



HAL
open science

Temperature modulate disease susceptibility of the Pacific oyster *Crassostrea gigas* and virulence of the Ostreid herpesvirus type 1

Lizenn Delisle, Bruno Petton, Jean François Burguin, Benjamin Morga, Charlotte Corporeau, Fabrice Pernet

► To cite this version:

Lizenn Delisle, Bruno Petton, Jean François Burguin, Benjamin Morga, Charlotte Corporeau, et al.. Temperature modulate disease susceptibility of the Pacific oyster *Crassostrea gigas* and virulence of the Ostreid herpesvirus type 1. *Fish and Shellfish Immunology*, 2018, 80, pp.71-79. 10.1016/j.fsi.2018.05.056 . hal-02619276

HAL Id: hal-02619276

<https://hal.science/hal-02619276>

Submitted on 25 May 2020

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

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

Temperature modulate disease susceptibility of the Pacific oyster *Crassostrea gigas* and virulence of the Ostreid herpesvirus type 1

Delisle Lizenn¹, Petton Bruno², Burguin Jean Francois¹, Morga Benjamin³, Corporeau Charlotte¹, Pernet Fabrice^{1,*}

¹ Ifremer, LEMAR UMR 6539, Technopole de Brest-Iroise, 29280, Plouzané, France

² Ifremer, LEMAR UMR 6539, Presqu'île du vivier, 29840, Argenton, France

³ Ifremer, Laboratoire de génétique et Pathologie des Mollusques Marins (LGPM), Avenue de Mus de Loup, 17390, La Tremblade, France

* Corresponding author : Lizenn Delisle, email address : fabrice.pernet@ifremer.fr

Abstract :

Temperature triggers marine diseases by changing host susceptibility and pathogen virulence. Oyster mortalities associated with the Ostreid herpesvirus type 1 (OsHV-1) have occurred seasonally in Europe when the seawater temperature range reaches 16–24 °C. Here we assess how temperature modulates oyster susceptibility to OsHV-1 and pathogen virulence. Oysters were injected with OsHV-1 suspension incubated at 21 °C, 26 °C and 29 °C and were placed in cohabitation with healthy oysters (recipients) at these three temperatures according to a fractional factorial design. Survival was followed for 14 d and recipients were sampled for OsHV-1 DNA quantification and viral gene expression. The oysters were all subsequently placed at 21 °C to evaluate the potential for virus reactivation, before being transferred to oyster farms to evaluate their long-term susceptibility to the disease. Survival of recipients at 29 °C (86%) was higher than at 21 °C (52%) and 26 °C (43%). High temperature (29 °C) decreased the susceptibility of oysters to OsHV-1 without altering virus infectivity and virulence. At 26 °C, the virulence of OsHV-1 was enhanced. Differences in survival persisted when the recipients were all placed at 21 °C, suggesting that OsHV-1 did not reactivate. Additional oyster mortality followed the field transfer, but the overall survival of oysters infected at 29 °C remained higher.

Highlights

► High temperature (29 °C) decreases the susceptibility of oysters to the virus. ► There was no alteration of its infectivity and virulence. ► At 26 °C, the pathogen virulence was enhanced. ► Disease mitigation measures based on temperature are discussed.

Keywords : Bivalve, Health, Marine disease, Mortality risk, Temperature, Virulence

27 Introduction

28 The risk of disease outbreaks in the marine environment are governed by interactions between
29 host, parasite, and environmental factors [1]. Of all the environmental factors, seawater
30 temperature plays a decisive role in triggering diseases [2]. Temperature modulates the aptitude
31 of a parasite to colonize its host and the ability of the host to defend itself. For example, mass
32 mortalities of shrimps and sea-stars caused by pathogenic viruses occurred during the warm
33 season [3–5]. Similarly, when abalones were exposed to the pathogenic bacteria *Vibrio harveyi*,
34 an increase of seawater temperature of one degree during the spawning season altered the host–
35 parasite relation in favor of the pathogen and lead to epidemic disease [6].

36 In warm-blooded species, fever, which consists of an increase in body temperature, makes it
37 possible to fight against viral and bacterial infections. Although marine animals are mostly
38 ectotherms (cold blooded), the application of high temperature treatments has proven efficient to
39 treat diseases. For instance, shrimps infected with the white spot syndrome virus at 32°C showed
40 no mortality, whilst they all died at 25°C [3,7].

41 There is a range of possible influences of high temperature effects on the infectivity of parasites
42 (replication, virulence) and host susceptibility. For instance, high temperature improves shrimp
43 resistance without altering the infectivity of the white spot syndrome virus [3,8]. However, the
44 stability of some shellfish pathogens is altered at high temperature, as reported for the abalone
45 herpesvirus [9].

46 This study focuses on the effect of high temperatures on the interaction between the Pacific
47 oyster *Crassostrea gigas* and the Ostreid herpesvirus type 1 (OsHV-1). Indeed, OsHV-1
48 outbreaks have caused mass mortalities in young oysters along the European coastline since 2008

49 and the virus has spread to Australia and New Zealand [10–13]. Seawater temperature defines the
50 start and the end of OsHV-1 epizootics. In France, the optimal seawater temperature range for
51 disease transmission and subsequent mortalities is between 16°C and 24°C in the field [14,15]. In
52 the laboratory, oyster survival of individuals acclimated to temperatures between 13°C and 29°C
53 exposed to OsHV-1 were much higher when temperatures were exceeded 26°C [16]. An
54 Australian study comparing survival of oysters injected with OsHV-1 at 14°C, 18°C, 22°C and
55 26°C shows that it was the lowest at 26°C [17]. Furthermore, the infectivity of OsHV-1 kept for
56 54 h at 25°C is lower than at 16°C [18].

57 Although the temperature clearly influences the risk of oyster mortality caused by OsHV-1, it is
58 not known at this time whether the temperature affects the host, by modifying its susceptibility to
59 the virus, the pathogen, by acting on virulence, or both. Also, we do not know if oysters that have
60 survived a high temperature infection are able to transmit the virus to healthy animals and if they
61 remain protected against a second infection. The answer to these questions is crucial to develop
62 OsHV-1 control measures from heat treatment.

63 Here we tested the effect of three temperatures on disease susceptibility of oysters and virulence
64 of OsHV-1. The control was 21°C as this temperature is permissive to OsHV-1 replication and
65 close to the optimal temperature for oyster growth and reproduction [19]. The tested temperatures
66 were 26°C, a temperature whose effects on the survival of the oyster remains to be clarified, and
67 29°C, a temperature at which the survival of oysters exposed to the virus is improved [16,17].
68 These two temperatures are in the thermal range of the oyster since the filtration stops only at
69 33°C and mortality occurs at 38°C [19] (*Petton com pers*).

70 The first experiment was designed to investigate the effects of high temperatures on OsHV-1
71 transmission, infection and mortality of oysters. Specific-pathogen-free (SPF) oysters were

72 injected with OsHV-1 suspension (pathogen donors) or synthetic seawater (controls) and placed
73 in cohabitation with SPF oysters hereafter called “pathogen recipients” or “control recipients”
74 respectively (Fig. 1). These recipients were previously acclimated or directly dipped (not-
75 acclimated) at 21°C, 26°C or 29°C. Their survival was followed for 14 d and the infectivity of
76 OsHV-1 was evaluated (experiment 1A). Next, the recipients were all placed at 21°C, and new
77 SPF oysters were added to the tanks in order to evaluate the potential for virus reactivation and
78 transmission (experiment 1B). Finally, recipients were transferred to a farming area where
79 OsHV-1 induced mortalities were occurring to evaluate the long-term susceptibility to the disease
80 (experiment 1C). Concomitantly, a second experiment (experiment 2) evaluated the effect of
81 temperature on the OsHV-1 suspension *per se*. The viral suspension and synthetic seawater
82 (control) were incubated at 21°C, 26°C and 29°C prior injection in oysters at 21°C. Survival of
83 recipients cohabited with injected oysters was followed for 14 d.

84 **Experimental procedures**

85 *Animals and maintenance*

86 Two cohorts of specific-pathogen-free (SPF) oysters were produced under controlled conditions
87 [20,21]. Briefly, wild oysters were collected in Fouras (Marennes-Oleron, France; 46° 00' 43.2'
88 'N, 1° 07' 02.9'' W) in August 2015 and were transferred to the Ifremer facilities in Argenton
89 (Brittany, France; 48°34'30''N, 4°36'18'' W) for conditioning. These animals were held in 500 L
90 flow through tanks with seawater kept at a constant temperature of 17°C that was enriched with a
91 phytoplankton mixture. Seawater was UV treated and filtered through 1 µm mesh. Fertilization
92 was performed by stripping the gonads from 100 individuals (1/3 males, 2/3 females) on 23
93 August 2015 (cohort 1) and 23 February 2016 (cohort 2). The fertilization rate was up to 90%.

94 The embryos developed in 150l tanks at 21°C for 48h, and D-larvae were transferred to flow-
95 through rearing systems at 25°C. After 13 days, competent larvae were collected and allowed to
96 settle in downwellers. On 1st October 2015 and 1st April 2016, oysters were transferred to
97 Ifremer facilities in Bouin for nursery where seawater was UV treated and filtered through 1
98 μm mesh (Vendée, France, 46°57'15.5"N 2°02'40.9"W).

99 The two cohorts of SPF oysters were moved back to Argenton on 28 April 2016 and split into
100 five 500 L tanks at 21°C prior to starting the experiments in open flow systems. At this time,
101 oysters from the first cohort were 8 months old, with a mean weight of 1.48 g. Oysters from the
102 second cohort were 2 ½ months old with a mean weight of 0.80 g. The oysters were screened for
103 the herpesvirus by qPCR at all the different production steps it was always undetected [22]. They
104 were fed with a mixture of *Chaetoceros muelleri* (CCAP 1010/3) and *Tisochrysis lutea* (CCAP
105 927/14) (1:1 in dry weight). Food concentration was set at $1500\mu\text{m}^3\cdot\mu\text{l}^{-1}$ of microalgae at the
106 outlet pipe of the tank so that oysters were fed *ad libitum* [23]. Temperature, salinity, pH and
107 oxygen were controlled daily with the WTW probes xi3101, cond340, pH3310 and FDO 925,
108 respectively.

109 Oysters from the first cohort were either injected with a suspension of OsHV-1 (pathogen donors)
110 or synthetic seawater (controls), or they were not injected and used as recipients in both
111 experiments 1 and 2. Oysters from the second cohort served as new recipients in experiment 1B.

112 *Experimental design*

113 Acclimation of oysters

114 On 9 May 2016, oysters for injection (N=3000 individuals) were either left at 21°C (control
115 temperature), or gradually increased to 26°C and 29°C at 2°C day^{-1} in three 500 L tanks (one for

116 each temperature, Fig. S1). At the same time, some of the recipient oysters (N=3600 individuals)
117 were transferred to the experimental room, distributed into twenty-five 45 L tanks and either left
118 at 21°C or gradually increased to 26°C or 29°C (15 tanks were set at 21°C, 5 tanks at 26°C and 5
119 tanks at 29°C, see Table 1). The remaining recipients were left undisturbed in 500 L tank at 21°C
120 and later served as non-acclimated recipients. Acclimation lasted for 11 days up until 19 May.

121 Each tank was equipped with a thermostat and heating resistor (Biotherm Ecco Hobby and
122 SCHEIGO Titane 300W, Europrix, France) to maintain the seawater at the desired temperature
123 (Fig. S2). Three tanks (one for each temperature) were equipped with a high-frequency
124 temperature data logger (iButton DS1922L) with 0.1°C resolution and a final accuracy of \pm
125 0.3°C. A light bubbling and a circulation pump (Aquapower 200 superfish 200L/H, Europrix,
126 France) were added in each 45 L tanks to maintain >90% oxygen saturation and seawater
127 homogenization. Seawater salinity was 35‰. The oyster filtration rate was measured daily in
128 each tank as reported in [23] (Fig. S3). The water flow was set at 70 L h⁻¹ in the 500 L tanks and
129 12 L h⁻¹ in the 45 L tank. Throughout this time, there was no oyster mortality.

130 *Infection*

131 On 19 May 2016, oysters for injection were myorelaxed in MgCl₂ solution at their respective
132 acclimation temperature until valve opening [24]. Pathogen donors were injected with 100µl of
133 viral suspension containing 6.9×10^6 copies of OsHV-1 µVar in the adductor muscle, while
134 controls were injected with the same volume of sterile synthetic seawater. Donors and controls
135 were further incubated for 5 hours at their respective acclimation temperature.

136 The viral suspension was obtained from 10 infected oysters. The gills and mantle of these oysters
137 were placed in a sterile 50 ml tube containing 10 volumes of artificial seawater (ASW, 9 mL g⁻¹

138 tissue). Tissues were ground on ice using an Ultraturax (3 × 5 s) mixer. After centrifugation
139 (1000 g, 5 min, 4°C), the supernatant was transferred to a new tube and diluted in 4 volumes of
140 ASW. The homogenate was filtered under sterile conditions using syringe filters at pore sizes of
141 5 µm, 2 µm, 0.45 µm and 0.22 µm (Millipore, Billerica, USA).

142 *Effect of high temperatures on OsHV-1 transmission, infection, and mortality of oysters*
143 *(experiment 1-part A)*

144 Injected oysters (pathogen donors and controls) were transferred into the 45 L tanks to cohabit
145 with the recipients acclimated and maintained at 21°C, 26°C and 29°C. Donors were distributed
146 in triplicate tanks while controls were in duplicates for each temperature (Table 1). At the same
147 time, the non-acclimated recipients left at 21°C were added to the tanks that contained pathogen
148 donors or controls, and the volume of water was reduced to 30 L and left stagnant for 12 hours.
149 Dead donors were removed 24 h post-injection (hpi) and all the donors were removed 72 hpi. The
150 biomass of controls and pathogen donors was 230 g while that of recipients was 350 g (Fig. 1).

151 Survival of acclimated and non-acclimated recipients was monitored for 14 days. Thirty living
152 recipients (both acclimated and non-acclimated, 15 individuals for each group) were sampled in
153 each tank at 0, 12, 24, 48 and 96 h post-cohabitation (hpc). Whole tissues of 9 recipients out of 15
154 were removed from the shells, pooled together, flash frozen and stored in liquid nitrogen. Then,
155 oyster tissues were crushed in liquid nitrogen with a MM400 homogenizer (Retsch, Eragny,
156 France) and divided for OsHV-1 DNA and viral gene expression.

157 *Effect of previous temperature treatments on survival of recipients at 21°C (experiment 1-part B).*

158 On 2 June 2016, the temperature of all recipient tanks was set at 21°C and new SPF oysters were
159 added. Recipient and new SPF survival was monitored for 11 days up until 13 June.

160 *Effect of previous temperature treatments on susceptibility of oysters to a second infection in a*
161 *farming area (experiment 1-part C).*

162 On 13 June 2016, the recipients surviving the experiment 1A and B were transferred into the Bay
163 of Brest at Pointe du Chateau in (48° 20' 06.19" N, 4° 19' 06.37" W) where mass mortalities of
164 oysters caused by OsHV-1 were occurring [25], and their survival was followed for 302 days.

165 *Effect of incubation temperature on the OsHV-1 suspension (experiment 2).*

166 On 19 May 2016, the viral suspension and the synthetic seawater were incubated at 21°C, 26°C
167 and 29°C for 5 hours before injecting 100 µl into the adductor muscle of both pathogen donors
168 and controls kept at 21°C. Virus suspension remain infectious for 48 h in seawater at 20°C but
169 infectivity decreases after 24 h [26]. These injected animals remained for 5 h in six separate tanks
170 (one for each combination of injection type and incubation temperature). Injected oysters
171 (pathogen donors and controls) were transferred to the 45 L tanks to cohabit with the recipients
172 acclimated at 21°C. Donors were distributed in triplicate tanks while controls were in duplicates
173 for each temperature (Table 1). The volume of water was reduced to 30 L and left stagnant for 12
174 hours. Dead donors were removed 24 hpi and all the donors were removed 72 hpi. The biomass
175 of controls and pathogen donors was 230 g while that of recipients was 350 g (Fig. 1). Controls
176 and pathogen donors at 21°C were common to experiments 1 and 2 (Table 1).

177 **OsHV-1 DNA quantification**

178 Level of OsHV-1 DNA was quantified in both control and pathogen recipients, acclimated or
179 non-acclimated, sampled at 0, 12, 24, 48 and 96 hpc in experiments 1A and 2. These analyses
180 were conducted by the Laboratoire Departemental Veterinaire de l'Herault (Montpellier, France)
181 using oyster powder homogenized in sterile artificial seawater [27]. Total DNA was then

182 extracted with a QIAamp tissue mini kit (Qiagen) according to the manufacturer's protocol. The
183 extract was stored at -20°C before detection and quantification according to a real-time PCR
184 protocol based on SYBR Green chemistry [27] with specific primers validated by [28]. The
185 results were expressed as the log of OsHV-1 DNA copies per mg of wet tissue.

186 Viral gene expression

187 Viral gene expression was quantified in acclimated pathogen recipients sampled at 24 and 48 hpc
188 in experiments 1A and 2.

189 *Total RNA extraction and cDNA synthesis.*

190 Total RNA was isolated using Extrac-all (Eurobio, Courtaboeuf, France) at a concentration of 1.5
191 mL 30 mg^{-1} powder, and treated with DNase I (Sigma, $1\text{ U } \mu\text{g}^{-1}$ total RNA). Samples were then
192 treated with DNase (DNase Max™ Kit, MO BIO laboratories, Inc) using ($1\text{ U } \mu\text{g}^{-1}$ total RNA)
193 to remove genomic DNA. Quality of RNA and quantity were determined using a NanoDrop 2000
194 (Thermo Scientific). First strand cDNA synthesis was performed using the iScript™ cDNA
195 Synthesis Kit (BIO-RAD) with $1\mu\text{g}$ of RNA [29]. A no reverse transcription was performed after
196 each DNase treatment using real time PCR to control for the absence of oyster and virus
197 genomic DNA.

198 *Real time PCRs and relative expression.*

199 Six viral genes (ORFs 27, 38, 41, 67, 87 and 99) were selected among the 39 ORFs described by
200 [30]. These ORFs encoded for different protein functions and expressed differently during an
201 OsHV-1 replication cycle [30].

202 The real-time PCR assay was performed in triplicate with 5µl cDNA (1/10 dilution) in a total
203 volume of 10µL. The concentrations of the reaction components were as follows: 10µM of each
204 primer, 1.5µl H₂O, 7.5µl of iQ™ SYBR Green Supermix (BIO-RAD). Real time PCR cycling
205 conditions were as follows: activation at 95°C for 5min followed by 45 cycles of 30s at 95°C, 1
206 min at 60°C, and a melting curve program from 95 to 70°C by decreasing the temperature by
207 0.5°C every 10s. Each run included a positive cDNA control (a pool of the 30 cDNA samples of
208 the present experiment analyzed in each amplification plate) and in all cases negative controls
209 (without cDNA) were included to rule out DNA contamination. The elongation factor-1 (EF)
210 from *C. gigas* was chosen to normalize the viral gene expression. PCR efficiency (E) was
211 determined by drawing standard curves from a serial dilution analysis of the pool of cDNA to
212 ensure that E ranged from 99% to 108% for each primer pair.

213 The calculation of the relative mRNA levels was based on a comparative Ct method [31]. No
214 differences between Ct values were observed for EF among temperatures and between injection
215 types and times. Therefore, the relative quantification value of the samples was normalized with
216 EF and relative to the positive control, and was expressed as $2^{-\Delta\Delta Ct}$, $\Delta Ct = [Ct(cDNA\ sample) -$
217 $Ct(positive\ cDNA\ control)]$ and $\Delta\Delta Ct = \Delta Ct\ of\ cDNA - \Delta Ct\ of\ EF$.

218 Standard curves were performed for each primer pair using serial dilutions of total DNA, with
219 PCR efficacy ($E=10^{(-1/slope)}$) being subsequently calculated thanks to these curves [32].

220 Statistical analysis

221 Survival functions were computed according to [33]. Survival time was measured in hours
222 (experiments 1A and 2) or days (experiment 1C) from the onset of each experiment phase. The
223 data were read as the number of dead oysters within each tank at each count. Survival time curves

224 of recipients were compared using the Cox model (1972) after adjusting for temperature effect
225 (21°C, 26°C, 29°C, experiments 1A, 1C and 2), acclimation (acclimated vs non acclimated,
226 experiment 1A) or injection (OsHV-1 vs SSW, experiments 1C). In experiment 1A, the survival
227 of control recipients was not included in the statistical model because it was 100 %. The
228 proportionality of hazards (PH) was checked with Martingale residuals [34]. Because the PH
229 assumption was violated, time dependent covariates representing the interaction of the original
230 covariates and times were added to the model. Time (t) was defined as dichotomous: $t \leq 120$ h or
231 $t > 120$ h. Custom hazard ratios were produced by means of contrasts.

232 Mixed-design ANOVAs were performed to assess differences in (i) OsHV-1 DNA in pathogen
233 recipients (experiments 1A and 2), depending on temperature (3 levels, main plot), acclimation (2
234 levels, subplot) and time (4 levels, sub-subplot) and (ii) the viral gene expression in acclimated
235 pathogen recipients (experiments 1A and 2), depending on temperature (3 levels, main plot) and
236 time (2 levels subplot). The replication unit was the tank in which the temperature and infection
237 treatments were applied. All mutual interactions among factors were tested, and Tukey's HSD
238 was used as a *post hoc* test. The normality of residuals and homogeneity of variances were
239 graphically checked, and the data were $\log(x+1)$ transformed where necessary. Statistical
240 analyses were performed in SAS 9.4 software (SAS institute).

241

242 **Results**

243 *Effect of high temperatures on OsHV-1 transmission, infection, and survival of oysters*
244 *(experiment 1A)*

245 The oysters injected with OsHV-1 suspension (pathogen donors) showed significant mortalities
246 48 hpi at the three tested temperatures (Fig. S1). Their final survival at 72 hpi was ranked as 29°C
247 ($72 \pm 2.1\%$) > 26°C ($62 \pm 2.1\%$) > 21°C ($46 \pm 2.6\%$).

248 The survival of control recipients (oysters living in cohabitation with SSW injected oysters) was
249 $100 \pm 0\%$ irrespective of temperature and acclimation treatments (data not shown), but low
250 levels of OsHV-1 DNA were occasionally detected in these animals ($<10^3$ cp mg⁻¹ wet tissue,
251 Table S1). Although control recipients were no longer considered SPF, absence of mortality
252 suggest that they were healthy, and only the pathogen recipients were considered hereafter.

253 At the end of the cohabitation trial, the survival of pathogen recipients acclimated at 29°C was
254 higher ($85.7 \pm 2.0\%$) than at 21°C and 26°C, where survival rates were $52.4 \pm 3.1\%$ and $43.9 \pm$
255 3.1% respectively (Fig. 2A). Survival of non-acclimated pathogen recipients was 5-9% lower
256 than that of their acclimated counterparts irrespective of temperature. Mortality started 72 h post-
257 cohabitation (hpc) at 26°C and 120 hpc at 21°C (Fig. 2A). Overall, mortality risk was lowest at
258 29°C (Table S2).

259 At the onset of the experiment, OsHV-1 DNA was not detected in SPF oysters. Between 0-24
260 hpc, the level of OsHV-1 DNA in pathogen recipients increased above 10^4 cp.mg⁻¹ irrespective of
261 temperatures, but rates of increase were the highest at 26°C and 29°C (Fig. 2B, Table S3).
262 Between 24-48 hpc, the level of OsHV-1 DNA continued to increase at 21°C, remained high at
263 26°C, and initiated a decrease at 29°C. Therefore, at 48 hpc, the level of OsHV-1 DNA ranked as
264 26°C = 21°C > 29°C. Finally, between 48-96 hpc, OsHV-1 DNA decreased at 26°C and 29°C
265 while it remained stable at 21°C. Therefore, at 96 hpc, OsHV-1 DNA ranked as 21°C > 26°C =
266 29°C. Interestingly, maximum values of OsHV-1 DNA were similar among temperatures

267 $(1.9 \times 10^6 \text{ cp.mg}^{-1}$ at 21°C 48 hpc; $3.4 \times 10^6 \text{ cp.mg}^{-1}$ at 26°C 24 hpc and at 29°C , $4.0 \times 10^5 \text{ cp.mg}^{-1}$ 24
268 hpc) but these maximum values were observed for longer at 26°C and 21°C than at 29°C .

269 The six viral Open Reading Frames (ORFs) selected in our study were expressed in pathogen
270 recipients at the three tested temperatures. Three of them were modulated by temperature (ORFs
271 27, 38 and 87). Gene expression levels were highest at 26°C , except ORF 87 at 48 hpc, which
272 exhibited the highest values at 21°C . At 29°C , viral gene expressions of these three ORFs were
273 lower than at 26°C and 21°C . Viral gene expression of the other ORFs (41, 67 and 99) increased
274 between 24 and 48 hpc (Fig. 2C and D).

275 *Effect of previous temperature treatments on recipient survival at 21°C (experiment 1B).*

276 The survival of both control and pathogen recipients placed at 21°C for 11 days was $100 \pm 0 \%$
277 irrespective of previous temperature treatments (data not shown). Moreover, the new SPF oysters
278 placed in cohabitation with them showed no mortality.

279 *Effect of previous temperature treatments on oyster susceptibility to a second infection in a*
280 *farming area (experiment 1C).*

281 Both pathogen and controls recipients transferred to a farming area where OsHV-1 occurred
282 showed high mortalities. However, the survival of pathogen recipients was higher ($75.9 \pm 5.3\%$)
283 than that of control recipients ($44.9 \pm 9.9\%$, Fig. 3, Table S4). There was no effect of previous
284 temperature treatments on the survival of control recipients. In contrast, the survival of the
285 pathogen recipients infected at 29°C was lower ($67.9 \pm 10.8\%$) than those infected at 21°C and
286 26°C , where survival was $79.3 \pm 3.7\%$ and $80.6 \pm 5.5\%$ respectively. Throughout the whole
287 experiment 1, the survival of the pathogen recipients previously exposed to donors at 29°C

288 remained higher (56.6%) than at 21°C and 26°C, where survival was 41.9% and 32.0%
289 respectively.

290 *Effect of incubation temperature on the OsHV-1 suspension (experiment 2).*

291 As observed in experiment 1, the survival of control recipients was 100 ± 0 % but low levels of
292 OsHV-1 DNA were occasionally detected ($<10^2$ cp mg⁻¹ wet tissue, Table S1). However, given
293 the absence of mortality and temperature effect on control recipients, only the pathogen recipients
294 were considered hereafter.

295 Pathogen recipients showed significant mortalities after 72 hpc (Fig. 4A, Table S5). At the end of
296 experiment 2, the survival of pathogen recipients ranked as a function of incubation temperature
297 of the viral suspension: 26°C (46 ± 3 %) < 21°C = 29°C (52 ± 2 %). The level of OsHV-1 DNA
298 and viral gene expression in pathogen recipients increased during the experiment but were not
299 influenced by the incubation temperature of the viral suspension (Fig. 4B and C).

300 **Discussion**

301 The major result of this study is that the survival of recipient oysters exposed to the virus at 29°C
302 (85.7% survival) was markedly higher than at 21°C and 26°C (52.4% and 43.9% respectively),
303 reflecting changes in host susceptibility and/or virus virulence. This concurs with a previous
304 study where recipients cohabited with field-infected donors [16]. These differences in survival
305 persisted when the pathogen recipients were all placed at 21°C, the optimal temperature for
306 OsHV-1 replication, and when they were re-exposed to OsHV-1 in the field. This result opens
307 new perspectives for mitigation measures using high temperature.

308 The differences in survival between recipients exposed to OsHV-1 at 21°C and 29°C coincided
309 with differences in viral replication in the host. Although levels of OsHV-1 DNA increased from
310 undetectable amounts to values higher than 10^4 DNA cp mg⁻¹ (the threshold at which mortality
311 generally occurs) for both temperatures during the first 24 hpc, it then decreased markedly in
312 recipients at 29°C whereas it continued to increase at 21°C. A similar pattern was observed in
313 SPF oysters having cohabited with field infected donors at these two temperatures [16]. In our
314 study, the expression of three viral genes (ORF 27, 38 and 87) involved in regulation of a ring
315 finger motif, DNA repairing and apoptosis inhibition [30] were lower in recipients at 29°C than
316 at 21°C. High temperature may reduce the expression of some viral genes that could be essential
317 to the development of the disease and viral cycle. Also, equally high expression levels of ORF
318 41, 67 and 99 at 21°C and 29°C suggest the induction of the OsHV-1 lytic cycle irrespective of
319 temperature [35].

320 Survival of pathogen donors at 29°C was higher than at 21°C, as observed in pathogen
321 recipients. This suggests that high temperature (29°C) decreased the susceptibility of oysters to
322 OsHV-1. This, however, raises the possibility that viral shedding of pathogen donors at 29°C was
323 lower than that at 21°C, which could have contributed to the increased survival of the pathogen
324 recipients at 29°C.

325 We also found that these temperatures (21°C and 29°C) had no effect on the virus suspension *per*
326 *se*. Indeed, the survival, level of OsHV-1 DNA and virus gene expression of recipients at 21°C
327 having cohabited with donors injected with viral suspension incubated were all similar at 21°C or
328 29°C. Therefore, high temperature (29°C) had no effect on the infectivity (a measure of the
329 ability of a disease agent to establish itself in the host) or the virulence (a measure of the severity
330 of a disease) of OsHV-1.

331 Therefore, high temperature (29°C) decreased the susceptibility of oysters to OsHV-1 without
332 altering virus infectivity and virulence; however, the underlying mechanism remains unknown.
333 Temperature impacts all aspects of ectotherm physiology, including their immunity [36]. For
334 instance, the antiviral response of oysters is stimulated at 22°C compared to 12°C [37]. High
335 temperatures (32°C) induce overexpression of heat shock proteins 70 involved in the repression
336 of white spot syndrome virus replication in shrimps [38].

337 At 26°C, the mortality of pathogen recipients started earlier than at 21°C and 29°C, survival was
338 lowest, levels of OsHV-1 DNA were highest (between 12-48 hpc) and the expression of three
339 viral genes was enhanced. Furthermore, the survival of pathogen recipients having cohabited with
340 donors injected with viral suspension incubated at 26°C was lowest, but levels of OsHV-1 DNA
341 and viral gene expression were similar among temperature treatments. Therefore, it appears that
342 the virulence of OsHV-1 was enhanced at 26°C, although an increase in susceptibility of the host
343 cannot be ruled out.

344 In our study, the survival of pathogen recipients at 26°C is the lowest as reported by [17].
345 However, in a previous study, survival of oysters exposed to OsHV-1 at 21°C was lower than at
346 26°C [16]. Discrepancies may reflect different methods of infection among studies. Here, the
347 donors were injected with a viral suspension, whereas in [16] they were naturally infected by a
348 short exposure to field conditions where OsHV-1 induced mortalities of oysters were occurring.
349 Therefore, their study probably encompasses other microorganisms naturally encountered in the
350 marine environment, including populations of pathogenic vibrios that influence oyster survival
351 [22,39].

352 When temperature of pathogen recipients decreases from 29°C to 21°C, a temperature permissive
353 for OsHV-1 replication, there was neither additional mortality nor disease transmission to the
354 new SPF oysters. Although high temperature increases elimination of several viruses in oysters
355 [40], herpesviruses are generally incurable and complete elimination from the host is unlikely
356 [41]. Our results suggest that OsHV-1 did not reactivate at 21°C, or at least not sufficiently to
357 induce mortality. However, OsHV-1 can persist in oysters for several months at low temperatures
358 (<13°C) and can reactivate and kill the host after a thermal elevation to 21°C [42].

359 Recipients showed additional mortalities following the second exposure to OsHV-1 in the field.
360 The survival of pathogen recipients previously infected at 29°C was lower than those infected at
361 21°C and 26°C, likely reflecting the persistence of a greater number of susceptible hosts at 29°C.

362 Throughout the whole experiment, the survival of recipients exposed to pathogen donors at 29°C
363 was on average 20% higher than at 21°C and 26°C. Oysters first infected at 29°C seemed less
364 susceptible to the virus than those first infected at 21°C and 26°C. For the second infection, the
365 oysters were 1 ½ month older and therefore potentially less susceptible to the pathogen [43].

366 Also, the first exposure to OsHV-1 may have lowered the susceptibility of oysters to a second
367 infection. A non-lethal exposure to a pathogen can enhance invertebrate immunity by immune
368 priming [44,45]. Immune priming against OsHV-1 has already been reported for oysters injected
369 with a synthetic viral analogue [46].

370 Oysters exposed to the virus at 29°C were less susceptible to OsHV-1 than at 21°C and 26°C
371 over the long term, thus opening new perspectives for mitigation measures. Hyperthermia was
372 already suggested as preventive method in shrimp farming to minimize the risk of a viral
373 outbreak [47]. Regarding the potential of high temperature treatment to mitigate disease risk in

374 oyster farms, several questions need to be answered. What is the sanitary status of the oysters
375 exposed to OsHV-1 at high temperature (OsHV-1-free or asymptomatic carrier)? What is the
376 optimal treatment duration and temperature to optimize survival and minimize energy costs?
377 How is it possible to practically implement a high temperature treatment on a production farm?
378 Finally, what are the economic efficiency (i.e. the cost of disease control measures compared to
379 ensuing benefits) and acceptability (the willingness of organizations to put into action disease
380 control measures) of high temperature treatment?

381 **Acknowledgements**

382 We thank the Ifremer staff involved in oyster and algae production at Argenton and Bouin. We
383 are grateful to Mathias Hubert for helping with the experimental set-up and to Melaine Gourault,
384 Valérian Le Roy, Margaux Mathieu-Resuge, Isabelle Quéau and Claudie Quéré for their help
385 with sampling. We also thank Maelenn Le Roy for helping with the RNA analyses, Amelie
386 Segarra for discussions, and Amelia Curd for her help with English editing. This work was
387 supported by the EU funded project VIVALDI (H2020 program, n°678589) and the TEMPO
388 project funded by the French Ministry of Environment, Energy and Sea (Convention DPMA
389 2016 16/1212569).

390 **References**

- 391 [1] C.A. Burge, C. Mark Eakin, C.S. Friedman, B. Froelich, P.K. Hershberger, E.E. Hofmann,
392 L.E. Petes, K.C. Prager, E. Weil, B.L. Willis, S.E. Ford, C.D. Harvell, *Climate Change*
393 *Influences on Marine Infectious Diseases: Implications for Management and Society*, *Ann.*
394 *Rev. Mar. Sci.* 6 (2014) 249–277. doi:10.1146/annurev-marine-010213-135029.
- 395 [2] C. Harvell, C. Mitchell, J. Ward, S. Altizer, A.P. Dobson, R.S. Ostfeld, M.D. Samuel,

- 396 Climate warming and disease risks for terrestrial and marine biota, *Science*. 296 (2002)
397 2158–2163. doi:10.1126/science.1063699.
- 398 [3] O.M. Vidal, C.B. Granja, F. Aranguren, J.A. Brock, M. Salazar, A profound effect of
399 hyperthermia on survival of *Litopenaeus vannamei* juveniles infected with white spot
400 syndrome virus, *J. World Aquac. Soc.* 32 (2001) 364–372. doi:10.1111/j.1749-
401 7345.2001.tb00462.x.
- 402 [4] A. Staehli, R. Schaerer, K. Hoelzle, G. Ribí, Temperature induced disease in the starfish
403 *Astropecten jonstoni*, *Mar. Biodivers. Rec.* 2 (2009) 10–14.
404 doi:10.1017/S1755267209000633.
- 405 [5] W.T. Kohl, T.I. McClure, B.G. Miner, Decreased temperature facilitates short-term sea
406 star wasting disease survival in the keystone intertidal sea star *Pisaster ochraceus*, *PLoS*
407 *One*. 11 (2016) 1–9. doi:10.1371/journal.pone.0153670.
- 408 [6] M.A. Travers, O. Basuyaux, N. Le Goïc, S. Huchette, J.L. Nicolas, M. Koken, C. Paillard,
409 Influence of temperature and spawning effort on *Haliotis tuberculata* mortalities caused by
410 *Vibrio harveyi*: An example of emerging vibriosis linked to global warming, *Glob. Chang.*
411 *Biol.* 15 (2009) 1365–1376. doi:10.1111/j.1365-2486.2008.01764.x.
- 412 [7] C.B. Granja, L.F. Aranguren, O.M. Vidal, L. Aragon, M. Salazar, Does hyperthermia
413 increase apoptosis in white spot syndrome virus (WSSV) infected *Litopenaeus vannamei*?,
414 *Dis. Aquat. Organ.* 54 (2003) 73–78. doi:10.3354/dao054073.
- 415 [8] C.B. Granja, O.M. Vidal, G. Parra, M. Salazar, Hyperthermia reduces viral load of white
416 spot syndrome virus in *Penaeus vannamei*, *Dis. Aquat. Organ.* 68 (2006) 175–180.
417 doi:10.3354/dao068175.

- 418 [9] S. Corbeil, L.M. Williams, J. Bergfeld, M.S.J. Crane, Abalone herpes virus stability in sea
419 water and susceptibility to chemical disinfectants, *Aquaculture*. 326–329 (2012) 20–26.
420 doi:10.1016/j.aquaculture.2011.11.031.
- 421 [10] V. Barbosa Solomieu, T. Renault, M.A. Travers, Mass mortality in bivalves and the
422 intricate case of the Pacific oyster, *Crassostrea gigas*, *J. Invertebr. Pathol.* 131 (2015) 2–
423 10. doi:10.1016/j.jip.2015.07.011.
- 424 [11] EFSA, Scientific opinion on the increased mortality events in Pacific oysters, *Eur. Food*
425 *Saf. Auth. J.* 8 (2010) 1–60. doi:10.2903/j.efsa.2010.1894.
- 426 [12] F. Pernet, C. Lupo, C. Bacher, R.J. Whittington, Infectious diseases in oyster aquaculture
427 require a new integrated approach, *Philos. Trans. R. Soc. B Biol. Sci.* 371 (2016)
428 20150213. doi:10.1098/rstb.2015.0213.
- 429 [13] F.J. Jenkins C, Hick P, Gabor M, Spiers Z, Fell SA, Gu X, Read A, Go J, Dove M,
430 O'Connor W, Kirkland PD, Identification and characterisation of an ostreid herpesvirus-1
431 microvariant (OsHV-1 μ -var) in *Crassostrea gigas* (Pacific oysters) in Australia., *Dis.*
432 *Aquat. Organ.* 105 (2013) 109–126. doi:doi.org/10.3354/dao02623.
- 433 [14] F. Pernet, J. Barret, P. Le Gall, C. Corporeau, L. Dégremont, F. Lagarde, J.F. Pépin, N.
434 Keck, Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and
435 vary with farming practices in the Mediterranean Thau lagoon, France, *Aquac. Environ.*
436 *Interact.* 2 (2012) 215–237. doi:10.3354/aei00041.
- 437 [15] T. Renault, L. Bouquet, J. Maurice, C. Lupo, Ostreid Herpesvirus 1 Infection among
438 Pacific Oyster (*Crassostrea gigas*) Spat : Relevance of Water Temperature to Virus
439 Replication and Circulation Prior to the Onset of Mortality, *Appl. Environ. Microbiol.* 80

- 440 (2014) 5419–5426. doi:10.1128/AEM.00484-14.
- 441 [16] B. Petton, F. Pernet, R. Robert, P. Boudry, Temperature influence on pathogen
442 transmission and subsequent mortalities in juvenile pacific oysters *Crassostrea gigas*,
443 Aquac. Environ. Interact. 3 (2013) 257–273. doi:10.3354/aei00070.
- 444 [17] M. de Kantzow, P. Hick, J. Becker, R. Whittington, Effect of water temperature on
445 mortality of Pacific oysters *Crassostrea gigas* associated with microvariant ostreid
446 herpesvirus 1 (OsHV-1 μ Var), Aquac. Environ. Interact. 8 (2016) 419–428.
447 doi:10.3354/aei00186.
- 448 [18] C. Martenot, L. Denechère, P. Hubert, L. Metayer, E. Oden, S. Trancart, E. Travaille, M.
449 Houssin, Virulence of Ostreid herpesvirus 1 μ Var in sea water at 16°C and 25°C,
450 Aquaculture. 439 (2015) 1–6. doi:10.1016/j.aquaculture.2015.01.012.
- 451 [19] S. Bougrier, P. Geairon, J.M. Deslous-Paoli, C. Bather, G. Jonquikres, Allometric
452 relationships and effects of temperature on clearance and oxygen consumption rates of
453 *Crassostrea gigas* (Thunberg), Aquaculture. 134 (1995) 143–154. doi:10.1016/0044-
454 8486(95)00036-2.
- 455 [20] B. Petton, P. Boudry, M. Alunno-Bruscia, F. Pernet, Factors influencing disease-induced
456 mortality of Pacific oysters *Crassostrea gigas*, Aquac. Environ. Interact. 6 (2015) 205–
457 222. doi:10.3354/aei00125.
- 458 [21] F. Le Roux, K.M. Wegner, M.F. Polz, Oysters and Vibrios as a Model for Disease
459 Dynamics in Wild Animals, Trends Microbiol. 24 (2016) 568–580.
460 doi:10.1016/j.tim.2016.03.006.
- 461 [22] B. Petton, M. Bruto, A. James, Y. Labreuche, M. Alunno-Bruscia, F. Le Roux,

- 462 *Crassostrea gigas* mortality in France: The usual suspect, a herpes virus, may not be the
463 killer in this polymicrobial opportunistic disease, *Front. Microbiol.* 6 (2015) 1–10.
464 doi:10.3389/fmicb.2015.00686.
- 465 [23] M. Fuhrmann, B. Petton, V. Quillien, N. Faury, B. Morga, F. Pernet, Salinity influences
466 disease-induced mortality of the oyster *Crassostrea gigas* and infectivity of the ostreid
467 herpesvirus 1 (OsHV-1), *Aquac. Environ. Interact.* 8 (2016) 543–552.
468 doi:10.3354/aei00197.
- 469 [24] M. Suquet, G. De Kermoisan, R.G. Araya, I. Queau, L. Lebrun, P. Le Souchu, C.
470 Mingant, Anesthesia in Pacific oyster, *Crassostrea gigas*, *Aquat. Living Resour.* 22 (2009)
471 29–34. doi:10.1051/alr/2009006.
- 472 [25] E. Fleury, J. Normand, A. Lamoureux, J.-F. Bouget, C. Lupo, N. Cochenec-Laureau, S.
473 Petton, B. Petton, Pouvreau Stephane, National monitoring network of mortality and
474 growth rates of the sentinel oyster *Crassostrea gigas*, SEANOE. (2018).
475 doi:10.17882/53007.
- 476 [26] P. Hick, O. Evans, R. Looi, C. English, R.J. Whittington, Stability of Ostreid herpesvirus-1
477 (OsHV-1) and assessment of disinfection of seawater and oyster tissues using a bioassay,
478 *Aquaculture.* 450 (2016) 412–421. doi:10.1016/j.aquaculture.2015.08.025.
- 479 [27] J.F. Pepin, A. Riou, T. Renault, Rapid and sensitive detection of ostreid herpesvirus 1 in
480 oyster samples by real-time PCR, *J. Virol. Methods.* 149 (2008) 269–276.
481 doi:10.1016/j.jviromet.2008.01.022.
- 482 [28] S.C. Webb, A. Fidler, T. Renault, Primers for PCR-based detection of ostreid herpes virus-
483 1 (OsHV-1): Application in a survey of New Zealand molluscs, *Aquaculture.* 272 (2007)

- 484 126–139. doi:10.1016/j.aquaculture.2007.07.224.
- 485 [29] A. Huvet, J.. Daniel, C. Quéré, S. Dubois, M. Prudence, A. Van Wormhoudt, D. Sellos,
486 J.F. Samain, J. Moal, Tissue expression of two α -amylase genes in the Pacific oyster
487 *Crassostrea gigas*. Effects of two different food rations, *Aquaculture*. 228 (2003) 321–333.
488 doi:10.1016/S0044-8486(03)00323-5.
- 489 [30] A. Segarra, N. Faury, J. Pépin, T. Renault, Transcriptomic study of 39 ostreid herpesvirus
490 1 genes during an experimental infection, *J. Invertebr. Pathol.* 119 (2014) 5–11.
491 doi:10.1016/j.jip.2014.03.002.
- 492 [31] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time
493 quantitative PCR and the $2^{-\Delta\Delta CT}$ method, *Methods*. 25 (2001) 402–408.
494 doi:10.1006/meth.2001.1262.
- 495 [32] R. Rasmussen, Quantification on the LightCycler, in: *Rapid Cycle Real-Time PCR*,
496 Springer, Berlin, Heidelb., 2001.
- 497 [33] E.L. Kaplan, P. Meier, Nonparametric Estimation from Incomplete Observations, *Am.*
498 *Stat. Assoc.* 53 (1958) 457–481. doi:10.2307/2281868.
- 499 [34] D.Y. Lin, L.J. Wei, Z. Ying, Checking the Cox model with cumulative sums of
500 martingale-based residuals, *Biometrika*. 80 (1993) 557–572. doi:10.1093/biomet/80.3.557.
- 501 [35] A. Segarra, Etude des interactions hôte/virus chez l’huître creuse, *Crassostrea gigas*, et
502 son virus Ostreid herpesvirus 1, PhD dissertation, Université de Bretagne Sud, 262pp,
503 2015.
- 504 [36] P.W. Hochachka, G.. Somero, Biochemical adaptation: mechanism and process in

- 505 physiological evolution, Oxford Uni, 2002.
- 506 [37] T.J. Green, C. Montagnani, K. Benkendorff, N. Robinson, P. Speck, Ontogeny and water
507 temperature influences the antiviral response of the Pacific oyster, *Crassostrea gigas*, Fish
508 Shellfish Immunol. 36 (2014) 151–157. doi:10.1016/j.fsi.2013.10.026.
- 509 [38] Y.R. Lin, H.C. Hung, J.H. Leu, H.C. Wang, G.H. Kou, C.F. Lo, The Role of Aldehyde
510 Dehydrogenase and Hsp70 in Suppression of White Spot Syndrome Virus Replication at
511 High Temperature, J. Virol. 85 (2011) 3517–3525. doi:10.1128/JVI.01973-10.
- 512 [39] A. Lemire, D. Goudenège, T. Versigny, B. Petton, A. Calteau, Y. Labreuche, F. Le Roux,
513 Populations, not clones, are the unit of vibrio pathogenesis in naturally infected oysters,
514 ISME J. 9 (2015) 1523–1531. doi:10.1038/ismej.2014.233.
- 515 [40] C. Choi, D.H. Kingsley, Temperature-Dependent Persistence of Human Norovirus Within
516 Oysters (*Crassostrea virginica*), Food Environ. Virol. 8 (2016) 141–147.
517 doi:10.1007/s12560-016-9234-8.
- 518 [41] M.A. Jarvis, J.A. Nelson, Human cytomegalovirus persistence and latency in endothelial
519 cells and macrophages, Curr. Opin. Microbiol. 5 (2002) 403–407.
520 doi:https://doi.org/10.1016/S1369-5274(02)00334-X.
- 521 [42] F. Pernet, D. Tamayo, B. Petton, Influence of low temperatures on the survival of the
522 Pacific oyster (*Crassostrea gigas*) infected with ostreid herpes virus type 1, Aquaculture.
523 445 (2015) 57–62. doi:10.1016/j.aquaculture.2015.04.010.
- 524 [43] L. Dégremont, P. Boudry, M. Ropert, J.F. Samain, E. Bédier, P. Soletchnik, Effects of age
525 and environment on survival of summer mortality by two selected groups of the Pacific
526 oyster *Crassostrea gigas*, Aquaculture. 299 (2010) 44–50.

- 527 doi:10.1016/j.aquaculture.2009.12.009.
- 528 [44] T.J. Little, A.R. Kraaijeveld, Ecological and evolutionary implications of immunological
529 priming in invertebrates, *Trends Ecol. Evol.* 19 (2004) 58–60.
530 doi:10.1016/j.tree.2003.11.011.
- 531 [45] B. Milutinović, J. Kurtz, Immune memory in invertebrates, *Semin. Immunol.* 28 (2016)
532 328–342. doi:10.1016/j.smim.2016.05.004.
- 533 [46] T.J. Green, C. Montagnani, Poly I: C induces a protective antiviral immune response in the
534 Pacific oyster (*Crassostrea gigas*) against subsequent challenge with Ostreid herpesvirus
535 (OsHV-1 μ var), *Fish Shellfish Immunol.* 35 (2013) 382–388.
536 doi:10.1016/j.fsi.2013.04.051.
- 537 [47] M.M. Rahman, C.M. Escobedo-bonilla, M. Corteel, J.J. Dantas-lima, Effect of high water
538 temperature (33 °C) on the clinical and virological outcome of experimental infections
539 with white spot syndrome virus (WSSV) in specific pathogen-free (SPF) *Litopenaeus*
540 *vannamei*, *Aquaculture.* 261 (2006) 842–849. doi:10.1016/j.aquaculture.2006.09.007.

541

542

543 **Table 1.** List of all treatment combinations used in experiments 1A, 1B and 2 in the laboratory.
544 Abbreviations: SSW, synthetic seawater; A, acclimated; NA, non-acclimated.

Seawater temperature	Injection	Injection temperature	Replicate	Tank	Acclimation	Experiment
-------------------------	-----------	--------------------------	-----------	------	-------------	------------

Seawater temperature	Injection	Injection temperature	Replicate	Tank	Acclimation	Experiment	
21°C	SSW	21°C	1	1	A	1-2	
			2	2	A	1-2	
		26°C	1	3	A	2	
			2	4	A	2	
		29°C	1	5	A	2	
			2	6	A	2	
	OsHV-1	21°C	1	7	A-NA	1-2	
			2	8	A-NA	1-2	
			3	9	A-NA	1-2	
		26°C	1	10	A	2	
			2	11	A	2	
			3	12	A	2	
29°C		1	13	A	2		
		2	14	A	2		
		3	15	A	2		
26°C		SSW	21°C	1	16	A	1
				2	17	A	1
		OsHV-1	21°C	1	18	A-NA	1
	2			19	A-NA	1	
	3			20	A-NA	1	
	29°C	SSW	21°C	1	21	A	1

Seawater temperature	Injection	Injection temperature	Replicate	Tank	Acclimation	Experiment
			2	22	A	1
	OsHV-1	21°C	1	23	A-NA	1
			2	24	A-NA	1
			3	25	A-NA	1

545

546

547 **Figure legends**

548 **Fig. 1.** Experimental design to investigate the effect of temperature on disease susceptibility of
549 the Pacific oyster *Crassostrea gigas* and virulence of the Ostreid herpesvirus type 1.
550 Abbreviations: OsHV-1, Ostreid herpesvirus type 1; SPF, specific-pathogen free oyster; SSW,
551 synthetic seawater.

552 **Fig. 2.** (A) Survival of oysters (pathogen recipients) having cohabited with donors injected with
553 OsHV-1 at 21°C, 26°C and 29°C with or without prior acclimation. (B) Quantification of OsHV-
554 1 DNA in pathogen recipients as a function of temperature. Data of acclimated and non-
555 acclimated oysters were averaged due to temperature × acclimation × time interaction not being
556 significant. Inset represents the significant interaction of temperature × acclimation. Data were
557 log (x+1) transformed. (C and D) Viral gene expression expressed as a delta threshold cycle
558 number (Ct) of OsHV-1 open reading frames (ORFs) relative to *C. gigas* elongation factor in live
559 acclimated pathogen recipients as a function of temperature. Inset represents the main effect of
560 time averaged for the six tested ORFs. The gene expression analyses were conducted only at 24
561 hpc and 48 hpc. For all analyses, values are means ± SE (n=3 tanks), and different letters indicate
562 significant differences. Only significant effects are represented.

563 **Fig. 3.** Survival of pathogen recipient after being transferred to a farming area where OsHV-1
564 occurred as a function of temperature during the first exposure to OsHV-1 in the laboratory.

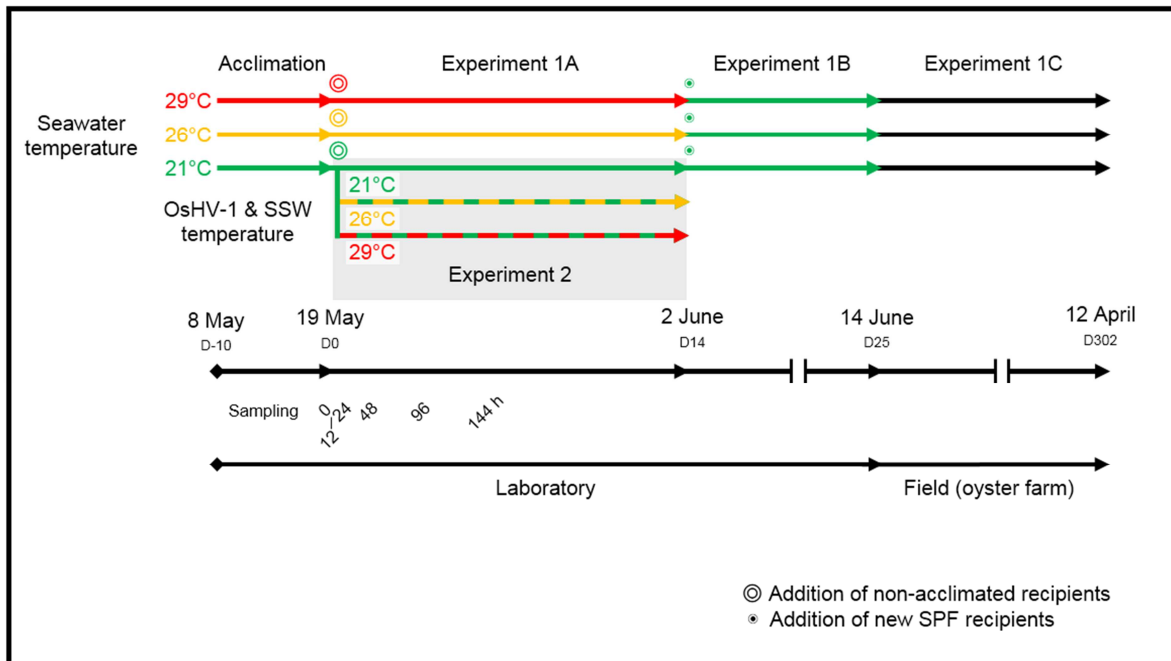
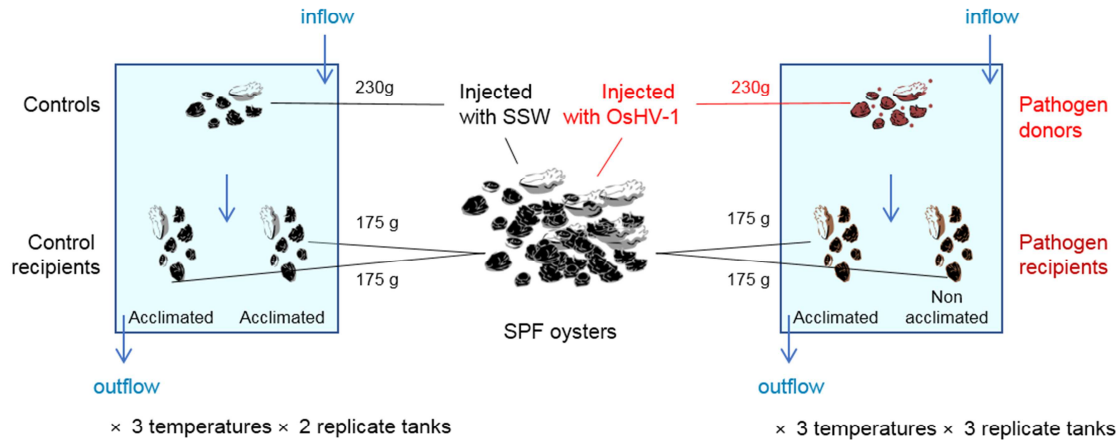
565 **Fig. 4.** (A) Survival of oysters (pathogen recipients) having cohabited with donors injected with
566 OsHV-1 incubated at 21°C, 26°C and 29°C for 5 hours. (B) Quantification of OsHV-1 DNA in
567 pathogen recipients as a function of incubation temperature of the viral suspension. Data of
568 recipients at 21°C, 26°C and 29°C were averaged because the interaction of temperature × time
569 was not significant (black line). Data were log (x+1) transformed. (C) Viral gene expression

570 expressed as a delta threshold cycle number (Ct) of OsHV-1 open reading frames (ORFs) relative
571 to *C. gigas* elongation factor 1 in live acclimated recipients as a function of time. The gene
572 expression analyses were conducted only at 24 hpc and 48 hpc. For all analyses, values are means
573 \pm SE (n=3 tanks), and different letters indicate significant differences. Only significant effects are
574 represented.

575

ACCEPTED MANUSCRIPT

Fig. 1.



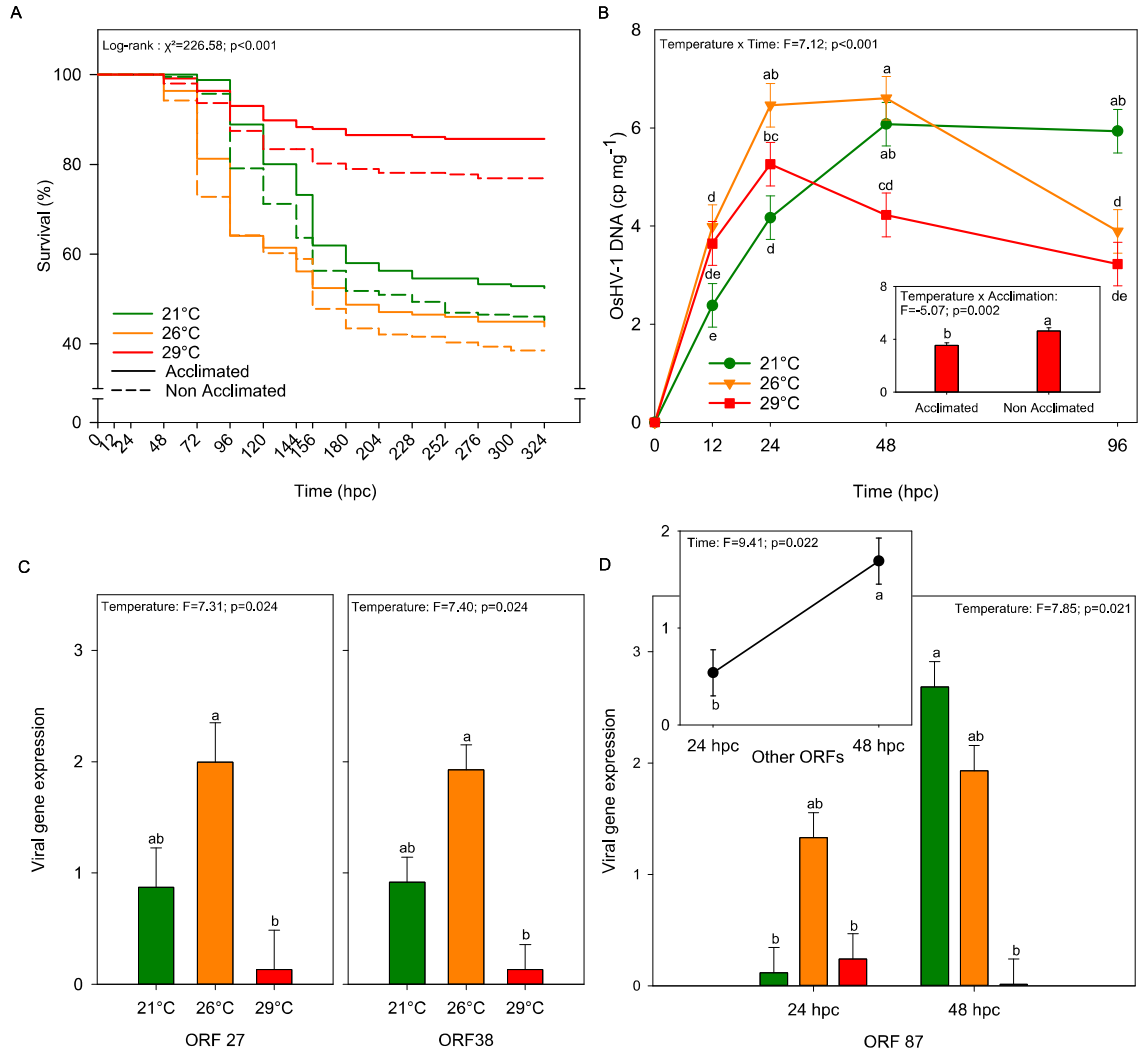


Fig. 3.

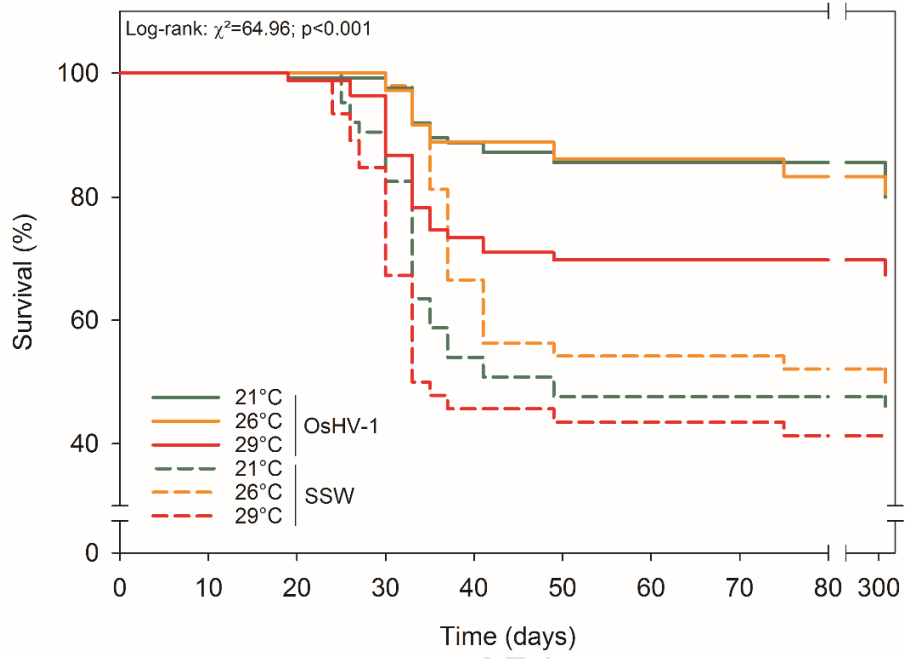


Fig. 4.

