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Original Article

Farmer monitoring reveals the effect of tidal height on mortality risk of oysters during a herpesvirus outbreak

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The intertidal zone is characterized by a sharp vertical gradient of environmental stress, which structures species distribution and their interactions. Few studies, however, have examined the influence of tidal height on host–pathogen interactions. Here, we investigated how the tidal height influence outbreak of the Ostreid herpesvirus type 1 (OsHV-1) affecting the Pacific oyster. A volunteer network composed of 20 oyster growers monitored the survival of 28 batches of oysters during an epizootic event in Southern Brittany, France. Oysters were spat from wild collection or hatchery production. The sampling sites were spread over a 150-km^2 area with a tidal height ranging from 0.98 to 2.90 m. Concomitantly, we followed survival of oyster spats in relation with OsHV-1 DNA detection at two sites and conducted risk analysis. We found that tidal height was associated with a lower risk of mortality. This effect was higher for hatchery than for wild oysters probably reflecting differences in health status. Our study opens perspectives for mitigation strategies based on tidal height and emphasizes the value of volunteer science in marine epidemiological studies.

Keywords: aquaculture, citizen science, marine epidemiology and health, OsHV-1, risk analysis.

Introduction

Since the mid-1970s, disease epidemics and mass mortalities have been occurring in marine environments at a historically unprecedented rate (Harvell *et al.*, 1999) with consequences for fisheries and aquaculture (Lafferty *et al.*, 2015). The risk of disease outbreak depends on interactions between hosts, pathogens, and the environment, and any change in one or more of these components may potentially increase or decrease this risk (Burge *et al.*, 2014). The intertidal zone is characterized by a vertical gradient of environmental stress with increasing elevation which structure species distribution, zonation, and community dynamics (Connell, 1972). This feature makes it an important model system for marine ecological studies. Nevertheless, few studies have examined the influence of tidal height on host–pathogen interaction (but see Burrell *et al.*, 1984; Ben-Horin *et al.*, 2013; Malek and Breitburg, 2016; Malek and Byers, 2017). It is likely that host species that spend less time underwater are less exposed to pathogens vectored by the seawater. Also, replication or proliferation of obligate parasites that depend on the host cell machinery is probably altered because host metabolism and growth are lowered (Widdows *et al.*, 1979; Somero, 2002).

One of the most striking examples of recent disease emergence is the Pacific Oyster Mortality Syndrome (POMS), killing up to 100% of the farmed oysters locally every year since 2008 in France (EFSA AHAW Panel, 2015; Barbosa Solomieu *et al.*, 2015; Pernet *et al.*, 2016; Alfaro *et al.*, 2018). Almost all French production areas were severely hit by POMS, thus resulting in a supply shortage and a rise in prices (Le Bihan *et al.*, in press). This syndrome is caused by the ostreid herpesvirus 1 (OsHV-1) which alters the immune state of oysters and leads to fatal bacteraemia

and death (de Lorgeril *et al.*, 2018). Epizootics caused by OsHV-1 occur every year when seawater temperature is between 16°C and 24°C in France (Pernet *et al.*, 2012; Renault *et al.*, 2014). Infection starts when viral particles come into contact with susceptible hosts via suspension feeding. There is a threshold dose for infection and a dose–response effect of OsHV-1 on mortality (Paul-Pont *et al.*, 2015; Petton *et al.*, in revision). Like other herpesviruses, latent and asymptomatic OsHV-1 infections are able to persist in hosts (Segarra *et al.*, 2014). In this case, virus reactivation can occur several weeks to months after initial exposure (Pernet *et al.*, 2015; Petton *et al.*, 2015), viral particles are shed into the water column and dispersal to new hosts occurs via water currents (Pernet *et al.*, 2012). The economic costs associated with increased oyster mortalities have promoted investigation of disease risk factors to improve management of shellfish farms, but the effect of tidal height has rarely been investigated (Pernet *et al.*, 2016).

A review of early experiments conducted in France during the first disease outbreaks suggest that tidal height can be associated with lower mortality of oysters (Soletchnik *et al.*, 2011). This hypothesis has only been partially confirmed. High rearing height is related to reduced mortality in adults but not in spats for which only a mortality delay occurs (Paul-Pont *et al.*, 2013; Whittington *et al.*, 2015a). More recently, tidal height is associated with a lower risk of mortality in only 9 out of 40 tested oyster spat families (Azema *et al.*, 2017). Broad-scale epidemiological studies reveal apparently conflicting results. A survey conducted in Irish oyster farms suggests that emersion time is negatively associated with mortality (Peeler *et al.*, 2012) but another one carried out in Australia shows no relation (de Kantzow *et al.*, 2017).

Mortality risk factors associated with OsHV-1 have recently been identified along an inshore-offshore gradient in permanently immersed animals, but not in inshore farming areas where animals are held at various tidal heights and are regularly emerged (Pernet *et al.*, 2018). Here, we supplemented this epidemiological study focusing on the farming areas. The objective was to investigate the effect of tidal height on mortality risk of oysters. An original aspect to the present study is that survival of farmed oysters was monitored by a network of growers to obtain simultaneous data from multiple remote sites. The results obtained by oyster growers were strengthen by the Ifremer observatory network of oyster mortality. While the roots of citizen science go back to the very beginnings of modern science, projects in which volunteers associate with scientists are new in the field of marine epidemiology (Foster-Smith and Evans, 2003; Silvertown, 2009; Dickinson *et al.*, 2010).

Material and method

Study site

The study sites covered a surface area of ca. 150 km^2 located in South Brittany, France, and encompass six production areas located in inlets, rivers, or gulfs where oysters were hit by OsHV-1 every year since 2008 (Figure 1) (Fleury *et al.*, 2018). While the number of oyster companies in this area has decreased by 25% since 2001 partly because of the virus, there were 241 companies in 2012 that supported 522 full-time jobs (Agreste, 2014). The coastline is heterogenous, alternating bedrock, sandy beaches, and sandy-loamy to sandy-muddy tidal flats. However, the large majority of oysters (70%) are cultivated off-bottom in mesh bags attached to iron tables (Buestel *et al.*, 2009). This area is influenced by semi-diurnal tides and the tidal range was between 0.22 and 5.88 m during the study period.

Farmer network

The experimental design was set up in collaboration with local oyster farmers. Oyster growers were informed by the regional shellfish committee that participatory monitoring of large-scale oyster mortalities was taking place and that volunteers were needed to survey their own concessions. A meeting was held with all respondents in mid-April 2013 to outline the monitoring protocol. Twenty farmers agreed to participate in the experiment. Farmers were in charge of one ($N = 14$ farmers), two ($N = 4$), or three ($N = 2$) batches of oysters for a total of 28 batches (#1–28). The sentinel oysters were chosen among the farmed stock by the farmers themselves. To be included in the experimental design, the sentinel oysters needed be at the spat stage and free of abnormal mortality since they arrived at the monitoring site. At the onset of the monitoring, sentinel oysters were 10–25 mm shell length and they had spent between 8 and 205 days on the farm (mean = 70 ± 64 days). The sentinel oysters were deployed after 15 October 2012 when seawater temperature was <16°C, so that OsHV-1 infection at the monitoring site was unlikely. They originated from hatcheries or from natural collection in the wild. Oysters from hatcheries were not genetically selected for disease resistance. At the time of this study, work on OsHV-1 oyster resistance in France was conducted on an experimental scale only. In fact, some private hatcheries and the National Shellfish Committee launched their own breeding programmes in 2013–14 (Dégremont *et al.*, 2015). Wild oysters were settled on limed tiles or plastic tubes during the preceding summer and the spat were removed during the fall or early winter according to standard practices (Héral, 1990). Wild oysters generally spent more time on the farm before the start of monitoring than those from hatcheries (116 vs. 40 days, respectively). A subsample ($n = 100$) of the farmed stock selected as sentinels was transferred into small mesh bags ($22 \times 20 \times 2$ cm, Ø = 6.0 mm) attached to iron tables. Farmers provide exact positions of each oyster bag, the date of arrival on the rearing site, and the origin of the oysters (wild or hatchery). Bathymetric data were provided by the Service Hydrographique et Océanographique de la Marine (SHOM). The survival monitoring started on 8 May 2013, before the onset of the epizootics, and lasted for 133 days until 18 September 2013. Live and dead animals were counted twice a month by the farmer, generally during spring tides (22 and 29 May, 12 and 26 June, 10 and 24 July, 8 and 22 August, and 6 and 18 September).

Observatory network

The Ifremer observatory network followed the survival of two batches of oyster spats originating from both wild or hatchery production at two sites located within the farming areas (#29–32, Figure 1). Oysters consisted of (i) wild spat collected on limed tiles in Arcachon Bay during summer 2012 or (ii) 3-month-old animals produced by a private hatchery. These two batches of oysters were gathered at the Ifremer laboratory (La Trinité-sur-Mer, France) between 10 and 26 March 2013. Before deployment in the field, a subsample of 50 oysters from each batch were individually screened for OsHV-1 DNA by qPCR and exposed to a thermal elevation at 21°C for 1 month in cohabitation with healthy spats to reveal both disease expression and transmission (Petton *et al.*, 2015). Wild oysters were asymptomatic carriers of

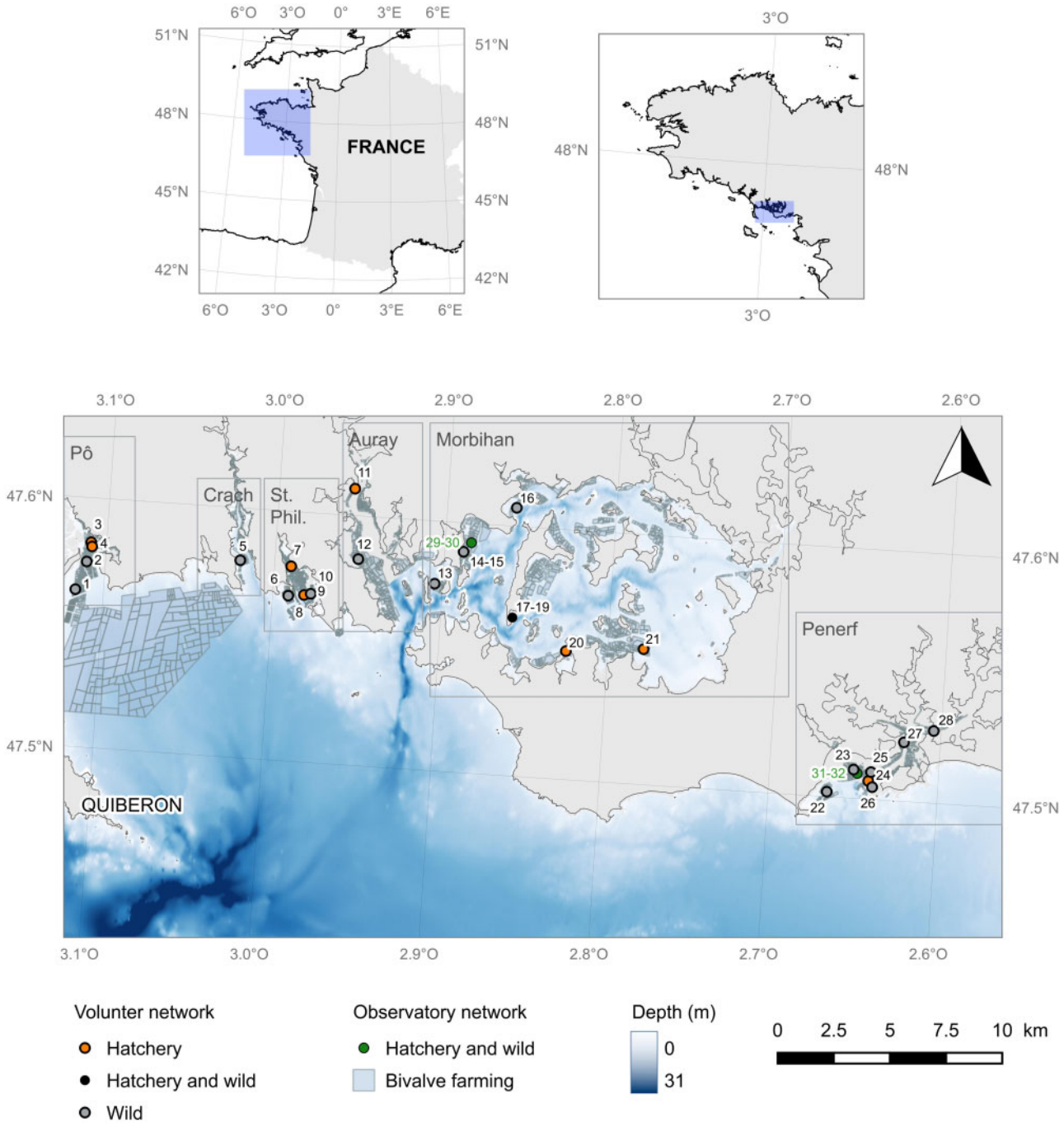

Figure 1. Spatial distribution of oyster survival monitoring sites.

OsHV-1 as the virus was detected in 3 out of 50 individuals and mortalities associated with OsHV-1 occurred in both the tested and the cohabited healthy oysters after thermal elevation at 21°C (see table S1, batch n°42 in Petton *et al.*, 2015). In contrast, oysters from the hatchery were considered specific pathogen free because OsHV-1 DNA was not detected and no mortality occurred after thermal elevation at 21°C (see table S1, batch n°40 in Petton *et al.*, 2015).

These two batches of oysters were placed in three regular-sized mesh bags per site (350 individuals per bag). Live and dead animals were counted twice a month during spring tides from April to December in one of the three bags. Only survival data collected at the same time as the farmers were considered. Three pools of three live hatchery oysters were collected from early May to mid-September in each bag and analysed for the detection and quantification of OsHV-1 DNA (one pool per bag) by LABOCEA (Quimper, France) using a standard real-time PCR protocol (Pepin *et al.*, 2008). Seawater temperature was measured every 15 min using temperature probes placed in one oyster bag at each site.

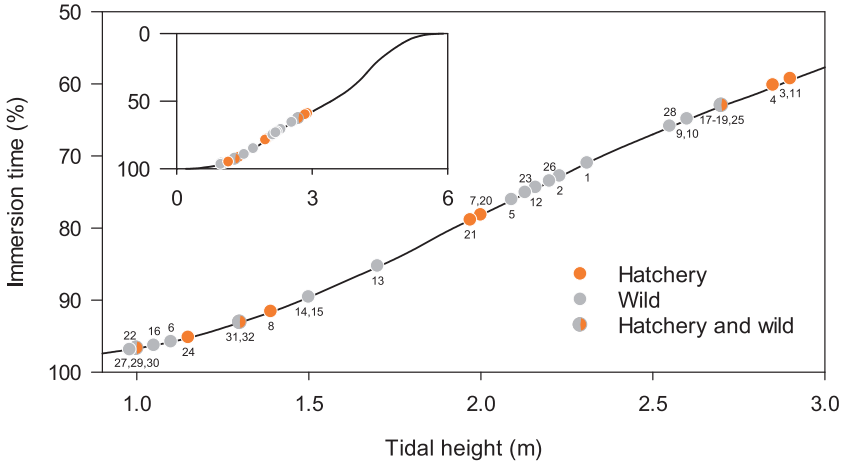

Figure 2. Relationship between immersion time and bathymetry. Circles represent the position of the oyster survival monitoring sites. Inset shows the entire bathymetric range during the period of study.

Statistics

Non-parametric estimates of the survivor function were computed using the Kaplan–Meier method (Kaplan and Meier, 1958). Survival time was measured as number of days after 8 May, the onset of the monitoring period, until 18 September 2013. The data were read as the number of dead animals within each bag at each time interval. Survival curves were presented for each oyster bag as a function of rearing site and origin of oysters. A Frailty model (Hougaard, 1995) was fitted to survival data to investigate the effect of tidal height, origin, and their interaction. The Frailty model is a proportional Cox regression model (Cox, 1972) with a random effect (site). Then, survival probabilities predicted by the model were plotted for every set of significant covariates or their interactions. These analyses were conducted with the LIFETEST and PHREG procedures of the SAS software package (SAS 9.4, SAS Institute).

Results

Survival was measured on 32 wild ($n = 20$ batches) or hatchery ($n = 12$ batches) oyster batches, spread over 22 sites within 6 production areas. Tidal height for these sites ranges from 0.98 to 2.90 m (mean ± SD = 1.94 ± 0.67 m), corresponding to an immersion time varying between 59.3% and 96.9% (mean ± SD = 78.9 ± 13.3%, Figure 2).

The oysters were hit by the mass mortality event at all sites (Figure 3). Mortality was first observed 35–49 days after the onset of the monitoring (i.e. 12–26 June) for 20 batches out of 32. Survival functions of oysters differed depending on their origin (Figure 2, log-rank test, χ^2=20.0, $p < 0.001$). Indeed, mortality occurred earlier for wild than for hatchery oysters. Final survival varied widely from 20% to 97% (mean = 51.0 ± 20.4%) and it was similar between origin (wild: 49.5% vs. hatchery: 53.5%, χ^2=1.8, $p = 0.187$). The observatory network revealed that these mortalities occurred while seawater temperature exceeded 16°C and coincided with the detection of high levels of OsHV-1 DNA in oyster tissues ($>10^6$ cp mg g^{-1} wet tissue, Figure 4).

The relationship between the mortality risk and site, tidal height, and origin of oysters was investigated using the Cox regression model (Table 1). Oyster origin and tidal height exerted a major influence on survival. Tidal height was associated with a lower mortality risk, with a greater effect on oysters originating from hatcheries than for those from the wild (Table 1). Any 1 m increase in tidal height led to a 48.8% reduction in mortality hazard for hatchery oysters compared to only 15.7% for wild oysters. Hazard ratios were 0.512 (95% confidence interval [CI] = 0.452–0.580) and 0.843 (0.764–0.930), respectively. Survival probabilities predicted by the model at the end of the monitoring of hatchery oysters held at 0, 0.98, 1.95 and 2.90 m were respectively 19%, 40%, 61% and 76%, compared to 42%, 44%, 47% and 50% for wild oysters (Figure 5a). Therefore, tidal height had more influence on the survival of hatchery oysters than on wild oysters. The mortality risk was the same for both wild and hatchery oysters when tidal height was 1.2 m (hazard ratio = 0.999, 95%CI [0.878–1.111]), corresponding to an average immersion time of 94.6% (Figure 5b). For tidal heights above 1.2 m, any increase reduced the risk of wild oyster mortality to a lesser extent than that of hatchery oysters, which meant that the survival of wild oysters was lower than that of hatchery oysters. Conversely, at tidal height below 1.2 m, any decrease increased the risk of hatchery oyster mortality to a greater extent than that of wild ones, conferring a higher survival on hatchery compared to wild oysters.

Discussion

Here, we validate the hypothesis that increasing tidal height decreases the risk of oyster mortality during an OsHV-1 epizootic, as indicated for adult oysters in Australia and some spat families in France (Paul-Pont *et al.*, 2013; Whittington *et al.*, 2015a; Azema *et al.*, 2017). Improved survival with increasing tidal height can be explained by a shorter immersion time, leading to a lower exposure to viral particles in the water. Indeed, survival of healthy oysters in laboratory conditions decreases with cohabitation time with infected oysters and reflects the cumulative exposure to OsHV-1 (Petton *et al.*, in revision). In line with this, survival of oysters decreases with biomass of infected oysters and increases with seawater renewal, two parameters that influence pathogen concentrations (Petton *et al.*, 2015). Also, survival of oysters injected with OsHV-1 decreases with increasing viral particle concentration (Paul-Pont *et al.*, 2015; Segarra *et al.*, 2016). Therefore, oysters that spend less time immerged will be less exposed to OsHV-1 particles vectored by seawater and show higher survival.

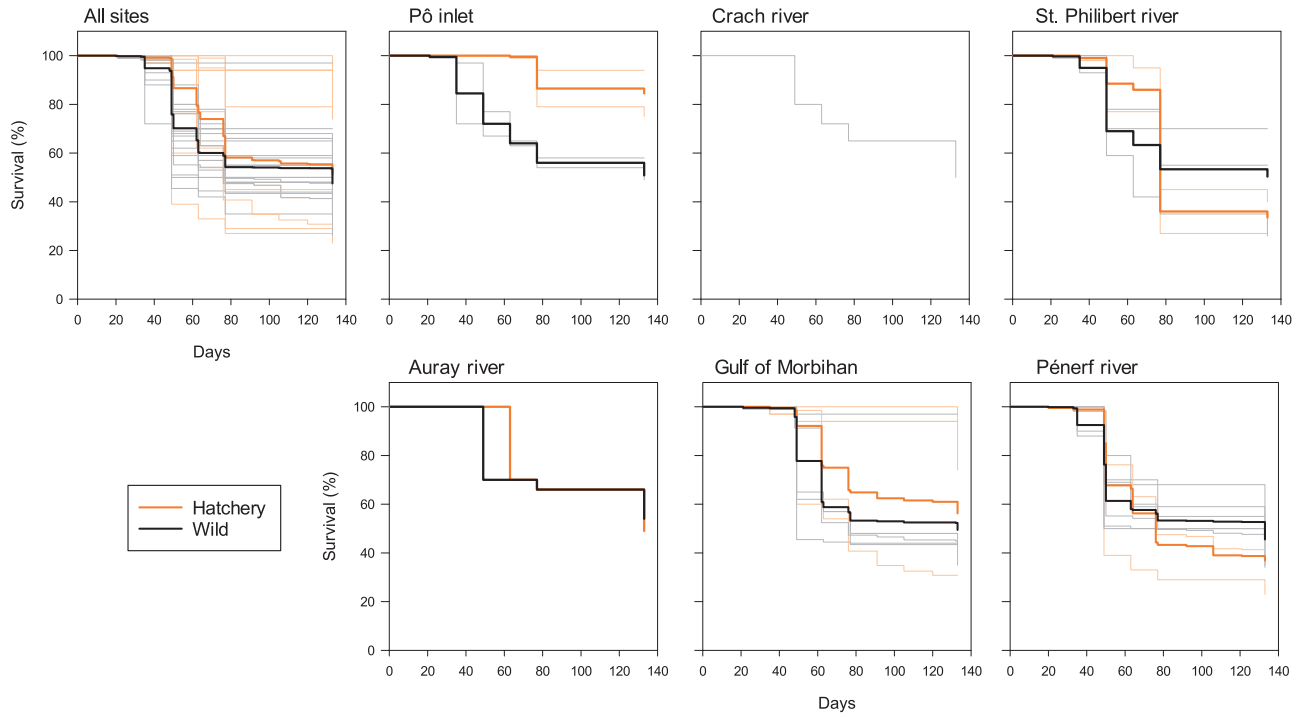

Figure 3. Survival of oysters. Thick lines represent the average survival per origin. Day 0 corresponds to 8 May 2013.

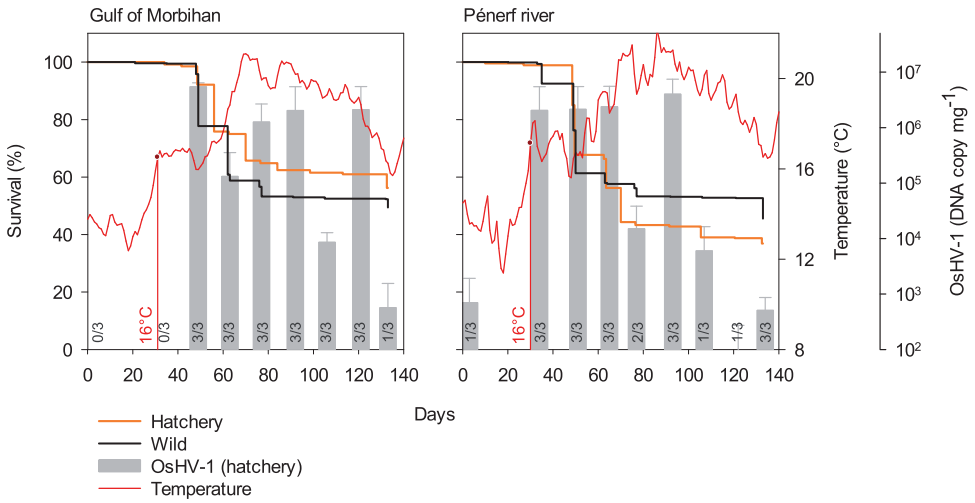

Figure 4. Oyster survival in two sites from the Ifremer observatory network in relation with seawater temperature and OsHV-1 DNA detection. Left axis: survival functions of wild and hatchery oysters. Right axis: evolution of seawater temperature and levels of OsHV-1 DNA in oyster tissues collected in hatchery oysters (mean ± SD, $n = 3$ pools of 3 individuals). Ratios indicate the number of positive samples out of the total number analysed. Day 0 correspond to 8 May 2013.

In addition, since herpesviruses replication is directly dependent on host cell activity, it may be reduced during emersion as metabolism and growth of the host decline (Widdows *et al.*, 1979; Somero, 2002). For instance, virus replication in shrimp depends on cellular growth and proliferation of the host (Su *et al.*, 2014). Further to this, fast-growing oysters are more susceptible to OsHV-1 than slow growers (Pernet *et al.* unpublished data).

Finally, differences in temperature regime between low and high tidal levels could also contribute to differences in survival of oysters exposed to OsHV-1. Indeed, during daytime exposure at low tide, the body temperature of a shelled molluscs can rapidly rise from that of the ambient seawater to 15°C above air temperature (e. g. Helmuth, 1999). In parallel, high temperature (>26°C) decreased the susceptibility of oysters to OsHV-1 (Petton *et al.*, 2013; de Kantzow *et al.*, 2016; Delisle *et al.*, 2018). It is possible that longer exposure to high air temperature at low tide makes oysters less susceptible to the virus at high tidal level. Lower virus exposure, reduction of metabolic rate, and exposure to high air temperature during emersion are not mutually exclusive hypotheses, and further studies are necessary to identify which mechanism prevails.

Interestingly, we found that the mortality risk of wild oysters was generally less influenced by the tidal height than that of hatchery ones. A likely and logical hypothesis relate to differences in their initial health status. Indeed, wild oysters used in the observatory network were asymptomatic carriers of the virus whereas hatchery oysters were free of OsHV-1. This observation is in line with a major study showing that 60% of the wild oyster batches tested in France are asymptomatic carriers of OsHV-1 during their first winter, exhibiting mortality after thermal elevation and infecting cohabiting healthy hatchery oysters, whereas those from hatcheries and nurseries are free of OsHV-1 (Petton *et al.*, 2015). Indeed, wild oysters are unpredictably exposed to the pathogen during their early life history whereas those from the hatcheries are generally protected from disease by means of prophylactic measures (Petton *et al.*, 2015; Whittington *et al.*, 2015b). It is therefore highly likely that a majority of wild oyster batches used in the volunteer network were initially asymptomatic carriers of the disease while hatchery oysters were free of OsHV-1.

Lower influence of tidal height on the mortality risk in wild oysters compared to that of hatchery oysters could also be explained by the fact that (i) tidal height decreased the risk of oyster mortality by reducing viral exposure during emersion and (ii) the mortality risk of wild animals, which were presumably asymptomatic carriers of the virus, was less dependent on external sources of virus particles than that of hatchery oysters which were presumably free of OsHV-1. Indeed, mortality risk of oysters is associated with the cumulative exposure to OsHV-1 (Petton *et al.*, in revision) and the reactivation of the virus in asymptomatic carriers does not require additional exposure to the pathogen; a thermal elevation beyond the permissivity threshold (16°C in Europe) is sufficient (Pernet *et al.*, 2015; Petton *et al.*, 2015).

More particularly, survival of hatchery oysters was higher than that of wild oysters at tidal heights >1.2 m (immersion time <89.6%) but for tidal heights <1.2 m the opposite was true. At tidal heights >1.2 m, regular emersion may have partially protected hatchery oysters from pathogens, whereas wild oysters most likely died because the virus reactivated when seawater temperature increased to 16°C. Conversely, at lower tidal levels, hatchery oysters were at higher risk of mortality because the presumed protection from emersion was decreased, and their susceptibility to disease was greater than that of wild oysters. As wild oysters are more likely to be exposed to the pathogen than their hatchery counterparts, they are also more likely to be selected for greater resistance to the disease (Dégremont, 2011; Pernet *et al.*, 2012). It is possible, however, that confounding effects other than health status were associated with oyster origins and contributed to the interactive effect of tidal height and the origin of oysters.

We also found that the onset of mortality in wild oysters occurred earlier than in hatchery oysters. Similarly, OsHV-1 outbreaks are first manifest in wild oysters followed by those from hatcheries and nurseries (Pernet *et al.*, 2010; Degremont and Benabdelmouna, 2014). This likely reflects the reactivation of the virus in wild oysters (asymptomatic carriers) as soon as the seawater temperature reached 16°C, thus leading to an earlier death of the host. In hatchery oysters (presumably free of OsHV-1), mortality would appear not only above a certain temperature threshold, but after exposure to the virus and infection. Therefore, the lag between mortality outbreaks of asymptomatic carriers and healthy oysters probably corresponds to the time required for disease transmission, infection, and expression once environmental conditions have become permissive.

Here, we showed a beneficial effect of tidal height on oyster survival during an OsHV-1 epizootic. However, increasing tidal

Table 1. Model parameter estimates from the Cox regression model.

Parameter	Level	df	Estimate	SE	χ^2	*p*
Origin	Hatchery	1	0.597	0.127	21.9	<0.0001
Tidal height	–	1	−0.171	0.050	11.5	0.0007
Tidal height × origin	Hatchery	1	−0.498	0.075	44.0	<0.0001
Site	–	–	–	–	58.5	<0.0001

The columns display the parameter name, the degrees of freedom that are associated with the parameter (df), the estimated parameter value, the standard error of the parameter estimate (SE), the Wald chi-square statistic, and the associated *p*-value for testing the significance of the parameter.

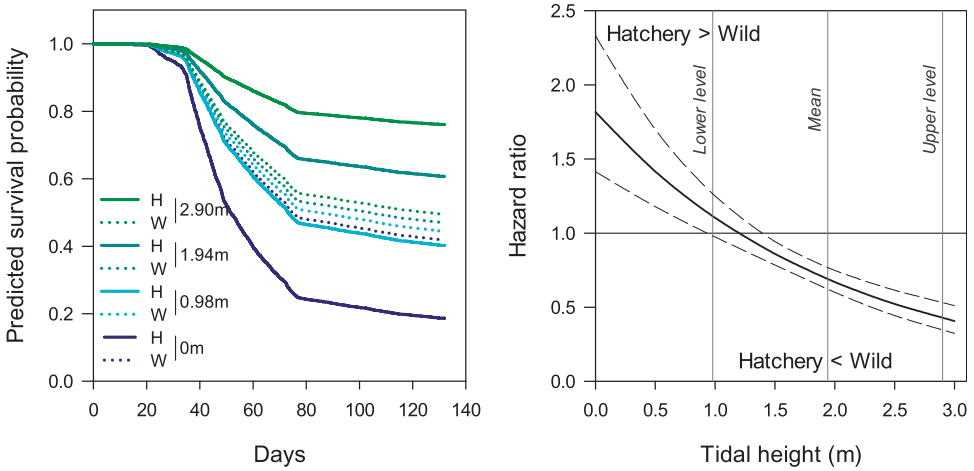

Figure 5. (a) Predicted survival probability curves of oysters as a function of tidal height and origin of oysters. (b) Hazard ratio of hatchery vs. wild oysters as a function of tidal height. Day 0 corresponds to 8 May 2013. Tidal height of 0 m represents permanent immersion whereas 0.98, 1.94, and 2.9 m were the lowest, the average, and the highest level recorded in our study. Values predicted at tidal height <0.98 m are extrapolations. Abbreviations: H, hatchery and W, wild.

height is not always associated with higher oyster survival (Paul-Pont *et al.*, 2013; Whittington *et al.*, 2015a). Indeed, tidal height delays the onset of spat mortality without increasing final survival (Whittington *et al.*, 2015a). While there is no apparent reason for delaying mortality if there is no survival gain at the end, this is a first step toward increasing survival and restoring the health of ecosystems. Therefore, studies to define the risk factors for disease management should consider the survival dynamics rather than the end point.

We therefore propose that farmers may mitigate oyster mortality by temporarily raising the culture height during the OsHV-1 mortality period, when seawater temperature is above 15°C. Oyster mortality mitigation strategies based on tidal height have already been suggested (Peeler *et al.*, 2012; Paul-Pont *et al.*, 2013; Whittington *et al.*, 2015a). Such a practice would be particularly relevant for oysters under 1-year old which are the most susceptible to the virus, and for oysters that are initially free of OsHV-1. However, to limit stunting due to the reduced feeding time that comes with increased emersion, farmers may consider lowering the culture height during the second summer, once oysters have become more resistant to the virus.

In line with this, rearing height of oyster spat in Australia was changed in response to OsHV-1 on 47% of leases, and 26% of these observed higher mortality when oysters were held low in the intertidal zone (Ugalde *et al.*, 2018). Yet, farmers consider this factor to be moderately important in limiting the losses caused by the virus. Also, a survey of 93 growers spread over two French farming areas (including the region covered by this study) conducted in 2014 reveals that 75% of them think that changing the rearing height is not an effective measure for controlling oyster mortalities caused by the virus (Le Bihan, Lupo, and Pernet, unpublished data). This probably reflects that the effect of tidal height on oyster mortality is not straightforward as it interacts with the origin of oysters, and more particularly with their initial health status (OsHV-1 free or asymptomatic carrier, this study) or their genetic make-up (Azema *et al.*, 2017). These interactions could skew the point of view of the growers.

Finally, we must evaluate the economic efficiency of such a measure. Does the survival gain compensate for reduced growth and increased handling? Then, we must assess acceptability, that is the willingness of farmers to put into action disease control measures. In France, oyster leases at high tide are usually dedicated to storing commercial oysters to limit their growth until they are shipped to markets. As a result, increasing rearing height of oyster spat could create an unwanted competition for space on the upper foreshore.

This study is based on the observation of a large number of oyster batches spread over the entire farming area at different tidal heights and over several sampling periods. This effort was made possible by the establishment of a volunteer network, thus highlighting its usefulness and power in marine epidemiology studies. In comparison, conventional experimental approaches are generally conducted on a more local scale and involve comparing final survival at two or three tidal heights in a restricted number of batches. However, associating observed mortalities with OsHV-1 was possible owing to the simultaneous presence of a long-term oyster mortality monitoring network with a scientific experiment carried out in the same location. Further hypothesis-driven controlled experiments are needed to elucidate the mechanism of increased disease resistance with tidal height. While citizen science has proven useful for marine epidemiology, more local hypothesis-driven research remains essential to uncover mechanisms underlying ecological patterns (Dickinson *et al.*, 2010).

Acknowledgements

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