The Effect of Elevated Water Temperature Stress on the Mussel *Mytilus edulis* (L.) Survival and Genetic Characteristics

N. LeBlanc, T. Landry, J. Davidson, R. Tremblay, Mary McNiven

Oceans and Science Branch
Fisheries and Oceans
Gulf Region
P.O. Box 5030
Moncton, N.B.
Canada E1C 9B6

2010

Canadian Technical Report of

Fisheries and Aquatic Sciences 2900

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Canadian Technical Report of Fisheries and Aquatic Sciences

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by

N. LeBlanc[1], Thomas Landry, Jeff Davidson[2], Réjean Tremblay[3], Mary McNiven[2]

Oceans and Science Branch
Fisheries and Oceans
Gulf Region
P.O. Box 5030
Moncton, N.B.
Canada E1C 9B6

[1]Joint R&D Division, Dept. of Virology, Immunology and Parasitology. The National Veterinary Institute (SVA) & Dept. of Biomedical Sciences and Veterinary Public Health, The Swedish University of Agricultural Sciences (SLU), SE-751 89 Uppsala, Sweden

[2]Atlantic Veterinary College, 550 University Avenue, Charlottetown P.E.I., Canada, C1A 4P3

[3]Institut des sciences de la mer, Université du Québec à Rimouski, 310 allée des Ursulines, Rimouski, QC, Canada G5L 3A1

Cat. No. Fs 97-6/2900E ISSN 0706-6457

Correct citation for this publication is:

LeBlanc, N., Landry, T., Davidson, J., Tremblay, R., McNiven, M. 2010. The Effect of Elevated Water Temperature Stress on the Mussel *Mytilus edulis* (L.), Survival and Genetic Characteristics. Can. Tech. Rep. Fish. Aquat. Sci. 2900: vii + 19p.

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ABSTRACT

Two cohorts of *Mytilus edulis* (L.) were sampled from seed lines in St. Peter's Bay, P.E.I. and culled using either elevated water temperature or air exposure. The objective of this study was to determine if the survivors had a detectable increase in heterozygosity from the selective treatments. Increasing the level of heterozygosity in mussels may produce stocks with increased growth and survival, traits that are important in mussel aquaculture. Mortality level from the elevated water temperature stress was greater for the larger size cohort compared to the smaller size cohort. These results suggest that the difference in mortality may be due to physiological condition, genetic characteristics or a combination of these factors. This study showed that culling of naturally collected seed can increase heterozygosity, which has been shown to be an indicator of mussel fitness.

RÉSUMÉ

Des naissains de moules *Mytilus edulis* (L.) provenant de deux cohortes différentes, ont été échantillonnés sur des collecteurs de naissains à St. Peter's Bay, I.P.E. et sélectionnées en fonction de leurs survie dans l'eau à température élevée ou exposé à l'air. L'objectif de cette étude était de déterminer si les moules sélectionnées démontraient un changement au niveau de l'hétérozygosité, selon ces deux traitements. Un niveau d'hétérozygosité élevé chez les moules pourrait avoir un effet positif sur la croissance et la survie, qui sont des caractéristiques importantes pour la mytiliculture. La mortalité induite par l'exposition à l'eau chaude était supérieure chez les moules de la cohorte à tailles supérieures comparativement à celle de tailles inférieures. Les résultats suggèrent que les niveaux de mortalité différents peuvent être attribués à la condition physiologique, aux caractéristiques génétiques ou à une combinaison de ces deux facteurs. Cette étude préliminaire démontre que la sélection de naissains provenant de collecte naturelle peut augmenter le niveau d'hétérozygosité, indicateur de santé physiologique chez les moules.

INTRODUCTION

Previous research, including studies in the Magdalen Islands, found that the degree of heterozygosity, measured on allozyme loci implicated in anabolic and catabolic processes, can be an indicator for the performance of a stock (Tremblay et al., 1998a; 1998b). In fact, the relationship between heterozygosity and physiological fitness indicators such as growth and fecundity has been well documented in several taxa including molluscs (Koehn and Gaffney, 1984; Rodhouse et al., 1986; Beaumont and Zouros, 1991; Hawkins and Bayne, 1991; Mitton, 1993; Bayne and Hawkins, 1997; Myrand et al., 2000). Tremblay et al. (1998b) found that the degree of heterozygosity tended to decrease after wild mussel spat were transferred to a suspension-cultured environment. They suggested that this loss was due to the fall-off of the faster growing mussels with higher heterozygosity, due to harsher physical conditions, such as wave action, once they have grown outside the sock. Historically, large scale mass mortality events have been reported in the mussel aquaculture industry on the temperate north-east coast of North America (Incze et al., 1980; Mallet et al., 1990; Myrand and Gaudreault, 1995). Results from several studies suggest that seed quality is an important factor in relation to these mortalities (Dickie et al., 1984; Mallet et al., 1990; Myrand and Gaudreault, 1995).

Culling practices are commonly used in hatcheries with the objective of producing a higher crop yield. Culling is defined as a process of exposing organisms to a stress which will selectively induce mortality in a portion of the organisms deemed less resistant (Lester, 1983). Currently, the mussel aquaculture industry relies on wild seed collection. This limits methods for improving seed quality to selection techniques as opposed to breeding programs (Gosling, 2003).

The selection techniques applied in this study are based on physiological characteristics. These characteristics were the ability to withstand elevated water temperature or air exposure. The objective of these experiments was to determine the effect of elevated water temperature and air exposure selection treatments on the heterozygosity of the surviving mussels, with the goal of eliminating the lower heterozygotes for the purpose of improving the performance traits, i.e. growth rate in aquaculture conditions.

MATERIALS AND METHODS

Sampling and size distribution

Juvenile mussels were collected, by diving, from commercial seed lines in St. Peter's Bay, . Water temperature and salinity during sampling were -1.0±0.5°C and 29.2 ppt, respectively. The specimens were immediately placed in coolers and transported to the Biological Station in Ellerslie, PEI within six hours of collection to avoid temperature variation. At the station, samples were placed in an acclimation tank (AT) with water at 1°C and 29.6 ppt. The tank dimensions were 99 cm by 113 cm and filled to a height of 41 cm. The water supply was derived from the Bideford River filtered at 1 micron and was aerated. Specimens were not fed and mortality remained low in the AT (< 5%) throughout the course of the experiments. The AT contained separate compartments (upweller tubes) for each treatment and control. To reduce thermal shock, acclimation of the mussels was performed by allowing water temperature in the AT to rise gradually from 1°C to 19°C over a period of 74 hours (0.25±0.19°C/hr).
The size distribution of a large sample of the mussels (n=2100) collected was analyzed to determine whether the sample consisted of one or more cohorts. Size was calculated by measuring the length of the shell, umbo to farthest point on the posterior edge. Random sub-samples were selected from the large sample for exposure to stress treatments and as controls for heterozygosity analysis.

Aerial exposure

The aerial exposure experiment was performed on five hundred mussels randomly selected from the original sample population. The experiment was started after three days of acclimation, when water temperature in the AT had reached 15.3°C. To ensure uniform conditions, all individual mussels were lightly dried with a paper towel before being placed on the laboratory counter-top where experiment was carried out. Mortality was determined by visual observations based on gaping and weak or non-response to tactile stimulation of the mantle. A weak response was defined as the inability to completely close the valves. It was expected that after a period of gaping the valves would become closed with mussels surviving for several days until the weaker mussels would start to die (Coleman, 1971; Eertman, 1993). Mortality checks were made twice daily for the first three days, and three times daily afterward. The air temperature during the experiment was 22.5°C ± 2°C and relative humidity was 61% ± 5%.

Elevated water temperature tolerance

The water temperature tolerance experiment was conducted after four days of acclimation when temperature in the AT was 19°C. Four hundred and fifty mussels were randomly selected and transferred into a stress tank supplied with water at 27±0.2°C. The salinity was maintained at approximately 30 ppt because it is considered optimal for

thermal tolerance (Wallis, 1975). Due to technical difficulties, temperature could not be maintained and varied between 24°C and 31°C (Figure 1). Mortality was determined by visual observations based on gaping and weak or non-response to tactile stimulation of the mantle. Mortality checks were performed three times daily for the eight days of the experiment. The tank was drained and sampled after eight days and mortality was calculated.

In keeping with other research on use of lethal stress for selection purposes, the level of mortality for this project was based on the LT_{50} (Wallis, 1975; Cotter et al., 1982; Myrand et al., 2002).

Allele frequency determination

Random samples of several hundred mussels that survived the elevated water temperature stress and control specimens were transported on dry ice to the MAPAQ marine lab in Grande-Rivière (Québec) where they were stored at -80°C until processed.

As described in Tremblay et al. (1998b), mussels were thawed and measured (to 0.01 mm) from the umbo to the farthest point on the posterior edge. A small piece of the digestive gland (all the tissue for small mussels) was homogenized in 200 ul (100ul for mussels <20 mm) of homogenization buffer [Tris-HCl, pH 8.0 with 30% sucrose, 5 mM DL-dithiolthreitol, 1% polyvinylpolypyrrolidone, 1mM phenylmethylsulphonyl fluoride, 0.1% B-nicotinamide adenine dinucleotide, 0.1% B-nicotinamide adenine dinucleotide phosphate] (Hebert and Beaton, 1989). The buffer/tissue solution was centrifuged at 15000 g at 4°C for 30 minutes. The supernatant was extracted and applied to a horizontal cellulose acetate gel (Hebert and Beaton, 1989). The polymorphic enzymes studied were mannosephosphate isomerase (MPI, EC 5.3.1.8), phosphoglucomutase (PGM, EC 2.7.5.1), octopine dehydrogenase (ODH, EC 1.5.1.11), glucose phosphate isomerase (GPI, EC 5.3.1.9), and leucine aminopeptidase (LAP, EC 3.4.11). Enzymes were stained using the method described by Hebert and Beaton (1989). A standard of all possible alleles was prepared by mixing homogenates of individuals until all genotypes were represented. The standard was used on each gel to aid in exact allele identification.

Statistical analyses

The distribution of the mussel population was analyzed using length frequency histograms and overlapping distribution curves (Chase and Bailey, 1999). Differences in size among treatments were analyzed using a two-sample t-test and Mann-Whitney tests (non-parametric).

Allelic frequencies and heterozygosity analyses, expected and observed under the assumption of the Hardy-Weinberg equilibrium were determined using BIOSYS-1 software (release 1.7; Swofford and Selander, 1989). Allelic frequencies among treatments were compared with multiple heterogeneity X^2 Monte Carlo simulations (Roff and Bentzen, 1989) using the REAP program (McElroy et al., 1991).

RESULTS

Frequency distribution

Examination of the size frequencies of the mussel population revealed the presence of two cohorts with mean lengths of 11.66 and 29.8 mm (Table 2 (controls), Figure 2). The log normal distribution fit best on the smaller peak while the normal curve fit best on the larger peak. Based on visual analysis, 20 mm was selected as the separation point between these two cohorts.

Aerial exposure

Shortly after emersion, gaping was observed in approximately 20% of the individuals. Within 12 hours of their initial exposure to the air, all mussels were closed tightly. After five days very little mortality was detected based on permanent gaping. On day 6, a mortality count of the entire sample was conducted, revealing 12% mortality (59 mussels). Although an additional 10% of specimens appeared dead (47 mussels) on day 7, the presence of noxious odour leads us to suspect that gaping was not the best indication of mortality for these small mussels exposed to air. Closer examination of 30 specimens, by opening them, revealed that they were all dead. The remaining specimens were all placed in water overnight and subsequent examination confirmed 100% mortality. Due to these results, there were no further analysis on this stressor.

Water temperature stress

After the start of the experiment, visual inspection of mussels in the tank and manual inspection found that mortality remained low (< 10 mussels per day) during the initial seven days. Water temperature only remained consistently above 27°C from day six onwards (Figure 1). Dead mussels were removed immediately to avoid contamination of the tank. Mortality levels increased rapidly after the temperature rose above 30°C at the end of the seventh day and continued for the next 18 hours, when the experiment was terminated. A comparison of the mortality levels of the two cohorts using a test for a difference of proportions found that the larger size cohort had a significantly higher mortality than the smaller size cohort ($P < 0.01$). The mortality results for the two cohorts after exposure in the water temperature stress are presented in Table 1.

Size of mussels exposed to elevated water temperature

In the smaller size cohort (< 20 mm), the control specimens were significantly larger than the survivors of the elevated water temperature stress ($P = 0.01$) (Table 2). This was not the case for the larger size cohort (> 20 mm) in which no difference was found between the control mussels and the survivors of the elevated water temperature ($P = 0.73$) (Table 2).

Genetic characteristics for the two cohorts

Comparing heterozygosity between the two cohorts was done by examining the control samples from each age group. The observed heterozygosity (H_o) for the smaller size cohort was 0.416 and 0.327 for the larger size cohort (Table 3). Analysis using degree of heterozygosity (mean heterozygosity calculated for each locus for each mussel group) found no significant difference between the two cohorts, although again the larger size cohort had a lower value (1.58) than the smaller size cohort (2.04) (Table 4). Significant deficits in heterozygosity (at the 5% level) at three loci (GPI, MPI, ODH) were found in both cohorts. Allelic frequency differences were detected at two loci, PGM and LAP between the two cohorts (Table 5).

Selective mortality from the exposure to elevated water temperature had an effect on the heterozygosity in both cohorts. Significant deficits (at the 5% level) at three loci (GPI, MPI, ODH) in the control group for each cohort were not present in the survivors of the elevated water temperature treatment. However in the larger size cohort a deficit at the PGM locus was found in the survivors of the exposure to elevated water temperature that was not present in the control group. The overall observed heterozygosities for the treated mussels in both cohorts were closer to expected levels than were the control groups (Table 3). Changes in mean heterozygosity were more pronounced in larger size cohort compared to smaller size cohort after exposure to elevated temperature treatment. However, analysis comparing degree of heterozygosity revealed no significant differences between treatment and control within or between cohorts ($P = 0.24$, Table 4).

Frequency analysis among the four groups (treatment and control in both cohorts) found differences at LAP and PGM loci (Table 5). As stated above, the control groups from the two cohorts differed significantly from each other at the LAP locus ($P = 0.001$) and PGM locus ($P = 0.001$). Also, in the smaller size cohort, survivors of the water temperature stress had significant differences at the LAP locus ($P = 0.01$) and PGM locus ($P = 0.01$) compared to its control group (Table 5).

DISCUSSION

In the aerial exposure experiment only a small number of individual mussels showed signs of mortality through gaping. The majority of mussels remained closed throughout the course of the experiment and when checked after seven days, the mortality was 100%. Although Eertman (1993) reported that mussels did not always gape upon death and smell sometimes had to be used as an indicator, this was reported to happen only occasionally. One explanation for the difference in gaping experienced in this case comes from the aerial conditions used. Although our experimental conditions were not constant, the temperature was 22.5± 2°C and the relative humidity of 61 ± 5%. In comparison, the conditions Eertman (1993) reported were constant temperature and humidity of 18°C and 83% respectively. Coleman (1971) did not report the air temperature used but stated that air temperatures were the same as the sea water temperatures where the animals were kept and the relative humidity was maintained between 85-90%. The warmer, dryer experimental conditions in our study may have had a desiccating effect on the mussels' tissues which held the shell closed even after the

adductor muscles failed. This effect may have been exacerbated by the small size of the mussels used in this experiment and could be responsible for the lack of gaping in moribund individuals. Clearly, relying on gaping to indicate mortality is unreliable under the conditions employed, especially in low humidity.

Although not measured for statistical analysis, monitoring mortality in the water temperature exposure experiment demonstrated that death occurred when temperatures were at or above 27°C. This result agrees with other elevated water stress experiments on marine mussels (Read and Cumming, 1967; Gonzalez and Yevich, 1976). As water temperature approached and surpassed 30°C, mortality increased dramatically within a few hours. Comparison between the smaller size and larger size cohorts revealed a significant difference in mortality. The smaller cohort survived the elevated water temperature exposure better than the larger cohort. This result does not agree with Wallis (1975) who reported that at any lethal temperature, smaller mussels succumbed more quickly than larger mussels. In that experiment smaller mussels were less than 3 cm and larger mussels were greater than 5 cm. This differs from the current experiment where all the mussels were under 5 cm in length. This finding by Wallis (1975) does not agree with Bayne (1984) who found that smaller individuals were at an energetic advantage in responding to the elevated temperature, as physiological measurements to determine scope for growth supported this conclusion. Wallis (1975) did not conduct genetic or physiological tests that could explain his results. The reason for the survival advantage of the smaller cohort in our sample may be two-fold. The smaller cohort may have had a genetic advantage as well as a physiological one. This theory may be supported by the higher heterozygosity observed in the smaller cohort compared to the larger cohort. The smaller cohort had an overall observed heterozygosity (H_o) of 0.416 while the larger cohort's overall H_o was 0.327. Although not statistically significant (likely due to relatively small sample size of larger cohort control group), the largest gap in degree of heterozygosity was found between these two groups: the smaller cohort control group had an average score of 2.04 and the larger cohort control group had an average score of 1.58. Theoretically, this indicates that the smaller cohort may have been genetically more robust and could better survive the stress of exposure to elevated water temperature.

Aside from the initial difference in heterozygosity mentioned above, the two cohorts differed in other genetic characteristics. Comparison of allelic frequencies between the control groups for each cohort found differences at two loci, LAP and PGM. Differences in allelic frequencies among mussels from the same area was observed (Lassen and Turano, 1978). The differences in frequency may have arisen from selection that occurred on the older cohort before the younger cohort was present. Variation in salinity has been shown to have a selective effect at the LAP locus, causing large changes in allele frequency (Koehn and Hilbish, 1987). Furthermore, both PGM and LAP loci have heat-sensitive allelomorphs (Theisen, 1978; Gardner and Skibinski, 1990). As with salinity, selective thermal conditions could have affected the older cohort before larval development and settlement of the younger cohort or at a time when the smaller cohort had a physiological advantage in survival due to smaller size. Another possibility is that the younger cohort experienced selective mortality in the pre-settlement stage.

Within the larger size cohort, a survival advantage for smaller mussels was not found. There was no difference in size between mussels exposed to elevated water temperature and those from the control. There was, however, a difference in size

between the treatments and controls of the smaller size cohort. The survivors of the elevated water temperature stress were significantly smaller than the control sample. These results, along with those of Wallis (1975), suggest that there may be size ranges over which differences in thermal resistance exist. Our results found that the advantage was always present in smaller mussels. However, the findings in Wallis (1975) which used a broader size range found the opposite. Although this study was designed to look at the effect of treatments based on the quantitative genetic characteristics of mussel stocks, our results show that single factors such as size or age can have significant impacts on the effects of selection treatments. The difference in mortality rate from the elevated temperature treatment between the cohorts along with the effect on size within the smaller size cohort strongly indicates that individual mussel size is an important factor in determining survival time in elevated water temperature. A study by LeBlanc et al. (2005) had similar results. In a population of seed mussels (less 10 mm in length), smaller mussels survived longer in lethal elevated water temperature than larger mussels.

This study found that there was a link between heterozygosity and survival in elevated water temperatures. Heterozygous mussels have been found to have lower protein turnover which results in energy conservation (Bayne and Hawkins, 1997). Survival of mussels in stressful conditions has been attributed to the higher fitness of heterozygotes due to lower metabolic needs resulting in better stamina under stress (Myrand et al., 2002). In our study, the more heterozygous individuals survived the elevated water temperature stress. This is evident by the increase in overall heterozygosity, in both cohorts, compared to the Hardy-Weinberg expected values. In both cohorts, significant deficits at three loci (GPI, MPI, ODH) disappeared after the elevated water temperature treatment (Table 3). The results indicated that there was a selective effect against homozygotes under elevated water temperature stress. Thus, heterozygotes appear to have an advantage under this stressful condition. Aside from Myrand et al. (2002), Beaumont and Toro (1996) found that in *Mytilus edulis* juveniles under copper stress the heterozygotes survived longer than their homozygote counterparts.

So far the results discussed show that the elevated water temperature stress had different effects on the cohorts based on size but similar genetic results. However some genetic results were cohort specific. In the larger size cohort a deficit in heterozygosity developed at the PGM locus after the treatment. In the smaller size cohort the elevated water temperature treatment caused significant frequency shifts in the LAP and PGM loci. The reasons for these different effects are unknown. They indicate that even with a relatively simple treatment, it will take time and effort to recognize all the important impacts selection may have on a mussel population.

One result that was different from what has often been reported in the literature is the larger H_o of the smaller sized cohort compared to the larger size cohort. Under the general theory of underdominance for the pre-settlement stage and overdominance after metamorphosis (Gosling, 1992), the expectation would be that because of longer exposure to natural stresses the older population would be more heterozygous. There has been one study in the region that has found decreasing heterozygosity in cultured mussel populations (Tremblay et al., 1998c). The explanation provided in that study was that heterozygotes move to the outside of the cluster of mussels on the long-line and are therefore more susceptible to loss from falling off. The decrease was found for a single

population. In this study, two separate cohorts were present but they were in a suspended environment. The difference in heterozygosity may simply be the result of two cohorts producing genetically different offspring or differential selective mortality during development. It is possible that one or more of these factors may have contributed the difference in these populations.

CONCLUSION

The results of this study are important to the mussel industry, especially when considered along with other experiments. As previously mentioned, Tremblay et al. (1998c) found that cultured mussel populations tended to become more homozygous than their wild counterparts. The explanation for the loss in heterozygosity relates to the long-line culture method. The theory is that the more heterozygous mussels move to better feeding positions on the outside of the mussel sock and are more susceptible to fall-off during bad weather. Further, Tremblay (1998a & b) and Myrand et al. (2000, 2002) determined that homozygous populations are more susceptible to summer mortality, which has been a problem in the mussel culture industry. As mentioned before, the theory for this difference in survival is that heterozygous populations have more energy to survive stressful conditions.

This study demonstrated that artificially induced stress can increase the heterozygosity of mussel stocks. These findings reveal the importance of genetic characterization and the need to further study selection techniques that can increase or maintain suitable levels of genetic variation in cultured mussels.

The selection technique of elevated water temperature had an effect on the genetic structure of mussel populations. In elevated water temperature stress, physiological factors as well as genetics play a role in survival. These findings are important to the mussel aquaculture industry because it proves that it is possible to manipulate the seed population without using a hatchery. Furthermore, this study found that both genotype and phenotype are important factors in determining the outcome of selection using elevated water temperature.

ACKNOWLEDGEMENTS

Mussels for this study were kindly provided by P.E.I. Mussel King, courtesy of Russell Dockendorf. Funding and logistical support were provided the Department of Fisheries and Oceans and the Province of Quebec.

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Table 1. Mortality of mussels exposed to elevated water temperature.

Replicate	**N**	**No. of Live Specimens after Treatment**	**No. of Dead Specimens after Treatment**	**% Mortality (S.E.)**
Cohort < 20 mm	84	51	33	39.2 (5.3)
Cohort > 20 mm	366	154	212	57.9 (2.6)

Table 2. Mean size of surviving mussels, exposed to elevated water temperature and controls, from two cohorts (< 20 mm, > 20 mm).

Group	N	Mean Size (mm)	Median (mm)	Standard error
Control < 20 mm	163	11.66	10.92	0.32
Temperature < 20 mm	51	9.86	8.34	0.66
Control > 20 mm	287	29.80	29.68	0.28
Temperature > 20 mm	154	29.95	29.98	0.37

Table 3. Number of individuals used for analysis, allelic (A-F) frequencies, observed (H_o) and expected (H_e) heterozygosities at five polymorphic loci from two cohorts before and after exposure to elevated water temperature. *Bold indicates significant difference at the 5% level. †GPI-glucose phosphate isomerase, MPI-mannose phosphate isomerase, ODH-octopine dehydrogenase, LAP-Leucine aminopeptidase, PGM- Phosphoglucomutase.

Cohort	**< 20 mm**	**< 20 mm**	**> 20 mm**	**> 20 mm**
Treatment	**control**	**Elevated temp.**	**control**	**Elevated temp.**
GPI				
N	107	36	41	100
A	0.117	0.083	0.085	0.11
B	0.019	0.042	0.024	0.015
C	0.22	0.25	0.195	0.155
D	0.364	0.514	0.537	0.455
E	0.014	0	0.012	0.035
F	0.266	0.111	0.146	0.23
H_o	0.561	0.639	0.537	0.69
H_e	0.734	0.662	0.653	0.706
D	**-0.239**	-0.034	**-0.178**	-0.023
MPI				
N	110	40	42	95
A	0.105	0.125	0.167	0.132
B	0.877	0.85	0.821	0.853
C	0.018	0.025	0.012	0.016
H_o	0.191	0.2	0.167	0.232
H_e	0.22	0.265	0.301	0.257
D	**-0.133**	-0.244	**-0.446**	-0.098
ODH				
N	95	39	41	99
A	0.032	0.013	0.024	0.03
B	0.895	0.936	0.878	0.934
C	0.074	0.051	0.098	0.035
H_o	0.147	0.128	0.146	0.111
H_e	0.194	0.123	0.222	0.125
D	**-0.241**	0.043	**-0.34**	-0.114
LAP				
N	116	38	42	100
A	0.177	0.079	0.06	0.095
B	0.453	0.368	0.321	0.29
C	0.366	0.526	0.583	0.595
D	0.004	0.026	0.036	0.02
H_o	0.681	0.605	0.381	0.61
H_e	0.632	0.588	0.558	0.555
D	**0.077**	0.029	-0.318	0.099
PGM				
N	90	38	42	98
A	0.183	0.053	0.048	0.107
B	0.539	0.684	0.738	0.658
C	0.278	0.263	0.214	0.224
D	0	0	0	0.01
H_o	0.5	0.342	0.405	0.408
H_e	0.599	0.466	0.412	0.507
D	-0.17	-0.266	-0.017	**-0.196**
All loci				
H_o	0.416	0.383	0.327	0.41
H_e	0.477	0.421	0.429	0.43

Table 4. Degree of heterozygosity for mussels treated with elevated water temperature and those untreated for two cohorts.

Treatment/cohort	N	Degree of heterozygosity	Standard error
Control/> 20 mm	40	1.58	1.08
Control/< 20 mm	73	2.04	1.03
Temp./> 20 mm	92	2.01	1.14
Temp./< 20 mm	33	1.94	1.27

Table 5. Multiple heterogeneity X^2 tests calculated with Monte-Carlo simulations to compare the allelic frequencies of mussels treated with elevated water temperature and controls from the two cohorts.

Locus	Cohort	Treatment	X^2	P
MPI	small	control	3.21	0.82
	small	temp.		
	large	control		
	large	temp.		
PGM	small	control	19.31	**0.02**
	small	temp.		
	large	control		
	large	temp.		
GPI	small	control	20.87	0.07
	small	temp.		
	large	control		
	large	temp.		
ODH	small	control	4.70	0.53
	small	temp.		
	large	control		
	large	temp.		
LAP	small	control	22.01	**0.01**
	small	temp.		
	large	control		
	large	temp.		
PGM	small	control	11.47	**0.001**
	large	control		
LAP	small	control	16.84	**0.001**
	large	control		
PGM	small	temp.	3.61	0.2
	large	temp.		
LAP	small	temp.	1.51	0.66
	large	temp.		
PGM	small	control	9.02	**0.01**
	small	temp.		
LAP	small	control	9.47	**0.01**
	small	temp.		
PGM	large	control	3.73	0.3
	large	temp.		
LAP	Large	control	1.82	0.62
	Large	temp.		

*Bold indicates significant difference at the 5% level.

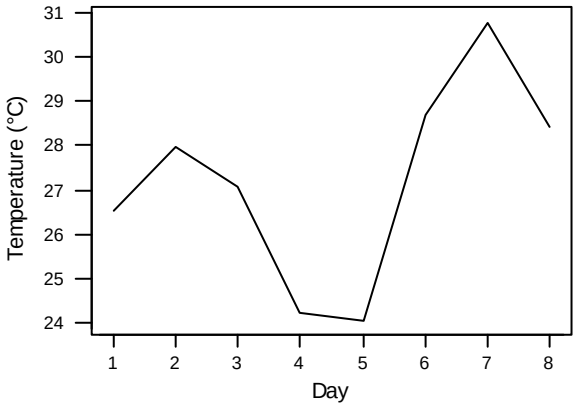

Figure 1. Daily average water temperature in elevated temperature stress tank.

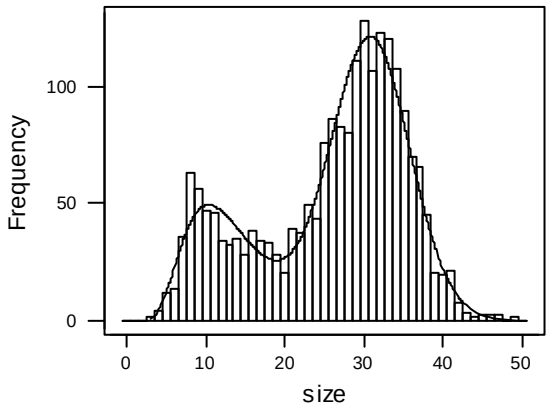

Figure 2. Size (mm) frequency distribution of mussels (N= 2100).