New protocol for the metagenomic analysis of the eukaryotic species included in the diet of *Mytillus galloprovincialis* mussels

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Background

- Mussel aquaculture is one of the most important industry in EU and Spain is the major producer. The Mediterranean mussel (*Mytilus galloprovincialis*) is the main cultivated species and 95% of its production takes place in Galicia (North-West corner of Spain), where mussel products from this area has a Protected Designation of Origin (POD) since 2008, certifying quality and traceability according to the EU seafood policy standards.
- To meet the EU traceability standards, besides a correct identification of species, the correct certification of the product origin is essential, more in the case of products with POD certification as *M. galloprovincialis*.
- Microbiota composition or the study of diet content throughout next-generation sequencing has been proposed as a good tool for tracing geographical origin. However, to apply this tool is essential to have a good protocol to amplify the target organisms avoiding the host DNA.

Objective

Here we tested different DNA extraction protocols from digestive gland of *M. galloprovincialis* and designed new primers to get a new protocol for the metagenomic analysis of the eukaryotic species included in the diet of *M. galloprovincialis* mussels that optimize the amplification and sequencing of eukaryotic organisms avoiding mussel DNA.

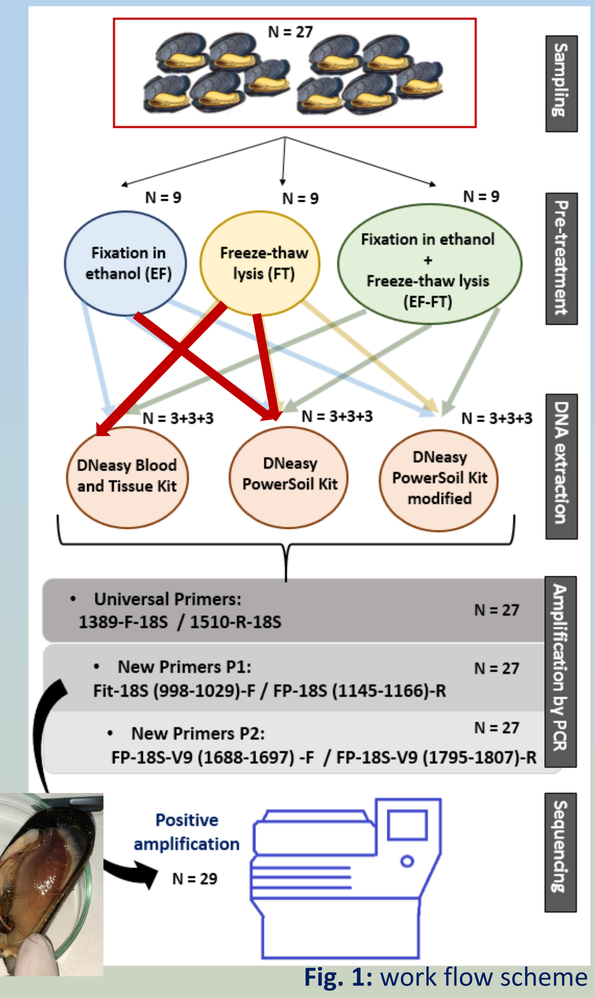


Fig. 1: work flow scheme

Main results

- Only three DNA extraction protocols (red Fig.1) resulted in enough DNA and good amplification to continue with the analysis. **Power soil kit showed the best results** after sequencing combined with FT pre-treatment.

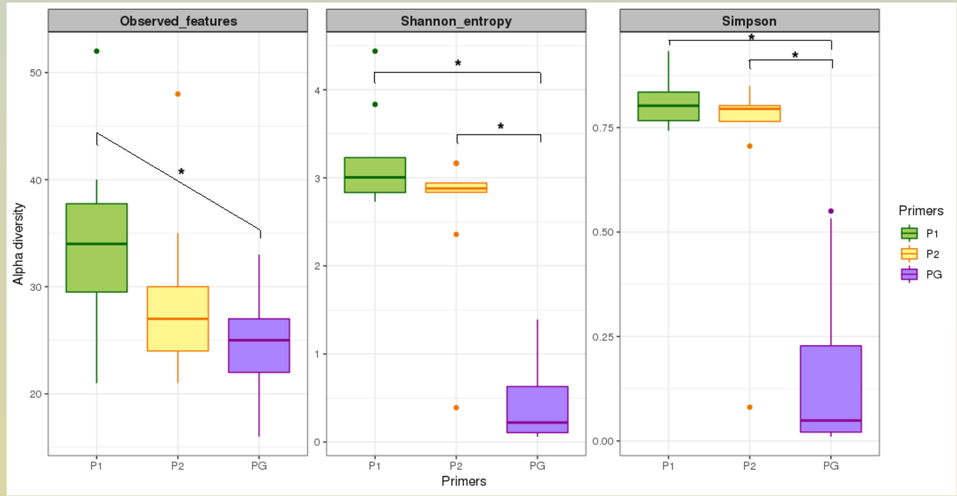
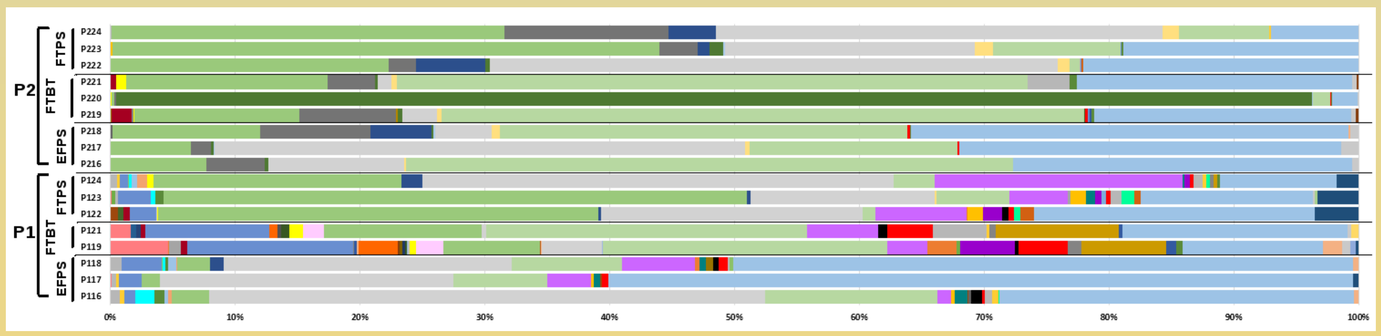


Fig. 2: (Left) Bar plot of the relative abundances of taxa amplified with the three primer sets: (PG) universal primers, (P1) new P1 primers, (P2) new P2 primers. **(Right)** Alpha diversity of the samples represented by primer set. The significant comparisons are marked with an asterisk.

- Significant reduction of Mytiloida amplification (black) and higher alpha diversity when we use new primer sets.



Higher abundance in P1

- Chlorodendrales (Chlorophyta)
- Mamiellophyceae (Chlorophyta)
- Chlorophyta
- Alveolata (SAR)
- Thraustochytriaceae (SAR-Stramenopiles)
- Thecofilosea (SAR-Cercozoa)
- NovelClade2 (SAR-Cercozoa)
- Ebriacea (SAR-Cercozoa)
- Cercozoa (SAR)
- Stramenopiles (SAR)
- MAST1 (SAR-Stramenopiles)
- Pirellulaceae (Bacteria)

Higher abundance in P2

- Gymnodiniphycidae (SAR-Dinoflagellata)
- Gymnodinium clade (SAR-Dinoflagellata)
- Thoracosphaeraceae (SAR-Dinoflagellata)

Fig. 3: (Left) Bar plot of the relative abundances of taxa, amplified with the new primer sets. The samples are ordered by DNA extraction protocol within each couple of primers used. **(Right)** Significant taxa in Kruskal-Wallis test.

- Higher differences between primer sets than DNA extraction protocol. The eukaryotic community recovered with primer sets P1 and P2 was weakly different, with P1 primer set showing an enrichment of some organisms.

CONCLUSIONS

The **FTPS protocol recovers efficiently the DNA** of the eukaryotic organisms present in the digestive gland of mussel.

New primer sets avoid significantly the amplification of the mussel DNA, being more efficient to amplify the eukaryotic organisms preset in the digestive gland than the universal primer set.

The complete protocol is adequate to be applied in metagenetic studies to evaluate the potential of eukaryotic diet as traceability tool **to determinate the geographical origin** of *M. galloprovincialis*.

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