



Stazione  
Zoologica  
Anton Dohrn  
Napoli



Ollscoil  
Teicneolaíochta  
an Atlantaigh  
Atlantic  
Technological  
University

# Illumina vs Oxford Nanopore: a multi-marker comparative study on fish eDNA metabarcoding

Maddalena Tibone, Sergio Stefanni, Jacopo Aguzzi, Bernadette O'Neill, Luca Mirimin

2<sup>nd</sup> Australia & New Zealand Environmental DNA conference

20<sup>th</sup> of February 2025

# Key aspects of environmental DNA metabarcoding

 Markers

Sequencing platform 

12S rRNA

Cytochrome Oxidase  
C Subunit I (COI)



Illumina



Nanopore

Are they comparable?

Are they comparable?

Is multi-marker better?

Longer amplicon = higher taxonomic resolution?



# Samples (n=20)



## Mock

Genomic DNA

13 species

n=4



## Mesocosm

Ray tank    Ocean tank

5 species    5 species

n=4

n=4



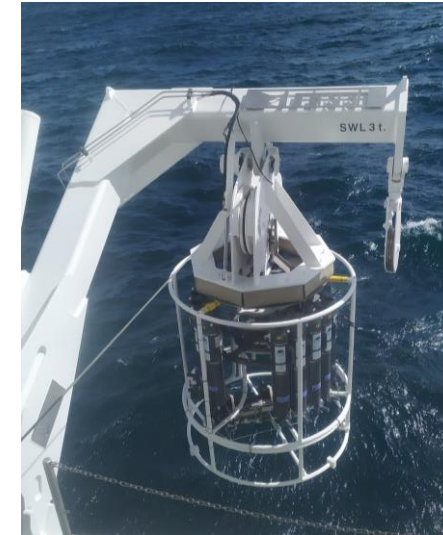
## Field

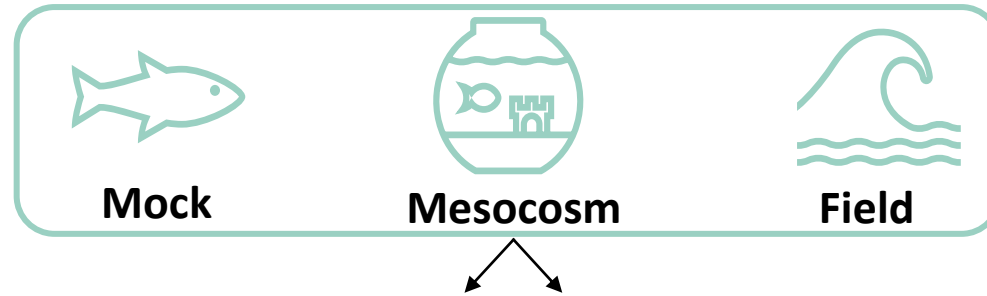
Pelagic    Mesopelagic

Unknown species

n=4

n=4





### **Nanopore Sequencing**

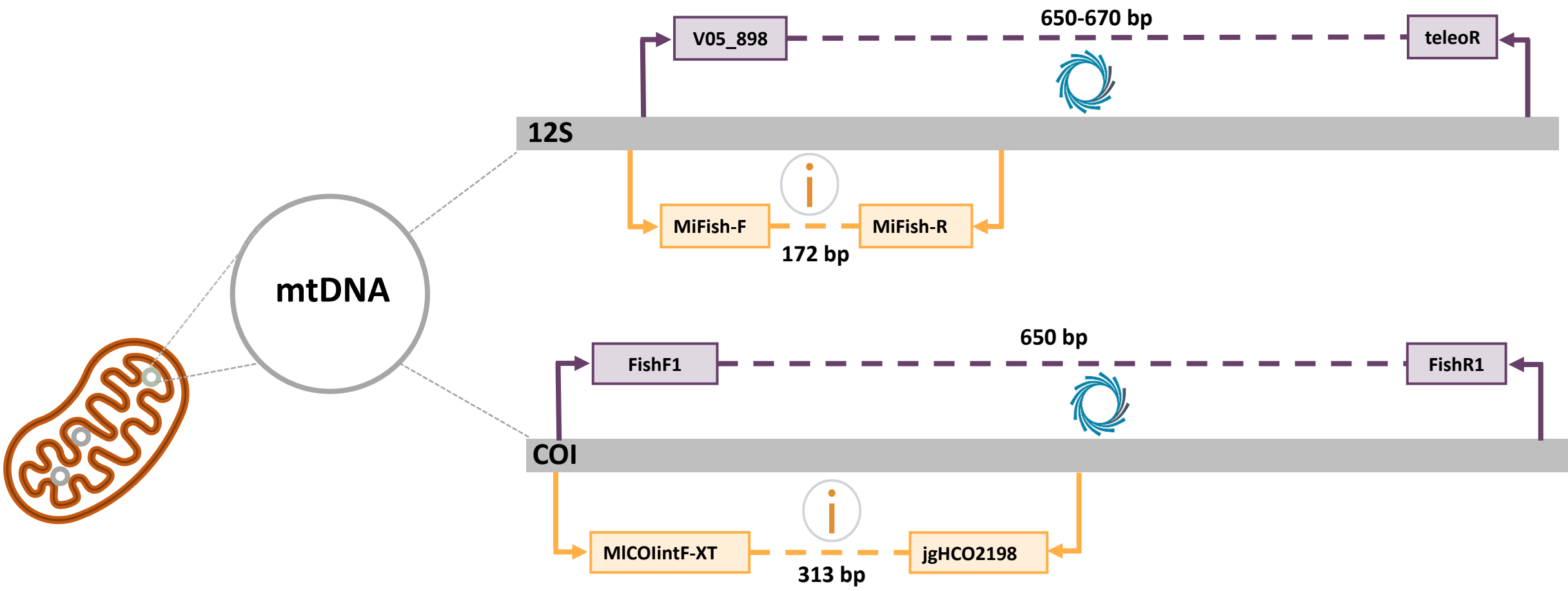
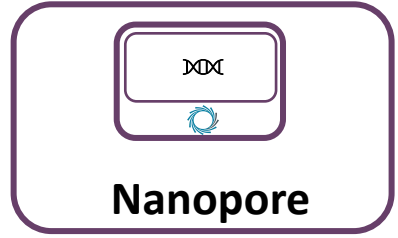
Kit 110	Kit 114
long 12S n=20	long 12S n=20
long COI n=20	long COI n=20

### **Illumina Sequencing**

NovaSeq PE250

short 12S n=20	short COI n=20
----------------	----------------



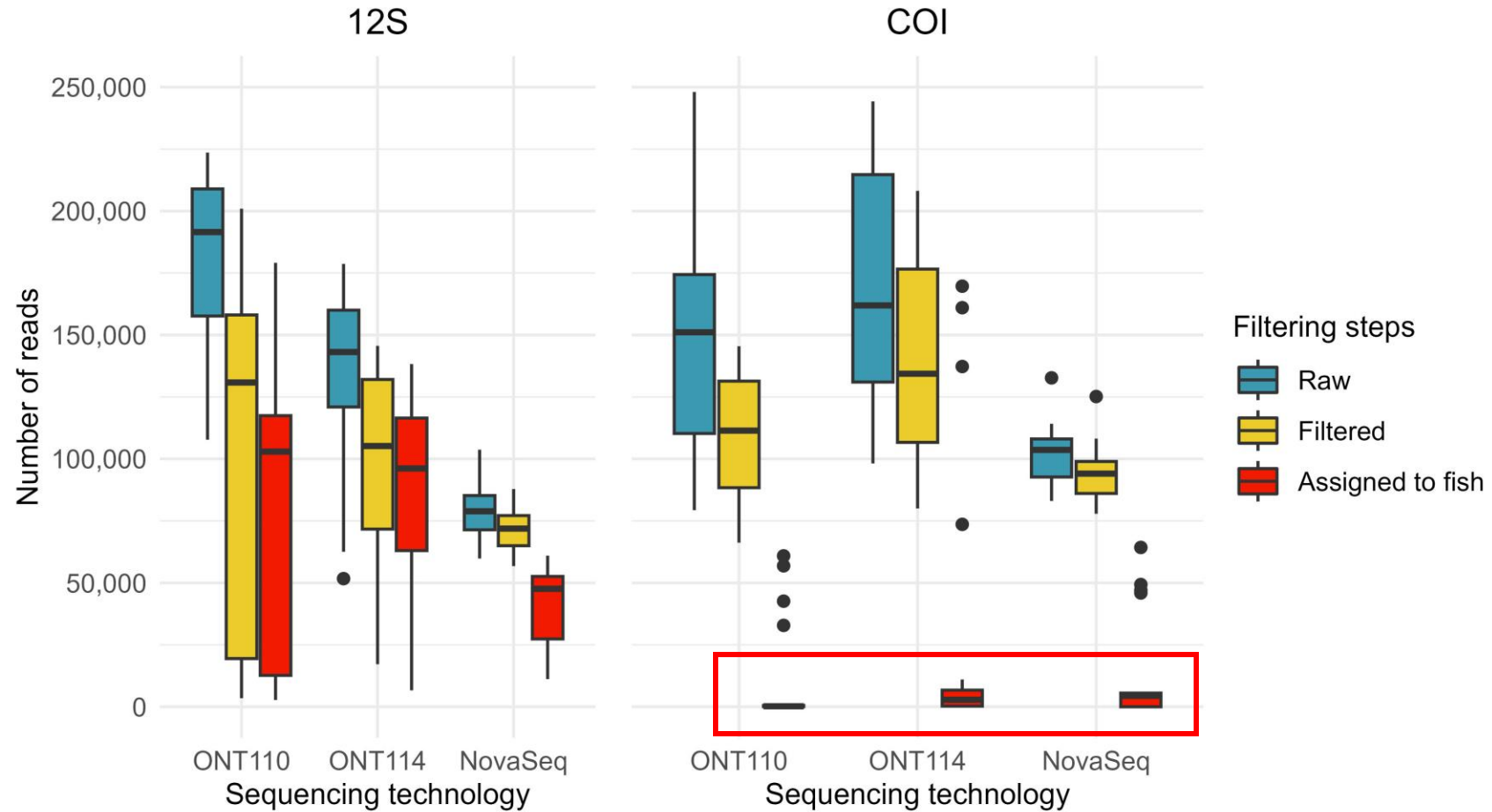


# Markers

12S > COI

Significantly more species recovered

Increased species richness with **multi-marker** approach



# Sequencing platforms



110



114



>30

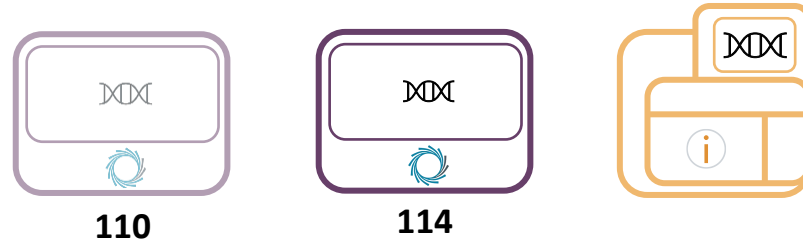
q-score

≈10

≈10

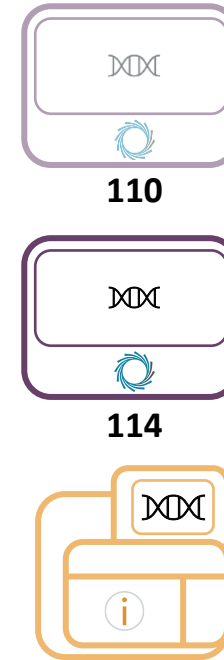


# Sequencing platforms



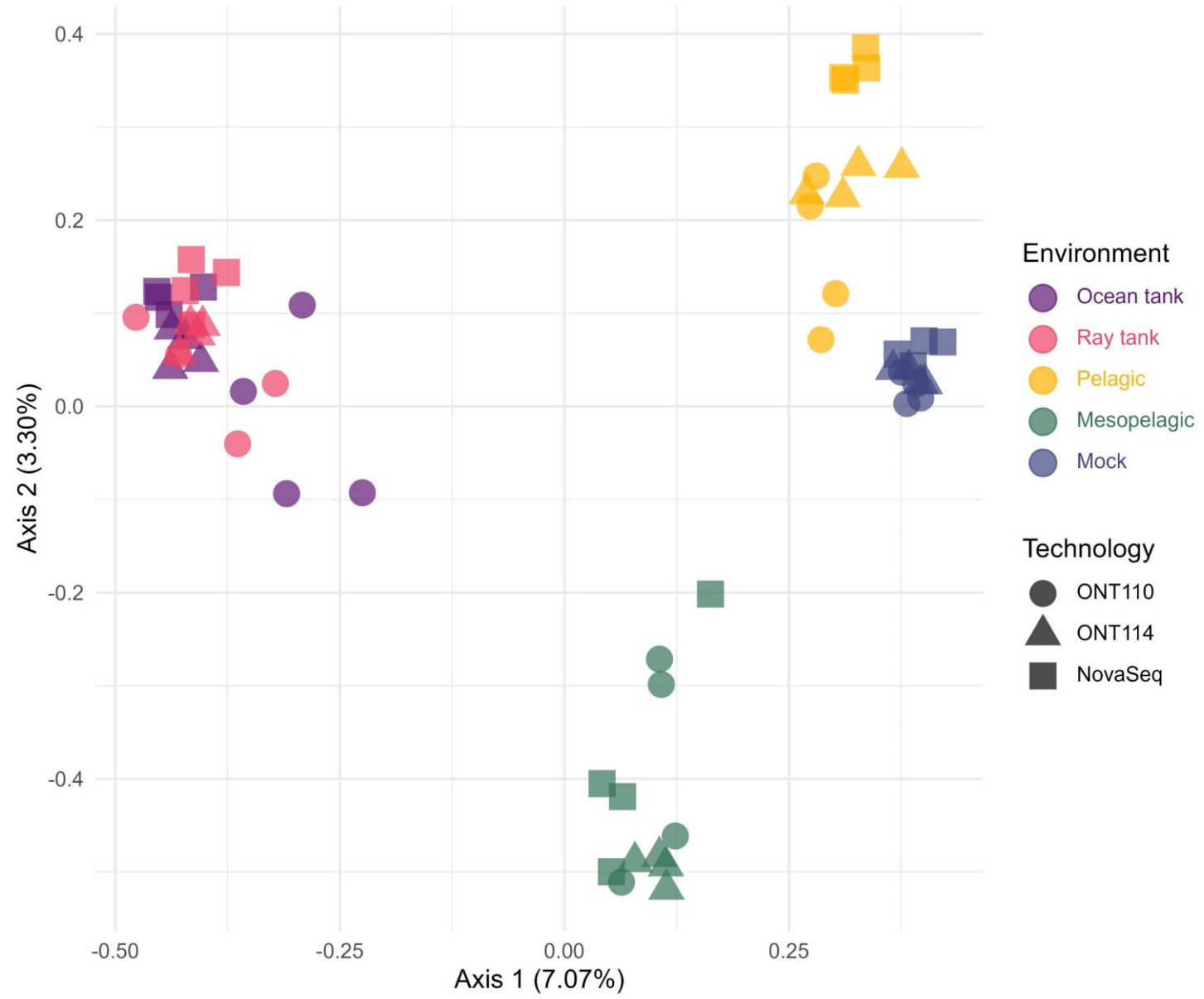
Species richness  
(12S+COI)

	*	*
*		
*		



# Sequencing platforms

Pres./abs.  
matrices  
(12S+COI)



# Mock communities

## Species detected



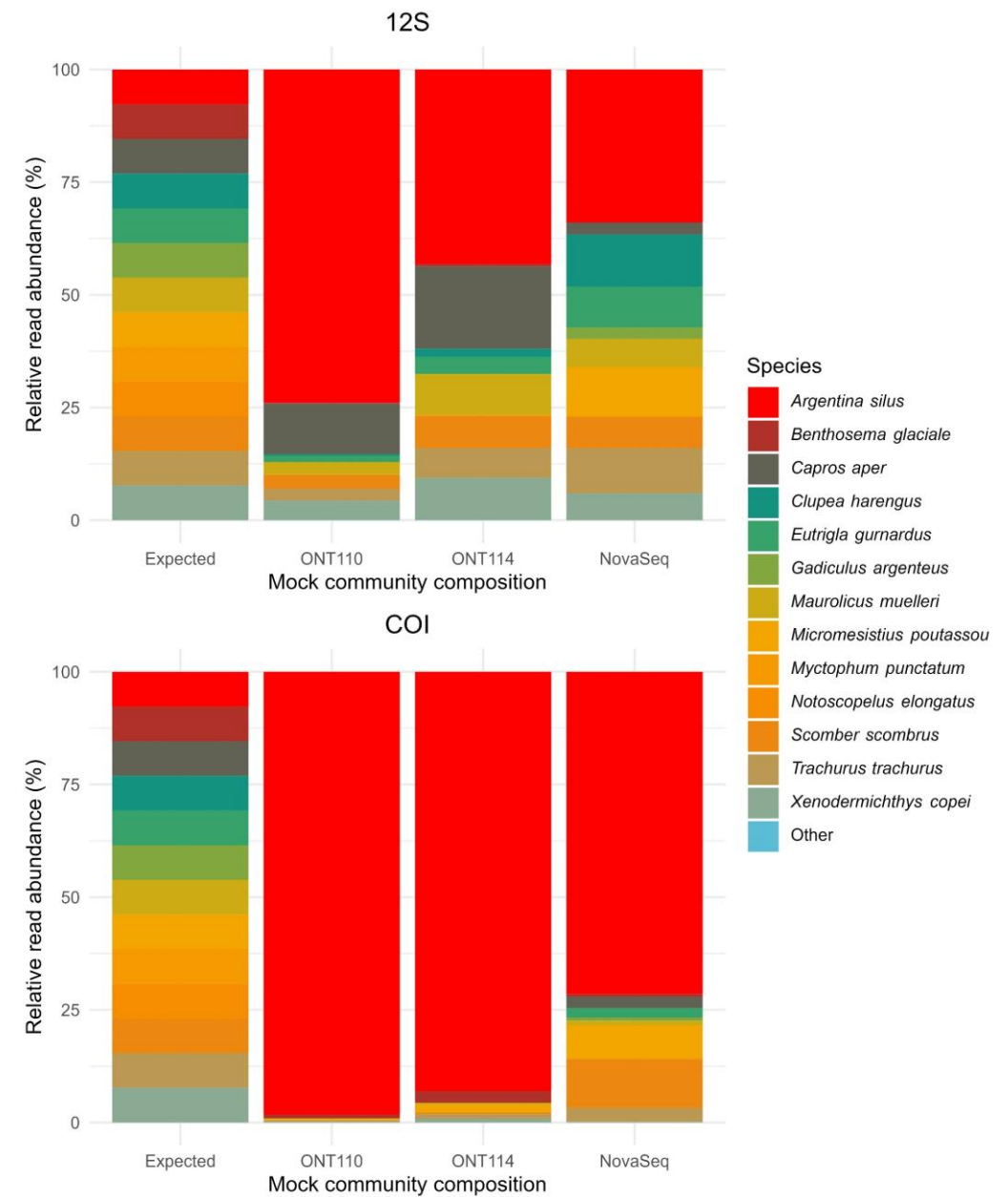
110



114

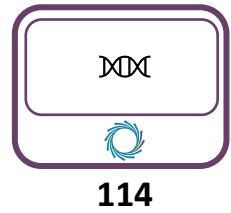
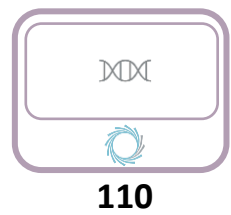


	12S	COI	12S+COI
110	11/13	6/13	11/13
114	12/13	9/13	12/13
112	10/13	11/13	12/13



# Aquarium tanks

## Species detected



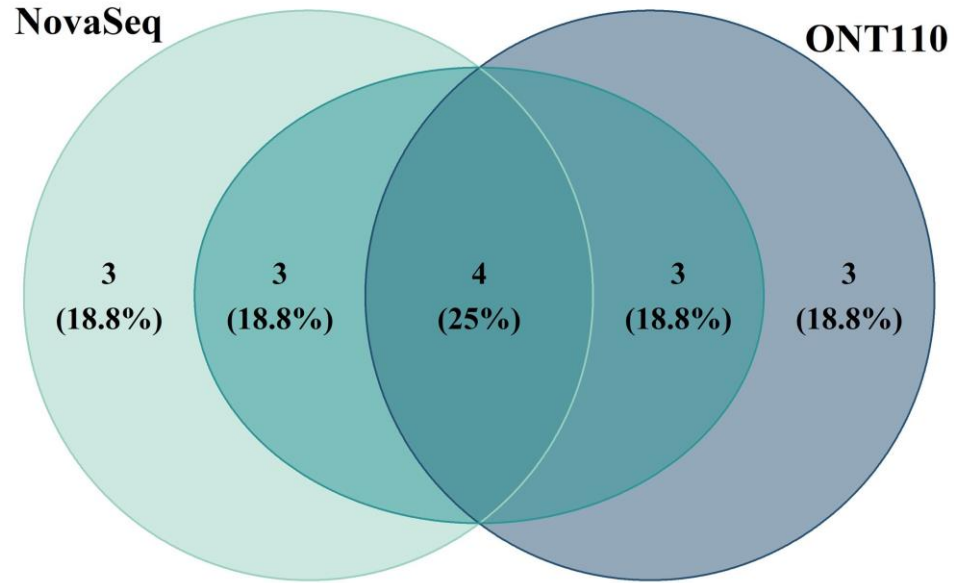
	12S	COI	12S+COI
110	9/10	5/10	10/10
114	9/10	5/10	10/10
114	8/10	10/10	10/10



# Field samples



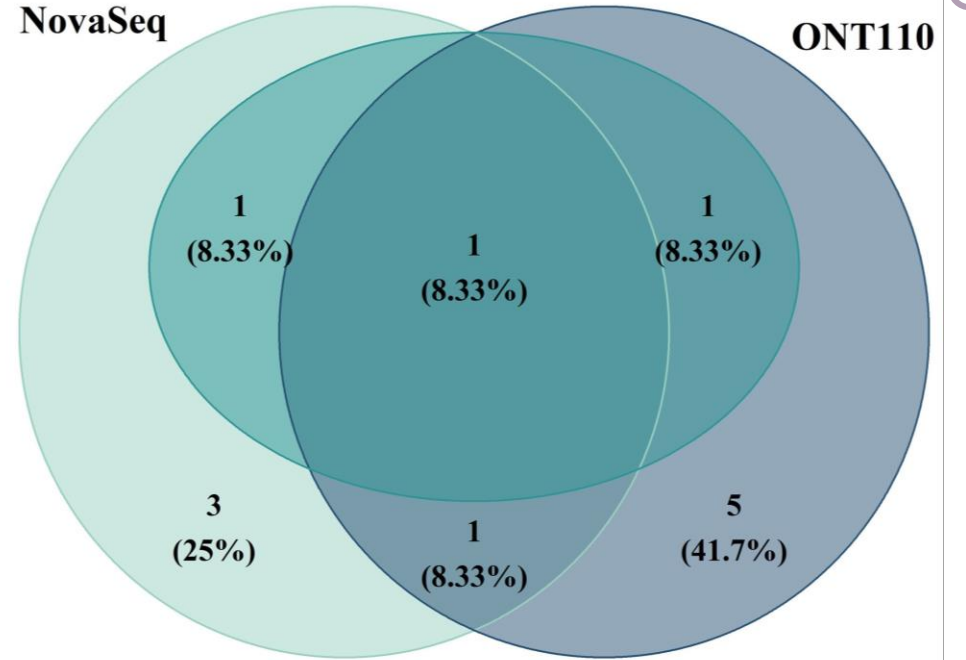
## Pelagic 12S



## ONT114



## Mesopelagic 12S



## ONT114

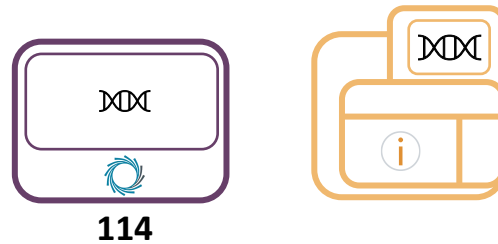


# Conclusions

Markers  
12S > COI but multi-marker preferable  
high non-target amplification with COI

Long fragments =  
higher taxonomic resolution

Sequencing platforms



Equivalent for species  
richness recovered

Most suited for field-based studies





Stazione  
Zoologica  
Anton Dohrn  
Napoli



Ollscoil  
Teicneolaíochta  
an Atlantaigh  
Atlantic  
Technological  
University



**fsbi**  
An International Society  
for Fish Biology

Travel grant

Sergio Stefanni, Jacopo Aguzzi,  
Bernadette O'Neill, Luca Mirimin

Thank you for your attention!



@MaddalenaTibone

maddalena.tibone@research.atu.ie

