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Author(s): Lionel Dégremont, Patrick Soletchnik and Pierre Boudry

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SUMMER MORTALITY OF SELECTED JUVENILE PACIFIC OYSTER *CRASSOSTREA GIGAS* UNDER LABORATORY CONDITIONS AND IN COMPARISON WITH FIELD PERFORMANCE

LIONEL DÉGREMONT,^{1*} PATRICK SOLECHNIK² AND PIERRE BOUDRY³

¹Ifremer, Laboratoire de Génétique et Pathologie, 17390 La Tremblade, France; ²Ifremer, Laboratoire Environnement Ressources de Poitou-Charentes, 17390 La Tremblade, France; ³Ifremer, UMR M100 Physiologie et Ecophysiologie des Mollusques Marins, 29280 Plouzané, France

ABSTRACT This study reports mortality under laboratory conditions in unselected controls and 2 lines of juvenile Pacific oysters *Crassostrea gigas* previously selected for their high or low survival in the field during the summer period. Oysters were also deployed in field conditions, and mortality between both conditions was then compared. In the laboratory, mortality was observed in all experiments and it always lasted for a week, indicating that mortality under laboratory conditions was a short-term event. It was also shown that mortality could be repeated for a batch in several experiments using oysters that never experienced any abnormal mortality. This approach could facilitate further studies to investigate the causes of mortality by allowing repeated trials during a summer. Differences in mortality between the resistant and the susceptible selected lines confirmed the positive response to selection under laboratory conditions. Batches that performed well in the laboratory also showed high survival in the field, and the results of those exhibiting low survival in the laboratory trials were also mirrored in the field. Finally, challenging oysters with heat stress is proposed as a useful method for estimating the survival capacity of hatchery-produced and wild-caught spat used by the oyster industry.

KEY WORDS: summer mortality, *Crassostrea gigas*, laboratory, field, oyster, juvenile

INTRODUCTION

Mortality in oysters, and particularly the summer mortality syndrome of *Crassostrea gigas*, is a major problem affecting the industry in several countries. Researchers have investigated the possible causes of this phenomenon in Japan (Imai et al. 1965, Koganezawa 1975), on the west coast of the United States (Glude 1975, Beattie et al. 1980, Cheney et al. 2000), in France (Maurer et al. 1986, Goulletquer et al. 1998, Soletchnik et al. 2005) and, more recently, in Australia (Li et al. 2007) and Ireland (Malham et al. 2009). Nevertheless, few studies have investigated summer mortality in *C. gigas* using parallel field and laboratory trials.

In France, the Morest program adopted a multidisciplinary approach to investigating summer mortality in *C. gigas* to assess the causes of the phenomenon and to propose solutions that maximize survival (Samain & McCombie 2008). One of the major results of this program was the high genetic basis observed for survival in young *C. gigas* (less than 1 y old) tested in the field during the summer period (Dégremont et al. 2007). The high heritability of this trait was also confirmed over successive years and generations through divergent selection, suggesting that selective breeding programs should efficiently improve survival during the summer period (Dégremont et al. 2010a). However, field testing does not allow for continuous monitoring of mortality and thus cannot be used to determine the timing of the onset of mortality, and length and severity of mortality events.

The production of experimental lines of oysters exhibiting high and low mortality not only provides valuable information for selective breeding, but also an opportunity to understand better the etiology of summer mortalities (Perdue et al. 1981), because these contrasting lines were also used in comparative studies of physiology and immunology (Samain et al. 2007). As a result, the possibility of making a laboratory-based study of these contrasting lines, known to show diverging patterns of

survival in the field, should make investigations simpler to conduct than field testing.

The main objectives of the current study were to investigate summer mortality in juvenile *C. gigas* under laboratory conditions and to compare these results with those obtained in the field. This study reports mortality in seven laboratory experiments in which selected lines and controls were monitored. These oysters had either been transferred directly from the nursery to the laboratory or deployed for several weeks in the field before being transferred to the laboratory. Experiments were then conducted either with or without heat stress, because it was found that selected families of *C. gigas* showed greatly improved survival both in the field and in elevated temperature laboratory trials (Beattie et al. 1980). This study also uses data from five previously reported field studies, in which mortality was recorded after the summer period on the same oyster stocks (Boudry et al. 2008, Dégremont et al. 2010a), for comparison with our laboratory results.

MATERIALS AND METHODS

Biological Material

Two sets (set 1 and set 2) of oyster stocks were produced in 2002 and an additional 3 sets (set 3, set 4, and set 5) in 2003 at the Ifremer hatchery in La Tremblade (Charente Maritime, France; Table 1). The selection strategy and crossing details are described in Dégremont et al. (2010a), where set 1 and set 2 are named G2 and G2c and set 3, set 4, and set 5 are named G3, G3c², and G3c, respectively. Briefly, selection was based on survival recorded in October 2001 in juvenile oysters younger than 1 y old exposed to field conditions during the summer period. For each set, 2 selected lines—1 resistant and 1 sensitive to the summer mortality phenomenon (hereafter referred to as R and S)—were produced, each containing several selected batches, all produced from families selected in the first generation (Dégremont et al.

*Corresponding author. E-mail address: lionel.degremont@ifremer.fr

TABLE 1.
Key dates of the 7 laboratory experiments (L1–L7) and the 5 field experiments (F1–F5).

Set	Year	Experiment	Spawning	Nursery	Field Start	Laboratory Start	Laboratory End	Field End	Field Name*
1	2002	L1	Mar. 18 th	May 2 nd	—	Jun. 13 th	Jul. 2 nd	—	—
1	2002	L2	Mar. 18 th	May 2 nd	—	Jul. 4 th	Aug. 5 th	—	—
1	2002	L3	Mar. 18 th	May 2 nd	Jul. 11 th	Aug. 22 nd	Sep. 4 th	—	Ronce
2	2002	L4	Apr. 29 th	Jun. 18 th	Aug. 8 th	Sep. 4 th	Sep. 17 th	—	Ronce
3	2003	L5	Feb. 17 th	Apr. 10 th	—	Jun. 25 th	Sep. 4 th	—	—
4	2003	L6	Mar. 24 th	May 15 th	—	Jul. 9 th	Sep. 4 th	—	—
5	2003	L7	Apr. 28 th	Jun. 16 th	—	Jul. 24 th	Sep. 4 th	—	—
1	2002	F1	Mar. 18 th	May 2 nd	Jul. 11 th	—	—	Oct. 8 th	Ronce, RA, BDV
2	2002	F2	Apr. 29 th	Jun. 18 th	Aug. 8 th	—	—	Oct. 8 th	Ronce
3	2003	F3	Feb. 17 th	Apr. 10 th	Jul. 1 st	—	—	Oct. 10 th	RA
4	2003	F4	Mar. 24 th	May 15 th	Jul. 16 th	—	—	Oct. 10 th	Ronce
5	2003	F5	Apr. 28 th	Jun. 16 th	Jul. 31 st	—	—	Oct. 10 th	Ronce

* Ronce (1°10' W, 45°48' N), Rivière d'Auray (RA; 2°57' W, 44°45' N), and Baie des Veys (BDV; 1°06' W, 49°23' N).

R and S indicate lines selected to be resistant and susceptible for summer mortality, respectively. 2N and 3N indicate diploid and triploid controls, respectively.

2010a). Unselected diploid (2N) controls were also produced for the 5 sets, and triploid (3N) controls were produced for set 2 and set 3 by crossing unselected diploid females and tetraploid males. In addition, 2 set 3 batches of triploid oysters named T3NR and T3NS were produced by crossing selected R or S diploid females with unselected tetraploid males. The main purpose of including triploids in our study was to assess their survival performance alongside the selected lines and diploid control, because 90% of the hatchery-produced oyster spat in France are triploid. Larval and spat culture methods were described in Dégremont et al. (2005), Dégremont et al. (2007) and Dégremont et al. (2010a). To summarize, larval culture and settlement were performed at the hatchery in La Tremblade, France. After spat had reached 2 mm, they were transferred to the Ifremer nursery facilities in Bouin (Vendée, France) for an intensive growth period until either deployment in the field or transfer to the laboratory (Table 1). The nursery conditions can be summarized as high water flow at ambient temperature, *ad libitum* feeding, and no emersion (Bacher & Baud, 1992).

Laboratory Experiments

All laboratory experiments were performed in raw seawater, which was first naturally decanted by gravity and then filtered

through sand. Cultured algae were added as feed. Seven laboratory experiments, hereafter referred to as L1–L7, were conducted during summer at the Laboratoire de Génétique et Pathologie in La Tremblade. Experiments L1–L4 were done in 2002 and experiments L5–L7 in 2003 (Table 1). Oysters were either transferred directly from the nursery to the laboratory (L1, L2, L5–L7) or transferred temporarily to the field at Ronce-les-Bains (Ronce) in Marennes-Oléron Bay (1°10' W, 45°48' N), before testing in the laboratory (L3 and L4). Key dates for each laboratory experiment and transfers are shown in Table 1. For all experiments, shell length ranged from 5–25 mm. The numbers of batches per stock (R line, S line, and controls) tested in each experiment, rearing methods (volume of the holding tank and type of container), numbers of replicates per batch, and density per replicate are shown in Table 2. To mimic field conditions, where all batches are grown together, selected and unselected batches were placed at random within 1 tank for 5 of the laboratory experiments (Table 2). For L3 and L4, batches were randomly placed in several tanks because the volume of the tank, combined with the size of the oysters, did not allow all batches to be tested in just one. Complete water replacement took place over each hour, and water temperatures were continuously monitored using a YSI (Yellow Springs Instruments, Yellow Springs, OH) or Thermo Track probe (Progesplus, 59780, Willems, France).

TABLE 2.
Number of batches tested per stock and growing method used in the laboratory experiments.

Experiment	No. of Batches				No. of Tanks	Volume of Tanks	Growing Method		
	R	S	T2N	T3N			Container (cm)	No. of Containers (replicate) per Batch	Density per Container
L1	9	9	4	1	1	120 L	Cylindrical basket, 7 × 7	3	100
L1, control	4	6	1	1	1	120 L	Tray, 30 × 40	1	200–600
L2	6	5	4	1	1	15 m ³	Small bag, 25 × 25	3	100
L3	11	11	4	0	2	120 L	Tray, 30 × 40	1	100
L4	10	8	3	0	7	120 L	Tray, 30 × 40	3	150
L5	12	12	1	3	1	15 m ³	Medium bag, 27 × 100	3	75
L6	12	11	1	0	1	15 m ³	Medium bag, 27 × 100	3	150
L7	12	12	1	0	1	15 m ³	Medium bag, 27 × 100	3	150

Finally, all batches in L1 were heat shocked for 2 h at 40°C 1 wk after the beginning of the experiment. As a control for L1, extra juveniles from 12 of the batches tested were not heat shocked, but instead simply held in a tank next to that holding the L1 batches. At the age of 3 mo, L1 and L1-control oysters were small (5–10 mm), and in these conditions, it is possible to keep up to 5,000 spat in a tray without any effect on growth and survival caused by food supplies. Mortality counts were made twice a week for L1–L4, and once a week from August 2003 for L5–L7.

Field Experiments

Field mortality results are not presented in full detail in the current article, because we instead emphasize their comparison with results recorded in the laboratory experiments. More details about the field testing are given in Boudry et al. (2008) and Dégremont et al. (2010a). Field tests of sets 1–5 are referred to as F1–F5, respectively, in the current study and their correspondence with laboratory experiments L1–L7 is given in Table 1. Oysters tested in F1 were deployed in 3 intertidal sites along the French coasts: Ronce (1°10'W, 45°48'N), Rivière d'Auray (RA; 2°57'W, 44°45'N), and Baie des Veys (BDV; 1°06'W, 49°23'N). In field experiments F2, F4, and F5, oysters were deployed only at Ronce, whereas F3 was deployed only at RA. All batches tested in laboratory experiments were also tested in the field, except for F1 in RA and BDV, which had 2 and 3 fewer batches than L1, and 3 and 4 fewer batches than L3, respectively. For all field experiments at each site, each batch was represented by 3 bags (100 × 27 cm) of 150 oysters (shell height, 13–25 mm). Bags were randomly attached to racks and mortality was recorded on October 8, 2002, for F1 and F2, and on October 10, 2003, for F3–F5.

Statistical Analyses

All analyses were conducted using SAS version 9. The replicate level (i.e., container, when appropriate, Table 2) was found nonsignificant ($P > 0.05$) and was therefore dropped from the models. Differences in mortality among stocks (R line, S line, diploid control, selected and unselected triploid controls) and among batches within stock recorded at the end of each laboratory experiment were analyzed by a logistic regression for binomial data using the GENMOD procedure in SAS (McCullagh & Nelder 1989, SAS Institute Inc. 1995) through the following model:

$$\text{Logit}(Y_{ijk}) = \mu + \text{stock}_i + \text{batch}_{j(i)} + E_{ijk}$$

where Y_{ij} is mortality of the k spat of the l batch within the i stock, and μ is the intercept, and all effects were fixed.

Comparisons among stocks were conducted using the least squares mean statements of the GENMOD procedure (Littell et al. 2002). For L1 and its control, only batches tested in both treatments (heat shocked and not heat shocked) were included in the statistical analyses. The following model was used:

$$\text{Logit}(Y_{ij}) = \mu + \text{stock}_i + \text{treatment}_j + (\text{stock}_i \times \text{treatment}_j)$$

where Y_{ij} is the probability of mortality for the i th stock (R, S, controls) at the j th treatment, and μ , stock, treatment, and stock × treatment are the intercept, stock, treatment, and interaction effects, respectively.

Batches tested in both laboratory and field conditions were used to calculate the Pearson's correlation coefficient for mortality; this was done using the CORR procedure.

RESULTS

Laboratory Experiments

Water temperature ranged from 18–22°C in 2002 and from 19–26°C in 2003 (Fig. 1). In all laboratory experiments, mortality was found to be significantly different among batches within stock ($P < 0.0001$); however, the results described here focus on the stock effect (R line, S line, controls).

In L1, oysters showed low mortality during the first week (Fig. 2, L1). Mortality began the day after the heat shock and remained high for the next week. At the end of the L1 experiment, significant differences in mean mortality were found among stocks, with the highest mortality for the S line (53%), intermediate levels for the diploid and triploid controls (40% and 44%, respectively), and the lowest for the R line (28%; chi-square = 147.3, $P < 0.0001$; Fig. 2, L1). It is interesting to note that mortality mostly occurred during a 1-wk period, and 80% of the final mortality occurred in only 4 days. For the L1-control (not heat shocked), mean mortality reached 10% for the R line, 24% for the triploid control, 26% for the diploid control, and 26% for the S line, whereas values were 24%, 44%, 51%, and 54% for the oyster from the same stocks that were heat shocked. Interaction between treatment and stock was not significant (chi-square = 0.47, $P = 0.93$), whereas stocks of oysters that had received a heat shock had significant higher mortality than those that had not (chi-square = 16.97, $P < 0.0001$), and mortality among stocks was also significantly different (chi-square = 37.66, $P < 0.0001$).

In L2, where oysters were tested in the laboratory only (Table 1), no mortality was observed during the first week, and most occurred between July 15th and July 25th (Fig. 2, L2). Significant differences in mortality were found among stocks (chi-square = 117.0, $P < 0.0001$), with similar low mortality in the

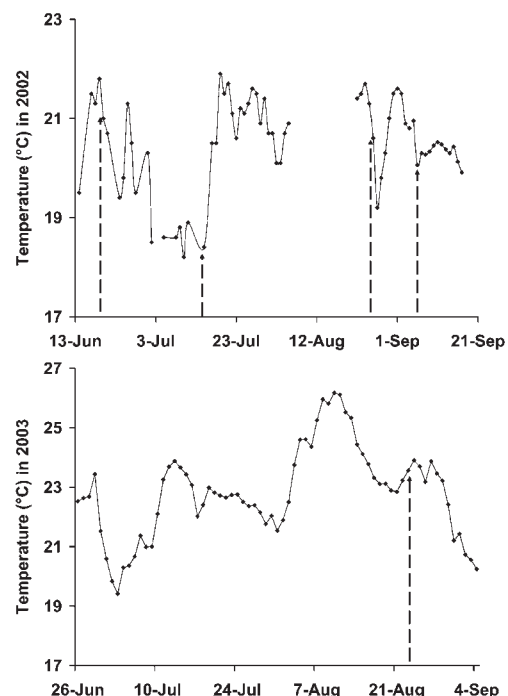


Figure 1. Water temperature in the laboratory experiments in 2002 (top) and 2003 (below). Dashed arrows indicate the onset of heavy mortality.

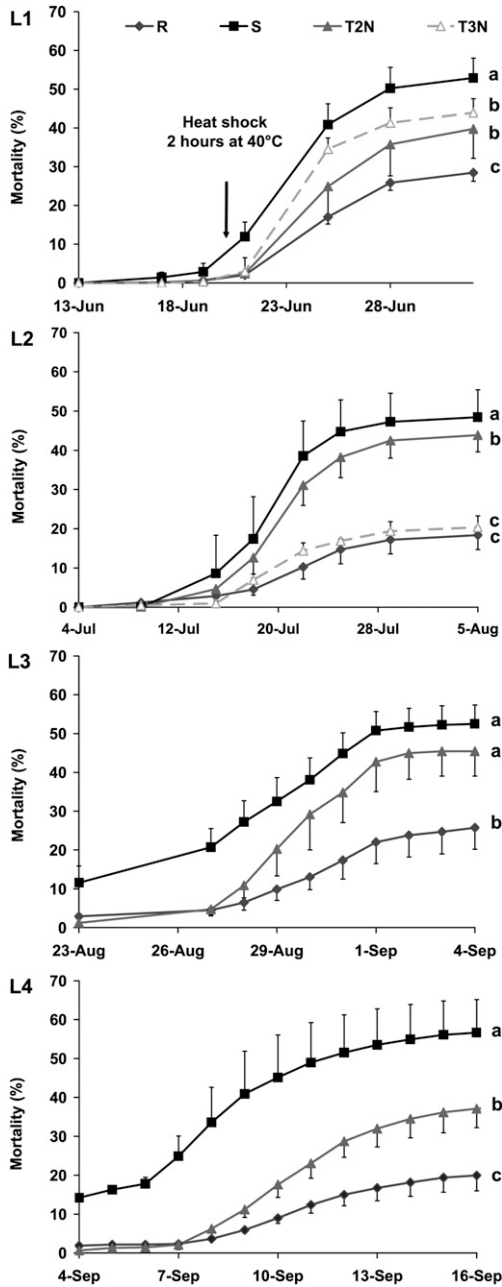


Figure 2. Cumulative mortality of the R and S lines, diploid controls (T2N; mean and SE among batches), and triploid control (T3N; mean and SE among replicates) for L1, L2, L3, and L4 in 2002. Stocks with different letters were significantly different ($P < 0.05$).

R line (19%) and triploid control (21%), whereas the S line (49%) and diploid control (44%) showed higher mortality (Fig. 2, L2). As in L1, most of the mortality (70%) occurred during 4 days.

In L3, mortality recorded after 6 wk of testing in the field was 3%, 12%, and 1% for the R line, S line, and diploid control, respectively. There was no significant difference in mortality among these stocks prior to their transfer to the laboratory (chi-square = 4.6, $P = 0.10$). Four days later, a mortality outbreak occurred in the laboratory between August 27th and September 1st, with 77% of final mortality observed in the laboratory phase occurring during this period (Fig. 2, L3). Daily sampling revealed that the onset of the heavy mortality episode differed

among batches by no more than 1 or 2 days. There was a significant difference in mortality among stocks at the end of the experiment (chi-square = 123.6, $P < 0.0001$), with similar high mortality obtained for the S line (53%) and the diploid control (45%), whereas the R line had lower mortality (26%).

As in L3, oysters used in L4 were brought into the laboratory after being tested for 1 mo in the field at Ronce, where mean mortality was low (6%) and no significant difference was found among stocks (chi-square = 4.7, $P = 0.10$). A major mortality event occurred 2 days after their transfer to the laboratory, between September 6th and September 11th (Fig. 2, L4). At the end of the L4 trial, mortality ranged from 9–52% for the R batches, 23–90% for the S batches, and 29–44% for the diploid control. Significant differences in mortality were found among stocks (chi-square = 246.8, $P < 0.0001$), with the highest mean mortality in the S line (57%), an intermediate level in the diploid control (38%), and the lowest in the R line (21%). As in the previous experiments, most of the mortality (70%) was recorded during a short period of time (4 days).

Mortality rates in L5, tested only in the laboratory, were less than 5% for each stock until August 23rd (Fig. 3, L5). Three days later, massive mortality affected all stocks, with 83% of the final mortality occurring during this period. On September 4th,

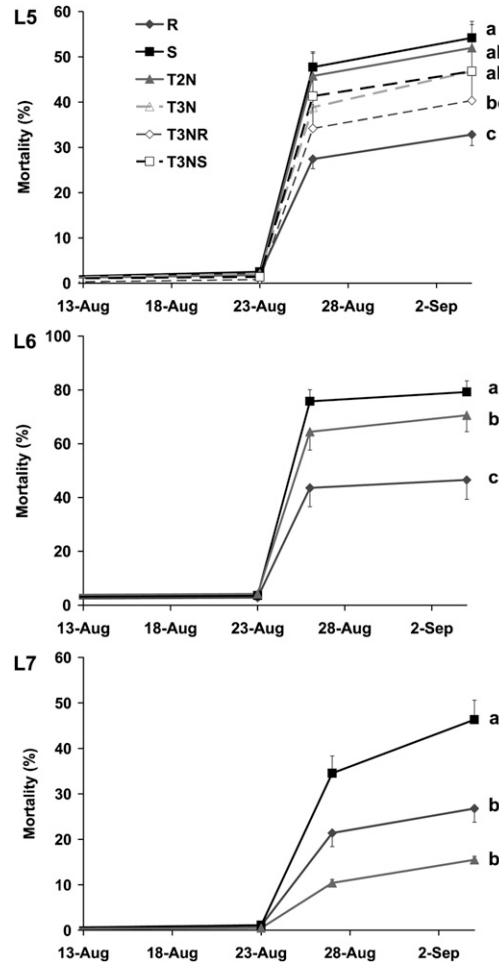


Figure 3. Cumulative mortality of the R and S lines (mean and SE among batches), diploid controls (T2N), triploid control (T3N), and selected triploids (T3NR and T3NS; mean and SE among replicates) for L5, L6, and L7 in 2003. Stocks with different letters were significantly different ($P < 0.05$).

mortality was 33% for the R line, 40% for T3NR, 47% for T3N and T3NS, 52% for the diploid control, and 54% for the S line, with significant differences in mortality among stocks (chi-square = 69.6, $P < 0.0001$; Fig. 3, L5).

In L6, tested only in the laboratory (Table 1), no unusual mortality was recorded from July 9th to August 23rd. As in L5, heavy mortality occurred for all stocks between August 23rd and August 26th, representing 89% of the final cumulative mortality. Significant differences in mortality were found among stocks (chi-square = 177.2, $P < 0.0001$), with the lowest mean mortality in the R line (48%), intermediate mortality in the diploid control (75%), and the highest mortality in the S line (82%; Fig. 3, L6).

Similar results were found for L7 in the final laboratory trial, with no significant mortality from July 24th to August 23rd, whereas heavy mortality occurred between August 23rd and August 26th, representing 73% of the final mortality (Fig. 3, L7). Significant differences in mortality were observed among stocks (chi-square = 65.2, $P < 0.0001$), with the lowest mortality (16%) for the diploid control, intermediate for the R line (27%), and highest for the S line (46%).

Field and Laboratory Comparisons

Mortality levels recorded in the field for the R line, S line, and controls are given in Table 3. For set 1, the comparison of mortality between L1 and F1 and between L3 and F1 revealed positive correlations in all sites, ranging from $r = 0.13$ – 0.63 , which were significant in Ronce and RA (Figs. 4 and 5). Mortality was also positively correlated between L2 and F1, but was only significant in RA ($r = 0.58$, $P < 0.05$; Fig. 6). For set 2, mortality recorded in L4 and F2 was significantly and positively correlated ($r = 0.94$, $P < 0.0001$; Fig. 7). For all sets produced in 2003, correlations between field and laboratory mortality were positive, high, and significant ($P < 0.0001$). Correlations were 0.85 between F3 and L5 (Fig. 8), 0.94 between F4 and L6 (Fig. 9), and 0.84 between F5 and L7 (Fig. 10).

DISCUSSION

Laboratory Experiments

The laboratory experiments reported herein allowed us to make a detailed description of the temporal kinetics of summer

TABLE 3.

Mortality (%) of the selected lines (R, S) and controls (diploids, T2N; triploids, T3N) recorded in the field.

Experiment (or Set)	Site	R Line	S Line	T2N	T3N
F1 (S1)	Ronce*	4/5/4	25/19/33	8/8/8	2/2/–
F1 (S1)	Rivière d'Auray*	7/6/7	39/41/43	24/24/24	7/7/–
F1 (S1)	Baie des Veys*	7/7/7	21/10/19	5/5/5	6/6/–
F2 (S2)	Ronce	3	34	4	–
F3 (S3)	Rivière d'Auray†	26	71	47	36/50/58
F4 (S4)	Ronce	35	73	62	–
F5 (S5)	Ronce	29	62	24	–

* The 3 mortality rates recorded in the field are given for the batches tested in the L1, L2, and L3 experiments, respectively.

† Mortalities of T3N are given for T3NR, T3N control, and T3NS stocks, respectively.

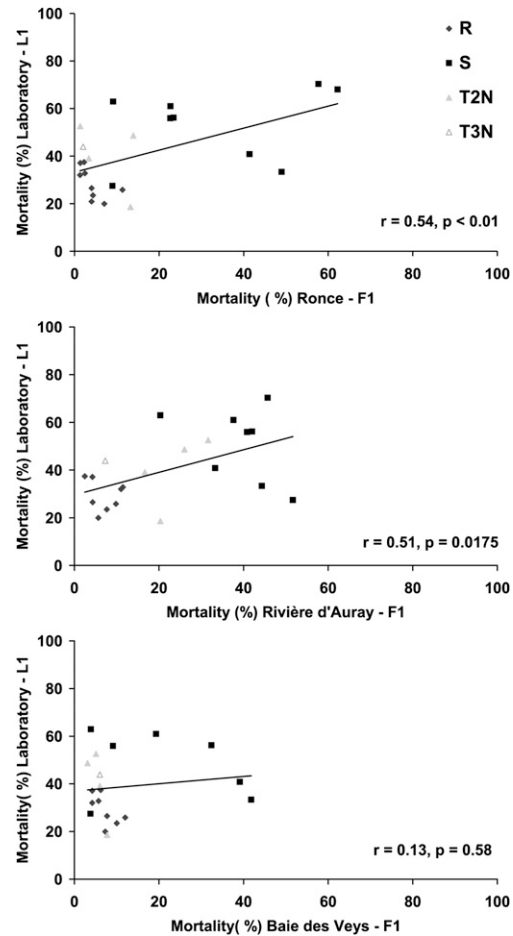


Figure 4. Relationship between field (F1) and laboratory (L1) mortality. Correlation coefficients and their significance are given.

mortality in juvenile *C. gigas*, as well as to understand better the effect of a number of factors hypothesized to be potentially involved in summer mortality: genetics (selection and ploidy), stress resulting from handling, temperature, and response to heat stress.

Kinetics of Mortality

Mortality was observed in all the laboratory experiments, but the timing of the onset when it occurred varied. In L3–L4, mortality started soon after transfer to the laboratory, but in L7 it started 2 mo later (Figs. 2 and 3, Table 1). Each time, mortality occurred within a 1-wk period, with 80% of total mortality occurring during a narrower 4-day period. Our results show that summer mortality is a short-term event in young *C. gigas* when they are tested under laboratory conditions, and are in agreement with those reported in juvenile (Sauvage et al. 2009) and adult oysters (Lipovsky & Chew 1972). Although this mortality could be the result of a viral or a bacterial infection, it is unlikely to be caused by a parasite, because longer development cycles usually observed in such cases (e.g., *Bonamia ostreae*-induced mortalities in the European flat oyster *Ostrea edulis* (Lallias et al. 2008)). Supporting the hypothesis of the involvement of a pathogen in the summer mortality observed in our laboratory experiments, the herpes virus OsHV-1 was detected during the

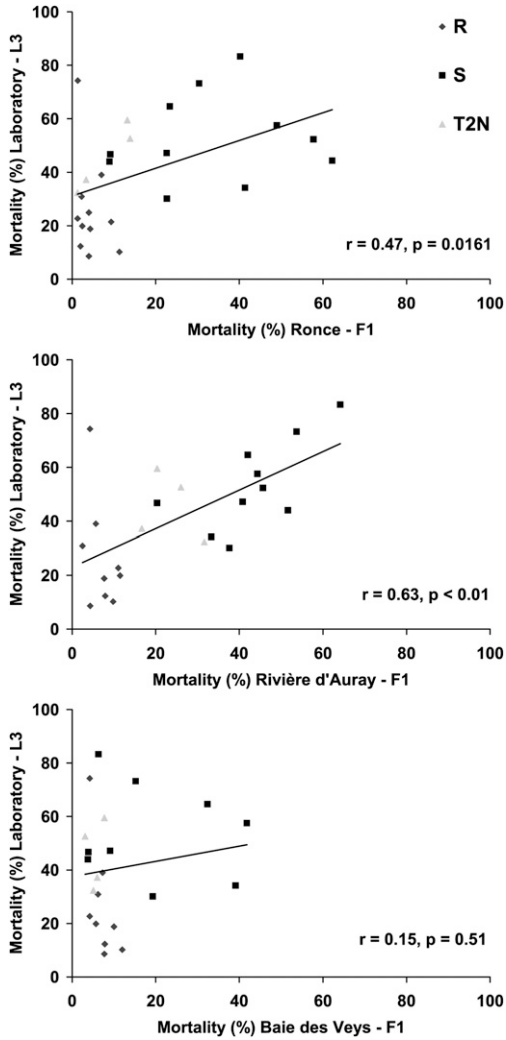


Figure 5. Relationship between field (F1) and laboratory (L3) mortality. Correlation coefficients and their significance are given.

peak of mortality in L4–L7 (data not shown). This result is in agreement with results found by several authors who reported bacteria and viruses associated with some cases of summer mortality in *C. gigas* (Friedman et al. 1991, Lacoste et al. 2001, Le Roux et al. 2002, Nicolas et al. 2008, Sauvage et al. 2009). Heavy mortality occurred in S batches in all experiments, whereas R batches in the same tanks did not suffer such high mortality. If a bacterium or virus was a major causal factor of the mortality observed in our laboratory experiments, this could indicate that horizontal transmission was limited, as suggested by Lacoste et al. (2001) and Sauvage et al. (2009), but could also show that R oysters are genetically more resistant to this pathogen.

Because we wanted to mimic field conditions, where all batches are grown together, batches from selected and unselected stocks were placed in random positions within tanks. One single tank was used for culturing all stocks, batches, and replicates in 6 of the laboratory experiments, but several were used for L3 and L4, because the volume of the tank combined with the size of the oysters in these trials did not allow us to test all batches within a single tank (Table 2). The onset of the mortality always occurred in all replicates and tanks within a 24–48-h period in the laboratory experiment. Similarly, the

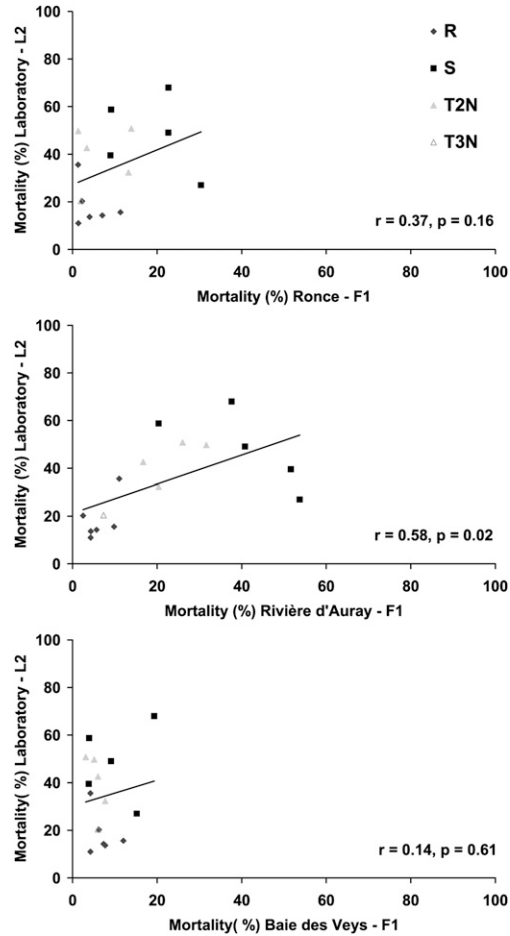


Figure 6. Relationship between field (F1) and laboratory (L2) mortality. Correlation coefficients and their significance are given.

onset of mortality was observed from August 23rd in L5–L7 in laboratory experiments (Fig. 3). Considered together, these results suggest that either seawater could have carried an agent, such as a toxin or a pathogen, which then spread to the batches tested, or that some of the oysters were already infected by a pathogen before the experiments, and proliferation began when environmental conditions allowed it, contaminating the other oysters in the tank. However, further investigations are needed to assess the involvement of such an agent in the summer mortality phenomenon of juvenile *C. gigas*. Batches should be

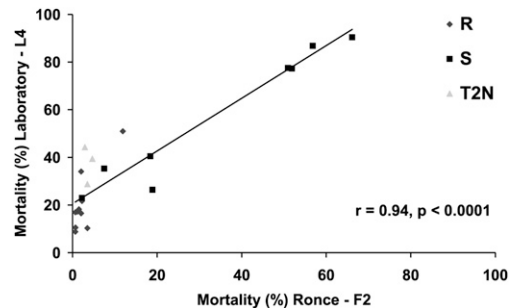


Figure 7. Relationship between field (F2) and laboratory (L4) mortality. Correlation coefficient and its significance are given.

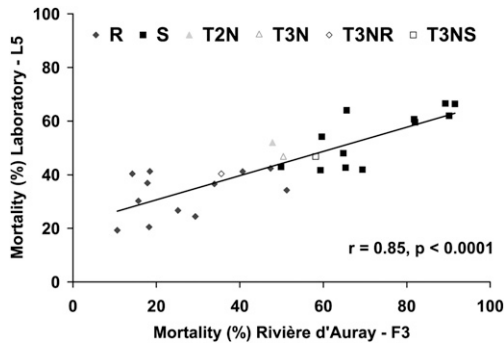


Figure 8. Relationship between field (F3) and laboratory (L5) mortality. Correlation coefficient and its significance are given.

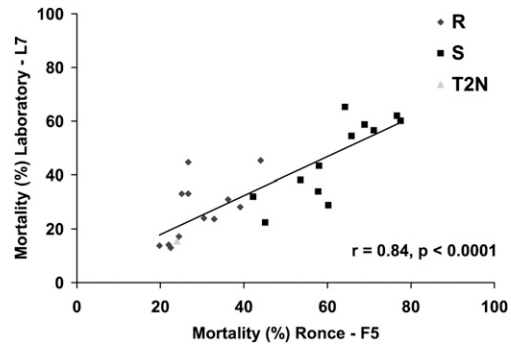


Figure 10. Relationship between field (F5) and laboratory (L7) mortality. Correlation coefficient and its significance are given.

tested in both communal (several batches in a tank) and separate (1 batch per tank) conditions in tanks for which seawater is either changed (flow-through system) or not (recirculating system), and in either sterilized (ultraviolet, chlorine, heat treatment) or untreated seawater.

Finally, consistent findings were obtained with set 1 in L1, L1-control, L2, and L3, with the lowest mortality in the R line, intermediate in the controls, and highest in the S line (Fig. 2). Thus, mortality can be induced several times in the laboratory by using oysters that were held in a site where they did not experience mortality. Dégremont et al. (2010b) found that juvenile *C. gigas* could be protected from mortality during their first year by keeping them in a nursery. Thus, studies investigating the causes of the phenomenon could be done under laboratory conditions before, during, and after the field mortality outbreak, with easy access to the animals (nursery relative to field), and results could be confirmed replicating laboratory experiments through time.

Genetics

Response to Selection

The R lines had lower mortality than the corresponding S lines in all laboratory experiments, indicating a positive response to selection to decrease or increase summer mortality in juvenile *C. gigas*. Our laboratory results confirmed those observed in the field (Boudry et al. 2008, Dégremont et al. 2010a), meaning that some of the causes of the mortality observed in RA and Ronce in 2001, involving oysters on which

selection had been carried out, were also found under laboratory conditions. Interestingly, in L5, T3NR (40%) had lower mortality than T3N and T3NS (47% for both batches), whereas mortality was 33% for the R line, 54% for the S line, and 52% for the diploid control (Fig. 3, L5). Similar trends were found under field conditions in RA in 2003, with summer mortality of 36%, 50%, and 58% for the T3NR, T3N, and T3NS, respectively, whereas mortality was 26%, 47%, and 71% for the R line, diploid control, and S line, respectively (Table 3). Thus, positive response to selection was also evident in triploid oysters through their selected diploid female parents, although these had only contributed a third of the genome of these progeny. Further improvement should therefore be possible when triploid oysters are produced using both selected tetraploid and diploid parents.

Ploidy

Triploid controls had high mortality in L1 (41%) and L5 (47%), which followed the mortality patterns of the diploid controls (40% and 52% in L1 and L5, respectively), suggesting that triploidy per se does not confer any survival advantage at the juvenile stage in *C. gigas* under our laboratory conditions, whereas it can be the case under field conditions (Boudry et al. 2008). Nevertheless, further investigations are needed to determine better the effect of ploidy on survival at the juvenile stage, particularly as around 90% of commercial hatchery-produced spat in France are now triploids.

Handling

Survival was greater than 98% during the first week of experiments L1, L2, L5, L6, and L7, indicating that neither transportation of the spat from the nursery to the laboratory nor the handling involved in this transfer caused significant mortality. This observation supports the findings observed in the first generation of field experiments in 2001 (Dégremont et al. 2005). Furthermore, it was shown that oysters could be protected from mortality events during their first year when they were grown in the nursery in Bouin, whereas those deployed in the field had significant summer mortality in the same year (Dégremont et al. 2010b). Consequently, oysters in the nursery can either be considered to be in good health, allowing them to be manipulated without the induction of mortality, and/or to have been protected from risk factors implicated in the summer mortality phenomenon. In L3 and L4, oysters were transferred from nursery to the field and then from the field to the laboratory, and mortality

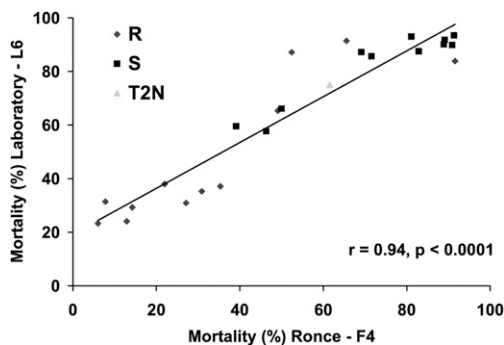


Figure 9. Relationship between field (F4) and laboratory (L6) mortality. Correlation coefficient and its significance are given.

occurred 4 and 2 days after the beginning of these experiments, respectively. Oysters might, therefore, be more fragile to handling and/or laboratory conditions after having been deployed in the field for several weeks during the summer.

Temperature

When mortalities occurred in our laboratory experiments, seawater temperatures ranged between 18°C and 24°C (Fig. 1). Our results support the mortality risk period defined by Samain and McCombie (2008), whereby summer mortality can occur after seawater temperatures reach a threshold value of 19°C. Nevertheless, on a finer scale, no trends were found between seawater temperature recorded in our laboratory experiments and the onset of mortality (Fig. 1). In L5, L6, and L7, water temperature reached a peak at 26°C on August 10, 2003, but mortality did not occur before August 23rd, indicating that high temperatures themselves did not induce immediate mortality. However, it is clear that summer mortality is related to seawater temperature, and previous temperature exposure is a factor affecting mortalities (Lipovsky & Chew 1972, Soletchnik et al. 2006, Li et al. 2007).

Experimental Heat Shock and Environmental Heat Stress

Thermal stress increased mortality in juvenile *C. gigas* and increased the difference between oyster stocks compared with those that had not been heat shocked, suggesting a difference in thermal tolerance. This is in agreement with results obtained by Shamseldin et al. (1997), who reported differences in thermal tolerance among stocks of *C. gigas*. Interestingly, oysters of set 1 tested in L2, L3, and also at the RA site, where high summer mortalities are reported in juvenile *C. gigas* (Dégremont et al. 2005, Dégremont et al. 2010b), had similar results to those heat shocked and tested in L1: The highest mortality was in the S line, intermediate in the unselected diploid controls, and the lowest in the R line. Nevertheless, one difference appeared concerning the triploid stock, because it had higher mortality than the R line in L1, whereas mortality of the R line and the triploid batch were similar and low in the field (Table 3) and in L2, meaning that heat stress more clearly revealed the survival capability of the triploid batch tested under L1 conditions. Similarly, the 3 batches of triploids tested in L5 had higher mortality than the R group (Fig. 3, L5). Seawater temperature during August 2003 was unusually warm because of a heat wave in France, reaching 25–26°C for a week, 2 wk before the onset of mortality (Fig. 1). This heat wave could have acted in a similar way to a heat stress in L5, L6, and L7. Clegg et al. (1998) found that thermal stress induces heat shock protein (HSP) synthesis during the following 2 wk. The production of HSPs requires high energy expenditure, but, in this case, comes at the time of year when oyster energy reserves are at their lowest (Heude-Berthelin 2000, Li et al. 2007). Furthermore, S oysters showed a large HSP70 increase under hypoxia, in contrast to R oysters, suggesting that S oysters are more susceptible to stress than R oysters (Samain et al. 2007). Thus, a management strategy could be proposed that would consist of testing wild-caught spat and the hatchery-produced spat younger than 6 mo old under laboratory conditions with a heat stress, to predict their survival performance prior to their deployment in the field. Finally, thermal tolerance may represent a selective trait that can be used to improve

resistance to a factor associated with summer mortality, as suggested by Beattie et al. (1980), Hershberger et al. (1984), and, more recently, Lang et al. (2009).

Comparison Between Field and Laboratory Experiments

In 2003, correlations between field and laboratory mortalities were very high ($r = 0.84$ – 0.94 ; Figs. 8, 9, and 10) and there is, therefore, general agreement between laboratory and field results. Batches that performed well in the laboratory also showed high survival in the field, and those showing low survival in the laboratory exhibited low survival in the field. Similar results were also found in adult *C. gigas* (Beattie et al. 1980) and eastern oyster *C. virginica* (Oliver et al. 2000). This also suggested that field and laboratory conditions favored mortality, but to a lesser extent for the R line, and they could be considered as a “bad” environment for survival in juvenile *C. gigas* younger than 1 y old.

In 2002, correlations between field and laboratory performance were lower than in 2003. This could be the result of (1) the grow-out sites used to test the oysters in 2002 and 2003, (2) the environmental conditions encountered during these years, or (3) the culture methods used to test the batches. With regard to (1), selection to produce batches with increased or decreased mortality had been made based on mortality results in RA, because the summer mortality was the highest at that site, whereas it was intermediate at Ronce and the lowest at BDV (Dégremont et al. 2005, Dégremont et al. 2010a). This is in agreement with the correlations found in our study comparing laboratory (L1, L2, L3) and field (F1) conditions, which were the highest in RA, intermediate in Ronce, and lowest in BDV (Figs. 4–6). The comparison between field and laboratory suggests that laboratory conditions themselves favor mortality in juvenile *C. gigas*.

With regard to (2), mortality in juvenile *C. gigas* in the field was lower in 2002 than in 2003 (Table 3), which was also confirmed in juvenile oysters all along the French coast (Fleury et al. 2005). This unusually high mortality encountered in juvenile *C. gigas* in 2003 was related to the long and exceptional heat wave that year, during which the highest minimum and maximum air temperatures were recorded for the last 50 y in France (Bessemoulin et al. 2004). Consequently, seawater temperature reached a record high in August 2003, with 26°C in Ronce and 24°C in RA (Ropert et al. 2008), and oysters were exposed to an even higher temperature at the low tide in the field. Thus, heat stress from the heat wave may have caused high mortality in both field and laboratory conditions in 2003, leading to high correlations between both conditions and providing a very effective challenge to identify survival capability of the different batches of oysters. In 2002, however, field conditions did not result in such high mortality (Table 3), whereas laboratory conditions did. Dégremont et al. (2010a) reported that in a “good year” and/or a “good environment” (i.e., low summer mortality in juvenile *C. gigas*), selective pressure is low and differences in mortality among groups appeared minor; alternatively, in a “bad year” and/or a “bad environment” (i.e., high summer mortality in juvenile *C. gigas*), only genetically superior batches are likely to show limited mortality. Thus, summer mortality remained low in the field except for a few S batches in 2002, whereas laboratory conditions led to higher mortality and greater differences in mortality among groups, indicating that our laboratory conditions could be considered a stressful environment for juvenile oysters. This

finding emphasizes the potential value of testing wild-caught spat and hatchery-produced spat in the laboratory (with or without heat stress) prior to their deployment to the field, to predict their survival performance.

Finally, with regard to (3), the culture methods used to test the batches were different between 2002 and 2003, and were different between batches tested in laboratory and field conditions. It is known that culture practices have a significant impact on summer mortality in the field (Soletchnik et al. 2005). Similar culture practices (i.e., bags fixed on iron racks) were used in 2003 to compare batches tested in field and laboratory conditions (Table 2). It was not the case in 2002, when baskets, trays, or small bags were used in laboratory and bags fixed on racks were used in the field. However, no studies have yet directly investigated the impact of laboratory growing methods on mortality. Nevertheless, if pathogens, such as viruses and bacteria, are the main causes of the mortality observed in laboratory, the containers in which the oysters were held would not have any significant impact on the mortality observed, because density per container remained low (100–150 oysters per replicate), oysters were cleaned every day, and the position of the containers within the tanks was changed daily to avoid microenvironmental effects such as food depletion, which could disfavor the oysters that were farther from the water inflow.

CONCLUSIONS

Our results revealed that summer mortality in young oysters can be observed under laboratory conditions, with or without

a thermal stress. Our study is the first report of such a finding in oysters younger than 1 y, and supports previous studies on older individuals (Lipovsky & Chew 1972, Sauvage et al. 2009). Kinetics of mortality revealed that around 80% of the cumulative summer mortality occurred within a period of 4 days, and involvement of pathogens was, therefore, highly likely. Interestingly, mortality levels in the laboratory experiments matched those recorded in the field, especially in 2003, for which the heat wave may have had an impact on survival performance in juvenile *C. gigas* grown in the field. This finding, along with easy access to oysters in the laboratory, will permit further studies in genetics, physiology, or pathology to understand better the etiology of summer mortality. Laboratory-based challenges could also be used in selective breeding programs to improve resistance to summer mortality and to characterize the quality of wild-caught and hatchery-produced spat used by oyster growers.

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