

Tomas Cedhagen
Assoc. Professor, Fil. Dr.
Aarhus University,
Department of Bioscience, Section of Aquatic Biology,
Building 1135, Ole Worms allé 1,
DK-8000 Aarhus C, Denmark
cedhagen@biology.au.dk / cedhagen@bios.au.dk

Environmental barcoding as a tool for monitoring impact associated with salmon farming on community of benthic Foraminifera.

Jan Pawlowski¹, Philippe Esling¹, Franck Lejzerowicz¹, Tomas Cedhagen², Xavier Pochon³, Susanna A. Wood³, Nigel Keeley³, Thomas A. Wilding⁴

¹ Department of Genetics and Evolution, University of Geneva, CH

² Department of Biological Sciences, Marine Ecology, Aarhus University, DK

³ Coastal & Freshwater Group, Environmental Technologies, Cawthron Institute, NZ

⁴ Ecology Department, Scottish Marine Institute, Oban, Argyll, UK

cedhagen@biology.au.dk

The problem

Current biomonitoring and bioassessment of marine aquaculture use benthic macrofauna as bioindicators. The identification of macrofaunal species is exclusively based on morphological characters, i.e.:

- it requires an excellent taxonomic expertise,
- it overlooks large proportion of morphologically indistinguishable juvenile and life-cycle stages,
- it is time consuming, and
- it is expensive.

Traditional vs molecular monitoring



Morphological analysis
of macrofauna in 10 kg
sediment sample



Molecular analysis of
micro- and meiofauna in
2 g sediment sample



Sampling

2 salmon farms near Oban,
Scotland



4 salmon farms in Marlborough
Sounds, New Zealand



Focus on benthic foraminifera

Benthic foraminifera are particularly suitable for detecting environmental changes and to be used as environmental indicators because of their:

- Abundance
- High diversity
- Small size
- Short life-cycles

Marine Pollution Bulletin 58 (2009) 1297–1309



Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

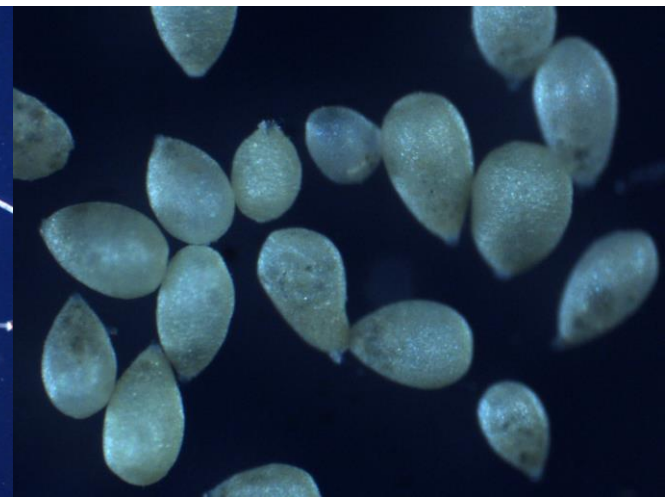


Impact of fish farming on foraminiferal community, Drvenik Veliki Island, Adriatic Sea, Croatia

Jelena Vidović^{a,*}, Vlasta Ćosović^a, Mladen Juračić^a, Donat Petricoli^b

^a Department of Geology, Faculty of Science, Horvatovac 102a, HR-10 000 Zagreb, HR, Croatia

^b DII Vd.o.o. for Sea Ecology, Avenija Vječeslava Holjevca 20, HR-10 000 Zagreb, Croatia



The principle of environmental barcoding

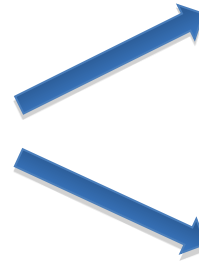


Environmental sample

DNA/RNA extraction



DNA/RNA of all species present in the environment



Searching for selected taxa (PCR, Sanger)

Next generation sequencing (NGS) of global diversity



identifying particular species or community of species present in environmental samples

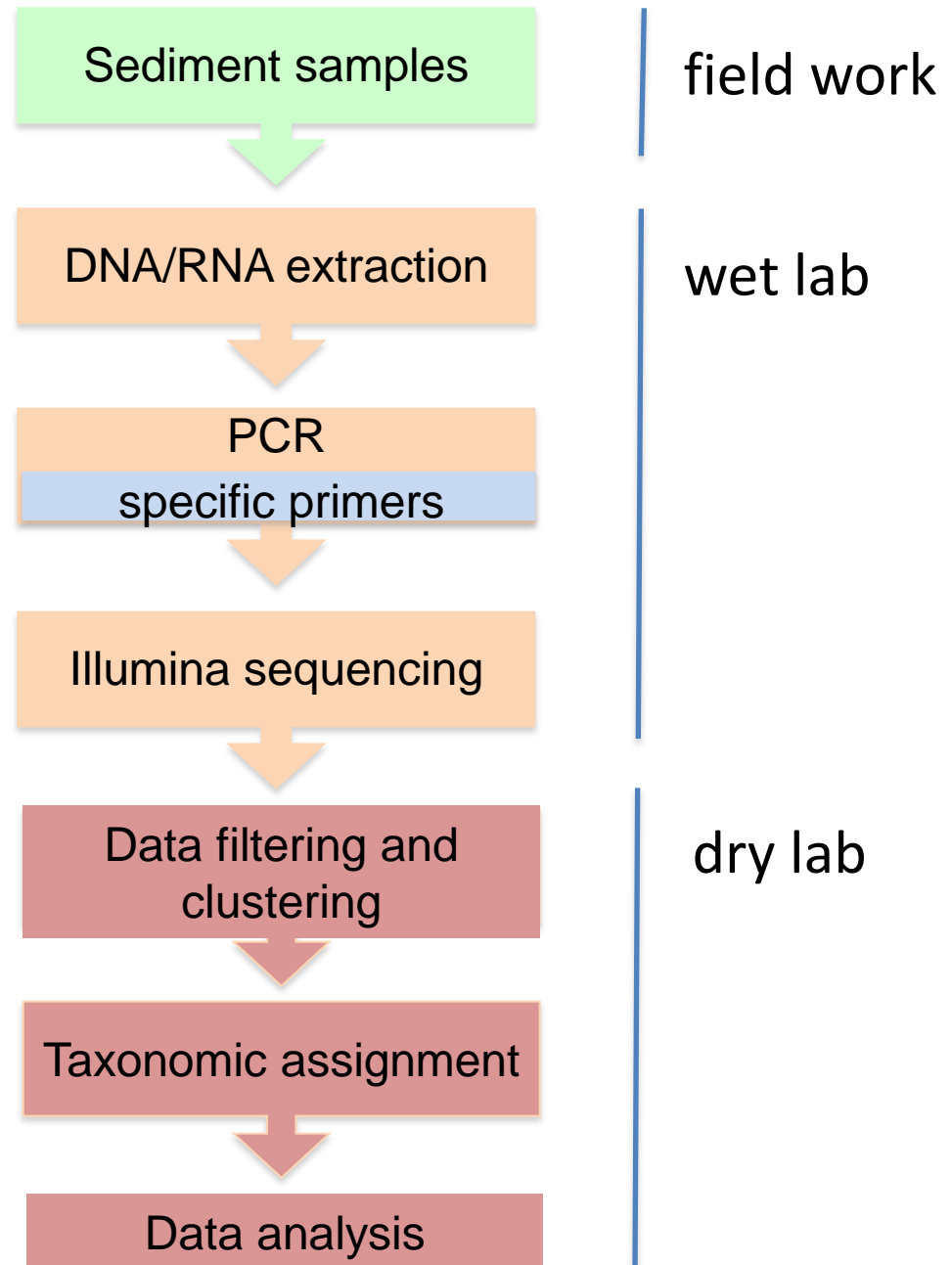
Methodology

Targeting micro- and meio-eukaryotes:

- foraminifera (18S 37f)
- other protists (18S V4)
- meiofauna (18S, COI)

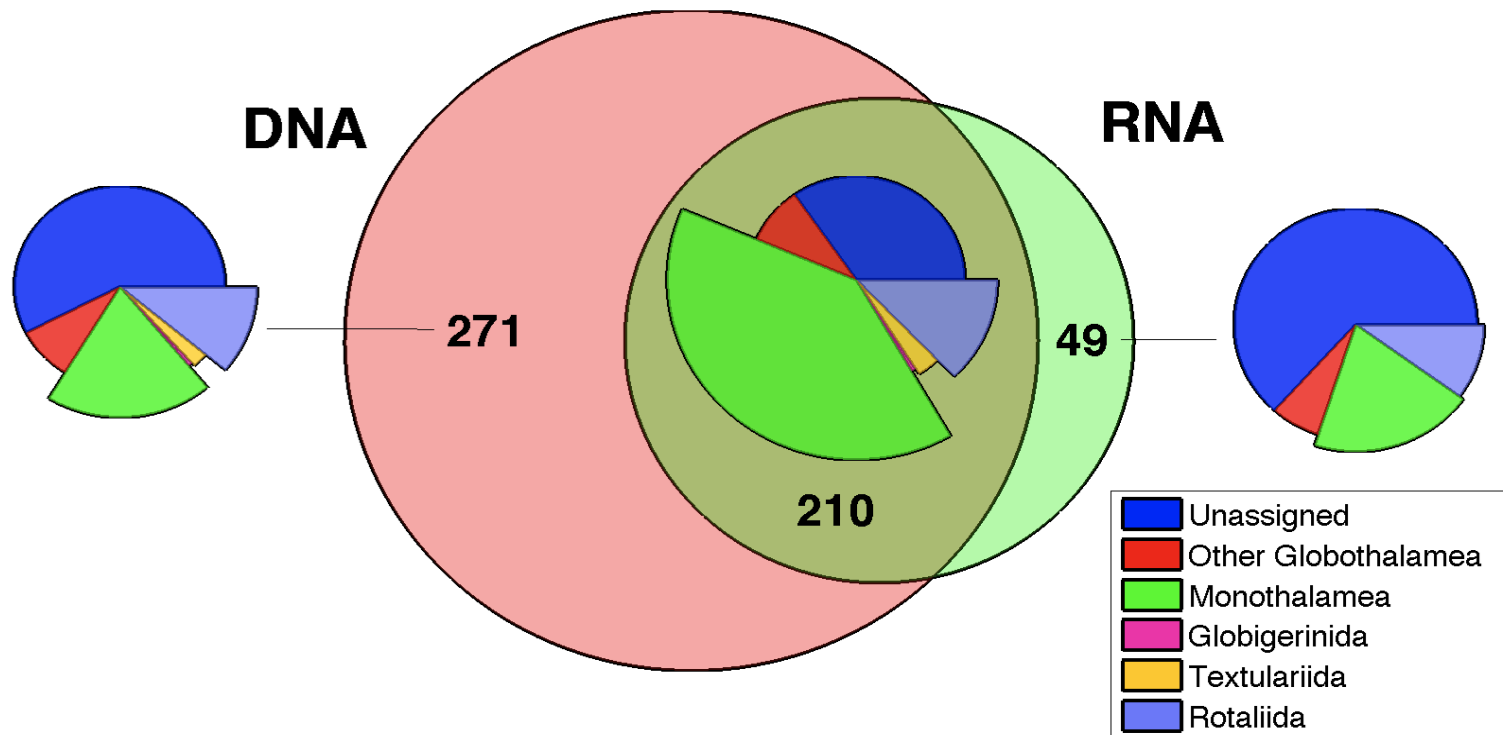


Illumina MiSeq next generation sequencer



DNA vs RNA

We analysed only the OTUs present in both DNA and RNA samples to avoid the presence of sequences derived from extracellular DNA and inactive cells.



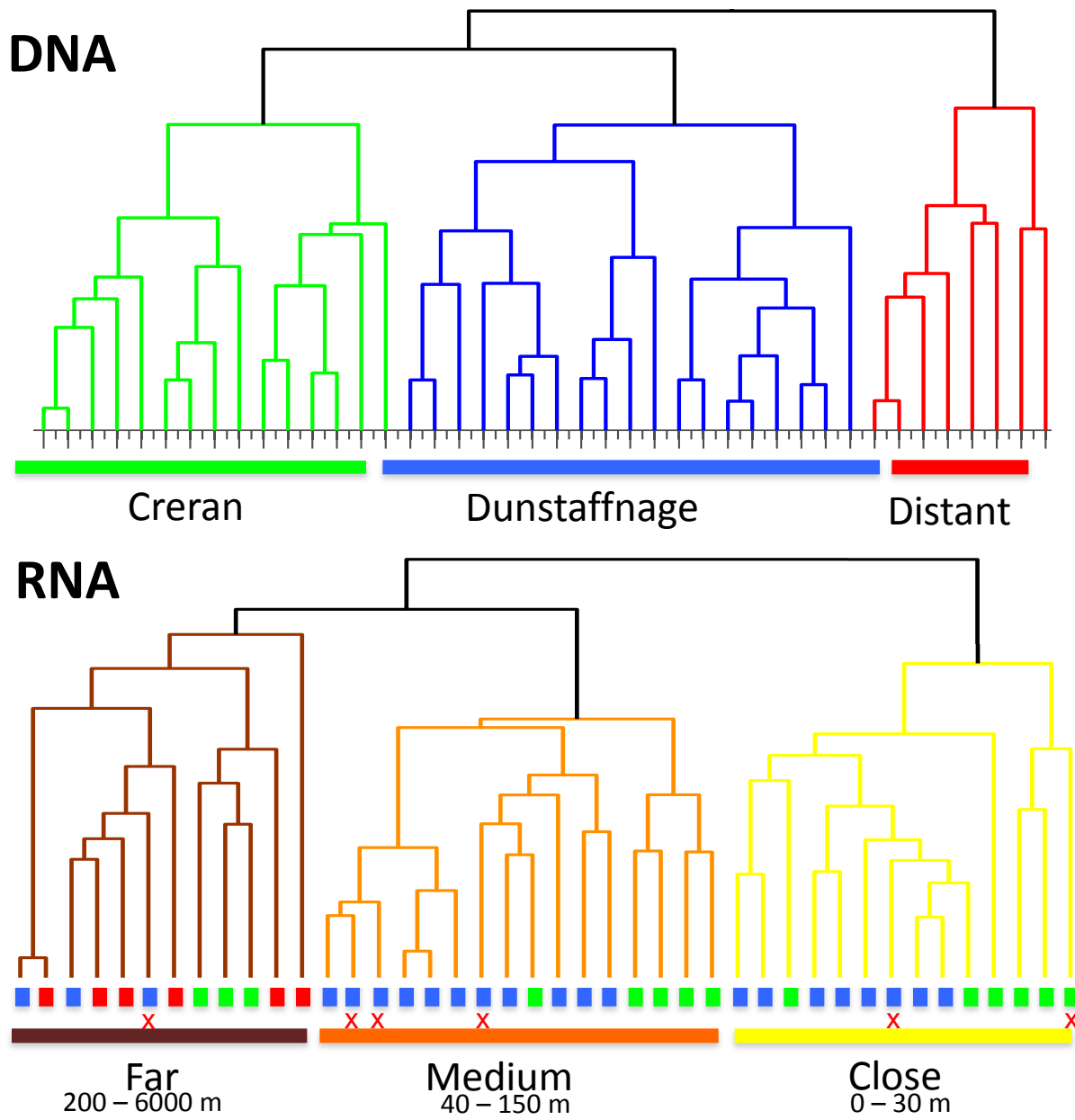
Morphospecies vs OTUs

Most abundant morphospecies are among the OTUs with the highest number of reads.

Class/Order	Species	Reads	Specimens
Monothalamea	Psammophaga spp.	1918634 (21%)	289 (8%)
Monothalamea	CladeY allogromiid (Hippo)	1791343 (20%)	?
Monothalamea	MON3	957471 (11%)	?
Monothalamea	CladeC allogromiid (orange blob)	311804 (3%)	387 (10%)
Globothalamea	Leptohalysis scotti	301358 (3%)	504 (14%)
Monothalamea	Micrometula spp.	259323 (3%)	841 (23%)
Monothalamea	CladeY allogromiid (HabSip27)	244612 (3%)	?
Globothalamea	Ammonia spp.	236030 (3%)	84 (2%)
Monothalamea	Bathysiphon argenteus	213827 (2%)	235 (6%)
Monothalamea	Bathysiphon flexilis	198243 (2%)	130 (4%)

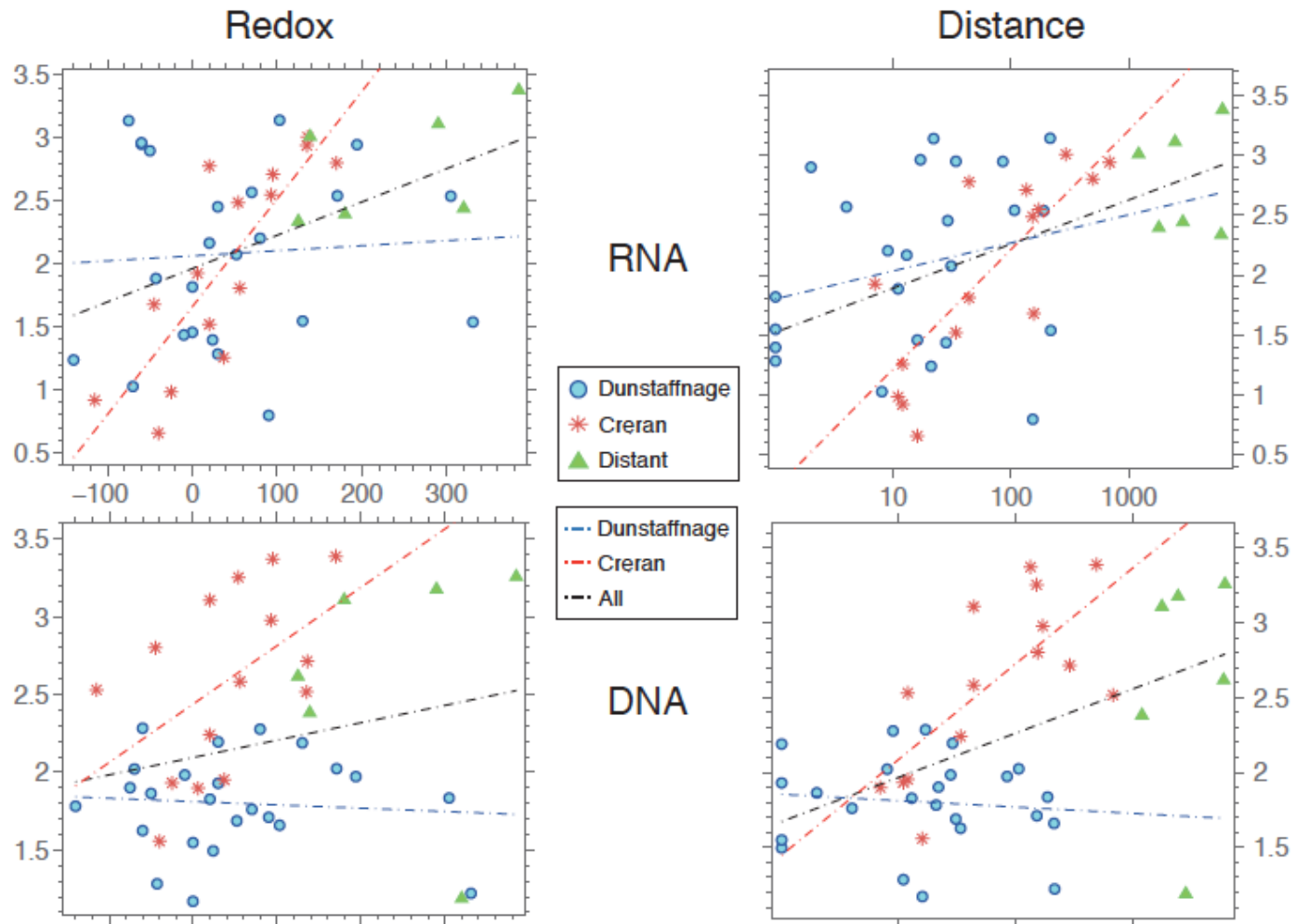
Community structure

Foraminiferal communities were well grouped together according to locality in DNA data and according to the distance to cages in RNA data.

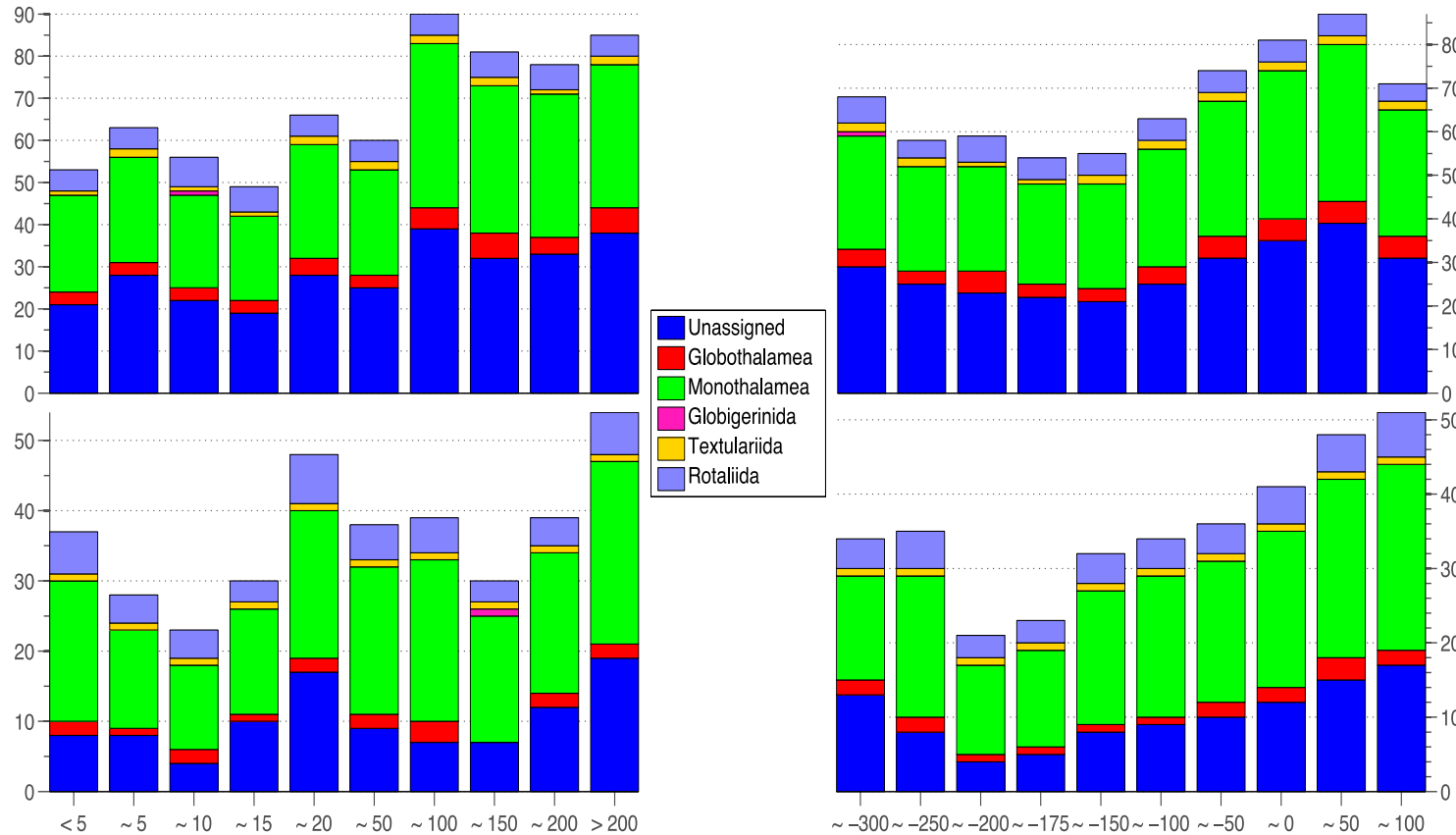


Species richness

Foraminiferal species richness increases with distance from cages and sediment oxygenation

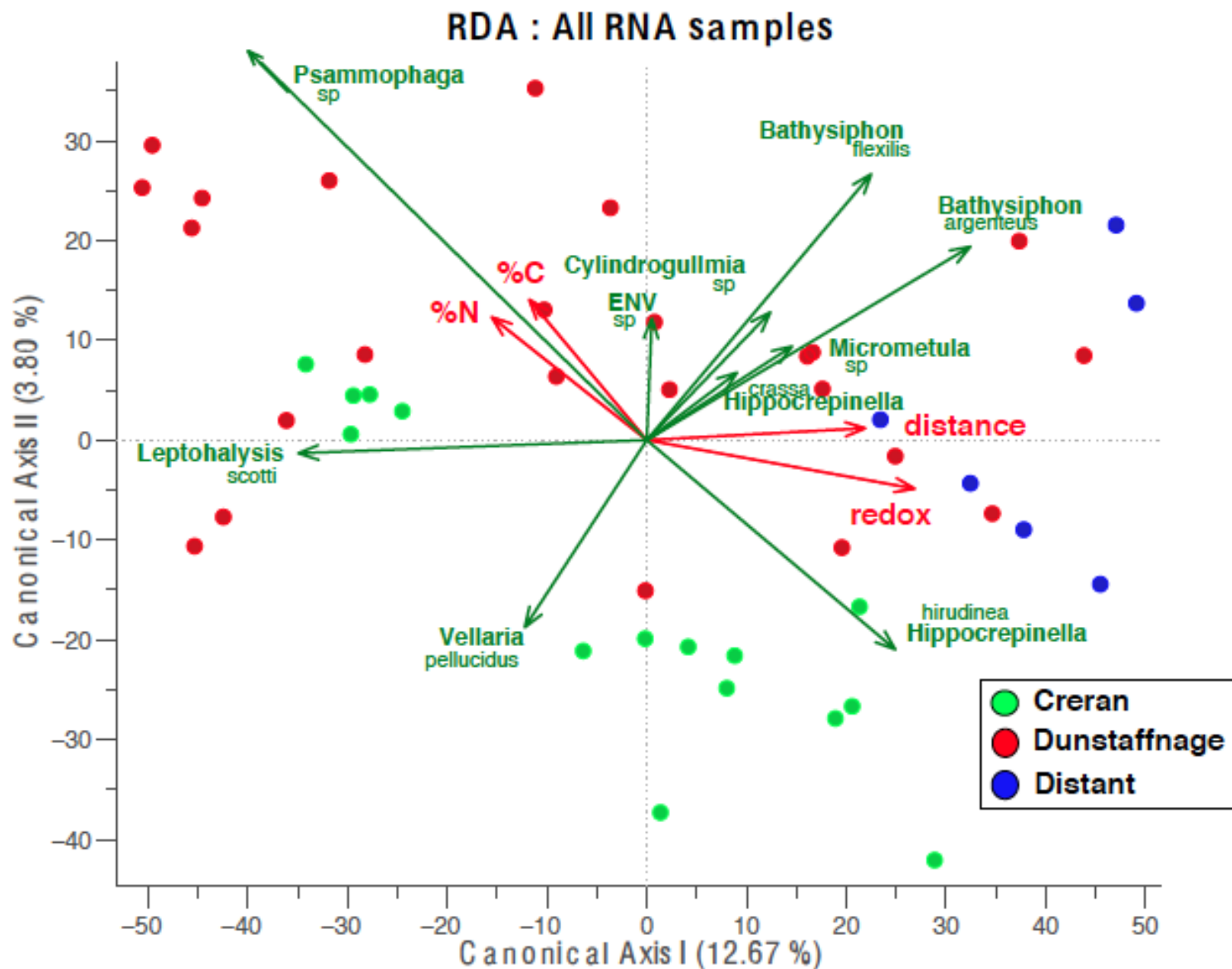


Species richness



Foraminiferal richness increases with distance to cages and with Redox values.

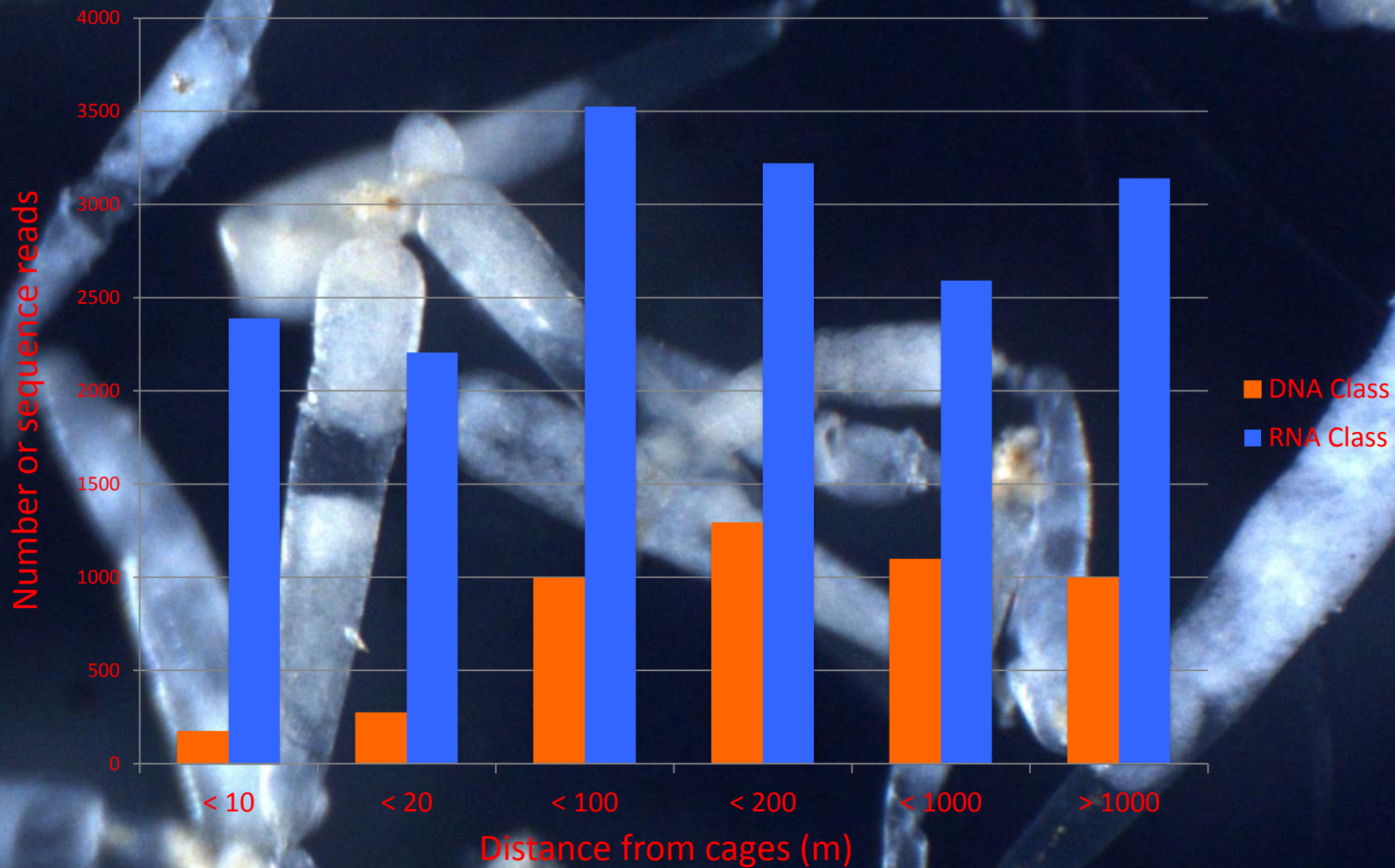
Detection of potential bioindicators



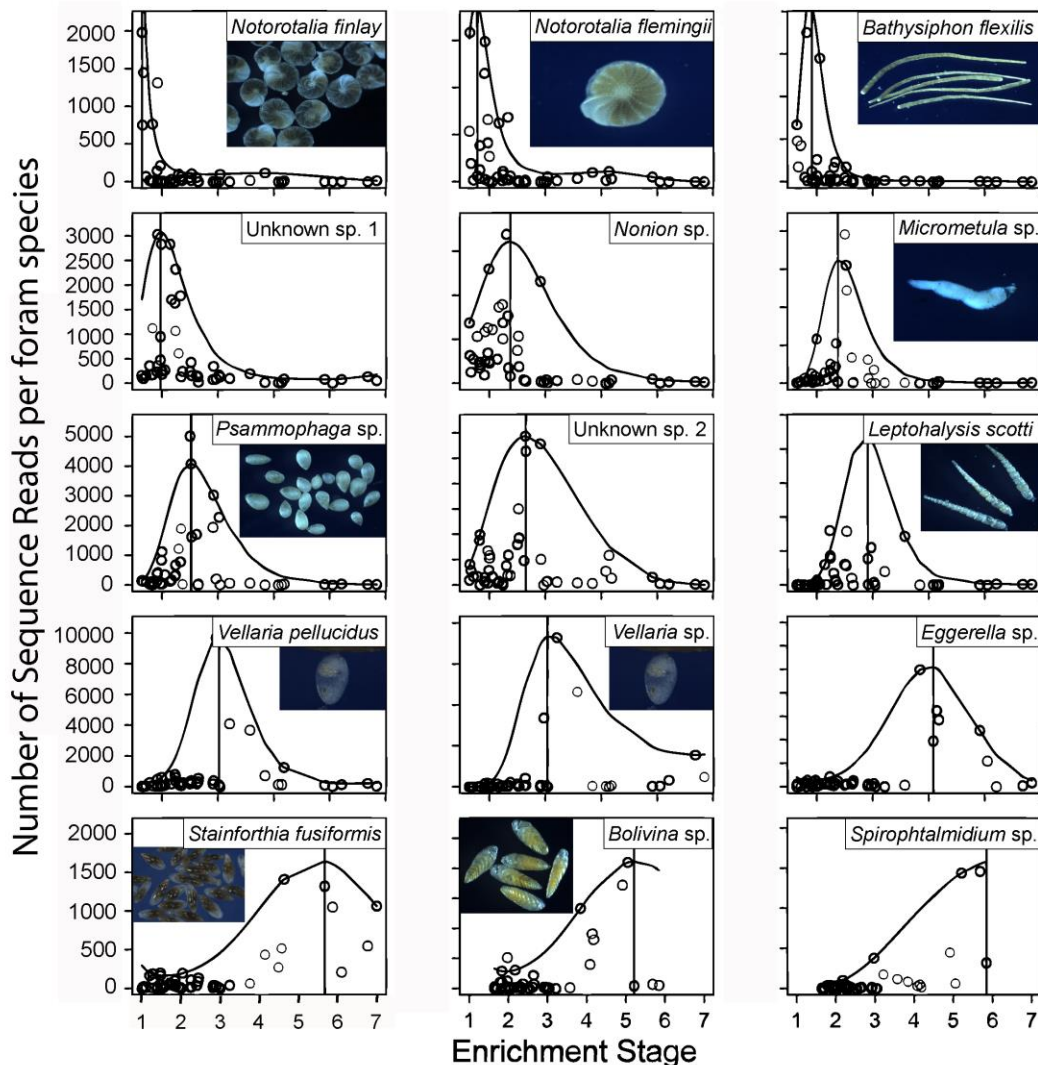
Psammophaga sp.



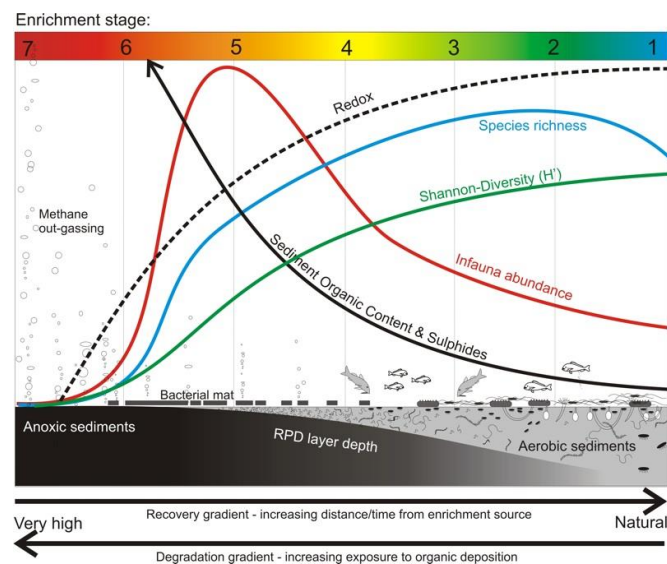
Micrometula sp.



Forams abundance vs enrichment plots



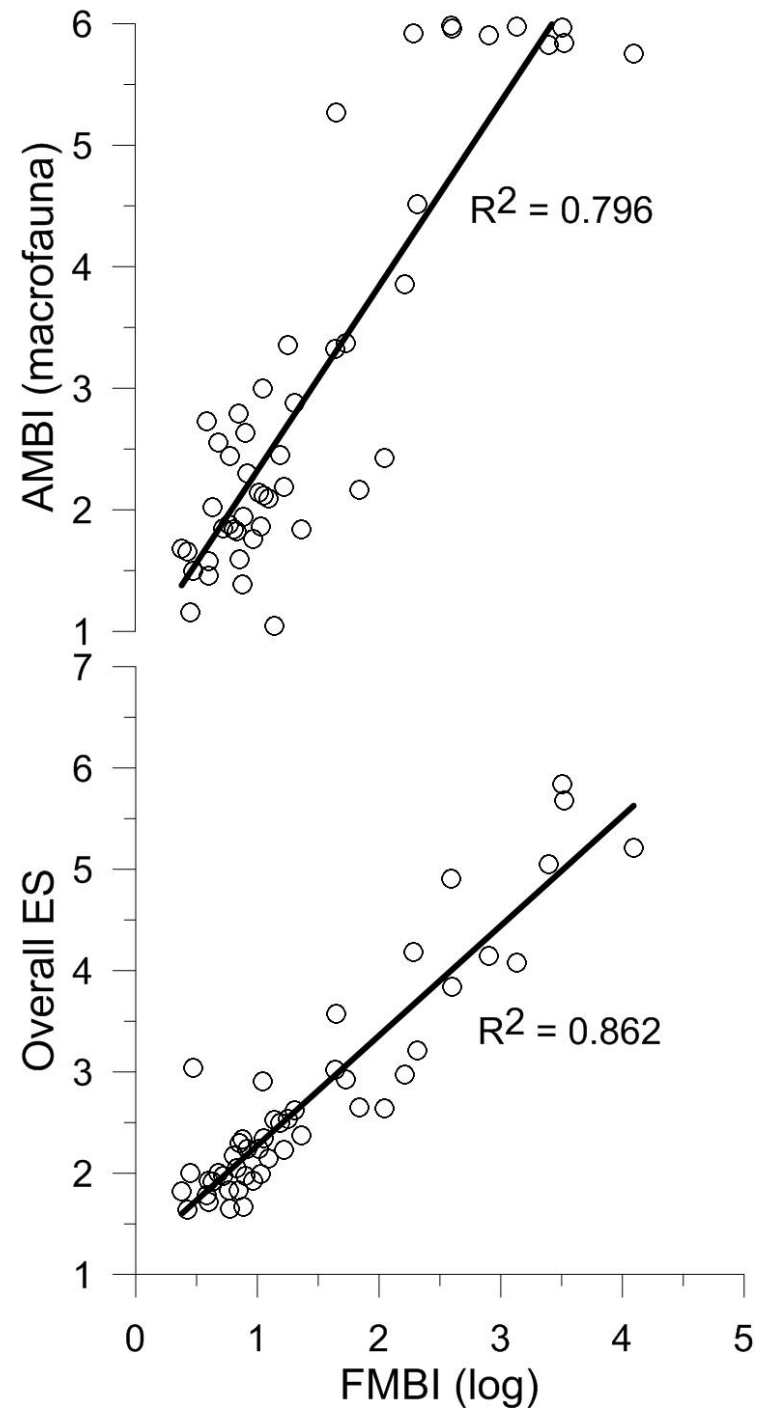
We identified 15 key bioindicator species showing different reads abundance in relation to Enrichment Stage



Forams index vs AMBI and ES

Correlation between
Forams Index
(**FMBI**), macrofauna
index (**AMBI**) and
enrichment stage
index (**ES**)

(Nigel Keeley,
Cawthron)



Summary

- Most of common foraminiferal species identified morphologically were recovered by eDNA/RNA approach.
- The foraminiferal species richness shows correlation to distance to cages and redox values (especially in RNA).
- Some foraminiferal species seem useful as bioindicators of enrichment.

Potential of environmental barcoding

- provides information on the **global diversity**, including inconspicuous, hard to identify species
- **increases the sensitivity** of bioassessment tests
- speeds up the process of species identification, allowing much **greater sample coverage and replication**;
- reduces time and cost of sample processing

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§



**UNIVERSITÉ
DE GENÈVE**

Franck Lejzerowicz
Philippe Esling
Loic Pillet

Tom Wilding



Tomas Cedhagen

Nigel Keeley



Xavier Pochon



Susie Wood





Prof. Jan
Pawlowski

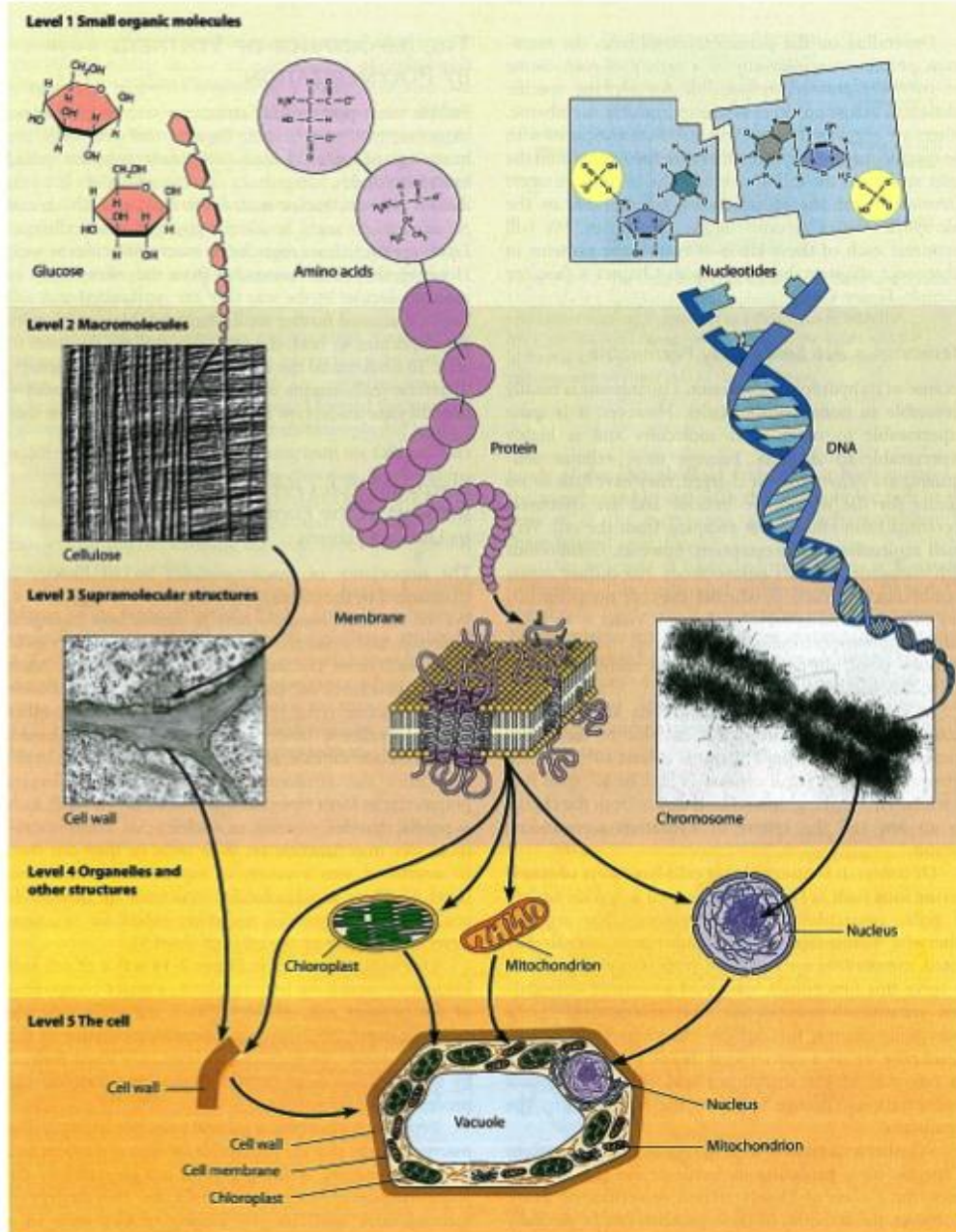


Figure 2-14 The Hierarchical Nature of Cellular Structures and Their Assembly. Small organic molecules (level 1) are synthesized from simple inorganic substances and are polymerized to form macromolecules (level 2). The macromolecules then assemble

into the supramolecular structures (level 3) that make up organelles and other subcellular structures (level 4) and, ultimately, the cell itself (level 5). The supramolecular structures shown as level 3 are more complex in their chemical composition than the figure suggests.

Chromosomes, for example, contain proteins as well as DNA—in about equal amounts, in fact. Similarly, membranes contain not only lipids, but also a variety of proteins; and cell walls contain not just cellulose, but also other carbohydrates and proteins.

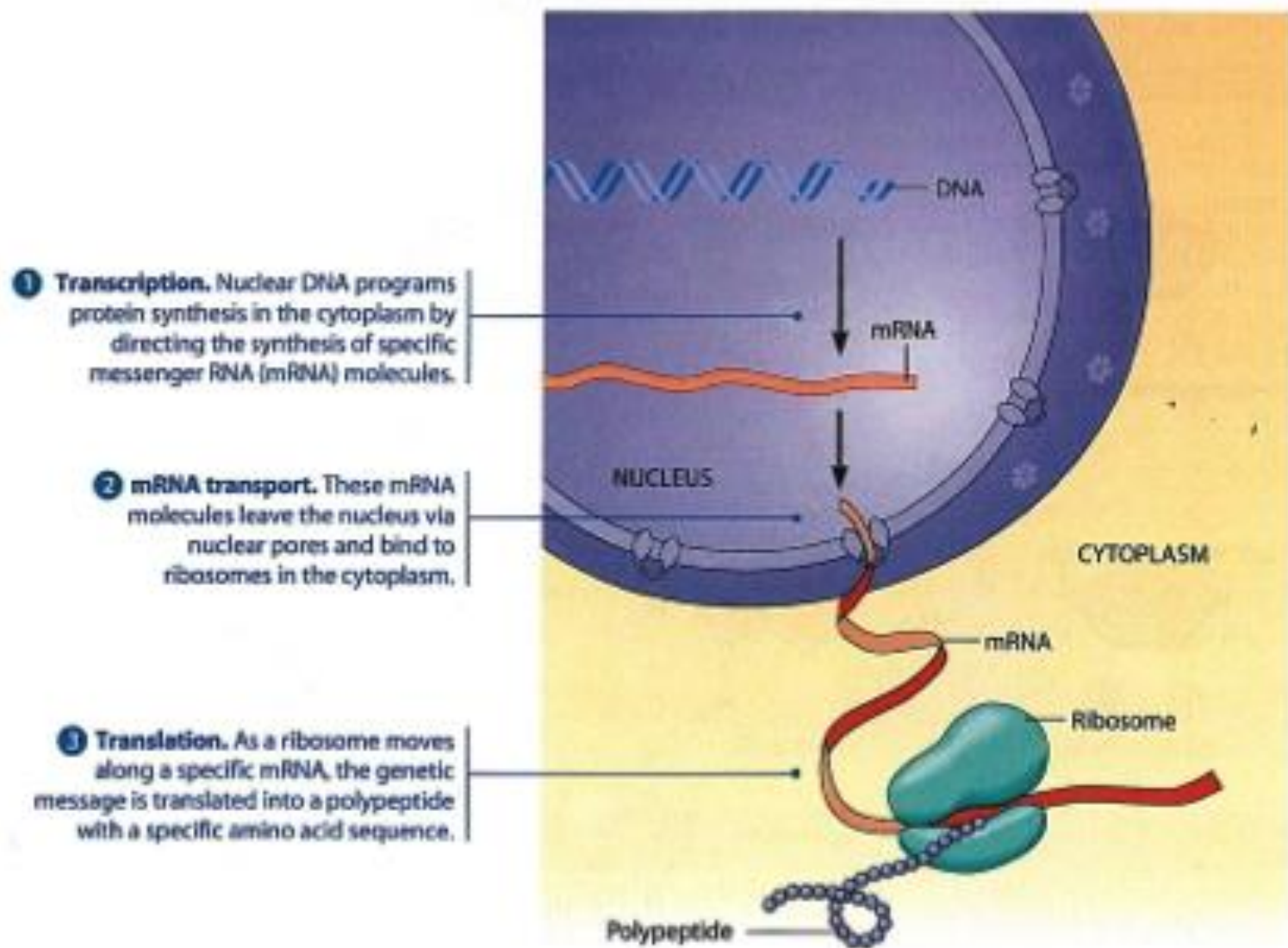


Figure 3-14 Genetic information is Stored in the Nucleotide Sequences of DNA Molecules. In eukaryotes, most of the DNA in a cell is located in the nucleus. This DNA contains instructions for **1** the synthesis of a complementary messenger RNA (mRNA) that then **2** travels to the cytoplasm, where it **3** is used by the ribosome to synthesize a protein.