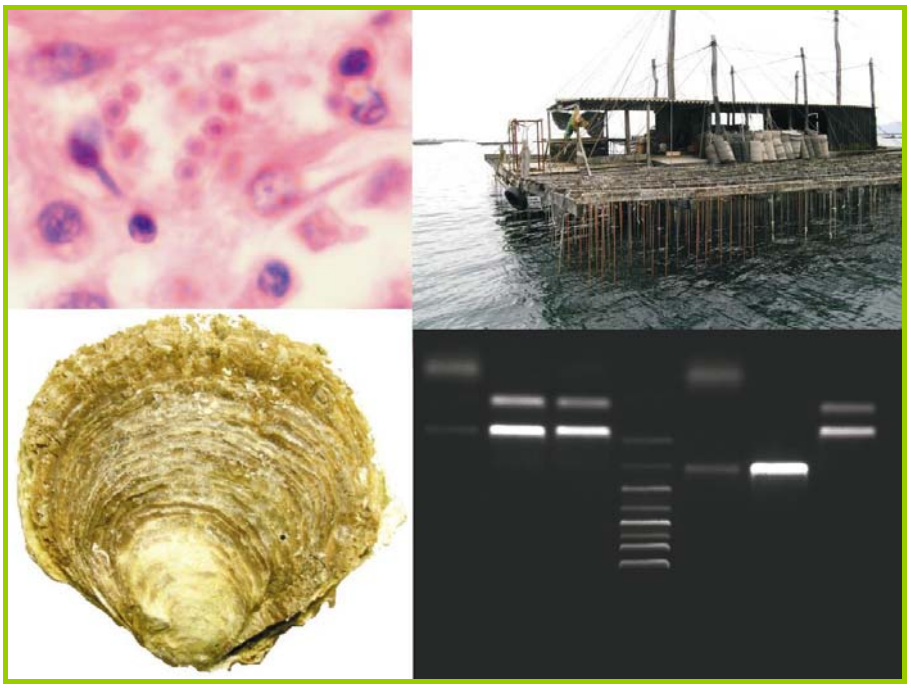
REPORT OF THE

Annual Meeting of the National Reference Laboratories for Mollusc Diseases

Nantes, 18-19 March 2008



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National Reference Laboratories for Mollusc Diseases
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Executive summary

Follow highlights of the discussions, expert opinion and recommendations formulated during the 2008 Annual Meeting of National Reference Laboratories (NRLs) for Diseases of Molluscs.

- In 2007, major epidemiological changes in EU were (1) the notification of *Bonamia exitiosa* in flat oysters *Ostrea edulis* in Galicia, Spain and its detection in flat oysters from the Italian adriatic coast (2) the spread of bonamiosis in two previously free areas, Loch Sween and Loch Talbert on the Western Scottish coast (3) the suspicion of bonamiosis in flat oysters from Norway which could not be confirmed.

- Following the notification of *Bonamia exitiosa* in *Ostrea edulis* in Spain and considering that *Bonamia* is routinely detected by histology and/or tissue imprints within the European Union, which do not allow to determine the parasite species, it appears necessary to investigate the possible presence of *B. exitiosa* in areas usually considered infected with *Bonamia ostreae.* A work plan has been submitted by the CRL with the support of the European Commission to the NRL network in order to characterize *Bonamia* isolates detected in Europe in 2008.

- The situation of mussels regarding infection by *Marteilia refringens* has been discussed during the first session. Considering that both mussels *Mytilus edulis* and *M. galloprovincialis* are listed as susceptible species to *M. refringens*, these bivalve species should be included in surveillance programmes of marteiliosis. The taxonomic position of both *Marteilia maurini* and *M. refringens* is not yet fixed but present available results on this issue let think that both parasites belong to a same species *M. refringens*. However, two types can be identified type M and type O which can be more or less host specific depending on studies.

- Size of samples is often questioned. Sample size and number of sites investigated should be determined by taking into account several parameters including the surface of the zone and the true values of the specificity and sensitivity of the techniques used for the detection of the pathogens.

- In 2007, few Member States reported abnormal mortality cases. An effort should be made to improve communication between farmers and people involved in sampling and to improve mortality reporting. Mortality reports constitute the first alarm signal in the case of the emergence and /or introduction of a listed and/or important pathogen.

- Host range especially for listed pathogens is not always well defined. Sometimes host species have been demonstrated to be susceptible on the basis of some experimental transmissions which mimic more or less the natural pathways or on the basis of some histological or molecular analysis which do not allow alone to confirm the pathogen species. Combined and multidisciplinary approach including histology, molecular biology, ecology, risk analysis and experimental assays need to be encouraged in order to define host range with more transparency. EFSA has been asked to review the present list of susceptible species.

- The article 17 of the new Directive introduces the notion of vector species potentially able to transfer a pathogen without being susceptible to the disease. This article relies

on a list of vector species (and development stages) for listed diseases. Because of the lack of data concerning these vector species, EFSA has been asked to prepare this list and used a risk based approach. The approach used to prepare the list was presented during the fourth session.

- A guideline to provide the Member States with guidance on how to implement the requirements of Article 10 on the establishment of a risk-based animal health surveillance scheme has been presented. In the framework of this scheme, inspections must be carried out with some recommended frequencies determined on the basis of the status of the concerned zone and the general risk the farm poses in relation to spreading and contracting diseases.

- Importance of having updated pictures of mollusc production map was reminded. Indeed, without such data, it is difficult to put in place relevant health surveillance systems. The lack of data regarding the distribution of flat oysters in Europe especially in some areas like the Black Sea has been tackled as well as the northwards extension of *Crassostrea gigas* observed since several years. To date, it is not possible to know if the *C. gigas* spread is due to an adaptation of the species or environmental changes.

- Global change or at least modification of some environmental parameters might have an impact on the distribution and the susceptibility of host species but also on the survival and the virulence of some pathogens. New research works based on flow cytometry tests allowed to study the impact of temperature and salinity on the survival of *Bonamia ostreae*. Results should help to understand the present distribution of the pathogen and to forecast potential modification of spread according to the evolution of the environment.

- Definition of pathogen species relies on several criteria including ultrastructural features and molecular data. The genome of parasites of the genus *Bonamia* is poorly known and until recently only ribosomal genes were available under geenbank. However, taxonomical studies require multiple gene approach. In that context, works have been done in order to characterize actin genes of *Bonamia ostreae*. These new molecular data allowed to confirm previous taxonomy results and to develop new diagnostic tool.

- Transcriptomic approach is more and more used to investigate the host response to some infections. Studying non-specific defence mechanisms (innate immunity) is of great interest especially for molluscs which do not have antibodies and thus do not develop acquired immunity. Results have been presented concerning the modification of gene expression in *Crassostrea gigas* in response to infection by OsHV-1.

- The conclusions of the European project WOPER (Workshop for the analysis of the impact of perkinsosis on the European shellfish industry) were presented during the last session. WOPER was a 1 year EU project aiming at addressing the threat of perkinsosis to European shellfish industry. It gave the opportunity of bringing concerned persons around the table during a workshop which took place in Vigo in September 2007.

- Results of the inter laboratory proficiency test organised in 2007 to test the capacity of NRLs to detect some pathogens by histology were presented and discussed. These

results highlight the need of more training especially for the detection of *Perkinsus marinus* and *Mikrocytos mackini*. Ring tests are not only interesting to assess the capacity of NRLs but also to estimate the confidence of results in the context of the surveillance of mollusc diseases.

- Regarding the need of training in histology, the CRL would like to give NRLs access to some collection of scanned histological slides. Such tool does not aim at replacing workshop or distribution of reference material but could offer alternative way of practising histology.
- The CRL proposes to plan the next annual meeting for the beginning of 2009 (March-April) in La Tremblade combined with a technical workshop. NRLs are invited to propose topics on which they are working or for which they need information and/or training.

Introduction

The 2008 Annual Meeting of National Reference Laboratories for Mollusc Diseases was held in Nantes on the 18th and the 19th of March 2006. 40 participants from 18 countries (Bulgaria, Belgium, Croatia, Czech Republic, Denmark, France, Germany, Italy, Ireland, Malta, The Netherlands, Norway, Poland, Romania, Slovenia, Spain, Sweden and United-Kingdom) attended the meeting.

The Annual Meeting was planned on two days and included seven sessions: 1) Diagnosis and survey of mollusc diseases 2) Host range definition 3) *Bonamia exitiosa* notification 4) Legislation and collaborative issues 5) Distribution of oysters cultivated in Europe 6) News from the bench 7) News from the Community Reference Laboratory.

This report provides summaries of the questions discussed during the meeting and outstanding facts for follow-up activities. It contains collective expert opinion and recommendations made during the meeting.

SESSION I: DIAGNOSIS AND SURVEY OF MOLLUSC DISEASES

(Chair: I. Arzul)

Current epidemiological situation in the Member States

National delegates participating in the Annual Meeting briefly presented outstanding issues and major changes with regards to the mollusc health status in their country. The detailed reports received from NRLs are given in annex of this report (Annex C).

Croatia: In 2007, 124 farms produced 81 t of flat oysters, *Ostrea edulis* and 3496 t of mussels. An experimental hatchery produces some flat oysters in zone III. No *Marteilia refringens* or *Bonamia ostreae* was detected in flat oysters in 2007. *Marteilia* sp. was reported

in all the mussel samples analysed from the three main production areas. No mortality event was reported in 2007.

Slovenia: The Slovenian mollusc production is represented by mussels, *Mytilus galloprovincialis*, (250 tons) and 3 farms. In 2007, mussels were collected monthly from two sampling sites, Seča and Strunjan. No pathogen was detected and no mortality was recorded.

Italy: The Italian mollusc production mainly relies on mussels, *Mytilus galloprovincialis* and manila clams *Ruditapes philippinarum* with 100 000 tons and 48 000 tons produced in 282 and 338 farms respectively in 2007. In addition to these farms, there are 4 hatcheries and 8 nurseries producing manila clams. There is no official data concerning transports of molluscs from and towards other member states and third countries.
Concerning the survey of flat oysters, 119 individuals were collected between zones I and II (Friuli-Venezia Giulia and Veneto) in June, July and September. No *Bonamia* parasite was detected while *Marteilia* sp. was detected in 30 individuals. Molecular characterization of this parasite was made by the CRL and confirmed that the parasite was *Marteilia refringens* type O. 6710 mussels were analysed in 2007. *Marteilia refringens* was detected at low prevalence in two samples originating from Friuli-Venezia Giulia and Liguria (7/1050 and 1/150 individuals were found infected)**.** *Perkinsus olseni* is present in manila clams *Ruditapes philippinarum* from Friuli-Venezia Giulia, Veneto and Emilia-Romagna areas with 44%, 45% and 14% of detection frequencies respectively. The detection of *P. olseni* is performed by histology and culture using thioglycollate. Culture using thioglycollate is more sensitive than histology but both techniques present concordant results when infection levels are high. No mortality event was reported in 2007.

Bonamia sp. was detected by histology in the context of a study carried out on flat oysters originated from Adriatic Sea (but collected from a dispatch centre). Molecular works allowed determining that B. exitiosa was present in these flat oysters.

Romania: *Mytilus galloprovincialis* is, according to the Romanian Reference Laboratory the only mollusc species farmed in Romania. 3 samples were collected in August (179 individuals) and one sample was collected in October (36 individuals). Histological examination did not reveal any presence of parasite. No mortality event was reported in 2007.

Bulgaria: *Mytilus galloprovincialis* (750 t) and *Rapana* sp. (8500 t) are the main mollusc production in Bulgaria. In 2007, 150 mussels were tested by histology and imprints and did not reveal presence of parasite. No mortality event was reported in 2007.

France: *Crassostrea gigas* is the most important mollusc production in France with 115 500 tons produced per year. *Mytilus edulis* and *M. galloprovincialis* constitute the second most important mollusc production with 67 500 produced in 2007. The official recognition of the free status of French zones is still under progress. However, due to the detection of *Bonamia ostreae* in flat oysters from Granville in 2006 and due to the difficulty of access to animals (poor density, wild population) in zone X, the surveillance of bonamiosis and marteiliosis was suspended for 2007 in these two areas.
In 2007, 390 mussels were collected from 6 mussel producing areas in order to determine the distribution of marteiliosis in mussels in France. This survey was initiated in 2006. In 2007, *Marteilia refringens* was detected in mussels from 2 sites: Baie des Veys and Thau Lagoon with less than 10% of detection frequencies. Molecular investigations revealed the presence of

Marteilia type M in Thau lagoon (it was not possible to perform molecular test on mussels found infected in Baie des Veys probably because of the very low level of infection observed by histology).
72 abnormal mortality outbreaks of Pacific oysters, *Crassostrea gigas*, were reported in 2007 and were mainly observed during summer. 52 cases concerned spat and juveniles, 2 cases concerned larvae and 18 cases were reported on adults. OsHV-1 and/or Vibrio strains could be detected in some of these mortality events.
Moreover, in 2007, 5 mortality events were reported in mussels and *Haplosporidium sp.* was detected in 1 sample (4/30 individuals). One case of mortality was reported in *Haliotis tuberculata* and two bacterial strains, *Vibrio harveyi* and *V. splendidus* could be isolated from moribund individuals.

Belgium: There is a small production of mussels (5 t), cupped oysters and flat oysters (50 t) in Belgium. However, no sample was tested for the presence of listed diseases. No mortality event was reported in 2007.

Spain: Most of the Spanish production of shellfish is located in Galicia. Cockles and clams represented 45 and 41% of the total mollusc production respectively in 2007. In Galicia, 510 flat oysters were collected in spring and autumn from 9 sites and tested regarding the presence of *Bonamia* sp. and *Marteilia refringens*. *Bonamia* sp. was detected in the 9 investigated areas. Moreover, *Bonamia exitiosa* was reported in one raft from the Ria de Arousa sometimes with *B. ostreae* in the same individuals. *Marteilia refringens* was not reported in Galicia but could be detected in Alicante where 150 flat oysters were collected in autumn from 1 site.
570 mussels were collected from 19 sites in Galicia and 270 mussels from 1 site in Ceuta in order to study the distribution of *Marteilia refringens* in *Mytilus galloprovincialis.* Both regions were found infected by the parasite.
Perkinsus olseni was detected in *Ruditapes decussatus, R. philippinarum* and *Venerupis pullastra* collected in Galicia and in *R. decussatus* from Austurias.
No mortality event was reported in 2007.

The Netherlands: *Mytilus edulis* is the most important mollusc production in The Netherlands with 31 260 tons produced per year. *Bonamia ostreae* is still detected in flat oysters, *Ostrea edulis*, from Grevelingen with a prevalence of 15% (17/116) but not from Oosterschelde where only 2 individuals could be collected and appeared free of the parasite. In addition, 1 flat oyster from Grevelingen showed some rickettsia like organisms.
Granulocytoma and trematode metacercaria were reported on mussels.
One mortality case was reported in April in mussels from Oosterschelde. Despite the high frequency of *Mytilicola* sp., the presence of gregarine (6%) and granulocytoma (18%) no veterinary cause of the mortality was found.

Germany: In 2007, there was no investigation on mollusc diseases in Germany. One mortality event was reported on *Crassostrea gigas* in lower Saxony Germany. Oysters and also blue mussels bred near oysters were sampled for analysis using cell lines (CHSE-214). Based on this observation, a viral infection was suspected and analysis was performed using cell lines.

Poland: There is a wild population of *Mytilus edulis* on the Polish cost of the Baltic Sea. 30 individuals were collected from two sites, Puck Bay and the open sea. These individuals were tested regarding the presence of *Marteilia refringens*. No parasite was recorded in these

samples. No mortality event was reported in 2007.

Denmark: *Mytilus edulis* is the most important mollusc production in Denmark with 57 335 tons produced in 2007. The production of flat oysters, located in Limfjorden, reached 1 212 tons in 2007. 30 to 33 individuals were collected in spring and autumn 2007 in three sites from the Limflorden including the hatchery present in this place. Investigations for 2007 were not finished at the meeting dates. Denmark is officially recognised *Bonamia ostreae* and *Marteilia refringens* free on the Limfjorden area since December 2004. No mortality event was reported in 2007.

Sweden: The Swedish shellfish production is represented by mussels, *Mytilus edulis*, with about 2 800 tons cultivated in farms (2 larger farms and 5 smaller) and 300 tons harvested from natural beds and *Ostrea edulis* with 1 ton produced in 2007 in 2 production units and 20 tons collected by divers from natural beds.
In 2007, 150 flat oysters were collected from 10 sites (15 oysters per site) in June-July and September-October. Neither *Bonamia* sp. nor *Marteilia refringens* was reported in the 300 tested individuals. No mortality event was reported in 2007.

Norway: In 2007, 3 000 tons of *Mytilus edulis* (on long lines), 5 tons of *Ostrea edulis* and 3 tons of *Pecten maximus* were produced in Norway. In addition 800 tons of scallops were harvested from natural beds. Norway was officially recognized as a free zone in 2004**.** Norway is considered as one zone. 30 to 40 flat oysters were collected from 6 sites in spring and autumn 2007 and were processed for histology. No *Marteilia refringens* was detected while *Bonamia* -like structures were observed in haemocytes of several oysters from a wild population in Southern Norway. PCR and *in situ* hybridization were performed on suspect individuals by the CRL but did not yield positive results. In 2008, a sampling of 150 oysters (instead of 30) is planned in the site where *Bonamia* like structures were observed.
No mortality event was reported in 2007.

United Kingdom: Most of the shellfish production in UK relies on mussels with 28 100 tons produced in 2007 and in a lesser concern on *Crassostrea gigas, Ostrea edulis*, scallops and clams and cockles (1370, 90, 20 and 10 tons respectively). In addition, 670 tons of *Ostrea edulis*, 24 500 tons of scallops and 16 000 tons of cockles were harvested from natural beds.
In 2007, 92 samples of 30 flat oysters were tested regarding the presence of *Bonamia ostreae* and *Marteilia refringens* in spring and autumn. *Bonamia* sp. was detected in all the previously reported infected zones but was also reported for the first time in Loch Sween and Loch Talbert. New cases have also been reported from North Kent (England) and Strangford Lough (Northern Ireland).
Three cases of mortality were reported in 2007, one in a clam hatchery (>90%), one in a nursery producing *Crassostrea gigas* (60-70%) and the last case concerned wild cockles (>90%). No disease agent was observed except some irido-like virus particles reported in some *C. gigas* juveniles.

Ireland: The entire coast of Ireland is an approved zone for marteiliosis. The entire coastline except Cork Harbour, Galway Bay, Clew Bay, Ballinakill Harbour, Achill Sound, Blacksod Bay, Lough Foyle & Lough Swilly is approved regarding bonamiosis.
In 2007, no *Marteilia refringens* was observed in investigated oysters. *Bonamia* sp. was reported in Clew Bay (6%), Lough Swilly (10%) and Blacksod Bay (11%).
In 2007, five mortality cases were reported in *Crassostrea gigas*. In one case (Lough Foyle)

the detection of OsHV-1 was possible and was associated with the presence of an algal bloom of *Karenia mikimotoi.*

Malta and **Czech Republic** attended the meeting but did not have data to present.

This session highlights, once again, the difficulty to obtain production and transfer data. However, it was reminded that these data are important and necessary to establish sampling strategy and to investigate mortality cases, listed pathogen presence or suspicion.

Mollusc disease surveillance focuses on farmed animals and concerned very rarely wild animals. However, wild animals are considered as aquatic animals in the European legislation and should thus be included in the mollusc health surveillance systems.

Very few mortality cases were reported in 2007 and these cases were declared in few member states. An effort should be made to improve communication between farmers and people involved in sampling (competent authority or laboratory according to the organisation in place in Member States). Mortality reports constitute the first alarm signal in the case of the emergence and /or introduction of a listed and/or important pathogen.

Size of samples is still a topic of interest and feeds some discussion. Indeed, regarding the current legislation, which refers to the OIE aquatic manual and code, 150 individuals should be collected from each site identified in the sampling programme in zones under agreement process and in approved zone for bonamiosis and/or marteiliosis. However, depending on the size of the zone, more than one site should be investigated. Moreover this sample size does not take into account the true values of the specificity and sensitivity of the techniques used for the detection of the pathogens.

One of the most new information is the detection of Bonamia exitiosa in Galicia, Spain and in Adriatic sea in Italy. This listed pathogen was not reported in association with abnormal mortality but this could be due to the lack of mortality reporting especially for flat oyster production. At the moment there is no explanation concerning the detection of this "exotic" pathogen. This pathogen was observed by histology and/or imprints and the species was assessed using molecular tools including PCR-RFLP and sequencing.

The distribution of bonamiosis is still increasing and in 2007 the disease affected two previously free areas: Loch Sween and Loch Talbert in Scotland. No clear information is available at the moment to explain the emergence of the disease in this place.
Some Bonamia -like organisms were observed by histology within haemocytes of flat oysters from Norway. However due to the very low level of infection it was not possible to confirm the presence of the parasite and to determine the species. In 2008, the size of the sample will be increased in this area.

During these presentations another point of discussion focused on the way mussels should be included in the surveillance programme regarding freedom of marteiliosis. Indeed, Mytilus edulis and M. galloprovincialis are listed as susceptible species to Marteilia refringens. However, most countries approved free for marteiliosis do not test mussels regarding the presence of the parasite. Moreover, some Marteilia refringens infected zone/country export mussels to free zone/country. Marteilia refringens is currently or was already detected in flat oysters from Italy, Spain, Portugal, France, Greece and in mussels from UK, France, Spain,

Italy, Croatia and Greece.
Marteilia refringens is not usually associated with mortality of mussels. However, in 2007, a non typical location of the parasite was reported in the connective tissue of mussels cultivated in Normandy, France in association with mortality.

SESSION II: HOST RANGE DEFINITION
(Chair: I. Arzul)

Pathogenesis of OsHV-1 in cultured juvenile *Ostrea edulis*.

Steve Feist

Oyster Herpesvirus (OsHV) has long been known as a problem in bivalve hatcheries, affecting both larvae and spat. Several species of bivalves can be affected including oysters, clams and scallops. It is known that adult oysters can be carriers but little is known about the pathogenesis of infection in on-growing sites. Monthly sampling of juvenile *Ostrea edulis* took place between August 2005 and July 2006, fifty oysters were sampled each month from three distinct areas in the Fleet and Portland harbour area of the South West coast of the United Kingdom. Size (length) and condition details were recorded and samples taken for histopathology and electron microscopy. During this study OsHV-1 was detected for the first time in *Ostrea edulis* in the UK. Data collected enabled the prevalence, severity and effects of ecological parameters such as temperature and exposure on the virus to be examined. Unlike other species where warmer water increases the prevalence of a virus, the optimal temperature for OsHV-1 in *O. edulis* was 6 - 12 °C. Prevalence of infection peaked at 16% in Portland harbour with mortalities amongst the groups reaching approximately 14%. All groups showed slow growth over the study period.

The presence of other bivalve species around the infected flat oysters was questioned. Both Crassostrea gigas and Pecten maximus have been present locally but not in the immediate vicinity to the flat oysters but their status regarding the presence of OsHV-1 was not investigated.

Recent research on *Bonamia ostreae*: an investigation into the role of Molluscs other than *Ostrea edulis* as possible carriers or this parasite.

Sarah Culloty

Molluscs such as Pacific oysters, scallops and abalone have become of increasing importance to the Irish shellfish industry in recent years. Due to concerns about the possibility of these molluscs transferring *Bonamia ostreae* with them when being moved between areas, a project, funded by the Marine Institute was developed to investigate the possibility of these species acting as potential hosts or carriers of *B. ostreae*. Previous studies have investigated the susceptibility of a range of bivalves to this parasite and their potential to transmit the parasite back to *O. edulis*. To date no evidence of susceptibility to this parasite has been detected in Pacific oysters, cockles, clams and mussels. However, these studies have relied on light microscopy techniques for detection of the parasite. In this study these techniques were again

employed along with molecular based techniques for detection of the parasite.
Through a series of natural exposure trials in the field and the laboratory, *C.gigas*, *P. maximus* and *H. discus hanai* were naturally exposed to *B. ostreae*. In a series of laboratory trials naturally exposed Pacific oysters and scallops were returned to the laboratory and held in tanks with naïve *O. edulis*. In a second set of laboratory trials naturally infected *O. edulis* were held in tanks with either pacific oysters, or scallops or abalone.
In field trials *B. ostreae* DNA was detected in both tissues and shell cavity fluid of *C. gigas*. In the laboratory, *B. ostreae* DNA was detected in tissue and shell cavity fluid of *C. gigas* returned to the laboratory and in the shell cavity fluid of *P. maximus*. When held in tanks with infected *O. edulis*, *B. ostreae* DNA was detected in the tissues and shell cavity fluid of *C. gigas* and in the shell cavity fluid of *P. maximus* and one abalone. In the Pacific oysters which demonstrated positive results for PCR screening of the tissues, positive in situ hybridisation was also achieved on these tissues and microcells were observed intracellularly.

Such studies are very interesting and need to be combined to a risk analysis in order to measure the probability of transmission of Bonamia ostreae from Crassostrea gigas, Pecten maximus and abalone to flat oysters according to epidemiological, zootechnical and environmental criteria.
Mike Hine reported that some Microcell-like structures were observed in some Crassostrea gigas from South Australia.

Looking for the intermediate host of *Marteilia refringens*

N. Carrasco, I. López-Flores, M. Alcaraz, M.D. Furones, F.C.J. Berthe and I. Arzul

Since the first description of *Marteilia refringens* (Paramyxea) in flat oysters *Ostrea edulis* in 1968 in the Aber Wrach, Brittany (France), the life cycle of this parasite has remained unknown. Following the evidence from studies that indicated the need for intermediate hosts, the calanoid copepod *Acartia (Paracartia) grani* was proposed to be involved in the life cycle of the parasite infecting flat oysters growing in ponds ("the claires") with a low biodiversity. Nevertheless, after several attempts, experimental transmission of the parasite through the copepod has failed, which has led to the possibility of new studies concerning other similar species and their potential role in the life cycle and transmission of *M. refringens*.
No complex natural environments, with high diversity, such as bays or estuaries, have been studied for this purpose. Therefore, a survey for the presence of the protozoan *Marteilia* was conducted by PCR on the zooplankton community of a natural ecosystem, the Alfacs and Fangar bays in the Delta de l'Ebre (NW Mediterranean). First results had reported the presence of the parasite in zooplankton from the bays of this area, which is a more complex and natural estuarine environment than that of the claire system. Correlation between new infections in mussels and zooplankton parasitization could be observed during the transmission period. Consequently, the dynamics of *Marteilia* in the zooplankton community from one of the bays, Alfacs Bay, as well as the dynamics of the parasite in cultivated mussels during one complete year, were studied.
Results suggested that the dynamics of the parasite in the zooplankton community and in cultivated mussels were linked. Furthermore, six different zooplankton taxa appeared to be parasitized by *Marteilia refringens*, including copepods (three Calanoida, *Acartia discaudata*, *A. clausi* and *A. italica*; one Cyclopoida, *Oithona* sp.; and one Harpacticoida, *Euterpina acutifrons*), and larval stages of decapod crustaceans (zoea larvae of Brachyura, probably

Portumnus sp.). These taxa are thus proposed as potential new intermediate hosts, since they appear to be parasitized and linked to the infection process of mussels by *Marteilia*.

These results need to be completed by some experimental assays in order to test the potential role of zooplancton species into the life cycle of the parasite. Moreover, the detection of Marteilia was performed by PCR and not by histology or in situ hybridization.

The lists of susceptible species to the notifiable diseases given in the European legislation or the OIE manual are used to be considered as exhaustive host ranges for these pathogens. However, sometimes these species have been demonstrated to be susceptible on the basis of some experimental transmissions which mimic more or less the natural pathways or on the basis of some histological or molecular analysis which do not allow alone to confirm the pathogen species. Combined and multidisciplinary approach including histology molecular biology, ecology, risk analysis and experimental assays need to be encouraged in order to define host range with more transparency.

SESSION III: BONAMIA EXITIOSA NOTIFICATION

(Chair: C. Francois)

Bonamia exitiosa: dispersal and reductive life-cycles

Mike Hine

Three groups of *Bonamia* may be distinguished ultrastructurally. *Bonamia perspora* is currently unique, in that it sporulates and has claw-like prongs on the end of spore filaments. *Bonamia ostreae* comprises a small uni-nucleate stage with a central nucleus that undergoes binary fission to form a bi-nucleate stage that divides into further uni-nucleate stages, rarely, a diplokaryon forms and/or plasmodia with 4-6 nuclei. *Bonamia exitiosa* is similar to *B. ostreae* in the small uni-nucleate stages, and binary fission to bi-nucleate stages, but differs in that the small uni-nucleate stage frequently grows into an amoeboid large uni-nucleate stage, with parallel arrays of smooth endoplasmic reticulum, that is endocytotic. It also possesses a vacuolated stage, with the vacuole derived by swelling of a mitochondrion.
If it is assumed that, like *Haplosporidium* spp. and *Minchinia* spp., *Bonamia* spp. were initially spore-forming, the life cycles of asporous species may have developed by abandoning sporulation, and reduction in plasmodial size to 4-5 µm, so that all developmental stages were intra-cellular. Thus, large extra-cellular plasmodia of spore-forming species that used haplosporosomes to damage surrounding cells, permitting feeding, and did not transmit directly host-to-host, developed into small intra-cellular cells that did not sporulate, did not eject haplosporosomes, and transmitted directly host-to-host.
B. exitiosa occurs in Australia (*Ostrea angasi*), New Zealand (*Ostrea chilensis*), and probably Argentina (*Ostrea puelchana*), and a *B. exitiosa*-like parasite occurs in Chile (*O. chilensis*). This pattern of distribution may be attributed to evolution of a *B. roughleyi/B. exitiosa* parasite in Australia, that spread in rafted *O. angasi* or *Saccostrea glomerata* to northern New Zealand, where it became established in *O. chilensis* and *Ostrea stentina*, before rafting to Chile, ~18,000 yBP. The origin of Argentinian *B. exitiosa* is unknown, but may it be a shipping introduction.

F. Berthe points out that scientists will find more and more intermediate forms of Bonamia sp. and wonder how we shall address that in terms of taxonomy. M. Hine answers that the

genus Haplosporidium is very wide and experts in molecular phylogeny do agree that this group is a poly-phylogenetic one. F. Berthe remembers the question on taxonomy in Marteilia genus and wonders if scientists are confident in their criteria to distinguish and give a name for those pathogens.
I. Arzul underlines the importance to have the same criteria to avoid multiplying the development of different diagnostic methods. I. Arzul and M. Hine suggest to use sequencing with more than one targeted gene. In human pathology, four genes are used to differentiate one species to another.
F. Berthe says that scientists lack epidemiological studies and that one or two cases in a research study are not enough. M. Hine confirms this opinion considering that generally it deals with very light infection.
M. Hine thinks that environmental parameters like temperature and salinity should be more investigated in case of Bonamia sp. infection. I. Arzul answers that CRL has worked on it (talk of session VI).
I. Arzul and M. Hine wonder about the distinction between oysters species (O. edulis / O. angasi).

Detection of *Bonamia exitiosa* in Spain and confirmation by CRL

Isabelle Arzul

Before october 2007, *Bonamia ostreae* used to be detected in *Ostrea edulis* in Northern hemisphere and *Bonamia exitiosa* in *Ostrea chilensis* in New Zealand and *O. angasi* in Australia. Some *Bonamia exitiosa*-like isolates have also been reported in *O. chilensis* from Chile, *O. puelchana* in Argentina, *Crassostrea arienkensis* in U.S.A.

Gross signs and histological features are not specific enough to help discriminating between these different microcells including *Mikrocytos* /*Bonamia roughleyi* described in *Saccostrea commercialis* in Australia.

Studies based on ultrastructural examination of these different species show some differences in terms of number and size of organelles which might help to differentiate between *Bonamia* species. However, caution should be taken regarding the difficulty to obtain good fixed material and the need of expert eyes to conclude about the species. Moreover, these studies usually consider very few infected individuals.

Molecular data are restricted to genes belonging to the ribosomal RNA gene cluster, especially the 18S and the ITS1. These data, even scarce, allowed to perform some phylogenetic analysis and to develop some diagnostic tools discriminating between *Bonamia* species more precisely between *B. ostreae*, *B. roughleyi* and *B. exitiosa* like group. However, sequencing remains necessary before any conclusion.

In October 2007, Spain notified the presence of *Bonamia exitiosa* in some flat oysters collected from Ria de Arousa, in Galicia. The CRL was asked to perform some confirmatory analysis. Histological features were very similar to infection with *Bonamia* parasites but the central location of nuclei and the size of parasites could suggest that it was *B. exitiosa***.**

Restriction profiles as well as 18S gene and ITS1 sequences were similar to *Bonamia exitiosa* ones**.** These results confirmed the presence of *B. exitiosa* in flat oysters *Ostrea edulis* from Spain.

Following this notification and considering that *Bonamia* is routinely detected by histology and/or imprints within the European Union, which do not allow determining the species, it appears necessary to investigate the possible presence of *B. exitiosa* in areas usually considered as infected with *Bonamia ostreae.*

The characterization of Bonamia in Ostrea angasi in Australia feeds some debate. Indeed, some study based on molecular work reported the presence of Bonamia exitiosa in this oyster species while other study based on ultrastructural examination showed some specific features distinct from B. exitiosa ones. I. Arzul asks M. Hine if this parasite was characterized by the mean of biomolecular tools. M. Hine answers that they did not characterized the species in that way. M. Hine adds that the infection with Bonamia exitiosa looks totally different in histology / transmission electron microscopy depending on the host. More precisely, there were not many infiltrations reported in infected Ostrea angasi compared to infected Ostrea chilensis.

I. Arzul would like to know what the impact of Bonamia exitiosa in Ostrea edulis is.
F. Berthe asks if the NRL representatives and M. Hine have information about mortalities in O. edulis infected by Bonamia species. I. Arzul explains that B. exitiosa has been detected in a dispatch center in Italy. I. Arzul adds that CRL received samples from UK when new areas were suspected to be infected by Bonamia but at this stage it was only suspected to be Bonamia ostreae.
F. Berthe thinks that the "sequencing approach" should be completed by an epidemiological approach.

Workplan to characterise *Bonamia* parasites in Europe
Pedro Rosado-Martin

In October 2007, Spain has reported to the European Commission and the OIE occurrence of *Bonamia exitiosa* in European flat oysters, *O. edulis.*

This is the first time the pathogen is reported in the EU, and detected in *Ostrea edulis*.
The **objectives** of the work-plan are:

1) Increase the knowledge of the spread of *Bonamia exitiosa* in the EU

Before the application of Directive 2006/88/EC, it is necessary to increase the knowledge of the *Bonamia exitiosa* situation in the Community.

2) Raise the awareness of the Competent Authorities and national laboratories as regards the risk posed by exotic diseases.

For the time being, the EU has been considered as free from certain mollusc pathogens. That is the case with *Bonamia exitiosa*. However, detection of *Bonamia ostreae* within the European Union is classically based on heart or gill imprints or histology which are not able to distinguish those exotic species from the endemic ones (*B. ostreae*). It is thus necessary to use diagnostic techniques including PCR-RFLP and sequencing, when appropriate, suitable to establish a clear differential diagnosis.
The **actions** to be carried out to confirm the presence/absence of *Bonamia exitiosa* in Member States with oyster production are:

- in the framework of the *Bonamia ostreae* routine monitoring programmes to be conducted during 2008, emphasis should be given to the differentiation of *B. ostreae* and *B. exitiosa*, especially in known *B. ostreae* positive areas;
- in *Bonamia ostreae* endemic areas, samples should be taken for screening by means of gill or heart tissue imprints or histology;
- in addition, material should be obtained to ensure the possibility to perform PCR, PCR-RFLP and ISH to ensure a proper differential diagnosis of the two *Bonamia* species;

- in case of positive findings of *Bonamia spp* (by tissue imprints or histology) appropriate samples should be sent to the NRLs. In accordance with the specifications of the CRL, NRLs will perform PCR RFLP and sequencing **after cloning** which are needed to ensure a proper differential diagnosis. If the techniques are not available in the NRL, the CRL will provide support to establish a diagnosis or the samples may be sent directly to the CRL;
- epidemiological and sequence data will be collected by the CRL and should be collated under a database format if possible;
- by 1st July 2008, MSs and the CRL should submit a report on the *B. exitiosa* situation to the Commission.

Concerning the EU list of notifiable infection, M. Hine thinks it will be dangerous to de-list Bonamia exitiosa. P. Rosado-Martin answers that EC will adopt a precautionary approach because EC thinks that not enough data will be available in July.
F. Berthe wonders if there is maybe only one species of Bonamia with a certain biodiversity and asks if we are sure that it exists different species. Mike Hine replies that Bonamia individuals are different at the structural point of view. F. Berthe would like to know if TEM's images of B. exitiosa have been taken by CRL in Spanish samples. I.Arzul answers that CRL hasn't the opportunity and thinks that the Spanish colleagues would like to perform TEM themselves.
F. Berthe would like to know how the disease was introduced in Spain.
P. Rosado-Martin said that EC does not know how the parasite has been introduced in Spain and is also waiting for the confirmation of the infection in Italy. At this point, EC does not know if both detections are linked.

SESSION IV: LEGISLATION AND COLLABORATIVE ISSUES

(Chair: L. Miossec)

Virtual research environment: a new culture of networking

Franck Berthe

Networks have long been identified as an absolute requirement to scientific activities. This is particularly true for completion of Community Reference Laboratories functions and duties. A new approach of internet, and open sources, may contribute to overcome some of the natural difficulties of networking. With this in mind, it is proposed here to review an experience of Virtual Research Environment (Mollusc Health Laboratory, http://vre.upei.ca/mhl/), as a way to identify opportunities for improvement of web based networking. VRE-MHL-α is a based on the concept of open research space founded on the idea that the future of science is open. The project explores the opening of the research process and this may apply to the community of mollusc health. VRE-MHL project is still in a development phase. It offers a common, transparent, honest and shared platform to an open scientific community. This involves scientists and researchers, students, professionals as well as officers of National administration. VRE members are registered users and owners of the web site. Users may join for a specific project as MHL can provide a home base for a group or a project. Groups are private areas within the VRE. The functionalities that are currently available include on line chat, interactive lab book, bibliography, long distance histology, collection database, private messaging system. With help of rss feeds and selective registration to specific groups, users may monitor and follow the activities ongoing in the

common workspace. They may control their level of involvement and contribution. Several of these functions will be demonstrated.

Steve Feist pointed out that networks already exist referring to the PANDA network but also mentioned similar developments in the US where usage of such networks is more established. According to Franck Berthe, this tool is more flexible. A ring-test could be included. Moreover they plan to put a slide collection on line. This tool needs to be kept alive to be helpful for the scientific community.

Possible vector species and live stages of susceptible species not transmitting disease as regards certain molluscan diseases

Franck Berthe

Following a request from the European Commission, the Panel on Animal Health and Welfare (AHAW) of the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on possible vectors for diseases of molluscs, crustaceans and fishes listed in Council Directive 2006/88/EC (Question No EFSA-Q-2007-061). The mandate was composed of two questions: i) which species may be responsible for the transmission of a specific disease by acting as vector species for a disease listed, and in particular which life stages and under which conditions those species can transmit diseases, and, ii) which life stages of the susceptible species listed may not transmit the diseases listed in the same annex.

Concerning the question of life stages of susceptible species not being able to transmit the listed diseases it was concluded that any life stage (with the possible exception of gametes, eggs and larvae) of the listed susceptible species is susceptible. Current practices in hatcheries do not prevent contamination of the commodities via water and fomites, and it is recognised that this may lead to disease transmission.

Article 17 of the Directive that regulates the introduction of live aquaculture animals of vector species into disease-free areas requires that a list of vector species is drawn up. Published scientific literature and practical evidence from farming and trading practices combined with disease surveillance demonstrate that certain non-susceptible aquatic animal species are not vectors of the listed diseases. Following a qualitative release and exposure assessment, potential species or groups of species and the conditions under which they may act as vectors for listed pathogens were identified. The likelihood of transfer and also the establishment of the hazards ranked from very low to moderate under stated conditions. Significant lack of data on prevalence, distribution and infectivity of the listed diseases/agents, as well as pathogen survival parameters outside the host, contributed to a high degree of uncertainty about the likelihood estimates of transfer and establishment of the hazards. Although the consequences of establishment were assumed to be high, the actual acceptable level of risk did not form part of the mandate.

During the development of the report, a number of significant issues were identified which were relevant to the mandate but were not included in the terms of reference. These issues were also presented.

Mike Hine asks what the definition of the vector is. Does a vector imply movement, as shipping for example? Franck Berthe says no, regarding the limit of Directive, birds and ship are not included as a vector. An intermediate host is a susceptible species. Hitchhikers and ballast water are not taking into consideration. So they took into account the intervalvar content, the interior of the gut for example.

Sven Bergman wants to know what the situation for an agent replicating in the vector is. Franck Berthe answers that it is not a vector but a susceptible species.
According to Tristan Renault, the general definition of a vector in pathology could include an animal in which the pathogen could replicate, as insects for example. Franck Berthe agrees that the definition is not easy to get and discussion could continue.
Pedro Rosado-Martin says that according to the Directive, a vector is an aquaculture species in order to limit movement if contamination is observed (risk mitigation).
Sven Bergman wonders what a carrier is. This concept is not in the Directive
Cyrille François would like to know if we could get funding from EFSA. Franck Berthe says no, the goal of EFSA is to push scientists to identify specific issues which need to be investigated, then to submit these issues to the EU Commission which can publish a call for proposal.

Risk based surveillance of mollusc diseases: theory and practice

Pedro Rosado-Martin

Council Directive 2006/88/EC lays down animal health requirements for aquaculture animals and products thereof and contains provisions on the prevention and control of certain diseases in aquatic animals.
Chapter II of the Directive introduces a series of minimum preventive measures aimed at increasing the awareness and preparedness of the competent authorities, aquaculture production business operators and others related to this industry, for diseases in aquaculture animals. These measures include obligations for farms/farming areas to apply animal health surveillance as appropriate for the type of production (Article 10).
The Commission is drafting guidelines to provide the Member States with guidance on how to implement the requirements of Article 10 on the establishment of a risk-based animal health surveillance scheme, in particular on the risk classification of aquaculture farms and mollusc farming areas.
This general animal health surveillance scheme shall aim at the detection of:

- any increased mortality (relevant for all farms and mollusc farming areas);
- listed diseases (relevant for farms and mollusc farming areas keeping species susceptible to the listed diseases).

In addition to conduct a general check of the health status of the animals of the farm or farming area, the purpose of Article 10 is to advise the aquaculture production business operators on aquatic animal health issues, and where necessary, undertake the necessary veterinary measures.
In the framework of this scheme, inspections must be carried out, either by the competent authorities or by any private veterinarian or other qualified aquatic animal health service. Part B of Annex III to the Directive lays down recommended frequencies of inspection. The frequencies are determined by two factors:

- The health status of the concerned zone or compartment in relation to the listed non-exotic diseases (categories I – V).
- The general risk the farm poses in relation to spreading and contracting diseases.

At each inspection it may be appropriate to go through the records of the farm in particular the mortality records, to get a picture of the health status evolution and health history of the farm. A representative selection of all production unites should be inspected. If the outcome of this examination leads to suspicion of infection with a listed disease, the animals should be subject to laboratory testing. This examination should in particular aim at detecting infection with the suspected listed diseases.

Hege Hellberg asks who will be implied in these controls. *Pedro Rosado-Martin answers that concerns both competent authorities and aquatic specialists. The guideline on risk based surveillance is now finalised.*
Isabelle Arzul would like to know how the targeted surveillance will be implemented. Pedro Rosado-Martin says that they started with fish diseases. It is in progress but not finalised. For the next annual meeting, they will have the next guideline finalized.
Isabelle Arzul points out that the exercise is not easy to perform because of lack of data on persistence of pathogens in water for some diseases. Pedro Rosado-Martin says the guideline was established on a general basis in order to complain with all pathogens and diseases.

SESSION V: DISTRIBUTION OF OYSTERS CULTIVATED IN EUROPE

(Chair: C. Garcia)

Distribution of flat oysters, *Ostrea edulis*, in Europe

Stein Mortensen

Oysters have been a valuable seafood resource ever since man started collecting food along the coastlines. If kept cold and humid, oysters may be kept alive for long periods of time. They have therefore always been moved – both short and long distance.
The European flat oyster, *Ostrea edulis*, is distributed along most European and Mediterranean coastlines, from Norway to the Black Sea. Today, stocks are threatened by disease, predation and loss of habitats. In order to face these threats, there is a need for a common platform for research based management and protection of our flat oyster resources. This platform must be based on knowledge about the different oyster populations, their distribution and state.
A future, sustainable flat oyster must be based on the establishment of local productions, using indigenous oyster populations, minimizing the need for introductions and transfers – thus minimizing the risk of disease spreading.
This presentation has a focus on the history of the oyster, transfers and the present distribution of the European flat oyster. The participants at the meeting are challenged, in order to present – or start collecting – relevant information from their countries.

The flat oyster, Ostrea edulis, is the native oyster in Europe and is almost present along the entire European coastline. Meanwhile, its real distribution along European coastline is not perfectly known; so, to better know its distribution, Stein Mortensen suggested that each National Reference Laboratory fills a map in order to represent the flat oyster distribution along its own coastline. This information coupled with genetic information about oyster origins would permit to improve our knowledge about the oyster history especially about oyster transfers / movements. Indeed, it was noted that one of the main factors of species dispersion are human activities.
In Western Europe, its distribution and its movements are relatively well known whereas in Eastern Europe, little information is available, particularly along the Black Sea. The presence of wild flat oyster beds is reported in Black Sea but also a decrease of this population. The origin of this decrease is not really determined but environmental problems are suspected specially the presence of abundant deposit.
The observation of empty shells in an area even if no live population is reported, is also relevant information in term of human life history because in the past, oysters were the food

of the poor.

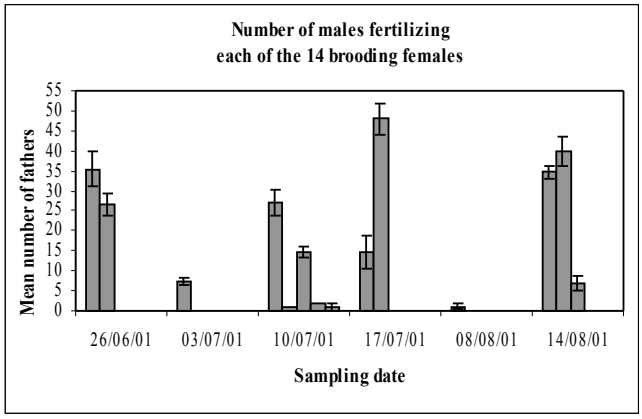
Genetic impact of the reproduction dynamics in the European flat oysters, *Ostrea edulis*.

Sylvie Lapègue, Nicolas Taris, Delphine Lallias, François Bonhomme, Pierre Boudry

The European flat oyster (*Ostrea edulis* L.) is a marine bivalve whose natural geographical distribution ranges along the Eastern Atlantic coast from Norway to Morocco, in addition to the Mediterranean and Black Seas. The comparison between the genetic differentiation between populations obtained by nuclear and mitochondrial markers led us to initiate studies at a finer scale, in order to estimate the effective number of breeders and the temporal dynamics of reproduction and, more specially, recruitment. Several experiments were performed to document (1) the variance in allele frequencies during a natural settlement period, (2) the paternal contribution to fertilization by analyzing larvae sampled at the brooding stage within individual females, (3) the variance of individual reproductive success within an experimental population.

First, 3 sets of collectors were successively deployed every two weeks and one set during the whole recruitment period in 2001. Adults oysters were also sampled among which 14 brooding females (i.e. females presenting larvae in their palleal cavity). Their larvae were sampled and preserved in ethanol. Mitochondrial (12S fragment) and microsatellites (four loci) analyses were performed.

Although the temporal cohorts did not exhibit any differentiation on the basis of the microsatellite markers, a slight but significant differentiation was observed with the mitochondrial marker : 5.7 % between one cohort and the adults. Moreover, our data on the genetic variability of single-female progeny show that females can be fertilized by a highly variable number of males, which can be, in some cases, very low (See Figure). In such cases, a temporary low effective size could lead to a level of inbreeding (even low) able to explain the correlation between growth and heterozygosity often observed in bivalves.



More investigations are needed to determine whether each male fertilizes several females or not. If they do, variability in environmental conditions should increase female more than male variance in reproductive success, thus explaining the reduced mitochondrial relative to nuclear effective size that was observed in the study of the genetic structure of the populations along the species range.

The relative impact of the movements of oysters on the genetic structure of flat oysters has been discussed.

Invasiveness of an introduced species in France: *Crassostrea gigas*.

Laurence Miossec

The Pacific oyster *Crassostrea gigas* has been introduced worldwide for aquaculture purposes since the beginning of the last century. In most of the countries the species established broadly, emphasizing its ability to adapt to a wide range of environmental conditions.
In Europe the Pacific oyster was massively introduced in the 1970s especially into France after mass mortalities of the Portuguese oyster *Crassostrea angulata* affected by a viral disease. Good environmental conditions in some French areas on the Atlantic coast induced successful recruitments in the following years. The population became established in the mid 70's. Illegal introductions in 1966 in Marennes Oleron were suspected to be the vector of the disease which affected the Portuguese oyster. This situation emphasized the risk of introducing unwanted organisms and disease agents when transplanting shellfish stocks without any precaution.
Recent published studies demonstrated that *Crassostrea gigas* has taking advantage of last warm summers to expand northwards into new areas. Increases in oyster abundance were simultaneously registered in the newly colonized sites. This situation has been observed in France, UK, Germany and Netherlands but also in Norway since the mid of the 90's. The dynamics of oyster population is clearly related to recruitment success. First results underlined that this species is acting as an invader due to its extensive recruitment. The species compete for space with mussels populations. But whole negative ecological consequences are still under investigation. Moreover the colonisation of new areas by *Crassostrea gigas* involves social and economical consequences. Some of them could be beneficial as for example the development of new spat harvesting areas for shellfish industry. Others could be detrimental for oyster culture due to trophic and spatial competition between wild and cultured molluscs. A 3-year national programme PROGIG, lead by the European Institute of Marine Science (university of West Brittany), is in progress to evaluate the importance of the expansion of this species along the French coasts, the dynamics of its proliferation and its ecological and economic consequences.

The question of impact of coast invasiveness by Crassostrea gigas on the native oyster Ostrea edulis was raised: could C. gigas proliferation be a barrier to the flat oyster settlement? This problem does not seem to exist because no settlement competition is observed between these two oysters. C. gigas is an interdital species whereas O. edulis is a subtidal species.

The northwards spreading of the Pacific oyster.

Stein Mortensen

The Pacific oyster is considered an adaptive species, which may become a pest, threatening the original balance in the shallow water ecosystems. In Denmark the Pacific oysters are forming reefs in the Wadden Sea and in the western part of Limfjorden. In the central part of Limfjorden and in Isefjorden the species is reported in low densities, and in 2007 the species is observed at two locations on the west coast of Jutland. On the Swedish west coast, a recent survey revealed dense populations (more than 300 per square meter at some sampling points)

of recently settled oysters at several shallow water sites. Some specimens have also been found on the Norwegian south coast.
Oysters collected in Denmark, on the Swedish west coast and Norwegian southern coast were attached to local substrates, indicating that the oysters settled there and have grown to adult size on the sites. A larval drift has thus occurred. The origin of the larvae is not described, but Swedish and Norwegian oysters might have the same origin. The oysters stocks in Denmark are probably a stepping stone in the dispersal of the species, releasing larvae drifting with coastal currents to Norway and Sweden. The circulation pattern in Skagerrak may support a larval drift from both the western coast of Denmark and from Kattegat.
Examination of oysters in a former oyster farm in western Norway revealed acini filled with ripe eggs and sperm. This verifies that the Pacific oyster may reproduce in Norwegian waters, as far north as 60°N. Remaining specimens from the live storage of oysters might have been the genitors of the younger generations.
The present work describes the first spatfall and reproduction of Pacific oysters in Scandinavia. This species is considered an alien in the Scandinavian fauna, and the establishment should be monitored – preferably as collaboration between Scandinavian research institutes.

An extension of Crassostrea gigas both in the South of Norway and in Denmark is observed since several years. An analysis of the environmental data of the last years including temperature data is in progress in order to know if environmental changes have been noted and if they could explain this spread.
Both in Norway and Denmark, C. gigas spawn are regularly observed but C. gigas settlement is not systematic; it is only noted following warm summers.
To date, it is not possible to know if the C. gigas spread is due to an adaptation of the species or environmental changes. Meanwhile, the temperature seems to have an important role; in Norway, C. gigas spawn are frequently observed in the southern lagoons where the seawater temperature is warm whereas, no spawn is reported in Bergen area where the temperature is colder.

SESSION VI: NEWS FROM THE BENCH

(Chair: J.P. Joly)

Antiviral immunity in the Pacific oyster, *Crassostrea gigas*: last development and perspective.

Tristan Renault, N. Faury, V. Barbosa Solomieu, K. Moreau, P. Haffner and J.-F. Pepin

Herpes-like viruses have been reported last decades in various marine mollusc species in association with mortality outbreaks throughout the world (Renault & Novoa 2004). One of these viruses isolated during French outbreaks has been characterised as an unassigned member of the *Herpesviridae* family and named ostreid herpes virus 1 (OsHV-1) or Pacific oyster herpes virus (Davison et al. 2005). Studying anti-viral non-specific defence mechanisms (innate immunity) is of great interest, because they constitute the only one in molluscs. Therefore, innate immunity has been investigated in the Pacific cupped oyster, *Crassostrea gigas*. The main aim of the work was focused on the identification and the characterisation of genes induced by OsHV-1 in the Pacific cupped oyster. In turn, this could be of benefit to the control of viral diseases of mollusc species.

Suppression Subtractive Hybridization (SSH) has been used to characterize genes involved in anti-viral response using OsHV-1 and Pacific oyster as a disease model. The subtracted library was obtained using RNA from oyster haemocytes (control and after virus contact). Clones identified as differentially expressed using SSH were sequenced and compared to sequences available in databases. Of the clones showing homology with known genes, several of them have functions connected to the innate immune response: macrophage expressed protein, molluscan defense molecule precursor or laccase 1. The full-length cDNA of these three genes was obtained using 5' and 3' RACE PCR. The predicted amino sequences were analysed for domain features and conserved signature motifs (Smart). Multiple alignment (ClustalW) and homology analysis of the deduced amino acid sequences of laccase 1, molluscan defence molecule precursor and macrophage expressed protein gene homologues were carried out. A phylogenic analysis was performed with MEGA program (version 3.1) based on amino acids alignment. The expression pattern of transcripts in healthy and OsHV-1 challenged oysters was studied by RT qPCR for laccase, molluscan defence molecule precursor and macrophage expressed protein gene homologues. The expression of the three selected genes was up-regulated and reached a maximum 48 hours after OsHV-1 challenge.

Davison, A.J., Trus, B.L., Cheng N., Steven, A.C., Watson, M.S., Cunningham, C., Le Deuff, R.M., Renault, T., 2005. A novel class of herpesvirus with bivalve hosts. J. Gen. Virol. 86, 41-53.

Renault, T., Novoa, B., 2004. Viruses infecting bivalve molluscs. Aquat. Living Ressour. 17, 397-409.

Mike Hine says that he could sometimes see capsides inside larvae without mortality.
Sven Bergman asks 1) if oysters are resistant to infection or resistant to the disease and 2) if the virus was observed inside ovocytes? Tristan Renault says that we don't know yet and we actually don't know if the virus can be responsible for mortalities in adult oysters and it's difficult to detect the virus inside the gametes with RT-PCR (question: is the virus DNA integrated in the oyster genome?).

Effect of salinity and temperature on *Bonamia ostreae* survival.

Isabelle Arzul, Céline Bond, Béatrice Gagnaire, Bruno Chollet, Benjamin Morga, Sylvie Ferrand, Maeva Robert and Tristan Renault

Bonamiosis due to the intrahaemocytic protistan parasite *Bonamia ostreae* is a European endemic disease affecting flat oysters *Ostrea edulis*. The parasite has been described in different ecosystems from estuaries to open sea and no clear correlations could be demonstrated between the development of the disease and environmental parameters such as temperature or salinity. The parasite life cycle, including its survival outside the host is not completely known. Nevertheless, the infection can be directly transmitted by cohabitation between infected and non-infected oysters suggesting that the parasite does not need intermediate host to complete its cycle. In the present study, the impact of temperature and salinity on the survival of purified parasites maintained in sea water was investigated by flow cytometry.
Purified parasites were incubated in three different 0.22 µm filtered sea water medium (artificial sea water; natural sea water from "La Seudre" Charente Maritime, France; underground salty water) and were subjected to three temperatures (4, 15 and 25°C). Then, purified parasites maintained in underground salty water were subjected to a range of salinity

(5, 15, 20, 25, 30, 35, 40 and 45 g/l). Parasites were collected after 12, 48 hours and 1 week of incubation for flow cytometry analyses including estimation of parasite mortality and non -specific esterase activities. All experiments were performed three times.
The parasite showed a significant higher survival in underground and natural sea water compared to artificial medium. Parasite survival and non specific esterase activities were lower at 25°C than at 4°C or 15°C. High salinities (35, 40 and 45 g/l) appeared to favour parasite survival and esterase activities (Fig. 1). No significant variation of parasite survival could be identified between 12 and 48 h after incubation starting. After one week, parasite cells appeared generally too damaged to allow good cytometry result interpretation. However, up to 58% of parasite survival could be observed after one week in underground salty water at 15°C.

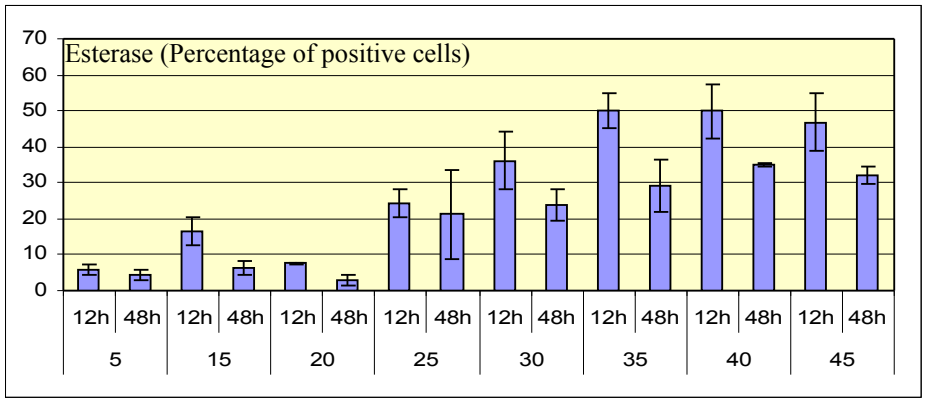


Figure 1. Percentage of positive parasites for non specific esterases evaluated by flow cytometry after an in vitro 12h or 48h incubation period in underground salty water at different salinities (5, 15, 20, 25, 30, 35, 40 and 45 g/l). Values are mean for three replicates. Bars represent standard error.

Steve Feist asked if morphology of dead cells was studied. Isabelle Arzul answers that it was not studied but testing infecting capacity of Bonamia cells after 1 week in sea water would be interesting.

Vibrio strains isolated during mortality events in France

Philippe Haffner

Vibrio are ubiquitous marine bacteria that represent a major source of concern in aquaculture facilities because of the discovery of increasing number of strains and species pathogenic for fish and shellfish causing severe loss of production. In France, the rearing of the Pacific oyster *Crassostrea gigas* is the main aquaculture activity and has recurrently suffered large scale summer mortality phenomenon for the last 15 years. Several *Vibrio sp* strains belonging to *V. aestuarianus* and *V. splendidus* species have been found to be associated with diseased juvenile oysters suffering summer mortality syndrome. Most of these strains exhibit a harmful effect when living bacteria or their extracellular products are experimentally injected to healthy oysters confirming their pathogenicity.
In order to precise the epidemiological relevance of these *Vibrio* species in mortality events occurring in cultivated mollusks in France, a study was conducted aiming to characterize bacterial strains isolated in a four years (2003, 2004, 2005 and 2007) bacteriological survey

performed by the French network of shellfish pathology (REPAMO) from IFREMER. During 2003-2007, 93 samples from different origins were collected in a context of abnormal mortality events concerning 4 molluscs species (*Crassostrea gigas* mainly but also abalone *Haliotis tuberculata*, scallop *Pecten maximus* and clam *Ruditapes philippinarum*) and more than 30 locations along Atlantic or Mediterranean French coast. Around 180 bacterial strains were isolated from diseased animals, selecting dominant bacterial isolates from each sample. Strains were taxonomically characterized by phylogenetical analysis sequencing a fragment of 16S rRNA gene and for *V. splendidus* related strains GyrB, a housekeeping gene encoding a DNA gyrase essential for chromosomes replication. Taxonomically representative bacterial strains were also characterized phenotypically, evaluating their virulence status by experimental infection.
Concerning *C. gigas*, this study revealed that the majority of the strains (54% or 62:115) isolated by REPAMO network during 2003-2007 belongs to *V. splendidus* and *V. aestuarianus*. Furthermore several strains of both species were found virulent in experimental infection of oysters confirming the pathogenicity of these two *Vibrio* species. A biotest predictive of the virulence potential was developed quantifying the metalloprotease like activity found in bacterial culture supernantants obtained from different strains. Besides *V. aestuarianus* and *V. splendidus*, virulence potential of other not yet documented bacterial strains associated to mortality events in oysters and belonging to *V. mytili* or *Shewanella colwelliana* species was evaluated by experimental infection of oysters because of their significant representativeness.
Concerning bacterial strains associated to mortality of other molluscs species, *V. splendidus*, *V. aestuarianus* and *S. colwelliana* were the most frequently isolated bacteria in clam *R. philippinarum* and scallop *P. maximus*, contrarily to abalone *H. tuberculata* where *V. harveyi* and *V. splendidus* were predominantly isolated. Because current diagnostic tests aiming to identify *V. species* are often time consuming and can give erroneous results, as phenotypic methods applied to *V. splendidus* characterization, reliable molecular based assays have been developed using classical or quantitative PCR to identify rapidly *V. species* associated to oysters disease outbreaks.

Sven Bergman asked how he could avoid punching contamination. Philippe Haffner answered that he took 2 punches from a blank filter to avoid contamination.
Steve Feist pointed out that Shewanella causes pathology in some fish and wondered if members of this genus might also be mollusc pathogens? Philippe Haffner answered that the experimental infection with this bacteria was negative.

Characterization of actin genes in *Bonamia ostreae*
I. Lopez-Flores, M. Robert, V.N. Suarez-Santiago, D. Longet, D. Saulnier, B. Chollet and I. Arzul

Bonamia ostreae is a protozoan parasite that infects the European flat oyster *Ostrea edulis* causing systemic infections and resulting in massive mortalities. Previously, isolation of the gene coding the SSU rRNA allowed to performe phylogenetic analysis which placed *Bonamia* within the Haplosporidia. Moreover, genes coding proteins like actin genes have a different evolutionary rate compared to those governing ribosomal genes and in this respect, may contribute to a wider knowledge of relationships within the haplosporidian species. In this work *B. ostreae* actin genes were characterized and their sequences were used for a phylogenetic analysis and for the development of a quantitative PCR assay. Characterization of the sequences allowed to identify two sequence types encoding two proteins and suggested

that two paralogous actin genes are present in the parasite genome. Actin phylogeny based on nucleotide sequences supported previous studies based on SSU rDNA sequences and placed *B. ostreae* in a clade with *Minchinia tapetis*, *Minchinia teredenis* and *Haplosporidium costale* as its closest relatives. Also, primers were designed on the actin gene sequences in order to develop a real time PCR aiming at quantifying parasite burden in oysters.

Mike Snow suggested the use of RT-PCR for quantifying target RNA and increasing the sensitivity.

SESSION VII: NEWS FROM THE COMMUNITY REFERENCE LABORATORY

(Chair: I. Arzul)

EU projects involving the CRL/NRL network: WOPER

I. Arzul

Some species of the genus *Perkinsus* (*P. olseni and P. mediterraneus*) are known to affect bivalve molluscs of the European coasts and other species (*P. marinus* and *P. chesapeaki*) are exotic to Europe. The goal of the European project WOPER was to bring together industry, research community and administration in a 3 day workshop to address the threat of perkinsosis to European shellfish industry.
The following topics were discussed:

- **Parasites of the genus *Perkinsus*:** life cycle and transmission ways; host species; taxonomy and phylogeny; physiology/metabolism; diagnostic tools.
- **Epizootiology:** geographic range; environmental influence; temporal patterns of disease dynamics; modelling for prediction.
- **Host-pathogen interaction:** host immune reaction; pathogen virulence factors; pathogen mechanism to elude host response; effects on host physiology.
- **Effects on shellfish industry:** socio-economic aspects of clam industry, with emphasis in Europe; losses due to *Perkinsus* in Europe and out of Europe; risk of introduction of exotic *Perkinsus spp.* Into Europe through shellfish imports and other ways.
- **Control and fighting strategies:** international rules for controlling mollusc traffic; selection programmes for resistance; production of *Perkinsus*-free seed; therapeutics; culture/fishery management; bed restoration.

The workshop took place in Vigo, Galicia, Spain on the 12th, 13th and 14th of September 2007. Proceedings of the Workshop available for downloading at the following website http://www.cetmar.org/woper.

Proficiency test 2007 for National Reference Laboratories for mollusc diseases

C. Garcia, B. Chollet, J.P. Joly, I. Arzul

A proficiency test aims at establishing that the examination of a given sample leads to the same conclusions in any laboratory within the National Reference Laboratory network. It also permits (1) to determine a laboratory's capability to conduct specific diagnostic tests, (2) to check or certify the performance of individual operators, (3) to harmonise existing test methods (4) to resolve interlaboratory differences and (5) to evaluate new test methods.

In 2007, it was the sixth proficiency test organised by the Community Reference Laboratory and it was composed of two slide collections (30 slides per collection). These objectives were:

- For collection 1, the detection of EU listed diseases in Directive 91/67/EC, bonamiosis due to *Bonamia ostreae* and marteiliosis due to *Marteilia refringens* in the European flat oyster *Ostrea edulis* by histology and cytology,
- For collection 2, the detection of listed or not listed exotic pathogens presently described in oysters (*Crassostrea virginica* and *C. gigas*) from North America by histology.

18 laboratories participated in both collections and 2 laboratories only in collection 1.
The laboratory results for collection 1 were similar to those of the previous proficiency tests; the mean of all laboratories was 84.37%, lightly superior to this of 2005 proficiency test. The main difficulties were encountered for the detection of light parasite infestations particularly for the parasite *Bonamia ostreae*.
The results of collection 2 highlighted the difficulty of laboratories to detect exotic pathogens, specially the parasite *Perkinsus marinus* in *Crassostrea virginica*; confusion exists between *P. marinus* and *Haplosporidium nelsoni* and the light parasite infestation are often misdiagnosed.
A disparity was observed between laboratories particularly for the detection of exotic pathogens; this disparity emphasizes the need for training in diagnosis by means of histology notably for the detection of *Perkinsus marinus*. So, training session will be organized in 2009 during the next workshop and will focus specially on this parasite.
Moreover, as histology and cytology techniques are often not sufficient to demonstrate free status and molecular tools, including PCR, in diagnostic procedures are more and more used, a new proficiency test was implemented in 2008 and was based on the detection of *Bonamia ostreae* by PCR. The goal is to alternate between histological and molecular biology proficiency tests according to the years.

I. Arzul reminds that results of ring test need to be taken into account into the interpretation of the results of the mollusc disease surveillance. An average of 80% of good responses or plus is good; an average of good responses between 60 and 80 % is good but results obtained during surveillance programme should be taken with caution; an average of good responses of 60% or less is not enough and requires additional training.

Some NRLs expressed their interest in participating in specific training in histology in addition to the workshop organised every two years by the CRL. The idea would be to offer training periods to a small group of colleagues and to focus on endemic and on exotic listed pathogens.

Working prospects of the Community Reference Laboratory for mollusc diseases
I. Arzul and B. Chollet

In 2007, the NRLs network for mollusc diseases included 22 NRLs from Member States and 3 NRLs from other countries (Norway, Croatia and Turkey).
Functions and duties of the CRL for mollusc diseases are given by the Directive 95/70/EC.
One of the main aims of the CRL is to harmonize diagnosis within the EU. For this purpose the CRL has created and maintains a collection of pathogens available for laboratories in Member States. A CD ROM on histology and anatomo-pathology has also been developed since 2002. The CD-ROM proposes illustrations and comments believed to be valuable for mollusc diseases diagnostic, especially diseases notifiable to the EU and OIE. The first edition was focused on the two oyster species *Ostrea edulis* and *Crassostrea gigas* and is subject to permanent reviewing and updating. The second edition (version 1.1), available in 2003,

includes oyster species and, now mussels, pearl oysters and abalone. It has been updated in 2007 and includes now clams and was distributed to NRLs .
Inter-laboratory proficiency tests are regularly organised to test the ability of laboratories to identify listed and important pathogens by histology. A sixth ring test started in February 2007 and includes 30 histological-cytological sections of *Ostrea edulis* and 30 histological sections to test capacity of NRLs to detect pathogens reported in North America.
In January 2008, a ring test for the detection of *Bonamia* sp. by PCR was organised for the first time and inclded 17 participants. Results are not analysed yet.
An advisory group in Quality Assurance was created in 2004. Exchange of information between NRLs during annual meetings or by e-mails allowed the CRL to write quality assurance documents that could be used by NRLs wishing to build their Quality System. These Standard Operating Procedures are available on the CRL website (http://www.ifremer.fr/crlmollusc/). Two SOPs were prepared in 2007 concerning the detection of *Mikrocytos mackini* and *Perkinsus marinus* by histology.
The CRL also aims at collecting and collating information on the mollusc disease situation in Member States and worldwide. A new template of the epidemiological report has been used to collect 2006 and 2007 data. This template will need to be modified in order to take into account the new Directive 2006/088/EC.

The CRL assists the NRLs in the diagnosis of disease outbreaks in Member States and provides opportunities of training and retraining through trainees, technical workshop and annual meetings. It is proposed to have the 2009 annual meeting joint to a technical workshop in March-April 2009.

The CRL carries out several studies on listed pathogens. In 2007, works have been done to characterize new genes in the genome of *Bonamia ostreae*. In 2007, the CRL was also involved in studies on the life cycle and host range of *Marteilia refringens*.

A new tool for training in histology has been presented. This tool relies on some virtual slides which could be easily accessible from the mollusc CRL website. These slides could include the previous ring test slides, some specific slide collections. The NanoZoomer System developed by Hamamatsu Photonics can scan tissue glass slides at magnification 20 or 40 and convert them to high resolution digital slides. Through computer connected to the internet, digital slides can be observed with “NDP serve” ® software. This new tool does not aim at replacing workshop and/or reference material but aims at providing additional support for improving histological diagnosis.

Annex A: Agenda of the Annual Meeting of the National Reference Laboratories for Mollusc Diseases, Nantes 18-19 March 2008

Tuesday 18th March

9.00 - 9.30 *Registration for the annual meeting*
Welcome address and announcements
P. Rosado Martin (European Commission)
I. Arzul (Community Reference Laboratory for Mollusc Diseases)

SESSION I: DIAGNOSIS AND SURVEY OF MOLLUSC DISEASES
Chairman I. Arzul

9.30 - 13.00 Representatives from each Member State present the disease situation in their respective country in 2007, emphasising mollusc diseases listed in the EU Directives 91/67 and 95/70, and/or the diagnosis of pathogens of special interest

(10.30 - 10.50 *Coffee Break*)

13. 00 - 14.00 *Lunch*

SESSION II: HOST RANGE DEFINITION
Chairman I. Arzul

14.00 - 14.20 Genetic impact of the reproduction dynamics in the European flat oyster *Ostrea edulis*. ***S. Lapegue***

14.20 - 14.40 Pathogenesis of OsHV in cultured juvenile *Ostrea edulis*. ***S. Feist***

14.40 - 15.10 Recent research on *Bonamia ostrea*e: an investigation into the role of Molluscs other than *Ostrea edulis* as possible carriers or this parasite. ***S. Cullotty***

15.10 - 15.30 Looking for the intermediate host of *Marteilia refringens*. ***I. Arzul***

15.30 – 15.50 Discussion

15.50 -16.10 Coffee Break

SESSION III: BONAMIA EXITIOSA NOTIFICATION
Chairman C. François

16.10 - 17.10 *Bonamia exitiosa:* dispersal and reductive life-cycles. ***M. Hine.***

19.30 Dinner downtown

SESSION III: BONAMIA EXITIOSA NOTIFICATION (continuation)

Chairman C. François

9.30 - 9.50 Detection of *Bonamia exitiosa* in Spain and confirmation by CRL. ***I. Arzul***

9.50 - 10.10 Workplan to characterise *Bonamia* parasites in Europe. ***P. Rosado-Martin***

SESSION IV: LEGISLATION AND COLLABORATIVE ISSUES

Chairman L. Miossec

10.10 - 10.30 Tools for collaborative work available on internet and perspectives for reflabnet. ***F. Berthe***

10.30 - 10.50 Coffee break

10.50 - 11.20 Possible vector species and live stages of susceptible species not transmitting disease as regards certain molluscan diseases. ***F. Berthe***

11.20 - 11.50 Risk based surveillance of mollusc diseases: theory and practice ***P. Rosado-Martin***

SESSION V: DISTRIBUTION OF OYSTERS CULTIVATED IN EUROPE

Chairman C. Garcia

11.50 - 12.10 Distribution of flat oysters in Europe. ***S. Mortensen***

12.10 - 12.30 Invasiveness of an introduced species in France: *Crassostrea gigas* ***L. Miossec***

12.30 - 14.00 Lunch

14.00 -14.20 The northwards spreading of the Pacific oyster. ***S. Mortensen***

SESSION VI: NEWS FROM THE BENCH

Chairman J.P. Joly

14.20 - 14.40 Antiviral immunity in the Pacific oyster, *Crassotrea gigas*: last development and perspective. ***T. Renault***

14.40 - 15.00 Effects of salinity and temperature on *Bonamia ostreae* survival. ***I. Arzul***

15.00 - 15.20 *Vibrio* strains isolated during mortality events in France. ***P. Haffner***

15.20 - 15.40 Characterization of actin genes in *Bonamia ostreae*. ***I. Arzul***

15.40 – 16.00 Coffee break

SESSION VII: NEWS FROM THE CRL
Chairman I. Arzul

16.00 - 16.20 EU Projects involving the CRL/NRL network WOPER ***I. Arzul***

16.20 - 16.40 Ring test 2007 ***C. Garcia***

16.40 - 17.00 Working prospects of the Community Reference Laboratory for mollusc diseases. ***I. Arzul and B. Chollet***

End

Annex B: List of participants

Anders Alfjorden Avdelning för vilt, fisk och miljö Fisksetionen S-751 89 Uppsala Sweden	(46) 18674191	(46) 18674044	Anders.alfjorden@sva.se
Raquel Aranguren Ruiz Instituto de Investigaciones Marinas, CSIC C/ Eduardo Cabello, n°6 36208 Vigo Spain	34 986 231930 ext 285	34 986 292762	arangur@iim.csic.es
Giuseppe Arcangeli Istituto zooprofilattico sperimentale delle venezie Sezione Di Adria Via Leonardo da Vinci 39 45011 Adria Italy	39 (0) 42621841	39(0) 426901411	garcangeli@izsvenezie.it
Isabelle Arzul Community Reference Laboratory for molluscs Ifremer - LGP Ronce-Les-Bains 17390 La Tremblade France	(33) 5 4676 2647	(33) 5 4676 2611	iarzul@ifremer.fr
Sven M Bergmann Friedrich-Loeffler-Institut Federal Research Institute for Animal Health Institute of Infectology Südufer 10 17493 Greifswald – Insel Riems Germany	49 38351 7103	49 38351 7226 49 38351 7275	sven.bergmann@fli.bund.de
Franck Berthe Animal Health and Welfare Unit European Food Safety Authority - EFSA Largo N. Palli 5/A I 43100 Parma Italy	390 521 036 870		Franck.BERTHE@efsa.europa.eu

Deborah Cheslett Fish Health Unit Marine Institute, Oranmore Rinville C.o Galway Ireland	353 91387 200	353 91 387 236	deborah.cheslett@marine.ie
Vanya Chikova National Veterniary Service 15A Plencho Slaveikov blvd. 1606 Sofia Bulgaria	359 2 91 59820	359 2 95 49593	vchikova@abv.bg
Bruno Chollet Community Reference Laboratory for molluscs Ifremer - LGP Ronce-Les-Bains 17390 La Tremblade France	(33) 5 4676 2651	(33) 5 4676 2611	Bruno.Chollet@ifremer.fr
Mihaela Costea Institute for Diagnosis and Animal Health 63 Dr Staicovici Street Sector 5, cod 050557 RO - 76 202 Bucharest Romania	40 21 410 12 99	40 21 411 33 94	costea.mihaela@idah.ro
Sarah Culloty Department of Zoology, Ecology & Plant Science, University College Enterprise Building, North Mall Cork Ireland	00 353 214904646	00 353 21 4904664	s.culloty@ucc.ie
Marc Engelsma Central Veterinary Institute CVI P.O. Box 65 8200 AB Lelystad The Netherlands	31 320 238 729	31 320 238 153	marc.engelsma@wur.nl
Stephen Feist CEFAS Weymouth Laboratory Barrack Road, The Nothe Dorset, DT48UB, Weymouth England United- Kingdom	1305 206 600	1305 206 601	Stephen.feist@cefas.co.uk

Sylvie Ferrand Community Reference Laboratory for molluscs Ifremer – LGP Ronce Les Bains 17390 La Tremblade France	(33) 5 4676 2650	(33) 5 4676 2611	Sylvie.Ferrand@ifremer.fr
Jean-Baptiste Fouquet ULG Faculté de Medecine Vétérinaire 20 bd de Colonster B-4000 Liège Belgium	32 (0)4 366 4226	32 (0) 366 4244	jbfouquet@ulg.ac.be
Cyrille François Community Reference Laboratory for molluscs Ifremer – LGP Ronce Les Bains 17390 La Tremblade France	(33) 5 4676 2647	(33) 5 4676 2611	Cyrille.francois@ifremer.fr
David Fraser FRS Marine Laboratory PO Box 101 Victoria Road Aberdeen AB11 9DB Scotland – United-Kingdom	44(0)1224876544	44(0)1224295511	fraserdi@marlab.ac.uk
Céline Garcia Communauty Reference Laboratory for molluscs Ifremer – LGP Ronce Les Bains 17390 La Tremblade France	(33) 5 4676 2645	(33) 5 4676 2611	Celine.Garcia@ifremer.fr
Mark Gatt Malta Centre for Fisheries Sciences - Fort San Lucjjan Marsylokk Malta BBG 1308	222 93325		Mark.gatt@gov.mt
Camino Gestal Instituto de Investigaciones Marinas, CSIC C/ Eduardo Cabello n°6 36208 Vigo Spain	34 986231930 ext285	34 986292762	cgestal@iim.csic.es

Philippe Haffner Ifremer – LGP Ronce Les Bains 17390 La Tremblade France	(33) 5 4676 2627	(33) 5 4616 2611	Philippe.Haffner@ifremer.fr
Hege Hellberg National Veterinary Institute of Bergen- PO Box 1263 Sentrum N5811 Bergen Norway	47 55 363 838	47 55 321 880	hege.hellberg@vetinst.no
Anders Hellstrom National Veterinary Institute of Sweden, Department of Animal Health and Microbial Strategies 751 89 Uppsala Sweden			anders.hellstrom@sva.se
Mike Hine 73 rue de la Fée au Bois 17450 Fouras France	(33) 5 46841639		vinet.hine@orange.fr
Vlasta Jencic National Veterinary Institute of Ljubljana, Laboratory for Fish Diseases Gerbiceva 60 1000 Ljubljana Slovenia	00 38614779145	00 386 1283224	Vlasta.jencic@vf.uni-lj.si
Jean-Pierre Joly Community Reference Laboratory for molluscs Ifremer - LGP Ronce-Les-Bains 17390 La Tremblade France	33 5 46 76 26 51	33 5 46 76 26 11	Jean.pierre.joly@ifremer.fr

Frantisek Kostka Statni Veterinarny ustav Rantirovska 93 586 05 Jihlava Czech Republic	420 567143218	420 567143284	kostka@svujihlava.cz
Ian Laing CEFAS Weymouth laboratory Barrack road The Nothe Weymouth, Dorset DT4 8UB England, United Kingdom	44 1305 206711	44 1305 206602	ian.laing@cefas.co.uk
Sylvie Lapègue Ifremer – LGP Ronce Les Bains 17390 La Tremblade France	(33) 5 4676 2631	(33) 5 4676 2611	Sylvie.Lapegue@ifremer.fr
Lone Madsen Technical University of Denmark National Institute of Aquatic Resources, Fish Diseases Laboratory Stigbøjlen 4 1870 Frederiksberg C Denmark	45 35 33 27 63		lm@aqua.dtu.de
Laurence Miossec Community Reference Laboratory for molluscs Ifremer - LGP Ronce-Les-Bains 17390 La Tremblade France	(33) 5 46 76 26 51	(33) 5 46 76 2611	lmiossec@ifremer.fr
Stein Mortensen Institute of Marine Research PO Box 1870 , Nordnes 5817 Bergen Norway	47 55 23 63 69	47 55 23 63 79	Stein.mortensen@imr.no
Drazen Oraic Croatian Veterinary Institute Dept. For Fish Diseases and Aquaculture Savska 143 10000 Zagreb Croatia	385 1 6123 614	385 1 6190 841	oraic@irb.hr

Agnieszka Pekala National Veterinary Research Institute Department of Fish Diseases NVRI, 24-100 Pulawy, Ul.Partyzantow 57 Poland	48 81 889 3375	48 81 886 25 95	A.Pekala@piwet.pulawy.pl
Tristan Renault Community Reference Laboratory for molluscs Ifremer - LGP Ronce-Les-Bains 17390 La Tremblade France	(33) 5 4676 2653	(33) 5 4676 2611	Tristan.renault@ifremer.fr
Maeva Robert Community Reference Laboratory for molluscs Ifremer - LGP Ronce-Les-Bains 17390 La Tremblade France	(33) 5 46 76 26 50	(33) 5 46 76 26 11	Maeva.robert@ifremer.fr
Pedro Rosado Martin European Commission Office DG SANCO-D1 Rue Froissart 101, F101 B-03/76 1040 Brussels Belgium	+32-2-2958916		pedro.rosado-martin@ec.europa.eu
Mike Snow Molecular Genetics, Fisheries Research Services, Marine Laboratory 375 Victoria Road AB11 9 DB Aberdeen United-Kingdom	441 224295611	441 224295667	m.snow@marlab.ac.uk
Stefano Sotgia FVO SANCO EC Grange Ireland	00 353 46 9061867		Stefano.sotgia@ec.europa.eu
Snjezana Zrncic Croatian Veterinary Institute Dept for Fish Diseases and Aquaculture Savska 143, 10000 Zagreb Croatia	385 1 6123 614	385 1 6190 841	zrncic@irb.hr

Belgium 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Federal Agency for the Safety of the Food Chain**	**Laboratoire de microbiologie des denrées alimentaires**
Address :	WTC III Boulevard Simon Bolivar 30 B-1000 Bruxelles	Boulevard de Colonster n°20, Bat B43bis, 4000 Liège, Belgique
Web site :	www.afsca.be	http://www.mdaoa.ulg.ac.be/fr/lnr/index.html
Contact :	Chantal Rettigner	Jean-Baptiste Fouquet
Phone :	32 (0)2 208 38 18	32 (0) 4 366 42 26
E-mail :	chantal.rettigner@afsca.be	jbfouquet@ulg.ac.be

General data

Technical data	**North Sea Oostende**	**North Sea**										**National data (official)**
Farms	1	4										
hatcheries - nurseries												

Production data												**National data (official)**	**FAO 2003**
Mytilus edulis		5											
Crassotrea gigas	20												
Ostrea edulis													

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	■	■	■								
	Histology	■	■	■			■			■		■
	PCR	■	■	■				■	■			■
	RFTM culture							■	■			
	Other											
Presumptive	Cytology	■	■	■								■
	Histology	■	■	■			■	■	■	■		■
	TEM											■
	PCR	■	■	■				■	■	■		
	RFTM culture							■	■			
	ISH		■									
	Other											
Confirmatory	Histology		■				■			■		■
	ISH	■	■	■						■		■
	TEM	■		■			■					
	PCR	■	■	■				■	■	■		■
	PCR-RFLP	■		■								
	Sequencing		■				■	■	■			■
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

■ Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
North Sea Oostende			0	

Results	
Bonamia	Marteilia

No monitoring in 2007.

Comments

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals
North Sea					0	
Oostende					0	

No abnormal mortality notified in 2007

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments

Additionnal comments

Croatia 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**MINISTRY OF AGRICULTURE, FORESTRY AND WATER MANAGEMENT**	**CROATIAN VETERINARY INSTITUTE**
Address :	AV VUKOVAR 78, 10000 ZAGREB	SAVSKA 143, 10000 ZAGREB
Web site :	www.mps.hr	www.veinst.hr
Contact :	Veterinary Directorate	Dept. Of Pathology, Laboratory for Fish Pathology
Phone :	38516109207	38516123614
E-mail :		zrncic@irb.hr

General data

Technical data	Zone I – Istra (Limski Bay, Vabriga, Catching area Zub)	Zone II – River Krka Estuary	Zone III – Malostonski Bay									National data (official)
Farms	9	10	105									124
hatcheries - nurseries			1-experimental									

Production data	Zone I – Istra (Limski Bay, Vabriga, Catching area Zub)	Zone II – River Krka Estuary	Zone III – Malostonski Bay									National data (official)	FAO 2005
Ostrea edulis	10	1	70									81	
Mytilus galloprovincialis	200	450	2700									3350	3000
	50	10	5									65	

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	Yes	Yes									
	Histology	Yes	Yes									
	PCR											
	RFTM culture											
	Other											
Presumptive	Cytology	Yes	Yes									
	Histology	Yes	Yes									
	TEM											
	PCR											
	RFTM culture											
	ISH											
	Other											
Confirmatory	Histology											
	ISH											
	TEM											
	PCR											
	PCR-RFLP											
	Sequencing											
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

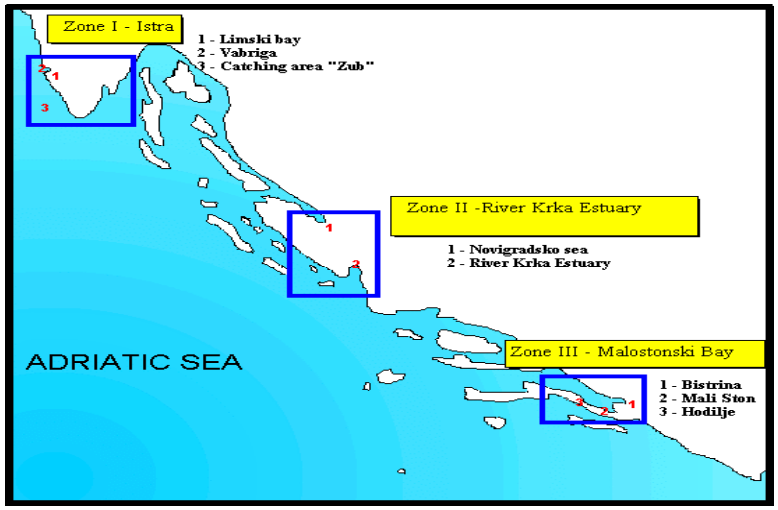
Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
Zone I –	02/07-04/07		2	100
Zone I –	00/09/07		1	50
Zone II –	00/04/07		1	30
Zone II –	00/09/07		1	30
Zone III –	00/03-05/07		5	250
Zone III –	00/09-10/07		5	250

Results	
Bonamia	Marteilia
0/100	
	0/50
0/30	
	0/30
0/250	
	0/250



Comments
No informations about abnormal mortalities during 2007.

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	Decree on the measures of animal health protection against inf. Diseases
Type :	Official program
Aim :	Detect pathogen
Duration :	Annual programme
Mollusc species concerned	Mytilus galloprovincialis
Targeted pathogens	Marteilia refringens
Objectives and Brief summary of the program / project	

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
Zone I	00/09-10/07	*Mytilus galloprovincialis*			3	90	*Marteilia spp.*	13,30%	
Zone II	00/09/07	*Mytilus galloprovincialis*			1	30	*Marteilia spp.*	6,70%	
Zone III	00/10/07	*Mytilus galloprovincialis*			3	90	*Marteilia spp.*	5,60%	
Other	00/09-10/07	*Mytilus galloprovincialis*			3	90	*Marteilia spp.*	7,80%	

Additionnal comments

Accreditation process in the frame of the CARDS 2002 project and under the supervision of SINAL experts (Italy) has started some Laboratories of CVI (Dept, of Public Health are accredited) while Laboratories which are performing diagnostic work are planned to achieve accreditation in September 2008.

Denmark 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Danish Veterinary and Food Administration, Animal Health Division**	**University of Denmark, National Institute of Aquatic Resources, Fish Disease**
Address :	Mørkhøj Bygade 19, DK-2860 Søborg, Denmark	Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark
Web site :	www.fvst.dk	www.aqua.dtu.dk
Contact :	Anders Højgaard	Lone Madsen
Phone :	4533956000	4535332763
E-mail :	1kontor@fvst.dk	lm@aqua.dtu.dk

General data

Technical data	Limfjorden											National data (official)
Farms												3
hatcheries - nurseries												1

Production data	Limfjorden											National data (official)	FAO 2003
Ostrea edulis	1212											1212	
Mytilus edulis	33286											57335	
Cardium spp.	1											39	
Spisula solida	0											6	
Chlamys opercularis	0											1	

Laboratory data

Diagnostic technics		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	Yes										
	Histology	Yes		*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*
	PCR											
	RFTM culture											
	Other											
Presumptive	Cytology	Yes										
	Histology	Yes	Yes									
	TEM											
	PCR											
	RFTM culture											
	ISH											
	Other											
Confirmatory	Histology											
	ISH											
	TEM											
	PCR											
	PCR-RFLP											
	Sequencing											
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

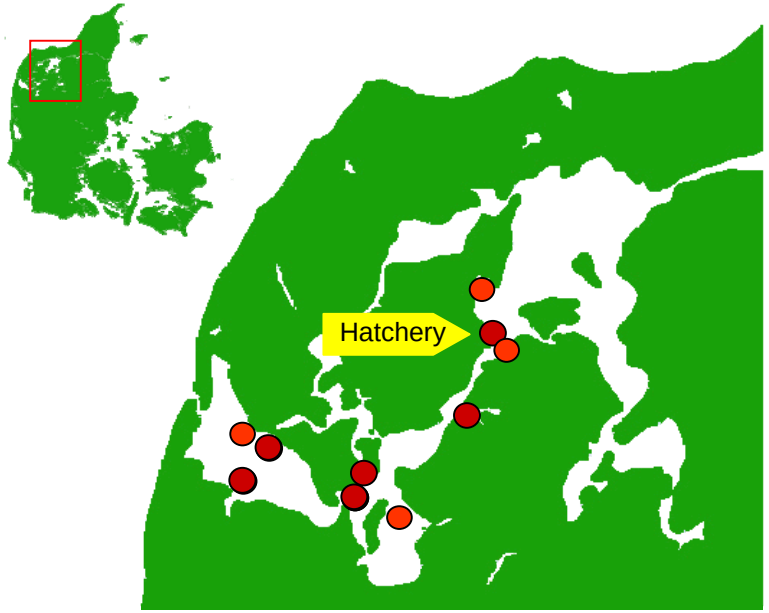
Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
Limfjorden	12/06/2007		3	30
Limfjorden	06/11/2007		3	30

Results	
Bonamia	Marteilia
0	0



Comments
Investigations for 2007 not finished

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments

Additionnal comments

France 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**DPMA**	**Laboratoire de Génétique et Pathologie, IFREMER La Tremblade**
Address :	3 place Fontenoy, 75007 Paris	Avenue Mus du Loup, 17390 La tremblade
Web site :		http://www.ifremer.fr/drvlgp/
Contact :	Djahne Montabord	Tristan Renault
Phone :	01 49 55 82 01	05 46 76 26 10
E-mail :	DPMA@equipement.gouv.fr	tristan.renault@ifremer.fr

General data

Technical data	**Zone I**	**Zone II**	**Zone III**	**Zone IV**	**Zone V**	**Zone VI**	**Zone VII**	**Zone VIII**	**Zone IX**	**Zone X**		**National data (official)**
Farms (2005 data)	54		635		376	1257	377	747	282	23		no
hatcheries - nurseries (2005 data)				1		3	>4	4	4			no

Production data	**Zone I**	**Zone II**	**Zone III**	**Zone IV**	**Zone V**	**Zone VI**	**Zone VII**	**Zone VIII**	**Zone IX**	**Zone X**		**National data (professional data 2005)**	**FAO 2003**
Crassotrea gigas	10000 tons				8500 tons	27500 tons	9500 tons	46000 tons	27000 tons			128500 tons	115000 tons
Ostrea edulis								1700 tons				1700 tons	2000 tons
Mytilus galloprovincialis	6000 tons											6000 tons	13000 tons
Mytilus edulis						13100 tons		21500 tons	22000 tons			56600 tons	55000 tons
Ruditapes decussatus												3000 tons	750 tons
Ruditapes philippinarum													750 tons
Cerastoderma edule												2500 tons	1000 tons
Pecten maximus												No data	No data
Haliotis tuberculata												No data	No data

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	Yes	Yes									
	Histology	Yes	Yes						*Yes*	*Yes*		*Yes*
	PCR	Yes										*Yes*
	RFTM culture								*Yes*			
	Other											
Presumptive	Cytology	Yes	Yes	Yes	Yes	Yes						
	Histology	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	TEM											
	PCR											Yes
	RFTM culture							Yes	Yes			
	ISH											
	Other											
Confirmatory	Histology											
	ISH	Yes	Yes	Yes	Yes	Yes	Yes			Yes	Yes	Yes
	TEM	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	PCR	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	PCR-RFLP	Yes	Yes	Yes	Yes			Yes	Yes			
	Sequencing	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods (A,B) in Manual of Diagnostic Tests for Aquatic Animals 2006

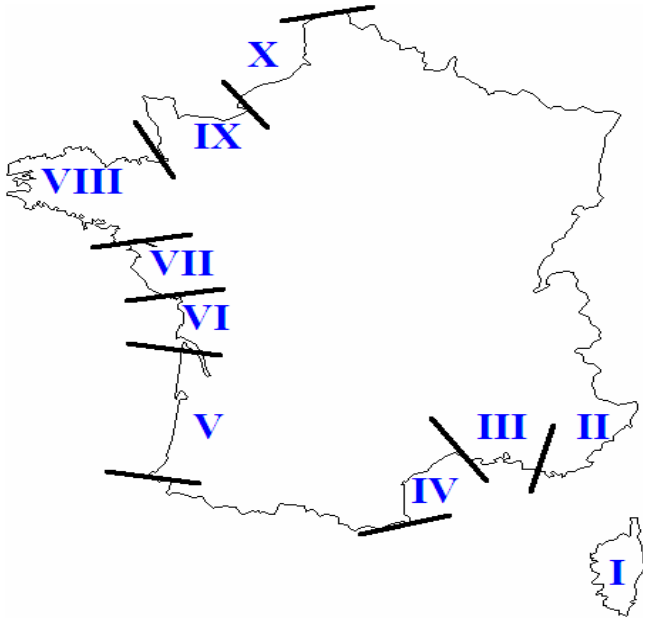
Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
Zone I	15/01/2007	harvested	1	100
	25/06/2007	harvested	1	30
Zone VIII	29/01/2007	cultured	1	78
	22/05/2007	wild	1	31
	22/05/2007	wild	1	31
	30/07/2007	cultured	1	30
	28/08/2007	harvested	1	24
	08/10/2007	cultured	1	60

Results	
Bonamia	Marteilia
0	
3	0
5	
4	
1	
15	
5	0
9	



Comments
Zone X is supposed Marteilia and Bonamia free but it is very difficult to obtain samples. No Ostrea edulis sample was performed in 2007 for this area.

Study of increased mortality

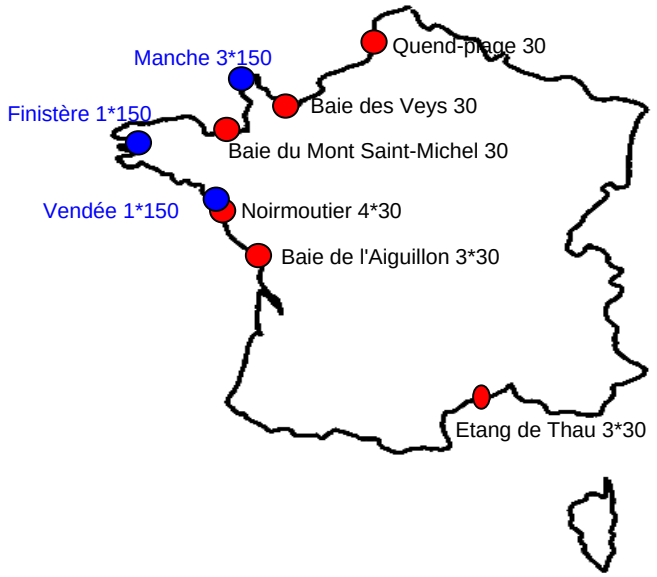
Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals
Zone III	05/02/2007	Mytilus galloprovincialis	Juvenile	cultured	3	30
	14/05/2007	Crassotrea gigas	Spat	cultured	1	63
	14/05/2007	Crassotrea gigas	Spat	cultured	1	65
	14/05/2007	Crassotrea gigas	Spat	cultured	1	30
	14/05/2007	Crassotrea gigas	Spat	cultured	1	25
	14/05/2007	Crassotrea gigas	Spat	cultured	1	30
	21/05/2007	Crassotrea gigas	Spat	cultured	2	30
	06/06/2007	Crassotrea gigas	Juvenile	cultured	1	65
	06/06/2007	Crassotrea gigas	Juvenile	cultured	1	33
	06/06/2007	Crassotrea gigas	Juvenile	cultured	1	30
	06/06/2007	Crassotrea gigas	Juvenile	cultured	1	45
	08/06/2007	Crassotrea gigas	Juvenile	cultured	2	60
Zone IV	27/06/2007	Crassotrea gigas	Spat	cultured	1	35
Zone V	22/05/2007	Crassotrea gigas	Spat	cultured	2	60
	04/06/2007	Crassotrea gigas	Adult	cultured	1	30
	21/08/2007	Crassotrea gigas	Adult	cultured	2	35
	28/08/2007	Crassotrea gigas	Adult	cultured	1	29
	30/08/2007	Crassotrea gigas	Juvenile	cultured	1	30
Zone VI	24/04/2007	Crassotrea gigas	Spat	cultured	1	5
	06/06/2007	Crassotrea gigas	Spat	cultured	1	33
	06/06/2007	Crassotrea gigas	Spat	cultured	1	49
	27/06/2007	Crassotrea gigas	Adult	cultured	2	35
	13/07/2007	Crassotrea gigas	Adult	cultured	2	33
	14/08/2007	Crassotrea gigas	Spat	cultured	13	1 pool
	14/08/2007	Crassotrea gigas	Spat	cultured	1	11
	14/08/2007	Crassotrea gigas	Spat	cultured	2	20
	14/09/2007	Crassotrea gigas	Spat	cultured	1	1 pool
	24/09/2007	Crassotrea gigas	Adult	cultured	1	10
	11/10/2007	Crassotrea gigas	Adult	cultured	1	30
	19/10/2007	Crassotrea gigas	Spat	cultured	1	2 pools
Zone VII	13/06/2007	Crassotrea gigas	Adult	cultured	1	35
	13/06/2007	Crassotrea gigas	Adult	cultured	2	30

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequenc y	Comments
Mytilicola	24	42/1208	For OsHV-1, the individuals are pooled (5 individuals/pool)
Rickettsia	24	32/1208	
Myicola	24	9/1208	
Neoplasia	24	1/1208	
Microsporidia	24	4/1208	
Papova-like virus	24	23/1208	
Haplosporidium nelsoni	24	2/1208	
Haplosporidium	24	4/1208	
Trematod	24	3/1208	
OsHV1	21	30/116 (po	
Vibrio splendidus	30	32/151	
Vibrio aestuarianus	30	16/151	
Vibrio harveyi	1	1/3	

	19/06/2007	Crassotrea gigas	Spat	cultured	1	1 pool
	19/06/2007	Crassotrea gigas	Spat	cultured	1	2 pools
	05/07/2007	Crassotrea gigas	Spat	cultured	1	3 pools
	12/07/2007	Crassotrea gigas	Spat	cultured	1	2 pools
	12/07/2007	Crassotrea gigas	Spat	cultured	1	1 pool
	25/07/2007	Crassotrea gigas	Spat	cultured	2	1 pool
	31/07/2007	Crassotrea gigas	Larva	cultured	1	1 pool
	30/08/2007	Crassotrea gigas	Spat	cultured	1	1 pool
	15/10/2007	Crassotrea gigas	Spat	cultured	1	1 pool
	29/11/2007	Crassotrea gigas	Spat	cultured	1	1 pool
Zone VIII	03/04/2007	Crassotrea gigas	Adult	cultured	1	30
	14/06/2007	Crassotrea gigas	Spat	cultured	1	65
	14/06/2007	Crassotrea gigas	Spat	cultured	1	65
	01/08/2007	Mytilus edulis	Spat	cultured	1	30
	01/08/2007	Mytilus edulis	Adult	cultured	1	30
	15/10/2007	Crassotrea gigas	Juvenile	cultured	1	35
Zone IX	12/08/2007	Crassotrea gigas	Adult	cultured	1	35
	20/08/2007	Crassotrea gigas	Larva	cultured	1	2 pools
	20/08/2007	Crassotrea gigas	Spat	cultured	1	2 pools
	30/08/2007	Crassotrea gigas	Adult	cultured	1	30
	11/09/2007	Crassotrea gigas	Adult	cultured	1	34
	20/09/2007	Crassotrea gigas	Adult	cultured	1	35
	11/09/2007	Haliotis tuberculata	Adult	wild	1	16

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	Official program
Aim :	Detect pathogen
Duration :	2 years
Mollusc species concerned	Mytilus edulis
	Mytilus galloprovincialis
	Haliotis tuberculata
Targeted pathogens	Marteilia refringens
	Candidatus Xenohaliotis californiensis
Objectives and Brief summary of the program / project	The parasite Marteilia refringens was detected in mussels along the French coasts but its real prevalence and distribution is unknown. Sampling strategy: 2 years sampling in main production areas (see the red spots on map). Until now, no detection of Candidatus Xenohaliotis californiensis in French abalones ; in 2006, the abalone wild beds were samplied and no detection of withering syndrome. In 2007, the



Mussels

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
Zone III	17/09/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia refringens	4/30	
	17/09/2007	Mytilus galloprovincialis	Spat	cultured	1	30	Marteilia refringens	3/30	
	08/10/2007	Mytilus galloprovincialis	Adult	wild	1	30	Marteilia refringens	2/30	
Zone VI	13/08/2007	Mytilus edulis	Adult	cultured	1	30	Marteilia refringens	0	
	13/08/2007	Mytilus edulis	Adult	wild	1	30	Marteilia refringens	0	
	13/08/2007	Mytilus edulis	Spat	cultured	1	30	Marteilia refringens	0	
Zone VII	29/08/2007	Mytilus edulis	Adult	cultured	1	30	Marteilia refringens	0	
	29/08/2007	Mytilus edulis	Spat	cultured	1	30	Marteilia refringens	0	
	30/08/2007	Mytilus edulis	Adult	cultured	1	30	Marteilia refringens	0	
	03/09/2007	Mytilus edulis	Adult	wild	1	30	Marteilia refringens	0	
Zone VIII	11/09/2007	Mytilus edulis	Adult	cultured	1	30	Marteilia refringens	0	
Zone IX	10/09/2007	Mytilus edulis	Adult	cultured	1	30	Marteilia refringens	3/30	
Zone X	29/08/2007	Mytilus edulis	Adult	cultured	1	30	Marteilia refringens	0	

Abalones

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Detect pathogen	Comments
Zone VII	01/10/2007	*Haliotis tuberculata*	Juvenile	cultured	1	150	*datus Xenohaliotis californi*	in process	
Zone VIII	19/10/2007	*Haliotis tuberculata*	Juvenile	cultured	1	150	*datus Xenohaliotis californi*	in process	
Zone IX	29/10/2007	*Haliotis tuberculata*	Juvenile	cultured	3	150	*datus Xenohaliotis californi*	in process	

Additionnal comments

Ireland 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Department of Communications, Marine & Natural Resources**	**Marine Institute**
Address :	Adelaide Road, Dublin 2, Ireland	Rinville, Oranmore, Co. Galway
Web site :	www.dcmnr.gov.ie	www.marine.ie
Contact :	Ms Rebecca Minch	Deborah Cheslett
Phone :	35316782000	35391387200
E-mail :	rebecca.minch@dcmnr.gov.ie	deborah.cheslett@marine.ie

General data

Technical data												**National data (official)**
Farms												
hatcheries - nurseries												

Production data												**National data (official)**	**FAO 2003**
Crassotrea gigas													
Ostrea edulis													
Mytilus edulis													

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	Yes										
	Histology		Yes	*Yes*		*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*
	PCR		Developing	*Developing*								*Yes*
	RFTM culture											
	Other											
Presumptive	Cytology											
	Histology	Yes					Yes	Yes	Yes	Yes		Yes
	TEM											
	PCR	Yes	Refer to CRL	Refer to CRL				Developing				
	RFTM culture											
	ISH		Refer to CRL									
	Other											
Confirmatory	Histology											
	ISH	Yes										Yes
	TEM											
	PCR	Refer to CRL	Refer to CRL	Refer to CRL				Refer to CRL	Refer to CRL	Refer to CRL		Yes
	PCR-RFLP	Developing		Developing								
	Sequencing	Developing	Refer to CRL	Developing			Refer to CRL	Refer to CRL	Refer to CRL			Yes
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

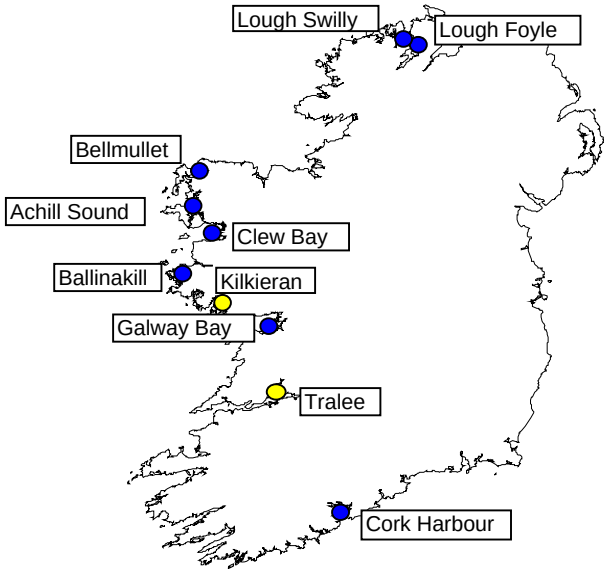
Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

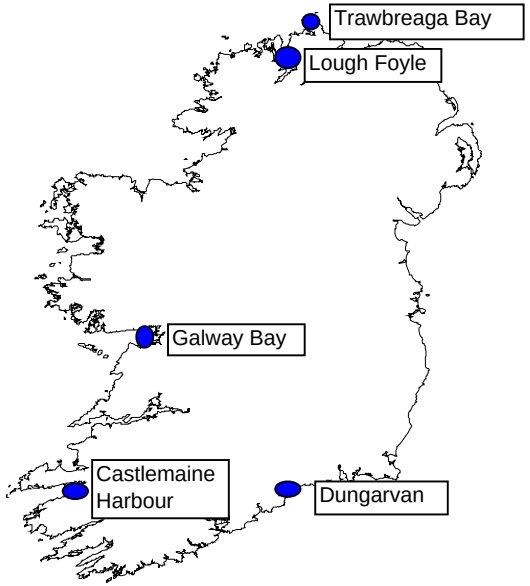
Analysis effort						Results	
Zones	Date	Zootechnics	Sample	Individuals		Bonamia	Marteilia
Lough Foyle	22/02/2007	wild	3	30		0	n/a
Kilkieran Bay	15/03/2007	wild	3	30		0	n/a
Clew Bay	23/03/2007	wild	5	30		28	n/a
Tralee	27/03/2007	wild	3	30		0	n/a
Lough Swilly	13/04/2007	wild	3	Other (precis	50	9	n/a
Lough Swilly	19/09/2007	wild	2	Other (precis	75	20	0
Clew Bay	09/10/2007	wild	2	30		3	0
Lough Foyle	10/10/2007	wild	3	30		13	0
Galway Bay	05/11/2007	wild	1	30		0	0
Blacksod Bay	06/11/2007	wild	3	30		10	0
Kilkieran Bay	08/11/2007	wild	3	30		0	0
Tralee	07/12/2007	wild	3	30		0	0



Comments
Positve sites in Blue, negative sites in Yellow. Spring sampling focused mainly on the negative sites and those sites where B. ostreae has only recently been found. No samples were taken in 2007 from Lissadell or Cork as neither site was culturing *O. edulis in 2007.* No samples were received from Ballinakill or achill either as no fishing taking place in Ballinakill and very little was fished around Achill.

Study of abnormal mortality

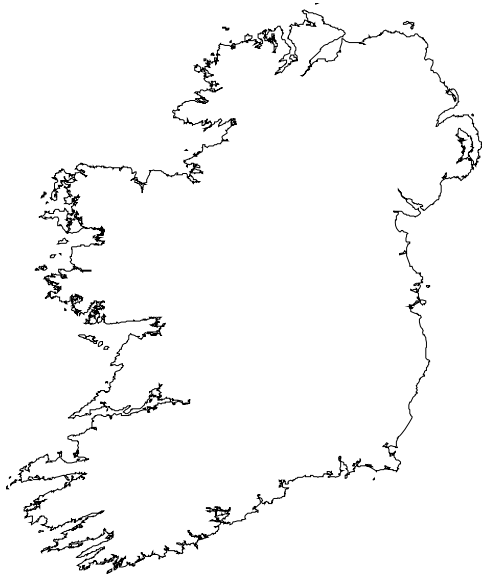
Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals
Harbour	29/08/2007	Crassotrea gigas	Adult	cultured	1	16
Bay	30/08/2007	Crassotrea gigas	Adult	cultured	2	12
Dungarvan	13/09/2007	Crassotrea gigas	Adult	cultured	1	117
Lough Foyle	10/10/2007	Crassotrea gigas	Adult	cultured	1	37
Harbour	12/10/2007	Crassotrea gigas	Adult	cultured	1	32
Galway Bay	05/12/2007	Crassotrea gigas	Adult	cultured	1	30



Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments
			a virus with sequence similarity to OsHV1 was isolated from Lough Foyle

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	



Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments

Additionnal comments

Italy 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Ministero della Salute Sanità Pubblica Veterinaria, Nutrizione, Sicurezza Alimenti**	**IZSVe - Laboratorio Patologia Molluschi**
Address :	**Via Giorgio Ribotta 5, 00144 ROMA**	**Via della Roggia 102, 33030 Basaldella di Campoformido (UDINE)**
Web site :		
Contact :	**Dott. UGO SANTUCCI**	**Dott. Giuseppe Ceschia**
Phone :	**0039-06-599467734**	**0039-0432-561196**
E-mail :	**u.santucci@sanita.it**	**gceschia@izsvenezie.it**

General data

Technical data	Friuli-Venezia Giulia	Veneto	Emilia-Romagna	Marche	Abruzzo	Molise	Puglia	Basilicata	Calabria	Campania	Lazio	Toscana	Liguria	Sardegna	Sicilia
Farms mussels	107	46	22	12	5	2	*	*	*	*	4		86	*	*
Farms clams	6	272	60												
Farms oysters C. gigas												1			
Hatcheries clams	2	2													
Nurseries clams	3	5													

Production data *	Friuli-Venezia Giulia	Veneto	Emilia-Romagna	Marche	Abruzzo	Molise	Puglia	Basilicata	Calabria	Campania	Lazio	Toscana	Liguria	Sardegna	Sicilia
Ostrea edulis															
Crassotrea gigas															
Mytilus galloprovincialis															
Ruditapes philippinarum															

*** NO OFFICIAL DATA**

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Marteilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	**Yes**	**Yes**									
	Histology	**Yes**	**Yes**						***Yes***			
	PCR											
	RFTM culture								***Yes***			
	Other											
Presumptive	Cytology	**Yes**	**Yes**									
	Histology	**Yes**	**Yes**						**Yes**			
	TEM											
	PCR								**Yes**			
	RFTM culture								**Yes**			
	ISH											
	Other											
Confirmatory	Histology											
	ISH											
	TEM											
	PCR											
	PCR-RFLP		**Refer to CRL**									
	Sequencing											
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

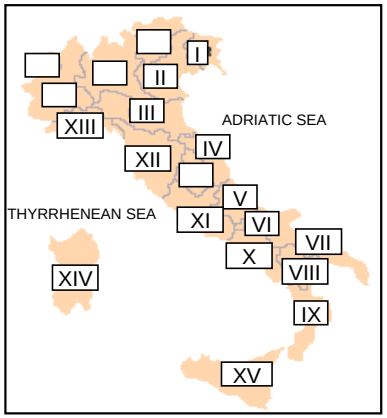
Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
I	**15/06/2007**	**wild**	**1**	**80**
I	**20/07/2007**	**wild**	**1**	**14**
II	**18/09/2007**	**wild**	**1**	**25**

Results	
Bonamia	Marteilia
0	**16**
0	**14**
0	**0**



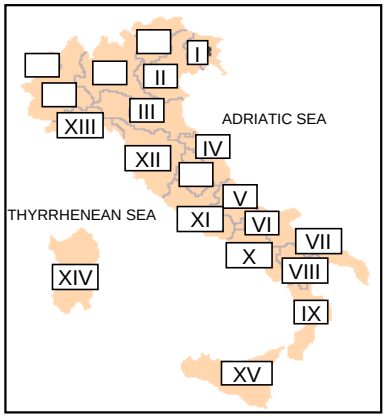
I. Friuli-Venezia Giulia
II. Veneto
III. Emilia Romagna
IV. Marche
V. Abruzzo
VI. Molise
VII. Puglia
VIII. Basilicata
IX. Calabria
X. Campania
XI. Lazio
XII. Toscana
XIII. Liguria
XIV. Sardegna
XV. Sicilia

Comments

Study of abnormal mortality *

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals

*** NO OFFICIAL DATA**

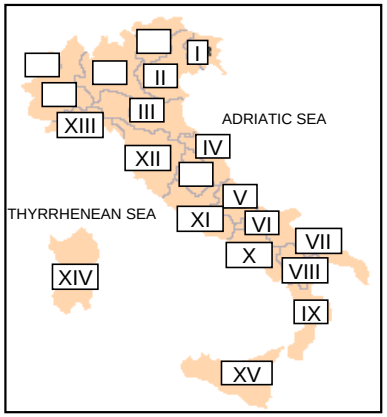


I. Friuli-Venezia Giulia
II. Veneto
III. Emilia Romagna
IV. Marche
V. Abruzzo
VI. Molise
VII. Puglia
VIII. Basilicata
IX. Calabria
X. Campania
XI. Lazio
XII. Toscana
XIII. Liguria
XIV. Sardegna
XV. Sicilia

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	In some regions a survey program concerning animal welfare is in place



I. Friuli-Venezia Giulia
II. Veneto
III. Emilia Romagna
IV. Marche
V. Abruzzo
VI. Molise
VII. Puglia
VIII. Basilicata
IX. Calabria
X. Campania
XI. Lazio
XII. Toscana
XIII. Liguria
XIV. Sardegna
XV. Sicilia

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
I	**august**	***M. galloprovincialis***	**adult**	**cultured**	**7**	**1050**	***Marteilia refringens***	**7/1050**	
II	**june august**	***M. galloprovincialis***	**adult**	**cultured**	**31**	**3960**	***Marteilia refringens***	**0/3960**	
III	**may**	***M. galloprovincialis***	**adult**	**cultured**	**6**	**300**	***Marteilia refringens***	**0/300**	
IV	**november**	***M. galloprovincialis***	**adult**	**cultured**	**5**	**750**	***Marteilia refringens***	**0/750**	
V	**june**	***M. galloprovincialis***	**adult**	**cultured**	**2**	**340**	***Marteilia refringens***	**0/340**	
VI	**june**	***M. galloprovincialis***	**adult**	**cultured**	**1**	**160**	***Marteilia refringens***	**0/160**	
XIII	**october**	***M. galloprovincialis***	**adult**	**cultured**	**3**	**150**	***Marteilia refringens***	**1/150**	
I	**october november**	***R. philippinarum***	**adult**	**cultured**	**9**	**414**	***Perkinsus olseni***	**182/414**	
II	**february december**	***R. philippinarum***	**adult**	**cultured**	**110**	**5595**	***Perkinsus olseni***	**2511/5595**	
III	**july**	***R. philippinarum***	**adult**	**cultured**	**6**	**300**	***Perkinsus olseni***	**42/300**	

Additionnal comments

Netherlands 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Ministry of Agriculture, Nature and Food Quality; Department of Fisheries**	**Central Veterinary Institute**
Address :	PO Box 20401, 2500 EK Den Haag, The Netherlands	PO Box 65, 8200 AB Lelystad, the Netherlands
Web site :	www.minlnv.nl	www.cvi.wur.nl
Contact :	Dr. ir. A.J. Rothuis	Marc Engelsma
Phone :	+31-70-378 4924	+31-320-238729
E-mail :	a.j.rothuis@minlnv.nl	marc.engelsma@wur.nl

General data

Technical data	**M. edulis**	**C. gigas O. edulis**										**National data (official)**
Farms	~93	~40										
hatcheries - nurseries	1 (experimental)											

Production data	**Waddenzee**	**Grevelingen**	**Oosterschelde**									**National data (official)**	**FAO 2003**
Mytilus edulis													
Crassotrea gigas													
Ostrea edulis													

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Marelia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	No	No									
	Histology	Yes	Yes									
	PCR	No	No									
	RFTM culture											
	Other											
Presumptive	Cytology	No	No									
	Histology	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
	TEM											
	PCR	No	No									
	RFTM culture											
	ISH		No									
	Other											
Confirmatory	Histology	No	No									
	ISH	Yes										
	TEM	No										
	PCR	Yes	No							Yes	Yes	
	PCR-RFLP	No										
	Sequencing	Yes	Yes	Yes	Yes					Yes	Yes	
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
Grevelingen	05/06/2007	wild	6	25
Oosterschelde	04/06/2007	cultured	1	2
Grevelingen	05/11/2007	wild	6	25
Oosterschelde	06/11/2007	cultured	2	2

Results	
Bonamia	Marteilia
18	0
0	0
to be determined	
to be determined	

Comments
The O. edulis population in the Oosterschelde is virtually non-existent. If O. edulis individuals are found during the sampling of C. gigas they are collected. Hence, the low numbers of O. edulis sampled from the Oosterschelde .

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals
Oosterschelde	06/04/2007	Mytilis edulis	Adult	cultured	7	25

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments
			High mortality of mussels up to 89% at a selected number of lots in the Oosterschelde. No listed pathogens found. Very high prevalence of *Mytilicola* sp. but also in reference samples form nearby area without mortalities.

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	Annual surveillance and monitoring of shellfish diseases
Type :	Official program
Aim :	Detect pathogens and collect reference material for abnormal mortalities
Duration :	
Mollusc species concerned	Crassotrea gigas Mytilus edulis
Targeted pathogens	General health status All pathogens, Ostracoblabe, Polydora
Objectives and Brief summary of the program / project	In addition to the O. edulis samples, samples of other shellfish are taken in the same program in order to estimate the health status of the animals and to serve as reference material for possible abnormal mortalities at later date.

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
Grevelingen	05/06/2007	Crassotrea gigas	Adult	cultured	1	25	All pathogens		
Oosterschelde	04/06/2007	Crassotrea gigas	Adult	cultured	3	25	All pathogens		
Oosterschelde	04/06/2007	Mytilus edulis	Adult	cultured	1	25	All pathogens		
Grevelingen	05/11/2007	Crassotrea gigas	Adult	cultured	1	25	All pathogens		
Oosterschelde	06/11/2007	Crassotrea gigas	Adult	cultured	3	25	All pathogens		
Oosterschelde	06/11/2007	Mytilus edulis	Adult	cultured	1	25	All pathogens		

Additionnal comments

Production data not yet available for 2007.

Norway 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Food safety authority**	**National Veterinary Institute Bergen**
Address :	Felles postmottak, P.O. Box 383, N-2381 Brumunddal, Norway	P.O. Box 1263 sentrum, N-5811 Bergen, Norway
Web site :	www.mattilsynet.no	www.vetinst.no
Contact :	Maria Melstokkå	Hege Hellberg
Phone :	(+47) 55 21 57 21	(+47) 55 36 38 19
E-mail :	maria.melstokkaa@mattilsynet.no	hege.hellberg@vetinst.no

General data

Technical data												**National data (official)**
Farms	Directorate of Fisheries: 696 sites registered in 2007, 374 sites in active use in 2006 (various species of molluscs)											374
hatcheries - nurseries	1 O. edulis	1 P. maximus										2

Production data												**National data (official)**	**FAO 2005**
Mytilus edulis												1049	4311
Crassotrea gigas												none	
Pecten maximus												3	3
Ostrea edulis												5	2

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology											
	Histology	Yes	Yes	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*
	PCR											
	RFTM culture											
	Other											
Presumptive	Cytology											
	Histology	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	TEM											
	PCR	Developing	Developing									
	RFTM culture											
	ISH											
	Other											
Confirmatory	Histology											
	ISH											
	TEM											
	PCR	Developing	Developing									
	PCR-RFLP											
	Sequencing											
	RFTM culture											
	Other	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL	Refer to CRL

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

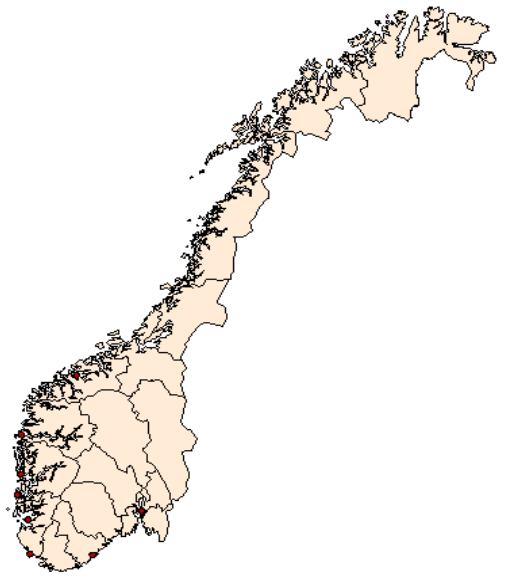
Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
	12/06/2007	cultured		28
	19/06/2007	cultured		30
	19/06/2007	cultured		30
	06/07/2007	wild		30
	18/09/2007	wild		30
	11/10/2007	cultured		30
	22/10/2007	wild		30
	02/11/2007	cultured		30

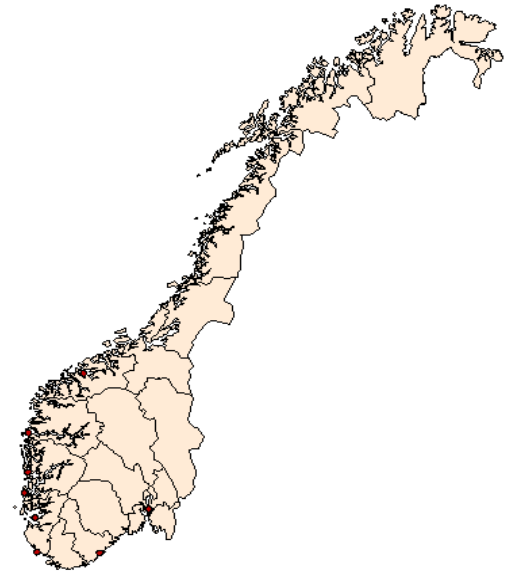
Results	
Bonamia	Marteilia
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0



Comments
Norway is considered as one zone. In 2007, a total of 238 oysters from five sampling points were examined. Six sampling points were included in the sampling plan 2007. However, one point was not sampled and two points were only sampled spring or autumn.

Study of abnormal mortality

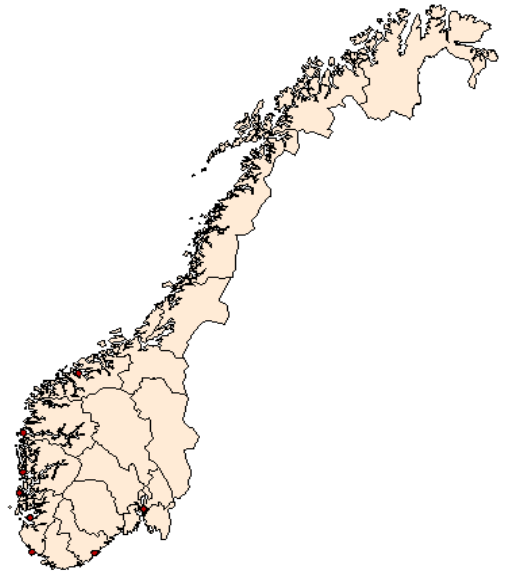
Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals



Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments
			No cases of abnormal mortality were reported to the NRL in 2007.

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	



Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments

Additionnal comments

Poland 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Agnieszka Pekala**	**National Veterinary Research Institute; Department of Fish Diseases**
Address :	Al.. Partyzantow 57; 24 - 100 Pulawy	Al. Partyzantow 57; 24 - 100 Pulawy
Web site :	www.piwet.pulawy.pl	www.piwet.pulawy.pl
Contact :	National Veterinary Research Institute; Department of Fish Diseases; Al.Partyzantow 57; 24 - 100 Pulawy	National Veterinary Research Institute; Al.Partyzantow 57; 24 - 100 Pulawy
Phone :	(48) 81 889 30 00	(48) 81 889 30 00
E-mail :	A.Pekala@piwet.pulawy.pl	

General data

Technical data												**National data (official)**
Farms	0											
hatcheries - nurseries	0											

Production data												**National data (official)**	**FAO 2003**
0												0	0

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	■	■	■								
	Histology	■	■	■			■			■		■
	PCR	■	■	■				■	■			■
	RFTM culture							■	■			
	Other											
Presumptive	Cytology	■	■	■								■
	Histology	■	■	■			■	■	■	■		■
	TEM											■
	PCR	■	■	■				■	■	■		
	RFTM culture							■	■			
	ISH		■									
	Other											
Confirmatory	Histology		■				■			■		■
	ISH	■	■	■						■		■
	TEM	■		■			■					
	PCR	■	■	■				■	■	■		■
	PCR-RFLP	■		■								
	Sequencing		■				■	■	■			■
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

■ Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals

Results	
Bonamia	Marteilia

Comments
The plans of surveillance and monitoring of Bonamia ostrea and Marteilia refringens in Ostrea edulis is impossible due to the fact that this species does not occur by the polish seaside.

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments
	0	0	We have not observed any abnormal mortality among molluscs wild population

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
	07/07/2008	Mytilus edulis	Adult	wild	2	30	Marteilia refringens		Diagnostic methods we used were cytology and histology

Additionnal comments

Wild population of Mytilus edulis from polish cost of Baltic sea have been sampling for diagnosis of non exotic agents for the first time in July in Poland. Two sampling points have been chosen. One was located on the Puck Bay and another on the open sea. 30 individuals have been taken from each point

Portugal 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Direcção Geral de Veterinária**	**Instituto Nacional de Recursos Biológicos (INRB/IPIMAR, I.P.)**
Address :	Largo da Academia Nacional de Belas Artes, 2 - 1249-105 Lisboa	IPIMAR - Laboratório de Patologia - Av de Brasília 1449-006 Lisboa
Web site :		www.ipimar.pt
Contact :	Susana Graszina Freitas	Francisco Ruano
Phone :	351 21 3239500	351 21 3027000
E-mail :	sfreitas@dgv.min-agricultura.pt	fruano@ipimar.pt

General data

Technical data	Zone (Algarve I, II,III)	Zone (Lisboa)	Zone (Centro)	Zone Norte								National data (official)
Farms	1500	25	20									
hatcheries - nurseries												

Production data	Zone (Algarve I, II,III)	Zone (Lisboa)	Zone (Centro)	Zone Norte								National data (official)	FAO 2003
Crassotrea angulata		6											3
Ruditapes decussatus	3000		10										3002
Crassotrea gigas	400		20										323
Mytilus edulis		250	nd	nd									279
Cerastoderma edule	50	22	60										23
													3630

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni/atlanticus*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	Yes	Yes									
	Histology	Yes	Yes				*Yes*			*Yes*		
	PCR	Developing	Developing					*Developing*	*Developing*			
	RFTM culture							*Yes*	*Yes*			
	Other											
Presumptive	Cytology											
	Histology	Yes	Yes					Yes	Yes			
	TEM											
	PCR	Developing	Developing									
	RFTM culture											
	ISH											
	Other											
Confirmatory	Histology											
	ISH											
	TEM											
	PCR	Developing	Developing						Developing			
	PCR-RFLP											
	Sequencing											
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
(Algarve I,	07/07/2007	wild	1	40
(Algarve I,	04/09/2007	wild	1	30
(Lisboa)	06/07/2007	cultured	1	40
Norht	12/10/2007	wild	1	40

Results	
Bonamia	Marteilia
0	24
0	20
0	7
0	10

Comments

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments
			None mortality episode had been detected or reported in the species surveyed and in the sites mentioned.

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	Rastreio Sanitário de espécies bivalves
Type :	Official program
Aim :	Detect pathogen
Duration :	1 year (applaing for 5 years program)
Mollusc species concerned	Ostrea edulis
	Mytilus edulis
	Crassotrea gigas Cerastoderma edule
	Crassotrea angulata
	Ruditapes decussatus
Targeted pathogens	All pathogens
Objectives and Brief summary of the program / project	Identify the most important pathogens in bivalves populations commercially exploited and qualifying their role in commercial operations.

effort	Date	Host	Age-class	Zootechnics	Sample		Results	
Sites						Individuals	Targeted pathogen(s)	Estimate prevalence
Algarve	06.07.2007	Ruditapes decussatus	Adult	cultured	3	75	All pathogens	Estimate prevalence
	04.09.2007	Cerastoderma edule	Adult	cultured	2	60	All pathogens	Estimate prevalence
	06.07.2007	Crassotrea gigas	Adult	cultured	1	30	All pathogens	Estimate prevalence
Sado	09.06.07	Crassotrea angulata	Adult	wild	2	60	All pathogens	Estimate prevalence
Lisboa	05.06.07	Mytilus edulis	Adult	cultured	2	60	All pathogens	Estimate prevalence
Aveiro	05.05.07	Mytilus edulis	Adult	cultured	1	30	All pathogens	Estimate prevalence

Additionnal comments

As result of this survey we detected high prevalence of Perkinsus in clams(65 to 70%) The cockles populations cultured together with clams shows 50% of prevalence of *Perkinsus* and 40% of prevalence of a digenetic trematode, Bucephalus sp. This nosological frame had been associated with severe mortalities occurred in both species in the sites surveyed: Natural populations of Portuguese oysters are quite healthy showing low (10%) prevalence of gill lesions. *Martelia* shows 40% of prevalence in the cultured mussels in Lagoa de Albufeira (Lisboa).

Romania 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**National Sanitary Veterinary and Food Safety Authority**	**Institute for Diagnosis and Animal Health**
Address :	1B, Negustori Street, sector 2, code 023951,Bucharest	63, Dr. Staicovici street, sector 5, code 050557, Bucharest
Web site :	www.ansv.ro	www.idah.ro
Contact :	Prof. dr. Ontanu Gheorghe	Dr. Biol. Mihaela Costea
Phone :	004021 3072399	40214109945
E-mail :	office@ansv.ro	costea.mihaela@idah.ro

General data

Technical data	**North**	**South**										**National data (official)**
Farms	1 farm											1 farm
hatcheries - nurseries	0											0

Production data												**National data (official)**	**FAO 2003**
Mytilus galloprovincialis	no official data											no official data	

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Martelia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology		YES									
	Histology		YES									
	PCR											
	RFTM culture											
	Other											
Presumptive	Cytology											
	Histology											
	TEM											
	PCR											
	RFTM culture											
	ISH											
	Other											
Confirmatory	Histology											
	ISH											
	TEM											
	PCR											
	PCR-RFLP											
	Sequencing											
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

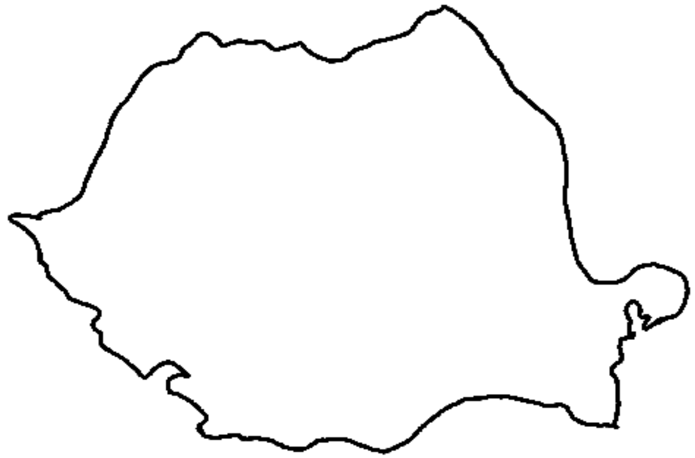
Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals

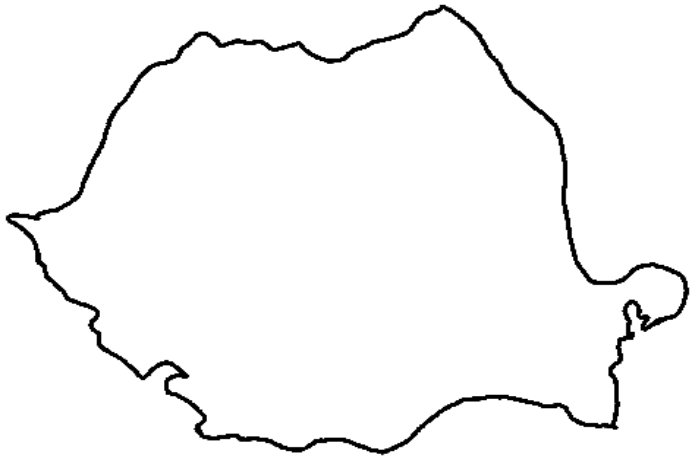
Results	
Bonamia	Marteilia



Comments

Study of abnormal mortality

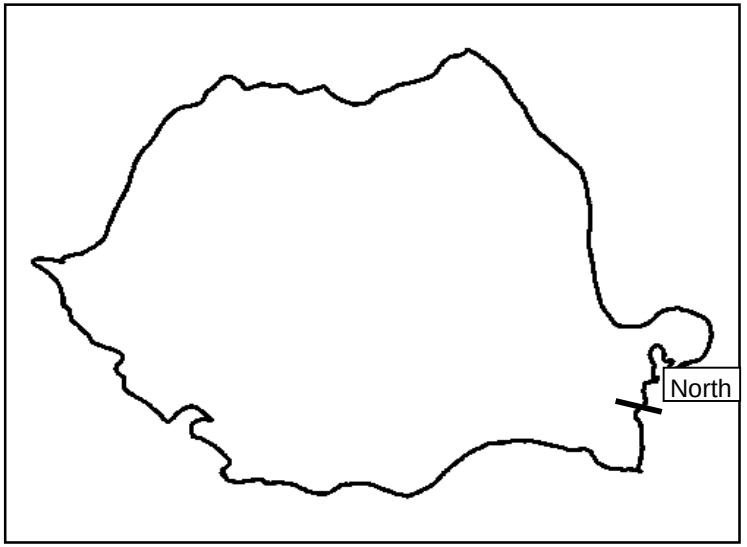
Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals



Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	Program for surveillance, prophylaxis and control activities for animal diseases, zoonosi
Type :	official program
Aim :	detect pathogen
Duration :	one year
Mollusc species concerned	Mytilus galloprovincialis
Targeted pathogens	Marteilia refringens
Objectives and Brief summary of the program / project	



Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
Zone North	07/08/2007	Mytilus galloprovincialis	adult	cultured	3	179	Marteilia refringens	Negative	Negative samples
Zone North	10/10/2007	Mytilus galloprovincialis	adult	cultured	1	36	Marteilia refringens	Negative	

Additionnal comments

Slovenia 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**National Veterinary Institute Veterinary Faculty**	
Address :	Gerbiceva 60, Ljubljana	Vlasta Jencic
Web site :	http://www.vf.uni-lj.si/veterina/index.htm	Mitja Gombac
Contact :	Vlasta Jencic	Rosvita Sitar
Phone :	38614779145	
E-mail :	vlasta.jencic@vf.uni-lj.si	

General data

Technical data												**National data (official)**
Farms												3
hatcheries - nurseries												0

Production data												**National data (official)**	**FAO 2003**
Mitylus galloprovincialis												250 tons	

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	■	■	■								
	Histology	■	■	■			■			■		■
	PCR	■	■	■				■	■			■
	RFTM culture							■	■			
	Other											
Presumptive	Cytology	■	■	■								■
	Histology	■	■	■			■	■	■	■		■
	TEM											■
	PCR	■	■	■				■	■	■		
	RFTM culture							■	■			
	ISH		■									
	Other											
Confirmatory	Histology		■				■			■		■
	ISH	■	■	■						■		■
	TEM	■		■			■					
	PCR	■	■	■				■	■	■		■
	PCR-RFLP	■		■								
	Sequencing		■				■	■	■			■
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

■ Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

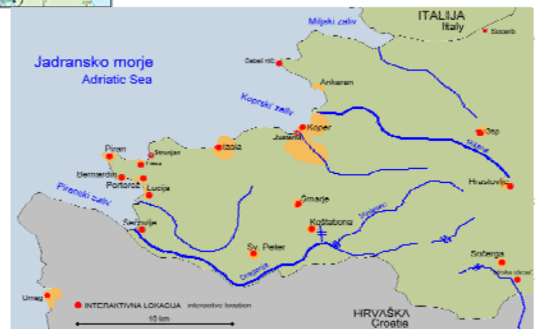
Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals

Results	
Bonamia	Marteilia



Comments

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments

Additionnal comments

Only mediterranean mussels (Mytilus galloprovincialis) are breed on three locations in Slovene Sea: Seča, Strunjan and Debeli rtič. Mussel seeds are bought or collected from natural beds, put in nets and cultured on ropes, which are hanging from rafts. Two sampling sites, i.e., Seča and Strunjan, were established in 2007 and both were monthly checked for excessive mortality. In 2007 no mortality was detected in neither of sampling sites.

Spain 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Ministerio de Agricultura, Pesca y Alimentación,**	**Instituto de Investigaciones Marinas, CSIC**
Address :	c/Alfonso XII nº 62 1º planta	c/ Eduardo Cabello nº6, 36208 vigo, Pontevedra
Web site :	http://www.mapa.es	http://www.iim.csic.es
Contact :	Lucio Ignacio Carbajo Goñi	Dr. Antonio Figueras Huerta
Phone :	00-34-913478295	00-34-986231930 ext 280
E-mail :	sganimal@mapya.es	pato1@iim.csic.es

General data

Technical data	Region I: Galicia	Region II: Asturias	Region III: Cantabria	Region IV: Cataluña	Region V: Islas Baleares	Region VI: Alicante	Region VII, Ceuta					National data (official)
Farms	Not available	0	Not available	Not available	Not available	1	7					
hatcheries - nurseries	Not available	2	Not available	Not available	Not available	0	0					

Production data	Region I: Galicia	Region II: Asturias	Region III: Cantabria	Region IV: Cataluña	Region V: Islas Baleares	Region VI: Alicante	Region VII, Ceuta					National data (official)	FAO 2003
Mytilus	Not availab	0	not availab	not availab	44,47	271,54	experimental					316,01	
Mytilus edulis	0	0	not availab	not availab	0	0	0					not available	
Ruditapes	662,3	2,7	not availab	not availab	0,8	0	0					665,8	
Ruditapes	1815,7	0	not availab	not availab	0	0	0					1815,7	
V. Rhomboides	714,9	0	not availab	not availab	0	0	0					714,9	
Venerupis pullastra	945,4	0	not availab	not availab	0	0	0					945,4	
Spisula solida	10,3	0	not availab	not availab	0	0	0					10,3	
Dosinia exoleta	66,7	0	not availab	not availab	0	0	0					66,7	
Donax trunculus	15,8	0	not availab	not availab	0	0	0					15,8	
Chamelea gallina	0	0	not availab	not availab	0	80,29	0					80,29	
Venerupis aurea	31,15	0	not availab	not availab	0	0	0					31,15	
Ostrea edulis	9,7	0	not availab	not availab	0,1	2,448	0					12,24	
Crassotrea gigas	not availab	275	not availab	not availab	0	0	0					275	
Ensis ensis	213,7	0	not availab	not availab	0	0	0					213,7	

Solen marginatus	26,2	6,9	not availab	not availab	0	0	0					33,1	
Phyllonotus	not availab	0	not availab	not availab	0	0,122	0					0,122	
Murex sp.	not availab	0	not availab	not availab	0	0,841	0					0,841	
Acanthocardia	90,8	0	not availab	not availab	0	0	0					90,8	
Cerastoderma edule	4693,1	0,5	not availab	not availab	0	0	0					4693,6	
Pecten maximus	226,2	0	not availab	not availab	0	0	0					226,2	
Chlamys varia	407,2	0	not availab	not availab	0	0	0					407,2	
Patella vulgata	0	2,4	not availab	not availab	0	0	0					2,4	
Litootina littorea	0	0,3	not availab	not availab	0	0	0					0,3	
glycymeris	39,4	0	not availab	not availab	0	0	0					39,4	
TOTAL	9968,5	287,8	not availab	not availab	44,57	355,241						10657	

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology	Yes	Yes									
	Histology	Yes	Yes	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*
	PCR	Yes							*Yes*			*Yes*
	RFTM culture							*Yes*	*Yes*			
	Other											
Presumptive	Cytology	Yes	Yes									
	Histology	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			Yes
	TEM								Yes			
	PCR	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	RFTM culture								Yes			
	ISH											
	Other											
Confirmatory	Histology		Yes									Yes
	ISH	Yes	Yes									Developing
	TEM											
	PCR	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	PCR-RFLP	Yes	Yes	Yes								
	Sequencing	Yes		Yes					Yes			
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort				
Zones	Date	Zootechnics	Sample	Individuals
region VI	24/10/2007	cultured	3	30
	06/11/2007	cultured	2	30
Region Y	09/04/2007	cultured	1	30
	10/04/2007	cultured	3	30
	10/04/2007	wild	1	30
	16/04/2007	wild	1	30
	18/04/2007	cultured	1	30
	07/05/2007	wild	1	30
	23/10/2007	cultured	2	30
	05/11/2007	wild	1	30
	07/11/2007	cultured	1	30
	07/11/2007	wild	1	30
	15/11/2007	wild	1	30
	26/11/2007	cultured	1	30
	10/12/2007	wild	1	30
	07/05/2007	wild	1	30

Results	
Bonamia	Marteilia
0	2
0	0
positive	0
3/3 positive	0
positive	0
positive	0
positive	0
positive	0
2/2 positive	0
positive	0
positive	0
positive	0
0	0
positive	0
positive	0
positive	0

Comments
Results of Bonamia sp. detection are given in number of positive sampled sites and not in number of positive individuals on each site.

Study of abnormal mortality no abnormal mortality was oficialy recorded

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments

Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	
Type :	
Aim :	
Duration :	
Mollusc species concerned	
Targeted pathogens	
Objectives and Brief summary of the program / project	

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
Region VII	01/01/2007	Mytilus galloprovincialis	Adult	cultured	2	30	Marteilia sp.	0	Positive results of Monitored pathogens are given in some cases in terms of positive or negative sampled sites, not in number of positive individuals. In line 215, the sampled molluscs species is Venerupis rhomboides.
	13/02/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0	
	21/03/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0	
	27/03/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0	
	19/06/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0	
	23/07/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	6,60%	
	29/11/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	6,60%	
	12/12/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	9,90%	
Region Y	19/03/2007	Mytilus galloprovincialis	Adult	cultured	2	30	Marteilia sp.	0	
	20/03/2007	Mytilus galloprovincialis	Adult	wild	3	30	Marteilia sp.	0	
	20/03/2007	Mytilus galloprovincialis	Adult	cultured	4	30	Marteilia sp.	3/4 sites positives	
	21/03/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	positive	
	22/03/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0	

	26/03/2007	Mytilus galloprovincialis	Adult	cultured	3	30	Marteilia sp.	1/3 sites positive			
	26/03/2007	Mytilus galloprovincialis	Adult	wild	1	30	Marteilia sp.	0			
	27/03/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0			
	28/03/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	positive			
	29/03/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0			
	03/04/2007	Mytilus galloprovincialis	Adult	cultured	1	30	Marteilia sp.	0			
	17/04/2007	Crassotrea gigas	Adult	wild	1	30	Perkinsus marinus	0			
	17/10/2007	Crassotrea gigas	Adult	cultured	2	30	Perkinsus marinus	0			
	23/10/2007	Crassotrea gigas	Adult	cultured	1	30	Perkinsus marinus	0			
	17/04/2007	Crassotrea gigas	Adult	wild	1	30	Haplosporidium nelsoni	0			
	17/10/2007	Crassotrea gigas	Adult	cultured	2	30	Haplosporidium nelsoni	0			
	23/10/2007	Crassotrea gigas	Adult	cultured	1	30	Haplosporidium nelsoni	0			
	17/04/2007	Crassotrea gigas	Adult	wild	1	30	Mikrocytos mackini	0			
	17/10/2007	Crassotrea gigas	Adult	cultured	2	30	Mikrocytos mackini	0			
	23/10/2007	Crassotrea gigas	Adult	cultured	1	30	Mikrocytos mackini	0			
	23/01/2007	Ruditapes decussatus	Adult	harvested	2	25	Perkinsus olseni	32,50%			
	20/03/2007	Ruditapes decussatus	Adult	harvested	2	25	Perkinsus olseni	16,50%			
	02/05/2007	Ruditapes decussatus	Adult	harvested	1	30	Perkinsus olseni	positive			
	08/05/2007	Ruditapes decussatus	Adult	harvested	1	30	Perkinsus olseni	0			
	16/05/2007	Ruditapes decussatus	Adult	harvested	1	30	Perkinsus olseni	positive			
	15/05/2007	Ruditapes decussatus	Adult	harvested	2	25	Perkinsus olseni	42,50%			
	15/06/2007	Ruditapes decussatus	Adult	harvested	2	25	Perkinsus olseni	45,00%			
	19/07/2007	Ruditapes decussatus	Adult	harvested	1	25	Perkinsus olseni	30,00%			
	14/05/2007	Ruditapes philippinarum	Adult	harvested	4	30	Perkinsus olseni	2/4 sites positive			
	31/01/2007	Venerupis pullastra	Adult	harvested	2	25	Perkinsus olseni	22,50%			
	09/04/2007	Venerupis pullastra	Adult	harvested	2	25	Perkinsus olseni	25,00%			
	07/05/2007	Venerupis pullastra	Adult	harvested	1	30	Perkinsus olseni	0			
	08/05/2007	Venerupis pullastra	Adult	harvested	2	30	Perkinsus olseni	2/2 positive			
	15/05/2007	Venerupis pullastra	Adult	harvested	2	25	Perkinsus olseni	44%			
	30/05/2007	Venerupis pullastra	Adult	harvested	2	30	Perkinsus olseni	1/2 sites positive			
	20/06/2007	Venerupis pullastra	Adult	harvested	1	25	Perkinsus olseni	45%			
	19/07/2007	Venerupis pullastra	Adult	harvested	1	25	Perkinsus olseni	60%			
	28/08/2007	Venerupis pullastra	Adult	harvested	2	25	Perkinsus olseni	17,50%			
	30/05/2007	Other (precise)	Adult	harvested	1	30	Perkinsus olseni	0			
Region II	01/04/2007	Ruditapes decussatus	Adult	harvested	2	100	Perkinsus olseni	2%			
	01/08/2007	Ruditapes decussatus	Adult	harvested	1	100	Perkinsus olseni	4%			

	01/08/2007	Ruditapes decussatus	Adult	harvested	1	100	Perkinsus olseni	8%			

Additionnal comments

When completing laboratory data, the lab is equiped and qualified to performe other confirmatory techniques (TEM, ISH, sequencing) of theexotic pathogens.

Sweden 2007

1. General data			
Production of the country	**European Oyster** (*O. edulis*): **Blue mussels** *(M. edulis***)**:	Farms: 1 ton/ year Natural beds: 20 tons / year Farms: 2800 tons/ year Natural beds : 300 tons/ year	
Zoning	Sweden / no notifiable molluscdiseases found		
Production of zones in the country	Production only on the swedish westcoast		
Number of farms within the country	Oyster production: 2 farms Blue mussel 7 farms		
Main exports for aquaculture purpose towards other Member States	No export	-	No
Main imports for aquaculture purpose from other Member States	No import	-	No
Main exports for aquaculture purpose towards Third Countries	No export		No
Main imports for aquaculture purpose from Third countries	No import		No
Number of hatcheries in the different zones with production	No hatcheries		No

2. Laboratory data	
Diagnostic methods used for *Bonamia ostreae*	Screening Methods: *Tissue imprints: heart* *Histology:* *Hepatopancreas, gills and mantel*
Diagnostic methods used for *Marteilia refringens*	Screening Methods *Histology: Digestive gland*
Diagnostic methods used for exotic pathogens	No screening
Diagnostic methods used for other diseases or pathogens monitored in the country	No screening

3. Epidemiological data			
SURVEILLANCE AND MONITORING OF *BONAMIA* / *MARTEILIA* :	Zone (Swedish westcoast)	June and July: September and October	150 specimens of Swedish oyster 150 specimens of Swedish oyster
SURVEILLANCE AND MONITORING OF OTHER PATHOGENS :	**Pathogen/Bivalve species (names)** No other monitouring during 2007		
STUDY OF ABNORMAL MORTALITY	Zone (Swedish west coast) No mortality recorded	Bivalve species	Sampling date
Number of positive results for *Bonamia* / *Marteilia*	Zone (Swedish westcoast)	0	
Number of positive results for other diseases / pathogen	No recorded findings in sweden	0	
Results regarding cases of abnormal mortality outbreaks	-		
Number of samples examined in total	300 specimens		

Additional comments: The investigations referred to are done as a 2 year projekt by the University of Gothenburg by Johanna Valero, and these presented data above are from the second year of this project. The intention is to continue the samplings and diagnostics at the swedish reference laboratory: National veterinay institute, Sweden (SVA).

United Kingdom 2007

	National competent authority	National Reference Laboratory for Mollusc diseases
Name :	**Department of Environment, Food and Rural Affairs (Defra)**	Cefas **/ FRS**
Address :	Nobel House, 17 Smith Square, London, SW1P 3JR	Barrack Road, Weymouth / **PO Box 101 Victoria Road, Torry, Aberdeen**
Web site :	http://www.defra.gov.uk	http://www.cefas.co.uk /**http://www.frs-scotland.gov.uk/**
Contact :	Veterinary Exotic Diseases, Research and Official Controls Division	Fish Health Inspectorate
Phone :	+44 (0)207 904 8722	+44 (0)1305 206673/4 / **+44(0)1224 876544**
E-mail :	helpline@defra.gsi.gov.uk	fish.health.inspectorate@cefas.co.uk /

General data

Technical data	Zone A	Zone B	Zone C	Zone F (SW Wales)	Zone D (rest of England, and Wales)	Northern Ireland	Zone D (rest of Scotland)	Zone E (Loch Sunart)	Zone G (West Loch Tarbert)			National data (official)
Farms	15	12	29	0	71	65	311	6	2			
hatcheries - nurseries	0	0	0	0	2	0	3	0	0			

Production data	Zone A	Zone B	Zone C	Zone F (SW Wales)	Zone D (rest of England, and Wales)	Northern Ireland	Zone D (rest of Scotland)	Zone E (Loch Sunart)	Zone G (West Loch Tarbert)			National data (official)	FAO 2003
Mytilus edulis	157	1 260	170		11 750	10 000	4 806						
Crassotrea gigas	306	170	171		168	346	208		3				
Ostrea edulis	8	1	51		2		22						
Ostrea edulis	36	620	15										
Cerastoderma edule		2											
Cerastoderma edule		600			14 955								
Ruditapes philippinarum		1				9							
Ruditapes philippinarum		386											
Mercenaria mercenaria			4										
Pecten maximus						2	2						
Chlamys opercularis							15						

Laboratory data

Diagnostic methods		non exotic agents *		exotic pathogens **								
		Bonamia ostreae	*Marteilia refringens*	*Bonamia exitiosa*	*Mikrocytos roughleyi*	*Mareilia sydneyi*	*Mikrocytos mackini*	*Perkinsus marinus*	*Perkinsus olseni*	*Haplosporidium nelsoni*	*Haplosporidium costale*	*Candidatus* Xenohaliotis californiensis
Screening	Cytology											
	Histology	Yes	Yes	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*
	PCR											
	RFTM culture							*Developing*	*Developing*			
	Other											
Presumptive	Cytology		Yes									
	Histology	Yes	Yes	Yes								
	TEM	Yes	Yes	Yes						Yes		
	PCR	Yes	Yes	Yes								
	RFTM culture											
	ISH		Refer to CRL									
	Other											
Confirmatory	Histology	Yes	Yes				Yes			Yes		Yes
	ISH	Yes	Refer to CRL	Refer to CRL						Refer to CRL		Refer to CRL
	TEM	Yes		Yes			Yes					
	PCR	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	PCR-RFLP	Yes		Yes								
	Sequencing	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	RFTM culture											
	Other											

* Pathogens responsible for listed diseases in annex A of Directive 91/67/CEE

** Pathogens responsible for listed diseases in annex D of Directive 95/70/CE

Recommanded methods in Manual of Diagnostic Tests for Aquatic Animals 2006

Three levels of examination procedures are proposed. **Screening** methods are those routinely performed for surveillance purpose. When abnormal mortalities occur, various **presumptive** diagnostic methods can be used in addition. When a pathogen is encountered during screening or mortality outbreaks, **confirmatory** methods should be used for specific identification, if available.

Epidemiological data

Surveillance and monitoring of *Bonamia ostreae* and *Marteilia refringens* in *Ostrea edulis*

Analysis effort					Results	
Zones	Date	Zootechnics	Sample	Individuals	Bonamia	Marteilia
Zone A	Aug, Sept &	cultured	3	30	8	0
	Sept	harvested	3	30	2	0
Zone B	Oct	harvested	18	30	14	0
Zone C	July & Sept	cultured	11	30	59	0
	Sept	harvested	2	30	5	0
Wales)	Oct & Nov	wild	3	30	20	0
of England	March	wild	5	30	0	0
of England	Sept & Nov	wild	5	30	0	0
Ireland	June & Sept	wild	3	30	0	0
Ireland	June & Sept	wild	1 (10 oysters	Other (precise)	0	0
Ireland	Dec	wild	1	30	1	0
of Scotland)	Spring	cultured	7	30	0	0
of Scotland)	Autumn	cultured	8	30	0	0
of Scotland)	Spring	wild			0	0
of Scotland)	Autumn	wild	7	30	0	0
Zone G	Spring	wild	2	30	10	0
	Autumn	wild	2	30	2	0
Zone E	Spring	wild	1	150	5	0
	Autumn	wild	5	30	0	0
Loch Sween	Spring	wild	4	30	1	0
	Autumn	wild	1	150	0	0

Comments

Study of abnormal mortality

Analysis effort						
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals
Hatchery	March	Ruditapes philippinarum	Spat	cultured	1	60
Hatchery	March	Ruditapes decussatus	Spat	cultured	1	60
Nursery	August	Crassotrea gigas	Juvenile	cultured	2	30
Burry Inlet	May	Cerastoderma edule	Adult	harvested	2	30
Burry Inlet	October	Cerastoderma edule	Adult	harvested	1	30

Summarized results			
Pathogen(s) isolated	Outbreaks	Frequency	Comments
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Surveillance and monitoring of other pathogens

Information about the program / project	
Name :	Survey of UK Hatcheries
Type :	Official program
Aim :	Detect pathogen
Duration :	Spring and Autumn
Mollusc species concerned	*Crassotrea gigas*
	Ruditapes philippinarum
	Ostrea edulis
	Ruditapes decussatus
Targeted pathogens	All pathogens
Objectives and Brief summary of the program / project	

Analysis effort							Results		
Sites	Date	Host	Age-class	Zootechnics	Sample	Individuals	Targeted pathogen(s)	Estimate prevalence	Comments
Hatchery 1	July & Nov	*Crassotrea gigas*	Juvenile	cultured	2	50	All pathogens	No positive samples	
	July	*Ostrea edulis*	Juvenile	cultured	1	50	All pathogens	No positive samples	
	July & Nov	*Ruditapes philippinarum*	Juvenile	cultured	2	50	All pathogens	No positive samples	
	July	*Ruditapes decussatus*	Juvenile	cultured	1	50	All pathogens	No positive samples	
	Nov	*Crassotrea gigas*	Larva	cultured	1	60	All pathogens	No positive samples	
Hatchery 2	June	*Crassotrea gigas*	Juvenile	cultured	1	60	All pathogens	No positive samples	
	Oct	*Crassotrea gigas*	Spat	cultured	1	60	All pathogens	No positive samples	

Additionnal comments

Hatchery clams - No disease agents were found in samples of clams taken from a hatchery experiencing temporary abnormal mortality.

Nursery oysters - One batch of Pacific oysters exhibited post-grading mortlaities of 60-70%. All sizes were affected. There was no mortality in other batches.
The initial examination, by histology, suggested the presence of oyster herpes virus. Further analysis of the sample was carried out using molecular biology and electron microscopy. Molecular biology results using OHV- specific primers were negative. Animals were examined using transmission electron microscopy which showed in some cases nuclear lesions similar to apoptotic cells, others with lesions usually described in association with viral infection like herpesvirus infection. IFREMER (the CRL) have examined the samples and consider that whilst there are virus-like particles present in some images submitted to them, they are not herpesvirus-like but may be more similar to irido-like viruses.

Wild cockles - Since July 2003 mass mortalities of 1 year old cockles and older have been observed in the Burry Inlet in South Wales. The same sequence of events is repeated annually: high spatfall in May and June culminates in densities approaching 9,000 individuals per square metre. From July onwards, mass mortalities of the previous year class occur, resulting in the obliteration of almost all 1-year-old cockles. A multi-disciplinary study has been initiated. The results so far do not give a clear indication of the possible cause of mortalities. High bacterial blood loads and low haemocyte counts in moribund cockles are probably an indication of a secondary, opportunistic infection rather than the prime cause. No significant pathogens have been detected.

Bonamia Scotland: Confirmed at two locations; Loch Sunart & West Loch Tarbert, movement controls remain in place. The Loch Sunart site is considered as fallow, however a confirmatory survey will be undertaken in 2008 within and out with the site. The site is to be left fallow for the foreseeable future. There are considerable wild populations of Ostrea edulis in West Loch Tarbert, clearance there is not considered an option. The aim is to contain the infection at both locations. Further epidemiological investigations are being considered in relation to Bonamia infection/transmission within Scotland
No abnormal mortalities in Shellfish populations, wild or aquaculture has been reported in Scottish waters in 2007.
Scottish shellfish hatcheries were unproductive in 2007, no health problems reported.