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Mass selection for survival and resistance to OsHV-1 infection in *Crassostrea gigas* spat in field conditions: response to selection after four generations

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Abstract :

The ostreid herpesvirus 1 (OsHV-1) is one of the major pathogens affecting the Pacific oyster *Crassostrea gigas*, and numerous mortality outbreaks have been observed worldwide. We report the first results of our experimental breeding program using mass selection focused on survival and resistance to OsHV-1 after four generations of selection for two lines. These lines originated from two stocks of adult wild oysters sampled from the Marennes-Oléron Bay in 2008. Each line was spawned in February 2009 to produce the base populations. Both lines were then either protected from OsHV-1 or tested in the field in 2009 where they were exposed to OsHV-1. For each line during 2010 to 2013, one generation per year was produced using either the survivors of the previous generation for the selected group or the oysters protected from OsHV-1 for the control group. After one generation of selection (G1) for both lines, the mean survival of the selected group was 34.5% compared with 12.3% in the control group. For the fourth generation of selection (G4), the survival of the selected group reached 69.0% and the survival of the control group was 7.3%. The gain in survival of the selected *C. gigas* spat over the control increased by 22.2%, 43.9%, 50.2% and 61.8% for the G1, G2, G3 and G4 generations, respectively. Our study demonstrates that mass selection for survival and OsHV-1 resistance was successful after four generations of selection, thus indicating a significant genetic improvement for the selected trait. A genotype x size interaction was observed with 55.1% of survival in G4 when selected spat were transferred at 1 g versus 89.9% of survival when they were transferred at 3 g. Our study is the first to provide some estimates of the realized heritability for disease resistance using a mass selection scheme in an oyster species with values ranging from 0.34 to 0.63 depending on the size of the oysters exposed to OsHV-1. Oysters selected for their higher resistance to OsHV-1 infection in G4 showed higher growth (58.4 mm – 19.4 g) than controls (51.4 mm – 15.2 g), and mass selection had significantly improved the yield for the selected oysters (13.3 kg) over the controls (1.2 kg). Mass selection could be easily implemented by a commercial hatchery that cannot afford family-based selection that requires the production of numerous families for the base population.

Highlights

► Mass selection to enhance survival and OsHV-1 resistance was successful in C. gigas spat. ► This is the first study to report realized heritability for disease resistance in an oyster species. ► The gain of survival per generation was 12.1% to 20.2% for 1 g and > 3 g oysters, respectively. ► The selected oysters grew faster and had a much higher yield than did the unselected oysters.

Statement of relevance

Strong impact on breeding program working on OsHV-1 resistance in *C. gigas*.

Keywords : survival, Crassostrea gigas, realized heritability, selection response, OsHV-1, disease resistance

1. **Introduction**

Massive disease-related mortality has been commonly reported worldwide in several major oyster species. One striking example concerns the mortality outbreaks related to the ostreid herpesvirus OsHV-1 infecting the Pacific cupped oyster *Crassostrea gigas*. During the last decade, such outbreaks were reported on the west coast of the USA (Burge et al., 2006), in New Zealand and Australia (Paul-Pont et al., 2013; Keeling et al., 2014) and in numerous countries in Europe (EFSA, 2010; Lynch et al., 2012; Roque et al., 2012; Domeneghetti et al., 2014). In France, which is the main European producer of *C. gigas*, OsHV-1 has regularly affected cultivated spat and juvenile oysters for several years (Renault et al., 1994; Dégremont, 2003; Garcia et al., 2011). However, since 2008, dramatic mortality outbreaks related to OsHV-1 have routinely occurred in *Crassostrea gigas* in France (Ségarra et al., 2010; Pernet et al., 2012; Dégremont, 2013), leading to decreased French oyster production from to 111 000 tons in 2008 to 82 000 tons in 2012 (FAO, 2014). In addition to environmental degradation and naturally higher susceptibility of the oyster to disease, French cultural practices could have favored the disease propagation and settlement over time among all growing areas. This increase in mortality could also be related to a higher virulence of the virus with the description of the genotype called OsHV-1 µvar (Ségarra et al., 2010), which was previously found in Normandy in 2004 (Martenot et al., 2012). Unfortunately, no evidence has been provided thus far to show a difference in virulence among OsHV-1 genotypes, although many of these strains were identified during mortality outbreaks dating back to 1993 (Lynch et al., 2012; Martenot et al., 2012; Renault et al., 2012; Lynch et al., 2013; Martenot et al., 2013). Nevertheless, severe production losses due to OsHV-1 have been reported in France and are related to a microvariant that has taken over as the predominant strain. This situation urges researchers to develop strategies to reduce the mortality in *C. gigas* spat.

One approach to combat this problem is a selective breeding program to enhance disease resistance, and thus, the survival of *C. gigas* spat. A first approach showed that the oysters selected for their higher or lower resistance to the summer mortality phenomena in 2001 were found to be correspondingly resistant or susceptible to OsHV-1 infection during mortality outbreaks observed in 2002 and 2003 (Dégremont, 2003; Dégremont et al., 2010a). The same findings were observed with subsequent generations of the selected oysters that were tested from 2009 to 2012, pointing to an underlying genetic basis for resistance to OsHV-1 infection in *C. gigas* (Dégremont, 2011; Dégremont, 2013). These findings were based on family selection for which numerous families are produced to estimate the genetic parameters and to obtain families containing the desirable genetic traits. Nevertheless, most of commercial hatcheries in France cannot afford to raise and test numerous oyster families. As an alternative, mass selection is simpler and less expensive because only one selected group is produced along with a control group to assess whether changes are caused by genetic modification or environmental variation. This selection method relies on high fecundity in the shellfish, enabling the use of a higher selection intensity compared to that used for terrestrial livestock (Gjedrem and Baranski, 2009). Nevertheless, without individual tagging and pedigree records, this selection scheme could lead to inbreeding and thus reduce the response to selection (Bentsen and Olesen, 2002). However, mass selection without a true control for inbreeding has been widely used in shellfish species to enhance growth or disease resistance.

Numerous mass selection trials have focused on growth and survival traits. Estimation of a realized heritability is reported for growth-related traits in oysters, clams and scallop species

(Toro and Newkirk, 1990; Hadley et al., 1991; Jarayabhand and Thavornyutikarn, 1995; Crenshaw et al., 1996; Toro et al., 1996; Ibarra et al., 1999; Zheng et al., 2004; He et al., 2008; Deng et al., 2009; Dobler and Hosken, 2009; Li et al., 2011; Zhao et al., 2012). Surprisingly, all studies regarding mass selection for survival related to disease resistance failed to provide such estimates, although a positive response to selection was always reported (Ford and Haskin, 1987; Naciri-Graven et al., 1998; Davis and Barber, 1999; Nell et al., 1999; Dove et al., 2013a; Frank-Lawale et al., 2014).

In our paper, we report our efforts to increase the survival of and resistance to OsHV-1 in *C. gigas* spat using mass selection by breeding disease survivors throughout four generations of selection. Enhanced survival as a result of this selection was investigated using two sizes of oyster, and the realized heritability for this trait was estimated for the first time in an oyster species. In addition, the growth parameters (individual shell height and individual whole weight) and yield (total weight of live oysters) were also recorded to estimate the effect that survival mass selection had on these important commercial traits.

2. Materials and methods

Five generations were produced in this study including one generation of multiplication using the wild oysters as parents and four generations of selection. To avoid confusion, the generations were numbered according to selection chronology as follows: G_0 for no selection, G_1 for the first generation of selection and so on.

2.1 Base population (G_0)

Wild oysters were sampled from two oyster reefs at Agnas (45°52’23’’N, 1°10’15’’W) and at La Tremblade (45°46'53" N, 1°7'19" W), both located in the Marennes-Oléron bay, in December 2008. We considered that the two oyster reefs were from the same genetic population, which was supported by a recent study that showed the lack of genetic differentiation between several French oyster populations (Rohfritsch et al., 2013). The two stocks of wild oysters constituted the parents of the base populations from which a mass selection on the basis of survival was carried out within each origin. The stock sampled at Agnas was named line A, and the stock sampled at La Tremblade was named line B. The two lines were placed in the conditioning room at the Ifremer hatchery in La Tremblade until spawning. The seawater temperature was gradually increased from ambient temperature to 21°C for one week, and the seawater was enriched with a cultured phytoplankton diet (*Isochrysis galbana, Tetraselmis suecica* and *Skeletonema costatum*) to favor gametogenesis.

Spawning occurred in February 2009 (Table 1). The oysters were opened, and those that were not ripe were discarded. The others were sexed using a microscope by spreading a small gonad tissue sample on a slide. For each parent, the gametes were collected by stripping the gonads, which were successively sieved to remove large (>60 µm) and small (<20 µm) tissues for the eggs and to remove only the large tissues for the sperm. For line A, seven males were each mated to a pool of eggs from 13 females, and for line B, 9 males were each mated to a pool of eggs from 21 females to produce the base population (G_0) (Table 1). The methodology used for the larvae and spat cultures was as described by Dégremont et al. (2007).

Each line was then assigned to one of two environments: (1) the G_0-CA and G_0-CB were held in our experimental facilities in La Tremblade and in Bouin to avoid disease-related mortality and to produce control (C) groups for the following generations, and (2) the G_0-SA and G_0-SB were exposed to OsHV-1 in field conditions where mortality outbreaks in the *C. gigas* spat populations have been reported (Fig. 1). The field deployment occurred during June 2009 (Table 2).

2.2 First generation (G_1)

Spawns occurred in March 2010 using the same protocols described for G_0. Two groups were produced for each line: the control group (C) G_1-C using G_0-C group, and the selected group (S) G_1-S using the survivors of the mortality outbreaks from the G_0-S group. For line A, ten males were each mated to a pool of eggs from 17 females to produce the G_1-CA group, while 10 males were each mated to a pool of 21 females to produce the G_1-SA group (Table 1). The same approach was used for line B by mating 13 males and 20 females to produce the G_1-CB group and 4 males and 28 females to produce the G_1-SB group (Table 1). For each line, control and selected groups were then deployed in the field four times during June, July, August and September 2010 (Table 2). Thus, G_1-CA and G_1-SA1 as well as G_1-CB and G_1-SB1 were tested in field conditions during the first deployment in June 2010 until October 2010 (Table 2). For the second deployment, G_1-CA and G_1-SA2 as well as G_1-CB and G_1-SB2 were tested in field conditions starting in July 2010 until October 2010 (Table 2), and so on until the fourth deployment (Table 2). Consequently, due to different dates of deployment, A1 to A4 were pseudo replicates of the same G_1-SA spawn using survivors of the G_0-SA from the line A selected group, and B1 to B4 were pseudo replicates from the same G_1-SB spawn using survivors of the G_0-SB from the line B selected group (Fig.1). This multiple deployment approach was used to strengthen the result for the response to selection and to investigate size-dependent responses of the oysters when exposed to OsHV-1 as it has been shown that larger oysters can exhibit greater disease resistance (Dégremont, 2011).

2.3 Second to fourth generations (G_2 to G_4)

Spawns occurred in February 2011, March 2012 and February 2013 for the G_2, G_3 and G_4, respectively (Table 1). For these generations, a pool of sperm was mated to a pool of eggs for each spawn. This process was different than that used for the two previous generations in which each male was mated to a pool of eggs. For each generation, two groups were also produced for each line, the control group C and the selected group S. For the control group, 10 males and 14 females of the G_1-CA group were mated to produce the G_2-CA group for line A, whereas 11 males and 19 females of the G_1-CB group were mated to produce the G_2-CB group (Fig. 1) (Table 1). In contrast to the G_1-S groups, the G_2-S groups for each line had four replicate spawns using different breeders from the G_1-S groups, and so generating 4 sub-lines in the selected group. Thus, for line A, the survivors of the G_1-SA1 were used to produce the G_2-SA1, the survivors of the G_1-SA2 were used to produce the G_2-SA2, the survivors of the G_1-SA3 were used to produce the G_2-SA3 and finally, survivors of the G_1-SA4 were used to produce the G_2-SA4 (Fig. 1). The same approach was used for line B, which also contained four sub-lines spawns named G_2-SB1 to G_2-SB4. For the subsequent generations G_3 and G_4, one control per line was produced, and each sub-line, A1 to A4 and B1 to B4, was generated using survivors of the previous generation (Fig. 1). To summarize, five spawns using different spawners (one control C and four selected sub-lines S) were produced for each line from the G_2 to G_4. Each spawn involved an average of 11 males and nine females, and the exact number of males and females for each spawn are provided in Table 1 along with the

inbreeding rate throughout all generations and spawns. As was done for G_1, all groups were deployed into the field four times during the summer season on a monthly basis from May to October (Table 2). For example, G_2-SA1 to A4 and G_2-SB1 to B4 along with their respective controls were deployed on 05/31/11, 07/07/11, 08/03/11, and 09/01/11. As indicated in the previous section, this approach of multiple oyster deployments was utilized to investigate the response to selection when exposed to OsHV-1 according to oyster size. Finally, the oysters used to produce the subsequent generation from the selected group were only those assessed during the first field deployment. Thus, for the selected group from line A, the G_4-S A1 was produced from the G_3-S A1 deployed on 06/20/12, which was produced from the G_2-S A1 deployed on 05/31/11 (Table 2) (Fig. 1). This strategy is explained by the low survival for this deployment, which increased the intensity of selection.

2.4 Field testing

For the second year in a row, mortality outbreaks related to OsHV-1 were observed along the entire French coast in 2009. Consequently, regulatory rules had forbidden oyster transfers between growing areas in the spring of 2009. As a result, the oysters could not be tested in our experimental farms located at Agnas in the Marennes-Oléron Bay as they were grown at the Ifremer nursery located in Bouin in the Bourgneuf Bay. As an alternative, we decided to test the G_0-S oysters in an interdital area in front of the nursery in the Bourgneuf Bay (46°56'26" N, 2°7'10" W). For each line, one bag (7 mm mesh size, 100 x55 cm) containing 2 kg of spat (mean individual weight of 1 g) was deployed in the interdital area where many other oyster farms surrounded our experimental farm. Mortality was recorded one month post-deployment as a check point and three months later as an endpoint (Table 2). The total weight of the live oysters at the endpoint date was recorded, giving the yield as the product of survival and growth.

The selected groups and control groups from each line spanning G_1 to G_4 were tested at the Ifremer experimental farm located at Agnas in the Marennes-Oléron Bay. For each deployment, two bags (7 mm mesh size, 100 x27 cm) per spawn containing 150 oysters each were tested, and the mean individual weight of the oysters ranged from 1 to 11 g (Table 3). The total weight of the live oysters was recorded at deployment and at the endpoint dates to estimate the yield, which was standardized to 1 kg of spat tested. The mortality was recorded in the fall, but an additional check-up was conducted one month post-deployment for some trials (Table 2). In addition to survival, 30 oysters per spawn were individually measured for shell height (in millimeters) and weight (in grams) at deployment and at the endpoint for G_4. The number of oysters was of course reduced for spawns showing heavy mortality at the endpoint. From 2010 to 2013, the seawater temperature was recorded using a YSI probe #6600 to monitor environmental fluctuations in the Pertuis Charentais (SAPERCHAIS/LERPC).

2.5 Detection and quantification of OsHV-1

No groups showed disease mortality prior to deployment, and OsHV-1 DNA had never been detected in any of the animals (several hundred) sampled from our hatchery and nursery prior to their deployment since 2008 (Dégremont and Benabdelmouna, 2014; Pernet et al., 2014). Thus, disease sampling was only focused on the period two weeks post-deployment when the first moribund oysters were observed, and this corresponded to the onset of mortality outbreaks related to OsHV-1 when *C. gigas* spat are deployed during the risk period when seawater temperatures is above 16°C (Pernet et al., 2012; Dégremont, 2013). Twelve

moribund oysters were sampled either in the S or C groups at two weeks post-deployment for the first deployment of all generations except for G_3. We focused on the first deployment as survivors of the selected group were used to produce the following generations. Detection and quantification of OsHV-1 DNA was carried out for each of the individual oysters using the SYBR® green real-time PCR protocol as described by Pépin et al. (2008) adapted for use with DPFor/DPRev primers to target the OsHV-1 DNA polymerase sequence (ORF 100). The results were expressed as the viral DNA copy number per mg of oyster tissue.

2.6 Data analyses

Statistical analyses are only presented for data collected at the endpoint. All statistics were performed using the SAS® 9.4 software.

2.6.1 Survival

A comparison of the survival between the C and S groups was analyzed within line and within generation for each deployment date by a binomial logistic regression using the GENMOD procedure with the following model:

$$\text{Logit}\,(Y_{il}) = \mu_i$$

For G_4, we fitted a complete model as follows:

$$\text{Logit}\,(Y_{ijkl}) = \mu_i + \mu_j + \mu_k + \mu_i \times \mu_j + \mu_i \times \mu_k + \mu_k \times \mu_j + \mu_i \times \mu_k \times \mu_j$$

where Y_{il} and Y_{ijkl} = the observed survival at the endpoint for oyster l in group i (control or selected) from line j (A or B) tested at deployment k (1 to 4). All effects and their interactions were fixed. When a significant interaction was observed, the SLICE option was used. This approach allows for a more powerful analysis than rerunning the model for each effect because the degrees of freedom are not reduced (Littell et al., 2002).

2.6.2 Estimation of the realized heritability of survival

This experiment is a mass selection based on a threshold trait. Thus, the survival trait is a function of an underlying variable, which is the level of resistance of the oyster to OsHV-1 infection, hereafter called the liability. Therefore, all oysters possessing a level of resistance to OsHV-1 infection higher than the threshold will survive, while the others will die. Unfortunately, this threshold is not fixed and is decreased for larger oyster size and to a lesser extent for older oysters (Dégremont, 2013). Additionally, the threshold could also vary according to field conditions, which is an uncontrolled environmental variable. However, the C (control) group produced for each generation in each line allowed us to assess the effects of changing environmental conditions during the course of the experiment (Roff, 1997). The response to selection was the difference in survival between the selected group and their respective controls within generations on the liability scale. The selection differential was the mean liability of the selected parents in the previous generation as it deviated from the mean liability of the population, given the intensity of selection i as reported in Appendix Table A by Falconer and Mackay (1996). The cumulative selection differential was then the sum of the selection differential for each generation. The realized heritability was estimated within and among the lines as the slope of the response to selection to the cumulative selection differential, which was forced through the origin for both selected and controls derived from

the same base population (Falconer and Mackay, 1996; Roff, 1997). The standard error of the realized heritability was the standard error of the slope from the simple linear regression, which was estimated by the REG procedure with the NOINT option. In detail, the data used for the estimation of the realized heritability are represented in bold and italics in Table 3, and correspond to the realized heritability to increased survival and OsHV-1 resistance for oysters having an average size of 1 g when exposed to the disease (only the survivors of the first deployment of G_2 and G_3 were used to produce the subsequent generation). In addition, the realized heritability was also estimated using the survival data obtained for the last deployment of G_4, which could be interpreted as an oyster size greater than 3 g.

2.6.3 Yield and growth

For yield and growth, only the results of the first deployment of the last generation G_4 were analyzed to focus on the potential effect of mass selection to improve oyster survival and resistance to OsHV-1 infection. Meanwhile, similar results were observed in the other deployments and in the earlier generations of selection (data not shown).

The yield, which was defined as the total weight of the live oysters at the endpoint, was standardized for 1 kg of spat tested, log transformed and analyzed using the GLM procedure by running an ANOVA using the following model:

$$\mathrm{Log}(Y_{ijkl}) = \mu + \mu_i + \mu_j + \mu_i \times \mu_j + \mu_{k(ij)} + \varepsilon_{ijkl}$$

where μ is the intercept, μ_i *and* μ_j are the fixed effect of the line (A or B) and group (control or selected), respectively, $\mu_{k(ij)}$ is the random variation in the bag, and ε_{ijkl} is the error term.

Similarly, the length and weight data were log transformed and analyzed using the GLM procedure by running an ANCOVA with time as a covariate.

3. Results

3.1 Seawater temperature

The seawater temperature in the Bourgneuf Bay was greater than 16°C during the field testing period in the Bourgneuf Bay. At Agnas, the seawater temperature from June 2010 to October 2013 is shown in Fig. 2 and was above 16°C during at least three weeks post-deployment for all deployment periods and generations, except the last deployment of G_3 in which the seawater temperature rapidly decreased below 16°C at the end of October 2012 (Fig. 2).

3.2 Detection and quantification of OsHV-1

For all generations, except for the G_3 for which no disease sample was obtained, OsHV-1 DNA was detected in all moribund oysters sampled during the peak of mortality. The viral load was high and exceeded 10^{+6} DNA copies per mg of fresh oyster tissue.

3.3 Survival

The onsets of mortality outbreaks were always observed within the two weeks post-deployment, except for the last G_3 deployment on October 2nd, 2012. When possible, comparison of the mortality recorded one month post-deployment with those recorded during the fall indicated that 90% of the cumulative mortality occurred during the first month post-deployment (data not shown).

At the endpoint, the survival for both lines of the base population G_0 was low, with 8.9% for line A and 22.4% for line B (Table 3).

For the G_1, the mean survival of the control group during each of the four deployments was again low with 4.7% and 19.9% for lines A and B, respectively, while it was 41.8% and 27.1% for the selected group (Table 3). For both lines, the survival of the selected group was significantly higher than that the control ($P < 0.0001$), except for the two last deployments of line B (Table 3). For the following generation G_2, the mean survival for the four deployments remained low for the control group with 11.0% and 18.3% for lines A and B, respectively. In contrast, the mean survival of the selected group was higher in G_2 than in G_1 with 55.2% and 61.8% for lines A and B, respectively. Within deployment and within line, the survival of the selected group was higher than the survival of the control group ($P < 0.0001$).

For the G_3 excluding the last deployment due to the absence of mortality, the mean survival of the control group was 8.7% and 5.6% for lines A and B, respectively, and 49.7% and 64.9% for the selected group (Table 3). Within deployment and within line, the survival of the selected group was higher than the survival of the control group ($P < 0.0001$).

For the G_4, the mean survival of the control group for the four deployments was still low with 6.3% and 8.3% for lines A and B, respectively, while it was higher for the selected group with 65.5% and 72.6%, respectively (Table 3). The overall survival analysis for G_4 revealed the presence of a significant group by deployment interaction ($P = 0.04$) (Table 4). At the group level, the selected group had a higher survival for the last deployment ($P < 0.0001$), while the control group had a similar survival whenever they were deployed in the field ($P = 0.46$) (Tables 3 and 4). No significant interaction was found between the group and the line ($P = 0.87$), and survival was found to be similar between lines ($P = 0.18$). In contrast, a significant difference in survival was found between groups, with the highest survival for the selected group and the lowest survival for the control group ($P < 0.0001$) (Table 4).

Overall, the survival across deployments of the selected group increased from 41.8% to 59.2% for the line A from the G1 to G4 respectively, and from 27.1% to 72.6 % for the line B while survival of the control group never exceeded 11.0% and 19.9% for lines A and B respectively (Table 3)

3.4 Realized heritability for resistance to OsHV-1 and response to selection according to size

For a 1-g oyster exposed to OsHV-1, the survival of the selected group over the survival of the control group regularly increased from +12.0% and +16.3% for lines A and B, respectively in the G1 the G_1 to +43.9% and +52.4% in the G_4 (Table 5). At each generation, the survival increased by 11.0% for line A and 13.1% for line B (Table 5). The realized heritability after four generations of mass selection to enhance survival in *C. gigas* spat, i.e., resistance to OsHV-1 infection, was moderate, with $h^2 = 0.34 \pm 0.05$ for line A, $h^2 = 0.52 \pm 0.03$ for line B and $h^2 = 0.41 \pm 0.03$ for both lines (Fig. 3ABC). With regards to the response to selection for larger oysters (>3 g), the gain of survival of the G_1 was high for the line A with +59.8%, while it was weak for the line B with +2.8%. Meanwhile, both lines showed high gains in survival in G_4 with +80.2% and +80.6% for lines A and B, respectively. The gains in survival per generation for oysters >3 g was higher than for 1-g oysters at 20.2% (Table 5). The realized heritability estimated using the data from the last deployment of G_4 revealed higher estimates than using the data of the first deployment of G_4 with $h^2 = 0.46 \pm 0.05$ for line A, $h^2 = 0.63 \pm 0.04$ for line B and $h^2 = 0.52 \pm 0.03$ for both lines (Table 5).

3.5 Yield and growth

For the first deployment of G_4, the mean individual shell height and weight for both lines at deployment were 17.7 mm and 1.0 g for the control group, respectively, and 17.1 mm and 0.9 g for the selected group. At the endpoint on October 8th, 2013, the mean individual shell height of the control group was 51.4 mm, and it was significantly lower than the mean individual shell height of the selected group at 58.4 mm (Fig. 4A) (Table 6). The same result was also observed for the whole oyster weight with a gain of 4 g for the selected group (19.4 g) over the control group (15.2 g) (Fig. 4B). The interaction between groups and lines was not significant, and both lines showed similar growth trends for both traits (Table 6).

The mean standardized yield, which is the total weight of all live oysters at the endpoint for 1 kg of spat tested, of each line and each group for the first deployment of G_4 in 2013 is presented in Fig. 4C. For 1 kg of spat deployed on May 29th, 2013, the standardized yield of the control group on October 8th, 2013 was 1.1 kg and 1.3 kg for lines A and B, corresponding to increases of 10% and 30%, respectively. In contrast, the standardized yield of the selected group was 11.5 kg and 15.0 kg for lines A and B, respectively. A significant difference in yield was found between groups ($P<0.0001$), and all other effects were not significant (Table 7).

4. Discussion

This study was conducted in the field at Agnas in the Marennes-Oléron Bay, where, in addition to OsHV-1, other pathogens or factors could have been involved in the mortality observed in our oysters. Meanwhile, the onset and kinetics of mortality combined with the high amount of OsHV-1 DNA found in moribund oyster tissues strongly supports that OsHV-1 could be considered as the main factor of the mortality outbreaks observed each year from 2009 to 2013. Furthermore, OsHV-1 is considered to be the main cause of mortality in *C.*

gigas when viral load exceeds 10^{+5} DNA copies per mg of fresh oyster tissue (Pépin et al., 2008; Sauvage et al., 2009; Oden et al., 2011; Schikorski et al., 2011).

Whatever the generation and the deployment, the survival of the control groups was usually lower than 20%, indicating that OsHV-1 was very active during the course of the experiment. For both lines and all deployments, survival of the selected *C. gigas* spat increased by 22.2%, 43.9%, 50.2% and 61.8% for G_1, G_2, G_3 and G_4, respectively, indicating that selection for resistance to OsHV-1 was successful (Table 3). These values are in the range obtained for other mass selection trials for survival to enhance disease resistance in oysters, such as resistance to *Martelia sydneyi* (QX disease) in *Saccostrea glomerata* (Nell and Hand, 2003; Nell and Perkins, 2006; Dove, et al., 2013a) and resistance to *Haplosporidium nelsoni* in *C. virginica* (Ford and Haskin, 1987).

Upon closer inspection, the mean gain of survival per generation ranged from 11.0% to 13.1% for oysters weighing 1 g, indicating that the mean survival of selected oysters could jump from 20% to 64-72% in only four generations of selection, which used to be the survival routinely observed in wild-caught *C. gigas* spat before 2008 in the Marennes-Oléron Bay (Mille et al., 2014). More interestingly, the size of the oysters when they were exposed to OsHV-1 had a significant impact on the response to selection, which was twice as much per generation for oysters heavier than 3 g in comparison with those weighing 1 g (Table 5). It was demonstrated that larger unselected *C. gigas* spat, and to a much lesser extent older oysters, are less susceptible to the disease than are the smaller spat, with a positive relationship between survival and size: 13%, 20% and 30% for oysters weighing 1 g, 5 g and 10 g, respectively (Dégremont, 2013). This finding suggest a higher resistance to the disease in larger oysters considering that the threshold did not change between sizes, allowing a higher proportion of oysters to survive. The underlying mechanisms of such resistance are still unknown. Consequently, in addition to selection, a culture strategy on oyster farms to maximize the survival against OsHV-1 could easily be implemented as already described (Dégremont, 2013) and was first described during the summer mortality phenomena during 2001-2003 (Dégremont et al., 2010c). One of these strategies could involve deployment of the selected *C. gigas* spat after the threat of exposure to OsHV-1 to utilize a site and cultural practices that favor rapid growth, and thus, promote the response to selection.

As the selection was completed using oysters weighing 1 g when exposed to OsHV-1 with the exception of the G_2, which used G_1 parents from each deployment (see Table 3), it is interesting to note that the gain in survival was higher for line B than for line A (Table 5). The hypothesis to explain this finding relied on a founder population effect. Indeed, the survival of the G_0 was higher for line B than line A indicating a higher resistance to OsHV-1 for line B as early as the G_0. Thus, genetic resistance was more rapidly developed in line B, even if the gain did not increase between G_3 and G_4 in contrast to line A (Table 5). Still, the gain of survival is expected to decrease for the following generations due to the decreased intensity of selection resulting in a higher survival rate of the selected oysters. However, even if all selected oysters were resistant to OsHV-1, survival of the selected group will reach a threshold that should be undeniably lower than 100%.

It is also worth noting that the mean gain in survival for the four deployments was quite similar between lines A and B from G_2 to G_4, but different in the G_1 with a mean gain in survival of 37.1% in line A compared with 7.2% in line B (Table 3). In a closer inspection of the data, this result is explained by a much higher survival of the G_1-SA3 (63.0%) and G_1-SA4 (70.3%) while their corresponding controls still had low survival (<9%) (Table 3). The

selected group of line A was able to develop OsHV-1 resistance in a very short period of time, and to date, this increase in size and age related to a sudden increase in resistance remains unexplained.

Our study is the first to report findings concerning the response to mass selection for survival and resistance to OsHV-1 infection in *C. gigas* spat. Furthermore, the realized heritability was estimated after four generations of selection to obtain an accurate estimate of heritability and to avoid the response of a single generation, which is frequently very variable (Roff, 1997). The realized heritability for OsHV-1 resistance estimated for oysters weighing 1 g when exposed to OsHV-1 was moderate, ranging from 0.34 to 0.52, and increased from 0.46 to 0.63 for larger oysters (Table 5). These results strongly support that a breeding program to enhance survival and OsHV-1 resistance would be successful due to a significant additive genetic variance. Unfortunately, there is not any value in comparing *C. gigas* to other oyster species because all mass selection for disease resistance failed to provide such estimates. Meanwhile, our estimates of realized heritability for OsHV-1 resistance in *C. gigas* spat are in agreement with those obtained for survival of this species in France (narrow sense heritabilities at 6 months of age ranged from 0.47 to 1.08; Dégremont et al., 2007, 2010b), on the West Coast of USA (h^2 broad sense at 6 months of age = 0.49 – 0.71; Evans and Langdon, 2006), in Japan (h^2 narrow sense at 1 year of age = 0.77; Usuki, 2002) and in Australia (h^2 at 21 months of age = 0.68; Ward et al., 2005). However, all results were not related to OsHV-1 infection due to the absence of disease sampling, but it is worth noting that OsHV-1 is present in all of these countries (Burge, et al., 2006; Garcia, et al., 2011; Shimahara et al., 2012; Jenkins et al., 2013). This was strongly supported through a similar mortality data for *C. gigas* spat in France obtained before and after 2008. Indeed, the same oyster families were tested, and OsHV-1 was detected in moribund oysters during several mortality outbreaks for all of the generations produced between 2001 and 2003. This suggested that OsHV-1 could have been one of the main causes of the summer mortality phenomenon for which the high heritability (narrow and realized) for survival in the *C. gigas* spat was found (Dégremont, et al., 2007; Dégremont, et al., 2010b).

Although there are no data to compare with other oyster species, the heritability of disease resistance has been reported in other important aquaculture species. Several studies reported low heritability such as the White Spot Syndrome Virus in shrimps (Gitterle et al., 2006; Cock et al., 2009), but others reported moderate to high heritability for disease resistance and/or survival in fish species (Henryon et al., 2005; Ødegård et al., 2007; Gjerde et al., 2009; Taylor et al., 2009). Our study is in agreement with those reporting high genetic variation for disease resistance and such findings seems common in aquatic species as suggested by Gjedrem and Baranski (2009) and confirmed for example by a recent review on disease resistance in salmonid species with heritability for resistance to *Aeromonas salmonicida* in *Salmo salar* ranging from 0.34 to 0.62 (Yáñez et al., 2014).

Our experimental breeding program is one strategy, along with family selection, mostly used by the French commercial hatcheries to enhance OsHV-1 resistance in *C. gigas* spat. Despite the fact that mass selection to improve survival of *C. gigas* spat was successful, it was done without a true control at the inbreeding level. Although the inbreeding level ranged from 0.13 to 0.15 after four generations of selection (Table 1), the true inbreeding rate is expected to be much higher. There is a high variance in reproductive success in the Pacific oyster, which was attributed to the gamete quality, sperm–egg interaction and differential viability among genotypes (Boudry et al., 2002). Their study also showed a 20% decrease of the effective population size under sperm competition, as done in our study from G_2 to G_4, in comparison

to no sperm competition, as done in our study for the G_0 and G_1. Our selection was made on survival by selecting OsHV-1-resistant oysters using high intensity of selection due to low survival rate of the selected oysters for both lines in G_0 and to a lesser extent for the other generations (Table 3). Simulations showed that the rate of inbreeding was higher when the heritability was high because a higher heritability increases the similarity between full-sib progeny and consequently increases the probability of selecting full-sibs as broodstock (Bentsen and Olesen, 2002). This is particularly true in fish and shellfish species with very high fecundity, such as *C. gigas*, which produce several millions eggs and billions of sperm. This may have quickly produced highly inbred lines because only a few parents are needed to reproduce each generation (Gjedrem and Baranski, 2009). Consequently, the moderate realized heritability (0.34 for line A and 0.52 for line B – Table 5) combined with the high intensity of selection and the high fecundity of oysters may have reduced the response to selection, and thus, the realized heritability.

Selection to improve survival and resistance to OsHV-1 resistance was successful after four generations of mass selection in *C. gigas* spat. The impact of selection on other growth traits was assessed for the first deployment of G_4 and was shown in this study. For both lines, the oysters of the control groups had a lower whole weight and lower shell height than did the selected groups (Fig. 4ab), suggesting that selection to improve resistance to OsHV-1 infection did not reduce but rather increased growth. Genetic correlations between growth and survival of the summer mortality phenomenon, for which OsHV-1 was highly suspected to be implicated, were null or low but positive, which could explain our results (Dégremont, et al., 2007). Similar results were also observed for the previous generations and latter deployments, but the data were not shown for the clarity of this paper. However, such traits should be recorded until market size is reached to confirm the lack of a negative relationship between survival and growth, but the results reported during the first year were already encouraging. Nevertheless, other studies investigating the growth and survival related to disease resistance until market size is reached never showed a lower growth of the selected lines over the controls, as observed for resistance to *Perkinsus marinus* and *H. nelsoni* in *C. virginica* (Frank-Lawale, et al., 2014), or for resistance to *Bonamia roughleyi* and *M. sydneyi* in *S. glomerata* (Dove, et al., 2013a; Dove et al., 2013b). Once again, care must be taken regarding the correlation between growth and survival related to disease resistance, as the breeders might also have been selected for growth too, such as in *S. glomerata* for which the breeding program was originally focused on mass selection of the largest individual growth before incorporating selection for QX resistance (Nell et al., 2000).

More interestingly, the yield of the selected groups was 10- to 15-fold higher than the yield of the control (Fig. 4c). Similar findings were observed for the same time period and using diploid and triploid oysters selected for their higher resistance to OsHV-1 after the mortality outbreak (Dégremont et al., 2014). As the yield is hugely affected by survival in sites where mortality outbreaks are reported whereas it is growth in sites without mortality (Evans and Langdon, 2006; Dégremont, et al., 2010b; Dégremont et al., 2012), selection to improve disease resistance will automatically improve the yield of the oysters. Meanwhile, such high performance of the selected groups (survival and growth) would result in splitting the oyster bag quite rapidly in order to not lose this advantage due to bag overcrowding, which was attained in our study at the endpoint. If not done, the selected oysters will reduce or stop their growth, and the correlation between growth and survival will switch from positive to negative due to inappropriate culturing practices.

In conclusion, our study clearly demonstrates that mass selection for survival and OsHV-1 resistance was successful after four generations of selection, indicating a significant genetic improvement for the selected trait. The gain per generation of selection was 12.1% for oysters weighing 1 g when exposed to OsHV-1, and the gain was almost twice as much for oysters heavier than 3 g at 20.2%. Our study is the first to provide estimates of the realized heritability for disease resistance using a mass selection scheme in an oyster species, with values ranging from 0.34 to 0.63, depending on the oyster size. Apparently, selection for resistance to OsHV-1 infection in our study improved growth in comparison with the controls, and this selection automatically improved the yield of *C. gigas,* which was 10- to 15-fold in our study. Mass selection could then be easily implemented by a commercial hatchery that cannot afford family-based selection that involves the production of numerous families for the base population.

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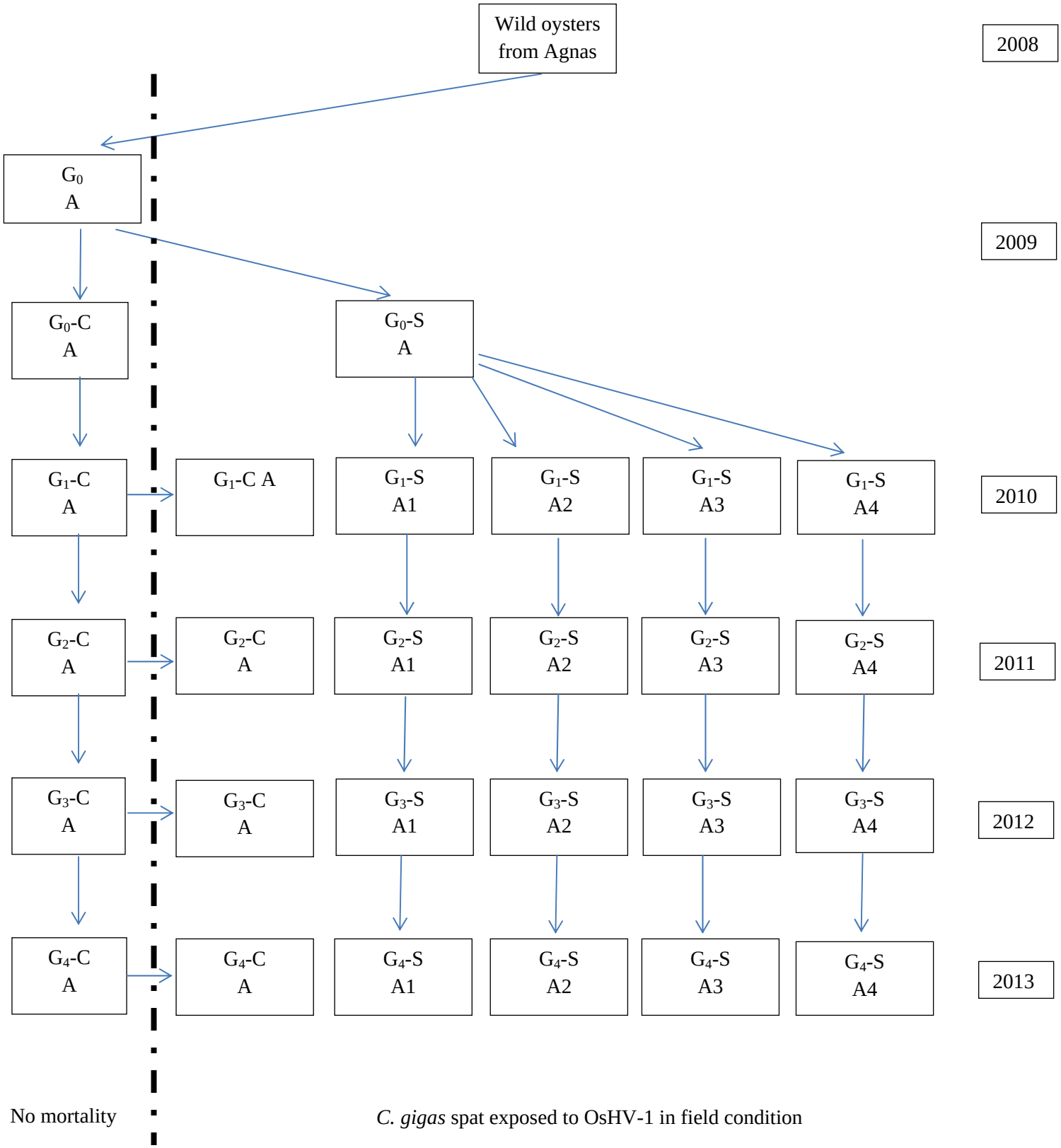


Fig. 1: Summary of the production of the selected (S) and control (C) groups of line A bred from wild oysters sampled at Agnas in the Marennes-Oléron Bay in 2008 (the same approach was used for line B but using wild oysters sampled at La Tremblade).

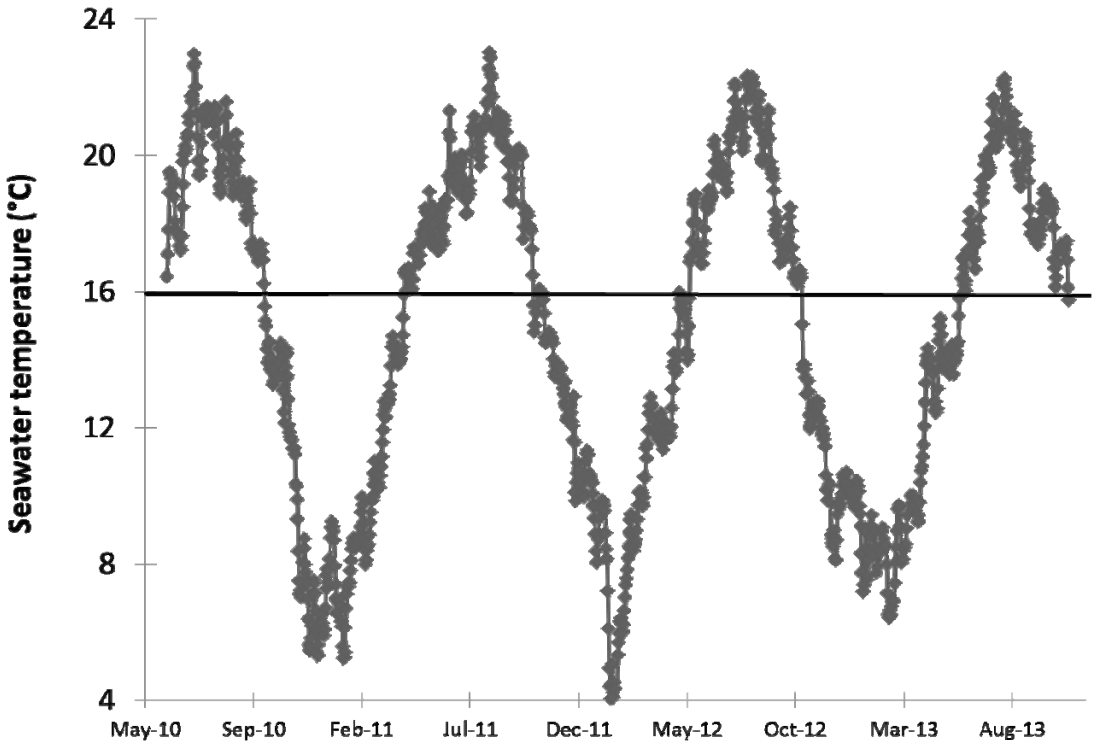
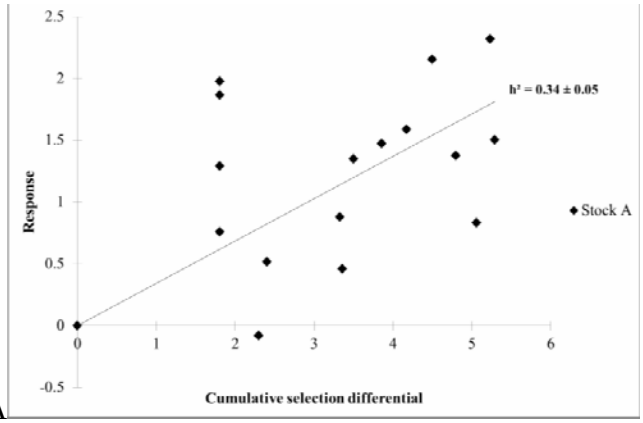
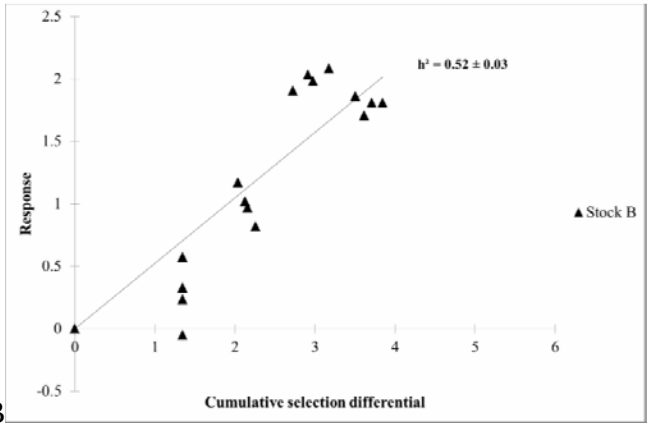


Fig. 2: Seawater temperature (°C) at Agnas from June 2010 to October 2013. The horizontal line represents the threshold of 16°C, beyond which mortality related to OsHV-1 is usually observed in our experimental field.

A



B



C

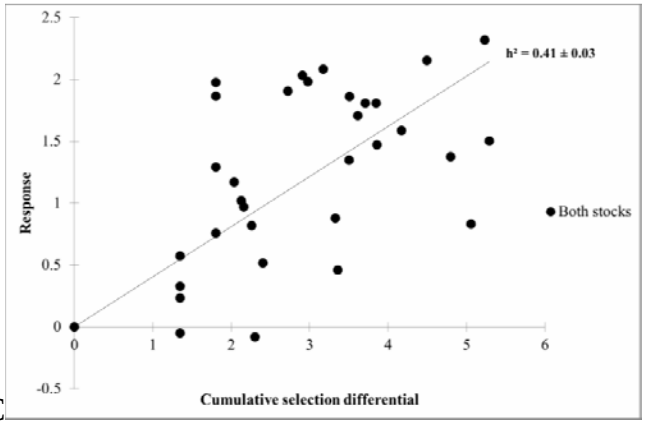


Fig. 3: Realized heritability (slope of the cumulative response according to the cumulative selection differential) to increase survival and resistance to OsHV-1 in C. gigas spat for each line (A & B) and both lines (C) for a size of 1 g when exposed to the disease.

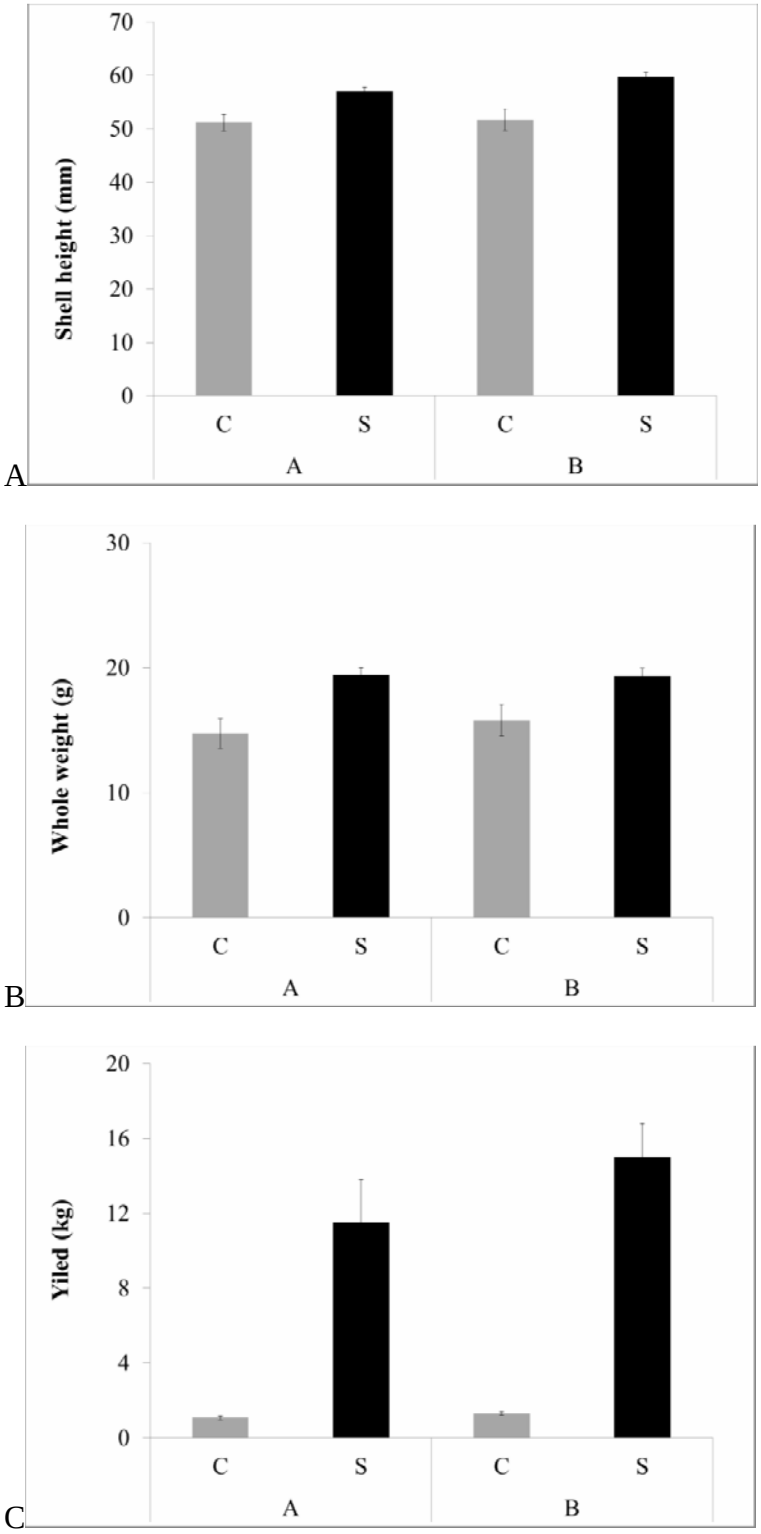


Fig. 4: Mean individual shell height (mm) (Fig. A), oyster weight (g) (Fig. B) and yield (kg) (Fig. C) of the control (C in grey) and selected (S in black) groups for both lines for the first deployment of G_4 at Agnas in October 2013. The yield is given for 1 kg of spat deployed on May 29th, 2013, and errors bars represent the SE.

Table 1 Summary of the production of each spawn, date of the spawn, number of females (Nf) and males (Nm), effective size (Ne) and rate of inbreeding (F) in both groups (control C and selected S) and both lines (line A and line B) from G_0 to G_4

				Line A					Line B				
Generation	Spawn date	Groups	Replicate[a]	Nf	Nm	Ne[b]	ΔF[c]	F[d]	Nf	Nm	Ne[b]	ΔF[c]	F[d]
G_0	02/23/09			13	7	18.2	0.03	0.03	21	9	25.2	0.02	0.02
G_1	03/15/10	C	X	17	10	25.2	0.02	0.05	20	13	31.5	0.02	0.04
		S	X	21	10	27.1	0.02	0.05	28	4	14.0	0.04	0.05
G_2	02/01/11	C	X	14	10	23.3	0.02	0.07	19	11	27.9	0.02	0.05
		S	X1	7	14	18.7	0.03	0.07	7	7	14.0	0.04	0.09
		S	X2	3	16	10.1	0.05	0.09	8	7	14.9	0.03	0.09
		S	X3	8	6	13.7	0.04	0.08	5	12	14.1	0.04	0.09
		S	X4	7	10	16.5	0.03	0.07	8	8	16.0	0.03	0.08
G_3	03/05/12	C	X	18	12	28.8	0.02	0.08	23	5	16.4	0.03	0.08
		S	X1	8	22	23.5	0.02	0.09	18	16	33.9	0.01	0.10
		S	X2	9	15	22.5	0.02	0.09	8	10	17.8	0.03	0.11
		S	X3	13	11	23.8	0.02	0.09	9	11	19.8	0.03	0.11
		S	X4	7	16	19.5	0.03	0.09	6	22	18.9	0.03	0.11
G_4	02/12/13	C	X	6	21	18.7	0.03	0.11	10	20	26.7	0.02	0.10
		S	X1	4	11	11.7	0.04	0.13	5	5	10.0	0.05	0.15
		S	X2	5	6	10.9	0.05	0.13	10	3	9.2	0.05	0.15
		S	X3	5	8	12.3	0.04	0.13	6	8	13.7	0.04	0.13
		S	X4	5	6	10.9	0.05	0.13	5	10	13	0.04	0.14

[a] The letter X should be replaced by the name of the line, either 'A' or 'B'. For the line A, the first replicate spawn of the selected group of the G_2-SA1 used 7 females and 14 males of the survivors of the G_1-SA1 (deployed in June 2010, see table 2). Then, 8 females and 22 males among the survivors of the G_2-SA1 were used to produce the G_3-SA1, and finally, 4 females and 11 males among the survivors of the G_3-SA1were used to produce the G_4-SA1.

[b] Ne is the effective size calculated as $Ne = 4\ Nf\ Nm / (Nf + Nm)$ (Falconer and Mackay, 1996),

[c] $\Delta F = 1/2Ne$ is the new inbreeding of the *t* (0 to 4) generation,

[d] the inbreeding rate F was calculated using the formula $Ft = \Delta F + (1- \Delta F)\ Ft\text{-}1$ (Falconer and Mackay,1996). This value is the minimum inbreeding rate, and extreme care must be taken as the inbreeding rate should be higher due to the high variance in reproductive success in the Pacific oyster (Boudry et al., 2002). In addition, because selection was made on the basis of survival by selecting OsHV-1 resistant oysters, and it is probable that some of them may be close relatives.

Table 2 Summary of the key dates (MM/DD/YY) for the field testing at each generation.

		Field testing				
Generation	Line	Deployment[a]	Groups tested[b]	Start date	Check date	End date
G_0	A & B	**1**	**SX**	**06/09/09**	07/08/09	09/06/09
G_1[c]	A & B	**1**	CX & **S X1**	**06/15/10**	07/13/10	10/20/10
		2	CX & **S X2**	**07/13/10**	08/09/10	10/20/10
		3	CX & **S X3**	**08/11/10**	09/10/10	10/20/10
		4	CX & **S X4**	**09/07/10**		10/20/10
G_2[d]	A & B	**1**	CX & **S X1 to 4**	**05/31/11**	06/26/11	08/30/11
		2	CX & S X1 to 4	07/07/11	08/17/11	08/30/11
		3	CX & S X1 to 4	08/03/11		09/06/11
		4	CX & S X1 to 4	09/01/11		10/06/11
G_3	A & B	**1**	CX & **S X1 to 4**	**06/20/12**	07/18/12	12/13/12
		2	CX & S X1 to 4	07/18/12	08/22/12	12/13/12
		3	CX & S X1 to 4	08/21/12	09/18/12	12/13/12
		4	CX & S X1 to 4	10/02/12		12/13/12
G_4	A & B	1	CX & S X1 to 4	05/29/13		10/08/13
		2	CX & S X1 to 4	06/25/13		10/08/13
		3	CX & S X1 to 4	07/23/13		10/08/13
		4	CX & S X1 to 4	08/20/13		10/08/13

[a] The oysters used to produce the next generation are in bold.

[b] The letter X should be replaced by the name of the line either 'A' or 'B'.

[c] For the first deployment of the G_1, lines A and B were tested in the field starting on 06/15/10. For each line, the control group C and the selected group S were exposed to OsHV-1, and the survivors of this deployment were spawned to produce the G_2-SA1 and G_2-SB1 for lines A and B, respectively. The same approach was used for the second deployment, but the survivors produced the spawns G_2-SA2 and G_2-SB2 for lines A and B, respectively, and so on until the fourth deployment.

[d] For the first deployment of the G_2, lines A and B were tested in the field. For each line, there was a control group C and the selected group S, which had four replicate spawns (SA1 to SA4 for line A, and SB1 to SB4 for line B). The survivors of this deployment were used to produce the next generation (in bold). For the other deployments of the G_2, the CA and CB of the control group, and SA1 to A4 for line A, and SB1 to SB4 for line B of the selected group were all tested at each deployment, but they were not used to produce the next generation.

Table 3 Survival at the endpoint for the control (C) and selected (S) groups of each line for each deployment of each generation, and gain in survival of the S group over the C group

Generation	Deployment	Group	At deployment		Line A			Line B			Lines A & B
			Age (months)	Size (g)	C (%)	S[c] (%)	Gain (%)	C (%)	S[c] (%)	Gain (%)	Mean gain (%)
G_0	1	S	4	1		**8.9**			**22.4**		
G_1	1	C & S1	3	1	**4.0**	**16.0**	12.0	**13.0**	**29.3**	16.3	
	2	C & S2	4	3	**1.3**	**15.0**	13.7	**10.3**	**17.0**	6.7	
	3	C & S3	5	6	**5.0**	**63.0**	58.0	**18.7**	**25.7**	7.0[ns]	
	4	C & S4	6	9	**8.7**	**70.3**	61.7	**37.7**	**36.3**	-1.4[ns]	
	1-4	C & S 1 to 4[b]		mean	4.7	41.8	37.1	19.9	27.1	7.2	22.2
G_2	1	C & S 1 to 4[a]	4	1	**17.0**	**31.6**	14.6	**16.3**	**50.0**	33.7	
	2	C & S 1 to 4[a]	5	3	6.7	46.4	39.7	17.7	63.6	45.9	
	3	C & S 1 to 4[a]	6	5	5.3	62.8	57.5	15.7	56.1	40.4	
	4	C & S 1 to 4[a]	7	7	15.0	80.1	65.1	23.7	77.6	53.9	
	1-4	C & S 1 to 4[b]		mean	11.0	55.2	44.2	18.3	61.8	43.5	43.9
G_3	1	C & S 1 to 4[a]	3	1	**2.0**	**34.5**	32.5	**2.7**	**54.8**	52.1	
	2	C & S 1 to 4[a]	4	3	3.0	35.0	33.0	2.3	60.2	57.9	
	3	C & S 1 to 4[a]	5	7	21.2	79.5	58.3	11.9	79.5	67.6	
	4	C & S 1 to 4[a]	6	11	98.7	98.8	0.1[ns]	98.7	98.6	-0.1[ns]	
	1-4	C & S 1 to 4[b]		mean[d]	8.7	49.7	41.0	5.6	64.9	59.3	50.2
G_4	1	C & S 1 to 4[a]	3	1	*6.7*	*50.6*	43.9	*7.2*	*59.6*	52.4	
	2	C & S 1 to 4[a]	4	2	4.3	61.7	57.4	5.0	77.8	72.8	
	3	C & S 1 to 4[a]	5	2	5.7	60.9	55.2	10.5	62.3	51.8	
	4	C & S 1 to 4[a]	6	4	*8.7*	*88.9*	80.2	*10.3*	*90.9*	80.6	
	1-4	C & S 1 to 4[b]		mean	6.3	65.5	59.2	8.3	72.6	64.3	61.8

[a] The survival of the S group is the mean of the four replicate spawns A1 to A4 for line A and B1 to B4 for line B.

[b] Mean survival of the four deployments within group (selected S or control C)

[c] In bold, the oysters used to produce the next generation for the selected group, and in bold and italic, the value used to estimate the realized heritability.

[d] No mortality observed for the last deployment of G_3. It was excluded for the calculation of the mean.

[ns] Not significant. All other comparisons showed a significantly higher survival of the S group ($P<0.0001$) within generation, deployment and line, but this is not indicated for the clarity of this table.

Table 4 Logit analysis of the survival of G_4 in 2013

Source	df	$\chi 2$	P
group	1	446.24	< 0.0001
line	1	1.81	0.18
deployment	3	13.29	0.0041
group x line	1	0.03	0.87
group x deployment	3	8.50	0.04
line x deployment	3	0.18	0.98
line x group x deployment	3	0.87	0.83
Slice option at the group level[a]			
Selected group	3	70.1	< 0.0001
Control group	3	2.57	0.46
Slice option at the deployment level[b]			
Deployment 1	1	37.65	< 0.0001
Deployment 2	1	49.73	< 0.0001
Deployment 3	1	40.78	< 0.0001
Deployment 4	1	102.89	< 0.0001

[a] Test for each group the difference in survival among the four deployments.

[b] Test for each deployment the difference in survival between the selected group and the control group.

Table 5 Gain of survival (% point higher than control) per generation of selection for each line and both lines according to size for oysters weighing 1 g or more than 3 g when exposed to OsHV-1

Generation	1g[ab]			>3g[ac]		
	Line A	Line B	Both lines	Line A	Line B	Both lines
G_1	+12.0%	+16.3%	+15.2%	+59.8%	+2.8%	+31.3%
G_2	+14.6%	+33.7%	+24.2%	+61.3%	+47.2%	+54.3%
G_3	+32.5%	+52.1%	+42.3%	+58.3%	+67.6%	+63.0%
G_4	+43.9%	+52.4%	+48.2%	+80.2%	+80.6%	+80.4%
Average per generation	+11.0%	+13.1%	+12.1%	+20.1%	+20.2%	+20.2%
Heritability realized	0.34±0.05	0.52±0.03	0.41±0.03	0.46±0.05	0.63±0.04	0.52±0.03

[a] The gain is the percentage points higher than the control. For example, when the survival of the control is 10%, the survival of the selected group for the line A and after four generations of selection should be 53.9% for oysters weighing 1 g when exposed to OsHV-1.

[b] The realized heritability was estimated using the G_0, the four G_1 deployments, and the first deployment of G_2, G_3 and G_4 (oysters weighing 1 g) (see Tables 2 and 3).

[c] The realized heritability was estimated using the G_0, the four G_1 deployments, the first deployment of G_2 and G_3, and the last deployment of G_4, which had a size of 4 g.

Table 6 Covariance analysis of shell height and oyster weight for the first deployment of G_4 at endpoint in October 2013

	Source	df	MS	F	P
Shell height	group	1	0.59	21.16	<0.0001
	line	1	0.01	0.07	0.79
	group x line	1	0.09	3.12	0.08
	error	576	0.03		
Oyster weight	group	1	4.27	26.89	<0.0001
	line	1	0.01	0.05	0.83
	group x line	1	0.14	0.90	0.34
	error	576	0.16		

Table 7 Variance analysis of the yield standardized for 1 kg of spat tested for the first deployment of G_4 at endpoint in October 2013

Source	df	MS	F	P
group	1	16.65	151.80	<0.0001
line	1	0.33	3.06	0.10
group x line	1	0.01	0.03	0.87
bag (line group)	4	0.05	0.22	0.92
error	11	0.01		