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Thirty-year history of Irish (Rossmore) *Ostrea edulis* selectively bred for disease resistance to *Bonamia ostreae*

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ABSTRACT: The protistan pathogen *Bonamia ostreae* was first detected in *Ostrea edulis* at Rossmore, Cork Harbour, on the south coast of Ireland in 1987. A selective breeding programme commenced in 1988 by Atlantic Shellfish Ltd. to produce *B. ostreae*-resistant oysters using 3 to 4 yr old survivors as broodstock for controlled spawning in land-based spatting ponds. On-growing of oyster spat settled on mussel cultch was carried out on designated beds within Cork Harbour. Oyster production subsequently increased successfully, resulting in 3 yr old Rossmore *O. edulis* being marketed from 1993 onwards and a record tonnage of 4 yr old oysters being produced in 1995 and 1996. *O. edulis* production, *B. ostreae* prevalence and oyster mortalities have been monitored and recorded at Rossmore for over 30 yr. The collation and analysis of this data from 52 samples and 3190 oysters demonstrate the introduction and progression of bonamiosis and subsequent interventions to ameliorate disease effects during this period at Rossmore. Results suggest that *O. edulis* mortalities are now negligible during the first 4 yr of growth, prevalence of *B. ostreae* infection is low, and no correlation exists between prevalence of infection and oyster mortalities. This study, when compared to other studies of bonamiosis-infected oyster populations, suggests that an intervention in the form of a selective breeding programme is required to reduce the impact of the disease.

KEY WORDS: Native European oyster · Protistan · Pathogen · Breeding programme · Disease management

INTRODUCTION

In Europe, the native flat oyster *Ostrea edulis* industry (along with naturally occurring *O. edulis* beds) have been in decline since the 18th and 19th centuries, with a significant reduction in their abundance during the 19th century (Korringa 1952, Yonge 1960, Edwards 1997, Wilkins 2004). The main causes of this decline include habitat destruction, over-exploitation with unsustainable harvesting rates, poor or irregular recruitment and the parasites *Marteilia refringens* and *Bonamia ostreae* during the 20th and 21st centuries (Edwards 1997, da Silva et al. 2005, OSPAR Commission 2006, Culloty & Mulcahy 2007).

Originally almost every harbour and bay around the Irish coast had beds of *Ostrea edulis*. Stocks were depleted due to over-fishing, resulting in a collapse in many fisheries in the mid-19th century (Wilkins 2004). The North Channel at Rossmore, Cork Harbour, was a long established public oyster bed until it went into decline at the end of the 19th century/beginning of the 20th century (Blake 1870, Browne 1904). Restocking of the beds began in the late 1960s; Atlantic Shellfish Ltd. obtained a fishery order for the beds and began to produce *O. edulis* spat in an

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experimental shore-based seawater pond. Spat, collected on mussel shell cultch, was subsequently relayed on the seabed at a density varying between 400 and 700 m^{-2}. The North Channel oyster population increased up to the summer of 1987, when the parasite *Bonamia ostreae* was initially detected in Rossmore oysters (McArdle et al. 1991). Subsequent screening of frozen heart material sampled in 1986 detected *B. ostreae* (Rogan et al. 1991), indicating that the parasite may have gone undetected for several years. Production, after the loss of 98% of the stock due to bonamiosis in 1987, recommenced in 1991, and by 1993, the tonnage of oysters ranged from 31.8 to 133.8 t up to 2003, when the fishery was closed due to sewage pollution. However, the selective breeding programme continues on a small scale.

The results of both field and laboratory trials during the 1990s indicate that a degree of *Bonamia ostreae* resistance had developed in the Rossmore oysters (Culloty et al. 2001). In another study undertaken by Culloty et al. (2004), the potential resistance of a number of European populations of *Ostrea edulis* was compared. Rossmore oysters had a lower prevalence and intensity of infection compared to the other oyster populations, especially when relayed and held at the northern European sites, however, they did not perform as well at the southern European site. An independent survey carried out at Rossmore in 2004 to determine the total stock level and survival showed that mortality in market-sized 4 yr old oysters was negligible (13%; E. Edwards unpubl. data). In the 10 dredge hauls undertaken in that survey, 2826 live oysters (87%) were caught and 414 (13%) oysters were dead. The dredge efficiency was calculated to be approximately 20%, thus indicating that a population of 16.9 oysters m^{-2} was present on the seabed (weighing 1.46 kg m^{-2} of bed) with an estimated population of 1 467 000 *O. edulis* (126.7 t) being present.

Several studies have been conducted to investigate the ability of oysters exposed to *Bonamia ostreae* to develop a resistance to this parasite (Elston et al. 1987, Martin et al. 1993, Boudry et al. 1996, Baud et al. 1997, Naciri-Graven et al. 1998, 1999, Culloty et al. 2001, 2004). Development of resistance may be hampered in natural populations by the dilution of 'resistance' genes because of susceptible stock contributing to the progeny (Culloty et al. 2004) and by the sale and consumption of resistant oysters before they contribute genes to future generations (Lauckner 1983).

The objectives of this study were to collate the *Bonamia ostreae* monitoring data gathered over 3 decades year to year for the Rossmore oysters and was targeted at different age groups at different times and on an irregular basis to assess the current status of this breeding programme and oyster population, in order to determine the impact on both the parasite and the oyster population during that time.

MATERIALS AND METHODS

Study site

The North Channel at Rossmore, Cork Harbour (51° 85′ N, 8° 26′ W), is located on the south coast of Ireland. The North Channel runs in an east–west direction, between the Great Island and the mainland. Depth ranges from 1 to 10 m, and the temperature at Rossmore varies between 5° and 20°C. The coldest temperatures occur in February and March and the warmest temperatures between June and August. The surface salinity varies between 26 and 33‰, depending on rainfall and tides (Lynch et al. 2007). The shape of the North Channel and the narrow exit via the East Passage make the ebb tide slightly longer than the flood tide (McManus 1988), and this narrow channel on the eastern side of Cobh Island obstructs the influx of water from the open sea into the North Channel. The reduced water flow and relatively sheltered conditions prevent oyster spat from being swept away by strong tidal flow. The major source of fresh water is the Midleton River that enters the northeastern part of the channel (Lynch et al. 2007).

Selective breeding programme

The breeding programme commenced in 1988 and continues at Rossmore with *Ostrea edulis* broodstock (initially 3 yr old stock, but then 4 and 5 yr old and even 10 yr old survivors) dredged from specific beds in the North Channel and allowed to spawn naturally in 1000 m^3 breeding ponds. Spat is cultched onto mussel shell and is scattered subtidally when a few millimetres in size, on beds assigned to separate year-classes. The total area of these beds is approximately 86 800 m^2. The beds are identified by wooden poles, which are set at 50 m intervals, to mark the oyster ground. With very few survivors after 1987, just 4 ponds with 600 broodstock were used initially at Rossmore in the selective breeding programme in 1988, while 21 ponds are currently available.

To distinguish progeny, oysters are classified into different year groups, e.g. R1+ are oysters that are at

least 1 yr old but have not yet reached their second year, and so on for the other age groups. To date, 6 successive generations (F1, F2 etc.) have been produced since the selective breeding programme commenced at Rossmore (Fig. 1).

Oyster samples

Age classes 0+ to 10+ (sample sizes n = 40 to 150) of Rossmore *Ostrea edulis* have been collected sporadically on a yearly basis from 1986 to 2012 for monitoring purposes and for use in field and laboratory trials (Table 1). Each oyster age group at Rossmore typically has an average whole weight of 10 g (1+), 30 g (2+), 55 g (3+) and 80 g (4+). Weight gain is approx. 20 g yr^{-1} (T. & D. Hugh-Jones pers. obs.), leading to a 140 g, 7 yr old oyster. By the end of the summer's growth, each age group will have added another 20 g, resulting in 4 yr old oysters reaching 100 g for the autumn market.

Mortality

Estimates of commercial oyster mortality were kept by Atlantic Shellfish Ltd. for most years (1984 to 2000) of operation at Rossmore by counting empty shells during the harvesting of market-sized oysters when dredging using a 180 cm blade. Each oyster bed contains only oysters of the same year class. An independent survey was carried out at Rossmore in 2007 (E. Edwards unpubl. data) and used 10 dredge hauls to determine both mortality and the total stock on the seabed by towing between 50 m bed markers.

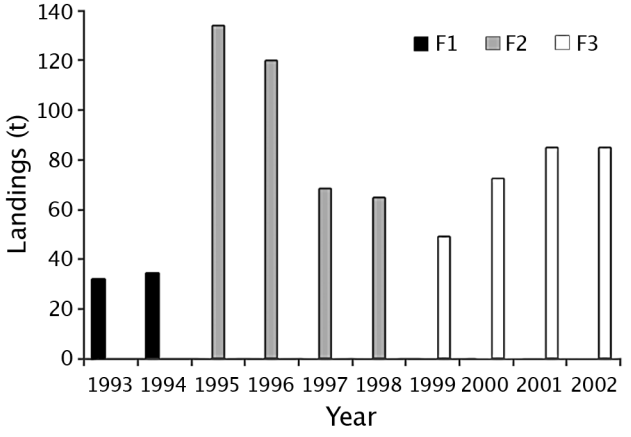

Fig. 1. *Ostrea edulis.* Landings per year of 3 oyster generations (F1, F2 and F3) selectively bred at Rossmore, Ireland, from 1993 to 2002

Diagnostic methods

Prevalence of *Bonamia ostreae* infection was measured using several diagnostic techniques recommended by the World Organisation for Animal Health (OIE 2006). Oysters were screened for *B. ostreae* infection by ventricular heart smears as previously described, and intensity of infection (Class 0 to Class 4) was recorded for certain samples (Bachère et al. 1982, Culloty et al. 1999). Molecular screening of oysters was carried out on samples collected from 2003 onwards (Table 1). DNA extraction methods included a phenol/chloroform extraction of gill tissue

Table 1. *Ostrea edulis.* Sampling data, including month, year, age group, sample size (per age group) and diagnostic method (heart smear [HS] and PCR) for the Rossmore oysters

Date	Oyster age	Sample size (n)	Diagnostic method
06/1986	3+ & 4+	60	HS
06/1987	4+	60	HS
04/1989	1+	60	HS
02/1990	2+	60	HS
05/1990	2+	60	HS
06/1990	2+	60	HS
07/1990	2+	60	HS
08/1990	1+	60	HS
09/1990	1+	60	HS
10/1990	1+	60	HS
11/1990	1+	60	HS
12/1990	1+	60	HS
01/1991	1+ & 2+	60	HS
02/1991	1+ & 2+	60	HS
03/1991	1+	60	HS
04/1991	1+	60	HS
05/1991	1+ & 2+	60	HS
07/1991	1+ & 2+	60	HS
08/1991	1+	60	HS
09/1991	1+ & 2+	60	HS
10/1991	1+	60	HS
11/1991	1+ & 2+	60	HS
12/1991	1+	60	HS
01/1992	1+ & 2+	60	HS
06/1998	5+	60	HS
09/1998	5+	60	HS
12/1998	5+	60	HS
03/1999	5+	60	HS
06/1999	5+	60	HS
10/1999	6+	60	HS
01/2000	6+	60	HS
07/2003	0+ & 1+	60	HS & PCR
08/2003	0+ & 1+	60	HS & PCR
10/2003	0+ & 1+	60	HS & PCR
12/2003	0+ & 1+	60	HS & PCR
03/2006	5+	60	HS & PCR
12/2006	5+	60	HS & PCR
06/2011	4+ & 10+	150 & 40	HS & PCR
07/2012	5+	60	HS & PCR

(Chomczynski & Sacchi 1987) from 2003 to 2006 and subsequently a Chelex-100 extraction (Walsh et al. 1991) of heart tissue. PCR was carried out using the primers BO/BOAS of Cochennec et al. (2000) with an expected product size of 300 bp and the primers C_F/C_R of Carnegie et al. (2000) with an expected product size of 709 bp. Positive and negative controls were used in each PCR reaction. Positive controls were obtained from *Ostrea edulis* heavily infected with *B. ostreae*. Negative controls consisted of double-distilled water (ddH_20). Direct sequencing of amplified products was routinely carried out to confirm the detection of *B. ostreae* (GenBank Accession AF192759 and AF262995).

Statistical analysis

Pearson's correlation coefficient was carried out to determine whether a correlation between mortality and *Bonamia ostreae* prevalence existed at Rossmore. Significant differences in the prevalence of infection between (1) seasons, (2) oyster age groups and (3) years were calculated using chi-square (χ^2) analysis with significance determined at $p < 0.05$.

RESULTS

In total, data from 52 samples screened consisting of 3190 *Ostrea edulis* of different age classes was available for analysis (between 1986 and 2012; Table 1).

Mortality (%) observed

Oyster mortality at Rossmore varied between years and between oyster age groups (R0+, R1+, R2+, R3+, R4+, R6+, R10+) from 1984 to 2000 (Figs. 2a & 3a). Older market sized oysters (R3+, R4+) experienced the highest mortalities in 1986 (90%) and 1987 (95%). In 1988, 55 to 75% mortality was observed in oysters of the younger age classes (1+, 2+, 3+), but by 1990, 2 yr after the breeding programme had commenced, a decrease to ≤32% was observed. Certain years such as 1991 saw an increase in mortality in young stock, but by 1999, there was significant survival in even 5 and 6 yr old stock. Since the start of 1999, mortalities in market sized 4 yr old oysters of 80–120 g have been negligible, with over 75% survival (D. Hugh-Jones unpubl. data)

Prevalence of infection (%) by heart smears and PCR

Over the years, 0 to 90% prevalence of infection by screening heart smears has been recorded, with an overall mean prevalence of 29.8% being observed in all oysters screened over that time period (Fig. 2b).

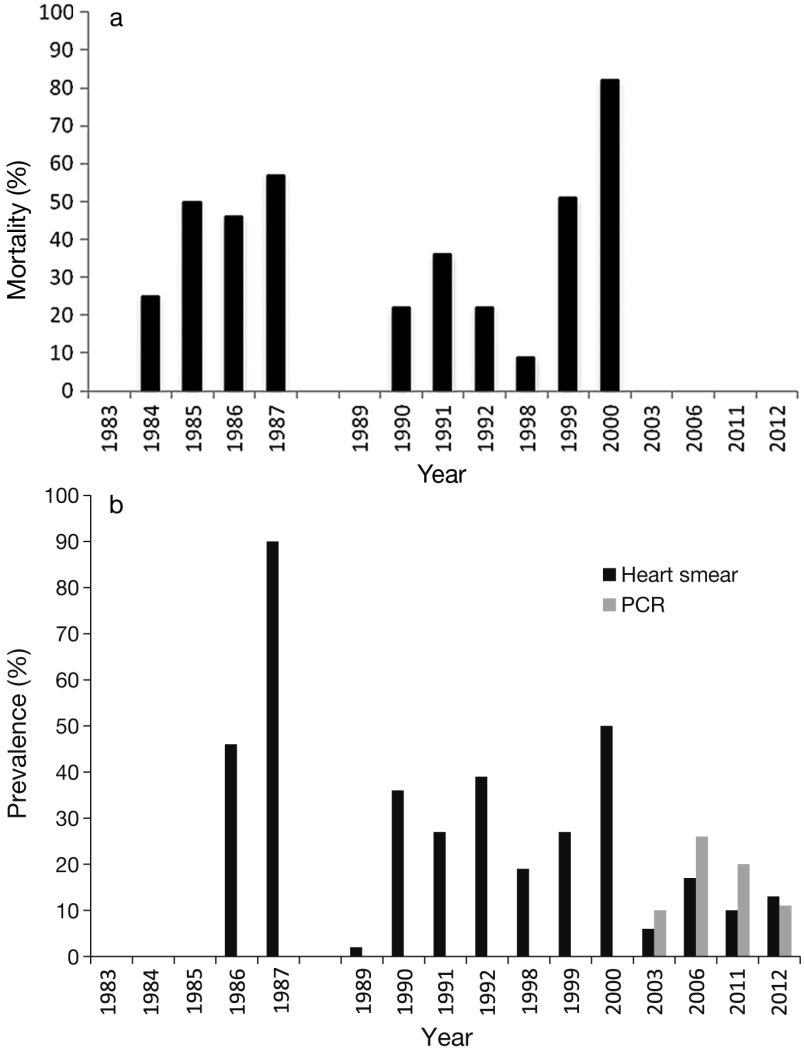

Fig. 2. *Ostrea edulis* infected by *Bonamia ostreae*. Mean (a) mortality and (b) prevalence of *B. ostreae* infection (screened by heart smear in 1986–2012 and PCR in 2003–2012) of all oyster age classes over the study period. In both panels, the gap on the *x*-axis highlights the start of the selective breeding programme in 1988

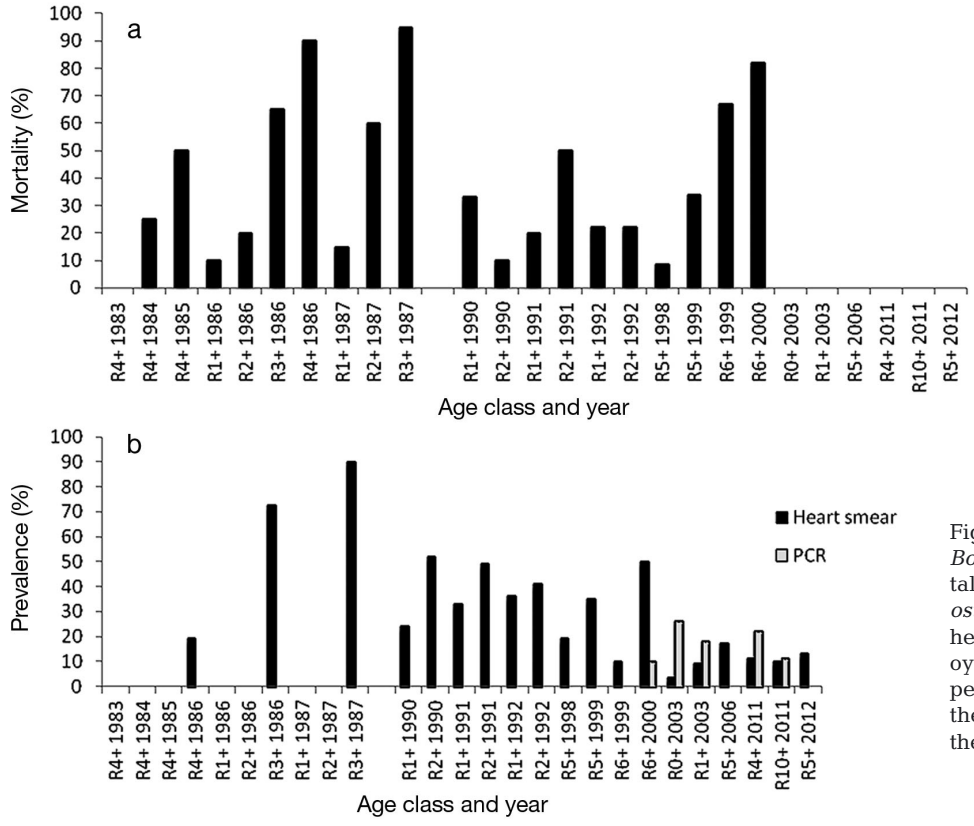

Fig. 3. *Ostrea edulis* infected by *Bonamia ostreae*. Mean (a) mortality and (b) prevalence of *B. ostreae* infection (screened by heart smear and PCR) in each oyster age class over the study period. In both panels, the gap on the *x*-axis indicates the start of the selective breeding programme in 1988

The highest mean prevalence of *Bonamia ostreae* was observed at Rossmore in 1987 (90%). During the 1990s, a mean prevalence of ≤39% was observed and remained constant in young Rossmore oysters with an increase to 50% being observed in 2000 in 6 yr old stock. Since 2000, the mean prevalence of infection has been ≤17%, with 4 of the 6 year-classes sampled being 4 to 10 yr olds (Figs. 2b & 3b). A test of significance was carried out between years (78 comparisons) for prevalence of infection. Prevalence of infection was significantly different in most year comparisons, although 22 of the year comparisons were not different. The time span between such year comparisons ranged from 1 to 15 yr. The most frequently observed time span was 8 yr (4 times) followed by 1 and 14 yr (both 3 times).

Mean prevalence of *Bonamia ostreae* was highest in spring (45.5%) followed by summer (37%) and winter (29.6%), with autumn (18.8%) having the lowest mean prevalence of this pathogen over the whole study period (Fig. 4). A significant difference in the prevalence of infection was observed in 4 of the 6 season comparisons: spring and autumn ($p = 0.00005$), summer and autumn ($p = 0.0026$), spring and winter ($p = 0.0285$) and autumn and winter ($p = 0.0469$). No difference was observed between summer and winter ($p = 0.2943$) and spring and summer ($p = 0.2501$).

In the ventricular heart smear screening, the prevalence of *Bonamia ostreae* observed in each age class was 0–6% (0+), 2–66% (1+), 18–80% (2+), 72% (3+), 10–90% (4+), 9–45% (5+), 10–50% (6+) and 10% (10+) (Fig. 5, Table 2). Eighteen comparisons of significant difference in prevalence of infection were carried out between the different age groups. All comparisons were significantly different except for 1+ and 5+ oysters ($p = 0.5198$), 1+ and 6+ oysters ($p = 0.1443$), 2+ and 4+ oysters ($p = 0.7736$) and 2+ and 6+ oysters ($p = 0.0771$).

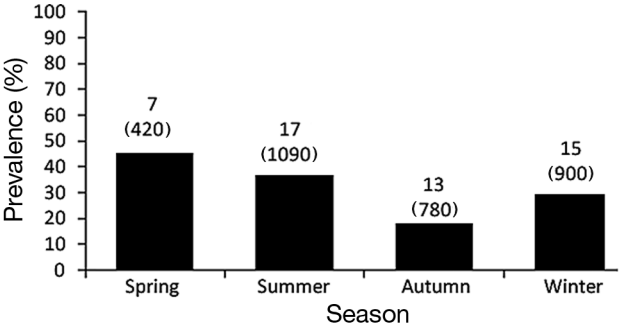

Fig. 4. *Bonamia ostreae* infecting *Ostrea edulis*. Mean prevalence (%) of *B. ostreae* in Rossmore oysters for each season for all oyster age groups screened from 1986 to 2012 (numbers above bars: no. of samples screened; in parentheses: total number of oysters screened)

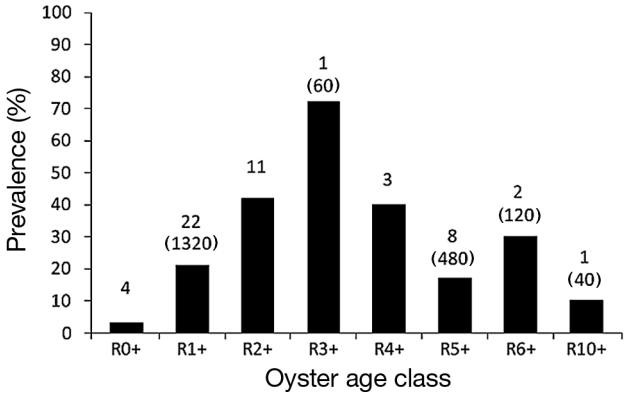

Fig. 5. *Bonamia ostreae* infecting *Ostrea edulis.* Mean prevalence (%) of *B. ostreae* in each Rossmore oyster age class in all oysters screened by heart smears from 1986 to 2012 (numbers above bars: no. of samples screened; in parentheses: total number of oysters screened)

Table 2. *Ostrea edulis.* Summary of prevalence results for the different oyster age groups showing the number of samples screened and the time period

Age group	Samples (n)	Time period	Prevalence (%) Range	Mean
0+	4	6 mo	0–6	3
1+	22	13 yr	2–66	21
2+	11	2 yr	18–80	42
3+	1	1 yr	72	72
4+	3	25 yr	10–90	40
5+	8	14 yr	9–45	17
6+	2	3 mo	10–50	30
10+	1	1 yr	10	10

A *Bonamia ostreae* prevalence range of 0 to 26% was recorded in the PCR screening at Rossmore from 2003 to 2012, with an overall mean prevalence of 15% (Figs. 2b & 3b). Detection by PCR was generally higher than actual clinical infections observed in these samples of oysters when screened by ventricular heart smears.

Intensity of infection

Intensity of infection observed in oysters at Rossmore has varied over time, and all intensities of infection have been recorded. At the initial stages of the breeding programme in the 1990s, a higher frequency of Class 3 and Class 4 intensities of infection were observed. In 1990 to 1991, Class 4 infections were observed in 7 to 27% of infected oysters, while 3 to 18% of oysters had Class 3 infections (Culloty & Mulcahy 1996). Much lower intensities of infection were observed 15 and 18 yr after the breeding programme began (2003 and 2006). In 2003, Class 3 infections were observed in 2% of oysters, while Class 4 infections were absent (Lynch et al. 2005). In 2006, Class 4 infections were not observed in screened oysters, while 7% had Class 3 infections (Lynch et al. 2008). In 2011 (n = 190) and 2012 (n = 60), 23 and 24 yr after the breeding programme commenced, only Class 1 (2–10%), Class 2 (3–8%) and Class 3 (2–3%) infections were observed in the screening (unpubl. data).

DISCUSSION

The results of this study indicate that although *Bonamia ostreae* is still present in Rossmore *Ostrea edulis*, since its introduction in the 1980s, its impact is much less significant than when first introduced. When the parasite was introduced, high mortalities and infection levels were observed. During the early 1990s, higher intensities of infection (Classes 3 and 4) were more prevalent, not only in the Rossmore stock, but also in other *O. edulis* stocks (naïve and exposed) that were relayed there from other Irish and European locations (Culloty & Mulcahy 1996, Culloty et al. 2004). Class 4 infections were absent and Class 3 infections were greatly reduced 15 to 24 yr after the breeding programme commenced. During the 1990s, a noticeable reduction in the prevalence of *B. ostreae* was observed in Rossmore *O. edulis*, even though oyster production, densities and disturbance due to dredging activity on the oyster beds returned to normal commercial intensity during this period. In the last decade, a continual reduction in the prevalence of infection has been observed.

Prior to the introduction of *Bonamia ostreae* at Rossmore, oyster mortalities were considered to be at acceptable levels for commercial production, i.e. less than 10%, and survival rates were good. Once the Rossmore oysters were exposed to the parasite, increasing mortalities were recorded for several years, which would be expected with the initial exposure of a parasite to a naïve, highly susceptible host population. By the time an F2 generation was being produced from survivors, oyster mortalities at Rossmore were considered to have returned to a commercially acceptable state. The exceptions to this were the years 1999 and 2000, and mortalities were associated with below normal mean air and sea temperatures (Met Eireann, www.met.ie/climate/Monthly Weather/clim-1999-Dec.pdf). At other sites such as

Lough Foyle, Ireland, 100% prevalence of infection was associated with significant mortalities and a large decrease in the population size (McGonigle et al. 2011).

Naïve oysters (spat and juveniles) have been relayed to Rossmore to investigate their performance and susceptibility to *Bonamia ostreae* compared to Rossmore spat and juveniles (Lynch et al. 2005). Rossmore spat showed the lowest prevalence of infection, although infection was observed in the initial sample. Naïve juveniles had acquired a 6% prevalence of infection within 6 mo of exposure, indicating that *B. ostreae* was present and virulent in Cork Harbour (Lynch et al. 2005). *B. ostreae* has been detected in non-typical hosts, such as the Pacific oyster *Crassostrea gigas* (Lynch et al. 2010), benthic macroinvertebrates and zooplankton (Lynch et al. 2007), at Rossmore from 2004 to 2007, which indicates that *B. ostreae* is present in the Rossmore environment (in the benthos and in the water column).

Rossmore oysters that have been exposed to *Bonamia ostreae* for an additional 2, 4 and 5 yr have a similar prevalence of infection to their younger counterparts. Several studies have observed that the impact of bonamiosis is more evident in oysters that are larger and older (Engelsma et al. 2010). Recently, a low prevalence of infection was observed in 10 yr old Rossmore oysters infected with Class 1 infections (unpublished data). This low intensity of infection might indicate that the parasite is being maintained at a sublethal level by older oysters, which readily recognise it and prevent it from proliferating to an intensity that would cause death.

The highest prevalence of infection at Rossmore was observed in spring, followed by summer, with significant difference in prevalence of infection being observed between these 2 seasons and winter and autumn. Different levels of physiological and metabolic activity in oysters, at different times of the year, may impact on the uptake of *Bonamia ostreae* cells, particularly in times like spring with increased feeding activity, which may result in a higher prevalence of infection in the oysters. Spring and summer environmental conditions, in particular temperature, may be more suitable for the proliferation of *B. ostreae* (Cochennec & Auffret 2002). Engelsma et al. (2010) observed the mean prevalence of infected oysters to be significantly higher in spring than in autumn in the Netherlands.

The initial broodstock used at Rossmore in 1988 was sourced from the survivors, i.e. the more resistant individuals available after the major 1987 mortality event, and had several years of exposure to increasing levels of *B. ostreae* (up to 90% prevalence). Such selection events have been observed in other oyster disease studies (Carnegie & Burreson 2011, Ford & Bushek 2012). In many host–pathogen studies, hosts are regarded as having resistant or susceptible genotypes, and the relative relationships among genotypes are fixed across environments (Lazzaro & Little 2009). Relative resistance or susceptibility in a host population is complex and involves genetic variation, environmentally mediated variation and genotype by environment interactions (de Jong 1990, Gomulkiewicz & Kirkpatrick 1992). In natural populations, hosts are more likely to be repeatedly exposed to the same parasites either within a generation or across consecutive generations (Tidbury et al. 2012); however, minor alterations in the abiotic environment may increase or diminish the effects of a pathogen.

Long-term epidemiological data sets are available for other oyster species, such as the eastern oyster *Crassostrea virginica* in the USA, which is infected by *Haplosporidium nelsoni*, the causative agent of Multinucleated Sphere Unknown disease (MSX; Andrews 1984). *H. nelsoni*, which has been present in *C. virginica* populations since the 1950s, now exists at very low levels of prevalence, due to naturally developed disease resistance to MSX disease and the possible influence of breeding programmes (Carnegie & Burreson 2009). Carnegie & Burreson (2011) found that wild *C. virginica* in Chesapeake Bay are developing resistance to *H. nelsoni* through strong selection pressures following exposure to high levels of parasites, resulting in more tolerant oysters that are making a significantly higher contribution to the progeny. Assuming that the tolerance has a genetic component, the tolerant trait will be selected for and could spread rapidly through the population. Other studies have demonstrated that without active intervention, e.g. selective breeding, minimal resistance will be observed in infected populations over an extended period of time (Culloty et al. 2004, Engelsma et al. 2010).

The results of our study highlight the importance of some kind of man-made intervention, such as a selective breeding programme for resistance against a particular pathogen, in the production of a commercial shellfish species. It also shows that the 'one size fits all' approach may not be feasible when certain factors such as local adaptation may be at play and that exposure to new abiotic or biotic factors may have an impact on the host–parasite relationship.

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