

Do oysters help mussels against crabs?

The influence of the Pacific oyster (*Crassostrea gigas*)
on the amount of Blue mussels (*Mytilus edulis*)
that survive the predation by Shore crabs (*Carcinus maenas*)



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Leeuwarden/ Den Hoorn, Texel, 03 July 2013

University of Applied Sciences



**VAN HALL
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PART OF WAGENINGEN UR



Royal Netherlands Institute for Sea Research

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Summary

Blue mussels (*Mytilus edulis*) create niches in the Wadden Sea and therefore they are important for the Wadden Sea. Because there are less mussel beds in the Wadden Sea than would be expected and because of their important role, they are monitored since the mid-nineties. It was found that some beds were overgrown by the alien bivalve Pacific oyster (*Crassostrea gigas*). Probably co-existence of the two bivalves has advantages like protection from predation; mussels migrating down between oysters for refuge when predators were abundant. One of these predators, the Shore crab (*Carcinus maenas*), uses various size-related techniques to open mussels. For any size of crab, there is an optimal size of mussel to predate on.

At the Royal Netherlands Institute for Sea Research (NIOZ), laboratory experiments have been conducted with mussel beds covered by oysters. The mussels may have settled themselves differently in the beds, than they would have done with predators around, like in the field. In the field they have anti-predatory responses caused by exposure to chemical cues from damaged conspecifics or from predators.

In the experiments will be shown how many mussels are eaten when they have the opportunity to draw back between the oysters compared to the amount of mussels that are eaten when they only could draw back between (dead) conspecifics when confronted with predators. Answering research questions about the influence of the oysters on the predation of mussels by crabs. Artificial mussel beds consisted of aquaria with the same four length classes were created with and without oysters. After 6 hours of predated by a small or a large crab, the amount of undamaged mussels was counted.

Several statistical tests were used to find the significant differences. Main differences were found between with and without oysters, and between length classes of mussels on beds without oysters. Also between the length classes of mussels when predated by small crabs compared to when predated by large crabs. The GLM resulted in a model, with interactions between some of the variables included, which explained a large part of the found differences.

The following conclusions are made. Fewer mussels are damaged when with oysters compared to without oysters, especially mussels of the three smallest length classes. Without oysters, the classes of mussels close to preferred prey size for the crab are predated a lot more. Large crabs manage to damage more mussels on beds with oysters than the small crabs. When no crab is present during acclimatization (the period of settling in the bed), more mussels survive the later predation. The final conclusion: the influence of the Pacific oyster is that more mussels survive predation by Shore crabs when oysters are present on mussel beds, especially interacting with the predation by small crabs.

Some results were the opposite of what was expected. Some of these results could be explained by interactions of variables like length class of mussels and size class of crabs. Other results that were not as expected could be explained by other researches. Like researches about the co-existence of the mussels and oysters and what are the triggers for mussels crawling between oysters.

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Chapter 1: Introduction

1.1: Problem description

Blue mussels (*Mytilus edulis*) have a quite important role in the Wadden Sea as one of the so-called “ecosystem engineers in marine benthic systems” (Borthagaray & Carranza, 2007). This means that they cause an accumulation of fine particles and organic material, which in the case of the mussel, results in a finer grain size of the sediment compared to bare sediment (Kochman et al., 2008). Due to this finer grain size, which leaves a harder substrate, together with the enrichment of the sediment with organic matter, mussels create niches for other benthic organisms (Markert, Wehrmann & Kröncke, 2010).

At the moment there are less mussel beds in the Wadden Sea than would be expected (Mosselwad, 2008). And because of their important role, the littoral mussel beds in the Dutch Wadden Sea have been monitored yearly to report their development since the mid-nineties. Van Zweedden et al. (2011) show that at the end of the nineties, the total biomass as well as the total surface of all littoral mussel beds in the Dutch Wadden sea was quite low, but started to rise again after 2001. After a small decrease around 2009, the numbers have stabilized again around 2011. This is also what Fey et al. found in their research (2012), focusing on mussel beds in the Eastern part of the Dutch Wadden Sea, where most of the mussel beds are situated. This was also mentioned by Van Zweedden et al. (2011); they show that, although some mussel beds disappeared through the last years, others have grown both in surface and biomass and form stable beds around the year 2011. But the situation is still not as good as when the yearly monitoring started. Some beds started to get overgrown by the alien bivalve Pacific oyster (*Crassostrea gigas*). It is still not known for 100% what kind of effects that can have on the population of mussels in the Wadden Sea.

To recover and manage the mussel beds in a sustainable way, more knowledge is needed, for example about the influence of the Pacific oyster. This bivalve, originating from the Japanese coast, has been introduced into the Northern Wadden Sea by aquaculture in 1986 (Eschweiler & Christensen, 2011; Reise, 1998), where it escaped from, and in 1964 already in the Easter Scheldt estuary (Markert, Wehrmann & Kröncke, 2010). The first time that oysters were sighted in the Dutch Wadden Sea was in 1983 near Oudeschild, Texel (Fey et al., 2006). Since then, the oysters have spread out over the Dutch Wadden Sea, and since 2004, the numbers of oysters are high enough to estimate their population development (Fey et al., 2006). They show that at some places in the Western Wadden Sea, like near Oudeschild and Zeeburg the oyster population stabilized around 2004, and that in the Eastern part, near Ameland for example, the densities kept rising.

Because of these growths of both the mussel and the oyster population, questions arise about the probable co-existence of the two bivalve species. Maybe they gain both positive effects from each other's presence.

Eschweiler & Christensen (2011) found that mussels in beds that were overgrown by oysters migrated down between the oysters for refuge when predators were abundant

which increases their survival. They did this even though it reduced their ability to grow compared to being positioned on top of the reef. This migration was only from top to bottom, vice versa migration did not occur. They also discovered that when mussels were located in the interspaces in an oyster reef in the German Wadden Sea, no loss or mortality in mussels was recorded. This was indicating that mussels were more protected from predation.

One of the predators on the mussel is the Shore crab (*Carcinus maenas*), which feeds on mussel banks during high tide (Crothers, 1968). When low tide is coming and the mussel beds are no longer covered by the tide, the crabs retreat under the tidemark. During their moult however, they do not feed themselves and their ability to move is low (Crothers, 1967). Crabs that moult frequently, and are in a normal or early intermoult have a green appearance or morphology. Crabs that are in a prolonged intermoult, which means that the period between moults is longer than normal (Crothers, 1968; Smallegange et al., 2009). To open the mussels, crabs use various size-related techniques like crushing, boring and edge-chipping, especially the males, who have one larger, more muscular chela or pincer (Elnor, 1978). And for any size of crab, there is an optimal size of mussel to predate on (Elnor & Hughes, 1978). For example, Elnor and Hughes observed during their research in 1978, that crabs of 6.0-6.5 cm preferred mussels with a shell-length of 1.75 cm. That size seems to be preferred until it is depleted or no longer reachable. Then crabs will take mussels sized below and above the optimal size. Knowing this, it can be concluded that not every crab is a danger for all mussels on a bed.

At the Royal Netherlands Institute for Sea Research (NIOZ), laboratory experiments have been conducted with artificial Blue mussel beds covered by Pacific oysters. During those experiments, Shore crabs of two size classes could forage on beds which contained a mixture of mussels in four length classes. During part one of that experiment, when the mussels were given the time to acclimatize and attach to the bottom, no predator was added. This is unlike the situation in the field, where predators are abundant at any moment. Due to the absence of predators like shore crabs, the mussels may settle themselves differently in the mussel beds, than they would have done with predators around. For example without crabs they can settle themselves at spots easier to reach by predators. Cheung et al. (2004), describe anti-predatory responses caused by exposure to chemical cues from damaged conspecifics or from predators. To create a situation closer to the situation in the field, a crab (feeding on damaged mussels) can be added during acclimatization of the mussels, without the crab being able to feed on the mussels of the beds. Eschweiler & Christensen used this information too when they were conducting experiments for their research in 2011.

Through experiments where the field situation is recreated as good as possible in an artificial environment, and all the information above is taken into account, it will be shown how many mussels are eaten when they have the opportunity to draw back between the oysters compared to the amount of mussels that are eaten when they only could draw back between (dead) conspecifics when confronted with predators.

1.2: Research questions and hypotheses

Mainquestion:

What is the influence of the Pacific oyster on the predation of the Blue mussel by Shore crabs on beds in the Wadden Sea?

Sub questions:

1:What is the difference between the predation by Shore crabs on Blue mussels on pure beds and on Blue mussels on beds overgrown by Pacific oysters?

2:What are the differences in the predation on Blue mussels in different length-classes by Shore crabs on beds overgrown by Pacific oysters as well as on pure mussel beds?

3:What is the difference in the predation on Blue mussels between mussels predated by large Shore crabs and Blue mussels predated by small Shore crabs?

4: What is the difference in the predation by Shore crabs between Blue mussels which have no Shore crab present during acclimatization and Blue mussels which have a Shore crab present during acclimatization?

Hypotheses:

The presence of the Pacific oyster on mussel beds in the Wadden Sea influences mussels survival from predation by Shore crabs.

1: On mussel beds overgrown by oysters, more mussels will survive the predation of the crabs compared to mussels on pure beds.

2: Small mussels have more space to hide between the oysters compared to the larger mussels, so on beds overgrown by oysters, less small mussels will be eaten; on pure beds there will be no significant difference between the length classes.

3: Small crabs will be capable to eat more mussels on mussel beds overgrown by oysters compared to large crab, and small crabs will predate less on the largest mussels compared to the large crab.

4: Mussels who were exposed to the chemical cues of damaged con-specifics during the acclimatization-period will be more prepared for a predator and hide themselves before the predation period starts, therefore they are less eaten.

1.3: Research aim

By describing the influence of the abundance of oysters on survival of Blue mussels of different length classes from predation by Shore crabs, there will be more knowledge

about the coexistence of mussels and oysters in the Wadden Sea. The research should result in a data-set that gives a good view of the influence of Pacific oysters on the survival of Blue mussels in four different length classes on beds overgrown by Pacific oysters, compared to the number of surviving Blue mussels on pure mussel beds.

Chapter 2: Materials and methods

2.1 Materials

- Kodak play sport waterproof camera, for taking pictures of the experiment.
- 20 Cages of 12 x 14 x 12 cm (see picture 1.)
- 20 water tubes with a diameter of 5 mm
- 20 oxygen tubes with a diameter of 5 mm
- 20 Experimental aquaria of 32.5 * 17.5 * 18.2 cm
- 6 storage aquaria of 185 * 50.5 * 36 cm
- 34 oxygen-stones
- Wadden Sea water of 15°C
- About 3 kg of death shell material (for creating the sediment)
- 7 lids of 3 mm thick transparent plastic with 3 holes in it for the water tubes and oxygen tubes, which cover 3 aquaria each.
- Electronic caliper; Mitutoyo model CD-15PKX
- Temperature log, for measuring the water temperature continuously
- pH measurement device



Picture 1. Crab cage used during acclimatization with caged crab, the net fits tighter around the frame than would be expected from the photo. This cage (12*14*12 cm) is placed on top of an experimental aquarium of 32.5*17.5*18.2 cm.

2.2 Methods

The Blue mussels (*Mytilus edulis*) were collected at dams at the North Sea coast of Texel near the village of Den Hoorn at beach pole 9. After a crude sorting the mussels were divided over 5 aquaria (185cm*50.5cm*36cm), each for every length class (6-9mm, 12-15mm, 18-21mm, 24-27mm) and one extra for all the mussels which were too big or too small for these classes, those ones are used as food for the crabs in the cages.

The Pacific oysters (*Crassostrea gigas*) were collected in the NIOZ harbour. They were rinsed with sea water and a soft brush and disposed of small animals in between, like small mussels, crabs and other invertebrates. Furthermore their volume was measured and they were labeled. For each label number, the volume was written down. With help of the labels, the right combinations of oysters could be made, so every experimental aquarium had more or less the same volume of oysters.

During the experiment, the mussels are put in 20 plastic aquaria (32.5cm*17.5cm*18.2cm). Ten of the aquaria are only filled with sediment of sand and dead shells, the other ten also contain Pacific oysters (500-550 ml). Each aquarium contains the same amount of mussels divided over the four length-classes, which are 50 individuals of 6-9 mm, 25 individuals of 12-15 mm, 13 individuals of 18-21 mm and 7 individuals of 24-27 mm sized mussels, based on the previous research at the NIOZ. The length of the mussels was measured with an electronic caliper, which measures in millimeters with two decimals. These calipers are linked to a laptop with excel, where every length is entered. The average length difference between aquaria was 0.1 mm from the other aquaria (averages per class are around 7.5/ 13.5/ 19.50/ 25.5). Each aquarium has a stream through of 4 liters of water per hour and is supplied with an oxygen stone. Every aquarium is blinded, except for the upper side, this is to prevent the (male) Shore crabs to have vision of each other during the predation period and are busier with posing to each other to show how big they are instead of hunting and eating. The aquaria are put in an empty basin, so that the water that is streaming out of each aquarium can be drained.

Shore crabs (*Carcinus maenas*) were caught with fyke-traps in the NIOZ harbour with small herring as bait in it or by trawling during the high tide period in the area of Balgzand in the Wadden Sea. After that they were measured and selected on sex and damage. Only the undamaged males with carapace-widths of 60.00-64.99 mm and 45.00-49.99 mm were selected. No difference was made between red and green individuals, because there were not enough green individuals available to cover the amount needed. The crabs were first kept in storage cages in the NIOZ harbour. Later they were stored in an aquarium of 185cm*50,5cm*36cm as well, when they were sorted on length. All the aquaria used for storage are provided with two oxygen stones and continuously running Wadden Sea water of 15°C. The air in the climate chamber with all aquaria, is kept at 15°C as well. To reproduce a day-night cycle, the aquaria are kept in a 12 hours light/12 hours dark cycle, which is controlled by a time switch. The experiments are carried out during the light period, which is from 08:00 until 20:00.

During experiment 1, also called “Peaceful acclimatization”, no crabs were present during the acclimatization period. In experiment 2, also known as “Caged crab”, a crab is present in a small cage (12cm*14cm*12cm) within every experimental aquarium where it will be fed with stored mussels, so the mussels are exposed to chemical cues. After the acclimatization period, a shore crab will be put in each aquarium, in total 10 small crabs (carapace or back shield-width of 45.00-49.99 mm) and 10 large crabs (carapace-width of 60.00-64.99 mm) predate on the mussels for 6 hours, which is about the same length of time as a high-tide period. After the experiment, the crabs are kept for observation for

one week, to check if they are not dying or moulting. This way reasons for not eating, other than the mussels being unreachable, can be found.

To measure control variables, the water temperature, a temperature log is used to keep track of the changes of water temperature and the data from that are listed in the computer. To measure the pH of the water, a pH measurement device from the Biology department is used. Before, during and after the experiment, small water samples were taken from the aquaria and analyzed in the pH measurement device. The data from this device were listed.

The mussel data are obtained by counting the unharmed mussels after the crab predation period of 6 hours. Each mussel that is not eaten will be measured in length and put in a data file in the computer. Also remarks like when they are crushed but not eaten or small edges of the shell chipped off were put into the files. For every aquarium is written down in the file if the mussel bed contained oysters or not.

2.3 Data analysis

2.3.1 The difference between the predation by Shore crabs on pure beds and on mussels on beds overgrown by Pacific oysters:

This is tested with an Independent Samples T-test and showed in a simple bar chart. The dependent variable of “the percentage of unharmed mussels” is put against the independent variable of “with oysters/without oysters”, the other independent variables; “size class of crabs”, “length class of mussels” and “no crab/caged crab during acclimatization” are pooled for this question. To test the equality of variances, the Levene’s Test is used. This showed p of 0.978, which means the variances are equal. For testing the normality, Shapiro-Wilk test is used, which resulted in an $p=0.001$ for with oysters, and an $p=0.848$ for without oysters. It can be concluded that the variables are equal of variances, but do not fulfil the demands for normality. Therefore the results are tested with the Mann-Whitney test.

Several post-hoc tests resulted in more detailed testing within the first test results, some of these detailed test results ended up in the first paragraph. For the tests of comparing per length class of mussels the results of beds with oysters and the beds without oysters, the Shapiro-Wilk test for testing the normality resulted in: for the mussels on beds with oysters: $p<0.001$ for 6-9 mm, 12-15 mm and 24-27 mm and $p=0.001$ for 18-21 mm and for mussels on the beds without oysters: $p=0.045$ for 6-9 mm, $p=0.098$ for 12-15 mm, $p=0.094$ for 18-21 mm and $p<0.001$ for 24-27 mm. The Levene’s test for homogeneity resulted in: $p=0.181$ 6-9 mm, $p=0.385$ for 12-15 mm, $p=0.863$ for 18-21 mm and $p=0.063$ for 24-27 mm. Although the aims for homogeneity are reached, for the normality they are not, therefore all are tested with the Mann-Whitney test.

To compare of the predation of mussels by small crabs on beds with oysters($n=72$) and beds without oysters($n=76$) the Kolmogorov-Smirnov test resulted in $p=0.001$ for small crabs on beds with oysters and $p=0.028$ for small crabs on beds without oysters. The Levene’s test resulted in $p=0.947$. Therefore it is tested with the Kruskal-Wallis test,

because the aim of normality is not reached. For the comparison between large crabs with oysters (n=80) and large crabs without oysters (n=80), Kolmogorov-Smirnov resulted in $p=0.002$ and $p=0.051$. The posthoc test of Games-Howell, for comparisons of groups with no normality, showed for this comparison to have no significant difference to be tested, for this the results from this posthoc test will be used. For this comparison the variables of “length class of mussels” and “no crab/caged crab during acclimatization” are pooled within the variable of “size class of crabs”.

For the comparison of the situation with no crab present during the acclimatization on beds with oysters and beds without oysters, and for the comparison of the situation with a caged crab is present during acclimatization on beds with oysters and beds without oysters are both the normality tested with the Kolmogorov-Smirnov test, which resulted in: $p<0.001$ for no crabs with oysters, no crab without oysters and caged crab with oysters, and $p=0.200$ for caged crab without oysters. The Levene’s test for homogeneity resulted in $p=0.005$ for the comparison of no crabs during acclimatization, and $p=0.032$ for the comparison of caged crabs during acclimatization. Because of the normality, both comparisons are tested with the Mann-Whitney test.

2.3.2 The differences in the predation on Blue mussels in different length-classes by Shore crabs on beds overgrown by Pacific oysters as well as on pure mussel beds:

These results are tested twice with an One-way ANOVA and the results of that are showed in two clustered bar charts. The dependent variable of “the percentage of unharmed mussels” is put against the independent variables of “length class of mussels” and “with oysters/without oysters”, for the variable last mentioned will it be tested and showed in charts for each case separate. The other independent variables; “size class of crabs” and “no crab/caged crab during acclimatization”, are pooled for this question. To test the normality, the Shapiro-Wilk test is run, which resulted as already mentioned in 2.3.1. The Levene test for homogeneity of variances resulted in an $p=0.008$ for length classes with oysters, which means it doesn’t reach the homogeneity aim. And an $p=0.277$ for length classes without oysters, which means the homogeneity criterion is reached. Because both test contained groups which do not reach the aim for normality, both are tested with the Kruskal-Wallis test.

Tests of results that came up after a posthoc test are present here as well. For the comparison of the length classes on beds with oysters, Shapiro-Wilk resulted as mentioned in 2.3.1. The posthoc test of Games-Howell showed that almost all the comparisons proved to have no significant difference, except for the comparisons between the length classes of 18-21 mm and 24-27 mm. For this difference the Levene’s test resulted in $p=0.035$. Therefore this differences are tested with the Mann-Whitney test and the other differences are shown from the Games-Howell test results.

For the comparisons on beds without oysters, between all the mussel length classes, the Shapiro-Wilk test resulted as mentioned in 2.3.1. The Levene’s test for homogeneity resulted in $p=0.177$ for 6-9 mm \leftrightarrow 12-15 mm, $p=0.066$ for 6-9 mm \leftrightarrow 18-21 mm, $p=0.943$ for 12-15 mm \leftrightarrow 24-27 mm and $p=0.701$ for 18-21 mm \leftrightarrow 24-27 mm. Therefore, all comparisons are tested with the Mann-Whitney test.

2.3.3 The difference in the predation on Blue mussels between mussels predated by large Shore crabs and Blue mussels predated by small Shore crabs:

These are both tested and showed in graphs thrice, both times it is tested with an One-way ANOVA and showed in a clustered bar chart. For the first test and chart the dependent variable of “the percentage of unharmed mussels” is put against the independent variables of “length class of mussels” and “size class of crabs”, the other independent variables; “with oysters/without oysters” and “no crab/caged crab during acclimatization”, are pooled. Before running the tests with the length classes of mussels combined with the large crab or small crab, the normality is tested. For testing the normality, the Shapiro-Wilk test is used. This resulted for the length classes predated by a small crab (n=37 for each length class of mussels) in: p=0.039 for 6-9 mm, p=0.001 for 12-15 mm, p=0.001 for 18-21 mm and p<0.001 for 24-27 mm. For the length classes (n=40 for each length class) predated by a large crab, the test resulted in: p<0.001 for 6-9 mm, p=0.095 for 12-15 mm, p=0.064 for 18-21 mm and p=0.001 for 24-27 mm. The Levene’s test for homogeneity resulted in p<0,001 with small crabs and p=0.001 with large crabs, therefore both will be tested with the Kruskal-Wallis test.

For the third test and chart, the dependent variable of “the percentage of unharmed mussels” is put against the independent variables of “size class of crabs” and “with oysters/without oysters”, the other independent variables; “length class of mussels” and “no crab/caged crab during acclimatization”, are pooled. To test the normality of these variables, the Kolmogorov-Smirnov test is run. This resulted for small crab with oysters (n=72) in p<0.001, large crab with oysters (n=80) in p=0.002, small crab without oysters (n=76) in p=0.028, large crab with oysters (n=80) in p=0.051. The Levene’s test for homogeneity resulted in p<0,001, therefore the Kruskal-Wallis test is used.

The tests that came up after the Posthoc test deal with the comparisons of length classes of mussels predated by small crabs and predated by large crabs. The results the Shapiro-Wilk tests for these groups are mentioned above. Levene’s test for homogeneity resulted for small crabs in: p=0.002 for 6-9 mm <> 24-27 mm, p<0.001 for 12-15 mm <> 24-27 mm, p=0.001 for 18-21 mm <> 24-27 mm. For the large crabs it was p=0.001 for 6-9 mm <> 12-15 mm, p<0.001 for 6-9 mm <> 18-21 mm and p=0.711 for 18-21 mm <> 24-27 mm. All these comparisons were therefore tested with Mann-Whitney. Again there were also some comparisons that did not pass the Posthoc test to be tested further, for those the results of the Games-Howell test are used. To groups are: 6-9 mm <> 12-15 mm, 6-9 mm <> 18-21 mm and 12-15 mm <> 18-21 mm for mussels predated by small crabs and 6-9 mm <> 24-27 mm, 12-15 mm <> 18-21 mm and 12-15 mm <> 24-27 mm for mussels predated by large crabs.

For the comparisons between large crabs and small crabs predated per length class of mussels, the Levene’s test result in p=0.005 for 6-9 mm, p=0.001 for 12-15 mm, p=0.529 for 18-21 mm and p<0.001 for 24-27 mm. Because of the normality of the results, all the comparisons are tested with the Mann-Whitney test.

For the comparison of small crabs on beds with oysters and large crabs on beds with oysters, Mann-Whitney is used, because of: Levene gives p<0.001 and Komogorov-

Smirnov resulted as mentioned in 2.3.2. For the comparison of small crabs on beds without oysters and large crabs on beds without oysters, also Mann-Whitney is used.

2.3.4 The difference in the predation by Shore crabs between Blue mussels which have no Shore crab present during acclimatization and Blue mussels which have a Shore crab present during acclimatization:

These results are also both tested and showed in graphs twice, both times it is tested with an One-way ANOVA and showed in a clustered bar chart. For the first test and chart, the dependent variable of “the percentage of unharmed mussels” is put against the independent variables of “length class of mussels” and “no crab/caged crab during acclimatization”, the other variables; “size class of crabs” and “with oysters/without oysters”, are pooled. To test the normality of these variables, the Shapiro-Wilk test is used. This resulted for the length classes with no crab during acclimatization (n=37 for each length class) in: p=0.014 for 6-9 mm, p=0.025 for 12-15 mm, p=0.002 for 18-21 mm and p<0.001 for 24-27 mm. For the length classes with a caged crab during acclimatization (n=40 for each length class) the results were: p=0.027 for 6-9 mm, p=0.035 for 12-15 mm, p=0.139 for 18-21 mm and p<0.001 for 24-27 mm. Levene resulted in p=0.005 for acclimatization with no crab and p=0.028 for acclimatization with caged crab. Therefore both are tested with Kruskal-Wallis.

For the third test and chart, the dependent variable of “the percentage of unharmed mussels” is put against the independent variables of “with oysters/without oysters” and “no crab/caged crab during acclimatization”, the other independent variables; “length class of mussels” and “size class of crabs”, are pooled. To test the normality for the variables, the Kolmogorov-Smirnov test was used. This resulted in: p<0,001 for No crab with oysters (n=72), p<0.001 for no crab without oysters (n=76), p<0.001 for caged crab with oysters (n=80) and p=0.004 for caged crab without oysters (n=80). Levene resulted in p=0.007. Therefore, Kruskal-Wallis is used.

The test that came up after the Posthoc test compare between the length classes of mussels per acclimatization situation. The results of the Shapiro-Wilk test are mentioned above. Levene resulted in p=0.011 for 12-15 mm <> 24-27 mm with no crab present during acclimatization. With a caged crab during acclimatization, Levene resulted in p=0.006 for 6-9 mm <> 12-15 mm, p=0.037 for 6-9 mm <> 18-21 mm, p=0.063 for 12-15 mm <> 24-27 mm and p=0.233 for 18-21 mm <> 24-27 mm. Therefore, all are tested with Mann-Whitney.

For the comparison of each length class of mussels in both acclimatization situations, Mann-Whitney is used: Levene resulted in p=0.784 for 6-9 mm, p=0.976 for 12-15 mm, p=0.874 for 18-21 mm and p=0.694 for 24-27 mm, but Kolmogorov-Smirnov resulted for normality as can be read in 2.3.2.

The comparisons of “no crabs present during acclimatization on beds with oysters” <> “caged crab present during acclimatization on beds with oysters” and “no crabs present during acclimatization on beds without oysters” <> “caged crab present during acclimatization on beds without oysters” are later tested too, with Mann-Whitney.

2.3.5 General Linear Model:

All the steps for testing with GLM are according to the steps from “Applied Logistic Regression” of David W. Hosmer, JR. and Stanley Lemeshow.

All independent variables are tested with a single analyses. For every variable the p-value was $p < 0.25$, therefore every independent variable was selected.

Before a GLM can be run “legally”, the normality of the residuals of the results unharmed mussels of the first GLM test run (with all the variables included), which were gathered through saving them, is tested with Kolmogorov-Smirnov. The outcome was $p < 0.001$, therefore officially the GLM cannot be run. However, due to the extra information the GLM still can provide when run, the choice was made to still run it.

The first run of the GLM was done with all the variables included, even if they did not show much significant differences in earlier separate tests. This showed for “size class of crab” to have no significant value. Therefore a second test was run without “size class of crabs”, in the outcome all the included variables proved to be significant. Because variables can affect each other, there was also chosen to see what the outcome would be when a third test was run. But this time excluding “Acclimatization” instead of “size class of crab”, because this variable had also a low significance compared to the other two. But without “acclimatization”, “size class of crab” still contained to be not significant. But when interactions were added, “size class of crab” was taken back into the test again due to the fact that interactions with that variable were $p < 0.10$. These interactions were `lengthclass*sizeclass` and `oysters*sizeclass`, and together with `oysters*lengthclass` they were selected for the final GLM test, which gave the largest Adjusted R Squared. The interactions of `acclimatization*oysters`, `acclimatization*lengthclass` and `acclimatization*sizeclass` had all $P > 0.10$ and were not further taken into the test. The residuals of the results of this final test were tested on normality with a Kolmogorov-Smirnov test, the outcome was $p < 0.001$ and on homogeneity with Levene’s resulting in $p = 0.013$, therefore the test can officially not be run.

Chapter 3: Results

To show the effect of the presence of oysters on mussel predation by crabs, various tests are run with the data, in this chapter will be dealt with the results of these tests.

Each paragraph shows the results linked to one of the research sub questions. In §3.1 till §3.3, the results of the experiments with no crabs present during acclimatization and with a caged crab present during acclimatization are combined (or pooled, as mentioned in §2.3). In §3.4, both acclimatization experiments will be compared.

3.1: *Mussel predation on beds with and without oysters*

To show the difference between mussels predated by crabs on mussel beds with oysters ($n=38$) and mussel beds without oysters ($n=39$), the beds are compared and tested. The results in figure 1 show a higher % of unharmed mussels on beds with oysters compared to beds without oysters.

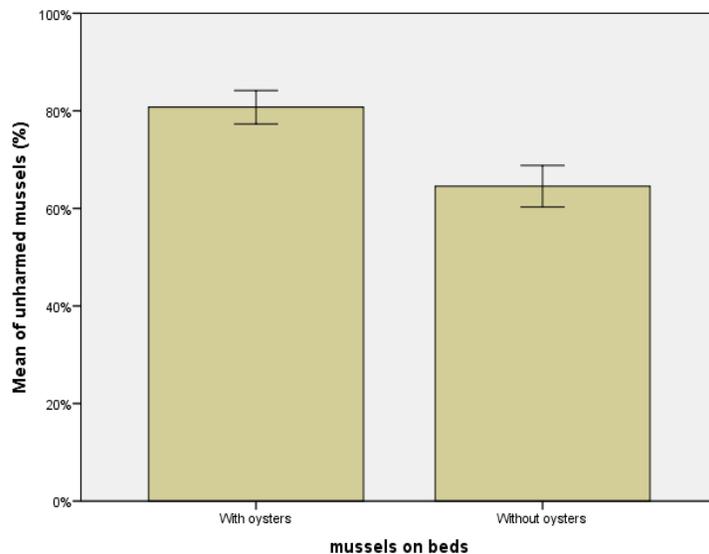


Figure 1. Unharmed mussels on beds with- and without oysters (mean % of 38 experimental mussel beds with oysters, and 39 experimental mussel beds without oysters). Error bars indicate the 95% confidence interval. The standard errors: $81 \pm 2.6\%$ for mussel beds with oysters and $63 \pm 2.8\%$ for without oysters. The difference of 17.69% is significant (Mann-Whitney; $p < 0.001$).

After this, the results were splitted up in several ways. Firstly in table 1, the results are splitted up, per length class of mussels. Except for the largest mussels, all the length classes show a significant difference between predation on beds with and without oysters. The results of this table are also visualised in graphs (see appendix I).

Table 1. Percentage of unharmed mussels per length class difference between beds with oysters(n=38) and beds without oysters(n=39). Differences are tested with Mann-Whitney.

| Groups | Difference in percentage of unharmed mussels | p-value Bold = significant difference |
|---------------------|---|--|
| Mussels of 6-9 mm | +14.2% with oysters | p<0.001 |
| Mussels of 12-15 mm | +29.6% with oysters | p<0.001 |
| Mussels of 18-21 mm | +13.70% with oysters | p=0.029 |
| Mussels of 24-27 mm | +7.4% with oysters | p=0.159 |

Secondly, the results are splitted up per size class of crabs: mussels predated by small crabs on beds with and without oysters(n=72 and 76 resp.) are compared and tested. As well as mussels predated by large crabs on beds with and without oysters(n=72 and 76 resp.). The results are shown in table 2, and also visualised in graphs(see appendix I).

Table 2. Difference in percentage of unharmed mussels per size class of predating crab between beds with oysters and beds without oysters. Differences are tested with Mann-Whitney.

| Groups | Difference in percentage of unharmed mussels | p-value Bold = significant difference |
|---------------|---|--|
| Small crabs | +26.8% with oysters | p=0.014 |
| Large crabs | +6.6% with oysters | p=0.113 |

Finally, the results are splitted up per type of acclimatization, i.e. with and without a caged crab present during acclimatization. Beds with and without oysters during both acclimatization are compared and tested. Table 3 shows the results of this and the results are also visualised in graphs(see appendix I).

Table 3. Difference in percentage of unharmed mussels per situation of acclimatization between beds with oysters(no crab: n=72, caged crab: n=80) and beds without oysters(no crab: n=76, caged crab: n=80). Differences are tested Mann-Whitney.

| Groups | Difference in percentage of unharmed mussels | p-value Bold = significant difference |
|---------------------------------|---|--|
| Acclimatization without crab | +13.7% with oysters | p=0.001 |
| Acclimatization with caged crab | +18.7% with oysters | p<0.001 |

To summarize the results so far; significant differences in the percentage of unharmed mussels between beds with- and without oysters are found:

- for all the results together
- for mussels within the length classes of 6-9 mm, 12-15 mm and 18-21 mm
- when predated by small crabs
- in both acclimatization situations

3.2: Mussel predation per length class of mussels

The results of all experiments are split up into the four length classes of mussels and compared to show the differences. With oysters present, the length classes are all predated differently (figure 2), but most of these differences are not significant(see table 4).

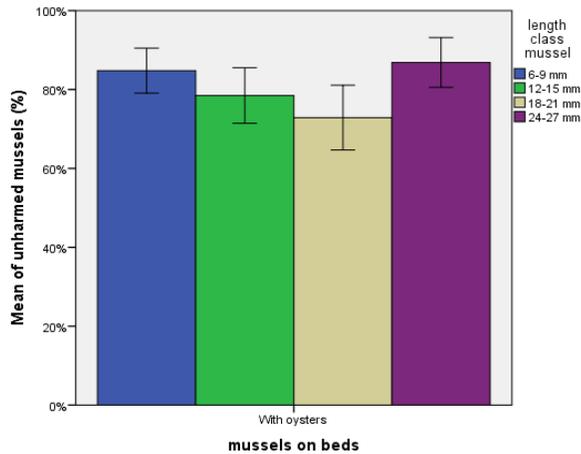


Figure 2. Unharmed mussels per length class of mussels on beds **with oysters** (mean % of n=38). Error bars indicate the 95% Confidence interval. The overall difference between the bars is significant (Kruskal-Wallis; p=0.011).

The same is done for beds with no oysters present (figure 3). Here the length classes differ a lot more compared to figure 2. Again the largest differences are found between the outer two length classes and the inner two. More charts of these specific differences are found in appendix I, for detailed differences and their significance see table 5.

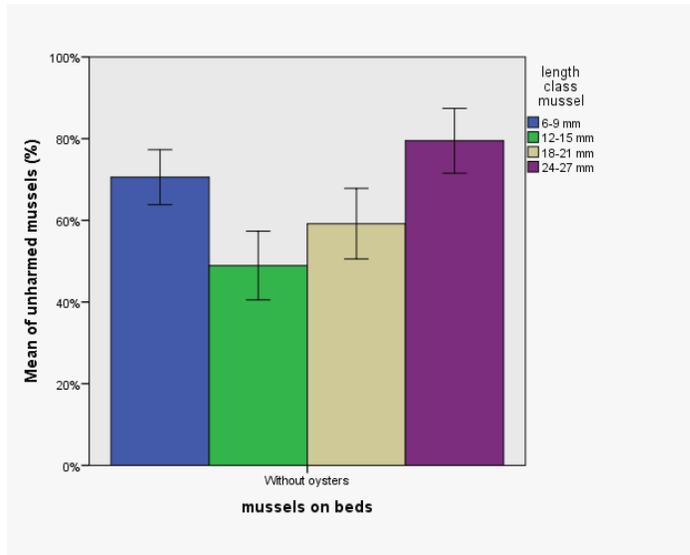


Figure 3. Unharmed mussels per length class of mussels on beds **without oysters** (mean % of n=39). Error bars indicate the 95% Confidence interval. The overall difference between the bars is significant (Kruskal-Wallis; $p < 0.001$).

Table 4 shows that on beds with oysters, the largest difference is found between the length classes of 18-21 mm and 24-27 mm (significant differences, p-value is shown in bold letters). For graphs of the differences see appendix I.

Table 4. The difference between the length classes of mussels. Percentage of unharmed mussels per length class of mussels on beds with oysters. Differences between length classes in % are tested with Mann-Whitney, when p-value is showed bold the difference is significant.

| Length class of mussels | 6-9 mm | 12-15 mm | 18-21 mm | 24-27 mm |
|-------------------------|-------------------------|--|---|---|
| 6-9 mm | | -6.3% ($p=0.261$) | -11.9% ($p=0.083$) | +2.1% ($p=0.129$) |
| 12-15 mm | +6.3% ($p=0.261$) | | -5.6% ($p=0.504$) | +8.4% ($p=0.010$) |
| 18-21 mm | +11.9% ($p=0.083$) | +5.6% ($p=0.504$) | | +14.0% ($p=0.003$) |
| 24-27 mm | -2.1% ($p=0.129$) | -8.4% ($p=0.010$) | -14.0% ($p=0.003$) | |

Table 5, resulting from comparing length classes of mussels on beds without oysters, shows: the largest differences are found between the length classes 6-9 mm and 12-15 mm and between 12-15 mm and 24-27 mm. These differences of 21.6% and 30.6%, together with the 20.3% of 18-21 mm <> 24-27 mm, are the only ones significant (Mann-Whitney; $p < 0.001$). For graphs of the differences see appendix I.

Table 5. The difference between the length classes of mussels. Percentage of unharmed mussels per length class of mussels on beds without oysters. Differences are tested with Mann-Whitney, when p-value is showed bold the difference is significant.

| Length class of mussels | 6-9 mm | 12-15 mm | 18-21 mm | 24-27 mm |
|-------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 6-9 mm | | -21.6% (p<0.001) | -11.4% (p=0.059) | +8.9% (p=0.025) |
| 12-15 mm | +21.6% (p<0.001) | | +10.2% (p=0.079) | +30.6% (p<0.001) |
| 18-21 mm | +11.4% (p=0.059) | -10.2% (p=0.079) | | +20.3% (p<0.001) |
| 24-27 mm | -8.9% (p=0.025) | -30.6% (p<0.001) | -20.3% (p<0.001) | |

To summarize the results of this paragraph; significant differences in the percentage of unharmed mussels between length classes of mussels are found:

- Between the length classes of 18-21 mm & 24-27 mm on beds with oysters.
- Between the length classes of 6-9 mm & 12-15 mm; 12-15 mm & 24-27 mm; 18-21 mm & 24-27 mm on beds without oysters.

3.3: Mussel predation per size class of crabs

The results are split up per size class of predating crabs and compared and tested to show the differences between predation by the two crab sizes. First, mussels of each length class (n=37) predated by small crabs are tested and charted (fig. 4). Each length class is predated differently by the small crabs. Especially the two smallest length classes are predated more than the larger classes. Those differences however, are only significant when the small classes of mussels are compared with the class of 24-27 mm. See table 6 for the detailed differences.

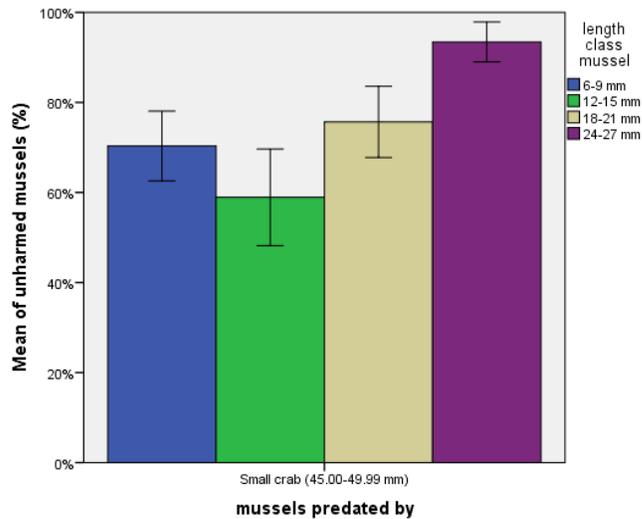


Figure 4. Unharmed mussels per length class of mussels on beds predated by **small crabs** (mean % of $n=148$ (= 37 experimental mussels beds multiplied by 4 length classes). Error bars indicate the 95% Confidence interval. The overall difference between the bars is significant (Kruskal-Wallis; $p<0.001$)

The same is done for the mussels predated by large crabs (fig. 5). Each length class is again predated differently by the large crabs and the differences are in occasion significant, especially between the length class of 6-9 mm and the two inner length classes (12-15 mm and 18-21 mm). See table 7 for detailed differences and tests. For separate graphs of all differences see appendix I.

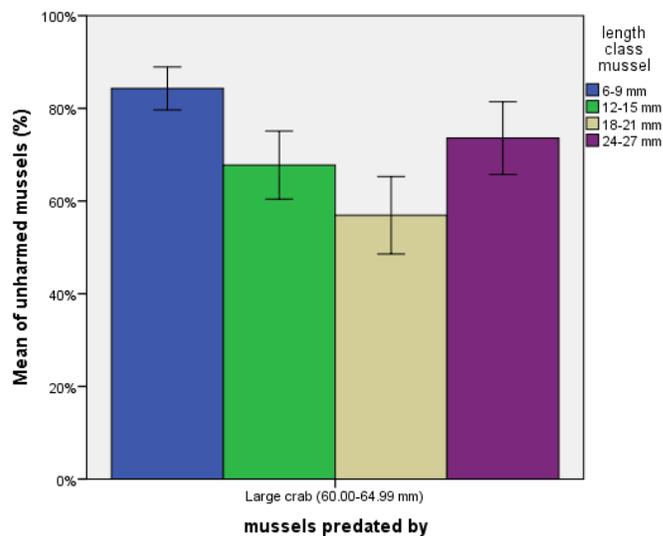


Figure 5. Unharmed mussels per length class of mussels on beds predated by **large crabs** (mean % of $n=160$ (=40 experimental mussels beds multiplied by 4 length classes). Error bars indicate the 95% Confidence interval. The overall difference between the bars is significant (Kruskal-Wallis; $p<0.001$)

Table 6 shows that on beds predated by small crabs the largest differences are between the length class of 24-27 mm and all the other length classes.

Table 6. The difference between the length classes of mussels. Percentage of unharmed mussels per length class of mussels on beds predated by small crabs. Differences are tested with Mann-Whitney, when p-value is showed bold the difference is significant.

| Length class of mussels | 6-9 mm | 12-15 mm | 18-21 mm | 24-27 mm |
|-------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 6-9 mm | | -11.4% (p=0.179) | +5.4% (p=0.177) | +23.1% (p<0.001) |
| 12-15 mm | +11.4% (p=0.179) | | +16.8% (p=0.024) | +34.5% (p<0.001) |
| 18-21 mm | -5.4% (p=0.177) | -16.8% (p=0.024) | | +17.8% (p<0.001) |
| 24-27 mm | -23.1% (p<0.001) | -34.5% (p<0.001) | -17.8% (p<0.001) | |

Table 7 shows that on beds predated by large crabs the largest differences are between mussel length classes of 6-9 mm and 12-15 mm, between 24-27 mm and 18-21 mm and between 6-9 mm and 18-21 mm.

Table 7. The difference between the length classes of mussels. Percentage of unharmed mussels per length class of mussels on beds predated by large crabs. Differences are tested with Mann-Whitney, when p-value is showed bold the difference is significant.

| Length class of mussels | 6-9 mm | 12-15 mm | 18-21 mm | 24-27 mm |
|-------------------------|---------------------------------|------------------------------|---------------------------------|------------------------------|
| 6-9 mm | | -16.6% (p=0.001) | -27.4% (p<0.001) | -10.7% (p=0.090) |
| 12-15 mm | +16.6% (p=0.001) | | -10.8% (p=0.058) | +5.8% (p=0.195) |
| 18-21 mm | +27.4% (p<0.001) | +10.8% (p=0.058) | | +16.6% (p=0.003) |
| 24-27 mm | +10.7% (p=0.090) | -5.8% (p=0.195) | -16.6% (p=0.003) | |

Table 8 shows that the largest differences in the predation of mussels by small crabs and by large crabs can be found in the length classes of 18-21 mm and 24-27 mm. Together with the the difference of 14.0% in the length class of 6-9 mm, do they show a significant difference.

Table 8. The difference between the percentage of unharmed mussels per length class of mussels on beds predated by small crabs and of mussels on beds predated by large crabs. Differences are tested with Mann-Whitney.

| Length class of mussels | Difference in percentage of unharmed mussels, small crab/large crabs are compared | p-value Bold = significant difference |
|-------------------------|---|---|
| 6-9 mm | +14.0% with large crab | p=0.008 |
| 12-15 mm | +8.8% with large crab | p=0.238 |
| 18-21 mm | +18.8% with small crab | p=0.002 |
| 24-27 mm | +19.9% with small crab | p<0.001 |

The results of all mussels are split up per size class of crabs as well as presence/absence of oysters and tested and compared (fig. 6). This to show the difference per size class of crabs per kind of mussel bed. The largest differences are found for the small crab and when small and large crabs on beds with oysters are compared. See table 9 for detailed differences, for charts of separate differences see appendix I.

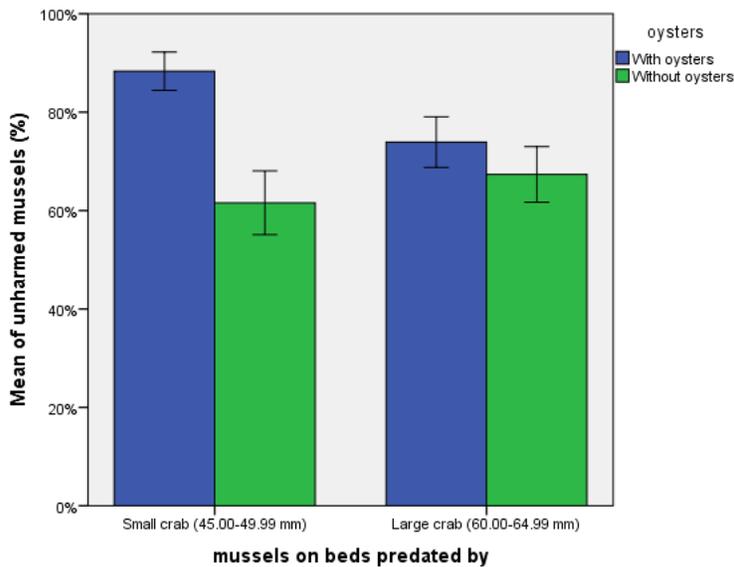


Figure 6. Unharmed mussels on beds with- and without oysters, predated by two size classes of crabs (mean % n=308 (=77 experimental mussel beds multiplied by 4 length classes). Error bars indicate the 95% Confidence interval. The overall difference between the bars is significant (Kruskal-Wallis; p<0.001)

Table 9 shows that present on mussel beds without oysters, the difference between the predation by small crabs and the predation by large crabs is quite small compared to mussel beds with oysters.

Table 9. The difference between the percentage of unharmed mussels of mussels predated by small crabs and of mussels predated by large crabs, per kind of mussel bed (with/without oysters present). Differences are tested with Mann-Whitney.

| Mussel beds | Difference in percentage of unharmed mussels | p-value Bold = significant difference |
|--------------------|---|--|
| With oysters | +14.4% with small crab | p<0.001 |
| Without oysters | +5.8% with large crab | p=0.217 |

To summarize the results of this paragraph; significant differences in the percentage of unharmed mussels between mussels predated by small crabs and mussels predated by large crabs are found:

- *Between the mussels of 24-27 mm and all the other length classes, and between 12-15 mm & 18-21 mm, when predated by small crabs.*
- *Between the length classes of 6-9 mm & 12-15 mm; between 6-9 mm & 18-21 mm; between 18-21 mm & 24-27 mm when predated by large crabs.*
- *In length classes of 6-9 mm, 18-21 mm and 24-27 mm when predation by small- and predated by large crab is compared.*
- *Between beds predated by small crabs & beds predated by large crabs when oysters are present.*

3.4: Mussel predation per type of acclimatization

The results of all mussels are split up per acclimatization type (no crab present/caged crab present) and length class and compared and tested. This to show the differences between the acclimatization types.

First this was done for the acclimatization with no crab present (fig. 7). The largest differences are found when the outer two length classes are compared with the inner two. For charts of all classes separately compared, see appendix I, for further details of the differences see table 10.

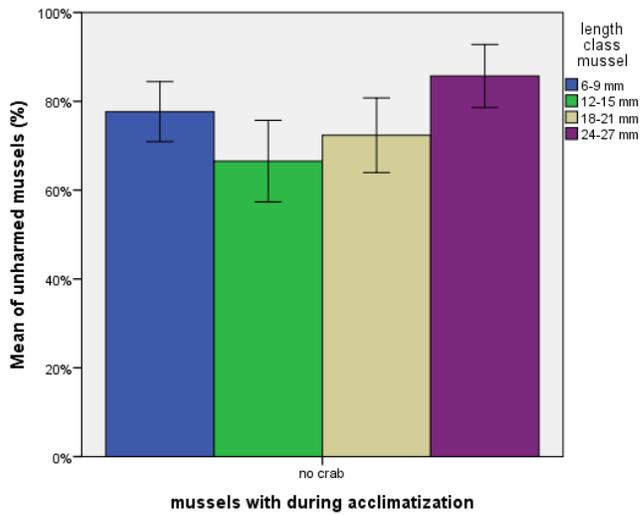


Figure 7. Unharmed mussels per length class on beds **with no crabs** during acclimatization (mean % n=148 (=37 experimental mussel beds multiplied by 4 length classes). Error bars indicate the 95% Confidence interval. The difference between bars is significant (Kruskal-Wallis; p=0.001)

The same was done for acclimatization with a caged crab (fig. 8). The largest differences are found when the outer two length classes are compared with the inner two. For charts of all classes separately compared, see appendix I, for further details of the differences see table 11.

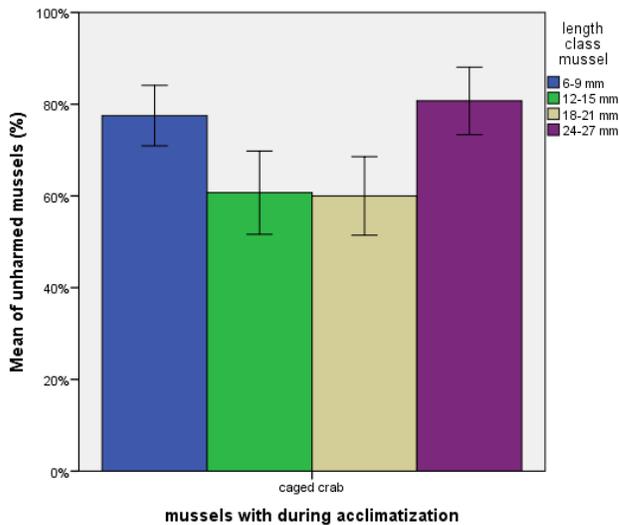


Figure 8. Unharmed mussels per length class on beds **with caged crabs** during acclimatization (mean % n=160 (=40 experimental mussel beds multiplied by 4 length classes). Error bars indicate the 95% Confidence interval. The overall difference between the bars is significant (Kruskal-Wallis; p<0.001)

Table 10 shows that the only significant difference between the length classes of mussels on beds is the difference between 24-27 mm and all other length classes after acclimatization without crab.

Table 10. The difference between the length classes of mussels. Percentage of unharmed mussels per length class of mussels on beds with no crab present during acclimatization period. Differences are tested with Mann-Whitney, when p-value is showed bold the difference is significant.

| Length class of mussels | 6-9 mm | 12-15 mm | 18-21 mm | 24-27 mm |
|-------------------------|-----------------------------|---------------------------------|------------------------------|---------------------------------|
| 6-9 mm | | -11.1% (p=0.177) | -5.3% (p=0.668) | +8.0% (p=0.008) |
| 12-15 mm | +11.1% (p=0.177) | | +5.8% (p=0.280) | +19.2% (p<0.001) |
| 18-21 mm | +5.3% (p=0.668) | -5.8% (p=0.280) | | +13.4% (p=0.005) |
| 24-27 mm | -8.0% (p=0.008) | -19.2% (p<0.001) | -13.4% (p=0.005) | |

Table 11 shows that after acclimatization with a caged crab there are multiple differences between the percentages of unharmed mussels per length class. The largest significant differences are between the length class of 24-27 mm and the two classes of 12-15 mm and 18-21 mm . Two smaller significant differences were found between the length class of 6-9 mm and the two classes of 12-15 mm and 18-21 mm.

Table 11. The difference between the length classes of mussels. Percentage of unharmed mussels per length class of mussels on beds with a caged crab present during acclimatization period. Differences are tested with Mann-Whitney, when p-value is showed bold the difference is significant.

| Length class of mussels | 6-9 mm | 12-15 mm | 18-21 mm | 24-27 mm |
|-------------------------|------------------------------|------------------------------|---------------------------------|---------------------------------|
| 6-9 mm | | -16.8% (p=0.008) | -17.5% (p=0.004) | +3.2% (p=0.211) |
| 12-15 mm | +16.8% (p=0.008) | | -0.7% (p=0.954) | +20.0% (p=0.001) |
| 18-21 mm | +17.5% (p=0.004) | +0.7% (p=0.954) | | +20.7% (p<0.001) |
| 24-27 mm | -3.2% (p=0.211) | -20.0% (p=0.001) | -20.7% (p<0.001) | |

Table 12 shows that between the two different situations of acclimatization, only one significant difference is found, of 12.3% in the length class of 18-21 mm. For the other length classes, the differences between the two acclimatization situations are proven to be not significant.

Table 12. The difference between the percentage of unharmed mussels per length class of mussels on beds with no crab present during acclimatization and of mussels on beds with caged crab present during acclimatization. Differences are tested with Mann-Whitney.

| Length class of mussels | Difference in percentage of unharmed mussels, acclimatization with/without crab is compared | p-value Bold = significant difference |
|--------------------------------|--|--|
| 6-9 mm | +0.2% with no crab | p=0.955 |
| 12-15 mm | +5.8% with no crab | p=0.317 |
| 18-21 mm | +12.3% with no crab | p=0.031 |
| 24-27 mm | +5.0% with no crab | p=0.241 |

The results of all mussels are split up for acclimatization without crab and with caged crab as well as by presence/absence of oysters and compared and tested. This to show the difference between acclimatization types per kind of mussel bed (with/without oysters) (fig. 9). The differences found in this case are larger between the kinds of mussel beds than between the acclimatization types. For the detailed numbers of the differences, see table 3 and table 13.

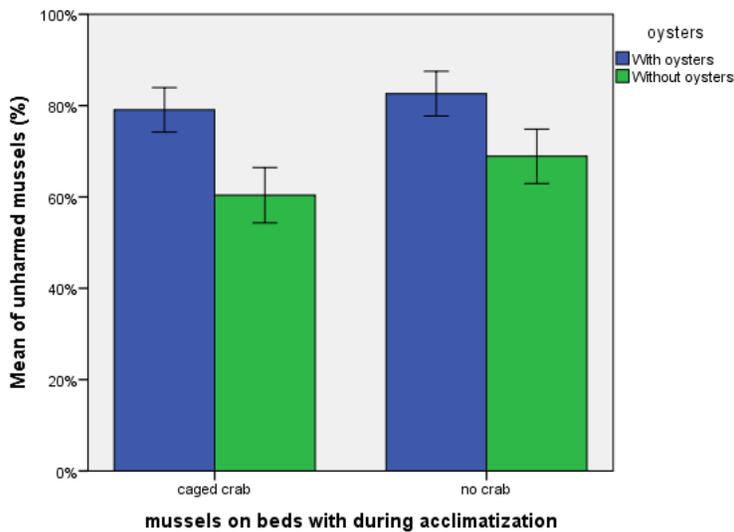


Figure 9. Unharmed mussels on beds with- and without oysters, and with no crab- and caged crab during acclimatization (mean % n=308 (77 experimental mussel beds multiplied by 4 length classes). Error bars indicate the 95% Confidence interval.

Table 13 shows that there are no significant differences between the mussel beds with oysters in both the acclimatization situation, as well as there is no significant difference between the mussel beds without oysters in both the acclimatization situations.

Table 13. The difference between the percentage of unharmed mussels on beds without crab during acclimatization and on beds with caged crabs during acclimatization, per type of mussel bed (with/without oysters present). Differences are tested with Mann-Whitney.

| Mussel beds | Difference in percentage of unharmed mussels, acclimatization with/without crab is compared | p-value Bold = significant difference |
|--------------------|--|--|
| with oysters | +3.5% with no crab | p=0.158 |
| without oysters | +8.5% with no crab | p=0.067 |

To summarize the results of this paragraph; significant differences in the percentage of unharmed mussels between mussels without crab during acclimatization and mussels with caged crab during acclimatization are found:

- *Between the length classes of 24-27 mm and all other length classes of mussels without crab during acclimatization.*
- *Between the length classes of 6-9 mm & 12-15 mm; between 6-9 mm & 18-21 mm; between 12-15 mm & 24-27 mm; between 18-21 mm & 24-27 mm of mussels with a caged crab during acclimatization.*
- *In length class of 18-21 mm when compared between acclimatization without crab and acclimatization with caged crab.*

3.5: General Linear Model

The final GLM was run for all the four variables, even though for all the four variables that contribute in the differences for the percentages of unharmed mussels, “size class of crab” was the only variable that did not show a significant contribution. But it was still included in the final test due to the contributing interactions were the variable was included. The model with the four variables had an Adjusted R squared=0.349, which can be interpreted as ±35% of the unharmed mussels being explained by this model.

Of the four variables, the variables of “Oysters” ($\eta_p^2=0.147$) and “length class mussel” ($\eta_p^2=0.139$) contributed the most to the explanation of the differences in unharmed mussels together with the interaction of size class of crabs with the length class of mussels ($\eta_p^2=0.127$)(fig. 10), followed by “acclimatization” ($\eta_p^2=0.024$). It resulted in p-values of $p<0.001$ for the variables of “Oysters” and “Length class mussel”, and $p=0.007$ and $p=0.054$ for “Acclimatization” and “size class crab”. The interaction that contributed the second most in explaining the differences was oysters with size class of crabs ($\eta_p^2=0.060$). Even though the interaction between with/without oysters and length class mussels ($\eta_p^2=0.038$) contributed less compared to the other two, it resulted in a clear chart (fig. 11). The p-values of the interactions are $p<0.001$ for size class crab with length class mussel, $p<0.001$ for size class crab with with/without oysters and $p=0.010$ for length class mussel with with/without oysters. For tables of the GLM see appendix II.

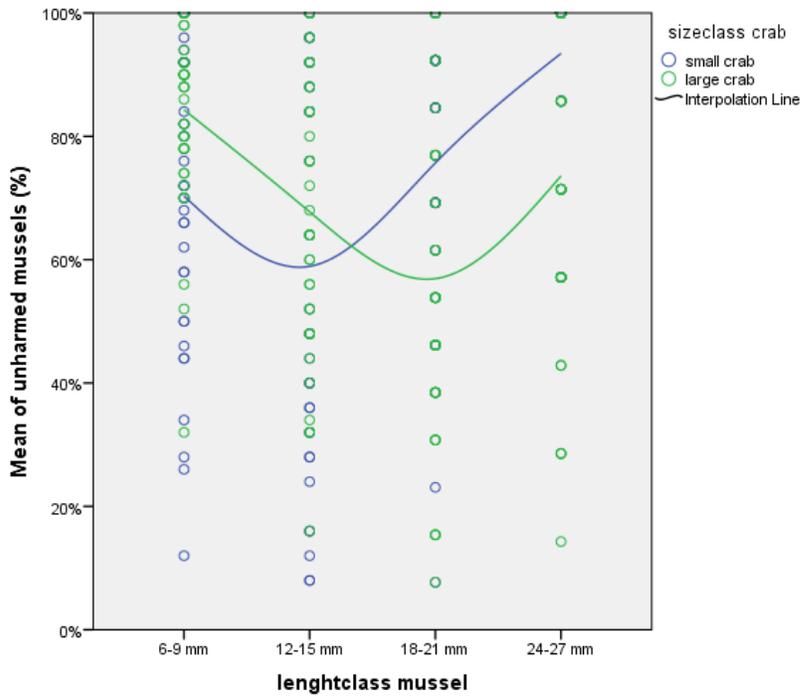


Figure 10. Interaction between size class of crabs and length class of mussels. The interaction is significant (GLM; $p < 0.001$). For both sizes of crabs, the preferred length class of mussel is recognizable in this chart.

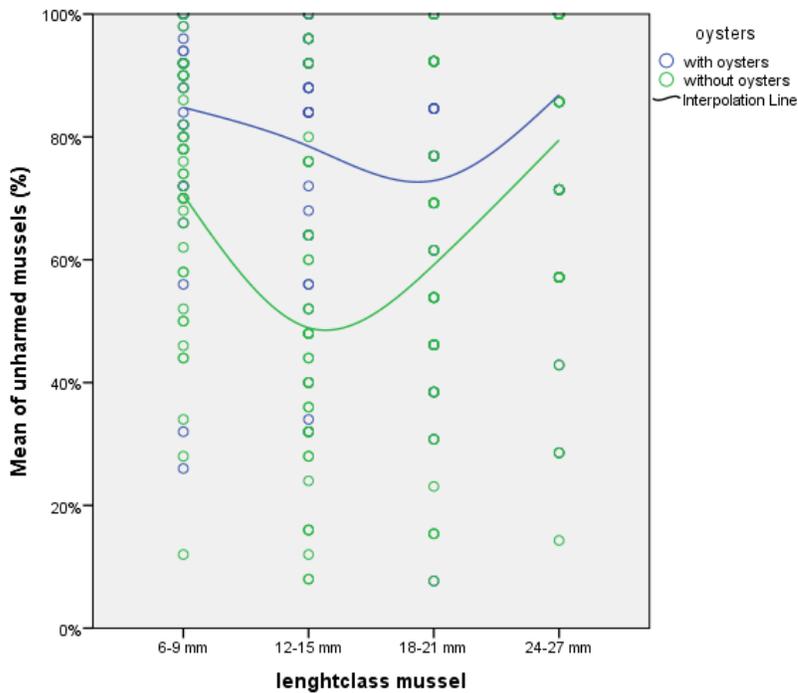


Figure 11. Interaction between with/without oysters and length class of mussels. The interaction is significant (GLM; $p < 0.001$). For each length class of mussels, the difference between with or without oysters is recognizable.

Chapter 4: Conclusion

The first sub question is considering the effect of oysters. From an early comparison between beds with oysters and without, the conclusion rises: more mussels stay unharmed on beds with oysters. When these results are split up per variable, the comparisons show again that more mussels stay unharmed on beds with oysters. This is the case for comparisons between the beds with and without oysters per every other variable, except for length class of 24-27 mm and large crabs, where the differences were too small to be significant. Therefore, the first sub question can be answered: fewer mussels are harmed/damaged when oysters are present compared to no oysters present, especially mussels of the three smallest length classes.

The second sub question is considering the effect of length class. The significant differences were between the largest length class (24-27 mm) and the two middle classes (12-15 mm and 18-21 mm) on beds with oysters. And between all length classes except 18-21 mm and the two smallest classes (6-9 mm and 12-15 mm) on beds without oysters. On both kinds of beds, less mussels of the largest and smallest class were harmed compared to the middle classes. But on the beds where no oysters were present, differences were larger, showing the two inner classes as most preferred preys. Concluding: when no oysters are present, the classes of mussels close to preferred prey size for the crabs are predated a lot more. And the differences between the length classes are larger.

The third sub question is considering the effect of size class crab. Conclusion from results: small crabs predate mainly on mussels of all but the largest length class, especially mussels of second smallest and smallest length class. Large crab on the other hand, predate mainly on mussels of all but the smallest length class, especially on mussels of second largest length class. In predation per length class, the two crab sizes differ a lot in all but the length class of 12-15 mm, there is the overlap in their predation. Another conclusion: large crabs manage to damage/harm more mussels on beds with oysters than small crabs.

The fourth sub question is considering the effect of acclimatization. The answer coming from the results is there is only one difference; when no crab is present during acclimatization, more mussels survive the later predation by shore crabs compared to acclimatization with a caged crab.

Having answered all the sub questions, there can be given a final conclusion as answer on main question. The influence of the Pacific oyster is: more mussels survive predation by Shore crabs when oysters are present on mussel beds, especially when predated by small crabs.

After running the GLM, there still can be given the conclusion that the abundance of the Pacific oysters has a positive effect on the survival of Blue mussels from predation. GLM gives the effect of acclimatization a stronger role than came forth of earlier tests, it's

interaction with the other variables is not a significant contribution to the differences. The test proves the strong influence of crab size when interacting with length class of mussels, but as single variable the size class of crabs does not contribute that much extra.

Chapter 5: Discussion

It was expected that for the smaller mussels as well as small crabs the differences in predation on beds with oysters and beds without oysters are proven, for the fact that every size of crab has an preferred prey size (Elner & Hughes, 1978, in their research to the optimal diet of the Shore crab). This helps to explain why the small crabs focused more on the smaller mussels. It also explains why the outcomes are not in line with what was expected. Because on beds without oysters the crabs have less difficulties in finding and eating their preferable sized mussels. Which means that on those beds there are in fact more differences in percentages of unharmed mussels between mussels of different length classes.

Murray et al.(2007), also found that crabs eat their preferable sized mussels.

Also it was expected that for predation by small crabs there would be less influence of the presence of oysters on the percentage of unharmed mussels than for predation by large crabs. The opposite however, seemed to be the case: the large crabs, feeding mostly on larger mussels, were less influenced by presence of oysters due to the fact that the mussel sizes they favored seemed to crawl not as deep between the oysters as the smaller mussels. This was observed, but not measured.

For acclimatization with and without crabs no differences were found. This can be explained by findings of Eschweiler & Christensen (2011), who researched to the migratory behavior of Blue mussels between Pacific oysters. They mention that the downward migration of mussels between the oysters is suggested to be caused more by physical contact with the crab rather than by exposure to chemical cues released by a crab. And even though the experiments with the caged crabs was more based on the chemical cues from damaged conspecifics(Cheung et al., 2004) and less on the cues from the crab, it still is worth to consider during future researches that mussels respond more on physical contact of the crab.

The GLM however, resulted for acclimatization having a significant influence, the opposite from what resulted before. Also, the size of crabs, which proved to have some influence, resulted in the GLM to have a less valued contribution as variable, but in interactions with others it contributed significant. But still, the two variables of “with/without oysters” and “length class of mussels” remained strong. On these, the focus lies. It could be possible that, due to the preferred prey size of the crabs, the variables of “size class crab” and “length class mussel” overlap each other a bit. Due to this interaction, the size class of crabs does not contain an added value to the differences in unharmed mussels. The GLM also showed trough interactions between oysters and the size class of crabs that small crabs indeed were more affected by the presence of oysters compared to large crabs. This was also recognizable in the interaction between the oysters and length class of mussels, where the two smaller length classes showed to be more influenced compared to the larger two. And considering the interaction between the length class of mussels and the size class of crabs, the similarities between those two interactions where the oysters were involved are something that could be expected.

In a few cases the amount of unharmed mussels was much lower than average, but no clear cause for it was found. One possible explanation can be that during the acclimatization period, some caged crabs managed to damage already some of the mussels through the fence, so later on it was easier for the predating crab to eat those mussels and that crab could eat extra mussels. But this was not proven by observations. Therefore the results of those beds were included in the analyses.

Observed but not measured, was the behavior of the crabs in the storage aquarium when they were fed. All crabs were showing no or almost no activity, even when mussels were thrown in the storage aquarium. But as soon as one mussel was caught by a crab or damaged mussels were thrown in, all crabs went immediately in aggressive behavior and the whole aquarium went into a kind of feeding frenzy. It could be possible that the crabs also react on chemical cues from damaged mussels, just like mussels can react on chemical cues. Crothers (1967), in his paper about the biology of the Shore crab, tells about chemoreception as one of the senses which crabs use to hunt. Therefore is it likely to think of the possibility for crabs being able to sense the cues. This could be an explanation for some lower percentages of unharmed mussels during the experiments with caged crabs during acclimatization. Because if the crabs in fact respond on the abundance of chemical cues in the aquaria and enter a kind of state of feeding frenzy, they eat more. Which leads to a lower percentage of unharmed mussels.

Chapter 6: Recommendations

For further studies to this subject, there could be recommended:

- Observations of the behavior of the caged crabs to see if they are capable to influence the experiments by damaging mussels through the fence of their cages.
- Add the possibilities for letting crabs crawl over the mussel beds during acclimatization period without being able to harm the mussels.
- Further statistic testing with the same dataset, like with non-linear models.
- Transformation of the dataset in statistics.

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Appendices

Appendix I: Graphs separate tests

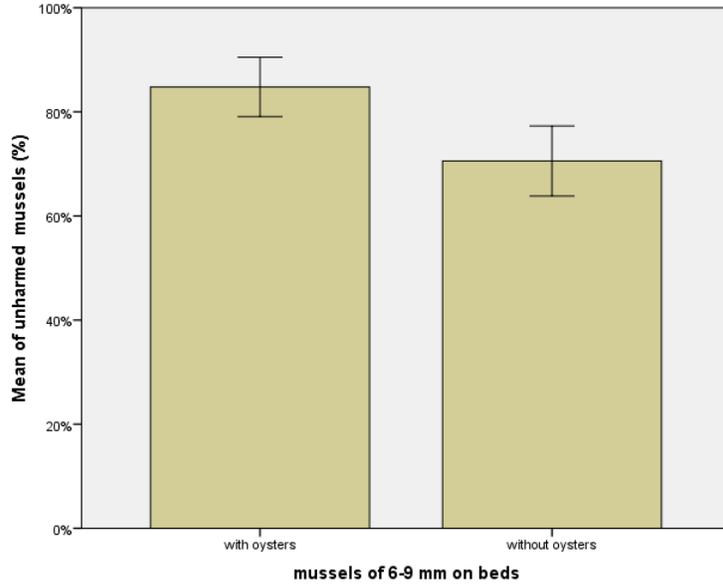


Figure 1A. Unharmed mussels of 6-9 mm on beds with oysters and without oysters (mean % of 38 experimental mussel beds with oysters, and 39 experimental mussel beds without oysters). The error bars indicate the 95% Confidence interval. The standard errors are $\pm 2.8\%$ for mussel beds with oysters and $\pm 3.3\%$ for without oysters. The difference between both bars is significant (Mann-whitney; $p < 0.001$).

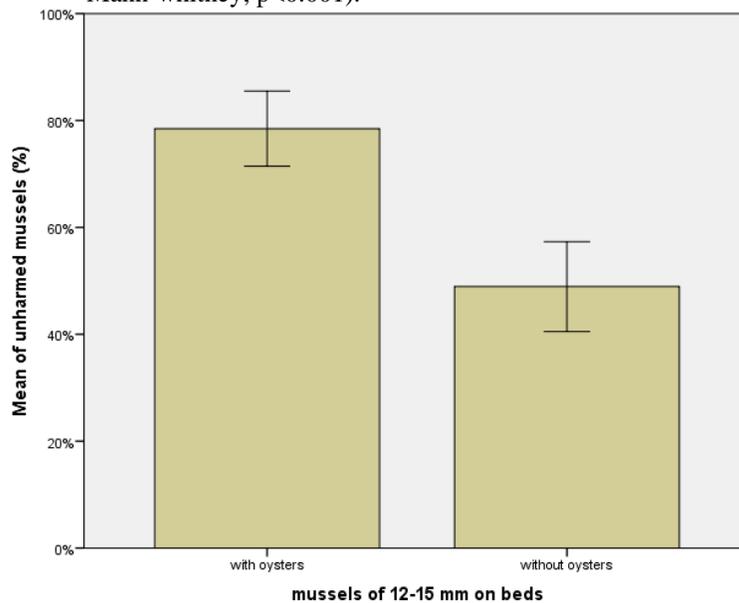


Figure 1B. Unharmed mussels of 12-15 mm on beds with oysters and without oysters (mean % of 38 experimental mussel beds with oysters, and 39 experimental mussel beds without oysters). The error bars indicate the 95% Confidence interval. The standard errors are $\pm 3.5\%$ for mussel beds with oysters and $\pm 4.2\%$ for without oysters. The difference between both bars is significant (Mann-whitney; $p < 0.001$).

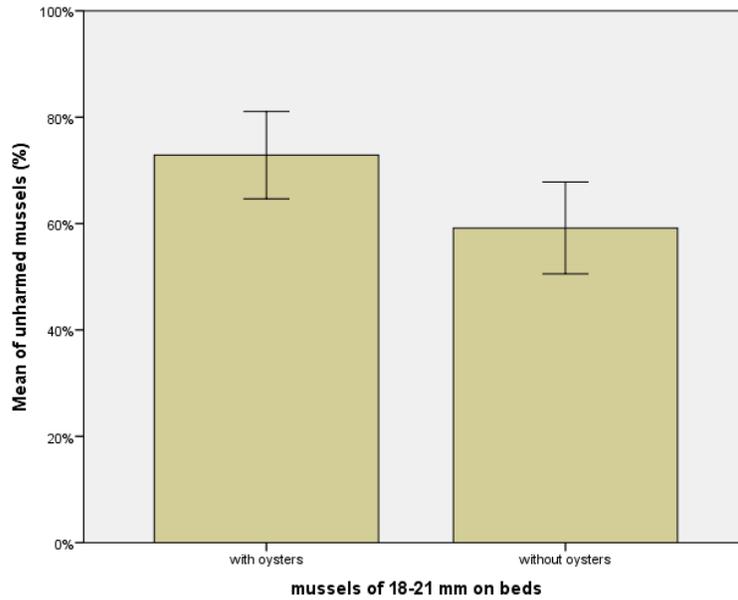


Figure 1C. Unharmed mussels of 18-21 mm on mussel beds with oysters and without oysters (mean % of 38 experimental mussel beds with oysters, and 39 experimental mussel beds without oysters). The error bars indicate the 95% Confidence interval. The standard errors are $\pm 4.0\%$ for mussel beds with oysters and $\pm 4.3\%$ for without oysters. The difference between both bars is significant(Mann-whitney; $p=0.029$).

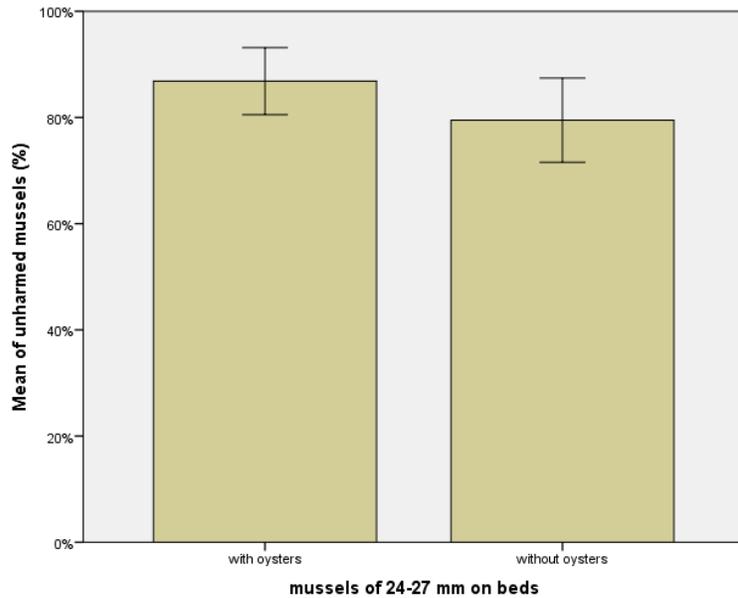


Figure 1D. Unharmed mussels of 24-27 mm on mussel beds with oysters and without oysters (mean % of 38 experimental mussel beds with oysters, and 39 experimental mussel beds without oysters). The error bars indicate the 95% Confidence interval. The standard errors are $\pm 3.1\%$ for mussel beds with oysters and $\pm 3.9\%$ for without oysters. The difference between both bars is not significant(Mann-whitney; $p=0.159$).

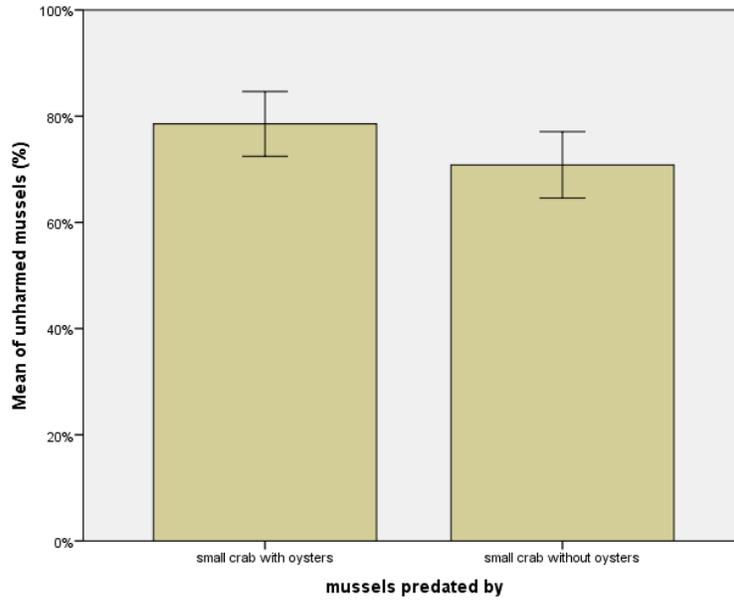


Figure 2. Unharmed mussels predated by small crabs on beds with oysters and without oysters (mean % of 72 cases(=18 experimental mussels beds with oysters multiplied by the four length classes), and 76 cases (=19 experimental mussels beds without oysters multiplied by the four length classes). The error bars indicate the 95% Confidence Interval. The standard errors are $\pm 3.1\%$ for the mussel beds with oysters and $\pm 3.1\%$ for without oysters. The difference between both bars is significant(Mann-whitney; $p=0.014$).

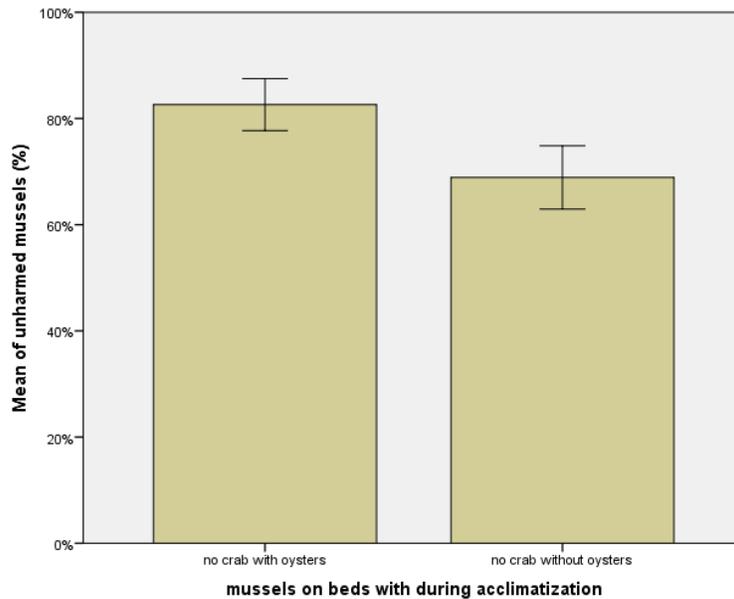


Figure 3A. Unharmed mussels with no crab present during acclimatization on beds with oysters and without oysters (mean % of 72 cases(=18 experimental mussels beds with oysters multiplied by the four length classes), and 76 cases (=19 experimental mussels beds without oysters multiplied by the four length classes)). The error bars indicate the 95% Confidence Interval. The standard errors are $\pm 2.5\%$ for mussel beds with oysters and $\pm 3.0\%$ for without oysters. The difference between both bars is significant(Mann-whitney; $p=0.001$).

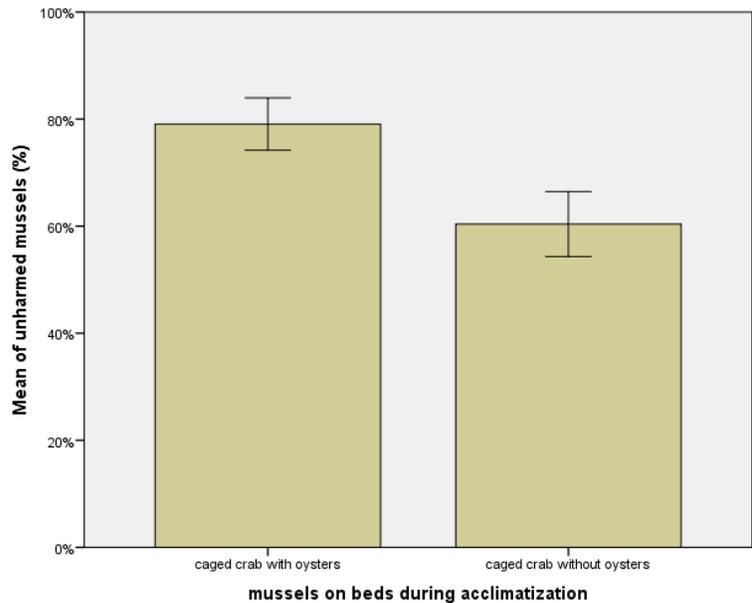


Figure 3B. Unharmed mussels with a caged crab present during acclimatization on beds with oysters and without oysters (mean % of 80 cases (=20 experimental mussels beds with oysters multiplied by the four length classes), and 80 cases (=20 experimental mussels beds without oysters multiplied by the four length classes)). The error bars indicate the 95% Confidence Interval. The standard errors are $\pm 2.5\%$ for mussel beds with oysters and $\pm 3.0\%$ for without oysters. The difference between both bars is significant (Mann-whitney; $p < 0.001$).

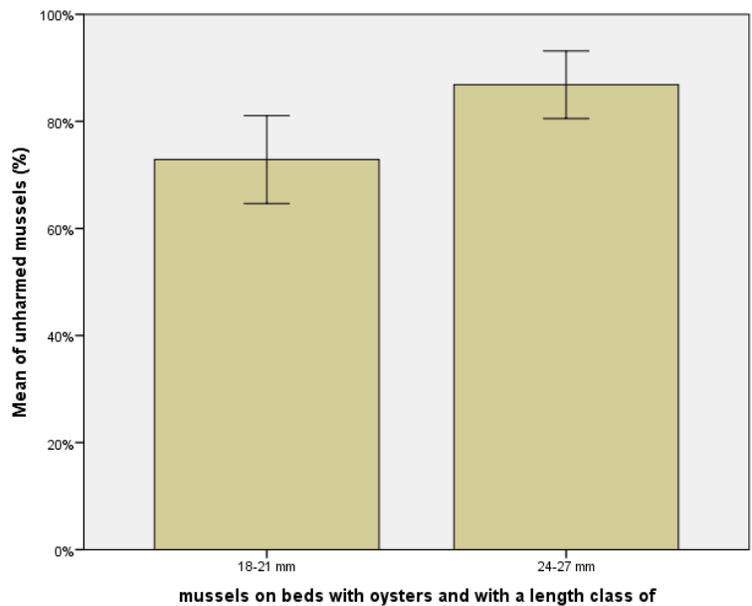


Figure 4A. Unharmed mussels of length classes of 18-21 mm and 24-27 mm on beds with oysters (mean % of 38 cases of 18-21 mm, and 38 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant (Mann-whitney; $p = 0.003$).

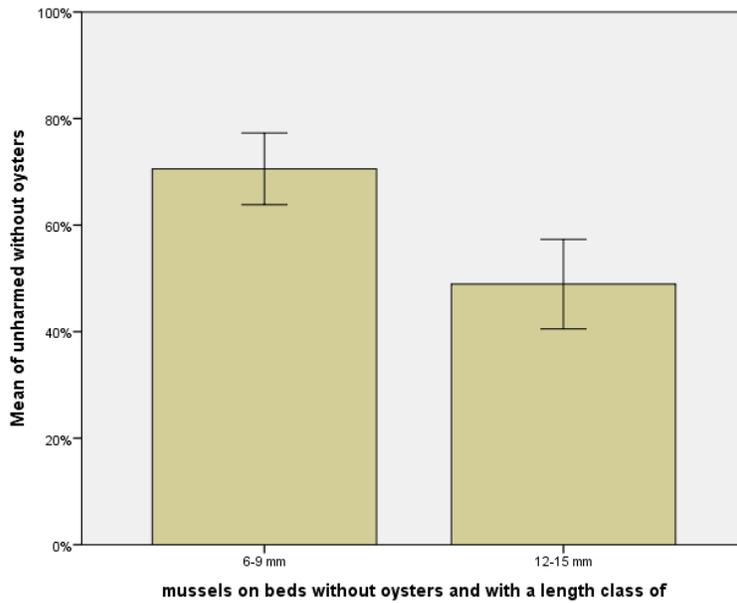


Figure 4B. Unharmed mussels of length classes of 6-9 mm and 12-15 mm on beds without oysters (mean % of 39 cases of 6-9 mm, and 39 cases of 12-15 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

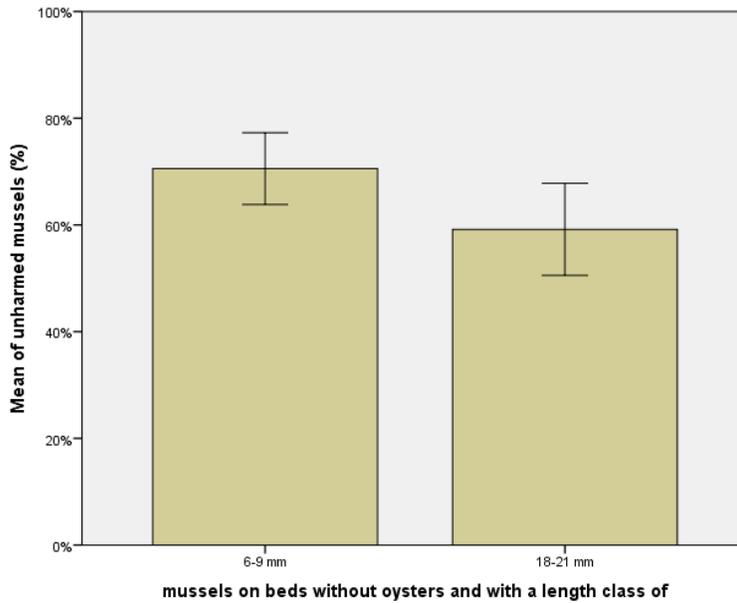


Figure 4C. Unharmed mussels of length classes of 6-9 mm and 18-21 mm on beds without oysters (mean % of 39 cases of 6-9 mm, and 39 cases of 18-21 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is not significant(Mann-whitney; $p = 0.059$).

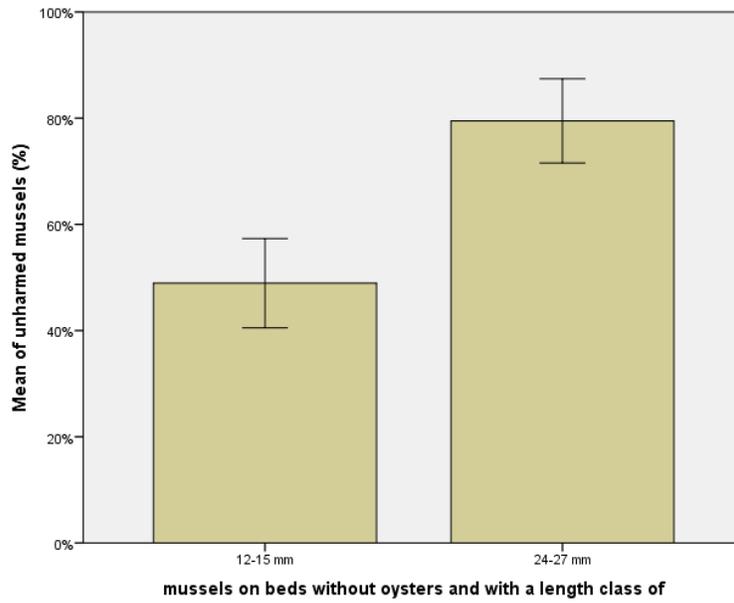


Figure 4D. Unharmed mussels of length classes of 12-15 mm and 24-27 mm on beds without oysters (mean % of 39 cases of 12-15 mm, and 39 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

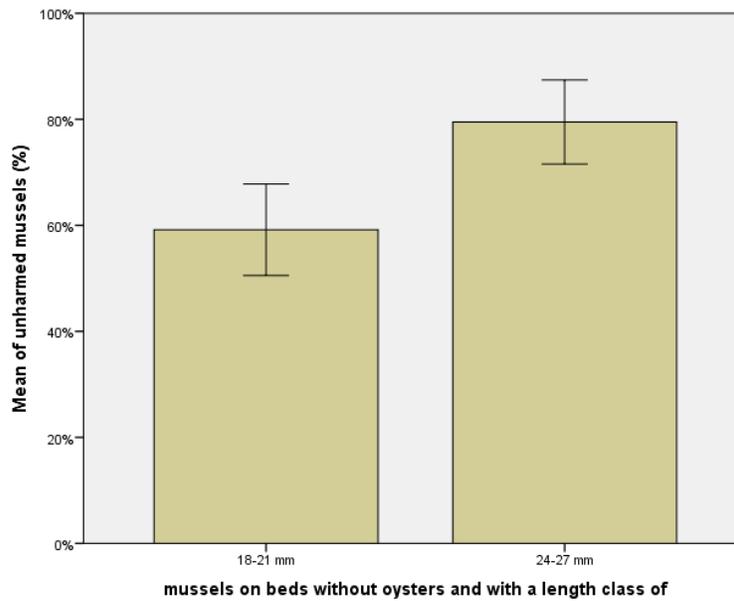


Figure 4E. Unharmed mussels of length classes of 18-21 mm and 24-27 mm on beds without oysters (mean % of 39 cases of 18-21 mm, and 39 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

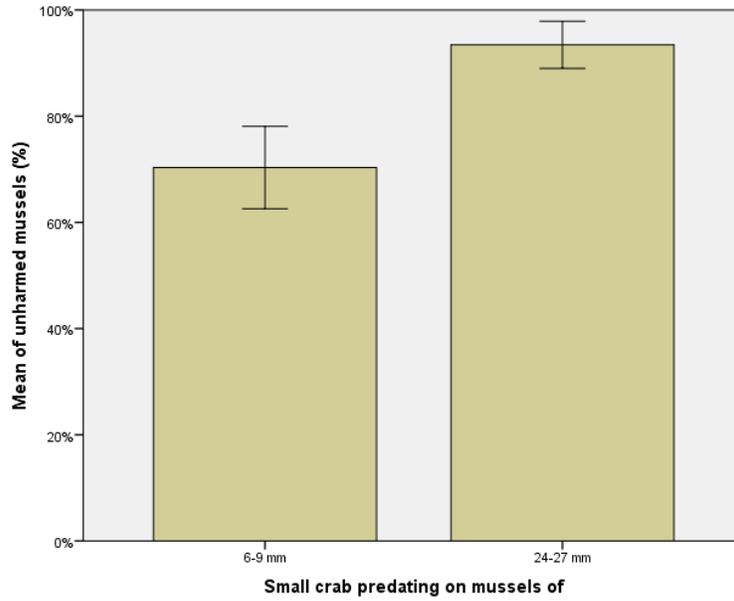


Figure 5A. Unharmed mussels of length classes of 6-9 mm and 24-27 mm on beds predated by small crabs (mean % of 37 cases of 6-9 mm, and 37 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

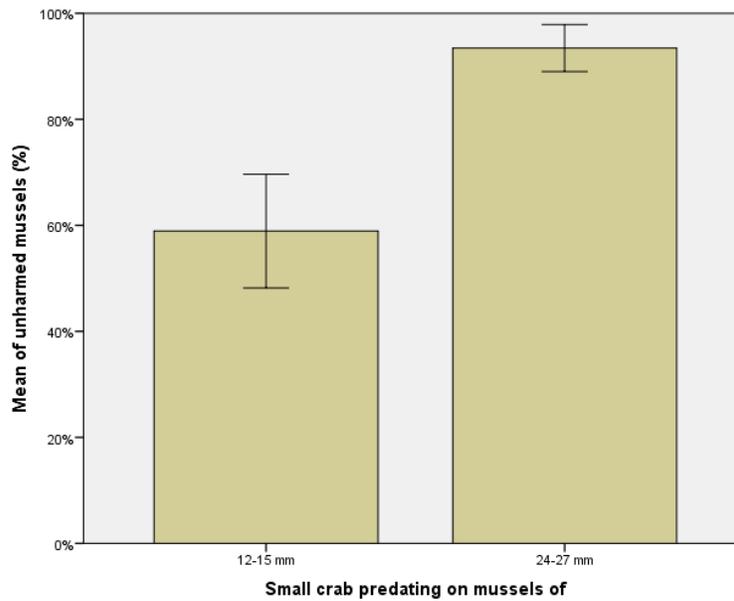


Figure 5B . Unharmed mussels of length classes of 12-15 mm and 24-27 mm on beds predated by small crabs (mean % of 37 cases of 12-15 mm, and 37 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

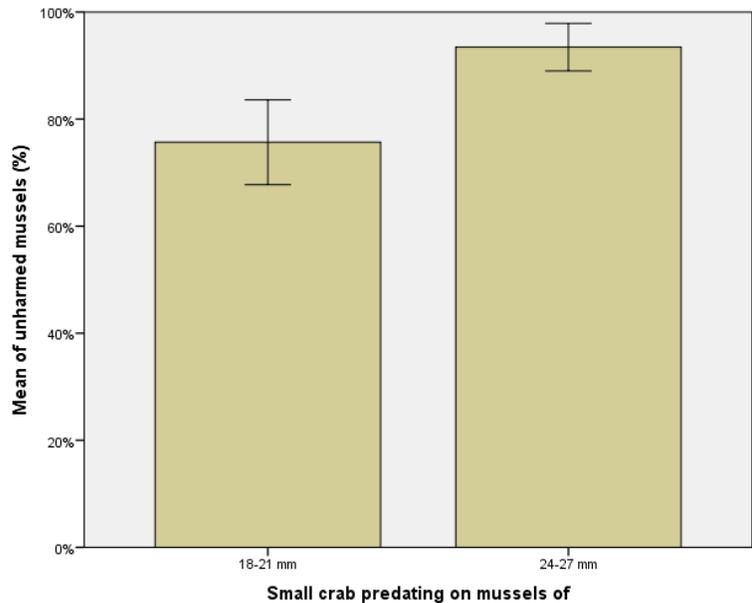


Figure 5C. Unharmed mussels of length classes of 18-21 mm and 24-27 mm on beds predated by small crabs (mean % of 37 cases of 18-21 mm, and 37 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

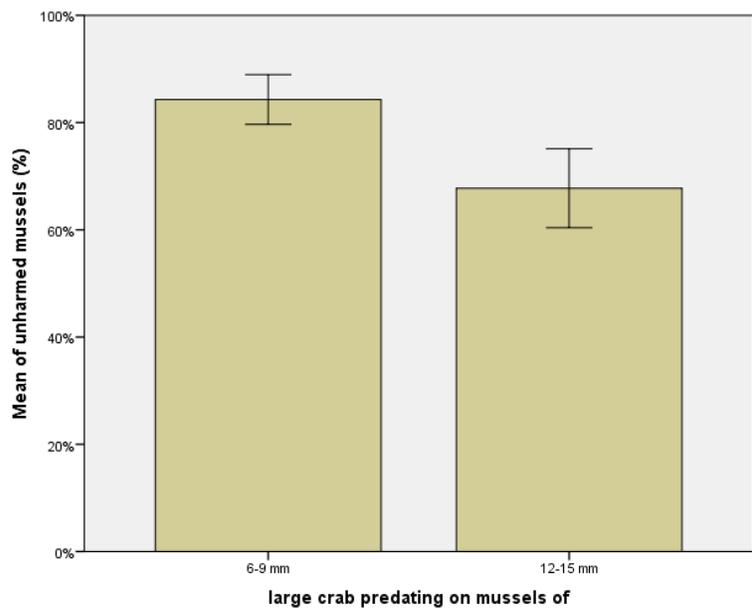


Figure 5D. Unharmed mussels of length classes of 6-9 mm and 12-15 mm on beds predated by large crabs (mean % of 40 cases of 6-9 mm, and 40 cases of 12-15 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p = 0.001$).

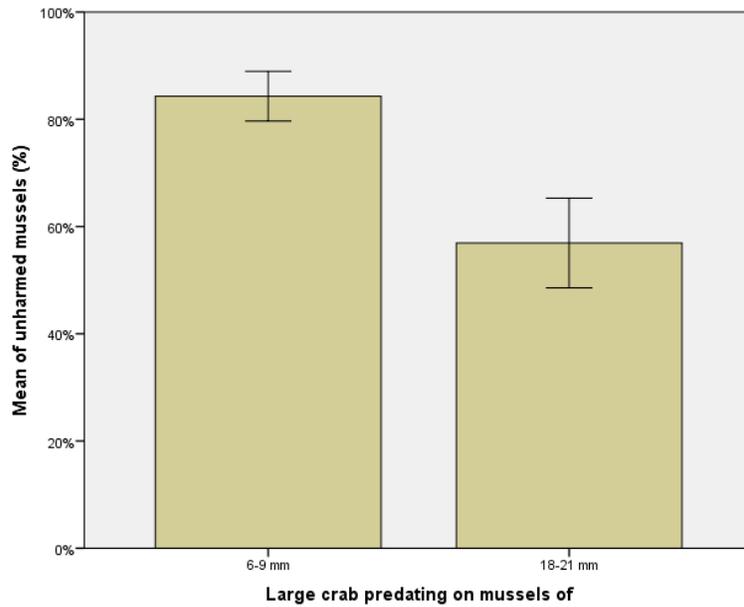


Figure 5E. Unharmed mussels of length classes of 6-9 mm and 18-21 mm on beds predated by large crabs (mean % of 40 cases of 6-9 mm, and 40 cases of 18-21 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

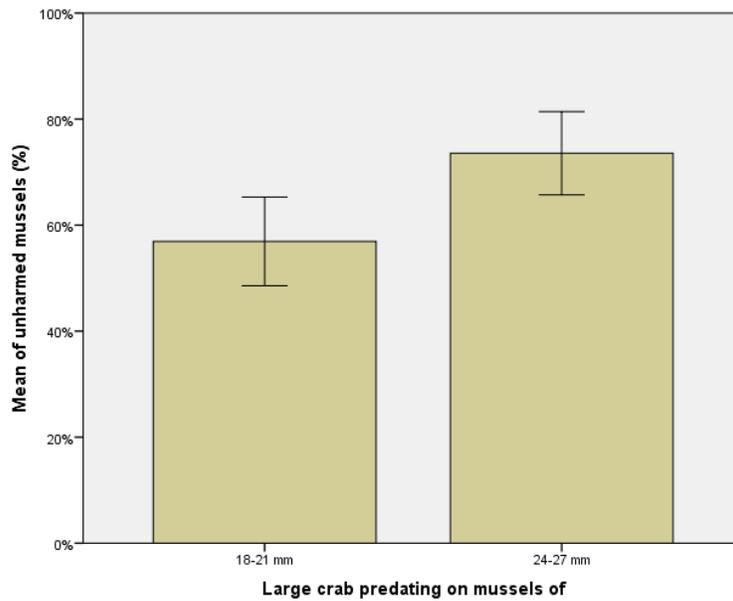


Figure 5F. Unharmed mussels of length classes of 18-21 mm and 24-27 mm on beds predated by large crabs (mean % of 40 cases of 18-21 mm, and 40 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

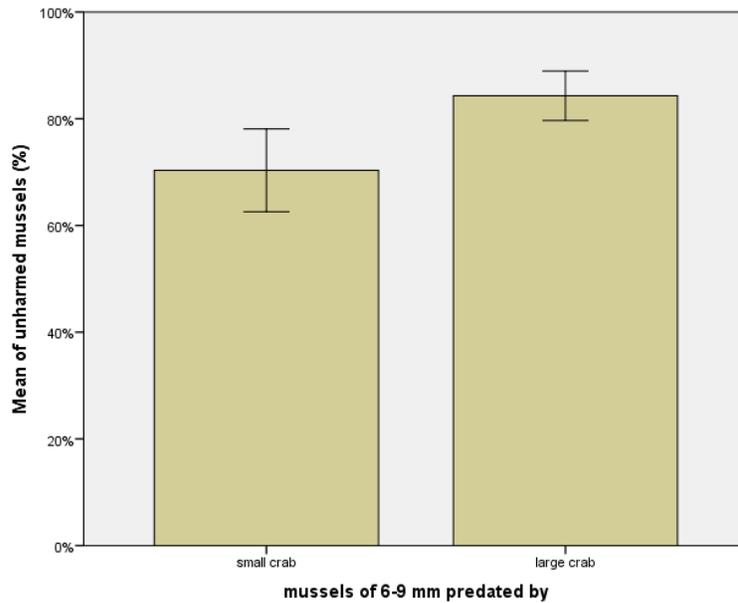


Figure 6A. Unharmed mussels of length classes of 6-9 mm on beds predated by small crabs and beds predated by large crabs (mean % of 37 cases of small crab, and 40 cases of large crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant (Mann-whitney; $p=0.008$).

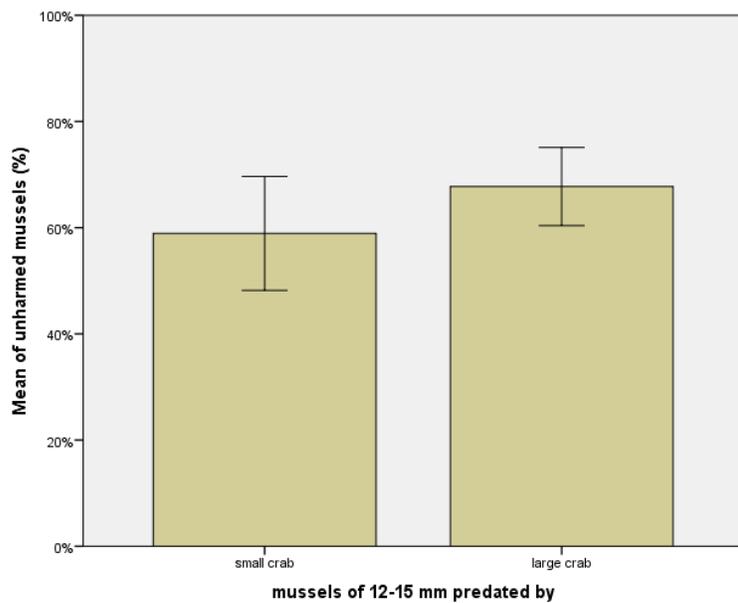


Figure 6B. Unharmed mussels of length classes of 12-15 mm on beds predated by small crabs and beds predated by large crabs (mean % of 37 cases of small crab, and 40 cases of large crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is not significant (Mann-whitney; $p=0.238$).

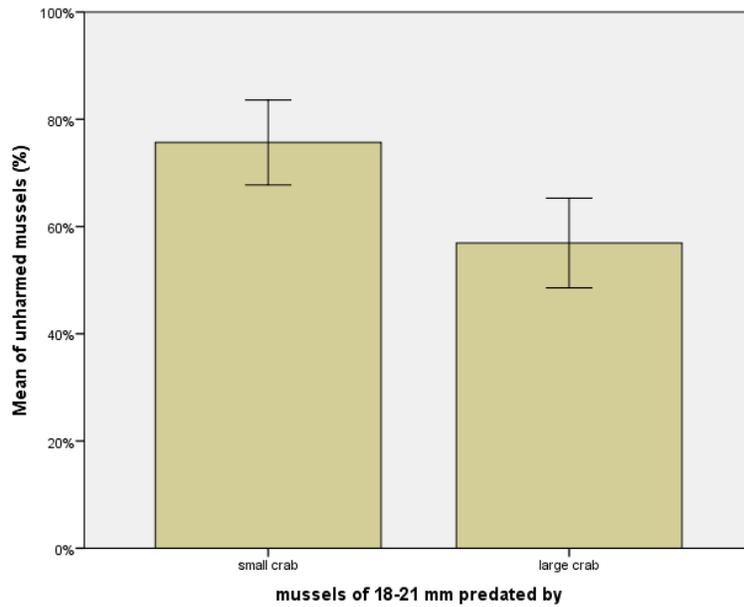


Figure 6C. Unharmed mussels of length classes of 18-21 mm on beds predated by small crabs and beds predated by large crabs (mean % of 37 cases of small crab, and 40 cases of large crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant (Mann-whitney; $p=0.002$).

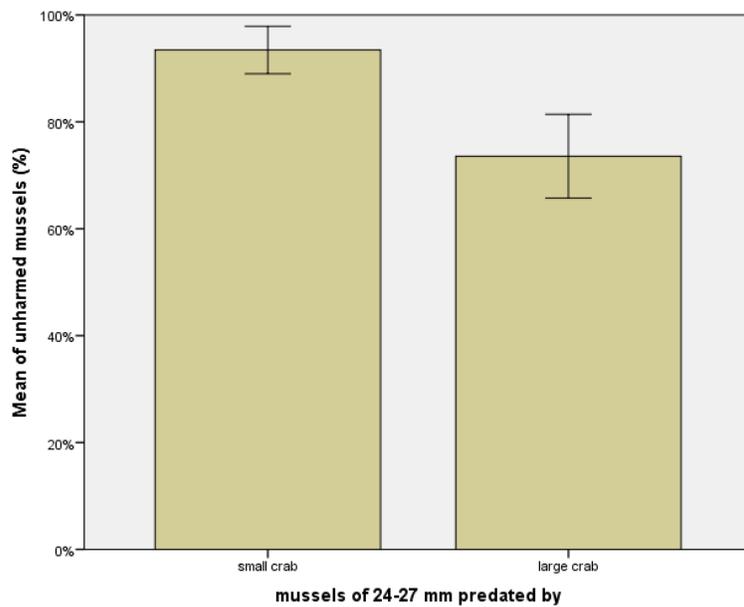


Figure 6D. Unharmed mussels of length classes of 24-27 mm on beds predated by small crabs and beds predated by large crabs (mean % of 37 cases of small crab, and 40 cases of large crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant (Mann-whitney; $p<0.001$).

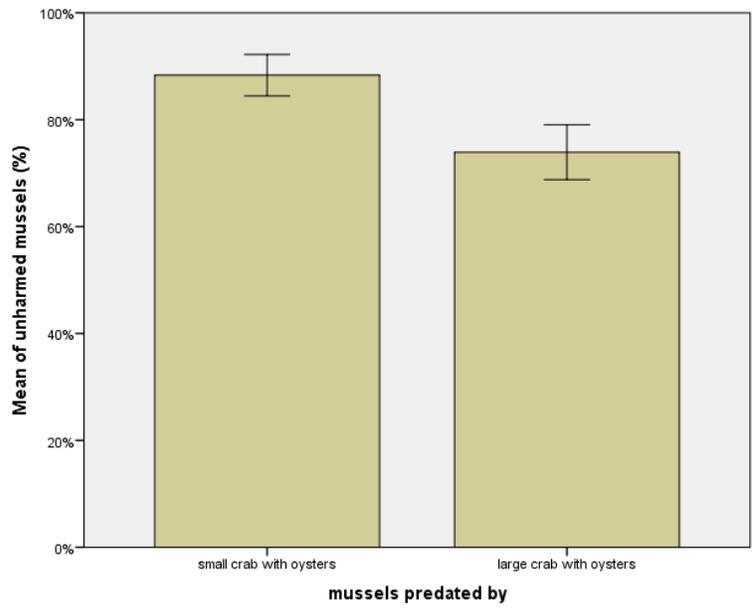


Figure 7. Unharmed mussels on beds with oysters, predated by small crabs and by large crabs (mean % of 72 cases of small crab(=18 beds multiplied by the four length classes), and 80 cases of large crab(=20 beds multiplied by the four length classes). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

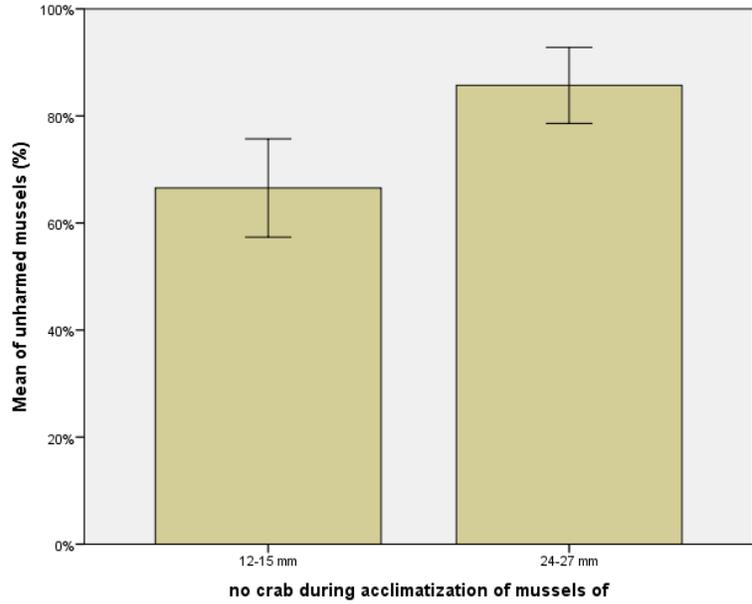


Figure 8A. Unharmed mussels of 12-15 mm and 24-27 mm with no crab present during acclimatization (mean % of 37 cases of 12-15 mm, and 37 cases of 24-27mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p < 0.001$).

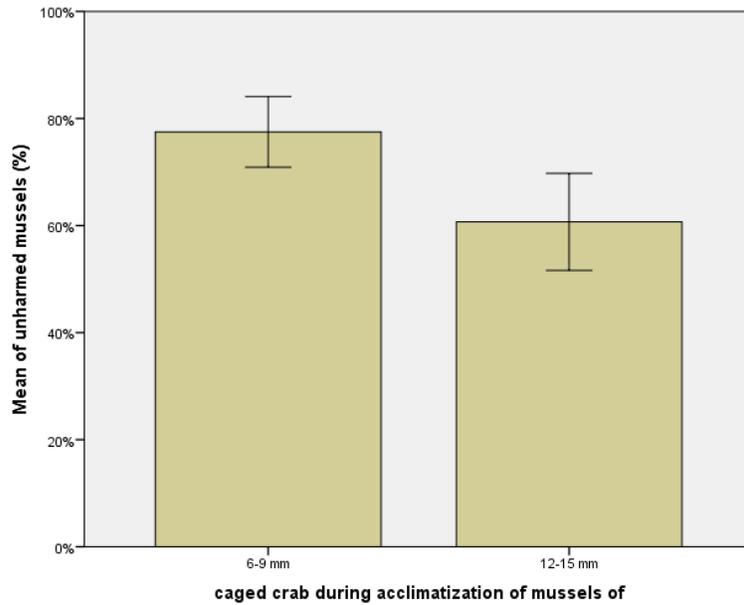


Figure 8B. Unharmed mussels of 6-9mm and 12-15 mm with a caged crab present during acclimatization (mean % of 40 cases of 6-9 mm, and 40 cases of 12-15 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p=0.008$).

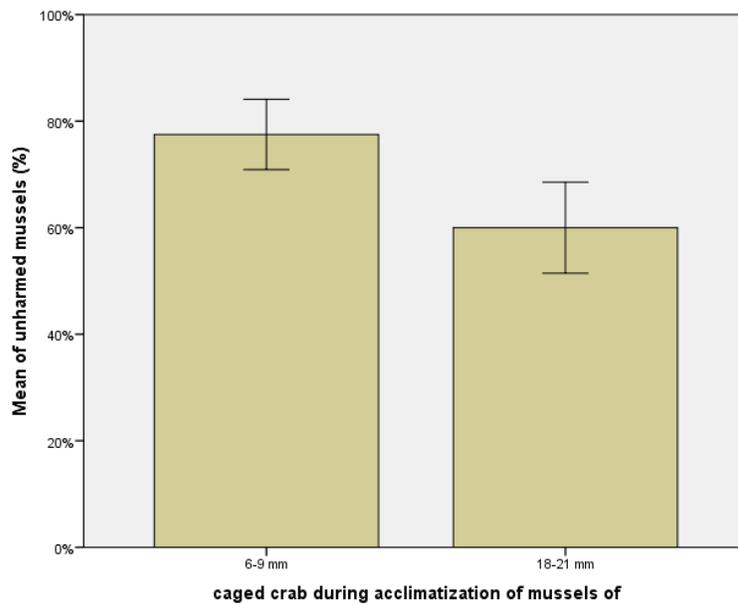


Figure 8C. Unharmed mussels of 6-9 mm and 18-21 mm with a caged crab present during acclimatization (mean % of 40 cases of 6-9 mm, and 40 cases of 18-21 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p=0.004$).

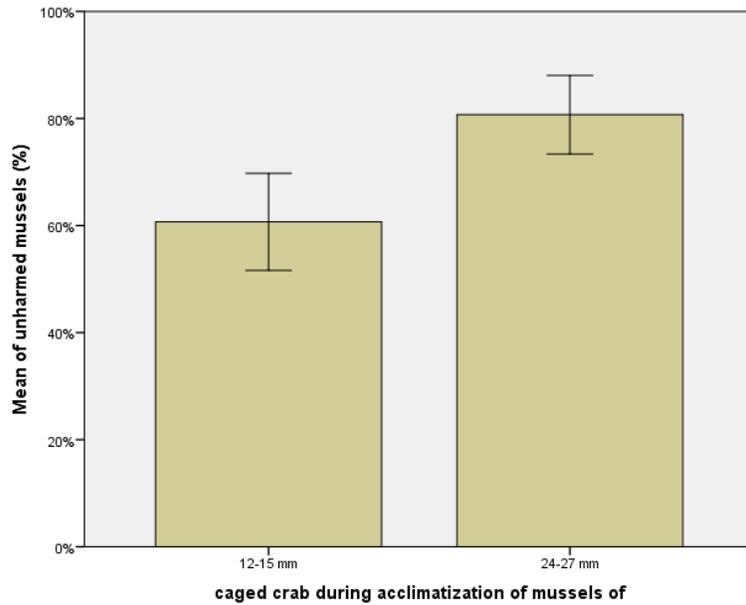


Figure 8D. Unharmed mussels of 12-15 mm and 24-27 mm with a caged crab present during acclimatization (mean % of 40 cases of 12-15 mm, and 40 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p=0.001$).

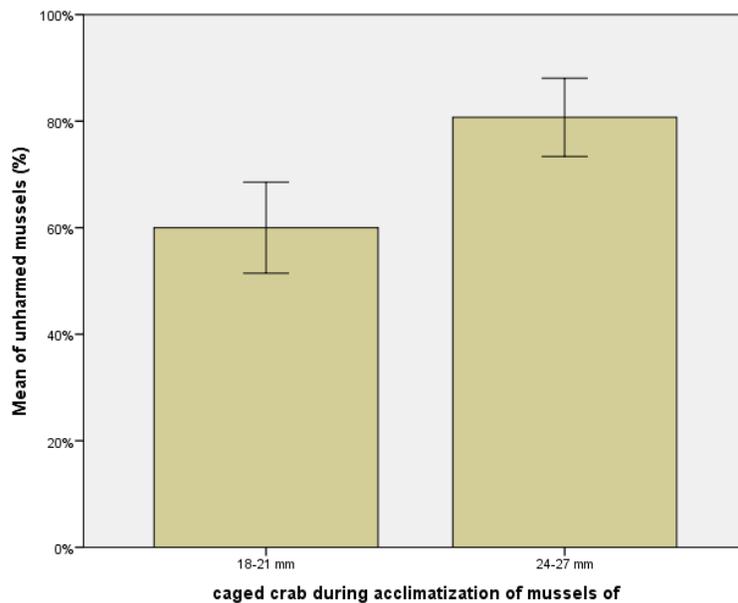


Figure 8F. Unharmed mussels of 18-21 mm and 24-27 mm with a caged crab present during acclimatization (mean % of 40 cases of 8-21 mm, and 40 cases of 24-27 mm). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p<0.001$).

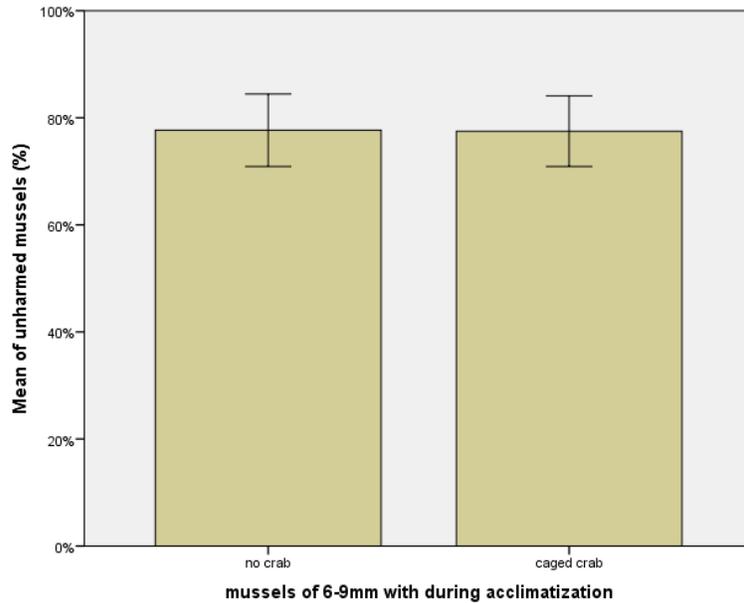


Figure 9A. Unharmed mussels of 6-9 mm with no crab and with a caged crab present during acclimatization on beds with oysters and without oysters (mean % of 37 cases for no crab, and 40 cases for caged crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is not significant(Mann-whitney; $p=0.955$).

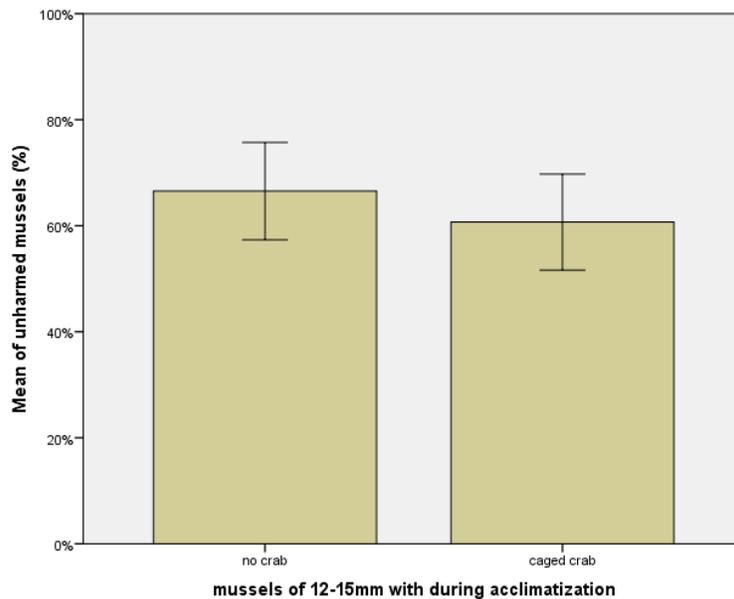


Figure 9B. Unharmed mussels of 12-15 mm with no crab and with a caged crab present during acclimatization on beds with oysters and without oysters (mean % of 37 cases for no crab, and 40 cases for caged crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is not significant(Mann-whitney; $p=0.317$).

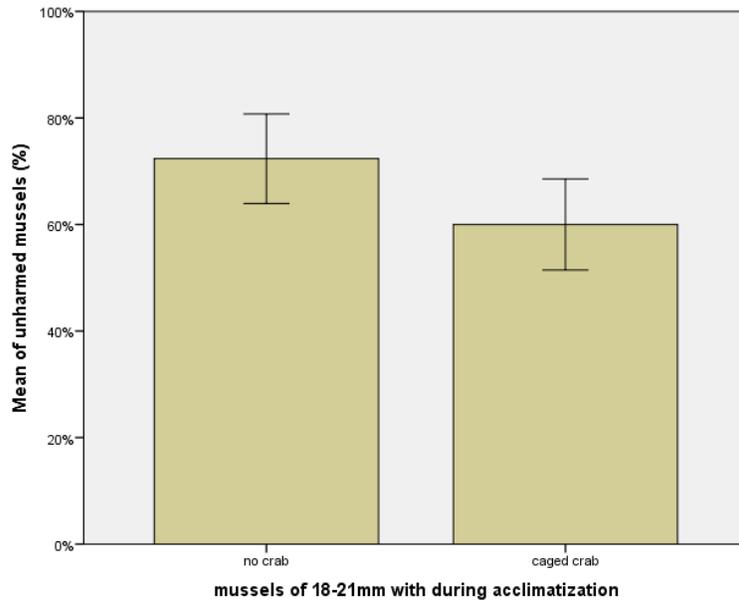


Figure 9C. Unharmed mussels of 18-21 mm with no crab and with a caged crab present during acclimatization on beds with oysters and without oysters (mean % of 37 cases for no crab, and 40 cases for caged crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is significant(Mann-whitney; $p=0.031$).

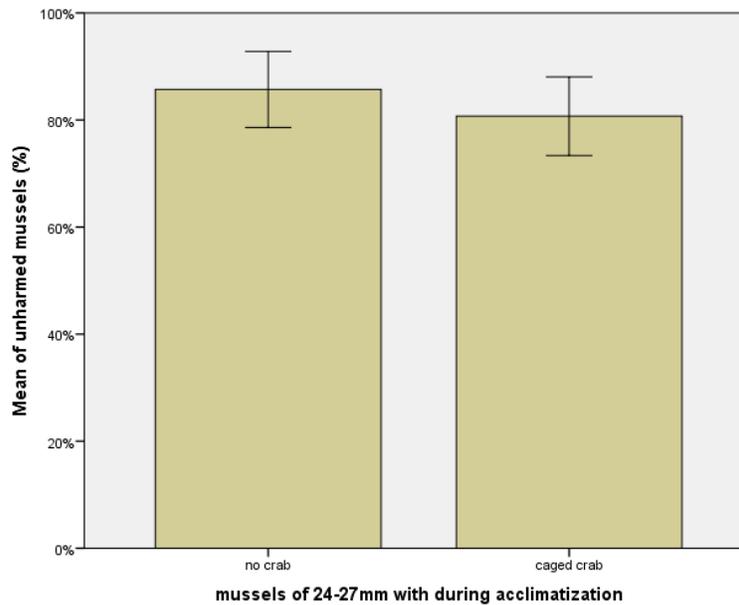


Figure 9D. Unharmed mussels of 24-27 mm with no crab and with a caged crab present during acclimatization on beds with oysters and without oysters (mean % of 37 cases for no crab, and 40 cases for caged crab). The error bars indicate the 95% Confidence Interval. The difference between both bars is not significant(Mann-whitney; $p=0.241$).

Appendix II: Final GLM output

Table 1A. GLM model with the significant interactions of lengthclassmussel*sizeclasscrab, oysters*sizeclasscrab and oysters*lengthclassmussel.

Tests of Between-Subjects Effects

Dependent Variable: unharmed

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|-----------------------------------|-------------------------|-----|-------------|----------|------|---------------------|
| Corrected Model | 76121,412 ^a | 13 | 5855,493 | 13,670 | ,000 | ,377 |
| Intercept | 1632066,251 | 1 | 1632066,251 | 3810,224 | ,000 | ,928 |
| oysters | 21572,277 | 1 | 21572,277 | 50,363 | ,000 | ,146 |
| lengthclassmussel | 20276,254 | 3 | 6758,751 | 15,779 | ,000 | ,139 |
| sizeclasscrab | 1606,765 | 1 | 1606,765 | 3,751 | ,054 | ,013 |
| Acclimatization | 3133,927 | 1 | 3133,927 | 7,316 | ,007 | ,024 |
| lengthclassmussel * sizeclasscrab | 18238,295 | 3 | 6079,432 | 14,193 | ,000 | ,127 |
| oysters * sizeclasscrab | 7981,294 | 1 | 7981,294 | 18,633 | ,000 | ,060 |
| oysters * lengthclassmussel | 4972,722 | 3 | 1657,574 | 3,870 | ,010 | ,038 |
| Error | 125931,570 | 294 | 428,339 | | | |
| Total | 1822554,516 | 308 | | | | |
| Corrected Total | 202052,983 | 307 | | | | |

a. R Squared = ,377 (Adjusted R Squared = ,349)

Table 1B. The effect size of the GLM model with the significant interactions of lengthclassmussel*sizeclasscrab, oysters*sizeclasscrab and oysters*lengthclassmussel.

Parameter Estimates

Dependent Variable: unharmed

| Parameter | B | Std. Error | t | Sig. | 95% Confidence Interval | | Partial Eta Squared |
|---|----------------|------------|--------|------|-------------------------|-------------|---------------------|
| | | | | | Lower Bound | Upper Bound | |
| Intercept | 71,413 | 4,354 | 16,403 | ,000 | 62,845 | 79,982 | ,478 |
| [oysters=1] | -2,076 | 5,235 | -,397 | ,692 | -12,378 | 8,226 | ,001 |
| [oysters=2] | 0 ^a | . | . | . | . | . | . |
| [lengthclassmussel=1] | 7,522 | 5,705 | 1,319 | ,188 | -3,706 | 18,750 | ,006 |
| [lengthclassmussel=2] | -16,727 | 5,705 | -2,932 | ,004 | -27,954 | -5,499 | ,028 |
| [lengthclassmussel=3] | -19,815 | 5,705 | -3,473 | ,001 | -31,043 | -8,587 | ,039 |
| [lengthclassmussel=4] | 0 ^a | . | . | . | . | . | . |
| [sizeclasscrab=1] | 10,180 | 5,264 | 1,934 | ,054 | -,181 | 20,540 | ,013 |
| [sizeclasscrab=2] | 0 ^a | . | . | . | . | . | . |
| [Acclimatization=1] | 6,391 | 2,363 | 2,705 | ,007 | 1,741 | 11,041 | ,024 |
| [Acclimatization=2] | 0 ^a | . | . | . | . | . | . |
| [lengthclassmussel=1] * [sizeclasscrab=1] | -33,754 | 6,677 | -5,055 | ,000 | -46,894 | -20,614 | ,080 |
| [lengthclassmussel=1] * [sizeclasscrab=2] | 0 ^a | . | . | . | . | . | . |
| [lengthclassmussel=2] * [sizeclasscrab=1] | -28,401 | 6,677 | -4,254 | ,000 | -41,541 | -15,261 | ,058 |
| [lengthclassmussel=2] * [sizeclasscrab=2] | 0 ^a | . | . | . | . | . | . |
| [lengthclassmussel=3] * [sizeclasscrab=1] | -1,026 | 6,677 | -,154 | ,878 | -14,166 | 12,115 | ,000 |
| [lengthclassmussel=3] * [sizeclasscrab=2] | 0 ^a | . | . | . | . | . | . |
| [lengthclassmussel=4] * [sizeclasscrab=1] | 0 ^a | . | . | . | . | . | . |
| [lengthclassmussel=4] * [sizeclasscrab=2] | 0 ^a | . | . | . | . | . | . |
| [oysters=1] * [sizeclasscrab=1] | 20,384 | 4,722 | 4,317 | ,000 | 11,090 | 29,677 | ,060 |
| [oysters=1] * [sizeclasscrab=2] | 0 ^a | . | . | . | . | . | . |

| | | | | | | | | |
|-------------------------------------|----------------|-------|-------|------|--------|--------|------|---|
| [oysters=2] * [sizeclasscrab=1] | 0 ^a | . | . | . | . | . | . | . |
| [oysters=2] * [sizeclasscrab=2] | 0 ^a | . | . | . | . | . | . | . |
| [oysters=1] * [lenghtclassmussel=1] | 6,414 | 6,672 | ,961 | ,337 | -6,717 | 19,546 | ,003 | |
| [oysters=1] * [lenghtclassmussel=2] | 21,812 | 6,672 | 3,269 | ,001 | 8,680 | 34,943 | ,035 | |
| [oysters=1] * [lenghtclassmussel=3] | 6,334 | 6,672 | ,949 | ,343 | -6,797 | 19,465 | ,003 | |
| [oysters=1] * [lenghtclassmussel=4] | 0 ^a | . | . | . | . | . | . | . |
| [oysters=2] * [lenghtclassmussel=1] | 0 ^a | . | . | . | . | . | . | . |
| [oysters=2] * [lenghtclassmussel=2] | 0 ^a | . | . | . | . | . | . | . |
| [oysters=2] * [lenghtclassmussel=3] | 0 ^a | . | . | . | . | . | . | . |
| [oysters=2] * [lenghtclassmussel=4] | 0 ^a | . | . | . | . | . | . | . |

a. This parameter is set to zero because it is redundant.

