FLOW CYTOMETRY TO MEASURE IMPACT OF TEMPERATURE AND SALINITY ON THE SURVIVAL OF *Bonamia ostreae*, PARASITE INFECTING FLAT OYSTER *Ostrea edulis*, IN SEAWATER

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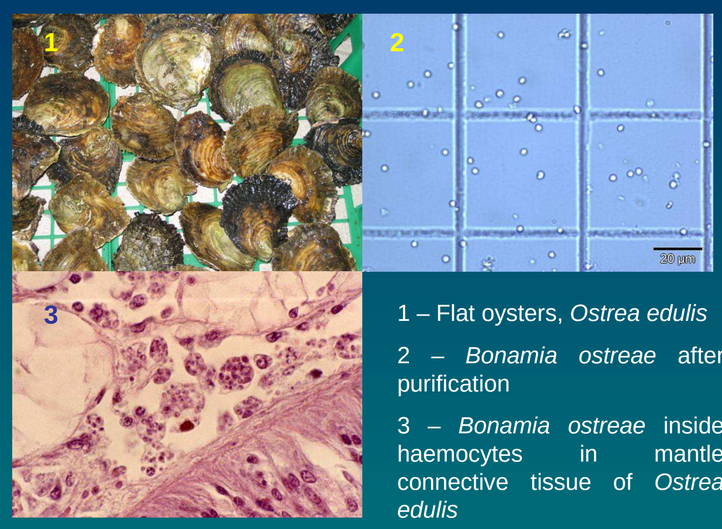
Introduction

The protozoan *Bonamia ostreae* (Fig 3) is a lethal parasite infecting flat oysters *Ostrea edulis* (Fig 1). It has been described in different ecosystems from estuaries to open sea and is presently reported in Europe, North America and Morocco. The life cycle is unknown but the disease can be transmitted directly by cohabitation. No clear correlations could be demonstrated between development of the disease and environmental parameters including temperature and salinity. However, previous works suggested an impact of the temperature on the parasite and / or on the defence capacity of oysters.
The parasite is not cultivable but it is possible to purify *Bonamia ostreae* from highly infected oysters. In this context, the objective of the present study was to test the survival of purified *Bonamia ostreae* in different sea water media (artificial, natural and underground salty sea water) according to temperature and salinity.

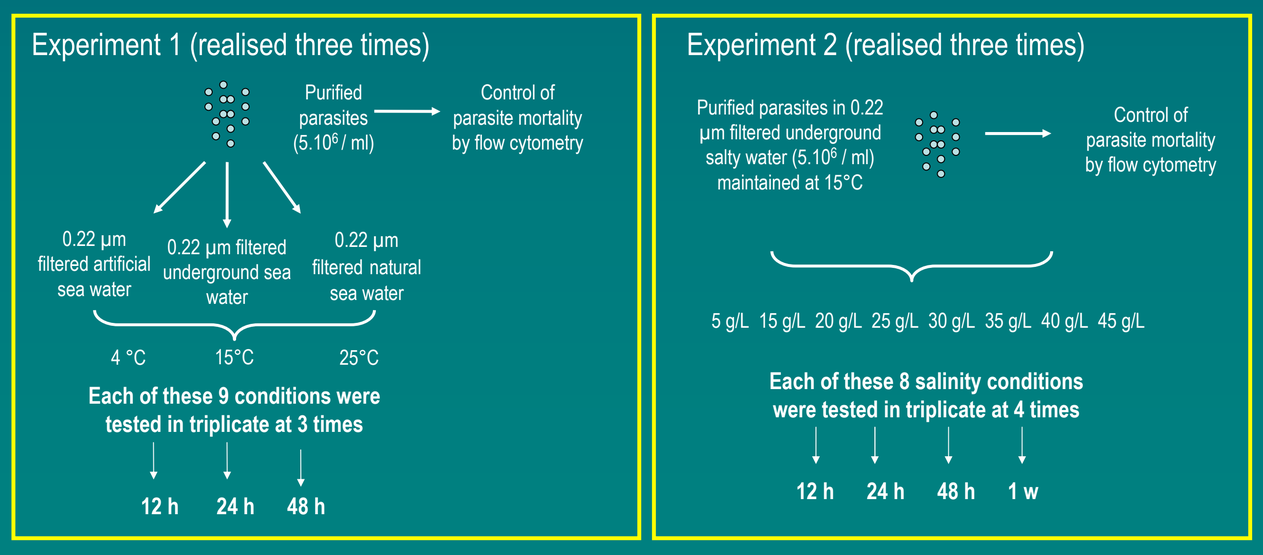


1 – Flat oysters, *Ostrea edulis*

2 – *Bonamia ostreae* after purification

3 – *Bonamia ostreae* inside haemocytes in mantle connective tissue of *Ostrea edulis*

Material and method



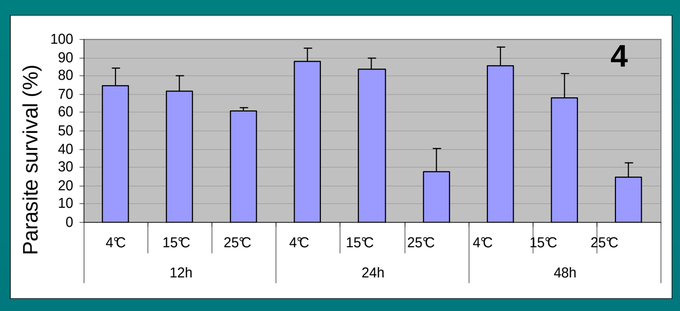
Purification performed according to Miahle et al. 1988.
Experiment 1 : incubation of purified parasites in three different media (artificial seawater; natural seawater and underground salty water) subjected to three temperatures (4, 15 and 25℃).
Experiment 2 : incubation of purified parasites at 15℃ in underground salty water subjected to a range of salinity (5, 15, 20, 25, 30, 35, 40 and 45 g/l).
Collection times : 12, 48 hours and 1 week of incubation.
Flow cytometry analyses : parasite mortality and non specific esterase activities.

Reference : Mialhe E, Bachere E, Chagot D, Grizel H (1988) Isolation and purification of the protozoan Bonamia ostreae (Pichot et al. 1980), a parasite affecting the flat oyster Ostrea edulis L. Aquaculture 71:pp. 293-299.

Results (experiment 1)

Lower parasite survival and lower esterase activities
- in artificial sea water
- at 25℃ especially 48 hours after purification (Fig 4 and 5).

Parasite is still alive 48 hours after purification (Fig 4 and 5).



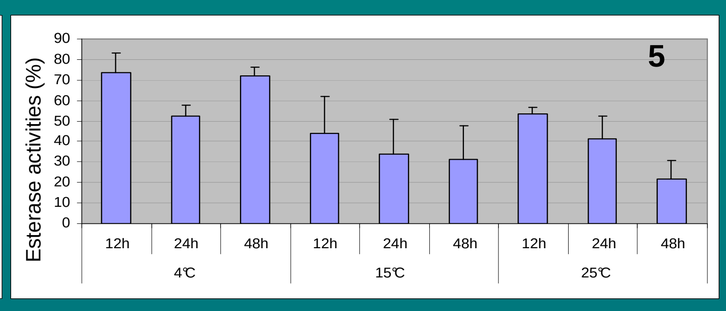
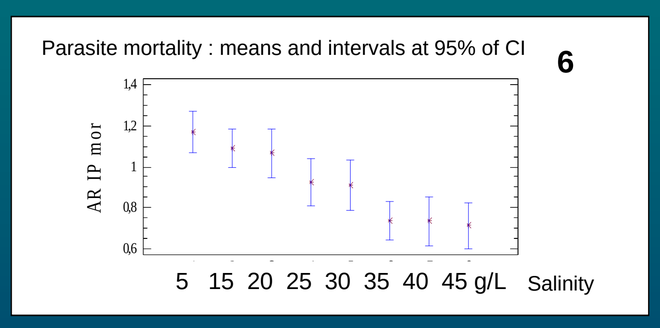


Fig 4 - Parasite survival in natural sea water according to the temperature and time of incubation (Values are mean of three replicates; Bars represent standard deviation; ** p=0.005; *** p<0.0001)

Fig 5 - Parasite esterase activities (% of positive cells) in underground salty water according to the temperature and time of incubation (Values are mean of three replicates; Bars represent standard deviation; ** p=0.0004)



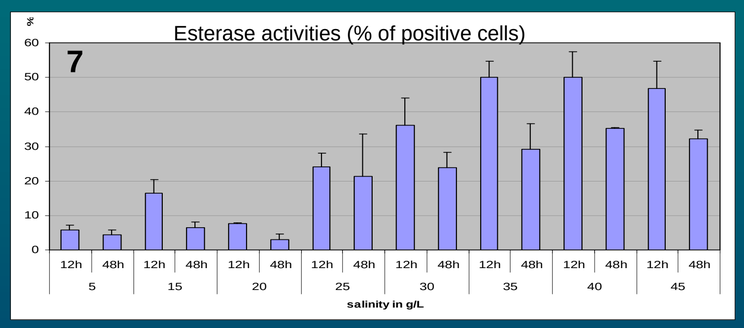


Fig 6 - Parasite mortality according to the salinity of underground salty water
Fig 7 - Parasite esterase activities in underground salty water according to the salinity and time of incubation (Values are mean of three replicates; Bars represent standard deviation; *** p=0.002 ;** p= 0.03)

Results (experiment 2)

Higher parasite mortality and lower esterase activities at 5, 15 and 20 g/L (Fig 6 and 7).

Significant impact of time of incubation on esterase activities for salinities higher than 35 g/L (Fig 7).

Conclusion

1- Better survival of purified *Bonamia ostreae* in natural filtered sea water (NSW) and in underground filtered salty water (USW) compared with artificial filtered sea water (ASW). This result could be explained by a difference of pH (pH of ASW 6.5- pH of NSW 8.06- pH of USW 7.06).
2- Water temperature of 25℃ does not appear suitabl e for parasite preservation.
3- Purified *Bonamia ostreae* show a better survival in hyper saline (≥35 g/L) than in hypo saline media (≤ 20 g/L).
4- Time of incubation did not appear to have a clear impact on parasite survival and activities in our study. It was possible to detect up to 58% of live cells after 1 week of incubation in underground salty water maintain at 4℃.

This study shows that flow cytometry is an interesting technique to investigate survival of small parasites like *Bonamia ostreae*. These results contribute to a better understanding of the disease but need to be validated by epidemiological surveys in the field.