Detecting two marine non-indigenous species in the Bay of Biscay threatening biodiversity and shellfish farming from the French coast using eDNA and molecular approaches

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Fig.1: *Rapana venosa* with oothecas on shell (©Ifremer-Pépin)

1 - Background

Recently, two marine **non-indigenous species (NIS)** were reported in Bay of Biscay on the Atlantic coast of France that could threaten shellfish farming and biodiversity locally (Fig.3).

-First is the veined rapa whelk ***Rapana venosa*** (Valenciennes, 1846) (Fig.1), a big predatory gastropod. Since one individual was reported in the Pertuis Breton area (Ré Island) in 2019, there have been dozens of reports in the Pertuis Charentais Sounds near the city of **La Rochelle**.

-Second NIS is a newly described Polycladida flatworm, ***Idiostylochus tortuosus*** *gen. nov., sp. nov.* (Gutiérrez et al., 2023, Fig.2), already observed for two years in **Arcachon Bay**. High abundances of this flatworm may have been associated with mussel mortalities. This predatory species was found feeding on farmed oysters and mussels in Arcachon Bay.

These benthic NIS are only visible during visits to the foreshore (intertidal, flatworm) or through accidental fishing in nets (subtidal, Rapana).

- In response to the concerns of fishermen and shellfish farmers, we initiated a scientific project -**RAPSODI**-

2 - Question is: Can we detect the DNA of these species in seawater samples?

-The objectives of RAPSODI project are (1) to raise awareness among local maritime stakeholders about these NIS, (2) **develop and validate protocols to detect these two NIS with molecular tools from environmental DNA (eDNA) samples**, and (3) **analyze samples collected *in situ***.

-At this time, there is no species-specific qPCR for the two targeted NIS. The joint use of eDNA and targeted quantitative PCR (qPCR) will help assess the spatio-temporal distribution of these NIS.

-Here, we present the protocol for the **development of two molecular tools** designed for future monitoring of the two NIS, based on **seawater eDNA**: one qPCR to detect ***Rapana venosa*** COI mtDNA, and one qPCR to detect ***Idiostylochus tortuosus*** 16S mtDNA.

-Better estimating the dynamics of these NIS is a prerequisite for preparing management measures.

Fig.2: Dorsal view of the flatworm *Idiostylochus tortuosus, 3 cm long* (©CAPENA-Vieira.J)

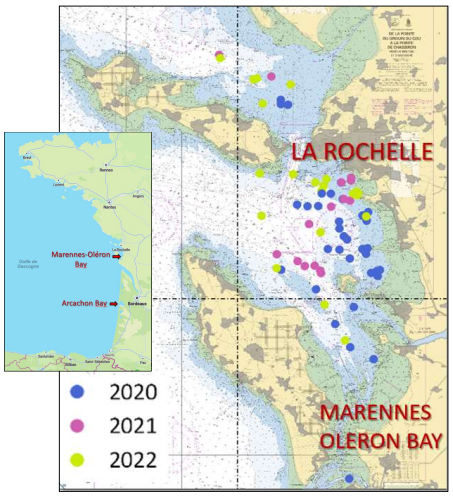


Fig.3: Map of main shellfish farming area impacted by the two NIS. Mapping of invasive *Rapana* reported by fishermen, according to the years (source CDPMEM-17-Roche). For each spot 1 to 10 Rapanas are observed

3 - Molecular tools : Design of 2 qPCR assays to detect the two targeted NIS DNA of *Rapana* or Flatworm *I. tortuosus* from seawater eDNA samples

Novel qPCR assays based on specific TaqMan-LNA probes

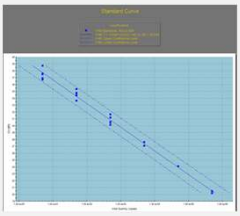
-**RAPCOI- qPCR assay** targeting mtDNA partial sequence of the *Rapana venosa* **Cytochrome Oxidase 1** gene (FAM/BHQ1 Probe, **116** bp amplicon size)

-**ITO16S- qPCR assay** targeting mtDNA partial sequence of the *Idiostylochus tortuosus* **16S gene** (HEX/BHQ1 Probe, **120** bp amplicon size).

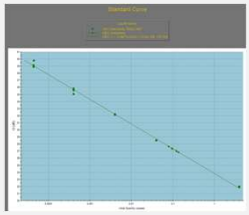
-**Both qPCR assays are highly specific** (multi bases LNA Probes, cross-testing with around 10 other species of marine molluscs and worms gave No CT value)

-**Both qPCR assays have a good Efficiency (>90%)**

-**Both qPCR assays were experimentally tested in Duplex PCR system with success, allowing detection of the two NIS in only one reaction**



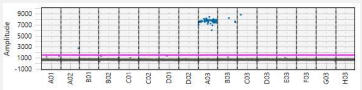
RAPCOI- qPCR assay



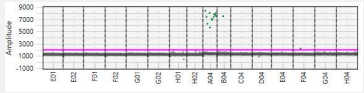
ITO16S- qPCR assay

-**Both qPCR assays developed were tested using BIORAD® QX200 Droplet digital PCR system (ddPCR) with success**

RAPCOI ddPCR assay



ITO16S ddPCR assay



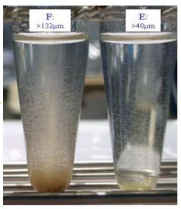
-Thanks to **digital-PCR** and "**absolute quantification experiments**" of NIS reference DNA samples for both probes sets, we were able to determine that the detection limit of our molecular tools is between **5 and 50 DNA copies per reaction**

Molecular tools : Seawater eDNA samples preparation

4 stations, 2 years: 2021+2022, March->Sept. Weekly sampled, 150 Seawater pellets samples

1500L filtered >40µm, Pellet collected in 50mL pillbox

50mL seawater pellet (-20°C)



Filtration of the pellet on 0.45µm

DNA extraction from filter using Qiagen Power Water Kit = **150 eDNA samples set**

4 - Results of qPCR tests to detect Rapana DNA from seawater eDNA samples

-150 eDNA samples were tested using the RAPCOI qPCR assay, 140 sub-surface sampling from oyster and mussel larval monitoring campaigns of **CAPENA**-Center for Aquaculture, Fisheries and the Environment of Nouvelle Aquitaine- and 10 bottom samples from fish nursery monitoring in Bay of Biscay of Ifremer "**NURSE**" campaign.

-Only four eDNA samples displayed Rapana DNA traces (3 from 2021, 1 from 2022), all other samples had No CT value.

-Probably, very few DNA traces of *Rapana* are present in the seawater of Marenne-Oléron Bay at sampling time and sub-surface seawater sampling seems poorly adapted compared to a bottom sampling near Rapana benthic habitat's.

-Concerning qPCR flatworm DNA detection, work is on progress.

5 - Conclusion

-**The present work demonstrates the efficiency of protocols associated with the molecular tools developed to detect the DNA of two marine NIS from seawater eDNA for future monitoring** of the gastropod, ***Rapana venosa*** and the Polyclad flatworm, ***Idiostylochus tortuosus***.

-The joint use of eDNA and targeted quantitative PCR (qPCR) will help assess spatio-temporal distribution of these two NIS.

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