

NOTE

What can the Limfjord tell us about limiting factors for *Bonamia ostreae* in northern Europe?

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Abstract

The Limfjord in Denmark is recognized as a unique production area for European flat oysters, *Ostrea edulis*, and is the only remaining area in Denmark where the native oyster stock still exists. 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. In the same period (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, thus preventing the spread of *Bonamia* parasites to the naïve flat oyster population in the Limfjord.

In Denmark, the Limfjord is recognized as a unique production area for European flat oysters (*Ostrea edulis*) and is the only remaining area in Denmark where the native oyster stock still exists. The Limfjord is an inlet (fjord) situated in Northern Jutland, covering around 1500 km² and connected to the North Sea in west (salinity 3.2-3.4%) and the Kattegat in east (salinity 1.9-2.5%). The net water flow is from west to

east mainly due to the tide and amounts to 6.8 km³ on annual basis (http://www.limfjord.dk/om_fjord.htm: Fakta om Limfjorden). In total, the water volume in the Limfjord is 7.4 km³, with an average depth of 4.9 m, with shallow areas along the coast line and and sounds with depths up to 28 m, all in all giving optimal conditions for oyster growth.

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The Limfjord has not always been directly connected with the North Sea. During the period 1200 to 1825 the Limfjord was separated from the North Sea by a narrow isthmus, which was broken through after a heavy storm. Until the penetration of the isthmus the water in the Limfjord was brackish with relative low salinity and, thereby, exhibited poor conditions for flat oyster production. The increasing salinity obtained after the event created suitable conditions for reappearance of the oysters in the Limfjord. After that period the oyster fishery in the area has been subjected to different kinds of regulation, e.g. prohibition of fishing during years with low stock density.

Due to climatic conditions, recruitment from the natural oyster stock has in periods not been high enough to keep the yield of fishing at an acceptable economical level, as reproduction of the natural stock only took place successfully during summers with water temperatures above 18-20 °C. Therefore, from the 1920's until the 1980's the fishery of the natural stocks was supplemented by relaying of oysters imported from e.g. the Netherlands, Norway, UK, USA and France.

During the 1980's the fishing yield of the natural oyster stock in the Limfjord was almost zero. Therefore, in order to fulfil the national demand oysters of almost marketing size were imported from France, UK and USA for "fattening" during summer time. The imported oysters were relayed on culture banks where they as far as possible were kept separate from each other and from the native Danish oyster stock.

Bonamiosis caused by *Bonamia ostreae* was observed in French flat oysters *O. edulis* in 1979

(Comps et al., 1980) with devastating results for French oyster production. In 1980, imported French oysters relayed in the Limfjord showed macroscopical changes corresponding to the gross pathology described from *Bonamia* infestations (see Comps et al., 1980). The oysters originated from Plougastel Douglas nearby Brest in Bretagne, France. The observation was done in oysters sampled for presentation at a workshop during an international conference arranged by the International Council for the Exploration of the Sea (ICES) in Copenhagen in 1980. Two out of 10 oysters showed fenestration of the gills suggesting infection with *Bonamia* parasites (Jørgen Kamp, personal communication). The workshop participants from France familiar with the diagnostics of bonamiosis immediately noticed the pathological changes. The potential diagnosis was verified by histology (Paul van Banning, personal communication). The findings resulted in attempts to clean the site by fishing up the whole batch of French oysters.

Another indication of that the oysters had been affected by bonamiosis was that the Danish importer had noted in his records that the mortality in this consignment of oysters was extremely high (78 %). The oysters were close to marketable size as they were imported in April 1980 and expected having reached marketable size during autumn/winter 1980.

In this case it was diseased oyster that were introduced to a disease-free area. From other studies it is known that oysters from disease-free areas can spend up to a year in infected waters before disease symptoms appear (Steins, 1997). According to Montes (1991) it may take at least 3 months before the parasite is observed in a disease-free batch that has been moved into an

infected zone. Larger oysters seem to be more affected than smaller oysters. The presence of *Bonamia* is more dependent on size than on age (Caceres-Martínez et al., 1995). In a study done by Caceres-Martinez et al. (1995), the parasite was first present when the oyster had a size of 4 cm, and from this length with increasing parasite prevalences, with mortalities exceeding 10 % at a size of 6 cm. All the above information is important, when trying to predict the risk of the parasite to having spread to the naïve population in a certain area.

Water temperature is another important feature influencing the spread of the parasite (Zabaleta and Barber, 1996). In 1980-81 as well as in 1984-85 and 1985-86, Denmark experienced winters with extremely low temperatures classified as “ice winters”, where Danish internal waters were covered by ice for longer periods. During these periods, the water temperature in the Limfjord was below 0 °C for several weeks (Table 1).

However, during the 1990's the natural flat oyster stock began increasing, especially due to warm summer periods, which gave a basis for successful reproduction. Giving hope for returning to former years' profitable oyster fishery and trade, a histological screening of flat oysters for *B. ostreae* and *Marteilia refringens* was initiated

in 1996 and repeated the following years. These investigations were conducted in old oysters with an estimated age of 8-12 years i.e. recruited in the period shortly after the introduction of the *Bonamia* infected oysters - without the finding of any signs of the parasites. A formalized surveillance programme was approved by the EU and started in 2000. In 2004, the Limfjord gained its approved status as being free from bonamiosis (as well as marteiliosis). Regular screening for both parasites has been performed and until today the parasites have not been found in flat oysters sampled from the Limfjord (AM Report, Mollusc diseases NRL, 2012 as well as AM reports from former years). The fishing of flat oysters has within the last decade risen with a factor 100 from 9 t to around 1000 t per year due to optimal climatic conditions with warm summers. All these observations suggest that *Bonamia* in this case has not been able to establish itself in the Danish naïve flat oyster population.

This event is probably the first instance where *Bonamia* has been introduced to an area with a naïve flat oyster population without devastating consequences. Introduction of *Bonamia* infected French oysters to UK, Ireland and the Netherlands has had serious consequences for the cultivation of flat oysters on the culture banks. In the 1980's, bonamiosis was introduced

Table 1. Water temperatures in the central Limfjord (Vildsund Bridge) during ice winters in the 1980'ies. (Source: Danish Meteorological Institute).

| Year | Weeks | Depth at registration | Temperature interval |
|---------|-------|-----------------------|----------------------|
| 1980-81 | 11 | 10 m | -0.14 °C - -1.17 °C |
| 1984-85 | 7 | 10 m | 0 °C - -1.02 °C |
| 1985-86 | 11 | 10 m | -0.4 °C - -1.17 °C |

to the Dutch flat oyster stocks with devastating consequences. Attempts to eliminate the disease by cleaning sites and restocking with disease free animals failed. Van Banning (1990 and 1991) demonstrated that, when an oyster bed was cleaned and fallowed, the naïve oysters, reintroduced into the area, quickly developed the infection, suggesting that a reservoir of infection had persisted in the area.

The *B. ostreae* parasites are believed to have a low survival rate during cold winters (Zabaleta & Barber 1996, Paul van Banning, personal communication), so the very low winter temperatures found during several winters in Denmark during the 1980's have probably contributed to an elimination of remaining parasites if any infected oysters were left after cleaning of the site. The low water temperature may also have eliminated the French oysters, themselves, as former observations in the Limfjord showed that during ice winter periods in e.g. 1939-42, relayed French and Norwegian oysters were totally eliminated while the natural stock survived (Spärck 1951) probably due to an evolutionary adaptation to such extreme climatic conditions. It has to be noted that the natural oyster stock during the 1980's was at its lowest level compared to the previous 30 years, and the areas chosen for relay were almost free from native oysters, which also may be a factor that may have influenced the elimination of bonamiosis.

The influence of water temperatures on the survival and sporulation of *B. ostreae* can be compared with other sites, where the parasite has been found. *B. ostreae* is known to have persisted in Casco Bay, Maine, USA, since 1998 (Carnegie, 2000). Twelve samples over a two year period from 1998 to 2000 showed preva-

lences from 1.8 % to 26.7 % with the average prevalence being 9 % (Carnegie, 2000). Most of the samples were studied by cytology, but the first sample taken 14 July 1998 was studied by histology and had a prevalence of 4.0 %, whereas the last sampling taken 4 June 2000 in the two year period was studied by cytology and had a prevalence of 26.7 %. More recent samplings from the same area (10 May 2012 and 21 September 2012) showed mean histological prevalences between two sites of 1.5 % and 6.5 %, respectively (D. Bouchard, University of Maine, personal communication). According to the website http://tidesandcurrents.noaa.gov/data_menu.shtml?stn=8418150%20Portland,%20ME&type=Meteorological%20Observations the average water temperatures in this area were around 5 °C with an all-time low at 0.5 °C during the winter 2012/13, while the temperatures at the same time during the winter 2011/12 were around 5 °C with an all-time low 3.5 °C. The minimum water temperatures during the years 1998 to 2012 were from 3.1 °C down to -1.2 °C. Only 5 out of the 15 years the minimum temperatures were below 0 °C, and during these years the water temperatures only stayed below 0 °C from one day and up to two weeks. In Norway, *B. ostreae* was found in wild flat oysters in Arendal in 2008, AM Report, Mollusc Diseases NRL (2010). No evidence of a clinical outbreak was seen and screening of oysters in years thereafter has not revealed *Bonamia* parasites (Mortensen et al., 2012). The website <http://www.mitrejsevej.dk/l/norge/vejret-arendal-vejruddsig-temperatur-klimatemp.html> shows that the coldest water temperatures found in Arendal in February and March are around 4 °C. Van Banning (pers. comm.) had observed lower incidence rate of *Bonamia* in Dutch waters after winters with very low water

temperatures. However, the above mentioned publications do not come up with information on the possible impact of low temperatures on the dynamics of the *Bonamia* reproduction. Only in the Danish case in the Limfjord, Table 1, the water temperatures were in longer periods (between 7 and 11 weeks) below 0 °C during winters in the 1980's, after *Bonamia* had been found in French relayed oysters.

In conclusion, it seems that elimination of a *Bonamia* infection after the introduction to an uninfected area (the Limfjord) was probably due to extreme climatic conditions like ice winters, which either directly eliminated the *Bonamia* parasites or indirectly eliminated the original host - the relayed French oysters - thus preventing the spread to the naïve flat oyster population in the Limfjord.

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