Ostrea conchaphila : a natural host of *Bonamia ostreae* ?

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Introduction

Bonamia ostreae is a protozoan parasite which has caused significant mortality of the European flat oyster, *Ostrea edulis*. It has been reported from Europe and North America. The observation of *Bonamia*-like organisms, speculated to be *B. ostreae,* in the vesicular connective tissue cells of Olympia oysters, *O. conchaphila*, from Oregon in 1969-1970 suggested that this species may be a natural host of the parasite (Farley et al. 1988). Considering that *B. ostreae* has been introduced to Europe by infected spats of *O. edulis* and, given that *O. edulis* is not a native species to North America, an hypothesis on origin of bonamiosis is to regard *O. conchaphila* as a natural and reservoir host for *B. ostreae*. However, the absence of investigation of the Oregon case at ultrastructural and molecular levels renders its interpretation highly speculative and does not bring much support to the working hypothesis (Elston 1990). Cohabitation experiments were performed to test the sensitivity of this non-indigenous species with regards to infection with *Bonamia ostreae*.

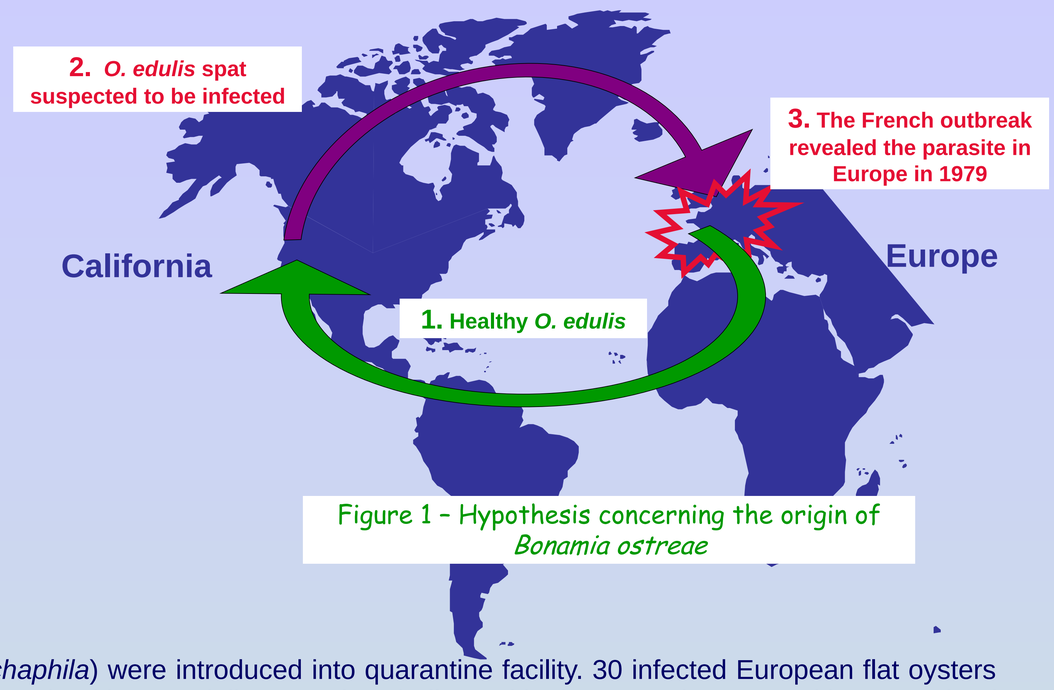


Figure 1 – Hypothesis concerning the origin of *Bonamia ostreae*

Material and Methods

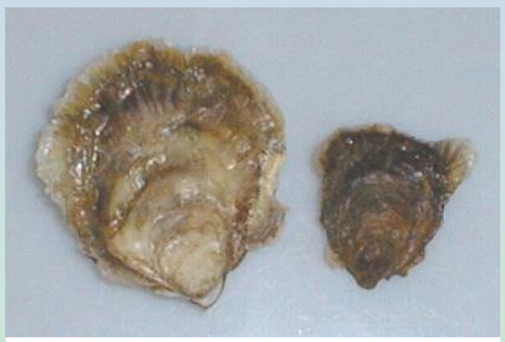


Figure 2 – *Ostrea edulis* (on the left), *Ostrea conchaphila* (on the right)

After careful initial examination, Olympia oysters (*Ostrea conchaphila*) were introduced into quarantine facility. 30 infected European flat oysters were put in contact with 30 Olympia oysters during 11 months. 30 healthy *O. edulis* were also challenged in the same time as positive controls of the study. 30 *O. edulis* and 30 *O. conchaphila* were kept in separated tanks during the assay as negative controls. All experiments were realized in triplicates (Figure 3). Temperature was checked daily in all tanks of experiment and did not present variation between tanks. Dead oysters were daily recorded and surviving animals were eventually sacrificed as the experiment ended. The presence of *B. ostreae* was checked by histology and heart imprints. Additional tests were done by PCR on challenged Olympia oysters according to the protocol described by Cochennec et al. (2000).

1 2 3	30 healthy *Ostrea conchaphila*	4 5 6	30 healthy *Ostrea edulis*	7 8 9	30 healthy challenged *Ostrea conchaphila* 30 initially infected *Ostrea edulis*	10 11 12	30 healthy challenged *Ostrea edulis* 30 initially infected *Ostrea edulis*

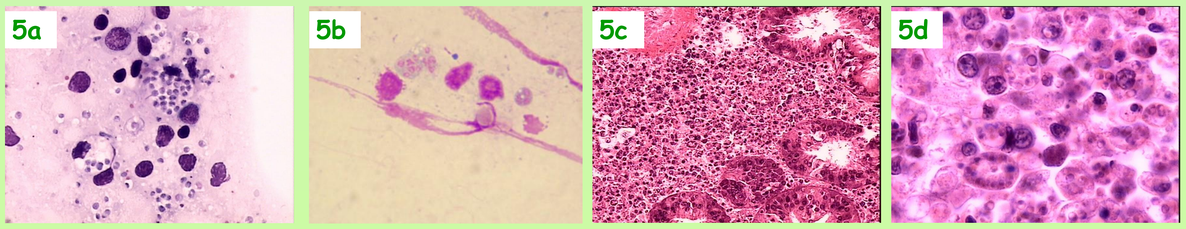
Figure 3- Experiment schedule. Each condition was repeated three times in three different tanks.

Results

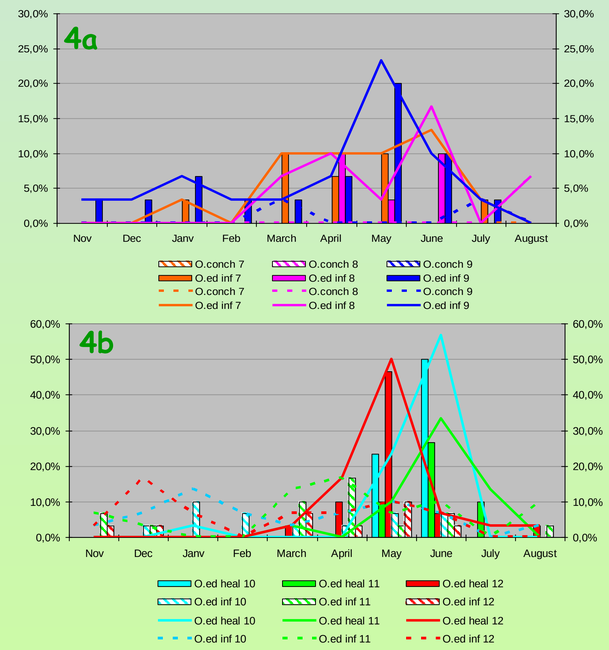
Mortality - During the assay, 5% of the challenged Olympia oysters and 76% of the challenged European oysters died. Total initially infected *Ostrea edulis* presented 57% of mortality during the experiment. Mortality rates in negative controls did not exceed 3% except in one tank (4) which exhibited 27% of mortality (but no pathogen detected).

Detection of *Bonamia ostreae* by histology/cytology - The evolution of *Bonamia ostreae* detection in initially infected and challenged *Ostrea edulis* dead during the experiment was well correlated with the evolution of mortalities (Figures 4a and 4b). At the end of the experiment, the parasite was detected in 64.4 % of challenged *O.edulis* whereas 40% of initially infected oysters were found positive. Most of the oysters detected positive presented a systemic distribution of the parasite (Figure 5c and 5c) with high infection level (Figure 5a). Numerous plasmodial stages could be observed by cytology in positive individuals (Figure 5b) suggesting a progressing infection. The parasite was detected neither in controls (both *Ostrea edulis* and *O. conchaphila*) nor in challenged *O. conchaphila*. Surprisingly, none of the *O. edulis* (both challenged and initially infected) sacrificed at the end of the experiment appeared positive.

Test of *Olympia oysters* by PCR - PCR assays carried out on challenged Olympia oysters confirmed the results obtained by histology/cytology : all the samples appeared negative.



Figures 5 - Challenged *Ostrea* edulis analysed by heart imprints (5a & 5b) and by histology (5c & 5d). 5a: numerous parasites in haemocytes and in extracellular position. 5b: plasmodial stages of the parasite. 5c: systemic infection associated with haemocytic infiltration. 5d : *Bonamia ostreae* present in haemocytes



Figures 4- Transmission assays between infected *Ostrea edulis* and 4a- healthy *O. conchaphila* (tanks 7, 8 and 9), 4b- healthy *O. edulis* (tanks 10, 11 and 12): evolution of mortality (lines) and dead oysters detected infected (histograms) during the experiments
O. ed heal : healthy *Ostrea edulis; O. ed* inf : infected *O. edulis*;
O. conch : Ostrea conchaphila

Conclusion

These results suggest that *Ostrea conchaphila* may not be a natural host of *Bonamia ostreae* and still leaves open the question of origin of bonamiosis. Investigations on other oyster or bivalve species present in California may help to understand the origin of the parasite. Moreover, while all species of the genus are usually considered as susceptible to *Bonamia ostreae*, *Ostrea conchaphila* appears to be resistant to the infection in our study.

References

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