



THE SURVEILLANCE AND CONTROL PROGRAMME FOR BONAMIOSIS AND MARTEILIOSIS IN EUROPEAN FLAT OYSTERS, *OSTREA EDULIS*, AND BLUE MUSSELS, *MYTILUS SP.* IN NORWAY IN 2018

Stein Mortensen, Cecilie Skår, Lisbeth Sælemyr, Mats Bøgwald and Anders Jelmert (Institute of Marine Research)



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The surveillance and control programme for bonamiosis and marteiliosis in European flat oysters, *Ostrea edulis*, and blue mussels, *Mytilus* sp. in Norway in 2018

Overvåkings- og kontrollprogram for bonamiose og marteiliose i flatøsters, *Ostrea edulis*, og blåskjell, *Mytilus* sp. i Norge i 2018 Rapport NOK østers 2018

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Authors:

Stein Mortensen, Cecilie Skår, Lisbeth Sælemyr, Mats Bøggwald and Anders Jelmert (Institute of Marine Research)

Research group leader(s): *Bjørn Olav Kvamme (Sykdom og smittespredning)* Approved by: Forskningsdirektørene: *Geir Lasse Taranger* Program leader(s): *Terje Svåsand*

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Summary (English):

The surveillance programme is carried out by the Institute of Marine Research according to a contract with the Norwegian Food Safety Authority. Samples were collected from four wild beds, two mussel farms and one oyster farm. Samples were collected in April/May and in October, in order to be able to detect *Bonamia* sp. and *Marteilia* sp. during the periods when the potential prevalence could be at the highest. No abnormal mortalities were observed in oyster populations during the surveillance. *Bonamia ostreae* / *B. exitiosa* were not detected. The results may be used as a background for an application for disease free status for Norwegian flat oysters.

There have been several reports on mortality or “disappearance” of mussels along the Norwegian coast. The reason(s) for the mortalities have not been determined. However, the parasite *Marteilia* sp. was detected for the first time in mussels, *Mytilus edulis* at Bømlo, western Norway, collected during the surveillance programme in 2016. This has been followed up with an extended survey in the IMR research project *Mussel mortalities* (83737-04). We have performed transmission experiments that identify the time period of infection and the progress of the infection in mussels. Analyses of samples collected in July 2018 indicate that the parasite may be present in an abandoned oyster lagoon at Espevik, Tysnes.

The results from the research project indicate that the *Marteilia* sp detected is limited to mussels. Flat oysters at the same site do not become infected. This is relevant to the listing of susceptible hosts for *Marteilia* spp. A genetic study of *Marteilia* spp. from the UK, Sweden and the present site at Aga has been included in a study in the EU-project VIVALDI. The name *Marteilia pararefringens* has been proposed, and there is strong evidence that *Marteilia refringens* and *Marteilia pararefringens* sp. nov. are distinct parasites of bivalves and have different European distributions. After the detection of *M. pararefringens* in mussels, it is important to obtain more data from mussels along the Norwegian coast. Mussels from Trøndelag in 2018 represented the first samples from mussels north of Bergen. In order to obtain a better set of data, we propose an extended surveillance that could be obtained through a revised surveillance programme combined with a new model for health control in mollusk farms.

Summary (Norwegian):

Overvåkingsprogrammet for sykdommene bonamiose og marteilliose i flatøsters og blåskjell utføres av Havforskningsinstituttet på oppdrag fra Mattilsynet. Det ble hentet skjell fra fire ville bestander, to blåskjellanlegg og ett østersanlegg. Høsten 2018 ble det for første gang inkludert prøver fra blåskjell fra Trøndelag. Prøver ble samlet inn i April/Mai og i Oktober, når prevalensen av parasittene *Bonamia* spp. og *Marteilia* spp. er høyest i smittede bestander. Det ble ikke observert unormal dødelighet verken vår eller høst. *Bonamia ostreae* / *B. exitiosa* ble ikke påvist. Resultatene kan danne bakgrunn for en søknad om etablering av fristatus for *Bonamia* spp. i norsk flatøsters.

Det er kommet inn en rekke rapporter om at blåskjell «forsvinner» mange steder langs kysten. Årsakene til dette er ikke kjent. Parasitten *Marteilia* sp. ble imidlertid for første gang påvist i blåskjell, *Mytilus edulis*, på Bømlo i 2016. Denne påvisningen er fulgt opp med en utvidet prøvetaking i HI-prosjekt *Blåskjell dødelighet* (83737-04) i 2017 og 2018. Det er gjort smittestudier som gir informasjon om smittetidspunkt og -forløp. Prøver samlet inn i Juli 2018 tyder på at parasitten også kan finnes i den nedlagte østerspollen i Espevik, Tysnes.

Resultatene viser at infeksjonen med *Marteilia* sp. er begrenset til blåskjell. Østers fra den samme lokaliteten blir ikke smittet. Genetiske studier av *Marteilia* spp. fra England, Sverige og Norge (Bømlo) er inkludert i en studie som er gjort i EU-prosjektet VIVALDI. *Marteilia* sp. fra disse områdene er ulike *Marteilia refringens* som forårsaker sykdom hos flatøsters og er foreslått gitt navnet *Marteilia pararefringens*. Det ser således ut til at *Marteilia refringens* og *Marteilia pararefringens* sp. nov. er ulike arter med ulike vertsarter (hhv østers og blåskjell). På bakgrunn av funnet av *M. pararefringens* er det

viktig å skaffe mer informasjon om helsestatus hos blåskjell langs hele kysten. Dette kan gjøres gjennom en utvidet helseovervåking, eventuelt gjennom en ny modell hvor overvåking og helsekontroll kobles, for å generere mer data.

Content

1	Introduction	6
2	Material and methods	7
3	Results	8
4	Discussion and conclusions	9
5	References	10

1 - Introduction

Norwegian populations of European flat oysters, *Ostrea edulis*, have been considered free from notifiable diseases. In 2006, microcells resembling the oyster parasite *Bonamia*

2 - Material and methods

The surveillance was performed according to EU directive 2006/88 and Decision 2015/1554. The sampling strategy, including wild beds and bivalve farms in operation, was revised in January 2015, and used as a background for the targeted surveillance also in 2018.

Sampling periods were defined according to the periods when the highest prevalence of *Bonamia ostreae* and *Marteilia* sp. (sporulating stage) have been detected in the northernmost areas where they have been detected (Engelsma et al. 2010; A. Alfjorden pers.comm). The selected sampling sites are shown in Figure 1 and listed in Table 1.

At Hafrsfjord and Langestrand, oysters and mussels were collected by skin-diving or wading in April and October and transported to the Institute of Marine Research (IMR) in Bergen. At Sveio, oysters and mussels were collected by the shellfish farmer and sent to IMR Bergen by over-night mail (Table 1). From Ytre Hvaler,

3 - Results

Bonamia spp. was not observed in any sample during 2018.

Langstrand, Aust-Agder.

The site was inspected by skin diving in May 2018. Dense oyster beds were observed down to approximately four-meter depth, with several cohorts present. There was no sign of abnormal mortality. Few adult Pacific oysters (*Crassostrea gigas*) were observed between the flat oysters. During sampling, Pacific oyster spat were observed on and in-between flat oyster shells and on pebbles in the inter-tidal zone.

Oysters: The reading of slides from oysters is delayed (see Table 1). During examination, gross morphology of shells and soft parts appeared normal. In the first 20 slides *Bonamia ostreae* / *B. exitiosa* or microcells resembling *Bonamia* spp. were not detected.

Mussels appeared normal, however most specimens had green pustules, presumably representing infections with the parasitic algae *Coccomyxa parasitica* (see Mortensen *et al.* 2005). *Marteilia* sp. was not observed.

Hafersfjord, Rogaland

Samples were collected in May and October (Table 1). Sampling was performed by Knut Magnus Persson in conjunction collection of flat oysters for a re-stocking programme off the coast of The Netherlands. No sign of abnormal mortality was reported. A few adult Pacific oysters (*Crassostrea gigas*) were observed between the flat oysters on shallow water. *Bonamia* sp. or *Marteilia* sp. were not observed in mussels or oysters. *Bonamia* PCR of oysters were negative. During examination of the flat oysters, perforations due to *Polydora* sp. infestations were observed in shells from most of the oysters. Gross morphology of soft parts appeared normal. Rickettsia-like organisms (RLO

4 - Discussion and conclusions

Examination of flat oysters

The wild flat oyster populations examined appears healthy, with a normal reproductive cycle pattern. Haemic neoplasia and the presence of intracellular Rickettsia-like colonies were occasionally observed, but at low prevalence and intensity. This is a common observation, and not considered a problem, although the neoplasia may cause problems and potentially induce winter mortalities of flat oysters in severe cases (Mortensen *et al.* 2013).

The oysters from Aga appear in relatively poor condition, with low condition index. This is probably due to food limitation. At Langestrand, several cohorts have been present throughout the study period. All samples since 2008 have been *Bonamia* negative (Mortensen *et al.* 2016). The situation has thus been stable since 2006. A 13 years long sub-clinical *Bonamia* infection seems unlikely, taking into account that this oyster bed experiences extremely variable conditions through the seasons. We consider the bivalves examined in 2018 as negative for *Bonamia ostreae* / *B. exitiosa*.

Marteilia spp. has not been detected in oysters by histological examination. The oysters collected in the lagoon at Aga, close to the *Marteilia*-infected mussels, were PCR negative. Oysters and mussels collected at Rog

5 - References

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HAVFORSKNINGSINSTITUTTET

Postboks 1870 Nordnes
5817 Bergen
E-post: post@hi.no
www.hi.no