MARINE MOLLUSC PATHOGENS

Fact sheet

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summary

SHEETS

MARINE MOLLUSC PATHOGENS

The ostreid herpesvirus type 1 (OsHV-1)

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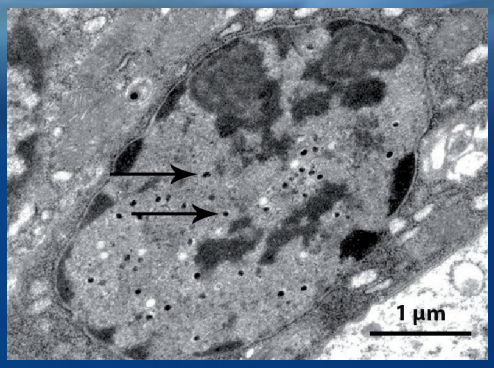
FACT SHEET 1

OsHV-1 is an enveloped double stranded DNA virus that infects oysters and other bivalve molluscs. Since the early 1990s, this virus has been regularly **associated with mortality events in the Pacific oyster** (*Crassostrea gigas*), especially in spat and juveniles. Since 2008, mortality outbreaks have reached proportions largely exceeding any previous oyster mortality events in France (all French oyster farming areas are affected). Adult oysters seem to be less susceptible to infection but can be asymptomatic carriers and might play a role in the spread of the disease. Two main genotypes of the virus have been associated with mass mortality events in France: the reference genotype, dominant before 2009, and the **µVar (microVariant) genotype**, dominant since 2009. This virus has also been detected outside mortality events.

The development of viral infections is influenced by temperature. In France, high OsHV-1 associated mortalities occur in spring and summer, when coastal waters become warmer.



©Ifremer/T. Renault

Presence of viral capsids in the nucleus of an infected Pacific oyster cell, observed under a transmission electronic microscope.

GEOGRAPHIC DISTRIBUTION OF THE OSHV-1 VIRUS

detected during mollusc mortality events

DIAGNOSTIC METHODS

Clinical signs are non-specific, although adductor muscles may be weak, making it difficult for the oyster to close its shell.

- The most common method for detecting the OsHV-1 virus: a quantitative polymerase chain reaction (qPCR) analysis to amplify and detect viral DNA in mollusc tissues.
- Observation of ultra-thin sections of (mollusc) tissue under a transmission electron microscope.

MAIN SUSCEPTIBLE SPECIES

*not found in Europe

Animal health regulations

EU

National regulations in Ireland and in the United Kingdom:

Article 43 of Council Directive 2006/88/EC, Commission Decision 2010/221/EU amended by Decisions 2013/213/EU, 2014/250/EU and 2016/169/EU.

OIE

Aquatic Animal Health Code (OIE, 2017)

Manual of Diagnostic Tests for Aquatic Animals, *chapter 2.4.5* (OIE, 2017)

OIE : World Organisation for Animal Health

Key dates

1972 — **United States of America:** first detection of a herpes-like virus in the eastern oyster *(Crassostrea virginica).*

1991 — **France:** mortality events in Pacific oysters, associated with the detection of a herpes-like virus (identified later), observed in 1991 in hatcheries, and in 1993 in the natural environment.

1993 — **France:** mortality events in Pacific oysters, associated with the detection of a herpes-like virus (identified later), observed in 1991 in hatcheries, and in 1993 in the natural environment.

1995 — Purification of OsHV-1 and partial characterisation of its genome.

2005 — Publication of the entire OsHV-1 genome.

2010 — Characterisation of the **µVar** genotype.

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MARINE MOLLUSC PATHOGENS

The *Vibrio aestuarianus* bacterium

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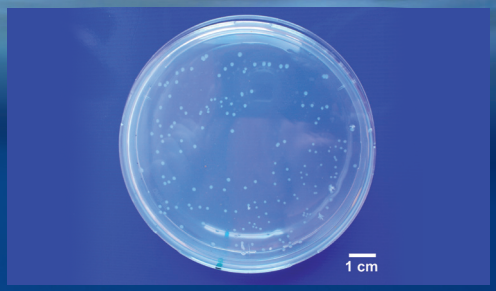
FACT SHEET 2

The bacterial species ***Vibrio aestuarianus*** infects **oysters**, as well as **other bivalve molluscs**. It is a rod-shaped bacterium that can swim in liquids. This species includes **virulent and non-virulent strains**. In France, mortality events of Pacific oysters (*Crassostrea gigas*) that occurred in 2001 were associated with this bacterial species. Its frequency of detection has increased since 2012, and this species is still associated with Pacific oyster mortality events. Susceptibility to this disease increases with oyster age and weight.

Several *V. aestuarianus* strains were also detected in cockles (*Cerastoderma edule*) in 2012 and 2015.



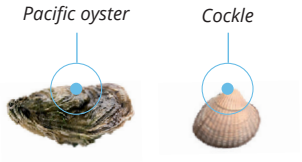
©Ifremer/M.-A. Travers

Colonies of *Vibrio aestuarianus* (each dot is a colony): observed on bacterial culture medium (agar supplemented with nutrients).

GEOGRAPHIC DISTRIBUTION OF *VIBRIO AESTUARIANUS*

detected during mollusc mortality events

MAIN SUSCEPTIBLE SPECIES



Animal health regulations

EU: Not regulated

OIE: Not regulated

OIE : World Organisation for Animal Health

DIAGNOSTIC METHODS

Clinical signs are non-specific, although adductor muscles may be weak, making it difficult for the oyster to close its shell.

- Mollusc tissues are ground and cultured to isolate the bacteria. A molecular biology technique, called polymerase chain reaction (PCR) is performed on the isolated bacteria to detect *Vibrio aestuarianus* DNA.
- The bacterial strain can be more precisely characterised by sequencing part of the bacterial genome.

Key dates

1983 — **United States of America:** isolation of the reference bacterial strain in oysters.

2001 — Detection of certain *Vibrio aestuarianus* strains, associated with mass mortality events of the Pacific oyster (*C. gigas*).

2006 — Detection of V. *aestuarianus* strains with non-characterised virulence in China and New Zealand.

2010 — First detection of V. *aestuarianus* strains associated with mortality of cockles (*C. edule*).

2012

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MARINE MOLLUSC PATHOGENS

Bacteria in the *Vibrio Splendidus* group

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FACT SHEET 3

The ***Vibrio splendidus*** group belong to the ***Vibrio* genus** and includes several bacterial species. They are small, motile, rod-shaped bacteria found in marine sediments, in seawater and in molluscs. This group contains **highly diverse species** (17 species are currently recognised), including **virulent and non-virulent strains**. The main species associated with mollusc mortality events are *V. splendidus, V. crassostreae and V. tasmaniensis.*

In France, the detection of this group of bacteria has often been associated with Pacific oyster (*Crassostrea gigas*) mortalities and mussel (*Mytilus edulis*) mortalities.

The *V. splendidus* group of bacteria seem to preferentially infect spat (oysters less than 1 year old) and part-grown (18 month-old) oysters.

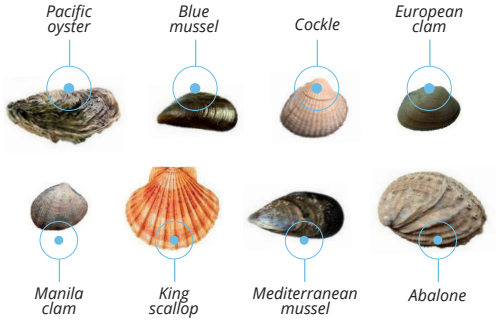
©Ifremer/C. Dubreuil

Colonies of *Vibrio splendidus* cultured on solid medium (agar supplemented with nutrients).

GEOGRAPHIC DISTRIBUTION OF THE *V. SPLENDIDUS* GROUP OF BACTERIA

detected during mollusc mortality events

MAIN SUSCEPTIBLE SPECIES



DIAGNOSTIC METHODS

Clinical signs are non-specific.

- Mollusc tissues are ground and cultured to isolate the bacteria.
- A molecular biology technique, called polymerase chain reaction (PCR), is performed on the isolated bacteria to detect *V. splendidus* group bacteria.
- The bacterial strains are then determined by sequencing part of the bacterial genome.

Animal health regulations

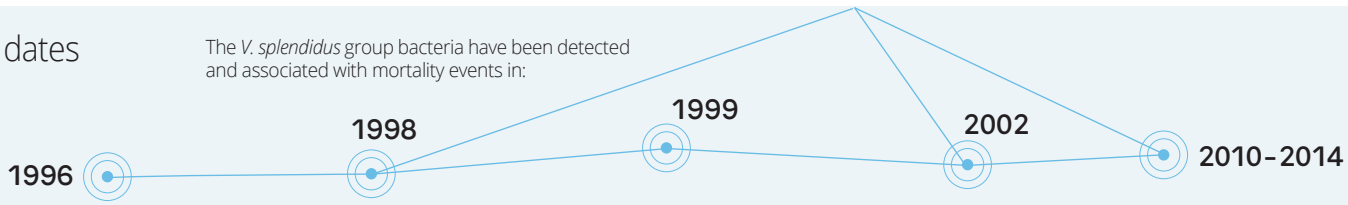
EU: Not regulated

OIE: Not regulated

OIE : World Organisation for Animal Health

Key dates

The *V. splendidus* group bacteria have been detected and associated with mortality events in:



in France: in the king scallop (*Pecten maximus*).

in Japan: in the Pacific oyster (*C. gigas*).

in France: in the Pacific oyster (*C. gigas*).

in France: in clams (*Ruditapes* sp.).

in France: in mussels (*Mytilus* sp.).

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MARINE MOLLUSC PATHOGENS

The protozoan parasite *Bonamia ostreae*

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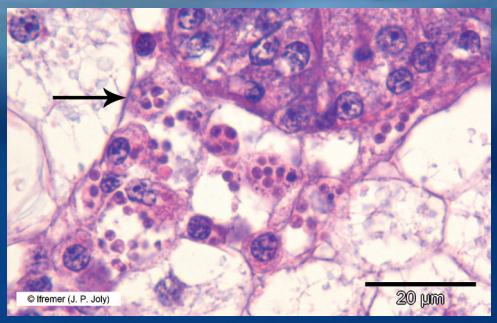
FACT SHEET 4

Bonamia ostreae is a unicellular parasite. This parasite is mainly intracellular and infects cells in the circulatory system (i.e. haemocytes, immune system cells) in the European flat oyster. It causes **bonamiosis**. In France, the first detection of this parasite (1979) was associated with **mass mortality in flat oysters** in Brittany (north-western France). This parasite can be observed in all life stages of the flat oyster, including larvae, spats and adults older than 1 year; nevertheless, adult oysters are more susceptible to this disease.

Bonamiosis prevalence* can peak at the end of winter. **This parasite is transmitted directly between oysters** via seawater.

***Disease prevalence** is a statistical concept referring to the number of cases of a disease that are present in a particular population at a given time.



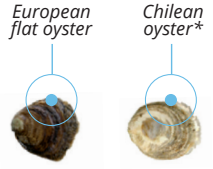
©Ifremer/J.-P. Joly

The parasite *Bonamia ostreae* observed inside haemocytes in the mantle (connective tissue) of a flat oyster (*Ostrea edulis*).

GEOGRAPHIC DISTRIBUTION OF *BONAMIA OSTREAE*

detected during mollusc mortality events

MAIN SUSCEPTIBLE SPECIES



*not found in Europe

DIAGNOSTIC METHODS

Clinical signs are generally non-specific, but lesions on gills can occur.

- Histological sections, particularly of gills and cardiac tissues, are observed under a light microscope to check for the presence* of *Bonamia* parasites.
- It is then possible to identify the species (*B. ostreae*) using molecular biology techniques, namely the polymerase chain reaction (PCR) and partial genome sequencing.

*The parasite can be more readily localised in host (oyster) tissues using *in situ* hybridisation (ISH).

Animal health regulations

EU

NOTIFIABLE DISEASE
Directive 2006/88/EC (Annex IV, Part II)

NOTIFIABLE DISEASE
Aquatic Animal Health Code (OIE, 2017).
Manual of Diagnostic Tests for Aquatic Animals, *chapter 2.4.3* (OIE, 2017).

OIE : World Organisation for Animal Health

Key dates

1979
France: high flat oyster mortality associated with the detection of *Bonamia ostreae*

1980's
Spreads along French and European coasts, with a substantial decline in flat oyster production.

1991
Becomes a **compulsorily notifiable non-exotic disease** (Council Directive 91/67/EEC).

2015
Detection of *Bonamia ostreae* in New Zealand

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MARINE MOLLUSC PATHOGENS

The protozoan parasite *Bonamia exitiosa*

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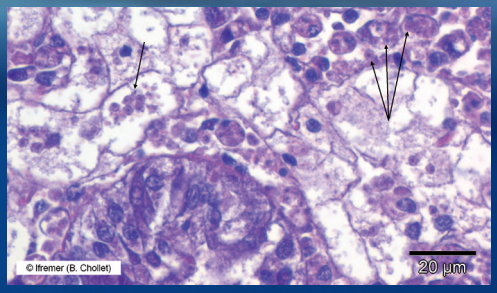
FACT SHEET 5

Bonamia exitiosa is a unicellular parasite. This parasite is mainly intracellular and infects cells in the circulatory system (i.e. haemocytes, immune system cells) in **bivalve molluscs**. It causes **bonamiosis**. This parasite was first detected in 1985 in New Zealand in association with mass mortality of the Chilean flat oyster (*Ostrea chilensis*).

This parasite was first **detected in Europe (Spain) in 2007** in the **European flat oyster** (*Ostrea edulis*), and subsequently in other European countries.

This parasite is **transmitted directly** between oysters via seawater.



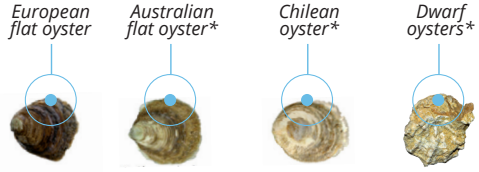
©Ifremer/B. Chollet

The parasite *Bonamia exitiosa* observed in haemocytes in the mantle (connective tissue) of a flat oyster (*Ostrea edulis*).

GEOGRAPHIC DISTRIBUTION OF *BONAMIA EXITIOSA*

detected during mollusc mortality events

MAIN SUSCEPTIBLE SPECIES



*not found in Europe

DIAGNOSTIC METHODS

Clinical signs are non-specific, but lesions on gills can occur.

- Histological sections, particularly of gills and cardiac tissues, are observed under a light microscope to check for the presence* of *Bonamia* parasites.
- It is then possible to identify the parasite species using molecular biology techniques: the polymerase chain reaction (PCR) and partial genome sequencing.

*The parasite can be more readily localised in host (oyster) tissues using *in situ* hybridisation (ISH).

Animal health regulations

EU

NOTIFIABLE DISEASE
Directive 2006/88/EC (Annex IV, Part II)

OIE

NOTIFIABLE DISEASE
Aquatic Animal Health Code (OIE, 2017).
Manual of Diagnostic Tests for Aquatic Animals, *chapter 2.4.2* (OIE, 2017).

OIE : World Organisation for Animal Health

Key dates

1985 — **New Zealand:** mass mortality of Chilean flat oysters (*Ostrea chilensis*) associated with the detection of *Bonamia exitiosa.*

1998 — Second epizootic event in New Zealand.

2006 — Becomes a **compulsorily notifiable exotic disease** (Council Directive 2006/88/EC).

2007 — **Spain:** detection in the European flat oyster (*Ostrea edulis*).

Since 2008 — Detection in France, Italy, the United Kingdom, Tunisia, Turkey, Portugal and Croatia.

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MARINE MOLLUSC PATHOGENS

The protozoan parasite *Marteilia refringens*

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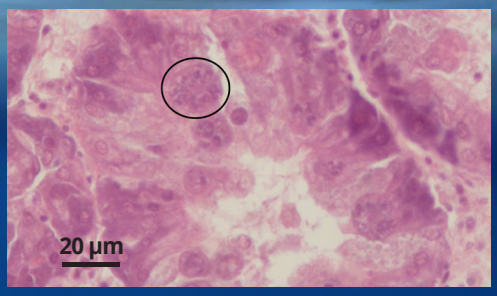
FACT SHEET 6

Marteilia refringens is a unicellular parasite that infects mainly the epithelium of the **digestive tract in bivalve molluscs. In France**, the first detection of this parasite (1967) was associated with **mass mortality of the flat oyster** (*Ostrea edulis*) in Brittany (north-western France).

Marteiliosis is a **seasonal disease** whose transmission seems to depend on temperature.

This parasite cannot be directly transmitted between bivalve molluscs. The transmission of the disease appears to require the infection of an intermediate host: a planktonic copepod (*Paracartia grani*) is suspected to host the intermediate developmental stages of *Marteilia refringens*.

There are two types of *Marteilia refringens*: the type O, more often detected in oysters, and the type M, more often detected in mussels. Recent studies suggest that those two types correspond to distinct species.



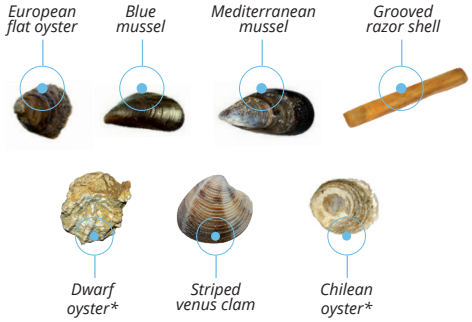
©Ifremer/B. Chollet

The parasite *Marteilia refringens* observed in digestive diverticula (in the epithelium) of a flat oyster (*Ostrea edulis*).

GEOGRAPHIC DISTRIBUTION OF *MARTEILIA REFRINGENS*

detected during mollusc mortality events

MAIN SUSCEPTIBLE SPECIES



*not found in Europe

Marteilia refringens has been reported in other bivalves species but host susceptibility was not always confirmed

DIAGNOSTIC METHODS

Clinical signs are non-specific, such as tissue necrosis, emaciation, pale digestive gland.

- Histological sections, particularly of the digestive gland, are observed under a light microscope to check for the presence* of *Marteilia* parasites.
- It is then possible to determine the species (*M. refringens*) using molecular biology techniques: the polymerase chain reaction (PCR) and partial genome sequencing.

*The parasite can be more readily localised in host (the bivalve mollusc) tissues using *in situ* hybridisation (ISH).

Animal health regulations

EU **NOTIFIABLE DISEASE**
Directive 2006/88/EC (Annex IV, Part II)

OIE **NOTIFIABLE DISEASE**
Aquatic Animal Health Code (OIE, 2017).
Manual of Diagnostic Tests for Aquatic Animals, *chapter 2.4.4* (OIE, 2017).

OIE : World Organisation for Animal Health

Key dates

1967
France: oyster (*Ostrea edulis*) mass mortality associated with the detection of *Marteilia refringens*.

1970
Spreads along French and Spanish coasts, with a substantial decline in flat oyster production.

1991
Becomes a **compulsorily notifiable non-exotic disease** (Council Directive 91/67/EEC).

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MARINE MOLLUSC PATHOGENS

The protozoan parasite *Mikrocytos mackini*

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FACT SHEET 7

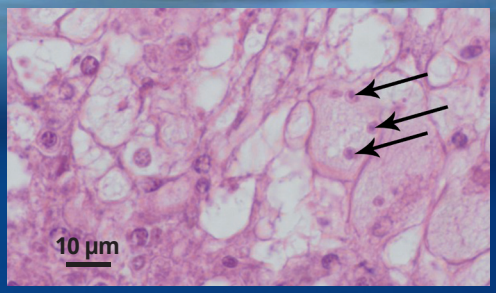
Mikrocytos mackini is a single-celled, intracellular parasite that infects various oyster species. It causes Denman Island disease in oysters. This parasite was first detected in 1960 in association with mortality events in Pacific oysters (*Crassostrea gigas*) on the Pacific coast of Canada (Denman Island).

Susceptibility to this disease increases with oyster age. The prevalence* of infection appears to be linked to water temperature of less than 10°C.

This parasite is **transmitted directly** between oysters via seawater.

This parasite has not been detected in Europe.

***Disease prevalence** is a statistical concept referring to the number of cases of a disease that are present in a particular population at a given time.



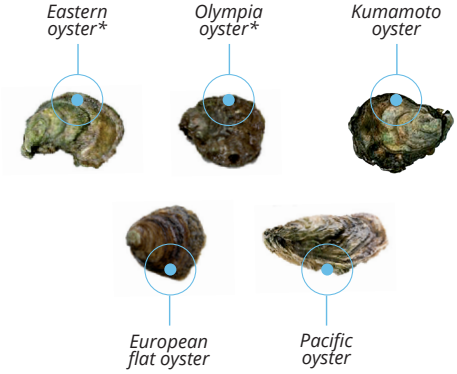
©Ifremer/B. Chollet

The parasite *Mikrocytos mackini* observed in labial palps (in connective tissues) of a Pacific oyster (*Crassostrea gigas*).

GEOGRAPHIC DISTRIBUTION OF *MIKROCYTOS MACKINI*

detected during mollusc mortality events

MAIN SUSCEPTIBLE SPECIES



*not found in Europe

DIAGNOSTIC METHODS

Clinical signs are non-specific, with pustules, abscesses or variation in organ colouration observed in severely affected individuals.

- Histological sections of mollusc tissues are observed under a light microscope to check for the presence* of *Mikrocytos* parasites.
- It is then possible to determine the species (*M. mackini*) using molecular biology techniques: the polymerase chain reaction (PCR) and partial genome sequencing.

*The parasite can be more readily localised in host (oyster) tissues using *in situ* hybridisation (ISH).

Animal health regulations

EU: **NOTIFIABLE DISEASE**
Directive 2006/88/EC (Annex IV, Part II)

OIE: Not regulated
OIE : World Organisation for Animal Health

Key dates

1960

Canada: first detection of *Mikrocytos mackini* associated with mass mortality in Pacific oysters (*Crassostrea gigas*).

2006

Becomes a **compulsorily notifiable exotic disease** (Council Directive 2006/88/EC).

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MARINE MOLLUSC PATHOGENS

The protozoan parasite *Perkinsus marinus*

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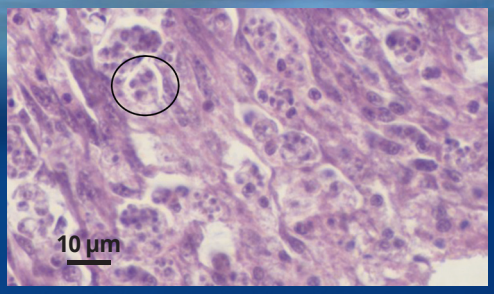
FACT SHEET 8

Perkinsus marinus is a single-celled, partially intracellular parasite that infects various oyster species. It causes **perkinsiosis** or **Dermo disease** (in reference to its original name, *Dermocystidium marinum*). This parasite, detected in the United States of America for the first time in 1946, has been associated with mass mortality events in Eastern oyster (*Crassostrea virginica*).

The prevalence* of infection appears to depend on water temperature and salinity. This parasite is **transmitted directly** between oysters via seawater.

This species, ***P. marinus*, has not been detected in Europe.**

***Disease prevalence** is a statistical concept referring to the number of cases of a disease that are present in a particular population at a given time.



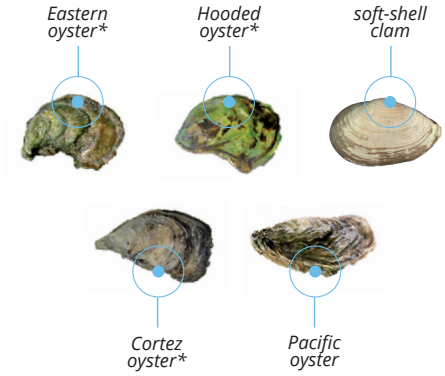
©Ifremer/B. Chollet

The parasite *Perkinsus marinus* observed in the digestive tract (epithelium) of an Eastern oyster (*Crassostrea virginica*).

GEOGRAPHIC DISTRIBUTION OF *PERKINSUS MARINUS*

detected during mollusc mortality events

MAIN SUSCEPTIBLE SPECIES



*not found in Europe

The American oyster is the most susceptible host

DIAGNOSTIC METHODS

Clinical signs are non-specific, with pale digestive gland, shrinkage of the mantle, occasional presence of pus-like pockets.

- Culturing mollusc tissues in Ray's fluid thioglycollate medium (RFTM) causes *Perkinsus* cells to swell.
- Histological sections of RFTM-cultured tissues are stained and observed under a light microscope*; *Perkinsus* cells are dilated and can be counted (although the species cannot be determined).
- It is then possible to determine the species (*P. marinus*) using molecular biology techniques: the polymerase chain reaction (PCR) and partial genome sequencing.

*The parasite can be more readily localised in host (oyster) tissues using *in situ* hybridisation (ISH).

Animal health regulations

EU **NOTIFIABLE DISEASE**
Directive 2006/88/EC (Annex IV, Part II)

OIE **NOTIFIABLE DISEASE**
Aquatic Animal Health Code (OIE, 2017).
Manual of Diagnostic Tests for Aquatic Animals, *chapter 2.4.6* (OIE, 2017).

OIE : World Organisation for Animal Health

Key dates

1946 — **United States of America (Gulf of Mexico):** mass mortality of American oyster (*Crassostrea virginica*).

1948 — Discovery of globules (spherical bodies) in the tissues of oysters affected by infections in 1946.

1952 — Development of a tissue culture technique by S.M. Ray (RFTM) to detect *Perkinsus* and evaluate the intensity of infection in oysters.

2006 — Becomes a **compulsorily notifiable exotic disease** (Council Directive 2006/88/EC).

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