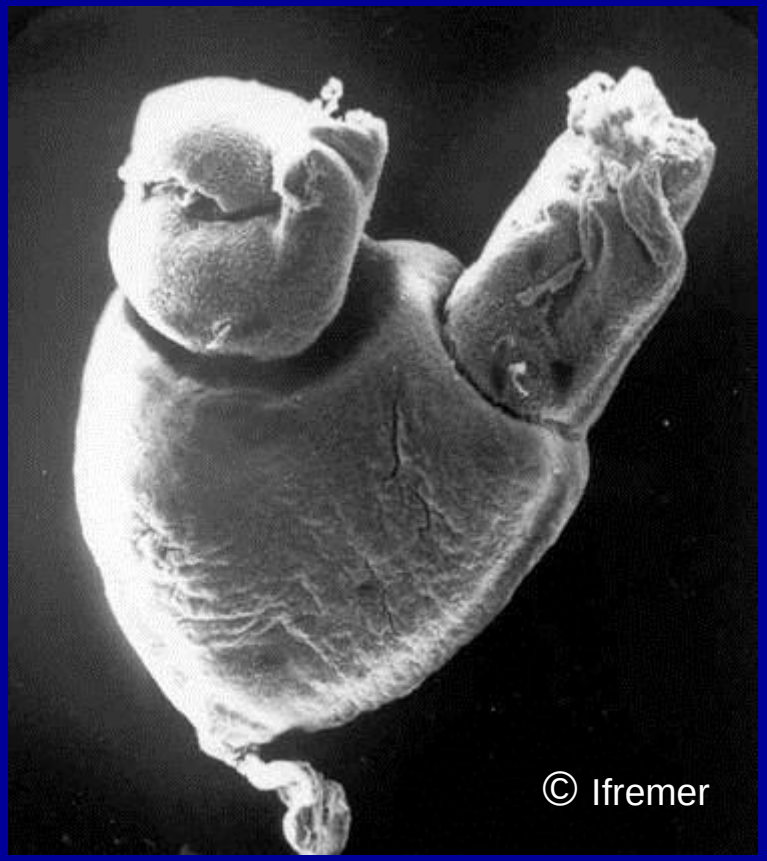
Infection with *Bonamia ostreae*



Heart from *Ostrea edulis* oyster

General information

➔ Category of the disease

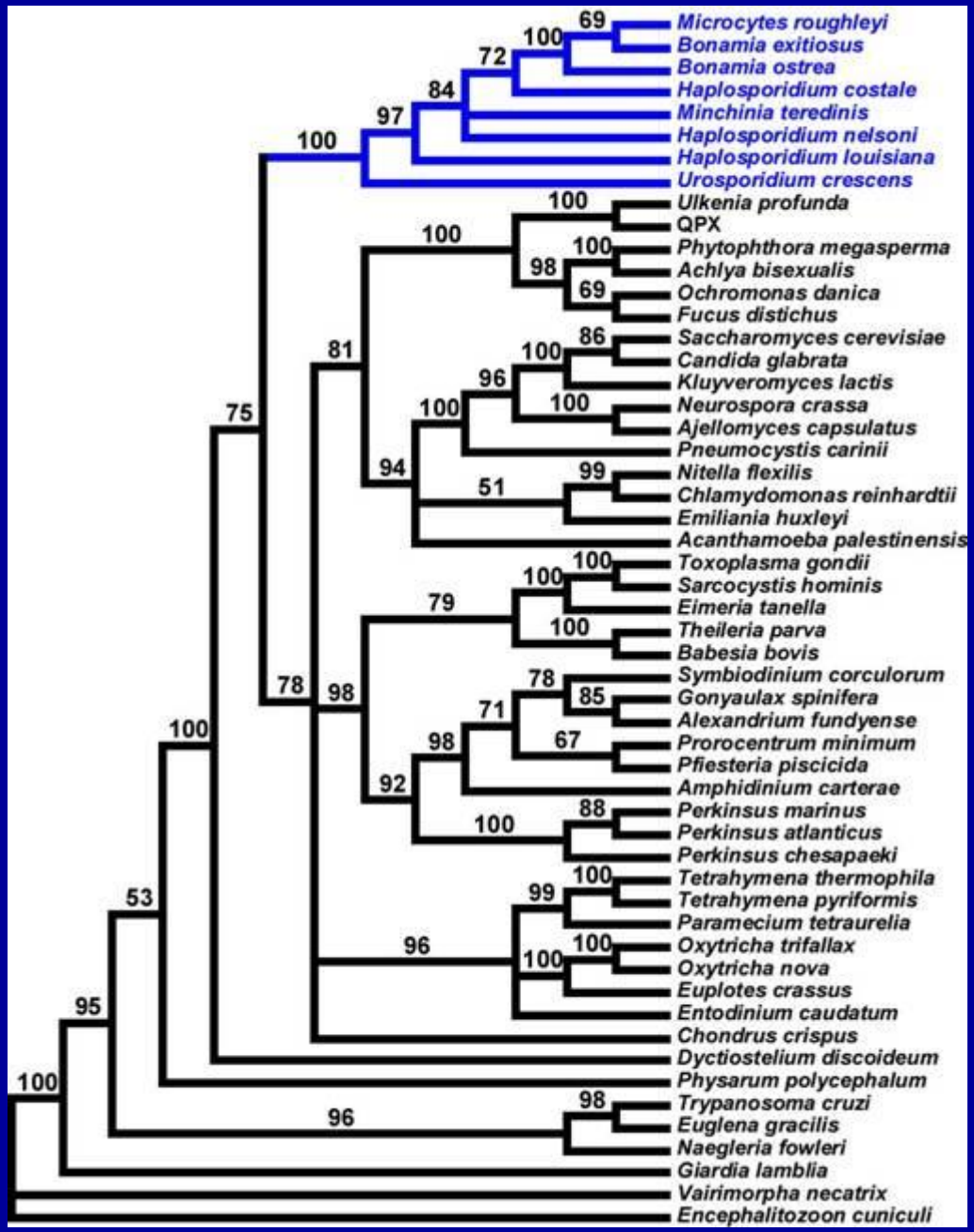
notifiable to the OIE and listed in Directive 2006-088

➔ Common, generally accepted names of the disease agent

Microcell disease, Bonamiasis, Haemocyte disease of flat oyster, Haemocytic parasitosis.

➔ Scientific name or taxonomic affiliation of the causative agent *Bonamia ostreae*.

Results of initial ulturastructural studies suggesting that this protist was affiliated with the Haplosporidia despite the lack of a spore stage (Bonami et al. 1985, Brehélin et al. 1982) were subsequently confirmed by DNA analysis (Carnegie & Cochennec 2004, Lopez-Flores et al. 2007)



Haplosporidian

Cochennec et al. 2003

Infection with *Bonamia ostreae*

Host species

➔Natural host

Ostrea edulis

➔Experimental transmission

Ostrea angasi, *O. chilensis*, (= *Tiostrea chilensis*, *T. lutaria*), *O. puelchana*

Low infectivity of *B. ostreae* to *Crassotrea ariakensis* (Audemard *et al.,* 2005)

Host species

➔ Other *Bonamia* species **:**

➔ *Bonamia exitiosa* and closely related parasites infects *Ostrea chilensis* in New Zealand and Chile, *Ostrea puelchana* in Argentina, *Ostrea angasi* in Australia, *Ostrea edulis* in Europe, *Ostrea stentina* (= *Ostreola equestris*) in Tunisia and North Carolina, U.S.A., *Crassostrea ariakensis* in the context of field trials in Florida, U.S.A. PCR positive results were obtained from *Crassostrea virginica* from Florida

➔ *Bonamia (Mikrocytos) roughleyi* infects *Saccostrea glomerata (commercialis)* in New South Wales, Australia

➔ *Bonamia perspora i*nfects *Ostrea stentina* (= *Ostreola equestris*) in North Carolina, U.S.A.

Host species

➔Species considered not susceptible to and not responsible for transmission

Pacific oyster, *Crassostrea gigas* (Renault et al. 1995), mussels, *Mytilus edulis* and *M. galloprovincialis*, and clams, *Ruditapes decussatus* and *R. philippinarum* do not appear to act as vectors nor alternate hosts for the parasite naturally nor experimentally (Culloty et al. 1999).

Geographical distribution

➔ Europe

along the Atlantic coast of Europe from Spain to Denmark, United Kingdom (excluding Scotland) and Ireland (parts of Ireland)

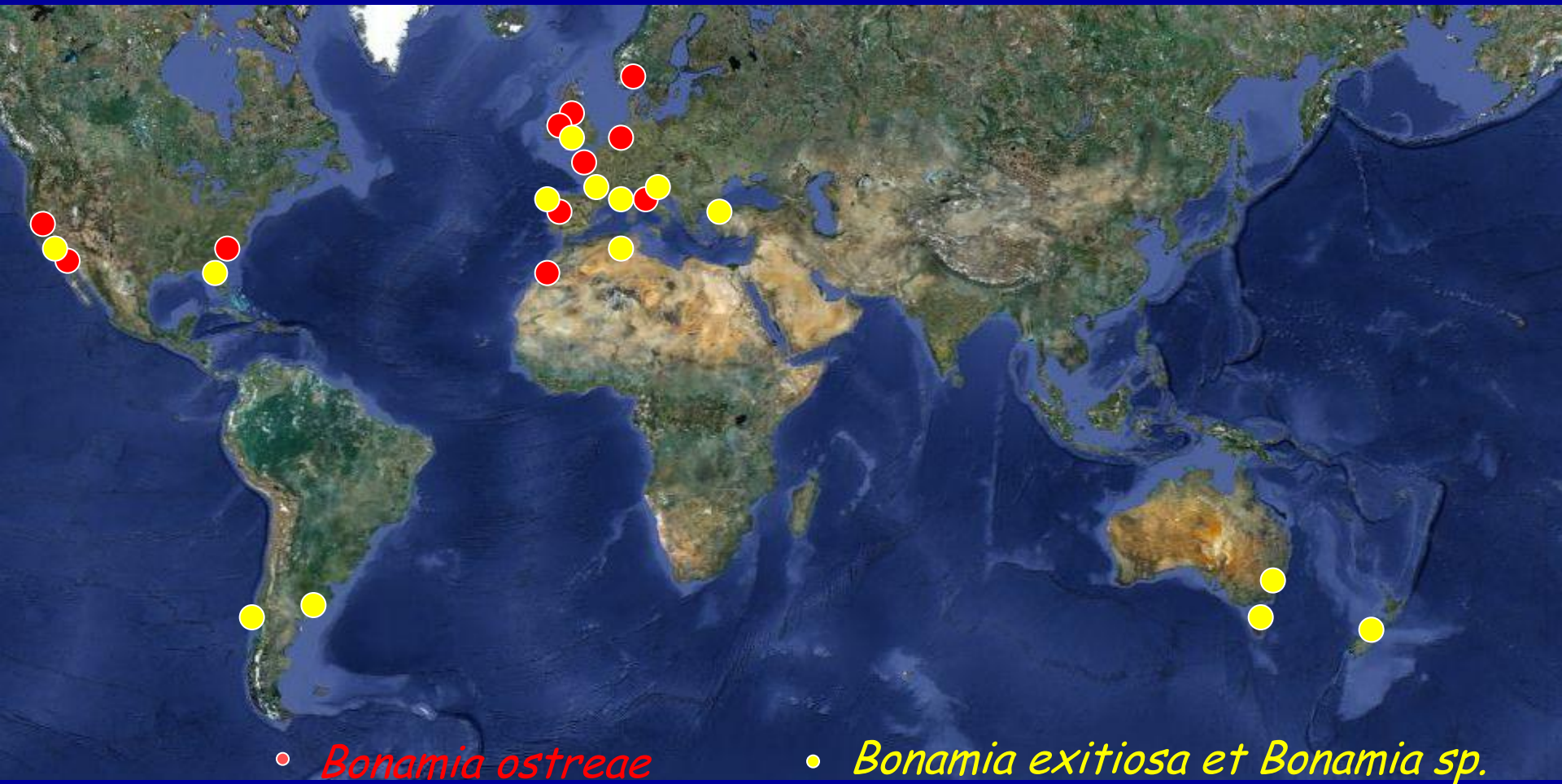
➔ North Africa

-In the lagune de Khnifiss, Morocco

➔ North America.

- In California, Washington state and Maine
- In both Washington and Maine, the prevalence of infection is usually low and heavy infections are rare.
- Current evidence suggests that *B. ostreae* was inadvertently introduced into Maine, Washington and Europe from California by the translocation of infected *Ostrea edulis* in the late 1970s (Grizel 1985, Elston et al. 1986, Friedman & Perkins 1994, Cigarría & Elston 1997).
- In British Columbia, Canada

Geographical distribution



Geographical distribution

Stocks were moved from California to France and Spain; the French outbreak revealed the parasite in Europe

The introduction is believed to have occurred with transfers of flat oysters, Ostrea edulis

Microcells in the vesicular connective tissue cells of Olympia oysters, *Ostrea conchaphila* (=*Ostrea lurida*) from Oregon, USA were speculated to be *B. ostreae* (Farley et al. 1988).

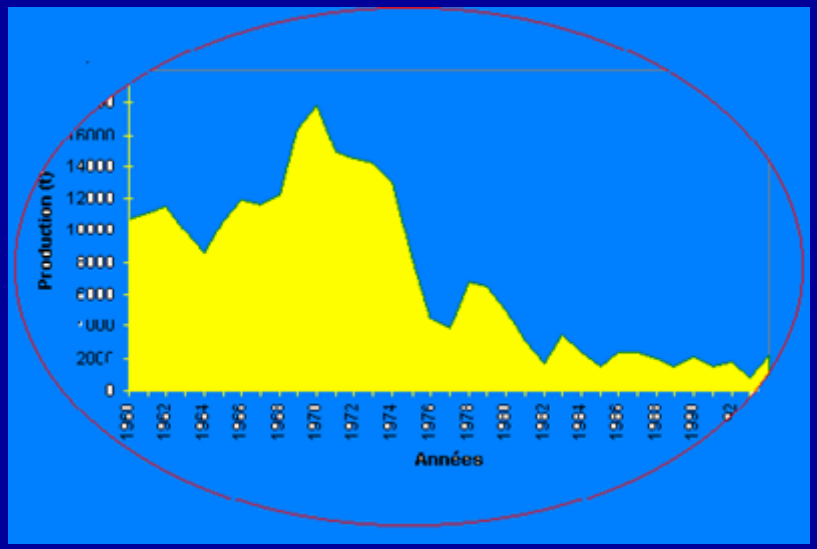
Elston (1990) indicated that although experiments suggest that *O. lurida* may contract the disease, infection has not been positively demonstrated.
Experiment performed in in La Tremblade showed a relative resistance of olympia oysters

Infection with *Bonamia ostreae*

Impact on the host

- *Bonamia ostreae*, in conjunction with earlier epizootics caused by *Marteilia refringens*, caused a drastic drop in the French production of *O. edulis* from 20,000 t per year in the 1970's to 1,800 t in 1995 (Grizel 1985).

- *Bonamia ostreae* has also had a significant negative impact on *O. edulis* production throughout its distribution range in Europe.

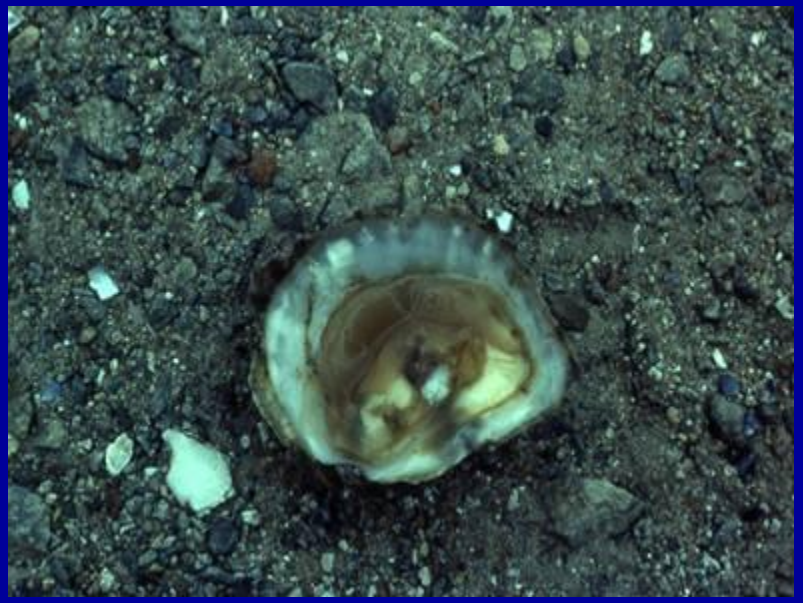


Losses are estimated at about 20% of employment, 240 millions US$ of turn over and 200 millions US$ of added value between 1980 and 1983

Infection with *Bonamia ostreae*

Impact on the host

➔ Although many infected oysters appear normal, others may have yellow discoloration and/or extensive lesions (i.e. perforated ulcers) in the connective tissues of the gills, mantle and digestive gland.



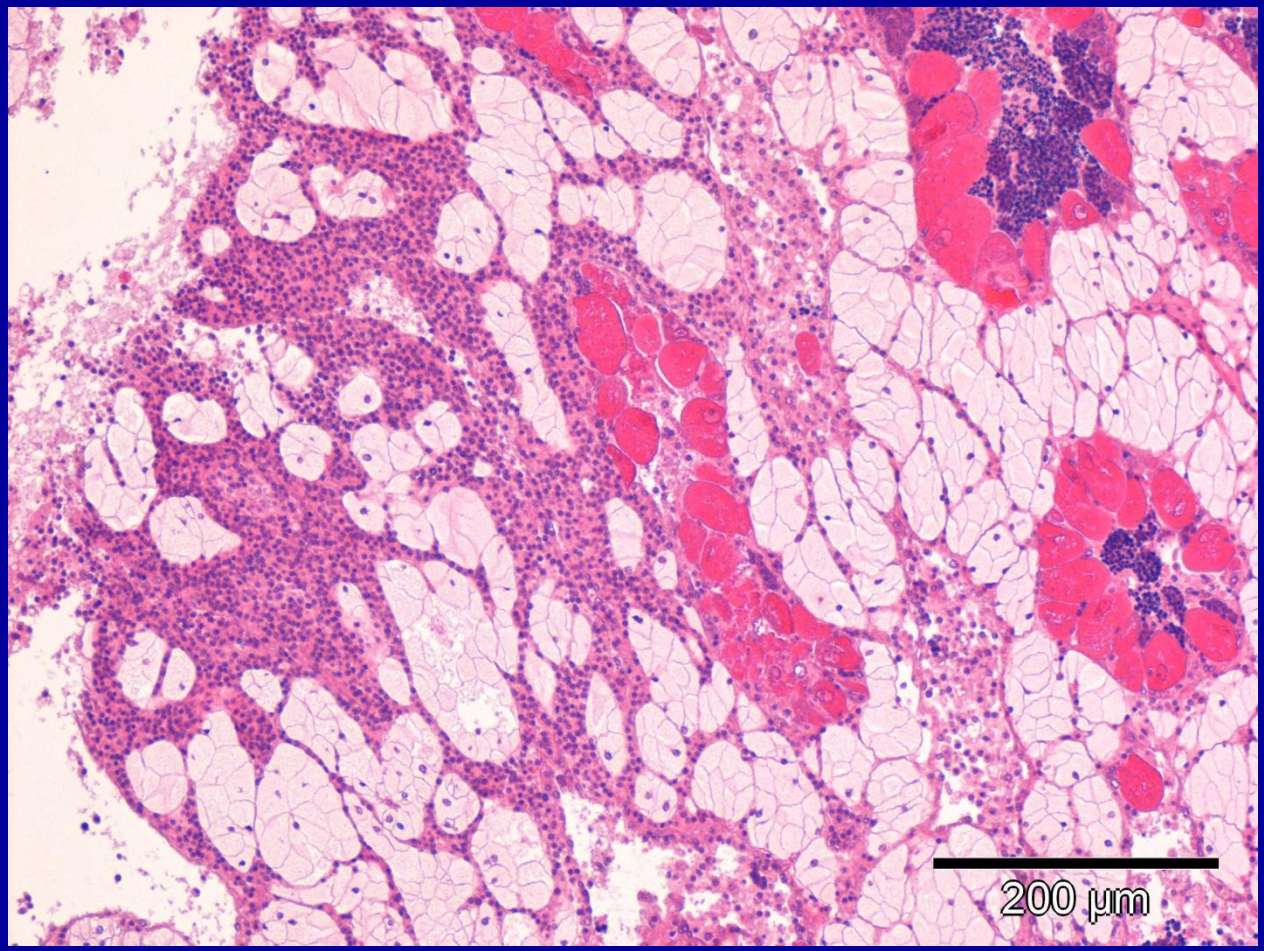
Infection with *Bonamia ostreae*

Impact on the host

- ➔ Actual pathology appears correlated to haemocyte destruction and diapedesis due to proliferation of *B. ostreae* (Balouet et al. 1983). Infection was demonstrated to result in the increase in the number of tissue infiltrating haemocytes (Cochennec-Laureau et al. 2003).

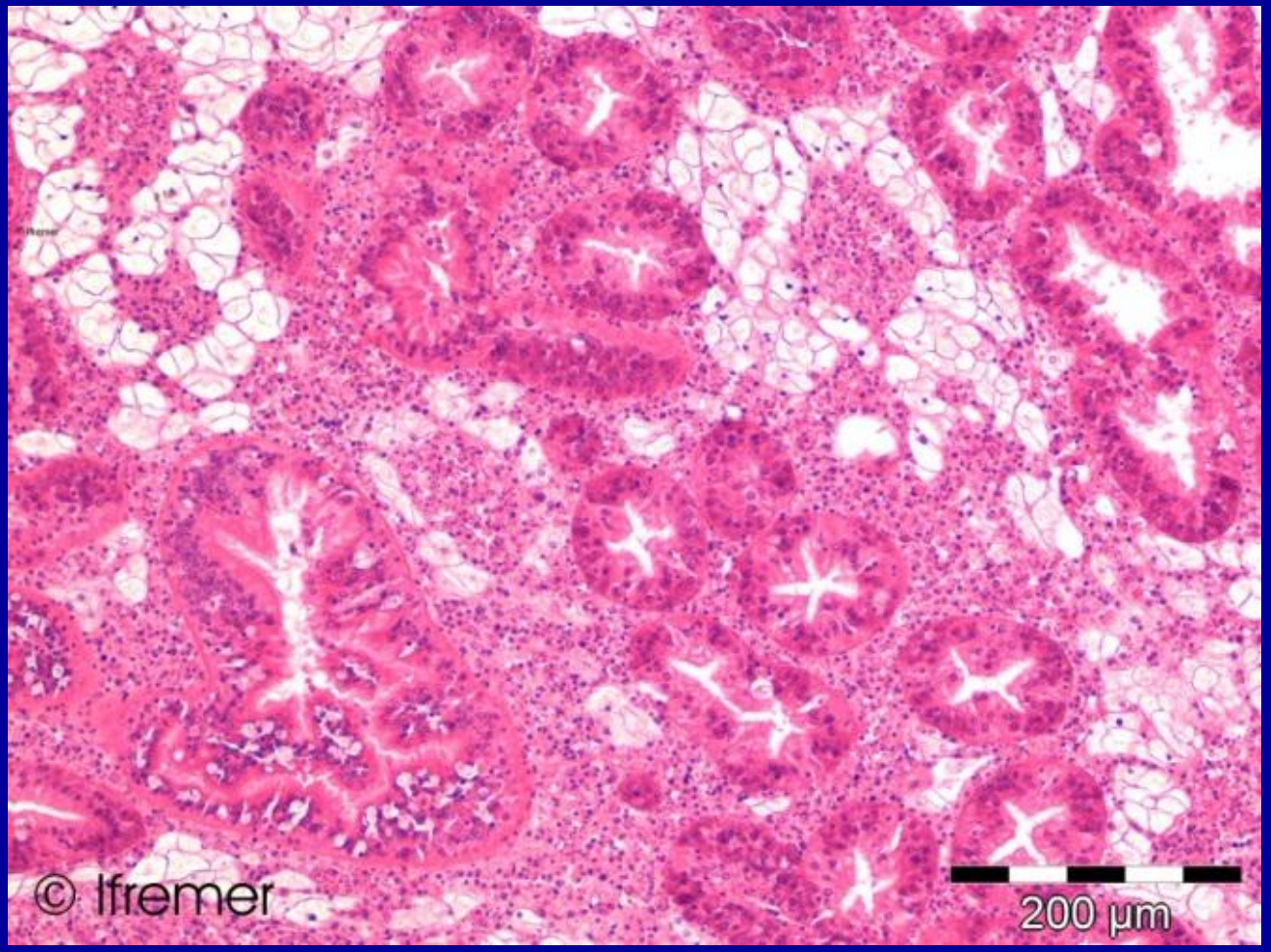
- ➔ Although some flat oysters die with light infections, others succumb to much heavier infections. Heavily infected oysters tend to be in poorer condition than uninfected oysters.

Haemocyte diapedesis and increase in the number of tissue infiltrating haemocytes



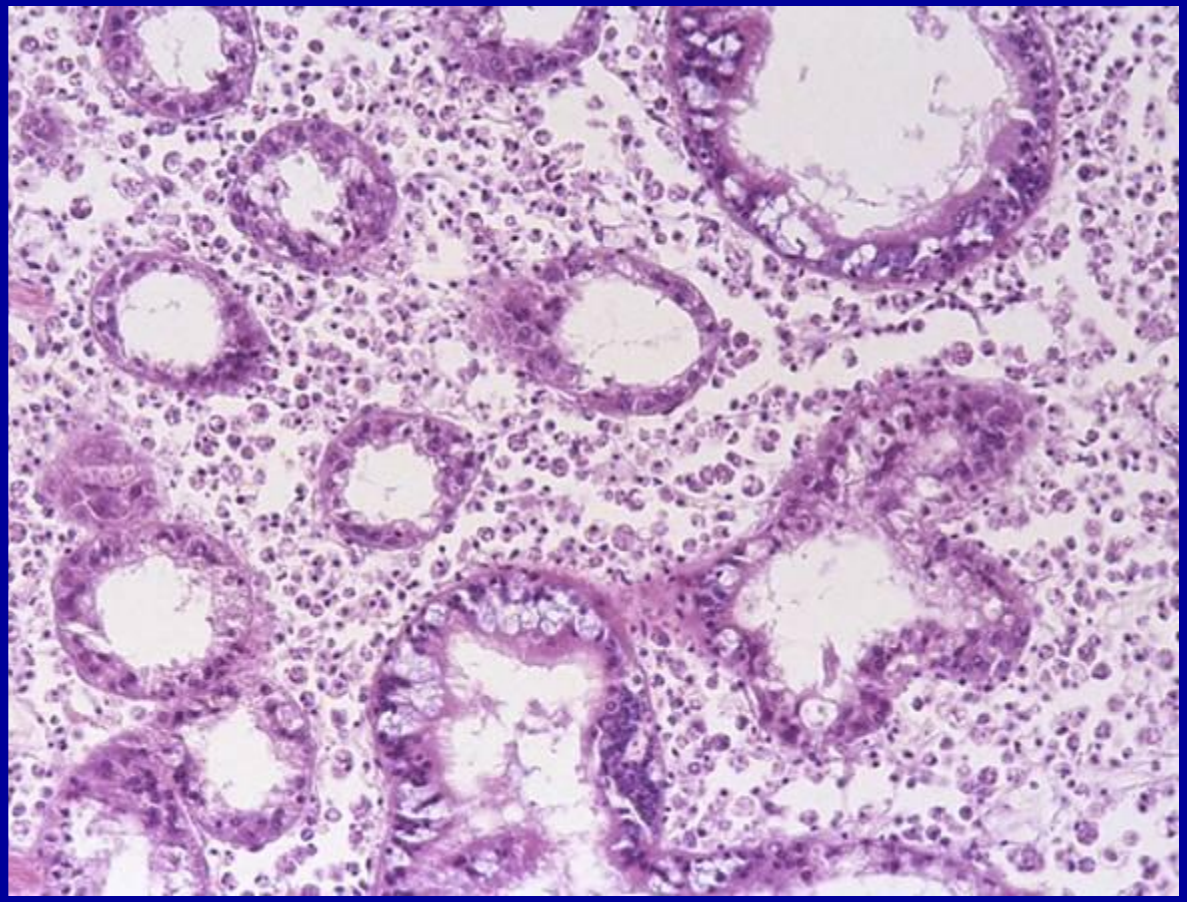
Infection with *Bonamia ostreae*

Haemocyte diapedesis and increase in the number of tissue infiltrating haemocytes



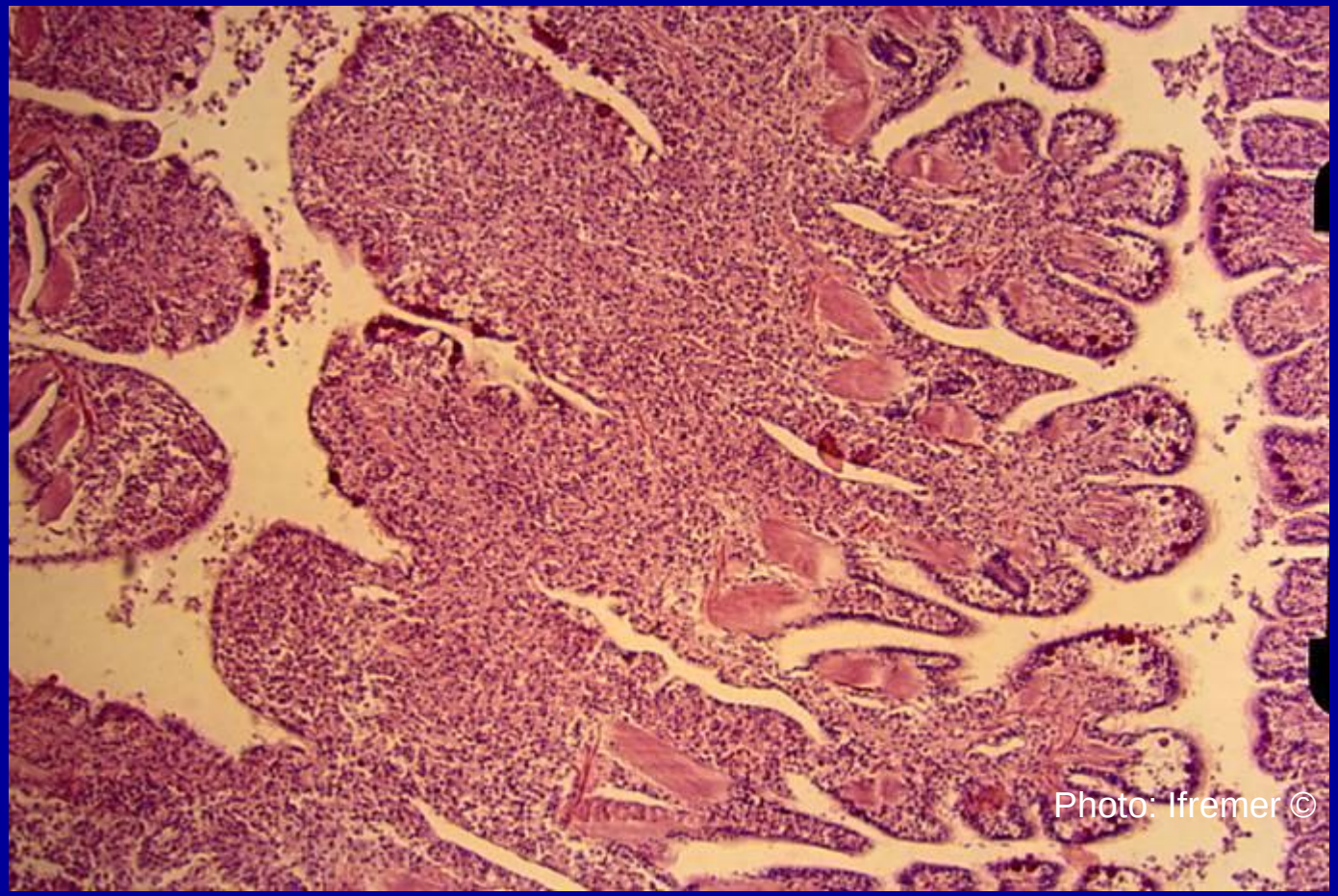
Infection with *Bonamia ostreae*

Haemocyte diapedesis and increase in the number of tissue infiltrating haemocytes



Infection with *Bonamia ostreae*

Haemocyte diapedesis and increase in the number of tissue infiltrating haemocytes



Infection with *Bonamia ostreae*

Impact on the host

- In one study, the presence of *Bonamia* was better related to size than to age of *O. edulis* and infection level was statistically independent of gonadal development stage (Cáceres-Martínez et al. 1995).

- However, Robert et al (1991) and Culloty and Mulcahy (1996) found that two years appeared to be the critical age for disease development in *O. edulis*. Males and females were equally affected (Culloty and Mulcahy 1996).

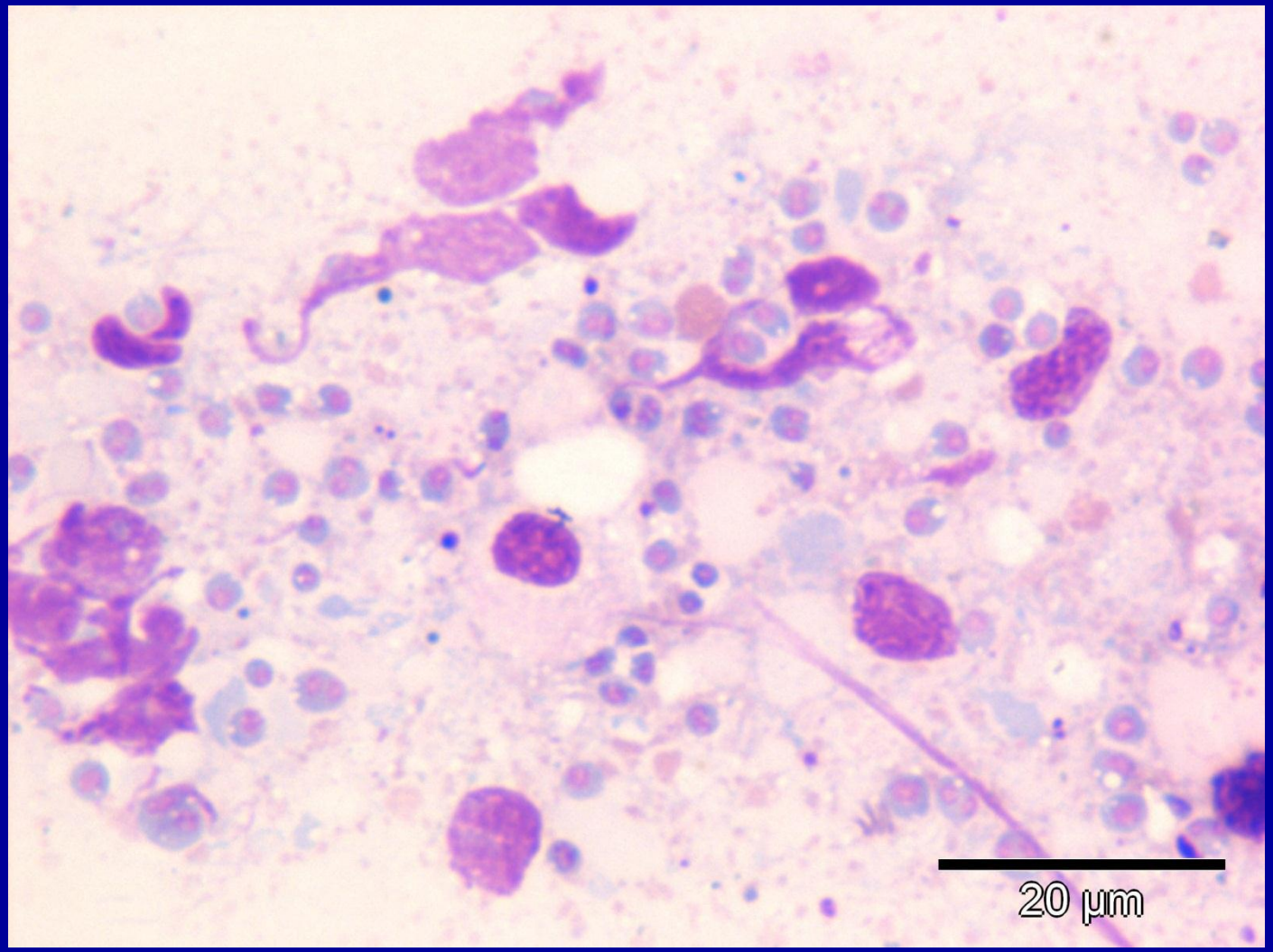
Diagnostic techniques

➔ Tissue Imprint :

Make acetone- (or methanol-) fixed impression smears from gill or heart tissue (preferably the ventricle since the auricles contain an abundance of serous cells which make detection of the parasite difficult). Stain with Wright, Wright-Giemsa or equivalent stain (e.g. Hemacolor, Merck; Diff-QuiK, Baxter). Examine for 2-5 µm spherical or ovoid organisms with a central nucleus within or outside the haemocytes.

Infection with *Bonamia ostreae*

Tissue imprint



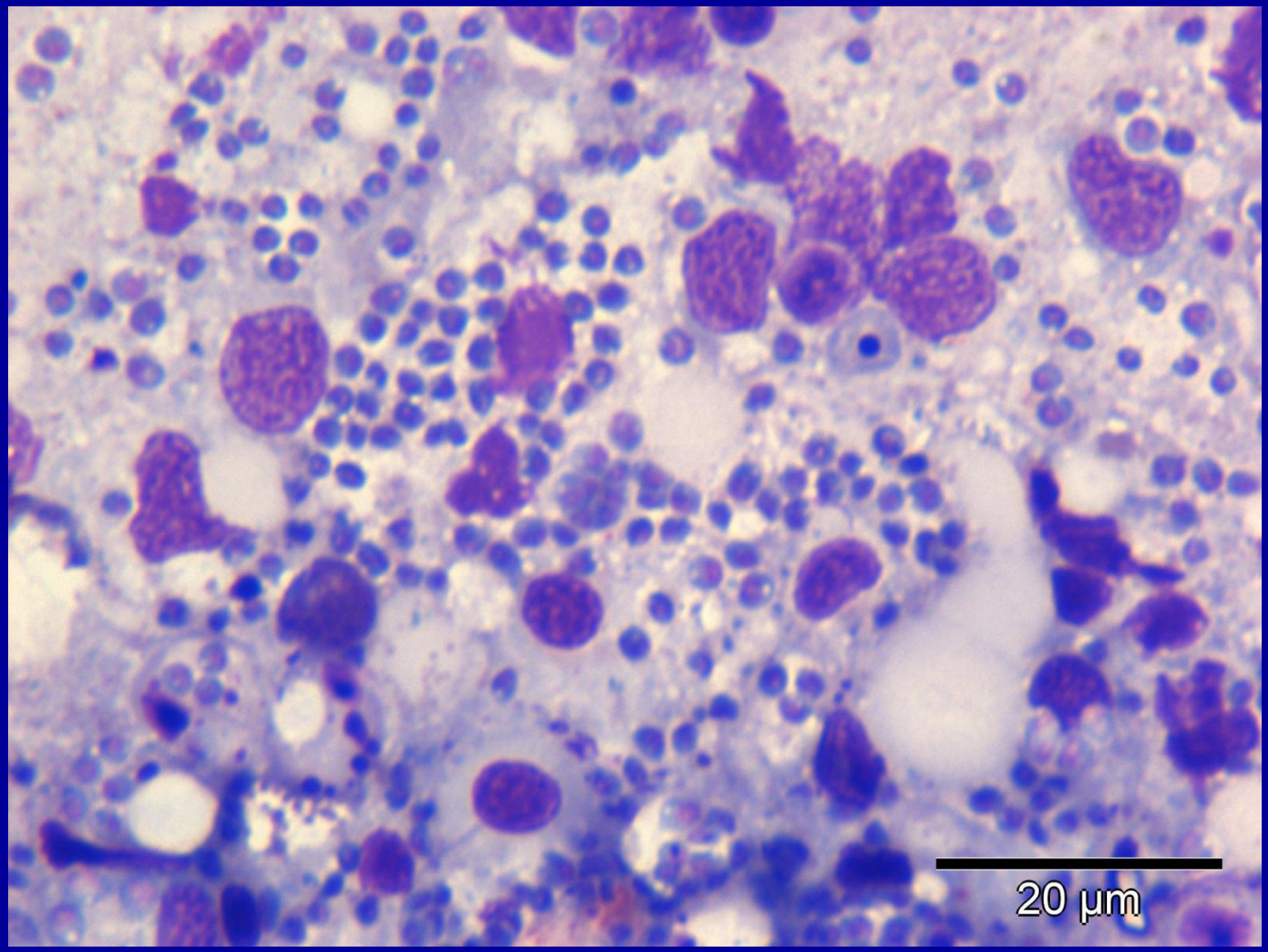
Infection with *Bonamia ostreae*

Diagnostic techniques

➔ Tissue Imprint:

- ➔ the organisms are enlarged by this method compared to those in fresh or histological preparations.
- ➔ Zabaleta and Barber (1996) observed that results obtained from the examination of stained haemolymph smears and histological preparations of an infected *O. edulis* populations were the same but suggested that histology was preferred for detecting light infections. O'Neill et al (1998) recommended that the ventricular heart smear technique be used in conjunction with either haemolymph smears or histology to increase the possibility of detecting light infections. Culloty et al. (2003) indicated that the stained heart smear technique is not reliable for detecting latent infections.

Tissue imprint

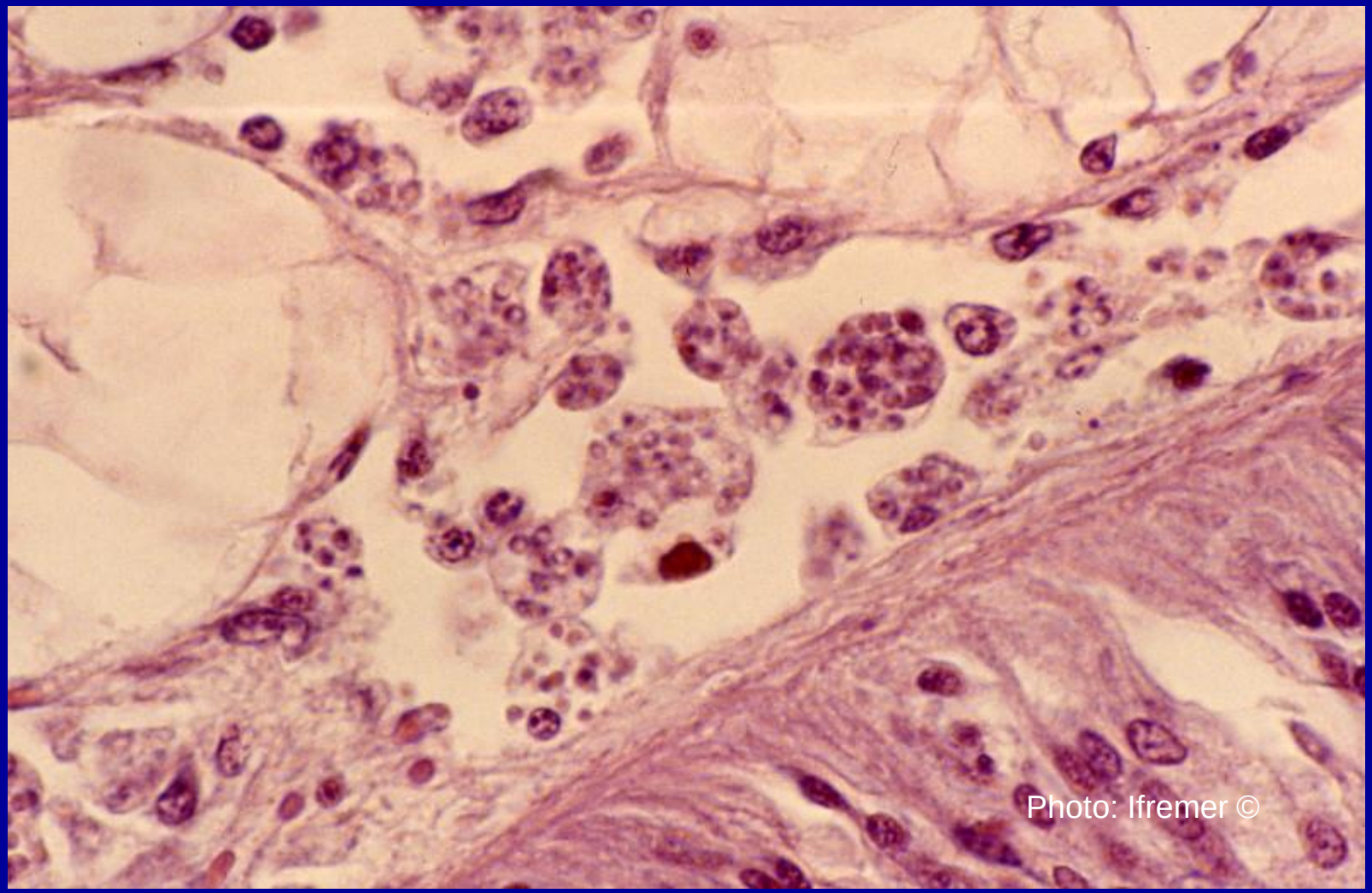


Infection with *Bonamia ostreae*

Diagnostic techniques

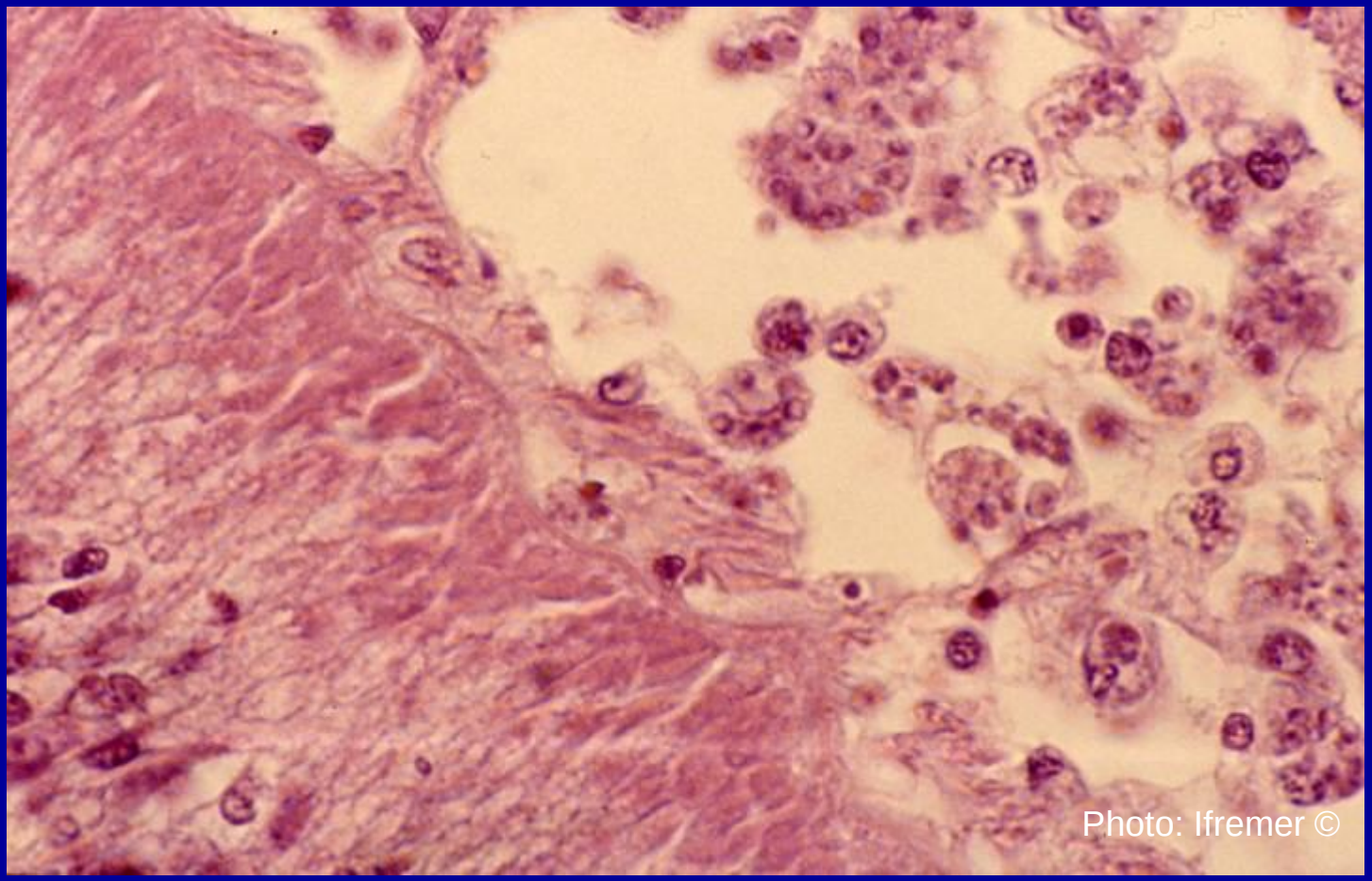
- Histology:
  - Examine haematoxylin and eosin stained tissue cross-sections for tiny protozoa (2-3 µm in diameter) within haemocytes.
  - *Bonamia ostreae* is distributed systemically in advanced infections. In early infections, *B. ostreae* are often observed within haemocytes, associated with dense focal haemocyte infiltrations in the connective tissue of the gill and mantle, and in the vascular sinuses around the stomach and intestine.
  - Van Banning (1990) proposed that *B. ostreae* was an ovarian tissue parasite for part of its life cycle.
  - Montes et al. (1994) observed *B. ostreae* within branchial epithelial cells of *O. edulis*.

Histology



Infection with *Bonamia ostreae*

Histology



Infection with *Bonamia ostreae*

Diagnostic techniques

➔ Electron Microscopy:

- Uninucleate, diplocaryotic and plasmodial stages have been described and illustrated (Pichot et al. 1980, Brehélin et al. 1982, Bonami et al. 1985, Montes et al. 1994).

- Intracellular structures include mitochondria, haplosporosomes, Golgi apparatus and persistent intranuclear microtubules. A stage contains a large vacuole derived from enlargement of one or more mitochondria. Haplosporosomes are formed from Golgi/nuclear material complexes and are similar in construction and structure to some viruses.

Electron Microscopy

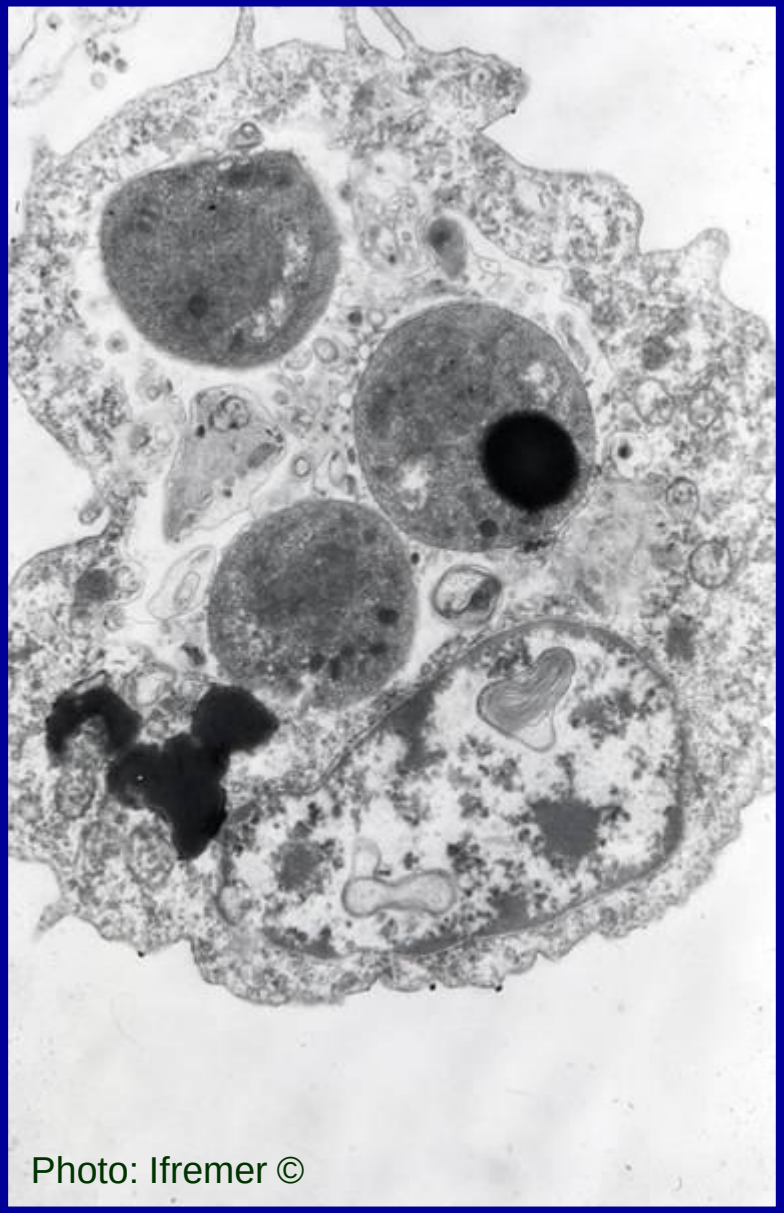


Photo: Ifremer ©

Photo: Ifremer ©

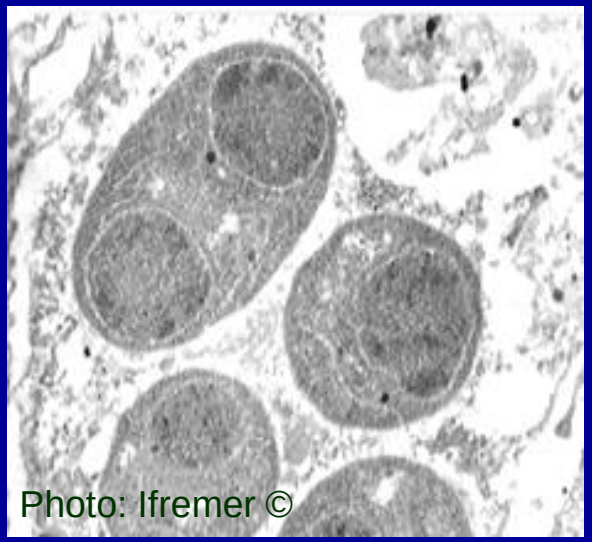


Photo: Ifremer ©

Infection with *Bonamia ostreae*

Electron Microscopy

➔ Dense forms can be used to differentiate between *B. exitiosa* and *B. ostreae* (Hine et al. 2001) :

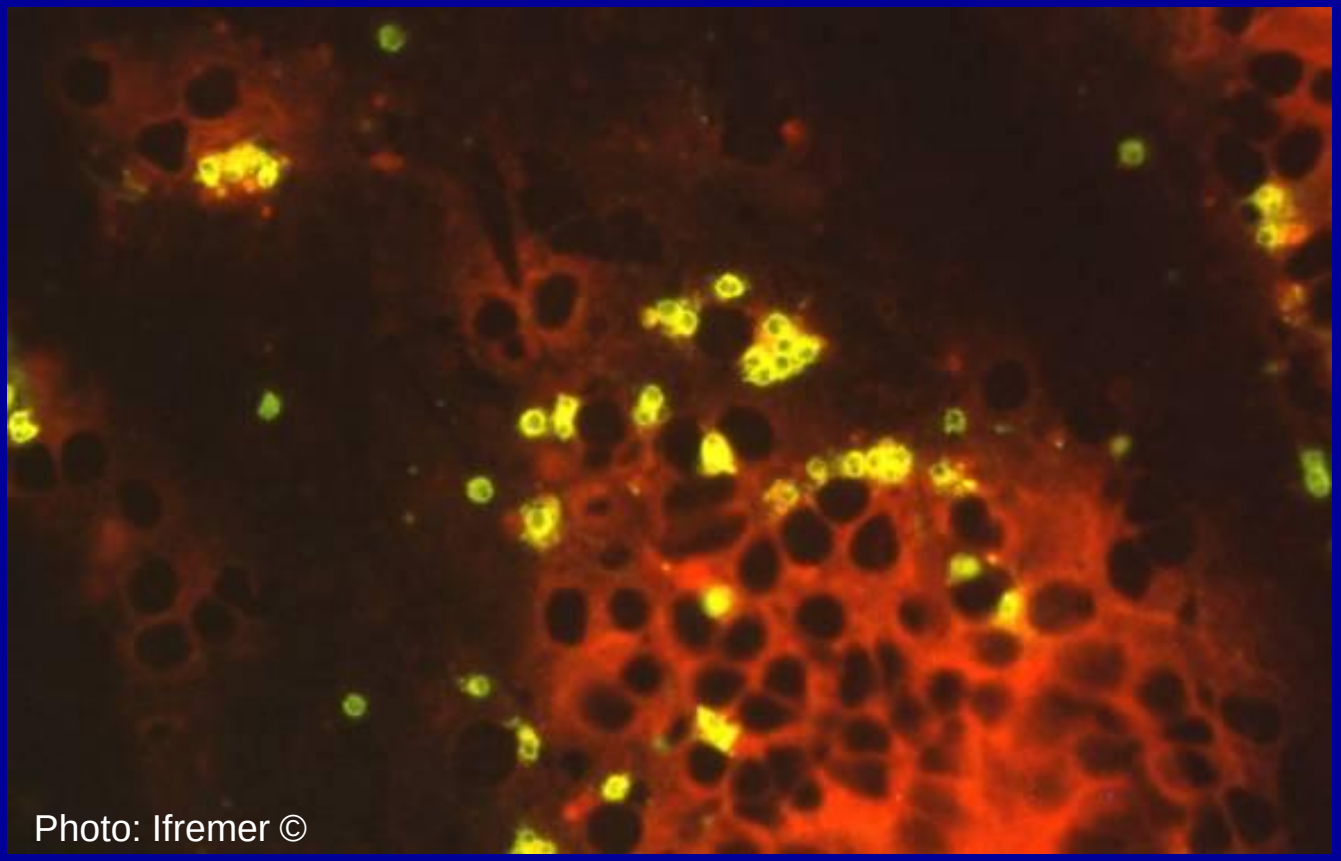
- Dense forms of *B. exitiosa* are less dense, slightly larger in size (3.0 ± 0.3 µm mean diameter n = 61 in comparison to *B. ostreae* with a mean diameter of 2.4 ± 0.5 µm, n =64), have more haplosporosomes, mitochondrial profiles and lipoid bodies per ultrastructure section, and have smaller tubulo-vesicular mitochondria than *B. ostreae*.

- In addition, dense forms of *B. ostreae* lack nuclear membrane-bound Golgi/nuclear cup complexes and a vacuolated stage

Diagnostic techniques

- Immunological Assay:
  - An immunofluorescent technique based on monoclonal antibodies was developed by Mialhe et al. (1988). However, this technique gave unclear results when tested extensively on oysters from Maine, USA (Zabaleta and Barber 1996).
  - Although direct monoclonal antibody sandwich immunoassay for the detection of B. ostreae in haemolymph samples of O. edulis was developed (Cochennec et al. 1992) and marketed commercially for a few years in the mid 1990s, it is no longer available on the market.

Monoclonal antibodies



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Infection with *Bonamia ostreae*

Diagnostic techniques

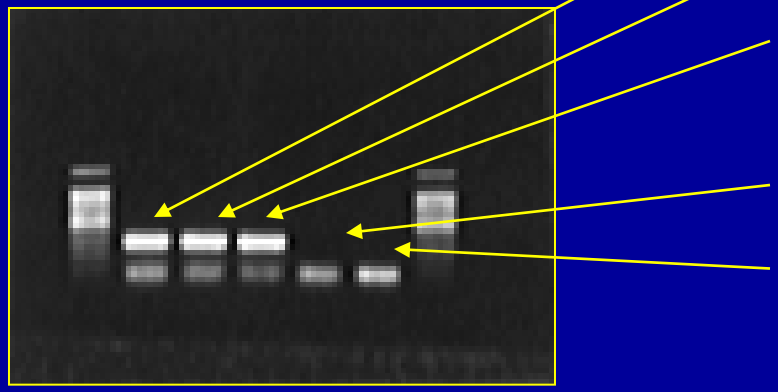
➔ Conventional PCR:

- Three conventional PCR protocols with three different primer pairs targeting the small subunit (SSU) rDNA have been developed for *Bonamia ostreae:*
  - The first primer pair, designated Bo-Boas, amplifies a 300 bp product (Cochennec et al., 2000).
  - The second primer pair, designated CF-CR, amplifies a 760 bp product (Carnegie et al., 2000).
  - The third primer pair, designated BoosF03 - BoosR03, amplifies a 352 bp product (Engelsma et al., 2010).

- Differenciation between *Bonamia ostreae, B. exitiosa* and *B. roughleyi* is possible by digesting Bo-Boas PCR products by *Bgl*I and *Hae*II (Cochennec *et al*., 2003; Hine *et al*., 2001)

- The PCR assay proved to be more sensitive, more specific and less ambiguous than standard histological and cytological (tissue imprint) techniques (Cochennec et al. 2000, Diggles et al. 2003).

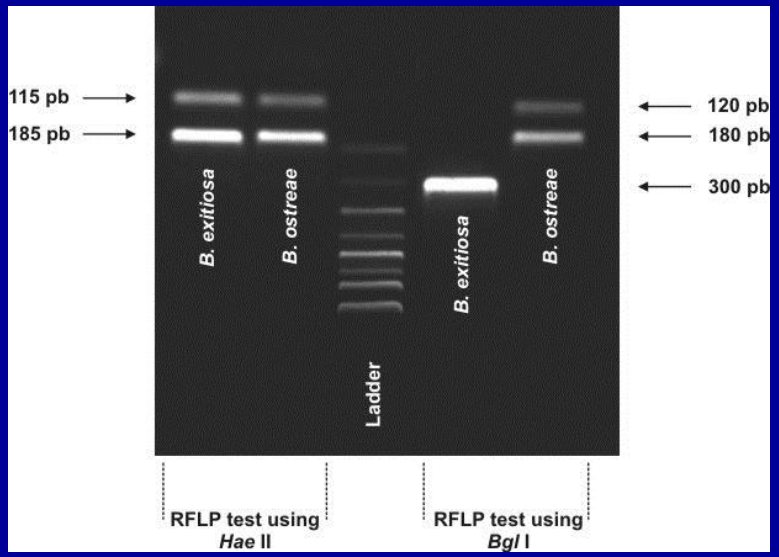
PCR RFLP



B. ostreae
B. exitiosa
B. roughleyi

M. mackini
negative controle

	B. ostr	*B. ex*	*B. roughl*
PCR	*300pb*	*304pb*	*304pb*
Bgl1	+	-	-
HaeII	+	+	-



Infection with *Bonamia ostreae*

Diagnostic techniques

➔ Real Time PCR:

- Two Taqman assays and one Sybergreen assay can be used to detect *Bonamia ostreae :*
  - A TaqMan PCR assay targeting the ITS1 (internal transcribed spacer) detects Bonamia spp. (Corbeil et al., 2006).
  - A TaqMan PCR assay targeting a small region (67 bp) of the small subunit (SSU) rDNA detects Bonamia spp. (Marty *et al.,* 2006).
  - A SYBR® Green real-time PCR assay targeting 201 bp of the actin 1 gene detects and quantifies *B. ostreae and not other related parasites* (Robert *et al.,* 2009).

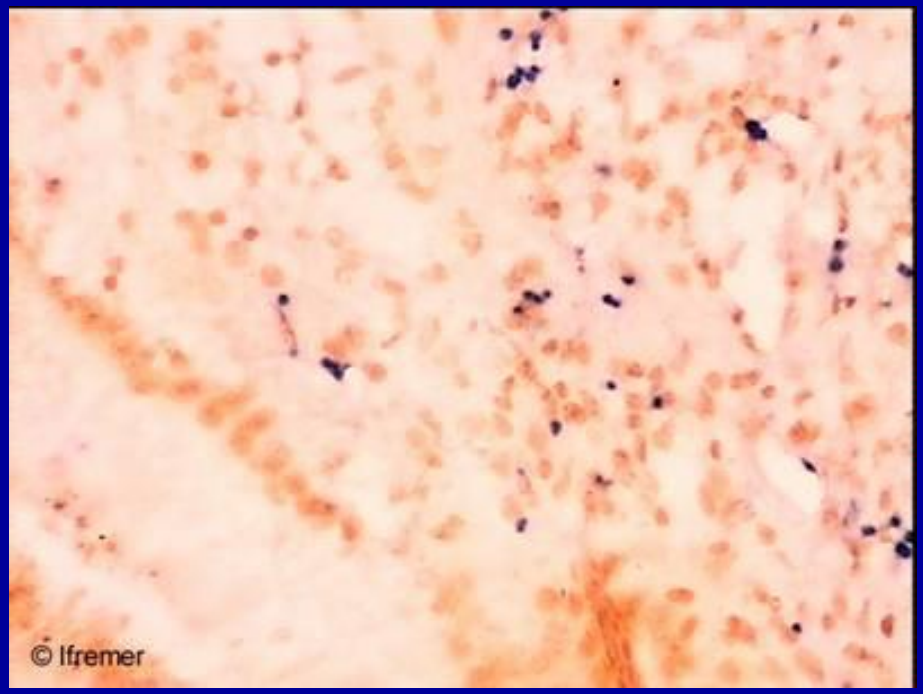
Diagnostic techniques

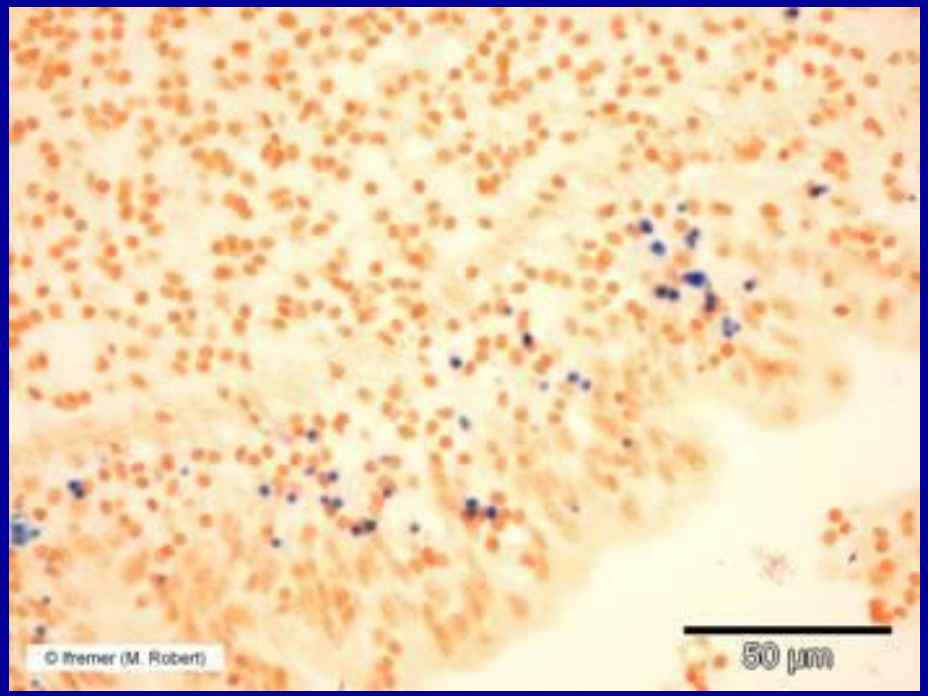
➔ *In situ* hybridization:

➔ Amplicons produced by conventional PCR are also used into *in situ* hybridization assays (Cochennec et al. 2000, Carnegie et al. 2001).

➔ In addition to detecting *B. ostreae*, the probe described by Cochennec et al. (2000) also detected *Bonamia exitiosa* and *Haplosporidium nelsoni*.

In situ hybridisation





Infection with *Bonamia ostreae*

Diagnostic techniques

➔ Culture:

- ➔ Limited multiplication of *B. ostreae* from explants of gills from heavily infected oysters was achieved after 3 days in vitro at 20 °C (Comps 1983).
- ➔ A protocol for the preparation of purified *B. ostreae* cell suspensions has been described by Mialhe et al. (1988) and these suspensions have been used in cytochemistry assays of the parasite (Hervio et al. 1991).

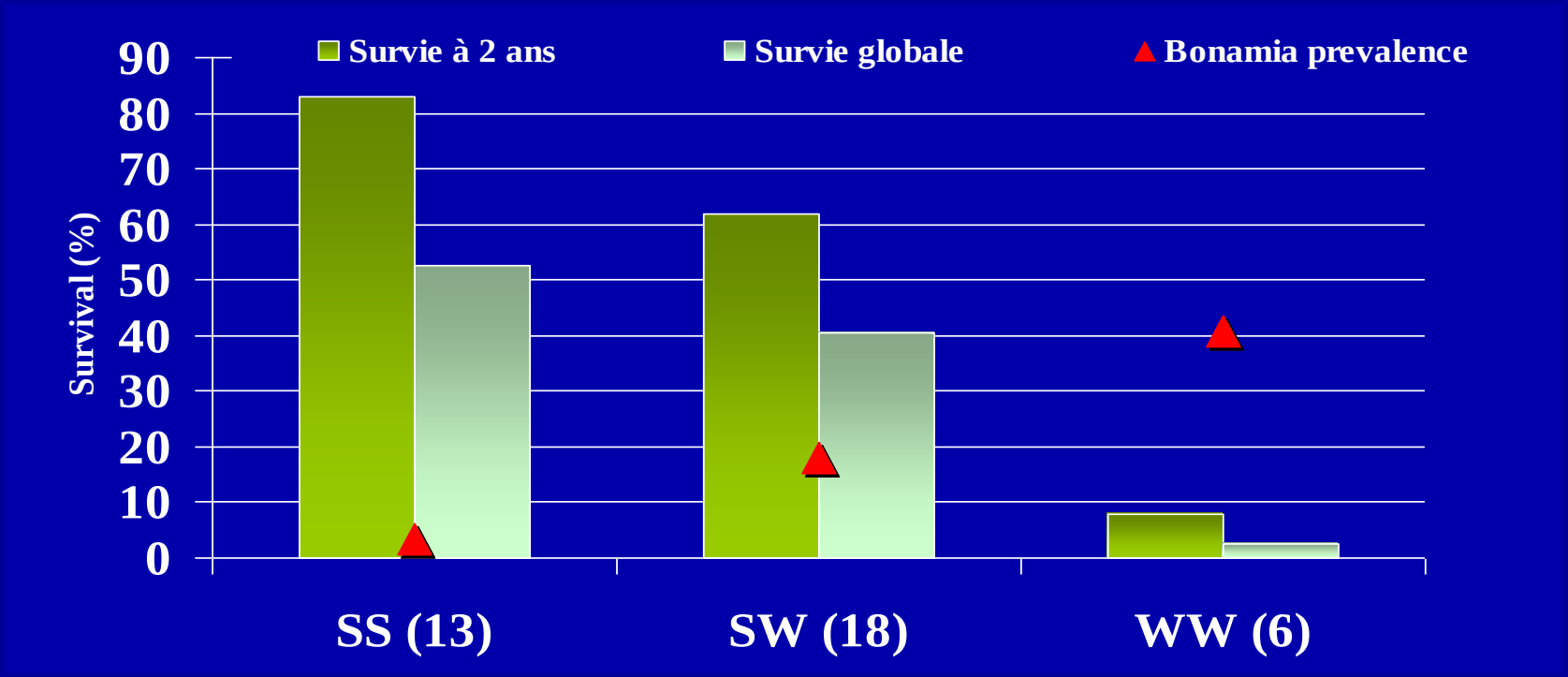
Methods of control

- Ensure that no flat oysters from the infected zones are introduced into areas where bonamiosis is not known to occur.
  - Some oysters from endemic areas may be asymptomatic and show no sign of *Bonamia* using routine detection techniques.
  - If infected animals are introduced into a naïve population, high mortalities can be expected for at least 6 years (van Banning 1985, 1991).
  - Experimental evidence indicates that *B. ostreae* can be transmitted directly between *O. edulis* (Grizel 1985, Culloty et al. 1999).
  - To date, there are no known eradication procedures.

Methods of control

➔ The breeding of bonamiosis-resistant flat oysters

➔ is reported to have some success (Martin et al. 1993, Boudry et al. 1996, Baud et al. 1997, Naciri-Graven et al. 1998, Naciri-Graven et al. 1999, Culloty et al. 2001).



Bédier et al. 2001

Methods of control

- Culture approach
  - Mortalities due to bonamiosis can be reduced using suspension culture and lower stocking densities.
  - Subtidal growing areas also appear to be less severely affected than intertidal areas.
  - Montes et al. (2003) observed that *O. edulis* could be successfully cultured in areas of Galicia, Spain, contaminated with *B. ostreae* if they were promptly marketed after about 15 to 18 months of culture.
  - Le Bec et al. (1991) suggested that culturing *O. edulis* with *C. gigas*, which are not naturally susceptible to infection, may help to reduce infection in *O. edulis.* However, in one study, the growth of *O. edulis* was reduced when they were cultured with *C. gigas* (Robert et al. 1991)

The end