

A literature review as an aid to identify strategies for mitigating ostreid herpesvirus 1 in *Crassostrea gigas* hatchery and nursery systems

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Abstract

An understanding of husbandry strategies and any associated risk factors is important for designing management control measures that can reduce mortality in Pacific oysters, *Crassostrea gigas*, caused by ostreid herpesvirus 1 (OsHV-1). The type of culture facility can be considered in relation to the potential pathways that could lead to the entry of a pathogen and its survival. In addition, the animal host (e.g. age, physiological state, selective breeding programmes), husbandry procedures (e.g. stocking density), the pathogen itself (e.g. pathogenicity, virulence) and environmental effects (e.g. temperature) represent other relevant interconnected factors. However, all these factors provide valuable background information for outlining the mitigation strategies needed by the industry, as well as in the context of surveillance and biosecurity programmes. These control mechanisms for hatchery or nursery areas are related to movement restrictions, water treatment, virus inactivation, the production calendar and practical farm management decisions. This comprehensive literature review compiles information related to such approaches and also includes the different existing guidelines suggested for control of OsHV-1. Therefore, the review represents a solid foundation for a more critical appraisal currently being developed to support recommendations for disease management strategies.

Key words: *Crassostrea gigas*, husbandry, management strategies, ostreid herpesvirus 1, oyster herpesvirus, risk factors.

Definitions used

Hatchery: Any installation that produces bivalve oyster seed for subsequent growth immersed directly in an open-water culture system

Nursery: An intermediate protected area for growth of oyster seed or spat prior to transplanting in an open-water culture system

Introduction

Husbandry strategies to reduce mortality in Pacific oysters, *Crassostrea gigas*, caused by ostreid herpesvirus 1 (OsHV-1) should be designed to prevent or manage the introduction and dissemination of the pathogen in bivalve aquaculture facilities. Potential specific pathways for the entry of a pathogen need to be identified, but they can be considered generally as: water (both hatcheries and open-water

facilities), bivalve broodstock movements (into hatcheries), other bivalve movements (inter- and intra-production sites), shared facilities and equipment (baskets, ropes, transport vessels), staff movements (inter- and intra-production sites), wildlife (incl. wildlife vectors) and feed (hatchery algae with uncontrolled source) (adapted from Georgiades *et al.* 2016). Many of the risk factors applying to open-water culture will also be relevant to hatcheries and nursery areas with unprotected water supplies (e.g. semi-closed systems). However, movement of marine aquatic organisms for aquaculture purposes raises many problems and challenges, as such movements may be deliberate, although they are often accidental, with bivalve spat and juveniles being transferred by the thousands or millions (Rodgers *et al.* 2015).

Bivalve molluscs do not have a conventional adaptive immune system that results in acquired immunity leading to immune memory and production of antibodies (Gestal

et al. 2008; Allam & Raftos 2015). Therefore, vaccination against pathogens is not feasible, and the use of drugs to treat any infection is limited in open-water production facilities (Renault 2016), since the rapid dilution of chemicals in water makes treatment impractical (Rodgers *et al.* 2015).

Crassostrea gigas originated from Asia and was intentionally introduced from the Pacific coast into a number of European countries in the 1970s (Rohfritsch *et al.* 2013; Rodgers *et al.* 2015). However, there are inherent risks associated with the transfer of shellfish, such as the introduction of diseases, including viruses, associated with any translocated species (Brenner *et al.* 2014). In addition, subsequent control measures rely on an understanding of factors such as the spatial distribution of mortalities, the hydrodynamics within a bay and other environmental aspects that, in the case of OsHV-1, still require scientific input to determine the relationships that lead to pathogen dissemination (Paul-Pont *et al.* 2014). Therefore, this review concentrates on the generic factors related to the animal host itself, aquaculture husbandry practices, the viral pathogen and associated environmental influences in the context of surveillance and biosecurity programmes, as well as any resultant mitigation strategies already published.

The purpose of the review is to reflect the diversity of various worldwide approaches that suggest mitigation measures for OsHV-1 according to local and national industry practices. These practices are faced with a global problem that is subjected to different environmental conditions according to the location of the specific sector. The review represents a solid foundation for a more critical appraisal that is currently being prepared which will use this information as a key element for recommending disease management strategies.

Animal host factors

Animal host factors can be related to the age of bivalves, their physiological state and growth rate, as well as any immunity resulting from exposure to a pathogen. Genetic resistance traits useful for developing selective breeding programmes can also be considered in this category. Table S1 summarizes the strategies detailed below related to animal host factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Host range

Ostreid herpesvirus 1 and its variants (see the section on other variants) have a wide bivalve host range, although they are associated with often severe mortalities in Pacific oyster *C. gigas* aquaculture facilities. The host-range species also include other *Crassostrea* spp. (i.e. *C. angulata* and *C. ariakensis*), as well as *Ostrea edulis*, *Ruditapes decussatus*,

R. philippinarum and *Pecten maximus* (Barbosa Solomieu *et al.* 2015). OsHV-1 has also been observed in mussels *Mytilus edulis* (Barbosa Solomieu *et al.* 2015) and *M. galloprovincialis* (Domeneghetti *et al.* 2014; Bivalife, pers. comm., 2014). A newly described variant of OsHV-1 (OsHV-1-SB) was recently reported to be associated with mass mortalities of blood clam (*Scapharca broughtonii*) broodstock in China (Xia *et al.* 2015). However, Arzul *et al.* (2001c) maintained that bivalve herpesviruses could be confined to a single host species in nature, but other factors, such as current intensive husbandry practices, may have led to transmission to new host species. Nevertheless, Burge *et al.* (2011) detected low levels of OsHV-1 DNA by PCR in *C. sikamea*, *C. virginica*, *M. galloprovincialis*, *O. edulis* and *Venerupis philippinarum* compared to higher levels in *C. gigas*.

Bivalve age

Mortality rates of *C. gigas* have been shown to vary between different production sites, and they depend on the age of oysters affected (Renault 2016; De Kantzow *et al.* 2017). The *C. gigas* age groups most susceptible to OsHV-1 infection and subsequent mortality are larvae and spat, whereas adults are less susceptible, although Petton *et al.* (2015b) showed that only juveniles carried and transmitted a detectable amount of OsHV-1 virus in field-exposed SPF animals. Nevertheless, OsHV-1 horizontal transmission has been demonstrated from both asymptomatic adults and infected spat to naïve spat (Dégremont *et al.* 2013; Dégremont & Benabdelmouna 2014).

Therefore, it is clear that all stages are susceptible to the virus, although to varying degrees, and Azéma *et al.* (2017a) even showed that the average mean mortality for naïve adults was 55%, although it could range from 0% to 100% depending on the family. The same authors also demonstrated that susceptibility to OsHV-1 infection decreased with age. Adults have also been shown to exhibit significantly lower mortality (Peeler *et al.* 2012), which was supported by De Kantzow *et al.* (2016) who showed in laboratory experiments that 8-month-old spat were 2.7 times more likely to die after challenge with OsHV-1 μ Var than 17-month-old adults.

However, adult Pacific oysters can also act as symptomatic carriers of the herpesvirus (Arzul *et al.* 2002; Paul-Pont *et al.* 2014; Petton *et al.* 2015b) that could then be capable of infecting the juvenile stages by cohabitation. Although vertical transmission is suspected in *C. gigas* (Barbosa-Solomieu *et al.* 2005), it has not been unequivocally demonstrated to date, except that *C. angulata* surviving an OsHV-1 mortality outbreak were shown to carry the virus and vertically transmit it to their offspring (López Sanmartín *et al.* 2016).

Nevertheless, in general terms, spat and juveniles, as well as rapidly growing *C. gigas*, have been reported to be more susceptible to OsHV-1, whereas decreased mortalities have actually been demonstrated in certain specific oyster families challenged by the virus (Anon 2011). Azéma *et al.* (2017a) found that a few tested families had a high resistance to OsHV-1 infection, whereas others had high susceptibility irrespective of the development stage. However, a majority of full-sib families exhibited increased resistance to infection from the spat to adult stage, emphasizing the importance of the life stage for resistance to OsHV-1 infection in *C. gigas*. Petton *et al.* (2015a) showed that the probability of mortality decreased with the age of oysters after first exposure, although it was also shown that the relationship between mortality and size was stronger than the relationship between mortality and age under field conditions (Dégremont 2013). Moreover, Azéma *et al.* (2017b) showed that smaller oysters had higher relative growth and higher mean mortality than larger oysters after testing 40 different families, indicating the importance of size in mortality associated with OsHV-1. In addition, although mortalities occurred in adults under experimental conditions, the adult stage was capable of managing infection despite apparent viral replication (Segarra *et al.* 2014a). In fact, Arzul *et al.* (2002) demonstrated that oyster herpesvirus could infect adults with high prevalence and the virus persisted after primary infection, whereas it was shown that juvenile and adult oysters could be a reservoir of putative pathogens (Petton *et al.* 2015b). Nevertheless, Pernet *et al.* (2012) found that Pacific oyster mortalities varied with farming practices in the Mediterranean. All this combined evidence indicates that selective breeding programmes could be a successful strategy for reducing *C. gigas* mortalities due to OsHV-1 μ Var infection (see the section on selective breeding programmes for more detail).

Antimicrobial response

Bivalves actually possess a wide-ranging number of responses to invading pathogens based on immune cells and haemocytes, as well as mucosal cells, that represent the first line of contact with their aquatic environment. According to Allam and Raftos (2015), bivalves respond to viral infections and the responses include reactions such as chemotaxis, encapsulation, apoptosis and the induction of antiviral states. In addition, oyster haemolymph has been shown to have virucidal activity against herpesviruses of mammals and fish (Olicard *et al.* 2005a,b; Carriel-Gomes *et al.* 2006). However, most important invertebrate infections actually initiate at mucosal surfaces (Allam & Raftos 2015).

It was assumed by Clegg *et al.* (2014) that prior exposure of seed or related broodstock to OsHV-1 μ Var in French hatcheries led to a likely genetic protective effect, since

C. gigas from French hatcheries showed less mortality than those from non-French hatcheries where there had been no previous exposure. The protective effect of prior exposure was also noted by other studies that showed a strong genetic basis for survival (Dégremont *et al.* 2007; Sauvage *et al.* 2009), as well as oysters selected for their higher survival that did not horizontally transmit OsHV-1 to non-resistant bivalves (Dégremont *et al.* 2013). Nevertheless, previous exposure is not related to a specific immune response because bivalve shellfish do not have an immunological memory, and they rely on innate immunity to fight pathogen-induced diseases (Clegg *et al.* 2014). Prado-Alvarez *et al.* (2016) also showed that the survival rate in previously exposed *C. gigas* was significantly higher than in naïve oysters after OsHV-1 μ Var challenge by intramuscular injection.

A study of gene transcript expression showed that OsHV-1 was able to replicate in a specific family of oysters, but they recovered from the infection, which indicated the virus could be able to enter into a persistence or latency phase (Segarra *et al.* 2014b).

One area that has received insufficient consideration to date is the effect of symbiotic microflora on host protection (Allam & Raftos 2015). This is either through indirect action, such as the production of probiotic antimicrobial compounds by haemolymph microbiota (Desriac *et al.* 2014), or direct action, such as killing of microorganisms by specific phage (Barr *et al.* 2013) and viral pathogen interference using a therapeutic virus (Paff *et al.* 2016).

It is clear that OsHV-1 infection can occur quickly, but the disease process only lasts a few days and apparently some oysters can effectively control the infection through a complex antiviral response, including control of OsHV-1 μ Var gene expression and reduction in virus load with subsequent survival after infection (Segarra *et al.* 2014b; He *et al.* 2015). Very few studies have concentrated on the ontogeny of the oyster immune system (Pernet *et al.* 2012), although the ability of immunocompetent cells to express immune-related genes has been suggested to explain the variability of susceptibility to infection during larval development (Tirapé *et al.* 2007). In addition, Green *et al.* (2014) used a herpes simplex virus (HSV-1) model to show that oysters relied on a cellular response for minimizing viral replication, which induced host cells into an antiviral state that, interestingly, was adversely affected by age and increased temperature.

The immunostimulant poly(I:C) is an analog of double-stranded RNA and can be used to mimic viral infection. It has been shown that poly(I:C) can decrease OsHV-1 replication in experimentally injection-infected *C. gigas* through induction of a protective antiviral immune response (Green & Montagnani 2013). Such studies related to RNA interference could provide valuable information concerning the

action of OsHV-1 in oyster disease processes (Petton *et al.* 2015b) and lead to early treatment of juvenile bivalves at the hatchery stage. In fact, Green *et al.* (2016) showed that larvae produced from poly(I:C)-treated parents had double the chance of surviving exposure to OsHV-1 compared to controls.

Selective breeding programmes

Based on the studies mentioned above, the role of innate immunity in *C. gigas* and the gene markers for OsHV-1 resistance will be of great value for attempting to develop specific breeding programmes. In addition, high genetic correlation for survival between different sites led Dégremont *et al.* (2007) to conclude that selective breeding at only a single site could be an effective management measure for improving survival, and therefore yield, in oysters (<1 year old) produced in all French coastal growing areas. Huvet *et al.* (2010) also suggested that resistant lines of *C. gigas* may survive better because it was shown that they were not as reproductively active as susceptible lines.

However, more information is needed for the metabolic processes and their pathways related to OsHV-1 infection in *C. gigas* that could be used to identify any biomarkers of disease resistance and thus develop antiviral control measures for mitigating the impact of mortalities (Corporeau *et al.* 2014). Nevertheless, selective breeding as a management strategy can be implemented easily for hatchery production to improve OsHV-1 resistance, and this will limit the spread of disease (Dégremont & Benabdelmouna 2014).

Dégremont *et al.* (2015c) suggested that mass selection after four generations of *C. gigas* originally derived from stocks of adult wild oysters could be an easier technique than much more costly family-based selection for commercial hatcheries. Nevertheless, another study demonstrated a large additive genetic variation for resistance to OsHV-1 infection in *C. gigas* that could also help selective breeding programmes (Dégremont *et al.* 2015b).

Specific *C. gigas* breeding programmes designed to produce genetically resistant oysters capable of withstanding OsHV-1 infection with reduced mortalities in production stocks are seen as an important addition to currently considered mitigation strategies. Dégremont *et al.* (2015a) considered that genetic improvement for disease resistance to pathogens would be an attractive option for reducing any impact on oyster production. In addition, producing resistant *C. gigas* in hatcheries can also be used subsequently in the development of restoration programmes in the field for wild oysters (Dégremont & Benabdelmouna 2014).

The selection of a family because of its resistance to OsHV-1-related summer mortality was shown to confer a survival advantage for juvenile *C. gigas* that was passed to descendant batches (Dégremont 2011), and OsHV-1-

resistant oyster lines were postulated as one way to reduce mortalities, as well as limit the spread of the disease and any potential reservoir role (Dégremont *et al.* 2013).

Renault (2016) indicated that studies to determine the genetic parameters for OsHV-1 infection resistance, as well as how these related to growth rates, were already making good progress, such as for the production of larvae with genetic resistance to OsHV-1 infection from selected adults (Dégremont *et al.* 2016b). Future genetic selection and selective breeding programmes need to form an important part of promoting resistance to mortalities caused by OsHV-1 and its variants that, once established, should increase the productivity of oysters in the production sector, since resistance has been demonstrated to be a highly heritable but variable trait (Sauvage *et al.* 2010; Dégremont 2013; Prado-Alvarez *et al.* 2016). Dégremont *et al.* (2005) indicated there was also a genetic basis for this trait, as there was a large variation in survival among families, although this was related to environmental factors rather than the presence of virus.

Nevertheless, Whittington *et al.* (2015a) cautioned that selective breeding programmes can have certain drawbacks, such as a long development time. However, selective breeding programs can actually determine how the selection of one trait will act on another trait, and Azéma *et al.* (2017a) found an absence of genetic correlation between resistance to OsHV-1 and resistance to *Vibrio aestuarianus* leading to the conclusion that selection for dual resistance could be possible. In addition, lines selected for their higher survival and higher resistance to OsHV-1 have been shown to have higher growth than unselected lines after four generations, although in practice this benefit would depend on the concomitant use of adequate culture techniques under actual production conditions (Dégremont *et al.* 2015c).

Aquaculture has already had a genetic impact on farmed oyster species, but care must be taken when choosing and characterizing the broodstock at the beginning of a breeding programme, as well as monitoring any new genetic diversity after a programme has been established (Rohfritsch *et al.* 2013). However, reducing mortalities using such programmes will have important economic benefits for the production sector, not only in the short term but in the future, since genetic improvement is inheritable and sustainable (Gjedrem *et al.* 2012). These programmes should be combined with a reconsideration of husbandry practices (e.g. oyster exchanges, stocking densities) (Rohfritsch *et al.* 2013) in order to offer solutions to the current mortality problems suffered by the sector.

Ploidy

Triploid *C. gigas* have been shown by Cheney *et al.* (2000) to undergo higher 'summer mortalities', even with no viral

association, compared to diploid oysters, although, in general, the oysters were subjected to chronic stress due to multiple environmental factors, such as low dissolved oxygen, high water temperatures and the presence of phytoplankton blooms. However, these factors often also occur at the time of OsHV-1 infections, which indicates that triploid oysters could be even more susceptible after the onset of the disease process according to the conditions at each specific production site.

Pernet *et al.* (2012) found that mortalities of diploid and triploid *C. gigas* were similar during spring, although they doubled in diploid oysters in summer and autumn. The same authors, unlike Cheney *et al.* (2000), commented that triploid oysters cultured in a French lagoon area had historically actually shown higher resistance to summer mortalities, which prior to 2008 were not necessarily linked to OsHV-1. Dégremont *et al.* (2010a) recorded similar mortality rates for both ploidy levels, whereas lower mortalities were shown in triploids (Gagnaire *et al.* 2006; Jenkins *et al.* 2013). Dégremont *et al.* (2016a) found that both diploid and chemically induced triploid *C. gigas* had similar mortality that could be high using unselected parents or low using parents selected for their higher resistance to OsHV-1. In Ireland, diploid *C. gigas* had a greater mortality risk (e.g. slightly higher cumulative mortalities) than triploid oysters following natural exposure to OsHV-1 μ Var (Clegg *et al.* 2014), whereas differences in ploidy have been found to have no significant effect on mortality (Peeler *et al.* 2012; De Kantzow *et al.* 2017). Therefore, according to the genetic material used, different studies have shown different results and the effect of ploidy is not clear, although Dégremont *et al.* (2016a) suggested that most studies confounded ploidy and family effects, although the absence of OsHV-1 susceptibility between diploids and triploids has been shown (Azéma *et al.* 2016; Dégremont *et al.* 2017). Therefore, it has been suggested that either tetraploid lines need to be produced from selected diploid broodstocks or hatcheries need to be provided with the best breeders that have the greatest resistance to OsHV-1 (Dégremont *et al.* 2016a).

Physiological and nutritional state

Whittington *et al.* (2015b) reported that well-fed oysters died due to OsHV-1, whereas poorly fed oysters did not, although it was suggested that this was probably associated with a lack of development of infection after removal of food from the water by filtration. However, Pernet *et al.* (2014b) showed that higher energy reserves in well-fed *C. gigas* coincided with a decreased risk of mortality, whereas Evans *et al.* (2015) maintained that cumulative mortality was higher and median time to death was lower in fed oysters compared with non-fed oysters. Nevertheless,

the opposite has also been reported, since high mortality was shown in unfed oysters challenged with OsHV-1 (Schikorski *et al.* 2011a,b). Therefore, the role of the physiological state of *C. gigas* exposed to OsHV-1 is unclear.

Pernet *et al.* (2014a) studied the energetic reserves and quality of available food in a *C. gigas* lagoon production area and concluded that the dynamics of spat mortality correlated with differences in their energetic condition, which was related to variable food quality and the role of diatoms in the diet. Pernet *et al.* (2012) previously showed that oysters cemented onto ropes had a better nutritional condition, as measured by triacylglycerol tissue levels, which enhanced their resistance to mortality compared to *C. gigas* in baskets.

Nevertheless, mass mortalities of *C. gigas* can occur only due to local environmental conditions without the involvement of any known pathogens. For instance, Bodoy *et al.* (1990) showed that ecological changes in the spring were associated with poor physiological condition of oysters growing at high densities, which coincided with high metabolic requirements, exhausted energetic reserves and scarce natural food resources that together caused mortalities. These conditions have subsequently also been shown to be factors in mass mortalities linked closely to OsHV-1 infections, with or without the involvement of *Vibrio* spp. Consequently, the potential for rapid OsHV-1 progression is likely to be exacerbated due to the coincidence of weakened oysters and rising water temperatures.

Petton *et al.* (2015b) considered that oyster physiology was a key feature of disease caused by OsHV-1 in *C. gigas* co-infected naturally with the herpesvirus and pathogenic vibrios, which linked physiology with associated microbial communities.

Husbandry factors

Husbandry factors can be related to the production cycle, including the culture systems used, stocking densities and the presence of co-cultured bivalves. Table S2 summarizes the strategies detailed below related to husbandry factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Type of culture

Whittington *et al.* (2015c) considered that improved husbandry was needed at all stages of the production cycle. However, Pernet *et al.* (2012) showed that *C. gigas* cemented onto ropes had approximately 30% mortality by the end of a production cycle compared to 80% held in Australian-type baskets that had a higher (6 \times) stocking density. This was attributed to enhanced water circulation around each oyster resulting in an increased flushing rate,

since the detection frequency and quantity of OsHV-1 DNA were similar in both culture systems.

Ostreid herpesvirus 1 was detected more frequently in sheltered inland facilities, such as nurseries and semi-enclosed areas (e.g. estuaries and rivers), which was significantly related to spat mortalities (Garcia *et al.* 2011). This was supported by Pernet *et al.* (2012) who showed *C. gigas* could be reared with no mortality in the open Mediterranean Sea compared to a nearby lagoon area, although the oysters remained susceptible to disease during the 2-year production cycle. In addition, the risk of mortality in sentinel oysters was shown to be higher inside a bivalve farming area than outside the area, leading Pernet *et al.* (2014b) to suggest that the infection pressure would be higher in intensive farming activity areas, and local differences in husbandry practices would have a significant role in the spatial and temporal dynamics of mortalities. Oyster leases are habitually concentrated in sheltered inshore areas where relatively low tidal currents represent favourable conditions for disease transmission and mortality (Petton *et al.* 2015a). Petton *et al.* (2015b) found that cultured oysters had a significantly higher mortality and transmitted more disease than wild oysters. However, it is generally considered that there must be a sufficient population of susceptible aquatic animals to support the spread of any infection; therefore, the start of an epidemic requires a minimum threshold of infected individuals that will lead to increasing disease spread (Murray 2009), which is related, in this case, to the density of cultured oysters.

The protective effect of high growing height on adult oysters but not spat was also confirmed by Whittington *et al.* (2015a), since age and growing height in the intertidal zone were important determinants of mortality related to OsHV-1 infection, and infection prevalence was lower in baskets than in trays. In contrast, the protective effect of a high growing height on spat oysters was observed by Azéma *et al.* (2017b), although the height had no effect on 31 of 40 families studied as these families showed no potential for adaptation to improved growing height practices. However, certain families showed extremely high mortality (>95%), whereas two families showed the lowest mortality (<40%), and there was a tendency in nine families towards higher mortality in oysters that emerged less (85% for a low height practice compared to 72% for high height). Nevertheless, a comparison between studies with variable results is difficult as they do not standardize on the same families, which highlights the importance of the germplasm due to the strong genetic basis for resistance to OsHV-1 in *C. gigas* (Azéma *et al.* 2017b).

It has also been determined that mortality associated with OsHV-1 depended on the position of oysters in the seawater column, since decreased mortality occurred for

adults in intertidal trays at a high height compared with a low height (Paul-Pont *et al.* 2013b).

Richez (2012) reported that oysters placed in ponds (e.g. French 'claires') were much less affected by mortalities during the summer due to a short 6 month production cycle starting in spring before any temperature increase and using very low densities (5 per m²), which gave *C. gigas* time to acclimatize. In addition, no mortality was seen in hatchery-produced oysters reared alone in ponds, although mortality was high following cohabitation with known positive wild-caught *C. gigas* (Dégremont & Benabdelmouna 2014).

In addition, hatcheries and nursery areas should be carefully located to avoid the direct influence of tidal currents that pass through known disease-positive zones. In fish farming areas, it has been shown that fewer, highly separated farms reduced overall losses compared to numerous smaller farms in close proximity (Salama & Murray 2011).

Density and handling

To understand the potential differences between hatchery and nursery/grow-out facilities that might influence the dissemination of OsHV-1 and its subsequent control, it is necessary to consider in general that the dynamics of aquatic infectious diseases are related to the density of host populations (Krkošek 2010). Any mitigation strategies (including possible eradication) depend on host density thresholds (Pernet *et al.* 2012) that need to consider the presence of the number of aquaculture facilities in a local epidemiological unit and how they interact. Low host densities decrease the contact rate defined by the relationship between the susceptible host and the host containing the pathogen, which can lead to slower pathogen dissemination and unsustainable infection (Pernet *et al.* 2012).

De Kantzow *et al.* (2017) showed that, even after allowing for time on farm, density and handling, baskets containing oysters with a greater average length had a lower mortality than baskets containing smaller oysters. In addition, handling oysters in the week before an outbreak led to higher mortality, indicating that handling should be avoided during designated high risk periods. Pernet *et al.* (2016) indicated that OsHV-1 disease risk increased in fast-growing *C. gigas* compared to slow-growing individuals. After a year, selected farm-reared small oysters were shown by Azéma *et al.* (2017b) to have twice the growth rate of large oysters and significantly higher mortality in 29 of 40 families tested. Therefore, it was hypothesized by the same authors that OsHV-1 could actively use oyster cellular mechanisms to replicate, which would lead to increased OsHV-1-associated mortality in fast-growing oysters, as the oyster daily growth rate is known to decrease from larvae

to adult that coincides with a decrease in susceptibility to OsHV-1 (Whittington *et al.* 2015a; Azéma *et al.* 2016).

Petton *et al.* (2015a) reported that the possibility of mortality in oysters decreased with water renewal and increased with the biomass of neighbouring infected animals, and that the early rearing history, timing and duration of exposure to the disease were important additional factors to consider (see also the section on the production calendar) together with density.

Presence of other species (incl. wild species and co-culture)

The presence of wild species in the same area as bivalve hatcheries or nursery areas, as well as on-site co-culture in aquaculture facilities, can be a potential source of pathogen transmission from non-susceptible to susceptible species. As OsHV-1 has been shown to have a wide host range that includes non-susceptible bivalve species (see the section on host range), these may have a direct effect on disease control strategies in hatcheries and nursery areas due to the potential for interspecies transmission (Arzul *et al.* 2001b,c; Barbosa Solomieu *et al.* 2015). In addition, Burge *et al.* (2011) also considered that various bivalve species could represent potential reservoirs for OsHV-1.

However, non-susceptible species could also be beneficial as their close proximity may lead to synergistic effects. For instance, Whittington *et al.* (2015a) found that the presence of a large number of non-susceptible adult Sydney rock oysters, *Saccostrea glomerata*, probably acted as a 'buffer' by removing OsHV-1 viral particles from the water close to cultivated *C. gigas* because of their filtration capacity. In addition, it has been shown that the mortality risk for sentinel oysters in mussel, *M. galloprovincialis*, facilities was lower than in oyster farms, indicating that they could possibly reduce the infection pressure on susceptible *C. gigas* (Pernet *et al.* 2014b).

Nevertheless, Pernet *et al.* (2016) considered that there were still information gaps concerning the definition of aquatic reservoirs and wild animal carriers of OsHV-1, which prevent an accurate evaluation of the effectiveness of disease management strategies. As OsHV-1 infections can start in wild oysters already infected in their native areas and rapid transmission can then occur to nursery areas, the virus cannot be eradicated from stocks of self-recruiting oysters (Dégremont & Benabdelmouna 2014).

Pathogen factors

The pathogen factors to consider for OsHV-1 can be related to its virulence, survival in the environment, life cycle, any latent period and pathogenicity. Table S3 summarizes the strategies detailed below related to pathogen

factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Life cycle

Domingo (2010) described three main steps for the processes of viral disease emergence: (i) introduction of a viral pathogen into a new host species; (ii) establishment of the pathogen in the new host; and (iii) dissemination of the pathogen in a large number of individuals of the new host.

Ostreid herpesvirus 1 satisfies these steps through its capability of direct transmission between hosts (Schikorski *et al.* 2011a,b). There are many reports that OsHV-1 is rapidly disseminated and causes explosive mortalities in *C. gigas*, which has led some authors to consider that the transmission phase of the life cycle cannot only be related to transfer between individual bivalve hosts. Paul-Pont *et al.* (2013a) suggested that the virus could be transmitted by specific planktonic vector particles in the water, since the spatial clustering of oyster mortalities is similar to that seen in planktonic aggregations (Whittington *et al.* 2015c). Therefore, Paul-Pont *et al.* (2014) considered that the most likely explanation for an Australian mass mortality event was synchronous exposure to a common environmental source capable of infecting most individuals at the same time.

It has also been considered that the induction of mortality events in *C. gigas* spat and larvae is probably multifactorial (Clegg *et al.* 2014; EFSA 2015). Nevertheless, Petton *et al.* (2015b) indicated that oysters were naturally co-infected by the herpesvirus and vibrios.

Pathogenicity and survival in the environment

The maximum survival time outside the described host bivalve species is still unknown (OIE 2016), and it is difficult to predict how pathogenicity can change over time (Castinel *et al.* 2013). Therefore, Pernet *et al.* (2016) considered there were information gaps related to the persistence of OsHV-1 outside its host.

Early experimental transmission showed OsHV-1 pathogenicity that was capable of causing 100% mortality within 6 days after infection (Renault *et al.* 1995). Later, Martenot *et al.* (2015) demonstrated that OsHV-1 μ Var could persist and remain infectious in seawater (54 h at 16°C), but high temperatures seemed to reduce its infectivity (33 h at 25°C), although pathogenicity could be modulated by biological, physical and chemical factors (e.g. plankton, water currents or pesticides). Hick *et al.* (2016) showed that OsHV-1 retained its infectivity in seawater for 2 days at 20°C. However, although OsHV-1 μ Var was shown to be significantly associated with mortality, indicating that it was therefore necessary although insufficient to

cause such events, it may also be the case that OsHV-1 is after all a sufficient cause, but there is a very strong dose effect (Whittington *et al.* 2013; Clegg *et al.* 2014). Nevertheless, it is also possible that OsHV-1 has low pathogenicity (Pande *et al.* 2015), although Burge *et al.* (2006) indicated that pathogenicity varied with the size of the host oyster. OsHV-1 μ Var has also been detected in the absence of mortality in imported *C. gigas* cultivated in Italy (Dundon *et al.* 2011).

Ostreid herpesvirus 1 μ Var DNA was detected in seawater by PCR and copy numbers in the first 48 h after injecting spat with virus were $1 \times 10^5 \text{ mL}^{-1}$, and a maximum of $1 \times 10^6 \text{ mL}^{-1}$ following infection after cohabitation with oysters (Schikorski *et al.* 2011a,b).

Virulence

Pernet *et al.* (2016) considered there were information gaps related to the minimum infective dose of OsHV-1, which would be data useful for constructing epidemiological models. Oden *et al.* (2011) defined a viral load threshold of 8.8×10^3 OsHV-1 DNA mg tissue⁻¹ above which there was a risk of oyster mortality. However, Petton *et al.* (2015b) showed that, although the quantity of OsHV-1 DNA was a predictor of mortality, in the absence of *Vibrio* spp. a high load of virus was not enough to induce full expression of the disease. Castinel *et al.* (2013) (citing Mortensen *et al.* 2007) considered that husbandry practices, such as stress or stock transfer, could lead to an increase in virulence of some pathogens. However, their direct role in disease outbreaks in the shellfish sector is unclear (Castinel *et al.* 2013).

It is too early to determine whether the newly described variants of OsHV-1 (see the section on other variants) have different virulence from OsHV-1 and OsHV-1 μ Var (Barbosa Solomieu *et al.* 2015).

Other variants

Gittenberger *et al.* (2016) considered the possibility that OsHV-1 μ Var and other variants could be more widespread than originally thought, especially in areas with wild stocks where there is no commercial oyster culture. In addition, Burioli *et al.* (2016) found nine different geographically distributed OsHV-1 μ Var genotypes in healthy wild *C. gigas* in Italy. The studies of Bai *et al.* (2015) and Martenot *et al.* (2013) both showed that OsHV-1 was distributed as widely as OsHV-1 μ Var and its other variants.

New variants μ Var $\Delta 9$ and μ Var $\Delta 15$ have been described in France associated with *C. gigas* (Martenot *et al.* 2013; Barbosa Solomieu *et al.* 2015), whereas new OsHV-1 virus types causing mortalities in clams, *S. broughtonii*, and scallops, *Chlamys farrieri*, have been characterized from China

(Bai *et al.* 2015; Xia *et al.* 2015). Lynch *et al.* (2012) described a previously undescribed OsHV-1 genotype in Ireland that was closely related to OsHV-1 μ Var, whereas Morrissey *et al.* (2015) detected another genotype closely related to OsHV-1 μ Var in approximately 21% of virus-infected bays.

Environment factors

The potential influence of environmental factors on OsHV-1, such as temperature, reservoir populations or water pollution, is important for hatchery and nursery areas due to the close association between each ecosystem compartment. Table S4 summarizes the strategies detailed below related to environment factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Temperature

In Europe, it has been shown that a seawater temperature of approximately 16°C triggers the onset of OsHV-1 infections (Dégremont 2013), and this is often followed by severe mortality outbreaks (up to 90–100%) in zones where the temperature increases rapidly in spring. This pattern occurs particularly in Atlantic (i.e. France) and Mediterranean (i.e. France and Spain but not Italy) coastal production sites.

Other, more northern countries, that have been reported positive for OsHV-1 μ Var (e.g. Ireland, UK-England), where the temperature reaches 16°C in the late spring-summer months, do not have sudden increases in water temperature in subsequent weeks and do not undergo such high mortality levels (Roque *et al.* 2012; Barbosa Solomieu *et al.* 2015). This indicates that viral infection must take place before seawater reaches a temperature of 16°C, as OsHV-1 virus DNA can be detected at <16°C before significant mortality events are reported (Renault *et al.* 2014). The upper limit for natural mortalities in the Thau Lagoon, France, has been shown to be approximately 22–25°C (Pernet *et al.* 2012).

Nevertheless, although OsHV-1-related mortality depends on seawater temperature, a lower threshold of 14°C has been observed, although this was linked to a higher temperature of 16°C during the previous 2 weeks (Dégremont *et al.* 2013). However, irrespective of temperature, mortalities are not consistent across sites, although, generally, higher temperatures equate to higher mortalities.

Low risk of infection in healthy oysters exposed to field conditions has been shown provided the daily average seawater temperature was <14.7°C, whereas OsHV-1 was transmitted to oysters when the temperature was between 14.7 and 15.8°C and mass mortalities then occurred at

>16°C (Petton *et al.* 2015a). De Kantzow *et al.* (2016) also showed the direct effect of water temperature on infection and disease by removing the field variables using laboratory challenge with OsHV-1 μ Var and they indicated that infection did not take place below a threshold temperature of 14–18°C.

In Australia, the seasonal risk factors are less certain, and the effects of temperature are not the same as those in Europe (Paul-Pont *et al.* 2013b), although stressful conditions (e.g. rearing techniques) favour viral infection. Whittington *et al.* (2015c) showed that when mortality outbreaks occurred, the water temperatures in New South Wales (NSW) were 20°C, which was approximately 4°C higher compared to, for instance, France. However, Paul-Pont *et al.* (2014) indicated a slightly higher temperature of 22°C was needed for onset of mortalities. Nevertheless, it has been pointed out that comparison with French farming sites should be treated with caution, as the density of cultured *C. gigas* is much lower in NSW coastal areas, which could explain why outbreaks in France are continuous in summer (Whittington *et al.* 2015c). The density and design of trays is also one of the factors that affects the circulation of water and the accessibility of oysters to viral particles in the water (Paul-Pont *et al.* 2013b). Close contact between animals facilitates infection and subsequent disease transmission as the temperature increases, which is augmented by high quantities of viral particles released by a larger number of infected hosts (Pernet *et al.* 2012; Whittington *et al.* 2015c), potentially leading to a higher probability of observing mortality at a lower temperature.

Nevertheless, irrespective of latitude, if oyster transfers occur during summer, this can result in virus transmission (OIE 2016). Table 1 shows the reported effect of

temperature on OsHV-1 infection and/or mortalities in more detail.

Viral particle attachment

The sudden explosive *C. gigas* mortality outbreaks associated with rapidly rising temperatures of >16°C often occur almost simultaneously in shallow-water oyster cultivation areas, such as embayments or lagoons. This indicates that the majority of oysters are almost certainly infected from a common environmental source, and that OsHV-1 is probably transmitted through water (Whittington *et al.* 2015c) and carried to susceptible oysters by plankton particles (Paul-Pont *et al.* 2013b; Evans *et al.* 2014, 2015). Viruses are too small to be efficiently retained by the gills of bivalves (Riisgård 1988), but suspension feeders can ingest picoplankton-sized particles embedded within aggregates (Kach & Ward 2008), indicating that OsHV-1 could also be associated with such marine accumulations (Whittington *et al.* 2015b).

It has also been hypothesized that mussels can bioaccumulate oyster pathogens attached to particles and aggregates, and may clear OsHV-1 from the water column in a lagoon environment, thus decreasing the infection pressure and mortality in susceptible oysters held in the vicinity of mussel farms (Pernet *et al.* 2014a,b; Ben-Horin *et al.* 2015).

Fouling organisms

Fitridge *et al.* (2012) considered that fouling organisms and microbial communities on cage netting could represent a health risk to cultured species by harbouring pathogenic

Table 1 OsHV-1 infection and/or mortalities related to temperature

Virus	Temp. range (°C)	Country	Infection/mortality	Reference
OsHV-1	13	France	No virus transmission	Petton <i>et al.</i> (2013)
OsHV-1	<16	France	Infection with no mass mortality	Renault <i>et al.</i> (2014)
OsHV-1 μ Var	16	France, Ireland, Spain	Lower threshold temperature above which natural mortality occurs	Clegg <i>et al.</i> (2014), Pernet <i>et al.</i> (2012), Petton <i>et al.</i> (2015a), Renault <i>et al.</i> (2014), Roque <i>et al.</i> (2012) and Bivalife (pers. comm., 2014)†
OsHV-1	16–22	France	Effective virus transmission	Petton <i>et al.</i> (2013)
OsHV-1 μ Var	19–24	Australia (NSW)	Disease occurrence‡	Paul-Pont <i>et al.</i> (2013a, 2014)
OsHV-1 μ Var	21–22	Australia (NSW)	Lower threshold temperature above which mortality occurs	Jenkins <i>et al.</i> (2013) and Paul-Pont <i>et al.</i> (2014)
OsHV-1 (μ Var)§	24	France	Upper threshold temperature above which natural mortality ceases to occur	Pernet <i>et al.</i> (2012)
OsHV-1	27–29	France	50% mortalities in oysters challenged by cohabitation	Petton <i>et al.</i> (2013)

†Bivalife: Controlling infectious diseases in oysters and mussels in Europe. FP7-KBBE-2010-4, grant agreement 266157.

‡DNA present at lower water temperature.

§OsHV-1 detected but probably the microvariant.

microorganisms. In addition, herpes-like particles have been seen in an estuarine thraustochytrid-like organism, as well as OsHV-1-like virions in a marine protist in experimental rearing tanks containing *C. gigas* larvae, and it is possible that this type of protist could act as a vector for OsHV-1 (Paul-Pont *et al.* 2013b).

Water hydrodynamics (incl. connectivity, tides/currents)

Pernet *et al.* (2016) considered there were information gaps related to the potential for long-distance dispersal of OsHV-1 associated with the influence of environmental factors. The siting of hatcheries and nursery areas is important because the hydrodynamic connectivity and biomass of potentially infected oysters can represent major drivers for disease in culture facilities (Salama & Murray 2011; Petton *et al.* 2013). Paul-Pont *et al.* (2014) considered that the exposure of oysters via tidal movements of water could explain a mass mortality, and Pernet *et al.* (2012) indicated that mortality spread between infected and healthy oysters as a result of hydrodynamic connectivity, which has major biosecurity implications for hatcheries with no protected water source or nurseries operating with semi-open systems.

Reservoir populations

A study by Whittington *et al.* (2013) showed that no species sampled (i.e. wild molluscs, decapods, gastropods and algae) could either be confirmed or ruled out as potential reservoirs for OsHV-1 and, as a result, they considered that the determination of environmental reservoirs of pathogens was inherently difficult.

Nevertheless, wild stocks of *C. gigas* are often used as broodstock and Petton *et al.* (2015a) suggested that OsHV-1 was maintained in wild oysters in most French farming areas. Therefore, translocation of wild oysters for use in hatcheries can be considered as an important risk factor for disease transmission that could lead to mortality of spat and juveniles once transferred to nursery and grow-out areas.

Watershed pollution (incl. pesticides/herbicides)

Moreau *et al.* (2015) showed that pesticides at environmentally relevant concentrations were able to cause adverse effects on Pacific oysters and they increased the susceptibility to OsHV-1 virus infection under experimental conditions. In addition, Gagnaire *et al.* (2007) reported that mortality was higher in pesticide-treated oysters compared to untreated oysters after challenge with *Vibrio splendidus*. This represents a problem for nursery areas sited in coastal areas, which are often shallow semi-enclosed lagoon-type ecosystems, because this type of watershed pollution is

inextricably linked to local agriculture and the seasonal use of herbicides or pesticides (Carafa *et al.* 2007, 2009). In addition, it has been shown that bioaccumulation of herbicides occurs in the water column, sediment, macroalgae and wild bivalves (Carafa *et al.* 2007, 2009).

Global warming/climate change

There is a link between global warming and the increased frequency of invertebrate mass mortality in littoral areas that leads to energetic constraints for suspension filter feeders, such as bivalves (Coma *et al.* 2009). Increased risk of mortality due to OsHV-1 has been linked to low energy reserves in *C. gigas* (Pernet *et al.* 2014b).

Wild and aquacultured populations can be connected by shared pathogens and any diseases are influenced by host densities, which in turn are affected by environmental alterations, such as climate change, that will moderate increased aquaculture production in the future (Krkošek 2010).

Designing management control measures

Some of the most important practical considerations for reducing *C. gigas* mortalities caused by OsHV-1 include the concepts of biosecurity and mitigation. Figure 1 shows potential management control strategies to reduce mortality considering the main compartments affected. However, prevention and establishment of the absence of a problem (e.g. a pathogen) should be the priority (Brenner *et al.* 2014), although early detection of disease trends or drivers relies on good quality surveillance data. Tables S5–S7 summarize the strategies detailed below related to surveillance, biosecurity and mitigation factors, respectively, for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Surveillance programmes

'Surveillance' is used to indicate the collection, analysis and dissemination of health information (OIE 2009). Carnegie *et al.* (2016) considered that solutions used to improve shellfish aquaculture health management should include the establishment of more broad-based surveillance programmes and application of risk analyses to improve regulation. The sustainability of bivalve shellfish production relies on the continuous provision of background scientific knowledge through surveillance programmes that can identify early trends in the development of emerging diseases. This is especially important because it is considered that the legislation has been slow to react to emerging diseases and the appearance of exotic pathogens in new areas (Rodgers *et al.* 2015), which is important for hatcheries and nursery areas.

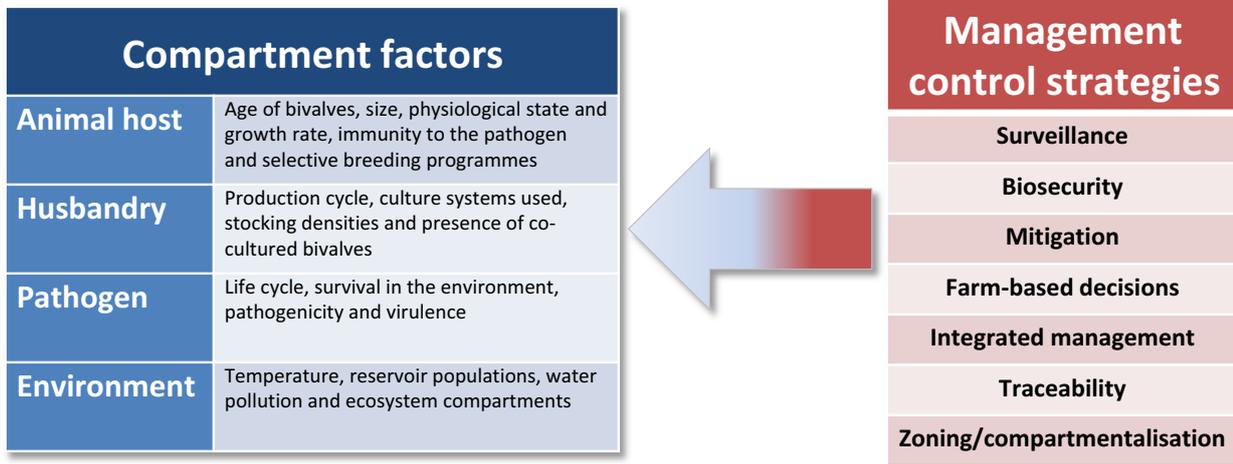


Figure 1 Management control strategies to reduce mortality considering the main compartments affected.

The importance of data collection through surveillance programmes and the creation of linked databases were reported by Soletchnik *et al.* (2007) who monitored oyster mortalities at multiple sites along the French coastline during a 10–12 year period. Mortality data were combined with information from environmental monitoring databases to show differences in different age classes for summer and spring mortality patterns in 1- and 2-year-old oysters, respectively, but without making reference to any specific pathogen.

However, it has been considered that there are data gaps in certain key areas (Rodgers *et al.* 2011) and a lack of basic consistent chronological data that could be used for generating real-time trends useful for informing decision-making (Peeler & Taylor 2011; Peeler *et al.* 2012; Rodgers *et al.* 2015). This is also supported by Brenner *et al.* (2014) who recommended: ‘...the need for comprehensive health surveillance strategies involving procedures to systematically look for early signs and assess the adverse effects on the health-status of a country...’, which has important implications for translocation of wild *C. gigas* into hatcheries as broodstock.

It is considered very important to establish farmers as the starting point of disease surveillance, so they can use their practical knowledge in conjunction with an awareness of the perceived surveillance benefits (Brugere *et al.* 2017), before involving policy makers and scientists in the development of necessary strategies and procedures that need to be developed through interdisciplinary collaboration (Brenner *et al.* 2014). This was also echoed by Castinel *et al.* (2015) who considered there was a need to work in partnership for developing practical and effective measures to manage bivalve diseases such as that caused by OsHV-1.

Nevertheless, the findings of a workshop recognized that passive farmer reporting was still the main system used to identify new outbreaks in all countries (Anon 2011), since OsHV-1 and its variants do not cause any listed (notifiable) diseases. Consequently, many mortality episodes are probably not reported unless high mortalities significantly affect production stocks or compensation schemes are in place to cover any losses.

However, the new European Animal Health Law (EU Regulation 2016/429; Anon 2016) emphasizes the importance of surveillance as a key element of animal health policy, whilst prioritizing prevention, early detection and a quick response, to enable more efficient control and eradication procedures (Brenner *et al.* 2014). The hope is that data collection, data analysis and the response to developing mortality outbreaks can be improved, although it is recognized that there are very few means to routinely collect useful risk factor data for trend identification. The Australian Aquavetplan (Anon 2015) also outlines the necessity for surveillance (see Annex S1).

Biosecurity programmes

Aquaculture facilities have an inherent risk of pathogen introduction and dissemination, but specified biosecurity measures can be used for their management (Georgiades *et al.* 2016). However, such measures are essentially more efficient if they are preventive, therefore, they should be proactive (early practical) rather than reactive (late responsive). Some basic concepts for biosecurity programmes are shown in Annex S2. In general, the identification and management of risks should be based on good epidemiological principles together with a logical, science-based approach to disease control (Subasinghe 2005). Castinel *et al.* (2015)

indicated what the most effective preventive strategies were for OsHV-1 considered by producers in Australia and New Zealand (see Annex S3).

Biosecurity in the context of the husbandry strategies for managing OsHV-1 in bivalve facilities (e.g. hatcheries and nursery areas) can be identified as a number of different measures designed to prevent or reduce the risk of transmission of the herpesvirus associated with disease. This approach helps to minimize the risk of disease transmission between neighbouring sites and protects a locally defined epidemiological unit from production losses.

Renault (2016) also considered that biosecurity measures for OsHV-1 could be designed to protect hatchery and nursery facilities, as well as the surrounding environment, from the introduction of the virus. However, after mortality outbreaks, the destruction of infected stock, disinfection of water and equipment, and fallowing can be used together with other biosecurity measures to limit the spread and prevent the reintroduction of OsHV-1 (Pernet *et al.* 2016). This was also supported by Normand *et al.* (2014) who considered that onshore rearing in hatcheries allowed closer control of the inputs related to the prevention of pathogen entry through the application of biosecurity-based management practices. The same authors considered that the use of large-sized hatchery spat in onshore nurseries would reduce the probability of disease outbreaks in closed production systems.

Sauvage *et al.* (2009) recommended screening for OsHV-1 in stocks of oysters during their transportation between different geographical zones or before transfer of larvae and seed to the field in order to help prevent the spread of the virus.

Coen and Bishop (2015) considered that biosecurity programmes were being increasingly used to reduce the translocation of diseases during aquaculture production in conjunction with measures such as cultivation using animals selectively bred for disease resistance. These authors (additionally citing Rodgers *et al.* 2015) indicated that biosecurity programmes involved:

- disease prevention (e.g. sourcing stock from certified disease-free locations or holding animals under quarantine conditions until they can be verified as disease-free);
- disease monitoring (e.g. regular assessment of the water quality and health of animals);
- cleaning and disinfection between production cycles (e.g. equipment);
- general security precautions (e.g. selective breeding).

Muehlbauer *et al.* (2014) supported that good husbandry and biosecurity practices were essential for successful prevention and control of disease, and they have additional benefits associated with increased production and profit. However, key complements to promoting biosecurity include education of the industry and the public as well

(Rodgers *et al.* 2015). In New Zealand, oyster and mussel farms put few biosecurity measures into practice but, as a result of OsHV-1, they now recognize the need for better education of the industry concerning the importance of prevention, disease transmission mechanisms and the need for rapid action following disease detection (Sim-Smith *et al.* 2016).

Mitigation strategies

Pernet *et al.* (2016) considered that ‘...disease management relies on establishment and maintenance of disease freedom or control of established diseases or both...’, although it is recognized that this is impossible in open-water marine bivalve systems once infection is established (Rodgers *et al.* 2015). Nevertheless, Segarra *et al.* (2014b) indicated that control of OsHV-1 infection was a key element for maintaining the competitiveness and increasing the sustainability of the oyster industry. Castinel *et al.* (2015) recommended that disease prevention and control strategies should be included in business risk management plans for the shellfish farming industry. The Australian Aquavetplan (Anon 2015) considers disease control strategies for OsHV-1 μ Var (see Annex S1), whereas Richez (2012) recommended sanitary measures to reduce the dissemination of OsHV-1 μ Var (see Annex S4), and Abollo Rodríguez and Villalba García (2013) provided guidelines for control (see Annex S5). An International OsHV-1 μ Var Workshop (Anon 2011) recommended specific control measures and contingency plans (see Annex S6).

Movement restrictions

Unrestricted movement of oysters is associated with a high risk of spread of OsHV-1 (EFSA 2015). Pernet *et al.* (2016) indicated that the risk of OsHV-1 dissemination in France was much higher for wild oysters collected in infected areas than for those from hatcheries and nurseries. Therefore, movement restrictions on *C. gigas* derived only from aquaculture cannot prevent the introduction of OsHV-1 to previously uninfected areas (EFSA 2015). However, Murray *et al.* (2012) suggested that OsHV-1 impact could be reduced by ceasing movements for restocking in areas where there is no natural recruitment. Nevertheless, since pathogen dissemination can take place naturally through the water, movement restrictions of live animals might not be effective in all cases (Pernet *et al.* 2016).

Renault (2016) considered that controlling animal transfers was one of the most suitable ways to combat infectious diseases in bivalve molluscs. This was shown by restrictions on movements of oysters in the UK that contributed to limiting the spread of OsHV-1 after diagnosis of disease (Renault 2011; cited by Pernet *et al.* 2016).

In general, Rodgers *et al.* (2015) considered that global movements posed a great threat to OsHV-1 dissemination because non-commercial bivalve species may be carriers of the herpesvirus without showing symptoms of disease.

Water treatment

An EFSA report considered that one of the potential routes for transmission of infectious agents affecting bivalves was the discharge of untreated seawater from depuration plants or other bivalve holding facilities (EFSA 2015). Therefore, effective disinfection of seawater effluent from closed or semi-closed *C. gigas* hatchery and nursery installations, such as using UV light or filtration, would be feasible for minimizing the risk of transmission of OsHV-1, although it is generally accepted that wild or grow-out oysters cultivated in open waters cannot be treated. In addition, hatcheries importing *C. gigas* broodstock should apply effluent controls to prevent infection of local wild stocks (Whittington *et al.* 2015b).

A simple and practical method using pumped seawater filtered to 5 µm in an upwelling oyster nursery facility was shown to prevent mortality caused by OsHV-1 µV_{ar} without the need for UV irradiation of the water, which could be used for water even from infected areas during risk periods (Whittington *et al.* 2015b). The same authors reported that ageing seawater for 48 h could also prevent mortalities.

Inactivation of virus

UV irradiation at 254 nm has been shown to be effective for inactivating OsHV-1 (EFSA 2015), although Evans *et al.* (2016) reported that standard biofiltration and UV irradiation in a recirculation aquaculture system did not remove all detectable OsHV-1 DNA from seawater. The iodophor Buffodine[®], the surfactant Impress and calcium hypochlorite were shown to inactivate a similar herpesvirus (AbHV of abalone) (Corbeil *et al.* 2012). Whittington *et al.* (2015c) reported that the alkaline detergent Pyroneg used for cleaning medical instruments and heating seawater to 42°C did not inactivate OsHV-1, whereas heating to 50°C for 5 min, the quaternary ammonium compound Virkon-S[®] (1% v/v for 15 min), sodium hydroxide (20 g L⁻¹ for 10 min), iodine (0.1% for 5 min) and formalin (10% v/v for 30 min) were shown to be effective. The same authors showed that chlorine (50 ppm for 15 min) inactivated OsHV-1 in seawater but not after the addition of 10% foetal bovine serum.

Renault (2016) concluded that as OsHV-1 was a fragile virus due to its lipid-containing envelope, adapted treatments in bivalve hatcheries and nurseries could be used to control viral infection.

Production calendar

Dégremont *et al.* (2005) showed the importance of field placement timing in relation to oyster survival, growth and

yield, and suggested transferring hatchery-produced spat after the critical period for mortalities (e.g. in their case early August to early September), although this was related to either environmental conditions or an unspecified pathogen. Dégremont *et al.* (2010b) reported a reduction in mortalities in selected resistant oysters at the juvenile stage when they were protected from summer mortality with an unspecified cause. Dégremont (2013) also recommended that prudent management strategies, such as transferring juveniles after the threat of exposure to OsHV-1 has passed, according to the seawater temperature, would support more effective production and lead to reduced mortalities by avoiding the higher risk periods for mortalities.

Carrasco *et al.* (2017) in conjunction with the collaboration of local producers designed a successful production calendar by adjusting production activities to local water temperature dynamics. Essentially, this involved optimizing the spat immersion schedule into two periods: summer (temperatures $\geq 25^{\circ}\text{C}$) and the end of autumn and beginning of winter (temperatures $\leq 13^{\circ}\text{C}$). These authors reported that the introduction of such a timetable together with recommendations concerning spat immersion size, culture density and the cementing calendar, reduced oyster mortalities from 80% to 2–7.5% in successive seasons.

Transmission

Barbosa-Solomieu *et al.* (2005) considered that the relationship shown between the infective status of oyster broodstock was of interest for hatchery production of larvae, and screening for OsHV-1 could help to avoid poor hatching success and subsequent survival. However, Sauvage *et al.* (2009) indicated that improved knowledge concerning OsHV-1 transmission was required for developing practical recommendations for limiting the impact of viral infection. Schikorski *et al.* (2011a) showed that only a short contact time was sufficient for virus transmission from infected oysters to healthy oysters. Moreover, interspecies viral transmission has been reported, including *C. gigas* to *C. ariakensis* and *O. edulis*, *R. philippinarum* to *C. gigas* and *C. gigas* to *C. angulata* larvae, which may be influenced by intensive rearing conditions in bivalve hatcheries (Arzul *et al.* 2001a,b,c).

Epidemiology

Epidemiological models can be used to assess the effectiveness of disease control scenarios in oyster-farm ecosystems (Pernet *et al.* 2016) and, as such, can be applied to a consideration of strategies for OsHV-1 in hatchery and nursery systems. Data related to pathogen prevalence and distribution can help to determine the technical feasibility and cost of eradication (Peeler & Otte 2016), although this is only realistic in closed-water systems rather than open water with no protected water source. Nevertheless, knowledge

gaps exist for areas such as persistence outside the host and potential for pathogen dispersal related to environmental factors, which are important considerations for disease management scenarios (Murray 2009).

Disease control in hatcheries and nursery systems has to consider the concept of an epidemiological unit, which can be contemplated as a group of aquatic animals or aquaculture establishments that share the same risk of exposure to a pathogen within a defined location (OIE 2009). Once this is established, the management of a designated zone can be tailored towards reducing the risk shared by such a common area, providing all the enterprises within the same unit apply the same management plan.

Collaboration between all those involved in designing the correct management scenario is essential for successful avoidance of disease. In this context, Turnbull *et al.* (2011) considered that scientists have the best understanding of the causes of a disease, but farmers can identify the aspects of their farming practices that can be changed or modified to reduce the impact of disease. However, social epidemiology that considers education at all levels can be useful for improving risk management in the production sector and enhancing the awareness of the need for disease controls (Castinel *et al.* 2015).

Farm management decisions

Table S8 summarizes the strategies detailed below related to farm management decision factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Carnegie *et al.* (2016) considered that one of the solutions that could be used to improve shellfish aquaculture health management would be wider training to enable better on-farm management decisions to be made, since it is considered that farmers can influence disease dynamics through such decisions and other related behaviours (Lupo *et al.* 2014).

Castinel *et al.* (2015) considered that the management decisions of oyster farmers could have played a part in the extent of OsHV-1 mortality outbreaks in Australia and New Zealand. Consequently, Turnbull *et al.* (2011) suggested that reliable scientific data and practical time-series observations from the aquaculture production sector applicable to disease control strategies should be tested in field trials before being used.

Lupo *et al.* (2014) found that notification procedures were quite well known among farmers and a reporting system was well accepted overall but the aims of the system, including providing details of mortalities, lacked awareness and this led to late reporting. This highlighted that the dialogue proposed by Turnbull *et al.* (2011) between all parties was important in disease control process, in a similar

way to zone management. Nevertheless, it was found that financial compensation for oyster production losses appeared to be a more important benefit than reporting the early detection of a disease outbreak (Lupo *et al.* 2014).

However, more specific prevention strategies have been seen as stopping movements of stock and gear, and zoning of farming areas by OsHV-1 status, since these were considered more useful because of their perceived effectiveness and practicality (Castinel *et al.* 2015). On-farm exposure to OsHV-1 and subsequent mortalities have resulted in farmers in Australia and New Zealand changing their approach to growing oysters. This has necessitated modification of husbandry techniques and operational strategies, such as species diversification, more use of hatchery spat and new or more versatile infrastructure (Castinel *et al.* 2015). In addition, Petton *et al.* (2015a) proposed the regulation of oyster movements between sites, timing of seeding and spatial planning related to seawater temperature and seed origin, restrictions on livestock movements between production areas, and density regulation for oyster beds.

Integrated and adaptive management approaches

Table S9 summarizes the strategies detailed below related to integrated management factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Rice *et al.* (2005) considered that ‘...the alternative to rigid and inflexible management frameworks is adaptive management, and adaptive management is part of the ecosystem approach...’. Adaptive management can apply spatial and temporal distribution controls that include regulations for the localization of installations and closed areas for shellfisheries, but it requires supporting through assessment, monitoring and scientific research, thus providing structured feedback for decision-making (Rice *et al.* 2005).

Cranford *et al.* (2012) recommended that such an approach for bivalve aquaculture should be based on a tiered indicator monitoring system using knowledge-based management and an integrative management framework, since pathogens represent only one of the pressures on the system together with others such as introductions and harvesting.

Pernet *et al.* (2016) also explored the case for a protective integrated approach to the management of bivalve diseases such as OsHV-1 through the incorporation of multidisciplinary science for a holistic understanding of the disease process. This process needs to be considered in terms of the diverse nature of bivalve culture husbandry techniques and the variable risk of the impact of bivalve aquaculture on marine environments (e.g. Europe and Australasia) (Cranford *et al.* 2012). In addition, the carrying capacity is a useful concept for maximizing aquaculture

stocking biomass, as lower density can lead to less disease pressure, although the concept could consider the whole ecosystem and all the activities involved in the aquaculture process under an integrated management approach (Figueira *et al.* 2015). Murray (2013) considered that an understanding of transmission processes helped to group finfish sites into spatially separated disease management areas, thus reducing the risks of pathogen spread and disease emergence by reducing movements between the areas.

Traceability

Table S10 summarizes the strategies detailed below related to traceability factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

Hastein *et al.* (2001) considered that the lack of traceability in oyster farming was a major limiting factor for the identification of epizootic sources, routes of spread and application of control measures. A system that allows the traceability of movements is recommended by the OIE (2017) at the compartment level (see the section on zoning and compartmentalization). A compartment is generally considered to be ‘...one or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met...’ (OIE 2017). Rodgers *et al.* (2015) considered that rapid and efficient traceability could stop potentially infected consignments being immersed in disease-free areas that might contain bivalve nurseries or hatcheries.

Zoning and compartmentalization

Table S11 summarizes the strategies detailed below related to zoning and compartmentalization factors for potentially avoiding the entrance and survival of OsHV-1 in hatchery and nursery systems.

The OIE Aquatic Animal Health Code (OIE 2017) indicates that zoning and compartmentalization can help in local disease eradication, as well as limiting disease spread and preventing pathogen introduction. Compartmentalization considers management practices related to biosecurity (see the section on biosecurity programmes), whereas zoning is more geographically aligned, but spatial considerations and good management have integral roles in their application (OIE 2017). Therefore, the concepts could be used to protect hatcheries and nursery areas providing the disease status of origin zones and their stocks is known. As such, Pernet *et al.* (2016) suggested that compartmentalization was ideally suited for closed and semi-closed oyster farming systems (see also Zepeda *et al.* 2008).

Pernet *et al.* (2016) also considered the value of minimum separation distances between farms, ‘firebreaks’ between aquaculture zones, and density regulation of susceptible hosts to limit disease spread in terms of zoning and compartmentalization for OsHV-1.

Clear epidemiological separation of aquatic animals with different disease status is required for the efficient designation of a compartment; therefore, the potential sources of infection and the risk of spread of infection into a compartment have to be identified (Zepeda *et al.* 2008). For nursery areas, and especially hatcheries, a protected water supply (e.g. filtered and/or UV treated) is important, as is axenic or mixed algal culture from a certified source, the entry of fomites (e.g. transport crates, settlement media, nets) and the entry of staff working at other sites (Zepeda *et al.* 2008).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Strategies related to ANIMAL HOST factors.

Table S2. Strategies related to HUSBANDRY factors.

Table S3. Strategies related to PATHOGEN factors.

Table S4. Strategies related to ENVIRONMENT factors.

Table S5. Strategies related to SURVEILLANCE factors.

Table S6. Strategies related to BIOSECURITY factors.

Table S7. Strategies related to MITIGATION factors.

Table S8. Strategies related to FARM MANAGEMENT DECISION factors.

Table S9. Strategies related to INTEGRATED MANAGEMENT factors.

Table S10. Strategies related to TRACEABILITY factors.

Table S11. Strategies related to ZONING AND COMPARTMENTALISATION factors.

Annex S1. AQUAVETPLAN Disease Strategy: Infection with ostreid herpesvirus-1 microvariant, Australia (Anon 2015).

Annex S2. Options to strengthen on-farm biosecurity management for commercial and non-commercial aquaculture – Technical Paper No: 2016/47, Ministry for Primary Industries, Aquaculture New Zealand (abridged from Georgiades *et al.* 2016).

Annex S3. OsHV-1 mortalities in Pacific oysters in Australia and New Zealand: the farmer's story, Cawthron Report No. 2567 (Castinel *et al.* 2015).

Annex S4. Report on the impact of recent *Crassostrea gigas* mortality in France and its consequences to oyster farming in Northern Ireland (Richez 2012).

Annex S5. HERPEMOL Guidelines for autocontrol of Ostreid herpesvirus, Spain (Abollo Rodríguez & Villalba García 2013; translated from the Spanish original).

Annex S6. International OsHV-1 μ Var Workshop, Cairns, Queensland, Australia (Anon 2011).