

1 **Risk factors associated with increased mortality of farmed Pacific oysters in Ireland**  
2 **during 2011**

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16

17 **Abstract**

18

19 The Pacific oyster, *Crassostrea gigas*, plays a significant role in the aquaculture industry in  
20 Ireland. Episodes of increased mortality in *C. gigas* have been described in many countries,  
21 and in Ireland since 2008. The cause of mortality events in *C. gigas* spat and larvae is  
22 suspected to be multifactorial, with Ostreid herpesvirus 1 (OsHV-1, in particular OsHV-1  
23  $\mu$ var) considered a necessary, but not sufficient, cause. The objectives of the current study  
24 were to describe mortality events that occurred in *C. gigas* in Ireland during the summer of  
25 2011 and to identify any associated environmental, husbandry and oyster endogenous factors.

26 A prospective cohort study was conducted during 2010 to 2012, involving 80 study batches,  
27 located at 24 sites within 17 bays. All 17 bays had previously tested positive for OsHV1-  
28  $\mu$ var. All study farmers were initially surveyed to gather relevant data on each study batch,  
29 which was then tracked from placement in the bay to first grading. The outcome of interest  
30 was cumulative batch-level mortality (%). Environmental data at high and low mortality sites  
31 were compared, and a risk factor analysis, using a multiple linear regression mixed effects  
32 model, was conducted. Cumulative batch mortality ranged from 2% to 100% (median = 16%,  
33 interquartile range: 10% - 34%). The final multivariable risk factor model indicated that  
34 batches imported from French hatcheries had significantly lower mortalities than non-French  
35 hatcheries; sites which tested negative for OsHV-1  $\mu$ var during the study had significantly  
36 lower mortalities than sites which tested positive and mortalities increased with temperature  
37 until a peak was reached. There were several differences between the seed stocks from  
38 French and non-French hatcheries, including prior OsHV-1  $\mu$ var exposure and ploidy. A  
39 range of risk factors relating to farm management were also considered, but were not found  
40 significant. The relative importance of prior OsHV-1  $\mu$ var infection and ploidy will become  
41 clearer with ongoing selection towards OsHV-1  $\mu$ var resistant oysters. Work is currently  
42 underway in Ireland to investigate these factors further, by tracking seed from various  
43 hatchery sources which were put to sea in 2012 under similar husbandry and environmental  
44 conditions.

45

46 Key words: oysters, *Crassostrea gigas*, mortality, Ireland, risk factors

47

## 48 **1. Introduction**

49

50 The Pacific oyster, *Crassostrea gigas*, plays a significant role in the aquaculture industry in  
51 Ireland, both in terms of volume and value, with an annual production of over 7,000 metric  
52 tonnes (Bord Iascaigh Mhara (BIM) / the Irish Sea Fisheries Board, personal  
53 communication). With a current value of €28-30 million per year, *C. gigas* accounts for  
54 approximately 20% by volume of overall shellfish produced in Ireland (BIM, personal  
55 communication). The main method of cultivation for Pacific oysters used in Ireland is bag  
56 and trestle cultivation, which is an off-bottom culture method. This cultivation method allows  
57 the oysters to be placed in mesh bags on metal framed structures called trestles in the inter-  
58 tidal zone, which allows access to the stock during low tide (Tidwell et al., 2012). Over half  
59 of all current licensed aquaculture producers in Ireland are oyster farmers, with *C. gigas*  
60 being grown in 44 bays all around the coast. *C. gigas* seed is predominantly sourced from  
61 hatcheries / nurseries or harvested wild seed, which is imported mainly from France and to a  
62 much smaller extent from the UK and the Channel Islands. The main export market for  
63 Pacific oysters is France, although there is also a growing market in Asia.

64  
65 Since the 1950s, episodes of increased mortality in *C. gigas* have been described globally in  
66 all areas of production. In Europe, severe mortality events in cultured Pacific oyster were  
67 observed during the summers of 2008 and 2009 (Dégremont et al., 2013). These events have  
68 been grouped by life stage into summer mortality in adults, mortality in spat and hatchery-  
69 related mortality (European Food Safety Authority, 2010). Mortality in spat and larvae at  
70 hatcheries have been associated with detection of ostreid herpesvirus 1 (OsHV-1), a virus  
71 also associated with mortality in other farmed bivalves, including the European flat oyster  
72 (*Ostrea edulis*), scallop (*Pecten maximus*) and the Manila clam (*Ruditapes philippinarum*)  
73 (Renault et al., 2001; Arzul et al., 2002; Batista et al., 2007). In 2000, OsHV-1var, a variant  
74 strain of OsHV-1, was identified in French hatcheries (Arzul et al., 2001a,b). Although

75 OsHV-1var presents several modifications in the C region of the genome, where the most  
76 significant modifications in relation to OsHV-1 occur, and a 2.8 kb deletion, OsHV-1 and  
77 OsHV-1var are considered representative of a single viral species as the differences between  
78 the two genotypes were not great enough to establish a separate viral species (Arzul et al.,  
79 2001b). In 2008, the emergence of a third strain was described, OsHV-1  $\mu$ var, in association  
80 with abnormal mortality in *C. gigas* in France (Segarra et al., 2010; Dégremont et al., 2013).  
81 It has since been shown that mortality in *C. gigas* spat can be induced following experimental  
82 infection with OsHV-1  $\mu$ var (Schikorski et al., 2011). Further, mortality can be induced  
83 following horizontal transmission of infection from unselected asymptomatic adult to  
84 juvenile *C. gigas* (Dégremont et al., 2013). Since 2009, OsHV-1  $\mu$ var has been the  
85 predominant herpes virus strain during mortality events (European Food Safety Authority,  
86 2010). It is now believed that the cause of mortality events in *C. gigas* spat and larvae is  
87 multifactorial, with OsHV-1 infection (with OsHV-1  $\mu$ var now predominating) a necessary  
88 but not sufficient cause (Samain and McCombie, 2010). Other suspected risk factors include  
89 an increase or a sudden change in the temperature, husbandry practices such as introduction  
90 of possibly infected spat, and the movement and mixing of populations and age groups  
91 (European Food Safety Authority, 2010; Garcia et al., 2011). The European Food Safety  
92 Authority (2010) also noted that no outbreaks had been reported when the water temperature  
93 was below 16°C.

94

95 In Ireland, mortality events in *C. gigas* have been reported for some years (Malham et al.,  
96 2009), but linked, since 2008, to the presence of OsHV-1  $\mu$ var (D. Cheslett, personal  
97 communication). In that year, reports of mortality in Pacific oysters were received from three  
98 oyster producing bays, and the presence of the OsHV-1  $\mu$ var was confirmed in all three bays  
99 by PCR and sequence analysis of the amplicon (European Food Safety Authority, 2010). In

100 2009, extensive mortality and the presence of OsHV-1  $\mu$ var were reported from 15 oyster  
101 production areas, peaking in July with an average batch mortality of 37% and with mortality  
102 occurring, on average, over an 18 day period (Peeler et al., 2012). Although few clear  
103 associations between mortality and management factors were identified, the age of oysters  
104 when first infected with OsHV-1  $\mu$ var, the condition of the oysters, temperature, and other  
105 environmental factors each appeared important (Peeler et al., 2012). European Union  
106 legislation was subsequently introduced to prevent the spread of the virus to unaffected areas  
107 in Ireland and the United Kingdom, whilst still allowing trade to continue between infected  
108 areas (European Community, 2010), but noting that there was no realistic prospect of  
109 eliminating the virus (Peeler et al., 2010). OsHV-1  $\mu$ var related mortality has continued in  
110 Ireland each summer since the initial detection of OsHV-1  $\mu$ var in 2008, both in bays  
111 previously infected with this virus and coincident with spread of infection to new bays.

112

113 The objectives of the current study were to describe any mortality events that occurred in *C.*  
114 *gigas* in Ireland during the summer of 2011 and to identify any associated environmental,  
115 husbandry and endogenous oyster factors, thereby providing information which could assist  
116 oyster farmers in minimising batch mortality in endemically affected areas.

117

## 118 **2. Materials and methods**

119

### 120 **2.1 Study design and population**

121

122 A prospective cohort study was conducted during 2010 to 2012, from the time of first batch  
123 immersion (03 August 2010), when oysters were two mm in size. The oysters were followed  
124 until the date of last batch grading before data analysis, in spring/summer 2012 (12 April

125 2012), where oysters had been immersed for up to 18 months (subsequently termed the study  
 126 period). The oyster batch was the unit of interest, and was defined as a variable number of  
 127 oysters of similar size, originating from one hatchery, placed at a particular site within a bay  
 128 at one point in time. All 405 batches of farmed oysters that were in the sea in Ireland during  
 129 2011 were considered for inclusion in the study. Farms were selected for logistical reasons,  
 130 such as the accessibility of stock to allow for frequent monitoring and the capacity to monitor  
 131 individual batches among the stock throughout the season. From the selected farms batches  
 132 were chosen in order to include batches from different hatcheries, ploidy status and  
 133 immersion date within each bay.

134 The number of batches required for the study was estimated, based on a confidence interval  
 135 of 95% ( $\alpha = 0.05$ ), a power of 80% and assuming a mortality of 43% amongst batches in  
 136 OsHV-1 positive bays and 12% in OsHV-1 negative bays (based on reported site-level  
 137 mortality from the 2009 Irish survey (Peeler et al., 2010); data as presented for OsHV-  
 138 1 positive bays and as estimated for OsHV-1 negative bays). The initial sample size was  
 139 calculated using the following formula to detect a difference between two proportions  
 140 (Dohoo et al., 2009) :

$$141 \quad n = [ Z_{\alpha} \sqrt{(2pq)} - Z_{\beta} \sqrt{((p_1q_1)+(p_2q_2))}]^2 / (p_1 - p_2)^2$$

142 Where:  $Z_{\alpha} = 1.96$ , the value required for a confidence of 95%,  $Z_{\beta} = -0.84$  the value required  
 143 for a power of 80%,  $p_1$  = estimate of lower proportion of disease,  $p_2$  = estimate of higher  
 144 proportion of disease,  $q_1 = 1-p_1$ ,  $q_2 = 1-p_2$ ,  $p = (p_1 + p_2)/2$  and  $q = 1-p$ .

145 The sample size was further adjusted to account for other confounders. Assuming other  
 146 confounders were not strong confounders the sample size was increased by 15% (Dohoo et  
 147 al., 2009, page 50). Clustering within farms was accounted for using the following formula:

$$148 \quad n' = n (1 + \rho (m - 1))$$

149 Where:  $\rho$  is the intra-cluster correlation coefficient, assumed to be 0.5 and  $m$  is the average  
150 number of batches of oysters per farm, assumed to be 3. In total, the aim of the study was to  
151 sample around 80 batches.

152

## 153 2.2 Data collection

154

155 An initial survey of all study farmers was conducted to gather relevant data on each study  
156 batch, and to provide a framework for data collection at batch-level throughout the study  
157 period. The survey was administered in a joint effort by BIM regional officers and the Fish  
158 Health Unit of the Marine Institute (MI). Each study batch was tracked throughout the study  
159 period. To achieve this, BIM Regional Officers made regular visits to survey the batches and  
160 collect data on batch identification, risk factors of interest, batch mortality data, splitting,  
161 grading and handling frequency. For any batch where mortality occurred, details of the  
162 mortality event were recorded (i.e. start and finish date of the mortality episode, estimated  
163 percentage mortality in the batch, any predisposing factors). Data were recorded on  
164 specifically designed survey forms which were submitted to the MI and entered into a  
165 Microsoft Access database. Data collection sheets are available in Appendix A.

166

167 Samples of the stock (30 animals from one batch in each bay; Table 1) were tested for the  
168 presence of OsHV-1 in the Fish Health Unit laboratory at the Marine Institute. Cell lysis and  
169 nucleic acid extraction was carried out using QIAamp DNA Mini Kit (Qiagen) using Qiagen  
170 QIAcube, according to the manufacturer's instructions. DNA extracts obtained were  
171 subjected to an initial screening process for the presence of OsHV-1 using real-time PCR  
172 analysis based on a Sybr Green chemistry with C9/C10 primer set targeting the C region of  
173 the genome (Pepin et al., 2008). Confirmatory testing for OsHV-1  $\mu$ var was carried using

174 nested PCR where subsamples of real-time positive PCR products were analysed using nested  
175 conventional PCR, using C2/C6 primers (Arzul et al., 2002) in the first round of conventional  
176 PCR, and internal primer set F-int/R-int, with a 514bp expected product size in the second  
177 round of conventional PCR, using PCR conditions (unpublished, D. Stone, CEFAS, UK).

178

179 A total of 55 data loggers, at least one per bay, were deployed to obtain environmental data.  
180 These were deployed in May 2011 on a small number of sites, due to availability of  
181 equipment, with further deployments made in early June 2011 on the remaining sites. Five  
182 different types of data loggers (two large types and three small types) were deployed, each  
183 recording a number of different parameters. The smaller data loggers were deployed attached  
184 to the bags in the batch of interest, whereas the larger data loggers were deployed alongside  
185 the trestles which held the batch of interest. These data loggers were programmed to record  
186 environmental parameters every hour for the duration of the deployment, with regular  
187 maintenance and calibration on a monthly basis. An illustration of the location of data loggers  
188 at Dungarvan bay is shown in Figure 1.

189

190 <Figure 1 here>

191

192 2.3 Descriptive analysis of temperature at high mortality sites where start date of mortality  
193 recorded

194

195 A high mortality site was defined as a site where 1 or more batches experienced a cumulative  
196 batch-level mortality > 34% (being the upper mortality quartile) during the study period. A  
197 low mortality site was defined as a site where no batches experienced a cumulative batch  
198 mortality > 34%. At all high mortality sites with available data (reported start date of

199 mortality event and temperature data during the 30 preceding days), the median temperature  
200 within the previous 7 and 30 days prior to the recorded start of mortality were reported. At all  
201 low mortality sites, the median temperature during the same time periods as each of the high  
202 mortality batches was also reported, for comparison.

203

204 At all high mortality sites with available data, a visual assessment of temperature changes  
205 was made during the period when mortality events were first recorded. A low-pass filter  
206 using Matlab (MathWorks, Natick, MA, USA), was used to remove the tidal, diurnal and  
207 other high frequency signals from the temperature time series, in order to assess the overall  
208 trend in temperature during the period when mortality occurred, particularly with respect to a  
209 16°C threshold.

210

#### 211 2.4 Linear Mixed model analysis

212

213 The cumulative batch-level mortality from placement to first grading was the outcome of  
214 interest. The independent variables, for consideration during the risk factor analysis, are  
215 presented in Table 1.

216

217 <Table 1 here>

218

219 Due to the highly skewed nature of the mortality data, the natural logarithm was used to  
220 transform these to a normal distribution. In addition the appropriate transformation of the  
221 outcome was assessed based on the residuals from the final model, comparing the profile  
222 likelihood ratio to identify the optimal box-cox transformation (Dohoo et al., 2009).

223

224 A mixed linear regression model of cumulative batch-level mortality was developed using  
225 SAS version 9.1.3 (SAS Institute Inc., 2003). A univariable screening approach was used,  
226 where all variables with  $p < 0.2$  at the univariable stage became candidates for the  
227 multivariable model. The need for a 'farm' random effect was tested within the mixed model,  
228 using a likelihood ratio test (Dohoo et al., 2009), to account for clustering of batches within  
229 farms. A backward selection procedure was used to eliminate terms from the model based on  
230 an F-test ( $p > 0.05$ ). The relationship between continuous predictors and the outcome was  
231 examined using plots of the continuous predictors against the logarithm of batch mortality.  
232 Where no obvious linear or polynomial relationship was observed, continuous predictors  
233 were categorised into four groups based on the corresponding quartiles. Variables that were  
234 not significant at the univariable screening stage were added to the final model and tested  
235 using an F-test ( $p < 0.05$ ). In addition, variables that, when combined, represent an underlying  
236 effect were tested in combination as described by Cohen et al. (2003) by adding the combined  
237 variables to the final model. For example, the variables: 'number of times bags were turned'  
238 and 'handling at grading' when combined were considered to represent a 'bag handling' effect.  
239 The correlation between covariates was evaluated using a chi-square test between nominal  
240 variables and kendall's tau-b assessment of correlation between continuous variables. Plots of  
241 studentised residuals from the final model and influence plots were examined to identify lack  
242 of fit or any outliers from the final model.

243

### 244 **3. Results**

245

#### 246 3.1 Study population

247

248 A total of 80 study batches, from 28 farms, located at 24 sites within 17 infected bays were  
249 included in the study (Figure 2). The batches contained between 10 and 4,650 bags of oysters  
250 with a median number of 215 bags per batch. The median number of oysters per bag as at the  
251 end of May 2011 was 2,000; this varied between 210 to 15,000 oysters per bag between  
252 batches. Cumulative batch mortality ranged from 2% to 100%, with a median batch mortality  
253 of 16% and an interquartile range of between 10% and 34% (Figures 2 and 3).

254

255 <Figure 2 here>

256 <Figure 3 here>

257

258 3.2 Descriptive analysis of temperature at high mortality sites where start date of mortality  
259 recorded

260

261 There were 14 high mortality sites (mortality >34%), with a total of 20 high mortality batches  
262 (out of 52 batches at these sites), and 10 low mortality sites (no batches experienced a  
263 cumulative batch mortality > 34%), with a total of 28 batches. Of the high mortality sites, 10  
264 sites had a recorded start date for mortalities, however, three of these sites had missing  
265 temperature data (three sites in Ballymacoda, Bannow and Lough Foyle bays). The median  
266 temperature within 7 and 30 days of the reported start of mortality at the seven high mortality  
267 sites with a recorded start date of mortality and relevant temperature data is shown in Table 2.

268 The median temperatures at the 10 low mortality sites during the same time periods as each  
269 of the high mortality sites are also presented in Table 2. The interquartile range for the high  
270 mortality sites overlapped the interquartile range for the low mortality sites during the 7 and  
271 30 days time intervals, with the exception of the site at Lough Swilly, indicating that there

272 was little difference in temperature between the low and high mortality sites prior to the start  
273 of mortalities.

274

275 <Table 2 here >

276

277 A visual assessment of temperature data during the reported start of mortality at the seven  
278 high mortality sites with a recorded start date for mortalities and temperature data prior to the  
279 start of mortalities is presented in Appendix B. At four sites (Achill Sound, Dungloe,  
280 Trawbreaga Bay and Woodstown Strand), an increase in temperature over 16°C coincided  
281 with the time period when mortality events were first reported; at one site (Sherkin), no visual  
282 association was observed; and at two sites (Clew Bay, Lough Swilly), a substantial change in  
283 temperature, in this case a decline followed by an increase, was coincident with the time  
284 period when mortality was first reported.

285

### 286 3.3 Linear mixed effects regression model

287

288 All predictors with a p-value in the univariable analysis of <0.2 were considered in the initial  
289 linear mixed effects regression model (Table 3). The risk factors entered into the initial model  
290 were: size, ploidy, OsHV-1  $\mu$ var bay status, hatchery and the maximum summer temperature.  
291 The remaining significant variables after the backward selection process were: OsHV-1  $\mu$ var  
292 bay status and hatchery. Variables that were not significant at the univariable stage ( $p>0.2$ ),  
293 and combined variables representing an underlying effect were added to the model, however,  
294 none were significant. The studentised residuals were plotted against each of the variables not  
295 in the current model using a Loess smooth to identify any potential relationships. The Loess  
296 plot against 'maximum summer temperature' suggested a possible quadratic relationship. A

297 quadratic term of the 'maximum summer temperature', after centring the variable to account  
298 for correlation between the original value and the quadratic term (Dohoo et al., 2009), was  
299 significant when added to the model (Table 4). Residuals from the final model indicated no  
300 significant lack of fit and no significant outliers. The appropriate transformation of the  
301 outcome variable (cumulative batch-level mortality), based on residuals from the initial  
302 model (i.e. containing hatchery and OsHV-1  $\mu$ var bay status) and comparing the raw  
303 mortality, natural logarithm transformation, arcsine transformation and box-cox  
304 transformations were assessed. The natural logarithm transformation was deemed as the most  
305 appropriate based on residuals and assessment of box-cox transformations and comparing the  
306 profile log-likelihood (Dohoo et al., 2009). The 'farm' random effect was significant  
307 (likelihood ratio test:  $p = 0.023$ ) and 38% of the variance was between farms and 62% within  
308 farms.

309

310 <Table 3 here >

311

312 A plot of the best unbiased linear estimates of mortality against the 'maximum summer  
313 temperature' is shown in Figure 4, indicating an increase in mortality which appears to level  
314 off after a temperature peak was reached. The final model also showed that batches imported  
315 from French hatcheries 1, 2, 4 and 5 had significantly lower mortalities than batches imported  
316 from Non-French hatchery 2. Non-French hatchery 1 mortalities were not significantly  
317 different to those from Non-French hatchery 2. Further, batches grown in bays which tested  
318 OsHV-1  $\mu$ var negative during the study had significantly lower mortalities than batches  
319 grown in bays which had tested positive.

320

321 <Table 4 here >

322 <Figure 4 here>

323

#### 324 **4. Discussion**

325

326 In this work, we have sought to identify risk factors associated with increased mortality of  
327 farmed Pacific oysters in Ireland during 2011. Increased farmed oyster mortality has been an  
328 ongoing concern in Ireland for some years (Malham et al., 2009; Peeler et al., 2012), although  
329 there is evidence from the current study of a lower median batch mortality in 2011 (16%)  
330 compared with either 2009 (37%) or 2010 (32%). Three risk factors were significantly  
331 associated with mortality including the hatchery from which seed was sourced, the presence  
332 of OsHV-1  $\mu$ var detected in specific bays during 2011 and the maximum temperature  
333 observed between June and August 2011, inclusive. Each will be considered in turn.

334

335 We noted a strong association between hatchery of origin and mortality, with seed imported  
336 from French hatcheries experiencing markedly lower mortality compared with seed imported  
337 from non-French hatcheries, when placed in bays which were historically infected with  
338 OsHV-1  $\mu$ var. As illustrated in Table 3, the median cumulative mortality of batches from  
339 French hatcheries varied between 7 and 25%, and from non-French hatcheries between 76  
340 and 86%. There are several differences between these two seed stocks, which may at least  
341 partly explain this result. The first relates to prior OsHV-1  $\mu$ var exposure. This virus is  
342 endemic in France (Pernet et al., 2012). Therefore, prior exposure to this virus, either of the  
343 seed itself or of the related broodstock, can be assumed. In contrast, the non-French  
344 hatcheries are located in areas outside of Ireland which were not previously infected with  
345 OsHV-1  $\mu$ var. Prior exposure can lead to latency (Dégremont et al., 2013), a common feature  
346 of other herpesvirus infections (Arzul et al., 2002), and OsHV-1  $\mu$ var has been identified in

347 apparently healthy oysters (Arzul et al., 2002; Barbosa-Solomieu et al., 2004; Dégremont et  
348 al., 2013). However, prior exposure will not result in a specific immune response, noting that  
349 molluscs lack immunological memory, relying entirely on innate immunity to overcome  
350 diseases (Gestal et al., 2008; Renault, 2008). Rather, the protective effect of prior exposure is  
351 likely genetic (Dégremont et al., 2007; Sauvage et al., 2009; Huvet et al., 2010), with oysters  
352 surviving a mortality event being naturally selected for resistance to disease (Dégremont et  
353 al., 2010; Pernet et al., 2012). In recent years, considerable progress has been made in France  
354 towards selection for OsHV-1  $\mu$ var resistant oysters, particularly in the context of summer  
355 mortality in adults (Dégremont et al., 2010; Dégremont et al., 2013). The second difference  
356 between the seed stocks relates to ploidy: a substantial proportion of the French-derived  
357 batches in this study were triploid (88%), whereas most of the non-French-derived batches  
358 were diploid (75%). In the current study, there was a univariable association between ploidy  
359 and cumulative batch mortality (with diploid stock being at greater risk, Table 3), however,  
360 ploidy was not retained in the final multivariable model (Table 4). This result is at odds with  
361 an earlier Irish study, where triploid oysters were at greater mortality risk (Peeler et al.,  
362 2012), but consistent with studies on adults from France, prior to 2006 at least (Gagnaire et  
363 al., 2006; Samain, 2011), which found triploid oysters were more resistant to summer  
364 mortality. This latter effect varied by season (Pernet et al., 2012), possibly due to seasonal  
365 differences in reproductive effort and immunological parameters. Given the data available, it  
366 is not possible to disentangle the relative importance of ploidy and prior OsHV-1  $\mu$ var  
367 exposure in the current study. The association with ploidy could be a consequence of  
368 confounding, noting that most of the French seed was triploid and most of the seed imported  
369 from non-French hatcheries was diploid. The observed effect could be due to innate  
370 resistance among stock from French hatcheries to OsHV-1  $\mu$ var infection.

371 There was also some variation in the mortality of batches from different French hatcheries,  
372 with median mortality varying between 7% and 25% (Table 3), although the sample size was  
373 too small to conduct a formal analysis as to whether these differences in mortality were  
374 significant. In these hatcheries, diploid females are produced locally, whereas the male  
375 tetraploids are owned by the French government but moved from one hatchery to another for  
376 the purposes of fertilization. This may reflect a degree of genetic selection in specific  
377 hatcheries over the period since 2007/08 when the disease first appeared in France. A genetic  
378 component, leading to hatchery differences, has previously been noted for summer mortality  
379 in *C. gigas* adult oysters (Dégremont et al., 2007; Sauvage et al., 2009; Huvet et al., 2010).

380

381 In this study, the OsHV-1  $\mu$ var status of the bay was significantly associated with mortality,  
382 (median mortality of 20% and 10% among bays where OsHV-1  $\mu$ var was detected, or not,  
383 during 2011, respectively, Table 3). In this context, bay status is best interpreted in terms of  
384 viral load during 2011, as opposed to either presence or absence, noting that we used a  
385 sampling strategy to provide 95% confidence that virus would be detected, if present at a  
386 specified design prevalence of 10% or greater (Table 1). Each of the 17 study bays can be  
387 considered endemically infected: OsHV-1  $\mu$ var had been detected in each, either during 2011  
388 or previously, and the virus is known to persist in adult oysters following primary infection  
389 (Lipart and Renault, 2002; Dégremont et al., 2013). These observations are consistent with  
390 earlier work, highlighting increased mortality risk with increasing quantities of OsHV-1  $\mu$ var  
391 DNA, but frequently in the context of summer mortality in adults (Pepin et al., 2008; Sauvage  
392 et al., 2009; Schikorski et al., 2011; Garcia et al., 2011).

393

394 We found a quadratic relationship between the natural logarithm of the cumulative mortality  
395 and the maximum temperature observed between June and August 2011, noting that the

396 maximum temperature observed in this study may have occurred as a one day event rather  
397 than a sustained rise in temperature. In addition, for the seven high mortality sites at which  
398 mortality date and temperature data were available, temperature had exceeded 16°C in the  
399 week prior to mortalities at five of the sites. At the other 2 sites, temperatures had either  
400 exceeded 16°C in the previous month (Sherkin) or were around 16°C in the previous week  
401 (Woodstown Strand) (Appendix B). However, there was little difference in the average  
402 temperature in the time prior to the mortalities when compared to sites with low mortalities  
403 (Table 2). A number of studies have highlighted the multifactorial nature of mortality events  
404 in oyster spat and larvae, with the European Food Safety Authority (EFSA, 2010) stating that  
405 climatic factors were unlikely to be a sufficient cause for summer mortalities. However, this  
406 publication also noted the absence of mortality outbreaks when the water temperature had  
407 been below 16°C. Several authors have highlighted the potential role of increased water  
408 temperature in oyster mortality events. During 1998 to 2006 in France, OshV-1 was often  
409 detected when temperatures increased quickly, but was no longer detected once temperatures  
410 were stable even if they remained high (Garcia et al., 2011). Further, a temperature increase  
411 may lead to a re-activation of latent infection (Sauvage et al., 2009). According to data  
412 collected from M1-M5 Databuoys deployed around the Irish coast, water temperatures were  
413 significantly lower in 2011 than in previous years (Marine Institute,  
414 <http://www.marine.ie/home/publicationsdata/data/buoys/>). Although several authors have  
415 highlighted the role of farm management (Samain and McCombie, 2010; European Food  
416 Safety Authority, 2010), no relevant variables were significant in the current multivariable  
417 modelling.

418

419 There are a number of limitations to the current study which we note here. We were reliant on  
420 farmer recall to determine the start date of mortalities. This proved problematic, however,

421 both in terms of data validity and completeness. Further, the precision of these estimates is  
422 influenced by the frequency with which farmers check their stock. In most cases, due to tidal  
423 patterns which lead to site inaccessibility, stock can only be checked at best every two weeks,  
424 and in some cases stock may not be checked for up to a month. Similar challenges have been  
425 described previously among French oyster farmers, where yearly variation in reporting  
426 sensitivity has been observed. Increases in reporting sensitivity were found to occur  
427 concurrent with outbreak occurrence and with implementation of financial incentives to  
428 encourage farmer reporting of mortality events (Lupo et al., 2012). The cumulative mortality  
429 estimate for each batch in the study was based on the outcome of grading for each batch in  
430 early spring 2012. Some farmers with several study batches found it difficult to obtain  
431 grading data for individual batches as grading would usually be carried out on a stock basis  
432 rather than a batch basis. We also faced some gaps in the environmental data, noting that  
433 recordings were not taken during the same time period across all sites. In addition, although  
434 data were available on a range of environmental parameters, only the temperature data proved  
435 suitable for subsequent analysis. Extra environmental data were only recorded at a limited  
436 number of sites due to cost and availability of monitoring equipment. There were some issues  
437 with tracking batches once they had been split. Several batches were split during the season,  
438 resulting in new stocking densities with new bag mesh sizes, and several new locations for  
439 the split batch. This proved problematic when tracking split batches through the season, as  
440 once the original batch was split, all subsequent batches had a separate set of data, and were  
441 essentially a new batch. Initial sample size calculations were based on the difference in the  
442 proportion of mortality in two groups. However, for the analysis linear regression with  
443 cumulative mortality as the outcome was used. If sample size calculations had been based on  
444 the difference between two mortality rates as described by Lwanga and Lemeshow (1990)  
445 there would have been very little difference in the estimated sample size. Finally, the farms

446 and study batches were not chosen at random, primarily for logistical reasons. It is possible  
447 that these samples are not representative of the broader oyster farming population in Ireland.

448

449 The study provides some insights into mortality events affecting farmed Pacific oyster  
450 production in Ireland. Batch mortality was lower in 2011, compared with earlier years, in  
451 association with lower viral loads. Mortality was significantly associated with hatchery  
452 source, for reasons that are currently unclear, and with water temperature. Further work is  
453 needed to elucidate the basis for this effect. The relative importance of prior OsHV-1  $\mu$ var  
454 infection and ploidy will become clearer with ongoing selection towards OsHV-1  $\mu$ var  
455 resistant oysters. Work is currently underway in Ireland to investigate these factors further,  
456 by tracking seed from various hatchery sources which were put to sea in 2012 under similar  
457 husbandry and environmental conditions.

458

#### 459 **Acknowledgements**

460

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464

#### 465 **Conflicts of interest**

466 The authors do not have any conflict of interest.

467

#### 468 **Appendices A and B. Supplementary data**

469 Supplementary data associated with this article can be found, in the online version.

470

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592 Tables:

593

594 Table 1. Independent variables considered in the study

595

596 Table 2. The median and interquartile range of temperature recorded within 7 and 30 days of  
597 the reported start of mortalities at seven high mortality sites (those with 1 or more batches  
598 with a cumulative mortality > 34%) during summer 2011 in Ireland. The median and  
599 interquartile range of temperatures at the ten low mortality sites (those sites without any high  
600 mortality batches) during the same time periods is included for comparison.

601

602 Table 3. The median cumulative mortality of oyster batches in Ireland in 2011, along with the  
603 interquartile range by categorical risk factors that met the criteria for inclusion in the  
604 multivariable model ( $p < 0.2$ ).

605

606 Table 4. Linear mixed regression model of the log of batch mortality among 80 batches of  
607 oysters in Ireland in 2011

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609 Figures:

610

611 Figure 1. The location of study batches and environmental data loggers (Hydrolab DS5  
612 Multiparameter Datasonde loggers; \_CTD loggers (DGS – Dungarvan South and DGN –  
613 Dungarvan North); RCM9 loggers) in Dungarvan Bay during 2011-12

614

615 Figure 2. Location of the 80 study batches and 17 bays, and the cumulative batch mortality  
616 (average, range) in each bay, of farmed *C. gigas* in Ireland during summer 2011

617

618 Figure 3. Distribution of the cumulative mortality (%) among the 80 study batches in Ireland  
619 during summer 2011

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621 Figure 4. Predicted best linear unbiased estimator of the cumulative mortality from the  
622 multivariable mixed linear model plotted against the maximum summer temperature, for each  
623 hatchery in OshV-1  $\mu$ var positive bays

624

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624 Table 1. Independent variables considered in the study

625

Independent variable	Explanation
Time in water	Measured in days from date of immersion until date of final batch grading. Also grouped into time when placed in water ( <i>2010, Spring 2011 and Summer 2011</i> )
Age placed in water	<i>2-3, 4-7, &gt;7 (months)</i>
Ploidy	<i>Diploid, triploid</i>
Bay	17 bays
Position on shore	<i>Low, mid, high</i>
OsHV-1 $\mu$ var bay status <sup>a</sup>	OsHV-1 $\mu$ var detection in 2011 ( <i>positive/negative</i> )
Bag turning	Number of times each bag turned between May to August 2011
Transport	<i>Air, sea and road, road</i>
Journey duration	<i><math>\leq 24</math> hrs, <math>&gt; 24</math> and <math>&lt; 36</math> hrs, <math>\geq 36</math> hrs and <math>&lt; 42</math> hrs, <math>\geq 42</math> hrs</i>
Bag mesh size	<i><math>&lt; 4</math>, 4 and <math>&gt; 4</math> (mm)</i>
Hatchery	The 7 hatcheries from which the oysters originated
Handling at grading	<i>Manual, mechanical, both</i>
Sea bed type	<i>Gravel, gravel/sand, mud, mud/sand, sand</i>
Average stocking density	<i>Average no. of oysters per bag. If bags were split, oyster density was as estimated at the end of May 2011</i>
Bag split	Whether bags were split during the study into smaller number of oysters ( <i>Yes/No</i> )
Maximum summer temperature	Maximum temperature reached from the start of June until the end of August 2011, measured at 23 sites with between 1 and 8 batches

at each site.

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626 a. Sampling was carried out on a bay basis (i.e. 30 animals from one batch in each bay was  
627 sampled for the presence of OsHV-1, the sample size was based on detecting a design  
628 prevalence of 10% or greater with 95% confidence), however, the batch sampled may not  
629 have been part of this study. A bay was considered positive if at least one batch was infected.

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630 Table 2. The median and interquartile range of temperature recorded within 7 and 30 days of the reported start of mortalities at seven high  
 631 mortality sites (those with 1 or more batches with a cumulative mortality > 34%) during summer 2011 in Ireland. The median and interquartile  
 632 range of temperatures at the ten low mortality sites (those sites without any high mortality batches) during the same time periods is included for  
 633 comparison

Bay <sup>a</sup>	Reported start of mortality	At each high mortality site			Among the ten low mortality sites <sup>a</sup>		
		Temperature			Temperature		
		Median	interquartile range		Median	interquartile range	
			Q1	Q3		Q1	Q3
Within 7 days of start of mortalities							
Achill Sound	14-Jul-11	15.9	14.9	16.7	15.3	14.5	16.5
Clew Bay	20-Jul-11	14.8	13.9	15.8	15.0	13.7	16.7
Dungloe	03-Jul-11	15.7	15.3	16.9	14.4	13.5	15.7
Lough Swilly	22-Jul-11	18.8	15.5	20.0	14.4	13.4	15.4
Sherkin	11-Aug-11	14.8	14.5	15.1	15.9	15.4	16.3
Trawbreaga Bay	26-Jul-11	15.2	14.4	16.9	14.9	14.1	16.1
Woodstown Strand	26-Jul-11	14.8	14.4	15.6	14.9	14.1	16.1

Within 30 days of start of mortalities

Achill Sound	14-Jul-11	17.0	15.6	18.8	19.2	18.0	19.8
Clew Bay	20-Jul-11	15.0	14.2	15.8	15.3	14.0	18.5
Dungloe	03-Jul-11	15.5	15.0	16.2	19.3	18.4	19.9
Lough Swilly	22-Jul-11	20.5	17.4	22.9	14.8	13.7	16.0
Sherkin	11-Aug-11	14.5	13.7	14.9	15.8	14.8	16.5
Trawbreaga Bay	26-Jul-11	15.2	14.3	16.3	15.0	14.0	16.3
Woodstown Strand	26-Jul-11	15.1	14.6	15.7	15.0	14.0	16.3

634 a. The bay may also contain other sites

635 Table 3. The median cumulative mortality of oyster batches in Ireland in 2011, along with the  
 636 interquartile range by categorical risk factors that met the criteria for inclusion in the  
 637 multivariable model ( $p < 0.2$ )

Risk factor		No. of batches	Cumulative batch mortality			P- value <sup>a</sup>
			Q1	Median	Q3	
Size (mm)	2/3	3	25	39	45	0.083
	4/6	66	9	14	31	
	7 +	11	20	34	57	
Ploidy	Diploid	17	10	33	82	0.059
	Triploid	63	8.7	15	32	
OsHV-1 $\mu$ var bay status	Negative	13	8	10	12	0.199
	Positive	67	10	20	35	
Hatchery	French hatchery 1	43	9	13	23	0.005
	French hatchery 2	8	9	19	41	
	French hatchery 3	1	25	25	25	
	French hatchery 4	1	7	7	7	
	French hatchery 5	15	8	15	39	
	Non-French hatchery 1	7	12	86	100	
	Non-French hatchery 2	5	34	76	82	

638 a. P-value is based on a univariable linear mixed model using log of the final mortality as the  
 639 outcome variable and 'farm' as a random effect

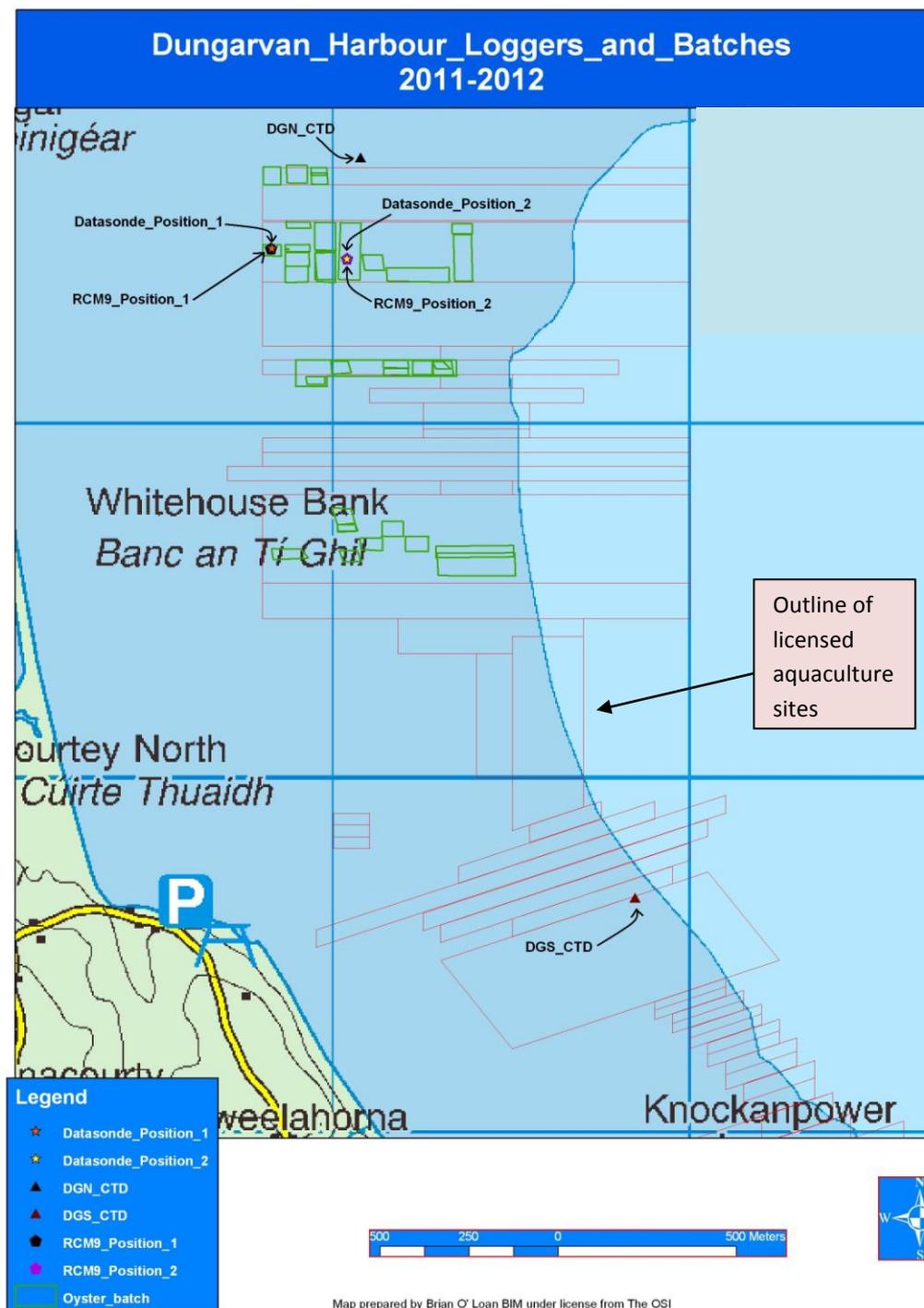
640 Table 4. Linear mixed regression model of the log of batch mortality among 80 batches of  
 641 oysters in Ireland in 2011

Variable	Categories	Estimate	Standard error	P value
Intercept		4.57	0.47	<0.001
Hatchery	French hatchery 1	-1.73	0.46	<0.001
	French hatchery 2	-1.37	0.52	0.010
	French hatchery 3	-1.20	0.84	0.157
	French hatchery 4	-1.97	0.87	0.026
	French hatchery 5	-1.33	0.50	0.011
	Non-French hatchery 1	-0.01	0.53	0.982
	Non-French hatchery 2	0.00	.	.
OsHV-1 $\mu$ var bay status	Negative	-0.85	0.37	0.035
	Positive	0.00	.	.
Max. summer temp		0.05	0.03	0.116
Max. summer temp <sup>2</sup>		-0.01	0.005	0.040
Random effects		Variance estimate	Standard error	
Farm		0.270	0.160	
Residual		0.443	0.095	

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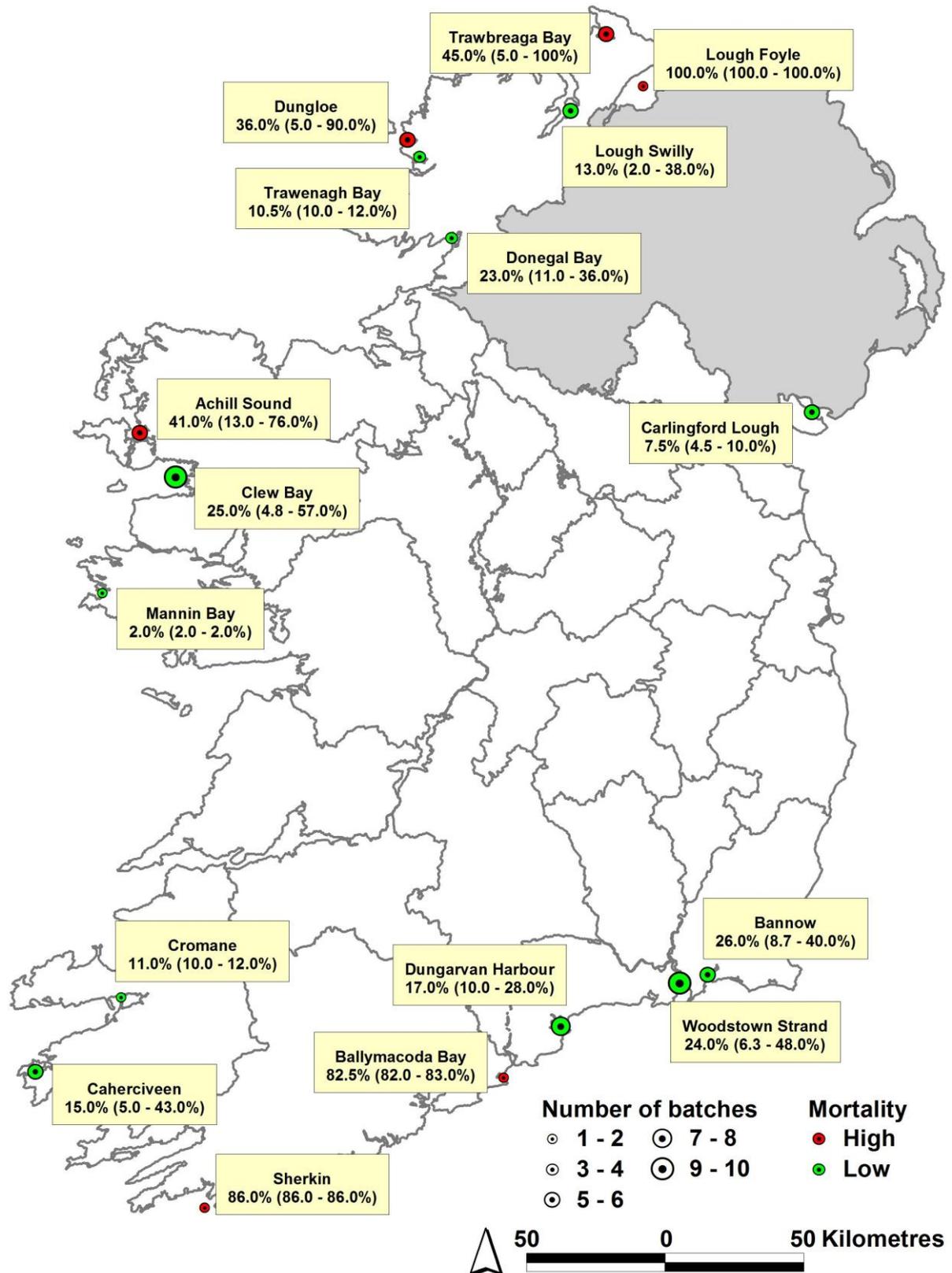
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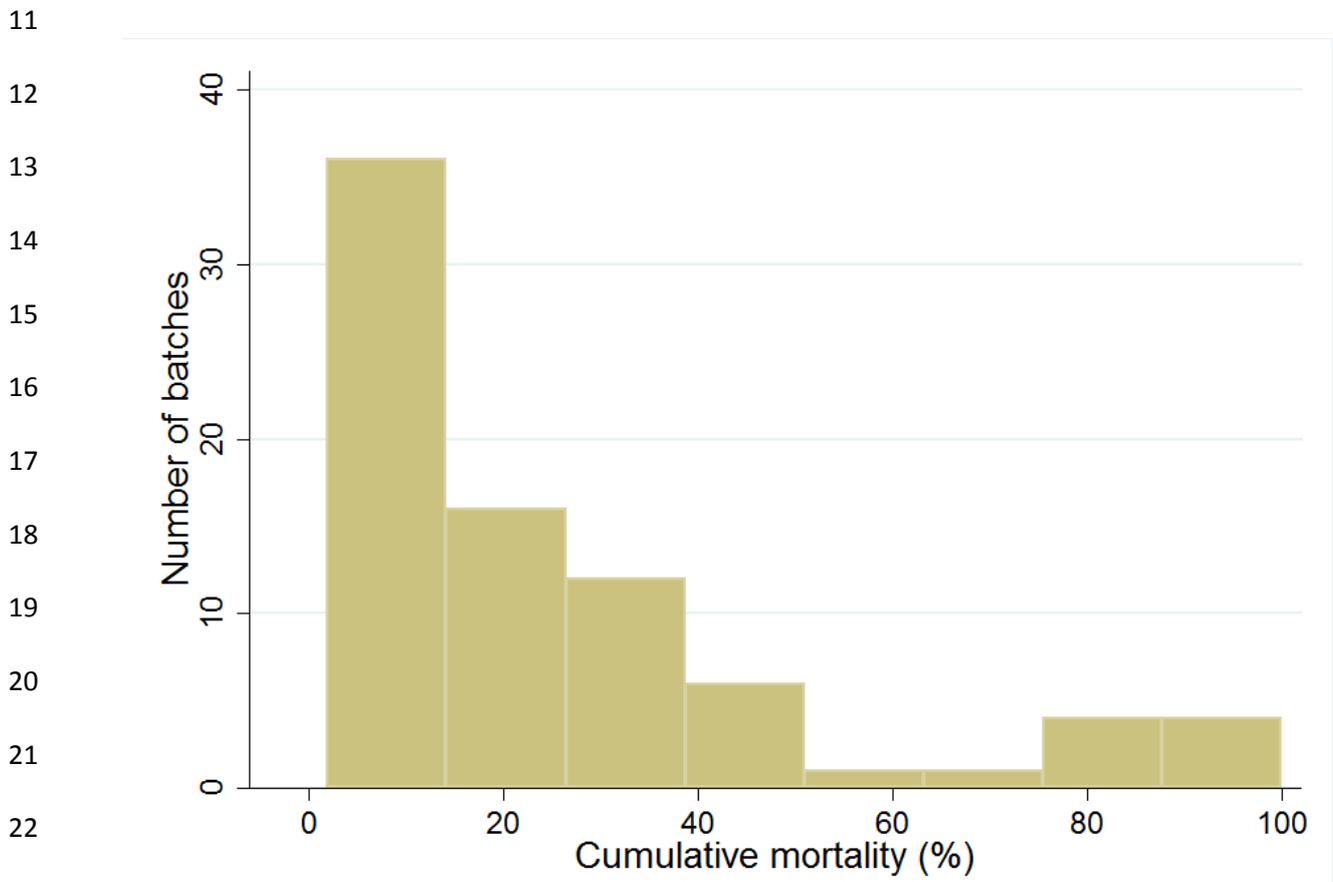
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