

## Report about mollusc diseases

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### About diagnostic laboratories for mollusc diseases in the region

Of the 75 laboratories contacted through the survey, 54 answered, 14 of which are partially or totally devoted to mollusc diseases. In fact, only 4 laboratories are devoted full-time to this work.

Of the 14 laboratories that declared to work on the diagnosis of mollusc diseases, 12 stated that they worked with mussels, 12 with oysters, 9 with clams and 8 with other mollusc species.

This is coherent with available production information. According to production data in 2001 (FAO source), the main mollusc species in the region are the blue mussel, *Mytilus edulis* (297,485 tonnes), the Mediterranean mussel, *Mytilus galloprovincialis* (131,014 tonnes), the Pacific cupped oyster, *Crassostrea gigas* (127,323 tonnes, an important part of which is produced on the Atlantic coast of France), the Japanese carpet shell clam, *Tapes* spp. (56,778 tonnes), and the European flat oyster, *Ostrea edulis* (5991 tonnes).

France (oysters and mussels), Spain (principally mussels and to a lesser extent clams) and Italy (mussels and clams) are the three main producing countries in the region.

Distribution per country is as follows: 3 laboratories in Spain, 2 in France, 2 in Greece, 1 in Italy, 1 in Portugal, 1 in Croatia, 1 in Romania, 1 in Israel, 1 in Morocco and 1 in Tunisia.

These laboratories include 1 OIE Reference Laboratory and Community Reference Laboratory for mollusc diseases, and 4 National Reference Laboratories (EU Directive 95/70/EC).

Table 1 presents an overview of techniques in the laboratories dealing with mollusc diseases. The different techniques in use in the laboratories show discrepancies when considering their availability versus effective implementation for diagnostic purpose.

Table 1. Overview of techniques in the laboratories dealing with mollusc diseases

Diagnostic methods	Available	Use for diagnostic
Clinical signs	12	10
Macroscopical examination	13	10
Microscopical observation of fresh samples	14	5 <sup>†</sup>
Haematological examination	6	2 <sup>†</sup>
Histopathology	13	11
Electron microscopy	7	3
Bacterial isolation	12	3
Bacterial biochemical identification	10	3
Immunohistochemistry	5	1
Fluorescent antibody technique	4	0
Agglutination	4	0
ELISA	4	0
Immunoblotting	1	0
PCR	6	4
Hybridization with DNA probes	4	2

<sup>†</sup>An ambiguity may come from those denominations as they could correspond to wet mounts, smears, gill/heart imprints or tissue imprints currently used for rapid diagnostic.

Some laboratories, although they have the equipment and skills, do not perform diagnostics. On the other hand, other laboratories, despite stating their involvement in mollusc disease investigations, did not provide evidence of their activity in terms of research or surveillance programmes. The final analysis of the survey is based on returns from 10 laboratories. A very limited number of these laboratories are involved in diagnostics of mollusc diseases on a routine basis; the number ranging between 1 and 5 depending on the disease under consideration. Other laboratories that are involved in research programmes with mollusc diseases were not contacted or did not answer during this survey.

Besides gross signs (macroscopical examination and clinical signs), the main technique used in diagnostic laboratories of the survey is histology (10/10). Other techniques are used but to a lesser extent (imprints 6/10, transmission electron microscopy (TEM) 3/10, bacteriology 3/10, PCR 3/10 and DNA probes 2/10). Efforts to improve the technical level of diagnostic laboratories appear necessary here.

## Main reported diseases

The main diseases reported in this survey study (years 1998, 1999 and 2000) and covered by the different laboratories are bonamiosis, marteiliosis, perkinsosis, haplosporidiosis, mytilicolosis, brown ring disease, larval/juvenile vibriosis and herpes-like virus infection. Other diseases were cited although their significance is difficult to establish; these were disseminated neoplasia of *Cerastoderma edule* and infection by *Haplosporidium tapetis* of *Tapes decussatus*. Table 2 gives an overview of the main diseases covered in the survey.

Table 2. Overview of the main diseases covered in the survey

Disease	Status based on survey
<b>Virus</b>	
Herpes-like virus infection	Diagnosed by 2 laboratories in mortality cases. Not reported in routine monitoring.
<b>Bacteria</b>	
Brown ring disease	Three laboratories from 3 countries were involved in the survey of brown ring disease. The disease was reported in both mortality cases and routine diagnosis.
Larval/juvenile fibrosis	Only 1 laboratory reported this disease, both in routine monitoring and in mortality cases.
<b>Parasites</b>	
Perkinsosis	Seven laboratories from 5 countries were involved in the survey of perkinsosis. The disease was diagnosed by 5 laboratories in routine monitoring and by 3 laboratories in mortality cases.
Marteiliosis	The parasite was diagnosed in oysters and mussels by 8 laboratories from 6 countries. It was reported by 4 laboratories in mortality cases and by 5 laboratories in routine diagnosis.
Bonamiosis	Diagnosed by a total of 5 laboratories from 3 countries, both in routine monitoring and in mortality cases.
Haplosporidiosis	The parasite was reported by 2 laboratories from 2 countries in routine monitoring.
Mytilicolosis	Only 1 laboratory reported this disease in routine monitoring.

Diseases affecting main mollusc species in the region are: (i) mussels – marteiliosis; (ii) clams – perkinsosis and brown ring disease; (iii) European flat oysters – bonamiosis and marteiliosis; and (iv) Pacific oysters – larval/juvenile vibriosis and herpes-like virus infection.

By crossing information available on production of molluscs in the region, activity of diagnostic laboratories included in the survey and current knowledge in molluscs pathology, it is possible to conclude that the *two major concerns for the region are perkinsosis of clams and marteiliosis of flat oysters and mussel*. The species susceptible to these diseases are of economic importance in the region and the impact of the disease has repercussions on aquaculture production and trade.

It is recalled that this survey does not form part of a disease/pathogen reporting system, and that validated information for country sanitary status, especially for notifiable diseases, should be obtained either from the relevant national authorities or the OIE.

## Research programmes

Current research programmes underway for mollusc disease investigations were listed by respondent laboratories: (i) bacterial infections of oyster juveniles (1 laboratory involved); (ii) *Bonamia ostreae* and resistance of flat oysters (2 laboratories); (iii) *Bonamia ostreae* and taxonomy of mikrocells (1 laboratory); (iv) taxonomy and life cycle of *Marteilia refringens* (2 laboratories); (v) diagnostic of oyster herpes-like virus, development and validation of molecular and immunological diagnostic tools (2 laboratories); (vi) perkinsosis of carpet shell clam, morphological characterisation, effects of disease and modulation of these effects by environmental conditions (1 laboratory); and (vii) study of disseminated neoplasia and other pathological conditions affecting cockle *Cerastoderma edule* populations (1 laboratory).

Again, other laboratories which are involved in research programmes with mollusc diseases were not contacted or did not answer during this survey. Therefore the above paragraph does not reflect the situation of research topics and skills outside the survey under consideration.

## General comments

To summarise:

(i) There is a certain consistency between species produced, producing countries, reported significant diseases, location and activities of diagnostic laboratories.

(ii) The overall picture is approximate and in the light of scientific literature a better knowledge of the health status of molluscs in the Mediterranean could be obtained.

(iii) Discrepancy in the surveillance effort and diagnostic methods implemented is noted among the laboratories involved in diagnostic of mollusc diseases.

Efforts may be directed towards:

(i) Organisation of training courses and ring testing of laboratories from the region.

(ii) Development of a database providing information on aquatic animals pathogens and diseases of concern in the region.

(iii) Enhancement of regional cooperation on questions of common interest by organisation of targeted workshops as joint events of forthcoming conferences and meetings.

(iv) Regional cooperation with a view to develop a convergent regulatory framework for mollusc aquaculture and trade with particular regard to diseases.

## General references about mollusc diseases

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## Herpes-like virus of oysters

The generally accepted name of the organism is herpes-type or herpes-like virus of oysters. It is not established if the herpes-like viruses reported from various species of oysters are the same or different virus. In Europe, herpes-like infection was reported from France and Ireland (oyster larvae and spat). It was detected in *Crassostrea gigas* and *Ostrea edulis* but also in *Tapes philippinarum*. It was associated with mass mortality outbreaks (80-90%) among *C. gigas* in France. Pathology may be related to poor husbandry such as crowding and/or environmental conditions such as high temperatures. Diagnostic techniques available are histology, TEM, PCR and *in situ* hybridisation. In histology, presumptive diagnosis can be made on observations of intranuclear inclusion bodies, Feulgen positive, abnormal chromatin pattern (usually marginated) and hypertrophied (enlarged) nuclei in various cells of the connective tissue. Confirmatory diagnosis is necessary by means of other techniques available. No methods of prevention or control are known. The disease caused by herpes-like virus is not listed by the OIE or the EU.

## Current status based on answers received

Based on answers to the questionnaires, only 2 laboratories reported the diseases in mortality cases; the disease was not reported in routine monitoring. Cases were more frequent in spat oysters. The agent appears to be a concern for the Pacific oyster, *C. gigas*. Data on yearly production do not reveal any reduction for this species since the early 90s when the herpes-like virus was described. The production of the susceptible species, *C. gigas*, is over-estimated due to the French contribution on its Atlantic coast. Therefore, this virus could appear not to be of major concern for the region. However, attention should be paid to the apparent low species specificity of the virus and reported infection in clam hatcheries. Clams are an important species for the region.

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## Brown ring disease

Brown ring disease (BRD) of Manila clams, is caused by *Vibrio tapetis* previously called *Vibrio* P1. The virulence of isolates varied depending on the bacterial strain and clam species assayed. Apparently different serotypes may exist. The known geographic distribution is Atlantic coasts of France, Spain, Portugal and Italy, and has recently been reported from England and Ireland. Brown ring disease was only reported from *Tapes* (= *Ruditapes*) *philippinarum* and *T. decussatus*. However, *V. tapetis* has also been isolated from *Venerupis* spp. and *Cerastoderma edule*. Bacteria adhere to the surface of the periostracal lamina at the mantle edge of the shell and progressively colonise the resulting secretion causing a brown deposit of organic material (a conchiolin deposit adhering to the inner surface of the shell), which is considered as symptomatic of the disease. Infection also disturbs the normal calcification process involved in shell deposition. Infected clams present a significant decrease in glycogen suggesting that mass mortalities could result from the degeneration of metabolic activity. Since 1987, brown ring disease has caused mass mortalities on various cultured clam beds along the west coast of France. Diagnostic techniques available are observation of gross signs, electron microscopy, immunoassays, culture and bacteriological identification. In terms of control, reducing density of clams appears to be beneficial. The current observation is that the disease is absent in enzootic areas when high summer temperatures occur as BRD is a cold-water disease. From a regulatory point of view, the disease is not covered by EU and OIE although it has been responsible for epizootics, and those clams that survive are usually unmarketable because of shrinkage and staining of the nacre.

## Current status based on answers received

Three laboratories from 3 countries were involved in the survey of brown ring disease. The disease was reported in both mortality cases and routine diagnosis. Although the disease appears to be absent from the Mediterranean, the target species are of regional importance.

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## Larval vibriosis

Commonly, this infection is named bacillary necrosis, larval necrosis, juvenile vibriosis or vibriosis. Several *Vibrio* species are associated: *Vibrio tubiashii*, *Vibrio anguillarum*, *Vibrio splendidus*, *Vibrio ordali*, *Vibrio alginolyticus* and *Vibrio* spp. But species of *Pseudomonas* and *Aeromonas* may also be involved in the disease. It may occur in all marine waters where bivalve hatchery and nursery culture is practised. *Crassostrea gigas*, *Ostrea edulis* and other cultured bivalve larvae including clams may be affected. Infections are usually initiated by the attachment of bacteria to the external shell surface. Attached bacteria form colonies that grow and contact the mantle resulting in necrosis of mantle epithelium and penetration of the bacteria into all soft tissues via the coelomic cavity. Systemic infection of the soft-tissues of the larvae and juveniles (spat or seed) result in tissue necrosis (due to production of exotoxin by the bacteria) and death. The signs of infection include the sudden onset, with affected larvae exhibiting reduced feeding rate, and erratic swimming behaviour possibly due to the velar damage. Definitive diagnosis of the disease as vibriosis or another resulting from other bacteria requires identification of the specific species or strain involved by appropriate biochemical, immunodiagnostic, or molecular methods. In histology, signs are tissue necrosis and presence of rod

shaped bacteria within the tissues of larvae, usually associated with damage to the velum. In juvenile oysters, the bacteria is initially attached to the external surface of the periostracum, with systemic invasion of the tissues. Immunological assays are available for some *Vibrio* species. Note that the use of inhibitory compounds may lead to the rapid development of bacterial resistance, potential elimination of beneficial organisms and possible emergence of other microbial pathogens. Current research is to select and test strains of bacteria for use as probiotics.

## Current status based on answers received

Based on answers to the questionnaires, a unique laboratory was involved in the diagnosis both in routine monitoring and mortality cases of this kind of infection with particular emphasis on *Crassostrea gigas*.

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## Perkinsosis, *Perkinsus atlanticus*, *P. olseni* and *P. marinus*

Molecular data available show that *Perkinsus atlanticus* infecting *Ruditapes philippinarum* and *R. decussatus* in Spain, Portugal, Korea and Japan are so similar to the original South Australian isolates of *P. olseni* and isolates from New Zealand that they can be considered to be con-specific. *Perkinsus olseni* and *P. atlanticus* should be recognised as one species. *Perkinsus olseni* was originally reported as the cause of mass mortalities among abalone (*Haliotis* spp.) in South Australia. Given these considerations, the parasite is therefore very widespread, and relatively non-host specific. It may affect a wide range of host species, *Tapes decussatus*, *T. philippinarum*, *Ruditapes semidecussatus*, *Venerupis aurea*, *V. pullastra* and many other species of molluscs. Another *Perkinsus* species was recently described in European flat oysters, *Ostrea edulis*, from the Mediterranean. In most clam species, the parasite

frequently induces the formation of visible milky white cysts or nodules on the gills, foot and mantle of heavily infected clams. The massive aggregation of *P. atlanticus* and haemocytes may form lesions that interfere with respiration. The impact of *P. olsenii*/*P. atlanticus* seems to vary between temperate regions, where it causes large-scale mortality in clams and abalones, and the tropics where it infects a wide range of hosts, but usually without causing apparent disease. In Europe, *Tapes philippinarum* seems to be more susceptible to infection than clam species native to Europe. Diagnostic techniques are histology, fluid thioglycollate medium (FTM) assay and PCR. PCR primers were designed for the diagnosis of *P. atlanticus* although their use is not recommended as long as comparison of these assays with standard diagnostic techniques is achieved. There are no methods of control in the natural environment. However, mortality can be minimised by avoiding stressful conditions such as high densities, harvesting stress or overcrowding in depuration plants during the warmer months. The *P. olsenii*/*P. atlanticus* complex is a component part of perkinsosis seen by the OIE as a notifiable disease. In the view of EU regulation, because of previous distinction between the two species *P. olsenii* and *P. atlanticus*, the disease is covered by EU Directive 95/70/EC. *Perkinsus olsenii*/*P. atlanticus* must be distinguished from other *Perkinsus* species, and more particularly *P. marinus* (listed by the OIE as the other causative agent of perkinsosis, and listed in the EU Directive 95/70/EC annex D).

### Current status based on answers received

This parasite is a major concern for the region. Clam diseases are covered by 9 laboratories out of the 14 responding laboratories working in the diagnosis of mollusk diseases. Seven laboratories from 5 countries were involved in the survey of perkinsosis. The disease was diagnosed by 5 laboratories in routine monitoring and by 3 laboratories in mortality cases. A study programme on the subject was reported in the survey.

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## **Marteiliosis, *Marteilia refringens*, *M. maurini* and *M. sydneyi***

Aber disease is associated with the paramyxean parasite *Marteilia refringens*. It occurs in France, Greece, Italy, Morocco, Portugal, Spain and Croatia. It infects European flat oysters, *Ostrea edulis*, blue mussels, *Mytilus edulis* and *M. galloprovincialis*. Since 1968, *M. refringens* has caused serious recurring mortality with a significant negative impact on the European *O. edulis* industry while mussels

appear less affected. Infection causes a poor condition index with glycogen loss (emaciation), discoloration of the digestive gland, cessation of growth, tissue necrosis and mortality. Mortality appears to be related to the sporulation of the parasite, which occurs in the epithelial cells of the digestive tubules. Earlier stages occur in the epithelia of the digestive ducts and possibly the gills (where it was punctually reported from Pacific oysters, *Crassostrea gigas*). *Marteilia refringens* is a major constraint on oyster and mussel farming in Europe, and it is a particular problem as it is difficult to distinguish from *M. maurini*. The two species overlap in host and geographical distribution, and a recently developed assay (PCR-RFLP) will be needed to clarify the host and geographic distributions in the region. Diagnostic methods are digestive gland imprints, histology, TEM, *in situ* hybridisation and PCR-RFLP when used for confirmatory diagnosis. There are no available methods of control. The parasite cannot be transmitted horizontally as it needs an intermediate host, as recently demonstrated. Knowledge of the life cycle of *Marteilia* could provide management strategies. *Marteilia refringens* is an OIE/EU listed agent (EU Directive 91/67/EC) together with its antipodean equivalent *M. sydneyi* (EU Directive 95/70/EC). *Marteilia sydneyi* is only known from mainland Australia, where it infects the Sydney rock oyster, *Saccostrea glomerata*.

### Current status based on answers received

This parasite, which is a major concern for the region, was diagnosed in oysters and mussels by 8 laboratories from 6 countries. It was reported by 4 laboratories in mortality cases and 5 laboratories in routine monitoring. Two laboratories are actively involved in research programmes on this pathogen.

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## **Bonamiosis, *Bonamia ostreae* and *B. exitiosus***

*Bonamia ostreae* is distributed on the Atlantic coast of Europe and the northern Mediterranean coast. It infects European flat oysters, *Ostrea edulis*. After being accidentally introduced into France

and Spain in *Ostrea edulis* imported from California, it caused epizootics in France, the Netherlands, England, Ireland and Spain. Present pathology appears correlated to haemocyte destruction due to proliferation of *B. ostreae*. Lesions occur in the connective tissues of the gills, mantle and digestive gland. Heavily infected oysters tend to be in poorer condition than uninfected oysters. Although many infected oysters appear normal, others may have yellow discoloration and/or lesions on the gills or mantle. Diagnostics are based on tissue (gill, heart) imprints, histology or TEM. More recently, DNA based assays have been developed for *B. ostreae* and *B. exitiosus*, a sister species. Bonamiosis, caused by *Bonamia exitiosus*, occurs around New Zealand, and a similar species occurs in southern Australia and Tasmania. It is closely related to, but not con-specific with, *Bonamia ostreae*. The two species appear identical under the light microscope but they can be differentiated by means of TEM or PCR-RFLP. There are no means of control. Both *B. ostreae* and *B. exitiosus* are listed by the OIE and the EU (EU Directives 91/67/EC and 95/70/EC).

## Current status based on answers received

Based on answers to the questionnaires, 5 laboratories from 3 countries reported this parasite both in routine monitoring and mortality cases in *Ostrea edulis*. The target species are of limited regional importance in terms of volume but should also be considered in terms of value.

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## **Haplosporidiosis, *Haplosporidium nelsoni*, *H. costale***

Haplosporidiosis refers to the disease caused by the parasites *Haplosporidium nelsoni* and *H. costale*. Haplosporidiosis occurs in eastern oysters, *Crassostrea virginica*, on the east coast of the United States. *Haplosporidium nelsoni* also occurs, but does not cause disease, in Pacific oysters, *Crassostrea gigas*, on the west and east coasts of the United States, in Japan, Korea and France. It is thought to have been introduced in the United States in *C. gigas* imported from Japan and jumped host into *C. virginica*. It was also probably introduced into France in *C. gigas*. It is unknown elsewhere. *Haplosporidium nelsoni* has caused massive mortality along the east coast of the United States. Diagnostic procedures are

based on histology, TEM as well as DNA based methods (when validated). These two parasites are listed by the OIE and the EU (EU Directive 95/70/EC).

Another *Haplosporidium* (= *Minchinia*) *tapetis* is occurring on the west coasts of France, Portugal and Spain, in *Tapes* (= *Ruditapes*) *decussatus*, *Venerupis aureus* and cultured *Tapes* (= *Ruditapes*) *philippinarum*. Prevalence of infection is usually low (about 4%). However, *H. tapetis* occurred in up to 59% of *T. decussatus* and 25% of *V. aureus* in Spain. The pathogenicity of the plasmodial stage in clams is minimal but the sporulation stage in the connective tissue causes important lesions in the digestive gland and gills. Although no mortality has been attributed to the parasite, the effect on clams or other bivalves in different environments is unknown and related species are highly pathogenic to oysters on the east coast of the United States. Histological diagnostic techniques are used. There are no known methods of prevention or control. This agent is not covered by current legislation. *Haplosporidium* spp. were also described in *Ostrea edulis* and *Mytilus galloprovincialis*.

## Current status based on answers received

Records of *Haplosporidium* spp. were obtained in 2 laboratories from 2 countries (if we except to consider histology as a non targeted examination). This is probably a critical situation given the potential importance of these parasites and possible detrimental effect on species of economic significance in the region.

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## **Mytilicolosis, *Mytilicola* spp.**

*Mytilicola intestinalis* is a copepod, it is not a worm. It occurs in Europe from Denmark to Italy. Host species are *Tapes decussatus*, *Cerastoderma (=Cardium) edule*, *Mytilus edulis*, *Mytilus galloprovincialis*, *Ostrea edulis* and a wide range of other marine bivalves. Pathogenicity to clams and cockles has not been reported. However, this parasitic copepod has been accused of causing disease and mortality in mussels but this assertion is even more controversial when regarding its significance for oysters. Juvenile mussels are rarely infected. Levels of infection appear directly correlated with size. There is evidence that growth in mussels, suffering *Mytilicola* infections, is severely retarded. Populations chronically affected with *M. intestinalis* frequently show prevalence of 100% infection and intensities of over 30 copepods per mussel. The effect of such infection appears related to adverse

growing conditions rather than to the actual pathogenicity of the copepod. The results of a 10-year study conducted in England during the late 70s and early 80s, showed that *M. intestinalis* exhibits the features of a commensal rather than of a harmful parasite. There is marked controversy with respect to the actual significance of this parasite to infected oysters. Some scientists believe that it causes poor growth and condition, extensive damage of the gut wall and sporadic mortality. Others believe that the effect of the copepod is worst during sub-optimal growing conditions. However, in most infections there is no evidence of pathology caused by these parasites. Diagnostic techniques are based on gross observations (the various species of *Mytilicola*, *M. intestinalis*, *M. orientalis* and *M. porrecta*, can be differentiated by external morphological characteristics), histology and enzyme extraction (digestion of tissues exposes copepods for easy quantification – this process is usually recommended for large scale surveys rather than diagnostics). No known methods of prevention or control except of good husbandry.

### Current status based on answers received

Only 1 laboratory reported this disease in routine monitoring.

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