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Response to selection for increasing resistance to the spring mortality outbreaks in *Mytilus edulis* occurring in France since 2014

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Abstract :

Massive spring mortality outbreaks have been reported in Mytilus spp. in France since 2014. The main areas affected are the Pertuis Charentais Sounds and the coast of the Pays de la Loire, which cultivate mainly *M. edulis*, and the putative causal agents remain unknown. We report the results of the first generation of mass selection focused on survival and resistance to the spring mortality in M. edulis. Two stocks of mussels were sampled in the Pertuis Charentais Sounds in 2014; one of these went through a spring mortality outbreak and the survivors were used to produce the selected stock, while the second stock was M. edulis considered to be naïve against the putative causal agents of the mortality outbreak and was used to produce the control stock. A first cohort was produced in 2015 and tested at one site in the Pertuis Charentais Sounds. In April and May 2016, a spring mortality outbreak was observed when seawater temperature ranged from 10 to 15 °C. In October 2016, the selected stock had a lower mortality (44%) than the control stock (78%). To confirm this result and investigate genotype by environment interaction, a second cohort was produced in 2016 and tested at six sites. Although no significant mortality was reported at the four sites in the Pertuis Charentais Sounds in 2017, the selected stock had a lower mortality (17–27%) than the control stock (61–74%) at the two sites along the coast of the Pays de la Loire. The survival had increased of 34–48% after one generation of mass selection and the realized heritability was high, ranging from 0.55 to 1.15, but further generations of selection are required to obtain a better estimation of the heritability. Our study showed a positive response to selection in three environments that were different to the site from which mussels were selected, suggesting the absence of strong genotype by environment interaction in sites where spring mortality outbreaks occurred. Selection to enhance M. edulis survival of the spring mortality should be efficient and should be capable of easy implementation through mass selection. Apparently, selection to enhance the survival of *M. edulis* did not affect the mussel growth in comparison with the controls, but automatically improved the yield of *M. edulis* in sites affected by the spring mortality outbreaks.

Highlights

► Mass selection to enhance the resistance to the spring mortality outbreak in *M. edulis* was successful. ► Significant genetic improvement after one generation of selection (+34–48%) ► No significant strong genotype by environment interaction ► Most of the mortality occurred at temperature ranging from 10 to 15 °C

Keywords : spring mortality, survival, *Mytilus edulis*, heritability, selection

1. Introduction

The French mussel production varied from 65 000 to 79 000 metric tons during the last decade (FAO, 2017). This production is based on diploid wild caught spat of the two species *Mytilus edulis* and *M. galloprovincialis* and their hybrids (Bierne et al., 2003; Faure et al., 2008). In March and April 2014, blue mussel production in the Pertuis Charentais Sounds faced sudden and unfamiliar mass mortality outbreaks (90–100%) affecting both juvenile and adult blue mussels (Polsenaere et al., 2017). Such mortalities were also reported the following years, as well as in northern sites mainly along the coast of the Pays de la Loire (Pépin et al., 2017). The mortality has been observed for cultivated mussels mainly in spring, but there is no information regarding such mortality for wild populations yet because of a lack of survey. The growing areas affected by these mortality events contain *M. edulis*, and could potentially involve hybrids between *M. edulis* of the Bay of Biscay and *M. galloprovincialis* of Brittany as the Pays the Loire area includes the boundary of the subpopulations of *Mytilus* spp. (Faure et al., 2008). This may suggest that (1) *M. galloprovincialis* could be less susceptible to the mortality observed on mussel farms than *M. edulis*, as observed recently under laboratory conditions by Benabdelmouna et al. (2018), or (2) the putative causal agents of the mortality are to date only present in these areas and have not spread to other areas yet.

A pathogenic strain of *Vibrio splendidus* was isolated from moribund mussels sampled in the Pertuis Charentais Sounds in 2014 (Oden et al., 2016), but its role in mortality outbreaks in French cultured mussel populations is still unknown. More recently, it was found that *M. edulis* that survived a mortality outbreak continued to show mortality in the following year, and naïve *M. edulis* adults had significant mortality when they were in contact with survivors of a mortality outbreak, suggesting a horizontal transmission of a putative causal agent (Benabdelmouna et al., 2018). Once again, no putative causal agent has been identified yet;

however, the bacteria belonging to the Splendidus clade were detected again (Benabdelmouna et al., 2018). Another study indicated that the mortality level of blue mussel stocks in France appeared to be significantly correlated to genomic abnormalities detected by flow cytometric analyses of hemolymph (Benabdelmouna and Ledu, 2016). Nevertheless, the etiology of the mortality remains unknown, which limits the assay using experimental infections. Numerous investigations are ongoing to identify the cause(s) of the blue mussel mortality and to propose solutions to reduce mortalities on mussel farms.

One of the proposed solutions relies on a selective breeding program to enhance survival when mussels are facing mortality outbreaks. As soon as the first mortality outbreak was reported in the Pertuis Charentais Sounds, survivors were sampled and used as broodstock to produce the first generation of mass selection. This scheme is simpler and less expensive than family-based selection, because only one selected group is produced, along with a control to assess the environmental change. This control used wild mussels that did not experience the mortality outbreak that occurred on mussel farms. The selection method relies on the high fecundity of shellfish, enabling the use of a higher selection intensity compared to terrestrial livestock (Gjedrem and Baranski, 2009).

Here, we report our efforts to increase the genetic resistance to the mortality outbreaks observed in *M. edulis* in the Pertuis Charentais Sounds and Pays de la Loire, both areas strongly affected by such mortality since 2014. Two cohorts of the first generation of mass selection were produced in 2015 and 2016 to increase survival, and so decrease mortality, but with a different intensity of selection in each year. Each cohort contained one selected stock and one control that were tested the following year on either mussel or oyster farms where a high spatfall of mussels occurs. We recorded the response to selection, the gain of survival (defined as a decrease in mortality of the selected group over the control group), and the realized heritability to increase the survival when mussels are facing a mortality outbreak. In

addition, growth parameters (individual shell height and individual whole weight) and yield (total weight of live mussels) were recorded to estimate the effect that mass selection for survival had on these important commercial traits.

2. Materials and methods

2.1. Biological material

2.1.1. Unselected stock

Spat of wild mussels (< 1 cm) were sampled on oyster gear at Agnas (45°52′23″ N, 1°10′15″ W) in the Pertuis Charentais Sounds in September 2014 (Fig. 1A). Approximately 1500 mussels were transferred to the Ifremer hatchery in La Tremblade. They were grown using UV-treated seawater enriched with a cultured phytoplankton diet (*Isochrysis galbana, Tetraselmis suecica*, and *Skeletonema costatum*) to favor growth. They were kept in tanks separately from all other stocks of mollusks held at the Ifremer hatchery. No abnormal mortality was reported for this stock when it was held in our facilities from September 2014 to January 2016 and these mussels were considered to be naïve against the putative causal agents of the mortality outbreak that occurred in mussel farms in 2014. The "naïve" mussels were used to produce the unselected stock. A PCR analysis showed that 15 individuals randomly sampled from this stock exhibited only a single band of 176 bp in length using the Me15 and Me16 primers, indicating that this stock was mainly *M. edulis* mussels (data not shown, see Benabdelmouna et al. (2018)).

2.1.2. Selected stock

Mussels from spatfall occurring in the Pertuis Charentais Sounds in 2013 were cultivated on rows of wooden poles, called "bouchots", by a mussel farmer at Yves (46°0′26″ N, 1°4′7″ W) (Fig. 1A). A mortality outbreak occurred in May 2014, reaching 50% by September 2014 (Robert et al., 2015). Approximately 1500 survivors were transferred to the Ifremer hatchery in La Tremblade and kept under the same conditions as the naïve mussels. A subset of the survivors was then used to produce the selected stock. PCR analysis of 15 mussels showed that they were all *M. edulis* (data not shown, see Benabdelmouna et al. (2018)), suggesting that this stock was mainly *M. edulis* mussels.

2.2. First cohort produced in 2015

Three hundred naïve mussels and 160 survivors of the field mortality outbreak were placed in the conditioning room to produce the first cohort (Table 1). Each stock was held in a separate tank to avoid the potential for horizontal transmission of putative causal agents of the mortality from survivors to the unselected stock (Benabdelmouna et al., 2018). Unheated UV-treated and filtered seawater (400 L per hour) was enriched with a cultured phytoplankton diet (*Isochrysis galbana*, *Tetraselmis suecica*, and *Skeletonema costatum*). When the gonads were ripe, mussels were induced to spawn in March 2015. For each stock, mussels were held in a mesh net and suspended in one 30 L tank filled with UV-treated seawater at 20 °C. Every 15 minutes, the water was sieved and fertilized eggs were collected on a 20 µm screen and transferred to a new tank of 150 L. The empty tank containing the parents was refilled in order for them to continue to spawn. This occurred until the end of the spawning event to maximize the effective size for both the unselected (hereafter called "15-control") and selected (hereafter called "15-selected)" stocks produced from the naïve and survivor mussels, respectively (Table 1). After spawning, the parents were returned to their respective tanks.

2.3. Second cohort produced in 2016

A second cohort was produced in January 2016 using the same two stocks of mussels sampled in 2014 (Table 1). While naïve mussels were kept without any abnormal mortality in our facilities, the mussels that survived the field mortality outbreak in 2014 experienced a new round of mortality (71%) in our facilities in 2015 meaning that the cumulative mortality (field and facilities) reached 86% when the surviving mussels were induced to spawn. The spawning involved 46 mussels that survived both mortality events and 540 mussels from the unselected stocks to produce the selected (hereafter called "16-selected)" and the unselected (hereafter called "16-control") stocks, respectively (Table 1). The spawning protocol in 2016 was similar to the one used in 2015. Thus, the selected stocks of the first and the second cohorts were both the first generation of mass selection to improve survival, but the intensity of selection was higher for the 16-selected stock than for the 15-selected stock.

2.4. Hatchery and nursery growing

For both cohorts, the 150 L tanks containing the embryos were filled with filtered and UV-treated seawater at 20 °C and this was changed three times per week. The larvae were fed daily with a mixture of algae (*I. galbana, Chaetoceros gracilis, S. costatum*). Larval density was established at 10 larvae mL^{-1} on day 1 post-fertilization and then progressively reduced to 5 and 3 larvae mL^{-1} on days 5 and 7 post-fertilization, respectively, to limit competition. At the pediveliger stage, the larvae were ready to settle; they were then transferred into 150-µm sieve-bottomed trays using a downweller system at 20 °C in the micro-nursery. The seawater was enriched with a mixture of four algal species routinely produced at the hatchery (*I.*

galbana, *C. gracilis*, *T. suecica*, and *S. costatum*). The sieves were washed daily and changed regularly depending on spat growth. When spat reached 1 cm, they were transferred into an upwelling nursery where they were grown until their deployment in the field which occurred during the fall.

2.5. Field testing

In November 2015, the first cohort was deployed in the field at La Floride in the Marennes-Oléron Bay, just in front of the hatchery (45°48′12″ N, 1°9′18″ W)(Fig. 1A). This site is dedicated to oyster culture but a large spatfall of mussels occurs in this area every year. For each stock (15-control and 15-selected), one mesh bag of 100 mussels were put into an oyster bag. This was replicated three times (Table 1). Mussels were checked regularly and mortality levels were recorded seven times between March 2016 and July 2016, and also in October 2016 and March 2017. In addition to monitoring survival, 30 mussels per stock were individually measured for shell height (in millimeters) and whole weight (in grams) at deployment and in October 2016, and the total weight of live mussels was also measured at these two dates for estimation of the yield. Seawater temperature was recorded hourly using two probes (Proges plus, 59780 Willems, France).

In order to investigate the genotype by environment interaction, both 16-control and 16-selected stocks of the second cohort were deployed at four sites in the Pertuis Charentais Sounds: La Floride, Agnas, Bay of Yves, and Bay of Aiguillon (46°17′18″ N, 1°18′12″ W), in November 2016 (Fig. 1A) (Table 2); and at two northern sites in the Pays de la Loire: Le Fiol in the Bay of Bourgneuf (47°2′22″ N, 2°1′55″ W) and La Plaine Sur Mer in the Loire estuary (47°13′46″ N, 2°14′45″ W), in September 2016 (Fig. 1B) (Table 2). Although the Agnas and La Floride sites are dedicated to oyster culture, the other sites are all dedicated to mussel

culture. The number of replicates (2 to 6) and the density (100 to 400) used for each stock at each site are given in Table 2. For the sites in the Bay of Bourgneuf and Loire estuary, survival was recorded monthly until July 2017. In addition, 20 mussels were individually measured for shell height (in millimeters) for each stock at deployment and 30 mussels per stock were measured at the endpoint in July 2017. For the sites in the Pertuis Charentais Sounds, survival was recorded in March/April, June, and October 2017, as well as the total weight of live mussels at deployment and at the endpoint (Table 2). Our experiment mimicked the production circle of mussel farmers.

2.6. Data analyses

All statistics were performed using SAS® 9.4 software (SAS Institute Inc., Cary, NC, USA).

2.6.1. Survival

Several bags were used to replicate stocks within cohort in order to test the variability of the mortality due to environmental factors. There was no significant replicate effect and therefore this was dropped from the analysis. For cohort 1, a comparison of the mortality between the 15-selected and 15-control stocks was analyzed for each monitoring date by a binomial logistic regression using the GENMOD procedure, with the following model:

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

where Y_{ij} = the observed mortality for mussel j in stock i (control or selected).

For cohort 2, the following model was used:

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

where Y_{ijk} = the observed mortality at the endpoint for mussel k in stock i (control or selected) at site j (6 sites). All effects and the interaction were fixed. Owing to a significant interaction,

the SLICE option was used; this allows 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

The low mortality observed for both stocks of cohort 2 in the Pertuis Charentais Sounds could be related to an environmental factor affecting the liability, such as a non-exposure to the causal agent of the mortality, that would invalidate the genetic analyses in terms of liability (Falconer and Mackay, 1996). Thus, the realized heritability to increase the survival after one generation of mass selection was only calculated for cohort 1 tested at La Floride in 2016, and for cohort 2 tested at Le Fiol and La Plaine sur Mer in 2017, using the mortality rates recorded in July of the corresponding year. This experiment involves a mass selection based on a threshold trait. The survival trait, and so the mortality trait, is a function of an underlying variable: the level of resistance of the mussels, hereafter called the liability. All mussels having a level of resistance higher than the threshold will survive, while the others will die. The threshold could vary in field conditions as the environment is uncontrolled. The control stock produced for each cohort allowed for assessing the effects of changing environmental conditions during the course of the experiment (Roff, 1997). The response to selection was the difference in survival (mortality) between the selected group and the control within a cohort on the liability scale. The selection differential was the mean liability of the selected parents in the previous generation as it deviates from their population mean liability, given the intensity of selection i as reported in Appendix Table A (Falconer and Mackay, 1996). With a survival of 50%, the intensity of selection was i = 0.798 for cohort 1, and with a survival of 14%, the intensity of selection was i = 1.590 for cohort 2 (Table 3). The realized heritability was estimated according to formula 18.2 and 18.3 in Falconer and Mackay (1996):

$h^2 = t/r$ with $t = \frac{Xc - Xs\sqrt{\left[1-(Xc^2-Xs^2)(1-(\frac{Xc}{i})\right]}}{i+Xs^2(i-Xc)}$

where the subscripts C and S refer to the control stock and the selected stock respectively, x is the normal deviate of the threshold from the mean, i is the mean deviation of the selected parents (the survivors) from the population mean, and r is the coefficient of relationship. When the relatives are offspring of two parents that survived the mortality outbreak, the appropriate 'r' is the regression on mid-parent, which is 1 (Falconer and Mackay, 1996).

2.6.3. Yield and growth

For each cohort, the shell length and the individual whole weight data were log transformed and analyzed using the MIXED procedure by running an ANCOVA with time as a covariate using the following model:

$$\text{Log}(Y_{ijk}) = \mu + stock_i + site_j + stock_i*site_j + time + time*stock_i + time*site_j + time*stock_i*site_j + \varepsilon_{ijk}$$

where μ is the intercept, *time* is the covariable, $stock_i$ is a fixed effect (15-control vs 15-selected for cohort 1 or 16-control vs 16-selected for cohort 2) as well as $site_j$ (only for cohort 2, La Plaine sur Mer and Le Fiol) and ε_{ijk} is the error term. The slopes of the relationships between the covariate (time) and either stock or/and site represent direct measures of growth. All effects containing the factor site were only tested for cohort 2. Owing to a significant $time*stock_i*site_j$ interaction, the model was rerun within site.

For each cohort and site, except at Le Fiol and La Plaine sur Mer where the total weight of live mussels at the endpoint was not recorded, the yield was log transformed, and analyzed using the GLM procedure by running an ANCOVA with the initial yield as a covariate using the following model:

$$\text{Log}(Y_{ij}) = \mu + \mu_i + \beta x_i + \varepsilon_{ij}$$

where μ is the intercept, μ_i *is* the fixed effect of the stock (selected or control), βx_i is the regression effect of the initial yield at the time of deployment (the covariate), and ε_{ij} is the error term.

3. Results

3.1. Seawater temperature

For the testing of the 15-control and 15-selected stocks, the seawater temperature in La Floride ranged from 22.9 °C in July 2016 to 3.1 °C in January 2017 (Fig. 2). For the sites located along the coast of the Pays de la Loire, seawater temperature decreased from 19 °C in September 2016 to 3.7 °C in January 2017. Then, seawater temperature gradually increased to 23.1 °C in June 2017 and remained above 18 °C until the endpoint (Fig. 3).

3.2. Survival of cohort 1

No mortality was reported from November 2015 to March 2016 (Fig. 2). The first dead mussels were observed on April 5, 2016, comprising 3% and 5% of the 15-selected and 15-control stocks, respectively. A significant mortality outbreak was then observed during April and May 2016, and mortality reached 37% and 75% for the 15-selected and 15-control stocks, respectively, on May 24, 2016 (Fig. 2). The mean cumulative mortality for the 15-selected and 15-control stocks were respectively 43% and 77% in July 2016, and 44% and 78% at the endpoint in March 2017 (Table 2). The mortality of the 15-control stock was significantly higher than the 15-selected stock (P <0.0001) from April 22, 2016 to the endpoint.

3.3. Survival of cohort 2

A significant interaction between sites and stocks was found due to the absence of mortality outbreaks in sites located in the Pertuis Charentais Sounds while it was observed in sites located in the Bay of Bourgneuf and the Loire estuary ($P < 0.0001$). The final cumulative mortality remained below 10% for both the 16-selected and 16-control stocks in the four sites in the Pertuis Charentais Sounds at the endpoint (Fig. 1A) (Table 3). Although no significant difference in mortality between stocks was observed in the Bay of Yves and the Bay of Aiguillon, a lower mortality for the 16-selected stock was observed at Agnas ($P < 0.0001$) and La Floride ($P = 0.04$).

At the two sites located in the Bay of Bourgneuf and the Loire estuary, the cumulative mortality showed similar patterns. No mortality was reported during the first two months after deployment in September 2016. Some mortality was reported from December 2016 to February 2017, especially for the 16-control stock, reaching 15% and 21% at Le Fiol and La Plaine sur Mer, respectively (Fig. 3). A peak of mortality was observed in April and May 2017 at both sites, when cumulative mortality values at Le Fiol and La Plaine sur Mer were respectively 52% and 61% for the 16-control stock, and 15% and 19% for the 16-selected stock. Subsequently, slight increase in mortality were still observed for both stocks at both sites until the endpoint. The final cumulative mortality values of the 16-control stock reached 61% and 74% at Le Fiol and La Plaine sur Mer, respectively, and were significantly higher than those of the 16-selected stock with 17% and 25% mortality at Le Fiol and La Plaine sur Mer, respectively ($P < 0.0001$) (Table 3; Fig. 3).

3.4. Response to selection to decrease the mortality in M. edulis

At La Floride, the mortality of the 15-selected stock was 34% lower than that of the 15-control stock at the endpoint (Table 3). The response to selection on the liability scale was 0.92, and the realized heritability after one generation of mass selection to decrease mortality, i.e., increase survival, in *M. edulis* was very high, with $h^2 = 1.15 \pm 0.09$ (Table 3). For the second cohort, the mortality of the 16-selected stock was lower than that of the 16-control stock by 44% and 48% at Le Fiol and La Plaine sur Mer, respectively, corresponding to a response to selection of 1.23 at Le Fiol and 1.32 at La Plaine sur Mer. For cohort 2, the realized heritability after one generation of mass selection to decrease mortality in *M. edulis* was again very high, with $h^2 = 0.78 \pm 0.05$ at Le Fiol and 0.83 ± 0.05 at La Plaine sur Mer (Table 3).

3.5. Growth

At deployment of the first cohort at La Floride, the mean individual shell length and whole weight were respectively 23.6 mm and 1.3 g for the 15-control stock, and 25.1 mm and 1.5 g for the 15-selected stock. In October 2016, the mean individual shell height was 40.7 mm for the 15-control stock and 41.2 mm for 15-selected stock (Fig. 4), and the mean individual whole weight was 5.2 g for the 15-control stock and 5.7 g for the 15-selected stock. The growth was not significantly different between the two stocks for shell length ($P = 0.33$) and weight ($P = 0.39$).

At deployment of the second cohort, the mean shell lengths for the 16-control and 16-selected stocks were respectively 27.5 mm and 25.3 mm at La Plaine sur Mer, and 26.5 mm and 26.4 mm at Le Fiol (Fig. 5). A significant interaction was found between the stocks and the sites ($P = 0.0004$). Thus, both stocks had a similar growth in shell length at Le Fiol with a final mean shell length of 40.7 mm for the 16-control stock and 39.9 mm for the 16-selected stock

(P = 0.49). In contrast, the 16-selected stock showed higher growth than the 16-control stock at La Plaine sur Mer (P < 0.0001) with final mean shell lengths of 42.8 mm (16-selected) and 42.4 mm (16-control) (Fig. 5).

3.6. Yield

For an easier comparison of the yield between stocks, the yield was standardized for 1 kg of mussels deployed in order to take in account for difference at deployment, which was likely related to the environment during the nursing period. For cohort 1, the mean yield (total weight of all live mussels) of the 15-control stock in October 2016 was 785 g for 1 kg of mussels deployed at La Floride in November 2015, whilst a significantly higher yield of 1834 g was observed for 15-selected stock (P = 0.0028).

For cohort 2, the 16-selected and 16-control stocks showed a similar yield at the four sites in the Pertuis Charentais Sounds (0.06 < P < 0.84), with average yields across sites of 2428 g (16-selected) and 2151 g (16-control) for 1 kg of mussels deployed. Unfortunately, the yield was not recorded at the two sites in the Pays de la Loire, but it was obvious that the selected stock had a higher yield than the control stock at both sites as shown by the photograph taken at the endpoint (Fig. 6).

4. Discussion

The mortality events observed at La Floride in 2016 and at the two sites in the Pays de la Loire in 2017 remain unexplained, along with all mortality events observed in mussel farms since 2014. In the Pertuis Charentais Sounds, mortality outbreaks were reported in our experimental site at La Floride in 2016 as well as in most of the mussel farms located in this

region. In 2017, no significant mortality was observed in our four experimental sites or in mussel farms in the Pertuis Charentais Sounds. In contrast, mortality outbreaks were reported in two of our sites along the coast of the Pays de la Loire in 2017 and also in mussel farms of this region. Thus, it could be assumed that the mortalities observed in our experimental sites could have the same etiology as those observed in the mussel farms. Our results also indicated that the putative causal agents of the mortality (pathogens, pollutant and/or environment) did not trigger mortality outbreaks in the Pertuis Charentais Sounds in 2017.

To the best of our knowledge, our study is the first to report findings on the response to selection for survival in *M. edulis*. The improvement in survival in the selected stock of *M. edulis* when they faced a mortality outbreak was high, with an increase of 34% compared to that in the control stock in 2016 and 44–48% in 2017 after one generation of mass selection (Table 3). The higher response to selection in 2017 could be in relation to the higher intensity of selection for the parents (Table 3). There is no available value for comparison in mussels and, although a response in a single generation is frequently variable (Roff, 1997), two independent spawns were used to estimate with a better accuracy the response to selection. Our values for the response to selection were two to four times higher than those obtained for mass selection trials for survival to enhance disease resistance in oyster species, such as resistance to *Marteilia sydneyi* in *Saccostrea glomerata* (Dove et al., 2013a; Nell and Hand, 2003) and resistance to *Haplosporidium nelsoni* in *Crassostrea virginica* (Ford and Haskin, 1987). Recently, the selected lines had a gain of survival of only 15% for the first generation of mass selection to improve survival and resistance to OsHV-1 in *Crassostrea gigas* and 48% for the fourth generation (Dégremont et al., 2015a), whereas such values were observed as early as the first generation of selection in *M. edulis*. This suggests that the resistance to mortality outbreaks in *M. edulis* could be controlled by only a few loci.

Our study could also suggest that high genetic variation to increase survival during a spring mortality outbreak might exist within a population of *M. edulis*. Thus, the high heritability, which must be taken with caution, could strongly indicate that a breeding program in *M. edulis* would easily be successful in enhancing survival against the putative causal agents of the spring mussel mortality that are still unidentified. Although the realized heritability was estimated from a single generation, which is frequently variable (Roff, 1997), the realized heritability estimated for both cohorts were high, ranging from 0.55 to 1.15 (Table 3). The heritability estimates from different sorts of relatives were reasonably consistent and gave no strong reason to doubt the adequacy of the liability model (Falconer and Mackay, 1996). Nevertheless, the heritability in excess of 100 per cent for cohort 1 is obviously unacceptable, and would suggest involvement of a single major gene (Falconer and Mackay, 1996). A second hypothesis to explain the heritability greater than one for cohort 1 concerns the different origins of the control and the selected stocks. Previous studies have demonstrated differential survival capabilities among stocks in *M. edulis* (Mallet et al., 1987) and in *M. galloprovincialis* (Fuentes et al., 1994). Thus, the control stock from Agnas could have been more susceptible than the selected stock, which was from Yves, before selection (Fig. 1). As a consequence, the response to selection (the numerator of the equation to estimate the realized heritability) would have been over-estimated, explaining the heritability greater than one for cohort 1. Meanwhile, such a result was not observed for cohort 2, but this could be explained by the higher intensity of selection, which is the denominator of the equation. In the near future, the response to selection could be estimated either (1) from the survivors of the control stock, which would need to be compared to their counterpart protected from the mortality, with both control and selected groups sharing the same origin; or (2) by testing several generations of selection to increase the survival in order to estimate the realized heritability using the slope of the response to selection to the cumulative selection differential,

which should not be forced through the origin as selected and control stocks do not derive from the same base population.

In addition to the previous hypothesis to explain the high response to selection and thus, the heritability, previous exposure to the putative causal agents of the mortality outbreaks could have set some epigenetic marks in *M. edulis*. Thus, increase resistance could have been transmitted to the subsequent generation through epigenetic alterations as recently demonstrated in *Caenorhabditis elegans* (Kishimoto et al., 2017). Thus, epigenetic selection, which, alone or combined with genetic selection, may increase the resistance to the spring mortality outbreaks in *M. edulis* which could also increase the reliability of producing animals with desired phenotypes (Gavery and Roberts, 2017). We also suggest that the high response to selection observed could be due to interspecific inheritance. A first investigation showed that *M. galloprovincialis* was less susceptible to *M. edulis* to the spring mortality outbreak at the spat stage under laboratory conditions (Benabdelmouna et al., 2018). Although 15 individuals per stock were confirmed as *M. edulis*, and that both stocks were originated from the Pertuis Charentais Sounds with predominantly *M. edulis*-like populations as showed by Bierne et al. (2003), some mussels could have been partially introgressed by alleles of *M. galloprovincialis*. In the case of a higher resistance to spring mortality for *M. galloprovincialis* than *M. edulis*, thus the richness of *M.edulis*-like for the selected stock should decreased over the subsequent generations of selection. The supposed resistance of *M. galloprovincialis* could be related to polygenic inheritance or/and major gene effects as observed for the resistance to leaf rust in poplars (Lefèvre et al., 1994).

The realized heritability to increase survival of *M. edulis* during a spring mortality outbreak was very high (> 0.55) (Table 3). Most of the heritabilities estimated in mussel species have been reported for growth parameters (Bai et al., 2017; Brichette et al., 2001; Nguyen et al., 2014; Toro et al., 2004), shell nacre color (Bai et al., 2017), mantle color, toxin accumulation

(Pino-Querido et al., 2015), and more recently calcification (Kingston et al., 2018). Regarding survival, Mallet et al. (1986) reported an additive genetic variance equal to zero in *M. edulis* adults, in contrast with our results. This is not surprising as mortality can have multiple causes, making it misleading to compare heritability for survival when the causes are different. For example, the heritability for survival was high when *C. gigas* was exposed to OsHV-1, but it was lower when oysters were exposed to *Vibrio aestuarianus* (Azéma et al., 2017).

Although, there are no available comparisons with other mussel species, our estimates of heritability are as high as those previously considered, obtained for survival related to disease resistance in mollusk and fish species such as resistance to OsHV-1 infection in *C. gigas* (Dégremont et al., 2015a; Dégremont et al., 2015b), the enteric redmouth disease in *Oncorhynchus mykiss* (Henryon et al., 2005), or amoebic gill disease in *Salmo salar* (Taylor et al., 2009). It is common to observe high genetic variation and very high genetic gains for improved disease resistance in aquatic species (Gjedrem and Rye, 2018). Thus, the high estimates of heritability may suggest that one of the putative causal agents of the spring mortality in *M. edulis* might be a pathogen.

Interestingly, the selected stock was improved from a spring mortality outbreak occurring in the Bay of Yves in 2014, and a positive response to selection was observed at La Floride in the Pertuis Charentais Sounds in 2016 and at both sites along the coast of the Pays de la Loire in 2017 (Fig. 1). This suggests an absence of strong genotype by environmental interaction for the selected trait in sites where spring mortality occurs, and that the putative causal agents of the mortality could be common to the different environments. Consequently, a single selective breeding program to enhance the survival should be efficient in *M. edulis* at the sites affected by the spring mortality, as demonstrated for the resistance to infection by OsHV-1 in *C. gigas* (Dégremont et al., 2016). Beyond that the French mussel production is only based on

natural spat collection, this study informs the capabilities to develop selected mussels to increase the survival in the context of future mortality outbreaks. This approach anticipates and gives keys for the mussel industry, as such phenomenon are expected to increase due to global warming, as recently demonstrated in *Crassostrea gigas* with actual exceptional mortality being likely to become the norm by ~2035 without any adaptive process (i.e. natural or artificial selection) (Yoann et al., 2018).

Our study highlighted that the etiology of the spring mortality outbreak in *M. edulis* is not confined to mussel farms. Indeed, such a mortality event was observed at our experimental sites in La Floride (Fig. 1), which is a site dedicated to oyster culture. Thus, the putative causal agents of the mortality might have already been dispersed throughout the Pertuis Charentais Sounds, either by currents as suggested by Polsenaere et al. (2017) or by the numerous shellfish transfers occurring in this area. This also highlights the risk of putative causal agents being spread along the entire coast of France, as the Pertuis Charentais Sounds play a critical role nationally, representing more than half of French oyster and mussel spat production (Goulletquer and Le Moine, 2002).

Our study has provided the first description of the spring mortality outbreaks in *M. edulis*. In La Floride, most of the mortality occurred in April and May 2016 when the seawater temperature ranged from 10 to 15 °C (Fig. 2). For both sites in the Pays de la Loire, mortality was observed for seawater temperature ranging from 4 to 23 °C; however, most of the mortality was observed at 5 to 15 °C during the spring (Fig. 3). Similar patterns of temperature-related mortality were observed under laboratory conditions in 2015 for *M. edulis* adults that survived or were naïve to the putative causal agents of the spring mortality (Benabdelmouna et al., 2018).

The effect of the mass selection on other growth traits was assessed in this study. Generally, both stocks had similar growth at all sites and similar yield at sites without significant

mortality, suggesting that selection to improve survival did not reduce the growth after one generation of mass selection. This is in agreement with the results of selection to increase OsHV-1 resistance in *C. gigas* (Dégremont et al., 2015a). In general, studies investigating growth and survival related to disease resistance until reaching market size have never shown a lower growth of the selected stocks compared to 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). In contrast, higher yield was obtained for the selected stocks in sites with high mortality, which is routine in marine mollusks as yield and survival are positively correlated in such conditions (Dégremont et al., 2005; Evans and Langdon, 2006). Thus, selection to improve survival against the spring mortality outbreak will automatically improve the yield of *M. edulis*.

In conclusion, our study clearly demonstrated that mass selection to increase survival and resistance to the spring mortality in *M. edulis* was successful after only one generation of selection, indicating a significant genetic improvement for the selected trait. The gain for the first generation of selection was very high with an increase in survival ranging from 34 to 48%. To the best of our knowledge, our study is the first to provide estimates of heritability for survival using a mass selection scheme in a mussel species, with values ranging from 0.55 to 1.15, depending on the cohort. Nevertheless, this value must be taken with care as it was estimated from a single generation, which is frequently variable. Further generations of mass selection are required to obtain a more reliable estimation of the heritability. The high response to selection is encouraging, and if this response is driven by loci related to resistance, thus mass selection could be implemented by the mussel industry and would have an economically positive effect in the case of massive mortality outbreaks. Nevertheless, it is crucial to identify the putative causal agents of the mortality that currently remain unknown.

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A

B

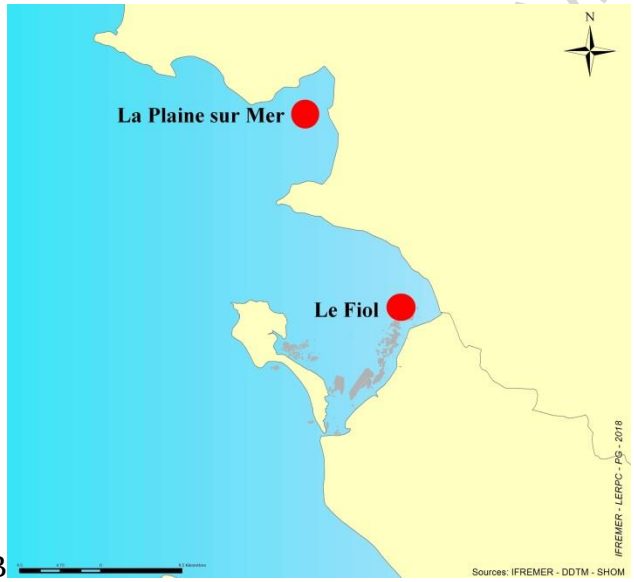


Figure 1 Locations of the mussels used to produce the selected stocks (Bay of Yves*) and the unselected stocks (Agnas**), and sites used for the field testing in the Pertuis Charentais Sounds (panel A) and along the coast of the Pays de la Loire (panel B).

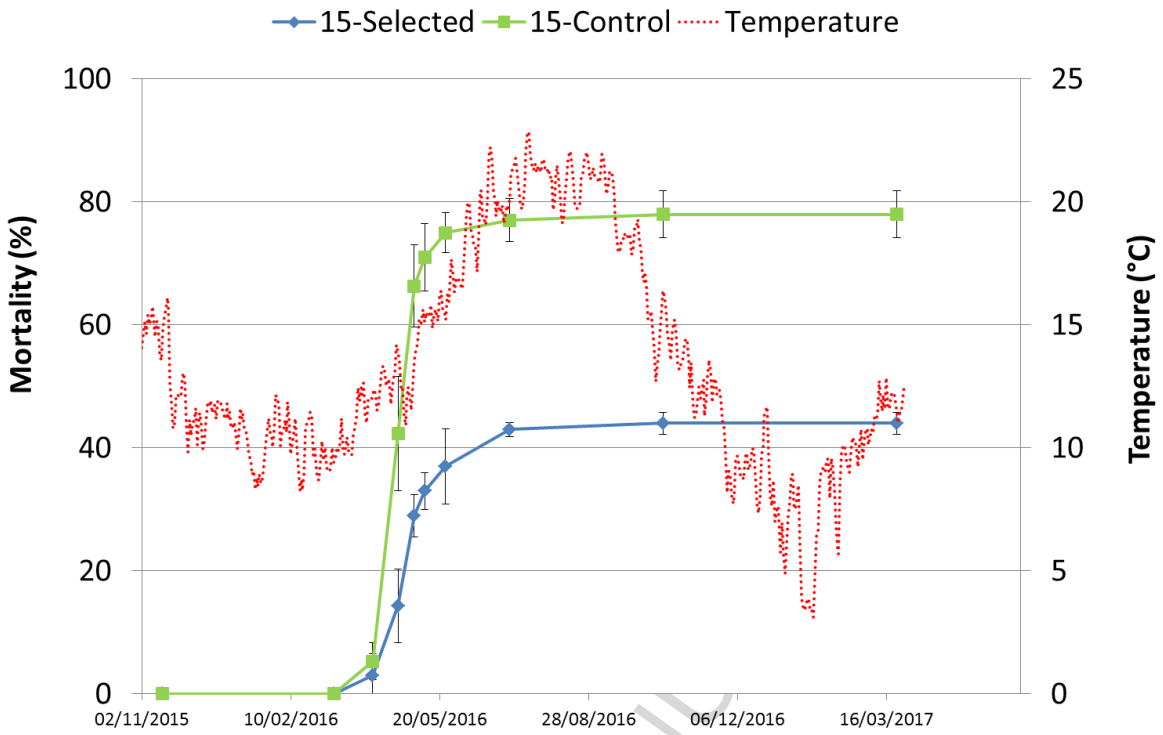
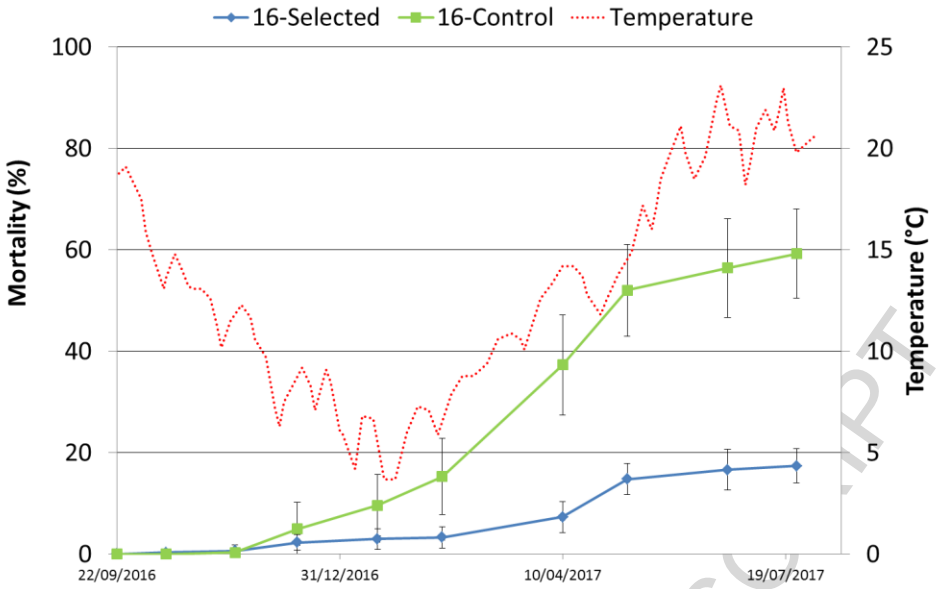


Figure 2 Mean cumulative mortality (% ± SE among replicates) of the 15-control and 15-selected stocks, and seawater temperature (°C) at La Floride from November 2015 to March 2017.

A



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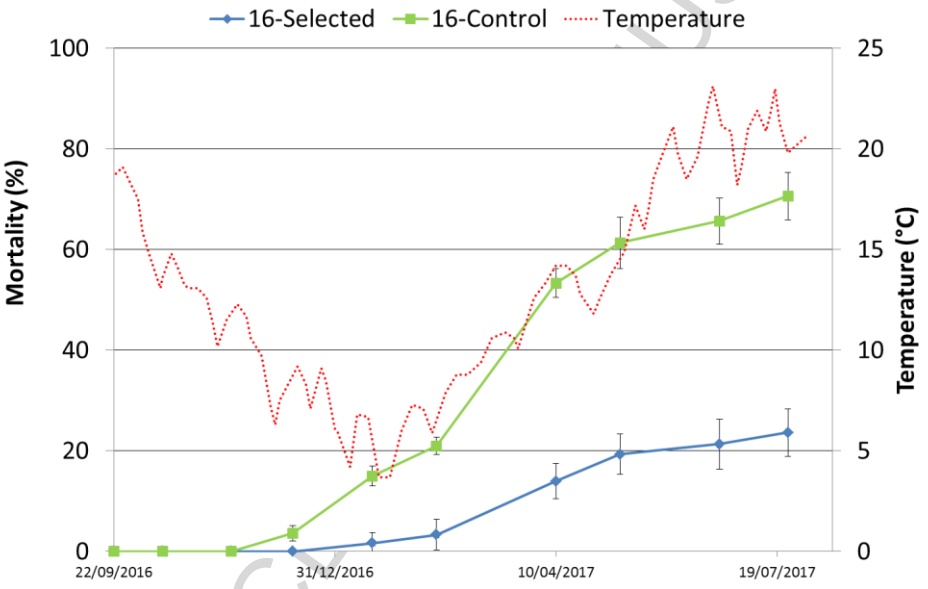

Figure 3 Mean cumulative mortality (% ± SE among replicates) of the 16-control and 16-selected stocks, and seawater temperature (°C) at Le Fiol in the Bay of Bourgneuf (panel A) and at La Plaine sur Mer in the Loire estuary (panel B) from September 2016 to July 2017.

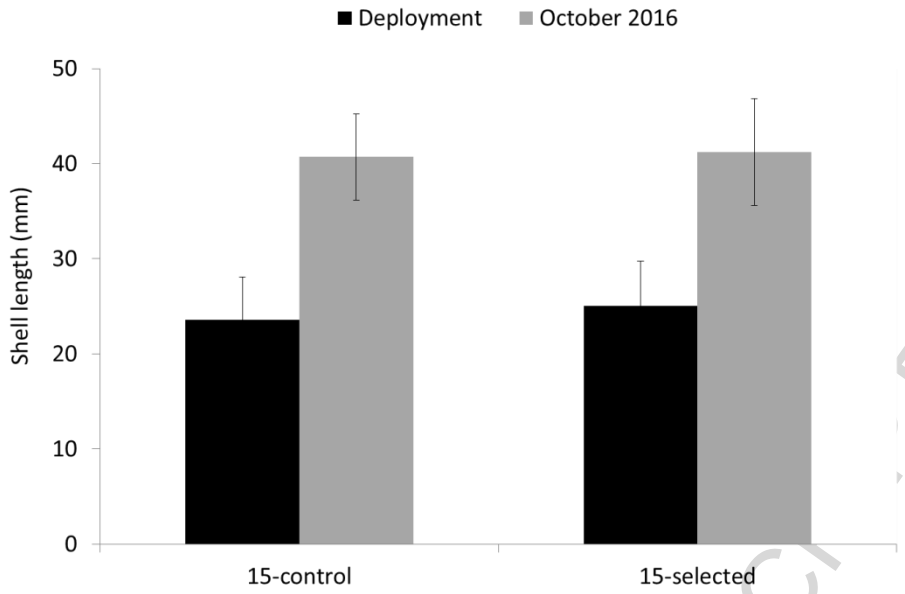

Figure 4 Mean shell length (± standard deviation) of the mussels at deployment in November 2015 and in October 2016 for the 15-control and 15-selected stocks of cohort 1 tested at La Floride.

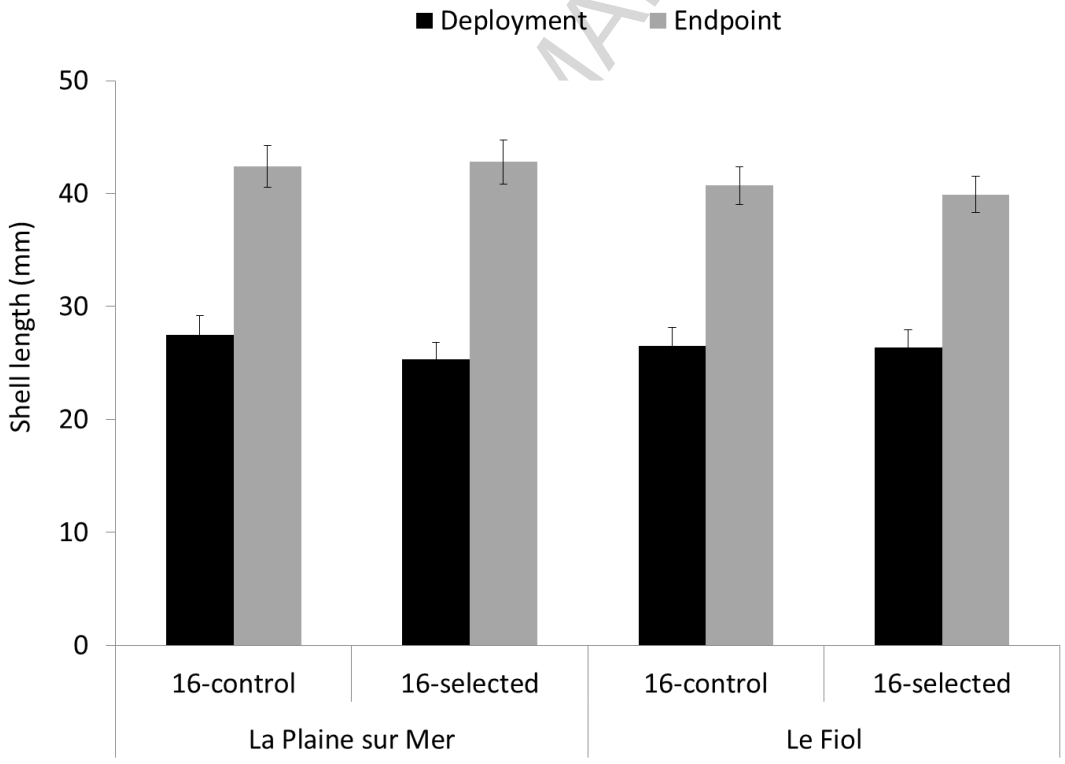

Figure 5 Mean shell length (± standard deviation) of the mussels at deployment in September 2016 and at the endpoint in July 2017 for the 16-control and 16-selected stocks of cohort 2 tested at La Plaine sur Mer and at Le Fiol.

Figure 6 Live mussels of the 16-control (right) and 16-selected (left) stocks at Le Fiol in the Bay of Bourgneuf (top) and at La Plaine sur Mer in the Loire estuary (bottom) in July 2017 (from 300 mussels used per stock and per site at deployment in September 2016).

Table 1 Summary of the key dates for the production of the first cohort in 2015, and the second cohort in 2016, of the first generation of mass selection to improve survival in *M. edulis*.

Cohort	Origin of the stock	Number of parents	Survival of the parents (%)	Spawn date	Stock name for testing
1	Agnas	300	100	March 2015	15-control
	Bay of Yves	160	50	March 2015	15-selected
2	Agnas	540	100	January 2016	16-control
	Bay of Yves	46	14	January 2016	16-selected

Table 2 Summary of the key dates for field testing of the mussels produced in 2015 and in 2016.

Cohort	Stock	Site	Start date	End date	Container	Number of replicates per stock	Density per replicate	Growing method
1	15-control 15-selected	La Floride	16/11/15	23/03/17	Mesh bag	3	100	Bag on racks
2	16-control 16-selected	Le Fiol	22/09/16	24/07/17	Mesh bag	3	100	Bag on wooden poles
		La Plaine sur Mer	22/09/16	24/07/17	Mesh bag	3	100	Bag on wooden poles
		La Floride	16/11/16	08/06/17	Mesh bag	6	100	Bag on racks
		Bay of Yves	16/11/16	05/10/17	Mesh bag	3	100	Bag on wooden poles
		Bay of Aiguillon	16/11/16	05/10/17	Mesh bag	2	400	Bag on wooden poles
		Agnas	16/11/16	12/10/17	Mesh bag	2	400	Bag on racks

Table 3 Mortality (± SE among replicates) of the selected and control stocks at the endpoint at each site, and gain of survival of the selected stock, response to selection, and realized heritability to increase the resistance to the spring mortality outbreaks in *M. edulis*.

Cohort[1]	End date	Site	Intensity of selection	Control (%)	Selected (%)	Gain of survival (%)	Response to selection	Realized heritability
1	23/03/17	La Floride	0.798	78.0 ± 4.0	44.0 ± 1.7	34	0.92	1.15 ± 0.09
2	24/07/17	Le Fiol	1.590	61.0 ± 6.2	17.3 ± 3.2	44	1.23	0.55 ± 0.05
2	24/07/17	La Plaine sur Mer	1.590	73.7 ± 4.0	25.3 ± 4.5	48	1.32	0.66 ± 0.05
2	08/06/17	La Floride	1.590	6.2 ± 1.3	3.3 ± 1.2			
2	05/10/17	Bay of Yves	1.590	5.7 ± 1.2	8.0 ± 2.6			
2	05/10/17	Bay of Aiguillon	1.590	6.9 ± 3.7	5.6 ± 0.9			
2	12/10/17	Agnas	1.590	7.8 ± 0.4	2.4 ± 1.9			

[1] The cohort 1 was produced in 2015, and the cohort 2 was produced in 2016.

- Mass selection to enhance the resistance to the spring mortality outbreak in *M. edulis* was successful.
- Significant genetic improvement after one generation of selection (+34-48%)
- No significant strong genotype by environment interaction
- Most of the mortality occurred at temperature ranging from 10-15°C