

Investigation of oyster herpes virus infection and oyster mortality in the Republic of Ireland in 2009 – a questionnaire survey

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Executive Summary by
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Executive Summary

1. Background to the Survey

In 2008 abnormally high levels of mortality were reported in *C.gigas* along the majority of the French coastline and in 3 bays in Ireland. According to French scientists, the mortality events were attributed to a combination of factors including the presence of a newly described variant genotype of the Ostreid herpes virus-1 termed OsHV-1 μ Var as well as a number of *Vibrio* species in combination with what were said to be exceptional environmental conditions. In 2009, there was a recurrence of the mortality events along the entire French coastline as well as in 17 bays in Ireland (including 2 cross border water catchments) and on the island of Jersey. Again the newly described genotype OsHV-1 μ Var was detected in the vast majority of samples analysed in all three countries.

In the Republic of Ireland a questionnaire based survey was designed and administered in 14 of the affected bays and 5 non-affected bays in order to characterise the pattern of mortality which had been experienced and to identify any associated management and environmental factors.

2. Materials & Methods

2.1. Design & Administration of the Survey and data collection

The questionnaire was designed in close collaboration between the Marine Institute (MI) and CEFAS and was administered by staff from the MI & BIM. Data was collected at three different levels i.e. (i)enterprise/ business (ii) site and (iii)batch and submitted to CEFAS for analysis. In total, data was collected on 346 batches of oysters from a total of 97 farm sites belonging to 70 enterprises.

2.2. Data Analysis

STATA 10 (Statacorp, 2009) was used for statistical analysis of the data which had been collected.

2.3. Age, size & stocking density of oysters

Oysters were categorised into spat ($\leq 5\text{g}$), juveniles (5-40g) and adults (40g+). Size of oysters was reported as gramme weights or grade such as G6, G8 (all G sizes were assumed to be less than 1g when converted to grammes). The product of weight x number of animals therefore allowed biomass or within bag density to be calculated.

3. Results

3.1 Production in Ireland

The survey was conducted in 19 of the 44 bays in the country where *C. gigas* production takes place. Most enterprises operated only one site. The number of enterprises / bay ranged from 1 to 10.

The majority of oysters were sourced from outside Ireland with 58% of stock originating in France, 76% of which was triploid. Batch size at introduction varied enormously between sites and the number of batches per site ranged from 1-24. Stocking densities varied between bays.

There was almost no variation in culture methods with the principle method of cultivation being bags and trestles.

3.2 Mortality during 2009

3.2.1 Historic

High levels of mortality were reported from 43 sites for years prior to 2009. These reports included the losses suffered through events such as the *Karenia mikimotoi* bloom in 2005 as well as mortality associated with the OsHV-1 μVar strain in 2008. OsHV-1 μVar was reported as a cause of mortality by three sites in 2008, of these 3 sites, two were confirmed as being infected with OsHV1-uvar and the other was untested in 2009.

3.2.2 Mortality in 2009

3.2.2.1 Rainfall and Temperature

High rainfall was recorded for 5 days immediately prior to the onset of mortality in Clew Bay. However, this association was not observed in other bays.

Water temperature data was only available for Castlemaine Harbour and Inver Bay (close proximity to Donegal Bay). In Castlemaine the mortality started the week following a spike in water temperature whilst in Donegal mortality only occurred after a second spike in temperature.

3.2.2.2 Site level analysis for mortality

Prior to 2009, there was no statistically significant difference in mortality levels between the affected and non-affected sites.

3.2.2.3 Spatio-temporal analysis

Plotting start date of mortality against end date revealed a pattern of mortality consistent with disease spread. This pattern could arise also from sequential introductions of new stock, however further evidence of contiguous spread was derived from mapping the spread of OsHV-1 between sites.

3.2.2.4 Mortality at the batch level

Analysis of mortality at the batch level showed a higher level of mortality in 2009 compared to previous years. There was considerable variation however in mortality at the batch level between bays.

Mortality was continuous in nearly all batches, ie there was one mortality event which occurred over a number of days or weeks and then stopped. In 2 batches, however, a second wave of mortality occurred.

Where mortality was reported in more than one year class, the first mortalities occurred in batches imported in autumn 2008 or spring 2009. Later mortalities occurred in batches that had been on site for longer periods which supports a

hypothesis of an introduction of an infectious agent with purchased stock which spreads to the established population.

3.2.2.5 Temporal Analysis

The average duration of mortality was 18.6 days. The earliest recorded onset was the 25/5/09 and the latest was 10/8/09 and peaked in late June. The longest duration was 63 days. Mortality was continuous in almost all sites.

3.2.3 Associations between batch level analyses and mortality

Mortality was significantly higher in spat than other age groups and there was a strong association between triploidy and higher mortality. 29% of triploid batches experienced $\geq 20\%$ mortality compared to 12% in diploids. Mortality in wild versus hatchery seed was also examined for batches from France. Whilst a greater proportion of wild stock suffered lower levels losses, the difference was not significant.

The effect of handling on mortality level was examined and manual compared with mechanical handling was associated with higher levels of losses (80% versus 50%).

From the data collected, no association was found between biomass per bag and total cumulative mortality.

There appears to be an association at batch level between the longest time spent out of the water and mortality. No batches which spent greater than 8 hours out of the water suffered mortality at levels greater than 40%.

3.2.4 Time on site before infection

It has been hypothesised that if stock is brought on site early in the year then it has longer to establish itself and therefore mortality will be lower. No evidence was found to back this up.

3.3 Explanatory variables for mortality at site level

3.3.1 Production

There was no association between production of other species of shellfish or level of oyster production and being an affected site.

3.3.2 Introduction of equipment to site

The data collected indicated that OsHV1-uvar related mortality was strongly related to the importation of trestles, but not bags. This is counter-intuitive and may be because introduction of equipment is often associated with expansion of production or other unknown risk factors.

3.3.2 Handling

There is no evidence that increased handling increases the risk of being affected. However, there appears to be a significant association between manual handling and the risk of being an infected site. The association could however be linked to a greater likelihood of disease being observed as a result of manual handling.

3.4 Bay level Analysis

3.4.1 Origin of movements

At the bay level, the introduction of oysters from France was strongly positively associated with the OsHV-1 status of the bay when compared with introductions from GB and Guernsey. In addition, all affected bays (14/14) received triploid stock from France and 9/14 received diploid stock from France.

4.0 Farmers Comments

Faster growing stocks (may be due to daily tidal immersion), water temperature, rainfall and handling stress were all noted as factors which were positively associated with mortality.

5.1-5.3 Discussion

Experience gained of OsHV-1 in France and of herpes viruses in general suggests that the OsHV-1 μ Var virus persists at low levels throughout the winter months and recurs once the water temperatures reach permissive levels. This study therefore aimed to inform us more about the introduction of the virus to bays in Ireland and to identify management factors which could be manipulated to minimise the impact of the disease in infected stocks, in the future.

The strong association between the introduction of stock from France where the virus was known to be present, compared with introductions from areas where the virus is absent, is consistent with the spread of the virus through infected stocks. Evidence accumulated since 2008 would indicate that whilst factors such as water quality and the presence of *Vibrio sp* may have a part to play in the mortality events the newly emerged variant of oyster herpes virus has a more central role. Investigation of the date of onset of mortality in bays with more than 5 sites provides strong evidence that the virus was introduced with stock and then spread to older established batches. The virus is most likely to have spread within bays on water currents, therefore further investigation of the direction of tidal and other currents within a bay may give further insights into spread.

It is clear that some sites within a bay do not experience mortality whilst others experience high levels. Mortality levels also appear to be highly variable. To explain why this occurs, it is necessary to consider “necessary” and “sufficient causes” for disease. The new μ Var of OsHV-1 is very likely to be necessary but not sufficient. A combination of necessary causes (infection, water temperature, susceptible age group) is needed to provide a sufficient cause. Ie whilst the virus must be present for the mortality to occur, but the virus on it's own cannot explain the absence of mortality in certain stocks or even the difference in mortality levels that have been observed. Different age groups for example show differing susceptibilities to the effects of the virus. It can be assumed that in areas such as Dungarvan most of the sites were exposed to the virus and yet some appeared unaffected. This indicates that either the virus is not highly infectious or that the oysters in unaffected areas were more resistant to the virus which may be age related. Environmental factors also quite likely have a role to play. This requires further analysis.

Handling and time out of the water were identified as potential risk factors for mortality. Oysters which were manually handled during thinning/ grading etc showed higher mortality levels than those that were mechanically handled. Oysters which were exposed for 8 hours or longer had lower levels of mortality -it is thought this may be related to the fact that these oysters are growing more slowly than those that are submerged in the water for longer periods.

5.4 Conclusions and Recommendations

Since 2008, it has become clear that OsHV-1 μ Var has played a crucial role in the mortality events which have been observed in France and Ireland. Reports of similar events have also emerged from Spain, Italy and most recently England. In all cases the newly emerged variant of oyster herpes virus has been isolated and links to imports of oysters from infected areas are evident. Whilst environmental conditions and the presence of other pathogenic agents such as *Vibrio* species may contribute to the overall levels of mortality the OsHV-1 μ Var virus is clearly required for the event to occur. This study provides further evidence of the critical role of the virus in the mortality events in Ireland in 2009

The study indicates that time out of the water and handling are possible management factors which could be used to reduce the mortality levels in OsHV-1 μ Var outbreaks. This too needs to be investigated further. Given the information which was made available for this study, it was not possible to fully investigate the role of stocking densities. This was due in part to the fact that a consistent approach to measuring density has not been formulated and that densities change over the life cycle of the oyster. In future studies a clear case definition needs to be developed.

The study confirms that levels of mortality within bays showed considerable variation, however the reasons for this remain unclear. Further studies may help to elucidate the causes for this. The MI will be participating in an FP7 funded study which is due to commence in 2011 which may help to give additional insights into this.

1. Introduction

1.1. Pacific oyster production

The Pacific oyster *Crassostrea gigas*, also known as the cupped oyster is the most important species of oyster cultivated in the Republic of Ireland (RoI) both in terms of volume and value, with an annual production of over 7,000 metric tonnes (mt) in 2007 (valued at over 15 million euros). This species is second only to the blue mussel in terms of the volume of production (18,270 mt worth 28.9 million euros in 2007), and greatly exceeds that of the native oyster *Ostrea edulis*.

Pacific oysters were introduced into the RoI, and into many other parts of Europe as a result of the decline in populations of the native oyster. Pacific oysters are easier to cultivate and are not susceptible to the major diseases of native oysters.

1.2. Summer mortality

France is the major producer of Pacific oysters in Europe. French oyster stocks have experienced a condition known as summer mortality for a number of years. This condition has also been observed in Pacific oysters in other parts of the world, notably in Japan and the USA since the 1980s. Whilst a precise aetiological agent has yet to be identified for summer mortality events, they are generally associated with the presence of oyster herpes virus (OsHV-1), and various *Vibrio* spp. (findings which are consistent from both USA and France). The mortalities are seasonal and may be influenced by environmental factors. The condition has been demonstrated to be transmissible. Mortality occurs mainly in juvenile stock but can affect all age classes including adults (Arzul and Renault, 2002).

In recent years mortalities of Pacific oysters in France have considerably increased; the levels of mortality in 2008 and 2009 have been particularly high. All of the major oyster growing areas in France, including both the Atlantic and Mediterranean coasts have been affected. The condition has shown a clear pattern of spread since 2008 (Miossec et al., 2009). Measures to control the spread of the disease in 2008 and

2009 have included prohibitions on movements of spat and juveniles but not of adult oysters.

Epidemiological work in France has been sparse with no rigorous analysis of the role of live animal movements, temperature, and other factors in the spread of the disease. A large scale questionnaire survey has been conducted but the data have yet to be analysed. Temperature is clearly important but the threshold for the initiation of mortalities appeared to vary considerably between regions in France in 2009¹ (Pepin et al., 2009a).

The OIE currently does not list OsHV-1 as a reportable disease, thus there is no recommended diagnostic methodology published in the Manual of Diagnostic Tests for Aquatic Animals (O.I.E., 2009). However the Community reference laboratory for molluscan diseases, IFREMER, La Tremblade, France has recommended a diagnostic methodology. A single round PCR (Renault and Arzul, 2001) has been developed which targets 896 base pairs (bp) of a part of the viral genome located in an inverted repeat and coding fragments of unknown proteins.

OsHV-1 has been identified in the majority (92%) of cases of clinical disease in France during the exceptional mortality events of 2008 and 2009 (Pepin et al. 2009, SCoFAH statement provided by France 4/9/09). Previous years data suggest that although OsHV is often found in Pacific oysters during mortality events, it has previously occurred at lower - 12% (4 out of 34 samples) in 2006 and 26% (19 out of 72 samples) in 2007 (NRL Meeting 2007, NRL Meeting 2008). OsHV-1 has been identified in the only mortality event investigated in Jersey (M. Gubbins, pers. comm.), and in the mortalities investigated in the RoI (D. Cheslett, pers. comm.). Experimental transmission studies have demonstrated that extracts from field infected oysters have induced mortality in spat and juvenile oysters (Pepin et al., 2009a).

¹ The pattern of spread in France in 2010 has to date been from south to north and consistent with increasing water temperature.

There is evidence from the scientific literature that OsHV-1 can persist in populations of surviving adult oysters following primary infection (Arzul and Renault, 2002; Lipart and Renault, 2002; Renault, 2008). This finding reflects the behaviour of other well studied herpesviruses found in vertebrate animal populations including those in the aquatic environment such as ictalurid and cyprinid herpesviruses (Gray et al., 1999; Goodwin et al., 2009).

1.3. *A new genotype of Oyster Herpesvirus*

The increase in virulence of the disease may be attributable to a new strain of the virus. Recent isolates of OsHV-1 from mortality events in France have shown distinct genetic differences to earlier isolates. The mortalities may be associated with a newly emerging and virulent μ Var of OsHV-1 (Pepin, 2009, SCoFCAH statement provided by France 4/9/09). Analysis of various target sequences within the viral genome present in infected batches of Pacific oysters demonstrated polymorphism where the DNA sequence exhibited only 96% sequence homology in the targeted C2 – C6 region (710 bp) with the reference OsHV-1 DNA.

This polymorphism has been characterised (Pepin *et al* 2009) and samples analysed at Cefas from a reported mortality in Jersey demonstrate similar polymorphism which includes deletions in a micro-satellite zone and substitutions in coding zones (Cefas 2009).

Among 38 batches of infected oysters dating from 1995, 2002, 2003, 2004, 2005, 2006 and 2007, and several geographical origins (France, USA, Japan, China), none displayed the identical profile to the new μ Var OsHV-1 found in France in 2008 and 2009 (Pepin et al., 2009b). From 2009 to date only the new μ Var OsHV-1 has been detected in France with 94% of samples from oyster mortalities being positive compared with a normal expected prevalence of 40%. The previously widely distributed original genotype seems to have disappeared (Pepin 2009). Pepin (2009) stated that the new biotype should 'be considered an emerging viral biotype associated with oyster mortalities'.

1.4. Position in Rol

There is an export trade in live Pacific oysters for on-growing from France to other parts of Europe. In 2009 reports of extensive mortalities of oysters were received from the Republic of Ireland (D. Cheslett, pers. comm.) and from Jersey (M. Gubbins, pers. comm.). The presence of OsHV-1 μ Var1 was confirmed in samples from both the Rol and Jersey, and in both cases, the oysters originated in France. There is an increasing amount of circumstantial evidence from areas where mortalities are occurring that infection can be transmitted from non-clinically affected surviving adult oysters to naive juvenile oysters. (F. Geoghegan pers. comm.).

In Ireland high mortality and the presence of OsHV1 μ Var1 were reported from oyster growing sites in 16 bays (D. Cheslett pers. comm.). Pacific oysters are cultured in 44 bays in the Rol, of which 21 introduced spat during 2009. Oysters from France had been imported during 2008 or 2009 to all but one of the bays where OsHV1 μ Var1 was detected (the other site had introduced oysters from another bay in the Rol which was OsHV1 μ Var1 positive). Anecdotally the level of mortality varied considerably between sites within the same bay.

1.5. Objectives of this study

This report summarises a questionnaire investigation of mortality in Pacific oysters during 2009. The study aimed to characterise the pattern of mortality and identify any associated management and environmental factors.

2. Material and Methods

2.1. Questionnaire design

The questionnaire (Appendix 1) was designed in close collaboration with staff from the Marine Institute (MI), Galway. A telephone conference was held (7th Oct 2009). Project members at Cefas and MI staff commented on drafts of the questionnaire. MI staff trialled the questionnaire with oyster farmers in 5 bays before final revisions were made.

2.2. Data collection and entry

Two MI and three BIM (Irish Sea Fisheries Board) staff were trained in administering the questionnaire; they conducted face to face interviews with oyster farmers during farm visits between November 2009 and January 2010. The questionnaire was formatted in MS-Excel to allow data to be entered electronically. Excel files were sent to Cefas where the survey data was extracted and collated. Data were collected at the level of the enterprise, site (within enterprise) and batch (within site). Data were available for 346 batches of oysters from a total of 97 farm sites belonging to 70 enterprises in 19 bays. The grid references of the licensing areas were noted (some sites spread over more than one licensing area).

2.3. Data analysis

2.4. Descriptive statistics and outcome variables

Data was extracted from the Excel questionnaires using a Visual Basic procedure for direct import into STATA 10 (StataCorp, 2009) for statistical analysis. Analysis has been conducted at the level of batch, site and bay. At the bay level presence of OsHV1 (at one or more sites) was used as the outcome variable. Sites reporting mortality were classified as positive. At the batch level reported mortality (cumulative prevalence) was used as the outcome as a continuous variable and categorised (in bivariable analyses mortality greater than 40% was treated as the positive outcome). The analysis at site and batch level was undertaken for all data and for observations from the subset of bays where OsHV1 had been detected. Scatter and boxplots were used to examine the distribution of continuous variables (box indicates inter-quartile range from Q1 to Q3 and line extensions represent the range with the outliers indicated by dots). Points on scatterplots may be moved slightly (jittered) to reveal multiple points where they coincide. Explanatory variables were screened for their association with the outcome (mortality) using t-tests, chi-squared tests and logistic regression. ArcMap (ESRI Corp, CA) was used to map the locations of sites within bays.

2.5. Age, size and stocking density of oysters

Oysters were categorised into three age/size groups: spat, juveniles and adults. Spat were defined as oysters equal to or less than 5 g, juveniles between 5 and 40 g and adults greater than 40 g. The size of oysters was reported in grammes or as a grade (G). The highest grade recorded was G12, which MI advised was equivalent to individual animal weight of less than one gramme. Records of oysters size by grade were converted to grammes (all recorded grades were <1g). Assigning nominal weights of 1g for all "grades", 5g for those apparently up to 5g, 20g for "juveniles" and 80g as an average for anything over 20g, the product of weight x number in a bag, as a measure of biomass or within-bag density was calculated (ranges from 0.4 to 40kg per bag).

3. Results

3.1. Oyster production in the RoI

The survey was conducted in 19 of the 44 bays in which Pacific oyster production takes place. Most (52) enterprises operated on one site, but some used up to four sites (Table 1).

Table 1 Oyster production sites per enterprise

Sites per enterprise	Number of enterprises
1	52
2	10
3	4
4	4

The number of enterprises per bay varied from 1 to 10, and the number of sites per bay from 1 to 19 sites (median = 5). Twelve sites reported no production in 2008, the average production of the remaining sites was 73.1 mt (median = 26 mt, range from 0.8 to 725 mt).

The large majority of oysters are sourced from outside of Ireland (Table 2). 58% of stock was triploid, 76% of which was imported from France (which supplies 60% of

all stock). 43 (12%) of batches had been screened for OsHV1 at source. Most sites (73%) imported stock from only one country (Table 3).

Table 2 Country of origin and genetic status of purchased stock

Country of origin	Diploid	Triploid	Total	(%)
France	53	152	205	60
England	58	9	67	20
Guernsey	23	12	35	10
Republic of Ireland	9	27	36	10
	143	200	343	100

Table 3 Sites by number of countries supplying stock

Number of countries supplying stock	Number of sites
One	70
Two	23
Three	2
Four	2
	97

Batch size (at time of introduction) was recorded as either number of animals or weight (mt), and is summarised in Table 4. There was a very large variation in size of batches (from 6,000 to 35 million animals). Batches of smaller numbers of animals are likely to be adult animals and batches of large numbers of animals spat. The large majority of batches were not split between sites (78%). Similarly, most batches (73%) were not mixed with other batches at the same site. 11% of batches were made up of oysters from more than one hatchery. 74 sites had ≤ 4 batches and the maximum number of batches per site was 24 (Table 5).

Stocking density (average number of animals per bag) varied between bays.

Information was available at the site level for average number of animals per bag for each age group (spat, juveniles, adults). No data were available on density of bags on trestles or variability within site. In general, a bag contained up to 4000 spat, up to 1000 juveniles or 150-200 adults. Higher densities were reported in Clew Bay, Achill Sound South and Trawbregga Bay (Clew: 10-40g @ 250; 10-30g @ 500; 10-30g @

300; 4-30g @ 600; 4-40g @ 500; Achill Sound South: 10-30g @ 500; 30-50g @ 300; 5-30g @ 350; and Trawbreaga: 35-40g @ 250; 45-65g @ 250).

Table 4 Batch size at introduction

	Animals	Metric tonnes
n (number of observations)	251	62
Mean	1,423,131	74
Median	450,000	19
first quartile	200,000	5
third quartile	10,000,000	51
Minimum	6,000	0.75
Maximum	35,000,000	1,600

Table 5 Summary of number of batches per site

Number of batches per site	Number of sites
One	19
Two	24
Three	19
Four	11
Five	6
Six	8
Seven	1
Eight	6
Nine	1
10 or more	3
total	97

The oldest site was established in 1974 and the newest in 2008 (median age of production was 13 years). There was almost no variation in culture methods (Q16): 94 sites use bags on trestles but the additional responses are not consistent; 3 sites report multiple methods (and no data was recorded for 3 sites). Three sites used bottom culture, one combining this with trestles. Four sites also report cages and

one site used "tumblers on frames." Fifteen sites produced other species of shellfish (mussels or abalone).

Table 6 Production sites per bay and OshV1 status

Bay	OshV1 status ¹	sites	batches	positive batches (%)
Achill Sound South	Y	3	12	2 (17)
Baltimore/Rillen	Y	1	3	2 (67)
Bertraghbhoy Bay	N	1	1	0
Caherciveen	Y	1	6	2 (33)
Carlingford	Y	6	23	2 (9)
CastlemaineH arbour	Y	13	35	5 (14)
ClewBay	Y	9	37	3 (8)
Donegal Bay	Y	5	30	3 (10)
Dungarvan Harbour	Y	19	64	2 (3)
Dungloe	Y	2	8	2 (25)
KenmareRiver/Sneem/Ardgroom	N	1	8	0
Loughras Beg	Y	1	6	4 (67)
Poulnasherry Bay	N	5	20	0
Shannon Estuary	N	2	3	0
Sherkin	N	1	6	0
Streamstown	N	5	13	0
Trawbreaga Bay	Y	11	37	6 (16)
Trawenagh Bay	Y	6	19	1 (5)
Waterford	Y	5	15	4 (27)
Total	13	97	346	38

¹Y = OshV1 test positive by PCR in 2009, N = OshV1 not found by PCR in 2009

3.2. Mortality

3.2.1. Historic

The level of observed mortality in years up to 2008 (Q8) ranged from 0-35% for spat (median 15), 0 to 30% for juveniles (median 5), and 0-50% for adults (median 5)

(Figure 1). 43 sites reported that there had been a particularly bad year with high levels of mortality before 2009: 17 noted 2008, 8 noted 2007 and 8 noted 2005. Some of the signs reported for earlier mortalities match signs reported in 2009 signs and three reported OHV as a cause of mortality in years prior to 2009; others indicated different causes. Of the three that reported OHV in 2008, two were confirmed and 1 was untested in 2009.

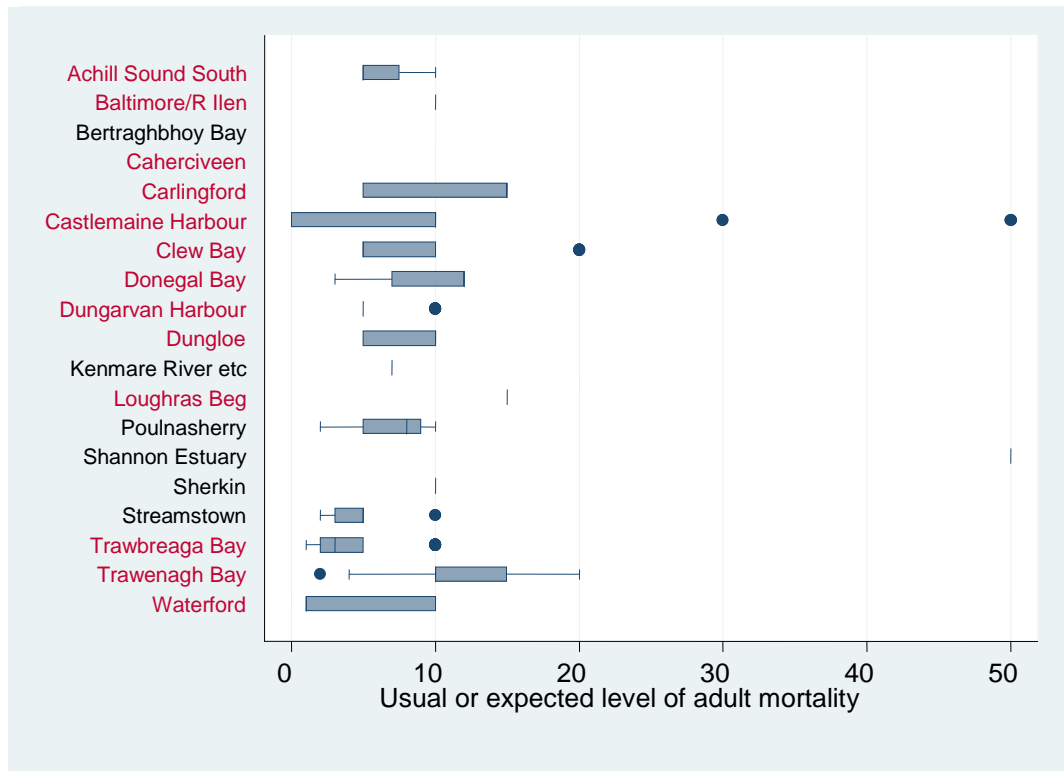


Figure 1 Adult oyster mortality in 2008 by bay (OshV1 positive bays in 2009 are coloured red)

3.2.2. Mortality during 2009

3.2.2.1 Rainfall and temperature

The temporal association between rainfall and mortality within a bay was investigated for bays where data were available. Rainfall was monitored in 2 locations in Clew Bay. High rainfall was recording during the first 5 days of July (Figure 2) and mortality started on July 6th 2009 in three sites. However, this

association was not observed in other bays where data from monitoring points in reasonable proximity to affected sites were available (including Castlemaine Harbour, Dungarvan Harbour and Carlingford Lough).

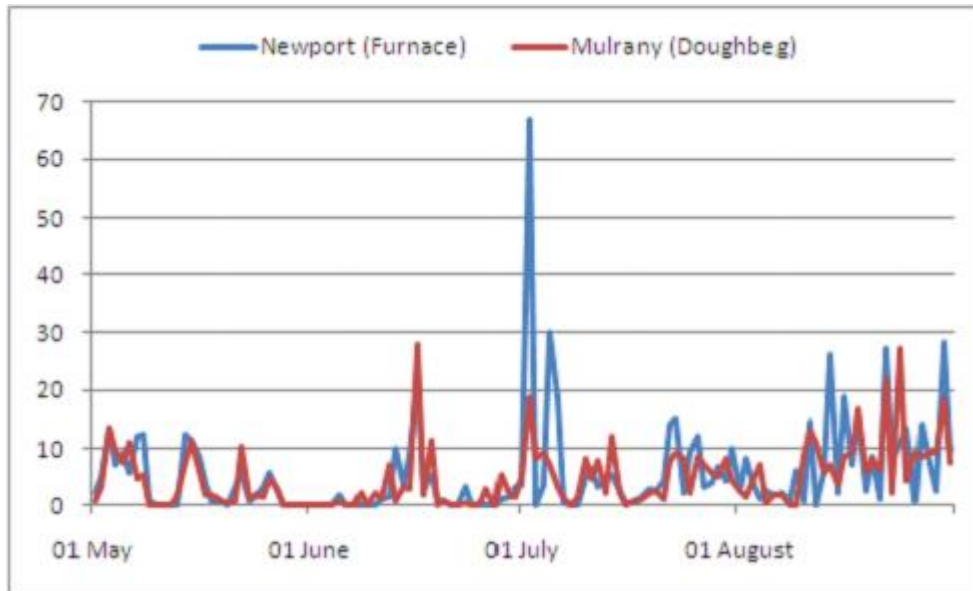


Figure 2 Rainfall in Clew Bay in 2009 (mm)

Water temperature were available for Castlemaine harbour (Figure 3) and Inver bay (Figure 4) (which is in close proximity to sites in Donegal bay). In Castlemaine harbour mortalities started the week following the spike in water temperature in early June. In Donegal Bay mortalities only occurred after the second spike in water temperature which started on 29th June.

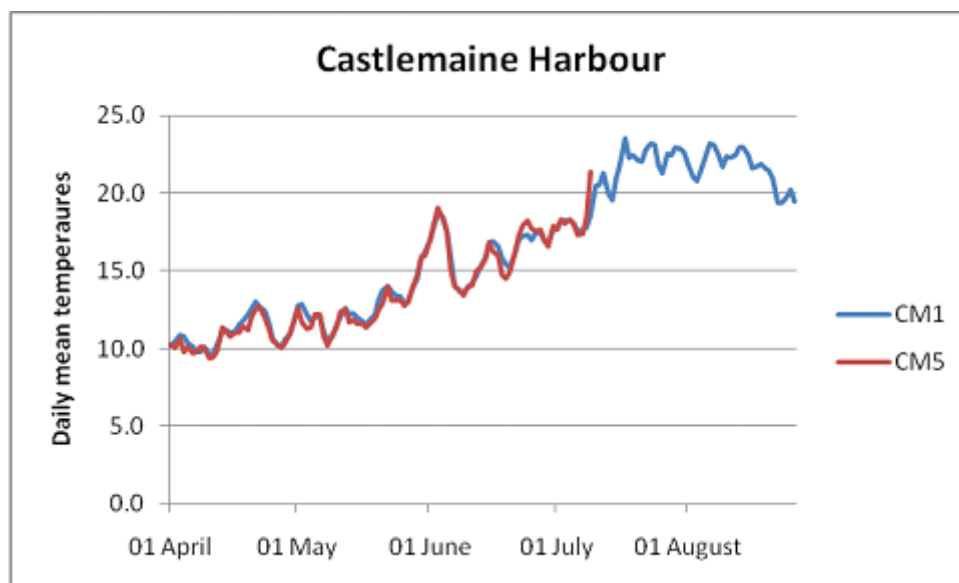


Figure 3 Water temperature data from data logging stations (CM1 & 2) in Castlemaine harbour in 2009

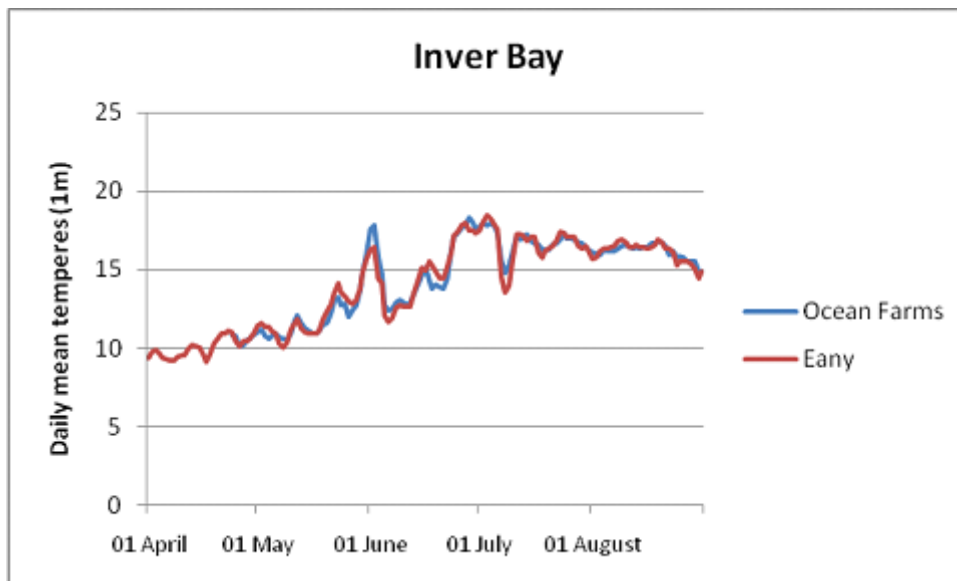


Figure 4 Water temperature records from logging stations in Inver Bay in 2009

3.2.2.2 Site level analysis

Sites were classified as diseased if mortalities had been reported (Q26: 43 sites) or a positive OHV test had been found for at least one batch (Q30: 1 extra site). This left 56 sites apparently disease-free and reporting no unusual mortality in Q26 & Q27. There was no statistically significant difference in the level of mortality reported during 2008 (Q10) or previous years (Q9) between affected and unaffected sites.

It needs to be noted that in Dungarvan Harbour the site reporting mortality early in the season had mortality in 07 stock and on grading in March / April 2009 empty shells were noticed – indicative of mortality during 2008.

Plotting the start date of mortality against end date at the level of the site (using data only from bays where OsHV1 had been detected) revealed a pattern (Figure 11) consistent with spread of disease from site to site. The pattern could arise from sequential introductions; however, further evidence of contiguous spread can be derived by mapping the spread of OsHV1 between sites. In Trawbreaga Bay three of the eleven sites in operation introduced stock from France between 15th May and 1st June 2009 (Figure 8). Any of these introductions may be considered as potential sources of virus introduction; all other stocks present during the investigation period were introduced from sources free of OsHV1 (i.e. from UK and Guernsey in 2009 or before, or other sites in Ireland before 2008) and none of the sites in the bay suffered increased mortality in 2008. All sites but one reported an onset of mortality between 29th June and 20th July 2009. OsHV1 infection in 3 stocks from sites with no oysters from potentially infected sources was confirmed by PCR. Eleven stocks of 37 present remained unaffected by disease, all but one of these were older stock introduced prior to 2009. Contiguous spread between sites (i.e. to stock from disease-free sources) was evident from similar analysis of movements and time of mortality onset in Clew bay.

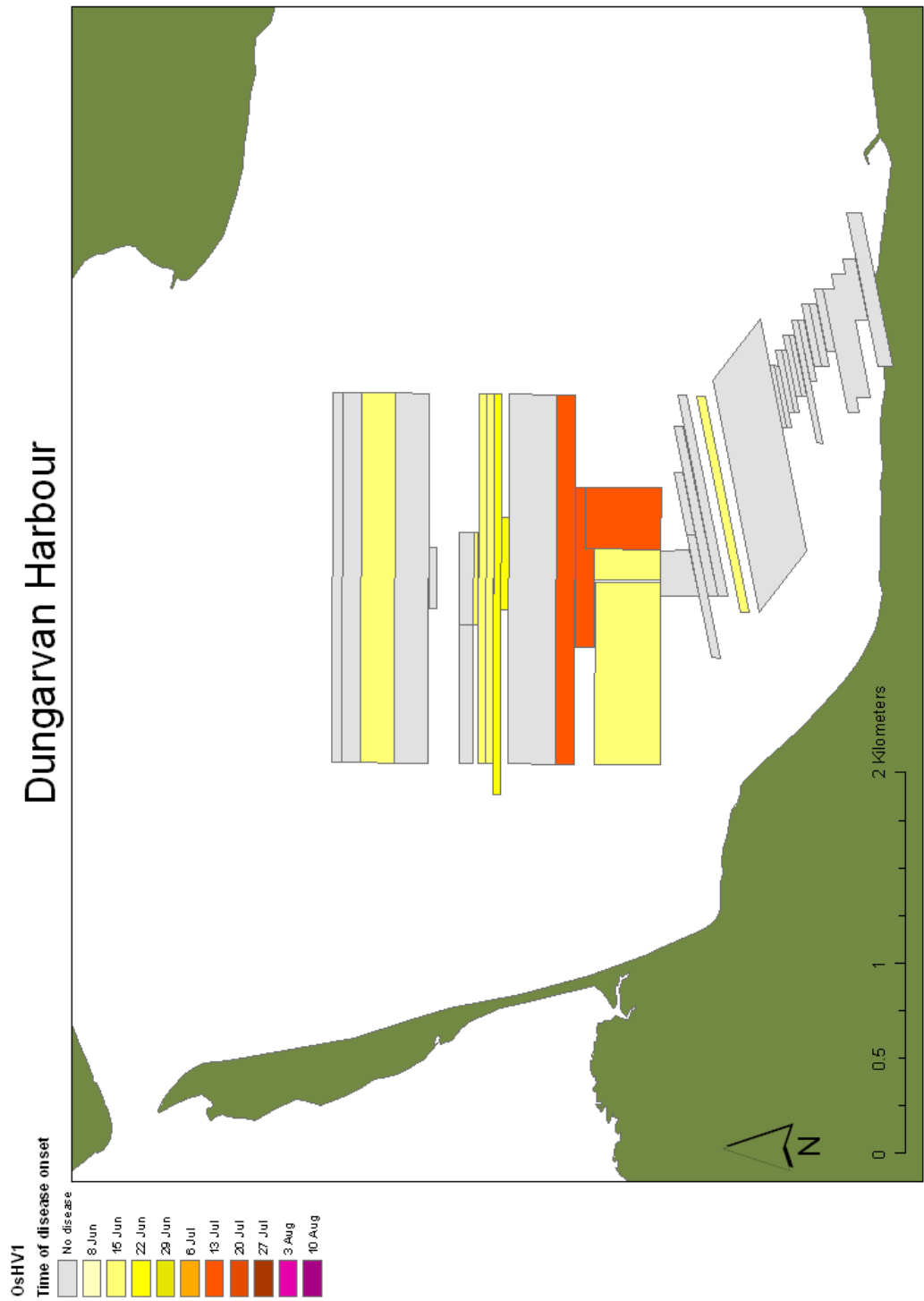


Figure 6 Oyster harvesting sites in Dungarven Harbour suffering mortality in 2009 (showing date when mortality was first observed)

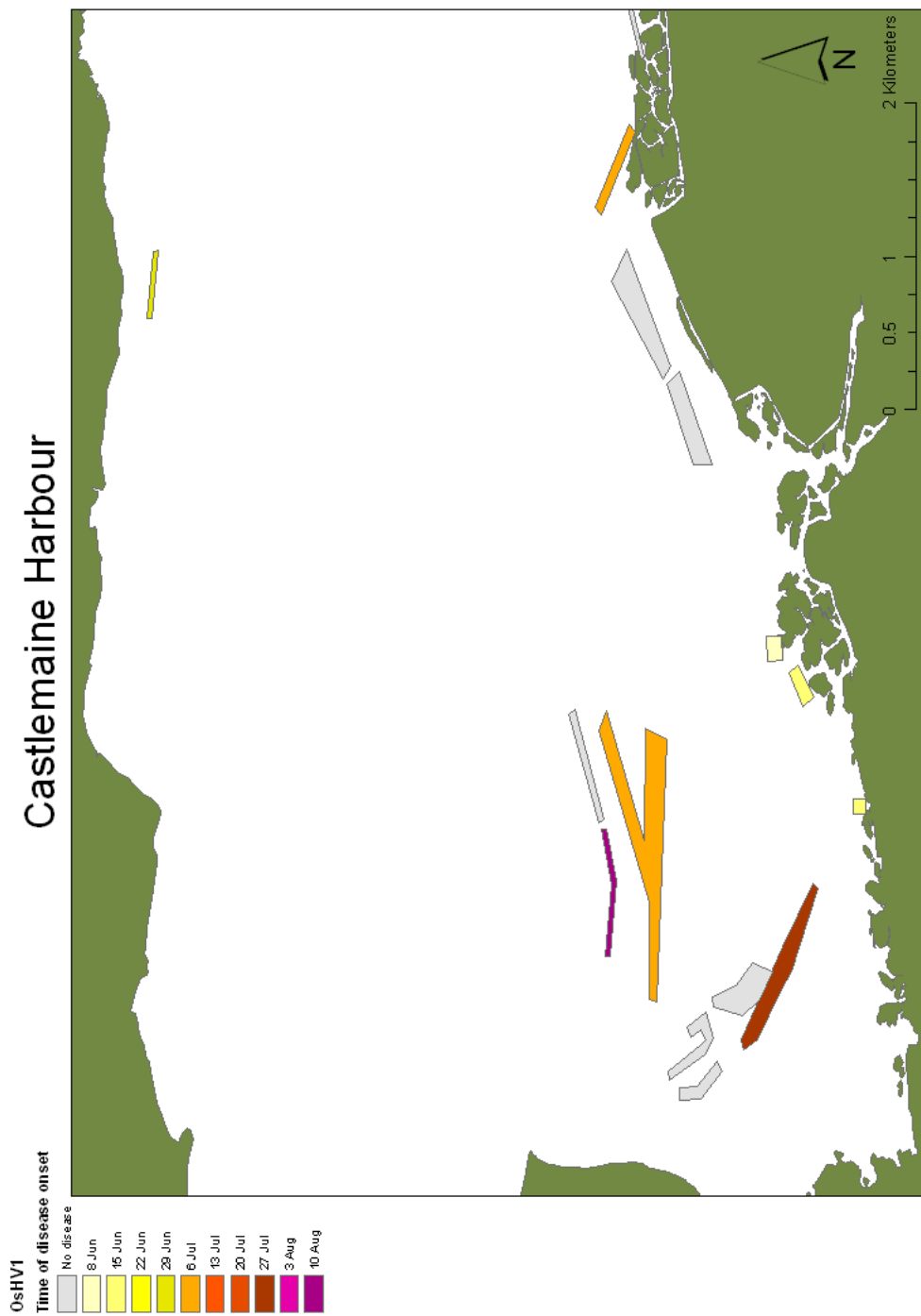


Figure 7 Oyster harvesting sites in Castlemaine Harbour suffering mortality in 2009 (showing date when mortality was first observed)

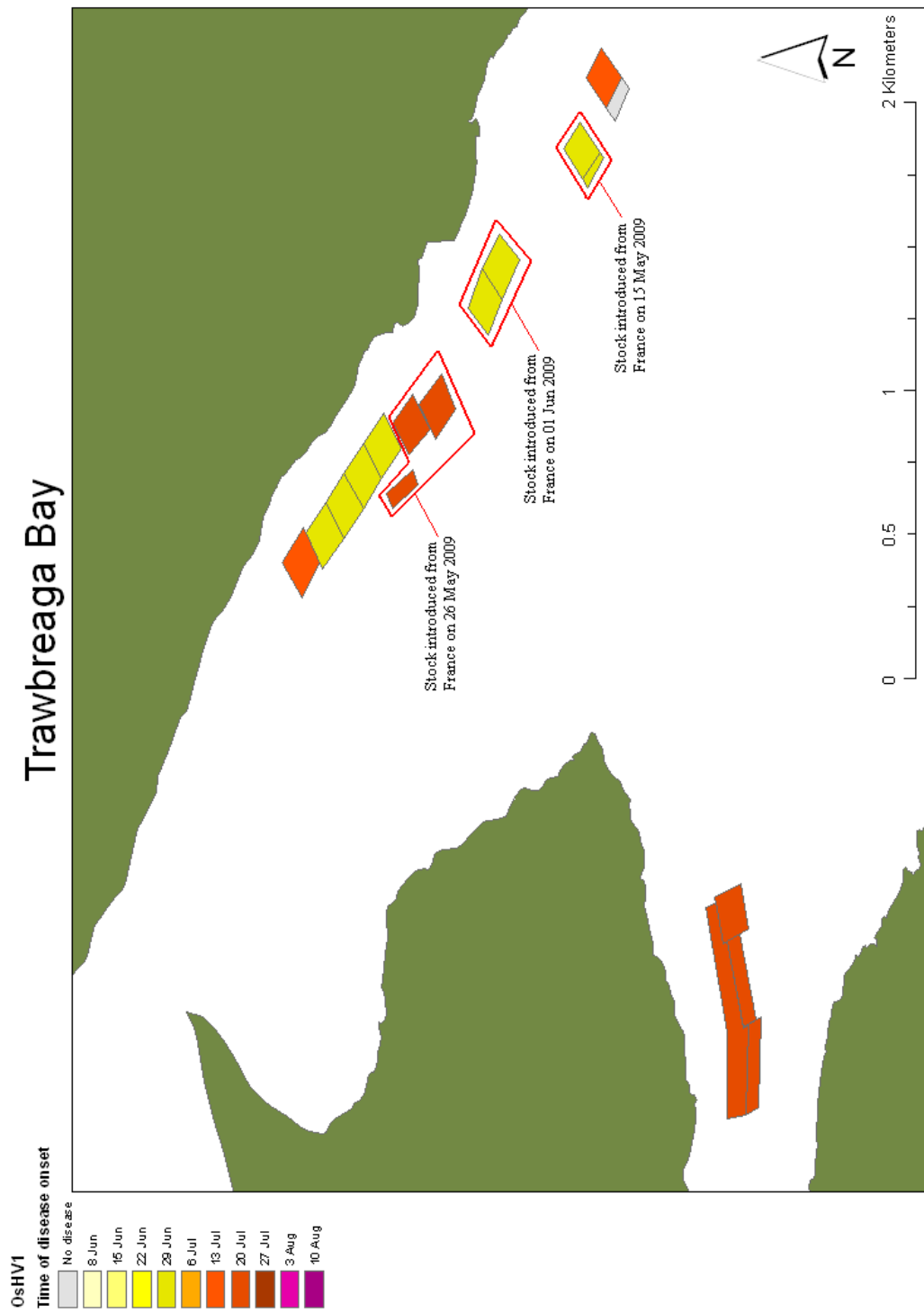


Figure 8 Oyster harvesting sites in Trawbreaga suffering mortality in 2009 (showing date when mortality was first observed)

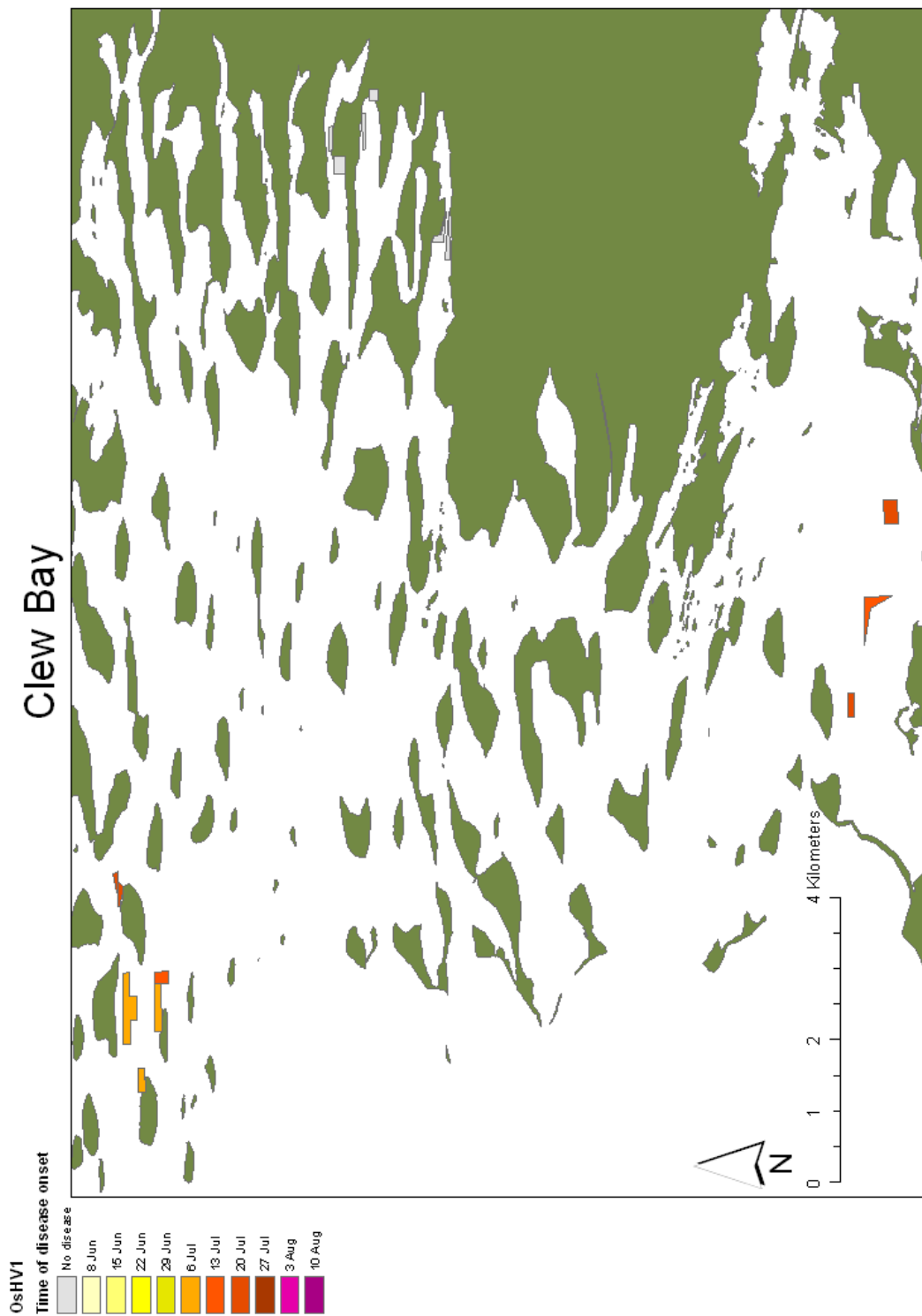


Figure 9 Oyster harvesting sites in Clew Bay suffering mortality in 2009 (showing date when mortality was first observed)

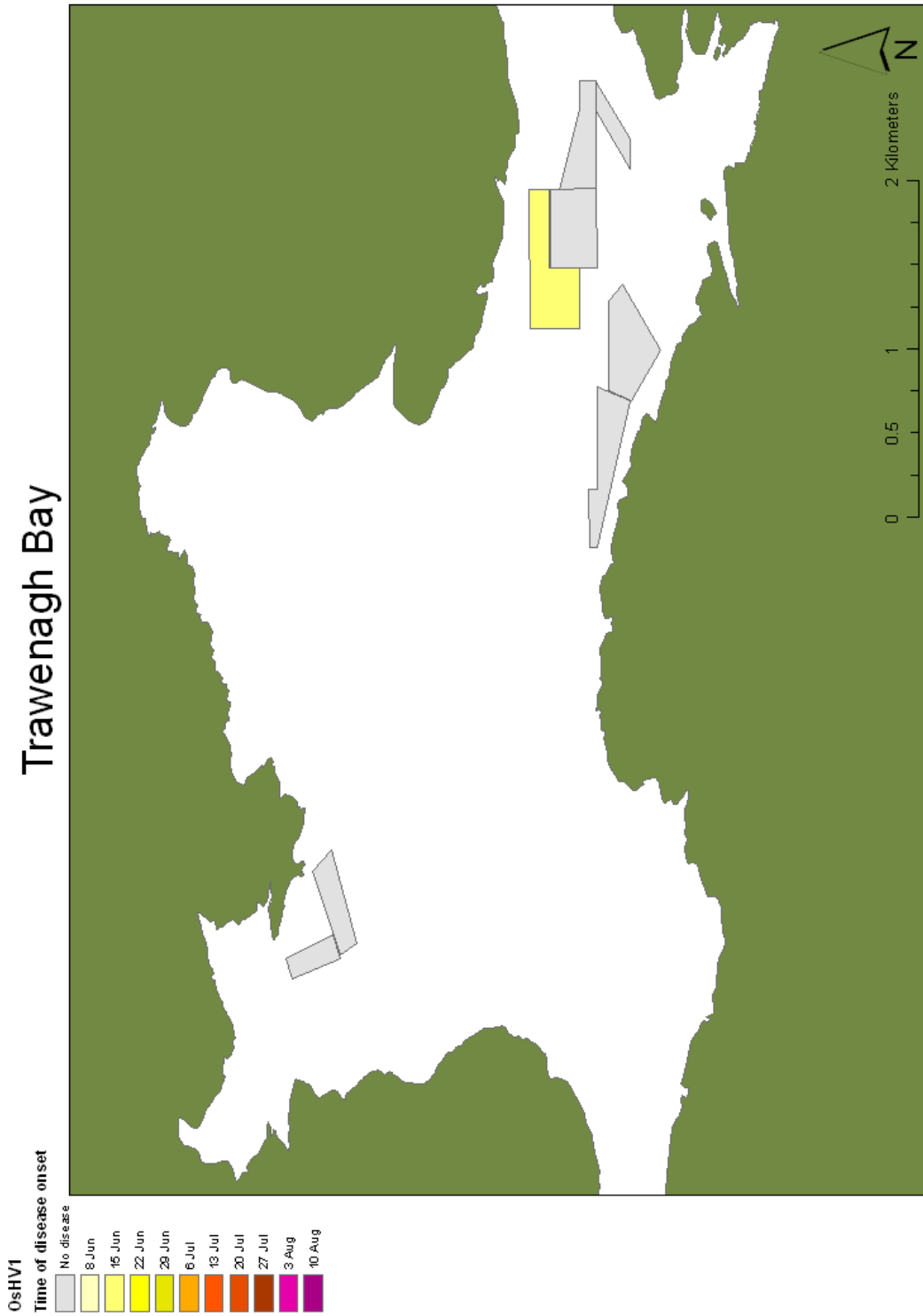


Figure 10 Oyster harvesting sites in Trawenagh suffering mortality in 2009 (showing date when mortality was first observed)

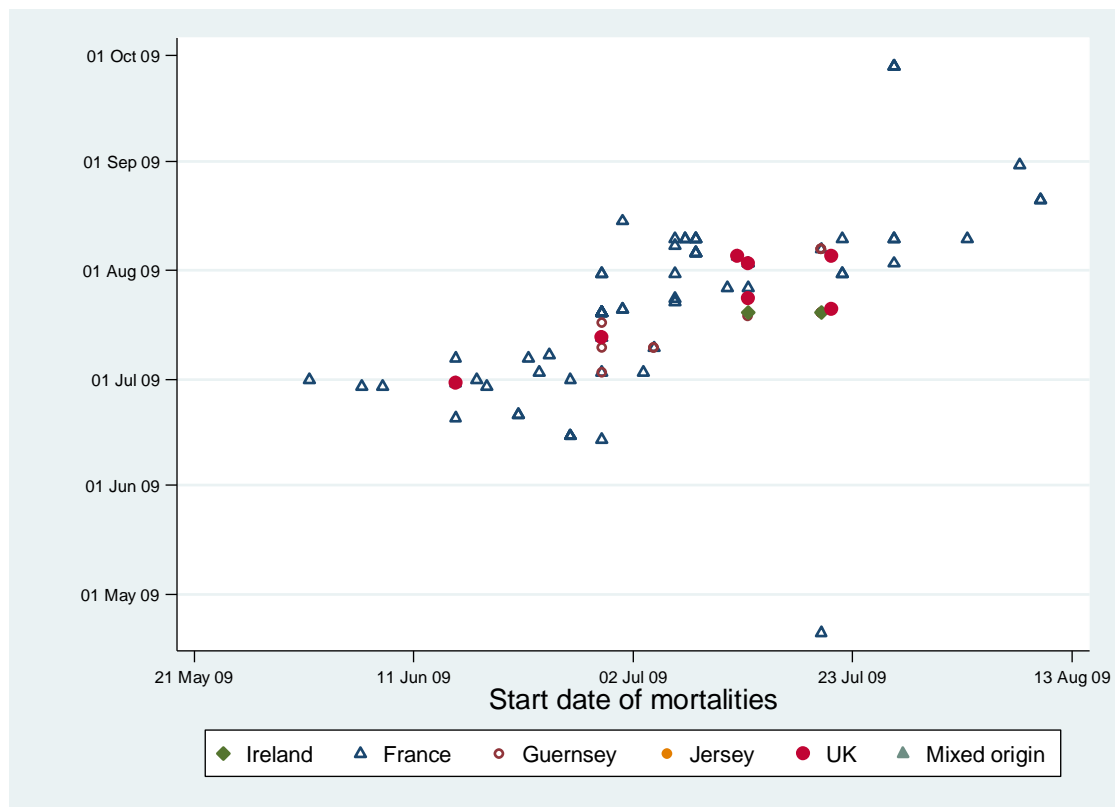


Figure 11 Start of mortality against end of mortality (for OsHV1 positive bays, symbol indicates country of origin)

3.2.2.4 Mortality at the level of the batch (all bays)

Mortality was observed in 109 batches from 47 sites (from a total of 346 batches and 97 sites). 104 of the 109 batches are located in bays where OsHV1 had been detected. Batch level analysis indicated a higher level of mortality in 2009 compared to previous years (Figure 12 and Figure 5). The median batch mortality was 37% (18-65% inter-quartile range) (the mean was 42%) (n=109) for all batches and 43% (median 38.5%, IQ range 20-62.5%) for batches in positive bays.. There was no statistically significant difference in mortality with test status. There was considerable variation in the level of mortality at the batch level between bays (Figure 12). Mortality was continuous in nearly all batches (in 2 batches it was reported as occurring in two waves).

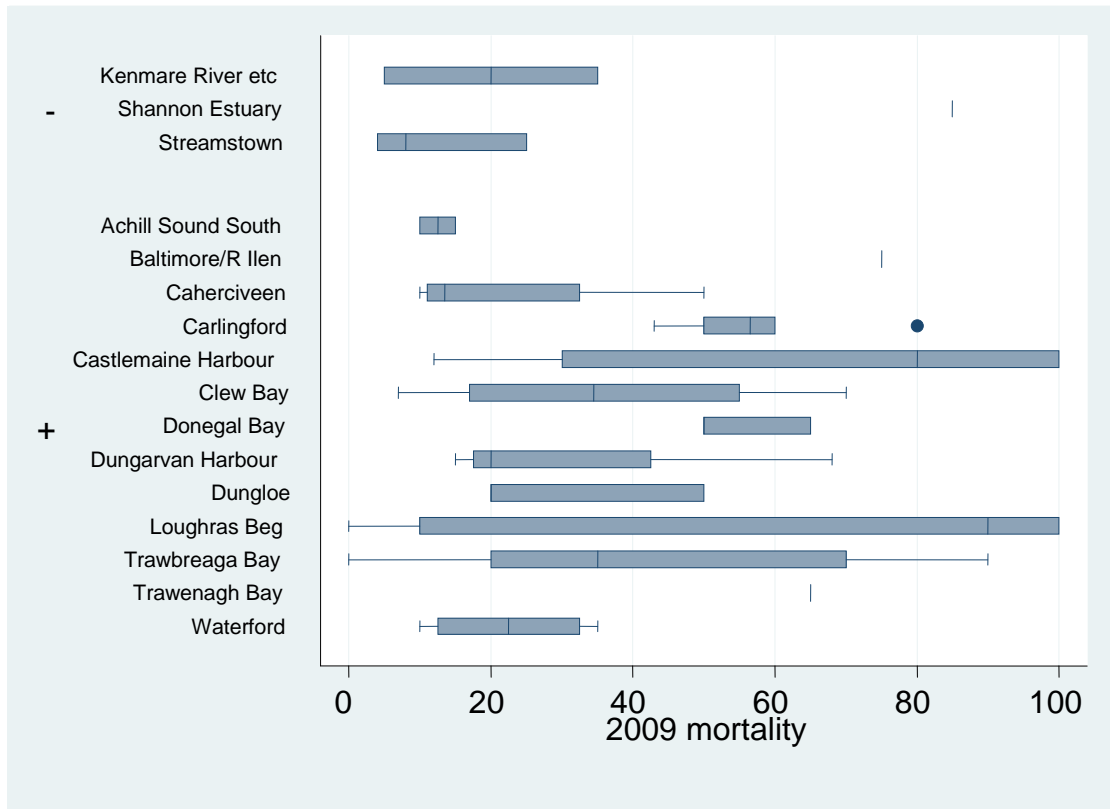


Figure 12 Batch level mortality in 2009 by bay (all bays, grouped OSHV positive and negative)

Mortalities that occurred early in 2009 were not tested and therefore it was not possible to demonstrate if they were OshV1 positive (Figure 13). However, the first mortalities were in batches imported in 2009 or 2008. Later, mortalities occurred in batches that had been on site for several years. This supports the hypothesis of an introduction of an infectious agent with purchased stock which subsequently spreads to the established population.

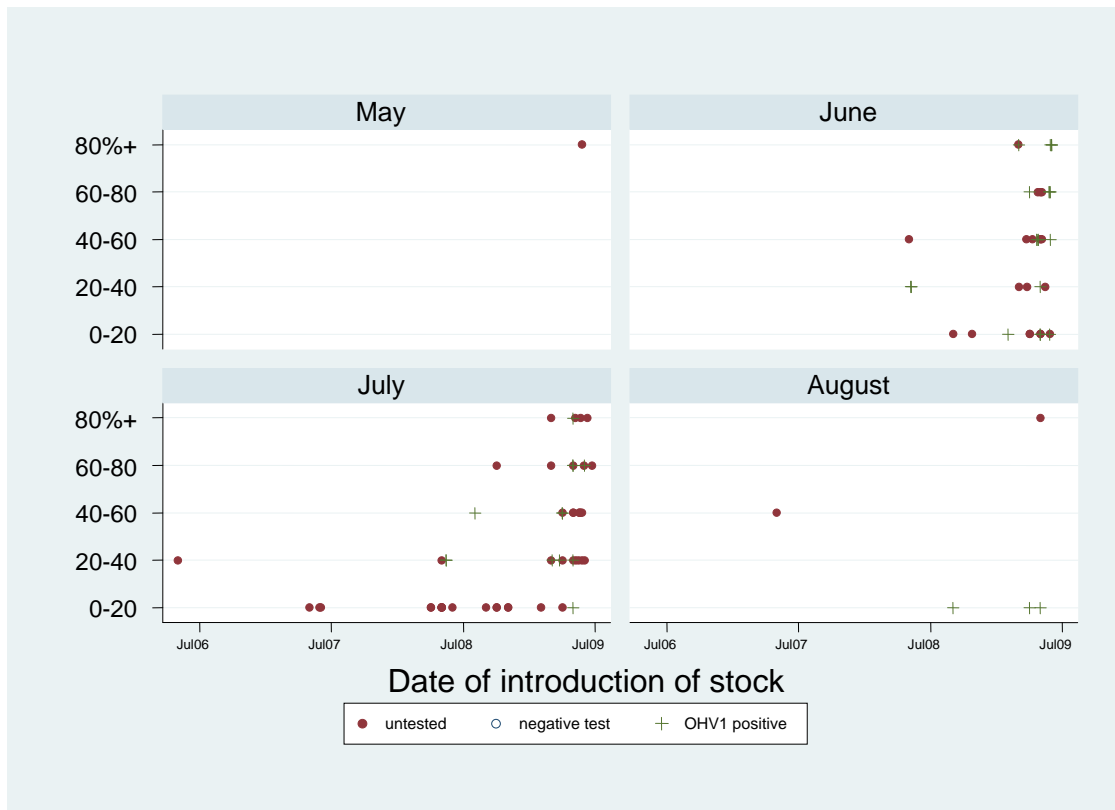


Figure 13 Mortality (batch level) split by date of introduction (positive bays) (Y-scale mortality codes: 1=0-20%, 2=>20-40%, 3=>40-60%, 4=>60-80% and 5=>80%)

Table 7 Categorized batch level mortality broken down by bay

Bay	No report	>0-20%	21-40%	41-60%	61-80%	>80%	Total
Achill Sound South	10	83%	2 17%	0 0%	0 0%	0 0%	12
Baltimore/ Rllen	1	33%	0 0%	0 0%	0 0%	2 67%	3
Bertraghbhoy Bay	1	100%	0 0%	0 0%	0 0%	0 0%	1
Caherciveen	2	33%	3 50%	0 0%	1 17%	0 0%	6
Carlingford	17	74%	0 0%	0 0%	5 22%	1 4%	23
Castlemaine Harbour	20	57%	3 9%	1 3%	3 9%	3 9%	35
ClewBay	19	51%	7 19%	5 14%	4 11%	2 5%	37
DonegalBay	21	70%	0 0%	0 0%	6 20%	3 10%	30
Dungarvan Harbour	56	88%	5 8%	1 2%	1 2%	1 2%	64
Dungloe	5	63%	2 25%	0 0%	1 13%	0 0%	8
KenmareRiveretc	6	75%	1 13%	1 13%	0 0%	0 0%	8
Loughras Beg	1	17%	2 33%	0 0%	0 0%	0 0%	6
Poulnasherry	20	100%	0 0%	0 0%	0 0%	0 0%	20
Shannon Estuary	2	67%	0 0%	0 0%	0 0%	0 0%	3
Sherkin	6	100%	0 0%	0 0%	0 0%	0 0%	6
Streamstown	10	77%	2 15%	1 8%	0 0%	0 0%	13
Trawbreaga Bay	15	41%	6 16%	9 24%	1 3%	2 5%	37
TrawenaghBay	18	95%	0 0%	0 0%	0 0%	1 5%	19
Waterford	7	47%	4 27%	4 27%	0 0%	0 0%	15
Total	237	68%	37 11%	22 6%	22 6%	15 4%	346

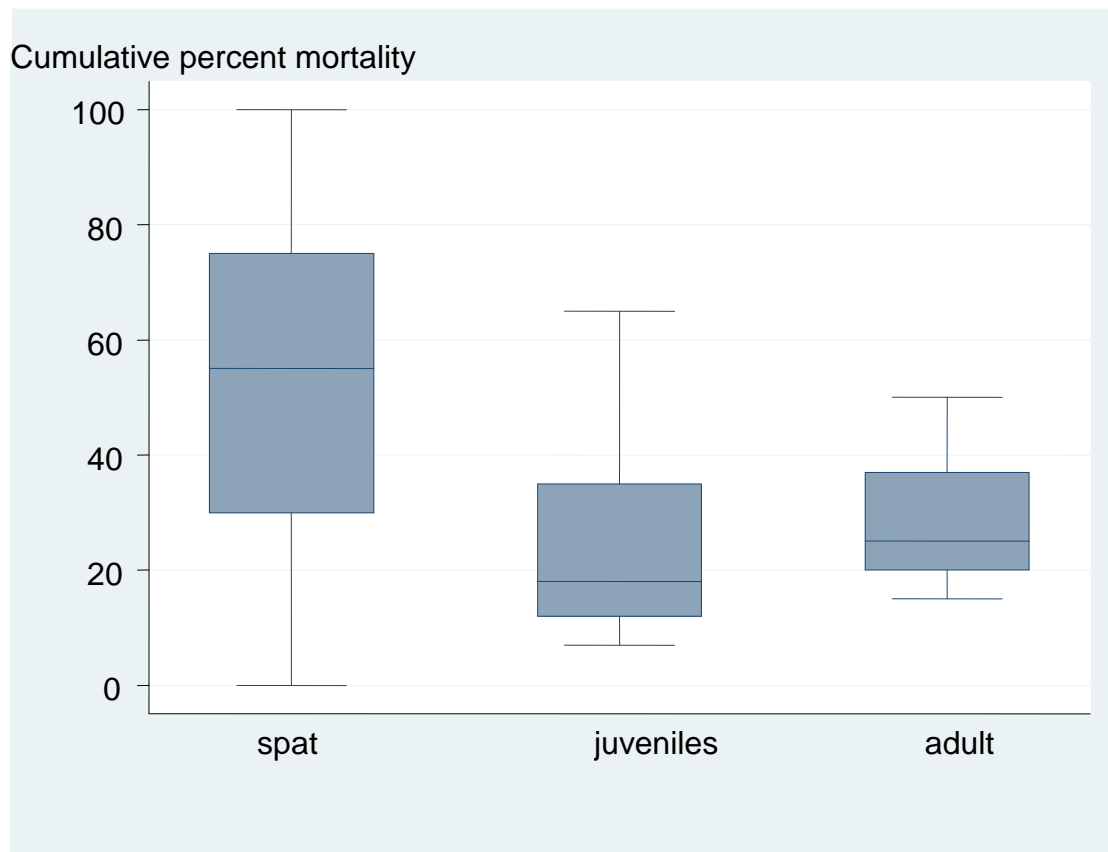


Figure 14 Analysis of cumulative mortality in 2009 by batch broken down by age group (OsHV1 positive bays only, n=104)

3.2.2.5 Temporal analysis – sites from bays where OsHV1 was detected

The average duration of mortality in batches of oysters from sites in bays where OsHV1 was detected was 18.6 days. The earliest recorded onset was 25/5/9 and the latest was 10/8/9 and peaked in late June (mortality starting in 9 sites during the week beginning 29/6) (Figure 15). The longest duration of mortality was 63 days (Figure 16).

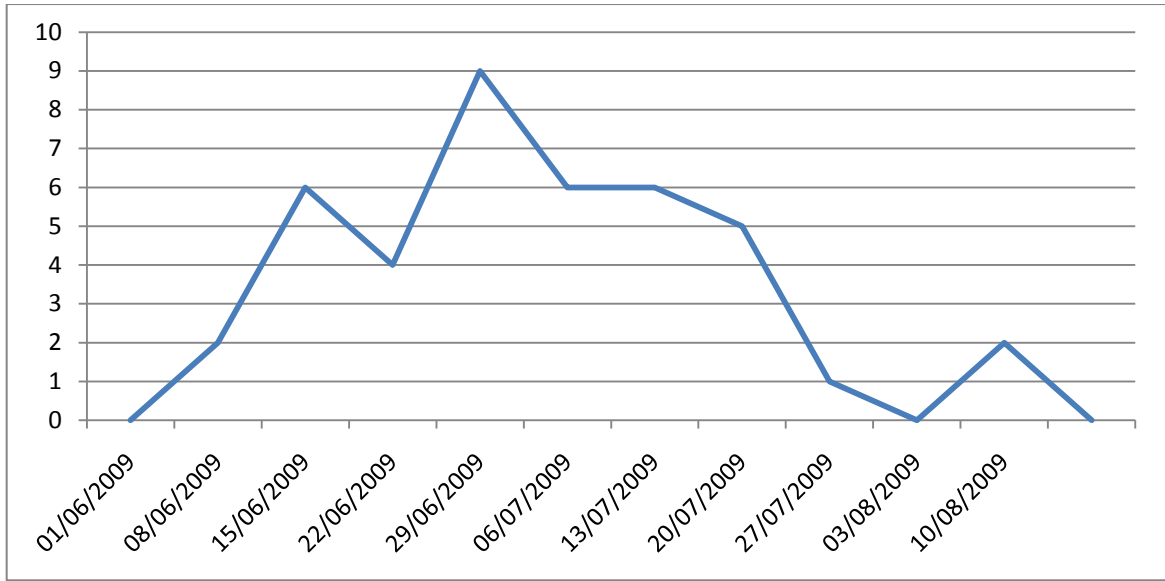


Figure 15 Onset of mortality by site over time (OshV1 positive bays only)

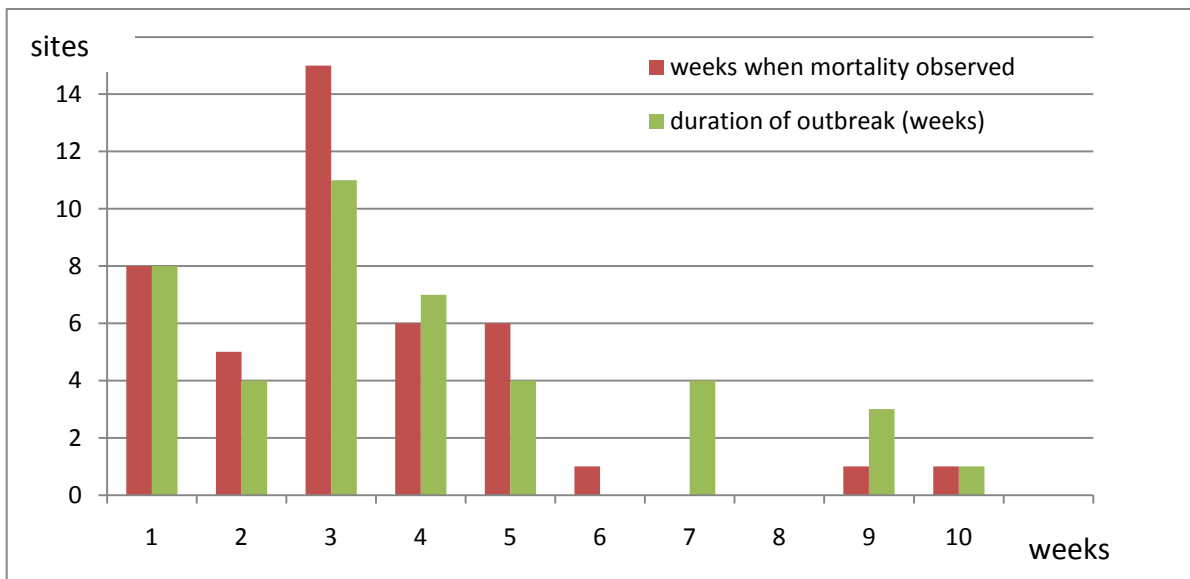


Figure 16 Sites by duration of outbreak and weeks with mortality (OshV1 positive bays only)

For the majority of sites for which batch level mortality had been provided mortality was continuous (35 of 43 sites), on the remaining 8 sites weeks when no mortality was recorded occurred during the outbreak (Q27). This finding is inconsistent with other data: growers reported that mortality was continuous in all but two batches. The duration of the outbreak (from the first to last week when mortality was recorded) and duration of mortality on most sites lasted between 1 and 5 weeks

(duration was longer on sites with a discontinuous pattern of mortality) (Figure 16). On average mortality was recorded over 3.3 weeks.

3.2.3. Associations between batch level variables and mortality (data from OSHV1 positive bays)

At the batch level the introduction of trestles had no effect. Mortality was significantly higher in spat compared with other age groups (Figure 14). There was a strong association between triploidy and higher batch level mortality (Table 8). 29% of triploid batches experienced >20% mortality compared with 12 % of diploid batches. These results produced a highly significant chi-squared value.

Table 8 Level of mortality (batch) broken down by diploid and triploid status

	Mortality (batch level)			Total
	<20%	20-40%	>40%	
Diploid	99	4	10	113
	87.61%	3.54%	8.85%	
Triploid	137	17	39	193
	70.98%	8.81%	20.21%	
Total	236	21	49	306

This was explored further by investigating mortality in wild sourced compared with hatchery origin batches from France (Table 9). Whilst a greater proportion of wild stock batches suffered lower levels of mortality, the difference was not significant.

Table 9 Level of mortality (batch) in French stock broken down by origin (wild or hatchery)

	Mortality (batch level)		Total
	0-40%	>40%	
Hatchery	138	33	171
	80%	20%	
Wild	32	3	35
	91%	9%	
Total			206

Manual compared with mechanical handling is associated with higher levels of mortality (~80 versus ~50%) (Figure 17). It is possible that manual handling provides more opportunity to observe and more accurately record mortality.

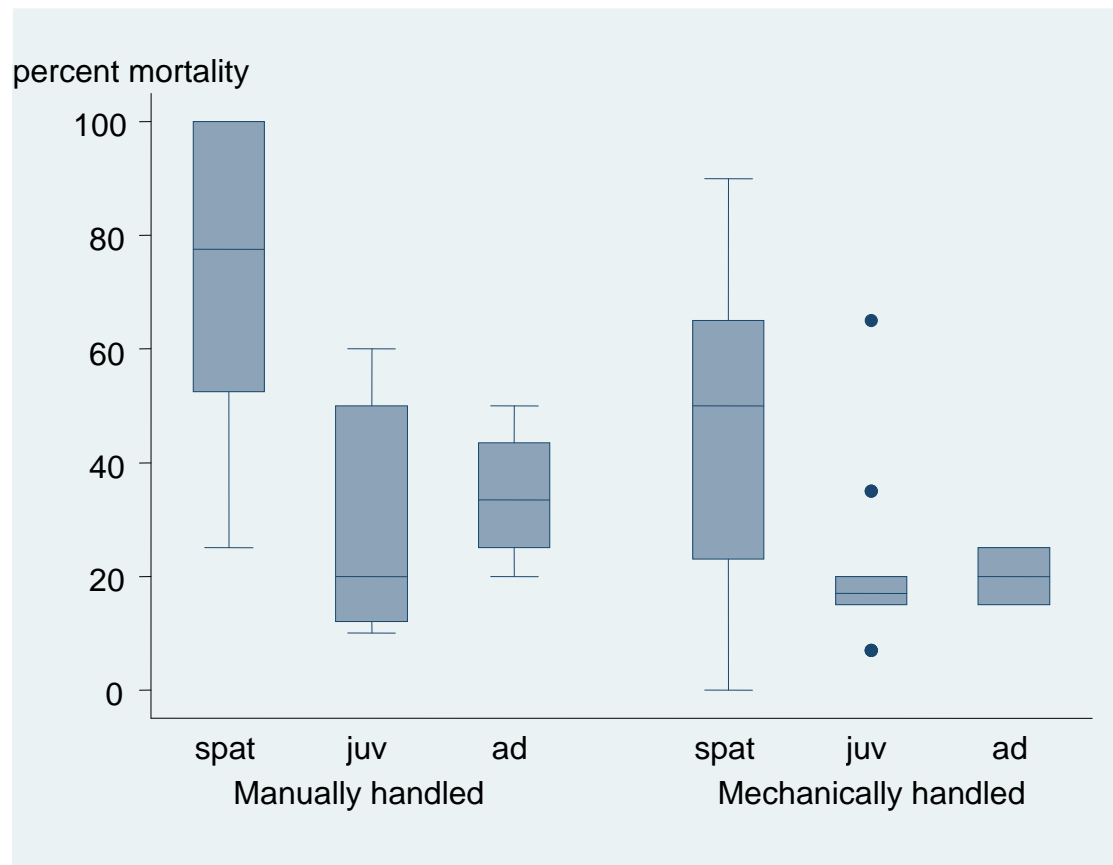


Figure 17. Association between handling and loss of stock by age (batch level analysis using OshV1 positive bays only)

Size when introduced, stocking density (biomass per bag) and grade (size) at start of mortality were investigated. No association was found between biomass per bag and total cumulative mortality (Figure 18).

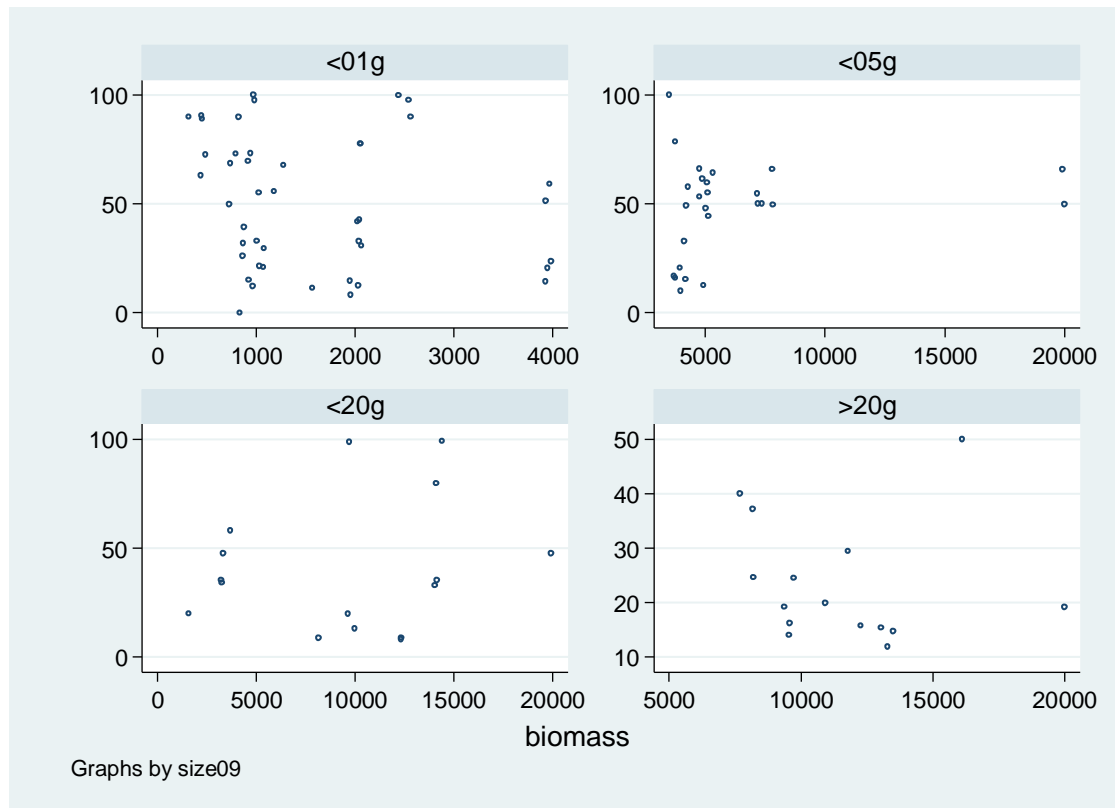


Figure 18 Cumulative mortality against biomass per bag at time of onset of mortality for different sizes of oysters

Animals introduced as spat (<1g) experienced more variable and higher levels of mortality compared with older animals, whilst animals introduced when greater than 20 gms had the lowest level of mortality (Figure 19). However, age at introduction is confounded with time of year (spat are more likely to be introduced in the winter). Similarly batches of larger (and therefore older) animals at the onset of mortality experienced lower levels of mortality compared with batches of smaller animals (Figure 20).

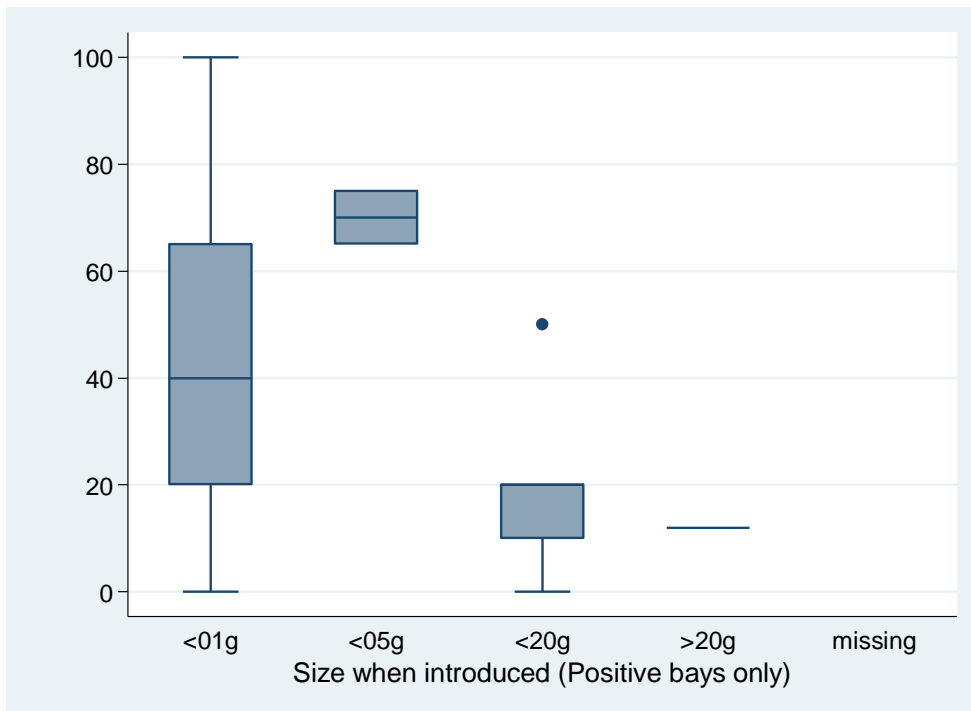


Figure 19 Cumulative mortality by size when introduced (batch level) (positive bays only)

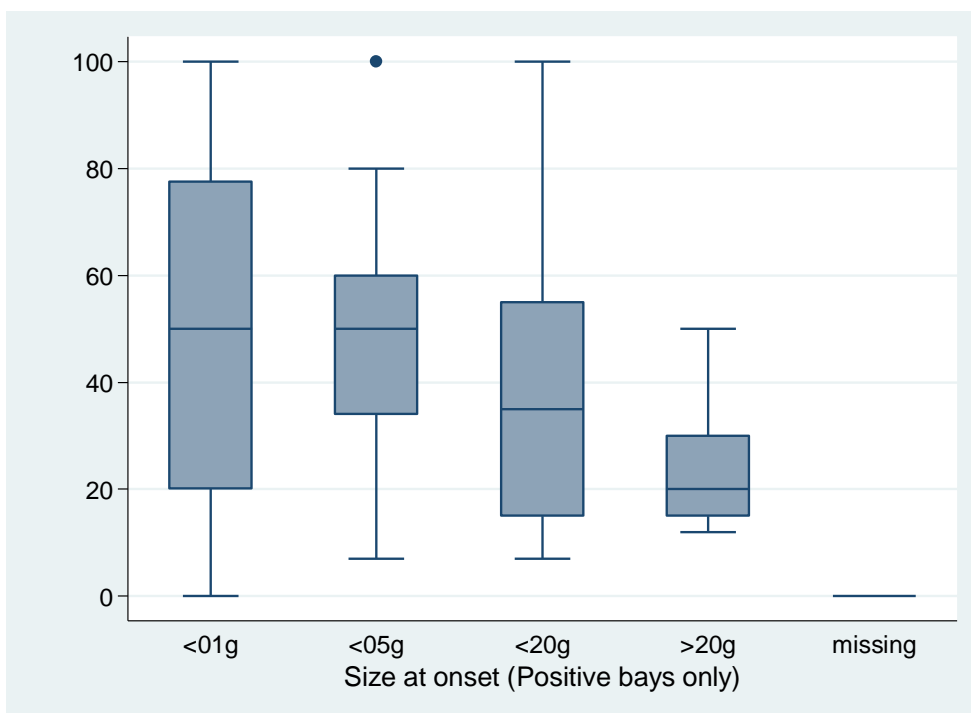


Figure 20 Cumulative mortality by size of oyster at onset of mortality (positive bays only)

When the data were categorized there appears to be an association at the batch level between the longest time spent out of water and mortality. No batches which spent more than 8 hour out of water suffered mortality at levels greater than 40% (Figure 21). There was no obvious association with the shortest time out of water.

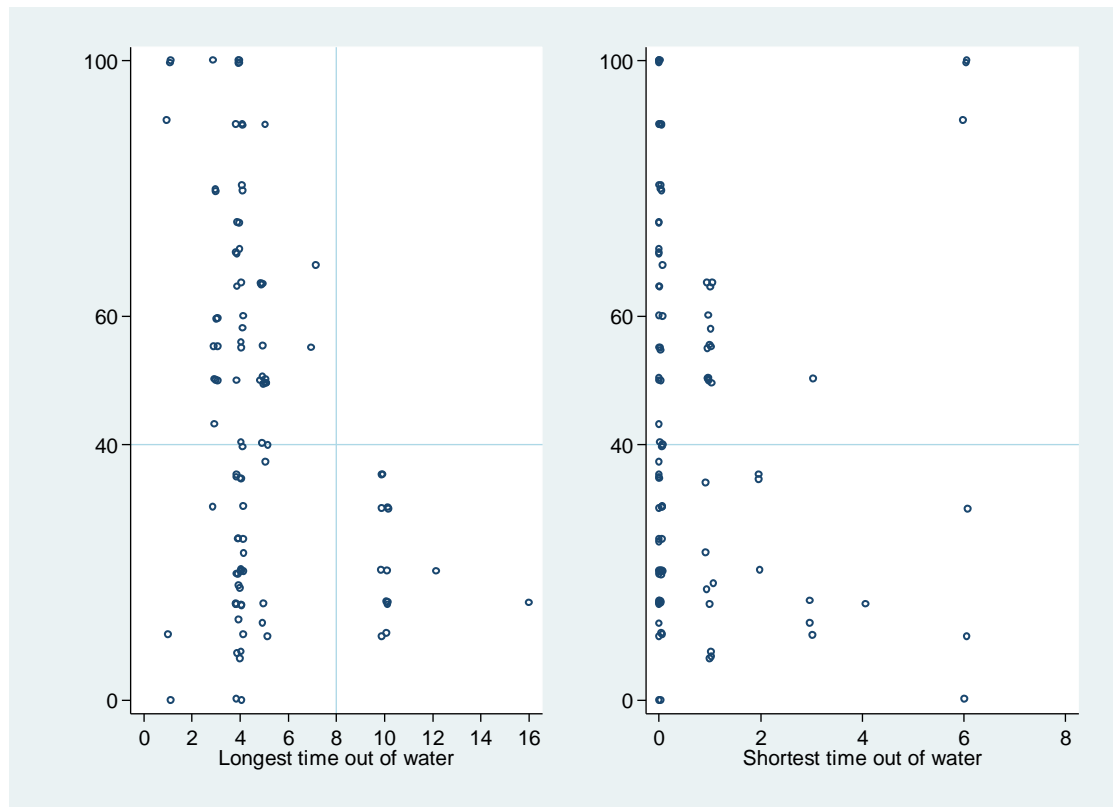


Figure 21 Time out of water against cumulative mortality (batch level)

3.2.4. Time on site before infection

The association between the number of days on site before the onset of mortality and the level of mortality was investigated at the batch level using data from infected bays. There was no indication, contrary to the views of some farmers, that the longer period period between introduction and onset of mortality, the lower the level of cumulative mortality (Figure 22).

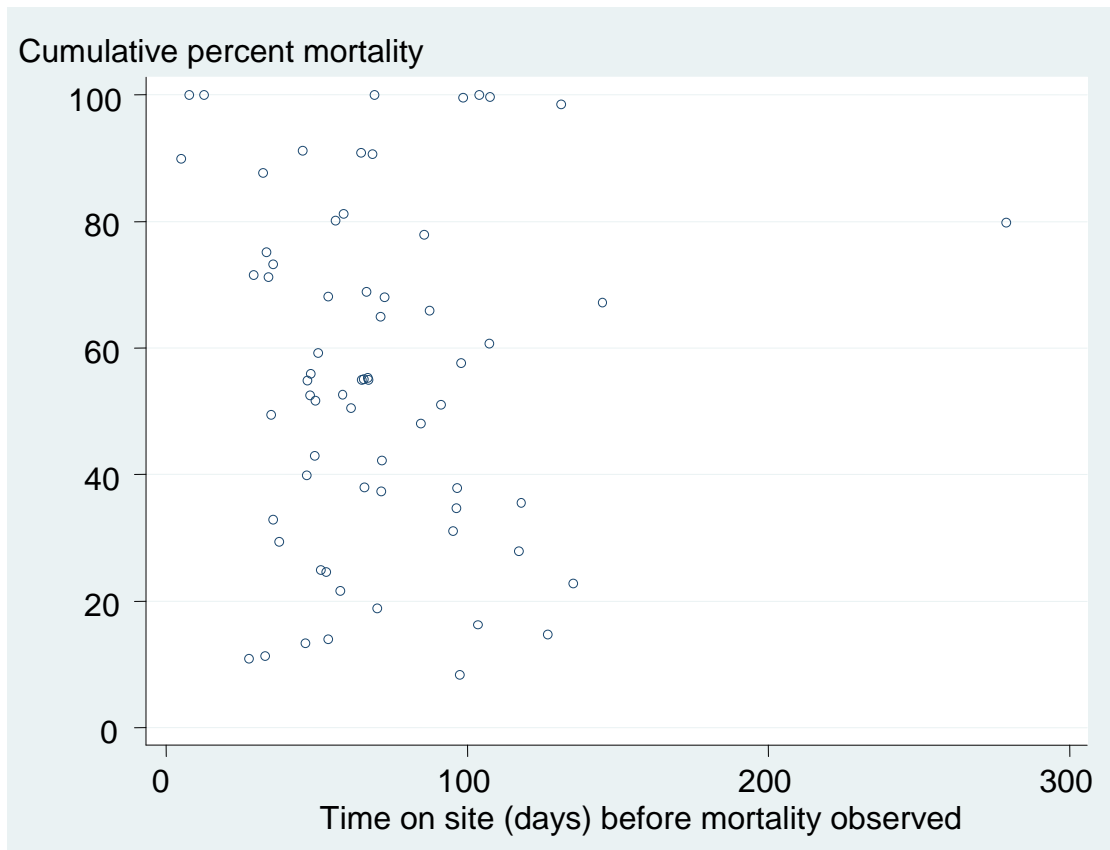


Figure 22 Time on site versus cumulative mortality in 2009 (batch level analysis)

3.3. Explanatory variables for mortality at the site level

3.3.1. Production

The production of other species of shellfish or the level of oyster production was not associated with being a case site (i.e. reporting abnormal levels of mortality), using all sites or only using sites from OsHV1 positive bays.

3.3.2. Introduction of equipment to sites

27 sites imported trestles (10 including bags); 19 were new sites. 19 sites imported bags; 13 were new. Being affected with disease was very strongly related to importing trestles (odds ratio = 4.85, $P=0.001$) but not bags; it was further linked to the equipment being new. This is counter-intuitive and may have arisen because introduction of equipment is associated with expansion of production or another risk factor. Materials were brought in from Ireland, UK, France and Spain. Repeating the

analysis for sites only from bays where OsHV1 had been detected gave a similar result.

3.3.3. Handling

3.3.3.1 Frequency

Q18 shows considerable variation in amount and pattern of inspection / handling. Four sites reported no handling at all, and eleven sites handled less than once monthly (12 or fewer in the year). At the other extreme, nine sites handled 360 or more times in the year, i.e. daily activity. At sites with more than one age group, the handlings for each age group are strongly correlated.

A logistic regression shows the total number of handlings as statistical significant ($p < 0.05$) as a predictor of the probability of being affected. However, the statistical significance disappears when the top points (two at 720 handlings/year) are omitted ($p > 0.15$). There is therefore no evidence that increased handling increases the risk of being affected, but clearly more frequent handling gives more opportunities for mortality to be observed. A delay in identifying losses may result in higher levels of cumulative mortality.

3.3.3.2 Method of handling

Q19 shows roughly 60:40 % mechanical to manual handling at all ages, but 20% of sites used both. Only one site marked the "not handled" column and the other three with zero handlings left the table blank, as did a site that carried out 120 handlings per year.

There was a striking association with manual handling and the risk of being an affected site. However, only 11 sites did not manually handle (and none was affected); 9 of the 11 sites were in OsHV1 positive bays. The association could again be linked to a greater likelihood that mortality and disease were observed. The same association was apparent when the analysis was repeated using only data from bays which were OsHV1 positive.

3.3.4. *Period of time exposed to air*

A logistic regression on the shortest time out of water at the site is completely non-significant, but a regression on the longest time out of water approaches significance but again this evaporates when the single largest observation (16h/day out of water) is excluded.

3.3.5. *Number of country sources of stock*

The distribution of affected and unaffected sites by number of countries from which a site had sourced stock (Table 10 & Table 11) was examined. Whilst positive sites were more likely to have introduced stock from more than one site (odds ratio>2) this was not found to be significant (chi-squared value not significant, $p = 0.14$).

Table 10 Sites broken down by number of countries supplying stock and disease status (all sites)

Number of countries supplying stock	Positive Sites	Negative sites
One	28	45
Two	11	12
Three	2	0
Four	2	0
	43	57

Table 11 Sites broken down by number of countries supplying stock and disease status (only sites from OsHV1 positive sites)

Number of countries supplying stock	Positive Sites	Negative sites
One	28	40
Two	10	9
Three	2	0
Four	1	0
	41	49

3.4. Bay level analysis

3.4.1. Origin of movements

At the level of the bay, introductions of oysters from France compared with GB, were strongly positively associated with the OsHV1 status of the bay (Table 12). A chi-squared test comparing movements to positive and negative bays from GB and Guernsey (OsHV1 free) compared with all other countries was highly significant (

Table 13).

All affected bays (14/14) received triploid stock and 9 of 14 received diploid stock from France. Of the unaffected bays 3 of 6 received French stock (1 diploid, 2 triploid). These findings are consistent with the hypothesis that French triploid spat is the source of infection (statistical analysis is not appropriate with such a small sample size).

Table 12 Introduction of stock by country of origin and OsHV1 status of the bay

Origin of stock	Number of batches introduced by	
	OsHV1 status of the bay	
	Positive bays	Negative bays
France	201	5
Ireland	32	4
Guernsey	36	4
GB	37	22

Table 13 Introduction of stock by country of origin grouped by country level OsHV1 status and OsHV1 status of the bay

Origin of stock	Number of batches introduced by	
	OsHV1 status of the bay	
	Positive bays	Negative bays
France/ Jersey/Ireland/other	235	12
GB & Guernsey	73	26

OR ~6, chi-squared 31.1 P<0.001

4. Farmers' comments

Farmers were invited to provide their own experiences or observations about the cause of mortality in 2009. The following factors were noted as positively correlated with mortality: faster growing stocks (may be due to daily tidal immersion), water temperature, rainfall and handling stress.

5. Discussion

5.1. Background to the study

Based on our knowledge of OsHV1 and experiences in France in 2008 and 2009, it is likely that the virus will persist at a low level in infected stock over the winter months. Disease is likely to recur when water temperatures are permissive in 2010. There are no realistic prospects of eliminating the virus. Protection of unaffected areas relies primarily on restriction of movements from infected areas. It is likely that farmers in affected areas will have to live with the disease. This study aimed to identify management factors which could be manipulated to minimise the impact of the disease in infected stocks. In addition, insights into environmental factors may help to inform management decisions, in particular the timing of stocking. A cross-sectional questionnaire based study was completed after the outbreak. This study

design has only limited capacity to assess factors which change over time (e.g. environmental parameters). In addition, the validity of the data relies on the recall and records of the farmers. Frequency of observations will vary between farmers, depending in part on their production system. Given the nature of oyster production, estimates of mortality can only be approximations. Therefore, the other key output from this study are recommendations for future longitudinal studies which can monitor environmental parameters (e.g. salinity, water temperature) and obtain more accurate estimates of mortality and infection.

5.2. Infectious aetiology and disease spread

The strong association between introduction of stock from areas where the new μ Var of OsHV-1 was known to be present (i.e. France), compared with stock from GB where to date the new μ Var of OsHV-1 has not been found, is consistent with spread of the virus through movements of stock from infected areas. The data suggests that French triploid stock may be more susceptible or likely to be infected compared with diploid stock. This was further supported by the finding that higher levels of mortality (>20%) were more commonly observed in triploid compared with diploid stock (the result was not statistically significant and merits further investigation).

The onset dates of mortality by site in bays with more than 5 sites (see maps) are consistent with spread of the virus between sites within the same bay. Further analysis at the batch level showed that introduced batches were first to have mortality (mostly starting in June) and batches of established oysters (introduced prior to 2009) starting dying in July. This is strong evidence that the virus was introduced with stock and then spread to older established batches. Further more detailed investigation in a prospective longitudinal study of the temporal relations between onset of observed mortality and the date of introduction at the batch level is required to determine whether stock were infected when introduced or after introduction.

The virus is most likely to spread between sites on water currents. To the best of our knowledge movement of stocks between sites within the same bay were minimal

(with the possible exception of enterprises running more than one site). Other routes of spread (shared equipment, personnel, birds) are not likely to be important. Thus analysis of the direction of the tidal and other currents may provide additional insights into spread. It is clear from the maps that some sites do not experience mortality whilst neighbouring areas experience high mortality. Secondly, highly variable levels of mortality were observed. To explain these observations it is useful to consider necessary and sufficient causes of disease. The new μ Var of OsHV-1 is very likely a necessary cause of disease (observed as mortality) but not sufficient. A combination of necessary causes (infection, water temperature, susceptible age group) is needed to provide a sufficient cause. . It can be assumed that within an area such as Dungarvan harbour most of the sites were exposed to the virus. The fact that some appeared unaffected indicates that the virus is not very highly infectious (oysters were not exposed to a sufficient viral challenge), or the oysters in unaffected areas were more resistant which may be age related (requires further analysis). It is also possible that management of some sites / batches may be protective (see following sections).

5.3. Risk factors for mortality associated with OsHV1

Risk factors at batch and site level were considered. The batch level analysis used cumulative mortality as an outcome and analysis was undertaken separately in bays where the virus was detected and all bays. At the site level reporting abnormal levels of mortality was the outcome used. Since many of the risk factors could be measured at the batch level, it is this analysis which provides a more accurate assessment of risk factors for mortality, compared with the site level analysis.

Manual handling of spat compared with mechanical handling is associated with higher levels of mortality (80 versus 50%). Similarly at the site level manual handling, compared with mechanical handling appears to be associated with mortality. However, it is very likely that this association is not causal and may be explained by the increased opportunity handling provides to detect and accurately record mortality. The finding that bringing in new trestles appeared to be associated with

increased risk of disease at the site level cannot be considered to be biologically plausible. The finding may be chance or introduction of trestles may be a proxy for another variable (which required further investigation).

There appeared to be a negative association between longest time out of water and mortality; no batches which was out of water for more than 8 hours suffered mortality higher than 40%. There may be a number of explanations for this. Firstly, there is a positive association between time spent immersed and rate of growth. Faster growing oysters may be more susceptible to disease. In addition exposure to infection will increase with time spent in water. Oysters positioned higher up the beach spend are longest out of water so potentially factors other than time in water may be confounded with position on the beach. However, most of the sites where oysters are spending long periods out of water originate from two bays – Dungarven and Waterford. It is possible that other bay level factors may be confounded with the length out of water and therefore this result needs to be treated with caution.

Further analysis is required to investigate the extent to which age determines mortality at the site level. Reports from France indicate high levels of mortality in juveniles and spat and our findings support this observation. Age or size at time of introduction of stock is confounded with month of introduction and time on farm before the onset of mortality. These associations could be more fully investigated in a prospective study and the analysis take into account associations between potential explanatory variables.. It proved difficult to assess the impact of stocking density since it is firstly difficult to measure consistently between sites and secondly is confounded with age / size.

5.4. Conclusion and recommendations

This study confirms that within bays where OsHV1 was detected there was considerable variation in the level of reported mortality per batch. However, few clear associations with management factors have been identified. It is plausible that the mortality due to OsHV1 infection will be determined in large part by i) age of oysters when first infected, ii) condition of the oysters, iii) temperatures, iv) other

environmental factors. The pattern of outbreaks in France, especially in 2009, indicated that other factors in addition to temperature were important in initiating outbreaks. Management practices were reasonably consistent across the industry and there were no practices which were very strongly associated with high levels of mortality. However, the results of this study indicate that maximum duration of the daily exposure to air (position on beach) and possibly handling are potential management factors which should be investigated in future studies. It was not possible to fully investigate stocking density, in part because a consistent approach to measuring density had not been formulated and densities change over time. In future studies a clear case definition needs to be developed. In this study mortality was studied at the batch level, and used as a continuous variable or categorised. There was a working assumption that in bays where the virus had been detected all sites and batches would have been exposed. The level of testing was not sufficient to verify this. It is possible that low mortality in some batches was attributable to a lack of exposure. In addition, a robust method to assess stocking should be developed and validated before the study. Variation within sites as well as over time should be assessed. A prospective longitudinal study is needed to fully investigate the influence on mortality OsHV1 infected stocks and their interaction of management and environmental factors. Analysis should take into account the hierarchical structure of oyster production (e.g. clustering of sites within bays) and associations between potential explanatory variables.

6. Acknowledgements

Members of the Fish Health Inspectorate, Cefas provided valuable advice on the design of the questionnaire. We acknowledge the work of the MI and BIM in collecting the data. Comments on early drafts of this report from the MI were invaluable.

7. References

Arzul, I., Renault, T., 2002. Herpesvirus infections in marine bivalves. *Herpesvirus et bivalves marins* 6, 169-174.

- Goodwin, A.E., Sadler, J., Merry, G.E., Marecaux, E.N., 2009. Herpesviral haematopoietic necrosis virus (CyHV-2) infection: Case studies from commercial goldfish farms. *Journal of Fish Diseases* 32, 271-278.
- Gray, W.L., Williams, R.J., Jordan, R.L., Griffin, B.R., 1999. Detection of channel catfish virus DNA in latently infected catfish. *Journal of General Virology* 80, 1817-1822.
- Lipart, C., Renault, T., 2002. Herpes-like virus detection in infected *Crassostrea gigas* spat using DIG-labelled probes. *Journal of Virological Methods* 101, 1-10.
- Miossec, L., Allain, G., Francoise, C., Garcia, C., Arzul, I., Cameron, A., 2009. Epidemiological study on *Crassostrea gigas* mass mortality observed in 2008 in France. In: . Report of the Annual Meeting and Seventh Combined Technical Workshop Meeting of the National Reference Laboratories for Mollusc Diseases, 16-19 March 2009. IFREMER, La Tremblade.
- O.I.E., 2009. Manual of Diagnostic Tests for Aquatic Animals. Office International des Epizooties Paris.
- Pepin, J.E., Segarra, A., Faury, N., Morga, B., Renault, T., 2009a. Summer oyster mortalities in 2008 and characterization of a new genotype in isolates of ostreid herpesvirus. In: Anon. (Ed.), 14th International Conference of the European Association of Fish Pathology. EAFP, Prague, p. 129.
- Pepin, J.F., Segarra, A., Faury, N., Schikorski, D., Cobret, L., Renault, T., 2009b. Summer Mortality in 2008 and Ostreid Herpesvirus Investigations. In: . Report of the Annual Meeting and Seventh Combined Technical Workshop Meeting of the National

Reference Laboratories for Mollusc Diseases. La Tremblade 16-19 March 2009.

Renault, T., 2008. Shellfish Viruses. Manuscript Number 781. IFREMER.

Renault, T., Arzul, I., 2001. Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: Specific viral DNA detection by PCR. *Journal of Fish Diseases* 24, 161-167.

StataCorp, 2009. Stata Statistical Software Release 10. StataCorp LP, College Station TX.

Appendix 1 Questionnaire

Oyster Herpes Virus Disease study Autumn 2009

The Marine Institute in Galway is investigating the possible origins and likelihood of spread of OHVD in this area. We would be grateful for your time and assistance to collect information about your enterprise, either from records you keep or your best recollection from the time. **Dark blue text indicates questions which should not be asked to the farmer. See separate sheet for information about the bay to be gathered by the MI.**

Section one should be completed for the enterprise by MI. Sections 2-6 need to be completed for each site¹ at which the grower operates.

Interviewer _____

Date _____

Contact information

MI to complete and check with farmer

1. Contact person and identification of enterprise (name, address, phone etc) ...
[Not used in analysis or results, but available if queries arise]
2. Enterprise number [allocated in office]
3. Assign ID to each site², note location (i.e. bay) and latitude, longitude

² The working definition of site is a discrete physical location where oysters are cultured.

Table 1

¹official ID could be used here

²If taken from records before visit, preferably draw or mark on a map and get farmer to confirm boundaries

	Site ID ¹	Bay ID	Georeference ²	
			latitude	longitude
1				
2				
3				
4				
5				
6				
7				

Sections 2 – 6 need to be completed **for each site** identified in section 1.

Site ID _____

Site information and history of disease and mortality

4. a) Year site started _____
OR

b) Year purchased from previous occupier _____

if b) name of previous occupier _____

5. Production of Pacific oysters in 2007 and 2008.

_____ tonnes (2007)

_____ tonnes (2008)

6. What species of shellfish do you farm or harvest *in the same bay* in addition to pacific oysters; at what level of production? **If none please go to question 7.**

Table 2

	Farmed / Harvested	Production tonnes 2008
Mussels		
Manila clams		
Flat oysters		
Other		
(if other please list)		

7. On the map for each site farmed please mark the following:

- Trestles (indicating length and distance apart) or location of oysters in bottom culture (indicating size of the area).
- Direction of prevalent water currents
- High and low spring (and neap?) tides.

8. In recent years (prior to 2009) what has been the expected (normal) level of mortality in your stock?
(*cumulative mortality by age group - % loss of stock during a year*)

Spat (less than 5g) _____%

Juveniles (5g to 40g) _____%

Adults (more than 40g) _____%

9. Before 2009 has there been a particularly bad year with high levels of oyster mortality?

y / n

10. If yes, what year was it? _____

(leave remaining questions in section 2 blank if no years are given)

11. What was the level of mortality in that year?

Spat (less than 5g) _____%

Juveniles (5g to 40g) _____%

Adults (more than 40g) _____%

12. What signs were observed?

13. Was a diagnosis made, and if yes what was it?

14. Were other species also affected?

If yes please list:

15. What other diseases were observed or diagnosed during 2008 and 2009? (if *none* please state).

Culture methods

16. What method of culture is used:

- a. Bags and trestles
- b. Bottom culture
- c. Bags and trestles **and** bottom culture
- d. Bags and lines
- e. Other

If other please describe:

17. Please describe in Table 3 any culture equipment brought onto the site in 2009, with or without stock (if none, please write none).

Table 3

*e.g. trestles, grading equipment, handling equipment, bags with oysters

Type of equipment* (describe)	Origin (country)	With stock? (Y / N)	Comments (was equipment dried, stored or disinfected before use)

18. How often was someone on site and may have identified a disease problem (if it was present)? Please estimate number of days each month when the oysters were inspected in or out of the bag ["within bag" operations include turning, "out of bag" operations include grading and thinning].

Table 4

		2009											
		J	F	M	A	M	J	J	A	S	O	N	D
Spat	Within bag												
	Out of bag												
Juv.	Within bag												
	Out of bag												
Adu.	Within bag												
	Out of bag												

19. List methods used to handle the stock?

Table 5

	Not handled	Manual	Mechanical	Other (please state)
Spat (less than 5g)				
Juveniles (5g to 40g)				
Adults (more than 40g)				

20. What is the longest and shortest period per day that any stock at this site is uncovered (i.e. exposed to air)?

Longest (i.e. at the top of the beach) _____ hrs

Shortest (i.e. at the bottom of the beach) _____ hrs (may be zero)

21. What is the stocking density on your site? Please provide the number of different sized oysters you use to stock your site (use the unit of size you routinely work with).

Table 6

¹ for example G-grade, mm, g

Size and unit ¹ of oyster	Number stocked per bag

22. What proportion (%) of trestles are stocked with a double layer of bags?

Details of stock on site during 2009

Site ID _____

23. Origins and status of 2009 stock: complete the following table for each batch present during 2009 *which remains identifiable*. Please record separately any batch that is split in a way that makes the 2 (or more) fractions epidemiologically distinct (i.e. batch 001 splits into 001A and 001B).

Table 7

*Enter the farm reference for the batch. Use this ID to cross reference batches with Tables 8, 9, and 10

¹Fr – France, UK – United Kingdom, J – Jersey, G - Guernsey , O – other

²Hatchery (H)/ Farmed (F)/ Wild (W)/ Unknown (U)

³Or age if size not known

⁴Diploid (D) / Triploid (T)/ Unknown (U)

⁵Harvested (H – give month of harvest) / Present (P)/ Dead (D)/ Moved off-site (M)

25. Complete Table 4.2 below for batches imported in 2009

Table 8

*cross reference batches with Table 7

¹ i.e. with batches from other sources bound for different sites

ID*	Method of transport (e.g. truck, boat, air)	Was the batch made up of oysters from more than one hatchery? y/n	Was there any chance of cross contamination during transport? ¹ y/n

8. Pacific Oyster mortality in 2009

26. What did you first notice? (signs of disease)

27. Tick periods in 2009 when mortality occurred?

Table 9

*cross reference batches with Table 7

	Week starting	Batch ID*	>>	
Before May				
May	04-May			
	11-May			
	18-May			
	25-May			
June	01-Jun			
	08-Jun			
	15-Jun			
	22-Jun			
July	29-Jun			
	06-Jul			
	13-Jul			
	20-Jul			
	27-Jul			
Aug	3-Aug			
	10-Aug			
	17-Aug			
	24-Aug			
Sep	31-Aug			
	7-Sep			
	14-Sep			
	21-Sep			
	28-Sep			
After Sept				

28. Did mortality start in i) one batch y/n
OR

ii) from the start right across the site? y/n

29. What was the date when stock was last handled and graded before mortalities were first noticed? *(if no unusually high mortalities write NA)*

Mortalities started on arrival _____ day / month / 2009

Handled in the bag _____ day / month / 2009

Graded _____ day / month / 2009

30. Please complete the table below for all batches present during 2009 (if no mortality was observed write NA in first column – [date start mortality] and leave remaining columns blank)

Table 10

*cross reference batches with Table 7

¹Whole batch removed

²On map please indicate how mortality varied over the site

Indicate which batch mortality started in on the map

³MI to complete

ID*	Date start mortality (NA if no mortality observed)	Date end of mortality	Grade or size (at start of mortality)	Stocking density (at start of mortality)	Cumulative (total loss) % mortality	Current status - still present, harvested, culled ¹ (give date of cull / harvest)	Was mortality continuous, or in two or more waves?	Did mortality vary over the site ² ?Y/ N	OsHV1 test positive?(yes / no / untested) ³

31. Were any affected batches culled? y / n

If yes please answer questions 32-34, if no go to question 35.

32. When were batches culled?

33. How long after mortality was detected in the batch was it culled? _____days

34. Did any other batches suffer mortality after the affected batches were culled?

y / n / NA

35. Was unusually high mortality reported in other shellfish species in the bay in 2009?

y / n / dk

36. If yes which species

If mortality started in one batch please answer following questions about that batch:

37. ID of batch (see Table 7) _____

38. Dates [and details] of handling from introduction to start of the mortality:
(e.g. relaying, turning, thinning, grading...etc).

Table 11

Handling description	Date

9. Grower observations

Fill in just once for comments that apply across the enterprise

39. Please describe any notable weather or environmental events over past twelve months: [eg rainwater flood, drought, algal bloom, gales, pollution, unseasonal temperatures] with approximate dates:

40. The remainder of the sheet (and following sheet) can be used to record any other observations the interviewee wishes to make about the pattern or possible causes of mortality in his stock during 2009.