



**HAL**  
open science

## Removal of oyster pathogens from seawater

R.J. Whittington, Paul Hick, Marine Fuhrmann, O. Liu, Ika Paul-Pont

► **To cite this version:**

R.J. Whittington, Paul Hick, Marine Fuhrmann, O. Liu, Ika Paul-Pont. Removal of oyster pathogens from seawater. *Environment International*, 2020, 410 (25), pp.106258. 10.1016/j.envint.2020.106258 . hal-03101568

**HAL Id: hal-03101568**

**<https://hal.science/hal-03101568>**

Submitted on 27 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Contents lists available at ScienceDirect

Environment International

journal homepage: [www.elsevier.com/locate/envint](http://www.elsevier.com/locate/envint)

Correspondence



## Removal of oyster pathogens from seawater

Industrial scale production of the Pacific oyster (*Crassostrea 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 1000g 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,

<https://doi.org/10.1016/j.envint.2020.106258>

Received 16 September 2020; Received in revised form 30 October 2020; Accepted 31 October 2020

Available online 23 November 2020

0160-4120/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

prior to sampling the membranes and retentates (Evans et al., 2017). Viral loads ranging between  $1.37 \times 10^1$  and  $2.86 \times 10^2$  OsHV-1 DNA copies per  $\text{cm}^2$  were detected on  $5 \mu\text{m}$  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  $100 \mu\text{m}$  or  $55 \mu\text{m}$  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  $5 \mu\text{m}$  and less than  $55 \mu\text{m}$  in diameter. Non-enveloped OsHV-1 virions are  $0.116 \mu\text{m}$  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 (Kapusinski 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  $20^\circ\text{C}$ . 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  $5 \mu\text{m}$  (nominal pore size) paper cartridges was also effective as a stand-alone measure. Filters with  $30 \mu\text{m}$  or  $55 \mu\text{m}$  (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  $0.02 \mu\text{m}$ ) 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 ( $5 \mu\text{m}$ ) 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  $5 \mu\text{m}$ ), (iv) ultrafiltration ( $0.2 \mu\text{m}$ ), (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.

#### References

- Azema, P., Mauouard, E., Lamy, J.B., Degremont, L., 2017. The use of size and growing height to improve *Crassostrea gigas* farming and breeding techniques against OsHV-1. *Aquaculture* 471, 121–129.
- Bitton, G., 1975. Adsorption of viruses onto surfaces in soil and water. *Water Res.* 9, 473–484.
- Burge, C.A., Griffin, F.J., Friedman, C.S., 2006. Mortality and herpesvirus infections of the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA. *Dis. Aquat. Org.* 72, 31–43.
- Cordier, C., Stavrakakis, C., Morga, B., Degremont, L., Voulgaris, A., Bacchi, A., Sauvade, P., Coelho, F., Moulin, P., 2020. Removal of pathogens by ultrafiltration from sea water. *Environ. Int.* 142, 105809.
- Cotter, E., Malham, S.K., O'Keefe, S., Lynch, S.A., Latchford, J.W., King, J.W., Beaumont, A.R., Culloty, S.C., 2010. Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the Irish Sea: The influence of growth, biochemistry and gametogenesis. *Aquaculture* 303, 8–21.
- Davison, A.J., Trus, B.L., Cheng, N.Q., Steven, A.C., Watson, M.S., Cunningham, C., Le Deuff, R.M., Renault, T., 2005. A novel class of herpesvirus with bivalve hosts. *J. Gen. Virol.* 86, 41–53.
- Degremont, L., Garcia, C., Allen, S.K., 2015. Genetic improvement for disease resistance in oysters: A review. *J. Invert. Pathol.* 131, 226–241.
- Dubert, J., Osorio, C., Prado, S., Barja, J., 2016. Persistence of antibiotic resistant *Vibrio* spp. in shellfish hatchery environment. *Microb. Ecol.* 72, 851–860.
- Evans, O., Hick, P., Alford, B., Whittington, R.J., 2017. Transmission of *Ostreid herpesvirus-1* microvariant in seawater: Detection of viral DNA in seawater, filter retentates, filter membranes and sentinel *Crassostrea gigas* spat in upwellers. *Aquaculture* 473, 456–467.
- Evans, O., Hick, P., Dhand, N., Whittington, R.J., 2015. Transmission of *Ostreid herpesvirus-1* in *Crassostrea gigas* by cohabitation: effects of food and number of infected donor oysters. *Aquacult. Environ. Interact.* 7, 281–295.
- Evans, O., Hick, P., Whittington, R.J., 2016. Distribution of *Ostreid herpesvirus-1* (OsHV-1) microvariant in seawater in a recirculating aquaculture system. *Aquaculture* 458, 21–28.
- Evans, O., Paul-Pont, I., Hick, P., Whittington, R.J., 2014. A simple centrifugation method for improving the detection of *Ostreid herpesvirus-1* (OsHV-1) in natural seawater samples with an assessment of the potential for particulate attachment. *J. Virol. Methods* 210, 59–66.
- FAO, 2020. *FAO Yearbook. Fishery and Aquaculture Statistics 2018. Food and Agriculture Organization of the United Nations, Rome.*
- Fletcher, M., Floodgate, G.D., 1973. Electron-microscopic demonstration of an acidic polysaccharide involved in adhesion of a marine bacterium to solid surfaces. *J. Gen. Microbiol.* 74, 325–334.
- Fuhrmann, M., Castinel, A., Cheslett, D., Furones Nozal, D., Whittington, R., 2019. The impacts of *Ostreid herpesvirus 1* microvariants on Pacific oyster aquaculture in the

- Northern and Southern Hemispheres since 2008. *Rev. Sci. Tech. Off. Int. Epiz.* 38, 491–509.
- Hick, P., Evans, O., Looi, R., English, C., Whittington, R.J., 2016. Stability of *Ostreid herpesvirus-1* (OsHV-1) and assessment of disinfection of seawater and oyster tissues using a bioassay. *Aquaculture* 450, 412–421.
- Hick, P.M., Evans, O., Rubio, A., Dhand, N.K., Whittington, R.J., 2018. Both age and size influence susceptibility of Pacific oysters (*Crassostrea gigas*) to disease caused by *Ostreid herpesvirus-1* (OsHV-1) in replicated field and laboratory experiments. *Aquaculture* 489, 110–120.
- Kapuscinski, R.B., Mitchell, R., 1980. Processes controlling virus inactivation in coastal waters. *Water Res.* 14, 363–371.
- Le Deuff, R.M., Renault, T., 1999. Purification and partial genome characterization of a herpes-like virus infecting the Japanese oyster, *Crassostrea gigas*. *J. Gen. Virol.* 80, 1317–1322.
- Liu, O., Paul-Pont, I., Rubio, A., Dhand, N., Whittington, R.J., 2020. Detection of *Ostreid herpesvirus-1* in plankton and seawater samples at an estuary scale. *Dis. Aquat. Org.* 138, 1–15.
- Marshall, K.C., 1975. Clay mineralogy in relation to survival of soil bacteria. *Annu. Rev. Phytopathol.* 13, 357–373.
- Martenot, C., Denechere, L., Hubert, P., Metayer, L., Oden, E., Trancart, S., Travaille, E., Houssin, M., 2015. Virulence of *Ostreid herpesvirus 1* mu Var in sea water at 16 degrees C and 25 degrees C. *Aquaculture* 439, 1–6.
- Martenot, C., Oden, E., Travaille, E., Malas, J.-P., Houssin, M., 2011. Detection of different variants of *Ostreid Herpesvirus 1* in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010. *Virus Res.* 160, 25–31.
- Mineur, F., Provan, J., Arnott, G., 2015. Phylogeographical analyses of shellfish viruses: inferring a geographical origin for ostreid herpesviruses OsHV-1 (*Malacoherpesviridae*). *Mar. Biol.* 162, 181–192.
- Mojica, K.D.A., Brussaard, C.P.D., 2014. Factors affecting virus dynamics and microbial host–virus interactions in marine environments. *FEMS Microbiol. Ecol.* 89, 495–515.
- Mori, K., 1979. Effects of artificial eutrophication on the metabolism of the Japanese oyster *Crassostrea gigas*. *Mar. Biol.* 53, 361–369.
- Paul-Pont, I., Dhand, N.K., Whittington, R.J., 2013. Spatial distribution of mortality in Pacific oysters *Crassostrea gigas*: reflection on mechanisms of OsHV-1 transmission. *Dis. Aquat. Org.* 105, 127–138.
- Paul-Pont, I., Evans, O., Dhand, N.K., Rubio, A., Coad, P., Whittington, R.J., 2014. Descriptive epidemiology of mass mortality due to *Ostreid herpesvirus-1* (OsHV-1) in commercially farmed Pacific oysters (*Crassostrea gigas*) in the Hawkesbury River estuary, Australia. *Aquaculture* 422–423, 146–159.
- Pernet, F., Lagarde, F., Jeanne, N., Daigle, G., Barret, J., Le Gall, P., Quere, C., D'orbcastel, E.R., 2014. Spatial and temporal dynamics of mass mortalities in oysters is influenced by energetic reserves and food quality. *Plos One* 9 (2). <https://doi.org/10.81371/journal.pone.0088469>.
- Pernet, F., Tamayo, D., Fuhrmann, M., Petton, B., 2019. Deciphering the effect of food availability, growth and host condition on disease susceptibility in a marine invertebrate. *J. Exp. Biol.* 222.
- Petton, B., Boudry, P., Alunno-Bruscia, M., Pernet, F., 2015. Factors influencing disease-induced mortality of Pacific oysters *Crassostrea gigas*. *Aquacult. Environ. Interact.* 6, 205–222.
- Ren, W., Chen, H., Renault, T., Cai, Y., Bai, C., Wang, C., Huang, J., 2013. Complete genome sequence of acute viral necrosis virus associated with massive mortality outbreaks in the Chinese scallop, *Chlamys farreri*. *Virol. J.* 10.
- Sainz-Hernández, J.C., Maeda-Martínez, A.N., 2005. Sources of *Vibrio* bacteria in mollusc hatcheries and control methods: a case study. *Aquac. Res.* 36, 1611–1618.
- Schikorski, D., Renault, T., Saulnier, D., Faury, N., Moreau, P., Pepin, J.F., 2011. Experimental infection of Pacific oyster *Crassostrea gigas* spat by *ostreid herpesvirus 1*: demonstration of oyster spat susceptibility. *Vet. Res.* 42, 27.
- Segarra, A., Pépin, J.F., Arzul, I., Morga, B., Faury, N., Renault, T., 2010. Detection and description of a particular *Ostreid herpesvirus 1* genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Res.* 153, 92–99.
- Soletchnik, P., Ropert, M., Mazurié, J., Gildas Fleury, P., Le Coz, F., 2007. Relationships between oyster mortality patterns and environmental data from monitoring databases along the coasts of France. *Aquaculture* 271, 384–400.
- Vincent-Hubert, F., Morga, B., Renault, T., Le Guyader, F.S., 2017. Adsorption of norovirus and ostreid herpesvirus type 1 to polymer membranes for the development of passive samplers. *J. Appl. Microbiol.* 122, 1039–1047.
- Whittington, R., Hick, P., Evans, O., Rubio, A., Alford, B., Dhand, N., Paul-Pont, I., 2015. Protection of Pacific oyster (*Crassostrea gigas*) spat from mortality due to *Ostreid herpesvirus-1* OsHV-1  $\mu$ Var) using simple treatments of incoming seawater in land-based upwellers. *Aquaculture* 437, 10–20.
- Whittington, R.J., Liu, O., Hick, P.M., Dhand, N., Rubio, A., 2019. Long-term temporal and spatial patterns of *Ostreid herpesvirus 1* (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management. *Aquaculture* 513, 734395.
- Whittington, R.J., Paul-Pont, I., Evans, O., Hick, P., Dhand, N.K., 2018. Counting the dead to determine the source and transmission of the marine herpesvirus OsHV-1 in *Crassostrea gigas*. *Vet. Res.* 49.
- Zhang, J., Li, Y., Ren, W., Cai, Y., 2010. Studies on phytoplanktons carrying and spreading AVNV. *J. Fish. China* 34, 1254–1259.

R.J. Whittington<sup>\*</sup>, P. Hick, M. Fuhrmann

School of Veterinary Science, The University of Sydney, Camden, NSW 2570, Australia

O. Liu

School of Veterinary Science, The University of Sydney, Camden, NSW 2570, Australia

Aquatic Pest and Health Policy, Animal Health Policy Branch, Biosecurity Animal Division, Australian Government Department of Agriculture, Water and the Environment, Canberra, ACT 2601, Australia<sup>1</sup>

I. Paul-Pont

School of Veterinary Science, The University of Sydney, Camden, NSW 2570, Australia

Laboratoire des Sciences de l'Environnement Marin (LEMAR), CNRS/UBO/IRD/IFREMER Institut Universitaire Européen de la Mer, 29280 Plouzane, France<sup>1</sup>

<sup>\*</sup> Corresponding author at: 425 Werombi Road, Camden, NSW 2570, Australia.

E-mail address: [richard.whittington@sydney.edu.au](mailto:richard.whittington@sydney.edu.au) (R.J. Whittington).

<sup>1</sup> Present address.