Restoring mussel bed

de Paoli, Hélène Claudine

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 29-01-2020
Chapter 5: Maladaptation of mussels (*Mytilus edulis*) limits active restoration of intertidal mussel beds

Authors:
Hélène de Paoli, Marjolijn Christianen, Elisa Elofer, Henk Bolhuis, Tjisse van der Heide, Johan van de Koppel
Abstract

In this paper, we show that maladaptation of the blue mussel (*Mytilus edulis*) – an important engineering species that provides crucial habitats for many intertidal species – explains the failure of mussel bed restoration attempts in the Wadden sea. In this area, extensive restoration efforts were undertaken to restore mussel beds through transplantation of mussels from subtidal areas into artificially created intertidal beds, an effort that failed within six-month time. We tested the hypothesis that maladaptation of the subtidal mussels to wave-exposed intertidal conditions could explain the swift failure of mussel bed restoration projects. Transplantation experiments with intertidal, subtidal, and acclimatized subtidal mussels clearly showed that subtidal mussels are not able to persist in the intertidal area, irrespective of the acclimatization. To explain these differences, both the phenotypic and genotypic characteristics of mussels from subtidal environments were compared to mussels living in an intertidal system. Subtidal mussels were shown to have a lighter shell and exhibited weaker attachment, both characteristics that would decrease their chances to persist hydrodynamic stress. We found no evidence for genetically distinct ecotypes between the subtidal and intertidal mussel populations, suggesting strong phenotypic and behavioral adaptation to the prior habitat as underlying causes. These results suggest that, similar to what is found for more developed vertebrate animals, transplantation of invertebrates can be hampered by phenotypic adaptations that reduce survival in the new habitat.
Introduction

Reintroduction or transplantation of species into a habitat that it formally occupied is a common tool in the conservation and restoration of natural systems, in particular for large, charismatic animals such as wolves and beavers (Bangs and Fritts 1996, Nolet and Rosell 1998, Ripple and Beschta 2003, 2012). Such restoration measures are often used to overcome establishment barriers and thresholds that either prevent the target organisms to reach their new habitat, or when establishment thresholds imposed by degraded environmental conditions or high chances of predation limit the success of the first arriving settlers (Peterson et al. 2003, Crain and Bertness 2006, Halpern et al. 2007, Borsje et al. 2011, Eklof et al. 2011). Especially when the organisms involved are ecosystem engineers or foundations species on which many other organisms depend, reintroduction or transplantation success is crucial to the restoration of ecosystems that have been degraded by human activity. For that reason, many millions of dollars have been spent in the past decades to reintroduces keystone species to their formally native habitat (Zedler 2000, Groot et al. 2013, Lamers et al. 2015, Silliman et al. 2015)

Reintroduction of a species can be difficult, as the individuals from the source population may not be well-adapted to the new conditions they will encounter. Organisms can face problems in overcoming and adapting to new environmental conditions, in finding food, or intense predation (Angert and Schemske 2005, Geber and Eckhart 2005, Sexton et al. 2009). Transplantation of engineering organisms (e.g., seagrass, freshwater and salt marsh plants, corals, oysters) is a technique widely used to restore ecosystems where protection from human activity has proved unsuccessful for reaching the conservation goals (Clark and Edwards 1995, Hashim et al. 2010). However, despite some successful cases (Green and Short 2003, Schulte et al. 2009), such transplantation programs all too often have limited success (Harriot and Fisk 1988, Fonseca et al. 1998, Henn et al. 2014). An important potential explanation for the lack of success is a
limited behavioral and phenotypic plasticity of the transplanted organisms to their new environment (Snell-Rood 2012, 2013). In coastal areas, for instance, where high predation pressure is one of the most determinant stressors for recruiting organisms, bivalves or gastropods grow thicker shells to avoid predation (Appleton and Palmer 1988, Palmer 1990, Trussell 1996, Leonard et al. 1999), and in more hydrodynamically exposed conditions marine and freshwater mussels produce more byssal threads for attachment (Young 1985, Rajagopal et al. 1996). To what extend maladaptation of transplanted organisms limit the effectiveness of restoration projects is poorly understood, especially in coastal areas.

In the Wadden Sea, extensive restoration efforts have been undertaken to restore mussel beds, key habitats for many intertidal species, by transplanting mussels (*Mytilus Edulis*) from subtidal areas into artificially created intertidal beds, an effort that failed within six months time (de Paoli et al. 2015). In the Wadden Sea, mussels are found in both subtidal and intertidal habitats, where they experience widely differing conditions. In the intertidal area, mussels are living under a high degree of hydrodynamic stress (Janssen-Stelder 2000). In addition, predation on adult mussels by birds and crabs, which are breaking the shell of the mussel to eat the flesh, can be high (Elner 1978, Reise 1985, Hilgerloh et al. 1997, Smallegange et al. 2009, van der Zee et al. 2012). In subtidal areas, wave exposure and the resulting hydrodynamic stress is much lower, whereas predation is driven by starfish who, rather than cracking the shell, inject their stomach into the mussel to digest the flesh inside the shell (Saier 2001). At present, knowledge is still lacking as to what extent mussels from subtidal areas are suitable as a source population for restoration of intertidal beds.

In this project, we tested for the consequences of adaptive differences of mussel originating from intertidal versus subtidal habitats for restoration of mussel beds. We first experimentally compared the persistence following transplantation of intertidal and subtidal mussels, and mussels that were acclimatized to the dynamic intertidal habitat. Then,
we related differences in survival to genetic, morphological and behavioral differences. Finally, we discuss how our results can be used as a baseline for restoration programs.

**Material and Methods**

To investigate the importance of mussel adaptation to specific habitat conditions for their persistence within restoration plots, we obtained and compared mussels from waves and wind-exposed intertidal beds and wave-protected subtidal beds. We obtained the intertidal mussels from an intertidal mussel bed located on the South of Schiermonnikoog, and subtidal mussels were collected from a mussel bed, nearby Terschelling (Figure 5.1). All of the field experiment took place on mudflats situated at the south of Schiermonnikoog (53°28’3.43”N, 6°14’13.40”E).

**Influence of provenance and acclimation on transplantation success**

One month prior to the experiment, subtidal mussels (3.8 ± 0.06 cm) were obtained from a low-dynamic area, and transported to the experimental mudflat, where they were transferred to netted bags (mesh: 0.8cm, 25kg mussels per bag), and fixed in the middle of an intertidal mussel bed adjacent to the experimental site (within ~200 m). To test for treatment effects, we exposed intertidal mussels (4.8 ± 0.12 cm), coming from the same mussel bed, to the same treatment.
Influence of habitat and acclimation on morphology

To understand differences in survival between subtidal and intertidal mussels, their morphological characteristics were measured and compared. Subtidal (N=37) and intertidal mussels (N=40) were collected in June 2013. In addition, to test for any effects of the acclimation treatment, mussels were also sampled and measured before and after acclimation. For each mussel, length, depth, and width were measured using a caliper. Mussel volume (cm³) was estimated assuming an ellipsoid shape: \[ V = \frac{4}{3} \pi \times \text{length} \times \text{depth} \times \text{width} \].

The soft tissue was separated from the shell, dried independently for 24 h at 70°C, and subsequently weighted. Both dry weight of the soft tissue and the shell were used to compare treatments.

Genetic differences

To test whether any differences found in the transplantation experiment could be explained by genetic differences, we collected subtidal and intertidal mussels at 8 different locations (4 subtidal; 4 intertidal) in the Wadden Sea (Figure 5.1). Mussels were frozen (-20°C) and stored immediately after collection. Prior to processing, mussels were thawed, and rinsed in autoclaved demineralized water.

Nucleic Acid Extraction

For DNA extraction, approximately 100 mg of thawed mussel muscle tissue was rinsed in sterile milliQ water to remove residual salts and debris. DNA was extracted using the MO-BIO UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Although this kit is initially developed for soil bacteria, it gave good results for mussel tissue with respect to yield and purity. The bead-beating step was performed for 10 minutes. The DNA concentration and purity was measured.

Figure 5.1: Sampling sites where we collected mussels for the laboratory and field experiments, the genetic analyses.

Next, we created small artificial beds (50 x 50cm) using subtidal and intertidal mussels, crossed with two acclimated mussel treatments, yielding a total of 4 treatments. First, non-acclimated subtidal mussels (3.9 ± 0.1 cm) were collected and transported by boat to the experimental site on the same day. Intertidal mussels (4.4 ± 0.2 cm) were collected within the same mussel bed used for acclimation. Artificial beds were built using 375 (+/- 5%) individual mussels (cover = 35%). As subtidal mussels and intertidal mussels do not weigh the same, the weight of mussels used to build the beds was different (4.5 kg of intertidal mussels or 2.5 kg of subtidal mussels) to end up with the same density. The experiment was set up in June 2013 and replicated 6 times, yielding 24 beds in total. Pictures of the plots were taken every day during 6 days to follow the evolution of mussel cover during the first days of experiment. After 20 days, remaining mussels on each plot were collected and weighed to measure the percentage of mussels surviving the transplantation procedure.
Influence of habitat and acclimation on morphology

To understand differences in survival between subtidal and intertidal mussels, their morphological characteristics were measured and compared. Subtidal (N=37) and intertidal mussels (N=40) were collected in June 2013. In addition, to test for any effects of the acclimation treatment, mussels were also sampled and measured before and after acclimation. For each mussel, length, depth and width were measured using a caliper. Mussel volume (cm$^3$) was estimated assuming an ellipsoid shape:

$$Volume = \frac{4}{3} \pi \cdot \text{Length} \cdot \text{Depth} \cdot \text{Width}$$

The soft tissue was separated from the shell, dried independently for 24h at 70°C, and subsequently weighted. Both dry weight of the soft tissue and the shell were used to compare treatments.

Genetic differences

To test whether any differences found in the transplantation experiment could be explained by genetic differences, we collected subtidal and intertidal mussels at 8 different locations (4 subtidal; 4 intertidal) in the Wadden Sea (Figure 5.1). Mussels were frozen (-20°C) and stored immediately after collection. Prior to processing, mussels were thawed, and rinsed in autoclaved demineralized water.

Nucleic Acid Extraction

For DNA extraction, approximately 100 mg of thawed mussel muscle tissue was rinsed in sterile milliQ water to remove residual salts and debris. DNA was extracted using the MO-BIO UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Although this kit is initially developed for soil bacteria, it gave good results for mussel tissue with respect to yield and purity. The bead-beating step was performed for 10 minutes. The DNA concentration and purity was
Sequence analysis

DNA sequence analyses were performed using Geneious version 6.1.8 by Biomatters. (http://www.geneious.com/). After ClustalW-based alignment and cluster analysis, phylogenetic trees were constructed using the maximum likelihood algorithm with 1000 bootstrap iterations.

Behavioral differences

To test for any behavioral differences between sub- and intertidal mussels, their movement was studied in a laboratory experiment, at the NIOZ in Yerseke. Subtidal and intertidal mussels were collected in June 2013. Small subtidal and intertidal mussel beds consisting of 250 mussels were set up in 120 x 80-cm tanks, supplied by fresh seawater pumped directly from the Eastern Scheldt, and continuously oxygenated with an air diffuser. Mussels were randomly placed on a hard substrate (stone tiles), and pictures were taken at the start of the experiment and after 5 hours, using a camera (canon powershot D10) mounted over the tanks. Using Matlab, we noted the starting position (X,Y) of a mussel, and its position after 5 hours, with which we calculated the total distance travelled by individual mussels within that period. The experiment was replicated 4 times (N=40).

To analyze organization time, we tracked the movement of 2 subtidal and 2 intertidal mussels over 15 h in a single experiment. Pictures were taken every minute using a time lapse camera (canon powershot D10) located over the tanks. If mussels moved less than 2 cm within an hour, we considered the movement involuntary. The time mussels stopped doing voluntary movement was estimated as organization time. This experiment was replicated 4 times (N=8).

Potential differences between sub- and intertidal mussels regarding attachment to substrate were studied in separate laboratory experiments. Small artificial beds (50 x 50 cm) made from sub- and intertidal mussels were set up in 2 tanks (van de Koppel et al. 2008). Mussels were randomly determined spectrophotometrically using the NanoDropTM ND-1000 spectrophotometer (NanoDrop products, Wilmington, DE, USA).

Table 5.1: Primers used for ecotyping

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFbis_F</td>
<td>5'ACAAGATGGACAATACCCGAACCACC 3'</td>
<td>Bierne et al 2003</td>
</tr>
<tr>
<td>EFbis_R2</td>
<td>5' CCTTCTGGATTTCATGAATCGG 3'</td>
<td>Bierne et al 2003</td>
</tr>
<tr>
<td>maca_F</td>
<td>5' GCTGTATTTCATCAATTTGTTGG 3'</td>
<td>Bierne et al 2003</td>
</tr>
<tr>
<td>mac-db_R</td>
<td>5' CGAAAAATTGTAGTCTAGTTTTGTG 3'</td>
<td>Bierne et al 2003</td>
</tr>
<tr>
<td>PLHaa_F</td>
<td>5' GAGCCCAAGTAGAATCCCG 3'</td>
<td>Heath et al., 1995</td>
</tr>
<tr>
<td>PLHaa_R</td>
<td>5' CCTTCGCATTTGAGATTTATT 3'</td>
<td>Heath et al., 1995</td>
</tr>
<tr>
<td>MAL-1_1H2_F</td>
<td>5' GCCGAGTGCTTTATTGAGACG 3'</td>
<td>Rawson et al., 1996</td>
</tr>
<tr>
<td>MAL-1_PR9_R</td>
<td>5' CTTCATGGGCGGTTTTGCTC 3'</td>
<td>Rawson et al., 1996</td>
</tr>
</tbody>
</table>

Ecotyping

To determine whether individual mussels belonged to the same or different ecotypes, four different nuclear loci (EFbis, mac-1, MAL-1 and PLIIa) were sequenced (Table 5.1), each of which are present as a single gene, and each of which were used in previous ecotyping studies (Heath et al. 1995, Ramon et al. 1996, Bierne et al. 2003). Primers for EFbis amplify the non-coding intron of elongation factor 1α (Bierne et al. 2003), primers for MAC-1 amplify the non-coding first intron of the actin gene (Bierne et al. 2003), MAL-1 primers amplify the coding part of the so called Mytilus anonymous locus 1 (a locus with unknown function) (Ramon et al. 1996) and the PLIIa primer set amplifies the coding part of the y-protamine-like sperm packaging protein (Heath et al. 1995). Primer generation and Sanger DNA sequencing was performed at BaseClear BV, Leiden, the Netherlands. A total of 42 samples were submitted for sequencing of the 4 different gene fragments. Heterozygous nucleotide differences were visible in the electropherograms as two overlapping fluorescent traces and were ignored for ecotyping analysis.
Sequence analysis

DNA sequence analyses were performed using Geneious version 6.1.8 by Biomatters. (http://www.geneious.com/). After ClustalW-based alignment and cluster analysis, phylogenetic trees were constructed using the maximum likelihood algorithm with 1000 bootstrap iterations.

Behavioral differences

To test for any behavioral differences between sub- and intertidal mussels, their movement was studied in a laboratory experiment, at the NIOZ in Yerseke. Subtidal and intertidal mussels were collected in June 2013. Small subtidal and intertidal mussel beds consisting of 250 mussels were set up in 120x80-cm tanks, supplied by fresh seawater pumped directly from the Eastern Scheldt, and continuously oxygenated with an air diffuser. Mussels were randomly placed on a hard substrate (stone tiles), and pictures were taken at the start of the experiment and after 5 hours, using a camera (canon powershot D10) mounted over the tanks. Using Matlab, we noted the starting position (X,Y) of a mussel, and its position after 5h, with which we calculated the total distance travelled by individual mussels within that period. The experiment was replicated 4 times (N=40).

To analyze organization time, we tracked the movement of 2 subtidal and 2 intertidal mussels over 15h in a single experiment. Pictures were taken every minute using a time lapse camera (canon powershot D10) located over the tanks. If mussels moved less than 2 cm within an hour, we considered the movement involuntary. The time mussels stopped doing voluntary movement was estimated as organization time. This experiment was replicated 4 times (N=8).

Potential differences between sub- and intertidal mussels regarding attachment to substrate were studied in separate laboratory experiments. Small artificial beds (50x50 cm) made from sub- and intertidal mussels were set up in 2 tanks (van de Koppel et al. 2008). Mussels were randomly
placed on a hard substrate (tile) and were allowed to aggregate for 24h. After this time, attachment to substrate was measured for 15 individual mussels using a Wagner Force DialTM FDK/FDN with peak force meter (WAGNER 2 INSTRUMENTS, Greenwich, CT, USA). Each mussel was pulled up vertically, and the maximum force used to detach the mussel from the bed was measured as attachment force. The experiment was replicated 4 times resulting in 60 replicate measurements.

**Statistical analyses**

All statistical tests were run in SPSS 22. For each test, normality and homogeneity of data or residuals were tested. Differences in survival were studied using Analysis of Variance (ANOVA), with biomass as response variable and habitat (subtidal or intertidal) and acclimation (acclimated or non-acclimated) as factors. The data were square root transformed to improve the normality of the residuals. Influence of habitat on shell height was studied with a Wilcoxon test, using shell weight as variable and habitat as factor. The effect of habitat on flesh weight was tested with a student t-test, and the effect of acclimation on shell weight was studied using a Wilcoxon signed-rank test, using acclimation as factor. Effect of acclimation on flesh weight was studied in a student t-test, using acclimation as factor. Differences in attachment strength, movement between subtidal and intertidal mussels were also tested using a t-test with square root transformed data to improve data normality. Differences in organization time, were tested using a t-test.

**Results**

**Influence of habitat and acclimation on transplantation success**

The results of the field experiment clearly showed that subtidal mussels are not able to persist in the dynamic intertidal environment.
Moreover, acclimation did not increase chances of persistence (Figure 5.2). After 20 days of experiment, only $1.6 \pm 1.6\%$ of the subtidal mussels persisted, while $66.9 \pm 6.1\%$ of the intertidal mussels persisted ($F_{1,24}=168.3$, $p<0.001$). We did not find any significant effect of the acclimation treatment ($F_{1,24}=2.45$, $p=0.133$), suggesting that acclimation of subtidal mussels to the intertidal environment is not relevant at the timescale of the experiment.

![Figure 5.2: Remaining mussel biomass after 20 days of experiment. Intertidal mussels exhibited much higher survival than subtidal mussels. We found no significant effect of the acclimation treatment. Mean +/- SE](image)

**Influence of habitat and acclimation on morphology**

Morphological parameters revealed clear differences between sub- and intertidal mussels. The flesh/shell ratio of intertidal mussels ($0.09 \pm 0.008$, Figure 5.3A) was found to be lower than for subtidal mussels ($0.19 \pm 0.008$, $t_{37}=9.054$, $p<0.001$). The shell of intertidal mussels ($595.6 \pm 2.8\,\text{mg/cm}^3$) was about 2 times heavier (Figure 5.3B) than the shell of subtidal mussels ($299.3 \pm 6\,\text{mg/cm}^3$, $t_{37}=-8.1$, $p<0.001$). Moreover, our measurements showed that intertidal mussels have less flesh ($47.8 \pm 2.8\,\text{mg/cm}^3$, Figure 5.3C) than subtidal mussels, ($56.5 \pm 2.8\,\text{mg/cm}^3$, $t_{37}=-2.05$, $p=0.03$). Even though the shell weight of intertidal mussels stayed constant over acclimation time, the flesh weight of acclimated intertidal mussels was...
reduced by 0.7 times compared to the control (33.46 ± 3.22 mg/cm³ and 47.8 ± 2.82 mg/cm³ respectively, tₙ₋₀=3.3, p=0.002), but this treatment did not affect the flesh and shell of subtidal mussels.

![Figure 5.3: Morphological differences between sub- and intertidal mussels. A: Flesh/Shell ratio. B: Flesh weight. C: Shell weight. Mean +/- SE](image)

**Genetic analysis**

Not all DNA sequences gave a readable electropherogram, potentially due to multiple copies of the locus on the genome, and resequencing these samples did not improve the result. Nevertheless, the dataset was sufficiently large and covered most sampling sites with a good distribution of mussels sampled from the sub- and intertidal zones. Although a few mutations were observed at the four loci, we could not detect divergence in the DNA sequences of those for loci between mussels from sub- and intertidal locations.

**Behavioral differences**

We found strong behavioral differences between sub- and intertidal mussels in the laboratory experiment. Intertidal mussels attached more than 2 times stronger than subtidal mussels (Figure 5.4A, 1.08N ± 0.06N...
and \(0.46 \pm 0.04N\) respectively, \(t_{68}=9.53, p<0.001\). The distance travelled by individual mussels within 5h did not differ between sub- and intertidal mussels (Figure 5.4B, \(2.99 \pm 0.03\) cm, \(t_{8}=0.83, p=0.41\)), indicating that the tendency to aggregate was similar for both groups. However, organization time (Figure 5.4C) was higher for intertidal mussels (9.2 ± 1.4h) than for subtidal mussels (4.6 ± 0.98h, \(t_{4}=2.65, p=0.019\)).

**Figure 5.4:** Behavioral differences between sub- and intertidal mussels in our laboratory experiment. A: Attachment force (N) after 24h. B: Distance travelled by individual mussels (cm) within 5h. C: Organization time (h). Mean +/- SE

### Discussion

Restoration of populations and habitats is a priority in the management of endangered ecosystems all over the world (Lotze et al. 2006, Durant et al. 2007, Halpern et al. 2008, Brierley and Kingsford 2009). Especially in areas dominated by habitat modifying species, transplantation of engineering organisms from healthy source areas is a widely applied restoration technique (Clark and Edwards 1995, Hashim et al. 2010, Silliman et al. 2015). Although a number for these transplantation projects have been successful (Green and Short 2003, Schulte et al. 2009), many have shown limited success (Harriott and Fisk 1988, Fonseca et al. 1998, Henn et al. 2014). The present study clearly shows that restoration of
Intertidal mussel beds by transplanting mussels from subtidal source areas (de Paoli et al. 2015) has – at least in part – failed due to maladaptation of subtidal mussels to the conditions that prevail on intertidal flats. The persistence of subtidal mussels in our field experiment was very low, while the majority of intertidal mussels persisted. More detailed comparisons of sub- and intertidal mussels revealed clear morphological and behavioral differences, where subtidal mussels had a lighter shell than intertidal mussels, and also showed weaker attachment to the abiotic substrate. Both of these factors are likely important for their persistence on intertidal flats, where mussels face high wave exposure and intense predation by birds and crabs. Hence, similar to what is observed in reintroductions with iconic vertebrate species, such as wolves or bears (Weaver 1978, Smith et al. 2003, Dax 2015), we observed that the restoration success of invertebrate mussels was limited by low adaptation of the source organisms to the conditions they are facing in their new habitat.

Restoration of intertidal mussel through transplantation of subtidal mussels has been attempted multiple times in the Wadden Sea, over decades, but without any success (Ens and Alting 1997, Pelt et al. 2003, Ens et al. 2004, de Paoli et al. 2015). Our results show that this failure can be explained by maladaptation of subtidal mussels to the harsher conditions in the intertidal environment. Similar to our findings, a study carried on horse mussels showed that transplantation success for this species can also be limited by different shell morphology (Fariñas-Franco et al. 2016), whereas attachment strength has similarly been shown to be important for zebra mussels (Rajagopal et al. 1996). Moreover, for blue mussels, aggregative behavior has been shown to be an important factor in mussel survival (van de Koppel et al. 2003). Hence differences in attachment strength, aggregative behavior, and shell thickness may provide a potential explanation for the limited success of bivalve transplantation in a wide range of restoration experiments.

Subtidal and intertidal mussels showed clear differences in morphology and behavior. The shell of intertidal mussels was about two
times heavier and attached twice as strong compared to subtidal mussels. The weak shells of subtidal mussels might be a limiting factor for their survival in intertidal areas, because mussels are heavily predated by birds (oystercatchers and gulls) and crabs that both crack the shell to reach the flesh (Elner 1978, Hilgerloh et al. 1997, Smallegange et al. 2009, van der Zee et al. 2012). In subtidal conditions, starfish pose the most prominent predatory threat to mussels, and their method of opening mussels by extruding their gut content into the mussels leads to little benefit of having a thick shell. In addition to shell strength, weak attachment to the sediment and conspecifics may lead to low survival due to higher losses as a result of dislodgement by waves and currents. In subtidal areas, where hydrodynamic stress (especially waves) is typically much lower, the required attachment strength in order to survive is lower. Hence, it appears that the traits that mussels adopt in subtidal habitat pose a disadvantage when they are transplanted to the intertidal.

The observed differences in morphology, behavior and resilience to the stress/force of water and susceptibility to predation of sub- and intertidal mussel populations could in principle have resulted from genetic, epigenetic or physiological adaptation to the extant environmental conditions. Genetic adaptation could have led to two genetically distinct populations (eco-types) that affect surface adhesion and self-clustering. When such genetic ecotypes exist, one would expect genetic diversification throughout the genome. We found, however, no evidence for such differences as genetic ecotyping did not link any of the variation found to the source being sub- or intertidal. This implies that the morphological and behavioral differences between intertidal and subtidal mussels in the Dutch Wadden Sea are most likely phenotypic (i.e. differences in gene expression developed in their native habitats), or result from selection on random, phenotypic variation in characteristics. Moreover, we did not find any adaptation of subtidal mussels when they were allowed to adjust to the intertidal environment, suggesting that phenotypic adaptation is either...
transferred over generations or happens early in development, and persisting afterwards.

The results of this study can be used as a baseline for future restoration projects. Our results show that direct transplantation of adult subtidal mussels to the intertidal has little chance of success. Transplantation of young mussels could potentially yield higher success rate, as they may acclimatize faster to a new environment (Smith and Jennings 2000). However, in any restoration program that encompasses the transplantation of organism in a different environment, we suggest it is useful to determine the potential success rate in pilot transplantations before scaling up. As restoration of intertidal mussel beds using subtidal mussels is, nowadays, not conceivable, it might be more important to (1) focus on protecting the remaining beds, by improving local conditions (limiting dredging or fishery activities) and (2) in case of restoration develop techniques to facilitate natural settlement by limiting predation and providing suitable settlement substrate (Van der Heide et al., 2014).
transferred over generations or happens early in development, and persisting afterwards. The results of this study can be used as a baseline for future restoration projects. Our results show that direct transplantation of adult subtidal mussels to the intertidal has little chance of success. Transplantation of young mussels could potentially yield higher success rate, as they may acclimatize faster to a new environment (Smith and Jennings 2000). However, in any restoration program that encompasses the transplantation of organisms in a different environment, we suggest it is useful to determine the potential success rate in pilot transplantations before scaling up. As restoration of intertidal mussel beds using subtidal mussels is, nowadays, not conceivable, it might be more important to (1) focus on protecting the remaining beds, by improving local conditions (limiting dredging or fishery activities) and (2) in case of restoration, develop techniques to facilitate natural settlement by limiting predation and providing suitable settlement substrate (Van der Heide et al., 2014).