REPORT OF THE

2014 Annual Meeting of the National Reference Laboratories for Mollusc Diseases

Nantes, 25-26 March 2014

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Executive summary

Highlights of the discussions, expert opinion and recommendations formulated during the 2014 Annual Meeting of National Reference Laboratories (NRLs) for Diseases of Molluscs are summarized below:

- Major information concerning **mollusc health situation** in EU in 2013 were: (1) a new *Mikrocytos* species, *Mikrocytos mimicus* detected and characterized from cupped oysters, *Crassostrea gigas* during mortality events in England (2) OsHV-1 µvar detected like previous years during mortality events of *Crassostrea gigas* in several countries and in locations previously considered free in Ireland and UK (3) *Vibrio aestuarianus* detected like in 2012 during several mortality cases of adults cupped oysters in France; the bacteria was also detected in Ireland but its involvement in mortality is not obvious (4) *Marteilia cochillia* involved in the collapse of wild beds of cockles *Cerastoderma edule* in Galicia (5) *Marteilia refringens* detected in farmed and wild mussels *Mytilus edulis* in Sweden in absence of movements with other infected farms (6) Massive mortalities of mussels reported in Italy and due to fragility of the byssus apparently increased by the ocean acidification (7) *Bonamia ostreae* detected sometimes with high prevalence in some Irish and UK locations and for the first time in New Zealand oyster, *Ostrea chilensis* in Menai Strait (England) (8) *Mikrocytos*-like parasite detected like previous years in association with mortality of *Donax trunculus* in France (9) an unidentified amoeba-like parasite recently detected in abalone *Haliotis discus* in Iceland.

- More and more NRLs investigate **presence of bacteria** when mortality of molluscs occur. During the last couple of years, the association between *Vibrio aestuarianus* and mortality of *Crassostrea gigas* raised some questions regarding its diagnosis. Indeed, isolation of bacteria prior to specific identification by real time PCR is time consuming in routine and isolation on TCBS is not suitable for this species. In this condition, it was proposed to directly use PCR on DNA extracted from tissue. However, in Ireland this approach led to the detection of *V. aestuarianus* in many samples without clear impact on the oysters. In addition, the relevance of using MALDI-TOF for bacteria identification was discussed considering that few laboratories are already working with this tool especially for fish bacteria. Exchange of spectra is already in place between some countries to improve datasets concerning fish and could be extent to molluscs bacteria.

- **Infection with Vibrio aestuarianus** has regularly been reported since 2001 in France but seems to be associated with more events of mortality these last years. It is more often detected in adults during warm periods of the year but is also present in juveniles and spat and all the year long. Considering the potential impact of this bacteria on *Crassostrea gigas* production in Europe, the relevance of listing this pathogen was discussed and evaluated checking the different criteria used at the European level to list diseases. *V. aestuarianus* fulfilled nearly all the criteria and could thus be a good candidate to be listed. The apparent emergence of a new *Mikrocytos* species, *Mikrocytos mimicus* in *C. gigas* in England also raised some concern about its potential spread in Europe.
• Experience from locations where susceptible species are present but some diseases absent is very informative. The situation of Limfjorden in Denmark regarding infection with *Bonamia ostreae* was presented suggesting that extreme climatic conditions prevented the spread of *Bonamia* parasites to flat oyster population in this place. However in these free zones, surveillance of exotic pathogen can require many samples and analyses. An exercise on *Mikrocytos mackini* was carried out in Charente Maritime in France. The approach aimed at identifying main **risks of introduction and establishment** of the disease and finally the most suitable space and time frames to target the pathogen in the context of a risk based surveillance approach.

• **Korea** is an important mollusc producing country especially for cupped oyster *Crassostrea gigas* and manila clam *Ruditapes philippinarum*. Recurrent mortalities of clams are reported in association with *Perkinsus olseni* and *Vibrio tapetis*. Mortality of *C. gigas* is also observed mostly during summer time and sometimes associated with parasites including *Marteiloïdes chungmensis*. The **impact of Perkinsus olseni** on clam population has been investigated since several years in Korea. These studies have shown that high levels of *P. olseni* infection interfere with spawning frequency and reduce egg production, which might result in long-term impacts on clam recruitment and population growth. The potential impact of **global warming** on the impact of the parasite.

• Different approaches including partial sequencing, microsatellite genotyping and complete genome sequencing have been used to better describe **OsHV-1 diversity** and allowed identifying subpopulations of this virus. Genotyping based on a microsatellite locus appeared as a powerful tool to study OsHV-1 polymorphism. This approach notably demonstrated that New Zealand and Australian variants belong to a group distinct from OsHV-1μvar detected in Europe. A study carried out in England in *Crassotrea gigas* outside mortality event revealed the presence of several variants of OsHV-1 distinct from OsHV-1 μvar. These results suggest that diversity of the virus is wider outside mortality events.

• A synthesis of the **early surveillance programme** for infection with OsHV-1 μvar in UK and Ireland was presented. The future of these programmes was discussed and will be included in the **mandate** sent by the European Commission to **EFSA**. This mandate also tackles the situation of *Crassostrea gigas* regarding infection with *Vibrio aestuarianus* and the potential role of purification centres in spreading some mollusc pathogens.

• Several **breeding programs** have recently been initiated in France at the professional scale to improve survival of oysters to face the dramatic impact of diseases to which resistance has been shown to be heritable. The detection of QTLs definitely appears useful to assist selection. On the other hand, understanding mechanisms involved in the oysters responses to diseases is also crucial. Recent works have shown that **apoptosis** is a pathway modulated by several pathogens including *Bonamia ostreae* in the flat oyster *Ostrea edulis*. Field studies also contribute to better evaluate interactions between micro-organisms, sometimes considered as pathogens, and bivalves. The health status of a wide range of bivalve species was recently evaluated in the Wadden Sea showing the presence of a diverse community of protozoans in the animals.
• According to the Directive 2006/88/EC, reference laboratories must be accredited. A review of the status of NRLs and the EURL concerning this requirement was done and presented. Most of NRLs hold **accreditation** for test methods used in the screening for the non-exotic listed diseases while there seems to be little consistency regarding accreditation for either the exotic diseases or non-listed diseases, particularly in relation to molecular diagnostic methods.

• **Interlaboratory Comparison** tests are compulsory for accreditation and are useful to test ability of participants to use a specific technique. In addition, such tests can present a good opportunity to compare different diagnostic techniques. In 2013, the EURL organized an ILC based on the detection of *Bonamia* sp. by PCR. This test also aimed at comparing several PCR assays currently available for the detection of these parasites. **Comparison study** is an interesting approach to evaluate performances of diagnosis techniques and finally to select tools to be recommended within the NRLs network.

• Next **Annual meeting** will be organised together with a **technical workshop** in March 2015. It is proposed that the technical workshop includes a session on the isolation and characterization of *Vibrio aestuarianus*. NRLs are invited to submit their suggestions for the annual meeting and technical workshop before next year.
The 2014 Annual Meeting of National Reference Laboratories for Mollusc Diseases was held in Ifremer facilities in Nantes on the 25th and 26th of March 2014. In total, 39 participants from 20 countries (Bulgaria, Croatia, Denmark, France, Germany, Greece, Iceland, Ireland, Italy, Lithuania, Montenegro, Poland, Portugal, Republic of Korea, Romania, Slovenia, Spain, Sweden, The Netherlands and United-Kingdom) and one representative from DG-Sanco (European Commission) attended the meeting.

The Annual Meeting included seven sessions: 1/ The current epidemiological situation in each Member State 2/ Vibrios: where are we? 3/ Emerging/listed diseases 4/ Mollusc health situation in Korea 5/ OsHV-1 6/ News from the bench 6/ EURL day life activities.

This report includes summaries of the questions discussed during the meeting and outstanding facts for follow-up activities. It also contains collective expert opinion and recommendations made during the meeting.
ANNUAL MEETING SESSION I : DIAGNOSIS AND SURVEY OF MOLLUSC DISEASES

(Chairperson: I. Arzul; Secretary : C. Garcia)

National delegates participating in the Annual Meeting 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 D). A summary of the reports is given here country by country followed by questions and discussion which took place at the end of each presentation.

Italy. G. Arcangeli

Production data: during 2013 (tons): Mussel (Mytilus galloprovincialis): 80,000; Manila clam (Ruditapes philippinarum): 30,000 Oysters (C. gigas): 50.

In 2013, a monitoring programme was carried out in order to determine the distribution of marteiliosis and bonamiosis in Italy. A total of 4,415 mussels were sampled in spring-summer in the Adriatic sea and Tyrrhenian sea: Marteilia refringens was detected in Tyrrhenian sea (Liguria, Campania) and Adriatic sea (Friuli). A total of 229 flat oyster (Ostrea edulis) were sampled in spring-summer in the Adriatic sea: all the samples were negative. Perkinsus olseni is confirmed to be present in Manila clams with high prevalence in lagoon.

Shellfish movements/transfers: the most important movement has been, as usual, the transfer in winter season of mussels from Spain, only for consumption purposes (purification process is done in Italy).

Epidemiological situation of OsHV-1 μvar in farmed C. gigas:
Sardinia - lagoon in the north-east: 50% mortality in juveniles and adults (french seed).
Liguria-La Spezia gulf, off shore farm: presence of OsHV-1 μvar in adults not associated with mortality- (Italian seed coming from a very small experimental hatchery located at Goro).
Veneto- Chioggia (lagoon): private hatchery: severe mortality in spats (breeding oysters coming from north Europe): now the hatchery is closed.
Marche : off shore: 60-70% mortality in juveniles.
Mass mortality in Manila clams: events in august, in Veneto - lagoons, attributable to excessive temperature. In february, mass mortality for abundant rainfall resulting in very low salinity values.
Other problems: the lose of grip in mussels farmed off-shore and in lagoon. The problem is more serious off-shore when, following sea storms, are possible losses of 50% of the production. In 2013 the problems was more severe than in other years.
Following the report of Mikrocytos sp. presence in manila clam in 2012 from The Netherlands, has been strengthened the research of this pathogen in Italy: all the samples were negative.

I. Arzul wondered how the sampling strategy was organized concerning the monitoring of Mikrocytos sp. G. Arcangeli answered that they did not perform specific sampling to detect Mikrocytos. However, they include positive Mikrocytos slides in their proficiency test in order to prepare laboratories to detect it.

I. Arzul also asked if they observed a relation between the increase of temperature and the increase of Perkinsus presence in clams in the context of the global warming. G. Arcangeli said that they do not observe a real change in Perkinsus detection. The increase of temperature does not affect clams whereas the ocean acidification has an impact on mussel particularly on byssus filament. The ocean
acidification seems to increase the fragility of the byssus and a mussel declumping is observed. Last year, important mussel mortalities occurred in Italy due to this phenomenon.

United Kingdom. M. Gubbins

The main species cultivated in the UK are mussels, Pacific oysters and native oysters, there are also a small number of farms holding clams. Pacific oysters clams and some native oysters are supplied from hatchery production, whilst most native oysters and all mussels are harvested from wild stock. There are a total of 452 aquaculture production businesses in the UK, producing a little over 27,358 tonnes of shellfish. Mussels made up the vast majority of this production with over 26,000 tonnes being recorded.

Surveillance and monitoring for Bonamia and Martelia has continued in Scotland, England, Wales and Northern Ireland with samples of native oysters (Ostrea edulis) and mussels (Mytilus edulis) being tested for the diseases. This programme did not detect the presence of B. exitiosa. However wild populations of New Zealand dredge oysters in the Menai Strait (Ostrea chilensis) tested positive for B. ostreae for the first time, and B.ostreae continues to be found in oysters from the infected zones.

The continued absence of Martelia refringens was demonstrated in Scotland Wales and Northern Ireland, and no positives were identified in England, movement controls remain in place on the Tamar for this parasite identified in mussels from this unfished area in 2010.

Abnormal mortalities were investigated on 4 occasions in Pacific oysters and mussels in Scotland; and on 7 occasions in England and Wales (all reports were of problems with Pacific oysters). One investigation in Poole Harbour identified the presence of OsHV-1μVar on a farm in Poole, although the epidemiological investigation did not identify the cause of the infection it was interesting to note that the virus from the samples was different from the previous two OsHV-1μVar outbreaks in England; and was identical to isolate 002 first identified in France during 2010 (JN 800 620). No listed pathogens were detected during any of the other follow up investigations, however three Pacific oyster sites in England reported increased mortality following purification of market-sized product, which when investigated was associated with a new species of Mikrocytos, now identified as M. mimicus. The event occurred in late spring and ceased during the summer, no recurrence has been observed. There were no reports of abnormal mortality in Northern Ireland.

The surveillance programmes for OsHV-1μVar in England, Wales, Scotland and Northern Ireland continued in 2013. In England 14 samples from 11 sites were analysed as part of the official programme to monitor the effectiveness of the containment areas. As a result of a mortality reported by a farmer in the late summer/ early autumn, one new positive for the μVar was identified in Poole Harbour, the event happened in October, later than the previously identified outbreaks, but was associated with elevated water temperatures not normally experienced at that time of year. The sequence of this positive was different from the previously identified UK positive sites in Kent and Essex, but as mentioned above was identical to a sequence reported from France in 2010 (accession no. JN800120).

Sites tested around the positive containment areas again identified OsHV-1, but not OsHV-1μVar. The sequence data from these positives was consistent with previously reported wild type strains of the virus. The identification of the virus was not associated with mortality events.

In summary, we can report a first finding of Mikrocytos mimicus in farmed Pacific oysters in North Norfolk; Bonamia ostreae was confirmed in North Wales where the parasite was found for the first time in a wild population of O. chilensis. There was no incidence of B. exitiosa in any of the samples examined. Ostreid herpesvirus (OsHV-1μVar) was identified in Pacific oysters from Poole Harbour for the first time, there was no evidence of spread from the site.
A. Kristmundson wanted to know what “cold temperature” means in UK. M. Gubbins answered that last winter, seawater temperatures decreased below 6°C during several months which is not frequent in UK.

I. Arzul was surprised by the high prevalence of bonamiosis (100%) reported in Lough Foyle (Northern Ireland). M. Gubbins agreed with I. Arzul, they never observed that before. Contrary to England, in Northern Ireland, screening of bonamiosis is performed by PCR and positive results are subsequently observed by histology. At this date, all histological analyses have not yet been performed.

Ireland. D. Cheslett

Mollusc production figures for 2013 estimate bottom mussel (M. edulis) production at 9833.6 tonnes, rope mussel (M. edulis) production at 5527 tonnes, C. gigas oysters production 8140.915 tonnes and O. edulis oysters production 459.2 tonnes. There are currently 314 authorised mollusc farms in Ireland, with the majority of these producing C. gigas oysters (166 farms) and M. edulis (116 farms).

The largest trade in live shellfish is in C. gigas between Ireland and France. In 2013, over 560 million C. gigas were imported into the country. 93% of these oysters came from France as seed or juveniles. Similarly, 98% of the 1295 tonnes of C. gigas exported for relaying were destined for France. Significant quantities of M. edulis also left Ireland with over 1060 tonnes of M. edulis being exported. 69% of this trade is to sites in NI.

The Marine Institute laboratories are accredited to the ISO 17025 Standard by Irish National Accreditation Board (INAB). For shellfish diagnostics accreditation is held for the screening of O. edulis using heart impression prints for Bonamia ostreae; and for the screening of O. edulis for the presence of Marteilia refringens by histology. In 2013 the MI also gained accreditation for the Detection of OsHV-1 by real-time Taqman PCR (Martenot et al. 2010). Non- accredited methods include general shellfish diagnostics using histology, detection of Vibrio species by classical bacteriology and numerous real-time and conventional PCR based methods.

O. edulis are sampled every year from Bonamia-free areas and from Lough Foyle and screened for the presence of Bonamia sp. and Marteilia refringens. Where possible 2-3 sites which are positive for B. ostreae are also sampled and screened for Bonamia ostreae in order to assess prevalence of parasite over time. In 2013, sampling took place in 5 sites in the autumn. Samples of 30 individual Ostrea edulis were taken from Tralee and Kilkieran and from 2 beds in Lough Foyle. All were negative for Marteilia refringens and both Kilkieran and Tralee remain free of B. ostreae. Lough Foyle-Southside bed (21/30) and Lough Foyle-Quigley’s point (8/30) both had a high prevalence of the parasite and oysters sampled presented with a pale digestive gland, though no mortality was reported. Larger samples were collected from Galway Bay and Lough Swilly as part of a Fisheries survey and were both found to be positive for Bonamia ostreae (4/50 and 127/150 respectively).

There were 14 mollusc growing areas remaining in the OsHV-1 µvar Surveillance Programme at the beginning of 2013 including 2 hatcheries. In 2013 the virus was detected in a further 3 growing areas and they were removed from the Programme. The virus was detected in association with mortality in 2 of these bays (Ballylongford and Dunmaus) and in the absence of mortality in the 3rd bay (Kinsale) although morality had been reported earlier in the Summer in Kinsale possibly as a result of V. aesturianus. No definitive source of infection was identified in any of these cases, although movements of personnel were suspected in one bay.

Mortality was reported to the MI from 20/29 bays previously known to be infected with OsHV-1µVar. In most of these reports mortality was confined to seed and juvenile oysters and timing of mortality was consistent with that expected for OsHV-1µVar infection. Samples were only
collected from 7 bays where patterns of mortality were atypical of that expected for OsHV-1µVar or sites were targeted because they had been categorised previously as high risk due to their movement patterns. *V. aesturianus* was frequently detected in these sites, but in the absence of pathology specific to *V. aesturianus* infection. The role of *V. aesturianus* in these mortality events remains unclear.

G. Arcangeli would like to know if the presence of the unknown bacteria observed in Manila clams was associated with macroscopic lesions. D. Cheslett answered that they did not observe specific macroscopic lesions except a brown ring on shell in some clams.

A. Kristmundsson asked about the surveillance strategy carried out on scallops in Ireland. D. Cheslett answered there is no specific strategy; they perform sampling only when mortality events are notified.

A. Kristmundsson explained that Ireland imports scallops from Scotland and that in 2007 Apicomplexan parasites were detected in scallops imported from Scotland in association with mortality. M. Gubbins specified that coccidian parasites were observed in scallops from the West coast of Scotland.

**Poland. E. Pazdzior**

Along the Polish costal zone of the Baltic Sea does not have the suitable condition to mollusc culture. Therefore the different species of molluscs (mainly *M. edulis* and *C. gigas*) are imported to Poland mainly from the European Community countries, for the purpose to human consumption. Authorization of the farms as well as farms categorization do not apply in Poland. During 2013 four samples of wild population of mussels (*M. edulis*) from different locations of the Baltic Sea were collected. A minimum of 30 individuals from each sampling points (Pucka Bay – 1 sample, open Baltic Sea – 3 samples) were collected in August. The water depth in the sampling point was from 1 m to 4 m, and the water temperature at the time was about 18°C. For *Marteilia refringens* detection tissue imprints and PCR analysis were carried out. All tested samples were negative. In 2013 any abnormal mortality of molluscs have not been observed. All diagnostic techniques used in the Polish NRL are under quality manager system, and tissue imprints has been accredited by Polish Centre for Accreditation according to ISO/IEC 17025. In the next year we are going to continue the sampling from indicated area of the Baltic Sea.

I. Arzul would like to know if there is other harvested mollusc species than mussel in Poland. E. Pazdzior answered that there is no other exploited mollusc. I. Arzul also asked if mollusk movements occur between Poland and Germany. E. Pazdzior answered no.

G. Arcangeli asked if there is a mollusc production in fresh waters. E. Pazdzior said no.

**The Netherlands. M. Engelsma**

*Mytilus edulis* is the most important shellfish species produced in the Netherlands, followed by the Pacific oyster *Crassostrea gigas* and a small production of the flat oyster *Ostrea edulis*. Annually a surveillance is carried out in spring and autumn for detection of shellfish diseases in these three species. In 2013 screening for *Bonamia ostreae* and *Marteilia refringens* was carried out using histology as well as PCR analysis. *Bonamia ostreae* has been present in *O. edulis* from Lake Grevelingen since 1988. *Bonamia ostreae* was observed in 8% of the investigated flat oysters in the spring of 2013 as analysed by histology. By PCR genetic material of *B. ostreae* was detected in 29% of the flat oysters. No *M. refringens* was observed in the investigated oysters and mussels. During 2013 no mortalities were reported in the mussel and oyster stocks.
S. Bergmann asked if they check mollusc coming from Germany. M. Engelsma answered that he checks different mollusc species but he does not know the origin.

Belgium. M. Engelsma

Belgium has 1 oyster farm. This farm had no production of oysters in 2013. As no production took place, no mollusc health surveillance scheme has been carried out in 2013. Furthermore, no abnormal mortalities of molluscs were reported. For 2014, depending on the production, a survey will be planned to analyse the health status of M. edulis with regard to the presence or absence of Marteilia refringens.

Denmark. L. Madsen

The blue mussel (Mytilus edulis) production/fishing in Denmark was 37,491 tons in 2013, which was roughly the same as the year before. Just above 15,000 tons originated from areas outside Limfjorden, whereas 22,000 tons had been fished in Limfjorden. There is no fishing of Crassostrea gigas, but the Pacific oyster can be found in Danish waters, especially the Wadden Sea. The fishing of Ostrea edulis, located in Limfjorden, is regulated by a quoting system as well as the fishing is only allowed in certain time periods during a year. The fishing amount of flat oysters was again lower in 2013 (142 tons) compared to the year before (296 tons). The reason for the lower fisheries is because the flat oyster stock has gone down dramatically. The stock has dropped in all areas of Limfjorden and the cause is believed to be the very cold winters for the past few years. There has been no recruitment of oysters for some years, which means that the fishing has been on the same cohort since 2005. In 2013 the screening of flat oysters consisted of samples of around 30 individuals collected in both spring and autumn 2013 in three sites from Limfjorden. Investigations for 2013 were not finished at meeting dates. Denmark has been officially recognized Bonamia ostreae and Marteilia refringens free in the Limfjorden area since December 2004. A blue mussel disease surveillance program in waters outside Limfjorden was founded in 2012. The program consists of four surveillance zones; Zone I: Kattegat; Zone II: Bælthavet; Zone III: Isefjorden; Zone IV: Storebælt and Smålandsfarvandet. From all four zones three samples, each consisting of 50 blue mussels, have been sampled in autumn both in 2012 and 2013 and then processed and screened by histology. Again in 2013 no Marteilia were found in the investigated blue mussels, wherefore the Danish Veterinary and Food Administration has sent an application to the EU, applying for disease-free status of the four mentioned areas, regarding Marteilia (and Bonamia).

S. Bergmann asked if they have contact with oyster farmers from Germany. L. Madsen answered no.

A. Kristmundsson would like to know which trematod species (metacercaria) was observed. L. Madsen answered that she could not specify the species because the observations were performed in histology and histology does not allow determining trematod species.

Germany. S. Bergman

S. Bergman explained that it is presently impossible to get information about mussels from the Wadden Sea.
Greece. V. Kosmidis

Mussels of the species *Mytilus Galoprovincialis* are cultivated in Greece and the production of 2013 was 22,000 tones. Also limited quantities of other species, are collected from natural beds located mainly in northern Greece. There are 89 officially licensed farms cultivating, 400 unauthorized farms which are also monitored for their practices from the prefectoral vet service, 11 purification centers, while hatcheries and nurseries do not exist in Greece. Farms are sustained by the natural brood movements and transfers take place only for consumption. Farms located in 5 areas, including thessaloniki, kavala, xanthi, preveza, komotini are classified in category V (they are known to be infected). Farms located in all other areas of the country are classified in category III. The screening method for *Martelia* sp detection is imprint cytology from the digestive gland. Confirmation is achieved by histology. As previously cytology and histology are used for the diagnosis and surveillance of marteiliosis. During in last year we examined 114 samples originating from 7 areas. Each sample consisted of 30 mussels and samplings were taken place on a yearly round basis. One out of 114 sample was found positive and the diagnosis was further based of histology. There is currently no surveillance program for other pathogens. Also we are not currently searching for HV-1, because we don’t have production of oysters. Increased mortality rates are currently addressed by prompt sampling with cytology and histology. No abnormal mortalities were reported in 2013.

The goals of our lab include

- Continue and expand surveillance for martelia sp
- Improve the diagnostic Facilities by adding molecular methods as confirmatory diagnostics
- And to obtain accreditation for Cytology –Histology and PCR, a process that is currently in progress

I. Arzul noted that it was the first country which mentioned its number of purification center. Few countries specify it and it is very difficult to have this kind of information whereas it is important information.

I. Arzul asked if there is a clam production in Greece. V. Kosmidis answered no.

Sweden. A. Alffjorden

The production in Sweden is still dominated by farmed blue mussels, *Mytilus edulis*. In 2013 the blue mussel production reached 1753 ton which is an increase compare to 2011. The production is concentrated to the southern parts of the mollusc harvest/production areas (Stenungsund up to Lysekil). During 2013 the harvest of blue mussels in the northerm harvest areas have been at low levels. The Swedish bluemussel industry has a production fluctuating around 2000 ton. The production of farmed flat oyster is still on very low levels in Sweden. One farm has both a hatchery and nursery but is producing spat at low levels. There are also one new small oyster farm starting up that intends to produce spat from natural beds.

Sweden has one zone, the Swedish west coast from the county of Halland to the border of Norway. During 2013, 150 adult oysters (1sample from farmed oyster and 4 from wild harvest sites/wild populations) were investigated for diseases and were found free of *Marteilia refringens* and *Bonamia spp*. In 2013 samples were also collected from 150 blue mussels for monitoring regarding marteiliosis. This program included three samples from mussel farms and two from wild stocks. In one of the samples from mussel farms, *Marteilia refringens* was detected at low levels (6,7%). In the northern parts of the westcoast there were only low levels of farmed mussels harvested 2013.
Instead wild blue mussels from natural stocks were collected for screening. One of these two wild populations sampled was found to be infected with *Marteilia refringens*. There is no farming activity in the area of this finding and further investigation will be done to search for the possible cause of this finding. Samples have been sent to the reference laboratory IFREMER for comparison with other isolates. The flat oyster investigations performed during 2013 is part of the Swedish screening program for Bonamia to get and maintain official freedom according to EU for this agent. The program to screen for Bonamios and Marteilios will continue during 2014 and samples are planned to be collected on the same levels as previous year.

L. Madsen asked if mortality was observed in mussel found infected with Marteilia refringens. A. Alfjorden answered no but it was difficult to know because it was a wild bed. They do not have the history of this wild bed, the density of this bed decreased but they have no information on the origin of this decrease. Meanwhile, no problem was observed on mussel farms in the area. A. Kristmundsson underlined the importance of marine current in the transfer of pathogen agents in natural stock and he was interested to know if they investigated the role of ballast waters. A. Alfjorden answered that they performed surveillance on flat oysters in the area and no Marteilia was observed. When Marteilia was observed in mussels, it was observed once in one particular area. They think there is no real connection between the infection of this mussel wild bed and ballast waters and if there was one, it is impossible to verify the impact of ballast waters. L. Madsen asked if they use real time PCR for Marteilia screening. A. Alfjorden answered yes, they use PCR for screening and they confirm their positive results by histology, particularly for Bonamia because this parasite is difficult to observe in histology.

**Portugal. F. Batista**

F. Ruano, F. Batista*, A. Grade

The aquaculture production of molluscs in Portugal in 2011 was 3,544 tons. A total of 1394 active farms/production sites were identified in Algarve region (96.0%), Lisbon region (1.8%) and Aveiro region (2.2%). The main produced species were the grooved carpet shell clam *Ruditapes decussatus* (66%), the Pacific oyster *Crassostrea gigas* (20%), the mediterranean mussel *Mytilus galloprovincialis* (7%), the Portuguese oyster *Crassostrea angulata* (3%) and the cockle *Cerastoderme edule* (3%). A total of 129 million individuals/spat of *C. gigas* were imported. In 2013, the main diseases observed were perkinsiosis and marteliosis, and high mortality associated with OsHV-1 μvar were observed. Perkinsiosis was the main concern for *R. decussatus* production and its endemic nature (prevalence between 60 and 70%) was associated with high mortality rates. Perkinsiosis was also observed in the manila clam *Ruditapes philippinarum* at Tagus estuary, Sado estuary and Albufeira lagoon (prevalence between 10 and 50%). However, no mortalities have been reported in *R. philippinarum* associated with perkinsiosis. OsHV-1 μvar was detected in *C. gigas* cultured in the southern coast of Portugal (offshore production) showing high mortality rates (between 43 and 73%). Marteliosis was observed in the flat oyster *Ostrea edulis* in wild populations at Formosa lagoon. The Mediterranean mussel *M. galloprovincialis* produced in rafts at Albufeira lagoon showed an endemic presence of Marteliosis but, apparently, without high mortalities associated with the disease. The populations of the Portuguese oyster *C. angulata* continue to show a healthy situation, both in Sado and Mira estuaries. The NRL has in progress a program for accreditation of general laboratorial procedures and aims to implement *in situ* hybridization for marteliosis, bonamiosis and OsHV-1.

I. Arzul asked if they determined the type of Marteilia refringens. F. Batista said no
**Bulgaria. V. Chikova**

The production of mollusc for 2013 in Bulgaria was 887.76 t. The main species is *Mytilus galloprovincialis* - the Mediterranean mussel. There is 19 registered farms uniformly distributed in the two areas along the coast of Black sea – the north zone (near Varna) and the south zone (southward of Burgas). The surveillance is passive and there is no available data on the movement of mussels. All aquaculture farms are in cat. III – not declared free but not known to be infected according Directive 2006/88/EC. Diagnostic techniques and research method used for diagnosis aren’t accredited in the NRL of mollusc diseases in Bulgaria but we intend to implemented of quality management system for the diagnosis of Marteilia sp. by histopathology. Diagnostic techniques are tissue imprint and histopathology of digestive gland. The Black Sea is suitable for both wild and farming mussels and can have stable future economic potential. The NRL is ready to meet the challenge of population growth and alerts of abnormal mortality in farms or natural harvest areas.

I. Arzul asked if Bulgaria would like to ask for freedom status regarding Marteilia infection. V. Chikova answered that they could be interested in the future.

**Romania. M. Costea**

For 2012 Romania registered a production of 10,953 kg of *M. galloprovincialis* and 588,424 kg of *Rapana venosa* from fishing activity. For 2013, no data is available yet. There is one farm of bivalve mollusc (*mussels Mytilus galloprovincialis*). NRL has accredited diagnostic techniques against 17025:2005 for detection of *Marteillia refringens* in mussels digestive gland imprints and detection of mollusc pathogens by histology. The accreditation was performed by National Accreditation Body - RENAR. Methods like « detection of *Perkinsus* sp. in thyoglicolate fluid » and PCR techniques are under quality management of 17025:2005 standard. For 2013 no abnormal mortality was registered. No sampling was performed.

I. Arzul asked if they would ask to be recognize as free regarding marteiliosis. M. Costea replied that for marteiliosis, it must be discussed. However they will probably ask for freedom status regarding bonamiosis because there is no susceptible species present in Romanian waters. The decision belongs to the Competent Authority.

I. Arzul would like to know on which bivalve species they look for Perkinsus. They test Perkinsus presence in *Crassostrea gigas* samples.

**Spain. R. Aranguren**

The Spanish mollusc production is represented mainly by the mussel, *Mytilus galloprovincialis*, aquaculture with more than 229.000 tn of production, followed by far by clams, cockles and oyster production. The 95 % of the total national production is achieved in the north west coast (Galicia). Due to the economic importance of the mollusc production in Galicia, a monitoring programme is carried out by the regional laboratory (INTERMAR, Xunta de Galicia) to estimate the prevalence of all listed pathogens including all symbionts and pathological conditions detectable by histology in the main commercial species in this region.
At a national level, in 2013 a surveillance monitoring programme was carried out in order to determine the distribution of marteiliosis and bonamiosis. Samples were analyzed by histology and tissue imprints. Positive samples according to these techniques were confirmed by PCR and PCR-RFLP. 4 different areas were analyzed distributed along the Atlantic and Mediterranean coasts; Galicia and Asturias in the Atlantic coast, and Comunidad Valenciana, Balearic Islands and Andalucía in the Mediterranean coast.

Regarding listed pathogens *Bonamia ostreae* and *Marteilia refringens*, Spain is officially not declared free but there are remaining areas where it is not known to be infected. Considering diagnosis and quality management, the Spanish National Reference Laboratory (NRL) is accredited by ENAC (entidad Nacional de acreditación) for cytology and histology diagnostic tests (*Bonamia* sp., *Marteilia* sp.) and for histology diagnostic test in clams (*Perkinsus* sp.) in accordance with UNE-EN ISO/IEC 17025 on ‘General requirements for the competence of testing and calibration laboratories’. All diagnostic tests realized in NRL are under quality management.

Important increased mortality events occurred in an oyster, *Crassostrea gigas*, producing areas in Galicia in summer time. Mortalities were severe and affected *Crassostrea gigas* spats and juveniles. No listed infectious agent was detected but OsHV-1 μvar. No other mortality episode was reported. For 2014, Spain will follow with the surveillance monitoring programme to estimate prevalence and distribution of listed pathogens.

I. Arzul wanted to know if mortality took place in both positive locations where Marteilia was found in cockles. R. Aranguren said that they observed mortality only in the first ria and not in the one recently found infected. No molecular characterization was yet performed on these samples; S. Zrnčič asked if the prevalence of Marteilia in Spain varies according to the years. R. Aranguren Ruiz answered that they do not observe a real change, Marteilia prevalence is always the same.

I. Arzul commented on the detection of Marteilia cochillia in Spain; this parasite was firstly detected in Catalonia and was also detected in one ria in Galicia where its detection was associated with important cockles mortality. They detected it again in another ria of Galicia, also associated with cockles mortality. R. Aranguren Ruiz confirmed this comment and added that this parasite was also detected in cockles in another ria but this time, no cockle mortality was observed. In the first affected Galician ria, the cockle production collapsed and now, the production level is very low.

Slovenia. *M. Gombač*, R. *Sitar*, V. *Jenčič*

Slovene Sea is part of the Gulf of Trieste in the northernmost end of The Adriatic Sea. Along 46.6 km long coast there are three mollusc breeding locations. The average water temperature is 15, 8 °C (0 - 30 °C), average salinity 37ppt (20 - 38 ppt) and the average oxygenation 7.5 mg O₂/l (6.9 - 11 mg O₂/l). The deepest point of the sea is 37 m and there is constant in flow of sweet water. A great amount of nutritive substances, borne by rivers, make it a rich home for numerous sea animals and plants (Rejec Brancelj, 2003). One hundred and six taxons of molluscs and 38 taxons of bivalves, including over 40 species, live naturally in the Slovene Sea (Lipej, 2004), but only Mediterranean mussels (*Mytilus galloprovincialis*) and recently introduced clams (*Venus verrucosa*) are farmed. All together there are 22 mollusc farms on three locations i.e., Seča, Strunjan and Debeli rtič. In 2012 the production was 438700 kg of Mediterranean mussels. Mollusc farms are monthly visited and inspected by veterinary service to determine possible increased mortality. In year 2013 no mortality was detected and no sampling was performed.

References


Croatia. S. Zrnčić

S. Zrnčić* & D. Oraić

During 2013. year totally 2705 tons of bivalve molluscs were cultivated and about 230 tons were collected along the Croatian coast. Main cultivated species are flat oysters (*Ostrea edulis*) and mussels (*Mytilus galloprovincialis*) which are raised in 135 farms. From the natural beds fishermen are collecting flat oysters, mussels, clams (*Venerupis decussatus*), cockles (*Cardium edule*), variegated scallop (*Chlamys varia*), scallop (*Pecten jacobeus*), wart venus (*Venus verucosa*) and Noah arch (*Navicula noah*). Farms are subjected to National surveillance programme which is organised in accordance with EU directive (2006/88/EC). There are 19 sampling points out of which 6 is in the Zone I (northern Adriatic), 7 in Zone II (middle Adriatic) and 6 in Zone III (southern Adriatic). Process of authorisation is still ongoing but 106 out of 135 farms are authorized. Sampling of oysters for detection of bonamiosis is performed from beginning of the April to June while samples of oysters and mussels are collecting from the end of August to October. Samples are analysed by means of cytology and PCR to the presence of *Bonamia ostreae* and *Marteilia refringens*. Positive samples are subjected to histological examination and sequencing. Categorization of mollusc farming areas reveals that all farming area with oyster cultivation (10) are category I concerning presence of *Bonamia* sp. Contrary, there are 19 mussels cultivation area and 16 of them are category V (infected) while 3 are category III.

Montenegro. B. Adzic

In Montenegro there is 16 farms of mediteranian mussels (*Mytilus galloprovincialis*). The intensification of mussels production started at the end of the last century. In 2013. total production of mussels was about 200 tones. All farms are situated in the Boka kotorska bay, which is the only fjord in Southern Europe. Three years ago we started with production of european oyster (*Ostrea edulis*). For now there is just one oyster farm, which is located in Kotorski zaliv (Kotor bay). We still do not have exact data of oysters production, but some estimates indicate that production is about 3 tones. First time last year Montenegro started with National surveillance programme on presence of *Bonamia ostreae* and *Marteilia refringens*. National referent laboratory is Diagnostic Veterinary Laboratory in Podgorica. Montenegro has carried zoning and the zones are Kotorsko-risanski bay and Tivatski bay. We had several sampling points for mussels collection and one for oysters collection. Sampling of oysters were conducted in April, and sampling of mussels in the autumn (September-October). Samples are analysed by means of cytology, histology and PCR to the presence of *Bonamia ostreae* and *Marteilia refringens*. During the surveillance we did not find positive case, but in the followed-up research, that Diagnostic Veterinary Laboratory has done for its needs, we found *Marteilia refringens* in 2% of sampled mussels at two sampling points. There was not any notification of abnormal mortality in 2013.

I. Arzul asked if there is trade between Croatia and Montenegro. B. Adzic answered that some mollusc exchanges occur.
Lithuania. D. Simkus

Lithuania – country in Northern Europe and has the coast of Baltic Sea (90.66 km). During 2013 years in Lithuania weren’t bivalve molluscs farming experience.

During 2013 years to Lithuania were imported certificated Gasteropoda (slug molluscs)/12.950 tons and otra (other) molluscs/37.19 tons from European Union. From Lithuania were exported certificated Gasteropoda (slug molluscs)/298.695 tons. Mollusc productions weren’t movement from/to the third world countries.

In National Food and Veterinary Risk Assessment Institute of Lithuania are used accredited microbiological tests - 2073/2005, 1441/2007 for mollusc's pathogens (E. Coli, Salmonella, Listeria monocytogenes) detection. During 2013 years weren’t test the samples for self and official control. Diagnostic techniques for other mollusc pathogens detection aren’t still used.

M. Engelsma asked which gastropod species are cultivated in Lithuania. D. Simkus answered there is no gastropod production, these animals are harvested from the wild and he did not know the English name.

Iceland. A. Kristmundsson

Mollusc farming in Iceland is a relatively new industry. Consequently, most of it is still at experimental basis. The total production in the year 2013 was less than 100 tn., the largest part being blue mussel, Mytilus edulis, produced at six different sites around Iceland. In addition there is some small scale farming of ocean quahog, Arctica islandica, as well as an experimental rearing of Japanese abalone, Haliotis discus, and Pacific oysters, Crassostrea gigas. The experimental farming of abalones has been practiced for several years and animals been regularly imported from both Japan and Ireland. The first import of Pacific oysters was in 2013.

Unlike mollusc farming, the catching of wild mollusc has a long history, the most valuable species being, Iceland scallop (Chlamys islandica), ocean quahog (Arctica islandica) and common whelk (Buccinum undatum).

Due to the novelty of mollusc farming in Iceland, no surveillance and monitoring system has yet been established. However, no notifiable parasites have been detected in a limited number of blue mussels using histological methods. No diseases or abnormal mortalities have been detected in any of the above mentioned species, except in the wild stock of Iceland scallop and abalone, Haliotis discus. An apicomplexan infection in the wild stock of Iceland scallops¹, which caused a total collapse in the stock in the early 2000’s, has been monitored for the last 12 years. Apparently, the stock is recovering and some experimental fishing has recently been allowed. In the abalone farming, an unidentified amoeba-like parasite has been causing problems and this case is presently being examined.


I. Arzul asked if the amoeba-organisms were detected in other countries. A. Kristmundsson said that these organisms were not reported in scallops in other country before.
E. Munro wondered if sequencing was performed on these amoeba parasites. A. Kristmundsson explained that amoeba constitutes a very wide and diverse group and it is very difficult to identify them. Amoeba taxonomy is very complex.

France. C. Garcia

C. Garcia *, Cyrille François, Coralie Lupo, Isabelle Arzul, Bruno Chollet, Christine Dubreuil, Delphine Serpin, Jean-Pierre Joly, Emmanuelle Omnes, Marie-Agnès Travers, Delphine Tourbiez, Philippe Haffner

The French mollusc production is mainly represented by Pacific cupped oyster Crassostrea gigas (82800 t) and mussels, Mytilus edulis and M. galloprovincialis (73900 t) [professional data, http://www.cnc-france.com/]. Since 2008, the oyster production has decreased due to massive spat mortalities and in another way, the mussel production has increased.

In France, the process of authorization of farms is on process of validation; the authorization will be given by mollusc farming areas and not by farms. For the moment, 69 mollusc farming areas are defined by mutual agreement between shellfish farmers and competent authority. Competent authority has planned to establish all the authorizations at the end of 2014. Concerning the categorization of the mollusc farming areas, France is officially not declared free but not known to be infected (category 3).

Considering diagnosis and quality management, the French National Reference Laboratory (NRL) is accredited by COFRAC (French accreditation body) for cytology and histology diagnostic tests (Perkinsus sp., Mikrocytos sp., Bonamia sp., Marteilia sp.) in accordance with EN ISO/IEC 17025. All other diagnostic tests realized in NRL (bacteriology, molecular analyses…) are under quality management. In France, the NRL coordinates a network of 9 official laboratories and 5 recognised laboratories. These laboratories perform all the analyses concerning OsHV-1 and Vibrio diagnostic on oysters. These different laboratories work under quality management and some of them are accredited for molecular analyses. The NRL performs all other analyses. In 2014, another laboratory network will be implemented and will perform the histological analyses for all molluscs. In 2012, the French surveillance system (network Repamo) was evaluated and it was noted it was necessary to improve certain axis of this network. One of these axes was to better define the network objectives. This work was done in 2013 and the new objective of the network was established as “Improving early detection of exotic or emergent shellfish diseases/infections of shellfish in France”.

Until 2012, 3 axes existed in French surveillance: 1- surveillance of bonamiosis and marteiliosis, 2- study of increased mortality and 3- surveillance of some other important pathogens.

In 2013, with this new objective, two complementary approaches were defined: one is an unspecific surveillance (= increased mortality events => passive surveillance) and the second one is based on a targeted surveillance (= listed diseases => planed/active surveillance).

In 2013, no active surveillance of bonamiosis and marteiliosis was performed, nevertheless in case of mortality events of susceptible species of these infections, sampling and diagnostic tests were realized. Regarding OsHV-1 µVar, no early detection program was submitted.

In 2013, surveillance only focused on the study of abnormal mortality. Surveillance strategy is based on notification of observed mortality by shellfish farmers to the Competent Authority. Currently, it is an individual investigation of abnormal mortality with samples collection for laboratory tests and a partial epidemiological survey. This strategy is going to change and to move towards a collective risk-assessment for a risk-based investigation in order to maximize chances of case detection and resource allocation.

As previous years, important increased mortality events occurred in most oyster producing areas in France, mainly in Spring-Summer. Mortalities mainly affected 6-18 month old Crassostrea gigas but adult outbreaks was also observed in summer. In oyster spat outbreaks, OsHV-1 virus was
frequently detected whereas, in adult outbreaks, the bacteria *Vibrio aesturianus* was detected in all adult cases and in some spat samples. Other mortality outbreaks occurred in 2013 and concerned mussels *Mytilus edulis*, wedge clam *Donax trunculus* and Manila clams *Ruditapes philippinarum*. In samples of *Donax trunculus*, a protozoan of the genus of *Mikrocytos* was detected as in the previous outbreaks. For 2014, France will remain its effort on mortality events of mollusc with a specific study to compare individual analyses and group analyses in some areas. It is planned to develop a method to estimate introduction and establishment risk of a new disease and also to define the surveillance strategy for diseases present in France.

**Turkey.**

*Turkish representative could not attend the meeting but sent a summary as well as the epidemiological report of their situation regarding mollusc diseases in 2013.*

The Turkish National Reference Laboratory (NRL) is accredited for cytological and histological diagnosis of some mollusc pathogens (Bonamia spp, Marteilia sp., Mikrocytos sp. Perkinsus spp.) by TURKAK, Turkish Accreditation Body, in accordance with TS EN ISO/IEC 17025. Data related to mollusc production in 2013 have not been officially published yet. *Tapes decussatus* production has occurred 292 tons. As no production took place in 2013, surveillance studies of *Bonamia* spp. and *Marteilia* sp. in spring and autumn couldn’t be carried out in flat oysters, *Ostrea edulis*, and mussels, *Mytilus galloprovincialis*. Diagnostic methods used for screening of *Perkinsus* spp. included culture and histology. Sampling in carpet shell clams (*Ruditapes decussatus*) was carried out from 2 stations in Izmir provincial zone in August. *Perkinsus* spp. was detected in all of 60 carpet clams sampled from 2 stations, with the prevalence of 100 %. No abnormal mortality was reported. Depending on the production, surveillance studies will be carried out in March and September for *Bonamia* spp. and in September for *Marteilia refringens* in flat oysters and mussels, and in August for *Perkinsus olseni/atlanticus* in carpet shell clams. Molecular methods such as PCR-RFLP and in situ hybridization will be used for confirmatory diagnosis of *Bonamia* and *Marteilia* spp.
ANNUAL MEETING SESSION II : VIBRIOS, WHERE ARE WE?

(Chairperson: A. Travers; Secretary : C. Lupo)

Vibrio aesturianus in Ireland. D. Cheslett

*V. aesturianus*, a marine bacterium, has been implicated in oyster mortality events for a number of years. Following the mass mortality events in oysters in France in 2008, *V. aesturianus* was implicated as part of a multifactorial process in which the pathogens OsHV-1 µVar and *V. splendidus* were also cited in conjunction with the unusual environmental conditions present at the time. The role of *V. aesturianus* in the mortalities affecting juveniles and spat which have occurred every summer since 2008 has never been fully elucidated. However, since the summer of 2012, *V. aesturianus* has been more implicitly linked to mortality events in adults and half-grown oysters in France. Whether a more virulent strain of *V. aesturianus* has emerged or whether the oysters have become more susceptible possibly due to prior exposure to OsHV-1, or because their increased tolerance to OsHV-1µVar since it’s emergence in 2008 has resulted in a loss of resistance to *V. aesturianus* is at present under debate.

The reports from France and the reliance on French stock by the Irish industry led to an increase in testing for *V. aesturianus* in Ireland in late 2012 and 2013. In late 2012 it was identified in oysters submitted from 2 sites which were tested retrospectively following reports from France. In 2013, a more targeted approach was taken with samples collected from any sites for which mortality reports did not fit with the classical pattern expected for OsHV-1µVar being subjected to testing for *V. aesturianus*. In previous years testing had focused on culturing *V. aesturianus* and testing any candidate colonies using a real time PCR assay. In 2013, taking a lead from IFREMER, a second approach has been followed. This involves direct screening of whole genomic DNA extracts from affected oysters. Where possible both methods have been used together so that the presence of viable bacteria can be demonstrated. Detection of the bacterium is significantly higher using DNA extracts for screening, suggesting that either this method is detecting non-viable bacteria at a significant level and / or that culture is underestimating its prevalence.

*V. aesturianus* was identified in numerous samples taken in 2013 but no pathology consistent with infection with *V. aesturianus* was observed. A retrospective study was therefore undertaken on samples collected between 2008 and 2012 with a view to gaining a better understanding of the distribution and the role of *V. aesturianus* in Ireland.
I. Arzul asked about movements between UK and Ireland. M. Gubbins answered that a same batch has been analyzed in UK and Ireland the first time the event had been detected. This was detected approximately at the same time in both hatcheries in June. It should be interesting to have a deeper look inside.

Mike Gubbins asked if this infection was rather acute or chronic. A. Travers explained that it depends on the seawater temperature, but that a wide range of temperature allows the development of the disease.

**Occurrence of Vibrio splendidus and other closely related Vibrio species in different bivalves in Italy. G. Caburlotto**

For several years *Vibrio aestuarianus* has been associated with summer mortalities affecting the production of the pacific cupped oyster (*Crassostrea gigas*) worldwide. Also *Vibrio splendidus* seems to be responsible of summer mortalities but its role is not clear. The present controversial status of *V. splendidus*, pathogenic or opportunistic, seems to persist above for other species of bivalves for which a few data exist. Moreover the high genetic variability within the clade *V. splendidus* and the very closely related *Vibrio* species makes difficult to define which one could be responsible of bivalves mortalities. For a better understanding of the ecology of these species a better range of bivalves should be considered.

**State of art in NRL of Italy.** From 2011 up to summer 2013 our Laboratory has analysed the presence of *Vibrio splendidus* and *Vibrio aestuarianus* in different bivalves such as *Ruditapes philippinarum*, *Mytilus galloprovincialis*, *Callista chione*, *Chamaelea gallina* consistently produced in the North Adriatic Sea and *C. gigas* harvested in different hatcheries of Italy. Samples were collected during monitoring programs and mass mortalities by the Local Veterinary Service. *V. splendidus* was isolated from the different bivalves. In specific, we observed the presence of *V. splendidus* through all the year, even at very low water temperature, during monitoring program and mass mortalities. Concerning oysters, the amount of *V. splendidus* differed in adult, larval and post larval stages. It’s interesting to observe that other *Vibrio* species not belonging to the clade *V. splendidus*, for example *V. tubiashii* or *V. neptunius* were isolated from samples and it would be interesting to investigate on their role.

In that context the lacking of complete reports of environmental parameters have made difficult to comprehend the ecology and the role of all these bacteria.

**Future perspectives.** Our aim is to develop rapid and accurate diagnostic tools to discriminate among members of *V. splendidus* clade and other related species. Moreover, for a better understanding of their ecology aimed to prevent bivalve mortalities, a complete reports of environmental parameters in the context of sampling may be contemplated.

B. Morga asked details about the host in which the two *Vibrio aestuarianus* were identified. G. Caburlotto answered that both *V. aestuarianus* were detected in the mussel *Mytilus galloprovincialis*. B. Morga added that the Maldi-TOF tool enables identifying only the bacteria that can be cultured, not all the bacteria present in the environment.

I. Arzul asked if different bacterial communities were observed between different mollusc species, especially if they live in different parts of the environment. G. Caburlotto answered that with the current number of available strains, it was not possible to compare, but the number of samples will be increased within two other years in the same geographic area. A. Travers added that the current identification tool for *V. splendidus* only allows identifying a large clade, thus it is not possible to obtain a detailed picture of this bacteria communities.

A. Travers asked if other NLRs used the MALDI-TOF tool. Three of them are currently using it. This is based on ribosomal protein and 16S, which are not very discriminatory. I. Arzul asked whether sharing of databases to be recommended. M. Engelsma precised that exchange of spectra
between Sweden, the Netherlands and Denmark was already scheduled to improve their own datasets concerning fish. Everyone agreed that speaking of this point for molluscs was also of interest.

*Vibrio aestuarianus* and Pacific oysters, *Crassostrea gigas* mortality in France: a new chapter in their relation. C. Garcia


The French network for the surveillance and monitoring of mollusc health (Repamo) ensures the surveillance of shellfish health status along French coasts according to the obligations of the European Directive 2006/088/EC, notably the monitoring and investigations of mollusc increased mortality.

In 2008, important mortality events associated with the detection of a new variant of the virus OsHV-1 were reported in Pacific oyster spat in France during spring and summer. In 2011-2012, a new mortality phenomenon was revealed by the Repamo network but this time, affecting adult Pacific oysters and apparently associated with the bacteria *Vibrio aestuarianus*. *V. aestuarianus* was detected for the first time in France in 2001 during *Crassostrea gigas* adult mortalities. Since this date, it has regularly been detected by the network during oyster mortalities in different French areas; it was detected in around 10-20% mortality cases and generally in oyster adults. Since 2011 and 2012, the detection frequency in analysed samples has increased from 29% in 2011 to 60% in 2012, reaching 77% in 2013. Moreover, in 2013, it can also be frequently detected in oyster spat. Considering the detection of *Vibrio aestuarianus* in the context of *Crassostrea gigas* mortality in France, Repamo data analysis was performed in order to evaluate the potential involvement of the bacteria in mortality events investigated in France since these last 10 years and to try to identify factors associated with these mortality outbreaks. Since 2012, according to the network data, it was noted a significant increase of its detection. It is usually detected when mortality rates are below 30% and mortality typology seems more progressive, less sudden on the field in comparison with OsHV-1 mortality. All age groups were found positive to *V. aestuarianus* but adults are more often found positive than juveniles and spat. It is significantly more detected in summer and it is also significantly more detected in adults during warm period and in juveniles in cold period. *V. aestuarianus* was detected in all French oyster production areas.

Different hypotheses have been advanced to explain this new phenomenon and notably the existence of a new genotype/strain of *V. aestuarianus*. To test this hypothesis, experimental infections were performed in oysters to compare the *V. aestuarianus* strains of 2001 and 2012. Preliminary results did not show any evidence of the apparition of a new strain. Monitoring towards *V. aestuarianus* will be maintained and other hypotheses will be explored.

K. Roenningen asked if pathological changes were observed in most of the cases of detection and if there was an association between the CT values and the reported mortality rates. C. Garcia answered that CT values were available at an individual level but not analyzed yet. She precised that defining thresholds that have biological significance should be of interest and that comparison of CT values with the number of strains identified in conventional bacteriology will be developed.

S. Zrnčič asked if the area of Poitou-Charentes has specificities. C. Garcia recalled the sampling bias of the study and explained that most of the French oyster stock is produced in this area. S. Zrnčič asked whether a low prevalence would decrease the pressure on mollusc farms. C. Garcia explained that there is too few data in France to answer without great uncertainty.

L. Madsen asked whether *V. aestuarianus* was primary or opportunistic. C. Garcia answered that this pathogen was a primary cause of infection.
B. Morga asked if Poitou-Charentes area was defined by specific environmental conditions. C. Garcia answered that this hypothesis was already tested in her analyses but no specific effect was found. F. Batista asked if the pathogen would be sensitive to the seawater salinity. C. Garcia explained that salinity was not really contrasted in France. F. Batista asked whether the poor growth of the bacteria on TCBS could be explained by the sensitivity of this bacteria to salinity. A. Travers explained that V. aestuarianus does not grow whatever is the salinity of the TCBS, thus there are difficulties to isolate this pathogen on TCBS.

Identification of Vibrio tubiashii subsp. Francisca Nov,a pathogen of bivalves and characterization of its extracellular products. M.A. Travers

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Since 2007, under the auspices of the French institutional surveillance network, REPAMO “REseau de PAthologie des MOLLusques, dominant bacterial isolates were obtained from different batches of Crassostrea gigas juveniles and adults experiencing high mortality rates in a French experimental hatchery/nursery. The aim of this work was (i) to fine-tune the taxonomic affiliation of those bacterial isolates, previously clustered with V. harveyi [1], (ii) to evaluate isolates virulence and toxicity of the extracellular products (ECPs) from a representative strain (07/118 T2 = LMG 27884T), and (iii) to characterize, in vitro and biochemically the major virulence secreted factors playing a potential role in the pathogenicity of this new subspecies of V.tubiashii. Based on multilocus sequence analysis (MLSA), DNA-DNA hybridization and phenotypic traits, bacterial isolates were classified as a novel subspecies: V. tubiashii subsp. francensis. The virulence of isolates (60 to 70% of mortality induced on C. gigas larvae and juveniles) and the toxicity of 07/118 T2 active ECPs (40 to 41% of mortality induced on C. gigas larvae and juveniles) were proved by experimental infection. Moreover, 07/118 T2 ECPs revealed their ability to inhibit C. gigas hemocytes- adhesion capacity and phagocytosis activity probably through the degradation of matrix structural proteins. Enzymatic activities and predominant proteins present in active ECPs were identified by biochemical analyses and mass spectrometry. This led to the characterization of zinc-dependent metalloenzymes-like classified under thermolysines family.


I. Arzul asked if QPCR was directly used on DNA extraction from samples. A. Travers said that the already published QPCR tool was not specific and that it could also detect V. coralliilyticus and V. neptunus, but the new one developed in the lab appeared specific and was used to directly screen stored DNA extract. It was possible to identify strains from USA, UK and France.
I. Arzul asked whether it would be possible to transfer this tool to other NLRs once published, and if mortalities associated with V. tubiashii detection were reported in other Member States. M. Gubbins recalled that in UK there was no routine screening for Vibrios in hatcheries but monitoring can quickly be implemented if recurrent mortalities occur, as in 2005. In France, V. tubiashii was detected in 14 batches in 2008 but this pathogen has never been detected since that time. In USA, this is considered as a re-emergent pathogen.

I. Arzul asked if V. tubiashii detected in environment in the Italian study were all pathogenic. G. Arcangeli answered that in some cases of larvae mortality in hatcheries, Vibrio spp. were isolated but there was no further characterization.

G. Caburlotto asked if an horizontal genetic transfer of the region near the gene coding for metalloprotease has been described. A. Travers was not aware of evidence that this gene may be transferred. However she specified that many proteins are linked to toxicity in a same species and the metalloprotease was not the only one.
ANNUAL MEETING SESSION III : EMERGING/LISTED DISEASES

(Chairperson: C. Garcia; Secretary : I. Arzul)

_Vibrio aestuarianus_ : a good candidate to be listed? C. Lupo and A. Travers

Increased mortality events are reported in France on adults of Pacific oysters, *Crassostrea gigas*, since 2001. In 2012, an apparent increase of these mortality events was observed, in association with the detection of _Vibrio aestuarianus_. As early detection of exotic or emerging diseases and rapid mitigation response are crucial in shellfish populations to prevent their spread and establishment in shellfish populations, a risk assessment of the spread of this pathogen is needed to inform if further measures are necessary, e.g. listing the infection.

The Council Directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals provides a number of criteria for listing non-exotic diseases (Annex IV, part I.B). These criteria were reviewed in the light of recent knowledge about _Vibrio aestuarianus_, the characteristics of the shellfish infection, the available diagnostic tools and the mitigation measures. Whereas some criteria for listing were met, information is still lacking to inform all the Directive’s criteria and more research is needed.

In perspective, more general questions were raised, notably as regards the optimal timing for sharing such information were raised (when it is still emergent or once it has emerged?), which is to be balanced with the trade implications for free compartments. The European Commission will soon ask the European Food Safety Agency for an updated scientific opinion on the increased mortality events in Pacific oysters, including a question on the importance of _Vibrio aestuarianus_. Thus, the listing of this pathogen is worth to be further evaluated.

_K. Roenningen would like to know how virulence of bacterial strain was defined. A. Travers explained that a bacterial strain was considered as virulent as soon as mortality was observed after experimental infection with low doses of bacteria (200 bacteria / animal) in C. gigas oysters._

_L. Madsen wanted to have details about the experimental conditions used to estimate if a strain is pathogenic or not. A. Travers said that different protocols were compared: (1) injection with low doses of bacteria (2) immersion in contaminated water: oysters were first injected with bacteria and then removed from the water. Subsequently naïve oysters were immersed in this water containing about 10^5 bacteria/ml for 24 hours and finally transferred in clean water._

_A. Travers added that in the context of Bivalife project, 10 sites were investigated regarding the presence of _V. aestuarianus_ : _V. aestuarianus_ was not detected in 5 sites, only non-pathogenic strains were detected in 3 sites and pathogenic ones were detected in 2 sites._

_M. Engelsma said that outside mortality events, it is difficult to detect _V. aestuarianus_ and thus it is difficult to conclude that a compartment is free of the bacteria._

_D. Cheslett added that the approach used in Bivalife project was first isolation and then identification. A. Travers explained that in this project there was a bias because the samples consisted of spat and not adult. However, at least it was possible to identify free and infected zones. She added that QPCR detects both pathogenic and non-pathogenic strains and does not allow differentiation between both._

_K. Roenningen had few comments : listing a disease is not an easy task, it aims at establishing legal basis to control and eventually eradicate a disease without going against the principle of easy trade. Thus almost all the criteria must be fulfilled. The impact of the disease must be demonstrated. From his point of view, considering the information currently available, _V. aestuarianus_ could be a
good candidate to be listed and the question is raised in the EFSA mandate. However he reminded that it is not only a scientific issue it is also political.

I. Arzul wondered if we could imagine having it listed as an emerging disease like it is done for some diseases by the OIE in order to share information without blocking trades.

Detection of a new species of *Mikrocytos* in *Pacific oysters* (*Crassostrea gigas*). D. Stone

In May 2013, a population of farmed Pacific oysters (*Crassostrea gigas*), held in at an intertidal shellfish farm on the North Norfolk coast was reported to be suffering from a mortality event, with up to 40% of the animals dead or dying. Animals had been exposed to a protracted period of cold weather, estimated to be below 10°C for up to three months. Affected animals were gaping with limited intra-valvular fluid. Affected individuals were considered to be thin and watery with obvious green pustules associated predominately with the surface of the mantle tissue and adductor muscle. Histopathology and electron microscopy results were consistent with an infection with the microcell, *Mikrocytosis mackini*

Investigations indicated similar mortalities at two other locations close to in index site. An initial designation (ID) was placed on all three sites restricting movements other than for direct human consumption and these were subject to normal hygiene regulations. No movements of oyster for aquaculture purposes was permitted

DNA was extracted from the mantle and adductor muscle of infected oysters for molecular analysis. A 980 nucleotide partial SSU sequence generated from a PCR amplicon produced using the mikrocytos generic primers shared only 79% nucleotide identity with *Mikrocytos mackini* (HM563060.1) suggesting a new species of mikrocytos. Phylogentic analysis using neighbour-joining methods placed the partial SSU sequence on a discrete branch within the same lineage as *Mikrocytos mackini* (HM563060.1) and two other mikrocytos species (DQ237912 and HM563061). The histopathology and molecular diagnostic results will be discussed.

A. Kristmundsson wondered if various forms of these micro parasites were observed. D. Stone said that no other stages was described until now.

Garcia asked about the temperature of the water in the location where the parasite was detected. D. Stone said that the water temperature was 6°C for several weeks. Oysters were located in an intertidal zone meaning that temperature could be very cold in winter time.

I. Arzul added that the sequence from *M. Mimicus* was different from the Mikrocytos found in *Donax trunculus* in France and in *Ruditapes philippinarum* in The Netherlands suggesting that they are different Mikrocytos species.

What can Limfjorden tell us about the limiting factors for *Bonamia ostreae* in northern Europe? L. Madsen

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Limfjorden in Denmark is recognized as a unique production area for European flat oysters, *Ostrea edulis*, being an inlet (fjord) that covers 1500 km² (depth between very shallow down to 28 m), as well as being connected to the North Sea in the west (salinity 3.2-3.4 %) and Kattegat in the east (salinity 1.9-2.5 %), net water flow going from west to east mainly due to the tide. Up to 1980, the (at that time) low stock of native oysters was supplemented with relaying of foreign flat oysters for fishing for human consumption. In 1980, macroscopic changes similar to those caused by *Bonamia ostreae* were observed in relayed French oysters, and the diagnosis was verified by histology. Attempts were made to clean the site by fishing up the whole batch of French oysters. During the 1980’s, Denmark experienced winters with extremely low water temperatures classified as “ice winters”, where the water temperatures stayed below 0 °C for up to 11 weeks. In the 1990’s, the native stock of flat oysters increased and a surveillance program for *B. ostreae* and *Marteilia refringens* was initiated. Neither of the parasites have until now been found. The introduction of *Bonamia*-infected oysters to other countries (e.g. the Netherlands) has had serious consequences for the cultivation of flat oysters, but this does not seem to have been the case in Denmark. From 1998 to 2012 *B. ostreae* has persisted in eastern waters of the USA (Maine). Here water temperatures have also been below 0 °C in some years, but only during one year the temperatures stayed below 0 °C for two weeks, where during other four years, the period with water temperatures below 0 °C was between 1 and 7 days. It is suggested that the elimination of *B. ostreae* in Danish waters is due to the extreme climatic conditions like ice winters, either by direct elimination of the parasite or by elimination of the host, the relayed French oysters (foreign oysters die as they are not adapted to such low temperatures like native oysters), thus preventing the spread of *Bonamia* parasites to the naïve flat oyster population in Limfjorden.


F. Batista asked about the salinity in the location where flat oysters are cultivated. L. Madsen said that variation of salinity ranged between 1.9 and 3.4% in the Limfjorden (see the above abstract). B. Chollet wondered if these oysters are genetically different from flat oysters in other European locations. L. Madsen told that Ifremer had had samples from Danish flat oysters in 2005 for genetic analyses. S. Lapegue explained that studies had shown that by using a set of markers developed in the laboratory LGPMM, S. Lapegue and co-workers identified six different groups of European flat oysters, among which one group from Northern Europe included flat oysters from Denmark.

*Mikrocytos mackini* exercice: introduction and establishment spatial risk assessment. C. Lupo

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As classical control measures are of limited use in marine open systems, successful control of diseases is very unlikely once established in shellfish populations. Thus, early detection of exotic or emerging diseases and rapid mitigation response are crucial. However, exotic disease introduction or emerging disease appearance are rare events, thus surveillance should account for maximizing the chances of detection. Risk-based surveillance is an interesting approach to focus surveillance on increased risk areas or time periods for such diseases introduction or establishment. In addition,
RBS can address the issue of resource allocation. The aim of the developed approach is to inform the design of a general RBS system for shellfish diseases in France, using spatial risk assessment tools for a spatial decision support system. Therefore this study is conducted to design an example approach on an exotic disease and to further assess its generalization to other diseases of interest. Its aim is to identify suitable areas for the introduction and the establishment of *Mikrocytos mackini* infection in the main oyster production area in France (Charente-Maritime bay). *Mikrocytos mackini* infection was chosen because this parasite of the Pacific oyster *Crassostrea gigas* represents a hazard to global oyster production in northern temperate regions. In addition, several recent detections of *Mikrocytos* sp. were reported in clams and *Donax* in Europe, and particularly a new *Mikrocytos mimicus* was described in Pacific oysters in UK.

GIS-based multi-criteria decision analysis (MCDA) will be used to define a set of weighted rules based on existing published or expert knowledge. The modeling process includes a number of steps:

1. Defining the objective of the modeling exercise;
2. Identifying the risk factors and retrieving related spatial data;
3. Generating the suitability map by combining the risk factors to obtain a final weighted estimate of suitability for each spatial unit in the study area;
4. Implementing a sensitivity analysis to account for the uncertainty of sparse data notably.

*I. Arzul wanted to know if all these sets of data would be required to conduct a such approach in another location. C. Lupo answered that this study should allow identifying the minimum required data to evaluate the risk of introduction and establishment of Mikrocytos mackini.*

*A. Kristmundsson said that water temperature in Iceland seems to be suitable for M. mackini all the yearlong and this parasite could thus present a threat for Iceland.*
Mollusc health situation in Korea. *K. Park*

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Mollusks have been traditionally used in a variety of foods in Korea, and various mollusk species are mass produced through farming and fishing. These commercially valued marine mollusks include the Pacific oyster, Manila clam, abalone, mussel, and squid. According to 2012 statistics (KOSIS), 284,856 tonne of Pacific oyster, 12,626 tonnes of Manila clams, and 6,564 tonne of abalones were produced through farming, while 181,408 tonne of squids were produced through squid jigging fishery. Of these marine mollusks, seasonal mass mortality of Manila clams are observed during the spring and early fall seasons; *Vibrio tapetis*, *Perkinsus olseni*, and trematodes have been detected as the main pathogens. Besides the pathogens, there is a sharp increase in the incidence of sediment hardening in the habitats of Japanese mud shrimps (*Upogebia major*) in Manila clam farms, which interferes with clam burrowing. Partial mortality events in Pacific oysters occur during the summer months, which rarely lead to mass mortality events, with *Marteilioides chungmuensis*, *Marteilia* sp., and *Gymnophalloides seoi* as the main known pathogens. An outbreak of the pathogen *M. chungmuensis* occurred in the fall of 2013. *G. seoi*, a zoonotic disease, has been detected only in wild Pacific oysters, but not in the farmed ones. In farmed abalones, the shell-perforating *Polydora* sp., which causes growth impediment, decreased market value, and mortality, was detected with an infection rate of 5-99% in 2012. *Anisakis* sp. was detected in squids, with an infection rate of 9.57% on the eastern coast, 72.37% on the southern coast, and 90.76% in the Yellow Sea (western coast) in 2010. While the production of mollusks in Korea shows a continued increase, sharp mortality-induced fluctuations in the annual yield have been observed, except in Pacific oysters. Pathogen infection, high-density farming, aging farms, and marine pollution have been identified as the causes of these fluctuations; various efforts by the government are under way to alleviate these problem.

*I. Arzul asked if there are many movements between Korea and other countries. K-I Park answered that most of transfers consist of movements of manila clams which are imported from China and exported to Japan. I. Arzul was surprised that herpesvirus was not detected during C. gigas mortality. K-I Park answered that it has already been detected during mortality events. I. Arzul wanted to know if there is a surveillance programme regarding abalone viral disease. K-I Park answered that he did not know.*
Research development on *Perkinsus olseni* in Korea. K. Park

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Perkinsosis is a major disease that affects commercially important marine mollusks globally, including oysters, abalone, clams, scallops, cockles, and mussels. Here, we report and discuss the spatial and temporal distribution, morphological features, and pathogenicity of *Perkinsus olseni* parasitizing Manila clams (*Ruditapes philippinarum*) distributed along the coasts of Korea. Infection prevalence of *P. olseni* ranged from 23% to 100% in Manila clams collected from 21 of 27 sites along Korea’s coasts during late 1990's. Average infection intensity at each sampling site varied from 12 to 3,924,309 *Perkinsus* cells per individual or 6 to 873,000 cells per gram tissue. More recent national wide survey (2010) showed that clams in all investigated site (28 sites) were infected with the pathogen and infection intensity increased greatly. Infection intensity and prevalence were high among the clams collected from commercial clam beds located on tidal flats along the west and south coasts of the country. In contrast, clams collected from sand beaches on the east coast were not infected by *P. olseni*. These results suggest that *Perkinsus* infection might be influenced by host density and sediment type. Biflagellated zoospores of the pathogen are ellipsoidal in shape (3.72 × 2.04 μm) with 2 flagella each; the anterior flagellum is 14.03 ± 1.03 μm long and has a unilateral array of mastigonemes, while the posterior flagellum is 6.96 ± 0.79 μm long with no mastigonemes. Liberated zoospores gradually transform into immature trophozoites by shedding the anterior flagella first and internalizing the posterior flagella later; deflagellation occurs at the distal end of the flagellar transition zone. Following deflagellation, the posterior flagellum gradually coils around the body of the zoospore and gets incorporated into the zoospore body. Transformation of zoospores into trophozoites takes approximately 2 weeks at 26°C. Mature trophozoites undergo schizogony by cleaving the cell, and a number of merozoites develop inside schizonts. Merozoites are finally released by rupture of the cellular membrane of the schizont within a week. High levels of *P. olseni* infection interfere with spawning frequency and reduce egg production, which might result in long-term impacts on clam recruitment and population growth. In addition, *P. olseni* infection also stimulates NO production, COX-2 activity, and allograft inflammatory factor (AIF-1) expression in Manila clams, suggesting that this pathogen induces an inflammatory response in the host clams. We believe that *P. olseni* is highly virulent and results in reduced reproduction in the Manila clam. Thus, mass mortalities of Manila clams observed in some major clam beds in Korea are partly associated with high levels of *P. olseni* infection.

G. Arcangeli said that in Italy they observe a similar situation. Last year, they observed mass mortalities of clams that could be explained by the farming techniques. In this case, Perkinsus olseni appeared more aggressive, and manila clam were less “resistant”.

G. Arcangeli wondered if different biotypes/strains of Perkinsus could explain this apparent difference of pathogenicity. K-I Park answered that they have not yet investigated this hypothesis.

I. Arzul added that in USA, it was shown that different strains of Perkinsus marinus can induce different level of mortalities.

K-I Park added that food availabitility seems to influence the intensity of manila clam mortalities in Korea : “When there is more food, there is less mortality caused by *P. olseni*”.

S. Bergmann wanted to have more details about the antibody used to investigate interactions between Perkinsus and the host. K-I Park explained that they used a polyclonal antibody produced in rabbit.
B. morga asked about the type of lectin recognized by the antibody. K-I Park answered that the antibody recognizes a C-Type and calcium dependent lectin.

S. Bergmann wondered if they measured lectin activities in not infected haemocytes. K-I Park explained that lectin activities was estimated by western blot and haemocytes agglutination test in infected and not infected cells.

I. Arzul wondered if in France or Italy, gross lesions including nodules have previously been reported in clams infected with perkinsus. G. Arcangeli answered that they have already reported some nodules in case of mass mortalities. C. Garcia said that in France it was not common. B. Chollet added that it has only been reported in Mediterranean Sea. Level of infection is not the same between both coasts (Mediterranean and Atlantic coasts), the level of infection is usually higher in South of France.
OsHV-1 diversity. T. Renault

OsHV-1 has been associated with mortality outbreaks in different bivalve species including the Pacific cupped oyster, *Crassostrea gigas*. Since 2008, massive mortality outbreaks are reported among *C. gigas* in several farming areas in Europe in relation to the detection of a newly described OsHV-1 variant called µVar. Although the reference type (a viral specimen collected in France in 1995 during a mortality event affecting *C. gigas* larvae, GenBank accession number AY509253) and the variant µVar were detected in association with mortality outbreaks in 2008 in France, detection of OsHV-1 since 2009 concerns mainly the variant µVar. These results raise questions about the emergence and the virulence of the variant µVar. In this context, different approaches including partial sequencing, microsatellite genotyping and complete genome sequencing have been used to better describe OsHV-1 diversity.

Determination of nucleotide sequences of PCR-amplified virus DNA fragments is the most accurate method for virus genotyping. The DNA sequencing approach has been used to characterize OsHV-1 specimens and virus variants were thus reported. The variant µVar showed several differences in two genome areas when compared with the reference type (GenBank accession n° AY509523) and all these differences need to be observed to define a viral specimen as the variant µVar. However, virus DNA sequencing is time-consuming in the high-scale format. The identification and genotyping of highly polymorphic microsatellite areas from vertebrate herpesviruses appears as a suitable approach. The variant µVar demonstrated a deletion of 12 bp in a microsatellite zone located up-stream of the ORF4.

A study targeting 3 different areas of the viral genome was carried out. Seventy-two OsHV-1 “specimens” collected mainly in France over an 18 year period, from 1993 to 2010 were analyzed. Additional samples were also collected in Ireland, USA, China, Japan and New Zealand. Three virus genome regions (ORF4, ORFs 35/36/37/38 and ORFs 42/43) were selected for PCR analysis and sequencing. Although ORF4 appeared as the most polymorphic genome area, distinguishing several genogroups, ORFs 35/36/37/38 and ORFs 42/43 also showed variations useful in grouping subpopulations of this virus.

During the course of Bivalife project, virus positive DNA samples collected from oysters or mussels in 2011 and 2012 (France, Ireland, Spain, the Netherlands) were selected in order to be determined regarding genotype status, namely the variant µVar or different variants, using sequencing with PCR products obtained from the 3 virus genome areas previously selected. Moreover, OsHV-1 positive samples collected in other countries (Australia, Brazil, China, Ireland, Japan, Korea, Mexico, Morocco, Netherlands, New Zealand, Spain, Sweden (*Ostrea edulis*), Tunisia, UK, and USA) were also analyzed. French specimens collected from 2008 to 2013 and all samples collected in other Members States (Ireland, Portugal, The Netherlands, Spain and UK) grouped together. The sequence of the variant µVar deposited in GenBank (accession n° HQ842610) was included in this group. It also integrated samples from New Zealand, Brazil and Korea. Although the C2/C6 fragment sequence for these specimens (New Zealand, Brazil and Korea) was quite similar to the sequence deposited in GenBank under the accession number HQ842610 (µVar), some differences were reported. A large deletion (605 bp) was reported for some PCR products obtained with the primer pair Del 36-37F2/Del 36-37R. The large deletion was observed for some French samples collected in 2008, all French samples collected after 2008, all samples collected in other Members States (Ireland, Portugal, The Netherlands, Spain and UK). This deletion was also noticed.
for samples collected in the USA, New Zealand and Tunisia.

A genotyping approach was developed for OsHV-1 specimens based on microsatellites. Microsatellites have been reported from different herpesviruses including human cytomegalovirus and used as molecular markers to define virus polymorphism.

In this context, during the course of the Aquagenet project (SOE2/P1/E287), 47 clinical OsHV-1 specimens were characterized targeting ORF 4 and its related upstream zone. DNA sequencing and genotyping of a microsatellite locus were performed. The results obtained with both techniques were compared and appeared to be equally useful to differentiate clinical OsHV-1 specimens. Genotyping based on a microsatellite locus appeared as a powerful tool to study OsHV-1 polymorphism and can offer a first level of discrimination between specimens in order to select best candidates for complete genome sequencing.

During the course of the Bivalife project, the work was pursued developing the genotyping approach targeting 6 microsatellite loci and a collection of 263 samples. Genotyping French samples using the 6 microsatellites identified 2 groups of samples (i) the first one containing samples collected in 1994/1995/1996 and in 2008/2009/2010 and (ii) a second one containing samples collected in 2005/2006 and 2007. Genotyping all samples using the 6 microsatellites identified 3 groups (i) one containing samples from Korea, New Zealand and Australia, (ii) samples collected in Europe and (iii) USA and Sweden. Genotyping samples considered as μVar or close related variants (microvariants) using the 6 microsatellites identified 3 groups (i) samples from New Zealand and Australia,( ii) samples collected in Europe and (iii) samples from Korea. The genotyping approach using the 6 microsatellites showed that all the samples from the collection collected since 2009 in Europe grouped together. Although based on partial sequencing the samples collected in New Zealand and in Australia have been previously identified as variants close from the variant μVar, genotyping using the 6 markers demonstrated a distinct group containing these samples.

G. Caburlotto asked details about the geographical distribution of the microvariants. T. Renault answered that some parts AUS and NZ specimen were very close to microvar and some other parts were very different. This is the first information. NZ and Australian specimen were not directly related to the European specimen. The hypothesis would be that close variants would have emerged at different places in the world at the same time.

K. Roenningen asked details about the microvariants isolated (mortalities, pathogenicity, environment...). T. Renault answered that NZ and AUS specimens were clearly isolated during massive mortalities whereas the Japanese specimen were not associated with mortality. More information is clearly needed, to see if different level of virulence may be related to different parts of the viral genome.

F. Batista asked if specimen before 1993 were analyzed. T. Renault answered that the first observed mortality associated with OsHV-1 in France was in 1992 and no specimen is available before this year. In NZ, specimens are available before mortality outbreaks in 2010. It was possible to demonstrate the presence of OsHV-1 in UK samples of Ostrea edulis (histological blocks) from the 1970s. Thus, other species could be infected and this raises the question of transfer between species. Next step will be to explore if the virus diversity is lower in Crassostrea gigas samples where no mortality occurs than in samples during mortality.

S. Bergmann asked about the complete sequencing of microvar and its similarity degree with the OsHV-1 referent. T. Renault said that deletions are observed in microvar, which is unusual to cause higher pathogenicity (for example vaccination is usually possible by removing virulence gene). Considering that most of herpesviruses can present latency status, S. Bergmann asked if genes involved in latency could be identified in OsHV-1 genome. T. Renault answered that this depends on herpesvirus species. About the latency, current data is scarce about involved genes. Maybe, speaking about persistence is more appropriate than latency. In addition, different oyster families
have shown different level of susceptibility and levels of replication, suggesting that oysters can manage the infection.

I. Arzul wondered if the recently described acute viral necrobiotic virus (AVNV) in Chlamys farreri in China is similar to OsHV-1. T. Renault answered that based on available data these two viruses are close together and could belong to the same viral species whereas the herpesvirus infecting abalone is clearly different.

Continuing investigations into the source of the OsHV-1 variants in the UK. D. Stone

The UK reported significant mortalities associated with OsHV-1 µVar in Whitstable Bay in the Thames estuary in July 2010, in the Blackwater Estuary in July 2012 and in Poole harbour in October 2013.

A molecular epidemiological investigation was undertaken following the outbreak in Poole harbour. All samples were identical in sequence and exhibited the 12bp deletion associated with the OsHV-1 µVar. However, there was a single nucleotide difference (Inserted G) when compared to the samples from Whitstable and Blackwater and reference OsHV-1 µVar sequence (accession no. HQ842610), but 100% nucleotide identity with Ostreid herpesvirus 1 isolate 002 from France in 2010 (accession no. JN800120). The initial results suggest that the outbreaks are not directly linked and the outbreak at Poole represents a separate introduction.

Further investigation on samples taken from the site on a second visit revealed three distinct OsHV-1 sequences. OsHV-1 µVar with an additional G insertion which shared 100% match to the µVar detected on the site previously, and 100% match with the sequence of a French sample from 2010. A wild type OsHV-1 with two additional AGT repeats compared to the reference wild type virus, and a wild type OsHV-1 with a single additional AGT repeat. Sequence data has been obtained for another two regions of the virus genome (ORF32 and between ORF49 and 50) for viruses originating from the original outbreak at Whitstable Bay, the Blackwater Estuary and Poole harbour and together with other wild type virus detected in some of the older stock. Six distinct genotypes of OsHV-1 were identified; three closely related to the reference OsHV-1 µVar and three more closely related to the reference wild type OsHV-1.

The results of the molecular epidemiological investigation, particularly the evidence for three separate introductions of the OsHV-1 µVar into the UK will be discussed.

G. Caburlotto wanted to know why Taqman assay was used for confirmation. D. Stone explained that when they tested these samples, they were concurrently participating in the Inter Laboratory Comparison organized by the EURL and thus took the opportunity to test these samples with the LNA taqman assay.

S. Bergman wondered how sequences were generated. D. Stone explained that sequences were consensus from duplicate. S. Bergman also asked how samples were tested. D. Stone said that samples were processed individually.

T. Renault wondered how fragments of the genome were selected. D. Stone answered that on 14 initially selected locations, 3 were finally interesting for this study.

F. Batista wanted to know from how many samples the 6 identified genotypes were found. D. Stone said that three sites appeared positive. From each site, 30 animals were tested by PCR and in total, 5-6 animals were used for sequencing.
I. Arzul wondered if these variants were detected in the context of mortality of oysters. D. Stone said that in this case, the detection of variants was done outside mortality event.
I. Arzul also asked if several genotypes could be identified from the same oyster. D. Stone answered that sequences were clear suggesting that only one genotype was found per oyster.

A summary of the OsHV-1µVar Surveillance in England and Wales 2009-2013
M. Gubbins

Following reports of large scale mortalities in farmed Pacific oysters in Europe in 2008 associated with a newly identified variant of ostreid herpesvirus, OsHV-1µVar, an initial survey of high risk sites was undertaken in England and Wales during 2009. No positives were found, and with the introduction of EC controls on the disease in 2010 a more comprehensive surveillance programme covering all farmed sites was implemented. After two years surveillance the UK applied for, and was granted, controls under Article 43 of 2006/88/EC for the entire coast except for two areas on the south east coast. In 2013 a third area was found positive on the south coast, and this was removed from the area under Article 43 measures.

114 sample sites were covered in the programme, from which a total of nearly 12,000 individual PCR tests were carried out. 8 tests from 3 areas were positive for OsHV-1µVar, and a further 7 tests identified other OsHV-1 variants.

The surveillance testing continues on a targeted basis, with monitoring around the positive areas to demonstrate effectiveness of the disease controls, and continued investigations of reported and observed mortality events.

I. Arzul wondered about the global cost of the program carried out in UK regarding OsV-1 µVar. M. Gubbins said that it was not estimated. T. Renault suggested to speak about reference type instead of wild type.
I. Arzul asked about the measures applied in case of detection of the reference type. M. Gubbins explained that there is no restriction in place in this situation. However, he said that there are not so many transfers of oysters in UK, it is thus easy to implement transfer restriction if necessary. T. Renault added that these locations are interesting to investigate OsHV-1 diversity.

OsHV-1µVar situation In Ireland. D. Cheslett

In the summer of 2008, severe mortality outbreaks were reported from all French production areas as well as three sites in Ireland. The outbreaks affected principally spat (oysters less than 1 year old) and juveniles (12 to 18 months old). Mortality was described as acute, with mortality rates of up to 100% being reported. Similar mortalities were again observed in 2009 in all French coastal regions as well as in Jersey and 16 sites in Ireland. Since 2009, there have been mortality events every summer in France and Ireland as well as in a number of other European Countries. The mortality has been associated with the emergence of a new strain of ostreid herpes virus (OsHV-1) termed OsHV-1µVar, which is characterised on the basis of partial sequence data exhibiting a systematic deletion of 12 base pairs in ORF 4 of the genome in comparison with OsHV-1 (GenBank # AY509253). Outbreaks are believed to be associated with an increase in the water temperature above 16°C.

In 2010, Ireland entered into a surveillance programme for the virus in those bays that had remained free of the virus in 2008 & 2009. At the beginning of the Surveillance Programme there were 25 surveillance sites in 8 compartments and 18 bays considered to be infected. During the course of 2010, a further 6 bays were added to the list of infected areas and removed from the Surveillance
Programme. The number of bays considered to be infected has increased annually and outbreaks of mortality have occurred in many of the infected sites whenever the water temperature has exceeded $16^\circ$C. By the end of 2013, the number of infected bays had increased to 32 and there are 11 bays remaining in the surveillance programme.

The sources of new infections have been investigated and, where possible, the most likely route of infection identified. These include imports of infected stock prior to 2008 where episodes of mortality prior to 2010 had gone unreported, proximity to depuration units processing stock from infected bays and bio-security failures on site. In four cases, however it has not been possible to identify any source of infection.

G. Arcangeli wondered who paid for this program. D. Cheslett said that it was paid by the government.

I. Arzul asked if only OsHV-1 μvar was detected. D. Cheslett explained that the reference type was detected once.

I. Arzul also asked if farmers were generally happy with the situation. D. Cheslett answered that some farmers would like to keep their free status regarding infection with OsHV-1 μvar whereas other farmers would prefer being allowed to import spat from zones known to be infected including France.

EFSA Mandate. K. Roenningen

Due to the increased mortality events in pacific oysters (*Crassostrea gigas*) in 2008 and 2009 the Commission asked EFSA for a scientific opinion. Based on this opinion the Commission laid down legislative measures to control the disease, currently expressed in Commission Decision 2010/221/EU approving national measures for limiting the impact of certain diseases in aquaculture animals and wild aquatic animals, Article 3a (OsHV-1μVar). These measures are, however, limited in time and a decision on further regime for eventual listing, surveillance programmes and trade restrictions must be done on the basis of a comprehensive scientific evaluation of the current situation and new knowledge and experience gained on this disease since 2010. In that context the Commission has asked EFSA for a new scientific opinion with the following terms of reference:

1. To update the previous EFSA's opinion with the latest scientific evidence on OsHV-1 μVar and possible other OsHV-1 microvariants associated with mortality events in Pacific oysters. This update should include an evaluation of the importance of other microvariants of the OsHV-1 detected in Australia, New Zealand and Asia and whether previous conclusions and recommendations are still valid.

2. Given the recent mortality events of adult *Crassostrea gigas* associated with *Vibrio aestuarianus* in France, EFSA is requested to evaluate the role of this agent. In case the assessment indicates a causative role of *Vibrio aestuarianus* in the mortality events, control measures should be recommended.

3. An evaluation of the effectiveness of current methods of water treatment in shellfish depuration plants in inactivating OsHV-1 and *V. aestuarianus*, and if appropriate give recommendations for other treatments.

4. To briefly review the feasibility, availability and effectiveness of the disease prevention and control measures laid down in current Union legislation as regards infection of oysters with OsHV-1 μVar In that respect, an assessment of the necessity of specific measures to prevent the introduction of other micro-variants of OsHV-1 into the Union should be done.
T. Renault had a comment regarding Vibrio aestuarianus: as it is an environmental bacteria, it seems difficult to list it. K. Roenningen explained that indeed, the possibility to list V. aestuarianus was discussed the day before and as soon as we would have a technique allowing to differentiate between virulent and non virulent strains, it could become a good candidate to be listed. T. Renault also asked about the eventuality to list specific variants versus the whole species. K. Roenningen recalled that regulation targets diseases and not pathogens. I. Arzul asked about the situation for fish virus: are the species or the types listed? K. Roenningen confirmed that serotypes/ genotypes are listed in that case.
ANNUAL MEETING SESSION VI : NEWS FROM THE BENCH

(Chairperson: C. Garcia; Secretary : B. Morga)

Application of high-throughput sequencing to the detection of regions of the cupped oyster genome involved in the resistance to spat summer mortality. S. Lapègue

S. Lapègue¹; F. Cornette¹, S. Heurtebise¹, E. Flahauw¹, M-T Auge², N. Bierne², L. Dégremont¹, P.A. Gagnaire²

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During the last years, several breeding programs have been initiated in France at the professional scale to improve survival of oysters to face the dramatic impact of diseases to which resistance has been shown to be heritable. The detection of QTLs (Quantitative Trait Loci) that are portions of the genome associated with the genetic variability of traits, is therefore crucial to understand the genetic architecture of those traits and potentially useful to assist selection.

We conducted a QTL mapping study in the Pacific oyster, Crassostrea gigas, facing heavy summer mortality associated with the virus OsHV-1 since 2008. We produced several F2 families segregating for survival and viral DNA load and performed experimental challenges on the foreshore or in raceways. We increased the number of markers available by first developing a medium throughput genotyping tool of 384 SNPs with the Golden Gate Illumina technology, then a high throughput tool of several tens of thousands of SNPs with the RAD-sequencing approach. Therefore we tremendously increased the resolution of existing genetic maps. New QTL analyses allowed confirming the location of some previously detected QTLs, showed their significance and interest for breeding. Furthermore additional QTLs were also detected both within a narrow genome window. This now allows a further investigation of those areas with the help of the whole oyster genome sequenced in 2012 in order to look for potential links with candidate genes that could be functionally related.

F. Batista wanted to know how much of the QTL explain the survival. S. Lapègue answered that more than 50% of the variance was explained for the survival regarding OsHV-1 infection. Similar QTLs were found during different studies carried out different years which supports that these QTLs are interesting.

I. Arzul asked if selection assisted by markers could realistically be used for molluscs. S. Lapègue said that she was optimistic considering that farmers are presently developing their own breeding programs. She added that the laboratory has developed and transferred a panel of markers that can be used for genotyping. Recently, farmers expressed their interest in including some of these markers in their programs.

C. Garcia wondered if this approach had already been used in other production. S. Lapègue answered that it is more often used when one gene is highly linked to one trait.
Health surveillance of mollusc populations in the Dutch Wadden Sea. M. Engelsma

Marc Engelsma, Betty van Gelderen, Ineke Roozenburg, Michal Voorbergen-Laarman

Central Veterinary Institute of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands

The Wadden Sea is an intertidal area stretching from the Netherlands and Germany to Denmark. The region is typified by extensive tidal mud flats and tidal creeks between the mainland and a string of islands. In 2009, the Dutch and German parts of the Wadden Sea were inscribed on UNESCO's World Heritage List. The Wadden Sea is characterized by its flora and fauna, especially birds, and popular for recreational activities. Although commercial mussel beds are present in the Dutch Wadden Sea, samples are not routinely taken for investigation of the health status of the mollusc population. In the Netherlands the focus of the annual surveillance for mollusc diseases is on the mollusc culture areas in the south-west of the Netherlands, in concordance with the potential risk of introduction of pathogens in these areas, from transfers of molluscs for aquaculture purposes. In the Wadden Sea, a number of studies have focused on the presence and the effects of parasites, in particular trematodes, on mollusc communities. However, much less is known on protozoan species associated with molluscs the Wadden Sea. In order to assess the health status of mollusc populations in the Wadden Sea, seven of the most abundant mollusc species were collected from different locations throughout the Dutch Wadden Sea in 2012 and 2013: blue mussels (*Mytilus edulis*), Pacific oysters (*Crassostrea gigas*), cockles (*Cerastoderma edule*), Baltic clams (*Macoma balthica*), peppery furrow shells (*Scrobicularia plana*), soft-shell clams (*Mya arenaria*) and American razor clams (*Ensis directus*). Histopathological and molecular analyses were used to screen for a wide range of pathogens and pathological conditions. The results of the histopathological analyses showed the presence of a large number of protozoan species associated with molluscs the Wadden Sea. Most prominent findings by histology were the observation of *Steinhausia* sp. in Baltic clams and the high prevalence of disseminated neoplasia in a batch of Baltic clams from a single location. Mussels and Pacific oysters were screened by real time PCR for the parasite *Marteilia refringens* and the bacterium *Nocardia crassostreae*, respectively. Both pathogens were not detected in the samples.

M. Gubbins wondered if signs of mortality or clinical signs were noticed in cockles. M. Engelsma said no.

C. Garcia asked if culture in thioglycollate was used to detect *Perkinsus* spp. in *Ruditapes*, *Macoma* or *Scrobicularia* species. M. Engelsma said no.

I. Arzul asked if this study would be continued for several years. M. Engelsma explained that they presently do not have money to extend this study. However, they would like including the Wadden Sea in the national monitoring program for mollusk diseases.

Modulation of apoptosis by stress factors including the protozoan parasite *Bonamia ostreae* in the flat oyster, *Ostrea edulis*. O. Gervais

Ophélie Gervais, Tristan Renault, Bruno Chollet and Isabelle Arzul

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Apoptosis is considered as a defense mechanism against stress factors including pathogens. In particular, studies performed on molluscs have shown that apoptosis takes an important place in protection against protozoan parasites. In previous works, flat oysters *Ostrea edulis* resistant to
bonamiosis showed an over-expression of some genes involved in apoptosis pathway suggesting that defense against the parasite partly relies on this process. However, intracellular pathogens such as *Bonamia ostreae* might modulate host apoptosis in order to favor their survival by ensuring against the death of the target cells, in this case haemocytes.

The apoptotic response of haemocytes from flat oysters was investigated after exposure to various stress factors like UV, dexamethasone and *B. ostreae*. Cellular modifications including phosphatidyl serine externalisation, intracytoplasmic calcium concentration, mitochondrial membrane potential and caspase activities were measured by flow cytometry. DNA fragmentation was evaluated by light microscopy using the TUNNEL assay. Lastly, ultrastructural modifications were observed by transmission electron microscopy.

Contrary to dexamethasone which did not significantly influenced the apoptotic response in haemocytes, exposure to UV and *B. ostreae* generally increased the different tested cellular activities. However, difference in the magnitude of the apoptotic response was noticed between haemocytes of flat oysters originating from zones displaying different status regarding bonamiosis. Our results show that apoptosis is an important mechanism developed by the flat oyster against stress and more particularly against bonamiosis, and that this mechanism seems to be dependent on the history and/or genetic of the oysters.
Laboratory accreditation has in recent years become an important process for many testing laboratories. The ability to demonstrate that results are consistent, reliable and repeatable as well as providing laboratories with better traceability has been a great driving force in laboratories pursuing accreditation despite the costs and demand on resources involved.

For many laboratories in the EURL-NRL network for fish and shellfish diseases the introduction of 2006/88/EC was instrumental in driving the process of accreditation forward. The majority of NRLs for Molluscan Diseases now hold accreditation for test methods used in the screening for the non-exotic diseases listed in 2006/88/EC. However, there seems to be little consistency across the NRL network regarding accreditation for either the exotic diseases or non-listed diseases, particularly in relation to molecular diagnostic methods.

S. Zrnčić underlined a mistake concerning this presentation. She specified that the Croatia NRL is not accredited but works under quality management.

I. Arzul wondered if each accredited NRL has an audit each year. All NRL members confirmed this point. Each year, there is an audit in order to maintain their accreditation.

I. Arzul also wanted to know if some accredited NRL has a flexible scope. D. Cheslett explained that in Ireland, it was not possible to have a flexible scope for molecular methods when they first began accrediting these methods as the accreditation body did not allow this. In UK, M. Gubbins said that their laboratory has a flexible scope. S. Bergmann explained that in Germany, all analyses performed in their laboratory are accredited but he did not know which scope is for each accredited analyses.

2013-ILC-01 test comparison study for the detection of Bonamia sp. by PCR.

I. Arzul

I. Arzul*, C. Dubreuil and C. Garcia

Infections with Bonamia ostreae and B. exitiosa are present in Europe and listed by the OIE and the European Commission. Diagnosis of bonamiosis relies on histo-cyto-pathology and more and more on PCR. Molecular tools are recommended as screening, presumptive and conformational techniques by the OIE. However, about twelve different PCR assays are currently available to detect Bonamia parasites. Most of these tools amplify 18S fragment while few of them are species specific and allow discrimination between B. ostreae and B. exitiosa. In this context, in 2013, the EURL organized an Inter Laboratory Comparison (ILC) test for the detection of Bonamia spp. by PCR. In addition, NRLs were invited to participate in a comparison study aiming at evaluating PCR assays for the detection of these parasites.

The ILC test consisted of 24 samples including 8 negatives, 8 B. ostreae and 8 B. exitiosa infected samples. Pools of gills suspensions fixed in ethanol were prepared from flat oysters previously characterized. These pools were aliquoted in order to prepare between 2 and 5 replicates per pool for each participant. Reference results were determined based on prior characterization results and
on results from analysis of one set of samples. Beginning of December 2013, the ILC was announced to NRLs. Registration was done through an electronic form and samples were sent mid December by express – mail. Participants were asked to send their results through an electronic form before the 3rd of February 2014. In total 17 participants registered and performed the test 2013-ILC-01. Most of participants had 100% of good answers or no more than 2 errors.

Less than half of participants in the 2013-ILC-01 also agreed participating in the comparison study. Considering that they mostly tested the Sybergreen assays developed by Ramilo et al. in 2013 for the detection of B. ostreae and B. exitiosa, comparison analysis focused on these assays and the Bo Boas PCR used for the ILC test. Relative specificity and sensitivity were better for the sybergreen assays. Higher agreement level and consistency were also obtained with the real time PCR assays compared to the conventional one. Comparison study is an interesting approach to evaluate performances of diagnosis techniques and finally to select tools to be recommended within the NRLs network.

I. Arzul presented the results of the interlaboratories study comparing different PCR techniques (conventional and real time PCR). Most laboratories tested the real time PCR developed by Ramilo et al. 2013. The Spanish laboratory has developed two real time PCR using SYBR®Green chemistry, one specific to B. ostreae and another one specific to B. exitiosa. The result of interlaboratories study concerning these two real time PCR were good and I. Arzul wanted to know if NRLs members would agree to recommend the use of these real time PCR assays as alternative methods to detect Bonamia parasites. NRLs did not express any objection against this suggestion. I. Arzul underlined that some laboratories prefer using real time PCR with TaqMan® chemistry but at this time, no such technique is available to distinguish both Bonamia species. The EURL is currently working on the development of multiplex Taqman assay to detect these two Bonamia species.

I. Arzul proposed to include real time PCR assays developed by Ramilo et al. (2013) in the first version of the European diagnostic manual for the detection of Bonamia parasites. Participants agreed with this proposition.

Working prospects of the European Reference Laboratory for mollusc diseases and concluding remarks. I.Arzul

In 2013, the network of National Reference laboratories (NRLs) for mollusc diseases included 23 laboratories. Among its different duties, the EURL has to collect data on mollusc disease situation within the EU. For that purpose an annual questionnaire is sent before and then discussed during the annual meeting. These data are included in the report of the meeting and are also available on the EURL website under the “NRLs’ section”.

The collection of reference material is also an important task of the EURL. It includes histological slides, paraffin blocks, suspensions of DNA, infected fixed tissue, bacterial strains. This material can be provided to laboratories in Europe and in other countries on request and depending on the availability of the material. Request should be, when possible, done through an electronic form available on the eurl website. In 2013, the EURL answered 26 requests of material. To facilitate access to histological material, the EURL has recently aquired a new software, Mscope which enables access to scanned histological slides through the EURL website. This tool can be used by NRLs, it includes all the previous ring test slides and a set of reference ones. The software also allows self evaluation.

The EURL has to assist Member states in the context of disease outbreaks. In 2013, the EURL answered 6 requests of assistance including double reading of histological slides, confirmatory diagnosis of infections with Marteilia refringens, Bonamia spp, Perkinsus spp.
Every year, the EURL organises interlaboratory comparison tests (ILC). In 2013, an ILC was organised and concerned the detection of *Bonamia* sp. by PCR. In 2014, the EURL will organize a new ILC based on histopathology.

The EURL is involved in the development and validation of methods for the detection and identification of listed pathogens. During these last years, the EURL has been involved in the development of Taqman assays. One Taqman multiplex assay was developed in order to detect and type *Marteilia refringens* and a new assay is currently under development for the detection of *B. ostreae* and *B. exitiosa*.

The EURL carries out various studies especially on endemic listed diseases. In 2013, investigations on *Bonamia ostreae* and congeneric species included the identification of new genes by RNAseq, the determination of routes of entry and release of the parasite by *in situ* hybridization. Investigations on *Marteilia refringens* focused on its life cycle and molecular diversity in different locations in Europe. In 2013 the EURL presented results from these different studies at international conferences (EAFP conference, Tampere, Finland) and was invited to participate at a workshop on Mollusc diagnosis in Geelong, Australia.

Lastly, the EURL provides opportunities for training. In 2013, 9 colleagues visited the laboratory for training on mollusc diseases. In addition, the EURL organised a technical workshop after the annual meeting. This workshop included one session on the detection of *Bonamia* sp. and *Marteilia refringens* by *in situ* hybridization and one session on the detection of mollusc pathogen by histopathology.

The next Annual meeting will be organised together with a technical workshop in March 2015 and should take place around La Tremblade. It is proposed that the technical workshop includes a session on the isolation and characterization of *Vibrio aestuarianus*. NRLs are invited to submit their suggestions for the annual meeting as soon as possible.

I. Arzul presented the working program of the EURL for 2014 and announced that there will be soon a histological ring test. This ring test will contain fewer slides than the previous ones (30 slides instead of 60). These slides will only include histological slides and not tissue imprint slides. In addition, I. Arzul proposed NRLs to have a ring test including tissue imprints slides (between 10 or 15 slides) using Mscope. I. Arzul asked NRLs if this proposition suited them. All NRL members agreed with this proposition. I. Arzul suggested reducing the time to perform the ring test because the slide number is lower than usual. She proposed two days to read the ring test. Most NRL members said that two days is too short to read 30 slides because in their laboratory, several people read the ring test slides. The ring test is also used by the NRLs to qualify their staff. After discussion, it was agreed that the time to read ring test slides would remain as usual, five days. S. Bergmann asked if the EURL could organize ring test for other laboratories than NRL. I. Arzul answered that it is difficult to include too many additional laboratories in the loop because the duration of a ring test is already long (=one year). Meanwhile, each NRL can organize this own ring test for other laboratories. The EURL can provide some slides or blocks to the NRLs if necessary.
Tuesday 25th of March  Nantes (Ifremer facilities)

SESSION I  DIAGNOSIS AND SURVEY OF MOLLUSC DISEASES
Chairperson: I. Arzul; Secretary: C. Garcia

9h00-12h30  Representatives from each Member State present the disease situation in their respective country in 2013, emphasising mollusc diseases listed in the EU Directive 2006/088, and/or the diagnosis of pathogens of special interest

10h30–11h00  Coffee Break

SESSION II  VIBRIO : WHERE ARE WE?
Chairperson: A. Travers; Secretary: C. Lupo

12h30-13h00  V. aestuarianus in Ireland Deborah Cheslett

13h00-14h00  Lunch Break

14h00-14h30  Occurrence of V. splendidus in different bivalve species in Italy. G. Caburlotto

14h30-15h00  Vibrio aestuarianus and Pacific oyster in France: a review of 10 years of surveillance Céline Garcia

15h00-15h30  Should we be afraid of Vibrio tubiashii? Agnès travers

15h30-16h00  Coffee Break

SESSION III  EMERGING/LISTED DISEASES
Chairperson: C. Garcia; Secretary: I. Arzul

16h00-16h30  Vibrio aestuarianus: a good candidate to be listed? Coralie Lupo & Agnès Travers

16h30-17h00  Mikrocytos mimicus David Stone

17h00-17h30  What can the Limfjord tell us about limiting factors for Bonamia ostreae in Northern Europe Lone Madsen

17h30-18h00  Mikrocytos mackini exercice Coralie Lupo

20h00-23h00  Dinner Downtown
SESSION IV  MOLLUSC HEALTH SITUATION IN KOREA
*Chairperson: I. Arzul; Secretary: B. Morga*

9h00-9h30  Mollusc health situation in Korea  Sunny Park

9h30-10h00  Research development on *Perkinsus olseni* in Korea  Sunny Park

SESSION V  OsHV-1
*Chairperson: B. Morga; Secretary: C. Lupo*

10h00-10h30  OsHV-1 diversity  Tristan Renault

10h30– 11h00  Coffee Break

11h00-11h30  Variants OsHV-1 in UK  David Stone

11h30-12h00  OsHV-1 situation UK and Ireland  Mike Gubbins & Deborah Cheslett

12h00-12h30  EFSA Mandate  Knut Roenningen

SESSION VI  NEWS FROM THE BENCH
*Chairperson: C. Garcia; Secretary: A. Travers*

12h30-13h00  Application of high-throughput sequencing to the detection of regions of the cupped oyster genome involved in the resistance to spat summer mortality.  Sylvie Lapègue

13h00– 14h00  Lunch Break

14h00-14-30  Survey carried out on parasites in mollusc populations in the Dutch Wadden Sea.  Marc Engelsma

14h30-15h00  Flat oyster, bonamiosis and apoptosis.  Ophélie Gervais

15h00-15h30  Coffee Break

SESSION VII  EURL daily life
*Chairperson: B. Morga; Secretary: C. Garcia*

15h30-16h00  QA status within the NRL network  Deborah Cheslett

16h00-16h30  Results from last ILC  Isabelle Arzul

16h30-17h00  Working programme and perspectives  Isabelle Arzul

*End of the Annual meeting*
**Annex B: List of Participants**

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<td>National Veterinary Service 15A Pencho Slaveikov blvd., 1606 Sofia Bulgaria</td>
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</tbody>
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Annex C : Evaluation of the Annual Meeting of NRLs for Mollusc Diseases 2014

A satisfaction survey form was distributed to the 39 participants in the Annual Meeting of NRLs for Mollusc Diseases. 24 responses were received from participants working for :

<table>
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<th>Your professional activity</th>
<th>Number of responses</th>
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<td>Administration</td>
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<td>Research Laboratory</td>
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<tr>
<td>Other</td>
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Level of satisfaction was expressed using a figure between 1 and 5 (1= "not satisfied" and 5 ="very happy"). Results are presented in the following table. For each item, the most frequent response is in bold.

The most frequent response was always « Satisfied » or « Very Happy »

Number of responses by level of satisfaction for each item
1 = « not satisfied»; 2 = « lightly satisfied »; 3 = « no opinion »; 4 = « satisfied »; 5 = « very happy »

<table>
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<tr>
<th>Item</th>
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<td>Session VII : EURL daily life</td>
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<td>Global satisfaction</td>
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Globally, the 2014 Annual Meeting of NRLs for Mollusc Diseases was well evaluated as demonstrated by the responses « satisfied » and “very happy” from all the participants.

Several comments emphasize this positive evaluation:
- « very well organized with relevant subjects and presentations» (2 responses)
- « meeting was satisfying, good organization and interesting »

Participants were also asked to express their suggestions or specific wishes for the next meeting which will be combined with a technical workshop.

The following comments were received regarding the organization:
- “In session 1, each representative should consider presenting only news from his country and not the whole data (production, status, diagnostic…)”
- “Session 1 is difficult to follow all the morning long….”.
- “At the end of session 1, it would be interesting to have a summary presenting what’s new in EU compared to previous year”
- “In 2015: annual meeting in May”

The following suggestions were made for next year:
- Present examples of application of national controls, case study
- Share some methods and protocols for the detection of vibrios
- Consider other Vibrios (not only V. splendidus and V. aestuarianus in oyster mortality events)
- Workshop on quantitative real time PCR
- Improve knowledge of histological lesions associated with OsHV-1, Vibrios, Nocardia…
• Histology of Bonamia and Marteilia and diagnostic of vibrios in molluscs
• If the 2014 histo ring test is finished, going through slides and results
• Coccidia in molluscs
• Bacteriology techniques

This evaluation and suggestions will be useful to prepare the agenda of our next Annual Meeting and Technical Workshop.

Thanks for your participation and your time