



THE SURVEILLANCE AND CONTROL PROGRAM FOR BONAMIOSIS AND MARTEILIOSIS IN EUROPEAN FLAT OYSTERS, *OSTREA EDULIS*, IN NORWAY IN 2024



Title (English and Norwegian):

The surveillance and control program for bonamiosis and marteiliosis in European flat oysters, *Ostrea edulis*, in Norway in 2024

Overvåkings- og kontrollprogrammet for bonamiose og marteiliose i europeisk flatøsters, *Ostrea edulis*, i Norge i 2024

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Summary (English):

The surveillance program for the molluscan diseases bonamiosis and marteiliosis is carried out by the Institute of Marine Research according to a contract with the Norwegian Food Safety Authority. In 2024, flat oysters were sampled from three locations: an abandoned breed poll where oyster spat was previously produced and two fjord systems with larger populations of flat oysters. Samples were collected in May and October, to be able to detect *Bonamia* sp. and *Marteilia* sp. during the periods when the potential prevalence is highest. No abnormal mortality was observed during the surveillance. *Bonamia ostreae* / *B. exitiosa* and *Marteilia refringens* were not detected. *Marteilia refringens* Type O and M are now divided into two species: *Marteilia refringens* in flat oysters and *Marteilia pararefringens* in blue mussels. Blue mussels are therefore no longer considered susceptible hosts for *M. refringens*, but our research project shows that the related *M. pararefringens* is widespread in certain mussel populations along the South and West coasts of Norway. *M. pararefringens* can have a serious impact on blue mussel populations, and consideration should be given to listing the parasite on a national list of notifiable diseases. The process of obtaining and maintaining a disease-free status for marteiliosis and bonamiosis is discussed.

Summary (Norwegian):

Overvåkingsprogrammet for sykdommene bonamiose og marteiliose i flatøsters utføres av Havforskningsinstituttet på oppdrag fra Mattilsynet. Det ble i 2024 hentet flatøsters fra tre lokaliteter: en brakklagt poll hvor det tidligere ble produsert østersengel og to fjordsystemer med større forekomster av flatøsters. Prøvene ble samlet inn i mai og oktober, som er de periodene prevalensen av parasittene *Bonamia* spp. og *Marteilia* spp. er vist å være høyest i smittede bestander. Det ble ikke observert unormal dødelighet. *Bonamia ostreae* / *B. exitiosa* ble ikke påvist. *Marteilia refringens* ble ikke påvist. *Marteilia refringens* Type O og M er nå splittet i to arter: *Marteilia refringens* i flatøsters og *Marteilia pararefringens* i blåskjell. Blåskjell er derfor ikke lenger regnet som mottakelig vert for *M. refringens*, men vårt forskningsarbeid viser at den beslektede *M. pararefringens* er utbredt i enkelte bestander langs Sør- og Vestlandet. *M. pararefringens* kan ha alvorlig effekt på blåskjellbestandene og det bør vurderes om parasitten skal listeføres på nasjonal liste over meldepliktige sykdommer. Prosessen med å oppnå og vedlikeholde sykdomsfri status for marteiliose og bonamiose er diskutert.

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1 - Introduction

Production and harvest of mollusks in Norway

Norway has a small shellfish industry, which is relatively stable, with approximately 60 active mussel farms, a few flat oyster farms and four dispatch centres distributing farmed as well as wild caught shellfish. In 2024, the commercial production (from aquaculture + harvest) was approximately 1 200 tons of mussels, *Mytilus* spp., 7 tons of flat oysters, *Ostrea edulis*, and 8 tons of wild Pacific oysters, *Magallana (Crassostrea) gigas*. Around 400 tons of scallops, *Pecten maximus*, were harvested from wild beds. There was also a small harvest of clams and horse mussels collected by divers. One oyster poll (lagoon) produced flat oyster seed. Most of the production was kept for on-growth at the site, around 1000 spat were sold. There was no export.

Monitoring of wild oyster populations

The Institute of Marine Research (IMR) carries out a surveillance of the wild oyster stocks to describe the dynamics of the oyster populations and detect changes – including mortality events and establishment of the invasive Pacific oyster, *M. gigas*, in mussel and flat oyster habitats. Reports from the surveillance is proposed as a basis for management and to obtain a sustainable harvest. This activity is linked to the disease surveillance programme.

The status of bonamiosis and marteiliosis in European flat oysters, *Ostrea edulis*, and blue mussels, *Mytilus* spp. in Norway

The health status of Norwegian flat oysters has been studied since 1989 (Mortensen 1993). A surveillance program for bonamiosis and marteiliosis in flat oysters was initiated in 1995. Since 2015, the surveillance program has been carried out by IMR according to a contract with the Norwegian Food Safety Authority.

Bonamia ostreae, *Bonamia exitiosa* and *Marteilia refringens* have never been detected in flat oysters during the surveillance. After observation of microcells in haemocytes in flat oysters from Langesand in Agder County, southern Norway in 2008, samples were sent to the European Reference Laboratory for mollusc diseases (EURL). Samples from two oysters tested PCR-positive for *B. ostreae*. A containment zone was established. An almost continuous targeted sampling and analysis of several thousand oysters since 2009 has never confirmed *Bonamia* at this site. Although the microcells may be observed in the oysters, they only appear as single cells, with low intensity and low prevalence. There is no sign of pathogen propagation, no pathology associated with the observed cells and no abnormal mortality in the population (see previous reports and Mortensen *et al.* 2020). Based on the results we concluded that the observed microcells are not *B. ostreae*.

The entire coastline of Norway is a disease-free zone with regard to *B. ostreae* with the exception of the zone in Agder. We have initiated an increased surveillance with a three-year annual sampling of 150 oysters every spring and a bi-annual sampling of a commercially exploited flat oyster population in the same region to fulfil the criteria for the re-establishment of disease-free status in compliance with EU 2020/689. However, Langesand has been extensively sampled since 2009, with 100 – 150 oysters sampled per year since 2015, with the exception of the period 2020 to 2022 (see Mortensen *et al.* 2016 and Appendix). The present sampling plan includes targeted sampling in 2023, 2024 and 2025.

The entire coastline of Norway is a disease-free zone also with regard to *Marteilia refringens*, with the exception of the containment zone in the municipality of Bømlo in Vestland County, southern Norway. The containment area was established in 2017 due to detection of *M. refringens* Type M in blue mussels. This type is now classified as a distinct species, *Marteilia pararefringens*, infecting mussels only (Kerr *et al.* 2018, Bøggwald *et al.* 2025). *M. refringens* is regarded as specific for flat oysters.

Due to the de-listing of mussels as susceptible species for *M. refringens*, mussels were not sampled in 2023 and 2024. However, based on recommendations from EFTAs Surveillance Authority (ESA), a three-year annual sampling of 150 oysters is performed also in this containment zone, to ensure that the oysters are not infected by *Marteilia* spp. and to enable a lift of the restrictions. This sampling commenced in 2023 and will be completed in 2025.

Infections with *M. pararefringens* have been detected in Norwegian blue mussels and may cause disease. This report gives a brief overview of the present situation, including a summary of the work done on *M. pararefringens* in mussels.

2 - Material and methods

Surveillance plans (2023 - 2025) have been designed for obtaining and maintain the disease-free statuses for *Bonamia* spp. and *Marteilia refringens*, as defined in Regulation (EU) 2016/429 and Delegated Regulation (EU) 2020/689.

Sample size and frequency (time of year) has been changed during the surveillance period. Bi-annual sampling of 150 specimens in two of the targeted populations (Hafrsfjord and Innerøyen) was initiated in 2021. Annual sampling of 150 specimens over a three-year period in the containment area in Agder was conducted between 2015 and 2017 and started again in 2023 (see Appendix).

In 2024, oysters were sampled from a wild oyster population in an abandoned oyster poll at Aga, Bømlo, Vestland County (where *M. pararefringens* was first detected), one wild oyster population in Langesand, Agder and one cultivated wild population in Arnevik, Agder (see Figure 1 and Table 1).

Surveillance included an on-site survey, as the state of the population (density, reproduction, signs of mortality) are considered important meta-data. Collection of oysters was done by swimmers/divers (NRL staff) and transported cold and humid directly to laboratory and kept in quarantine lab until dissection.

Oysters were processed at the IMR laboratory in Bergen, according to standard methodology. The samples from Bømlo and Langesand, each consisting of 154 specimens, were analyzed using histology using dorso-ventral cross sections, fixed in Davidson's fixative, embedded in paraffin, sectioned at 3 μ m, stained with Hematoxylin Erythrosin Saffron (HES), mounted with a cover slip and observed at 100 to 1000 x magnification.

Histology aiming at the detection of *Bonamia* spp. and *Marteilia* spp. is accredited and was carried out according to NS-EN ISO / IEC 17025 (MET.HIS.02: Prosessering av skjell - påvisning vha histologi og PCR / Påvisning av parasitter i skjell, vha histo-cytopatologi).

The sample from Arnevik was split in two and analyzed with histology and Polymerase Chain Reaction analysis (PCR)(Table 1). Tissue from 11 oysters from Arnevik where single microcells were observed and 33 oysters from Langesand with *post mortem* alterations in tissues were forwarded to *Bonamia* PCR.

Samples for *Bonamia* spp. detection were dissected from gill tissue and fixed in ethanol. Two PCR methods were run on all samples. One described as in Cochenne *et al.* (2020) and the other method was from the protocol from EURL, *Bonamia ostreae* and *Bonamia exitiosa* detection by Taqman® Real Time Polymerase Chain Reaction, Edition n° 2 .

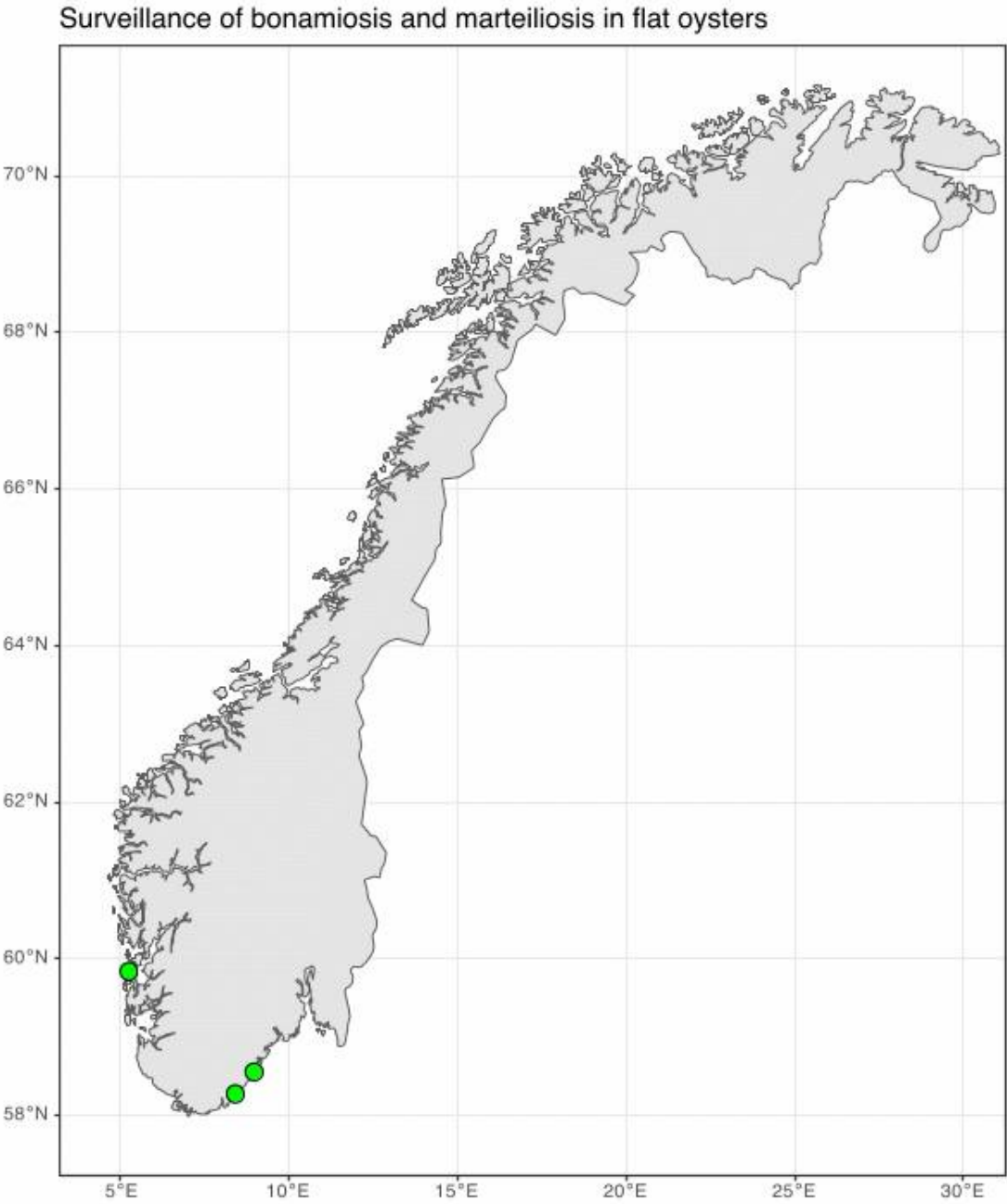


Figure 1. Sampling sites of flat oysters, *Ostrea edulis* in the surveillance program for *Bonamia* spp. and *Marteilia refringens* in 2024. See also Table 1.

Table 1. Sampling and surveillance of flat oysters (*Ostrea edulis*) in 2024. Sites are shown in Figure 1.

Sampling site	Sampling date	Sample size	Method
Agapollen, Vestland	16. October	154	Microscopy
Arnevik, Agder	26. May	154	75 obs by microscopy, 78 by <i>Bonamia</i> PCR
Langesand, Agder	27. May	152	Microscopy

3 - Results

No abnormal mortality was observed during the surveillance. *Bonamia ostreae* / *B. exitiosa* and *Marteilia refringens* were not detected. Results are briefly described below.

Sampling and examination of flat oysters

Langesand (58.5392, 8.9376) and the surrounding area have a large population of flat oysters. The site was previously included in the surveillance program and subjected to a targeted *Bonamia* survey (see Mortensen *et al.* 2020 and previous reports). The site has an apparent stable sub-population of flat oysters growing from just under the littoral zone, down to around six-meter depth. The population contain specimens of several year-classes with older specimens dominating in the deeper stratum. There was no sign of elevated mortality. 154 flat oysters were analyzed by histology and screened for bonamiosis and marteiliosis by microscopy; neither *Bonamia* nor *Marteilia* was detected in any of the samples. Ciliates were observed in one individual. Early-stage haemic neoplasia was observed in seven oysters. One trematode metacercaria was observed in one individual. An intra-cellular Rickettsia-like organism (RLO) was observed in one individual. There was no associated haemocytic response to the observations. Most flat oysters appeared in good condition, with immature to mature gonads, but several had been affected during transport, resulting in morbidity and postmortem changes that could be observed histologically. There were mild focal, non-specific inflammations in the digestive gland, mantle connective/storage tissue of 27 individuals. A granuloma was observed in one individual. Heavy inflammation in the gills was observed in one flat oyster, but no microcells or other pathogenic agents were observed in or surrounding the lesions. Gonad status and sex were described and assessed: 26% were hermaphrodites, 30% were males, 33% were females, and 11% were spent/immature.

Arnevik (58.2723, 8.4276) contains a small oyster bank, approximately 2000 m² in size, located near the river-mouth in the inner part of the fjord. The bank has a high density of flat oysters, ranging from 1 to 5 individuals per square meter to areas of almost complete cover. Pacific oysters are present in low numbers, along with a patchy occurrence of mussels. A part of the oyster bank is completely covered with oysters, with a high percentage of live specimens, while the surrounding area has a lower density of oysters. Although the area is limited, the density of oysters in this area is exceptionally high. The population is cultivated by the company Sørskjell AS, who harvest from the bottom and re-seed with empty oyster shells that act as substrate for oyster larvae.

The oysters were in an overall good condition, with immature to maturing gonads. A few single microcells were observed in 11 specimens. Haemocytes with enlarged nuclei, potentially early stages of haemic neoplasia, were observed in four oysters.

Aga (59.8399, 5.2475). Agapollen is a former production site which produced oyster seed during most of the period between 1882 and 2010. There is no oyster production today, but there are still wild oysters present in the poll. Agapollen is the site where we detected *Marteilia pararefringens* in mussels in 2016.

In 2024 we sampled local flat oysters in the poll. A total of 154 local flat oysters were analyzed by histology and screened for bonamiosis and marteiliosis by microscopy; neither *Bonamia* nor *Marteilia* was detected in any of the samples. Haemic neoplasia was observed in only one oyster, and mild or focal hemocyte aggregation was observed in the connective tissue of the digestive gland, mantle, or gonadal tissue in 10% of the samples. One

oyster sample had massive hemocyte aggregation (granulocytomas) in the connective tissue of the mantle. Moreover, high prevalence of RLO and ciliates were observed in the digestive gland in ca. 30% and 12% of the sections, respectively.

Gonadal status was also assessed and described as males (n=5), females (n=62), hermaphrodites (n=78) or spent (n=11). No gonadal abnormality was observed in any of the samples. Generally, the oysters were in good health condition with the digestive gland in adsorptive phase and intact gills.

4 - Discussion

The health status of Norwegian flat oysters

The over-all aim of the surveillance program is to gain knowledge on the health situation of farmed and commercially exploited Norwegian flat oysters and mussels and to contribute to the maintenance of disease freedom.

Norwegian populations of European flat oysters have been monitored since 1989 and are considered free from notifiable diseases (Mortensen 1993; Mortensen *et al.* 2016; 2020). *Marteilia refringens* has never been detected in Norway, and Norwegian flat oysters, *Ostrea edulis*, appear free from *Bonamia* spp.

Based on the results we have obtained so far; we conclude that the microcells or microcell-like structures observed in flat oysters from Agder are not *Bonamia ostreae*. Though they appear non-pathogenic, further research will be required to elucidate their nature or relationship to *Bonamia* or similar microcell parasites.

Healthy flat oysters are a valuable resource. It is important to monitor Norwegian stocks and disseminate the information on their health status to obtain a consensus on how to protect and care for this resource. We are monitoring Norwegian flat oyster populations and aim at using the data gained in a Nordic and European context. The monitoring of stocks is linked to national health surveillance, and through the contact with European scientists to both genetic studies (Alves Monteiro *et al.* 2024) and re-stocking programs (<https://noraeurope.eu/>).

There is a growing interest in the re-establishment of wild oyster beds in Europe. The restoration is dependent on the availability of flat oysters free from *Bonamia* spp. and *M. refringens* (Sas *et al.* 2020). Both the oyster farming industry and re-stocking projects therefore focus on where to find naïve flat oyster populations that are free from *Bonamia* spp., as well as other pathogens that may affect the populations. In the present situation – and after the re-occurrence of *B. ostreae* in Limfjorden, Denmark, in 2014 (Madsen & Thomassen 2015) – safe sources of oysters can only be found in Sweden and Norway.

There is a small commercial harvest of wild flat oysters along the south and south-western coast of Norway. During this harvest, half-grown oysters are sometimes collected and used as seed in oyster farms. One example is Sørskjell Ltd, located in Arnevik, which was included in the surveillance in 2022 and 2024. Here, the shellfish farmer produces both mussels and oysters in suspended culture and uses local beds of oysters both as a bottom culture and as a source of half-grown oysters for the farm. The production appears efficient and sustainable and demonstrates the link between wild and farmed oysters.

Marteilia infections in mussels

Mussels are not included in the surveillance program since they are not susceptible hosts for *M. refringens* or *Bonamia* spp. However, *Marteilia pararefringens* is present in several populations. Bøgwald & Mortensen (2024) have shown that the parasite can cause severe infections across its range and has a much greater (though restricted) distribution than previously thought.

5 - Conclusions and recommendations

Obtaining disease-free status

The targeted surveillance will be continued in 2025, according to the plan discussed with the Norwegian Food Safety Authority, and with the aim of fulfilling the criteria needed to apply for a disease-free status with regard to *Bonamia ostreae* and *Marteilia refringens* in Vestland and Agder Counties. If the parasites are (still) not detected, this will “close the gap” and the Competent Authority may apply for a disease-free status for *Bonamia ostreae* and *Marteilia refringens* in the containment zones. When disease freedom is granted, the annual, targeted three-year surveillance may be discontinued, in accordance with (EU) 2020/689, Article 81.

Maintaining disease-free status after 2025

In order to maintain the disease-free status, the surveillance program must be re-designed in compliance with (EU) 2016/429 Article 41. Surveillance must include both passive and active surveillance.

Passive surveillance requires the implementation of the monitoring and notification model used in fish aquaculture and a rapid response from the NRL in the case of abnormal mortality or suspicion of disease.

Active surveillance requires a risk-based surveillance of farms or groups of farms/populations in accordance with (EU) 2020/689, Annex VI, Part II, chapter 3, section 4. There are few farms and commercially exploited populations in Norway, and these may be grouped epidemiologically.

Health inspections and sampling must be carried out in accordance with (EU) 2020/689, Annex VI, part 1, chapter 1, pt 1,2.

If surveillance is terminated or does not fulfill the requirements, the disease-free status will be withdrawn “without undue delay” (EU) 2016/429 Article 42 .

Listing of *Marteilia pararefringens* in mussels, *Mytilus* spp.

Mussels are no longer included in the surveillance program for bonamiosis and marteiliosis, but *M. pararefringens* is still present along the Norwegian coast. It is pathogenic to mussels, potentially causing mass mortality and reduced condition. It can be diagnosed and seems to be restricted to certain areas, but the complete geographic range is still not fully known. Due to the parasite's putative life cycle involving several hosts, it cannot be eradicated once introduced or spread. Therefore, it is important to maintain control of *M. pararefringens* and monitor it to ensure that it does not spread, both to wild and cultivated mussel populations. The parasite fulfills the criteria to be put on the national list of notifiable diseases.

The need for a new management model for flat oysters, *Ostrea edulis*

Health surveillance is linked to monitoring of stocks, oyster genetic studies and European oyster projects, strengthening the scientific basis for a strong and adequate management of the few remaining, healthy flat oyster populations in Europe. It is also important to avoid the introduction of Pacific oysters into sites that are used as sources of disease-free flat oyster seed for aquaculture purposes. A management plan for Norwegian

flat oysters will be proposed in 2025, with a special focus on how to protect some of the dense wild oyster populations in Southern Norway.

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