eDNA based approach to investigate *Marteilia refringens* and *Bonamia ostreae* lifecycle

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Objective

- □ Study the distribution of *M. refringens* and *B. ostreae* in **sediment**, **seawater**, **plankton** and **benthic fauna** regarding their development in **flat oysters**
- Use previously developed eDNA approaches in a field study
- Take the opportunity of a natural site infected by both parasites and associated with long-term context data
- Analyze results regarding environmental data

eDNA based approach to investigate Marteilia refringens and Bonamia ostreae lifecycle



Method



Seasonal sampling of 5 environmental compartments (Rade of Brest, Brittany, France)

- Sediment
- Seawater < 20 μm
- Plankton > 20 μm
- Benthic fauna
- Bivalves (including flat oysters)

DNA extraction using dedicated extraction kits

Real-time PCR analysis

- TaqMan [®] multiplex Bonamia sp. / Marteilia refringens real-time PCR

Results

- *Quantitative* \rightarrow Seawater < 20 μ m, sediment
- *Qualitative* : Positive (Ct <= 37) / Negative (Ct < 37) \rightarrow other compartments



VAD

Results : Parasites DNA detection in flat oysters





Results : DNA detection of *M. refringens* in other compartments



DNA detection of *M. refringens* in seawater < 20 µm (threshold = 1000 parasites per filter)





18 17-16 15-14 13e 12ē 11-Number of detected parasites a 10-< 10 **5** 9from 10 to 50 8-> 50 7-6-5-4-3-2jul-18 iul-19 apr-18 oct-18 ian-19 apr-19 Campaign

- DNA detection of *M. refringens* in environmental compartments globally follows its development in flat oyster

- January 2019 : DNA is no longer detected in scallops, plankton and seawater contrary to sediment where DNA detection is maximal





Conclusions





Perspectives

- 2 last campaigns to analyze (Oct. 2019) and to perform (Jan. 2020)
- Compare 2018-2019 vs 2019-2020 results
- Analyze global dataset regarding
 - Other monitoring data of parasites prevalence in flat oysters
 - Environmental context (temperature, salinity, chlorophyll a)
- Complete results for positive detections of *M. refringens* :
 - RNA analyzes (seawater and sediment): characterize parasite status
 - In Situ Hybridization (plankton, benthic metazoans): localize the parasite



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EURL for Molluscs Diseases



