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# Environmental monitoring through next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic macro- and meiofauna communities

*Speaker: Tomas Cedhagen*

JAN PAWLOWSKI,\* PHILIPPE ESLING,\*† FRANCK LEJZEROWICZ,\* TOMAS CEDHAGEN‡ and THOMAS A. WILDING §

\*Department of Genetics and Evolution, University of Geneva, Sciences 3, 30, Quai Ernest Ansermet, CH-1211, Geneva 4, Switzerland,

†IRCAM, UMR 9912, Université Pierre et Marie Curie, Paris, France,

‡Department of Biological Sciences, Aquatic Biology, Aarhus University, Aarhus, Denmark,

§ Ecology Department, SAMS, Scottish Marine Institute, Oban, Argyll, UK

# What is the NGS eDNA survey?

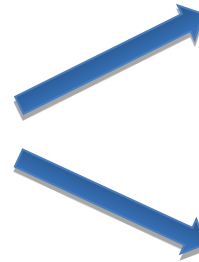


Environmental sample (water sediment)

DNA/RNA extraction



DNA/RNA of all species present in the environment



1. Detecting selected species (PCR, Sanger sequencing)

2. Inventory of global diversity using next generation sequencing (NGS)

Identifying particular species or community of species present in environmental samples



# The problem

**Current benthic monitoring is exclusively based on morpho-taxonomic species identification, i.e.:**

- it requires an excellent taxonomic expertise,
- it overlooks the morphologically indistinguishable juvenile and life-cycle stages of macrofauna and small-sized organisms (meiofauna, protists)
- it is time consuming, and
- it is expensive
- it is today a limiting factor for certification.

# The objective

**Replacing the morphotaxonomic inventories by NGS eDNA surveys !**



# The proof-of concept studies

## Application of NGS eDNA surveys for benthic monitoring of salmon farms

The NGS eDNA tests were based the genetic inventory of:

- Foraminifera (18S rDNA 37f region)
- Metazoa:macro- and meiofauna (18S rDNA V4 region)





# Sampling



In most of the studies the eDNA and eRNA samples were taken in parallel to traditional macrofauna samples

# Sampling

2 salmon farms near Oban,  
Scotland



4 salmon farms in Marlborough  
Sounds, New Zealand





# Forams results

**Objective:** Evaluate the use of benthic foraminifera as indicators of fish-farming impact

MOLECULAR ECOLOGY  
RESOURCES

Molecular Ecology Resources (2014)

doi: 10.1111/1755-0998.12261

**Environmental monitoring through protist next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic foraminifera communities**

JAN PAWLOWSKI,\* PHILIPPE ESLING,\*† FRANCK LEJZEROWICZ,\* TOMAS CEDHAGEN‡ and THOMAS A. WILDING§

**Next-Generation Environmental Diversity Surveys of Foraminifera: Preparing the Future**

J. PAWLOWSKI<sup>1,\*</sup>, F. LEJZEROWICZ<sup>1</sup>, AND P. ESLING<sup>2</sup>

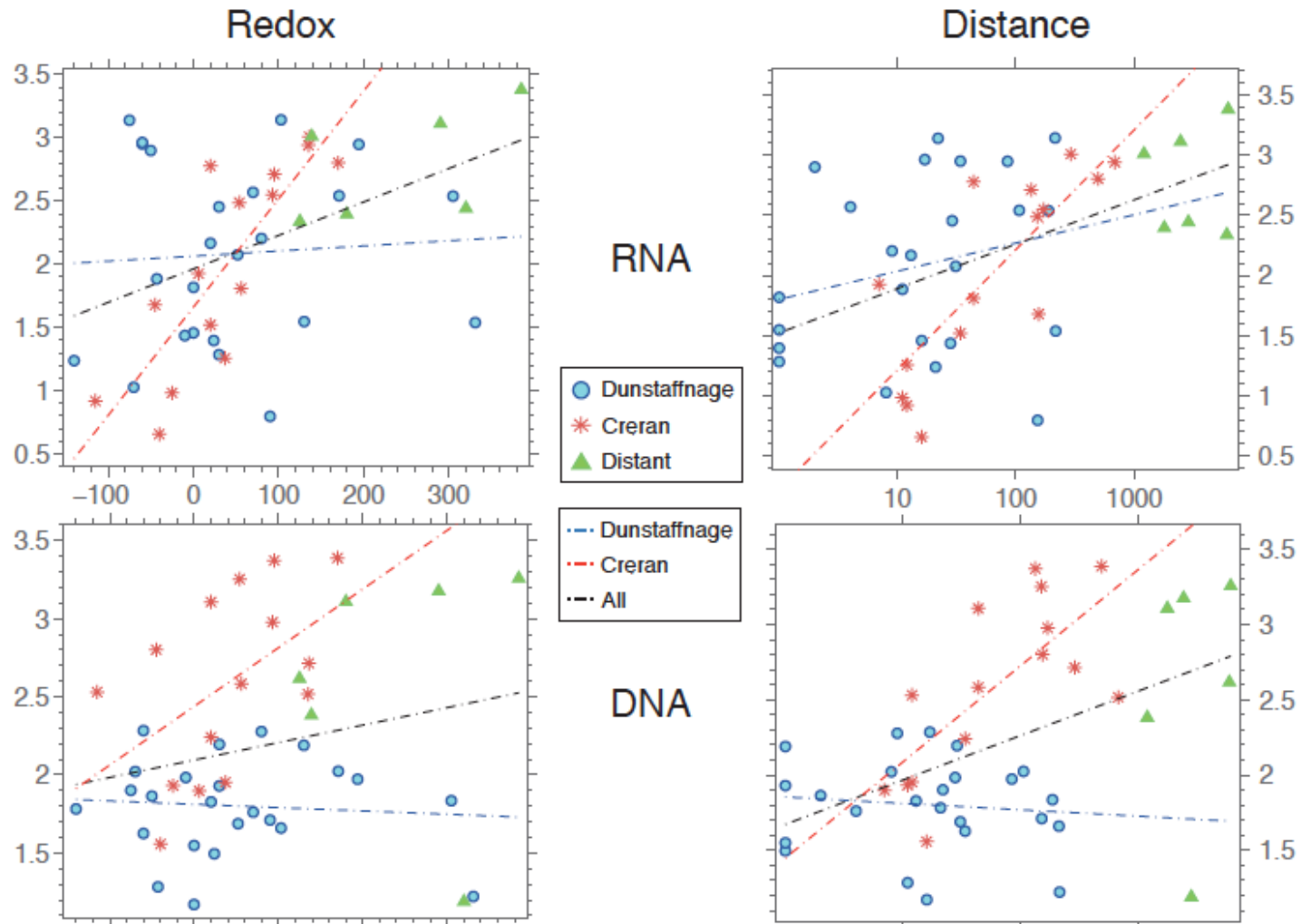
<sup>1</sup>*Department of Genetics and Evolution, University of Geneva, Switzerland; and* <sup>2</sup>*IRCAM, UMR 9912, Université Pierre et Marie Curie, Paris, France*

Biol. Bull 2014

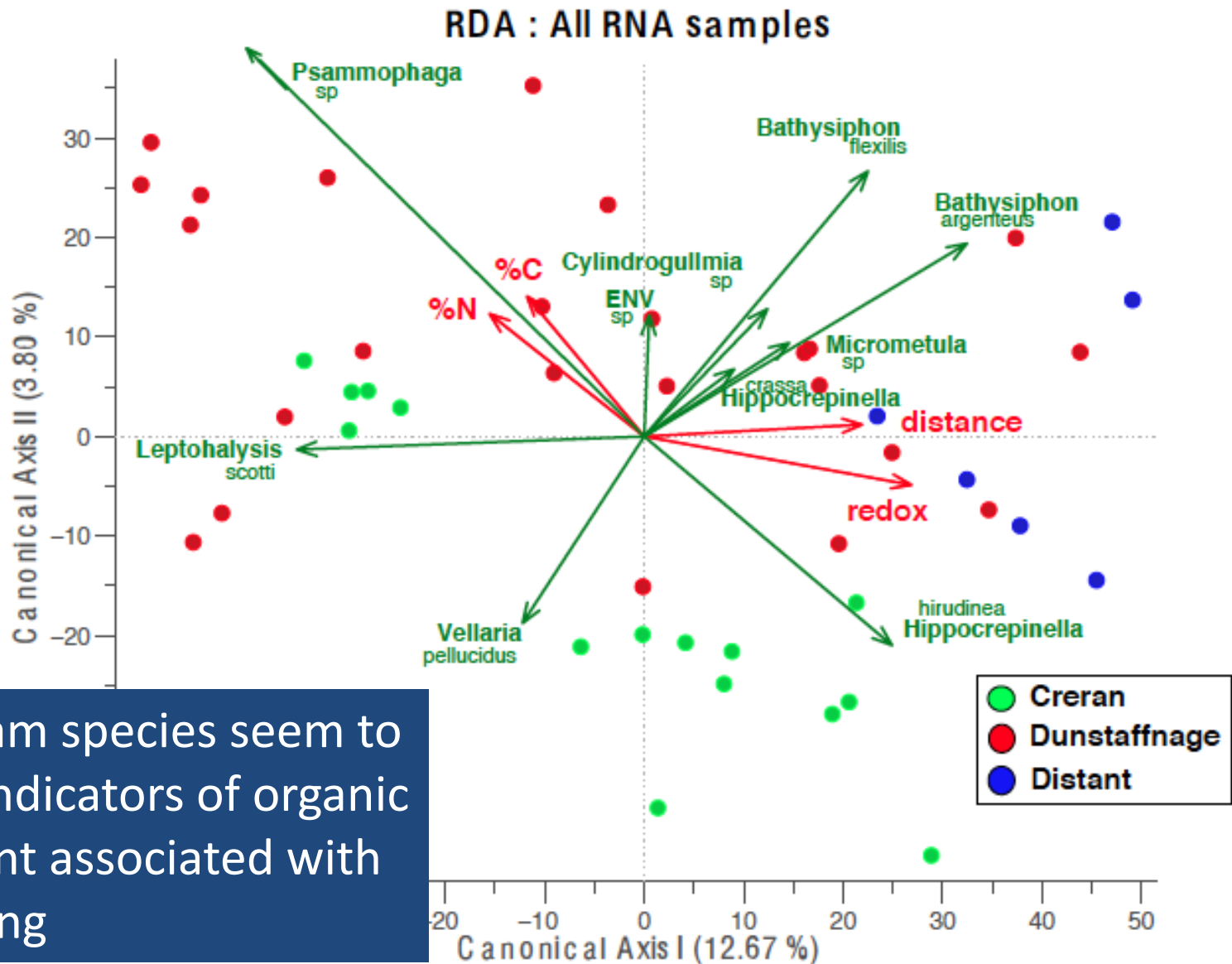


# Forams results

Foraminiferal species richness increases with distance from cages and sediment oxygenation



# Forams results



Some foram species seem to be good indicators of organic enrichment associated with fish-farming

# Forams results: *Psammophaga* n.sp.



# Forams studies - conclusions

- Most of common foraminiferal species identified morphologically were recovered by eDNA/RNA approach.
- The foraminiferal OTUs/species richness shows correlation to distance to cages and redox values (especially in RNA).
- Some foraminiferal species are potentially useful bioindicators of enrichment stage.
- Forams Community Index correlates with macrofaunal indices

# SCIENTIFIC REPORTS



OPEN

## High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems

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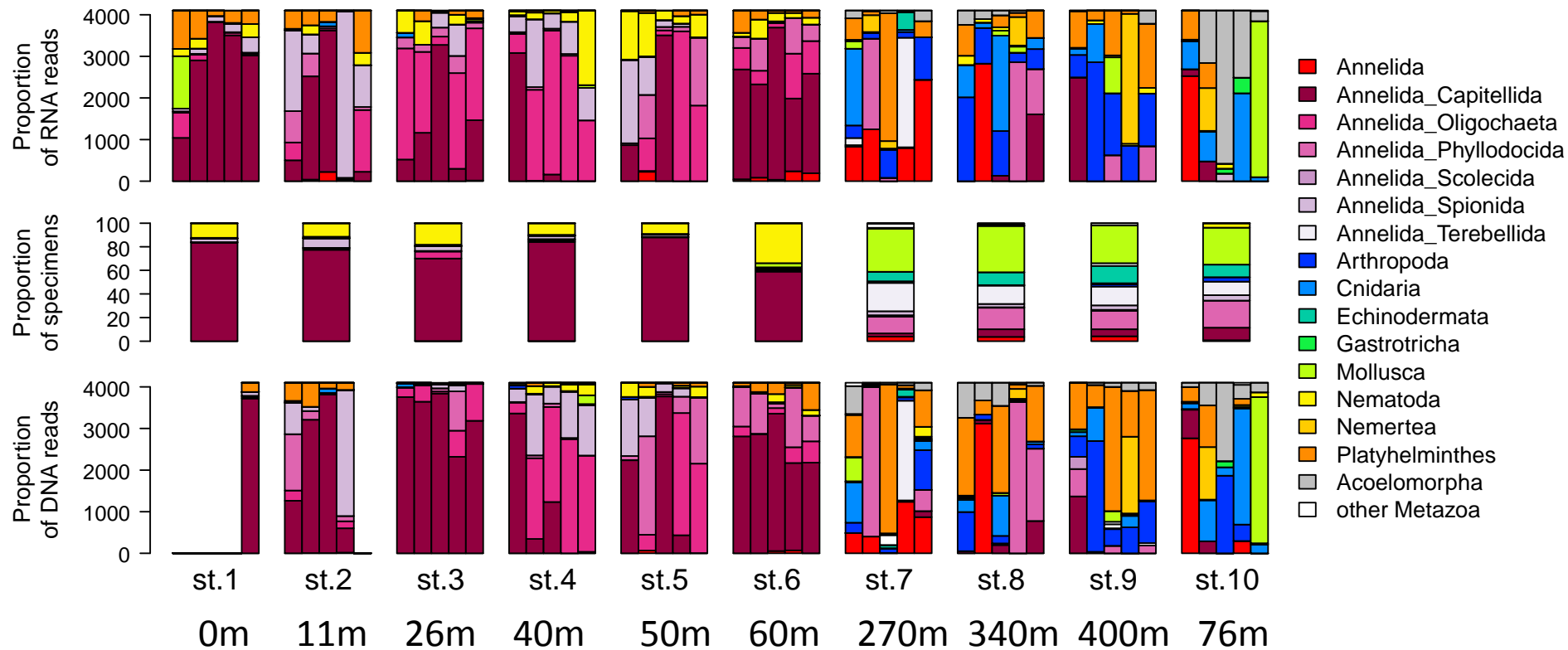
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Franck Lejzerowicz<sup>1,†</sup>, Philippe Esling<sup>1,2</sup>, Loïc Pillet<sup>1,3</sup>, Thomas A. Wilding<sup>4</sup>,  
Kenneth D. Black<sup>4</sup> & Jan Pawlowski<sup>1</sup>

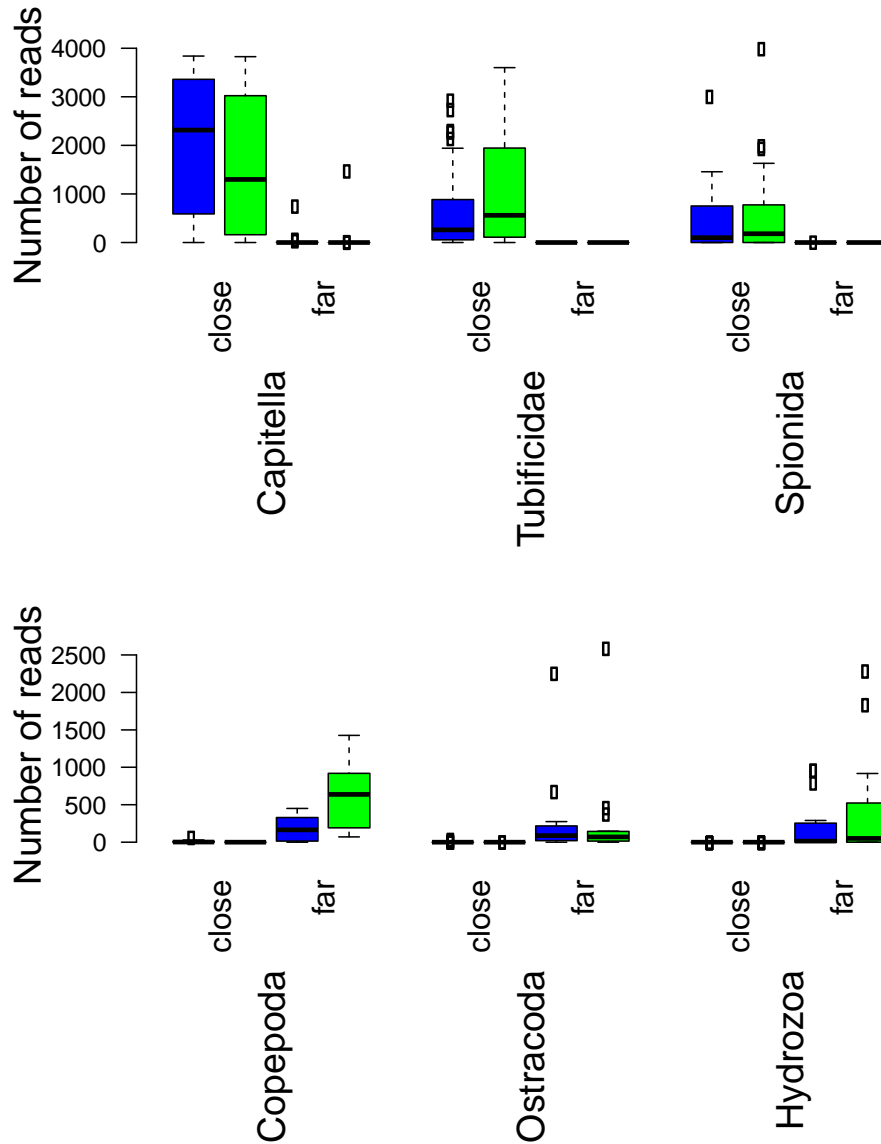


# Metazoan results



Taxonomic composition of samples with taxa identified morphologically (middle panel), and genetically by RNA (upper panel) and DNA (lower panel)

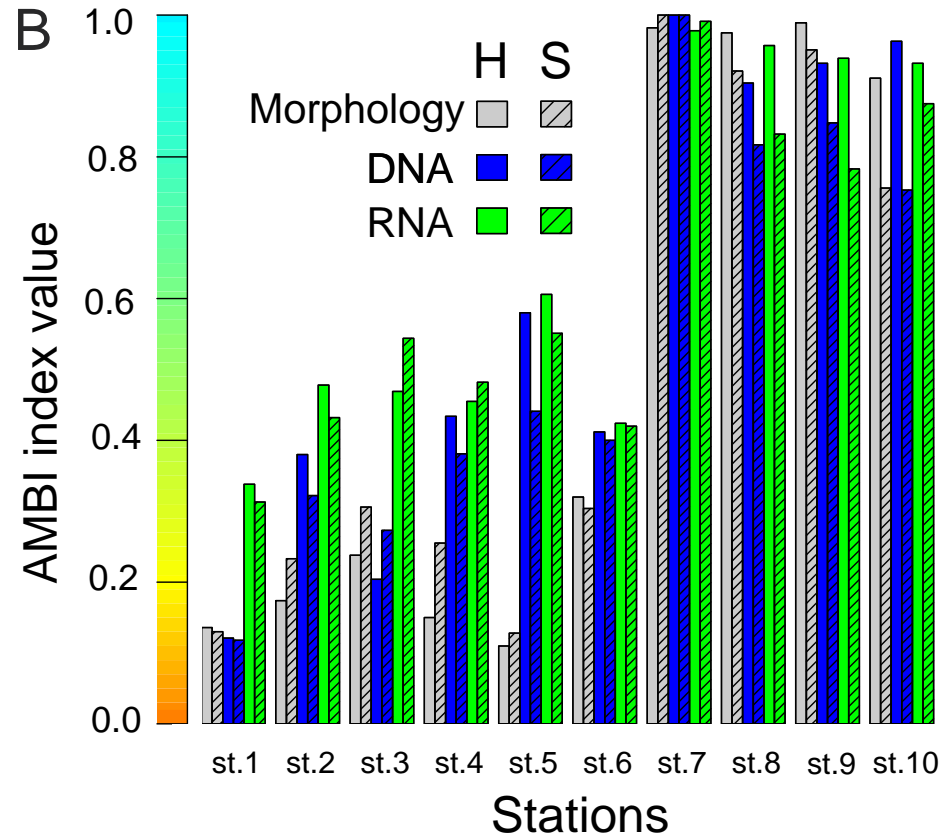
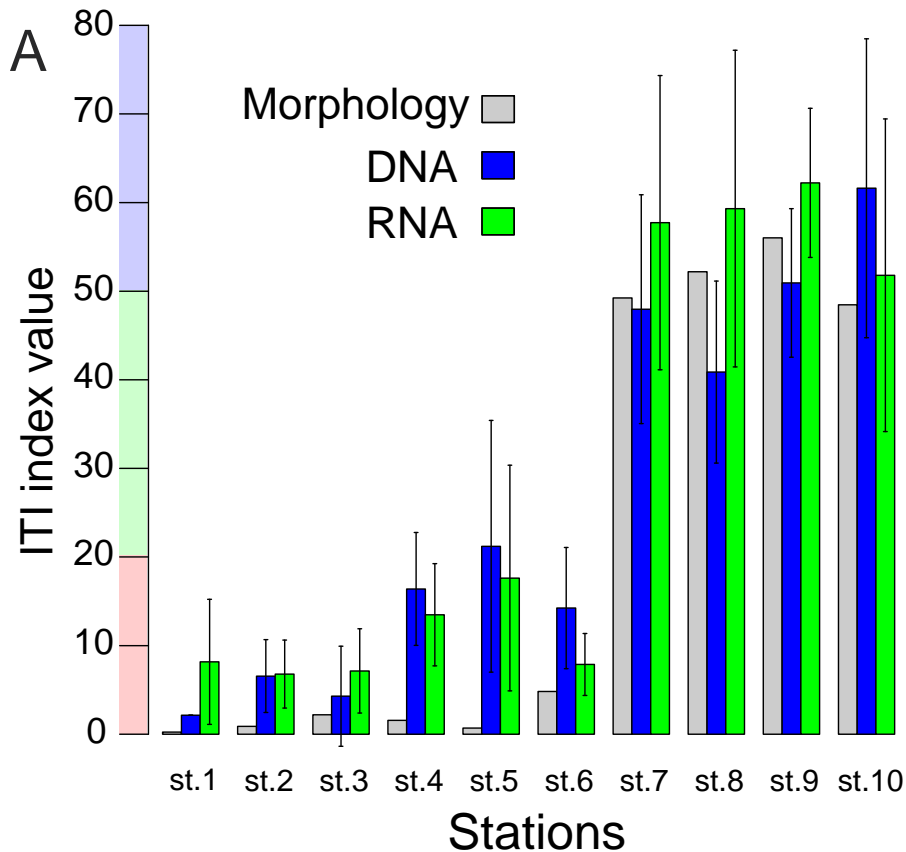
# Metazoan results



DNA ■  
RNA ■

Quantitative analysis of NGS data provides some information about the abundance of common metazoan taxa.

# Metazoan results



The ITI and AMBI indices inferred from NGS and morpho-taxonomic data provides very similar bioassessment values

# Metazoan studies - conclusions

- A broad range of metazoan taxa can be identified in eDNA/RNA NGS data.
- There is a good correlation between morphological and eDNA data for common species (especially Annelida).
- The values of biotic indices inferred from NGS data are very similar to the values recovered by benthic macrofauna surveys.

# Advantages of NGS eDNA surveys

	<b>eDNA/RNA</b>	<b>Morphotaxonomy</b>
time	fast (1-2 weeks)	slow (> 3 months)
material	2-5 g samples	grab sample
coverage	high (many replicates)	limited
biodiversity	macro-, meiofauna, protists, bacteria	macrofauna
identification accuracy	depend on reference database	depend on taxonomic expertise
sensitivity	higher	lower
workflow	automatized	manual



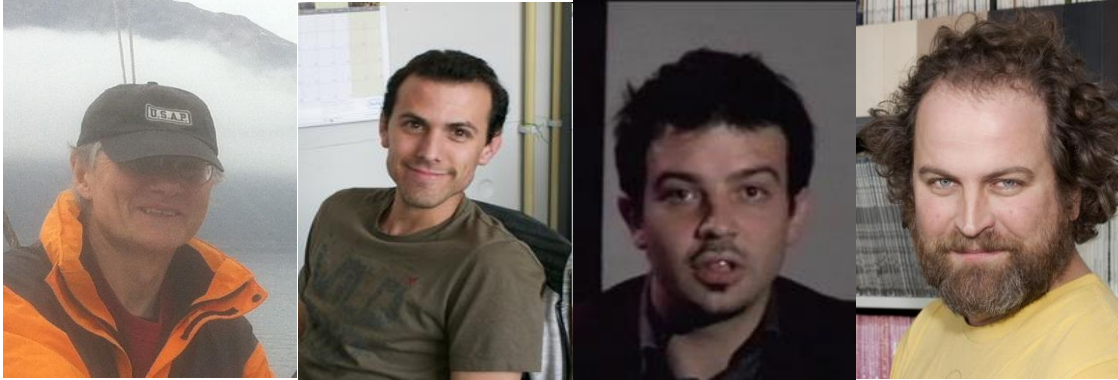
# Future validation studies

Main goals:

- to adapt the sampling procedure to enhance the presence of macrofauna in NGS data (by working on sieved samples)
- to develop a new category of bio-indicators (meiofauna, forams), which will fit better to the NGS eDNA surveys (sediment samples),
- to improve the accuracy of NGS data analysis



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Jan Pawlowski  
Franck Lejzerowicz  
Philippe Esling  
Loic Pillet

Nigel Keeley

Xavier Pochon

Susie Wood



Tom Wilding





Thank you  
very much

