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Removal of oyster pathogens from seawater

Industrial scale production of the Pacific oyster *(Crassotrea gigas)* in aquaculture is important globally, meeting consumer demand for food, supporting the economies of coastal communities and providing ecosystem services. Global production reported in 2018 was about 650,000 tonnes per annum valued at US$1.37b (FAO, 2020). As is the case in all farming systems, *C. gigas* production can be severely impacted by infectious diseases. This was apparent historically as reflected in early literature (Mori, 1979; Soletchnik et al., 2007), but since 2008 scientific endeavour to understand, control and prevent infectious diseases in *C. gigas* has been motivated by the emergence and international spread of the highly virulent microvariant genotypes of *Ostreid herpesvirus 1* (OsHV-1) (Martenot et al., 2011; Mineur et al., 2015; Segarra et al., 2010; Whittington et al., 2018). OsHV-1 is particularly pathogenic in young oysters (Hick et al., 2018; Petton et al., 2015), with life history stages in hatcheries and nurseries being the most susceptible. Mortality rates in young oysters are commonly >80% (Paul-Pont et al., 2014). The response by industry has been remarkably consistent internationally, including enterprise diversification and consolidation, programs to select *C. gigas* for genetic resistance to the disease and modifications to husbandry (Degremont et al., 2015; Fuhrmann et al., 2019). Therefore, the recent publication in Environment International on removal of pathogens including OsHV-1 from seawater by ultrafiltration (Cordier et al., 2020), is of interest because of its potential industrial application in hatcheries where there are millions of susceptible larvae and juveniles. However, prior observations on the epidemiology of OsHV-1, its inactivation and filterability and on laboratory techniques to detect it have informed such research on water treatments to protect aquaculture facilities.

In 2012, it was observed that the pattern of mortality in *C. gigas* on Australian oyster farms was inconsistent with uniform distribution of OsHV-1 in the water column and it was proposed that the virus was attached to particles in the plankton community - used here in the general sense to describe the mobile, pleomorphic aggregation of mostly tiny animals, plants and other particles upon which oysters feed (Paul-Pont et al., 2013) and which also includes abiotic particles. In a laboratory infection model, *C. gigas* exposed to OsHV-1 via cohabitation (i.e. OsHV-1 released from infected oysters into the seawater) experienced greater mortality if they were fed microalgae compared to not being fed (Evans et al., 2015). Indeed, the marine herpesvirus acute viral necrosis virus (AVNV), which was eventually shown to be a variant of OsHV-1 (DNA sequence homology 97%; amino acid sequence homology 94–100%) (Ren et al., 2013), had already been shown to associate with microalgae and to be infectious to scallops through feeding (Zhang et al., 2010). In the experimental designs in the above studies, besides exposure to the virus through ingestion of vector particles, there could be a nutritional influence on disease outcome in fed compared to non-fed animals. Investigating nutritional physiological factors, Pernet et al. (2014) showed that diatoms in the diet of *C. gigas* contributed to energetic reserves which decreased the risk of mortality (Pernet et al., 2014) and later that high food levels and growth were associated with a higher risk of mortality, while energy reserves were associated with a lower risk (Pernet et al., 2019). The influence of growth on mortality was supported by some studies (Azema et al., 2017; Cotter et al., 2010) but not others (Burge et al., 2006; Hick et al., 2018; Whittington et al., 2019). Further research on the pathophysiology of OsHV-1 infection is required to better understand the interactions of exposure *per se* and nutritional factors, as both can be modified to benefit aquaculture production.

Microalgae are an important component of the plankton, present within a size range that will sediment in still water, and like other plankton components can be pelleted under a centrifugal force. Laboratory experiments confirmed that the rate of detection by real time PCR of OsHV-1 in natural seawater is enhanced by centrifuging samples at 1000*g* for 20 min and then testing the resulting pellet. Under these centrifugation conditions it was estimated that the particles with which OsHV-1 associated were in the order of 7–12 μm in diameter (Evans et al., 2014). However, the range of particle types to which a virus could attach in seawater is large. Indeed, as viruses are charged particles they can be adsorbed on the surface of inorganic and organic particles including sediments, sand, clay, plankton and bacteria (Kapuscinski and Mitchell, 1980; Liu et al., 2020; Mojica and Brussaard, 2014). While some viruses adsorb to bacteria (Bitton, 1975), bacteria in turn may adsorb to other components including abiotic particles, such as clay and other solid surfaces (Fletcher and Floodgate, 1973; Marshall, 1975). The adsorption of viruses to particles changes according to temperature and factors that modulate the ionic environment such as salinity and pH, and is affected by organic matter size, shape and concentration (Kapuscinski and Mitchell, 1980; Mojica and Brussaard, 2014). OsHV-1 has been detected on microplastics collected in an active oyster farm in France during summer mortalities and investigations are on-going to clarify the role of plastic particles in transmitting the disease through water (unpublished data). Due to viral adsorption, removing inorganic and organic particles from seawater by filtration or sedimentation can contribute to virus removal.

Evans et al. (2014) performed research on OsHV-1 using cellulose acetate and polyethersulfone membrane filtration (pore sizes 0.22–5 μm) and small volumes of seawater in an attempt to confirm the particle attachment hypothesis. While OsHV-1 was detectable both on the filter membranes and in the filtrates, the patterns of viral depletion in the filtrates were inconsistent; experimental variables such as the type of membrane, the seawater sampling method and the potential flocculation of virus during sample handling required further investigation. Subsequently, these researchers pumped large volumes (35–38 L per min for 24 h) of seawater that was naturally contaminated with OsHV-1 through cartridges containing pleated paper or polyester fabric filter membranes,

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prior to sampling the membranes and retentates (Evans et al., 2017). Viral loads ranging between 1.37×10^1 and 2.86×10^2 OsHV-1 DNA copies per cm^2 were detected on 5 μm pore membranes and OsHV-1 DNA was also detected in retentates and in the seawater, showing that OsHV-1 had been retained and had accumulated on the filter membrane. In the same study, OsHV-1 DNA was not detected in the retentates of 100 μm or 55 μm stacked polypropylene disc filters, indicating that the particles with which OsHV-1 was associated had passed through. Overall, these results suggest that OsHV-1 associates with particles greater than 5 μm and less than 55 μm in diameter. Non-enveloped OsHV-1 virions are 0.116 μm in diameter (Davison et al., 2005; Le Deuff and Renault, 1999). They appear to associate with a range of substances. Vincent-Hubert et al. (2017) demonstrated adsorption of OsHV-1 to zetapor, gauze, nylon, low-density polyethylene (LDPE) and polyvinylidene difluoride (PVDF) membranes. While the epidemiological, experimental disease transmission and physico-laboratory evidence makes it highly likely that OsHV-1 attaches to particles in seawater, the virus may also exist in tissue debris released from a diseased host as aggregates of viral particles and as free viral particles (Evans et al., 2016, 2014). This would be the case just after its release from infected oyster cells and prior to its attachment to particles, which enables the transmission cycle to continue (see Fig. 8 in Whittington et al. (2018)).

The duration of infectivity of viruses including OsHV-1 in seawater is limited and temperature dependent (Kapuscinski and Mitchell, 1980; Martenot et al., 2015). Using a bioassay with highly susceptible *C. gigas* spat, Hick et al. (2016) showed that OsHV-1 was inactivated in seawater after 2 days at 20 °C. It was also susceptible to inactivation by ultraviolet radiation and a range of chemicals (Hick et al., 2016; Schikorski et al., 2011). This information, together with knowledge that the virus is attached to particles, informs processes for water treatment to protect aquaculture.

The options for treatment of seawater include aging (time), sedimentation, filtration and disinfection. The first three of these approaches was tested in temporally replicated, controlled experiments in upwellers in a purpose built nursery in an estuary in which OsHV-1 was endemic (Whittington et al., 2015). Seawater that was naturally contaminated with OsHV-1 was rendered safe for rearing highly susceptible *C. gigas* spat (2 mm shell length) simply by holding the seawater for 2 days to allow sedimentation of particles and inactivation of the virus over time. Filtration of the seawater through 5 μm (nominal pore size) paper cartridges was also effective as a stand-alone measure. Filters with 30 μm or 55 μm (nominal sizes) pores were not effective, as mortality due to OsHV-1 occurred in spat kept in water downstream of such filters. These findings on water holding and filtration have been adopted and successfully applied in hatcheries and nurseries at commercial industrial scale to produce *C. gigas* spat in both Australia and New Zealand.

The recent finding that ultrafiltration (hollow fibre, polyethersulphone, nominal pore size 0.02 μm) of OsHV-1 contaminated seawater prevents mortality of *C. gigas* (Cordier et al., 2020) is consistent with the earlier research and provides another option for water treatment. Like conventional filtration (5 μm) and sedimentation/aging of seawater, ultrafiltration did not completely remove OsHV-1 from seawater but was sufficient to reduce infectious doses below the pathogenic threshold for immersion exposure of *C. gigas* (Cordier et al., 2020; Evans et al., 2017; Whittington et al., 2015). As PCR was used in all these studies as a proxy for viral detection, another explanation is that the signal detected in filtrates was inactive virus, viral fragments or free viral DNA.

There is an important question about the cost effectiveness and practicality of the evidence-based options for rendering seawater safe for production of *C. gigas* and other shellfish. A wide range of pathogens needs to be considered and evidence may be limited or contradictory. For some pathogens such as bacteria, the many sources of contamination beyond seawater must be identified and some treatments such as conventional filtration may be ineffective (Sainz-Hernández and Maeda-Martínez, 2005). To obtain adequate biosecurity for viral pathogens such as OsHV-1, and to minimize the need for antimicrobial treatments in hatcheries that can lead to antibiotic resistance (Dubert et al., 2016), treatment of incoming seawater and effluent is often warranted. But under what circumstances and in what combinations/sequence would (i) aging/sedimentation of seawater, (ii) conventional pre-filtration of seawater (sand filter), (iii) fine filtration (1 or 5 μm), (iv) ultrafiltration (0.2 μm), (v) ultraviolet radiation and (vi) chemical disinfection (ozone, chlorine, other) be recommended? These processes are applicable for closed (recirculating) and semi-open hatchery/nursery production systems that use relatively low volumes of seawater and that house early life history stages of *C. gigas*. However, because of the large volumes of water required, they cannot be used in open production systems in which highly susceptible young oysters and rapidly growing juveniles are placed after leaving the nursery. Consequently tens of millions of individual oysters remain vulnerable on estuarine farms (Paul-Pont et al., 2014). A further consideration for hatcheries and nurseries is the negative impact on oyster nutrition and growth when the food present in natural seawater is removed by an industrial water treatment (Whittington et al., 2015). Can this be overcome economically?

CRediT authorship contribution statement

R.J. Whittington: Writing - review & editing. **P. Hick:** Writing - review & editing. **M. Fuhrmann:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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