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

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ABSTRACT

The Pacific oyster, *Crassostrea gigas*, plays a significant role in the aquaculture industry in Ireland. Episodes of increased mortality in *C. gigas* have been described in many countries, and in Ireland since 2008. The cause of mortality events in *C. gigas* spat and larvae is suspected to be multifactorial, with ostreid herpesvirus 1 (OsHV-1, in particular OsHV-1 µvar) considered a necessary, but not sufficient, cause. The objectives of the current study were to describe mortality events that occurred in *C. gigas* in Ireland during the summer of 2011 and to identify any associated environmental, husbandry and oyster endogenous factors. A prospective cohort study was conducted during 2010–2012, involving 80 study batches, located at 24 sites within 17 bays. All 17 bays had previously tested positive for OsHV-1 µvar. All study farmers were initially surveyed to gather relevant data on each study batch, which was then tracked from placement in the bay to first grading. The outcome of interest was cumulative batch-level mortality (%). Environmental data at high and low mortality sites were compared, and a risk factor analysis, using a multiple linear regression mixed effects model, was conducted. Cumulative batch mortality ranged from 2% to 100% (median = 16%, interquartile range: 10–34%). The final multivariable risk factor model indicated that batches imported from French hatcheries had significantly lower mortalities than non-French hatcheries; sites which tested negative for OsHV-1 µvar during the study had significantly lower mortalities than sites which tested positive and mortalities increased with temperature until a peak was reached. There were several differences between the seed stocks from French and non-French hatcheries, including prior OsHV-1 µvar exposure and ploidy. A range of risk factors relating to farm management were also considered, but were not found significant. The relative importance of prior OsHV-1 µvar infection and ploidy will become clearer with ongoing selection towards OsHV-1 µvar resistant oysters. Work is currently underway in Ireland to investigate these factors further, by tracking seed from various hatchery sources which were put to sea in 2012 under similar husbandry and environmental conditions.

1. Introduction

The Pacific oyster, *Crassostrea gigas*, plays a significant role in the aquaculture industry in Ireland, both in terms of volume and value, with an annual production of over 7000 metric tonnes (Bord Iascaigh Mhara (BIM)/the Irish

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Sea Fisheries Board, personal communication). With a current value of €28–30 million per year, *C. gigas* accounts for approximately 20% by volume of overall shellfish produced in Ireland (BIM, personal communication). The main method of cultivation for Pacific oysters used in Ireland is bag and trestle cultivation, which is an off-bottom culture method. This cultivation method allows the oysters to be placed in mesh bags on metal framed structures called trestles in the inter-tidal zone, which allows access to the stock during low tide (Tidwell, 2012). Over half of all current licensed aquaculture producers in Ireland are oyster farmers, with *C. gigas* being grown in 44 bays all around the coast. *C. gigas* seed is predominantly sourced from hatcheries/nurseries or harvested wild seed, which is imported mainly from France and to a much smaller extent from the UK and the Channel Islands. The main export market for Pacific oysters is France, although there is also a growing market in Asia.

Since the 1950s, episodes of increased mortality in *C. gigas* have been described globally in all areas of production. In Europe, severe mortality events in cultured Pacific oyster were observed during the summers of 2008 and 2009 (Dégremont et al., 2013). These events have been grouped by life stage into summer mortality in adults, mortality in spat and hatchery-related mortality (European Food Safety Authority, 2010). Mortality in spat and larvae at hatcheries have been associated with detection of ostreid herpesvirus 1 (OsHV-1), a virus also associated with mortality in other farmed bivalves, including the European flat oyster (*Ostrea edulis*), scallop (*Pecten maximus*) and the Manila clam (*Ruditapes philippinarum*) (Renault and Arzul, 2001; Arzul et al., 2002; Batista et al., 2007). In 2000, OsHV-1var, a variant strain of OsHV-1, was identified in French hatcheries (Arzul et al., 2001a,b). Although OsHV-1var presents several modifications in the C region of the genome, where the most significant modifications in relation to OsHV-1 occur, and a 2.8 kb deletion, OsHV-1 and OsHV-1var are considered representative of a single viral species as the differences between the two genotypes were not great enough to establish a separate viral species (Arzul et al., 2001b). In 2008, the emergence of a third strain was described, OsHV-1 μvar, in association with abnormal mortality in *C. gigas* in France (Segarra et al., 2010; Dégremont et al., 2013). It has since been shown that mortality in *C. gigas* spat can be induced following experimental infection with OsHV-1 μvar (Schikorski et al., 2011). Further, mortality can be induced following horizontal transmission of infection from unselected asymptomatic adult to juvenile *C. gigas* (Dégremont et al., 2013). Since 2009, OsHV-1 μvar has been the predominant herpes virus strain during mortality events (European Food Safety Authority, 2010). It is now believed that the cause of mortality events in *C. gigas* spat and larvae is multifactorial, with OsHV-1 infection (with OsHV-1 μvar now predominating) a necessary but not sufficient cause (Samain and McCombie, 2010). Other suspected risk factors include an increase or a sudden change in the temperature, husbandry practices such as introduction of possibly infected spat, and the movement and mixing of populations and age groups (European Food Safety Authority, 2010; Garcia et al., 2011). The European Food Safety Authority (2010) also noted that no outbreaks had been reported when the water temperature was below 16 °C.

In Ireland, mortality events in *C. gigas* have been reported for some years (Malham et al., 2009), but linked, since 2008, to the presence of OsHV-1 μvar (D. Cheslett, personal communication). In that year, reports of mortality in Pacific oysters were received from three oyster producing bays, and the presence of the OsHV-1 μvar was confirmed in all three bays by PCR and sequence analysis of the amplicon (European Food Safety Authority, 2010). In 2009, extensive mortality and the presence of OsHV-1 μvar were reported from 15 oyster production areas, peaking in July with an average batch mortality of 37% and with mortality occurring, on average, over an 18 day period (Peeler et al., 2012). Although few clear associations between mortality and management factors were identified, the age of oysters when first infected with OsHV-1 μvar, the condition of the oysters, temperature, and other environmental factors each appeared important (Peeler et al., 2012). European Union legislation was subsequently introduced to prevent the spread of the virus to unaffected areas in Ireland and the United Kingdom, whilst still allowing trade to continue between infected areas (European Community, 2010), but noting that there was no realistic prospect of eliminating the virus (Peeler et al., 2010). OsHV-1 μvar related mortality has continued in Ireland each summer since the initial detection of OsHV-1 μvar in 2008, both in bays previously infected with this virus and coincident with spread of infection to new bays.

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

2. Materials and methods

2.1. Study design and population

A prospective cohort study was conducted during 2010–2012, from the time of first batch immersion (03 August 2010), when oysters were 2 mm in size. The oysters were followed until the date of last batch grading before data analysis, in spring/summer 2012 (12 April 2012), where oysters had been immersed for up to 18 months (subsequently termed the study period). The oyster batch was the unit of interest, and was defined as a variable number of oysters of similar size, originating from one hatchery, placed at a particular site within a bay at one point in time. All 405 batches of farmed oysters that were in the sea in Ireland during 2011 were considered for inclusion in the study. Farms were selected for logistical reasons, such as the accessibility of stock to allow for frequent monitoring and the capacity to monitor individual batches among the stock throughout the season. From the selected farms batches were chosen in order to include batches from different hatcheries, ploidy status and immersion date within each bay.

The number of batches required for the study was estimated, based on a confidence interval of 95% (alpha = 0.05),