwater	Fish	Stagnant water; flowing water; surface sediment	Fyke net	Higher diversity	Shaw et al. (2016)
Freshwater	Invertebrates	Water column; surface sediment	Sediment collected using a Van Veen grab	Higher diversity	Gardham, Hose, Stephenson, and Charlton (2014)
Freshwater	Fish/Diptera	Surface and bottom water column	Long-term data, electrofishing (fish) and emerging traps (Diptera) at the time of eDNA sampling	Higher diversity compared to sampling but lower diversity compared to long-term data	Lim et al. (2016)
Marine	Fish	Surface and bottom water column	Long-term observation	Complementary	Yamamoto et al. (2017)
Marine	Fish	Bottom water column	Trawl catch data	Similar family richness	Thomsen et al. (2016)
Marine	Fish	Water column	Scuba diving	Higher diversity	Port et al. (2015)
Terrestrial	Plants	Honey	Melissopalynology (i.e., pollen grains retrieved from honey are identified morphologically)	Complementary	Hawkins et al. (2015)
Terrestrial	Mammals, plants	Midden pellets	Historical surveys	Higher diversity	Murray et al. (2012)
Terrestrial	Mammals	Saliva	Local knowledge (i.e., physical evidence) and camera data	Complementary	Hopken et al. (2016)
Terrestrial	Birds, invertebrates, plants	Top soil	Invertebrates: leaf litter samples and pitfall traps; reptiles: pitfall traps and under artificial ground covers; birds: distance sampling method; plants: above-ground surveys	Complementary for plants and invertebrates	Drummond et al. (2015)
Terrestrial	Earthworms	Top soil	Irrigated quadrats with 10 L of allyl isothiocyanate solution and hand-collected emerging worms	Complementary	Pansu et al. (2015)
Terrestrial	Plants	Top soil	Historical surveys	Complementary	Jørgensen et al. (2012)
Terrestrial	Plants	Top soil	Above-ground surveys	Complementary and better taxonomic resolution	Yoccoz et al. (2012)
Terrestrial	Vertebrates	Top soil	Local knowledge from safari parks, zoological gardens and farms; visual observations; historical surveys	Complementary	Andersen et al. (2012)

^aComplementary means the two survey methods detected different diversity, but does not exclude that some of the diversity was detected by both methods. Higher diversity means the study found more diversity was detected compared to conventional, but does not exclude that some of the diversity was not detected by both methods. Better taxonomic resolution means that sequence-based identifications could be resolved to a lower taxonomic rank compared with the conventional method.

¿Qué método de biomonitordeo es mejor?

Metodología

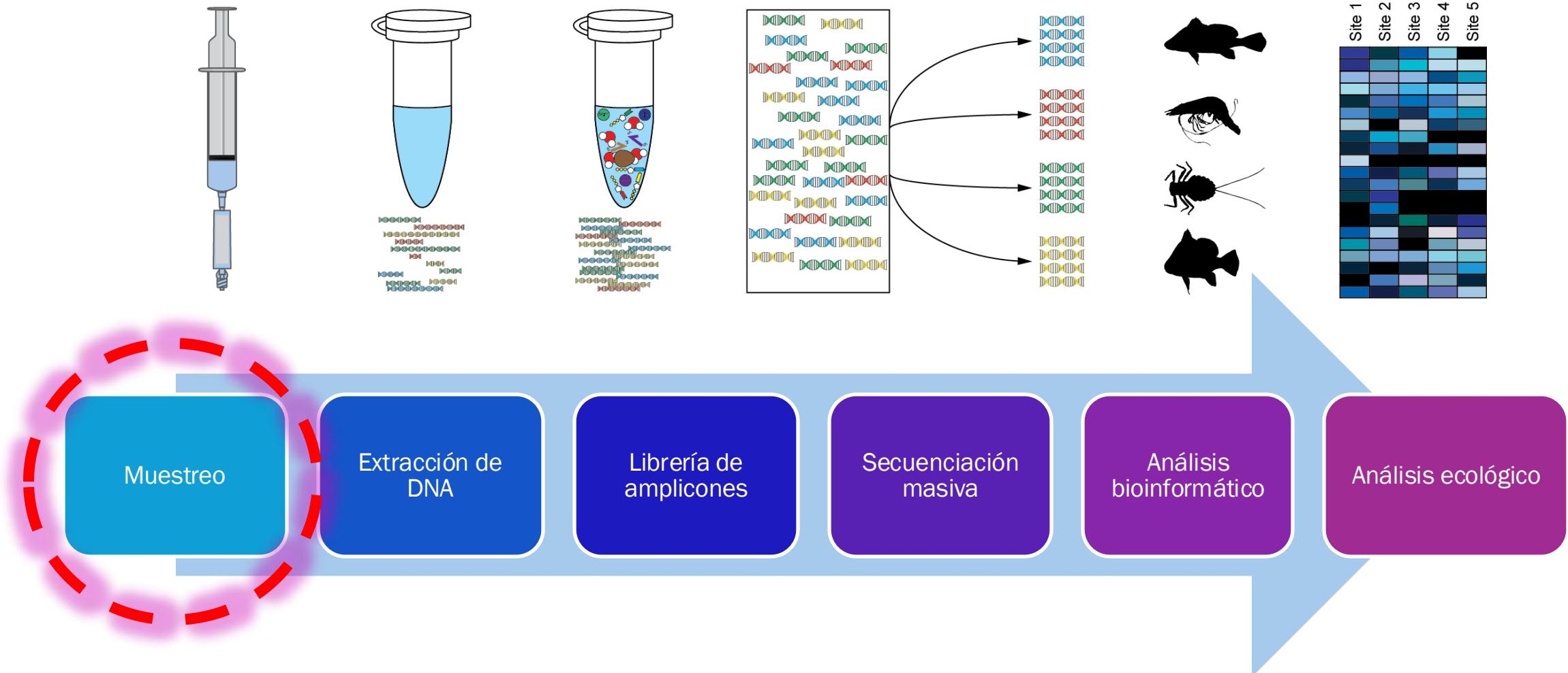
Metabarcoding: flujo de trabajo general



Diseño de estrategia

- Preguntas de investigación: qué grupo taxonómico, cambios en el tiempo o espacio
- Necesitas resultados cuantitativos de una especie? O presencia/ausencia de múltiples especies

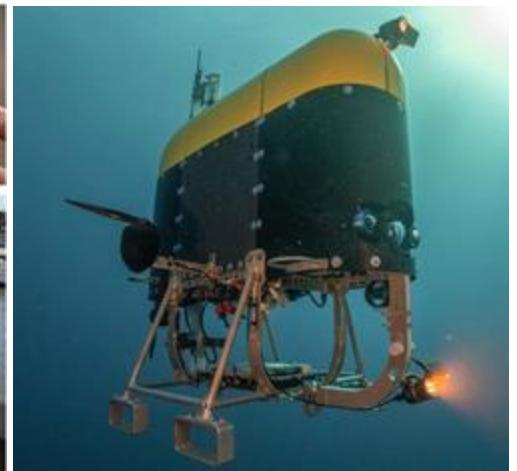
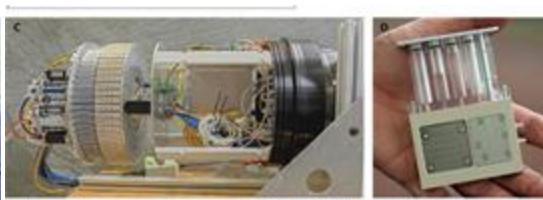
Metabarcoding: flujo de trabajo general



Muestreo

fácil

difícil



Yamahara et al. 2019

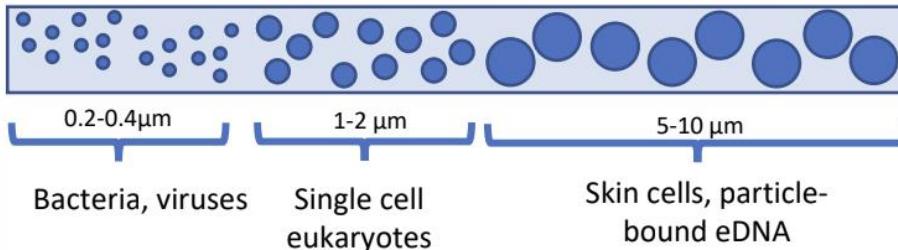
Muestreo

DNA collection methods



DNA Filter

Pore size:



Material: cellulose nitrate, glass fiber filters, polyethersulfone, mixed cellulose esters, polycarbonate

Filtration: vacuum, gravity, peristaltic, hand pump/manual, passive sampling

Preservative: Ethanol, RNAlater, silica gel, Longmire's buffer or lysis buffer, self-preserving filters, freezing

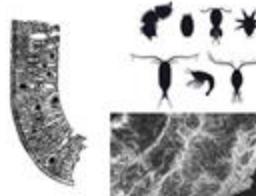
Filtración: concentración de ADN en membranas



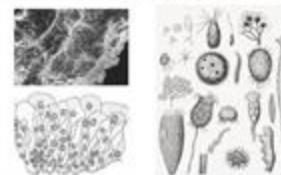
11/9/2025
Bruce et al. 2021

Physical components of eDNA sample

Whole organisms, tissue and biofilm aggregates



Smaller tissue and biofilm aggregates, cells, large organelles



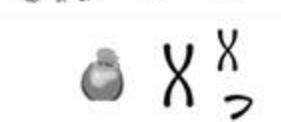
Eukaryotic and microbial cells, organelles, chromosomes



Microbial cells, organelles, cellular debris, chromosomes and fragments



Cellular debris, chromosomes and fragments



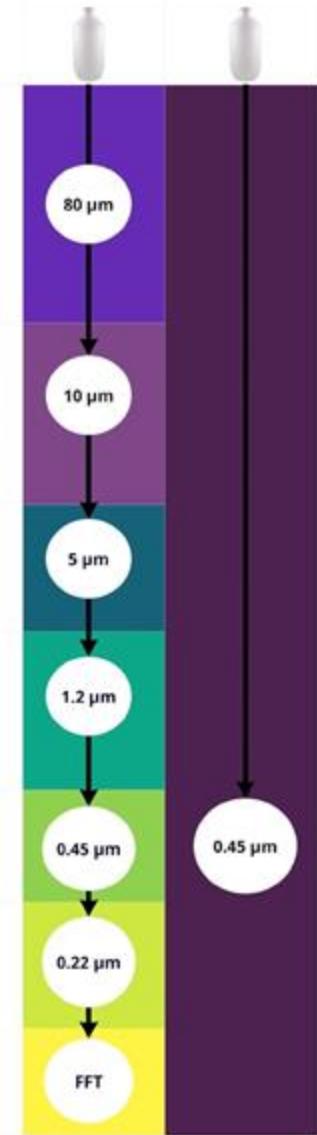
Cellular debris, fragmented chromosomes and extracellular DNA



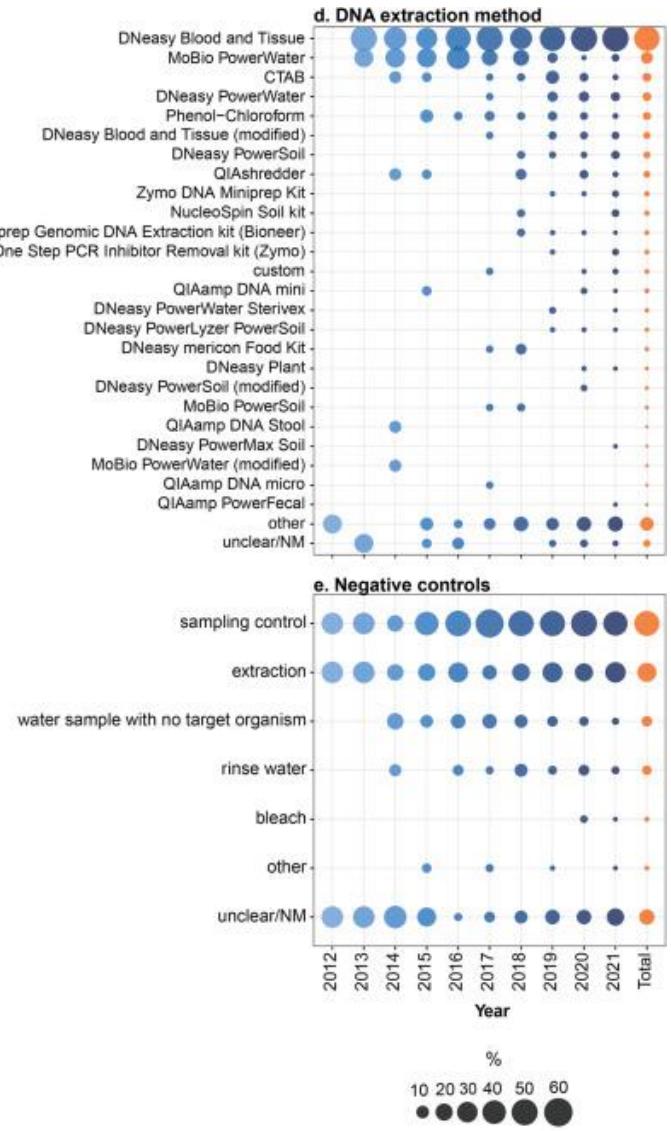
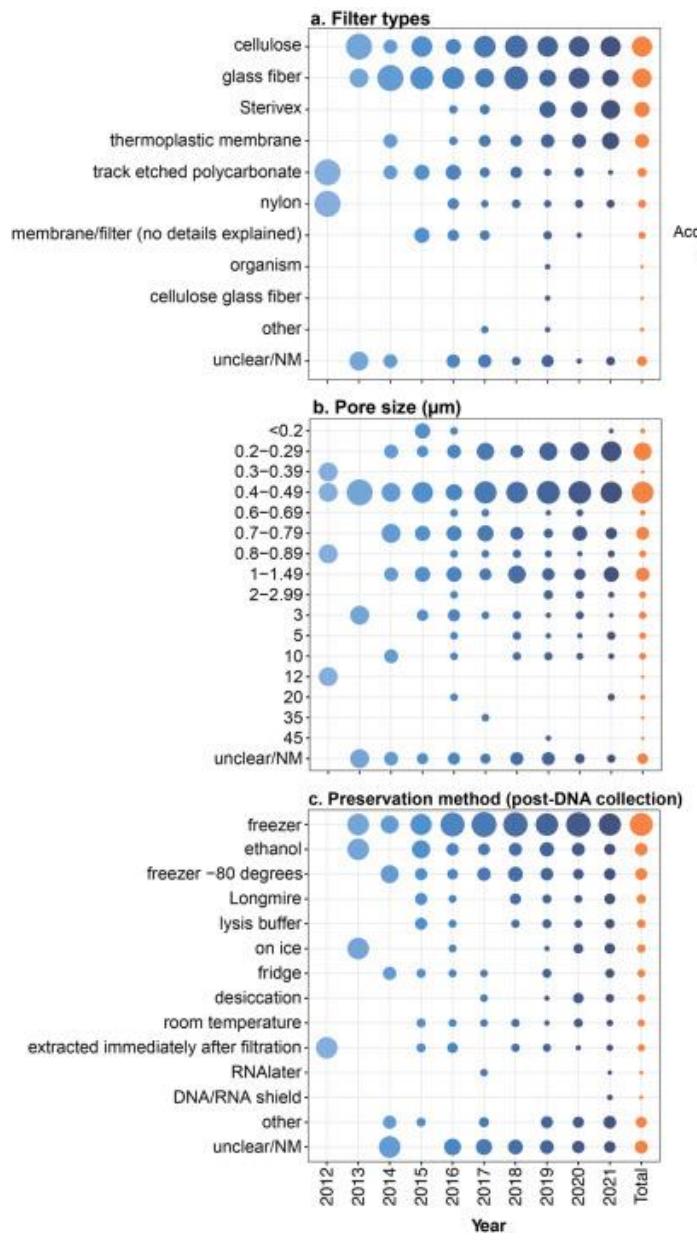
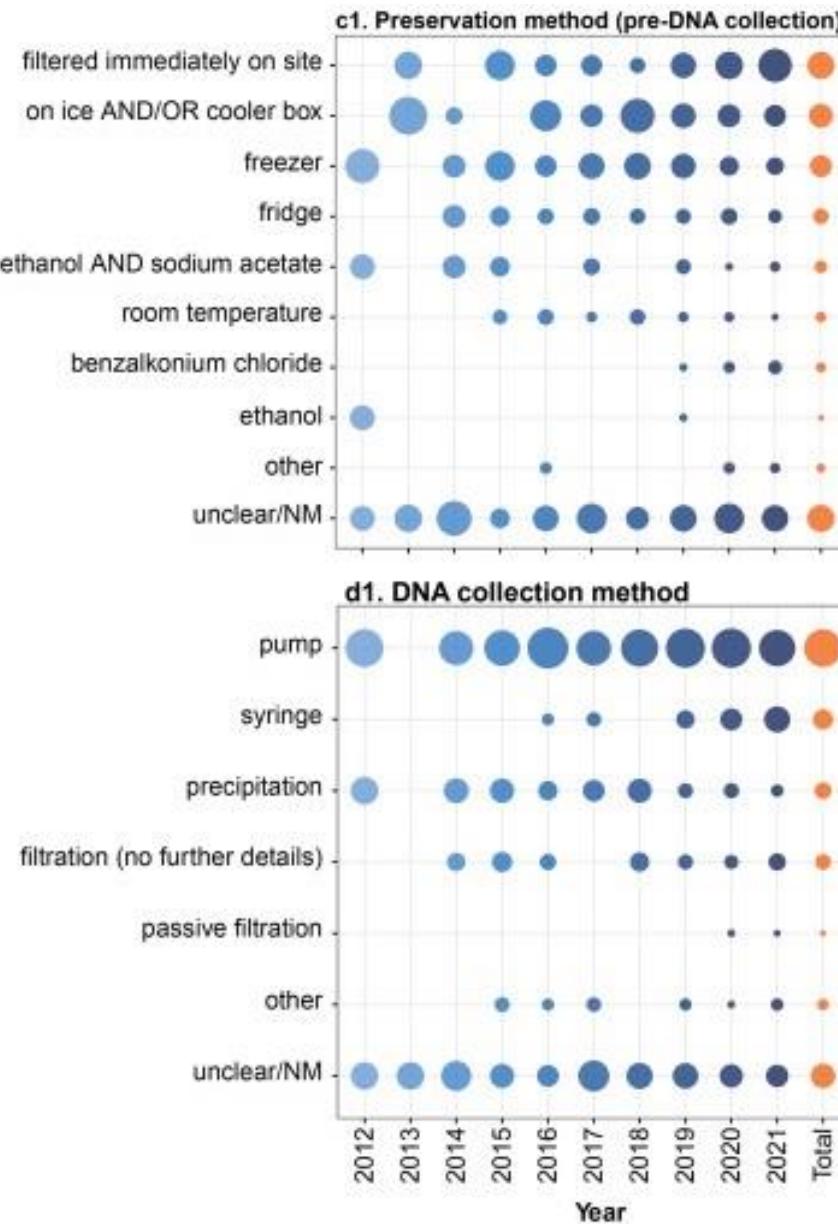
Extracellular DNA



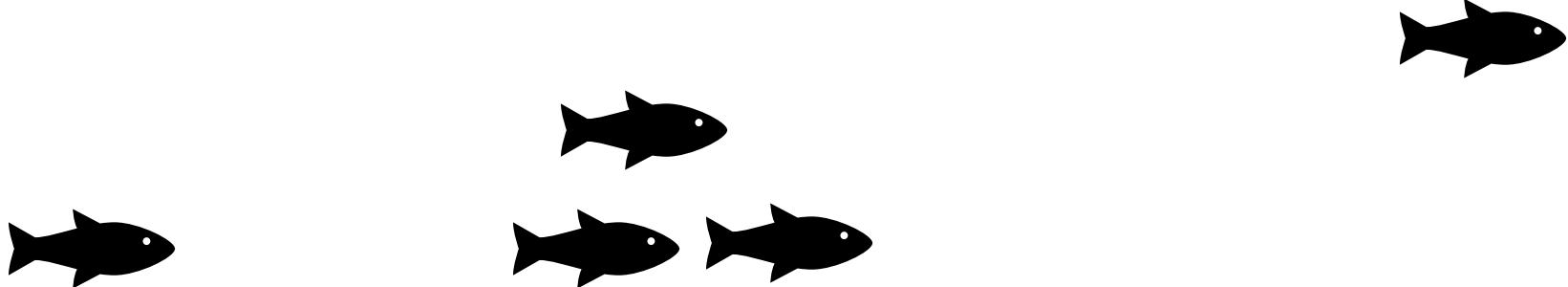
Serial filtering Standard eDNA sampling



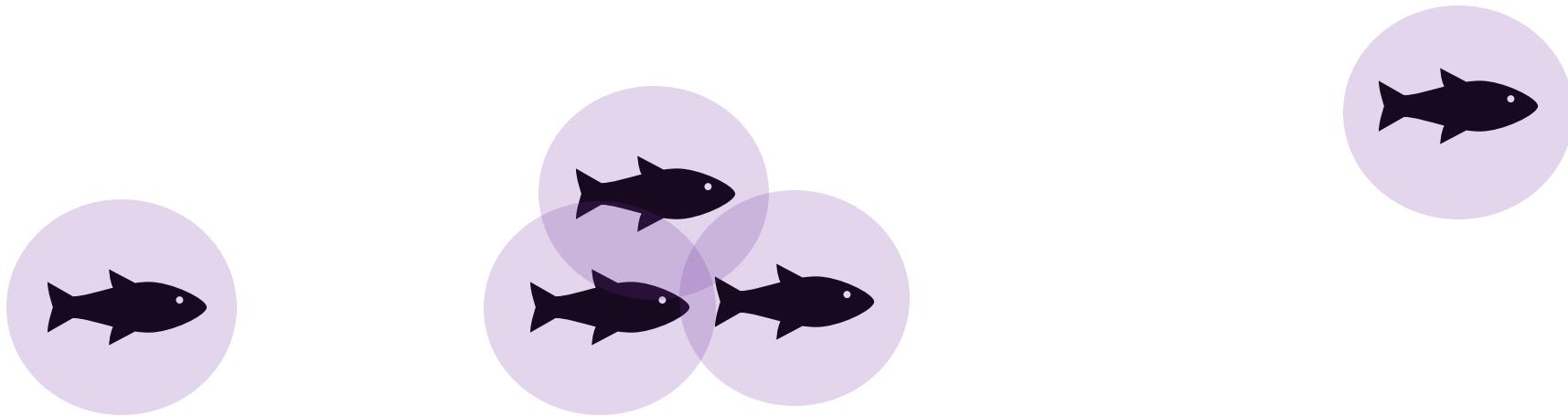
67 Power et al. 2023



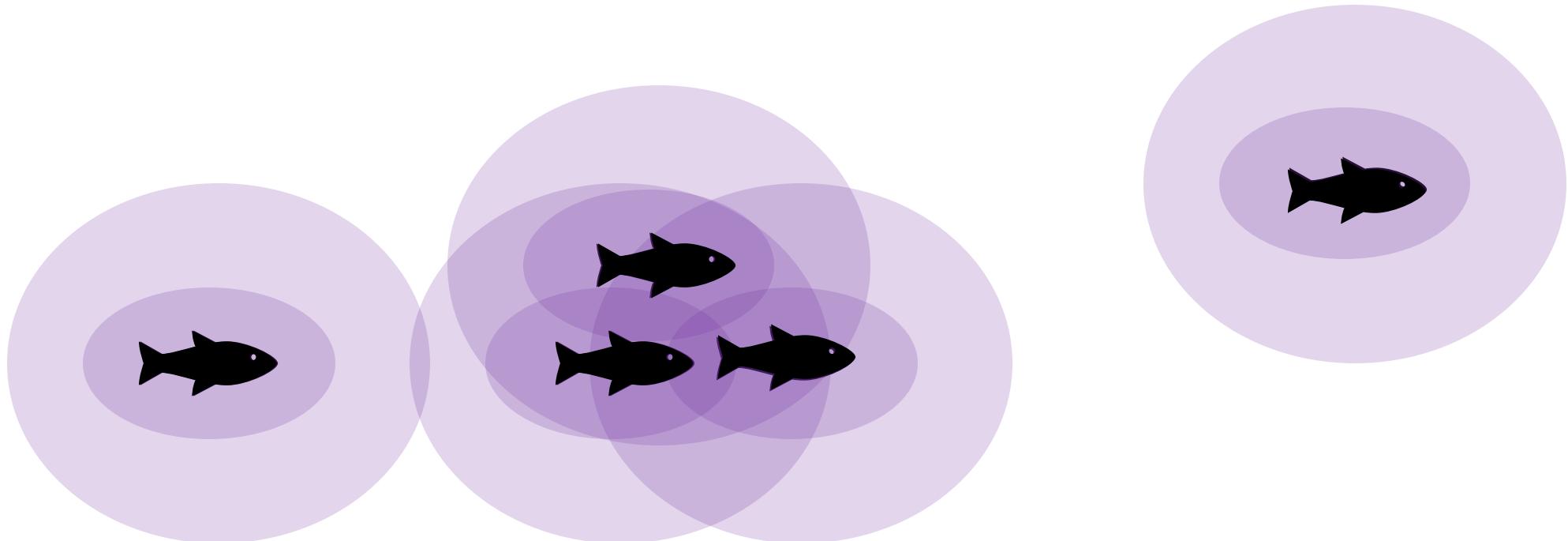
Muestreo: en dónde muestrear?



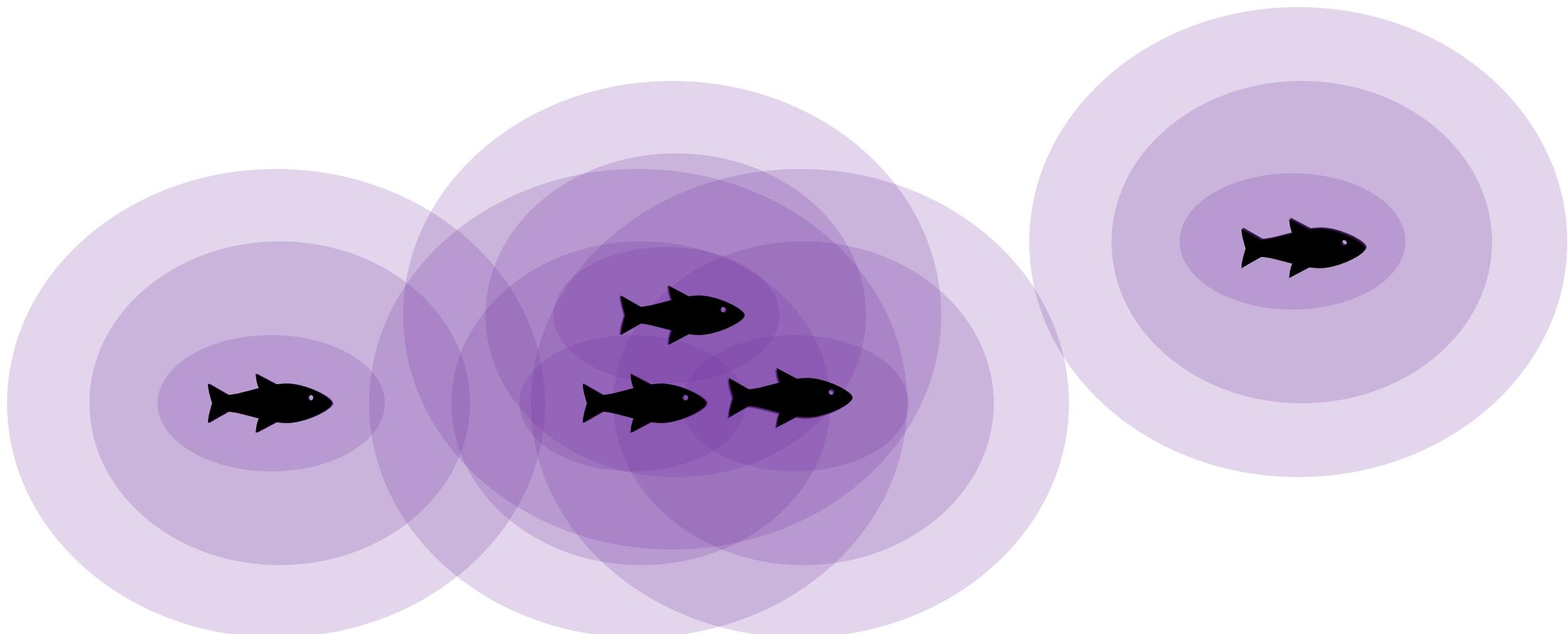
Muestreo: en dónde muestrear?



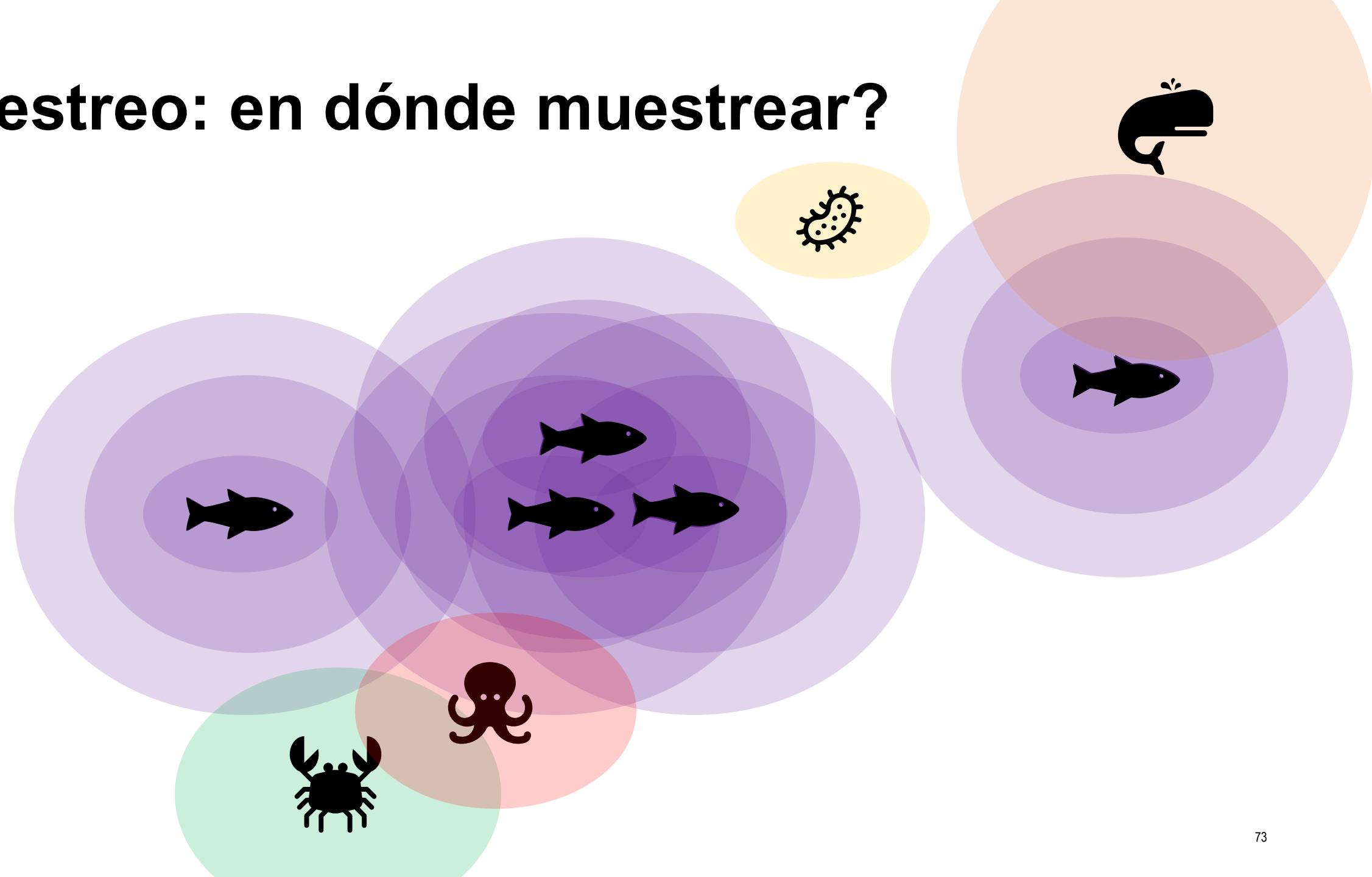
Muestreo: en dónde muestrear?



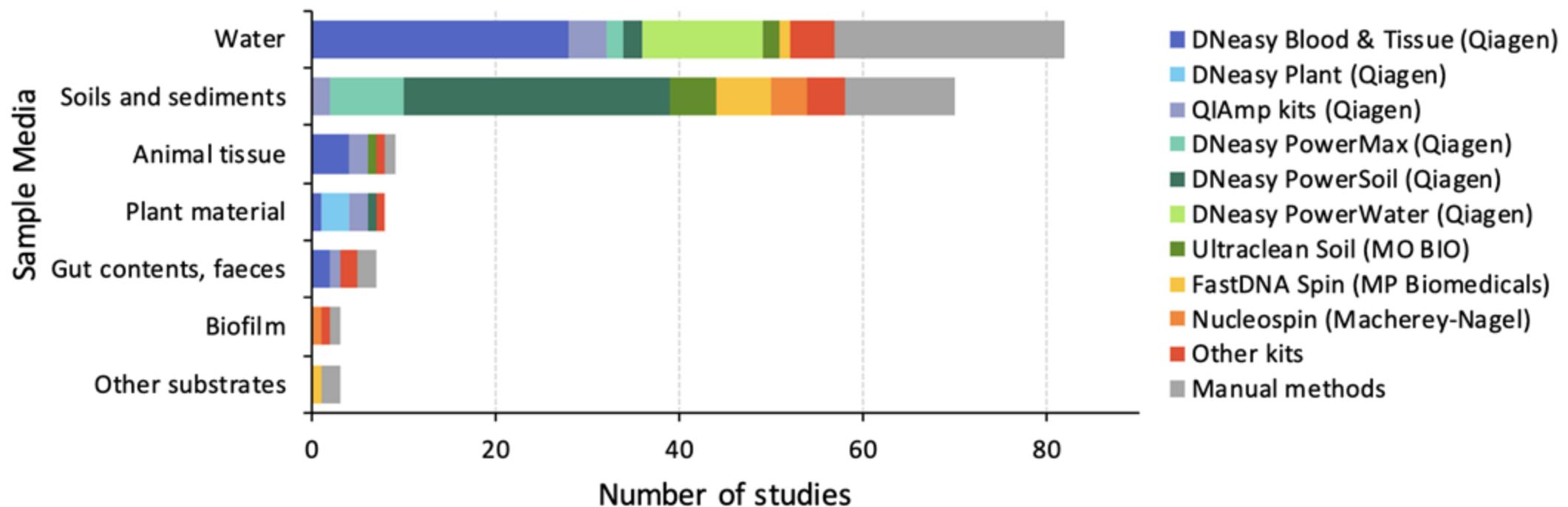
Muestreo: en dónde muestrear?



Muestreo: en dónde muestrear?



Extracción de ADN



Lear et al. 2018

¿Qué marcador usar?

- Qué nivel taxonómico nos interesa

18S > 16S > COI > **12S**

diferente resolución taxonómica

- Qué tan representado está nuestro barcode en las bases de datos de referencia
- Genebank, BOLD, etc.
- ¿Tenemos que reforzar la base de datos?
- Enfoques libres de taxonomía

¿Qué marcador usar?

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- Qué tan representado está nuestro barcode en las bases de datos de referencia
- Genebank, BOLD, etc.
- ¿Tenemos que reforzar la base de datos?
- Enfoques libres de taxonomía

Además:

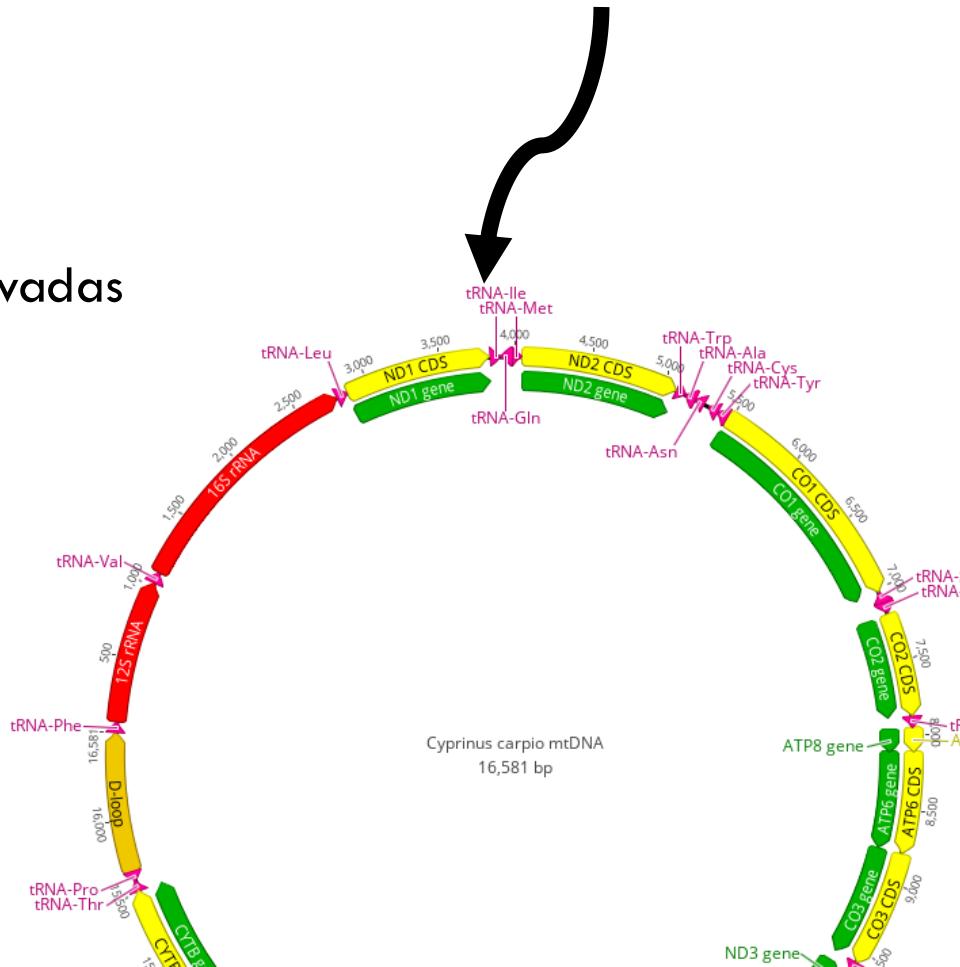
- Qué nivel taxonómico nos interesa
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- Qué tan representado está nuestro barcode en las bases de datos de referencia
- Genebank, BOLD, etc.
- ¿Tenemos que reforzar la base de datos?
- ¿Enfoques libres de taxonomía?

Barcode ideal



Genoma mitocondrial
n=1000
Muy conservado
COX I
12S

1. Variable inter, conservada intra
2. Estándar
3. Filogenéticamente informativa
4. Robusta, zonas flanqueantes conservadas
5. Región corta de fácil amplificación



Características de un barcode

“Such an ideal DNA marker has not yet been found or, perhaps, does not even exist. As a consequence... the various categories of users (e.g. taxonomists, ecologists, etc.) will not give the same priority to the five criteria listed above ...1,2,3 for taxonomists (DNA barcoding *sensu stricto*), 4 y 5 for ecologists working with environmental samples (DNA barcoding *sensu lato*).”

Valentini et al 2009

Elección de primer

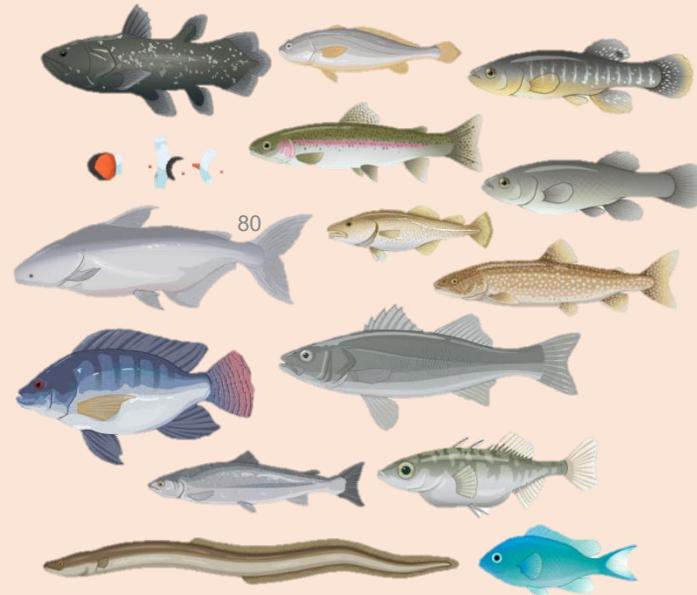
D-loop

control region
(Dlp1.5H, Oordlp4)
Baker et al 2018



MiFishU

12S
(MiFish-F, MiFishU-R)
Miya et al 2015



MarVer1

12S
(MarVer1-F, MarVer1-R)
Valsechhi et al 2020



Elección de primer

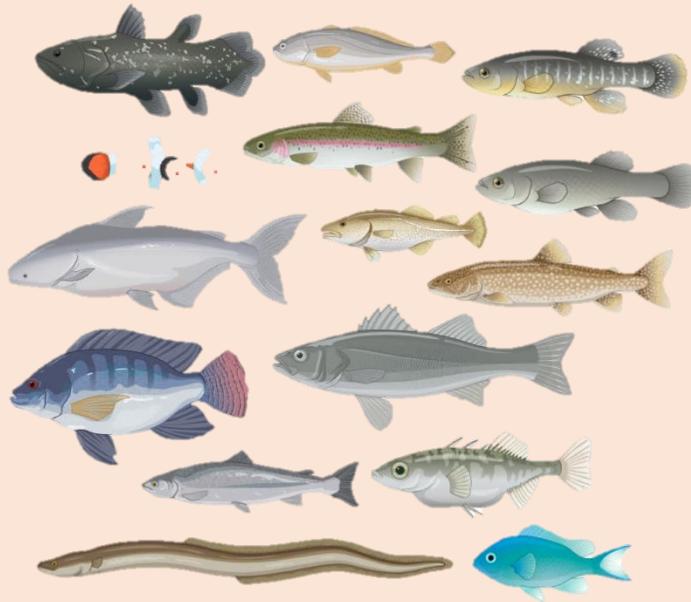
D-loop

control region
(Dlp1.5H, Oordlp4)
Baker et al 2018



MiFishU

12S
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Miya et al 2015

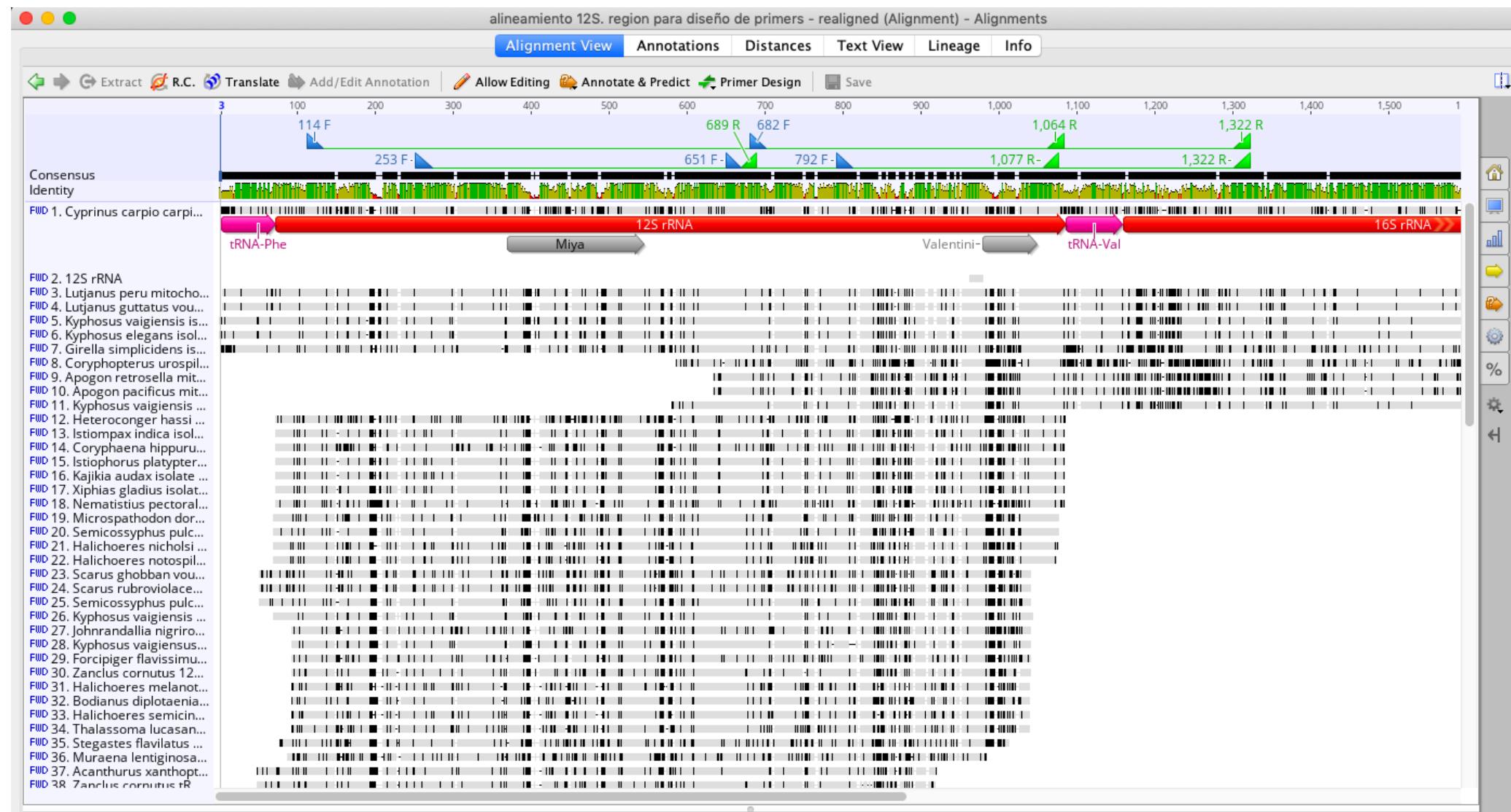


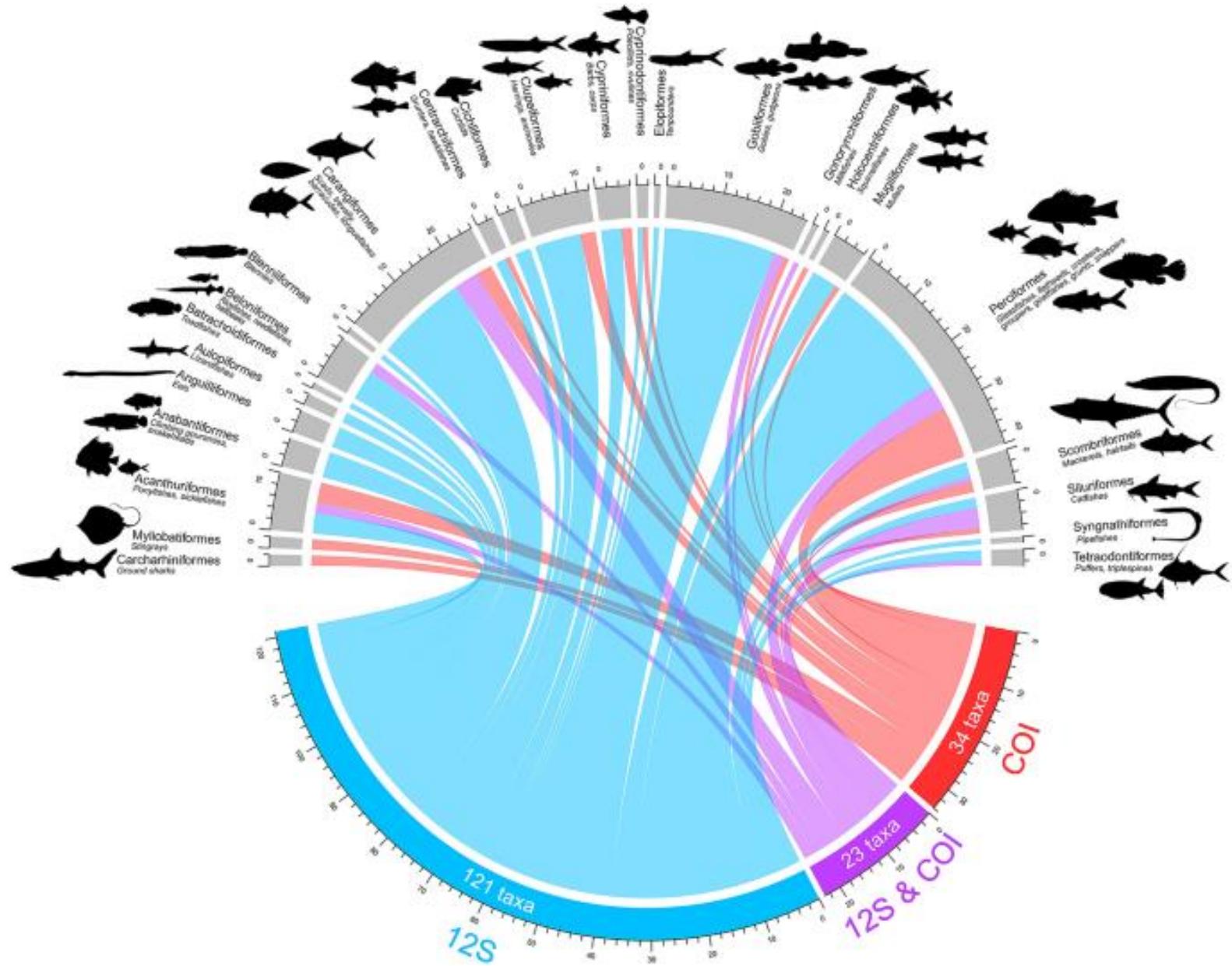
MarVer1

12S
(MarVer1-F, MarVer1-R)
Valsechhi et al 2020



Importante



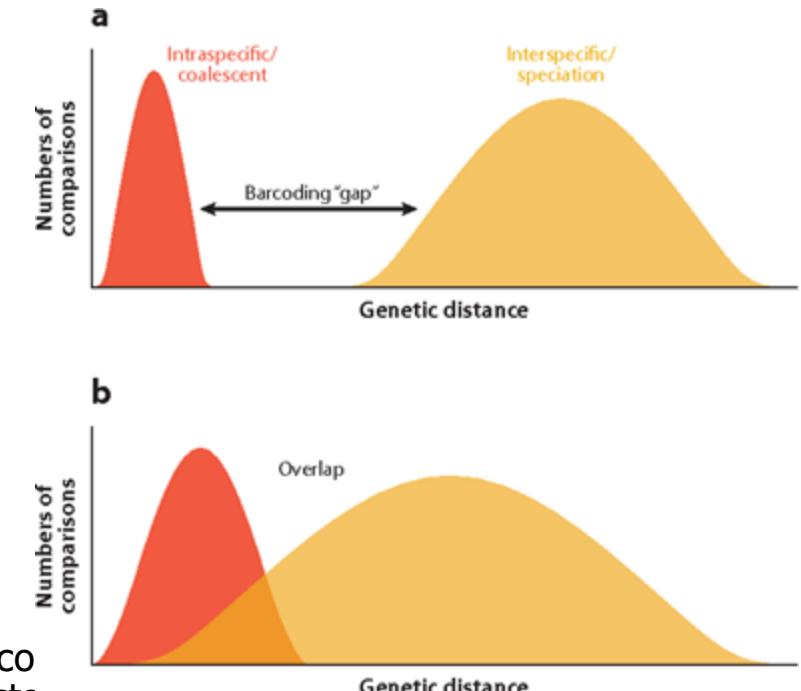


Elección y diseño de marcador y primers

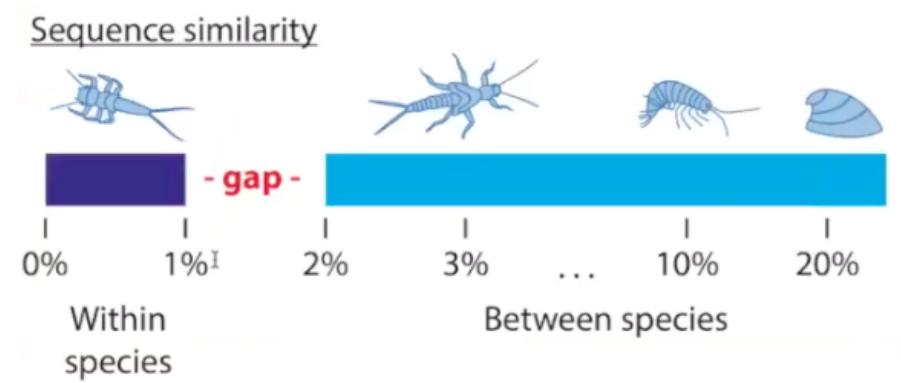
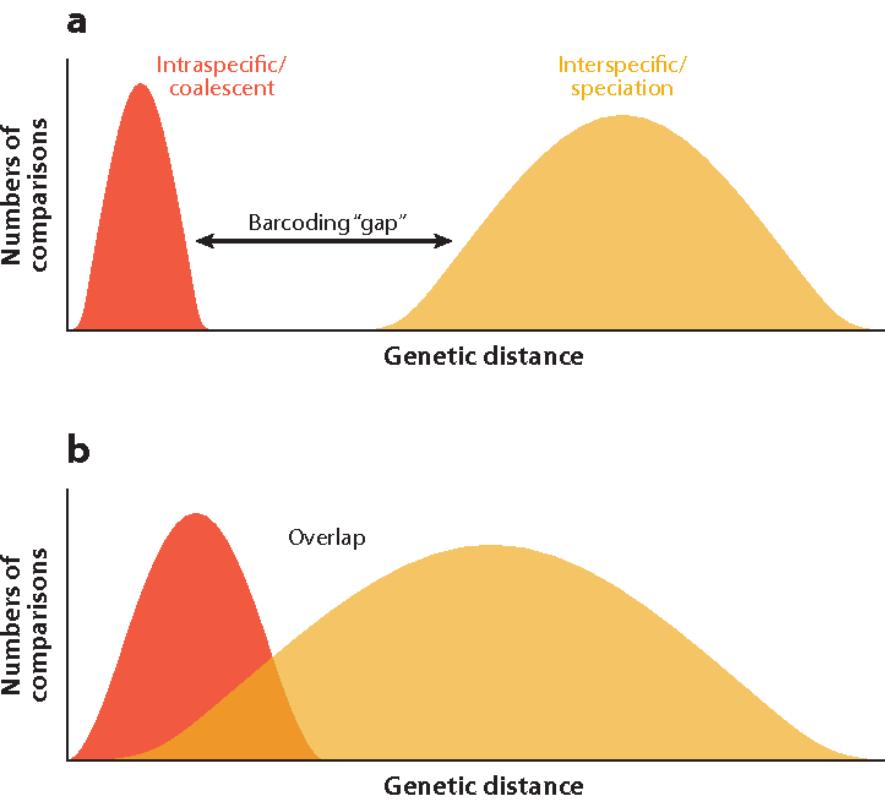
1. Variable inter, conservada intra
2. Estándar
3. Filogenéticamente informativa
4. Robusta, zonas flanqueantes conservadas
5. Región corta de fácil amplificación

"Such an ideal DNA marker has not yet been found or, perhaps, does not even exist. As a consequence, different users (e.g. taxonomists, ecologists, etc.) will not give the same priority to the five criteria listed above ... 1, 2, 3 for taxonomists (DNA barcoding *sensu stricto*), 4 y 5 for ecologists working with environmental samples (DNA barcoding *sensu lato*)."

Valentini et al 2009

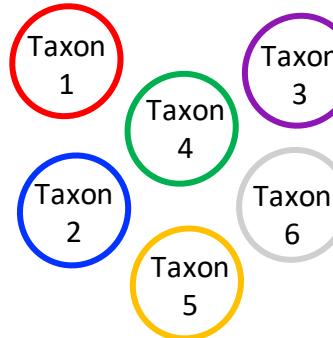


“Barcode gap”

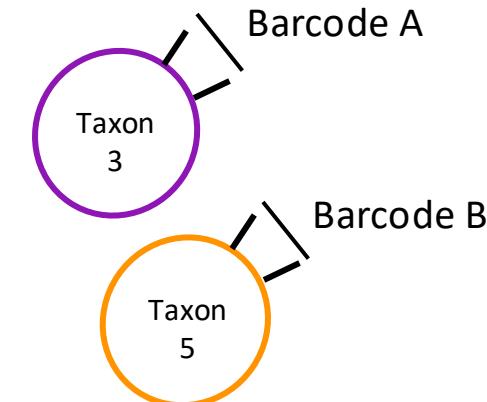


Metabarcoding

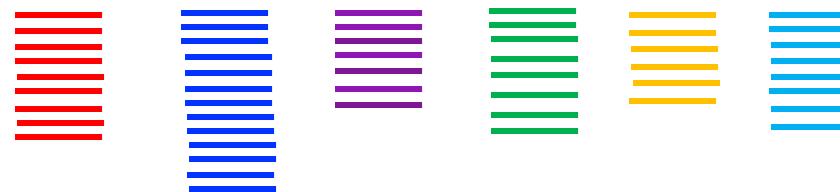
Muestra ADN ambiental



PCR



Secuenciación masiva
(metabarcoding)



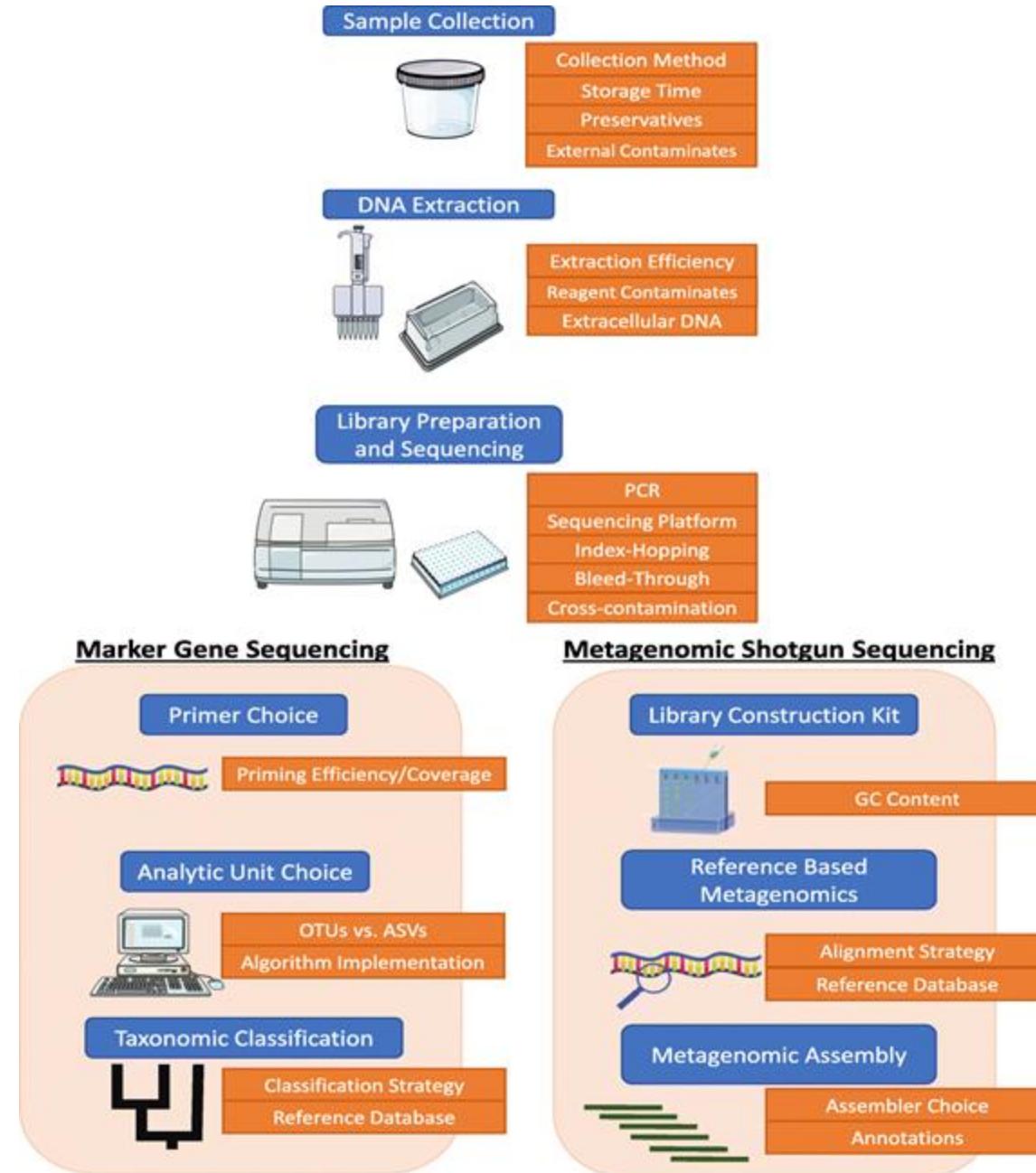
Taxon 1 Taxon 2 Taxon 3 Taxon 4 Taxon 5 Taxon 6

Taxon	Muestra A	Muestra B	Muestra C	Muestra C	Muestra D
1	0.25	0.25	0.01	0.2	0.6
2	0.05	0.05	0.1	0.2	0.1
3	0.12	0.18	0.5	0.01	0.05
4	0.3	0.4	0.01	0.3	0.2
5	0.28	0.12	0.38		

Limitaciones

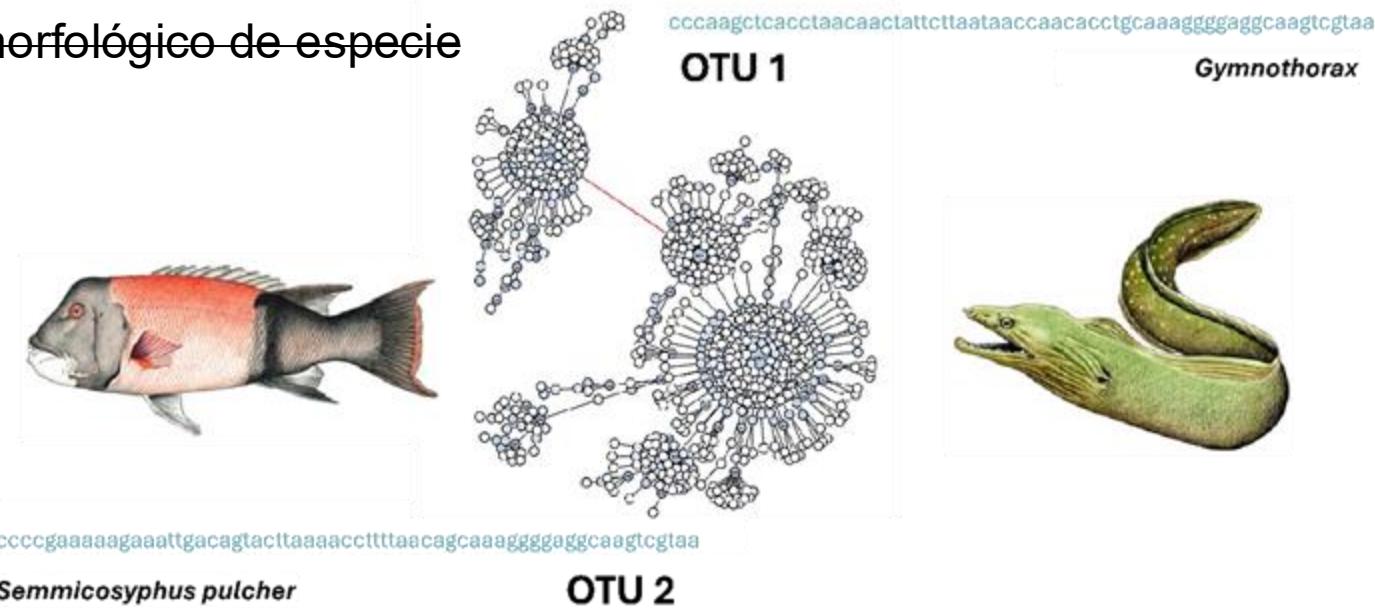
- Abundancias relativas (a lo secuenciado)
- Sesgos en la amplificación

Factores técnicos



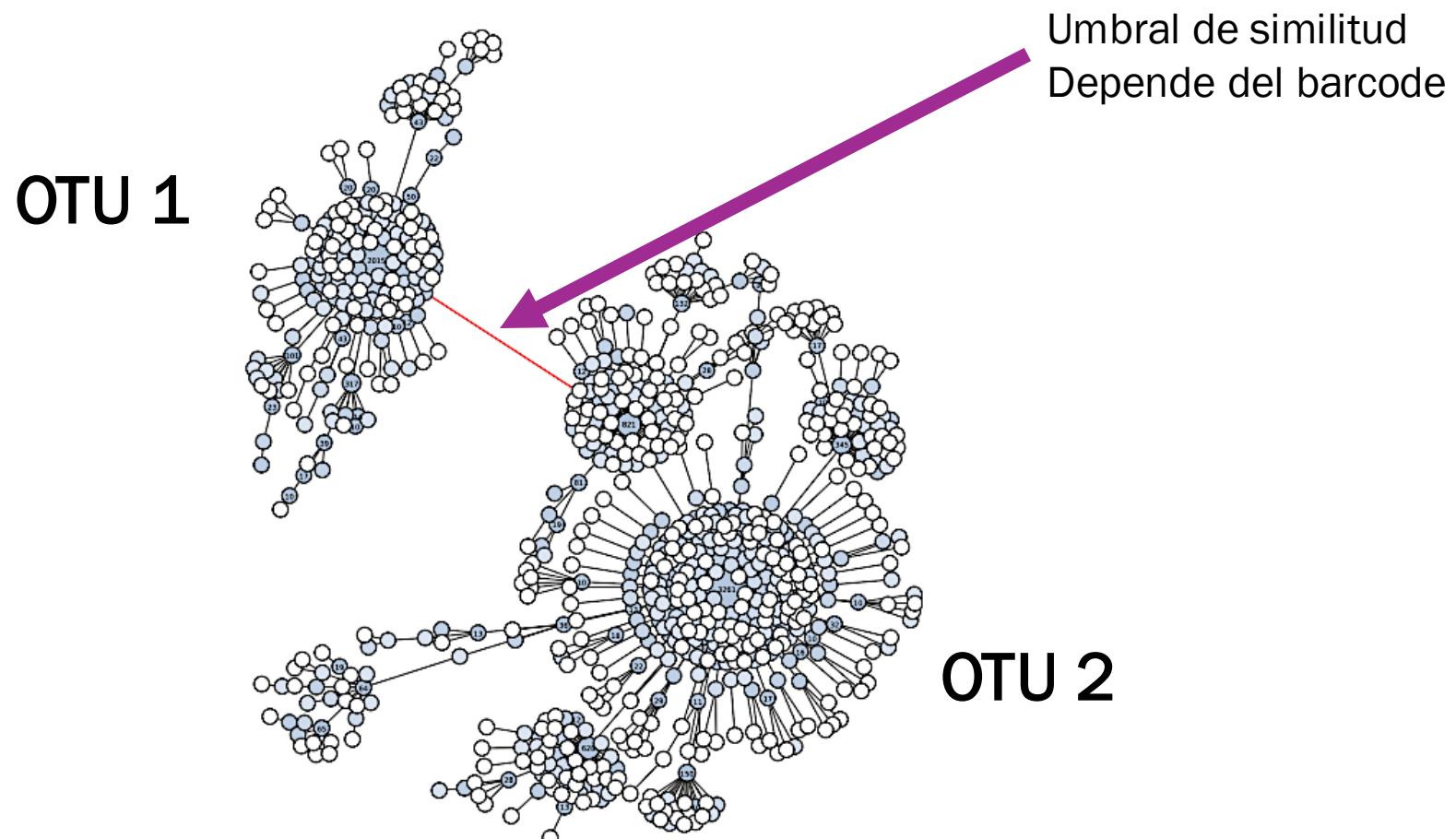
Asignación taxonómica de las secuencias OTUs, ASV,... etc.

OJO: Concepto morfológico de especie



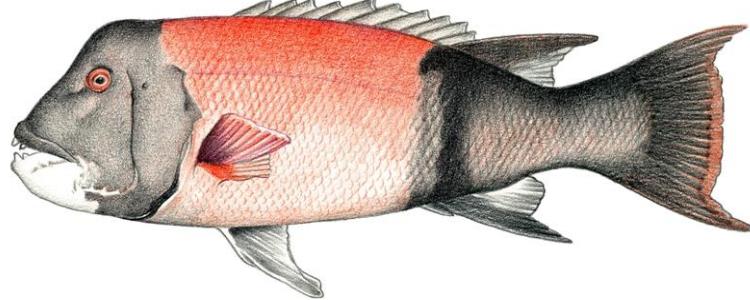
- **OTU (Operational Taxonomic Unit):** Constructo artificial usado para agrupar secuencias en unidades, facilitando su análisis. Los OTUs ayudan a manejar los errores de secuenciación inherentes a la tecnología.
- **ASV (Amplicon Sequence Variant):** Son secuencias resultantes de metodologías más recientes que consideran las tasas de error de secuenciación, y representan secuencias biológicas verdaderas análogas a los haplotipos.

Análisis bioinformático y asignación taxonómica



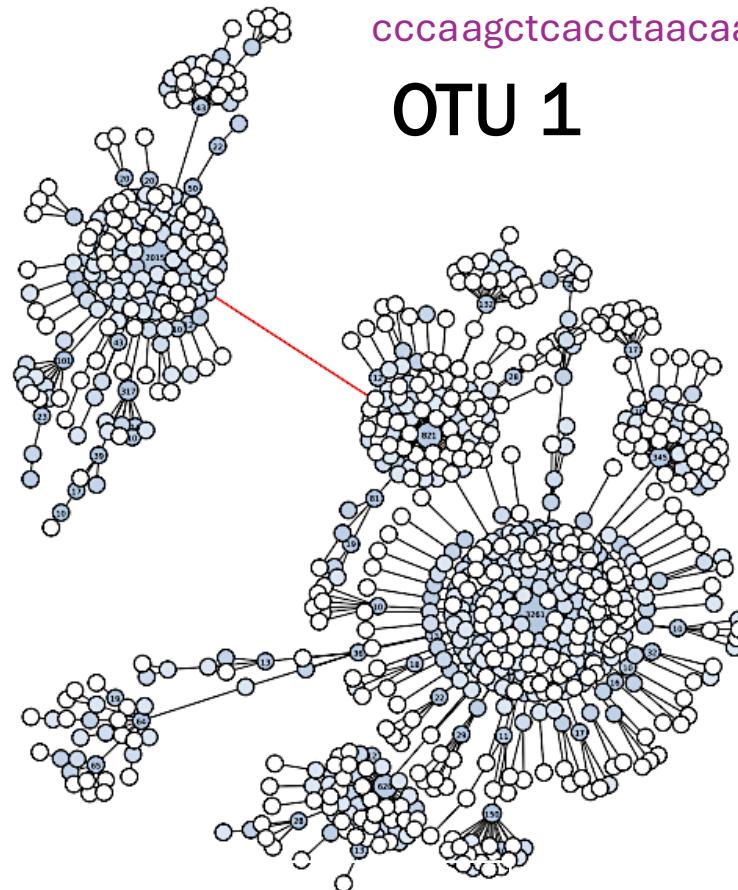
Operational Taxonomic Units (OTU) ~ especie
OJO: Concepto morfológico de especie

Análisis bioinformático y asignación taxonómica



ccccgaaaaagaaattgacagtactaaaacctttaacagcaaaggaggcaagtctaa

Semicossyphus pulcher

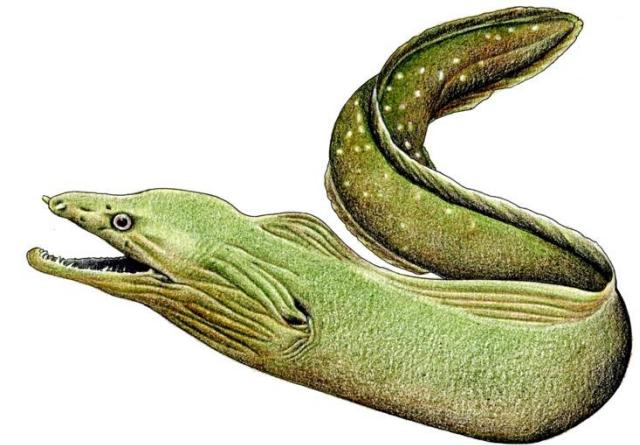


OTU 2

cccaagctcacctaacaactattcttaataaccacaccctgcaaaggaggcaagtctaa

OTU 1

Gymnothorax castaneus



LABORATORIO

CREACIÓN LIBRERÍAS DE AMPLICONES

Preparación de librerías de amplicones para metabarcoding

Primera PCR, 35 ciclos

Primer Forward

eDNA12SV-F

5' TCGTCGGCAGCGTC AGATGTGTATAAGAGACAG **ACACCGCCCGTCACTCT** [] **CTTCCGGTACACTTACCATG** AGATGTGTATAAGAGACAG **GTCTCGTGGGCTCGG** 3'

**Adapter
used for
2nd PCR**

**3' end of
Illumina
adapter**

**Universal 12S
Forward
Primer teleo_F**

**Universal 12S
Reverse Primer
teleo_R**

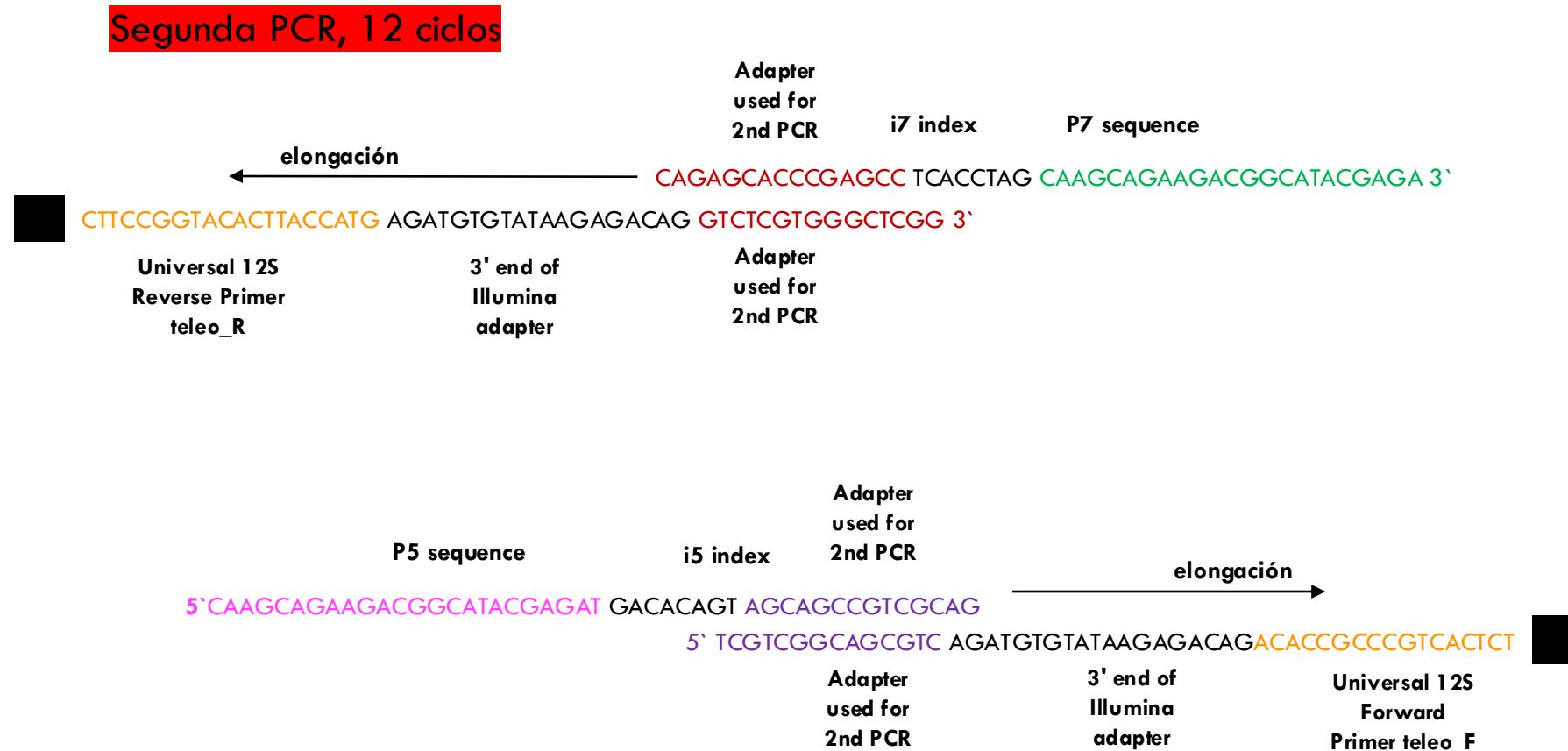
**3' end of
Illumina
adapter**

Primer Reverse

eDNA12SV-R

**Adapter
used for
2nd PCR**

Preparación de librerías de amplicones para metabarcoding



Preparación de librerías de amplicones para metabarcoding

Librería para secuenciación

5' CAAGCAG AAGACGGCATACGAGAT GACACAGT AGCACGCCGTCGCAG AGATGTGATAAGAGACAG ACACCGCCCGTCACTCT AMPLICÓN CTTCCGGTACACTTACCATG AGATGTGATAAGAGACAG CAGAGCACCCGAGCC TCACCTAG CAAGCAG AAGACGGCATACGAGA 3'
5' CAAGCAG AAGACGGCATACGAGAT GACACAGT AGCACGCCGTCGCAG AGATGTGATAAGAGACAG ACACCGCCCGTCACTCT AMPLICÓN CTTCCGGTACACTTACCATG AGATGTGATAAGAGACAG CAGAGCACCCGAGCC TCACCTAG CAAGCAG AAGACGGCATACGAGA 3'

P5 sequence	i5 index	Adapter used for 2nd PCR	3' end of Illumina adapter	Universal 12S teleo_F	Universal 12S teleo_R	3' end of Illumina adapter	Adapter used for 2nd PCR	i7 index	P7 sequence
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i5 index

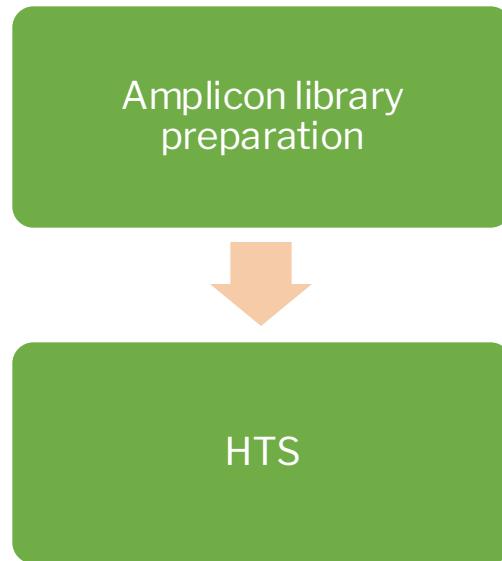
GACACAGT
GCATAACG
ACAGAGGT
CCACTAAG
TGTTCCGT
GATACTG
AGCCGTA
CTCCTGAA

$$8 \times 12 = 96$$

i7 index

TCACCTAG
CAAGTCGT
CTGTATGC
AGTCGCA
ATCGGAGA
AAGTCCTC
TGGATGGT
AGGTGTTG
GACGAAC
GTTCTCG
TTCGCCAT
CAACTCCA

Detección de multiples especies: Metabarcoding



Library preparation:

12S rRNA 65 bases (Valentini)

PCR 1

ACACCGCCCGTCACTCTCCCCAAGCTCCGGCCCTAATTAACCTAAACCCCTATAACTGCAAAGGGAGGAAAGTCGTAACTGGTAAGTGTACCGGAAG

Primer F

PCR 2

ACTGTGTCAACACCGCCCGTCACTCTCCCCAAGCTCCGGCCCTAATTAACCTAAACCCCTATAACTGCAAAGGGAGGAAAGTCGTAACTGGTAAGTGTACCGGAAGCTGTCTTTACATCTCTAGGTGA

Index i5

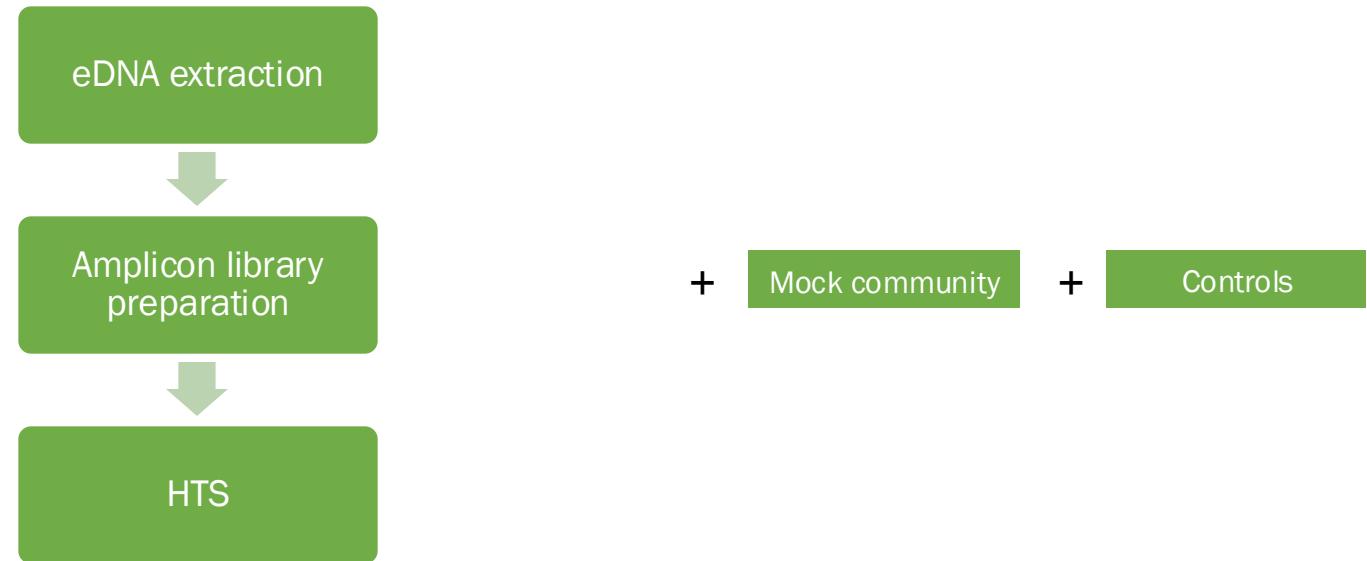
Primer F

Primer R

Primer R

Illumina Adapter Index i7

eDNA extraction, library construction and HTS



Library preparation:

12S rRNA 65 bases (Valentini)

PCR 1

ACACCGCCCGTCACTCTCCCCAAGCTCCGGCCCTAATTAACCTAAACCTATAACTGCAAAGGAGAGGAAAGTCGTAACTGGTAAGTGTACCGGAAG

Primer F

PCR 2

ACTGTGTCACACCGCCCGTCACTCTCCCCAAGCTCCGGCCCTAATTAACCTAAACCTATAACTGCAAAGGAGAGGAAAGTCGTAACTGGTAAGTGTACCGGAAGCTGTCTTTACACATCTCTAGGTGA

Index i5

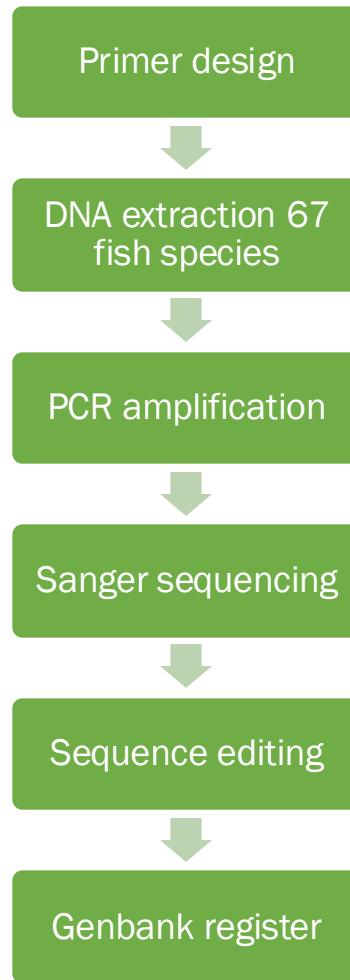
Primer F

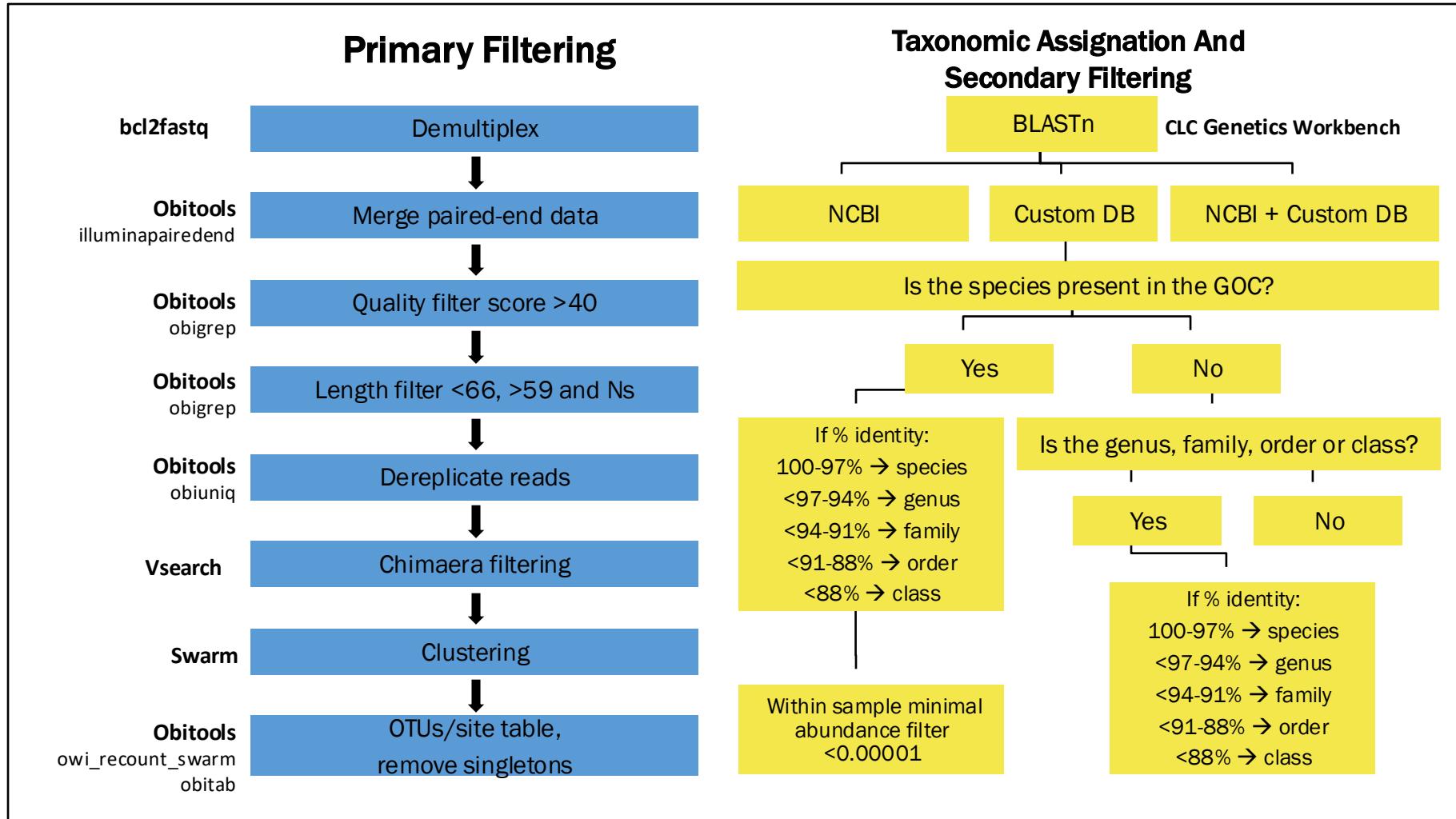
Primer R

Primer R

Illumina Adapter Index i7

Local reference database construction

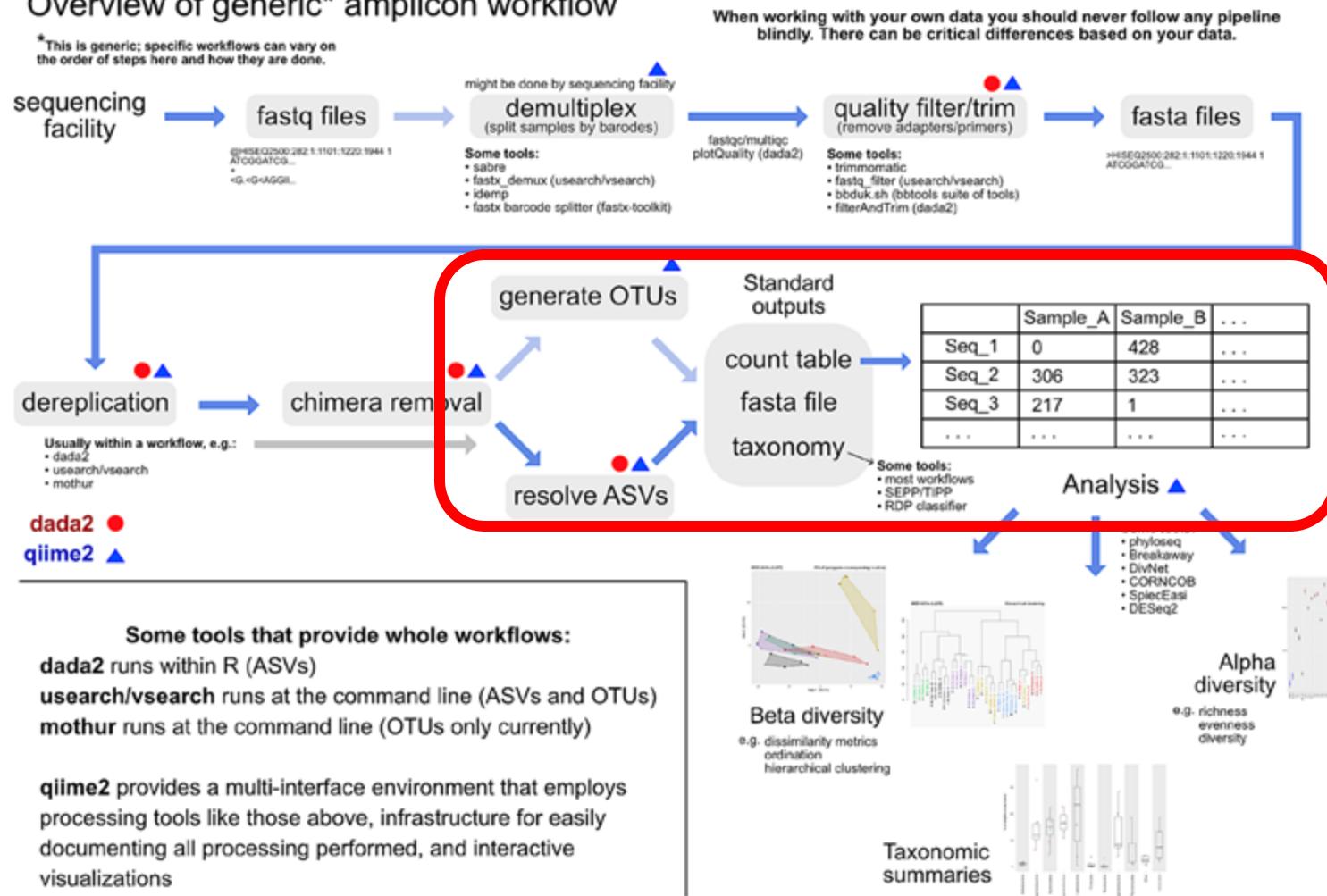




Detección de multiples especies: Metabarcoding

Overview of generic* amplicon workflow

*This is generic; specific workflows can vary on the order of steps here and how they are done.

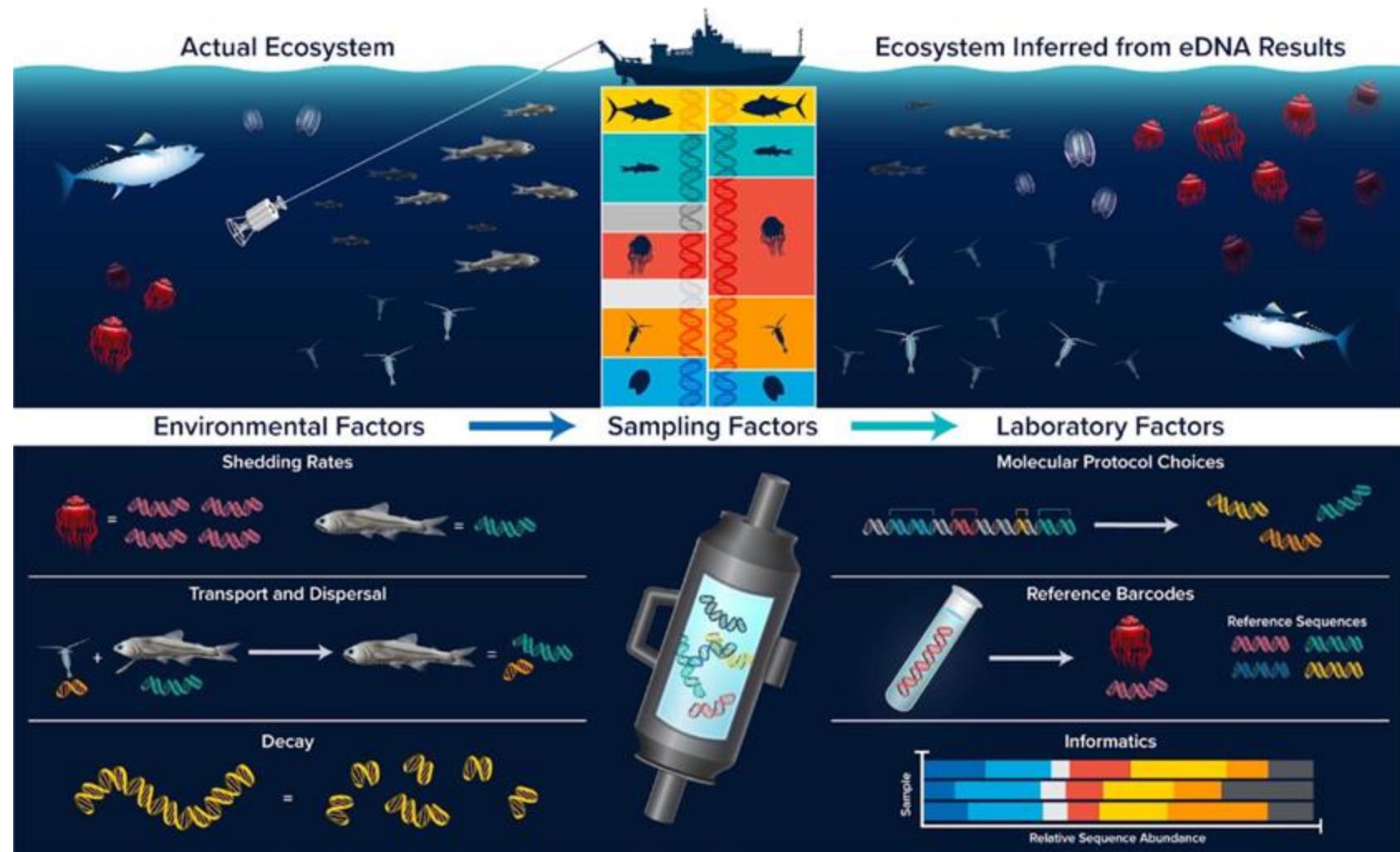


OTU vs ASV

	OTU	ASV
Sesgo de referencia	Puede estar sujeto a sesgo de referencia.	La referencia no se utiliza hasta la asignación taxonómica.
Comparabilidad	No comparable entre estudios	Puede compararse entre estudios
Representación	Representado por una secuencia consenso.	Representado por una secuencia exacta.
Diversidad de especies	Puede representar múltiples especies con secuencias diferentes.	Si representa múltiples especies, es porque comparten la identidad de secuencia.
Secuencias químicas	Propenso a secuencias químicas; la detección puede ser compleja y requerir sesgo de referencia.	Propenso a secuencias químicas; la detección es sencilla y libre de referencia.

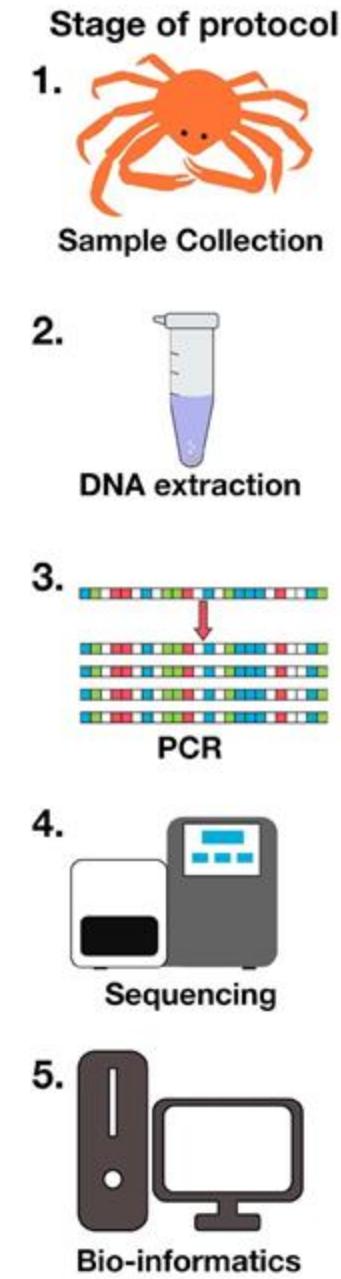
CUANTITATIVO

¿Entonces, lo que detectamos con eDNA es lo que hay en el mar?



¿El metabarcoding es cuantitativo?

- Durante la amplificación de ADN, ciertas secuencias pueden amplificarse más que otras debido a diferencias en la eficiencia de los primers, lo que puede sesgar los resultados.
- Algunos fragmentos de ADN pueden ser secuenciados más eficientemente que otros, lo que también introduce un sesgo en la representación de especies en los resultados finales.
- En una muestra con muchas especies, las secuencias de especies menos abundantes pueden quedar subrepresentadas o incluso no ser detectadas debido a la competencia con secuencias más abundantes

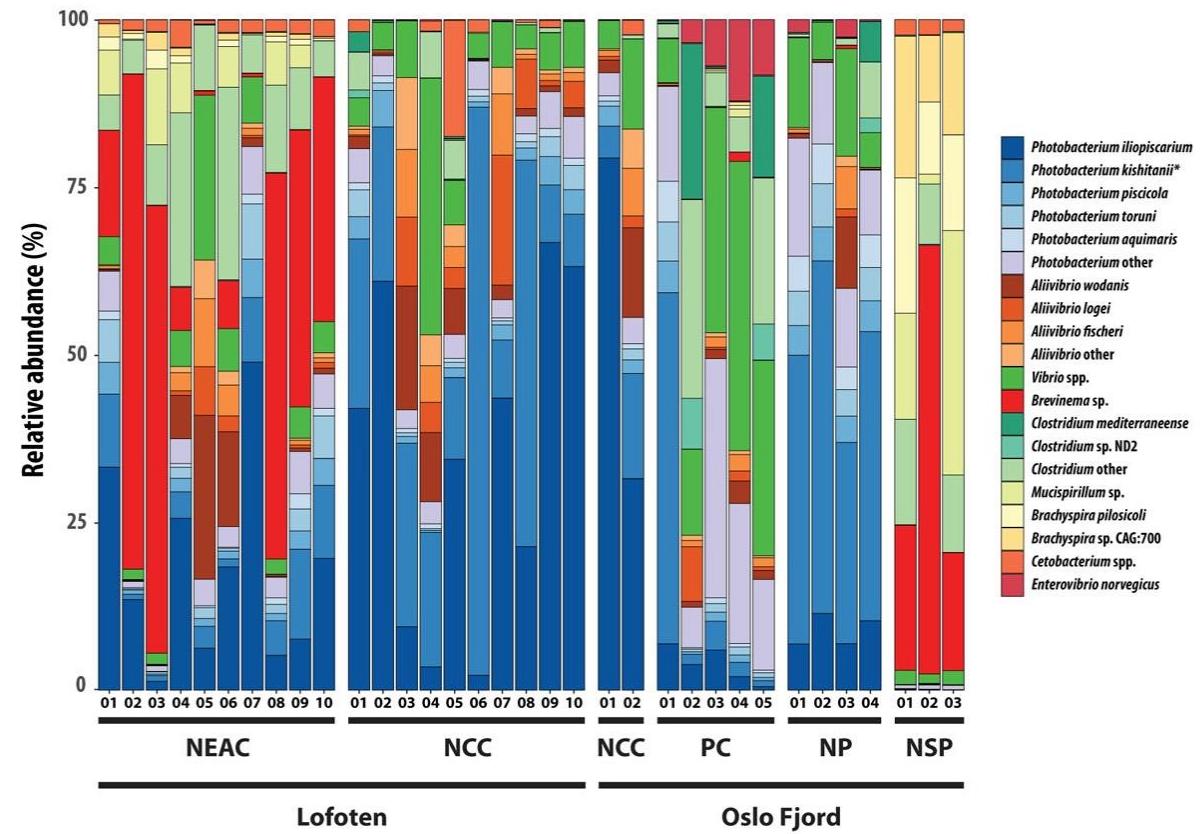


Implicaciones cuantitativas

Presencia/ausencia



Datos cuantitativos en términos de proporciones Abundancias relativas



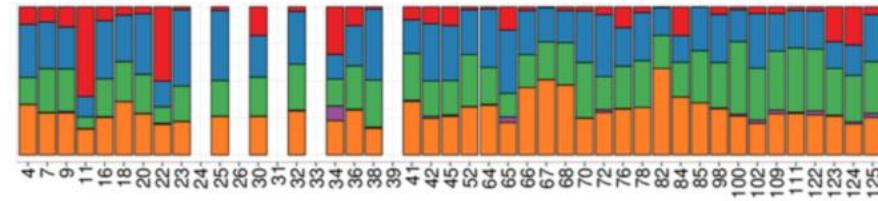
Implicaciones cuantitativas

eDNA DNA extraorganismo



Presencia/ausencia

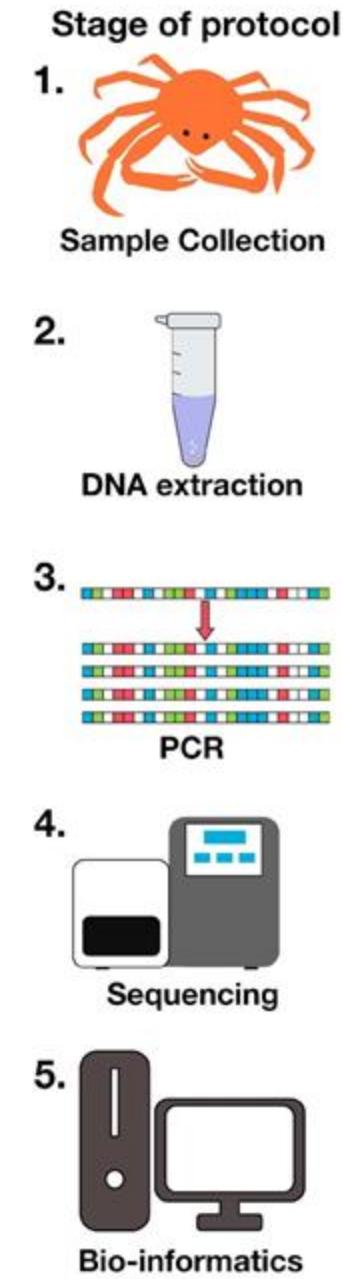
DNA de comunidades



Datos cuantitativos
Abundancias relativas

¿El metabarcoding es cuantitativo?

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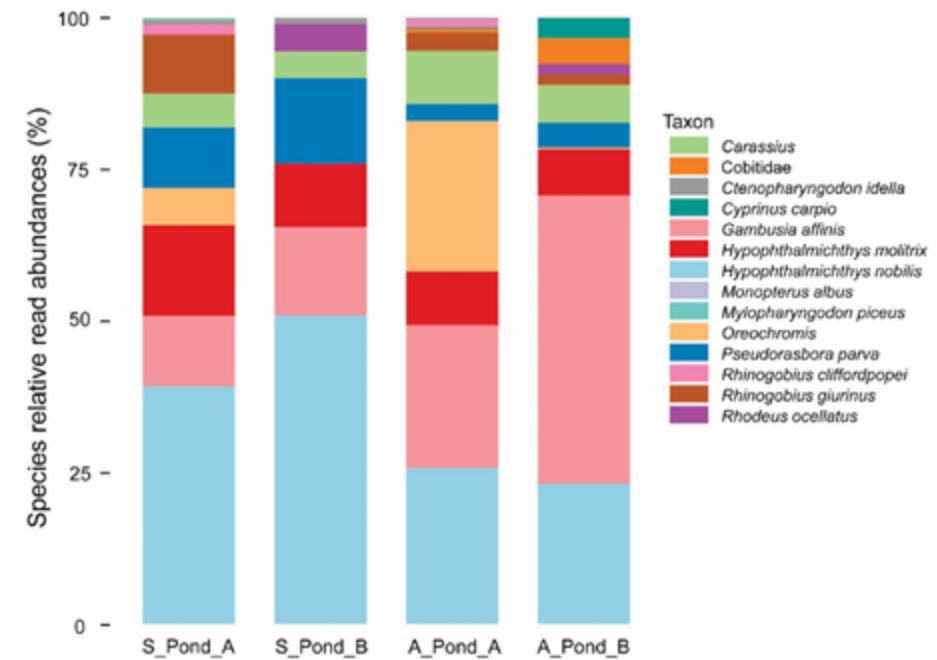
Los resultados de metabarcoding son composicionales

Los datos de metabarcoding son composicionales porque reflejan la estructura relativa de las secuencias de ADN en una muestra, no sus cantidades absolutas. Esto es una consecuencia de la forma en que se obtienen y procesan los datos, donde las proporciones entre especies son lo que realmente se mide y analiza. Para trabajar con estos datos, es importante usar métodos estadísticos que tengan en cuenta esta naturaleza composicional para evitar interpretaciones erróneas.

Sequencer takes a random sample from the total pool of available sequences in a PCR reaction (say ~100k per sample).

Consequences of compositional data

1. Destroys any information about absolute abundance for taxa
2. You cannot model species independently.
- increasing one species means other species decrease



Shu et al.
2022

$$A_i = c_i(1 + a_i)^{N_{PCR}}$$

A_i = amplicons produced (species i)

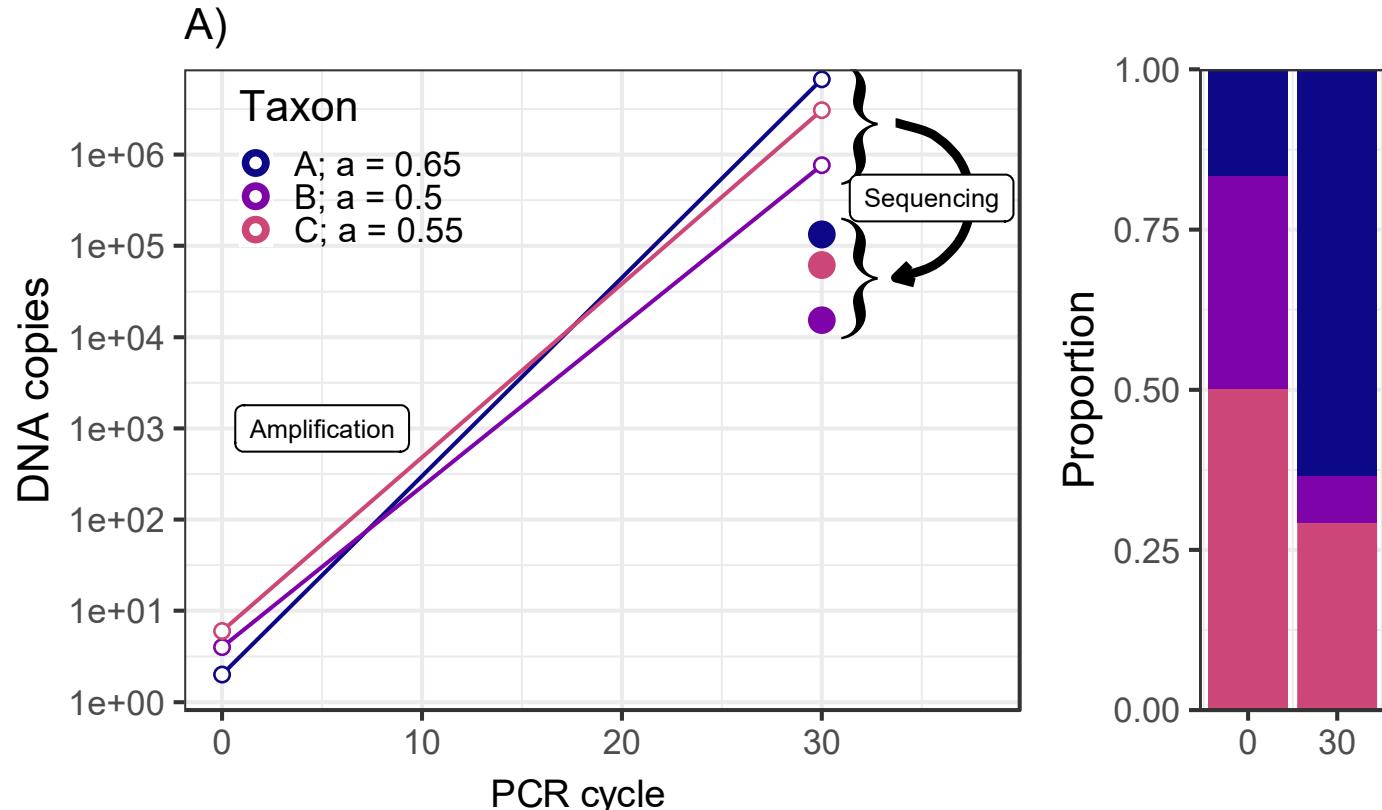
c_i = initial number of DNA copies (species i)

a_i = amplification efficiency (species i)

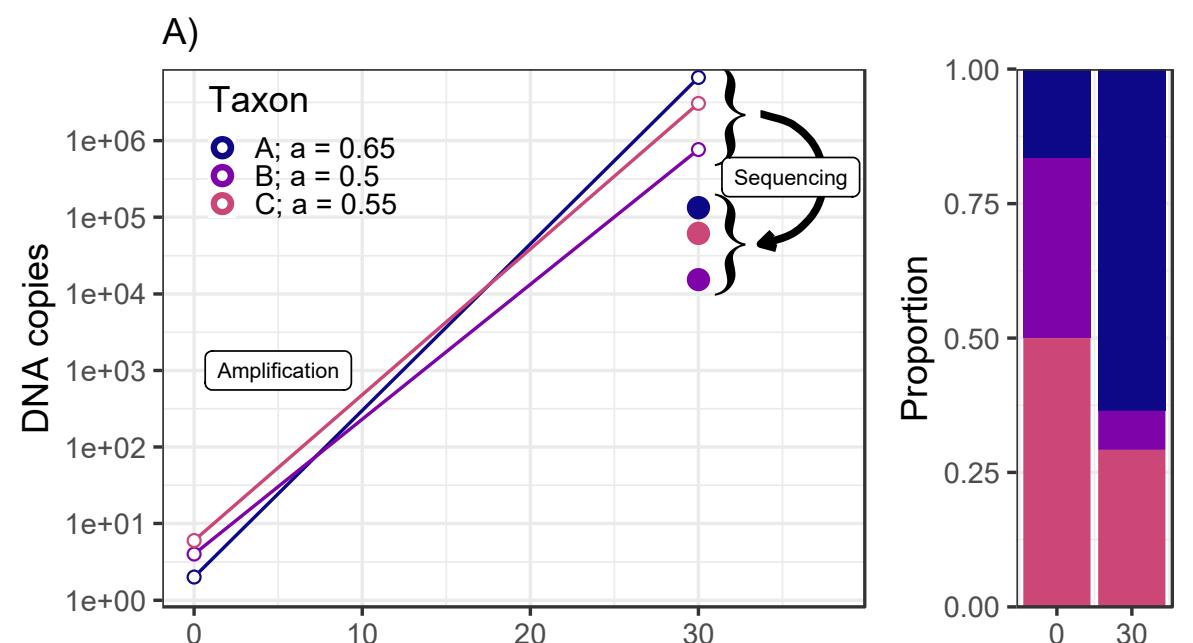
N_{PCR} = Number of PCR cycles.

Assumptions

- PCR has not saturated at N_{PCR} (still in the exponential phase of PCR).
- Amplification efficiencies a_i are a trait of the taxon-primer pair and protocol.
 - Do not depend on the other species present, sequencing run, other things



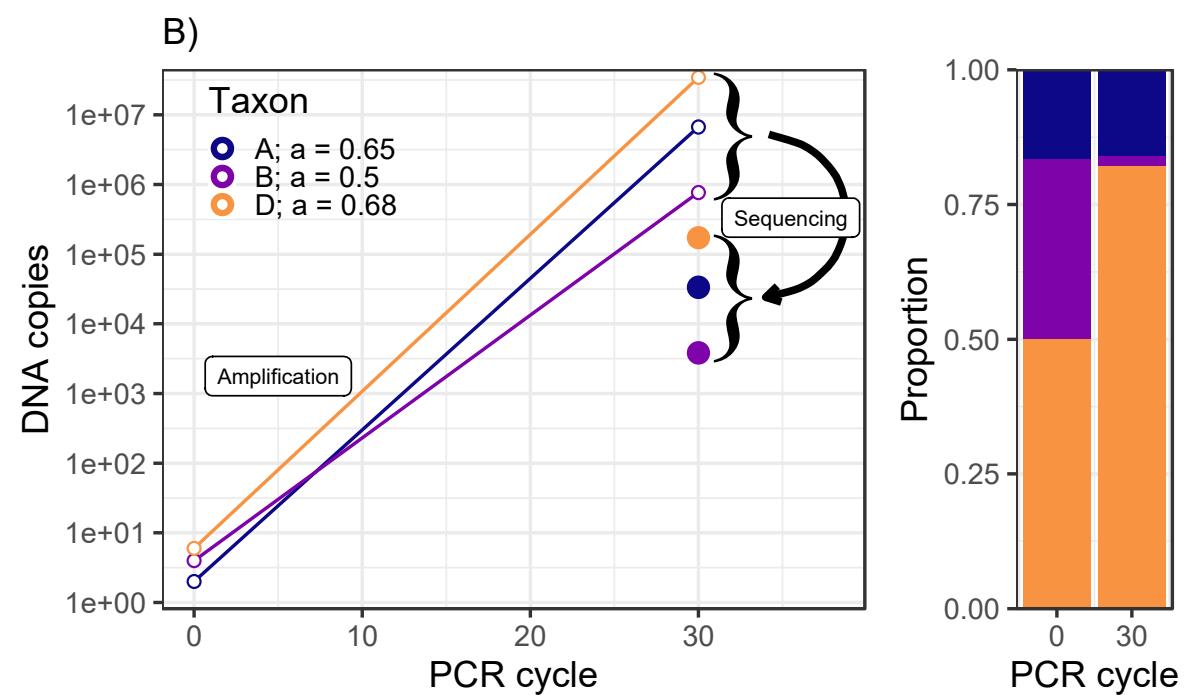
Example with three species (A,B,C)



Swap species D in for species C:

Nothing has changed about species A or B, but the observed proportions after PCR are radically different

"A" increases in the top panel but decreases in the bottom panel.



That's a fair amount of detail.

Metabarcoding is compositional;

Best you can expect is proportional abundances without additional information.

We can account for common source of bias in metabarcoding data

We know when it doesn't work well

1. Species of interest have low amplification bias
2. Highly diverse samples (too many species, too many rare sequences)
3. A species was not in the mock community.

A couple of points

This is a simplification, of course.

With DNA you are seeing the shadows / echoes of creatures

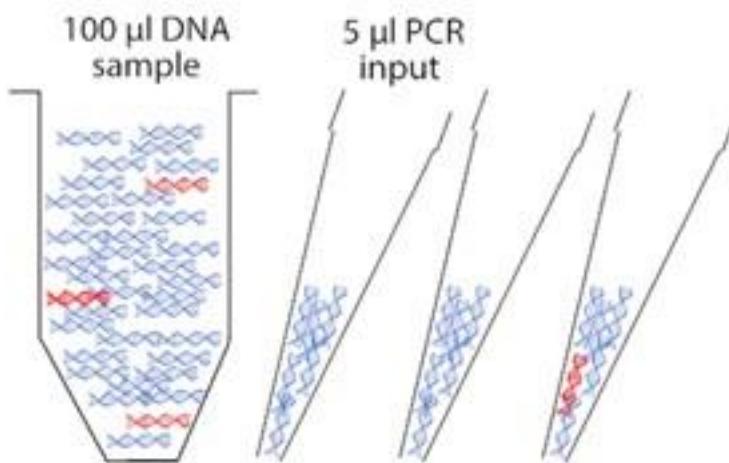
- spatial scale of couple hundred of meters*
- temporal scale of hours to days*

Units of are DNA copies per volume, do not include abundance or biomass

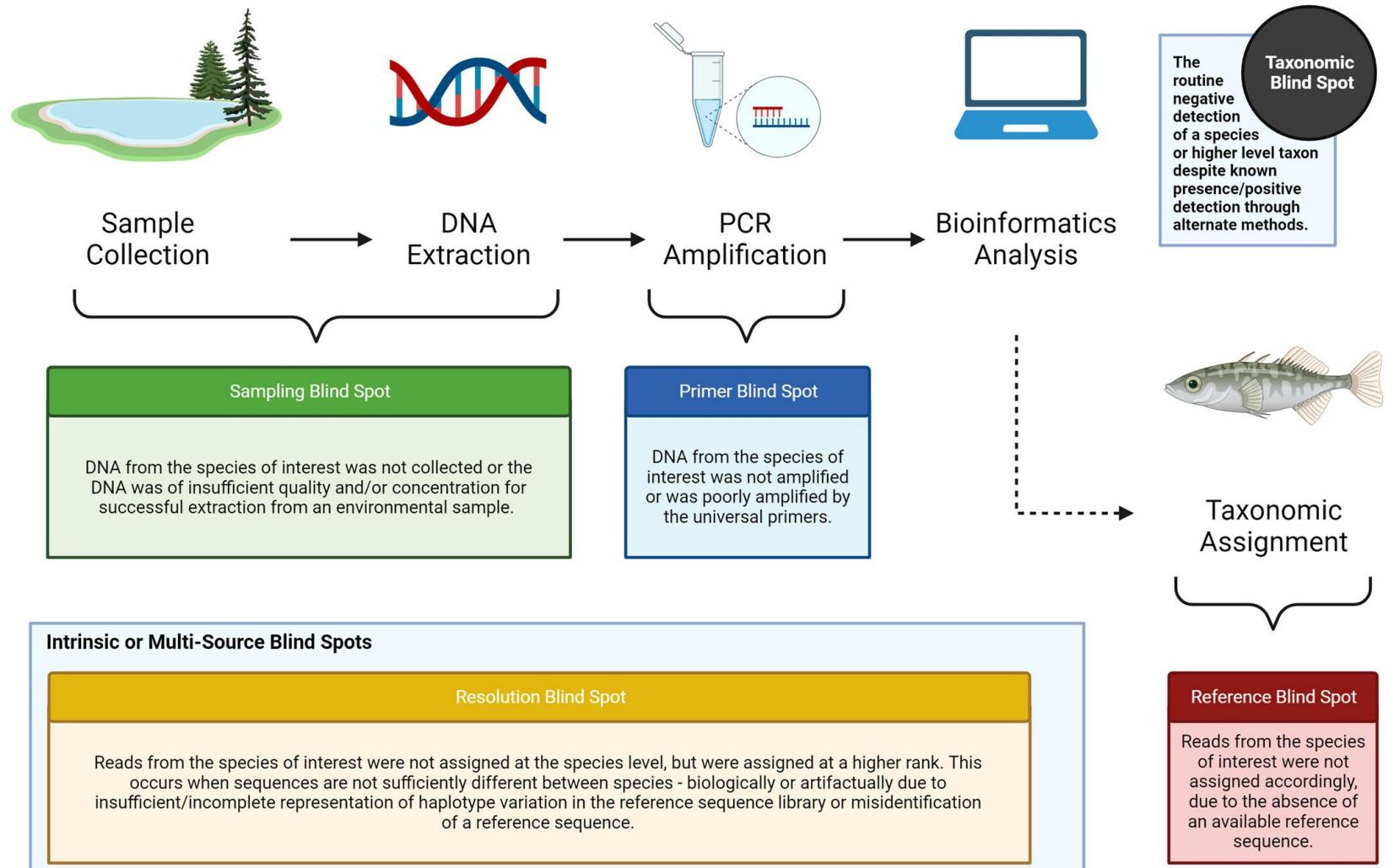
- process for mapping DNA back to abundance is not obvious

*Depends on sensitivity of your assay and how hard you are looking

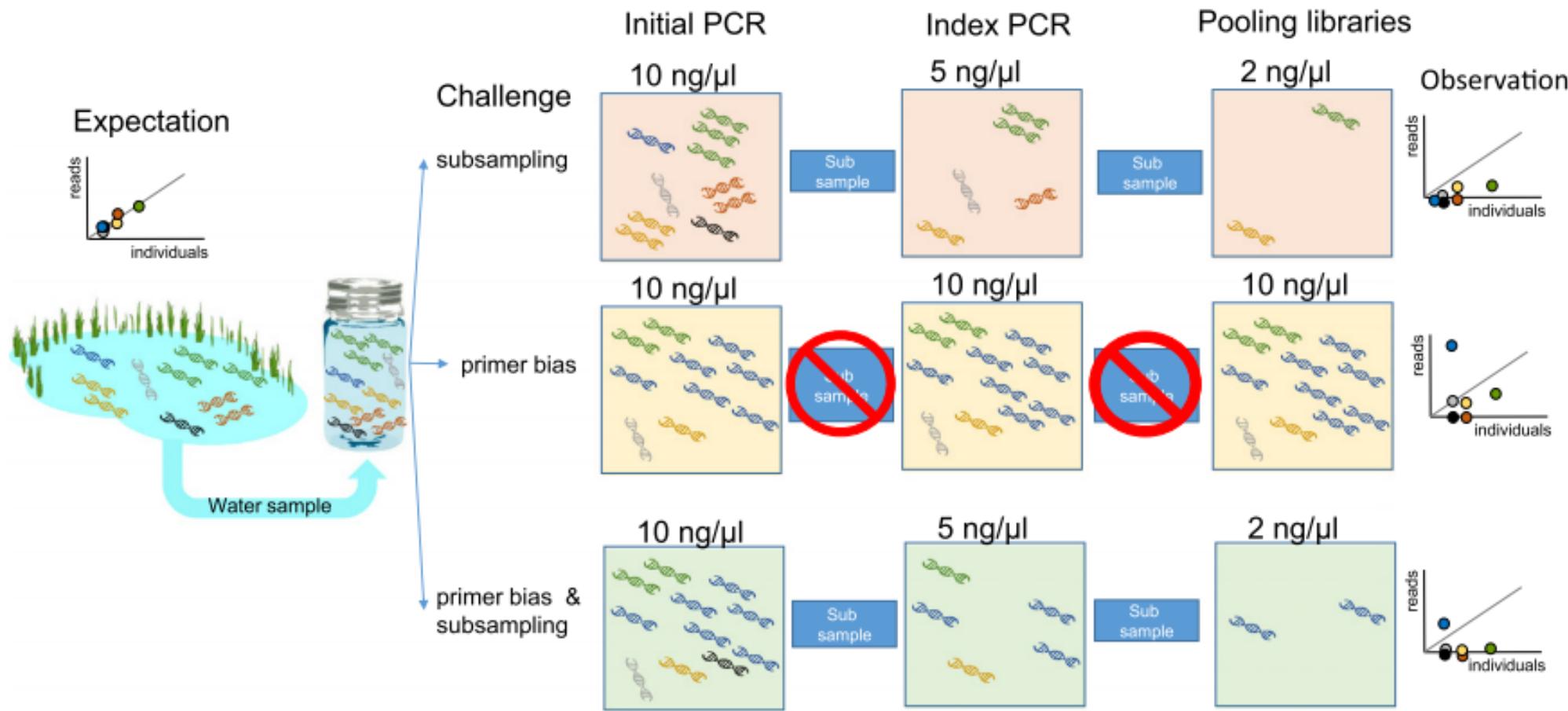
Poisson Distribution (Law of Small Numbers)



eDNA copies/L	Metabarcoding detection (1 L water)
10,000	
1,000	reproducible
100	
10	variable
1	
0.1	
0.01	rare or absent
0.001	



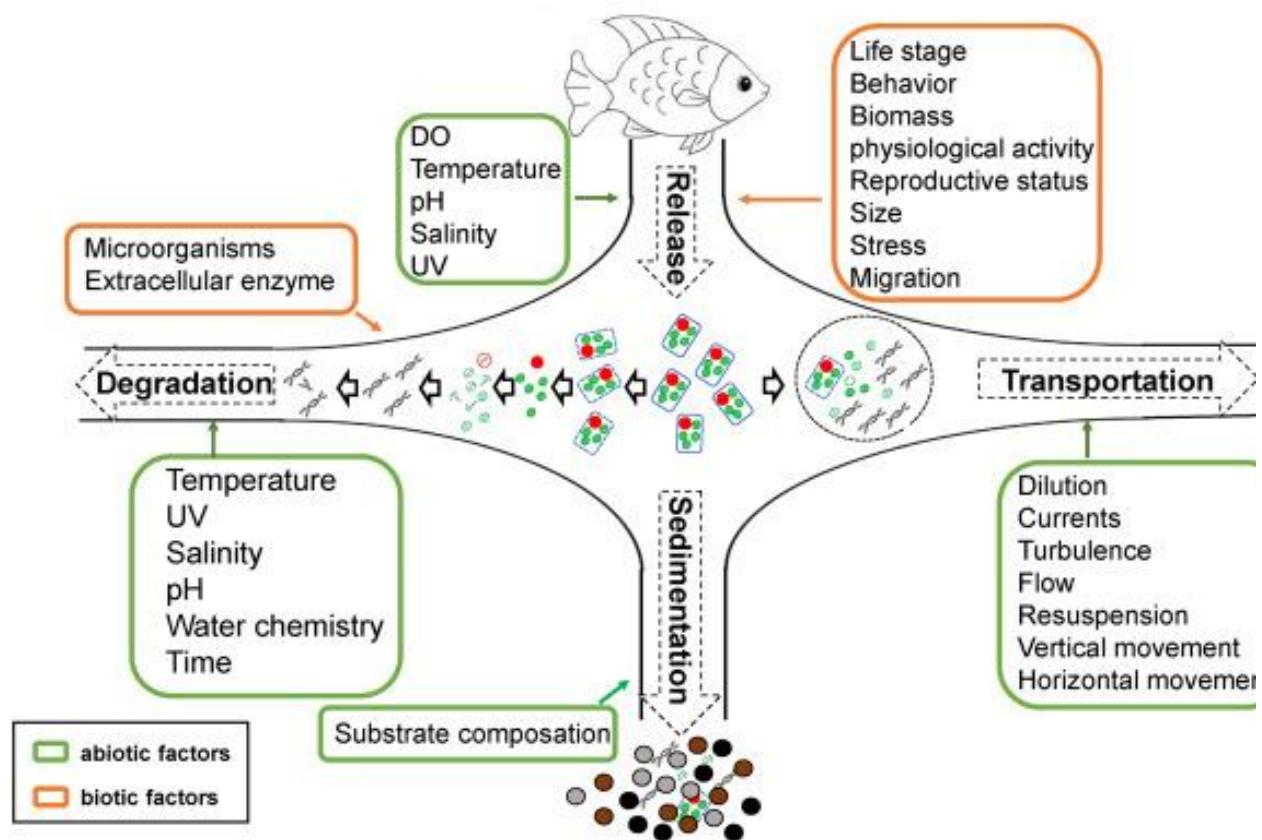
Otros factores que afectan la detección



DETECCIÓN

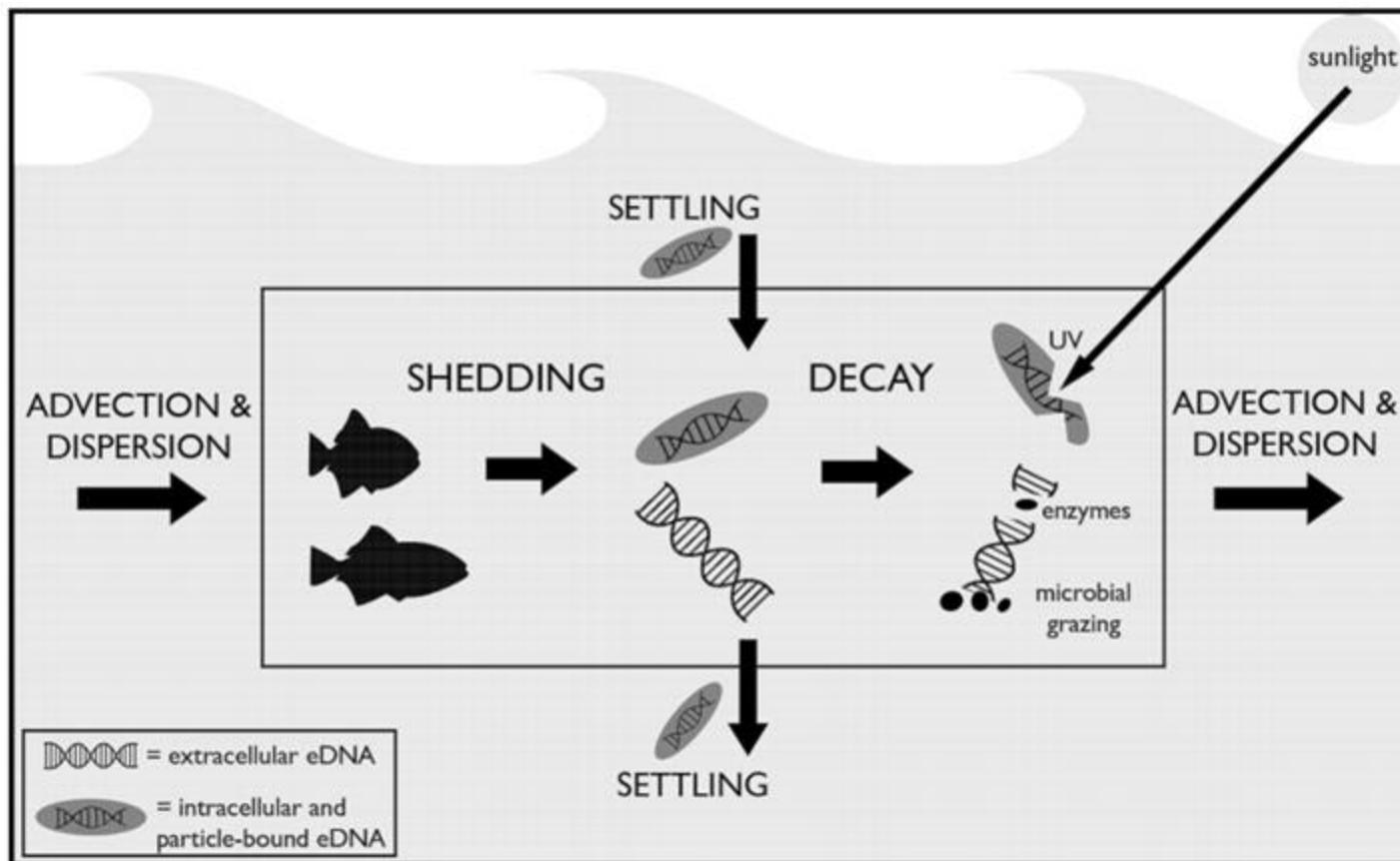
Factores que afectan la detección de eDNA

- Procesos determinísticos:
Degradación
- Procesos estocásticos:
Abióticos: Factores ambientales (temperatura, pH, transporte-advección, UV).
Bióticos: degradación microbiana, biofiltración.
- Biología, ecología y estacionalidad de los organismos objetivo.
- Especies móviles > liberación eDNA
- Especies sedentarias > liberación más localizada
- Reproducción, ej. Desove = boom de eDNA



Ecología del eDNA

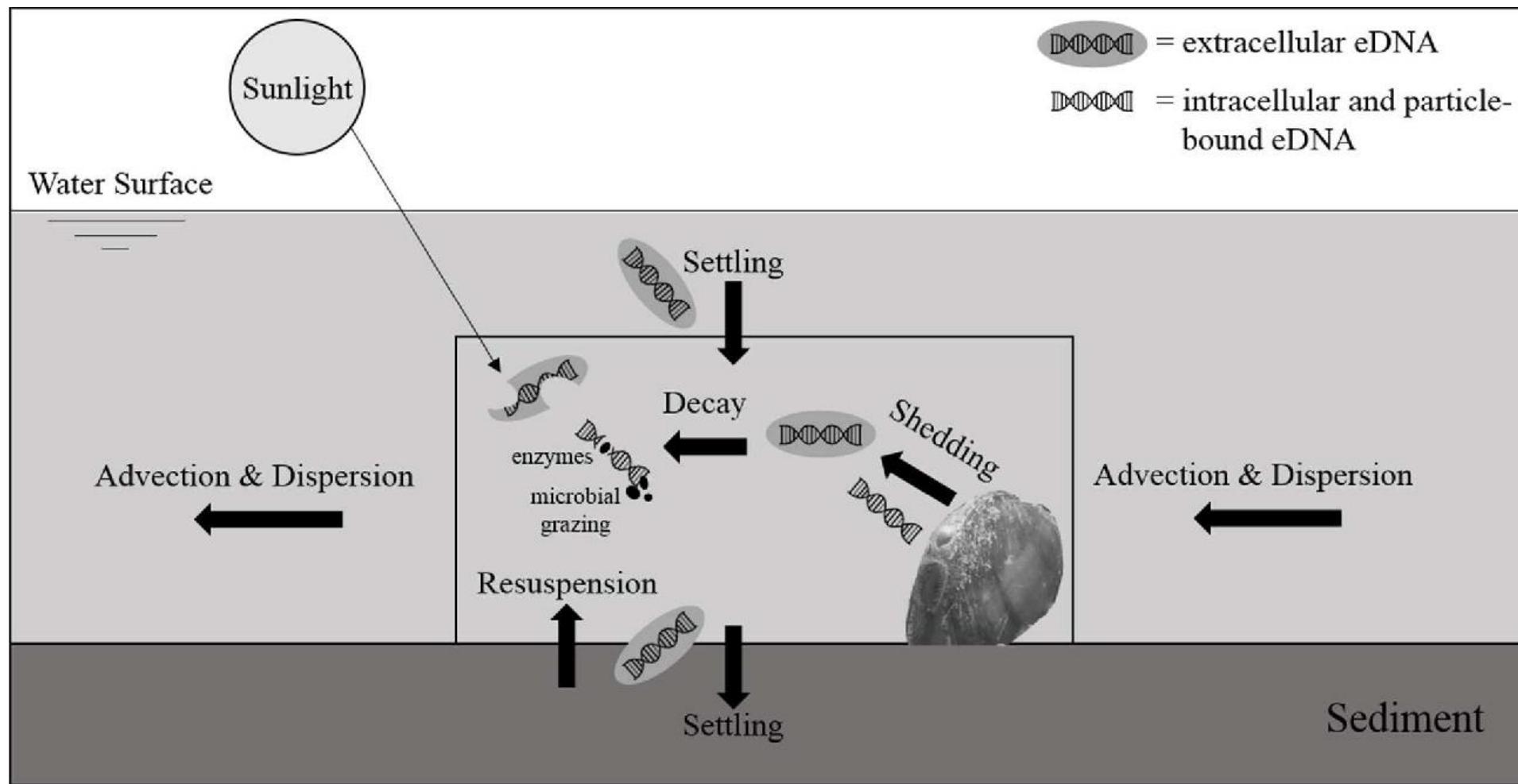
Cómo las características oceanográficas efectan la detección de especies



- temperatura
- profundidad
- corrientes
 - mareas
- hora del día
- tipo de hábitat

Probabilidad de detección

Ecología del eDNA*



Terms

Environmental DNA (eDNA)

DNA captured from an environmental sample without first isolating any target organisms (Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012). Traces of DNA can be from faeces, mucus, skin cells, organelles, gametes or even extracellular DNA. Environmental DNA can be sampled from modern environments (e.g., seawater, freshwater, soil or air) or ancient environments (e.g., cores from sediment, ice or permafrost, see Thomsen & Willerslev, 2015).

Community DNA

DNA is isolated from bulk-extracted mixtures of organisms separated from the environmental sample (e.g., soil or water).

Macro-organism environmental DNA

Environmental DNA originating from animals and higher plants.

Barcode

First defined by Hebert et al. (2003), the term refers to taxonomic identification of species based on single specimen sequencing of diagnostic barcoding markers (e.g., COI, *rbcL*).

Metabarcoding

Taxonomic identification of multiple species extracted from a mixed sample (community DNA or eDNA) which have been PCR-amplified and sequenced on a high-throughput platform (e.g., Illumina, Ion Torrent).

High-throughput sequencing (HTS)

Sequencing techniques that allow for simultaneous analysis of millions of sequences compared to the Sanger sequencing method of processing one sequence at a time.

Community DNA metabarcoding

HTS of DNA extracted from specimens or whole organisms collected together, but first separated from the environmental sample (e.g., water or soil).

eDNA ecology

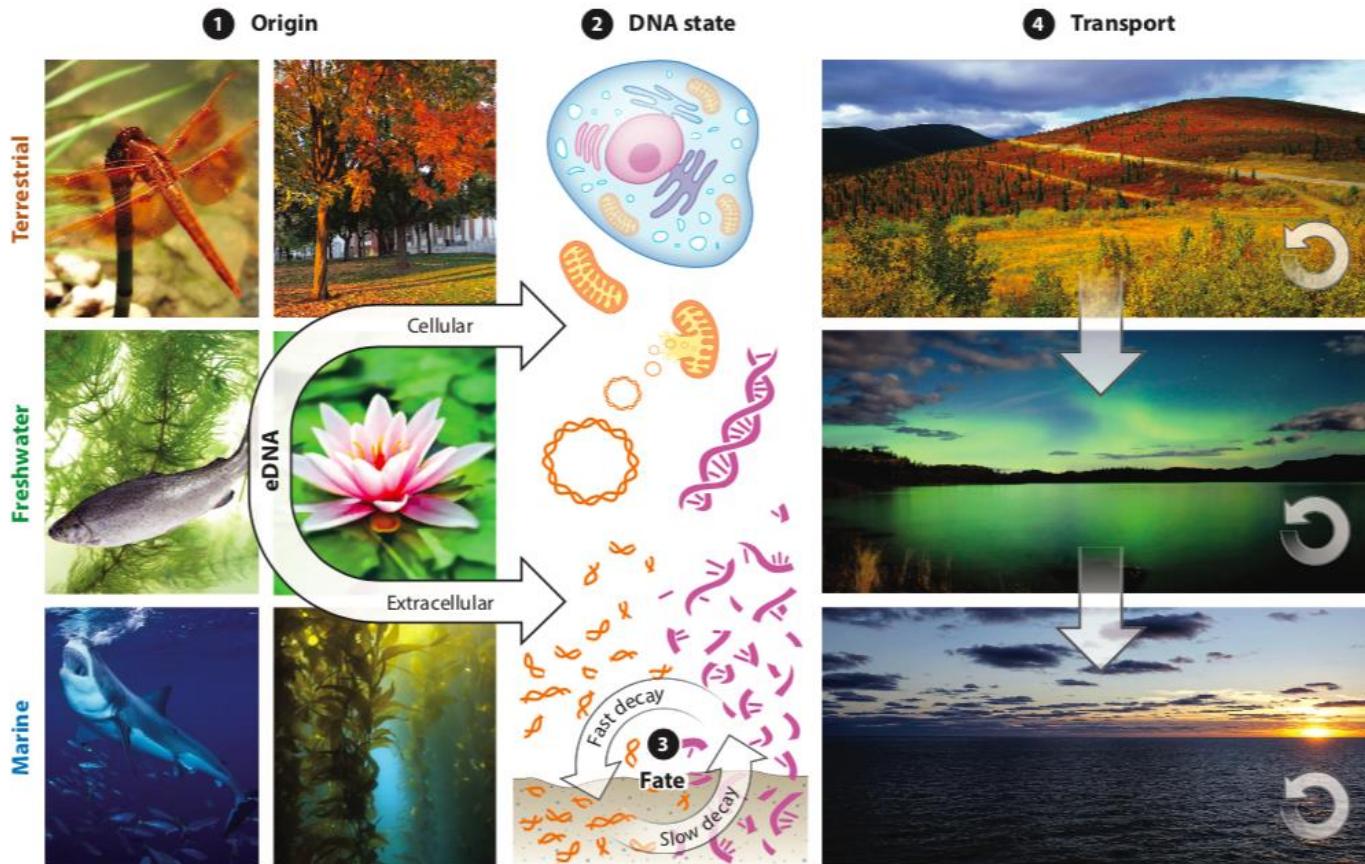
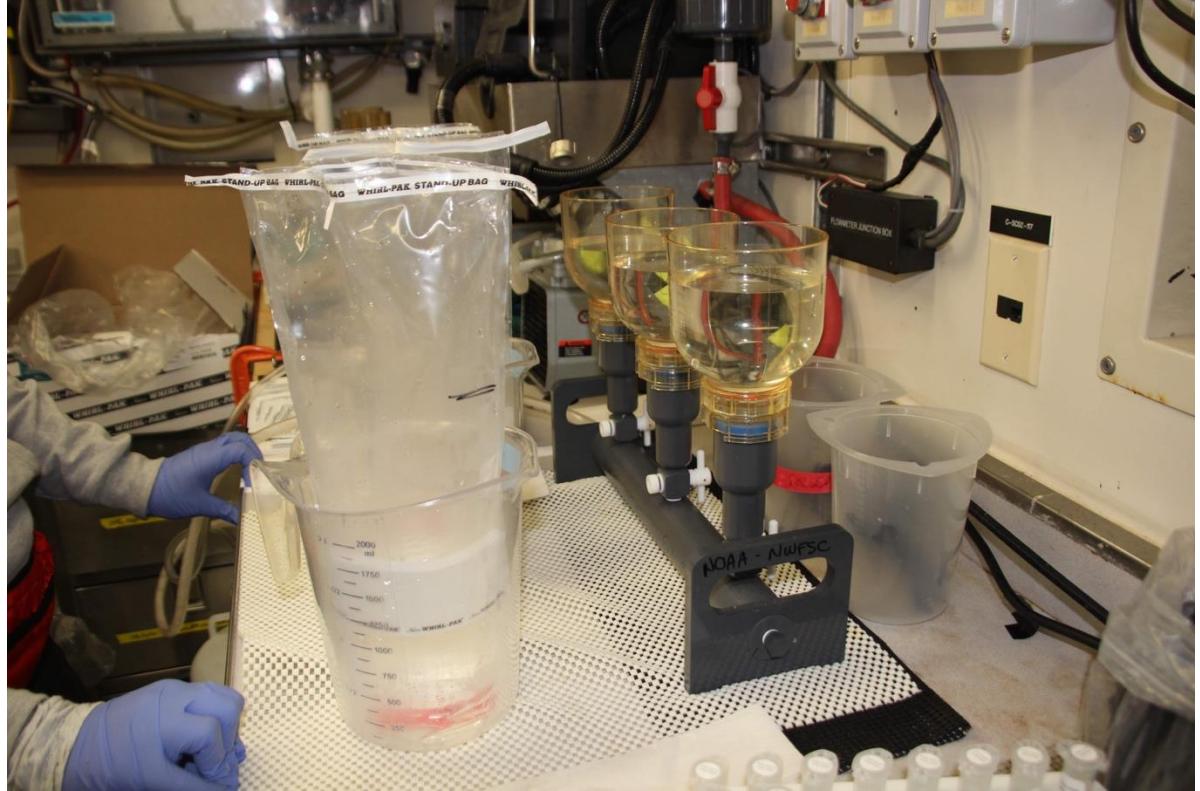
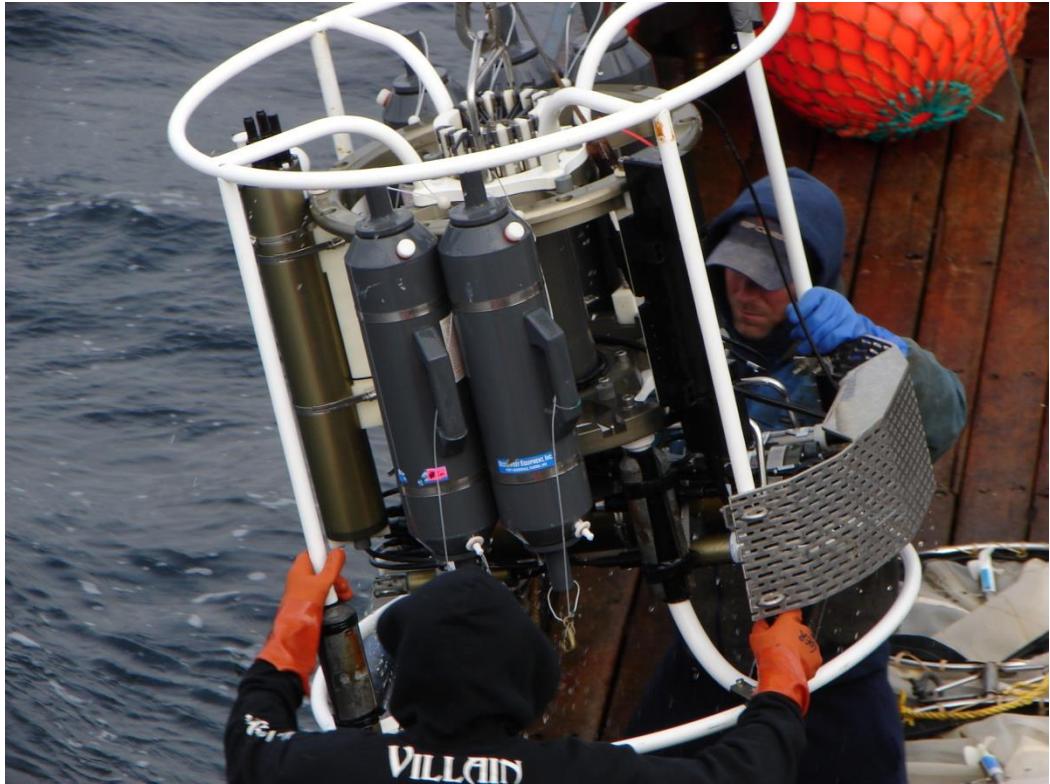


Figure 2

Ecology of environmental DNA (eDNA): its (1) origin (derived from resident organisms), (2) state (cellular and extracellular form; free or particulate), (3) fate (slow decay in sediments or fast decay in water column), and (4) transport within and across environments (by wind, water currents, biotic activity, etc.).

Barnes & Turner 2016; Cristescu & Hebert 2018

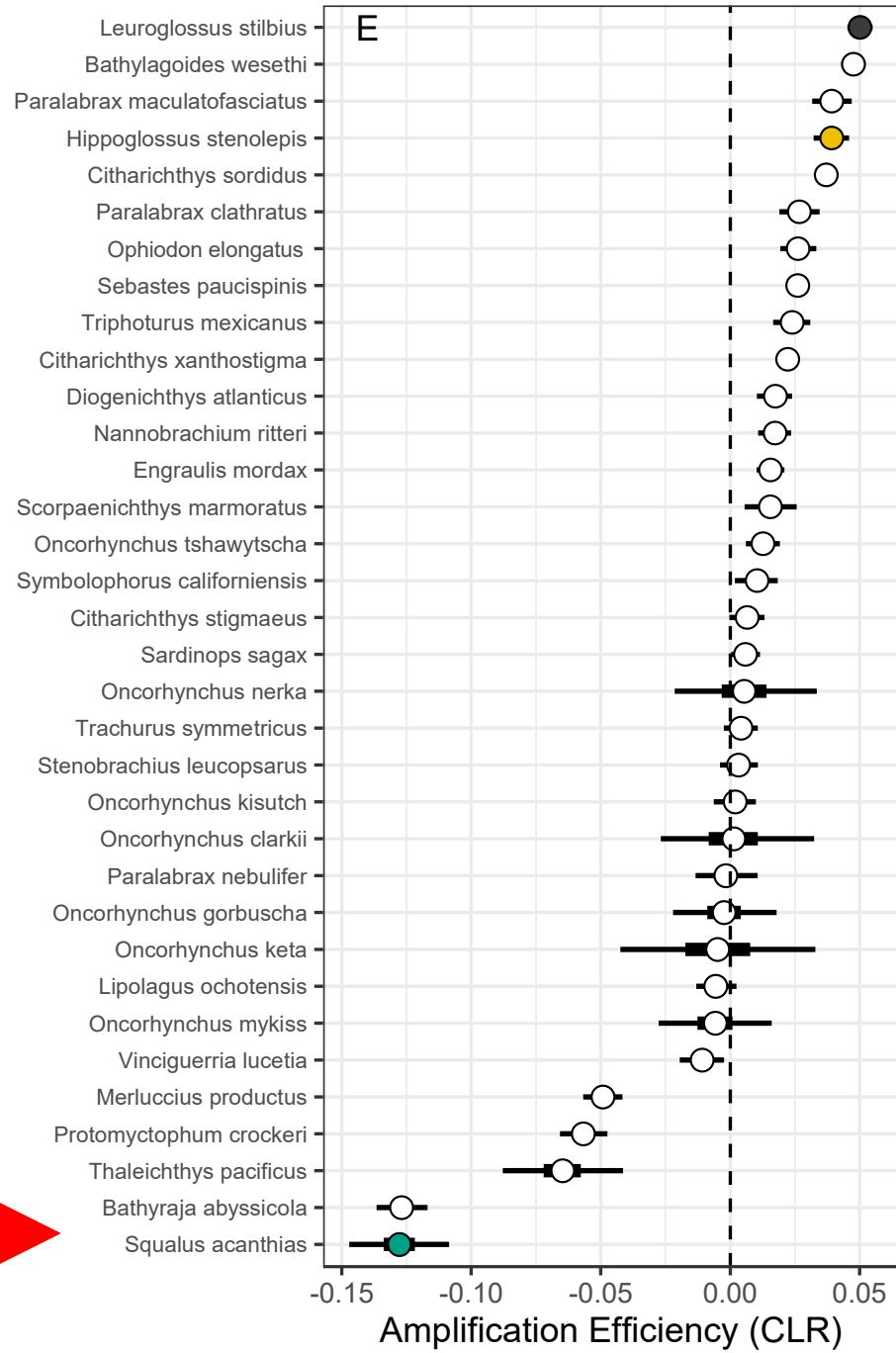
I'd like to see more than one species at a time.

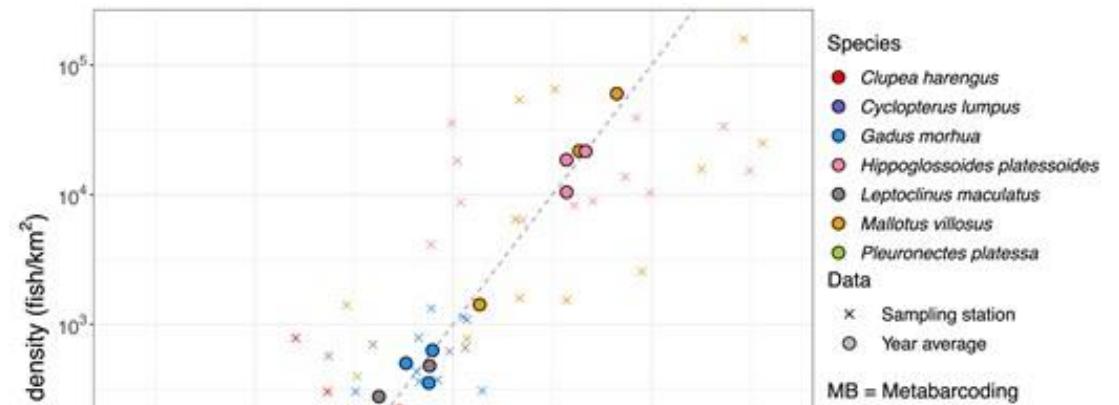
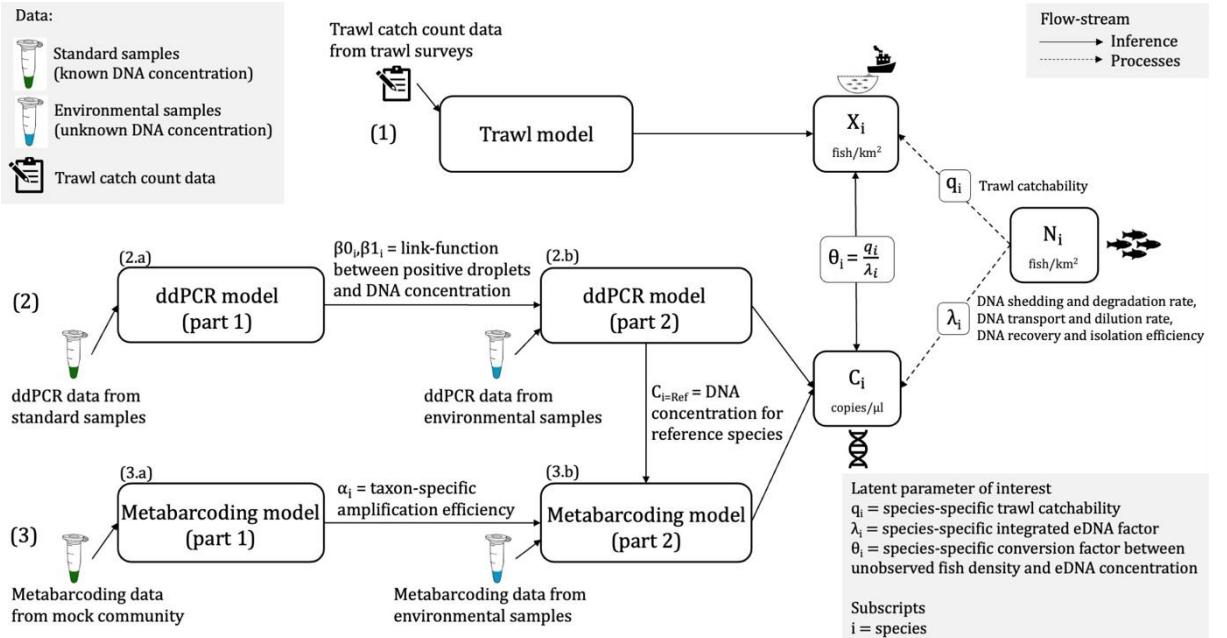
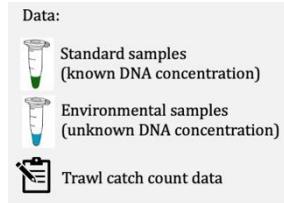


I can re-use those existing water samples!

Among-species variation in amplification

SHARKS!





Predicting trawl catches using environmental DNA

Gledis Guri ^{1,2,*}, Andrew Olaf Shelton³, Ryan P. Kelly⁴, Nigel Yoccoz⁵, Torild Johanse
Kim Præbel^{1,2}, Tanja Hanebrekke¹, Jessica Louise Ray⁶, Johanna Fall ^{1,7}, Jon-Ivar Westgaard¹

¹Norwegian Institute of Marine Research, Department Trawl, 0007, Norway

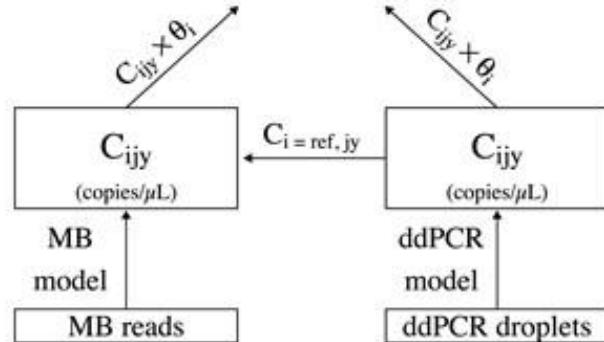
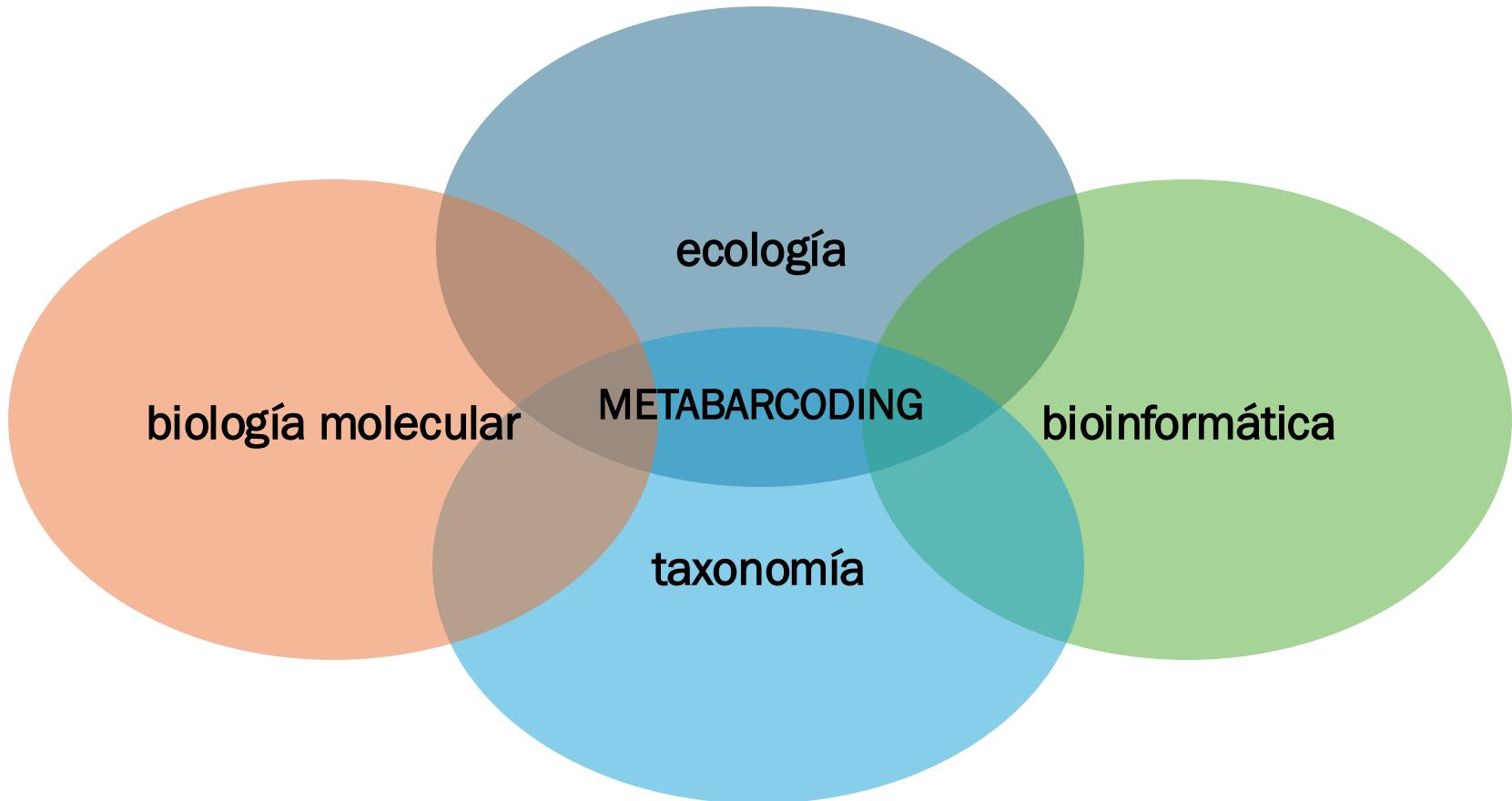


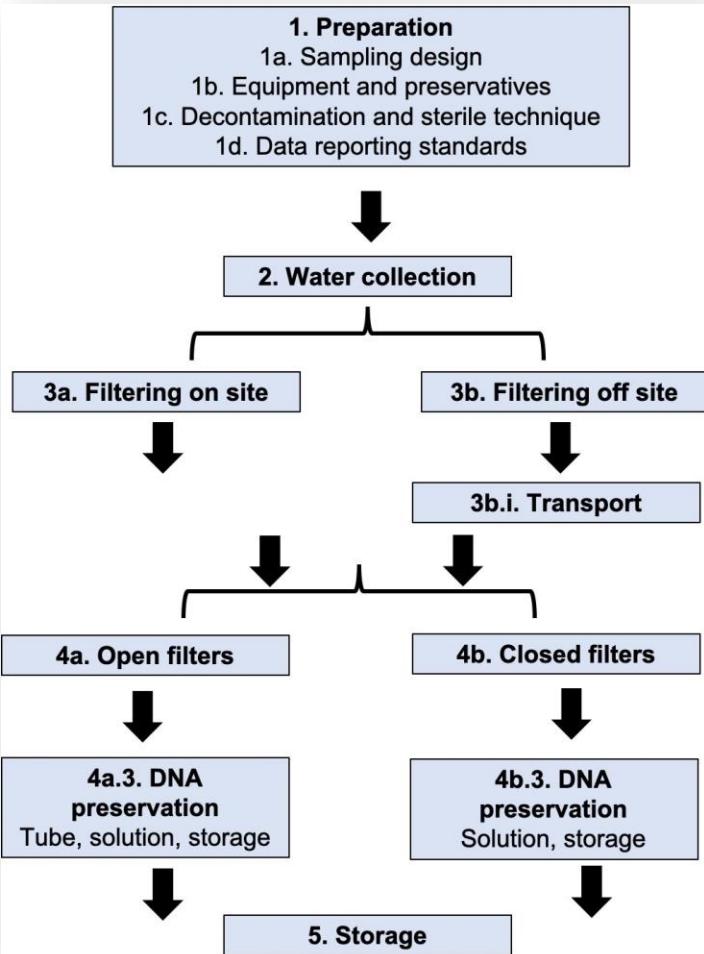
Table 2. Recommendations for conducting environmental DNA studies

Recommendations for eDNA sampling, analysis and reporting
Pilot study
<ul style="list-style-type: none">• Implement field sampling protocol and evaluate detection rates with sampling and site data (e.g. filter material and pore size, sample volume, number of samples, spatial distribution of samples)• Test extraction and analysis protocols• Validate eDNA assays <i>in silico</i>, <i>in vitro</i> and <i>in situ</i>
Field
<ul style="list-style-type: none">• Collect negative controls• Employ strict decontamination protocols for all equipment and clothing that is reused• Collect multiple samples at each site to address false negatives and estimate detection probabilities
Laboratory
<ul style="list-style-type: none">• Process samples only in a dedicated clean laboratory (completely separated from PCR products) with restricted access, regular decontamination (bleach, UV) and use of filtered tips• Use probe-based qPCR if target is a few well-characterized species; for many target or unknown species, use high-throughput sequencing• For qPCR, use technical replicates (≥ 3), and internal positive control to test for inhibition• Archive samples at -20 or -80°C
Reporting
<ul style="list-style-type: none">• Report quantification values as copy #/volume sampled• Acknowledge challenges inferring: across space/time, presence vs. viable population and confounding sources of eDNA• Maintain archived database with collection date and exact geographic location

Disciplinas y habilidades a desarrollar:



Water eDNA sampling SOP



- 1. Preparation**
- Sampling design
 - Sample container
 - Sterilized bottle (1L Nalgene or similar)
 - Sterile Whirlpak (or similar)
 - Enteral feeding bag
 - Filter choice
 - Pore size: Dependent on application. Recommended 0.2-0.45um filter for microbial applications, although 1um may be suitable for large volume collection.
 - Composition: Cellulose nitrate (CN), Polyethersulphone (PES), Polycarbonate (PC) or other hydrophilic materials demonstrated to perform equivalently.
 - Sterility: When possible, an enclosed filter unit (e.g. Sterivex) is preferred over an open filter unit (e.g. Stericup or Swinnex). All filters should be sterile and DNA-free.
 - Volume to collect/filter
 - Volume to collect is determined by a combination of detection sensitivity, resources to filter/analyze multiple replicates, spatial coverage, oligotrophic/eutrophic conditions, etc.
 - More replicates and larger volumes will provide greater probability of detection.
 - Default: collect 1L samples and filter the maximum volume possible.
 - Replicates
 - Replicates help increase detection probability and support statistical comparisons.
 - Default: 3 replicates.
 - If resources are limited, replicates may be combined for analyses.
 - Field blanks
 - The number of field blanks collected is determined by both cost and importance of detecting contamination at regular intervals to protect impacted samples. See [SOP 1c](#) for more guidance.
 - Default: 1 field blank per sampling outing.
- b. Preservatives**
- Preservation solution
 - 95-100% molecular grade Ethanol
 - RNAlater
 - Silica beads
 - Lysis buffer (e.g. Longmire's Solution, bead solution)
 - Sterilization and sterile technique (please see [SOP 1c](#)).
 - Data reporting standards (please see [SOP 1d](#)).
- 2. Water collection**
- 3. Grab sample:**
- Rinse sterile bottle 3x with sample water, discarding rinse water downstream or offshore. Submerge bottle in water to collect sample, ideally with sampling pole to minimize risk of contamination. Sterile Whirlpaks (or similar) can also be used for water collection.
- 3a. Filtering on site**
Proceed to filtration steps below.
- 3b. Filtering off-site**
- Unfiltered water samples should be stored on ice and in the dark. Filtration should occur within 4-8 hours of sample collection. Record storage conditions and time between sample collection and filtration.
- 4a. Open filter**
- Load filter onto filter holder using sterile tweezers.
 - Invert sample gently to homogenize. Load filter onto filter holder using sterile tweezers. Filter sample until volume is completed or filter clogs. Pass ~10cc air through filter to dry. Record volume filtered. Remove filter from filter holder, gently roll filter using clean forceps; two sets of forceps can be used if necessary. Place filter in sterile tube (Falcon or Eppendorf appropriate for size) for preservation.
 - If using liquid preservation, the filter must be thoroughly submerged in the preservation solution. Label the tube and place it in a labeled bag.
- 4b. Closed filter**
- Invert sample gently to homogenize.
 - Filter sample until volume is completed or filter clogs. Pass ~10ml air through filter to dry. Record volume filtered.
 - If using liquid preservation, load preservation solution into the filter unit using a sterile pipette, ensuring the membrane is fully covered, and place the filter and housing in a labeled bag.
- 5. Storage**
- Keep tubes with filters (with or without preservation solution) on ice, dry ice, or liquid nitrogen until they are transported to -20°C or -80°C freezer for storage.
- TERMS AND DEFINITIONS**
- REFERENCES**
- Working draft of CEN Standard
- Links to training videos:**
- Table with part numbers for ordering:**

Metadata Reporting

Metadata reporting standards (SOP 1d)

Introduction:

Metadata helps to provide the context to your DNA data. High-quality metadata allows others to search, discover, and compare your data with those collected by other users. Thinking about metadata documentation at the initiation of a project will help make this process easier and increasingly routine.

Different types of metadata are generated throughout the process of collecting and analysing DNA samples (Figure 1). It is useful to consider each of these stages, and their associated metadata, in developing a metadata template for your project.

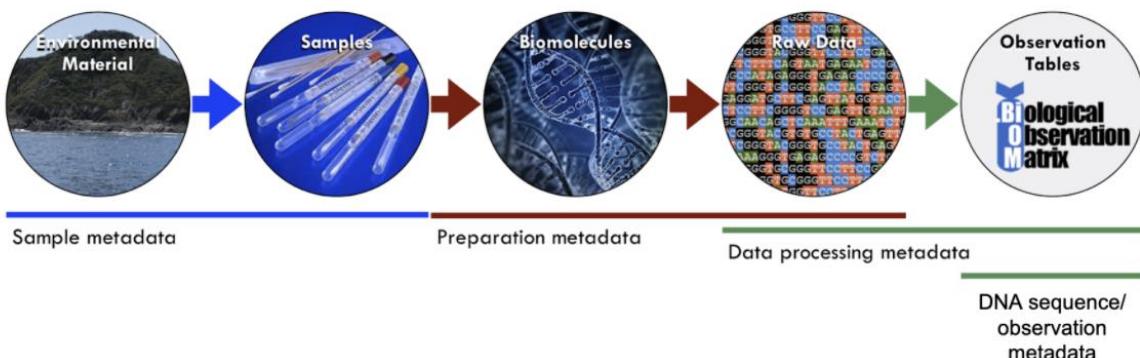


Figure 1. Sample metadata (modified from (Thompson et al., 2020)).

I. Examples of pre-existing data reporting standards and related data reporting resources

Standard	Link	DataTypes
MIQE/dMIQE	http://rdml.org/index.html	qPCR/ddPCR data Minimally required for qPCR and ddPCR data
MixS	https://gensc.org/mixs/	Minimally required for metagenomic sequencing data
Species BARCODE data standard	https://www.ebi.ac.uk/ena/submit/species-barcode-checklist	DNA sequence data
FAIR data principles	https://www.go-fair.org/fair-principles/	All
CEDEN vocabulary	http://ceden.org/ceden_namewcodes.shtml and there is a request form for new templates http://ceden.org/vocabulary_request.shtml	All
SWAMP	http://swamp.ornl.gov/introduction-to-metadata-and-ontologies/	Field data
NMDC	https://microbiomedata.org/introduction-to-metadata-and-ontologies/	All, but not probe-based
QIITA metadata wizard	https://qiita.ucsd.edu/qimap/	All
II. Recommended minimum metadata for DNA samples.		
Category	Field	
Environmental material	Latitude/Longitude	
	Environmental package (Seawater/lake/river/etc)	
	Date and time of sample collection	
	Name and contact info of sample collector	
	Volume filtered	
	Water chemistry data (Temp/salinity/pH)	
	Flow velocity	
	Recent weather, precipitation, flow events	
Samples	Filter details (make, model, pore size, lot number)	
	Preservation solution	
	Field sample conditions during transport	
	Field/lab filtered or preserved	
	Date and time of filtration and preservation	
	Storage conditions (e.g. -20C, -80C, liquid nitrogen)	
	DNA extract sample volume	
	DNA concentration	
	Date of storage	
	Temperature of storage	
Data processing metadata: Probe-based qPCR	Design and validation methods Primer/probe sequences, amplicon length Positive and negative controls Inhibition detection and handling	

Estudio	Especie objetivo / Aplicación	Niveles de validación	Tecnología utilizada	Principales aportes metodológicos
Ficetola et al. (2016)	Diversas especies acuáticas	In silico	cPCR / qPCR	Establecen criterios para diseño de primers específicos y evaluación bioinformática.
Bustin & Huggett (2017)	Estándares de qPCR	In vitro	qPCR	Proponen parámetros de calidad: eficiencia, R ² , LoD, LoQ y reproducibilidad.
Valentini et al. (2016)	Biodiversidad en ríos europeos	In situ	qPCR / metabarcoding	Validan desempeño de ensayos en campo con controles y réplicas ambientales.
Minamoto et al. (2021)	Peces de agua dulce en Japón	In vitro / in situ	qPCR / ddPCR	Comparan sensibilidad entre tecnologías y proponen protocolos estandarizados.
De Brauwer et al. (2022)	Especies marinas tropicales	In silico / in vitro	qPCR	Evalúan especificidad de primers mitocondriales y condiciones óptimas de PCR.

Cool examples:

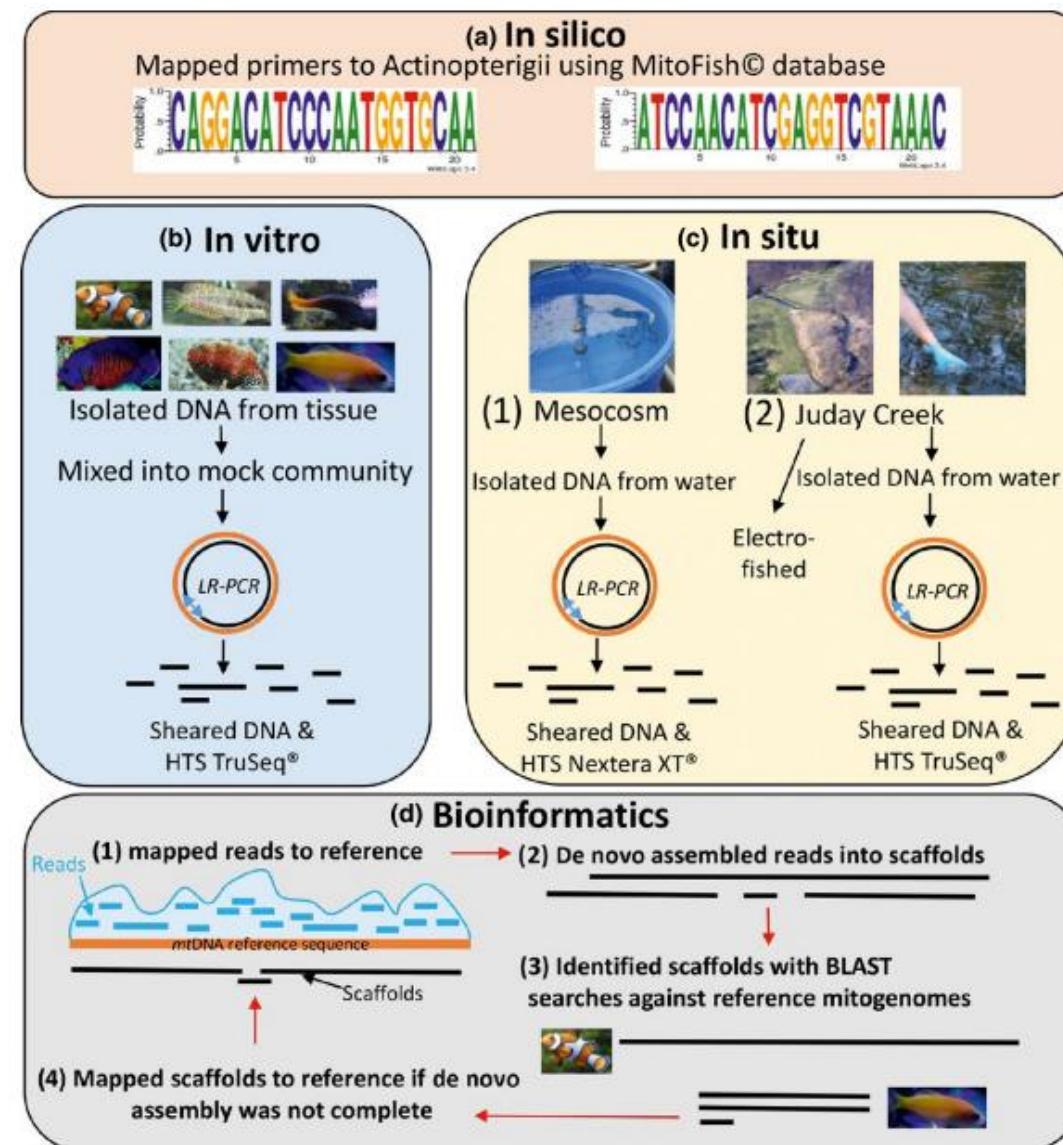


FIGURE 1 Methods overview for laboratory workflow used to design and test long-range PCR (LR-PCR) for sequencing of whole mitochondrial genomes from environmental DNA. Each box (a-d) represents the steps used in silico, in vitro and in situ to validate the method. High-throughput sequencing on the Illumina MiSeq is abbreviated as HTS

RESEARCH ARTICLE

Long-range PCR allows sequencing of mitochondrial genomes from environmental DNA

Kristy Deiner* | Mark A. Renshaw* | Yiyuan Li | Brett P. Olds | David M. Lodge | Michael E. Pfrender

Cool examples: Looking for the Loch Ness Monster



- >1000 observations
- Different scientific expeditions since 1934 camera and binoculars, sonar, surveys, under water drones, 2018 (eDNA).
- The search of Loch Ness is about so much more than mysterious water monsters.



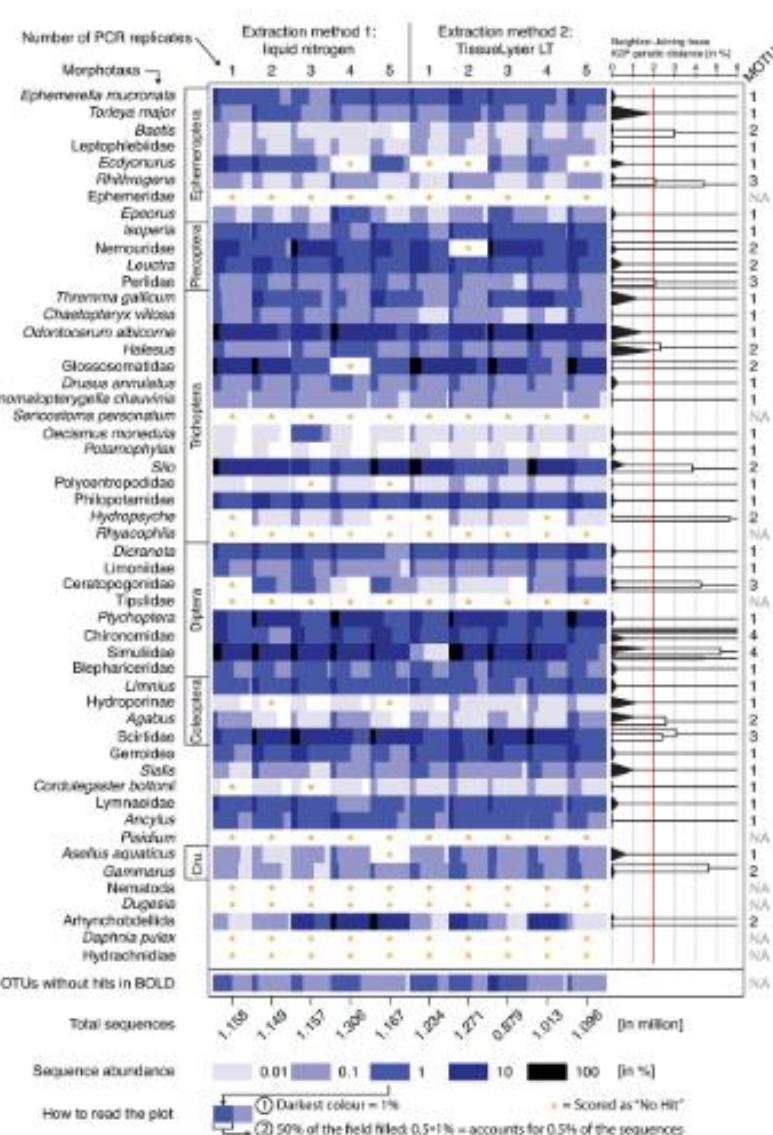
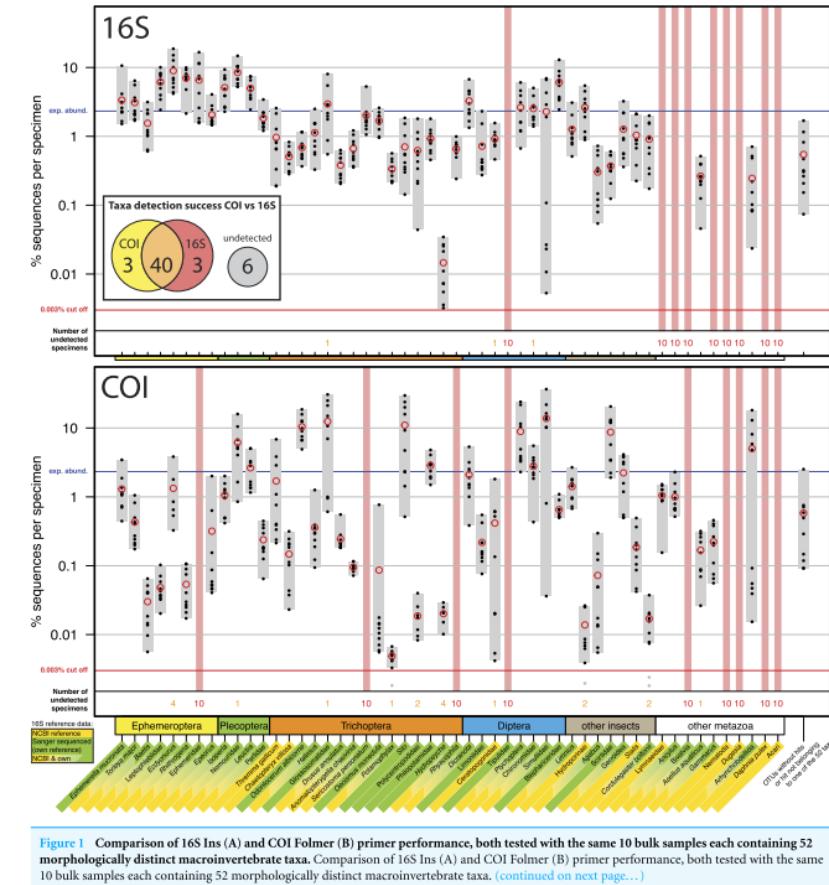
Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding Protocol

Vasco Elbrecht, Florian Leese*

Department of Animal Ecology, Evolution and Biodiversity, Ruhr University Bochum, Universitaetsstrasse 150, D-44801 Bochum, Germany

* florian.leese@rub.de

Extraction and markers



Primer efficiency is highly species-specific, which would prevent straightforward assessments of species abundance and biomass in a sample.

Thus, PCR-based metabarcoding assessments of biodiversity should rely on presence-absence metrics.

Cool examples:

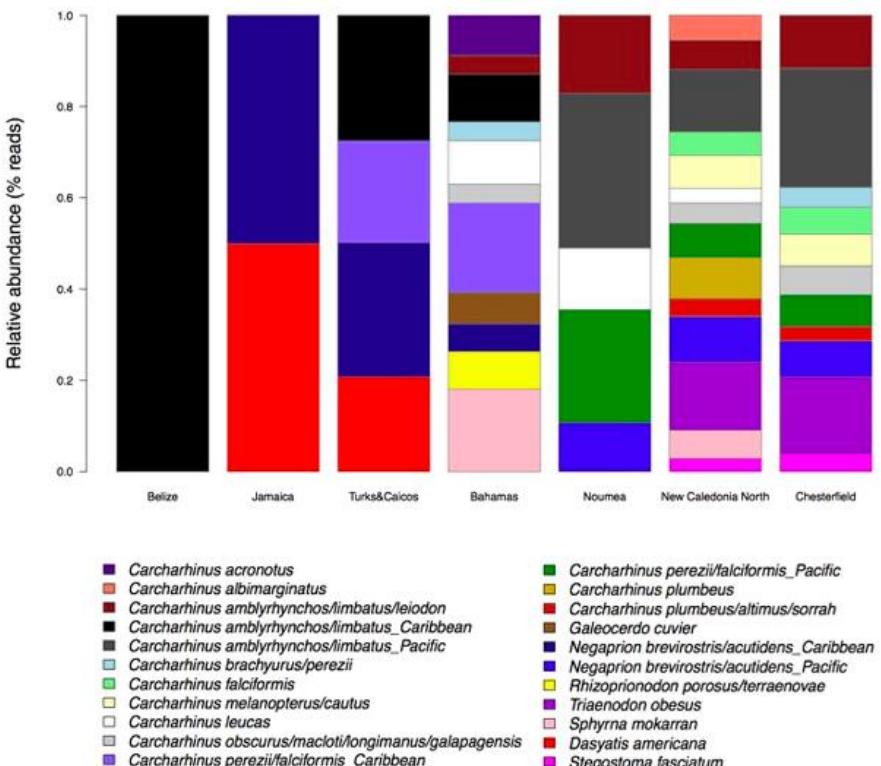
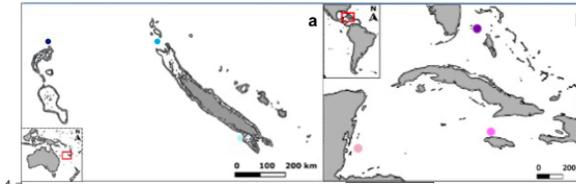
Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact

- Predict and compare elasmobranch diversity in the Caribbean and New Caledonian locations impacted by various levels of anthropogenic pressures.
- mtDNA COI 127bp
- 22 species of shark (12 Caribbean, 16 New Caledonia and 9 in both)
- ✓ eDNA good for species richness and relative abundance
- ✓ Good for monitoring purposes
- ✓ The patterns of MOTU richness and abundance of sequence reads follow the level of anthropogenic impact in each location.

Caribbean: Long history of elasmobranch exploitation and high anthropogenic pressures
 Belize
 Jamaica
 Turks & Caicos

Bahamas: Gillnet and long line fishing prohibited since 1991, and is a shark sanctuary

New Caledonian: Elasmobranchs do still occur in relatively high numbers around remote, isolated locations such as coral reefs on uninhabited atolls
 Noumea
 New Caledonia
 Chesterfield Id



Una especie Monitoreo de merluza con eDNA



Environmental DNA provides quantitative estimates of Pacific hake abundance and distribution in the open ocean

Andrew Olaf Shelton¹, Ana Ramón-Laca⁴, Abigail Wells², Julia Clemons³, Dezheng Chu³, Blake E. Feist¹, Ryan P. Kelly⁵, Sandra L. Parker-Stetter^{3,6}, Rebecca Thomas¹, Krista M. Nichols¹ and Linda Park¹

¹Conservation Division, Northwest Fisheries Science Center, Seattle, Washington, United States of America

A workflow for the relative quantification of multiple fish species from oceanic water samples using environmental DNA (eDNA) to support large-scale fishery surveys

Ana Ramón-Laca , Abigail Wells, Linda Park

Published: September 27, 2021 • <https://doi.org/10.1371/journal.pone.0257773>

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0257773>

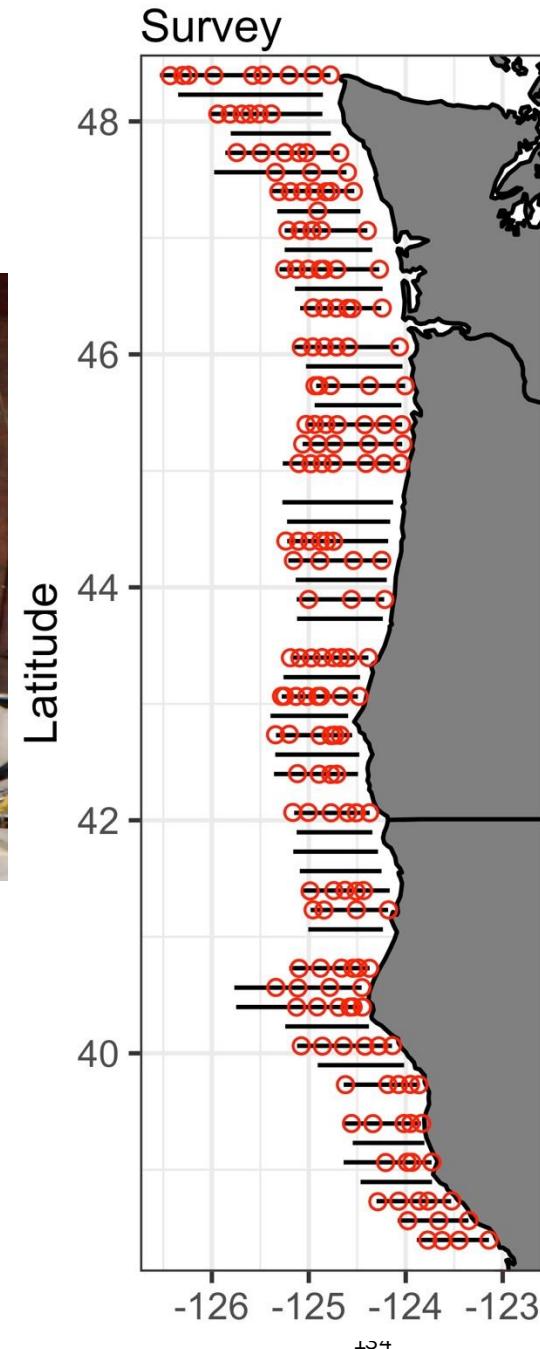
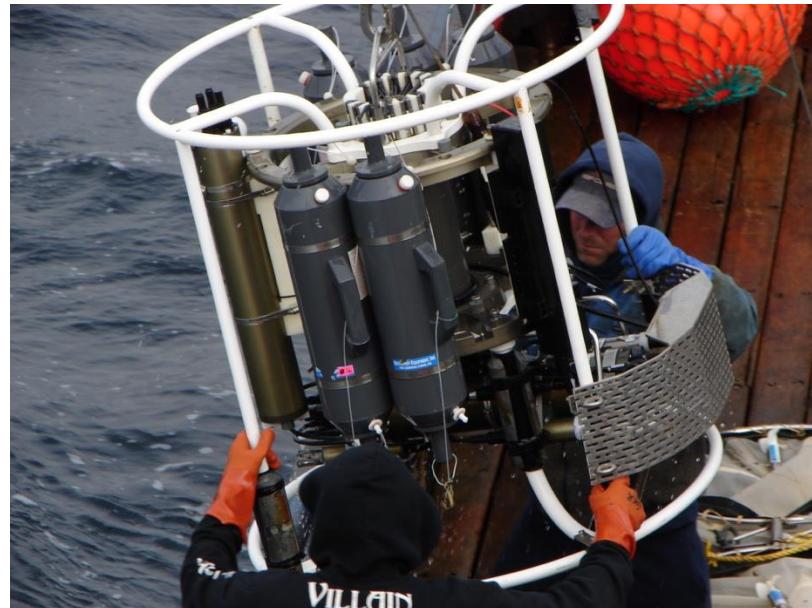
<https://royalsocietypublishing.org/doi/10.1098/rspb.2021.2613>

<https://www.fisheries.noaa.gov/feature-story/traces-dna-can-accurately-assess-fish-ocean>

Photo: Jeff Bash, NOAA Fisheries West Coast

eDNA (2019, 2021, 2023, ...)

- 2019: 186 stations sampled, 1972 water samples (night)
 - San Francisco to Canadian border
- 2 x 2.5 L seawater filtered from depth
 - 3m, 50m, 100m, 150m, 300m, 500m
- Single-species qPCR primer
 - Pacific hake DNA concentration



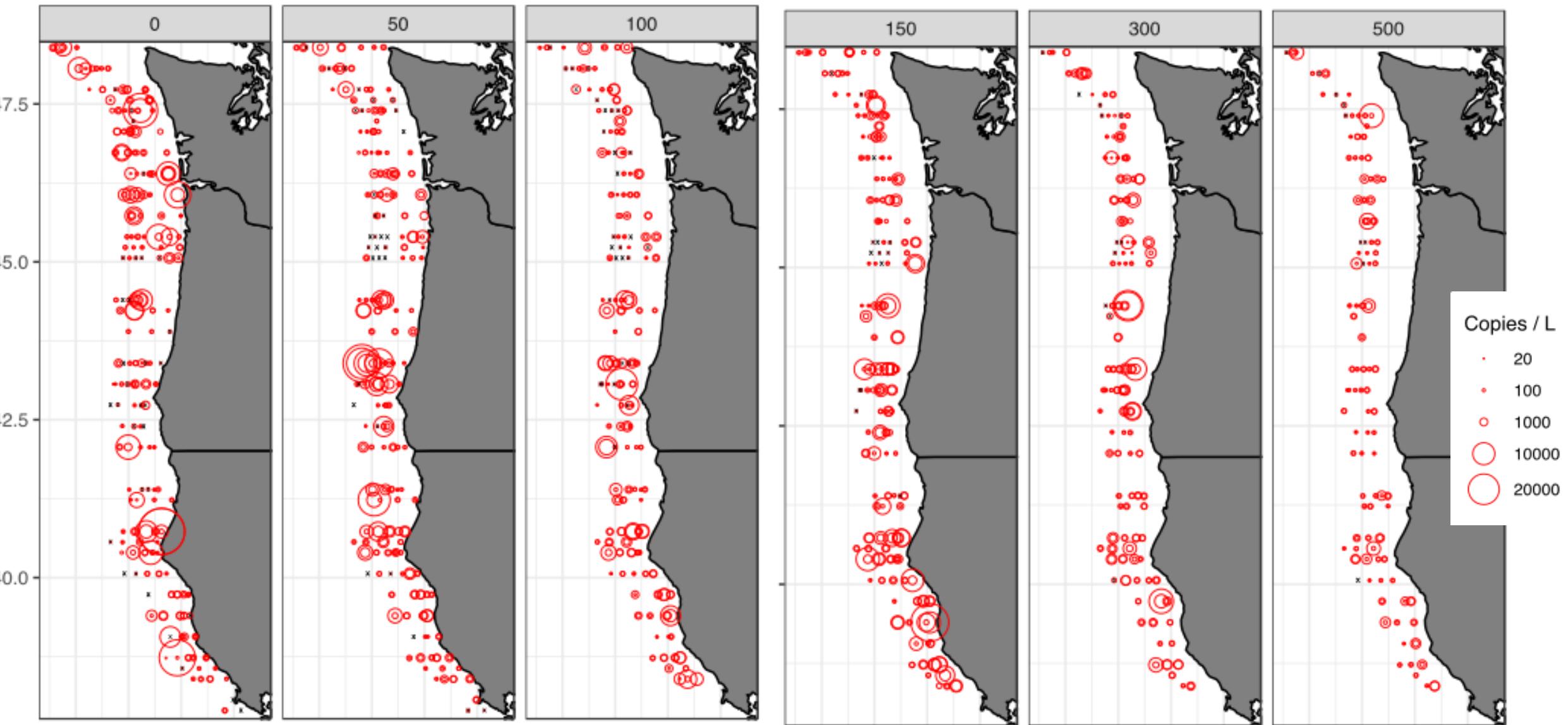
Acoustic-Trawl

- Daytime sampling.

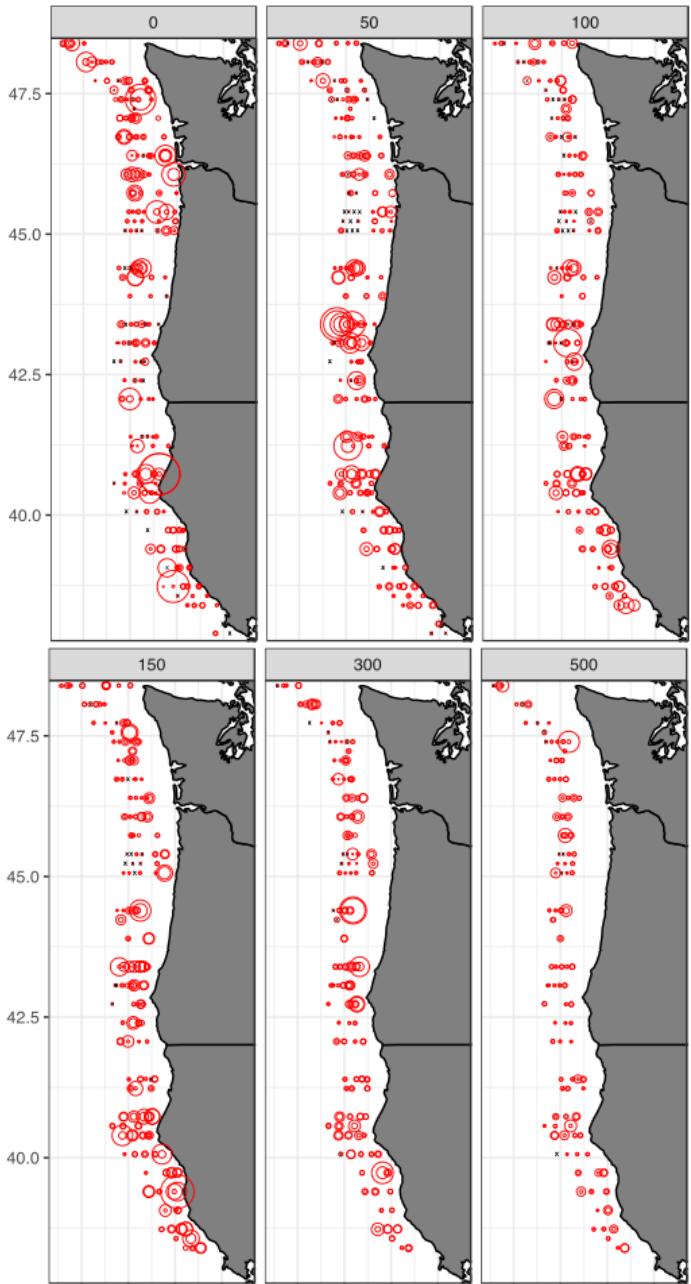
Largest eDNA sampling effort in the world to date.

**Collecting and processing water for eDNA is feasible at scale...
but not a trivial investment of time or resources**

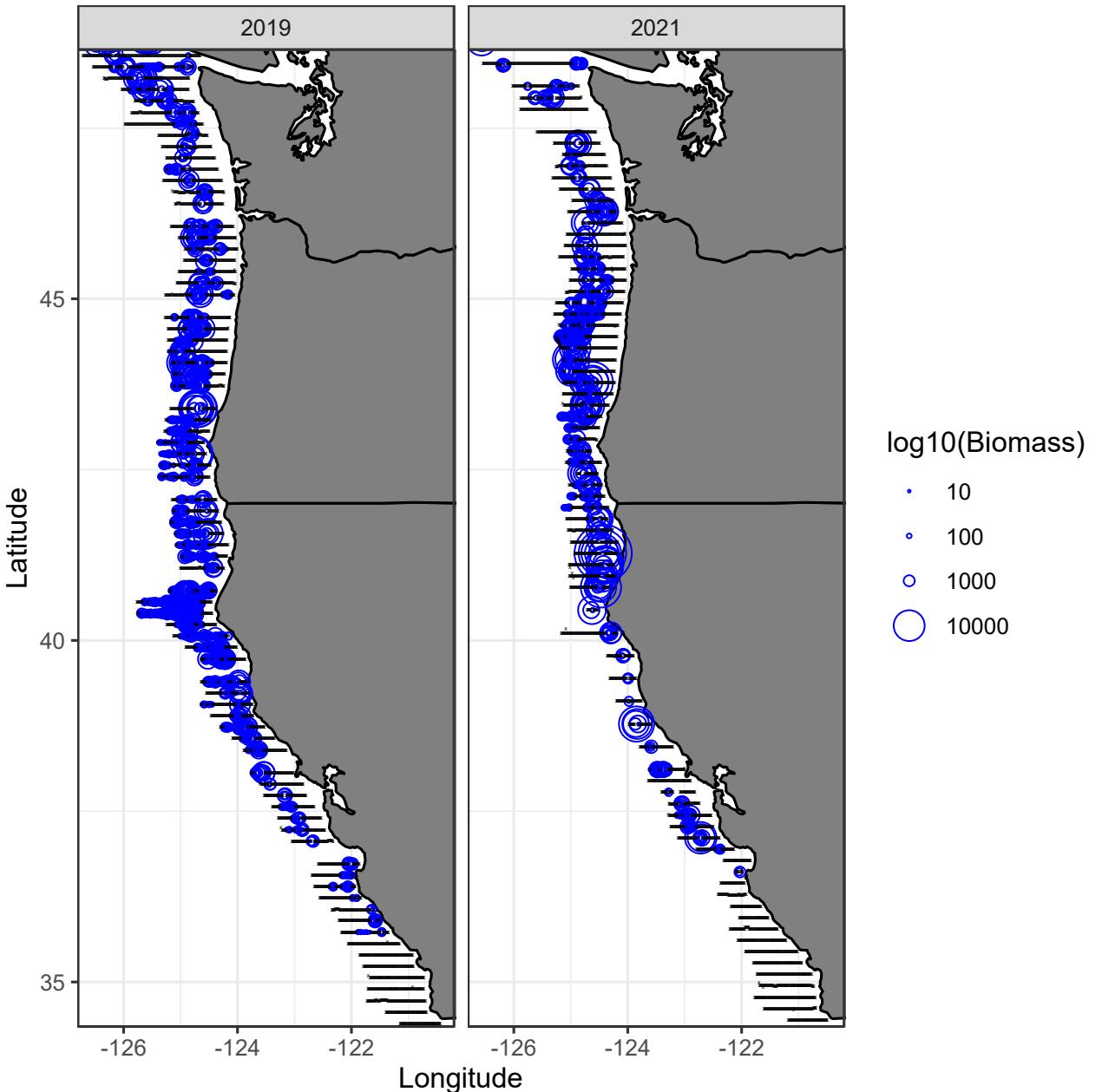
Spatial and depth patterns of hake DNA



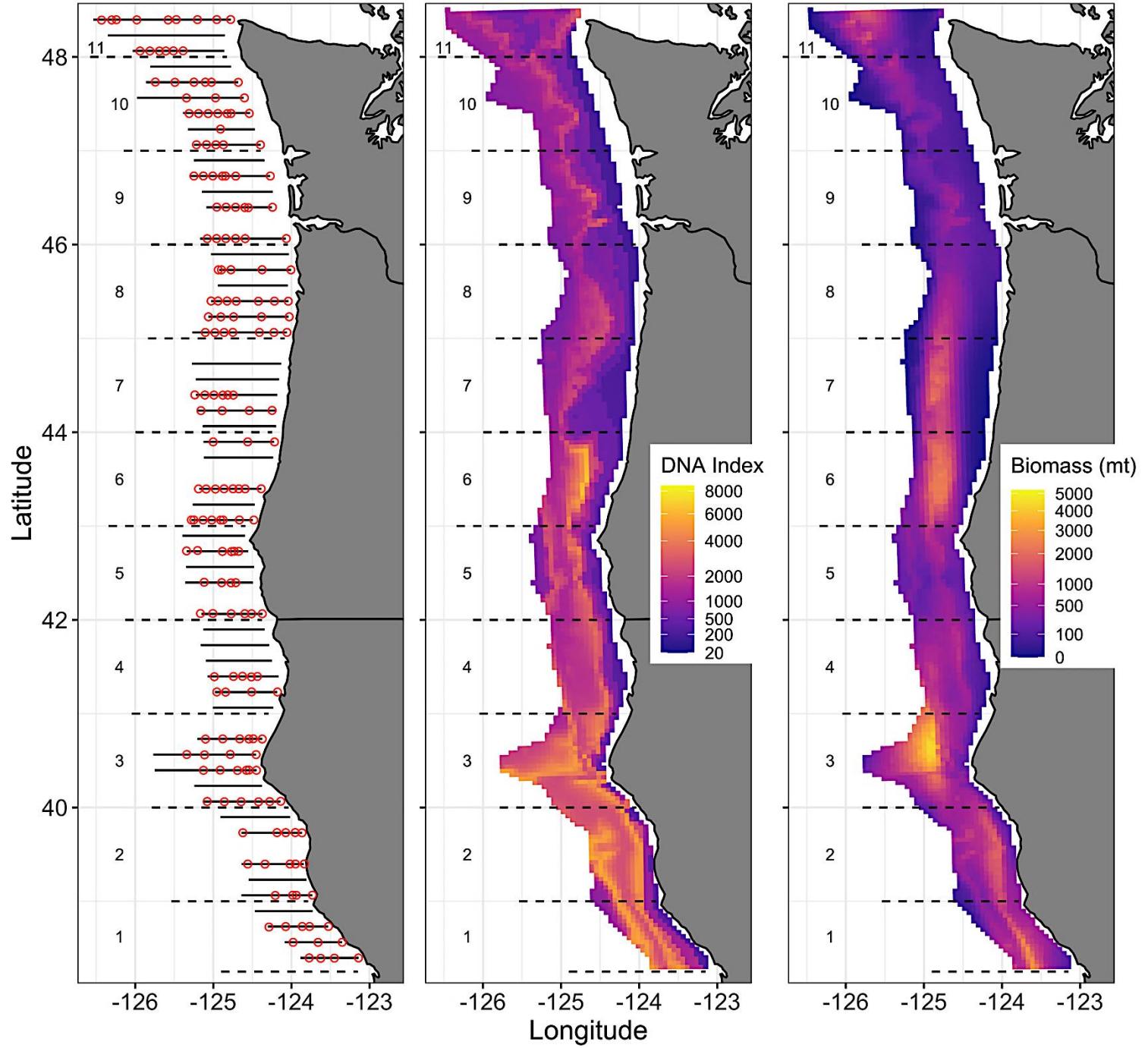
eDNA



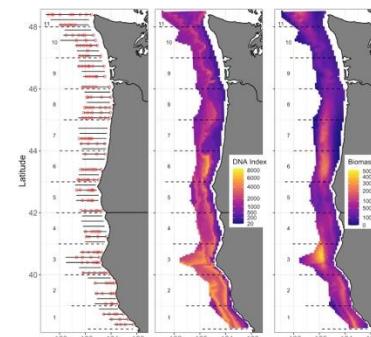
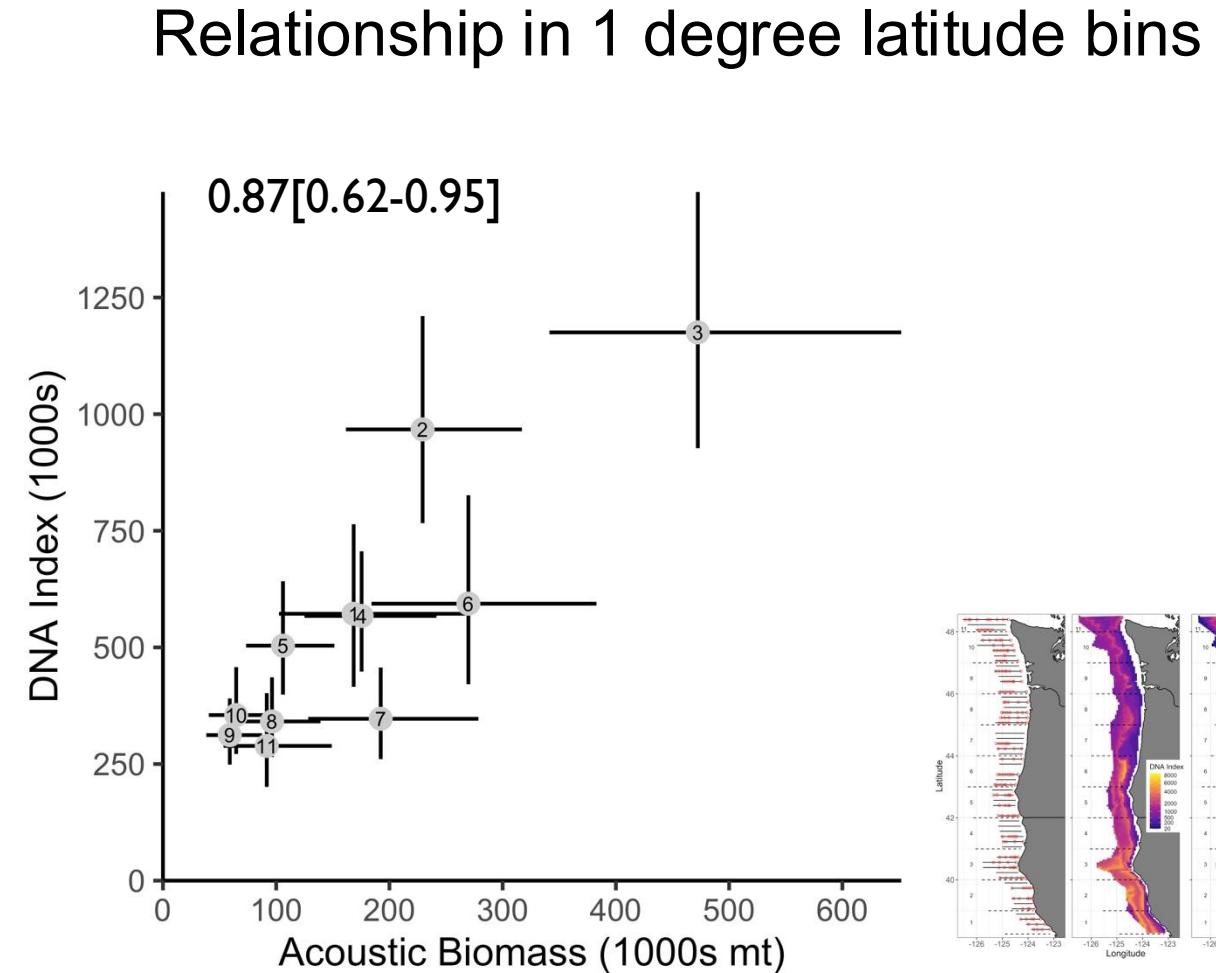
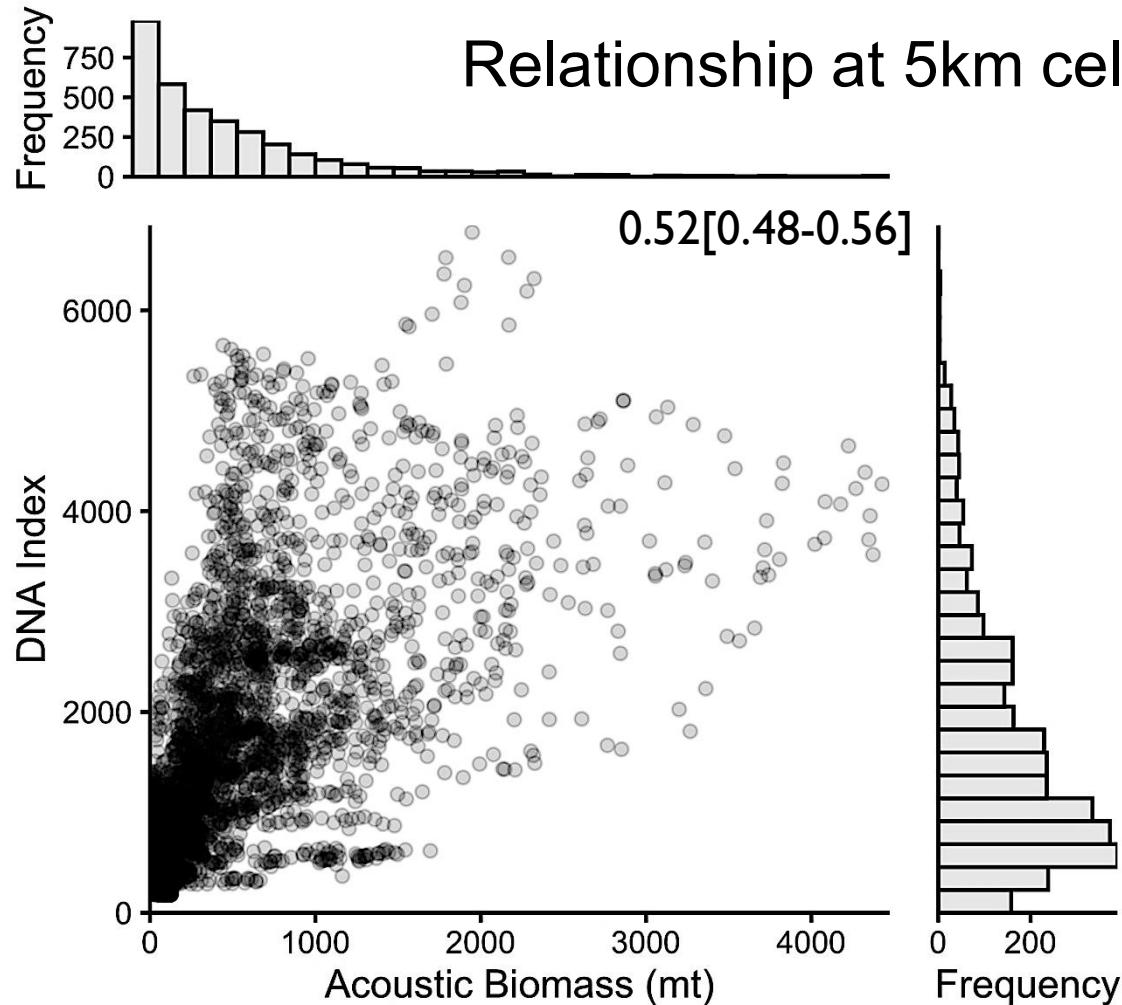
Acoustics



Generate indices of abundance comparable to acoustic-trawl surveys

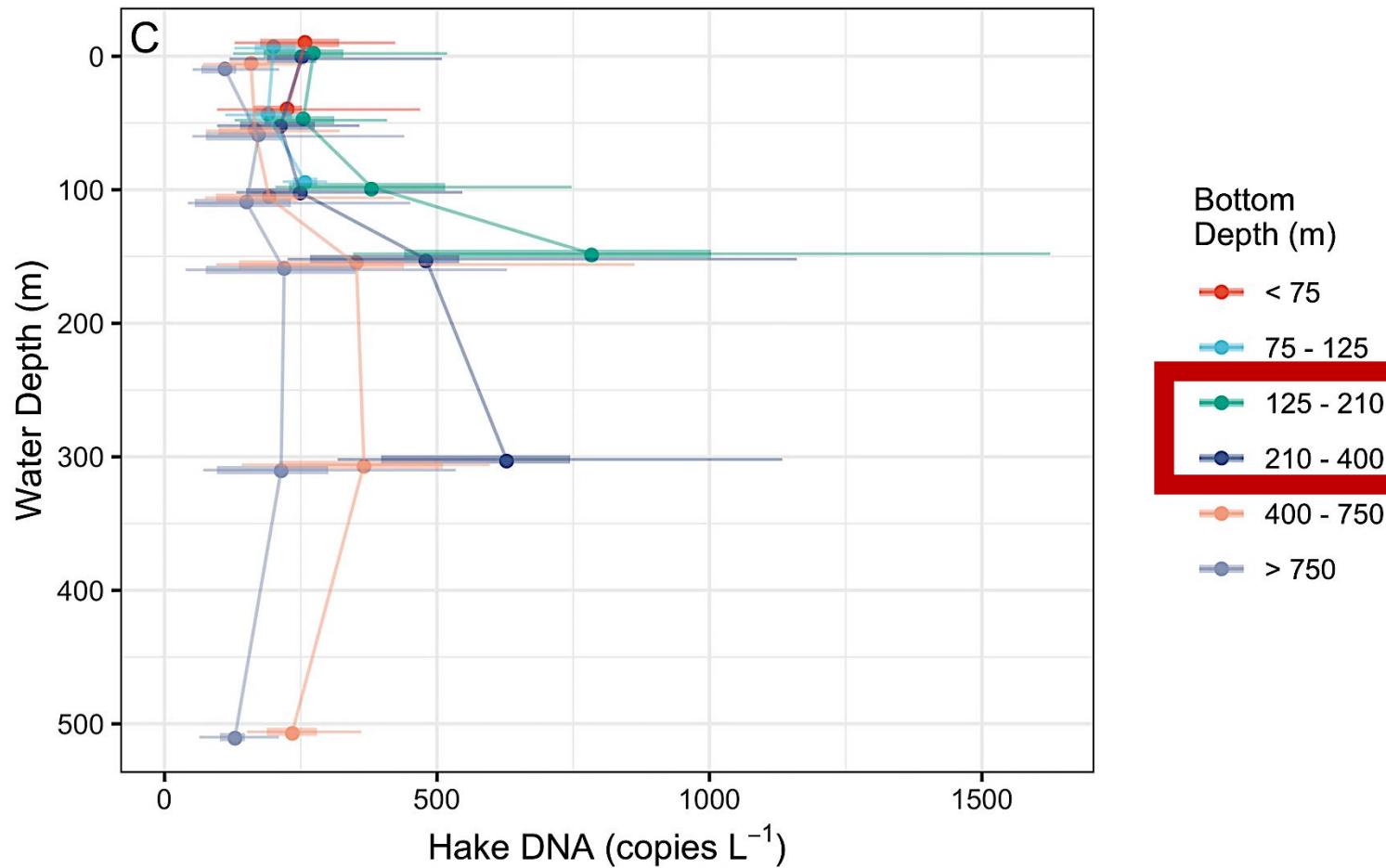


Estimates of abundance are strongly correlated (at large spatial scales)

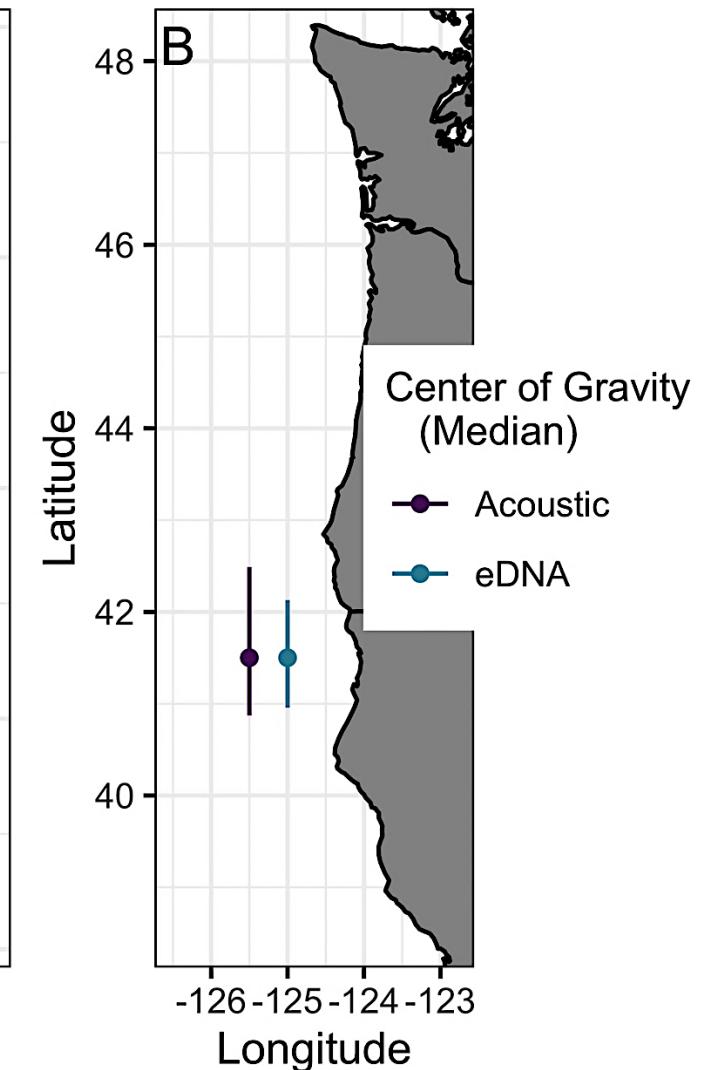
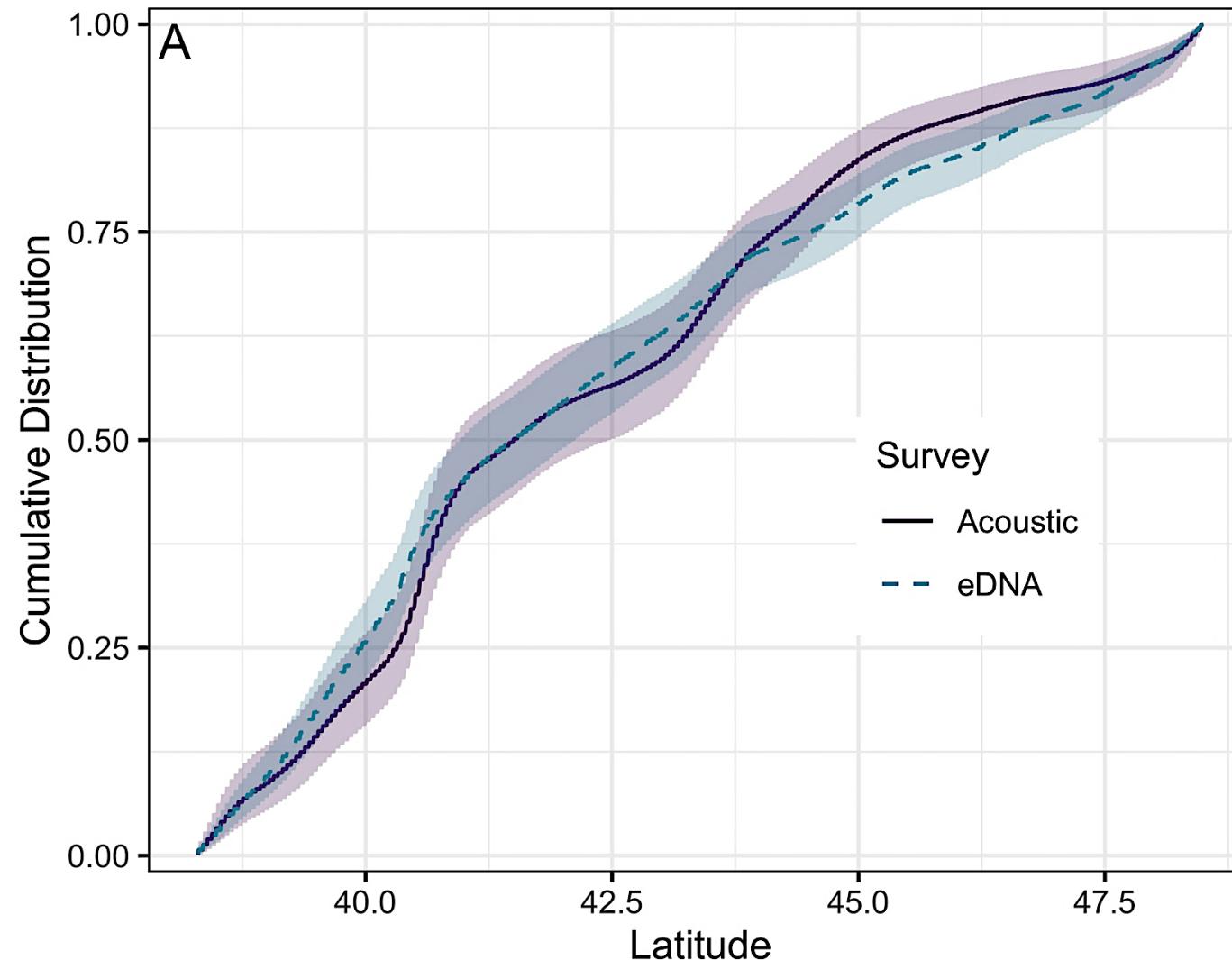


Depth-distribution of DNA correspond to published estimates for hake

- Largest hake DNA concentrations:
- Moderate water depths
- -100-300m sampling depth
- Along the continental shelf break
- - 150-300m bottom depth



Similarities extend to estimates of species distribution

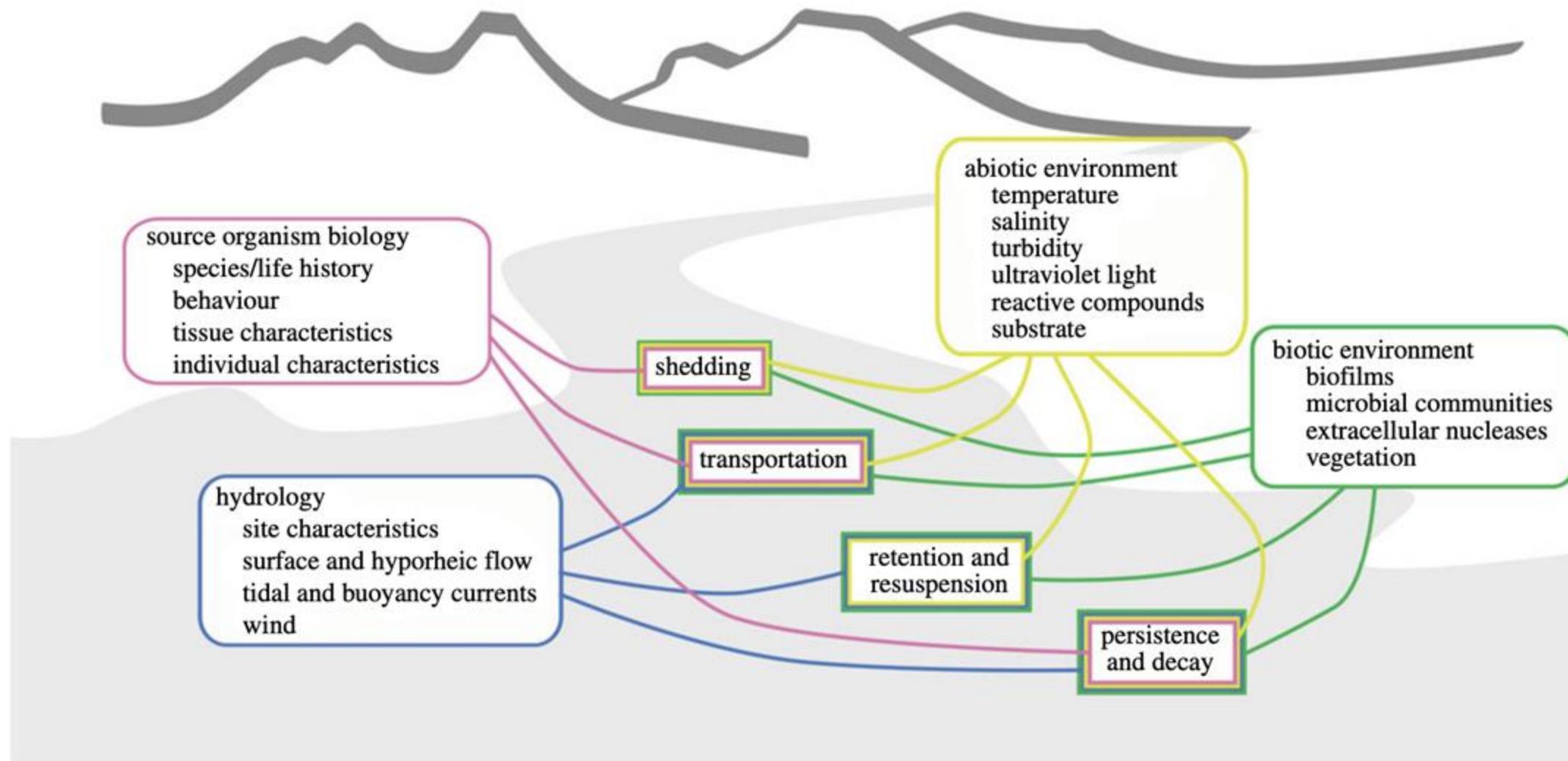


eDNA Fate and Transport: *Where did you come from, where did you go?*

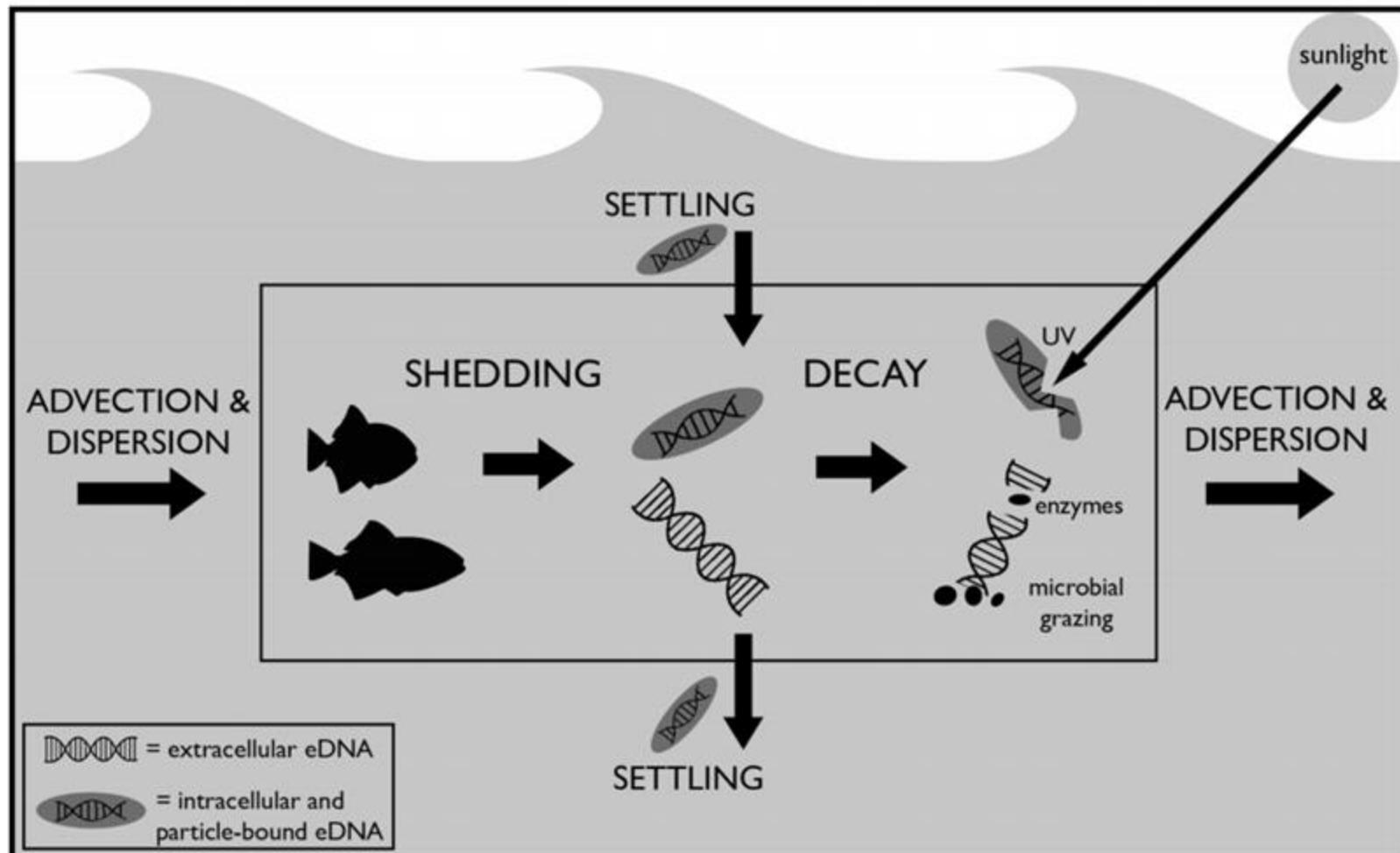
Eily Allan

25 January 2023

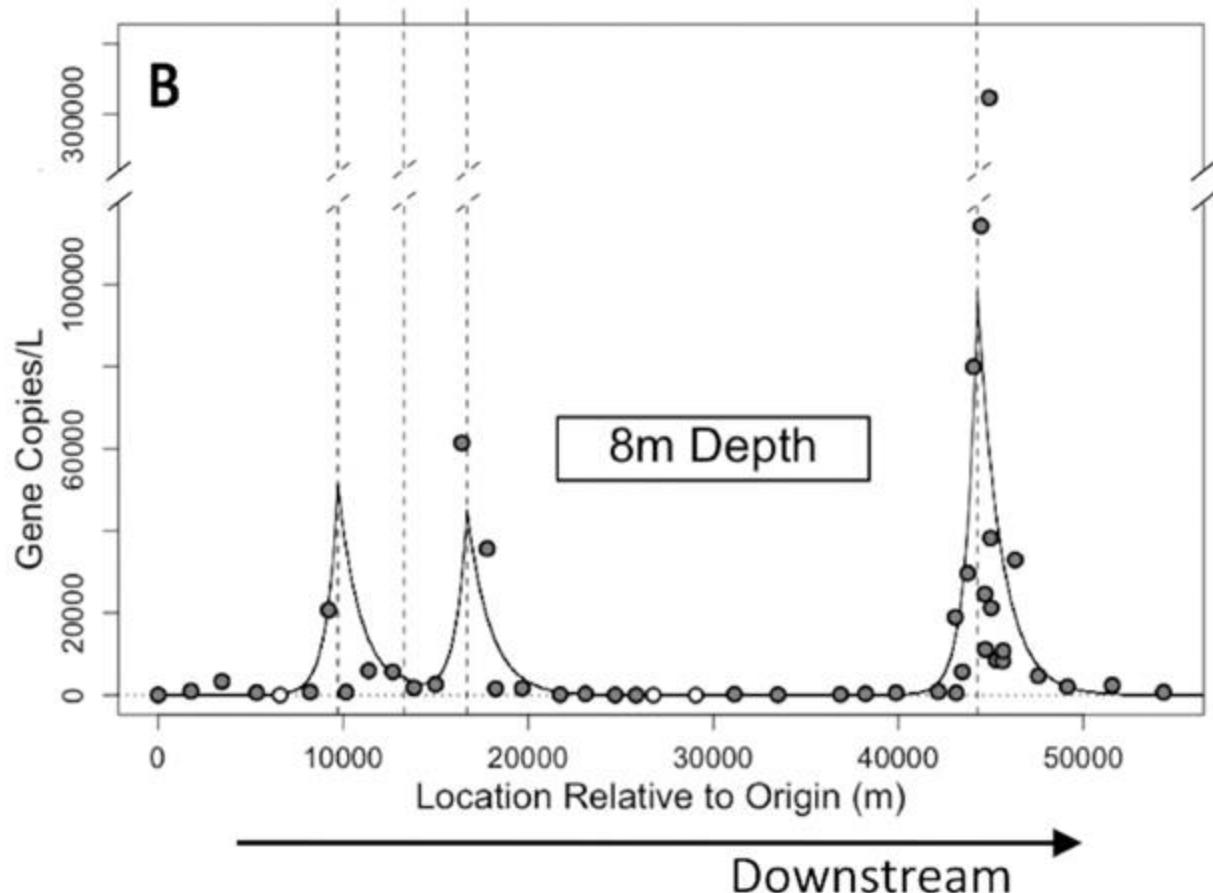
General conceptual figure



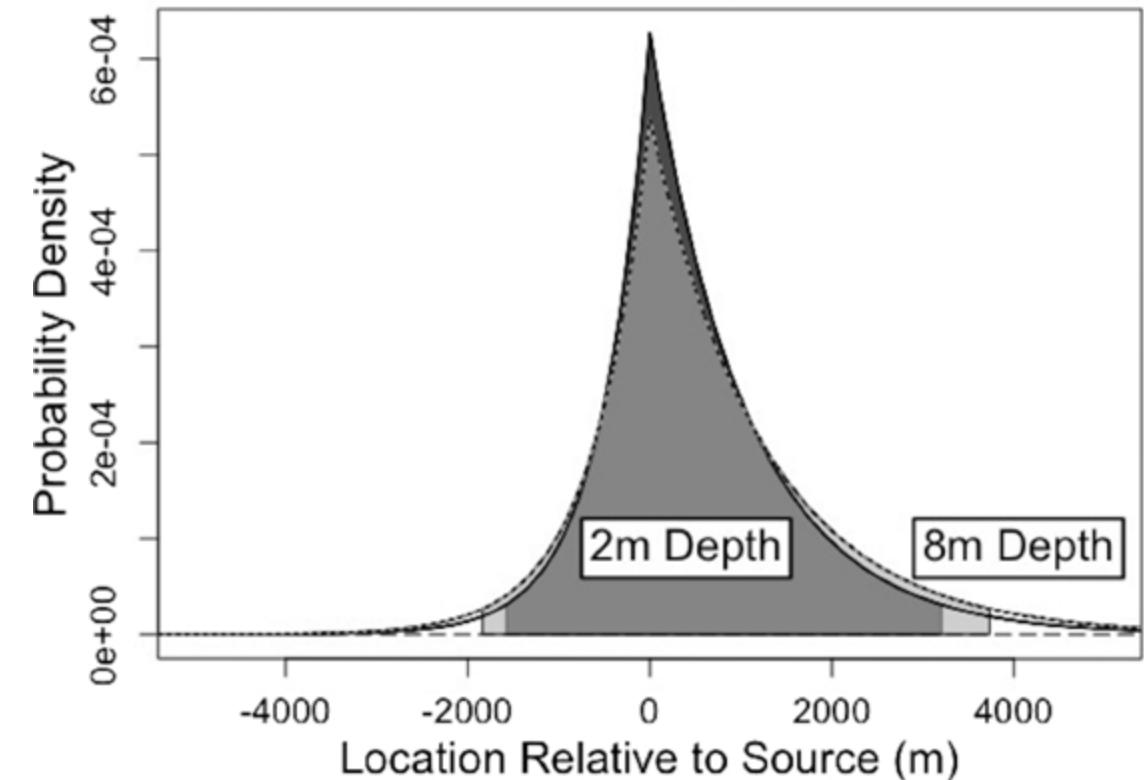
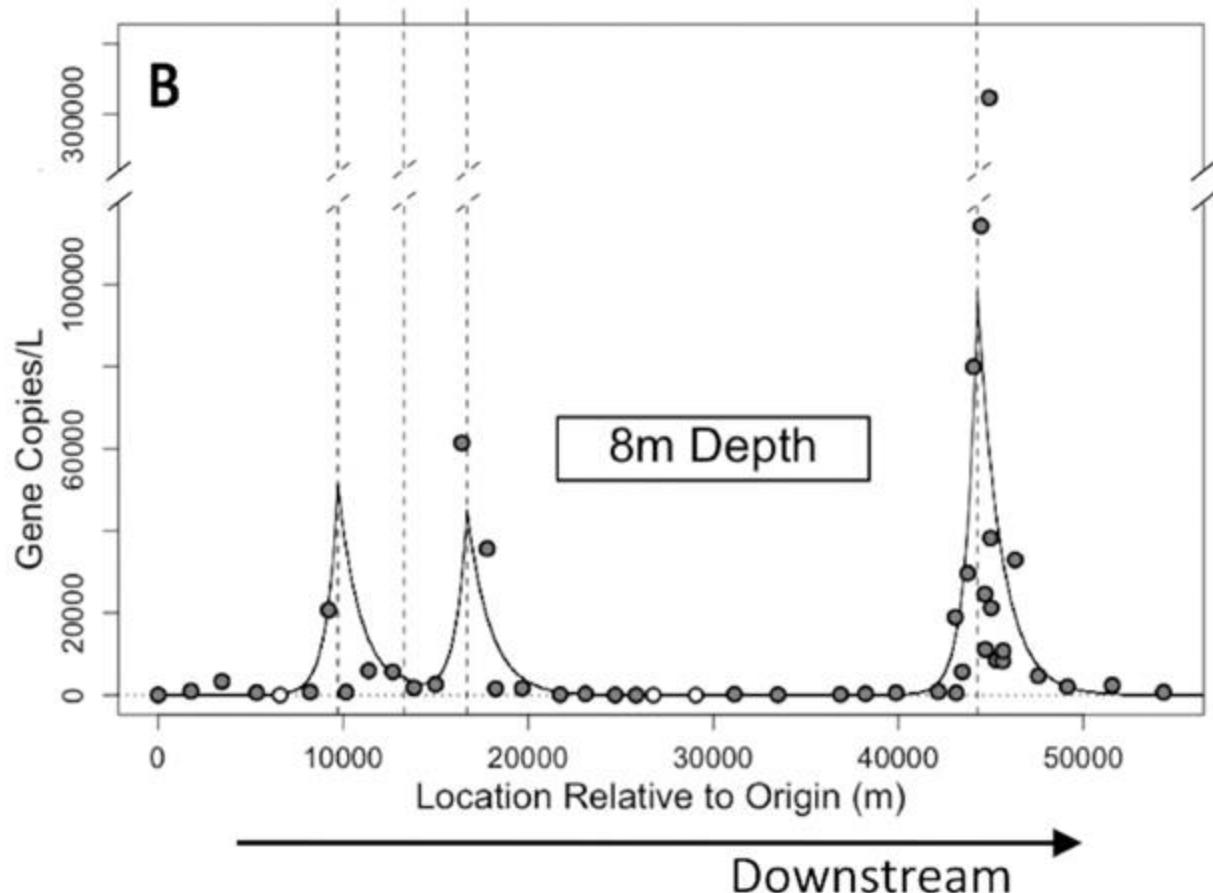
Mass balance of eDNA in the ocean



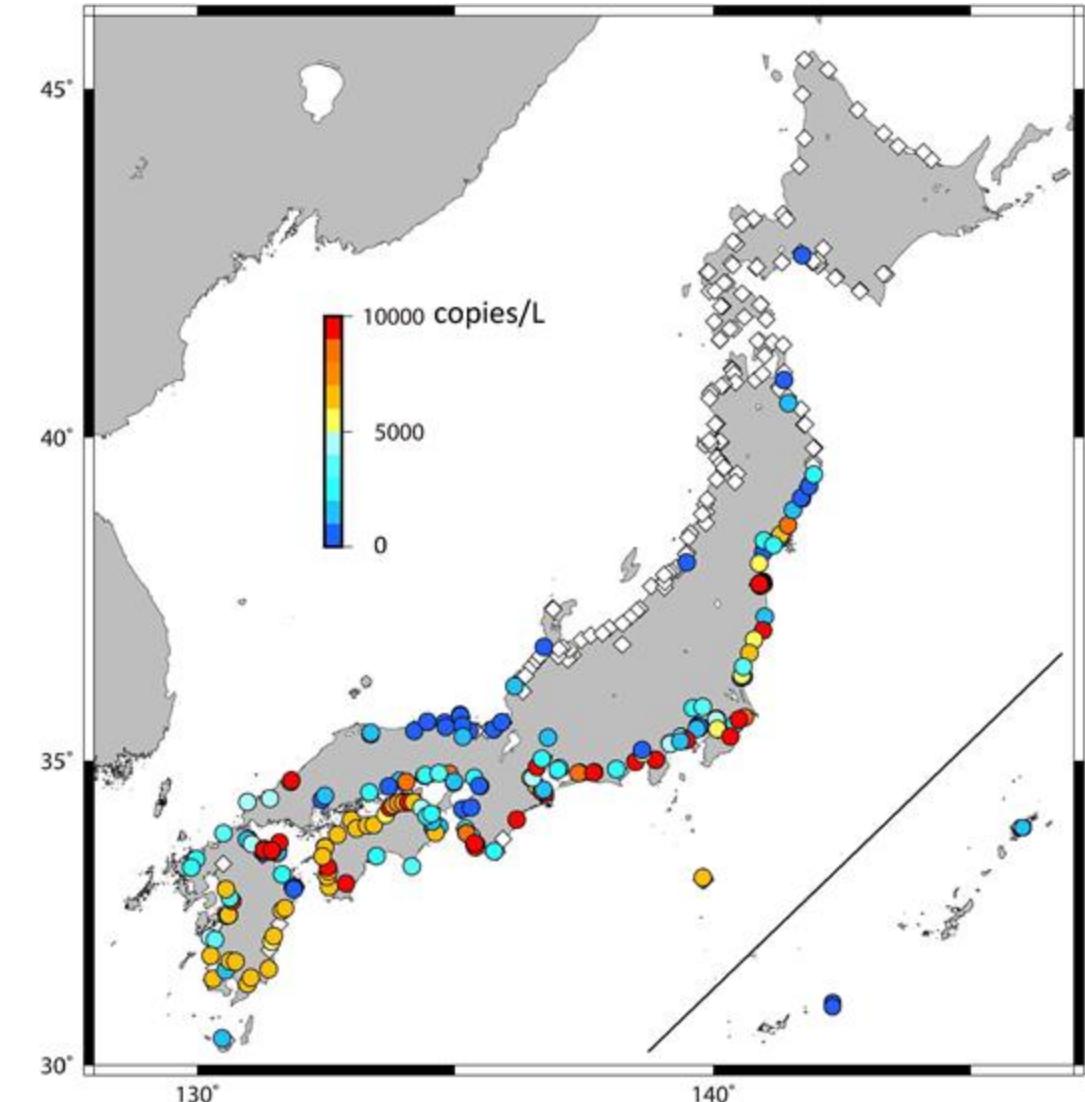
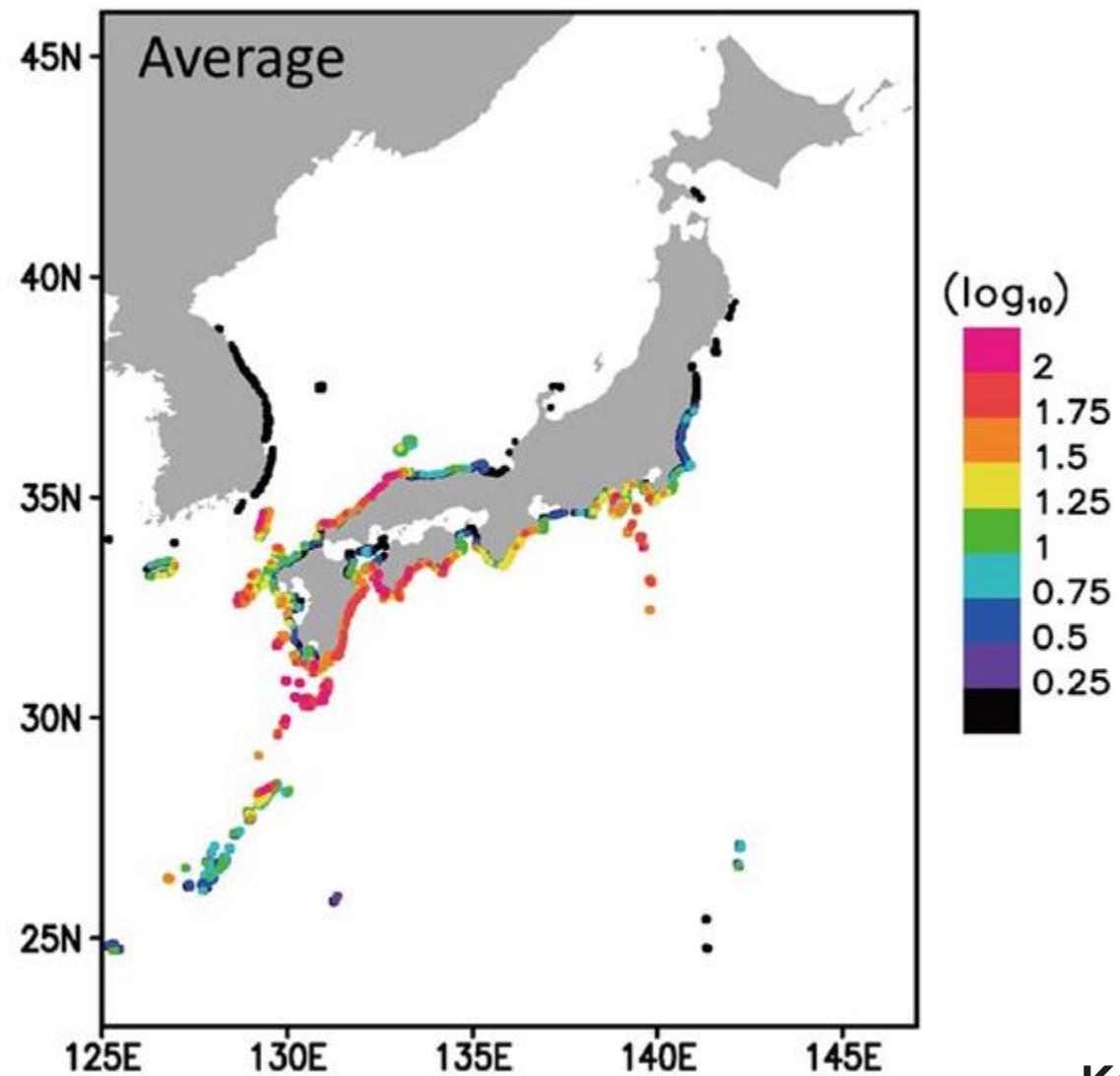
Observations: marine transport



Observations: marine transport

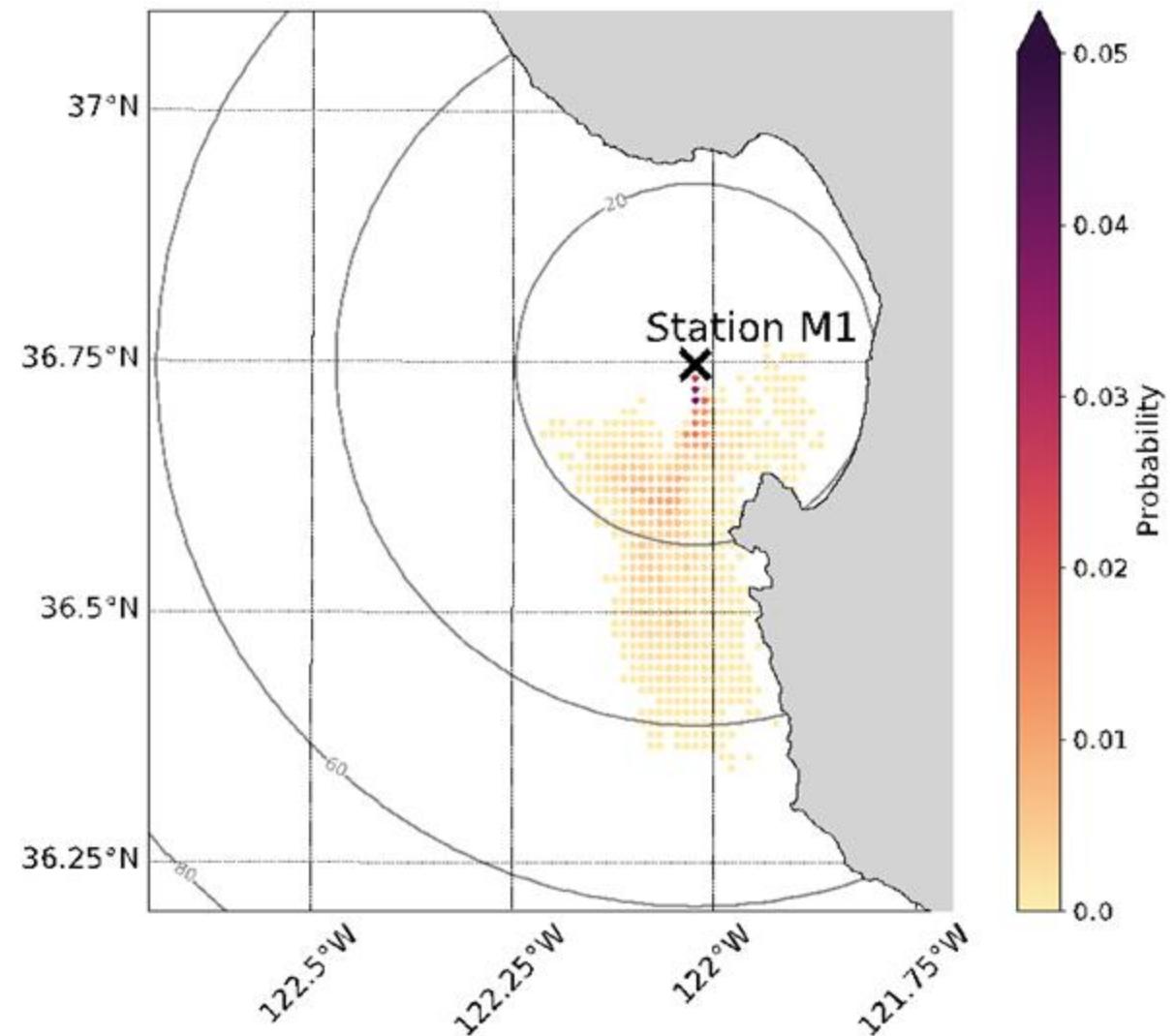


Observation + model: larvae, eDNA

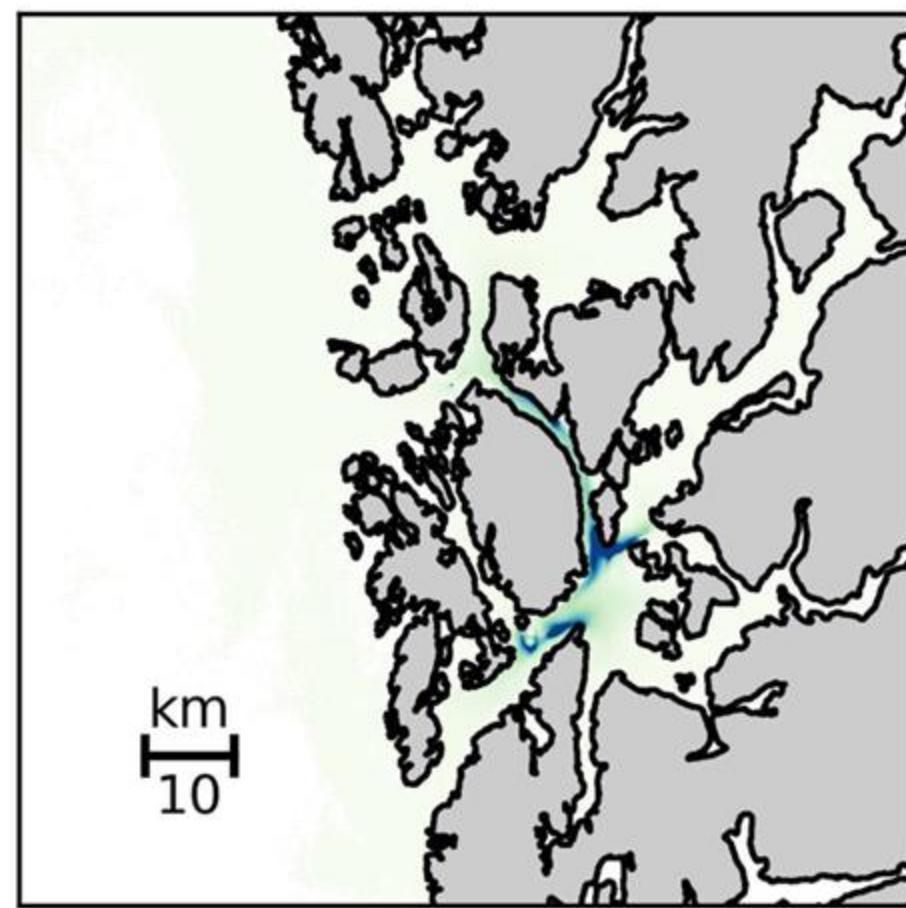


Numerical ocean models + eDNA

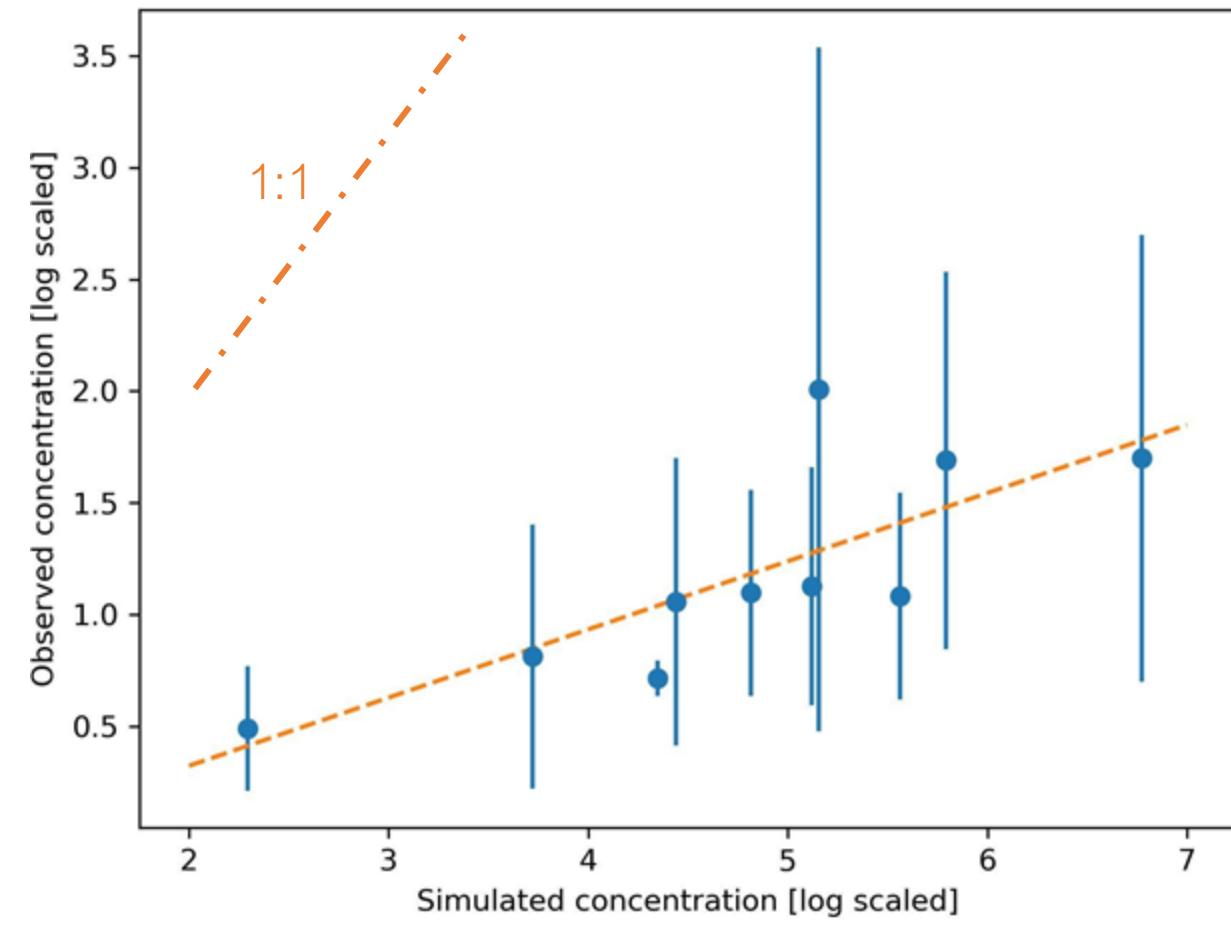
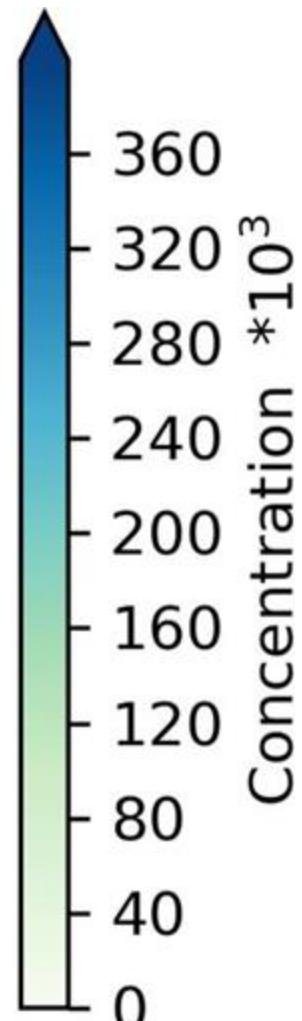
- Using relevant **time-scales**, predict **length-scales** of objects in ocean or atmosphere^{1,2}
- Other applications:
 - oil spills¹
 - search and rescue¹
 - larvae, plankton²⁻⁴
 - microplastics⁵



Models + Observations + Mesocosms



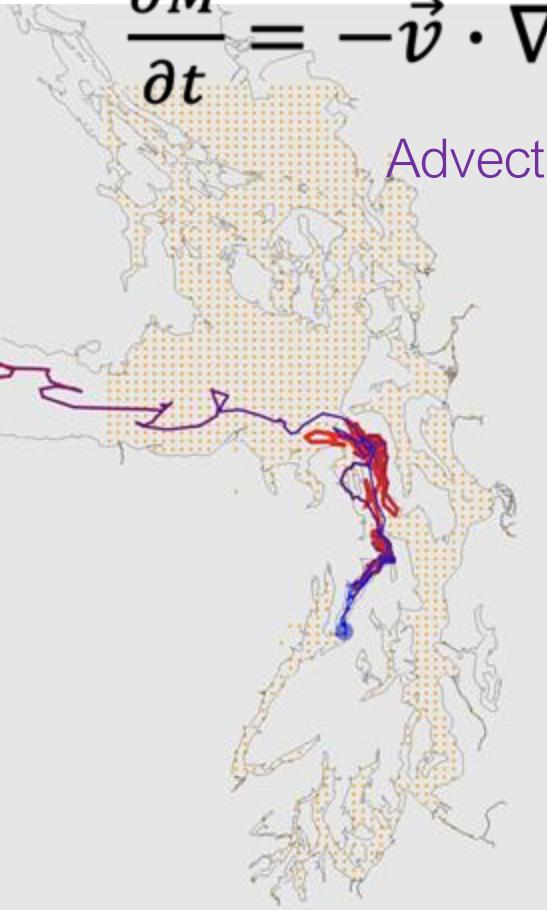
Lagrangian Advection and Diffusion Model



Mass balance for particle tracking

$$\frac{\partial M}{\partial t} = -\vec{v} \cdot \nabla M + \kappa_H \nabla_H^2 M + \frac{\partial}{\partial z} \left(\kappa_V \frac{\partial M}{\partial z} \right) + w_s \frac{\partial M}{\partial z} - kM + S$$

Advection Horizontal diffusion Vertical diffusion Settling Decay Shedding



Mass balance for particle tracking

$$\frac{\partial M}{\partial t} = -\vec{v} \cdot \nabla M + \kappa_H \nabla_H^2 M + \frac{\partial}{\partial z} \left(\kappa_V \frac{\partial M}{\partial z} \right) + w_s \frac{\partial M}{\partial z} - kM + S$$

Advection Horizontal diffusion Vertical diffusion Settling Decay Shedding

physical ocean model eDNA properties

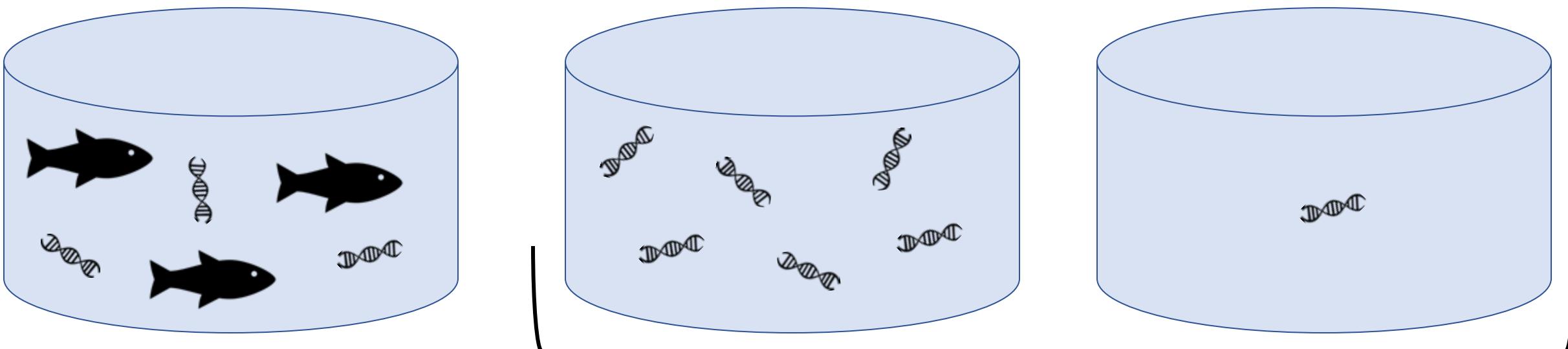
✓ ? ?

Advection and Diffusion

- Jilian!

? Decay: CSTR tank experiments

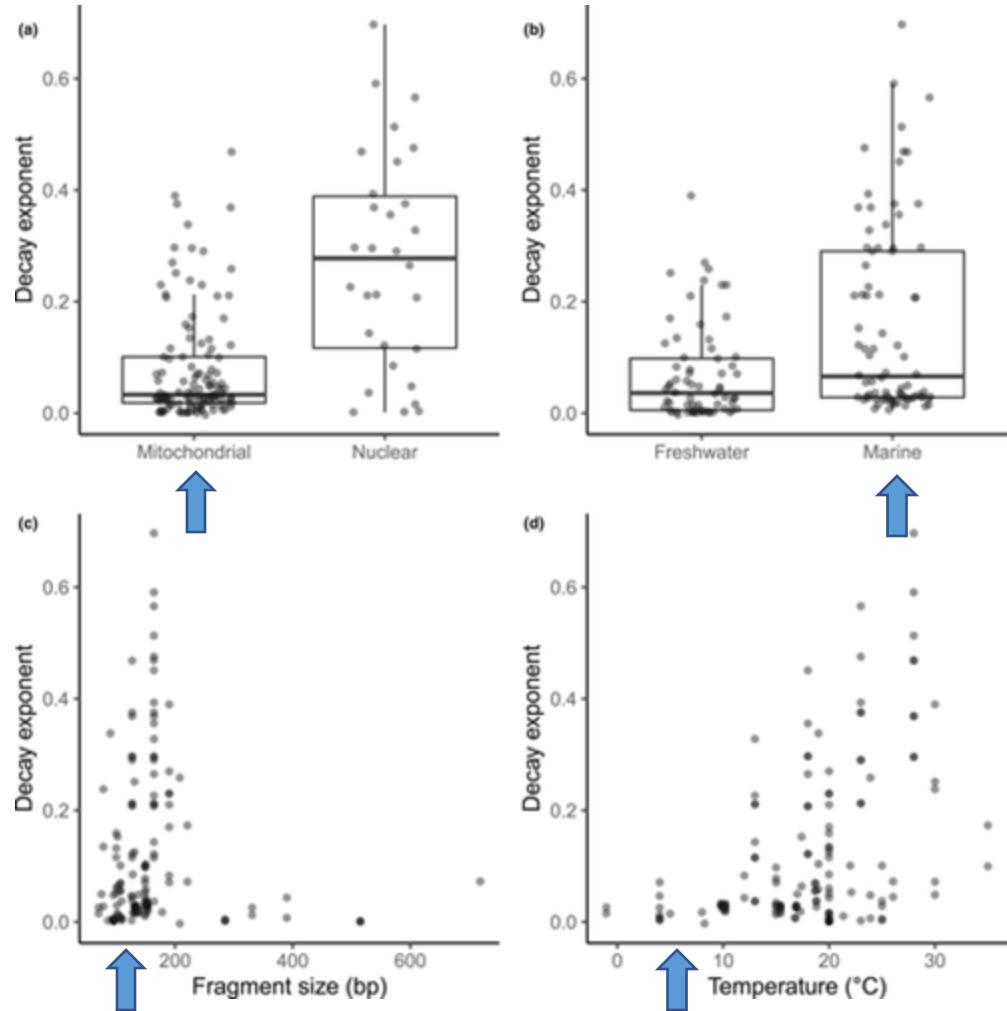
$$V \frac{dC}{dt} = S - kCV$$



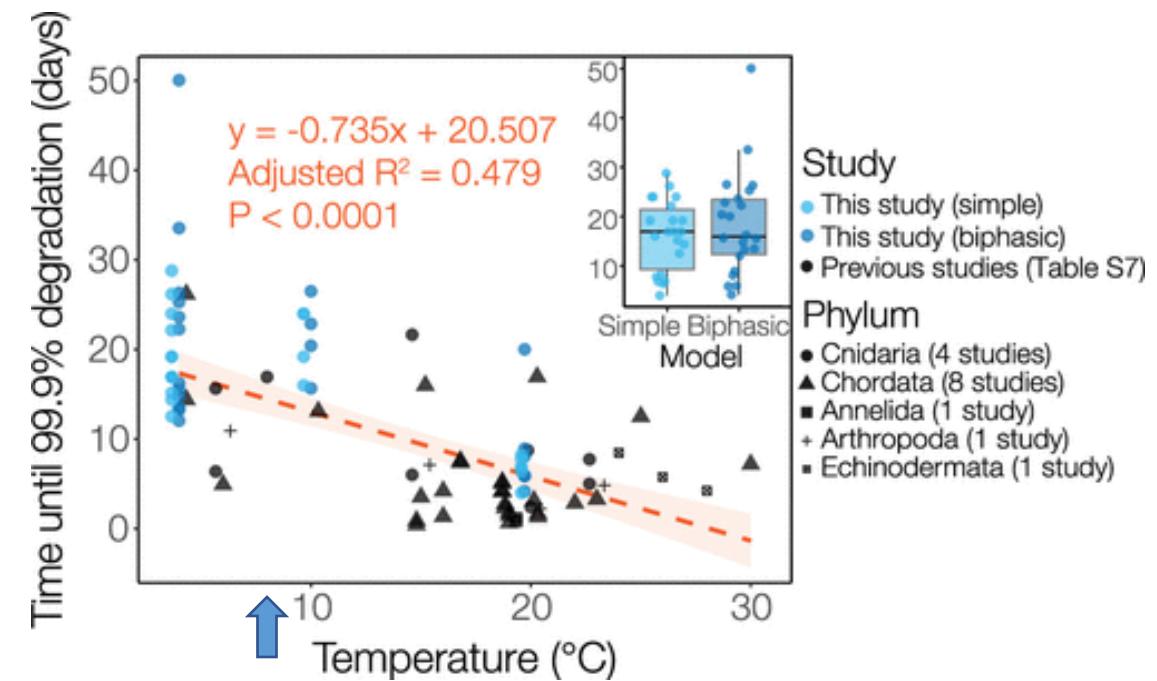
$$\cancel{V \frac{dC}{dt} = S - kCV}$$

1. Measure dC/dt
2. Solve for k

? Decay: Literature review



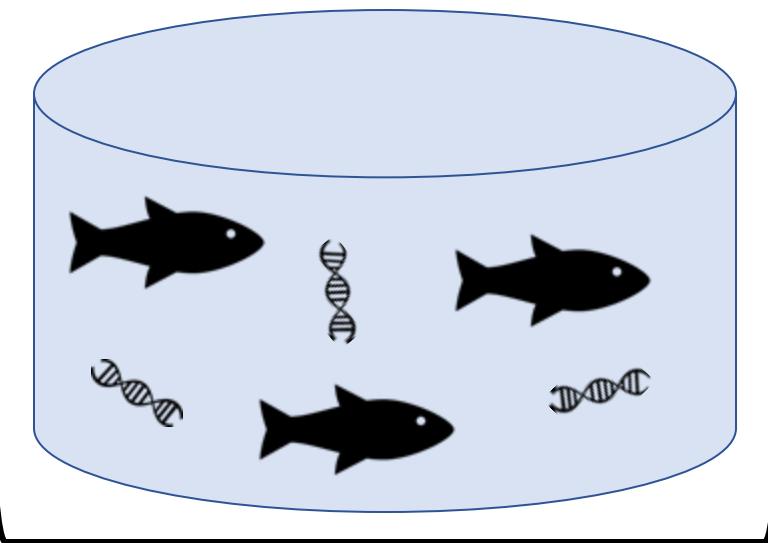
Lamb et al. (2022)



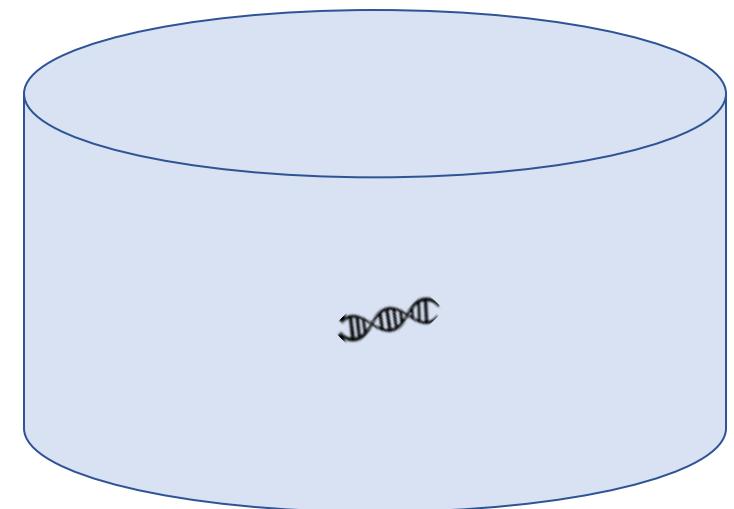
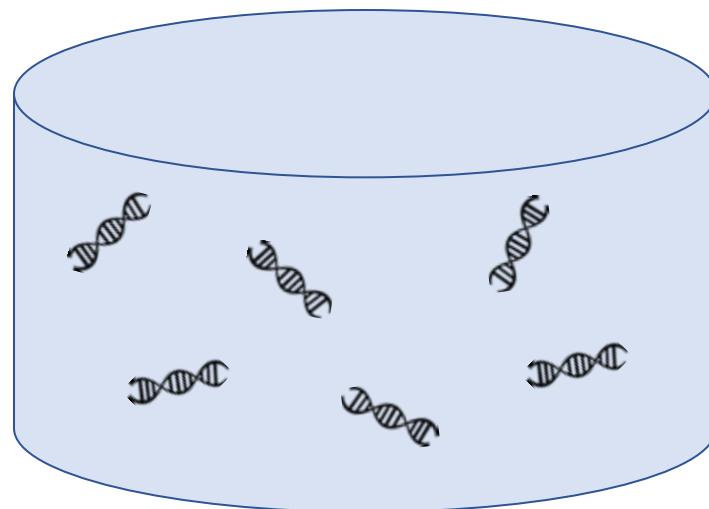
McCartin et al. (2022)

? Sheding: CSTR tank experiments

$$V \frac{dC}{dt} = S - kCV$$



$$\cancel{V \frac{dC}{dt} = S - kCV}$$



1. Let tank get to steady state.
2. Use k to solve for S.

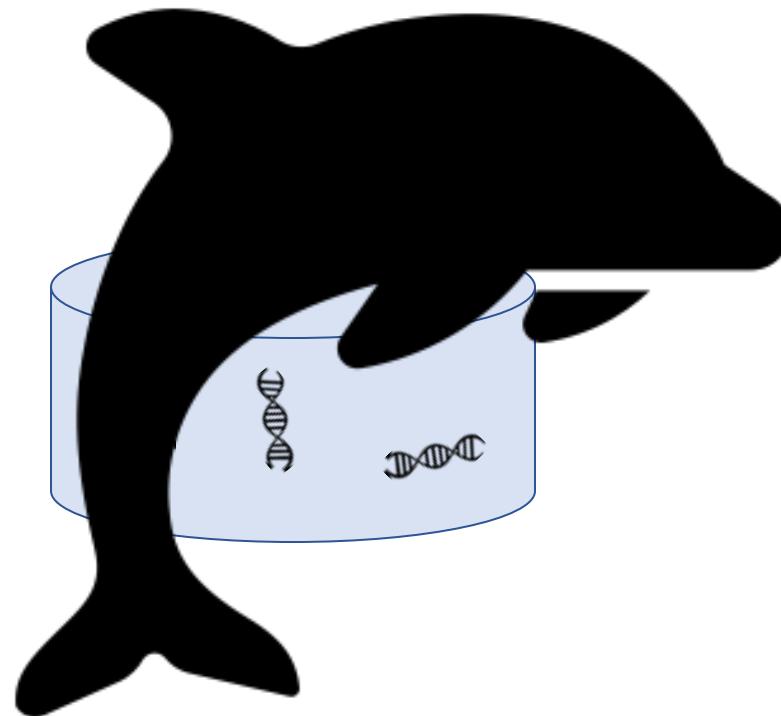
? Shredding: Literature review

5 orders of magnitude??

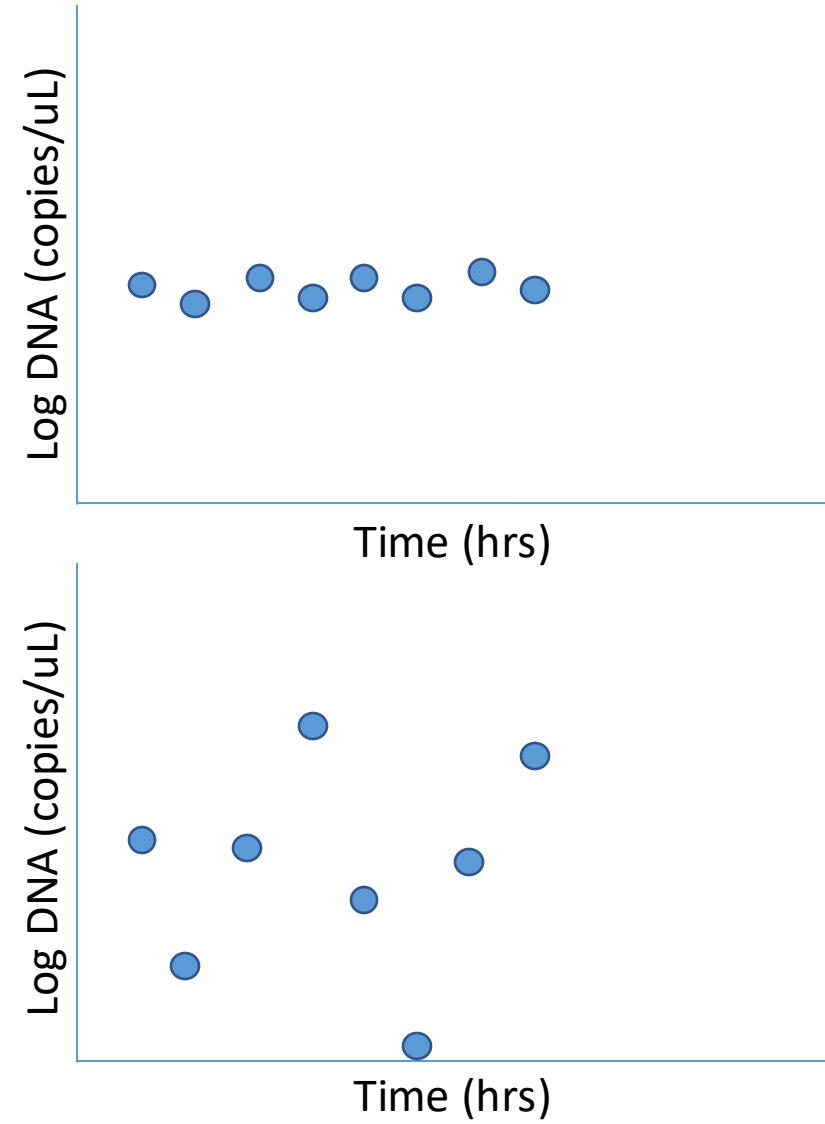
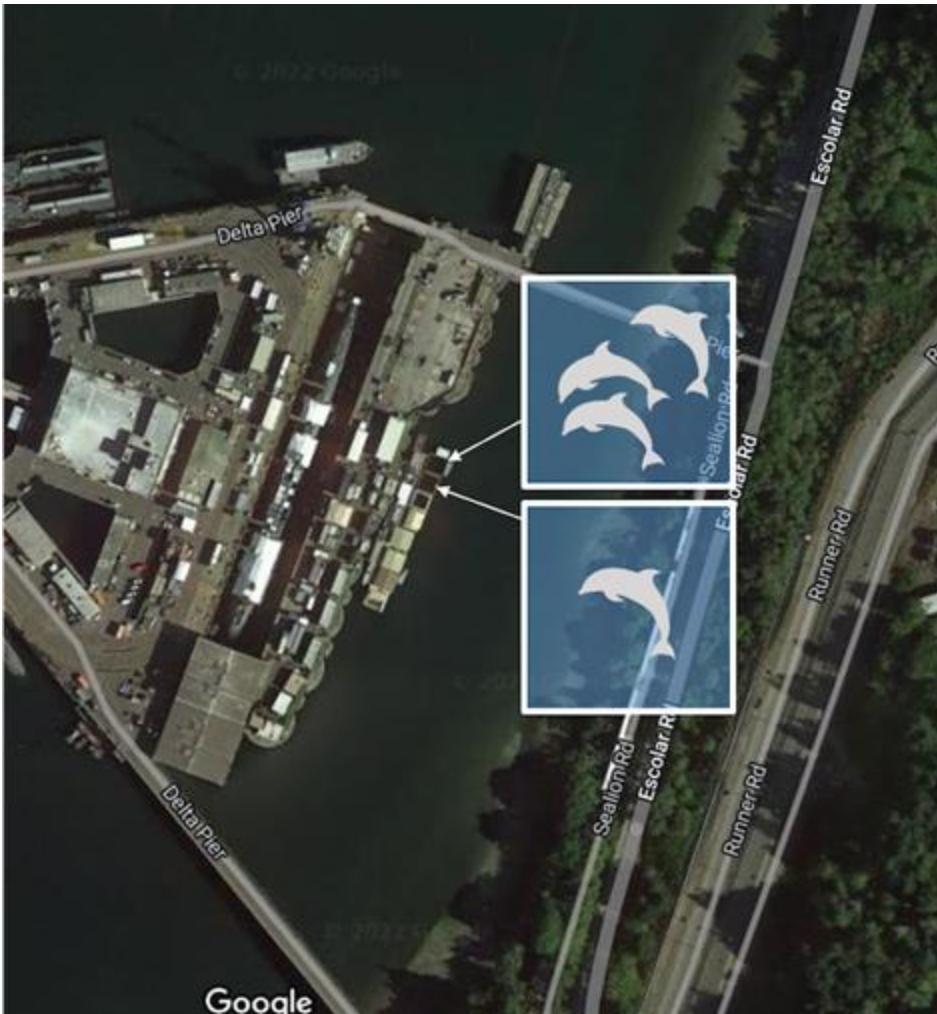
Citation	Study description	Organism Class	Shedding rate (Order of magnitude)	Major conclusions/Drivers
This Study	Marine-tank	Actinopterygii Scyphozoa	10^3 - 10^5 pg/hr 10^2 - 10^4 pg/hr/ind	<ul style="list-style-type: none"> Tanks are not real life mostly fish / small things not the same environment stressed bumping up against tank mostly starved
Sassoubre et al. (2016)	Marine-tank	Actinopterygii	10^5 - 10^7 pg/hr 10^2 - 10^3 pg/hr/g 10^3 - 10^5 pg/hr/ind	<ul style="list-style-type: none"> Effect of taxa type on shedding rate; Normalization of pg/hr, pg/hr/individual, pg/hr/g changes rank order of shedding rate Normalization of pg/hr, pg/hr/individual, pg/hr/g changes rank order of shedding rate; Presence of multiple species in tank does not affect shedding rate
Klymus et al. (2015)	Fresh-tank	Actinopterygii	10^4 - 10^9 copy/hr	<ul style="list-style-type: none"> What to normalize by?? time, # individuals, biomass Temporal variability
Maruyama et al. (2014)	Fresh-tank	Actinopterygii	10^5 - 10^6 copy/hr/g 10^7 copy/hr	<ul style="list-style-type: none"> Life stage/normalization: juveniles shedding 4x than adults when normalized by biomass, adults shedding 12x than juveniles when normalized by # of individuals
Sansom and Sassoubre (2017)	Fresh-tank	Actinopterygii	10^{5-6} copy/hr 1 copy/hr/g 10^3 - 10^4 copy/hr	<ul style="list-style-type: none"> scales: seconds, minutes, hours, days, months, years Density: no effect; Normalization of copy/hr, copy/hr/individual, copy/hr/g changes rank order of shedding rate
Jo, et al. (2019)	Marine-tank	Actinopterygii	10^5 - 10^9 copy/hr 10^3 - 10^4 copy/hr/g	<ul style="list-style-type: none"> processes: pee/poop, hunt/be hunted, grow, reproduce, die Biomass: higher biomass, higher shedding rate; Temperature: higher temperature, higher shedding rate
Nevers et al. (2018)	Fresh-tank	Actinopterygii	10^5 copy/hr 10^5 copy/hr/g 10^6 copy/hr/ind	<ul style="list-style-type: none"> Abundance/biomass: no effect; Temperature: no effect
Minamoto et al. (2017)	Marine-tank	Scyphozoa	10^8 copy/hr/ind.	Variability: shedding rate between individuals is highly variable



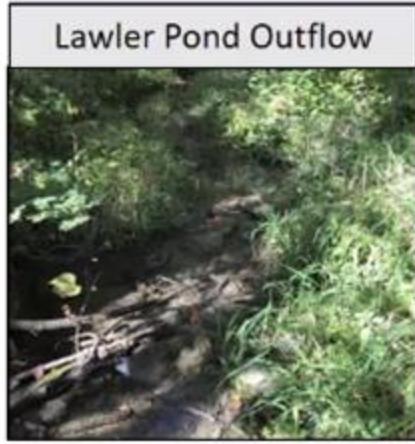
? Shedding: dolphins hard to put in tank...



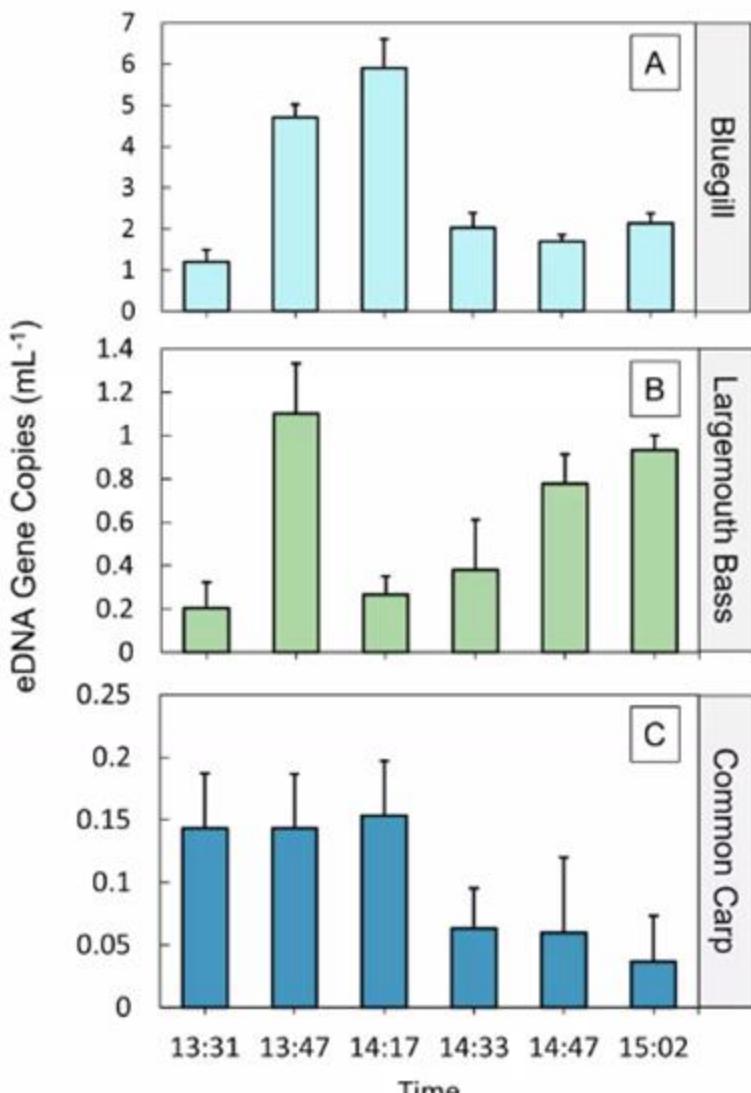
? Shedding: observations



Ambient eDNA method assessment



- @ pond outlet: collected water samples over time.
- For three fish species, outflow [eDNA] varied over time among samples taken 15 minutes apart.
- Fluctuation indicates eDNA is not well-mixed within the pond.
- Take home: can't measure eDNA removal rates in outflow stream using ambient pond eDNA in this pond → stream system.





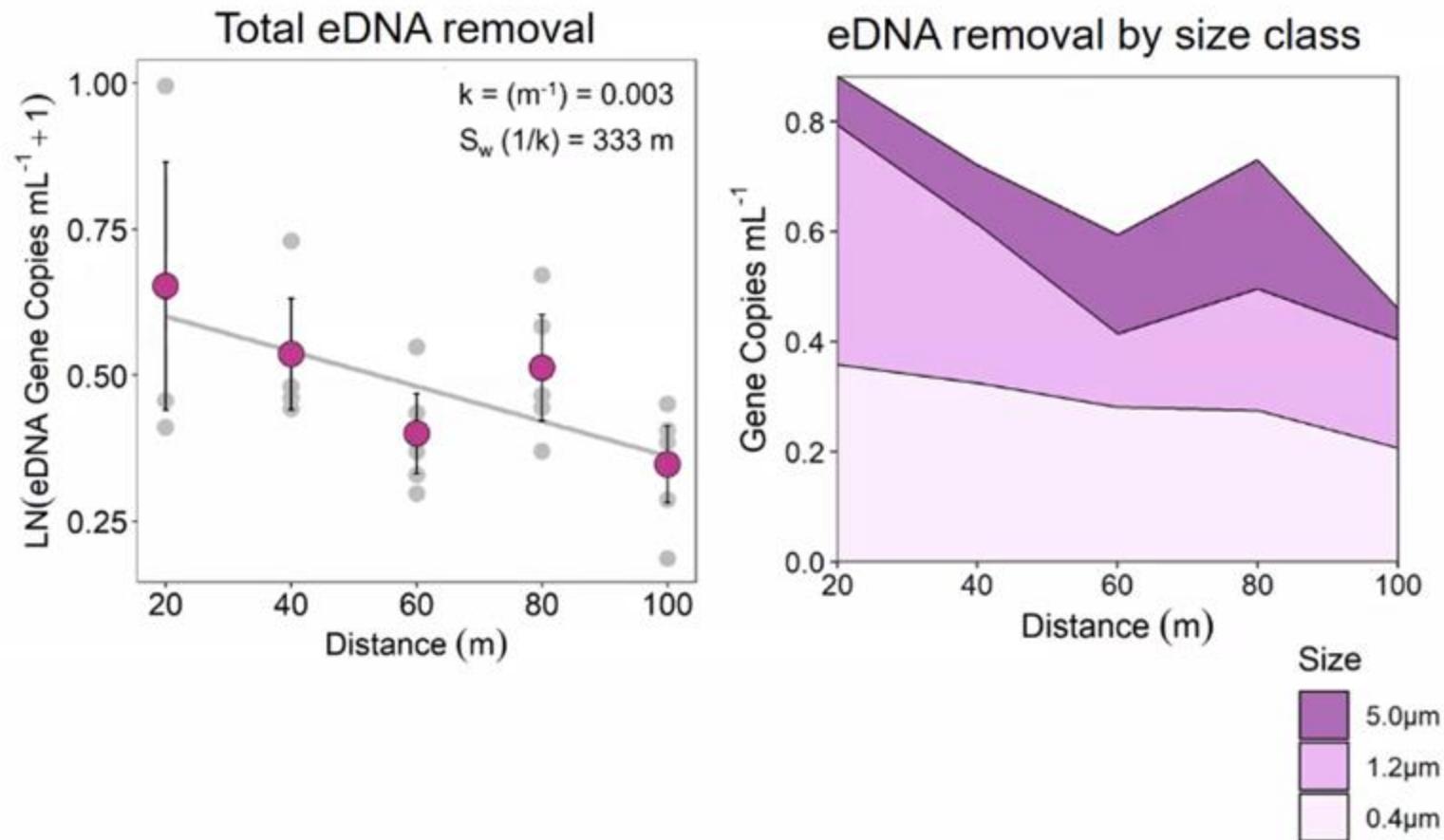
Lawler Pond Outflow

Elise Snyder

Experimental eDNA additions to quantifying eDNA transport and removal rates at FCTC

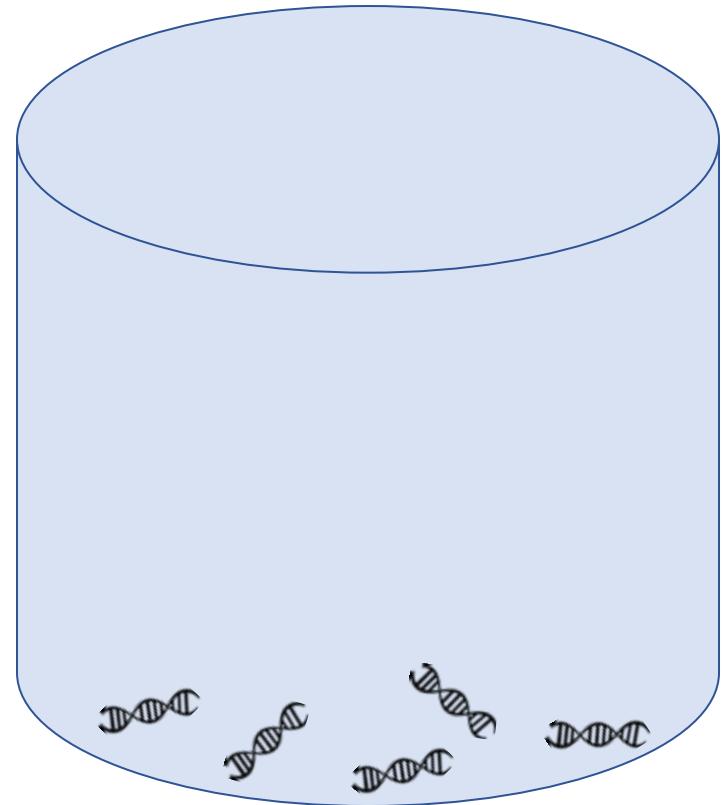
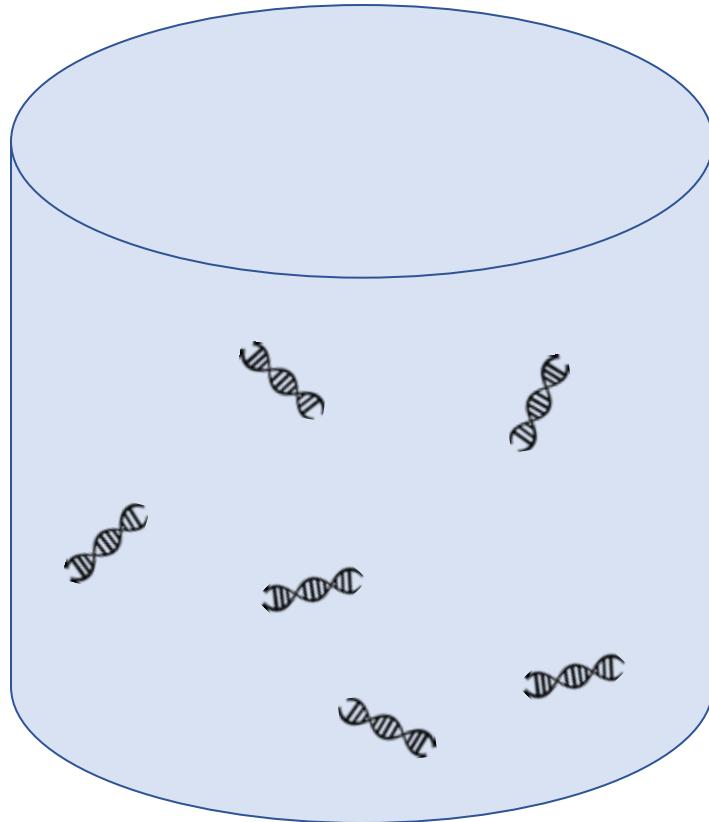
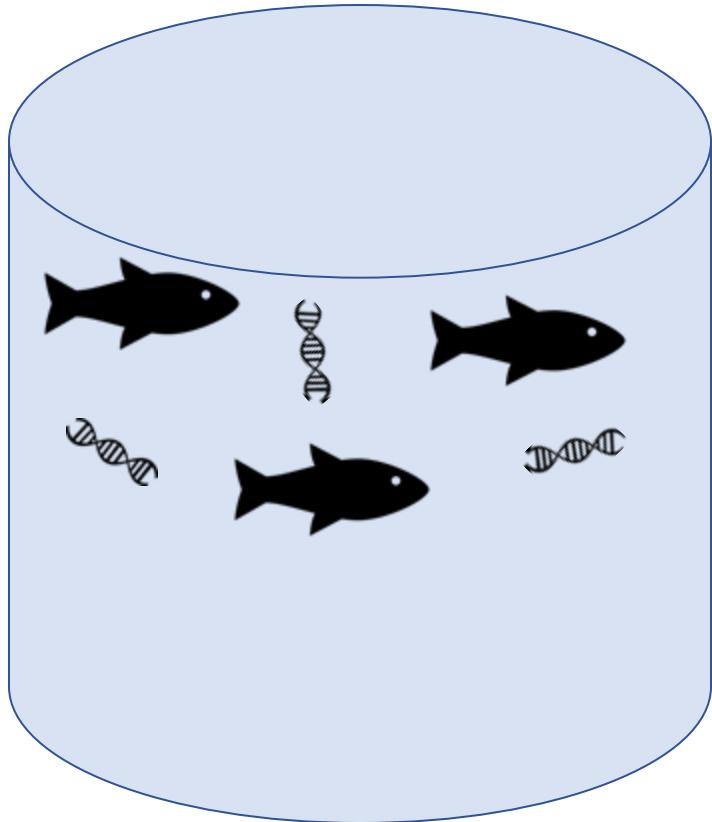
Steelhead (*O. mykiss*)

- **November 2022:** used short-term eDNA additions of Steelhead Trout to quantify removal rates by measuring eDNA decline along a 100 m stream reach.
- Success! Average eDNA travel distance before being removed from the water column (S_w) was 333 m.
- But removal rates varied by eDNA size class.



?

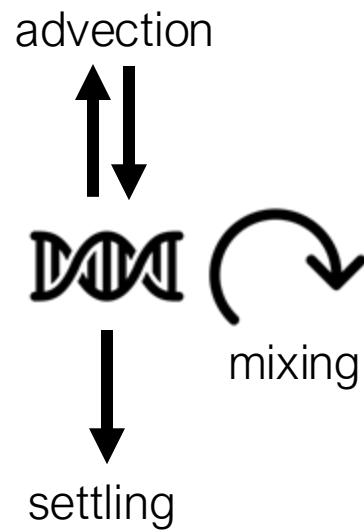
Settling: tank experiments practically difficult



?

Settling: model results

Timescale of decay controls
length-scale of transport



$$t_{90} = 38 \text{ hr}$$

$$L_{settle} = w_s t_{90} = 10 \frac{\text{m}}{\text{day}} * 38 \text{ hr} = 16 \text{ m}$$

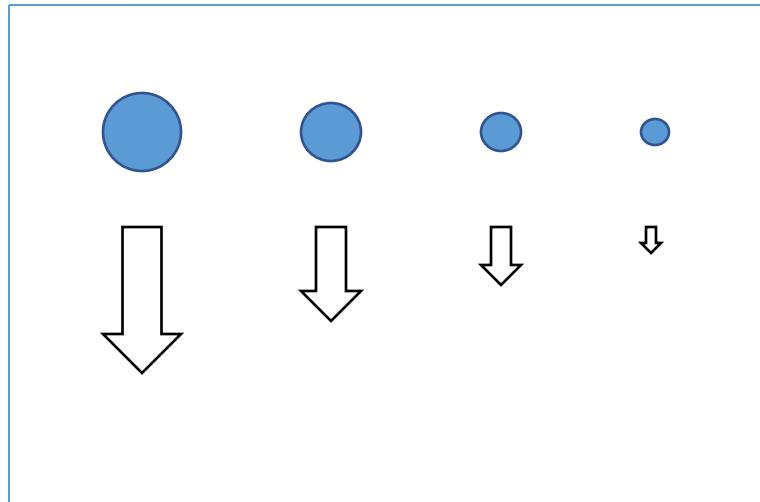
$$L_{advect} = v t_{90} = 10^{-4} \frac{\text{m}}{\text{s}} * 38 \text{ hr} = 13 \text{ m}$$

$$L_{mix} = \sqrt{\kappa_v t_{90}} = \sqrt{10^{-3} \frac{\text{m}^2}{\text{s}} * 38 \text{ hr}} = 12 \text{ m}$$

?

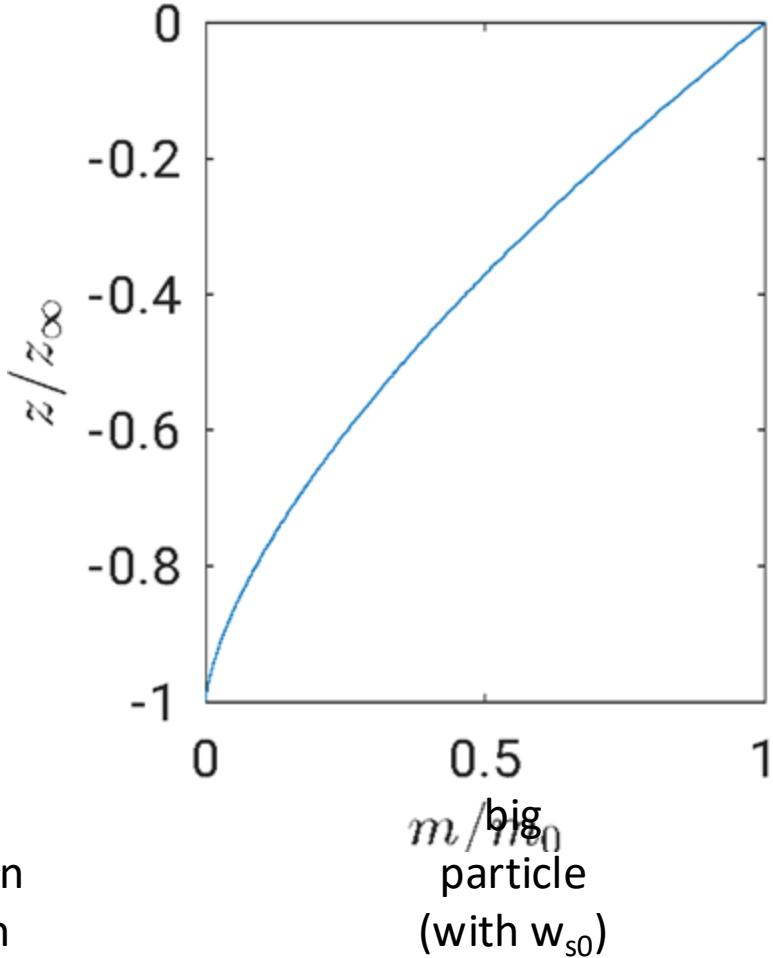
Settling: model results – with breakdown

$$w_s = \frac{1}{18} \frac{\frac{\rho_p}{\rho} - 1}{\nu} g d^2.$$



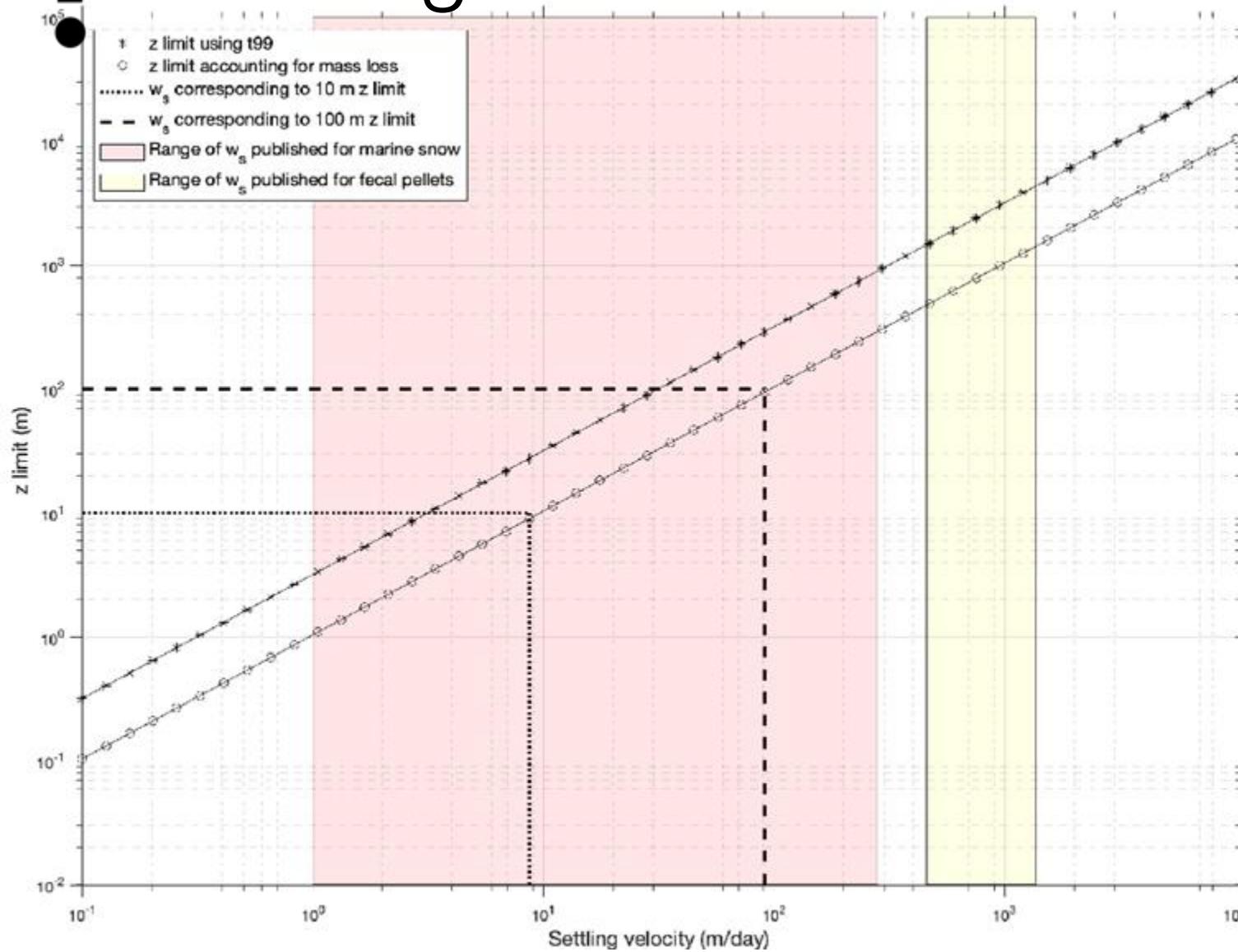
$$m = m_0 e^{-(k+r)t}$$

fully
broken
down



?

Settling: sensitive to model, but what to use?



Mass balance for particle tracking

$$\frac{\partial M}{\partial t} = -\vec{v} \cdot \nabla M + \kappa_H \nabla_H^2 M + \frac{\partial}{\partial z} \left(\kappa_V \frac{\partial M}{\partial z} \right) + w_s \frac{\partial M}{\partial z} - kM + S$$

Advection Horizontal diffusion Vertical diffusion Settling Decay Shedding

physical ocean model eDNA properties

✓ ? ?

Mass balance for particle tracking

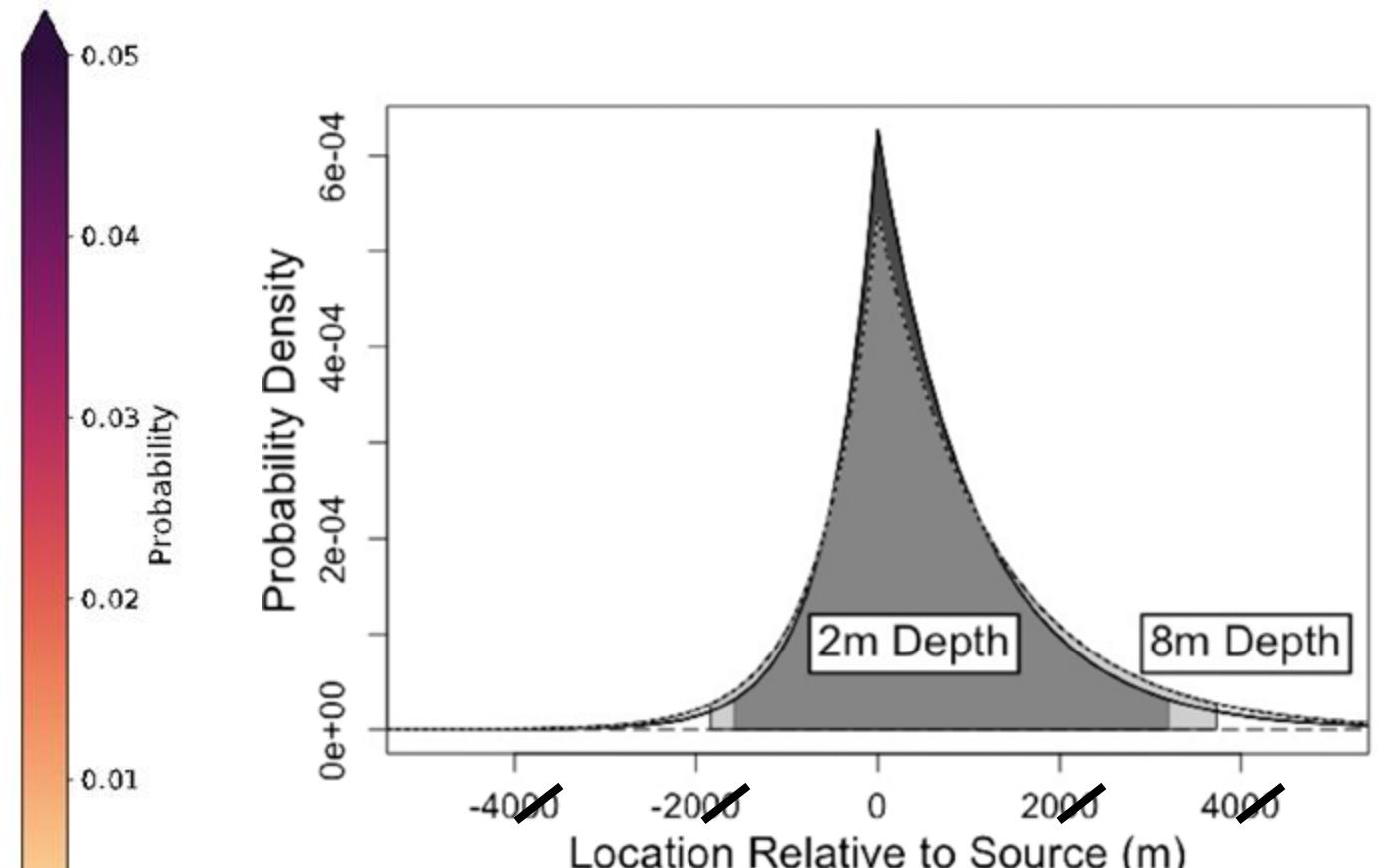
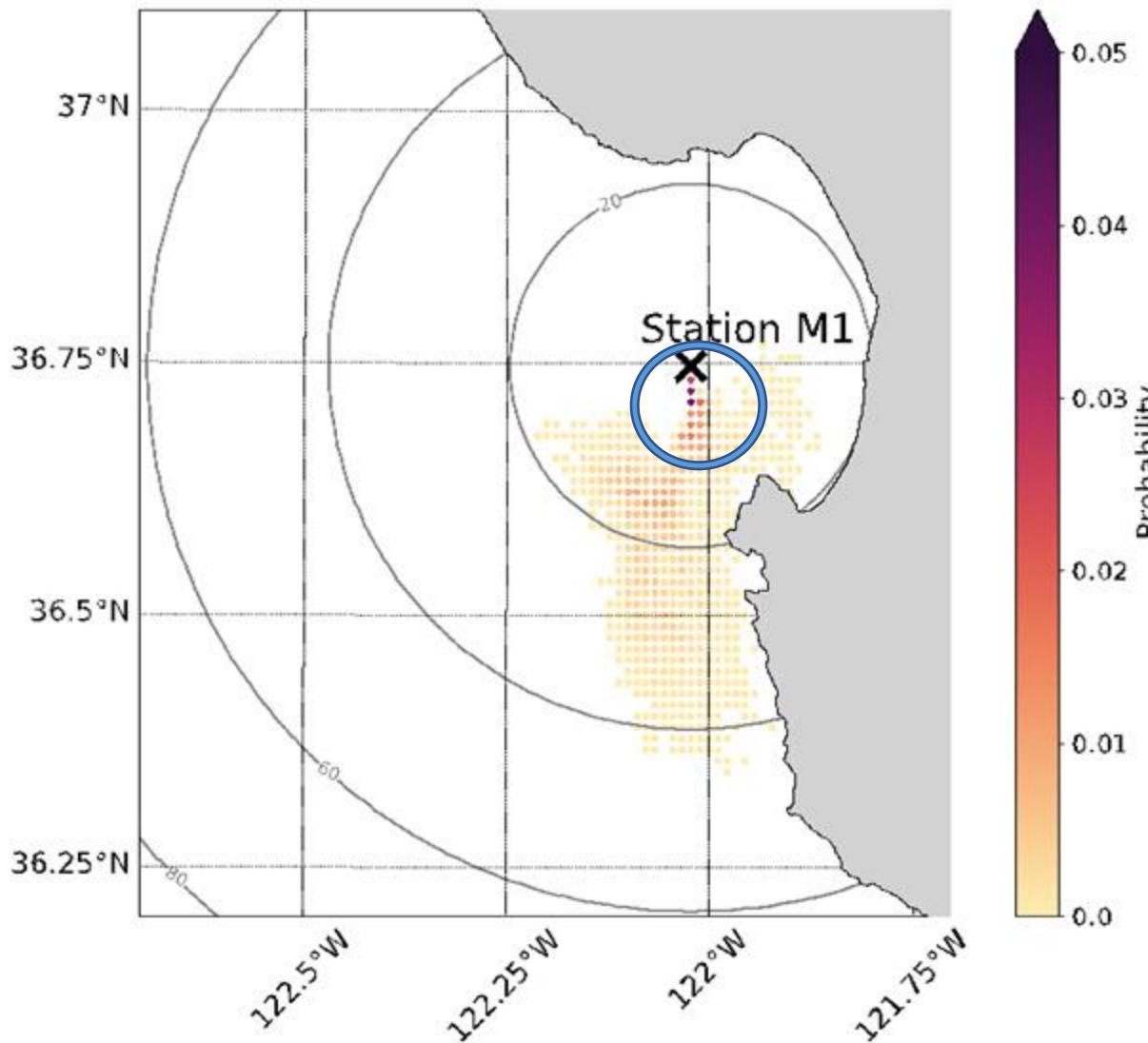
$$\frac{\partial M}{\partial t} = -\vec{v} \cdot \nabla M + \kappa_H \nabla_H^2 M + \frac{\partial}{\partial z} \left(\kappa_V \frac{\partial M}{\partial z} \right) + w_s \frac{\partial M}{\partial z} - kM + S$$

Advection Horizontal diffusion Vertical diffusion Settling Decay Shedding

physical ocean model eDNA properties

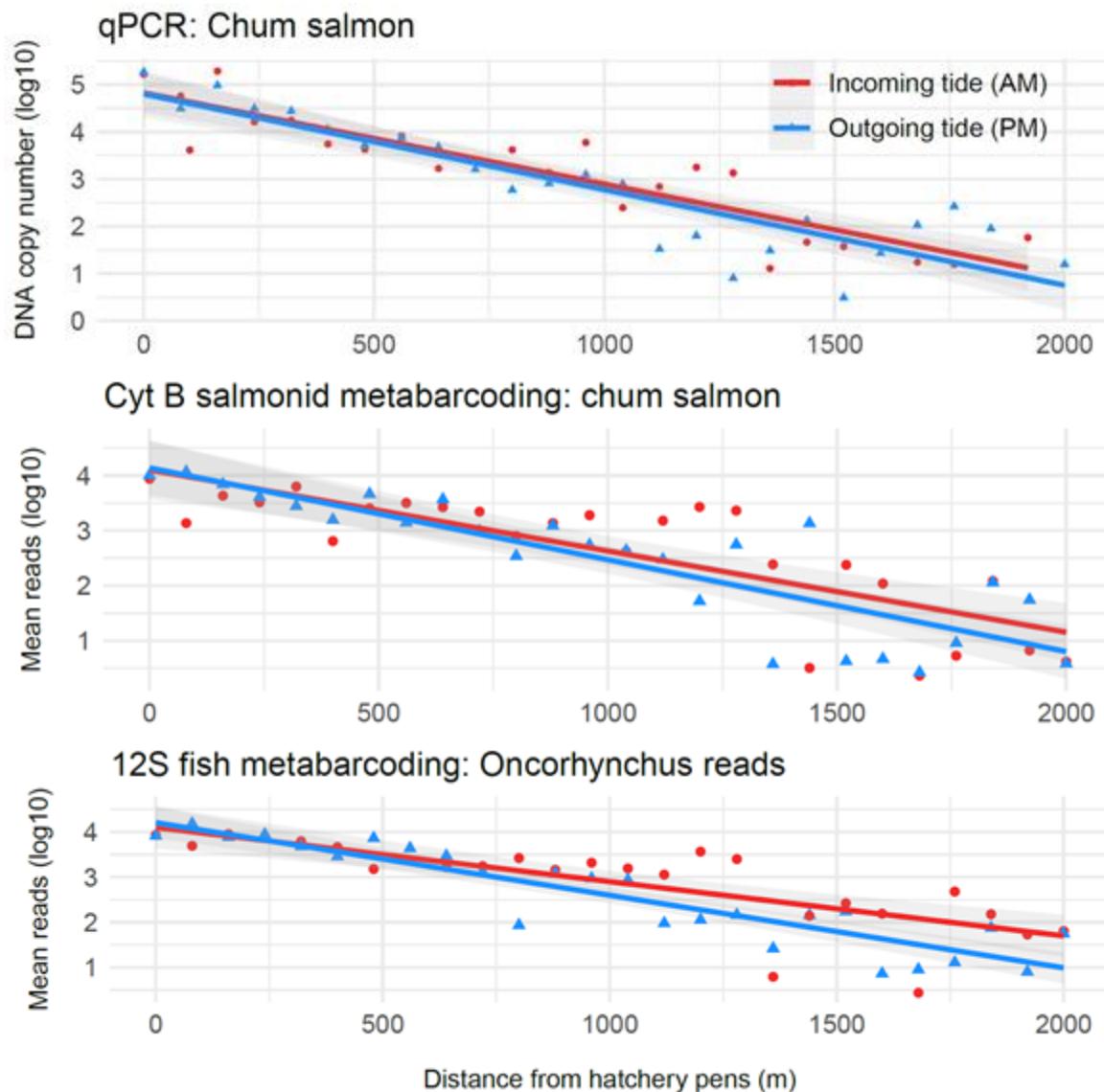
? ? ?

What might the future look like?

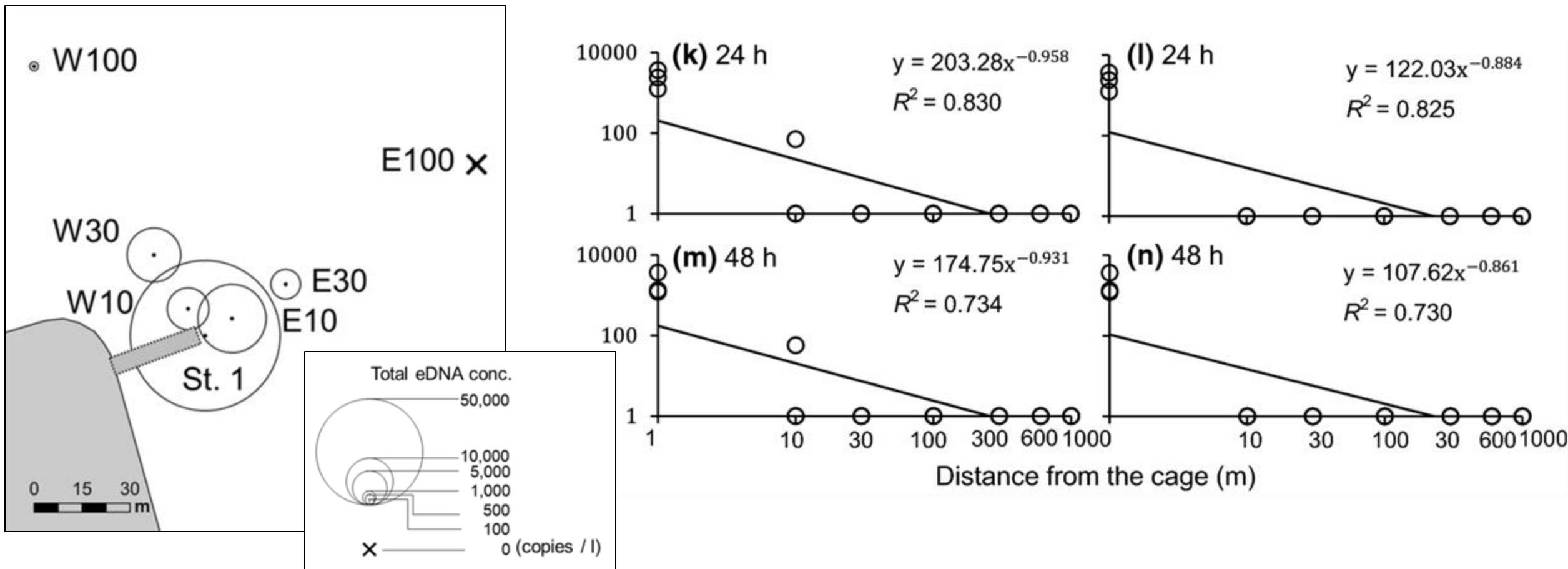


Thanks! Questions?

Observations: marine transport

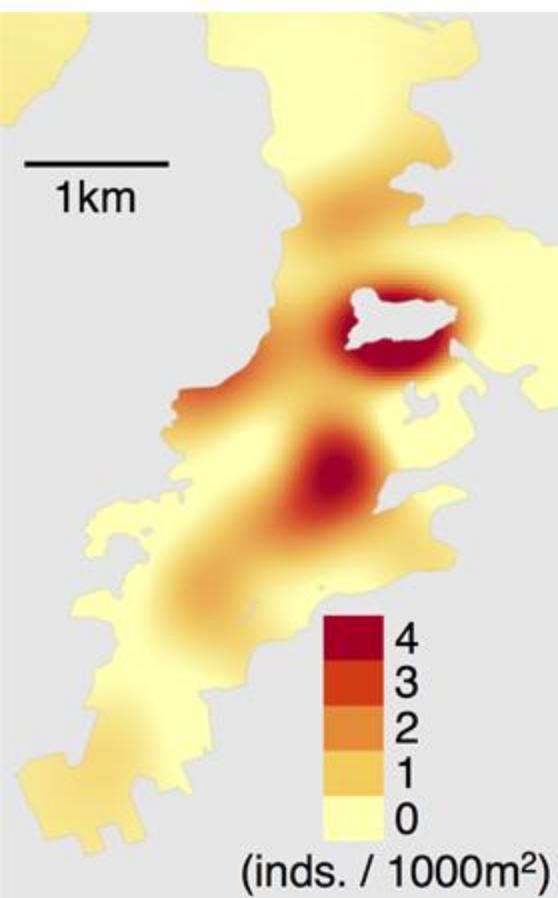


Observations: marine transport – caged fish

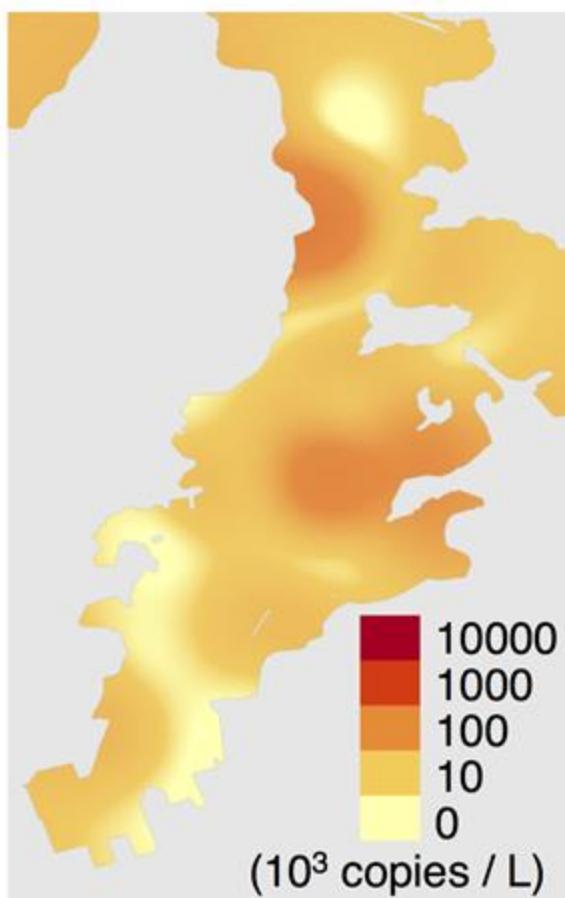


Observations: eDNA + visual observations

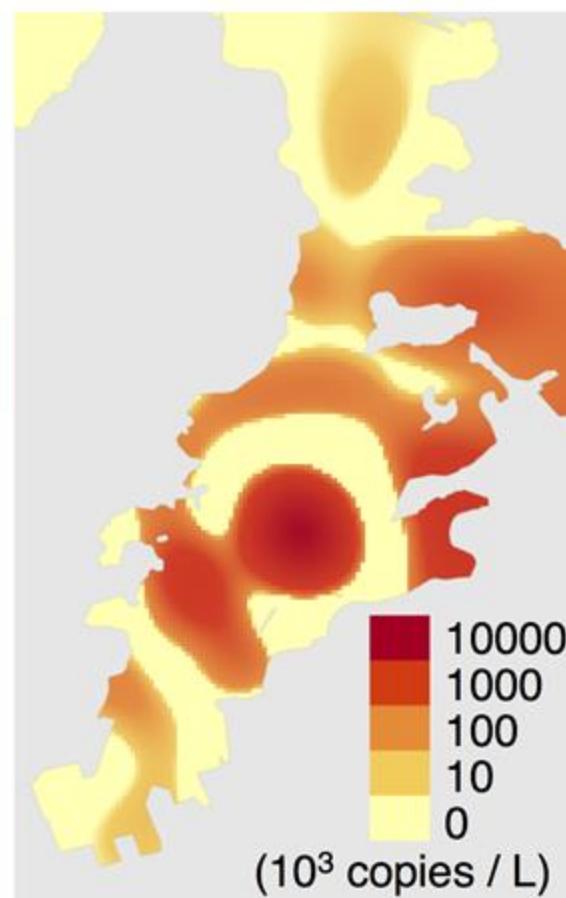
(a)



(b)



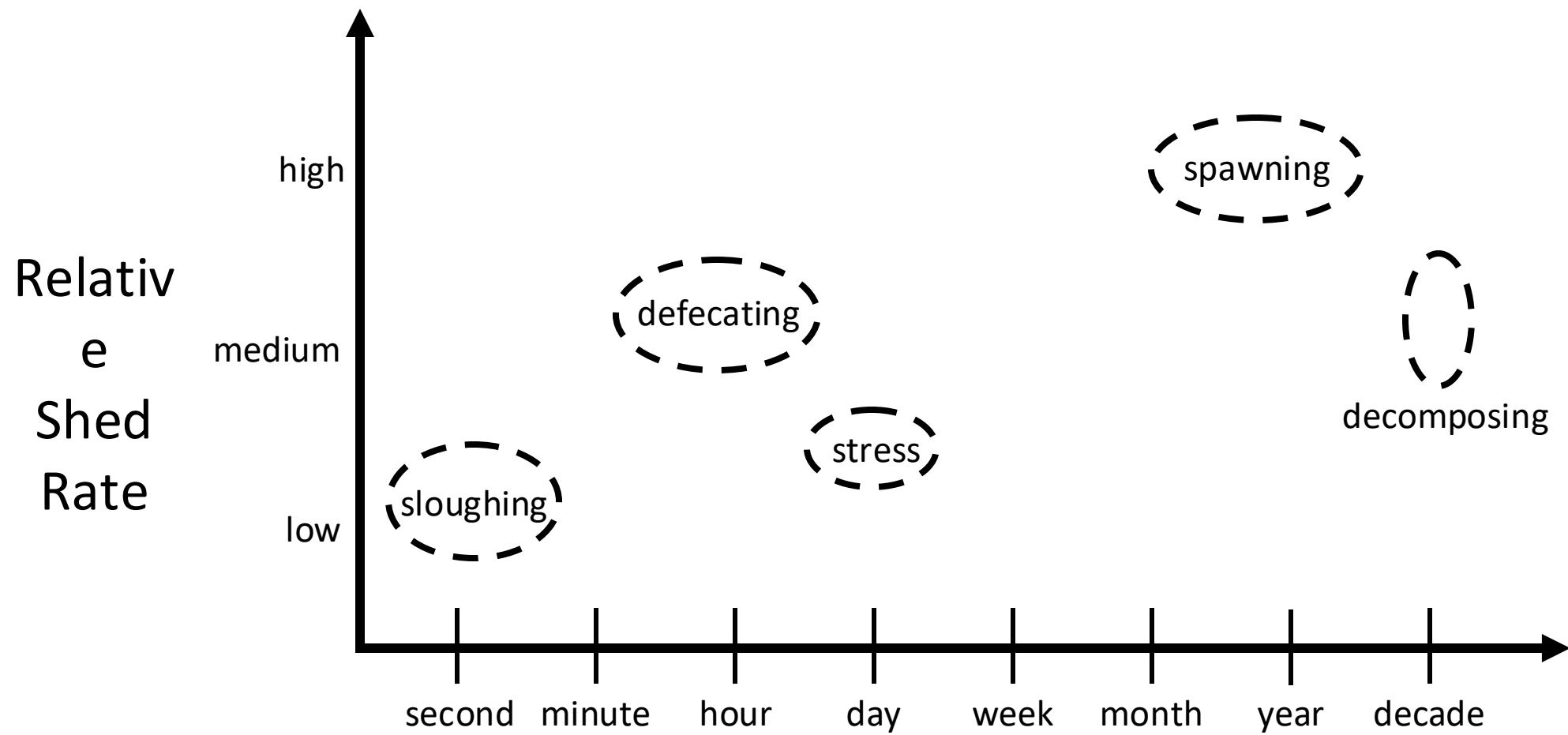
(c)



Observed individuals

eDNA (surface)

eDNA (bottom)

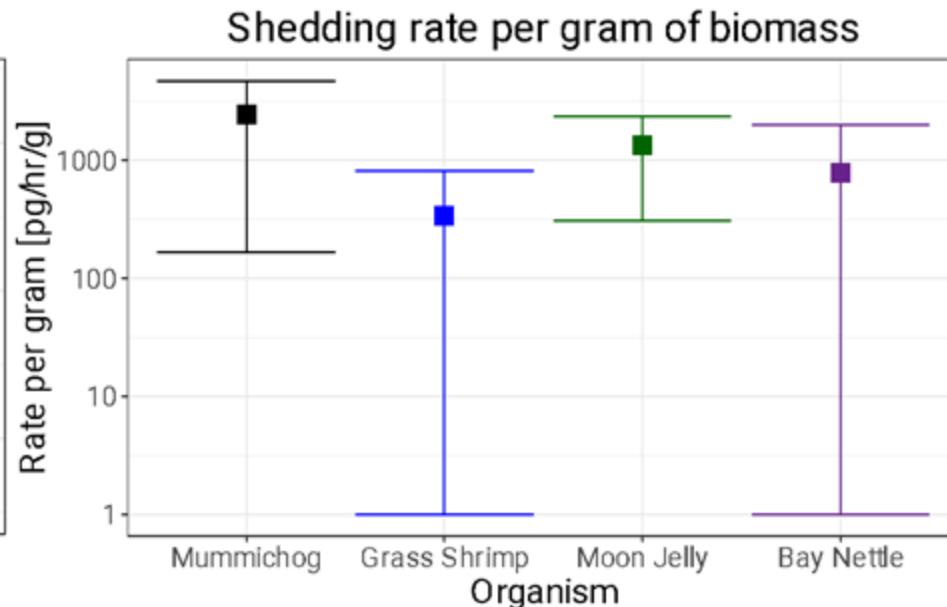
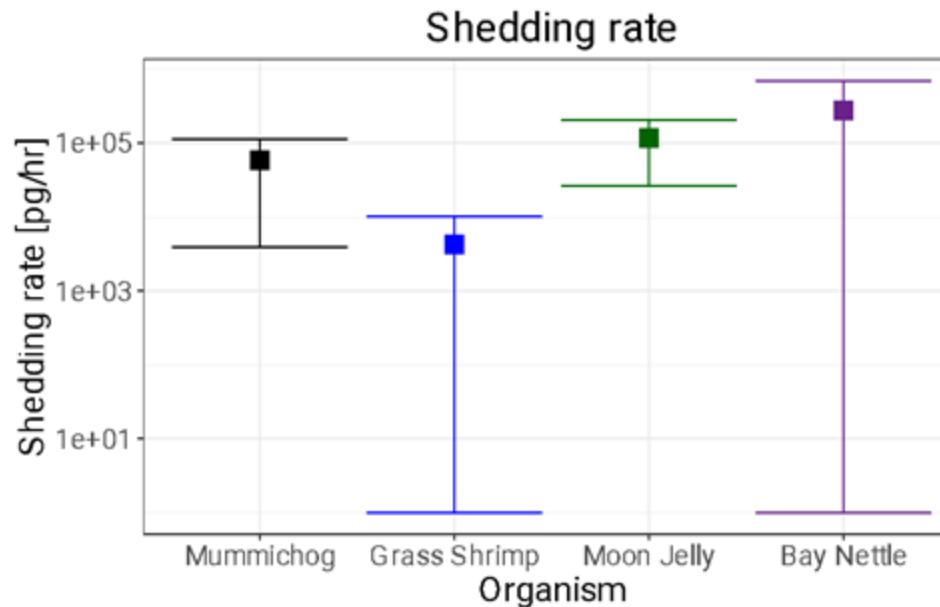


Temporal Frequency of Process (1/time)

Not all shedding is equal...



*note log scale



1. Bay nettle
2. Moon jelly
3. Mummichog
4. Shrimp

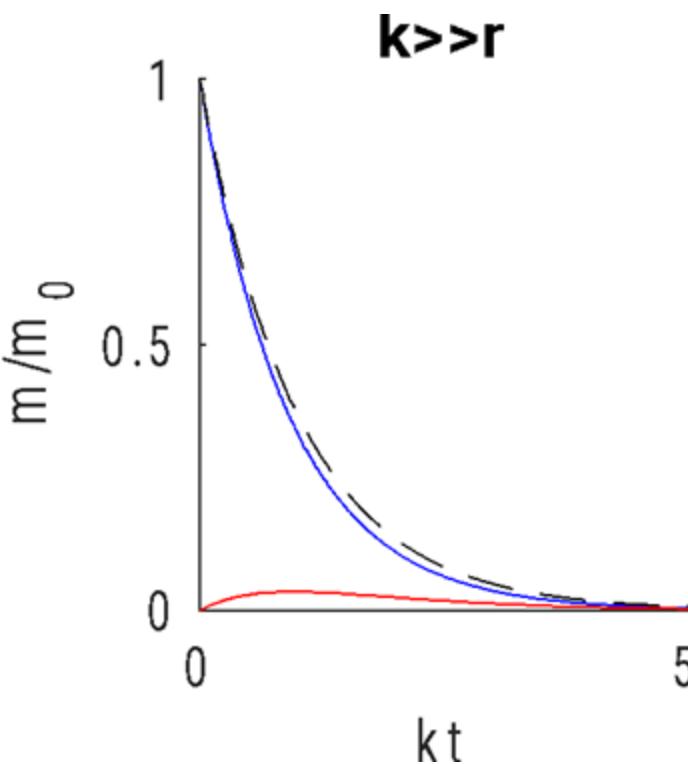
1. Mummichog
2. Moon jelly
3. Bay nettle
4. Shrimp

$$m = m_0 e^{-(k+r)t}$$

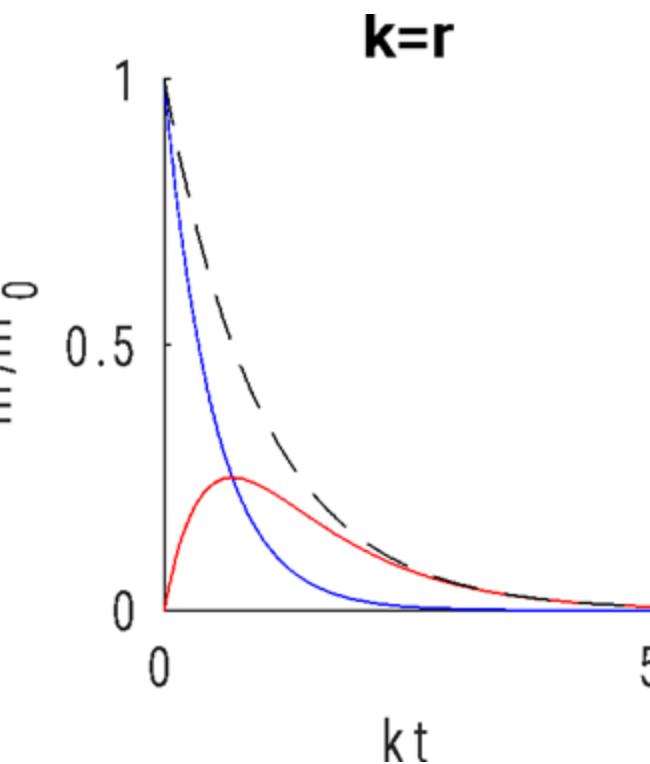
k is decay rate; **r** is breakdown rate

$$m_r = m_0 e^{-kt} [1 - e^{-rt}]$$

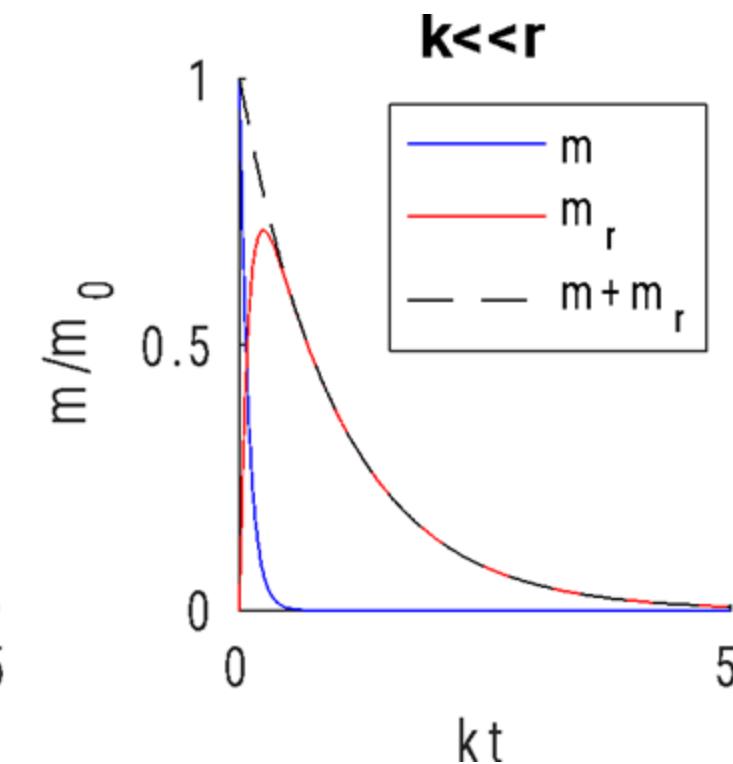
$$m + m_r = m_0 e^{-kt}$$



Large particles decay faster than breaking into smaller particles



Both decay and breakdown are important



Large particles break down very quickly into small particles and then small particles decay

MODEL avg k: 0.06 1/hr (changes w T)

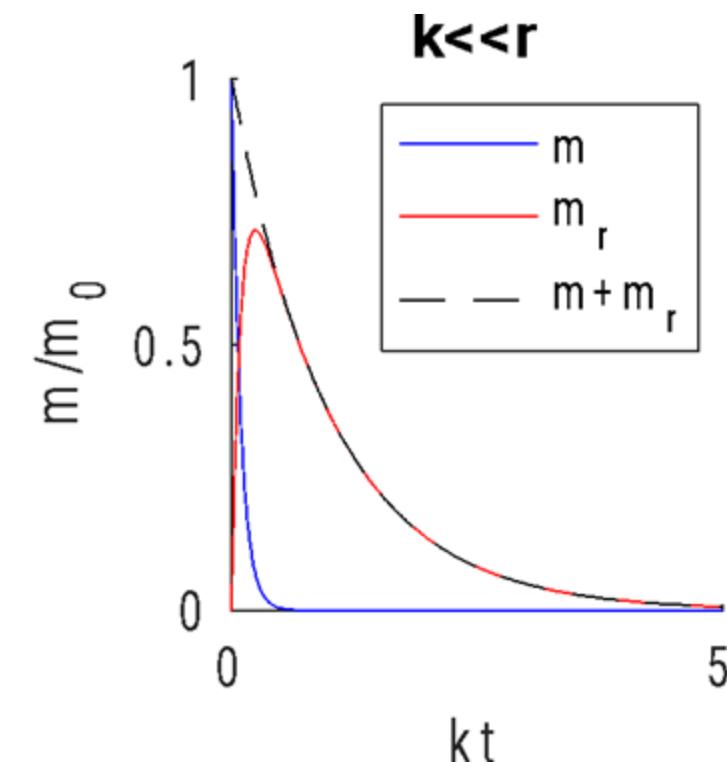
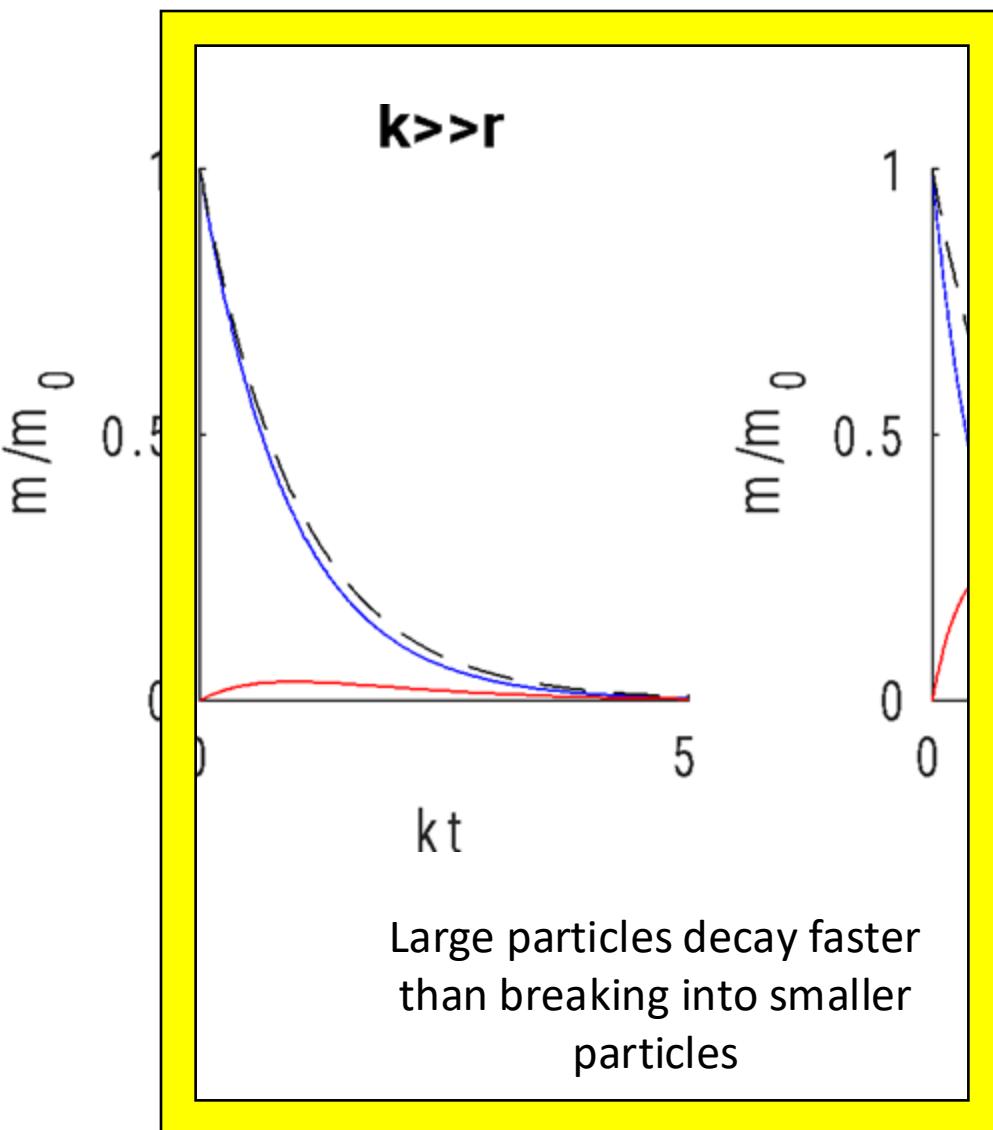
MODEL r: 0.008 1/hr (not change)

$$m = m_0 e^{-(k+r)t}$$

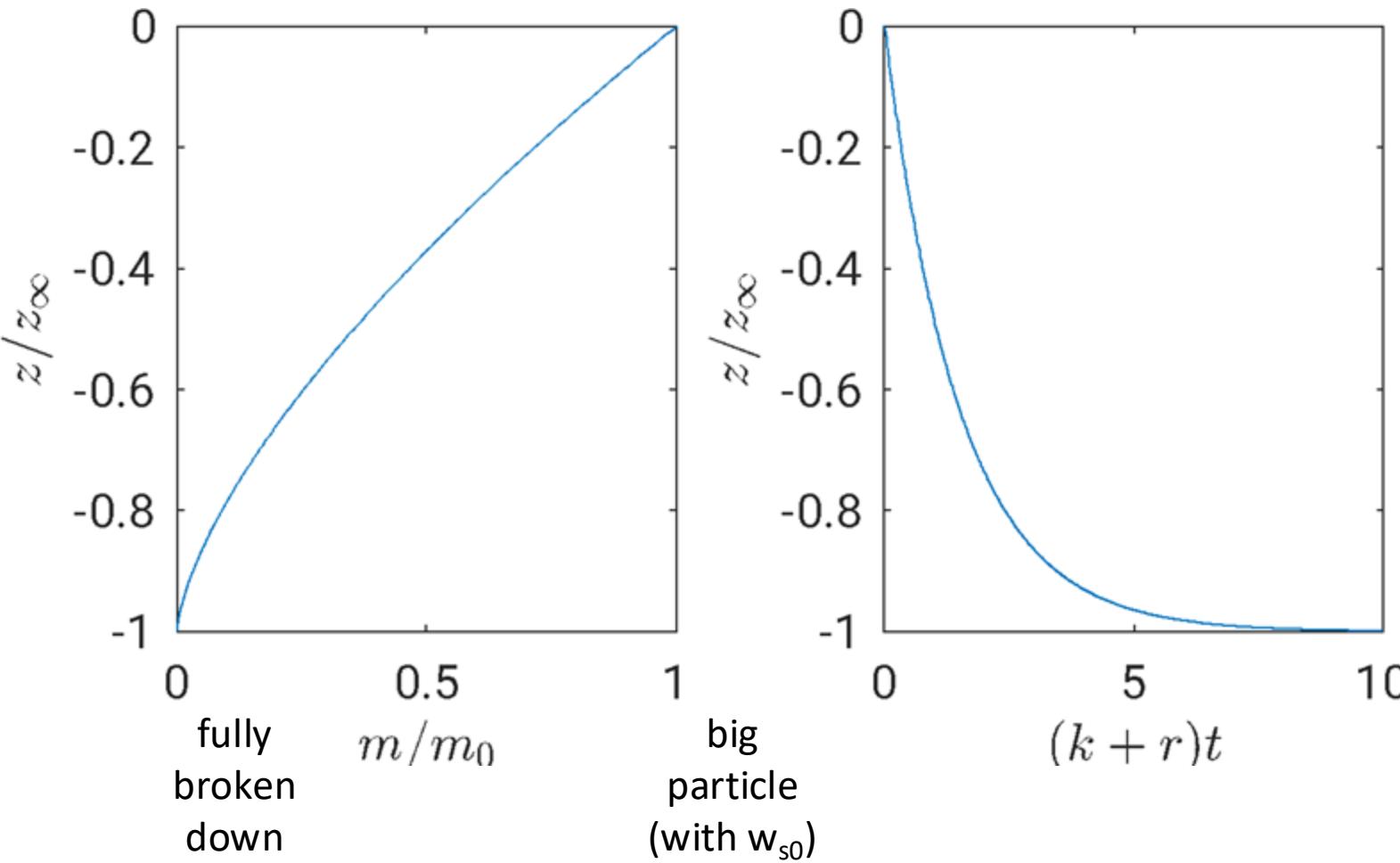
k is decay rate; r is breakdown rate

$$m_r = m_0 e^{-kt} [1 - e^{-rt}]$$

$$m + m_r = m_0 e^{-kt}$$



As particles breakdown, settle slower



$$w_s = \frac{1}{18} \frac{\frac{\rho_p}{\rho} - 1}{\nu} g d^2.$$

$$d = 2 \left(\frac{3}{4\pi} \frac{m}{\rho_p} \right)^{1/3}$$

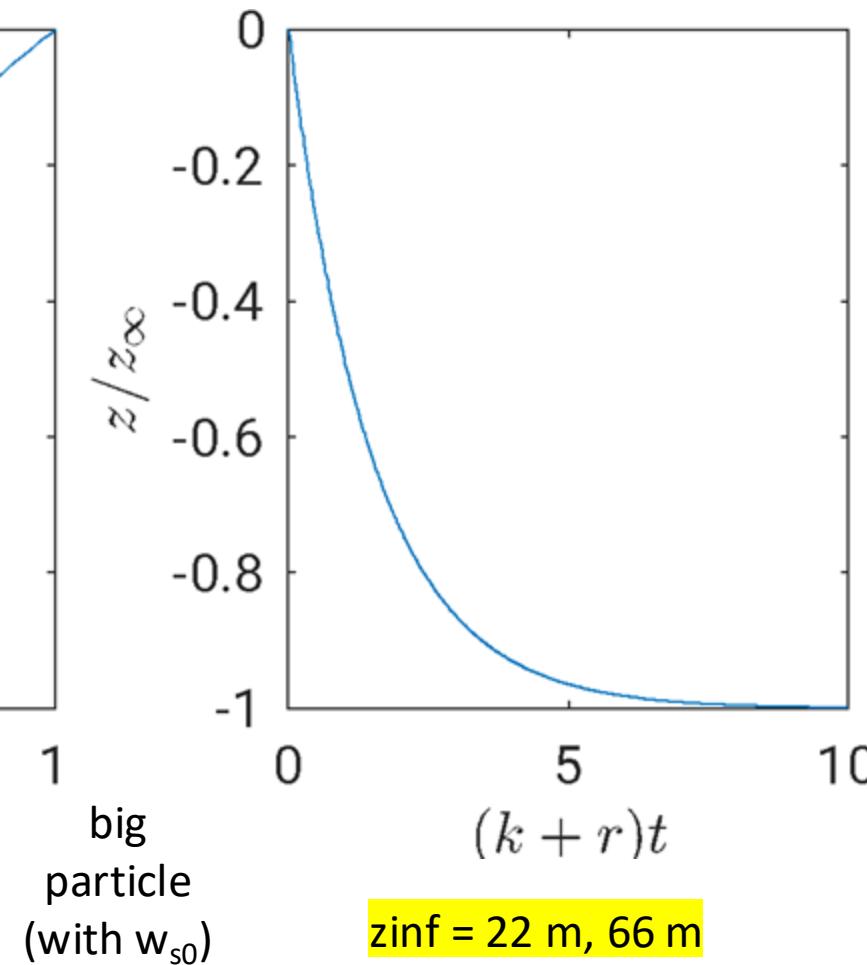
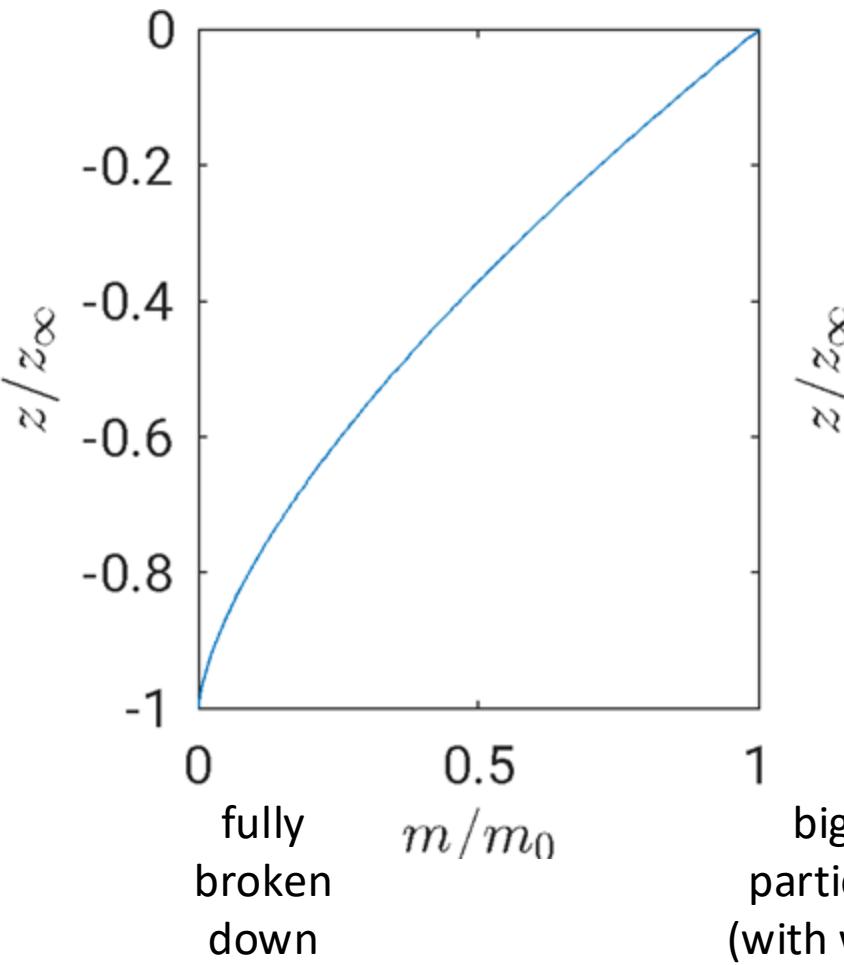
$$\frac{w_s}{w_{s0}} = \left(\frac{m}{m_0} \right)^{2/3}.$$

$$w_s = w_{s0} e^{-2/3(k+r)t}.$$

$$z_\infty = \int_0^\infty w_s dt = w_{s0} \int_0^\infty e^{-2/3(k+r)t} dt$$

$$z_\infty = \frac{3}{2} \frac{w_{s0}}{k+r}$$

As particles breakdown, settle slower



$$w_s = \frac{1}{18} \frac{\frac{\rho_p}{\rho} - 1}{\nu} g d^2.$$

$$d = 2 \left(\frac{3}{4\pi} \frac{m}{\rho_p} \right)^{1/3}$$

$$\frac{w_s}{w_{s0}} = \left(\frac{m}{m_0} \right)^{2/3}.$$

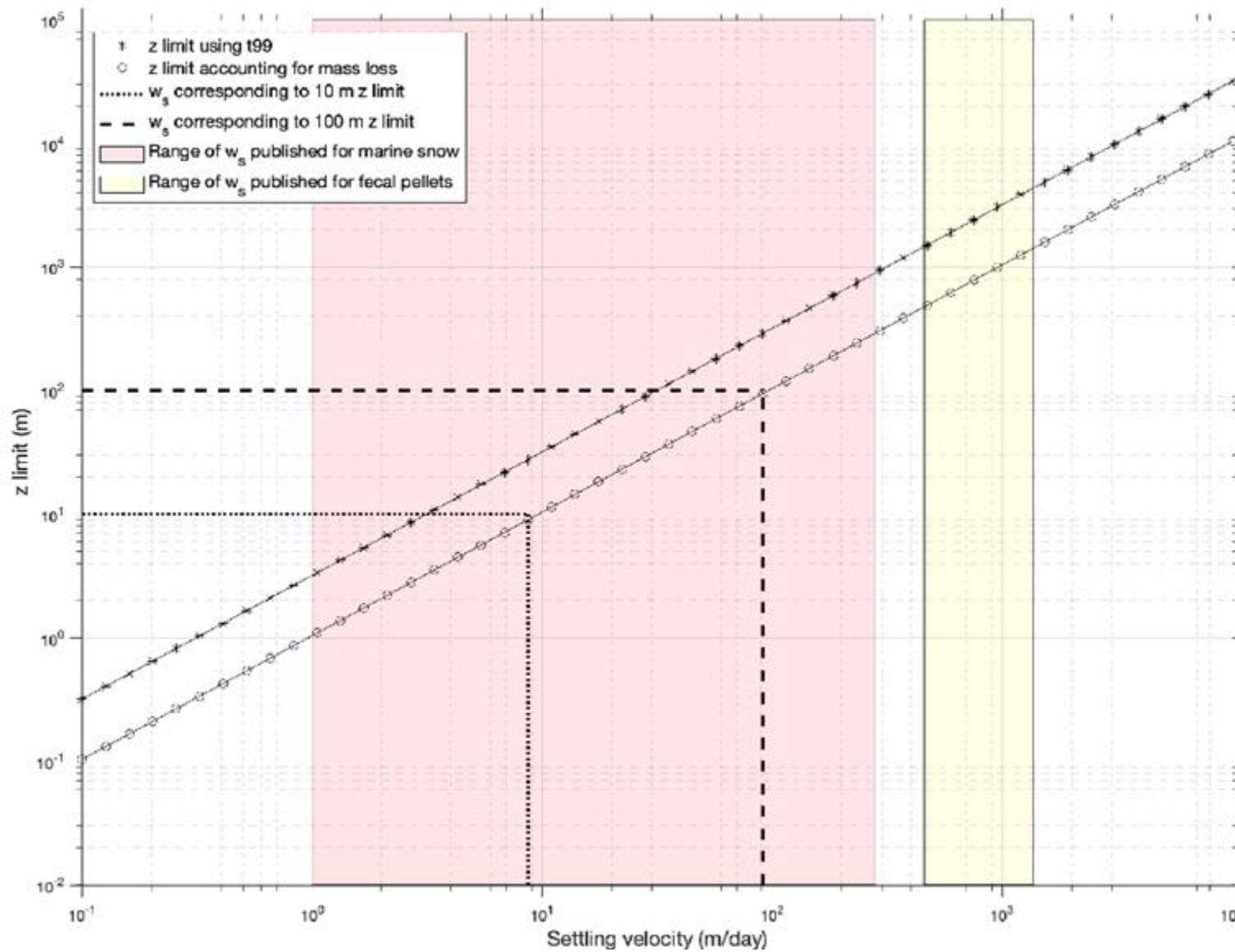
$$w_s = w_{s0} e^{-2/3(k+r)t}.$$

$$z_\infty = \int_0^\infty w_s dt = w_{s0} \int_0^\infty e^{-2/3(k+r)t} dt$$

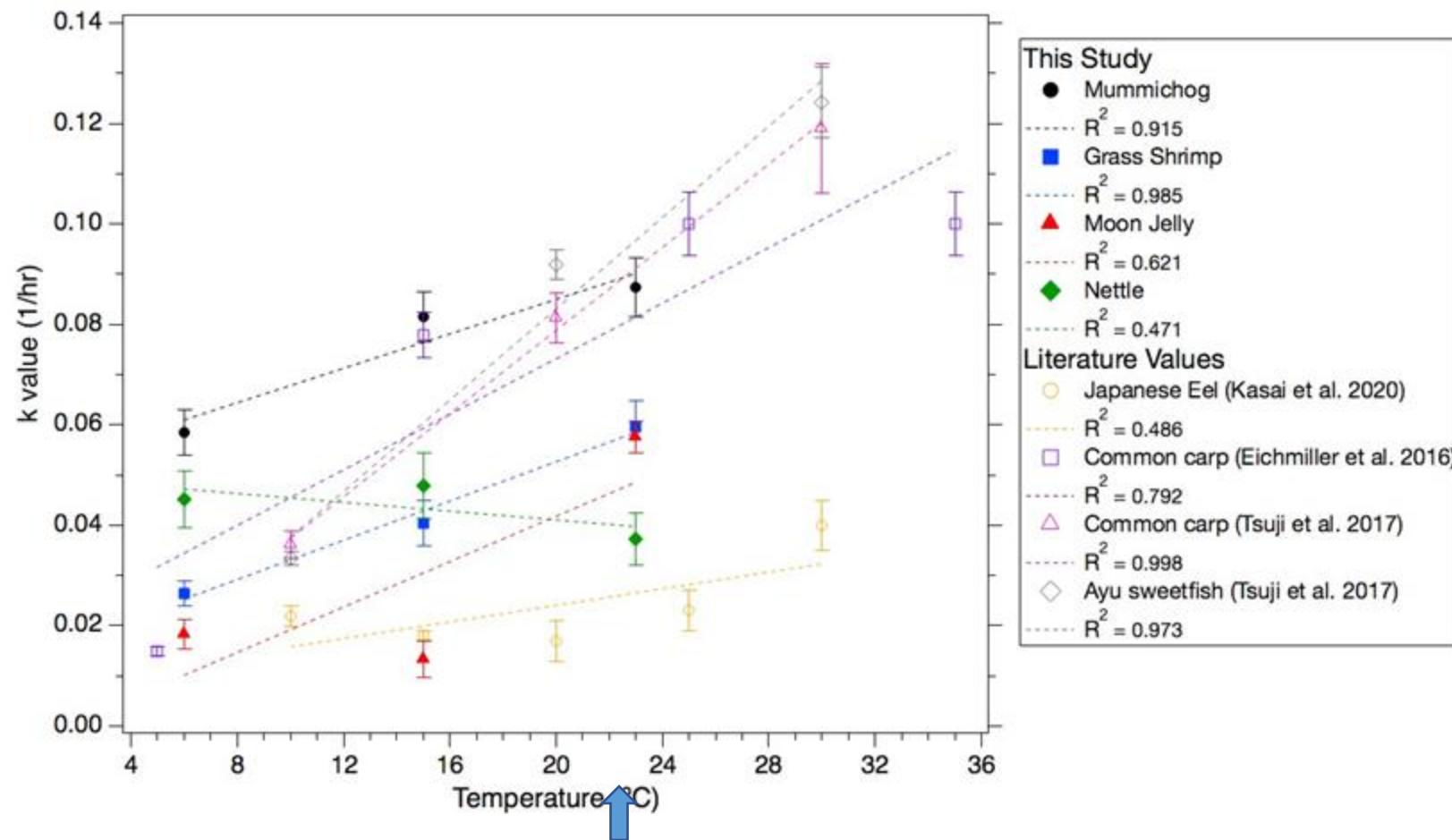
$$z_\infty = \frac{3}{2} \frac{w_{s0}}{k+r}$$

MODEL avg k: 0.06 1/hr (changes w T)
 MODEL r: 0.008 1/hr (not change)
 MODEL w_{s0} : 0, 25 m/day, 75 m/day

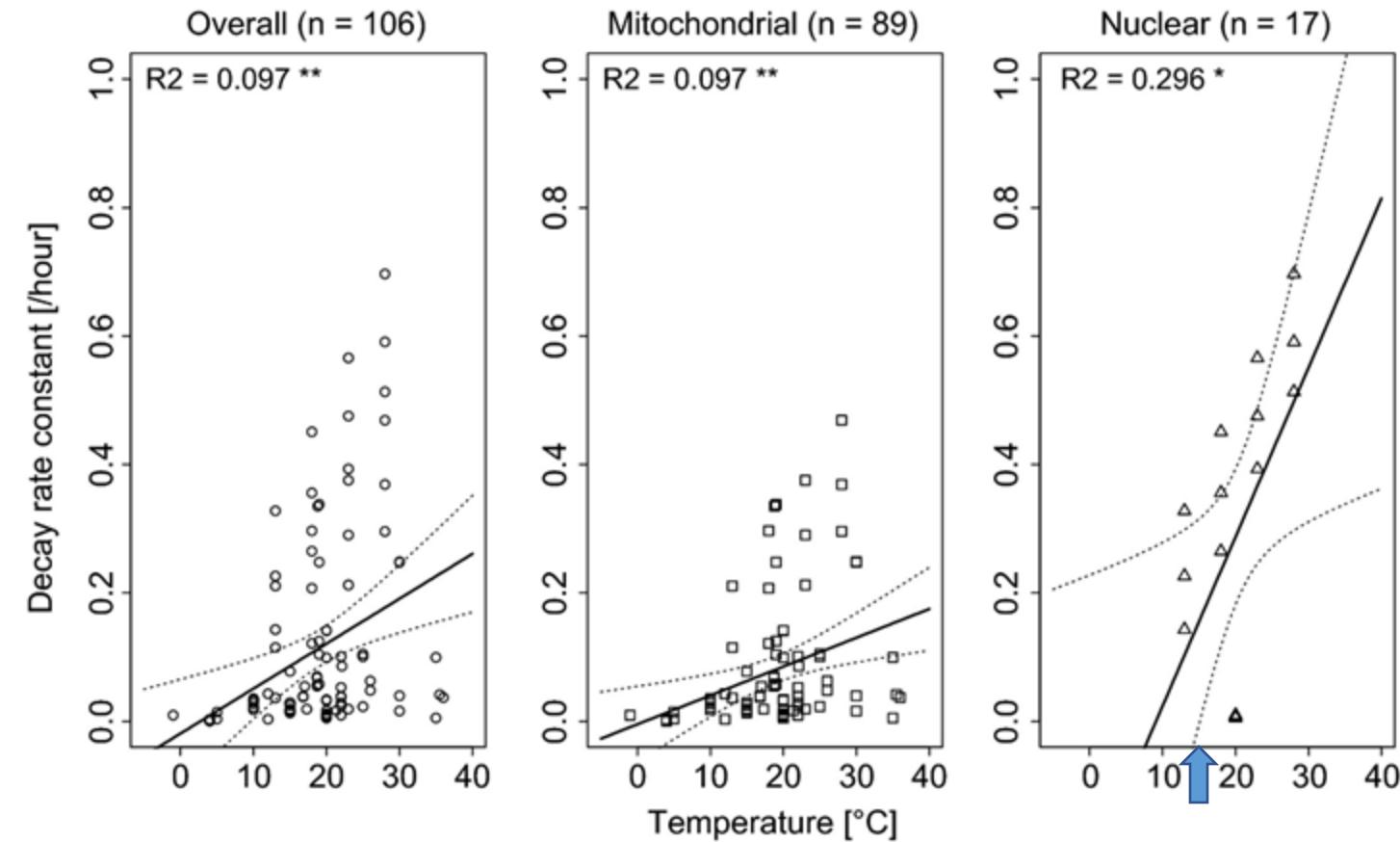
Side note: settling velocity is actually a sensitive parameter - but what estimate to use?



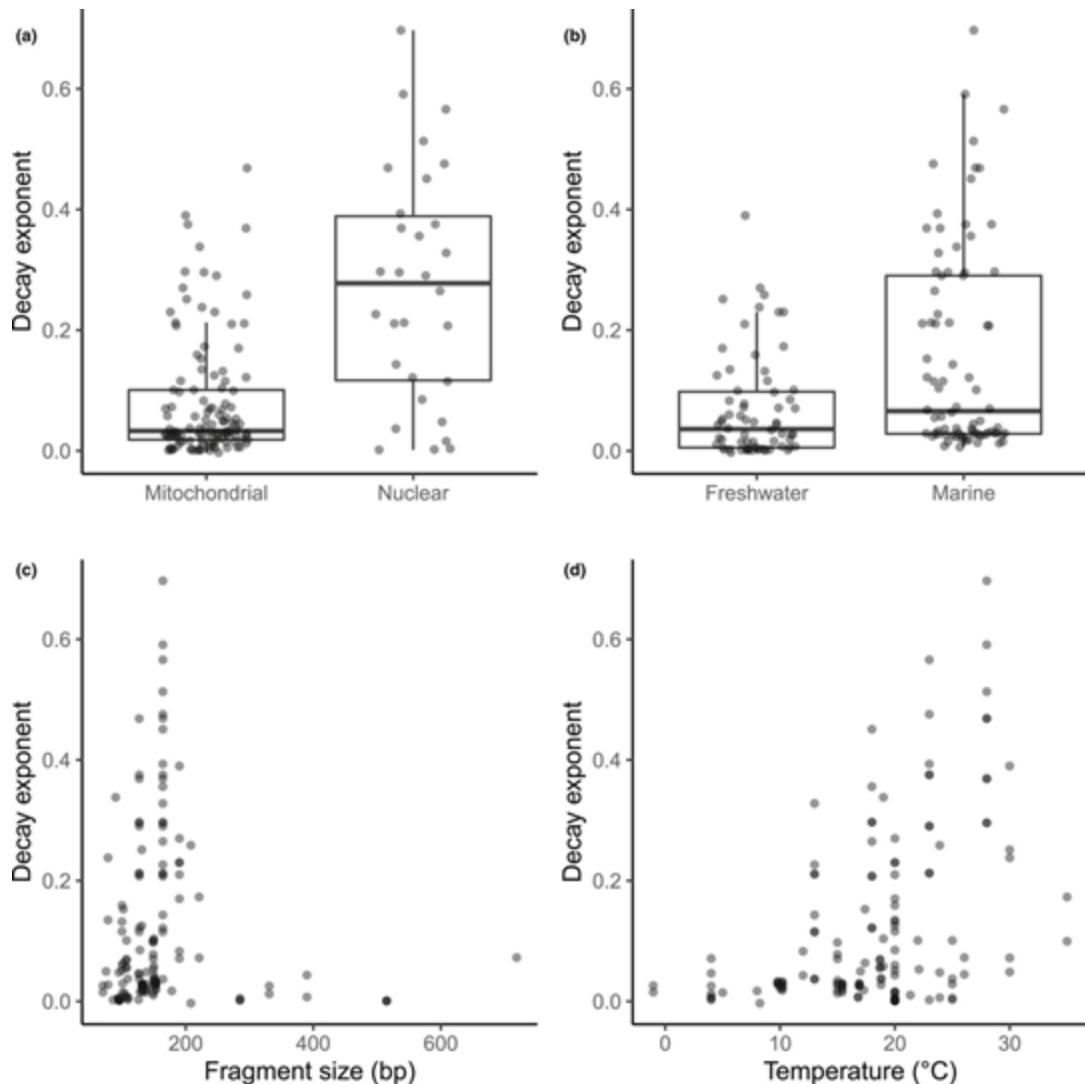
Decay: Literature review



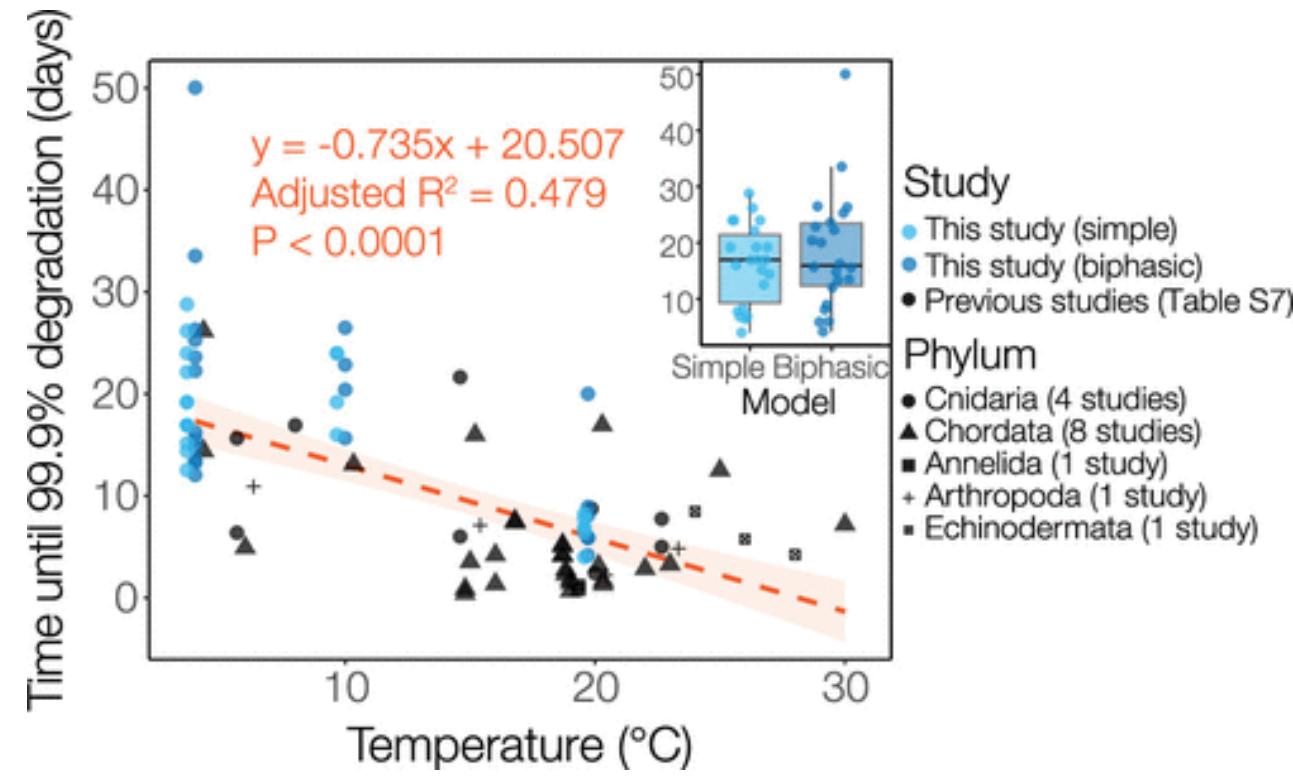
Decay: Literature review



eDNA decay rates



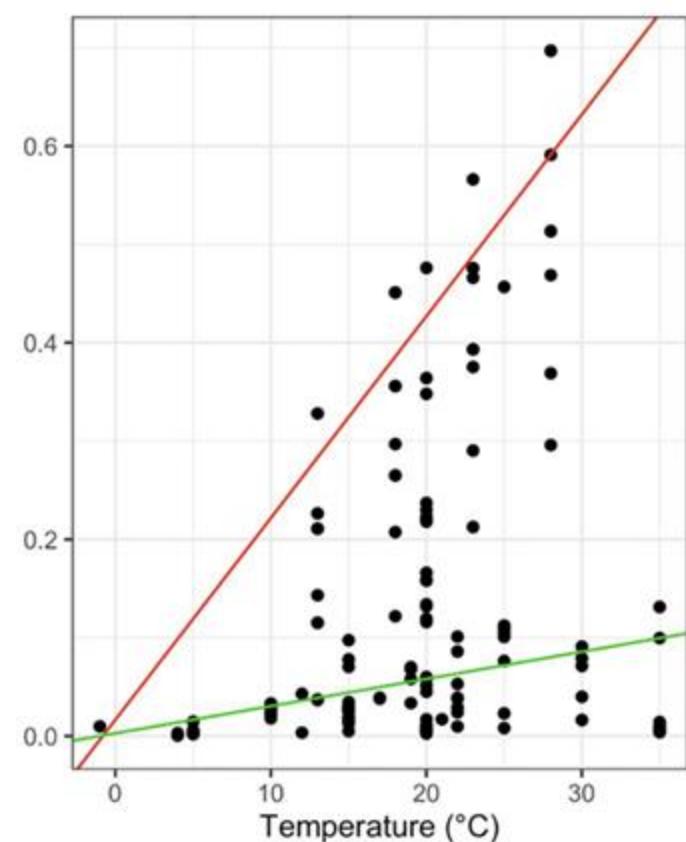
Lamb et al. (2022)



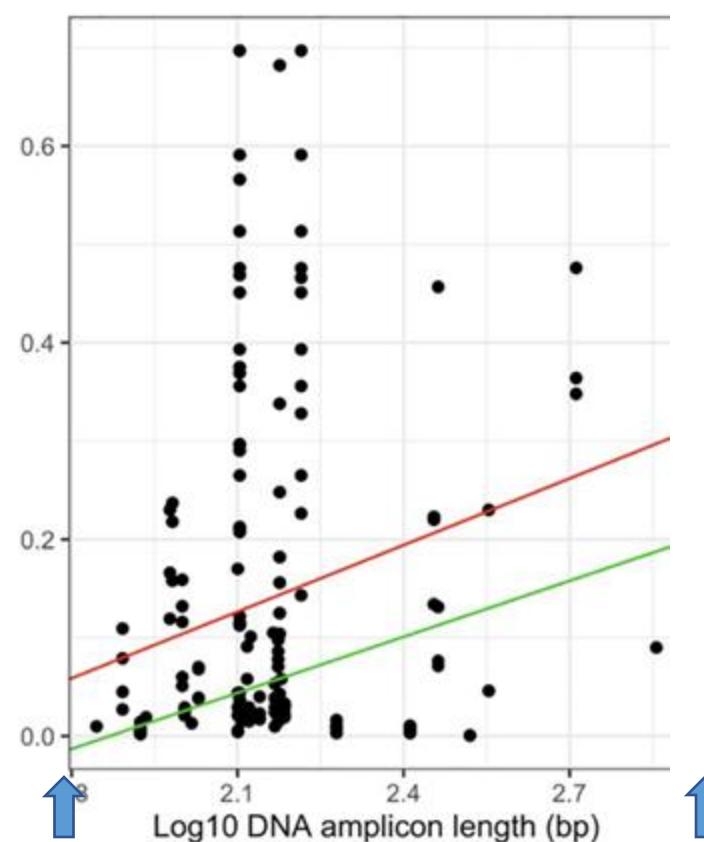
McCartin et al. (2022)

Decay: Literature review

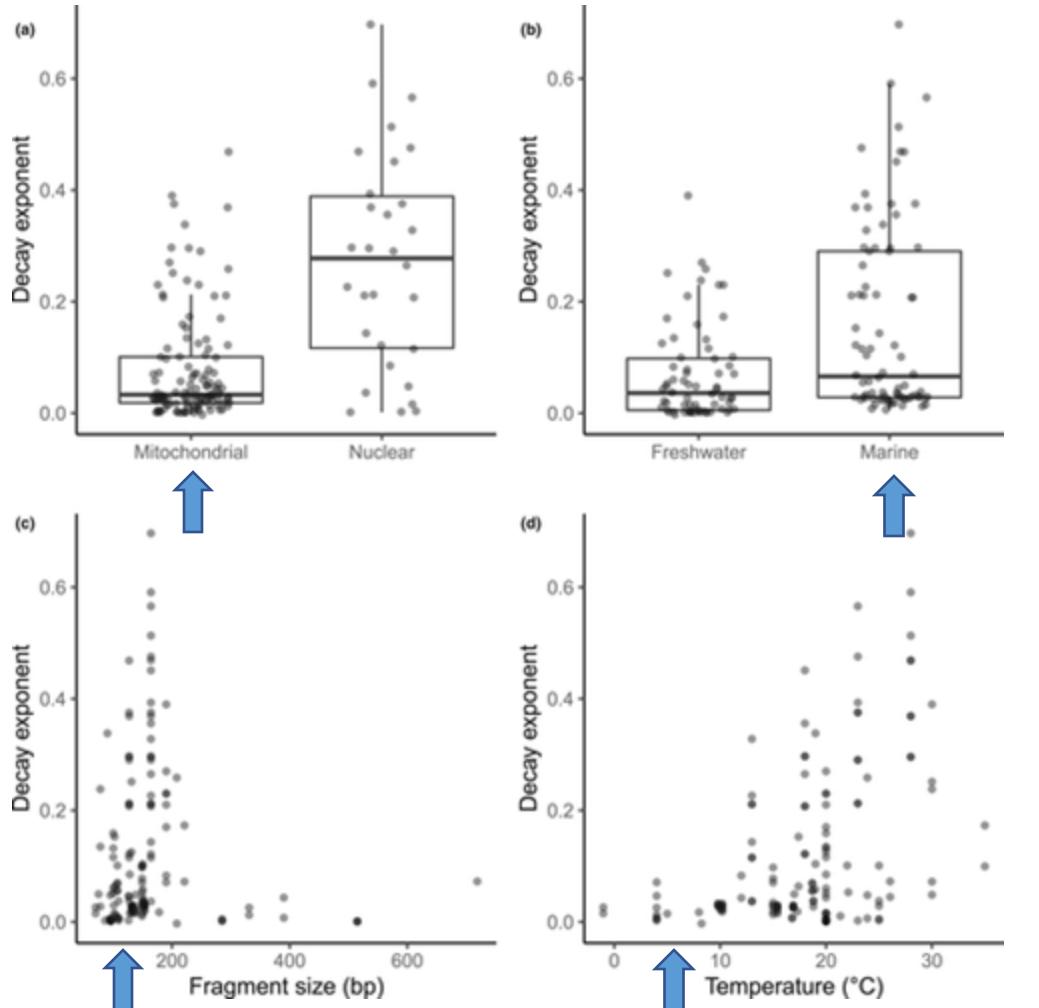
A water temperature



B DNA amplicon length



Decay: Literature review



	Author	H ₂ O	DNA	Temperature (°C)	Fragment length (bp)	Weighting (%)	Decay exponent
2020	Villacorta-Rath <i>et al.</i>	F	M	30 – 35	221	0.3	●
	Curtis <i>et al.</i>	F	M	8.2 – 23.9	208	0.1	●
	Skinner <i>et al.</i>	M	M	17.4	102	0	●
	Kasai <i>et al.</i>	F	M	10 – 30	138	4.4	●
	Sakata <i>et al.</i>	F	M	17.3	132	0.2	●
	Kutti <i>et al.</i>	M	M	8	178	0.2	●
	Wood <i>et al.</i>	M	M	19	90 – 150	0.3	●
2019	Jo <i>et al.</i>	M	N	13 – 28	164	4.2	●
	Takahara <i>et al.</i>	F	M	4 – 25	94 – 126	1.8	●
	Sengupta <i>et al.</i>	F	M	23	86	0.2	●
	Moushomi <i>et al.</i>	F	B	20	101 – 128	0.7	●
	Jo <i>et al.</i>	M	M	13 – 28	127	2.4	●
	Ladell <i>et al.</i>	F	B	23.9	95 – 110	0.4	●
	Collins <i>et al.</i>	M	M	9.8 – 16.9	132 – 153	10.4	●
2018	Nukazawa <i>et al.</i>	F	M	21.4 – 22.1	149	0.2	●
	Nevers <i>et al.</i>	F	M	12 – 19	150	0.3	●
	Cowart <i>et al.</i>	M	M	-1	70	0.4	●
	Byllemans <i>et al.</i>	F	B	20	95 – 515	15.1	●
	Minamoto <i>et al.</i>	M	M	18.8	151	1.1	●
	Weltz <i>et al.</i>	M	M	4	331	0.5	●
	Jo <i>et al.</i>	M	M	26	127 – 719	0.2	●
2017	Tsuji <i>et al.</i>	F	M	10 – 30	78 – 131	0.4	●
	Hinlo <i>et al.</i>	F	M	4 – 20	390	0.5	●
	Lance <i>et al.</i>	F	M	4 – 30	190	47.4	●
	Andruszkiewicz <i>et al.</i>	M	M	16.8	107	2.9	●
	Eichmiller <i>et al.</i>	F	M	5 – 35	149	2.7	●
	Sassoubre <i>et al.</i>	M	M	18.7 – 22	107	1	●
	Forsström <i>et al.</i>	M	M	17	75	0.1	●
2014	Maruyama <i>et al.</i>	F	M	20	100	0.4	●
	Thomsen <i>et al.</i>	M	M	15	101 – 104	1.1	●

Retos

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- ✓ Bases de datos de referencia
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- ✓ Metodológicos, técnicos
- ✓ Ambiente complejo, dinámico
- ✓ Diversidad genética



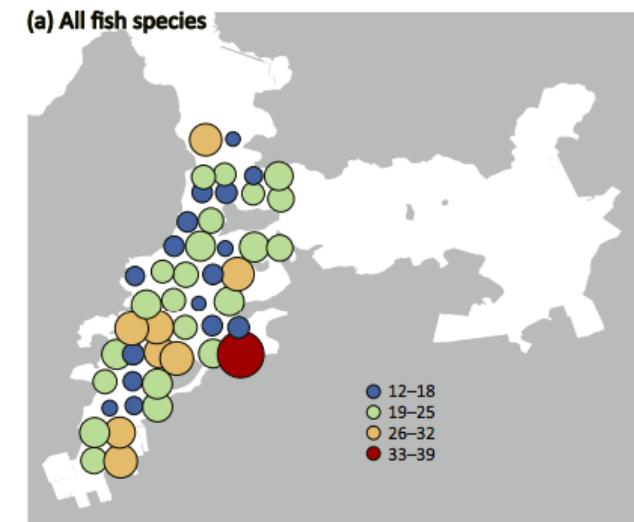
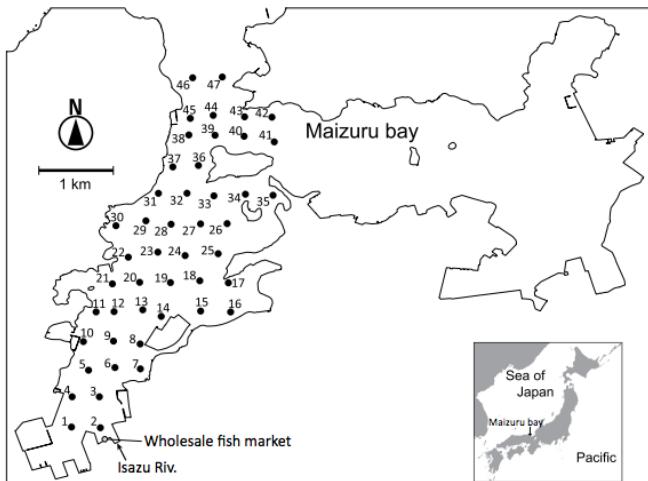
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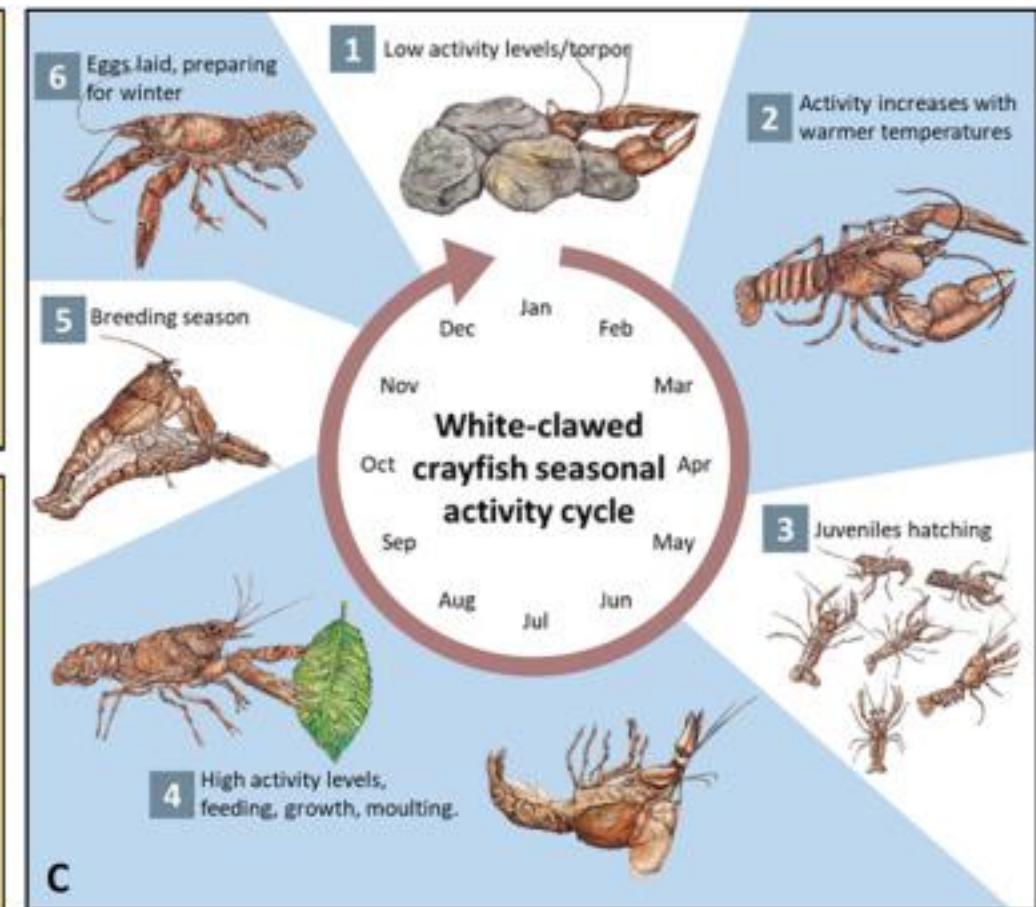
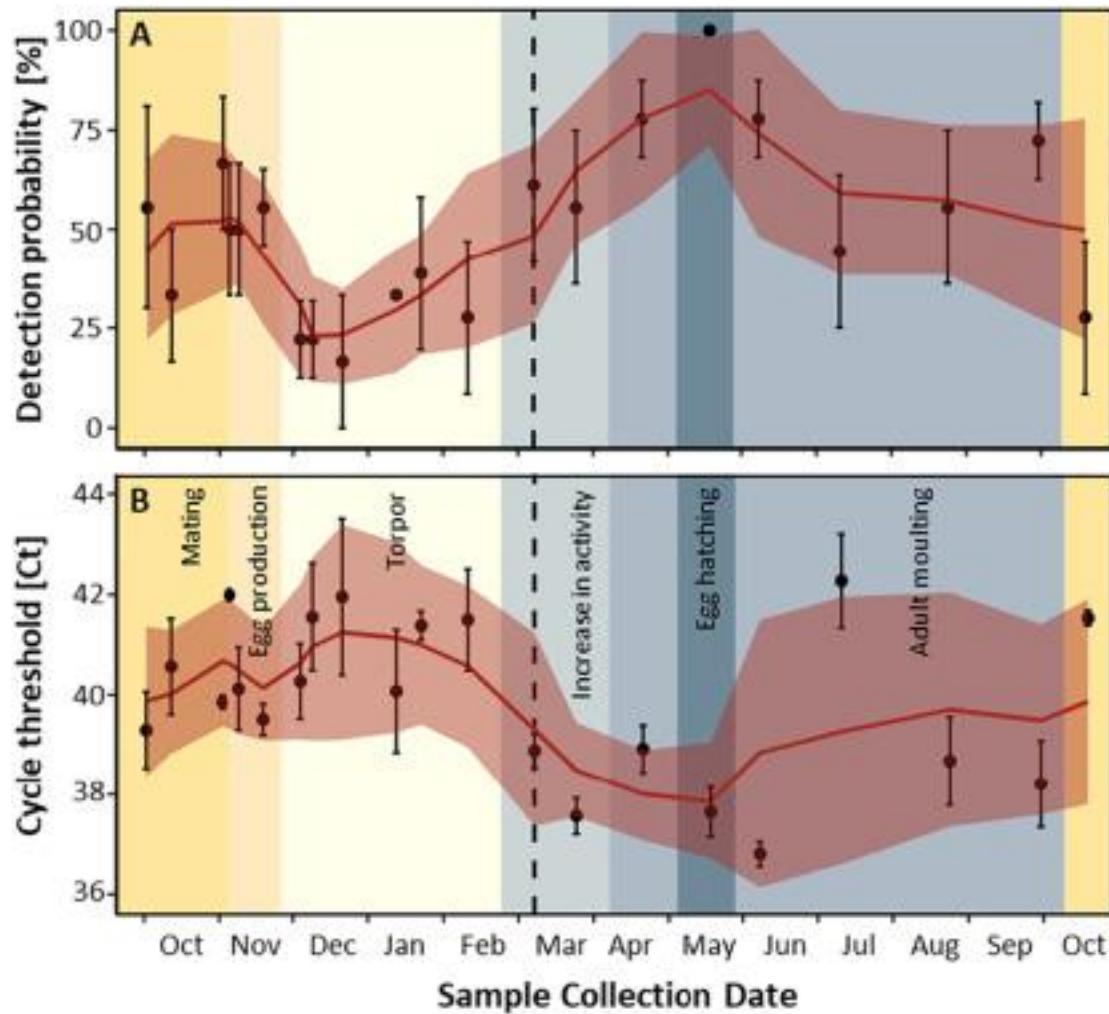
Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea

Received: 06 June 2016
Accepted: 06 December 2016
Published: 12 January 2017

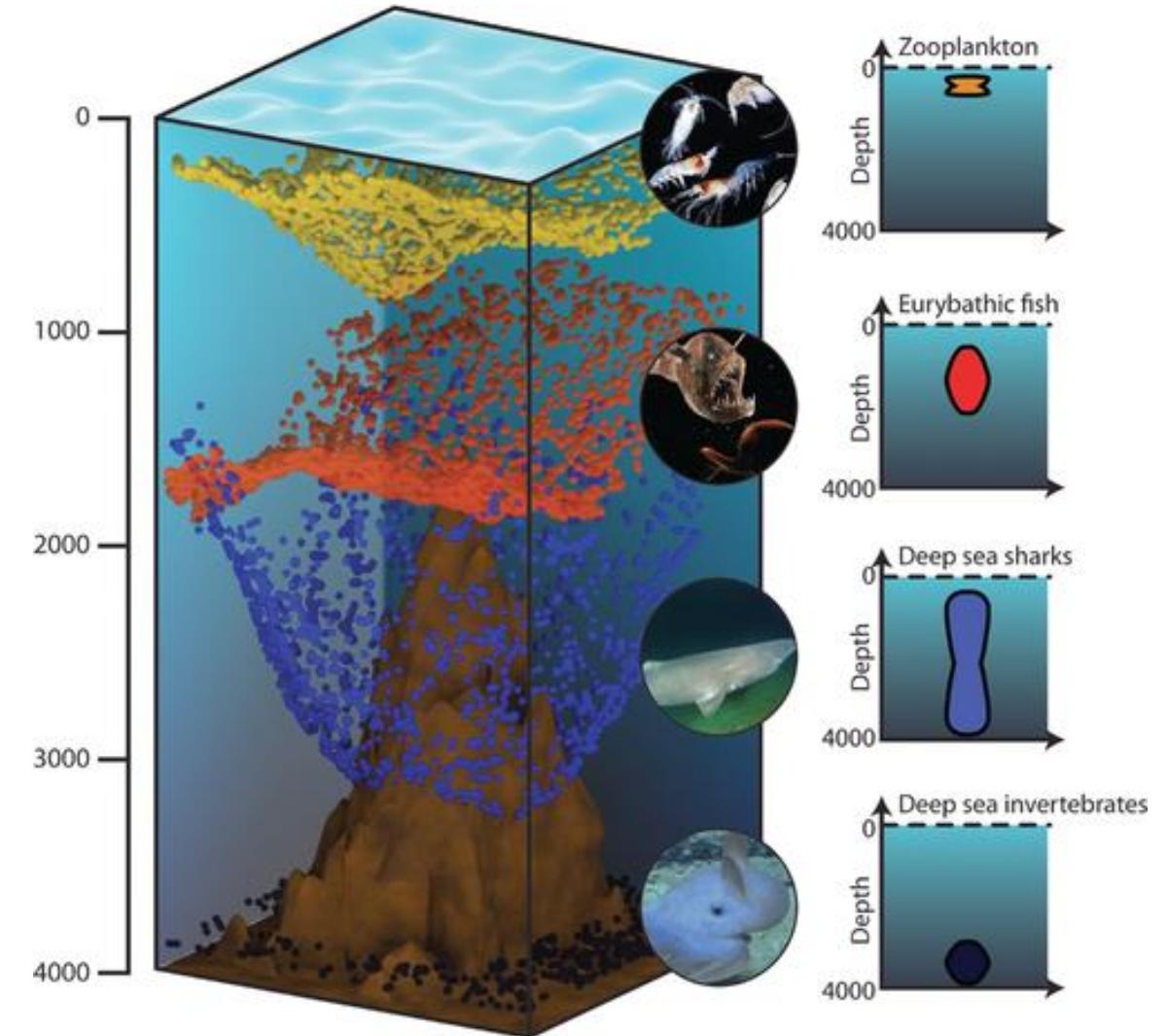
Satoshi Yamamoto¹, Reiji Masuda², Yukuto Sato³, Tetsuya Sado⁴, Hitoshi Araki⁵, Michio Kondoh⁶, Toshifumi Minamoto¹ & Masaki Miya⁴

- 47 stations ~11 km² Maizuru Bay, Japan
- 1 L sea water filtered, 12S rRNA
- 147 species
- ✓ Over 14 years of underwater visual censuses (140 censuses), 73,709 individuals belonging to 80 species were recorded.
- ✓ Metabarcoding detected 62.5 % of visually detected species



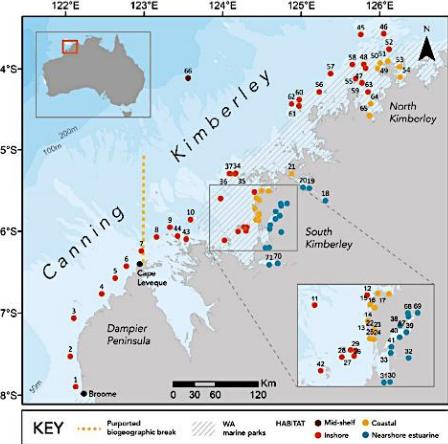
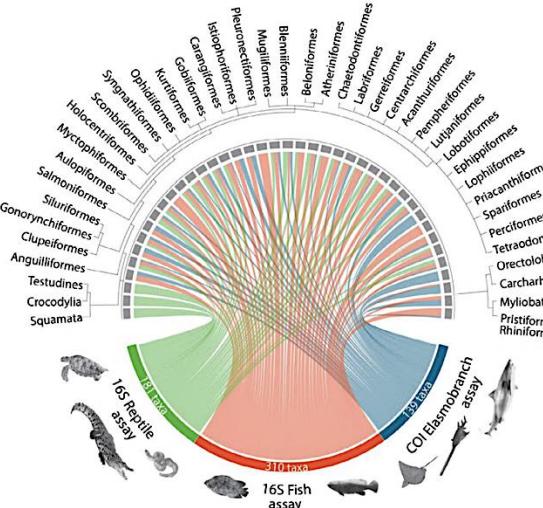


Troth et al 2021



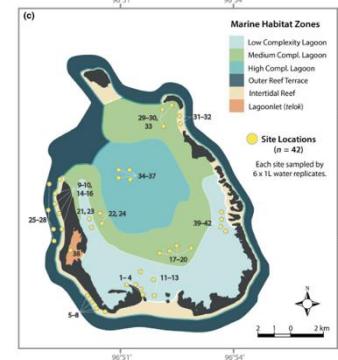
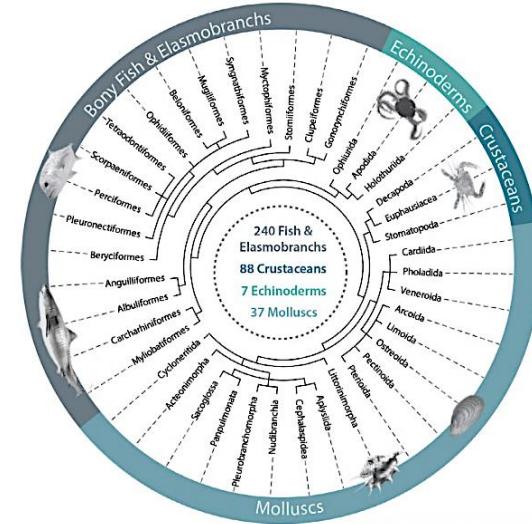
Levin et al. 2017 Conservation letters

Gran escala



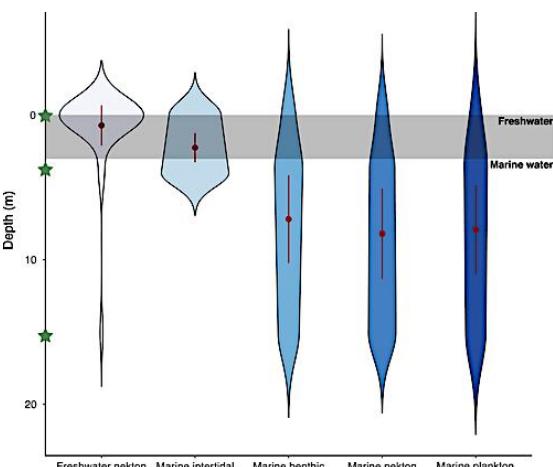
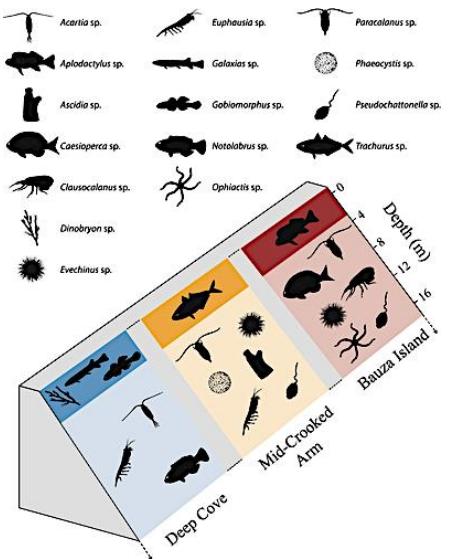
West et al 2020 C

Escala fina



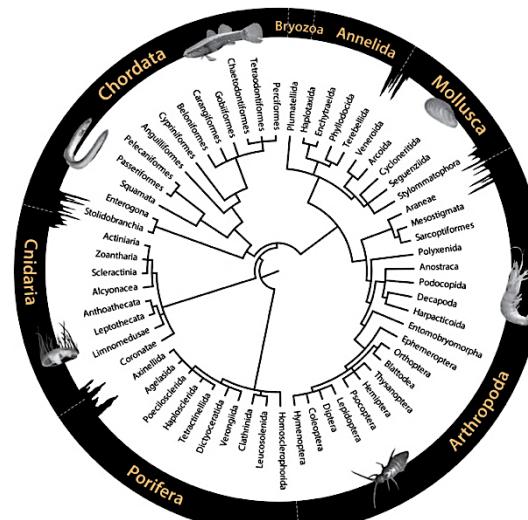
West et al 2020 A

Zonación vertical



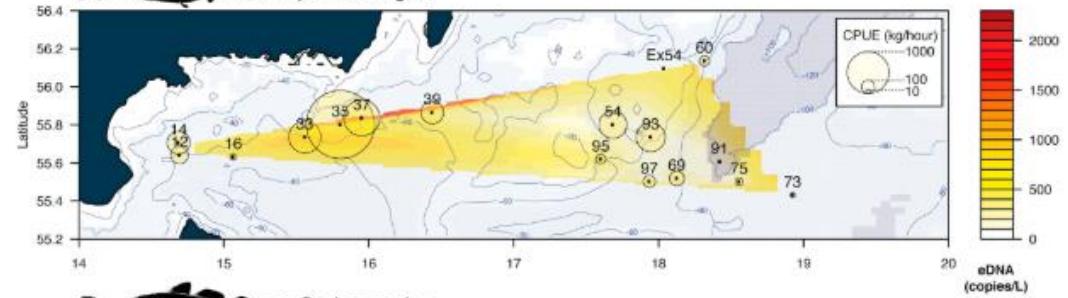
Jeunen et al 2019

Ambientes extremos

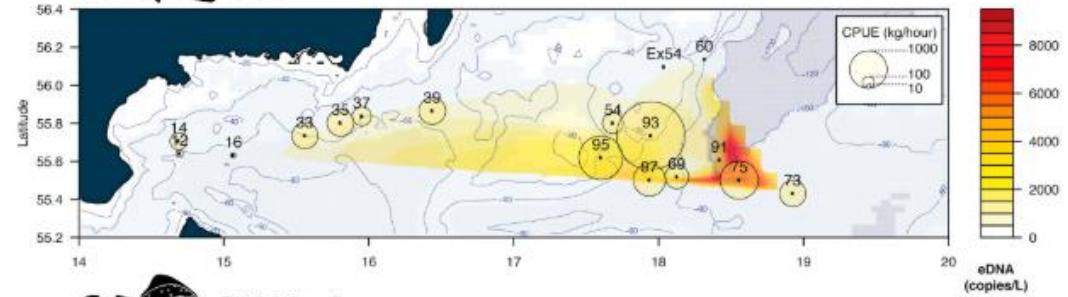


West et al 2020 B

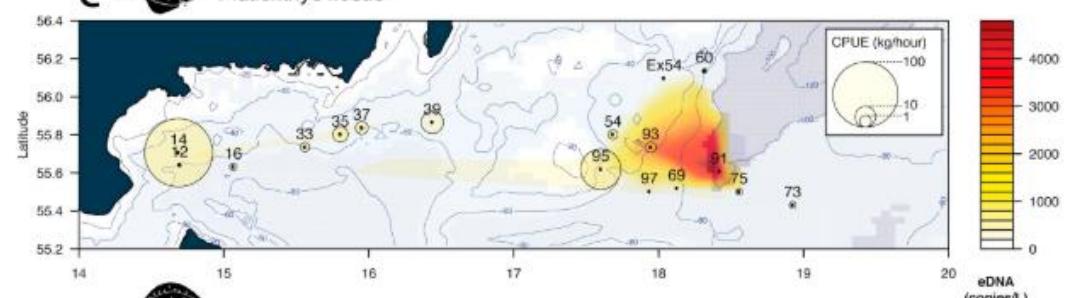
A  *Clupea harengus*



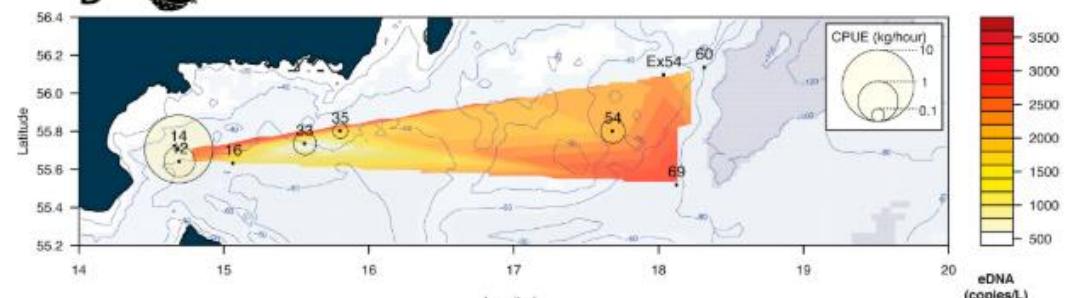
B  *Gadus morhua*



C  *Platichthys flesus*



D  *Pleuronectes platessa*



¿Nos da información de abundancia?

Journal of Experimental Marine Biology and Ecology 510 (2019) 31–45



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journal homepage: www.elsevier.com/locate/jembe

Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea^{a,*}

Steen Wilhelm Knudsen^{a,b,c,*}, Rasmus Bach Ebert^a, Martin Hesselsoe^{b,d}, Franziska Kuntke^{b,e}, Jakob Hassingboe^f, Peter Bondgaard Mortensen^f, Philip Francis Thomsen^{b,h}, Eva Egelyng Sigsgaard^{b,g}, Brian Klitgaard Hansenⁱ, Einar Eg Nielsen^j, Peter Rask Møller^k

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^c DTU Aqua, Roskilde, Nykøbing Sjælland, DK-4600 Roskilde, Denmark

^d NIBAS A/S, Øster Henningsgade 12, DK-9000 Aalborg, Denmark

^e Department of Chemistry and Bioscience, Aalborg University, Frederik Bajers Vej 7H, DK-9220 Aalborg East, Denmark

^f Eurofins Milje A/S, Ladehøjlandsvæj 85, DK-6600 Vojens, Denmark

^g Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen East, Denmark

ⁱ Danish Technical University, Section for Marine Living Resources, Vejlbyvej 39, DK-8600 Silkeborg, Denmark



¿En dónde estamos parados en México?



PROGRAM 1
BARCODE 500K
Cost: \$125 million
Timeline: 2010-2015
Status: Complete in August 2015
Goals:

- Deliver DNA barcode coverage for 0.5 million species.
- Develop the informatics platform and analytical protocols required for the development of the DNA barcode reference library.
- Establish a core facility to provide sequencing and informatics support.



Paul Herbert

PROGRAM 2
BIOSCAN
Cost: \$180 million
Timeline: 2019-2026
Status: Launched June 2019
Goals:

- Deliver DNA barcode coverage for 2 million species.
- Activate biomonitoring for one or more ecoregions in each participating nation & codify species interactions for these sites.
- Develop informatics support for high-throughput sequencing.
- Promote [applications](#) of DNA barcoding.

PROGRAM 3
PLANETARY BIODIVERSITY MISSION
Cost: \$500 million
Timeline: 2026-2045
Status: Activation in January 2026
Goals:

- Complete the census of all multicellular species.
- Establish a global biosurveillance program.
- Construct a 'library of life' by preserving DNA extracts from all species.



eDNA para la conservación

Para proteger hay que conocer

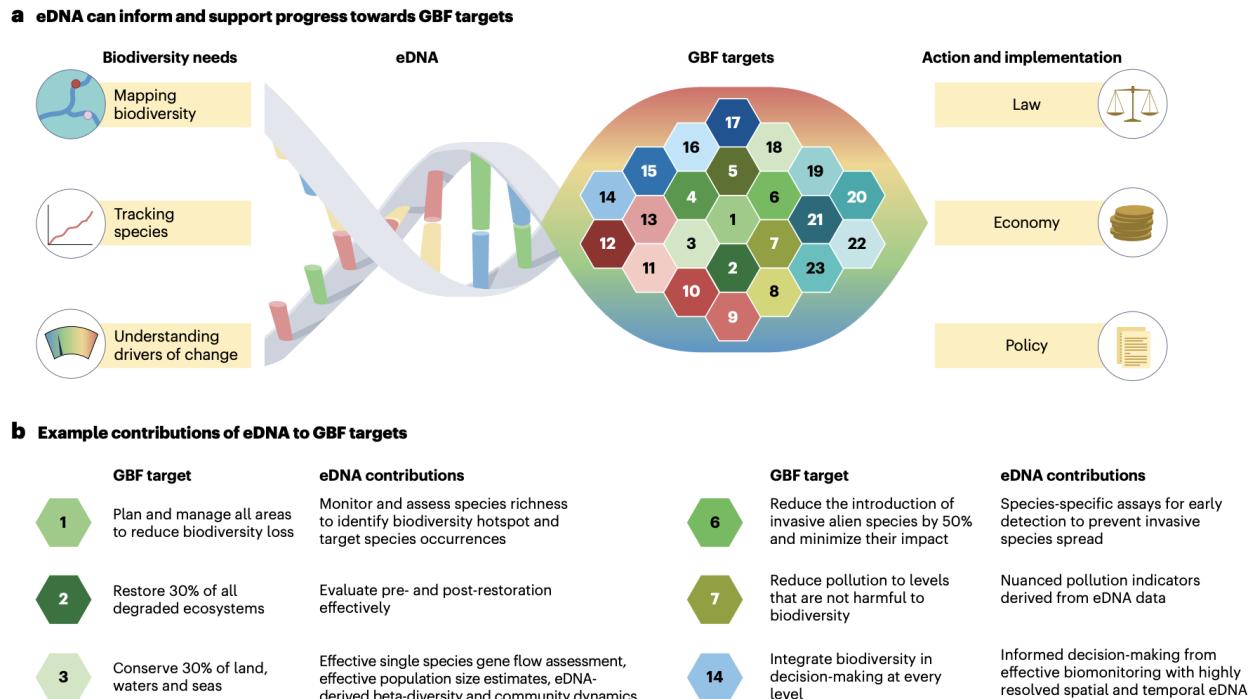


Fig. 1 | Contribution of eDNA to the Kunming–Montreal Global Biodiversity Framework. a, The Kunming–Montreal Global Biodiversity Framework (GBF) identified 23 action-oriented global targets to be reached by 2030 (ref. 6). Many of these targets require information on the state, change and trends of biodiversity for scientific, political and economic decision-making. Using environmental DNA (eDNA) to provide information on biological diversity offers a potentially

universal approach that can support GBF targets by providing baseline data and action–response information to guide decision-making. Establishing, tracking and assessing biodiversity hotspots, biodiversity trends and change is particularly important. b, Multiple targets can be directly assessed using existing eDNA technologies, which are sufficiently developed for implementation but not yet routinely used in most countries.

Herramienta transversal para enfrentar la crisis de biodiversidad

Permite evaluar rápida y simultáneamente a todo el espectro taxonómico en ecosistemas acuáticos—desde microbios hasta mamíferos—y así generar los datos que exigen los objetivos del Marco Global Kunming–Montreal (GBF) para 2030 (por ej., conservar el 30 % del océano, restaurar ecosistemas degradados, frenar extinciones).

Contribución a metas para la conservación

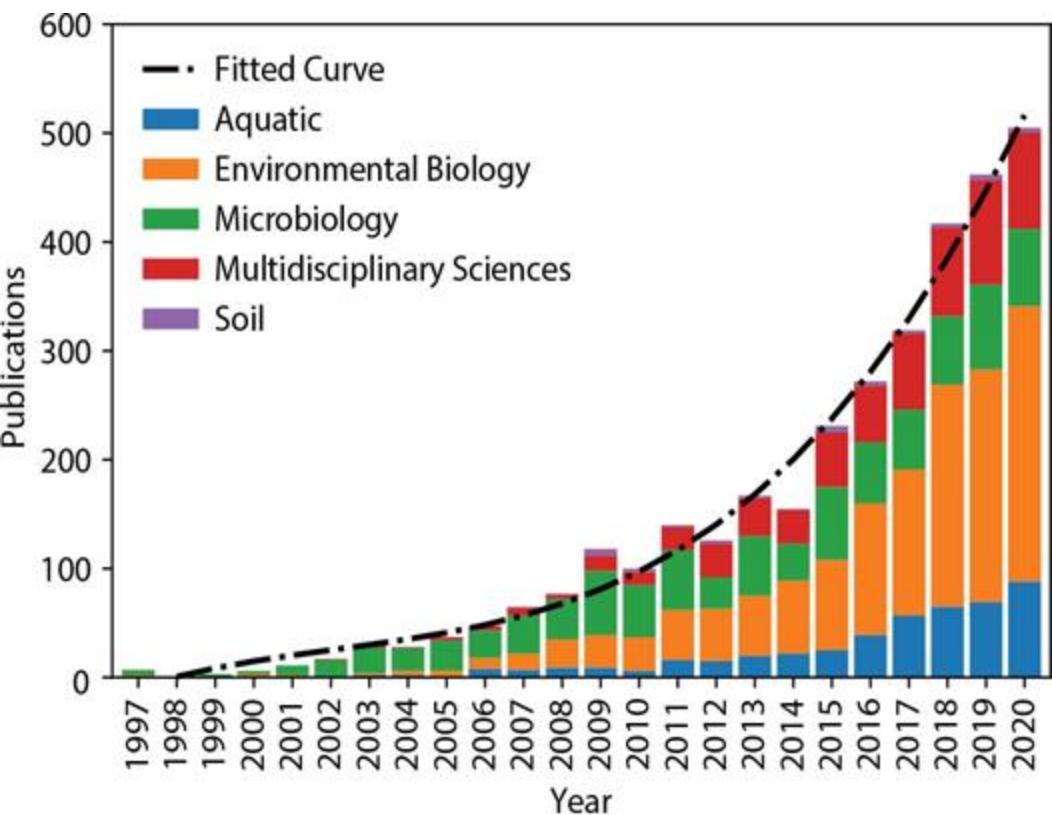
- **mapear** biodiversidad
- **rastrear** especies clave
- **evaluar** respuestas de los ecosistemas a presiones humanas.

áreas de aplicación y oportunidades

- Exploración oceánica
- Monitoreo de la biodiversidad
- Mejora en la detección de especies raras y difíciles de encontrar (crípticas)
 - Detección de especies invasoras o en peligro de extinción
 - Identificación de algas nocivas y otros microbios
 - Cambios estacionales
 - Evaluación de impactos ambientales y restauración
 - Pesquerías
 - Impacto ambiental mínimo
 - Biomonitoring a gran escala
 - Recolección automatizada de muestras

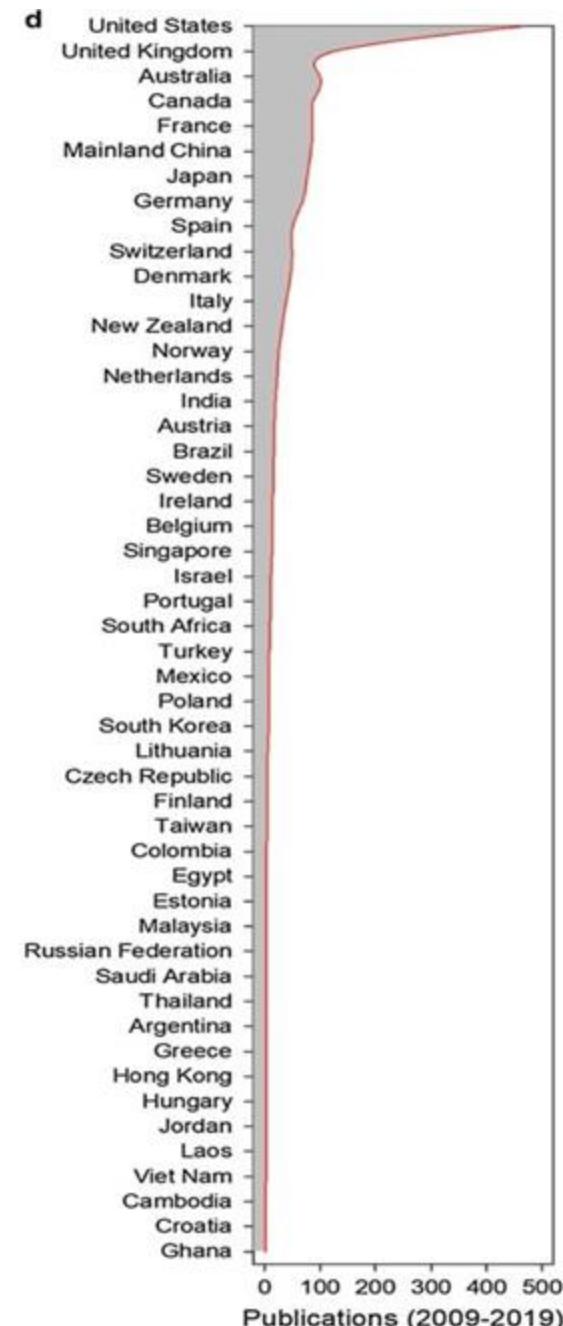
Aplicaciones y oportunidades

- **Industria alimentaria:** identificación-verificación de especies
- **Acuacultura:** detección de microorganismos, enfermedades, microbioma, paternidad
- **Impacto ambiental:** monitoreo de biodiversidad, especies indicadoras
- **Manejo de residuos:** especies microbianas indicadoras, detección de SARS-CoV-2
- **Ecotoxicología:** cambios en biodiversidad por contaminantes, monitoreo de especies indicadoras, efectos subletales en comunidades acuáticas, identificación de especies resilientes
- **Cambio climático:**
 - distribución de las especies
 - temporadas reproductivas
 - distribución y abundancia de stocks pesqueros
 - biodiversidad: riqueza, diversidad, estructura de las comunidades
- Entre otras posibilidades...



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11/9/2025

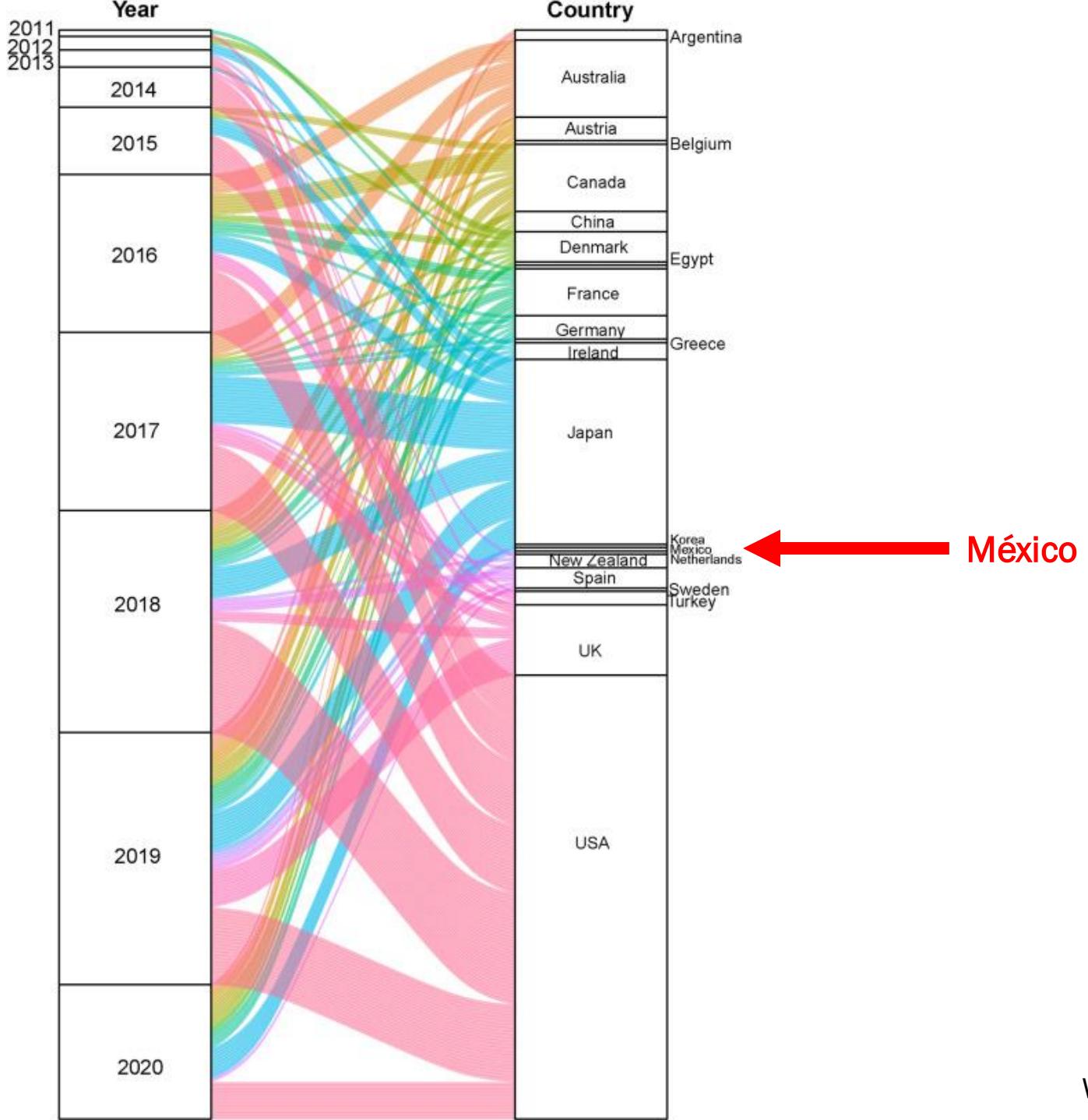


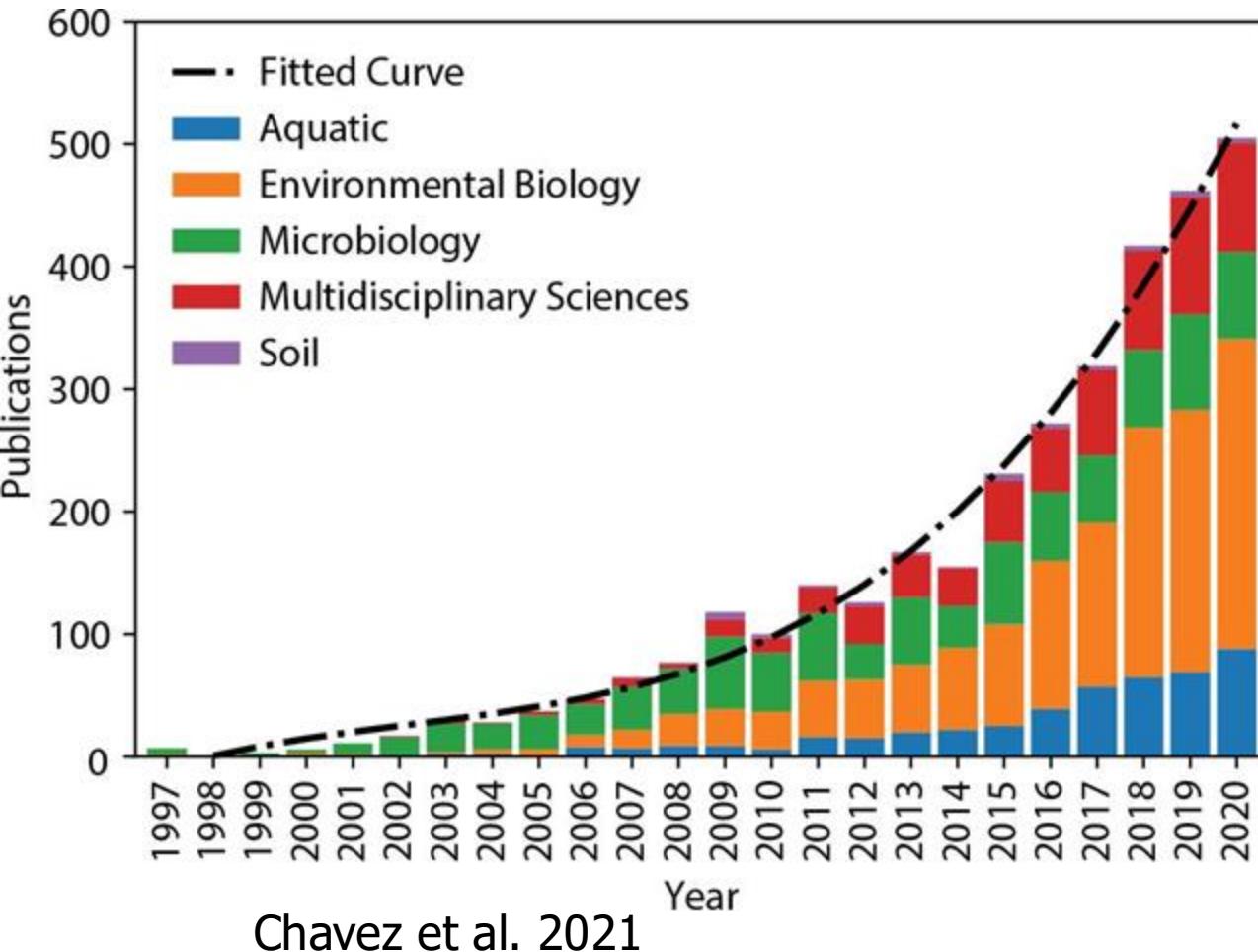
Garlapati et al. 2019

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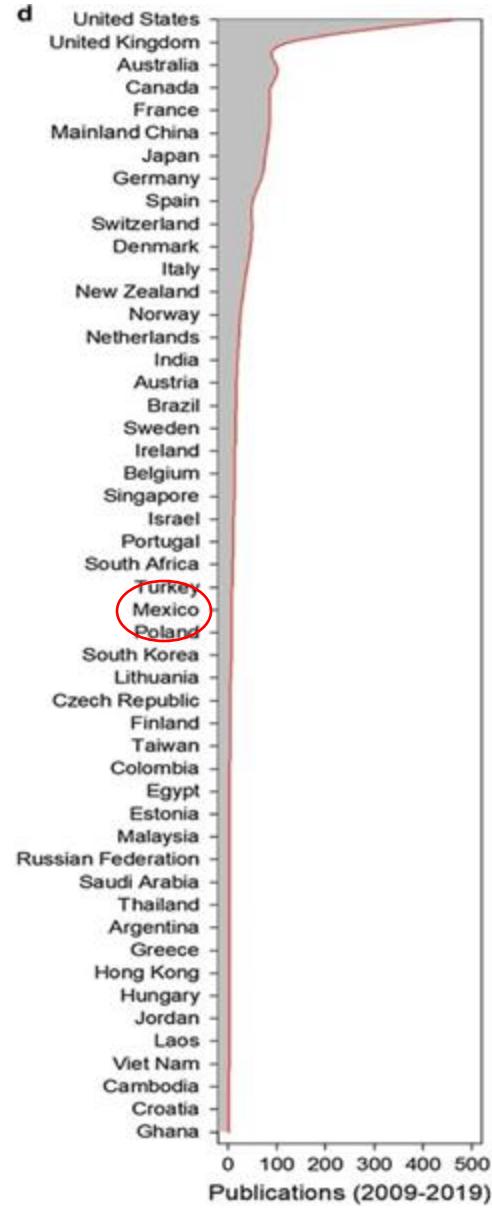
áreas de aplicación

- **Exploración oceánica y monitoreo de biodiversidad**
- **Detección de especies crípticas e invasoras**
- **Especies difíciles de encontrar o en peligro.**
- **Identificación de organismos microscópicos:** Algas nocivas y microbios patógenos.
- **Monitoreo de cambios estacionales:** Migraciones y ciclos ecológicos.
- **Evaluación de impactos ambientales:** Salud de los ecosistemas, detección de especies clave.
- **Pesquerías y acuicultura:** Monitoreo de species y microbioma en el sector pesquero y acuícola.
- **Ecotoxicología:** Efectos de contaminantes en la biodiversidad y la resistencia de especies.
- **Cambio climático:** Impacto del cambio climático en la distribución y abundancia de especies.
- **Biomonitoring a gran escala y recolección automatizada:** Permite monitoreos amplios y eficientes con recolección automatizada de muestras.





Chavez et al. 2021



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