# *Bonamia ostreae* and *Ostrea edulis*: A Stable Host-Parasite System in France?

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## Abstract

Bonamiosis due to the intrahaemocytic protistan parasite *Bonamia ostreae* is a European endemic disease affecting flat oysters *Ostrea edulis*. After its first description in June 1979 in L'Ile Tudy, Brittany, the parasite rapidly spread to all French oyster farming areas and in other European countries through transfers of live molluscs. Bonamiosis and marteiliosis, another protozoan disease appeared in the seventies, drastically reduced the French flat oyster production from 20 000 tonnes in 1970 to less than 2000 tonnes after 1981. In 2001, about 1650 t of flat oysters were marketed in France. The production is restricted to several areas specialised in reproduction, growth and/or marketing, and depends on transfers of animals. Most important production areas of flat oysters have been surveyed regarding the presence of the parasite *Bonamia ostreae* since the first appearance of the disease until nowadays. This long term data series has been analysed revealing some fluctuations of detection frequencies. Differences could be noted according to animal age and sampling season. Indeed, adult oysters are usually more often detected infected than juveniles and winter or spring corresponds to the maximum recorded detection frequencies within a year. Potential impact of environmental factors including temperature and salinity has been investigated and will be discussed in order to understand the evolution of the disease. Although the disease is enzootic in France since 1980, the production of flat oyster still exists suggesting a stable host-parasite system.

## Introduction

The flat oyster *Ostrea edulis* was the flagship of the Breton oyster production until two diseases due to the protozoans *Bonamia ostreae* and *Marteilia refringens* spread in the 1970's. These diseases drastically reduced the French flat oyster production from nearly 20,000 t per year in 1970 to less than 2,000 t nowadays.

The parasite *Bonamia ostreae* was first described in June 1979, in L'Ile Tudy, South Brittany, France, in association with mass mortality of flat oysters, *Ostrea edulis* (Pichot *et al*., 1979). This pathogen rapidly spread to most of the Breton and, then, other French and European oyster production areas through transfers of live animals. Losses in France were estimated at about 20% of employment, 240 millions US$ of turn over and 200 millions US$ of added value between 1980 and 1983 (Meuriot & Grizel, 1984).

Although the flat oyster production was drastically affected by diseases, the production still exists and is localised in few specific areas. A national census concerning shellfish culture in France was carried out in 2002 by the statistic service of the French Ministry of Agriculture with collaboration of Ifremer and the National Shellfish-farming Committee (Girard et al. 2005). The objectives were (1) to quantify the shellfish production according to species (oyster, mussel, clam, cockle), life stages and local cultural practices and (2) to estimate shellfish transfers between farms and shellfish areas within the country. We present herein data concerning the French flat oyster production.

Quiberon bay, South Brittany, France (Figure 1) constitutes an interesting site regarding the surveillance of bonamiosis because of the simultaneous presence of all flat oyster age stages (spat • 1 year; juveniles > 1 year and • 2 years; adults > 2 years). We analyse and present the long term series of data collected in this area. In Quiberon bay, on the period 1980-2003, more than 30 000 oysters were sampled for different purposes in the context of the French surveillance and monitoring of mollusc health programme, REPAMO, and were tested by heart imprints and sometimes by histology for the detection of *Bonamia ostreae*. Data from Quiberon bay were also compared to those from Cancale, North Brittany (Figure 1) which is one of the main French growing flat oyster areas and which is also affected by bonamiosis.

Although the disease is enzootic in Quiberon bay since 1979, the production of flat oyster is still present in this area. One can wonder how environment and cultural practices can influence the evolution of the disease and the oyster defence mechanisms while the parasite appears to inescapably lead to the death of infected oysters.

#### *Objective*

This analysis has two objectives: the first one is to draw an updated and dynamic picture of the French flat oyster production from data collected during the national shellfish culture census carried out in 2002 in France, and the second one is to study the evolution of bonamiosis in an endemic area (Quiberon Bay, Brittany) from pathological data collected between 1980 and 2003 through the French surveillance and monitoring of mollusc health programme, REPAMO and to compare this area to Cancale, the biggest French flat oyster growing area.

## Methods

### *National shellfish culture census*

The national shellfish culture census was realised by the statistic service of the French Ministry of Agriculture with collaboration of Ifremer and the National Shellfish-farming Committee. Data were collected between April and June 2002 through a standardized questionnaire filled by trained investigators. Requested data concerned shellfish farm activities in 2000 (spat production) and 2001 (other life stages - economical data). All the French shellfish farms (all rearing farms including hatcheries and nurseries) were investigated.

### *Sampling considerations*

Two areas in which population size is considered as infinite were selected for our study: Quiberon bay, South Brittany and Cancale, North Brittany (Figure 1). Oyster sampling was carried out for different purposes but all the oysters were collected randomly.

In Quiberon bay, on the period 1980-1988, 18 804 oysters were sampled and analysed. Unfortunately only partial data concerning the number and size of samples were available. In the same area, on the period 1989-2003, 242 samples corresponding to 15 073 oysters were collected. Each sample contained between 10 and 200 oysters, 50% of samples included 50 individuals or more.

In Cancale area, 142 samples were collected on the period 1989-2004. Each sample contained between 15 and 100 individuals, 50% of samples included 50 individuals or more.

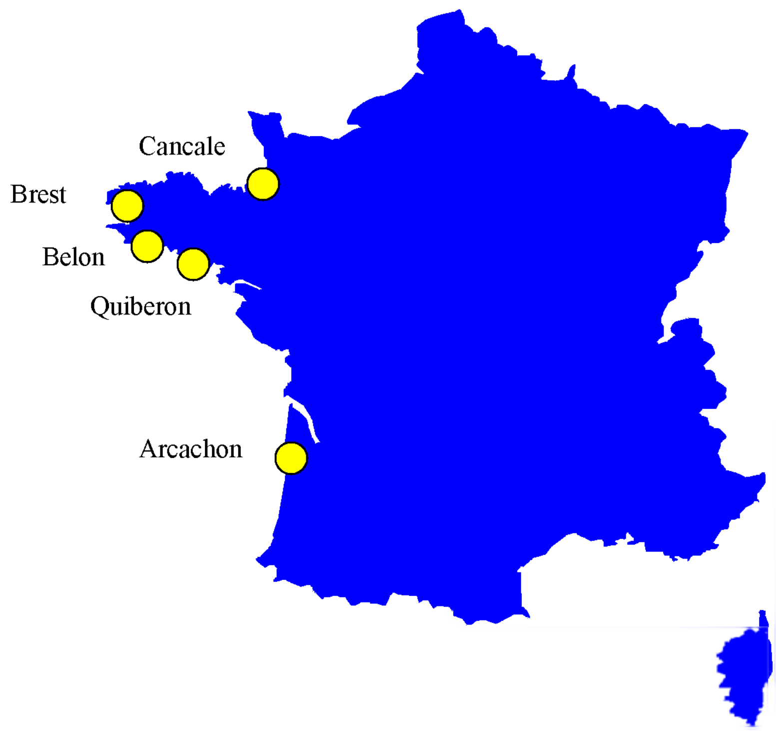


**Figure 1. Location of flat oyster production areas cited in the text**

*Diagnostic methods*

In Quiberon bay, on the period 1980-1988, most individuals were tested by using heart imprints and sometimes histology. In the same area, on the period 1989-2003, 25 samples were tested by using histology and 217 samples by using heart imprints. In Cancale area, 21 samples were tested by using histology and 126 samples by using heart imprints. Heart imprints as well as histological sections observations were performed by experienced people and thus we consider that there is no difference of sensitivity between results obtained by both techniques. Heart imprint examination is usually considered more sensitive since it is easier to detect the parasites even at low level of infections. However, this difference decreases when the analysis is done by experienced people.

Briefly, for heart imprints, after drying tissues on an absorbent paper, several imprints are made on a glass slide. Then, slides are air-dried, fixed in methanol and stained using a Hemacolor® kit (Merck), in accordance with the manufacturer's instructions. After rinsing in tap water, drying them, slides are mounted with a cover-slip using an appropriate synthetic resin. Observations are firstly made with a 10-20X objective and then under oil immersion with a 100X objective.

For histology, sections of tissue that include gills, digestive gland, mantle, gonad are fixed for 24 hours in Davidson's fixative followed by normal processing for paraffin embedding and staining with haemoatoxylin and eosin. Observations are made at increasing magnifications to 1000X.

*Statistical analyses*

Pearson's correlation coefficients between Quiberon winter detection frequencies and winter or previous summer temperature and salinity were determined using XLSTAT-Pro software

(Addinsoft, 2004). Winter detection frequencies were preferred to year detection frequencies or other seasons since they correspond to the maximum value within a year. The same analysis was realised to compare adult, juvenile or year detection frequencies between Quiberon and Cancale.

## Results

The flat oyster spat production was estimated at 375 $10^6$ units in 2000. Most of spat is naturally collected in Quiberon bay (87,8%) and in a lesser concern in Brest (12,2%). All Brest spat production and 1/3 of Quiberon bay spat production is moved to Cancale, North Brittany, when spat is 10 months. In 2001, the production of flat oyster was estimated at 1653 tonnes. Most of oysters grow in Cancale (North Brittany) and Quiberon bay (South Brittany) while marketing also takes place in Belon (North Brittany) and Arcachon bay (Aquitaine).

In a first step, detection frequencies of bonamiosis in Quiberon bay were analysed per year on the period 1980-2003 without considering spat samples (Figure 2). Indeed, heart imprints or histology do not seem to be the most efficient diagnostic tools to detect the parasite in young oyster stages (Lynch et al. 2005) and spat is very rarely detected infected using these techniques. In this context, detection frequencies fluctuate by waves with a mean of 13,2%.

In a second step, detection frequencies were analysed by age class and season on the period 1989-2003. Very few spats were found infected (4/2534). Juveniles usually appear less infected than adults. Detection frequencies (without including spat) also present some fluctuations within a year and usually show minimum value in summer and maximum value in winter.

Winter detection frequencies and previous summer salinity were significantly positively correlated ($r$= 0.793, $p$<0.05) while winter detection frequencies and previous summer temperature were significantly negatively correlated ($r$= -0.767, $p$<0.05).

Lastly, Quiberon bay data were compared with detection frequencies from Cancale on the 1989-2003 period. Cancale presents less variation than Quiberon and lower detection frequencies with a mean estimated at 6,7%. However, no significant correlation was observed between adult, juvenile or year detection frequencies between both areas.

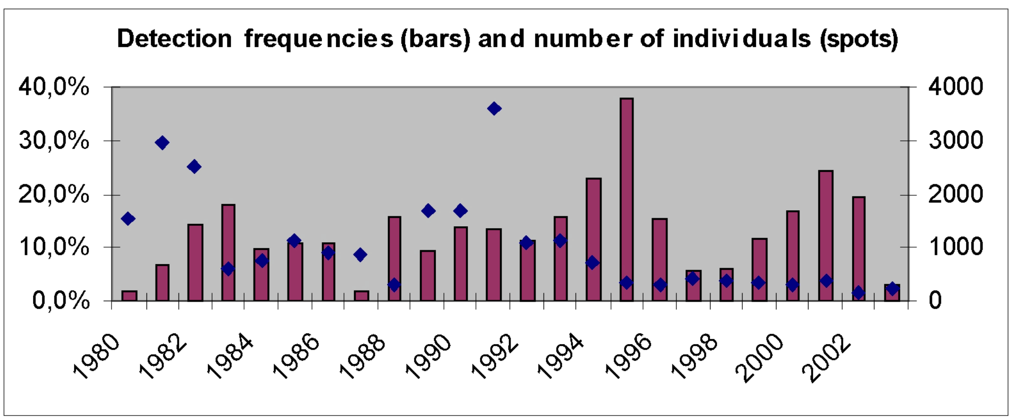


**Figure 2. Evolution of detection frequencies in Quiberon bay on the period 1980-2003 without considering spat samples. Number of individuals analysed per year are indicated by the blue spots.**

## Discussion

Data collected through the national census show that the French flat oyster production is located in few specialised areas: 2 spat collection areas (Brest and Quiberon bays); 2 growth areas (Quiberon and Cancale) and 4 main marketing areas (Cancale, Belon, Quiberon and Arcachon). The French flat oyster production still implies transfers. Indeed, spat is moved from Brest or Quiberon bays to Cancale for growth and 83% of adults are moved from a farm to another before marketing. These cultural practices tend to take advantage of the environmental characteristics specific for each production site but present some risks regarding the propagation of new pathogens notably through transfers of live oysters.

Detection frequencies fluctuate on the studied period (1980-2003) and also within a year. Because the disease induces mortality of oysters older than 2 years, the detection frequencies are influenced by oyster mortalities. Unfortunately mortality data are not available on this period in this area. First analyses concerning detection frequencies and environmental parameters like temperature or salinity in Quiberon bay show significative correlations between winter detection frequencies and previous summer lower temperature and higher salinity. These results need to be confirmed.

Most of Cancale oysters originate from Quiberon bay. Thus both Quiberon and Cancale juveniles and adults included in this study share the same origin. However, detection frequencies recorded in both areas were not significantly correlated suggesting that environmental parameters and cultural practices have more impact on the evolution of the disease than initial parasite burden.

Although bonamiosis is enzootic in Quiberon bay since 1980, the production of flat oyster is still present. Different explanations can be advanced to understand this fact. Bonamiosis kills oysters older than 2 years but flat oysters can reproduce from year 1. Moreover, Quiberon oyster bed is regularly harvested for further growth or marketing which allows the elimination of highly infected oysters. Lastly, after 25 years of cohabitation, the host-parasite system might have acquired a certain stability which can be partly explained by the development of a tolerance or resistance of the flat oyster to the infection and/or the impact of the parasite.

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# *Bonamia ostreae* and *Ostrea edulis*: a stable host-parasite system in France?

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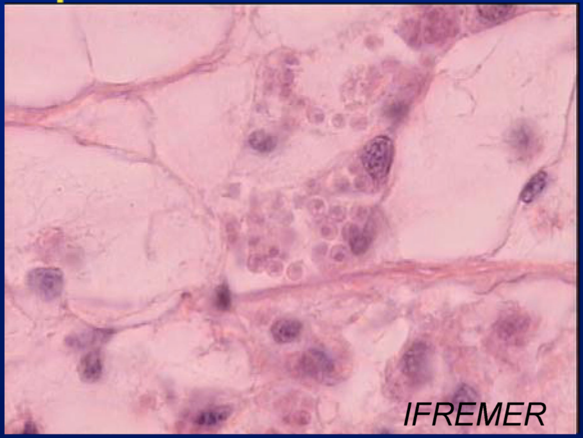
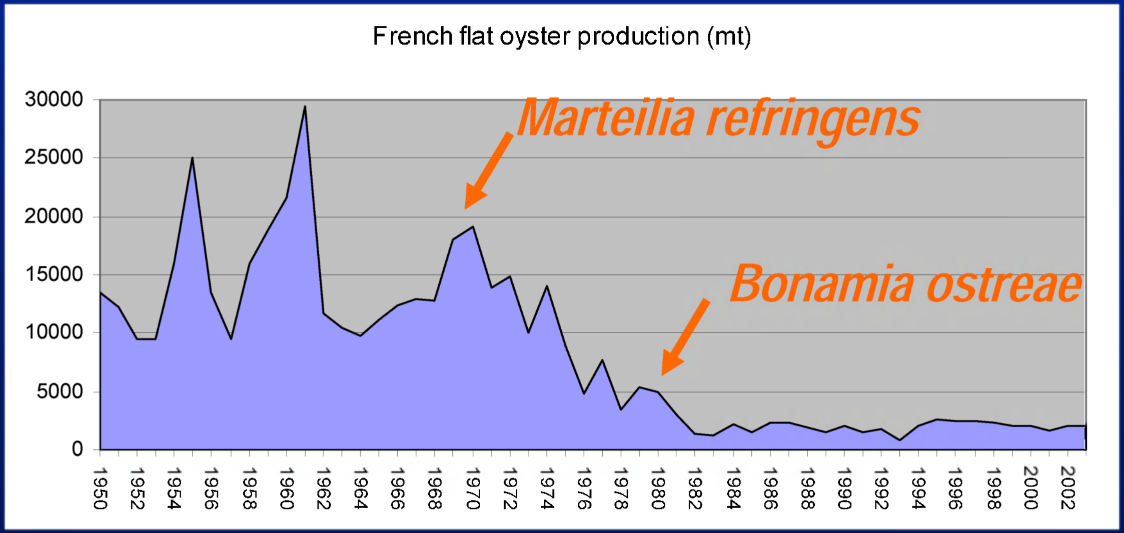
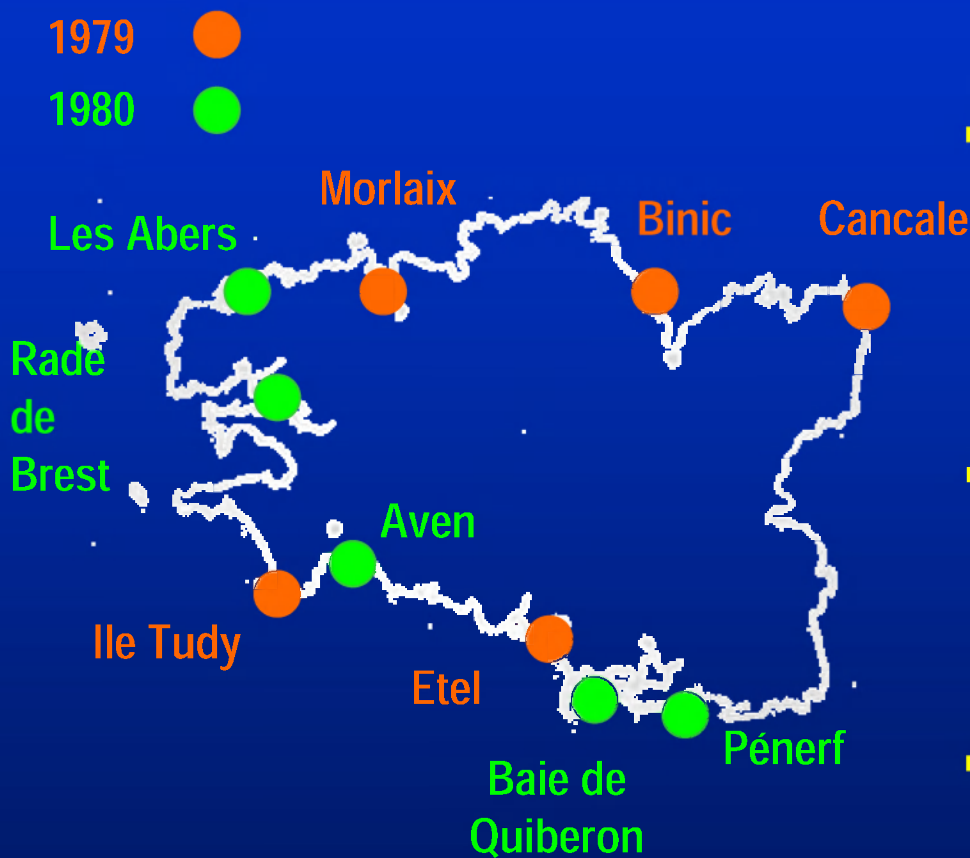


# Introduction

- The flat oyster *Ostrea edulis* was the flagship of the Breton oyster production until two diseases due to the protozoans *Bonamia ostreae* and *Marteilia refringens* spread in the 1970's.
- These diseases drastically reduced the flat oyster production from nearly 20,000 t per year in 1970 to less than 2,000 t nowadays.
- Losses were estimated at about 20% of employment, 240 millions US$ of turn over and 200 millions US$ of added value between 1980 and 1983.



# Introduction




- The protozoan *Bonamia ostreae* was first reported in June 1979, in oyster farms of Tudy Island, Brittany, in association with abnormal mass mortalities (80-90%) (Pichot *et al.*, 1979).
- During the following months, the same parasite was detected in all the Brittany farming centres and then has rapidly spread to most European oyster stocks (both reared and wild).
- The introduction is believed to have occurred with transfers of flat oysters, *Ostrea edulis* moved from California to France and Spain.

# Introduction


- Bonamiosis due to *Bonamia ostreae* is an OIE and EU listed disease.
- *Ostrea edulis* is the only natural host presently identified.
- *Bonamia ostreae* is present in Europe, North America and Morocco.
- The life cycle is unknown. However, the disease can be transmitted directly by cohabitation.
- The parasite infects haemocytes but can be found free in some epithelia. The infection is usually associated with haemocyte infiltration.
- A "latent period" of 3-5 months is reported before detecting the parasite by histology and cytology.
- Bonamiosis tolerant flat oysters were developed with success in the context of genetic selection programmes.

# Aims of the study

- To draw an updated and dynamic picture of the French flat oyster production from data collected during the national shellfish culture census carried out in 2002 in France.

- To study the evolution of the disease in an endemic area (Quiberon Bay, South Brittany) and to compare these data with the main French flat oyster growing area (Cancale, North Brittany).

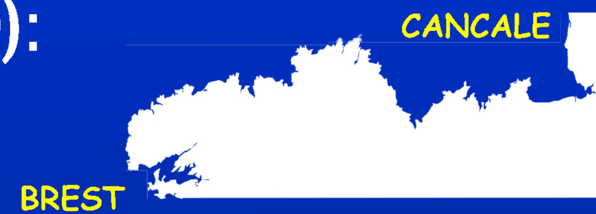
# National census: material and methods

- Data were collected through a standardized questionnaire filled by trained investigators between April and June 2002.
- Collected data concerned shellfish farm activities in 2000 (for spat production) and 2001 (for other life stages and economical data).
- All French shellfish farms were investigated.

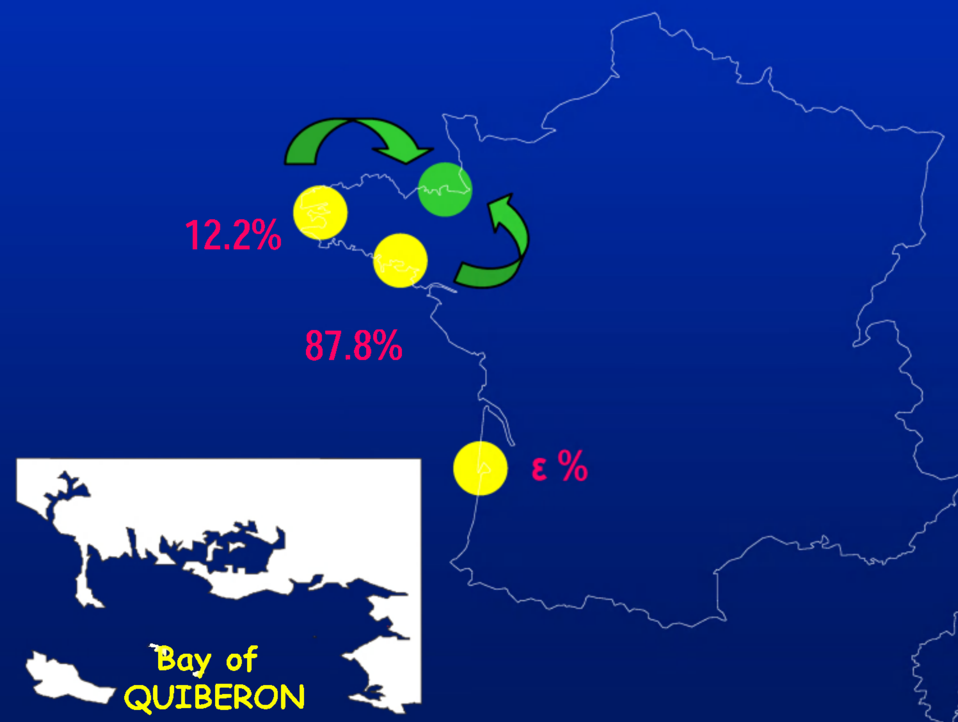
# National census: results (1)

## Flat oyster spat production (2000):

**375 $10^6$ units**




- 99.4% of spat was naturally collected on limed tiles or bags of mussel shells.
- 2.2 $10^6$ units were produced by 3 hatcheries in 2000.
- Most Brest spat production and 1/3 of Quiberon bay spat production is moved to Cancale, North Brittany, when spat is 10 months old.

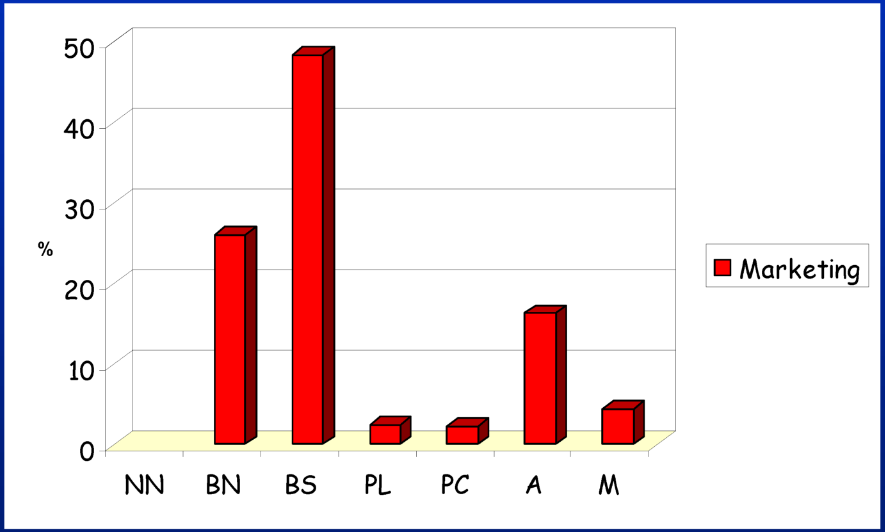


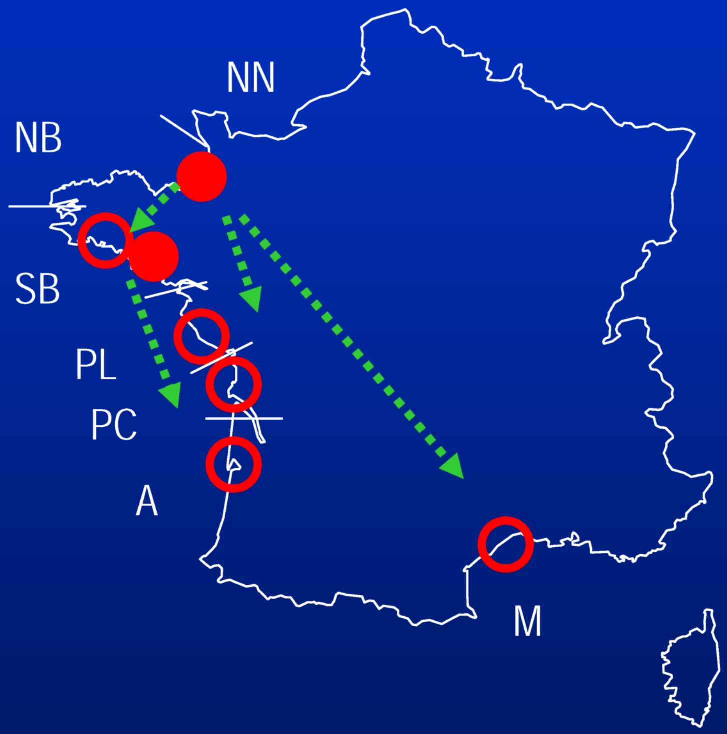

*2001 data from national census (Agreste)*

# National census: results (2)

## Flat oyster marketing (2001):

**1653 t**




*2001 data from national census (Agreste)*

# Quiberon study : Material and methods

## Sampling considerations

Oysters were collected randomly for different purposes with different sampling strategies through the French surveillance and monitoring of mollusc health programme (REPAMO).

**Cancale**

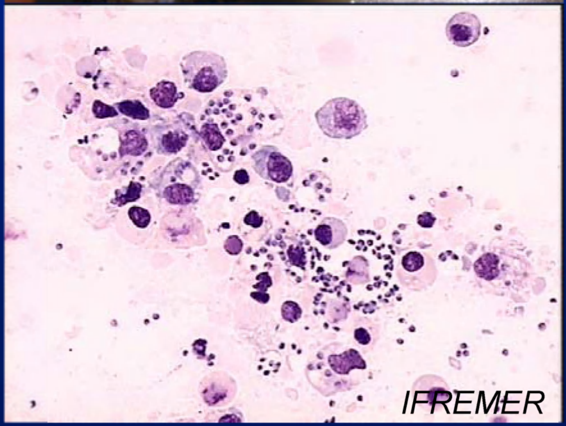
- Presence of adults
- 1989-2004: 7 672 oysters sampled (= 147 samples containing between 15 and 100 oysters - 50% of samples included 50 individuals or more).

**Quiberon Bay**

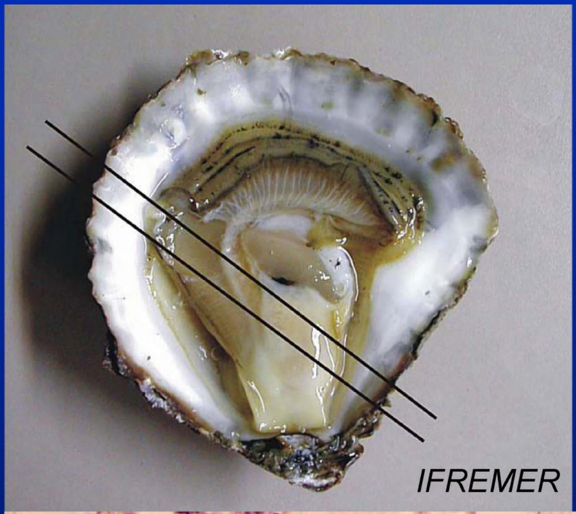
- Simultaneous presence of all flat oyster age stages
- 1980-1988: 18 804 oysters sampled
- 1989-2003: 15 073 oysters sampled (= 242 samples containing between 10 and 200 oysters - 50% of samples included 50 individuals or more).

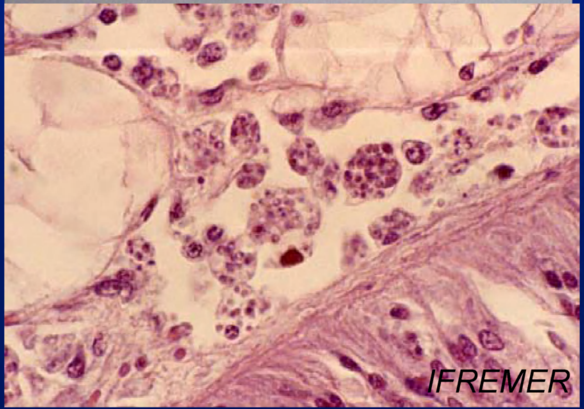
# Quiberon study : Material and methods

## Diagnostic methods


- Most oysters were tested by using heart imprints (88%) and sometimes histology (12%).
- No specificity and sensitivity data to compare these both techniques.
- Heart imprints are usually considered more sensitive. However, when performed by trained people, these techniques present similar results.



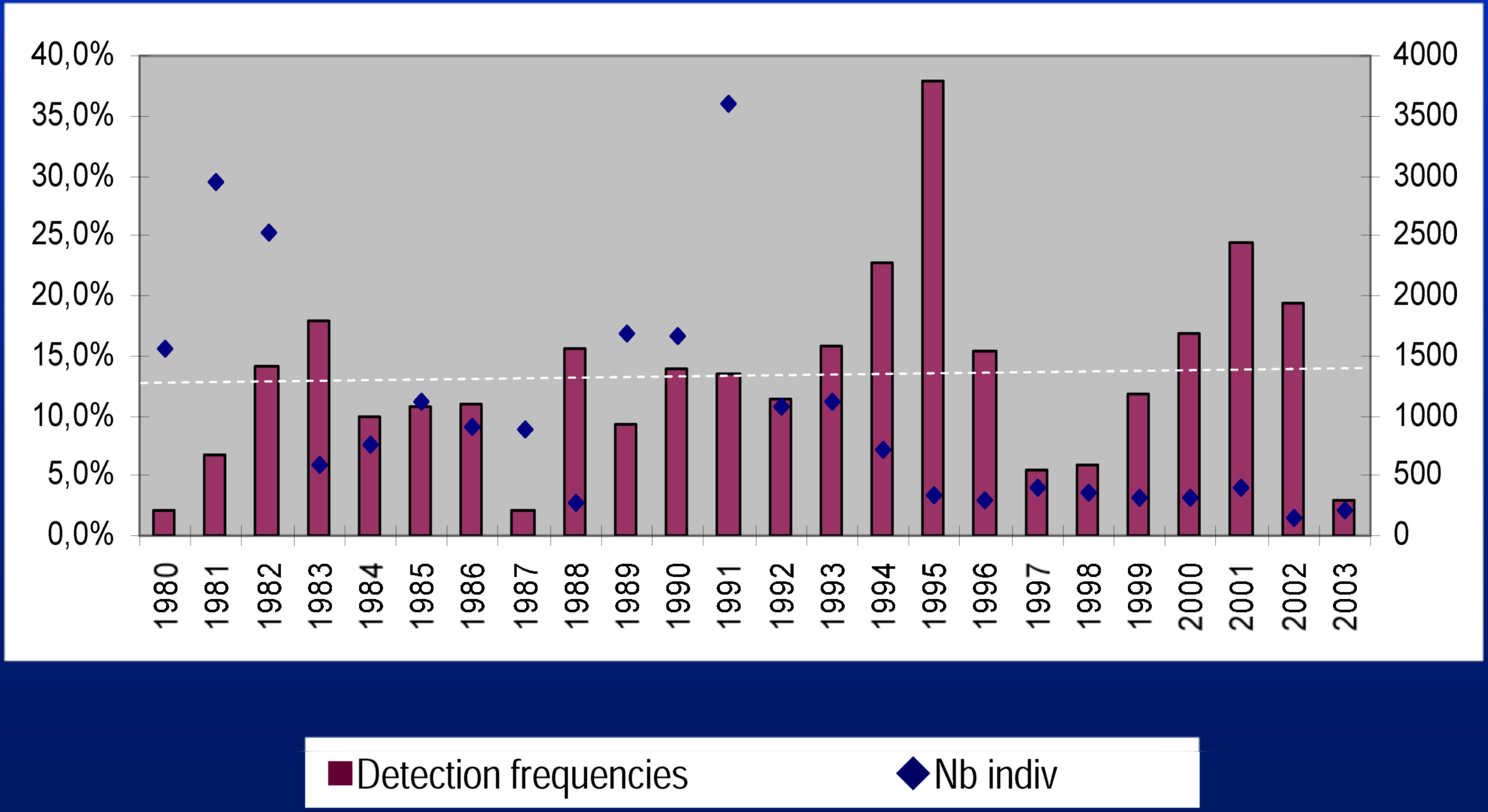
# Quiberon study : Material and methods

## Data analysis

- Considering biases induced by the sampling method, we just aim at drawing tendencies and identifying potential correlations
- Data (detection frequencies) were analysed by year, season and age class
- Data were compared using ANOVA with a single factor and a Tuckey's test (SAS)
- Correlations between environmental data including temperature and salinity and between both production areas (Quiberon and Cancale) were analysed using Pearson correlation coefficient (XLSTAT)

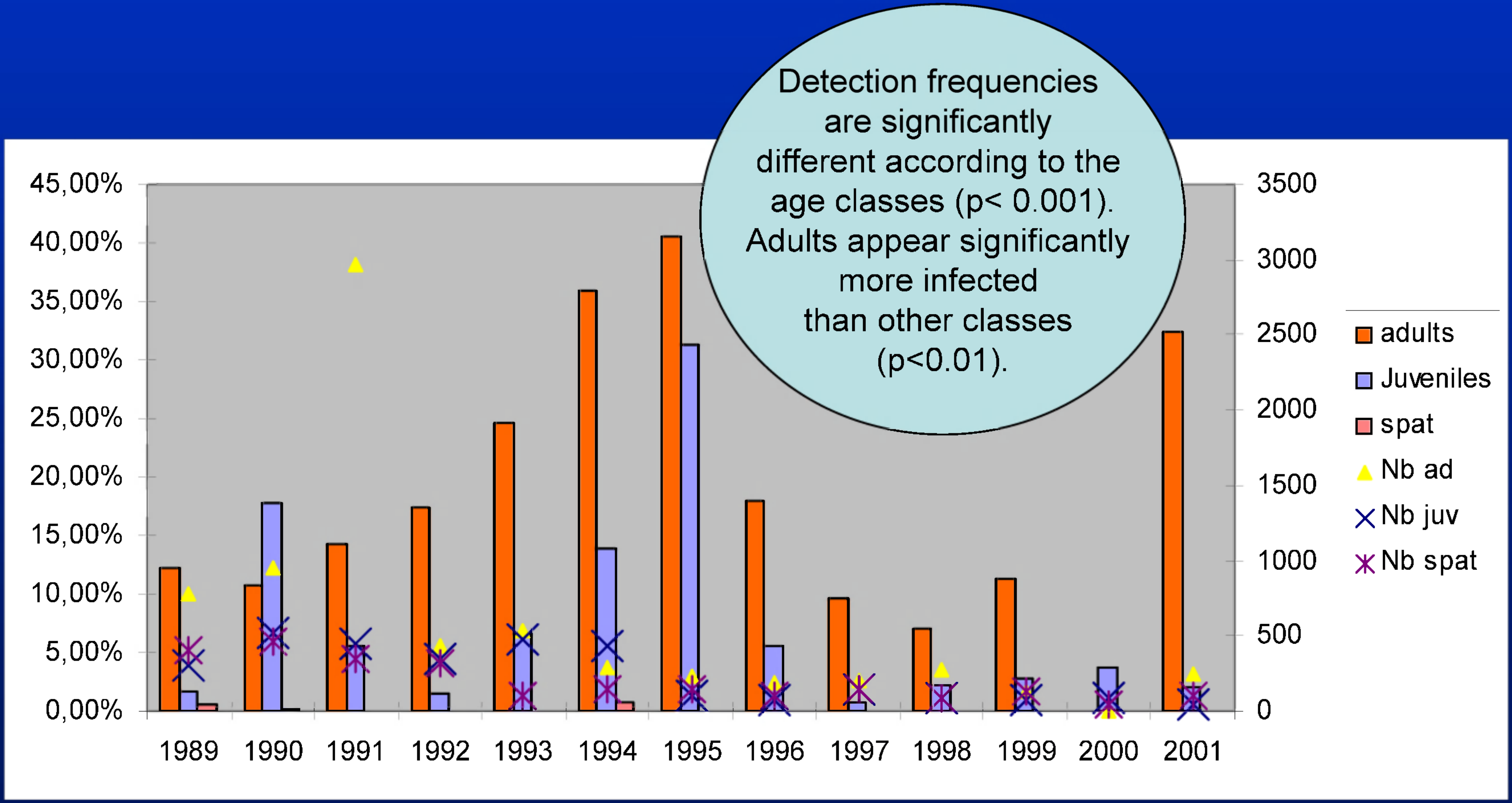
# Quiberon study: Results (1)

## Evolution of the detection frequencies/year (1980-2003)



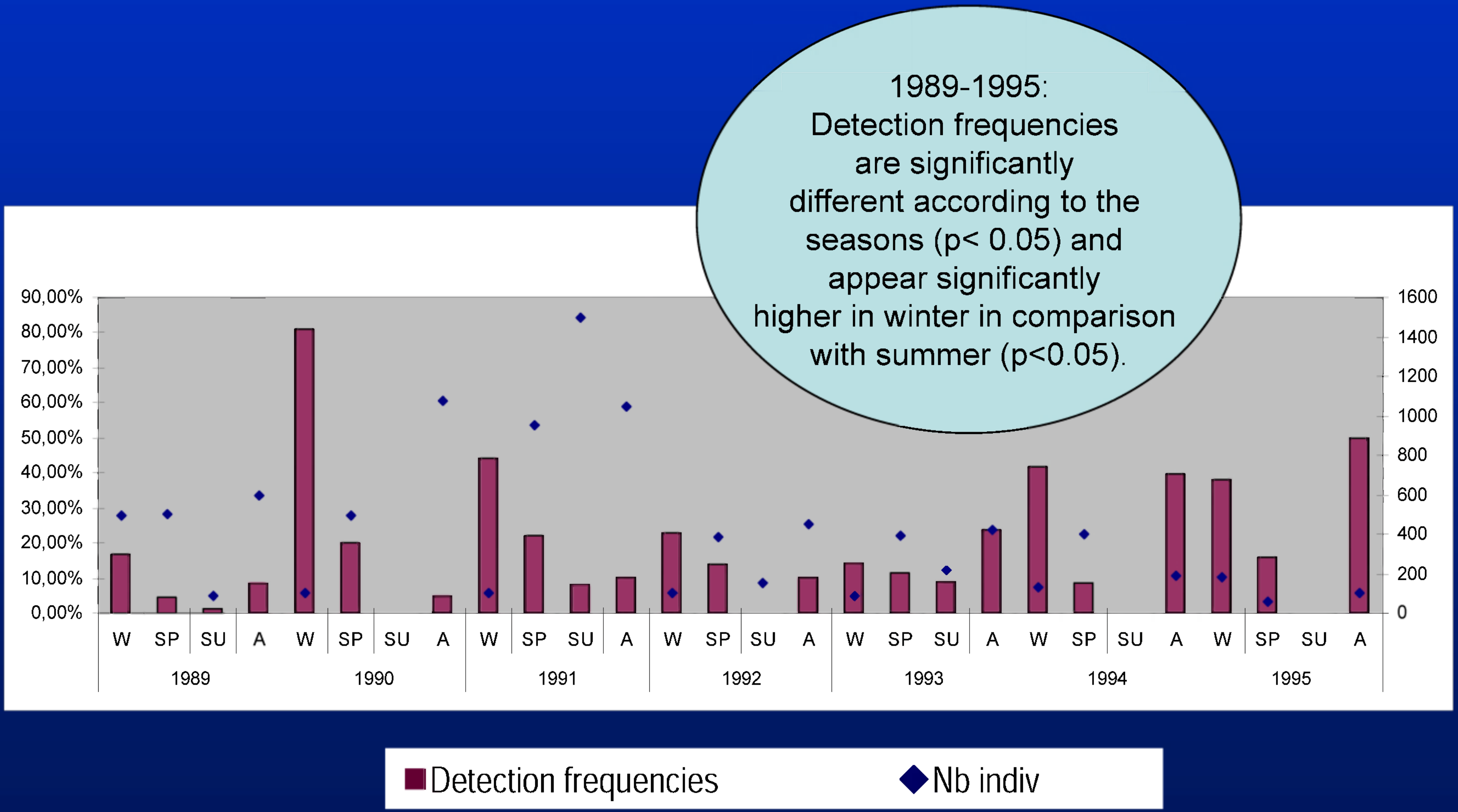
# Quiberon study: Results (2)

## Evolution of the detection frequencies/age (1989-2001)



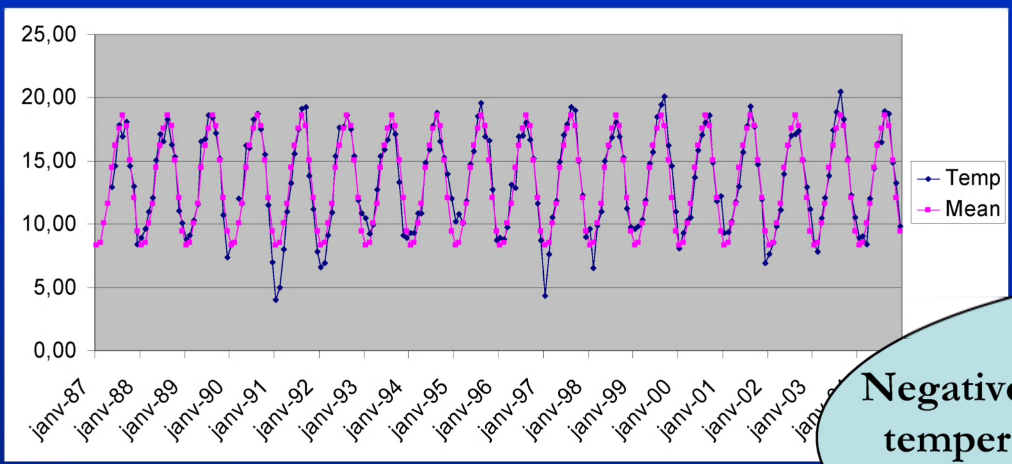
# Quiberon study: Results (3)

## Evolution of the detection frequencies/season (1989-1995)



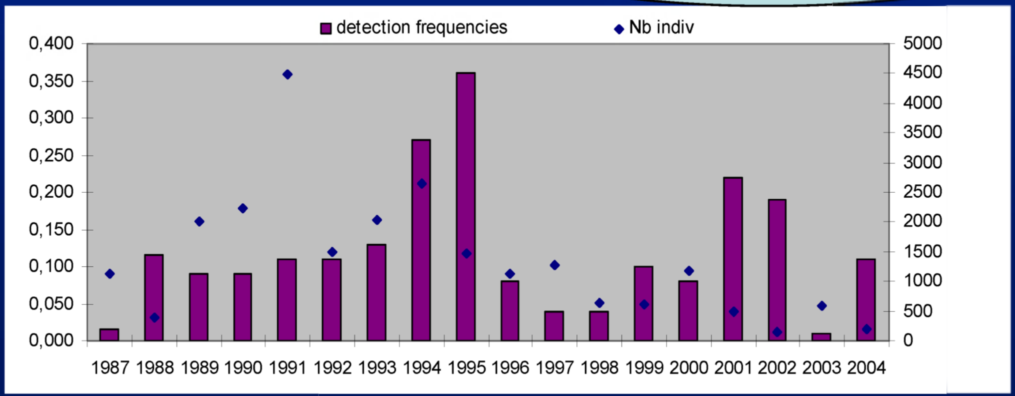
# Quiberon study: Results (4)

## Potential impact of temperature (Pearson correlation coefficient)



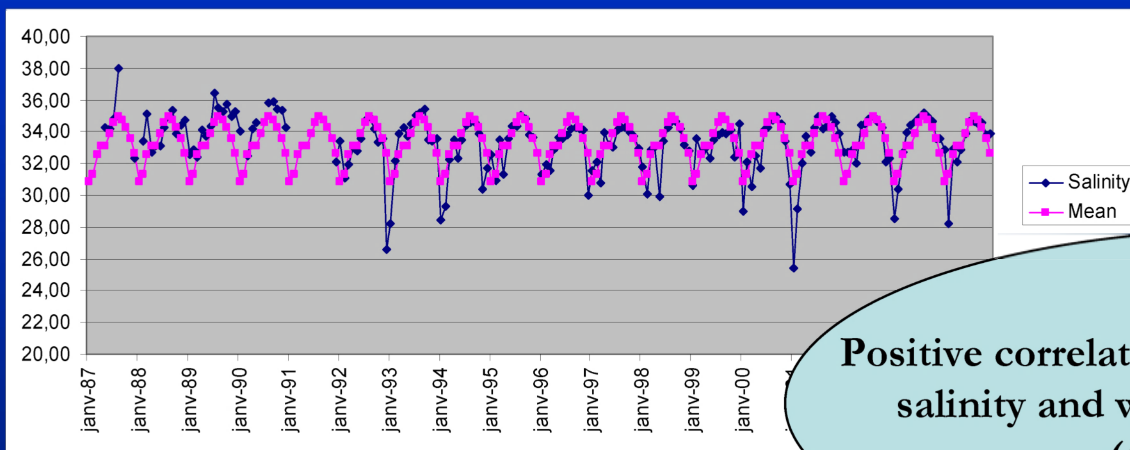

*REPHY data*

**Negative correlation between previous summer temperature and winter detection frequencies (r=- 0.767, p<0.01)**



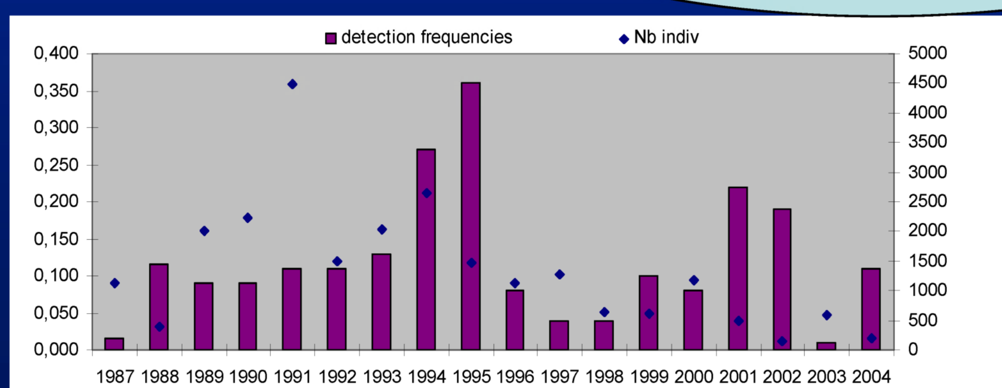
# Quiberon study: Results (5)

## Potential impact of salinity (Pearson correlation coefficient)




*REPHY data*

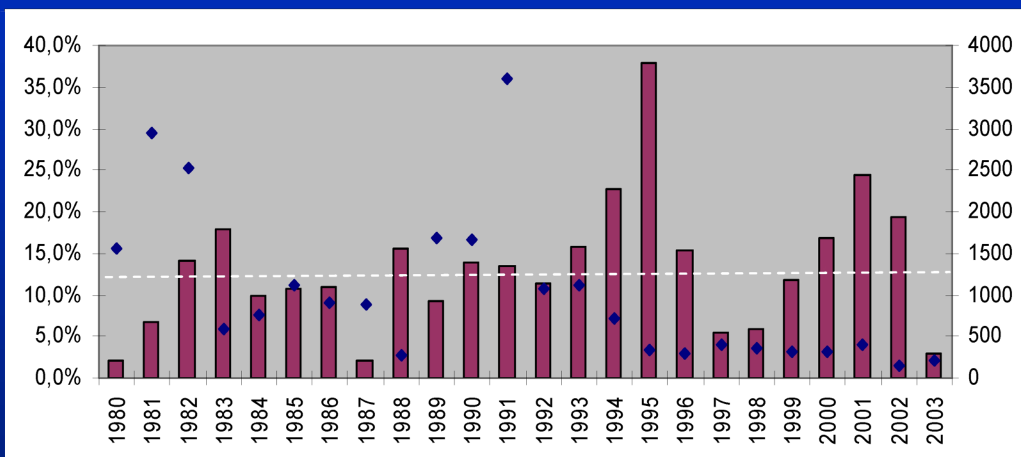
**Positive correlation between previous summer salinity and winter detection frequencies ($r=0.793$, $p<0.01$)**



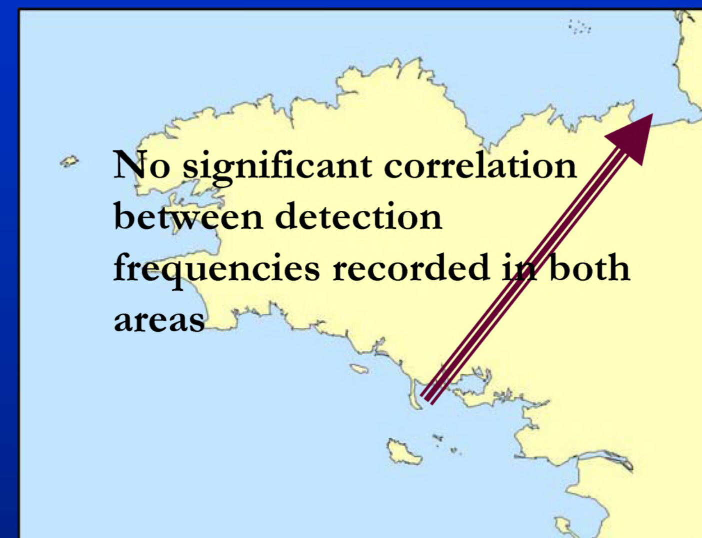
# Quiberon study: Results (6)

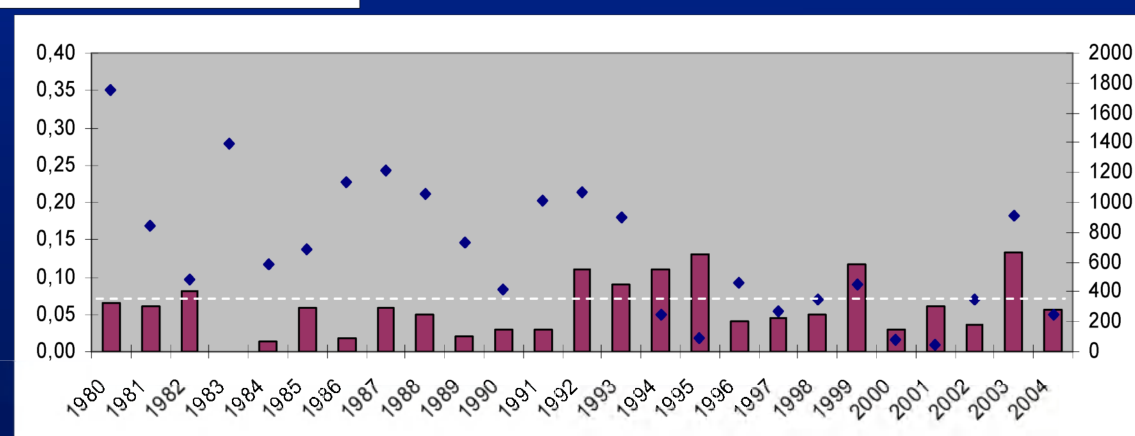
## Comparison with Cancale area

(Pearson correlation coefficient)




Quiberon




Cancale

# Discussion (1)


The French flat oyster production is located in few specialised areas:

- 2 spat collection areas: Brest and Quiberon bays
- 2 growth areas: Quiberon and Cancale
- 4 main marketing areas: Cancale, Belon, Golfe du Morbihan and Arcachon


The French flat oyster production implies transfers:

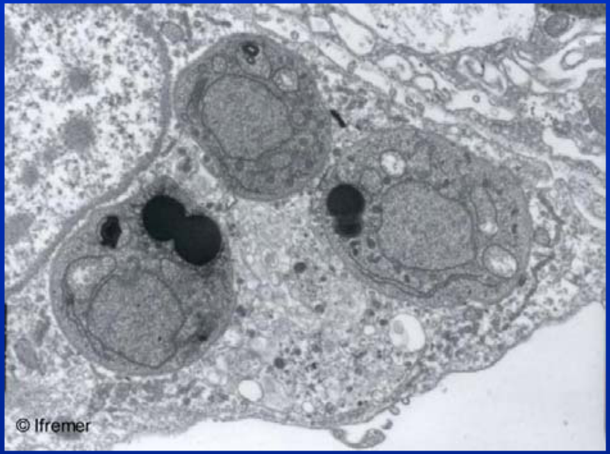
- Spat is moved from Brest or Quiberon bays to Cancale for growth
- 83% of adults are moved from a farm to another before marketing

# Discussion (2)

## Quiberon study

- Oysters were collected randomly but for different purposes and with different strategies. Consequently, data present some biases and results need to be confirmed by further studies.
- Detection frequencies fluctuate between years and within a year (seasonal variations).
- Mortality data are not available for the studied period. However, according to farmers, mortality mainly occurs on 2 years old oysters at the beginning of summer.
- Summer temperature and salinity seem to be significantly correlated with winter detection frequencies.
- Most Cancale oysters originate from Quiberon bay. However, no correlation could be identified between detection frequencies recorded on both areas (environment and cultural practices >initial parasite burden?).

# Discussion (3)



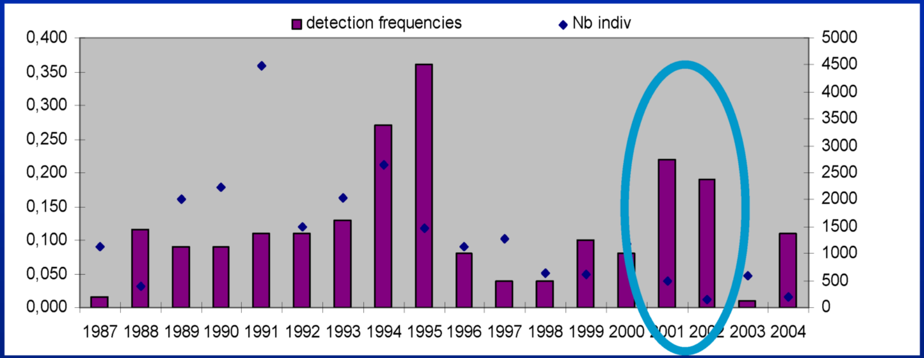


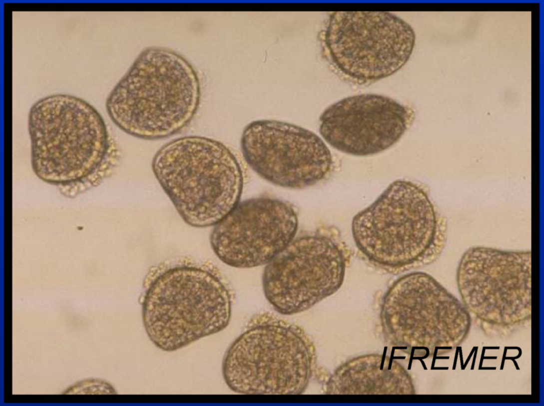
Bonamiosis is enzootic in Quiberon bay since 1980 :

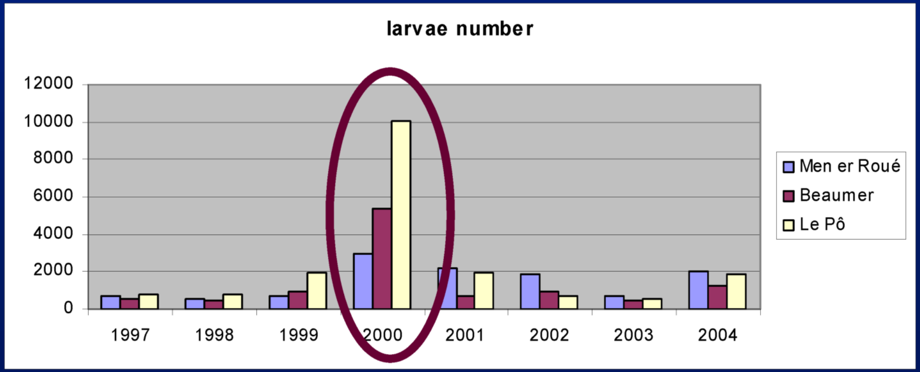
- mortality mainly affects 2 years old oysters (which have already spawned)
- oyster bed is regularly harvested for further growth and marketing and part of highly infected oysters is eliminated
- oysters have probably developed a natural tolerance regarding the infection with *Bonamia ostreae*

# Perspectives

- Survey of bonamiosis and mortality
- Density data, larvae production and spat collection data



- Thanks for your attention
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