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Competition for food reduces disease susceptibility in a marine invertebrate

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Abstract. Competition between organisms interfere in host and pathogen dynamics in ways that are difficult to predict. By one side, competitors can reduce the food supply and cause nutritional stress. Such stress could further modulate the susceptibility to infection by altering immune response or metabolic rate of the host. Alternatively, competitors may trap pathogens before they reach the focal host, and therefore reduce, enhance, or have no effect on infection according to the competitor's susceptibility to the infection. To better understand how competition influences host and pathogen interactions, we experimentally assessed the relative importance of competition for pathogens and resources on the severity of a viral disease infecting the Pacific oyster *Crassostrea gigas*. We designed an open-flow system where food enriched seawater flowed to filter-feeding competitors (or empty controls) before being delivered to recipient oysters. We tested a range of competing species that exhibit both low (ascidians, European oysters, mussels) and high (Pacific oysters) susceptibility to the virus. We assessed the physiological condition of the recipient oysters during acclimation, we added virus-contaminated seawater upstream of the distribution system, and we monitored host and pathogen dynamics. We found that the presence of competitors, regardless of susceptibility to the virus, indirectly reduced the infection rate of hosts by decreasing their food ingestion and growth rates. Although competitors can reduce viral particles from the seawater, this had no effect on the host population. Our data suggest that the effect of competition for food overwhelmed that of competition for pathogens, thus emphasizing the importance of considering resource availability in host and pathogen dynamics. More particularly, resource availability can have positive effects at the individual level, fostering physiological condition and growth, but negative effects at the population level, increasing magnitude of epidemics.

Key words: competition; *Crassostrea gigas*; disease ecology; filtration; ostreid herpesvirus 1; pathogen.

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INTRODUCTION

Food provisioning influence disease risk and outcome in two ways. On the one hand, food availability improves the physiological condition of the host and lowers their susceptibility to infectious disease, reflecting a tradeoff between immunity and other functions (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000). On the other hand, food scarcity limits the resources available to the pathogen and slows the growth and metabolism of the host on which the pathogen depends to proliferate (Murray and Murray 1979, Smith et al. 2005, Ayres and Schneider

2009, Hall et al. 2009*b*, Civitello et al. 2018). Therefore, food availability can have both positive and negative effects on the severity of infectious diseases.

In natural ecosystems, competitors reduce the food availability to the host and potentially modulate infection dynamics. However, predicting the effect of competition on infection dynamics is difficult because not only food resources are affected, but also pathogens (Dallas et al. 2016). For instance, the competitor may trap microbes before they reach the focal host. If the competitor is less susceptible to the pathogen than the focal host, then it may reduce pathogen transmission and infection according to the dilution effect (Keesing et al. 2006, Ostfeld et al. 2006, Hall et al. 2009*a*, Johnson and Thieltges 2010, Strauss et al. 2015). Conversely, if the competitor is more susceptible than the host species, it may increase the pathogen population size and infection rate of the focal host. Thus, competitor may enhance, reduce, or have no net effect on susceptible host density and infection prevalence, according to the relative susceptibility of the competitor to pathogen.

Here, we specifically assessed the relative importance of competition as a consumer of resources or as a consumer of pathogens on the severity of a viral disease affecting oysters. Since 2008, farmed stock of juvenile oysters has suffered mortalities associated with the detection of ostreid herpesvirus 1 (OsHV-1) variants worldwide (Segarra et al. 2010, Barbosa Solomieu et al. 2015). In Europe, OsHV-1 outbreaks every year during the spring and the summer season when seawater temperature is between 16°C and 24°C (Pernet et al. 2012*a*). Infection starts when viral particles come into contact with susceptible oysters via filter feeding. Then, viral particles are directed toward the digestive gland and the hemolymphatic system and rapidly spread to other organs (Schikorski et al. 2011, Segarra et al. 2016). Infected cells transcribe viral genes, which leads to replication and shedding of new viral particles within 24 h (Segarra et al. 2014*a*). Mortality can occur only two days after exposure to the virus (Schikorski et al. 2011) and affect 0–100% of the host population depending on its resistance to the pathogen (de Lorgeril et al. 2018). Mortality generally plateau after 10 d of exposure while virus DNA is no longer detected in the seawater (Schikorski et al. 2011, Petton et al. 2019). There is a threshold dose for infection and a dose-response effect of OsHV-1 on mortality (Paul-Pont et al. 2015, Segarra et al. 2016, Petton et al. 2019). Oysters naturally coexist with a wide diversity of competing species that have rarely been considered in host–pathogen interactions (Ben-Horin et al. 2015, Pernet et al. 2016).

Our main hypothesis is that competing filter-feeders could potentially reduce disease severity in oysters. For instance, we know that food availability and virus load increase mortality risk in oysters exposed to OsHV-1 (Paul-Pont et al. 2015, Pernet et al. 2019, Petton et al. 2019), suggesting that both food restriction and pathogen dilution act in the same direction. Food restriction slows the growth and metabolism of oysters (Pernet et al. 2019) on which the virus depends to proliferate (Jouaux et al. 2013, Segarra et al. 2014*b*). We therefore expected the competitors to reduce the availability of food for the oysters, their growth rate, and thereby, their mortality risk (food restriction effect). One consequence of this would be that the duration of exposure to the competitor which causes food restriction and reduced growth should help limit the risk of mortality. Also, we expected that the competitors would reduce the pathogen load, the transmission, and the mortality risk of oysters (dilution effect) and that this effect would increase with the retention efficiency for small particles of the competitor. Although viruses are very small particles, filter-feeders can clear them from the water column (Faust et al. 2009, Welsh et al. 2020). Secondarily, we tested the hypotheses that the increased susceptibility of the competitor would increase mortality risk in oysters because the dilution effect of pathogens is reduced.

Here, we designed an open-flow system where food enriched seawater flowed to filter-feeding competitors (or empty controls) before being delivered to recipient oysters. We tested a range of competitors that exhibit both low (ascidians, European oysters, mussels) and high (Pacific oysters) susceptibility to the virus. After 10 d, we added seawater contaminated with OsHV-1 upstream of the distribution system. At this time, we also added oysters that were fed ad libitum in the recipient tanks. These individuals underwent shorter exposure to competitors than those placed 10 d before in the system. We measured

growth and lipid reserves of oysters, and we monitored daily survival in the host population and pathogen load and prevalence.

Materials and Methods

Animals and maintenance

Specific pathogen-free (SPF) oysters were produced under controlled conditions (Petton et al. 2015, Le Roux et al. 2016). Briefly, on 9 August 2016, 60 adult oysters that were partially mature were transferred to the experimental Ifremer facilities located at Argenton (Brittany, France, 48°48′24.49″ N, 3°0′22.84″ W), where they were acclimated for two weeks in 500-L flow-through tanks with seawater maintained at 17°C and supplied with phytoplankton ad libitum. On 23 August 2016, the oysters were fully mature and gametes from 45 individuals (1/3 males, 2/3 females) were collected by stripping and fertilized. The embryos developed in 150-L tanks at 21°C for 48 h, and D-larvae were transferred to flow-through rearing systems at 25°C. After 15 d, competent larvae were allowed to settle in downwellers. On 4 October 2016, when the oysters were >2 mm shell length, they were transferred to the Ifremer nursery facility at Bouin (Vendée, France, 46°57′15.5″ N 2°02′40.9″ W). On 27 January 2017, they were transferred back at Argenton and kept at 13.5°C in 500-L flow-through tanks until the onset of the experiment. On 2 May 2017, the oysters were 8 months old and 0.68 g wet mass. The oysters were screened using an OsHV-1-specific quantitative PCR assay at the different stages of production and no OsHV-1 DNA was detected. These juvenile oysters were used as filter-feeding competitors and recipients.

Ascidians (*Ciona intestinalis* and *Ascidiella aspersa*), European oysters (*Ostrea edulis*), mussels (*Mytilus* sp.), and wild adult Pacific oysters (*Crassostrea gigas*) were collected in the bay of Brest in April 2017. The ascidians were collected on April 13 from submerged ropes at the harbor (48°22′44.86″ N, 4°19′06.370″ W). The European oysters were collected on April 20 by scuba divers at Anse du Roz (48°19′547″ N, 4°19′841″ W). Wild mussels and adult oysters were taken on April 24 nearby an oyster farm (48°20′06.19″ N, 4°19′06.37″ W). These animals were kept at 13.5°C in 45-L flow-through rearing tanks until the start of the experiment.

At Argenton, animals were kept in UV-sterilized, 1-μm filtered seawater supplied with a mixture of *Chaetoceros muelleri* (CCAP 1010/3) and *Tisochrysis lutea* (CCAP 927/14; 1:1 in dry weight). Food concentration was set at 1500 $\mu m^3/\mu L$ of microalgae at the outlet pipe of the tank so that oysters were fed ad libitum. Cell concentration was measured daily using an electronic particle counter (Multisizer 3) equipped with a 100-μm aperture tube. Temperature (13.5°C), salinity (35.2 psu), pH_{NBS} (8.2), and oxygen (>99%) were monitored daily with the WTW probes xi3101, cond340, pH3310 and FDO 925, respectively (Fisher scientific, Illkirch-Graffenstaden, France). Oysters were either injected with a suspension of OsHV-1 (pathogen donors), or they were not injected and used as competitors or recipients.

Experimental design

We experimentally tested the effect of competitors on disease susceptibility of oysters. We used a three-level open-flow system where virus-contaminated seawater supplied with phytoplankton (level 1) was distributed amongst 12 experimental units consisting of one tank containing the competitors (level 2) connected to one recipient tank containing the SPF oysters (level 3, Fig. 1). The tested competitors were an ascidian community, European oysters, mussels, and Pacific oysters (adult or juvenile). One tank remained empty as control (no food competition). Each treatment tank was run in duplicate spread over two blocks (six experimental units per block). Each block was subdivided in two groups of three experimental units. Each group was connected to the seawater supply, to the food supply, and to the source of infection by flexible tubes fitted inside a peristaltic pump. The seawater flow was set at 300 mL/min at the entry of each experimental unit. Each tank was 45 L, renewal rate of the seawater was 0.8 h^{-1}, and seawater was homogenized by means of a vigorous air bubbling and a recirculation pump. The experiment consisted of two successive phases: acclimation and virus exposure (Appendix S1: Fig. S1).

The competitors.—We first used the ascidians *C. intestinalis* and *A. aspersa* because they are abundant species living with *C. gigas* (Mazouni et al. 2001), they are among the most efficient filter-feeders for retaining small particles like pathogens (Randløv and Riisgård 1979, Riisgard

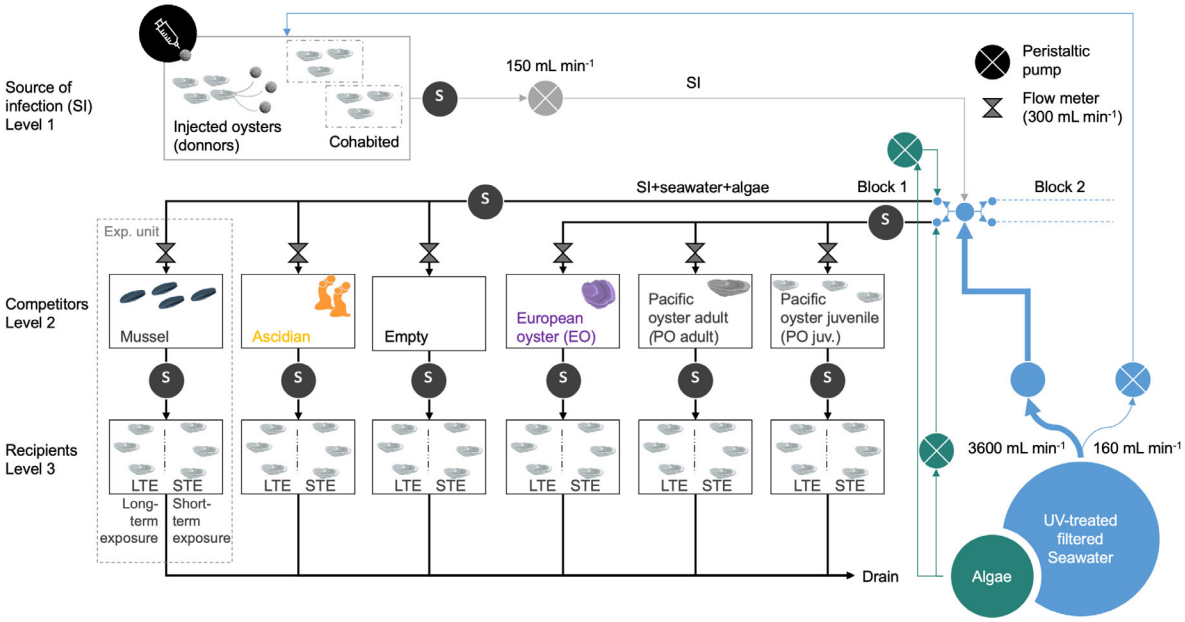

Fig. 1. Schematic of the experimental design. Only one block out of two is represented here. Arrows indicate direction of water flow, and black circles labeled with a letter S indicate seawater sampling for OsHV-1 DNA detection analyses. juv, juvenile; OsHV-1, ostreid herpesvirus 1.

1988, Bayne 2017 for review), and they are presumably not susceptible to OsHV-1. There is indeed no abnormal mortality of ascidians in the wild when OsHV-1 outbreak (F. Pernet, *personal observation*).

We also tested mussels *Mytilus* sp. because they naturally compete for food with *C. gigas* (Riera et al. 2002, Pernet et al. 2012*b*) and they are much less susceptible to OsHV-1 than *C. gigas*. Although OsHV-1 DNA is occasionally detected in mussels, viral load is generally low, there is no evidence of virus transmission to *C. gigas*, and no mortality was reported (Burge et al. 2011, Domeneghetti et al. 2014).

We included European oysters *O. edulis* because this species is more susceptible to OsHV-1 than mussels. The virus can replicate and induces significant mortalities in laboratory conditions (López Sanmartín et al. 2016). There is however no information associating OsHV-1 with abnormal mortality rates of European oysters in the wild.

We used the focal host *C. gigas* as a disease susceptible filter-feeding competitor. We distinguished adult and juvenile because disease susceptibility is generally much higher in the latter (Barbosa Solomieu et al. 2015, EFSA 2015, Pernet et al. 2016).

The only known pathogen affecting juvenile *C. gigas* in France is OsHV-1 (Barbosa Solomieu et al. 2015 and see data from the REPAMO network at https://wwz.ifremer.fr/sante_mollusques/Documentation/Bulletins-de-Surveillance).

Competitors were therefore unlikely to introduce other pathogens affecting juvenile oysters.

Acclimation.—On 28 April 2017, each treatment was randomly assigned to one tank per block. The biomass in each tank was adjusted to reach ~80% of the incoming seawater filtered by the animals. The phytoplankton concentration was set at ~4000 $\mu m^3/\mu L$ at the tank inlet, and the biomass of animal was adjusted to obtain ~750 $\mu m^3/\mu L$ at the outlet (Appendix S1: Table S1). Cell concentrations were checked daily using the particle counter over the course of the experiment between 2 May and 31 May 2017. The average phytoplankton concentrations (±1 standard deviation [SD]) at the inlet and outlet of the tanks containing the competitors were, respectively, 4107 (±34) and 744 (±250) $\mu m^3/\mu L$ (Appendix S1:

Fig. S2). The volume of water cleared of phytoplankton particles (clearance rate) was 14.7 L/h ± 1.1, corresponding to 81.8% ± 6.2 of the incoming water filtered by the animals (mean ± SD across treatments, Appendix S1: Table S1). For the control (empty) tank, the phytoplankton concentration at the tank inlet (4155 $\mu m^3/\mu L \pm 8$) was similar to that measured at the outlet (4090 $\mu m^3/\mu L \pm 33$, means ± SD between two replicate tanks, Appendix S1: Fig. S2). Overall, the food condition of oysters exposed to competitors covered their maintenance costs, whereas the control oysters were fed ad libitum. These conditions were similar to the low and high food regimes used in Pernet et al. (2019).

Between 28 April and 12 May 2017, seawater temperature was gradually increased from 13.5 to 21.0°C at a rate of 0.5°C/d in all tanks (Appendix S1: Fig. S1) to reach the optimum temperature for disease transmission (Petton et al. 2013). On 2 May, some of the SPF oysters were transferred to the recipient tanks for long-term exposure (LTE) to competitors. The average biomass of LTE oysters in each tank was 67.7 g ± 0.1, corresponding to 94 ± 5 individuals. The remaining recipients later served as short-term exposed oysters to competitors (STE). They were also exposed to the temperature ramping protocol and fed ad libitum (see previous section). Both LTE and STE oysters were kept in the closest possible conditions (food concentration and water renewal) but in different volumes of water (500 L vs. 40 L) and in different rooms. We therefore cannot rule out potential confounding effects with the time of exposure to competitors. Survival of oysters and other competitors was 100% during acclimation.

Virus exposure.—On 11 May 2017, SPF oysters for injection were myorelaxed in $MgCl_2$ (50 g/L in a mixture of seawater and distilled water 40/60 v/v) at 21°C. A total of 3000 oysters (2.25 kg) were individually injected with 25 μL of viral suspension containing 3.2×10^4 copies of OsHV-1 μVar/μL in the adductor muscle (Schikorski et al. 2011). They were kept in a 200-L tank in static seawater for 24 h where they shed viral particles in the seawater. The seawater surrounding the donors became contaminated with the virus and used as the source of infection. A subsample population of 110 pathogen donors were gathered in a mesh bag for daily survival monitoring. Also, two groups of 91 SPF oysters not injected with OsHV-1 were added to the tank to monitor disease transmission and mortality.

On 12 May 2017, 24 h after virus injection, the source of infection was connected to the seawater distribution network by flexible tubes fitted inside a peristaltic pump (Fig. 1). For each tank, the water flow from the source of infection was 4.2% of the total water flow. At this time, new SPF oysters that were fed ad libitum were added in the recipient tanks (Appendix S1: Fig. S1). These individuals underwent a short-term exposure to competitors (STE). Comparing STE and LTE oysters provides information about the effect of the host's metabolism (STE oysters were fed ad libitum before virus exposure while LTE oysters were not) while controlling the dilution of the pathogens (LTE and STE oysters were placed in the same tanks). The average biomass of STE oysters in each tank was 75.2 g ± 0.1, corresponding to 94 ± 7 individuals. Survival of oysters placed in the virus-contaminated seawater (level 1), in the competitor tanks (level 2), and in the recipient tanks (level 3) was monitored daily for 19 d, and dead animals were removed. The virus was successfully transmitted from donors to recipients through seawater (Appendix S2). The water input from the source of infection was removed after 4 d of exposure at the onset of recipient mortality (Petton et al. 2019). For the remainder of the experiment, the organisms were supplied with UV filtered seawater without viral contamination. Due to logistical constraints, there was no uninfected control. In our experimental conditions, the survival of these controls is always 100%, so that they are generally excluded from the survival analyzes (e.g., Fuhrmann et al. 2016, Delisle et al. 2018, Pernet et al. 2019, Petton et al. 2019).

Sampling and analyses

Ingestion rate.—Ingestion rate, the volume of microalgae consumed per minute, was measured daily over the course of the experiment in each tank containing the recipient oysters using the following formula:

$$\text{Ingestion rate}\,(\mu m^3/\text{min}) = ([\text{Cell}_{\text{inlet}}] - [\text{Cell}_{\text{outlet}}]) \times \text{waterflow}$$

where the variables were the concentrations of microalgae at the inlet and outlet of the tank

([$Cell_{inlet}$] and [$Cell_{outlet}$] in μm^3 of algae per μL of seawater) and the water flow in the rearing tank (300 mL/min).

Lipid reserves of oysters.—Both LTE and STE oysters were weighted and sampled in each tank ($N = 10$ individuals) on 12 May, just before pathogen exposure. Soft tissues were removed from the shells, pooled together, flash frozen in liquid nitrogen and stored at −80°C. Samples were then ground in liquid nitrogen with a MM400 homogenizer (Retsch, Eragny, France), and the resulting oyster powder was subsampled (~150 mg) and placed in 6-mL glass vials containing 3 mL of chloroform-methanol (2:1, v/v) and stored at −20°C until quantification of neutral lipids. Samples were sonicated for 5 min, spotted on activated silica plates using a CAMAG (Muttenz, Switzerland) automatic sampler, and the plates were eluted in hexane-diethylether acetic acid (20:5:0.5 v/v/v) followed by hexane-diethylether (97:3, v/v). Lipid classes appeared as black spots after plates were dipped in a $CuSO_4$–H_3PO_4 solution and heated. Plates were read by scanning at 370 nm, and black spots were quantified using Wincats software (CAMAG). This method allows the separation of free fatty acids, alcohols, mono-diacylglycerols, triacylglycerols (TAG), and sterols (ST). Because TAG are mainly reserve lipids and ST are structural constituents of cell membranes, the TAG/ST ratio was used as an index of the relative contribution of reserve to structure (Pernet et al. 2019).

OsHV-1 DNA detection.—The level of OsHV-1 DNA was determined (1) in seawater samples collected with sterile 15-mL Falcon tubes at the outlet of the tank containing the pathogen donors, at the inlet and at the outlet of the tanks containing the competitors 18-, 114-, and 402-hour post-infection (hpi), and (2) in alive oysters sampled 114 hpi at the onset of mortality. Samples were stored at −20°C.

For seawater, analyses were conducted on aliquots of 200 μL taken from a sample of 10 mL seawater. For oysters, tissues of five LTE and five STE individuals were sampled in each tank. The sample size (5 out of 100 oysters) corresponds to the sample size for detecting the presence of disease at a 95% confidence level, considering that the minimum expected disease prevalence is 50% (Pfeiffer 2010, see equation 7.3 p:76). Oyster tissues were individually homogenized in sterile artificial seawater, and total DNA was then extracted with a QIAamp tissue mini kit (QIAGEN, Hilden, Germany). The specificity and sensitivity of the detection test using these primers are similar to those reported by Pepin et al. (2008). The results were expressed as the log of OsHV-1 DNA copies per mL of seawater or per mg of wet oyster tissue. Virus detection and quantification analyses were conducted by Labocea, a French public diagnostic laboratory (Quimper, France), in compliance with approved quality management system ISO 17025 and COFRAC.

Statistics

Survival time curves of oysters were computed by the Kaplan–Meier method (Kaplan and Meier 1958) and compared using multiple comparisons for log-rank tests. Survival time was measured as days from 12 May, the onset of the exposure to pathogens. Combinations of competitor, duration of exposure to competitors, and tank were used as strata, and the survival estimates were compared by using the log-rank test of homogeneity of strata. Between-tank survival estimates for each treatment combination were not different (Appendix S3). We therefore used combinations of competitor and exposure duration as strata.

The survival time curves of oysters exposed to OsHV-1 were compared using the Cox regression model (Cox 1972) considering the effect of competitors, exposure duration, and their interaction. Each tank was considered as cluster using the sandwich method to obtain robust parameter estimates. The proportionality of hazards (PH) was checked with martingale residuals (Lin et al. 1993). Covariates related to physiological condition of LTE oysters were tested before adjustment for fixed effects (competitors and exposure duration).

The differences in oyster total body mass, ingestion rate, and physiological condition (triglyceride to sterol ratio) at the end of the acclimation period across treatments were analyzed by general linear models (GLMs), and correlations among the dependent variables were tested. Daily ingestion rates were averaged over the period from May 2nd to May 12th inclusively.

General linear mixed models (split-plot) were used to determine (1) the effect of competitors

(main plot) and sampling time (subplot, repeated measurement) on the virus concentration in the seawater at the outlet of the tanks containing the competitors and (2) the effect of competitors (main plot) and exposure duration (subplot) on the virus prevalence (binomial distribution) and concentration in individual recipient oysters. The unit of replication was the tank in which the treatments were applied. To examine the influence of oyster total body mass, ingestion rate, and physiological condition on virus prevalence and concentration in LTE oysters, we used logistic (logit link) and linear regression models, respectively.

Interactions among the factors were tested, and Tukey's HSD was used as a post hoc test. The normality of residuals and homogeneity of variance was graphically checked, and virus concentration in oysters was $\log_{10}(\times/10^4 + 1)$ transformed to meet the normality assumption. These statistical analyses were conducted using LIFETEST, PHREG, GLM, MIXED, GLIMMIX, REG, and LOGISTIC procedures of the SAS software package (SAS 9.4; SAS Institute, Cary, North Carolina, USA).

Results

Survival of recipient oysters placed downstream of competing filter-feeders for 10 d (LTE) and further exposed to OsHV-1 was 97.2% (±1.6%, SD among competitors) compared with only 76.4% in controls (Fig. 2). Competitors decreased the amount of food available to the recipient oysters thereby reducing their growth and their lipid reserves (Fig. 3). At the onset of the virus exposure, total body mass and lipid reserves (expressed as the TAG/ST ratio) were, respectively, 1.3 and 2.3× higher in controls than in oysters held at the outlet of competitor tanks, reflecting the amount of ingested food particles. Therefore, competitors decreased mortality risk, food availability, growth rate, and lipid reserves in LTE oysters (Table 1; Appendix S1: Table S2).

Survival of recipient oysters placed with the competitors at the onset of the virus exposure (short-term exposed, STE) was lower than that of LTE, but there was no effect of exposure duration on controls (Fig. 2, Table 1). Differences in survival between LTE and STE recipients were particularly high for those held at the outlet of tanks containing adult Pacific oysters or mussels. Survival of STE recipients held at the outlet of mussel tanks was similar to that of controls (Fig. 2, Table 1). Values of total body mass and TAG/ST of STE oysters (0.85 g and 2.9, respectively) seemed higher than those of oysters exposed to competitors over the long term (LTE oysters: 0.84 g and 1.7, respectively), but lower than those of LTE control oysters without competitors (1.07 g and 3.9, respectively).

Compared with control, the concentration of virus in seawater (estimated by the number of OsHV-1 DNA copies detected by qPCR/mL) downstream of ascidians decreased by 39% and 70% 18- and 114-hpi, respectively (Fig. 4). A reduction in virus concentration was also observed to a lower extent at the outlet of Pacific oysters 18 hpi but not 114 hpi. Virus concentrations downstream of European oysters and mussels were similar to those in the control tank. The concentration of virus in seawater downstream of Pacific oysters 114 hpi was higher than observed at the outlet of ascidians, European oysters and mussels. At the end of the experiment (402 hpi), OsHV-1 DNA was not detected in the seawater of the tanks.

Virus DNA was detected in the tissues of 36 recipient oysters out of 120 sampled, 114 hpi. Virus DNA was detected in oysters under all conditions except those placed downstream of ascidians (Fig. 5). However, virus DNA detection in oysters (prevalence and concentration) did not differ significantly among conditions (Appendix S1: Table S3). The concentration of OsHV-1 DNA in oysters reached values higher than 10^8 DNA cp/mg. Virus prevalence and concentration in LTE oysters were weakly but consistently and positively correlated with total body mass, food ingestion, and lipid reserves (Appendix S1: Table S4).

Discussion

Here, we determined the net effect of competition on host population by assessing the relative importance of competition as a consumer of resources or as a consumer of pathogens on the severity of the disease induced by the OsHV-1, a pathogen that infects the oyster *C. gigas*.

We found that competition benefits susceptible host population exposed to the virus. For instance, their survival was increased in the

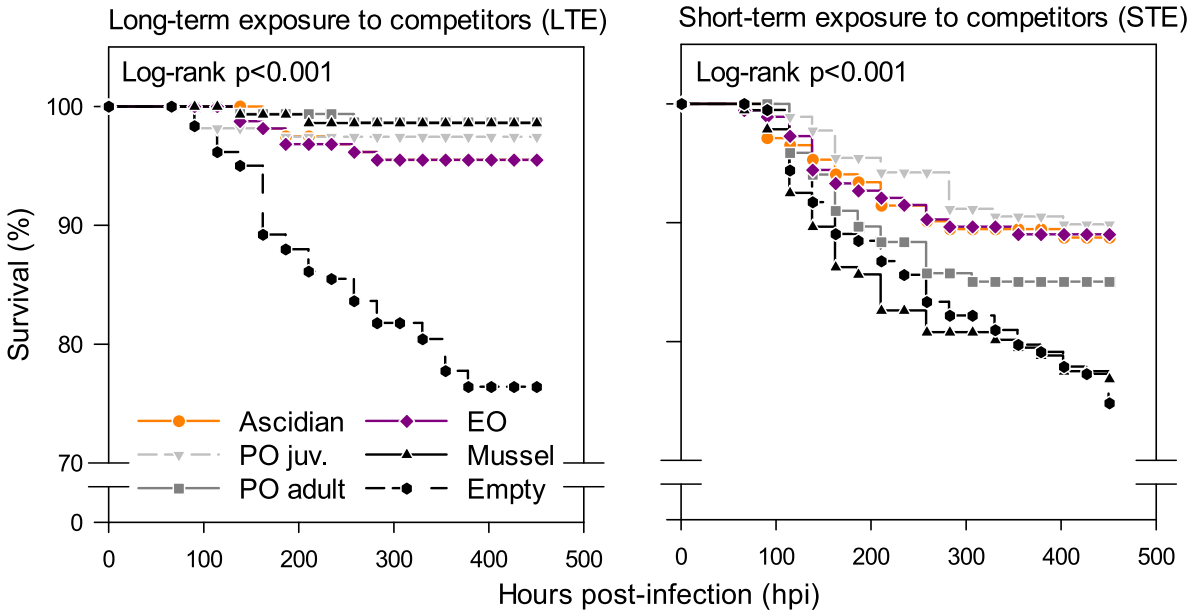

Fig. 2. Survival of recipient oysters exposed to OsHV-1 as a function duration of competitor exposure. PO, Pacific oyster *Crassostrea gigas*; EO, European oyster *Ostrea edulis*; juv, juvenile; OsHV-1, ostreid herpesvirus 1.

presence of a competitor, and this was regardless of the competitor's susceptibility to the pathogen. We also showed that competitors reduced food availability and growth of the host, a mechanism that possibly explains their lower susceptibility to the disease in the presence of filter feeders. In a previous study, food restriction increased the survival of oysters exposed to the virus by decreasing their growth rate (Pernet et al. 2019). Like other viruses, OsHV-1 uses the host's cell machinery to replicate, even stimulating the cellular growth of the host to maximize its growth potential (Jouaux et al. 2013, Segarra et al. 2014*b*). Our data support this, as the prevalence and concentration of OsHV-1 in oysters exposed to competitors over the long term (LTE) were positively associated with growth (body mass), food ingestion, and lipid reserves of oysters. In contrast to studies showing that competitors cause a nutritional stress that increases susceptibility to infection (Pulkkinen and Ebert 2004, Dallas et al. 2016), we provide evidence that competition for food resources can help susceptible host population by decreasing infection though decreased growth.

We also found that exposure duration to competitors plays a major role in the host response to the pathogen. For instance, the survival of recipient oysters placed with the competitors at the onset of the virus exposure (short-term exposure, STE) was always lower than the survival of their counterparts exposed over the long term (LTE). Differences in survival between LTE and STE oysters were not attributable to pathogen dilution since these oysters were placed in the same tanks. It might rather reflect that the STE oysters were fed ad libitum until they were exposed to the virus whereas LTE oysters were food restricted due to competition. Therefore, their increased metabolism due to higher food ingestion likely increased their susceptibility to the pathogen as compared to the LTE oysters. Although other confounding effects were possible (STE and LTE oysters were maintained separately during acclimation), mortality of LTE and STE in empty controls was remarkably similar. This suggests that susceptibility of oysters to OsHV-1 was not influenced by the separate housing during acclimation and that confounding effects were probably of minor importance.

We also observed that the survival of STE oysters varied greatly depending on the competing species placed upstream. These differences in survival were not explained by food availability.

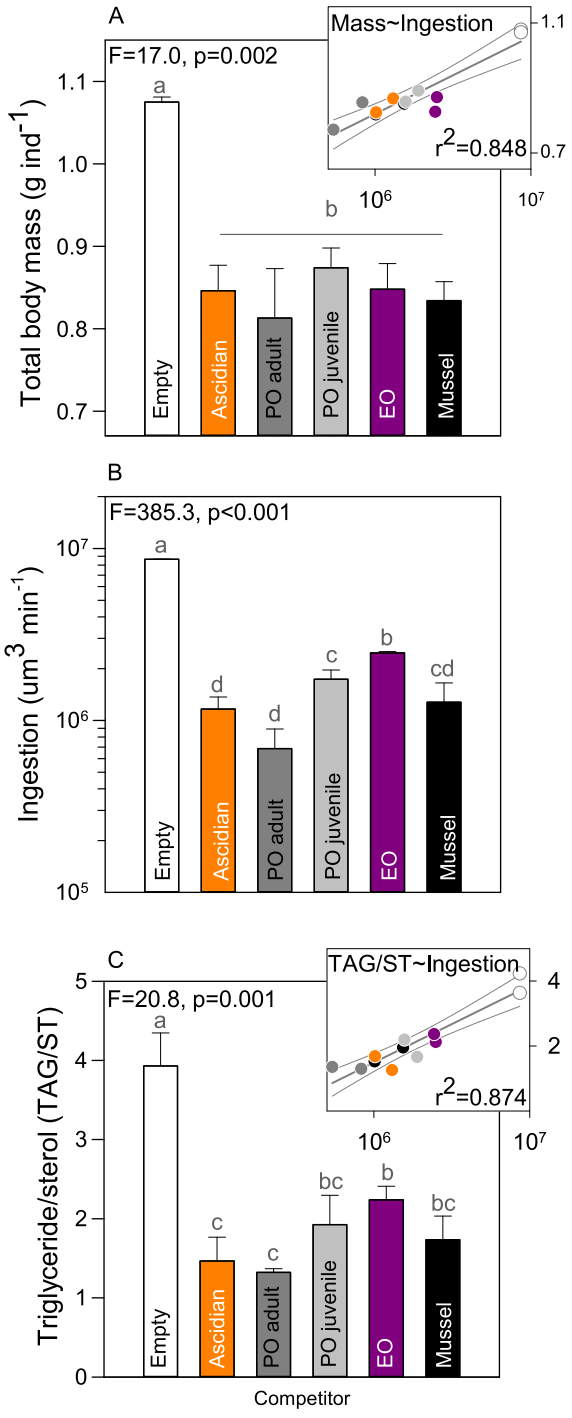

Fig. 3. Total body mass, ingestion and lipid reserves of oysters measured at the end of the acclimation period, before the exposure to the virus. Inset shows the relationships between body mass or lipid reserves (TAG/ST) and ingestion rate. Letters indicate significant differences (data are means ± standard deviation, $n = 2$ replicate tanks). PO, Pacific oyster *Crassostrea gigas*; EO, European oyster *Ostrea edulis*; juv, juvenile; TAG, triacylglycerols; ST, sterols.

For instance, survival of control oysters that were fed ad libitum was similar to that of oysters maintained downstream of mussel tanks that were food-restricted. We hypothesize that competitors harbor species-specific bacterial microflora (Schmitt et al. 2012, Vezzulli et al. 2018) that temporarily destabilizes the host microbiota, its immune response and its susceptibility to disease. In support to this hypothesis, transplantation of oysters to new habitats is accompanied by shifts in microbiota composition that potentially leads to mortality (Lokmer et al. 2016). The reshuffling of the oyster microbiota is indeed an integral part of the infectious process induced by OsHV-1 (de Lorgeril et al. 2018). For instance, OsHV-1 triggers an immunosuppression followed by microbiota destabilization and fatal bacteremia by opportunistic bacterial pathogens (de Lorgeril et al. 2018). Furthermore, as the characteristics of the microbiota of oysters are indicative of their health status, these could be predictive of oyster mortality events associated with disease (Clerissi et al. 2020).

Our data also suggest that the reduction in food availability forced by competitors was exceedingly more influential than their removal of pathogens from the environment. Although ascidians reduced the concentration of virus in seawater by 2×, they did not provide any additional protective effect on the oyster against the virus. For instance, OsHV-1 DNA concentration in the seawater remained high (>10^6 cp/mL), and mortality risk of STE oysters to ascidians was similar to their counterparts exposed to European oysters that had no significant effect on pathogen concentration in the seawater. It is however likely that a higher reduction in the viral load in water, by increasing the biomass of ascidians, could have a favorable effect on the survival of oysters. The relationship between competitor biomass, viral load in seawater, and host survival requires further investigation.

We also observed that the increased susceptibility of the competitor did not increase mortality risk in the recipient host. For instance, survival of

Table 1. Cox regression model.

Variable	DF	Estimate	SE	χ^2	*P*	Odds ratio
Bloc	1	0.291	0.050	33.9	<0.001	1.3
Competitor†						
Ascidian	1	−1.750	0.051	1162.5	<0.001	0.2
PO adult	1	−2.983	0.136	482.9	<0.001	0.1
PO juvenile	1	−2.249	0.492	20.9	<0.001	0.1
Mussel	1	−2.926	0.590	24.6	<0.001	0.1
EO	1	−1.750	0.059	872.9	<0.001	0.2
STE						
Exposure duration‡	1	0.073	0.057	1.7	0.194	
Competitor × exposure						
Ascidian	1	0.934	0.228	16.8	<0.001	
PO adult	1	2.459	0.132	346.0	<0.001	
PO juvenile	1	1.261	0.549	5.3	0.022	
Mussel	1	2.882	0.716	16.2	<0.001	
EO	1	0.889	0.102	76.7	<0.001	
Contrast						
Exposure duration by competitor						
Empty STE vs. LTE	1	0.073	0.057	1.7	0.194	1.1
PO juv. STE vs. LTE	1	1.334	0.546	6.0	0.015	3.8
PO adult STE vs. LTE	1	2.533	0.121	441.1	<0.001	12.6
Ascidian STE vs. LTE	1	1.008	0.221	20.9	<0.001	2.7
Mussel STE vs. LTE	1	2.955	0.713	17.2	<0.001	19.2
EO STE vs. LTE	1	0.962	0.084	130.9	<0.001	2.6
Competitor by exposure duration						
Empty LTE vs. other LTE	1	11.659	0.816	204.1	<0.001	115,679.0
Empty STE vs. [Ascidian + PO + EO] STE	1	3.189	0.212	225.9	<0.001	24.3
Empty STE vs. Mussel STE	1	0.044	0.126	0.1	0.728	1.0
Mussel STE vs. other STE	1	3.014	0.544	30.8	<0.001	20.4
PO juv. STE vs. [Ascidian + PO adult + EO] STE	1	−0.765	0.260	8.6	0.003	0.5
PO juv. STE vs. [Ascidian + EO] STE	1	−0.300	0.227	1.7	0.187	0.7
PO juv. STE vs. PO adult STE	1	−0.465	0.057	66.9	<0.001	0.6
Covariate						
Ingestion rate	1	2.773	0.328	71.6	<0.001	16.0
Body mass	1	9.440	1.147	67.8	<0.001	12,579.7
TAG/ST	1	0.974	0.088	123.0	<0.001	2.6

Notes: PO, Pacific oyster *Crassostrea gigas*; EO, European oyster *Ostrea edulis*; STE, short-term exposure to competitors; LTE, long-term exposure to competitors; juv, juvenile; TAG, triacylglycerols; ST, sterols; SE, standard error. Covariates were not adjusted for fixed effects and relate only to LTE to competitors.

† Oysters held at the outlet of empty tanks (control with no competitor) served as reference level.

‡ Acclimated recipient oysters served as reference level.

oysters exposed to susceptible juvenile oysters over the long term (LTE) was similar to that of oysters exposed to other non-susceptible competitors. This further supports the idea that the effect of reduced food availability probably overwhelmed that of dilution or amplification of the pathogen in the environment.

We confirm that filter feeders can remove viral particles from the water column, as reported for clams and avian influenza viruses (Faust et al. 2009). Analyses of the viral DNA concentrations at the outflow of the tanks containing competing filter feeders revealed that, among the species tested, ascidians were the most likely to retain the viral particles, thus reflecting their higher retention efficiency for small particles (Randløv and Riisgård 1979, Riisgard 1988, Bayne 2017 for review). Likewise, among a wide range of organisms tested, the breadcrumb sponge is the one that most effectively reduces viral abundance (Welsh et al. 2020). Note, however, that viruses can exist not only free floating in the water

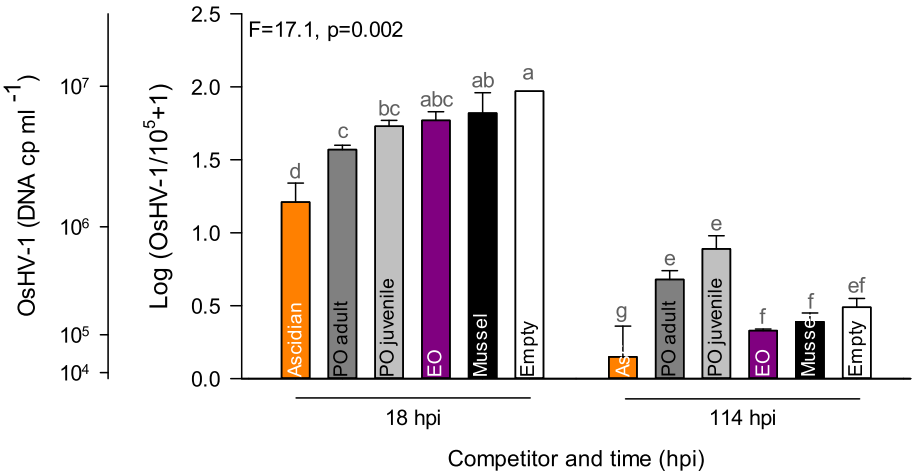

Fig. 4. Levels of OsHV-1 DNA in the seawater at the outlet of tanks containing competitors 18- and 114-h post-infection (data are means ± standard deviation, $n = 2$ replicate tanks). Letters indicate significant differences. PO, Pacific oyster *Crassostrea gigas*; EO, European oyster *Ostrea edulis*; juv, juvenile.

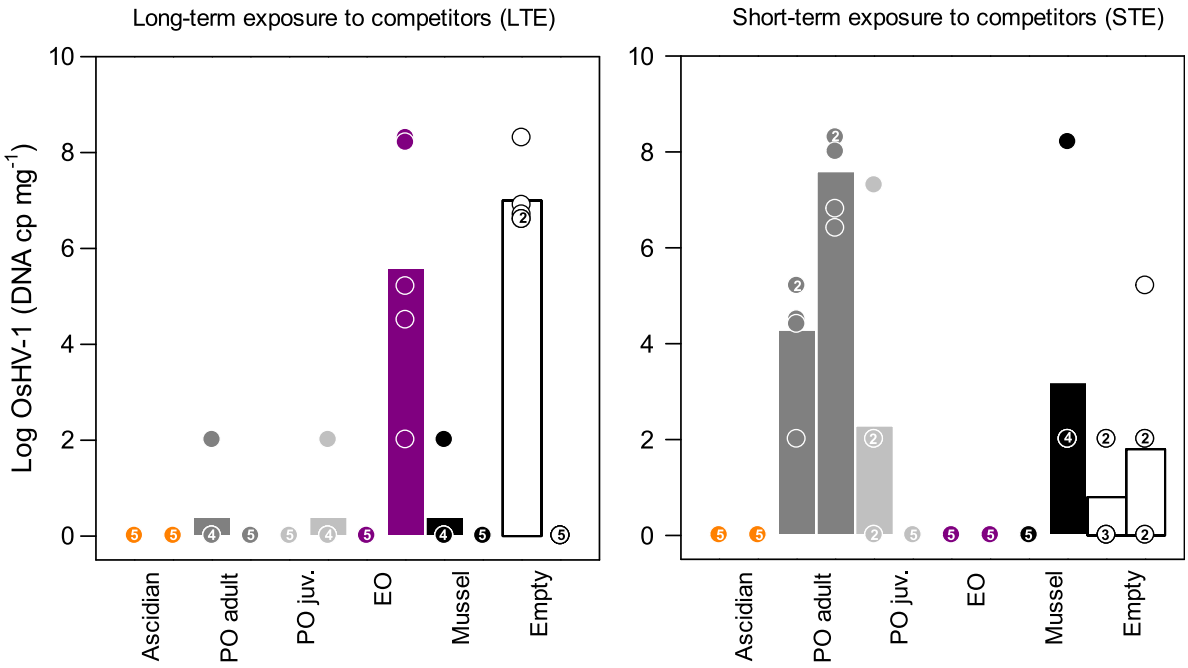

Fig. 5. Levels of OsHV-1 DNA in recipient oysters 114 h post-infection as a function of competitors and duration of competitor exposure for each tank ($n = 2$ tanks). Circles indicate individual measurements, and shaded bars show mean values ($N = 5$ oysters per tank and condition). The numbers in the circles indicate the number of overlapping samples. PO, Pacific oyster *Crassostrea gigas*; EO, European oyster *Ostrea edulis*; juv, juvenile; OsHV-1, ostreid herpesvirus 1.

column, but also attached to large organic aggregates that are more efficiently retained by filter feeders (Lyons et al. 2005, Froelich et al. 2013). Like other viruses, OsHV-1 is more probably carried on particles rather than being uniformly distributed in water. For instance, the removal of

large particles reduces the infectivity of OsHV-1-contaminated seawater and increases survival of the host (Whittington et al. 2015). Our study suggests, however, that removing the phytoplankton particles did not necessarily reduce OsHV-1 DNA concentration in seawater. Indeed, mussels and European oysters cleared ~80% of the phytoplankton biomass but the viral load in seawater remained similar to the control.

Unlike survival, detection of virus DNA (concentration and prevalence) did not differ depending on competitors. This probably reflects that the number of sampled oysters did not provide a robust estimate of disease prevalence. For example, it is surprising that OsHV-1 was not detected in oysters held with ascidians while mortalities were recorded. In this case, we probably misdiagnosed an infected population as non-diseased. This is very likely with relatively small sample fractions, that is, about 1–5% of the source population (Pfeiffer 2010). Considering that the expected disease prevalence was 0.5 and that all animals tested were negative then the source population could still contain a maximum of 13 diseased oysters (Pfeiffer 2010, see equation 7.5, p:77). We therefore cannot exclude the presence of OsHV-1 in oysters held with ascidians.

For reasons of logistical limitations, our experimental design was not full-factorial. Indeed, a missing treatment is a reduction in food availability without a competitor (or a competitor with food compensation) to decipher the effect of the competitor per se from that of the availability of food. This treatment was however tested in a previous study and resulted in an increase in survival which is consistent with what we observed here in the presence of competitors (Pernet et al. 2019). We also compared the survival of the juvenile oysters used as a competitor with that of the juvenile oysters placed downstream and with that of controls (Appendix S2). In this case, the competitors and the recipients were of the same species, same cohort and same life story. In addition, the competitor had little effect on the concentration of the pathogen while it had a major effect on food availability. Consequently, the only known difference between the competitors and the recipients, and between the two types of recipients (controls vs. juvenile oysters), was the availability of food, thus making it possible to apprehend this factor alone. Survival of food restricted recipient oysters was much lower than that of their upstream counterparts fed ad libitum. We are therefore confident that the lack of these treatments did not limits inference from the study.

Here, we specifically assessed the relative importance of competition as a consumer of pathogens or as a consumer of resources on the severity of the disease, thus relating dilution theory to competition for food resources (Hall et al. 2009*b*, Dallas et al. 2016). We suggest that competition benefits susceptible host population, not by diluting the pathogens, but by decreasing food availability and growth of the host on which the pathogen depends to proliferate. Our study emphasizes the importance of considering that resource availability can have positive effects at the individual level, fostering physiological condition and growth, but negative effects at the population level, increasing disease severity and magnitude of epidemics.

This finding opens perspectives for managing marine diseases. Although filter feeders can dilute pathogens in aquatic ecosystems (Burge et al. 2016), they can also limit the growth of the host and contain the epidemic risk. However, additional experimental studies are needed to evaluate the relationship between the strength of competition and infection in the host population, and to cross this effect with the dose and the type of pathogens. Such experiments should initially be carried out in laboratory conditions, but further field observations and experiments (i.e., removal experiments) are necessary for validation and scaling-up to more natural systems.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3435/full