In situ trophic ecology of benthic marine suspension feeders

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Ifremer

Zones Ateliers
LTSER FRANCE BREST IROISE

Context

- Food is a key factor for recruitment and growth success of wild and cultivated benthic invertebrates and contributes to the structuring of benthic communities

- Importance of food sources (quantity, quality, availability) AND physiological processes related to invertebrate feeding

- Suspension filter feeders have a preference for living phytoplankton and phytobenthos cells, and *a priori* for diatoms

- At lower taxonomic levels, knowledge is more limited, especially *in situ*

  ⇨ This can be partly explained by methods available/classically used to study *in situ* trophic ecology in benthic communities

Methods used *in situ* to study trophic ecology

Direct observation

- Not easy to identify preys due to degradation
- Bias in interpreting results
- Impossible to work on larvae

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Food Webs

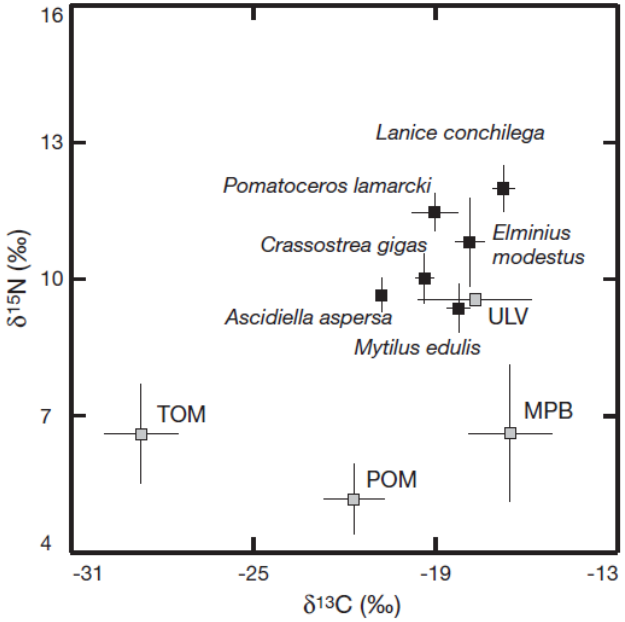
journal homepage: www.journals.elsevier.com/food-webs

There's no harm in having too much: A comprehensive toolbox of methods in trophic ecology

Nabil Majdi [a,b,c,1], Nicolas Hette-Tronquart [a,d,*,1], Etienne Auclair [a,e], Alexandre Bec [a,f], Tiphaine Chouvelon [a,g], Bruno Cognie [a,i], Michael Danger [a,h], Priscilla Decottignies [a,i], Aurélie Dessier [a,j], Christian Desvilettes [a,f], Stanislas Dubois [a,k], Christine Dupuy [a,j], Clémentine Fritsch [a,l], Cédric Gaucherel [a,m], Mickaël Hedde [a,n], Franck Jabot [a,o], Sebastien Lefebvre [a,p,q], Martin P. Marzloff [a,k], Benjamin Pey [c], Nathalie Peyrard [a,e], Thibaut Powolny [a,l], Régis Sabbadin [a,e], Elisa Thébault [a,r], Marie-Elodie Perga [a,s]

Indirect measurement

C and N isotope ratios for different benthic species and food sources



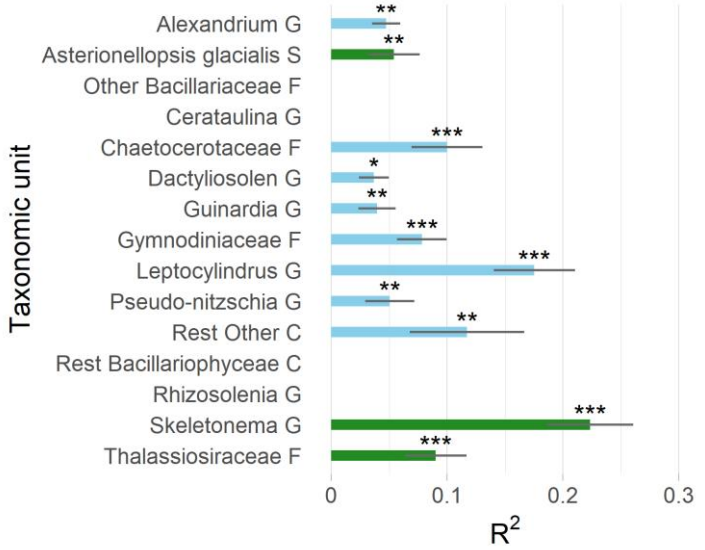
Dubois et al., 2007

e.g. stable isotopes, fatty acids

- Long term assimilation patterns
- Low taxonomic resolution

Statistical analysis, ecophysiological modelling

Correlation between Pacific oyster growth rate and phytoplankton taxonomic units



Gangnery et al., in prep

- Tells nothing about mechanisms

Remaining questions

- How trophic resource is used (sharing, competition) by benthic communities at a small spatial scale?
- Which preys are key for which predators? Why?
- How these preys vary over the long term?
- What consequences might the rarefaction/proliferation of some preys or their phenological modification have in the current context of climate change and the erosion of biodiversity?

A promising method: DNA tracing with metabarcoding

- Ability to detect DNA from degraded prey
- Better taxonomic resolution
- Short-term feeding patterns
- Continuous improvement of next-generation sequencing techniques leading to lower costs

MOLECULAR ECOLOGY
Molecular Ecology (2012) 21, 1931–1950 doi: 10.1111/j.1365-294X.2011.05403.x

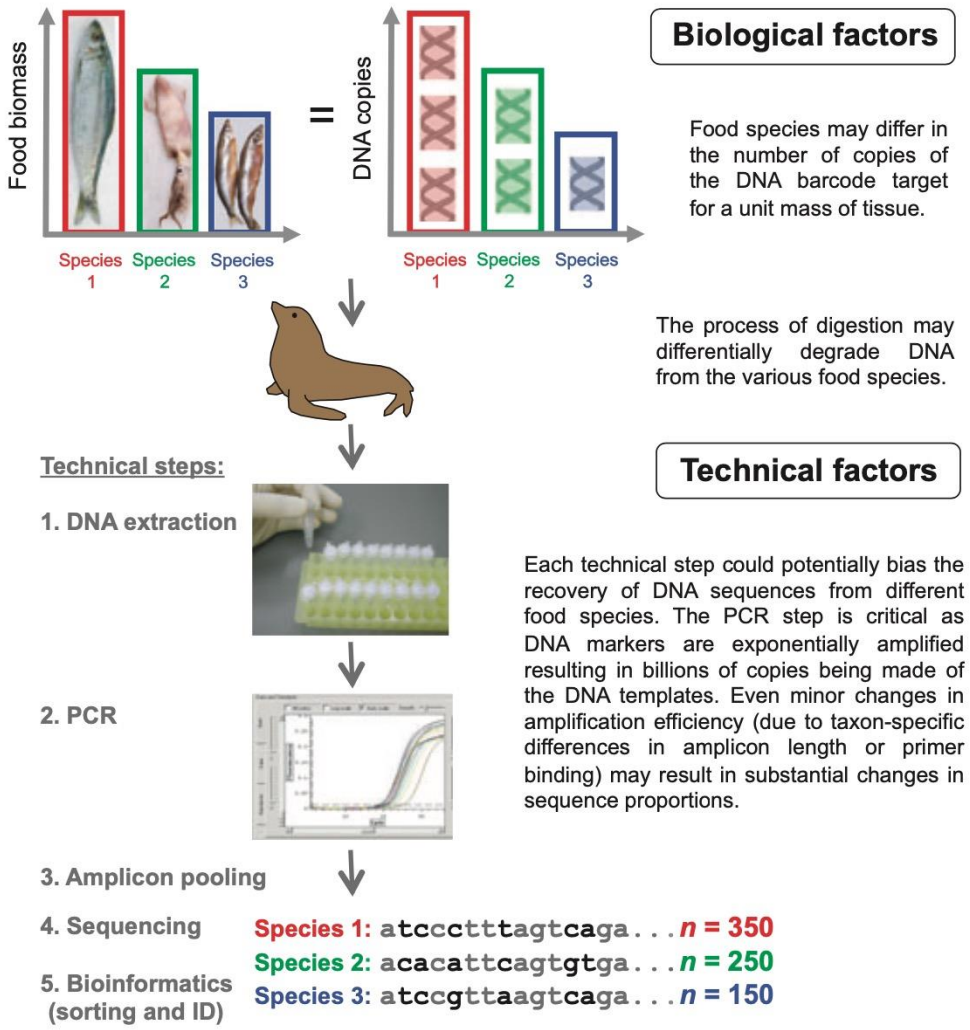
INVITED REVIEW

Who is eating what: diet assessment using next generation sequencing

FRANCOIS POMPANON,*[1] BRUCE E. DEAGLE,†‡[1] WILLIAM O. C. SYMONDSON,§[1] DAVID S. BROWN,§ SIMON N. JARMAN† and PIERRE TABERLET*

- Several possible biases at different stages
- Crucial technical choices

- A growing literature over the past 2 decades
- Only 10 papers on suspension feeders (to our knowledge):
  - 2 on species from deep or freshwater ecosystems
  - 3 on larvae of coastal species
  - 5 on adults of coastal species



A one shot experimental set up: objectives

Use of trophic resource by a community of suspension-feeders associated with flat oyster reef structures and sharing a similar trophic niche

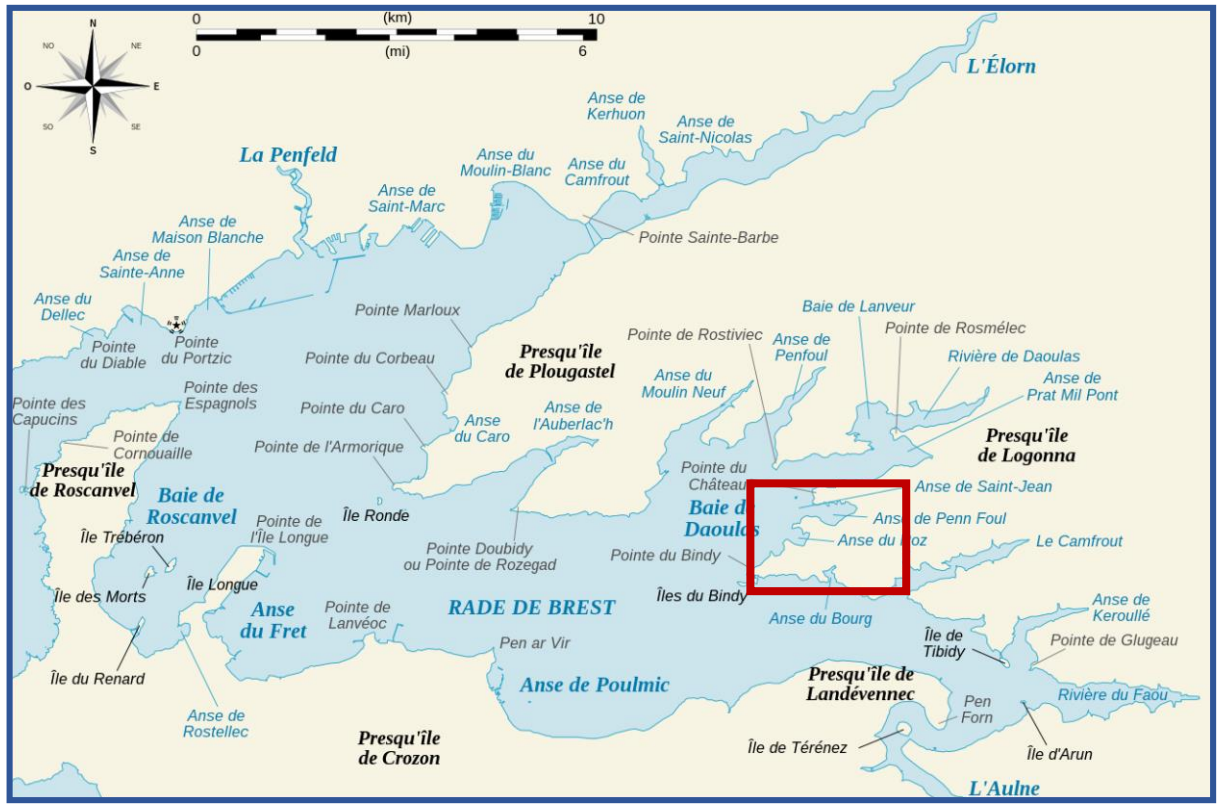
1. **Solve technical aspects**

   - Quality of DNA extraction and amplification from different host matrices (digestive tissues, feces)
   - Choice of molecular markers
   - Choice of methods for blocking host DNA replication

2. **Investigate trophic ecology and food sharing**

   - Which taxa are ingested?
   - Which taxa are not/poorly assimilated?
   - With which taxonomic resolution preys are identified?
   - Can sequencing data be used semi-quantitatively by comparing the contents of surrounding water and host matrices?

Experimental set up: benthic species



10 natural flat oyster aggregates and associated fauna collected in 'Baie de Daoulas', 'Rade de Brest', Brittany on 27 June 2023

Flat oyster, *Ostrea edulis*

Variegated scallop, *Mimachlamys varia*

Red tubeworm, *Serpula vermicularis*

Long clawed porcelain crab, *Pisidia longicornis*

Experimental set up: benthic species

Flat oyster
Ostrea edulis

Variegated scallop
Mimachlamys varia

Red tubeworm
Serpula vermicularis

Long clawed porcelain crab
Pisidia longicornis

↘ Individual size, density and biomass
↘ Complexity of feeding process

Experimental set up: analysed matrices

Digestive tissues
(ingested food)


n=5 ; digestive gland + gonad

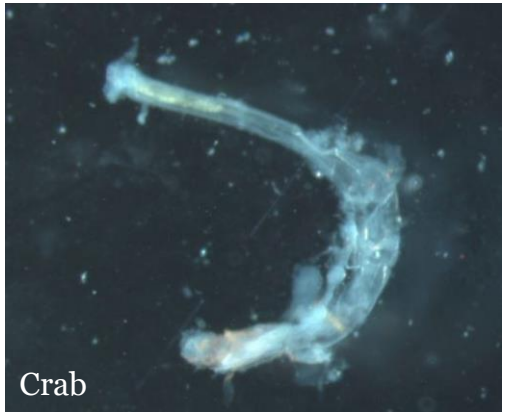

n=5 ; digestive gland


n=5 ; portion of tissue between the plume and the beginning of the intestine


n=4 ; stomach content, under binocular


Oyster

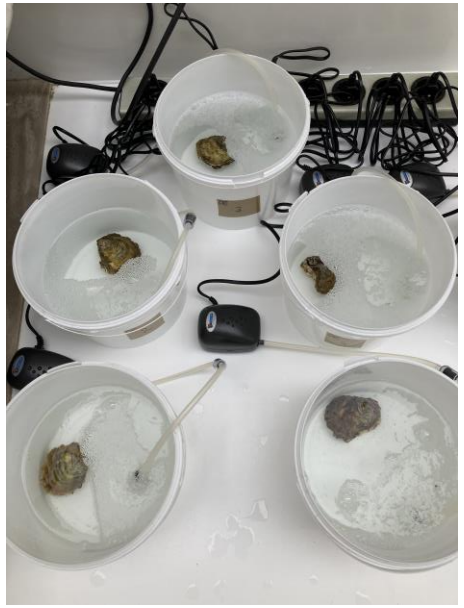

Scallop


Crab


Worm

Feces
(± assimilated food)

n=5 per species



1 individual per aquarium filled with seawater pre-filtered to 1 µm and UV-treated

Collection after ≈ 18-40 h

Experimental set up: food sources

- Simultaneously with suspension feeder aggregates, duplicates of surrounding water was sampled (± 2h HT)

- Assumption: digestion times are short enough for this water sampling to be a good proxy for available food

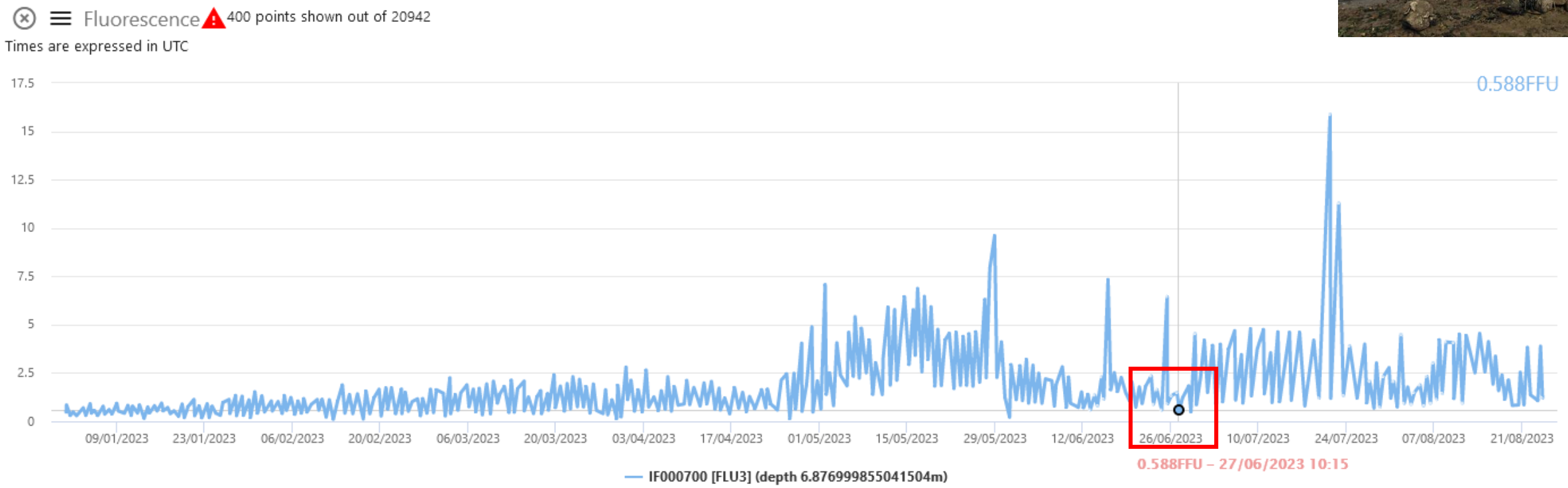
- Date of experiment: avoid dominance of any one taxon (not during bloom period), relatively diverse flora (diatoms, dinoflagellates), average abundance.

  ⇨ retrospective analysis of a temporal series of flora observed at a nearby site (Pointe du Château) over the period 2009-2022

- Targeted food sources: phytoplankton and phytobenthos
  - ❖ Light microscopy analysis: microplankton (> 20 µm or < 20 µm but forming chains)
  - ❖ DNA analysis using 2 different protocols:
    - 3 size classes: 0.2-3 µm [pico-]; 3-20 µm [nano-]; > 20 µm [micro]
    - 1 size class: > 0.22 µm

Characterization of food sources

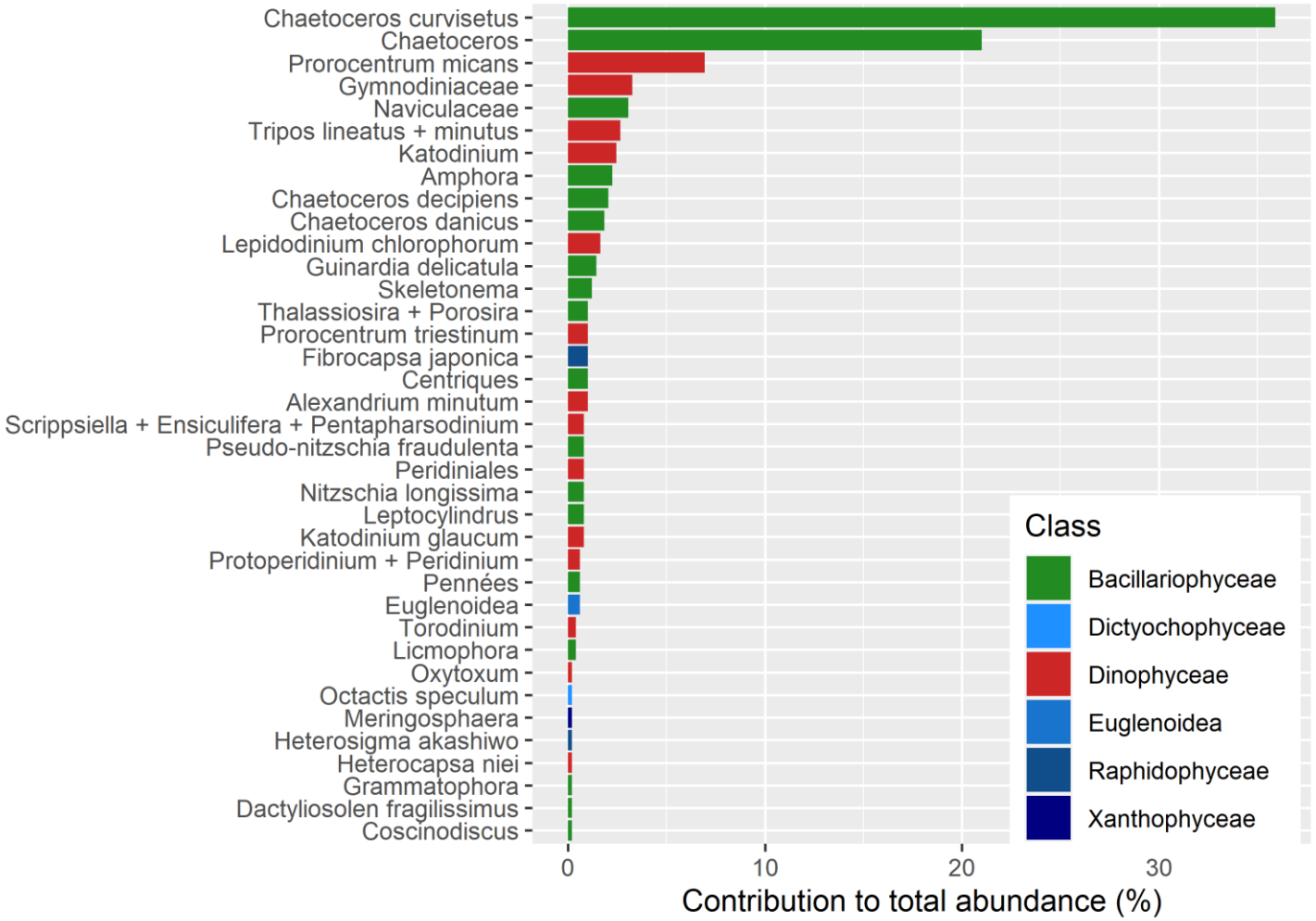
Fluorescence measured on 06/27/2023



Probe SNO Coast HF - Smart Daoulas
https://data.coriolis-cotier.org/

Characterization of food sources

Composition of the micro-phytoplankton flora on 27/06/2023



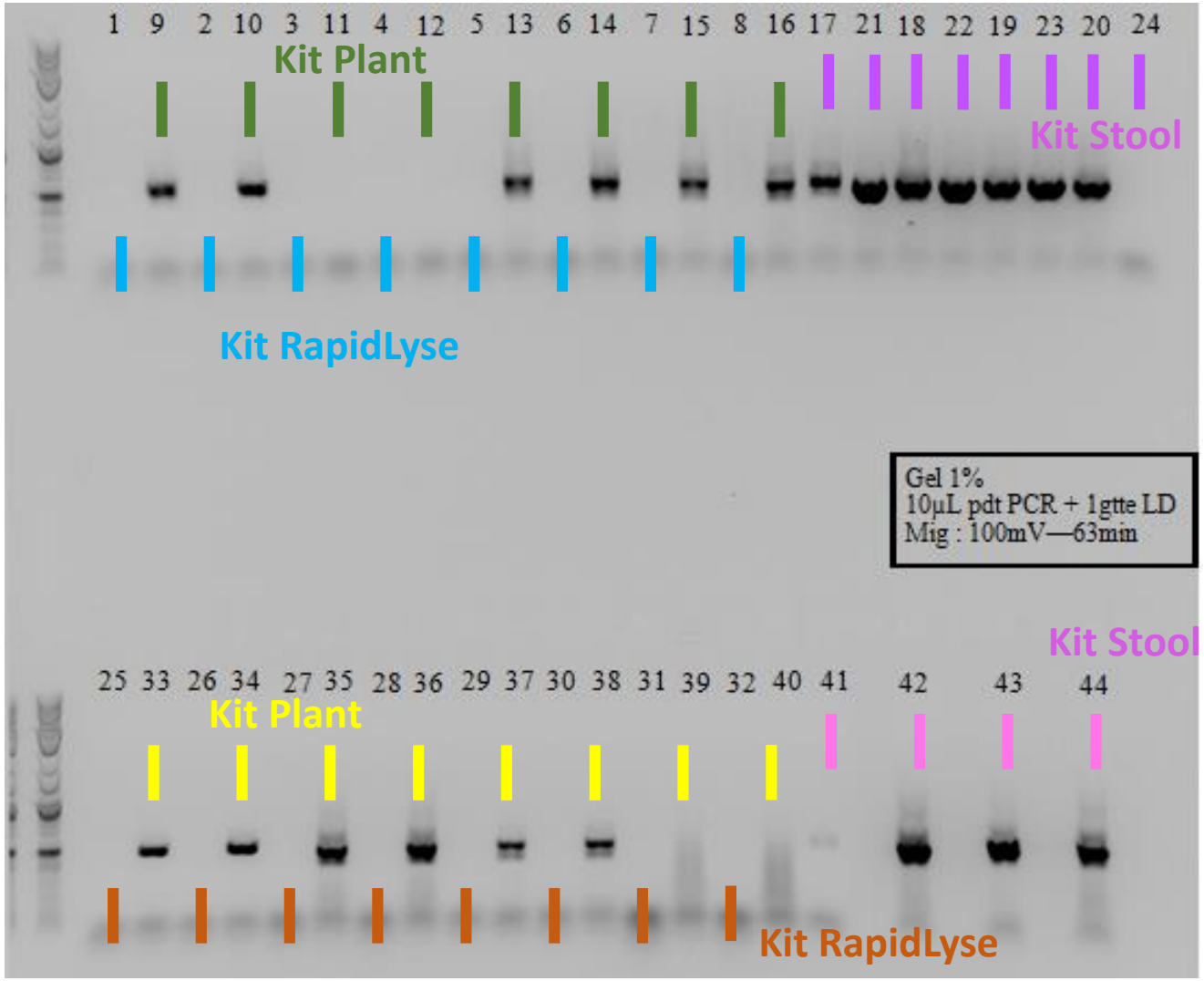
- **37 taxa**
- **Total abundance** = 49 000 cells.l^{-1}
  75% diatoms / 22% dinoflagellates
- **Dominance**
  Berger-Parker = 0,36 (*Chaetoceros curvisetus*)
  0,6 (*Chaetoceros* genus)
- **Richness** = 31 taxa identified at genus level
  15 diatoms / 12 dinoflagellates
- Forage taxa / harmful taxa

Optimization of DNA extraction (amplification on 23S gene)

Preliminary test on 17/04/2023

FECES

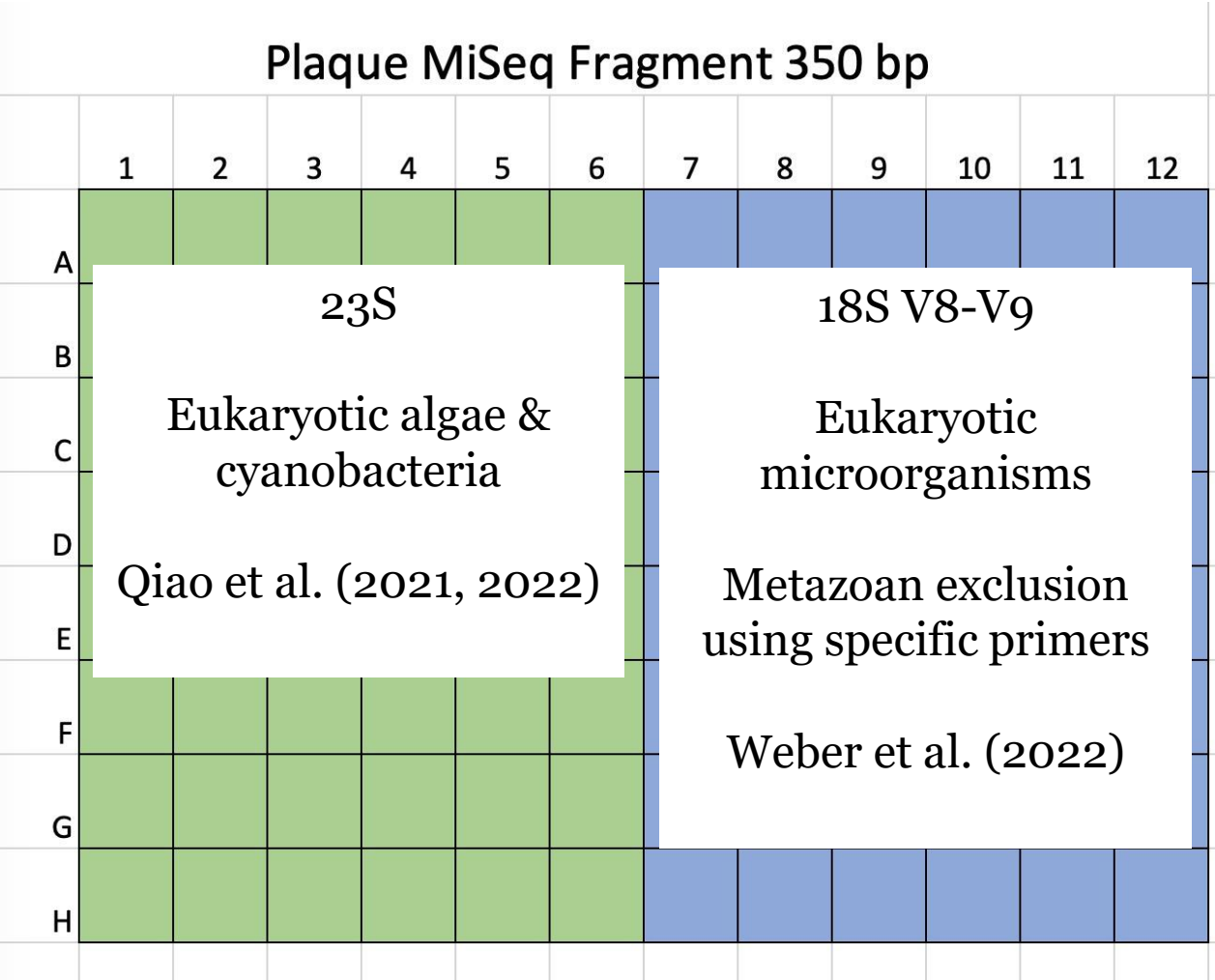
DIGESTIVE TISSUES

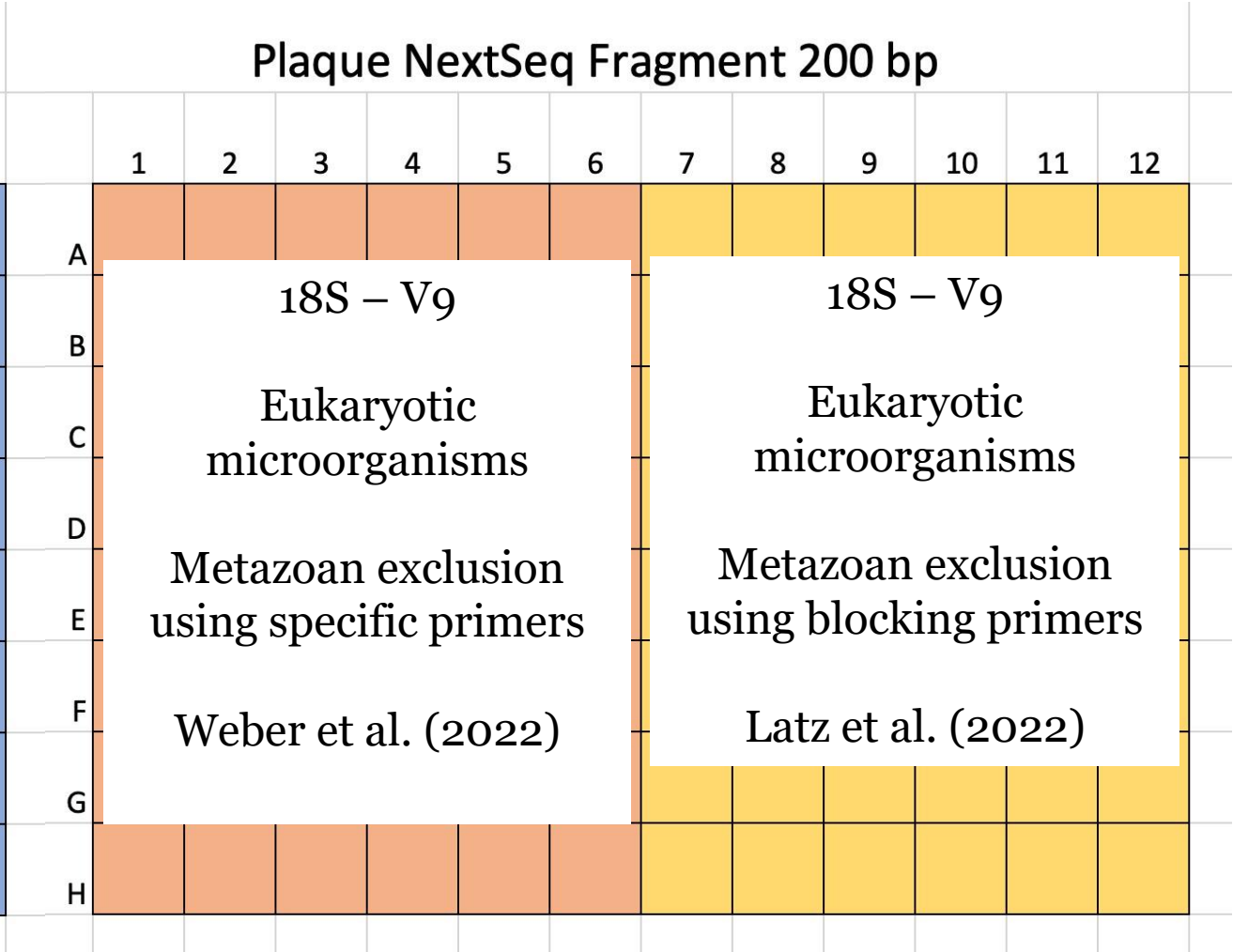


- NucleoSpin Rapid Lyse
  Proteinase K
- NucleoSpin Plant II
  CTAB
- Nucleospin Stool
  Glass bead milling plus enzymatic lysis

Sequencing strategy (work in progress)

1. Long vs. short fragment
2. 2 different target genes
3. 2 methods for blocking host DNA replication





Sequencing strategy (work in progress)

23S

Chloroplasts
Only plants are amplified

18S

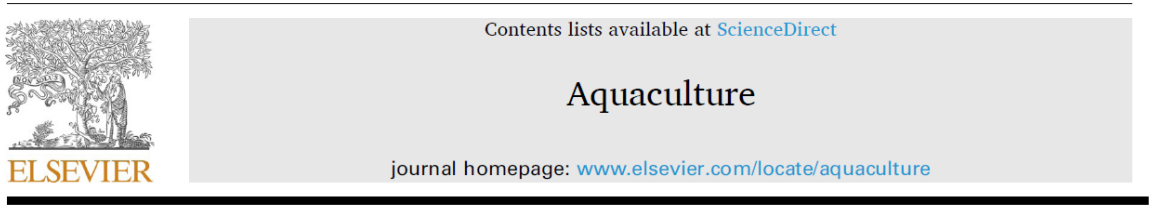
Weber 2022 (V8-V9)
Primers exclude metazoans

Weber 2022 (V9)
Primers exclude metazoans

Latz 2022 (V9)
Universal primers
+
"blocking primers" host-specific

Perspectives

- This is a work in progress: sequencing will be completed by the end of 2023, data will be analyzed in the first half of 2024 (bioinformatics and ecological aspects)
- The ambition is to set up a larger-scale project aimed at answering the questions identified at the beginning of this presentation
- And today: the objective is to examine possible future interaction or even collaboration with Japanese and other French colleagues interested in this thematic/method combo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Aquaculture

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

Factors driving the settlement of Pacific oyster *Crassostrea gigas* larvae in Hiroshima Bay, Japan

Tadashi Matsubara [a,*], Mineo Yamaguchi [a], Kazuo Abe [a], Goh Onitsuka [a], Katsuyuki Abo [a], Tomohiro Okamura [a], Takafumi Sato [b], Ken-ichiro Mizuno [c], Franck Lagarde [d], Masami Hamaguchi [a,e]