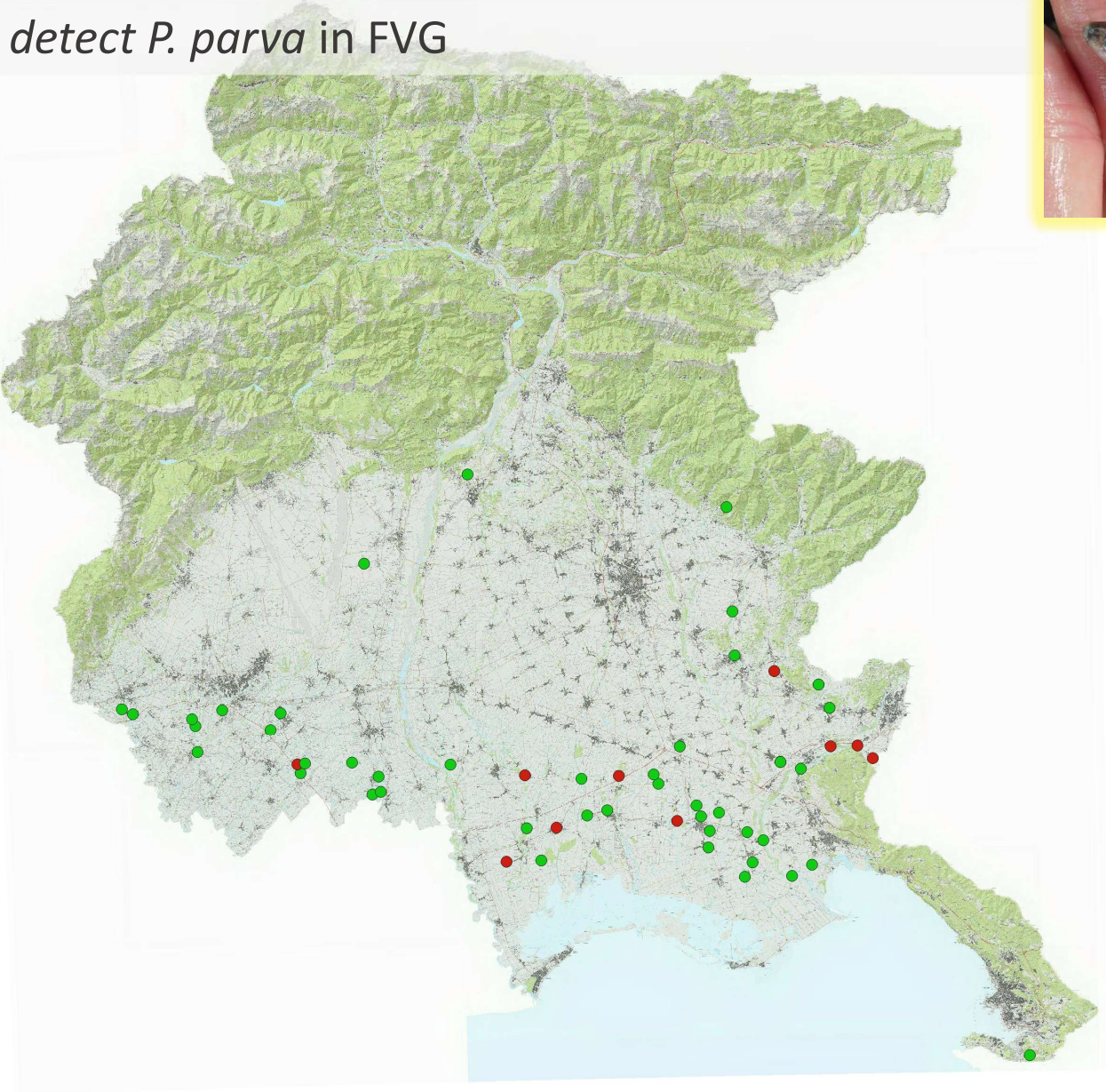


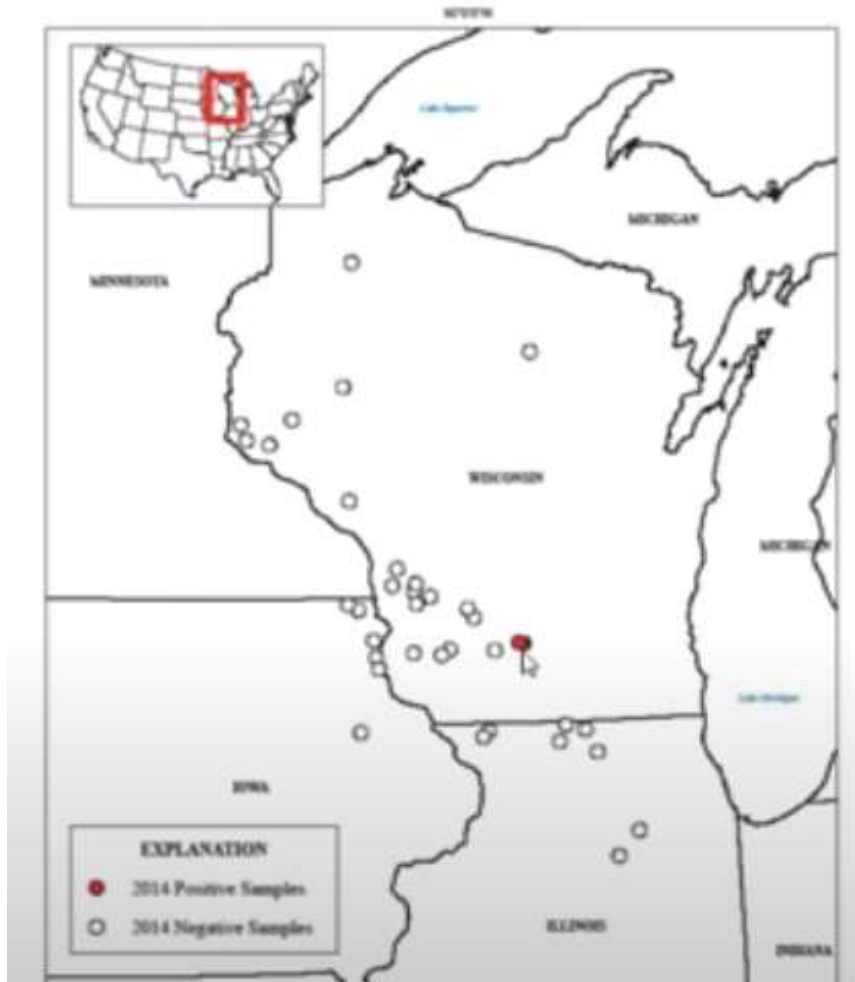
Zoogeography

Lesson 19

eDNA analyses to detect P. parva in FVG



- New Zealand mud snail (*Potamopyrgus antipodarum*) in Wisconsin



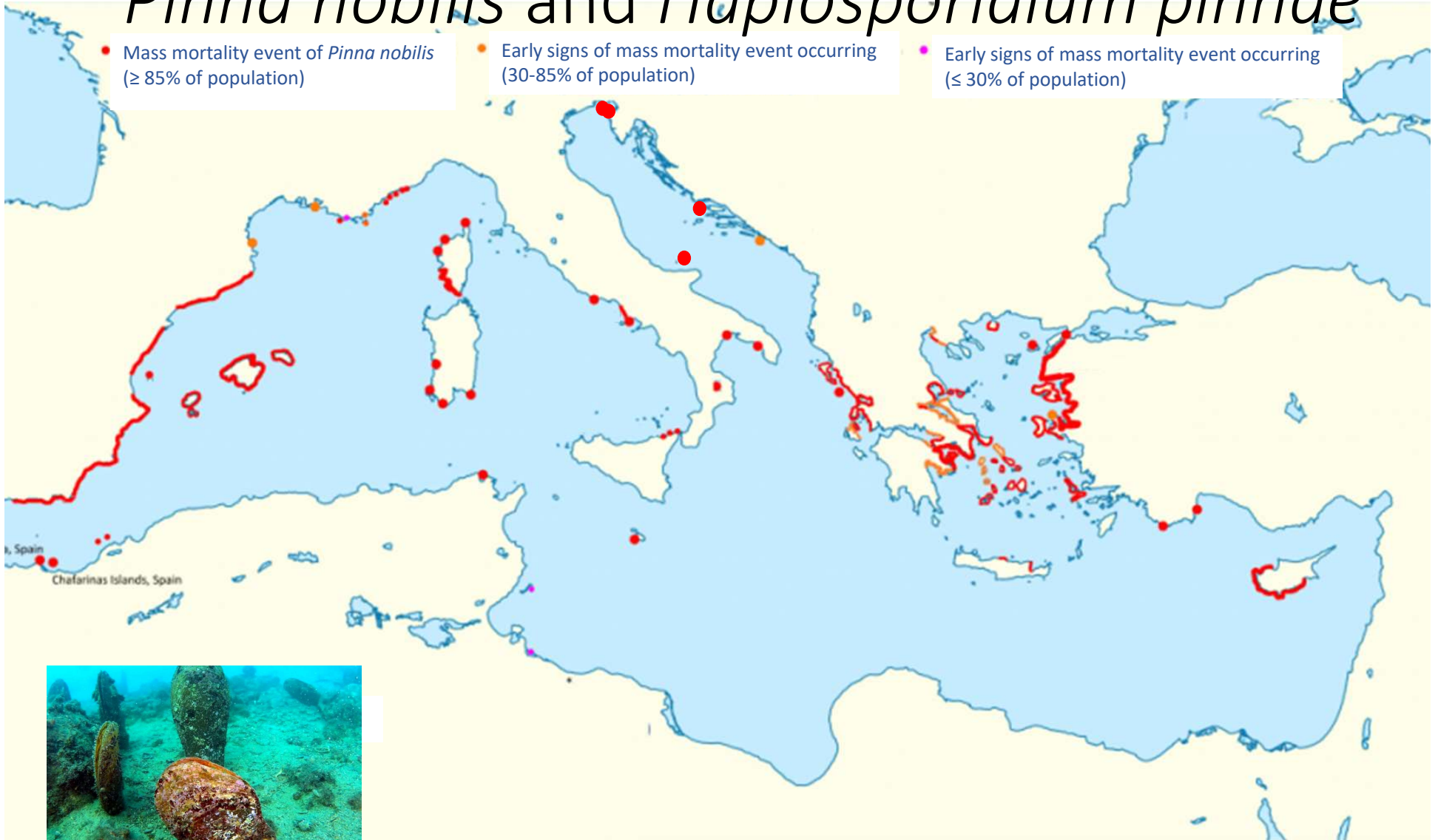
- They test 46 other stations across the states of Wisconsin, Minnesota and Illinois
- They find no other sites positive for the presence of New Zealand mud snails 3 years after the study (2017)
- they identify new positive stations

Pinna nobilis and *Haplosporidium pinnae*

• Mass mortality event of *Pinna nobilis* ($\geq 85\%$ of population)

• Early signs of mass mortality event occurring (30-85% of population)

• Early signs of mass mortality event occurring ($\leq 30\%$ of population)

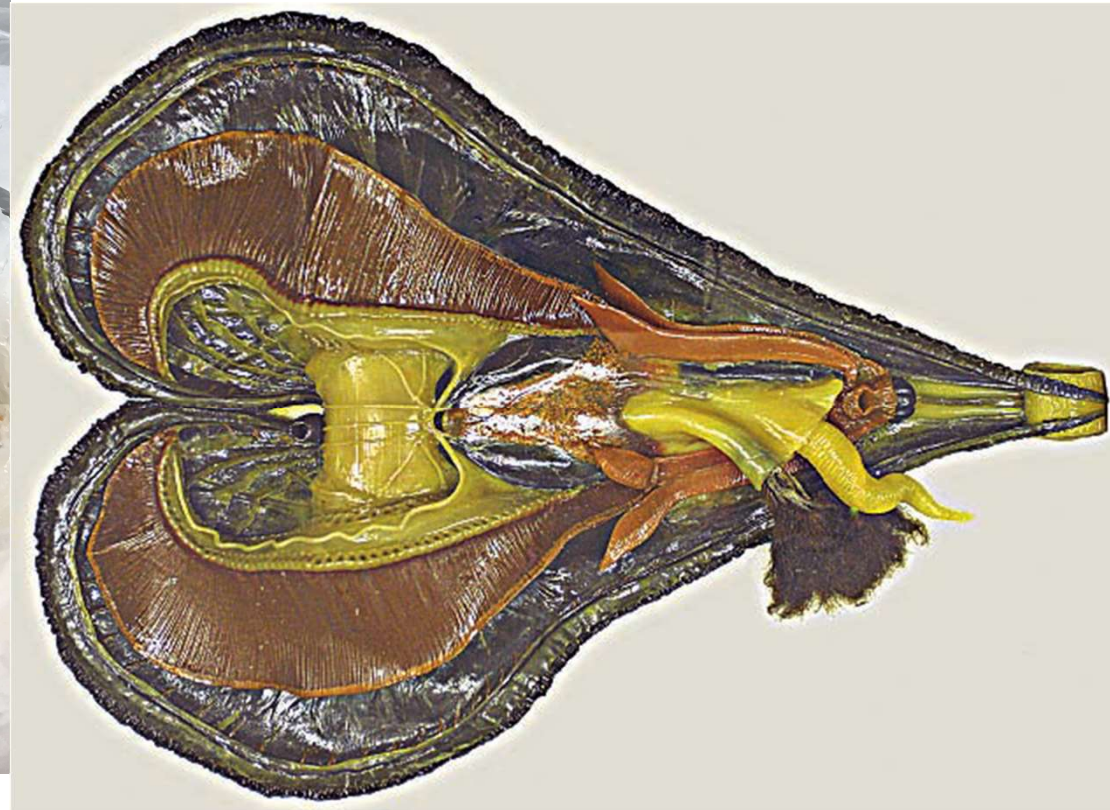


Positive sample



18 specimens analysed

A wax model of *Pinna nobilis* from the Poli's collection of anatomical wax models of molluscs, currently housed in the Muséum National d'Histoire Naturelle in Paris.



RESEARCH ARTICLE

Real-Time PCR based test for the early diagnosis of *Haplosporidium pinnae* affecting fan mussel *Pinna nobilis*

Monserrat López-Sanmartín^{1*}, Gaetano Catanese^{2,3}, Amalia Grau^{2,3}, José María Valencia^{2,3}, Jose Rafa García-March⁴, José Ignacio Navas¹

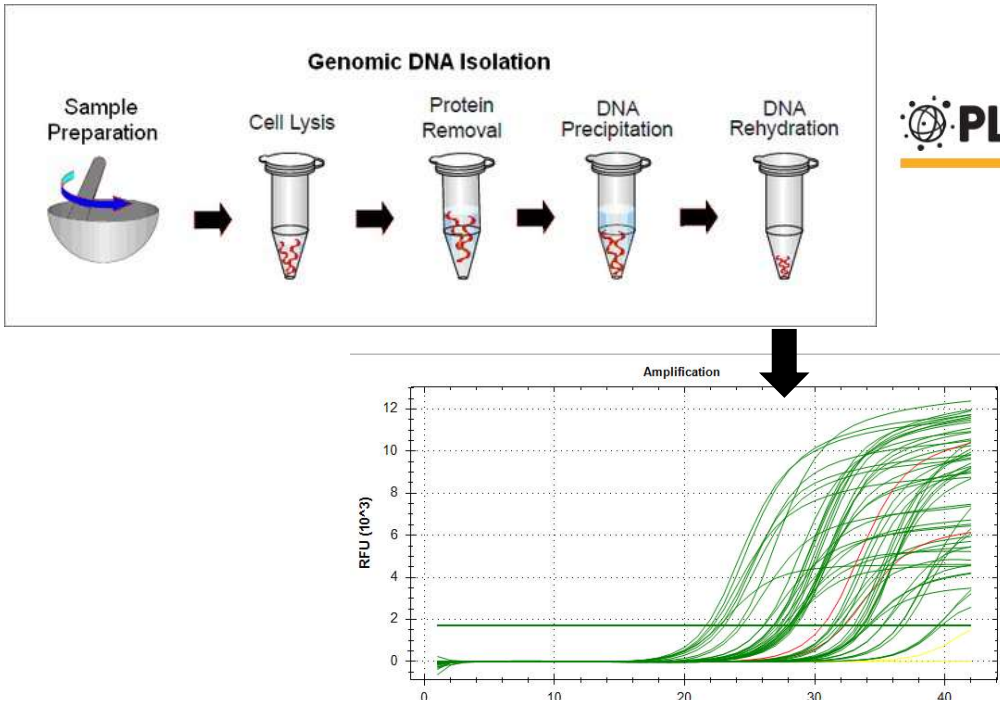
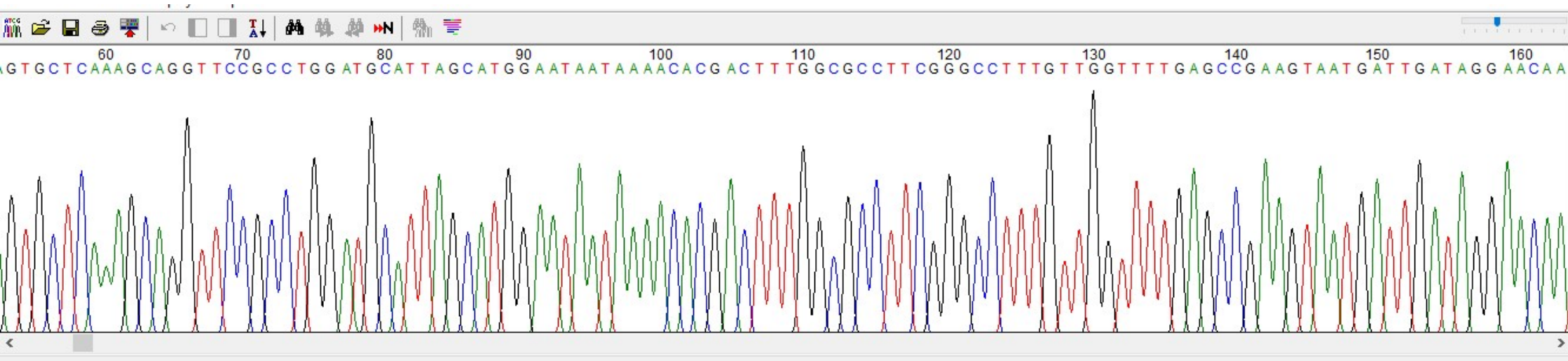


Table 2. Characteristics of the primers used for PCRs.

Primer	Sequence (5' - 3')	Fragment size (bp)	Temperature annealing (°C)	Reference
18SFr	CGAGCAATAACAGGTCTGTG	200	50°C	Mauri et al 2011
18SRw	GGCAGGGACTTAATCAA			
HPNF3	CATTAGCATGGAATAATAAACACGAC	600	55°C	Catanese et al 2018
HPNR3	GCGACGGCTATTTAGATGGCTGA			
HpF	GGTACGGAGAATCCGGGGTT	1409	55°C	This study
HpR	ACTTGTCCCTTCCTCTAATAATAAGG			
HpF3	GCGGGCTTAGTTCAGGGG	165	60°C	This study
HpR3	ACTTGTCCCTTCCTCTAATAATAAGG			

Results for *Haplosporidium pinnae*



Sequences producing significant alignments

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select all 100 sequences selected

[GenBank](#)

[Graphics](#)

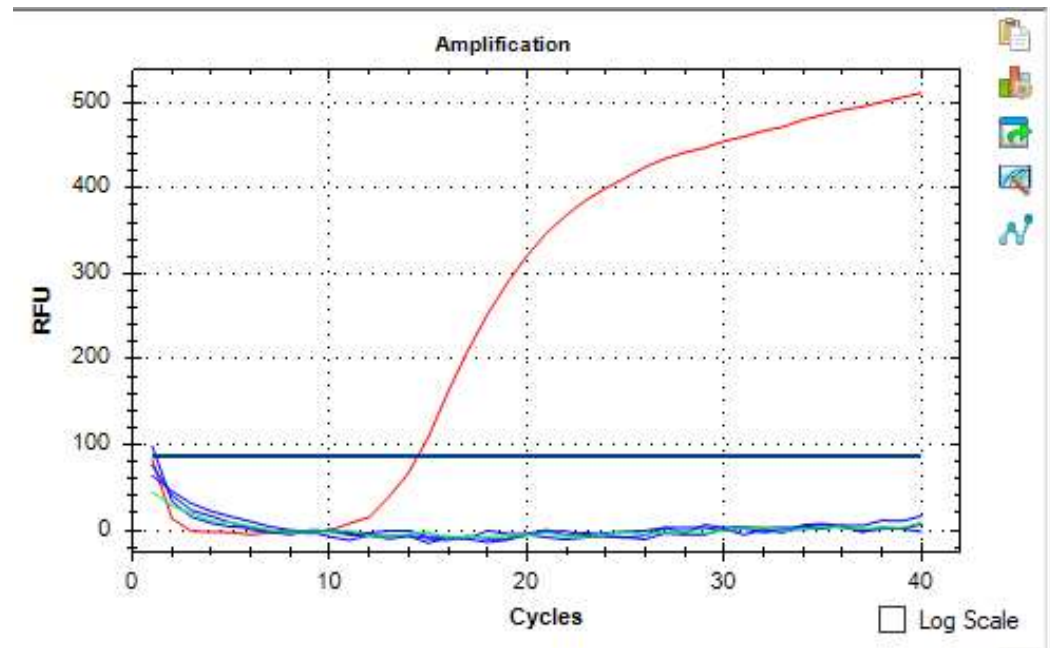
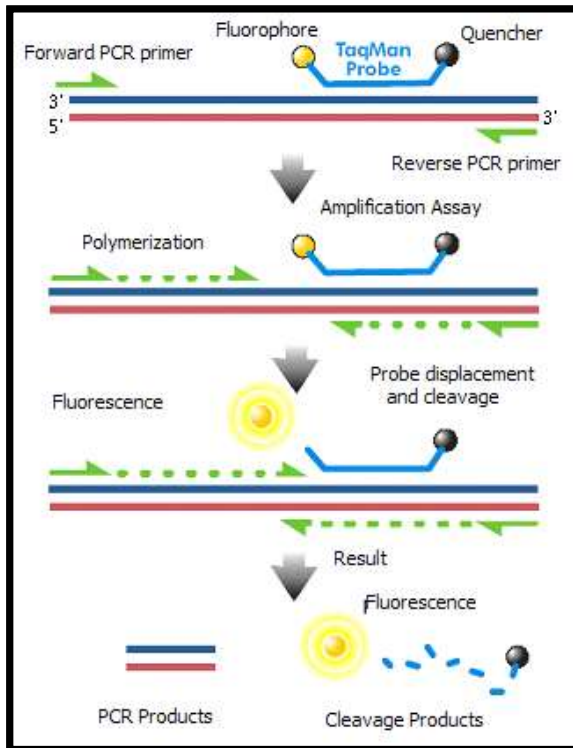
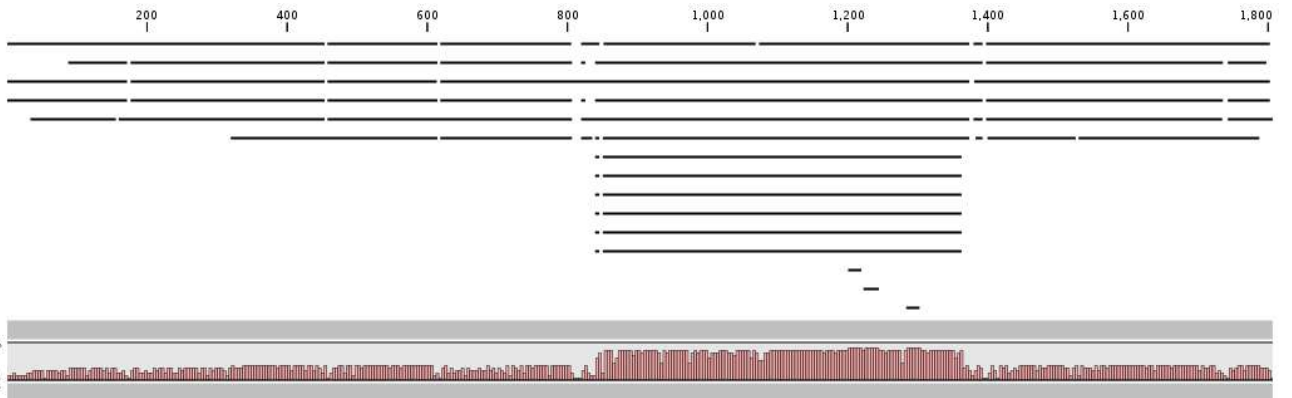
[Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Haplosporidium pinnae isolate PN1 small subunit ribosomal RNA gene, partial sequence	1842	1842	96%	0.0	99.80%	MN104247.1
<input checked="" type="checkbox"/>	Haplosporidium pinnae gene for small subunit ribosomal RNA, partial sequence	1838	1838	97%	0.0	99.41%	LC338065.1

eDNA approach

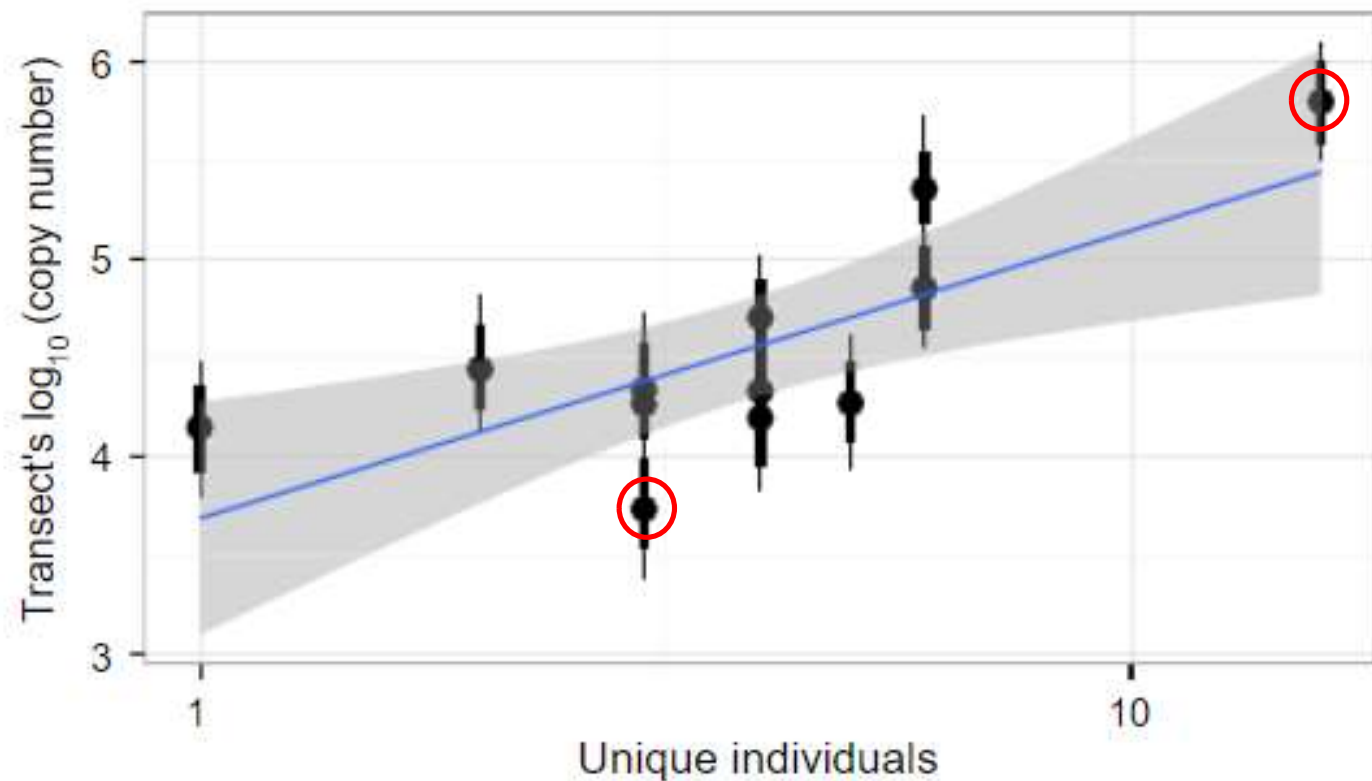
JX185413_Haplosporidium_littoralis
 KC578010_Haplosporidium_costale
 JN368430_Haplosporidium_tuxtliensis
)_Haplosporidium_sp_ex_S_glomerata
 KJ534587_Haplosporidium_patagon
 LC338065_Haplosporidium_pinnae
 MK142774_Haplosporidium_pinnae
 MK142775_Haplosporidium_pinnae
 MK142776_Haplosporidium_pinnae
 MK142777_Haplosporidium_pinnae
 MK142778_Haplosporidium_pinnae
 MK142779_Haplosporidium_pinnae

Hp_CM_short_F
 Hp_CM_probe
 Hp_CM_short_R_rc
 Consensus
 Conservation
 100%
 0%
 2.00kcs



Detecting the movement and spawning activity of bigheaded carps with environmental DNA

RICHARD A. ERICKSON,^{*a} Christopher B. Rees,^{*1a} ALISON A. COULTER,^{†2a}
CHRISTOPHER M. MERKES,^{*} SUNNIE G. MCCALLA,^{*a} Katherine F. Touzinsky,[†] Liza Walleser,^{*3}
Reuben R. Goforth^{†b} and JON J. AMBERG^{*b}
**U.S. Geological Survey Upper Midwest Environmental Sciences Center, La Crosse, WI, USA, †Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA*



Detecting spawning/ behavioural activity

- Planning the opening and/or closure of some barriers
- Interfere with reproduction of invasive species
- Limiting the collection of target species in particular period of the year or encourage it if they are exotic species
- Activating monitoring actions on target species



Detecting the abundancies changes of species

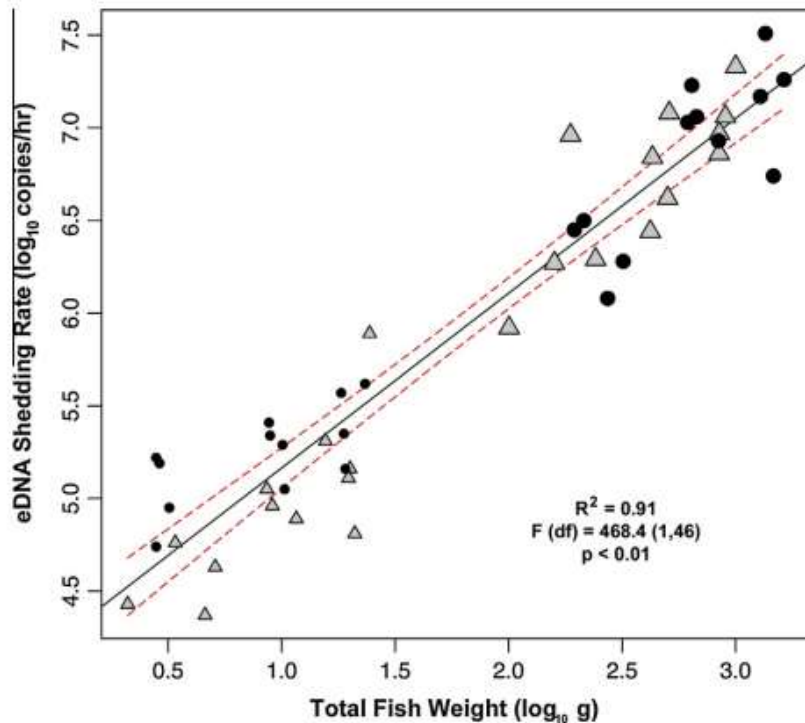


Fig. 2. Combined scatter plots of eDNA shedding rate against biomass of fish in tanks. (Bighead carp sub-adults – large, black circles; bighead carp juveniles – small, black circles; silver carp sub-adults – large, grey triangles; silver carp juveniles – small, grey triangles). Dashed line indicates 95% CI.

Special Issue Article: Environmental DNA

Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix* [☆]

Katy E. Klymus ^{a,*}, Catherine A. Richter ^b, Duane C. Chapman ^b, Craig Paukert ^c

^a Missouri Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife, University of Missouri, Columbia, MO, USA

^b U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO, USA

^c U.S. Geological Survey, Missouri Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife, University of Missouri, Columbia, MO, USA

More biomass, more eDNA



Caution with this statement

Detecting the abundancies changes of species

[eDNA] = released DNA – degraded DNA

There are factors that influence the release and degradation rates of the DNA:

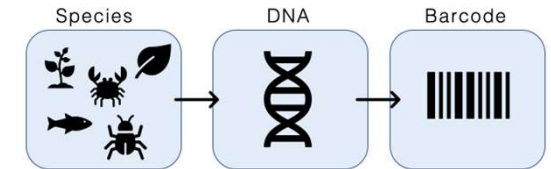
- pH
- Temperature
- Food availability
- Seasonality
- Turbidity
- UVs exposition



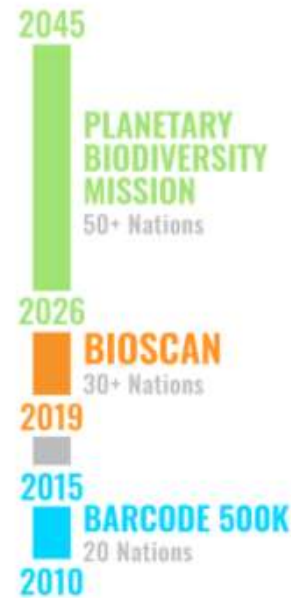
Determining species assemblies



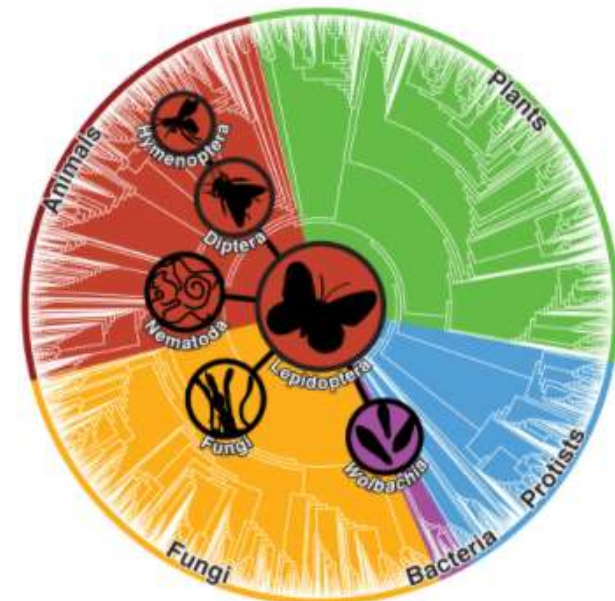
DNA barcoding: species identification from short DNA fragments



TRACKING ECOSYSTEMS



TIMELINE

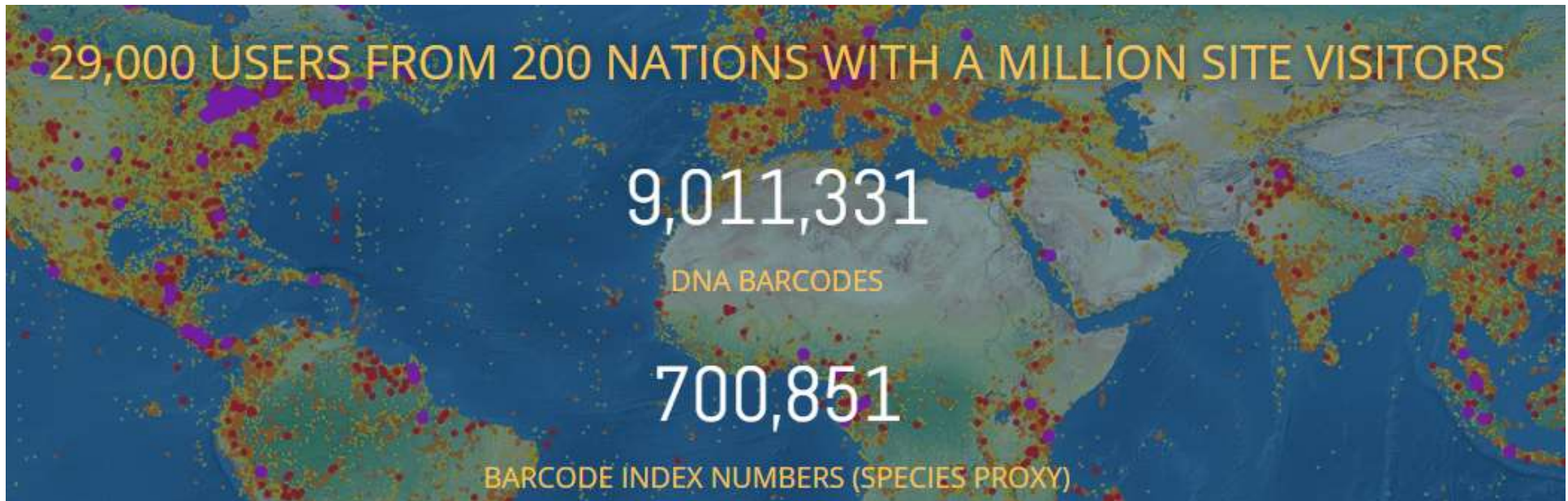
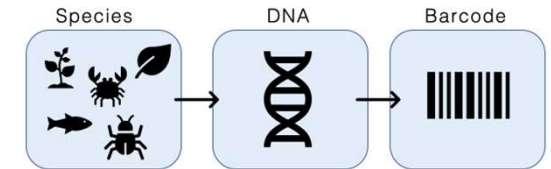


REVEALING SYMBIOMES

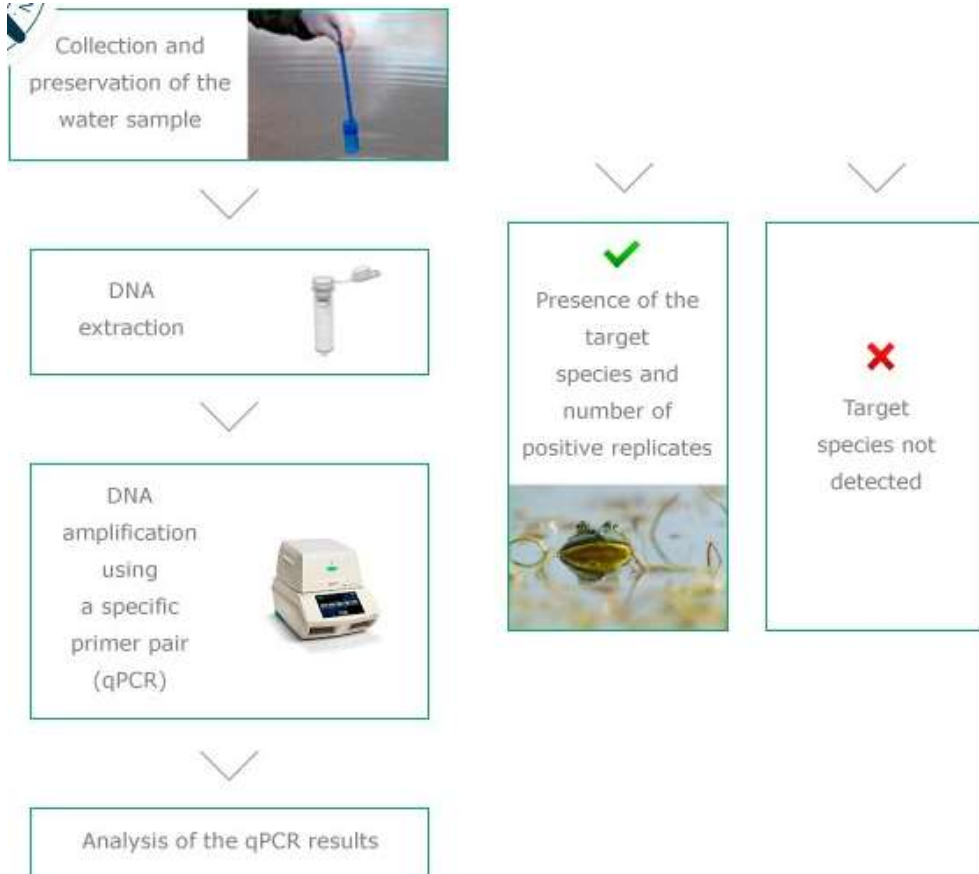
Determining species assemblies



DNA barcoding: species identification from short DNA fragments



DNA barcoding



DNA metabarcoding



Not only recent DNA....Paleoecology

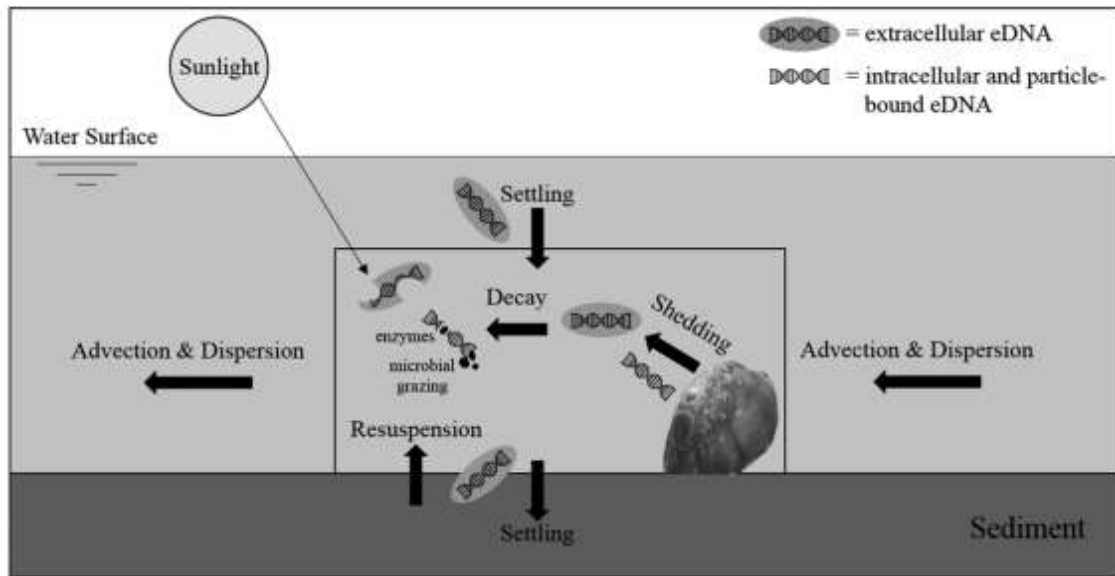


Figure 1. Conceptual model of the source, transport, and fate of eDNA from a freshwater mussel in a stream environment. Processes

Evaluating monitoring actions

Invasive species control/eradication



Restore natural habitats



In both cases eDNA can be used to assess the actions undertaken

Create Databases

- eDNA samples can be used to trace different species
- Multiple testes can be done on the same sample
- Species to monitor today, may not need to be monitored tomorrow
- Samples can be stored frozen or freeze-dried
- Archival samples can be reanalysed years later
- Different laboratories can analyse the same samples



Svalbard Global Seed Vault (Norway)

eDNA: not only research, but chance to develop new employment realities



Monitoring of target taxonomic groups in aquatic ecosystems

This approach has been developed since 2011 by SPYGEN and its partners. It is based on the use of universal primer pairs* and Next Generation Sequencing technologies. This **non-invasive** method enables **blind detection** of all species of a target group present on the study site and thus represents a powerful **environmental monitoring tool**. It enables improved detection of rare species (in comparison with conventional methods), a **reduction in survey costs** and the **avoidance of any risk of introduction of pathogens or invasive species** during sampling.

For more information, you can download: [Valentini et al. 2016.pdf](#)

**Patented technology (CNRS – Université Grenoble 1)*



Diet analysis from faecal samples

This **non invasive** method was developed in 2007 by the Laboratoire d'Ecologie Alpine in order to improve our knowledge on the **biology** of threatened animal species and their **interactions with the ecosystem**. It is based on the extraction of DNA from faecal samples and its amplification using an universal primer pair*. The amplified DNA is then sequenced (Next Generation Sequencing) and the obtained sequences are compared to the GenBank international reference database using bioinformatics tools.

For more information, you can download: [Soininen et al. 2009.pdf](#)

**Patented technology (CNRS – Université Grenoble 1)*

eDNA: not only research, but chance to develop new employment realities



Plant composition from a honey sample

Developed in 2010 by the Laboratoire d'Ecologie Alpine, this method is based on the use of an universal primer pair for plants* and Next Generation Sequencing technologies. Using bees as "**environmental samplers**", it is possible to assess the **plant biodiversity** in a study site and to follow its evolution through time, by regularly analysing honey samples. It also represent a powerful tool for the assessment of honey **geographical origin**.

For more information, you can download: [Valentini et al. 2010.pdf](#)

*Patented technology (CNRS – Université Grenoble 1)

Next Steps

Instead of spending endless hours doing this...



Develop molecular and bioinformatics methods to analyse massive sequencing



Next Steps

$$[eDNA] = \text{released DNA} - \text{degraded DNA}$$

There are factors that influence DNA release and degradation rates:

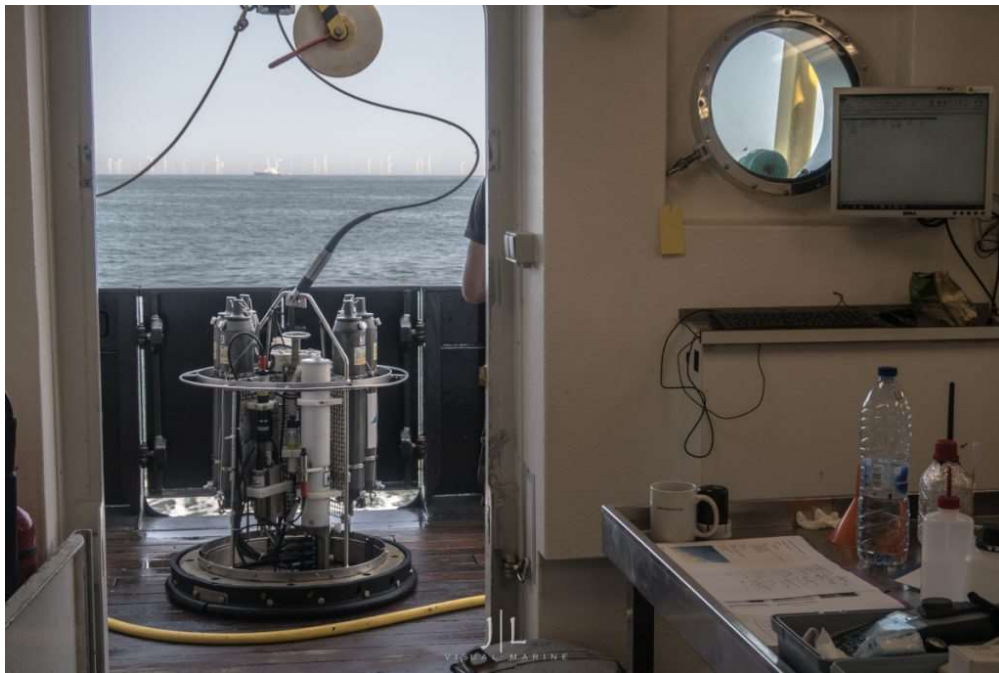
- pH
- Temperature
- Food availability
- Seasonality
- Turbidity
- Uvs exposition



We have to improve the formula!

Next Steps

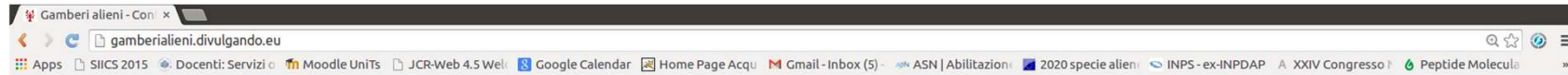
Greater automation for continuous monitoring and long-term data collection



Next Steps



<http://gamberialieni.divulgando.eu/>



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e contribuisci al controllo
del Gambero Rosso
in Italia



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dal tuo computer o
dal tuo dispositivo mobile
con un semplice click

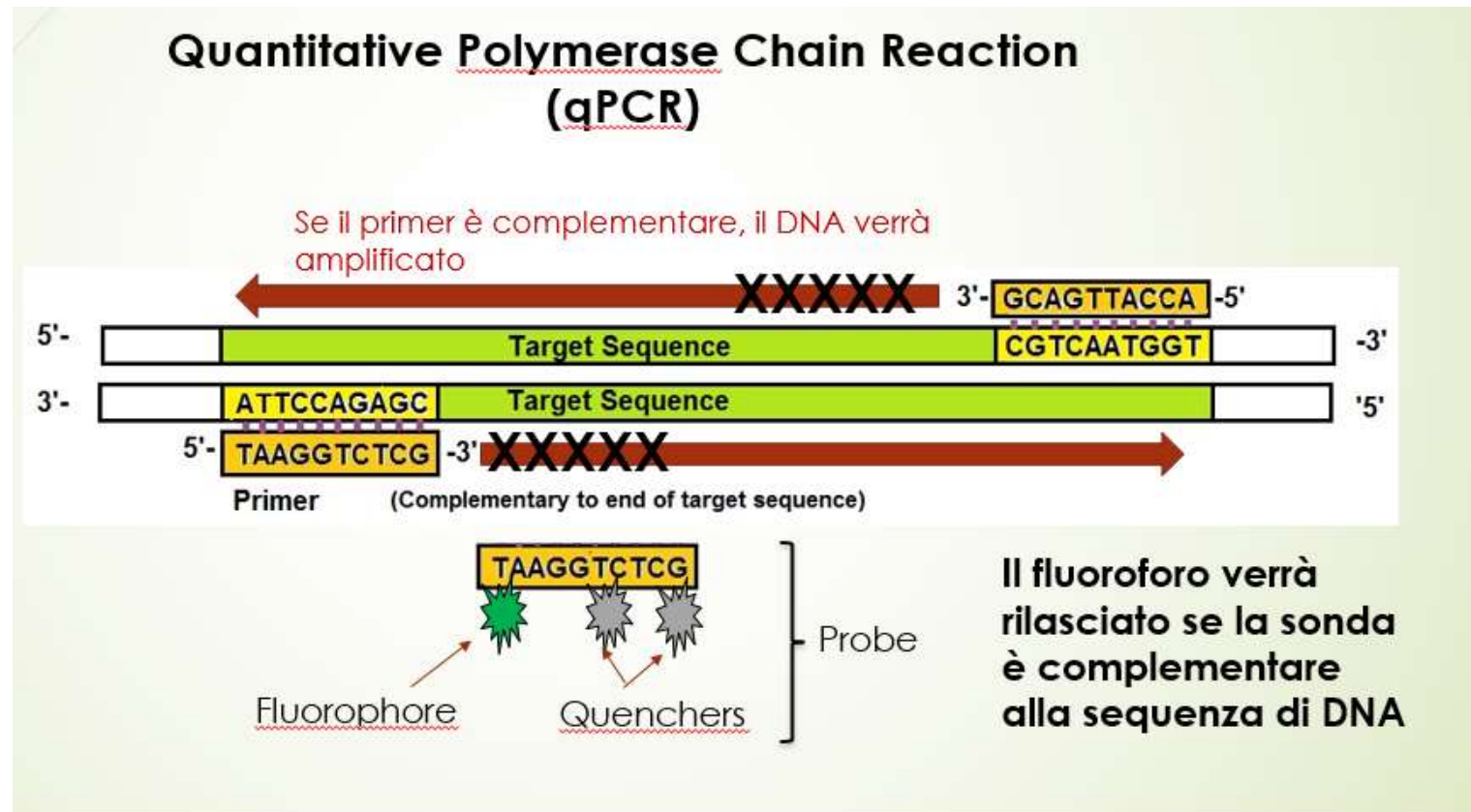
Real demonstration of eDNA sampling

<https://vimeo.com/278562904>

<https://vimeo.com/352017863>

<https://www.youtube.com/watch?v=VQ8zZDPR7OY>

Primer and probe design for qPCR



Databases and target species

NCBI Resources How To chmanf My NCBI Sign Out

NCBI National Center for Biotechnology Information

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NCBI News & Blog

NCBI Virus: Test drive our new SARS-CoV-2 interactive data dashboard! 03 Dec 2020

Are you looking for SARS-CoV-2 sequence data? Look no further! The

December 9 Webinar: Using BLAST+ in

- <https://www.eurofinsgenomics.eu/en/ecom/tools/qpcr-assay-design/>

Design Parameters [open](#) / [close](#)

Target Region (default: complete sequence): Start: End:

Primer 3' clamp (default: C/G): ▼

Max. Tm difference [°C] between the primers: (default: 2°C)

qPCR Probe Criterias [open](#) / [close](#)

	Minimum	Maximum
Probe Length (mer):	<input type="text" value="9"/>	<input type="text" value="40"/>
Probe GC (%):	<input type="text" value="20"/>	<input type="text" value="80"/>

Orientation [open](#) / [close](#)

Primer Criterias [open](#) / [close](#)

	Minimum	Maximum
Length (mer):	<input type="text" value="18"/>	<input type="text" value="22"/>
Primer GC (%):	<input type="text" value="40"/>	<input type="text" value="55"/>
Tm (°C):	<input type="text" value="50"/>	<input type="text" value="65"/>

Amplicon Criterias [open](#) / [close](#)

	Minimum	Maximum
Size (bp):	<input type="text" value="100"/>	<input type="text" value="300"/>
GC Content (%):	<input type="text" value="20"/>	<input type="text" value="80"/>
Tm (°C):	<input type="text" value="70"/>	<input type="text" value="95"/>

Reaction Conditions [open](#) / [close](#)

Output Parameters [open](#) / [close](#)

* These fields are mandatory

https://bioinfo.ut.ee/primer3/

Primer3web version 4.1.0 - Pick primers from a DNA sequence.

[disclaimer](#)

[code](#)

[cautions](#)

Select the [Task](#) for primer selection

[Template masking](#) before primer design ([available species](#))

[Select species](#)

[Nucleotides to mask in 5' direction](#)

[Primer failure rate cutoff](#) <

[Nucleotides to mask in 3' direction](#)

Paste source sequence below (5'→3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a [Mispriming Library \(repeat library\)](#)

Pick left primer, or use left primer below Pick hybridization probe (internal oligo), or use oligo below Pick right primer, or use right primer below (5' to 3' on opposite strand)

[Sequence Id](#) A string to identify your output.

[Targets](#) E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the [source sequence](#) with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.

[Overlap Junction List](#) E.g. 27 requires one primer to overlap the junction between positions 27 and 28. Or mark the [source sequence](#) with -: e.g. ...ATCTAC-TGTCAT.. means that primers must overlap the junction between the C and T.

[Excluded Regions](#) E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the [source sequence](#) with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.

[Pair OK Region List](#) See manual for help.

[Included Region](#) E.g. 20,400: only pick primers in the 400 base region starting at position 20. Or use { and } in the [source sequence](#) to mark the beginning and end of the included region: e.g. in ATC{TTC...TCT}AT the included region is TTC...TCT.

[Start Codon Position](#)

[Internal Oligo Excluded Region](#)

[Force Left Primer Start](#) [Force Right Primer Start](#)

[Force Left Primer End](#) [Force Right Primer End](#)

[Sequence Quality](#)



Molecular Evolutionary
Genetics Analysis

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MEGA X 10.2 for Windows (32 and 64 bit) is now available.

This is a bug fix release. Linux and macOS versions will be released soon.

