

# Infection Of *Perkinsus olseni* In Clams As An Example of The New French Surveillance Programme.

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## Abstract

The French network for the surveillance and monitoring of mollusc health (REPAMO) was officially set up in 1992 to control the shellfish status along the French coasts according to the European directives 91/67/EEC and 95/70/EC. The objectives are 1) the surveillance of notifiable diseases present in France (Bonamiosis and Marteiliosis of flat oysters *Ostrea edulis*), 2) the surveillance of health status of cultured and wild populations of molluscs, 3) the study of abnormal mortality and 4) the control of exchanges from European and third countries.

In this context, a new surveillance programme, called Active Targeted Survey, part of the REPAMO, was applied in 2004 in order to improve the monitoring of the health status of the French mollusc populations (objective 2). The objective is to provide evidence of freedom regarding notifiable exotic diseases (OIE list and directive 95/70/EC annexe D) in susceptible host species. The new strategy, designed from data collected between 1990 and 2002 on the main harvested and farming species, is an active surveillance approach targeting a new mollusc species every two years. The sampling strategy including sampling method, sampling points, frequency, is defined according to the objective, the mollusc species and the targeted pathogen.

In the context of this new strategy we have chosen to start with perkinsosis in clams along the French coasts.

We present the theoretical approach and the data collected in 2004-2005 for clam species (*Ruditapes decussatus* and *R. philippinarum*) regarding infection with the protozoan *Perkinsus olseni*. Five areas, located along the French coasts, were investigated in autumn. In 2004, data demonstrated that no clam area was disease free and that prevalence and intensity of *Perkinsus* infection were higher in the South of France than in the English Channel.

## Introduction

The European bivalve mollusc production is estimated at 760 000 tonnes a year. France is among the leading countries with a production estimated at 198 000 tons in 2004. Main cultivated species are the cupped oyster *Crassostrea gigas*, the flat oyster *Ostrea edulis*, 2 mussel species *Mytilus edulis* and *M. galloprovincialis*, 2 clam species *Ruditapes decussatus* and *R. philippinarum* and one cockle species *Cerastoderma edule*.

In the last century, the European shellfish production has experienced several outbreaks which have deeply affected the French oyster culture. In the sixties and the seventies high mortalities of the Portuguese oyster *Crassostrea angulata* were associated with 2 iridoviruses (Renault and Novoa, 2004); moreover in the late seventies and in the eighties two major disease-causing organisms *Marteilia refringens* and *Bonamia ostreae* have significantly affected the flat oyster production (Berthe et al., 2004 ; Pichot et al., 1979).

Following these events, European regulations were laid down to prevent the shellfish industry from outbreaks and massive mortality. Council directive 91/67/EEC establishes the animal health conditions governing the placing on the market of aquaculture animals and products. Council

directive 95/70/EC introduces minimum Community measures for the control of certain diseases affecting bivalve molluscs.

In response to these obligations, French authority has put in place a surveillance system. The surveillance and monitoring of mollusc health programme, REPAMO, was implemented in 1992 in accordance to EU regulations. The objectives are 1) the surveillance of notifiable diseases present in France (Bonamiosis and Marteiliosis in *Ostrea edulis*), 2) the surveillance of health status of cultured and wild populations of molluscs, 3) the study of abnormal mortality and 4) the control of exchanges from European and third countries.

### **Objective**

The objective of this study is to explain how we implemented in 2004 a surveillance programme, called Active Targeted Survey. This programme is devoted to the objective 2 of the REPAMO. A classification of the pathogen-host pairs, based on information already collected on the presence or prevalence of the listed pathogens in French shellfish, was established. Each pair will be successively investigated during a two or three year survey, starting with pairs for which we have very few information. Consequently, *Perkinsus olseni* in clams along the French coasts was selected to start with. Then we present the results collected in 2004-2005 on the health status of 2 clam species, the Manila clam *Ruditapes philippinarum* and the European clam *R. decussatus* regarding the presence of *Perkinsus olseni* in farming and natural harvested beds.

### **Methods**

#### *Design of the new surveillance programme*

Data collected on shellfish pathogens in the main shellfish areas between 1990 and 2002 were analysed in order to evaluate health status of the main cultivated mollusc species regarding the presence of diseases included in the OIE list and directive 95/70/EC (i.e. oysters *Crassostrea gigas* regarding the presence of *Mikrocytos mackini*, *Perkinsus marinus* and *Haplosporidium nelsoni*, mussels *Mytilus edulis*, *M. galloprovincialis* regarding *Marteilia* sp. and clams *Ruditapes philippinarum* and *R. decussatus* regarding *Perkinsus olseni*).

#### *Perkinsosis survey on clams in 2004-2005*

The objective was to detect *Perkinsus olseni* in clams, cultivated and harvested along the French coasts, at a 95% confidence level with a minimum prevalence of 2%. Main clam areas were selected using recent published data on production (Girard *et al.*, 2005): 5 areas were investigated in 2004, one in the English Channel, 2 in the Atlantic Ocean and 2 in the Mediterranean Sea. Four additional areas were sampled in 2005, two in the English Channel and two along the Atlantic coast. 30 individuals were sampled in 3 to 6 sampling points in each area in 2004 where the population was estimated infinite. The sampling was reduced the second year according to the first results. It was systematic with a random sub-sampling in each sampling point. The sampling frequency was once a year, in September-October according to the life cycle of the pathogen. The detection of *Perkinsus olseni* was made on clam gills using the ray's Fluid Thioglycolate Method (Ray, 1966). We assumed that the sensitivity and specificity of this method were perfect.

### **Results**

The main objective of this Active Targeted Survey was to detect pathogen with a minimum prevalence of 2% at a 95% confidence level. This survey was developed exclusively in shellfish areas because the number of shellfish farms is very high in France (4113). For technical constraints, it is impossible to monitor simultaneously all the cultivated and harvested mollusc species in all

shellfish areas. We proposed to rank them according to the previous collected data, pathogens listed by OIE and EU legislation and the risk status of the shellfish areas based on production and shellfish movements. Table 1 summarizes data collected between 1990 and 2002 for the main cultivated mollusc species along the French coasts and the resulting classification.

Shellfish species	N samples*	Pathogens	Presence	Rank	Comments
<i>Ruditapes decussatus</i>	86	<i>Perkinsus olseni</i>	yes	1	Few data
<i>Ruditapes philippinarum</i>	150	<i>Perkinsus olseni</i>	yes		
<i>Mytilus edulis</i>	158	<i>Marteilia</i> sp.	yes	2	Few data
<i>Mytilus galloprovincialis</i>	39	<i>Marteilia</i> sp.	yes		
<i>Crassostrea gigas</i>	682	<i>Mikrocytos mackini</i>	no	3	Relevant data collected in 2000
		<i>Perkinsus marinus</i>	no		
		<i>Haplosporidium nelsoni</i>	yes		

Table 1: Summary of the data collected in previous studies and in the REPAMO between 1990 and 2002 (\* each sample included approximately 30 individuals).

The new proposed survey targets a new mollusc species every two or three year depending of the cultivated cycle of the species. The pair *Perkinsus olseni*/*Ruditapes* sp. was first selected because few data were available on the health status of the 2 species of clams and the sampling was done in a limited number of areas. Moreover the pathogen was recently listed in the directive 95/70/EC.

The result of the two-year survey on *Perkinsus olseni* infection in clams emphasized the presence of *Perkinsus olseni* in all the studied areas; the prevalence ranged between 1.7 and 100% (table 2). High prevalences were specially observed in Arcachon bay and along the Mediterranean Sea. Lower prevalences were registered in other areas along the Atlantic coast and English Channel. Detection frequencies were slightly reduced in 2005 compared to 2004.

	Areas	2004			2005		
		N sampling points	N individuals	Prevalence of <i>Perkinsus olseni</i> (%)	N sampling points	N individuals	Prevalence of <i>Perkinsus olseni</i> (%)
English Channel	Agon				2	60	86.7
	Chausey Island	4	160	58.8	2	60	41.7
Atlantic ocean	Abers				4	120	1.7
	Pont L'Abbé river				3	86	31.4
	Gulf of Morbihan	5	150	51.3	5	150	37.3
	Marennnes Oleron				5	149	12.1
	Arcachon bay	5	150	100	2	60	91.7
Mediterranean sea	Salse Leucate	3	90	100	3	69	95.7
	Thau lagoon	5	150	88.7	3	82	75.6

Table 2: Prevalence of *Perkinsus olseni* in clams collected in 2004 – 2005 along the French coasts

## Discussion

*Perkinsus olseni* is present in all clam areas with very high prevalence in the South of France but even in the English Channel. However, no abnormal mortality was registered during this survey period. This could be related to the moderate level of infection intensity (data not shown). Low temperatures observed in the Abers could explain the low detection frequency of *P. olseni* in this area. Further analyses will investigate possible correlations between prevalence of the parasite and environmental parameters. Moreover further studies will be developed in order to evaluate the pathogenicity of the different *Perkinsus* isolates and consequently the risk associated with the presence of this pathogen in these mollusc species.

The future EU regulation recommends to develop a risk based animal health surveillance in order to have an overview of the disease situation, and to facilitate rapid reaction in case of suspicion of a disease. The Active Targeted Survey implemented in France in 2004 could fit on this concept of surveillance. In 2006-2007, the couple Mussels/*Marteilia* sp. will be investigated in order to better understand the geographical distribution of *Marteilia maurini* and *M. refringens* in *Mytilus edulis* and *M. galloprovincialis* in French waters.

## References

- Berthe F.C.J., F. Le Roux, R.D. Adlard and A. Figueras (2004). Marteiliosis in molluscs: a review. *Aquat. Living Resour.* 17, 433-448.
- S. Girard, J.A. Pérez Agúndez, L. Miossec, N. Czerwinski (2005) Recensement de le Conchyliculture 2001, *Agreste Cahier n°1*, Ed. Ministère de l'Agriculture, de la Pêche et de la Ruralité, 89 pages.
- Pichot, Y., M. Comps, G. Tigé, H. Grizel and M.A. Rabouin. (1979). Recherches sur *Bonamia ostreae* gen. n., sp. n., parasite nouveau de l'huître plate *Ostrea edulis* L. *Revue des Travaux de l'Institut des Pêches Maritimes.* 43: 131-140.
- Ray S.M. (1966). A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proc. Natl. Shellfish Assoc.* 54: 55-69.
- Renault T. and B. Novoa (2004). Viruses infecting bivalve molluscs. *Aquat. Living Resour.* 17, 397-409.



## Infection of clams by *Perkinsus olseni* as an example of the new French Surveillance Programme

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## Introduction (1)

In the XX's, the European shellfish industry has experienced several outbreaks:

- *Crassostrea angulata* (Portuguese oyster): 2 **iridoviruses**
- *Ostrea edulis* (Flat oyster): 2 parasites, **Marteilia refringens** and **Bonamia ostreae**

European legislation was laid down to prevent the shellfish industry from outbreaks, mass mortality and spread of diseases:

- \* Directive 91/67/EEC
- \* Directive 95/70/EC



## Introduction (2)

In 1992, implementation of the French surveillance system, called **REPAMO**, in accordance with EU regulation, with 3 objectives:

1. The surveillance of notifiable diseases present in France (Bonamiosis and Marteiliosis in *Ostrea edulis*)
2. The surveillance of health status of cultured and wild populations of molluscs
3. The study of abnormal mortality



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## Introduction (3)

In 2004 we improved the surveillance of health status of cultured and wild populations of molluscs (objective 2) in compliance with Article 4 of the Directive 95/70/EC which recommends to keep track of the health situation of the stocks.

### The Active Targeted Survey



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## Objectives of this presentation

- ✓ To explain how we implemented the **Active Targeted Survey**
- ✓ To present the results collected in 2004-2005 on the infection of 2 clam species *Ruditapes philippinarum* and *Ruditapes decussatus* by *Perkinsus olseni* in farming and natural harvested areas



## Design of the *Active Targeted Survey* (1)

**Objective:** to provide evidence of freedom regarding notifiable diseases or pathogens (OIE list and Directive 95/70/EC Annexe D) in susceptible host species.

### *Pathogens*

*Mikrocytos mackini*  
*Perkinsus marinus*  
*Haplosporidium nelsoni*

*Marteilia sp.*

*Perkinsus olseni*

### *Hosts present in France*

*Crassostrea gigas*

*Mytilus edulis*  
*Mytilus galloprovincialis*

*Ruditapes philippinarum*  
*Ruditapes decussatus*

Pathogen-host pairs

## Design of the *Active Targeted Survey* (2)

**Where:** In areas, selected according to their risk status based on shellfish production and shellfish movements

*(high number of shellfish farms: n=4113)*

**In wild and farmed shellfish**

**For how long duration:** Two or three year survey according to the production cycle of the shellfish species

**Which pathogen-host pair:**

Data collected between 1990 and 2002 in the main shellfish areas



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## Design of the *Active Targeted Survey* (3)

972 samples\* of shellfish collected between 1990 and 2002

### *Crassostrea gigas*

682 samples

*Mikrocytos mackini*: no

*Perkinsus marinus*: no

*Haplosporidium nelsoni*: yes

Relevant data collected  
in 2000

3

### *Mytilus edulis*

158 samples

*Mytilus galloprovincialis*

39 samples

*Marteilia sp.*: yes

Few data

2

### *Ruditapes decussatus*

86 samples

*R. philippinarum*

150 samples

*Perkinsus olseni*: yes

Few data and recently  
listed in the Directive  
95/70/EC

1

\* each sample included approximately 30 individuals

## *Perkinsus* survey in clams in 2004-2005 (1)

### Objective

To detect *Perkinsus olseni* in 2 clam species *Ruditapes philippinarum* and *Ruditapes decussatus*, cultivated and harvested along the French coasts



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## Methods - Sampling strategy

✓ Sampling designed to detect infected animals at a 95% confidence level and minimum prevalence of 2% (assuming that the sensitivity and specificity are 1)

First year: **150** individuals in each monitored area (**5 points** of **30 individuals**)

Second year: sampling **adjusted** according to the first results

Systematic selection of sampling points in each area and random sampling in each sampling point

✓ Studied population: adults of both species

✓ Sampling frequency: **once a year**, in September-October

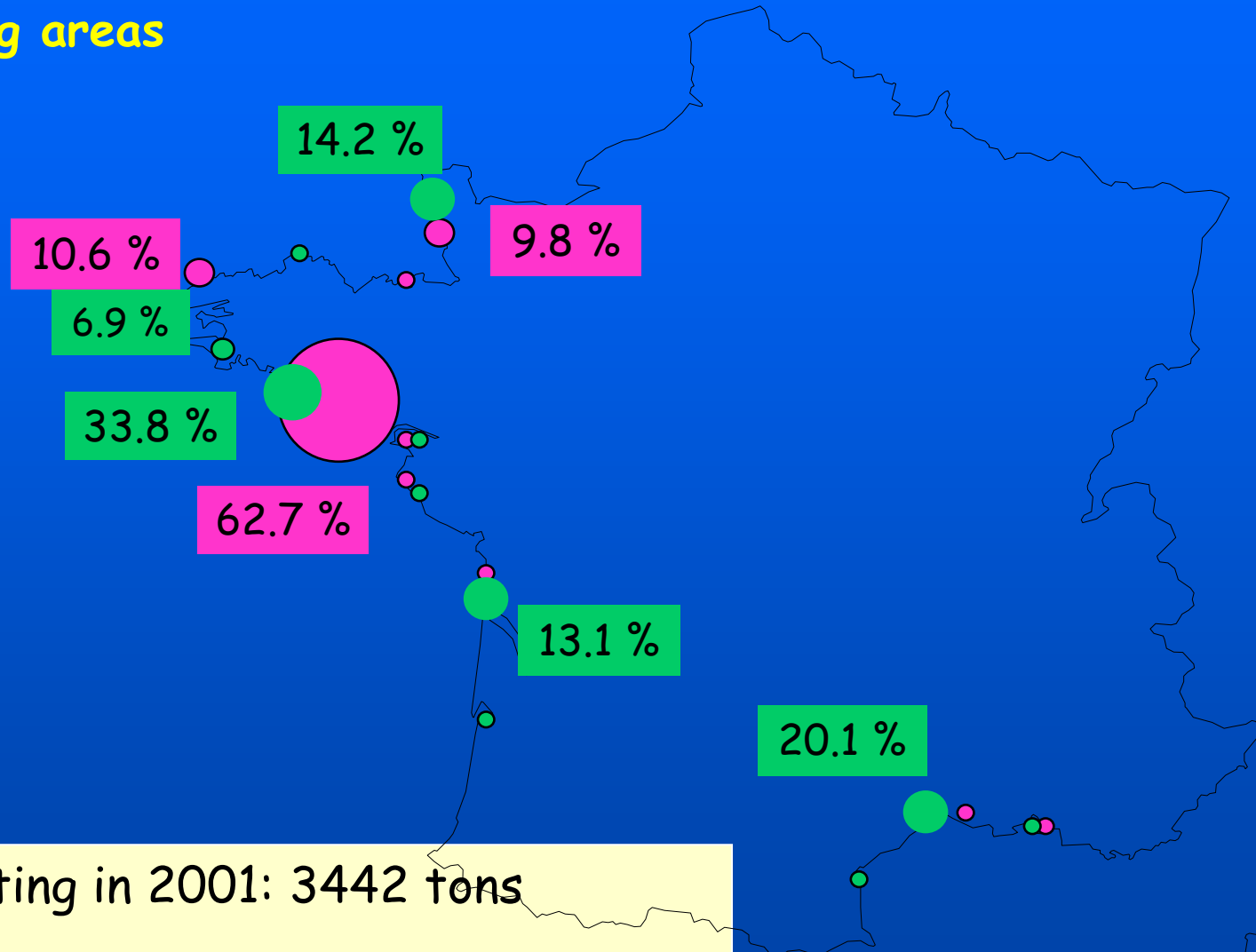
✓ **2 year** survey

## Methods - Parameters and analytical method

- Clam identification and biometry
- Detection and quantification of *Perkinsus olseni* on gills using the Ray's Fluid Thioglycolate Method (RFTM)
- Calculation of prevalences and 95% CI done using the software Survey toolbox

# Perkinsus survey in clams in 2004-2005 (4)

## Methods - Sampling areas



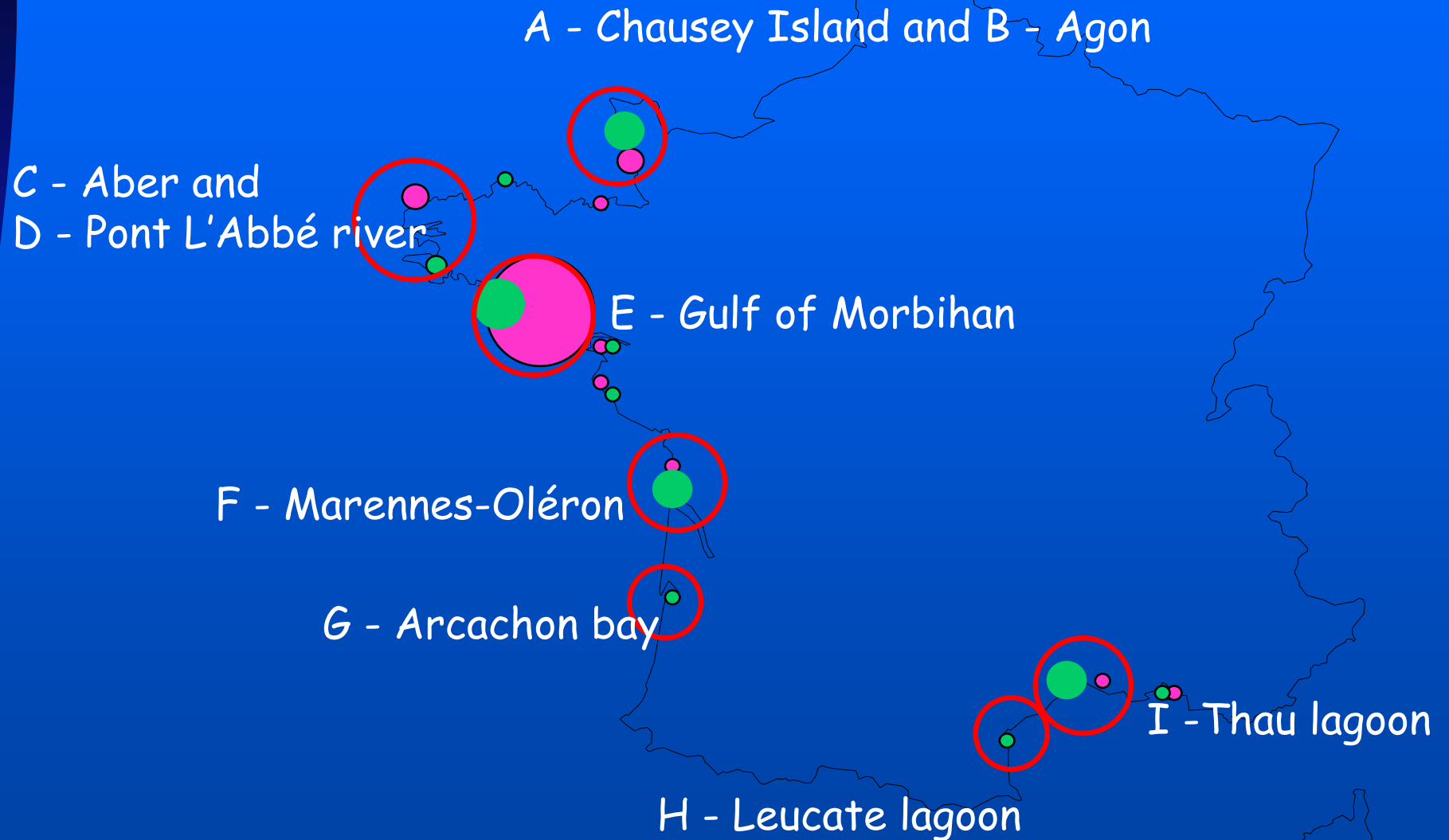
Clam marketing in 2001: 3442 tons

Farmed : 2034 tons 59.1%

Harvested : 1408 tons 40.9%



Sampling areas



Perkinsus survey in clams in 2004-2005 (6)

Results

*Ruditapes philippinarum* versus *R. decussatus*

A (n=220): *R. philippinarum* 100%

B (n=60): *R. philippinarum* 100%

C (n=120):  
*R. philippinarum* 100%

D (n=86):  
*R. philippinarum* 72.1%

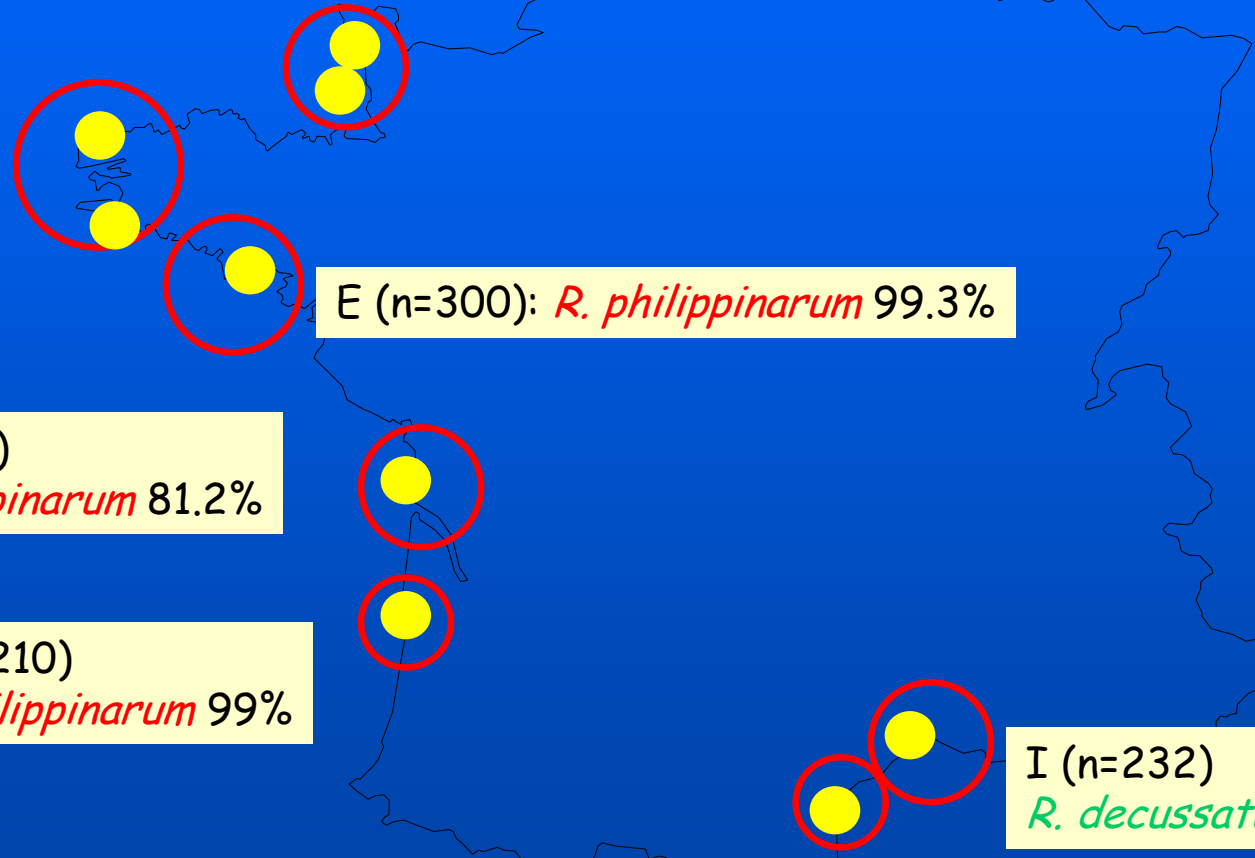
E (n=300): *R. philippinarum* 99.3%

F (n=178)  
*R. philippinarum* 81.2%

G (n=210)  
*R. philippinarum* 99%

H (n=99)  
*R. decussatus* 100%

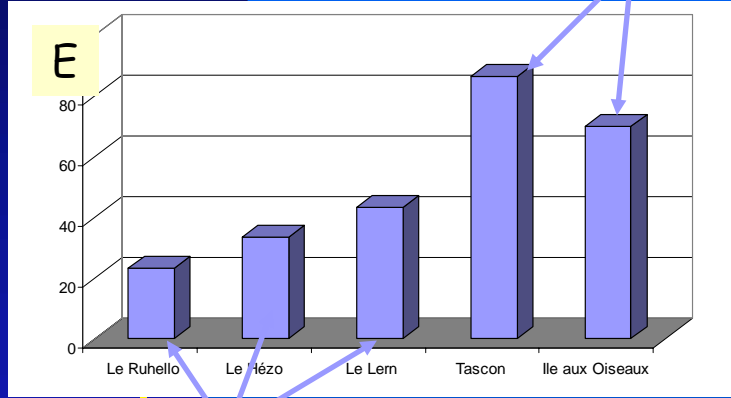
I (n=232)  
*R. decussatus* 99.6%



# Perkinsus survey in clams in 2004 (7)

## Results

P = 78.3%  
95% CI 62.0 - 94.7  
N=2 n=60

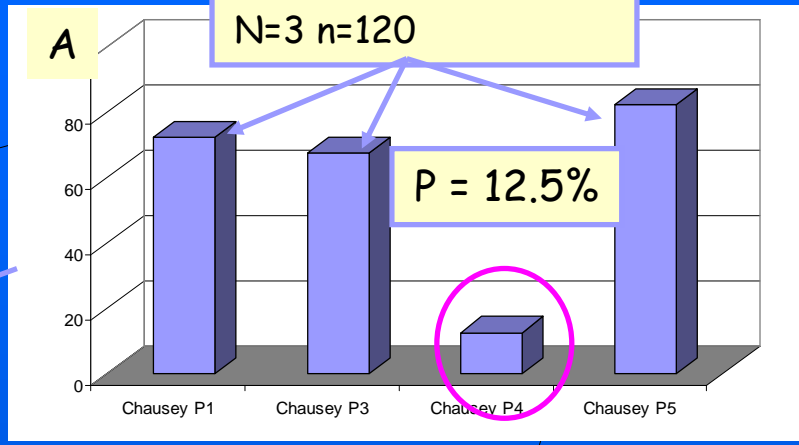


P = 33.3%  
95% CI 22.0 - 44.6  
N=3 n=90

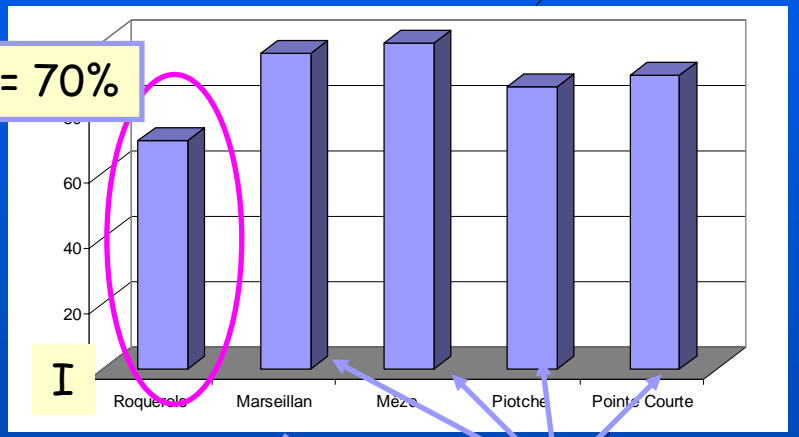
G P = 100%  
N=5 n=150

H P = 100%  
N=3 n=90

P = 73.3%  
95% CI 66.2 - 80.5  
N=3 n=120



P = 70%



P = 93.3%  
95% CI 87.4 - 99.2  
N=4 n=120

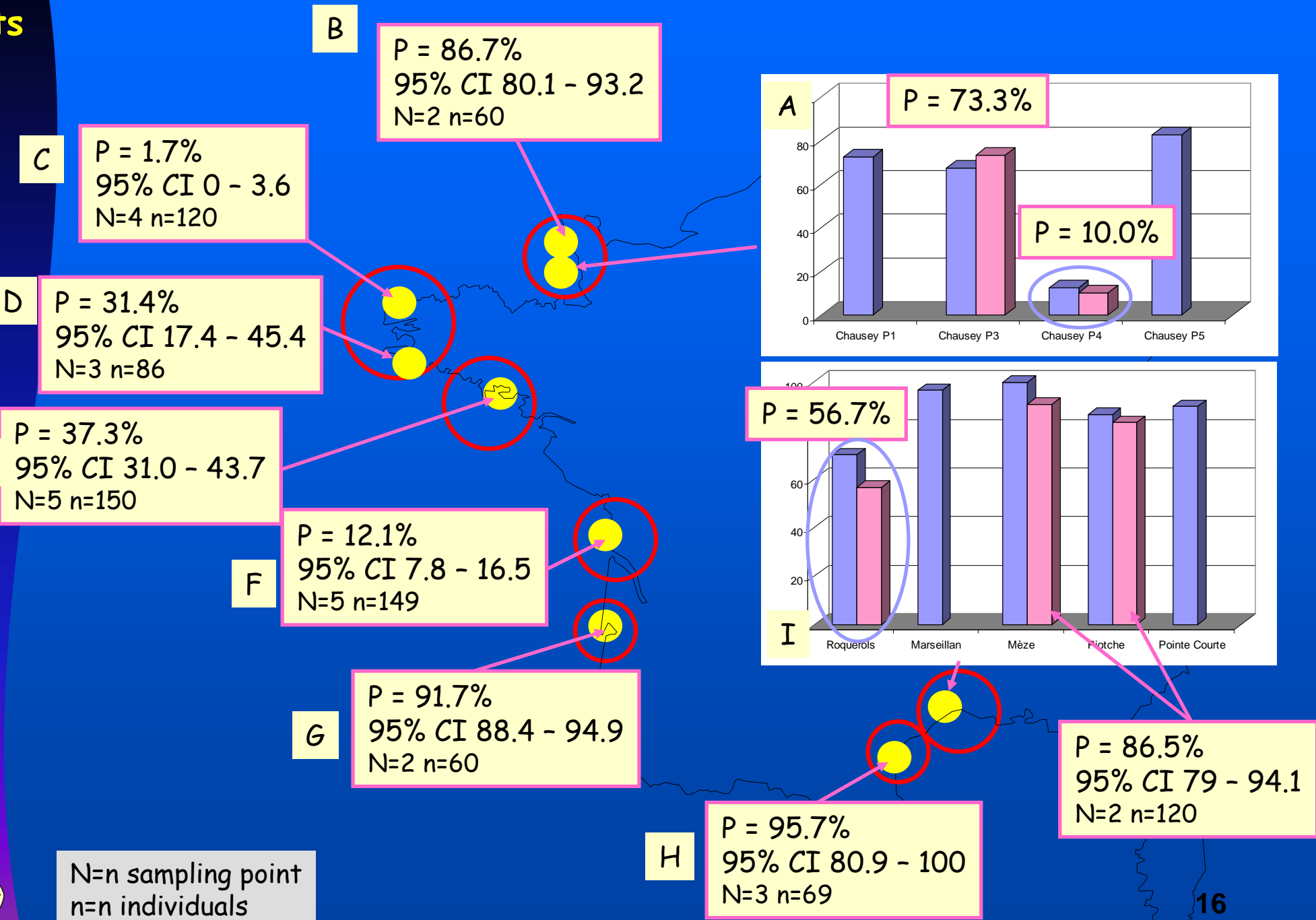


N=n sampling point  
n=n individuals

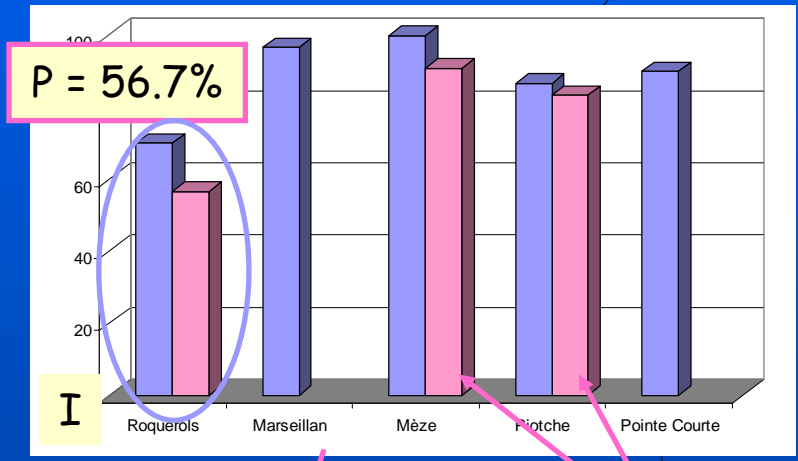
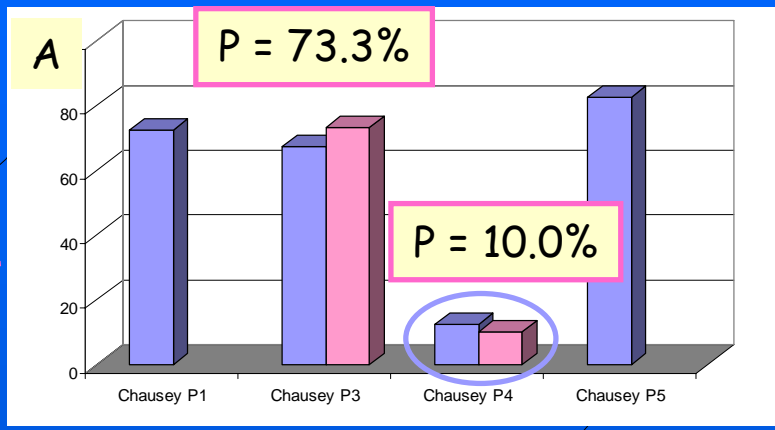
# Perkinsus survey in clams in 2005 (8)

## Results

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N=n sampling point  
n=n individuals



## Discussion - conclusion (1)

*Perkinsus olseni* was present in all clam areas

- ✓ Even in the English Channel
- ✓ Low prevalence in the Far West of France (West Brittany, low temperature too)
- ✓ High prevalence in the South of France (high salinity too)
- ✓ Nevertheless, no abnormal mortality registered during this survey period (could be related to the moderate level of infection intensity)

The detection frequencies seem to be similar in 2005 compared to 2004 except in two areas (Gulf of Morbihan and Arcachon bay) where the prevalence was slightly reduced in 2005 compared to 2004.

Further analyses on collected data (on environmental parameters, on shellfish density, on *Perkinsus* isolates,...)



## Discussion - conclusion (2)

The **Active Targeted Survey**: a risk-based strategy in accordance with the recommendations of the future EU regulation

In 2006 - 2007 the next pathogen/host pair:

### *Pathogens*

*Marteilia maurini*  
*Marteilia refringens*

### *Hosts*

*Mytilus edulis*  
*Mytilus galloprovincialis*



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Thank you for your attention

