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FEATURE ARTICLE

Increased growth metabolism promotes viral infection in a susceptible oyster population

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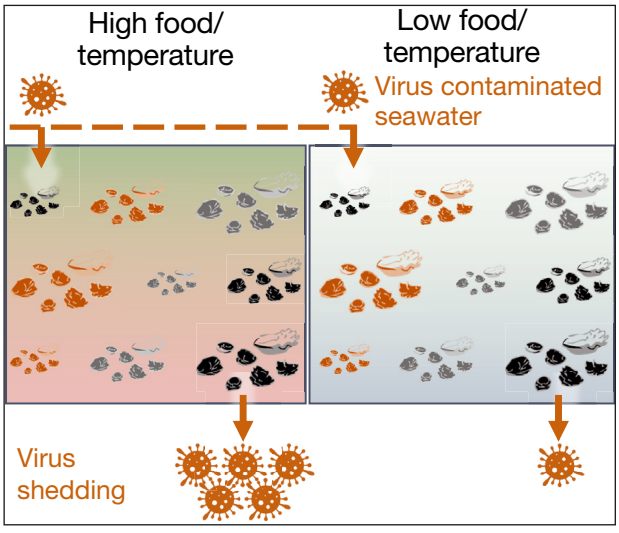
ABSTRACT: The magnitude of an epidemic depends on host susceptibility to the disease, a trait influenced by the genetic constitution of the host and its environment. While the genetic basis of disease susceptibility is often associated with immune capacities, environmental effects generally reflect complex physiological trade-offs. We suggest here that in the case of obligate pathogens whose proliferation depends on the cellular machinery of the host (e.g. viruses), disease susceptibility is directly influenced by host growth. To test our hypothesis, we focussed on a viral disease affecting an ecologically relevant model exploited worldwide, the Pacific oyster *Crassostrea gigas*. Oysters originating from 3 lines with contrasting resistance to the disease were divided into 3 groups displaying different growth rates and acclimated to 3 food levels and 2 temperatures to generate different growth rates. These oysters were then exposed to the virus, and survival and viral shedding were measured. Finally, we developed a risk model to rank the relative importance of temperature, food, genetic selection and growth on disease-induced mortality. We found that increasing growth through temperature, food level or selection of fast-growing animals all increased mortality, especially in host populations where susceptible phenotypes dominated. Food provisioning was the most influential factor associated with higher viral shedding, followed by temperature, resistance phenotype and growth rate. We suggest that growth-forcing factors may promote the development of obligate intracellular pathogens and epidemic risk, thus opening up avenues for disease management based on the manipulation of host metabolism.

KEY WORDS: Epidemiology · Growth · Health · Metabolism · Physiological trade-off · Temperature



Oysters exposed to a virus show increased viral shedding if they have inherently higher growth rates and are exposed to high temperature and food levels.

Graphic: Fabrice Pernet, Ifremer

1. INTRODUCTION

Epidemics are occurring at an unprecedented historical rate, mainly reflecting the effects of climate change and anthropogenic factors (Harvell et al. 1999). In this context, there is an urgent need to identify and prioritise disease risk factors to implement effective management measures. We know that the magnitude of an epidemic depends on host susceptibility to the disease, a complex trait that reflects the genetic constitution of the host and its environment. The genetic basis of disease resistance has been established for most of the diseases affecting major

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domestic animal species and is generally mediated by innate and acquired immunity (Cock et al. 2009, Bishop et al. 2010).

Food and temperature are particularly important environmental factors that can positively or negatively influence host susceptibility. For instance, unrestricted food supply may improve the physiological condition of the host and lowers its susceptibility to infectious disease, reflecting an allocation tradeoff between energy available to mount immune response and energy for other fitness-related functions (Lochmiller & Deerenberg 2000). Alternatively, food scarcity may limit the pathogen and slow the growth and metabolism of the host on which the pathogen depends to proliferate (Smith et al. 2005, Civitello et al. 2018). In ectothermic organisms, increased temperature accelerates growth within the thermal tolerance window of the species, as does increased food level. When these organisms are exposed to obligate intracellular parasites, like viruses, that depend on the cellular machinery of the host to replicate and proliferate, it is likely that increased temperature and food promote pathogen proliferation. This leads us to hypothesize that factors forcing host growth promote pathogen proliferation and epidemic risk.

To test our hypothesis, we focussed on the Pacific oyster mortality syndrome (POMS) affecting juveniles of *Crassostrea gigas*, a keystone species exploited worldwide. POMS is a polymicrobial disease with an initial and necessary step of infection by the Ostreid herpesvirus OsHV-1 µVar (de Lorgeril et al. 2018). OsHV-1 uses the host cell machinery to replicate (Segarra et al. 2014, Delisle et al. 2020) and creates an immune-compromised state evolving towards a fatal bacteraemia (de Lorgeril et al. 2018).

Seawater temperature, food availability, oyster genetics and growth all influence oyster–virus interaction and disease outcome (Petton et al. 2021). In Europe, epizootics initiated by OsHV-1 occur when seawater temperature ranges between 16 and 24°C (Pernet et al. 2012). Above 16°C, temperature, metabolic rates and susceptibility to OsHV-1 may be related. In addition, oysters exposed to high food levels or selected for fast growth rate are more susceptible to OsHV-1 than slow-growing individuals (Pernet et al. 2019). Finally, resistance to OsHV-1 is a highly heritable trait based on control of viral replication that may not be genetically correlated with growth or associated with differences in metabolic rates (Dégremont et al. 2010, Dégremont 2011, Haure et al. 2021).

Here, we acclimated oysters at 2 temperatures permissive for the virus and at 3 food regimes. To integrate the resistance factor related to genetics, we used 3 oyster lines exhibiting highly contrasted resistance phenotypes with regard to the POMS. Each line was divided into 3 groups according to individual growth rates. We then exposed these oysters to OsHV-1 and followed host survival and virus production. This experimental design was inspired by Pernet et al. (2019), who assessed the effects of food availability, growth and host condition on virus susceptibility of oysters. The experiment described herein, conducted 4 yr later, is different from Pernet et al. (2019) in that temperature and genetics are incorporated and starvation has been added to dietary levels. This new design makes it possible to extend the investigation to the overall effect of growth metabolism (and not just food) on disease susceptibility, and also to determine the relative importance of temperature, food supply, genetics and growth as disease risk factors. Part of this experimental setup was deployed in a natural infectious ecosystem to verify the consistency with the results obtained in the laboratory.

2. MATERIALS AND METHODS

2.1. Oysters

The 3 oyster lines with contrasting OsHV-1 resistance (grey box in Fig. 1) were produced from genitors issued from 0, 1 or 2 rounds of mass selection to the disease. Briefly, a wild population of adult oysters was matured and spawned under laboratory conditions in April 2016, and the resulting progeny (G0) were transferred to the field 3 mo later, where they suffered 92% mortality due to OsHV-1 (Fig. 1). Survivors were spawned in March 2017, and their progeny (G1) were transferred to the field in the same period, where they suffered 57% mortality due to OsHV-1. The surviving G1 oysters were spawned in April 2018 to produce the G2 oysters used in the present study. The same procedure was repeated in 2017 and 2018 to produce the G1 and G0 oysters, respectively, used in the present study (Fig. 1).

The wild population of adult oysters used for reproduction consisted of 28–35 females and 10–15 males collected in the natural environment in the Bay of Brest (Pointe du Château, Brittany, France, 48° 20′ 06″ N, 4° 19′ 06″ W) and transferred to the Ifremer facilities in Argenton (Brittany, 4° 31′ 16″ N, 4° 46′ 2″ W) in February of each year for conditioning periods of 6 wk in 500 l flow-through tanks at 17.5°C

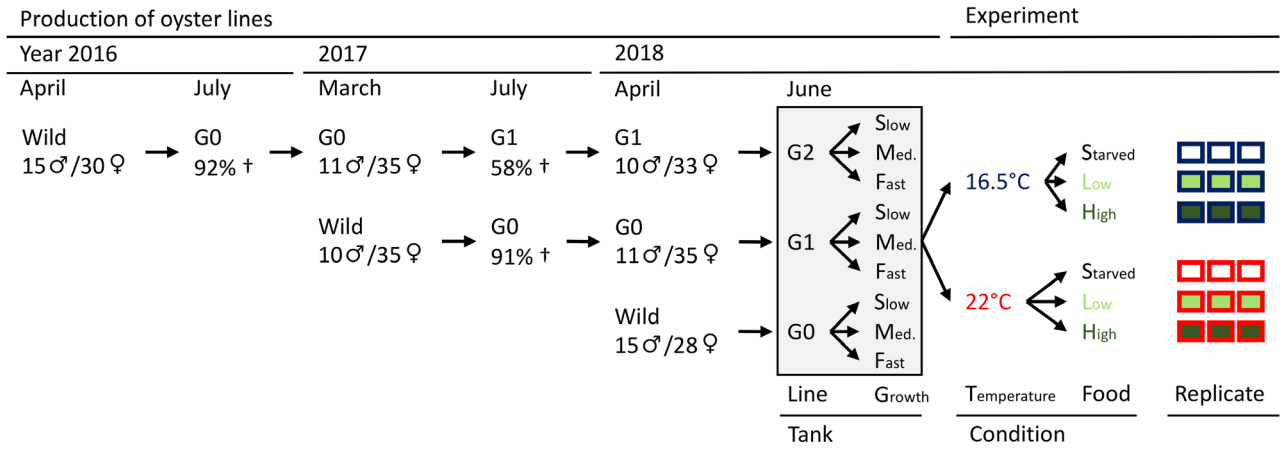

Fig. 1. Timeline for production of oyster lines and experimental design. G: generation number; %†: mortality during the spring/summer season due to an outbreak of OsHV-1 in the field

(Petton et al. 2015). Seawater was treated with UV and filtered through 1 µm mesh. Diet consisted of *Tisochrysis lutea* (CCAP 927/14) and *Chaetoceros muelleri* (CCAP 1010/3). Gametes were obtained by stripping and were mixed at 10 spermatozoids per oocyte. Embryogenesis occurred in 150 l tanks filled with 1 µm filtered and UV-treated seawater at 21°C for 48 h. Fecundation success and hatching rates ranged from 91 to 98 % and 28 to 73 %, respectively. D-larvae were reared in flow-through cylinders (5 l) at 25°C. After 15 d, competent larvae were allowed to settle on cultch in trays fitted with a 125 µm nylon mesh on the bottom. After 10 d, the oysters were collected on 400 µm mesh and maintained at 20°C until they reached 2 mm shell length and then transferred to a nursery at the Ifremer marine station located in Bouin Vendée, France (46° 57′ 16″ N, 2° 02′ 41″ W) and transferred again at the age of 3 mo to a grow-out site located at Pointe du Château. OsHV-1 occurs there each year when seawater temperature reaches 16°C during the spring–summer period and causes massive mortality of young oysters (Petton et al. 2015). The survival of each line of oysters was reported before the conditioning period to assess the selection pressure of POMS.

Phytoplankton concentration was measured twice daily using an electronic particle counter (Beckman Coulter counter Multisizer 3 equipped with a 100 µm aperture tube) at the inflow and the outflow of each tank. Cell concentration was maintained at ~1500 μm^3 μl^{-1} at the outflow during all rearing stages and expressed in μm^3 min^{-1} g^{-1} wet mass of oyster. Temperature, salinity and oxygen were measured daily with the WTW probes xi3101, cond340 and FDO 925 (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/q015p019_supp.pdf).

2.2. Experimental design

2.2.1. Selection of slow-, medium- and fast-growing oysters

Slow-, medium- and fast-growing oysters were obtained following the method described by Pernet et al. (2019). On 16 May 2018, 43 d after spawning, G0, G1 and G2 oysters were sorted into 3 size classes using sieves with 2 and 4 mm mesh. Oysters retained on mesh between 2 and 4 mm represented the majority of the total population and were used for the experiment. On 24 May, oysters from each line were again sorted into 3 sizes classes (2–4, 4–6 and 6–8 mm mesh) and divided into 3 tanks (50 l capacity) per size class and line and acclimated for 7 d. Mean individual mass among all 3 oyster lines (G0, G1 and G2) was 15 ± 2 mg for the slow growers, 44 ± 4 mg for the medium growers and 116 ± 38 mg for the fast growers (error values are ±SD throughout the article).

2.2.2. Acclimation to temperature and food regimes

On 1 June, oysters were exposed to 2 temperatures and 3 food regimes, i.e. 6 experimental treatments. Each treatment was applied to 3 flow-through tanks (50 l) at ~200–360 ml min^{-1} (total = 18 tanks, Fig. 1). The flow rate was adjusted daily to maintain the oxygen level at a saturation level above 90 % while keeping the desired temperature (Fig. S1). A subsample of oysters from each line and each size class was placed in a flat-bottomed basket nested in the tank. The basket was divided into 9 compartments of equal area and each combination of line × growth was randomly assigned to a compartment. In each compartment, we placed an av-

erage of 90 fast-growing oysters and only 64 medium- or slow-growing oysters because fewer individuals were available. Overall, the initial oyster biomass was 39.6 ± 0.3 g per tank. Fast-growing oysters were 2.4 times larger than medium-growing oysters, which were 2.2 times larger than slow-growing oysters (Fig. 2A). There was no line effect on oyster size except for the fast-growing G1, which were larger than their G0 and G2 counterparts.

Oysters were acclimated at 16.5 and 22°C because these temperatures are both permissive to infection by OsHV-1 (Pernet et al. 2012, Petton et al. 2013), but growth and metabolic rates of oysters at these temperatures differ (Bougrier et al. 1995). Each tank was equipped with a thermostat and heating resistor (Biotherm Ecco Hobby and SCHEIGO Titane 300 W, Europrix) to maintain the seawater at the desired temperature.

Oysters were either starved, fed at a low level to cover their maintenance costs (i.e. almost zero growth) or fed at a high level. The average phytoplankton concentration at the outlets of tanks was 459 ± 86 for the low food condition and 1860 ± 387 $\mu m^3\ \mu l^{-1}$ for the high food condition during the acclimation period, similar to a previous study (Pernet et al. 2019). The phytoplankton concentration at the tank inflow was gradually increased over time to compensate for the increasing grazing rate of the oysters, especially as the temperature and the food ration were high. No oyster mortality occurred during the acclimation period.

2.2.3. Exposure to OsHV-1

We used a method that consists of producing seawater contaminated with OsHV-1 by injecting the

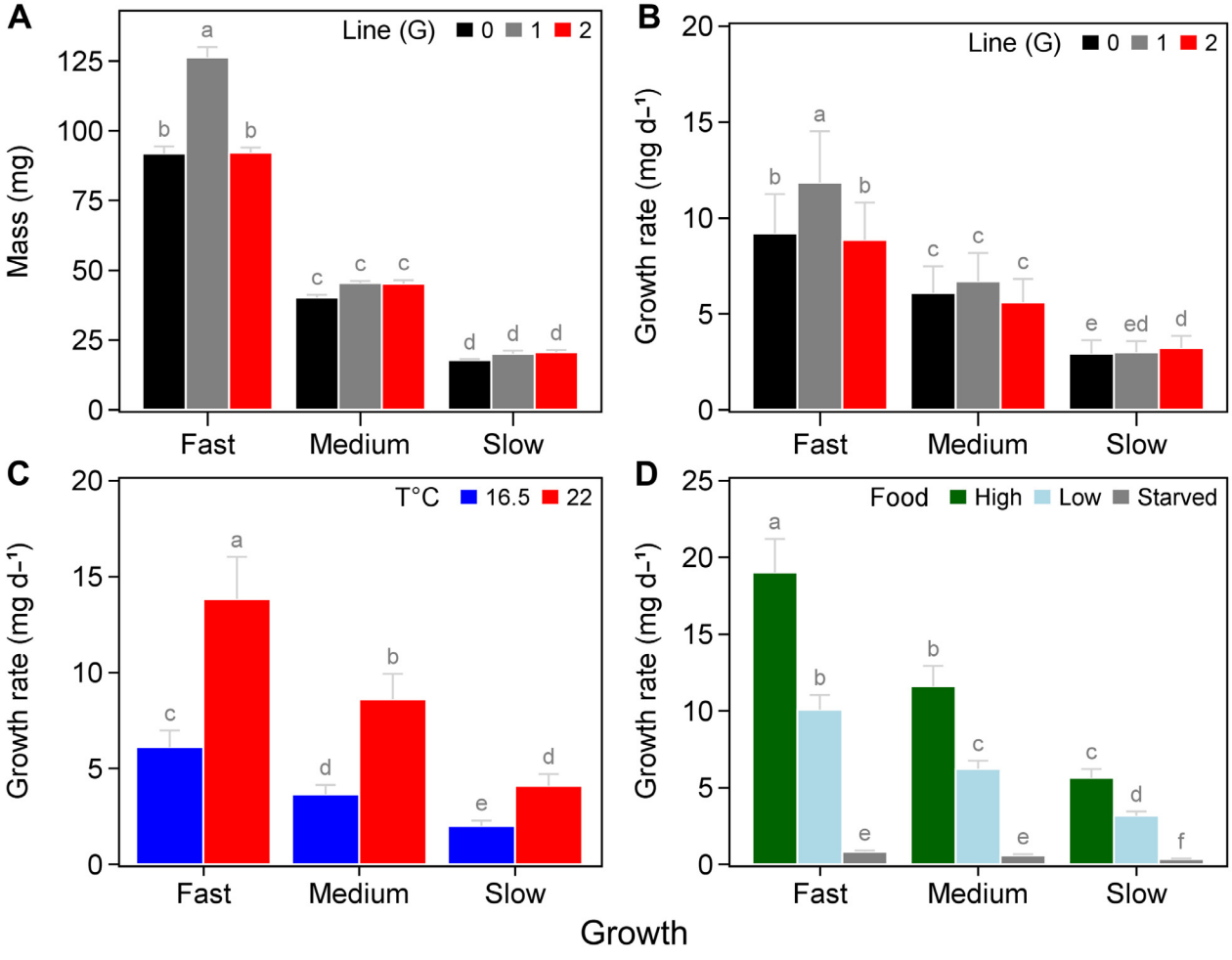

Fig. 2. Individual oyster (A) mass at the beginning of the acclimation period, (B) growth rate during the acclimation period and growth rates of oysters as a function of (C) temperature × line and (D) food × growth (D) for fast-, medium- and slow-growing oysters selected from each line (G0, G1, G2). Data are means ± SE (n = 3 tanks for each combination of food × temperature). Lowercase letters indicate significant differences. G: generation

virus into the muscle of donor oysters and distributing it to the recipient oysters placed in the 18 experimental tanks (Pernet et al. 2021). On 14 June 2018, the oysters for injection were myorelaxed in $MgCl_2$ at 21°C (Suquet et al. 2009). A total of ca. 600 specific-pathogen-free oysters (1.4 kg) produced in January 2018 were injected in the adductor muscle with 50 µl of viral suspension, containing 1.0×10^4 copies of OsHV-1 µVar μl^{-1} (Schikorski et al. 2011). The injected oysters were kept in a 45 l tank in static oxygenized seawater for 24 h where they shed viral particles. On 15 June, the outlet of this tank was connected to the seawater network by flexible tubes to distribute the virus-contaminated seawater for 7 d (Pernet et al. 2021). The survival of recipient oysters was monitored daily for at least 14 d. Oysters held at 16.5°C or starved were monitored for 5 more days in case mortality was delayed. Dead animals were removed daily. We did not use uninfected controls in our experiment, because their survival is always 100%, and they are generally not considered for analyses (Pernet et al. 2021).

2.3. Field trial

In order to evaluate the realism of the viral challenge conducted under laboratory conditions, we compared survival of oysters naturally exposed to OsHV-1 in the wild with those of laboratory challenged animals. On 1 July 2018, we transferred a subsample of the fast-growing oysters belonging to the G0 and G2 lines maintained under the high food condition into the Bay of Brest (48° 20′ 06″ N, 4° 19′ 06″ W), where disease-induced mortality was occurring among local oysters. Oysters were evenly distributed in 2 oyster bags per condition (N = 50 to 200 individuals per line, growth rate and bag). We monitored survival of oysters daily for the first 8 d and every 3–4 d thereafter until 15 d. We also recorded seawater temperature at the vicinity of the oyster bags every 15 min using an SP2T probe (NKE Instrumentation). Seawater temperature was on average 20.5°C ± 0.4 during the field challenge (S. Petton et al. 2022).

2.4. Sampling and analyses

We sampled 1 ml of seawater at 0, 20, 44, 65, 89, 113 and 161 h post infection (hpi) at the inlet and outlet of the tanks containing the oysters for OsHV-1 DNA qPCR analyses. Seawater was stored in sterile 1.5 ml Eppendorf tubes at −20°C. The detection and quantification of OsHV-1 DNA was carried out by a public laboratory (Labocea) following the methods of Martenot et al. (2010). The results are expressed as the number of OsHV-1 DNA copies μl^{-1}. Twenty pools of 5 dead oysters were analysed for the presence of OsHV-1 DNA in the tissues. They were all positive, with levels higher than 10^6 DNA copies mg^{-1}.

We also sampled 5 oysters per condition on 15 June (Day 0) just before pathogen exposure. The whole oysters, including the shell, were pooled, frozen in liquid nitrogen and stored at −80°C. Samples were then ground in liquid nitrogen with a mixer mill (MM400, Retsch). The resulting oyster powder was used for quantification of organic matter, neutral lipids and carbohydrates as described by Pernet et al. (2019). We selected 18 samples to investigate main effects (no interaction) of temperature (16.5 vs. 22°C, high food, G0, fast-growers), food regime (starved vs. low vs. high, 22°C, G0, fast-growers), line (G0 vs. G1 vs. G2, 22°C, high food, fast-growers) and growth (slow vs. medium vs. fast, 22°C, high food, G0).

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

Lipid reserves were assessed based on the triacylglycerol (TAG) to sterol (ST) ratio, an index of the relative contribution of reserve (TAG) to structural (cell membrane, ST) lipids (Pernet et al. 2019). Oyster powder (ca. 150 mg) was placed in 3 ml of chloroform–methanol (2:1, v/v), sonicated and spotted on silica plates that were then eluted with 2 solvent mixtures of increasing polarity made of hexane-diethylether acetic acid (Pernet et al. 2019). The plates were dipped in a $CuSO_4$–H_3PO_4 solution to reveal lipid spots and scanned at 370 nm (CAMAG) to quantify neutral lipid classes based on external standards.

Finally, carbohydrate content was determined spectrophotometrically at 490 nm according to DuBois et al. (1956). Oyster powder (ca. 200 mg) was placed in Eppendorf tubes containing 1.5 ml of nanopure water, homogenised with an ultra Turrax (IKA) and diluted 10 times. The diluted powder (250 µl) 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 before measuring absorbance. Total carbohydrate

concentration was then calculated using a standard calibration curve using glucose and expressed in mg g^{-1} organic matter.

2.5. Statistics

2.5.1. Survival analyses

Kaplan-Meier survival curves were computed for each combination of temperature × food level × line × growth rate. Survival time was measured as hpi or degree-hour (°C h), a metric that reflects the thermally dependent physiological time in ectothermic organisms.

2.5.2. Cox model

The survival of oysters was compared using a Cox regression considering the effect of temperature, food level, line, growth and their mutual interactions. The most important sources of variation were selected according to the Bayesian information criterion (BIC, where lower values are considered better). The proportionality of hazards (PH) was checked with martingale residuals. Because the PH assumption was violated, time-dependent covariates representing the interaction of the original covariates and log time were added to the model.

2.5.3. General linear models

Mixed-model ANOVAs (split-plot) were conducted on biomass and growth rate of oysters respectively at the onset and at the end of the acclimation period (Days 0 and 14). The tested effects were temperature and food level (main plot), and line and growth rate (subplot). Mixed-model ANOVAs were also conducted on food ingestion and OsHV-1 DNA concentration in the seawater. The tested effects were temperature and food level (main plot), and time and source (tank inlet vs. outlet for OsHV-1 only) (subplot). Tank was used as a random factor in all of these models. Because increasing temperature and food level increased the biomass of oysters at the end of the acclimation period (Table A1 in the Appendix) and could have influenced viral shedding (increased oyster biomass results in higher viral shedding), we compared model outputs with or without biomass as a covariate on viral concentration at the tank outlet (Table A2). Oyster biomass in the tank had no effect on viral concentration in the seawater, and the addition of this covariate reduced the quality of the model according to Akaike's information criterion (AIC). Therefore, oyster biomass was not further considered.

Finally, ANOVAs were used to determine differences in % organic matter, carbohydrate and TAG:ST ratio, according to temperature, food level, line and growth rate. Only main effects were investigated here. For models with statistical significance ($p < 0.05$), LSMEAN multiple comparison tests were used to determine differences among treatments. The normality of residuals and homogeneity of variance were graphically checked, and data were $\log(x + 1)$ transformed to meet the normality assumption where necessary. Statistics and graphing were all conducted using the SAS 9.4 software package (SAS Institute).

3. RESULTS

3.1. Production of oysters with contrasting growth-related metabolism

During the acclimation period, the growth rate of oysters ranked as fast > medium > slow growers (Fig. 2B,C, Table 1), increased with higher temperature and food level (Fig. 2C,D) and corresponded to higher ingestion of phytoplankton (Fig. 3). At the end of the acclimation period, food consumption of oysters ranked as: 22-high food > 16.5-high food = 22-low food > 16-low food > 22-starved = 16-starved.

3.2. Food is the most important risk factor, followed by temperature

At the end of the acclimation period, oysters with different growth metabolism were exposed to OsHV-1 to investigate their susceptibility to the disease. Final survival of oysters varied widely, ranging from 100% in starved oysters to as low as 16.4% for G0 oysters under high food level at 22°C (Fig. S2). Mortalities occurred later at 16.5°C than at 22°C (Fig. S2). Conversion of calendar time to degree–hours partly compensated for the effect of temperature on the onset of disease-induced mortality (Fig. S3).

We compared the survival curves of oysters exposed to OsHV-1 using the Cox regression model and found that the most important source of variation was food level, followed by temperature, oyster line,

Table 1. Summary of generalized linear mixed-effects model to assess the effects of temperature (Temp, 16.5 vs. 22°C), food level (starved, low and high), line (G0, G1 and G2) and growth (fast, medium, slow) on growth rate of Pacific oysters measured during the acclimation period. Data were log(x + 1) transformed. Significant p-values ($p < 0.05$) are in **bold**

Effects	df	F	p
Temp	1	570.0	**<0.001**
Food	2	3440.6	**<0.001**
Temp × Food	2	122.6	**<0.001**
Error a: Tank (Temp × Food)	12		
Line	2	20.4	**<0.001**
Temp × Line	2	0.1	0.909
Food × Line	4	3.5	**0.021**
Temp × Food × Line	4	2.8	**0.048**
Error b: Line × Tank (Temp × Food)	24		
Growth	2	498.0	**<0.001**
Temp × Growth	2	3.5	**0.037**
Food × Growth	4	48.6	**<0.001**
Temp × Food × Growth	4	2.2	0.083
Line × Growth	4	5.8	**<0.001**
Temp × Line × Growth	4	2.3	0.071
Food × Line × Growth	8	1.3	0.242
Temp × Food × Line × Growth	8	1.4	0.232
Error c: Growth × Line × Tank (Temp × Food) + Growth × Tank (Temp × Food)	72		

growth rate and their interactions (food × temperature and line × growth, Fig. S4). The Cox regression model was:

Survival (time, censoring) ~ food + temperature + line + growth + food × temperature + line × growth (1)

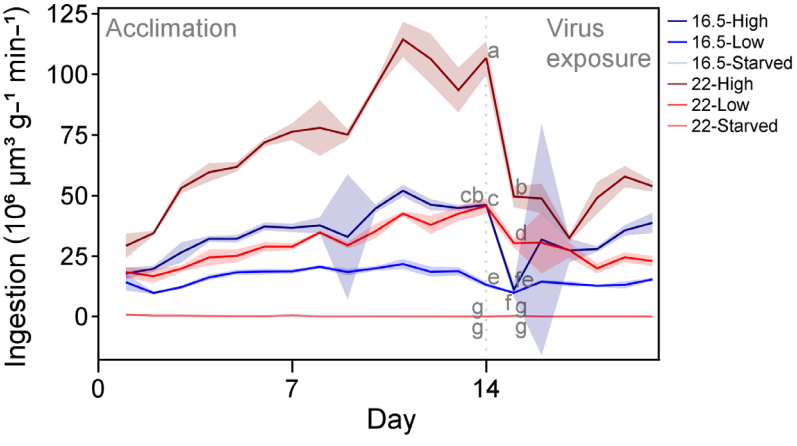

Fig. 3. Ingestion rate of phytoplankton for oysters as a function of temperature (16.5 and 22°C) and food level during the acclimation period (0–14 d) and virus exposure (14–20 d). Values are means ± 95% confidence intervals (n = 3 tanks). Lowercase letters indicate significant differences occurring at the onset of virus exposure. Data from oysters kept under starvation at both temperatures are indistinguishable and close to zero

We included time-dependent covariates for food level and temperature because the risk functions were not proportional for these factors (Table 2).

3.3. Starvation and lower temperature decrease mortality risk

Starved oysters had a much higher survival than fed ones, regardless of temperature (Fig. 4A). At 16.5°C, survival probability at low food level was lower than at high food level. The ratio in the risk functions (low vs. high food) increased until about 200°C h, and then decreased thereafter until 5542°C h, where the risk functions become equal. At 22°C, the survival probability was higher at low than at high food levels. However, the ratio in the risk functions became gradually equal up until 5638°C h. All else being equal, the probability of survival at 16.5°C was much higher than that observed at 22°C.

3.4. Higher growth rate increases mortality risk in susceptible populations

In G0 oysters, higher growth rate was clearly associated with decreased survival and ranked as fast < medium < slow growers (Fig. 4B). In G1 and G2 oysters, survival probability was markedly increased compared to G0, and the effect of growth rate was attenuated. All else being equal, survival probability of oysters increased with increasing the number of parental generations exposed to OsHV-1 (Fig. 4B). The greatest survival gain was acquired between G0 and G1.

3.5. Early exposure to the virus coincides with a decrease in food intake in the host

We monitored the concentration of virus in seawater (estimated by the number of OsHV-1 DNA copies μl^{-1}) at the inlet and at the outlet of oyster tanks. All tanks were exposed to the same concentration of virus in seawater (ANOVA, tank effect, $F_{17,108} = 0.45$, $p = 0.967$). At the tank inlet, virus concentration increased between 0 and 20 hpi from undetected to 46 DNA

Table 2. Time-varying Cox regression model. The tested effects were selected using the Bayesian information criterion (BIC). The retained model includes temperature, food level, line and growth rate. Reference levels were 22°C, high food, G0, fast growth. Significant p-values ($p < 0.05$) are in **bold**

Effect	Level		df	Estimate	SE	χ^2	p	Odds ratio
Temperature	16.5		1	−9.376	1.195	61.5	**<0.001**	0.000
Food	Starved		1	−230.066	18.771	150.2	**<0.001**	0.000
	Low		1	−1.554	0.433	12.9	**<0.001**	0.211
Temp × Food	16.5	Starved	1	−2146.527	340.349	39.8	**<0.001**	0.000
	16.5	Low	1	5.310	1.257	17.9	**<0.001**	202.424
Line	G1		1	−1.684	0.125	182.2	**<0.001**	0.186
	G2		1	−1.967	0.129	233.8	**<0.001**	0.140
Growth	Medium		1	−0.241	0.038	40.5	**<0.001**	0.786
	Slow		1	−0.754	0.084	80.1	**<0.001**	0.470
Line × Growth	G1	Medium	1	0.417	0.148	7.9	**0.005**	1.517
	G1	Slow	1	0.032	0.389	0.0	0.934	1.033
	G2	Medium	1	−0.306	0.222	1.9	0.167	0.736
	G2	Slow	1	0.488	0.209	5.5	**0.019**	1.629
Temp × log(time)	16.5		1	0.001	0.000	42.9	**<0.001**	1.001
Food × log(time)	Starved		1	0.027	0.002	141.7	**<0.001**	1.028
	Low		1	0.000	0.000	10.3	**0.001**	1.000
Temp × Food × log(time)	16.5	Starved	1	0.275	0.044	39.9	**<0.001**	1.317
	16.5	Low	1	−0.001	0.000	16.8	**<0.001**	0.999

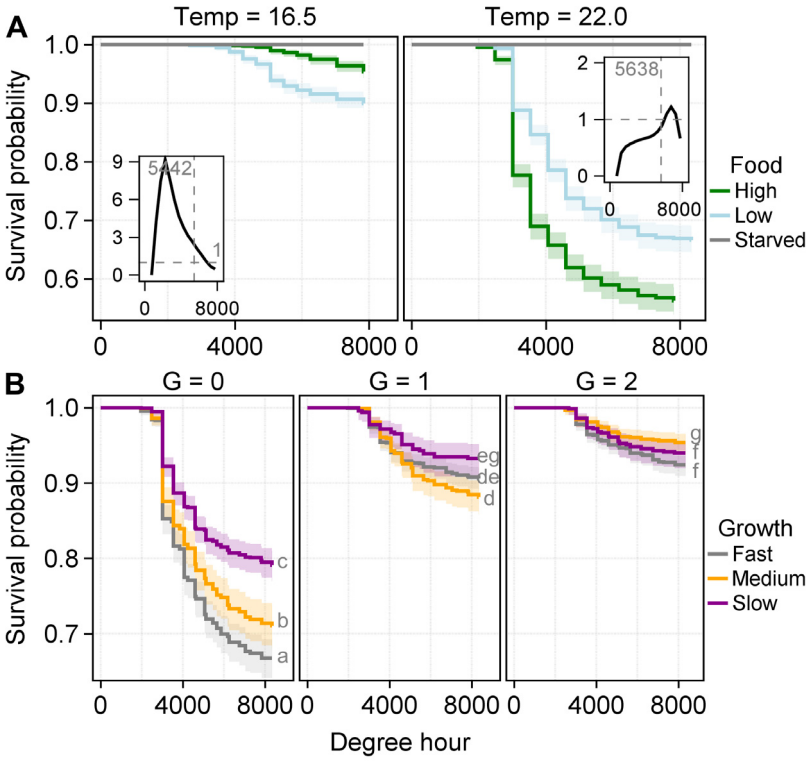

Fig. 4. Survival probability of oysters exposed to OsHV-1 for the effects used in the Cox regression model: (A) temperature × food and (B) line × growth. Insets show odds ratios of low vs. high food level as a function of time for each temperature. Vertical reference line indicates the time when the risks between food levels are equal. Values are means ± 95% confidence intervals. Lowercase letters indicate significant differences. G: generation

copies μl^{-1} (±0.3), and decreased gradually (Fig. 5). We concomitantly found that food consumption decreased markedly at 24 hpi, ranging from 76 to 54% and 34 to 25% for oysters exposed to high and low food levels, respectively (Fig. 3).

3.6. Virus production increases with food and to a lesser extent with temperature

We investigated the effect of temperature and food level on virus shedding (Fig. 5) and found that temperature, food level, time and source (tank inlet vs. outlet) interacted in their effects on OsHV-1 DNA concentration in seawater (Table 3). Because it is difficult to interpret a 4-way interaction, we focus on the significant 3-way interactions.

Among these interactions, the most significant was food × source × time (Fig. 5, Table 3). Under high food condition, the viral concentration increased between 20 and 89 hpi, and gradually decreased thereafter while

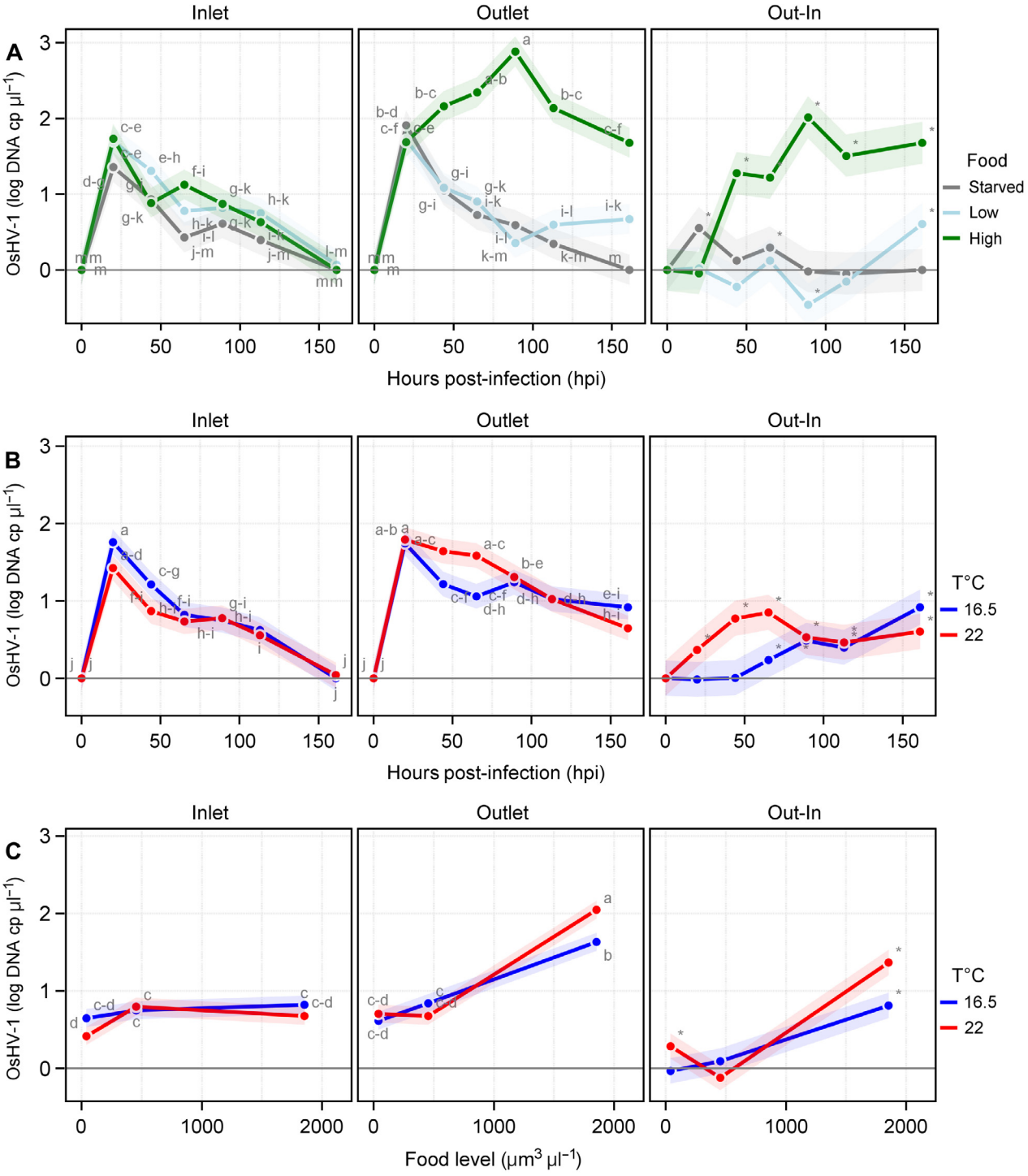

Fig. 5. Levels of OsHV-1 DNA in seawater. Data are means ± 95% confidence intervals (n = 6 tanks for food or temperature effect and n = 3 tanks for temperature × food). Interactions of (A) food and time, (B) temperature and time and (C) temperature and food. Data were measured at the inlet (left column) and outlet (centre column) of the tank. Lowercase letters indicate significant differences. Pairwise comparisons between the outlet and the inlet are represented in the right column (asterisks indicate statistical significance at α = 0.05). Food levels reported on the *x*-axis corresponded to starved, low and high levels

remaining high (Fig. 5A). Under low food condition and starvation, the viral concentration decreased from 20 hpi onwards. We statistically compared the viral concentration between outlet and inlet for

Table 3. Summary of generalized linear mixed-effects model to assess the effects of temperature (Temp, 16.5 vs. 22°C), food level (starved, low and high), source (tank inlet vs. outlet) and time on levels of OsHV-1 DNA concentration in seawater. Data were log(x + 1) transformed. Significant p-values ($p < 0.05$) are in **bold**

Effects	df	*F*	p
Temp	1	0.01	0.942
Food	2	187.86	**<0.001**
Temp × Food	2	4.73	**0.031**
Error a: tank (Temp × Food)	12		
Source	1	171.65	**<0.001**
Temp × Source	1	13.13	**0.003**
Food × Source	2	127.67	**<0.001**
Temp × Food × Source	2	13.66	**0.001**
Error b: Source × Tank (Temp × Food)	12		
Time	6	185.03	**<0.001**
Temp × Time	6	2.21	**0.045**
Food × Time	12	17.16	**<0.001**
Temp × Food × Time	12	1.27	0.245
Source × Time	6	9.48	**<0.001**
Temp × Source × Time	6	5.52	**<0.001**
Food × Source × Time	12	15.71	**<0.001**
Temp × Food × Source × Time	12	2.17	**0.016**
Error c: time × Source × Tank (Temp × Food) + Source × Tank (Temp × Food)	143		

each time level and food condition. In the high food condition, the viral concentration was statistically higher at the outlet than at the inlet of tanks from 44 hpi onwards (1.3 to 2.0 log higher). In the low food condition, this occurred only once at the end of the experiment. In the absence of food, there was no significant difference between the viral concentrations measured at the outlet and inlet of the tanks (excepted at the early beginning of the virus exposure), suggesting that there was no significant shedding of viral particles.

We also found that increasing temperature marginally increased viral concentrations in seawater punctually at 65 hpi at the outlet of the oyster tanks (Fig. 5B, Table 3, temperature × source × time effect). At 22°C, the viral concentration at the tank outlet became higher than at the tank inlet at 20 hpi. At 16.5°C, this occurred later at 65 hpi. Thus, the tested temperatures slightly influenced viral excretion by oysters.

Finally, we found that temperature, food and source interacted in their effects on virus concentration (Table 3, Fig. 5C). Overall, virus concentration increased with food level, but this effect was exacerbated by increasing temperature.

3.7. Food is the main driver of energy reserves

We analyzed the main effects of temperature, food level, oyster line and growth rate on the energy reserves of oysters though quantification of organic matter, TAG:ST ratio and carbohydrates. Energy reserves can vary according to the different treatments and influence the survival of oysters exposed to OsHV-1. We found that increasing food level from starvation to full ration increased organic matter and the TAG:ST ratio in oysters by ~2 and ~10 times, respectively, but not carbohydrates (Fig. 6, Table 4). In addition, we found that oysters at 22°C had a 26% higher organic matter content compared to those at 16.5°C, and fast-growing oysters had a 42% higher TAG:ST ratio than the others. Finally, the 3 lines of oysters differed in their carbohydrate content, which was 4.7 and 1.8 times higher in G1 than in G0 and G2, respectively.

3.8. Survival of oysters exposed to the virus is similar between the lab and the wild

In order to evaluate the realism of the viral challenge conducted under laboratory conditions, we compared survival curves of oysters naturally exposed to OsHV-1 in the wild with those of laboratory challenged animals (Fig. 7). Despite a slightly higher risk of mortality in the wild than in the laboratory for the fast-growing G0 and the slow-growing G2 oysters, the survival dynamics were very close or even indistinguishable under the other conditions (Fig. 7). These results confirm that the disease that developed in our experimental set-up resembles the disease contracted in the natural environment.

4. DISCUSSION

In this study, we deciphered the complex interactions between food availability, temperature, genetics and growth rate of the host on disease susceptibility, focussing on a polymicrobial disease caused primarily by a virus affecting Pacific oysters. We found that increasing the host growth rate by using elevated temperature, providing more food or selecting fast-growing animals facilitates viral replication and disease development, especially in host populations where susceptible phenotypes dominate. These effects are indeed attenuated in resistant host populations. Risk analysis shows that food level is the most influential factor on disease severity, followed by

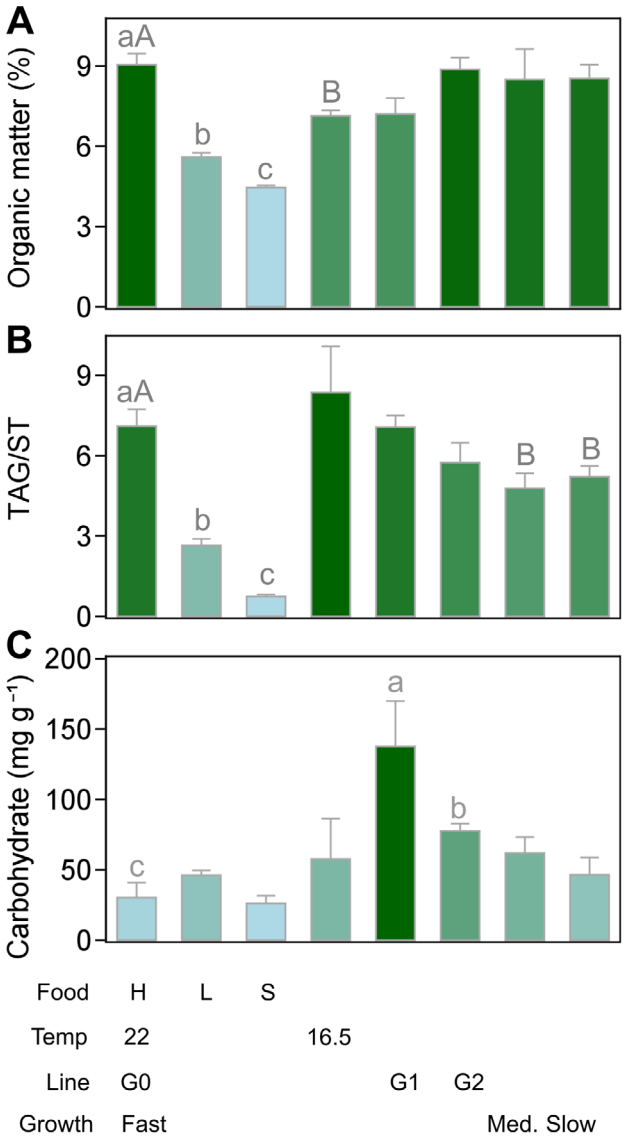

Fig. 6. Organic matter (% of total body mass), lipid reserves (triacylglycerol:sterol ratio, TAG:ST) and carbohydrate content (mg per g of organic matter) measured at the end of the acclimation period, before the exposure to the virus. Analyses were conducted on a subset of the samples to investigate main effects only (no interaction). Fast-growing G0 oysters held at 22°C and fed *ad libitum* (high food level, H) were used as a reference and compared to their counterparts exposed to low food (L) or starved (S), held at 16.5°C, from G1 and G2 and with medium and slow growth rate, everything else being equal. Lowercase letters indicate significant differences, and capitalization distinguishes effects when 2 factors were significant. G: generation

temperature, resistance phenotype and growth rate. It is striking that starvation offers total protection against the disease.

We found that increased food intake promoted host growth and viral shedding. In contrast, starvation or food restriction slowed the growth-related metabolism of the host on which the pathogen depends to proliferate. We also found that oysters reduced food intake at the onset of the viral challenge. Loss of appetite is common in animals with infectious diseases and is considered a conserved adaptive strategy to increase survival (Murray & Murray 1979, Kyriazakis et al. 1998). However, the relationship between diet restriction and immunity is complicated and varies with pathogen (Ayres & Schneider 2009, Wang et al. 2016). For example, in fruit flies, anorexia is beneficial for the host in some infections but not all (Ayres & Schneider 2009). In mice, fasting metabolism is protective in bacterial, but not viral, inflammation (Wang et al. 2016).

We also found that increasing temperature leads to increased host growth and viral shedding. However, compared to increased food supply, higher temperature greatly enhances the risk of mortality but only moderately increases viral shedding. Although both food and temperature increase growth rate and disease susceptibility, they most likely act on distinct metabolic pathways, which induce different effects on disease expression.

One possibility is that increased temperature may influence the proliferation or the virulence of opportunistic bacterial pathogens responsible for the secondary infection occurring during the disease (de Lorgeril et al. 2018, Petton et al. 2021). In support of this hypothesis, we know that (1) temperature shapes the oyster microbiota and influences oyster health (Lokmer & Wegner 2015), and (2) temperature exerts a strong influence on bacterial communities in marine waters and, in some cases, increases the abundance and virulence of bacterial pathogens (Vega Thurber et al. 2009, Vezzulli et al. 2010). For example, *Vibrio* concentrations in Mediterranean waters are correlated with sea surface temperature, with few vibrios below 18°C and a sharp increase in abundance above 22°C (Vezzulli et al. 2010). This example is particularly interesting here because vibrios are considered part of the oyster pathobiota during OsHV-1 infection (Bruto et al. 2017, de Lorgeril et al. 2018, Lasa et al. 2019).

Another non-exclusive explanation could be that starvation or food restriction induces autophagy, a cellular degradation pathway by which cytoplasmic cellular constituents are directed to the lysosome (Bagherniya et al. 2018). Autophagy can be an immune mechanism reducing intracellular pathogen load (Desai et al. 2015). In oysters, the autophagy pathway is activated during starvation and plays a protective role in oysters against OsHV-1 (Moreau et

Table 4. Summary of ANOVAs. The tested effects were temperature (16.5 vs. 22°C), food level (starved, low and high), line (G0, G1 and G2) and growth rate (fast, medium, slow). Data of triacylglycerol:sterol ratios (TAG:ST) were log(x + 1) transformed. Significant p-values ($p < 0.05$) are in **bold**

Variable	Effect	df	*F*	p
Organic matter	Temperature	1,4	17.04	**0.015**
	Food	2,6	84.94	**<0.001**
	Line	2,6	4.27	0.070
	Growth	2,6	0.16	0.852
TAG:ST	Temperature	1,4	0.47	0.532
	Food	2,6	69.46	**<0.001**
	Line	2,6	1.61	0.276
	Growth	2,6	5.21	**0.049**
Carbohydrate	Temperature	1,4	0.80	0.420
	Food	2,6	2.13	0.200
	Line	2,6	7.40	**0.024**
	Growth	2,6	1.86	0.236

al. 2015). Those last authors found that oysters maintained without food for 4 wk to stimulate autophagy survived OsHV-1 infection better than fed animals. When the autophagy pathway was artificially inhibited by a chemical, the survival of starved oysters during OsHV-1 infection was lower, suggesting that the benefit of starvation depends on autophagy (Moreau et al. 2015).

Concomitantly, starvation decreases host reserves and thus may limit the resources available to the pathogen. Like their hosts, pathogenic organisms require resources to proliferate. Resource limitation may constrain pathogen proliferation (Smith et al. 2005, Civitello et al. 2018) as reported in food-restricted zooplankton exposed to fungal parasites (Hall et al. 2009). As a corollary, decreasing food consumption in response to infection is considered an adaptive response by the host to limit the resources available to the parasite (Murray & Murray 1979, Ayres & Schneider 2009). However, we do not have evidence here that the physiological condition of the host increases disease susceptibility by providing additional resources to the virus. In fact, the physiological condition of oysters is generally associated with better survival of OsHV-1 infection (Pernet et al. 2019 and references therein), likely reflecting a trade-off between immunity and other energy-demanding functions (Sheldon & Verhulst 1996, Lochmiller & Deerenberg 2000). The outcome of the disease, i.e. survival or death of the host, may depend on whether the host or the pathogen makes the best use of energy reserves.

We observed that survival of oysters exposed to OsHV-1 increases markedly with the number of mass selection rounds performed in the field where the disease naturally occurs. This agrees with the fact that resistance to OsHV-1 is a highly heritable trait (Dégremont et al. 2010). Nevertheless, differences in resistance between oyster lines were not associated with differences in growth rate or physiological condition. This is consistent with the idea that this trait is not genetically correlated with growth or associated with differences in metabolic rates (Dégremont et al.

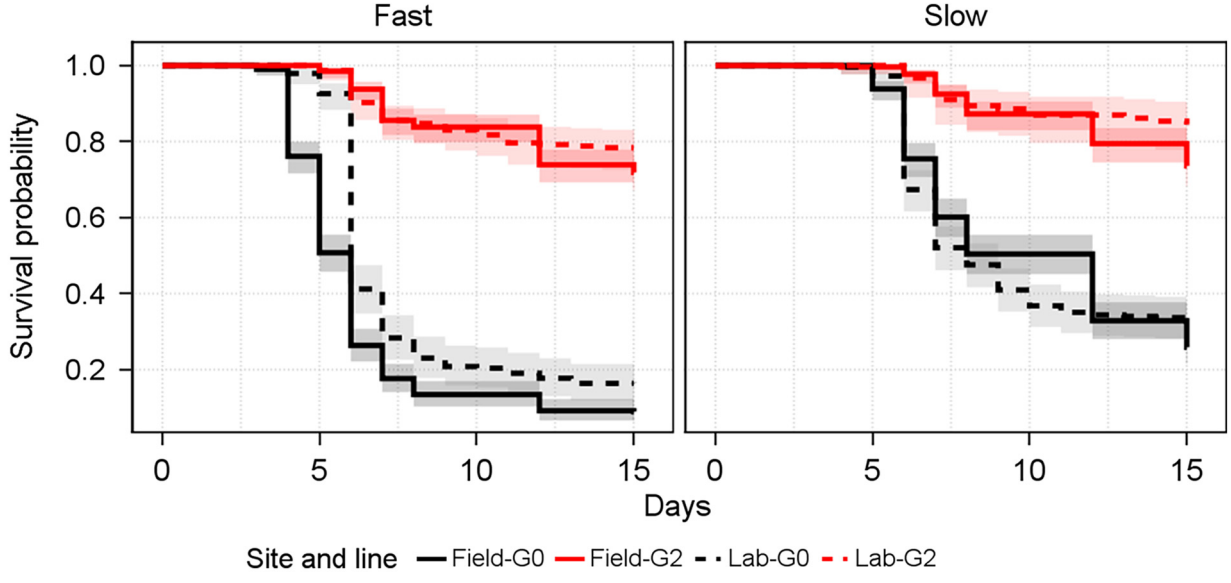

Fig. 7. Survival probability of oysters exposed to OsHV-1 under field or laboratory conditions as a function of growth rate (fast and slow) and line (G0 and G2). Values are means ± 95% confidence intervals (n = 2–3 tanks or oyster bags). G: generation

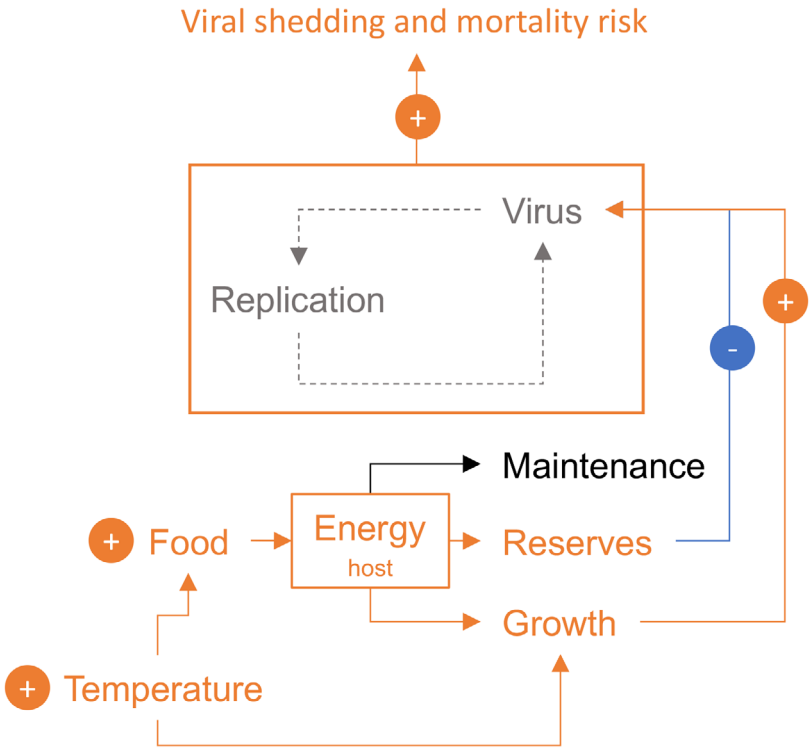

Fig. 8. Food availability acts both as an enabling factor for mortality by increasing oyster growth and virus replication, likely providing materials for virus construction, but also as a limiting factor by increasing energy reserves of the host. The net effect of food availability is an increased risk of mortality of oysters exposed to the virus. Temperature increases both growth rate and food ingestion, and thus favours viral replication and shedding in seawater and mortality risk in the host population. Orange, blue and black lines indicate positive, negative and neutral feedback, respectively, dashed grey lines indicate virus replication in the host

2010, Dégremont 2011, Haure et al. 2021). It is likely that selection for the resistant phenotype operates more on immune-related traits rather than on growth, energetics or overall metabolism. Indeed, global transcriptomic analysis of resistant and susceptible oyster families revealed a more rapid antiviral response and increased basal expression of immune genes in resistant phenotypes compared to susceptible ones (de Lorgeril et al. 2018, 2020).

Future studies need to identify the metabolic pathways that are modulated when manipulating temperature or food or when selecting fast-growing animals and which explain the differences in survival against pathogens. The metabolic pathways that are altered when manipulating food, temperature or selecting for growth are different, which would explain why they cause different effects on viral replication and host survival.

In conclusion, our results emphasise the importance of considering growth-related metabolism of the host in the context of viral infections. Faster-growing oysters die sooner than slower-growing oysters upon virus exposure: factors contributing to increased growth rates, such as temperature, food availability or selection of fast-growing individuals, increase virus production and mortality in the host population (Fig. 8); however, the effect of food remains ambivalent. Not only does the food supply support growth, but it can also contribute to the needs of the pathogen while allowing the host to acquire the energy needed to fight infection. Moreover, the risk of mortality of oysters selected for the resistance phenotype was lower, as expected, and less influenced by host growth. The physiological basis for selection of the resistant phenotype is likely not related to growth, but rather relies on selection for increased antiviral activity.

Our study provides new perspectives for managing marine diseases based on the manipulation of host metabolism. Considering that rapid host growth increases virus production, it is better to optimise growth rather than maximise it. In marine bivalves like oysters, this can be easily achieved through feed limitation by raising the animals higher on the foreshore or by favouring co-culture with trophic competitors not susceptible to the pathogen. These approaches might be especially useful in infections that cannot be controlled by vaccines or drugs. Currently, the majority of studies using metabolism to fight disease focus on autoimmunity or cancer; however, a focus on viral infections could be a fruitful field of investigation (Sumbria et al. 2021).

Data accessibility. Data are deposited at the SEANOE Digital Repository: https://www.seanoe.org/data/00808/92042/ (B. Petton et al. 2022).

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Appendix. Supplementary statistics

Table A1. Biomass of oysters in the tanks at the end of the acclimation period. Data are means ± SD (n = 3 tanks)

Temp (°C)	Food	Mean ± SD (g)
16.5	High	104.9 ± 0.7
	Low	81.5 ± 1.6
	Starved	44.5 ± 0.2
22	High	202.5 ± 4.7
	Low	124.4 ± 2.3
	Starved	45.0 ± 0.6

Table A2. Generalized linear mixed-effects model selection used to assess the effects of temperature (Temp, 16.5°C vs. 22°C), food level (starved, low and high), and time on levels of OsHV-1 DNA in seawater at the tank outlet. Data were log (x + 1) transformed

	Model without covariate			Model with covariate		
Effects	df	*F*	p	df	*F*	p
Biomass	0			1	0.0	0.949
Temp	1	5.3	0.040	1	0.1	0.761
Food	2	235.6	<0.001	2	25.9	<0.001
Temp × Food	2	11.5	0.002	2	5.3	0.025
Error a: tank (Temp × Food)	12			11		
Time	6	85.9	<0.001	6	85.3	<0.001
Temp × Time	6	4.7	0.001	6	4.6	0.001
Food × Time	12	24.5	<0.001	12	24.0	<0.001
Temp × Food × Time	12	2.3	0.017	12	2.2	0.019
Error b: Time × Tank (Temp ×Food)	72			72		
Model AIC	64.7			71.5		

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