Bonamia ostreae-induced mortalities in one-year old European flat oysters *Ostrea edulis* : experimental infection by cohabitation challenge

D. Lallias[1,2], I. Arzul[1], S. Heurtebise[1], S. Ferrand[1], B. Chollet[1], M. Robert[1], A.R. Beaumont[2], Pierre Boudry[1*], B. Morga[1] and S. Lapègue[1]

[1] Ifremer, Laboratoire de Génétique et Pathologie, La Tremblade, France
[2] School of Ocean Sciences, University of Wales, Bangor, UK
* Present address: Ifremer, UMR M100, Plouzané, France

CONTEXT OF THE STUDY

- Flat oyster *Ostrea edulis*: endemic European species
- Marteiliosis (caused by *Marteilia refringens*) and bonamiosis (caused by *Bonamia ostreae*) : two parasitic diseases
  → Drastic decline in its aquaculture production (Figure 1)
- Selective breeding programme undertaken by Ifremer since 1985 to produce families of oysters tolerant to *B. ostreae*
  - 1st stage: Production of two oyster strains (S85 and S89) by mass spawning (Naciri-Graven et al. 1998)
  - 2nd stage: Production of biparental families combined with within-family selection (Launey 1998)
  → These families showed enhanced survival and lower prevalence of the parasite (Lapègue et al. 2004)


O. edulis

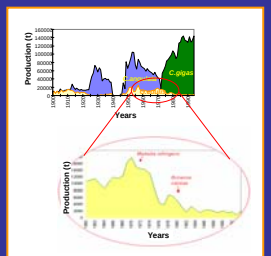


Figure 1

6-MONTHS COHABITATION CHALLENGE EXPERIMENT

Aim of the study: transmit the disease from wild oysters (injected 1 x 10^6 cells of parasite) to two families of oysters originating from the Ifremer selective breeding programme and to follow the dynamics of mortality in association with the detection of *B. ostreae*.

- Two tested families of oysters originating from the selective breeding programme (Figure 2):
  - Family 1: 20 month-old at the start of experiment
  - Family 2: 8 month-old at the start of experiment
- Cohabitation challenge experiment (Figure 3):
  - Each raceway: 4 trays in stacks of two
  - 5 raceways containing Family 1
    - Each upper basket: 22 wild oysters
    - Each lower basket: 50 tested oysters
  - 5 raceways containing Family 2
    - Each upper basket: 22 wild oysters
    - Each lower basket: 55 tested oysters
  - Phytoplankton-enriched sea water (150 $l.h^{-1}$)
  - Mortalities checked daily
  - Ventricular heart smears on dying oysters
  → Detect level of infection to *B. ostreae*

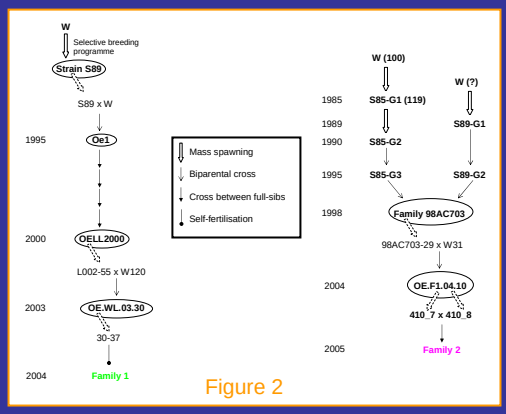
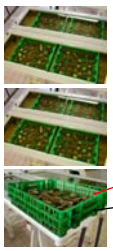


Figure 2


Cohabitation between artificially infected wild oysters and two tested families originating from the selective breeding programme (Figure 2)

Artificially infected wild oysters

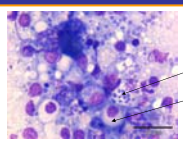
Tested oysters (**Family 1** or **Family 2**)

Figure 3

RESULTS

- **Cumulative mortality (Figure 4):**
  - No significant raceway effect: data pooled
  - 4 months before first infections in the tested oysters
  - Total cumulative mortality: 58% (wild oysters), 9% (Family 1), 20% (Family 2)
  - Detection of the parasite in dying oysters: 67% (wild oysters), 68% (Family 1), 89% (Family 2)

  → Mortality significantly higher in Family2 (χ^2=20.87, p<0.001, 1 d.f.)
  → Level of infection significantly higher in Family2 (χ^2=24.34, p<0.001, 4 d.f.)
- **Kinetics of bonamiosis development: (Figure 5):**
  - Wild oysters: moderately to heavily infected, died during the whole course of the experiment
  - Family 1: low to moderate level of infection, most deaths after day 130
  - Family 2: moderate to high level of infection, most deaths after day 130



Ventricular heart smear

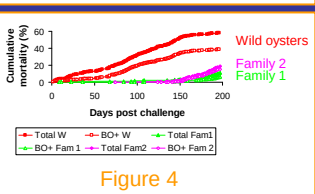


Figure 4

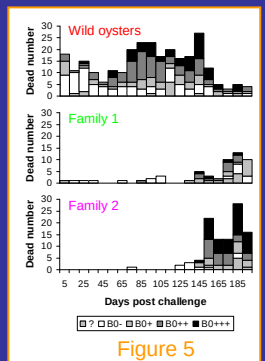


Figure 5

Prespawning oysters as young as one year-old can become infected with *B. ostreae* and die from bonamiosis

DISCUSSION

- Cohabitation challenge experiment with injected wild oysters: efficient way to study the kinetics of bonamiosis development in experimental conditions
- Heart imprints: lower sensitivity than PCR methodology, but semi-quantitative scale for the level of infection with the parasite can be applied
- Most probable cause of the discrepancy in the development of the disease between the two tested families: a difference in their genetic background
- One-year old oysters can die due to *B. ostreae* infection: potential implications for the management of flat oysters stocks

REFERENCES

Lapègue S., Bédier E., Goyard E., Dégremont L., Baud J.-P., Gérard A., Goulletquer P., Boudry P., 2004, Apport d'un programme de génétique à une filière de production aquacole: l'exemple de l'ostréiculture. In: Styli 2003 Trente ans de crevetticulture en Nouvelle-Calédonie. Actes Colloq. IFREMER 38, 113-121.

Launey S., 1998, Marqueurs microsatellites chez l'huître plate *Ostrea edulis* L. : caractérisation et applications à un programme de sélection pour une résistance au parasite *Bonamia ostreae* et à l'étude de populations naturelles. Thèse de doctorat. Institut national agronomique Paris Grignon, pp. 305 p.

Naciri-Graven Y., Martin A.-G., Baud J.-P., Renault T., Gérard A., 1998, Selecting the flat oyster *Ostrea edulis* (L.) for survival when infected with the parasite *Bonamia ostreae*. J. Exp. Mar. Biol. Ecol. 224, 91-107.