Deciphering the effect of food availability, growth and host condition on disease susceptibility in a marine invertebrate

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RESEARCH ARTICLE

Deciphering the effect of food availability, growth and host condition on disease susceptibility in a marine invertebrate

Fabrice Pernet[1,*], David Tamayo[1,2], Marine Fuhrmann[1,3] and Bruno Petton[1]

ABSTRACT

Food provisioning influences disease risk and outcome in animal populations in two ways. On the one hand, unrestricted food supply improves the physiological condition of the host and lowers its susceptibility to infectious disease, reflecting a trade-off between immunity and other fitness-related functions. 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. Here, we investigated how food availability, growth rate and energetic reserves drive the outcome of a viral disease affecting an ecologically relevant model host, the Pacific oyster, *Crassostrea gigas*. We selected fast- and slow-growing animals, and we exposed them to high and low food rations. We evaluated their energetic reserves, challenged them with a pathogenic virus, monitored daily survival and developed a mortality risk model. Although high food levels and oyster growth were associated with a higher risk of mortality, energy reserves were associated with a lower risk. Food availability acts both as an enabling factor for mortality by increasing oyster growth and as a limiting factor by increasing their energy reserves. This study clarifies how food resources have an impact on susceptibility to disease and indicates how the host's physiological condition could mitigate epidemics. Practically, we suggest that growth should be optimized rather than maximized, considering that trade-offs occur with disease resistance or tolerance.

KEY WORDS: Energetics, Health, Herpesvirus, Nutrition, Physiological trade-off

INTRODUCTION

Infections, immune responses and nutrition are intimately related and influence disease risk and outcome in animal populations (Chandra and Newberne, 1977; Gershwin, 2012). Indeed, restricted access to food sources decreases the general physiological condition of the host, and animals in poor condition are generally more susceptible to disease because they lack the necessary resources to mount an adequate immune response (Beldomenico and Begon, 2010; Moret and Schmid-Hempel, 2000). This reflects a trade-off between immunity and other fitness-related functions such as growth, maintenance and reproduction under energy-limited conditions (Lochmiller and Deerenberg, 2000; Moller et al., 1998; Sheldon and Verhulst, 1996; Zuk and Stoehr, 2002). Therefore, the amount of available food and the physiological condition of the host are often associated with higher resistance or tolerance to infectious diseases.

However, in some cases, restricted food availability and the subsequent diminished physiological condition of the host can increase immune responses and disease resistance. Like their hosts, pathogenic organisms require energy resources to grow and reproduce. In food-restricted hosts, these resources may be reduced to levels that can limit pathogen growth (Civitello et al., 2018; Smith et al., 2005). For example, food-restricted zooplankton exposed to virulent fungal parasites died more slowly and exhibited fewer parasites than did well-fed ones (Hall et al., 2009). From this perspective, decreasing food consumption in response to infection is considered an adaptive response by the host to limit the resources available to the parasite (Ayres and Schneider, 2009; Murray and Murray, 1979).

Also, food availability can increase host metabolic rate, and therefore amplify the replication and proliferation of pathogens that depend on its cellular machinery. In line with this, the rate of pathogenesis, i.e. the time between infection and first symptoms or death, measured across a wide range of bird and mammalian hosts, is associated with body size and reflects the scaling of host metabolism (Cable et al., 2007). At the individual scale, virus replication depends on cellular growth and proliferation of the host, and any alteration to these pathways reduces virus gene expression and replication (Su et al., 2014).

Therefore, food availability has both positive and negative effects on the severity of infectious diseases (Beisel, 1982). A recent literature survey revealed that food availability is more likely to increase parasite abundance in invertebrate than in vertebrate hosts (Cressler et al., 2014). However, the fundamental mechanisms that influence the relationship of food availability, energetic reserves and growth with disease resistance have yet to be identified.

We first hypothesized that increasing food availability promotes energy resources of the host and therefore enhances disease resistance or tolerance. Alternatively, food and energetic reserves of the host have either no influence or increase disease susceptibility by providing additional resources to the pathogen. We also hypothesized that increasing the growth rate of the host (by providing more food or by selecting fast-growing animals) enhances the host-cell metabolism and, therefore, stimulates pathogen proliferation and disease susceptibility. These hypotheses are not mutually exclusive.

We experimentally tested these hypotheses by focusing on a disease affecting one of the major invertebrate species harvested globally, the Pacific oyster, *Crassostrea gigas*. Since 2008, farmed stock of juvenile oysters has suffered mortalities associated with the recurrent detection of ostreid herpesvirus 1 (OsHV-1) variants worldwide (EFSA, 2015; Segarra et al., 2010). In addition to being socio-economically devastating, oyster diseases affect overall ecosystem productivity and health (Coen et al., 2007). The oyster *C. gigas* has become a model species for studies in marine

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epidemiology and is well suited for investigating the energetic trade-off between growth and disease resistance.

Fed oysters have a higher risk of death than starved animals (Evans et al., 2015; Moreau et al., 2015). Paradoxically, the energy reserves of oysters, which reflect food availability, are associated with increased resistance or tolerance to OsHV-1 (Pernet et al., 2012, 2018, 2014). Oyster farmers report that fast-growing oysters appear more susceptible to OsHV-1 (Pernet et al., 2016). Growth rates amongst individual oysters are highly variable and are to a large extent controlled by food availability and genetic makeup (Bayne, 2004).

Here, we selected fast- and slow-growing oysters and exposed them to high and low food rations. We assessed their energetic reserves, challenged them with OsHV-1 and monitored their daily survival. Then, we developed a mortality risk model based on food availability, growth rate and energetic content of the host. Deciphering the role of these three factors in oyster mortality risk would inform on mitigation strategies based on farming practices.

MATERIALS AND METHODS

Oysters and maintenance

On 16 January 2014, 60 adult oysters (3 years old, 80 mm shell length) were transferred to the Ifremer facilities in Argenton (Brittany, France; 48°31′16″N, 4°46′2″W) for a conditioning period of 6 weeks (Petton et al., 2015). These animals were held in 500 l flow-through tanks with seawater held at a constant temperature of 17°C and enriched with a phytoplankton mixture. Seawater was treated with UV and filtered through 1 µm mesh. Daily mixed diet consisted of *Isochrysis affinis galbana* (CCAP 927/14) and *Chaetoceros gracilis* (UTEX LB2658) 1:1 dry mass at a ration equivalent to 6% of oyster dry mass. Once the oysters were reproductively mature, gametes from 45 individuals (1/3 males, 2/3 females), obtained by stripping, were mixed in a 5 l jar in a proportion of 10 spermatozoids per oocyte. The fertilized oocytes completed their embryonic development in 150 l tanks filled with 1 µm-filtered and UV-treated seawater at 21°C for 48 h. The D-larvae were then reared in flow-through rearing systems (Rico-Villa et al., 2008). At the end of the pelagic phase (15 days), competent larvae were collected on a 100 µm sieve and allowed to settle on cultch in downwellers made of rectangular plastic trays (14×45×35 cm H×L×W) fitted with a 125 µm nylon mesh on the bottom. Downwellers were placed in a rectangular raceway and supplied with phytoplankton-enriched seawater at 120 ml min^{-1}. After 10 days, the cultchless seed was collected on 400 µm mesh and maintained in downwellers for 15 days until reaching >2 mm shell length. During the larval and post-larval stages, the oysters were reared at 25°C. Then, they were transferred to 50 l tanks at 21°C for the duration of the experiment.

Phytoplankton concentration was measured twice a day at the inflow and the outflow of each tank and was maintained at 1500–2000 $µm^3$ $µl^{-1}$ at the outflow during adult conditioning and larval and juvenile rearing. Cell counts were made using an electronic particle counter (Beckman Coulter Counter Multisizer 3 equipped with a 100 µm aperture tube). Phytoplankton consumption is expressed as cell volume per gram of oyster per minute ($µm^3$ min^{-1} g^{-1} wet mass). Temperature, salinity and oxygen were measured twice daily with WTW probes (xi3101, cond340 and FDO 925, WTW GmbH, Weilheim, Germany).

Experimental design

Selection of slow- and fast-growing oysters

Slow- and fast-growing oysters were obtained by repetitive selection of individuals according to their size. On 29 April 2014, oysters were sorted into five size classes using sieves of 1, 2, 4, 6 and 8 mm mesh (Fig. S1). Oysters retained on 4–6 mm mesh represented 38% of the total population biomass and were used for the experiment (ca. 7500 oysters, with a mean individual mass of 0.040 g). These oysters were placed in three 50 l tanks for 14 days. Then, on 12 May 2014, the oysters were sorted in two sizes classes (4–6 and 6–8 mm mesh) and divided into two 50 l tanks per size class for 7 days. The average oyster biomass per tank was 81.5 g for the 4–6 mm oysters and 94.0 g for the 6–8 mm oysters. On 19 May 2014, the average oyster biomass per tank increased by 37–38% and 64–69%, respectively, so that the oysters that were initially retained on the 4–6 mm mesh were considered as slow growers whereas those retained on the 6–8 mm mesh were fast growers. Mean shell length and individual mass were 10.6±0.5 mm and 0.064 g for the slow growers and 15.1±0.9 mm and 0.173 g for the fast growers.

Exposure to low and high food regimes

From 20 May 2014 onwards, slow- and fast-growing oysters were continuously exposed to low and high food regimes. The objective was to test the effect of different-sized food rations on fast- and slow-growing oysters. Animals corresponding to each of the experimental groups [slow growing–low food (SL), slow growing–high food (SH), fast growing–low food (FL) and fast growing–high food (FH)] were maintained in three 50 l flow-through tanks at 360 ml min^{-1}. The average phytoplankton concentration at the tank outlet was 387 ±95 $µm^3$ $µl^{-1}$ for the low food condition and 2017±300 $µm^3$ $µl^{-1}$ for the high food condition (Fig. S2). The phytoplankton concentration at the tank inflow was held almost constant at 965±27 $µm^3$ $µl^{-1}$ for the low food regime. For the high food regime, it was gradually increased over time (from ca. 3700–4400 to 7400–8000 $µm^3$ $µl^{-1}$) to compensate for the increasing grazing rate of the growing oysters (Fig. S2). The low food condition covered the maintenance costs of the oysters (almost zero growth), whereas the oysters exposed to the high food condition were fed *ad libitum*. Initial biomass per tank was 34.9±0.2 g for the slow-growing oysters (540 individuals) and 48.9±0.1 g for the fast-growing ones (280 individuals). The total oyster biomass was recorded in each tank every 3–4 days for the next 45 days. The individual oyster wet mass was calculated as the ratio of the total oyster biomass to the number of individuals in each tank. On 17 June 2014, the SH oysters attained the same mass as the FL oysters (Fig. 1A).

Exposure to OsHV-1

We applied a widely used method of infection that consists of placing specific pathogen-free (SPF) oysters called ‘recipients’ in cohabitation in the laboratory with diseased donor oysters that were naturally infected in the field (de Lorgeril et al., 2018; Petton et al., 2019; Petton et al., 2013). Briefly, SPF oysters were transferred on 10 June 2014 to a farming area in the Bay of Brest (48°20′06.19″N, 4°19′06.37″W) where oyster mortality was occurring (de Lorgeril et al., 2018; Petton et al., 2015). After a week of exposure to field conditions, a few dead oysters were observed (less than 1%), and live individuals were moved back to the Ifremer facilities in Argenton. These oysters tested positive for the OsHV-1, and the level of viral DNA was $1.9×10^8$ copies mg^{-1} fresh mass (mean of 3 pools of 5 oysters). These oysters were used as pathogen donors.

On 17 June 2014, when the SH oysters attained the same mass as the FL oysters, subsamples of oysters from each tank (130 individuals for the slow growers and 80 individuals for the fast growers) were transferred into 12 new 50 l tanks to cohabit with 125 pathogen donors and were then referred to as recipients (de Lorgeril et al., 2018; Petton et al., 2019; Petton et al., 2013). The remaining

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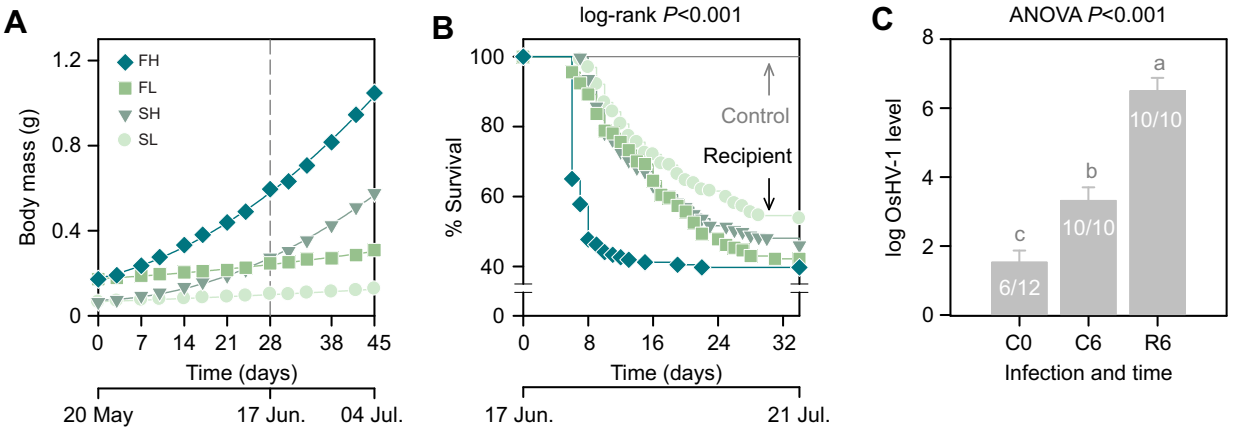

Fig. 1. Exposure of fast- and slow-growing oysters to high and low food regimes and pathogens. (A) Growth of control oysters selected for fast (F) and slow (S) growth exposed to high (H) and low (L) food level (mean individual body mass of 2–3 replicate tanks). The regression models fitted to the data are available in Table S1. The dashed line represents the onset of the exposure to pathogens. (B) Survival curves of control (C) and recipient (R) oysters exposed to pathogens for each treatment combination (means of 2–3 replicate tanks). (C) OsHV-1 DNA detection (copies mg^{-1} wet tissue) in control and recipient oysters at the onset of the cohabitation trial and at mortality (0 versus 6 days). Ratios in bars indicate the number of positive samples out of the total analysed. Different letters indicate significant differences. Only significant effects are shown.

oysters were left undisturbed in their tanks and used as controls. The pathogen donors were left in cohabitation with the recipients until recipient mortality began, i.e. for 6 days. Survival of controls and recipients was monitored daily for 34 days until equilibrium, and dead recipient oysters were removed from the tanks.

Sampling and analyses

Control and recipient oysters were sampled in each tank on day 0 just before pathogen exposure and on day 6, when mortality started in recipients. The number of individuals sampled was 40 for SL, 15 for SH and FL and 10 for FH, corresponding to an average biomass of 4.7 g per group. The whole oysters, including the shell, were pooled, flash frozen in liquid nitrogen and stored at −80°C. Samples were then ground in liquid nitrogen with an MM400 homogenizer (Retsch), and the resulting oyster powder was subsampled for quantification of OsHV-1 DNA, organic matter, carbohydrates and neutral lipids. Two samples of control oysters (one SH and one FL) collected on day 6 were lost and were excluded from laboratory analyses.

OsHV-1 DNA

A subsample of oyster powder (ca. 100 mg) was homogenized in sterile artificial seawater and total DNA was then extracted with a QIAamp tissue mini kit (Qiagen) according to the manufacturer's protocol. The extract was stored at −20°C before detection and quantification according to a real-time PCR protocol based on SYBR® Green chemistry (Pepin et al., 2008) with specific primers (Webb et al., 2007). These analyses were performed by LABOCEA (Quimper, France). The results were expressed as the number of OsHV-1 DNA copies per mg of wet tissue.

Organic matter

Oyster powder (ca. 200 mg) was placed in pre-weighed aluminium cups, weighed on a microbalance (Mettler-Toledo), dried for 72 h at 60°C and weighed again. The samples were then combusted at 500°C for 24 h and the remaining ash weighed. The organic matter content was determined as ash-free dry mass relative to the total body mass of the oyster (%).

Carbohydrates

Oyster powder (ca. 200 mg) was placed in Eppendorf tubes containing 1.5 ml of nanopure water, homogenized for 30 s on ice with a T10 basic ultra Turrax (IKA) and stored at −80°C until analysis (Pernet et al., 2012). Samples were diluted 10 times and 250 µl of the diluted powder was mixed with 500 µl phenol solution (5% m/v) and 2.5 ml H_2SO_4 (96% v/v) and incubated for 20 min (DuBois et al., 1956). The subsample was then placed in a double-beam UV-visible spectrophotometer (UVmc2 SAFAS), and absorbance was measured at 490 nm. Total carbohydrate concentration was then calculated using a standard calibration curve with pure glucose and expressed in mg g^{-1} organic matter.

Neutral lipids

Oyster powder (ca. 150 mg) was placed in 6 ml glass vials containing 3 ml chloroform–methanol (2:1 v/v) and stored at −20°C until analyses. Samples were sonicated for 5 min, spotted on activated silica plates using a CAMAG 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 versus structure (Fraser, 1989).

Statistics

Survival analyses

Non-parametric estimates of the survivor function 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 17 June 2014, the onset of the cohabitation trial (exposure to pathogens). Combinations of growth rate×food level were used as strata and the resulting survival estimates were compared by using the log-rank test of homogeneity of strata. Recipient oysters from two tanks (one FH and one FL) were inadvertently mixed during cleaning and were excluded from further analyses.

Cox model

The survival time curves of oysters exposed to OsHV-1 were compared using the Cox regression model (Cox, 1972) after

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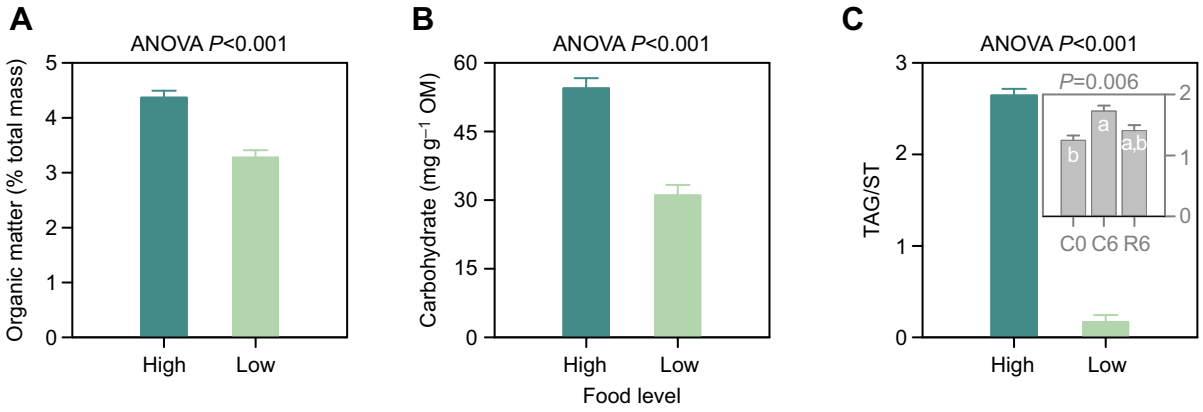

Fig. 2. Effect of food level on oyster organic matter, carbohydrate and lipid ratios. (A) Organic matter, (B) carbohydrate concentration (as a fraction of organic matter, OM) and (C) triglyceride to sterol ratio (TAG/ST) in oysters as a function of food level. Inset shows the effect of infection at the onset of the cohabitation trial and at mortality (0 versus 6 days) in control (C) and recipient (R) oysters. Values are means±s.e.m. of 2–3 replicate tanks. Only significant effects are shown.

adjustment for the effect of oyster growth, food level and their interaction. The survival of control oysters (not exposed to diseased oysters) was not included in the statistical models because it was always 100%. The proportionality of hazards (PH) was checked with Martingale residuals (Lin et al., 1993). Because the PH assumption was violated, time-dependent covariates representing the interaction of the original covariates and log(time) were added to the model (Quantin et al., 1996). Additional covariates related to oyster energy levels (percentage organic matter, carbohydrate concentration and triglyceride to sterol ratio measured at day 0 and 6) were tested one by one. Significant explanatory covariates (P<0.05) were selected using a stepwise method and tested in the multivariate Cox regression model. These analyses were conducted using the SAS software package (SAS 9.4, SAS Institute).

General linear models

General linear models (GLMs) were used to determine differences in the level of OsHV-1 DNA, percentage organic matter, carbohydrate and TAG/ST ratio, according to growth rate (fast versus slow), food level (high versus low), time (day 0 versus day 6) and infection (control versus recipient) and their interactions. Infection was nested within time. Associations between final survival of oysters and growth rate, food level and their interactions were tested using logistic (logit link) regression models. For models with statistical significance (P<0.05), LSMEAN multiple comparison tests were used to determine differences among treatments.

RESULTS

At the onset of the cohabitation trial (exposure to pathogens), body mass of fast growers in the high food treatment (FH) was 2.2-, 2.4- and 5.8-fold higher than that of slow growers in the high food treatment (SH), fast growers in the low food treatment (FL) and slow growers in the low food treatment (SL), respectively (Fig. 1A; Table S1).

The survival of control oysters was 100±0% irrespective of growth rate and food level (Fig. 1B). Although low levels of OsHV-1 DNA were detected occasionally at the onset of the cohabitation trial and systematically thereafter (Fig. 1C; Table S2), the absence of mortality infers that oysters were healthy.

Survival of recipients varied with oyster growth and food ration (Fig. 1B). The FH oysters died earlier and faster than those from other groups. Overall, the risk of death ranked as FH>FL, FL=SH, SH=SL and FL>SL (multiple comparisons for log-rank tests, P<0.05). At the end of the trials, survival of fast-growing oysters (39.7% and 42.2% for FH and FL, respectively) was lower than that of slow-growing animals (46.0% and 53.2% for SH and SL, respectively, logistic regression model for growth effect: χ^2=8.2, P<0.004). The detection of OsHV-1 DNA in recipients increased markedly between the onset of pathogen exposure and mortality, with no effect of food level and growth rate (Fig. 1C; Tables S2 and S3).

Organic matter, carbohydrate and TAG/ST ratio were greater in oysters from the high food treatment than in those from the low food treatment, regardless of growth rate (Fig. 2; Table S3). This effect was particularly pronounced for TAG/ST ratio, which was 15-fold greater in oysters from the high food treatment. Concomitantly, TAG/ST increased in controls (+38%) but not significantly in recipients (see Fig. 2C, inset). Overall, the mortality risk of recipients increased with high food levels and oyster growth rate but it decreased with increasing lipid reserves (Fig. 3, Table 1; Table S4).

DISCUSSION

In this study, we deciphered the complex interaction of food availability, growth and physiological condition of host on disease susceptibility, focusing on a viral disease affecting the oyster *Crassostrea gigas*. Current thinking is that food availability improves the physiological condition of the host and lowers their

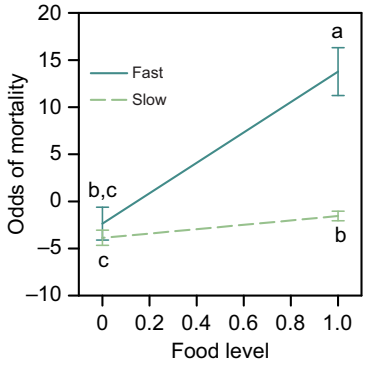

Fig. 3. Odds of mortality of recipient oysters as a function of growth rate and food level. Odds of mortality (means±s.e.m.) at the average of the TAG/ST ratio at day 6. Different letters indicate significant differences.

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Table 1. Summary of the Cox regression model adjusted for the effect of oyster growth, food level, time and their interactions and covariates on the odds of oyster mortality

Parameter	d.f.	Estimate±s.e.	χ^2	*P*	Odds ratio
Growth (fast versus slow)	1	1.498±1.814	0.68	0.409	
Food (high versus low)	1	2.311±0.935	6.11	**0.013**	
Fast×high food	1	13.830±4.008	11.91	**0.001**	
Fast×log(time)	1	−0.409±0.700	0.34	0.559	0.664
High food×log(time)	1	−0.315±0.382	0.68	0.409	0.729
Fast×high food×log(time)	1	−6.230±1.721	13.11	**<0.001**	0.002
TAG/ST	1	−0.521±0.091	32.70	**<0.001**	0.594

The tested covariates were percentage organic matter, carbohydrate concentration and triglyceride to sterol ratio (TAG/ST) in oysters at day 6.

susceptibility to infectious disease, reflecting a trade-off between immunity and other functions (Lochmiller and Deerenberg, 2000; Moller et al., 1998; Sheldon and Verhulst, 1996; Zuk and Stoehr, 2002). However, 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 (Ayres and Schneider, 2009; Cable et al., 2007; Civitello et al., 2018; Hall et al., 2009; Murray and Murray, 1979; Smith et al., 2005; Su et al., 2014).

Here, we found that food availability improved the physiological condition of the host while increasing its susceptibility to infectious disease. Indeed, food availability concomitantly increased energy reserves and mortality risk of oysters during pathogen exposure. However, there was no evidence that the host's physiological condition increased disease susceptibility by providing additional resources to the pathogen. On the contrary, increasing the lipid reserves (triglycerides) of the host was associated with a lower mortality risk, which is in line with previous studies suggesting a trade-off between immunity and other energy-demanding functions in oysters (Pernet et al., 2012, 2018, 2014). Although food levels increased energy reserves, these two factors showed antagonistic effects on oyster mortality risk during viral exposure.

The positive association between food level and mortality risk confirms previous studies on oysters (Evans et al., 2015; Moreau et al., 2015) and probably reflects improved growth and metabolic rates of the host, which in turn amplifies pathogen replication. In line with this, we concomitantly found that, with a large food ration, fast growers were more susceptible to the disease than slow growers. Oysters exposed to a high food level or selected for fast growth rate were more at risk than slow-living ones. Like other viruses, OsHV-1 uses the host cell machinery to replicate (Jouaux et al., 2013; Segarra et al., 2014), and stimulation of cellular growth of the host would amplify viral gene expression and replication (Su et al., 2014).

In our study, however, there was no evidence of increased virus replication in response to increased food availability and growth rate of the host. Indeed, OsHV-1 DNA levels in host tissues at the onset of mortality were similarly high in all conditions. This suggest that food availability and growth rate of oysters influenced disease tolerance but not resistance. Resistance refers to the ability of a host to limit pathogen growth and can be measured as the inverse of the pathogen load, whereas tolerance is a measure of the ability of a host to survive an infection at a given pathogen load (Rauw, 2012; Roy and Kirchner, 2000).

Nevertheless, we cannot exclude that virus replication and shedding were affected by food ration or host growth rate. Here, we measured the level of viral DNA at the onset of oyster mortality in the host tissues as a proxy of virus production. Although the values measured at this time certainly corresponded to the maximum viral loads reached in live oysters for each treatment (de Lorgeril et al., 2018; Petton et al., 2019), analysing the temporal dynamics of viral shedding in the seawater would probably provide a better estimate of virus production by the host.

Furthermore, we have recently discovered that the oyster disease triggered by OsHV-1 is polymicrobial (de Lorgeril et al., 2018). For instance, OsHV-1 creates an immune-compromised state in oysters, evolving towards subsequent bacteraemia by opportunistic bacterial pathogens, leading to oyster death. Bacteraemia plays a major role in the outcome of the disease. We cannot exclude that food availability, growth rate and lipid reserves of the host act on the susceptibility of oysters to bacteraemia.

Finally, the lower mortality risk at low food levels may also reflect induced autophagy, an evolutionarily conserved cell recycling process that is activated in response to stress such as starvation (Desai et al., 2015). Autophagy also controls microbial infections, both through direct destruction of the pathogen and as one of the key mediating factors in the host defence mechanisms of innate and adaptive immunity (Desai et al., 2015). The autophagy pathway is functional in oysters and explains why starvation reduces mortality during OsHV-1 infection (Moreau et al., 2015).

In conclusion, food ration and oyster growth were associated with a higher risk of mortality while energy reserves were associated with a lower risk. Food availability acts both as an enabling factor for mortality by increasing the growth of the oyster and also as a limiting factor by allowing the host to acquire energy reserves. Further investigations are needed to accurately assess whether these factors affect viral proliferation, bacteraemia or autophagy. Considering that trade-offs occur between oyster growth and disease resistance or tolerance, growth should be optimized rather than maximized (Bayne, 2004), as suggested for other livestock production (Rauw, 2012; van der Most et al., 2011). A simple way to reduce the growth rate of oysters is to increase the tidal height (or air exposure time) (Gillmor, 1982). This has proven to be effective for increasing the survival of oysters against the virus (Azéma et al., 2017; Paul-Pont et al., 2013; Pernet et al., 2019).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: F.P., D.T., M.F., B.P.; Methodology: F.P., D.T., M.F., B.P.; Investigation: F.P., D.T., M.F., B.P.; Writing - original draft: F.P.; Writing - review & editing: D.T., M.F., B.P.; Project administration: F.P.; Funding acquisition: F.P.

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Supplementary information
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