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Assessment of the impact of a pathogen, *Bonamia ostreae*, on *Ostrea edulis* oyster stocks with different histories of exposure to the parasite in Ireland

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ABSTRACT

The protozoan parasite *Bonamia ostreae* has decimated *Ostrea edulis* stocks throughout Europe over the past four decades. A study of two stocks of *O. edulis* in Ireland with varying periods of exposure to *B. ostreae*, 5 years and 22 years, was undertaken. The objective of the study was to determine if varying lengths of exposure would translate into observations of differing susceptibilities to *B. ostreae*. A number of oyster beds within each area were screened. The study was carried out over 13 months to investigate seasonality and the role of environmental parameters, population density and size on disease development. Of particular interest was the fact that prevalence of infection in both stocks was very similar. The stock that had been exposed for 22 years had a similar prevalence, intensity and seasonality of infection as the stock infected for 5 years.

B. ostreae was detected in both stocks throughout the year with the highest prevalence in spring, possibly related to the increase in water temperature and/or oysters directing their energy towards gametogenesis. The study indicated that oyster stocks can maintain themselves over extended periods of time in *B. ostreae* endemic areas. However, prevalence of *B. ostreae* will remain relatively stable within the stock without some intervention to improve resistance levels e.g. by breeding for resistance over a number of years. Some natural resistance to infection will build up in individual oysters but in natural populations this will continually be diluted by cross fertilisation with more susceptible oysters.

1. Introduction

The European flat oyster's (*Ostrea edulis*; Linnaeus, 1758) natural range extends from the coast of Norway, south to Morocco and into the Mediterranean basin as far as the Black Sea coast (FAO, 2010). Due to overfishing, habitat destruction and exotic diseases (Culloty and Mulcahy, 2007), wild populations are now scarce throughout Europe with disease free, natural beds, only occurring in isolated regions along the west coasts of Ireland, Scotland, Scandinavia and in the Limfjord region of Denmark.

Over the last forty years, European aquaculture production of *O. edulis* has shown a drastic decline from a peak output of nearly 30,000 tons in 1961 to 6000 tons today, mainly due to the impact of two parasitic diseases, *Marteilia refringens* (Grizel et al., 1974) and *Bonamia ostreae* (Pichot et al., 1979) (Lallias et al., 2009). With ongoing infection of *B. ostreae* in populations and a considerable shift to *Crassostrea gigas* (Thunberg, 1793) farming, the industry has been unable to recover to production figures achieved in the 1960s, despite numerous efforts including prohibiting relaying of oysters, fallowing beds and cleansing sites (Culloty and Mulcahy, 2007; FAO, 2010; Hugh-Jones, 1994). Ireland, as with the rest of Europe suffered a dramatic decline in the species, even before the introduction of parasitic diseases, possibly due to overexploitation and poor management of fisheries (Culloty and Mulcahy, 2007).

One of the major reasons for the decline of *O. edulis* is *B. ostreae*, an intracellular haplosporidian parasite found in flat oysters in Europe (Balouet et al., 1983; McArdle et al., 1991; van Banning, 1991) and on the Atlantic and Pacific coasts of the USA (Elston et al., 1986; Friedman and Perkins, 1994; Friedman et al., 1989). The parasite was first described in Europe in Brittany, France, by Pichot et al. (1979). *B. ostreae* was first detected in *O. edulis* in Cork harbour, on the south coast of Ireland in 1987 (McArdle et al., 1991). A report of the presence of *B. ostreae* in a consignment of oysters from Clew Bay on the west coast exported to France was made in 1988. Later screening of 1500 Clew Bay *O. edulis* in 1991 failed to confirm the presence of *B. ostreae* (see: McArdle et al., 1991). Presence of the parasite in the bay was finally confirmed in 1994, but infections occurred at a very low prevalence (Marine Institute et al., 2006). On the north coast of Ireland, Lough Foyle was the stock in which *B. ostreae* was most recently detected, in spring 2005, with 43% prevalence of infection in one of three beds screened (Loughs Agency, 2011).

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A range of epidemiological studies on *B. ostreae* and its host have indicated differences in seasonality in this disease with size, age and environmental conditions all impacting on prevalence and intensity of infection observed at different sites (Cáceres-Martínez et al., 1995; Culloty and Mulcahy, 1996; Engelsma et al., 2010). Long term epidemiological studies of other oyster species indicate that some changes in host parasite dynamics (the nature of which have not always been elucidated) can occur over an extended period of time, with changes in host susceptibility, phenotypic and genotypic variation in the host and parasite and changes in environmental conditions all contributing to different disease patterns being observed in populations (Carnegie and Burreson, 2012).

The aim of this study was to investigate whether exposure to *B. ostreae* over varying periods of time (5 and 22 years) in wild, natural oyster stocks would result in changes in the host–parasite dynamics. It was considered that this reduction in host susceptibility might occur following exposure over an extended period of time thereby affecting prevalence and intensity of infection and thus subsequent impacts on stocks. The role of environmental parameters, population density and size classes for host–parasite relationships were also assessed, when information was available. To achieve these objectives, two stocks — Clew Bay with one of the earliest records of the disease in Ireland and Lough Foyle, the stock with the most recent detection of the parasite, were chosen to monitor infection over a 13-month period, encompassing spatial and seasonal variations.

2. Materials and methods

2.1. Study areas

Two stocks in Ireland were selected; Clew Bay, a wild fishery that has been affected by *B. ostreae* since 1988 (McArdle et al., 1991), situated on the west coast and Lough Foyle, a wild fishery where *B. ostreae* was first detected in 2005 (Loughs Agency, 2011), found on the north coast of Ireland.

Clew Bay (53°83′33. N, 9°80′00. W) is a westerly facing bay in Co. Mayo on the West Coast of Ireland and is dominated by a drowned drumlin field. The bay covers an area of approximately 16,000 ha. The inner bay is shallow with an average depth of 10 m increasing seawards to 20 m. The seabed is a mixture of sand, gravel, mud and boulders. The tidal range is 5 m. Fourteen rivers flow into the bay. It has been categorised as a class A area under Directive 91/492/EEC — Classification of Shellfish Production Areas (http://sfpa-ie.access.secure-ssl-servers.biz/index.php?q=shellfish). The bay is characterised by the presence of 117 islands and there are numerous oyster beds seeded from natural settlement.

Lough Foyle (55°11′67. N, 7°08′33. W) is a northerly facing bay bordering Co. Donegal and Co. Derry on the north coast of Ireland. The Lough covers an area of approximately 18,600 ha. The average depth of the Lough is 5 m, reaching a max of 15 m. The seabed is a mixture of poorly sorted sands, gravels and mudflats. The tidal range is 3 m. Three rivers flow into the Lough. It has been categorised as a class B area under Directive 91/492/EEC — Classification of Shellfish Production Areas. There are a total of 16 productive native oyster beds seeded from natural settlement in the bay, covering an area of 7081 ha.

2.2. Sampling

Samples of live *O. edulis* were collected from the two stocks, Clew Bay and Lough Foyle, at three monthly intervals, beginning October 2010 and finishing in October 2011 (October 2010, January 2011, April 2011, July 2011, October 2011). The samples were taken from seven oyster beds within Clew Bay (Table 1) and five oyster beds within Lough Foyle (Table 1). Three of the beds sampled in Lough Foyle, Site A, B and C, all form part of a larger bed termed Southside. 8–60 oysters were collected on each bed on each sampling date. The sample size depended on the availability of oysters on the bed with a desired sample size being 60 individuals per bed. In total 2475 oysters were collected and examined over the 13 month period — 1097 from Clew Bay and 1378 from Lough Foyle. Oysters were processed immediately upon arrival at the laboratory with the whole weight (g) and length (cm) of each oyster being recorded before oysters were opened.

Table 1
Name and location of sampling beds in Clew Bay and in Lough Foyle.

Stock	Site no.	Name of bed	Grid reference	
Clew Bay	1	Rosgibbilean	53°53′22.10″N	9°35′47.00″W
	2	Rosbeg	53°51′24.80″N	9°34′54.00″W
	3	Rosbarnagh	53°52′29.80″N	9°35′34.40″W
	4	Newport River	53°52′56.90″N	9°35′38.80″W
	5	Friar's Island	53°53′8.10″N	9°37′48.00″W
	6	Inishloy North	53°51′53.00″N	9°35′14.08″W
	7	Inishloy South	53°51′39.10″N	9°35′15.10″W
Lough Foyle	1	Sandy ridge	55° 8′0.20″N	7° 4′5.94″W
	2	Flat ground	55° 5′6.82″N	7° 6′8.55″W
	3	Site A	55° 6′9.88″N	7° 4′3.42″W
	4	Site B	55° 6′7.62″N	7° 4′3.77″W
	5	Site C	55° 7′1.69″N	7° 4′3.07″W

2.3. Cytological processing and examination

Diagnosis of *B. ostreae* infection was carried out using ventricular heart imprints. The imprints were air dried before being fixed in methanol for 2 min and stained with Hemacolor 2 and 3 (Merck) (Culloty et al., 1999) and washed prior to mounting in DPX. The imprints were examined under light microscopy at 400× magnification. The intensity of *B. ostreae* infection was determined using the following scale:

Class 0: No *B. ostreae* cells observed.
Class 1: 1–10 *B. ostreae* cells observed.
Class 2: 11–100 *B. ostreae* cells observed.
Class 3: *B. ostreae* cells observed in all fields of vision.
Class 4: *B. ostreae* cells observed in all cells (Bachère et al., 1982; Culloty et al., 2004).

2.4. DNA isolation and polymerase chain reaction analysis (PCR)

A small sample of gill was collected from each animal and frozen at −20 °C for DNA based diagnosis. In cases where heart imprints could not be obtained or could not be subsequently read, PCR analysis was used to confirm *B. ostreae* DNA presence. DNA was extracted from the tissue using the Chelex-100 method (Lynch et al., 2008; Walsh et al., 1991) and stored at −20 °C. PCR analyses were performed with primers Bo Boas, which amplified 300 bp from the SSU rRNA gene, using the method of Cochennec et al. (2000). All PCR assays included positive controls (*B. ostreae* DNA from infected oysters) and a negative control (double distilled water). The PCR products were run on 2% agarose gel in TE buffer gel (110 V and for 40 min) stained with ethidium bromide (10 mg/L).

2.5. Environmental data

Temperature and salinity data were collected by Bord Iascaigh Mhara (BIM) for Clew Bay and by the Loughs Agency for Lough Foyle (www.loughs-agency.org). Star Oddi loggers, deployed at several intertidal sites in Clew Bay north, acquired Clew Bay temperature and salinity data. The loggers recorded on the hour every hour. Electronic instruments that recorded every 15 min acquired Lough Foyles' temperature and salinity data. Several technical issues occurred during the gathering of data in Clew Bay so environmental data was compiled from information from several loggers in the north of the bay. In Lough Foyle, as a result of technical difficulties, no environmental data were available from July 2011 to October 2011.