

Fish, crayfish & mussels in environmental DNA

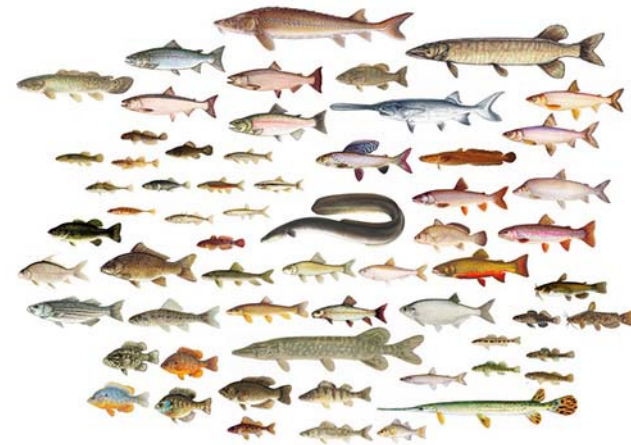
Laboratory analyses & results.



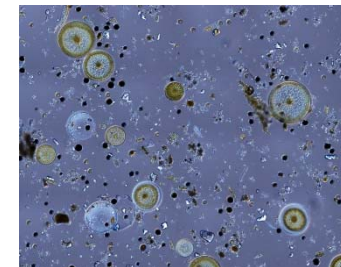
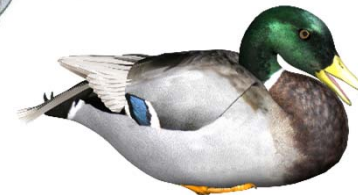
Rodrigo Esparza-Salas
Centre for Genetic Identification/Swedish
Museum of Natural History
December 2014

Objectives

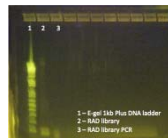
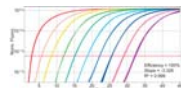
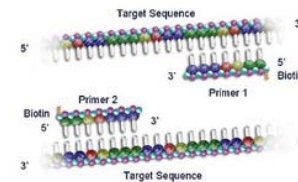
- Optimize sampling protocols
- Use available NGS methods for Fish DNA barcoding
- Develop simple methods for the detection of crayfish
- Develop methods for the detection of large mussel species in parallel (NGS)
- Develop NGS protocols for the correct identification of cyprinid fish



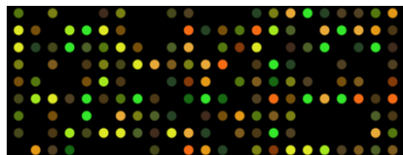
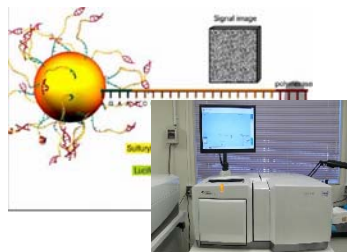
Environmental DNA



Environmental DNA



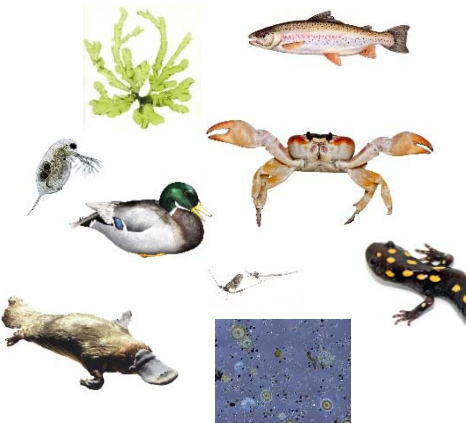
1 - E-gel 146 Plus DNA ladder
2 - H2O control
3 - MO library PCR



Environmental DNA (eDNA) is a powerful tool for monitoring biodiversity in aquatic ecosystems. It allows researchers to detect the presence of various species, including rare and elusive ones, by analyzing DNA fragments shed into the water. This method is non-invasive and can provide early warnings of species invasions or declines. The process involves collecting water samples, extracting DNA, and using sensitive detection methods like qPCR or metabarcoding to identify the species present. eDNA has been successfully used to monitor fish, amphibians, and invertebrates in rivers, lakes, and oceans.

The development of eDNA detection methods has been a significant advancement in environmental monitoring. Key factors for successful eDNA detection include sample collection, DNA extraction efficiency, and the use of highly sensitive and specific detection techniques. Researchers are continuously improving these methods to increase their accuracy and reliability. The integration of eDNA with other monitoring tools, such as remote sensing and traditional field surveys, provides a more comprehensive understanding of ecosystem health and biodiversity.

eDNA has numerous applications in environmental science and conservation. It is used to monitor the presence of invasive species, assess the impact of human activities on ecosystems, and track the recovery of endangered species. The method is also valuable for monitoring water quality and detecting harmful algal blooms. By providing a rapid and cost-effective way to detect species, eDNA is becoming an essential tool for environmental managers and conservationists.



DNA extraction

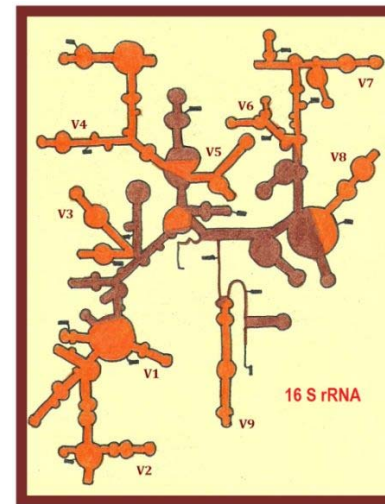
- Potential problems
 - Amount of recovered DNA
 - Inhibiting compounds in DNA sample (minerals, salts, proteins)
 - Sample cross-contamination

MoBio Power Water DNA
isolation kit

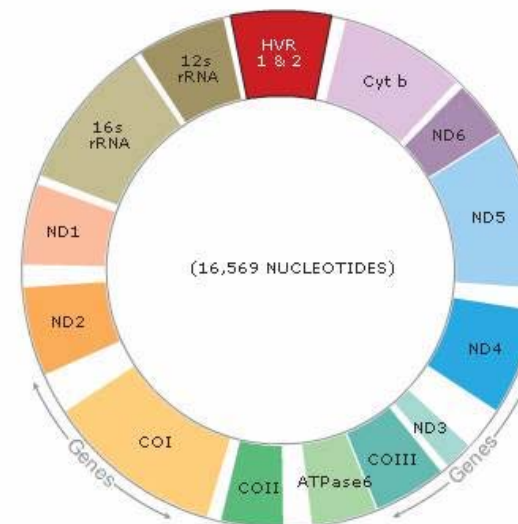


Barcoding genes

- Mitochondrial DNA
 - 16s
 - Cytochrome Oxidase I (COI)
 - Cytochrome B
 - ND2, etc.



- Nuclear DNA
 - 18s
 - ITS
 - Etc



Choice of DNA markers

- Next-generation sequencing
- Fish
 - 300 base pair long section of 16s rDNA gene
- Mussels
 - 170 base pair section of 16s rDNA
- Crayfish
 - 170bp species-specific section of COI

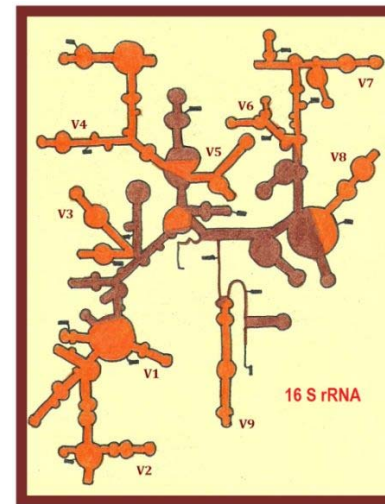
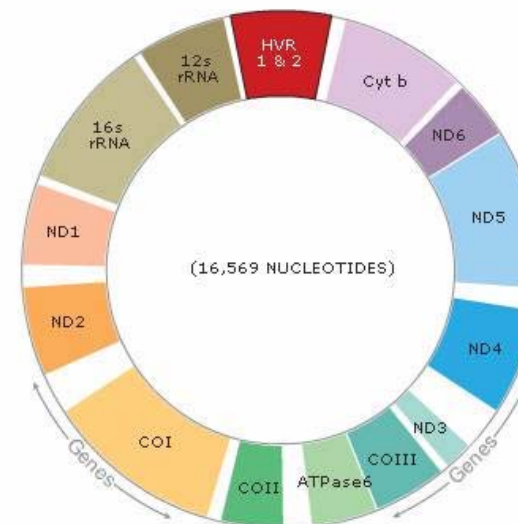
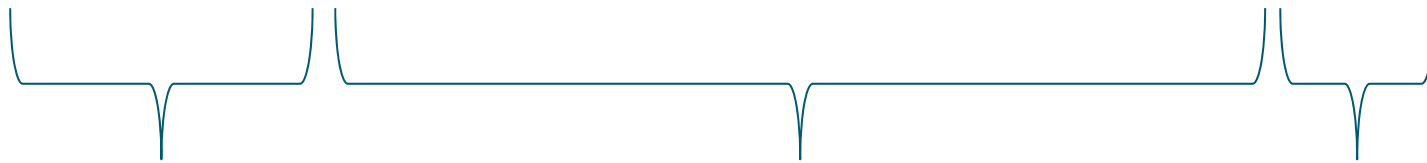
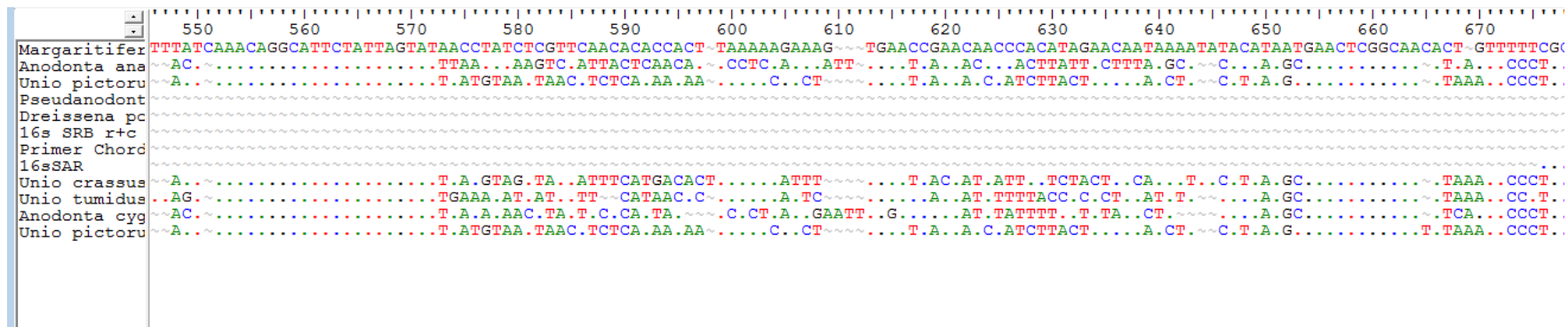


Illustration by Lieth-Peters



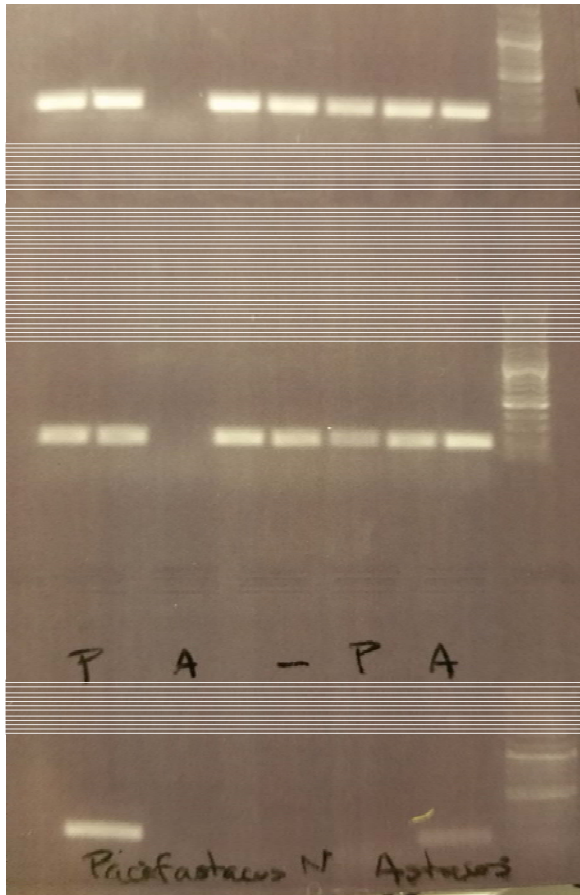
Choice of DNA markers



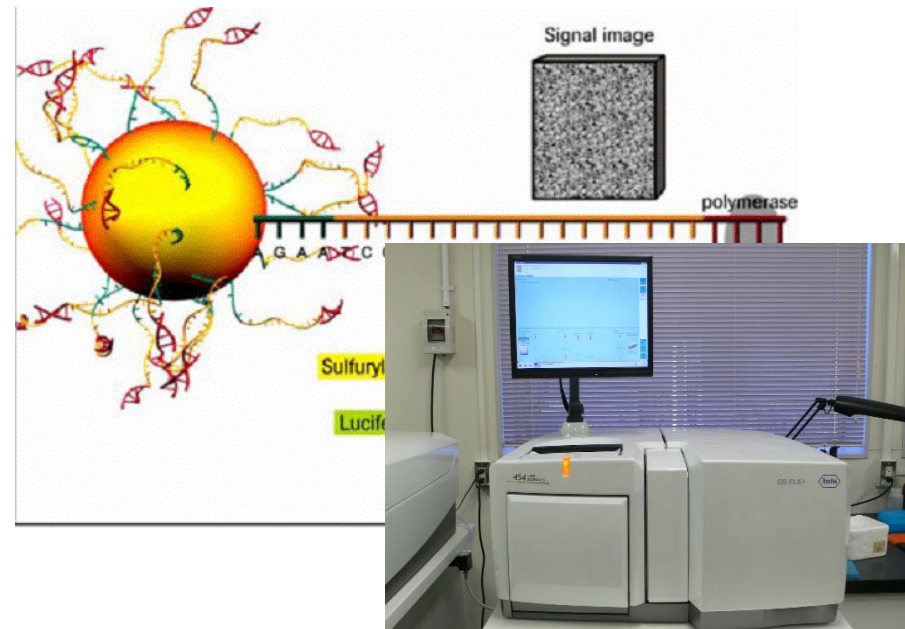
Conserved region
(PCR primer)

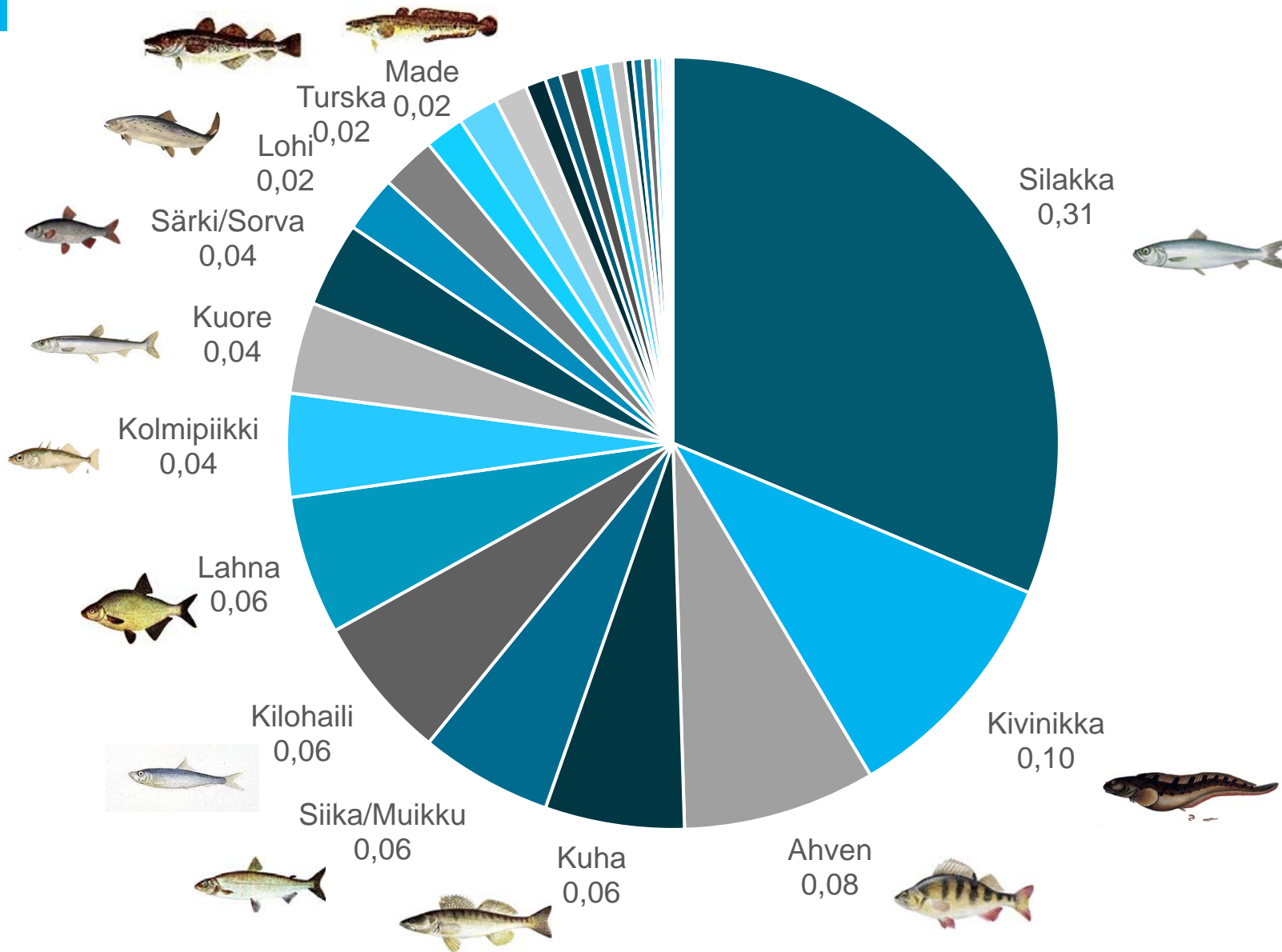
Variable region
(Target sequence)

Conserved region
(PCR primer)



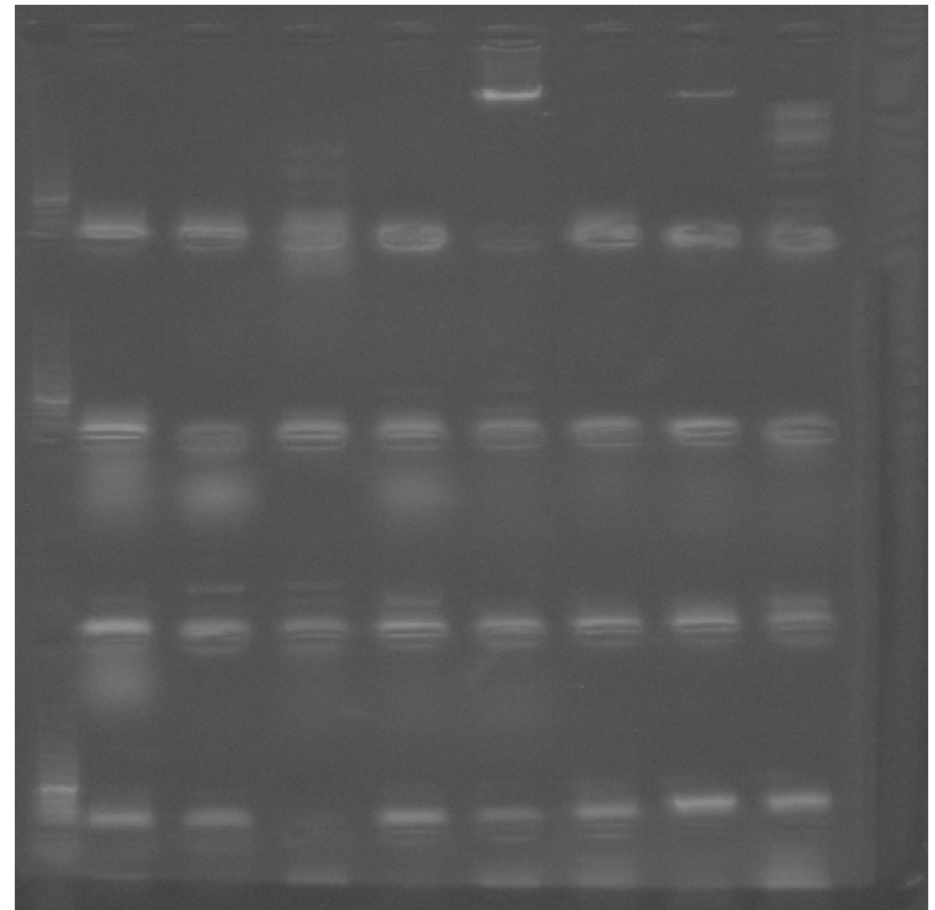
- Pyrosequencing for Fish and mussels
- PCR + gel electrophoresis for crayfish





Some results

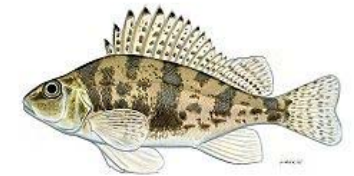
- Fish DNA
 - Positive PCR product in 12/55 samples for first marker
 - Non-specific amplification for second marker
- Mussel DNA
 - Positive PCR products in 13/55 samples
- Crayfish DNA
 - Negative



Mussel PCR ~170 bp

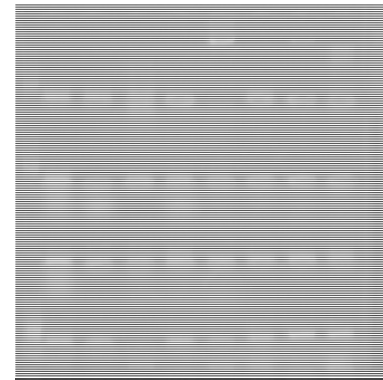
Next-generation sequencing

- First attempt using fish and Mussel PCR products
 - ~84 000 total DNA sequences
 - 66% low quality reads
 - 30% adaptor-dimers (non-specific)
 - 0,06% target sequences
- Other non-target organism DNA
 - Bacteria, microalgae, protozoans, vertebrates, crustaceans, annelids,



Next steps (2014)

- Re-sequencing with modified protocol for NGS
 - PCR reaction
 - Selection of target PCR products
- Develop a specific marker for Zebra mussel (*Dreissena polymorpha*)
 - test with field samples



Next steps (2015)



Illumina MiSeq IonTorrent PGM Roche GS Junior

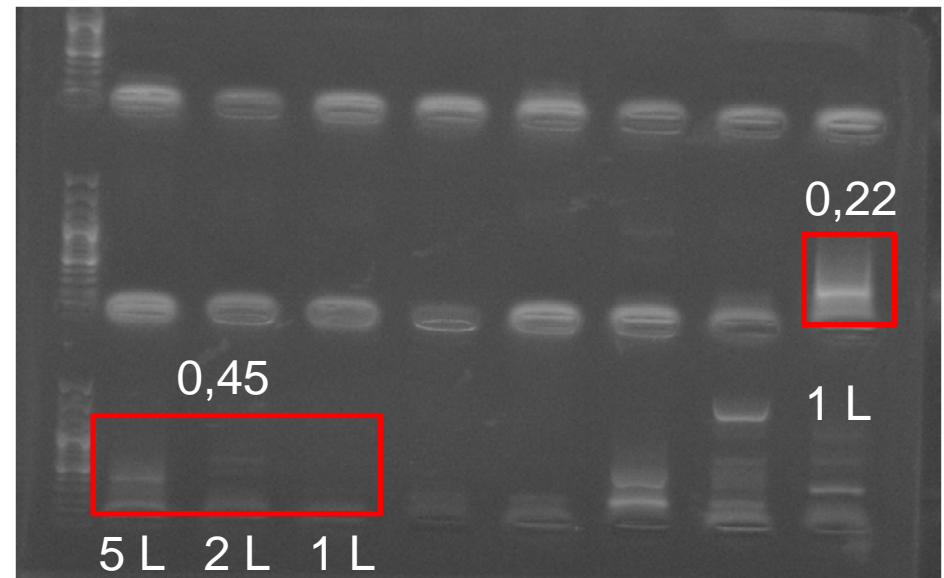
Instrument price		x	
Read length			x
Throughput	x		
Run time		x	
Price/run		x	
Hands-on time	x		
Read quality	x		
Assembly: coverage			x
Assembly: fewest pieces			x
Assembly: gene space	x		

Reads 24 M 100 000



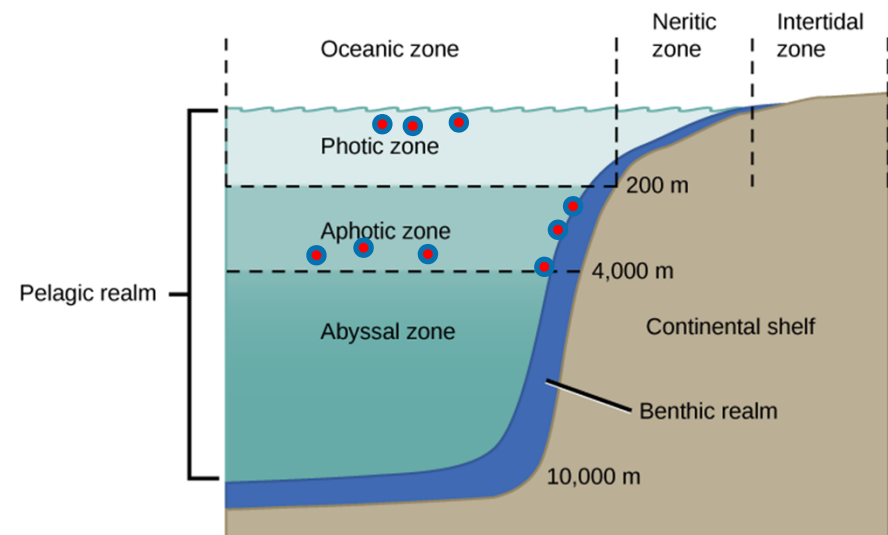
Sampling recommendations

- Double filtration protocol
 - Pre-filtration (e.g. glass-fiber filter)
 - Second filtration with 0,22 micrometer membrane



Sampling recommendations

Targeted sampling to maximise DNA recovery



Objectives

- Optimize sampling protocols
- Use available NGS methods for Fish DNA barcoding
- Develop simple methods for the detection of crayfish
- Develop methods for the detection of large mussel species in parallel (NGS)
- Develop NGS protocols for the correct identification of cyprinid fish

